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Original article

Design, synthesis and antifungal activity of isosteric analogues of benzoheterocyclic *N*-myristoyltransferase inhibitors

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ABSTRACT

N-myristoyltransferase (NMT) has been a promising new target for the design of novel antifungal agents with new mode of action. A series of benzoxazole and indole derivatives were designed and synthesized as isosteric analogues of benzoheterocyclic NMT Inhibitors. *In vitro* antifungal assay indicated that the benzoxazole derivatives were far more potent than the indoles. Molecular docking studies revealed that the hydrogen bonding interaction between the benzoheterocyclic core and NMT might be essential in the orientation of the inhibitor to a proper position. The antifungal activity of benzoxazole derivative **8f** was comparable or superior to that of fluconazole, which can serve as a good starting point for further studies of structural diversity of the benzoheterocyclic NMT inhibitors.

1. Introduction

During the past two decades, the incidence of systemic fungal infections has been increasing dramatically due to the increasing number of immunocompromised hosts, such as patients undergoing anticancer chemotherapy or organ transplants, and patients with AIDS [1]. Clinically, candidosis, aspergillosis and cryptococcosis have been identified as three major fungal infections [2,3]. The mortality rate associated with invasive *Candida* is approximately 40% [4], whereas associated with invasive *Aspergillus* reaches 100% in solid organ transplant recipients [5]. In contrast to the large number of antibacterial antibiotics, there are very few antifungal agents that can be used for life-threatening fungal infections. These drugs are amphotericin B [6], 5-fluorocytosine, azoles (such as fluconazole and itraconazole) [7], and echinocandins (such as caspofungin and micafungin) [8]. However, current antifungal therapy is far from satisfaction, because currently available antifungal agents have several drawbacks, such as drug related toxicity, severe drug resistance, non-optimal pharmacokinetics, and serious drug–drug interactions. Moreover, severe resistance of antifungal drugs has also been observed [9]. Therefore, there is an emergent need to develop new antifungal drugs with novel modes of action.

Myristoyl-CoA: protein *N*-myristoyltransferase (NMT) is a cytosolic monomeric enzyme that catalyzes the transfer of the myristoyl group from myristoyl-CoA to the N-terminal glycine of a number of eukaryotic cellular and viral proteins [10,11]. Genetic experiments have shown that NMT is a promising target enzyme for the development of novel fungicidal drugs having a broad antifungal spectrum [12]. Up to now, peptidomimetic inhibitors [13–16], myristic acid analogues [17,18], benzofuran inhibitors [19–23] and benzothiazole inhibitors [23,24] have been reported to possess NMT inhibitory activity. Among them, only the benzofuran and benzothiazole inhibitors showed high selectivity and good antifungal activity. They can serve as a good starting point for the discovery of novel antifungal agents. Therefore, the extension of structure–activity relationships (SARs) of these NMT inhibitors is of great importance.

Analysis of the chemical structure of the benzofuran and benzothiazole inhibitors reveals that they share three important chemical elements (Fig. 1), i.e. a benzoheterocyclic core, a secondary amine at its C-4 side chain, a hydrophobic group attached to its C-2 position through various linkers. Crystal structure of NMT inhibitor complex [25] and our docking studies [26] found that the benzoheterocyclic core was located at the center of the active site,

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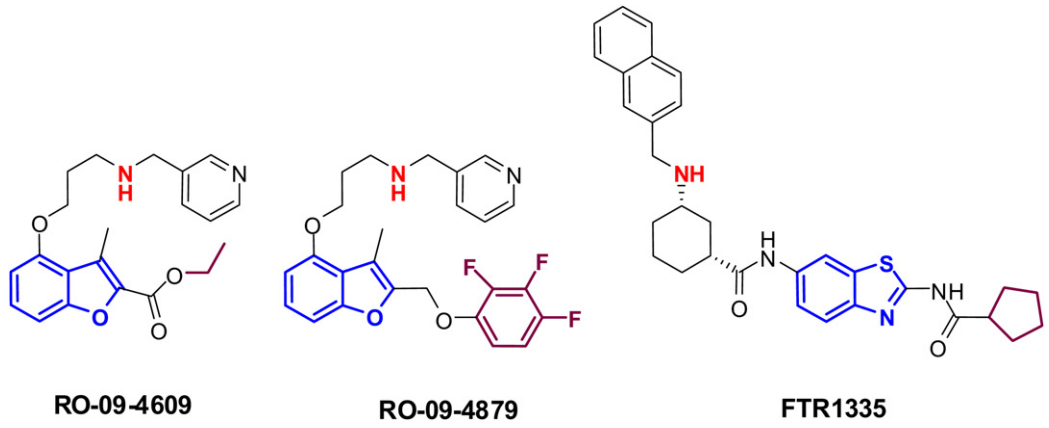


Fig. 1. Representative structures of the benzofuran and benzothiazole NMT inhibitors.

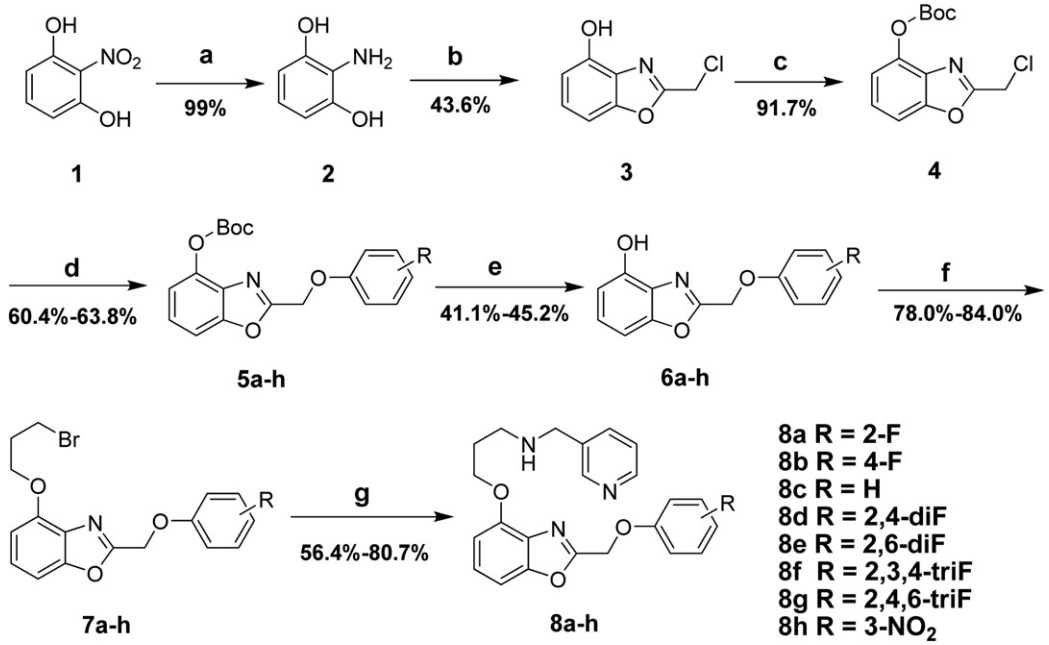
surrounded by some hydrophobic residues, such as Tyr225, Tyr354 and Leu394. In an effort to investigate the importance of the benzoheterocyclic core on the antifungal activities, we designed and synthesized a series of benzoxazole and indole analogues as isosteric analogues of benzoheterocyclic NMT Inhibitors.

2. Chemistry

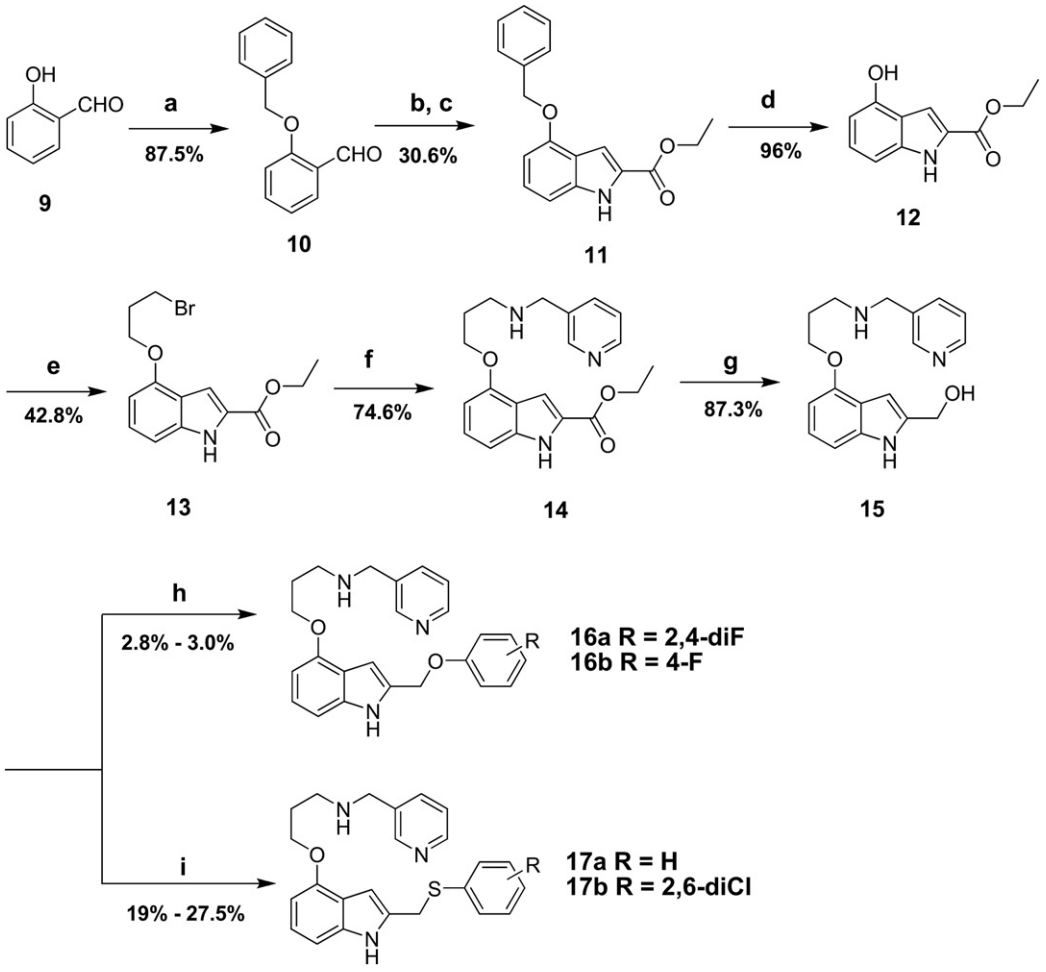
The synthetic route of benzoxazole derivatives was outlined in Scheme 1. 2-Nitroresorcinol (**1**) was reduced to 2-aminoresorcinol (**2**) by catalytic hydrogenation with H_2 and 10% Pd/C in EtOH in 99% yield. Reaction of **2** with ethyl chloroacetimidate gave 2-(chloromethyl)benzoxazol-4-ol (**3**). The hydroxyl group of **3** was protected by $(Boc)_2O$ to give **4**. In the presence of NaH, treatment of **4** with various substituted phenols in DMF at room temperature provided the ethers **5a**–**h**. After removing the protecting group of compounds **5a**–**h** using CF_3COOH, alkylation of the resulting **6a**–**h** with excess 1,3-dibromopropane gave bromopropanyl derivatives **7a**–**h** in good yields. The target benzoxazoles **8a**–**h** were prepared by reacting **7a**–**h** with 3-(aminomethyl)pyridine in EtOH at 75 °C with moderate to good yields.

Starting from salicylaldehyde (**9**), the indole intermediate **12** was synthesized by a four-step procedure (Scheme 2). Initially, the hydroxyl group of **9** was protected by benzyl bromide. Then, compound **10** was condensed with ethyl azidoacetate to form an intermediate azide. By the thermal cycliation of the azide in toluene at reflux, indole **11** was obtained, which was deprotected to give key intermediate **12**. Treatment of **12** with excess 1,3-dibromopropane in the presence of potassium carbonate in DMF gave *O*-bromopropanyl derivative **13**. Amination of **13** with 3-(aminomethyl)pyridine at 75 °C in EtOH gave amino derivative **14** without affecting the ester group. The target compounds **16a**–**b** were prepared by Mitsunobu reaction of appropriate phenols with alcohol **15** [20], which was obtained by the reduction of ester **14** with $LiAlH_4$.

Compounds **20a**–**e** were prepared following the route depicted in Scheme 3. Ester **13** was hydrolyzed by lithium hydroxide, and the resulting acid **18** was converted into its acid chloride by treatment with oxalyl chloride, which was immediately subjected to the next anilide formation reaction. The resulting amides **19a**–**e** was heated to 75 °C with 3-aminomethylpyridine in EtOH to give **20a**–**e**.



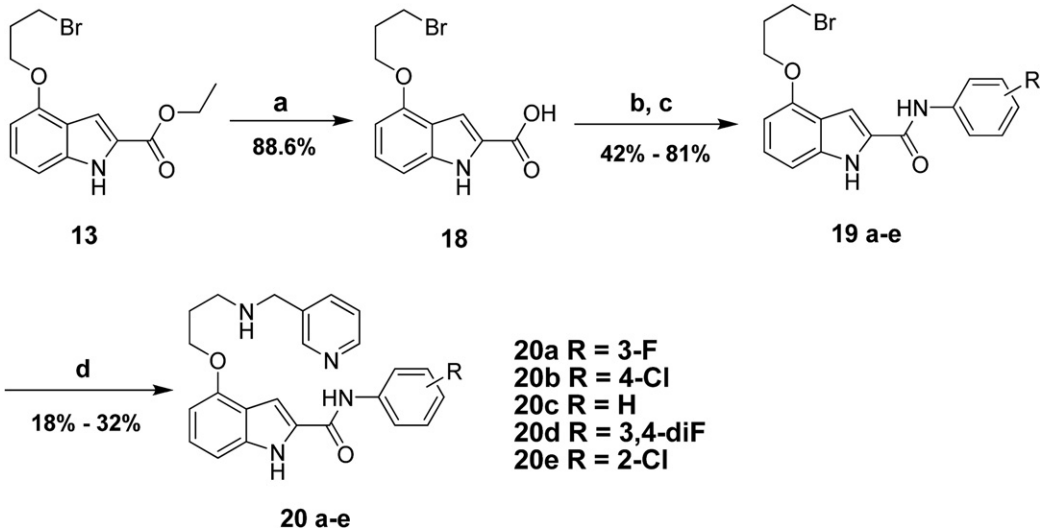
Scheme 1. Reagents and conditions: a: H_2, 10% Pd/C, EtOH; b: $ClCH_2C(=NH)OC_2H_5 \cdot HCl$, 0 °C; c: Boc_2O, CH_2Cl_2; d: ROH, NaH, DMF; e: CF_3COOH, CH_2Cl_2; f: 1,3-dibromopropane, K_2CO_3, DMF, rt; g: 3-(Aminomethyl)pyridine, EtOH, 75 °C.



Scheme 2. Reagents and conditions: a: benzyl bromide, 70 °C, reflux; b; Ethyl azidoacetate, NaOEt, EtOH, −10 °C; c: toluene, 120 °C, reflux; d: H_2, 10% Pd/C, EtOH, 50 °C; e: 1,3-dibromopropane, K_2CO_3, DMF, rt; f: 3-(Aminomethyl)pyridine, EtOH, 75 °C, reflux; g: $LiAlH_4$, THF, 0 °C; h: TBP, ADDP, phenols, CH_2Cl_2, −20 °C to rt. i: CF_3COOH, benzenethiols, CH_2Cl_2, rt.

3. Pharmacology

In vitro antifungal activity was measured by means of the MIC using the serial dilution method in 96-well microtest plates. Fluconazole and benzofuran NMT inhibitors (RO-09-4609 and RO-09-4879) were used as reference drugs. Test fungal strains were obtained from the ATCC or were clinical isolates. The MIC determination was performed according to the National Committee for Clinical



Scheme 3. Reagents and conditions: a: LiOH, HCl, THF/MeOH, 0 °C, 6 h; b: Oxalyl chloride, Et_3N, CH_2Cl_2, rt, 12 h; c: substituted-anilines, CH_2Cl_2, rt, 3 h; d: 3-(Aminomethyl)pyridine, EtOH, 75 °C, reflux,12 h.

Laboratory Standards (NCCLS) recommendations. The detailed experimental protocols can be found in our previous studies [27].

4. Results and discussion

4.1. Antifungal activity of the benzoxazole derivatives

The antifungal activity of each compound was expressed as the minimal inhibitory concentration (MIC) that achieved 80% inhibition of the tested fungi. *In vitro* antifungal activity assay (Table 1) indicated that the benzoxazole derivatives **8a**–**h** showed moderate to good potency against *Candida* spp. In general, these compounds were more active against *Candida parasilosis* and *Candida tropicalis* than *Candida albicans*. All the compounds showed better activity against *C. tropicalis* than fluconazole with their MIC values in the range of 1 μg/mL to 0.0625 μg/mL. Moreover, compound **8a** was more active against *C. tropicalis* (MIC = 0.0625 μg/mL) than benzofuran inhibitor RO-09-4609 and RO-09-4879. On the *C. parasilosis* strain, compounds **8d** and **8f** (MIC = 0.25 μg/mL) were more potent than fluconazole and RO-09-4609. However, they were 4-fold less potent than RO-09-4879. Most of the compounds showed moderate activity against *C. albicans* except for compound **8f**. For *C. albicans*, the MIC value of compound **8f** was 0.25 μg/mL, which was comparable to that of fluconazole. However, it was 2-fold less active than RO-09-4879. As compared with *Candida* spp., the inhibitory activity of the compounds against *Cryptococcus neoformans* was decreased. The MIC values for the most of the compounds were in the range of 16 μg/mL to 64 μg/mL. Moreover, most of the compounds were inactive against *Aspergillus fumigatus* and only compound **8g** showed marginal inhibitory activity (MIC = 64 μg/mL). Among the benzoxazole derivatives, compound **8f** was the most potent one with broad antifungal spectrum, which was worthy of further optimization.

4.2. Antifungal activity of the indole derivatives

When the benzoxazole scaffold of compounds **8a**–**h** was changed to indole, the antifungal activity was decreased to the large extent (Table 2). The loss of the antifungal activity against deep fungal pathogens (i.e. *C. albicans*, *C. parasilosis* and *C. tropicalis*) was observed for compounds **16a** and **16b**. Only compound **16a** showed marginal activity against *C. neoformans* (MIC = 64 μg/mL). Furthermore, we tested the inhibitory activity of indole derivatives against dermatophytes. Compounds **16a** and **16b** showed moderate activity against *Microsporum gypseum* and *Trichophyton rubrum* with their MIC values in the range of 8 μg/mL to 128 μg/mL. We further varied the linker between the phenyl group and indole ring. The replacement of ether group by the thio-ether group led to the slightly improvement of the antifungal activity. Compounds **17a** and **17b** show marginal activity against deep fungal pathogens. For the dermatophytes, their MIC values were in the range of 4 μg/mL to 64 μg/mL. They showed better inhibitory activity against *M. gypseum* than fluconazole. If the thio-ether group is replaced by the amide group (compounds **20a**–**e**), their antifungal activity against deep fungal pathogens is lost again. Most of compounds 20a-e only showed moderate activity against dermatophytes with MIC values in the range of 2 μg/mL to 64 μg/mL.

Table 1
Antifungal *in vitro* activities of the benzoxazole derivatives (MIC_{80}, μg mL^{-1}).[a]

Compounds	*C. alb.*	*C. par.*	*C. tro.*	*C. neo.*	*A. fum.*
8a	4	1	0.0625	16	>64
8b	16	1	1	16	>64
8c	16	1	0.25	64	>64
8d	1	0.25	1	16	>64
8e	64	4	1	64	>64
8f	0.25	0.25	0.25	64	>64
8g	16	1	1	16	64
8h	>64	4	0.25	>64	>64
RO-09-4609	4	4	1	64	>64
RO-09-4879	0.125	0.0625	0.125	0.5	16
FLZ	0.25	4	4	1	>64

[a] Abbreviations: *C. alb. Candida albicans*; *C. par. Candida parasilosis*; *C. tro. Candida tropicalis*; *C. neo. Cryptococcus neoformans*; *A. fum. Aspergillus fumigatus;* FLZ: Fluconazole.

Table 2
Antifungal *in vitro* activities of the indole derivatives (MIC_{80}, μg mL^{-1}).[a]

Compounds	*M. gyp.*	*T. rub.*	*C. alb.*	*C. neo.*	*A. fum.*
14	32	64	>128	>128	>128
16a	8	32	>128	64	>128
16b	32	128	>128	128	>128
17a	8	64	128	64	>128
17b	4	16	64	64	>128
20a	32	64	128	128	>128
20b	32	128	>128	>128	>128
20c	32	64	>128	>128	>128
20d	16	32	>128	128	>128
20e	2	128	>128	>128	>128
RO-09-4609	4	4	1	64	>64
RO-09-4879	2	1	0.125	0.5	16
FLZ	16	1	0.25	2	>64

[a] Abbreviations: *M. gyp. Microsporum gypseum*; *T. rub. Trichophyton rubrum*; *C. alb. Candida albicans*; *C. neo. Cryptococcus neoformans*; *A. fum. Aspergillus fumigatus*; FLZ: Fluconazole.

4.3. SARs of the benzoxazole and indole derivatives

In vitro antifungal assay indicated that the benzoheterocyclic core of the benzofuran and benzothiazole NMT inhibitors was important for the antifungal activity. When the benzofuran scaffold was replaced by benzoxazole, the antifungal was retained. The antifungal activity of compound **8f** was slightly less than that of RO-09-4879. However, the antifungal activity of corresponding indole analogues was almost lost. For the benzoxazole derivatives, the substitutions on the phenyl ring were important for the antifungal activity. In general, a nitro group at position 3 of the phenyl group (compound **8h**) was unfavorable for the antifungal activity. When the phenyl group of compound **8c** was substituted with one or more fluorine atoms, the antifungal activity was increased. In most cases, the antifungal activity increased as the increase of the number of the fluorine atoms on the phenyl ring. For example, compound **8f** (2,3,4-trifluoro derivative) was the most active compound with broad antifungal spectrum. However, fluorine substitutions at both position 2 and 6 of the phenyl group led to the decrease of the antifungal activity. For example, the movement of the fluorine atom from position 4 (compound **8d**) to position 6 (compound **8e**) resulted in obvious decrease of the antifungal activity. The similar results can be found in compounds **8f** and **8g**. For the indole derivatives, the variation of the linker of C-2 side chain can slightly improve the antifungal activity. The thio-ether group was more favorable than ether or amide group.

4.4. Molecular docking studies

In an attempt to correlate the SARs with the binding mode of the synthesized compounds, two representative compounds, **8f** and **16a**, were docked into the crystal structure of NMT from *C. albicans* (CaNMT) [25]. Our previous modeling studies have proved that the affinity method within InsightII 2000 software package [28] was an effective method to investigate the binding mode of the NMT inhibitors [26]. The docking results of compounds **8f** and **16a** were shown in Fig. 2. The overall conformation of compound **8f** in the

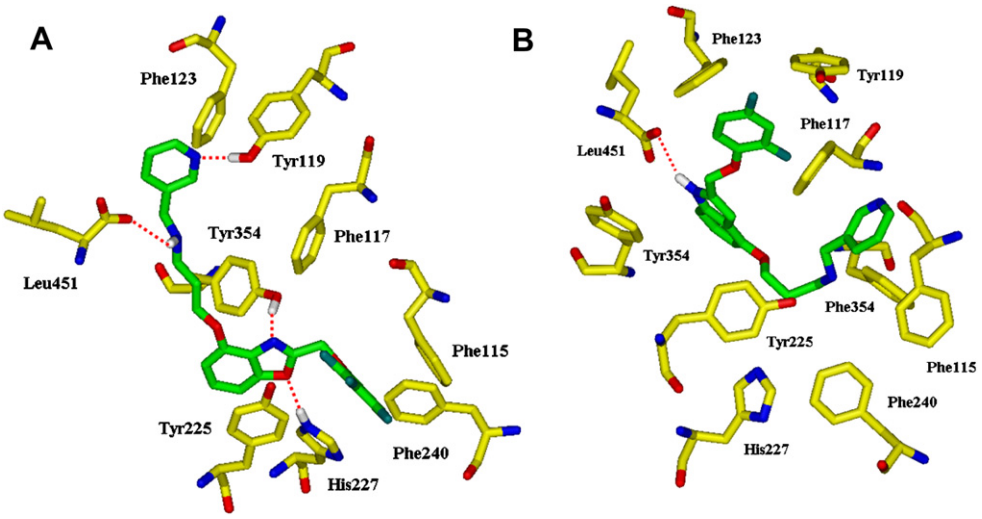


Fig. 2. Stereoview of the docking conformation of compound **8f** (**A**) and **16a** (**B**) in the active site of CaNMT. The residues interacting with compound **8f** and **16a** are shown and hydrogen bonds are displayed through red dotted lines (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

active site of CaNMT was very similar to that of the benzofuran inhibitors (Fig. 2A). The benzoxazole ring was located at the center of the active site, surrounded by some hydrophobic residues, such as Tyr225, Tyr354 and Leu394. The phenyl part of the benzoxazole ring could form π-π interaction with Tyr225. The substituted phenyl group of C-2 side chain formed hydrophobic interaction with Phe115 and Phe240. The terminal pyridinyl group C-4 side chain was surrounded by hydrophobic aromatic residues, such as Tyr119 and Phe123. Four hydrogen bonds were observed between compound **8f** and CaNMT. The second amine group of C-4 side chain made a hydrogen bond with C-terminal carboxylate of Leu451, which is an important functional residue in the catalytic cycle of CaNMT [25]. The oxygen and nitrogen atom of the benzoxazole ring formed two hydrogen bonds with His227 and Tyr354, respectively. Another hydrogen bond was formed between the pyridine nitrogen of C-4 side chain and Tyr119 hydroxyl group. Among them, hydrogen bonding interactions with His227, Tyr119 and Leu451 of compound **8f** are identical to those of benzofuran inhibitor RO-09-4879. In the crystal complex of CaNMT [25], the ether oxygen of C-2 side chain made a hydrogen bond with Asn392. However, this hydrogen bond was lost for compound **8f**, mainly because of the additional hydrogen bond between benzoxazole nitrogen and Tyr354. As compared with compound **8f**, RO-09-4879 has a C-3 methyl group, which can form additional *van der Waals* interaction with CaNMT. As a result, the interaction energy of RO-09-4879 with CaNMT was a little lower than compound **8f**.

It is interesting that compound **16a** shared a completely different conformation in the active site of CaNMT (Fig. 2B). Instead of the C-4 secondary amine, the indole nitrogen formed a weak hydrogen bond with Leu451. As a result, important hydrogen bonding and hydrophobic interactions observed for compound **8f** were lost. The docking result is supported by the weak antifungal activity of compound **16a**. Based on the above docking results, it is supposed that the hydrogen bonding interactions between the benzoheterocyclic core and CaNMT might be essential for orienting the inhibitor to the correct direction in the active site.

5. Conclusion

In summary, we have extended the SARs of the benzofuran and benzothiazole NMT inhibitors. The importance of benzoheterocyclic core on the antifungal activity was investigated by the isostreric design of the benzoxazole and indole derivatives. *In vitro* antifungal assay indicated that the replacement of benzofuran scaffold by benzoxazole led to a slight decrease of the antifungal activity. However, the antifungal activity of the indole derivatives was almost lost. They only showed moderate activity against dermatophytes. The binding mode of the synthesized compounds was explored by flexible molecular docking, which indicated the importance of the hydrogen bonding interaction between the benzoheterocyclic core and NMT in the orientation of the inhibitor to a proper position. The benzoxazole derivative **8f** showed good antifungal activity with broad spectrum, which was comparable or superior to fluconazole. Although compound **8f** is slightly less potent than RO-09-4879, it is more drug-like with lower molecular weight and hydrophobicity, which can serve as a good starting point for further studies of structural diversity of the NMT inhibitors.

6. Experimental protocols

6.1. Chemistry

Melting points (mp) were determined by microscope melting point apparatus with aromatic temperature control system (XT4A). Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker 500 spectrometer with TMS as an internal standard and d_6-DMSO as solvent. Chemical shifts (δ values) and coupling constants (J values) are given in ppm and Hz, respectively. ESI mass spectra were performed on an API-3000 LC–MS spectrometer. High-resolution mass spectroscopy measurements were performed on a Kratos-concep mass spectrometer under electron impact ionization (EI) conditions. Elemental analyses were performed with a MOD-1106 instrument and were consistent with theoretical values within ±0.4%. TLC analysis was carried out on silica gel plates GF254 (Qindao Haiyang Chemical, China). Silica gel column chromatography was performed with Silica gel 60 G (Qindao Haiyang Chemical, China). Commercial solvents were used without any pretreatment.

6.1.1. Chemical synthesis of 2-aminobenzene-1,3-diol (**2**)

10% Pd–C (2.0 g) was added to a solution of 2-nitrobenzene-1,3-diol (31.0 g, 0.2 mol) in EtOH (500 mL), then the reaction mixture was put into the high-pressure reaction vessel under H_2

atmosphere at 4 atm and stirred at room temperature for 10 h. The mixture was filtered though celite and the resulting solution was dried over anhydrous $MgSO_4$, concentrated under reduced pressure to give **2** as brown solid (49.5 g, 99% yield). ^{1}H NMR δ (ppm): 8.82 (s, 2H, OH), 6.21–6.27 (m, 3H, Ar-H), 3.82 (s, 2H, NH_2). MS (ESI) *m*/*z*: 126 (M + 1).

*6.1.2. 2-(Chloromethyl)benzoxazol-4-ol (**3**)*

Ethyl 2-chloroacetimidate hydrochloride (31.6 g, 0.26 mol) was added to a solution of compound **2** (25.0 g, 0.2 mol) in CH_2Cl_2 (500 mL) at 0 °C and the mixture was stirred for 2 h. Then, the reaction mixture was heated to room temperature and stirred for 24 h. After the reaction was completed, the resulting solution was diluted with CH_2Cl_2 (500 mL) and washed with water (500 mL × 3). The organic layer was separated, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/EtOAc = 10:1, v/v) to give **3** as white crystalline solid (16.0 g, 43.6% yield). mp: 148–149 °C. ^{1}H NMR δ (ppm): 10.42 (s, 1H, OH), 7.23 (t, 1H, *J* = 8.0 Hz, Ar-C7H), 7.15 (d, 1H, *J* = 8.0 Hz, Ar-C6H), 6.77 (d, 1H, *J* = 8.0 Hz, Ar-C5H), 5.03 (s, 2H, CH_2Cl). MS (ESI) *m*/*z*: 184 (M + 1).

*6.1.3. Tert-butyl 2-(chloromethyl)benzoxazol-4-yl carbonate (**4**)*

Boc_2O (2.5 g, 11.48 mmol) and dimethylaminopyridine (DMAP, 2.5 mg, 0.02 mmol) was added to a solution of compound **3** (1.835 g, 10 mmol) in CH_2Cl_2 (50 mL). The reaction mixture was stirred at room temperature for 30 min. The organic layer was washed with 1N HCl (80 mL), H_2O (80 mL), saturated $NaHCO_3$ (80 mL) and saturated brine (80 mL), then dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give crude product as yellow liquid which was purified by silica gel column chromatography (hexane/EtOAc = 20:1, v/v) to give **4** as colorless oil (2.6 g, 91.7% yield). ^{1}H NMR δ (ppm): 7.72 (d, 1H, *J* = 8.0 Hz, Ar-C7H), 7.49 (t, 1H, *J* = 8.0 Hz, Ar-C6H), 7.29 (d, 1H, *J* = 7.5 Hz, Ar-C5H), 5.09 (s, 2H, CH_2Cl), 1.51 (s, 9H, *tert*-butyl). MS (ESI) *m*/*z*: 284 (M + 1).

*6.1.4. General procedure for the synthesis of compounds **5a**–**h***

Substituted phenol (1.03 mmol) was added to a suspension of NaH (60%, 400 mg, 10 mmol) in dried DMF (20 mL) and was stirred for 30 min. Compound **4** (2.6 g, 9.2 mmol) was added to the reaction mixture and stirred for 3 h. The resulting mixture was diluted with EtOAc (100 mL) and washed with H_2O (100 mL × 2). The organic layer was separated, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/EtOAc = 20:1, v/v) to give compounds **5a**–**h**.

*6.1.5. General procedure for the synthesis of compounds **6a**–**h***

Trifluoroacetic acid (15 mL) was added to a solution of compound **5a**–**h** (5.85 mmol) in CH_2Cl_2 (15 mL). The reaction mixture was stirred at room temperature for 20 min. Trifluoroacetic acid was evaporated under reduced pressure. The residue was diluted with CH_2Cl_2 (100 mL), washed with H_2O (100 mL), saturated $NaHCO_3$ (100 mL), and saturated brine (100 mL). Then the organic layer was separated, dried over anhydrous Na_2SO_4, concentrated under reduced pressure to give crude product which was purified by silica gel column chromatography (hexane/EtOAc = 4:1, v/v) to give compounds **6a**–**h**.

*6.1.6. General procedure for the synthesis of compounds **7a**–**h***

Compounds **6a**–**h** (1.16 mmol), anhydrous K_2CO_3 (690 mg, 4 mmol) and 1,3-dichloropropane were suspended in dried DMF (15 mL). The reaction mixture was stirred at room temperature for 5 h, then was diluted with EtOAc (50 mL) and washed with H_2O (50 mL). The organic layer was separated, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/EtOAc = 50:1, v/v) to give compounds **7a**–**h**.

*6.1.7. General procedure for the synthesis of compound **8a**–**h***

3-(Aminomethyl)pyridine (0.92 mmol) was added to a solution of compound **7a**–**h** (0.92 mmol) in dried DMF (15 mL). The reaction mixture was stirred at room temperature for 24 h, then was quenched by the addition of H_2O (100 mL) and extracted with EtOAc (100 mL). The organic layer was separated, dried over anhydrous Na_2SO_4, concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH/Et_3N = 50:1:0.2, v/v) to give compounds **8a**–**h**

*6.1.8. 3-{2-[(2-Fluorophenoxy)methyl]benzoxazol-4-yloxy}-N-(pyridin-3-ylmethyl) propan-1-amine (**8a**)*

White solid (280 mg, 74.7% yield). mp: 47–48 °C. ^{1}H NMR δ (ppm): 8.62 (s, 1H, pyridinyl-C2H), 8.51 (d, 1H, *J* = 3.5 Hz, pyridinyl-C6H), 7.86 (d, 1H, *J* = 7.5 Hz, pyridinyl-C4H), 6.95–7.40 (m, 8H, Ar-H), 5.50 (s, 2H, CH_2OPh), 4.31 (t, 2H, *J* = 6.0 Hz, $O\underline{CH_2}CH_2CH_2NH$), 3.98 (s, 2H, $NHCH_2$pyridinyl), 2.92 (t, 2H, *J* = 6.5 Hz, $OCH_2CH_2\underline{CH_2}NH$), 2.04–2.10 (m, 2H, $OCH_2\underline{CH_2}CH_2NH$). ^{13}C NMR δ (ppm): 159.53 (C), 153.96 (C), 152.57 (C), 150.96 (C), 149.70 (CH), 148.50 (CH), 145.84 (C), 136.01 (CH), 135.01 (C), 130.38 (C), 126.39 (CH), 124.40 (CH), 123.39 (CH), 122.78 (CH), 116.46 (CH), 107.66 (CH), 103.74 (CH), 67.66 (CH_2), 64.30 (CH_2), 51.10 (CH_2), 46.25 (CH_2), 29.27 (CH_2). HRMS (*m*/*z*): calcd. for $C_{23}H_{22}FN_3O_3$: 407.1645; found: 407.1634. Anal. calcd. for $C_{23}H_{22}FN_3O_3$: C, 67.80; H, 5.44; N, 10.31. Found: C, 67.66; H, 5.45; N, 10.33.

*6.1.9. 3-{2-[(4-Fluorophenoxy)methyl]benzoxazol-4-yloxy}-N-(pyridin-3-ylmethyl) propan-1-amine (**8b**)*

Pale yellow solid (264 mg, 70.4% yield). mp: 48–49 °C. ^{1}H NMR δ (ppm): 8.53 (s, 1H, pyridinyl-C2H), 8.42 (d, 1H, *J* = 5.0 Hz, pyridinyl-C6H), 7.74 (d, 1H, *J* = 7.5 Hz, pyridinyl-C4H), 6.94–7.36 (m, 8H, Ar-H), 5.40 (s, 2H, CH_2OPh), 4.29 (t, 2H, *J* = 6.0 Hz, $O\underline{CH_2}CH_2CH_2NH$), 3.73 (s, 2H, $NHCH_2$Pyridinyl), 2.69 (t, 2H, *J* = 6.5 Hz, $OCH_2CH_2\underline{CH_2}NH$), 1.95 (p, 2H, *J* = 6.5 Hz, $OCH_2\underline{CH_2}CH_2NH$). ^{13}C NMR δ (ppm): 159.76 (C), 158.82 (C), 154.01 (C), 152.51 (C), 151.00 (C), 149.62 (CH), 148.37 (CH), 135.87 (CH), 135.41 (C), 130.37 (C), 126.32 (CH), 123.35 (CH), 116.14 (CH), 107.62 (CH), 103.60 (CH), 67.66 (CH_2), 63.51 (CH_2), 51.18 (CH_2), 46.27 (CH_2), 29.43 (CH_2). HRMS (*m*/*z*): calcd. for $C_{23}H_{22}FN_3O_3$: 407.1645; found: 407.1638. Anal. calcd. for $C_{23}H_{22}FN_3O_3$: C, 67.80; H, 5.44; N, 10.31. Found: C, 67.87; H, 5.46; N, 10.27.

*6.1.10. 3-[2-(Phenoxymethyl)benzoxazol-4-yloxy]-N-(pyridin-3-ylmethyl)propan-1-amine (**8c**)*

White solid (245 mg, 68.5% yield). mp: 48–49 °C. ^{1}H NMR δ (ppm): 8.54 (d, 1H, *J* = 1.5 Hz, pyridinyl-C2H), 8.44 (dd, 1H, *J*1 = 5.0 Hz, *J*2 = 1.5 Hz, pyridinyl-C6H), 7.76 (d, 1H, *J* = 7.5 Hz, pyridinyl-C4H), 6.94–7.34 (m, 9H, Ar-H), 5.42 (s, 2H, CH_2OPh), 4.29 (t, 2H, *J* = 6.5 Hz, $O\underline{CH_2}CH_2CH_2NH$), 3.78 (s, 2H, $NHCH_2$Pyridinyl), 2.73 (t, 2H, *J* = 7.0 Hz, $OCH_2CH_2\underline{CH_2}NH$), 1.97 (p, 2H, *J* = 6.5 Hz, $OCH_2\underline{CH_2}CH_2NH$). ^{13}C NMR δ (ppm): 160.04 (C), 157.93 (C), 152.54 (C), 150.94 (C), 149.70 (CH), 148.48 (CH), 135.98 (CH), 135.01 (C), 130.45 (C), 129.59 (CH), 126.23 (CH), 123.37 (CH), 121.86 (CH), 114.89 (CH), 107.76 (CH), 103.69 (CH), 67.70 (CH_2), 62.78 (CH_2), 51.08 (CH_2), 46.24 (CH_2), 29.30 (CH_2). HRMS (*m*/*z*): calcd. for $C_{23}H_{23}N_3O_3$: 389.1739; found: 389.1750. Anal. calcd. for $C_{23}H_{23}N_3O_3$: C, 70.93; H, 5.95; N, 10.79. Found: C, 71.07; H, 5.92; N, 10.76.

*6.1.11. 3-{2-[(2,4-Difluorophenoxy)methyl]benzoxazol-4-yloxy}-N-(pyridin-3-yl methyl)propan-1-amine (**8d**)*

White solid (302 mg, 77.2% yield). mp: 48–49 °C. ^{1}H NMR δ (ppm): 8.52 (s, 1H, pyridinyl-C2H), 8.42 (dd, 1H, *J*1 = 3.5 Hz,

*J*2 = 1.0 Hz, pyridinyl-C6H), 7.73 (d, 1H, *J* = 7.5 Hz, pyridinyl-C4H), 6.95–7.39 (m, 7H, Ar-H), 5.48 (s, 2H, CH_2OPh), 4.29 (t, 2H, *J* = 6.5 Hz, $O\underline{CH_2}CH_2CH_2NH$), 3.72 (s, 2H, $NHCH_2Pyridinyl$), 2.68 (t, 2H, *J* = 6.5 Hz, $OCH_2CH_2\underline{CH_2}NH$), 1.94 (p, 2H, *J* = 6.5 Hz, $OCH_2\underline{CH_2}CH_2NH$). HRMS (*m*/*z*): calcd. for $C_{23}H_{21}F_2N_3O_3$: 425.1551; found: 425.1537. Anal. calcd. for $C_{23}H_{21}F_2N_3O_3$: C, 64.93; H, 4.98; N, 9.88. Found: C, 64.73; H, 4.99; N, 9.86.

6.1.12. 3-{2-[(2,6-Difluorophenoxy)methyl]benzoxazol-4-yloxy}-N-(pyridin-3-yl methyl)propan-1-amine (**8e**)

Pale yellow solid (221 mg, 56.4% yield). mp: 48–49 °C. ^{1}H NMR δ (ppm): 8.53 (d, 1H, *J* = 1.5 Hz, pyridinyl-C2H), 8.43 (dd, 1H, *J*1 = 5.0 Hz, *J*2 = 1.5 Hz, pyridinyl-C6H), 7.74 (d, 1H, *J* = 8.0 Hz, pyridinyl-C4H), 6.94–7.38 (m, 7H, Ar-H), 5.40 (s, 2H, CH_2OPh), 4.28 (t, 2H, *J* = 6.5 Hz, $O\underline{CH_2}CH_2CH_2NH$), 3.73 (s, 2H, $NHCH_2Pyridinyl$), 2.51 (t, 2H, *J* = 7.0 Hz, $OCH_2CH_2\underline{CH_2}NH$), 1.94 (m, 2H, $OCH_2\underline{CH_2}CH_2NH$). ^{13}C NMR δ (ppm): 159.21 (C), 156.89 (C), 154.91 (C), 152.54 (C), 150.83 (C), 149.65 (CH), 148.45 (CH), 136.27 (CH), 133.90 (C), 130.24 (C), 126.35 (CH), 123.90 (CH), 123.34 (CH), 112.16 (CH), 107.66 (CH), 103.58 (CH), 67.52 (CH_2), 50.53 (CH_2), 45.83 (CH_2), 28.66 (CH_2). HRMS (*m*/*z*): calcd. for $C_{23}H_{21}F_2N_3O_3$: 425.1551; found: 425.1534. Anal. calcd. for $C_{23}H_{21}F_2N_3O_3$: C, 64.93; H, 4.98; N, 9.88. Found: C, 64.80; H, 4.97; N, 9.90.

6.1.13. N-(Pyridin-3-ylmethyl)-3-{2-[(2,3,4-trifluorophenoxy)-methyl]benzoxazol-4-yloxy}propan-1-amine (**8f**)

White solid (310 mg, 76.1% yield). mp: 49–51 °C. ^{1}H NMR δ (ppm): 8.52 (s, 1H, pyridinyl-C2H), 8.41 (d, 1H, *J* = 3.5 Hz, pyridinyl-C6H), 7.73 (d, 1H, *J* = 7.5 Hz, pyridinyl-C4H), 6.95–7.37 (m, 6H, Ar-H), 5.55 (s, 2H, CH_2OPh), 4.29 (t, 2H, *J* = 6.0 Hz, $O\underline{CH_2}CH_2CH_2NH$), 3.73 (s, 2H, $NHCH_2Pyridinyl$), 2.68 (t, 2H, *J* = 6.5 Hz, $OCH_2CH_2\underline{CH_2}NH$), 1.94 (p, 2H, *J* = 6.5 Hz, $OCH_2\underline{CH_2}CH_2NH$). ^{13}C NMR δ (ppm): 158.84 (C), 152.55 (C), 151.01 (C), 149.59 (CH), 148.34 (CH), 147.80 (C), 145.95 (C), 143.60 (C), 142.01 (C), 135.82 (CH), 135.55 (C), 130.28 (C), 126.60 (CH), 123.33 (CH), 110.51 (CH), 110.18 (CH), 107.62 (CH), 103.61 (CH), 67.68 (CH_2), 64.98 (CH_2), 51.24 (CH_2), 46.30 (CH_2), 29.47 (CH_2). HRMS (*m*/*z*): calcd. for $C_{23}H_{20}F_3N_3O_3$: 443.1457; found: 443.1440. Anal. calcd. for $C_{23}H_{20}F_3N_3O_3$: C, 62.30; H, 4.55; N, 9.48. Found: C, 62.42; H, 4.53; N, 9.45.

6.1.14. N-(Pyridin-3-ylmethyl)-3-{2-[(2,4,6-trifluorophenoxy)-methyl]benzoxazol-4-yloxy}propan-1-amine (**8g**)

Pale yellow solid (256 mg, 62.8% yield). mp: 48–49 °C. ^{1}H NMR δ (ppm): 8.51 (d, 1H, *J* = 1.5 Hz, pyridinyl-C2H), 8.42 (dd, 1H, *J*1 = 5.0 Hz, *J*2 = 1.5 Hz, pyridinyl-C6H), 7.73 (d, 1H, *J* = 8.0 Hz, pyridinyl-C4H), 6.94–7.38 (m, 6H, Ar-H), 5.35 (s, 2H, CH_2OPh), 4.28 (t, 2H, *J* = 6.5 Hz, $O\underline{CH_2}CH_2CH_2NH$), 3.72 (s, 2H, $NHCH_2Pyridinyl$), 2.66 (t, 2H, *J* = 6.5 Hz, $OCH_2CH_2\underline{CH_2}NH$), 1.93 (p, 2H, *J* = 6.5 Hz, $OCH_2\underline{CH_2}CH_2NH$). ^{13}C NMR δ (ppm): 158.82 (C), 156.79 (C), 154.85 (C), 152.51 (C), 150.94 (C), 149.41 (CH), 148.13 (CH), 135.70 (CH), 135.17 (C), 131.32 (C), 130.20 (C), 126.35 (CH), 123.14 (CH), 112.26 (CH), 107.57 (CH), 100.68 (CH), 67.65 (CH_2), 50.89 (CH_2), 45.99 (CH_2), 29.19 (CH_2). HRMS (*m*/*z*): calcd. for $C_{23}H_{20}F_3N_3O_3$: 443.1457; found: 443.1445. Anal. calcd. for $C_{23}H_{20}F_3N_3O_3$: C, 62.30; H, 4.55; N, 9.48. Found: C, 62.23; H, 4.56; N, 9.49.

6.1.15. 3-{2-[(3-Nitrophenoxy)methyl]benzoxazol-4-yloxy}-N-(pyridin-3-ylmethyl) propan-1-amine (**8h**)

Pale yellow solid (322 mg, 80.7% yield). mp: 48–49 °C. ^{1}H NMR δ (ppm): 8.53 (s, 1H, pyridinyl-C2H), 8.42 (d, 1H, *J*1 = 3.5 Hz, *J*2 = 1.5 Hz, pyridinyl-C6H), 7.94 (d, 1H, *J* = 2.0 Hz, pyridinyl-C4H), 6.95–7.88 (m, 8H, Ar-H), 5.61 (s, 2H, CH_2OPh), 4.30 (t, 2H, *J* = 6.5 Hz, $O\underline{CH_2}CH_2CH_2NH$), 3.73 (s, 2H, $NHCH_2Pyridinyl$), 2.69 (t, 2H, *J* = 7.0 Hz, $OCH_2CH_2\underline{CH_2}NH$), 1.95 (p, 2H, *J* = 6.5 Hz, $OCH_2\underline{CH_2}CH_2NH$). HRMS (*m*/*z*): calcd. for $C_{23}H_{22}N_4O_5$: 434.1590; found: 434.1603. Anal. calcd. for $C_{23}H_{22}N_4O_5$: C, 63.59; H, 5.10; N, 12.90. Found: C, 63.46; H, 5.11; N, 12.91.

6.1.16. 2-(Benzyloxy)benzaldehyde (**10**)

Salicylaldehyde (5.25 mL, 49.1 mmol) and benzyl bromide (6.45 mL, 54.0 mmol) were added to a suspension of K_2CO_3 (29.9 g, 216 mmol) in MeOH (300 mL) and $CHCl_3$ (600 mL) under N_2 atmosphere. Then, the reaction mixture was refluxed for 24 h and filtrated. The filtrate was concentrated under reduced pressure. 1N HCl (200 mL) was added to the residue. Then the mixture was extracted with CH_2Cl_2 (300 mL × 2). The combined organic layer was washed with saturated brine (300 mL × 2), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was recrystallized from absolute EtOH to give **10** as white needles (9.1 g, 87.5% yield). mp: 49–51 °C. MS (ESI) *m*/*z*: 213 (M + 1).

6.1.17. Ethyl 4-(benzyloxy)-1H-indole-2-carboxylate (**11**)

Compound **10** (15.8 g, 74 mmol) and a solution of α-ethyl 2-azidoacetate (50 mL) in EtOH (20 mL) was added to a suspension of Na (7 g) in absolute EtOH (200 mL) at −10 °C. After the addition over a period of 2 h, the mixture was stirred for additional 8 h, cooled to room temperature, and then quenched by the addition of H_2O (1500 mL). The precipitate was filtrated, washed with H_2O (500 mL) and dried under vacuum. The crude product was dissolved in toluene (250 mL). The solution was heated to reflux at 125 °C for 3 h, then cooled to room temperature and filtrated. The filtrate was concentrated under reduced pressure. The residue was recrystallized from $CHCl_3$/hexane to give **11** as white needles (6.7 g, 30.6% yield). mp: 169–172 °C. ^{1}H NMR δ (ppm): 11.90 (s, 1H, indole-NH), 6.63–7.52 (m, 9H, Ar-H), 5.23 (s, 2H, OCH_2Ph), 4.32 (q, 2H, *J* = 7.0 Hz, $COO\underline{CH_2}CH_3$), 1.33 (t, 3H, *J* = 7.0 Hz, $COOCH_2\underline{CH_3}$). MS (ESI) *m*/*z*: 296 (M + 1).

6.1.18. Ethyl 4-hydroxy-1H-indole-2-carboxylate (**12**)

Compound **11** (3.0 g, 10.17 mol) and 10% Pd–C (300 mg) were suspended in EtOAc (200 mL) and EtOH (300 mL) under H_2 atmosphere (4 atm) and stirred at 50 °C for 6 h. The reaction mixture was filtered off using celite. The filtrate was concentrated under reduced pressure to give crude solid. The crude product was recrystallized from absolute EtOH (10 mL) to give **12** as grey solid (2.0 g, 96% yield). mp: 160–162 °C. ^{1}H NMR δ (ppm): 11.70 (s, 1H, indole-NH), 9.67 (s, 1H, OH), 6.38–7.20 (m, 4H, Ar-H), 4.32 (q, 2H, *J* = 7.0 Hz, $COO\underline{CH_2}CH_3$), 1.33 (t, 3H, *J* = 7.0 Hz, $COOCH_2\underline{CH_3}$). MS (ESI) *m*/*z*: 206 (M + 1).

6.1.19. Ethyl 4-(3-bromopropoxy)-1H-indole-2-carboxylate (**13**)

Compound **12** (2.05 g, 10 mmol), anhydrous K_2CO_3 (6.9 g, 50 mmol) and 1,3-dibromopropane (5 mL, 50 mmol) were suspended in DMF (25 mL). The reaction mixture was stirred at room temperature for 3 h, then was diluted with EtOAc (300 mL) and washed with H_2O (300 mL × 2). The combined organic layer was dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/EtOAc = 50:1, v/v) to give **13** as white solid (1.5 g, 42.8% yield). ^{1}H NMR δ (ppm): 11.90 (s, 1H, indole-NH), 6.55–7.17 (m, 4H, Ar-H), 4.33 (q, 2H, *J* = 7.0 Hz, $COO\underline{CH_2}CH_3$), 4.20 (t, 2H, *J* = 6.0 Hz, $O\underline{CH_2}CH_2CH_2Br$), 3.76 (t, 2H, *J* = 6.5 Hz, $OCH_2CH_2\underline{CH_2}Br$), 2.33 (p, 2H, *J* = 6.0 Hz, $OCH_2\underline{CH_2}CH_2Br$), 1.34 (t, 3H, *J* = 7.0 Hz, $COOCH_2\underline{CH_3}$). MS (ESI) *m*/*z*: 327 (M + 1).

6.1.20. Ethyl 4-[3-(pyridin-3-ylmethylamino)propoxy]-1H-indole-2-carboxylate (**14**)

3-(Aminomethyl)pyridine (3 mL, 30 mmol) was added to a solution of compound **13** (1.61 g, 4.94 mmol) in absolute EtOH (30 mL). The reaction mixture was stirred at 70 °C for 24 h, then was cooled to room temperature and concentrated under reduced

pressure. The residue was diluted with EtOAc (50 mL) and H_2O (50 mL). The organic layer was separated, washed with saturated NH_4Cl (100 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford yellow oil, which was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 50:1 → 20:1, v/v) to give **14** as grey solid (1.3 g, 74.6% yield). ^{1}H NMR δ (ppm): 11.88 (s, 1H, indole-NH), 8.56 (s, 1H, pyridinyl-C2H), 8.45 (m, 1H, pyridinyl-C6H), 7.77 (d, 1H, J = 7.5 Hz, pyridinyl-C4H), 7.33 (dd, 1H, $J1$ = 7.5 Hz, $J2$ = 2.5 Hz, pyridinyl-C5H), 6.52–7.17 (m, 4H, Ar-H), 4.33 (q, 2H, J = 7.0 Hz, COO$\underline{CH_2}CH_3$), 4.16 (t, 2H, J = 6 Hz, O$\underline{CH_2}CH_2CH_2NH$), 3.80 (s, 2H, NHC$H_2$Pyridinyl), 2.78 (t, 2H, J = 7.0 Hz, $OCH_2CH_2\underline{CH_2}NH$), 1.99 (p, 2H, J = 6.5 Hz, $OCH_2\underline{CH_2}CH_2NH$), 1.35 (t, 3H, J = 7.0 Hz, $COOCH_2\underline{CH_3}$). MS (ESI) m/z: 354 (M + 1).

6.1.21. {4-[3-(Pyridin-3-ylmethylamino)propoxy]-1H-indol-2-yl}-methanol (**15**)

$LiAlH_4$ (1.5 g, 39.5 mmol) was added in small portions to a solution of compound **14** (2.6 g, 7.37 mmol) in THF (30 mL) at 0 °C. The reaction mixture was stirred for 1 h. Then H_2O (1 mL) was added to get rid of excess $LiAlH_4$. The mixture was stirred for 1 h, dried with anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 50:1, v/v) to give **15** as yellow oil (1.4 g, 87.3%). ^{1}H NMR δ (ppm): 10.95 (s, 1H, indole-NH), 8.53 (s, 1H, pyridinyl-C2H), 8.43 (d, 1H, J = 4.5 Hz, pyridinyl-C6H), 7.74 (d, 1H, J = 8.0 Hz, pyridinyl-C4H), 7.31 (dd, 1H, $J1$ = 7.5 Hz, $J2$ = 5.0 Hz, pyridinyl-C5H), 6.22–6.91 (m, 4H, Ar-H), 5.16 (s, 1H, OH), 4.56 (s, 2H, CH_2OH), 4.11 (t, 2H, J = 6.0 Hz, O$\underline{CH_2}CH_2CH_2NH$), 3.73 (s, 2H, NHC$H_2$Pyridinyl), 2.69 (t, 2H, J = 7.0 Hz, $OCH_2CH_2\underline{CH_2}NH$), 1.92 (m, 2H, $OCH_2\underline{CH_2}CH_2NH$). MS (ESI) m/z: 312 (M + 1).

6.1.22. General procedure for the synthesis of 3-{2-[(substituted phenoxy)methyl]-1H-indol-4-yloxy}-N-(pyridin-3-yl methyl)-propan-1-amine (**16a–b**)

1,1-(Azodicarbonyl)dipiperidine (0.9 mmol) was added to a solution of compound **15** (0.6 mmol), tri-*tert*-butylphosphine (TBP, 0.9 mmol) and substituted phenol (0.6 mmol) in dried CH_2Cl_2 (2 mL) which was cooled to −20 °C under N_2 atmosphere and stirred for 15min. The reaction mixture was stirred at room temperature for 24 h, then was poured into saturated $NaHCO_3$ (40 mL) and extracted with EtoAc (40 mL). The combined organic layer was washed with saturated brine (40 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 50:1 → 20:1, v/v) to give compounds **16a–b**.

6.1.23. 3-{2-[(2,4-Difluorophenoxy)methyl]-1H-indol-4-yloxy}-N-(pyridine-3-ylmethyl) propan-1-amine (**16a**)

Yellow solid: (7.0 mg, 2.8% yield). ^{1}H NMR δ (ppm): 11.31 (s, 1H, indole-NH), 8.53 (s, 1H, pyridinyl-C2H), 8.42 (d, 1H, J = 3.5 Hz, pyridinyl-C6H), 7.74 (d, 1H, J = 7.5 Hz, pyridinyl-C4H), 6.47–7.38 (m, 8H, Ar-H), 5.22 (s, 2H, CH_2OPh), 4.13 (t, 2H, J = 6.0 Hz, O$\underline{CH_2}CH_2CH_2NH$), 3.73 (s, 2H, NHC$H_2$Pyridinyl), 2.69 (t, 2H, J = 6.0 Hz, $OCH_2CH_2\underline{CH_2}NH$), 1.93 (p, 2H, J = 6.5 Hz, $OCH_2\underline{CH_2}CH_2NH$). HRMS ($m/z$): calcd. for $C_{24}H_{23}F_2N_3O_2$: 423.1758; found: 423.1774. Anal. calcd. for $C_{24}H_{23}F_2N_3O_2$: C, 68.07; H, 5.47; N, 9.92. Found: C, 68.20; H, 5.48; N, 9.89.

6.1.24. 3-{2-[(4-Fluorophenoxy)methyl]-1H-indol-4-yloxy}-N-(pyridine-3-ylmethyl) propan-1-amine (**16b**)

Pale yellow solid (8.0 mg, 3.0% yield). ^{1}H NMR δ (ppm): 11.29 (s, 1H, indole-NH), 8.53 (s, 1H, pyridinyl-C2H), 8.42 (d, 1H, J = 3.5 Hz, pyridinyl-C6H), 7.74 (d, 1H, J = 7.5 Hz, pyridinyl-C4H), 7.31 (dd, 1H, $J1$ = 8.0 Hz, $J2$ = 5.0 Hz, pyridinyl-C5H), 6.44–7.15 (m, 8H, Ar-H), 5.15 (s, 2H, CH_2OPh), 4.13 (t, 2H, J = 6.5 Hz, O$\underline{CH_2}CH_2CH_2NH$), 3.74 (s, 2H, NHC$H_2$Pyridinyl), 2.70 (t, 2H, J = 7.0 Hz, $OCH_2CH_2\underline{CH_2}NH$), 1.93 (p, 2H, J = 6.5 Hz, $OCH_2\underline{CH_2}CH_2NH$). HRMS ($m/z$): calcd. for $C_{24}H_{24}FN_3O_2$: 405.1853; found: 405.1840. Anal. calcd. for $C_{24}H_{24}FN_3O_2$: C, 71.09; H, 5.97; N, 10.36. Found: C, 70.88; H, 5.98; N, 10.38.

6.1.25. General procedure for the synthesis of compounds **17a–b**

Substituted thiol (0.2 mmol) was added to a stirring solution of compound **15** (0.2 mmol) and trifluoroacetic acid (0.2 mL) in dried CH_2Cl_2 (5 mL). The reaction mixture was stirred at room temperature for 2 h, then was diluted with CH_2Cl_2 (30 mL), washed with 0.1 mol/L NaOH (30 mL), H_2O (30 mL) and saturated brine (30 mL). The organic layer was separated, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 50:1, v/v) to give compounds **17a–b**.

6.1.26. [3-(2-(Phenylthiomethyl)-1H-indol-4-yloxy)]-N-(pyridine-3-ylmethyl)propan-1-amine (**17a**)

Colorless oil (22 mg, 27.5%). ^{1}H NMR δ (ppm): 11.34 (s, 1H, indole-NH), 8.52 (s, 1H, pyridinyl-C2H), 8.42 (d, 1H, J = 3.5 Hz, pyridinyl-C6H), 7.74 (d, 1H, J = 7.5 Hz, pyridinyl-C4H), 6.50–7.39 (m, 10H, Ar-H), 4.68 (d, 1H, J = 13.5 Hz, CH_2S), 4.45 (d, 1H, J = 13.5 Hz, CH_2S), 4.12–4.15 (m, 2H, O$\underline{CH_2}CH_2CH_2NH$), 3.73 (s, 2H, NHC$H_2$Pyridinyl), 2.69–2.73 (m, 2H, $OCH_2CH_2\underline{CH_2}NH$), 1.93 (t, 2H, J = 6.5 Hz, $OCH_2\underline{CH_2}CH_2NH$). HRMS ($m/z$): calcd. for $C_{24}H_{25}N_3OS$: 403.1718; found: 403.1705. Anal. calcd. for $C_{24}H_{25}N_3OS$: C, 71.43; H, 6.24; N, 10.41. Found: C, 71.57; H, 6.22; N, 10.39.

6.1.27. 3-{2-[(2,6-Dichlorophenylthio)methyl]-1H-indol-4-yloxy}-N-(pyridine-3-yl methyl)propan-1-amine (**17b**)

Colorless oil (18 mg, 19% yield). ^{1}H NMR δ (ppm): 11.22 (s, 1H, indole-NH), 8.52 (s, 1H, pyridinyl-C2H), 8.42 (d, 1H, J = 4.5 Hz, pyridinyl-C6H), 7.73 (d, 1H, J = 7.5 Hz, pyridinyl-C4H), 7.54 (d, 1H, J = 8.0 Hz, pyridinyl-C5H), 6.41–7.40 (m, 7H, Ar-H), 4.54 (d, 1H, J = 13.5 Hz, CH_2S), 4.31 (d, 1H, J = 13.5 Hz, CH_2S), 4.10–4.14 (m, 2H, O$\underline{CH_2}CH_2CH_2NH$), 3.72 (s, 2H, NHC$H_2$Pyridinyl), 2.69 (d, 2H, J = 2.5 Hz, $OCH_2CH_2\underline{CH_2}NH$), 1.93 (p, 2H, J = 6.0 Hz, $OCH_2\underline{CH_2}CH_2NH$). HRMS ($m/z$): calcd. for $C_{24}H_{23}Cl_2N_3OS$: 471.0939; found: 471.0957. Anal. calcd. for $C_{24}H_{23}Cl_2N_3OS$: C, 61.02; H, 4.91; N, 8.89. Found: C, 60.89; H, 4.92; N, 8.90.

6.1.28. 4-(3-Bromopropoxy)-1H-indole-2-carboxylic acid (**18**)

$LiOH\cdot H_2O$ (560 mg, 13.3 mmol), H_2O (35 mL) and MeOH (12 mL) were added to a solution of compound **13** (1.63 g, 5.0 mmol) in THF (45 mL) at 0 °C. The reaction mixture was stirred at room temperature for 6 h and was added 1N HCl (11 mL). After removing the solvent under reduced pressure, the residue was extracted with EtOAc (120 mL). The EtOAc solution was washed with saturated brine (120 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford crude solid, which was washed with haxene/EtOAc (4:1, v/v) and dried under vacuum to give **18** as grey solid (1.32 g, 88.6% yield). ^{1}H NMR δ (ppm): 12.79 (s, 1H, COOH), 11.75 (s, 1H, indole-NH), 6.54–7.14 (m, 4H, Ar-H), 4.20 (t, 2H, J = 6.0 Hz, O$\underline{CH_2}CH_2CH_2Br$), 3.75 (t, 2H, J = 6.5 Hz, $OCH_2CH_2\underline{CH_2}Br$), 2.33 (p, 2H, J = 6.0 Hz, $OCH_2\underline{CH_2}CH_2Br$). MS (ESI) m/z: 299 (M + 1).

6.1.29. General procedure for the synthesis of compounds **19a–e**

Oxalyl chloride (0.5 mL) and a drop of triethylamine were added to a stirring solution of **18** (0.67 mmol) in dried CH_2Cl_2 (5 mL). The reaction mixture was stirred at room temperature for 12 h, and then was added substituted aniline (0.67 mol). The mixture was stirred at room temperature for 3 h and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2) to give compounds **19a–e**.

*6.1.30. General procedure for the synthesis of compounds **20a–e***

3-(Aminomethyl)pyridine (0.28 mol) was added to a solution of compound **19a–e** (0.28 mmol) in absolute EtOH (5 mL). The reaction mixture was stirred at 80 °C for 12 h and evaporated under reduced pressure. The residue was dissolved in EtOAc (50 mL) and H_2O (50 mL). The organic layer was separated, washed with saturated NH_4Cl (50 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 20:1, v/v) to give compounds **20a–e**.

*6.1.31. N-(3-Fluorophenyl)-4-[3-(pyridin-3-ylmethylamino)-propoxy]-1H-indole-2-carboxamide (**20a**)*

Yellow solid (30 mg, 25% yield). ^{1}H NMR δ (ppm): 11.77 (s, 1H, indole-NH), 10.35 (s, 1H, CONH), 8.55 (s, 1H, pyridinyl-C2H), 8.42 (d, 1H, J = 4.5 Hz, pyridinyl-C6H), 7.81 (d, 1H, J = 7.0 Hz, pyridinyl-C4H), 6.54–7.75 (m, 9H, Ar-H), 4.18 (t, 2H, J = 6.0 Hz, O$\underline{CH_2}CH_2CH_2$NH), 3.75 (s, 2H, NHC$\underline{H_2}$Pyridinyl), 2.73 (t, 2H, J = 6.5 Hz, OCH$_2$CH$_2\underline{CH_2}$NH), 1.98 (p, 2H, J = 6.5 Hz, OCH$_2\underline{CH_2}$CH$_2$NH). HRMS (m/z): calcd. for $C_{24}H_{23}FN_4O_2$: 418.1805; found: 418.1817. Anal. calcd. for $C_{24}H_{23}FN_4O_2$: C, 68.88; H, 5.54; N, 13.39. Found: C, 69.01; H, 5.52; N, 13.40.

*6.1.32. N-(4-Chlorophenyl)-4-[3-(pyridin-3-ylmethylamino)-propoxy]-1H-indole-2-carboxamide (**20b**)*

Yellow solid (36 mg, 30% yield). ^{1}H NMR δ (ppm): 11.74 (s, 1H, indole-NH), 10.29 (s, 1H, CONH), 8.54 (s, 1H, pyridinyl-C2H), 8.42 (d, 1H, J = 4.5 Hz, pyridinyl-C6H), 7.86 (d, 1H, J = 9.0 Hz, pyridinyl-C4H), 6.53–7.75 (m, 9H, Ar-H), 4.18 (t, 2H, J = 6.5 Hz, O$\underline{CH_2}CH_2CH_2$NH), 3.75 (s, 2H, NHC$\underline{H_2}$Pyridinyl), 2.73 (t, 2H, J = 6.5 Hz, OCH$_2$CH$_2\underline{CH_2}$NH), 1.98 (p, 2H, J = 6.0 Hz, OCH$_2\underline{CH_2}$CH$_2$NH). HRMS (m/z): calcd. for $C_{24}H_{23}ClN_4O_2$: 434.1510; found: 434.1499. Anal. calcd. for $C_{24}H_{23}ClN_4O_2$: C, 66.28; H, 5.33; N, 12.88. Found: C, 66.08; H, 5.32; N, 12.91.

*6.1.33. N-Phenyl-4-[3-(pyridin-3-ylmethylamino)propoxy]-1H-indole-2-carboxamide (**20c**)*

Pale yellow solid (32 mg, 29% yield). ^{1}H NMR δ (ppm): 11.71 (s, 1H, indole-NH), 10.17 (s, 1H, CONH), 8.54 (s, 1H, pyridinyl-C2H), 8.42 (d, 1H, J = 4.5 Hz, pyridinyl-C6H), 7.82 (d, 1H, J = 8.5 Hz, pyridinyl-C4H), 6.53–7.75 (m, 10H, Ar-H), 4.18 (t, 2H, J = 6.5 Hz, O$\underline{CH_2}CH_2CH_2$NH), 3.76 (s, 2H, NHC$\underline{H_2}$Pyridinyl), 2.75 (t, 2H, J = 6.0 Hz, OCH$_2$CH$_2\underline{CH_2}$NH), 1.99 (p, 2H, J = 6.5 Hz, OCH$_2\underline{CH_2}$CH$_2$NH). HRMS (m/z): calcd. for $C_{24}H_{24}N_4O_2$: 400.1899; found: 400.1516. Anal. calcd. for $C_{24}H_{24}N_4O_2$: C, 71.98; H, 6.04; N, 13.99. Found: C, 71.83; H, 6.03; N, 14.01.

*6.1.34. N-(3,4-Difluorophenyl)-4-[3-(pyridin-3-ylmethylamino)-propoxy]-1H-indole-2-carboxamide (**20d**)*

Yellow solid (39 mg, 32% yield). ^{1}H NMR δ (ppm): 11.79 (s, 1H, indole-NH), 10.38 (s, 1H, CONH), 8.55 (s, 1H, pyridinyl-C2H), 8.43 (d, 1H, J = 3.5 Hz, pyridinyl-C6H), 7.98–8.02 (m, 1H, J = 8.5 Hz, pyridinyl-C4H), 6.54–7.76 (m, 8H, Ar-H), 4.18 (t, 2H, J = 6.0 Hz, O$\underline{CH_2}CH_2CH_2$NH), 3.76 (s, 2H, NHC$\underline{H_2}$Pyridinyl), 2.74 (t, 2H, J = 6.5 Hz, OCH$_2$CH$_2\underline{CH_2}$NH), 1.99 (p, 2H, J = 6.5 Hz, OCH$_2\underline{CH_2}$CH$_2$NH). HRMS (m/z): calcd. for $C_{24}H_{22}F_2N_4O_2$: 436.1711; found: 436.1700. Anal. calcd. for $C_{24}H_{22}F_2N_4O_2$: C, 66.05; H, 5.08; N, 12.84. Found: C, 66.18; H, 5.06; N, 12.42.

*6.1.35. N-(2-Chlorophenyl)-4-[3-(pyridin-3-ylmethylamino)-propoxy]-1H-indole-2-carboxamide (**20e**)*

Flavo-green solid (22 mg, 18% yield). ^{1}H NMR δ (ppm): 11.75 (s, 1H, indole-NH), 10.04 (s, 1H, CONH), 8.54 (s, 1H, pyridinyl-C2H), 8.42 (d, 1H, J = 3.5 Hz, pyridinyl-C6H), 7.75 (d, 1H, J = 8.0 Hz, pyridinyl-C4H), 6.53–7.59 (m, 9H, Ar-H), 4.18 (t, 2H, J = 6.0 Hz, O$\underline{CH_2}CH_2CH_2$NH), 3.76 (s, 2H, NHC$\underline{H_2}$Pyridinyl), 2.74 (t, 2H, J = 7.0 Hz, OCH$_2$CH$_2\underline{CH_2}$NH), 1.98 (p, 2H, J = 6.5 Hz, OCH$_2\underline{CH_2}$CH$_2$NH). HRMS (m/z): calcd. for $C_{24}H_{23}ClN_4O_2$: 434.1510; found: 434.1494. Anal. calcd. for $C_{24}H_{23}ClN_4O_2$: C, 66.28; H, 5.33; N, 12.88. Found: C, 66.01; H, 5.34; N, 12.90.

6.2. Molecular docking

The crystallographic coordinates of CaNMT [25] (0.32 Å resolution, R_{cryst} = 0.284) were obtained from the Brookheaven Protein Databank as entries 1IYL. The chemical structures of compounds **8f** and **16a** were built by InsightII 2000 software package. Followed by energy minimization, compounds **8f** and **16a** were docked into the active site of CaNMT using the Affinity module within InsightII 2000 software package. The detailed docking protocols are similar to that in our previous modeling studies on CaNMT [26].

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Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejmech.2010.03.007.

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