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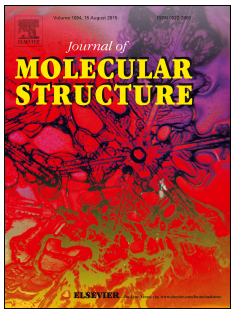

Synthesis and structural studies of 3,6-disubstituted-bis-1,2,4-triazolo-[4,3-*b*][3′,4′-*f*]pyridazines

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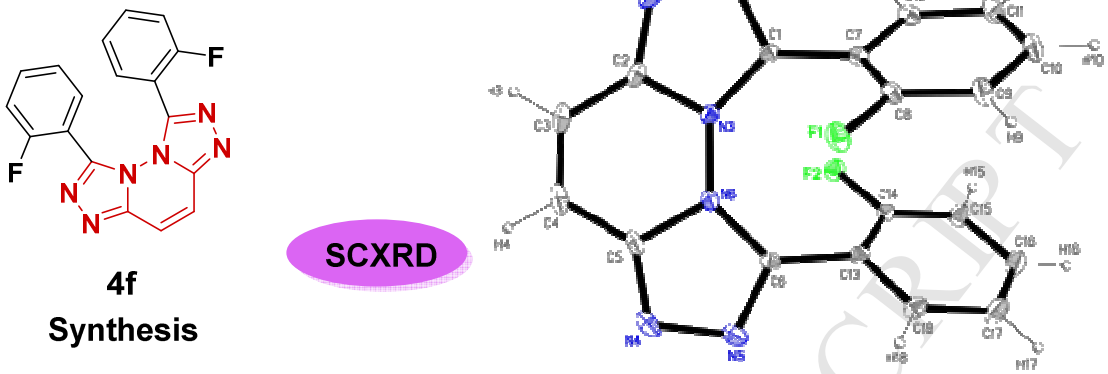
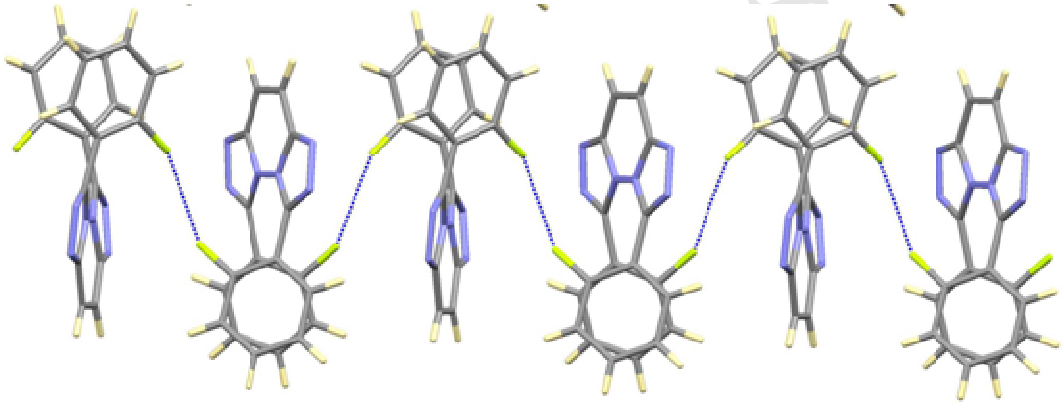
Graphical abstract





Perspective view of F---F interactions

Synthesis and structural studies of 3,6-disubstituted-bis-1,2,4-triazolo-[4,3-*b*][3',4'-*f*]pyridazines

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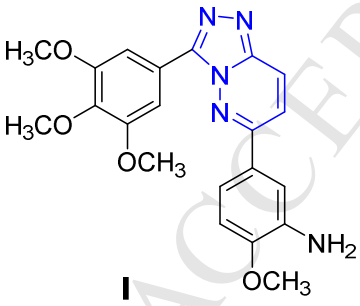
Abstract: Synthesis of a series of 3,6-disubstituted-bis-1,2,4-triazolo-[4,3-*b*][3',4'-*f*]pyridazines (**4**) was accomplished by the oxidative intramolecular cyclization of 6-arylidenehydrazino-3-aryl-1,2,4-triazolo[4,3-*b*]pyridazine (**3**) using iodobenzene diacetate (IBD), as a green oxidant, in dichloromethane at room temperature. The compounds **3** and **4** were characterized by IR, NMR (^{1}H and ^{13}C), mass spectral data and elemental analyses. X-ray crystal analysis of sterically strained 3,6-di-(2'-fluorophenyl)-bis-1,2,4-triazolo-[4,3-*b*][3',4'-*f*]pyridazine **4f** and 3,6-di-(4'-fluorophenyl)-bis-1,2,4-triazolo-[4,3-*b*][3',4'-*f*]pyridazine **4g** indicated that pyridazine ring has twisted conformation leading to nonplanar tricyclic core. Both compounds crystallized in orthorhombic $P2_12_12_1$ space group, containing one single molecule per asymmetric unit. The studies reveal that the compound **4f** is associated with weak, centrosymmetric F···F interactions (distance of 2.882(3) Å), with *cis* geometry, between adjacent molecules which are responsible for the formation of chains along *a* axis. Additionally, compounds **4** were screened for their cytotoxic activity against the human cervical carcinoma (HeLa) cell line using MTT assay, however, with not much significant activity.

Keywords: 6-Hydrazino-1,2,4-triazolo[4,3-*b*]pyridazine; 6-Arylidenehydrazino-3-aryl-1,2,4-triazolo[4,3-*b*]pyridazine; Bis-1,2,4-triazolo-[4,3-*b*][3',4'-*f*]pyridazines; Iodobenzene diacetate; X-ray crystallography.

1. Introduction

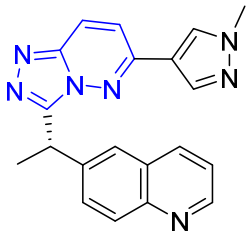
1,2-Diazine, commonly referred as pyridazine, is heteroaromatic compound containing two nitrogen atoms with free electron pairs that permit the pyridazine ring to act like as an effective and stable complexing agent for transition metal ions and to be used for the development of new π-conjugated organic compounds [1]. The chemistry of 1,2,4-triazoles fused with pyridazine ring has received considerable attention owing to their versatile pharmacological properties such as anticonvulsant [2], antitubercular [3], antitublin [4], antiproliferative [5],

antimicrobial [6, 7], anxiolytic [8], and cognition enhancing activity [9]. These compounds also act as c-Met kinase inhibitors [10], tankyrase inhibitors [11], pan-phosphodiesterase (PDE) inhibitors [12, 13] besides being highly selective antagonists of canbinoid receptors [14]. Meticulously, 3,6-diaryl-[1,2,4]triazolo[4,3-*b*]pyridazine (**I**) (Figure 1) exhibited significant antiproliferative effects (IC_{50} = 0.008-0.014 μM) against SGC-7901, A549 and HT-1080 cell lines and cell cycle studies revealed that compound **I** arrested cell cycle progression at G2/M phase in A549 cells [4]. (*S*)-6-(1-(6-(1-Methyl-1*H*-pyrazol-4-yl)-[1,2,4]triazolo[4,3-*b*]pyridazin-3-yl)ethyl)quinoline (**II**) was reported as selective c-Met inhibitor which inhibited tumor growth in GTL-16 xenograft tumor model besides being pan-PDE family inhibitor [10]. Very recently our group reported anticancer potential of [1,2,4]triazolo[4,3-*b*]pyridazines against three human cancer cell lines namely SB-ALL, NALM-6 and MCF-7. Among them 6-chloro-3-(1*H*-indole-3'-yl)-[1,2,4]triazolo[4,3-*b*]pyridazine (**III**) displayed potent cytotoxic activity with IC_{50} values ranging from 1.14 to 3.55 μM and induced apoptosis of NALM-6 cells *via* caspase 3/7 activation [15]. Additionally, pyridazine derivatives play an imperative role in chemistry because of their unique property of forming and strengthening of supramolecular assembly [16-19].



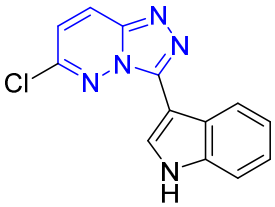
I

IC_{50} = 0.014 μM (SGC-7901)
= 0.008 μM (A549)
= 0.012 μM (HT-1080)



II

c-MET Ki 0.0103 μM
c-MET cell IC_{50} = 0.006 μM



III

IC_{50} = 1.64 ± 0.74 μM (SB-ALL)
= 1.14 ± 0.24 μM (NALM-6)
= 3.55 ± 0.54 μM (MCF-7)

Figure 1. Representative [1,2,4]triazolo[4,3-*b*]pyridazine derivatives with anticancer activity.

The DNA photocleavage and antifungal activities of a series of 1-aryl-4-methyl-1,2,4-triazolo[4,3-*b*]quinoxalines [20, 21] has been reported by our group and the study further extended to synthesis of 3,10-disubtituted-bis-1,2,4-triazolo[4,3-*a*][3',4'-*c*]-quinoxalines [22]. Inspired by biological importance of 1,2,4-triazoles fused with pyridazine ring it was envisioned to extend our work to the synthesis of bis-1,2,4-triazolopyridazines. The most widely used method for the synthesis of bis-1,2,4-triazolopyridazines involves the cyclization of 6-arylidenehydrazino-3-aryl-1,2,4-triazolo[4,3-*b*]pyridazine into bis-1,2,4-triazolo-[4,3-*b*][3',4'-*f*]pyridazine upon treatment with Br_2/AcOH or lead tetracetate [23], or Me_4NBr/oxone® [24], thereby constructing the second triazole ring. Whereas simultaneous formation of bis-triazole has been reported by oxidative cyclization of 3,6-bis-(arylidenehydrazino)pyridazines using Br_2/AcOH or lead tetraacetate [23] and by the reaction of 3,6-dichloropyridazine with two moles of acid hydrazides [25]. However, these methods suffer from one or more disadvantages such as harsh reaction conditions, use of toxic and expensive reagents, higher reaction temperature, tedious workup reaction condition and longer reaction time. Recently, iodobenzene diacetate (IBD) emerged as a powerful oxidizing reagent for various synthetic transformations replacing toxic metal based oxidants, such as Tl(III), Pb(IV) and Hg(II), because of its similarity in oxidation reactions, comparative less toxic nature, ready availability, ease of handling and easy reaction workup condition.

Keeping in view the significance of 1,2,4-triazolopyridazines and in continuation of our ongoing research work on hypervalent iodine reagents in organic transformations [26-30], we herein report the facile synthesis of a series of 3,6-disubstituted-bis-1,2,4-triazolo-[4,3-*b*][3',4'-*f*]pyridazines from hydrazone precursors using iodobenzene diacetate, structural studies by X-ray crystallography and *in vitro* cytotoxicity study.

2. Experimental

2.1. Materials and methods

Melting points were determined in digital melting point apparatus MEPA and are uncorrected. IR spectra were recorded on a Buck Scientific IR M-500 spectrophotometer in KBr pellets (ν_{max} in cm^{-1}). ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra for analytical purpose were recorded on a Bruker Avance II 400 NMR spectrometer using DMSO-d_6 or $CDCl_3$ as a solvent; chemical shifts are expressed in δ units (parts per million) downfield from TMS as an internal reference. The coupling constants (*J*) are given in Hz. Mass spectra were measured in EI mode on a Kratos MS-50 spectrometer at MS Facilities at Sophisticated Analytical Instrument Facility (SAIF), Panjab University, Chandigarh, India. Elemental analyses were also performed at SAIF, Panjab University, Chandigarh, India.

*2.2. Synthesis of 6-hydrazino-3-aryl/heteroaryl-1,2,4-triazolo[4,3-b]pyridazine (**2a-l**)*

6-Chloro-3-aryl/heteroaryl-1,2,4-triazolo[4,3-*b*]pyridazine (**1**) (2.0 mmol) was dissolved in 10 mL of ethanol and then hydrazine hydrate (0.1 g, 2.0 mmol) was added. The resulting mixture was refluxed for 3 hours. After cooling to room temperature, a solid residue separated out which was filtered off, washed with water and dried to afford hydrazine derivative **2**.

*2.2.1. 6-Hydrazino-3-phenyl-1,2,4-triazolo[4,3-b]pyridazine (**2a**)*

Yield 70%; m.p. 245 ºC; IR (KBr, cm^{-1}): 3233 (br NH str.), 1602 (C=N str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.32 (bs, 2H, -NH_2), 6.85-6.88 (d, 1H, *J*=9.88 Hz, pyridazine-5H), 7.46-7.48 (m, 1H, Ph-4'-H), 7.51-7.55 (m, 2H, Ph-3', 5'-H), 7.87-7.90 (d, 1H, *J*= 9.84 Hz, pyridazine-4H), 8.48 (s, 1H, -NH), 8.52-8.54 (m, 2H, Ph-2', 6'-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 114.96, 123.51, 126.86, 126.95, 128.30, 129.08, 143.11, 145.03, 156.35. Anal. Calcd. for $C_{11}H_{10}N_6$: C, 58.40; H, 4.46; N, 37.15. Found: C, 58.12; H, 4.14, N, 37.39.

*2.2.2. 6-Hydrazino-3-(2'-chlorophenyl)-1,2,4-triazolo[4,3-b]pyridazine (**2b**)*

Yield 69%; m.p. >315 ºC; IR (KBr, cm^{-1}): 3448 (br NH str.), 1610 (C=N str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.10 (bs, 2H, -NH_2), 6.90-6.92 (d, 1H, *J*=9.88 Hz, pyridazine-5H), 7.48-7.52 (dt, 1H, J_o=7.44 Hz, J_m=1.4 Hz, Ph-4'-H), 7.54-7.59 (dt, 1H, J_o=7.42 Hz, J_m=1.8 Hz, Ph-5'-H), 7.62-7.65 (dd, 1H, J_o=8.02 Hz, J_m=1.24 Hz, Ph-3'-H), 7.71-7.73 (dd, 1H, J_o=7.54 Hz, J_m=1.76 Hz, Ph-6'-H), 7.93-7.95 (d, 1H, *J*=9.84 Hz, pyridazine-4H), 8.42 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 115.52, 123.41, 126.24, 126.89, 129.73, 131.36, 132.46, 133.63, 143.16, 145.58, 156.45. MS: *m/z* 261.05 $[M+1]^+$, 263.04 $[M+1+2]^+$ (3:1). Anal. Calcd. for $C_{11}H_9ClN_6$: C, 50.68; H, 3.48; N, 32.24. Found: C, 50.54; H, 3.83, N, 32.06.

*2.2.3. 6-Hydrazino-3-(4'-chlorophenyl)-1,2,4-triazolo[4,3-b]pyridazine (**2c**)*

Yield 70%; m.p. 231 ºC; IR (KBr, cm^{-1}): 3336 (br NH str.), 1612 (C=N str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.32 (bs, 2H, -NH_2), 6.85-6.88 (d, 1H, *J*=9.88 Hz, pyridazine-5H), 7.53-7.56 (dd, 2H, J_o=7.0 Hz, J_m=1.72 Hz, Ph-3', 5'-H), 7.89-7.91 (d, 1H, *J*=9.84 Hz, pyridazine-4H), 8.53 (s, 1H, -NH), 8.56-8.58 (m, 2H, Ph-2', 6'-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 115.16, 123.63, 125.71, 128.42, 128.59, 133.96, 143.98, 145.05, 156.46. Anal. Calcd. for $C_{11}H_9ClN_6$: C, 50.68; H, 3.48; N, 32.24 Found: C, 50.23; H, 3.10, N, 32.06.

*2.2.4. 6-Hydrazino-3-(3'-bromophenyl)-1,2,4-triazolo[4,3-b]pyridazine (**2d**)*

Yield 68%; m.p. 263 ºC; IR (KBr, cm^{-1}): 3244 (br NH str.), 1605 (C=N str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.35 (bs, 2H, -NH_2), 6.88-6.90 (d, 1H, *J*=9.88 Hz, pyridazine-5H), 7.47-7.51 (t, 1H, *J*=7.96 Hz, Ph-5'-H), 7.63-7.65 (m, 1H, J_o=8.02 Hz, J_m=1.0 Hz, Ph-6'-H), 7.92-7.95 (d, 1H, *J*=9.88 Hz, pyridazine-4H), 8.57 (s, 1H, -NH), 8.59-8.61 (m, 1H, J_o=8.0 Hz, J_m=1.28 Hz, Ph-4'-H), 8.66-8.67 (m, 1H, J_m=1.72 Hz, Ph-2'-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 115.34, 121.83, 123.78, 125.64, 128.91, 129.03, 130.93, 132.06, 144.17, 144.50, 156.60. MS: *m/z* 305.03

$[M+1]^+$, 307.03 $[M+1+2]^+$ (1:1). Anal. Calcd. for $C_{11}H_9BrN_6$: C, 43.30; H, 2.97; N, 27.54. Found: C, 43.75; H, 3.12, N, 27.28.

*2.2.5. 6-Hydrazino-3-(4'-bromophenyl)-1,2,4-triazolo[4,3-b]pyridazine (**2e**)*

Yield 71%; m.p. 224.5 ºC; IR (KBr, cm^{-1}): 3248 (br NH str.), 1636 (C=N str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.25 (bs, 2H, -NH_2), 6.86-6.88 (d, 1H, *J*=9.84 Hz, pyridazine-5H), 7.66-7.68 (d, 2H, *J*=8.56 Hz, Ph-3', 5'-H), 7.85-7.88 (d, 1H, *J*=9.8 Hz, pyridazine-4H), 8.48-8.50 (d, 2H, *J*=8.6 Hz, Ph-2', 6'-H), 8.51 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 115.16, 122.65, 123.59, 126.06, 128.62, 131.48, 144.00, 145.13, 156.44. MS: *m/z* 305.01 $[M+1]^+$, 307.02 $[M+1+2]^+$ (1:1). Anal. Calcd. For $C_{11}H_9BrN_6$: C, 43.30; H, 2.97; N, 27.54. Found: C, 43.15; H, 2.45, N, 27.24.

*2.2.6. 6-Hydrazino-3-(2'-fluorophenyl)-1,2,4-triazolo[4,3-b]pyridazine (**2f**)*

Yield 66%; m.p. 252.5 ºC; IR (KBr, cm^{-1}): 3564 (br NH str.), 1601 (C=N str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.34 (bs, 2H, -NH_2), 6.83-6.86 (d, 1H, *J*=9.84 Hz, pyridazine-5H), 7.23-7.25 (m, 1H, Ph-5'-H), 7.65-7.67 (m, 1H, Ph-3'-H), 7.88-7.91 (d, 1H, *J*=9.84 Hz, pyridazine-4H), 8.29-8.30 (m, 1H, Ph-4'-H), 8.51 (s, 1H, -NH), 8.57 (m, 1H, Ph-6'-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 114.98, 115.47, 123.41, 125.21, 127.05, 127.60, 132.01, 143.15, 144.29, 156.54, 159.11. Anal. Calcd. for $C_{11}H_9FN_6$: C, 54.10; H, 3.71; N, 34.41. Found: C, 54.24; H, 3.80, N, 34.56.

*2.2.7. 6-Hydrazino-3-(4'-fluorophenyl)-1,2,4-triazolo[4,3-b]pyridazine (**2g**)*

Yield 69%; m.p. 258.5 ºC; IR (KBr, cm^{-1}): 3672 (br NH str.), 1607 (C=N str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.32 (bs, 2H, -NH_2), 6.84-6.86 (d, 1H, *J*=9.84 Hz, pyridazine-5H), 7.29-7.33 (t, 2H, J_o=8.84 Hz, Ph-3', 5'-H), 7.89-7.92 (d, 1H, *J*=9.88 Hz, pyridazine-4H), 8.51 (s, 1H, -NH), 8.58-8.61 (dd, 2H, J_o=8.8 Hz, $J_{(m)\ HF}$=5.6 Hz, Ph-2', 6'-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ:

115.04, 115.38-115.59 (d, $^{2}J_{C\text{-}F}$=21 Hz, Ph-3', 5'-C), 123.48, 123.64, 129.10-129.18 (d, $^{3}J_{C\text{-}F}$=8.0 Hz, Ph-2', 6'-C), 143.81, 145.21, 156.44, 161.28-163.74 (d, $^{1}J_{C\text{-}F}$=246 Hz, Ph-4'-C). Anal. Calcd. for $C_{11}H_9FN_6$: C, 54.10; H, 3.71; N, 34.41. Found: C, 54.42; H, 3.44, N, 34.10.

*2.2.8. 6-Hydrazino-3-(4'-methoxyphenyl)-1,2,4-triazolo[4,3-b]pyridazine (**2h**)*

Yield 68%; m.p. 258.5 ºC; IR (KBr, cm^{-1}): 3317 (br NH str.), 1609 (C=N str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.85 (s, 3H, -OCH_3), 4.29 (bs, 2H, -NH_2), 6.81-6.83 (d, 1H, *J*=9.88 Hz, pyridazine-5H), 7.06-7.08 (d, 2H, *J*=8.96 Hz, Ph-3', 5'-H), 7.86-7.88 (d, 1H, *J*=9.84 Hz, pyridazine-4H), 8.44 (s, 1H, -NH), 8.47-8.49 (d, 2H, *J*=8.92 Hz, Ph-2', 6'-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 55.11, 113.86, 114.67, 119.47, 123.65, 128.44, 143.41, 148.89, 156.32, 159.41. Anal. Calcd. for $C_{12}H_{12}N_6O$: C, 56.24; H, 4.72; N, 32.79. Found: C, 56.58; H, 4.40, N, 32.25.

*2.2.9. 6-Hydrazino-3-(2',5'-dimethoxyphenyl)-1,2,4-triazolo[4,3-b]pyridazine (**2i**)*

Yield 67%; m.p. 125 ºC; IR (KBr, cm^{-1}): 3317 (br NH str.), 1612 (C=N str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.71 (s, 3H, Ph-5'-OCH_3), 3.77 (s, 3H, Ph-2'-OCH_3), 4.16 (bs, 2H, -NH_2), 6.86-6.89 (d, 1H, *J*= 9.84 Hz, pyridazine-5H), 7.05-7.12 (m, 3H, Ph-3', 4', 6'-H), 7.87-7.90 (d, 1H, *J*= 9.88 Hz, pyridazine-4H), 8.34 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 55.51, 56.22, 113.28, 115.13, 116.45, 116.59, 116.65, 123.40, 143.08, 145.64, 152.07, 152.77, 156.22. Anal. Calcd. for $C_{13}H_{14}N_6O_2$: C, 54.54; H, 4.93; N, 29.35. Found: C, 54.16; H, 4.62, N, 29.79.

*2.2.10. 6-Hydrazino-3-(3',4'-dimethoxyphenyl)-1,2,4-triazolo[4,3-b]pyridazine (**2j**)*

Yield 69%; m.p. 192 ºC; IR (KBr, cm^{-1}): 3217(br NH str.), 1624 (C=N str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.87 (s, 3H, Ph-3'-OCH_3), 3.91 (s, 3H, Ph-4'-OCH_3), 4.17 (bs, 2H, -NH_2), 6.81-6.84 (d, 1H, *J*=9.8 Hz, pyridazine-5H), 7.06-7.08 (d, 1H, J_o=8.36 Hz, Ph-5'-H), 7.86-7.88 (d, 1H, *J*=9.92 Hz, pyridazine-4H), 8.13-8.16 (dd, 1H, J_o=8.48 Hz, J_m=1.88 Hz, Ph-6'-H), 8.18 (d, 1H,

J_m=1.88 Hz, Ph-2'-H), 8.48 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 55.36, 55.48, 109.93, 111.40, 114.64, 119.51, 119.77, 123.65, 143.62, 145.82, 148.36, 149.57, 156.30. Anal. Calcd. for $C_{13}H_{14}N_6O_2$: C, 54.54; H, 4.93; N, 29.35. Found: C, 54.25; H, 5.04, N, 29.67.

*2.2.11. 6-Hydrazino-3-(thiophen-2'-yl)-1,2,4-triazolo[4,3-b]pyridazine (**2k**)*

Yield 67%; m.p. 248.5 ºC; IR (KBr, cm^{-1}): 3402 (br NH str.), 1630 (C=N str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.38 (bs, 2H, -NH_2), 6.83-6.86 (d, 1H, J=9.88 Hz, pyridazine-5H), 7.24-7.26 (dd, 1H, $J_{4', 5'}$ =5.0 Hz, $J_{3', 4'}$ =3.72 Hz, thiophene-4'-H), 7.69-7.71 (dd, 1H, $J_{4', 5'}$ =5.0 Hz, $J_{3', 5'}$ =1.0 Hz, thiophene-5'-H), 7.92-7.94 (d, 1H, J=9.88 Hz, pyridazine-4H), 8.30-8.32 (m, 1H, $J_{3', 4'}$ =3.64 Hz, $J_{3', 5'}$ =1.0 Hz, thiophene-3'-H), 8.58 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 115.00, 123.48, 127.12, 127.50, 127.62, 127.69, 143.13, 143.32, 156.58. MS: *m/z* 233.09 [M]$^+$. Anal. Calcd. for $C_9H_8N_6S$: C, 46.54; H, 3.47; N, 36.18. Found: C, 46.25; H, 3.86, N, 36.52.

*2.2.12. 6-Hydrazino-3-(furan-2'-yl)-1,2,4-triazolo[4,3-b]pyridazine (**2l**)*

Yield 65%; m.p. 226 ºC; IR (KBr, cm^{-1}): 3232 (br NH str.), 1615 (C=N str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.33 (bs, 2H, -NH_2), 6.70-6.71 (dd, 1H, $J_{3', 4'}$ =3.38 Hz, $J_{4', 5'}$ =1.8 Hz, furan-4'-H), 6.87-6.89 (d, 1H, J=9.88 Hz, pyridazine-5H), 7.65-7.66 (d, 1H, $J_{3', 4'}$ =3.32 Hz, furan-3'-H), 7.84-7.85 (d, 1H, $J_{3', 5'}$ =1.08 Hz, furan-5'-H), 7.90-7.93 (d, 1H, J=9.88 Hz, pyridazine-4H), 8.67 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 105.01, 107.15, 116.23, 125.70, 142.30, 143.21, 154.12, 163.14. Anal. Calcd. for $C_9H_8N_6O$: C, 50.00; H, 3.73; N, 38.87. Found: C, 49.85; H, 3.80, N, 38.60.

*2.3. Synthesis of 6-arylidenehydrazino-3-aryl-1,2,4-triazolo[4,3-b]pyridazine (**3a-l**)*

6-Hydrazino-3-aryl/heteroaryl-1,2,4-triazolo[4,3-*b*]pyridazine (**2a-l**) (3.0 mmol) was dissolved in 15 mL of ethanol and the solution was heated for 10 min. After complete dissolution of **2a-l** in ethanol, corresponding aldehyde (0.31 g, 3.0 mmol) was added. The reaction mixture

was then refluxed for 30 min. The reaction was monitored by TLC. On completion of the reaction, the crude product was filtered, washed with cold ethanol, and recrystallized using ethanol to afford **3a-l**.

*2.3.1. 6-Benzylidenehydrazino-3-phenyl-1,2,4-triazolo[4,3-b]pyridazine (**3a**)*

Yield 75%; m.p. 266.5 ºC; Lit m.p. [23]. ^{13}C NMR (100 MHz, DMSO-d_6) δ: 113.13, 124.84, 126.23, 126.64, 126.92, 128.19, 128.37, 128.96, 129.29, 134.43, 142.24, 146.20, 153.53. Anal. Calcd. For $C_{18}H_{14}N_6$: C, 68.78; H, 4.49; N, 26.93. Found: C, 68.23; H, 3.99; N, 26.75.

*2.3.2. 6-(2'-Chlorobenzylidenehydrazino)-3-(2'-chlorophenyl)-1,2,4-triazolo[4,3-b]pyridazine (**3b**)*

Yield 70%; m.p. 293.5 ºC; IR (KBr, cm^{-1}): 3152 (NH str.), 1602 (C=N str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.37-7.39 (m, 2H, Ph-H), 7.46-7.48 (m, 1H, Ph-H), 7.54-7.58 (m, 1H, Ph-H), 7.62-7.66 (m, 1H, Ph-H), 7.70-7.74 (m, 3H, Ph-H), 8.04-8.06 (d, 1H, *J*=9.36 Hz, pyridazine-5H), 8.30-8.32 (d, 1H, *J*=10 Hz, pyridazine-4H), 8.44 (s, 1H, -CH), 11.99 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 113.44, 125.09, 126.17, 126.31, 126.90, 126.99, 129.55, 130.09, 131.61, 131.81, 132.44, 132.54, 134.06, 138.14, 143.36, 145.71, 153.87. MS: *m/z* 383.01 [M+1]$^+$, 385.02 [M+1+2]$^+$, 387.01 [M+1+4]$^+$ (9:6:1). Anal. Calcd. for $C_{18}H_{12}Cl_2N_6$: C, 56.41; H, 3.16; N, 21.93. Found: C, 56.14; H, 3.20, N, 21.67.

*2.3.3. 6-(4'-Chlorobenzylidenehydrazino)-3-(4'-chlorophenyl)-1,2,4-triazolo[4,3-b]pyridazine (**3c**)*

Yield 72%; m.p. 187 ºC; IR (KBr, cm^{-1}): 3355 (NH str.), 1606 (C=N str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.53-7.55 (d, 2H, *J*=8.64 Hz, Ph-H), 7.63-7.65 (d, 2H, *J*=8.56 Hz, Ph-H), 7.74-7.76 (d, 1H, *J*=8.4 Hz, pyridazine-5H), 7.88-7.90 (d, 2H, *J*=8.64 Hz, Ph-H), 8.19-8.21 (d, 1H, *J*=9.8 Hz, pyridazine-4H), 8.52-8.54 (d, 2H, *J*=8.44 Hz, Ph-H), 8.69 (s, 1H, -CH), 11.03 (s, 1H, -NH);

^{13}C NMR (100 MHz, DMSO-d_6) δ: 113.55, 124.76, 127.87, 128.23, 128.59, 128.89, 129.84, 132.47, 134.22, 136.08, 139.92, 143.24, 145.11, 160.46. Anal. Calcd. for $C_{18}H_{12}Cl_2N_6$: C, 56.41; H, 3.16; N, 21.93. Found: C, 56.64; H, 3.45, N, 21.46.

2.3.4. 6-(3'-Bromobenzylidenehydrazino)-3-(3'-bromophenyl)-1,2,4-triazolo[4,3-b]pyridazine ***(3d)***

Yield 73%; m.p. 305 ºC; IR (KBr, cm^{-1}): 3155 (NH str.), 1608 (C=N str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.35-7.38 (t, 1H, *J*=7.84 Hz, Ph-H), 7.51-7.55 (m, 4H, Ph-H), 7.67-7.69 (d, 1H, *J*=7.96 Hz, pyridazine-5H), 7.73-7.75 (d, 1H, *J*=7.76 Hz, Ph-H), 7.95-7.97 (m, 1H, Ph-H), 8.13 (s, 1H, -CH), 8.14-8.17 (d, 1H, *J*=9.9 Hz, pyridazine-4H), 8.48-8.50 (m, 1H, Ph-H), 8.84 (s, 1H, -NH). MS: *m/z* 470.96 $[M+1]^+$, 472.94 $[M+1+2]^+$, 474.95 $[M+1+4]^+$ (1:2:1); Anal. Calcd. for $C_{18}H_{12}Br_2N_6$: C, 45.79; H, 2.56; N, 17.80. Found: C, 45.33; H, 2.21, N, 17.31.

2.3.5. 6-(4'-Bromobenzylidenehydrazino)-3-(4'-bromophenyl)-1,2,4-triazolo[4,3-b]pyridazine ***(3e)***

Yield 76%; m.p. 211 ºC; IR (KBr, cm^{-1}): 3124 (NH str.), 1611 (C=N str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.59-7.61 (d, 2H, *J*=8.32 Hz, Ph-H), 7.66-7.69 (m, 4H, Ph-H), 7.75-7.77 (d, 1H, *J*=8.4 Hz, pyridazine-5H), 7.80-7.82 (d, 2H, *J*=8.32 Hz, Ph-H), 8.45-8.47 (d, 1H, *J*=8.36 Hz, pyridazine-4H), 8.66 (s, 1H, -CH), 11.77 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 113.11, 124.71, 125.39, 125.93, 128.23, 128.46, 130.06, 131.56, 131.63, 131.84, 133.80, 144.38, 145.44, 153.16. Anal. Calcd. for $C_{18}H_{12}Br_2N_6$: C, 45.79; H, 2.56; N, 17.80. Found: C, 45.91; H, 2.80, N, 17.60.

2.3.6. 6-(2'-Fluorobenzylidenehydrazino)-3-(2'-fluorophenyl)-1,2,4-triazolo[4,3-b]pyridazine ***(3f)***

Yield 72%; m.p. 235 ºC; IR (KBr, cm^{-1}): 3132 (NH str.), 1615 (C=N str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.01-7.02 (m, 3H, Ph-H), 7.30-7.62 (m, 5H, Ph-H), 8.02-8.04 (d, 1H, *J*=9.6 Hz, pyridazine-5H), 8.28-8.30 (d, 1H, *J*=9.2 Hz, pyridazine-4H), 8.37 (s, 1H, -CH) 11.61 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 113.15, 115.22, 115.35, 115.43, 115.56, 124.97, 128.17, 128.26, 128.96, 129.04, 131.12, 141.16, 143.98, 144.04, 145.21, 161.39. Anal. Calcd. for $C_{18}H_{12}F_2N_6$: C, 61.71; H, 3.45; N, 23.99. Found: C, 61.36; H, 3.89, N, 23.56.

2.3.7. 6-(4'-Fluorobenzylidenehydrazino)-3-(4'-fluorophenyl)-1,2,4-triazolo[4,3-b]pyridazine ***(3g)***

Yield 76%; m.p. 249 ºC; IR (KBr, cm^{-1}): 3132 (NH str.), 1620 (C=N str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.19-7.24 (t, 2H, *J*=8.76 Hz, Ph-H), 7.35-7.39 (t, 2H, *J*=8.84 Hz, Ph-H), 7.59-7.61 (d, 1H, *J*=9.68 Hz, pyridazine-5H), 7.75-7.79 (dd, 2H, J_o=8.6 Hz, $J_{(m)\ HF}$=5.6 Hz, Ph-H), 8.14 (s, 1H, -CH), 8.16-8.19 (d, 1H, *J*=10 Hz, pyridazine-4H), 8.53-8.56 (dd, 2H, J_o=8.8 Hz, $J_{(m)\ HF}$=5.6 Hz, Ph-H), 11.62 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 113.29, 115.39-115.61 (d, $^{2}J_{C\text{-}F}$=22 Hz, Ph-3', 5'-C), 115.49-115.71 (d, $^{2}J_{C\text{-}F}$=22 Hz, Ph-3', 5'-C), 123.28, 125.17, 128.32-128.41 (d, $^{3}J_{C\text{-}F}$=8.0 Hz, Ph-2', 6'-C), 128.98-129.06 (d, $^{3}J_{C\text{-}F}$=9.0 Hz, Ph-2', 6'-C), 131.13, 141.23, 144.04, 145.21, 153.40, 161.11, 162.92. Anal. Calcd. for $C_{18}H_{12}F_2N_6$: C, 61.71; H, 3.45; N, 23.99. Found: C, 61.63; H, 3.71, N, 23.62.

2.3.8. 6-(4'-Methoxybenzylidenehydrazino)-3-(4'-methoxyphenyl)-1,2,4-triazolo[4,3-b]pyridazine ***(3h)***

Yield 73%; m.p. 278 ºC; IR (KBr, cm^{-1}): 3155 (NH str.), 1614 (C=N str.); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.86 (s, 3H, $-OCH_3$), 3.89 (s, 3H, $-OCH_3$), 6.94-6.96 (d, 2H, J_o=8.8 Hz, Ph-H), 7.04-7.06 (dd, 2H, J_o=8.88 Hz, J_m=1.92 Hz, Ph-H), 7.61-7.64 (d, 2H, J_o=8.8 Hz, Ph-H), 7.63-7.65 (d, 1H, *J*=9.96 Hz, pyridazine-5H), 7.82 (s, 1H, -CH), 8.04-8.06 (d, 1H, *J*=9.96 Hz, pyridazine-4H),

8.38-8.39 (m, 2H, Ph-H), 8.40 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 54.98, 55.01, 112.89, 113.62, 113.90, 119.23, 124.73, 127.14, 127.43, 128.41, 142.13, 160.12, 160.16. Anal. Calcd. for $C_{20}H_{18}N_6O_2$: C, 64.16; H, 4.85; N, 22.45. Found: C, 64.32; H, 4.95, N, 22.80.

*2.3.9. 6-(2',5'-Dimethoxybenzylidenehydrazino)-3-(2',5'-dimethoxyphenyl)-1,2,4-triazolo[4,3-b]pyridazine (**3i**)*

Yield 74%; m.p. 250.5 ºC; IR (KBr, cm^{-1}): 3240 (NH str.), 1606 (C=N str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.71-3.79 (m, 12H, -OCH_3), 6.90-7.04 (m, 4H, Ph-H), 7.33-7.34 (d, 1H, *J*=2.84 Hz, Ph-H), 7.48-7.49 (d, 1H, *J*=2.84 Hz, Ph-H), 7.56-7.58 (d, 1H, *J*=9.88 Hz, pyridazine-5H), 7.99-8.01 (d, 1H, *J*=9.96 Hz, pyridazine-4H), 8.29 (s, 1H, -CH), 11.42 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 55.29, 55.46, 56.03, 56.20, 112.66, 113.01, 113.12, 116.08, 116.53, 116.69, 119.04, 122.22, 124.69, 137.40, 143.25, 145.60, 151.50, 152.15, 152.87, 153.06, 153.22, 156.40. MS: *m/z* 435.19 $[M+1]^+$. Anal. Calcd. for $C_{22}H_{22}N_6O_4$: C, 60.82; H, 5.10; N, 19.34. Found: C, 60.51; H, 4.98, N, 19.22.

*2.3.10. 6-(3',4'-Dimethoxybenzylidenehydrazino)-3-(3',4'-dimethoxyphenyl)-1,2,4-triazolo[4,3-b]pyridazine (**3j**)*

Yield 72%; m.p. 247 ºC; IR (KBr, cm^{-1}): 3233 (NH str.), 1621 (C=N str.); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.93 (s, 3H, -OCH_3), 3.95 (s, 3H, -OCH_3), 3.98 (s, 3H, -OCH_3), 3.99 (s, 3H, -OCH_3), 6.88-6.90 (d, 1H, *J*=8.32 Hz, Ph-H), 6.97-6.99 (d, 1H, *J*=8.48 Hz, Ph-H), 7.10-7.13 (dd, 1H, J_o=8.3 Hz, J_m=1.8 Hz, Ph-H), 7.34-7.35 (d, 1H, J_m=1.76 Hz, Ph-H), 7.61-7.64 (d, 1H, *J*=9.96 Hz, pyridazine-5H), 7.84 (s, 1H, -CH), 7.96-7.97 (d, 1H, J_m=1.8 Hz, Ph-H), 8.05-8.07 (d, 1H, *J*=9.96 Hz, pyridazine-4H), 8.09-8.12 (dd, 1H, J_o=8.42 Hz, J_m=1.96 Hz, Ph-H); 8.55 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 55.39, 55.46, 55.48, 55.71, 107.63, 110.51, 110.67, 110.74,

113.07, 119.34, 120.07, 120.76, 124.50, 127.34, 143.83, 146.22, 148.37, 148.83, 149.78, 149.95. Anal. Calcd. for $C_{22}H_{22}N_6O_4$: C, 60.82; H, 5.10; N, 19.34. Found: C, 60.25; H, 5.36, N, 19.56.

*2.3.11. 3-(Thiophen-2'-yl)-6-(2-(thiophen-2'-ylmethylidene)hydrazino)-1,2,4-triazolo[4,3-b]pyridazine (**3k**)*

Yield 72%; m.p. 90 ºC; IR (KBr, cm^{-1}): 3122 (NH str.), 1603 (C=N str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.08-7.10 (t, 1H, $J_{4',5'}$ =4.8 Hz, $J_{3',4'}$ =3.8 Hz, thiophene-4'-H), 7.26-7.28 (m, 1H, $J_{4',5'}$ =4.8 Hz, $J_{3',4'}$ =3.8 Hz, thiophene-4'-H), 7.33-7.34 (d, 1H, $J_{3',4'}$ =3.2 Hz, thiophene-3'-H), 7.43-7.46 (d, 1H, *J*=9.8 Hz, pyridazine-5H), 7.50-7.51 (d, 1H, $J_{4',5'}$ =4.9 Hz, thiophene-5'-H), 7.68-7.69 (d, 1H, $J_{4',5'}$ =4.64 Hz, thiophene-5'-H), 8.14-8.15 (d, 1H, *J*= 6.04 Hz, pyridazine-4H), 8.28-8.29 (d, 1H, $J_{3',4'}$ =3.12 Hz, thiophene-3'-H), 8.36 (s, 1H, -CH), 11.55 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 113.07, 125.12, 126.89, 127.52, 127.69, 128.95, 137.87, 139.46, 143.54. Anal. Calcd. for $C_{14}H_{10}N_6S_2$: C, 51.52; H, 3.09; N, 25.75. Found: C, 51.36; H, 3.36, N, 23.54.

*2.3.12. 3-(Furan-2'-yl)-6-(2-(furan-2'-ylmethylidene)hydrazino)-1,2,4-triazolo[4,3-b]pyridazine (**3l**)*

Yield 70%; m.p. 94 ºC; IR (KBr, cm^{-1}): 3122 (NH str.), 1618 (C=N str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.59 (m, 1H, furan-4'-H), 6.77 (m, 1H, furan-4'-H), 6.82-6.83 (d, 1H, $J_{3',4'}$ =3.08 Hz, furan-3'-H), 7.49-7.51 (m, 2H, pyridazine-5H, furan-H), 7.75 (s, 1H, furan-H), 7.91 (s, 1H, -CH), 8.05 (s, 1H, furan-H), 8.18-8.21 (d, 1H, *J*=10 Hz, pyridazine-4H), 11.60 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 111.43, 111.72, 112.14, 113.37, 125.31, 132.98, 140.28, 140.94, 143.40, 144.47, 144.58, 149.53, 153.56. MS: *m/z* 295.10 $[M+1]^+$. Anal. Calcd. for $C_{14}H_{10}N_6O_2$: C, 57.14; H,3.43; N, 28.56. Found: C, 57.31 H, 3.11, N, 28.30.

*2.4. Synthesis of 3,6-disubstituted-bis-1,2,4-triazolo-[4,3-b][3',4'-f]pyridazine (**4a-l**)*

To a solution of 6-benzylidenehydrazino-3-phenyl-1,2,4-triazolo[4,3-*b*]pyridazine (**3a**) (0.62 g, 2.0 mmol) in dichloromethane (20 mL), iodobenzene diacetate (0.70 g, 2.2 mmol) was added in small portion and the reaction mixture was stirred at room temperature for 1-2 hour or until the completion of reaction as monitored by TLC. Solid separated out was filtered, washed and recrystallized with ethanol to afford **4a**.

*2.4.1. 3,6-Diphenyl-bis-1,2,4-triazolo-[4,3-b][3',4'-f]pyridazine (**4a**)*

Yield 82%; m.p. 274 ºC; IR (KBr, cm^{-1}): transparent in the region of –NH str., 1638 (C=N str.); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.06-7.17 (m, 3H, 2×Ph-3', 4', 5'-H), 7.29-7.32 (m, 2H, 2×Ph-2', 6'-H), 7.71 (s, 1H, 2×pyridazine-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 118.10, 126.30, 128.55, 128.76, 130.73, 146.70, 148.25. Anal. Calcd. for $C_{18}H_{12}N_6$: C, 69.22; H, 3.87; N, 26.91. Found: C, 69.49; H, 3.42, N, 26.63.

*2.4.2. 3,6-Di-(2'-chlorophenyl)-bis-1,2,4-triazolo-[4,3-b][3',4'-f]pyridazine (**4b**)*

Yield 78%; m.p. 201.5 ºC; IR (KBr, cm^{-1}): transparent in the region of –NH str., 1602 (C=N str.); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.93-6.95 (d, 1H, *J*=8.0 Hz, 2×Ph-H), 7.09-7.13 (dt, 1H, *J*=7.94 Hz, *J*=1.4 Hz, 2×Ph-H), 7.17-7.21 (dt, 1H, *J*=7.56 Hz, *J*=0.84 Hz, 2×Ph-H), 7.74 (s, 1H, 2×pyridazine-H), 7.79-7.81 (dd, 1H, *J*=7.62 Hz, *J*=1.48 Hz, 2×Ph-2'-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 118.16, 126.07, 127.17, 129.32, 132.17, 132.91, 134.42, 145.55, 146.27. MS: *m/z* 381.01 $[M+1]^+$, 383.02 $[M+1+2]^+$, 385.06 $[M+1+4]^+$, (9:6:1). Anal. Calcd. for $C_{18}H_{10}Cl_2N_6$: C, 56.71; H, 2.64; N, 22.05. Found: C, 56.92; H, 2.51, N, 22.39.

*2.4.3. 3,6-Di-(4'-chlorophenyl)-bis-1,2,4-triazolo-[4,3-b][3',4'-f]pyridazine (**4c**)*

Yield 74%; m.p. 244.5 ºC; IR (KBr, cm^{-1}): transparent in the region of –NH str., 1623 (C=N str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.12-7.14 (d, 2H, *J*=8.4 Hz, 2×Ph-H), 7.39-7.41 (d, 2H, *J*=8.4 Hz, 2×Ph-H), 8.06 (s, 1H, 2×pyridazine-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 118.13, 125.57,

128.26, 130.77, 135.35, 146.11, 146.98. Anal. Calcd. for $C_{18}H_{10}Cl_2N_6$: C, 56.71; H, 2.64; N, 22.05. Found: C, 56.39; H, 2.30, N, 22.20.

*2.4.4. 3,6-Di-(3'-bromophenyl)-bis-1,2,4-triazolo-[4,3-b][3',4'-f]pyridazine (**4d**)*

Yield 72%; m.p. 190 ºC; IR (KBr, cm^{-1}): transparent in the region of –NH str., 1613 (C=N str.); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.08-7.12 (t, 1H, *J*=7.84 Hz, 2×Ph-H), 7.34-7.36 (dd, 1H, *J*=8.12 Hz, *J*=0.96 Hz, 2×Ph-H), 7.38 (m, 1H, 2×Ph-H), 7.41-7.43 (m, 1H, 2×Ph-H), 7.75 (s, 1H, 2×pyridazine-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 118.34, 123.08, 127.12, 127.92, 130.37, 131.39, 132.92, 146.51, 146.74. Anal. Calcd. for $C_{18}H_{10}Br_2N_6$: C, 45.99; H, 2.14, N, 17.88. Found: C, 45.72; H, 2.01, N, 17.46.

*2.4.5. 3,6-Di-(4'-bromophenyl)-bis-1,2,4-triazolo-[4,3-b][3',4'-f]pyridazine (**4e**)*

Yield 74%; m.p. 197 ºC; IR (KBr, cm^{-1}): transparent in the region of –NH str., 1629 (C=N str.); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.15-7.17 (d, 2H, *J*=8.52 Hz, 2×Ph-H), 7.29-7.31 (d, 2H, *J*=8.52 Hz, 2×Ph-H), 7.72 (s, 1H, 2×pyridazine-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 117.98, 124.41, 125.73, 130.71, 131.18, 146.21, 146.84. MS: *m/z* 468.92 $[M+1]^+$, 470.93 $[M+1+2]^+$, 473.93 $[M+1+4]^+$, (1:2:1). Anal. Calcd. for $C_{18}H_{10}Br_2N_6$: C, 45.99; H, 2.14, N, 17.88. Found: C, 45.69; H, 2.33, N, 17.59.

*2.4.6. 3,6-Di-(2'-fluorophenyl)-bis-1,2,4-triazolo-[4,3-b][3',4'-f]pyridazine (**4f**)*

Yield 70%; m.p. 257.5 ºC; IR (KBr, cm^{-1}): transparent in the region of –NH str., 1631 (C=N str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.82-7.62 (m, 4H, 2×Ph-H), 7.98 (s, 1H, 2×pyridazine-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 115.62-115.82 (d, $^2J_{C\text{-}F}$=20 Hz), 118.20-118.29 (d, *J*=9 Hz), 124.54-126.62 (d, *J*=8 Hz), 130.73, 131.79, 133.62-133.70 (d, *J*=8 Hz), 141.97, 142.35, 146.54, 157.48-159.96 (d, $^1J_{C\text{-}F}$=248 Hz). Anal. Calcd. for $C_{18}H_{10}F_2N_6$: C, 62.07; H, 2.89, N, 24.13. Found: C, 62.36; H, 2.99, N, 24.33.

*2.4.7. 3,6-Di-(4'-fluorophenyl)-bis-1,2,4-triazolo-[4,3-b][3',4'-f]pyridazine (**4g**)*

Yield 72%; m.p. 292.5 ºC; IR (KBr, cm^{-1}): transparent in the region of –NH str., 1610 (C=N str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.87-6.92 (t, 2H, J_o=8.8 Hz, 2×Ph-3', 5'-H), 7.45-7.48 (dd, 2H, J_o=8.76 Hz, $J_{(m)\ HF}$=5.32 Hz, 2×Ph-2', 6'-H), 8.17 (s, 1H, 2×pyridazine-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 115.15-115.37 (d, $^{2}J_{C\text{-}F}$=22 Hz, Ph-3', 5'-C), 118.04, 123.31-123.34 (d, J=3), 131.47-131.56 (d, $^{3}J_{C\text{-}F}$=9 Hz, Ph-2', 6'-C), 146.23, 146.89, 161.49-163.96 (d, $^{1}J_{C\text{-}F}$=247 Hz, Ph-4'-C). Anal. Calcd. for $C_{18}H_{10}F_2N_6$: C, 62.07; H, 2.89, N, 24.13. Found: C, 62.50; H, 2.61, N, 24.01.

*2.4.8. 3,6-Di-(4'-methoxyphenyl)-bis-1,2,4-triazolo-[4,3-b][3',4'-f]pyridazine (**4h**)*

Yield 75%; m.p. 225 ºC; IR (KBr, cm^{-1}): transparent in the region of –NH str., 1612 (C=N str.); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.76 (s, 3H, 2×Ph-4'-OCH_3), 6.57-6.59 (m, 2H, 2×Ph-3', 5'-H), 7.04-7.07 (m, 2H, 2×Ph-2', 6'-H), 7.64 (s, 1H, 2×pyridazine-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 54.89, 113.53, 117.60, 118.89, 130.42, 146.52, 147.23, 160.15. Anal. Calcd. for $C_{20}H_{16}N_6O_2$: C, 64.51; H, 4.33, N, 22.57. Found: C, 64.24; H, 4.25; N, 22.22.

*2.4.9. 3,6-Di-(2',5'-dimethoxyphenyl)-bis-1,2,4-triazolo-[4,3-b][3',4'-f]pyridazine (**4i**)*

Yield 73%; m.p. >315 ºC; IR (KBr, cm^{-1}): transparent in the region of –NH str., 1617 (C=N str.); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.44 (s, 3H, 2×Ph-5'-OCH_3), 3.70 (s, 3H, 2×Ph-2'-OCH_3), 6.33-6.36 (d, 1H, J_o=9.3 Hz, 2×Ph-3'-H), 6.66-6.70 (dd, 1H, J_o=9.3 Hz, J_m=3Hz, 2×Ph-4'-H), 7.13-7.14 (d, 1H, J_m=3Hz, 2×Ph-6'-H), 7.62 (s, 1H, 2×pyridazine-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 55.25, 56.02, 111.20, 115.05, 116.41, 117.87, 118.69, 146.31, 146.62, 151.26, 153.43. MS: *m/z* 433.16 $[M+1]^+$. Anal. Calcd. for $C_{22}H_{20}N_6O_4$: C, 61.10; H, 4.66, N, 19.43. Found: C, 61.38; H, 4.39, N, 19.68.

*2.4.10. 3,6-Di-(3',4'-dimethoxyphenyl)-bis-1,2,4-triazolo-[4,3-b][3',4'-f]pyridazine (**4j**)*

Yield 73%; m.p. 226 ºC; IR (KBr, cm^{-1}): transparent in the region of –NH str., 1606 (C=N str.); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.78 (s, 3H, 2×Ph-4'-OCH_3), 3.83 (s, 3H, 2×Ph-3'-OCH_3), 6.51-6.53 (d, 1H, J_o=8.32 Hz, 2×Ph-5'-H), 6.74-6.75 (d, 1H, J_m=1.8 Hz, 2×Ph-2'-H), 6.98-7.01 (dd, 1H, J_o=8.28 Hz, J_m=1.88 Hz, 2×Ph-6'-H), 7.67 (s, 1H, 2×pyridazine-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 55.87, 55.92, 110.87, 111.16, 117.95, 118.67, 121.35, 146.59, 148.14, 148.33, 150.71. Anal. Calcd. for $C_{22}H_{20}N_6O_4$: C, 61.10; H, 4.66, N, 19.43. Found: C, 61.15; H, 4.90, N, 19.23.

*2.4.11. 3,6-Di-(thiophen-2'-yl)-bis-1,2,4-triazolo-[4,3-b][3',4'-f]pyridazine (**4k**)*

Yield 72%; m.p. 231 ºC; IR (KBr, cm^{-1}): transparent in the region of –NH str., 1611 (C=N str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.72-6.74 (t, 1H, $J_{4',5'}$ =4.8 Hz, $J_{3',4'}$ =4.0 Hz, 2×thiophene-4'-H), 7.16-7.18 (d, 1H, $J_{3',4'}$ =3.6 Hz, 2×thiophene-3'-H), 7.53-7.54 (d, 1H, $J_{4',5'}$ =5.2 Hz, 2×thiophene-5'-H), 7.86 (s, 1H, 2×pyridazine-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 118.11, 126.48, 127.76, 129.26, 131.10, 142.64, 146.83. Anal. Calcd. for $C_{14}H_8N_6S_2$: C, 51.84; H, 2.49; N, 25.91. Found: C, 51.52; H, 2.88, N, 25.61.

*2.4.12. 3,6-Di-(furan-2'-yl)-bis-1,2,4-triazolo-[4,3-b][3',4'-f]pyridazine (**4l**)*

Yield 70%; m.p. 225 ºC; IR (KBr, cm^{-1}): transparent in the region of –NH str., 1610 (C=N str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.38 (m, 1H, 2×furan-4'-H), 6.89-6.90 (d, 1H, $J_{3',4'}$ =3.08 Hz, 2×furan-3'-H), 7.17 (m, 1H, 2×furan-5'-H), 8.22 (s, 1H, 2×pyridazine-H). Anal. Calcd. for $C_{14}H_8N_6O_2$: C, 57.54; H, 2.76; N, 28.76. Found: C, 57.23; H, 2.40, N, 28.53.

2.5. X-ray crystallography

To study the effect of substituents on the planarity of the molecule three candidates viz. 3,6-di-(2'-fluorophenyl)-bis-1,2,4-triazolo-[4,3-*b*][3',4'-*f*]pyridazine **4f**, 3,6-di-(4'-fluorophenyl)-bis-1,2,4-triazolo-[4,3-*b*][3',4'-*f*]pyridazine **4g** and 3,6-di-(2',5'-dimethoxyphenyl)-bis-1,2,4-triazolo-[4,3-*b*][3',4'-*f*]pyridazine **4i** were selected for X-ray diffraction studies. Compounds **4f**

and **4g** have fluorine as substituent at ortho and para position of aryl ring, respectively. In compound **4i** two methoxy groups are present at ortho and meta positions thereby further increasing the steric crowding. Single crystals of **4f**, **4g** and **4i** were obtained by slow evaporation of a DCM-ethanol (7:3) solution containing 2-3 drops of petroleum ether of the initial solid product at ambient temperature over a period of about 3 days. The molecular structures of **4f** and **4g** were determined by single crystal X-ray diffraction. However, unfortunately the crystals of the compound **4i** did not diffract X-rays and its structure could not be obtained through X-ray.

Crystallographic data for compounds **4f** and **4g**, can be obtained from CAI de Difracción de Rayos-X, Universidad Complutense de Madrid (Madrid, Spain).

2.5.1. X-Ray data collection and structure refinement

Data collection for **4f** and **4g** was carried out at room temperature on a Bruker Smart CCD diffractometer using in all cases graphite-monochromated Mo-Kα radiation (λ = 0.71073 Å) operating at 50 kV and 35 mA, with exposure times of 20 s in omega. The details of the fundamental crystal structure and data refinement parameters of the compounds **4f** and **4g** are given in Table 1. The structures were solved by direct methods and refined by full-matrix least-squares procedures on F^2 using the SHELXL-97 [31]. All non-hydrogen atoms were refined anisotropically. The hydrogen atoms were included in their calculated positions and refined riding on the respective carbon atoms.

Table 1

Crystal data and structure refinement details for compounds **4f** and **4g**.

Crystal Data	**4f**	**4g**
CCDC code	1850032	1850033
Empirical formula	$C_{18}H_{10}F_2N_6$	$C_{18}H_{10}F_2N_6$
Formula weight	348.32	348.32

Crystal system	Orthorhombic	Orthorhombic
Space group	$P2_12_12_1$	$P2_12_12_1$
a/Å	10.293(1)	6.620(2)
b/Å	10.989(1)	14.886(3)
c/Å	13.939(2)	15.601(4)
α (°)	90.0	90.0
β (°)	90.0	90.0
γ (°)	90.0	90.0
V/Å^3	1578.6(3)	1537.3(6)
Z	4	4
D_c /g/cm^3	1.467	1.505
μ(Mo-Kα) /mm^{-1}	0.110	0.113
F *(000)*	712	712
θ range (°)	2.36 to 25.0	1.89 to 26.00
Index ranges	-12,-13,-14 to 12, 12, 16	-8,-18,-19 to 7, 18, 16
Reflections collected	12050	12688
Unique reflections	2769	3001
[R(int)]	[R(int) = 0.0535]	[R(int) = 0.0531]
Completeness to theta %	99.8%	99.3%
Data/restraints/parameters	2769 / 0 / 235	3001/ 0 / 235
Goodness-of-fit on F^2	0.996	0.998
R1 (reflns obsd) [I>2σ(I)] [a]	0.0332 (1720)	0.0310 (2131)
wR2 (all data) [b]	0.0517	0.0657

[a] $R1=\Sigma||F_o|-|F_c||/\ \Sigma|F_o|$

[b] $wR2=\{\Sigma[w(F_o^2-F_c^2)^2]/\ \Sigma[w(F_o^2)^2]\}$

CCDC 1850032 and 1850033 contain the supplementary crystallographic data for the compound **4f** and **4g**, respectively. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

2.6. Cytotoxic activity

2.6.1. Cell Culture

Human cervical carcinoma (HeLa) cell lines were cultured in RPMI-1640 medium supplemented with 10% fetal serum albumin (FBS), 100 units/ml penicillin, and 100 mg/ml streptomycin. The cell lines were maintained in an incubator containing 5% of CO_2 at 37 ºC.

2.6.2. Cell Viability Assay

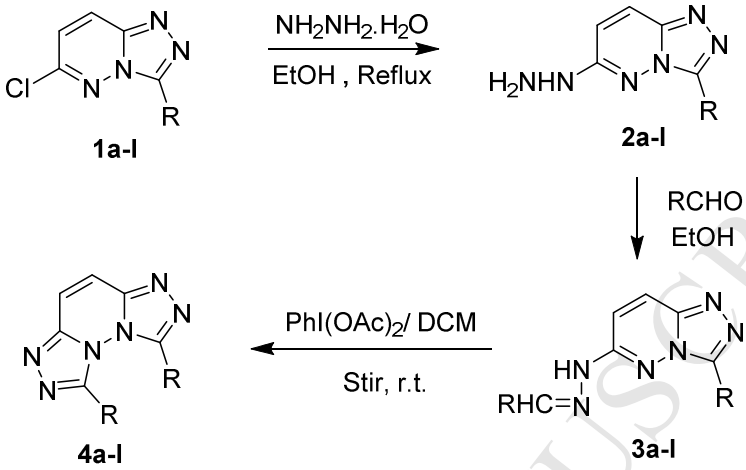
The cells were seeded at a density of 2×10^5 per mL in RPMI solution by transferring 200 µL into each well on a 96-well plate and incubated for 24 hr. Cells were treated with various compounds at different concentration (50 µM and 100 µM in DMSO) and incubated over a period of 48 h at 37 ºC. After this 20 µL of MTT reagent (5 mg/mL in phosphate-buffered saline) was added to each well and incubated for 4 hr at 37 °C. 200 µL DMSO (MTT solvent) was added and absorbance was read using a plate reader, with the optical density of the purple formazan A_{550} being proportional to the number of viable cells. The mean values and the standard error of the mean (SEM) of all the experiments for each compound were then used to plot graphs using GraphPad Prism program and Excel. The concentration required for 50% inhibition of cell viability (IC_{50}) was calculated using the GraphPad Prism.

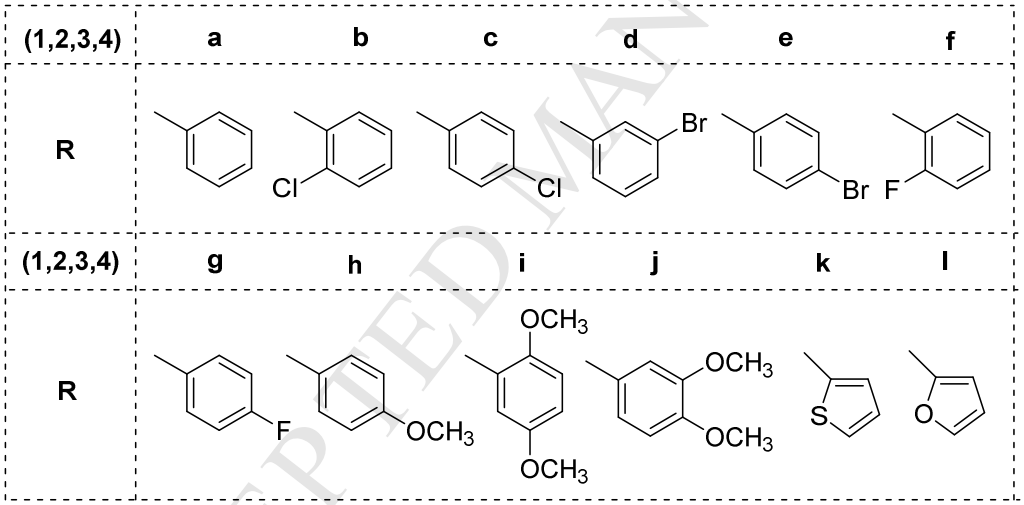
3. Results and Discussion

3.1. Chemistry

Synthesis of 3,6-disubstituted-bis-1,2,4-triazolo-[4,3-*b*][3',4'-*f*]pyridazine derivatives (**4a-l**) is depicted in Scheme 1. Precursor 6-hydrazino-3-aryl/heteroaryl-1,2,4-triazolo[4,3-*b*]pyridazines (**2a-l)** were prepared from reaction of different substituted 6-chloro-1,2,4-triazolo[4,3-*b*]pyridazines (**1a-l)** with hydrazine hydrate in refluxing ethanol. The key intermediate 6-arylidenehydrazino-3-aryl-1,2,4-triazolo[4,3-*b*]pyridazines (**3a-l**) were obtained by condensation of compounds **2a-l** with aldehydes in ethanol. Hydrazones (**3a-l)** upon oxidative

cyclization with 1.1 equivalents of IBD in dichloromethane at room temperature afforded the corresponding bis-1,2,4-triazolo-[4,3-*b*][3',4'-*f*]pyridazines (**4a-l)**.





Scheme 1. IBD-mediated synthesis of 3,6-disubstituted-bis-1,2,4-triazolo-[4,3-*b*][3',4'-*f*]pyridazines (**4**).

The structures and purity of all the compounds were established on the basis of a careful comparison of their spectral data (IR, ^{1}H NMR, ^{13}C NMR) as well as high resolution mass. ^{1}H NMR spectra of compounds **2a-l** exhibited a broad singlet signal at δ 3.33-4.38 ppm for two proton intensity corresponding to NH_2 and a sharp singlet signal at δ 8.34-8.67 ppm for one proton intensity corresponding to NH proton. In addition, ^{1}H NMR spectra of compounds **2a-l**

displayed two doublets of one proton intensity at δ 6.81-6.92 ppm and 7.85-7.95 ppm attributed to pyridazine protons H-5 and H-4, respectively having the coupling constant *J*= ~ 9.8 Hz.

IR spectra of compounds **3a-l** showed characteristic absorption band in the region ~3122-3355 cm^{-1} for N-H stretching vibration and 1602-1621 cm^{-1} for C=N stretching vibration. ^{1}H NMR spectra of compounds **3a-l** exhibited a sharp singlet at δ 7.82-8.69 ppm for aldehydic proton and a broad singlet at δ 8.40-11.99 ppm for NH. Moreover, in compounds **3** a pair of doublets of one proton intensity was obtained at δ 7.43-8.06 ppm and 7.99-8.47 ppm corresponding to pyridazine protons H-4 and H-5, respectively. IR spectra of **4a-l** were found to be transparent in the region of NH stretch and bend, thus confirm the oxidation of **3** to **4**. An important characteristic feature in the ^{1}H NMR spectra of **4a-l** was disappearance of the signals at δ 7.82-8.69 ppm and 8.40-11.99 ppm for aldehydic proton and for NH, respectively, which were present in the spectra of the intermediate hydrazones **3a-l**, thus suggesting the cyclization of hydrazones **3** into bis-triazoles **4**. Interestingly, in ^{1}H NMR spectra of compounds **4** a sharp singlet signal was observed at δ 7.62-8.22 ppm for pyridazine protons H-4 and H-5 in contrast to two doublets of one proton intensity as obtained in **2** and **3**. In conjunction with ^{1}H NMR, ^{13}C NMR spectra also exhibited symmetrical pattern of **4.**

The most striking feature of NMR (^{1}H and^{13}C) spectra of bis-triazoles **4** indicated that the molecule is totally symmetrical. It is evident particularly from the spectra of **4h**, **4i** and **4j**, all having methoxy substituents that number of expected peaks in aliphatic region is just half. Similarly pyridazine proton and aryl protons also appear in half proportion. This observation seems to be quite unusual as the two aryl groups are present in a very narrow space and it is expected that they will be distorted in space to accommodate each other. Therefore, it was planned to study the structure of bis-triazoles by X-ray diffraction studies.

3.2. Crystallographic studies

To rationalize the planarity of tricyclic system **4**, perspective MM2-minimum energy conformational analysis of **4f**, **4g** and **4i** was carried out using the software *ChemBioDrawUltra*, Cambridge Soft, 2014 [32]. It seems that tricyclic ring is not planar and phenyl rings are parallel (Figure 2). In case of **4i**, triazolopyridazine rings are oriented differently due to more steric bulk of methoxy substituents on phenyl ring.

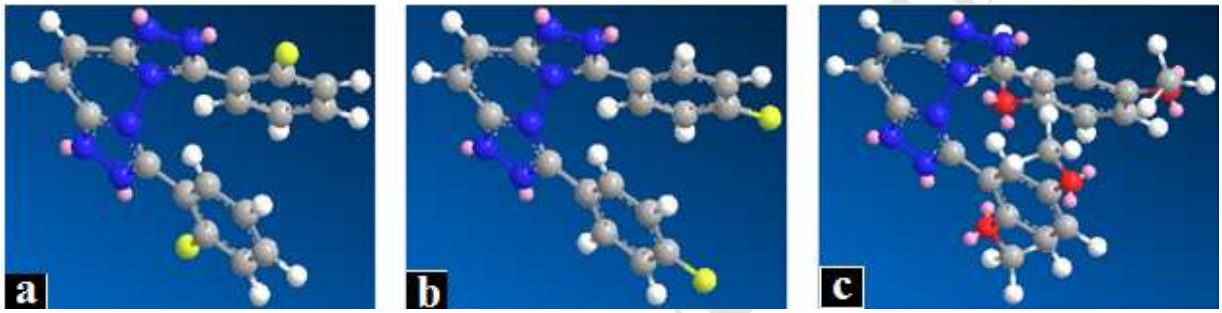


Figure 2. (a), (b) and (c) Perspective MM2-mimimum energy conformational analysis of compound **4f**, **4g** and **4i**, respectively.

Suitable crystals of **4f** and **4g** were studied by single crystal X-ray diffraction. Experimental details for structural analyses are summarized in Table 1. The selected bond distances and bond angles for compound **4f** and **4g** are given in Table 2. Both compounds crystallize in orthorhombic $P2_12_12_1$ space group, containing one single molecule per asymmetric unit. The ORTEP plot of **4f** and **4g** with the labelling of their asymmetric units is depicted in Figures 3 and 4, respectively.

The structures for compounds **4f** and **4g** show similarities. The molecules in both compounds are not planar, as expected [33], because of the twist of the aromatic rings on the bistriazole units in order to minimize their steric hindrance (Figure 5). In that way, the phenyl rings form a dihedral angle of about 50º with their corresponding triazole rings, concretely 56.0(2)º and 51.9(2)º in compound **4g**, while they are a bit lower for **4f**, 48.2(3)º and 47.5(3)º.

The presence of the fluorine atoms in the *ortho* position in the later compound seem not to affect significantly to this angle, as they are located opposite each other and towards the outside of the theoretical molecular plane.

Table 2

Selected bond length and bond angles (Å, °) of compound **4f** and **4g** with esd's in parenthesis.

Bond length	**4f**	**4g**	**Bond angle**	**4f**	**4g**
C(1)-N(2)	1.316(3)	1.321(2)	N(2)-C(1)-N(3)	108.8(2)	107.23(17)
C(1)-N(3)	1.371(3)	1.380(2)	N(2)-C(1)-C(7)	120.3(2)	122.79(18)
C(2)-N(1)	1.311(3)	1.314(2)	N(3)-C(1)-C(7)	130.8(2)	129.68(16)
C(2)-N(3)	1.383(3)	1.380(2)	N(1)-C(2)-N(3)	109.4(2)	109.04(17)
C(2)-C(3)	1.427(4)	1.421(3)	N(1)-C(2)-C(3)	129.9(3)	129.8(2)
C(3)-C(4)	1.326(3)	1.327(3)	N(3)-C(2)-C(3)	120.5(3)	120.1(2)
C(4)-C(5)	1.432(4)	1.435(3)	C(4)-C(3)-C(2)	119.8(3)	119.7(2)
C(5)-N(4)	1.312(3)	1.312(2)	C(3)-C(4)-C(5)	119.7(3)	119.49(18)
C(5)-N(6)	1.380(3)	1.378(2)	N(4)-C(5)-N(6)	109.4(2)	108.73(16)
C(6)-N(5)	1.318(3)	1.318(2)	N(4)-C(5)-C(4)	129.8(3)	130.24(17)
C(6)-N(6)	1.377(2)	1.372(2)	N(6)-C(5)-C(4)	120.6(3)	119.96(18)
N(3)-N(6)	1.388(2)	1.3818(19)	N(5)-C(6)-N(6)	108.2(2)	107.96(15)
N(2)-N(1)	1.381(3)	1.379(2)	N(5)-C(6)-C(13)	120.5(2)	124.52(16)
N(4)-N(5)	1.375(3)	1.390(2)	N(6)-C(6)-C(13)	131.1(2)	127.07(16)
			C(8)-C(7)-C(12)	117.4(2)	119.40(19)
			C(8)-C(7)-C(1)	122.9(2)	121.68(17)
			C(12)-C(7)-C(1)	119.4(2)	118.59(18)
			C(14)-C(13)-C(18)	117.3(2)	119.09(16)
			C(14)-C(13)-C(6)	123.1(2)	120.30(16)
			C(18)-C(13)-C(6)	118.8(2)	120.37(17)
			C(1)-N(3)-C(2)	105.6(2)	106.65(15)
			C(1)-N(3)-N(6)	135.1(2)	133.66(15)
			C(2)-N(3)-N(6)	119.2(2)	119.37(15)
			C(1)-N(2)-N(1)	108.6(2)	109.62(16)
			C(2)-N(1)-N(2)	107.4(2)	107.32(16)
			C(6)-N(6)-C(5)	105.8(2)	106.84(15)
			C(6)-N(6)-N(3)	135.2(2)	134.21(14)
			C(5)-N(6)-N(3)	119.0(2)	118.86(15)
			C(5)-N(4)-N(5)	107.5(2)	107.59(15)
			C(6)-N(5)-N(4)	109.0(2)	108.68(14)

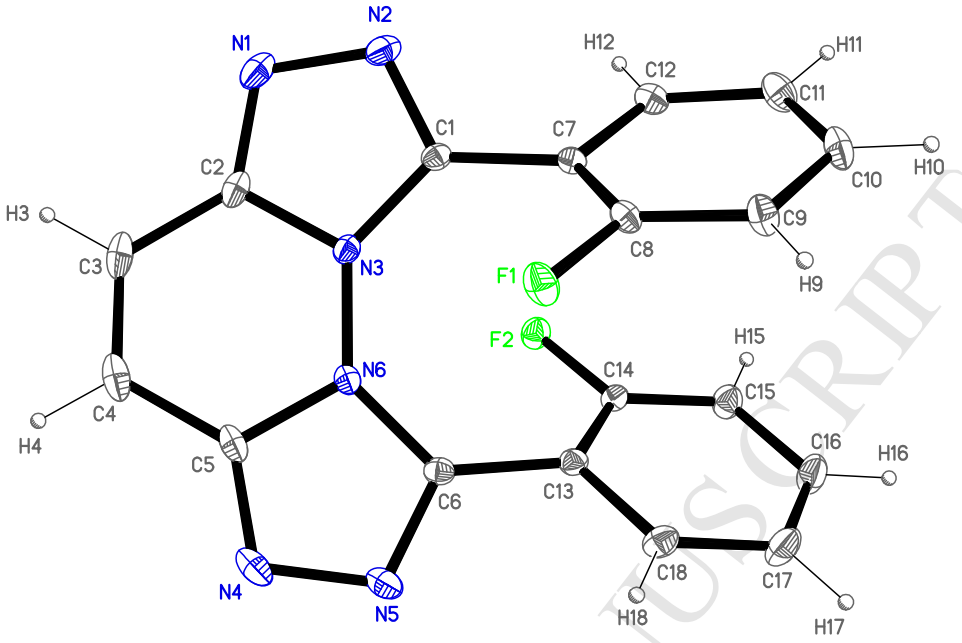


Figure 3. ORTEP plot (20% probability for the ellipsoids) of **4f** showing the labelling scheme of its asymmetric unit.

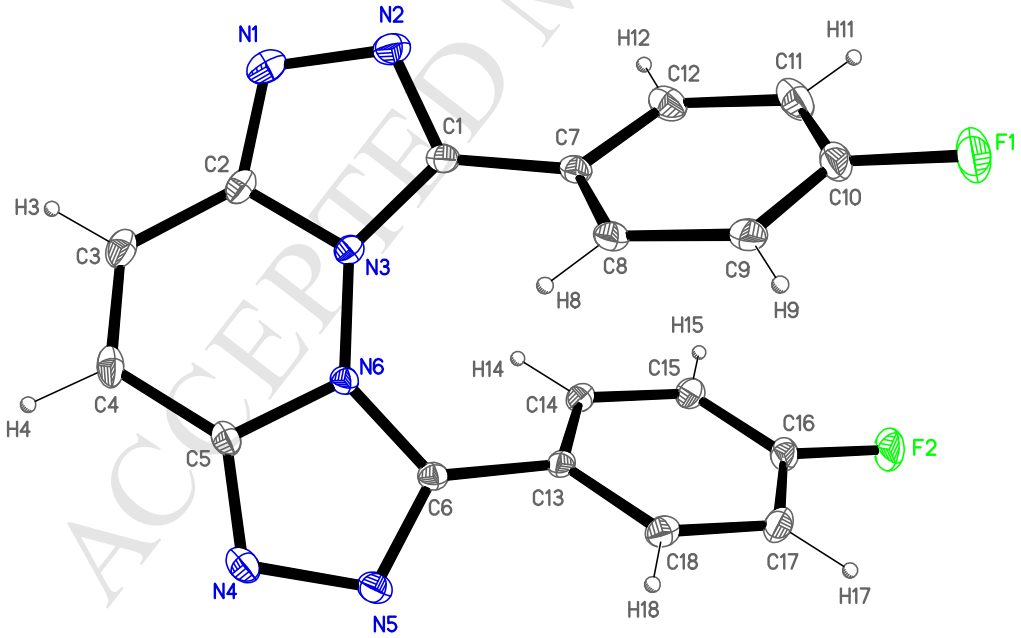


Figure 4. ORTEP plot (20% probability for the ellipsoids) of **4g** showing the labelling scheme of its asymmetric unit.

Additionally, the phenyl rings on both triazole units are very close, inducing a distortion in the bistriazolopyridazine that deviates from the planarity; this feature agrees with the bond distances and angles found for both compounds, due to a partial electronic delocalization observed for this moiety. In this sense, in compound **4f**, one of the triazole and the pyridazine rings are almost coplanar. This moiety forms a dihedral angle about 12º with the other triazole ring, being the electronic delocalization disrupted in the C6N6N3C1 fragment (Figure 5a). In compound **4g** the distortion is more accused with dihedral angles of 13.2(2)º and 14.3(2)º between each triazole fragment and the pyridazine ring, and a greater deviation from the planarity located on the N4N5C6 fragment (Figure 5b).

In both derivatives the phenyl rings are almost parallel, in order to minimize their steric hindrance, since they are linked to C1 and C6, two atoms separated by a distance comparable to the sum of their van der Waals radii. For this reason, the substituting *ortho* or *para* fluorine atoms do not have influence in the molecular structure and only seem to do it on the intermolecular interactions. In this context, compound **4f** shows the fluorine atoms pointing towards opposite directions in order to avoid very short intramolecular contacts, giving rise instead to the formation of weak F···F contacts (distance of 2.882(3) Å) between adjacent molecules leading to the formation of chains along *a* axis, as depicted in Figure 6. These intermolecular fluorine interactions can be considered as weak if they are compared with the sum of the van der Waals radii of 2.94 Å that it is mostly considered the borderline, but they are in the usual range for this type of contacts. They are centrosymmetric in *cis* disposition (Type I, cisoid) and CF···FC angles of 144.8(3)º and 146.6(3)º [34]. No significant interaction between the chains has been found. Nevertheless, in compound **4g** the fluorine atoms in *para* positions precludes the intermolecular halogen bonding interactions. Moreover, no significant contacts

between the molecules of **4g** have been found apart from the usual van der Waals forces (Figure 7).

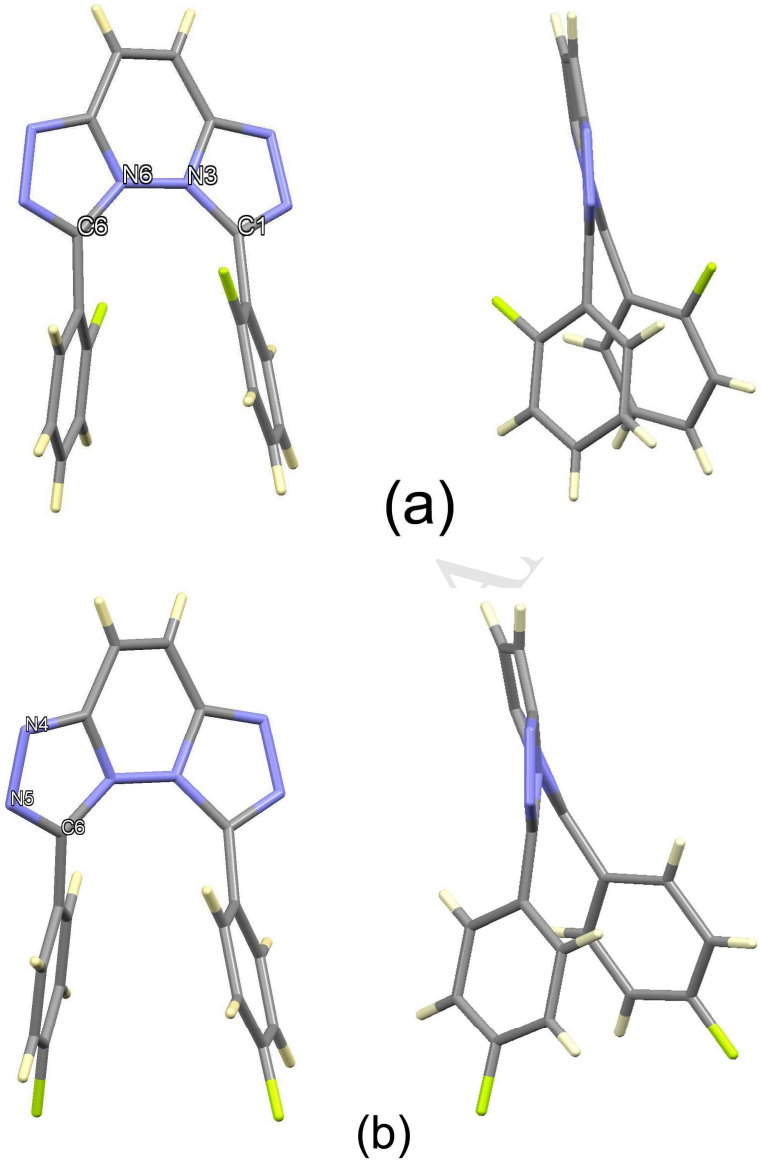


Figure 5. Different views of the molecules for (a) compound **4f** and (b) for **4g**.

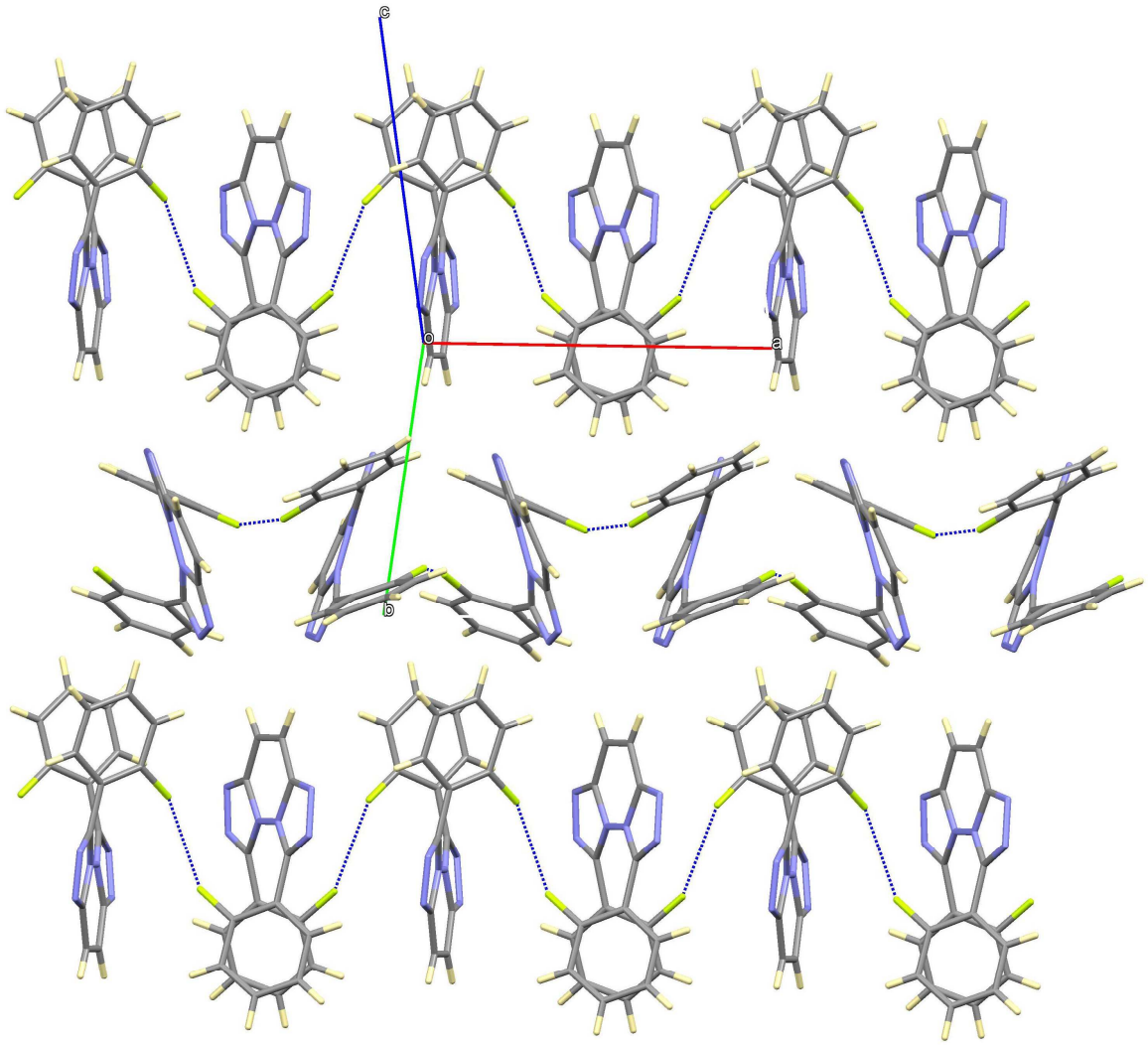


Figure 6. Perspective view of the chains formed along *a* axis in **4f** showing weak F···F interactions.

A significant distortion of the valence angles is also observed due to the steric hindrance between the aryl substituents. For instance, in compound **4f** the exocyclic angles N(3)C(1)C(7) 130.8(2)º and N(6)C(6)C(13) 131.1(2)º are appreciably greater, and the angles N(2)C(1)C(7) 120.3(2)º and N(5)-C(6)-C(13) 120.5(2)º are appreciably smaller than the ideal value of 126º for the five-membered ring. The C(1)-N(3)-N(6) 135.1(2)º and C(6)-N(6)-N(3) 135.2(2)º angles are also increased. The angles C(1)-N(3)-C(2) 105.6(2)º and C(6)-N(6)-C(5) 105.8(2)º in the triazole

rings, and the C(2)-N(3)-N(6) 119.2(2)º and C(5)-N(6)-N(3) 119.0(2)º angles in the pyridazine ring, however, are smaller than the ideal values for the regular pentagon and hexagon (108 and 120º, respectively).

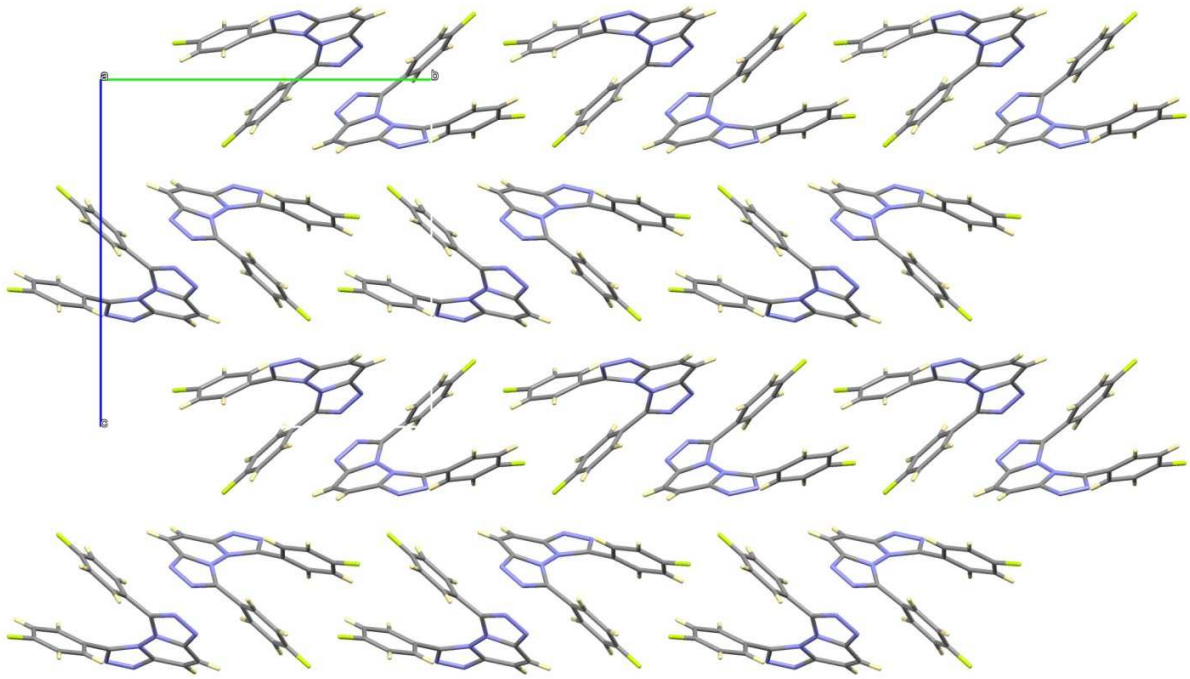


Figure 7. Perspective view of the crystal packing of **4g** along *a* axis.

3.3. Cytotoxicity studies

The antitumor activities of bis-1,2,4-triazolopyridazine **4** were evaluated *in vitro* against HeLa cervical cancer cells with doxorubicin as positive reference. Most of the compounds could not be screened for their cytotoxic activity because of poor solubility in buffer medium. However, four compounds **4c**, **4d**, **4e** and **4i** were screened for their cytotoxic effect using colorimetric MTT assay. As shown in the Table 3 and Figure 8, compounds **4c**, **4d**, **4e** and **4i** exhibited cytotoxicity against HeLa cell line with IC_{50} values in the range 19.37-56.16 µM which is not promising. Therefore, no further studies were carried out to evaluate mechanism.

Table 3

IC_{50} values of compounds **4c**, **4d**, **4e** and **4i** against HeLa cancer cell line.

Compd	R	HeLa IC_{50}[a] ± SEM (µM)
4c	-4-ClC_6H_4	37.02 ± 1.53
4d	-3-BrC_6H_4	56.16 ± 6.80
4e	-4-BrC_6H_4	19.37 ± 1.08
4i	-2,5-$(OCH_3)_2C_6H_3$	40.79 ± 4.37
Doxorubicin		4.8 ± 0.8

[a] IC_{50} = half maximal concentration represents the concentration of drug able to inhibit by 50% the *in vitro* growth. Each value represents mean ± SD of three experiments.

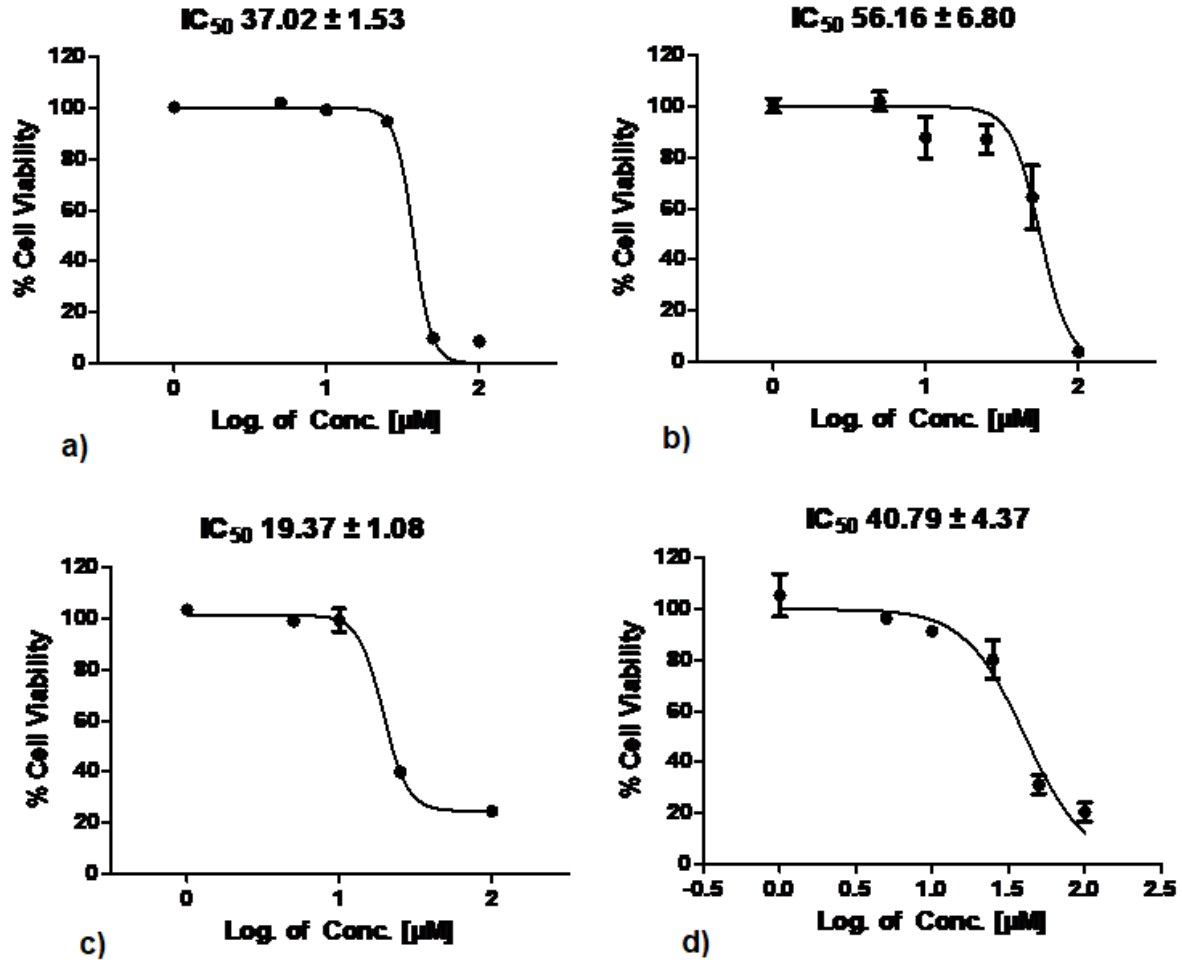


Figure 8. Graph representing % cell viability versus logarithm of the concentration and IC_{50} values obtained a) for **4c**, b) for **4d**, c) for **4e**, and d) for **4i**.

4. Conclusion

In summary, IBD offers an effective, convenient and environmentally benign approach to the synthesis of bis-triazolopyridazines **4**. Compared to other conventional methods the present protocol is remarkably superior in terms of ease of handling, faster reaction rates, better yields and operational simplicity. X-ray analysis of **4f** and **4g** indicated that pyridazine ring has twisted conformation as a result of steric strain between two aryl groups leading to nonplanar tricyclic core. The crystal packing of compound **4f** is constituted of chains along *a* axis formed by weak, centrosymmetric F···F interactions, with *cis* geometry, between adjacent molecules. The *para* position of the fluorine atoms in compound **4g** precludes any interaction. The cytotoxicity of some of the compounds (**4c**, **4d**, **4e** and **4i**) was also evaluated against HeLa cancer cell line, however, results are not very significant.

Acknowledgments

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Supplementary data

Supplementary data related to this article can be found attached.

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Highlights

- A series of new bistriazolopyridazines **4** has been synthesized by using eco-friendly oxidant and structurally characterized.
- X-ray single crystal structures of **4f** and **4g** were determined.
- Crystal studies show that tricyclic bistriazolopyridazine ring has twisted conformation due to presence of two aryl groups.
- Both **4f** and **4g** crystallized in the orthorhombic crystal system, space group $P2_12_12_1$, containing one single molecule per asymmetric unit.
- Formation of chains along *a* axis due to weak, centrosymmetric F···F interactions, with *cis* geometry, between adjacent molecules in **4f**.