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Bioorganic & Medicinal Chemistry 12 (2004) 2013–2020
Bioorganic & Medicinal Chemistry

Design, synthesis, and biological evaluation of novel 2-pyridinyl-[1,2,4]triazoles as inhibitors of transforming growth factor β1 type 1 receptor

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Received 30 January 2004; revised 2 March 2004; accepted 2 March 2004

Abstract—A series of 2-pyridinyl-[1,2,4]triazoles have been synthesized and evaluated for their ALK5 inhibitory activity in the luciferase reporter assays. Compound **12b** showed significant ALK5 inhibition (SBE-Luciferase, 73%; p3TP-Luciferase, 85%) at a concentration of 5 μM that is comparable to that of SB-431542 (SBE-Luciferase, 79%; p3TP-Luciferase, 88%), but weak p38α MAP kinase inhibition (4%) at a concentration of 10 μM that is much lower than that of SB-431542 (54%). The binding mode of **12b** generated by flexible docking studies revealed that the structure of **12b** is a good fit into the (NPC-30345)-binding cavity of ALK5.

1. Introduction

Progressive tissue fibrosis in the major organs such as kidney, liver, lung, heart, and skin can lead to significant organ dysfunction and resulting patient morbidity and mortality. Many cytokines and growth factors are involved in fibrogenesis, 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] Upon ligand induced oligomerization, TβR-II hyperphosphorylates serine/threonine residues in the GS region of the TβR-I or activin-like kinase 5(ALK5), which leads to activation of TβR-I by creating 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] On the basis of these findings, we have synthesized a series of 2-pyridinyl-[1,2,4]triazoles **6a**–**f**, **7a**–**f**, **11a**–**d**, and **12a**–**d**, and evaluated their ALK5 inhibitory activity in the luciferase reporter assays.

Keywords: 2-Pyridinyl-[1,2,4]triazoles; Inhibitors; TGF-β1 type 1 receptor; ALK5.
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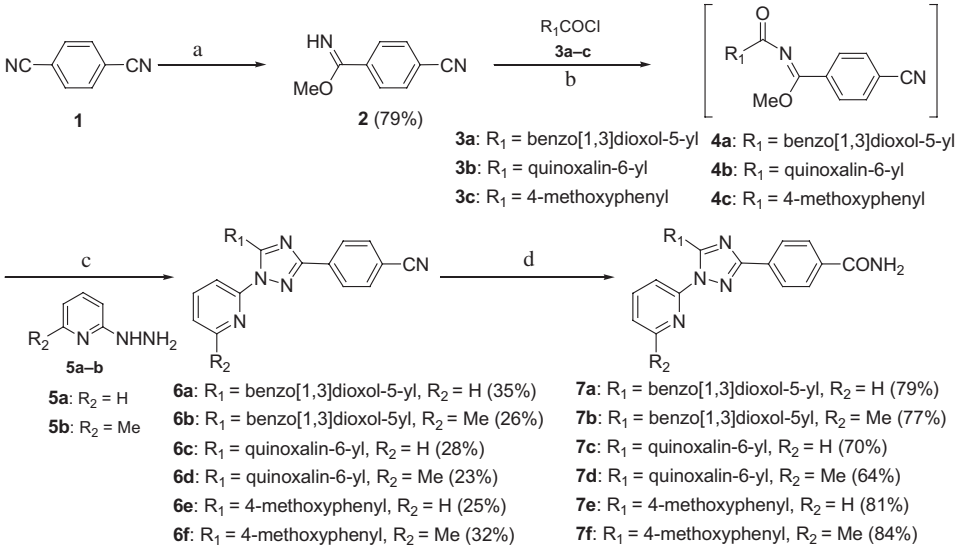
doi:10.1016/j.bmc.2004.03.004

2. Results and discussion

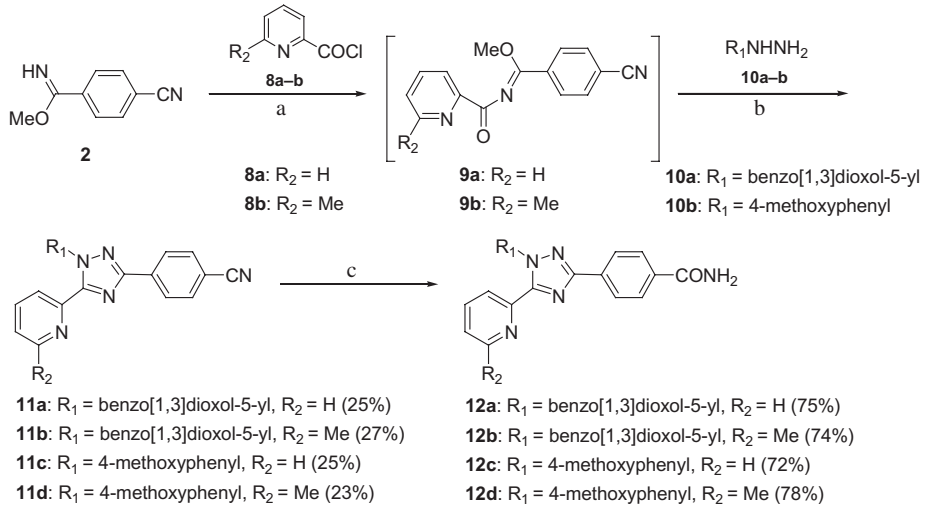
The 1-(2-pyridinyl)-[1,2,4]triazoles **6a**–**f** and **7a**–**f** and the 5-(2-pyridinyl)-[1,2,4]triazoles **11a**–**d** and **12a**–**d** were prepared as shown in Schemes 1 and 2, respectively.

Treatment of 1,4-dicyanobenzene (**1**) with NaOMe in MeOH gave 4-cyanobenzimidic acid methyl ester (**2**)[6] in 79% yield. Coupling of the imidate **2** with the aroyl chlorides **3a**–**c**[7] in CH_2Cl_2 in the presence of Et_3N and a catalytic amount of DMAP followed by cyclization of the resulting unstable *N*-aroylimidates **4a**–**c** with 2-hydrazinopyridine (**5a**) or 2-hydrazino-6-methylpyridine (**5b**)[8] according to the method of Baccar and Barrans[9] yielded the 1-(2-pyridinyl)-[1,2,4]triazols **6a**–**f** in 23–35% yields. Conversion of the nitrile functionality of the compounds **6a**–**f** to carboxamide was accomplished by treatment with 28% H_2O_2 and 6 N NaOH solution to afford the triazoles **7a**–**f** in 64–84% yields.

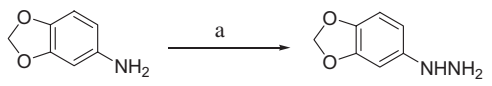
The imidate **2** was reacted with picolinoyl chloride (**8a**) or 6-methylpicolinoyl chloride (**8b**) in toluene in the presence of Et_3N to give *N*-picolinoylimidates **9a**–**b**, which were subsequently treated with benzo[1,3]dioxol-5-ylhydrazine (**10a**) or 4-methoxyphenylhydrazine (**10b**) to afford the 5-(2-pyridinyl)-[1,2,4]triazoles **11a**–**d** in 23–27% yields. The carboxamides **12a**–**d** were produced from **11a**–**d** in 72–78% yields in the same reaction condition shown at Scheme 1. The unknown hydrazine **10a** was prepared by diazotization of 3,4-(methylenedioxy)aniline (**13**) with $NaNO_2$ and concentrated HCl followed by reduction of the resulting diazonium salt



Scheme 1. Reagents and conditions: (a) NaOMe (1.0 equiv), MeOH, rt, 22 h; (b) R_1COCl (**3a**–**c**) (1.0 equiv), Et_3N (1.1 equiv), DMAP (cat.), CH_2Cl_2, 30 °C, 6 h; (c) **5a**–**b** (1.0 equiv), rt, 6 h; (d) 28% H_2O_2, 6 N NaOH, 95% EtOH, 55 °C, 3 h.



Scheme 2. Reagents and conditions: (a) **8a**–**b** (1.2 equiv), Et_3N (2.0 equiv), toluene, 40 °C, 12 h; (b) R_1NHNH_2 (**10a**–**b**) (1.0 equiv), CH_2Cl_2, rt, 6 h; (c) 28% H_2O_2, 6 N NaOH, 95% EtOH, 55 °C, 3 h.



13 **10a** (26%)

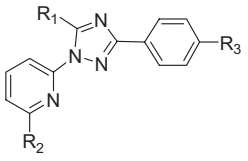
Scheme 3. Reagents and conditions: (a) (i) $NaNO_2$ (2.5 equiv), concd HCl (10 equiv), H_2O, 0 °C, 30 min, (ii) $SnCl_2{\cdot}2H_2O$ (2.3 equiv), concd HCl, 15 °C.

with $SnCl_2{\cdot}2H_2O$ in concentrated HCl in 26% yield (Scheme 3).

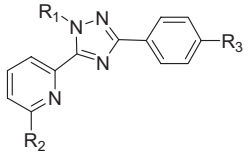
To investigate whether these potential inhibitors could inhibit TGF-β-induced downstream transcriptional activation to ALK5 signaling, two different luciferase reporter genes, SBE-Luc reporter construct containing four tandem copies of the CAGA Smad binding element cloned upstream of the adenovirus major 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).

Among the 1-(2-pyridinyl)-[1,2,4]triazoles, the benzo-[1,3]dioxolyl analogue **7b** (SBE-Luciferase, 54%; p3TP-Luciferase, 67%) and the quinoxalinyl analogue **7d** (SBE-Luciferase, 45%; p3TP-Luciferase, 60%) displayed modest ALK5 inhibition at 5 μM compared to that of control. Of the 5-(2-pyridinyl)-[1,2,4]triazoles, the benzo[1,3]dioxolyl analogue **11b** displayed modest ALK5 inhibition (SBE-Luciferase, 52%; p3TP-Luciferase, 59%), but its carboxamide derivative **12b** showed significantly improved ALK5 inhibitory activity (SBE-Luciferase, 73%; p3TP-Luciferase, 85%) that is comparable to that of SB-431542 (SBE-Luciferase, 79%; p3TP-Luciferase, 88%). P38α MAP kinase is the only kinase to be significantly affected in vitro on a panel of

Table 1. Effect of 2-pyridinyl-[1,2,4]triazoles on the activity of TGF-β-induced ALK5 and p38α MAP kinase

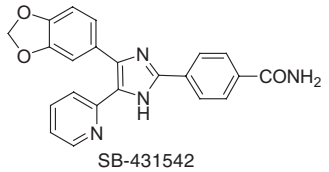


6a–f, 7a–f



11a–d, 12a–d

Compound	R_1	R_2	R_3	Activity (% control)[a]		
				SBE-Luciferase[b]	p3TP-Luciferase[b]	p38α MAP Kinase[c]
6a	Benzo[1,3]dioxol-5-yl	H	CN	86	80	98
6b	Benzo[1,3]dioxol-5-yl	Me	CN	77	99	97
6c	Quinoxalin-6-yl	H	CN	88	83	102
6d	Quinoxalin-6-yl	Me	CN	87	91	105
6e	4-Methoxyphenyl	H	CN	66	74	95
6f	4-Methoxyphenyl	Me	CN	105	64	99
7a	Benzo[1,3]dioxol-5-yl	H	$CONH_2$	75	79	103
7b	Benzo[1,3]dioxol-5-yl	Me	$CONH_2$	46	33	106
7c	Quinoxalin-6-yl	H	$CONH_2$	95	55	108
7d	Quinoxalin-6-yl	Me	$CONH_2$	55	40	101
7e	4-Methoxyphenyl	H	$CONH_2$	93	67	100
7f	4-Methoxyphenyl	Me	$CONH_2$	94	43	110
11a	Benzo[1,3]dioxol-5-yl	H	CN	93	72	96
11b	Benzo[1,3]dioxol-5-yl	Me	CN	48	41	94
11c	4-Methoxyphenyl	H	CN	91	75	98
11d	4-Methoxyphenyl	Me	CN	62	53	106
12a	Benzo[1,3]dioxol-5-yl	H	$CONH_2$	77	76	98
12b	Benzo[1,3]dioxol-5-yl	Me	$CONH_2$	27	15	96
12c	4-Methoxyphenyl	H	$CONH_2$	86	78	95
12d	4-Methoxyphenyl	Me	$CONH_2$	56	34	102
				21	12	46



SB-431542

[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.

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. All the 2-pyridinyl-[1,2,4]triazoles tested exhibited weak or no measurable inhibition for p38α MAP kinase (<10%), thus showing high selectivity (Table 1).

Previous study suggested that key features of triarylimidazole ALK5 inhibitors are the 2-pyridinyl moiety and a substituent containing terminal hydrogen bond donor group (carboxylic acid or carboxamide).[4] The X-ray structure of ALK5 complexed with inhibitor NPC-30345 revealed that the inhibitor occupies not only the space for ATP but also the backside of the ATP binding pocket by contacting Leu278 and Ser280.[12] This region is poorly conserved in other kinases, which commonly have larger residue at the position equivalent to Ser280 of ALK5. Therefore, it was suggested that the interaction with Ser280 residue gives the specificity of NPC-30345 for ALK5.[12] To obtain an insight into the selective ALK5 inhibition of **12b**, we built a docking model of ALK5:**12b** complex using the flexible docking algorithms (FlexiDock and FlexX). As demonstrated in Figure 1, the inhibitor **12b** lies in the same binding pocket of ALK5 as is occupied by NPC-30345 in the X-ray structure.[12] All three substituents attached to the central triazole ring of **12b** form various hydrogen bonds (H-bonds) with amino acid residues in the binding pocket, and the 6-methyl group of pyridine moiety is positioned close to the hydrophobic residues (Leu278, Trp249, and Phe262), possibly forming a hydrophobic interaction (Fig. 1B). The carboxamide group of phenyl substituent form H-bonds with backbone amide of Ile211 and Ser287. Two oxygen atoms of 3,4-(methylenedioxy)phenyl ring form strong H-bonds with the positively charged amino protons of the Lys232 side chain. Finally, the nitrogen atom in the 2-pyridinyl moiety forms H-bond with the OH of Ser280, which is critical for the selectivity of **12b** for ALK5 over p38α MAP kinase. Considering that Thr106 of p38 MAP kinase is located at the position equivalent to Ser280 of ALK5,[13] the interaction between methyl-substituted 2-pyridinyl moiety and Thr106 would not be formed in the binding pocket of p38 MAP kinase due to the steric clash. In addition, the interaction between 6-methyl substituent of 2-pyridinyl and the hydrophobic residues (Leu278, Trp249, and Phe262) would also play a crucial role in selective inhibition of ALK5 activity. As shown in Table 1, the attachment of methyl group at the R_2 position of the compounds consistently improves the ALK5 inhibitory activity. The docking model suggests that further modification of the R_2 position with a substituent, which induces π–π stacking interactions with the aromatic amino acid residues (Trp249 and Phe262) may increase the activity.

Conclusively, the binding mode of **12b** generated by flexible docking studies revealed that the structure of the molecule is a good fit into the (NPC-30345)-binding cavity of ALK5, and well elucidated the selective activity of **12b** for ALK5. On the basis of these results, compound **12b** has been selected as a preclinical candidate, and further toxicological and pharmacological evaluation is currently undergoing in our laboratory.

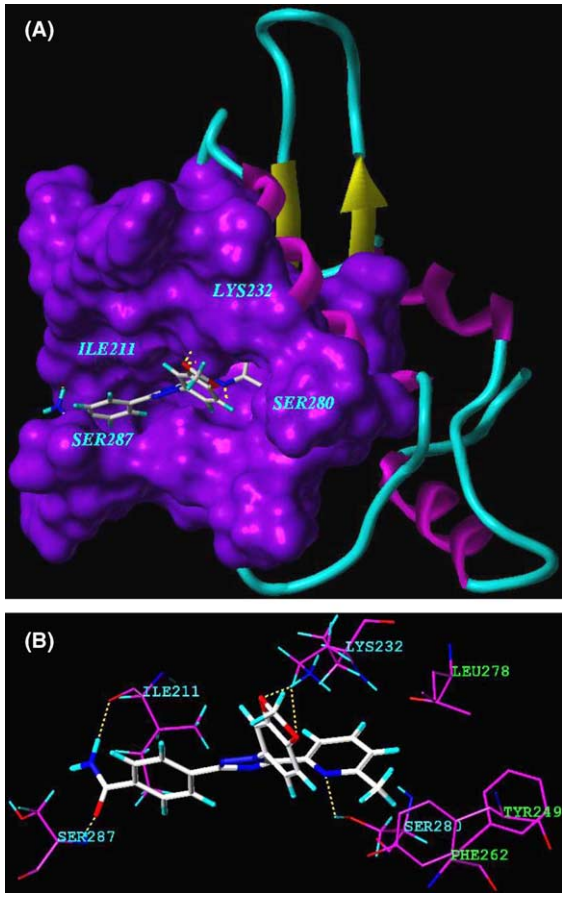


Figure 1. (A) Secondary structure of ALK5 docked with **12b**. The binding pocket for the ligand is demonstrated by Connolly surface (purple). The ligand is rendered in capped stick. Oxygen atoms of the ligand are red, and nitrogen atoms are blue. (B) The binding mode of **12b** observed in the docking model. The amino acid residues within the ALK5 binding site are represented in line form. Carbon atoms of amino acids are colored in magenta. Yellow dotted lines are hydrogen bonding interactions (<2.5 Å).

3. Experimental

^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Unity 400 spectrophotometer. The chemical shifts for ^{1}H NMR spectra are reported in parts per million (ppm) relative to internal tetramethylsilane in $CDCl_3$ or CD_3OD. When $CDCl_3$ or DMSO-d_6 was used as solvent for ^{13}C NMR spectra, it served as the internal standard at δ 77.0 or 39.5, respectively. Electrospray ionization mass spectra (ESI-MS) were obtained on a Q-Tof2 mass spectrometer (Micromass, Manchester, UK). Analytical thin-layer chromatography (TLC) was performed on Merck silica gel 60F-254 glass plates. Medium-pressure chromatography (MPLC) was performed using Merck silica gel 60 (230–400 mesh) with a VSP-2200 ceramic pump (Eyela). Elemental analyses were performed on a Carlo Erba 1106 elemental analyzer.

3.1. General procedure for the preparation of the 1-(2-pyridinyl)-[1,2,4]triazols 6a–f

To a stirred solution of 4-cyanobenzimidic acid methyl ester (**2**) (4.00 mmol) in CH_2Cl_2 (25 mL) at room temperature were added aroyl chloride **3a**–**c** (4.00 mmol), Et_3N (4.40 mmol) and a catalytic amount of DMAP (0.40 mmol). The mixture was warmed to 30 °C and stirred for 6 h, and to it, 2-hydrazinopyridine (**5a**) or 2-hydrazino-6-methylpyridine (**5b**) (4.00 mmol) was added. The reaction mixture was stirred for an additional 6 h at room temperature, and to it, water (30 mL) was added. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (2×25 mL). The combined CH_2Cl_2 solution was washed with brine (50 mL), dried (anhydrous Na_2SO_4), filtered, and evaporated to dryness under reduced pressure. The residue was purified by MPLC on silica gel using a mixture of Et_2O and CH_2Cl_2 as eluent to afford the titled compound **6a**–**f** as a solid.

3.1.1. 4-(5-Benzo[1,3]dioxol-5-yl-1-pyridin-2-yl-1*H*-[1,2,4]-triazol-3-yl)benzonitrile (6a). Yield 35%; ^{1}H NMR ($CDCl_3$) δ 5.99 (s, 2H), 6.79 (d, 1H, $J = 8.4$ Hz), 7.03 (d, 1H, $J = 1.2$ Hz), 7.06 (dd, 1H, $J = 8.4$ Hz, 1.2 Hz), 7.39 (dd, 1H, $J = 8.0$ Hz, 4.8 Hz), 7.58 (d, 1H, $J = 8.4$ Hz), 7.73 (d, 2H, $J = 8.4$ Hz), 7.88 (td, 1H, $J = 8.0$ Hz, 1.6 Hz), 8.33 (d, 2H, $J = 8.4$ Hz), 8.52 (dd, 1H, $J = 4.8$ Hz, 1.6 Hz); ^{13}C NMR ($CDCl_3$) 101.84, 108.68, 109.59, 119.05, 119.79, 124.10, 124.43, 127.36, 132.66, 139.18, 149.39; MS (EIS) m/z 368.10 (MH^+). Anal. Calcd for $C_{21}H_{13}N_5O_2$: C, 68.66; H, 3.57; N, 19.06. Found: C, 68.82; H, 3.40; N, 18.88.

3.1.2. 4-[5-Benzo[1,3]dioxol-5-yl-1-(6-methylpyridin-2-yl)-1*H*-[1,2,4]triazol-3-yl]benzonitrile (6b). Yield 26%; ^{1}H NMR ($CDCl_3$) δ 2.54 (s, 3H), 5.99 (s, 2H), 6.77 (dd, 1H, $J = 8.4$ Hz, 0.8 Hz), 7.05 (s, 1H), 7.07 (dd, 1H, $J = 8.4$ Hz, 0.8 Hz), 7.26 (t, 2H, $J = 7.6$ Hz), 7.73 (m, 3H), 8.32 (d, 2H, $J = 8.0$ Hz); ^{13}C NMR ($CDCl_3$) δ 24.40, 101.79, 108.51, 109.69, 113.00, 116.99, 119.06, 121.82, 124.15, 124.39, 127.39, 132.62, 135.15, 139.20, 147.85, 149.54, 150.23, 159.25, 160.36; MS (EIS) m/z 382.12 (MH^+). Anal. Calcd for $C_{22}H_{15}N_5O_2$: C, 69.28; H, 3.96; N, 18.36. Found: C, 69.01; H, 4.05; N, 18.24.

3.1.3. 4-(1-Pyridin-2-yl-5-quinoxalin-6-yl-1*H*-[1,2,4]triazol-3-yl)benzonitrile (6c). Yield 28%; ^{1}H NMR ($CDCl_3$) δ 7.41 (m, 1H), 7.79 (d, 2H, $J = 8.0$ Hz), 7.83 (dd, 1H, $J = 8.0$ Hz, 1.6 Hz), 7.96 (td, 1H, $J = 8.0$ Hz, 1.6 Hz), 8.04 (dd, 1H, $J = 8.0$ Hz, 1.6 Hz), 8.16 (d, 1H, $J = 8.0$ Hz), 8.35 (d, 1H, $J = 1.6$ Hz), 8.40 (m, 3H), 8.90 (dd, 2H, $J = 8.0$ Hz, 1.6 Hz); ^{13}C NMR ($CDCl_3$) δ 113.33, 118.91, 118.96, 124.60, 127.41, 129.86, 130.29, 130.64, 130.84, 132.76, 134.77, 139.46, 142.61, 143.68, 146.04, 146.28, 149.01, 150.72, 154.90, 160.84; MS (EIS) m/z 376.12 (MH^+). Anal. Calcd for $C_{22}H_{13}N_7$: C, 70.39; H, 3.49; N, 26.12. Found: C, 70.30; H, 3.53; N, 26.00.

3.1.4. 4-[1-(6-Methylpyridin-2-yl)-5-quinoxalin-6-yl-1*H*-[1,2,4]triazol-3-yl]benzonitrile (6d). Yield 23%; ^{1}H NMR ($CDCl_3$) δ 2.38 (s, 3H), 7.25 (d, 1H, $J = 8.0$ Hz), 7.54 (d, 1H, $J = 8.0$ Hz), 7.78 (m, 3H), 8.05 (dd, 1H, $J = 8.8$ Hz, 2.0 Hz), 8.13 (d, 1H, $J = 8.8$ Hz), 8.38 (m, 3H), 8.90 (dd, 2H, $J = 5.2$ Hz, 2.0 Hz); ^{13}C NMR ($CDCl_3$) δ 24.09, 113.21, 115.81, 118.97, 124.20, 127.41, 129.52, 130.37, 130.84, 130.87, 132.70, 134.85, 139.47, 142.52, 143.61, 146.00, 146.23, 149.92, 154.79, 158.87, 160.67; MS (EIS) m/z 390.16 (MH^+). Anal. Calcd for $C_{23}H_{15}N_7$: C, 70.94; H, 3.88; N, 25.18. Found: C, 71.23; H, 3.54; N, 24.87.

3.1.5. 4-[5-(4-Methoxyphenyl)-1-pyridin-2-yl-1*H*-[1,2,4]triazol-3-yl]benzonitrile (6e). Yield 25%; ^{1}H NMR ($CDCl_3$) δ 3.84 (s, 3H), 6.87 (m, 2H), 7.37(ddd, 1H, $J = 7.6$ Hz, 5.2 Hz, 1.2 Hz), 7.48 (m, 2H), 7.53 (d, 1H, $J = 7.6$ Hz), 7.72 (m, 2H), 7.85 (td, 1H, $J = 7.2$ Hz, 2.0 Hz), 8.32 (dd, 2H, $J = 6.8$ Hz, 2.0 Hz), 8.51 (dd, 1H, $J = 5.2$ Hz, 2.0 Hz); ^{13}C NMR ($CDCl_3$) δ 55.58, 113.01, 114.16, 116.29, 119.06, 119.79, 120.42, 124.33, 127.17, 127.38, 130.86, 132.63, 135.15, 139.09, 149.37, 151.12, 156.15, 160.49, 161.40; MS (EIS) m/z 354.18 (MH^+). Anal. Calcd for $C_{21}H_{15}N_5O$: C, 71.38; H, 4.28; N, 19.82. Found: C, 71.25; H, 4.35; N, 19.75.

3.1.6. 4-[5-(4-Methoxyphenyl)-1-(6-methylpyridin-2-yl)-1*H*-[1,2,4]triazol-3-yl]benzonitrile (6f). Yield 32%; ^{1}H NMR ($CDCl_3$) δ 2.51 (s, 3H), 3.80 (s, 3H), 6.85 (dt, 2H, $J = 8.8$ Hz, 2.4 Hz), 7.23 (t, 2H, $J = 7.2$ Hz), 7.49 (dt, 2H, $J = 8.8$ Hz, 2.4 Hz), 7.70 (m, 3H), 8.32 (dt, 2H, $J = 8.4$ Hz, 2.0 Hz); ^{13}C NMR ($CDCl_3$) δ 24.37, 55.57, 112.93, 113.99, 116.96, 119.08, 119.79, 120.49, 124.04, 127.41, 130.92, 132.59, 135.28, 139.14, 150.38, 156.11, 159.20, 160.40, 161.35; MS (EIS) m/z 368.16 (MH^+). Anal. Calcd for $C_{22}H_{17}N_5O$: C, 71.92; H, 4.66; N, 19.06. Found: C, 71.74; H, 4.85; N, 18.89.

3.2. General procedure for the preparation of the 1-(2-pyridinyl)-[1,2,4]triazols 7a–f

To a stirred solution of **6a**–**f** (0.25 mmol) in 95% EtOH (10 mL) at room temperature were added 28% H_2O_2 (1.15 mmol) and 6 N NaOH (0.09 mmol) solution. The mixture was warmed to 55 °C and stirred for 3 h, and to it, 1 N HCl solution was added to adjust to pH 7 at 0 °C. The ethanol solvent was evaporated off under reduced pressure, and the residue was dissolved in CH_2Cl_2 (40 mL). The CH_2Cl_2 solution was washed with brine (20 mL), dried (anhydrous Na_2SO_4), filtered, and evaporated to dryness under reduced pressure. The residue was purified by MPLC on silica gel using a mixture of MeOH and CH_2Cl_2 as eluent to afford the titled compound **7a**–**f** as a solid.

3.2.1. 4-(5-Benzo[1,3]dioxol-5-yl-1-pyridin-2-yl-1*H*-[1,2,4]-triazol-3-yl)benzamide (7a). Yield 79%; ^{1}H NMR (CD_3OD) δ 6.04 (s, 2H), 6.85 (d, 1H, $J = 8.4$ Hz), 7.04 (s, 1H), 7.07 (dd, 1H, $J = 8.0$ Hz, 1.6 Hz), 7.54 (dd, 1H, $J = 8.0$ Hz, 4.8 Hz), 7.73 (d, 1H, $J = 8.4$ Hz), 8.04 (m,

3H), 8.27 (d, 2H, $J = 8.4$ Hz), 8.52 (dd, 1H, $J = 4.4$ Hz, 0.8 Hz); ^{13}C NMR (CD_3OD) δ 100.99, 107.27, 108.14, 119.23, 120.54, 122.86, 123.82, 125.46, 127.12, 132.55, 133.86, 138.67, 146.97, 147.97, 148.71, 149.82, 154.75, 159.75, 161.88, 162.18, 162.47, 168.94; MS (EIS) m/z 386.10 (MH^+). Anal. Calcd for $C_{21}H_{15}N_5O_3$: C, 65.45; H, 3.92; N, 18.17. Found: C, 65.38; H, 4.13; N, 18.01.

3.2.2. 4-[5-Benzo[1,3]dioxol-5-yl-1-(6-methylpyridin-2-yl)-1*H*-[1,2,4]triazol-3-yl]benzamide (7b). Yield 77%; ^{1}H NMR ($CDCl_3$) δ 2.53 (s, 3H), 5.85 (br s, 1H), 5.98 (s, 2H), 6.14 (br s, 1H), 6.77 (d, 1H, $J = 8.4$ Hz), 7.07 (m, 2H), 7.23 (d, 1H, $J = 8.0$ Hz), 7.27 (d, 1H, $J = 8.0$ Hz), 7.71 (t, 1H, $J = 8.0$ Hz), 7.88 (d, 2H, $J = 8.4$ Hz), 8.29 (d, 2H, $J = 8.4$ Hz); ^{13}C NMR ($CDCl_3$) δ 24.39, 101.74, 108.48, 109.74, 117.00, 122.02, 124.01, 124.15, 127.11, 127.88, 134.05, 134.32, 139.15, 147.79, 149.41, 150.32, 155.64, 159.15, 161.04, 169.23; MS (EIS) m/z 400.12 (MH^+). Anal. Calcd for $C_{22}H_{17}N_5O_3$: C, 66.16; H, 4.29; N, 17.53. Found: C, 66.43; H, 4.21; N, 17.39.

3.2.3. 4-(1-Pyridin-2-yl-5-quinoxalin-6-yl-1*H*-[1,2,4]triazol-3-yl)benzamide (7c). Yield 70%; ^{1}H NMR ($CDCl_3$) δ 5.71 (br s, 1H), 6.11 (br s, 1H), 7.36 (m, 1H), 7.81 (d, 1H, $J = 8.4$ Hz), 7.92 (m, 3H), 8.02 (dd, 1H, $J = 8.8$ Hz, 2.0 Hz), 8.12 (d, 1H, $J = 8.8$ Hz), 8.34 (m, 4H), 8.86 (dd, 2H, $J = 6.0$ Hz, 2.0 Hz); ^{13}C NMR ($CDCl_3$) δ 118.93, 124.46, 127.16, 128.04, 129.82, 130.54, 130.78, 130.81, 134.00, 134.32, 146.00, 146.21, 148.97, 161.55, 168.97; MS (EIS) m/z 394.16 (MH^+). Anal. Calcd for $C_{22}H_{15}N_7O$: C, 67.17; H, 3.84; N, 24.92. Found: C, 66.92; H, 3.92; N, 24.83.

3.2.4. 4-[1-(6-Methylpyridin-2-yl)-5-quinoxalin-6-yl-1*H*-[1,2,4]triazol-3-yl]benzamide (7d). Yield 64%; ^{1}H NMR ($CDCl_3$) δ 2.31 (s, 3H), 5.71 (br s, 1H), 6.13 (br s, 1H), 7.17 (d, 1H, $J = 7.6$ Hz), 7.49 (d, 1H, $J = 8.4$ Hz) 7.72 (d, 1H, $J = 7.6$ Hz), 7.88 (d, 2H, $J = 8.4$ Hz), 8.00 (dd, 1H, $J = 8.4$ Hz, 2.0 Hz), 8.07 (d, 1H, $J = 8.4$ Hz), 8.31 (m, 3H), 8.83 (dd, 2H, $J = 4.4$ Hz, 1.6 Hz); ^{13}C NMR (DMSO-d_6) δ 23.21, 115.42, 123.58, 125.72, 127.78, 128.56, 129.67, 129.79, 130.01, 132.43, 134.96, 139.30, 141.52, 142.59, 145.88, 146.08, 149.22, 153.71, 157.49, 160.29, 167.62; MS (EIS) m/z 408.20 (MH^+). Anal. Calcd for $C_{23}H_{17}N_7O$: C, 67.80; H, 4.21; N, 24.06. Found: C, 68.12; H, 4.04; N, 23.72.

3.2.5. 4-[5-(4-Methoxyphenyl)-1-pyridin-2-yl-1*H*-[1,2,4]-triazol-3-yl]benzamide (7e). Yield 81%; ^{1}H NMR ($CDCl_3$) δ 3.84 (s, 3H), 5.71 (br s, 1H), 6.16 (br s, 1H), 6.90 (dt, 2H, $J = 8.8$ Hz, 2.0 Hz), 7.39 (ddd, 1H, $J = 7.4$ Hz, 5.0 Hz, 1.0 Hz), 7.53 (dt, 2H, $J = 8.8$ Hz, 2.0 Hz), 7.58 (d, 1H, $J = 8.0$ Hz), 7.88 (td, 1H, $J = 7.2$ Hz, 2.0 Hz), 7.91 (d, 2H, $J = 8.8$ Hz), 8.34 (dt, 1H, $J = 8.0$ Hz, 2.0 Hz), 8.53 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 55.58, 114.13, 119.80, 119.79, 120.66, 124.17, 127.10, 127.90, 130.88, 134.07, 134.34, 139.04, 149.23, 155.94, 161.19, 161.30, 169.10; MS (EIS) m/z 372.16 (MH^+). Anal. Calcd for $C_{21}H_{17}N_5O_2$: C, 67.91; H, 4.61; N, 18.86. Found: C, 67.73; H, 4.85; N, 18.83.

3.2.6. 4-[5-(4-Methoxyphenyl)-1-(6-methylpyridin-2-yl)-1*H*-[1,2,4]triazol-3-yl]benzamide (7f). Yield 84%; ^{1}H NMR (CD_3OD) δ 2.48 (s, 3H), 3.78 (s, 3H), 4.32 (br d, 2H), 6.85 (d, 2H, $J = 7.6$ Hz), 7.42 (d, 2H, $J = 7.2$ Hz), 7.73 (t, 1H, $J = 6.4$ Hz), 7.90 (d, 2H, $J = 7.6$ Hz), 8.20 (d, 2H, $J = 7.6$ Hz); ^{13}C NMR (CD_3OD) δ 24.18, 55.75, 114.43, 117.65, 120.16, 124.80, 127.23, 128.47, 131.15, 134.08, 134.74, 139.89, 150.31, 156.43, 159.61, 161.34, 161.87, 170.95; MS (EIS) m/z 386.18 (MH^+). Anal. Calcd for $C_{22}H_{19}N_5O_2$: C, 68.56; H, 4.97; N, 18.17. Found: C, 68.78, H, 4.62; N, 17.98.

3.3. Benzo[1,3]dioxol-5-ylhydrazine (10a)

To a stirred solution of benzo[1,3]dioxol-5-ylamine (4.08 g, 30 mmol) in H_2O (20 mL) and concentrated HCl (31 mL, 300 mmol) at 0 °C was added $NaNO_2$ (2.47 g, 36 mmol) dissolved in H_2O (8 mL), and the mixture was stirred for 30 min. The reaction mixture was poured into a stirred solution of $SnCl_2{\cdot}2H_2O$ (15.5 g, 68 mmol) in concentrated HCl (38 mL) at 15 °C, and stirred for an additional 30 min. The precipitates were filtered, washed with H_2O, and suspended in H_2O (80 mL) and 6 N NaOH (10 mL) solution. The suspension was stirred for 30 min at room temperature and filtered, and the filtered solution was extracted with CH_2Cl_2 (200 mL). The CH_2Cl_2 solution was washed with brine (50 mL), dried (anhydrous $MgSO_4$), filtered, and evaporated to dryness under reduced pressure. The residue was purified by MPLC on silica gel using a mixture of MeOH and CH_2Cl_2 (5:95) as eluent to afford **10a** (1.15 g, 26%) as a solid: ^{1}H NMR ($CDCl_3$) δ 3.50 (br s, 2H), 5.00 (br s, 1H), 5.86 (s, 2H), 6.24 (dd, 1H, $J = 8.4$ Hz, 2.4 Hz), 6.44 (d, 1H, $J = 2.4$ Hz), 6.67 (d, 1H, $J = 8.4$ Hz); ^{13}C NMR ($CDCl_3$) δ 95.79, 101.06, 104.12, 108.62, 147.29; MS (EIS) m/z 153.04 (MH^+). Anal. Calcd for $C_7H_8N_2O_2$: C, 55.26; H, 5.30; N, 18.41. Found: C, 55.03; H, 5.35; N, 18.32.

3.4. General procedure for the preparation of the 5-(2-pyridinyl)-[1,2,4]triazoles 11a–d

To a stirred solution of **2** (0.56 mmol) in toluene (4 mL) at room temperature were added picolinoyl chloride (**8a**) or 6-methylpicolinoyl chloride (**8b**) (0.67 mmol) and Et_3N (1.12 mmol). The mixture was warmed to 40 °C, stirred for 12 h, and filtered. The filtered solution was evaporated to dryness, and the residue was dissolved in CH_2Cl_2 (4 mL), and to it, benzo[1,3]dioxol-5-ylhydrazine (**10a**) or 4-methoxyphenylhydrazine (**10b**) (0.56 mmol) was added. The mixture was stirred for 6 h at room temperature and evaporated to dryness, and the residue was purified by MPLC on silica gel using a mixture of EtOAc and hexane as eluent to afford the titled compound **11a–d** as a solid.

3.4.1. 4-(1-Benzo[1,3]dioxol-5-yl-5-pyridin-2-yl-1*H*-[1,2,4]-triazol-3-yl)benzonitrile (11a). Yield 25%; ^{1}H NMR ($CDCl_3$) δ 6.00 (s, 2H), 6.76 (dd, 1H, $J = 8.2$ Hz, 1.4 Hz), 6.83 (m, 1H), 6.89 (dd, 1H, $J = 5.4$ Hz, 1.8 Hz), 7.29 (m, 1H), 7.70 (m, 1H), 7.75 (m, 1H), 7.87 (m, 1H),

8.07 (m, 1H), 8.28 (m, 2H), 8.51 (m, 1H); MS (EIS) m/z 368.10 (MH^+). Anal. Calcd for $C_{21}H_{13}N_5O_2$: C, 68.66; H, 3.57; N, 19.06. Found: C, 68.93; H, 3.40; N, 18.83.

3.4.2. 4-[1-Benzo[1,3]dioxol-5-yl-5-(6-methylpyridin-2-yl)-1*H*-[1,2,4]triazol-3-yl]benzonitrile (11b). Yield 27%; ^1H NMR ($CDCl_3$) δ 2.41 (s, 3H), 6.02 (s, 2H), 6.78 (dd, 1H, $J = 8.4$ Hz, 1.6 Hz), 6.85 (m, 1H), 6.92 (d, 1H, $J = 1.6$ Hz), 7.16 (m, 1H), 7.62 (m, 2H), 7.71 (m, 2H), 8.31 (m, 2H); MS (EIS) m/z 382.12 (MH^+). Anal. Calcd for $C_{22}H_{15}N_5O_2$: C, 69.28; H, 3.96; N, 18.36. Found: C, 69.00; H, 4.23; N, 18.29.

3.4.3. 4-[1-(4-Methoxyphenyl)-5-pyridin-2-yl-1*H*-[1,2,4]-triazol-3-yl]benzonitrile (11c). Yield 25%; ^1H NMR ($CDCl_3$) δ 3.82 (s, 3H), 6.91 (m, 2H), 7.29 (m, 1H), 7.32 (m, 2H), 7.71 (d, 2H, $J = 8.4$ Hz), 7.76 (dd, 1H, $J = 7.8$ Hz, 1.2 Hz), 7.84 (d, 1H, $J = 7.8$ Hz), 8.32 (d, 2H, $J = 8.4$ Hz), 8.52 (d, 1H, $J = 4.4$ Hz); ^{13}C NMR ($CDCl_3$) δ 55.77, 112.89, 114.38, 119.04, 124.62, 124.75, 127.02, 127.23, 131.72, 132.65, 135.16, 136.99, 147.34, 149.81, 153.98, 160.07, 160.13; MS (EIS) m/z 354.20 (MH^+). Anal. Calcd for $C_{21}H_{15}N_5O$: C, 71.38; H, 4.28; N, 19.82. Found: C, 71.52; H, 4.02; N, 19.69.

3.4.4. 4-[1-(4-Methoxyphenyl)-5-(6-methylpyridin-2-yl)-1*H*-[1,2,4]triazol-3-yl]benzonitrile (11d). Yield 23%; ^1H NMR ($CDCl_3$) δ 2.40 (s, 3H), 3.83 (s, 3H), 6.91 (m, 2H), 7.15 (dd, 1H, $J = 6.6$ Hz, 1.8 Hz), 7.33 (m, 2H), 7.60 (m, 2H), 7.72 (d, 2H, $J = 8.8$ Hz), 8.33 (d, 2H, $J = 8.8$ Hz); MS (EIS) m/z 368.16 (MH^+). Anal. Calcd for $C_{22}H_{17}N_5O$: C, 71.92; H, 4.66; N, 19.06. Found: C, 72.23; H, 4.44; N, 18.98.

3.5. General procedure for the preparation of the 5-(2-pyridinyl)-[1,2,4]triazoles 12a–d is the same as for the compounds 7a–f

3.5.1. 4-(1-Benzo[1,3]dioxol-5-yl-5-pyridin-2-yl-1*H*-[1,2,4]-triazol-3-yl)benzamide (12a). Yield 75%; ^1H NMR ($CDCl_3$) δ 5.81 (br s, 1H), 6.02 (s, 2H), 6.17 (br s, 1H), 6.78 (d, 1H, $J = 8.0$ Hz), 6.85 (dd, 1H, $J = 8.0$ Hz, 2.0 Hz), 6.92 (d, 1H, $J = 2.0$ Hz), 7.29 (ddd, 1H, $J = 8.0$ Hz, 4.8 Hz, 1.2 Hz), 7.77 (td, 1H, $J = 8.0$ Hz, 1.6 Hz), 7.88 (m, 3H), 8.29 (d, 2H, $J = 8.4$ Hz), 8.53 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 102.19, 107.32, 108.21, 119.71, 124.66, 124.71, 127.00, 127.94, 132.85, 134.06, 134.26, 137.00, 147.46, 148.11, 148.26, 149.79, 153.86, 160.84, 169.19; MS (EIS) m/z 386.12 (MH^+). Anal. Calcd for $C_{21}H_{15}N_5O_3$: C, 65.45; H, 3.92; N, 18.17. Found: C, 65.30; H, 4.13; N, 17.88.

3.5.2. 4-[1-Benzo[1,3]dioxol-5-yl-5-(6-methylpyridin-2-yl)-1*H*-[1,2,4]triazol-3-yl]benzamide (12b). Yield 74%; ^1H NMR ($CDCl_3$) δ 2.46 (s, 3H), 5.60 (br s, 1H), 6.05 (s, 2H), 6.10 (br s, 1H), 6.82 (d, 1H, $J = 8.0$ Hz), 6.90 (dd, 1H, $J = 8.0$ Hz, 1.6 Hz), 6.98 (d, 1H, $J = 1.6$ Hz), 7.19 (m, 1H), 7.66 (m, 2H), 7.91 (d, 2H, $J = 8.0$ Hz), 8.33 (d, 2H, $J = 8.0$ Hz); ^{13}C NMR ($CDCl_3$) δ 24.57, 102.13, 107.57, 108.05, 109.31, 119.97, 121.64, 124.38, 127.02, 127.91, 133.95, 134.37, 137.10, 146.62, 158.83, 160.79; MS (EIS) m/z 400.14 (MH^+). Anal. Calcd for $C_{22}H_{17}N_5O_3$: C, 66.16; H, 4.29; N, 17.53. Found: C, 65.88; H, 4.43; N, 17.35.

3.5.3. 4-[1-(4-Methoxyphenyl)-5-pyridin-2-yl-1*H*-[1,2,4]-triazol-3-yl]benzamide (12c). Yield 72%; ^1H NMR ($CDCl_3$) δ 3.86 (s, 3H), 5.70 (br s, 1H), 6.18 (br s, 1H), 6.94 (m, 2H), 7.32 (m, 1H), 7.37 (m, 2H), 7.77 (td, 1H, $J = 8.0$ Hz, 2.0 Hz), 7.90 (m, 3H), 8.34 (d, 2H, $J = 8.0$ Hz), 8.55 (m, 1H); MS (EIS) m/z 372.16 (MH^+). Anal. Calcd for $C_{21}H_{17}N_5O_2$: C, 67.91; H, 4.61; N, 18.86. Found: C, 68.25; H, 4.38; N, 18.64.

3.5.4. 4-[1-(4-Methoxyphenyl)-5-(6-methylpyridin-2-yl)-1*H*-[1,2,4]triazol-3-yl]benzamide (12d). Yield 78%; ^1H NMR ($CDCl_3$) δ 2.39 (s, 3H), 3.82 (s, 3H), 5.80 (br s, 1H), 6.19 (br s, 1H), 6.89 (m, 2H), 7.13 (dd, 1H, $J = 6.2$ Hz, 2.6 Hz), 7.34 (m, 2H), 7.59 (m, 2H), 7.87 (d, 2H, $J = 8.4$ Hz), 8.29 (d, 2H, $J = 8.4$ Hz); ^{13}C NMR ($CDCl_3$) δ 24.50, 55.80, 114.11, 121.61, 124.28, 126.98, 127.27, 127.88, 132.00, 133.93, 134.42, 137.03, 146.71, 154.05, 158.80, 159.96, 160.77, 169.21; MS (EIS) m/z 386.19 (MH^+). Anal. Calcd for $C_{22}H_{19}N_5O_2$: C, 68.56; H, 4.97; N, 18.17. Found: C, 68.50; H, 5.12; N, 17.98.

3.6. P38 kinase inhibition assay

P38α kinase assay was performed according to the instruction manual of assay kit provided by the manufacturer (Upstate Biotechnology). Briefly, to activate MAPKAP kinase-2, 10 μL of reaction mixture contained 200 ng of inactive MAPKAP kinase-2, 0.06 unit of purified p38α kinase and 2 μL of magnesium/ATP cocktail (75 mM $MgCl_2$/500 μM cold ATP) were mixed by vortexing and incubated for 15 min at 30 °C with agitation. Before activation reaction was started, inhibitors dissolved in DMSO were added to the reaction tube with 10 μM of final concentration. After activation, 10 μCi [γ-^{32}P]ATP and 10 μL of 0.86 mM MAPKAP kinase-2 substrate were added, and the mixture was incubated for 10 min at 30 °C with agitation. Then, 40 μL of reaction mixture was transferred into P81 phosphocellulose paper. After extensively washing the paper three times with 40 mL of 0.75% phosphoric acid, the bound radioactivity was determined by liquid scintillation counter.

3.7. Flexible docking

The FlexiDock module in sybyl 6.9 (SYBYL molecular modeling software, Tripos Inc.: St. Louis, MO, USA, 2003) was used to dock **12b** onto the active site of ALK5. To ensure similar starting geometry of the ligand in the binding site of known inhibitor NPC-30345, the crystal structure of ALK5:NPC-30345 complex (pdb entry = 1IAS)[12] was used as a reference. (Since the chemical structure of NPC-30345 is not revealed in the refined X-ray structure, the electron density for NPC-30345

was viewed with molecular graphics program *O*[14] and used to locate the docking position for **12b**.) In the initial docked structure of ALK5:**12b** complex, Lys232 and Ser287 are positioned within the distance that could form H-bonds with the acceptor atoms of **12b**. The binding site was defined as all residues within 6.5 Å distance from **12b**, and 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. Docking was performed with the introduction of constraints for formation of hydrogen bond between selected donor–acceptor atoms (i.e., donor in protein: Lys232 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. The selected structure of ALK5:**12b** complex was used as a receptor for further flexible docking of **12b** to build a final optimized model. The FlexX docking and subsequent scoring were performed using the default parameters of the FlexX program implanted in the sybyl. For the docking of **12b** into the target active site, the main settings are 1000 solutions per iteration during the incremental construction algorithm and a maximum protein–ligand atom–atom overlap of 2.5 Å.[3] Final scores for all FlexX solutions (up to 1000) per compound were calculated by a standard scoring function, and used for database ranking. Top-ranked conformer of **12b** complexed with ALK5 was selected as a final model shown in Figure 1.

Acknowledgements

This work was supported by the Ewha Womans University Research Grant of 2002 and a grant from KISTEP, Korea (M1-0310-43-0000).

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