Bioorganic & Medicinal Chemistry 21 (2013) 5480–5487

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Oxazolopyridines and thiazolopyridines as monoamine oxidase B inhibitors for the treatment of Parkinson's disease

Hye Ri Park [a,†], Jiyoon Kim [a,b,†], Taekeun Kim [c], Seonmi Jo [c], Miyoung Yeom [a,b], Bongjin Moon [b], Il Han Choo [d], Jaeick Lee [e], Eun Jeong Lim [a], Ki Duk Park [a], Sun-Joon Min [a], Ghilsoo Nam [a], Gyochang Keum [a], C. Justin Lee [c,f,*,‡], Hyunah Choo [a,*,‡]

[a] Center for Neuro-Medicine, Korea Institute of Science and Technology, Hwarangro 14-gil-5, Seongbuk-gu, Seoul 136-791, Republic of Korea
[b] Department of Chemistry, Sogang University, Mapo-gu, Seoul 121-742, Republic of Korea
[c] Center for Functional Connectomics, Korea Institute of Science and Technology, Seongbuk-gu, Seoul 136-791, Republic of Korea
[d] Department of Neuropsychiatry, School of Medicine, Chosun University, Pilmoondaero 309, Dong-gu, Kwangju 501-759, Republic of Korea
[e] Doping Control Center, Korea Institute of Science and Technology, Seongbuk-gu, Seoul 136-791, Republic of Korea
[f] Center for Neural Science, Korea Institute of Science and Technology, Seongbuk-gu, Seoul 136-791, Republic of Korea

ARTICLE INFO

Article history:
Received 23 April 2013
Revised 28 May 2013
Accepted 29 May 2013
Available online 11 June 2013

Keywords:
Monoamine oxidase B
MAO-B
Parkinson's disease
Oxazolopyridine
Thiazolopyridine

ABSTRACT

In Parkinson's disease, the motor impairments are mainly caused by the death of dopaminergic neurons. Among the enzymes which are involved in the biosynthesis and catabolism of dopamine, monoamine oxidase B (MAO-B) has been a therapeutic target of Parkinson's disease. However, due to the undesirable adverse effects, development of alternative MAO-B inhibitors with greater optimal therapeutic potential towards Parkinson's disease is urgently required. In this study, we designed and synthesized the oxazolopyridine and thiazolopyridine derivatives, and biologically evaluated their inhibitory activities against MAO-B. Structure–activity relationship study revealed that the piperidino group was the best choice for the R^1 amino substituent to the oxazolopyridine core structure and the activities of the oxazolopyridines with various phenyl rings were between 267.1 and 889.5 nM in IC_{50} values. Interestingly, by replacement of the core structure from oxazolopyrine to thiazolopyridine, the activities were significantly improved and the compound **1n** with the thiazolopyridine core structure showed the most potent activity with the IC_{50} value of 26.5 nM. Molecular docking study showed that van der Waals interaction in the human MAO-B active site could explain the enhanced inhibitory activities of thiazolopyridine derivatives.

1. Introduction

As the life expectancy is increasing, aging and age-related neurodegenerative diseases have become important health problems in developed countries. Among the age-related neurodegenerative diseases with no currently available cure, Parkinson's disease is the second most common one after Alzheimer's disease.[1] This disease is characterized by the impairment of motor function such as tremor, rigidity, bradykinesia, gait dysfunction, and postural instability.[2] The motor symptoms of Parkinson's disease are mainly caused by the death of dopaminergic neurons in the substantia Nigra pars compacta.[3] Accordingly, current medications for ameliorating Parkinson's symptoms mostly target dopamine depletion in the brain.[4] Dopamine is a monoamine neurotransmitter which is synthesized by direct conversion of L-3,4-dihydroxyphenylalanine (L-DOPA), and degraded by catechol-O-methyl transferase (COMT) or monoamine oxidase (MAO).[5] There are two isozymes of MAO, which are identified as MAO-A and MAO-B.[6] Among these, elevated enzymatic activity of MAO-B results in Parkinson's disease-like pathology[7] and motor impairments.[8]

MAO-B is a flavin adenine dinucleotide (FAD)-containing enzyme, and is located in the mitochondrial outer membranes of glial cell types and some neuronal population.[9] Because MAO-B degrades dopamine and at the same time produces toxic by-products such as hydrogen peroxide and ammonia,[10] inhibition of MAO-B is effective in alleviating the symptoms of Parkinson's disease.[11] Indeed, selegiline[12] and rasagiline,[13] which are selective and irreversible MAO-B inhibitors, have been prescribed to patients with Parkinson's disease. However, these inhibitors are known to cause undesirable adverse effects such as hallucination and headache.[14,15] Moreover, neurotoxic or ineffective metabolites are generated from these inhibitors, which limit the long term use of those inhibitors.[11,16] Therefore, development of alternative MAO-B inhibitors with greater optimal therapeutic potential towards Parkinson's disease is urgently required.

* Corresponding authors. Tel.: +82 2 958 5157; fax: +82 2 958 5189.
E-mail addresses: cjl@kist.re.kr (C.J. Lee), hchoo@kist.re.kr (H. Choo).
† The first two authors contributed equally to this work.
‡ These two corresponding authors contributed equally to this work.

0968-0896/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2013.05.066

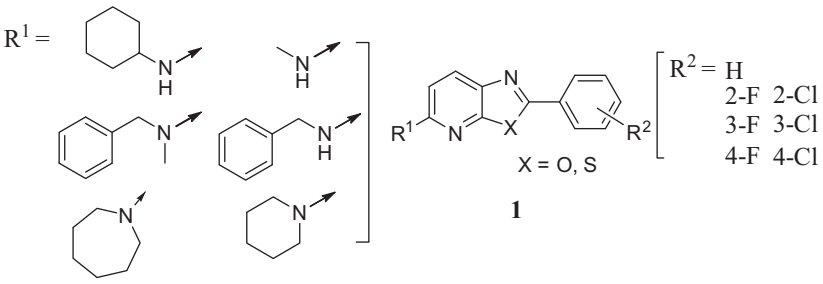


Figure 1. Designed oxazolopyridines and thiazolopyridines **1**.

In this study, we focused on oxazolopyridines and thiazolopyridines to find new MAO-B inhibitors which possibly can be applied to patients with Parkinson's disease in the future. Previously, oxazolopyridine derivatives with aryl or heteroaryl substitution were employed as inhibitors of the MAO-B, and as molecular probes for assessing MAO-B levels in living patients via positron emission tomography (PET) tracer technology.[17] However, other derivatives of oxazolopyridines and any derivatives of thiazolopyridines have not been tested on MAO-B yet. Thus, in this study, we prepared novel oxazolopyridine and thiazolopyridine derivatives **1** by introduction of amino functionalities and halogen substituents to the oxazolopyridine core and the 2-phenyl substituent of the oxazolopyridine scaffold, respectively (Fig. 1). Herein, we report preparation of a novel series of oxazolopyridine or thiazolopyridine derivatives and evaluation of their inhibitory activities against MAO-B.

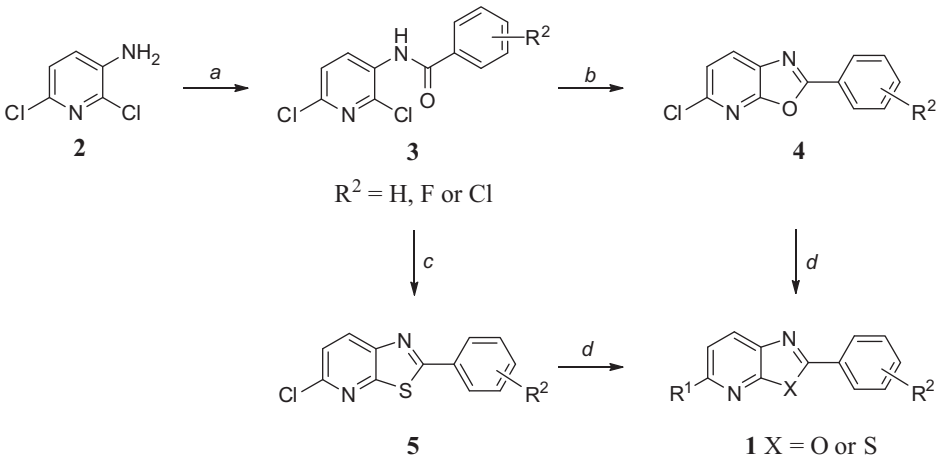
2. Results and discussion

2.1. Chemistry

Oxazolopyridine or thiazolopyridine derivatives were synthesized in three steps as shown in Scheme 1. 2,6-Dichloro-pyridin-3-amine **2** underwent acylation with various substituted benzoyl chlorides in acetonitrile to afford *N*-(2,6-dichloropyridin-3-yl)benzamide derivatives **3** in 59–99% yields. To synthesize the oxazolopyridines, the benzamides **3** were cyclized in the presence of sodium carbonate in DMF to give oxazolopyridines **4** in 60–95% yields. The oxazolopyridines **4** were converted to the desired oxazolopyridines **1** by reaction with various amines. However, the compound **1a** with *N*-methylamino group as R^1 was obtained from the oxazolopyridine **4** upon treatment with *N*,*N*-benzylmethylamine followed by debenzylation under acidic conditions. The benzamides **3** were also treated with Lawesson's reagent under basic conditions, which led to thiazolopyridines **5** in 50–80% yield.[18] With the same procedure as amination of oxazolopyridines **4**, the desired thiazolopyridines **1** (X = S) were obtained in 7–80% yields.

2.2. Inhibition of human MAO-B

The synthesized compounds were biologically evaluated against human MAO-B (hMAO-B). The potential effects of the compounds were investigated by measuring the amount of H_2O_2 produced by MAO-B from a human MAO-B substrate (benzylamine) and our test compound using the Amplex Red® MAO assay kit.[19] The prepared compounds were initially screened at 10 μM, and



Compd	X	R^1	R^2	Compd	X	R^1	R^2
1a	O	*N*-methylamino	H	**1i**	O	1-piperidino	4-F
1b	O	*N*-cyclohexylamino	H	**1j**	O	1-piperidino	2-Cl
1c	O	*N*-benzylamino	H	**1k**	O	1-piperidino	3-Cl
1d	O	*N*,*N*-benzylmethylamino	H	**1l**	O	1-piperidino	4-Cl
1d	O	1-azepino	H	**1m**	S	1-piperidino	H
1f	O	1-piperidino	H	**1n**	S	1-piperidino	2-F
1g	O	1-piperidino	2-F	**1o**	S	1-piperidino	2-Cl
1h	O	1-piperidino	3-F	**1p**	S	1-piperidino	4-Cl

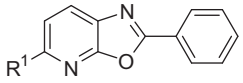
Scheme 1. Synthesis of oxazolopyridines and thiazolopyridines. Reagents and conditions: (a) $R^2PhCOCl$, CH_3CN, 80 °C, 59–99%; (b) Na_2CO_3, DMF, 110 °C, 60–95%; (c) Lawesson's reagent, DBU, toluene, 110 °C then K_2CO_3, DMF, 160 °C, 50–80%; (d) amines with R^1 group, DMF, reflux, or *N*,*N*-benzylmethylamine, DMF, reflux then H_2SO_4, 7–80%.

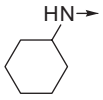
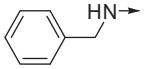
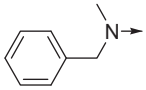
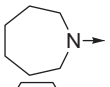
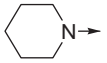
the IC_{50} values were estimated only for the compounds of which %inhibitions at this concentration were higher than 50%.

2-Phenyloxazolopyridine derivatives **1a**–**1f** with various amino substituents were initially tested against hMAO-B, and the results are shown in Table 1. The compounds **1a**, **1c**, and **1d** with small or aromatic R^1 substituents such as *N*-methylamino, *N*-benzylamino, and *N*,*N*-benzylmethylamino groups showed no inhibitory activities against hMAO-B. In contrast, amino groups with aliphatic rings like *N*-cyclohexylamino and 1-piperidino as R^1 substituents provided the corresponding oxazolopyridines (**1b** and **1f**) with significantly increased inhibitory activities. The IC_{50} values of the compounds **1b** and **1f** were 2539.2 and 1372.8 nM, respectively. The compound **1e** with 1-azepino group as R^1 showed only marginal activity with 57.9% inhibition at 10 μM. Among the compounds **1a**–**1f**, the compound **1f** is the most active. Therefore, we fixed R^1 as 1-piperidino group and modified the phenyl ring on the compound **1f** to obtain more active compounds against hMAO-B.

To improve inhibitory potency against hMAO-B, positional scanning of the halogen substitution on the phenyl ring of the compound **1f** with piperidino group was conducted and the results are summarized in Table 2. The oxazolopyridines **1g**–**1l** with a halogen substituent showed better inhibitory activities against hMAO-B than the compound **1f** with a simple phenyl ring. In the case of the compounds **1g**–**1i** with a fluorine substituent, substitution at the *ortho*-position led to slightly improved activity with an IC_{50} value of 665.1 nM. Interestingly, the fluorine atom substituted at the *meta*- and *para*-position provided the resulting compounds **1h** and **1i** with high hMAO-B inhibitory activities (IC_{50} = 271.8 and 270.3 nM, respectively). On the other hand, when a chlorine substituent was introduced, the 4-substituted derivative **1l** showed the lowest inhibitory activity among the series with an IC_{50} value of 889.5 nM. The compound **1j** with the chlorine substituent at the *ortho*-position of aromatic ring was more potent (IC_{50} = 267.1 nM) than the compound **1l**. The compound **1k** with *meta*-chloro substituent showed similar IC_{50} value as that of the compound **1j**.

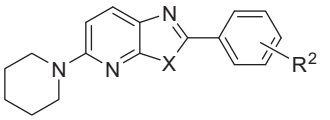
Table 1
Activities of the oxazolopyridines **1a**–**1f** with a phenyl ring against hMAO-B



Compd	R^1	%Inhibition at 10 μM[a]	IC_{50}[b] (nM)
1a		19.0	ND[c]
1b		79.1	2539.2 ± 62.4
1c		4.8	ND[c]
1d		3.7	ND[c]
1e		57.9	ND[c]
1f		86.8	1372.8 ± 48.4

[a] %Inhibition against hMAO-B at 10 μM.
[b] All IC_{50} values shown in the table are expressed as mean ± SEM from four experiments.
[c] Not determined.

Table 2
hMAO-B inhibition results for the synthesized compounds **1f**–**1p**



Compd	X	R^2	%Inhibition at 10 μM[a]	IC_{50}[b] (in μM)	Fold increase[c]
1f	O	H	86.8	1372.8 ± 48.4	1.0
1g	O	2-F	94.9	665.1 ± 8.5	2.1
1h	O	3-F	89.7	271.8 ± 15.4	5.1
1i	O	4-F	93.4	270.3 ± 22.1	5.1
1j	O	2-Cl	96.3	267.1 ± 2.7	5.1
1k	O	3-Cl	91.7	334.5 ± 4.8	4.1
1l	O	4-Cl	69.8	889.5 ± 24.2	1.5
1m	S	H	95.5	356.0 ± 21.6	3.9
1n	S	4-F	92.7	26.5 ± 1.3	51.8
1o	S	2-Cl	95.1	171.4 ± 5.7	8.0
1p	S	4-Cl	84.2	357.9 ± 19.3	3.8
Selegiline			97.4	1.3 ± 0.03	—[d]

[a] %Inhibition against hMAO-B at 10 μM.
[b] All IC_{50} values shown in the table are expressed as mean ± SEM from four experiments.
[c] Fold increase = IC_{50} of **1f**/IC_{50} of the compound modified (**1g** ~ **1p**, respectively).
[d] Not determined.

Finally, we introduced sulfur atom to the core oxazolopyridine ring to provide the thiazolopyridine derivatives **1m**–**1p**. The biological results are shown in Table 2. The compound **1m** showed more potent inhibitory activity than its oxazolopyridine congener **1f** with an IC_{50} value of 356 nM. Other compounds (**1n**–**1p**) showed similar increase in inhibitory activity compared with their oxazolopyridine counterparts. The hMAO-B inhibitory activities of the thiazolopyridine derivatives **1o** and **1p** with a chlorine substituent were considerably higher (IC_{50} = 171.4 and 357.9 nM, respectively) than the corresponding oxazolopyridines **1j** and **1l** (IC_{50} = 267.1 and 889.5 nM, respectively). Interestingly, the thiazolopyridine 1n with a fluorine substituent at the *para*-position of the phenyl ring showed the most potent hMAO-B activity with an IC_{50} value of 26.5 nM. Based on the increased activities of the thiazolopyridines, it could be assumed that the change of atom size and/or electronegativity at the core structure caused by substitution of oxygen with sulfur might influence the bioactivity against hMAO-B. These compounds **1f**–**1p** were also assayed for hMAO-A inhibitory activity. All tested compounds showed no inhibitory activities against hMAO-A at 10 μM. Therefore, the oxazolopyridines **1f**–**1l** and the thiazolopyridines **1m**–**1p** are determined as selective hMAO-B inhibitors.

2.3. Molecular docking study

For molecular docking study, two compounds **1n** and **1i** were selected. The compound **1n** is the most potent MAO-B inhibitor in these series of the compounds and the compound **1i** is exactly the same as the compound **1n** except with oxazolopyridine core structure. Comparison between the binding modes of the two compounds **1n** and **1i** could provide insights to figure out the improved inhibitory activities by replacement of the core structure from oxazolopyridine to thiazolopyridine. Molecular docking study was carried out in order to understand the energetically preferred conformations adopted by the compounds **1i** and **1n** inside the hMAO-B binding pocket and thereby the key factor for the improved inhibitory activities of the thiazolopyridines. Crystallographic structure of hMAO-B (PDB code 2V60)[20] from the Protein Data Bank was used as a template for docking experiments because the ligand inside of the crystal structure of MAO-B is a coumarin derivative which is a reversible inhibitor like our MAO-B inhibitors

1i and **1n** and has similar structural properties: a fused ring system and similar size.

The MAO-B inhibitors **1i** and **1n** were successfully docked to the MAO-B active site, of which the GLIDE energies are −29.2 and −34.2 kcal/mol, respectively and the two inhibitors occupied the entrance hydrophobic cleft formed by Pro102, Cys172, Ile199, Ile 316, Tyr326, and Phe343 (Fig. 2). The binding modes of the two inhibitors **1i** and **1n** are shown in Figure 2. The two inhibitors were shown to form no specific interaction except van der Waals interaction as a key interaction between the inhibitors and hMAO-B. Due to different van der Waals radii of oxygen atom and sulfur atom, the van der Waals surfaces of the compounds **1i** and **1n** are distinctive, and Cys172 in the active site of hMAO-B plays an important role in distinguishing binding modes of the two compounds. The sulfur atom in Cys172 is nearest to the oxygen atom in the compound **1i** with a distance of 3.8 Å (Fig. 2a) and the sulfur atom in the compound **1n** with a distance of 3.3 Å (Fig. 2b), respectively. The van der Waals surfaces of the sulfur atom in Cys172 and the sulfur atom in the compound **1n** are closer to each other than the corresponding surfaces in the binding mode of the compound **1i** (Fig. 2c), which indicates that the compound **1n** has stronger van der Waals interaction with Cys172 than the compound **1i** and thereby more potent inhibitory activity against hMAO-B.

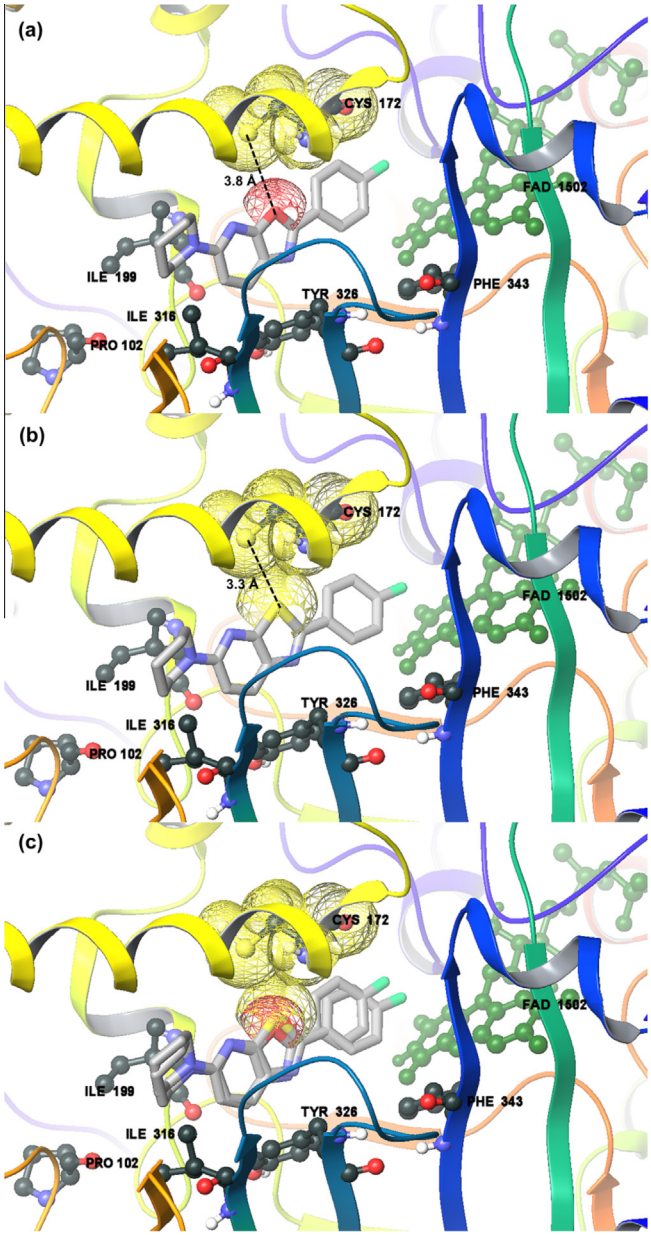


Figure 2. Binding modes of (a) **1i** and (b) **1n** inside of the hMAO-B active site. (c) Two compounds **1i** and **1n** were overlapped to compare binding modes and van der Waals surface. Van der Waals surfaces of Cys172, the oxygen of **Ii**, and the sulfur of **In** were expressed with net shapes.

3. Conclusion

The oxazolopyridine and thiazolopyridine derivatives **1a–1p** were designed, synthesized and biologically evaluated as inhibitors of MAO-B enzyme. The structure–activity relationship study revealed that the piperidino group was the best choice as R^1 group, and the activities of the oxazolopyridines with various aromatic rings were between 267.1 and 889.5 nM in IC_{50} values. Interestingly, by replacement of the core structure from oxazolopyridine to thiazolopyridine, the activities were significantly improved and the most potent compound was **1n** of which an IC_{50} value was 26.5 nM. Molecular docking study showed that van der Waals interaction in the hMAO-B active site could explain the enhanced inhibitory activities of thiazolopyridine derivatives. Based on this study, an extensive SAR (structure–activity relationship) study of the compound **1n** to obtain improved pharmacological profiles with in vivo activity in Parkinon's disease animal model is warranted.

4. Experimental section

4.1. Chemicals and instrumentation

All starting materials and reagents were purchased from Sigma–Aldrich, TCI, Fluka, Lancaster and Acros, and used without further purification. Analytical thin layer chromatography was performed on silica gel 60 F254 purchased from Merck. Column chromatography was performed using silica gel grade 230–400 (Merck Kieselgel 60 Art 9385). Nuclear magnetic resonance spectra were recorded on Bruker Advance 400 (or 300) spectrometer at 400 MHz (or 300 MHz) for ^{1}H NMR and at 100 MHz for ^{13}C NMR with tetramethylsilane as an internal standard. Chemical shifts are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) or br s (broad singlet). Coupling constants are reported in hertz (Hz). The chemical shifts are reported as parts per million (δ) relative to the solvent peak. The high-resolution mass spectrum was performed on a LTQ Orbitrap (Thermo Electron Corporation).

4.2. General procedure for preparation of 3a–3g

Substituted benzoyl chloride (1.1 equiv) was added dropwise to a solution of the 2.6-dichloro-pyridin-3-amine (1 equiv) in acetonitrile at 0 °C. The resulting mixture was stirred for 6 h at 80 °C. After cooling down to room temperature, the solvent was removed under reduced pressure. The crude compound was dissolved in methanol, and then white solid was recrystallized from methanol to give desired product.

4.2.1. *N*-(2,6-Dichloropyridin-3-yl)benzamide (3a)

The desired product was obtained in 90% yield: ^{1}H NMR (400 MHz, MeOD) δ 8.29 (d, J = 8.4 Hz, 1H), 8.00–7.98 (m, 2H), 7.62 (t, J = 6.1 Hz, 1H), 7.57–7.50 (m, 3H).

4.2.2. *N*-(2,6-Dichloropyridin-3-yl)-2-fluorobenzamide (3b)

The desired product was obtained in 95% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.15 (d, J = 17.2 Hz, 1H), 8.93 (d, J = 8.8 Hz,

1H), 8.16 (td, *J* = 8.0, 2.0 Hz, 1H), 7.60–7.54 (m, 1H), 7.35–7.30 (m, 2H), 7.22 (dd, *J* = 12.4, 0.8 Hz, 1H).

4.2.3. *N*-(2,6-Dichloropyridin-3-yl)-3-fluorobenzamide (3c)

The desired product was obtained in 60% yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.93 (d, *J* = 8.5 Hz, 1H), 8.37 (s, 1H), 7.68 (t, *J* = 7.5 Hz, 2H), 7.57 (q, *J* = 8.0 Hz, 1H), 7.41–7.34 (m, 2H).

4.2.4. *N*-(2,6-Dichloropyridin-3-yl)-4-fluorobenzamide (3d)

The desired product was obtained in 94% yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.72 (d, *J* = 4.5 Hz, 1H), 8.24 (s, 1H), 7.72 (d, *J* = 8.0 Hz, 2H), 7.46 (d, *J* = 8.1 Hz, 2H), 7.28 (d, *J* = 8.0 Hz, 1H).

4.2.5. 2-Chloro-*N*-(2,6-dichloropyridin-3-yl)benzamide (3e)

The desired product was obtained in 75% yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.98 (d, *J* = 8.6 Hz, 1H), 8.68 (s, 1H), 7.80 (d, *J* = 6.7 Hz, 1H), 7.56–7.52 (m, 2H), 7.49–7.45 (m, 1H), 7.39 (d, *J* = 8.6 Hz, 1H).

4.2.6. 3-Chloro-*N*-(2,6-dichloropyridin-3-yl)benzamide (3f)

The desired product was obtained in 59% yield: ^{1}H NMR (400 MHz, MeOD) δ 8.22 (d, *J* = 8.3 Hz, 1H), 7.97 (t, *J* = 1.9 Hz, 1H), 7.90–7.88 (m, 1H), 7.62–7.61 (m, 1H), 7.5 (dd, *J* = 7.9, 11.6 Hz, 2H).

4.2.7. 4-Chloro-*N*-(2,6-dichloropyridin-3-yl)benzamide (3g)

The desired product was obtained in 99% yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.93 (d, *J* = 4.7 Hz, 1H), 8.34 (s, 1H), 7.89 (d, *J* = 8.3 Hz, 2H), 7.56 (d, *J* = 8.3 Hz, 2H), 7.39 (d, *J* = 8.6 Hz, 1H).

4.3. The general procedure for preparation of 4a–4g

Na_2CO_3 (1 equiv) was added to a solution of substituted *N*-(2,6-dichloropyridin-3-yl)benzamide (1 equiv) in DMF. The resulting mixture was stirred at 160 °C for 24 h. After completion of the reaction, as monitored by TLC, the reaction mixture was cooled to room temperature and partitioned between ethyl acetate and water. The mixture was extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (hexane:EtOAc = 20:1) on silica gel to obtain the desired product.

4.3.1. 5-Chloro-2-phenyloxazolo[5,4-*b*]pyridine (4a)

The desired product was obtained in 95% yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.30 (d, *J* = 6.4 Hz, 2H), 8.05 (d, *J* = 8.2 Hz, 1H), 7.67–7.57 (m, 3H), 7.43 (d, *J* = 8.2 Hz, 1H).

4.3.2. 5-Chloro-2-(2-fluorophenyl)oxazolo[5,4-*b*]pyridine (4b)

The desired product was obtained in 95% yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.24–8.23 (m, 1H), 8.13–8.09 (m, 1H), 7.67–7.59 (m, 1H), 7.47–7.29 (m, 3H).

4.3.3. 5-Chloro-2-(3-fluorophenyl)oxazolo[5,4-*b*]pyridine (4c)

The desired product was obtained in 80% yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.07 (d, *J* = 8.1 Hz, 2H), 7.97 (d, *J* = 9.1 Hz, 1H), 7.57 (q, *J* = 8.0 Hz, 1H), 7.44 (d, *J* = 8.2 Hz, 1H), 7.36–7.30 (m, 1H).

4.3.4. 5-Chloro-2-(4-fluorophenyl)oxazolo[5,4-*b*]pyridine (4d)

The desired product was obtained in 73% yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.32–8.26 (m, 2H), 8.04 (d, *J* = 8.2 Hz, 1H), 7.43 (d, *J* = 8.2 Hz, 1H), 7.32–7.21 (m, 2H).

4.3.5. 5-Chloro-2-(2-chlorophenyl)oxazolo[5,4-*b*]pyridine (4e)

The desired product was obtained in 75% yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.20 (d, *J* = 8.6 Hz, 1H), 7.99 (d, *J* = 8.2 Hz, 1H), 7.65 (d, *J* = 8.1 Hz, 1H), 7.45–7.33 (m, 3H).

4.3.6. 5-Chloro-2-(3-chlorophenyl)oxazolo[5,4-*b*]pyridine (4f)

The desired product was obtained in 63% yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.29 (s, 1H), 8.50 (dd, *J* = 2.9, 4.6 Hz, 1H), 8.06 (d, *J* = 8.2 Hz, 1H), 7.63–7.51 (m, 2H), 7.45 (d, *J* = 8.2 Hz, 1H)

4.3.7. 5-Chloro-2-(4-chlorophenyl)oxazolo[5,4-*b*]pyridine (4g)

The desired product was obtained in 60% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (dd, *J* = 1.8, 5.1 Hz, 2H), 8.01 (d, *J* = 8.2 Hz, 1H), 7.54 (dd, *J* = 1.8, 5.2 Hz, 2H), 7.40 (d, *J* = 8.2 Hz, 1H)

4.4. General procedure for preparation of 5a–d

Lawesson's reagent (1.5 equiv) was added to a solution of substituted *N*-(2,6-dichloropyridin-3-yl)benzamide (1 equiv) in toluene. The resulting mixture was stirred for 5 h at 110 °C and monitored with TLC. After cooling down to room temperature, 1.8-diazabicylo[5.4.0]undec-7-ene (3 equiv) was added and the reaction mixture was allowed to stir for 60 h. The solvent was removed under reduced pressure, and the residue was dissolved in DMF. After adding K_2CO_3 (3 equiv), the reaction mixture was stirred for 3 h at 160 °C. After completion of the reaction, as monitored by TLC, the reaction mixture was cooled to room temperature and partitioned between ethyl acetate and water. The mixture was extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (hexane:EtOAc = 20:1) on silica gel to obtain the desired product.

4.4.1. 5-Chloro-2-phenylthiazolo[5,4-*b*]pyridine (5a)

The desired product was obtained in 50% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (d, *J* = 8.5 Hz, 1H), 8.09–8.07 (m, 2H), 7.55–7.50 (m, 3H), 7.45 (d, *J* = 8.5 Hz, 1H).

4.4.2. 5-Chloro-2-(4-fluorophenyl)thiazolo[5,4-*b*]pyridine (5b)

The desired product was obtained in 73% yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.24 (d, *J* = 8.5 Hz, 1H), 8.14–8.08 (m, 2H), 7.49 (d, *J* = 8.5 Hz, 1H), 7.30–7.21 (m, 2H).

4.4.3. 5-Chloro-2-(2-chlorophenyl)thiazolo[5,4-b]pyridine (5c)

The desired product was obtained in 80% yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.26–8.21 (m, 1H), 8.15 (d, *J* = 8.6 Hz, 1H), 7.61–7.57 (m, 1H), 7.51–7.44 (m, 2H), 7.36 (d, *J* = 8.6 Hz, 1H).

4.4.4. 5-Chloro-2-(4-chlorophenyl)thiazolo[5,4-*b*]pyridine (5d)

The desired product was obtained in 73% yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.10–8.00 (m, 3H), 7.56–7.44 (m, 3H)

4.5. *N*-Methyl-2-phenyloxazolo[5,4-*b*]pyridin-5-amine (1a)

To a solution of **4a** (200 mg, 0.9 mmol) in DMF (3 ml), *N*-benzylmethylamine (1.2 ml, 9.0 mmol) was added and stirred at 135 °C for 4 h. After completion of the reaction, as monitored by TLC, the reaction mixture was cooled to room temperature, and then solvent was removed reduced pressure. The mixture was diluted with saturated $NaHCO_3$ solution and extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated. The residue was dissolved in 95% H_2SO_4 (0.5 ml) and refluxed for 12 h. After cooling to room temperature, the reaction flask was basified with diluted sodium hydroxide solution until a pH of 10. The reaction mixture was extracted with CH_2Cl_2, the combined organic layers were dried over $MgSO_4$, filtered, and evaporated to give afford **1a** (109 mg, 0.5 mmol, 54% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.24–8.20 (m, 2H), 7.83 (d, *J* = 8.6 Hz, 1H), 7.55–7.51 (m, 3H), 6.46 (d, *J* = 8.6 Hz, 1H), 4.75 (s, 1H), 3.07 (d, *J* = 5.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 159.43, 158.76, 157.12, 130.54, 129.22, 128.40, 127.41, 126.87, 124.21, 104.22,

29.11; HRMS (ESI) m/z calcd for $C_{13}H_{12}N_3O$ $[M+H]^+$ 226.0975; found: 226.0976.

4.6. *N*-Cyclohexyl-2-phenyloxazolo[5,4-*b*]pyridin-5-amine (1b)

To solution of **4a** (50 mg, 0.2 mmol) in DMF (2 ml), cyclohexylamine (0.2 ml, 2.0 mmol) was added and stirred at 35 °C for 4 h. After completion of the reaction, as monitored by TLC, the reaction mixture was cooled to room temperature, and then solvent was removed reduced pressure. The mixture was diluted with saturated $NaHCO_3$ solution and extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (hexane:CH_2Cl_2:EtOAc = 20:1:1) on slica gel to give afford **1b** (20 mg, 0.07 mmol, 34% yield): ^{1}H NMR (300 MHz, MeOD) δ 8.12–8.11 (m, 2H), 7.70 (d, J = 8.7 Hz, 1H), 7.57–7.53 (m, 3H), 6.54 (d, J = 8.7 Hz, 1H), 3.79 (s, 1H), 2.08 (d, J = 12.2 Hz, 2H), 1.83 (dd, J = 3.9, 5.7 Hz, 2H), 1.71 (d, J = 13.2 Hz, 1H), 1.52–1.42 (m, 2H), 1.30 (t, J = 12.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 159.64, 158.75, 156.05, 130.74, 129.71, 128.84, 127.48, 126.81, 124.17, 105.51, 50.41, 33.34, 25.79, 24.89; HRMS (ESI) m/z calcd for $C_{18}H_{20}N_3O$ $[M+H]^+$ 294.1601; found: 294.1604.

4.7. *N*-Benzyl-2-phenyloxazolo[5,4-*b*]pyridin-5-amine (1c)

To solution of **4a** (100 mg, 0.9 mmol) in DMF (3 ml), benzylamine (0.4 ml, 4 mmol) was added and stirred at 35 °C for 4 h. After completion of the reaction, as monitored by TLC, the reaction mixture was cooled to room temperature, and then solvent was removed reduced pressure. The mixture was diluted with saturated $NaHCO_3$ solution and extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (hexane:CH_2Cl_2:EtOAc = 20:1:1) on slica gel to give afford **1c** (30 mg, 0.1 mmol, 25% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.24–8.21 (m, 2H), 7.82 (d, J = 8.6 Hz, 1H), 7.55–7.51 (m, 3H), 7.46–7.31 (m, 5H), 6.46 (d, J = 8.6 Hz, 1H), 5.04 (s, 1H), 4.67 (d, J = 5.7 Hz, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 159.49, 159.14, 156.41, 138.75, 130.92, 129.89, 128.89, 128.76, 127.50, 127.37, 126.90, 124.87, 105.46, 46.57; HRMS (ESI) m/z calcd for $C_{19}H_{16}N_3O$ $[M+H]^+$ 302.1288; found: 302.1290.

4.8. *N*-Benzyl-*N*-methyl-2-phenyloxazolo[5,4-*b*]pyridin-5-amine (1d)

To solution of **4a** (200 mg, 0.4 mmol) in DMF (3 ml), *N*-benzylmethylamine (1.2 ml, 9.0 mmol) was added and stirred at 35 °C for 4 h. After completion of the reaction, as monitored by TLC, the reaction mixture was cooled to room temperature, and then solvent was removed reduced pressure. The mixture was diluted with saturated $NaHCO_3$ solution and extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (hexane:CH_2Cl_2:EtOAc = 20:1:1) on slica gel to give afford **1d** (218 mg, 0.7 mmol, 80% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25–8.21 (m, 2H), 7.85 (d, J = 8.8 Hz, 1H), 7.54–7.50 (m, 3H), 7.39–7.27 (m, 5H), 6.58 (d, J = 8.8 Hz, 1H), 4.91 (s, 2H), 3.21 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 159.37, 158.96, 156.68, 138.05, 130.83, 129.75, 128.88, 128.68, 128.25, 127.45, 127.18, 127.04, 126.86, 123.88, 103.70, 53.97, 37.02; HRMS (ESI) m/z calcd for $C_{20}H_{18}N_3O$ $[M+H]^+$ 316.1444; found: 316.1446.

4.8.1. 5-(Azepan-1-yl)-2-phenyloxazolo[5,4-*b*]pyridine (1e)

To solution of **4a** (97 mg, 0.4 mmol) in DMF (2 ml), hexamethyleneimine (0.5 ml, 4.0 mmol) was added and stirred at 35 °C for 4 h. After completion of the reaction, as monitored by TLC, the reaction mixture was cooled to room temperature, and then solvent was removed reduced pressure. The mixture was diluted with saturated $NaHCO_3$ solution and extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (hexane:CH_2Cl_2:EtOAc = 20:1:1) on slica gel to give afford **1e** (70 mg, 0.2 mmol, 57% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.24–8.20 (m, 2H), 7.82 (d, J = 8.8 Hz, 1H), 7.54–7.50 (m, 3H), 6.54 (d, J = 8.9 Hz, 1H), 3.74 (t, J = 5.9 Hz, 4H), 1.88 (s, 4H), 1.63–1.59 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 162.33, 156.34, 130.60, 129.56, 128.81, 127.60, 126.76, 123.06, 103.10, 48.35, 27.70, 27.11; HRMS (ESI) m/z calcd for $C_{18}H_{20}N_3O$ $[M+H]^+$ 294.1601; found: 294.1603.

4.9. General procedure for preparation of 1f–1p

To solution of substituted phenyloxazolopyridine or phenylthiazolopyridine (1 equiv) in DMF, piperidine (10 equiv) was added and stirred at 35 °C for 4 h. After completion of the reaction, as monitored by TLC, the reaction mixture was cooled to room temperature, and then solvent was removed reduced pressure. The mixture was diluted with saturated $NaHCO_3$ solution and extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (hexane:CH_2Cl_2:EtOAc = 20:1:1) on slica gel to obtained the desired product.

4.9.1. 2-Phenyl-5-(piperidin-1-yl)oxazolo[5,4-*b*]pyridine (1f)

The desired product was obtained in 26% yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.22–8.16 (m, 2H), 7.80 (d, J = 8.7 Hz, 1H), 7.83–7.47 (m, 3H), 6.70 (d, J = 8.7 Hz, 1H), 3.63 (s, 4H), 1.68 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 159.33, 159.19, 157.32, 130.85, 129.65, 128.88, 127.42, 126.86, 123.93, 104.80, 46.91, 25.50, 24.65; HRMS (ESI) m/z calcd for $C_{17}H_{18}N_3O$ $[M+H]^+$ 280.1444; found: 280.1446.

4.9.2. 2-(2-Fluorophenyl)-5-(piperidin-1-yl)oxazolo[5,4-*b*]pyridine (1g)

The desired product was obtained in 9% yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.20 (t, J = 1.6 Hz, 1H), 7.89 (d, J = 8.9 Hz, 1H), 7.53–7.46 (m, 1H), 7.33–7.24 (m. 2H), 6.73 (d, J = 8.9 Hz, 1H), 3.67 (s, 4H), 1.72 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.49 (J = 257 Hz), 159.01, 157.51, 155.20 (J = 6 Hz), 132.20 (J = 8 Hz), 129.88 (J = 30 Hz), 129.72, 124.40 (J = 3 Hz), 123.71, 117.00 (J = 21 Hz), 115.81 (J = 10 Hz), 104.91, 46.83, 25.49, 24.63; HRMS (ESI) m/z calcd for $C_{17}H_{17}FN_3O$ $[M+H]^+$ 298.1350; found: 298.1352.

4.9.3. 2-(3-Fluorophenyl)-5-(piperidin-1-yl)oxazolo[5,4-*b*]pyridine (1h)

The desired product was obtained in 19% yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00 (d, J = 7.8 Hz, 1H), 7.90 (d, J = 8.0 Hz, 1H), 7.84 (d, J = 8.9 Hz, 1H), 7.53–7.18 (m, 1H), 7.21 (t, J = 5.9 Hz, 1H), 6.73 (d, J = 8.9 Hz, 1H), 3.67 (s, 4H), 1.72 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 162.97 (J = 245 Hz), 159.36, 157.85, 130.56 (J = 8.1 Hz), 129.84, 129.57, 123.75, 122.52, 117.74 (J = 21.2 Hz), 105.01, 46.86, 25.50, 24.64; HRMS (ESI) m/z calcd for $C_{17}H_{17}FN_3O$ $[M+H]^+$ 298.1350; found: 298.1352.

4.9.4. 2-(4-Fluorophenyl)-5-(piperidin-1-yl)oxazolo[5,4-*b*]pyridine (1i)

The desired product was obtained in 11% yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (d, J = 9. 1 Hz, 2H), 7.92 (d, J = 8.2 Hz, 1H), 7.33 (t, J = 8.1 Hz, 1H), 7.36 (d, J = 9.1 Hz, 2H), 3.43 (s, 4H), 1.72 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 164.40 (J = 250 Hz), 162.33, 158.33, 157.29, 129.60, 128.97 (J = 8.7 Hz), 123.82, 123.78, 116.12 (J = 22.0 Hz), 104.83, 46.91, 25.48, 24.63; HRMS (ESI) m/z calcd for $C_{17}H_{17}FN_3O$ $[M+H]^+$ 298.1350; found: 298.1353.

4.9.5. 2-(2-Chlorophenyl)-5-(piperidin-1-yl)oxazolo[5,4-*b*]pyridine (1j)

The desired product was obtained in 18% yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.19–8.16 (m, 1H), 7.91 (d, J = 8.9 Hz, 1H), 7.59–7.56 (m, 1H), 7.44–7.41 (m, 2H), 6.73 (d, J = 8.9 Hz, 1H), 3.67 (s, 4H), 1.72 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 159.10, 157.59, 156.67, 132.87, 131.47, 131.19, 131.15, 130.19, 126.88, 126.18, 123.62, 104.93, 46.56, 25.51, 24.66; HRMS (ESI) m/z calcd for $C_{17}H_{17}ClN_3O$ $[M+H]^+$ 314.1055; found: 314.1057.

4.9.6. 2-(3-Chlorophenyl)-5-(piperidin-1-yl)oxazolo[5,4-*b*]pyridine (1k)

The desired product was obtained in 56% yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.22 (s, 1H), 8.09 (d, J = 6.5 Hz, 1H), 7.84 (d, J = 8.8 Hz, 1H), 7.47 (s, 2H), 6.72 (d, J = 8.9 Hz, 1H), 3.67 (s, 4H), 1.72 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 159.36, 127.67, 157.46, 135.03, 130.70, 130.16, 129.83, 129.17, 126.85, 124.79, 123.74, 105.02, 46.86, 25.49, 24.63; HRMS (ESI) m/z calcd for $C_{17}H_{17}ClN_3O$ $[M+H]^+$ 314.1055; found: 314.1057.

4.9.7. 2-(4-Chlorophenyl)-5-(piperidin-1-yl)oxazolo[5,4-*b*]pyridine (1l)

The desired product was obtained in 17% yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.14 (d, J = 8.7 Hz, 2H), 7.83 (d, J = 8.9 Hz, 1H), 7.50 (d, J = 8.7 Hz, 2H), 6.72 (d, J = 8.9 Hz, 1H), 3.67 (s, 4H), 1.72 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 162.34, 158.15, 157.37, 136.93, 129.72, 129.24, 128.04, 125.94, 123.80, 104.95, 46.87, 25.50, 24.64; HRMS (ESI) m/z calcd for $C_{17}H_{17}ClN_3O$ $[M+H]^+$ 314.1055; found: 314.1058.

4.9.8. 2-Phenyl-5-(piperidin-1-yl)thiazolo[5,4-*b*]pyridine (1m)

The desired product was obtained in 73% yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (d, J = 9.2 Hz, 3H), 7.51–7.48 (m, 3H), 6.84 (d, J = 9.2 Hz, 1H), 3.67 (s, 4H), 1.71 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 161.81, 157.72, 157.58, 139.55, 134.25, 131.22, 130.24, 128.96, 126.85, 106.53, 46.73, 25.63, 24.71; HRMS (ESI) m/z calcd for $C_{17}H_{18}N_3S$ $[M+H]^+$ 296.1216; found: 296.1220.

4.9.9. 2-(4-Fluorophenyl)-5-(piperidin-1-yl)thiazolo[5,4-*b*]pyridine (1n)

The desired product was obtained in 35% yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04–7.99 (m, 3H), 7.18 (t, J = 6.4 Hz, 2H), 6.84 (d, J = 9.2 Hz, 1H), 3.69 (d, J = 4.8 Hz, 4H), 1.72 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 164.00 (J = 249 Hz), 160.50, 157.76, 157.58, 139.51, 131.16, 130.63 (J = 3.3 Hz), 128.70 (J = 8.4 Hz), 116.03(J = 21.9 Hz), 106.53, 46.72, 25.61, 24.69; LC/MS (ESI) m/z calcd for $C_{17}H_{17}FN_3S$ $[M+H]^+$ 314.1122; found: 314.1124.

4.9.10. 2-(2-Chlorophenyl)-5-(piperidin-1-yl)thiazolo[5,4-*b*]pyridine (1o)

The desired product was obtained in 7% yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.19–8.13 (m, 1H), 8.08 (d, J = 9.2 Hz, 1H), 7.57–7.51 (m, 1H), 7.45–7.36 (m, 2H), 6.87 (d, J = 9.2 Hz, 1H), 3.70 (s, 4H), 1.72 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 159.47, 157.63, 154.07, 148.84, 131.94, 131.03, 130.45, 130.37, 123.97, 123.48, 121.13, 105.22, 46.77, 25.50, 24.64; HRMS (ESI) m/z calcd for $C_{17}H_{17}ClN_3S$ $[M+H]^+$ 330.0826; found: 330.0829.

4.9.11. 2-(4-Chlorophenyl)-5-(piperidin-1-yl)thiazolo[5,4-*b*]pyridine (1p)

The desired product was obtained in 35% yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03–7.95 (m, 3H), 7.47 (d, J = 8.4 Hz, 3H), 7.47 (d, J = 8.4 Hz, 2H), 6.84 (d, J = 9.2 Hz, 1H), 3.68 (s, 4H), 1.72 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 160.26, 157.81, 157.62, 139.49, 136.11, 132.82, 131.25, 129.15, 127.97, 106.63, 46.70, 25.61, 24.68; HRMS (ESI) m/z calcd for $C_{17}H_{17}ClN_3S$ $[M+H]^+$ 330.0826; found: 330.0828.

4.10. Human MAO-B assay

Human recombinant MAO-B enzyme prepared from insect cells was obtained from Sigma-Aldrich. Benzylamine hydrochloride (Sigma) was used as substrate for the enzyme. Sodium phosphate buffer (0.05 M, pH 7.4) was used for all the enzyme reactions and dilutions. The final volume of the enzyme reactions was 200 μl, and each reaction contained benzylamine hydrochloride, various concentrations of the test inhibitors (0.01–100 μM) and 1% DMSO as co-solvent. The potential effects of the test drugs on hMAO-B activity were investigated by measuring their effects on the production of H_2O_2 from benzylamine, using the Amplex Red® MAO assay kit (Invitrogen).

Briefly, 0.1 ml of sodium phosphate buffer (0.05 M, 7.4 pH) containing the test drugs (new compounds or reference inhibitors) in various concentrations and adequate amounts of recombinant hMAO-B (0.5 μl, 71 U/mg) were incubated for 1 h at 37 °C in a 96-well plate. After this incubation period, the reaction was started by adding (final concentrations) 200 μM Amplex Red® reagent, 1 U/ml horseradish peroxidase, and 1 mM benzylamine hydrochloride. The production of H_2O_2 and, consequently, of resorufin, was quantified at 37 °C in a multidetection microplate fluorescence reader (TECAN) based on the fluorescence generated (absorbance, 570 nm).

Control experiments were carried out simultaneously by replacing the test drugs (new compounds and reference inhibitors) with appropriate dilutions of the vehicles. In addition, the possible capacity of the above test drugs to modify due to nonenzymatic inhibition (e.g., for directly reaction with Amplex Red® reagent) was determined by adding these drugs to solutions containing only the Amplex Red® reagent in a sodium phosphate buffer.

The specific fluorescence absorbance (used to obtain the final results) was calculated after subtraction of the background activity, which was determined from vials containing all components except the hMAO-B, which was replaced by a sodium phosphate buffer solution.

4.11. Docking study

All docking studies were performed with Maestro 7.5 package (Schrödinger, Inc.) and carried out on the X-ray crystal structure of hMAO-B (PDB code 2V60) which was obtained from the Protein Data Bank. Three dimensional structures of the compounds **1i** and **1n** were prepared by build module of Maestro 7.5 and then the constructed 3D structures of **1i** and **1n** were minimized with conjugate gradient method, OPLS-AA (Optimized Potentials for Liquid Simulations-All Atom) force field, and GB/SA continuum water model. The crystal structure of hMAO-B was prepared for docking with the Protein Preparation Wizard workflow of Maestro 7.5 to assign the charge state of ionizable residues, add hydrogens, and carry out energy minimization. The docking was performed by using GLIDE 4.0 (http://www.schrodinger.com). The grid was made by applying a van der Waals radii scaling factor of 1.00 with a partial charge cut-off of less than 0.25e and the co-crystal ligand was used to center docking box with a size capable of accommodating ligands with a length of $\leqslant$20 Å. The docking calculations were performed with Glide standard precision (SP) mode, and ten best poses for each ligand were retained.

Acknowledgement

This research was supported by the KIST Institutional Program (2E24183 and 2E23770).

References and notes

1. de Lau, L. M. L.; Breteler, M. M. B. *Lancet Neurol.* **2006**, *5*, 525.
2. Jankovic, J. *J. Neurol. Neurosurg. Psychiatry* **2008**, *79*, 368.
3. German, D. C.; Manaye, K.; Smith, W. K.; Woodward, D. J.; Saper, C. B. *Ann. Neurol.* **1989**, *26*, 507.
4. Gardian, G.; Vecsei, L. *Int. J. Clin. Pharmacol. Ther.* **2010**, *48*, 633.
5. Fahn, S. *Mov. Disord.* **2008**, *23*, S497.
6. Bach, A. W. J.; Lan, N. C.; Johnson, D. L.; Abell, C. W.; Bembenek, M. E.; Kwan, S. W.; Seeburg, P. H.; Shih, J. C. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4934.
7. Mallajosyula, J. K.; Kaur, D.; Chinta, S. J.; Rajagopalan, S.; Rane, A.; Nicholls, D. G.; Di Monte, D. A.; Macarthur, H.; Andersen, J. K. *PLoS ONE* **2008**, *3*, e1616.
8. Lieu, C. A.; Chinta, S. J.; Rane, A.; Andersen, J. K. *PLoS ONE* **2013**, *8*, e54200.
9. Levitt, P.; Pintar, J. E.; Breakefield, X. O. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 6385.
10. Bortolato, M.; Chen, K.; Shih, J. C. *Adv. Drug Delivery Rev.* **2008**, *60*, 1527.
11. Fernandez, H. H.; Chen, J. J. *Pharmacotherapy* **2007**, *27*, 174S.
12. Pålhagen, S.; Heinonen, E.; Hagglund, J.; Kaugesaar, T.; Maki-Ikola, O.; Palm, R. *Neurology* **2006**, *66*, 1200.
13. Rabey, J. M.; Sagi, I.; Huberman, M.; Melamed, E.; Korczyn, A.; Giladi, N.; Inzelberg, R.; Djaldetti, R.; Klein, C.; Berecz, G. *Clin. Neuropharmacol.* **2000**, *23*, 324.
14. Vezina, P.; Mohr, E.; Grimes, D. *Can. J. Neurol. Sci.* **1992**, *19*, 142.
15. Stern, M. B.; Marek, K. L.; Friedman, J.; Hauser, R. A.; LeWitt, P. A.; Tarsy, D.; Olanow, C. W. *Mov. Disord.* **2004**, *19*, 916.
16. Dimpfel, W.; Hoffmann, J. *BMC Pharmacol.* **2011**, *11*, 2.
17. Barrow, J.; Harrison, S.; Mulhearn, J.; Sur, C.; Williams, D. L.; Wolkenberg, S. WO 155,017, **2009**, A2.
18. Kempson, J.; Spergel, S. H.; Guo, J.; Quesnelle, C.; Gill, P.; Belanger, D.; Dyckman, A. J.; Li, T.; Watterson, S. H.; Langevine, C. M.; Das, J.; Moquin, R. V.; Furch, J. A.; Marinier, A.; Dodier, M.; Martel, A.; Nirschl, D.; Kirk, K. V.; Burke, J. R.; Pattoli, M. A.; Gillooly, K.; McIntyre, K. W.; Chen, L.; Yang, Z.; Marathe, P. H.; Wang-Iverson, D.; Dodd, J. H.; McKinnon, M.; Barrish, J. C.; Pitts, W. J. *J. Med. Chem.* **2009**, *52*, 1994.
19. Chimenti, F.; Secci, D.; Bolasco, A.; Chimenti, P.; Bizzarri, B.; Granese, A.; Carradori, S.; Yánez, M.; Orallo, F.; Ortuso, F.; Alcaro, S. *J. Med. Chem.* **2009**, *52*, 1935.
20. Binda, C.; Wang, J.; Pisani, L.; Caccia, C.; Carotti, A.; Salvati, P.; Edmondson, D. E.; Mattevi, A. *J. Med. Chem.* **2007**, *50*, 5848.