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Identification of novel non-peptide CXCR4 antagonists by ligand-based design approach

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ABSTRACT

The design and synthesis of novel non-peptide CXCR4 antagonists is described. The peptide backbone of highly potent cyclic peptide-based CXCR4 antagonists was entirely replaced by an indole framework, which was expected to reproduce the disposition of the key pharmacophores consistent with those of potential bioactive conformations of the original peptides. A structure–activity relationship study on a series of modified indoles identified novel small-molecule antagonists having three pharmacophore functional groups through the appropriate linkers.

Chemokines are a family of small proteins with chemotactic and proactivatory effects on leukocytes. Chemokines mediate their biological effects by binding to the specific G-protein coupled receptor subtypes that are differentially and widely expressed in blood cells. Among these chemokine receptors, CXCR4 has a broad tissue distribution and the activation by its endogenous ligand CXCL12 (SDF-1, stromal cell-derived factor 1) leads to chemotaxis, immunomodulation, and other regulatory functions including progenitor cell migration during embryologic development of the cardiovascular, hematopoietic, and central nervous systems. In addition to its physiological roles, CXCR4 also plays important roles in pathological conditions. These include tumor growth and metastasis[1] and rheumatoid arthritis (RA).[2] CXCR4 has also been reported to act as a major co-receptor involved in the entry of T-cell-line-tropic human immunodeficiency virus type 1 (HIV-1) strains into target cells.[3] Thus, CXCR4 is considered as an important therapeutic target for multiple diseases. Inhibitory compounds of CXCL12 or HIV-1 binding to CXCR4 could be novel classes of anti-cancer, anti-RA, and anti-HIV-1 drugs. Previously, we found highly potent peptide-based CXCR4 antagonists such as **1**, **2**, and **3** (Fig. 1).[4,5]

1 H-Arg^1-**Arg^2**-**Nal^3**-Cys^4-**Tyr^5**-Arg^6-Lys^7(-D-Lys^8-Pro^9-)-S-S-HO-**Arg^{14}**-Cys^{13}-Cit^{12}-Arg^{11}-Tyr^{10}

2 cyclo(-D-Tyr^1-**L-Arg^2**-**L-Arg^3**-**L-Nal^4**-L-Gly^5-)

3 cyclo(-**D-Tyr^1**-D-Arg^2-**L-Arg^3**-**L-Nal^4**-L-Gly^5-)

Figure 1. Structures of **1** and its downsized peptides **2** and **3**. Bold residues are the indispensable residues for the potent CXCR4-antagonistic activity. Nal, L-3-(2-naphthyl)alanine; Cit, L-citrulline.

Peptide **1** and its derivatives effectively blocked X4-HIV-1 entry to the cell by specifically binding to CXCR4,[6] and also showed an anti-metastatic effect against breast cancer[7] and anti-RA activity[8] in mouse models.

Although peptides are excellent lead molecules for development of pharmaceutical agents, special drug delivery systems are usually required for their clinical use because of the poor bioavailability and instability against enzymes. Whereas several peptide-based CXCR4 antagonists have been reported, only small numbers of small-molecule CXCR4 antagonists have been

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reported.[9] These prompted us to design novel non-peptide CXCR4 antagonists based on the SAR and conformational studies on peptide ligands **1**–**3**.

Cyclic pentapeptide-based CXCR4 antagonists **2** and **3** were identified by screening of cyclic pentapeptide libraries, which were designed based on SAR studies of peptide **1**. The constrained backbone of the cyclic peptide was utilized as a template for positioning the key functional groups in space as is found in parent peptide **1**. Subsequent conformational analysis of **2** permitted us to determine the topology of the four indispensable residues, then, rational approach toward the de novo design of non-peptide antagonists may be envisaged.[10]

Our previous SAR studies on **2** and its derivatives have shown that at least three functional groups on the peptide side-chains are required: (1) an aromatic ring such as 2-naphthyl- or 3-indolyl group at position 4; (2) a guanidino group at position 3; (3) a guanidino group at position 2 or a phenol group at position 1.[11] However, it was difficult to determine the spatial relationships between these functional groups due to the free rotation of the side-chain torsion (χ) angles. In our structural analyses, peptide **2** adopted a

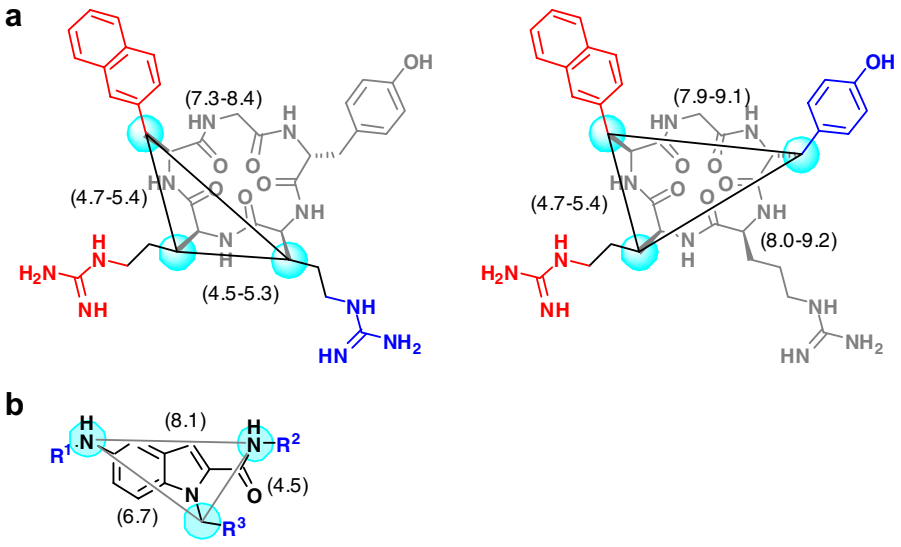
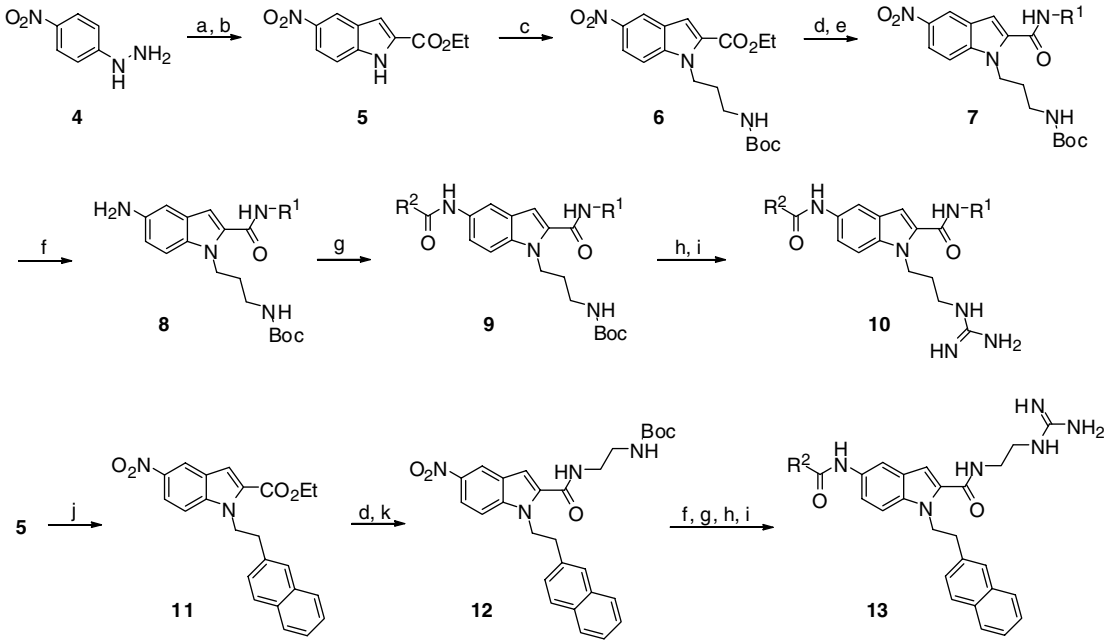


Figure 2. Design of indole-based CXCR4 antagonists based on molecular dynamics calculation of **2**. Distances (Å) between Cβ atoms bearing three essential functional groups during 1000 ps MD calculation of **2** (a) and between two key atoms of energy-minimized 5-acetamido-1-methylindole-2-carboxamide (b) are shown in parentheses. R^1–R^3 include naphthyl, indolyl, guanidino, and phenol groups.



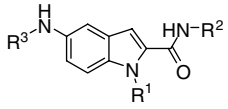
Scheme 1. Reagents and conditions; (a) ethyl pyruvate, EtOH, reflux; (b) polyphosphoric acid, xylene, 130°C; (c) NaH, *N*-Boc-3-bromopropylamine, DMF, 70 °C; (d) 1 N NaOH aq., EtOH-THF; (e) R^1-NH_2, HATU, Et_3N, DMF; (f) NH_4CO_2H, Pd/C, EtOH-THF, reflux; (g) R^2-CO_2H, HATU, Et_3N, DMF; (h) 95% TFA-H_2O; (i) 1-*H*-pyrazolecarboxamidine hydrochloride, Et_3N, DMF; (j) 2-(2-naphthyl)ethyl bromide, NaH, DMF, 70 °C; (k) *N*-Boc-ethylenediamine, HATU, Et_3N, DMF.

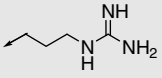
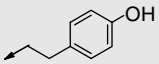
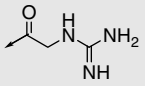
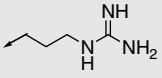
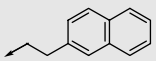
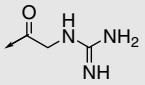
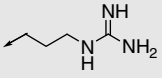
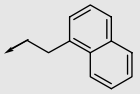
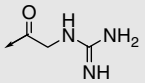
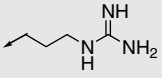
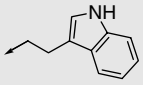
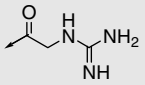
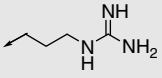
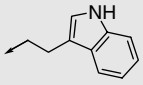
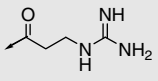
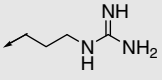
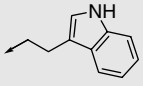
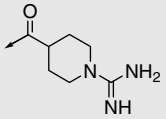
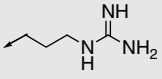
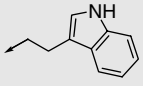
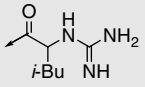
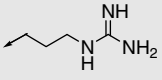
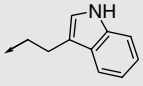
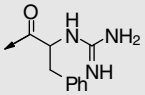
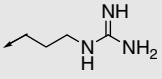
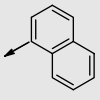
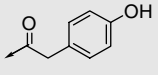
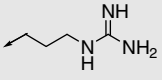
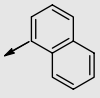
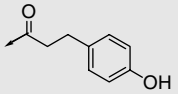
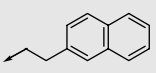
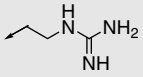
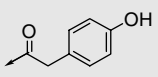
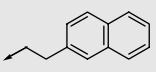
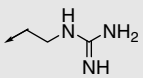
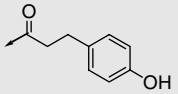
variety of global conformations, in which the distances between indispensable functional groups were variable. On the other hand, relatively rigid cyclic peptide backbone and fixed distances between Cβ atoms, which append key functional groups, were observed.[5] Hence, we envisioned that introduction of crucial functional moieties for receptor binding onto a bicyclic heterocycle scaffold, which mimics the relatively fixed cyclic pentapeptide backbone of **2**, would provide non-peptide CXCR4 ligands. In this letter, we report a part of our ongoing research to develop novel non-peptide small molecule CXCR4 antagonists.

Among several molecular scaffold candidates, we first selected 5-aminoindole-2-carboxylic acid for the following reasons: (1) molecular modeling suggested that it met the spatial requirements for displaying the three key substituents (Fig. 2);[12] (2) accessible synthetic approaches were available for attachment of the three substituents; (3) indoles represent an important class of bioactive compounds and the physicochemical properties in terms of medicinal chemistry are well-documented.

Syntheses of indole-based compounds were achieved as shown in Scheme 1. (4-Nitrophenyl)hydrazine **4** was converted to the corresponding hydrazone, which was subjected to Fischer ring closure reaction to produce an indole **5**. Alkylation of N^1 position of the indole **5** with *N*-Boc-3-bromopropylamine gave **6**. This was hydrolyzed using 1 N aqueous sodium

Table 1
Inhibitory activities of indole derivatives **10a–j** and **13a–b** against binding of ^{125}I-SDF-1α to CXCR4



Compound	R^1	R^2	R^3	% inhibition at 10 μM
10a				23
10b				63
10c				61
10d				88
10e				70
10f				86
10g[a]				77
10h[a]				72
10i				62
10j				55
13a				51
13b				53

[a] Evaluated as a racemic mixture.

hydroxide in EtOH-THF and the resulting free carboxylic acid was coupled with amines using *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) as coupling reagent to give **7**. The nitro group of **7** was reduced to amine **8** upon treatment with Pd-C and ammonium formate in EtOH. The aminoindole **8** was then coupled with carboxylic acids to give **9**. Deprotection of Boc group(s) by 95% TFA and guanylation of the free amino group(s) produced the target compounds **10**. Another series of compounds **13** were synthesized from **5** using similar reaction sequences described for **10**.

All indole-based compounds listed in Tables 1 and 2 were purified by preparative reverse-phase HPLC (purity >95%) and characterized by MALDI-TOF-MS. These compounds were tested for competitive binding inhibition in human CXCR4 transfected Chinese hamster ovary (CHO) cells using [^{125}I]SDF-1 as a radioligand, with the results given as percentage inhibition at 10 μM. IC_{50} values of selected compounds are shown in Table 2.

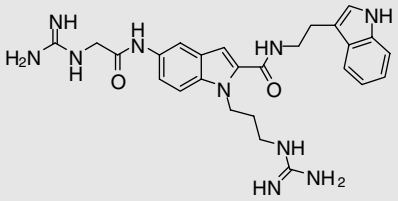
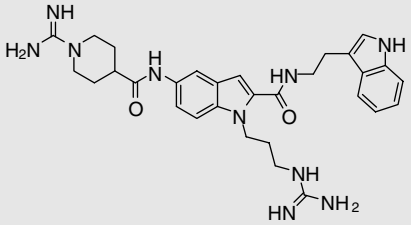
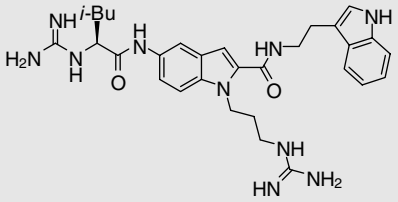
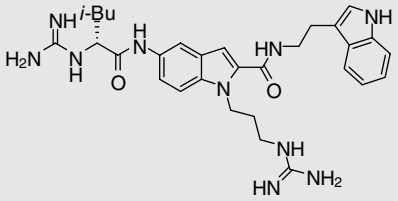
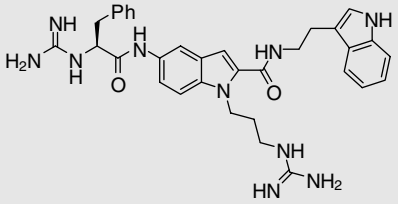
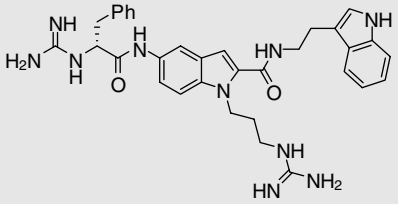
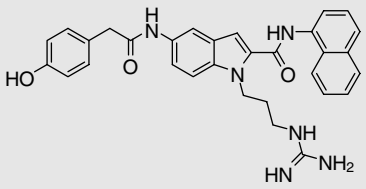
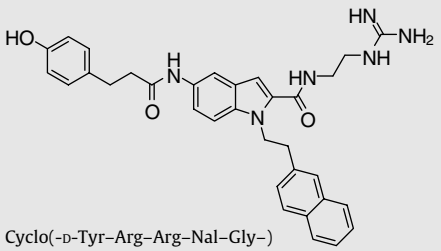
Compound **10d** with 2-(3-indolyl)ethyl group at the R^2 position showed 88% inhibition at 10 μM (IC_{50} = 3.0 μM) and was more potent than compounds having (4-hydroxyphenyl)-, (1-naphthyl)- or (2-naphthyl)-alkyl group at the R^2 position (compounds **10a–c**, 23–63% inhibition at 10 μM). Further SAR studies based on **10d** were undertaken. Chain elongation of the guanidinoacetyl group (R^3) of **10d** caused slight decrease in the affinity (**10e**). The use of *N*-amidinopiperidine-4-carbonyl was also acceptable for high potency [IC_{50} (**10f**) = 3.0 μM]. Introduction of an isobutyl or benzyl group into the α-carbon of guanidinomethyl carbonyl group of **10d** did not cause significant drop in binding affinity (compounds **10g** and **10h**). Compounds with *S*-configuration at the chiral center showed more potent CXCR4 antagonistic activity as compared with the corresponding *R*-isomers. (***S***)-**10g** was identified as the most potent compound [IC_{50} ((***S***)-**10g**) = 1.2 μM].[13] Compound (***S***)-**10g** also showed potent anti-HIV-1 activity (IIIB strain, inhibition of HIV-1 induced cytopathogenicity: EC_{50} = 5.4 μM). The IC_{50} value of (***S***)-**10g** is 34-fold lower as compared with parent peptide **2**. This is probably due to the absence of phenol functionality in (***S***)-**10g** which corresponds to D-Tyr side-chain of peptide **2**. Decreased number of amide bond in (***S***)-**10g** might also lead to the lower affinity. We have previously showed the importance of backbone amide functionalities of **2** for CXCR4 antagonistic activity by using reduced-amide isosteres or (*E*)-alkene dipeptide isosteres.[14]

Indole-based compounds having a phenol group at R^3 position showed moderate CXCR4-binding affinity (**10i**, **10j**, **13a**, and **13b**). Interestingly, these compounds did not show complete inhibition even at higher concentrations in the binding inhibition experiments, while compounds having a guanidino group at R^3 position (**10c**, **10d**, and (***S***)-**10g**) achieved complete inhibition (Fig. 3). These results suggest that **10c**, **10d** and (***S***)-**10g** are inhibitors that competitively bind to the SDF-1 binding site of CXCR4, while **10i**, **10j**, **13a**, and **13b** may bind to an allosteric site of CXCR4 and partially antagonize the SDF-1 binding.

Comparison of energy-minimized structures of (***S***)-**10g** and previously reported solution conformation of **2** revealed that three functional groups on the indole template well overlapped the three pharmacophore residues of **2** as expected. In this model, indole scaffold favorably mimicked the backbone of Arg–Arg–Nal sequence of **2** (see Fig. 4).

In summary, a series of indole-based compounds were designed, synthesized, and characterized as a novel class of CXCR4 antagonists. Although their IC_{50} values are in the μM range, these indole derivatives could serve as a useful lead for further medicinal chemistry programs.

Table 2
IC_{50} values of selected indole derivatives

Compound	Structure	IC_{50} (μM)
10d		3.0
10f		3.0
(***S***)-**10g**		1.2
(***R***)-**10g**		2.2
(***S***)-**10h**		1.7
(***R***)-**10h**		3.7
10i		4.8
13b		2.7
2	Cyclo(-D-Tyr–Arg–Arg–Nal–Gly–)	0.035

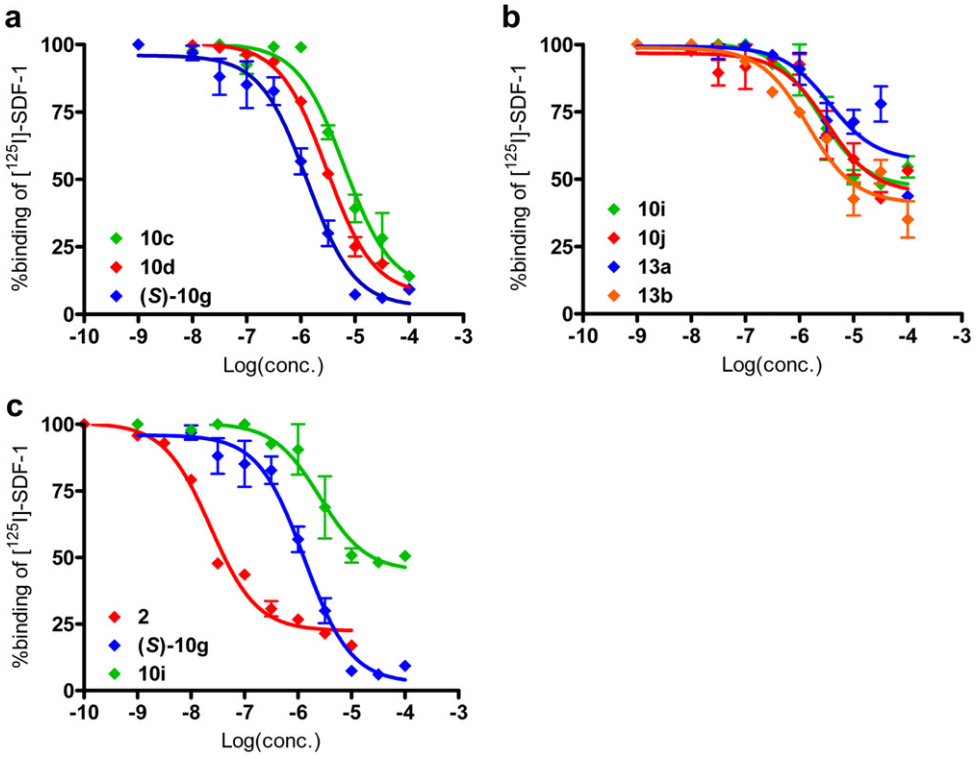


Figure 3. Ligand binding dose response of the compounds (a) having two guanidino pharmacophores and (b) having a phenol pharmacophore, and (c) the comparison of the two subsets with the parent peptide **2**.

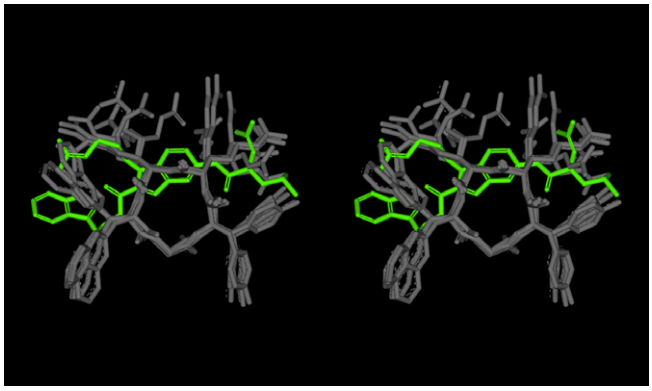


Figure 4. Overlay of a low-energy structure of (***S***)-**10g** (green) and **2** (gray). The molecular modeling of (***S***)-**10g** was performed using MacroModel-Program (Ver. 8.1) with 'MMFF' force field.

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12. Distances (Å) between β-carbons of **2** during 1000 ps MD calculation; D-Tyr^1–Arg^3: 8.0–9.2, D-Tyr^1–Nal^4: 7.9–9.1, Arg^3–Nal^4: 4.7–5.4, Arg^2–Arg^3: 4.5–5.3, Arg^2–Nal^4: 7.3–8.4, Arg^3–Nal^4: 4.7–5.4. Distances (Å) between two key atoms of energy-minimized 5-acetamido-1-methylindole-2-carboxamide:

(acetamide N)-(*N*-methyl C), 6.7; (acetamide N)–(carboxamide N), 8.1; (methyl C)-(carboxamide N), 4.5.

13. Compound (***S***)-**10g**: $[\alpha]_D^{23} - 6.4$(*c*0.35, CH_3OH); ^1H NMR (500 MHz, DMSO-d_6): δ = 0.93 (d, *J* = 6.0 Hz, 3H), 0.95 (d, *J* = 5.9 Hz, 3H), 1.60–1.76 (m, 3H), 1.92 (tt, *J* = 6.6, 7.2 Hz, 2H), 2.97 (t, *J* = 7.6 Hz, 2H), 3.14 (dt, *J* = 6.2, 6.6 Hz, 2H), 3.55 (dt, *J* = 6.7, 7.0 Hz, 2H), 4.26–4.41 (m, 1H), 4.55 (t, *J* = 7.2 Hz, 2H), 6.86–7.53 (brm, 8H), 6.99 (m, 1H), 7.03–7.11 (m, 2H), 7.20 (br, 1H), 7.33–7.43 (m, 2H), 7.55 (d, *J* = 9.0 Hz, 1H), 7.59 (d, *J* = 7.9 Hz, 1H), 7.73 (t, *J* = 5.4 Hz, 1H), 7.90 (d, *J* = 9.0 Hz, 1H), 7.95–7.99, (br, 1H), 8.65 (t, *J* = 5.7 Hz, 1H), 10.08 (s, 1H), 10.82 (s, 1H); LRMS (FAB): 573 (MH^+, base peak), 444; HRMS (FAB): calcd for $C_{30}H_{41}N_{10}O_2$ (MH^+) 573.3414; found 573.3418. Compound (***S***)-**10h**: $[\alpha]_D^{23}$ 3.3(*c*0.34, CH_3OH); ^1H NMR (500 MHz, DMSO-d_6): δ = 1.91 (tt, *J* = 7.2, 7.4 Hz, 2H), 2.96 (t, *J* = 7.3 Hz, 2H), 2.97–3.04 (m, 1H), 3.13 (dt, *J* = 5.4, 7.2 Hz, 2H), 3.20–3.27 (m, 1H), 3.54 (dt, *J* = 6.0, 7.3 Hz, 2H), 4.54 (t, *J* = 7.4 Hz, 2H), 4.56–4.62 (m, 1H), 6.78–7.64 (brm, 8H), 6.98 (m, 1H), 7.03–7.10 (m, 2H), 7.19 (m, 1H), 7.21–7.27 (m, 1H), 7.27-7.40 (m, 6H), 7.55 (d, *J* = 8.9 Hz, 1H), 7.58 (d, *J* = 7.7 Hz, 1H), 7.71 (t, *J* = 5.3 Hz, 1H), 7.85 (d, *J* = 8.9 Hz, 1H), 7.89–7.92, (br, 1H), 8.64 (t, *J* = 6.0 Hz, 1H), 10.12 (br, 1H), 10.82 (br, 1H); LRMS (FAB): 607 (MH^+, base peak), 444; HRMS (FAB): calcd for $C_{33}H_{39}N_{10}O_2$ (MH^+) 607.3257; found 607.3251.
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