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Synthesis and Study of a Series of 3-Arylcoumarins as Potent and Selective Monoamine Oxidase B Inhibitors

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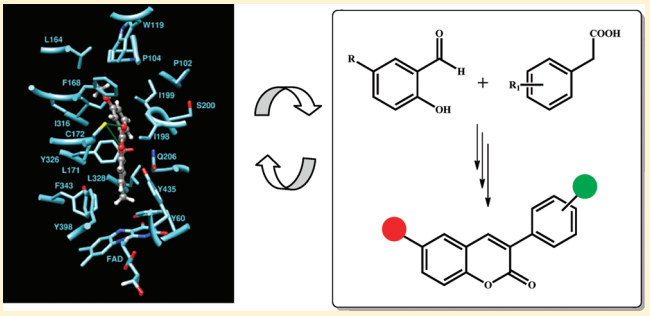
ABSTRACT: New series of 6-substituted-3-arylcoumarins displaying several alkyl, hydroxyl, halogen, and alkoxy groups in the two benzene rings have been designed, synthesized, and evaluated in vitro as human monoamine oxidase A and B (hMAO-A and hMAO-B) inhibitors. Most of the studied compounds showed a high affinity and selectivity to the hMAO-B isoenzyme, with IC_{50} values on nanomolar and picomolar range. Ten of the 22 described compounds displayed higher MAO-B inhibitory activity and selectivity than selegiline. Coumarin **7** is the most active compound of this series, being 64 times more active than selegiline and also showing the highest hMAO-B specificity. In addition, docking experiments were carried out on hMAO-A and h-MAO-B structures. This study provided new information about the enzyme−inhibitor interaction and the potential therapeutic application of this 3-arylcoumarin scaffold.



INTRODUCTION

Coumarins are a wide family of compounds present in remarkable amounts in the nature.[1,2] Representative compounds occur for instance in the vegetable kingdom, either in free or combined state.[1,2] Because of their structural variability, these heterocyclic compounds occupy an important role not only in organic chemistry but also in medicinal chemistry.[3,4] Therefore, coumarins have been attracting considerable interest because of their numerous biological activities. In the literature, coumarin derivatives have been described as anticancer,[5] antioxidant, or anti-inflammatory,[6] vasorelaxant,[7] antimicrobial,[8,9] antiviral,[10,11] and enzymatic inhibitors.[12−14] In particular, some coumarins had been previously described as monoamine oxidase inhibitors.[15−17]

Monoamine oxidases (MAOs) are FAD-depending enzymes found in the outer mitochondrial membrane of neuronal, glial, and other mammalian cells.[18] These enzymes are responsible for catalyzing the oxidative deamination of neurotransmitters and dietary amines, regulating intracellular levels of biogenic amines in the brain and the peripheral tissues.[19,20] Two enzymatic isoforms, named MAO-A and MAO-B, have been identified on the basis of their amino acid sequences,[21] three-dimensional structure,[22] tissue distribution,[23] inhibitor selectivity,[24] and substrate preferences.[25] The hMAO-A isoform has a higher affinity for serotonin and noradrenaline, whereas hMAO-B isoform preferentially deaminates β-phenylethylamines and benzylamine.[26,27] On the other hand, dopamine and tyramine are common substrates for both isoforms. These physiologic properties determine the clinical interest of MAO inhibitors. Selective MAO-A inhibitors, such as clorgyline (irreversible) and moclobemide (reversible), are used for the treatment of neurological disorders like depression and anxiety,[28] while selective and irreversible MAO-B inhibitors, such as selegiline and rasagiline, are used in the therapy of Parkinson's disease.[29,30] All of these aspects have led to an intensive search for novel MAO inhibitors. Additionally, the description of the crystal structure of the two MAO isoforms by Binda et al. has provided relevant information about the selective interactions and the pharmacophoric requirements needed for the design of potent and selective inhibitors.[31−34] In recent years, interest in selective hMAO-B inhibitors has significantly intensified due to the discovery that expression levels of this isoenzyme in neuronal tissue increase 4-fold with age, resulting in an increment of dopamine metabolism, as well as of production of hydrogen peroxide, which cause oxidative stress and may play a relevant role in the etiology of neurodegenerative diseases.[35] As a result, MAO-B inhibitors would be useful as adjuvant for the treatment of Parkinson's and Alzheimer's diseases. For instance, it has been reported that selegiline protects neuronal cells from the consequences of the oxidative stress in these patients.[36]

In the past few years, a broad consensus has been reached concerning the necessity for the research of new, more potent, and less toxic MAO-B selective inhibitors. This research has resulted in a significant number of compounds with different

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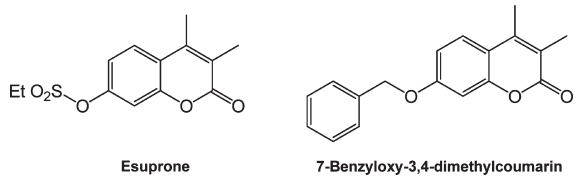


Figure 1. Coumarin-based MAO-A and MAO-B inhibitors.

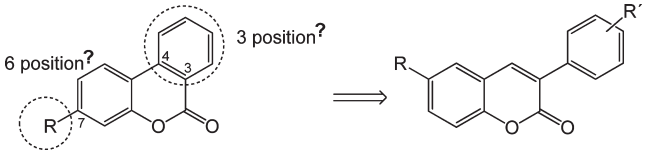


Figure 2. Planed modifications and newly synthesized coumarins.

structural scaffolds.[37−42] Therefore, the coumarin nucleus has emerged in the 1990s as a promising scaffold for MAO inhibitors.[43] Structure−selectivity relationship studies about coumarin derivatives suggested that selectivity was mainly determined by the nature of the linkage between the coumarin and the lipophilic aryl groups in the position 7.[44] Therefore, the alkylsulfonyloxy group provided MAO-A inhibitors like esuprone, while a benzyloxy substituent led to potent and selective MAO-B inhibitors, such as 7-benzyloxy-3,4-dimethylcoumarin (Figure 1).[45]

In relation to this research, we have previously reported interesting MAO inhibitory properties for several 7-substituted coumarins containing a benzene ring fused at 3,4 positions (Figure 2).[46] This condensation, in general, increased the MAO-A and MAO-B inhibitory activities, and when an additional acetonyloxy group was present in the 7 position, this resulted in compounds with very high MAO-B selectivity. These results led us to analyze the importance of the aryl substituent under the pyrone ring and the above-mentioned C7 substituent, synthesizing a series of 3-arylcoumarin derivatives (Figure 2). The substitution at C7 was removed and a smaller substituent was introduced at C6 and the 3,4-condensed benzene ring has moved at C3, including different substituent groups on it.[47−49] The high MAO-B selectivity found for this new 3-arylcoumarin scaffold encouraged us to synthesize and study the MAO inhibitory activity of new analogues, in which a variety of groups with different size, electronic, and lipophilic properties were introduced in both aromatic rings, in order to clarify the influence of the substitution pattern in the MAO inhibitory activity and selectivity of the 3-arylcoumarin skeleton.

Finally, on the basis of the significant structural information currently available on MAOs,[50−52] and to analyze the structural requirements for MAO activity and selectivity, docking experiments were carried out on hMAO-A and hMAO-B crystallographic structures.

CHEMISTRY

The coumarin derivatives **1**−**22** were efficiently synthesized according to the protocol outlined in Scheme 1. The chemical structure of the compounds is organized in Table 1. The general reaction conditions and the compounds characterization are described in the Experimental Section.

Perkin condensation of different *ortho*-hydroxybenzaldehydes with the adequate arylacetic acid, using *N*,*N*′-dicyclohexylcarbodiimide (DCC) as dehydrating agent,[47,48] afforded the 3-arylcoumarins **1**, **2**, and **6**−**14**. The treatment of the 3-(methoxyphenyl)-6-methylcoumarins **9**,[47] **10**,[48] **12**, and **14** with *N*-bromosuccinimide (NBS), in carbon tetrachloride (CCl_4), under reflux, using 2,2′-azo-bis-iso-butyronitrile (AIBN) as catalyst,[49] afforded the bromomethoxy derivatives **15**−**18**. In addition, compounds **3**, **19**−**21** were obtained by acidic hydrolysis of the respective methoxy derivatives **2**, **9**, **10**, and **11**, using hydriodic acid 57% in the presence of acetic acid and acetic anhydride.[48] Finally, the Williamson reaction[45] of the hydroxycoumarin **3** with chloroacetone or cyclopentyl bromide, gave the corresponding ethers **4** and **5**, respectively. The Williamson reaction of the hydroxycoumarin **20** with a chloroacetone gave the corresponding derivative **22**.

BIOCHEMISTRY

The biological evaluation of the test drugs on hMAO activity was investigated by measuring their effects on the production of hydrogen peroxide (H_2O_2) from *p*-tyramine (a common substrate for h-MAO-A and hMAO-B), using the Amplex Red MAO assay kit (Molecular Probes, Inc., Eugene, Oregon, USA) and microsomal MAO isoforms prepared from insect cells (BTI-TN-5B1−4) infected with recombinant baculovirus containing cDNA inserts for hMAO-A or hMAO-B (Sigma-Aldrich Química SA, Alcobendas, Spain) were used as source for the two microsomal MAO isoforms.[53] The production of H_2O_2 catalyzed by the two MAO isoforms can be detected using 10-acetyl-3,7-dihydroxyphenoxazine (Amplex Red reagent), a nonfluorescent and highly sensitive probe that reacts with H_2O_2 in the presence of horseradish peroxidase to produce a fluorescent product, resorufin. New compounds and reference inhibitors were unable to react directly with the Amplex Red reagent, which indicates that these drugs do not interfere with the measurements. On the other hand, in our experiments and under our experimental conditions, hMAO-A displayed a Michaelis constant (K_m) equal to 457.17 ± 38.62 μM and a maximum reaction velocity (V_{max}) in the control group of 185.67 ± 12.06 (nmol *p*-tyramine/min)/mg protein, whereas hMAO-B showed a K_m of 220.33 ± 32.80 μM and V_{max} of 24.32 ± 1.97 (nmol *p*-tyramine/min)/mg protein (n = 5). Most tested compounds, concentration-dependently inhibited this enzymatic control activity (Table 1).

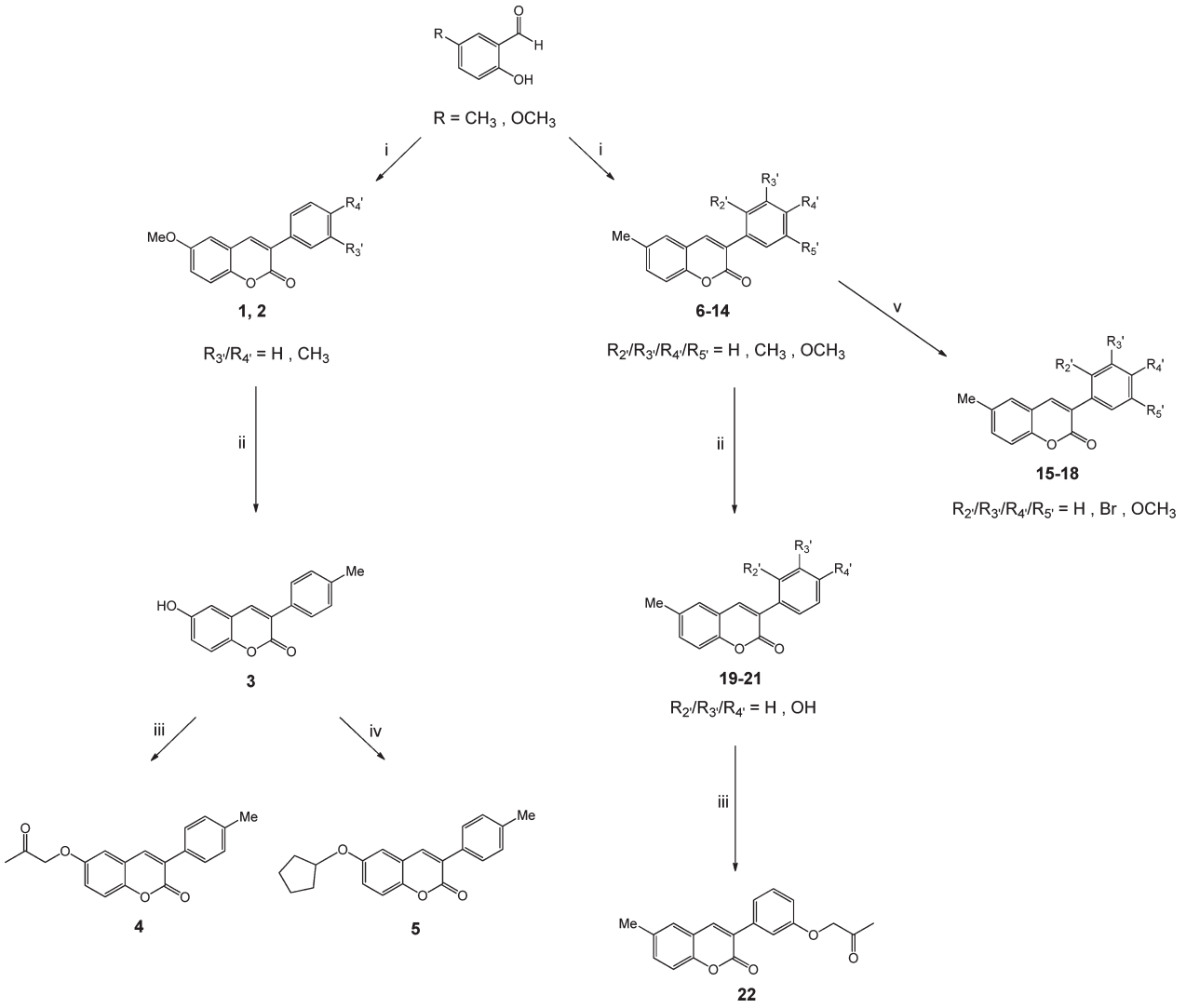
DOCKING STUDIES

To obtain information about enzyme−inhibitor interactions that help us to explain the structural requirements for MAO activity and selectivity of 3-arylcoumarin scaffold, a docking study was performed. The crystallographic structure of MAO-B in complex with a coumarin inhibitor (pdb code 2V61)[52] and of MAO-A in complex with harmine (pdb code 2ZX5)[51] were used to dock the derivates under study. The docking simulations were carried out using the DOCK v. 6.3 package.[54]

To validate our docking protocol, we used the 7-(3-chlorobenzyloxy)-4-[(methylamino)methyl]coumarin cocrystallized with the MAO-B enzyme,[52] as well as two other MAO inhibitors (3-[(4-fluorophenyl)carbamoyl]coumarin and 3-[(4-methanesulfonylphenyl)carbamoyl]coumarin), all of them previously subjected to molecular modeling studies and having bulky substituents at the 3-position as is the case for the new coumarin derivates reported in our manuscript (Figure 3).[15]

Although the proposed docking methodology is able to reproduce the crystallographic orientation of the MAO-B

Scheme 1[a]



[a] Reagents and conditions: (i) substituted phenylacetic acid, DCC, DMSO, 110 °C, 24 h; (ii) HI, AcOH, Ac_2O, reflux, 3 h; (iii) chloroacetone, K_2CO_3, acetone, reflux, 16 h; (iv) cyclopentyl bromide, K_2CO_3, acetone, reflux, 24 h; (v) NBS, AIBN, CCl_4, reflux, 18 h.

coumarinic inhibitor (pdb code 2V61), the successful redocking of the crystallographic inhibitor does not warrant a realistic binding prediction of the new inhibitors proposed. To study whether our methodology is able or not to reproduce the previously described binding mode of noncrystallographic inhibitors, we redocked the last two above-mentioned compounds. This yielded that the obtained binding modes are in agreement with the results previously reported by Chimenti et al.[15]

During the validation of the docking protocol, we also included 3, 5, and 6 structural water molecules in the receptor structure, yielding similar scoring and orientations for the compounds as in the water depleted receptor structure. This is the main reason we did not use any structural water molecule in the modeling of the compounds included in the paper. Furthermore, the use of the water depleted receptor would allow the ligands to explore the lower region of the binding pocket. In this way, the docking algorithm is able to explore orientations of the inhibitors with the 3-substituent oriented to the upper subpocket and to the FAD cofactor. Despite the fact that water molecules were not considered during the ligands orientation step, the predicted poses were rescored using the DOCK Amber-based scoring function, which takes into account the solvent effect using a continuous solvent model and account for small structural rearrangements on the predicted complexes structures.[55,56] These results support the use of our protocol for the prediction and analysis of the binding mode of the coumarin derivatives described in this study.

The molecular docking studies were done for different compounds of the studied series. Although we mainly focused the present results on two representative compounds, the coumarin derivatives **9** and **10**, with substituents in *para* and *meta* positions (Figure 4). They interact with the isoenzyme in the well-known binding pocket,[32,33,50,52] with the substituent at C6 pointing to the FAD cofactor, Phe343, and Tyr60. The coumarin ring is oriented toward the bottom of the substrate cavity, interacting with the FAD cofactor as well as with Tyr398, Tyr435, and Gln206 through van der Waals and hydrophobic interactions, including $\pi-\pi$ interactions with these tyrosine residues. In addition, the predicted orientation of the coumarin moiety allows the interaction of its oxygen atoms of the coumarin moiety

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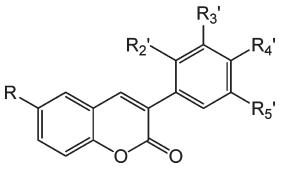
Table 1. Structure and hMAO Inhibitory Activity in Vitro of the 3-Arylcoumarin Derivatives 1−22 and Reference Compounds[a]



compd	R	R2′	R3′	R4′	R5′	IC_{50} hMAO-A (μM)	IC_{50} hMAO-B	SI[d]
1	OCH_3	H	CH_3	H	H	*b*	17.05 ± 0.88 nM	>5882[e]
2	OCH_3	H	H	CH_3	H	*b*	1.52 ± 0.08 nM	>6789[e]
3	OH	H	H	CH_3	H	24.90 ± 1.33	67.10 ± 2.99 nM	372
4	$C_3H_5O_2$	H	H	CH_3	H	*b*	5.52 ± 0.29 μM	>18[e]
5	C_5H_9O	H	H	CH_3	H	*b*	14.47 ± 0.78 μM	>6.9[e]
6	CH_3	H	H	H	H	*b*	283.75 ± 19.90 nM	>352[e]
7	CH_3	H	H	CH_3	H	*b*	0.31 ± 0.02 nM	>333333[e]
8	CH_3	H	CH_3	H	H	*c*	15.01 ± 0.83 nM	>6667[e]
9	CH_3	H	H	OCH_3	H	*b*	13.05 ± 0.90 nM	>7663
10	CH_3	H	OCH_3	H	H	*b*	0.80 ± 0.05 nM	>125000
11	CH_3	OCH_3	H	H	H	*b*	*b*	
12	CH_3	H	OCH_3	H	OCH_3	*b*	8.98 ± 1.42 nM	>11136[e]
13	CH_3	H	OCH_3	OCH_3	H	25.14 ± 1.68	2.73 ± 0.12 nM	9209
14	CH_3	H	OCH_3	OCH_3	OCH_3	*b*	160.64 ± 1.01 nM	>623[e]
15	CH_3	H	Br	OCH_3	H	*b*	0.74 ± 0.02 nM	>135870[e]
16	CH_3	H	OCH_3	Br	H	*b*	3.25 ± 0.17 nM	>31250[e]
17	CH_3	Br	OCH_3	H	OCH_3	*b*	54.03 ± 3.91 μM	>1.9[e]
18	CH_3	Br	OCH_3	OCH_3	OCH_3	*b*	*b*	
19	CH_3	H	H	OH	H	*b*	155.59 ± 17.09 nM	>643[e]
20	CH_3	H	OH	H	H	35.04 ± 1.88	650.03 ± 34.82 nM	54
21	CH_3	OH	H	H	H	21.58 ± 1.16	120.02 ± 6.43 nM	180
22	CH_3	H	$C_3H_5O_2$	H	H	*c*	180.04 ± 9.65 nM	>555[e]
Selegiline						67.25 ± 1.02	19.60 ± 0.86 nM	3431
Iproniazide						6.56 ± 0.76	7.54 ± 0.36 μM	0.87

[a] Each IC_{50} value is the mean ± SEM from five experiments (n = 5). [b] Inactive at 100 μM (highest concentration tested), at higher concentrations the compounds precipitate. [c] 100 μM inhibits the corresponding hMAO activity by approximately 40−45%, at higher concentrations the compounds precipitate. [d] Selectivity index: MAO-B selectivity ratios [IC_{50} (MAO-A)]/[IC_{50} (MAO-B)] for inhibitory effects of both new compounds and reference inhibitors. [e] Values obtained under the assumption that the corresponding IC_{50} against MAO-A is the highest concentration tested (100 μM).

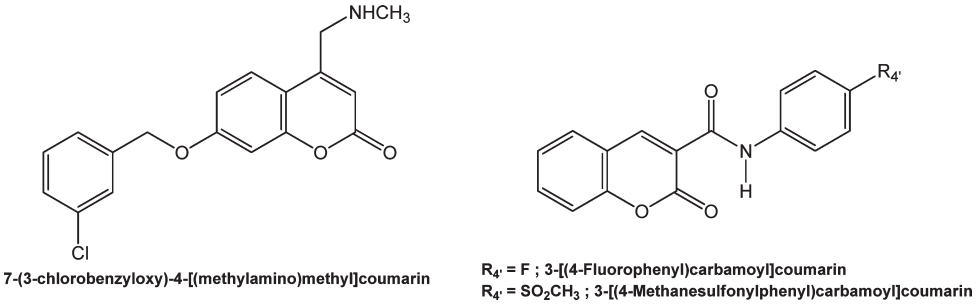


Figure 3. Compounds used to validate the docking protocol.

with Cys172, showing a favorable geometry for the formation of hydrogen bonds between the ligand and the receptor. Moreover, the coumarin core also interacts with Ile198, Ile199, and Leu171. Finally, the substituted 3-phenyl ring is oriented to an entrance cavity, an hydrophobic subpocket existing only in the MAO-B isoform, which is defined by Leu171, Ile199, Tyr326, Phe168, Ile316, Trp119, Pro102, and Pro104. This is probably one explanation to the MAO-B selectivity of the described compounds.

RESULTS AND DISCUSSION

All described coumarins in this report (compounds **1**−**22**) were efficiently synthesized and evaluated for their ability to

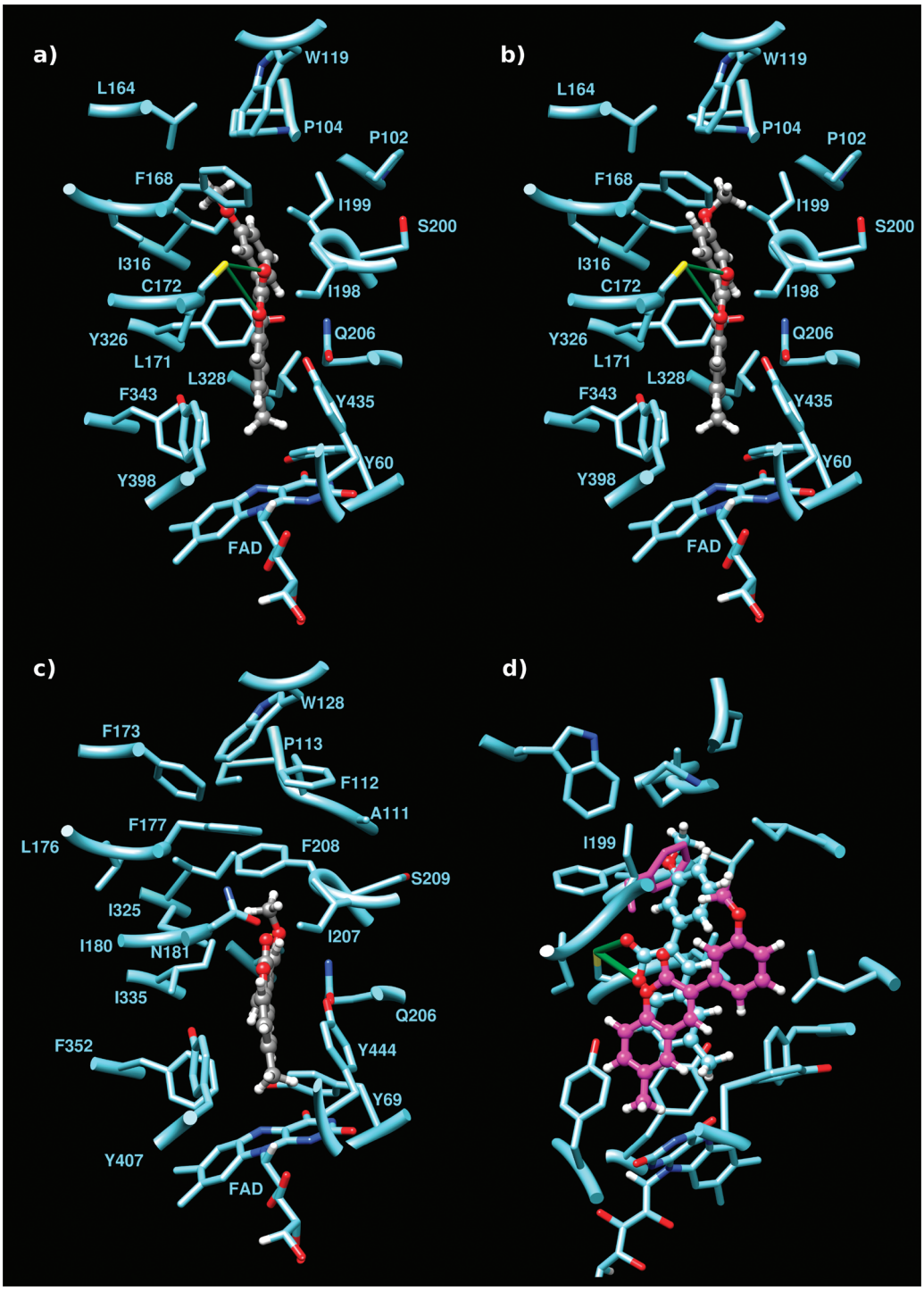


Figure 4. Molecular docking studies of coumarin derivatives **9** and **10**. Most stable binding poses of compound **10** (a) and **9** (b) into MAO-B binding site, and compound **10** (c) into MAO-A binding site. Compounds are colored by atom type, and predicted H-bonds are represented as green pseudobonds. Superposition of binding poses of compound **10** (d) to both isoenzymes, MAO-A (ligand and Phe208 in magenta), and MAO-B.

inhibit the A and B isoforms of hMAO. The corresponding IC_{50} values and MAO-B selectivity ratios [IC_{50} (MAO-A)]/[IC_{50} (MAO-B)] are shown in Table 1. The chemical structures of the newly designed compounds, as well as the biological and docking results, can help us with an interesting SAR study. From the experimental results, it can be observed that most of the tested compounds are selective inhibitors toward MAO-B, with IC_{50} values in the low micro, nano, and picomolar range. Ten of the 22 tested compounds have similar or better IC_{50} than the selegiline (IC_{50} = 19.60 nM, reference MAO-B inhibitor). Two of the most active compounds are the coumarin derivatives **7** and **10**, with IC_{50} against hMAO-B of 0.31 and 0.80 nM, respectively. Both compounds **7** and **10** are *para* or *meta* substituted in the 3-phenyl ring with a methyl or a methoxy group, respectively. With the aim

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of introducing a group with different physicochemical properties under the 3-phenyl ring of the coumarin moiety, we have brominated the different 3-methoxyphenyl derivatives. Therefore, compound **15** has the *para* and *meta* positions substituted with a methoxy and a bromo groups, respectively. This compound proved to be one of the three most active compounds, with IC_{50} against hMAO-B of 0.74 nM. This new substitution makes this compound a better MAO-B inhibitor than the corresponding derivative without the bromo atom, compound **9**, with an IC_{50} of 13.05 nM. On the other hand, compound **16**, with the *para* and *meta* positions substituted with a bromo and a methoxy groups, respectively, seems to lose MAO-B inhibitory activity. So, we can infer that the presence of an electron donor at the *para* position is an important modification to improve the activity. From the biological data, the coumarin **7**, with a *p*-methyl substituent in the 3-phenyl ring and a 6-methyl substituent in the coumarin aromatic ring, was the most potent and selective one against MAO-B isoenzyme. This compound was 63 times more active than the selegiline, resulting also much more selective than it (selectivity MAO-B index >333333).

The activity data of new reported compounds point out that almost all the substitutions at *ortho* position of the 3-phenyl ring seem to be unfavorable for the activity. Compounds **11** and **18**, with a methoxy or a bromo substituent, respectively, at the *ortho* position are inactive at 100 μM (highest concentration tested). The corresponding compound **14**, compared with compound **18** but without the *ortho*-bromo atom, has an activity in the nanomolar range. Compound **17**, also with a bromo atom at *ortho* position, has an MAO-B inhibitory activity in the high micromolar range, while their correspondent nonbrominated compound **12**, with a "free" *ortho* position, has an activity against MAO-B in the low nanomolar range. It seems clear that the presence of different substituents in the *ortho* position annulated or strongly decreased the inhibitory MAO-B activity. An exception occurs when the *ortho* position is substituted with a hydroxyl group (compound **21**). In this case, the methoxy derivative (compound **11**) has no activity against MAO-B and the hydroxyl one has an IC_{50} in the nanomolar range. This is going to be explained later in the discussion of the Docking Studies. Moreover, inhibitory MAO-B activity increases when the small lipophilic groups (methoxy or methyl) are included at *meta* or *para* positions. Nevertheless, the dimethoxy-substituted compound **13** is less active than the *meta*-monosubstituted one (compound **10**). In addition, a hydroxyl group incorporated in *para*- or *meta*-positions of the phenyl ring of the moiety seems to be less favorable for the MAO-B inhibition than a methoxy or methyl group at the same position, as it can be seen by comparing the IC_{50} values of compounds **7**−**10** with those of compounds **19** and **20**, respectively. Finally, a methyl group in the 6 position of the coumarin moiety (compound **7**) provides better MAO-B inhibitory activity than a methoxy group (compound **2**) or a hydroxyl group (compound **3**). Also, bulkier alkoxy substituents at C6, such as an 2-oxopropoxy group (compound **4**) or a cyclopentyloxy group (compound **5**), significantly reduced the activity, being better tolerated in the 3-phenyl ring, in particular in the *meta* position (compound **22**). Almost all the studied compounds with substituents in the 3-phenyl ring proved to be most active against the MAO-B than the nonsubstituted 6-methyl-3-phenylcoumarin **6** (IC_{50} = 283 nM). Compounds substituted in the *ortho* position (excepting compound **21**, already mentioned), compound **20** and compounds bulky substituted (compounds **4** and **5**) in position 6 were the only exception.

On the basis of the docking results, the proposed binding mode for this series of compounds in the pocket of hMAO-B is consistent with the observation that only small substituents are tolerated at position 6, as can be seen analyzing the results of the compounds **4** and **5**. The increase in the size of substituents at C6 results in a disruption of the proposed binding mode because part of the space occupied by the coumarin ring will be used by this bulky substituent, resulting in the loss of the key interactions to stabilize the ligand−enzyme complex.

Additionally, the poses predicted by the docking simulations show a small angle between the planes defined by the coumarin and the 3-phenyl ring. These conformations, which are sterically prohibited when a methoxy group is included at 2′ position of the 3-phenyl ring, are however permitted for a hydroxyl group at the same position. These conformational changes can explain why the *ortho* position substitutions of the 3-phenyl ring considerably decreased or abolished the activity and why the compound **21** has activity against MAO-B, whereas the corresponding methoxy derivative **11** is inactive at the highest tested concentration against the same enzyme (Table 1). Moreover, the docking results indicate that a substituent at *para*- or *meta*-position of the 3-phenyl ring makes the compound occupy the above-mentioned hydrophobic subpocket (Figure 4a and 4b).

Finally, the comparison between the predicted binding mode of compound **10** to MAO-A (Figure 4c) with its binding mode to MAO-B (Figure 4d) corroborates that entrance cavity only exits in MAO-B because in MAO-A its formation is prevented by the presence of the Phe208 residue instead of the Ile199 present in the same position of MAO-B.

On the basis of the docking studies information, the high MAO-B selectivity of these series of compounds could be explained by taking into account two factors: the ability of the compounds to recognize and exploit the entrance cavity in MAO-B and the fact that, according to predicted binding mode, in MAO-A these compounds should occupy a more distant position into the binding pocket to fit in the cavity and interact with the binding points (ligand in magenta ball and sticks, Figure 4d). This movement to a deeper region in the cavity results in the displacement of some water molecules conserved in the X-ray structures of the MAOs, which is not necessary for the binding to MAO-B, according to the proposed models. This displacement of the water molecules is an energetically expensive process that negatively influences the formation of stable complexes between the inhibitors and MAO-A.

CONCLUSION

In this paper, we have used the Perkin reaction as a key step of a good methodology for the efficient and general synthesis of a selected series of 3-arylcoumarins. Most of the studied compounds show a high affinity and selectivity for the MAO-B isoenzyme. Several compounds of these series proved to have much higher MAO-B inhibitory activity and selectivity than the selegiline. The docking studies performed on these compounds show that their interactions with the MAO-B binding pocket are more intense than those with the MAO-A one. The docking results are in accord with the biological data and allowed us to support an interesting SAR study. Additionally, all the results show that a small substitution at C6 (methyl or methoxy groups) of the coumarin core seems to be important when a phenyl group is located at C3. A bulkier group at C6 (compounds **4** and **5**) drastically decreases the MAO-B inhibitory activity. With a small

substituent at C6, both kind and position of substituents included in the 3-aryl group play a crucial role in activity and selectivity. *Meta* and *para* substituents were the most favorable positions for the desired activity. Currently, further research is going to be conducted on the 3-arylcoumarin scaffold as potential agents for the treatment of Parkinson's disease.

EXPERIMENTAL SECTION

Chemistry. Melting points were determined using a Reichert Kofler thermopan or in capillary tubes on a Büchi 510 apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 1640FT spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX spectrometer at 300 and 75.47 MHz, respectively, using TMS as internal standard (chemical shifts in δ values, J in Hz). Mass spectra were obtained using a Hewlett-Packard 5988A spectrometer. Elemental analyses were performed using a Perkin-Elmer 240B microanalyser and were within ±0.4% of calculated values in all cases. Silica gel (Merck 60, 230−00 mesh) was used for flash chromatography (FC). Analytical thin layer chromatography (TLC) was performed on plates precoated with silica gel (Merck 60 F254, 0.25 mm). The purity of compounds **1**−**22** was assessed by HPLC and was found to be higher than 95%.

General Procedure for the Preparation of 3-Phenylcoumarins (1, 2, 6−14). A solution of 2-hydroxy-6-methylbenzaldehyde/2-hydroxy-6-methoxybenzaldehyde (7.34 mmol) and the corresponding phenylacetic acid (9.18 mmol) in dimethyl sulfoxide (15 mL) was prepared. *N,N′*-Dicyclohexylcarbodiimide (11.46 mmol) was added, and the mixture was heated in an oil bath at 110 °C for 24 h. Ice (100 mL) and acetic acid (10 mL) were added to the reaction mixture. After keeping it at room temperature for 2 h, the mixture was extracted with ether (3 × 25 mL). The organic layer was extracted with sodium bicarbonate solution (50 mL, 5%) and then water (20 mL). The solvent was evaporated under vacuum, and the dry residue was purified by FC (hexane/ethyl acetate 9:1).

6-Methoxy-3-(3-methylphenyl)coumarin (***1***). Yield 79%; mp 98−99 °C. ^{1}H NMR ($CDCl_3$) 2.46 (s, 3H, $-CH_3$), 3.90 (s, 3H, $-OCH_3$), 7.01 (d, 1H, H-5, J = 2.8), 7.14 (dd, 1H, H-7, J = 9.0, J = 2.9), 7.25−7.41 (m, 3H, H-5′, H-4′, H-8), 7.50−7.54 (m, 2H, H-2′, H-6′), 7.79 (s, 1H, H-4). ^{13}C NMR ($CDCl_3$) 21.5, 55.8, 109.9, 117.4, 119.0, 120.0, 125.7, 128.3, 128.8, 129.2, 129.6, 134.7, 138.0, 139.6, 147.9, 156.1, 160.7. MS m/z 267 ([M + 1]$^+$, 30), 266 (M$^+$, 100), 238 (38), 195 (25), 167 (14), 165 (17), 152 (24). Anal. Calcd for $C_{17}H_{14}O_3$: C, 76.68; H, 5.30. Found: C, 76.72; H, 5.38.

6-Methoxy-3-(4-methylphenyl)coumarin (***2***). Yield 76%; mp 130−131 °C. ^{1}H NMR ($CDCl_3$) 2.40 (s, 3H, $-CH_3$), 3.86 (s, 3H, $-OCH_3$), 7.01 (d, 1H, H-7, J = 3.9), 7.13−7.17 (m, 2H, H-5, H-8), 7.30−7.35 (m, 2H, H-3′, H-5′), 7.65 (d, 2H, H-2′, H-6′, J = 8.1), 7.78 (s, 1H, H-4). ^{13}C NMR ($CDCl_3$) 21.3, 55.8, 109.8, 117.4, 118.9, 120.1, 128.4, 128.6, 129.2, 131.9, 138.9, 139.0, 147.9, 156.1, 160.8. MS m/z 267 ([M + 1]$^+$, 20), 266 (M$^+$, 100), 238 (25), 195 (18), 165 (9), 152 (16). Anal. Calcd for $C_{17}H_{14}O_3$: C, 76.68; H, 5.30. Found: C, 76.67; H, 5.23.

6-Methyl-3-(4-methylphenyl)coumarin (***7***). Yield 72%; mp: 139−140 °C. ^{1}H NMR ($CDCl_3$) 2.40 (s, 6H, $(-CH_3)_2$), 7.21−7.30 (m, 5H, H-5, H-7, H-8, H-3′, H-5′), 7.60 (d, 2H, H-2′, H-6′, J = 8.2), 7.72 (s, 1H, H-4). ^{13}C NMR ($CDCl_3$) 21.1, 21.6, 116.4, 119.7, 127.9, 128.4, 128.7, 129.4, 132.2, 132.5, 134.3, 139.0, 139.4, 139.5, 151.8, 161.2. MS m/z 251 ([M + 1]$^+$, 32), 250 (M$^+$, 100), 222 (56), 221 (24), 178 (27), 150 (12), 136 (12), 124 (19). Anal. Calcd for $C_{17}H_{14}O_2$: C, 81.58; H, 5.64. Found: C, 81.52; H, 5.60.

6-Methyl-3-(3-methylphenyl)coumarin (***8***). Yield 75%; mp 84−85 °C. ^{1}H NMR ($CDCl_3$) 2.39 (s, 6H, $-(CH_3)_2$), 6.98−7.02 (m, 3H, H-4′, H-7, H-8), 7.13−7.24 (m, 4H, H-2′, H-5, H-5′, H-6′), 7.72 (s, 1H, H-4). ^{13}C NMR ($CDCl_3$) 20.8, 21.5, 116.1, 119.4, 125.6, 127.6, 128.3, 129.2, 129.5, 132.3, 134.1, 134.8, 138.0, 139.5, 139.8, 151.6, 160.8. MS m/z 251 ([M + 1]$^+$, 27), 250 (M$^+$, 100), 222 (72), 221 (39), 178 (32), 150 (10), 136 (10), 124 (10). Anal. Calcd for $C_{17}H_{14}O_2$: C, 81.58; H, 5.64. Found: C, 81.56; H, 5.62.

General Procedure for the Preparation of 3-(Bromomethoxyphenyl)coumarins (15−18). A solution of 3-(methoxyphenyl)-coumarins (3.76 mmol), NBS (4.51 mmol), and AIBN (cat.) in CCl_4 (5 mL) was stirred under reflux for 18 h. The resulting solution was filtered to remove the succinimide. The solvent was evaporated under vacuum and purified by flash chromatography (hexane/ethyl acetate 95:5).

3-(3-Bromo-4-methoxyphenyl)-6-methylcoumarin (***15***). Yield: 41%; mp 206−207 °C. ^{1}H NMR ($CDCl_3$) 2.42 (s, 3H, $-CH_3$), 3.94 (s, 3H, $-OCH_3$), 6.97 (d, 1H, H-5′ J = 8.7), 7.23−7.35 (m, 3H, H-2′, H-6′, H-5), 7.69−7.74 (m, 2H, H-7, H-8), 7.89 (s, 1H, H-4). ^{13}C NMR ($CDCl_3$) 20.8, 56.3, 111.5, 111.6, 116.1, 119.3, 126.3, 127.6, 128.5, 129.0, 132.5, 133.1, 134.2, 139.1, 151.5, 156.2, 160.6. MS m/z (%) 347 (18), 346 (98), 345 ([M + 1]$^+$, 19), 344 (M$^+$, 100), 303 (45), 301 (45), 275 (11), 250 (17), 222 (13), 194 (11), 178 (13), 165 (58), 163 (11), 139 (15), 132 (42), 82 (18), 76 (14), 63 (19), 50 (14). Anal. Calcd for $C_{17}H_{13}BrO_3$: C, 59.15; H, 3.80. Found: C, 59.10; H, 3.72.

3-(4-Bromo-3-methoxyphenyl)-6-methylcoumarin (***16***). Yield: 51%; mp 139−140 °C. ^{1}H NMR ($CDCl_3$) 2.49 (s, 3H, $-CH_3$), 3.89 (s, 3H, $-OCH_3$), 6.49 (dd, 1H, H-7, J = 7.4, J = 1.3), 7.08−7.12 (m, 3H, H-2′, H-6′, H-8), 7.32−7.42 (m, 2H, H-5, H-5′), 7.74 (s, 1H, H-4). ^{13}C NMR ($CDCl_3$) 33.1, 55.4, 114.2, 114.7, 117.1, 119.7, 120.9, 126.8, 128.2, 128.7, 129.6, 132.2, 134.3, 135.7, 139.4, 153.2, 159.5. MS m/z (%) 346 (45), 345 ([M + 1]$^+$, 10), 344 (M$^+$, 100), 266 (49), 265 (45), 238 (24), 237 (67), 194 (42), 165 (29), 133 (28). Anal. Calcd for $C_{17}H_{14}O_2$: C, 59.15; H, 3.80. Found: C, 59.17; H, 3.83.

3-(2-Bromo-3,5-dimethoxyphenyl)-6-methylcoumarin (***17***). Yield: 46%; mp 178−179 °C. ^{1}H NMR ($CDCl_3$) 2.40 (s, 3H, $-CH_3$), 3.79 (s, 3H, $-OCH_3$), 3.87 (s, 3H, $-OCH_3$), 6.51 (s, 2H, H-4′, H-6′), 7.26−7.33 (m, 3H, H-5, H-7, H-8), 7.60 (s, 1H, H-4). ^{13}C NMR ($CDCl_3$) 20.8, 55.6, 56.4, 100.1, 104.3, 107.4, 116.4, 118.7, 127.9, 129.0, 132.8, 134.2, 137.6, 142.3, 152.1, 157.0, 159.7. MS m/z (%) 377 (42), 376 ([M + 1]$^+$, 18), 375 (M$^+$, 100), 282 (59), 279 (14), 239 (11) 208 (9), 152 (10), 118 (9), 58 (12). Anal. Calcd for $C_{18}H_{15}BrO_4$: C, 57.62; H, 4.03. Found: C, 57.61; H, 4.08.

3-(2-Bromo-3,4,5-trimethoxyphenyl)-6-methylcoumarin (***18***). Yield: 50%; mp 167−168 °C. ^{1}H NMR ($CDCl_3$) 2.40 (s, 3H, $-CH_3$), 3.83 (s, 3H, $-OCH_3$), 3.90 (s, 6H, $-(OCH_3)_2$), 6.72 (s, 1H, H-6′), 7.25 (d, 1H, H-8, J = 8.4), 7.29 (d, 1H, H-5, J = 1.9), 7.34 (dd, 1H, H-7, J = 8.4, J = 2.0), 7.63 (s, 1H, H-4). ^{13}C NMR ($CDCl_3$) 20.8, 55.6, 61.1, 61.1, 110.1, 110.4, 116.4, 118.7, 127.8, 128.4, 131.2, 132.9, 134.3, 142.7, 143.4, 151.2, 152.0, 152.7, 160.1. MS m/z (%) 408 (32), 407 ([M + 1]$^+$, 12), 406 (M$^+$, 68), 404 (5) 326 (22), 325 (59), 282 (15), 264 (15) 169 (9), 139 (10). Anal. Calcd for $C_{19}H_{17}BrO_5$: C, 56.31; H, 4.23. Found: C, 56.38; H, 4.28.

General Procedure for the Preparation of Hydroxy-3-phenylcoumarins (3, 20−21). A solution of substituted 6-methoxy-3-phenylcoumarin (0.50 mmol) in acetic acid (5 mL) and acetic anhydride (5 mL), at 0 °C, was prepared. Hydriodic acid 57% (10 mL) was added dropwise. The mixture was stirred, under reflux temperature, for 3 h. The solvent was evaporated under vacuum, and the dry residue was purified by CH_3CN crystallization.

6-Hydroxy-3-(4-methylphenyl)coumarin (***3***). Yield 61%; mp 214−215 °C. ^{1}H NMR (DMSO-d_6) 2.32 (s, 3H, $-CH_3$), 7.01 (d, 1H, H-7, J = 8.8, J = 2.9), 7.07 (d, 1H, H-5, J = 2.8), 7.21−7.26 (m, 3H, H-3′, H-5′, H-8), 7.59 (d, 2H, H-2′, H-6′, J = 8.1), 8.08 (s, 1H, H-4), 9.77 (s, 1H, −OH). ^{13}C NMR (DMSO-d_6) 21.3, 113.0, 117.1, 120.0, 120.5, 127.2, 128.8, 129.2, 132.3, 138.5, 140.4, 146.7, 154.2, 160.5. MS m/z (%) 253 ([M + 1]$^+$, 51), 252 (M$^+$, 100), 225 (35), 224 (96), 223 (62) 195 (13), 181 (17), 165 (19), 152 (31), 139 (10), 125 (15), 115 (15). Anal. Calcd for $C_{16}H_{12}O_3$: C, 76.18; H, 4.79. Found: C, 76.11; H, 4.77.

*3-(3-Hydroxyphenyl)-6-methylcoumarin (**20**).* Yield 53%; mp 160−161 °C. ^{1}H NMR (DMSO-d_6) 2.32 (s, 3H, $-CH_3$), 6.77 (dd, 1H, H-4′, J = 7.1, J = 2.4), 7.05−7.07 (m, 2H, H-5, H-8), 7.21−7.25 (m, 2H, H-5′, H-6′), 7.38 (dd, 1H, H-7, J = 8.5, J = 1.8), 7.50 (s, 1H, H-2′), 8.08 (s, 1H, H-4), 9.52 (s, 1H, −OH). ^{13}C NMR (DMSO-d_6) 20.8, 115.9, 116.1, 119.6, 127.3, 128.7, 129.7, 133.0, 134.2, 136.4, 140.8, 151.5, 157.5, 160.2. MS m/z 254 (12), 253 ([M + 1]$^+$, 63), 252 (M$^+$, 73), 251 (15), 225 (46), 223 (53), 195 (33), 194 (18), 181 (29), 178 (17), 166 (12), 165 (42), 152 (43), 139 (13), 126 (24), 111 (19), 63 (16), 51 (16). Anal. Calcd for $C_{16}H_{12}O_3$: C, 76.18; H, 4.79. Found: C, 76.20; H, 4.83.

*3-(2-Hydroxyphenyl)-6-methylcoumarin (**21**).* Yield 56%; mp 167−168 °C. ^{1}H NMR (DMSO-d_6) 2.38 (s, 3H, $-CH_3$), 6.30−6.35 (m, 2H, H-3′, H-5′), 7.22−7.24 (d, 1H, H-5, J = 1.5), 7.29−7.31 (m, 2H, H-4′, H-6′), 7.34 (d, 1H, H-8, J = 8.4), 7.44 (dd, 1H, H-7, J = 8.4, J = 1.5), 7.97 (s, 1H, H-4), 9.60 (s, 1H, −OH). ^{13}C NMR (DMSO-d_6) 20.3, 115.6, 118.7, 119.0, 122.3, 125.9, 128.0, 129.6, 130.8, 132.3, 133.6, 141.8, 151.2, 155.0, 159.5. MS m/z 253 ([M + 1]$^+$, 18), 252 (M$^+$, 100), 251 (10), 234 (12), 223 (58), 195 (35), 194 (5), 181 (18), 165 (17), 152 (18), 126 (5), 63 (6), 51 (6). Anal. Calcd for $C_{16}H_{12}O_3$: C, 76.18; H, 4.79. Found: C, 76.20; H, 4.81.

General Procedure for the Preparation of 2-Oxopropoxy-6-phenylcoumarin (4, 22). Under a suspension of anhydrous K_2CO_3 (0.25 mmol) and the corresponding hydroxycoumarin (0.13 mmol), in anhydrous acetone (3 mL), the chloroketone (0.25 mmol) was added. The suspension was stirred, at reflux temperature, for 16 h. The mixture was cooled, and the precipitate was recovered by filtration and washed with anhydrous acetone (3 × 40 mL). The solvent was evaporated under vacuum, and the dry residue was purified by FC (hexane/ethyl acetate 85:15).

*3-(4-Methylphenyl)-6-(2-oxopropoxy)coumarin (**4**).* Yield 74%; mp 128−129 °C. ^{1}H NMR ($CDCl_3$) 2.33 (s, 3H, $-CH_3$), 2.42 (s, 3H, $-CH_3$), 4.64 (s, 2H, $-CH_2$), 6.96 (d, 1H, H-5, J = 2.9), 7.14 (dd, 1H, H-7, J = 9.0, J = 2.9), 7.28−7.33 (m, 3H, H-3′, H-5′, H-8), 7.60−7.65 (m, 2H, H-2′, H-6′), 7.74 (s, 1H, H-4). ^{13}C NMR ($CDCl_3$) 21.3, 26.6, 73.5, 111.0, 117.7, 119.2, 120.2, 128.4, 129.0, 129.2, 131.7, 138.6, 139.1, 148.4, 154.2, 160.6, 204.7. MS m/z 309 ([M + 1]$^+$, 31), 308 (M$^+$, 100), 266 (12), 265 (50), 236 (15), 235 (26), 207 (31) 195 (12), 179 (16), 178 (16), 165 (11), 153 (12), 129 (20). Anal. Calcd for $C_{19}H_{16}O_4$: C, 74.01; H, 5.23. Found: C, 73.90; H, 5.18.

*6-Methyl-3-[3-(2-oxopropoxy)phenyl]coumarin (**22**).* Yield 71%; mp 107−108 °C. ^{1}H NMR ($CDCl_3$) 2.31 (s, 3H, $-CH_3$), 2.43 (s, 3H, $-CH_3$), 4.61 (s, 2H, $-CH_2$), 6.94 (d, 1H, H-5, J = 1.2), 7.24−7.42 (m, 6H, H-7, H-8, H-2′, H-4′, H-5′, H-6′), 7.77 (s, 1H, H-4). ^{13}C NMR ($CDCl_3$) 20.8, 26.6, 73.1, 114.8, 115.0, 116.1, 119.2, 121.9, 127.7, 129.7, 132.6, 134.2, 136.4, 140.2, 151.6, 157.6, 160.6, 205.3. MS m/z 309 ([M + 1]$^+$, 14), 308 (M$^+$, 70), 266 (55), 265 (50), 237 (29), 236 (21), 235 (20), 178 (24), 165 (10). Anal. Calcd for $C_{19}H_{16}O_4$: C, 74.01; H, 5.23. Found: C, 74.08; H, 5.30.

Preparation of 6-(2-Cyclopentyloxy)-3-(4-methylphenyl)-coumarin (5). Under a suspension of anhydrous K_2CO_3 (0.25 mmol) and the 6-hydroxycoumarin **3** (0.13 mmol), in anhydrous acetone (3.0 mL), the cyclopentyl bromide (0.25 mmol) was added. The suspension was stirred, at reflux temperature, for 24 h. The mixture was cooled, and the precipitate was recovered by filtration and washed with anhydrous acetone (3 × 40.0 mL). The solvent was evaporated under vacuum, and the dry residue was purified by FC (hexane/ethyl acetate 9:1). Yield 58%; mp 147−148 °C. ^{1}H NMR ($CDCl_3$) 1.37−1.99 (m, 8H, $(-CH_2)_4$), 2.52 (s, 3H, $-CH_3$), 4.89 (s, 1H, H-1″), 7.05 (d, 1H, H-5, J = 2.7), 7.17 (dd, 1H, H-7, J = 9.0, J = 2.7), 7.36−7.40 (m, 3H, H-3′, H-5′, H-8), 7.72 (d, 2H, H-2′, H-6′, J = 8.1), 7.84 (s, 1H, H-4). ^{13}C NMR ($CDCl_3$) 21.3, 24.0, 32.8, 80.0, 111.8, 117.3, 120.1, 120.3, 128.4, 128.4, 129.1, 132.0, 138.8, 139.2, 147.6, 154.6, 160.9. MS m/z 321 ([M + 1]$^+$, 7), 320 (M$^+$, 28), 253 (19), 252 (100), 224 (38), 223 (13), 152 (11). Anal. Calcd for $C_{21}H_{20}O_3$: C, 78.75; H, 6.29. Found: C, 78.69; H, 6.24.

Determination of hMAO Isoform Activity. Briefly, 0.1 mL of sodium phosphate buffer (0.05 M, pH 7.4) containing different concentrations of the test drugs (new compounds or reference inhibitors) in various concentrations and adequate amounts of recombinant hMAO-A or hMAO-B required and adjusted to obtain in our experimental conditions the same reaction velocity, i.e., to oxidize (in the control group) the same concentration of substrate: 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 incubated for 15 min at 37 °C in a flat-black-bottom 96-well microtest plate and placed in the dark fluorimeter chamber. After this incubation period, 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 test drugs (new compounds and reference inhibitors) with appropriate dilutions of the vehicles. In addition, the possible capacity of the above test drugs to modify the fluorescence generated in the reaction mixture due to nonenzymatic 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. To determine the kinetic parameters of hMAO-A and hMAO-B (K_m and V_{max}), the corresponding enzymatic activity of both isoforms was evaluated (under the experimental conditions described above) in the presence of a number (a wide range) of *p*-tyramine concentrations.

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. In our experimental conditions, this background activity was practically negligible.

Molecular Docking Simulations. The crystallographic structure of MAO-B in complex with a coumarin inhibitor (pdb code 2V61)[52] and the MAO-A in complex with harmine (pdb code 2ZX5)[51] were used to dock the coumarins under study. For both isoenzymes, hydrogen atoms and charges were added to the receptor structure using the UCSF Chimera,[57] and the atomic charges of the FAD cofactor were calculated using GAMESS.[58]

Three-dimensional conformers for the compounds were generated using the OMEGA software.[59] A maximum of 50000 conformations per molecule were generated using an energy window of 10 kcal. All rotatable bonds were considered during the torsion search, using the Merck Molecular Force Field (MMFF), and duplicate conformers were discarded based on a root-mean-square (rms) value of 0.5 Å. A maximum number of 300 conformers were saved for each compound. Afterward, AM1-BCC charges were added to each conformer using the MOLCHARGE program, which is part of the QUACPAC package.[60]

Rigid docking was carried out for each ligand conformer using the DOCK 6.3 package.[54] A maximum of 5000 orientations per ligand was assayed. The energy grid-based scoring function was selected for poses quality evaluation. The five lowest scored poses for each ligand conformer were saved, allowing for a maximum of 1500 saved poses for each compound. Afterward, the five lowest scored conformers of each ligand were rescored, using the DOCK Amber-based scored function, considering only the flexibility of the ligand. Finally, the pose with the lowest Amber-based scoring value for each compound was rescored, with the same scoring function, considering both the ligand and the binding. We chose the Amber-based scoring function to take

into account small structural rearrangements on both ligand and receptor as well as the effect of the solvent through a GB/SA continuum model for the solvation free energy.[55,56] Molecular graphics images were produced using the UCSF Chimera package from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (supported by NIH P41 RR001081).

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DEDICATION

This article is dedicated to the memory of Prof. Francisco Orallo

ABBREVIATIONS USED

hMAO, human monoamine oxidase; FAD, flavin adenine dinucleotide; IC_{50}, half maximal inhibitory concentration; cDNA, cDNA; SAR, structure−activity relationship; PDB, Protein Data Bank; HPLC, high-performance liquid chromatography

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