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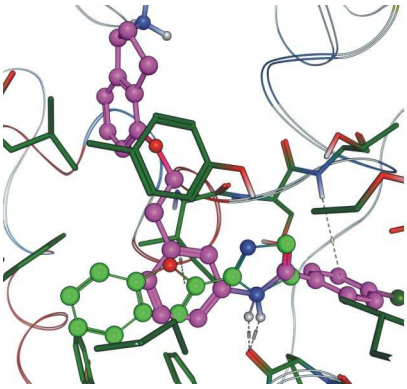
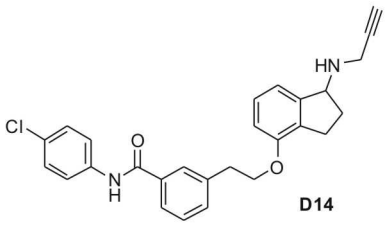
Graphical abstract





D14

	D14 (racemic)	rasagiline (*R*)
IC_{50} (hMAO-B)	20 nM	10 nM
IC_{50} (hMAO-A)	9330 nM	1010 nM
Selectivity (A/B)	466.5	101

Design, Synthesis and Bioevalucation of Novel 2,3-Dihydro-1*H*-inden-1-amine Derivatives as Potent and Selective Human Monoamine Oxidase B Inhibitors Based on Rasagiline

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Abstract

Parkinson's disease (PD) is associated with elevated levels of hMAO-B in the brain, and MAO-B has been recognized a successful target for developing anti-PD drugs. Herein we report rasagiline derivatives as novel potent and selective hMAO-B inhibitors. They were designed by employing fragment-based drug design strategy to link rasagiline and hydrophobic fragments, which may target a hydrophobic pocket in the entrance cavity of hMAO-B. Different linkers such as $-OCH_2-$, $-SCH_2-$, $-OCH_2CH_2-$, $-OCH_2CH_2O-$, $-OCH_2CH_2CH_2O-$ were tried. A promising selective hMAO-B inhibitor **D14** with similar inhibitory activity as rasagiline and improved isoform selectivity was yielded. The selectivity profile of compounds reported herein suggests that we can further develop more potent hMAO-B inhibitors with high isoform selectivity through this strategy.

Keywords: hMAO-B, isoform selectivity, fragment-based drug design, structure activity relationship

1. Introduction

Monoamine oxidase (MAO, EC 1.4.3.4), an enzyme of the protein family of flavin-containing amine oxidoreductases, catalyzes the oxidative deamination of monoamines and then influences their levels in brain by virtue of their roles in neurotransmitter breakdown [1]. In humans there are two types of MAO, hMAO-A and hMAO-B which share ~70% sequence identities. Both of them can be found in the central nervous system, such as neurons and astroglia [2, 3]. hMAO-A catalyzes the oxidative deamination of serotonin, adrenaline, and noradrenaline; whereas, hMAO-B deaminates preferentially β-phenethylamine and benzylamine [4].

hMAO-A and hMAO-B have appealed lots of interests for developing drugs in the fields of neurodegenerative conditions, including depression, Parkinson's disease (PD), Alzheimer's disease (AD), etc. [5-8]. hMAO-A inhibitors, for example, clorgiline (**1** in Fig. 1) are used for depression; while hMAO-B inhibitors display a critical role in the treatment of PD which are associated with elevated levels of hMAO-B in the brain. Therefore, developing novel, potent, selective hMAO-B inhibitors has gained extensive attentions in the treatment of these two diseases [9-18].

<Fig. 1>

Currently, several selective hMAO-B inhibitors had been approved including selegiline (**2**), rasagiline (**3**) and safinamide (**4**) [19, 20]. Among them, selegiline and rasagiline are irreversible inhibitors, utilizing the propargyl group to covalently bind to the flavin ring of the cofactor, flavin adenine dinucleotide (FAD). In this way, the

bound enzymes fail to work until the cell makes new enzymes. In contrast to selegiline and rasagiline, safinamide is reversible inhibitor [21, 22].

Fragment based drug discovery (FBDD) is a rational design method by growing or combining small chemical fragments having weak affinities to produce a lead with a higher affinity [23, 24]. It has emerged as one of the most promising alternatives to the traditional methods of drug development. In our lab, we employed a FBDD strategy to link two biomedical interesting fragments, resveratrol and coumarin, and got a series of novel hMAO-A and hMAO-B selective inhibitors with a scaffold of 3-((*E*)-3-(2-((*E*)-styryl)phenyl)acryloyl)-2H-chromen-2-one [25].

A crystal structure of hMAO-B-rasagiline complex was reported, in which, a fragment is in the entrance cavity [26]. We believed that linking fragments binding in the entrance cavity to rasagiline could increase the potency and/or selectivity against hMAO-B. In this work, we report our first endeavor to link chemical fragments into rasagiline, which yielded a potent and selective hMAO-B inhibitor, **D14**, with an IC_{50} value of 20 nM against hMAO-B and a selectivity index (SI) of 466.5 of hMAO-B over hMAO-A. The enzyme inhibitory activity of **D14** is comparable with approved drug rasagiline, and its selectivity is significantly increased. All result indicated that **D14** could be a candidate for further study.

2. Results

The crystal structure of rasagiline binding to hMAO-B has been deposited in the Protein Database Bank (PDB ID: 2FXQ) [26]. In this complex, a fragment of

2-(2-benzofuranyl)-2-imidazoline (2-BFI) is located in the entrance cavity that is distinct from the substrate-binding cavity (Fig. 2A). 2-BFI inhibits recombinant hMAO-B with a K_i of 8.3 μM, and increases binding energy of -3.9 kcal/mol to tranylcypromine against hMAO-B. The benzofuran moiety of 2-BFI is in a hydrophobic pocket composed of residues of Phe103, Trp119, Leu164, Leu167, Phe168, Leu171, Ile199, Ile316, and Tyr326 and the imidazoline moiety forms two hydrogen bonds with Tyr326 and Pro102, in addition, weak hydrophobic interactions with Leu88 by the edge of imidazoline could be observed.

It is intuitive that linking 2-BFI or similar fragments to rasagiline at appropriate positions may enhance potency and/or selectivity. When aligning the structures of hMAO-A (PDB ID: 2Z5X [27]) and hMAO-B (PDB ID: 2FXQ), it was found that there are two different residues in that hydrophobic pocket: Leu164 and Ile199 of hMAO-B against Phe173, Phe208 of hMAO-A, respectively (Fig. 2B), leading to a bulker hydrophobic pocket in hMAO-B than in hMAO-A. Therefore, compounds targeting this pocket of hMAO-B may not only have strong enzyme inhibitory activity, but have excellent isoform selectivity between hMAO-A and hMAO-B [28]. Meanwhile, selectivity of hMAO-A and hMAO-B inhibitors could be caused by the structural differences arising from Ile-335 in hMAO-A versus Tyr326 in MAO-B [27, 29].

<Fig. 2>

Based on these observation, we designed novel hMAO-B inhibitors by introducing various benzene ring and its analogs to occupy the hydrophobic pocket with different

carbon linkers such as -OCH_2-, -SCH_2-, -OCH_2CH_2-, -OCH_2CH_2O-, -$OCH_2CH_2CH_2O$- according to the strategy as shown in Fig. 3. We hope that a candidate could be found as follow as the summary of SAR. From the crystal structure of 2FXQ, it can be clearly seen that the best place in the 2,3-dihydro-1H-indene moiety of rasagiline to extend a hydrophobic fragment is the 4 position.

<Fig. 3>

The designed hMAO-B inhibitors **D1-D25** were then synthesized according to the reactions shown in **Scheme 1** and **Scheme 2** (the groups represented by R_1 and R_2 are presented in **Table 1**). The intermediates **b1-b10, b16** and **b18-b25** were synthesized by introducing benzyl bromide or several substituted benzyl bromide to the hydroxyl of **a1** with potassium carbonate. While, **b11-b14** and **b17** were synthesized by Mitsunobu reaction with substituted phenethyl alcohol. Additionally, **b15'** was reacted with benzyl mercaptane, affording intermediate **b15** by Buchwald-Hartwig reaction. Then, final compounds **D1-D25** were obtained from **b1-b25** by reacting with propargylamine according to Borch Reduction (specific experimental procedures are supplied in Supporting Information). All these target compounds (**D1-D25**) are racemic.

<Scheme 1>

<Scheme 2>

Compounds **D1-D25** were evaluated for their inhibitory activities against hMAO-A and hMAO-B. The results are summarized in **Table 1**, in which IC_{50}s of hMAO-A

and hMAO-B and the SIs to hMAO-B ([IC_{50}(hMAO-A)]/[IC_{50}(hMAO-B)]) of **D1**-**D25** and the positive control compounds clorgyline (**1**) [30], and rasagiline (**3**) are listed.

<Table 1>

In our assay, compound **D1**, which introduces -OCH_2Ph to the 4-position of rasagiline (racemic mixture) showed IC_{50} values of 15.44 and 1.97 μM against hMAO-A and hMAO-B respectively with a SI of 7.84. Substitution at the meta-position on the benzene ring of **D1** by halogens or methyl group (**D2**-**D5**) led to significantly improved inhibitory activities against hMAO-B and selectivity against hMAO-A. Among them, **D2** had an IC_{50} value of 0.28 μM against hMAO-B (~55 fold increment) and a SI of 18.57 (~2 fold increment). Substitution at the para-position on the benzene ring (**D6**-**D9**) also resulted in activity improvement but not so significantly as **D2**-**D5**. **D8** displayed a SI of > 43.17. Replacement of the benzene ring of **D1** by a naphthalene moiety (**D10**) decreased inhibitory activities against both hMAO-A and hMAO-B. **D15** is a compound using a sulfur atom to replace the oxygen atom in the linker of **D1**. This replacement led to ~2.5 fold increment of potency against hMAO-B.

When the linker was -OCH_2CH_2- (**D11**-**D13**), the potency against hMAO-B did not changed considerably with **D1**. Surprisingly, the activity of compound **D14** was enhanced remarkably with an IC_{50} value of 0.02 μM against hMAO-B and a SI of 466.5. In our assay, rasagiline, the positive drug, exhibited an IC_{50} value of 0.01 μM against hMAO-B and a SI of 101. Considering the compounds reported here are

racemic, whereas rasagiline is a (*R*)-enantiomer, **D14** played comparable inhibitory activity with rasagiline, meanwhile, the SI was enhanced ~4.6-fold. Modeling study indicated that the phenyl group of **D11** could insert into the hydrophobic pocket in the entrance cavity, but only partly occupying the area around the furan moiety of 2-BFI, while the rasagiline part of **D14** keeps the interactions rasagiline has in the crystal complex (see more information in the docking study section). When attaching 4-chloro-benzamide into the meta-position of the phenyl ring (**D14**), the amide group could form a hydrogen bond with Pro102, mimicking the imidazoline of 2-BFI. At the same time, the oxygen atom of the amide group in **D14** is close to the hydroxyl group of Tyr326, implying a possible hydrogen bond between them. It was reported that selectivity of hMAO-A and hMAO-B inhibitors could be caused by the structural differences arising from Ile-335 in hMAO-A versus Tyr326 in MAO-B [27, 29], which is consistent with our finding reported herein. The 4-chloro-phenyl moiety on the one hand forms hydrophobic interactions with Leu88 and Pro102, and on the other hand, has NH-π interaction [31] with Thr201 (Fig. 4). These extra interactions of **D14** compensate for the entropic lose induced by not totally fitting the hydrophobic pocket that 2-BFI occupies. Compared with **D11**, the inhibitory activity against hMAO-B of **D14** was enhanced around 200-fold, and the SI was improved about 200-fold, indicating **D14** is a more potent and highly selective hMAO-B inhibitor.

<Fig. 4>

Prolonging the linker to four atoms (-OCH_2CH_2O-), the gotten compounds **D16**-**D19** did not demonstrate improved activity against hMAO-B. Though **D18** also

has an amide group at the meta-position of the phenyl ring, it did not show as potent as **D14**. Additionally, when the linker was five atoms (-$OCH_2CH_2CH_2O$-), compounds **D20**-**D25** showed reduced inhibitory activities and SIs.

In the crystal structure of 2XFQ, rasagiline covalently binds to the cofactor FAD, which challenged our docking study. To facilitate docking our compounds into the active site of hMAO-B, structures of both of receptor and ligands were adjusted according to Fig. 5 so that the ligands could be dockable. In this way, modified rasagiline can interact with FAD by a hydrogen bond but not a covalent bond. A hydrogen bond with Gln206 of hMAO-B observed in the crystal structure also could be kept. Glide 6.7 SP of the Schrödinger suite was used for docking. When docking compounds into the active site, constraints that docked compounds had to form two hydrogen bonds with adjusted FAD and Gln206 were defined. Through this strategy, modified rasagiline was docked into the active site of hMAO-B successfully and the conformation of rasagiline in the crystal structure was reproduced with an RMSD value of 0.1597, which guaranteed our modeling work.

<Fig. 5>

3. Conclusion

hMAO-B has been recognized a successful target for developing anti-PD drugs. Indeed, two irreversible and one reversible hMAO-B inhibitors are in the market for the treatment of PD. However, these approved drugs could relief but not cure the PD patients, and they have side effects. For example, rasagiline may cause severe

hypertension, make people sleepy and motor control worse in some people. Therefore, developing more potent and selective hMAO-B inhibitors has great significances for therapeutic purposes.

In this article, we report novel potent and selective hMAO-B inhibitors, which are rasagiline derivatives and were designed by employing a FBDD strategy to link rasagiline and simple hydrophobic moieties, which may target a hydrophobic pocket in the entrance cavity of hMAO-B. In a crystal structure of hMAO-B-rasagiline complex, a fragment of 2-BFI is located in this pocket and inhibits recombinant hMAO-B with a K_i of 8.3 μM. Linkers having different lengths such as -OCH_2-, -SCH_2-, -OCH_2CH_2-, -OCH_2CH_2O-, -$OCH_2CH_2CH_2O$- were tried. This strategy led to the identification of a promising selective hMAO-B inhibitor **D14**. This racemic compound showed comparable inhibitory activity with (*R*)-rasagiline. At the same time, **D14** demonstrated improved isoform selectivity over rasagiline.

This is our first endeavor to link fragments into rasagiline to get more potent and/or selective hMAO-B inhibitors. The simplest fragment, benzene ring and its analogs were employed. Actually, we screened a fragment library to try to get simpler fragments than 2-BFI which can interact with hMAO-B as 2-BFI. We hope we can link these simpler fragments into rasagiline to get more novel selective hMAO-B inhibitors by employing the strategy reported herein, which can also be called a diversity evolution strategy [32].

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Appendix A. Supplementary data

Supplementary data related to this article can be found at

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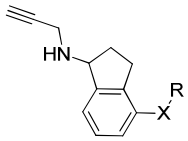
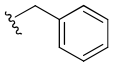
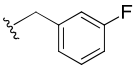
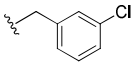
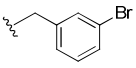
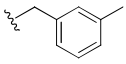
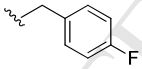
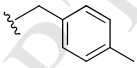
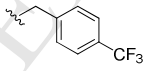
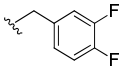
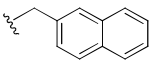
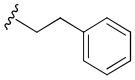
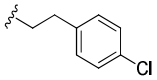
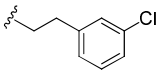
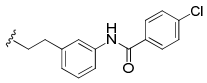
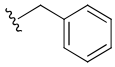
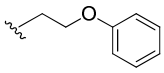
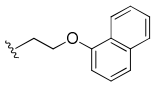
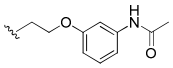
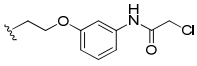
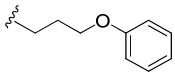
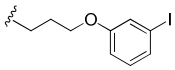
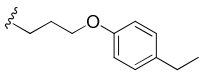
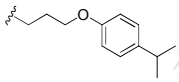
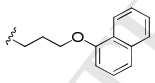
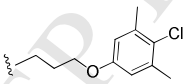
Table 1. Inhibitory activities of rasagiline derivatives **D1**-**D25** against hMAO-A and hMAO-B and selectivity of these compounds to hMAO-B.[a]



Compd	X	R	IC_{50} (μM, hMAO-A)	IC_{50} (μM, hMAO-B)	Selectivity to hMAO-B (A/B)
D1	O		15.44	1.97	7.84
D2	O		5.20	0.28	18.57
D3	O		7.00	0.33	21.21
D4	O		9.55	0.65	14.69
D5	O		11.20	0.55	20.36
D6	O		7.80	1.87	4.17
D7	O		4.33	1.09	3.97
D8	O		NA[b]	1.39	>43.17
D9	O		7.22	1.41	5.12
D10	O		NA	6.21	>9.66
D11	O		9.10	3.87	2.35
D12	O		10.21	2.51	4.07
D13	O		NA	5.87	>10.22

D14	O		9.33	0.02	466.5
D15	S		NA	6.24	>9.62
D16	O		14.09	4.57	3.08
D17	O		NA	3.39	>17.70
D18	O		8.30	5.52	1.5
D19	O		NA	6.91	>8.68
D20	O		NA	10.22	>5.87
D21	O		NA	18.01	>3.33
D22	O		31.40	10.49	2.99
D23	O		42.67	5.38	7.93
D24	O		NA	NA	-
D25	O		NA	6.91	>8.68
Clorgyline (**1**)	-	-	0.189	NA	<0.0031
Rasagiline (**3**)	-	-	1.01	0.010	101

[a]Each IC_{50} is the mean ± SEM from three experiments. Standard error of the IC_{50} was generally less than 10%. [b]IC_{50} is more than 60 μM.

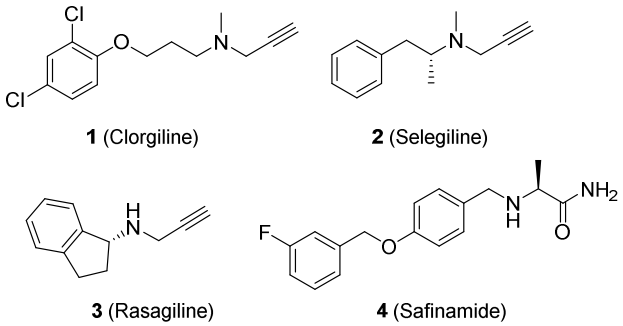


Fig. 1. Selected hMAO inhibitors in clinical use. Clorgiline (**1**) is a selective hMAO-A inhibitor, and selegiline (**2**), rasagiline (**3**) and safinamide (**4**) are selective hMAO-B inhibitors.

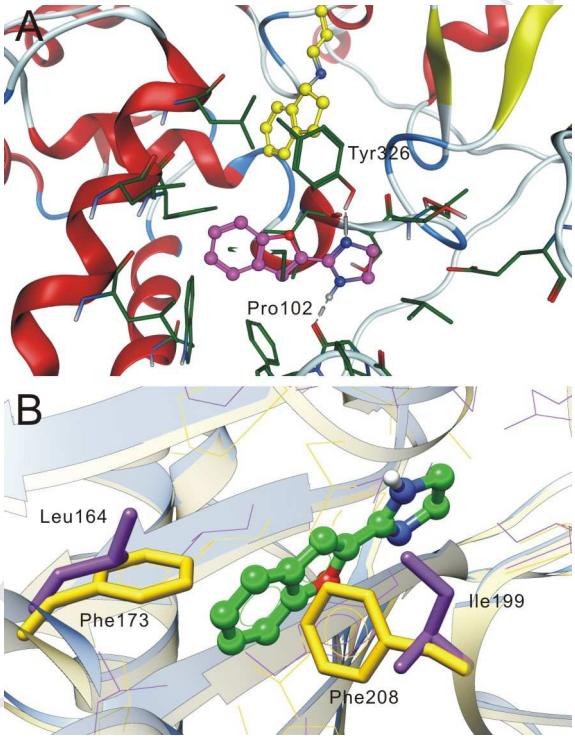


Fig. 2. (A) Crystal structure of rasagiline (yellow) binding to hMAO-B, in which, a fragment of 2-BFI (purple) is located in the entrance cavity. (B) Structural differences in the entrance cavity between hMAO-A and hMAO-B. Residues of Phe173 and Phe208 of hMAO-A are colored in yellow, while, their corresponding residues Leu164 and Ile199 in hMAO-B are colored in purple.

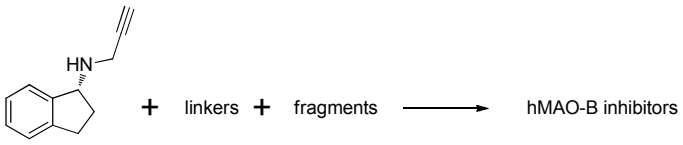


Fig. 3. Design of novel hMAO-B inhibitors by employing a fragment-based drug design strategy and rasagiline as the lead compound.

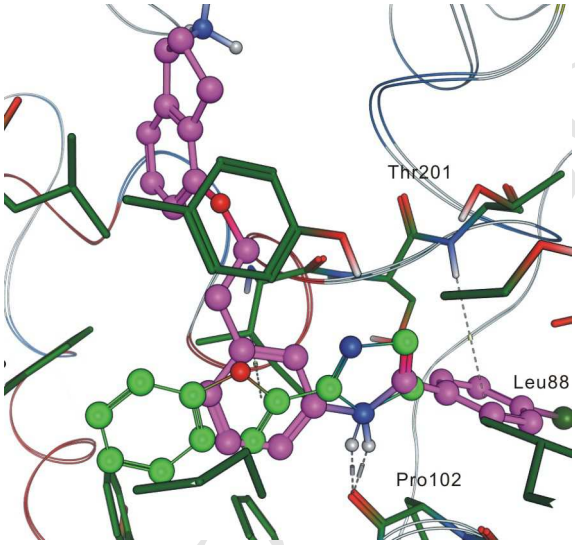


Fig. 4. Putative binding mode of **D14** shown as purple ball and stick mode. 2-BFI is presented as green line and stick mode.

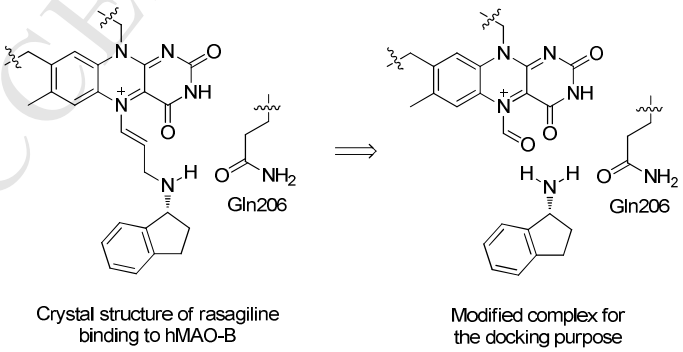
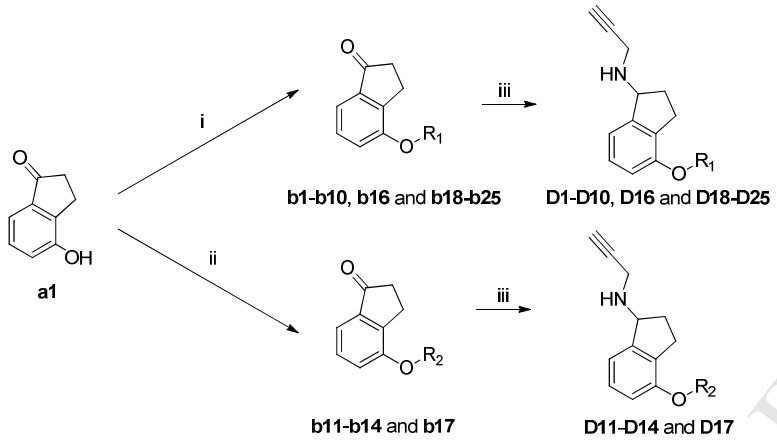
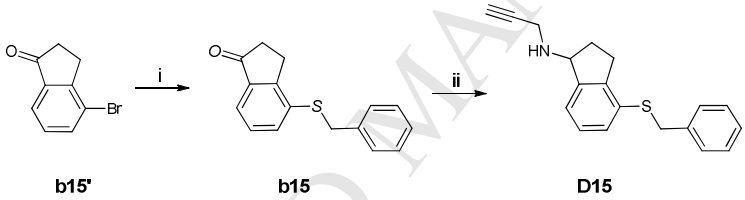


Fig. 5. For the docking purpose, covalently bound rasagiline and the cofactor FAD were adjusted.



Scheme 1. *Regents and conditions*: (i) R_1Br, potassium carbonate, acetonitrile, 85 □, 3 h; (ii) R_2OH, Ph_3P, DBAD, THF, 30 □, 12 h; (iii) mono-propargylamine, acetic acid, STAB, CH_2Cl_2, 12 h.



Scheme 2. *Regents and conditions*: (i) benzyl mercaptane, $Pd_2(dba)_3$, xantphos, cesium carbonate, 1,4-dioxane, 100 □, 13 h; (ii) mono-propargylamine, acetic acid, STAB, CH_2Cl_2,12 h.

Highlights:

- FBDD strategy was employed to design novel selective hMAO-B inhibitors.
- Selective hMAO-B inhibitors were gotten by linking rasagiline and fragments.
- A hMAO-B inhibitor with greatly improved isoform selectivity was yielded.
- More hMAO-B inhibitors with high selectivity can be developed by this strategy.