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# *N*-Sulfonylanthranilic Acid Derivatives as Allosteric Inhibitors of Dengue Viral RNA-Dependent RNA Polymerase

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**Abstract:** A novel class of compounds containing *N*-sulfonylanthranilic acid was found to specifically inhibit dengue viral polymerase. The structural requirements for inhibition and a preliminary structure−activity relationship are described. A UV cross-linking experiment was used to map the allosteric binding site of the compound on the viral polymerase.

The World Health Organization has estimated that the four serotypes of the dengue virus (DENV[a]) cause 50−100 million human infections annually worldwide.[1] Approximately 500 000 of the infected individuals progress to dengue hemorrhagic fever, leading to 22 000 deaths.[2] DENV is mosquito-borne and threatens up to 2.5 billion people in more than 100 endemic countries. Although the morbidity of dengue infection is low, its socioeconomic impact is high.[3] Currently there is no vaccine or effective antiviral treatment available.[4] As a result, development of a safe, effective therapeutic agent for DENV is urgently needed.

DENV is a member of the genus *flavivirus* in the family *Flaviviridae*.[5] The viral genome is a single-stranded RNA (∼10.7 kb) of positive polarity. The viral genome encodes three structural proteins and seven nonstructural proteins. The nonstructural protein 5 (NS5) functions as a methyltransferase and an RNA-dependent RNA polymerase (RdRp) that are essential for viral replication.[6] The RdRp is responsible for the synthesis of both minus-sense and positive-sense viral RNAs during replication. Similar to other polymerases, the structure of DENV RdRp exhibits a canonical right-hand conformation and consists of fingers, palm, and thumb domains.[7] The active site of the enzyme is defined by a conserved GDD motif in the palm domain.

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[a]Abbreviations: DENV, dengue virus; RdRp, RNA-dependent RNA polymerase; NS5, nonstructural protein 5; SPA, scintillation proximity assay; GTP, guanine triphoshpate.

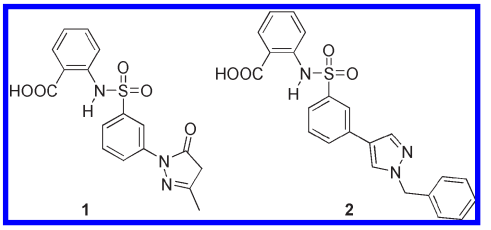
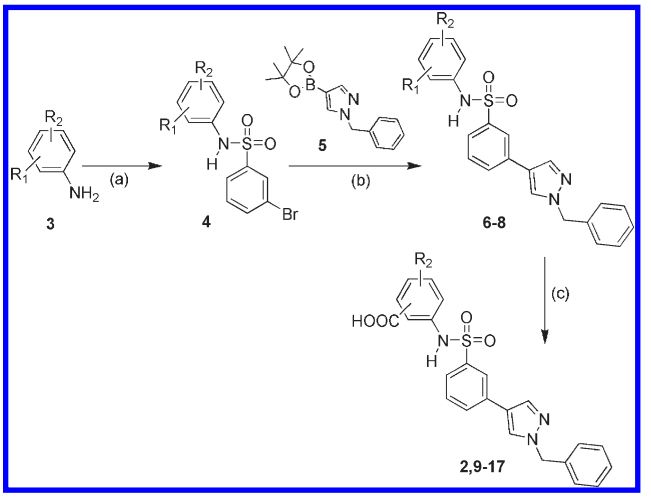



**Figure 1.** High throughput hit **1** and *N*-sulfonylanthranilic acid lead **2**.

**Scheme 1.** Synthesis of Inhibitors **2** and **6**−**17**[a]




[a]Reagents and conditions: (a) 3-bromobenzenesulfonyl chloride, pyridine; (b) $Pd(Ph_3)_4$, $K_2CO_3$, DMF; (c) LiOH, MeOH.

The DENV RdRp is a preferred antiviral target because of the established druggability of this class of viral enzymes,[8] its essentiality for viral replication, its conservation among all four serotypes, and the lack of counterparts in mammalian cells.[9] To date, no non-nucleoside inhibitor of the DENV RdRp has been reported.

In our search for DENV RdRp inhibitors, we developed a scintillation proximity assay (SPA), using a DENV-2 full-length recombinant NS5, polyG as a primer, and biotinylated-oligo C as a template.[10] The assay measured the incorporation of radioactive $^3$H-GTP into the polyC/biotinylated-oligo G complex that was captured on the streptavidin-coated scintillation beads. High throughput screening of the Novartis corporate compound archive led to the identification of 2-[3-(3-methyl-5-oxo-4,5-dihydropyrazol-1-yl)benzenesulfonylamino]benzoic acid (**1**) with an $IC_{50}$ of 7.2 $\mu$M. Derivatization of **1** rapidly produced *N*-sulfonylanthranilic acid (**2**), which had a $IC_{50}$ of 0.7 $\mu$M (Figure 1). In this communication, we report the synthesis and biological activity of this new series of *N*-sulfonylanthranilic acids.

Scheme 1 depicts the general procedure for the synthesis of **2** and derivatives with a fixed benzylpyrazole fragment. 3-Bromobenzenesulfonyl chloride was reacted with appropriate anilines **3** to form sulfonamides **4**, and subsequent coupling with commercially available benzyl pyrazole boronic ester (**5**) through a palladium catalyzed Suzuki reaction[11] gave the desired *N*-sulfonylanthranilic acid analogues.

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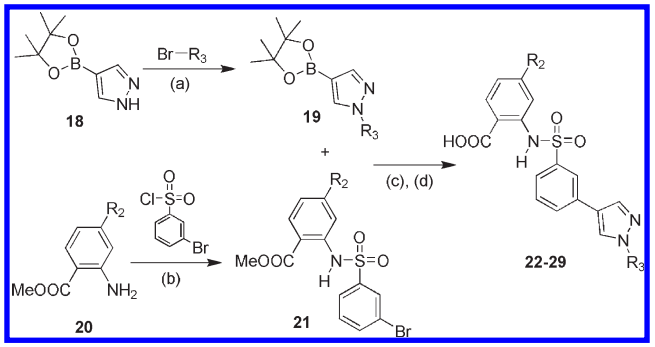
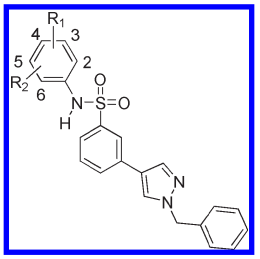
**Scheme 2.** Synthesis of Inhibitors **22**−**29**[a]




[a] Reagents and conditions: (a) $CsCO_3$, $CH_3CN$; (b) pyridine; (c) $Pd(Ph_3)_4$, $K_2CO_3$, DMF; (d) LiOH, MeOH.

**Table 1.** Substitution Effect on the Phenyl Ring of *N*-Sulfonylanthranilic Acids



| compd | $R_1$ | $R_2$ | $IC_{50}$ ($\mu$M) |
|---|---|---|---|
| **2** | 2-COOH | H | 0.7 |
| **6** | H | H | > 20 |
| **7** | 2-COOMe | H | > 20 |
| **8** | 2-$CONH_2$ | H | > 20 |
| **9** | 3-COOH | H | > 20 |
| **10** | 4-COOH | H | > 20 |
| **11** | 2-COOH | 6-Cl | 18.02 |
| **12** | 2-COOH | 5-Cl | 0.77 |
| **13** | 2-COOH | 4-Cl | 2.56 |
| **14** | 2-COOH | 3-Cl | > 20 |
| **15** | 2-COOH | 5-F | 0.37 |
| **16** | 2-COOH | 5-methoxy | 9.99 |
| **17** | 2-COOH | 4,5-dimethoxy | > 20 |

The carboxylic acid targets were produced by hydrolysis of the corresponding methyl esters.

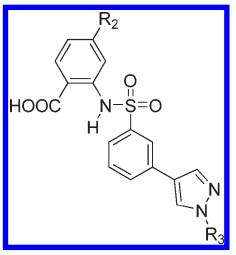
Compounds **22**−**29** were synthesized as shown in Scheme 2. Modifications of the pyrazole were achieved by direct alkylation of commercially available pyrazole boronic ester (**18**) in the presence of cesium carbonate. Suzuki coupling reaction of the modified pyrazole boronic esters **19** with 3-bromobenzenesulfonamides **21** followed by hydrolysis furnished the desired *N*-sulfonylanthranilic acids.

Efforts to understand the structure−activity relationships of the lead molecule started with the carboxylic acid moiety.

As shown in Table 1, removal (**6**) or replacement of carboxylic acid with functional groups such as ester and amide (**7** and **8**) resulted in lower potency compounds, indicating that the carboxylic acid is essential for the activity. Moreover, compounds having the carboxylic acid functional group in meta- and para-position (**9** and **10**) were inactive against RdRp, demonstrating the importance of the position of the carboxylic acid (Table 1).

The effect of substituents on the phenyl ring adjacent to the carboxylic acid was explored subsequently (Table 1). A chlorine atom was well tolerated in the 5-position (**12**), while substitution at the 4- (**13**), 6- (**11**), and 3- (**14**) position led to progressively inferior inhibition. On the basis of this initial structure−activity information, further work focused on the 5-position of the phenyl ring. Replacing the chlorine with the stronger electron-withdrawing fluorine (**15**) improved the activity slightly, while an electron-donating methoxy group (**16**) reduced the activity significantly. These results suggest that electron-withdrawing groups may be generally preferred at the 5-position of the phenyl ring. The results suggest that the electron withdrawing group increases the acidity of the carboxylic acid which may be critical for the interaction with the polymerase enzyme. In agreement with this hypothesis, significant loss of potency was observed when a second methoxy group was introduced at the 4-postion (**17**) (Table 1).

**Table 2.** Replacement of Benzyl on Pyrazole



| compd | $R_2$ | $R_3$ | $IC_{50}$ ($\mu$M) |
|---|---|---|---|
| **2** | H | benzyl | 0.7 |
| **22** | H | H | > 20 |
| **23** | H | Me | > 20 |
| **24** | H | isobutyl | > 20 |
| **25** | H | isopentyl | 6.77 |
| **26** | H | ethylmorpholine | > 20 |
| **27** | H | methyl-2-naphthalene | 0.37 |
| **28** | F | methyl-2-naphthalene | 0.26 |

With 2-[3-(1*H*-pyrazol-4-yl)benzenesulfonylamino]benzoic acid as a fixed fragment, replacement of the benzyl group on the pyrazole ring was investigated to identify the minimum scaffold capable of inhibiting the dengue NS5 polymerase (Table 2). Removal (**22**) or replacement of the benzyl group with small hydrophobic substituents such as methyl (**23**) on pyrazole was detrimental to the activity. Interestingly, while the isobutyl derivative (**24**) was not able to inhibit the dengue polymerase, the compound with an isopentyl replacement (**25**) (one carbon longer aliphatic chain) showed activity at low micromolar range. Moreover, no activity at the concentration tested was observed when a hydrophilic morpholine (**26**) was introduced.

Therefore, the structural feature that gives rise to potency is likely to be a hydrophobic terminal group linked to the headgroup by a defined chain length. This could be demonstrated by the potency increase of the methyl-2-naphthalene replacement (**27**) that extends the hydrophobic substituent. A further (albeit small) improvement in potency was made through a combination of structural fragments showing the best activity. Compound **28** with fluoro substitution on the phenyl ring of carboxylic acid and methyl-2-naphthalene on pyrazole displayed an $IC_{50}$ of 0.26 $\mu$M against the dengue polymerase (Table 2).

Inhibitors **2**, **15**, and **28** were assessed for selectivity against several other polymerases, including human DNA polymerase α and $\beta$ and hepatitis C RdRp. The inhibitors did not inhibit these polymerases, exhibiting $IC_{50}$ of > 20 $\mu$M

**Table 3.** Selectivity Panel

| | $IC_{50}$ ($\mu$M) | | | |
|---|---|---|---|---|
| compd | dengue RdRp | HCV RdRp | human DNA Pol α | human DNA Pol $\beta$ |
| **2** | 0.7 | > 20 | > 20 | > 20 |
| **15** | 0.37 | > 20 | > 20 | > 20 |
| **28** | 0.26 | > 20 | > 20 | > 20 |

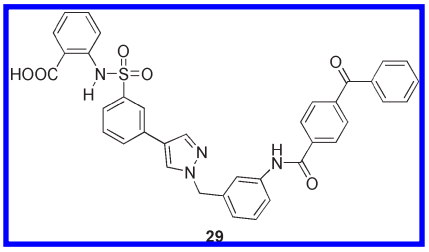



**Figure 2.** Structure of inhibitor **29**.

(Table 3). These results demonstrate that these novel inhibitors specifically suppress DENV RdRp.

*N*-Sulfonylanthranilic acid derivatives seem to inhibit the dengue RdRp during elongation; they can bind to the RdRp alone or to the RNA-RdRp complex, suggesting that compound binding does not interfere with template binding and vice versa (unpublished results). In an effort to map the site of association between the compound and the polymerase, a low micromolar inhibitor (**29**) (Figure 2) (RdRp $IC_{50}$ = 1.5 $\mu$M) tethered with the photoalkylating group[12] benzophenone was synthesized. Under ultraviolet light irradiation, the compound reacted with the protein and irreversibly inhibited the RdRp activity in our assay. On the basis of MALDI MS, a peptide spanning from amino acid position 313 to 326 of DENV-2 NS5 polymerase (QTGSASSMVNGVVR) was covalently linked to **29** ($[M + H]^+$ $m/z$ 2048.8), resulting in a mass increment of 656.17 (data not shown). Since unmodified N-terminal $b_5$ and C-terminal $y_6$ ions were observed in the corresponding MS−MS spectrum, the modification is located within the sequence SSM of the above amino acid sequence.

On the basis of the known reactivity of the n,$\pi$* triplet state of benzophenone[13] and the presence of methionine in the identified peptide sequence (e.g., $m/z$ 1376.7, $-CH_2CH_2S^+$), we assumed that amino acid M − 320 is most likely the cross-linking site.

A computational study was performed to rationalize the photoaffinity labeling result and to identify the possible compound binding site. In the X-ray crystallographic structure of the DENV RdRp (PDB code 2J7U),[7] residues 311−317, 408−415, and 454−466 were not observed. As a consequence, the three-dimensional atomic coordinates for these amino acid sequences were obtained by constructing a homology structure for the DENV RdRp using MOE.[14] The structure 2J7U was used as the primary template, and the Cartesian coordinates for the backbone atoms of the missing amino acids were obtained from the crystal structure of the West Nile virus RdRp (PDB code 2HCN).[15] The rmsd for the backbone atoms between the X-ray structure 2J7U and the homology structure was 1.03 Å. Inhibitor **29** (with the attached benzophenone moiety) was docked into the polymerase binding pocket of the homology model using GOLD.[16] The docked pose that best explained the overall SAR presented above was chosen as a reasonable initial orientation. This modeled complex of the docked ligand and

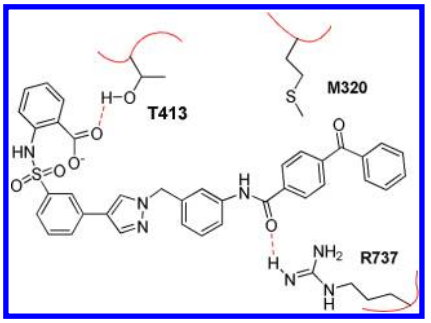



**Figure 3.** Ligand interaction map for inhibitor **29** in the binding pocket. The red dotted lines show the hydrogen bonding interactions between **29** and T413, R737. The backbone of the specific residues are shown as red lines.

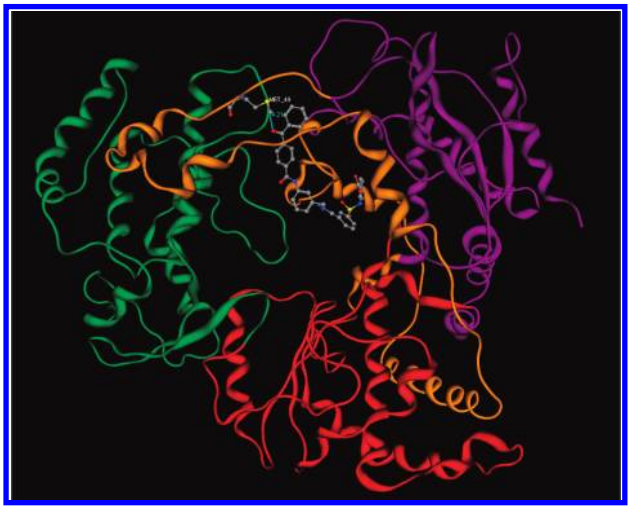


**Figure 4.** Proposed allosteric binding site of *N*-sulfonylanthranilic acid derivative **29** in the dengue polymerase domain.

protein structure was subjected to a molecular dynamics study in which only the loops of the protein and the ligand were allowed to move. As the photo-cross-linking experiments were done at 277 K, a NVT ensemble was used with an initial heating phase of 10 ps. The AMBER99[17] force field in MOE was used for the dynamics simulation with a time step of 1 fs with sampling at every 0.5 ps over a 1 ns equilibration period. A further simulation phase of 0.5 ns was calculated using the same time parameters as before. The distance from the carbonyl O atom in the benzophenone moiety to the S in M320 in the protein was measured for each of the saved structures. It was found that this distance varied between 4.0 and 5.0 Å for 82% of the complex structures during the dynamics, and for 18% of the time of observation, this distance fluctuated between 3.0 and 4.0 Å. The average distance between the O and S atoms during the dynamics was 4.21 Å (Figure 3). The side chain of R737 formed a strong hydrogen bonding donor interaction with the carbonyl bond of the carboxamide linker moiety of **29** at a distance of 2.03 Å. Another such interaction, at a distance of 2.4 Å, was observed between the carboxylate/acid group and the hydroxyl group in the side chain of T413. The hydrogen bond between carobxylate/acid and the hydroxyl group of T413 is likely strengthened by electron withdrawing effect of the additional group at the 5-position of phenyl ring, which is consistent with initial structure−activity relationship study. These two interactions were maintained during the course of the dynamics

simulations, indicating an importance in the stabilization of the compound in the allosteric binding pocket. On the basis of these results, we hypothesize that the reported compounds bind to the dengue RdRp at an allosteric pocket that is located between the finger and the thumb regions as shown in Figure 4.

We have identified *N*-sulfonylanthranilic acid derivatives that can selectively inhibit dengue viral polymerase. Our results suggest that these compounds may bind to a novel allosteric site located between the finger and the thumb regions in dengue RdRp.

While the reported compounds are not potent enough for development, they have revealed an allosteric binding site that may be explored for future dengue NS5 polymerase inhibitors.

**Acknowledgment.** We thank Peiting Zeng for analytical support.

**Supporting Information Available:** Structures, experimental methods, and characterization data for compounds **2**−**29**, UV cross-linking experiment, computational experimental methods, and 3D structure (PDB data file). This material is available free of charge via the Internet at http://pubs.acs.org.

## References

(1) (a) Mackenzie, J. S.; Gubler, D. J.; Petersen, L. R. Emerging flaviviruses: the spread and resurgence of Japanese encephalitis, West Nile and dengue viruses. *Nat. Med.* **2004**, *10* (12, Suppl.), S98–S109. (b) Gubler, D. J. Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. *Trends Microbiol.* **2002**, *10* (2), 100–103. (c) Jacobs, M. G.; Young, P. R. Dengue: a continuing challenge for molecular biology. *Curr. Opin. Infect. Dis.* **1998**, *11*, 319. (d) Monath, T. P. Dengue: the risk to developed and developing countries. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 2395.

(2) (a) Guzman, M. G.; Kouri, G. Dengue: an update. *Lancet Infect. Dis.* **2002**, *2* (1), 33–42. (b) Gubler, D. J. The global emergence/resurgence of arboviral diseases as public health problems. *Arch. Med. Res.* **2002**, *33* (4), 330–342.

(3) Clark, D. V.; Mammen, M. P., Jr.; Nisalak, A.; Puthimethee, V.; Endy, T. P. Economic impact of dengue fever/dengue hemorrhagic fever in Thailand at the family and population levels. *Am. J. Trop. Med. Hyg.* **2005**, *72* (6), 786–791.

(4) (a) Gubler, D. J. Dengue and dengue hemorrhagic fever. *Clin. Microbiol. Rev.* **1998**, *11*, 480–496. (b) Halstead, S. B. Dengue. *Curr. Opin. Infect. Dis.* **2002**, *15* (5), 471–476.

(5) Blok, J. Genetic relationships of the dengue virus serotypes. *J. Gen. Virol.* **1985**, *66*, 1323–1325.

(6) Khromykh, A. A.; Kenney, M. T.; Westaway, E. G. Trans-complementation of Flavivirus RNA Polymerase Gene NS5 by using Kunjin virus replicon-expressing BHK Cells. *J. Virol.* **1998**, *72* (9), 7270–7279.

(7) Yap, T. L.; Xu, T.; Chen, Y. L.; Malet, H.; Egloff, M. P.; Canard, B.; Vasudevan, S. G.; Lescar, J. Crystal structure of the dengue virus RNA-dependent RNA polymerase catalytic domain at 1.85-angstrom resolution. *J. Virol.* **2007**, *81* (9), 4753–4765.

(8) (a) De Clercq, E. Recent highlights in the development of new antiviral drugs. *Curr. Opin. Microbiol.* **2005**, *8*, 552–560. (b) De Francesco, R.; Carfi, A. Advances in the development of new therapeutic agents targeting the NS3-4A serine protease or the NS5B RNA-dependent RNA polymerase of the hepatitis C virus. *Adv. Drug Delivery Rev.* **2007**, *59*, 1242–1262.

(9) Rawlinson, S. M.; Pryor, M. J.; Wright, P. J.; Jans, D. A. Dengue virus RNA polymerase NS5: a potential therapeutic target? *Curr. Drug Targets* **2006**, *7*, 1623–1638.

(10) The reaction was performed in 96-well, half area plates. Compounds were incubated at various concentrations with 50 nM purified recombinant dengue 2 NS5 polymerase and annealed poly(C)/biotin oligo G as the template, in 50 mM Tris-HCl, pH 7.0, 10 mM KCl, 2 mM $MgCl_2$, 2 mM $MnCl_2$, 1 mM $\beta$-mercaptoethanol, and 0.05% CHAPS buffer for 1 h at room temperature. The reaction was initiated by addition of 0.4 $\mu$M GTP and 0.5 $\mu$Ci [$^3$H]GTP in a total reaction volume of 50 $\mu$L for 1 h at room temperature. The reaction was stopped by adding equal volumes of 25 mM Tris-HCl, pH 7.5, 75 mM NaCl, 20 mM EDTA, and 0.15 mg of streptavidine coated scintillation beads per well. After 1 h, the reaction was quantified using a scintillation counter. Each concentration was tested in duplicate, and the data were reported as an average of at least two experiments.

(11) Miyaura, N; Suzuki, A. Palladium-catalyzed cross-coupling reactions of organoboron compounds. *Chem. Rev.* **1995**, *95*, 2457–2483.

(12) Al Mawsawi, L. Q.; Fikkert, V.; Dayam, R.; Witvrouw, M.; Burke, T. R., Jr.; Borchers, C. H.; Neamati, N. Discovery of a small-molecule HIV-1 integrase inhibitor-binding site. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 10080–10085.

(13) Prestwich, G. D.; Dorman, G.; Elliott, J. T.; Marecak, D. M.; Chaudhary, A. Benzophenone photoprobes for phosphoinositides, peptides and drugs. *Photochem. Photobiol.* **1997**, *65*, 222–234.

(14) *Molecular Operating Environment*, version 2007.0902; Chemical Computing Group, Inc.: Montreal, Quebec, Canada, 2007.

(15) Malet, H.; Egloff, M.-P.; Selisko, B.; Butcher, R. E.; Wright, P. J.; Roberts, M.; Gruez, A.; Sulzenbacher, G.; Vonrhein, C.; Bricogne, G.; Mackenzie|, J. M.; Khromykh, A. A.; Andrew, D.; Davidson, A. D.; Canard, B. Crystal structure of the RNA polymerase domain of the West Nile virus non-structural protein 5. *J. Biol. Chem.* **2007**, *282* (14), 10678–10689.

(16) Jones, G.; Willett, P.; Glen, R. C. Molecular recognition of receptor sites using a genetic algorithm with a description of desolvation. *J. Mol. Biol.* **1995**, *245*, 43–53.

(17) Cieplak, P.; Caldwell, J.; Kollman, P. Molecular mechanical models for organic and biological systems going beyond the atom centered two body additive approximation: aqueous solution free energies of methanol and *N*-methyl acetamide, nucleic acid base, and amide hydrogen bonding and chloroform/water partition coefficients of the nucleic acid bases. *J. Comput. Chem.* **2001**, *22*, 1048–1057.