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Discovering Potent Small Molecule Inhibitors of Cyclophilin A Using de Novo Drug Design Approach

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Abstract: This work describes an integrated approach of de novo drug design, chemical synthesis, and bioassay for quick identification of a series of novel small molecule cyclophilin A (CypA) inhibitors (**1**−**3**). The activities of the two most potent CypA inhibitors (**3h** and **3i**) are 2.59 and 1.52 nM, respectively, which are about 16 and 27 times more potent than that of cyclosporin A. This study clearly demonstrates the power of our de novo drug design strategy and the related program LigBuilder 2.0 in drug discovery.

Cyclophilin A (CypA[a]) plays a critical role in many biological processes such as enhancing the rate of folding/unfolding of proteins via its peptidyl-prolyl isomerase (PPIase) activity,[1,2] inhibiting the serine/threonine phosphatase activity of calcineurin as the target of cyclosporin A (CsA),[3,4] facilitating viral replication and infection via its binding to the HIV-1 Gag polyprotein[5] and nucleocapsid protein of SARS coronavirus (SARS-CoV),[6] and inducing neuroprotective/neurotrophic effects when presented at high levels in the brain.[7,8] In addition, recently, it was reported that CypA was overexpressed in cancer cells, especially in solid tumors. This suggests that CypA is an important regulator in tumorigenesis and tumor apoptosis.[9]

Given the importance of CypA in regulation of numerous biological processes and related disease development, significant efforts have focused on discovering CypA inhibitors in the past decade.[10−20] However, survey of the existing CypA inhibitors reveals that they have very limited structural diversity and less potency. The currently known CypA inhibitors were mainly derived from nature products (e.g., CsA,[10] FK506,[11] rapamycin,[12] and sanglifehrin A[13]) or peptide analogues,[14] which are usually structural complex molecules and possess poor pharmacokinetic and physicochemical characteristics (for peptides) in a certain extent. Therefore, clearly seeking potent, structurally novel, small molecule inhibitors represents an important direction in the field.

In the present study, we report the discovery of a new class of small molecule CypA inhibitors with nanomolar inhibitory potencies by using a newly upgraded de novo drug design package, LigBuilder 2.0. The previous LigBuilder 1.2 version is a de novo drug design program based on the three-dimensional protein structures. With the structural constraints of a target protein, LigBuilder 1.2 can build up ligands iteratively using a library of organic fragments.[21] To increase the efficiency and success rate of designed compounds, great efforts have been made in the new version of LigBuilder (version 2.0) to overcome the crucial limitations of de novo drug design methods. There are two significant improvements in LigBuilder 2.0 as compared to LigBuilder 1.2. First, a synthesis accessibility analysis module was introduced that contained an embedded chemical reaction databases and a retrosynthesis analyzer. This module checks all the compounds designed for their synthesis accessibility and can give reasonable synthetic routes. Second, a newly developed cavity detection procedure is used to accurately detect the locations of possible binding pockets in the targets and predict their druggability. Ligands are designed inside the detected cavities and overgrowth can be eliminated.

The crystal structure of CypA in complex with sanglifehrin macrolide (SFM) (PDB entry 1NMK)[13] was used as a starting structural model for de novo drug design. The cavity module of LigBuilder 2.0 was employed to put the protein coordinates into lattice space, detect the cavity location and boundary, and analyze the physicochemical properties of the binding site.

As a part of our continuing effort to identify potent small molecule CypA inhibitors for developing immunosuppressive agents, several scaffolds as general CypA inhibitor templates were identified.[15−18] In addition, we have described the pharmacophore of these inhibitors, noting that most of the potent small molecule inhibitors contained an amide fragment as the key linker between two terminal fragments (Figure 1),[15−18,22] and this amide linker contributes to inhibitory activity via forming 2−3 hydrogen bonds with residues Arg55, Gln63, and Asn102 around the "saddle" between the two sub-binding pockets of CypA (site A and site B, Figure 1).[17,22] Recently, Guichou et al.[20] reported a series of urea-based CypA inhibitors (Figure 1). Inspired by these two linkers, we proposed and designed acylurea as a seed (linker) to interact with the three residues of CypA mentioned above (Figure 1), and then determined the preliminary conformation and position of this seed structure according to the known and putative CypA−ligand interactions. Taking this seed as a starting point, LigBuilder 2.0 was used to generate new molecules according to the shape and properties of CypA binding sites. In this case, the seed structure grew from both ends to occupy the two sub-binding pockets of CypA (Figure 1). To adjust the pose of the designed molecules and eliminate the possible effects of the preassigned seed location, LigBuilder 2.0 employed a stochastic optimization method to optimize the intramolecular energy of a ligand by rotating the

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[a] Abbreviations: CypA, cyclophilin A; PPIase, peptidyl-prolyl isomerase; CsA, cyclosporin A; HIV, human immunodeficiency virus; SARS-CoV, severe acute respiratory syndrome-associated coronavirus; ADME/T, absorption, distribution, metabolism, excretion, toxicology; SAR, structure−activity relationship.

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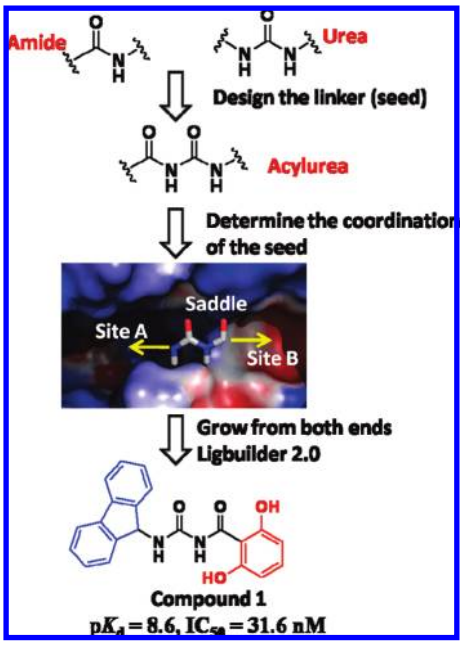


Figure 1. Discovery process of the lead compound (**1**) based on de novo drug design.

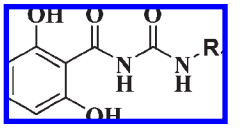
newly introduced fragment and the intermolecular binding energy by ligand pose perturbation. Because of the stochastic nature of genetic algorithm, we ran 50 procedures to sample the solution space. Genetic algorithm population size was set to 2000, and 20 genetic generations were carried out per procedure. Molecules generated were ranked according to the scores of binding affinity, biological availability, shape complementarity, and synthesizability. Finally, the top 98 molecules (Supporting Information, Table S1) were selected for further analysis and 37 of them (38%) were found to share a common scaffold (compound **1**, Figure 1) with a predicted binding affinity around 8.6 (pK_d value). Accordingly, we selected and synthesized compound **1** and evaluated its ability to inhibit the *cis*−*trans* isomerase activity of cyclophilin A. Encouragingly, compound **1** turned out to be a strongly potent CypA inhibitor with IC_{50} of 31.6 ± 2.0 nM, even more potent than CsA (IC_{50} = 40.7 ± 3.0 nM).

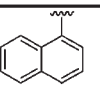
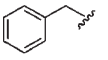
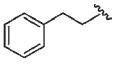
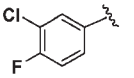
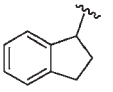
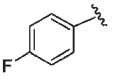
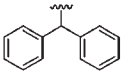
To provide expedient and significant structure−activity relationship (SAR) information and improve inhibitory activity of the lead compound, chemical modifications were performed on the two terminal fragments of compound **1**, i.e., the 9*H*-fluorene ring (blue, Figure 1) and the 2,6-dihydroxyphenyl moiety (red, Figure 1). First, we used groups with various sizes to replace the 9*H*-fluorene ring (Figure 1, Table 1) and obtained eight analogues (**2a**−**h**, Table 1). Second, by maintaining the 9*H*-fluorene ring and replacing the 2,6-dihyroxy substituents with other polar and hydrophobic ones, compounds **3a**−**s** were designed and synthesized (Table 2). The details for synthesis and purity analyses of compounds **1**−**3** are described in the Supporting Information. All compounds **1**−**3** were confirmed ≥95% purity (Supporting Information, Table S2).

The effects of compounds **2a**−**h** and **3a**−**s** in inhibiting the PPIase activity of CypA were determined using the standard chymotrypsin-coupled assay (Tables 1 and 2).[23] The results indicated that 12 derivatives, i.e., **2b**, **2d**, **3a**−**3c**, **3f**−**i**, **3k**, **3p**, and **3r**, were the CypA inhibitors with IC_{50} values ranging from 1.52 to 1910 nM. Remarkably, the PPIase inhibitory activities of derivatives **3h** and **3i** have increased up to 2.59 ± 0.20 and 1.52 ± 0.10 nM, respectively, which are about 16 and 27 times more potent than that of CsA. To our knowledge, these two compounds are the most potent small molecule CypA inhibitors reported so far. An analysis of the data listed in Tables 1 and 2 reveals some noteworthy observations of the SAR for compounds **1**−**3**: (1) replacement of 9*H*-fluorene ring with smaller aromatic cyclic structures (**2a**, **2e**, and **2g**) significantly reduces the biological activities, which was confirmed both experimental inhibitory activities (Table 1) and calculated affinities (Supporting Information, Table S2); (2) displacement of 9*H*-fluorene ring with flexible structures (**2b**−**c**) or nonplanar ring (**2h** and **2f**) are not beneficial (Table 1 and Supporting Information Table S2), the reason refers to the following docking study; (3) the substitutions on the phenyl ring substantially affect the potency of compounds. In general, multiple-halogen (**3g**−**i**) and single electron-withdrawing (**3a**−**c**, **3f**, and **3r**) substituents are favorable to the activities of these compounds (Table 2 and Supporting Information Table S3); (4) introduction of electron-donating substitutions on the phenyl ring is not beneficial (**3d**−**e** and 3l−**o**) (Table 2).

To address more information for SAR of the discovered CypA inhibitors and to gain clues for further structural

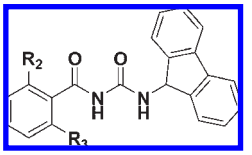
Table 1. Chemical Structures of Compounds **2a**−**h** and Their Activities



compd	R_1	enzyme inhibition assay (IC_{50}, nM)[a]
2a		> 10000
2b		370 ± 12
2c		Inactive
2d		136 ± 13
2e		>10000
2f		>10000
2g		>10000
2h		>10000

[a] Data are the mean value of three independent experiments and the deviations are < 10% of the mean value.

Table 2. Chemical Structures of Compounds **3a**−**s** and Their Activities



entry	R_2, R_3	enzyme inhibition assay (IC_{50}, nM)[a]	entry	R_2, R_3	enzyme inhibition assay (IC_{50}, nM)[a]
3a	Cl, H	103 ± 5	**3k**	MeS, F	1910 ± 100
3b	CN, H	71.2 ± 3.0	**3l**	MeO, F	inactive
3c	F, H	159 ± 7	**3m**	MeO, Cl	> 10000
3d	MeO, H	> 10000	**3n**	MeS, H	> 10000
3e	Me, H	inactive	**3o**	BnO, F	inactive
3f	CF_3, H	364 ± 17	**3p**	BnO, H	12.1 ± 0.4
3g	F, F	263 ± 24	**3q**	OH, H	> 10000
3h	Cl, Cl	2.59 ± 0.20	**3r**	NO_2, H	620 ± 32
3i	Cl, F	1.52 ± 0.10	**3s**	NH_2, H	> 10000
3j	H, H	inactive			

[a] Data are the mean value of three independent experiments and the deviations are < 10% of the mean value.

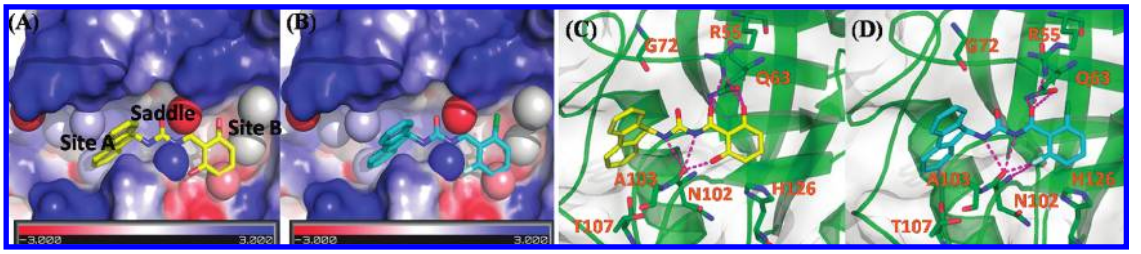


Figure 2. (A,B) Docking models of compounds **1** (A, yellow) and **3i** (B, cyan) into the active sites of CypA. The CypA surface was colored by electrostatic potential. The color balls are the pharmacophore of binding site. Red ball indicates the hydrogen bond acceptor area, blue ball is hydrogen bond donor area, and the white ball is hydrophobic area. The light-colored ball indicates weaker property of corresponding color. (C, D) Detailed interactions of compounds **1** (C, yellow) and **3i** (D, cyan) to the binding sites of CypA; hydrogen bonds are represented by magenta dotted lines. These images were generated using the PyMol program (http://www.pymol.org/).

optimization, the binding models of the lead compound **1** and the most potent inhibitor **3i** to CypA were generated by using AUTODOCK 3[24] by applying a Lamarckian genetic algorithm, with a grid space set to be 0.375 Å (Figure 2). The docking binding mode of compound **1** is very close to the original binding mode designed by LigBuilder 2.0, with a rmsd of 1.5 Å. The binding sites of CypA could be divided into three subareas,[15−17] denoted as site A, saddle, and site B (Figure 2A). To clearly describe the inhibitor−protein interactions, we assign pharmacophore features to the binding sites based on the algorithm of Pocket V.2,[25] as represented by color balls: red, hydrogen-bond acceptor area; blue, hydrogen-bond donor area; white, hydrophobic area. The light-colored ball indicates weaker property of corresponding color. It is obvious that the binding model of compound **1** to CypA is very similar to that of **3i** to CypA, the planar fluorene ring inserts vertically into the hydrophobic gorge area in site A and forms favorable hydrophobic interactions (while the nonplanar cyclic moiety, such as 2,3-dihydro-1*H*-indene ring of **2f**, can not fit well with site A); the phenyl ring of 2,6-disubstitued phenyl moiety fits the hydrophobic pharmacophore in site B; owing to these two types of appropriate terminal fragments, the acylurea linker of compounds **1** and **3i** adopted a proper orientation and interacted well with the saddle site (Figure 2A,B). The interaction models also revealed that both compounds **1** and **3i** form seven hydrogen bonds with residues Arg55, Gln63, and Asn102 in the saddle site (Figure 2C,D). These hydrogen bonds may directly lead to the higher inhibitory activities of these two compounds, which is in good agreement with our design idea, namely tandem amide (acylurea) would contribute to more hydrogen bond interactions and possess more potency than amide or urea does (Figure 1). Furthermore, compound **3i** contains a chlorine atom that may impart hydrophobic interaction (Figure 2B). The additional interaction was regarded as one of the key factors leading to ∼20 times potency improvement of compound **3i** compared to compound **1**.

In summary, we have discovered highly potent small molecule CypA inhibitors by using de novo drug design approach combined with structural optimization, organic synthesis, and bioassay. On the basis of our previous studies and Guichou's results, we designed acylurea as a seed fragment for CypA inhibitor construction using LigBuilder 2.0. From this one-round inhibitor generation, a highly potent lead compound (**1**) with IC_{50} of 31.6 ± 2.0 nM against CypA PPIase was identified. Further SAR studies of compound **1** resulted in two nanomolar level CypA inhibitors, **3h** and **3i**. Notably, to our knowledge, compounds **3h** and **3i** are the most potent CypA inhibitors reported up to date.

Recently, de novo drug design methods have been receiving much attention for designing novel active compounds. Compared to virtual screening, de novo drug design approaches can produce novel molecules independent of known compound structures and can easily produce "clean" molecules only with required pharmacological profiles. A number of de novo drug design programs have been developed in recent years.[26−28] Current de novo drug design methods, however, are faced with problems caused by the uncertainty of novel designed chemical entities, which are

the reason that de novo drug design techniques in practice are not as widely spread as virtual screening techniques. One of the most severe limitations is that a majority number of candidates produced by de novo drug design methods are hard to be synthesized. Great efforts have been made in LigBuilder 2.0 to overcome these limitations. In the present study, we have successfully used LigBuiler2.0 to design highly potent CypA inhibitors. We are expecting more successful stories of drug design from scratch using de novo design programs like LigBuilder 2.0.

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Supporting Information Available: General information for chemical agents and analytical measurements, brief introduction of the LigBuilder 2.0, chemical structures of top 98 molecules, LigBuilder 2.0 predicted binding affinities of synthesized compounds, detailed synthetic procedures and related spectroscopic data for the designed compounds **1**−**3**, HPLC reports for the purity check of the active compounds **1**−**3**, and bioassay method for determining the inhibitory activity. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Galat, A. Peptidylprolyl cis/trans isomerases (immunophilins): biological diversity−targets−functions. *Curr. Top. Med. Chem.* **2003**, *3*, 1315–1347.
(2) Dornan, J.; Taylor, P.; Walkinshaw, M. D. Structures of immunophilins and their ligand complexes. *Curr. Top. Med. Chem.* **2003**, *3*, 1392–1409.
(3) Liu, J.; Farmer, J. D.; Lane, W. S.; Friedman, J.; Weissman, I.; Schreiber, S. L. Calcineurin is a common target of cyclophilin−cyclosporin A and FKBP−FK506 complexes. *Cell* **1991**, *66*, 807–815.
(4) Zou, X. J.; Matsumura, Y. J.; John, P.; Ryo, S.; Alberto, M.; Jack, M.; Stanley, C. J. Cytokine gene expression in rejecting and tolerant rat lung allograft models: analysis by RT-PCR. *Transplant. Immunol.* **1995**, *3*, 151–161.
(5) Luban, J.; Bossolt, K. L.; Franke, E. K.; Kalpana, G. V.; Goff, S. P. Human immunodeficiency virus type 1 Gag protein binds to cyclophilins A and B. *Cell* **1993**, *73*, 1067–1078.
(6) Luo, C.; Luo, H.; Zheng, S.; Gui, C.; Yue, L.; Yu, C.; Sun, T.; He, P.; Chen, J.; Shen, J.; Luo, X.; Li, Y.; Liu, H.; Bai, D.; Shen, J.; Yang, Y.; Li, F.; Zuo, J.; Hilgenfeld, R.; Pei, G.; Chen, K.; Shen, X.; Jiang, H. Nucleocapsid protein of SARS coronavirus tightly binds to human cyclophilin A. *Biochem. Biophys. Res. Commun.* **2004**, *321*, 557–565.
(7) Capano, M.; Virji, S.; Crompton, M. Actopaxin is phosphorylated during mitosis and is a substrate for cyclin B1/cdc2 kinase. *Biochem. J.* **2002**, *363*, 29–36.
(8) Dawson, T. M.; Steiner, J. P.; Lyons, W. E.; Fotuhi, M.; Blue, M.; Snyder, S. H. The immunophilins, FK506 binding protein and cyclophilin, are discretelylocalized in the brain: relationship to calcineurin. *Neuroscience* **1994**, *62*, 569–580.
(9) Choi, K. J.; Piao, Y.; Lim, M.; Kim, J.; Ha, J.; Choe, W.; Kim, S. Overexpressed cyclophilin A in cancer cells renders resistance to hypoxia- and cisplatin-induced cell death. *Cancer Res.* **2007**, *67*, 3654–3662.
(10) Handschumacher, R. E.; Harding, M. W.; Rice, J.; Drugge, R. J.; Speicher, D. W. Cyclophilin: a specific cytosolic binding protein for cyclosporin A. *Science* **1984**, *226*, 544–547.
(11) Siekierka, J. J.; Hung, S. H.; Poe, M.; Lin, C. S.; Sigal, N. H. A cytosolic binding protein for the immunosuppressant FK506 has peptidyl-prolyl isomerase activity but is distinct from cyclophilin. *Nature* **1989**, *341*, 755–757.
(12) Clane, R. Y.; Lim, S.; Samaan, A.; Collier, D. S.; Pollard, S. G.; White, D. J. G.; Thiru, S. Rapamycin for immunosuppression in organ allografting. *Lancet* **1989**, *334*, 227.
(13) Sedrani, R.; Kallen, J.; Martin Cabrejas, L. M.; Papageorgiou, C. D.; Senia, F.; Rohrbach, S.; Wagner, D.; Thai, B.; Jutzi Eme, A. M.; France, J.; Oberer, L.; Rihs, G.; Zenke, G.; Wagner, J. Sanglifehrin−cyclophilin interaction: Degradation work, synthetic macrocyclic analogues, X-ray crystal structure, and binding data. *J. Am. Chem. Soc.* **2003**, *125*, 3849–3859.
(14) Li, Q.; Moutiez, M.; Charbonnier, J. B.; Vaudry, K.; Menez, A.; Quemeneur, E.; Dugave, C. Design of a gag pentapeptide analogue that binds human cyclophilin A more efficiently than the entire capsid protein: New insights for the development of novel anti-HIV-1 drugs. *J. Med. Chem.* **2000**, *43*, 1770–1779.
(15) Li, J.; Chen, J.; Gui, C.; Zhang, L.; Qin, Y.; Xu, Q.; Zhang, J.; Liu, H.; Shen, X.; Jiang, H. Discovering novel chemical inhibitors of human cyclophilin A: virtual screening, synthesis, and bioassay. *Bioorg. Med. Chem.* **2006**, *14*, 2209–2224.
(16) Li, J.; Chen, J.; Zhang, L.; Wang, F.; Gui, C.; Zhang, L.; Qin, Y.; Xu, Q.; Liu, H.; Nan, F.; Shen, J.; Bai, D.; Chen, K.; Shen, X.; Jiang, H. One novel quinoxaline derivative as a potent human cyclophilin A inhibitor shows highly inhibitory activity against mouse spleen cell proliferation. *Bioorg. Med. Chem.* **2006**, *14*, 5527–5534.
(17) Li, J.; Zhang, J.; Chen, J.; Luo, X.; Zhu, W.; Shen, J.; Liu, H.; Shen, X.; Jiang, H. Strategy for discovering chemical inhibitors of human cyclophilin A: focused library design, virtual screening, chemical synthesis and bioassay. *J. Comb. Chem.* **2006**, *8*, 326–337.
(18) Wang, F.; Chen, J.; Liu, X.; Shen, X.; He, X.; Jiang, H.; Bai, D. Synthesis and peptidyl-prolyl isomerase inhibitory activity of quinoxalines as ligands of cyclophilin A. *Chem. Pharm. Bull.* **2006**, *54*, 372–376.
(19) Wu, Y.; Belyakov, S.; Choi, C.; Limburg, D.; Thomas, B. E., IV; Vaal, M.; Wei, L.; Wilkinson, D. E.; Holmes, A.; Fuller, M.; McCormick, J.; Connolly, M.; Moeller, T.; Steiner, J.; Hamilton, G. S. Synthesis and biological evaluation of non-peptidic cyclophilin ligands. *J. Med. Chem.* **2003**, *46*, 1112–1115.
(20) Guichou, J. F.; Viaud, J.; Mettling, C.; Subra, G.; Lin, Y. L.; Chavanieu, A. Structure-based design, synthesis, and biological evaluation of novel inhibitors of human cyclophilin A. *J. Med. Chem.* **2006**, *49*, 900–910.
(21) Wang, R.; Gao, Y.; Lai, L. LigBuilder: a multiple-purpose program for structure-based drug design. *J. Mol. Model.* **2000**, *6*, 498–516.
(22) Fan, F.; Zhu, J.; Ni, S.; Cheng, J.; Tang, Y.; Kang, C.; Li, J.; Jiang, H. Pharmacophore refinement and 3D-QSAR studies of inhibitors of cyclophilin A containing amide linker. *QSAR Comb. Sci.* **2009**, *28*, 183–193.
(23) Kofron, J. L.; Kuzmic, P.; Kishore, V.; Colon-Bonilla, E.; Rich, D. H. Determination of kinetic constants for peptidyl prolyl cis−trans isomerases by an improved spectrophotometric assay. *Biochemistry* **1991**, *30*, 6127–6134.
(24) Goodsell, D. S.; Morris, G. M.; Olson, A. J. Automated docking of flexible ligands: applications of AutoDock. *J. Mol. Recognit.* **1996**, *9*, 1–5.
(25) Chen, J.; Lai, L. H. Pocket v.2: Further developments on receptor-based pharmacophore modeling. *J. Chem. Inf. Model.* **2006**, *46*, 2684–2691.
(26) Schneider, G.; Fechner, U. Computer based de novo design of drug-like molecules. *Nature Rev. Drug Discovery* **2005**, *4*, 649–663.
(27) Schneider, G.; Hartenfeller, M.; Reutlinger, M.; Tanrikulu, Y.; Proschak, E.; Schneider, P. Voyages to the (un)known: adaptive design of bioactive compounds. *Trends Biotechnol.* **2009**, *27*, 18–26.
(28) Mauser, H.; Guba, W. Recent developments in de novo design and scaffold hopping. *Curr. Opin. Drug Discovery Dev.* **2008**, *11*, 365–374.