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Design, synthesis and biological evaluation of novel chiral oxazino-indoles as potential and selective neuroprotective agents against $A\beta_{25-35}$-induced neuronal damage

Jing Chen [a,†], Ling-Xue Tao [b,†], Wei Xiao [c,†], Sha-Sha Ji [d], Jian-Rong Wang [a], Xu-Wen Li [a,*], Hai-Yan Zhang [b,*], Yue-Wei Guo [a,*]

[a] State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu Chong Zi Road, Zhangjiang Hi-Tech Park, Shanghai 201203, PR China
[b] CAS Key Laboratory of Receptor Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, PR China
[c] Jiangsu Kanion Pharmaceutical Co. Ltd, Lianyungang 222001, PR China
[d] School of Biological Science and Technology, Weifang Medical University, 7166 Bao Tong West Street, Weifang, Shandong 261053, PR China

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ABSTRACT

A series of chiral oxazino-indoles have been synthesized via a key intermolecular oxa-Pictet–Spengler reaction. These compounds exhibited significant and selective neuroprotective effects against $A\beta_{25-35}$-induced neuronal damage. This is the first report of evaluating the influence of chiral diversity of oxazino-indoles on their neuroprotective activities, with the structure–activity relationship been analyzed. The highly active compounds **3f**, **3g**, **4g**, **4h**, and **6b** all performed over 90% cell protection, providing a new direction for the development of neuroprotective agents against Alzheimer's disease.

Alzheimer's disease (AD) is one of the most common dementia occurring in elderly people. The pathogenesis of AD is still unknown, while multiple hypotheses[1–3] have been proposed based on a series of evidence. Among them, the amyloid cascade hypothesis[2] is the prevailing one, which suggested that the production and the accumulation of aggregates of β-amyloid peptide (Aβ) may be a key process. According to this hypothesis, Aβ was produced by proteolytic cleavage of the amyloid precursor protein (APP)[4–6], and based on such evidence, various anti-Aβ strategies including lowering the production of the peptide, preventing the formation of Aβ aggregates, and increasing the rate of Aβ clearance from the brain have been pursued.[7–10] Although many neuroprotective compounds with various structure features have been reported, the progress of the new and effective drug discovery in this field is still slow and the novel specific bioactive compounds investigation are urgently merited.[11–15]

Indole-containing compounds are pharmacologically attractive due to their inherently biological activities and physicochemical properties.[16–18] In this superfamily, *N*-fused indoles[19,20], especially those incorporated with oxazino (Fig. 1), have drawn considerable attention due to the rigid skeleton and concurrently varied biological activities. 3,4-Dihydro-1*H*-[1,4]oxazino[4,3-*a*]indoles, a series representative *N*-fused bicyclic indoles, was firstly investigated by Humber and coworkers as the antidepressant reagents.[21] Due to their promising biological activities and pharmaceutical privileged scaffolds, a large number of synthetic methods[22,23] have been developed to construct such skeleton. In 2010, Xiao[24] described a general approach to oxazino[4,3-*a*]indole skeleton by applying vinyl sulphonium salts as the reactants. Later on, Gharpure[25] firstly developed stereoselective method for the synthesis of oxazino[4,3-*a*]indoles, employing an oxa-Pictet–Spengler reaction.[26,27] Inspired by the previous works and in order to screen the bioactive small molecules for drug discovery, we envisaged the possibility of developing a simple and effective way to synthesize chiral oxazino[4,3-*a*]indoles by employing an intermolecular oxa-Pictet–Spengler reaction. With regard to the stereochemistry-based activity relationship, we speculated that the chiral methyl group of the starting material could induce the new chiral center and

* Corresponding authors.
E-mail addresses: xwli@simm.ac.cn (X.-W. Li), hzhang@simm.ac.cn (H.-Y. Zhang), ywguo@simm.ac.cn (Y.-W. Guo).
† Authors contributed equally to this work.

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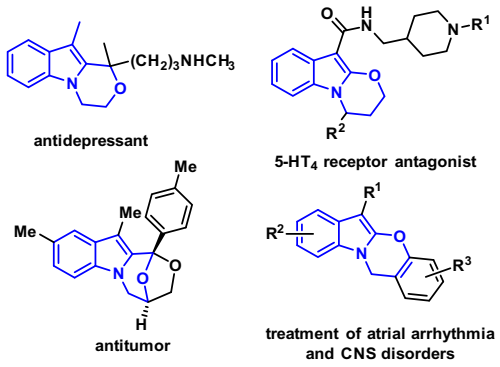
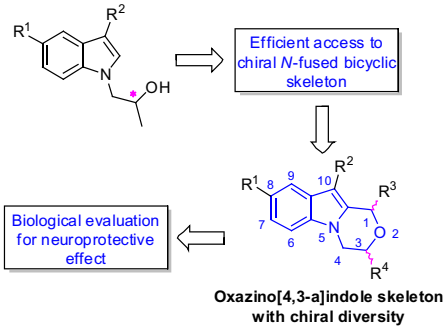


Figure 1. Oxazino incorporated indoles in bioactive molecules.



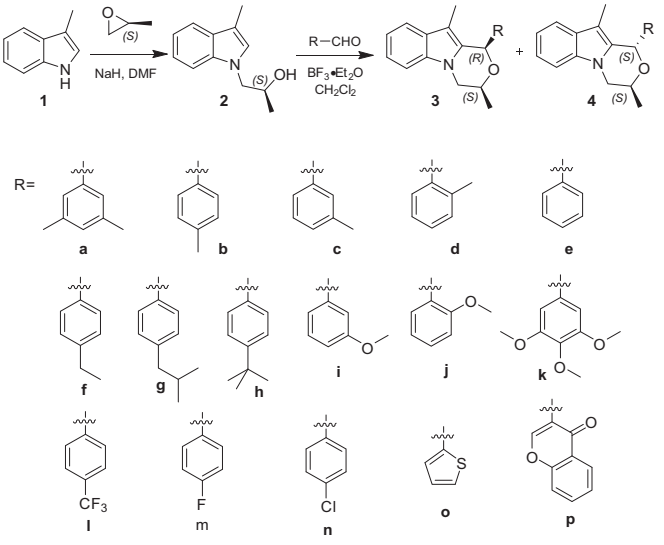
Scheme 1. Efficient access to neuroprotective chiral oxazino[4,3-*a*]indoles.

influence the physicochemical property and biological activity of the indole derivatives (Scheme 1). Aromatic aldehydes were applied in the reaction, since the additional aryl group could probably increase the molecular rigidity and have π–π stacking to the enzyme, which usually play important role for the biological activities. In addition, aromatic aldehydes were easier to be introduced than the aliphatic ones for an industrial applicable sake. Herein, we report the construction of chiral oxazino[4,3-*a*]indoles via a simple synthetic access, their neuroprotective bioassays, and the structure–activity relationship (SAR) study.

A novel synthetic route to prepare 3,4-dihydro-1*H*-[1,4]oxazino [4,3-*a*]indoles was developed. As shown in Scheme 2, 3-methyl-1*H*-indole (**1**) was deprotonated by sodium hydride (NaH) in dry DMF, followed by a nucleophilic addition to (*S*)-epoxypropane, affording (*S*)-1-(3-methyl-1*H*-indol-1-yl) propan-2-ol (**2**), which then underwent an intermolecular oxa-Pictet–Spengler reaction to afford the desired compounds **3** and **4** by reacting with various aldehydes in dichloromethane with the Lewis acid $BF_3{\cdot}Et_2O$ as a catalyst. In fact, the chiral methyl group was designed to direct the unique configuration of the newly generated chiral center of the R group in **3** or **4**. However, the selectivity was unsatisfactory, and the two diastereomers were generated with almost the same ratio. Compared to the excellent diastereoselectivity achieved in the intramolecular oxa-Pictet–Spengler reaction [25], the poor selectivity in our reaction are probably attributed to the lack of an CH_2CO_2Et group as a stereoselective directing group, of which the ester might be easier to occupy the pseudo-equatorial orientation in the transition state than the aromatic groups in our case, in order to avoid 1,3-diaxial interaction with R^4 group (Scheme 1). Fortunately, the diastereomers **3** and **4** were easy to be purified via column chromatography, leading to the configuration diversity of the molecule for further biological evaluation. The configurations of **3e** and **4e** were further confirmed by X-ray diffraction as shown in Fig. 2[28] to be (1*R*, 3*S*) and (1*S*, 3*S*), respectively, by which the configurations of their analogs were determined accordingly.

In order to obtain the chiral diversity of our designed products for bioassay, the syntheses of compounds **6a**, **6b**, **7a** and **7b** were carried out by using the similar approach as that of **3** and **4**, while (*R*)-epoxypropane as one of the reactants instead of (*S*)-epoxypropane, to investigate the effect of absolute configuration on the neuroprotective activity. Meanwhile, the de-3-methyl indole derivatives were also prepared by reacting with 2-bromoethanol, followed by oxa-Pictet–Spengler reaction to obtain compounds **9a** and **9b** as shown in Scheme 3.

With the aim to improve the water solubility of these compounds and investigate the substitution variety of the indole moiety, commercially available compound 5-methoxytryptamine (**10**) was utilized as the starting material to prepare the tryptamine derivatives as shown in Scheme 4. Compound **10** was subjected



Scheme 2. Synthesis of 3,4-dihydro-1*H*-[1,4]oxazino[4,3-*a*]indoles comprising various phenyl rings by a key intermolecular oxa-Pictet–Spengler reaction.

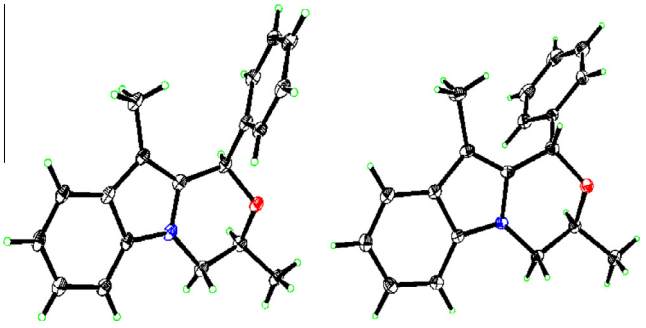
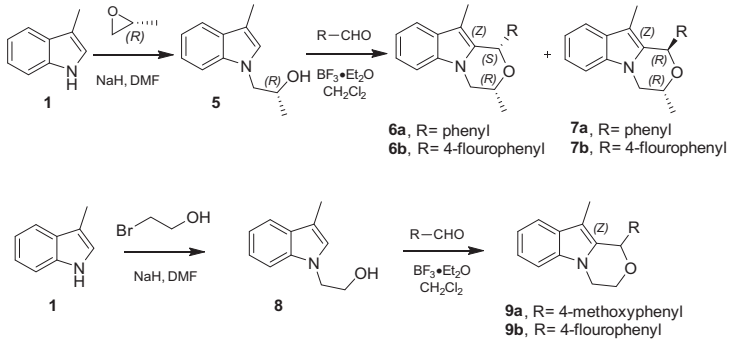


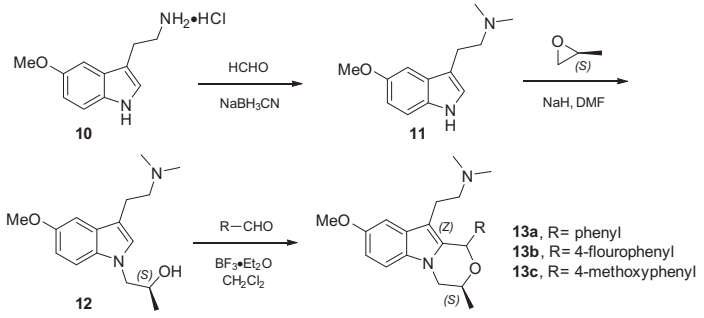
Figure 2. Crystal structure of **3e** and **4e**.

to methylation reaction in the presence of formaldehyde and $NaBH_3CN$ to produce intermediate **11**, which was then converted to compound **12** by reacting with (*S*)-epoxypropane in the presence of NaH in DMF. Finally, the oxazine ring was constructed by oxa-Pictet–Spengler reaction with the same conditions as presented above to get the target compounds **13a**–**13c**. All these synthesized products were characterized by extensive spectroscopic analyses of MS, ^1H and ^{13}C NMR.

With all the target compounds in hand, their neuroprotective properties were assessed aiming at discovering bioactive agents to prevent $A\beta_{25-35}$-induced neuronal damage in SH-SY5Y neuroblastoma cell lines. All the synthesized compounds were tested for



Scheme 3. Synthesis of (3*R*)-3,4-dihydro-1*H*-[1,4]oxazino[4,3-*a*]indoles and de-methyl indole derivatives.



Scheme 4. Synthesis of compounds **13a**–**13c**.

the biological activity at the concentration of 10 μM and the results are summarized in Table 1. Interestingly, Compounds **3a** and **4a**, a pair of diastereomers differing at C-1 position, displayed the highly variant biological results. **3a**, with (1*R*)-configuration, was inactive whereas **4a**, with (1*S*)-configuration, exhibited significant neuroprotection with cell viability recovered to 87.1% of control as compared with Aβ-alone group (62.1%). This surprising result stimulated our interest for further SAR studies in the following aspects: (i) The influence of the stereochemistry; (ii) The importance of the methyl substitution at the 3,10-position; (iii) The effect of substitution at the benzene ring. Compared to **4a**, compounds **3b**–**e**, and **4b**–**e** bearing mono-methyl substitution at *o*-, *m*-, *p*-position, respectively, or no substitution (for **3e** and **4e**), did not perform any protecting activity. Compounds **3f**–**h** and **4f**–**h** with bulkier alkyl substitution at *para*-position of benzene ring were synthesized and evaluated. Compounds **3f**–**h**, **4g**–**h** showed 93.0%, 108.4%, 84.5%, 96.2%, 92.1% cell viability, respectively, similar as that of the positive control epigallocatechin gallate (EGCG) (98.6%). This series of compounds presented significant activity except for compound **4f**, which indicated that the size of the substituent at the *para*-position might be important for the biological activity. The different electronic features (electron-withdrawing or electron-donating) of the benzene ring were further investigated. Among compounds **3i**–**k** and **4i**–**k**, bearing electron-donating groups on the benzene ring, only *o*-methoxysubstitution with (*S*)-configuration at C-1 position (**4j**) displayed 77.7% cell viability. Furthermore, compounds **3l**–**n**, **4l**–**n** with electron-withdrawing groups on the benzene ring were synthesized and evaluated, while only *para*-chloro substituted derivative **3n** with the (*R*)-configuration at C-1 position displayed 74.4% cell viability, while its diastereoisomer **4n** with (*S*)-configuration was not active. At last, compounds **3o**–**p** and **4o**–**p** with heteroaromatic ring instead of benzene ring on C-1 position did not show any neuroprotective activity. In addition, thiophene and chromone featured by their influence on a wide range of pharmacological properties[29], have been introduced on the C-1 position to obtain compounds **3o**–**p** and **4o**–**p**. Unfortunately, no neuroprotective effect has been observed for the above compounds.

In order to further evaluate the influence of the stereochemistry on the neuroprotective activity, compounds **6** and **7** with (*R*)-configuration at C-3 position were synthesized (Scheme 3) by using the same procedure as that of **3** and **4**, while with (*R*)-epoxypropane as the reagent to introduce the diversity of the stereochemistry. All the four compounds **6a**, **6b**, **7a** and **7b** were biologically evaluated. In the bioassay, **6a** and **7a** did not exhibit neuroprotective activity. Interestingly, compound **6b** displayed remarkable activity with cell viability remained as 90.6% of control, while its diastereoisomer **7b** was inactive. It is worth to note that among all the isomers (**3m**, **4m**, **6b** and **7b**), only **6b** was active, indicating the impact of absolute configuration was essential on the neuroprotective activity of such structure. In addition, compounds **9a** and **9b**, with methyl group removed at C-3 position, was proved to be inactive. Finally, 5-methoxytryptamine was utilized as the starting material to design and synthesize compounds **13a**–**c** characterized by an 8-methoxyl substitution and an *N*,*N*-dimethylethanamine at C-10 position, with the aim to improve their neuroprotective activity and water solubility as a drug candidate. Unfortunately, all the three compounds were inactive, despite the improving of the water solubility.

All the target compounds were also subjected to bioassays of H_2O_2 induced damage and oxygen glucose deprivation (OGD) induced injury in SH-SY5Y, with no effect observed, suggesting that such compounds are selectively neuroprotective against the $A\beta_{25-35}$-induced neuronal cell damage. We also examined the effects of the most active compounds **3f**, **3g**, **6b**, **4g and 4h** on cell viability and cell proliferation under Aβ-free condition by using MTT and sulforhodamine B (SRB) assay, respectively. The results showed that none of the above compounds have the influence on the cell proliferation.

Table 1
The neuroprotective of compounds **3** and **4** against $A\beta_{25-35}$-induced neurotoxicity in SH-SY5Y cells

Compd	R	Cell viability[a] (%)	Compd	R	Cell viability (%)
3a	3,5-Dimethylphenyl	N.A.[b]	**4a**	3,5-Dimethylphenyl	87.1
3b	4-Methylphenyl	N.A.	**4b**	4-Methylphenyl	N.A.
3c	3-Methylphenyl	N.A.	**4c**	3-Methylphenyl	N.A.
3d	2-Methylphenyl	N.A.	**4d**	2-Methylphenyl	N.A.
3e	Phenyl	N.A.	**4e**	Phenyl	N.A.
3f	4-Ethylphenyl	93.0	**4f**	4-Ethylphenyl	N.A.
3g	4-Isobutylphenyl	108.4	**4g**	4-Isobutylphenyl	96.2
3h	4-*t*Bu-phenyl	84.5	**4h**	4-*t*Bu-phenyl	92.1
3i	3-Methoxyphenyl	N.A.	**4i**	3-Methoxyphenyl	N.A.
3j	2-Methoxyphenyl	N.A.	**4j**	2-Methoxyphenyl	77.7
3k	3,4,5-trimethoxyphenyl	N.A.	**4k**	3,4,5-Trimethoxyphenyl	N.A.
3l	4-Trifluoromethylphenyl	N.A.	**4l**	4-Trifluoromethylphenyl	N.A.
3m	4-Fluorophenyl	N.A.	**4m**	4-Fluorophenyl	N.A.
3n	4-Chlorophenyl	74.4	**4n**	4-Chlorophenyl	N.A.
3o	2-Thiophene	N.A.	**4o**	2-Thiophene	N.A.
3p	3-Chromone	N.A.	**4p**	3-Chromone	N.A.
6a[c]	Phenyl	N.A	**7a**[c]	Phenyl	N.A.
6b[c]	4-Fluorophenyl	90.6.	**7b**[c]	4-Fluorophenyl	N.A.
			EGCG		98.6

[a] The neuroprotective effect of these compounds on $A\beta_{25-35}$-induced neurotoxicity in SH-SY5Y cells. The cell viability in control was taken as 100%, and the average value of cell viability under $A\beta_{25-35}$ exposure was 62.1 ± 2.2%. The positive control is epigallocatechin gallate (EGCG).
[b] N.A. means not active.
[c] With *R*-configuration at 3-position.

The scaffold of *N*-fused indoles has attracted intensive attention due to its frequently existence in natural products and pharmaceutical agents. In this study, we effectively constructed bicyclic chiral oxazino[4,3-*a*]-indoles by the application of intermolecular oxa-Pictet–Spengler reaction. More than 40 derivatives have been synthesized and evaluated for their neuroprotective effect against $A\beta_{25-35}$-induced neuronal cell damage. The results clearly indicated that five compounds, namely **3f**, **3g**, **4g**, **4h**, and **6b**, exhibited robust neuroprotective effects against Aβ-induced neurotoxicity. Furthermore, the results of SRB assay indicate that the beneficial pharmacological profiles of above active compounds are attributed to their ability on battling Aβ-associated neurotoxicity rather than promoting the cell proliferation. The preliminary SAR analysis suggested that bulky alkyl substitution of the *para*-position of the benzene ring plays a crucial role in the neuroprotection. In addition, the influence of stereochemistry on the bioactivity varied by different substitutions at the core structure. For instance, among the compounds with 3,5-dimethyl group (**3a** and **4a**) and 2-methoxyl group (**3j** and **4j**), those with (1*S*, 3*S*)-configuration (**4a** and **4j**) exhibited strong neuroprotective effect, whereas the ones with (1*R*, 3*S*)-configuration (**3a** and **3j**) were inactive. Similar result was also observed on the 4-fluorophenyl substituted compounds (**3m**, **4m**, **6b** and **7b**), of which only **6b** with (1*S*, 3*R*)-configuration showed significant neuroprotective activity. It is worth to mention that the strong activities of 1,3-*trans*-isomers **4a** and **4j** and the 1,3-*cis*-isomer **6b**, suggesting that the *trans*-configuration combined with the nucleophilic substitution at C-1 for **4a** and **4j**, whereas the *cis*-configuration combined with the electrophilic substitution at C-1 for **6b**, were probably important for the activities.

In conclusion, the above described interesting chemical and biological results provided a fascinating clue for the discovery of more potent *N*-fused indole derivatives with neuroprotective activities. More-in-depth investigation should be conducted towards the most bioactive compounds, such as **3f**, **3g**, **4g**, **4h**, and **6b**, including chemical modification, molecular action mechanism, and in vivo biological study.

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