UNIVERSITA' DEGLI STUDI DI CAGLIARI

SCUOLA DI DOTTORATO IN SCIENZE E TECNOLOGIE FARMACEUTICHE

Coordinatore: Prof. Elias Maccioni

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Desarollo de derivados de 4-hidroxicumarina con diferente actividad farmacológica.

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"Dico ai giovani: non pensate a voi stessi, pensate agli altri. Pensate al futuro che vi aspetta, pensate a quello che potete fare, e non temete niente. Non temete le difficoltà: io ne ho passate molte, e le ho attraversate senza paura, con totale indifferenza alla mia persona."

Rita Levi Montalcini

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*A mis amigos "*santiagueses*" por haberme acompañado en esa experiencia inolvidable y especial.*

A todos ellos gracias!

I Índice

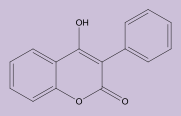
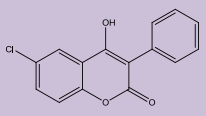
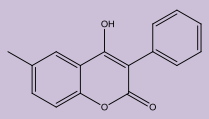
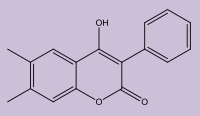
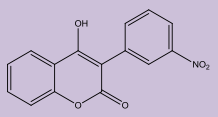
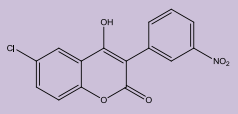
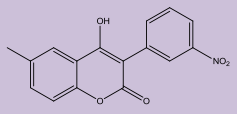
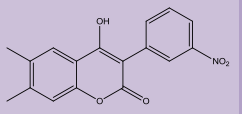
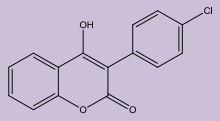
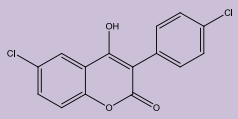
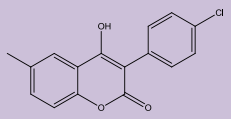
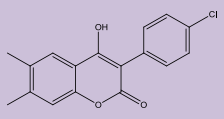
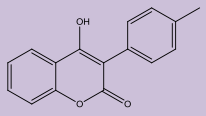
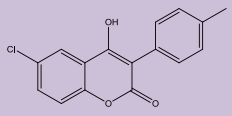
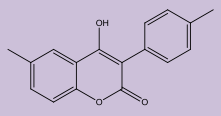
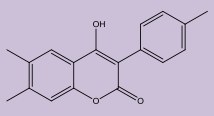
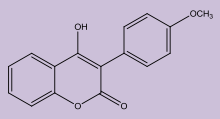
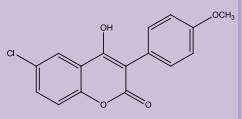
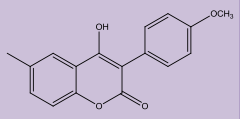
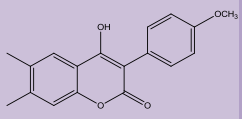
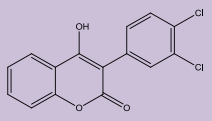
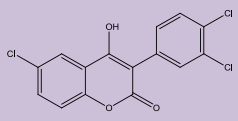
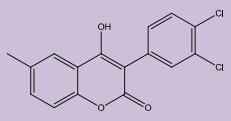
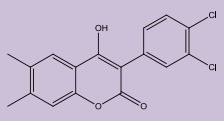
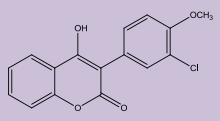
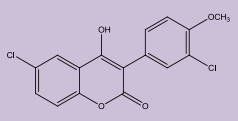
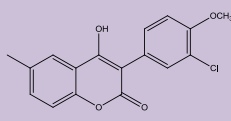
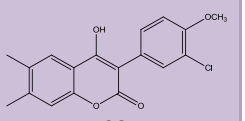
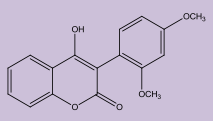
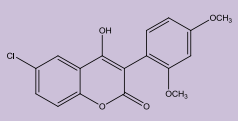
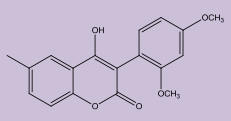
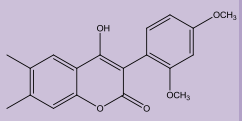
II Relación de abreviaturas

AAPH	2,2′-azobis(2-amidino-propano)dihidrocloruro
AcOEt	acetato de etilo
AcOH	ácido acético
ADN	ácido desoxirribonucleico
Ar	argón
AUC	área bajo de la curva
^{o}C	grados Celsius
δ	desplazamiento químico en ppm
d	doblete
dd	doble doblete
DCM	diclorometano
DME	dimetoxietano
DOPA	dopamina
DMSO	dimetilsulfóxido
EtOH	etanol
FL	fluoresceína
G	gramo
µg	microgramo
h	hora
HDL	lipoproteínas de alta densidad
Hz	hertzio
iMAO	inhibidores monoaminooxidasa
J	constante de acoplamiento
m	multiplete
M	molaridad
µM	micromolar
MAO	monoaminooxidasa
MeOH	metanol
mg	milígramo
min	minuto
mL	mililitro
mmol	milimol
8-MOP	8-metoxi-psoraleno
m/z	relación masa/carga
µL	microlitros
ORAC	capacidad de absorción de radicales de oxígeno
ORAC-FL	capacidad de absorción de radicales de oxígeno con fluoresceína
Pf	punto de fusión
ppm	partes por millón
QSAR	relación cuantitativa estructura-actividad
REA	relación estructura-actividad
RMN	resonancia magnética nuclear
s	singlete
t	triplete
TLC	cromatografia en capa fina
TMS	tetrametilsilano

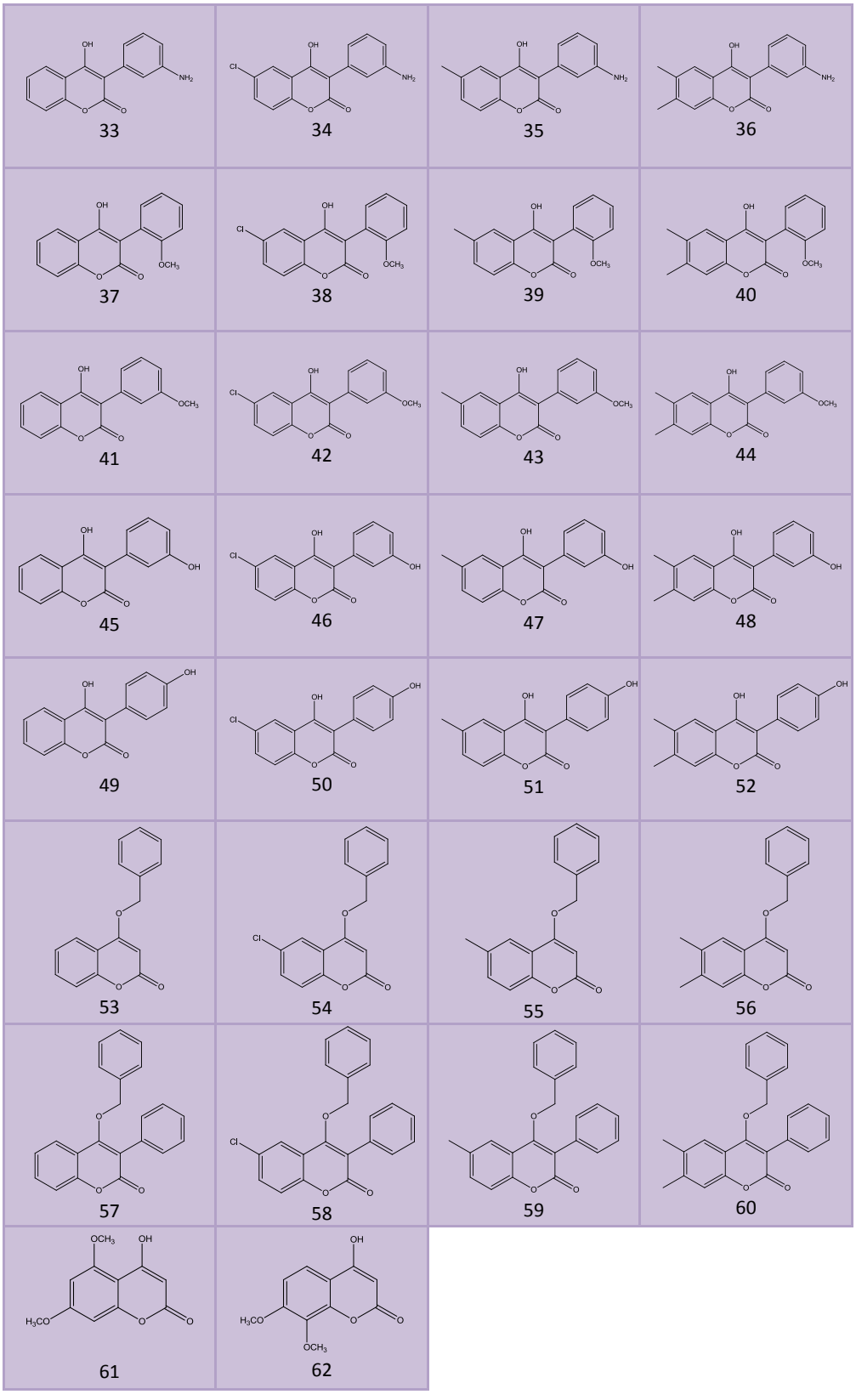
UV	radiación ultravioleta

III Relación de compuestos finales obtenidos

Relación de compuestos finales obtenidos

 1	 2	 3	 4
 5	 6	 7	 8
 9	 10	 11	 12
 13	 14	 15	 16
 17	 18	 19	 20
 21	 22	 23	 24
 25	 26	 27	 28
 29	 30	 31	 32

Relación de compuestos finales obtenidos



1. Introducción

1.1. Las cumarinas

Las cumarinas conocidas también como benzopironas, son una familia de compuestos de origen natural y sintético que han suscitado desde mucho tiempo un gran interés debido a sus posibles aplicaciones biológicas.[1,2,3,4]

En las plantas se pueden encontrar en general en las raíces, las hojas, los frutos y las flores (**Figura 1**).

Fig.1: *Galium odoratum* (Rubiaceae), *Illicium verum* (Illiciaceae), *Neochamaolea pulverulenta* (Ecneoraceae).

La primera cumarina (**Figura 2**), fue aislada de las semillas del árbol *Coumarona Odorata Aube*[5] y de ello deriva el nombre de la familia de estos compuestos con estructuras más o menos complejas.[6,7,8]

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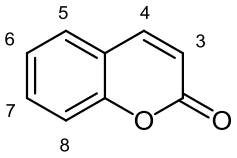

Fig. 2: 2H-chromen-2-one (cumarina)

1.2. Actividad biológica de las cumarinas

Debido a la gran variedad estructural de estas moléculas son muchas las propiedades farmacológicas asociadas a dicho anillo, entre otras: antimicrobianas,[9,10] antiinflamatorias,[11,12] antiespasmódicas, antivirales,[13,14] antihelmínticas, antioxidantes,[15] o inhibidoras enzimáticas.[16,17] Existen además derivados tricíclicos o tetracíclicos de cumarinas que se comportan como intercalantes del ADN y, por tanto, tienen interés como antitumorales o bien como agentes fotoquimioterápicos en el tratamiento de la psoriasis (8-MOP)[18,19] (**Figura 3**).

La warfarina y el carbocromeno, ambos utilizados actualmente en clínica, son dos conocidos ejemplos de la familia de las cumarinas con actividad cardioprotectora, debido a su acción vasodilatadora[20] e inhibidora de la agregación plaquetaria.[21,22]

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Hay que destacar además la actividad antimicrobiana de compuestos como la novobiocina y la clorobiocina así como la actividad antiviral de compuestos 4-fenilcumarinicos[23] y 7,8-piranocumarínicos(**Figura 3**).[24]

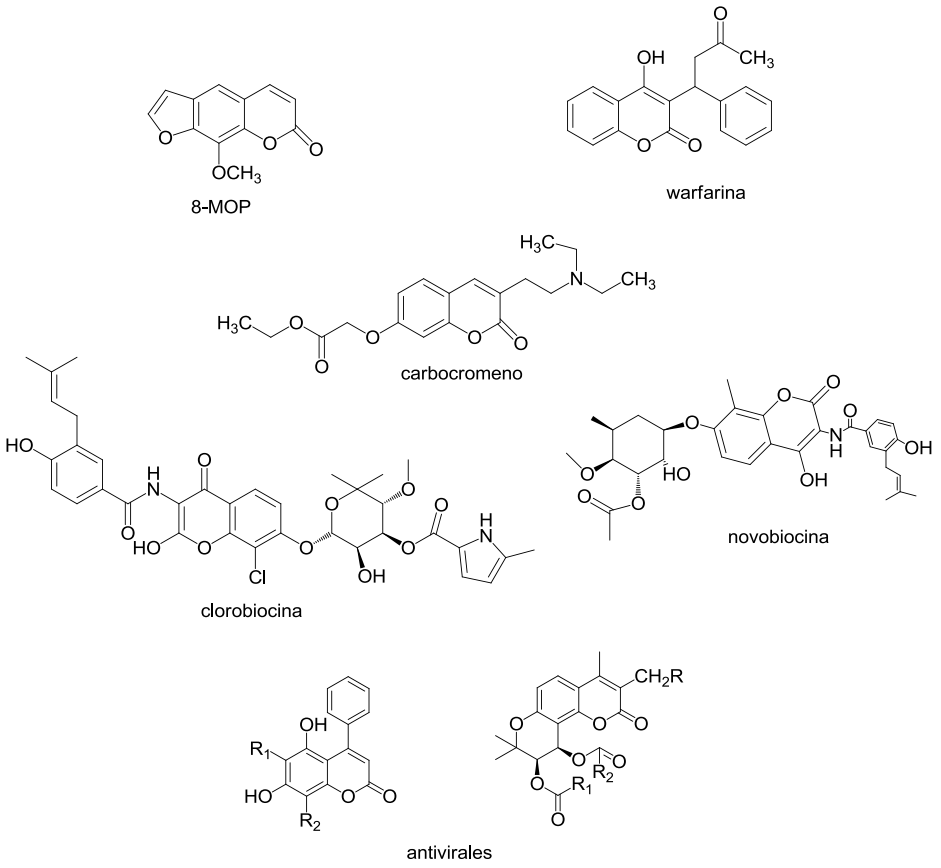

Fig. 3

En los últimos años nuestro grupo de investigación ha sintetizado por primera vez análogos cumarínicos para los que ha encontrado una muy interesante actividad inhibidora de la monoaminooxidasa (iMAO).[25] La MAO, es una flavoenzima implicada en la degradación de aminas y presenta dos isoformas, conocidas como MAO-A y MAO-B. Están localizadas a nivel de las membranas

[23]Bedoya, L.; Beltran, M.; Sancho, R.; Olmedo, D. A.; Del Olmo, E.; Lopez-Perez, J. L.; San Feliciano, A. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4447.
[24]Xie, L.; Yu, D.; Wild, C.; Allaway, G.; Turpin, J.; Smith, P. C.; Lee, K. *J. Med. Chem.* **2004**, *47*, 756.
[25](a) Matos, M. J.; Viña, D.; Quezada, E.; Picciau, C.; Delogu, G.; Orallo, F.; Santana, L.; Uriarte, E. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3268 ; (b) Delogu, G.; Picciau, C.; Ferino, G.; Quezada, E.; Podda, G. Uriarte, E.; Viña. D. *Eur. J. Med. Chem.* **2011**, *46*, 1147.

mitocondriales de las células neuronales e intervienen en la desaminación de neurotransmisores como la serotonina, adrenalina y noradrenalina, de ahí las posibles aplicaciones terapéuticas[26,27] como iMAO, por ejemplo en el tratamiento de la enfermedad de Parkinson (iMAO-B) o en el tratamiento de la depresión (iMAO-A).

Además de estas actividades mencionadas anteriormente, algunas cumarinas han mostrado actividad inhibidora frente a la tirosinasa. En los mamíferos, la tirosinasa está implicada en la oxidación de L-tirosina a dopaquinona la cual es un intermedio clave en la biosíntesis de la melanina,[28] pigmento implicado en diversos procesos biológicos tales como la hiperpigmentación de la piel y aparición de melanomas. Por otra parte, en la industria alimentaria juega también un importante papel en la conservación y en la estabilidad de frutas y vegetales.[29]

Es por ello que el estudio de los inhibidores de la tirosinasa tiene hoy en día un gran interés no solo terapéutico en el tratamiento de enfermedades de la piel, sino un gran impacto económico e industrial debido a su utilidad como agentes cosméticos despigmentantes.[30]

Es bien conocida la actividad inhibidora de la tirosinasa de la arbutina y del ácido kojico, considerados los primeros compuestos de referencia si bien el primero ha sido prohibido debido a sus efectos secundarios. Estrechamente relacionados, los flavonoides así como sus precursores biosintéticos tipo chalcona han sido descritos como inhibidores de tirosina.[31]

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[26]Chimenti, F.; Secci, D.; Bolasco, A.; Chimenti, P.; Bizzarri, B.; Granese, A.; Carradori, S.; Yánez, M.; Orallo, F.; Ortuso, F.; Alcaro, S. *J. Med. Chem.* **2009**, *52*, 1935.

[27]Brühulmann, C.; Ooms, F.; Carrupt, P. A.; Testa, B.; Catto, M.; Leonetti, F.; Altomare, C.; Carotti, A. *J. Med. Chem.* **2001**, *44*, 3195.

[28]Ando H., Matsui M. S., Ichihashi M. *Int. J. Mol. Sci.* **2010**, *11*, 2566.

[29]Friedman M. *J. Agric. Food Chem.* **1996**, 44 (3), 631.

[30]Hassan Khan M. T. *Pure Appl. Chem*. **2007**, Vol. 79, No. 12, 2277.

[31](a) Khatib, S.; Nerya, O.; Musa, R.; Shmuel, M.; Tamir, S.; Vaya, J. *Bioorg. Med. Chem*. **2005**, *13*, 433; (b) Kim, S. J.; Son, K. H.; Chang, H. W.; Kang, S. S.; Kim, H. P. *Biol. Pharm. Bull*. **2003**, *26*, 1348.

Por otra parte, se han descrito recientemente las benzilidenbenzofuranonas (auronas) isómeros de las flavonas para las que se ha encontrado una interesante actividad inhibidora de dicho enzima (**Figura 4**).[32]

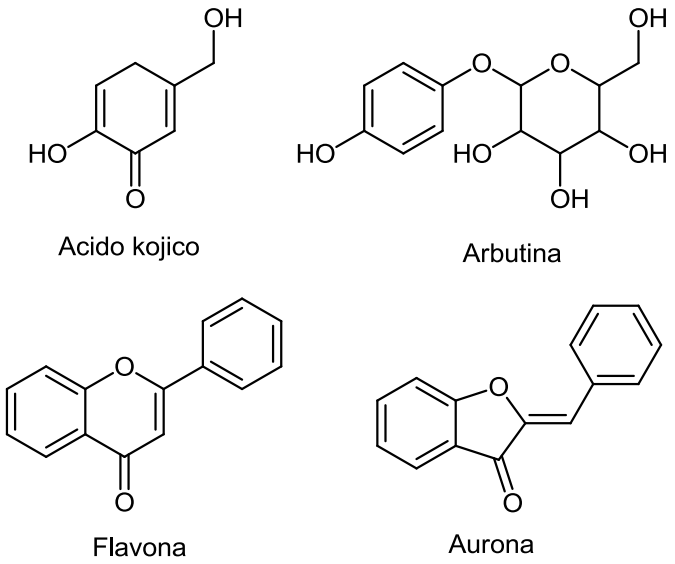
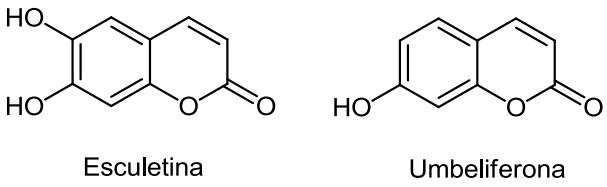


Fig. 4

Muy estrechamente relacionadas con las estructuras mencionadas, Masamoto *et al*.[33] han propuesto la estructura cumarínica como un nuevo prototipo de potencial actividad inhibidora de la tirosinasa. El estudio de la relación estructura-actividad de 18 análogos cumarínicos ha revelado que la mayor actividad inhibidora de todos los compuestos estudiados corresponde a la esculetina. En contraste con ese estudio, Sollai *et al*.[34] han demostrado que la esculetina es un substrato de la tirosinasa más que un inhibidor, y el análogo monohidroxilado, la umbeliferona es un inhibidor de dicha enzima (**Figura 5**).

[32]Okombi, S.; Rival, D.; Bonnet, S.; Mariotte, A.; Perrier, E.; Boumendjel, A. *J. Med. Chem*, **2006**, *49*, 329.
[33]Masamoto, Y.; Murata, Y;. Baba, K; Shimoishi, Y;, Tada, M.; Takahata, K. *Biol. Pharm. Bull.* **2004,** *27*, 422.
[34]Sollai, F.; Zucca, P.; Sanjust, E.; Steri, D.; Rescigno, A. *Biol. Pharm. Bull.* **2008**, *31*, 2187.



Esculetina Umbeliferona

Fig. 5

1.3. El resveratrol

El resveratrol[35,36] es una fitoalexina de origen natural producida por algunas especies de espermatofitas, como las vides en respuesta a un daño sufrido, por ejemplo una infección por hongos o bajo condiciones de estrés, como la pérdida de las hojas (**Figura 6**).[37]

Se encuentra en la piel de la uva y no en la pulpa, por lo cual existe en mayor concentración en el vino tinto que en el vino blanco (donde el tiempo de contacto con la piel de la uva en la fermentación es mucho más grande-mayor tiempo de maceración).

Fig. 6: Vitis Vinifera

En el 1976 los ingleses Langcake y Pryce[38] descubrieron la estructura de esta molécula. Se trata del 3,5,4′-trihidroxi estilbeno, cuyo isómero *trans*, mayoritario, es la forma más activa. El *trans*-resveratrol posee propiedades espectroscópicas ultravioletas y de fluorescencia características (**Figura 7**).

[35]Leiro, J.; Álvarez, E.; Arranz, J. A.; Laguna, R.; Uriarte, E.; Orallo, F. *J. Leukoc. Biol.* **2004**, *75*, 1156.
[36]Orallo, F.; Álvarez, E.; Camiña, M; Leiro, J. M.; Gómez, E.; Fernández, P. *Mol. Pharmacol.* **2002**, *61*, 294.
[37]Orsini, F.; Pelizzoni, F.; Verotta, L.; Aburjai, T. *J. Nat. Prod*. **1997** *60*, 1082.
[38]Langcake, P.; Pryce, R. J. *Physiol. Plant Pathol*. **1976** *9*, 77.

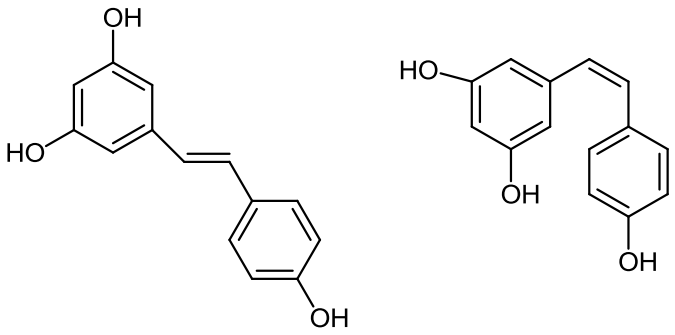


Fig.7: Formas isomericas del resveratrol (*trans* y *cis*-resveratrol)

El interés por este compuesto y sus derivados se fue incrementando a medida que los estudios epidemiológicos establecieron una relación inversa entre el consumo de vino tinto y la incidencia de enfermedades cardiovasculares (paradoja francesa),[39] además de las propiedades hemostáticas y del aumento del HDL circulante descritas para el etanol.[40]

1.4. Actividad biológica del resveratrol

Por su gran interés farmacológico en los últimos años se ha estudiado ampliamente esta molécula.[41] Después de un extenso estudio de farmacocinética de este compuesto, se concluye que su biodisponibilidad por administración oral es bastante satisfactoria. Un consumidor moderado de vino tinto absorbe cuantidad suficiente para explicar su efecto beneficioso en la salud humana.[42]

El resveratrol presenta propriedades antiinflamatorias, antioxidantes y cardioprotectoras (vasodilatador e inhibidor de la agregación plaquetaria).[43]

[39]Renaud, S.; De Lorgeril, M. *Lancet*. **1992**, *339*, 1523.
[40]Leger, A. S.; Cochrane, A. L.; Moore, F. *Lancet*. **1979**, *1*, 1017.
[41]Andrus, M.; Liu. J. *Tetrahedron Lett.* **2006**, *47*, 5811.
[42]Orallo, F. Biological Effects of *Cis*- versus *Trans*-Resveratrol, in: B. B. Aggarwal, S. Shishodia (Eds), *Resveratrol in Health and Disease*, CRC Press, Boca Raton, USA, **2005**, 577.
[43]Frémont, L. *Life Sci.* **2000**, *66*, 663.

Además de las propiedades descritas, y más relacionado con nuestra línea de investigación, se ha demostrado una interesante actividad del resveratrol como inhibidor enzimático de la MAO.

El *cis*-resveratrol resulta ser menos efectivo que el *trans*-resveratrol sobre dicha enzima.[44]

El resveratrol ha mostrado una actividad inhibidora DOPA oxidasa más elevada que el ácido kojico utilizado como referencia.[45]

1.5. Los flavonoides: flavonas e isoflavonas.

Los flavonoides constituyen una de las subfamilias de polifenoles naturales a las que la comunidad científica ha dedicado más atención en los últimos años. Sus múltiples propiedades biológicas observadas experimentalmente y su abundancia en la dieta, junto con su presencia en numerosos remedios de la medicina tradicional, los convierten en posibles candidatos a explicar la asociación encontrada entre el consumo de determinados productos de origen vegetal y la disminución del riesgo de presentar determinadas enfermedades crónicas.

Con el nombre de flavonoides se conoce a un gran número de compuestos naturales de bajo peso molecular, la mayoría con estructura de fenil-benzopirona (o fenil-cromona), productos del metabolismo secundario vegetal. En las plantas, los flavonoides se encuentran en estado simple o en forma de heterósidos. La estructura química básica de los flavonoides consiste en un esqueleto carbonado C6-C3-C6, donde los componentes C6 son anillos aromáticos unidos por tres átomos de carbono que pueden formar o no un tercer anillo pirano o pirona (anillos A-C). Las distintas clases de flavonoides se diferencian en la concentración de saturación y en los sustituyentes del anillo C (**Figura 8**), mientras que los compuestos individuales, dentro de cada uno de estos grupos, se distinguen por la diferente sustitución de los anillos A y B. De esta forma, se

[44]Yánez, M.; Fraiz, N.; Cano, E.; Orallo, F. *Biochem. Biophys. Res. Commun.* **2006**, *344*, 688.
[45]Likhitwitayawuid K. *Current Science* **2008**, *94*, 44.

han identificado hasta 4.000 compuestos diferentes. Entre ellos se pueden distinguir:

- **Flavonas**, derivados de la estructura 2-fenilcromen-4-ona (2-fenil-1,4-benzopirona).
- **Isoflavonas**, derivados de la estructura 3-fenilcromen-4-ona (3-fenil-1,4-benzopirona).

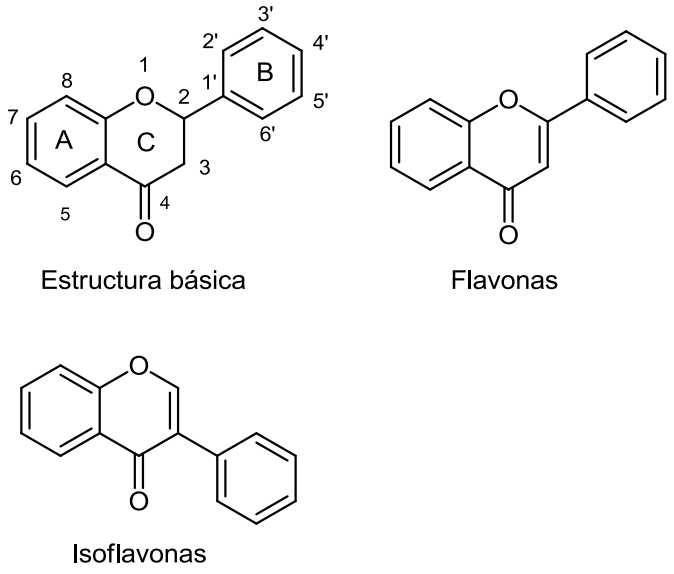


Fig.8

1.6. Actividad biológica de flavonoides

Son muchas las actividades biológicas asociadas a estos compuestos,[46,47] entre ellas, además de la mencionada inhibición de la tirosinasa, se han descrito como antiinflamatorios y analgésicos, antioxidantes, antitumorales, antimicrobianos, así como relacionadas con el sistema cardio-vascular:

- como cardiotónicas tienen un efecto tónico sobre el corazón, potenciando el músculo cardíaco y mejorando la circulación;

[46]Martínez-Flórez, S.; González-Gallego, J.; Culebras, J. M.; Tuñón, M. J. *Nutr. Hosp*. **2002**, *6*, 271.
[47]Álvarez-Castro, E.; Orallo-Cambeiro, F. *OFFARM* **2003**, *22*,130.

- como protectores capilares, los flavonoides con mejores resultados en este campo son la hesperidina, la rutina y la quercetina;
- como antitrombóticas;
- como hipocolesterolémicos.

2. Antecedentes y objetivos

Desde hace tiempo el Laboratorio de Química Orgánica de la Facultad de Farmacia de la USC, en colaboración con el Laboratorio de Química Orgánica de la Facultad de Farmacia de la Universidad de Cagliari viene trabajando en el diseño, síntesis y estudio farmacológico de nuevas moléculas con diferentes actividades biológicas.

Uno de los prototipos estudiados ha sido el ***trans*-resveratrol** y otros análogos estilbénicos debido a las ya mencionadas propiedades farmacológicas, entre ellas el potente efecto vasodilatador. Además de los conocidos efectos cardioprotectores del resveratrol, se conoce también la actividad vasorelajante y vasodilatador coronaria del anillo **cumarínico**, estructura que posee un amplio número de actividades farmacológicas tal y como hemos expuesto con anterioridad.

Así, debido a la amplia experiencia de nuestro grupo de investigación en ambos tipos de estructuras, en los últimos años hemos llevado a cabo el diseño de moléculas híbridas **resveratrol-cumarina** (**I**)[48,49] con objeto no solo de potenciar su actividad cardioprotectora[50] sino de explorar además otras actividades farmacológicas.

Las moléculas preparadas mostraron efectivamente actividad cardioprotectora, y además sometidos a estudios de inhibición enzimática, dichos híbridos mostraron, una muy interesante actividad inhibidora de las monoaminooxidasas MAOs, muchos de ellos con una actividad en el rango sub nM-pM, con la ventaja de ser muy selectivos a la MAO-B y por tanto de gran interés como fármacos anti-Parkinson.[51] Dichos compuestos se han mostrado además muy efectivos como inhibidores de la tirosinasa.

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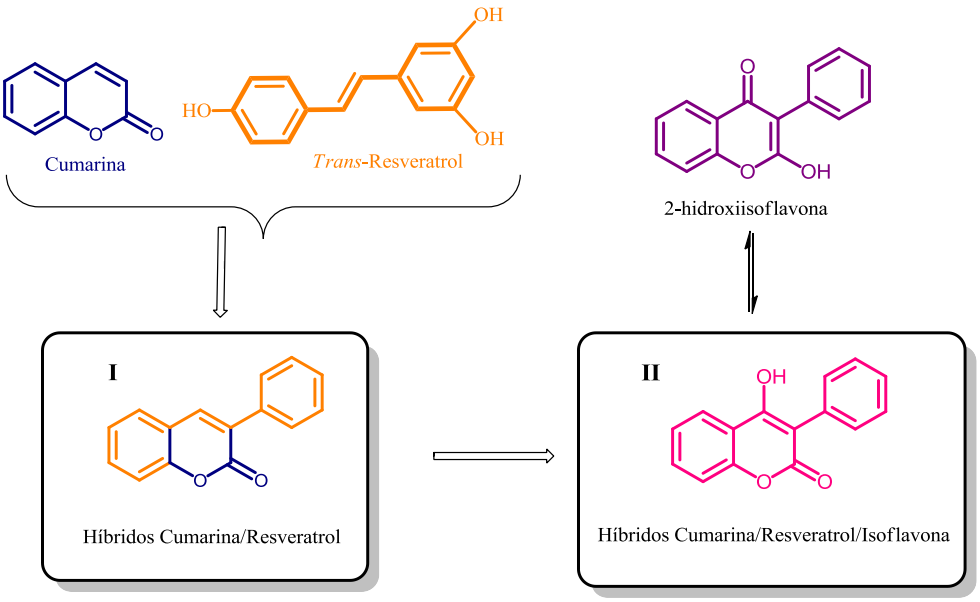
[48]Vilar, S.; Quezada, E.; Santana, L.; Uriarte, E.; Yánez, M.; Fraiz, N.; Alcaide, C.; Cano, E.; Orallo, F. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 257.

[49]Vilar, S.; Quezada, E.; Alcaide, C.; Orallo, F.; Santana, L.; Uriarte, E. *Qsar Comb. Sci.* **2007**, *26*, 317.

[50]Orallo, F.; Alvarez, E.; Camina, M.; Leiro, J. M.; Gomez, E.; Fernandez, P. *Mol. Pharmac.* **2002**, *61*, 294.

[51](a) Santana, L.; González-Díaz, H.; Quezada, E.; Uriarte, E.; Yánez, M.; Viña, D.; Orallo, F. *J. Med. Chem.* **2008**, *51*, 6740; (b) Matos, M. J.; Viña, D.; Janeiro, P.; Borges, F.; Santana, L.; Uriarte. E. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5157; (c) Matos, M. J.; Terán, C.; Pérez-Castillo, Y.; Uriarte, E. Santana, L.; Viña, D. *J. Med. Chem.* **2011**, *54*, 7127; (d) Viña, D.; Matos, M. J.; Yáñez, M.; Santana, L.; Uriarte, E. *Med. Chem. Comm.* **2012**, *3*, 213.

Teniendo en cuenta estos aspectos, y con objeto de incrementar dichas actividades y aportar nuevos datos sobre las relación estructura-actividad (REA) en cada campo, en el presente trabajo nos propusimos el diseño de nuevos análogos (**II**) en los que se incorpora un grupo hidroxílico en la posición 4 del prototipo híbrido 3-arilcumarínico (**Esquema I**).



Esquema I

Dicho objetivo nos permitirá:

1) Aproximarnos estructuralmente a otro tipo de estructuras: los flavonoides muy abundantes en la naturaleza tales como **flavonas** e **isoflavonas** de conocido interés en enfermedades cardiovasculares tal y como hemos señalado anteriormente.

2) Estudiar los efectos de la incorporación del grupo hidroxilo sobre la actividad y/o selectividad MAO-A/B y su potencial aplicación al tratamiento de enfermedades neurodegenerativas tales como el Parkinson o el Alzheimer.

3) Poner a punto un procedimiento sintético sencillo, eficiente y versátil que nos permita acceder a este tipo de compuestos.

4) Preparar, purificar y caracterizar series de 4-hidroxi-3arilcumarinas.

5) Estudiar la actividad de estos compuestos frente a diferentes dianas farmacológicas.

3. Parte teórica y resultados

3.1. Síntesis de cumarinas

La síntesis de cumarinas y de sus derivados es un área de gran y permanente interés por el gran numero de compuestos tanto de origen natural como sintético que presentan este anillo heterocíclico y porque son a veces utilizadas como intermediarios en la síntesis de furocumarinas,[21] piranocumarinas[52] o 2-acil-resorcinoles[53,54] etc.

En el transcurso de los años, varios procesos de síntesis han sido estudiados y probados para la obtención de estos derivados, y así como al principio los primeros métodos presentaban demasiados pasos y en general bajos rendimientos[55], hoy las rutas sintéticas más utilizadas recurren a reacciones de condensación de Pechmann, Perkin, Reformatsky, Knoevenagel o Wittig a partir de fenoles convenientemente sustituidos.

Sin embargo, es cada vez mayor el uso de la "Green Chemistry" que tiende a disminuir el uso de disolventes potencialmente tóxicos y/o caros recurriendo a la utilización de resinas o de líquidos iónicos o a técnicas de irradiación con microondas.[56]

De las varias alternativas sintéticas, en esta memoria hemos decidido recurrir a un método alternativo: la reacción de Suzuki,[57,58] catalizada por paladio y utilizando como especie electrófila el fenil-yodonio zwitterión para sintetizar las 4-hidroxi-3-arilcumarinas.

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[52]Moskvina, V. S.; Khilya, V. P. *Chem. Nat. Comp.* **2008**, *44*, 16.
[53]Singh, P. R.; Singh, De V. U.; Samant, S. D. *Synl*ett. **2004**, *11*, 1909.
[54]Quezada, E.; Delogu, G.; Viña, D.; Santana, L.; Podda, G.; Matos, M. J.; Picciau, C. *Helv. Chim. Acta* **2009**, *92*, 1309.
[55]Roman, J.; Kozhkov, V.; Larock, R. C. *J. Org. Chem*. **2003**, *68*, 6314.
[56](a) Tuanli, Y.; Dawei, Y.; Larock, R. C. *J. Org. Chem.* **2005**, *70*, 9985; (b) Bogdal, D. *J. Chem. Research* **1998**, *(S)*, 468; (c) Hoz, A.; Moreno, A.; Vázquez, E. *Synlett*. **1999**, *5*, 608; (d) Kidwai, M.; Kumar, P. *J. Chem. Research* **1997**, *(S)*, 178.
[57]Zhu, Q.; Wu, J.; Fathi, R.; Yang, Z. *Org. Lett.* **2002**, *4*, 3333.
[58](a) Miyaura, N. *et al. Tetrahedron Lett.* **1979**, *20*, 3437; (b) Miyaura, N.; Suzuki, A. *Chem. Commun.* **1979**, *19*, 866; (c) Suzuki, A. *J. Organometallic Chem.* **1999**, *576*, 147.

3.2. Discusión del planteamiento sintético

Con el nombre de reacción de Suzuki se denominó en 1979 la primera síntesis de biarilos por acoplamiento cruzado de ácidos aril borbónicos con halogenuros de arilo realizada por Suzuki y Miyaura. La reacción se generalizó posteriormente, abarcando los acoplamientos entre compuestos organoborónicos y sustratos como halogenuro de alquenilo, alquinilo y arilo. Los primeros acoplamientos llevados a cabo por Suzuki consistieron en la condensación de acido fenilbóronico con varios halogenuros de arilo, utilizando como catalizador Pd $(PPh_3)_4$. Posteriormente se han introducido numerosas modificaciones en este procedimiento original, como por ejemplo el uso de otros catalizadores de paladio como $PdCl_2(PPh_3)_4$ u otros sin ligandos tipo fosfina. Por otra parte se han utilizado otras bases como trietilamina, bicarbonato sódico en combinación en algunos casos con catalizadores de transferencia de fase como Bu_4NCl o éteres corona. Además se han empleado diferentes disolventes tales como dimetoxietano (DME). La reacción transcurre como un ciclo (**Figura 9**) por lo tanto se pueden distinguir distintas etapas: 1) adición oxidativa del paladio (0) a la especie electrófila; 2) adición de la base que actúa activando el complejo de paladio, es decir, favoreciendo la formación de la especie $RPd(Nu)L_2$ a partir de $RPdXL_2$ y facilitando de esta forma la siguiente etapa; 3) transmetalación con organoboranos; 4) eliminación reductiva con liberación del compuesto biarilo y regeneración del paladio (0).

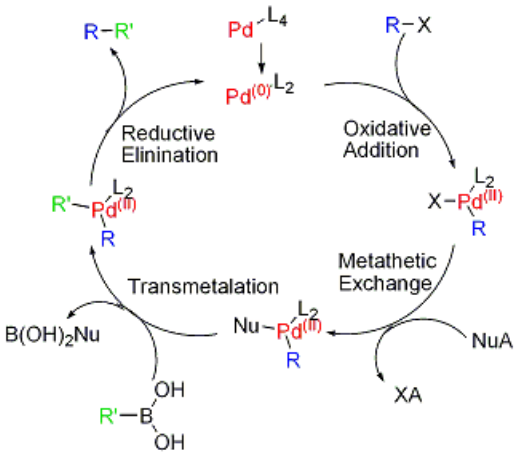
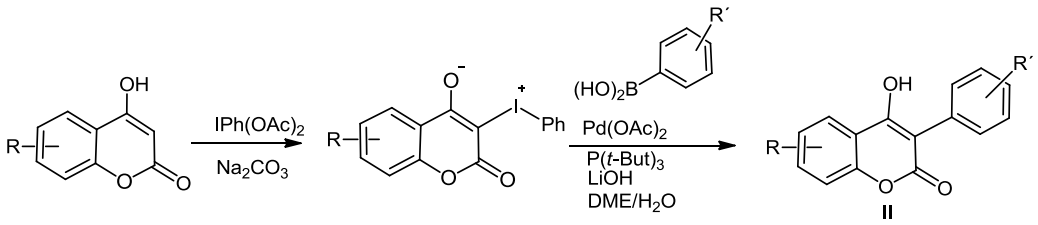


Fig.9

En nuestro caso la introducción del grupo arilo en la posición 3 del anillo cumarínico se ha llevado a cabo mediante la formación inicial de una especie intermedia de fenil-yodonio y posterior reacción de acoplamiento con el correspondiente ácido arilborónico, ambos convenientemente sustituidos. (**Esquema II**).[57]



Esquema II

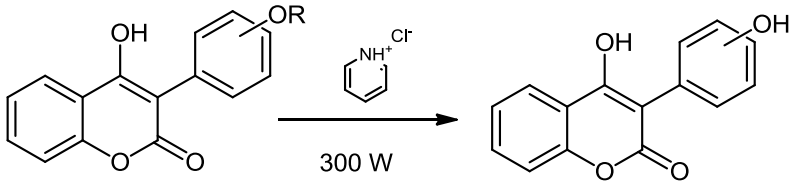
La síntesis de la especie intermedia de 3-fenil-yodonio zwitterión se ha llevado a cabo por reacción de distintas 4-hidroxicumarinas con el yodobenzodiacetato. Esos intermedios actúan como buenos electrófilos y se utilizan sin purificación posterior en la siguiente reacción de Suzuki. Los productos finales obtenidos (**II**) son sólidos fácilmente purificables por cromatografía de columna.

Con este método rápido y sencillo hemos obtenido una serie de 44 compuestos con modificaciones en las posiciones 6 y 7 del esqueleto cumarínico y en las

posiciones 2', 3', 4'del arilo, con unos rendimientos que varían entre 40% y 80% dependiendo de los sustituyentes de los reactivos de partida.

Los derivados mono-metoxilados **17-20** y **41-44** han sido hidrolizados para la obtención de los correspondientes hidroxi derivados **45-52**. Los métodos más comunes[59,60] para esta etapa recurren a reacciones en la presencia de un ácido de Lewis como el MgI_2, el $AlCl_3$, el BBr_3 o el $AlBr_3$. Como fuentes de protones son utilizados ácidos o alcoholes, siendo de uso común el HCl, el MeOH o el EtOH. Estas reacciones se llevan a cabo, en su gran mayoría, utilizando diclorometano, acetonitrilo, acetona, tetrahidrofurano o piridina como disolventes.

Otra forma de hidrolizar, aunque de más difícil elaboración y purificación, es la reacción con el ácido yodhídrico, en presencia de ácido acético y anhídrido acético. Esta reacción se lleva a cabo a reflujo, durante 4-5 horas. En este trabajo los derivados hidroxilados se han obtenido por hidrólisis en microondas de los correspondientes compuestos metoxilados en presencia de cloruro de piridinio a 300W durante solo 6 minutos (**Esquema III**).[61] Hemos decidido aplicar este método por la sencillez y rapidez del procedimiento que nos ha permitido sintetizar los derivados desiderados con buenos rendimientos.

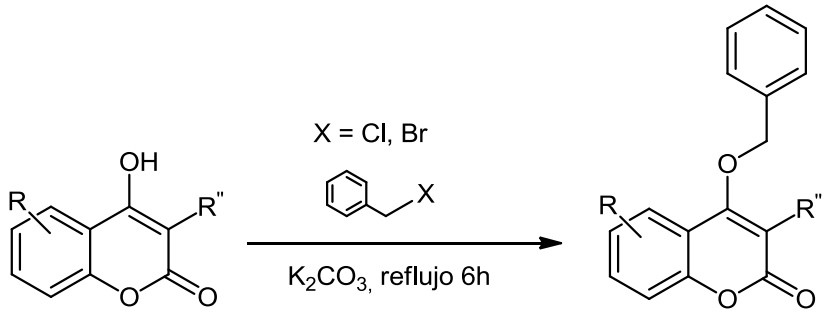


Esquema III

La síntesis de los compuestos **53-60** nos ha permitido introducir un grupo éter sobre el grupo 4-hidroxi, costante en todos lo derivados sintetizado hasta ahora,

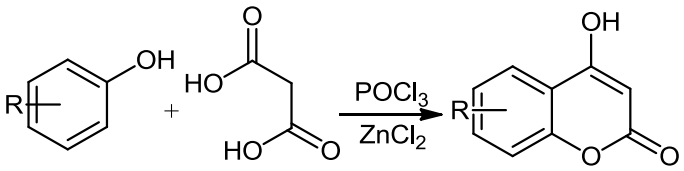
[59]Fraginals R.; Schaeffer J. M.; Stampf J. L.; Benezra C. *J. Med. Chem.* **1991**, *34*, 1024.
[60]Kulkarni P.; Kadam A. J.; Mane R. B.; Uday V.; Desai, P.; Wadgaonkarb Shivaji P. *J. Chem. Research* **1999**, *(S)*, 394.
[61]Horie T.; Tominaga H.; Kawamura Y.; Hada T.; Ueda N.; Amano Y.; Yamamoto S. *J. Med. Chem.* **1991**, *34*, 2169.

con el objectivo de ver como ese cambio pueda afectar la actividad biológica. De esa forma hemos preparado distintas 4-benciloxicumarinas. De todas las reacciones para la obtención de dicho anillo, la reacción de Williamson es la alternativa más eficaz para la mayoría de los autores. Transcurre a través del mecanismo de tipo SN_2 a partir de un haloalcano y un alcoxido o un alchol en medio básico. En nuestro caso hemos utilizado haluro de bencilo y K_2CO_3 como base (**Esquema IV**).[62]



Esquema IV

En este momento, estamos intentando incrementar la versatilidad de nuestra quimioteca, con objeto de explorar sobre todo el efecto de los sustituyentes en la posición 7 del anillo cumarínico. Así pues, intentamos la preparación de las 4-hidroxicumarinas 5,7 o bien 7,8 disubstituidas **61** y **62** mediante reacción de los correspondientes fenoles con ácido malónico en presencia de una mezcla de $POCl_3$/ $ZnCl_2$ seco (**Esquema V**).



Esquema V

[62](a) Raghu Ram, S.; Krupadanam, G. L. D.; Srimannarayana, G. *Synthetic Communications* **1998**, *28*, 2421; (b) Majumdar, K. C.; Khan, A. T.; Chattopadhyay, S. K. *Indian J. Chem. A.* **1990**, *29b*, 483; (c) Stefanachi, A.; Favia, A. D.; Nicolotti, O.; Leonetti, F.; Pisani, L.; Catto, M.; Zimmer, C.; Hartmann, R. W.; Carotti, A. *J. Med. Chem.* **2011**, *54*, 1613.

Desafortunadamente los resultados no corresponden a los esperados ya que si bien hemos preparado los derivados **61** y **62**, los rendimientos son bastantes bajos (26-35 %). Lo que nos obliga a poner a punto una nueva estrategia sintética para la preparación de nuevos derivados.

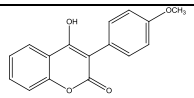
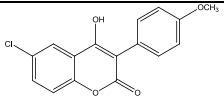
3.3. Discusión de la actividad biológica

3.3.1. Actividad iMAO

Sobre la base de los buenos resultados previos obtenidos por el grupo con las 3-arilcumarinas, hemos decidido de evaluar la actividad inhibidora de la monoaminooxidasa de los compuestos sintetizados en ese proyecto y de estudiar la influencia que los distintos sustituyentes introducidos en varias posiciones pueden aportar en dicha actividad.

Se trata de una serie de derivados que presenta distintos grupos (hidrogeno, metilo, halógeno) en posición 6, hidrogeno y/o metilo en 7 del esqueleto de la cumarina y el anillo 3-bencenico simple o substituido en posición 3' y/o 4' con grupos metoxi y/o halógeno.

La actividad iMAO fue evaluada in vitro utilizando como compuestos de referencia el R-(-)-deprenilo y la iproniazida. (**Tabla 1**).

COMPUESTOS	IC_{50} IMAO-A (μM)	IC_{50} IMAO-B (μM)	S.I.
 17	**	69.59 ± 4.70	> 1.4
 18	**	**	---

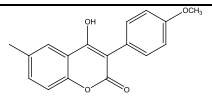
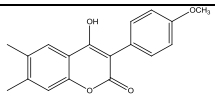
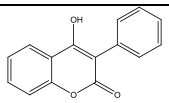
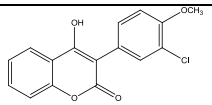
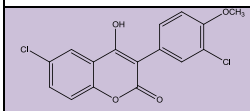
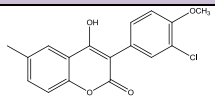
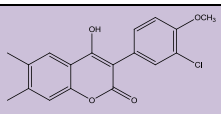
 19	**	32.04 ± 2.16	> 3.1
 20	**	**	---
 1	**	**	---
 25	**	9.26 ± 0.63	> 10
 **26**	**	**2.79 ± 0.19**	**> 36**
 27	**	42.68 ± 2.88	> 2.3
 **28**	**	**7.48 ± 0.51**	**> 13**
R-(-)-Deprenilo	68.73 ± 4.21	0.017 ± 0.0019	4,043
Iproniazida	6.56 ± 0.76	7.54 ± 0.36	0.87

Tabla 1: Actividad i-MAO

Cada valor de IC_{50} es la media ± S.E.M. de cinco experimentos (n = 5).

* Inactivos a 1 mM (la concentración más alta ensayada).

** Inactivos a 100 µM (la concentración más alta ensayada).

SI: el índice de selectividad iMAO-B = $IC_{50\ (\text{iMAO-A})}$ / $IC_{50\ (\text{iMAO-B})}$.

Algunos de los compuestos ensayados presentan actividad inhibidora y además son MAO-B selectivos.

El compuesto **28** tiene una IC_{50} similar a la iproniazide con la ventaja de que es mucho más selectivo que ésta frente la MAO-B. El compuesto más activo es el **26** que lleva el cloro en posición 6 y 3' y un grupo metoxi en posición 4'. Ninguno de los compuestos presenta actividad inhibidora MAO-A.

De los resultados obtenidos se deduce que la combinación de los sustituyentes metoxi y halógeno parece importante para incrementar la actividad i-MAO.

También se ha llevado a cabo el estudio computacional de docking (**Figura 10**) para determinar cuales son las configuraciones energéticamente favorecidas adoptadas por los 3 compuestos más activos **26**, **27**, **28** cuando interaccionan con en el centro activo de la MAO-B. La entrada de la cavidad del sitio de unión es ocupada por el núcleo de la 4-hidroxicumarina que presenta el anillo 3-arilo situado frente al cofactor FAD. El aminoácido Cys172 juega un papel importante en la estabilización del complejo enzima-cumarina, formando un enlace H con el oxígeno del carbonilo en todas las tres cumarinas. El aumento de la actividad de los compuestos **26** y **28**, en comparación con el **27**, podría explicarse en parte por la presencia de otro enlace H, esta vez vinculante. Sólo los derivados **27** y **28** lo presentan, respectivamente entre el grupo 4-hidroxi y el aminoácido Ile199. Por otra parte, los tres compuestos presentan interacciones de tipo π-π del anillo 3-arilo con el aminoácido Tyr326. Curiosamente, el grupo *p*-metoxi ocupa la misma posición en las tres cumarinas, mientras que la posición adoptada por el átomo de cloro en el anillo 3-arilo parece ser menos importante en la modulación de la actividad inhibitoria de la isoforma MAO-B, ya que supone una orientación opuesta en los dos compuestos más activos **27** y **28**. Además se observan para los tres compuestos interacciones de Van der Waals y electrostáticas con los aminoácidos Pro102, Ile316 y Phe343.

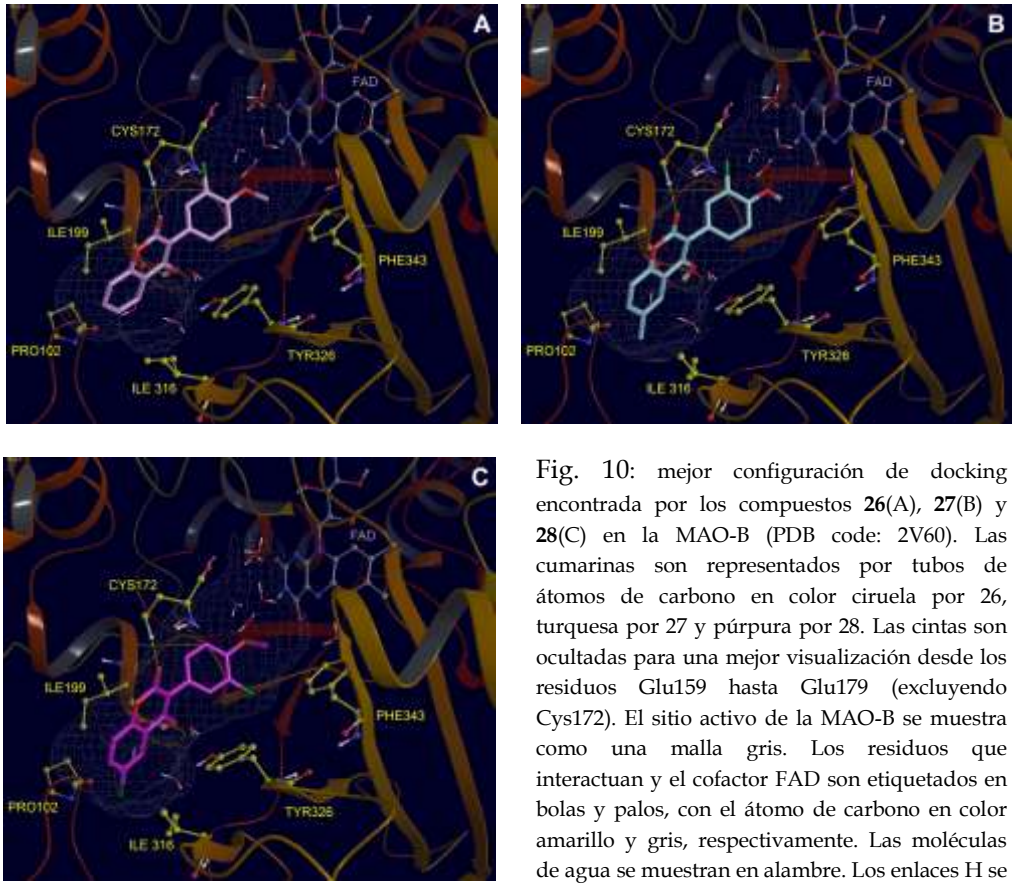


Fig. 10: mejor configuración de docking encontrada por los compuestos **26**(A), **27**(B) y **28**(C) en la MAO-B (PDB code: 2V60). Las cumarinas son representados por tubos de átomos de carbono en color ciruela por 26, turquesa por 27 y púrpura por 28. Las cintas son ocultadas para una mejor visualización desde los residuos Glu159 hasta Glu179 (excluyendo Cys172). El sitio activo de la MAO-B se muestra como una malla gris. Los residuos que interactuan y el cofactor FAD son etiquetados en bolas y palos, con el átomo de carbono en color amarillo y gris, respectivamente. Las moléculas de agua se muestran en alambre. Los enlaces H se muestran en líneas de puntos amarillo. Los hidrógenos no polares se omiten.

3.3.2. Actvidad antimicrobiana

En segundo lugar a causa del amplio rango de actividades farmacológicas atribuidas en el curso de los años a las cumarinas, las chalconas y los estilbenos derivados del resveratrol se decidió de evaluar los compuestos sintetizados en ese proyecto frente a distintas cepas bacterianas patógenas [*Escherichia coli* CECT434, *Pseudomonas aeruginosa* CECT108 Agar Mueller Hinton (MHA), *Staphylococcus aureus* CECT435].

Los ensayos se realizaron en el Departamento de Microbiología y Parasitología, Facultad de Biología, de la Universidad de Santiago de Compostela. La actividad se valoró por el método de difusión en placa, siguiendo las recomendaciones del National Committee for Clinical Laboratory Standards (2006) (NCCLS) 4. Con este fin, se impregnaron discos estériles (Liofilchem Bacteriology Products, Italia) con los compuestos sintetizados a concentraciones finales de 10 y 100 mg/disco y

se aplicaron sobre la superficie de placas de Agar Mueller Hinton (Difco) previamente inoculadas con las cepas bacterianas. Las placas se incubaron a 37 ^{o}C durante 24 horas y se determinó el diámetro de la zona de inhibición del crecimiento de las bacterias utilizadas como un índice de su sensibilidad a los antimicrobianos probados. Se ensayaron un total de 46 compuestos, realizándose dos réplicas por cada microorganismo probado.

Los resultados obtenidos indican que:

•10 de los 46 productos evaluados (**32%**) presentaron actividad antimicrobiana frente a *S. Aureus;*

•ninguno de los compuestos probados mostró capacidad para inhibir el crecimiento de *E. coli* y *P. Aeruginosa.*

En base a la experiencia y a los estudios de las enfermedades bacterianas en acuicultura marina realizados en el grupo de investigación del Departamento de Microbiología y Parasitología, decidimos evaluar las 4-hidroxi-3-arilcumarina sintetizadas frente a la bacteria *Tenacibaculum Maritimum*, responsable de la enfermedad de los peces conocida como flexibateriosis marina.

La técnica que se utilizó para hacer ese ensayo es la misma que se describió anteriormente. Debemos destacar los excelentes resultados obtenidos ya que el 90% de los compuestos evaluados frente a dicha bacteria resultaron ser activos, selectivos y muchos de ellos más activos que los compuestos utilizados como referencia ampicilina y acido oxolínico. En estos momentos se está llevando a cabo la determinación de las IC_{50} para los compuestos más interesantes, si bien no podemos aportar datos concretos debido a la confidencialidad impuesta por la tramitación de una patente que hemos solicitado.

3.3.3. Actividad antioxidante

Posteriormente en colaboración con el grupo de investigación del Departamento de Química Inorgánica y Analitica de la Universidad de Chile hemos llevado a

cabo también el estudio de la capacidad antioxidante de una pequeña serie de compuestos obteniéndose resultados bastante satisfactorios.

Para dicho estudio se empleó la técnica de medida de la Capacidad de Absorción de Radicales de Oxígeno con fluoresceína (ORAC-FL).

El ORAC-FL es un ensayo de tipo cinético utilizado para estimar la capacidad antioxidante de compuestos hidrófilos y usa una tecnología basada en la detección de la fluorescencia. En este ensayo, la oxidación ocurre por exposición del florífero al radical peroxyl AAPH que conduce al decaimiento de la emisión de la fluorescencia con el tiempo. En presencia de un compuesto que tenga propiedades antioxidantes, el decaimiento de la fluorescencia es retrasado. Para poder evaluar la capacidad antioxidante de los compuestos ensayados se utiliza un compuesto de referencia que normalmente es el Trolox, análogo de la vitamina A.

La fluorescencia fue registrada cada minuto para 90 minutos. En cada ensaye se utilizaron también un blanco con FL y AAPH, utilizando el tampón de fosfato de sodio en vez de la solución de antioxidante, y cinco soluciones de calibración de Trolox (0.5, 1.0, 1.5., 2.0 y 2.5 µM) como antioxidante (**Figura 11**). La capacidad de inhibición fue expresada como equivalentes de Trolox (M) y fue cuantizada por integración del área bajo la curva (AUC). Todas las mezclas de reacción estuvieron preparadas tres veces y se realizaron para cada muestra al menos tres ensayes.

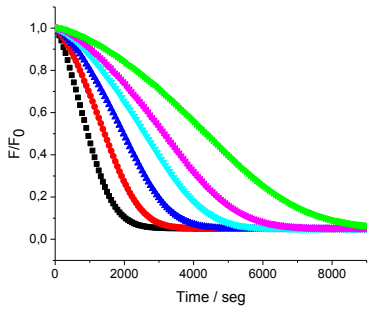


Fig. 11: Perfil cinético del consume de fluorescencia producido por radicales peroxil en presencia del derivado **5**.

La área bajo de la curva del descenso de la fluorescencia fue calculada integrando el decaimiento de la florescencia dónde F0 es la florescencia inicial leída en el minuto 0 y F es la fluorescencia leída en el tiempo. La red AUC correspondiente a la muestra fue calculada restando el AUC correspondiente al blanco (**Figura 12**).

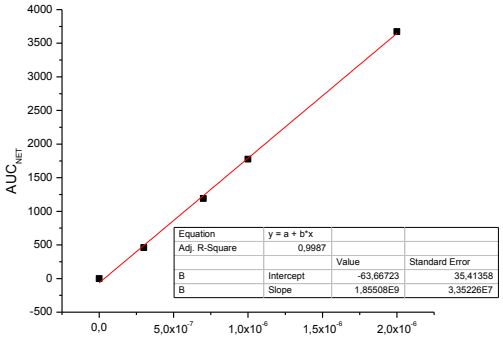


Fig. 12: AUC del compuesto **6**.

Los valores de índice de ORAC-FL para estos compuestos mostraron que las 4-hidroxicoumarinas con sustituyentes en la posición 3 tienen el índice ORAC más grande que la 4-hidroxicumarina simple, esto está relacionado con la mejor deslocalización del radical semiquinonico en la parte del esqueleto del arilo (**Tabla 2**).

Cumarinas	Index ORAC-FL
1	4,4
4-OH	4,2
6	7,7
5	6,5
13	3,9
21	5,7

Tabla 2

En los derivados con sustituyentes en el arilo (compuestos **5**, **6**, **13**, **21**) la presencia de grupos electro-atractores o electro-donadores influencia el aumento o la disminución de los valores de índice de ORAC. El compuesto **21** presenta la mejor capacidad antioxidante comparada con la de los análogos **1** y **13** con hidrogeno y cloro respectivamente en su estructura. La capacidad antioxidante también es dependiente del número de los grupos hidroxilos en el esqueleto de la cumarina.

3.3.4. Actividad citotóxica y anticáncer.

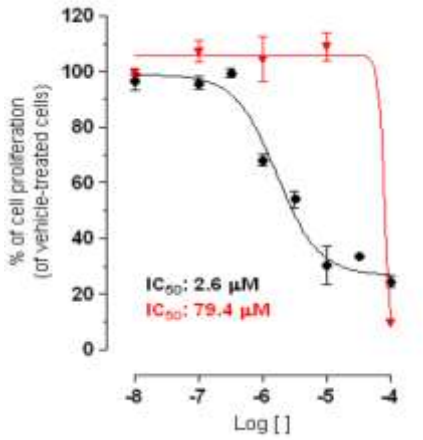
Debido a que en la literatura existen buenos resultados de la actividad anticáncer de diferentes derivados de la 4-hidroxicumarina hemos llevado a cabo en collaboración con el grupo de investigación del Instituto de Bioquímica y Medicina Molecular de la Universidad de Berna un estudio preliminar de la actividad citotóxica de los compuestos sintetizados. La inhibición de la proliferación/viabilidad célular de estas moléculas fue evaluada in vitro frente las dos líneas celulares tumorales: MCF-7 (células humanas de cáncer de mama) y HL-60 (células humanas de leucemia promielocítica).

La proliferación célular fue medida usando el reactivo de proliferación celular WST-1 (4-[3-(4-yodofenil)-2-(4-nitrofenil)-2H-5-tetrazol]-1,3-benceno disulfonato) (Roche, Mannheim, Alemania) sobre la base de la división celular del WST-1 a formazán.

La inhibición de la viabilidad celular se evaluó como el porcentaje de reducción de la absorción ultravioleta de las células tratadas en comparación con los controles (células tratadas con vehículo). Por los compuestos más activos se calcoló la concentración inhibitoria del 50% del crecimiento celular (IC_{50}). Cada valor se obtuvo a partir de tres experimentos independientes realizados por triplicado.

Como resultado 4 de los compuestos ensayados han mostrado buena actividad frente la línea celular MCF-7 mientras que solo 2 derivados resultan ser activos sobre la línea HL-60.

El compuesto bioactivo más potente es el **61**; ha mostrado misma potencia en ambas líneas celulares de cáncer probadas, con IC_{50} de 2 μM. Es más activo que el tamoxifeno, pero menos en respecto al paclitaxol, utilizados como compuestos de referencia. Curiosamente el compuesto **62**, su isómero estructural, no es activo frente las células MCF-7, mientras que es débilmente activo frente las HL60. En consecuencia, el compuesto **61** es treinta veces más activo que el compuesto **62** en la actividad citotóxica frente las células MCF-7 (**Figura 13**).



(Fig. 13): Curva de porcentaje de proliferación célular frente MCF-7 de compuesto **61** y de Compuesto **62** .

La diferente potencia de acción mostrada por los dos isómeros estructurales es muy interesante. Como conclusión, estos resultados preliminares nos animan a continuar los esfuerzos hacia la optimización del perfil farmacológico de esta molecula como prototipo importante en el ámbito de las enfermedades tumorales.

En estos momentos se están llevando a cabo estudios más profundos de todas las actividades ensayadas, así como de la actividad inhibidora de la enzima tirosinasa.

4. Parte experimental

4.1 Aspectos generales

Reactivos y disolventes

Todos los reactivos químicos utilizados se han comprado en Aldrich Chemical Company, Fluka o Merck.

Todas las reacciones se han llevado a cabo bajo atmósfera de argón desoxigenado y seco, salvo que se indique lo contrario. El argón se secó haciéndolo pasar por columnas de $CaCl_2$, lentejas de NaOH y P_2O_5.

Cromatografía

La identificación cualitativa de los compuestos y el seguimiento de las reacciones se monitorizó mediante cromatografía de capa fina (TLC, *thin layer chromatografy*). Las placas para TLC utilizadas contienen sílica gel como fase estacionaria (Merck sílica gel $60F_{254}$). Como eluyentes se utilizaron un disolvente o mezcla de ellos y sus relaciones se indicarán en fracciones de volumen:volumen. La visualización de los productos de reacción se realizó bajo luz UV (254-366 nm) para los compuestos que absorben a estas longitudes de onda o por revelado al calor de los cromatofolios de capa fina previamente tratados con el agente revelador adecuado.

Para la purificación de los compuestos por cromatografía en columna se utilizó gel de sílice Merck tipo 60, con tamaño de partícula de 35-70 μm.

Técnicas analíticas

Los puntos de fusión se determinaron en un aparato Stuart Scientific de lectura digital y no están corregidos.

Los espectros de resonancia magnética nuclear (RMN) se registraron en un aparato BRUKER DPX 250 (250 MHz), un aparato VARIAN MERCURY 300 (300 MHz) y en un aparato Varian Inova 500 (500 MHz) a temperatura ambiente en $CDCl_3$ o DMSO-d_6 y utilizando como referencia interna la señal del TMS o la del disolvente. En la descripción de los espectros ^1H RMN se indican los

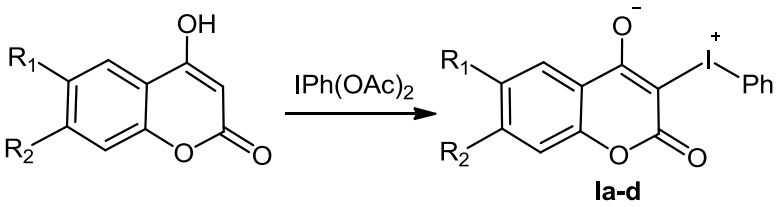
desplazamiento químicos (δ) en ppm. Las constantes de acoplamientos (*J*) se indican en Hertzios (Hz).

Los espectros de masas de baja resolución se realizaron en un sistema Hewlett-Packard 5972-MSD operando a 70 eV.

Los análisis elementales se realizaron en analizadores elementales CARLO ERBA, modelo EA 1108.

La irradiación de microondas se ha llevado a cabo utilizando un horno de microondas comercial (Samsung M1727).

4.2. Síntesis de 3-(4-Hidroxicumarinil)fenilyodonio, sales internas (Ia-d)



Ia-d

	R_1	R_2
Ia	H	H
Ib	Cl	H
Ic	CH_3	H
Id	CH_3	CH_3

En un matraz de fondo redondo se prepara una solución de 1 mmol de Na_2CO_3 en 10 mL de H_2O, donde se suspende 1 mmol de yodobenzenodiacetato. Se deja bajo agitación magnética en atmósfera de argón durante 2 horas. A continuación, se añade la mezcla de una solución de 1 mmol de Na_2CO_3 en 10 mL de H_2O con 1 mmol de la correspondiente 4-hidroxicumarina. Se agita a temperatura ambiente durante 14 horas y se deja bajo atmósfera de argón. Se filtra el precipitado, se lava con agua y se seca al vacío.

3-(4-Hidroxicumarinil)fenilyodonio, sal interna **(Ia)**

Rto: 98,6%.

PF: 137-139 °C.

^{1}H NMR (DMSO-d_6) δ: 7.18-7.30 (m, 2H, H6, H8), 7.40 (t, J = 7.5 Hz, 2H, H3', H5'), 7.47-7.60 (m, 2H, H7, H4'), 7.79-7.92 (m, 3H, H5, H2', H6').

^{13}C NMR (DMSO-d_6) δ: 82.52, 99.44, 115.48, 116.76, 120.33, 123.95, 126.16, 131.20, 131.62, 133.36, 133.59, 154.25, 158.59, 161.37, 172.88.

MS *m/z* (%): 363 (M^+, 98), 204 (30), 165 (48), 88 (24), 77 (52), 76 (24), 51 (29).

Anal. Elem. Calculado para $C_{15}H_9IO_3$: C 49.98, H 2.49; encontrado: C 49.97, H 2.46.

3-(6-Cloro-4-hidroxicumarinil)fenilyodonio, sal interna **(Ib)**

Rto: 98%.

PF: 162-164 °C.

^{1}H NMR (DMSO-d_6) δ: 7.20-7.70 (m, 5H, H7, H8, H3', H4', H5'), 7.72-7.95 (m, 3H, H5, H2', H6').

^{13}C NMR (DMSO-d_6) δ: 82.66, 99.77, 115.59, 119.08, 121.69, 125.21, 128.15, 131.30, 131.66, 133.03, 133.75, 138.43, 153.03, 161.02, 171.75.

MS *m/z* (%): 398 (M^+, 100), 274 (31), 271 (95), 231 (43), 199 (31), 152 (26), 87 (25), 77 (48).

Anal. Elem. Calculado para $C_{15}H_8ClIO_3$: C 45.20, H 2.02; encontrado: C 45.22, H 1.99.

3-(4-Hidroxi-6-metilcumarinil)fenilyodonio, sal interna **(Ic)**

Rto: 99%.

PF: 181-183 °C.

^{1}H NMR (DMSO-d_6) δ: 2.32 (s, 3H, CH_3), 7.15 (d, *J* = 8.3 Hz, 1H, H8), 7.31-7.44 (m, 3H, H7, H3', H5'), 7.51 (t, *J* = 7.3 Hz, 1H, H4'), 7.66 (s, 1H, H5), 7.79-7.86 (m, 2H, H2', H6').

^{13}C NMR (DMSO-d_6) δ: 20.75, 82.68, 115.52, 116.56, 120.01, 125.84, 131.17, 131.59, 133.06, 133.51, 134.07, 140.87, 148.53, 152.51, 161.59, 173.13.

MS *m/z* (%): 378 (M^+, 98), 251 (100), 211 (37), 179 (27), 77 (29).

Anal. Elem. Calculado para $C_{16}H_{11}IO_3$: C 50.82, H 2.93; encontrado C 50.86 H, 2.96.

3-(4-Hidroxi-6,7-dimetilcumarinil)fenilyodonio, sal interna **(Id)**

Rto: 98%.

PF: 182-184 °C.

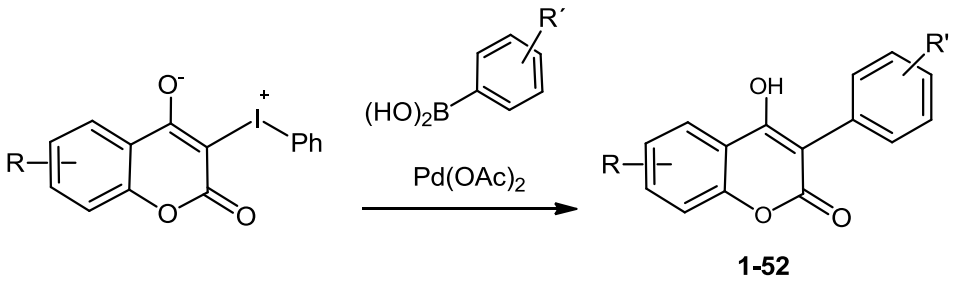
^{1}H NMR (DMSO-d_6) δ: 2.23 (s, 3H, CH_3), 2.27 (s, 3H, CH_3), 7.07 (s, 1H, H8), 7.40 (t, *J* = 7.4 Hz, 2H, H3', H5'), 7.51 (t, *J* = 7.3 Hz, 1H, H4'), 7.61 (s, 1H, H5), 7.82 (d, *J* = 7.2 Hz, 2H, H2', H6').

^{13}C NMR (DMSO-d_6) δ: 19.18, 20.11, 82.31, 112.66, 115.49, 117.13, 117.97, 126.14, 131.13, 131.57, 132.16, 133.44, 138.44, 142.73, 152.70, 161.75, 173.25.

MS *m/z* (%): 392 (M^+, 98), 265 (100), 225 (32), 77 (25).

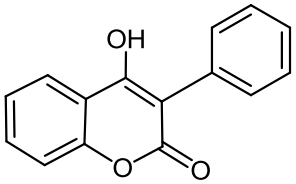
Anal. Elem. Calculado para $C_{17}H_{13}IO_3$: C 52.06, H 3.34; encontrado C 52.09, H 3.33.

4.3. Síntesis de 3-fenil-4-hidroxicumarinas, (1-44)



Se prepara una mezcla de 0.55 mmol de aril yodonio, de 1.65 mmol de LiOH y de 0.028 mmol de $Pd(OAc)_2$. Se le añade una solución de 1,21 mmol de ácido arilborónico y de 0.011 mmol de $P(t\text{-}Bu)_3$ en DME/H_2O (10 mL/2.5 mL). Se deja bajo agitación en atmósfera de argón a temperatura ambiente durante 48 horas. El sólido obtenido se purifica mediante cromatografía en columna utilizando una mezcla 6:4 de Hexano:AcOEt.

3-Fenil-4-hidroxicumarina, **(1)**



1

Rto: 63%.

PF: 237-239 °C.

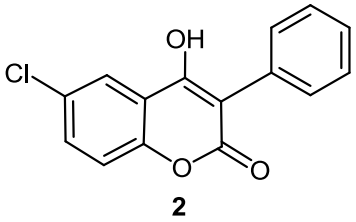
^{1}H NMR (DMSO-d_6) δ: 7.26-7.46 (m, 7H, H6, H8, H2', H3', H4', H5', H6'), 7.60-7.68 (m, 1H, H7), 7.99 (d, *J* = 7.9, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 106.5, 116.6, 116.8, 124.3, 124.4, 127.9, 128.4, 130.4, 131.4, 132.5, 132.7, 134.5, 152.7, 160.7, 162.4.

MS *m/z* (%): 238 (M^+, 46), 152 (26.12), 118 (99), 93 (31), 90 (31), 89 (35), 77 (24), 65 (45), 63 (35).

Anal. Elem. Calculado para $C_{15}H_{10}O_3$: C 75.60, H 4.23; encontrado C 75.62, H 4.22.

6-Cloro-3-fenil-4-hidroxicumarina, **(2)**



2

Rto: 68%.

PF: 263-265 °C.

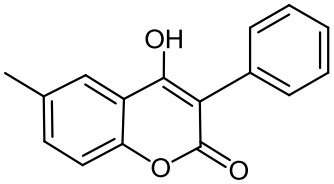
^{1}H NMR (DMSO-d_6) δ: 7.20-7.51 (m, 5H, H2', H3', H4', H5', H6'), 7.61-7.83 (m, 2H, H7, H8), 8.09-7.88 (m, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 162.20, 159.79, 151.57, 134.73, 132.62, 132.36, 131.58, 130.67, 128.7, 128.34, 128.02, 123.64, 119.04, 118.67, 107.64.

MS *m/z* (%): 273 (M^+, 95.22), 165 (32.25), 163 (30.65), 78 (29.17).

Anal. Elem. Calculado para $C_{15}H_9ClO_3$: C 66.07, H 3.33; encontrado C 66.03, H 3.30.

3-Fenil-4-hidroxi-6-metilcumarina, **(3)**



3

Rto: 57%.

PF: 243-245 °C.

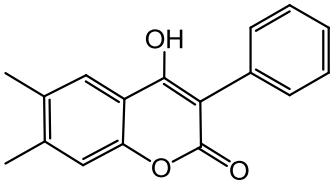
^{1}H NMR (DMSO-d_6) δ: 2.39 (s, 3H, CH_3), 7.20-7.51 (m, 7H, H7, H8, H2', H3', H4', H5', H6'), 7.79 (s, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 21.2, 106.7, 116.6, 116.7, 123.9, 128.0, 128.1, 128.7, 131.7, 132.8, 133.8, 133.9, 134.7, 151.1, 160.8, 162.7.

MS *m/z* (%): 252 (M^+, 41), 251 (84), 165 (38), 135 (95), 118 (87), 89 (100), 77 (74), 63 (42), 51 (46).

Anal. Elem. Calculado para $C_{16}H_{12}O_3$: C 76.18, H 4.79; encontrado C 76.21, H 4.78.

3-Fenil-4-hidroxi 6,7-dimetilcumarina, **(4)**



4

Rto: 46%.

PF: 253-255 °C.

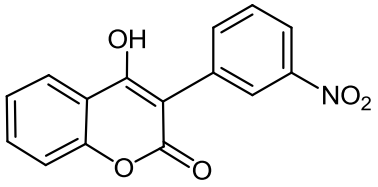
^{1}H NMR (DMSO-d_6) δ: 2.27 (m, 6H, 2xCH_3), 7.17 (s, 1H, H8), 7.25-7.60 (m, 5H, H2', H3', H4', H5', H6'), 7.71 (s, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 19.6, 20.3, 105.9, 114.4, 117.2, 124.2, 128.0, 128.6, 131.9, 132.8, 133.0, 142.7, 148.7, 151.4, 161.0, 162.8.

MS *m/z* (%): 266 (M^+, 81), 149 (67), 118 (73), 77 (94), 63 (31).

Anal. Elem. Calculado para $C_{17}H_{14}O_3$: C 76.68, H 5.30; encontrado C 76.68, H 5.28.

4-Hidroxi-3-(3′-nitrofenil)cumarina, **(5)**



5

Rto: 40%.

PF: 275-277 °C.

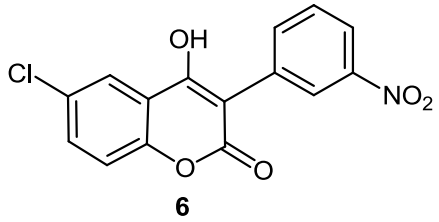
^{1}H NMR (DMSO-d_6) δ: 7.35-7.51 (m, 2H, H6, H8), 7.62-7.78 (m, 2H, H7, H6′), 7.90 (d, *J* = 7.7 Hz, 1H, H5′), 8.04 (d, *J* = 7.9 Hz, 1H, H5), 8.20 (d, *J* = 8.2 Hz, 1H, H4′), 8.31 (s, 1H, H2′).

^{13}C NMR (DMSO-d_6) δ: 91.1, 104.4, 116.9, 117.2, 122.8, 124.4, 124.6, 126.2, 129.9, 133.3, 134.6, 138.5, 148.0, 152.9, 161.9, 162.2.

MS *m/z* (%): 283 (M^+, 57), 121 (100), 120 (27).

Anal. Elem. Calculado para $C_{15}H_9NO_5$: C 63.61, H 3.20; encontrado C 63.58, H 3.23.

6-Cloro-4-hidroxi-3-(3′-nitrofenil)cumarina, **(6)**



6

Rto: 50%.

PF: 300-302 °C.

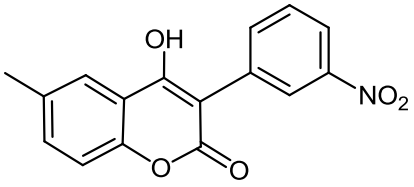
^{1}H NMR (DMSO-d_6) δ: 7.38-7.50 (m, 1H, H8), 7.65-7.75 (m, 2H, H7, H6′), 7.91 (d, *J* = 6.5 Hz, 1H, H5′), 8.00-8.06 (m, 1H, H5), 8.18 (d, *J* = 8.2 Hz, 1H, H4′), 8.33 (s, 1H, H2′).

^{13}C NMR (DMSO-d_6) δ: 92.0, 104.5, 117.7, 122.5, 123.6, 125.9, 128.4, 129.7, 132.6, 134.6, 138.2, 147.9, 151.5, 161.3, 164.8.

MS *m/z* (%): 317 (M^+, 75), 157 (30), 156 (34), 155 (100), 154 (86), 105 (36).

Anal. Elem. Calculado para $C_{15}H_8ClNO_5$: C 56.71, H 2.54; encontrado C 56.73, H 2.56.

4-Hidroxi-6-metil-3-(3′-nitrofenil)cumarina, **(7)**



7

Rto: 41%.

PF: 276-278 °C.

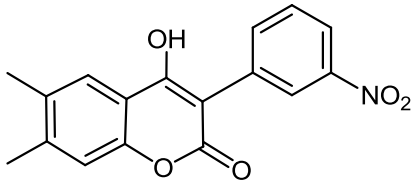
^{1}H NMR (DMSO-d_6) δ: 2.38 (s, 3H, CH_3), 7.23-7.89 (m, 5H, H5, H7, H8, H5′, H6′), 8.10-8.40 (m, 2H, H2′, H4′).

^{13}C NMR (DMSO-d_6) δ: 21.2, 104.6, 116.6, 116.8, 122.9,124.2, 126.5, 130.1, 134.0, 134.3, 134.8, 138.6, 148.2, 151.3, 162.0, 162.5.

MS *m/z* (%): 297 (M^+, 51), 135 (100), 134 (69).

Anal. Elem. Calculado para $C_{16}H_{11}NO_5$: C 64.65, H 3.73; encontrado C 64.68, H 3.76.

4-Hidroxi-6,7-dimetil-3-(3′-nitrofenil)cumarina, **(8)**



8

Rto: 40%.

PF: 296-298 °C.

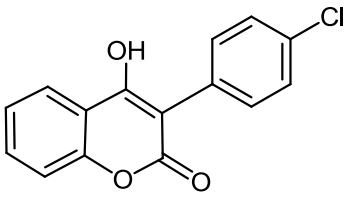
^{1}H NMR (DMSO-d_6) δ: 2.30 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 7.24 (s, 1H, H8), 7.69 (s, 1H, H5), 7.79 (s, 1H, H6′), 7.89 (d, *J* = 7.7 Hz, 1H, H5′), 8.18 (d, *J* = 9.6 Hz, 1H, H4′), 8.28 (s, 1H, H2′).

^{13}C NMR (DMSO-d_6) δ: 19.4, 20.2, 103.5, 114.2, 117.1, 122.5, 124.2, 126.2, 129.7, 132.9, 134.8, 138.2, 142.9, 147.8, 151.3, 162.2, 162.4.

MS *m/z* (%): 311 (M^+, 70), 149 (100), 148 (73).

Anal. Elem. Calculado para $C_{17}H_{13}NO_5$ C 65.59, H 4.21; encontrado C 65.58, H 4.20.

3-(4'-Clorofenil)-4-hidroxicumarina, **(9)**



9

Rto: 52%.

PF: 267-269 °C.

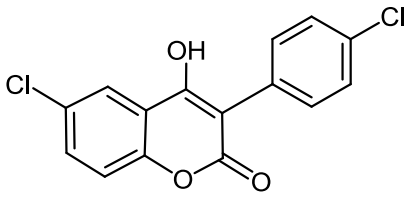
^{1}H NMR (DMSO-d_6) δ: 7.32-7.52 (m, 6H, H6, H8, H2', H3', H5', H6'), 7.66 (t, *J* = 7.8 Hz, 1H, H7), 8.00 (d, *J* = 6.6 Hz, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 105.3, 116.6, 116.7, 123.7, 124.2, 124.5, 124.6, 131.6, 132.5, 132.8, 133.2, 133.3, 152.7, 161.0, 162.2.

MS *m/z* (%): 272 (M^+, 80), 152 (64), 121(100).

Anal. Elem. Calculado para $C_{15}H_9ClO_3$: C 66.07, H 3.33; encontrado C 66.04, H 3.27.

6-Cloro-3-(4′-clorofenil)-4-hidroxicumarina, **(10)**



10

Rto: 66%.

PF: 314-316 °C.

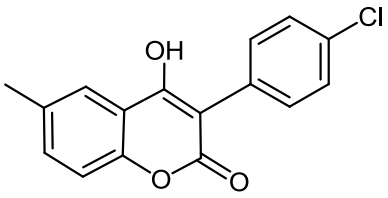
^{1}H NMR (DMSO-d_6) δ: 7.34-7.51 (m, 5H, H8, H2′, H3′, H5′, H6′), 7.67 (d, *J* = 8.8 Hz, 1H, H7),7.99 (s, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 99.7, 105.9, 118.6, 118.8, 123.5, 123.6, 128.5, 131.3, 132.5, 132.6, 133.2, 133.3, 151.4, 160.2, 161.89.

MS *m/z* (%): 306 (M^+, 63), 155 (64), 154 (37), 152 (100).

Anal. Elem. Calculado para $C_{15}H_8Cl_2O_3$: C 58.66, H 2.63; encontrado C 58.64, H 2.67.

3-(4′-Clorofenil)-4-hidroxi-6-metilcumarina, **(11)**



11

Rto: 54%.

PF: 305-307 °C.

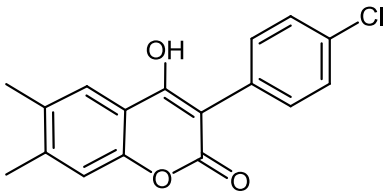
^{1}H NMR (DMSO-d_6) δ: 2.37 (s, 3H, CH_3), 7.28 (d, *J* = 8.4 Hz, 1H, H8), 7.35-7.53 (m, 5H, H7, H2′, H3′, H5′, H6′), 7.78 (s, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 20.8, 105.3, 116.5, 116.6, 123.8, 131.5, 132.5, 133.2, 133.4, 133.6, 133.7, 133.8, 150.9, 161.0, 161.2, 162.33.

MS *m/z* (%): 286 (M+, 95), 152 (52), 135 (100), 134 (53), 77 (24).

Anal. Elem. Calculado para $C_{16}H_{11}ClO_3$: C 67.03, H 3.87; encontrado C 67.05, H 3.88.

3-(4′-Clorofenil)-4-hidroxi-6,7-dimetilcumarina, **(12)**



12

Rto: 41%.

PF: 300-302 °C.

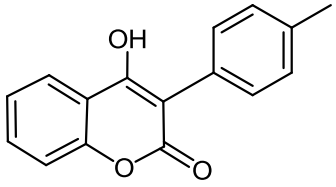
^{1}H NMR (DMSO-d_6) δ: 2.16 (s, 3H, CH_3), 2.20 (s, 3H, CH_3), 7.09 (s, 1H, H8), 7.18-7.42 (m, 4H, H2′, H3′, H4′, H5′), 7.62 (s, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 19.4, 20.2, 104.6, 116.8, 124.1, 128.5, 128.6, 131.5, 131.7, 132.5, 132.9, 133.4, 133.6, 142.7, 151.2, 161.2, 162.7.

MS *m/z* (%): 300 (M+, 100), 149 (100).

Anal. Elem. Calculado para $C_{17}H_{14}ClO_3$: C 67.89, H 4.36; encontrado C 67.86, H 4.37.

4-Hidroxi-3-(4′-metilfenil)cumarina, **(13)**



13

Rto: 46%.

PF: 226-228 °C.

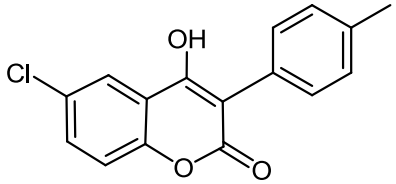
^{1}H NMR (DMSO-d_6) δ: 2.33 (s, 3H, CH_3), 7.23 (dt, *J* = 8.5 Hz, 4H, H2′, H3′, H5′, H6′), 7.32-7.41 (m, 2H, H6, H8), 7.58-7.69 (m, 1H, H7), 7.97 (d, *J* = 7.9 Hz, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 21.2, 106.7, 116.7, 124.0, 128.0, 128.1, 128.7, 130.7, 131.7, 132.7, 133.8, 133.9, 134.7, 151.1, 160.9, 162.7.

MS *m/z* (%): 252 (M^+, 100), 132 (80), 121 (48).

Anal. Elem. Calculado para $C_{16}H_{12}O_3$: C 76.18, H 4.79; Encontrado C 75.89, H 4.79.

6-Cloro-4-hidroxi-3-(4'-metilfenil)-cumarina, **(14)**



14

Rto: 66%.

PF: 303-305 °C.

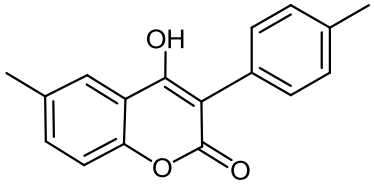
^{1}H NMR (DMSO-d_6) δ: 2.34 (s, 3H, CH_3), 7.14-7.37 (m, 4H, H2', H3', H5', H6'), 7.38-7.50 (m, 1H, H8), 7.60-7.76 (m, 1H, H7), 7.97 (s, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 21.3, 107.9, 118.5, 118.7, 123.3, 127.9, 128.3, 129.0, 131.1, 131.3, 132.2, 132.4, 137.3, 151.2, 159.5, 162.00.

MS *m/z* (%): 286 (M+, 49), 152 (52), 132 (100), 77 (24).

Anal. Elem. Calculado para $C_{16}H_{11}ClO_3$: C 67.03, H 3.87; encontrado C 67.02, H 3.88.

4-Hidroxi-3-(4'-metilfenil)-6-metilcumarina, **(15)**



15

Rto: 50%.

PF: 225-227 °C.

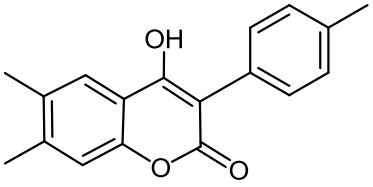
^{1}H NMR (DMSO-d_6) δ: 2.34 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 7.16-7.33 (m, 5H, H8, H2', H3', H5', H6'), 7.44 (d, *J* = 8.4 Hz, 1H, H7), 7.77 (s, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 20.9, 21.3, 106.3, 116.3, 116.5, 123.0, 128.9, 129.1, 129.4, 129.5, 131.2, 133.4, 133.5, 137.0, 151.0, 160.4, 162.42.

MS *m/z* (%): 266 (M^+, 79), 135 (58), 132 (100).

Anal. Elem. Calculado para $C_{17}H_{14}O_3$: C 76.68, H 5.30; encontrado C 76.68, H 5.33.

4-Hidroxi-6,7-dimetil-3-(4′-metilfenil)cumarina, **(16)**



16

Rto: 44%.

PF: 265-269 °C.

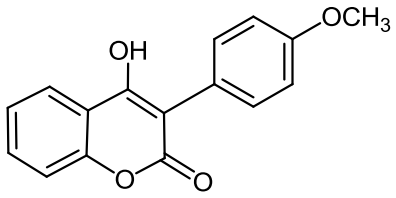
^{1}H NMR (DMSO-d_6) δ: 2.28 (s, 3H, CH_3), 2.30-2.41 (m, 6H, 2xCH_3), 7.10-7.37 (m, 5H, H6, H2′, H3′, H5′, H6′), 7.72 (s, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 19.6, 20.3, 21.6, 105.8, 106.0, 114.4, 117.2, 124.1, 129.3, 129.8, 130.2, 131.5, 131.7, 133.0, 137.2, 142.6, 151.3, 160.95.

MS *m/z* (%): 280 (M^+, 34), 279 (76), 149 (100), 132 (73), 91 (42), 77 (39).

Anal. Elem. Calculado para $C_{18}H_{16}O_3$: C 77.12, H 5.75; encontrado C 77.10, H 5.74.

4-Hidroxi-3-(4′-metoxifenil)cumarina, **(17)**



17

Rto: 65%.

PF: 255-257 °C.

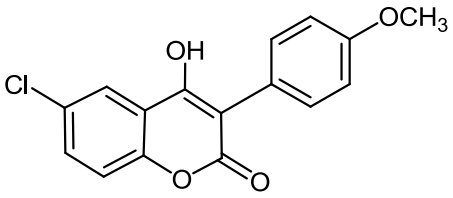
^{1}H NMR (DMSO-d_6) δ: 3.79 (s, 3H, OCH_3), 6.90-7.03 (m, 2H, H3′, H5′), 7.25-7.44 (m, 4H, H6, H8, H2′, H6′), 7.63 (t, *J* = 7.7 Hz, 1H, H7), 7.97 (d, *J* = 7.9 Hz, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 55.5, 106.0, 113.9, 116.5, 116.9, 118.6, 124.0, 124.3, 124.4, 132.5, 132.6, 152.6, 158.9, 159.1, 160.4, 162.49.

MS *m/z* (%): 268 (M^+, 100), 148 (70), 121 (48), 78 (26), 63 (34).

Anal. Elem. Calculado para $C_{16}H_{12}O_4$: C 71.64, H 4.51; encontrado C 71.62, H 4.52.

6-Cloro-4-hidroxi-3-(4'-metoxifenil)cumarina, **(18)**



18

Rto: 56%.

PF: 258-260 °C.

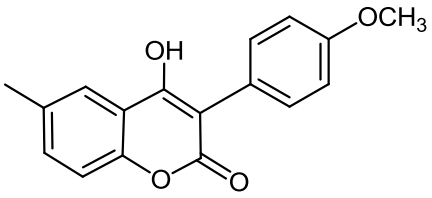
^{1}H NMR (DMSO-d_6) δ: 3.78 (s, 3H, OCH_3), 6.97 (d, *J* = 8.6 Hz, 2H, H3', H5'), 7.30 (d, *J* = 8.6 Hz, 2H, H2', H6'), 7.43 (d, *J* = 8.8 Hz, 1H, H8), 7.66 (d, *J* = 8.8, 1H, H7), 7.96 (s, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 55.2, 99.4, 106.7, 114.0, 114.3, 118.5, 122.9, 124.9, 126.5, 129.8, 130.2, 130.5, 131.0, 151.1, 159.2, 162.1.

MS *m/z* (%): 302 (M^+, 100), 155 (42), 148 (97), 120 (25).

Anal. Elem. Calculado para $C_{16}H_{11}ClO_4$: C 72.33, H 5.00; encontrado C 72.36, H 5.03.

4-Hidroxi-6-metil-3-(4′-metoxifenil)cumarina, **(19)**



19

Rto: 50%.

PF: 228-230 °C.

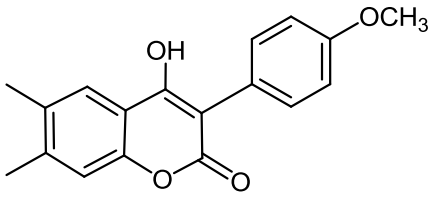
^{1}H NMR (DMSO-d_6) δ: 2.38 (s, 3H, CH_3), 3.78 (s, 3H, OCH_3), 6.92-7.0 (m, 2H, H3′, H5′), 7.23-7.34 (m, 3H, H7, H8, H6′), 7.43 (d, *J* = 8.4 Hz, 1H, H2′), 7.76 (s, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 21.1, 55.2, 105.3, 114.1, 114.2, 116.8, 117.1, 124.6, 127.1, 130.0, 130.1, 132.3, 135.6, 149.4, 159.6, 160.4, 162.1.

MS *m/z* (%): 283 (27), 282 (M^+, 95), 148 (100), 135 (82), 77 (22).

Anal. Elem. Calculado para $C_{17}H_{14}O_4$: C 72.33, H 5.0; encontrado C 72.31, H 4.98.

4-Hidroxi-6,7-dimetil-3-(4′-metoxifenil)cumarina, **(20)**



20

Rto: 42%.

PF: 221-223 °C.

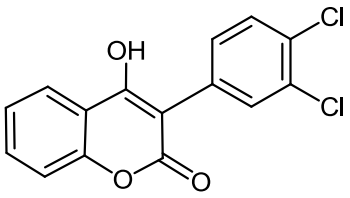
^{1}H NMR (DMSO-d_6) δ: 2.28 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 3.75 (s, 3H, OCH_3), 6.96 (d, *J* = 8.8 Hz, 2H, H3′, H5′), 7.19 (s, 1H, H8), 7.28 (d, *J* = 8.7 Hz, 2H, H2′, H6′), 7.71 (s, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 19.4, 20.1, 55.5, 105.4, 114.0, 116.9, 123.7, 123.9, 124.5, 132.6, 142.1, 151.0, 159.9, 159.0, 160.5, 162.7.

MS *m/z* (%): 296 (M^+, 84), 245 (33), 149 (69), 148 (100).

Anal. Elem. Calculado para $C_{18}H_{16}O_3$ C 72.96, H 5.44; encontrado C 72.94, H 5.41.

3-(3',4'-Diclorofenil)-4-hidroxicumarina, **(21)**



21

Rto: 51%.

PF: 321-323 °C.

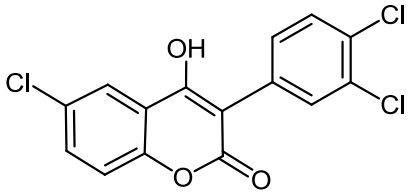
^{1}H NMR (DMSO-d_6) δ: 7.30-7.51 (m, 3H, H6, H2', H6'), 7.54-7.79 (m, 3H, H7, H8, H5'), 7.90-8.05 (m, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 103.9, 116.3, 123.8, 124.0, 126.9, 130.5, 131.4, 132.6, 132.9, 133.0, 134.1, 135.8, 152.3, 161.2, 161.5.

MS *m/z* (%): 307 (M^+, 26), 306 (45), 185 (28), 172 (30), 121 (100).

Anal. Elem. Calculado para $C_{15}H_8Cl_2O_3$: C 58.66, H 2.63; encontrado C 58.64, H 2.60.

6-Cloro-3-(3′,4′-diclorofenil)-4-hidroxicumarina, **(22)**



22

Rto: 43%.

PF: 347-350 °C.

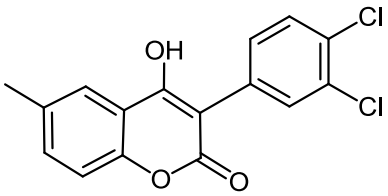
^{1}H NMR (DMSO-d_6) δ: 7.34-7.54 (m, 2H, H2′, H6′), 7.58-7.77 (m, 3H, H7, H8, H5′), 7.91-8.06 (m, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 104.2, 118.1, 118.4, 123.1, 127.9, 130.1, 130.5, 131.3, 132.2, 132.8, 134.0, 135.6, 150.9, 160.4, 161.2.

MS *m/z* (%): 307 (M^+, 74), 340 (75), 228 (65), 188 (72), 155 (100), 98 (39), 57 (30).

Anal. Elem. Calculado para $C_{15}H_7Cl_3O_3$: C 52.74, H 2.07; encontrado C 52.72, H 2.10.

3-(3',4'-Diclorofenil)-4-hidroxi-6-metilcumarina, **(23)**



23

Rto: 48%.

PF: 307-310 °C.

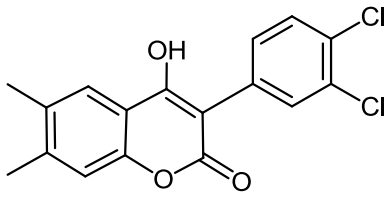
^{1}H NMR (DMSO-d_6) δ: 2.38 (s, 3H, CH_3), 7.25-7.53 (m, 3H, H8, H2', H6'), 7.61-7.73 (m, 2H, H6, H5'), 7.79 (s, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 161.63, 161.08, 150.51, 135.75, 134.11, 133.48, 133.31, 133.04, 132.95, 131.46, 130.51, 130.11, 129.98, 123.39, 116.08, 115.87, 103.58, 20.33.

MS *m/z* (%): 321 (M^+, 59), 135 (100), 134 (69).

Anal. Elem. Calculado para $C_{16}H_{10}Cl_2O_3$: C 59.84, H 3.14; encontrado C 59.81, H 3.11.

3-(3′,4′-Diclorofenil)-4-hidroxi-6,7-dimetilcumarina, **(24)**



24

Rto: 48%.

PF: 311-314 °C.

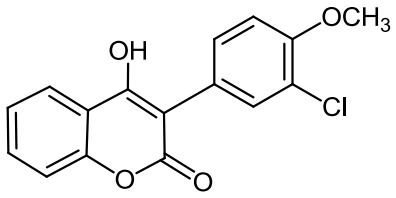
^{1}H NMR (DMSO-d_6) δ: 2.28 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 7.21 (s, 1H, H8), 7.31-7.49 (m, 2H, H6′, H2′), 7.61-7.78 (m, 2H, H5, H5′).

^{13}C NMR (DMSO-d_6) δ: 18.9, 19.7, 102.3, 113.6, 116.6, 123.6, 129.1, 130.1, 131.5, 133.0, 133.2, 142.5, 150.8, 161.3, 161.8.

MS *m/z* (%): 335 (M^+, 64), 334 (64), 228 (28), 150 (36), 149 (100), 148 (85), 77 (30).

Anal. Elem. Calculada para $C_{17}H_{12}Cl_2O_3$: C 60.92, H 3.61; encontrado C 60.90, H 3.59.

3-(3′-Cloro-4′-metoxifenil)-4-hidroxicumarina, **(25)**



25

Rto: 80%.

PF: 298-300 °C.

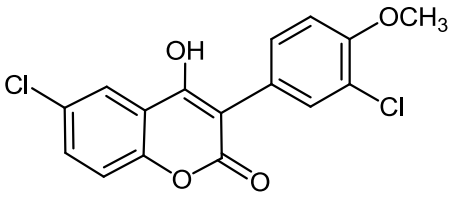
^{1}H NMR (DMSO-d_6) δ: 3.94 (s, 3H, OCH_3), 7.21 (s, 1H, H2′), 7.28-7.49 (m, 3H, H6, H8, H5′), 7.56-7.83 (m, 2H, H7, H6′), 7.98 (d, *J* = 7.6, 1H, H5), 15.7 (s, 1H, OH).

^{13}C NMR (DMSO-d_6) δ: 56.2, 105.0, 112.8, 116.6, 116.9, 120.7, 124.2, 124.4, 125.6, 131.5, 132.7, 132.8, 152.7, 154.3, 161.0, 162.3.

MS *m/z* (%): 302 (M^+, 100), 182 (64), 121 (95).

Anal. Elem. Calculado para $C_{16}H_{11}ClO_4$: C 63.48, H 3.66; encontrado C 63.45, H 3.69.

6-Cloro-3-(3′-cloro-4′-metoxifenil)-4-hidroxicumarina, **(26)**



26

Rto: 62%.

PF: 321-323 °C.

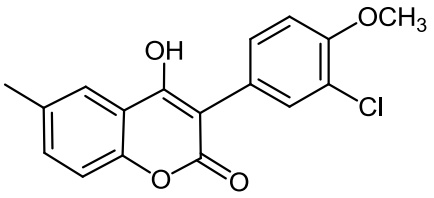
^{1}H NMR (DMSO-d_6) δ: 3.90 (s, 3H, OCH_3), 7.0-7.57 (m, 3H, H8, H2′, H5′), 7.60-7.84 (m, 2H, H7, H6′), 8.0 (s, 1H, H5), 15.7 (s, 1H, OH).

^{13}C NMR (DMSO-d_6) δ: 56.8, 113.0, 119.0, 121.1, 123.6, 125.5, 128.7, 131.7, 132.6, 132.8, 135.2, 136.1, 151.5, 154.6, 160.2, 162.2

MS *m/z* (%): 338 (M^+, 59), 336 (100), 182 (75), 155 (88), 142 (26), 57 (31).

Anal. Elem. Calculado para $C_{16}H_{10}Cl_2O_4$: C 57.00, H 2.99; encontrado C 57.03, H 3.02.

3-(3′-Cloro-4′-metoxifenil)-4-hidroxi-6-metilcumarina, **(27)**



27

Rto: 80%.

PF: 301-304 °C.

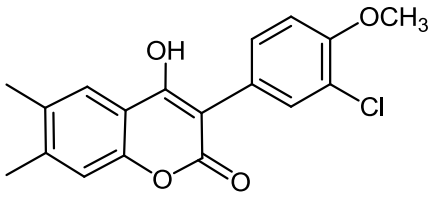
^{1}H NMR (DMSO-d_6) δ: 2.45 (s, 3H, CH_3), 3.90 (s, 3H, OCH_3), 7.14-7.24 (m, 1H, H8), 7.25-7.37 (m, 3H, H7, H2′, H5′), 7.40-7.52 (m, 1H, H6′), 7.80 (s, 1H, H5), 15.4 (s, 1H, OH).

^{13}C NMR (DMSO-d_6) δ: 20.9, 56.6, 105.0, 112.8, 116.4, 120.8, 123.8, 125.6, 129.0, 131.5, 132.7, 133.6, 133.6, 150.8, 154.3, 160.9, 162.4.

MS *m/z* (%): 316 (M^+, 59), 182 (80), 135 (100), 97 (36), 83 (37), 71 (51), 57 (91).

Anal. Elem. Calculado para $C_{17}H_{13}ClO_4$: C 64.46, H 4.14; encontrado C 64.46, H 4.13.

3-(3′-Cloro-4′-metoxifenil)- 4-hidroxi-6,7-dimetilcumarina, **(28)**



28

Rto: 58%.

PF: 314-315 °C.

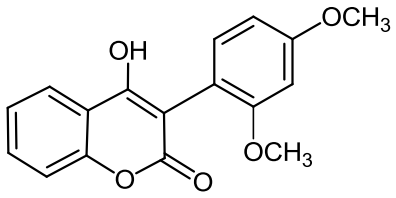
^{1}H NMR (DMSO-d_6) δ: 2.20-2.30 (s, 3H, CH_3), 2.31-2.39 (s, 3H, CH_3), 3.81-3.96 (s, 3H, OCH_3), 7.09-7.25 (m, 2H, H2′, H5′), 7.27-7.37 (m, 1H, H8), 7.38-7.49 (m, 1H, H6′), 7.68-7.80 (s, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 19.3, 20.1, 56.5, 104.6, 112.7, 114.6, 117.0, 120.7, 124.0, 125.8, 131.5, 132.7, 132.8, 142.4, 151.1, 154.1, 161.3, 161.6.

MS *m/z* (%): 330 (M^+, 81), 182 (55), 149 (100).

Anal. Elem. Calculado para $C_{18}H_{15}ClO_4$: C 65.36 H 4.57; encontrado C 65.38 H 4.55.

4-Hidroxi-3-(2′,4′-dimetoxifenil)cumarina, **(29)**



29

Rto: 40%.

PF: 277-279 °C.

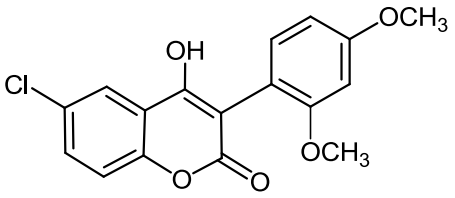
^{1}H NMR (DMSO-d_6) δ: 3.62-3.74 (s, 3H, OCH_3), 3.75-3.86 (s, 3H, OCH_3), 6.51-6.67 (m, 2H, H3′, H5′), 7.02-7.15 (d, *J* = 8.1 Hz, 1H, H6′), 7.28-7.45 (m, 2H, H6, H8), 7.57-7.70 (m, 1H, H7), 7.85-7.97 (m, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 55.7, 55.8, 99.0, 102.8, 105.4, 112.9, 116.5, 116.7, 123.9, 124.3, 132.4, 133.4, 152.7, 159.3, 160.7, 161.2, 162.10.

MS *m/z* (%): 298 (M^+, 100), 178 (63), 149 (30), 121 (56).

Anal. Elem. Calculado para $C_{17}H_{14}O_5$: C 68.45, H 4.73; encontrado C 68.42, H 4.73.

6-Cloro-4-hidroxi-3-(2′,4′-dimetoxifenil)cumarina, **(30)**



30

Rto: 71%.

PF: 317-319 °C.

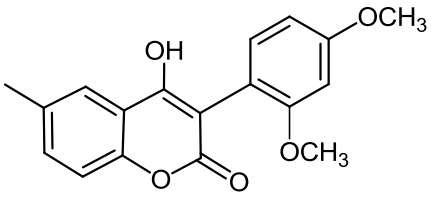
^{1}H NMR (DMSO-d_6) δ: 3.62-3.72 (s, 3H, OCH_3), 3.77-3.92 (s, 3H, OCH_3), 6.49-6.86 (m, 2H, H3′, H5′), 7.01-7.24 (m, 1H, H6′), 7.36-7.56 (m, 1H, H8), 7.60-7.79 (m, 1H, H7), 7.81-7.99 (s, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 55.9, 56.1, 99.2, 104.0, 105.7, 112.7, 118.5, 118.9, 123.4, 128.6, 132.3, 133.5, 151.6, 159.5, 159.8, 161.6, 162.00.

MS *m/z* (%): 333 (M^+, 100), 299 (56), 178 (75), 155 (35), 142 (57), 98 (32), 57 (42), 55 (33).

Anal. Elem. Calculado para $C_{17}H_{13}ClO_5$: C 61.36, H 3.94; encontrado C 61.37, H 3.90.

4-Hidroxi-6-metil-3-(2′,4′-dimetoxifenil)cumarina, **(31)**



31

Rto: 57%.

PF: 274-277 °C.

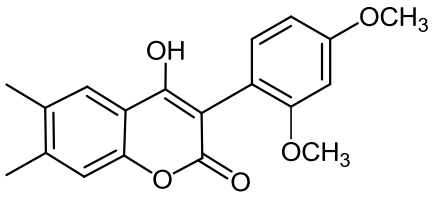
^{1}H NMR (DMSO-d_6) δ: 2.30-2.44 (s, 3H, CH_3), 3.63-3.74 (s, 3H, OCH_3), 3.75-3.86 (s, 3H, OCH_3), 6.49-6.68 (m, 2H, H3′, H5′), 7.02-7.13 (m, 1H, H6′), 7.21-7.32 (m, 1H, H8), 7.37-7.51 (m, 1H, H7), 7.66-7.76 (s, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 21.1, 55.9, 56.1, 99.2, 103.0, 105.6, 113.2, 116.5, 116.6, 123.9, 133.5, 133.7, 133.8, 151.2, 159.5, 161.0, 161.4, 162.5.

MS *m/z* (%): 312 (M^+, 59), 137 (100), 97 (36), 83 (37), 71 (51), 57 (79).

Anal. Elem. Calculado para $C_{18}H_{16}O_5$: C 69.22, H 5.16; encontrado C 69.24, H 5.19.

4-Hidroxi-6,7-dimetil-3-(2′,4′-dimetoxifenil)cumarina, **(32)**



32

Rto: 42%.

PF: 279-281 °C.

Rto: (42%).

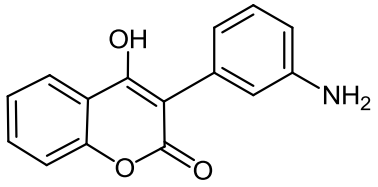
^{1}H NMR (DMSO-d_6) δ: 2.22-2.30 (s, 3H, CH_3), 2.31-2.35 (s, 3H, CH_3), 3.62-3.72 (s, 3H, OCH_3), 3.75-3.85 (s, 3H, OCH_3), 6.50-6.67 (m, 2H, H3′, H5′), 6.99-7.10 (d, *J* = 8.1 Hz, 1H, H6′), 7.14-7.22 (s, 1H, H8), 7.60-7.71(m, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 19.30, 20.1, 55.7, 55.8, 99.0, 101.2, 105.3, 113.1, 114.2, 116.8, 123.8, 132.6, 133.4, 142.0, 151.2, 159.3, 161.0, 161.1, 162.4.

MS *m/z* (%): 326 (M^+, 100), 178 (94), 149 (72).

Anal. Elem. Calculado para $C_{19}H_{18}O_5$: C 69.93, H 5.56; encontrado C 69.95, H 5.59.

3-(3′-Aminofenil)-4-hidroxicumarina**, (33)**



33

Rto: 52%.

PF: 259-261 °C.

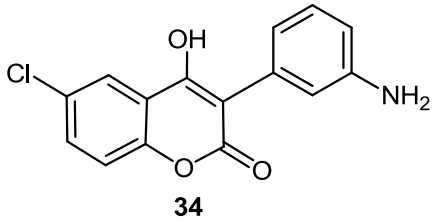
^{1}H NMR (DMSO-d_6) δ: 6.51-6.65 (m, 3H, H2′, H4′, H6′), 7.20 (m, 1H, H5′), 7.45-7.47 (m, 2H, H6, H8), 7.63 (m, 1H, H7), 7.81 (m, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 105.0, 110.7, 114.2, 116.4, 117.4, 118.9, 123.3, 125.4, 128.3, 133.3, 133.7, 148.3, 152.5, 160.4, 161.9.

MS *m/z* (%): 253 (M^+, 26), 184 (100), 162 (48), 121 (60), 120 (64), 92 (52).

Anal. Elem. Calculado para $C_{15}H_{11}NO_3$: C 71.14, H 4.38; encontrado C 71.13, H 4.38.

3-(3'-Aminofenil)-6-cloro-4-hidroxicumarina, **(34)**



34

Rto: 65%.

PF: 313-316 °C.

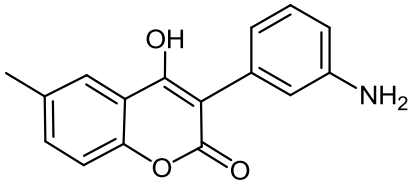
^{1}H NMR (DMSO-d_6) δ: 6.68-6.45 (m, 3H, H2', H4', H6'), 7.20 (m, 1H, H5'), 7.38 (m, 1H, H8), 7.68 (m, 1H, H7), 8.04 (s, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 105.0, 114.2, 116.9, 118.8, 188.9, 122.9, 126.8, 129.5, 131.0, 133.3, 133.7, 148.3, 150.6, 160.4, 161.9.

MS *m/z* (%): 287 (M^+, 45), 196 (41), 184 (100), 155 (39), 154 (91), 126 (36), 98 (34), 63 (32).

Anal. Elem. Calculado para $C_{15}H_{10}ClNO_3$: C 62.62, H 3.50; encontrado C 62.65, H 3.48.

3-(3′-Aminofenil)-4-hidroxi-6-metilcumarina, **(35)**



35

Rto: 44%.

PF: 282-284°C.

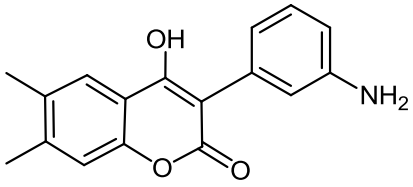
^{1}H NMR (DMSO-d_6) δ: 2.49 (s, 3H, CH_3), 6.56 (d, *J* = 7.9 Hz, 1H, H4′), 6.77 (d, *J* = 7.6 Hz, 1H, H6′), 6.83 (s, 1H, H2′), 7.09 (t, *J* = 7.8 Hz, 1H, H5′), 7.31 (t, *J* = 7.3 Hz, 1H, H8), 7.41 (t, *J* = 7.5 Hz, 1H, H7), 7.53 (d, *J* = 7.9 Hz, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 21.7, 105.0, 110.7, 114.2, 116.9, 117.3, 118.9, 127.0, 132.0, 133.3, 133.7, 135.1, 148.3, 149.5, 160.4, 161.9.

MS *m/z* (%): 267 (M^+, 62), 169 (66), 149 (63), 71 (62), 69 (61), 57 (100).

Anal. Elem. Calculado para $C_{16}H_{13}NO_3$: C 71.90, H 4.90; encontrado C 71.92, H 4.89.

3-(3′-Aminofenil)-4-hidroxi-6,7-dimetilcumarina, **(36)**



36

Rto: 41%.

PF: 289-290 °C.

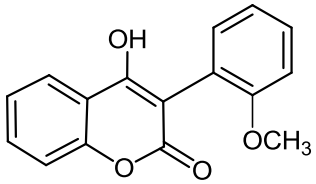
^{1}H NMR (DMSO-d_6) δ: 2.43 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 6.44-6.66 (m, 3H, H2′, H4′, H6′), 7.02 (s, 1H, H8), 7.21 (m, 1H, H5′), 7.45 (s, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 19.0, 19.2, 105.0, 110.4, 114.7, 114.8, 117.2, 119.9, 121.0, 126.7, 128.5, 133.3, 133.5, 133.7, 142.1, 147.3, 148.5, 160.2, 161.7.

MS *m/z* (%): 281 (M^+, 70), 184 (100), 57 (22).

Anal. Elem. Calculado para $C_{17}H_{15}NO_3$: C 72.58, H 5.37; encontrado C 72.59, H 5.38.

4-Hidroxi-3-(2′-metoxifenil)cumarina, **(37)**



37

Rto: 47%.

PF: 245-247 °C.

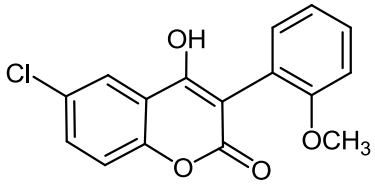
^{1}H NMR (DMSO-d_6) δ: 3.71 (s, 3H, OCH_3), 6.99 (t, *J* = 7.4 Hz, 1H, H3′), 7.04-7.1 (m, 1H, H5′), 7.19 (dd, *J* = 7.4, 1H, H4′), 7.34-7.41 (m, 3H, H6, H8, H6′), 7.64 (t, *J* = 7.0 Hz, 1H, H7), 7.92 (m, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 56.7, 103.7, 111.0, 116.3, 117.4, 119.4, 120.9, 123.6, 125.4, 128.5, 128.6, 130.1, 155.3, 157.6, 160.8, 161.7.

MS *m/z* (%): 268 (M^+, 65), 174 (49), 149 (76), 148 (71), 121 (100), 85 (32), 71 (50), 57 (92), 55 (45).

Anal. Elem. Calculado para $C_{16}H_{12}O_4$: C 71.64, H 4.51; encontrado C 71.62, H 4.54.

6-Cloro-4-hidroxi-3-(2'-metoxifenil)cumarina, **(38)**



38

Rto: 56%.

PF: 266-268 °C.

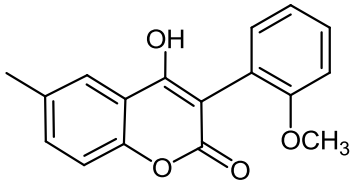
^{1}H NMR (DMSO-d_6) δ: 3.71 (s, 3H, CH_3). 6.98 (t, *J* = 7.5 Hz, 1H, H3'), 7.06 (d, *J* = 8.6 Hz, 1H, H5'), 7.18 (d, *J* = 7.2 Hz, 1H, H4'), 7.33-7.39 (m, 1H, H8), 7.44 (d, *J* = 8.7 Hz, 1H, H6'), 7.68 (d, *J* = 8.8 Hz, 1H, H7), 7.91 (s, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 56.6, 103.6, 111.4, 118.3, 119.8, 121.0, 122.9, 126.8, 129.4, 129.5, 132.0, 150.6, 157.0, 160.7, 161.0.

MS *m/z* (%): 302 (M^+, 44), 149 (80), 97 (31), 85 (34), 83 (30), 71 (50), 69 (35), 57 (100), 55 (44).

Anal. Elem. Calculado para $C_{16}H_{11}ClO_4$: C 72.33, H 5.00; encontrado C 71.30, H 5.00.

4-Hidroxi-6-metil-3-(2′-metoxifenil)cumarina, **(39)**



39

Rto: 50%.

PF: 233-235 °C.

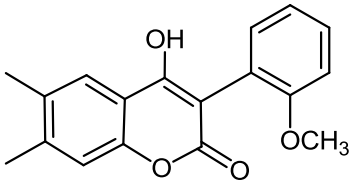
^{1}H NMR (DMSO-d_6) δ: 2.30-2.41 (s, 3H, CH_3), 3.64-3.74 (s, 3H, OCH_3), 7.03-7.06 (d, *J* = 9.0 Hz, 1H, H3′), 7.14-7.20 (d, *J* = 7.6 Hz, 1H, H5′), 7.41-7.46 (d, *J* = 7.7 Hz, 1H, H4′), 7.53-7.56 (d, *J* = 7.6 Hz, 1H, H8), 7.63-7.68 (t, *J* = 4.5 Hz, 2H, H7, H6′), 7.69-7.72 (s, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 21.4, 56.2, 103.4, 11.4, 116.3, 117.0, 119.4, 120.6, 127.2, 128.9, 129.3, 132.5, 135.0, 149.3, 157.8, 160.5, 161.7.

MS *m/z* (%): 282 (M^+, 35), 135 (43), 85 (54), 83 (40), 71 (72), 69 (43), 57 (100), 55 (48).

Anal. Elem. Calculado para $C_{17}H_{14}O_4$: C 72.33 H 5.00; encontrado C 72.35, H 4.99.

4-Hidroxi-6,7-dimetil-3-(2′-metoxifenil)cumarina, **(40)**



40

Rto: 44%.

PF: 227-229 °C.

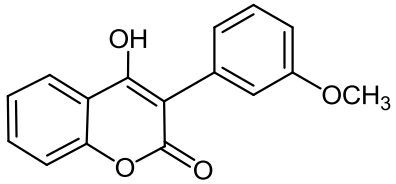
^{1}H NMR (DMSO-d_6) δ: 1.99-2.14 (s, 3H, CH_3), 2.21-2.39 (s, 3H, CH_3), 3.99-428 (s, 3H, OCH_3), 6.98-7.09 (m, 1H, H3′), 7.20-7.26 (m, 1H, H5′), 7.51-7.80 (m, 4H, H5, H8, H4′ H6′).

^{13}C NMR (DMSO-d_6) δ: 18.8, 19.2, 56.4, 103.7, 114.0, 114.6, 117.4, 120.9, 121.0, 126.9, 128.3, 129.7, 133.6, 142.1, 147.5, 157.9, 160.5, 161.9.

MS *m/z* (%): 296 (M^+, 21), 149 (51), 85 (42), 69 (63), 57 (100), 55 (70).

Anal. Elem. Calculado para $C_{18}H_{16}O_3$: C 72.96, H 5.44; encontrado C 72.95, H 5.41.

4-Hidroxi-3-(3′-metoxifenil)cumarina, **(41)**



41

Rto: 57%.

PF: 245-247 °C

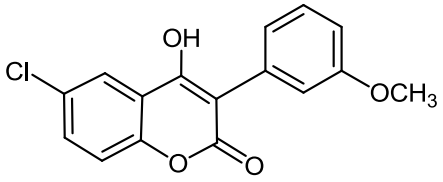
^{1}H NMR (DMSO-d_6) δ: 3.71 (s, 3H, OCH_3), 6.99 (t, *J* = 7.4 Hz, 1H, H4′), 7.04-7.10 (m, 1H, H6′), 7.19 (s, 1H, H2′), 7.34-7.41 (m, 3H, H5′, H6, H8), 7.68 (m, 1H, H7), 7.950-7.95 (m, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 56.7, 104.7, 11.0, 117.4, 119.9, 120.9, 123.6, 125.4, 128.5, 128.6, 155.3, 156.6, 160.8, 161.7.

MS *m/z* (%): 268 (M^+, 71), 148 (100), 121 (92), 98 (24), 85 (32), 57 (32), 55 (23).

Anal. Elem. Calculado para $C_{16}H_{12}O_4$: C 71.64, H 4.51; encontrado C 71.64, H 4.50.

6-Cloro-4-hidroxi-3-(3'-metoxifenil)cumarina, **(42)**



42

Rto: 68%.

PF: 279-280 °C.

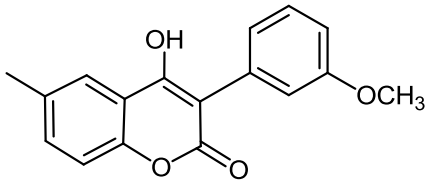
^{1}H NMR (DMSO-d_6) δ: 3.83 (s, 3H, OCH_3), 6.86 (t, J = 7.1 Hz, 2H, H4', H6'), 7.14-7.25 (m, 1H, H2'), 7.47-7.58 (m, 1H, H8), 7.64-7.80 (m, 2H, H7, H5'), 7.99 (s, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 92.1, 107.3, 115.2, 118.7, 121.9, 123.3, 128.4, 129.5, 131.3, 151.3, 152.5, 157.4, 159.4, 161.8, 162.9.

MS *m/z* (%): 302 (M^+, 65), 155 (73), 154 (26), 134 (100).

Anal. Elem. Calculado para $C_{16}H_{11}ClO_4$: C 63.48, H 3.66; encontrado C 63.49, H 3.64.

4-Hidroxi-6-metil-3-(3′-metoxifenil)cumarina, **(43)**



43

Rto: 48%.

PF: 243-245 °C.

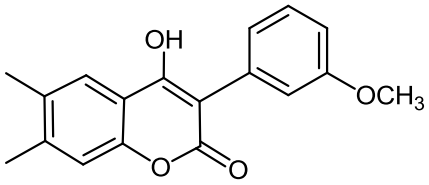
^{1}H NMR (DMSO-d_6) δ: 2.30-2.41 (s, 3H, CH_3), 3.64-3.70 (m, 3H, OCH_3), 7.00-7.03 (d, *J* = 9.0 Hz, 1H, H6′), 7.20-7.24 (d, *J* = 7.6 Hz, 1H, H4′), 7.46-7.51 (s, 1H, H2′), 7.56-7.58 (s, 1H, H5), 7.63-7.68 (m, 2H, H7, H8), 7.69-7.72 (m, 1H, H5′).

^{13}C NMR (DMSO-d_6) δ = 21.4, 56.2, 103.4, 111.4, 116.3, 117.0, 119.4, 120.6, 127.2, 128.9, 129.3, 132.5, 135.0, 149.3, 157.8, 160.5, 161.7.

MS *m/z* (%): 282 (M^+, 60), 239 (54), 148 (65), 135 (48), 98 (71), 84 (49), 74 (43), 71 (57), 69 (43), 57 (100), 55 (70).

Anal. Elem. Calculado para $C_{17}H_{14}O_4$: C 72.33, H 5.00; encontrado C 72.34, H 4.99.

4-Hidroxi-6,7-dimetil-3-(3′-metoxifenil)cumarina, **(44)**



44

Rto: 52%.

PF: 251-253 °C.

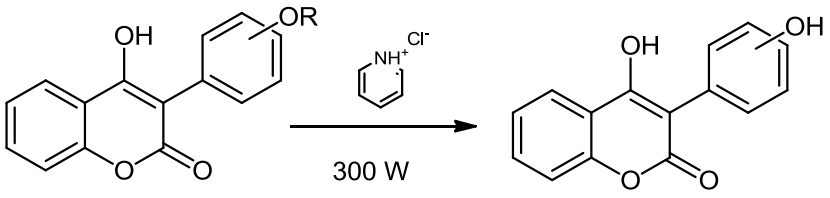
^{1}H NMR (DMSO-d_6) δ: 2.28 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 3.75 (s, 3H, OCH_3), 6.96 (d, *J* = 8.8 Hz, 2H, H4′, H6′),), 7.18 (m, 2H, H8, H2′), 7.20 (s, 1H, H5), 7.71 (m, 1H, H5′).

^{13}C NMR (DMSO-d_6) δ: 19.4, 20.1, 55.5, 105.4, 113.2, 114.3, 116.9, 123.7, 123.9, 124.5, 132.6, 132.7, 142.1, 151.0, 158.9, 159.0, 160.5, 162.7.

MS *m/z* (%): 296 (M^+, 97), 149 (90), 148 (100), 57 (25).

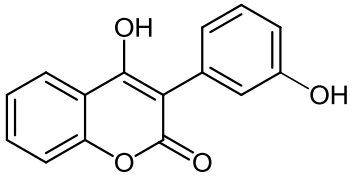
Anal. Elem. Calculado para $C_{18}H_{16}O_3$: C 72.96, H 5.44; encontrado C 72.94, H 5.46.

4.4. Hidrólisis de los derivados metoxilados, (45-52)



En un matraz de fondo redondo se prepara una mezcla de 0.38 mmol del compuesto metoxilado disuelto en 1 mL de DCM y 3.8 mmol de cloruro de piridinio. Se deja a 300W en el microondas por 6 minutos. Se enfría y se trata con hielo y agua. Se extrae en éter etílico. Se concentra la fase orgánica. Se purifica en columna utilizando como eluyente DCM y/o se cristaliza en EtOH.

3-(3′-Hidroxifenil)-4-hidroxicumarina, **(45)**



45

Rto: 48%.

PF: 265-267 °C.

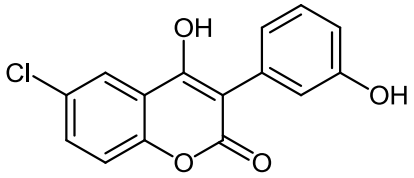
^{1}H NMR (DMSO-d_6) δ: 7.94 (s, 1H, H5), 7.41 (m, 5H, H6, H7, H8, H2′, H5′), 6.76 (m, 2H, H4′, H6′).

^{13}C NMR (DMSO-d_6) δ: 80.3, 93.0, 101.2, 106.7, 115.1, 116.7, 118.3, 122.1, 124.2, 124.5, 129.5, 132.8, 152.7, 157.5, 160.1.

MS *m/z* (%): 254 (M^+, 48), 134 (26), 121 (100), 65 (28).

Anal. Elem. Calculado para $C_{15}H_{10}O_4$: C 70.86, H 3.96; encontrado C 70.88, H 3.98.

6-Cloro-3-(3'-hidroxifenil)-4-hidroxicumarina, **(46)**



46

Rto: 65%.

PF: 283-285 °C.

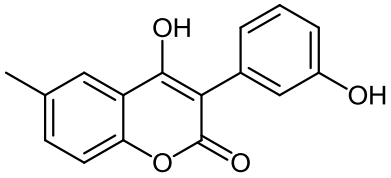
^{1}H NMR (DMSO-d_6) δ: 6.76 (t, *J* = 7.1 Hz, 2H, H4', H6'), 7.14-7.25 (m, 1H, H2'), 7.37-7.48 (m, 1H, H8), 7.64-7.80 (m, 2H, H7, H5'), 7.96 (s, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 92.1, 107.3, 115.2, 118.7, 121.9, 123.3, 128.4, 129.4, 132.3, 151.3, 152.5, 157.4, 159.4, 161.8, 164.9.

MS *m/z* (%): 288 (M^+, 65), 155 (63), 154 (26), 134 (100).

Anal. Elem. Calculado para $C_{15}H_9ClO_4$: C 62.41, H 3.14; encontrado C 62.39, H 3.12.

3-(3'-Hidroxifenil)-4-hidroxi-6-metilcumarina, **(47)**



47

Rto: 68%.

PF: 244-246 °C.

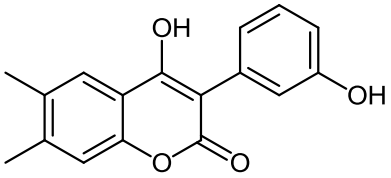
^{1}H NMR (DMSO-d_6) δ: 2.49 (s, 3H, CH_3), 6.74 (d, J = 8.4 Hz, 3H, H2', H4', H6'), 7.14-7.34 (m, 2H, H7, H8), 7.39-7.51 (m, 1H, H5'), 7.76 (s, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 20.6, 91.2, 106.4, 115.0, 116.3, 118.3, 122.0, 123.7, 129.1, 129.3, 132.9, 150.8, 157.4, 160.1, 162.3, 166.0.

MS *m/z* (%): 268 (M^+, 58), 135 (100), 134 (33).

Anal. Elem. Calculado para $C_{16}H_{12}O_4$: C 71.64, H 4.51; encontrado C 71.63, H 4.53.

3-(3′-Hidroxifenil)-4-hidroxi-6,7-dimetilcumarina, **(48)**



48

Rto: 40%.

PF: 273-275 °C.

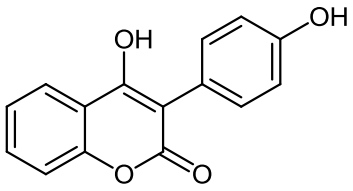
^{1}H NMR (DMSO-d_6) δ: 2.28 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 6.74 (t, J = 8.3 Hz, 3H, H2′, H4′, H6′), 7.18 (t, J = 8.0 Hz, 2H, H8, H5′), 7.71 (s, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 19.4, 19.7, 105.0, 112.7, 114.7, 115.6, 116.7, 121.7, 126.7, 129.3, 132.1, 133.7, 142.0, 148.0, 157.4, 160.1, 162.3.

MS m/z (%): 282 (M^+, 39), 149 (100), 148 (36).

Anal. Elem. Calculado para $C_{17}H_{14}O_4$: C 72.33, H 5.00; encontrado C 72.35, H 5.02.

3-(4'-Hidroxifenil)-4-hidroxicumarina, **(49)**



49

Rto: 60%.

PF: 265-267 °C.

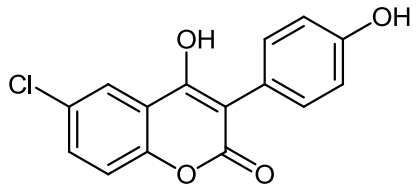
^{1}H NMR (DMSO-d_6) δ: 6.73-6.85 (dd, *J* = 9.0, 2H, H3', H5'), 7.14-7.24 (m, 2H, H6, H8), 7.31-7.43 (m, 2H, H2', H6'), 7.54-7.67 (m, 1H, H7), 7.90-8.01 (m, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 105.0, 115.6, 115.8, 116.2, 117.8, 123.6, 125.3, 125.9, 128.4, 130.2, 130.3, 152.8, 157.3, 160.4, 161.8.

MS *m/z* (%): 254 (M^+, 99), 134 (83), 121 (100), 71 (28), 57 (55).

Anal. Elem. Calculado para $C_{15}H_{10}O_4$: C 70.86, H 3,96; encontrado C 70.89, H 3.98.

6-Cloro-3-(4'-hidroxifenil)-4-hidroxicumarina, **(50)**



50

Rto: 65%.

PF: 299-300 °C.

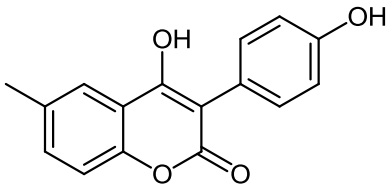
^{1}H NMR (DMSO-d_6) δ: 6.73-6.91 (m, 1H, H5'), 7.13-7.28 (m, 1H, H3'), 7.33-7.53 (m, 2H, H2', H6'), 7.56-7.81 (m, 2H, H7, H8), 7.87-8.05 (s, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 105.0, 115.8, 116.2, 117.8, 122.8, 125.3, 126.9, 129.3, 130.0, 130.2, 131.3, 150.8, 157.3, 160.5, 161.9.

MS *m/z* (%): 289 (M^+, 66), 288 (94), 231 (33.93), 155 (100), 134 (92), 97 (45), 81 (29), 77 (36), 69 (52), 57 (72), 55 (45).

Anal. Elem. Calculado para $C_{15}H_9ClO_4$: C 62.41, H 3.14; encontrado C 62.43, H 3.13.

3-(4′-Hidroxifenil)-4-hidroxi-6-metilcumarina, **(51)**



51

Rto: 54%.

PF: 264-266 °C.

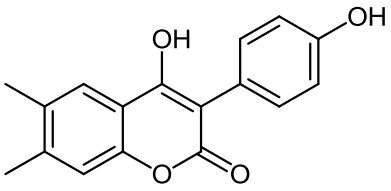
^{1}H NMR (DMSO-d_6) δ: 2.33-2.41 (s, 3H, CH_3), 6.74-6.84 (m, 2H, H3′, H5′), 7.14-7.20 (m, 2H, H7, H8), 7.23-7.31 (m, 1H, H6′), 7.38-7.50 (m, 1H, H2′), 7.67-7.77 (s, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 21.9, 105.0, 115.6, 116.2, 117.8, 125.9, 128.4, 130.5, 130.6, 132.2, 136.3, 149.5, 157.3, 160.4, 161.8.

MS *m/z* (%): 268 (M^+, 82), 135 (100), 134 (62).

Anal. Elem. Calculado para $C_{16}H_{12}O_4$: C 71.64, H 4.51; encontrado C 71.64, H 4.53.

3-(4′-Hidroxifenil)-4-hidroxi-6,7-dimetil-cumarina, **(52)**



52

Rto: 45%.

PF: 289-282 °C.

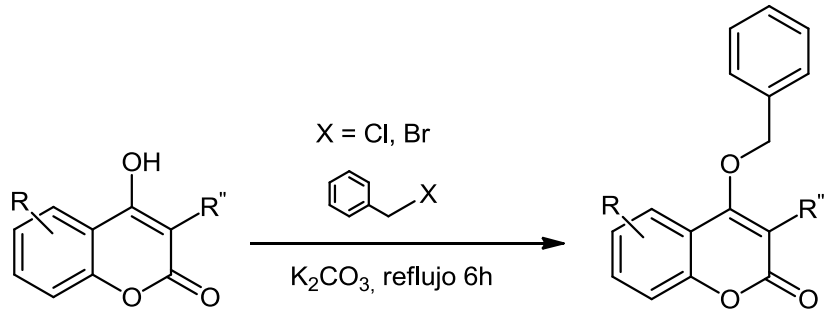
^{1}H NMR (DMSO-d_6) δ: 2.20-2.29 (s, 3H, CH_3), 2.29-2.43 (s, 3H, CH_3), 6.67-6.87 (m, 3H, H8, H3′, H5′), 7.13-7.19 (m, 2H, H2′, H6′), 7.63-7.77 (s, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 19.2, 20.0, 105.0, 115.6, 115.8, 116.2, 117.8, 125.0, 126.3, 130.2, 130.7, 133.8, 142.4, 147.0, 157.8, 160.4, 161.9.

MS *m/z* (%): 282 (M^+, 70), 149 (100).

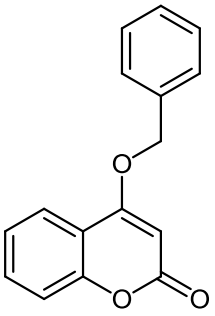
Anal. Elem. Calculado para $C_{17}H_{14}O_4$: C 72.33, H 5.00; encontrado C 72.36, H 5.02.

4.5. Síntesis de las 4-benciloxicumarinas, (53-60)



A una solución de 4-hidroxicumarina (1.5 mmol) en apropriado solvente (EtOH, acetona etc), se le añade K_2CO_3 (4.5 mmol) y haluro de bencilo (4.5 mmol). La reacción se mantiene bajo agitación y a reflujo durante 6 horas en atmósfera de nitrógeno. Después de que la solución se ha enfriado, se filtra el K_2CO_3 y se evapora a sequedad. Se trata el resultante residuo aceitoso con éter y se obtiene un precipitado que luego se filtra. Se purifica mediante FC con una mezcla 7:3 de Hexano:AcOEt o por cristalización.

4-Benciloxicumarina, **(53)**



53

Rto: 50%.

PF: 230-232°C.

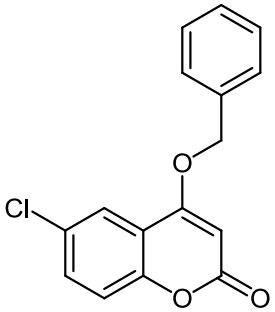
^{1}H NMR (DMSO-d_6) δ: 3.88 (s, 2H, CH_2), 5.35 (s, 1H, H3), 5.59 (s, 1H, H3'), 7.03-8.06 (m, 8H, H4', H5', H6, H7, H2', H6', H8, H5).

^{13}C NMR (DMSO-d_6) δ: 69.0, 87.8, 116.2, 116.5, 123.5, 125.7, 127.1, 127.2, 127.6, 128.4, 128.6, 128.9, 136.3, 152.7, 161.7, 166.9.

MS *m/z* (%): 356 (M^+, 21), 149 (77), 119 (35), 105 (72), 91 (100), 71 (47), 57 (72), 55 (36).

Anal. Elem. Calculado para $C_{16}H_{12}O_3$: C 76.18, H 4.79; encontrado C, 76.19, H 4.78.

4-Benciloxi-6-clorocumarina, **(54)**



54

Rto: 52%.

PF: 234-236°C.

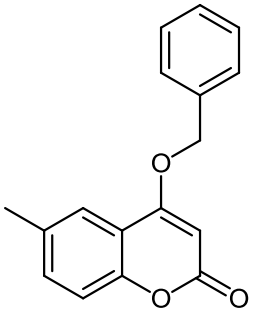
^{1}H NMR ($CDCl_3$) δ: 3.59 (s, 2H, CH_2), 4.52 (s, 1H, H3), 7.03-7.16 (m, 3H, H3', H-4', H5'), 7.22 (s, 1H, H8), 7.29-7.39 (m, 2H, H7, H6'), 7.64-7.72 (m, 2H, H5, H2').

^{13}C NMR ($CDCl_3$) δ: 68.9, 87.5, 118.9, 122.9, 126.8, 127.0, 127.1, 127.6, 128.8, 128.9, 129.5, 131.1, 136.3, 150.8, 162.4, 169.9.

MS *m/z* (%): 286 (M^+, 100), 155 (54), 154 (74), 131 (30), 126 (29), 91 (54), 77 (26).

Anal. Elem. Calculado para $C_{16}H_{11}ClO_3$: C 67.03, H 3.87; encontrado: C 67.06, H 3.89.

4-Benciloxi-6-metilcumarina, **(55)**



55

Rto: 45%.

PF: 220-223 °C.

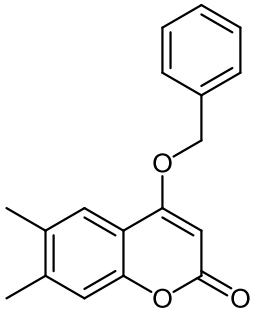
^{1}H NMR ($CDCl_3$) δ: 2.40 (s, 3H, CH_3), 3.45-3.74 (s, 1H, H3), 4.25-4.70 (m, 2H, CH_2), 6.79-7.03 (m, 2H, H7, H8), 7.04-7.33 (m, 4H, H2', H3', H4', H5'), 7.39-7.74 (m, 2H, H5, H6').

^{13}C NMR ($CDCl_3$) δ: 21.9, 69.0, 87.5, 116.9, 117.5, 127.0, 127.1, 127.2, 127.6, 128.8, 128.9, 132.1, 135.4, 136.3, 149.8, 162.4, 169.9.

MS *m/z* (%): 266 (M^+, 100), 135 (57), 134 (65), 91 (52), 77 (27).

Anal. Elem. Calculado para $C_{17}H_{14}O_3$: C 76.68, H 5.30; encontrado: C 76.66, H 5.28.

4-Benciloxi-6,7-dimetilcumarina, **(56)**



56

Rto: 46%.

PF: 227-229 °C.

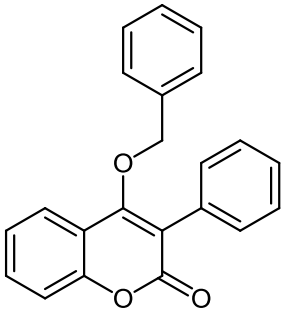
^{1}H NMR ($CDCl_3$) δ: 2.36-2.60 (s, 6H, CH_3), 3.45-3.65 (s, 2H, CH_2), 4.40-4.43 (s, 1H, H3), 6.78-6.86 (s, 2H, H8, H3'), 6.92-7.05 (m, 1H, H4'), 7.06-7.15 (m, 1H, H5'), 7.20-7.26 (m, 1H, H5), 7.52-7.44 (m, 2H, H-2', H-6').

^{13}C NMR ($CDCl_3$) δ: 18.9, 19.2, 68.9, 87.5, 114.9, 117.5, 127.0, 127.1, 127.2, 127.6, 128.8, 128.9, 133.5, 136.3, 142.1, 147.8, 162.4, 169.9.

MS *m/z* (%): 280 (M^+, 100), 149 (69), 148 (55), 91 (46).

Anal. Elem. Calculado para $C_{18}H_{16}O_3$: C 77.12, H 5.75; encontrado: C 77.15, H 5.77.

4-Benciloxi-3-fenilcumarina, **(57)**



57

Rto: 46%.

PF: 204-206 °C.

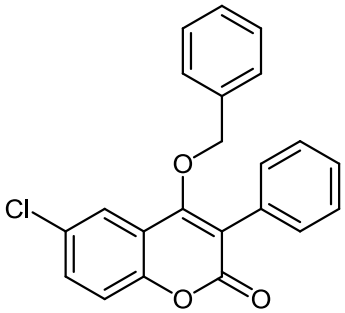
^{1}H NMR ($CDCl_3$) δ: 4.65 (s, 2H, CH_2), 7.11-7.18 (m, 2H, H2', H6'), 7.34-7.24 (m, 5H, H4', H6, H3'', H4'', H5''), 7.37 (d, J = 8.3, 1H, H8), 7.45-7.41 (m, 1H, H2''), 7.48 (t, J = 7.4, 2H, H3', H5'), 7.55 (d, J = 7.0, 2H, H6'', H7), 7.84 (d, J = 8.0, 1H, H5).

^{13}C NMR ($CDCl_3$) δ: 69.0, 100.3, 115.9, 117.6, 123.4, 125.3, 127.1, 127.3, 127.6, 127.8, 128.0, 128.4, 128.5, 128.6, 128.7, 128.8, 128.9, 132.6, 136.3, 152.4, 161.8, 166.9.

MS *m/z* (%): 328 (M^+, 100), 238 (45), 208 (44), 152 (32), 92 (65), 91 (61).

Anal. Elem. Calculado para $C_{22}H_{16}O_3$: C 80.47, H 4.91; encontrado: C 80.48, H 4.89.

4-Benciloxi-6-cloro-3-fenilcumarina, **(58)**



58

Rto: 56%.

PF: 175-177 °C.

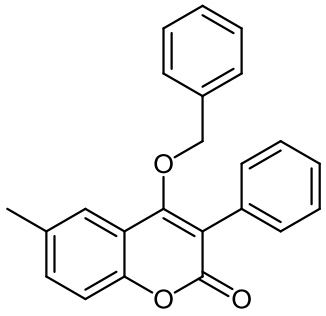
^{1}H NMR ($CDCl_3$) δ: 4.64 (s, 2H, CH_2), 7.14 (s, 1H, H2'), 7.23-7.38 (m, 7H, H8, H3', H4', H6', H3'', H4'', H5''), 7.43-7.57 (m, 4H, H5', H2'', H6'', H7), 7.79 (s, 1H, H5).

^{13}C NMR ($CDCl_3$) δ: 69.1, 100.2, 118.5, 122.9, 126.9, 127.0, 127.1, 127.6, 127.8, 128.4, 128.6, 128.6, 128.7, 128.8, 128.9, 131.1, 132.6, 135.4, 136.3, 150.8, 161.7, 166.9.

MS *m/z* (%): 362 (M^+, 36), 92 (34), 91 (100), 65 (22).

Anal. Elem. Calculado para $C_{22}H_{15}ClO_3$: C 72.83, H 4.17; encontrado: C 72.86 H 4.19.

4-Benciloxi-3-fenil-6-metilcumarina, **(59)**



59

Rto: 50%.

PF: 173-175 °C.

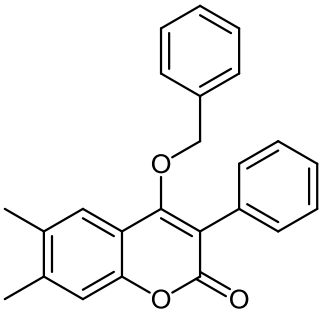
^{1}H NMR ($CDCl_3$) δ: 2.40 (s, 3H, CH_3), 4.64 (s, 2H, CH_2), 7.13-7.16 (m, 2H, H7, H-2'), 7.26 (s 3H, H4', H6', H8), 7.30-7.34 (m, 3H, H3'', H4'', H5''), 7.47 (t, J = 7.52, 2H, H-3', H5'), 7.53 (m, 2H, H2'', H6''), 7.57 (s, 1H, H5).

^{13}C NMR ($CDCl_3$) δ: 21.9, 69.1, 100.3, 116.8, 117.5, 127.0, 127.1, 127.2, 127.6, 127.8, 128.4, 128.6, 128.6, 128.7, 128.8, 128.9, 132.1, 132.6, 135.4, 136.3, 149.8, 161.7, 166.9.

MS *m/z* (%): 342 (M^+, 73), 208 (24), 92 (30), 91 (100).

Anal. Elem. Calculado para $C_{23}H_{18}O_3$: C 80.68, H 5.30; encontrado: C, 80.66, H 5.28.

4-Benciloxi-3-fenil-6,7-dimetilcumarina, **(60)**



60

Rto: 40%.

PF: 193-195°C.

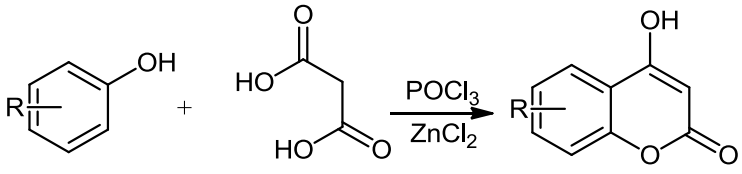
^{1}H NMR ($CDCl_3$) δ: 2.36 (s, 6H, 2xCH_3), 4.64 (s, 2H, CH_2), 6.89 (s, 1H, H8), 7.03 (m, 1H, H6'), 7.07 (m, 1H, H5'), 7.14 (s, 1H, H4'), 7.27 (s, 3H, H3'', H4'', H5'), 7.31 (s, 1H, H5), 7.46 (s, 1H, H2''), 7.50-7.56.(m, 1H, H6'').

^{13}C NMR ($CDCl_3$) δ: 18.9, 19.3, 69.1, 100.3, 114.5, 117.5, 126.7, 127.1, 127.2, 127.6, 127.8, 128.4, 128.5, 128.6, 128.7, 128.8, 128.9, 132.1, 133.6, 136.3, 142.7, 147.8, 161.7, 166.9.

MS *m/z* (%): 356 (M^+, 21), 149 (77), 119 (35), 105 (72), 91 (100), 71 (47), 57 (72), 55 (36).

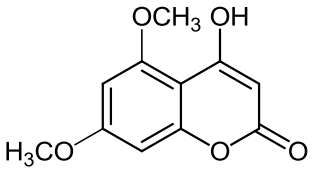
Anal. Elem. Calculado para $C_{24}H_{20}O_3$: C 80.88, H 5.66; encontrado C 80.8; H 5.68.

4.6. Síntesis de las 4-hidroxicumarinas, (61-62)



En un matraz de fondo redondo se prepara una mezcla de 1.06 mmol de fenol, 1.06 mmol de ácido malónico y 3.18 mmol de $ZnCl_2$ anhidro en 10 mL de $POCl_3$. Se deja por 48 h con agitación y bajo atmosfera de argón a 80 °C. Luego se enfría y se trata con hielo y agua. El crudo se disuelve en Na_2CO_3 10%, se filtra y se acidifica. Se columna con una mezcla 9:1 de Hexano:AcOEt y se cristaliza en EtOH o MeOH.

4-Hidroxi-5,7-dimetoxicumarina, **(61)**



61

Rto: 26%.

PF: 160-162 °C.

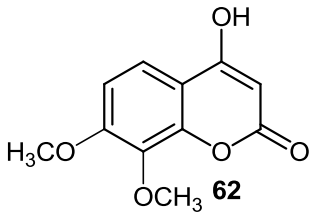
^{1}H NMR (DMSO-d_6) δ: 3.90-3.83 (s, 6H, 2xOCH_3), 6.46-6.38 (s, 1H, H3), 6.62-6.53 (s, 1H, H6), 6.70-6.63 (s, 1H, H8).

^{13}C NMR (DMSO-d_6) δ: 55.8, 55.9, 91.4, 101.3, 119.8, 136.3, 152.8, 157.3, 162.4, 166.8.

MS *m/z* (%): 296 (M^+, 21), 149 (51), 85 (42), 69 (63), 57 (100), 55 (70).

Anal. Elem. Calculado para $C_{11}H_{10}O_5$: C 59.46, H 4.54; encontrado C 59.43, H 4.52.

4-Hidroxi-7,8-dimetoxicumarina, **(62)**



62

Rto: 34%.

PF: 190-192 °C.

^{1}H NMR (DMSO-d_6) δ: 3.85-3.76 (s, 3H, OCH_3), 3.98-3.88 (s, 3H, OCH_3), 6.79-6.69 (s, 1H, H3), 7.28-7.15 (m, 1H, H6), 7.67-7.52 (m, 1H, H5).

^{13}C NMR (DMSO-d_6) δ: 56.8, 60.9, 91.4, 111.3, 120.8, 136.3, 148.0, 149.5, 162.4, 166.1, 162.4.

MS *m/z* (%): 296 (M^+, 21), 149 (51), 85 (42), 69 (63), 57 (100), 55 (70).

Anal. Elem. Calculado para $C_{11}H_{10}O_5$: C 59.46, H 4.54; encontrado C 59.48, H 4.53.

5. Conclusiones

✓ Se ha desarrollado un método sencillo y generalizable para la preparación con buen rendimiento de derivados híbridos cumarina-resveratrol hidroxilados en la posición 4.

✓ Se han obtenido, purificado y caracterizado una serie de 52 distintos derivados 4-hidroxi-3-arilcumarínicos en cantidad suficiente para realizar ensayos farmacológicos frente a diferentes dianas biológicas.

✓ Del mismo modo se ha puesto a punto una ruta de síntesis eficiente, directa y generalizable para obtener una serie de 8 distintas 4-benciloxicumarinas.

✓ La evaluación iniciales de los compuestos sintetizados ofrece muy diferentes e interesantes perfiles farmacológicos.

✓ De los ensayos de inhibición enzimática frente a las dos isoformas MAO-A y MAO-B, algunos de los compuestos son muy activos y selectivos frente a la MAO-B. Los primeros estudios de relación estructura-actividad i-MAO de los compuestos evaluados, nos permiten concluir que la actividad de los mismos está ligada a la presencia de grupos metoxi.

✓ Algunos de los compuestos evaluados como antibacterianos han resultados activos frente la cepa *Staphylococus aureus* y la mayoría frente a *Tenacibaculum Maritimum.*

✓ Muchos de los compuestos ensayados han mostrado interesante actividad antioxidante.

✓ De los ensayos de la actividad citotóxica de las líneas celulares MCF-7 y HL-60 se evidencia un comportamiento "contrario" de los compuestos **61** y **62**, isómeros estructurales.

IV Anexo

Artículos publicados

2012

Tyrosine-like condensed derivatives as tyrosinase inhibitors

Maria João Matos,* Lourdes Santana, Eugenio Uriarte, Silvia Serra, Marcella Corda, Maria Benedetta Fadda, Benedetta Era and Antonella Fais JPP-11-0933.R1 Accept (05-Jan-2012).

Hydroxycoumarins as selective MAO-B inhibitors

Serra, S.;* Ferino, G.; Matos, M. J.; Vázquez-Rodríguez, S.; Delogu, G.; Viña, D.; Cadoni, E.; Santana, L.; Uriarte, E. *Bioorg. Med. Chem. Lett.* **2012**, *22,* 258.

2011

Synthesis of various substituted 3-aryl-4-hydroxycoumarins as new possible drugs of the tenacibaculosis disease

Serra, S;* Vázquez-Rodríguez, S.; Delogu, G.; Fuentes-Edfuf, C.; Santos, Y; Santana, L.; Uriarte, E. 15 th ECSOC.

2010

Efficient synthesis of coumarin-chalcones hybrids as new scaffold with antibacterial interest

Saleta Vazquez-Rodriguez *, Silvia Serra , Ysabel Santos and Lourdes Santana 14th ECSOC.

Synthesis of new possible monoamine oxidase inhibitors

Silvia Serra,* Maria Joao Matos, Giovanna Delogu, Lourdes Santana and Eugenio Uriarte 14th ECSOC.

2009

Coumarin-Chalcone Hybrids as new scaffolds in drug discovery

Saleta Vazquez Rodriguez *, Silvia Serra, Lourdes Santana and Eugenio Uriarte

13th ECSOC.

Journal of Pharmacy and Pharmacology

Tyrosine-like condensed derivatives as tyrosinase inhibitors

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Keywords:	Drug Discovery and Characterisation < Drugs from Natural Sources, Structure/Activity Relationships < Biomedicinal Chemistry, Bioassay Approaches < Pharmaceutical Analysis
Abstract:	With the aim to find out structural features for the tyrosinase inhibitory activity, in the present communication we report the pharmacological evaluation of a new series of 3-aminocoumarins differently substituted with hydroxyl groups, which have been synthesized because they included in their structures the tyrosine fragment (tyrosine-like compounds). The synthesized compounds 4 and 7-9 were evaluated in vitro as mushroom tyrosinase inhibitors. Two of the described compounds show lower IC50 than the umbelliferone, using as reference compound. Compound 7 (IC50 = 53 μM) is the best tyrosinase inhibitor of this small series, having an IC50 10-fold lower than umbelliferone. Compound 7 (3-amino-7-hydroxycoumarin) has precisely the amino and hydroxyl groups mimicking the same positions that both groups occupy on the tyrosine molecule.

Tyrosine-like condensed derivatives as tyrosinase inhibitors

Maria João Matos,[a,*] Lourdes Santana,[a] Eugenio Uriarte,[a] Silvia Serra,[a,b] Marcella Corda,[c] Maria Benedetta Fadda,[c] Benedetta Era[c] and Antonella Fais[c]

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Abstract

Objectives With the aim to find out structural features for the tyrosinase inhibitory activity, in the present communication we report the pharmacological evaluation of a new series of 3-aminocoumarins differently substituted with hydroxyl groups, which have been synthesized because they included in their structures the tyrosine fragment (tyrosine-like compounds).
Methods The synthesized compounds **4** and **7-9** were evaluated *in vitro* as mushroom tyrosinase inhibitors.
Key findings Two of the described compounds show lower IC_{50} than the umbelliferone, using as reference compound.
Conclusions Compound **7** (IC_{50} = 53 μM) is the best tyrosinase inhibitor of this small series, having an IC_{50} 10-fold lower than umbelliferone. Compound **7** (3-amino-7-hydroxycoumarin) has precisely the amino and hydroxyl groups mimicking the same positions that both groups occupy on the tyrosine molecule.
Keywords Tyrosinase inhibition; depigmenting agents; tyrosine-like compounds; mixed-type inhibitor.

Introduction

Tyrosinase (EC 1.14.18.1) is a multifunctional dinuclear copper centre metalloenzyme widely distributed in nature.[1] This enzyme catalyses two distinct reactions of conversion of the tyrosine: 3'-hydroxylation of L-tyrosine into L-3,4-dihydroxyphenylalanine (L-DOPA) and oxidation of the resultant L-DOPA into DOPA quinone, which further polymerizes spontaneously into melanin.[2] Most melanin-biosynthesis inhibitors are phenol or catechol analogues, which are structurally similar to tyrosinase substrates: tyrosine or L-DOPA.[3] Therefore, tyrosine-like molecules could be an interesting scaffold to the tyrosinase inhibition process. Tyrosinase is

mainly involved in the formation of pigments such as melanins and other polyphenolic compounds.[1] Tyrosinase oxidizes phenols and diphenols using a catalytic mechanism dependent on the presence of copper at its active site.[1,2] This enzyme is responsible for unwanted browning of fruits and vegetables.[4] Therefore, it is involved in the process to maintain the appearance, flavour, texture and nutritional value of many fresh-cut products.[4] Tyrosinase is also responsible for the colouring of skin, hair and eyes in animals, including humans.[5] In fact, tyrosinase inhibitors have been used as depigmenting agents for the treatment or prevention of hyperpigmentation disorders.[6] In the last years, many tyrosinase inhibitors have been reported, including vitamin C (ascorbic acid), kojic acid, umbelliferone, resveratrol, hydroquinone and oxyresveratrol.[7-12] Because of the structural similarity, the umbelliferone was used like reference inhibitor (Figure 1).

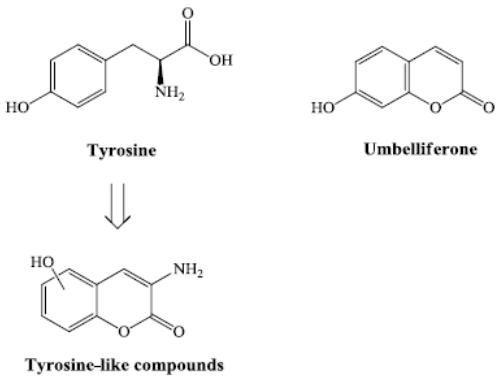


Figure 1. Chemical structures of tyrosine (tyrosinase substrate), tyrosine-like condensed molecules and umbelliferone (tyrosinase inhibitor).

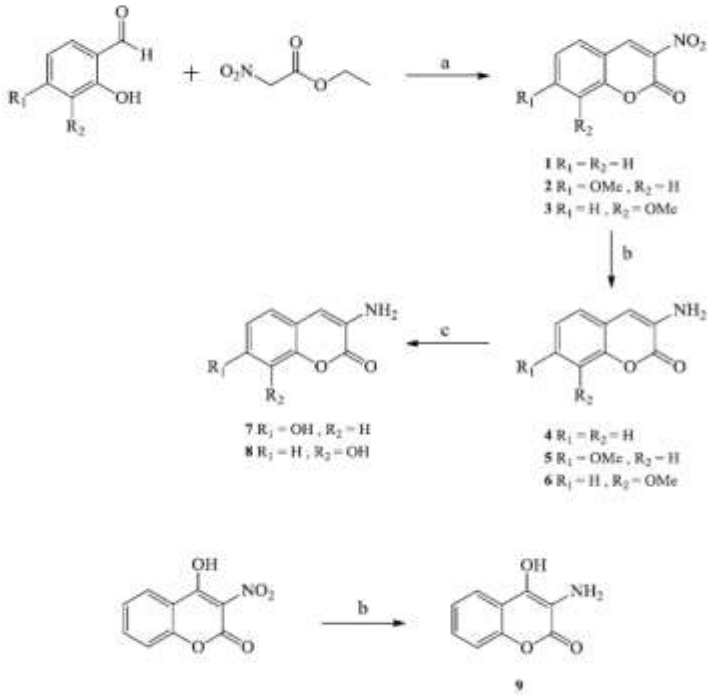
Coumarins are a large family of compounds, of natural and synthetic origin, which presents different pharmacological activities.[13] Chemically they are lactones of the cinnamic acid. Their structural variety is responsible for the important place that they occupy in the natural products and synthetic organic chemistry realm.[14] Some important studies pay special attention to their antioxidative, anticancer anti-inflammatory, cardioprotective and enzymatic inhibitory properties.[15-21] In recent studies, some coumarins proved to be mushroom tyrosinase inhibitors.[22,23] In those studies, esculetin (6,7-dihydroxycoumarin) and umbelliferone (7-hydroxycoumarin) exhibited some of the strongest inhibitory activities of the tested series. In fact, esculetin proved to be the strongest inhibitor of those series.[22] Recently, and in contrast with the first findings, Sollai *et al.* have shown that esculetin is considered to be a tyrosinase substrate rather than an inhibitor, whereas umbelliferone seems to be an inhibitor of the mentioned enzyme.[24] These recent studies revealed that tyrosinase affinity can be efficiently modulated by appropriate substitutions in the coumarin moiety. The introduction of different number of hydroxyl groups in different positions of the

coumarin is one of the most important modifications.[22,23] Also, until the last years, polyphenols were the largest scaffold in tyrosinase inhibition.[25,26]

Tyrosinase inhibitors could have broad applications. As the ideal drug candidate has not been attained, an intensive search for new and innovative tyrosinase inhibitors is still needed. This effort has considerably increased in the recent years. In this context, and in an attempt to develop novel tyrosinase inhibitors, we had previously synthesized and described 3-arylcoumarin derivatives in which both the coumarin and the resveratrol templates were present.[23,27] Based on these results, and with the aim of finding new structure-activity features, in the present communication we propose the study of a series of tyrosine-like condensed molecules (Figure 1). The similarity of these compounds with the tyrosinase substrate is the novelty of this study. The proposed compounds are structurally related to the amino acid tyrosine, the natural substrate of this enzyme. Tyrosinase inhibitors based on aminocoumarin scaffold have not been previously studied.

Materials and Methods

In the present work we synthesized (Scheme 1) and evaluated a small series of 3-aminocoumarins. We decided to explore the importance of the position of a hydroxyl group under the 3-aminocoumarin moiety, based on the idea of mimic the molecular structure of the tyrosinase substrate.



Scheme 1. *Reagents and conditions:* (a) acetic anhydride, NaH, r. t., 3 h; (b) H_2, EtOH, Pd/C, r. t., 5 h; (c) HI, AcOH, Ac_2O, 110 °C, 5 h.

The coumarin derivatives **4**, **7-9** [28-30] were efficiently synthesized according to the synthetic protocol outlined in scheme 1. Starting from different substituted commercially available salicylaldehydes and the ethyl nitroacetate, we afforded 3-nitrocoumarins **1-3**, in good yields (75-95 %). They were synthesized in a dry Schlenk tube, with acetic anhydride as solvent, in the presence of sodium hydride, at room temperature, for three hours. The reaction mixture was purified by flash chromatography, using hexane/AcOEt, in a proportion of 9:1, as eluent. The 3-aminocoumarins **4-6** were prepared from previously synthesized 3-nitrocoumarins, in EtOH, with Pd/C as catalyst, under H_2 atmosphere. The obtained products were purified by crystallization in AcOEt to give the desired 3-aminocoumarins, in a yield of 95 %. The hydroxy derivatives **7** and **8** were obtained from the methoxy ones (compounds 5 and 6 respectively) by a hydrolysis reaction with hydriodic acid, in the presence of acetic acid and acetic anhydride, at 110 °C, for five hours. The residue was purified by crystallization of acetonitrile, and the hydroxy derivatives were obtained in a yield of 60 %. Compound **9** [31] was obtained via reduction reaction, in the same conditions as above described, of the commercially available 4-hydroxy-3-nitrocoumarin, in a yield of 93 %.

The biological assays were carried out following the protocol described below. Pre-incubation with the enzyme: 1/15 M phosphoric acid buffer solution (pH 6.8, 1.8 mL), an aqueous solution of mushroom tyrosinase (1000 U/mL, Sigma Chemical Co., 0.1 mL) and DMSO (0.1 mL) with or without the sample. The mixture was incubated at 25 °C for 10 min. Then, a 1.5 mM of L-3,4-dihydroxyphenylalanine (L-DOPA) solution (1 mL) was added and the reaction was monitored at 475 nm, for 5 min. The percent of tyrosinase activity inhibition was calculated as: inhibition (%) = (A – B) / A x 100, where A represents the difference in the absorbance of control sample between 0.5 and 1.0 min, and B represents the difference in absorbance of the test sample between 0.5 and 1.0 min. The mushroom tyrosinase activity was determinate by spectrophotometric assays (Varian Cary 50). Umbelliferone was used as a reference tyrosinase inhibitor.

Statistical Methods

All experiments were carried out three times. Continuous variables were expressed as mean ± SD. The IC_{50} value, a concentration giving 50 % inhibition of tyrosinase activity, was determined by interpolation of dose-response curves and all data were statistically evaluated using Student's *t*-test or Mann Whitney test (Statistica 6, Statsoft Tulsa; Oklahoma). In order to assess the slopes of curves in figure 2 was used the Kruskal-Wallis test followed by Dunn's post-hoc test (Statistica 6, Statsoft Tulsa; Oklahoma). The criterion for statistical significance was generally taken as $P < 0.05$.

Results

The tyrosinase inhibitory activity of compounds **4** and **7-9** was evaluated *in vitro* by the measurement of the enzymatic activity of mushroom tyrosinase enzyme extracted from the mushroom specie *A. bisporus*. Then, the IC_{50} values for inhibitory effects of the new compounds were calculated (Table 1).

Table 1. Inhibitory effects of compounds **4**, **7-9** and umbelliferone on mushroom tyrosinase activity.

Compounds	IC_{50} (mM) (L-DOPA 0.5 mM)
4	> 5.0
7	0.05±0.01
8	> 5.0
9	0.25±0.003
Umbelliferone	0.42[a]

[a] Obtained from the data in ref.[23]

These results are average results of three experiments.

In the presence of the compound **7**, kinetic studies on mushroom tyrosinase, using a Lineweaver–Burk double reciprocal plot (Figure 2), showed that compound **7** was a mixed-type inhibitor. It can be seen an increasing of the concentration of **7** resulted in a family of lines with different slope and intercept, which intersected in the second quadrant. This behaviour showed that compound **7** can bind not only with free enzyme, but also with the enzyme-substrate complex, and their equilibrium constants are different. The inhibition constants for the inhibitor binding with free enzyme, K_I, (2.14 mM) and with enzyme-substrate complex, K_{IS}, (0.78 mM) were obtained from the linear secondary plots of $1/V_{max}$ and K_m/V_{max} versus the concentration of compound **7**, respectively.

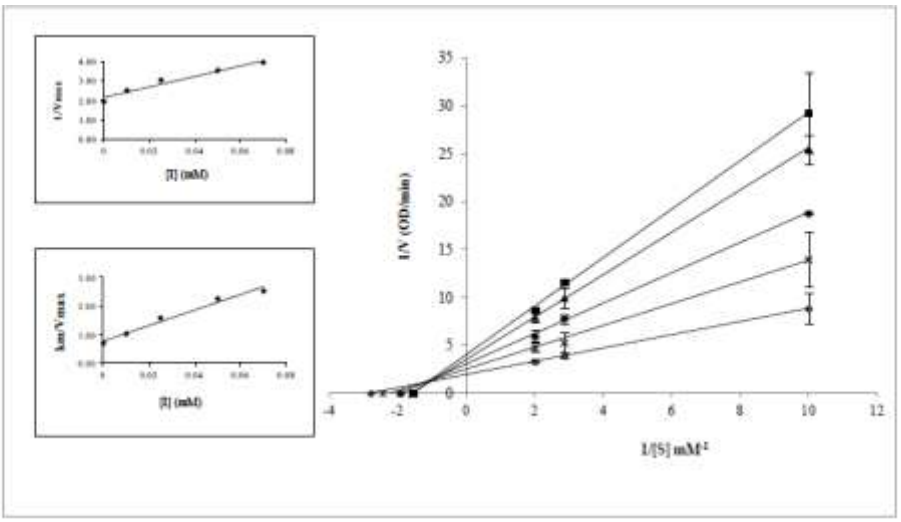


Figure 2: Lineweaver–Burk plots for inhibition of compound 7 on mushroom tyrosinase for catalysis of L-DOPA. Inhibitor concentrations were 0 (♦), 0.010 mM (●), 0.025 mM (x), 0.050 mM (▲), 0.070 mM (■). The insets are the secondary plots of $1/V_{max}$ and K_m/V_{max} versus concentration of compound 7, respectively.

Discussion

In the present communication, the possible tyrosinase inhibitory effect of coumarins that incorporate a portion of tyrosine on their skeletons was described. Therefore, the introduction of a hydroxyl substituent into different positions of the 3-aminocoumarin moiety was studied. By this way it was obtained a small series of compounds that are both tyrosine (tyrosinase substrate) and umbelliferone (tyrosinase inhibitor) analogues.

It is known that umbelliferone (7-hydroxycumarin) has tyrosinase inhibitor effect (IC_{50} = 0.42 mM), despite having no amino group in its position 3. The 3-aminocoumarin (compound **4**) was synthesized and evaluated and did not show tyrosinase inhibitory activity. Then, other coumarins were prepared maintaining the amino group in 3-position, incorporating also a hydroxyl group in different positions of the coumarin skeleton.

Different synthetic methodologies were carried out to obtain compounds **7** and **8**, which have the hydroxyl group in positions 7 and 8, respectively, of the coumarin nucleus (benzene ring) and compound **9**, with the hydroxyl group in position 4 of the coumarin (pyrone ring). As shown in the Table 1, compound **7** is the most active compound of this series, with IC_{50} in the micromolar range (IC_{50} = 53 μM). This compound is more than 10 times more active than umbelliferone, the reference compound. Compound **7** has precisely the amino and hydroxyl groups mimicking the same positions that both groups occupy on the tyrosine molecule. Compound **8** is inactive against tyrosinase whereas compound **9** was active against this enzyme (IC_{50} = 0.26 mM). Compound **9** presents only slightly higher tyrosinase inhibitory activity than umbelliferone.

Therefore, it can be inferred that the activity depends on the position of the hydroxyl group in the coumarin moiety. Also, the presence of the hydroxyl group at the same position of the tyrosine and the umbelliferone is important to the inhibitory activity.

Conclusions

In conclusion, in the present study it was shown that some of the synthesized tyrosine-like condensed derivatives have inhibitory activity against mushroom tyrosinase. The two active compounds present tyrosinase inhibitory activity in the micromolar range. The presence of a hydroxyl group in seven position of the 3-aminocoumarin, the same position as tyrosine and umbelliferone, improves the inhibitory activity respect to the other synthesized derivatives and the reference compound. So, the introduction of hydroxyl groups improves the pharmacological potential of these 3-aminocoumarins, confirming that this lead could be effectively optimized in a candidate for the treatment of some hyperpigmentation skin diseases. These finds have encouraged us to continue the efforts towards the optimization of the pharmacological profile of these coumarins.

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Hydroxycoumarins as selective MAO-B inhibitors

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ABSTRACT

A series of 3-aryl-4-hydroxycoumarin derivatives was synthesized with the aim to find out the structural features for the MAO inhibitory activity and selectivity. Methoxy and/or chloro substituents were introduced in the 3-phenyl ring, whereas the position 6 in the coumarin moiety was not substituted or substituted with a methyl group or a chloro atom due to their different electronic, steric and/or lipophilic properties. Most of the synthesized compounds presented MAO-B inhibitory activity. The presence of methoxy and chloro groups, respectively in the *para* and *meta* positions of the 3-phenyl ring, have an important influence on the inhibitory activity. Moreover, the presence of a chloro atom in the six position of the moiety (compound **7**) improved the inhibitor activity as well as its selectivity against MAO-B compared with iproniazide, used as reference compound. Docking experiments were carried out to understand which are the most energetically preferred orientations adopted by compounds **5**, **6** and **7** inside the MAO-B binding pocket.

Neurodegenerative diseases (ND) constitute the third most important health problem in developed countries. Alzheimer's disease (AD) is the most prevalent of the ND followed by Parkinson's disease (PD). AD is a neurodegenerative and progressive disorder associated, in the most of the cases, with senile dementia. It seems to have a multiple etiology although the main cause it is thought to be the accumulation of β-amyloid plaques in brain that can provoke a degeneration or atrophy of the cholinergic neurons. PD is also a chronic and progressive neurodegenerative disorder, characterized by a predominant motor symptomatology, usually accompanied by non motor symptoms such as depression and anxiety. It seems to be caused by a decrease in the dopamine levels in neuronal synapses. In the treatment of this disease, dopaminergic agonists are generally employed. However, other therapeutic alternatives can be employed, such as the use of monoamine oxidase B inhibitors (MAOI-B), or the use of antioxidant compounds in order to prevent the oxidative cells damage.

MAO is an important FAD-containing enzyme (flavoenzyme) present in the outer mitochondrial membrane of neuronal, glial and many other cells.[1,2] It exists in two isoforms namely as MAO-A and MAO-B that have been identified based on their amino acid sequences, three-dimensional structure, substrate preference and inhibitor selectivity.[3,4] MAO-A preferentially deaminates serotonin and noradrenaline, whereas MAO-B has a higher affinity to phenylethylamine and benzylamine.[5,6] Therefore, these isoenzymes play a vital role in the monoamines degradation and, as consequence, in the inactivation of neurotransmitters. Their regulation determines the interest of the MAOI compounds as drugs used in the treatment of neurodegenerative and neurological disorders. In particularly, MAO-A inhibitors are effective in the treatment of depression,[7,8] while MAO-B inhibitors are useful in the treatment of PD.[9,10] In the last years, it has been proved that in patients who suffer ND the activity of brain MAO-B is increased. This fact provokes an increase of free radicals that are responsible for the oxidative stress, neuronal cell death and also for the development of the β-amyloid plaques.[11] In this sense, it is an accepted theory that the beneficial effects of the MAO-B inhibitors in the prevention of the neuronal damage is mostly derived to the decrease of hydrogen peroxide generated by the inhibition of this enzyme.

Coumarins are an important group of organic compounds from natural and/or synthetic origin, that show, due to their structural diversity, numerous biological activity.[12–14] There are coumarins described as anticancer, anti-inflammatory, antimicrobial, cardioprotective, vasorelaxant, and antioxidant agents.[15–20] Recently studies pay special attention to the MAO inhibitory properties of the 3-arylcoumarin derivatives.[21–26]

Flavonoids are naturally occurring polyphenolic compounds that are found in diets and medicinal herbs. The physiological benefits of dietary flavonoids (flavones and isoflavones) have been attributed to their antioxidant and free radical-scavenging abilities.[27] Additionally, flavonoids have been revealed to exhibit

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anti-amiloidogenic properties such as inhibitory effects on β-secretase enzimes[28,29] and also MAO-B inhibitory activity.[30]

In previous works, we reported the synthesis and biological evaluation of a series of 3-arylcoumarins as potent and selective MAO-B inhibitors.[23–25] These interesting results prompted us to synthesize and evaluate new 3-arylcoumarin derivatives, in which we introduce a hydroxyl group in the 4 position of coumarin skeleton (Fig. 1).

This modification allows us considering the similarity of the core structure of the 4-hydroxycoumarin (B) comparing with the 2-hydroxyisoflavone (C). Taking into account these data we could establish a potential therapeutic application of these analogues flavonoids/coumarins as a new interesting scaffold with interest against age-related neurodegeneration such as PD or AD.

The synthesis of 3-aryl-4-hydroxycoumarin derivatives (**1–7**)[31–33] is outlined in Scheme 1.

The key step for the synthesis of the 3-arylcoumarin skeleton was achieved by a palladium-catalyzed coupling Suzuki reaction between phenyliodonium zwitterions and aryl conveniently substituted phenyl boronic acids. Initially we have synthesized the different phenyliodonium coumarinate species (I–III).[31,32] They are electrophilic molecules with a positive charge at iodine, compensated by a internal negative charge that can be localized formally at the α-carbon or delocalized to a neighboring oxygen. Then we have carried out the palladium-catalyzed coupling reaction using $Pd(OAc)_2$ as catalyst and $P(t\text{-}Bu)_3$ as ligand to afford the compounds (**1–7**) with good yields.

By ^1H and ^{13}C NMR spectroscopy studies we have found out that the synthesized compounds (**1–7**) were presented in the 4-hydroxycoumarin tautomeric form. Therefore we have centered on their analysis at the physiologic pH conditions. In the biological inhibitory assays it was also used the appropriate solvent that guaranties the presence of this structure.

The MAO inhibitory activity of compounds **1–7** was evaluated in vitro by the measurement of the enzymatic activity of human recombinant MAO isoforms expressed in BTI insect cells infected with baculovirus.[34] Subsequently, the IC_{50} values and MAO-B selectivity indexes [IC_{50} (MAO-A)]/[IC_{50} (MAO-B)] for inhibitory effects of both new types of compounds and iproniazide (used as reference inhibitor) were calculated (Table 1).[34,35]

In the present Letter, we have studied the effect in the MAOI activity of the introduction of alkyl, alkoxy or halogen substituents in different positions of the 3-aryl-4-hydroxycoumarin nucleus. We have found that the simple 4-hydroxy-3-phenylcoumarin did not present MAOI activity (compound **1**). The introduction of a methoxy substituent in the *para* position of the 3-phenyl ring led to a compound **2** with inhibitory activity against MAO-B. So we take this compound as a point of reference for the subsequent modifications. Due to the fact that the 6 position of the coumarin skeleton is particularly relevant to the MAO-B inhibitor activity and selectivity,[36,37] we have studied the effect of the introduction of a methyl group or a chloro atom in this position. The introduction of a methyl group (compound **3**) slightly improved the activity. However, the replacement for a chloro atom at the same position decreases the activity (compound **4**). In addition, when we analyze the second series (compounds **5–7**) where a chloro atom has been introduced at the *meta* position of the *p*-methoxy-3-phenyl ring, the MAO-B inhibitory activity, in most of the cases, improves. In this case, if we introduce a methyl group in the 6 position (compound **6**) the MAOI-B activity did not change, whereas if the substituent is a chloro atom (compound **7**) the MAO-B inhibitory activity considerably improves. Therefore compound **7** is more active against MAO-B isoenzyme than the

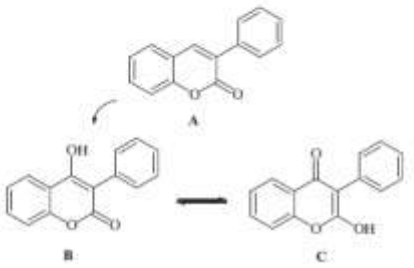
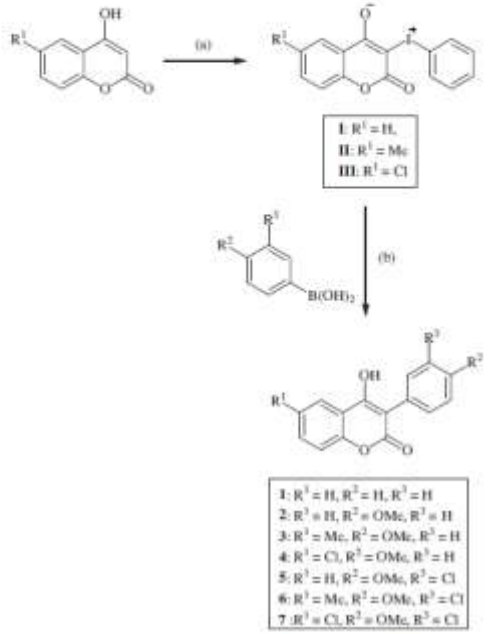


Figure 1. Chemical structures of the 3-phenylcoumarin (A), 4-hydroxy-3-phenylcoumarin (B) and 2-hydroxyisoflavone (C).



Scheme 1. Reagents and conditions: (a) $PhI(OAc)_2$, Na_2CO_3, H_2O, rt, 14 h; (b) $Pd(OAc)_2$, $P(t\text{-}Bu)_3$, LiOH, DME/H_2O, rt, 24–48 h.

Table 1
MAO-A and MAO-B inhibitory activity results for the synthesized compounds **1–7** and reference inhibitor

Compounds	MAO-A IC_{50} (μM)	MAO-B IC_{50} (μM)	Selectivity index
1	[a]	[a]	—
2	[a]	68.59 ± 4.70	>1.4[b]
3	[a]	32.04 ± 2.16	>3.1[b]
4	[a]	[a]	—
5	[a]	9.26 ± 0.63	>10[b]
6	[a]	42.68 ± 2.88	>2.3[b]
7	[a]	2.79 ± 0.19	>36[b]
Iproniazide	6.56 ± 0.76	7.54 ± 0.36	0.87
R-(−)-Deprenyl	68.73 ± 4.21	$17x10^{-3} \pm 1.9 \times 10^{-3}$	4.043

All IC_{50} values shown in the table are expressed as means ± SEM from five experiments. Level of statistical significance:

[a] Inactive at 100 μM (highest concentration tested).

[b] Values obtained under the assumption that the corresponding IC_{50} against MAO-A is the highest concentration tested (100 μM).

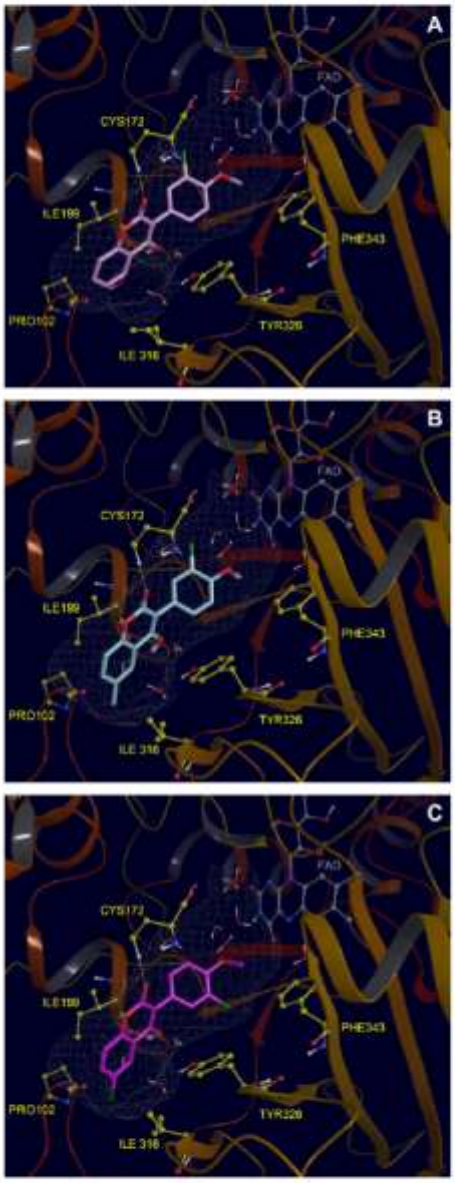


Figure 2. Best docking poses retrieved for compounds **5** (a), **6** (b) and **7** (c) into the MAO-B (PDB code: 2V60). Coumarins are represented in tube with carbon atoms colored in plum for **5**, turquoise for **6** and purple for **7**. Hidden ribbons for a better visualization from residues Glu158 to Glu179 (excluding Cys172). MAO-B binding site displayed as gray mesh style. Interacting residues and FAD cofactor labeled in ball and stick, with carbon atom colored in yellow and gray respectively. Water molecules depicted in wire rendering. H-bonds displayed in yellow dot line. Nonpolar hydrogens are omitted.

iproniazide but not than the *R*-(−)Deprenyl, used as reference compounds. None of the described compounds showed MAO-A inhibitory activity for the highest concentration tested (100 μM).

A computational approach, through docking calculations, was used to better understand which are the most energetically preferred orientations adopted by compounds **5**, **6** and **7** inside the MAO-B binding pocket.

High resolution crystal structure of hMAO-B (PDB code 2V60)[4] was taken as target for docking experiments. Protein Preparation Wizard of Maestro 9.1[38] was used to set-up protein before run calculations. With this helpful tool co-crystallized ligand was removed, water molecules beyond 5 Å from the ligand were deleted, hydrogen atoms were added and then minimized using OPLS_2005 force field. Compounds **5**, **6** and **7** were prepared with LigPrep[39] tool using MMFFs force field. The QM-Polarized Ligand Docking (QPLD)[40] protocol was used for docking calculations. In a first step, ligands were docked into the known[20] MAO-B binding site using Glide[41] standard precision (SP); the initial charges were calculated by semiempirical methods and 8 best poses for each ligand were retained. In the second step the Quantum Mechanical (QM) treatment of charges, calculated by Jaguar[42], allowed to take into consideration the polarization of the charges in the ligand by the protein. The final step consisted to redock the ligand, with improved charges, with Glide extra precision (XP) mode, retaining the 4 best poses for each ligand. With the aim of minimizing the protein–ligand complexes and return the estimate binding free energy for each ligand, the 4 output complexes derived from QPLD were subjected to MacroModel-eMBrAcE[43] minimization. With this flexible-receptor tool all residues with a distance not exceeding 5Å from the ligand were allowed to move freely, while residues outside this shell were frozen. The complexes were minimized using OPLS_2005 force field. Results were retrieved using Energy difference mode which calculates separately the energy associated first on the receptor, than on the ligand and finally on the complex using the follow equation:

$$\Delta E = E_{\text{complex}} - E_{\text{ligand}} - E_{\text{protein}}$$

The most stable binding solution for compounds **5**, **6** and **7** shows the same pattern (Fig. 2).

The entrance cavity of binding site is occupied by the hydroxycoumarin moiety leaving the 3-arylcoumarin rings directed toward FAD cofactor. Cys172 plays a significant role for the complex stabilization, forming an H-bond with carbonyl oxygen of all the three coumarins. The higher activity showed by **5** and **7**, compared to **6**, could be partially due to involvement of another H-bond in the binding mode; in this case only **5** and **7** form an H-bond between the 4-hydroxy group and Ile199. Furthermore, 3-arylcoumarin rings of **5**, **6** and **7** share a π–π stacking interaction with Tyr326. Interestingly, the *p*-methoxy group occupies the same position in the three coumarins, while the position adopted by the chloro atom in the 3-phenyl ring appears to be unimportant for the modulation of MAO-B inhibitor activity, because it assumes an opposite orientation in the two most actives compounds **5** and **7**. Good van der Waals and electrostatic interactions with Pro102, Ile316 and Phe343 were also observed for all the docked molecules.

As conclusion, these preliminary results allow us a better understanding of the molecular fragments that are essential to maintain and/or improve the MAO activity and selectivity. These findings encourage us to continue the efforts towards the optimization of the pharmacological profile of this structural moiety as an important scaffold for the potential treatment of ND.

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33. *General procedure for the preparation of 3-aryl-4-hydroxycoumarins*: a degassed solution of appropriated phenyl boronic acid (2.2 equiv) and $P(t\text{-}Bu)_3$ (27 mL) in DME and H_2O (4:1, 12.5 mL) was added to a mixture of iodonium ylide (0.55 mmol), LiOH/H_2O (3 equiv) and $Pd(OAc)_2$ (6.2 mg) under argon at room temperature. After being stirred at the same temperature for 24–48 h. The resulting mixture was purified by FC (hexane/ethyl acetate, 7:3) to give the desired compound.
3-(3′-Chloro-4′-methoxyphenyl)-4-hydroxycoumarin (5). It was obtained with yield 80%. Mp: 298–300 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 3.94 (s, 3H, OCH_3), 7.21 (s, 1H, H2′), 7.28–7.49 (m, 3H, H6, H8, H5′), 7.56–7.83 (m, 2H, H7, H6′), 7.98 (d, 1H, H5, J = 7.6), 15.7 (s, 1H, OH). ^{13}C NMR (DMSO-d_6) δ (ppm): 56.2, 105.0, 112.8, 116.6, 116.9, 120.7, 124.2, 124.4, 125.6, 131.5, 132.7, 132.8, 152.7, 154.3, 161.0, 162.3. MS m/z (%): 302 (M^+, 100), 182 (64), 121 (95). Anal. Calcd for $C_{16}H_{11}ClO_4$: C, 63.48; H, 3.66. Found: C, 63.45; H, 3.69.
3-(3′-Chloro-4′-methoxyphenyl)-4-hydroxy-6-methylcoumarin (6). It was obtained with yield 80%. Mp: 301–304 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 2.45 (s, 3H, CH_3), 3.90 (s, 3H, OCH_3), 7.14–7.24 (m, 1H, H8), 7.25–7.37 (m, 3H, H7, H2′, H5′), 7.40–7.52 (m, 1H, H6′), 7.80 (s, 1H, H5), 15.4 (s, 1H, OH). ^{13}C NMR (DMSO-d_6) δ (ppm): 20.9, 56.6, 105.0, 112.8, 116.4, 120.8, 123.8, 125.6, 129.0, 131.5, 132.7, 133.6, 133.6, 150.8, 154.3, 160.9, 162.4. MS m/z (%): 316 (M^+, 59), 182 (80), 135 (100), 97 (36), 83 (37), 71 (51), 57 (91). Anal. Calcd for $C_{17}H_{13}ClO_4$: C, 64.46; H, 4.14. Found: C, 64.46; H, 4.09.
6-Chloro-3-(3′-chloro-4′-methoxyphenyl)-4-hydroxycoumarin (7). It was obtained with yield 62%. Mp: 321–323 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 3.90 (s, 3H, OCH_3), 7.0–7.57 (m, 3H, H8, H2′, H5′), 7.60–7.84 (m, 2H, H7, H6′), 8.0 (s, 1H, H5), 15.7 (s, 1H, OH). ^{13}C NMR (DMSO-d_6) δ (ppm): 56.8, 113.0, 119.0, 121.3, 123.6, 125.5, 128.7, 131.7, 132.6, 132.8, 135.2, 136.1, 151.5, 154.6, 160.2, 162.2. MS m/z (%): 338 (M^+, 59), 336 (100), 182 (75), 155 (88), 142 (26), 57 (31). Anal. Calcd for $C_{16}H_{10}Cl_2O_4$: C, 57.0; H, 2.99. Found: C, 57.05; H, 3.05.
34. Determination of human monoamine oxidase (hMAO) isoform activity. The effects of the tested compounds on hMAO isoform enzymatic activity were evaluated by a fluorimetric method. Briefly, 0.1 mL of sodium phosphate buffer (0.05 M, pH 7.4) containing the tested drugs in several concentrations and adequate amounts of recombinant hMAO-A or hMAO-B required and adjusted to obtain in our experimental conditions the same reaction velocity [165 pmol of *p*-tyramine/min (hMAO-A: 1.1 μg protein; specific activity: 150 nmol of *p*-tyramine oxidized to *p*-hydroxyphenylacetaldehyde/min/mg protein; hMAO-B: 7.5 μg protein; specific activity: 22 nmol of *p*-tyramine transformed/min/mg protein)] were placed in the dark fluorimeter chamber and incubated for 15 min at 37 °C. The reaction was started by adding (final concentrations) 200 μM Amplex® Red reagent, 1 U/mL horseradish peroxidase and 1 mM *p*-tyramine. The production of H_2O_2 and, consequently, of resorufin was quantified at 37 °C in a multidetection microplate fluorescence reader (FLX800™, Bio-Tek® Instruments, Inc., Winooski, VT, USA) based on the fluorescence generated (excitation, 545 nm; emission, 590 nm) over a 15 min period, in which the fluorescence increased linearly. Control experiments were carried out simultaneously by replacing the tested drugs with appropriate dilutions of the vehicles. In addition, the possible capacity of the above tested drugs for modifying the fluorescence generated in the reaction mixture due to non-enzymatic inhibition (e.g., for directly reacting with Amplex® Red reagent) was determined by adding these drugs to solutions containing only the Amplex® Red reagent in a sodium phosphate buffer. The specific fluorescence emission (used to obtain the final results) was calculated after subtraction of the background activity, which was determined from vials containing all components except the hMAO isoforms, which were replaced by a sodium phosphate buffer solution.
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Synthesis of various substituted 3-aryl-4-hydroxycoumarins as new possible drugs of the tenacibaculosis disease

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Abstract

Bacterial diseases of freshwater fish epithelia have been extensively studied; however in marine fishes relatively few bacterial skin and gill diseases have been characterized. *Tenacibaculum Maritimum*, a Gram-negative and filamentous bacterium, has been described as the etiological agent of tenacibaculosis in marine fish. Since 1990, as the farming of fish became a steadily growing industry, this disease has been widely distributed in several countries and is considered a limiting factor for the culture of many species of commercial value in the world. Among the drugs used in the past few years enrofloxacin proved to be the most useful compound in the control of tenacibaculosis outbreaks, although the rapid appearance of resistant strains has already been described. Therefore, it is important to discover new classes of selective antimicrobial drugs.

Coumarin derivatives are a class of compounds that present a wide range of pharmacological activities. Among others, recent studies pay special attention to the antibiotic properties of the coumarin moiety. In order to contribute to the development of new agents, in the present communication we have synthesized new 3-arylcoumarin derivatives, structurally related with well know antibiotic novobiocin.

On this scaffold we introduced substituents with different electronic, steric and/or lipophilic properties in different positions of the aromatic rings.

The compounds have been prepared via a palladium-catalyzed coupling type Suzuki reaction of phenyliodonium zwitterion species previously synthesized by us. Further, were evaluated for antibacterial activity against bacterial strains. The preliminary results of biological activity and/or selectivity against Tenacibaculum Maritimum are presented.

Key words: coumarin, *Tenacibaculum Maritimum*, Suzuki reaction.

Introduction

The rapid expansion of the aquaculture industry in the last decade has increased the losses caused by systemic bacterial infections in marine fish farming throughout the world. Bacterial diseases of freshwater fish epithelia have been extensively studied; however in marine fishes relatively few bacterial skin and gill diseases have been characterized. Marine tenacibaculosis, which is caused by *Tenacibaculum Maritimum* (formerly *Flexibacter maritimus*), is an economically important disease in a great variety of European cultured fish.[1] This pathogen, a Gram-negative and filamentous bacterium, primarily attacks skin, mouth, fins and tail of fish, causing severe necrotic and ulcerative lesions on the body surface. The manifestations of this infection depend on the species and age of the fish.

Up to now, most treatments were proposed for the tenacibaculosis outbreaks. Among the drugs used in the past few years the antibiotic enrofloxacin proved to be the most useful compound in the control of tenacibaculosis, although the rapid appearance of resistant strains has already been described.[2] Therefore, it is important to discover new classes of selective antimicrobial drugs.

Coumarins are a large family of compounds, of natural and/or synthetic origin, that presents different pharmacological activities.[3-7] Due to their structurally variability, they occupy an important place in the realm of natural products and synthetic organic chemistry. Among others, recent studies pay special attention to the antibiotic properties of the coumarin moiety.[8-10]

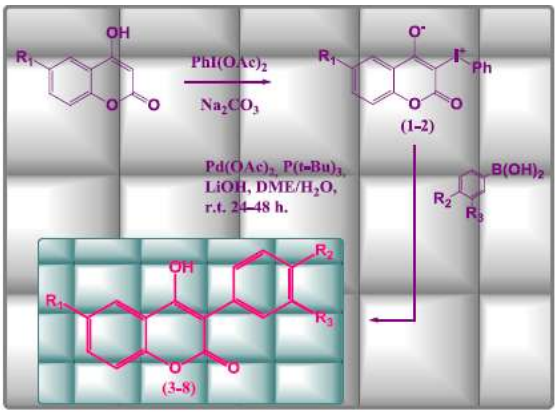
Whit the aim to contribute to the development of new agents, in the present communication we report the synthesis of new 3-arylcoumarin derivatives, via a palladium-catalyzed coupling type Suzuki reaction.

Results and discussion

In the present work we have designed and evaluated a series of 3-phenylcoumarin derivatives with different number of chloro substituent in both the 3-aryl ring and coumarin nucleus. The compounds were synthesized according to Scheme 1 and details are given in the Experimental section.[11]

The key step for the synthesis of the 3-arylcoumarin skeleton was achieved by a palladium-catalyzed coupling Suzuki reaction between phenyliodonium zwitterions species previously synthesized by us and aryl conveniently substituted boronic acids.

We have used 2 differents commercial coumarins to produce two coumarin-based phenyliodonium zwitterions (**1**-**2**). Then we have carried on the palladium-catalyzed coupling reaction to introduce the aryl group at the 3 position of coumarin scaffold (Compounds **3**-**8**, Table 1).



Scheme 1

Compounds	R1	R2	R3	Yield %	M.P.
3	H	H	H	63	237-239 °C
4	Cl	H	H	68	263-265 °C
5	H	Cl	H	52	267-269 °C
6	Cl	Cl	H	66	314-316 °C
7	H	Cl	Cl	51	321-323 °C
8	Cl	Cl	Cl	43	347-349 °C

Table 1

All the compounds were screened for their in vitro antibacterial activity by disk diffusion method. Most of them were inactive against different species of Gram-pos. and Gram-neg. organisms, but surprisingly all tested compounds have be found to exhibit antibacterial activity against *Tenacibaculum Maritimum,* showing significant zone of inhibition of the growth of the bacteria in the agar plates (Table 2). The general procedure[12] and details are given in the Experimental section.

The compound **6** shows the best activity with a zone of inhibition of 38 mm. (Photo 1)

Compounds	Diameter of the zone of inhibition of the growth of ***T. Maritimum***
3	27 mm
4	18 mm
5	20 mm
6	38 mm
7	25 mm
8	21 mm

Table 2: Evaluation of diameter of the zone of inhibition of *Tenacibaculum Maritimum* inhibitory of compounds **3–8.**

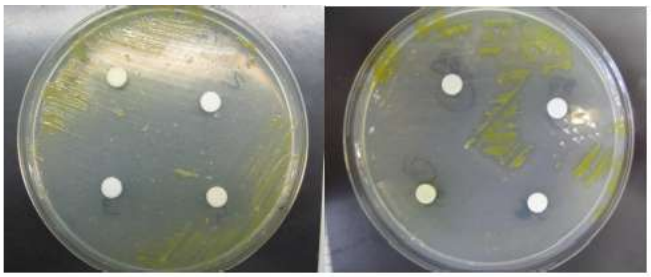


Photo1: zone of inhibition of compound **3** and **6** respectively.

Conclusions

In summary, we report a versatile methodology for the synthesis of a new library of coumarin derivatives in a short number of steps and satisfactory yields. The compounds were then evaluated for antibacterial activity against different bacterial and fungal strains. The preliminary results indicate that most of the compounds showed remarkable antibacterial activity and interesting selectivity against *Tenacibaculum Maritimum*, especially compound 6 with an inhibition zone of 38 mm. These results suggest that this new scaffold can be further optimized for building potent and selective antibacterials used in Tenacibaculosis disease.

Experimental section

Chemistry

All reactions were carried out under dry and deoxygenated argon atmosphere. Identification of the compounds and course of the reactions were visualized using TLC plates (Merck, silica gel 60F254) under UV light (254-366 nm).

Melting points were determined using a Reichert Kofler thermopan or in capillary tubeson a Buchi 510 apparatus and are uncorrected.

General procedure for the preparation of 3-phenyliodonium coumarinates (1-2).

Iodobenzene diacetate (10 mmol) was suspended in a solution of Na_2CO_3 (10 mmol) in water (100 mL) and was stirred for 30 min at room temperature. To this solution was added a mixture of the corresponding 4-hydroxycoumarin (10 mmol) and Na_2CO_3 (10 mmol) in water (100 mL). The mixture was stirred at room temperature for 14 h, the precipitate was collected by filtration, washed with water (5 x 20 mL) and dried under vacuum. The resulting white solid was used in the next reaction without further purification.

General procedure for the preparation of 3-aryl-4-hidroxycoumarins (3-8).

A degassed solution of appropriated phenyl boronic acid (2.2 equiv.) and P(*t*-But)$_3$ (27 mL) in DME and H_2O (4:1, 12.5 mL) was added to a mixture of iodonium ylide (0.55 mmol), LiOH/H_2O (3 equiv.) and

$Pd(OAc)_2$ (6.2 mg) under argon at room temperature and stirred for 24-48 h. The mixture was then purified by FC (hexane:ethyl acetate, 7:3) to give the desired compound. Yields 43-68 %.

Biological assay

The Antibacterial activity was assessed by disk diffusion method, following the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) (2006). [12] To this aim, were recoated sterile discs (Liofilchem Bacteriology Products, Italy) with the synthesized compounds at final concentrations of 10 and 100 μg/disc and were applied on the surface of agar plates Mueller Hinton Agar (Difco) previously inoculated with the bacterial strains. The plates were incubated at 37 °C during 24h and it was determined the diameter of the growth zone of inhibition as an index of their sensitivity to the antimicrobials tested.

Acknoledgments: We are grateful to the Spanish Ministerio de Sanidad y Consumo (PS09/00501) and to Xunta da Galicia (CSA030203PR). Silvia Serra is grateful to Programma Master and Back PR_MABA2009-613 Regione Sardegna. S.V.R. thanks to Ministerio de Educación y Ciencia for a PhD grant (AP2008-04263).

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Efficient synthesis of coumarin-chalcones hybrids as new scaffold with antibacterial interest.

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Abstract: Due to the potential antibacterial activity of the chalcone and coumarin moieties, hybrid compounds containing both structures have been synthesized in good yield using the Knoevenagel reaction as the key step.

Keywords: Coumarin, Chalcone, Knoevenagel , Antibacterial

Introduction

Chemotherapy, in its most general sense, is the treatment of diseases by chemicals especially by killing micro-organisms or cancerous cells. Nowadays are known a wide range of different chemotherapeutic agents. In this sense, the emergence of multyidrug-resistance bacteria has made treatment of infectious diseases difficult. This means that it is necessary the discovery of novel antibacterial agents.

One of our aims in the last years has been the development of new tools and methodologies for drug discovery. The molecular manipulation of promising lead compounds is still a major line of approach to develop new and efficient drugs. Following this aim we designed hybrid molecules coming from two natural occurring compounds: coumarin and chalcones. Coumarin derivatives have well known

pharmacological activities such as antibacterial, antitumor, anti-inflammatory, antithrombotic, cardio protectors or enzymatic inhibitors.[1-5]

Chalcones (a,β-unsaturated ketones) are an important group of natural or synthetic flavonoids that are know to exhibit an impressive array of biological properties[6-8]. Particularly, their antimicrobial and antifungal action is attributed to the reactive enone moiety[9]. As Michael acceptor enone, reactions of chalcones are modulated by electron withdrawing/donating character of sustituents at the *p*-positions of the aromatic groups.

Therefore in the present study, the chalcone functionality has been attached in a coumarin nucleus. The new scaffold incorporate two Michel enones in a single molecule and introducing different sustituents in the aromatic ring allow us to modulate the acceptor character for the thiol nucleophilic attack of microbial proteins.

Using this strategy a series of 3-cinnamoylcoumarin derivatives has been synthesized as potential antibacterial compounds.

Results and Discussion

The aim of this work has been to synthesize new coumarin-chalcone hybrids containing different sustituents in the aromatic rings that can potentially be used as new lead compounds in drug discovery, particularly as antimicrobial agents.

Compounds were synthesized using a two steps synthetic strategy that allows us to obtain the desired compounds in good yields (***Figure 1***)

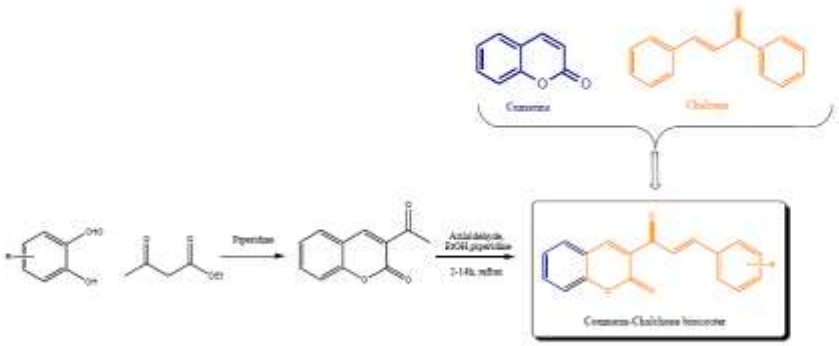
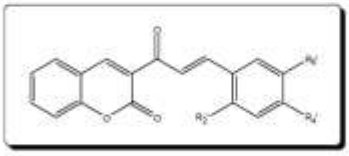


Figure 1*. Synthetic route used to hybrids of coumarin-chalcone.*

The 3-acetylcoumarin precursor was prepared by a Knoevenagel reaction in basic conditions in 89% yield. The final step was a Claisen-Schmidt aldolic condensation in basic conditions that allow us to obtain the final compounds in good yield (***Table 1***)

Table 1. *Synthesized compounds*



Compound	R_2'	R_4'	R_5'	Yield (%)
2	H	H	H	60
3	H	OMe	H	61
4	OMe	OMe	H	80
5	OMe	OMe	OMe	50
6	OMe	NO_2	H	67
7	H	H	NO2	53

The followed methodologies in a parallel synthesis way, bring the opportunity of synthesize structural related compounds with punctual modifications in the aromatic rings depending on the starting materials.

Biological assays as antibacterial agents will be further presented.

General Experimental Procedure

All reactions were carried out under dry and deoxygenated argon atmosphere. Solvents were used as anhydrous by reflux of each solvent over an appropriate dryer agent and further distillate under argon atmosphere.

Qualitative identification of the compounds and course of the reactions were visualized using TLC plates (Merck, silica gel $60F_{254}$) under UV light (254-366 nm). Melting points were determined using a Reichert Kofler thermopan or in capillary tubes on a Büchi 510 apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a Bruker WM-250 at 250 MHz using TMS as internal standard (chemical shifts in δ values, *J* in Hz).

Synthesis of 3-acetylcoumarin: A mixture of salicilaldehyde (1 eq.), ethyl acetoacetate (1 eq.) and a few drops of piperidine were mixed for 5 min. at room temperature without

any solvent. Reaction was neutralized with HCl (1M) and finally the product was isolated by filtration. The final compound was then recristalized in EtOH[10].

3-Acetylcoumarin (1): Yield 89%. **Mp.:** 119-121 ºC. **^{1}H NMR** (250 MHz, $CDCl_3$) δ ppm 8.34 (s, 1H), 7.54 – 7.42 (m, 2H), 7.26 – 7.12 (m, 2H), 2.56 (s, 3H).

General procedure for the synthesis of 3-cinnamoylcoumarins (2-7): A mixture of 3-acetylcoumarin (1 eq.) and te corresponding benzaldehyde (1.2 eq.) in EtOH was stired with a few drops of piperidine under reflux during 2-12h. Mixture was cooled and the resulting solid was filtered and purified by recristalization or flash chromatography. Purification of compounds **2-5** was made by recristalization in MeOH, while compounds **6-7** were purified by flash chromatography using a 8:2 mixture of Hexane:AcOEt as eluent.

3-Cinnamoylcoumarin (2): Yield 60% **Mp.:** 202-204 **^{1}H-NMR** (250 MHz, $CDCl_3$) δ ppm 8.60 (s, 1H), 7.92 (d, *J* = 7.9 Hz, 2H), 7.73 – 7.61 (m, 4H,), 7.45 – 7.35 (m, 5H).

3-(4'-Methoxicinnamoyl)cumarin (3): Yield 60% **Mp:** 202-203 ºC. **^{1}H NMR** (250 MHz, $CDCl_3$) δ ppm 8.40 (s, 1H), 7.71 (d, *J* = 15.8 Hz, 1H), 7.62 (d, *J* = 15.8 Hz, 1H) 7.48 (m, 4H), 7.29 – 7.11 (m, 2H), 6.76 (d, *J* = 8.8 Hz, 2H), 3.69 (s, 3H).

3-(2',4'-Dimethoxicinnamoyl)cumarin (4): Yield 80% **Mp.:**192-194ºC. **^{1}H NMR** (250 MHz, $CDCl_3$) δ ppm 8.37 (s, 1H), 8.00 (d, *J* = 15.8 Hz, 1H), 7.72 (d, *J* = 15.8 Hz, 1H), 7.56 – 7.36 (m, 3H), 7.29 – 7.11 (m, 2H), 6.36 (dd, *J* = 8.6, 2.2 Hz, 1H), 6.29 (d, *J* = 2.2 Hz, 1H), 3.73 (s, 3H), 3.69 (s, 3H).

3-(2',4',5'-Trimethoxicinnamoyl)cumarin (5): Yield 50%. **Mp:** 190-192 ºC. **^{1}H NMR** (250 MHz, $CDCl_3$) δ ppm 8.38 (s, 1H), 8.05 (d, *J* = 15.8 Hz, 1H), 7.65 (d, *J* = 15.8 Hz, 1H), 7.55-7.40 (m, 2H), 7.28 – 7.12 (m, 2H), 7.01 (s, 1H), 6.33 (s, 1H), 3.78 (s, 3H), 3.74 (s, 3H), 3.73 (s, 3H).

3-(2'-Methoxy-4'-nitrocinnamoyl)coumarin (6): Yield 57% **Mp:** 227-229 ºC. **^{1}H NMR** (250 MHz, $CDCl_3$) δ ppm 8.57 (s, 1H), 7.96 (d, *J* = 12.6 Hz 1H), 7.84-53 (m, 5H), 7.42 (d, *J* = 8.8 Hz, 2H) 7. 32 (d, *J* = 12.6 *Hz*, 1H), 3.86 (s, 3H).

3-(3'-Nitrocinnamoyl)coumarin (7): Yield 49% **Mp:** 205-207 ºC. . **^{1}H NMR** (250 MHz, $CDCl_3$) δ ppm 7,98 (d, *J* = 7.68 Hz 1H), 7.90 (d, *J* = 1.8 Hz), 7.85 (s,1H) 7.45 (t, *J* = *6.57 Hz*, 2H), 7.40 – 7.30 (m, 1H), 7.28-7.17 (m, 5H).

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Synthesis of new possible monoamine oxidase inhibitors.

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Abstract:

We have generated different resveratrol-coumarin hybrids with the aim of evaluate their biological applications and their pharmacological properties, particularly the inhibitory activity of monoamine oxidase enzyme. According to that, a first series of 3-aryl-4-hydroxycoumarins has been synthesized starting from aryl boronic acids and phenyliodonium zwitterions precursors by a palladium-catalyzed coupling reaction.

Keywords: coumarin, MAO, Suzuki reaction.

Introduction:

Coumarins are an important group of organic compounds from natural and synthetic origins which have different pharmacological activities as for example antibacterial,[1,2] cardioprotector, vasodilator,[3] anti-inflammator,[4] anti-HIV,[5] monoamine oxidase (MAO) inhibitor.[6,7]

MAO exists in two isoforms MAO-A and MAO-B, which differ according to the substrate specificity, MAO-A preferentially deaminates serotonin and norepinephrine. MAO-B acts preferentially on fenilethylamine. Because of the vital role that MAO plays in the inactivation of neurotransmitters, the regulation of MAO-A and MAO-B activity has been an important target for the treatment of the pathologies. In particularly MAO-B inhibitors are used in the therapy of Alzheimer's and Parkinson's diseases, while MAO-A inhibitors are used as antidepressants and anti-anxiety agents.

So far, among several MAO inhibitors, some coumarin derivatives have been described as good inhibitory MAO activity[8-12]. Related to that, in the last years our research group first investigated the MAO inhibitory activity 7-substituted coumarin derivatives (**A**) in which the activity and selectivity can be modulated depending of the substituents in the pyrone ring. (Figure 1)

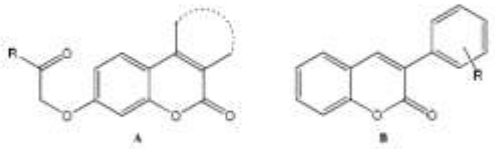


Figure 1: coumarins with MAO inhibitory activity.

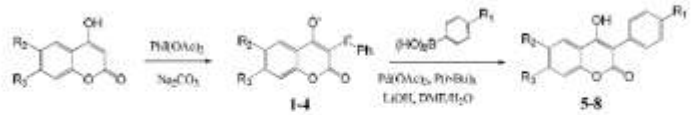
Starting from these studies, in the last years our research group has carried out the synthesis of different 3-arylcoumarins (**B**) with an interesting MAO-B inhibitor activity.[13-16] On basis of that, we can conclude that substituents in the 7-position are not essentials for the MAO-B inhibition, if in the 3-position of the coumarin an aryl group is present. This results encouraged us to produce a series of 3-arylcoumarin where a hydroxyl group have been incorporated at the 4 position of the coumarin scaffold. We also pretend to introduce substituents in one or two aromatic rings with different electronic, steric and/or lipophilic properties in order to study the effects on the possible activity and/or selective MAO-A/B.

These compounds can be synthesized by construction of the substituted coumarin moiety or alternatively, by arylation of the coumarin scaffold. In our case, 3-substituted coumarins have been obtained using the last methodology introducing the aryl group by palladium-catalyzed Suzuki-Type coupling strategy.[17]

Result and discussion:

Coumarins have been obtained synthesizing initially the electrophilic specie, the pheniliodonium coumarinate. This is a molecule with a positive charge at iodine compensated by a internal negative charge that is localized formally at the α-carbon or delocalized to a neighboring oxygen. We have used 4 differents type of commercial coumarins to produce 4 coumarin-based phenyliodonium zwitterions (**1-4**). Then we introduce the aryl group at the 3 position of coumarin scaffold using a palladium-catalyzed Suzuki-Type coupling reaction with 4-methoxyphenyl boronic acid under conditions indicated in scheme 1.[17] The mild reaction condictions and the commercial availability of both 4-hydroxycoumarin and boronic acids make this method a valuable tool for generating

diversified 3-aryl-4-hydroxy-coumarins in order to essay as vasodilatadors, antioxidants and monoamino oxidase inhibitors.



Scheme 1: synthetic strategy for the prepared compounds

We have prepared derivatives containing different substituents in positions 3', 6 and 7.

Aryl coumarins	R1	R2	R3
5	OCH_3	H	H
6	OCH_3	Cl	H
7	OCH_3	CH_3	H
8	OCH_3	CH_3	CH_3

General experimental procedure :

All reactions were carried out under dry and deoxygenated argon atmosphere. Identification of the compounds and course of the reactions were visualized using TLC plates (Merck, silica gel 60F254) under UV light (254-366 nm).

Melting points were determined using a Reichert Kofler thermopan or in capillary tubeson a Buchi 510 apparatus and are uncorrected. 1H-NMR spectra were recorded on a Bruker WM-250 at 250 MHz using TMS as internal standard (chemical shifts in δ values, *J* in Hz).

General procedure for the preparation of 3-phenyliodonium coumarinates (1-4): Iodobenzene diacetate (1 mmol) was suspended in a solution of Na_2CO_3 (1mmol) in water (10 mL) and stirred for 2 hours at room temperature. To this solution was added a mixture of 4-hydroxycoumarin (1mmol) in water (10 mL). After the mixture was stirred at room temperature for 14 hours, the precipitate was collected by filtration, washed with water (10 mL) and dried under vacuum. The resulting white solid was used without further purification.

Following this conditions we have synthesized:

4-Hydroxy-3-(4-metoxiphenyl)iodonium coumarinate (1).

6-Chloro-4-hydroxy-3-(4-metoxiphenyl)iodonium coumarinate (2).

4-Hydroxy-6-methyl-3-(4-metoxiphenyl)iodonium coumarinate (3).

4-Hydroxy-6,7-dimethyl-3-(4-metoxiphenyl)iodonium coumarinate (4).

General procedure for cross coupling reaction: A degassed solution of boronic acid (2.2 equiv) and P(*t*-But)$_3$ (27 µL) in DME and water (4:1, 12.5 mL) was added to a mixture of iodonium ylide (0,55 mmol) LiOH/H_2O (3.0 equiv) and $Pd(OAc)_2$ (6.2 mg) under argon at room temperature. After being stirred at the same temperature for 24-48 hours the mixture was purified on column (exane:ethylacetate 8:2).

Following this conditions we have synthesized:

4-Hydroxy-3-(4-metoxiphenyl)coumarin (5). Yield 65%, MP: 255-257 °C

6-Chloro-4-hydroxy-3-(4-metoxiphenyl)coumarin (6). Yield 56%, MP: 258-260 °C

4-Hydroxy-6-methyl-3-(4-metoxiphenyl)coumarin (7). Yield 50%, MP: 228-230 °C

4-Hydroxy-6,7-dimethyl-3-(4-metoxiphenyl)coumarin (8). Yield 42%, MP:221-223 °C

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Coumarin-Chalcone Hybrids as new scaffolds in drug discovery

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Abstract: The first hydroxilated series of coumarin-chalcone derivatives has been synthesize starting from the corresponding salicyl aldehyde and β-ketoester precursors by a Knoevenagel reaction in order to obtain the methoxy derivatives which have been further hydrolyzed with a Lewis acid.

Keywords: Coumarin, Chalcone, Knoevenagel

Introduction

Coumarins and chalcone are a family of natural and synthetic compounds that have raised an enormous interest for a long time due to its biological applications. One of the biggest applications of coumarins resides on the wide range of pharmacological activities such as antimicrobial, antiviral, antioxidant, antiplatelet, vasorelaxant or enzymatic inhibitors[1,2,3,4] so this coumarin scaffold give us a hint in the discovery and development of new drugs. Besides coumarins, chalcones share a wide range of this biological properties like antiproliferative, cardioprotector, or anti-inflammatory activity[5,6,7] and in the present year chalcones have been proposed as valid scaffolds for monoamino oxidases inhibitors[8].

On the other hand, many studies suggest that polyphenols may be involved in neurotoxic or cyclooxygenase activity as well as they play a role as chemopreventive and tumor growth inhibition agents and their protective properties against oxidative stress.[9,10,11] It is for these reasons that we have synthesized a novel series of hydroxylated coumarin-based compounds containing a chalcone moiety in order to make different biological essays and test their potential pharmacological activities.

Results and Discussion

The aim of this work has been to synthesize new hydroxy coumarin-chalcone hybrids which contain the hydroxyl sustituents in the aromatic rings that can potentially be used as new lead compounds in drug discovery.

Compounds were synthesized using a two steps synthetic strategy that allows us to obtain methoxy derivatives which were further hydrolyzed using a Lewis acid. (***Figure 1***)

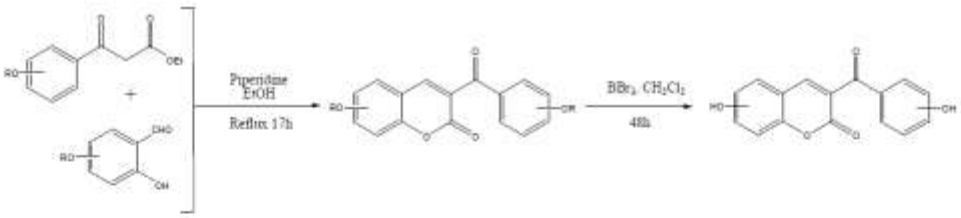
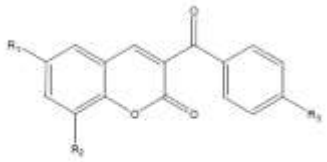


Figure 1. *Synthetic route used to prepare hydroxy coumarin-chalcone hybrids.*

The main scaffold with methoxy substituents was prepared by a Knoevenagel reaction in basic conditions in 82-93% yields. First synthesized derivatives have the methoxy sustituens placed in positions 8 and 4', (***Table 1***)

Table 1. *Coumarin-chalcone hybrids*

Compound	1	2	3	4
R_1	-H	-Br	-H	-Br



R_2	-H	-OMe	-H	-OH
R_3	-OMe	-H	-OH	-H

Hydroxy derivatives were further prepared from the ether precursors by catalysis with a Lewis acid, BBr_3 in CH_2Cl_2[12]. Purification of compounds **1-2** was made by recristalization in MeOH, while compounds **3-4** were purified by flash chromatography.

The followed methodologies to prepare compounds **1-2**, in a parallel synthesis way, bring the opportunity of synthesize structural related compounds with punctual modifications in the aromatic rings depending on the starting materials.

Biological assays as vasodilatators, antioxidants and monoaminooxidase inhibitors are currently being carried out and results will be further presented.

General Experimental Procedure

All reactions were carried out under dry and deoxygenated argon atmosphere. Solvents were used as anhydrous by reflux of each solvent over an appropriate dryer agent and further distillate under argon atmosphere.

Qualitative identification of the compounds and course of the reactions were visualized using TLC plates (Merck, silica gel $60F_{254}$) under UV light (254-366 nm). Melting points were determined using a Reichert Kofler thermopan or in capillary tubes on a Büchi 510 apparatus and are uncorrected. ^1H NMR spectra were recorded on a Bruker WM-250 at 250 MHz using TMS as internal standard (chemical shifts in δ values, *J* in Hz).

General procedure for the preparation of 3-benzoylcoumarins: In a round bottom flask the appropiated salicylaldehyde (1 equiv.unless otherwise noted) and ethyl benzoylacetate (1 eq. unless otherwise indicated) were dissolved in dry EtOH. Were then added 5 drops of piperidine, and the reaction was subjected to reflux. The reaction was monitored by TLC and after the end of it, the solution was cooled on ice and the precipitate was filtered and washed with ether. The solid obtained was purified by recrystallization in MeOH: CH2Cl2 and / or column chromatography.

General procedure for the preparation of hydroxy 3-benzoylcoumarins: Ether derivatives of 3-benzoylcoumarins (1 eq.) were dissolved in dry DCM and BBr_3 (20 eq.) was added dropwise at room temperature in a sealed tube. Mixture was stired and heated at 80ºC for 48h. After cooling to room temperature, the crude reaction mixture was quenched carefully with ice, water and 1 N HCl. The aqueous layer was extracted with AcOEt (3 × 50 cm3) and the combined organic extracts were washed with saturated aqueous NaHCO3 and brine, dried ($MgSO_4$) and concentrated to dryness.

3-(*p*-methoxybenzoyl)coumarin (1): Yield: 93%. ^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.34 (s, 1H, H-4), 7.92 (d, J = 8.8 Hz, 2H, H-2', H-6'), 7.83 (d, J = 8.6 Hz, 1H, H-5), 7.70 (d, J = 7.26 Hz, 1H, H-8), 7.55-7.33 (m, 2H, H-6, H-7), 7.05 (d, J = 8.8 Hz, 2H, H-3', H-5'), 3.85 (s, 3H, -CH_3). MS m/z (%):281 ([M+1]$^+$, 19), 280 ([M]$^+$, 82), 135 (100), 77 (20).

3-benzoyl-5-bromo-8-methoxycoumarin (2): Yield: 82%. ^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 7.78 (s, 1H, H-4), 7.69 (d, J = 7.3, 2H, H-2', H-6'), 7.51-7.24 (m, 3H, H-3', H-4', H-5'), 7.14 (s, 1H, H-5), 7.09 (s, 1H, H-7), 3.82 (s, 3H, -CH_3) MS m/z (%):360 ([M+1]$^+$, 18), 359 ([M]$^+$, 58), 358 (18), 357 (58), 105 (100), 77 (70).

3-(p-hydroxybenzoyl)coumarin (3): Yield: 67%. ^{1}H NMR: (250 MHz, *DMSO-d_6*) δ ppm 10.6 (s, 1H, -OH), 8.3 (s, 1H, -H4), 7.8 (d, J =8.5 Hz, 4H, -H2', -H3', -H5', -H6'), 7.7 (t, J = 7.50 Hz, 1H, -H5), 7.5-7.3 (m, 2H, H6, H7), 6.9 (d, J =8.5 Hz, 1H, -H8). MS m/z 267 ([M+1]$^+$, 12), 266 ([M]$^+$, 57), 249 (22)

3-benzoyl-5-bromo-8-hydroxycoumarin (4): Yield: 52%. ^{1}H NMR: (250 MHz, *DMSO-d_6*) δ ppm 10.8 (s, 1H, -OH), 7.8 (d, J =7.9 Hz, 2H, H2', H6') 7.6-7.3 (m, 3H, -H3', -H4', -H5'), 7.0 (s, 1H, -H5), 6.8 (s, 1H, H7). MS m/z 346 ([M+1]$^+$, 18), 345 ([M]$^+$, 62), 344 (18), 343 (53), 105 (100).

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Presentaciones a congresos nacionales e internacionales

2012

SISOC IX, Tenerife (España) **"Synthesis and antioxidant analysis of new 4-hydroxycoumarin derivatives"** (comunicación oral) Giovanna Delogu, Saleta Vázquez-Rodríguez, Eugenio Uriarte, Fernanda Perez-Cruz, Claudio Olea-Azar and Silvia Serra; "**Phenylbenzofurans: Inhibitors and/or Activators for Mushroom Tyrosinase?**" Benedetta Era, Antonella Fais, Marcella Corda, Maria Benedetta Fadda, Stefania Utzeri, Carmen Picciau, Silvia Serra, Giovanna Delogu.

2011

***"GEN XXXII"*, Galicia (España) "Nuevos derivados cumarínicos como inhibidores de sistemas enzimáticos implicados en enfermedades neurodegenerativas"** (comunicación oral) Maria João Matos, Dolores Viña, Giulio Ferino, Saleta Vazquez Rodriguez, Silvia Serra, Lourdes Santana and Eugenio Uriarte.

***2nd Iberic Meeting on Medicinal Chemistry*, Porto (Portugal):"A new series of 4-hydroxy-3-phenylcoumarins as MAO inhibitors**" Silvia Serra, Giovanna Delogu, Saleta Vazquez-Rodriguez, Maria João Matos, Dolores Viña, Eugenio Uriarte; **"Phenylbenzofurans: inhibitors and/or activators for mushroom tyrosinase?"** B. Era, C. Picciau, S. Serra, M.B. Fadda, M. Corda, S. Utzeri, A. Fais and G. Delogu ; **"Synthesis of 2-phenylbenzofurans. Structures with rigid trans-resveratrol's core"** Giovanna Delogu, Carmen Picciau, Elias Quezada, Silvia Serra, Lourdes Santana, Dolores Viña; **"Arylcoumarins as new tyrosinase inhibitors"** Maria João Matos, Silvia Serra, Lourdes Santana, Marcella Corda, Maria Benedetta Fadda, Benedetta Era and Antonella Fais**.**

2010

7*th* *ERA Flash Conference-Bioinspired Chemistry* Santiago de Compostela (España): "**Design and Synthesis of New 3-Aryl-4-Hydroxycoumarins as Pharmacological Agents"** Silvia Serra,* Maria Joao Matos, Giovanna Delogu, and Eugenio Uriarte; "**Semirigid trans-Chalcones Through Coumarin Scaffolds. New Antibacterial Agents"** Saleta Vazquez-Rodriguez,* Silvia Serra, Cristina Fuentes-Edfuf, Ysabel Santos, Eugenio Uriarte, and Lourdes Santana.

Segundo Simposio de Quimica Organica (SIBEAQO-II), Santiago de Compostela (España): **"Versatilidade de metologias sintéticas na praparação de séries de cumarinas 3-substituídas"** Uriarte E, Matos MJ, Janeiro P, Vazquez-Rodriguez S, Serra S, Borges F, Santana L, Sobarzo-Sánchez E.

EFMC-ISMC 2010 XXIst International Symposium on Medicinal Chemistry, Bruselas, Belgica: ***"Development of Multi-task models QSAR for research of tyrosinase inhibitors***" Ilaria Caddia, Silvia Serra, Antonella Fais, Benedetta Era, Giovanna Delogu, Lourdes Santana and Riccardo Concu.

III Microbiologia Clinica, Avila (España): **" *Nueva aproximacion al desarollo de potenciales farmacos antibacterinos*"** D. Rey-González, S. Vazquez-Rodriguez, C. Fuentes-Edfuf, M. J. Matosb, S. Serra, Y. Santos, A. Muñoz Crego, L. Santana y E. Uriarte.

European School of Medicinal Chemistry organizado por European Federation for medicinal Chemistry, Società Chimica Italiana-Divisione Chimica Farmaceutica, Urbino (Italia).

2009

"Escuela de verano de la SEQT – Desarrollo de nuevos fármacos", Toledo (España): "***New Oxoisoaporphine Derivatives With Cytotoxic And Antiplasmoidal Activities***" Silvia Serra, Eduardo Sobarzo-Sánchez,* Mohamed

Haddad, Lourdes Santana,Humberto González-Díaz, Joelle Quetin-Leclercq, Eugenio Uriarte.


9th Spanish-Italian Symposium
on Organic Chemistry
SISOC-IX
Tenerife
February 10th-14th 2012

FLASH PRESENTATIONS

FP-7 (PO-79)

Synthesis and Antioxidant Analysis of New 4-Hydroxycoumarin Derivatives

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Coumarins are one of the most abundant groups of naturally occurring heterocyclic compounds. Because of their structural features they are important building blocks in the natural product and synthetic chemistry areas. In addiction a lot of biological properties were described about these compounds.[1] Many coumarin derivatives have shown a strong antioxidant activity, due to their ability to neutralize reactive oxygen species (ROS) considered the main cause of cardiovascular disease, cancer, atherosclerosis and aging processes.[2-5] If these highly reactive species are not neutralized by antioxidant systems, can cause oxidative damage to cellular components such as membrane lipids, proteins and DNA.
In the present work we have used the 4-hydroxycoumarin moiety as the molecular template in the design and synthesis of new derivatives (Figure 1).

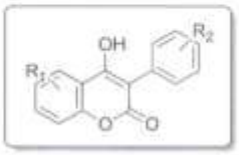

Figure 1: generic structure of 4-hydroxycoumarin derivatives.

All the coumarins were efficiently synthesized. Their antioxidant capacity was then calculated by the oxygen radical absorbance capacity method using fluorescence measurements (ORAC-FL experiments). As reference compound was used TROLOX, a hydrosoluble vitamin E derivative. The synthesized coumarin derivatives have shown good ORAC values and results will be further presented.

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PO-21

Phenylbenzofurans: Inhibitors and/or Activators for Mushroom Tyrosinase?

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Tyrosinases, better termed polyphenoloxidases (EC 1.14.18.1; monophenol, *o*-diphenol: oxygen oxidoreductase), are copper-containing monooxygenases catalyzing the *o*-hydroxylation of monophenols to the corresponding catechols (monophenolase activity) and the oxidation of catechols to the corresponding *o*-quinones (diphenolase activity).[1]

Quinone is a highly reactive compound and can polymerize spontaneously to form melanin by a series of non-enzymatic reactions. The plants from which are extracted the tyrosinase inhibitors are numerous, among these the Morus lhou (S.) Koidz. is well renowned. This a polyphenol-rich plant that is one of the most ubiquitous traditional herbal medicines in East Asia. Previous workers reported that this species contains tyrosinase inhibiting flavanones, flavones, stilbenes and phenylbenzofurans.[2,3]

Benzofuran derivatives are an important class of organic compounds that occur in natural products because of their biological activities, including antitumoral properties.

In this work we describe the synthesis of a new series of 2-phenylbenzofurans suitably modified. The 2-phenylbenzofuran scaffold study have different substitutions like methoxyl, hydroxyl and/or bromo on differents positions (Figure 1).

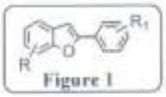

Figure 1

The key step for the formation of the benzofuran backbone was achieved by an intramolecular Wittig[4] reaction between triphenylphosphonium salts and the appropriate benzoyl chloride. The tyrosinase activity of compounds synthesized was evaluated *in vitro* by the measurement of the enzymatic activity of mushroom tyrosinase enzyme extracted from the mushroom.

This preliminary study indicates that the type and the position of groups may play an important role in determining the kind of activity.

Ackowledgements: This work was funded by PRIN 2008, prot. F21J10000010001

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GEN 32

XXXII REUNIÓN ANUAL DEL GRUPO ESPAÑOL DE NEUROTRANSMISIÓN Y NEUROPROTECCIÓN

Santo Estevo de Ribas de Sil (Ourense)

14-17 de diciembre de 2011

Nuevos derivados cumarínicos como inhibidores de sistemas enzimáticos implicados en enfermedades neurodegenerativas

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El aumento de la esperanza media de vida de la población en las sociedades actuales, ha puesto de manifiesto la importancia del descubrimiento de nuevos fármacos para el tratamiento de enfermedades neurodegenerativas (EN) asociadas en buena medida a la edad entre las que destacan por su prevalencia el Alzheimer o Parkinson.[1] El desarrollo de tratamientos neuroprotectores efectivos que puedan tratar los síntomas y/o detener la progresión de estas enfermedades en las etapas más tempranas de su evolución es uno de los principales retos de los investigadores en esta área. Considerando su naturaleza multifactorial, el desarrollo de fármacos se orienta en los últimos años hacia fármacos que actúan sobre múltiples dianas farmacológicas. Tanto la acetilcolinesterasa (AChE) como la monoamino oxidasa B (MAO-B) desempeñan un papel relevante en estas patologías. Así, los inhibidores selectivos o duales de estas enzimas son estructuras químicas de gran interés (figura 1).[1-3]

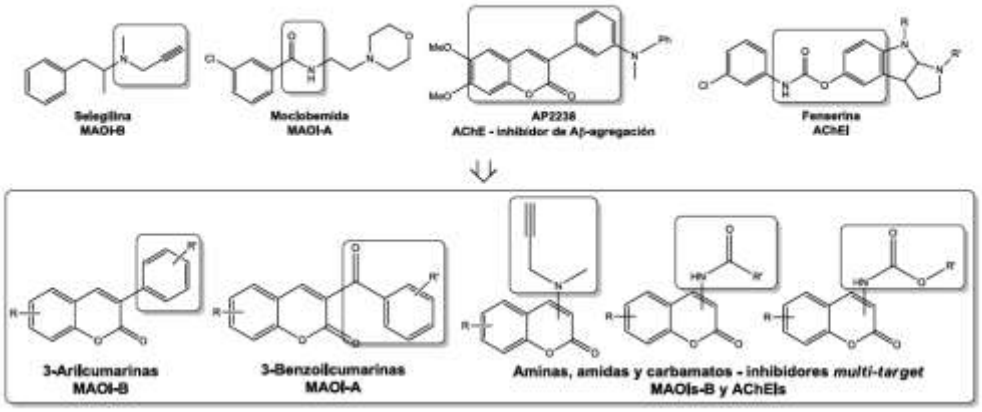

Figura 1 – Estructura química de los derivados de cumarina incluyendo rasgos estructurales de los inhibidores de referencia de MAO y AChE. (inhibidores de referencia y compuestos sintetizados).

Las cumarinas son una importante familia de compuestos naturales y/o sintéticos que, por sus múltiples propiedades, ocupan un lugar importante en el área de los productos naturales y la química orgánica.[4] En el presente trabajo hemos desarrollado metodologías sintéticas que nos han permitido obtener nuevas series de cumarinas diferentemente sustituidas (figura 1). Algunas 3-arilcumarinas han demostrado ser inhibidores de la MAO-B muy potentes y selectivos.[1] Basándonos en estos resultados hemos diseñado nuevos derivados introduciendo grupos amida y carbamato con la finalidad de encontrar inhibidores enzimáticos duales inhibidores de la AChE, MAO-A y MAO-B.[2]

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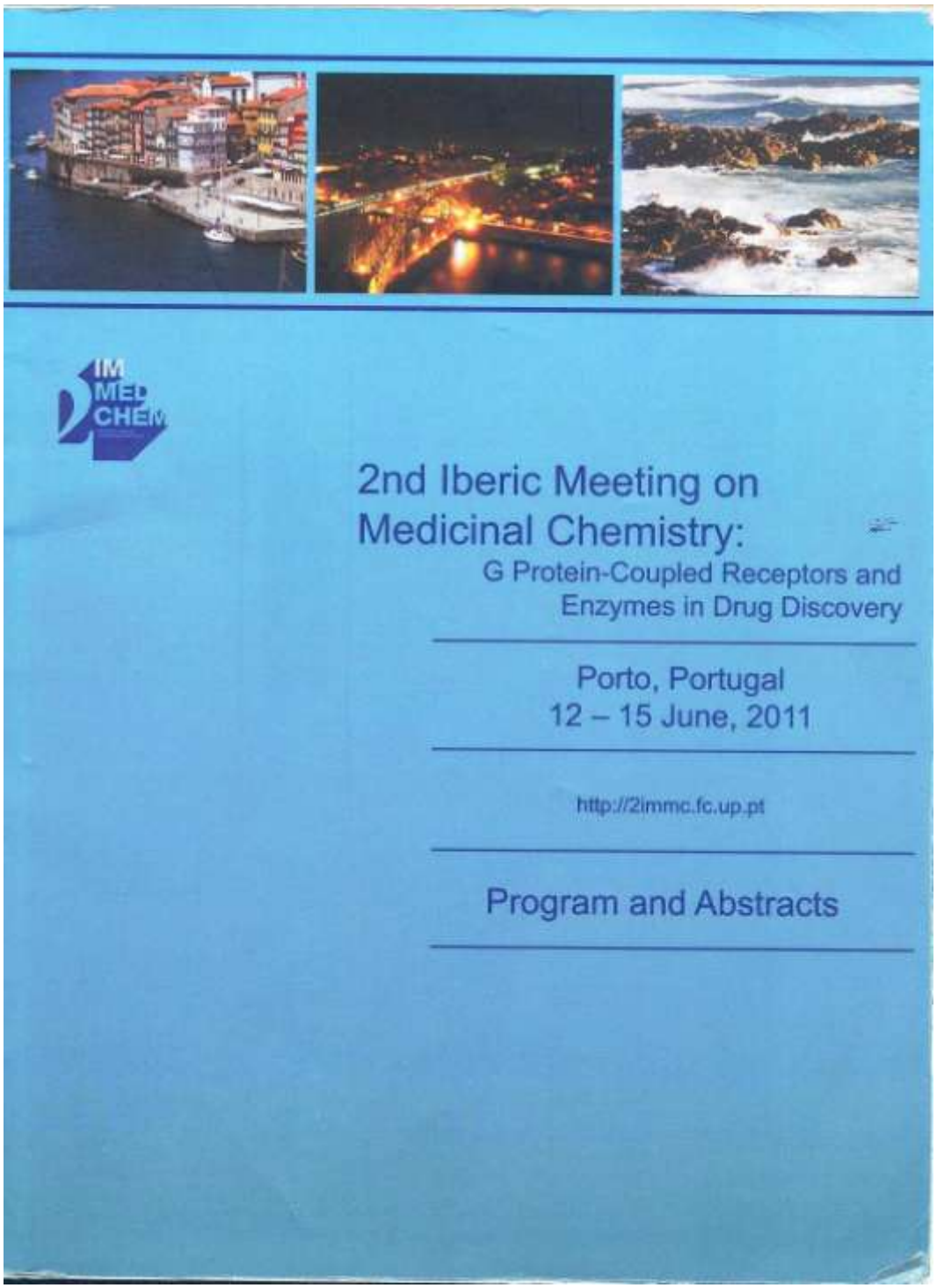
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P58

A new series of 4-hydroxy-3-phenylcoumarins as MAO inhibitors

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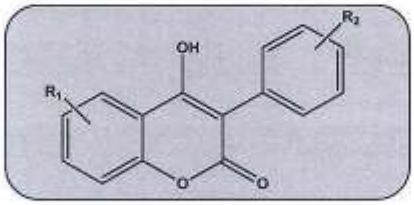
Coumarins are an important group of organic compounds, of natural and synthetic origin, that present a broad range of biological activities like antioxidant,[1] anti-inflammatory,[2] antibacterial,[3] cardioprotective, vasorelaxant,[4] anti-HIV and anticarcinogenic.[5] Recent study pay special attention to their monoamine oxidase (MAO) inhibitor property. MAO is an important FAD-containing enzyme present in the mitochondrial outer membrane of glial, neuronal and other cells. It exists in two isoforms MAO-A and MAO-B and plays a vital role in the monoamines degradation and in the inactivation of neurotransmitters. MAO-A preferentially deaminates serotonin and noradrenaline, while MAO-B preferentially deaminates phenylethylamine and benzylamine. These properties determine the interest of MAO inhibitors. Related to that, first in the last years our research group synthesized and investigated the MAO inhibitory activity of 7-substituted coumarin derivatives.[6] Then we studied the MAO-inhibitor activity of coumarin-resveratrol hybrids incorporating an aryl group in the 3 position of the coumarin skeleton.[7] Based on the SAR studies we can concluded that substituents at the 7-position are not essential for the MAO activity, when an aryl group is present at the 3-position of the coumarin. Then, in order to evaluate the effect of the introduction of π-excedent heteroaryl groups in the biological activity, we synthesized series of 3-indolyl and 3-thiophenylcoumarins. The good MAO inhibitor activity found for these compounds[8] encouraged us to synthesize a new series of 3-phenyl coumarins introducing two modifications: substituents with different electronic and/or steric properties and incorporation of an additional hydroxyl group at the 4 position of the coumarin scaffold.



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P5

PHENYLBENZOFURANS: INHIBITORS AND/OR ACTIVATORS FOR MUSHROOM TYROSINASE?

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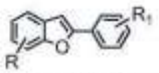
Tyrosinase (EC 1.14.18.1) is an enzyme catalysing the rate-limiting reaction in melanin biosynthesis[1]. The enzyme catalyses two different reactions. The first one is hydroxylation of monophenol to *o*-diphenol (monophenolase activity), and the second is oxidation of *o*-diphenol to *o*-quinone (diphenolase activity). Therefore, tyrosinase is known as a key enzyme implicated in the anabolism of melanin in melanocytes, and its inhibitors have become increasingly important in medicinal and cosmetic products in relation to hyperpigmentation[2, 3].
In previous studies, phenylbenzofurans were isolated from Morus lhou, belonging to the family of Moraceae, plant that is one of the most ubiquitous traditional herbal medicines in East Asia[4]. Interestingly, a phenylbenzofuran *Moracin M* displayed significant inhibitory activities against tyrosinase. For this reason, we have synthesized the new phenylbenzofurans like inhibitors of the mushroom tyrosinase.
The preparation of phenylbenzofurans was successfully achieved by a Wittig reaction between the appropriate triphenylphosphonium salt and the corresponding benzoyl chloride[5].
Tyrosinase activity assays were performed with L-DOPA as substrate, as previously described[6] with slight modifications.



R = H, Me, Br, OMe, OEt
R1 = H, OMe,OH

The compound 2-(4'-metossiphenyl)-7-bromobenzofuran, is the most active compound of this series. Surprisingly, the compound 2-(4'-hydroxyphenyl)-7-bromobenzofuran that has a hydroxyl group instead of a methoxy group shows a different activity.
This preliminary result indicates that the type and the position of groups may play an important role in determining the kind of activity.
The different activities of these compounds that differ in only one group require further study.

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P18

Synthesis of 2-phenylbenzofurans. Structures with rigid *trans*-resveratrol's core

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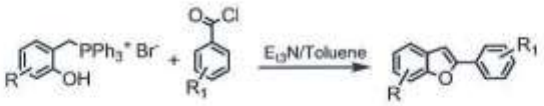
Benzo[b]furan derivatives are an important class of organic compounds that occur in many compounds and natural products because of their biological activities, including antitumor properties. They can be used as inhibitors of 5-lipoxygenase, antagonists of the angiotensin II receptor, blood coagulation factor Xa inhibitors, ligands of adenosine A1 receptor and so forth.[1]
In this work we describe the synthesis of a new series of 2-phenylbenzofurans suitably modified. These compounds included in their structure the resveratrol's nucleus, always in their *trans* configuration. Resveratrol is a natural substance produced by several plants, as vines. A number of beneficial health effects are associated to this compound. Anticancer, antiviral, anticoagulant, neuroprotective, antiaging, antiinflammatory and life-prolonging effects have been reported.[2] Recently 3-phenylcoumarins (also with the *trans*-resveratrol's core in their structure) have showed a antioxidative, anticancer, and enzymatic inhibition properties. Some coumarins proved to be monoamine oxidase inhibitors (MAOI).[3]
With the aim to find out the structural features for the MAO inhibitory activity and selectivity, in the present work we report the synthesis and pharmacological evaluation of a new series of 2-phenylbenzofurans.
The synthesis of benzofurans was successfully achieved by a Wittig reaction between the appropriate triphenylphosphonium salt and the corresponding benzoyl chloride. The desired Wittig reagent was readily prepared from the conveniently substituted 2-hydroxy-benzyl alcohol and triphenylphosphine hydrobromide.[4]



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P39

Arylcoumarins as new tyrosinase inhibitors

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Tyrosinase (EC 1.14.18.1) is a multifunctional dinuclear copper centre enzyme widely distributed in nature and mainly involved in the formation of pigments such as melanins and other polyphenolic compounds.[1,2] Coumarins (figure 1), the moiety which we are deeply exploring, are a large family of compounds of natural and/or synthetic origin that proved to have a large variety of pharmacological properties,[3] such us different enzymatic inhibition.

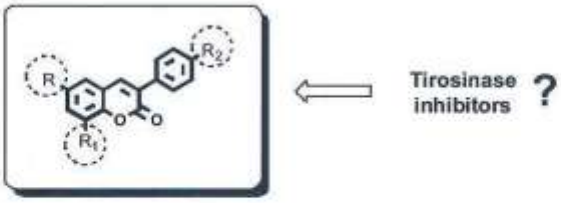

Figure 1. Structural studied coumarin moiety.

With the aim to find out structural features for the tyrosinase inhibitory activity, in the present communication we report the synthesis and pharmacological evaluation of a new series of 3-arylcoumarin derivatives with different number of hydroxyl or ether groups and bromo substituent in the scaffold. The synthesized compounds were evaluated as mushroom tyrosinase inhibitors showing, two of them, lower IC_{50} than the umbelliferone, used as reference compound. Posterior studies suggested us that these compounds are non-competitive tyrosinase inhibitors. The bromo and hydroxyl substitutions, which we are studying more deeply, proved to be one of the important modifications to improve coumarin´s biological profile.

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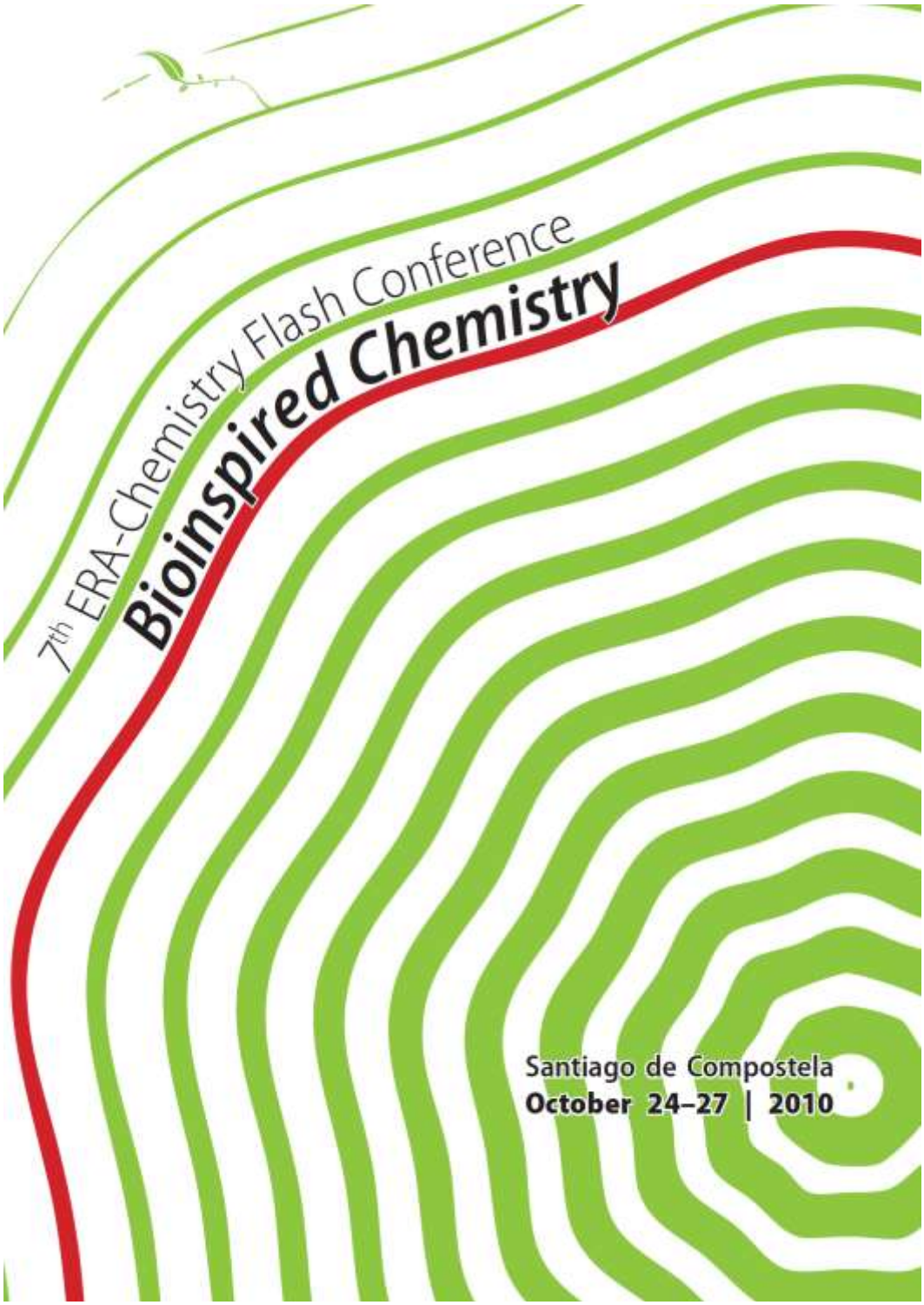
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Design and Synthesis of New 3-Aryl-4-Hydroxycoumarins as Pharmacological Agents

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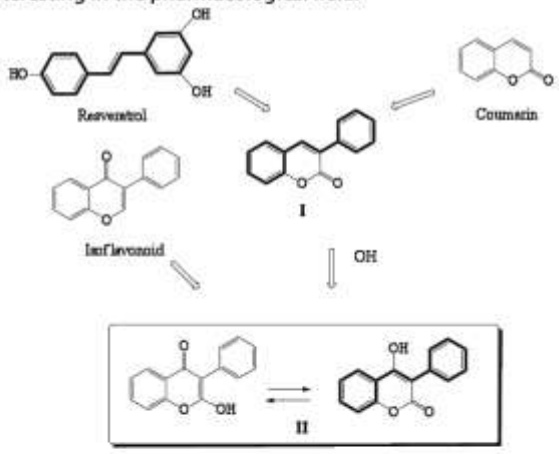
A synthesis of new compounds with an hydroxyl group incorporated at 4 position of the aryl-coumarin scaffold has been developed with the aim of evaluate their activies.

For years our research group have been working on designing, synthesing and evaluate molecules with different biological activities. One of the prototypes we have studied has been the *trans*-resveratrol,[1] and other stilbenes due to theirs pharmacological properties.[2] On the other hands, due to the large experience of our researching group in the coumarin field, during the last years we have carried out the design of hybrid molecules such resveratrol-coumarin (**I**), which have not only a cardio protector activity, but also an interesting inhibitory activity of the monoamineoxidase (MAO).[2]

Considering these aspects and in order to increase and/or explore other activities, in these project we proposed the desing of new analogues (**II**) in which is a hydroxyl group have been incorporated at 4 position of the 3-arylcoumarin scaffold.

This structure allows to approach another type of flavonoid structures abundant in nature such as flavonoids and isoflavonoids interesting in the pharmacological field.



In these prototypes we pretend to incorporate in one or two aromatic rings different substituents with different electronic, steric and/or lipophilicity properties in order to study the effects on the activity and/or selective MAO-A/B and carry out preliminary studies of possible antimicrobial activity of this prototypes.

A palladium-catalyzed coupling reaction has been used on the key step in the synthesis of this compounds, using phenyliodonium zwitterions and aryl boronic acids as precursors.[3]

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3 a) M. J. Matos, D. Viña, E. Quezada, C. Picciau, G. Delogu, F. Orallo, L. Santana, E. Uriarte, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3268; b) Q. Zhu, J. Wu, R. Fathi, Z. Yang, *Org. Lett.* **2002**, *4*, 3333.

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Semirigid *trans*-Chalcones Through Coumarin Scaffolds. New Antibacterial Agents

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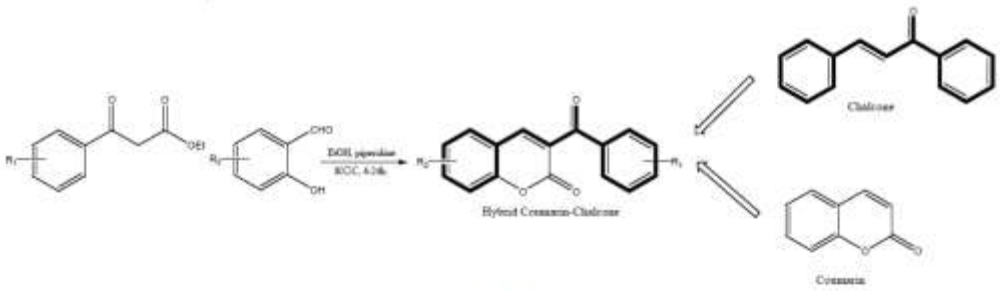
Coumarin and chalcones are natural origin compounds with a wide range of pharmacological activities. Fusing both scaffolds we have been designed and synthesized in a parallel type reaction a series of hybrid molecules, and have been further tested as antimicrobian agents.

The ability of bacteria to adquire resistance to already existing chemotherapies is one of the biggest challenges of the 21st century medicine, and therefore the discovery of new selective and potent antibacterial agents is a crucial endeavour.

The design of hybrid molecules based on well-known scaffolds is becoming a common tool in drug discovery. Coumarins and chalcone are a family of natural origin compounds which have arisen a big interest due to their wide range of biological properties such as antimicrobial, antiviral, anti-inflammatory, enzymatic inhibitors or antioxidant activities.[1,2,3] For this reason, we thought about the potential activity of hybrid molecules cointaining both scaffolds.



In the present work, we show the developed methodology and the synthetic strategy followed to synthesize a series of 3-benzoylcoumarins as well as the preliminary evaluation as antibacterial agents. The key step to prepare the hybrid compounds was a Knoevenagel reaction. Using this synthetic strategy, we were able to obtain the desired compounds in just one step. The main feature of this hybrids is the ability of stablish a *trans* geometry in the chalcone moiety through the coumarin scaffold. Antibacterial evaluation was carried out following the standard methodology[4] and compounds were tested against three strains of bacteria *Escherichia coli* CECT434, *Pseudomonas aeruginosa* CECT108 and Staphylococcus aureus CECT435. Promising results will be shown in the poster.

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DEVELOPMENT OF MULTI-TASK MODELS QSAR FOR RESEARCH OF TYROSINASE INHIBITORS

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Quantitative Structure Activity Relationship (QSAR) is a well known tool with a very large application range[1,2]. For instance QSAR approaches are used to predict protein function[3], antimicroblal compounds[4], toxicity[5] and so on. Developments of new compounds for the modulation[7] of tyrosine hydroxylase is a major goal for the medicinal chemistry, because the product of the reaction catalyzed by the enzyme is the tyrosine, an aminoacid involved in several biological processes. In fact, is not only precursor of a wide range of hormones and catecholamines such as dopamine, noradrenaline and adrenaline, but also pigments such as melanine. In human beings, its synthesis comes from another essential aminoacid, phenylalanine, which is acquired through the diet. In the suprarenal gland, tyrosine is transformed by the enzyme tyrosine in L-dopa, an intermediate in the biosynthetic pathway of dopamine, which is involved in the Parkinson. Thus, any disorder of this enzyme provokes several heavy diseases such as vitiligo, pitiriasis or melanoma. In this work we develop the first multi-target (mt) QSAR to optimize the synthesis of new molecules for the modulation of the activity of the tyrosine hydroxylase[6]. A large database of 700 compounds tested against the mushroom tyrosinase and L-DOPA has been selected from the literature. Then a mt-QSAR model was obtained that correctly classified the 80% of the compounds presents in the dataset. In conclusion, the final goal of the present model is predict the activity and direct the synthesis of new compounds with inhibitor activity against one or both enzymes.

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Santiago de Compostela, España

VERSATILIDADE DE METOLOGIAS SINTÉTICAS NA PRAPARAÇÃO DE SÉRIES DE CUMARINAS 3-SUBSTITUÍDAS

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Um elevado número de compostos naturais e sintéticos análogos de cumarinas apresenta um amplo espectro de propriedades biológicas. Nos últimos anos o nosso grupo de investigação está a estudar 3-fenilcumarinas e 3-benzoilcumarinas, derivados sintéticos estruturalmente híbridos cumarina-resveratrol e cumarina-chalcona, respectivamente. Em ambos derivados a ligação dupla 3,4 da cumarina fixa, na sua estrutura, a dupla ligação tanto o núcleo do resveratrol, como da chalcona, na sua disposição *trans*.
Interessa-nos, para isso, optimizar procedimentos sintéticos directos, simples e generalizáveis, que nos permitam aceder a ambas séries deste tipo de compostos em quantidades suficientes para a sua avaliação farmacológica. No presente trabalho descrevemos diferentes metodologias para a preparação destas famílias de compostos, partindo de um mesmo salicilaldeído convenientemente substituído, utilizando reacções de Perkin e Knoevenagel como etapas chave (figura 1).[1-3]

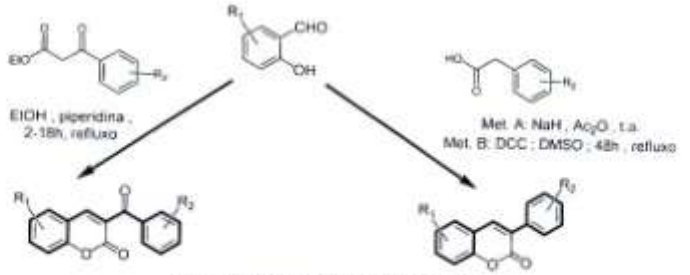

Fig. 1 – Esquema sintético e condições de reacção

Diferentes compostos deste tipo, que incorporam numa só estrutura dois importantes farmacóforos, estão a demonstrar excelentes propriedades farmacológicas em diversos campos.

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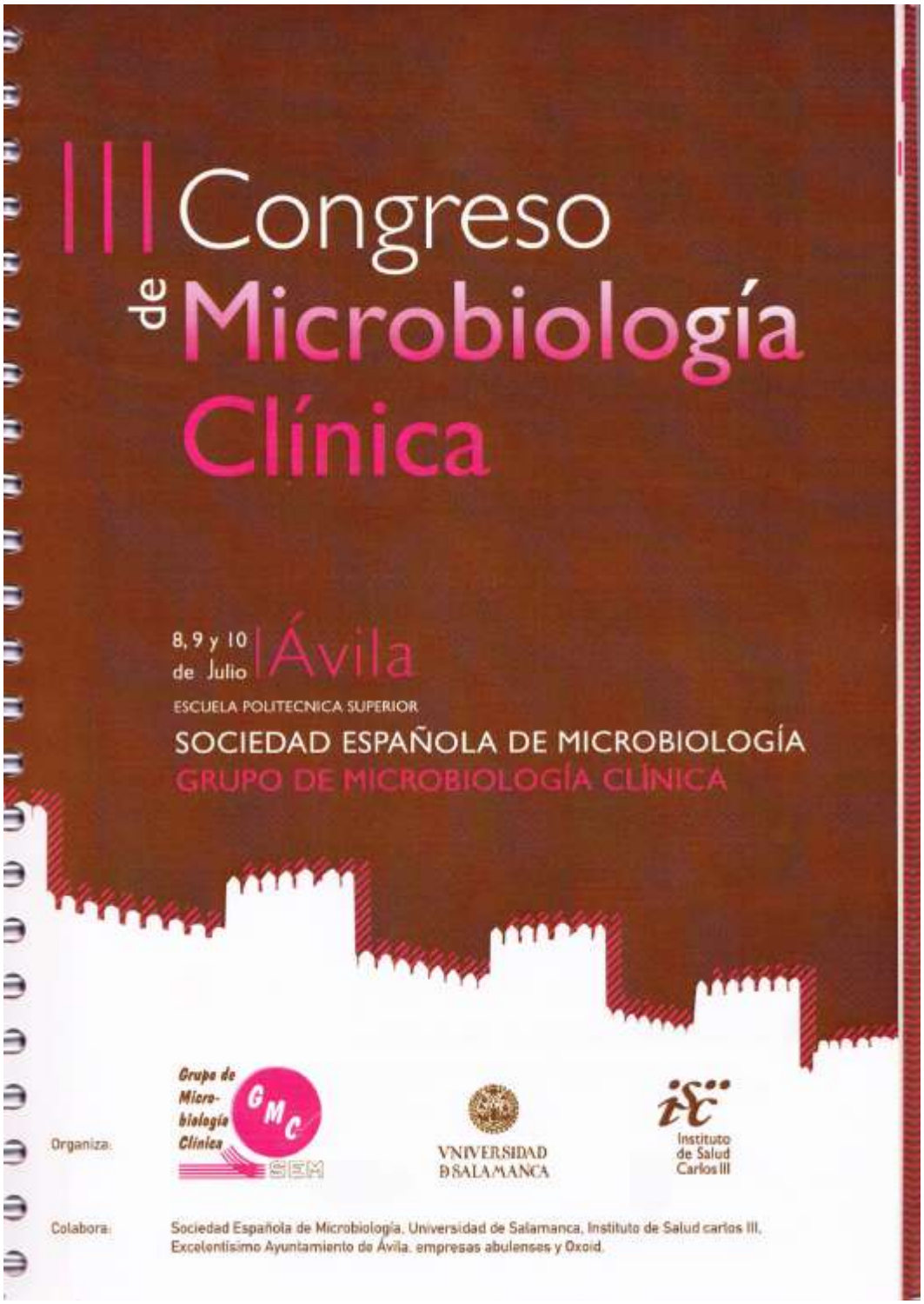
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NUEVA APROXIMACIÓN AL DESARROLLO DE POTENCIALES FÁRMACOS ANTIBACTERIANOS

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La capacidad de las bacterias para adquirir resistencia a quimioterapias ya existentes es uno de los grandes retos a los que se enfrenta la medicina en el siglo XXI. Por otra parte, al igual que en el caso de las infecciones bacterianas, las infecciones fúngicas presentan un gran riesgo para la salud, particularmente para aquellos individuos que tienen el sistema inmunológico deprimido. El descubrimiento de nuevas clases de agentes antifúngicos y antibacterianos selectivos, debe ser por tanto tratado como algo primordial.

Las cumarinas, las chalconas y los estilbenos derivados de resveratrol son familias de compuestos de origen natural o sintético, que han suscitado durante mucho tiempo un gran interés debido sobre todo a sus aplicaciones biológicas. Debido a la gran diversidad estructural de las moléculas con núcleo cumarínico, son muchas las propiedades farmacológicas que se asocian con dicho anillo, pudiendo encontrarse en casi todas las categorías fármaco-terapéuticas: antimicrobianos, antivirales, antihelmínticos, anti-inflamatorios, antiespasmódicos, inhibidores enzimáticos, anticoagulantes, antioxidantes, entre otros (Orallo et al., 2002; Leiro et al., 2004; Matos et al., 2009). Por este motivo, se ha escogido la cumarina como esqueleto central para sintetizar nuevos derivados que, además de contener esta estructura básica, pudiesen incluir estructuras derivadas de las chalconas y de los estilbenos derivados del resveratrol (Figura 1).

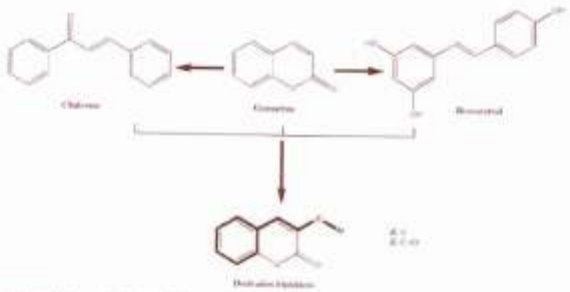

Figura 1. Estructura básica de los derivados sintéticos.

Basándonos en ésto, se han sintetizado nuevos análogos de las estructuras mencionadas con potencial actividad antimicrobiana. Estos compuestos híbridos incorporan núcleos farmacofóricos de cumarina-estilbenos y de cumarina-chalconas. Los compuestos mencionados fueron sintetizados con buen rendimiento siguiendo distintas rutas sintéticas, basadas en las reacciones de Perkin y Knoevenagel modificadas (Matos et al., 2009). La actividad antibacteriana de dichos derivados fue evaluada por el método de difusión en placa, siguiendo las recomendaciones del National Committee for Clinical Laboratory Standards (2006) (NCCLS). Con este fin, se impregnaron discos estériles (Liofilchem Bacteriology Products, Italy) con los compuestos sintetizados a concentraciones finales de 10 y 100µg/disco y se aplicaron sobre la superficie de placas de Agar Mueller Hinton (Difco) previamente inoculadas con las cepas bacterianas de referencia de *Escherichia coli* CECT434, *Pseudomonas aeruginosa* CECT108 y *Staphylococcus aureus* CECT435. Las placas se incubaron a 37ºC durante 24h y se determinó el diámetro de la zona de inhibición del crecimiento de las bacterias utilizadas como un índice de su sensibilidad a los antimicrobianos probados. Se ensayaron un total de 46 compuestos, realizándose dos réplicas por cada microorganismo probado.

Los resultados obtenidos indican que 9 de los 46 (20%) productos evaluados presentaron actividad antimicrobiana, siendo seis de ellos muy activos frente a *S. aureus* (Figura 2). Ninguno de los compuestos probados mostró capacidad para inhibir el crecimiento de *E. coli* y *P. aeruginosa*.

Es necesario evaluar un mayor número de cepas de *S. aureus*, incluyendo cepas resistentes a la meticilina, con el fin de valorar la utilidad de los compuestos sintetizados en el control de las infecciones causadas por este microorganismo.

Figura 2. Ensayo de difusión en agar usando algunos de los compuestos sintetizados y como cepa patrón S. aureus CECT435.

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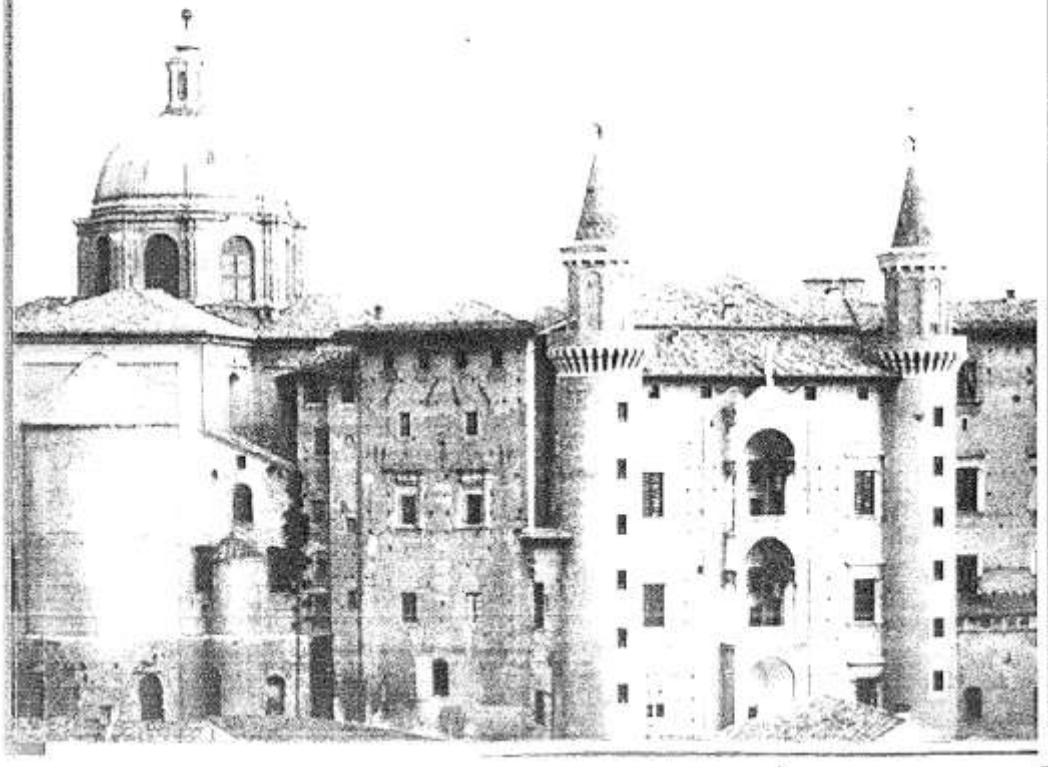


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NEW OXOISOAPORPHINE DERIVATIVES WITH CYTOTOXIC AND ANTIPLASMOIDAL ACTIVITIES

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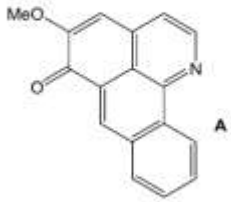
Oxoisoaporphine alkaloids[1] are less known than their isomers, oxoaporphines, which possess interesting cytotoxic properties.[2] Eight oxoisoaporphine alkaloids were synthesized and, in order to compare their cytotoxic activities with those of previously isolated natural oxoaporphines, were evaluated against four cancer and one non-cancer cell lines. Results showed that oxoisoaporphines exhibited similar cytotoxic activities than those of their isomers, oxoaporphines, and indicated a selective effect of some of them towards different cell lines. Furthermore, some of them were evaluated for their *in vitro* antiplasmodial activity. Compound **A** was found to exhibit the stronger antiplasmodial activity (IC_{50} = 1.45 μM). These pharmacological results were compared with the electronic properties of the compounds by the density-functional theory (DFT) and the Quantitative Structure-Activity Relationship (QSAR) method which provided a new insight for the cytotoxic and antimalarial activities of these heterocycles.



A

IC_{50} = 1.45 μM

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