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# Synthesis and biological evaluation of novel 2-pyridinyl-[1,2,3]triazoles as inhibitors of transforming growth factor β1 type 1 receptor

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**Abstract**—A series of 2-pyridinyl-[1,2,3]triazoles have been synthesized and evaluated for their ALK5 inhibitory activity in the luciferase reporter assays. Compound **8d** showed significant ALK5 inhibition (SBE-luciferase activity, 25%; p3TP-luciferase activity, 17%) at a concentration of 5 μM that is comparable to that of SB-431542 (SBE-luciferase activity, 21%; p3TP-luciferase activity, 12%), but weak p38α MAP kinase inhibition (13%) at a concentration of 10 μM that is much lower than that of SB-431542 (54%).


Chronic tissue fibrosis in the major organs such as kidney, liver, lung, heart, and skin present major medical problems ranging from disfigurement to progressive disability and death. Much attention is focused on the roles of the many cytokines and growth factors, among them, transforming growth factor β1 (TGF-β1) plays a central role in excessive accumulation of extracellular matrix by both stimulating the expression of matrix components such as collagens, fibronectin, and matrix proteoglycans and inducing inhibitors of matrix degrading metalloproteinases and plasminogen-activator inhibitor.[1] TGF-β1 transduces signals through two highly conserved single transmembrane serine/threonine kinases, the type I and type II TGF-β receptors (TβR-I and TβR-II, respectively).[2] TβR-II activates TβR-I upon formation of the ligand–receptor complex by hyperphosphorylating serine/threonine residues in the GS region of the TβR-I or activin-like kinase (ALK5), which creates a binding site for Smad proteins. The activated TβR-I in turn phosphorylates Smad2/Smad3 proteins at the C-terminal SSXS-motif thereby causing dissociation from the receptor and heteromeric complex formation with Smad4. Smad complexes translocate to the nucleus, assemble with specific DNA-binding co-factors and co-modulators to finally activate transcription of extracellular matrix components, and inhibitors of matrix-degrading proteases.[2] Therefore, it becomes evident that inhibition of ALK5 phosphorylation of Smad2/Smad3 could reduce TGF-β1-induced excessive accumulation of extracellular matrix. The 4-pyridyl substituted triarylimidazoles are known to be selective inhibitors of p38 MAP kinase since a 4′-nitrogen atom is involved in a required hydrogen bond to the ATP binding site of p38 MAP kinase.[3] Callahan et al. have recently reported that the 2-pyridyl substituted triarylimidazoles are selective inhibitors of ALK5 over p38 MAP kinase, suggesting that there may be an alternative ATP binding site available to ALK5 inhibitors.[4] SB-431542, one of the leading 2-pyridyl substituted triarylimidazole, inhibits the in vitro phosphorylation of immobilized Smad3 with an $IC_{50}$ of 94 nM.[4] It inhibits also the activin type 1 receptor ALK4 and the nodal type 1 receptor ALK7, which are very highly related to ALK5 in their kinase domains, but has no effect on the other ALK family members that recognize bone morphogenetic proteins, on components of the ERK, JNK, or p38 MAP kinase pathways, or on components of the signaling pathways activated in response to serum.[5] The 4(5)-(2-pyridinyl)-[1,2,3]triazoles having no substituent, a methyl or an ethyl substituent at the 2-position of the triazole ring have recently been claimed as selective ALK5 inhibitors, but their specific ALK5 inhibitory activity has not been disclosed.[6] On the basis of these findings, we have synthesized a series

*Keywords*: 2-Pyridinyl-[1,2,3]triazoles; Inhibitors; TGF-β1 type 1 receptor; ALK5.
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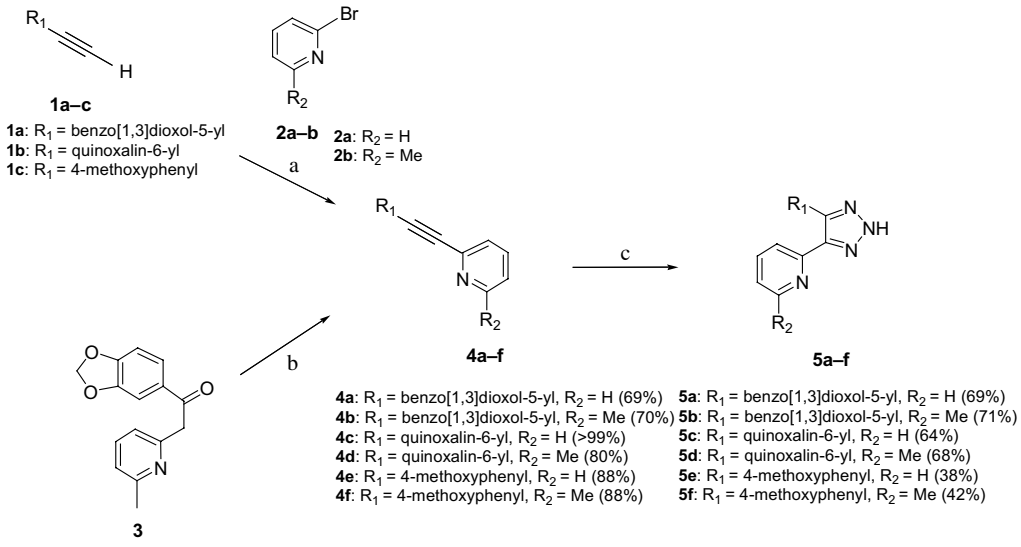
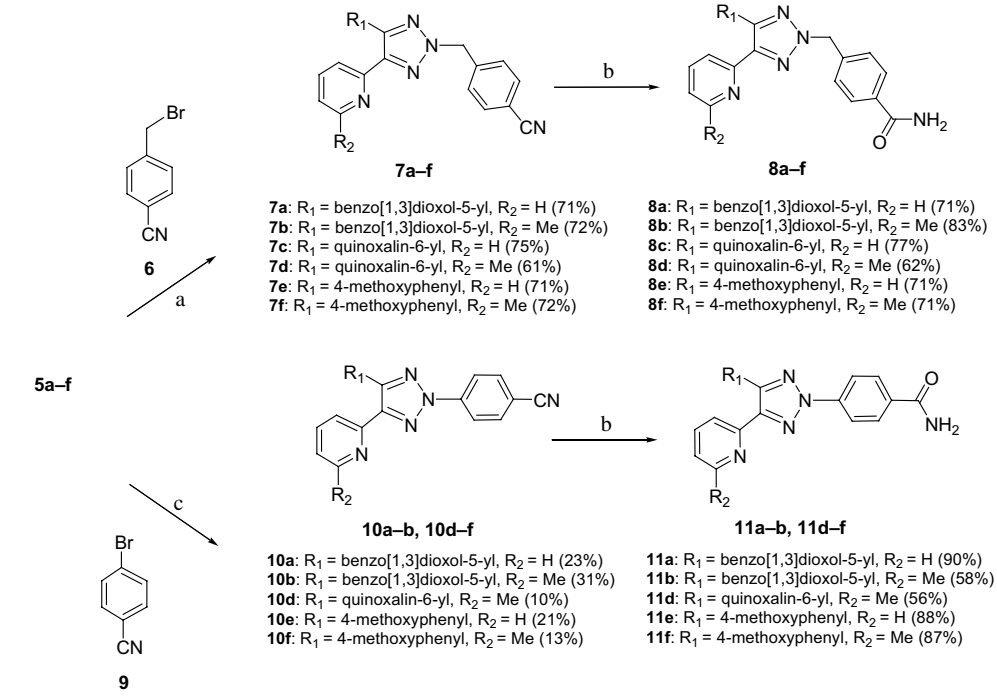
of 4-aryl-5-(2-pyridinyl)-2-(4-substituted-benzyl)-[1,2,3]-triazoles **7a**–**f** and **8a**–**f** and 4-aryl-5-(2-pyridinyl)-2-(4-substituted-phenyl)-[1,2,3]triazoles **10a**–**b**, **10d**–**f**, **11a**–**b**, and **11d**–**f**, and evaluated their ALK5 inhibitory activity in the luciferase reporter assays.

The 4-aryl-5-(2-pyridinyl)-[1,2,3]triazoles **5a**–**f** were prepared as shown in Scheme 1. Coupling of 5-ethynylbenzo[1,3]dioxolane (**1a**),[7] 6-ethynylquinoxaline (**1b**),[8] or 1-ethynyl-4-methoxybenzene (**1c**) with either 2-bromopyridine (**2a**) or 2-bromo-6-methylpyridine (**2b**) in a mixture of THF and TMEDA in the presence of CuI and $Pd(PPh_3)_4$ catalysts gave the disubstituted acetylene intermediates **4a** and **4c**–**f** in good yields. Reaction of 1-benzo[1,3]dioxol-5-yl-2-(6-methylpyridin-2-yl)ethanone (**3**)[9] with trifluoromethanesulfonic anhydride and *N*,*N*-diisopropylethylamine in $CH_2Cl_2$ produced the disubstituted acetylene **4b** in 70% yield. Cycloaddition of the acetylenes **4a**–**f** with $TMSN_3$ in DMF afforded the [1,2,3]-triazoles **5a**–**f** in 38–71% yields.[6] Alkylation of **5a**–**f** with 4-cyanobenzyl bromide (**6**) using NaH as a base and *n*-$Bu_4NI$ in THF yielded 2-(4-cyanobenzyl)-




**Scheme 1.** Reagents and conditions: (a) CuI, $Pd(PPh_3)_4$, TMEDA, THF, 55 °C, 10 h; (b) $(CF_3SO_2)_2O$, *N*,*N*-diisopropylethylamine, $CH_2Cl_2$, reflux, overnight; (c) $TMSN_3$, DMF, reflux, 14 h.



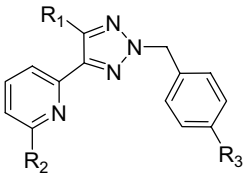

**Scheme 2.** Reagents and conditions: (a) 60% NaH, *n*-$Bu_4NI$, THF, rt, 1 h; (b) 28% $H_2O_2$, 6 N NaOH, 95% EtOH, 55 °C, 3 h; (c) $K_2CO_3$, DMSO, 150 °C, 14 h.

triazoles **7a**–**f** as major positional isomers (61–75% yields). Conversion of the nitrile functionality of the compounds **7a**–**f** to carboxamide was accomplished by treatment with 28% $H_2O_2$ and 6 N NaOH solution to afford the triazoles **8a**–**f** in 62–83% yields. Alternatively, compounds **5a**–**f** were tried to alkylate with 4-bromobenzonitrile (**9**) with $K_2CO_3$ in DMSO at 150 °C. Although **5c** failed to give the desired alkylated product under this condition, other 2-(4-cyanophenyl)triazoles **10a**–**b** and **10d**–**f** could be obtained in rather low yields (10–31%). The carboxamides **11a**–**b** and **11d**–**f** were produced from **10a**–**b** and **10d**–**f** in 56–90% yields in the same reaction condition as for **8a**–**f** (Scheme 2).

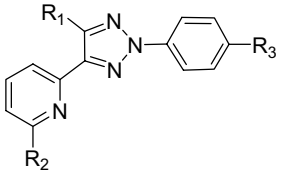
To investigate whether these potential inhibitors could inhibit TGF-β-induced downstream transcriptional activation to ALK5 signaling, two different luciferase reporter genes, SBE-Luc reporter construct containing four tandem copies of the CAGA Smad binding element cloned upstream of the adenovirus major late promoter (MLP)[10] and p3TP-Lux reporter construct containing three AP-1 binding elements and the plasminogen activator inhibitor-1 (PAI-1) promoter[11] were used for this analysis. HepG2 cells were transiently transfected with either SBE-Luc reporter construct or p3TP-Lux reporter construct. Immediately after transfection, cells were treated with DMSO alone or 5 μM of inhibitors dissolved in DMSO followed by TGF-β (5 ng/mL). After 24 h incubation, luciferase activity in cell lysates was determined by using a luciferase assay system (Promega) (Table 1).

Of the 4-aryl-5-(2-pyridinyl)-2-(4-substituted-benzyl)-[1,2,3]triazoles **7a**–**f** and **8a**–**f**, the benzo[1,3]dioxolyl

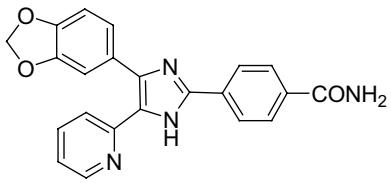
**Table 1.** Effect of 2-pyridinyl-[1,2,3]triazoles on the activity of TGF-β-induced ALK5 and p38α MAP kinase



**7a–f, 8a–f**



**10a–b, 10d–f, 11a–b, 11d–f**

| Compound | $R_1$ | $R_2$ | $R_3$ | Activity (% control)[a] | | |
|---|---|---|---|---|---|---|
| | | | | SBE-luciferase[b] | p3TP-luciferase[b] | p38α MAP kinase[c] |
| **7a** | Benzo[1,3]dioxol-5-yl | H | CN | 28 | 18 | 85 |
| **7b** | Benzo[1,3]dioxol-5-yl | Me | CN | 25 | 25 | 89 |
| **7c** | Quinoxalin-6-yl | H | CN | 62 | 35 | 81 |
| **7d** | Quinoxalin-6-yl | Me | CN | 27 | 16 | 92 |
| **7e** | 4-Methoxyphenyl | H | CN | 70 | 98 | 102 |
| **7f** | 4-Methoxyphenyl | Me | CN | 53 | 55 | 98 |
| **8a** | Benzo[1,3]dioxol-5-yl | H | $CONH_2$ | 68 | 39 | 82 |
| **8b** | Benzo[1,3]dioxol-5-yl | Me | $CONH_2$ | 26 | 18 | 80 |
| **8c** | Quinoxalin-6-yl | H | $CONH_2$ | 60 | 46 | 80 |
| **8d** | Quinoxalin-6-yl | Me | $CONH_2$ | 25 | 17 | 87 |
| **8e** | 4-Methoxyphenyl | H | $CONH_2$ | 68 | 60 | 96 |
| **8f** | 4-Methoxyphenyl | Me | $CONH_2$ | 40 | 28 | 94 |
| **10a** | Benzo[1,3]dioxol-5-yl | H | CN | 84 | 60 | 91 |
| **10b** | Benzo[1,3]dioxol-5-yl | Me | CN | 103 | 109 | 105 |
| **10d** | Quinoxalin-6-yl | Me | CN | 51 | 37 | 95 |
| **10e** | 4-Methoxyphenyl | H | CN | 76 | 110 | 102 |
| **10f** | 4-Methoxyphenyl | Me | CN | 78 | 75 | 99 |
| **11a** | Benzo[1,3]dioxol-5-yl | H | $CONH_2$ | 60 | 39 | 82 |
| **11b** | Benzo[1,3]dioxol-5-yl | Me | $CONH_2$ | 27 | 21 | 70 |
| **11d** | Quinoxalin-6-yl | Me | $CONH_2$ | 33 | 17 | 93 |
| **11e** | 4-Methoxyphenyl | H | $CONH_2$ | 79 | 61 | 96 |
| **11f** | 4-Methoxyphenyl | Me | $CONH_2$ | 49 | 33 | 96 |
| SB-431542 |  | | | 21 | 12 | 46 |

[a] Activity is given as the mean of triplicate determinations relative to control incubations with DMSO vehicle.
[b] Luciferase activity was determined with 5 μM of inhibitor.
[c] Kinase activity was determined with 10 μM of inhibitor.

analogues **7a** (SBE-luciferase activity, 28%; p3TP-luciferase activity, 18%), **7b** (SBE-luciferase activity, 25%; p3TP-luciferase activity, 25%), and **8b** (SBE-luciferase activity, 26%; p3TP-luciferase activity, 18%), and the quinoxalinyl analogues **7d** (SBE-luciferase activity, 27%; p3TP-luciferase activity, 16%) and **8d** (SBE-luciferase activity, 25%; p3TP-luciferase activity, 17%) displayed significant ALK5 inhibition at 5 μM compared to that of control, that is comparable to that of SB-431542 (SBE-luciferase activity, 21%; p3TP-luciferase activity, 12%). However, the 4-methoxyphenyl analogues **7e**, **7f**, **8e**, and **8f** showed much lower ALK5 inhibition compared to the benzo[1,3]dioxolyl analogues and the quinoxalinyl analogues. Introduction of a methyl substituent at the 6-position of the pyridine ring moiety markedly increased their ALK5 inhibitory activity (**7c** vs **7d**; **7e** vs **7f**; **8a** vs **8b**; **8c** vs **8d**; **8e** vs **8f**). Although compound **7a** showed much higher ALK5 inhibition compared to **8a**, most of the carbonitrile analogues showed the similar level of ALK5 inhibition compared to their corresponding carboxamide derivatives (**7b** vs **8b**; **7c** vs **8c**; **7d** vs **8d**). Among the 4-aryl-5-(2-pyridinyl)-2-(4-substituted-phenyl)-[1,2,3]triazoles **10a**–**b**, **10d**–**f**, **11a**–**b**, and **11d**–**f**, the benzo[1,3]dioxolyl analogue **11b** (SBE-luciferase activity, 27%; p3TP-luciferase activity, 21%) and the quinoxalinyl analogue **11d** (SBE-luciferase activity, 33%; p3TP-luciferase activity, 17%) displayed significant ALK5 inhibition, but inhibitory activity of most this series of compounds were lower than those of the corresponding 2-(4-substituted-benzyl) series of compounds. The most potent compound **8d** was chosen and its ALK5 inhibition was determined at different concentrations using HepG2 cells transiently transfected with SBE-Luc reporter construct. As shown in Figure 1, **8d** inhibited ALK5 in a dose-dependent manner (SBE-luciferase activity: 0.05 μM, 93%; 0.1 μM, 70%; 1.0 μM, 31%; 5.0 μM, 24%). P38α MAP kinase is the only kinase to be significantly affected in vitro on a panel of 24 kinases unrelated to the ALKs by SB-431542.[5] Therefore, in vitro kinase assay on this enzyme was performed in the presence of 10 μM of inhibitors. SB-431542 displayed 54% inhibition at this concentration, however, the most inhibitory compound **11b** showed 30% inhibition, and the rest of compounds including **8d** (13%) also exhibited weak or no measurable inhibition for this enzyme (<20%), thus showing high selectivity (Table 1).

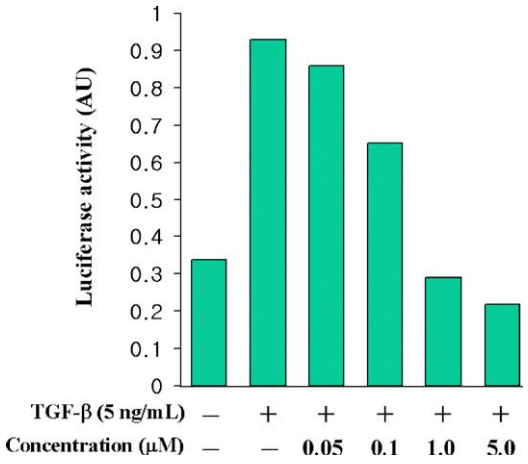



**Figure 1.** Effect of **8d** on the activity of TGF-β-induced ALK5. HepG2 cells were transiently transfected with SBE-Luc reporter construct. Luciferase activity was determined in the presence of different concentrations of **8d** and is given as the mean of triplicate determinations relative to control incubations with DMSO vehicle.

To examine a possible binding mode of **8d** at the active site of ALK5, we built a docking model of ALK5:**8d** complex using the flexible docking algorithms (FlexiDock and FlexX).[12] As demonstrated in Figure 2, the inhibitor **8d** lies in the same binding pocket of ALK5 as is occupied by NPC-30345 in the X-ray structure.[13] All three substituents attached to the central triazole ring of **8d** form various hydrogen bonds (H-bonds) with amino acid residues in the binding pocket. The carboxamide group of phenyl substituent forms H-bonds with backbone amide of Ile211 and Ser287. The nitrogen atom in the 2-pyridinyl moiety forms strong H-bond with the

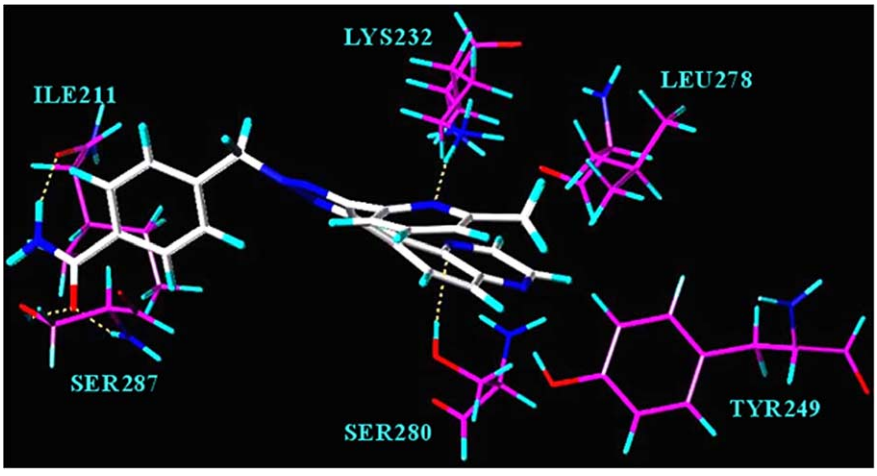



**Figure 2.** The binding mode of **8d** observed in the docking model. The amino acid residues within the ALK5-binding site are represented in line form. Carbon atoms of amino acids are colored in magenta. The ligand is rendered in capped stick. Yellow dotted lines are hydrogen bonding interactions (<2.5 Å) between **8d** and the amino acid residues (Ile211, Lys232, Ser280, and Ser287).

positively charged amino protons of the Lys232 side chain. Finally, a nitrogen atom of quinoxalinyl ring forms H-bond with the OH of Ser280. In addition, quinoxalinyl ring is in close contact with Leu278, and the 6-methyl group of 2-pyridinyl moiety occupies a hydrophobic cavity consisted of Leu278 and Phe262, possibly forming hydrophobic interactions. Taking into account that Leu278 and Ser280 residues are poorly conserved in other kinases,[14] the observed interactions between these residues and quinoxalinyl ring and 6-methyl group of 2-pyridinyl moiety of **8d** would play a crucial role in selective inhibition of ALK5 activity. The binding mode of **8d** generated by flexible docking studies revealed that the structure of ligand is compatible with the (NPC-30345)-binding cavity of ALK5,[13] and supported the selective activity of **8d** for ALK5.

On the basis of these results and its excellent aqueous solubility data (**8d**, >100 mg/mL; SB-431542, <1 mg/mL in 1 N HCl), compound **8d** has been selected as a preclinical candidate, and further toxicological and pharmacological evaluation is currently undergoing in our laboratory. These data will be published in due course in elsewhere.

## Acknowledgements

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12. *Preparation of 3D—molecular structure and conformational search*: The structure of the ligand (**8d**) was prepared in. MOL2 format using the sketcher module of sybyl 6.9 (SYBYL molecular modeling software, Tripos Inc.: St. Louis, MO, USA, 2003), Gasteiger–Huckel charges were assigned to the ligand atoms, and then energy-minimized until converged to a maximum derivative of 0.001 kcal $mol^{-1}$ $Å^{-1}$. To obtain a global minimum energy conformation of **8d**, molecular dynamics was run with a simulated annealing protocol. The calculation followed the temperature protocol beginning at 700 K and gradually cooling down to 200 K for 1000 fs and was run for five cycles. The dynamics result was analyzed and 200 conformers were randomly selected. The selected conformers were briefly minimized and the final coordinates were saved into a database.
*Docking*: The X-ray crystal structure of ALK5:NPC-30345 complex (pdb entry = 1IAS)[13] was retrieved from the Protein Data Bank (PDB). The active site was defined as all the amino acid residues enclosed within 6.5 Å radius sphere centered by the bound ligand, NPC-30345. For the docking of the conformer library of **8d** into the target active site, the FlexX docking and subsequent scoring were performed using the default parameters of the FlexX program implanted in the sybyl 6.9. Final score for FlexX solutions per conformer was calculated by a standard scoring function, and used for ranking. Top-ranked conformer of **8d** complexed with ALK5 was selected, and then FlexiDock was run to build a final optimized model. The binding site was defined as all residues within 6.5 Å distance from **8d**. (All basic amino acid residues lining the binding site were positively charged.) Rotatable bonds of these residues, primarily the side chain single bonds, were allowed conformational flexibility in the docking process, while the backbone and remaining bonds were held rigid. In the initial FlexX-docked structure of ALK5:**8d** complex, Ser280, and Ser287 are positioned within the distance that could form H-bonds with **8d**. Therefore, FlexiDock was performed with the introduction of constraints for formation of hydrogen bond between selected donor–acceptor atoms (i.e., donor in protein: Ser280 and Ser287) in the active site. Flexidock provided nearly 20 (maximum number of generations to allow: 30,000) solutions for each docking experiment. Each of these structures was minimized to eliminate bad electronic and/or steric contacts, and the structure with lowest energy was selected as a final model shown in Figure 2.
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