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Discovery, Biological Evaluation, and Structure—Activity and —Selectivity Relationships of 6'-Substituted (E)-2-(Benzofuran-3(2H)-ylidene)-N-methylacetamides, a Novel Class of Potent and Selective Monoamine Oxidase Inhibitors

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(5) Supporting Information

ABSTRACT: The use of selective inhibitors of monoamine oxidase A (MAO-A) and B (MAO-B) holds a therapeutic relevance in the treatment of depressive disorders and Parkinson's disease (PD), respectively. Here, the discovery of a new class of compounds acting as monoamine oxidase inhibitors (MAO-Is) and bearing a 6'-substituted (*E*)-2-(benzofuran-3(2*H*)-ylidene)-*N*-alkylacetamide skeleton is reported. 6'-sulfonyloxy derivatives exhibited outstanding affinities to MAO-A (7.0 nM < IC₅₀ < 49 nM, much higher than moclobemide) and a pronounced MAO-A/B selectivity. The corresponding 6'-benzyloxy derivatives showed potent MAO-B inhibition and inverted selectivity profile. The rigid *E*-geometry of the exocyclic double bond allowed a more efficient binding conformation compared to more flexible and less active 2-(1-benzofuran-3-yl)-*N*-methylacetamide isomers and 4-*N*-



methylcarboxamidomethylcoumarin analogues. Focused structural modifications and docking simulations enabled the identification of key molecular determinants for high affinity toward both MAO isoforms. These novel MAO-Is may represent promising hits for the development of safer therapeutic agents with a potential against depression, PD, and other age-related neurodegenerative pathologies.

INTRODUCTION

Monoamine oxidases (MAO, EC 1.4.3.4, amine-oxygen oxidoreductase) are FAD-dependent enzymes bound to the outer mitochondrial membrane, where they catalyze the oxidative deamination of both exogenous and endogenous amines.¹ Since MAOs are involved in the catabolic transformation of several neurotransmitters, including serotonin, histamine, and catecholamines, they have been considered attractive therapeutic targets in neuropharmacology.² Several decades after the seminal discovery of the antidepressant action of MAOs inhibitors, recombinant DNA technology has allowed the isolation, purification, and structural characterization of two distinct enzymatic isoforms, named MAO-A and MAO-B. Although exerting the same biological function and showing nearly 70% homology, the two isoenzymes differ in their threedimensional structure, substrate specificity, and sensitivity to inhibitors. MAO-A predominates in catecholaminergic neurons, deaminates preferentially serotonin, and is selectively inhibited by clorgyline, whereas the MAO-B isoform prevails in serotonergic neurons, metabolizes 2-phenylethylamine (PEA)

preferentially, and is blocked by low concentrations of selegiline.³ Dopamine, adrenaline, and noradrenaline are nonselectively degraded by both isoenzymes.⁴ The two isoforms are unevenly present in most mammalian cell types but erythrocytes,⁵ with the proportion and distribution varying from species to species. The different density and distribution in human brain regions have been mapped through positron emission tomography (PET) studies.⁶ The highest enzymatic activity has been found in hypothalamus and basal ganglia (striatum), where the prevalent isoform is MAO-B (80% of total human brain MAO). To a major extent, the mood-elevating effect of MAOs inhibitors is mediated by the elevation of serotonin level, as a consequence of the inhibition of MAO-A in glial cells.³

The X-ray crystallographic resolution of the enzymeinhibitors complexes has elucidated the three-dimensional structures of both MAO isoforms and delineated the binding

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mode of reversible and irreversible inhibitors.⁷⁻¹⁰ The main structural differences of MAOs have been found in their active sites because of the diverse shape and volume of the substrates/ inhibitors binding pockets. In hMAO-A there is a single hydrophobic cavity of nearly 550 Å³ volume in humans (450 Å³ in the rMAO-A). By contrast, the active site of hMAO-B is characterized by a smaller entrance cavity (290 Å³ volume) and a wider substrate cavity of 420 Å³ volume.^{10,11} In both isoenzymes these cavities are narrow pockets lined with hydrophobic residues, with the flavin ring of FAD at the distal end. The replacement of Ile199 and Tyr326 residues in hMAO-B (correspondingly, Phe208 and Ile335 residues in hMAO-A), which can be observed within the region that separates the two cavities, represents a key structural feature for isoform-selective molecular recognition.¹⁰ In addition, Ile199 acts as a flexible "gating switch", changing conformation according to the bound ligand and moving from an open to a closed arrangement with the protein backbone.¹² On this basis, selective inhibitors have been designed by exploiting the structural differences of the "gate-keeper" residues and of the shape of MAO-A substratebinding site that looks less elongated and not as flat as the

MAO-B substrate cavity. Since the 1950s, MAOs have attracted the attention of medicinal chemists. Iproniazid (Chart 1), initially conceived as antituberculosis drug, was the first MAO-I developed as an antidepressive agent. The first generation of MAO-Is (e.g., tranylcypromine, Chart 1) was represented by irreversible and nonselective compounds, whose severe side effects (e.g., hypertensive crisis triggered by the so-called "cheese effect" 13 and the hepatotoxicity of hydrazine-containing compounds^{14,15}) hampered their further development. Later, a second generation of MAO-Is came into the market. They were selective but still irreversible inhibitors, typically bearing a propargylamine moiety (e.g., clorgyline and selegiline, Chart 1). The last generation MAO-Is were selective and reversible, with potentially lower adverse effects. Currently, reversible and selective MAO-AIs (RIMA) represent the third/fourth line treatment of depressive disorders¹⁶ (e.g., moclobemide, Chart 1), whereas some irreversible and selective MAO-BIs are clinically used in the therapy of Parkinson's disease (PD) as monotherapy or as L-DOPA adjuvants (e.g., selegiline and rasagiline).

More recently, MAO-Is have been re-evaluated as potential therapeutics against many age-related pathologies,¹⁸ for which neurotoxicity, protein misfolding and/or aggregation, iron accumulation, mitochondrial damage, and oxidative stress have been described as major downstream causes, e.g., in Alzheimer's disease (AD), PD, amyotrophic lateral sclerosis (ALS, Lou Gehrig's disease), and Huntington's disease (HD). Several studies reported an increase of MAO-B activity in the brain and blood platelets of patients suffering from PD and AD.^{19,20} Inhibition of MAOs has the potential of modulating the level of key neurotransmitters involved in the pathogenesis or triggering the cognitive decline and motor disabilities of some neurodegenerative disorders (NDs).²¹ Furthermore, toxic radicals are produced by the MAO catalytic cycle itself, leading to hydrogen peroxide that works as a harmful precursor of reactive oxygen species (ROS).²² These mechanisms underlie the considerable interest toward MAOs in the search of novel therapeutic options against oxidative stress-related pathologies.^{23,24} The inhibition of MAOs can reduce ROS production directly or indirectly by limiting the Fenton reaction.²

Article

Aiming at the discovery of novel and effective diseasemodifying agents to cope with NDs, the attention of many researchers has been focused on MAO-Is by following two main research lines. One consists of the study of already known inhibitors (e.g., rasagiline, Chart 1) and of looking for additional neuroprotective properties.^{26,27} Another elegant, as well-promising, strategy is the design of multitarget directed ligands (MTDLs) showing MAO inhibition as the core activity²⁸ (e.g., ladostigil, Chart 1).^{29,30} To date, the lack of a disease-modifying therapy for NDs and other severe neurological illnesses linked to aging, as well as the safety issues associated with currently available antidepressive and anti-PD agents, stresses the urgency for novel, efficient, and less toxic MAO-Is.^{3,16,31,32}

Over the years, large numbers of heterocyclic scaffolds have been exploited to design inhibitors targeting MAOs.^{33–39} Among them, nature-inspired oxygen heterocycles have occupied a prominent role.^{40–45} In the past decade, our research group has been successfully involved in the discovery and optimization of novel MAO inhibitors (i.e., indeno-fused azines^{46,47} and coumarins⁴⁸), drawing helpful structure–activity and structure–selectivity relationships (SARs and SSRs).^{49,50} The introduction of small basic moieties at position 4 of the Scheme 1. General Procedure for the Synthesis of 2H-1-Benzofuran-6-yl Derivatives 20–38, 43, and Benzofurans 39, 40, and 44^a



^{*a*}Reagents and conditions. (a) For $Y = SO_2$: triethylamine, R_2SO_2Cl , dry acetonitrile, room temperature, 18 h. For $Y = CH_2$: Cs_2CO_3 , R_2CH_2Cl , dry *N*,*N*-dimethylformamide, room temperature, 24 h. (b) For **20–36**: suitable 4-chloromethylcoumarin **3–19**, methylamine 2.0 N in THF, dry THF, room temperature, 18 h. For **38**: **4**, isopropylamine, dry THF, room temperature, 24 h. For **43**: **42**, methylamine 2.0 N in THF, dry THF, room temperature, 3 h. (c) For **39–40**: **21** (for **39**) or **37** (for **40**), DBU, dry THF, room temperature, 24 h. For **44**: **43**, DBU, dry THF, reflux, 8 h.

Scheme 2. Hypothetic Reaction Mechanism for the Reaction of 7-Benzyloxy- and 7-Sulfonyloxy-4-chloromethylcoumarins with Primary Amines in THF



coumarin ring has led to the discovery of potent and selective MAO-B inhibitors (e.g., NW-1772, Chart 1) with an outstanding biopharmacological profile.^{51,52}

Using the same design strategy, we tried to improve the pharmacokinetic properties of diversely substituted 7-sulfonyloxycoumarins, previously reported as potent, selective, but highly lipophilic MAO-A inhibitors.⁵⁰ While working on the synthesis of the designed molecules, we faced an unexpected side reaction and discovered serendipitously a novel and effective scaffold for the inhibition of MAOs, that is (E)-2-(benzofuran-3(2H)-ylidene)-N-methylacetamide. Depending on the substituents placed at positions 6' and 3' of the benzofuran backbone, potent and extremely selective MAO-A or, to a lesser extent, MAO-B inhibitors were obtained, with IC₅₀ values in the nanomolar range. Herein, we report the synthesis, structural assignment, and biological evaluation as MAO-Is of a novel series of 6'-substituted-(E)-2-[benzofuran-3(2H)-ylidene]-N-alkylacetamides, along with a modeling study aimed at highlighting key molecular determinants for high MAO affinity. In addition, more flexible 2-(1-benzofuran-3-yl)-N-methylacetamide isomers and 4-N-methylcarboxamidomethylcoumarin analogues were prepared to extend our understanding of the SARs and SSRs.

CHEMISTRY

The preparation of starting 4-chloromethyl-7-hydroxycoumarins 1^{51} and **2** was carried out by the well-known von Pechmann cyclocondensation reaction with slight modifications.⁵³ Compounds 1 and 2 were reacted with the suitable (hetero)arylsulfonyl chloride or alkylsulfonyl chloride and subsequently with appropriate amines. In place of the expected nucleophilic substitution reaction, the coupling between 4-chloromethyl derivatives 3-19 and primary amines yielded compounds 20-38 (Scheme 1) as the unique products, deriving from the opening reaction of the lactonic ring followed by a cyclization reaction through the intramolecular nucleophilic attack of the phenoxide anion on the chloromethyl group (Scheme 2). The exocyclic double bond always exhibited an *E*-geometry. A similar reaction was previously observed by others but under more drastic conditions, and it led to benzofuran-3-acetic acids.⁵⁴

When the same reaction was performed on 7-(3'chlorobenzyloxy)-4-chloromethylcoumarin **42**, the nucleophilic substitution product, which is the already known MAO-BI NW-1772,⁵¹ was isolated along with a side product (**43**) as described for compounds **20–38** (Scheme 1). The different reaction outcome can be attributed to the different electronic effect of the substituent at position 7 of the coumarin ring. In fact, this group modulates the electrophilic reactivity of the coumarin lactonic carbonyl. To assess the stereochemistry of the exocyclic double bond of 6'-substituted-(*E*)-2-(benzofuran-3(2*H*)-ylidene)-*N*-alkylacetamides, NOESY NMR experiments were performed (see Supporting Information). A strong NOE effect between the exocyclic vinylic proton and the amide

Scheme 3. Synthesis of Amidocoumarin Derivatives 49 and 50^a



^aReagents and conditions: (a) 2.0 N CH_3NH_2 in THF, 90 °C, sealed vessel, 96 h; (b) triethylamine, 3-chlorobenzenesulfonyl chloride, dry acetonitrile, room temperature, 5–24 h.

Scheme 4. Synthesis of 4-Aminomethylcoumarin Derivatives 52 and 53^a



"Reagents and conditions: (a) *N*-(diphenylmethyl)methylamine, anhydrous K₂CO₃, TBAI (cat.), dry THF, reflux, 7 h; (b) TES, TFA, reflux, 4 h; (c) (i) hexamethylenetetramine, dry chloroform, reflux, 48 h; (ii) HCl 6% in ethanol, reflux, 4 h.

proton as well as with the endocyclic methylene along with the absence of spatial coupling signal between the vinylic proton and H4 of benzofuran ring fully confirmed the *E*-geometry. By treatment of derivatives **21**, **37**, and **43** with a strong non-nucleophilic base such as 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), the aromatization to the corresponding less rigid benzofuran-3-acetamides **39**, **40**, and **44** was accomplished in high yields (Scheme 1). Compound **41** was prepared by reacting the commercially available 6-hydroxy-1-benzofuran-3(2H)-one with 3-chlorobenzenesulfonyl chloride as detailed in Experimental Section.

In order to extend the SAR study of these newly discovered scaffolds, a small number of amido- and aminocoumarin congeners were also synthesized. The synthetic route to amido derivatives **49** and **50** started from the condensation of resorcinol with diethyl 1,3-acetonedicarboxylate or diethyl oxalacetate sodium salt to obtain intermediates **45** and **46**, respectively. These esters underwent aminolysis with methylamine in a sealed vessel before final sulfonylation to yield compounds **49** and **50** (Scheme 3). To synthesize compound **52**, a protected form of methylamine was required to circumvent the lactone opening reaction. To this purpose, *N*-(diphenylmethyl)methylamine was chosen as the starting material, being a hindered secondary amine more amenable to nucleophilic substitution rather than to lactonic ring-

opening. The reductive cleavage of benzydryl group with trifluoroacetic acid (TFA) in the presence of triethylsilane (TES) as radical scavenger furnished the desired amine (Scheme 4). Primary amine **53** was prepared through a Delepine reaction⁵⁵ by hydrolyzing urotropine salt of chloride **4** under acidic conditions (Scheme 4).

ENZYME INHIBITION ASSAYS

MAO inhibition data of compounds 20-41, 43, 44, 49, 50, 52, and 53 are reported in Tables 1 and 2. The inhibition of monoamine oxidases A and B activity was measured in vitro through a spectrophotometric method,⁵⁶ by using crude rat brain mitochondrial suspensions as previously reported.⁵⁰

RESULTS AND DISCUSSION

Nineteen 6'-sulfonyloxy-(E)-2-(benzofuran-3(2*H*)-ylidene)-*N*-alkylacetamides (**20**–**38**) were designed, synthesized, and tested as MAO-Is. Most of them showed MAO-A inhibition values in the nanomolar range and a clear-cut selectivity over MAO-B (Table 1). To gain deeper insights into the SARs, focused structural modifications were introduced on the benzofuran ring at position 6 (sulfonate group) and at position 3 (amide *N*-substituent). In addition, the effect of a methyl group at position 7 was studied in a few compounds.

Table 1. Biological Data of 2*H*-1-Benzofuran-6-yl Derivatives 20–38 and 43, Benzofurans 39, 40, and 44, and Benzofuran-3-one 41



41

20-40, 43-44

compd	Y	R ₁	R ₂	R ₃	double bond ^a	MAO-A ^b	MAO-B ^b
20	SO ₂	Н	phenyl	Me	exo	34 ± 3.9	0%
21	SO_2	Н	3'-chlorophenyl	Me	exo	41 ± 3.3	0%
22	SO_2	Н	3'-fluorophenyl	Me	exo	41 ± 3.2	$6 \pm 0.5\%^{c}$
23	SO ₂	Н	3'-cyanophenyl	Me	exo	19 ± 2.5	0%
24	SO ₂	Н	4'-chlorophenyl	Me	exo	12 ± 2.6	6%
25	SO ₂	Н	4'-cyanophenyl	Me	exo	45 ± 3.5	$10 \pm 2.1\%^{c}$
26	SO ₂	Н	4'-nitrophenyl	Me	exo	37 ± 2.9	13%
27	SO ₂	Н	4'-acetylphenyl	Me	exo	29 ± 3.2	0%
28	SO ₂	Н	4'-methylsulfonylphenyl	Me	exo	19 ± 3.1	0%
30	SO ₂	Н	5'-chlorothien-2'-yl	Me	exo	16 ± 3.1	$6 \pm 1.4\%$
31	SO ₂	Me	phenyl	Me	exo	9.8 ± 1.4	$5 \pm 0.5\%$
32	SO ₂	Me	3'-chlorophenyl	Me	exo	24 ± 4.7	$2 \pm 0.5\%$
33	SO ₂	Me	4'-cyanophenyl	Me	exo	15 ± 2.7	$6 \pm 0.5\%$
29	SO ₂	Н	4'-methoxyphenyl	Me	exo	11 ± 2.2	0%
34	SO ₂	Me	4'-methoxyphenyl	Me	exo	7.0 ± 2.1	0%
35	SO ₂	Н	3′,3′,3′-trifluoropropyl	Me	exo	9.1 ± 0.9	$17 \pm 2.4\%$
36	SO ₂	Me	3',3',3'-trifluoropropyl	Me	exo	11 ± 2.8	$5 \pm 0.8\%$
37	SO_2	Н	3'-chlorophenyl	Et	exo	4200 ± 1100	0%
38	SO ₂	Н	3'-chlorophenyl	ⁱ Pr	exo	$14 \pm 2.3\%$	$1 \pm 0.3\%$
39	SO ₂	Н	3'-chlorophenyl	Me	endo	409 ± 8.2	$4 \pm 0.7\%$
40	SO ₂	Н	3'-chlorophenyl	Et	endo	$31 \pm 2.0\%$	0%
41 ^d	SO ₂		3'-chlorophenyl			$17 \pm 2.6\%$	0%
43	CH_2	Н	3'-chlorophenyl	Me	exo	427 ± 5.9	36 ± 2.0
44	CH_2	Н	3'-chlorophenyl	Me	endo	3100 ± 800	284 ± 7.4
MOC ^e						10000 ± 2400	0%

^{*a*}Exocylic (exo) or endocyclic (endo) double bond position. ^{*b*}MAO-A and MAO-B inhibitory activities are expressed as $IC_{50} \pm SD$ (nM), unless otherwise expressed as percentage of inhibition $\pm SD$, measured at 10 μ M. Values are the mean of two or three independent experiments. ^{*c*}Values are expressed as percentage of inhibition $\pm SD$ measured at 20 μ M by mean of two or three independent experiments. ^{*d*}Exact structure in table heading. ^{*e*}MOC = moclobemide.

Table 2. Biological Data of Coumarin Congeners 49, 50, 52, and 53

	Cl	0, s, 0 , s, 0	R X ^{NH}	
compd	Х	R	MAO-A ^a	MAO-B ^a
49	-CH ₂ CO-	Me	111 ± 8.3	8300 ± 2100
50	-CO-	Me	$8.0 \pm 0.9\%$	$9.0 \pm 1.7\%$
52	-CH2-	Me	5300 ± 800	1700 ± 500
53	-CH2-	Н	997 ± 14	3300 ± 500

^{*a*}MAO-A and MAO-B inhibitory activities are expressed as IC₅₀ \pm SD (nM) unless otherwise expressed as percentage of inhibition \pm SD, measured at 10 μ M. Values are the mean of two or three independent experiments.

The modulation of MAO-A inhibitory activity of N-methyl substituted amides was pursued by introducing a series of

substituents with varying lipophilic, electronic, and steric properties on the phenyl ring of the sulfonate group at position 6 (derivatives 20-29, Table 1). The MAO-A inhibitory potencies and MAO-A over MAO-B selectivities of compounds 21, 22, 25, 26, and 27 remained close to those observed for the unsubstituted compound 20, which showed $IC_{50} = 34$ nM at MAO-A and no MAO-B inhibition at 10 μ M. Interestingly, 4'methoxy- (29), 4'-chloro- (24), and to a lesser extent 3'-cyano-(23) and 4'-methylsulfonyl-benzenesulfonate (28) derivatives exhibited a slightly higher MAO-A activity than 20. The different stereoelectronic and lipophilic properties of these substituents did not allow the derivation of significant insights on the SARs and SSRs. As a further structural variation, the 3'chlorophenyl group of 21 was replaced with the isosteric 5'chlorothien-2'-yl group, affording compound 30 with a significantly improved MAO-A affinity (IC₅₀ = 16 nM vs 41) nM) and a high MAO-A selectivity.

The introduction of a methyl group at position 7 was conceived keeping in mind the remarkable change of affinity against both MAO isoforms resulting from the introduction of a methyl substituent at position 8 of 4,7-disubstituted coumarin



Figure 1. Key pharmacophoric elements for MAO-A inhibition.

derivatives.³⁰ The working idea was rooted on the fact that position 7 of benzofurans might be considered topologically equivalent to position 8 of coumarins. Even though the change of MAO-A affinity was not very pronounced, all of the 7methyl-substituted derivatives proved to be more active than the corresponding 7-unsubstituted analogues (compare 32 vs 21, 33 vs 25, and 34 vs 29, Table 1). This structural modification led to the most active compound of the series (34, $IC_{50} = 7.0$ nM), bearing a 4'-methoxybenzenesulfonate moiety. This MAO-AI was nearly 1000-fold more potent than moclobemide (IC₅₀ = 10 μ M), which represents one of the most popular RIMA used in the therapy of depression. The positive effect of the methyl group at position 7 was not confirmed by derivative 36 that was slightly less active than its demethylated analogue 35 (IC₅₀ = 11 nM and IC₅₀ = 9.1 nM, respectively), endowed with a flexible fluoroalkylsulfonate group. Compounds 35 and 36, designed as safer and less metabolically labile analogues of arylsulfonates, exhibited outstanding potencies and an excellent MAO-A selectivity. The homologation of the amide methyl group to ethyl causes a dramatic drop (~100-fold) of MAO-A affinity (37 vs 21). A further increased steric hindrance in the isopropyl homologue 38 led to the complete loss of affinity to both MAO isoforms.

The biological evaluation of MAO inhibition was extended to the benzofuran isomers **39** and **40** to address the importance of a rigid geometry, allowed by the *E*-configuration of the exocyclic double bond, for a regiospecific interaction at the MAO-A binding site. Indeed, the increased flexibility of the amide lateral chain at position 3 resulted in a consistent drop of MAO-A affinity (**39** vs **21**, **40** vs **37**), proving the importance of the rigid "folded" conformation determined by the *E*-double bond for a high inhibitory potency. Like their precursors **21** and **37**, both benzofuran isomers **39** and **40** were not able to inhibit MAO-B.

A comparison between compound **21** and its benzofuran-3one analogue **41** (Table 1), lacking the exocyclic α , β unsaturated amide moiety and showing a very poor (as for MAO-A) or totally absent (as for MAO-B) inhibitory activity, highlighted the importance of the rigid 3-propenamide chain for a strong binding interaction at the enzymatic active site.

To further validate this novel scaffold, a small series of congeners of compound **21** was designed by replacing the benzofuran bicyclic core of derivatives 20-38 with a coumarin nucleus and by introducing small nitrogen-containing groups (i.e., amine and amide groups) at position 4 while maintaining a *m*-chlorobenzenesulfonate ester moiety at position 6. In our

design strategy, compound 21 was chosen as the reference compound because of its closer similarity with previously reported potent coumarin MAO inhibitors^{50,51} that bear a mchlorophenyl ring as substituent at position 7. 4-N-Methylcarboxamidomethylcoumarin 49 showed lower MAO-A inhibitory potency (IC₅₀ = 111 nM, Table 2) than its 2,3dihydrobenzofuran analogue 21 but greater than its benzofuran analogue 39 (IC₅₀ = 34 nM and IC₅₀ = 409 nM, respectively), and consequently, its selectivity ratio over MAO-B was much lower. Conversely, the less flexible amido derivative 50 was almost inactive on both isoenzymes when tested at 10 μ M. The insertion of a basic, protonatable group at position 4 of the coumarin core led to moderate MAO inhibitors 52 and 53 exhibiting micromolar affinities toward MAO-A and MAO-B and poor selectivity (Table 2). Interestingly, compound 52 showed a reversed, albeit low, selectivity, being more active on MAO-B. Biological data found for the benzyloxy derivative 43 (IC₅₀ = 427 nM and IC₅₀ = 36 nM for MAO-A and MAO-B, respectively, Table 1) confirm our previous findings concerning the key role of the substituent at position 7 in modulating the isoform selectivity for coumarin derivatives.⁵⁰ A comparison between inhibitor 43 and the corresponding benzofuran 44 points out that the exocyclic double bond is preferred for a high MAO-B binding affinity in this series, too.

The *E*-geometry of the double bond freezes the compound in a "folded" molecular shape, which is essential for an optimal fitting into the MAOs substrate cavity. The unusual deshielding of the benzofuran H4′ in 2,3-dihydrobenzofuran-3-yl compounds ranging from 8.68 to 8.97 ppm can be attributed to the strong anisotropic effect of the amide carbonyl that points toward H4′ of the aromatic ring. As a matter of fact, the same proton was found at 7.50 ppm in an open chain *trans*-cinnamic acid derivative (see Supporting Information).

The SARs discussed above for these novel, potent, and selective sulfonate MAO-A inhibitors allowed the identification of the key structural requirements for an efficient MAO-A binding, which are summarized in a pictorial representation in Figure 1. The nature and size of the bridge linking the aromatic or alkyl moiety to position 6 or 7 of the benzofuran and coumarin rings, respectively, influenced mainly selectivity and represented a crucial pharmacophoric feature along with the rigid and folded *E*-geometry of the propenamide moiety, which allowed an optimal spatial orientation of the molecule for productive binding interactions within the MAO active site and was essential for a high enzymatic affinity. The substituents on the amide nitrogen impact a sterically sensitive enzymatic

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region. In fact, groups larger than methyl drastically reduced the MAO-A inhibitory potency. Stringent lipophilic, electronic, and steric requirements are absent in the arylsulfonyloxy group, where electron-withdrawing and electron-donating substituents in para as well as in meta position did not produce any relevant shifts of affinity. On this basis, this region can be properly modified in order to improve the pharmacokinetic properties of this class of inhibitors.

Molecular Modeling Study. A molecular modeling study was carried out to shed light on peculiar binding interactions modulating MAO-A affinity, with the aim of explaining at least the dramatic drop of affinity resulting from the introduction of large-sized *N*-amide substituent at position 3 (i.e., changing the *N*-substituent of the 3-propenamide group from methyl to isopropyl, that is, from **21** to **38**) and supporting the relevance of maintaining an exocyclic double bond (i.e., a propenamide moiety at position 3) for high MAO-A inhibitory potency (compare **21** vs **39**).

Since inhibition data were determined on mitochondria MAOs from rat brain homogenates, docking simulations were run on a rat model of the MAO-A isoform. The 3D structure for rMAO-A was retrieved from the PDB (coded as 105W). As reported in our previous papers,^{50,51} a number of structural water molecules were explicitly considered in the docking simulations carried out using GOLD. Among those available in GOLD, the ChemScore scoring function proved to better rank the binding affinity of ligands in the simulated enzyme—inhibitor complexes.⁵⁷ As a result, a good agreement was observed between experimental inhibitory activities and docking scoring values.

The first purpose of the docking study was that of explaining the dramatic fall of MAO-A activity originated by increasing the size of the N-substituent on the 3-propenamide group (compare 21-NMe with 37-NEt and 38-NⁱPr derivatives). As shown in Figure 2a,b, docking simulations showed that the posing of 21 and 38 returned a similar binding mode with the benzofuran moiety facing the region beneath the FAD and the 3'-chlorophenyl moiety placed in the cavity entrance, lined by residues having a more pronounced hydrophobic character. This binding topology was actually observed in all the poses occurring during GOLD simulation. However, N-substituents bulkier than methyl were less tolerated, presumably because of a high chance of steric clashes with structural water molecules in a protein region characterized by a number of tyrosine residues. Very satisfactorily, docking simulations disclosed that moving from methyl to isopropyl (21 vs 38) lowered the score from 21.37 to 12.61 kJ/mol. In addition, the steric hindrance of the bulkier isopropyl group could hinder the formation of the hydrogen bond (HB) between the 3-propenamide carbonyl and the Tyr444 hydroxyl along with other reinforcing interactions mediated by two surrounding structural water molecules (Figure 2b).

The second goal of the docking investigation was that of elucidating the key role of the exocyclic double bond to strengthen the binding interactions with MAO-A. Indeed a significant drop of inhibitory activity was observed going from an exocyclic to an endocyclic double bond in tautomeric compounds belonging to both the 6'-sulfonyloxy and 6'benzyloxy classes (compare 21 vs 39 and 43 vs 44, respectively). However, docking simulations were limited to inhibitors 21 and 39 for which posing and scoring were compared. As can be seen in Figure 2c, derivative 39, bearing the endocyclic double bond, adopted a reversed binding mode



Figure 2. Docking pose of inhibitor 21 (a), 38 (b), and 39 (c) into rMAO-A binding site. Relevant amino acid residues, FAD cofactor, and inhibitors are represented as stick models colored according to the following atom code: C atoms in white, yellow, and green for amino acid residues, cofactor, and inhibitor, respectively. Red dashed lines indicate the HB network established by the ligand with protein residues and structural water molecules. The protein structure is rendered as a white cartoon model.

with the 3'-chlorophenyl moiety facing the FAD while the benzofuran ring occupied the cavity entrance surrounded by a number of phenylalanine residues. The reversed binding topology shown by **39** was, to some extent, not completely unexpected, as it was already observed in recent investigations for some 7-substituted coumarin derivatives.^{50,51} As far as inhibitor **39** is concerned, the observed binding topology was likely assumed to ensure a larger room for the 3-carboxamidomethyl group which is much more flexible than the *E*-propenamide moiety of **21**. The reversed posing of **39** was characterized by a lower score compared to **21** (i.e., 13.55

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kJ/mol vs 21.37 kJ/mol) in agreement with the observed inhibitory potency. Unlike **21**, the HB network involved the 6'sulfonyloxy group of the bridge joining the two aromatic moieties with the effect of moving the 3'-chlorophenyl ring into a less hydrophobic region mostly formed by structural water molecules and tyrosine residues. On the other hand, the amide NH group of **39** established an HB with the carbonyl backbone of Phe208 in the entrance cavity of the enzyme.

CONCLUSION

During the course of our investigation aimed at enhancing the pharmacokinetic features of already reported MAO-Is,⁵ an unexpected lactone opening reaction of 7-substituted coumarins with primary amines was observed. Serendipitously, this reaction constituted a straightforward and very efficient synthetic method for a scaffold hopping to 6'-substituted (E)-2-(benzofuran-3(2H)-ylidene)-N-methylacetamides as a novel class of MAO-Is. The nature and the size of the 6'-substituents modulate MAO affinity and isoform selectivity. In particular, in this report we described several (hetero)aryl sulfonate esters showing outstanding affinity (IC₅₀ in the nanomolar range) and selectivity toward MAO-A (approximately, selectivity index of \gg 500), comparable to that of esuprone (7-hydroxy-3,4dimethylcoumarin ethanesulfonate),⁵⁸ taken as the reference coumarin compound, and much greater than moclobemide, which is a prototype reversible MAO-A inhibitor and one of the most widely used antidepressive agents.

Structural modifications mainly addressing the 6-arylsulfonate group and the N-amide substituents at position 3 enabled the detection of the crucial pharmacophoric elements modulating MAO-A inhibitory potency, fully supported by a docking study. The high MAO-A selectivity of the arylsulfonates could be ascribed to the folded molecular shape, better accommodated in MAO-A substrate-binding site, which is wider and less flat than MAO-B. The obtainment of MAO-Atargeting inhibitors devoid of dietary restrictions and toxic side effects is still a desirable goal in the therapy of depressive disorders. Remarkably, the substitution of the chemically and metabolically sensitive, and even toxic, arylsulfonate moiety with a fluoroalkylsulfonate group, afforded compounds 35 and 36 with a potentially safer pharmacological and toxicological profile. The outstanding MAO-A inhibitory potency and MAO-A over MAO-B selectivity of 35 and 36 should warrant further preclinical investigation to exploit their potential as antidepressive agents.

Moreover, the new class of selective MAO-B inhibitors (such as compound 43) reported herein would also deserve further attention for their potential use in the treatment of behaviorand age-related disorders associated with MAO activity and/or with MAO byproducts toxicity.

A hit-to-lead development process to improve chemical and metabolic stability of these novel scaffolds is ongoing and will be reported in due time.

EXPERIMENTAL SECTION

Chemistry. Starting materials, reagents, and analytical grade solvents were purchased from Sigma-Aldrich (Europe). The purity of all the intermediates, checked by ¹H NMR and HPLC, was always better than 95%. Flash chromatographic separations were performed on a Biotage SP1 purification system using flash cartridges prepacked with KP-Sil 32–63 μ m, 60 Å silica. All reactions were routinely checked by TLC using Merck Kieselgel 60 F₂₅₄ aluminum plates and visualized by UV light or iodine. Regarding the reactions requiring the

use of dry solvents, the glassware was flame-dried and then cooled under a stream of dry argon before use. Elemental analyses were performed on the EuroEA 3000 analyzer only on the final compounds tested as MAOs inhibitors and are reported in Supporting Information. The measured values for C, H, and N agreed to within $\pm 0.40\%$ of the theoretical values. Nuclear magnetic resonance spectra were recorded at 300 MHz on a Varian Mercury 300 instrument at ambient temperature in the specified deuterated solvent. Chemical shifts (δ) are quoted in parts per million (ppm) and are referenced to the residual solvent peak. The coupling constants *J* are given in hertz (Hz). The following abbreviations were used: s (singlet), d (doublet), dd (doublet of doublet), dq (doublet of quadruplet), dt (doublet of triplet), t (triplet), q (quadruplet), m (multiplet), br (broad signal). Signals due to OH and NH protons were located by deuterium exchange with D₂O.

Synthesis of 4-(Chloromethyl)-7-hydroxy-8-methyl-2*H*-chromen-2-one (2). A slight modification of a reported procedure was followed.⁵¹ 2-Methylresorcinol (4.1 g, 33 mmol) was dissolved in concentrated H₂SO₄ (40 mL) while cooling at 0 °C, and ethyl 4-chloroacetoacetate (4.1 mL, 30 mmol) was slowly added in 15 min via syringe. The mixture was kept at room temperature under magnetic stirring for 24 h and then poured onto crushed ice (600 g) and filtered. The resulting solid was crystallized from methanol/water. Yield: 62%. ¹H NMR (300 MHz,DMSO-*d*₆) δ : 2.14 (s, 3H), 4.93 (s, 2H), 6.40 (s, 1H), 6.87 (d, *J* = 8.8 Hz, 1H), 7.52 (d, *J* = 8.8 Hz, 1H), 10.54 (s, 1H, disappears with D₃O).

General Procedure for the Synthesis of Intermediates 3–19. The suitable 7-hydroxycoumarin derivative 1^{51} (50 mmol) or 2 (10 mmol) was suspended in anhydrous acetonitrile (200 mL in the case of 1; 40 mL in the case of 2) followed by the addition of triethylamine (7.7 mL, 55 mmol in the case of 1; 1.5 mL, 11 mmol in the case of 2). After complete dissolution of the starting coumarin derivatives, the suitable sulfonyl chloride (50 mmol in the case of 1, 10 mmol in the case of 2) was added. The mixture was stirred at room temperature for 18 h. After removal of the solvent under vacuum, the crude solid was purified by flash chromatography (gradient eluent, different mixtures of ethyl acetate in *n*-hexane).

4-(Chloromethyl)-2-oxo-2*H***-chromen-7-yl Benzenesulfonate** (3). 3 was purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane 0% \rightarrow 40%). Yield: 90%. ¹H NMR (300 MHz, DMSO-*d*₆) δ : 4.98 (s, 2H), 6.68 (s, 1H), 7.12 (dd, *J*₁ = 2.2 Hz, *J*₂ = 8.8 Hz, 1H), 7.17 (d, *J* = 2.2 Hz, 1H), 7.66–7.71 (m, 2H), 7.82–7.87 (m, 2H), 7.91–7.94 (m, 2H).

4-(Chloromethyl)-2-oxo-2*H***-chromen-7-yl 3-Chlorobenzenesulfonate (4). 4** was purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane 0% → 50%). Yield: 86%. ¹H NMR (300 MHz, DMSO-*d*₆) δ: 4.99 (s, 2H), 6.69 (s, 1H), 7.17 (dd, *J*₁ = 2.2 Hz, *J*₂ = 8.8 Hz, 1H), 7.28 (d, *J* = 2.2 Hz, 1H), 7.68–7.73 (m, 1H), 7.88 (d, *J* = 8.8 Hz, 1H), 7.91–7.94 (m, 2H), 8.00–8.01 (m, 1H).

4-(Chloromethyl)-2-oxo-2*H***-chromen-7-yl 3-Fluorobenzenesulfonate (5).** 5 was purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane 0% → 40%). Yield: 94%. ¹H NMR (300 MHz, DMSO-*d*₆) δ: 4.99 (s, 2H), 6.69 (s, 1H), 7.16 (dd, *J*₁ = 2.5 Hz, *J*₂ = 8.8 Hz, 1H), 7.27 (d, *J* = 2.5 Hz, 1H), 7.71–7.78 (m, 3H), 7.83–7.87 (m, 1H), 7.87 (d, *J* = 8.8 Hz, 1H).

4-(Chloromethyl)-2-oxo-2*H***-chromen-7-yl 3-Cyanobenzene**sulfonate (6). 6 was purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane 0% → 30%). Yield: 88%. ¹H NMR (300 MHz, DMSO- d_6) δ : 5.00 (s, 2H), 6.69 (s, 1H), 7.15 (dd, $J_1 = 2.5$ Hz, $J_2 = 8.8$ Hz, 1H), 7.26 (d, J = 2.5 Hz, 1H), 7.85 (t, J = 8.0 Hz, 1H), 7.86 (d, J = 8.8 Hz, 1H), 8.16 (dq, $J_1 = 1.4$ Hz, $J_2 = 8.3$ Hz, 1H), 8.29 (dt, $J_1 = 1.4$ Hz, $J_2 = 8.0$ Hz, 1H), 8.46 (t, J = 1.4 Hz, 1H).

4-(Chloromethyl)-2-oxo-2*H***-chromen-7-yl 4-Chlorobenzene-sulfonate (7).** 7 was purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane 0% → 30%). Yield: 91%. ¹H NMR (300 MHz, DMSO- d_6) δ: 4.99 (s, 2H), 6.69 (s, 1H), 7.14 (dd, J_1 = 2.5 Hz, J_2 = 8.8 Hz, 1H), 7.25 (d, J = 2.5 Hz, 1H), 7.73–7.78 (m, 2H), 7.87 (d, J = 8.8 Hz, 1H), 7.90–7.95 (m, 2H).

4-(Chloromethyl)-2-oxo-2H-chromen-7-yl 4-Cyanobenzenesulfonate (8). 8 was purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane $0\% \rightarrow 30\%$). Yield: 81%. ¹H NMR (300 MHz, DMSO- d_6) δ : 4.99 (s, 2H), 6.70 (s, 1H), 7.15 (dd, $J_1 = 2.5$ Hz, $J_2 = 8.8$ Hz, 1H), 7.29 (d, J = 2.5 Hz, 1H), 7.87 (d, J = 8.8 Hz, 1H), 8.11 (d, J = 8.8 Hz, 2H), 8.17 (d, J = 8.8 Hz, 2H).

4-(Chloromethyl)-2-oxo-2*H***-chromen-7-yl 4-Nitrobenzene-sulfonate** (9). 9 was purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane 0% → 50%). Yield: 77%. ¹H NMR (300 MHz, DMSO- d_6) δ : 4.99 (s, 2H), 6.70 (s, 1H), 7.16–7.19 (m, 1H), 7.28–7.30 (m, 1H), 7.87 (d, *J* = 8.5 Hz, 1H), 8.19 (d, *J* = 8.3 Hz, 2H), 8.45 (d, *J* = 8.3 Hz, 2H).

4-(Chloromethyl)-2-oxo-2H-chromen-7-yl 4-Acetylbenzenesulfonate (10). 10 was purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane 0% → 40%). Yield: 95%. ¹H NMR (300 MHz, DMSO-*d*₆) δ: 2.64 (s, 3H), 4.98 (s, 2H), 6.69 (s, 1H), 7.15 (dd, J_1 = 2.2 Hz, J_2 = 8.8 Hz, 1H), 7.25 (d, J = 2.2 Hz, 1H), 7.87 (d, J = 8.8 Hz, 1H), 8.07 (d, J = 8.5 Hz, 2H), 8.18 (d, J = 8.5 Hz, 2H).

4 - (Chloromethyl) - 2 - oxo - 2*H* - chromen - 7 - yl 4-(Methylsulfonyl)benzenesulfonate (11). 11 was purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane 0% → 50%). Yield: 62%. ¹H NMR (300 MHz, DMSO- d_6) δ : 3.30 (s, 3H), 4.99 (s, 2H), 6.70 (s, 1H), 7.17 (dd, J_1 = 2.5 Hz, J_2 = 8.8 Hz, 1H), 7.32 (d, J = 2.5 Hz, 1H), 7.88 (d, J = 8.8 Hz, 1H), 8.21 (s, 4H).

4-(Chloromethyl)-2-oxo-2H-chromen-7-yl 4-Methoxybenzenesulfonate (12). 12 was purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane 0% → 40%). Yield: 91%. ¹H NMR (300 MHz, DMSO-*d*₆) δ: 3.85 (s, 3H), 4.98 (s, 2H), 6.68 (s, 1H), 7.10 (dd, J_1 = 2.5 Hz, J_2 = 8.8 Hz, 1H), 7.16 (d, J = 2.5 Hz, 1H), 7.17 (d, J = 9.1 Hz, 2H), 7.83 (d, J = 9.1 Hz, 2H), 7.85 (d, J = 8.8 Hz, 1H).

4-(Chloromethyl)-2-oxo-2H-chromen-7-yl 5-Chlorothiophene-2-sulfonate (13). 13 was purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane $0\% \rightarrow 30\%$). Yield: 57%. ¹H NMR (300 MHz, DMSO- d_6) δ : 5.01 (s, 2H), 6.72 (s, 1H), 7.22 (dd, J_1 = 2.5 Hz, J_2 = 8.8 Hz, 1H), 7.33 (d, J = 2.5 Hz, 1H), 7.41 (d, J = 4.1 Hz, 1H), 7.80 (d, J = 4.1 Hz, 1H), 7.92 (d, J = 8.8 Hz, 1H).

4-(Chloromethyl)-8-methyl-2-oxo-2*H*-chromen-7-yl Benzenesulfonate (14). 14 was purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane $0\% \rightarrow 40\%$). Yield: 85%. ¹H NMR (300 MHz, DMSO- d_6) δ : 1.99 (s, 3H), 5.00 (s, 2H), 6.70 (s, 1H), 7.10 (d, *J* = 8.8 Hz, 1H), 7.68–7.76 (m, 3H), 7.85–7.93 (m, 3H).

4-(Chloromethyl)-8-methyl-2-oxo-2*H***-chromen-7-yl 3-Chlorobenzenesulfonate (15). 15** was purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane 0% → 50%). Yield: 84%. ¹H NMR (300 MHz, DMSO- d_6) δ : 2.07 (s, 3H), 5.00 (s, 2H), 6.71 (s, 1H), 7.11 (d, *J* = 9.1 Hz, 1H), 7.71–7.76 (m, 2H), 7.88–7.92 (m, 1H), 7.95–7.98 (m, 1H), 8.00–8.01 (m, 1H).

4-(Chloromethyl)-8-methyl-2-oxo-2*H*-chromen-7-yl 4-Cyanobenzenesulfonate (16). 16 was purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane $0\% \rightarrow 30\%$). Yield: 87%. ¹H NMR (300 MHz, DMSO-*d*₆) δ : 2.05 (s, 3H), 5.00 (s, 2H), 6.72 (s, 1H), 7.10 (d, *J* = 8.8 Hz, 1H), 7.74 (d, *J* = 8.8 Hz, 1H), 8.13 (d, *J* = 8.3 Hz, 2H), 8.19 (d, *J* = 8.3 Hz, 2H).

4-(Chloromethyl)-8-methyl-2-oxo-2H-chromen-7-yl 4-Methoxybenzenesulfonate (17). 17 was purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane $0\% \rightarrow 60\%$). Yield: 69%. ¹H NMR (300 MHz, acetone- d_6) δ : 2.10 (s, 3H), 3.95 (s, 3H), 4.98 (s, 2H), 6.65 (s, 1H), 7.14 (d, J = 8.8 Hz, 1H), 7.20 (d, J = 9.1Hz, 2H), 7.76 (d, J = 8.8 Hz, 1H), 7.87 (d, J = 9.1 Hz, 2H).

4-(Chloromethyl)-2-oxo-2*H***-chromen-7-yl 3,3,3-Trifluoropropane-1-sulfonate (18). 18** was purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane $0\% \rightarrow 40\%$). Yield: 71%. ¹H NMR (300 MHz, DMSO-*d*₆) δ : 2.90–3.06 (m, 2H), 3.95–4.01 (m, 2H), 5.04 (s, 2H), 6.73 (s, 1H), 7.43 (dd, $J_1 = 2.5$ Hz, $J_2 = 8.8$ Hz, 1H), 7.59 (d, J = 2.5 Hz, 1H), 7.96 (d, J = 8.8 Hz, 1H).

4-(Chloromethyl)-8-methyl-2-oxo-2*H***-chromen-7-yl 3,3,3-Trifluoropropane-1-sulfonate (19).** 19 was purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane 0% → 60%). Yield: 81%. ¹H NMR (300 MHz, acetone- d_6) δ: 2.43 (s, 3H), 2.99–3.15 (m, 2H), 3.98–4.04 (m, 2H), 5.01 (s, 2H), 6.69 (s, 1H), 7.45 (d, *J* = 8.8 Hz, 1H), 7.85 (d, *J* = 8.8 Hz, 1H).

General Procedure for the Synthesis of Final Compounds 20–38. A suitable sulfonate intermediate, 3–19 (2.0 mmol), was

suspended in dry THF (8.0 mL), and a commercially available 2.0 N solution of methylamine (for compounds 20-36) or ethylamine (for compound 37) in THF (10 mL, 20 mmol) was added. For the synthesis of compound 38 isopropylamine (1.7 mL, 20 mmol) was previously dissolved in dry THF (2.0 mL) before addition to the reaction mixture. The resulting mixture was stirred at room temperature until completion of the reaction as checked by TLC control (3–24 h). The inorganic precipitate was filtered off, and the solution was concentrated to dryness and purified by flash chromatography (gradient eluent, different mixtures of ethyl acetate in *n*-hexane).

(3*E*)-3-[2-(Methylamino)-2-oxoethylidene]-2,3-dihydro-1benzofuran-6-yl Benzenesulfonate (20). 20 was purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane 20% → 80%). Yield: 86%. ¹H NMR (300 MHz, DMSO-*d*₆) δ : 2.63 (d, *J* = 4.7 Hz, 3H), 5.22 (d, *J* = 2.5 Hz, 2H), 5.84 (t, *J* = 2.5 Hz, 1H), 6.50–6.54 (dd, *J*₁ = 2.2 Hz, *J*₂ = 8.5 Hz, 1H), 6.62 (d, *J* = 2.2 Hz, 1H), 7.64–7.69 (m, 2H), 7.78–7.89 (m, 3H), 8.05 (br s, 1H, disappears with D₂O), 8.82 (d, *J* = 8.5 Hz, 1H).

(3*E*)-3-[2-(Methylamino)-2-oxoethylidene]-2,3-dihydro-1benzofuran-6-yl 3-Chlorobenzenesulfonate (21). 21 was purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane 10% → 80%). Yield: 91%. ¹H NMR (300 MHz, DMSO- d_6) δ : 2.64 (d, *J* = 4.7 Hz, 3H), 5.23 (d, *J* = 2.5 Hz, 2H), 5.86 (t, *J* = 2.5 Hz, 1H), 6.58 (dd, J_1 = 2.2 Hz, J_2 = 8.8 Hz, 1H), 6.70 (d, *J* = 2.2 Hz, 1H), 7.66-7.71 (m, 1H), 7.82-7.85 (m, 1H), 7.89-7.95 (m, 2H), 8.04 (br q, *J* = 4.7 Hz, 1H, disappears with D₂O), 8.86 (d, *J* = 8.8 Hz, 1H).

(3*E*)-3-[2-(Methylamino)-2-oxoethylidene]-2,3-dihydro-1benzofuran-6-yl 3-Fluorobenzenesulfonate (22). 22 was purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane 10% → 80%). Yield: 93%. ¹H NMR (300 MHz, DMSO-*d*₆) δ : 2.64 (d, *J* = 4.4 Hz, 3H), 5.24 (d, *J* = 2.5 Hz, 2H), 5.86 (t, *J* = 2.5 Hz, 1H), 6.58 (dd, *J*₁ = 2.2 Hz, *J*₂ = 8.8 Hz, 1H), 6.69 (d, *J* = 2.2 Hz, 1H), 7.69–7.73 (m, 3H), 7.78–7.80 (m, 1H), 8.04 (br q, *J* = 4.4 Hz, 1H, disappears with D₂O), 8.85 (d, *J* = 8.8 Hz, 1H).

(3*E*)-3-[2-(Methylamino)-2-oxoethylidene]-2,3-dihydro-1benzofuran-6-yl 3-Cyanobenzenesulfonate (23). 23 was purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane 10% → 90%). Yield: 83%. ¹H NMR (300 MHz, DMSO-*d*₆) δ: 2.64 (d, *J* = 4.6 Hz, 3H), 5.23 (d, *J* = 2.6 Hz, 2H), 5.86 (t, *J* = 2.6 Hz, 1H), 6.60 (dd, *J*₁ = 2.2 Hz, *J*₂ = 8.5 Hz, 1H), 6.74 (d, *J* = 2.2 Hz, 1H), 7.85 (t, *J* = 8.0 Hz, 1H), 8.05 (br q, *J* = 4.6 Hz, 1H, disappears with D₂O), 8.16 (dq, *J*₁ = 1.4 Hz, *J*₂ = 8.3 Hz, 1H), 8.29 (dt, *J*₁ = 1.4 Hz, *J*₂ = 8.0 Hz, 1H), 8.46 (t, *J* = 1.4 Hz, 1H), 8.85 (d, *J* = 8.5 Hz, 1H).

(3*E*)-3-[2-(Methylamino)-2-oxoethylidene]-2,3-dihydro-1benzofuran-6-yl 4-Chlorobenzenesulfonate (24). 24 was purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane 10% → 80%). Yield: 87%. ¹H NMR (300 MHz, DMSO- d_6) δ : 2.64 (d, *J* = 4.7 Hz, 3H), 5.23 (d, *J* = 2.5 Hz, 2H), 5.85 (t, *J* = 2.5 Hz, 1H), 6.55 (dd, J_1 = 2.2 Hz, J_2 = 8.5 Hz, 1H), 6.67 (d, *J* = 2.2 Hz, 1H), 7.74 (d, *J* = 8.8 Hz, 2H), 7.88 (d, *J* = 8.8 Hz, 2H), 8.04 (br q, *J* = 4.7 Hz, 1H, disappears with D₂O), 8.85 (d, *J* = 8.5 Hz, 1H).

(3E)-3-[2-(Methylamino)-2-oxoethylidene]-2,3-dihydro-1benzofuran-6-yl 4-Cyanobenzenesulfonate (25). 25 was purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane 20% \rightarrow 90%). Yield: 98%. ¹H NMR (300 MHz, DMSO-*d*₆) δ : 2.64 (d, *J* = 4.7 Hz, 3H), 5.22 (d, *J* = 2.5 Hz, 2H), 5.86 (t, *J* = 2.5 Hz, 1H), 6.56 (dd, *J*₁ = 2.2 Hz, *J*₂ = 8.8 Hz, 1H), 6.71 (d, *J* = 2.2 Hz, 1H), 8.03–8.08 (m, 3H, 1 proton disappears with D₂O), 8.15 (d, *J* = 8.5 Hz, 2H), 8.85 (d, *J* = 8.8 Hz, 1H).

(3*E*)-3-[2-(Methylamino)-2-oxoethylidene]-2,3-dihydro-1benzofuran-6-yl 4-Nitrobenzenesulfonate (26). 26 was purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane 20% → 80%). Yield: 90%. ¹H NMR (300 MHz, DMSO- d_6) δ : 2.64 (d, *J* = 4.7 Hz, 3H), 5.24 (d, *J* = 2.5 Hz, 2H), 5.86 (t, *J* = 2.5 Hz, 1H), 6.56 (dd, J_1 = 2.2 Hz, J_2 = 8.5 Hz, 1H), 6.74 (d, *J* = 2.2 Hz, 1H), 8.04 (br q, *J* = 4.7 Hz, 1H, disappears with D₂O), 8.13–8.18 (m, 2H), 8.41–8.45 (m, 2H), 8.87 (d, *J* = 8.5 Hz, 1H).

(3*E*)-3-[2-(Methylamino)-2-oxoethylidene]-2,3-dihydro-1benzofuran-6-yl 4-Acetylbenzenesulfonate (27). 27 was purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane 20% → 90%). Yield: 97%. ¹H NMR (300 MHz, DMSO-*d*₆) δ: 2.63 (d, *J* = 4.7 Hz, 3H), 2.64 (s, 3H), 5.23 (d, *J* = 2.5 Hz, 2H), 5.85 (t, *J* = 2.5 Hz, 1H), 6.54 (dd, *J*₁ = 2.2 Hz, *J*₂ = 8.8 Hz, 1H), 6.69 (d, *J* = 2.2 Hz, 1H), 8.01-8.05 (m, 3H, 1 proton disappears with D₂O), 8.16 (d, *J* = 8.8 Hz, 2H), 8.83 (d, *J* = 8.8 Hz, 1H).

(3*E*)-3-[2-(Methylamino)-2-oxoethylidene]-2,3-dihydro-1benzofuran-6-yl 4-(Methylsulfonyl)benzenesulfonate (28). 28 was purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane 30% → 90%). Yield: 88%. ¹H NMR (300 MHz, DMSO-*d*₆) δ: 2.64 (d, *J* = 4.7 Hz, 3H), 3.32 (s, 3H), 5.24 (d, *J* = 2.5 Hz, 2H), 5.86 (t, *J* = 2.5 Hz, 1H), 6.59 (dd, *J*₁ = 2.5 Hz, *J*₂ = 8.5 Hz, 1H), 6.73 (d, *J* = 2.5 Hz, 1H), 8.05 (br q, *J* = 4.7 Hz, 1H, disappears with D₂O), 8.16 (d, *J* = 8.8 Hz, 2H), 8.20 (d, *J* = 8.8 Hz, 2H), 8.86 (d, *J* = 8.5 Hz, 1H).

(3*E*)-3-[2-(Methylamino)-2-oxoethylidene]-2,3-dihydro-1benzofuran-6-yl 4-Methoxybenzenesulfonate (29). 29 was purified by flash chromatography (gradient eluent, ethyl acetate in *n*hexane 20% → 90%). Yield: 94%. ¹H NMR (300 MHz, DMSO-*d*₆) δ : 2.63 (d, *J* = 4.7 Hz, 3H), 3.85 (s, 3H), 5.22 (d, *J* = 2.5 Hz, 2H), 5.84 (t, *J* = 2.5 Hz, 1H), 6.50 (dd, *J*₁ = 2.2 Hz, *J*₂ = 8.8 Hz, 1H), 6.62 (d, *J* = 2.2 Hz, 1H), 7.15 (d, *J* = 9.1 Hz, 2H), 7.78 (d, *J* = 9.1 Hz, 2H), 8.02 (br q, *J* = 4.7 Hz, 1H, disappears with D₂O), 8.82 (d, *J* = 8.8 Hz, 1H).

(3*E*)-3-[2-(Methylamino)-2-oxoethylidene]-2,3-dihydro-1benzofuran-6-yl 5-Chlorothiophene-2-sulfonate (30). 30 was purified by flash chromatography (gradient eluent, ethyl acetate in *n*hexane 10% → 70%). Yield: 80%. ¹H NMR (300 MHz, DMSO- d_6) δ: 2.65 (d, *J* = 4.7 Hz, 3H), 5.28 (d, *J* = 2.5 Hz, 2H), 5.87 (t, *J* = 2.5 Hz, 1H), 6.64 (dd, J_1 = 2.2 Hz, J_2 = 8.8 Hz, 1H), 6.75 (d, *J* = 2.2 Hz, 1H), 7.38 (d, *J* = 4.4 Hz, 1H), 7.75 (d, *J* = 4.4 Hz, 1H), 8.06 (br q, *J* = 4.7 Hz, 1H, disappears with D₂O), 8.90 (d, *J* = 8.8 Hz, 1H).

(3*E*)-7-Methyl-3-[2-(methylamino)-2-oxoethylidene]-2,3-dihydro-1-benzofuran-6-yl Benzenesulfonate (31). 31 was purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane 20% → 80%). Yield: 94%. ¹H NMR (300 MHz, DMSO- d_6) δ : 1.82 (s, 3H), 2.64 (d, *J* = 4.7 Hz, 3H), 5.24 (d, *J* = 2.2 Hz, 2H), 5.84 (t, *J* = 2.2 Hz, 1H), 6.43 (d, *J* = 8.8 Hz, 1H), 7.66-7.71 (m, 2H), 7.81-7.90 (m, 3H), 8.02 (br s, 1H, disappears with D₂O), 8.68 (d, *J* = 8.8 Hz, 1H).

(3*E*)-7-Methyl-3-[2-(methylamino)-2-oxoethylidene]-2,3-dihydro-1-benzofuran-6-yl 3-Chlorobenzenesulfonate (32). 32 was purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane 10% → 80%). Yield: 85%. ¹H NMR (300 MHz, DMSO-*d*₆) δ : 1.89 (s, 3H), 2.65 (d, *J* = 4.7 Hz, 3H), 5.25 (d, *J* = 2.5 Hz, 2H), 5.85 (t, *J* = 2.5 Hz, 1H), 6.51 (d, *J* = 8.8 Hz, 1H), 7.69–7.75 (m, 1H), 7.85–7.87 (m, 1H), 7.93–7.96 (m, 2H), 8.03 (br q, *J* = 4.7 Hz, 1H, disappears with D₂O), 8.71 (d, *J* = 8.8 Hz, 1H).

(3*E*)-7-Methyl-3-[2-(methylamino)-2-oxoethylidene]-2,3-dihydro-1-benzofuran-6-yl 4-Cyanobenzenesulfonate (33). 33 was purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane 20% → 90%). Yield: 90%. ¹H NMR (300 MHz, DMSO-*d*₆) δ : 1.88 (s, 3H), 2.64 (d, *J* = 4.7 Hz, 3H), 5.25 (d, *J* = 2.5 Hz, 2H), 5.85 (t, *J* = 2.5 Hz, 1H), 6.49 (d, *J* = 8.8 Hz, 1H), 8.02 (br q, *J* = 4.7 Hz, 1H, disappears with D₂O), 8.08 (d, *J* = 8.5 Hz, 2H), 8.17 (d, *J* = 8.5 Hz, 2H), 8.69 (d, *J* = 8.8 Hz, 1H).

(3*E*)-7-Methyl-3-[2-(methylamino)-2-oxoethylidene]-2,3-dihydro-1-benzofuran-6-yl 4-Methoxybenzenesulfonate (34). 34 was purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane 30% → 90%). Yield: 97%. ¹H NMR (300 MHz, DMSO- d_6) δ : 1.85 (s, 3H), 2.64 (d, *J* = 4.7 Hz, 3H), 3.86 (s, 3H), 5.24 (d, *J* = 2.5 Hz, 2H), 5.83 (t, *J* = 2.5 Hz, 1H), 6.48 (d, *J* = 8.5 Hz, 1H), 7.17 (d, *J* = 8.8 Hz, 2H), 7.80 (d, *J* = 8.8 Hz, 2H), 8.01 (br q, *J* = 4.7 Hz, 1H, disappears with D₂O), 8.68 (d, *J* = 8.5 Hz, 1H).

(3*E*)-3-[2-(Methylamino)-2-oxoethylidene]-2,3-dihydro-1benzofuran-6-yl 3,3,3-Trifluoropropane-1-sulfonate (35). 35 was purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane 20% → 70%). Yield: 86%. ¹H NMR (300 MHz, DMSO d_6) δ : 2.66 (d, *J* = 4.7 Hz, 3H), 2.85–3.01 (m, 2H), 3.86–3.91 (m, 2H), 5.28 (d, *J* = 2.5 Hz, 2H), 5.88 (t, *J* = 2.5 Hz, 1H), 6.92 (dd, *J*₁ = 2.2 Hz, *J*₂ = 8.5 Hz, 1H), 6.99 (d, *J* = 2.2 Hz, 1H), 8.06 (br q, *J* = 4.7 Hz, 1H, disappears with D₂O), 8.97 (d, *J* = 8.5 Hz, 1H).

(3E)-7-Methyl-3-[2-(methylamino)-2-oxoethylidene]-2,3-dihydro-1-benzofuran-6-yl 3,3,3-Trifluoropropane-1-sulfonate (36). 36 was purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane 20% \rightarrow 90%). Yield: 98%. ¹H NMR (300 MHz, DMSO-*d*₆) δ : 2.12 (s, 3H), 2.66 (d, *J* = 4.7 Hz, 3H), 2.89–3.01 (m, 2H), 3.95–4.01 (m, 2H), 5.28 (d, *J* = 2.5 Hz, 2H), 5.87 (t, *J* = 2.5 Hz, 1H), 6.92 (d, *J* = 8.5 Hz, 1H), 8.03 (br q, *J* = 4.7 Hz, 1H, disappears with D₂O), 8.79 (d, *J* = 8.5 Hz, 1H).

($3\tilde{E}$)-3-[2-(Ethylamino)-2-oxoethylidene]-2,3-dihydro-1-benzofuran-6-yl 3-Chlorobenzenesulfonate (37). 37 was purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane 10% → 80%). Yield: 90%. ¹H NMR (300 MHz, DMSO-d₆) δ : 1.02 (t, *J* = 7.2 Hz, 3H), 3.08-3.17 (m, 2H), 5.23 (d, *J* = 2.2 Hz, 2H), 5.80-5.88 (m, 1H), 6.58 (dd, *J*₁ = 2.2 Hz, *J*₂ = 8.5 Hz, 1H), 6.70 (d, *J* = 2.2 Hz, 1H), 7.67-7.70 (m, 1H), 7.83 (d, *J* = 8.0 Hz, 1H), 7.91 (d, *J* = 8.5 Hz, 1H), 7.94 (s, 1H), 8.10 (br t, *J* = 5.2 Hz, 1H, disappears with D₂O), 8.86 (d, *J* = 8.5 Hz, 1H).

(3*E*)-3-[2-(Isopropylamino)-2-oxoethylidene]-2,3-dihydro-1benzofuran-6-yl 3-Chlorobenzenesulfonate (38). 38 was purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane 10% → 60%). Yield: 51%. ¹H NMR (300 MHz, DMSO- d_6) δ : 1.06 (d, *J* = 6.6 Hz, 6H), 3.80-3.90 (m, 1H), 5.23 (d, *J* = 1.9 Hz, 2H), 5.80-5.88 (m, 1H), 6.58 (dd, J_1 = 2.2 Hz, J_2 = 8.8 Hz, 1H), 6.70 (d, *J* = 1.6 Hz, 1H), 7.66-7.70 (m, 1H), 7.84 (d, *J* = 7.7 Hz, 1H), 7.91 (d, *J* = 8.0 Hz, 1H), 7.94 (s, 1H), 7.98 (br d, *J* = 7.4 Hz, 1H, disappears with D₂O), 8.86 (d, *J* = 8.8 Hz, 1H).

General Procedure for the Synthesis of Benzofurans 39 and 40. The suitable derivative **21** or **37** (0.5 mmol) was dissolved in dry THF (5.0 mL), and DBU (0.075 mL, 0.5 mmol) was added. The mixture was stirred at room temperature for 24 h. The solvent was removed under reduced pressure and the resulting oil was purified by flash chromatography (gradient eluent, different mixtures of ethyl acetate in *n*-hexane), yielding the desired final products.

3-[2-(Methylamino)-2-oxoethyl]-1-benzofuran-6-yl 3-Chlorobenzenesulfonate (39). 39 was purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane 20% \rightarrow 80%). Yield: 88%. ¹H NMR (300 MHz, DMSO-*d*₆) δ : 2.56 (d, *J* = 4.4 Hz, 3H), 3.46 (s, 2H), 6.95 (dd, *J*₁ = 1.9 Hz, *J*₂ = 8.5 Hz, 1H), 7.37 (d, *J* = 1.9 Hz, 1H), 7.58 (d, *J* = 8.5 Hz, 1H), 7.64–7.70 (m, 1H), 7.79–7.82 (m, 1H), 7.89 (s, 1H), 7.92–7.93 (m, 2H), 8.00 (br q, *J* = 4.4 Hz, 1H, disappears with D₂O).

3-[2-(Ethylamino)-2-oxoethyl]-1-benzofuran-6-yl 3-Chlorobenzenesulfonate (40). 40 was purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane 20% \rightarrow 90%). Yield: 60%. ¹H NMR (300 MHz, DMSO-*d*₆) δ : 0.99 (t, *J* = 7.2 Hz, 3H), 3.00–3.10 (m, 2H), 3.45 (s, 2H), 6.95 (dd, *J*₁ = 2.2 Hz, *J*₂ = 8.5 Hz, 1H), 7.37 (d, *J* = 2.2 Hz, 1H), 7.58 (d, *J* = 8.5 Hz, 1H), 7.64–7.69 (m, 1H), 7.79– 7.83 (m, 1H), 7.88–7.94 (m, 3H), 8.08 (br s, 1H, disappears with D₂O).

Synthesis of 3-Oxo-2,3-dihydro-1-benzofuran-6-yl 3-Chlorobenzenesulfonate (41). Commercially available 6-hydroxy-1benzofuran-3(2*H*)-one (0.83 g, 5.5 mmol) was suspended in dry THF (18 mL), and potassium carbonate (0.42 g, 3.0 mmol) was added. 3-Chlorobenzenesulfonyl chloride (0.70 mL, 5.0 mmol) was dissolved in dry THF (7.0 mL) and added dropwise while cooling at 0 °C with an external ice bath. The mixture was slowly kept at room temperature and then the stirring was continued for an additional 18 h. The solvent was evaporated under reduced pressure, and the resulting mixture was purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane 20% \rightarrow 40%). Yield: 39%. ¹H NMR (300 MHz, CDCl₃) δ : 4.67 (s, 2H), 6.73 (dd, $J_1 = 1.9$ Hz, $J_2 = 8.3$ Hz, 1H), 6.89 (d, J = 1.9 Hz, 1H), 7.52 (t, J = 8.0 Hz, 1H), 7.62 (d, J = 8.3 Hz, 1H), 7.66–7.70 (m, 1H), 7.75–7.78 (m, 1H), 7.88–7.90 (m, 1H).

Synthesis of 7-[(3-Chlorobenzyl)oxy]-4-(chloromethyl)-2*H*-chromen-2-one (42). 7-Hydroxycoumarin derivative 1^{51} (1.1 g, 5.0 mmol) was dissolved in dry DMF (25 mL), and then Cs₂CO₃ (1.9 g, 6.0 mmol) and 3-chlorobenzyl bromide (0.65 mL, 5.0 mmol) were added. The mixture was stirred at room temperature for 24 h and then poured onto crushed ice. The precipitate was washed several times with water and used without further purification for the next steps. Yield: 79%. Spectroscopic data are in agreement with those already reported in the literature.^{8,51}

Synthesis of (2*E*)-2-[6-[(3-Chlorobenzyl)oxy]-1-benzofuran-3(2*H*)-ylidene]-*N*-methylacetamide (43). Intermediate 42 (0.67 g, 2.0 mmol) was dissolved in dry THF (4.0 mL), and the commercially available 2.0 N solution of methylamine was added (10 mL, 20 mmol). The resulting mixture was stirred at room temperature for 3 h. The inorganic precipitate was filtered off and the solution was concentrated to dryness and purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane 20% \rightarrow 70%), isolating the nonfluorescent spot at higher R_f (0.7, in ethyl acetate/*n*-hexane 8/2 v/v). Yield: 31%. ¹H NMR (300 MHz, DMSO- d_6) δ : 2.64 (d, *J* = 4.7 Hz, 3H), 5.14 (*s*, 2H), 5.17 (d, *J* = 2.2 Hz, 2H), 5.67 (t, *J* = 2.2 Hz, 1H), 6.56–6.60 (m, 2H), 7.36–7.42 (m, 3H), 7.50 (s, 1H), 7.87 (br q, *J* = 4.7 Hz, 1H, disappears with D₂O), 8.83 (d, *J* = 8.8 Hz, 1H).

Synthesis of 2-{6-[(3-Chlorobenzyl)oxy]-1-benzofuran-3-yl}-N-methylacetamide (44). Derivative 43 (0.099 g, 0.30 mmol) was dissolved in dry THF (2.0 mL), and DBU (0.045 mL, 0.30 mmol) was added. The mixture was heated at reflux for 8 h. The solvent was removed under reduced pressure and the resulting oil was purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane 20% \rightarrow 90%), yielding the desired final product 44. Yield: 62%. ¹H NMR (300 MHz, DMSO-d₆) δ : 2.57 (d, *J* = 4.7 Hz, 3H), 3.42 (s, 2H), 5.15 (s, 2H), 6.95 (dd, *J*₁ = 2.2 Hz, *J*₂ = 8.5 Hz, 1H), 7.23 (d, *J* = 2.2 Hz, 1H), 7.35–7.42 (m, 3H), 7.46 (d, *J* = 8.5 Hz, 1H), 7.51 (s, 1H), 7.68 (s, 1H), 7.97 (br q, *J* = 4.7 Hz, 1H, disappears with D₂O).

Synthesis of Ethyl (7-Hydroxy-2-oxo-2*H*-chromen-4-yl)acetate (45). Resorcinol (0.66 g, 6.0 mmol) was suspended in diethyl 1,3-acetonedicarboxylate (1.2 mL, 6.6 mmol), and a few drops of concentrated H₂SO₄ were added. The mixture was heated at 100 °C for 45 min with vigorous stirring, then cooled at room temperature and crystallized from hot acetic acid, furnishing the desired ester as an off-white solid. Yield: 61%. ¹H NMR (300 MHz, DMSO-*d*₆) δ : 1.15 (t, *J* = 7.2 Hz, 3H), 3.90 (s, 2H), 4.08 (q, *J* = 7.2 Hz, 2H), 6.21 (s, 1H), 6.71 (d, *J* = 2.2 Hz, 1H), 6.77 (dd, *J*₁ = 2.2 Hz, *J*₂ = 8.8 Hz, 1H), 7.48 (d, *J* = 8.8 Hz, 1H), 10.57 (s, 1H, disappears with D₂O).

Synthesis of Ethyl 7-Hydroxy-2-oxo-2*H*-chromene-4-carboxylate (46). Resorcinol (0.66 g, 6.0 mmol) was suspended in absolute ethanol (15 mL), and diethyl oxalacetate sodium salt (3.8 g, 18 mmol) was added. The mixture was refluxed for 96 h, cooled at room temperature, and concentrated to dryness. The resulting solid mixture was treated with chloroform, and the insoluble residue was filtered-off. The solution was concentrated under vacuum and purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane 10% \rightarrow 70%), thus furnishing the desired ester as a yellow solid. Yield: 43%. ¹H NMR (300 MHz, DMSO-*d*₆) δ : 1.32 (t, *J* = 7.2 Hz, 3H), 4.36 (q, *J* = 7.2 Hz, 2H), 6.58 (s, 1H), 6.76 (d, *J* = 2.2 Hz, 1H), 6.82 (dd, *J*₁ = 2.2 Hz, *J*₂ = 8.8 Hz, 1H), 7.89 (d, *J* = 8.8 Hz, 1H), 10.75 (s, 1H, disappears with D₂O).

General Procedure for the Synthesis of Amides 47 and 48. Ester 45 or 46 (2.0 mmol) and commercially available 2.0 N solution of methylamine in THF (30 mmol, 15 mL) were stirred at 90 °C in a closed Pyrex vessel for 96 h. After cooling, the crude mixture was concentrated to dryness and treated with CHCl₃. The resulting precipitate was filtered and washed several times with CHCl₃, thus obtaining the desired product with a satisfactory purity.

2-(7-Hydroxy-2-oxo-2H-chromen-4-yl)-N-methylacetamide (47). Yield: 89%. ¹H NMR (300 MHz, DMSO- d_6) δ : 2.57 (d, J = 4.7 Hz, 3H), 3.60 (s, 2H), 6.14 (s, 1H), 6.70 (d, J = 2.5 Hz, 1H), 6.77 (dd, $J_1 = 2.5$ Hz, $J_2 = 8.8$ Hz, 1H), 7.57 (d, J = 8.8 Hz, 1H), 8.06 (br s, 1H, disappears with D₂O), 10.53 (s, 1H, disappears with D₂O).

7-Hydroxy-N-methyl-2-oxo-2H-chromene-4-carboxamide (48). Yield: 73%. ¹H NMR (300 MHz, DMSO- d_6) δ : 2.77 (d, J = 4.7 Hz, 3H), 6.22 (s, 1H), 6.72 (d, J = 2.5 Hz, 1H), 6.77 (dd, $J_1 = 2.5$ Hz, $J_2 = 8.8$ Hz, 1H), 7.55 (d, J = 8.8 Hz, 1H), 8.73 (br q, J = 4.7 Hz, 1H, disappears with D₂O), 10.72 (br s, 1H, disappears with D₂O).

General Procedure for the Synthesis of Final Compounds 49 and 50. Amide 47 or 48 (1.0 mmol) was suspended in dry acetonitrile (8.0 mL), and triethylamine (0.14 mL, 1.0 mmol) was added, followed by 3-chlorobenzenesulfonyl chloride (0.17 mL, 1.2 mmol). The mixture was stirred at room temperature for 5–24 h. After evaporation of the solvent, the resulting crude oil was purified as indicated below. **4-[2-(Methylamino)-2-oxoethyl]-2-oxo-2H-chromen-7-yl 3-Chlorobenzenesulfonate (49). 49** was purified by crystallization from hot absolute ethanol. Yield: 84%. ¹H NMR (300 MHz, DMSO- d_6) δ : 2.56 (d, *J* = 4.7 Hz, 3H), 3.68 (s, 2H), 6.45 (s, 1H), 7.13 (dd, *J*₁ = 2.2 Hz, *J*₂ = 8.8 Hz, 1H), 7.23 (d, *J* = 2.2 Hz, 1H), 7.67–7.73 (m, 1H), 7.77 (d, *J* = 8.8 Hz, 1H), 7.86–7.95 (m, 2H), 8.00–8.01 (m, 1H), 8.07 (br s, 1H, disappears with D₂O).

4-[(Methylamino)carbonyl]-2-oxo-2*H*-chromen-7-yl 3-Chlorobenzenesulfonate (50). 50 was purified by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane 20% → 70%). Yield: 80%. ¹H NMR (300 MHz, DMSO-*d*₆) δ : 2.76 (d, *J* = 4.7 Hz, 3H), 6.59 (s, 1H), 7.11 (dd, *J*₁ = 2.5 Hz, *J*₂ = 8.8 Hz, 1H), 7.31 (d, *J* = 2.5 Hz, 1H), 7.67–7.73 (m, 1H), 7.79 (d, *J* = 8.8 Hz, 1H), 7.81–7.88 (m, 1H), 7.92–7.95 (m, 1H), 8.00–8.01 (m, 1H), 8.83 (br q, *J* = 4.7 Hz, 1H, disappears with D₂O).

Synthesis of 4-[[(Diphenylmethyl)(methyl)amino]methyl]-2oxo-2*H*-chromen-7-yl 3-Chlorobenzenesulfonate (51). Intermediate 4 (0.39 g, 1.0 mmol) was dissolved in dry THF (10 mL) before the addition of K_2CO_3 (0.14 g, 1.0 mmol), tetrabutylammonium iodide (TBAI) (0.0070 g, 0.020 mmol), and *N*-(diphenylmethyl)methylamine (0.22 g, 1.1 mmol). The mixture was kept under reflux for 7 h, and then the solvent was evaporated under reduced pressure. Purification by flash chromatography (gradient eluent, ethyl acetate in *n*-hexane 0% \rightarrow 30%) yielded the desired product as an amorphous solid. Yield: 56%. ¹H NMR (300 MHz, DMSO- d_6) δ : 2.08 (s, 3H), 3.62 (s, 2H), 4.65 (s, 1H), 6.70 (s, 1H), 7.09 (dd, J_1 = 2.5 Hz, J_2 = 8.8 Hz, 1H), 7.16–7.23 (m, 5H), 7.26–7.31 (m, 4H), 7.46–7.48 (m, 3H), 7.67–7.72 (m, 1H), 7.85–7.95 (m, 2H), 7.99–8.00 (m, 1H).

Synthesis of 4-[(Methylamino)methyl]-2-oxo-2*H*-chromen-7-yl 3-Chlorobenzenesulfonate (52). Coumarin derivative 51 (0.22 g, 0.40 mmol) was preliminarily dissolved in trifluoroacetic acid (5.0 mL) under irradiation with ultrasounds. Then triethylsilane (0.13 mL, 0.80 mmol) was added and the mixture was refluxed for 4 h. After dilution with saturated Na₂CO₃ (50 mL), the aqueous phase was extracted with ethyl acetate (3 × 30 mL). The organic layers were collected, dried over Na₂SO₄, concentrated to dryness, and the obtained crude solid was purified by flash chromatography by using ethyl acetate as the eluent. Yield: 72%. ¹H NMR (300 MHz, DMSO d_6) δ : 2.31 (s, 3H), 3.82 (s, 2H), 6.47 (s, 1H), 7.09 (dd, J_1 = 2.5 Hz, J_2 = 8.8 Hz, 1H), 7.23 (d, J = 2.5 Hz, 1H), 7.67–7.73 (m, 1H), 7.85– 7.95 (m, 2H), 7.86 (d, J = 8.8 Hz, 1H), 7.99–8.01 (m, 1H), NH not detected.

Synthesis of 4-(Aminomethyl)-2-oxo-2H-chromen-7-yl 3chlorobenzenesulfonate Hydrochloride (53). Hexamethylenetetramine (0.28 g, 2.0 mmol) was added to a suspension of intermediate 4 (0.77 g, 2.0 mmol) in dry chloroform (10 mL), and the mixture was refluxed for 48 h. After the mixture was cooled to room temperature, the obtained precipitate was separated from the solution by filtration and used without further purification. Urotropine salt of intermediate 4 (0.80 g, 1.5 mmol) was refluxed in 6% HCl in ethanol (10 mL) until complete dissolution, and the mixture was heated for an additional 1 h and then cooled to room temperature. The resulting precipitate was filtered, dissolved in saturated Na₂CO₃ (40 mL), and extracted with ethyl acetate $(3 \times 60 \text{ mL})$. The organic layers were collected, washed with brine (1 \times 30 mL), dried over NaSO₄, and concentrated to dryness. The resulting oil was treated with the commercially available solution of 4.0 N HCl in dioxane to obtain the desired product as a hydrochloride salt. Overall yield: 69%. ¹H NMR (300 MHz, DMSO-*d*₆) δ: 4.35 (s, 2H), 6.54 (s, 1H), 7.17 $(dd, J_1 = 2.5 Hz, J_2 = 8.8 Hz, 1H), 7.34 (d, J = 2.5 Hz, 1H), 7.68-7.74$ (m, 1H), 7.85 (d, J = 8.8 Hz, 1H), 7.87–7.96 (m, 2H), 8.00–8.01 (m, 1H), 8.50 (s, 3H, disappears with D_2O).

Monoamine Oxidases Inhibition Assays. MAO inhibitory activity of compounds in Tables 1 and 2 was assessed using a continuous spectrophotometric assay,⁵⁶ monitoring the rate of oxidation of the nonselective nonfluorescent MAO substrate kynuramine to 4-hydroxyquinoline. Briefly, male Sprague–Dawley rats (200–250 g) were sacrificed by decapitation. The brains were immediately removed and washed in an ice-cold isotonic $Na_3HPO_4/$

KH₂PO₄ buffer (pH 7.40) containing sucrose. A crude brain mitochondrial fraction was then prepared by differential centrifugaand stored at -40 °C in an isotonic Na₂HPO₄/KH₂PO₄ buffer tion (pH 7.4) containing KCl. MAO-A and MAO-B activities in mitochondrial preparations (1 mg/mL) were assayed using as selective and irreversible inhibitors clorgyline (250 nM) and (-)-L-deprenyl (250 nM), respectively. After a preincubation for 5 min with the assayed compound dissolved in DMSO at a final concentration of 5% (v/v), kynuramine was added at a concentration equal to the corresponding $K_{\rm M}$ value (90 μ M for MAO-A and 60 μ M for MAO-B). Then the rate of formation of 4-hydroxyquinoline was monitored at 314 nm for 5 min. Finally, IC_{50} values were determined by nonlinear regression of MAO inhibition vs -log of the concentration plots, using the program Origin, version 6.0 (Microcal Software Inc., Northampton, MA).

Docking Simulations. GOLD, a genetic algorithm-based software,⁶⁰ was used for a docking study selecting ChemScore as a fitness function. ChemScore was derived empirically from a set of 82 protein-ligand complexes for which measured binding affinities were available.61 ChemScore estimates the total free energy change that occurs on ligand binding by summing components that are the product of a term dependent on the magnitude of a particular physical contribution to free energy (e.g., hydrogen bonding) and a scale factor determined by regression. Empirical parameters used in the fitness function (hydrogen bonds energies, atom radii and polarizabilities, torsion potentials, hydrogen bonds directionalities, etc.) are taken from the GOLD parameter file. The protein input file may be the entire protein structure or a part of it comprising only the residues that are in the region of the ligand binding site. In the present study, GOLD was allowed to calculate interaction energies within a sphere of a 12 Å radius centered on phenolic oxygen atom of Tyr444 and Tyr435 in rMAO-A and rMAO-B, respectively.

Docking simulations toward MAO-A were carried out by allowing torsions and flexibility to Gln215 and Phe352, respectively. As explained elsewhere,^{50,51} a number of structural water molecules⁶² were explicitly considered in all the docking runs.

ASSOCIATED CONTENT

S Supporting Information

Elemental analysis data for compounds 20–41, 43, 44, 49, 50, 52, 53; synthesis and spectroscopic characterization of open chain cinnamic acid derivatives; NOESY spectral data for compounds 29 and 38. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

HB, hydrogen bond; HD, Huntington's disease; L-DOPA, L-3-(3,4-dihydroxyphenyl)alanine; MAO-A, monoamine oxidase A; MAO-B, monoamine oxidase B; MAO-I, monoamine oxidase inhibitor; MTDL, multitarget directed ligand; ND, neurodegenerative disease/disorder; PEA, 2-phenylethylamine; RIMA, reversible inhibitor of monoamine oxidase A; SSR, structure–selectivity relationship; TBAI, tetrabutylammonium iodide; TES, triethylsilane

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