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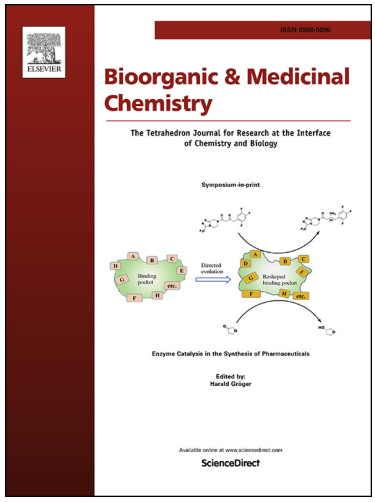

Discovery, synthesis, biological evaluation and molecular docking study of (R)-5-methylmellein and its analogs as selective monoamine oxidase A inhibitors

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PII: S0968-0896(18)31749-8
DOI: https://doi.org/10.1016/j.bmc.2019.03.060
Reference: BMC 14852

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 11 October 2018
Revised Date: 7 March 2019
Accepted Date: 31 March 2019

Please cite this article as: Huang, C., Xiong, J., Guan, H-D., Wang, C-H., Lei, X., Hu, J-F., Discovery, synthesis, biological evaluation and molecular docking study of (R)-5-methylmellein and its analogs as selective monoamine oxidase A inhibitors, *Bioorganic & Medicinal Chemistry* (2019), doi: https://doi.org/10.1016/j.bmc.2019.03.060

Graphical Abstract

Discovery, synthesis, biological evaluation and molecular docking study of (*R*)-5-methylmellein and its analogs as selective monoamine oxidase A inhibitors

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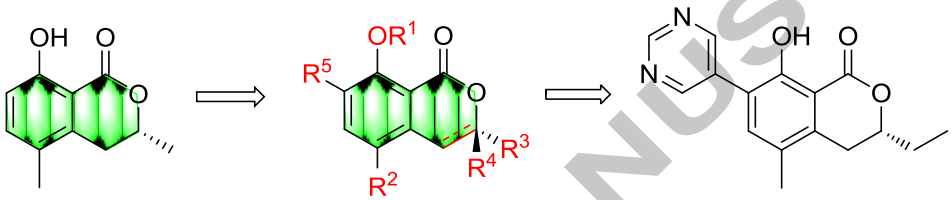

(*R*)-5-Methylmellein isolated from *Xylaria nigripes*
MAO-A, IC_{50}: 4.6 μM
MAO-B, IC_{50}: 38.5 μM

Up to 42 synthetic analogs

The optimal compound (**13aR**)

MAO-A, IC_{50}: 0.06 μM
MAO-B, IC_{50}: > 50 μM

Bioorganic & Medicinal Chemistry
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Discovery, synthesis, biological evaluation and molecular docking study of (*R*)-5-methylmellein and its analogs as selective monoamine oxidase A inhibitors

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ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
Natural product
5-Methylmellein
Synthetic analogs
Monoamine oxidase
Inhibitor

ABSTRACT

(*R*)-5-Methylmellein (5-MM), the major ingredient in the fermented mycelia of the medicinal fungus *Xylaria nigripes* (called Wuling Shen in Chinese), was found to be a selective inhibitor against monoamine oxidase A (MAO-A) and might play an important role in the clinical usage of this edible fungus as an anti-depressive traditional Chinese medicine (TCM). Based on the discovery and hypothesis, a variety of (*R*)-5-MM analogs were synthesized and evaluated *in vitro* against two monoamine oxidase isoforms (MAO-A and MAO-B). Most synthetic analogs showed selective inhibition of MAO-A with IC_{50} values ranging from 0.06 to 29 μM, and compound **13aR** is the most potent analog with high selectivity (IC_{50}, MAO-A: 0.06 μM; MAO-B: > 50 μM). Interestingly, the enzyme kinetics study of **13aR** indicated that this ligand seemed to bind in the MAO-A active site according to so-called "tight-binding inhibition" mode. The molecular docking study of **13aR** was thereafter performed in order to rationalize the obtained biological results.

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1. Introduction

Monoamine oxidases (MAOs, EC 1.4.3.4, amine−oxygen oxidoreductase), a family of flavin-containing amine oxidoreductases, are mostly found in the central nervous system (CNS) and located in the mitochondrial outer membrane[1]. Their enzymatic function is to catalyze the oxidative deamination of monoamine neurotransmitters, such as dopamine, serotonin, and norepinephrine[2]. There are two types of MAOs in mammals, MAO-A and MAO-B, defined by the cysteine amino acid (Cys406 in MAO-A and Cys397 in MAO-B) bound covalently to their co-factor flavin adenine dinucleotide (FAD)[3]. Despite the 70% sequence identity in their compositions, the two isoenzymes still show significant difference in their three dimensional structures[4], substrate specificities, sensitivities to inhibitors[5] and distributions in tissues and cells[6].

Due to their abilities to degrade neurotransmitters, MAOs have been demonstrated to be enormously valuable therapeutic targets in neuropharmacology. In addition, the relationship between neurotransmitter level and MAOs activity proves to be linked with neurological and psychiatric disorders[7]. Currently, MAO-A inhibitors are used for the treatment of anxiety and depression, while MAO-B inhibitors could potentially be used as therapeutic agents in Parkinson's and Alzheimer's diseases[8].

Although some MAO-A inhibitors, including phenelzine, isocarboxazid, tranylcypromine, iproniazid, moclobemide and toloxatone (Fig. 1)[9,10], have made great contributions to the depressive patients, their clinic usage showed some limitation due to potential side effects, food and drug interactions, and introduction of other classes of drugs[11]. As a result, the development of new generation of MAO-A inhibitors attracted significant attention recently, aiming at safer and better-tolerated therapeutic agents[12].

An innegligible source for novel lead molecules in this aspect is to investigate natural products, especially those herbal ingredients from folk medicine used to treat various neurological disorders. *Xylaria nigripes* (Koltz.) Sacc. (family Xylariaceae), under the folklore name of Wuling Shen in Chinese, is a precious and editable medicinal fungus used in traditional Chinese medicine (TCM) as a nerve tonic agent for a long history[13]. Although wild fungus is hard to acquire due to its growth around abandoned termite (*Odontotermes formosanus*) nests, its fermented alternative can be manufactured in large scale. Notably, Wuling Capsule, made from fermented product, was approved in 1999 by the China Food and Drug Administration (CFDA) as a First Grade New TCM in the treatment of insomnia, anxiety, and depression[14].

In recent years, the fermented mycelia of *X. nigripes* was studied regarding their active chemical constituents and their anti-depression mechanisms. In our previous work, some novel natural products were obtained from the EtOH extract of fermented mycelia of *X. nigripes*. Unfortunately, these minor components, such as the spiroketal pyrrole-derived alkaloids (*i.e.*, xylapyrrosides), did not exhibit any anti-depressive activities in our well-established assays using neurological disorder model[15a-b]. Interestingly, (*R*)-5-methylmellein (5-MM)[15c-f] (**9aR**, Figure 1), the major secondary metabolite (yield 0.026%) of *X. nigripes*, was found to display promising anti-depression efficacy in the primary forced swimming test (FST, @ 1 mg/kg) and tail suspension test (TST, @ 3 mg/kg) using mouse anti-depressive models[16]. It was reported that 5-MM, isolated from unidentified fungal strain 8082, could inhibit mouse brain MAOs in a dose-dependent manner with an IC_{50} value of 1.06 μM[17]. The absolute configuration at the stereo center was not defined in the paper, so we hypothesized that the enantiomer (*R*)-5-MM (**9aR**) from Wuling Capsule might be the active inhibitor to demonstrate anti-depressive effect.

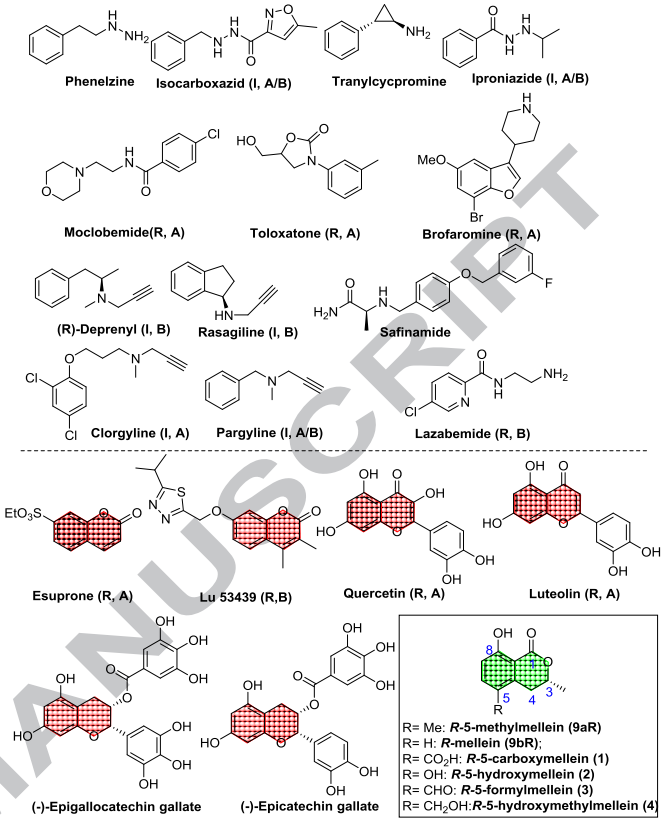
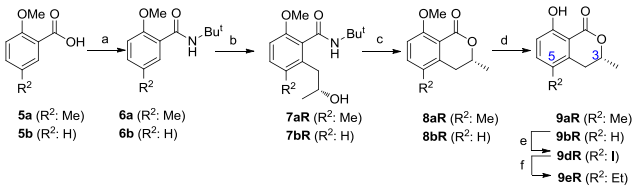


Figure 1. The structures of some representative MAO inhibitors including approved drugs and reported natural products. **I**: irreversible; **R**: reversible; **A**: MAO-A; **B**: MAO-B. Compounds **1**–**4**, **9aR** and **9bR** were isolated from *X. nigripes*.

The isocoumarin core structure of six natural products isolated from *X. nigripes* is quite popular in nature, and has attracted significant attention in the field of drug discovery due to their interesting and diversified biological activities[18]. Moreover, the bioisosteres of this heterocyclic scaffold are also privileged structures for the rational discovery of new MAO inhibitors (Figure 1), such as coumarin (esuprone), flavones (quercetin) and chroman (epicatechin gallate)[19]. Herein, we describe the synthesis and structure-activity-relationship (SAR) around compound **9aR**.

2. Results and discussion

2.1. Chemistry



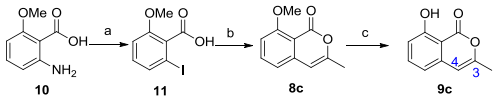
Reagents and conditions: a) $SOCl_2$, *t*-$BuNH_2$/THF, 96%; b) *n*-BuLi, TMEDA, (*R*)-(+)-propylene oxide, THF, -78 °C, 47%; c) *p*-TsOH·H_2O/toluene, reflux, 60%; d) BBr_3/CH_2Cl_2, -78 °C to 0 °C, 96%; e) NIS, $FeCl_3/CH_3CN$, rt, 60%; f) 1.0 M $ZnEt_2$, $Pd(dppf)Cl_2$, 1,4-dioxane, 110 °C, 63%

Scheme 1. The synthesis of (*R*)-methyl mullein (**9aR**) and C5-substituted analogs.

Despite the known synthesis of racemic 5-MM[20], its asymmetric version has not been reported. Inspired by J. Clayden's work on the asymmetric synthesis of (*R*)-mellein[21], our

asymmetric total synthesis of (*R*)-5-MM was efficiently accomplished in 4 steps, as shown in Scheme 1. Starting from the acid **5a**, the amide **6a** was prepared through the coupling of the corresponding acyl chloride and *t*-$BuNH_2$. **6a** was next ortholithiated with *n*-BuLi and subsequently reacted with the optical active epoxide [(*R*)-(+)-propylene oxide] to afford the chiral alcohol **7aR**, which was transformed into (*R*)-5-MM (**9aR**) via cyclization and deprotection. The overall yield was up to 26% with an excellent enantiomeric excess (ee: 98%).

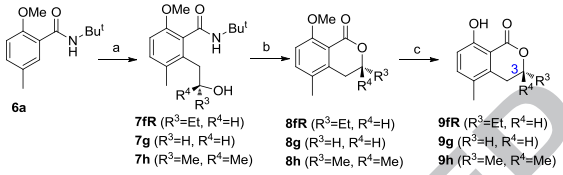
In order to probe the influence of the stereo configuration of C-3 on the inhibition of MAOs, the enantiomer **9aS** was prepared using the same approach, where the ortholithiated species from **6a** reacted with (*S*)-(–)-propylene oxide. Similarly, (*R*)-mellein, (*S*)-mellein and (*R*)-5-ethylmellein (**9bR**, **9bS**, **9eR**) were also successfully obtained (Scheme 1).



Reagents and conditions: a) $NaNO_2$, HCl, KI, H_2O/acetone (1/1), 7–10 °C, 85%; b) 2,4-pentanedione, CuI, Cs_2CO_3, DMSO, 120 °C, 49%; c) BBr_3/CH_2Cl_2, -78 °C to 0 °C, 51%.

Scheme 2. The synthesis of the aromatized analog (**9c**).

The attempt to aromatize **8aR** or **8bR** with a variety of oxidants failed to introduce a carbon-carbon double bond, thus the aromatized products (**8c** and **9c**) were prepared via an iodo intermediate (**11**)[22] as depicted in Scheme 2.

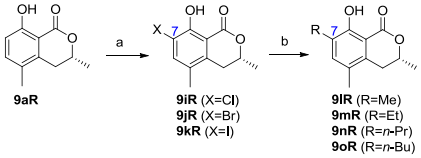


Reagents and conditions: a) *n*-BuLi, TMEDA, THF, -78 °C, for **7fR** with (2*R*)-(+)-1,2-epoxybutane, 36%, for **7g** with 1,2-epoxy ethane, 45%, for **7h** with 1,1-dimethylethylene oxide, 18%; b) *p*-TsOH·H_2O/toluene, reflux, **8fR**: 77%, **8g**: 43%, **8h**: 42%; c) BBr_3/CH_2Cl_2, -78 °C to 0 °C, **9fR**: 85%, **9g**, 90%, **9h**: 94%.

Scheme 3. The synthesis of C3-substituted analogs.

In order to probe the bulky tolerance at the C3-position, a series of analogs (**9fR**, **9g**, **9h**) modified at this position were prepared as shown in Scheme 3.

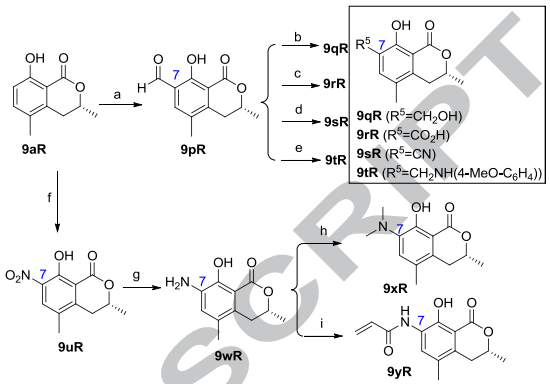
With readily available **9aR** in hand, a series of C7-halo analogs (**9iR**, **9jR** and **9kR**) were synthesized using SO_2Cl_2[23], NBS and NIS as halogenating reagents, respectively. As shown in Scheme 4, the iodide **9kR** was coupled with several organozinc reagents through Negishi coupling reaction to afford C7-alkylated analogs (**9lR**–**9oR**).



Reagents and conditions: a) $FeCl_3/CH_3CN$, halogenating reagents, rt, for **9iR** with SO_2Cl_2, 87%, for **9jR** with NBS, 96%, for **9kR** with NIS, 94%; b) Pd(dppf)Cl_2, 1,4-dioxane, 110 °C, for **9lR** with Me_2Zn: 81%, for **9mR** with Et_2Zn: 63%; for **9nR** with *n*-PrZnI: 31%, for **9oR** with *n*-BuZnI: 17%.

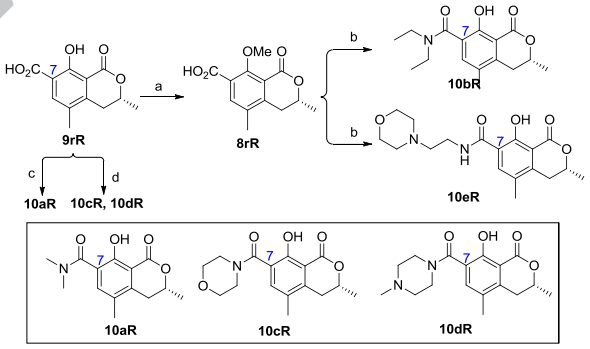
Scheme 4. The synthesis of C7-halo and C7-alkyl analogs

In order to introduce polar substituents to C7-position, **9aR** was initially formylated at the C7 position to provide **9pR** through Friedel-Crafts reaction[24], and through some conventional reactions, like reduction, oxidation, condensation/oxidation[25] or reductive amination, **9pR** could be transformed into **9qR**, **9rR**, **9sR** and **9tR**, respectively (Scheme 5). In addition, **9aR** was subjected to nitration to introduce nitro group at the C7 position, and the desired product **9uR** was further reduced to the amine **9wR**. Upon *N*-alkylation or *N*-amidation, **9wR** was smoothly converted into **9xR** and **9yR**, respectively.



Reagents and conditions: a) Cl_2CHOCH_3, $TiCl_4$, anhydrous CH_2Cl_2, -10 °C, 85%; b) H_2, 10% Pd/C, EtOH/AcOEt (1/1), rt, 93%; c) $NaClO_2$,30% H_2O_2, NaH_2PO_4, THF/H_2O (1/1), rt, 51%; d) NH_2OH•HCl, $FeCl_3$, DMF, 160 °C, 43%; e) *p*-anisidine, *p*-toluenesulfonic acid; $NaBH(OAc)_3$, $ClCH_2CH_2Cl$, rt, 66%; f) $[NO_2]^+BF_4^-$, CH_2Cl_2, -20 °C, 36%; g) H_2, 10% Pd/C, EtOH/CH_2Cl_2 (1/1), rt, 79%; h) Me_2SO_4, NaOH, DMF/H_2O (1/2), rt, 16%; i) acryloyl chloride, Et_3N, CH_2Cl_2, rt, 46%.

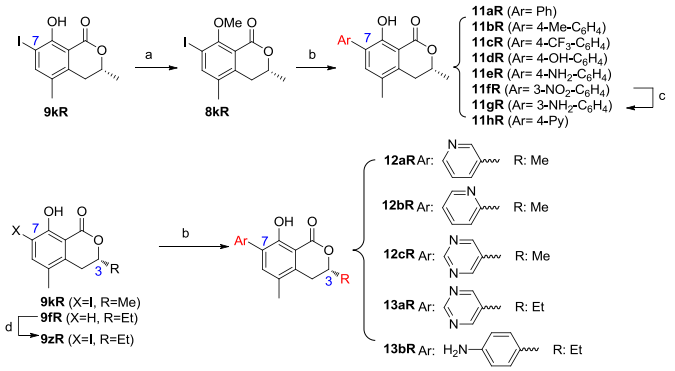
Scheme 5. The synthesis of the analogs modified at C7-position.



Reagents and conditions: a) MeI, K_2CO_3, DMF/H_2O (1/2), rt, 85%; b) EDCI/HOBt, amine (Et_2NH or 2-morpholinoethanamine), CH_2Cl_2, rt, ii. BBr_3/CH_2Cl_2, -78 °C to 0 °C, for **10bR**: 71%, for **10eR**: 67%; c) i. $SOCl_2$, ii. Me_2NH (aqueous solution), rt, for **10aR**: 72%; d) EDCI/HOBt, amines, CH_2Cl_2, rt, for **10cR**: 52%, for **10dR**: 53% (based on **9rR**).

Scheme 6. The synthesis of several C7-substituted analogs with nitrogen-containing side chain

Considering the importance of amide or amine fragments in the known MAO inhibitors, several amide or amine fragments were introduced into the side chain, through amidation with the acid **9rR**, resulting in the production of **10aR**–**10eR**, as shown in Scheme 6.



Reagents and conditions: a) MeI, K_2CO_3, DMF/H_2O (1/2), rt, 85%; b) i. $Pd(PPh_3)_4$, K_2CO_3, ArB(OH)$_2$, 1,4-Dioxane for MeO-**11aR** to MeO-**11fR**; $PdCl_2(PPh_3)_2$, CuI, 4-(tri-*n*-butylstannyl)pyridine, CsF, DMF; for MeO-**11hR**; for **12aR**–**13aR**, $Pd(OAc)_2$, dppf, CuCl, ArB(OH)$_2$, $CsCO_3$, DMF, 110 °C; $Pd(PPh_3)_4$, K_2CO_3, ArB(OH)$_2$, toluene/ethanol/H_2O (10/1/0.2), 90 °C for **13bR**; ii. BBr_3/CH_2Cl_2, -78 °C to 0 °C, two steps for **11aR**: 75%; for **11bR**: 58%; for **11cR**: 42%; for **11dR**: 54%; for **11eR**: 58%; for **11fR**: 56%; for **11hR**: 14%; for **12aR**: 7%, for **12bR**: 10%, for **12cR**: 7%; for **13aR**: 13%, for **13bR**: 28%; c) H_2 (1 atm), 10% Pd/C, MeOH, rt, 92%; d) $FeCl_3/CH_3CN$, NIS, rt, 94%.

Scheme 7. The synthesis of several C7-substituted analogs containing aryl or heteroaryl fragments

Various aryl and heteroaryl groups were introduced to C-7 position to probe the potential π-π interaction. As shown in Scheme 7, these analogs (**11aR**–**11hR**, **12aR**–**12cR**, and **13aR**–**13bR**) were prepared through Pd-catalyzed coupling reactions with 7-iodo derivatives (**9kR** and **9zR**).

2.2. Biological activity

(*R*)-5-MM and the synthetic analogs were evaluated for inhibitory activities using commercially available human MAO-A and MAO-B, with clorgyline and pargyline as reference inhibitors of MAO-A or MAO-B, respectively. The enzymatic inhibition assay was performed using kynuramine as the substrate for MAOs and 4-hydroxyquinoline as a marker. The inhibitory activities were analyzed using the sensitive UPLC-ESI-MS/MS method[26] with acetyl aminophenol as internal standard, by which the quantity of 4-hydroxyquinoline was measured compared with the blank. The results were shown in Table 1 and Table 2, and the selectivity index data (SI, MAO-A over MAO-B) were also listed in the tables if necessary.

As shown in tables, the synthetic (*R*)-5-MM (**9aR**) exhibited inhibitory activity against MAO-A with about 10-fold selectivity over MAO-B, almost the same result compared with the natural product isolated from Wuling Capsule (IC_{50}, MAO-A: 4.6 μM; MAO-B: 38.5 μM). Although (*R*)-5-MM was indeed a selective MAO-A inhibitor, it was less potent than the known MAO-A inhibitor clorgyline (IC_{50}, MAO-A: 0.025 μM). Most analogs exhibited comparable inhibition against MAO-A with IC_{50} values in the 0.06–29 μM range and high selectivity over MAO-B ranging from 1.7-fold to more than 830-fold. Among them, compound **13aR** was the most potent and selective MAO-A inhibitor (IC_{50}, MAO-A: 0.06 μM; MAO-B: > 50 μM).

2.3 Structure activity relationship (SAR) study

Based on a variety of (*R*)-5-MM analogs and their MAO inhibitory activities, we could obtain some insights on the structure-activity relationship. As shown in Table 1, masking the phenolic hydroxyl group of **9aR** with methyl group resulted in a dramatic loss in activity of both MAO-A and MAO-B, exemplified as **8aR**, **8aS** and **8bR**, implying that the essential hydroxyl group (as a hydrogen-bonding donator) might form hydrogen-bonding interaction with MAO.

The stereo-configuration at the C3-position appeared to have little effect on the activity and selectivity, because both (*R*)- and (S)-5-MM (IC_{50} of MAO-A/-B for **9aR** *vs* **9aS**: 4.6/38.5 μM *vs* 4.1/40.9 μM) showed almost the same activity and selectivity, and the similar results were also found in the case of **9bR** and **9bS** (IC_{50} of MAO-A/-B for **9bR** *vs* **9bS**: 6.2/~50 μM vs 7.5/24.0 μM). However, the aromatized product **9c** displayed obviously reduced activity (IC_{50} of MAO-A *vs* -B: 23.4 *vs* > 50 μM). Further analysis suggested that one suitably sized group at sp^3-hybrized C3 position might be required to keep both good activity and selectivity. Removal of C3 methyl group (**9g**) maintained similar activity of MAO-A in spite of an evident sacrifice in selectivity (IC_{50} of MAO-A *vs* -B: 3.2 vs 5.5 μM). Additionally, dimethyl substitution at the C3 position was unbeneficial to both MAO-A and MAO-B inhibition because **9h** lost both MAO-A and -B inhibition. However, replacement of methyl with ethyl group (**9fR**) could keep the activity against MAO-A, and enhance selectivity (IC_{50} of MAO-A *vs* -B: 8.0 *vs* > 50 μM).

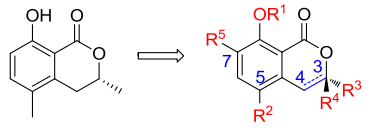
The SAR at C5 position revealed that only small substituent (Me and H) was permitted, with **9aR** and **9bR** displaying similar activity and selectivity. Larger or hydrophilic substituent, such as Et (**9eR**), CO_2H (**1**), OH (**2**) and CH_2OH (**4**), resulted in a loss of activity against MAO-A or a reduced selectivity over MAO-B. As a result, methyl group is the most optimal at the C-5 position.

The investigation on the C7-position indicated that this site was tolerable to a variety of substitutions. For example, compared with the parent compound **9aR**, halogen or $C_{1–4}$ short chain alkyl group introduced into this site (**9iR**–**9qR**) gave comparable activity against MAO-A as well as good selectivity over MAO-B. The polar substituents, such as NO_2, NH_2 and NMe_2 (**9uR**–**9xR**), also seemed to be tolerated. Interestingly, both CO_2H and CN substitutions (**9rR**–**9sR**) led to total loss of both MAO-A and MAO-B activities.

Although amido group with small, bulky or hydrophilic chain side was always unbeneficial to the activities, leading to a dramatic loss of inhibition (**9yR, 10aR**–**10eR**), arylaminomethyl group (**9tR**) could keep the activity against MAO-A along with a better selectivity, implying that there might be a large space or π-π interaction in this binding pocket.

Further SAR exploration was performed on the aryl groups at the C7-position (Table 2). The introduction of hydrophobic benzene ring, such as Me and $-CF_3$ (**11aR**–**11cR**), significantly enhanced the activity against MAO-B (IC_{50}, increased up to 1.0 μM from 38.4 μM of **9aR**) rather than that of MAO-A. However, benzene ring bearing polar substituents, such as *p*-OH, and *p*-NH_2, *m*-NO_2, evidently enhanced the activity against MAO-A with an improved selectivity over MAO-B (**11dR**–**11fR**). Notably, *m*-NH_2 on benzene ring (**11gR**) could further enhance the activity against MAO-A with an excellent selectivity over MAO-B (IC_{50} of MAO-A *vs* -B: 0.2 *vs* > 50 μM). The substitution with heterocycles was also tolerated at this position. 3-Pyridyl at the C7-position (**12aR**) provided the optimal potency and an excellent selectivity (IC_{50} of MAO-A *vs* -B: 0.2 *vs* > 50 μM), compared with 2- and 4-pyridyl (**11hR, 12bR**). Interestingly, the pyrimidyl analog (**12cR**, IC_{50} of MAO-A *vs* -B: 0.14 *vs* > 50 μM) was comparable to the 3-pyridyl analog (**12aR**). Further analog of **12cR** with ethyl substitution at the stereocenter led to the most optimal compound **13aR** (IC_{50} of MAO-A *vs* -B: 0.06 *vs* > 50 μM).

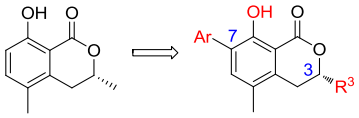
Table 1. Inhibitory activities of synthesized compounds against monoamine oxidases.

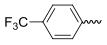
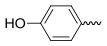
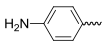
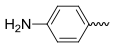


Compound	C3,4-bond	R^1	R^2	R^3	R^4	R^5	IC_{50} (μM)[a] (MAO-A)	IC_{50} (μM)[a] (MAO-B)	SI[c]
9aR[d]	—	H	Me	Me	H	H	4.6 ± 0.0	38.5 ± 1.4	8.3
8a	—	Me	Me	Me	H	H	> 50	> 50	-[g]
8aS	—	Me	Me	H	Me	H	> 50	> 50	-[g]
8bR	—	Me	H	Me	H	H	> 50	> 50	-[g]
9aS	—	H	Me	H	Me	H	4.1 ± 0.1	40.9 ± 5.6	10.0
9c[e]	=	H	Me	H	Me	-	23.4 ± 0.1	> 50	> 2.0
9fR	—	H	Me	Et	H	H	8.0 ± 0.7	> 50	> 6.2
9g	—	H	Me	H	H	H	3.2 ± 0.3	5.5 ± 0.6	1.7
9h	—	H	Me	Me	Me	H	> 50	> 50	-[g]
9bR	—	H	H	Me	H	H	6.2 ± 0.3	~ 50	8.1
9bS	—	H	H	H	Me	H	7.5 ± 0.4	24.0 ± 0.9	3.2
9eR	—	H	Et	Me	H	H	＞ 50	16.1 ± 1.4	< 0.3
1[f]	—	H	COOH	Me	H	H	＞ 50	＞ 50	-[g]
2[f]	—	H	OH	Me	H	H	17.1 ± 1.2	＞ 50	＞ 2.9
4[f]	—	H	CH_2OH	Me	H	H	＞ 50	＞ 50	-[g]
9iR	—	H	Me	Me	H	Cl	2.2 ± 0.1	＞ 50	> 22
9jR	—	H	Me	Me	H	Br	28.6 ± 1.2	＞ 50	> 1.7
9kR	—	H	Me	Me	H	I	15.9 ± 0.6	＞ 50	> 3.1
9lR	—	H	Me	Me	H	Me	3.8 ± 0.1	＞ 50	> 13
9mR	—	H	Me	Me	H	Et	2.9 ± 0.3	＞ 50	> 17
9nR	—	H	Me	Me	H	*n*-Pr	4.5 ± 0.4	＞ 50	> 11
9oR	—	H	Me	Me	H	*n*-Bu	18.3 ± 0.3	＞ 50	> 2.7
9qR	—	H	Me	Me	H	CH_2OH	19.2 ± 0.5	＞ 50	> 2.6
9rR	—	H	Me	Me	H	CO_2H	＞ 50	＞ 50	-[g]
9sR	—	H	Me	Me	H	CN	＞ 50	＞ 50	-[g]
9tR	—	H	Me	Me	H	$NHCH_2(4\text{-}MeO\text{-}C_6H_4)$	4.4 ± 0.2	＞ 50	>11
9uR	—	H	Me	Me	H	NO_2	15.8 ± 1.3	＞ 50	3.1
9wR	—	H	Me	Me	H	NH_2	4.1 ± 0.4	＞ 50	> 11
9xR	—	H	Me	Me	H	NMe_2	13.6 ± 0.8	＞ 50	> 3.6
9yR	—	H	Me	Me	H	$NHCOCH{=}CH_2$	＞ 50	＞ 50	-[g]
10aR	—	H	Me	Me	H	$CONMe_2$	＞ 50	＞ 50	-[g]
10bR	—	H	Me	Me	H	$CONEt_2$	＞ 50	＞ 50	-[g]
10cR	—	H	Me	Me	H	$CO\text{-}[N(CH_2CH_2)_2O]$	＞ 50	＞ 50	-[g]
10dR	—	H	Me	Me	H	$CO\text{-}[N(CH_2CH_2)_2NMe]$	＞ 50	＞ 50	-[g]
10eR	—	H	Me	Me	H	$COCH_2CH_2\text{-}[N(CH_2CH_2)_2O]$	＞ 50	＞ 50	-[g]
Clorgyline[b]		-	-	-	-	-	0.025 ± 0.01	-[g]	-[g]
Pargyline[b]		-	-	-	-	-	-[g]	5.0 ± 0.6	-[g]

[a]All the values are expressed as the mean ± SEM of triplicate determinations; [b]Positive control; [c]SI = [IC_{50} MAO-B (μM)] / [IC_{50} MAO-A (μM)]; [d] Both the sample isolated from Wuling capsule and synthetic sample displayed almost the same values; [e]C3,4-double bond; [f]The structures of the compounds (**1**, **2** and **4**) are shown in Figure 1; [g] means not determined.

Table 2. IC_{50} values of C7-aryl or heteroaryl analogs against monoamine oxidases.



Compound	R^3	Ar	IC_{50} (μM)[a] (MAO-A)	IC_{50} (μM)[a] (MAO-B)	SI[c]
9aR	Me	H	4.6 ± 0.0	38.5 ± 1.4	8.3
11aR	Me		10.5 ± 1.5	1.0 ± 0.1	0.1
11bR	Me		12.4 ± 2.8	4.1 ± 0.3	0.3
11cR	Me		13.7 ± 3.5	1.0 ± 0.3	0.1
11dR	Me		1.1 ± 0.1	38.9 ± 5.9	38
11eR	Me		0.8 ± 0.1	21.0 ± 2.7	26
11fR	Me		3.0 ± 0.4	> 50	> 17
11gR	Me		0.20 ± 0.0	> 50	> 250
11hR	Me		2.7 ± 0.2	> 50	> 18
12aR	Me		0.20 ± 0.1	> 50	> 250
12bR	Me		2.1 ± 0.1	> 50	> 24
12cR	Me		0.14 ± 0.3	> 50	> 350
13aR	Et		0.06 ± 0.04	> 50	> 830
13bR	Et		0.3 ± 0.1	> 50	> 167
Clorgyline[b]	-	-	0.025 ± 0.005	-[d]	-[d]
Pargyline[b]	-	-	-[d]	5.0 ± 0.6	-[d]

[a]All the values are expressed as the mean ± SEM of triplicate determinations; [b]Positive control; [c]SI = [IC_{50} MAO-B (μM)]/[IC_{50} MAO-A (μM)]; [d] "-" means not determined.

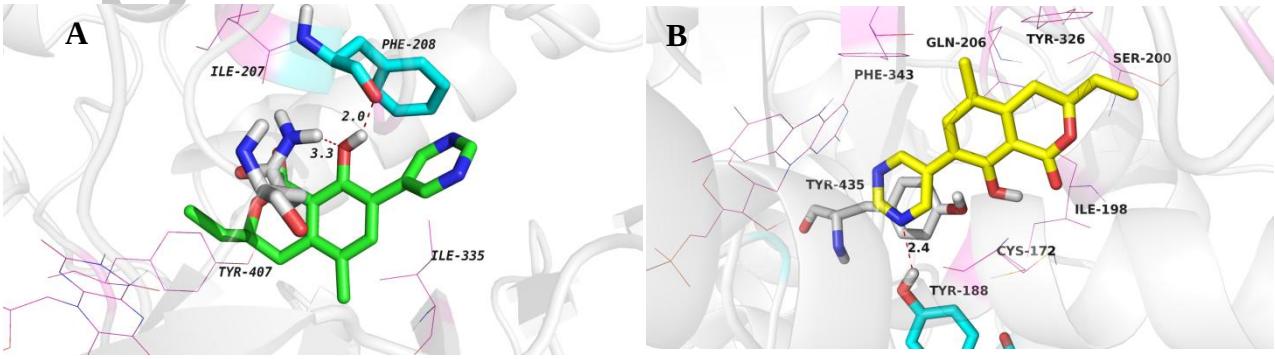


Figure 2. (A) The binding mode of **13aR** (green sticks) with MAO-A (PDB code: 2Z5Y); (B) The binding mode of **13aR** (yellow stick) with MAO-B (PDB code: 6FVZ). The pictures were generated by Pymol.

2.4 The kinetic study of enzyme MAO-A and the mode of MAO-A inhibition

Currently reversibility is an important characteristic of MAO inhibitors due to a number of potential side effects from irreversible MAO inhibitors[11]. Thus, the kinetic study of enzyme MAO-A and the mode of MAO-A inhibition were subsequently carried out with some representative compounds such as **11gR**, **12aR**, **12cR** and the most potent compound (**13aR**) using reported protocols[8e]. From the substrate-dependent kinetic experiments, both the corresponding progression curves and the Linewever-Burk plots were generated. The initial rates of the MAO-A-catalyzed oxidation of kynuramine were measured at eight different substrate concentrations in the absence and in the presence of two different concentrations of the tested compounds. The results are depicted in Fig. 3.

Based on the kinetic data of the enzyme MAO-A reactions, the linear Eadie-Hofstee plots exhibited that MAO-A catalyzed reactions were conformed by Michaelis-Menten equation (Fig.3A). The enzyme kinetic analysis was also consistent with the experimental results using the purified recombinant human MAO-A. The Km and Vmax values for MAO-A were calculated to be 87.79 ± 6.76 μ M and 492.00±17.52 μM/min/mg, respectively.

The Lineweaver-Burk plots of the inhibitory activities of the representative compounds (**13aR**, **12aR**, **12cR** and **11gR)** on *h*MAO-A indicated a noncompetitive mechanism. As shown in Figure 3B-E, all the lines are linear and intersect on the *x*-axis, showing a decrease of substrate V_{max} with constant Km. These results appeared to be similar to another natural product luteolin, which is known as a weak noncompetitive *h*MAO-A inhibitor[27].

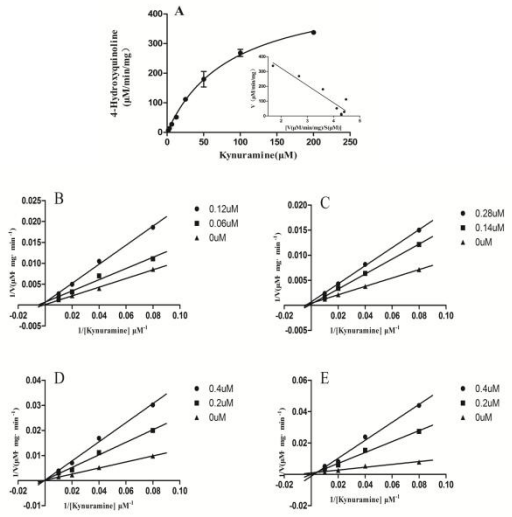


Figure 3. The kinetic study (A) and the mode of MAO-A inhibition with the representative compounds including **13aR** (B), **12aR** (C), **12cR** (D) and **11gR** (E).

Recently, it was reported[8a], some chromone analogs could display non-competitive behavior when ligands bind to the enzyme active site in a way that is defined as "tight-binding inhibition"[28]. In this case, the standard steady-state kinetic model used to describe the mechanism of inhibition is no longer valid. Instead, higher concentrations of substrate are needed in order to compete with the inhibitor in terms of binding to the enzyme active site. To figure out the exact mechanism of our compounds, we measured IC_{50} values of **13aR** using different substrate concentrations.

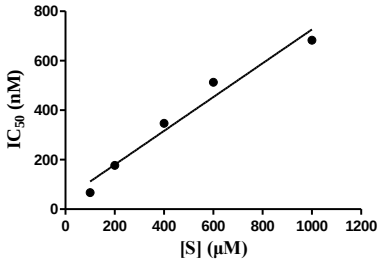


Figure 4. Effect of substrate (S) concentration on the IC_{50} values for **13aR**.

We applied a protocol by plotting IC_{50} values of **13aR** at different kynuramine concentration from 50 μM to 1000 μM. As shown in Fig. 4, the linear correlation between IC_{50} values and substrate concentration indicated that our compound was in full agreement with tight-binding competitive inhibition, confirming our compound should bind in the MAO-A active site.

2.5 Molecular docking study

With this insight in mind, in order to explain these biological results, a preliminary molecular docking study was performed using the most potent and selective compound **13aR** as the ligand and HRM/MAO-A complex structure (PDB code: 2Z5Y) as the reference due to the structural similarity of the ligand HRM.[19a] Aiding by Schrodinger (Maestro suite) and Pymol, one possible binding mode of the best compound **13aR** and MAO-A was described as shown in Figure 2A.

Similar to HRM, **13aR** would stay into the same pocket of MAO-A, and the binding site was close to co-factor FAD. Two critical hydrogen bonds between **13aR** and MAO-A were observed: one from the phenol hydroxyl as hydrogen bond donor and Phe208 residue as hydrogen bond acceptor; and the other from the amino of the Gln215 residue as hydrogen bond donor and the carbonyl oxygen atom of the lactone as hydrogen bond acceptor. Both hydrogen bonds should give essential contribution to the activity of (*R*)-5-MM and its analogs. In addition, the pyrimidine ring at the C-7 position of **13aR** appeared to have a π–π interaction with the phenyl ring of Phe208, making extra contribution to the activity.

To explain the selective inhibition of compound **13aR** toward *h*MAO-A, molecular docking study of **13aR** with *h*MAO-B (PDB code: 6FVZ)[8a] was carried out. As shown in Figure 2B, **13aR** displayed an opposite orientation in *h*MAO-B, leading to the loss of two important hydrogen bonds and one π–π interaction with Phe208 in the binding site of *h*MAO-B. On the other hand, the pyrimidine ring in **13aR** can not only form one hydrogen bond with Tyr188, but also establish one π–π interaction with Tyr435 in the pocket of *h*MAO-B. However, the docking score of **13aR** with *h*MAO-A and *h*MAO-B were -9.824 and -8.873 respectively, which means stronger binding affinity of **13aR** to the binding sites of *h*MAO-A than *h*MAO-B.

3. Conclusion

In continuation of our efforts to investigate the active chemical constituents of the fermented mycelia of *X. nigripes* and their anti-depression mechanisms, we discovered (*R*)-5-MM was a selective inhibitor against MAO-A. A variety of analogs around (*R*)-5-MM were synthesized and evaluated *in vitro* for their inhibitory potency against MAO-A and MAO-B, with most of them displaying selective MAO-A inhibitory activities in the 0.06–29 μM range. Particularly, compound **13aR** was the most potent MAO-A inhibitor with high selectivity (IC_{50}, MAO-A:

0.06 μM; MAO-B: > 50 μM). The enzyme kinetics study of **13aR** indicated that this ligand seemed to bind in the MAO-A active site according to so-called "tight-binding inhibition" mode. The molecular docking study of **13aR** was thereafter performed in order to rationalize the obtained biological results, which supported one possible binding mode of the potent inhibitor with MAO-A.

4. Experimental

General. All chemicals were purchased from Adamas, SCRC, Alfa Aesar, and used without further purification. Deuterated solvents were purchased from Cambridge Isotope Laboratories. All non-aqueous reactions were carried out using oven-dried (110 °C) or heat gun dried glassware under a positive pressure of anhydrous argon unless otherwise noted. THF and dichloromethane were purified by distillation and dried by passage over activated 4 Å molecular sieves under an argon atmosphere. ^{1}H, ^{13}C and ^{19}F NMR data were recorded on a Varian Model Mercury 400 MHz or Bruker 600 MHz spectrometers using solvent signals ($CDCl_3$: δ_H 7.26/δ_C 77.0; $CFCl_3$: δ_F 0) as references. ^{1}H NMR chemical shifts (δ) are given in ppm (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet) downfield from Me_4Si. LC-MS data were recorded on an Agilent 1260/6120 quadrupole LC/MS spectrometer, and high resolution mass spectra obtained on an AB SCIEX Triple TOF™ 5600+ mass spectrometer. IR spectra were recorded on a Nicolet AVATAR 360 FT-IR spectrometer; Optical rotations were acquired on a Rudolf Autopol IV automatic polarimeter.

*4.1.1. General procedure for the synthesis of compounds (**6a** and **6b**)*

The appropriate benzoic acid (**5a** or **5b**) (19.9 mmol) was converted into its corresponding acid chloride by treatment with thionyl chloride (22.0 mL, 284 mmol), and this intermediate was reacted with *tert*-butylamine (8.0 mL, 74.0 mmol) at room temperature for 0.5 h. The reaction mixture was concentrated under reduced pressure and dissolved in CH_2Cl_2, washed with water, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography with petroleum ether (PE)/EtOAc to yield pure product.

*4.1.1.1. N-(tert-butyl)-2-methoxy-5-methylbenzamide (**6a**).* Colorless liquid (yield: 96%); R_f = 0.43 (PE/EtOAc 20/1). IR (film) ν_{max}: 3395, 2925, 1659, 1540, 1495, 1393, 1246, 1025, 810 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.11 (1H, d, J = 8.2 Hz), 6.74─6.66 (1H, m), 5.85 (1H, s), 4.69 (1H, s), 3.77 (3H, s), 3.72 (1H, s), 2.86 (1H, d, J = 13.9 Hz), 2.62─2.51 (1H, m), 2.26 (3H, s), 1.44 (9H, s), 1.00 (3H, t, J = 7.2 Hz). ESI-MS (*m/z*): 202.3 $[M+H]^+$.

4.1.2. *General procedure for the synthesis of compounds (**7aR**–**bR**, **7fR**, **7g** and **7h**).*

To a solution of **6a** or **6b** (4.83 mmol) in anhydrous THF (20.0 mL) was added TMEDA (1.6 mL, 10.6 mmol) at -78 °C. To the resulting mixture was added dropwise a solution *n*-BuLi in hexane (2.5 M in hexane, 19.3 mmol) at -78 °C. After 2 h, to the resulting mixture was added dropwise the epoxide (5.80 mmol) at -78 °C. The reaction mixture was stirred at -78 °C for 7 h. The resulting mixture was quenched with saturated aqueous NH_4Cl, concentrated *in vacuo* and extracted with CH_2Cl_2. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel chromatography.

4.1.2.1. (*R*)*-N-(tert-butyl)-2-(2-hydroxypropyl)-6-methoxy-3-methylbenzamide (**7aR**).* White solid (yield: 47%); R_f = 0.39 (PE/EtOAc 3/1). mp 130─132 °C. $[\alpha]^{20}_D$ -165 (*c* 0.40, $CHCl_3$). IR (film) ν_{max}: 3285, 2964, 2925, 1637, 1468, 1365, 1261, 1082 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.11 (1H, d, J = 8.2 Hz), 6.69 (1H, d, J = 8.2 Hz), 5.85 (1H, s), 4.69 (1H, s), 3.77 (3H, s), 3.72 (1H, s), 2.86 (1H, d, J = 13.9 Hz), 2.62─2.51 (1H, m), 2.26 (3H, s), 1.44 (9H, s). ESI-MS (*m/z*): 280.2 $[M+H]^+$.

*4.1.3. General procedure for the synthesis of compounds (**8aR**–**bR**, **8fR**, **8g** and **8h**).*

To a solution of the raw materials (**7aR**–**bR**, **7fR**, **7g** or **7h**) (2.32 mmol) in toluene (30.0 mL) was added *p*-TsOH·H_2O (662 mg, 3.48 mmol). The reaction mixture was refluxed and stirred for 2 h. The resulting mixture was concentrated *in vacuo*. The residue was purified by silica gel chromatography.

*4.1.3.1. (R)-N-(tert-butyl)-2-(2-hydroxypropyl)-6-methoxy-3-methylbenzamide (**8aR**).* White solid (yield: 60%); R_f = 0.44 (PE/EtOAc 10/1). mp: 89–90 °C. $[\alpha]^{20}_D$ -234 (*c* 0.20, $CHCl_3$). IR (film) ν_{max}: 2954, 2924, 1682, 1673, 1463, 1375, 1236, 1219, 1118, 807 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (d, J = 8.4 Hz, 1H), 6.81 (d, J = 8.4 Hz, 1H), 4.48 (s, 1H), 3.90 (s, 3H), 2.86 (d, J = 16.3 Hz, 1H), 2.73–2.60 (m, 1H), 2.21 (s, 3H), 1.48 (d, J = 5.8 Hz, 3H). ESI-MS (*m/z*): 207.2 $[M+H]^+$.

*4.1.4. General procedure for the synthesis of compounds (**9aR**–**bR**, **9aS**, **9bS**, **9fR**, **9g** and **9h**).*

To a solution of the raw materials (**8aR**–**bR**, **8fR**, **8g** and **8h**) (7.12 mmol) in anhydrous CH_2Cl_2 (20.0 mL) was added dropwise boron tribromide (437 μL, 4.46 mmol) at -78 °C. After 1 h, the reaction mixture was raised at 0 °C and stirred for 1 h. The resulting mixture was quenched with saturated aqueous NH_4Cl, and extracted with CH_2Cl_2. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*.The residue was purified by silica gel chromatography.

4.1.4.1. (*R*)*-8-hydroxy-3,5-dimethylisochroman-1-one (**9aR**).* White solid (yield: 96%); R_f = 0.23 (PE/EtOAc 20/1). ee% = 98%. $[\alpha]^{20}_D$ -110 (*c* 0.20, $CHCl_3$). mp: 127–128 °C. IR (film) ν_{max}: 2923, 1662, 1377, 1366, 1294, 803 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.00 (s, 1H), 7.29 (d, J = 8.7 Hz, 1H), 6.81 (d, J = 8.5 Hz, 1H), 4.69 (s, 1H), 2.95 (d, J = 16.6 Hz, 1H), 2.78–2.65 (m, 1H), 2.20 (s, 4H), 1.55 (d, J = 6.1 Hz, 3H). ESI-MS (*m/z*): 193.1 $[M+H]^+$.

*4.1.4.2. (R)-8-hydroxy-3-methylisochroman-1-one (**9bR**).* White solid (yield: 96%); R_f = 0.21 (PE/EtOAc 20/1). $[\alpha]_D^{20}$ -99.1 (*c* 0.20, $CHCl_3$). IR (film) ν_{max}: 2955–2854, 1681, 1464, 1377, 1292, 1237, 1220, 1119, 807 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.02 (brs, 1H), 7.40 (t, J = 7.9 Hz, 1H), 6.88 (d, J = 8.4 Hz, 1H), 6.68 (d, J = 7.4 Hz, 1H), 4.72 (m 1H), 2.92 (d, J = 7.1 Hz, 2H), 1.52 (d, J = 6.3 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.9, 162.1, 139.4, 136.1, 117.9, 116.2, 108.3, 76.1, 34.6, 20.7. ESI-MS (*m/z*): 179.1 $[M+H]^+$.

4.1.4.3. (*S*)*-8-hydroxy-3,5-dimethylisochroman-1-one (**9aS**).* White solid (yield: 95%). R_f = 0.23 (PE/EtOAc 20/1). ee% = 86%. $[\alpha]^{20}_D$ 95.9 (*c* 0.20, $CHCl_3$). mp: 126–128 °C. IR (film) ν_{max}: 3408, 2925, 1597, 1451, 1384, 1295, 1206, 1130, 1089, 1053, 803 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.00 (s, 1H), 7.29 (d, J = 8.7 Hz, 1H), 6.81 (d, J = 8.5 Hz, 1H), 4.69 (s, 1H), 2.95 (d, J = 16.6 Hz, 1H), 2.78–2.65 (m, 1H), 2.20 (s, 4H), 1.55 (d, J = 6.1 Hz, 4H). ESI-MS (*m/z*): 193.1 $[M+H]^+$.

4.1.4.4. (*S*)*-8-hydroxy-3-methylisochroman-1-one (**9bS**).* White solid (yield: 96%); R_f = 0.21 (PE/EtOAc 20/1). $[\alpha]_D^{20}$ 85.3 (*c* 0.20, $CHCl_3$). IR (film) ν_{max}: 2955–2854, 1681, 1464, 1377, 1292, 1237, 1200 , 1119, 807 cm^{-1}. ^{1}H NMR(400MHz, $CDCl_3$) δ 11.02 (br s, 1H), 7.40 (t, J = 7.9 Hz, 1H), 6.88 (d, J = 8.4 Hz,

1H), 6.68 (d, *J* = 7.4 Hz, 1H), 4.72 (m, 1H), 2.92 (d, *J* = 7.1 Hz, 2H), 1.52 (d, *J* = 6.3 Hz, 3H). ESI-MS (*m/z*): 179.1 $[M+H]^+$.

4.1.4.5. (*R*)*-3-ethyl-8-hydroxy-5-methylisochroman-1-one* ***(9fR)***. White solid (yield: 85%); R_f = 0.32 (PE/EtOAc 20/1). $[\alpha]_D^{20}$ -87.9 (*c* 0.20, $CHCl_3$). mp: 83–85 °C. IR (film) ν_{max}: 2963–2925, 1674, 1609, 1478, 1385, 1219, 1172, 1126, 1043, 826 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 11.00 (s, 1H), 7.28 (d, *J* = 8.5 Hz, 1H), 6.81 (d, *J* = 8.5 Hz, 1H), 4.52–4.41 (m, 1H), 2.92 (dd, *J* = 16.6, 3.3 Hz, 1H), 2.73 (dd, *J* = 16.6, 11.7 Hz, 1H), 2.19 (s, 3H), 1.98–1.86 (m, 1H), 1.86–1.75 (m, 1H), 1.10 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.5, 137.8, 137.1, 124.9, 115.6, 108.3, 80.2, 29.7, 28.0, 18.1, 9.3. ESI-MS (*m/z*): 207.1 $[M+H]^+$, HRESI-MS (*m/z*): $[M+H]^+$ Calcd. for $C_{12}H_{15}O_3$: 207.1016 found: 207.1017.

4.1.4.6. 8-hydroxy-5-methylisochroman-1-one ***(9g)***. White solid (yield: 90%); R_f = 0.30 (PE/EtOAc 20/1). IR (film) ν_{max}: 2955–2924, 1667, 1605, 1588, 1475, 1408, 1384, 1263, 1246, 1206, 1165, 1130, 1043, 829 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 10.93 (s, 1H), 7.29 (d, *J* = 8.5 Hz, 1H), 6.82 (d, *J* = 8.5 Hz, 1H), 4.56 (t, *J* = 6.1 Hz, 2H), 2.95 (t, *J* = 6.1 Hz, 2H), 2.20 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.6, 137.9, 137.3, 124.9, 115.8, 108.5, 67.5, 24.8, 18.0. ESI-MS (*m/z*):179.1 $[M+H]^+$, HRESI-MS (*m/z*): $[M+H]^+$ Calcd. for $C_{10}H_{11}O_3$: 179.0703 found: 179.0700.

4.1.4.7. 8-hydroxy-3,3,5-trimethylisochroman-1-one ***(9h)***. White solid (yield: 94%) R_f = 0.28 (PE/EtOAc 20/1). IR (film) ν_{max}: 2983–2925, 1669, 1607, 1477, 1387, 1373, 1247, 1220, 1180, 1112, 1045, 834 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 11.13 (s, 1H), 7.29 (d, *J* = 8.5 Hz, 1H), 6.81 (d, *J* = 8.5 Hz, 1H), 2.89 (s, 2H), 2.18 (s, 3H), 1.48 (s, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.4, 138.0, 136.0, 125.4, 115.6, 107.7, 81.4, 36.4, 27.5, 18.1. ESI-MS (*m/z*): 207.1 $[M+H]^+$, HRESI-MS (*m/z*): $[M+H]^+$ Calcd. for $C_{12}H_{15}O_3$: 207.1016 found: 207.1014.

4.2.1. The procedure for the synthesis of compound ***(11)***.

The 2-amino-6-methoxybenzoic acid **10** (2.0 g, 11.9 mmol) was added to the solution with ice water (28.0 mL), HCl (10 M, 7.2 mL) and acetone (10.0 mL). The suspension was cooled to 0 °C followed by the addition of sodium nitrite (1.66 g, 24.0 mmol) in 12.0 mL of water. The reaction was stirred for 30 min at 0 °C. Then KI (3.98 g, 24.0 mmol) was added to the solution. The reaction was heated to 90 °C for 6 h. The reaction was cooled and quenched with saturated aqueous NH_4Cl, and extracted with CH_2Cl_2. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel chromatography.

4.2.1.1. 8-methoxy-3-methyl-1H-isochromen-1-one ***(11)***. White solid (yield: 85%); R_f = 0.24 (CH_2Cl_2/EtOAc 1/1). ^1H NMR (400 MHz, $CDCl_3$) δ 12.17 (s, 1H), 11.35 (s, 1H), 7.41 (t, *J* = 8.4 Hz, 1H), 6.72 (d, *J* = 8.5 Hz, 1H), 6.49 (d, *J* = 8.3 Hz, 1H), 4.07 (s, 3H). ESI-MS (*m/z*):278.1 $[M+H]^+$.

4.2.2. The procedure for the synthesis of compounds ***(8c)***.

Acetylacetone (1.1 mL, 10.8 mmol) was added to a suspension of **11** (1.50 g, 5.40 mmol), CuI (103 mg, 0.54 mmol) and Cs_2CO_3 (1.76 g, 5.40 mmol) in anhydrous DMSO (10 mL) in a 50 mL Schlenk tube under an Argon atmosphere and stirred at 120 °C overnight. The resulting mixture was quenched with saturated aqueous NH_4Cl, and extracted with CH_2Cl_2. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel chromatography.

4.2.2.1. 8-methoxy-3-methyl-1H-isochromen-1-one ***(8c)***. Yellow solid (yield: 49%); R_f = 0.28 (PE/EtOAc 2.5/1). ^1H NMR (400 MHz, $CDCl_3$) δ 10.99 (s, 1H), 7.55 (t, *J* = 7.9 Hz, 1H), 6.91 (d, *J* = 8.3 Hz, 1H), 6.79 (d, *J* = 7.7 Hz, 1H), 6.27 (s, 1H), 2.27 (s, 3H). ESI-MS (*m/z*): 177.1 $[M+H]^+$.

4.2.3. The procedure for the synthesis of compound ***(9c)***.

The same as *4.1.3*.

4.2.3.1. 8-hydroxy-3-methyl-1H-isochromen-1-one ***(9c)***. White solid (yield: 51%); R_f = 0.25 (PE/EtOAc 5/1). ^1H NMR (400 MHz, $CDCl_3$) δ 10.99 (s, 1H), 7.55 (t, *J* = 7.9 Hz, 1H), 6.91 (d, *J* = 8.3 Hz, 1H), 6.79 (d, *J* = 7.7 Hz, 1H), 6.27 (s, 1H), 2.27 (s, 3H). ESI-MS (*m/z*): 177.1 $[M+H]^+$.

4.3.1. General procedure for the synthesis of compounds ***(9dR,9iR–kR)***.

To a solution of **9aR** or **9bR** (0.52 mmol) in CH_2Cl_2 (10.0 mL) was added NXS (X = Br, I) (0.57 mmol) and $FeCl_3$ (4.20 mg, 0.03 mmol) at room temperature, then stirred for 1 h. The resulting mixture was quenched with saturated aqueous NH_4Cl, and extracted with CH_2Cl_2. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel chromatography (PE/EtOAc 20/1), giving **9dR**, **9jR** and **9kR**, respectively. **9iR** was prepared according to the known method[23].

4.3.1.1. (R)-8-hydroxy-5-iodo-3-methylisochroman-1-one ***(9dR)***. White solid (yield: 20%). R_f = 0.39 (PE/EtOAc 20/1). $[\alpha]_D^{20}$ -65.0 (*c* 0.30, $CHCl_3$). mp: 144–146 °C. IR (film) ν_{max}: 3407, 2930, 1676, 1598, 1451, 1384, 1295, 1210, 1130, 1093, 1054, 801 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 11.26 (s, 1H), 7.83 (d, *J* = 8.9 Hz, 1H), 6.73 (d, *J* = 8.8 Hz, 1H), 4.83–4.59 (m, 1H), 3.07 (dd, *J* = 16.9, 3.1 Hz, 1H), 2.81 (dd, *J* = 16.7, 11.8 Hz, 1H), 1.57 (d, *J* = 6.3 Hz, 4H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 162.6, 145.5, 142.0, 118.7, 110.0, 84.9, 75.5, 40.0, 20.7. ESI-MS (*m/z*): 304.0 $[M+H]^+$.

4.3.1.2. (*R*)*-7-chloro-8-hydroxy-3,5-dimethylisochroman-1-one* ***(9iR)***. White solid (yield: 87%). R_f = 0.17 (PE/EtOAc 25/1). $[\alpha]_D^{20}$ 11.4 (*c* 0.30 $CHCl_3$). mp: 93–95 °C. IR (film) ν_{max}:2955–2853, 1682, 1480, 1455, 1293, 1216, 1201, 1168, 803, 764 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 11.54 (brs, 1H), 7.40 (s, 1H), 4.77–4.63 (m, 1H), 2.93 (dd, *J* = 16.6, 3.4Hz, 1H), 2.71 (dd, *J* = 16.6, 11.6Hz, 1H), 2.19 (s, 3H), 1.56 (d, *J* = 6.2 Hz, 4H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.9, 156.0, 137.6, 135.8, 125.7, 120.0, 109.3, 75.7, 31.7, 20.8, 17.9. ESI-MS (*m/z*): 227.1, $[M+H]^+$. HRESI-MS (*m/z*): $[M+H]^+$ Calcd. for $C_{11}H_{12}ClO_3$: 227.0469, found: 227.0468.

4.3.1.3. (*R*)*-7-bromo-8-hydroxy-3,5-dimethylisochroman-1-one* ***(9jR)***. White solid (yield: 96%); R_f = 0.20 (PE/EtOAc 20:1). $[\alpha]_D^{20}$ -69.1 (*c* 0.20, $CHCl_3$). mp: 165–167 °C. IR (film) ν_{max}: 2953–2851, 1662, 1366, 1377, 1294, 802, 770 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 11.66 (br s, 1H), 7.57 (s, 1H), 4.89–4.56 (m, 1H), 2.92 (dd, *J* = 3.2, 16.8 Hz, 1H), 2.69 (dd, *J* = 11.7, 16.8 Hz, 1H), 2.20 (s, 3H), 1.56 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.7, 159.2, 146.7, 137.7, 127.1, 108.2, 82.6, 75.6, 31.7, 20.8, 17.8. ESI-MS (*m/z*): 270.0 $[M+H]^+$. HRESI-MS (*m/z*): $[M+H]^+$ Calcd. for $C_{11}H_{12}BrO_3$: 270.9964, found: 270.9962.

4.3.1.4. (*R*)*-8-hydroxy-7-iodo-3,5-dimethylisochroman-1-one* ***(9kR)***. White solid (yield: 94%). R_f = 0.24 (PE/EtOAc 15/1). $[\alpha]_D^{20}$ -63.5 (*c* 0.30, $CHCl_3$). mp: 164–168 °C. IR (film) ν_{max}: 2953–2851, 1660, 1389, 1364, 1293, 1216, 804 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 11.84 (br s, 1H), 7.79 (s, 1H), 4.77–4.61 (m, 1H), 2.92 (dd, *J* = 3.2,16.8 Hz, 1H), 2.69 (dd, *J* = 16.8, 11.7 Hz, 1H), 2.18 (s, 3H), 1.55 (d, *J* = 6.3 Hz, 3H).^{13}C NMR (150 MHz, $CDCl_3$) δ 169.7, 159.2, 146.7, 137.7, 127.1, 108.2, 82.6, 75.5,

31.7, 20.8, 17.7. ESI-MS (*m/z*): 319.0 $[M+H]^+$. HRESI-MS (*m/z*): $[M+H]^+$ Calcd. for $C_{11}H_{12}IO_3$: 318.9826, found: 318.9825.

*4.3.2. General procedure for the synthesis of compounds (**9eR** and **9lR**–**9oR**).*

To a solution of **9kR** or **9dR** (0.24 mmol) in 10.0 mL of anhydrous 1,4-dioxane charged with argon was added organic zinc reagent (0.72mmol) and $Pd(dppf)Cl_2$ (27.0 mg, 0.04 mmol). The reaction mixture was refluxed under argon until TLC analysis showed complete consumption of the starting material. The resulting mixture was quenched with saturated aqueous NH_4Cl, concentrated *in vacuo* and extracted with CH_2Cl_2. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel chromatography.

4.3.2.1. (*R*)*-5-ethyl-8-hydroxy-3-methylisochroman-1-one (**9eR**).* White solid (yield: 63%). R_f = 0.27 (PE/EtOAc 30/1). $[\alpha]^{20}_D$ -67.0 (*c* 0.20, $CHCl_3$). mp: 91–93 °C. IR (film) ν_{max}: 2963, 2927, 1674, 1478, 1385, 1220, 1130, 1071, 836 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 11.04 (s, 1H), 7.31 (d, *J* = 8.6 Hz, 1H), 6.85 (d, *J* = 8.6 Hz, 1H), 4.75–4.61 (m, 1H), 3.00 (dd, *J* = 16.4, 3.1 Hz, 1H), 2.75 (dd, *J* = 16.4, 11.5 Hz, 1H), 2.54 (q, *J* = 7.5 Hz, 2H), 1.55 (d, *J* = 6.3 Hz, 3H), 1.15 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.6, 136.6, 136.4, 131.1, 116.0, 108.1, 75.4, 31.4, 25.0, 20.9, 14.9. ESI-MS (*m/z*): 207.1 $[M+H]^+$.

4.3.2.2. (*R*)*-8-hydroxy-3,5,7-trimethylisochroman-1-one **9lR**.* R_f = 0.26 (PE/EtOAc 40/1). White solid (yield: 81%). $[\alpha]^{20}_D$ -103 (*c* 0.20, $CHCl_3$). mp: 178–180 °C. IR (film) ν_{max}: 2921, 1724, 1663, 1622, 1468, 1385, 1295, 1187, 1134, 1027, 801 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 11.22 (s, 1H), 7.16 (s, 1H), 4.74–4.57 (m, 1H), 2.91 (dd, *J* = 16.4, 3.2 Hz, 1H), 2.68 (dd, *J* = 16.4, 11.7 Hz, 1H), 2.21 (s, 3H), 2.16 (s, 3H), 1.53 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.7, 158.8, 138.9, 134.1, 124.6, 124.0, 107.3, 75.6, 31.8, 20.9, 17.9, 15.3. ESI-MS (*m/z*):207.2 $[M+H]^+$, HRESI-MS (*m/z*): $[M+H]^+$ Calcd. for $C_{12}H_{15}O_3$: 207.1016 found: 207.1014.

4.3.2.3. (*R*)*-7-ethyl-8-hydroxy-3,5-dimethylisochroman-1-one (**9mR**).* White solid (yield: 63%). R_f = 0.24 (PE/EtOAc 30/1). $[\alpha]^{20}_D$ -84.6 (*c* 0.30, $CHCl_3$). mp: 70–72 °C. IR (film) ν_{max}: 2962–2926, 1667, 1616, 1456, 1386, 1292, 1244, 1182, 1139, 1048, 807 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 11.24 (s, 1H), 7.17 (s, 1H), 4.73–4.60 (m, 1H), 2.91 (brd, *J* = 16.3 Hz, 1H), 2.73–2.60 (m, 3H), 2.18 (s, 3H), 1.54 (d, *J* = 6.1 Hz, 3H), 1.20 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.8 , 158.6, 137.4, 134.1, 130.6, 124.1, 107.5, 75.5, 31.8, 22.6, 20.9, 18.0, 13.8. ESI-MS (*m/z*): 221.2 $[M+H]^+$, HRESI-MS (*m/z*): $[M+H]^+$ Calcd. for $C_{13}H_{17}O_3$: 221.1172, found: 221.1177.

4.3.2.4. (*R*)*-8-hydroxyl-3,5-dimethyl-7-propylisochroman-1-one (**9nR**).* White liquid (yield: 31%). R_f = 0.19 (PE/EtOAc 30/1). $[\alpha]^{20}_D$ -74.6 (*c* 0.10, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 11.24 (s, 1H), 7.15 (s, 1H), 4.73–4.59 (m, 1H), 2.91 (dd, *J* = 16.6, 3.3 Hz, 1H), 2.69 (dd, *J* = 16.5, 11.8 Hz, 1H), 2.58 (t, *J* = 7.8 Hz, 3H), 2.17 (s, 3H), 1.64–1.59 (m, 2H), 1.54 (d, *J* = 6.2 Hz, 3H), 0.95 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.8, 158.7, 138.2, 134.2, 129.1, 124.0, 107.5, 75.5, 31.8, 31.5, 22.7, 20.9, 18.0, 14.0. ESI-MS (*m/z*): 235.2 $[M+H]^+$, HRESI-MS (*m/z*): $[M+H]^+$ Calcd. for $C_{14}H_{19}O_3$: 235.1329, found: 235.1336.

4.3.2.5. (*R*)*-7-butyl-8-hydroxy-3,5-dimethylisochroman-1-one (**9oR**).* Gray liquid (yield: 17%). R_f= 0.26 (PE/EtOAc 30/1). $[\alpha]^{20}_D$ -81.4 (*c* 0.10, $CHCl_3$). IR (film) ν_{max}: 2928, 1668, 1456, 1386, 1225, 1181, 1139, 807 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 11.24 (s, 1H), 7.15 (s, 1H), 4.66 (s, 1H), 2.91 (dd, *J* = 16.5, 2.8 Hz, 1H), 2.69 (dd, *J* = 16.5, 11.7 Hz, 1H), 2.60 (t, *J* = 7.4 Hz, 2H), 2.17 (s, 3H), 1.58 (m, 2H), 1.54 (d, *J* = 6.3 Hz, 3H), 1.38 (dt, *J* = 14.7, 7.3 Hz, 2H), 0.93 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.8, 158.7, 138.1, 134.2, 129.4, 124.0, 107.5, 75.5, 31.8, 31.7, 29.1, 22.6, 20.9, 18.0, 13.9. ESI-MS (*m/z*): 249.2 $[M+H]^+$, HRESI-MS (*m/z*): $[M+H]^+$ Calcd. for $C_{17}H_{18}NO_3$: 249.1485 found: 249.1487.

*4.4.1. The procedure for the synthesis of compound (**9pR**).*

4.4.1.1. (*R*)*-8-hydroxy-7-formyl-3,5-dimethylisochroman-1-one (**9pR**).* Yellow solid (yield: 85%). R_f = 0.25 (PE/EtOAc 8/1). $[\alpha]^{20}_D$ -137 (*c* 0.20, $CHCl_3$). mp: 152–154 °C. IR (film) ν_{max}: 2955–2858, 1659, 1613, 1453, 1478,1402, 1392, 1260, 1183, 1139, 1038, 806 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 11.72 (s, 1H), 10.44 (s, 1H), 7.84 (s, 1H), 4.81–4.65 (m, 1H), 3.00 (dd, *J* = 17.2, 3.4 Hz, 1H), 2.77 (dd, *J* = 17.3, 11.5 Hz, 1H), 2.24 (s, 3H), 1.57 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (150MHz, $CDCl_3$) δ 188.3, 169.7, 162.8, 144.8, 135.6, 125.6, 122.6, 109.5, 75.3, 32.3, 20.8, 18.0. ESI-MS (*m/z*): 221.0 $[M+H]^+$, 243.0 $[M+Na]^+$, HRESI-MS (*m/z*): $[M+H]^+$ Calcd. for $C_{11}H_{13}O_3$: 221.0808, found: 221.0806.

*4.4.2. The procedure for the synthesis of compounds (**9qR**).*

A mixture of **9pR** (300.0 mg, 1.36 mmol), 10% Pd/C (30.0 mg) in mixture solution of EtOH and EtOAc (1:1) (10 mL) was stirred under H_2 at room temperature and atmospheric pressure for 3.5 h. The resulting mixture was quenched with saturated aqueous NH_4Cl, then filtered, concentrated *in vacuo* and extracted with CH_2Cl_2. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel chromatography.

4.4.2.1. (*R*)*-8-hydroxy-7-(hydroxymethyl)-3,5-dimethylisochroman-1-one (**9qR**).* White solid (yield: 93%). R_f = 0.22 (PE/EtOAc 30/1). $[\alpha]^{20}_D$ -89.6 (*c* 0.30, $CHCl_3$). mp: 122–124 °C. IR (film) ν_{max}: 3397, 2924, 1664, 1618, 1452, 1478,1430, 1386, 1295, 1182, 1138, 1058, 1019, 804 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 11.35 (s, 1H), 7.33 (s, 1H), 4.68 (s, 3H), 2.94 (dd, *J* = 16.6, 2.7 Hz, 1H), 2.71 (dd, *J* = 16.5, 11.7 Hz, 1H), 2.52 (s, 1H), 2.19 (s, 3H), 1.54 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.5, 158.3, 136.9, 136.3, 127.2, 124.7, 107.9, 75.7, 61.0, 31.8, 20.6, 18.0. ESI-MS (*m/z*): 221.1 $[M-H]^-$, HRESI-MS (*m/z*): $[M+Na]^+$ Calcd. for $C_{12}H_{15}O_4$: 245.0784, found: 245.0784.

*4.4.3. The procedure for the synthesis of compounds **9rR**.*

To a solution of *(**9pR**)* (100.0 mg, 0.46 mmol) in THF/H_2O (1/1, *v*/*v*) (20.0 mL) was added Na_2HPO_4 (327 mg, 2.73 mmol), 30% H_2O_2 (0.14 mL, 1.36 mmol), $NaClO_2$ (123 mg, 1.36 mmol), stirred at room temperature for 2 h. The resulting mixture was quenched with saturated aqueous $Na_2S_2O_3$, concentrated in vacuo and extracted with CH_2Cl_2. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel chromatography.

4.4.3.1. (*R*)*-8-hydroxy-3,5-dimethyl-1-oxoisochromane-7-carboxylic acid (**9rR**).* White solid (yield: 51%). R_f = 0.34 (PE/EtOAc 1/3). IR (film) ν_{max}: 3277, 2963–2851, 1732, 1698, 1667, 1607, 1392, 1380, 1267, 1208, 1190, 1132, 1075, 821 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 13.22 (1H, s), 8.21 (1H, s), 4.78 (1H, m), 3.05 (1H, d, *J* = 17.2 Hz), 2.82 (1H, dd, *J* = 17.2, 11.8 Hz), 2.28 (3H, s), 1.61 (3H, d, *J* = 6.3 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.4, 164.5, 158.8, 143.9, 141.0, 127.04, 115.4, 109.0, 76.0, 32.0, 20.8, 18.0. ESI-MS (*m/z*): 237.1 $[M+H]^+$, HRESI-MS (*m/z*): $[M+H]^+$ Calcd. for $C_{16}H_{16}NO_3$: 237.0758 found: 237.0756.

*4.4.4. The procedure for the synthesis of compound (**9sR**).*

4.4.4.1. (*R*)*-8-hydroxy-3,5-dimethyl-1-oxoisochromane-7-carbonitrile (**9sR**).* White solid (yield: 43%). R_f = 0.21

(PE/EtOAc 5/2). $[\alpha]^{20}_D$ -87.8 (*c* 0.20, $CHCl_3$). mp: 206–208 °C. IR (film) ν_{max}: 2955–2923, 2225, 1670, 1618, 1395, 1369, 1308, 1271, 1199, 1039, 802 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 11.72 (s, 1H), 7.55 (s, 1H), 4.82–4.65 (m, 1H), 3.01 (dd, *J* = 17.2, 3.3 Hz, 1H), 2.78 (dd, *J* = 17.2, 11.5 Hz, 1H), 2.24 (s, 3H), 1.58 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.1, 162.0, 143.2, 140.2, 126.1, 115.1, 109.3, 100.1, 75.4, 32.0, 20.8, 18.0. ESI-MS (*m/z*): 218.1 $[M+H]^+$, HRESI-MS (*m/z*): $[M+H]^+$ Calcd. for $C_{12}H_{12}NO_3$: 218.0812, found: 218.0814.

*4.4.5. The procedure for the synthesis of compound (**9tR**).*

To a solution of **9pR** (70.0 mg, 0.30 mmol) in $ClCH_2CH_2Cl$ (20.0 mL) was added *p*-toluenesulfonic acid (52.0 mg, 0.30 mmol), *p*-anisidine (106.0 μL, 0.91 mmol), $NaBH(OAc)_3$ (193.0 mg, 0.91mmol). The reaction mixture was stirred at room temperature for 3.5 h under argon atmosphere. The resulting mixture was quenched with saturated aqueous $NaHCO_3$, concentrated in vacuo and extracted with CH_2Cl_2. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel chromatography.

4.4.5.1. (*R*)*-8-hydroxy-7-(((4-methoxyphenyl)amino)methyl)-3,5-dimethylisochroman-1-one (**9tR**).* Yellow solid (yield: 66%). R_f = 0.29 (PE/EtOAc 6/1). $[\alpha]^{20}_D$ -77.4 (*c* 0.30, $CHCl_3$). mp: 120–121 °C. IR (film) ν_{max}: 2925, 1664, 1617, 1512, 1455, 1386, 1293, 1364, 1234, 1180, 1084, 1137, 1034, 820 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 11.36 (s, 1H), 7.33 (s, 1H), 6.76 (d, *J* = 8.8 Hz, 2H), 6.62 (d, *J* = 8.8 Hz, 2H), 4.74–4.60 (m, 1H), 4.30 (s, 2H), 3.73 (s, 3H), 2.91 (dd, *J* = 16.6, 3.0 Hz, 1H), 2.69 (dd, *J* = 16.5, 11.7 Hz, 1H), 2.16 (s, 3H), 1.54 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.6, 158.5, 152.3, 142.2, 137.2, 135.7, 125.8, 124.5, 114.8, 114.6, 107.82, 75.6, 55.7, 43.9, 31.8, 20.9, 18.1. ESI-MS (*m/z*): 326.1 $[M-H]^-$, HRESI-MS (*m/z*): $[M+H]^+$ Calcd. for $C_{19}H_{22}NO_4$: 328.1543 found: 328.1542.

*4.4.6. The procedure for the synthesis of compound (**9uR**).*

To a solution of nitronium tetrafluoroborate (1.04 g, 7.81 mmol) in anhydrous CH_2Cl_2 (20.0 mL) was added dropwose the mixture of **9aR** (1.0 g, 5.21 mmol) in anhydrous CH_2Cl_2 (15.0 mL) at -20 °C. Then the reaction mixture was stirred at room temperature overnight. The resulting mixture was quenched with saturated aqueous NH_4Cl, concentrated in vacuo and extracted with CH_2Cl_2. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel chromatography.

*4.4.6.1. (R)-8-hydroxy-3,5-dimethyl-7-nitroisochroman-1-one (**9uR**).* Yellow solid (yield: 36%). R_f = 0.21 (PE/EtOAc 5/1). $[\alpha]^{20}_D$ -197 (*c* 0.20, $CHCl_3$). mp: 192–194 °C. IR (film) ν_{max}: 2926, 1674, 1582 1518, 1385, 1340, 1311, 1263, 1179, 1143, 1084, 1079, 764 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 12.39 (s, 1H), 8.03 (s, 1H), 4.84–4.66 (m, 1H), 3.04 (dd, *J* = 17.2, 2.7 Hz, 1H), 2.81 (dd, *J* = 17.1, 11.7 Hz, 1H), 2.28 (s, 3H), 1.59 (d, *J* = 6.2 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.2, 154.8, 144.4, 136.1, 132.9, 125.3, 110.8, 75.4, 32.1, 20.7, 18.0. ESI-MS (*m/z*):236.0 $[M-H]^-$, HRESI-MS (*m/z*): $[M+H]^+$ Calcd. for $C_{11}H_{12}NO_5$: 238.0710 found: 238.0711.

*4.4.7. The procedure for the synthesis of compound (**9wR**).*

The procedure is same as the preparation of **9qR** in 4.4.2.

4.4.7.1. (*R*)*-7-amino-8-hydroxy-3,5-dimethylisochroman-1-one(**9wR**).* Yellow solid (yield: 79%). R_f = 0.26 (PE/EtOAc 4/1). $[\alpha]^{20}_D$ -118 (*c* 0.20, $CHCl_3$). mp: 172–174 °C. IR (film) ν_{max}: 3370, 2926, 1664, 1605, 1486, 1385, 1324, 1181, 1138, 1049, 801 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 11.04 (s, 1H), 6.74 (s, 1H), 4.72–4.56 (m, 1H), 3.79 (s, 2H), 2.85 (dd, *J* = 16.4, 3.2 Hz, 1H), 2.63 (dd, *J* = 16.3, 11.5 Hz, 1H), 2.12 (s, 3H), 1.52 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.9, 148.3, 134.0, 125.3, 124.6, 122.3, 107.5, 76.2, 31.3, 20.9, 18.0. ESI-MS (*m/z*): 208.1 $[M+H]^+$, HRESI-MS (*m/z*): $[M+H]^+$ Calcd. for $C_{11}H_{14}NO_3$: 208.0968 found: 288.0966.

*4.4.8. The procedure for the synthesis of compound (**9xR**).*

To a solution of **9wR** (156.0 mg, 0.43 mmol) in DMF/H_2O (15.0 mL, 1/2) was added NaOH (102 mg, 2.54 mmol), the mixture stirred at room temperature for 0.5 h. Then added dimethyl sulfate (143 μL, 1.51 mmol) to the mixture and stirred at room temperature for 0.5 h. The resulting mixture was quenched with saturated aqueous NH_4Cl, concentrated in vacuo and extracted with CH_2Cl_2. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel chromatography.

*4.4.8.1. (R)-7-(dimethylamino)-8-hydroxy-3,5-dimethyl-3,4-dihydronaphthalen-1(2H)-one (**9xR**).* Yellow solid (yield: 16%). R_f = 0.27 (PE/EtOAc 6/1). $[\alpha]^{20}_D$ -91.2 (*c* 0.10, $CHCl_3$). Mp: 153–155 °C. IR(film)ν_{max}: 2918–2778, 1660, 1487, 1448, 1385, 1341, 1289, 1235, 1179, 1148, 1051, 804 cm^{-1}. ^1H NMR (400MHz, $CDCl_3$) δ 11.60 (s, 1H), 6.91 (s, 1H), 4.74–4.50 (m, 1H), 2.90 (dd, *J* = 16.4, 3.5 Hz, 2H), 2.81 (s, 6H), 2.67 (dd, *J* = 16.4, 11.6 Hz, 2H), 2.19 (s, 4H), 1.53 (d, *J* = 6.2 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.1, 154.1, 140.3, 128.8, 126.0, 124.2, 107.9, 75.8, 42.9, 31.6, 20.9, 18.3. ESI-MS (*m/z*): 236.3 $[M+H]^+$, HRESI-MS (*m/z*): $[M+H]^+$ Calcd. for $C_{13}H_{18}NO_3$: 236.1281 found: 236.1283.

*4.4.9. The procedure for the synthesis of compound (**9yR**).*

To a solution of **9wR** (60.0 mg, 0.29 mmol) in anhydrous CH_2Cl_2 (20.0 mL) was added acryl chloride (7.2 μL, 0.87 mmol). Then the mixture stirred at room temperature for 6 h. The resulting mixture was quenched with saturated aqueous NH_4Cl, concentrated in vacuo and extracted with CH_2Cl_2. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel chromatography.

*4.4.9.1. (R)-N-(8-hydroxy-3,5-dimethyl-1-oxoisochroman-7-yl)acrylamide (**9yR**).* White solid (yield: 46%). R_f = 0.26 (PE/EtOAc 3/1). $[\alpha]^{20}_D$ -96.2 (*c* 0.30, $CHCl_3$). mp: 126–128 °C. IR(film)ν_{max}: 3339, 2925, 1667, 1624, 1530, 1425, 1387, 1372, 1263, 1210, 1184, 1139, 1045, 801 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 11.43 (s, 1H), 8.51 (s, 1H), 7.85 (s, 1H), 6.42 (d, *J* = 16.7 Hz, 1H), 6.37–6.24 (m, 1H), 5.77 (d, *J* = 9.8 Hz, 1H), 4.68 (s, 1H), 2.91 (dd, *J* = 16.6, 2.8 Hz, 1H), 2.68 (dd, *J* = 16.5, 11.8 Hz, 1H), 2.19 (s, 3H), 1.53 (d, *J* = 5.8 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.5, 163.4, 149.1, 131.2, 131.0, 127.7, 127.6, 125.5, 125.1, 107.3, 76.1, 31.4, 20.8, 18.3. ESI-MS (*m/z*): 262.2 $[M+H]^+$, HRESI-MS (*m/z*): $[M+H]^+$ Calcd. for $C_{14}H_{16}NO_4$: 262.1074 found: 262.1076.

*4.5.1. The procedure for the synthesis of compound (**8rR**).*

A mixture of **9rR** (1.56 mmol), K_2CO_3 (744.0 mg, 4.68 mmol), and iodomethane (1.80 mmol) in DMF/H_2O (1/2, *v/v*) (10 mL) was stirred for 5 h at room temperature. The resulting mixture was quenched with saturated aqueous NH_4Cl, concentrated *in vacuo* and extracted with CH_2Cl_2. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel chromatography.

*4.5.2. General procedure for the synthesis of compounds (**10aR–eR**).*

Method 1. The appropriate benzoic acid (**9rR**) (0.32mmol) was converted into its corresponding acid chloride by treatment with thionyl chloride (4 mL, 56.0 mmol), then added this intermediate to the aqueous solution of amine (0.64 mmol) in CH_2Cl_2 (10.0 mL) at room temperature for 1 h. The resulting mixture was quenched with saturated aqueous NH_4Cl, concentrated in vacuo and extracted with CH_2Cl_2. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel chromatography.

Method 2. To a solution of **8rR** or **9rR** (0.32 mmol) in anhydrous CH_2Cl_2 (20.0 mL) was added 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI, 123.0 mg, 0.64 mmol), HOBt (0.96 mmol), amine (0.48 mmol). Then the mixture stirred at room temperature overnight. The resulting mixture was quenched with saturated aqueous NH_4Cl, concentrated *in vacuo* and extracted with CH_2Cl_2. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel chromatography.

*4.5.2.1. (R)-8-hydroxy-N,N,3,5-tetramethyl-1-oxoisochromane-7-carboxamide (**10aR**).* White solid (yield: 72%). R_f = 0.38 (CH_2Cl_2/EtOAc 10/1). $[\alpha]^{20}_D$ -82.7 (*c* 0.10, $CHCl_3$). mp: 156–158 °C. IR (film) ν_{max}: 2925, 1669, 1634, 1503, 1454, 1424, 1389, 1259, 1190, 1148, 1068, 1019, 861 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 11.34 (s, 1H), 7.35 (s, 1H), 4.75–4.62 (m, 1H), 3.12 (s, 3H), 2.99–2.92 (m, 4H), 2.73 (dd, *J* = 16.8, 11.5 Hz, 1H), 2.20 (s, 3H), 1.56 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.1, 167.8, 156.2, 138.6, 136.6, 125.4, 124.5, 108.3, 75.5, 38.2, 34.9, 31.8, 20.8, 18.0. ESI-MS (*m/z*): 264.1 $[M+H]^+$, HRESI-MS (*m/z*): 264.1 $[M+Na]^+$ Calcd. for $C_{14}H_{17}NO_4Na$: 264.1230 found: 264.1233.

*4.5.2.2. (R)-N,N-diethyl-8-hydroxy-3,5-dimethyl-1-oxoisochromane-7-carboxamide (**10bR**).* White solid (yield: 71%). R_f = 0.28 (CH_2Cl_2/EtOAc 30/1). $[\alpha]^{20}_D$ -68.6 (*c* 0.20, $CHCl_3$). Mp: 180–182 °C. IR (film) ν_{max}: 2925, 1669, 1620, 1432, 1379, 1362, 1372, 1253, 1210, 1184, 1146, 1072, 810 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 11.23 (s, 1H), 7.27 (s, 1H), 4.69 (m, 1H), 3.57 (q, *J* = 6.8 Hz, 2H), 3.22 (q, *J* = 7.2 Hz, 2H), 2.95 (dd, *J* = 16.8, 3.2 Hz, 1H), 2.72 (dd, *J* = 16.8, 11.6 Hz, 1H), 2.19 (s, 3H), 1.55 (d, *J* = 6.3 Hz, 3H), 1.24 (t, *J* = 7.2 Hz, 3H), 1.08 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.0, 167.2, 156.1, 138.0, 135.9, 125.2, 125.1, 108.3, 75.5, 42.9, 39.1, 31.8, 20.8, 18.0, 14.1, 12.9. ESI-MS (*m/z*): 292.2 $[M+H]^+$, HRESI-MS (*m/z*): $[M+Na]^+$ Calcd. for $C_{16}H_{21}NO_4Na$: 292.1543 found: 292.1542.

*4.5.2.3. (R)-8-hydroxy-3,5-dimethyl-7-(morpholine-4-carbonyl)isochroman-1-one (**10cR**).* White solid (yield: 52%). R_f = 0.32 (PE/EtOAc 2/3). $[\alpha]^{20}_D$ -88.3 (*c* 0.20, $CHCl_3$). Mp: 312–314 °C. IR (film) ν_{max}: 2957–2925, 1673, 1633, 1457, 1391, 1362, 1268, 1211, 1177, 1139, 1114, 1008, 810 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 11.37 (s, 1H), 7.37 (s, 1H), 4.69 (m, 1H), 3.77 (br s, 4H), 3.67 (brs, 2H), 3.33 (s, 2H), 2.96 (dd, *J* = 16.5, 2.8 Hz, 1H), 2.73 (dd, *J* = 16.5, 11.8 Hz, 1H), 2.20 (s, 3H), 1.56 (d, *J* = 6.2 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.0, 166.3, 156.1, 139.0, 136.9, 125.67, 123.4, 108.3, 75.5, 67.0, 66.8, 47.5, 42.4, 31.8, 20.8, 18.0. ESI-MS (*m/z*): 306.1 $[M+H]^+$, HRESI-MS (*m/z*): $[M+H]^+$ Calcd. for $C_{16}H_{20}NO_5$: 306.1336 found: 306.1341.

*4.5.2.4. (R)-8-hydroxy-3,5-dimethyl-7-(4-methylpiperazine-1-carbonyl)isochroman-1-one (**10dR**).* Yellow solid (yield: 53%). R_f = 0.24 (CH_2Cl_2/MeOH 40/1). ^1H NMR (400 MHz, $CDCl_3$) δ 11.32 (s, 1H), 7.35 (s, 1H), 4.69 (m, 1H), 3.82 (s, 2H), 3.34 (s, 2H), 2.96 (dd, *J* = 16.6, 3.5 Hz, 1H), 2.73 (dd, *J* = 16.6, 12.1 Hz, 1H), 2.48 (s, 2H), 2.39 (s, 2H), 2.31 (s, 3H), 2.20 (s, 3H), 1.56 (d, *J* = 5.9 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.0, 166.1, 156.1, 138.7, 136.7, 125.5, 123.8, 108.3, 75.5, 55.2, 54.6, 46.9, 46.0, 41.7, 31.8, 20.8, 18.0. ESI-MS (*m/z*): 319.2 $[M+H]^+$, HRESI-MS (*m/z*): $[M+H]^+$ Calcd. for $C_{17}H_{23}N_2O_4$: 319.1652 found: 319.1655.

*4.5.2.5. (R)-8-hydroxy-3,5-dimethyl-N-(2-morpholinoethyl)-1-oxoisochromane-7-carboxamide (**10eR**).* Gray solid (yield: 67%). R_f = 0.28 (MeOH/CH_2Cl_2 1/40). $[\alpha]^{20}_D$ -67.3 (*c* 0.20, $CHCl_3$). Mp: 160–162 °C. IR (film) ν_{max}: 2952–2851, 1672, 1641, 1525, 1454, 1396, 1354, 1255, 1116, 1019, 808 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 12.63 (s, 1H), 8.50 (s, 1H), 8.26 (s, 1H), 4.84–4.61 (m, 1H), 3.73 (m, 4H), 3.59 (q, *J* = 6.0 Hz, 2H), 3.00 (dd, *J* = 16.9, 3.3 Hz, 1H), 2.77 (dd, *J* = 16.9, 11.7 Hz, 1H), 2.59 (t, *J* = 6.2 Hz, 2H), 2.51 (s, 4H), 2.24 (s, 3H), 1.57 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.8, 164.1, 158.7, 141.2, 140.2, 125.6, 119.3, 75.6, 67.0, 56.9, 53.3, 36.5, 32.0, 20.8, 18.0. ESI-MS (*m/z*): 371.2 $[M+H]^+$, HRESI-MS (*m/z*): $[M+Na]^+$ Calcd. for $C_{18}H_{24}N_2O_5Na$: 371.1577 found: 371.1587.

*4.6.1. General procedure for the synthesis of compounds (**11aR–hR**, **12aR–cR**, **13aR–bR**).*

Method 1. General reaction for the synthesis of **11aR–gR** and **13bR** or **9zR** (0.30 mmol), boronic acid or its pinacol ester (0.39 mmol), K_2CO_3 (125 mg, 0.90 mmol) and $Pd(Ph_3P)_4$ (10 mol%) were suspended in 1,4-dioxane (10 mL) and water (2 drops) and vial was sealed tightly. The mixture was irradiated for 1 h at a pre-selected temperature of 100 °C, using a maximum irradiation power of 150 W. After the reaction, the vial was cooled to 60 °C by air jet cooling. The resulting mixture was quenched with saturated aqueous NH_4Cl, concentrated *in vacuo* and extracted with CH_2Cl_2. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel chromatography. Then the intermediate released free phenyl hydroxyl according to the reaction of *4.1.3*, producing the target compounds.

Method 2. General procedure–The Suzuki-Miyaura reaction for the synthesis of **13bR** in toluene/EtOH/H_2O. The mixture was refluxed at 110 °C overnight. The remaining steps were the similar to **Method** 1.

Method 3. General procedure–Conventional Stille coupling reaction for the synthesis of **11gR**. **8kR** (100 mg, 0.30 mmol), CuI (11.0 mg, 0.06 mmol), CsF (60.3 mg, 0.40 mmol), $PdCl_2(PPh_3)_2$ (17.0 mg, 0.03 mmol), 4-(Tri-*n*-butylstannyl)pyridine (100.0 mg, 0.27 mmol), were suspended in anhydrous DMF (7.0 mL) under argon atmosphere. The mixture was refluxed at 120 °C overnight. The resulting mixture was quenched with saturated aqueous NH_4Cl, concentrated *in vacuo* and extracted with CH_2Cl_2. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel chromatography.

Method 4. General procedure–Conventional Suzuki-Miyaura reaction for the synthesis of **12aR–13aR**. **9kR** or **9zR** (0.30 mmol), boronic acid or its pinacol ester (0.39 mmol), CuCl (31.3 mg, 0.31 mmol), dppf (18.0 mg, 0.03 mmol), $Pd(OAc)_2$ (7.3 mg, 0.01 mmol), $CsCO_3$ (410.6 mg, 1.27 mmol), were suspended in anhydrous DMF (7.0 mL) under argon atmosphere. The mixture was refluxed at 120 °C overnight. The resulting mixture was quenched with saturated aqueous NH_4Cl, concentrated *in vacuo* and extracted with CH_2Cl_2. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel chromatography.

*4.6.1.1. (R)-8-hydroxy-3,5-dimethyl-7-phenylisochroman-1-one (**11aR**).* Yellow solid (yield: 75%). R_f = 0.31 (PE/CH_2Cl_2 2/1). $[\alpha]^{20}_D$ -111 (*c* 0.20, $CHCl_3$). mp: 168–170 °C. IR (film) ν_{max}: 2928, 1663, 1617, 1458, 1421, 1385, 1322, 1263, 1194, 1131, 1057, 806 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 11.56 (s, 1H), 7.58 (d, *J* = 7.6 Hz, 2H), 7.43 (t, *J* = 7.6 Hz, 2H), 7.40–7.31 (m, 2H), 4.83–4.65 (m, 1H), 2.99 (dd, *J* = 16.6, 3.1 Hz, 1H), 2.77 (dd, *J* = 16.6, 11.7 Hz, 1H), 2.25 (s, 3H), 1.58 (d, *J* = 5.8 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.7, 157.8, 138.7, 136.9, 136.3, 129.2, 128.5, 128.2, 127.4, 124.8, 108.3, 75.5, 31.9, 20.9, 18.1. ESI-MS (*m/z*):269.2 $[M+H]^+$, HRESI-MS (*m/z*): $[M+H]^+$ Calcd. for $C_{17}H_{17}O_3$: 269.1172 found: 269.1178.

*4.6.1.2. (R)-8-hydroxyl-3,5-dimethyl-7-(p-tolyl)isochroman-1-one (**11bR**).* White solid (yield: 58%). R_f = 0.35 (PE/CH_2Cl_2 2/1). $[\alpha]^{20}_D$ -81.1 (*c* 0.20, $CHCl_3$). mp: 157–159 °C. IR (film) ν_{max}: 2925, 1723, 1471, 1385, 1317, 1270, 1195, 1151, 1063, 1041, 821 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 11.53 (s, 1H), 7.47 (d, *J* = 7.5 Hz, 2H), 7.37 (s, 1H), 7.25 (d, *J* = 7.9 Hz, 2H), 4.80–4.59 (m, 1H), 2.99 (dd, *J* = 16.5, 3.1 Hz, 1H), 2.77 (dd, *J* = 16.5,11.7 Hz, 1H), 2.40 (s, 3H), 2.25 (s, 3H), 1.58 (d, *J* = 5.8 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.7, 157.9, 138.6, 137.1, 136.0, 134.0, 129.1, 128.9, 128.6, 124.7, 75.5, 32.0, 21.2, 20.9, 18.1. ESI-MS (*m/z*): 297.1 $[M+H]^+$, HRESI-MS (*m/z*): $[M+H]^+$ Calcd. for $C_{18}H_{19}O_3$: 297.1485 found: 297.1496.

*4.6.1.3. (R)-8-hydroxyl-3,5-dimethyl-7-(4-(trifluoromethyl)phenyl)isochroman-1-one (**11cR**).* Yellow solid (yield: 42%). R_f = 0.37 (PE/CH_2Cl_2 2/1). $[\alpha]^{20}_D$ -72.7 (*c* 0.40, $CHCl_3$). mp: 141–143 °C. IR (film) ν_{max}: 2926, 1667, 1618, 1402, 1388, 1324, 1195, 1165, 1123, 1069, 1057, 845 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 11.63 (s, 1H), 7.73–7.64 (m, 4H), 7.38 (s, 1H), 4.82–4.61 (m, 1H), 3.00 (d, *J* = 16.7 Hz, 1H), 2.78 (dd, *J* = 16.7, 11.6 Hz, 1H), 2.26 (s, 3H), 1.58 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR(150 MHz, $CDCl_3$) δ 170.6, 157.8, 140.6, 138.5, 137.4, 129.5, 129.4, 127.0, 125.1, 108.6, 75.5, 31.9, 20.9, 18.1. ^{19}F NMR (376 MHz, $CDCl_3$) δ -62.97. ESI-MS (*m/z*): 335.0 $[M-H]^-$, HRESI-MS (*m/z*): $[M-H]^-$ Calcd. for $C_{18}H_{14}F_3O_3$: 335.0900 found: 335.0901.

*4.6.1.4. (R)-8-hydroxyl-7-(4-hydroxyphenyl)-3,5-dimethylisochroman-1-one (**11dR**).* Yellow solid (yield: 54%). R_f = 0.28 (PE/EtOAc 2/1). $[\alpha]^{20}_D$ -32.1 (*c* 0.20, $CHCl_3$). mp: 226–228 °C. IR (film) ν_{max}: 3332, 2923, 1649, 1611, 1578, 1520, 1422, 1392, 1264, 1199, 1152, 1106, 1056, 804 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 11.54 (s, 1H), 7.46 (d, *J* = 8.5 Hz, 2H), 7.35 (s, 1H), 6.90 (d, *J* = 8.6 Hz, 2H), 5.12 (s, 1H), 4.80–4.63 (m, 1H), 2.98 (dd, *J* = 16.6, 3.1 Hz, 1H), 2.76 (dd, *J* = 16.5, 11.6 Hz, 1H), 2.24 (s, 3H), 1.57 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (150 MHz MHz, $CDCl_3$) δ 170.8, 157.8, 155.0, 138.5, 135.8, 130.5, 129.4, 128.1, 124.7, 115.1, 108.3, 75.5, 31.9, 20.9, 18.1. ESI-MS (*m/z*): 285.0 $[M+H]^+$, HRESI-MS (*m/z*): $[M-H]^-$ Calcd. for $C_{18}H_{16}F_3O_3$: 283.0976 found: 283.0976.

*4.6.1.5 (R)-7-(4-aminophenyl)-8-hydroxy-3,5-dimethylisochroman-1-one (**11eR**).* Yellow solid (yield: 58%). R_f = 0.29 (PE/EtOAc 2/1). $[\alpha]^{20}_D$ -62.3 (*c* 0.10, $CHCl_3$). Mp: 91–93 °C. IR (film) ν_{max}: 3372, 2924, 1660, 1612, 1519, 1455, 1385, 1324, 1289, 1261, 1131, 1056, 831 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 11.52 (s, 1H), 7.40 (d, *J* = 8.1 Hz, 2H), 7.34 (s, 1H), 6.75 (d, *J* = 8.1 Hz, 2H), 4.71 (d, *J* = 5.3 Hz, 1H), 3.68 (br s, 2H), 2.96 (dd, *J* = 16.5, 3.1 Hz, 1H), 2.75 (dd, *J* = 16.5, 11.7 Hz, 1H), 2.23 (s, 3H), 1.57 (d, *J* = 6.2 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.8, 157.9, 145.8, 138.3, 135.3, 130.2, 128.6, 127.1, 124.6, 114.8, 108.2, 75.5, 31.9, 20.9, 18.1. ESI-MS (*m/z*): 284.2 $[M+H]^+$, HRESI-MS (*m/z*): $[M+H]^+$ Calcd. for $C_{17}H_{18}NO_3$: 284.1281 found: 284.1280.

*4.6.1.6. (R)-8-hydroxyl-3,5-dimethyl-7-(3-nitrophenyl)isochroman-1-one (**11fR**).* White solid (yield: 56%). R_f = 0.33 (PE/EtOAc 3/1). $[\alpha]^{20}_D$ -85.0 (*c* 0.30, $CHCl_3$). mp: 245–247 °C. IR (film) ν_{max}: 2926, 1664, 1619, 1526, 1457, 1385, 1349, 1324, 1197, 1136, 1057, 804 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 11.68 (s, 1H), 8.44 (s, 1H), 8.19 (d, *J* = 8.2 Hz, 1H), 7.96 (d, *J* = 7.7 Hz, 1H), 7.59 (dd, *J* = 8.2,7.7 Hz, 1H), 7.42 (s, 1H), 4.81–4.67 (m, 1H), 3.02 (dd, *J* = 16.7, 3.1 Hz, 1H), 2.80 (dd, *J* = 16.7, 11.6 Hz, 1H), 2.28 (s, 3H), 1.59 (d, *J* = 6.2 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.5, 157.7, 148.3, 138.5, 138.3, 137.8, 135.4, 129.0, 125.8, 125.3, 124.1, 122.1, 108.7, 75.6, 31.9, 20.9, 18.1. ESI-MS (*m/z*): 312.0 $[M-H]^-$, HRESI-MS (*m/z*): $[M+H]^+$ Calcd. for $C_{17}H_{14}NO_5$: 314.1023 found: 314.1023.

*4.6.1.7. (R)-7-(3-aminophenyl)-8-hydroxyl-3,5-dimethylisochroman-1-one (**11gR**).* Gray solid (yield: 92%). R_f = 0.18 (PE/EtOAc 3/1). $[\alpha]^{20}_D$ -75.5 (*c* 0.10, $CHCl_3$). mp: 160–162 °C. IR (film) ν_{max}: 2923–2851, 1661, 1615, 1455, 1417, 1386, 1327, 1257, 1190, 1131, 1056, 806 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 11.54 (s, 1H), 7.37 (s, 1H), 7.22 (t, *J* = 7.8 Hz, 1H), 6.97–6.89 (m, 2H), 6.69 (d, *J* = 7.7 Hz, 1H), 4.72 (m, 1H), 3.73 (s, 2H), 2.98 (dd, *J* = 16.6, 3.3 Hz, 1H), 2.76 (dd, *J* = 16.6, 11.6 Hz, 1H), 2.23 (s, 3H), 1.57 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.7, 157.8, 146.2, 138.7, 138.0, 136.2, 129.1, 128.7, 124.6, 119.6, 116.1, 114.3, 108.2, 75.5, 31.9, 20.9, 18.1. ESI-MS (*m/z*): 284.2 $[M+H]^+$, HRESI-MS (*m/z*): $[M+H]^+$ Calcd. for $C_{17}H_{18}NO_3$: 284.1281 found: 284.1287.

*4.6.1.8. (R)-8-hydroxyl-3,5-dimethyl-7-(pyridine-4-yl)isochroman-1-one (**11hR**).* White solid (yield: 14%). R_f = 0.25 (PE/EtOAc 5/1). $[\alpha]^{20}_D$ -48.2 (*c* 0.10, $CHCl_3$). mp: 142–144 °C. IR (film) ν_{max}: 2955–2853, 1668, 1615, 1593, 1544, 1455, 1386, 1332, 1267, 1195, 1134, 1058, 825 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 11.69 (s, 1H), 8.64 (d, *J* = 5.2 Hz, 2H), 7.53 (d, *J* = 5.2 Hz, 2H), 7.41 (s, 1H), 4.81–4.64 (m, 1H), 3.00 (dd, *J* = 16.7, 3.1 Hz, 1H), 2.78 (dd, *J* = 16.7, 11.6 Hz, 1H), 2.26 (s, 3H), 1.58 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.5, 157.9, 149.7, 144.6, 138.2, 138.1, 125.4, 125.2, 123.8, 108.7, 75.5, 32.0, 20.9, 18.1. ESI-MS (*m/z*): 270.1 $[M+H]^+$, HRESI-MS (*m/z*): $[M+H]^+$ Calcd. for $C_{17}H_{18}NO_3$: 270.1125 found: 270.1133.

*4.6.1.9. (R)-8-hydroxyl-3,5-dimethyl-7-(pyridine-3-yl)isochroman-1-one (**12aR**).* Yellow solid (yield: 7%). R_f = 0.22 (PE/EtOAc 2/1). $[\alpha]^{20}_D$ -146 (*c* 0.10, $CHCl_3$). mp: 150–152 °C. IR (film) ν_{max}: 2923, 1662, 1615, 1448, 1385, 1321, 1290, 1185, 1132, 1059, 809 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 11.59 (br s, 1H), 8.76 (s, 1H), 8.56 (s, 1H), 7.96 (d, *J* = 7.9 Hz, 1H), 7.38 (s, 1H), 7.34 (t, *J* = 6.3 Hz, 1H), 4.81–4.62 (m, 1H), 3.00 (d, *J* = 16.7 Hz, 1H), 2.78 (dd, *J* = 16.7, 11.7 Hz, 1H), 2.26 (s, 3H), 1.58 (d, *J* = 6.2 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.7, 158.1, 149.9 148.6, 138.5, 137.6, 136.9, 132.9, 125.4, 125.1, 123.2, 108.8, 75.8, 32.1, 21.1, 18.3. ESI-MS (*m/z*): 270.1 $[M+H]^+$, HRESI-MS (*m/z*): $[M+H]^+$ Calcd. for $C_{16}H_{16}NO_3$: 270.1125 found: 270.1127

*4.6.1.10. (R)-8-hydroxyl-3,5-dimethyl-7-(pyridine-2-yl)isochroman-1-one (**12bR**).* Yellow solid (yield: 10%). R_f = 0.18 (PE/EtOAc 2/1). $[\alpha]^{20}_D$ -120 (*c* 0.10, $CHCl_3$). mp: 148–150 °C. IR (film) ν_{max}: 2920, 1661, 1614, 1583, 1443, 1418, 1385, 1318, 1258, 1195, 1134, 1008, 807 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 12.35 (s, 1H), 8.67 (s, 1H), 8.04 (d, *J* = 8.0 Hz, 1H), 7.96 (s, 1H), 7.75 (t, *J* = 8.0 Hz, 1H), 7.24 (dd, *J* = 12.2, 5.9 Hz, 1H), 4.71 (dt, *J* = 10.9, 5.0 Hz, 1H), 2.99 (d, *J* = 16.7 Hz, 1H), 2.77 (dd, *J* = 16.7, 11.6 Hz, 1H), 2.26 (s, 3H), 1.56 (d, *J* = 6.2 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.9, 158.7, 154.5, 148.9, 138.5, 138.2, 136.2, 125.5, 125.0 124.3，122.0, 109.1, 75.2, 32.1, 20.9, 18.2. ESI-MS (*m/z*): 270.1 $[M+H]^+$, HRESI-MS (*m/z*): $[M+H]^+$ Calcd. for $C_{16}H_{16}NO_3$: 270.1125 found: 270.1131.

*4.6.1.11. (R)-8-hydroxyl-3,5-dimethyl-7-(pyrimidin-5-yl)isochroman-1-one (**12cR**).* Gray solid (yield: 7%). R_f = 0.18 (PE/EtOAc 4/1). $[\alpha]^{20}_D$ -31.7 (*c* 0.10, $CHCl_3$). mp: 181–183 °C. IR (film) ν_{max}: 2922, 1661, 1618, 1411, 1387, 1323, 1204, 1137, 1057, 999, 804 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 11.65 (s, 1H), 9.16 (s, 1H), 8.97 (s, 2H), 7.38 (s, 1H), 4.81–4.65 (m, 1H), 3.01 (dd, *J* = 16.8, 3.3 Hz, 1H), 2.79 (dd, *J* = 16.8, 11.6 Hz, 1H), 2.27 (s, 3H), 1.58 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.3, 157.8, 157.2, 156.6, 138.4, 137.7, 130.8, 125.6, 121.3, 108.8, 75.6, 31.9, 20.9, 18.1. ESI-MS (*m/z*): 271.1 $[M+H]^+$, HRESI-MS (*m/z*): $[M+H]^+$ Calcd. for $C_{16}H_{16}NO_3$: 271.1077 found: 271.1079.

*4.6.1.12. (R)-3-ethyl-8-hydroxy-5-methyl-7-(pyrimidin-5-yl)-3,4-dihydronaphthalen-1(2H)-One (**13aR**).* White solid (yield: 13%). R_f = 0.39 (PE/EtOAc 4/1). $[\alpha]^{20}_D$ -77.3 (*c* 0.20, $CHCl_3$). mp: 153–155 °C. IR (film) ν_{max}: 2921–2851, 1662, 1616, 1550, 1412, 1393, 1323, 1201, 1183, 1138, 1040, 806 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 11.67 (s, 1H), 9.18 (s, 1H), 8.98 (s, 2H), 7.38 (s, 1H), 4.61–4.46 (m, 1H), 2.99 (dd, *J* = 16.8, 3.4 Hz, 1H), 2.81 (dd, *J* = 16.8, 11.6 Hz, 1H), 2.29 (s, 3H), 2.04–1.91 (m, 1H), 1.91–1.80 (m, 1H), 1.13 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.4, 157.8, 157.2, 156.6, 138.5, 137.7, 130.8, 125.7, 121.2, 109.0, 80.4, 29.8, 27.9, 18.2, 9.3. ESI-MS (*m/z*): 283.1 $[M-H]^-$, HRESI-MS (*m/z*): $[M+H]^+$ Calcd. for $C_{16}H_{22}NO_4$: 285.1234 found: 285.1232.

*4.6.1.13. (R)-7-(4-aminophenyl)-3-ethyl-8-hydroxy-5-methylisochroman-1-one (**13bR**).* Gray solid (yield: 28%). R_f = 0.23 (PE/EtOAc 4/1). $[\alpha]^{20}_D$ -59.3 (*c* 0.10, $CHCl_3$). mp: 141–143 °C. IR (film) ν_{max}: 3367, 2958–2851, 1660, 1613, 1518, 1455, 1387, 1287, 1270, 1191, 1126, 1038, 834 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 11.54 (s, 1H), 7.40 (d, *J* = 8.4 Hz, 2H), 7.34 (s, 1H), 6.75 (d, *J* = 8.4 Hz, 2H), 4.57–4.41 (m, 1H),3.13 (brs, 2H), 2.94 (dd, *J* = 16.6, 3.3 Hz, 1H), 2.76 (dd, *J* = 16.6, 11.7 Hz, 1H), 2.23 (s, 3H), 2.00–1.87 (m, 1H), 1.88–1.77 (m, 1H), 1.12 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.9, 157.8, 145.7, 138.2, 135.4, 130.1, 128.5, 127.0, 124.6, 114.8, 108.4, 80.3, 29.7, 28.0, 18.1, 9.4. ESI-MS (*m/z*): 298.2 $[M+H]^+$, HRESI-MS (*m/z*): $[M+H]^+$ Calcd. for $C_{18}H_{20}NO_3$: 298.1438 found: 298.1440.

5. Monoamine oxidase (MAO-A and MAO-B) inhibition assay

Human recombinant MAO-A and MAO-B were purchased from BD Biosciences (Woburn, MA, USA). Clorgyline and Deprenyl were used as standard inhibitors of MAO-A and MAO-B, respectively. Kynuramine was used as substrate for MAO-A and MAO-B in incubations, and 4-hydroxyquinoline metabolized from Kynuramine by MAOs was used as marker to evaluate inhibitive activities of test compounds.

The test compounds or reference standard were dissolved in DMSO, and diluted to different concentration samples (from 100 μM to 0.0125 μM in pH 7.4 buffer solution, 50 μL). The enzyme reactions were carried out with the samples (50 μL), enzymes (0.0025 mg/mL, 5 μL) and substrates (25 μM in pH 7.4 buffer solution, 25 μL) in buffer solution (the final volume: 100 μL) at 37 °C for 10 min, and then quenched with a cooled solution of acetyl aminophenol in CH_3CN (200 μL). After eddy-mixing (1 min) and centrifugation (13000 g, 10 min), 10 μL supernatant was injected to the UPLC-ESI-MS/MS system to measure the quantity of 4-hydroxyquinoline related to the blank. The results for both reference drugs and test compounds were expressed as IC_{50} in Table 1 and Table 2, and the selectivity index (SI, MAO-A over MAO-B) was also given in the tables if necessary.

6. Molecular docking

The crystal structures of MAO-A (PDB code: 2Z5Y) and MAO-B (PDB code: 6FVZ) were obtained from the Protein Data Bank. Molecular modeling studies were performed using Schrodinger (Maestro suite). Protein structures were prepared by Protein Preparation Wizard (PrepWizard) of the Schrödinger suite to add missing hydrogen atoms, adjust bond orders, assign hydrogen bonds, and relax the protein−ligand complex. The other parameters were accepted as default.

Ligand sampling and preparation were performed using LigPrep of Schrödinger by using the OPLS2005 force field and charges. All possible ligand states at pH 7.4 were generated by Epik. The other parameters were accepted as default.

The compounds were then docked using Glide standard precision (SP) mode, and the top 10 poses were kept for analysis. The center of the docking receptor grids was defined as the coordinate of cognate ligands. The other parameters were accepted as default. The pictures were performed using Pymol software, and polar interactions were analyzed.

7. The kenetics study of enzyme MAO-A and the mode of MAO-A inhibition

The kinetics study of MAO-A, whose substrate was kynuramine, was represented by Michaelis-Menten kinetic experiment. The catalytic rates of human MAO-A enzyme were measured at eight different concentrations of substrate (1.5625, 3.125, 6.25, 12.5, 25, 50, 100 and 200 μM). The corresponding progression curve and the linear Eadie-Hofstee plot were generated using GraphPad Prism 5.0 (GraphPad software). In addition, the maximal velocity (Vmax) and the Michaelis constant (Km) were calculated using the same software version 5.0.

The mode of MAO-A inhibition by **13aR**, **12cR**, **11gR** and **12aR** were investigated by constructing a set of three Lineweaver-Burk plots. The first plot was constructed in the absence of inhibitor, while the other two plots were constructed in the presence of two different concentrations of the inhibitor (IC_{50} value and $2\times IC_{50}$ value). The substrate kynuramine was used at concentrations ranging from 12.5 to 100 μM, while the final concentration of MAO-A was 0.0025 mg/mL. All enzyme reactions and catalytic activity measurements were carried out as described above for the IC_{50} determinations. Linear regression analysis was performed using the Prism version 5.0 software package.

Conflict of interest

The authors confirm that this article content has no conflict of interest.

Acknowledgements

This research was supported by NSFC grants (No. 81773599, 21472024, 21472021) and a MOST grant (2018ZX09735008-005). The authors are grateful to Dr. Jie-Fei Cheng for his helpful discussion.

Supplementary Material

Supplementary data associated with this article can be found in Supporting Information.

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