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A novel series of 6-substituted 3-(pyrrolidin-1-ylmethyl)chromen-2-ones as selective monoamine oxidase (MAO) A inhibitors

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ABSTRACT

A series of 6-substituted 3-(pyrrolidin-1-ylmethyl)chromen-2-ones (coumarins) have been synthesized and their inhibitory activity to human monoamine oxidase A (MAO A) and B (MAO B) determined. Incorporation of a basic amino function in the C3 position together with substitution at the C6 position produced novel coumarin compounds with selectivity for the MAO A subtype. Substitution in the C6 position with small hydrophilic groups such as hydroxy (**19**, $IC_{50} = 1.46$ μM) or amino (**18**, $IC_{50} = 3.77$ μM) gave the most potent and selective compounds for MAO A. These compounds also showed excellent aqueous solubility properties. Compound **18** [6-amino-3-(pyrrolidin-1-ylmethyl)chromen-2-one] administrated *in vivo* induced in rat brain a neurotransmitter metabolite profile typical of MAO A inhibition: decreased 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindoleacetic acid (5-HIAA) but increased 3-methoxytyramine (3-MT) levels.

1. Introduction

Monoamine oxidase (MAO) is a flavoenzyme located intracellularly at the outer mitochondrial membrane responsible for the oxidative deamination of dietary amines, monoamine neurotransmitters and hormones. The main function of MAOs is to terminate the actions of neurotransmitter amines in the brain and peripheral tissues and also to protect the brain from a variety of trace amines such as benzylamine, β-phenylethylamine and tyramine [1]. The MAO mediated degradation of the monoamines serotonin (5-HT), norepinephrine (NE) and dopamine (DA) in the brain is essential for a correct function of synaptic neurotransmission. Two distinct types of MAOs were discovered by Johnston and co workers in the 1970s [2,3], namely MAO A and MAO B. They share 70% amino acid sequence homology and have different organ and tissue distribution, substrate specificity and sensitivity to inhibitors and therefore it appears that the two subtypes have diverse physiological functions. The development of selective MAO inhibitors has therefore led to important contributions in the therapy of several neuropsychiatric and neurological disorders [4].

The older MAOIs (e.g. iproniazid) were unselective and irreversible and had broad side effect profiles and dietary restrictions due to “the cheese reaction”, a severe hypertensive crisis upon consumption of food containing large quantities of tyramine. [5]. Newer reversible inhibitors of MAO A (RIMA) are easily displaced by ingested tyramine in the gut and thus do not cause the “the cheese reaction” and no dietary restrictions are needed. Inhibitors of MAO B are mainly associated with symptomatic treatment of Parkinson’s disease [6,7]. However, the age-related increase in MAO B activity, as well as the neuroprotective effects of MAO B inhibitors, have been considered as a rational to use MAO B inhibitors in Alzheimer’s disease [8] (e.g. safinamide [9], selegiline [R-(-)-deprenyl] [10] and rasagiline [11]). MAO A on the other hand is found in catecholaminergic neurons and is responsible for the metabolism of the major neurotransmitters 5-HT, NE and DA, offering a multi neurotransmitter strategy for the treatment of depression. The development of reversible inhibitors of MAO A (RIMAs) with better tolerability profile has sparked a renewed interest for this category of compounds, particularly in view of their efficacy in treatment resistant depression (e.g. moclobemide [12,13], brofaromine [12], toloxatone [14], amifuraline [15] and CX157 [16]) [17].

The coumarins are one of the structural scaffolds that have shown MAO inhibitory activity and in recent years the knowledge of how to develop selective MAO B ligands within this class has

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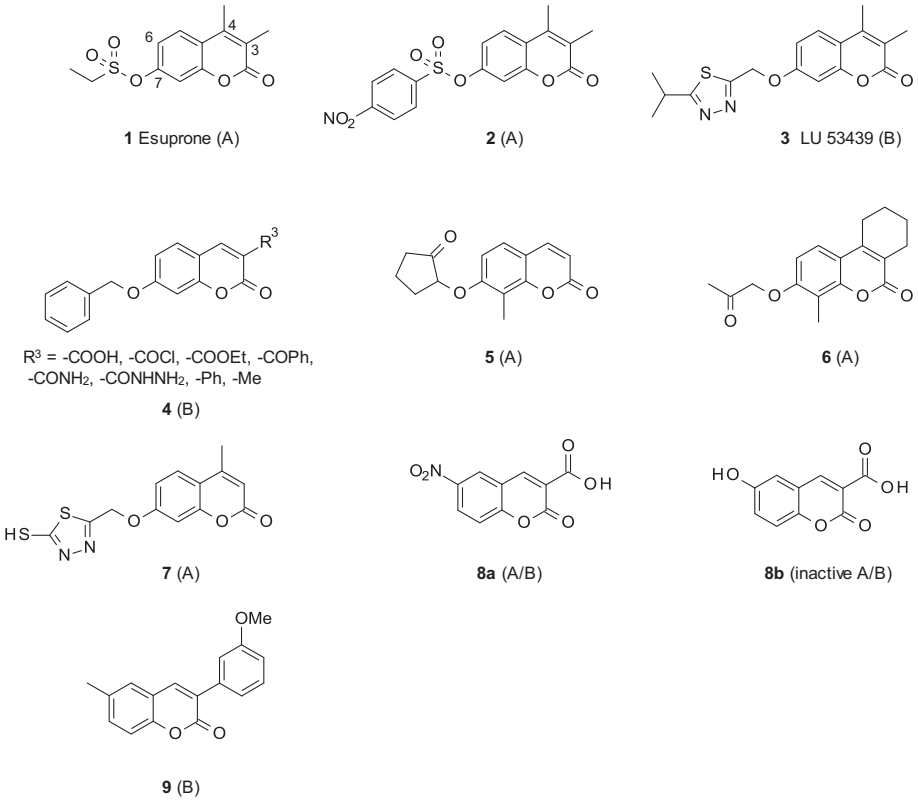


Fig. 1. Reversible MAO A (A) and MAO B (B) inhibitors in the coumarin class.

emerged. On the other hand only a few publications can be found describing MAO A selective coumarins [18]. In 1994, Rendenbach-Muller et al. [19] discovered the importance of the C7 position in simple 7-substitued 3,4-dimethylcoumarines (3,4-dimethylchromen-2-one) for the selectivity between the MAO A and B isoforms. A sulfonic ester linkage yielded MAO A selective inhibitors (**1**, esuprone, Fig. 1) and Gnerre et al. [20] identified the potent and selective MAO A ligand **2** (IC_{50} = 10.7 nM, Fig. 1), based on the same motif as **1**. However, a phenolic linker with a spaced heteroaromatic group was found to be preferred for MAO B selectivity (**3**, LU 53439, Fig. 1), while the corresponding intermediate 7-hydroxy-3,4-dimethylcoumarin was found to be inactive for both MAO A and MAO B. In addition, both compounds **1** and **3** were found to bind reversible to the enzyme [19]. Further studies on 7-substituted coumarins (**4**, Fig. 1) provided knowledge about a wide range of different groups (carboxylic acid, carboxylic ester, acyl, phenyl, benzyloxy, etc.) that are tolerated in position C3 with retained selectivity for MAO B [20–27]. In addition, simultaneous substitution at C3 and C4 with methyl were shown to improve MAO B inhibition, as did 3,4-annelation with 5- and 6-membered alkyl rings. However, Santana et al. [28] and Abdelhafez et al. [29,30] also demonstrated that within the C3-, C4- and C7-substituted coumarins, potent and selective MAO A inhibitors can be developed (i.e. **5**, **6** and **7**, Fig. 1). This in turn indicates that the selection of substituent(s), especially in C7 position, is crucial for the outcome of potency and selectivity for MAO A and B.

Among the existing publications on coumarins as MAO inhibitors only a few have reported the effect of substitution at the C6 position. Chimenti et al. [31] reported on a series of C6-substituted compounds with a polar acyl group at the C3 position, most of these derivatives turned out to be potent MAO B inhibitors with low selectivity over MAO A e.g. the nitro analog **8a** (pIC_{50} = 7.17 and 7.71 for MAO A and B, respectively). However, Secci et al. [25] reported on a similar series with a polar acyl group at position C3 with different substituents at position C5–C8 of the coumarin nucleus, the monosubstituted C6 analogs were found to yield low potent to inactive compounds e.g. **8b** (MAO A IC_{50} = 40.2 μM, MAO B inactive). Furthermore, Matos et al. [27] reported on a study where they have instead introduced lipophilic groups at position C3 and C6 such as a phenyl and methyl group e.g. compound **9** (Fig. 1), yielding a potent MAO B inhibitor (IC_{50} = 0.8 nM) without any MAO A activity.

In our search for novel compounds active on the dopaminergic system in the brain, we applied scaffold hopping from the known partial DA type 2 agonist (D_2), 3-[(benzylamino)methyl]-2,3-dihydro-1,4-benzodioxin-6-ol (**10**, Fig. 2) [32] to the 3-(aminomethyl)chromen-2-one (coumarin) scaffold (**11**, Fig. 2). We maintained a spaced basic amino function in C3 position and focused on different functionalities in C6 position (e.g. electron withdrawing and donating groups). The new compounds were screened *in vivo* for effects on the dopaminergic, adrenergic, and serotonergic system in the rat brain (i.e. striatum) and the aim was to identify compounds with a DA D_2 antagonistic property. However, the 3-(aminomethyl)chromen-2-one substituted ligands showed a different profile *in vivo* than expected and when we further analyzed the results we concluded that these new compounds turned out to be MAO A inhibitors.

Based on the generic structure **11** (Fig. 2) and different substituents (R^1, R^2 and R^6) listed in Table 1 (**12**–**22**), we hereby report a novel series of selective MAO A ligands. The new compounds were tested for *in vitro* activity for human MAO A and B and selected compounds were also screened for their selectivity against off-targets (i.e. monoaminergic, serotonergic receptors and their transporters). *In vivo* data (i.e. effects on 3,4-dihydroxyphenylacetic acid [DOPAC], 3-methoxytyramine [3-MT] and 5-hydroxyindo leacetic acid [5-HIAA] in rat brain, Fig. 1S) is also reported for a number of compounds which further support the *in vitro* finding.

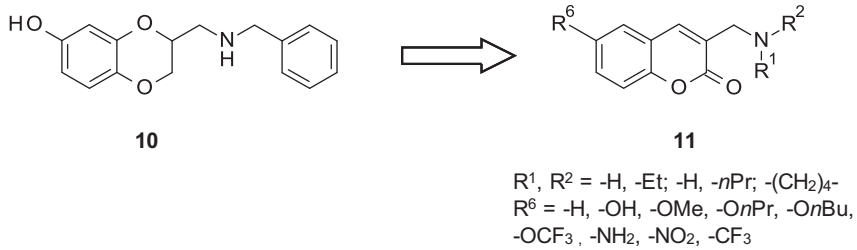
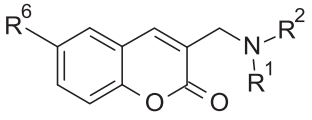


Fig. 2. Design strategy "scaffold hopping" from the dopamine agonist 1,4-benzodioxan core (**10**) to coumarin core (**11**, generic structure).

2. Chemistry

The different 6-substituted coumarin derivatives **12**–**14**, **17**, **18** and **20** were prepared by the Baylis-Hillman methodology, described by Kaye et al. [33], with minor modifications. The different salicylaldehydes (**23a**–**e**, Scheme 1) were benzylated under standard conditions using potassium carbonate as base (48–97%, **24a**–**e**). **23a** was made from 4-butoxyphenol with a magnesium mediated *ortho* specific formylation in excellent yield (98%) [34]. The benzylated derivatives (**24a**–**e**, Scheme 1) were mixed with methyl acrylate, 1,4-diazabicyclo[2.2.2]octane (DABCO) and deuterated chloroform and stirred at room temperature for 1–7 weeks yielding Baylis-Hillman products in good yield (73–97%, **25a**–**e**) [33]. When the salicylaldehyde was substituted with electron withdrawing groups (**24b**, **24c**) the reaction accelerated (1–2 weeks) and this has also been reported by others [35–37]. The conjugate addition was made with: ethylamine, propylamine and pyrrolidine using methanol as solvent, and producing excellent conversion (80–100%, **26a**–**f**). Debenzylation by hydrogenolysis achieved the ring opened hydroxyl derivatives (**27a**–**f**) which after filtration was stirred over night at ambient temperature, yielding spontaneous cyclization to coumarins **12**–**14**, **17**, **18** and **20** (21–62%, in some cases base catalyzed with potassium carbonate). In the nitro substituted **26c** concomitant reduction of the nitro functionality to the corresponding aniline was observed (**27c**).

Table 1
Monoamine oxidase inhibitory activity of compounds **12**–**22**.[a,b]



Compound	R^1, R^2	R^6	MAO B	MAO A	SI[c]
			IC_{50} (μM)	IC_{50} (μM)	
12	–H, –^{n}Pr	–H	62.3 ± 5.35	17.1 ± 2.19	3.6
13	–H, –Et	–O^{n}Bu	7.34 ± 1.06	1.95 ± 0.38	3.7
14	–$(CH_2)_4$–	–H	>100	6.32 ± 0.78	>15.8
15	–$(CH_2)_4$–	–NO_2	>100	>100	–
16	–$(CH_2)_4$–	–CF_3	>100	8.46 ± 3.36	>11.8
17	–$(CH_2)_4$–	–OCF_3	>100	4.24 ± 0.61	>23.5
18	–$(CH_2)_4$–	–NH_2	>100	3.77 ± 0.65	>26.5
19	–$(CH_2)_4$–	–OH	>100	1.46 ± 0.47	>68
20	–$(CH_2)_4$–	–OMe	>100	4.48 ± 0.50	>22.3
21	–$(CH_2)_4$–	–O^{n}Pr	22.3 ± 4.77	2.16 ± 0.16	10.3
22	–$(CH_2)_4$–	–OBn	>100	>100	–
Tranylcypromine	–	–	0.25 ± 0.02	0.24 ± 0.01	1

Abbreviations: Kynuramine, 3-(2-aminophenyl)-3-oxopropanamine; SI, selectivity index; SE, standard error.

[a] Inhibitory activity to human MAO A and MAO B with kynuramine as substrate [44].

[b] All values are expressed as the mean ± SE of duplicate determinations.

[c] The selectivity index is the selectivity for MAO A isoform and is given as the ratios of IC_{50} (MAO B)/IC_{50} (MAO A).

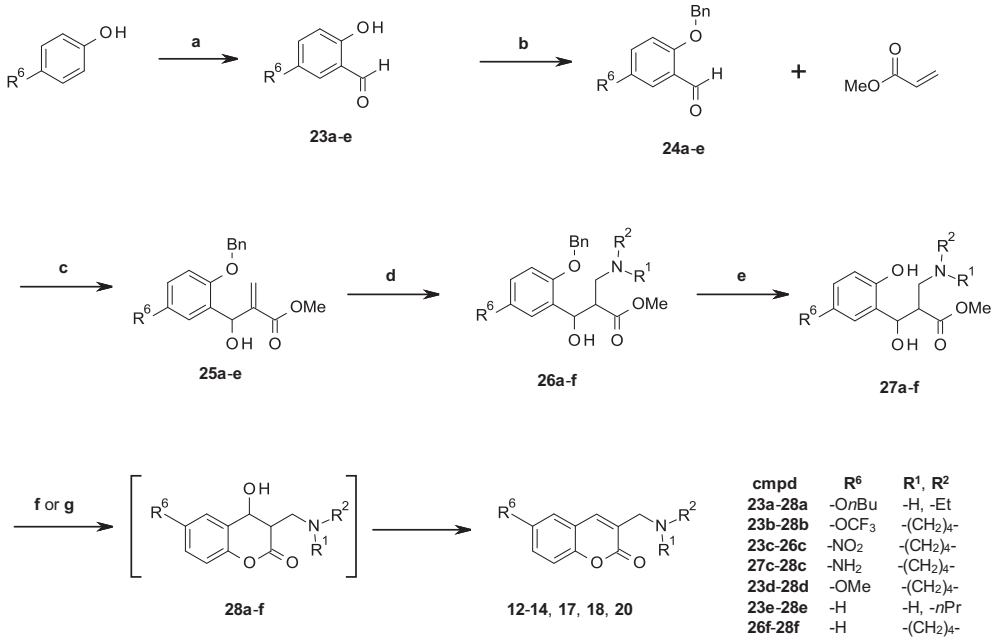
Compound **16** (Scheme 2), which is substituted with a 6-trifluoromethyl group, was synthesized using a modified version of the Baylis-Hillman methodology. In this case, using 2-tetrahydropyranyl (THP) as protecting group instead of a benzyl group was warranted, as the reactivity of the trifluoromethyl group is greatly enhanced in *p*-trifluoromethyl substituted phenols and thus benzylation of the starting material [2-hydroxy-5-(trifluoromethyl)benzaldehyde] under basic conditions leads to 1,6-elimination of hydrogen fluoride [38,39]. Using an acid labile protecting group, such as THP, solved this problem and 2-tetrahydropyran-2-yloxy-5-(trifluoromethyl)benzaldehyde (**29**, Scheme 2) was synthesized according to Geneste et al. [40] by directed *ortho*-lithiation of the THP protected 4-(trifluoromethyl) phenol. Compound **29** was further reacted with methyl acrylate and DABCO to yield the Baylis-Hillman product **30** with full conversion after five days (rate enhancement). Conjugate addition to **31** (Scheme 2) with pyrrolidine as base and deprotection under acidic conditions yielded the ring opened hydroxyl derivative **32** (Scheme 2). Correction of pH to basic conditions (triethylamine) and concomitant heating (microwave irradiation) gave ring closure to **16** in 40% yield (Scheme 2).

A few compounds were made by transformation or addition of functional groups at the C6-position of the aromatic ring (Scheme 3). Compound **14** was selectively nitrated at C6 in 45% yield (**15**) [41,42]. Furthermore, compound **20** with a 6-substituted methoxy group was demethylated with hydrobromic acid, producing **19** in 63% yield (as a hydrobromic salt). Attempts to further alkylate the 6-hydroxy compound (**19**) were made, the most favorable conditions were found to be large excess of triethylamine and benzyl bromide with refluxing acetonitrile as solvent (41%, **22**). The coumarins seems to be sensitive for nucleophile attack (C2, C4 and the exocyclic methylene carbon), as well as a competing quaternization reaction at the basic amine and this could explain the low yield and many byproducts seen when alkylating the coumarin with 1-iodopropane (9%, **21**) [43].

3. Biological assays

3.1. In vitro pharmacology

All the coumarins described in this report (**12**–**22**, Table 1) were evaluated for their ability to inhibit the A and B isoforms of hMAO (Table 1). Kynuramine, a MAO A and MAO B mixed substrate, served as substrate for inhibition studies with both enzymes. The determination of MAO catalytic rates in the presence of compounds **12**–**22** was accomplished by measuring the concentration of 4-hydroxyquinoline, the MAO catalyzed oxidation product of kynuramine, via LC-MS/MS [Cyprotex Discovery Ltd (Macclesfield, UK), see Supplementary material] [44]. The corresponding IC_{50} values and the MAO A selectivity [expressed as IC_{50} (MAO B)/IC_{50} (MAO A)] are reported in Table 1. A subset of the synthesized compounds (**12**, **15**–**18** and **20**) were tested for their ability to inhibit DA D_2 receptor functional response using human HEK cells with D_2-G_{qi5} clone in



Scheme 1. Ring synthesis of 6-substituted coumarin derivatives **12**–**14**, **17**, **18** and **20**. Reagents and conditions: (a) 1. magnesium methoxide 6–10% in methanol, 2. paraformaldehyde, toluene, 3. 10% hydrochloric acid; (b) benzyl bromide, K_2CO_3, acetonitrile, 80 °C; (c) DABCO, $CDCl_3$, rt, 1–7 weeks; (d) NR^1R^2: ethylamine, propylamine or pyrrolidine, methanol, rt; (e) H_2, Pd/C, methanol, rt; (f) methanol, rt; (g) K_2CO_3, methanol, rt; Abbreviations: DABCO, 1,4-diazabicyclo[2.2.2]octane.

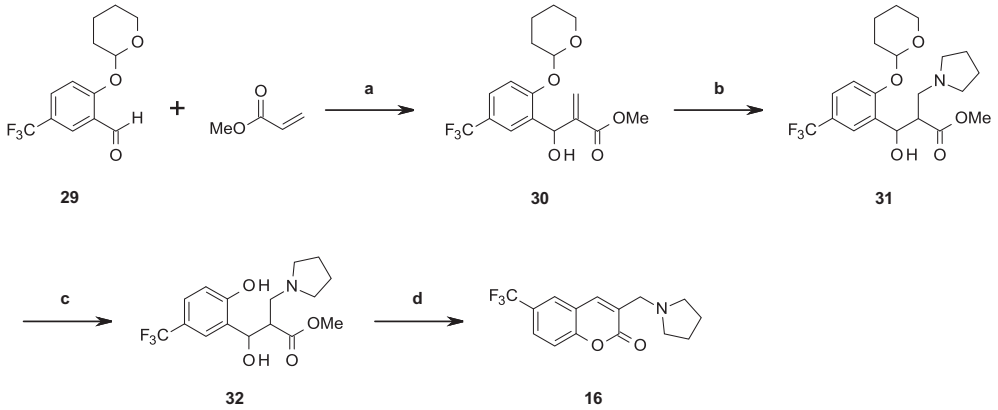
the presence of DA reported by Dyhring et al. [45] (Table 2S, Supplementary material). Two compounds (**16**, **18**) were further evaluated for their selectivity in competition binding assays by CEREP (Poitiers, France) for affinity to the serotonin type 1A receptor (5-HT_{1A}) [46], serotonin type 2A receptor (5-HT_{2A}) [47], adrenergic type 1 receptor (α_1) [48], adrenergic type 2 receptor (α_2) [49] and histaminergic type 1 receptor (H_1) [50], as well as the dopamine transporter protein (DAT) [51], serotonergic transporter protein (SERT) [52] and adrenergic transporter protein (NAT) [53] (Table 3).

3.2. In vivo pharmacology

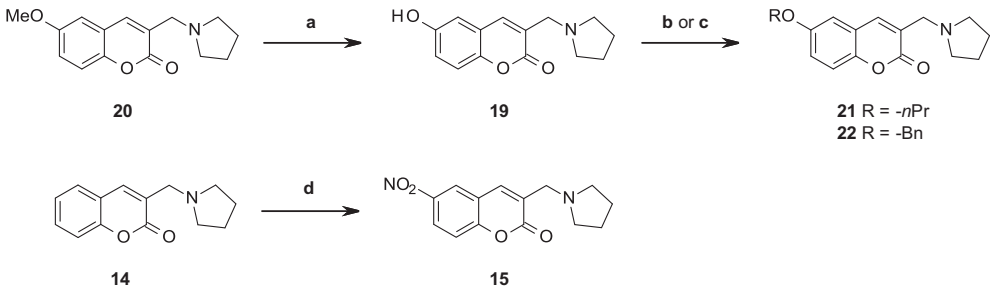
A selection of the synthesized compounds in Table 1 and the known MAO A and MAO B inhibitors, moclobemide (MAO A), selegiline (high dose MAO A/B) and tranylcypromine (MAO A/B) were administrated subcutaneously to freely moving rats that were sacrificed after 1 h. The post-mortem levels of 3-MT, DOPAC, and 5-HIAA in striatum were quantified using the method described in the Supplementary material. The results for compounds **12**, **14**–**16** and **18**–**20** are shown in Table 2.

3.3. Microdialysis

Microdialysis is an invasive sampling technique that makes it possible of continuous measurement of neurotransmitters (DA, 5-HT) and their metabolites (DOPAC, 5-HIAA and 3-MT) concentrations in the extracellular fluid in rat brain (*in vivo*). This is made by insertion of a microdialysis probe in the region of interest in living animals. Compound **18** was administrated subcutaneously to freely moving rats and the levels of DOPAC and 3-MT was measured *in vivo* by HPLC/EC system in striatum during 2 h (Fig. 4). The method used is described in Supplementary material and by Waters et al. [54].



Scheme 2. Ring synthesis of 3-(pyrrolidin-1-ylmethyl)-6-(trifluoromethyl)chromen-2-one (**16**). Reagents and conditions: (a) DABCO, $CDCl_3$, rt, 5 days; (b) pyrrolidine, methanol, rt; (c) conc. hydrochloric acid, methanol, rt; (d) triethylamine, methanol, microwave irradiation 100 °C. Compound **29** was synthesized according to Geneste et al. [40]. Abbreviations: DABCO, 1,4-diazabicyclo[2.2.2]octane.



Scheme 3. Functional group modifications on coumarin core to **15**, **19**, **21** and **22**. Reagents and conditions: (a) 48% hydrobromic acid, 110 °C; (b) benzyl bromide, triethylamine, acetonitrile, 70 °C; (c) 1-iodopropane, K_2CO_3, *N*,*N*-dimethylformamide, 80 °C; (d) conc. H_2SO_4/HNO_3, H_2O, rt.

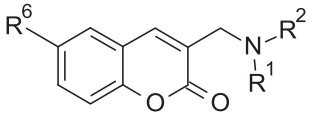
3.4. Molecular modeling

The modeling presented in this paper was performed in the Schrödinger 2012.04 suite of modeling tools [55]. The X-ray crystal complex of MAO A with the reversible inhibitor harmine (resolution 2.17 Å, PDB entry 2Z5Y [56]) was processed with the standard protein preparation protocol [55] including generating appropriate protonation states for the ligand, co-factor, and protein, optimizing the hydrogen bond network and orientation of Asn, Glu and His residue, and a restrained force field minimization using OPLS 2005 [57]. Ligand docking was performed using GLIDE, with standard settings for receptor grid generation and partial charges and docking procedure [58,59]. Ligands were prepared for docking using LigPrep [60] with standard settings for generation of protonation and tautomer states using OPLS 2005 force field.

4. Results and discussion

In the search for novel compounds with effects on DA D_2 receptors in the rat brain, the scaffold jumping from the 1,4-benzodioxan core to the coumarin ring led to a profile distinctly different from what would be expected. The compounds **12**, **15**–**18** and **20** were found to be completely abolished of any functional activity at the DA D_2 receptors (IC_{50} > 16,000 nM, Table 2S). This effect was found to be independent on which substituent was used in the coumarin C6 position or if the basic amino group was a secondary or tertiary amine (i.e. *n*-propylamine or pyrrolidine ring). The reason for the lack of affinity to the DA D_2 receptors is not easily explained but we speculate that the planar geometry of the coumarin ring forces the basic amino group into a position that is not optimal for the affinity to DA D_2 receptors (compared to the geometry for the benzodioxane ring in **10**) [61]. However, the new molecules were instead found to be MAO inhibitors with selectivity for MAO A. From Table 1, which shows the MAO inhibition data for all new compounds, it can be concluded that most of them display weak to moderate inhibitory activity at MAO A (range IC_{50} 1.46–17.1 μM), but with a clear selectivity towards MAO A. For the unsubstituted coumarin rings **12** and **14** the switch from the secondary amine (*n*-propylamine, **12**) to the tertiary pyrrolidine ring (**14**) improved the potency and selectivity for MAO A. The introduction of functional groups at position C6 led to various effects on both MAO A and B. Small electron donating groups such as amino (**18**) and hydroxyl (**19**) had a positive influence on the inhibitory activity at MAO A, with the hydroxyl compound (**19**) being the most potent within this series (IC_{50} = 1.46 μM) and with high selectivity towards MAO B (SI > 68). Adding small alkyl groups to the hydroxy group slightly reduced the activity at MAO A; methoxy (**20**, IC_{50} = 4.48 μM) and trifluoromethoxy group (**17**, IC_{50} = 4.24 μM) while the introduction of a bulky benzyloxy group (**22**) abolished all activity at both MAO A and B. However, intermediate size alkoxy groups such as *n*-propoxy (**21**, IC_{50} = 2.16 μM) and *n*-butoxy group (**13**, IC_{50} = 1.95 μM) was very well tolerated at MAO A, but the activity at MAO B was also enhanced and the selectivity towards MAO B was therefore reduced (SI = 4–10). The introduction of electron withdrawing groups at position C6 was also investigated and the trifluoromethyl group (**16**) had an IC_{50} of 8.46 μM at MAO A

Table 2
Ex vivo brain monoaminergic biochemistry in rats given various doses of compounds **12**, **14**–**16**, **18**–**20** and reference compounds.



Compound	R^1, R^2	R^6	Dose (μmol/kg)	DOPAC[a,b] % of control	3-MT[a,b] % of control	5-HIAA[a,b] % of control
12	–H, –^{n}Pr	–H	100	70*	89	116
14	–$(CH_2)_4$–	–H	100	58	164*	106
15	–$(CH_2)_4$–	–NO_2	100	105	75	118
16	–$(CH_2)_4$–	–CF_3	100	77	88	104
18	–$(CH_2)_4$–	–NH_2	86	45*	170*	72*
19	–$(CH_2)_4$–	–OH	80	47*	126	86*
20	–$(CH_2)_4$–	–OMe	100	65*	129	90
Moclobemide	–	–	37	18*	398*	80*
Tranylcypromine	–	–	0.14	20*	257*	87
Selegiline	–	–	53	36*	146*	96

*P-values <0.05 using student's t-test.
Abbreviations: DOPAC, 3,4-dihydroxyphenylacetic acid; 5-HIAA, 5-hydroxyindoleacetic acid; 3-MT, 5-methoxytryptamine; SEM, standard error of the mean.
[a] Post mortem neurochemistry (subcutaneous injection) analysis of striatal DOPAC, 3-MT, and 5-HIAA levels compared with saline control (n = 4).
[b] ±SEM are reported in supplementary material (Table 1S).

Table 3
In vitro selectivity data for a selection of serotonergic, adrenergic and histaminergic receptors as well as for dopamine, serotonin and norepinephrine transporters for compounds **16** and **18**.[a]

Cmpd	5-HT_{1A}[a] (%)	5-HT_{2A}[a] (%)	α_1[a] (%)	α_2[a] (%)	H_1[a] (%)	DAT[a] (%)	SERT[a] (%)	NAT[a] (%)
16	8	20	4	20	4	8	−4	1
18	0	−4	4[b]	2	13	0	1[b]	−2

Abbreviations: 5-HT, serotonin; α_1, adrenergic type 1 receptor; α_2, adrenergic type 2 receptor; H_1, histamine type 1 receptor; NAT, norepinephrine transporter protein; SERT, serotonin transporter protein; DAT, dopamine transporter protein.

[a] Inhibition of control specific binding at 1 μM reported with [^{3}H]8-OH-DPAT as ligand for h5-HT_{1A} (agonist), [^{3}H]ketanserin as ligand for h5-HT_{2A} (antagonist), [^{3}H]prazosin as ligand for rat α_1 (non selective, antagonist), [^{3}H]RX 821002 as ligand for rat α_2 (non selective, antagonist), histamine as ligand for hH_1 (antagonist), [^{3}H]BTCP as ligand for hDAT (antagonist), [^{3}H]imipramine as ligand for hSERT (antagonist) and [^{3}H]nisoxetine as ligand for hNAT (antagonist).

[b] Inhibition of control specific binding at 10 μM reported.

while the nitro (**15**) was found to be inactive at both MAO A and MAO B. To give some further explanation to the SAR findings in this new series of MAO A ligands, the new compounds were subjected to molecular modeling, based on the high resolution X-ray crystal complex of MAO A with the reversible inhibitor harmine (resolution 2.17 Å, PDB entry 2Z5Y [56]). The X-ray crystal structure of this entry was selected in spite of the presence of a single mutation (G110A) in the sequence of the protein. This mutation introduces only a minor local distortion of the backbone, and the introduced methyl (alanine) is pointing away from the active site (with a distance to harmine of >10 Å). However, one of the reasons for selecting this structure is that 2Z5Y has higher resolution than the structure of the wild type (PDB entry 2Z5X). Another reason is that 2Z5Y contains five water molecules in the active site instead of seven (2Z5X), and all water molecules are at identical positions to the corresponding waters in the wild type 2Z5X. The two missing waters may, therefore, be regarded as more easily replaced by ligands, and thus one could argue that the active site in 2Z5Y is slightly more suitable for docking. Flexible ligand docking using GLIDE was performed for the two most potent and selective ligands 6-amino (**18**) and 6-hydroxy (**19**), together with the 6-benzoloxy inactive analog (**22**). Similar types of binding modes were identified for **18** and **19**. However, GLIDE failed to produce a binding mode for **22**, neither when applying standard nor extra precision mode docking. In the extra precision mode only one binding mode of **19** (Fig. 3) and two similar modes of **18** were obtained. The top ranked binding mode of **18** is shown in Fig. 2S (Supplementary material). The binding mode of **19** revealed that the protonated amino group (C3) makes a hydrogen bond interaction to the amide group (oxygen) of the Gln-215 side chain. Moreover, a hydrogen bond between the 6-hydroxy group of **19** and the backbone carbonyl of Phe-208 was also observed. The Phe-208 residue is one of two amino acids residues (i.e. Phe-208, Ile-335) in the active site of MAO A responsible for substrate/inhibitor selectivity over MAO B [56,62,63]. By examining Fig. 3 it can be concluded that the inactive 6-benzyloxy analog (**22**) could not adopt the same binding mode to MAO A as **18** (Fig. 2S) and **19** (Fig. 3 and Fig. 3S in Supplementary material) since there is not enough space for such group in C6-position. The binding mode found (**18**, **19**) would not permit the phenyl ring to point towards the entrance of the cavity; instead the phenyl would clash into the protein. The opening would in any case be too narrow to accommodate a benzyl group. However, smaller more flexible alkyls like *n*-propyl (**21**) are more likely to fit within the opening and possibly enhance binding through hydrophobic interactions with Phe-208, Leu-97 and Val-210. In addition, other reversible MAO A inhibitors (i.e. other structural series) with a basic nitrogen have been found to make a hydrogen bond between the protonated amine and the amide group (oxygen) of the Gln-215

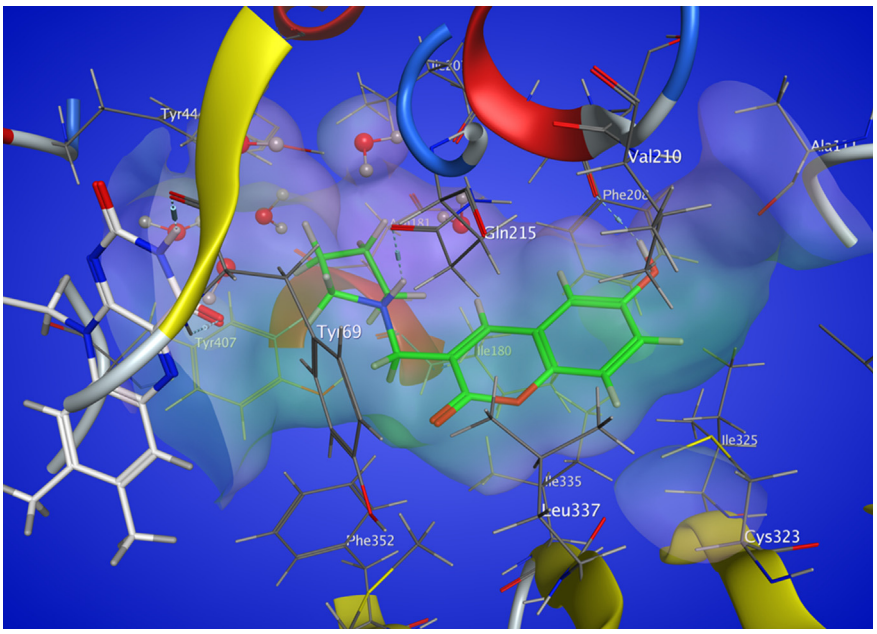


Fig. 3. Binding pose of compound **19** (thick bonds with green carbon atoms) in the active site of human MAO A (PDB: 2Z5Y) viewed alongside the active site. Part of FAD is shown in thick bonds with white carbon atoms. The molecular surface of the active site is shown as a transparent shape. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

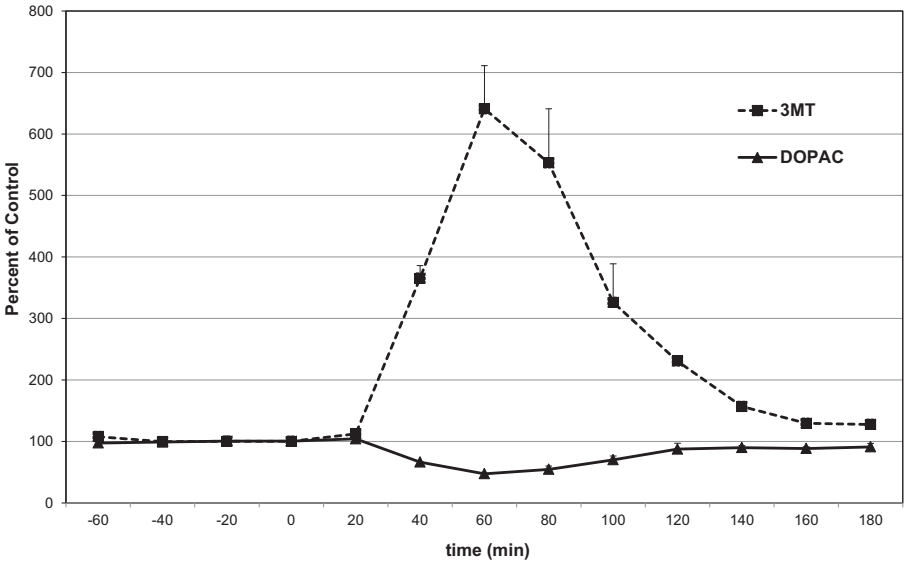


Fig. 4. Striatal levels of the dopaminergic metabolites 3-MT and DOPAC, measured by microdialysis in freely moving rats ($n = 1–2$) and expressed as percentage of saline control, after administration of **18** (43 μmol/kg) at 0 min. Abbreviations: DOPAC, 3,4-dihydroxyphenylacetic acid; 3-MT, 3-methoxytyramine.

side chain (Pettersson et al. [64], Gallardo-Godoy et al. [65] and La Regina et al. [66]). The coumarins are usually docked within the MAO A cavity with the coumarin core in the same mode as we found, with the heterocyclic ring closest to FAD cofactor. However, no reports of MAO A selective coumarins with a basic 3-amino side chain have been investigated on this core before. Recently Abdelhafez et al. [29,30] reported of potent MAO A selective coumarins on a C7 structural motif e.g. **7**, and was found by molecular modeling to have four different hydrogen bonding interactions within MAO A cavity (two each to Tyr-444 and Asn-181), and further claimed that Ile-335 and Phe-208 and the mono vs. dipartite cavity of MAO A respective MAO B are responsible for the selectivity seen over MAO B [29,30].

The *in vitro* findings were further supported by *in vivo* studies in rat brain (Table 2). The most potent and selective MAO A compounds in our series (**14**, **18**–**20**) administrated *in vivo* induced a neurotransmitter metabolite profile typical of MAO A inhibition: decreased DOPAC and increased 3-MT levels (Table 2). The major metabolite of 5-HT, 5-HIAA was only slightly altered by the most potent ligands, 6-amino (**18**) and 6-hydroxy (**19**). These data are in line with previously reported *in vivo* profiles for MAO A inhibitors such as moclobemide or a high dose of selegiline (non selective) [64,67]. One of the most potent ligand within this series, compound **18**, was further investigated by a microdialysis experiment in freely moving rats. A large increase in 3-MT along with a slight decrease of DOPAC was detected in striatum (Fig. 4), further supporting the inhibitory effect on MAO A [64,68]. In a test panel for off target affinity for various receptors and protein transporters which may have an impact on the *in vivo* profile, compound **18** did not show any significant affinity (Table 3) and we therefore conclude that the *in vivo* effects seen of **18** is most likely attributed to the inhibition of MAO A. We have not measured whether the new compounds (i.e. **18** and **19**) are reversible or irreversible MAO A inhibitors. However, due to the fact that these compounds share the chemical motif known for coumarins which are classified as reversible MAO inhibitors (plus that they lack reactive functional groups), it is most likely that these new coumarins also are reversible MAO A inhibitors [31,69].

One of the major drawbacks with the coumarins developed so far, are that the chemical properties such as low aqueous solubility and weak metabolic stability hampers further development of clinical candidates [18]. Therefore a search for new coumarins with improved pharmacokinetic properties and better aqueous solubility is ongoing. Recently, Pisani et al. [69] reported the discovery of a new potent and selective reversible MAO B inhibitor with improved pharmacokinetic and toxicity properties (NW-1772, **23**, Fig. 5) [69,70]. Introduction of the polar amino group seems to be in favor of previously developed MAO B coumarin inhibitors in terms of these properties. The new coumarins (**12**, **14**–**16** and **18**–**20**) were prepared as hydrochloric salts (except **19** which is a hydrobromic salt) and dissolved in saline solutions at room temperature and they were all found to be easily dissolved in concentrations up to 5–7 mg/mL (the highest amount tested). For compounds **15**, **16**, **18,** and **20** the temperature was raised to approx 40 °C to speed up the dissolution. Furthermore, compounds **12**, **14**, **17** and **18** were tested (conc. 5 μM) for enzymatic stability in the presence of rat liver microsomes (Table 3S, Supplementary material) and it was found that substitution at the C6 position greatly affected this stability. Compounds **12**, **14** and **17** were not metabolically stable (<5% remaining compound after 60 min) while compound **18** had significantly higher stability (80% remaining compound after 60 min).

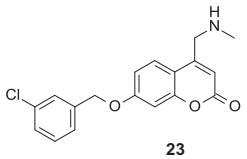


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Fig. 5. Reversible monoamine oxidase B methylamino spaced coumarin inhibitor.

5. Conclusion

A new series of MAO A selective inhibitors have been identified within the coumarin scaffold, with a basic amino function at the C3 position together with small functional groups at the C6 position. These new compounds have, as expected, high aqueous solubility (>5 mg/mL in saline solution) and the metabolic stability was found to be dependent on the substitution at the C6 position. The

most potent and selective coumarins are the 6-subsituted 3-(pyrrolidin-1-ylmethyl)chromen-2-ones with small substituents such as $-NH_2$, $-OH$, $-OMe$ and $-OCF_3$ in the C6 position. Among them the 6-amino (**18**) and 6-hydroxy (**19**) compounds were the most potent inhibitors of MAO A (IC_{50} 3.77 and 1.46 μM, respectively), while the corresponding benzyloxy (**22**) was found to be inactive. In addition, molecular modeling results were in line with the SAR found for the series. The *in vitro* findings were confirmed with *in vivo* data, by measuring the changes in DOPAC, 3-MT and 5-HIAA levels in rat brain (i.e. striatum). The most potent and selective MAO A inhibitors within this series (**18** and **19**) gave the expected *in vivo* MAO A profile (decrease in DOPAC and 5-HIAA levels with a concomitant increase in 3-MT). This indicates that it is possible to develop new selective MAO A inhibitors from this structural class and that these compounds may have potential for the treatment of depression and mood disorders.

6. Experimental section

6.1. Chemistry general

^1H and ^{13}C NMR spectra were recorded in CD_3OD, $CDCl_3$, or DMSO-d_6 at 300 and 75 MHz, respectively, using a Varian XL 300 spectrometer (Varian, Darmstadt, Germany), or at 400 and 100 MHz, respectively, using a Mercury Plus 400 spectrometer (Varian, Darmstadt, Germany). Chemical shifts are reported as δ values (ppm) relative to an internal standard (tetramethylsilane). Low-resolution mass spectra were recorded on a HP 5970A instrument (Agilent Technologies, Stockholm, Sweden) operating at an ionization potential of 70 eV. The mass detector was interfaced with a HP5700 gas chromatograph (Agilent Technologies, Stockholm, Sweden) equipped with a fused silica column (11 m, 0.22 mm i.d.) coated with cross-linked SE-54 (film thickness 0.3 mm, He gas, flow 40 cm/s). Electrospray ionization mass spectra were recorded on Agilent 1200 series liquid chromatography/mass selective detector (Agilent Technologies, Stockholm, Sweden). High resolution mass spectrometer was recorded on MaXis, Q-TOF type instrument (Bruker Daltonics Inc, Billerica, USA). The microwave heating was performed in a Smith synthesizer single-mode microwave cavity producing continuous irradiation at 2450 MHz (Personal Chemistry AB, Uppsala, Sweden). For further instructions see Alterman et al. [71] Elemental analyses were performed by MikroKemi AB (Uppsala, Sweden). Melting points were determined with Büchi 545 instrument (Kebo Lab, Goteborg, Sweden) and are uncorrected. For flash chromatography, silica gel 60 (0.040–0.063 mm, VWR, no. 109385) was used. The amine products were converted to the corresponding salts by dissolving the free base in methanol or ethanol and adding 1 equiv of ethanolic HCl solution. The solvent was removed azeotropically with absolute ethanol *in vacuo* followed by recrystallization from appropriate solvents. Purity of all target compounds where assessed as greater than 95% by elemental analysis (C, H, N) or HPLC/MS (one compound). Intermediates **24c** [35], **24d** [72], **24e** [33], **25e** [33,35], and **29** [40] are known and synthesized by published methods. Intermediate **25c** [35] are known but made by method reported within this publication.

6.1.1. General method for benzylation of phenols (*24a–d*)

Phenol (1 equiv) was dissolved in acetonitrile and then benzylbromide (1.1 equiv) and K_2CO_3 (2 equiv) were added. The reaction mixture was heated at 80 °C for 1–2 h, cooled to ambient temperature and K_2CO_3 was filtered off and subsequently washed with 2 × 50 mL acetonitrile. The combined organic phases were concentrated *in vacuo* and the residue was purified with flash chromatography using isooctane–ethyl acetate in appropriate ratios to give the title compounds (**24a–d**).

6.1.2. General method for "Baylis–Hillman products" methyl 2-[(2-benzyloxyphenyl)-hydroxy-methyl]prop-2-enoates derivatives (*25a–e*)

A mixture of 2-benzyloxybenzaldehyde (**24a–e**) (1 equiv, 5 mmol), methyl acrylate (1 equiv, 5 mmol) and DABCO (0.52 equiv, 2.63 mmol) in $CDCl_3$ (0.25 mL) was stirred for 1–7 weeks. The mixture was concentrated *in vacuo* to give an oily residue, which was purified by flash chromatography using isooctane–ethyl acetate–methanol in appropriate ratios to give the title compounds (**25a–e**).

6.1.3. General method for debenzylation and ring-closure to coumarins (*12–14, 17, 18, 20*)

A mixture of **25a–e** (1 equiv) and amine (1.5 equiv) in methanol was stirred over night at room temperature. Excess amine was evaporated *in vacuo* to give an oily residue (**26a–f**) which was redissolved in methanol (10 mL). Pd/C (0.1 wt%) was added under N_2 atmosphere and the reaction mixture was hydrogenated under H_2 (50 psi) for 0.5–1 h and debenzylated product is achieved (**27a–f**). The mixture was filtered through celite and washed with methanol (20 mL). The methanolic solution was stirred over night at room temperature, in some cases with addition of base K_2CO_3 (3 equiv) if the reaction stopped at "–OH intermediate" (**28a–f**). Filtration of K_2CO_3 and the solvent were removed *in vacuo* to give an oily residue, which was purified by flash chromatography using ethyl acetate–methanol in appropriate ratios as eluent to give the title compounds (**12–14**, **17**, **18**, **20**).

6.1.4. 6-Butoxy-3-(ethylaminomethyl)chromen-2-one (*13*)

The intermediate methyl 3-(2-benzyloxy-5-butoxy-phenyl)-2-(ethylaminomethyl)-3-hydroxy-propanoate (**26a**) was obtained in 100% yield ESIMS: *m/z* 416.0 $(M + H)^+$. Ring closure afforded the product in 45% yield by the general method described in Chapter 6.1.3. MS *m/z* (relative intensity, 70 eV) 275 (M^+, 5), 246 (bp), 231 (20), 190 (27), 176 (24). ESIMS: *m/z* 276.0 $(M + H)^+$. ^1H NMR (CD_3OD, 400 MHz) $\delta = 0.98$ (t, $J = 7.42$ Hz, 3H), 1.16 (t, $J = 7.22$ Hz, 3H), 1.50 (qt, $J = 7.52$, 7.27 Hz, 2H), 1.75 (dd, $J = 8.40$, 6.44 Hz, 2H), 2.69 (q, $J = 7.29$ Hz, 2H), 3.65 (s, 2H), 3.98 (t, $J = 6.44$ Hz, 2H), 7.01–7.15 (m, 2H), 7.21 (d, $J = 8.98$ Hz, 1H), 7.80 (s, 1H). ^{13}C NMR (CD_3OD, 101 MHz) $\delta = 14.10$, 14.56, 20.18, 32.32, 44.02, 47.32, 69.32, 111.75, 118.12, 120.62, 120.89, 127.21, 141.70, 148.69, 157.19, 163.15. The amine was converted to the HCl salt which was recrystallized in ethanol/diethyl ether, mp 174–176 °C. HRMS $C_{16}H_{21}NO_3$ $(M + H)^+$ calcd 276.1594, found 276.1594. Anal. ($C_{16}H_{21}NO_3 \cdot HCl$) C, H, N.

6.1.5. 5-Butoxy-2-hydroxy-benzaldehyde (*23a*)

4-Butoxyphenol (5.2 g, 31.28 mmol) was added to magnesium methoxide (35 mL of 6–10 wt.% solution in methanol, 18.76 mmol) and the mixture was heated to reflux. Approximately half the amount of methanol was distilled off and toluene (40 mL) was added to the residue. The azeotropic mixture of toluene and methanol was removed by fractional distillation, until the temperature of the reaction mixture rose to 95 °C. A slurry of paraformaldehyde powder (3.38 g, 112.6 mmol) in toluene (20 mL) was added to the reaction mixture at 95 °C with concurrent removal of volatile materials by distillation for 1 h, after which the mixture was cooled to room temperature and quenched with 10% hydrochloric acid and stirred over night at ambient temperature. Next day the mixture was extracted with ethyl acetate (3 × 100 mL) and the combined organic phases were dried ($MgSO_4$) and concentrated *in vacuo* to give crude **23a** (5.93 g, 98%): MS *m/z* (relative intensity, 70 eV) 194 (M^+, 25), 138 (bp), 137 (42), 120 (6) 92 (5). ^1H NMR ($CDCl_3$, 400 MHz) $\delta = 0.98$ (t, $J = 7.54$ Hz, 3H), 1.39–1.59 (m, 2H), 1.76 (dd, $J = 14.52$, 6.80 Hz, 2H), 3.94 (t, $J = 6.43$ Hz, 2H), 6.91 (d, $J = 9.19$ Hz, 1H), 6.99 (d, $J = 2.94$ Hz, 1H), 7.14 (dd, $J = 9.19$, 2.94 Hz,

1H), 9.83 (s, 1H), 10.64 (s, 1H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ = 13.77, 19.15, 31.22, 68.59, 116.15, 118.55, 120.03, 125.78, 152.21, 155.87, 196.18.

6.1.6. 2-Benzyloxy-5-butoxy-benzaldehyde (**24a**)

The product was obtained in 68% yield by the general method described under chapter 6.1.1. MS *m*/*z* (relative intensity, 70 eV) 284 (M^+, 11), 192 (9), 137 (6), 136 (6), 91 (bp). ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 0.96 (t, *J* = 7.41 Hz, 3H), 1.39–1.58 (m, 2H), 1.67–1.90 (m, 2H), 3.94 (t, *J* = 6.63 Hz, 2H), 5.13 (s, 2H), 6.98 (d, *J* = 8.97 Hz, 1H), 7.10 (dd, *J* = 8.97, 3.12 Hz, 1H), 7.24–7.51 (m, 6H), 10.50 (s, 1H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ = 13.78, 19.14, 31.21, 68.30, 71.23, 111.04, 115.02, 123.88, 125.48, 127.31, 128.18, 128.65, 136.28, 153.40, 155.65, 189.50.

6.1.7. Methyl 2-[(2-benzyloxy-5-butoxy-phenyl)-hydroxy-methyl] prop-2-enoate (**25a**)

Reaction during 7 weeks yielded the product in 73% yield, by the general method described under chapter 6.1.2. MS *m*/*z* (relative intensity, 70 eV) 370 (M^+, 13), 262 (29), 191 (55), 113 (26) 91 (bp). ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 0.96 (t, *J* = 7.29 Hz, 3H), 1.39–1.56 (m, 2H), 1.67–1.82 (m, 2H), 3.35 (d, *J* = 6.28 Hz, 1H), 3.73 (s, 3H), 3.91 (td, *J* = 6.53, 1.76 Hz, 2H), 5.02 (s, 2H), 5.68 (t, *J* = 1.26 Hz, 1H), 5.90 (d, *J* = 6.03 Hz, 1H), 6.28 (s, 1H), 6.76 (dd, *J* = 8.8, 2.8 Hz, 1H), 6.85 (d, *J* = 8.8 Hz, 1H), 6.97 (d, *J* = 3.2 Hz, 1H), 7.24–7.50 (m, 4H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ = 13.91, 19.27, 31.45, 51.93, 68.26, 68.41, 70.97, 113.31, 114.17, 114.38, 126.04, 127.42, 127.98, 128.58, 130.74, 137.03, 141.30, 149.81, 153.54, 167.06.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.ejmech.2013.11.035.

Abbreviations

DOPAC	3,4-dihydroxyphenylacetic acid
5-HIAA	5-hydroxyindoleacetic acid
3-MT	3-methoxytyramine

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