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Resorcinol-based hemiindigoid derivatives as human tyrosinase inhibitors and melanogenesis suppressors in human melanoma cells



Brayan Roulier^{a,1}, Inbal Rush^{b,1}, Leticia M. Lazinski^{a,c}, Basile Pérès^a, Hamza Olleik^d,
Guy Royal^c, Ayelet Fishman^b, Marc Maresca^d, Romain Haudecoeur^{a,*}

^a Univ. Grenoble Alpes, CNRS 5063, DPM, 38000, Grenoble, France

^b Department of Biotechnology and Food Engineering, Technion – Israel Institute of Technology, Haifa, 3200003, Israel

^c Univ. Grenoble Alpes, CNRS 5250, DCM, 38000, Grenoble, France

^d Aix Marseille Univ., CNRS, Centrale Marseille, iSm2, 13397, Marseille, France

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ABSTRACT

Human tyrosinase (*hsTYR*) catalyzes the key steps of melanogenesis, making it a privileged target for reducing melanin production *in vivo*. However, very few *hsTYR* inhibitors have been reported so far in the literature, whereas thousands of mushroom tyrosinase (*abTYR*) inhibitors are known. Yet, as these enzymes are actually very different, including at their active sites, there is an urgent need for new true *hsTYR* inhibitors in order to enable human-directed pharmacological and dermocosmetic applications without encountering the inefficiency and toxicity issues currently triggered by kojic acid or hydroquinone. Starting from the two most active compounds reported to date, *i.e.* a 2-hydroxypyridine-embedded aurone and thiamidol, we combined herein key structural elements and developed new nanomolar *hsTYR* inhibitors with cell-based activity. From a complete series of thirty-eight synthesized derivatives, excellent inhibition values were obtained for two compounds in both human melanoma cell lysates and purified *hsTYR* assays, and a promising improvement was observed in whole cell experiments.

1. Introduction

Human tyrosinase (*hsTYR*, EC 1.14.18.1) is a type-3 copper-containing metalloenzyme characterized by a coupled binuclear active site that performs both the *ortho*-hydroxylation of monophenols and the oxidation of catechols [1]. Especially, *hsTYR* carries out the hydroxylation of L-tyrosine to L-DOPA and the subsequent oxidation of L-DOPA to dopaquinone, thereby initiating the spontaneous synthesis of melanin pigments in specialized skin cells, *i.e.* the melanocytes [2]. Melanin plays a crucial role in cell protection against UV radiations and free radicals. Nonetheless, the production of these pigments, *i.e.* melanogenesis, can also induce abnormal levels of melanin accumulation, leading to hyperpigmentation disorders, melasma or skin lesions, and ultimately causing health issues and disfigurements, sometimes severe and profoundly affecting the quality of life [3,4]. In addition, melanoma is closely related to an uncontrolled synthesis of melanins, which promotes the emergence of resistance to almost all traditional anti-cancer therapies [5,6]. As a consequence, melanoma is the most deadly skin

cancer (more than 60% of the deaths related to skin cancers), while its incidence rate is low (1% of the skin cancers) [7]. This multiresistance could be bypassed by the administration of inhibitors as adjuvants in immuno-, chemo- or radiotherapy for sensitizing melanoma, thereby improving the prognosis of the disease [8,9]. Overall, the inhibition of *hsTYR* is a well-established strategy for lowering or suppressing melanogenesis *in vivo*, and numerous inhibitors have been developed in the past decade for therapeutic and/or dermocosmetic purposes (Fig. S7) [10–12]. However, the production of properly matured *hsTYR* in high amounts is a challenge, due to its multiple specific N-glycosylations unavailable in simple organisms such as *E. coli* for recombinant production, mostly jeopardizing large-scale inhibitor screenings.¹³ Hence, most studies only relied on *in vitro* inhibition assays using the cheap and readily-available mushroom tyrosinase from *Agaricus bisporus* (*abTYR*), despite a low homology between these two enzymes (22% identity) and very distinct interaction patterns [13,14]. As a consequence, even the most widely used compound in human-directed dermocosmetic applications, *i.e.* kojic acid (KA), is a hundred-fold less active against *hsTYR*

* Corresponding author.

E-mail address: Romain.Haudecoeur@univ-grenoble-alpes.fr (R. Haudecoeur).

¹ B.R. and I.R. have contributed equally to this work.

than *ab*TYR ($K_i = 350 \mu\text{M}$ vs. $4.3 \mu\text{M}$) [15], resulting in the use of high concentrations, thereby promoting associated adverse effects, such as dermatitis or erythema, as well as carcinogenicity. Generally, such activity gaps were observed for almost all TYR reference inhibitors, including hydroquinone, arbutin, benzoic acid and several simple resorcinol derivatives.¹³ Therefore, a development process involving an appropriate array of tests performed using human models appears as a requirement for producing melanogenesis suppressors that could act efficiently and safely in a human context.

In the recent years we have developed aurones as a new class of TYR effectors. Aurones are naturally occurring flavonoids with a promising medicinal potential [16]. They provide bright yellow flower pigmentation to some of the most advanced taxa in the plant kingdom, among other still little-known roles [17]. Some derivatives from this subclass were found initially as melanogenesis suppressors in cell-based assays [18]. A broader study showed that aurone analogues can actually adopt versatile behaviors, acting either as inhibitors, activators or alternative substrates of several isolated mushroom and bacterial TYRs, depending on their substitution pattern at the B-ring [19–21]. In addition, we showed recently that the aurone scaffold is particularly suited for building interactions with active site residues located relatively far from the dicopper center in *hs*TYR, yielding compound **1** as one of the best *hs*TYR inhibitors reported to date ($K_i = 0.35 \mu\text{M}$), but with membrane penetration and water solubility issues probably due to the presence of a 2-hydroxypyridine-*N*-oxide (HOPNO) moiety [22]. Thus, we propose to dismiss the HOPNO chelating group. In our previous studies, aurones bearing a resorcinol moiety, *i.e.* 2',4'-dihydroxyaurones, were always associated with an inhibitor behavior, regardless of the TYR source. This observation was consistent with the known beneficial effect of resorcinol moiety in the frame of TYR inhibition, as witnessed by the number of reported inhibitors containing this chemical group [23–28]. Indeed, if some resorcinols were previously found to undergo a slow oxidation process in the presence of TYR and additives [29,30], they act as true inhibitors in physiological conditions.¹⁴ Some of them are actually used for treating human hyperpigmentation disorders, such as 4-*n*-butylresorcinol (*i.e.* rucinol, marketed as Iklen®, Fig. 1) [25,26]. The resorcinol-based thiamidol (Fig. 1) seems especially very promising, regarding both *in vitro* ($K_i = 0.25 \mu\text{M}$ against *hs*TYR),¹⁴ and clinical contexts (facial hyperpigmentation improvement in 96% of treated

women with melasma after 12 weeks of treatment) [31]. Altogether, the introduction of a resorcinol moiety on an aurone scaffold, or on a related hemiindigoid core, could combine an efficient copper-chelating resorcinol head with a *hs*TYR-tailored hemiindigoid backbone, able to interact with the second coordination sphere of the enzyme.

Thus, we report herein a series of thirty-eight molecules designed starting from compound **1** and including a 2',4'-resorcinol moiety, or close analogues, at the B-ring. The best derivatives, identified through a preliminary screening using *hs*TYR-containing human melanoma MNT-1 cell lysates, were evaluated both on the isolated enzyme expressed in a mammalian-cell system, thus insuring the expression of a mammalian glycosylation profile, and against melanogenesis in a human whole-cell system. Potential cytotoxicity issues were finally examined, and a binding geometry was proposed through molecular modelling, using a recent homology model of *hs*TYR.

2. Results and discussion

Human melanoma cell lysates assay and structure-activity relationship. Successive generations of potential inhibitors were first screened against tyrosinase activity from human melanoma cell lysates. In a previous study, compound **1** was found only moderately active in this screening test ($\text{IC}_{50} = 16.6 \mu\text{M}$).²² A similar value was obtained from our slightly modified protocol ($\text{IC}_{50} = 15 \mu\text{M}$, Table 1). The direct replacement of the HOPNO-embedded part of aurone **1** by a 2',4'-resorcinol-based B-ring was successful, as it led to a ~2.5-fold improved activity ($\text{IC}_{50} = 6.4 \mu\text{M}$ for **5**). As already seen with the previous HOPNO series, 4-hydroxy and 4,6-dihydroxy substitution patterns were slightly less effective ($\text{IC}_{50} = 25 \mu\text{M}$ for **3**, $7.7 \mu\text{M}$ for **12**), as were several other alternative phenolic ($\text{IC}_{50} = 19 \mu\text{M}$ for **4**, $11.8 \mu\text{M}$ for **17**) or naked ($\text{IC}_{50} = 18 \mu\text{M}$ for **2**) A-rings. If the methylation of the 6-hydroxy group maintained a similar potency ($\text{IC}_{50} = 6.9 \mu\text{M}$ for **6**), the introduction of a longer chain dramatically compromised the activity ($\text{IC}_{50} > 100 \mu\text{M}$ for *O*-butyl **7**). Similarly, replacing the phenol group by a halogen or amino derivative at position 6 produced inactive analogues ($\text{IC}_{50} > 100 \mu\text{M}$ for **8–9**), including 4,6-disubstituted compounds such as **13–15**. Finally, the 7-hydroxy analogue **10** was found as the best inhibitor in the aurone series, with an IC_{50} value of $1.57 \mu\text{M}$ (a 10-fold gain of activity starting from compound **1**). Modifying the nature of the substituent at position 7

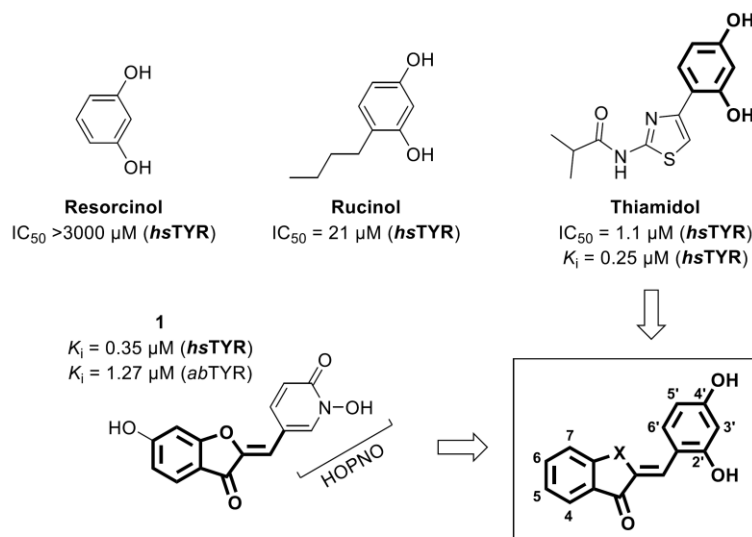
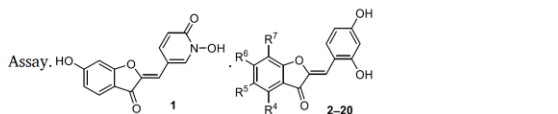


Fig. 1. Structures of classical *hs*TYR inhibitors with their K_i and/or IC_{50} values. In the box, a general structure of the new series designed in the frame of this study.

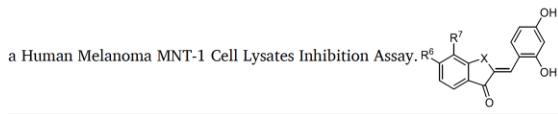
Table 1
Structures and Activities of 1 and Resorcinol-Based Aurone Derivatives 2–20 in Human Melanoma MNT-1 Cell Lysates Inhibition Assay.



compound	R ⁴	R ⁵	R ⁶	R ⁷	MNT-1 lysates IC ₅₀ (μM)
1					15 ± 1
2	H	H	H	H	18 ± 2
3	OH	H	H	H	25 ± 2
4	H	OH	H	H	19 ± 1
5	H	H	OH	H	6.4 ± 0.8
6	H	H	OMe	H	6.9 ± 0.4
7	H	H	OBu	H	>100
8	H	H	Cl	H	>100
9	H	H	NMe ₂	H	>100
10	H	H	H	OH	1.57 ± 0.09
11	H	H	H	OMe	80 ± 10
12	OH	H	OH	H	7.7 ± 0.8
13	OH	H	NMe ₂	H	18.4 ± 0.7
14	OMe	H	OMe	H	>100
15	F	H	F	H	88 ± 8
16	H	H	OH	OH	11.8 ± 0.7
17	H	H	OH	Me	80 ± 10
18	H	H	OH	I	32 ± 1
19	H	H	OMe	Me	>100
20	H	H	OMe	OMe	>100
KA					>100
Rucinol					8.3 ± 0.7

and combining it with OH or OMe groups at position 6 afforded underwhelming results in all cases (IC₅₀ > 11.8 μM for **16–20**). As expected, the well-known TYR inhibitor kojic acid was completely devoid of biological activity in the studied range of concentrations. Thus, given the superior potential of the 7-hydroxy substitution and, to a lesser extent, of the 6-hydroxy counterpart, we decided to explore the impact of C-ring modifications of **2**, **5** and **10**, first by replacing the intracyclic oxygen atom of the aurone skeleton with a carbon-, nitrogen or sulfur-based moiety. The methylene-based indanone series provided overall better results than the previous aurone series, and maintained the same order of inhibition potency (Table 2): the 7-hydroxy analogue **23** (IC₅₀ = 1.6 μM, vs. 1.57 μM for **10**) still appeared as superior to the 6-hydroxy **22** (IC₅₀ = 3.4 μM, vs. 6.4 μM for **5**), more active itself than the naked derivative **21** (IC₅₀ = 5.8 μM, vs. 18 μM for **2**). However, substituting the intracyclic carbon with a methyl group led to a decrease of the anti-*hsTYR* effect (IC₅₀ = 8.2 μM for racemic **24**), while introducing a second carbonyl group caused a complete loss of activity (IC₅₀ > 100 μM for **25**). Nitrogen- and sulfur-based counterparts, namely hemiindigos (HI)

Table 2
Structures and Activities of Resorcinol-Based Hemiindigoid Derivatives 21–30 in Human Melanoma MNT-1 Cell Lysates Inhibition Assay.



compound	R ⁶	R ⁷	X	MNT-1 lysates IC ₅₀ (μM)
21	H	H	CH ₂	5.8 ± 0.6
22	OH	H	CH ₂	3.4 ± 0.2
23	H	OH	CH ₂	1.6 ± 0.2
24	H	H	CHMe	8.2 ± 0.3
25	H	H	C=O	>100
26	H	H	NH	12.7 ± 0.7
27	OH	H	NH	2.3 ± 0.3
28	H	H	S	5.9 ± 0.4
29	OH	H	S	5.8 ± 0.4
30	H	OH	S	48 ± 3

and hemithioindigos (HTI), produced mixed results, but generally managed to retain the inhibition potency of the best aurones and indanones (IC₅₀ = 2.3–5.9 μM for **27–29**). Surprisingly, the 7-hydroxy HTI **30** did not share the greater potency of aurone and indanone counterparts (IC₅₀ = 48 μM, vs. 1.57–1.6 μM for **10** and **23**). Finally, several derivatives were synthesized in order to identify elements that are crucial for activity in the hemiindigoid scaffolds (Fig. 2). The evaluation of chalcone and flavone analogues of **2** confirmed the need to retain a five-membered C-ring, as both compounds were found less active against melanogenesis (IC₅₀ > 60 μM for **31** and **32**, vs. 18 μM for **2**). Similarly, the removal of the exocyclic double bond or the replacement of the ketone at position 3 afforded inactive products in all cases (IC₅₀ > 100 μM for **33–37**), highlighting the impact of hemiindigoid scaffolds themselves beyond substitution patterns. The B-ring resorcinol moiety was also questioned, but phloroglucinol analogues such as **38** and 4'-hydroxy-2'-methoxy derivatives such as **39** always led to a significant activity decrease, compared to parent compound **5** (IC₅₀ = 15 μM and >100 μM, vs. 6.4 μM, respectively).

Inhibition of purified human tyrosinase and *in silico* modeling.

The next step was to confirm the biomolecular target of resorcinol-based hemiindigoids. Thus, the best compounds identified in the human melanoma MNT-1 cell lysates assay were evaluated in an inhibition test involving purified *hsTYR*. The expression of a construct of *hsTYR* (intramelanosomal domain, residues 1–456 with a C-terminal HisTag) was performed in human HEK293T cells and the enzyme was purified using a HisTrap™ column. The obtained molecular weight was 60–75 kDa, a range attesting multiple glycosylations, as the amino-acid sequence alone is ~53 kDa. In addition, the use of a human expression system ensured the production of proper mammalian N-glycans, while only paucimannose structures are generated by insect cells. The kinetic parameters measured for *hsTYR*-mediated oxidation of L-DOPA were found as relevant ($K_m = 0.32 \pm 0.01$ mM, $V_{max} = 5.35 \pm 0.10$ μmol min⁻¹. mg⁻¹, $k_{cat} = 5.95$ s⁻¹, Fig. S4), in regard to those reported previously.¹⁵ Then, kinetic studies were performed with the two best compounds identified during the previous screening assay, **10** and **23**, using L-DOPA as the substrate and a readout at 475 nm. Both molecules displayed a very high level of anti-*hsTYR* activity, associated with inhibition constants in the submicromolar range ($K_i = 0.55$ μM and 0.25 μM, respectively, Table 3, Fig. S6). Thus, the hemiindigoid series showed a similar inhibition potency as the reference thiamidol ($K_i = 0.25$ μM). In addition, as expected the inhibition mechanisms of compounds **10** and **23** were identified as partially competitive, with alpha values of 3.0–3.5, consistent with a direct binding to the dicopper center. In comparison, kojic acid was found as a very weak inhibitor ($K_i = 1.1$ mM) with a mixed mechanism, as already revealed in different *hsTYR* constructs and expression systems.^{14,15} Overall, these results tend to confirm *hsTYR* as the main target of the hemiindigoid series in the MNT-1 lysates assay. Thus, we relied on a homology model of *hsTYR* [32], based on the crystal structure of *hsTYR*P1 [33], for proposing a binding geometry and a rationalization of interactions at the active site through molecular docking. The use of LeDock was endorsed by a recent report that pointed out the superior accuracy obtained by this docking software for metalloproteins [34]. The best pose identified for compound **23** is represented in Fig. 3A. As expected, the phenol group at position 4' is predicted to interact directly with copper atom A. In addition, several hydrogen bonds could occur, between the phenol group at position 2' and the backbone of Ser375, or between the 7-hydroxy substituent and the carboxylate of Glu203 (Fig. 3B). These elements could explain the higher potency of resorcinol compounds, and of 7-substituted compounds. The A-ring of hemiindigoid scaffolds was found well-positioned for the building of an edge-to-face π-stacking interaction with the aromatic ring of Phe347. Finally, the B-ring could establish a face-to-face π-stacking with His367, reminiscently of the interaction observed between the aromatic ring of L-tyrosine and His208 of tyrosinase from *Bacillus megaterium* in a reported crystallographic study (Fig. 3C) [35]. Indeed, the superimposition of **23** with L-tyrosine was almost perfect,

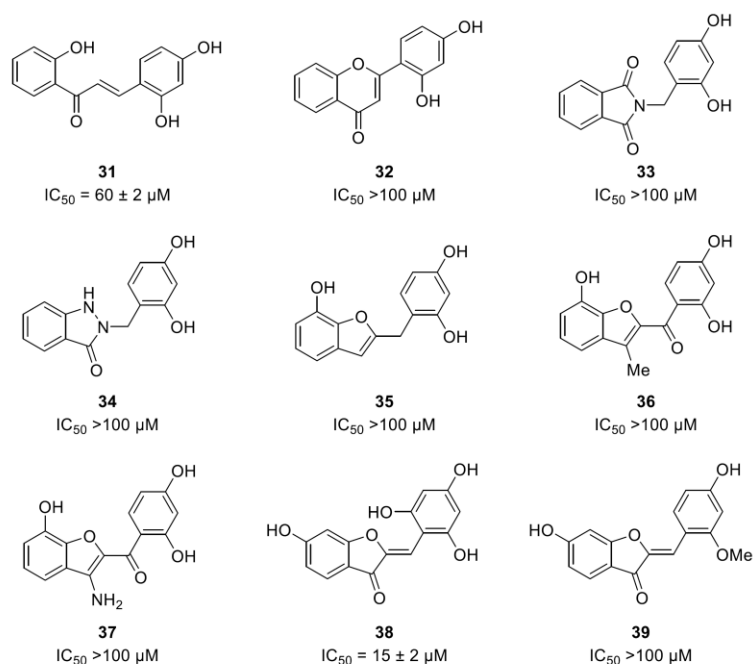


Fig. 2. Structures and activities of alternative derivatives 31–39 in a human melanoma MNT-1 cell lysates inhibition assay.

Table 3

Kinetic Parameters Measured using a Purified *hsTYR* Inhibition Assay, and MNT-1 Whole Cells Melanogenesis Inhibition and Cytotoxicity for 10, 23 and Kojic Acid (KA).

cd	purified <i>hsTYR</i>						MNT-1 whole cells	
	inhib. mech.	K_i (μM)	K_m (μM)	V_{\max} ($\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$)	k_{cat} (s^{-1})	alpha	IC_{50} (μM)	CC_{50} (μM)
10	PC ^a	0.55 ± 0.09	143 ± 7	4.86 ± 0.07	5.41	3.5 ± 0.2	32 ± 2	108 ± 3
23	PC ^a	0.25 ± 0.04	161 ± 6	5.18 ± 0.06	5.78	3.0 ± 0.1	29 ± 2	91 ± 2
KA	M ^b	1100 ± 300	310 ± 20	5.2 ± 0.1	5.8	0.16 ± 0.05	15000 ± 1000	34000 ± 600

^a partial competitive.

^b mixed.

with a similar interaction with the metallic center and an identical aromatic ring orientation (Fig. 3D). In addition, the intracyclic ketone of compound 23 was found very close to the carboxylate group of *L*-tyrosine.

Human melanoma whole cells assay and cytotoxicity. As *hsTYR* was confirmed as the biomolecular target of resorcinol hemiindigoids, we next evaluated the potential of the two best inhibitors of the series to operate in cell-based environments. Indeed, compound 1 revealed a very low activity against whole MNT-1 cells, despite its promising profile against the isolated enzyme. The cytotoxicity of compounds 10 and 23 on MNT-1 cells was explored first, and no significant effect was recorded until ~100 μM . The CC_{50} values obtained (108 μM for 10 and 91 μM for 23, Table 3) were consistent with previously reported polyhydroxylated aurones [36] and provided room for operating concentrations in the micromolar range. Thus, the melanin content was measured after a 96 h incubation of MNT-1 whole cells with either 10, 23 or kojic acid. The hemiindigoids induced a 50% inhibition of melanogenesis at 32 μM and 29 μM respectively (Table 3), a range of concentration where both compounds were found completely non-cytotoxic (see Supporting Information). While these values are still higher than those obtained from the MNT-1 lysates assay, compounds 10 and 23 exhibited a ~3-fold

improvement of cellular activity, compared to the parent compound 1. It is worth noting that kojic acid was found as a far weaker inhibitor in this assay, yielding an IC_{50} of 15 mM. Altogether, these results demonstrate a correlation between the values obtained from assays involving the purified *hsTYR* enzyme, MNT-1 cell lysates and MNT-1 whole cells, and endorse the development of more active *hsTYR* inhibitors as a way to find more efficient melanogenesis suppressors in cell-based environments.

Chemical synthesis. Globally, hemiindigoid derivatives were prepared either by cyclization of a corresponding chalcone, or through condensation of a benzaldehyde with an appropriate benzofuran-2(3*H*)-one or closely related five-membered cyclic ketone. Regarding 6-dimethylamino-substituted aurones 9 and 13, the prior synthesis of substituted chalcones was achieved starting from commercial phenolic compounds through a final condensation step with 2,4-dimethoxybenzaldehyde (Scheme 1). The other aurone and indanone derivatives were produced from corresponding bicyclic synthons, either purchased from commercial sources or prepared according to previously described methods (Scheme 2) [37]. Several sets of reaction conditions were employed, depending on the desired A-ring substitution pattern. Polyhydroxylated analogues were most often synthesized in basic medium,

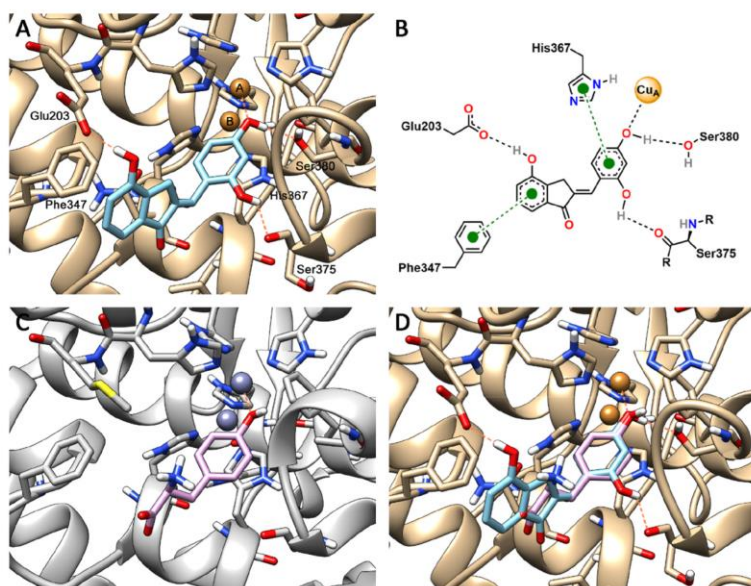
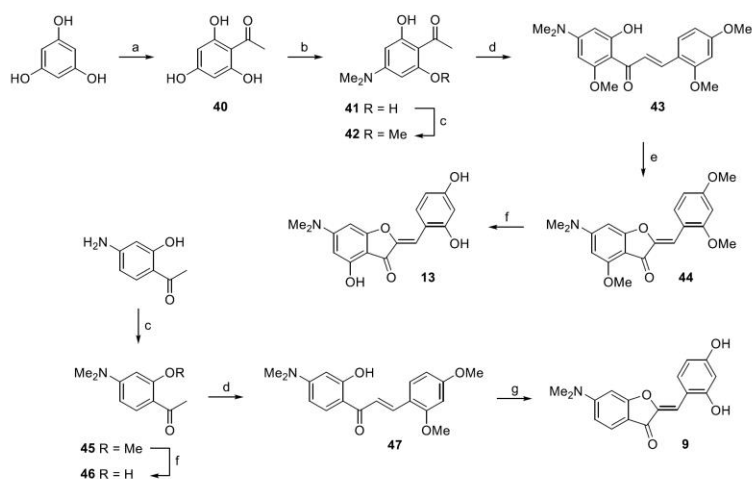


Fig. 3. Visual representation of a predicted binding mode of compound **23** at the active site of a homology model of *hsTYR*, provided by LeDock software (A). Schematic view of related key interactions (B). Visual representation of a crystal structure (PDB code 4p6r) of *l*-tyrosine bound to *Bacillus megaterium* tyrosinase, with copper atoms replaced by zinc atoms (C). Superimposition of *l*-tyrosine from the previous crystal structure with compound **23** at the active site of a homology model of *hsTYR* (D).

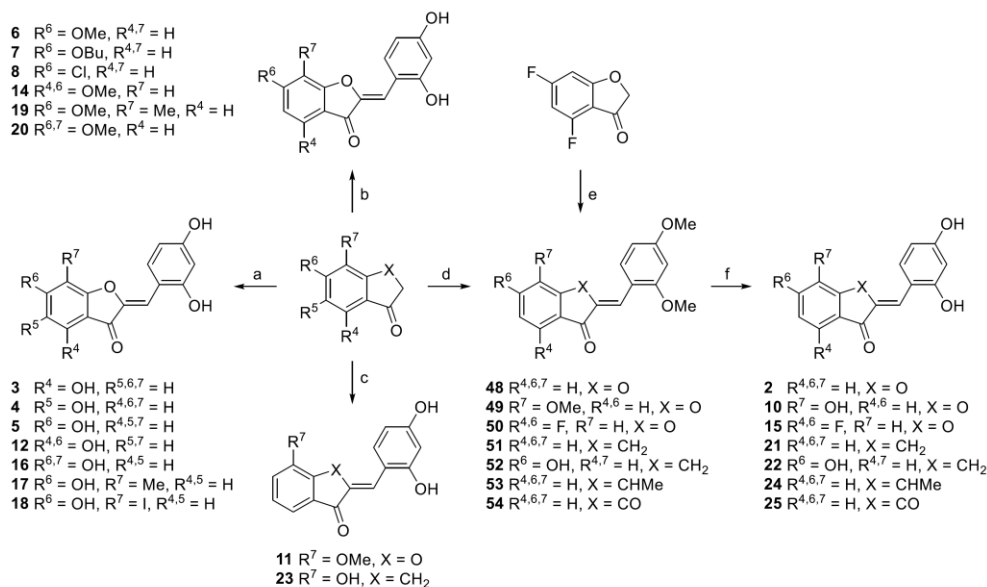


Scheme 1. Synthesis of 6-dimethylamino-substituted aurones.^a

^aReagents and conditions: (a) Ac_2O , $\text{BF}_3 \cdot \text{Et}_2\text{O}$, AcOEt , 50°C ; (b) $\text{HNMe}_2 \cdot \text{HCl}$, NaHCO_3 , H_2O , 70°C ; (c) Me_2SO_4 , K_2CO_3 , acetone, reflux; (d) 2,4-dimethoxybenzaldehyde, KOH , EtOH , H_2O , 25°C ; (e) $\text{Hg}(\text{OAc})_2$, pyridine, 110°C ; (f) BBr_3 , CH_2Cl_2 , $0-25^\circ\text{C}$; (g) $\text{Hg}(\text{OAc})_2$, pyridine, 110°C then BBr_3 , CH_2Cl_2 , $0-25^\circ\text{C}$.

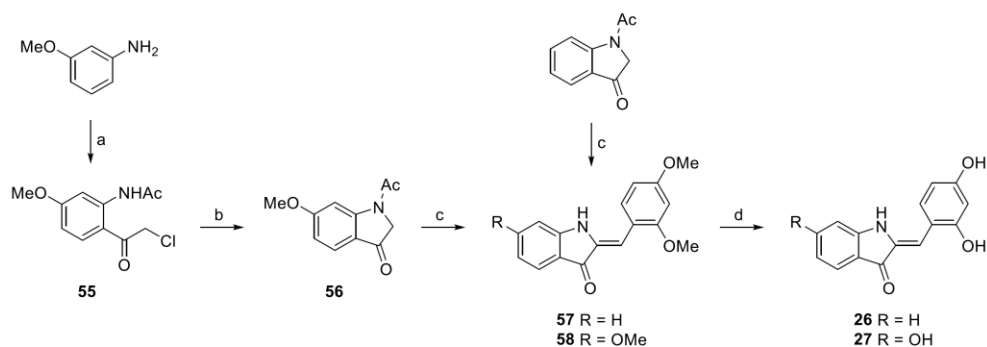
with KOH in a mixture of $\text{EtOH}/\text{H}_2\text{O}$ at 80°C , but some compounds required the use of a protected 2,4-dimethoxybenzaldehyde with milder conditions in MeOH , as the concurrent degradation process of the polyphenolic species was too fast (e.g. analogues **48**, **49**). This pathway was also applied to indanone and indanedione synthesis. A further deprotection with BBr_3 finally generated the 2',4'-dihydroxy substitution pattern from every 2',4'-dimethoxy intermediate. In some cases, especially when alkoxy or halide groups were targeted at the A-ring of aurones and indanones, three alternative conditions were used. A neutral aqueous medium-based method afforded very mild conditions for compounds **6-8**, **14**, **19**, **20**, with low yields, however [38]. Acidic conditions at room temperature with SOCl_2 in MeOH gave compounds

11 and **23**. Finally, the sensitivity of the fluoro groups to nucleophilic substitution during **50** synthesis dismissed the use of KOH in EtOH , and milder conditions with Al_2O_3 in CH_2Cl_2 followed by methoxy deprotection were preferred [39]. Classical basic condensation and deprotection sequences were also applied to the synthesis of **HI 26** and **27** (Scheme 3), and of **HTI 28** (Scheme 4), after preparation of the corresponding indole and benzothiophene ketones from 3-methoxyaniline and 3-methoxythiophenol respectively for the last two analogues. **HTI 29** and **30** were produced through an alternative route, by cyclization of 2'-nitrochalcones **60** and **61** in presence of sulfur and triethylamine in DMSO , followed by deprotection of the methoxy groups. The chalcone and flavone derivatives **31** and **32** were reached following classical



Scheme 2. Synthesis of aurones, indanones and indanediones.^a

^aReagents and conditions: (a) 2,4-dihydroxybenzaldehyde, KOH, EtOH, H₂O, 80 °C; (b) 2,4-dihydroxybenzaldehyde, H₂O, 100 °C; (c) 2,4-dihydroxybenzaldehyde, SOCl₂, MeOH, 25 °C; (d) 2,4-dimethoxybenzaldehyde, KOH, MeOH, H₂O, 25–65 °C; (e) 2,4-dimethoxybenzaldehyde, Al₂O₃, CH₂Cl₂; (f) BBr₃, CH₂Cl₂, 0–25 °C.



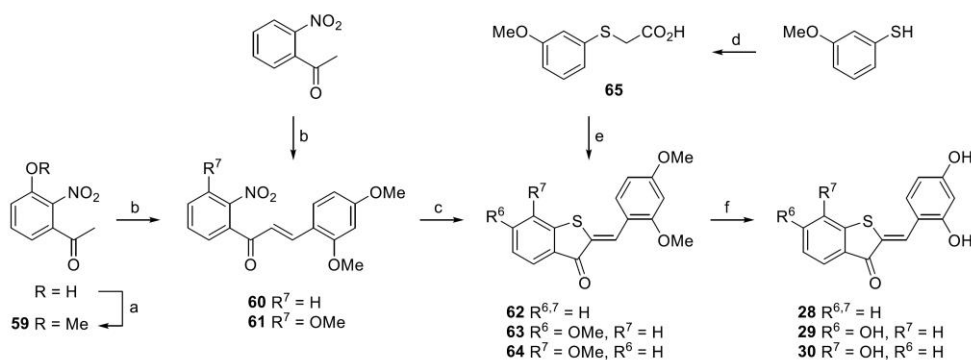
Scheme 3. Synthesis of hemiindigos.^a

^aReagents and conditions: (a) chloroacetonitrile, BCl₃, AlCl₃, PhNO₂, 80 °C then HCl, H₂O, 80 °C then Ac₂O, 80 °C; (b) NaH, DMF, 25 °C; (c) 2,4-dimethoxybenzaldehyde, KOH, MeOH, H₂O, 35 °C; (d) BBr₃, CH₂Cl₂, 0–25 °C.

procedures (Scheme 5). A condensation between 2-hydroxyacetophenone and 2,4-dimethoxybenzaldehyde afforded the protected chalcone **66**, which was either directly deprotected, or first cyclized into flavone **67**. The isoindoline analogue **33** was prepared by reaction of 2,4-dimethoxybenzylamine with phthalic anhydride, followed by a deprotection step. The synthesis of indazolone derivative **34** involved a Davis-Beirut reaction after substitution of the 1-(bromomethyl)-2-nitrobenzene by 2,4-dimethoxybenzylamine [40]. While benzofuran compound **35** was produced through direct reduction of the corresponding aurone with LiAlH₄ and further deprotection, the syntheses of derivatives **36** and **37** were performed through the substitution of 2-bromo-1-(2,4-dimethoxyphenyl)ethan-1-one by a phenolic compound, followed by intramolecular aldol condensation (Scheme 6). Finally, aurones **38** and **39** bearing alternative B-ring substituents were prepared according to the aforementioned classical KOH/MeOH procedure.

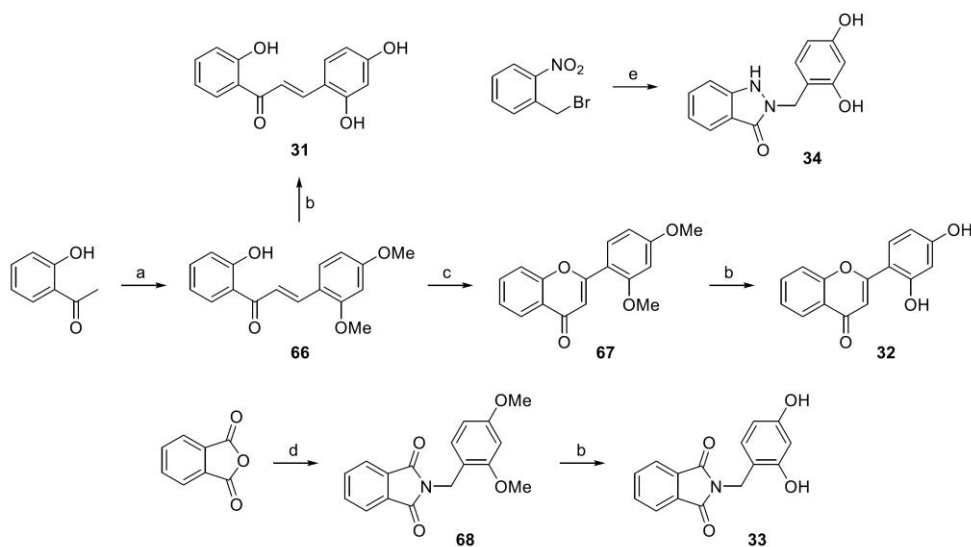
3. Conclusion

In conclusion, a series of thirty-eight resorcinol-based compounds have been synthesized and evaluated to determine their human tyrosinase inhibition activity through a set of complementary assays. Identified first *via* a screening assay using human melanoma MNT-1 cell lysates, compounds **10** and **23** were confirmed as potent inhibitors of *h*sTYR in a test involving the purified enzyme properly expressed in human HEK 293T cells, and thus associated with a native glycosylation pattern. Both compounds exhibited *K*_i values similar to the reference thiamidol with a partially competitive mechanism. The effect has been rationalized by molecular modeling experiments, supporting the hypothesis of binding to the copper center through the phenol at position 4' of the hemiindigoid scaffolds and suggesting a geometry consistent with the known binding mode of the natural substrate *L*-tyrosine. The



Scheme 4. Synthesis of hemithioindigos.^a

^aReagents and conditions: (a) Me₂SO₄, K₂CO₃, acetone, 60 °C; (b) 2,4-dimethoxybenzaldehyde, NaOH, EtOH, H₂O, 0 °C; (c) Et₃N, S, DMSO, 100 °C; (d) chloroacetic acid, NaOH, H₂O, 60 °C; (e) SOCl₂, CH₂Cl₂, 40 °C then AlCl₃, CH₂Cl₂, 25 °C then 2,4-dimethoxybenzaldehyde, Al₂O₃, CH₂Cl₂, 25 °C; (f) BBr₃, CH₂Cl₂, 0–25 °C.



Scheme 5. Synthesis of chalcone, flavone, isindoline and indazolone derivatives.^a

^aReagents and conditions: (a) 2,4-dimethoxybenzaldehyde, KOH, MeOH, H₂O, 65 °C; (b) BBr₃, CH₂Cl₂, 0–25 °C; (c) I₂, DMSO, 145 °C; (d) 2,4-dimethoxybenzylamine, AcOH, 120 °C; (e) 2,4-dimethoxybenzylamine, DIPEA, MeOH, 25 °C then KOH, MeOH, H₂O, 65 °C then BBr₃, CH₂Cl₂, 0–25 °C.

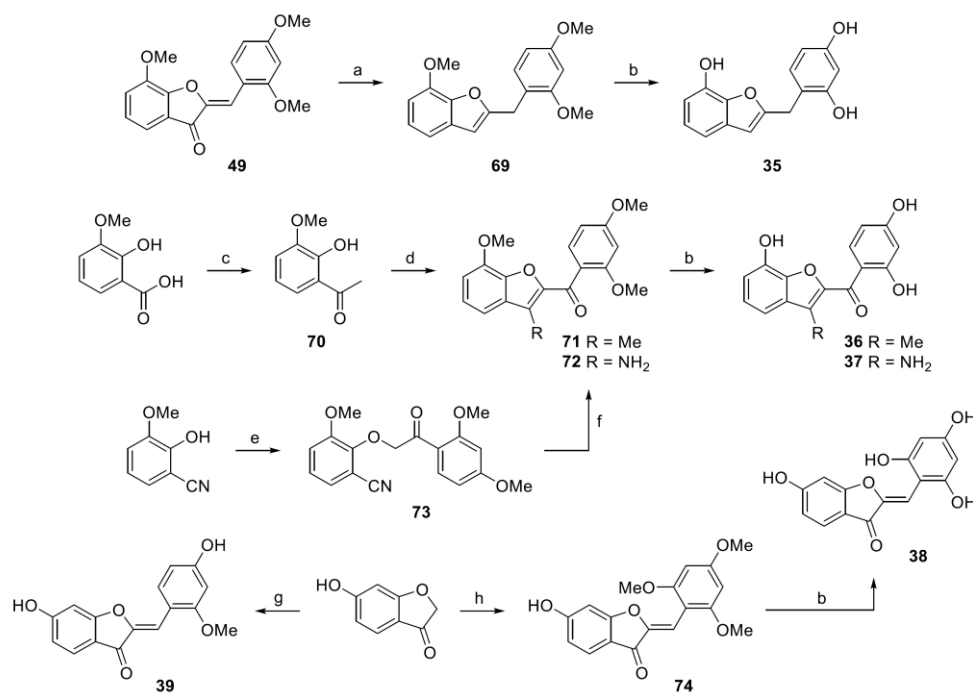
anti-*hs*TYR biological activities were confirmed in whole cells, yielding a far more promising profile than the parent compound **1** for further developments. Overall, the results obtained within this study will contribute to expand the currently very small *hs*TYR inhibitors portfolio and to enable human-directed applications in the dermocosmetic and therapeutic fields.

4. Experimental section

Chemistry. ¹H and ¹³C NMR spectra were recorded on an AC-400 instrument (Bruker, 400 MHz for ¹H, 100 MHz for ¹³C). Chemical shifts are reported in ppm relative to the solvent in which the spectrum was recorded. Electrospray ionization (ESI) high resolution mass spectra were acquired at the ICMG PSM Platform on a LTQ Orbitrap XL Thermo Scientific. All tested compounds had purity higher than 95%, measured either by combustion analysis or by HPLC analysis. Combustion analyses

were performed at the analysis facilities of the Molecular Chemistry Department. HPLC analyses were performed with a μ -bondapak-C18 analytical column (Waters Corporation, Milford, MA) on a Waters chromatographic system, equipped with two M – 510 pumps and a photodiode array detector Waters 996, using Millennium 32 software. Silica gel UV₂₅₄ plates (0.20 mm; Macherey-Nagel) were used for thin-layer chromatography (TLC), and silica gel 60 M (0.04–0.063 mm; Macherey-Nagel) was used for flash chromatography. Unless otherwise stated, reagents were obtained from commercial sources (mostly from Sigma, Alfa Aesar, Fluorochem) and were used without further purification. Compounds **3–5** and **12** have already been reported in a previous study.²⁰

General Procedure A. To a solution of a phenylethane-1-one or benzofuran-3(2*H*)-one derivative in EtOH or MeOH (3–10 mL/mmol) were added an aqueous solution of KOH (50%, 1–5 mL/mmol), NaOH (50%, 1–5 mL/mmol) or NaH (1 equiv), and a benzaldehyde derivative



Scheme 6. Synthesis of benzofurans and aurones with alternative B-ring substitution.^a

^aReagents and conditions: (a) LiAlH_4 , AlCl_3 , Et_2O , 25 °C; (b) BBr_3 , CH_2Cl_2 , 0–25 °C; (c) MeLi , THF, 65 °C; (d) 2-bromo-1-(2,4-dimethoxyphenyl)ethan-1-one, DBU, CH_2Cl_2 , 25 °C; (e) 2-bromo-1-(2,4-dimethoxyphenyl)ethan-1-one, K_2CO_3 , DMF, 25 °C; (f) NaOMe , MeOH, 25 °C; (g) 4-hydroxy-2-methoxybenzaldehyde, KOH , MeOH, H_2O , 65 °C; (h) 2,4,6-trimethoxybenzaldehyde, KOH , MeOH, H_2O , 65 °C.

(1–1.5 equiv). The solution was stirred at 25–80 °C for 2–5 h. After cooling, if a precipitation occurred, the solid was filtered, washed with the reaction solvent and dried to afford the crude product. Otherwise, EtOH or MeOH was removed under reduced pressure, and aqueous HCl (1–6 N) was added to the residue to adjust the pH to 7–8. The mixture was then extracted three times with AcOEt. The combined organic layers were washed with water and brine, dried over anhydrous MgSO_4 , filtered, and the filtrate was concentrated under reduced pressure to afford the crude product.

General Procedure B. To a solution of a methoxy derivative in anhydrous CH_2Cl_2 (5 mL/mmol) was added pure BBr_3 (10 equiv) at 0 °C. The solution was stirred at 25 °C for 2–4 days. Cold water was then added and the suspension was extracted three times with AcOEt. The combined organic layers were washed with water and brine, dried over anhydrous MgSO_4 , filtered, and the filtrate was concentrated under reduced pressure to afford the crude product.

General Procedure C. To a solution of a phenol derivative in acetone (5 mL/mmol) was added K_2CO_3 (1.3 equiv) and Me_2SO_4 (1.2–3.0 equiv) dropwise. The solution was refluxed for 5 h. After cooling, the solution was filtered and concentrated under reduced pressure to afford the crude product.

General Procedure D. To a solution of a benzofuran-3(2H)-one derivative in water (8 mL/mmol) was added 2,4-dihydroxybenzaldehyde (1 equiv). The solution was refluxed for 18 h. After cooling, the precipitate was filtered, washed with water and dried to afford the crude product.

General Procedure E. To a solution of a chalcone derivative in pyridine (16 mL/mmol) was added $\text{Hg}(\text{OAc})_2$ (1 equiv). The solution was refluxed for 15 h. After cooling, iced water was added and the mixture was extracted three times with AcOEt. The combined organic

layers were washed with water and brine, dried over anhydrous MgSO_4 , filtered, and the filtrate was concentrated under reduced pressure to afford the crude product.

(Z)-2-(2,4-Dihydroxybenzylidene)benzofuran-3(2H)-one (2). The crude product was prepared according to the general procedure B from **48** (300 mg, 1.06 mmol) to afford **2** (172 mg, 64%) as an orange solid. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 10.40 (s, 1H, OH), 10.13 (s, 1H, OH), 8.04 (d, 1H, $J = 9.3$ Hz, H_6), 7.76 (m, 2H, H_4 , H_6), 7.53 (d, 1H, $J = 8.5$ Hz, H_7), 7.30 (t, 1H, $J = 7.4$ Hz, H_5), 7.22 (s, 1H, =CH–), 6.43 (m, 2H, H_3 , H_5). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ 182.7 (C_3), 164.7, 161.5, 159.7 (C_8 , C_2 , C_4), 144.2 (C_2), 136.7 (C_6), 132.8 (C_6), 123.9 (C_4), 123.5 (C_5), 121.5 (C_9), 113.1 (C_7), 110.5 (C_1), 108.5 (C_5), 107.9 (=CH–), 102.3 (C_3). HRMS (ESI) m/z calc. for $\text{C}_{15}\text{H}_{11}\text{O}_4$ ($\text{M} + \text{H}$)⁺: 255.06519. Found: 255.06400.

(Z)-2-(2,4-Dihydroxybenzylidene)-6-methoxybenzofuran-3(2H)-one (6). The crude product was prepared according to the general procedure D from 6-methoxybenzofuran-3(2H)-one (100 mg, 0.609 mmol), and purified by column chromatography on silica gel (eluent CH_2Cl_2 –MeOH) to afford **6** (10 mg, 6%) as a yellow solid. ^1H NMR (500 MHz, MeOD) δ 8.09 (d, 1H, $J = 8.7$ Hz, H_6), 7.63 (d, 1H, $J = 8.6$ Hz, H_4), 7.35 (s, 1H, =CH–), 6.92 (d, 1H, $J = 1.6$ Hz, H_7), 6.79 (dd, 1H, $J = 8.6$, 1.6 Hz, H_5), 6.41 (dd, 1H, $J = 8.7$, 2.0 Hz, H_5), 6.34 (d, 1H, $J = 2.0$ Hz, H_3), 3.93 (s, 3H, OCH_3). ^{13}C NMR (125 MHz, MeOD) δ 184.5 (C_3), 169.4, 169.2, 162.8, 161.1 (C_6 , C_8 , C_2 , C_4), 147.2 (C_2), 134.5 (C_6), 126.2 (C_4), 116.2 (C_9), 113.6 (C_5), 112.8 (C_1), 110.0 (C_5), 109.4 (=CH–), 103.1 (C_3), 97.5 (C_7), 56.7 (OCH_3). HRMS (ESI) m/z calc. for $\text{C}_{16}\text{H}_{13}\text{O}_5$ ($\text{M} + \text{H}$)⁺: 285.07575. Found: 285.07520.

(Z)-2-(2,4-Dihydroxybenzylidene)-6-butoxybenzofuran-3(2H)-one (7). The crude product was prepared according to the general procedure D from 6-butoxybenzofuran-3(2H)-one (412 mg, 2.00 mmol),

and purified by column chromatography on silica gel (eluent CH₂Cl₂-MeOH) to afford **7** (20 mg, 3%) as a yellow solid. ¹H NMR (400 MHz, MeOD) δ 8.11 (d, 1H, *J* = 8.7 Hz, H₆), 7.65 (d, 1H, *J* = 8.6 Hz, H₄), 7.36 (s, 1H, =CH-), 6.92 (d, 1H, *J* = 2.0 Hz, H₇), 6.80 (dd, 1H, *J* = 8.6, 2.0 Hz, H₅), 6.42 (dd, 1H, *J* = 8.7, 2.2 Hz, H₅), 6.35 (d, 1H, *J* = 2.2 Hz, H₃), 4.13 (t, 2H, *J* = 6.4 Hz, OCH₂CH₂CH₂CH₃), 1.93-1.70 (m, 2H, OCH₂CH₂CH₂CH₃), 1.54 (m, 2H, OCH₂CH₂CH₂CH₃), 1.01 (t, 3H, *J* = 7.4 Hz, OCH₂CH₂CH₂CH₃). ¹³C NMR (100 MHz, MeOD) δ 184.5 (C₃), 169.5, 168.7, 162.8, 161.1 (C₆, C₈, C₂, C₄), 147.2 (C₂), 134.4 (C₆), 126.2 (C₄), 116.0 (C₉), 113.9 (C₅), 112.8 (C₁), 109.9 (C₅), 109.3 (=CH-), 103.1 (C₃), 97.9 (C₇), 69.9 (OCH₂CH₂CH₂CH₃), 32.2 (OCH₂CH₂CH₂CH₃), 20.2 (OCH₂CH₂CH₂CH₃), 14.1 (OCH₂CH₂CH₂CH₃). HRMS (ESI) *m/z* calc. for C₁₉H₁₉O₅ (M + H)⁺: 327.12270. Found: 327.12203.

(Z)-2-(2,4-Dihydroxybenzylidene)-6-chlorobenzofuran-3(2H)-one (8). The crude product was prepared according to the general procedure D from 6-chlorobenzofuran-3(2H)-one (200 mg, 1.19 mmol), and purified by column chromatography on silica gel (eluent CH₂Cl₂-MeOH) to afford **8** (42 mg, 9%) as an orange solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.44 (br s, 1H, OH), 10.24 (br s, 1H, OH), 8.00 (d, 1H, *J* = 8.5 Hz, H₆), 7.77 (d, 1H, *J* = 8.2 Hz, H₄), 7.74 (d, 1H, *J* = 1.4 Hz, H₇), 7.33 (dd, 1H, *J* = 8.2, 1.4 Hz, H₅), 7.23 (s, 1H, =CH-), 6.42 (m, 2H, H₃, H₅). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 181.4 (C₃), 164.9, 161.8, 159.9 (C₈, C₂, C₄), 144.3, 140.8 (C₂, C₆), 132.9 (C₆), 125.3 (C₄), 124.1 (C₅), 120.6 (C₉), 113.6 (C₇), 110.3 (C₁), 108.7 (=CH-), 108.6 (C₅), 102.3 (C₃). HRMS (ESI) *m/z* calc. for C₁₅H₁₀O₄Cl (M + H)⁺: 289.02621. Found: 289.02573.

(Z)-2-(2,4-Dihydroxybenzylidene)-6-(dimethylamino)benzofuran-3(2H)-one (9). To a solution of **47** (301 mg, 0.920 mmol) in pyridine (15 mL) was added Hg(OAc)₂ (731 mg, 2.30 mmol). The solution was refluxed for 15 h. After cooling, iced water was added and the mixture was extracted three times with AcOEt. The combined organic layers were washed with water and brine, dried over anhydrous MgSO₄, filtered, and the filtrate was concentrated under reduced pressure. To a solution of the residue in anhydrous CH₂Cl₂ (8 mL) was added pure BBr₃ (1.75 mL, 18.4 mmol) at 0 °C. The solution was stirred at 25 °C for 2 days. Cold water was then added and the suspension was extracted three times with AcOEt. The combined organic layers were washed with water and brine, dried over anhydrous MgSO₄, filtered, and the filtrate was concentrated under reduced pressure. The residue was washed with acetone to afford **9** (17 mg, 6%) as a black solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.17 (s, 1H, OH), 9.91 (s, 1H, OH), 7.97 (d, 1H, *J* = 8.6 Hz, H₆), 7.48 (d, 1H, *J* = 8.7 Hz, H₄), 6.93 (s, 1H, =CH-), 6.64-6.55 (m, 2H, H₅, H₇), 6.42-6.34 (m, 2H, H₃, H₅), 3.09 (s, 6H, N(CH₃)₂). ¹³C NMR (125 MHz, DMSO-*d*₆) δ 180.1 (C₃), 167.4, 160.3, 158.6, 156.5 (C₆, C₈, C₂, C₄), 146.0 (C₂), 132.1 (C₆), 124.9, 111.0 (C₁), 109.5 (C₅), 108.6 (C₉), 108.0 (C₅), 104.0 (=CH-), 102.3 (C₃), 92.7 (C₇), 40.2 (N(CH₃)₂). HRMS (ESI) *m/z* calc. for C₁₇H₁₆NO₄ (M + H)⁺: 298.10738. Found: 298.10749.

(Z)-2-(2,4-Dihydroxybenzylidene)-7-hydroxybenzofuran-3(2H)-one (10). The crude product was prepared according to the general procedure B from **49** (120 mg, 0.384 mmol), and purified by column chromatography on silica gel (eluent CH₂Cl₂-MeOH) to afford **10** (53 mg, 51%) as a yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.39 (s, 1H, OH), 10.38 (s, 1H, OH), 10.11 (s, 1H, OH), 8.14 (d, 1H, *J* = 9.2 Hz, H₆), 7.18 (m, 3H, H₄, H₆, =CH-), 7.08 (t, 1H, *J* = 7.7 Hz, H₅), 6.43 (m, 2H, H₃, H₅). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 183.2 (C₃), 161.4, 159.6, 153.5 (C₈, C₂, C₄), 144.2, 143.4 (C₂, C₇), 133.1 (C₆), 124.0 (C₉), 123.1 (C₆), 122.4 (C₅), 113.6 (C₄), 110.6 (C₁), 108.5 (C₅), 107.9 (=CH-), 102.2 (C₃). HRMS (ESI) *m/z* calc. for C₁₅H₁₁O₅ (M + H)⁺: 271.06010. Found: 271.05891.

(Z)-2-(2,4-Dihydroxybenzylidene)-7-methoxybenzofuran-3(2H)-one (11). To a solution of 7-methoxybenzofuran-3(2H)-one (200 mg, 1.22 mmol) in MeOH (6 mL) were added SOCl₂ (132 μL, 1.83 mmol) dropwise and 2,4-dihydroxybenzaldehyde (169 mg, 1.22 mmol). The solution was stirred at 25 °C for 2 h. The precipitate was then filtered,

washed with EtOH, dried, and purified by column chromatography on silica gel (eluent CH₂Cl₂-MeOH) to afford **11** (32 mg, 15%) as an orange solid. ¹H NMR (400 MHz, MeOD) δ 8.17 (d, 1H, *J* = 8.7 Hz, H₆), 7.49 (s, 1H, =CH-), 7.32 (m, 2H, H₄, H₆), 7.18 (t, 1H, *J* = 7.8 Hz, H₅), 6.44 (d, 1H, *J* = 8.7 Hz, H₅), 6.36 (s, 1H, H₃), 4.04 (s, 3H, OCH₃). ¹³C NMR (100 MHz, MeOD) δ 186.0 (C₃), 163.3, 161.6, 156.5 (C₈, C₂, C₄), 147.6, 146.3 (C₂, C₇), 134.8 (C₆), 125.0 (C₅), 124.6 (C₉), 119.5 (C₆), 116.1 (C₄), 112.8 (C₁), 111.6 (C₅), 109.6 (=CH-), 103.1 (C₃), 56.9 (OCH₃). HRMS (ESI) *m/z* calc. for C₁₆H₁₃O₅ (M + H)⁺: 285.07575. Found: 285.07543.

(Z)-2-(2,4-Dihydroxybenzylidene)-6-(dimethylamino)-4-hydroxybenzofuran-3(2H)-one (13). The crude product was prepared according to the general procedure B from **44** (754 mg, 2.12 mmol), and precipitated from AcOEt to afford **13** (208 mg, 31%) as a brown solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.43 (s, 1H, OH), 10.09 (s, 1H, OH), 9.83 (s, 1H, OH), 7.90 (d, 1H, *J* = 8.5 Hz, H₆), 6.78 (s, 1H, =CH-), 6.43-6.30 (m, 2H, H₃, H₅), 6.12 (d, 1H, *J* = 1.9 Hz, H₅), 5.83 (d, 1H, *J* = 1.9 Hz, H₇), 3.02 (s, 6H, N(CH₃)₂). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 178.1 (C₃), 167.4, 159.8, 158.2, 157.5, 157.3 (C₄, C₆, C₈, C₂, C₄), 146.2 (C₂), 131.8 (C₆), 111.2 (C₁), 107.9 (C₅), 102.4 (=CH-), 102.1 (C₃), 100.1 (C₅), 92.8 (C₉), 86.0 (C₇), 40.1 (N(CH₃)₂). HRMS (ESI) *m/z* calc. for C₁₇H₁₆NO₅ (M + H)⁺: 314.10230. Found: 314.10259.

(Z)-2-(2,4-Dihydroxybenzylidene)-4,6-dimethoxybenzofuran-3(2H)-one (14). The crude product was prepared according to the general procedure D from 4,6-dimethoxybenzofuran-3(2H)-one (150 mg, 0.772 mmol), and recrystallized from MeOH to afford **14** (17 mg, 7%) as an orange solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.26 (s, 1H, OH), 9.99 (s, 1H, OH), 7.93 (d, 1H, *J* = 8.6 Hz, H₆), 6.96 (s, 1H, =CH-), 6.67 (d, 1H, *J* = 1.7 Hz, H₇), 6.40 (d, 1H, *J* = 2.3 Hz, H₃), 6.37 (dd, 1H, *J* = 8.6, 2.3 Hz, H₅), 6.31 (d, 1H, *J* = 1.7 Hz, H₅), 3.90 (s, 3H, OCH₃), 3.87 (s, 3H, OCH₃). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 178.8 (C₃), 168.3, 167.7, 160.7, 158.9, 158.7 (C₄, C₆, C₈, C₂, C₄), 145.0 (C₂), 132.2 (C₆), 110.6 (C₁), 108.2 (C₅), 105.0 (=CH-), 104.5 (C₉), 102.3 (C₃), 94.2 (C₅), 89.7 (C₇), 56.4 (OCH₃), 56.1 (OCH₃). HRMS (ESI) *m/z* calc. for C₁₇H₁₅O₆ (M + H)⁺: 315.08631. Found: 315.08514.

(Z)-2-(2,4-Dihydroxybenzylidene)-4,6-difluorobenzofuran-3(2H)-one (15). The crude product was prepared according to the general procedure B from **50** (64 mg, 0.200 mmol), and purified by column chromatography on silica gel (eluent CH₂Cl₂-MeOH) to afford **15** (27 mg, 90%) as a yellow solid. ¹H NMR (400 MHz, MeOD) δ 8.05 (d, 1H, *J* = 8.8 Hz, H₆), 7.42 (s, 1H, =CH-), 7.08 (m, 1H, H₇), 6.81 (m, 1H, H₅), 6.41 (dd, 1H, *J* = 8.8, 2.4 Hz, H₅), 6.34 (d, 1H, *J* = 2.4 Hz, H₃). ¹³C NMR (100 MHz, MeOD) δ 180.5 (C₃), 173.0, 171.3 (d, 168.3 (dd) (C₄, C₆, C₈), 163.6, 161.7 (C₂, C₄), 160.5 (dd, C₉), 146.1 (C₂), 134.6 (C₆), 112.3 (C₁), 111.5 (=CH-), 109.6 (C₅), 103.1 (C₃), 100.3 (dd, C₅), 98.3 (dd, C₇). HRMS (ESI) *m/z* calc. for C₁₅H₇F₂O₄ (M - H)⁻: 289.0307. Found: 289.0309.

(Z)-2-(2,4-Dihydroxybenzylidene)-6,7-dihydroxybenzofuran-3(2H)-one (16). The crude product was prepared according to the general procedure A from 6,7-dihydroxybenzofuran-3(2H)-one (100 mg, 0.602 mmol) and 2,4-dihydroxybenzaldehyde (83 mg, 0.602 mmol), and purified by column chromatography on silica gel (eluent CH₂Cl₂-MeOH) to afford **16** (113 mg, 66%) as a yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.54 (s, 1H, OH), 10.26 (s, 1H, OH), 10.01 (s, 1H, OH), 9.54 (s, 1H, OH), 8.15 (d, 1H, *J* = 9.3 Hz, H₆), 7.09 (d, 1H, *J* = 8.3 Hz, H₄), 7.05 (s, 1H, =CH-), 6.71 (d, 1H, *J* = 8.3 Hz, H₅), 6.41 (m, 2H, H₃, H₅). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 181.9 (C₃), 160.9, 159.1, 154.7, 153.9 (C₆, C₈, C₂, C₄), 145.4 (C₂), 133.0 (C₆), 130.1 (C₇), 115.1 (C₉), 114.8 (C₄), 112.6 (C₅), 110.7 (C₁), 108.3 (C₅), 105.9 (=CH-), 102.2 (C₃). HRMS (ESI) *m/z* calc. for C₁₅H₁₁O₆ (M + H)⁺: 287.05501. Found: 287.05448.

(Z)-2-(2,4-Dihydroxybenzylidene)-6-hydroxy-7-methylbenzofuran-3(2H)-one (17). The crude product was prepared according to the general procedure A from 6-hydroxy-7-methylbenzofuran-3(2H)-one (100 mg, 0.609 mmol) and 2,4-dihydroxybenzaldehyde (168 mg, 1.22 mmol), and washed with water to afford **17** (27 mg, 16%) as a

yellow solid. ^1H NMR (400 MHz, DMSO- d_6) δ 10.88 (s, 1H, OH), 10.24 (s, 1H, OH), 9.99 (s, 1H, OH), 8.00 (d, 1H, $J = 8.7$ Hz, H₆), 7.43 (d, 1H, $J = 8.3$ Hz, H₄), 7.05 (s, 1H, =CH-), 6.75 (d, 1H, $J = 8.3$ Hz, H₅), 6.42 (m, 2H, H₃, H₅), 2.20 (s, 3H, CH₃). ^{13}C NMR (100 MHz, DMSO- d_6) δ 181.7 (C₃), 165.3, 163.3, 160.8, 159.1 (C₆, C₈, C₂, C₄), 145.3 (C₂), 132.4 (C₆), 122.3 (C₄), 113.1 (C₉), 111.7 (C₅), 110.7 (C₁), 108.4 (C₅), 107.4 (C₇), 105.6 (=CH-), 102.3 (C₃), 7.7 (CH₃). HRMS (ESI) m/z calc. for C₁₆H₁₃O₅ (M + H)⁺: 285.07575. Found: 285.07491.

(Z)-2-(2,4-Dihydroxybenzylidene)-6-hydroxy-7-iodobenzo-furan-3(2H)-one (18). The crude product was prepared according to the general procedure A from 6-hydroxy-7-iodobenzo-furan-3(2H)-one (168 mg, 0.609 mmol) and 2,4-dihydroxybenzaldehyde (168 mg, 1.22 mmol), and washed with water to afford **18** (48 mg, 20%) as an orange solid. ^1H NMR (400 MHz, DMSO- d_6) δ 11.83 (s, 1H, OH), 10.38 (s, 1H, OH), 10.11 (s, 1H, OH), 8.09 (d, 1H, $J = 8.6$ Hz, H₆), 7.56 (d, 1H, $J = 8.3$ Hz, H₄), 7.13 (s, 1H, =CH-), 6.79 (d, 1H, $J = 8.3$ Hz, H₅), 6.43 (d, 1H, $J = 2.3$ Hz, H₃), 6.39 (dd, 1H, $J = 8.6, 2.3$ Hz, H₅). ^{13}C NMR (100 MHz, DMSO- d_6) δ 181.2 (C₃), 167.4, 165.1, 161.2, 159.5 (C₆, C₈, C₂, C₄), 145.0 (C₂), 132.5 (C₆), 125.0 (C₄), 114.3 (C₉), 111.6 (C₅), 110.4 (C₁), 108.4 (C₅), 106.7 (=CH-), 102.3 (C₃), 67.4 (C₇). HRMS (ESI) m/z calc. for C₁₅H₁₀O₅I (M + H)⁺: 396.95674. Found: 396.95566.

(Z)-2-(2,4-Dihydroxybenzylidene)-7-methyl-6-methoxybenzofuran-3(2H)-one (19). The crude product was prepared according to the general procedure D from 7-methyl-6-methoxybenzofuran-3(2H)-one (76 mg, 0.430 mmol), and purified by column chromatography on silica gel (eluent CH₂Cl₂-MeOH) to afford **19** (41 mg, 32%) as a yellow solid. ^1H NMR (400 MHz, DMSO- d_6) δ 10.29 (s, 1H, OH), 10.03 (s, 1H, OH), 8.01 (d, 1H, $J = 8.3$ Hz, H₆), 7.61 (d, 1H, $J = 8.6$ Hz, H₄), 7.10 (s, 1H, =CH-), 6.96 (d, 1H, $J = 8.6$ Hz, H₅), 6.43 (m, 2H, H₃, H₅), 3.95 (s, 3H, OCH₃), 2.24 (s, 3H, CH₃). ^{13}C NMR (100 MHz, DMSO- d_6) δ 181.9 (C₃), 164.1, 163.9, 161.0, 159.2 (C₆, C₈, C₂, C₄), 145.1 (C₂), 132.5 (C₆), 122.6 (C₄), 114.6 (C₉), 110.6 (C₁), 109.1 (C₇), 108.4 (C₅), 107.1 (C₅), 106.2 (=CH-), 102.3 (C₃), 56.6 (OCH₃), 7.8 (CH₃). HRMS (ESI) m/z calc. for C₁₇H₁₃O₅ (M - H)⁻: 297.07685. Found: 297.07554.

(Z)-2-(2,4-Dihydroxybenzylidene)-6,7-dimethoxybenzofuran-3(2H)-one (20). The crude product was prepared according to the general procedure D from 6,7-dimethoxybenzofuran-3(2H)-one (115 mg, 0.592 mmol), and washed with water to afford **20** (29 mg, 16%) as a yellow solid. ^1H NMR (400 MHz, DMSO- d_6) δ 10.36 (s, 1H, OH), 10.10 (s, 1H, OH), 7.96 (d, 1H, $J = 9.1$ Hz, H₆), 7.48 (d, 1H, $J = 8.6$ Hz, H₄), 7.13 (s, 1H, =CH-), 7.01 (d, $J = 8.6$ Hz, 1H, H₅), 6.43 (m, 2H, H₃, H₅), 4.01 (s, 3H, OCH₃), 3.94 (s, 3H, OCH₃). ^{13}C NMR (100 MHz, DMSO- d_6) δ 181.5 (C₃), 161.2, 159.4, 158.4, 156.7 (C₆, C₈, C₂, C₄), 144.9 (C₂), 133.4 (C₇), 132.3 (C₆), 119.2 (C₉), 116.5 (C₄), 110.4 (C₁), 108.9 (C₅), 108.5 (C₅), 106.8 (=CH-), 102.3 (C₃), 60.7 (OCH₃), 56.8 (OCH₃). HRMS (ESI) m/z calc. for C₁₇H₁₅O₆ (M + H)⁺: 315.08631. Found: 315.08517.

(E)-2-(2,4-Dihydroxybenzylidene)-2,3-dihydro-1H-inden-1-one (21). The crude product was prepared according to the general procedure B from **51** (200 mg, 0.710 mmol), and purified by column chromatography on silica gel (eluent CH₂Cl₂-MeOH) to afford **21** (36 mg, 20%) as a red solid. ^1H NMR (400 MHz, MeOD) δ 8.15 (t, 1H, $J = 2.0$ Hz, =CH-), 7.79 (d, 1H, $J = 8.0$ Hz, H₆), 7.66-7.59 (m, 3H, H₄, H₅, H₇), 7.42 (m, 1H, H₆), 6.43 (dd, 1H, $J = 8.0, 2.4$ Hz, H₅), 6.37 (d, 1H, $J = 2.4$ Hz, H₃), 3.98 (d, 2H, $J = 2.0$ Hz, H₃). ^{13}C NMR (100 MHz, MeOD) δ 196.9 (C₁), 162.7, 161.5 (C₂, C₄), 151.6 (C₉), 139.5 (C₈), 135.6 (=CH-), 132.4 (C₅), 131.3 (C₂), 131.2 (C₆), 128.6 (C₇), 127.4 (C₆), 124.7 (C₄), 115.8 (C₁), 109.1 (C₅), 103.5 (C₃), 33.5 (C₃). HRMS (ESI) m/z calc. for C₁₆H₁₁NO₃ (M - H)⁻: 251.0714. Found: 251.0708.

(E)-2-(2,4-Dihydroxybenzylidene)-5-hydroxy-2,3-dihydro-1H-inden-1-one (22). The crude product was prepared according to the general procedure B from **52** (300 mg, 1.01 mmol), and purified by column chromatography on silica gel (eluent CH₂Cl₂-MeOH) to afford **22** (89 mg, 33%) as a yellow solid. ^1H NMR (400 MHz, DMSO- d_6) δ 10.48 (s, 1H, OH), 10.06 (s, 1H, OH), 9.87 (s, 1H, OH), 7.77 (s, 1H,

=CH-), 7.59 (d, 1H, $J = 8.3$ Hz, H₇), 7.53 (d, 1H, $J = 8.6$ Hz, H₆), 6.94 (s, 1H, H₄), 6.83 (dd, 1H, $J = 8.3, 1.9$ Hz, H₆), 6.41 (d, 1H, $J = 2.2$ Hz, H₃), 6.36 (dd, 1H, $J = 8.6, 2.2$ Hz, H₅), 3.88 (s, 2H, H₃). ^{13}C NMR (100 MHz, DMSO- d_6) δ 191.7 (C₁), 163.3, 160.5, 159.3 (C₅, C₂, C₄), 152.7 (C₉), 130.8 (C₆), 130.5 (=CH-), 129.9 (C₈), 126.2 (C₇), 125.4 (C₂), 115.8 (C₄), 113.8 (C₆), 111.9 (C₁), 107.8 (C₅), 102.5 (C₃), 32.0 (C₃). HRMS (ESI) m/z calc. for C₁₆H₁₃O₄ (M + H)⁺: 269.08084. Found: 269.07980.

(E)-2-(2,4-Dihydroxybenzylidene)-4-hydroxy-2,3-dihydro-1H-inden-1-one (23). To a solution of 4-hydroxy-2,3-dihydro-1H-inden-1-one (200 mg, 1.35 mmol) in MeOH (6 mL) were added SOCl₂ (146 μL , 2.03 mmol) dropwise and 2,4-dihydroxybenzaldehyde (186 mg, 1.35 mmol). The solution was stirred at 25 °C for 2 h. The precipitate was then filtered and washed with water, EtOH and MeOH to afford **23** (139 mg, 38%) as a red solid. ^1H NMR (400 MHz, MeOD) δ 9.26 (s, 1H, =CH-), 8.24 (d, 1H, $J = 9.0$ Hz, H₆), 7.85 (d, 1H, $J = 7.9$ Hz, H₇), 7.69-7.55 (m, 2H, H₆, H₃), 7.48 (dd, 1H, $J = 9.0, 2.2$ Hz, H₅), 7.31 (d, 1H, $J = 7.9$ Hz, H₅), 4.19 (s, 2H). ^{13}C NMR (100 MHz, MeOD) δ 176.8 (C₁), 169.7, 159.8, 156.3 (C₄, C₂, C₄), 150.6 (C₉), 137.9 (C₈), 134.5 (=CH-), 134.4 (C₆), 132.4 (C₂), 132.3 (C₆), 123.6 (C₅), 122.3 (C₁), 120.3 (C₇), 116.4 (C₅), 104.2 (C₃), 31.7 (C₃). HRMS (ESI) m/z calc. for C₁₆H₁₁O₄ (M - H)⁻: 267.06628. Found: 267.06604.

(E)-2-(2,4-Dihydroxybenzylidene)-3-methyl-2,3-dihydro-1H-inden-1-one (24). The crude product was prepared according to the general procedure B from **53** (129 mg, 0.440 mmol), to afford **24** (112 mg, 96%) as a yellow oil. ^1H NMR (400 MHz, MeOD) δ 8.07 (d, 1H, $J = 1.8$ Hz, =CH-), 7.78 (m, 1H, H₄), 7.66-7.59 (m, 2H, H₅, H₇), 7.52 (d, 1H, $J = 8.6$ Hz, H₆), 7.41 (m, 1H, H₅), 6.44 (dd, 1H, $J = 8.6, 2.4$ Hz, H₅), 6.38 (d, 1H, $J = 2.4$ Hz, H₃), 4.34 (m, 1H, H₃), 1.35 (m, 3H, CH₃). ^{13}C NMR (100 MHz, MeOD) δ 196.5 (C₁), 162.5, 161.2 (C₂, C₄), 157.8 (C₉), 138.2, 137.6 (C₂, C₈), 135.9 (=CH-), 133.2 (C₅), 131.6 (C₆), 128.7 (C₇), 126.3 (C₆), 124.5 (C₄), 114.8 (C₁), 108.9 (C₅), 103.4 (C₃), 38.1 (C₃), 19.0 (CH₃). HRMS (ESI) m/z calc. for C₁₇H₁₅O₃ (M + H)⁺: 267.10157. Found: 267.10136.

2-(2,4-Dihydroxybenzylidene)-1H-indene-1,3(2H)-dione (25). The crude product was prepared according to the general procedure B from **54** (25 mg, 0.085 mmol), and purified by column chromatography on silica gel (eluent CH₂Cl₂-AcOEt) to afford **25** (4 mg, 15%) as a red solid. ^1H NMR (400 MHz, MeOD) δ 9.12 (d, 1H, $J = 8.7$ Hz, H₆), 8.29 (s, 1H, =CH-), 7.90-7.88 (m, 4H, H₄₋₇), 6.46-6.39 (m, 2H, H₃, H₅). ^{13}C NMR (100 MHz, MeOD) δ 190.6, 189.3 (C₁, C₃), 166.1, 163.4 (C₂, C₄), 141.5, 140.2 (C₈, C₉), 139.1 (=CH-), 135.9 (C₆), 135.2, 135.0 (C₅, C₆), 122.5 (C₂), 122.4, 122.4 (C₄, C₇), 113.1 (C₁), 108.7 (C₅), 101.9 (C₃). HRMS (ESI) m/z calc. for C₁₆H₉O₄ (M - H)⁻: 265.04954. Found: 265.05060.

(Z)-2-(2,4-Dihydroxybenzylidene)indolin-3-one (26). The crude product was prepared according to the general procedure B from **57** (42 mg, 0.15 mmol), and purified by column chromatography on silica gel (eluent CH₂Cl₂-MeOH) to afford **26** (8 mg, 20%) as a red solid. ^1H NMR (400 MHz, MeOD) δ 7.62 (d, 1H, $J = 7.7$ Hz, H₄), 7.53 (d, 1H, $J = 8.6$ Hz, H₆), 7.49-7.44 (m, 1H, H₆), 7.21 (s, 1H, =CH-), 7.10 (d, 1H, $J = 8.2$ Hz, H₇), 6.89 (m, 1H, H₅), 6.44 (dd, 1H, $J = 8.6, 2.4$ Hz, H₅), 6.38 (d, 1H, $J = 2.4$ Hz, H₃). ^{13}C NMR (100 MHz, MeOD) δ 188.6 (C₃), 161.8, 159.8, 155.0 (C₈, C₂, C₄), 137.0 (C₂), 134.1 (C₆), 133.1 (C₆), 125.1 (C₄), 122.1 (C₅), 120.4 (=CH-), 114.9 (C₉), 113.5 (C₇), 111.7 (C₁), 109.2 (C₅), 103.6 (C₃). HRMS (ESI) m/z calc. for C₁₅H₁₂NO₃ (M + H)⁺: 254.0812. Found: 254.0808.

(Z)-2-(2,4-Dihydroxybenzylidene)-6-hydroxyindolin-3-one (27). The crude product was prepared according to the general procedure B from **58** (367 mg, 1.20 mmol), and purified by column chromatography on silica gel (eluent CH₂Cl₂-MeOH) to afford **27** (139 mg, 43%) as a red solid. ^1H NMR (400 MHz, MeOD) δ 7.50-7.46 (m, 2H, H₄, H₆), 7.07 (s, 1H, =CH-), 6.44-6.40 (m, 2H, H₅, H₇), 6.38-6.34 (m, 2H, H₃, H₅). ^{13}C NMR (100 MHz, MeOD) δ 186.6 (C₃), 167.1, 161.4, 159.4, 157.7 (C₆, C₈, C₂, C₄), 135.0 (C₂), 132.9 (C₆), 127.2 (C₄), 115.0, 114.9 (C₁, C₉), 110.5 (=CH-), 110.3 (C₅), 109.1 (C₅), 103.6 (C₃), 98.3 (C₇).

7.86–7.74 (m, 1H, H₄), 7.62 (ddd, 1H, *J* = 8.3, 7.3, 1.4 Hz, H₆), 7.45 (s, 1H, =CH–), 7.31 (d, 1H, *J* = 8.3 Hz, H₇), 7.23–7.16 (m, 1H, H₅), 6.63 (dd, 1H, *J* = 8.8, 2.4 Hz, H₅), 6.47 (d, 1H, *J* = 2.4 Hz, H₃), 3.89 (s, 3H, OCH₃), 3.88 (s, 3H, OCH₃). ¹³C NMR (100 MHz, CDCl₃) δ 184.5 (C₃), 165.7 (C₈), 162.9, 160.7 (C₂, C₄), 146.1 (C₂), 136.3 (C₆), 133.5 (C₆), 124.6 (C₄), 123.2 (C₅), 122.4 (C₉), 114.6 (C₁), 113.0 (C₇), 107.9 (=CH–), 105.9 (C₅), 98.1 (C₃), 55.8 (OCH₃), 55.6 (OCH₃). HRMS (ESI) *m/z* calc. for C₁₇H₁₅O₄ (M + H)⁺: 283.09649. Found: 283.09510.

(Z)-2-(2,4-Dimethoxybenzylidene)-7-methoxybenzofuran-3(2H)-one (49). The crude product was prepared according to the general procedure A from 7-methoxybenzofuran-3(2H)-one (200 mg, 1.22 mmol) and 2,4-dimethoxybenzaldehyde (203 mg, 1.22 mmol), to afford **49** (156 mg, 41%) as a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 8.34 (d, 1H, *J* = 8.8 Hz, H₆), 7.48 (s, 1H, =CH–), 7.39 (dd, 1H, *J* = 6.7, 2.0 Hz, H₄), 7.18–7.06 (m, 2H, H₅, H₆), 6.65 (dd, 1H, *J* = 8.8, 2.4 Hz, H₅), 6.46 (d, 1H, *J* = 2.4 Hz, H₃), 4.02 (s, 3H, OCH₃), 3.89 (s, 3H, OCH₃), 3.87 (s, 3H, OCH₃). ¹³C NMR (100 MHz, CDCl₃) δ 184.6 (C₃), 163.0, 160.7, 155.5 (C₈, C₂, C₄), 146.1, 146.0 (C₂, C₇), 133.8 (C₆), 123.8 (C₉), 123.6 (C₅), 118.1 (C₆), 115.9 (C₄), 114.6 (C₁), 108.4 (=CH–), 106.0 (C₅), 98.1 (C₃), 56.5 (OCH₃), 55.8 (OCH₃), 55.6 (OCH₃). HRMS (ESI) *m/z* calc. for C₁₈H₁₇O₅ (M + H)⁺: 313.10705. Found: 313.10555.

(Z)-2-(2,4-Dimethoxybenzylidene)-4,6-difluorobenzofuran-3(2H)-one (50). To a solution of 4,6-difluorobenzofuran-3(2H)-one (95 mg, 0.560 mmol) in anhydrous CH₂Cl₂ (11 mL) were added 2,4-dimethoxybenzaldehyde (139 mg, 0.840 mmol) and neutral Al₂O₃ (2.24 g). The suspension was stirred at 25 °C for 18 h. The solid was then removed by filtration, and the filtrate was concentrated under reduced pressure. The residue was recrystallized from MeOH to afford **50** (83 mg, 47%) as a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 8.17 (d, 1H, *J* = 8.8 Hz, H₆), 7.44 (s, 1H, =CH–), 6.83 (dd, 1H, *J* = 8.4, 0.9 Hz, H₇), 6.64–6.52 (m, 2H, H₅, H₅), 6.46 (d, 1H, *J* = 2.3 Hz, H₃), 3.89 (s, 3H, OCH₃), 3.88 (s, 3H, OCH₃). ¹³C NMR (100 MHz, CDCl₃) δ 179.2 (C₃), 169.7 (d), 167.2 (d), 166.6 (dd) (C₄, C₆, C₈), 163.4, 160.9 (C₂, C₄), 159.4 (dd, C₉), 145.8 (C₂), 133.5 (C₆), 114.0 (C₁), 108.8 (=CH–), 106.4 (C₅), 99.6 (dd, C₅), 98.2 (C₃), 97.2 (dd, C₇), 55.8 (OCH₃), 55.7 (OCH₃). HRMS (ESI) *m/z* calc. for C₁₇H₁₃O₄F₂ (M + H)⁺: 319.0776. Found: 319.0769.

(E)-2-(2,4-Dimethoxybenzylidene)-2,3-dihydro-1H-inden-1-one (51). The crude product was prepared according to the general procedure A from 2,3-dihydro-1H-inden-1-one (200 mg, 1.48 mmol) and 2,4-dimethoxybenzaldehyde (246 mg, 1.48 mmol), to afford **51** (405 mg, 97%) as a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 8.08 (t, 1H, *J* = 2.1 Hz, =CH–), 7.87 (d, 1H, *J* = 7.6 Hz, H₇), 7.61 (d, 1H, *J* = 8.6 Hz, H₆), 7.58–7.46 (m, 2H, H₄, H₅), 7.37 (m, 1H, H₆), 6.53 (dd, 1H, *J* = 8.6, 2.5 Hz, H₅), 6.45 (d, 1H, *J* = 2.5 Hz, H₃), 3.91 (d, 2H, *J* = 2.1 Hz, H₃), 3.85 (s, 3H, OCH₃), 3.82 (s, 3H, OCH₃). ¹³C NMR (100 MHz, CDCl₃) δ 194.2 (C₁), 162.4, 160.7 (C₂, C₄), 149.5 (C₈), 138.4 (C₉), 134.0 (C₅), 132.1 (C₂), 130.7 (C₆), 128.2 (C₇), 127.3 (C₆), 125.9 (C₄), 124.1 (=CH–), 117.5 (C₁), 105.2 (C₅), 98.2 (C₃), 55.5 (OCH₃), 55.4 (OCH₃), 32.5 (C₃). HRMS (ESI) *m/z* calc. for C₁₈H₁₇NO₃ (M + H)⁺: 281.1172. Found: 281.1170.

(E)-2-(2,4-Dimethoxybenzylidene)-5-hydroxy-2,3-dihydro-1H-inden-1-one (52). The crude product was prepared according to the general procedure A from 5-hydroxy-2,3-dihydro-1H-inden-1-one (200 mg, 1.35 mmol) and 2,4-dimethoxybenzaldehyde (336 mg, 2.03 mmol), to afford **52** (389 mg, 97%) as a yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.54 (s, 1H, OH), 7.74 (s, 1H, =CH–), 7.70 (d, 1H, *J* = 9.2 Hz, H₇), 7.61 (d, 1H, *J* = 8.3 Hz, H₆), 6.95 (d, 1H, *J* = 1.7 Hz, H₄), 6.84 (dd, 1H, *J* = 8.3, 2.1 Hz, H₅), 6.68–6.62 (m, 2H, H₆, H₃), 3.92 (s, 2H), 3.89 (s, 3H), 3.84 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 191.5 (C₁), 163.6, 162.1, 160.1 (C₅, C₂, C₄), 152.9 (C₈), 132.9 (C₂), 130.6 (C₆), 129.6 (C₉), 125.6 (C₇), 125.0 (=CH–), 116.4 (C₄), 116.0 (C₁), 111.9 (C₆), 106.0 (C₅), 98.4 (C₃), 55.8 (OCH₃), 55.5 (OCH₃), 31.8 (C₃). HRMS (ESI) *m/z* calc. for C₁₈H₁₇O₄ (M + H)⁺: 296.11214. Found: 296.11096.

(E)-2-(2,4-Dimethoxybenzylidene)-3-methyl-2,3-dihydro-1H-inden-1-one (53). The crude product was prepared according to the general procedure A from 3-methyl-2,3-dihydro-1H-inden-1-one (93 μL,

0.680 mmol) and 2,4-dimethoxybenzaldehyde (114 mg, 0.680 mmol), and purified by column chromatography on silica gel (eluent CH₂Cl₂-AcOEt) to afford **53** (129 mg, 64%) as a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 8.00 (d, 1H, *J* = 2.1 Hz, =CH–), 7.84 (m, 1H, H₆), 7.58–7.46 (m, 3H, H₄, H₆, H₇), 7.35 (m, 1H, H₅), 6.52 (dd, 1H, *J* = 8.6, 2.4 Hz, H₅), 6.43 (d, 1H, *J* = 2.4 Hz, H₃), 4.25 (m, 1H, H₃), 3.81 (s, 3H, OCH₃), 3.79 (s, 3H, OCH₃), 1.31 (m, 3H, CH₃). ¹³C NMR (100 MHz, CDCl₃) δ 194.0 (C₁), 162.5, 160.5 (C₂, C₄), 155.8 (C₈), 138.4, 137.3 (C₂, C₉), 134.3 (C₇), 131.5 (C₆), 128.7 (C₅), 127.5 (C₆), 124.9 (=CH–), 123.9 (C₄), 116.6 (C₁), 105.2 (C₅), 98.2 (C₃), 55.6 (OCH₃), 55.4 (OCH₃), 36.8 (C₃), 18.4 (CH₃). HRMS (ESI) *m/z* calc. for C₁₉H₁₉O₃ (M + H)⁺: 295.13287. Found: 295.13271.

2-(2,4-Dimethoxybenzylidene)-1H-indene-1,3(2H)-dione (54). The crude product was prepared according to the general procedure A from 1H-indene-1,3(2H)-dione (100 mg, 0.710 mmol) and 2,4-dimethoxybenzaldehyde (166 mg, 0.710 mmol), and recrystallized from MeOH to afford **54** (193 mg, 92%) as a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 9.25 (d, 1H, *J* = 9.0 Hz, H₆), 8.48 (s, 1H, =CH–), 8.00–7.92 (m, 2H, H₄, H₇), 7.80–7.71 (m, 2H, H₅, H₆), 6.64 (dd, 1H, *J* = 9.0, 2.4 Hz, H₅), 6.44 (d, 1H, *J* = 2.4 Hz, H₃), 3.93 (s, 3H, OCH₃), 3.92 (s, 3H, OCH₃). ¹³C NMR (100 MHz, CDCl₃) δ 190.4, 189.6 (C₁, C₃), 166.4, 163.0 (C₂, C₄), 142.3141.0, 140.0 (C₈, C₉ = CH–), 136.6 (C₆), 134.8, 134.6 (C₅, C₆), 125.5 (C₂), 122.3, 122.3 (C₄, C₇), 116.1 (C₁), 105.6 (C₅), 97.7 (C₃), 55.8 (OCH₃), 55.7 (OCH₃). HRMS (ESI) *m/z* calc. for C₁₈H₁₅O₄ (M + H)⁺: 295.0965. Found: 295.0960.

N-(2-(2-Chloroacetyl)-5-methoxyphenyl)acetamide (55). To a solution of *m*-anisidine (10.0 g, 81.2 mmol) in PhNO₂ (100 mL), were added BCl₃ in CH₂Cl₂ (89 mL, 1 M, 89.3 mmol), chloroacetonitrile (6 mL, 97.4 mmol) then AlCl₃ (12.0 g, 89.3 mmol) at 0 °C. The solution was stirred at 80 °C for 4 h then at 25 °C for an additional 18 h. Then, aqueous HCl (1 N, 150 mL) was added, and the mixture was stirred at 80 °C for 1 h. After cooling, the mixture was extracted three times with CH₂Cl₂. The combined organic layers were washed with water and brine, dried over anhydrous MgSO₄, filtered, and the filtrate was concentrated under reduced pressure. The residue was solubilized in acetic anhydride (50 mL) and the mixture was stirred at 80 °C for 1.5 h. After cooling, the mixture was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (eluent CH₂Cl₂) to afford **55** (6.48 g, 33%) as a brown solid. ¹H NMR (400 MHz, CDCl₃) δ 8.42 (d, 1H, *J* = 2.7 Hz, H₃), 7.71 (d, 1H, *J* = 9.0 Hz, H₆), 6.60 (dd, 1H, *J* = 9.0, 2.7 Hz, H₄), 4.68 (s, 2H, CH₂), 3.87 (s, 3H, OCH₃), 2.22 (s, 3H, CH₃). ¹³C NMR (100 MHz, CDCl₃) δ 193.0 (C=O), 169.9 (NH-C=O), 165.7 (C₅), 144.8 (C₁), 132.5 (C₃), 112.3 (C₂), 110.1 (C₆), 104.2 (C₄), 55.8 (OCH₃), 46.8 (CH₂), 25.7 (CH₃). HRMS (ESI) *m/z* calc. for C₁₁H₁₃NO₃Cl (M + H)⁺: 242.05785. Found: 242.05771.

1-Acetyl-6-methoxyindolin-3-one (56). To a solution of **55** (7.00 g, 33.6 mmol) in DMF (140 mL), NaH (674 mg, 28.0 mmol) was added portionwise under argon. The solution was stirred at 25 °C for 1.5 h. The mixture was then concentrated under reduced pressure, and the residue was solubilized in CH₂Cl₂. The solution was washed with saturated aqueous NH₄Cl, water and brine, dried over anhydrous MgSO₄, filtered, and the filtrate was concentrated under reduced pressure to afford **56** (1.10 g, 16%) as a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 8.05 (d, 1H, *J* = 2.2 Hz, H₇), 7.61 (d, 1H, *J* = 8.7 Hz, H₄), 6.72 (dd, 1H, *J* = 8.7, 2.2 Hz, H₅), 4.24 (s, 2H, H₂), 3.89 (s, 3H, OCH₃), 2.28 (s, 3H, CH₃). ¹³C NMR (100 MHz, CDCl₃) δ 192.5 (C₃), 168.4, 167.5 (C₆, N-C=O), 156.2 (C₈), 131.1 (C₄), 118.2 (C₉), 110.0 (C₅), 104.2 (C₇), 56.8 (OCH₃), 24.3 (CH₃). HRMS (ESI) *m/z* calc. for C₁₁H₁₂NO₃ (M + H)⁺: 206.08117. Found: 206.08084.

(Z)-2-(2,4-Dimethoxybenzylidene)indolin-3-one (57). The crude product was prepared according to the general procedure A from 1-acetylindolin-3-one (277 mg, 1.58 mmol) and 2,4-dimethoxybenzaldehyde (263 mg, 1.58 mmol), to afford **57** (42 mg, 10%) as a red solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.73 (d, 1H, *J* = 8.4 Hz, H₆), 7.56 (d, 1H, *J* = 7.6 Hz, H₄), 7.49 (m, 1H, H₆), 7.12 (d, *J* = 8.0 Hz, 1H, H₇), 6.93 (s, 1H, =CH–), 6.89 (m, 1H, H₅), 6.69–6.65 (m, 2H, H₃, H₅), 3.89 (s, 3H,

HRMS (ESI) m/z calc. for $C_{15}H_{12}NO_4$ (M + H)⁺: 270.07608. Found: 270.07596.

(Z)-2-(2,4-Dihydroxybenzylidene)benzo [b] thiophen-3(2H)-one (28). The crude product was prepared according to the general procedure B from **62** (2.00 g, 5.30 mmol), and purified by column chromatography on silica gel (eluent CH_2Cl_2 -MeOH) to afford **28** (201 mg, 14%) as an orange solid. ¹H NMR (400 MHz, MeOD) δ 8.25 (s, 1H, =CH-), 7.84 (dd, 1H, J = 7.8, 1.3 Hz, H₄), 7.77 (d, 1H, J = 7.8 Hz, H₆), 7.68 (m, 1H, H₆), 7.52 (m, 1H, H₇), 7.40 (m, 1H, H₅), 6.47-6.43 (m, 2H, H₃, H₅). ¹³C NMR (100 MHz, MeOD) δ 190.2 (C₃), 163.6, 162.0 (C₂, C₄), 147.6 (C₉), 136.1 (C₈), 132.6 (C₂), 132.3 (C₆), 131.5 (C₆), 127.4 (C₄), 126.5 (C₇), 126.2 (C₅), 125.1 (=CH-), 115.0 (C₁), 109.3 (C₅), 103.5 (C₃). HRMS (ESI) m/z calc. for $C_{15}H_9O_3S$ (M - H)⁻: 269.02669. Found: 269.02802.

(Z)-2-(2,4-Dihydroxybenzylidene)-6-hydroxybenzo [b] thiophen-3(2H)-one (29). The crude product was prepared according to the general procedure B from **63** (348 mg, 1.10 mmol), and purified by column chromatography on silica gel (eluent CH_2Cl_2 -MeOH) to afford **29** (35 mg, 11%) as a dark red solid. ¹H NMR (400 MHz, MeOD) δ 8.34 (s, 1H, =CH-), 7.73 (d, 1H, J = 8.5 Hz, H₄), 7.56 (d, 1H, J = 8.6 Hz, H₆), 6.92 (d, 1H, J = 2.1 Hz, H₇), 6.74 (dd, 1H, J = 8.5, 2.1 Hz, H₅), 6.43 (dd, 1H, J = 8.6, 2.4 Hz, H₅), 6.37 (d, 1H, J = 2.4 Hz, H₃). ¹³C NMR (100 MHz, MeOD) δ 189.0 (C₃), 165.9, 163.1, 161.6 (C₆, C₂, C₄), 150.6 (C₉), 132.4 (C₄), 130.0 (C₆), 129.4 (=CH-), 127.1 (C₈), 124.6 (C₂), 115.4 (C₇), 115.0 (C₁), 110.5 (C₅), 109.1 (C₅), 103.5 (C₃). HRMS (ESI) m/z calc. for $C_{15}H_{11}O_4S$ (M + H)⁺: 287.03726. Found: 287.03687.

(Z)-2-(2,4-dihydroxybenzylidene)-7-hydroxybenzo [b] thiophen-3(2H)-one (30). The crude product was prepared according to the general procedure B from **64** (368 mg, 1.12 mmol), and purified by column chromatography on silica gel (eluent CH_2Cl_2 -MeOH) to afford **30** (103 mg, 32%) as a dark orange solid. ¹H NMR (400 MHz, MeOD) δ 8.44 (s, 1H, =CH-), 7.68 (dd, 1H, J = 8.7 Hz, H₆), 7.39 (dt, 1H, J = 7.6, 1.3 Hz, H₆), 7.17 (td, 1H, J = 7.7, 1.4 Hz, H₅), 7.02 (dt, 1H, J = 7.9, 1.3 Hz, H₄), 6.47 (dd, 1H, J = 8.7, 2.4 Hz, H₅), 6.38 (d, 1H, J = 2.4 Hz, H₃). ¹³C NMR (100 MHz, MeOD) δ 191.0 (C₃), 163.6, 162.0 (C₂, C₄), 154.1 (C₇), 134.5 (C₉), 134.0 (C₈), 132.6 (=CH-), 131.5 (C₆), 127.7 (C₅), 127.0 (C₂), 120.5 (C₄), 118.3 (C₆), 115.1 (C₁), 109.3 (C₅), 103.4 (C₃). HRMS (ESI) m/z calc. for $C_{15}H_9O_4S$ (M - H)⁻: 285.02270. Found: 285.02241.

(E)-3-(2,4-Dihydroxyphenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (31). The crude product was prepared according to the general procedure B from **66** (210 mg, 0.740 mmol), and purified by column chromatography on silica gel (eluent CH_2Cl_2 -MeOH) to afford **31** (100 mg, 39%) as a yellow solid. ¹H NMR (400 MHz, MeOD) δ 8.18 (d, 1H, J = 15.4 Hz, H₁), 8.04 (dd, 1H, J = 8.1, 1.7 Hz, H₆), 7.81 (d, 1H, J = 15.4 Hz, H₄), 7.55 (d, 1H, J = 8.4 Hz, H₆), 7.48 (ddd, 1H, J = 8.6, 7.2, 1.7 Hz, H₄), 7.00-6.91 (m, 2H, H₃, H₅), 6.38 (dd, 1H, J = 8.4, 2.3 Hz, H₅), 6.36 (d, 1H, J = 2.3 Hz, H₃). ¹³C NMR (100 MHz, MeOD) δ 196.0 (C=O), 164.4, 163.3, 161.2 (C₂, C₂, C₄), 143.7 (C₁), 136.8 (C₄), 132.8 (C₆), 131.0 (C₆), 121.7 (C₁), 120.0 (C₅), 119.0 (C₄), 117.3 (C₃), 115.5 (C₁), 109.3 (C₅), 103.6 (C₃). HRMS (ESI) m/z calc. for $C_{15}H_{11}O_4$ (M - H)⁻: 255.0652. Found: 255.0657.

2-(2,4-Dihydroxyphenyl)-4H-chromen-4-one (32). The crude product was prepared according to the general procedure B from **67** (128 mg, 0.450 mmol), and purified by column chromatography on silica gel (eluent CH_2Cl_2 -MeOH) to afford **32** (28 mg, 25%) as a yellow solid. ¹H NMR (400 MHz, MeOD) δ 8.11 (dd, 1H, J = 8.0, 1.6 Hz, H₅), 7.86 (d, 1H, J = 8.8 Hz, H₆), 7.77 (m, 1H, H₇), 7.66 (dd, 1H, J = 8.5, 1.1 Hz, H₆), 7.45 (m, 1H, H₆), 7.35 (s, 1H, H₃), 6.48 (dd, 1H, J = 2.3 Hz, H₅), 6.44 (d, 1H, J = 2.3 Hz, H₃). ¹³C NMR (100 MHz, MeOD) δ 181.5 (C₄), 164.8, 163.7, 160.8, 158.1 (C₂, C₉, C₂, C₄), 135.5 (C₇), 131.5 (C₆), 126.5 (C₅), 126.3 (C₆), 124.6 (C₁₀), 119.5 (C₈), 111.2 (C₃), 110.5 (C₁), 109.5 (C₅), 104.5 (C₃). HRMS (ESI) m/z calc. for $C_{15}H_{11}O_4$ (M + H)⁺: 255.0652. Found: 255.0649.

2-(2,4-Dihydroxybenzyl)isoindoline-1,3-dione (33). The crude product was prepared according to the general procedure B from **68** (84

mg, 0.280 mmol), and purified by column chromatography on silica gel (eluent CH_2Cl_2 -MeOH) to afford **33** (38 mg, 50%) as a white solid. ¹H NMR (400 MHz, MeOD) δ 7.85-7.65 (m, 4H, H₄, H₅, H₆, H₇), 6.91 (d, 1H, J = 8.3 Hz, H₆), 6.29 (d, 1H, J = 2.4 Hz, H₅), 6.22 (dd, 1H, J = 8.3, 2.4 Hz, H₃), 4.72 (s, 2H, CH₂). ¹³C NMR (100 MHz, MeOD) δ 169.9 (C=O), 159.1, 157.2 (C₂, C₄), 135.3 (C₅, C₆), 133.3 (C₆), 131.0 (C₈, C₉), 124.1 (C₄, C₇), 115.1 (C₁), 107.6 (C₅), 103.7 (C₃), 37.5 (CH₂). HRMS (ESI) m/z calc. for $C_{15}H_{11}O_4NNa$ (M + Na)⁺: 292.0580. Found: 292.0576.

2-(2,4-Dihydroxybenzyl)-1,2-dihydro-3H-indazol-3-one (34). To a solution of 2-nitrobenzyl bromide (1.00 g, 4.75 mmol) in MeOH (15 mL), 2,4-dimethoxybenzylamine (2.00 g, 9.50 mmol) and DIPEA (2.45 g, 19.0 mmol) were added dropwise. The solution was stirred at 25 °C for 18 h. Water (8 mL) and KOH (1.00 g, 19.0 mmol) were then added and the mixture was stirred at 60 °C for 24 h. Then, water (50 mL) was added and MeOH was removed under reduced pressure. Water was then added and the mixture was extracted three times with CH_2Cl_2 . The combined organic layers were washed with water and brine, dried over anhydrous $MgSO_4$, filtered, and the filtrate was concentrated under reduced pressure. To a solution of the residue in anhydrous CH_2Cl_2 (10 mL) was added pure BBr_3 (1.60 mL, 16.4 mmol) at 0 °C. The solution was stirred at 25 °C for 2 days. Cold water was then added and the suspension was extracted three times with AcOEt. The combined organic layers were washed with water and brine, dried over anhydrous $MgSO_4$, filtered, and the filtrate was concentrated under reduced pressure to afford **34** (134 mg, 11%) as a white solid. ¹H NMR (400 MHz, MeOD) δ 7.76 (dd, 1H, J = 8.0, 1.2 Hz, H₄), 7.51 (ddd, 1H, J = 8.4, 7.0, 1.2 Hz, H₆), 7.24 (dd, 1H, J = 8.4, 0.9 Hz, H₇), 7.15 (ddd, 1H, J = 8.0, 7.0, 0.9 Hz, H₅), 6.97 (d, 1H, J = 8.3 Hz, H₆), 6.33 (d, 1H, J = 2.4 Hz, H₃), 6.26 (dd, 1H, J = 8.3, 2.4 Hz, H₅), 5.02 (s, 2H, CH₂). ¹³C NMR (100 MHz, MeOD) δ 162.5 (C₃), 159.9, 157.9 (C₂, C₄), 146.9 (C₈), 132.9 (C₆), 131.9 (C₆), 123.9 (C₄), 122.5 (C₅), 117.7 (C₉), 114.4 (C₁), 113.0 (C₇), 107.9 (C₅), 103.9 (C₃), 44.0 (CH₂). HRMS (ESI) m/z calc. for $C_{14}H_{13}O_3N_2$ (M + H)⁺: 257.09207. Found: 257.09145.

4-((7-Hydroxybenzofuran-2-yl)methyl)benzene-1,3-diol (35). The crude product was prepared according to the general procedure B from **69** (441 mg, 1.48 mmol), and purified by column chromatography on silica gel (eluent CH_2Cl_2 -MeOH) to afford **35** (146 mg, 38%) as a yellow oil. ¹H NMR (400 MHz, MeOD) δ 6.95-6.90 (m, 3H, H₄, H₆, H₄), 6.64 (dd, 1H, J = 7.1, 1.8 Hz, H₅), 6.38 (d, 1H, J = 2.5 Hz, H₃), 6.29 (dd, 1H, J = 8.2, 2.5 Hz, H₅), 6.24 (s, 1H, H₃), 3.98 (s, 2H, CH₂). ¹³C NMR (100 MHz, MeOD) δ 159.9, 158.2, 157.1 (C₂, C₂, C₄), 144.7, 142.9 (C₇, C₈), 132.2 (C₉), 132.0 (C₆), 124.1 (C₅), 116.4 (C₁), 112.6 (C₄), 110.6 (C₆), 107.7 (C₅), 104.0 (C₃), 103.5 (C₃), 29.0 (CH₂). HRMS (ESI) m/z calc. for $C_{15}H_{13}O_4$ (M + H)⁺: 257.08084. Found: 257.08068.

(2,4-Dihydroxyphenyl)(7-hydroxy-3-methylbenzofuran-2-yl)methanone (36). The crude product was prepared according to the general procedure B from **71** (60 mg, 0.180 mmol), and purified by column chromatography on silica gel (eluent CH_2Cl_2 -MeOH) to afford **36** (30 mg, 59%) as a yellow solid. ¹H NMR (400 MHz, MeOD) δ 8.46 (d, 1H, J = 9.0 Hz, H₆), 7.17 (dd, 1H, J = 7.9, 1.4 Hz, H₄), 7.16-7.11 (m, 1H, H₅), 6.92 (dd, 1H, J = 7.4, 1.4 Hz, H₆), 6.45 (dd, 1H, J = 9.0, 2.4 Hz, H₅), 6.33 (d, 1H, J = 2.4 Hz, H₃), 2.56 (s, 3H, CH₃). ¹³C NMR (100 MHz, MeOD) δ 188.0 (C=O), 168.0, 166.5 (C₂, C₄), 149.2, 144.9, 144.3 (C₂, C₇, C₈), 136.2 (C₆), 132.1 (C₉), 128.3 (C₃), 125.4 (C₅), 114.7 (C₆), 114.1 (C₁), 112.9 (C₄), 109.4 (C₅), 103.8 (C₃), 10.5 (CH₃). HRMS (ESI) m/z calc. for $C_{16}H_{11}O_5$ (M - H)⁻: 283.06120. Found: 283.06121.

(3-Amino-7-hydroxybenzofuran-2-yl)(2,4-dihydroxyphenyl)methanone (37). The crude product was prepared according to the general procedure B from **72** (101 mg, 0.310 mmol), and purified by column chromatography on silica gel (eluent CH_2Cl_2 -MeOH) to afford **37** (64 mg, 72%) as a yellow solid. ¹H NMR (400 MHz, MeOD) δ 8.76 (d, 1H, J = 9.0 Hz, H₆), 7.32 (dd, 1H, J = 7.9, 1.1 Hz, H₄), 7.08 (m, 1H, H₅), 6.96 (dd, 1H, J = 7.8, 1.1 Hz, H₆), 6.45 (dd, 1H, J = 9.0, 2.5 Hz, H₅), 6.29 (d, 1H, J = 2.5 Hz, H₃). ¹³C NMR (125 MHz, MeOD) δ 184.4 (C=O), 167.1, 164.8 (C₂, C₄), 146.4, 145.6, 144.4 (C₂, C₇, C₈), 134.8

(C₆), 134.5 (C₃), 124.2 (C₅), 123.8 (C₆), 116.6 (C₆), 113.5 (C₁), 112.8 (C₄), 108.7 (C₅), 103.8 (C₃). HRMS (ESI) *m/z* calc. for C₁₅H₁₁NO₅ (M - H)⁻: 284.05645. Found: 284.05645.

(Z)-2-(2,4,6-Trihydroxybenzylidene)-6-hydroxybenzofuran-3(2H)-one (38). The crude product was prepared according to the general procedure B from **74** (160 mg, 0.487 mmol), and purified by column chromatography on silica gel (eluent CH₂Cl₂-MeOH) to afford **38** (59 mg, 43%) as a yellow solid. ¹H NMR (400 MHz, MeOD) δ 7.60 (d, 1H, *J* = 8.5 Hz, H₄), 7.22 (s, 1H, =CH-), 6.68 (dd, 1H, *J* = 8.5, 2.0 Hz, H₅), 6.64 (d, 1H, *J* = 2.0 Hz, H₇), 5.94 (s, 2H, H₃, H₅). ¹³C NMR (100 MHz, MeOD) δ 183.7 (C₃), 168.6, 168.0 (C₆, C₈), 163.1 (C₄), 160.0 (C₂, C₆), 146.2 (C₂), 126.7 (C₄), 115.3 (C₉), 114.0 (C₅), 108.5 (=CH-), 101.4 (C₁), 99.3 (C₇), 96.1 (C₃, C₅). HRMS (ESI) *m/z* calc. for C₁₅H₉O₆ (M - H)⁻: 285.04046. Found: 285.04022.

(Z)-2-(4-Hydroxy-2-methoxybenzylidene)-6-hydroxybenzofuran-3(2H)-one (39). To a solution of 6-hydroxybenzofuran-3(2H)-one (100 mg, 0.660 mmol) in MeOH (2 mL) were added aqueous KOH (50%, 3 mL) and 4-hydroxy-2-methoxybenzaldehyde (100 mg, 0.660 mmol). The solution was refluxed for 5 h. After cooling, MeOH was removed under reduced pressure, and aqueous HCl (1–6 N) was added to the residue to adjust the pH to 7–8. The precipitate was filtered, washed with water and Et₂O and dried to afford **39** (90 mg, 48%) as a yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.04 (d, 1H, *J* = 8.6 Hz, H₆), 7.54 (d, 1H, *J* = 8.4 Hz, H₄), 6.98 (s, 1H, =CH-), 6.67 (s, 1H, H₇), 6.63 (d, 1H, *J* = 8.4 Hz, H₅), 6.55 (d, 1H, *J* = 8.6 Hz, H₅), 6.51 (s, 1H, H₃), 3.85 (s, 3H, OCH₃). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 180.6 (C₃), 168.4, 167.6 (C₆, C₈), 161.1, 159.8 (C₂, C₄), 145.9 (C₂), 132.3 (C₆), 125.4 (C₄), 113.7 (C₉), 111.9 (C₁), 111.6 (C₅), 108.5 (C₅), 104.0 (=CH-), 99.0 (C₃), 98.6 (C₇), 55.6 (OCH₃). HRMS (ESI) *m/z* calc. for C₁₆H₁₂O₅Na (M + Na)⁺: 307.05769. Found: 307.05799.

2,4,6-Trihydroxyacetophenone (40). To a solution of phloroglucinol (10.0 g, 79.4 mmol) and acetic anhydride (18 mL, 190 mmol) in AcOEt (40 mL), BF₃·Et₂O (12 mL, 97.2 mmol) was added dropwise. The solution was heated at 50 °C for 10 h. Water was then added and the mixture was extracted three times with AcOEt. The combined organic layers were washed with water and brine, dried over anhydrous MgSO₄, filtered, and the filtrate was concentrated under reduced pressure. The residue was recrystallized from water to afford **40** (9.61 g, 72%) as yellow crystals. ¹H NMR (400 MHz, DMSO-*d*₆) δ 5.80 (s, 2H, H₃, H₅), 2.54 (s, 3H, CH₃). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 202.5 (C=O), 164.8, 164.3 (C₂, C₄, C₆), 104.1 (C₁), 94.6 (C₃, C₅), 32.4 (CH₃). HRMS (ESI) *m/z* calc. for C₆H₆O₄ (M + H)⁺: 169.04954. Found: 169.04969.

4-Dimethylamino-2,6-dihydroxyacetophenone (41). To a solution of *N,N*-dimethylamine hydrochloride (10.0 g, 123 mmol) and NaHCO₃ (8.49 g, 101 mmol) in water (20 mL), **40** (1.00 g, 5.95 mmol) was added. The solution was heated to 70 °C and stirred for 10 h under argon. After cooling, water was then added and the mixture was extracted three times with AcOEt. The combined organic layers were washed with saturated aqueous Na₂CO₃ and brine, dried over anhydrous MgSO₄, filtered, and the filtrate was concentrated under reduced pressure to afford **41** (688 mg, 60%) as a purple solid. ¹H NMR (400 MHz, CDCl₃) δ 5.65 (s, 2H, H₃, H₅), 2.94 (s, 6H, N(CH₃)₂), 2.62 (s, 3H, CH₃). ¹³C NMR (100 MHz, CDCl₃) δ 201.6 (C=O), 155.5 (C₂, C₄, C₆), 101.9 (C₁), 91.2 (C₃, C₅), 39.8 (N(CH₃)₂), 32.1 (CH₃). HRMS (ESI) *m/z* calc. for C₁₀H₁₃NO₃ (M - H)⁻: 194.08117. Found: 194.08281.

4-(Dimethylamino)-2-hydroxy-6-methoxyacetophenone (42). The crude product was prepared according to the general procedure C from **41** (666 mg, 3.40 mmol), and purified by column chromatography on silica gel (eluent CH₂Cl₂) to afford **42** (495 mg, 70%) as a white solid. ¹H NMR (400 MHz, CDCl₃) δ 5.74 (d, 1H, *J* = 2.4 Hz, H₅), 5.60 (d, 1H, *J* = 2.4 Hz, H₃), 3.84 (s, 3H, OCH₃), 3.01 (s, 6H, N(CH₃)₂), 2.54 (s, 3H, CH₃). ¹³C NMR (100 MHz, CDCl₃) δ 201.4 (C=O), 167.0, 163.3, 155.7 (C₂, C₄, C₆), 103.0 (C₁), 92.0 (C₃), 86.4 (C₅), 55.3 (OCH₃), 40.1 (N(CH₃)₂), 32.6 (CH₃). HRMS (ESI) *m/z* calc. for C₁₁H₁₆NO₃ (M + H)⁺: 210.11247. Found: 210.11184.

(E)-3-(2,4-Dimethoxyphenyl)-1-(4'-(dimethylamino)-2'-

hydroxy-6'-methoxyphenyl)prop-2-en-1-one (43). The crude product was prepared according to the general procedure A from **42** (106 mg, 0.510 mmol) and 2,4-dimethoxybenzaldehyde (126 mg, 0.760 mmol), and recrystallized from AcOEt to afford **43** (66 mg, 36%) as a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 8.03 (d, 1H, *J* = 15.7 Hz, H_β), 7.90 (d, 1H, *J* = 15.7 Hz, H_α), 7.52 (d, 1H, *J* = 8.5 Hz, H₆), 6.50 (dd, 1H, *J* = 8.5, 2.4 Hz, H₅), 6.44 (d, 1H, *J* = 2.4 Hz, H₃), 5.80 (d, 1H, *J* = 2.4 Hz, H₅), 5.65 (d, 1H, *J* = 2.5 Hz, H₃), 3.90 (s, 3H, OCH₃), 3.86 (s, 3H, OCH₃), 3.83 (s, 3H, OCH₃), 3.03 (s, 6H, N(CH₃)₂). ¹³C NMR (100 MHz, CDCl₃) δ 191.6 (C=O), 168.0, 162.9, 162.6, 160.2, 155.6 (C₂, C₄, C₂, C₄, C₆), 136.9 (C_β), 130.3 (C₆), 126.2 (C_α), 118.4 (C₁), 105.6 (C₅), 103.5 (C₁), 98.6 (C₃), 92.5 (C₃), 87.2 (C₅), 55.7 (OCH₃, OCH₃), 55.7 (OCH₃), 40.1 (N(CH₃)₂). HRMS (ESI) *m/z* calc. for C₂₀H₂₄NO₅ (M + H)⁺: 358.16490. Found: 358.16428.

(Z)-2-(2,4-Dimethoxybenzylidene)-6-(dimethylamino)-4-methoxybenzofuran-3(2H)-one (44). The crude product was prepared according to the general procedure E from **43** (63 mg, 0.180 mmol), and precipitated from AcOEt to afford **44** (58 mg, 90%) as a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 8.17 (d, 1H, *J* = 8.8 Hz, H₆), 7.13 (s, 1H, =CH-), 6.55 (dd, 1H, *J* = 8.8, 2.5 Hz, H₅), 6.43 (d, 1H, *J* = 2.5 Hz, H₃), 5.99 (d, 1H, *J* = 1.9 Hz, H₅), 5.71 (d, 1H, *J* = 1.9 Hz, H₇), 3.93 (s, 3H, OCH₃), 3.85 (s, 3H, OCH₃), 3.83 (s, 3H, OCH₃), 3.07 (s, 6H, N(CH₃)₂). ¹³C NMR (100 MHz, CDCl₃) δ 179.6 (C₃), 168.6 (C₈), 161.8, 159.9, 159.5, 158.0 (C₄, C₆, C₂, C₄), 148.0 (C₂), 132.6 (C₆), 115.4 (C₁), 105.5 (=CH-), 103.2 (C₅), 101.8 (C₉), 98.1 (C₃), 89.3 (C₅), 86.9 (C₇), 56.0 (OCH₃), 55.7 (OCH₃), 55.6 (OCH₃), 40.7 (N(CH₃)₂). HRMS (ESI) *m/z* calc. for C₂₀H₂₂NO₅ (M + H)⁺: 356.14925. Found: 356.14852.

4-(Dimethylamino)-2-methoxyacetophenone (45). The crude product was prepared according to the general procedure C from 4-amino-2-hydroxyacetophenone (500 mg, 3.30 mmol) to afford **45** (321 mg, 64%) as a white solid. ¹H NMR (400 MHz, CDCl₃) δ 7.72 (d, 1H, *J* = 8.9 Hz, H₆), 6.16 (dd, 1H, *J* = 8.9, 2.3 Hz, H₅), 5.95 (d, 1H, *J* = 2.3 Hz, H₃), 3.79 (s, 3H, OCH₃), 2.93 (s, 6H, N(CH₃)₂), 2.45 (s, 3H, CH₃). ¹³C NMR (100 MHz, CDCl₃) δ 196.3 (C=O), 161.41, 154.6 (C₂, C₄), 132.2 (C₆), 115.6 (C₁), 103.9 (C₅), 93.4 (C₃), 54.8 (OCH₃), 39.8 (N(CH₃)₂), 31.5 (CH₃). HRMS (ESI) *m/z* calc. for C₁₁H₁₆NO₂ (M + H)⁺: 194.11756. Found: 194.11727.

4-(Dimethylamino)-2-hydroxyacetophenone (46). The crude product was prepared according to the general procedure B from **45** (321 mg, 1.66 mmol) to afford **46** (289 mg, 90%) as a white solid. ¹H NMR (400 MHz, CDCl₃) δ 12.88 (s, 1H, OH), 7.53 (d, 1H, *J* = 9.0 Hz, H₆), 6.21 (dd, 1H, *J* = 9.0, 2.5 Hz, H₅), 6.08 (d, 1H, *J* = 2.5 Hz, H₃), 3.04 (s, 6H, N(CH₃)₂), 2.49 (s, 3H, CH₃). ¹³C NMR (100 MHz, CDCl₃) δ 201.0 (C=O), 165.0, 156.0 (C₂, C₄), 132.5 (C₆), 110.5 (C₁), 104.0 (C₅), 98.0 (C₃), 40.1 (N(CH₃)₂), 25.7 (CH₃). HRMS (ESI) *m/z* calc. for C₁₀H₁₄NO₂ (M + H)⁺: 180.10191. Found: 180.10162.

(E)-3-(2,4-Dimethoxyphenyl)-1-(4'-(dimethylamino)-2'-hydroxyphenyl)prop-2-en-1-one (47). The product was prepared according to the general procedure A from **46** (289 mg, 1.61 mmol) and 2,4-dimethoxybenzaldehyde (402 mg, 2.42 mmol), and purified by column chromatography on silica gel (eluent cyclohexane-AcOEt) to afford **47** (317 mg, 60%) as an orange solid. ¹H NMR (400 MHz, CDCl₃) δ 8.05 (d, 1H, *J* = 15.5 Hz, H_β), 7.71 (d, 1H, *J* = 9.2 Hz, H₆), 7.61–7.51 (m, 2H, H₆, H_α), 6.51 (dd, 1H, *J* = 8.6, 2.4 Hz, H₅), 6.45 (d, 1H, *J* = 2.4 Hz, H₃), 6.22 (dd, 1H, *J* = 9.2, 2.6 Hz, H₅), 6.11 (d, 1H, *J* = 2.6 Hz, H₃), 3.88 (s, 3H, OCH₃), 3.83 (s, 3H, OCH₃), 3.03 (s, 6H, N(CH₃)₂). ¹³C NMR (100 MHz, CDCl₃) δ 190.9 (C=O), 166.2, 162.8, 160.3, 155.7 (C₂, C₄, C₂, C₄), 138.4 (C_β), 131.2 (C₆), 130.8 (C₆), 118.9 (C_α), 117.5 (C₁), 110.8 (C₁), 105.4 (C₅), 103.8 (C₅), 98.5 (C₃), 98.2 (C₃), 55.6 (OCH₃), 55.5 (OCH₃), 40.0 (N(CH₃)₂). HRMS (ESI) *m/z* calc. for C₁₉H₂₂NO₄ (M + H)⁺: 328.15433. Found: 328.15409.

(Z)-2-(2,4-Dimethoxybenzylidene)benzofuran-3(2H)-one (48). The crude product was prepared according to the general procedure A from benzofuran-3(2H)-one (300 mg, 2.24 mmol) and 2,4-dimethoxybenzaldehyde (372 mg, 2.24 mmol), to afford **48** (327 mg, 52%) as a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 8.30 (d, 1H, *J* = 8.8 Hz, H₆),

7.86–7.74 (m, 1H, H₄), 7.62 (ddd, 1H, *J* = 8.3, 7.3, 1.4 Hz, H₆), 7.45 (s, 1H, =CH–), 7.31 (d, 1H, *J* = 8.3 Hz, H₇), 7.23–7.16 (m, 1H, H₅), 6.63 (dd, 1H, *J* = 8.8, 2.4 Hz, H₅), 6.47 (d, 1H, *J* = 2.4 Hz, H₃), 3.89 (s, 3H, OCH₃), 3.88 (s, 3H, OCH₃). ¹³C NMR (100 MHz, CDCl₃) δ 184.5 (C₃), 165.7 (C₈), 162.9, 160.7 (C₂, C₄), 146.1 (C₂), 136.3 (C₆), 133.5 (C₆), 124.6 (C₄), 123.2 (C₅), 122.4 (C₉), 114.6 (C₁), 113.0 (C₇), 107.9 (=CH–), 105.9 (C₅), 98.1 (C₃), 55.8 (OCH₃), 55.6 (OCH₃). HRMS (ESI) *m/z* calc. for C₁₇H₁₅O₄ (M + H)⁺: 283.09649. Found: 283.09510.

(Z)-2-(2,4-Dimethoxybenzylidene)-7-methoxybenzofuran-3(2H)-one (49). The crude product was prepared according to the general procedure A from 7-methoxybenzofuran-3(2H)-one (200 mg, 1.22 mmol) and 2,4-dimethoxybenzaldehyde (203 mg, 1.22 mmol), to afford **49** (156 mg, 41%) as a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 8.34 (d, 1H, *J* = 8.8 Hz, H₆), 7.48 (s, 1H, =CH–), 7.39 (dd, 1H, *J* = 6.7, 2.0 Hz, H₄), 7.18–7.06 (m, 2H, H₅, H₆), 6.65 (dd, 1H, *J* = 8.8, 2.4 Hz, H₅), 6.46 (d, 1H, *J* = 2.4 Hz, H₃), 4.02 (s, 3H, OCH₃), 3.89 (s, 3H, OCH₃), 3.87 (s, 3H, OCH₃). ¹³C NMR (100 MHz, CDCl₃) δ 184.6 (C₃), 163.0, 160.7, 155.5 (C₈, C₂, C₄), 146.1, 146.0 (C₂, C₇), 133.8 (C₆), 123.8 (C₉), 123.6 (C₅), 118.1 (C₆), 115.9 (C₄), 114.6 (C₁), 108.4 (=CH–), 106.0 (C₅), 98.1 (C₃), 56.5 (OCH₃), 55.8 (OCH₃), 55.6 (OCH₃). HRMS (ESI) *m/z* calc. for C₁₈H₁₇O₅ (M + H)⁺: 313.10705. Found: 313.10555.

(Z)-2-(2,4-Dimethoxybenzylidene)-4,6-difluorobenzofuran-3(2H)-one (50). To a solution of 4,6-difluorobenzofuran-3(2H)-one (95 mg, 0.560 mmol) in anhydrous CH₂Cl₂ (11 mL) were added 2,4-dimethoxybenzaldehyde (139 mg, 0.840 mmol) and neutral Al₂O₃ (2.24 g). The suspension was stirred at 25 °C for 18 h. The solid was then removed by filtration, and the filtrate was concentrated under reduced pressure. The residue was recrystallized from MeOH to afford **50** (83 mg, 47%) as a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 8.17 (d, 1H, *J* = 8.8 Hz, H₆), 7.44 (s, 1H, =CH–), 6.83 (dd, 1H, *J* = 8.4, 0.9 Hz, H₇), 6.64–6.52 (m, 2H, H₅, H₅), 6.46 (d, 1H, *J* = 2.3 Hz, H₃), 3.89 (s, 3H, OCH₃), 3.88 (s, 3H, OCH₃). ¹³C NMR (100 MHz, CDCl₃) δ 179.2 (C₃), 169.7 (d), 167.2 (d), 166.6 (dd) (C₄, C₆, C₈), 163.4, 160.9 (C₂, C₄), 159.4 (dd, C₉), 145.8 (C₂), 133.5 (C₆), 114.0 (C₁), 108.8 (=CH–), 106.4 (C₅), 99.6 (dd, C₅), 98.2 (C₃), 97.2 (dd, C₇), 55.8 (OCH₃), 55.7 (OCH₃). HRMS (ESI) *m/z* calc. for C₁₇H₁₃O₄F₂ (M + H)⁺: 319.0776. Found: 319.0769.

(E)-2-(2,4-Dimethoxybenzylidene)-2,3-dihydro-1H-inden-1-one (51). The crude product was prepared according to the general procedure A from 2,3-dihydro-1H-inden-1-one (200 mg, 1.48 mmol) and 2,4-dimethoxybenzaldehyde (246 mg, 1.48 mmol), to afford **51** (405 mg, 97%) as a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 8.08 (t, 1H, *J* = 2.1 Hz, =CH–), 7.87 (d, 1H, *J* = 7.6 Hz, H₇), 7.61 (d, 1H, *J* = 8.6 Hz, H₆), 7.58–7.46 (m, 2H, H₄, H₅), 7.37 (m, 1H, H₆), 6.53 (dd, 1H, *J* = 8.6, 2.5 Hz, H₅), 6.45 (d, 1H, *J* = 2.5 Hz, H₃), 3.91 (d, 2H, *J* = 2.1 Hz, H₃), 3.85 (s, 3H, OCH₃), 3.82 (s, 3H, OCH₃). ¹³C NMR (100 MHz, CDCl₃) δ 194.2 (C₁), 162.4, 160.7 (C₂, C₄), 149.5 (C₈), 138.4 (C₉), 134.0 (C₅), 132.1 (C₂), 130.7 (C₆), 128.2 (C₇), 127.3 (C₆), 125.9 (C₄), 124.1 (=CH–), 117.5 (C₁), 105.2 (C₅), 98.2 (C₃), 55.5 (OCH₃), 55.4 (OCH₃), 32.5 (C₃). HRMS (ESI) *m/z* calc. for C₁₈H₁₇NO₃ (M + H)⁺: 281.1172. Found: 281.1170.

(E)-2-(2,4-Dimethoxybenzylidene)-5-hydroxy-2,3-dihydro-1H-inden-1-one (52). The crude product was prepared according to the general procedure A from 5-hydroxy-2,3-dihydro-1H-inden-1-one (200 mg, 1.35 mmol) and 2,4-dimethoxybenzaldehyde (336 mg, 2.03 mmol), to afford **52** (389 mg, 97%) as a yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.54 (s, 1H, OH), 7.74 (s, 1H, =CH–), 7.70 (d, 1H, *J* = 9.2 Hz, H₇), 7.61 (d, 1H, *J* = 8.3 Hz, H₆), 6.95 (d, 1H, *J* = 1.7 Hz, H₄), 6.84 (dd, 1H, *J* = 8.3, 2.1 Hz, H₅), 6.68–6.62 (m, 2H, H₆, H₃), 3.92 (s, 2H), 3.89 (s, 3H), 3.84 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 191.5 (C₁), 163.6, 162.1, 160.1 (C₅, C₂, C₄), 152.9 (C₈), 132.9 (C₂), 130.6 (C₆), 129.6 (C₉), 125.6 (C₇), 125.0 (=CH–), 116.4 (C₄), 116.0 (C₁), 111.9 (C₆), 106.0 (C₅), 98.4 (C₃), 55.8 (OCH₃), 55.5 (OCH₃), 31.8 (C₃). HRMS (ESI) *m/z* calc. for C₁₈H₁₇O₄ (M + H)⁺: 296.11214. Found: 296.11096.

(E)-2-(2,4-Dimethoxybenzylidene)-3-methyl-2,3-dihydro-1H-inden-1-one (53). The crude product was prepared according to the general procedure A from 3-methyl-2,3-dihydro-1H-inden-1-one (93 μL,

0.680 mmol) and 2,4-dimethoxybenzaldehyde (114 mg, 0.680 mmol), and purified by column chromatography on silica gel (eluent CH₂Cl₂–AcOEt) to afford **53** (129 mg, 64%) as a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 8.00 (d, 1H, *J* = 2.1 Hz, =CH–), 7.84 (m, 1H, H₆), 7.58–7.46 (m, 3H, H₄, H₆, H₇), 7.35 (m, 1H, H₅), 6.52 (dd, 1H, *J* = 8.6, 2.4 Hz, H₅), 6.43 (d, 1H, *J* = 2.4 Hz, H₃), 4.25 (m, 1H, H₃), 3.81 (s, 3H, OCH₃), 3.79 (s, 3H, OCH₃), 1.31 (m, 3H, CH₃). ¹³C NMR (100 MHz, CDCl₃) δ 194.0 (C₁), 162.5, 160.5 (C₂, C₄), 155.8 (C₈), 138.4, 137.3 (C₂, C₉), 134.3 (C₇), 131.5 (C₆), 128.7 (C₅), 127.5 (C₆), 124.9 (=CH–), 123.9 (C₄), 116.6 (C₁), 105.2 (C₅), 98.2 (C₃), 55.6 (OCH₃), 55.4 (OCH₃), 36.8 (C₃), 18.4 (CH₃). HRMS (ESI) *m/z* calc. for C₁₉H₁₉O₃ (M + H)⁺: 295.13287. Found: 295.13271.

2-(2,4-Dimethoxybenzylidene)-1H-indene-1,3(2H)-dione (54). The crude product was prepared according to the general procedure A from 1H-indene-1,3(2H)-dione (100 mg, 0.710 mmol) and 2,4-dimethoxybenzaldehyde (166 mg, 0.710 mmol), and recrystallized from MeOH to afford **54** (193 mg, 92%) as a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 9.25 (d, 1H, *J* = 9.0 Hz, H₆), 8.48 (s, 1H, =CH–), 8.00–7.92 (m, 2H, H₄, H₇), 7.80–7.71 (m, 2H, H₅, H₆), 6.64 (dd, 1H, *J* = 9.0, 2.4 Hz, H₅), 6.44 (d, 1H, *J* = 2.4 Hz, H₃), 3.93 (s, 3H, OCH₃), 3.92 (s, 3H, OCH₃). ¹³C NMR (100 MHz, CDCl₃) δ 190.4, 189.6 (C₁, C₃), 166.4, 163.0 (C₂, C₄), 142.3141.0, 140.0 (C₈, C₉, =CH–), 136.6 (C₆), 134.8, 134.6 (C₅, C₆), 125.5 (C₂), 122.3, 122.3 (C₄, C₇), 116.1 (C₁), 105.6 (C₅), 97.7 (C₃), 55.8 (OCH₃), 55.7 (OCH₃). HRMS (ESI) *m/z* calc. for C₁₈H₁₅O₄ (M + H)⁺: 295.0965. Found: 295.0960.

N-(2-(2-Chloroacetyl)-5-methoxyphenyl)acetamide (55). To a solution of *m*-anisidine (10.0 g, 81.2 mmol) in PhNO₂ (100 mL), were added BCl₃ in CH₂Cl₂ (89 mL, 1 M, 89.3 mmol), chloroacetonitrile (6 mL, 97.4 mmol) then AlCl₃ (12.0 g, 89.3 mmol) at 0 °C. The solution was stirred at 80 °C for 4 h then at 25 °C for an additional 18 h. Then, aqueous HCl (1 N, 150 mL) was added, and the mixture was stirred at 80 °C for 1 h. After cooling, the mixture was extracted three times with CH₂Cl₂. The combined organic layers were washed with water and brine, dried over anhydrous MgSO₄, filtered, and the filtrate was concentrated under reduced pressure. The residue was solubilized in acetic anhydride (50 mL) and the mixture was stirred at 80 °C for 1.5 h. After cooling, the mixture was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (eluent CH₂Cl₂) to afford **55** (6.48 g, 33%) as a brown solid. ¹H NMR (400 MHz, CDCl₃) δ 8.42 (d, 1H, *J* = 2.7 Hz, H₃), 7.71 (d, 1H, *J* = 9.0 Hz, H₆), 6.60 (dd, 1H, *J* = 9.0, 2.7 Hz, H₄), 4.68 (s, 2H, CH₂), 3.87 (s, 3H, OCH₃), 2.22 (s, 3H, CH₃). ¹³C NMR (100 MHz, CDCl₃) δ 193.0 (C=O), 169.9 (NH–C=O), 165.7 (C₅), 144.8 (C₁), 132.5 (C₃), 112.3 (C₂), 110.1 (C₆), 104.2 (C₄), 55.8 (OCH₃), 46.8 (CH₂), 25.7 (CH₃). HRMS (ESI) *m/z* calc. for C₁₁H₁₃NO₃Cl (M + H)⁺: 242.05785. Found: 242.05771.

1-Acetyl-6-methoxyindolin-3-one (56). To a solution of **55** (7.00 g, 33.6 mmol) in DMF (140 mL), NaH (674 mg, 28.0 mmol) was added portionwise under argon. The solution was stirred at 25 °C for 1.5 h. The mixture was then concentrated under reduced pressure, and the residue was solubilized in CH₂Cl₂. The solution was washed with saturated aqueous NH₄Cl, water and brine, dried over anhydrous MgSO₄, filtered, and the filtrate was concentrated under reduced pressure to afford **56** (1.10 g, 16%) as a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 8.05 (d, 1H, *J* = 2.2 Hz, H₇), 7.61 (d, 1H, *J* = 8.7 Hz, H₄), 6.72 (dd, 1H, *J* = 8.7, 2.2 Hz, H₅), 4.24 (s, 2H, H₂), 3.89 (s, 3H, OCH₃), 2.28 (s, 3H, CH₃). ¹³C NMR (100 MHz, CDCl₃) δ 192.5 (C₃), 168.4, 167.5 (C₆, N–C=O), 156.2 (C₈), 131.1 (C₄), 118.2 (C₉), 110.0 (C₅), 104.2 (C₇), 56.8 (OCH₃), 24.3 (CH₃). HRMS (ESI) *m/z* calc. for C₁₁H₁₂NO₃ (M + H)⁺: 206.08117. Found: 206.08084.

(Z)-2-(2,4-Dimethoxybenzylidene)indolin-3-one (57). The crude product was prepared according to the general procedure A from 1-acetylindolin-3-one (277 mg, 1.58 mmol) and 2,4-dimethoxybenzaldehyde (263 mg, 1.58 mmol), to afford **57** (42 mg, 10%) as a red solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.73 (d, 1H, *J* = 8.4 Hz, H₆), 7.56 (d, 1H, *J* = 7.6 Hz, H₄), 7.49 (m, 1H, H₆), 7.12 (d, *J* = 8.0 Hz, 1H, H₇), 6.93 (s, 1H, =CH–), 6.89 (m, 1H, H₅), 6.69–6.65 (m, 2H, H₃, H₅), 3.89 (s, 3H,

OCH₃), 3.84 (s, 3H, OCH₃). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 185.9 (C₃), 161.5, 159.6, 153.9 (C₈, C₂, C₄), 136.0 (C₂), 133.2 (C₆), 130.7 (C₆), 124.1 (C₄), 120.5 (C₅), 119.5 (=CH-), 115.6 (C₉), 112.7 (C₇), 106.2 (C₁-), 105.0 (C₅), 98.6 (C₃), 56.0 (OCH₃), 55.7 (OCH₃). HRMS (ESI) *m/z* calc. for C₁₇H₁₆NO₃ (M + H)⁺: 282.11247. Found: 282.11233.

(Z)-2-(2,4-Dimethoxybenzylidene)-6-methoxyindolin-3-one (58). The crude product was prepared according to the general procedure A from **56** (872 mg, 4.25 mmol) and 2,4-dimethoxybenzaldehyde (706 mg, 4.25 mmol), to afford **58** (367 mg, 28%) as a red solid. ¹H NMR (400 MHz, CDCl₃) δ 7.65 (d, 1H, *J* = 8.5 Hz, H₆), 7.41 (d, 1H, *J* = 8.4 Hz, H₇), 6.90 (s, 1H, =CH-), 6.56 (dd, 1H, *J* = 8.4, 2.4 Hz, H₅), 6.51 (d, 1H, *J* = 2.3 Hz, H₄), 6.46 (dd, 1H, *J* = 8.6, 2.1 Hz, H₃), 6.37 (d, 1H, *J* = 2.1 Hz, H₅), 3.91 (s, 3H, OCH₃), 3.85 (s, 3H, OCH₃), 3.84 (s, 3H, OCH₃). ¹³C NMR (100 MHz, CDCl₃) δ 181.7 (C₃), 166.6, 161.7, 158.6, 154.8 (C₆, C₈, C₂, C₄), 134.8 (C₂), 132.5 (C₆), 126.6 (C₄), 117.0 (=CH-), 115.6 (C₅), 108.3 (C₇), 107.6 (C₁-), 105.9 (C₅), 99.6 (C₃), 95.4 (C₉), 56.2 (OCH₃), 55.7 (OCH₃), 55.6 (OCH₃). HRMS (ESI) *m/z* calc. for C₁₈H₁₈O₄N (M + H)⁺: 312.12303. Found: 312.12261.

1-(3-Methoxy-2-nitrophenyl)ethan-1-one (59). To a solution of 1-(3-hydroxy-2-nitrophenyl)ethan-1-one (300 mg, 1.65 mmol) in acetone (8 mL) were added K₂CO₃ (296 mg, 2.15 mmol) and Me₂SO₄ (188 μL, 1.98 mmol) dropwise. The solution was refluxed for 5 h. The solid was filtered and the solution was concentrated under reduced pressure to afford **59** (312 mg, 97%) as a white solid. ¹H NMR (400 MHz, CDCl₃) δ 7.52 (m, 1H, H₅), 7.38 (d, 1H, *J* = 7.9 Hz, H₄), 7.26 (d, 1H, *J* = 8.5 Hz, H₆), 3.88 (s, 3H, OCH₃), 2.54 (s, 3H, OCH₃). ¹³C NMR (100 MHz, MeOD) δ 195.8 (C=O), 151.4 (C₃), 139.0 (C₂), 131.5 (C₅), 131.2 (C₁), 121.0 (C₄), 117.1 (C₆), 56.9 (OCH₃), 28.1 (CH₃). HRMS (ESI) *m/z* calc. for C₉H₁₀NO₄ (M + H)⁺: 196.06043. Found: 196.06045.

(E)-3-(2,4-Dimethoxyphenyl)-1-(2-nitrophenyl)prop-2-en-1-one (60). The crude product was prepared according to the general procedure A from 1-(2-nitrophenyl)ethan-1-one (500 mg, 3.03 mmol) and 2,4-dimethoxybenzaldehyde (503 mg, 3.63 mmol), to afford **60** (478 mg, 50%) as a yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.16 (dd, 1H, *J* = 8.2, 1.2 Hz, H₃), 7.88 (dd, 1H, *J* = 7.5, 1.2 Hz, H₅), 7.79 (ddd, 1H, *J* = 8.2, 7.5, 1.5 Hz, H₄), 7.74 (d, 1H, *J* = 8.5 Hz, H₆), 7.71 (dd, 1H, *J* = 7.5, 1.5 Hz, H₆), 7.56 (d, 1H, *J* = 16.3 Hz, H_β), 7.15 (d, 1H, *J* = 16.3 Hz, H_α), 6.65–6.57 (m, 2H, H₃, H₅), 3.83 (s, 3H, OCH₃), 3.82 (s, 3H, OCH₃). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 192.0 (C=O), 163.6, 160.0 (C₂, C₄), 147.0 (C₂), 141.1 (C_β), 135.7 (C₁), 134.2 (C₄), 131.2 (C₅), 130.7 (C₆), 129.0 (C₆), 124.5 (C₃), 123.0 (C_α), 115.1 (C₁), 106.5 (C₅), 98.3 (C₃), 55.9 (OCH₃), 55.6 (OCH₃). HRMS (ESI) *m/z* calc. for C₁₇H₁₆NO₅ (M + H)⁺: 314.10231. Found: 314.1021.

(E)-3-(2,4-Dimethoxyphenyl)-1-(3-methoxy-2-nitrophenyl)prop-2-en-1-one (61). The crude product was prepared according to the general procedure A from **59** (329 mg, 1.69 mmol) and 2,4-dimethoxybenzaldehyde (280 mg, 1.69 mmol), to afford **61** (385 mg, 66%) as a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 7.83 (d, 1H, *J* = 15.5 Hz, H_β), 7.56–7.44 (m, 2H, H₆, H₅), 7.29–7.19 (m, 2H, H₄, H₆), 7.16 (d, 1H, *J* = 15.5 Hz, H_α), 6.51 (dd, 1H, *J* = 8.6, 2.4 Hz, H₅), 6.44 (d, 1H, *J* = 2.4 Hz, H₃), 3.94 (s, 3H, OCH₃), 3.85 (s, 6H, OCH₃). ¹³C NMR (100 MHz, CDCl₃) δ 190.4 (C=O), 163.9, 160.9 (C₂, C₄), 151.7 (C₃), 143.4 (C_β), 134.9 (C₂), 131.9 (C₅), 131.6 (C₆), 122.0 (C₆), 120.8 (C_α), 116.6 (C₁), 115.5 (C₄), 105.8 (C₅), 98.7 (C₃), 56.9 (OCH₃), 55.8 (OCH₃), 55.8 (OCH₃). MS (ESI) *m/z* 344.11 (M + H)⁺.

(Z)-2-(2,4-Dimethoxybenzylidene)benzo [b] thiophen-3(2H)-one (62). To a solution of **77** (200 mg, 0.638 mmol) in DMSO (226 μL) were added sulfur (102 mg, 3.19 mmol) and Et₃N (431 μL, 3.19 mmol). The solution was stirred at 100 °C for 5 h. After cooling, water was added and the mixture was extracted three times with AcOEt. The combined organic layers were washed with water and brine, dried over anhydrous MgSO₄, filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (eluent cyclohexane–AcOEt) to afford **62** (128 mg, 67%) as a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 8.41 (s, 1H, =CH-), 7.95 (ddd, 1H, *J* = 7.7, 1.3, 0.7 Hz, H₄), 7.74 (d, 1H, *J* = 8.7 Hz, H₆), 7.55 (ddd, 1H, *J* =

8.2, 7.0, 1.3 Hz, H₅), 7.50 (dd, 1H, *J* = 7.7, 1.0 Hz, H₇), 7.30 (m, 1H, H₅), 6.62 (dd, 1H, *J* = 8.7, 2.4 Hz, H₅), 6.49 (d, 1H, *J* = 2.4 Hz, H₃), 3.91 (s, 3H, OCH₃), 3.88 (s, 3H, OCH₃). ¹³C NMR (125 MHz, CDCl₃) δ 188.5 (C₃), 163.2, 161.0 (C₂, C₄), 146.1 (C₉), 134.7 (C₆), 131.4 (C₆), 131.2 (C₈), 128.7 (C₄), 127.6 (C₂), 126.9 (C₇), 125.3 (C₅), 123.8 (=CH-), 116.8 (C₁-), 105.4 (C₅), 98.4 (C₃), 55.7 (OCH₃), 55.6 (OCH₃). HRMS (ESI) *m/z* calc. for C₁₇H₁₅O₃S (M + H)⁺: 299.0736. Found: 299.0731.

(Z)-2-(2,4-Dimethoxybenzylidene)-6-methoxybenzo [b] thiophen-3(2H)-one (63). To a solution of **65** (613 mg, 3.10 mmol) in CH₂Cl₂ (20 mL) were added SOCl₂ (292 μL, 4.01 mmol) and DMF (200 μL). The solution was refluxed for 2 h. After cooling, the solution was concentrated under reduced pressure, and the residue was dissolved in CH₂Cl₂ (20 mL), and AlCl₃ (455 mg, 3.41 mmol) was added at 0 °C. The solution was stirred at 25 °C for 18 h. Iced water was then added, and the mixture was extracted three times with CH₂Cl₂. The combined organic layers were washed with water and brine, dried over anhydrous MgSO₄, filtered, and the filtrate was concentrated under reduced pressure. The residue was dissolved in anhydrous CH₂Cl₂ (30 mL), and 2,4-dimethoxybenzaldehyde (273 mg, 1.65 mmol) and neutral Al₂O₃ (6.58 g) were added. The suspension was stirred at 25 °C for 18 h. The solid was then removed by filtration, and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (eluent CH₂Cl₂–AcOEt) to afford **63** (348 mg, 34%) as an orange solid. ¹H NMR (400 MHz, CDCl₃) δ 8.28 (s, 1H, =CH-), 7.83 (d, 1H, *J* = 8.6 Hz, H₄), 7.65 (d, 1H, *J* = 8.7 Hz, H₆), 6.90 (d, 1H, *J* = 2.2 Hz, H₇), 6.77 (dd, 1H, *J* = 8.6, 2.2 Hz, H₅), 6.56 (dd, 1H, *J* = 8.7, 2.4 Hz, H₅), 6.44 (d, 1H, *J* = 2.4 Hz, H₃), 3.86 (s, 6H, OCH₃), 3.83 (s, 3H, OCH₃). ¹³C NMR (100 MHz, CDCl₃) δ 187.0 (C₃), 165.3, 162.9, 160.7 (C₆, C₂, C₄), 148.8 (C₉), 131.2 (C₆), 128.3 (C₄), 128.2 (C₈), 127.6 (=CH-), 124.6 (C₂), 116.8 (C₁-), 113.1 (C₇), 107.5 (C₅), 105.3 (C₅-), 98.4 (C₃), 55.8 (OCH₃), 55.6 (OCH₃), 55.5 (OCH₃). HRMS (ESI) *m/z* calc. for C₁₈H₁₇O₄S (M + H)⁺: 329.08421. Found: 329.08361.

(Z)-2-(2,4-Dimethoxybenzylidene)-7-methoxybenzo [b] thiophen-3(2H)-one (64). To a solution of **61** (386 mg, 1.12 mmol) in DMSO (500 μL) were added sulfur (573 mg, 5.62 mmol) and Et₃N (248 μL, 5.62 mmol). The solution was stirred at 100 °C for 3 h. After cooling, water was added and the mixture was extracted three times with AcOEt. The combined organic layers were washed with water and brine, dried over anhydrous MgSO₄, filtered, and the filtrate was concentrated under reduced pressure to afford **64** (353 mg, 96%) as a yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.20 (s, 1H, =CH-), 7.72 (d, 1H, *J* = 8.6 Hz, H₆), 7.47 (d, 1H, *J* = 7.0 Hz, H₆), 7.37 (m, 2H, H₄, H₅), 6.78 (dd, 1H, *J* = 8.6, 2.5 Hz, H₅), 6.72 (d, 1H, *J* = 2.3 Hz, H₃), 3.96 (s, 3H, OCH₃), 3.93 (s, 3H, OCH₃), 3.87 (s, 3H, OCH₃). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 187.4 (C₃), 165.1, 163.4, 161.0, 160.7 (C₆, C₉, C₂, C₄), 131.5 (C₈), 130.9 (C₆), 128.0 (=CH-), 127.4 (C₅), 126.5 (C₂), 119.8 (C₄), 118.1 (C₆), 116.3 (C₁-), 106.8 (C₅), 98.6 (C₃), 56.2 (OCH₃), 56.1 (OCH₃), 55.7 (OCH₃). HRMS (ESI) *m/z* calc. for C₁₈H₁₇O₄S (M + H)⁺: 329.08421. Found: 329.08410.

2-((3-Methoxyphenyl)thio)acetic acid (65). To a solution of 3-methoxythiophenol (4.00 g, 28.6 mmol) in aqueous NaOH (2 M, 113 mL) was added chloroacetic acid (3.10 g, 33.1 mmol). The solution was stirred at 60 °C for 2 h. After cooling, aqueous HCl (1 N) was added to the mixture to adjust the pH to 1. The mixture was then extracted three times with AcOEt. The combined organic layers were washed with water and brine, dried over anhydrous MgSO₄, filtered, and the filtrate was concentrated under reduced pressure to afford **65** (5.18 g, 92%) as a brown oil. ¹H NMR (400 MHz, CDCl₃) δ 7.16 (m, 1H, H₅), 6.96–6.92 (m, 2H, H₂, H₄), 6.73 (m, 1H, H₆), 3.74 (s, 3H, OCH₃), 3.64 (s, 2H, CH₂). ¹³C NMR (100 MHz, CDCl₃) δ 174.4 (CO₂H), 159.4 (C₃), 135.7 (C₁), 129.6 (C₅), 121.1 (C₆), 114.3 (C₂), 112.3 (C₄), 54.8 (OCH₃), 35.6 (CH₂). HRMS (ESI) *m/z* calc. for C₉H₁₁O₃S (M + H)⁺: 199.04234. Found: 199.04237.

(E)-3-(2,4-Dimethoxyphenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (66). The crude product was prepared according to the general procedure A from 1-(2-hydroxyphenyl)ethan-1-one (300 mg, 2.20 mmol) and 2,4-dimethoxybenzaldehyde (366 mg, 2.20 mmol), and

recrystallized from cyclohexane to afford **66** (210 mg, 34%) as a yellow solid. ^1H NMR (400 MHz, CDCl_3) δ 13.08 (s, 1H, OH), 8.15 (d, 1H, $J = 15.5$ Hz, H_b), 7.89 (dd, 1H, $J = 8.1, 1.6$ Hz, H_c), 7.68 (d, 1H, $J = 15.5$ Hz, H_d), 7.56 (d, 1H, $J = 8.6$ Hz, H_6), 7.45 (ddd, 1H, $J = 8.1, 7.1, 1.6$ Hz, H_4), 6.99 (dd, 1H, $J = 8.1, 1.2$ Hz, H_3), 6.91 (ddd, 1H, $J = 8.1, 7.1, 1.2$ Hz, H_5), 6.53 (dd, 1H, $J = 8.6, 2.4$ Hz, H_5), 6.46 (d, 1H, $J = 2.4$ Hz, H_3), 3.91 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, CDCl_3) δ 194.5 (C=O), 163.7, 163.7, 160.9 ($\text{C}_2, \text{C}_2, \text{C}_4$), 141.5 (C_b), 136.1 (C_4), 131.7 (C_6), 129.8 (C_6), 120.5 (C_1), 118.8 (C_d), 118.7 (C_5), 118.3 (C_3), 117.1 (C_1), 105.8 (C_5), 98.7 (C_3), 55.8 (OCH_3), 55.7 (OCH_3). MS (ESI) m/z 284.97 ($\text{M} + \text{H}^+$).

2-(2,4-Dimethoxyphenyl)-4H-chromen-4-one (67). To a mixture of **66** (150 mg, 0.528 mmol) in DMSO (3 mL) was added I_2 (27 mg, 0.110 mmol). The suspension was stirred at 145 °C for 24 h. After cooling, the solid was filtered and the filtrate was extracted three times with AcOEt. The combined organic layers were washed with water and brine, dried over anhydrous MgSO_4 , filtered, and the filtrate was concentrated under reduced pressure to afford **67** (128 mg, 76%) as a pure yellow solid. ^1H NMR (400 MHz, MeOD) δ 8.05 (ddd, 1H, $J = 8.0, 1.7, 0.5$ Hz, H_5), 7.86 (d, 1H, $J = 8.7$ Hz, H_6), 7.73 (m, 1H, H_7), 7.56 (m, 1H, H_8), 7.41 (m, 1H, H_6), 7.06 (s, 1H, H_3), 6.61 (dd, 1H, $J = 8.7, 2.4$ Hz, H_5), 6.58 (d, 1H, $J = 2.4$ Hz, H_3), 3.91 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, MeOD) δ 181.0 (C_4), 165.4, 163.4, 161.5, 157.7 ($\text{C}_2, \text{C}_9, \text{C}_2, \text{C}_4$), 135.3 (C_7), 131.6 (C_6), 126.3 (C_5), 126.0 (C_6), 124.2 (C_{10}), 119.3 (C_8), 113.6 (C_1), 111.0 (C_3), 107.1 (C_5), 99.8 (C_3), 56.3 (OCH_3), 56.1 (OCH_3). HRMS (ESI) m/z calc. for $\text{C}_{17}\text{H}_{15}\text{O}_4$ ($\text{M} + \text{H}^+$): 283.0965. Found: 283.0966.

2-(2,4-Dimethoxybenzyl)isoindoline-1,3-dione (68). To a solution of phthalic anhydride (100 mg, 0.670 mmol) in acetic acid (5 mL) was added 2,4-dimethoxybenzylamine (124 mg, 0.740 mmol). The solution was refluxed for 4 days. After cooling, the solution was concentrated under reduced pressure, and the residue was dissolved in AcOEt. The organic layer was washed with water and brine, dried over anhydrous MgSO_4 , filtered, and the filtrate was concentrated under reduced pressure. The residue was recrystallized from a mixture of MeOH, AcOEt and cyclohexane (7/2/1) to afford **68** (84 mg, 42%) as a white solid. ^1H NMR (400 MHz, CDCl_3) δ 7.84–7.80 (m, 2H, H_4, H_7), 7.70–7.66 (m, 2H, H_5, H_6), 7.15 (dd, 1H, $J = 8.1, 0.7$ Hz, H_6), 6.42–6.37 (m, 2H, H_3, H_5), 4.81 (s, 2H, CH_2), 3.79 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, CDCl_3) δ 168.1 (C=O), 160.5, 158.2 (C_2, C_4), 133.8 (C_5, C_6), 132.3 (C_8, C_9), 129.9 (C_6), 123.2 (C_4, C_7), 116.7 (C_1), 103.9 (C_5), 98.5 (C_3), 55.5 (OCH_3), 55.3 (OCH_3), 36.6 (CH_2). HRMS (ESI) m/z calc. for $\text{C}_{17}\text{H}_{15}\text{NO}_4\text{Na}$ ($\text{M} + \text{Na}^+$): 320.0893. Found: 320.0891.

2-(2,4-Dimethoxybenzyl)-7-methoxybenzofuran (69). To a mixture of LiAlH_4 (84 mg, 2.20 mmol) in anhydrous Et_2O (20 mL) was added **49** (344 mg, 1.10 mmol). The solution was stirred at 25 °C for 1 h. AlCl_3 (293 mg, 2.20 mmol) was then added, and the solution was stirred at 25 °C for 18 h. The solid was filtered and the filtrate was diluted with AcOEt. The organic layer was washed with water and brine, dried over anhydrous MgSO_4 , filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (eluent AcOEt–cyclohexane) to afford **69** (115 mg, 35%) as a colorless oil. ^1H NMR (400 MHz, CDCl_3) δ 7.15 (d, 1H, $J = 8.3$ Hz, H_6), 7.11 (m, 1H, H_5), 7.08 (m, 1H, H_4), 6.75 (dd, 1H, $J = 7.5, 1.5$ Hz, H_6), 6.52 (d, 1H, $J = 2.4$ Hz, H_3), 6.47 (dd, 1H, $J = 8.3, 2.4$ Hz, H_5), 6.28 (s, 1H, H_3), 4.11 (s, 2H, CH_2), 4.01 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, CDCl_3) δ 159.9, 158.6, 158.2 ($\text{C}_2, \text{C}_4, \text{C}_4$), 144.9, 143.8 (C_7, C_8), 130.7 (C_9), 130.7 (C_6), 123.0 (C_5), 118.0 (C_1), 112.7 (C_4), 105.5 (C_5), 104.1 (C_6), 103.1 (C_3), 98.5 (C_3), 55.9 (OCH_3), 55.3 (OCH_3), 55.3 (OCH_3), 28.3 (CH_2). HRMS (ESI) m/z calc. for $\text{C}_{18}\text{H}_{19}\text{O}_4$ ($\text{M} + \text{H}^+$): 299.12779. Found: 299.12805.

1-(2-Hydroxy-3-methoxyphenyl)ethan-1-one (70). To a solution of 2-hydroxy-3-methoxybenzoic acid (2.00 g, 11.9 mmol) in THF (60 mL) was added dropwise a solution of MeLi (957 μL , 36.8 mmol) in THF (20 mL) at 0 °C. The suspension was stirred at 0 °C for 15 h, then refluxed for 18 h. After cooling, the solution was concentrated under

reduced pressure. The residue was purified by column chromatography on silica gel (eluent AcOEt–cyclohexane) to afford **70** (1.29 g, 65%) as a white solid. ^1H NMR (400 MHz, CDCl_3) δ 7.27 (dd, 1H, $J = 8.0, 1.4$ Hz, H_4), 6.99 (dd, 1H, $J = 8.0, 1.4$ Hz, H_6), 6.88–6.82 (m, 1H, H_5), 3.83 (s, 3H, OCH_3), 2.57 (s, 3H, CH_3). ^{13}C NMR (100 MHz, CDCl_3) δ 205.1 (C=O), 152.9, 149.0 (C_2, C_3), 122.0 (C_6), 119.8 (C_1), 118.4 (C_5), 117.1 (C_4), 56.3 (OCH_3), 27.1 (CH_3). HRMS (ESI) m/z calc. for $\text{C}_9\text{H}_9\text{O}_3$ ($\text{M} + \text{H}^+$): 167.07027. Found: 167.07003.

(2,4-Dimethoxyphenyl)(7-methoxy-3-methylbenzofuran-2-yl)methanone (71). To a solution of 2-bromo-1-(2,4-dimethoxyphenyl)ethan-1-one (321 mg, 1.78 mmol) and DBU (543 mg, 3.57 mmol) in CH_2Cl_2 (10 mL) were added **70** (298 mg, 1.78 mmol) and 3 Å molecular sieves. The solution was stirred at 25 °C for 18 h. Aqueous HCl (10%, 10 mL) was added, and the molecular sieves was removed by filtration. The organic layer was decanted, dried over anhydrous MgSO_4 , filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (eluent AcOEt–cyclohexane) to afford **71** (60 mg, 10%) as a white solid. ^1H NMR (400 MHz, CDCl_3) δ 7.51 (d, 1H, $J = 8.5$ Hz, H_6), 7.22–7.15 (m, 2H, H_5, H_6), 6.88 (dd, 1H, $J = 7.2, 1.6$ Hz, H_4), 6.54 (dd, 1H, $J = 8.5, 2.3$ Hz, H_5), 6.50 (d, 1H, $J = 2.3$ Hz, H_3), 3.92 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3), 2.49 (s, 3H, CH_3). ^{13}C NMR (100 MHz, CDCl_3) δ 185.4 (C=O), 163.7, 160.2 (C_2, C_4), 149.3, 145.8, 143.8 ($\text{C}_2, \text{C}_7, \text{C}_8$), 132.0 (C_6), 131.2 (C_9), 124.9 (C_3), 123.6 (C_5), 121.8 (C_1), 113.2 (C_4), 109.5 (C_6), 104.8 (C_5), 99.1 (C_3), 56.1 (OCH_3), 55.9 (OCH_3), 55.5 (OCH_3), 9.8 (CH_3). HRMS (ESI) m/z calc. for $\text{C}_{19}\text{H}_{19}\text{O}_5$ ($\text{M} + \text{H}^+$): 327.12270. Found: 327.12237.

(3-Amino-7-methoxybenzofuran-2-yl)(2,4-dimethoxyphenyl)methanone (72). To a mixture of **73** (128 mg, 0.390 mmol) in MeOH (10 mL) was added NaOMe (21 mg, 0.390 mmol). The solution was stirred at 25 °C for 2 h. Water was added and the solid was filtered, washed with water and MeOH and dried to afford **72** (101 mg, 79%) as a white solid. ^1H NMR (400 MHz, CDCl_3) δ 7.60 (d, 1H, $J = 8.4$ Hz, H_6), 7.15 (dd, 1H, $J = 7.9, 1.3$ Hz, H_4), 7.10 (m, 1H, H_5), 6.90 (dd, 1H, $J = 7.6, 1.3$ Hz, H_6), 6.55 (dd, 1H, $J = 8.4, 2.3$ Hz, H_5), 6.52 (d, 1H, $J = 2.3$ Hz, H_3), 5.81 (br s, 2H, NH_2), 3.91 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, CDCl_3) δ 184.3 (C=O), 163.0, 159.6 (C_2, C_4), 146.1, 144.1, 140.7 ($\text{C}_2, \text{C}_7, \text{C}_8$), 135.8 (C_3), 131.6 (C_6), 123.0 (C_9), 122.8 (C_5), 121.3 (C_1), 112.0 (C_4), 111.0 (C_6), 104.5 (C_5), 99.3 (C_3), 56.2 (OCH_3), 56.0 (OCH_3), 55.5 (OCH_3). HRMS (ESI) m/z calc. for $\text{C}_{18}\text{H}_{18}\text{NO}_5$ ($\text{M} + \text{H}^+$): 328.11795. Found: 328.11749.

2-(2-(2,4-Dimethoxyphenyl)-2-oxoethoxy)-3-methoxybenzotrile (73). To a solution of 2-hydroxy-3-methoxybenzotrile (119 mg, 0.799 mmol) in DMF (10 mL) were added K_2CO_3 (166 mg, 1.20 mmol) and 2-bromo-1-(2,4-dimethoxyphenyl)ethan-1-one (158 mg, 0.878 mmol). The solution was stirred at 25 °C for 2 h. Water was added and the solid was filtered, washed with water and MeOH and dried to afford **73** (128 mg, 49%) as a white solid. ^1H NMR (400 MHz, CDCl_3) δ 7.99 (d, 1H, $J = 8.8$ Hz, H_6), 7.15 (dd, 1H, $J = 7.1, 2.2$ Hz, H_6), 7.07–6.99 (m, 2H, H_4, H_5), 6.56 (dd, 1H, $J = 8.8, 2.3$ Hz, H_5), 6.43 (d, 1H, $J = 2.3$ Hz, H_3), 5.42 (s, 2H, CH_2), 3.88 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3). ^{13}C NMR (125 MHz, CDCl_3) δ 192.7 (C=O), 165.2, 161.2 (C_2, C_4), 151.6, 150.5 (C_2, C_3), 133.2 (C_6), 125.0 (C_6), 123.5 (C_5), 118.0 (CN), 117.2 (C_4), 116.7 (C_1), 106.6 (C_1), 105.8 (C_5), 98.2 (C_3), 78.2 (CH_2), 56.3 (OCH_3), 55.7 (OCH_3), 55.6 (OCH_3). HRMS (ESI) m/z calc. for $\text{C}_{18}\text{H}_{18}\text{NO}_5$ ($\text{M} + \text{H}^+$): 328.11795. Found: 328.11737.

(Z)-2-(2,4,6-Trimethoxybenzylidene)-6-hydroxybenzofuran-3(2H)-one (74). The crude product was prepared according to the general procedure A from 6-hydroxybenzofuran-3(2H)-one (100 mg, 0.670 mmol) and 2,4-dimethoxybenzaldehyde (131 mg, 0.670 mmol), to afford **74** (160 mg, 72%) as a yellow solid. ^1H NMR (400 MHz, DMSO- d_6) δ 11.05 (s, 1H, OH), 7.57 (d, 1H, $J = 8.5$ Hz, H_4), 6.68 (s, 1H, =CH-), 6.66 (dd, 1H, $J = 8.5, 1.9$ Hz, H_5), 6.61 (d, 1H, $J = 1.9$ Hz, H_7), 6.31 (s, 2H, H_3, H_5), 3.84 (s, 3H, OCH_3), 3.83 (s, 6H, OCH_3). ^{13}C NMR (100 MHz, DMSO- d_6) δ 181.2 (C_3), 167.6, 166.3, 162.8 ($\text{C}_6, \text{C}_8, \text{C}_4$), 159.5 (C_2, C_6), 147.0 (C_2), 125.8 (C_4), 113.2 (C_9), 112.5 (C_5), 103.8

(=CH-), 102.1 (C₁-), 98.1 (C₇), 91.1 (C₃, C₅), 55.9 (OCH₃), 55.5 (OCH₃). HRMS (ESI) *m/z* calc. for C₁₈H₁₇O₆ (M + H)⁺: 329.10196. Found: 329.10141.

MNT-1 Cell Culture. Human melanoma cells (MNT-1) were obtained from the National Institute of Health (NIH) and were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal calf serum and 1% antibiotics, in 75 cm² ventilated flask maintained at 37 °C in a 5% CO₂ incubator. Cells were routinely passed using trypsin-EDTA solution. Inhibition of tyrosinase by test compounds was first evaluated using cell lysate prepared from MNT-1 cells. Briefly, confluent MNT-1 cells cultivated on 75 cm² flasks were washed with phosphate buffer saline (PBS) and detached using trypsin-EDTA solution. Cells suspension was aliquoted in cryotubes (approximately 4.10⁶ cells per tube) and tubes were then centrifuged at 1200 rpm for 5 min. Supernatants were eliminated and cells pellets were snapfrozen in liquid nitrogen before being stored at -80 °C until tyrosinase assay.

Screening Against MNT-1 Lysates. Defrosted MNT-1 cell lysates were washed with 1 mL of PBS/Triton 1X and centrifuged three times at 3000 g for 3 min. The supernatant was then extracted and diluted in PBS/Triton 1X (10 mL). 96 µL of supernatant was seeded on 96-well plates, and treated with increasing concentrations of test compounds (dissolved in DMSO), all wells (including negative controls) receiving the same amount of vehicle (i.e. 2% final concentration of DMSO (v/v)). The plate was then incubated at 37 °C for 10 min, 100 µL of L-DOPA in PBS/Triton 1X (4.0 mM) was added to each well, and the melanin formation was monitored by measuring absorbance at 600 nm after 3 h using a SPECTRA MAX® ABS Plus microplate reader with SoftMax Pro 7.1 software. Each experiment was performed three times, and the obtained data were normalized and presented as curves defined between 0 and 100% activity, related to appropriate blank (without the lysate) and control (without the inhibitors) experiments.

MNT-1 Whole Cells Assay and Cytotoxicity. MNT-1 cells grown on 75 cm² flask were detached using trypsin-EDTA solution. Detached cells were then placed onto 96-wells plates at the initial density of 100,000 cells per cm² and incubated at 37 °C in a 5% CO₂ incubator. After two to three days, when confluence was reached, cell medium was changed to fresh medium and MNT-1 cells were treated with increasing concentrations of test compounds (dissolved in DMSO), all wells (including negative controls) receiving the same amount of vehicle (i.e. 0.1% final concentration of DMSO (v/v)). Cells were then incubated for 96 h at 37 °C in a 5% CO₂ incubator. At the end of the incubation, cells were washed three times with cold PBS and were then transferred to Eppendorf tubes. After centrifugation (at 1200 rpm for 5 min), cell pellets were finally lysed by adding 0.5 mL of NaOH (2 M) and repeated sonication/vortexing until melanin was completely solubilized. The use of NaOH for extracting and solubilizing melanin has been heavily reported. Cell-associated melanin contents were measured at 600 nm using pure melanin (purchased from Sigma-Aldrich) diluted in 2 M NaOH as standard. Protein contents were also measured using the Lowry's procedure, bovine serum albumin (BSA) being used as standard. Protein quantification was performed to normalize melanin contents and also to estimate the toxicity of the compounds since protein contents reflect the number of cells present in the wells.

Human Tyrosinase Construct. A truncated form of *hsTYR* (accession number: L8B082) comprising the signal sequence and part of the intra-melanosomal domain (amino acids 1–456) was cloned to plasmid pcDNA-3.1/myc-His(-)A (Invitrogen) in frame with a C-terminal His-Tag, using XhoI and HindIII restriction enzymes. The ligated plasmid was transformed to DH5α cells and selected on LB agar with 100 µg/mL ampicillin. Gene sequence and open reading frame with the HisTag were confirmed by sequencing.

Cell Growth, Transfection, Expression and Purification. HEK 293T cells were cultured at 37 °C with 5% CO₂ in Dulbecco's Modified Eagle's (DMEM) high-glucose medium (Sigma) supplemented with 10% heat-inactivated fetal bovine serum, 10,000 U/mL penicillin, 10 mg/mL streptomycin, 250 µg/mL amphotericin B and 1% non-essential amino

acids (all from Biological Industries). Cells were counted to obtain 8-10⁶ cells in each plate to reach 70% confluence at transfection. 18–24 h after counting, the cells were transiently transfected with 6.8–7.5 µg of plasmid using CalFectin reagent (SigmaGen Laboratories) following manufacturer's instructions. 18 h post transfection, the cells were washed twice with fetal bovine serum (Biological Industries) and then cultured in expression media containing DMEM high-glucose without phenol red and glutamine supplemented with 200 mM glutamine, 10,000 U/mL penicillin, 10 mg/mL streptomycin, 250 µg/mL amphotericin B, 1% non-essential amino acids (all from Biological Industries) and with 10 mM valproic acid (Sigma). After 5 days of incubation in the expression media, the supernatant containing secreted *hsTYR* was collected, concentrated in Centricon Plus-70, 30 kDa (Merck Millipore), equilibrated with binding buffer (20 mM Tris-HCl, pH 7.5, 500 mM NaCl, 20 mM imidazole) and purified by metal affinity chromatography on Ni²⁺-Sephacrose column (GE Healthcare). *hsTYR* was eluted in one-step elution at 500 mM imidazole, and 0.54 mg of purified enzyme with a specific activity of 3.17 units/mg were collected from 200 mL media. One unit (U) is defined as the amount of enzyme that releases 1 µmol of L-dopachrome per minute under these assay conditions.

SDS Page and Western Blotting. *hsTYR* presence in elution fractions was verified by 12% SDS-PAGE acrylamide gels stained with Coomassie blue and western blotting. For western blotting, anti-His-HRP mouse monoclonal IgG antibody (R&D Systems) was used in 1:4000 dilution in Tris-buffered saline (TBS).

Human Tyrosinase Activity Assay. The assay was performed as described previously for other TYR [41,42]. In short, the assay was done in 96-well plates containing 1.2 µg of *hsTYR*, 0.01 mM CuSO₄ and 1 mM L-DOPA in 50 mM sodium-phosphate buffer, pH 7.5–7.8. Absorbance was measured at 475 nm, following the formation of L-dopachrome ($\epsilon = 3600 \text{ M}^{-1} \text{ cm}^{-1}$), in 18 s intervals for 1 h in an EPOCH 2 microplate reader (BioTek) at 37 °C. Activity was deferred from the slopes of absorbance at 475 nm vs. time, and converted to units (U).

Human Tyrosinase Kinetic Assay with Inhibitors. The assay was performed as described above with adjustments: compounds **10** and **23** were dissolved in DMSO and screened in various concentrations (0, 0.5, 2 and 8 µM), maintaining a 2% DMSO final concentration (v/v). L-DOPA concentrations ranged between 65 and 1375 µM. KA was dissolved in dH₂O in concentrations of 0, 100, 300, 500 and 700 µM with L-DOPA concentrations of 85–1700 µM.

Kinetics Analysis. SigmaPlot software was used to analyze kinetic results by fitting the data to eight different inhibition models: competitive, non-competitive, un-competitive and mixed, each of them in complete (full) and partial models. Kinetic constants were obtained by SigmaPlot according to the best-fitting inhibition model. k_{cat} values were calculated using MW of 66.766 kDa as measured by Cordes et al. [43].

Molecular Modeling. A homology model of *hsTYR* built from the crystal structure of *hsTYRP1* was retrieved from Swiss Model Expaty.³² The docked compound and the protein were prepared using LePro tool. Compound **23** was then docked using LeDock software with standard settings, and a total of 20 poses were generated. Only poses with low energy conformations and plausible geometries were considered. UCSF Chimera 1.14 was used to display the small molecule ligand-protein docking results. The crystal structure of tyrosinase from *Bacillus megaterium* with bound tyrosine was retrieved from the Protein DataBank (PDB code 4p6r) and was superimposed with the best docking poses using UCSF Chimera 1.14.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ejmech.2022.114972>.

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