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SYNTHESIS AND CYTOTOXIC EVALUATION OF SOME NEW 1-(6-CHLOROPYRIDAZIN-3-YL)-3-*H*/ALKYL-4-PHENYL-1*H*-PYRAZOL-5-AMINES AND THEIR DERIVATIVES

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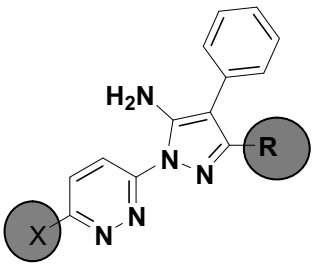
ABSTRACT Synthesis of new 1-(6-chloropyridazin-3-yl)-3-*H*/alkyl-4-phenyl-1*H*-pyrazol-5-amines **(3)** was accomplished by the reaction of 3-chloro-6-hydrazinopyridazine **(1)** with differently substituted β-ketonitriles **(2)** under reflux in ethanol. Subsequently, a series of 6-amino derivatives (**4**, **5** and **6**) was synthesized by amino-de-halogenation of **3** with secondry cyclic amines e.g. pyrrolidine, piperidine and morpholine respectively. Structures of all the compounds were established by IR, NMR (^{1}H, ^{13}C and ^{19}F) and mass spectral data, and elemental analysis. All the compounds were screened for their cytotoxic effects against three cancer cell lines (SB-ALL, NALM-6, MCF-7) using colorimetric MTT assay.



KEYWORDS 3-Chloro-6-hydrazinopyridazine, 3-Oxo-2-phenylalkanenitrile, 1-(6-Chloropyridazin-3-yl)-3-*H*/alkyl-4-phenyl-1*H*-pyrazol-5-amines, Amino-de-halogenation, Cytotoxicity

INTRODUCTION

5-Aminopyrazole moiety gained a special attention from organic chemists, because of their wide spread use in pharmaceuticals and agrochemicals. They display diverse type of biological properties such as antibacterial,[1,2] anti-inflammatory,[3] antitumor.[4] 5-Aminopyrazoles such as BIRB796 **(1a)** and RO3201195 **(1b)** emerged as a powerful lead molecules as highly selective and potent p38-MAPK inhibitors.[5,6] Further, literature study shows that 5-aminopyrazoles have been found to exhibit growth inhibitory activity against a panel of human cancer cell lines.[7,8] 1-(5-Amino-1-(4-chlorophenyl)-3-ethyl-1*H*-pyrazol-4-yl)-3-ethoxypropan-1-one **(1c)** showed anti-tumor activity for both leukemia HL-60 cell and liver cancer cells BEL-7402.[9]

Though, pyridazine nucleus exists rare in nature but it plays a vital role in drug discovery as it can improve the physiochemical profile of drug candidates by increasing their water solubility. Pyridazine derivatives have been reported to possess anticancer activity such as azamerone **(1d)** which is the second natural product containing pyridazine ring and exhibits cytotoxicity against mouse splenocyte populations of T-cells and macrophages with an IC_{50} value of 40 μM.[10] Pyrazol-4-ylpyridazinones, **(1f)** have

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been reported as a potent c-Met kinase inhibitors and anti-proliferative agent against Hs746T human gastric cancer cell line.[11] Further, pyridazine skelton bearing pyrrolidine, piperidine and morpholine moieties exhibits plethora of biological activities. [12-14] Recently our group reported a series of 1-(4',6'-dimethylpyrimidin-2'yl)-5-amino-3-arylpyrazoles and their derivatives exhibiting promising cytotoxicity against HL-60 cell line.[15]

Based on above observations and in continuation to our efforts toward the synthesis of new pharmacologically active heterocyclic compounds with improved biological activity,[16-19] we envisaged to synthesize title compounds **3**, **4**, **5** and **6** by replacing pyrimidine ring with isomeric pyridazine nucleus bearing chlorine, pyrrolidine, piperidine and morpholine at position-6. All the synthesized compounds were evaluated for their cytotoxicity against three cancer cell line namely SB-ALL, NALM-6 and MCF-7 using colorimetric MTT assay.

RESULTS AND DISCUSSION

Chemistry

The method utilized for the synthesis of 1-(6-chloropyridazin-3-yl)-3-*H*/alkyl-4-phenyl-1*H*-pyrazol-5-amines **3a-d**, 3-*H*/alkyl-4-phenyl-1-(6-(cyclicamine-1-yl)pyridazin-3-yl)-1*H*-pyrazol-5-amines **4a-d, 5a-d, 6a-d** is summarized in **Scheme1**.

The starting compound, 3-oxo-2-phenylalkanenirile **2a-d** were obtained by the Claisen condensation between α-phenylacetonitrile and appropriately substituted ethyl esters using sodium ethoxide as base.[20] Refluxing **2a-d** with 3-chloro-6-hydrazinopyridazine (**1**) in ethanol provided 1-(6-chloropyridazin-3-yl)-3-*H*/alkyl-4-phenyl-1*H*-pyrazol-5-amines (**3a-d**) as an exclusive product in excellent yield. Further, we replaced chlorine with cyclic amines, such as pyrrolidine, piperidine, and morpholine at 6-position of the pyridazine ring to study structure activity relationship (SAR). 3-*H*/Alkyl-4-phenyl-1-(6-(cyclicamin-1-yl)pyridazin-3-yl)-1*H*-pyrazol-5-amines **4a-d, 5a-d, 6a-d** were synthesized by amino-de-halogenation of **3** by piperidine, morpholine and pyrrolidine, respectively. The structure and purity of all the compounds were confirmed by TLC and corresponding spectral data (IR, MS, ^{1}H, ^{13}C and ^{19}F NMR) and elemental analysis.

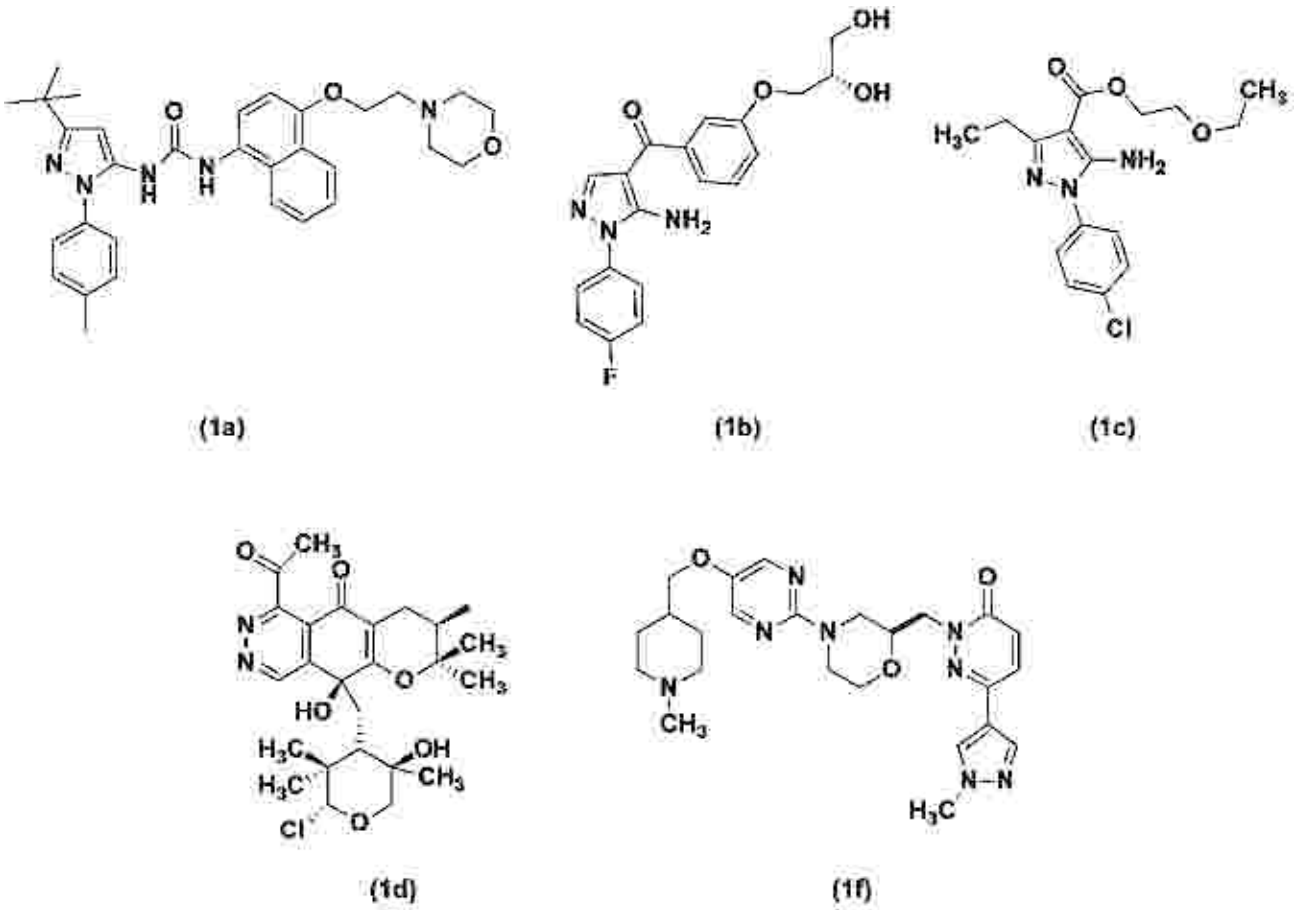
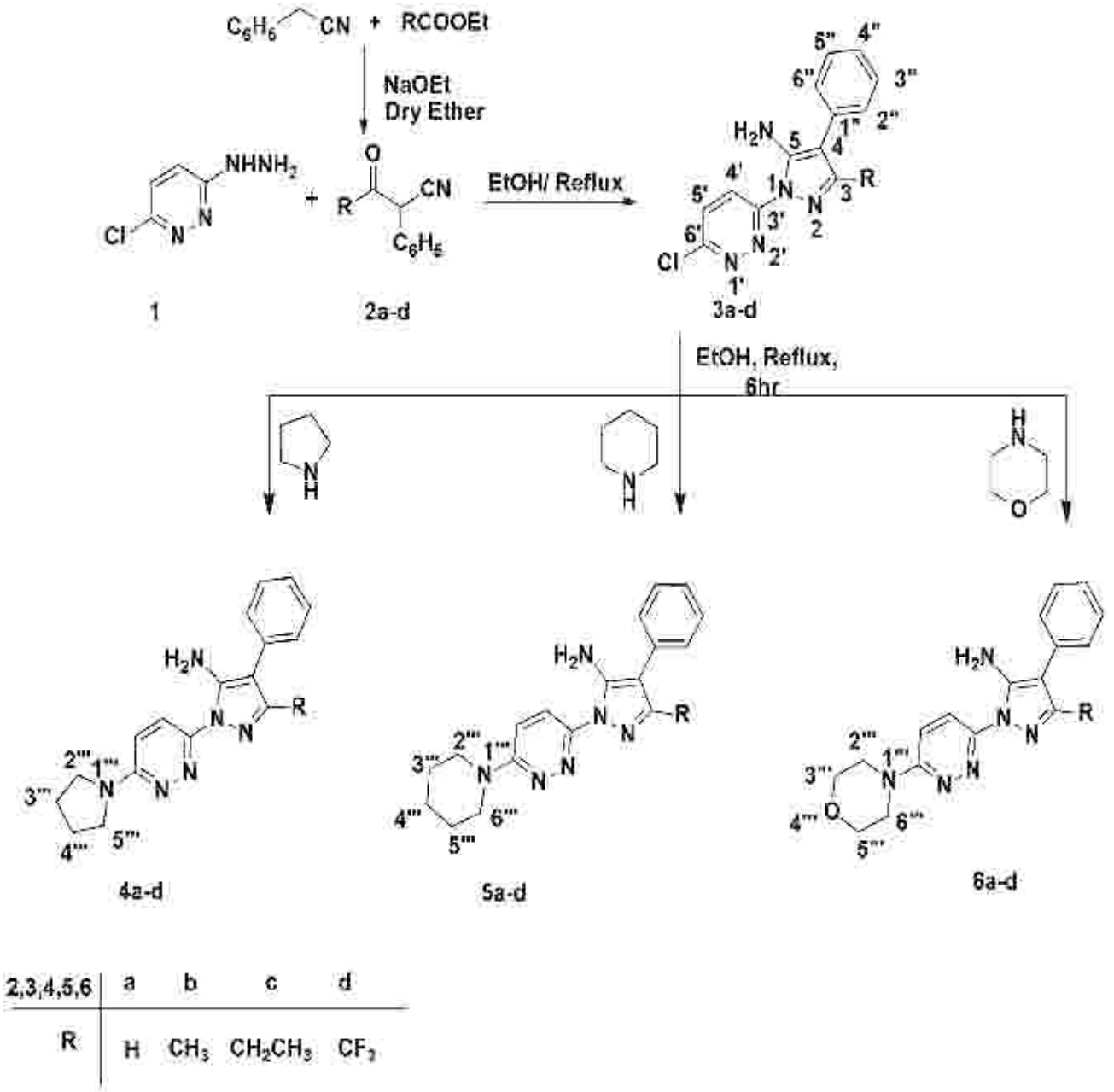


Fig. 1: Structure of 5-aminopyrazoles and pyridazine of pharmaceutical relevance



2,3,4,5,6	a	b	c	d
R	H	CH_3	CH_2CH_3	CF_3

Scheme-1: Synthesis of 5-aminopyridazinylpyrazoles 3a-d and their corresponding cyclic amine derivatives 4a-d, 5a-d and 6a-d derivatives.

The IR spectra of compounds **3a-d**, **4a-d**, **5a-d**, **6a-d** showed two sharp absorption bands in the range of 3256-3564 (symm.) and 3364-3610 cm^{-1} (asymm.) due to NH_2 stretchings. Presence of NH_2group was also confirmed by ^1H NMR spectra, which exhibited a broad singlet between δ 5.89-6.24 ppm. The corresponding signals for H-4' and 5' for compounds **3a-d** were obtained at δ 8.25-8.33 ppm and δ 7.55-7.69 ppm as a pair of doublets, respectively having a coupling constant ^3J= ~9.2 Hz. Replacement of chlorine with cyclic amine causes an upfield shift in **4a-d**, **5a-d** and **6a-d** and their signal appeared at δ 7.91-8.13 ppm and δ 6.83- 7.14 ppm for H-4' and H-5', respectively. A sharp singlet of one proton at δ 7.63 ppm for pyrazole H-3 was obtained in case of **3a-6a**. However, in case of **3b-6b** a sharp singlet of 3-protons appeared at δ 2.20 ppm for $-CH_3$ group. Further for compounds **3c-6c** a typical triplet-quartet pattern of ethyl group appeared at δ 1.16-1.19 ppm and 2.63-2.67 ppm.

^{13}C NMR spectral data of compounds **3a-d**, **4a-d**, **5a-d** and **6a-d** is given in **Table 1** and **Table 2** respectively. ^{19}F NMR spectra of compounds **3d-6d** showed a sharp singlet at δ ~ -61 ppm thus indicating the presence of CF_3 group at 3-position of pyridazine ring[1].

Table 1 ^{13}**C NMR δ (ppm) data for 1-(6-chloropyridazin-3-yl)-3-*H*/alkyl-4-phenyl-1*H*-pyrazol-5-amines (3a-d) and 3-*H*/Alkyl-4-phenyl-1-(6-(pyrrolidin-1-yl)pyridazin-3-yl)-1*H*-pyrazol-5-amines (4a-d)**

Compd.	3a	3b	3c	3d	4a	4b	4c	4d
C-3	142.57	145.84	145.84	131.14	139.94	144.91	144.90	130.38
C-4	105.17	104.22	103.68	102.83	104.86	103.72	103.14	102.62
C-5	145.19	151.22	152.38	147.21	144.25	148.29	150.88	146.03
C-3'	157.24	156.92	157.03	156.79	155.95	155.76	155.75	156.22
C-4'	130.77	130.58	130.50	129.34	125.65	125.97	126.08	127.40
C-5'	126.23	126.54	126.63	128.44	121.01	120.84	120.99	121.14
C-6'	153.08	152.46	156.30	154.13	150.94	150.76	153.55	150.22
C-1"	132.39	132.40	132.43	127.88	133.25	133.33	133.37	129.49
C-2",C-6"	129.24	129.12	129.11	129.48	129.07	128.92	128.91	129.49
C-3",C-5"	126.35	128.61	128.85	129.09	126.24	128.58	128.83	128.91
C-4"	121.70	121.40	121.55	127.88	115.88	115.77	115.72	115.85
C-2"', C-5"'	-		-	-	46.90	46.88	46.88	46.94
C-3"', C-4"'	-		-	-	25.48	25.48	25.48	25.47
-CH_2	-		20.79	-	-	-	20.73	-
-CH_3	-	13.28	12.79	-	-	13.19	13.23	-
-CF_3	-		-	142.31 (q, $J'_{C\text{-}F=132Hz}$	-	-	-	139.76 (q, $J'_{C\text{-}F}$=144 Hz

*Numbering of different carbon atoms is indicated in Scheme 1.

Table 2 ^{13}**C NMR δ (ppm) data for 3-*H*/Alkyl-4-phenyl-1-(6-(piperidin -1-yl)pyridazin-3-yl)-1*H*-pyrazol-5-amines (5a-d) and 3-*H*/Alkyl-1-(6-(morpholinopyridazin-3-yl)-4-phenyl-1*H*-pyrazol-5-amine (6a-d)**

Compd.	3a	3b	3c	3d	4a	4b	4c	4d
C-3	140.21	145.14	137.85	125.06	140.57	145.13	145.12	130.93
C-4	104.84	104.00	103.15	97.39	104.94	103.86	103.28	102.02
C-5	144.36	148.61	145.02	140.90	144.46	149.01	152.46	146.27
C-3'	158.31	158.12	158.17	153.34	158.28	158.09	158.03	158.63
C-4'	125.71	126.03	126.14	122.20	125.82	126.14	126.24	128.46
C-5'	121.02	120.82	120.98	115.94	121.25	121.04	121.19	121.41
C-6'	151.53	151.52	151.52	145.51	152.48	152.34	154.23	151.74
C-1"	133.16	133.33	133.26	-	133.01	133.07	133.11	130.15
C-2", C-6"	129.09	128.94	128.95	124.24	129.13	128.97	128.97	129.48
C-3",C-5"	126.24	128.58	128.82	123.69	126.26	128.59	128.84	129.44
C-4"	116.78	116.78	116.80	111.32	116.62	116.57	116.56	116.44
C-2"',C-6"'	46.65	46.71	46.74	41.26	45.78	45.88	45.90	45.57
C-3"',C-5"'	25.37	25.37	25.37	20.11	66.49	66.50	66.51	66.43
C-4"'	24.47	24.47	24.48	19.18	-	-	-	-
-CH_2	-	-	20.74	-	-	-	20.74	-
-CH_3	-	13.20	13.21	-	-	13.19	13.12	-
-CF_3	-	-	-	-	-	-	-	140.72 (q, J'

*Numbering of different carbon atoms is indicated in Scheme 1.

Biological Activity

Cytotoxic Activity

All the synthesized compounds were evaluated for their cytotoxicity in human lymphocytic leukemia (SB-ALL and NALM6), and human breast carcinoma (MCF-7) cell lines using the well-established MTT cell proliferation.[21,22] The three cell lines are efficient *in vitro* models for their cytotoxic evaluation of different types of chemotherapeutic agents such as DNA minor groove binders,[23] protein kinase inhibitors[24] and caspases inhibitors[25] among others.

SB-ALL and MCF-7 cell lines with an IC_{50} value 10.4 μM and 21.5 μM respectively. Compound **4a** exhibited satisfactory cytotoxicity against NALM-6 cell line with IC_{50} value 5.4 μM (**Table 4**). Although the result of cytotoxicity is not outstanding, there is possibility of improvement by further derivatization.Only one compound (**4a**) out of 15 compounds tested inhibited approximately, 50% cell survival at 10 μM final concentration. Results are tabulated in **Table 3**. Compound **3d** could not be screened for its cytotoxic activity because it precipitated out on addition of buffer. Compound **4a** was moderately active against

Cell Culture

SB-ALL and NALM-6 were cultured in RPMI-1640 supplemented with 10% fetul serum albumin and 50 μg/ml of penicillin and streptomycin. MCF-7 was cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetul serum albumin and 50 μg/ml of penicillin and streptomycin. All cell lines were maintained in an incubator containing 5% of CO_2 at 37 ºC.

Cell Viability Assay

Cells were seeded in a 96-well plate at a density of 100,000 per ml were treated with various compounds at a final concentration of 10 μM and incubated for 48 h. Cell viability assay was performed using a MTT cell proliferation kit from ATCC (American Type Culture Collection) (#30-1010K). In summary, 10 μl MTT reagent was added to each well, and cells were placed back in incubator for 4 hr. 100 μl of detergent (from kit) was added and absorbance data was collected at 570 nm using Biotek synergy 2 spectrophotometer, Data was calculated as percentage of cell survival using the following formula:

$$\% \text{ Cell survival} = (100/A_t * A_s)$$

Where A_t and A_s are the absorbance of wells treated with test compounds and solvent control, respectively.

Table 3 Percentage cell survival of compounds using MTT assay at 10 μM concentration against three cancer cell lines

Sr. No.	Compd.	% Cell Survival		
		SB-ALL	NALM-6	MCF-7
1	**3a**	76.4±5.4	68.4±11.5	94.5±2.9
2	**3b**	100.4±9.4	94.3±12.4	91.7±18.8
3	**3c**	95.9±10.4	99.1±3.9	92.1±18.9
4	**4a**	63.6±4.1	54.4±10.1	83.4±5.1
5	**4b**	77.1±17.1	67.4±5.4	90.3±11.0
6	**4c**	87.2±8.3	83.6±11.4	95.9±2.4
7	**4d**	100.4±12.7	94.2±3.9	83.6±2.7
8	**5a**	87.1±3.7	84.0±7.5	82.7±5.5
9	**5b**	75.4±13.2	78.2±11.0	69.2±2.0
10	**5c**	89.9±15.0	89.7±16.7	82.7±0.4
11	**5d**	91.3±11.7	90.7±9.9	83.9±9.5
12	**6a**	94.7±7.1	88.8±9.9	79.8±4.2
13	**6b**	91.0±9.7	98.2±5.8	90.3±11.0
14	**6c**	76.3±11.3	80.2±19.2	85.7±3.4
15	**6d**	74.4±10.4	67.2±7.8	87.8±7.7

Table 4 IC_{50} value of compound 4a against three cancer cell lines

Compd.	IC_{50} value for (in μM) against three cancer cell lines		
	SB-ALL	NALM-6	MCF-7
4a	10.4±1.2	5.4±0.8	21.5±5.5

EXPERIMENTAL

Melting points were determined in open capillaries and are uncorrected. IR spectra were recorded on a Buck Scientific IR M-500 spectrophotometer in KBr pellets (v max in cm^{-1}); ^{1}H and ^{13}C NMR spectra for analytical purpose were recorded in $CDCl_3$ on a Bruker instrument at 300 MHz and 400 MHz respectively; chemical shifts are expressed in δ-scale downfield from TMS as an internal standard. ^{19}F NMR spectra were run on DRX 300 at 376 MHz and the internal standard for ^{19}F spectra was fluorotrichloromethane, setting the $CFCl_3$ signal at δ 0.0. Elemental analyses were performed at Sophisticated Analytical Instrument Facility, Panjab University, Chandigarh, India.

3-Chloro-6-hydrazinopyridazine (**1**) was synthesized according to the literature procedure.[26,27]

Synthesis of 1-(6-chloropyridazin-3-yl)-4-phenyl-1*H*-pyrazol-5-amine (3a)

3-Chloro-6-hydrazinopyridazine **(1)** (0.14g, 1 mmol) was added to a solution of 3-oxo-2-phenylpropanenitrile **(2a)** (0.14g, 1 mmol) in ethanol (10 mL) and the reaction mixture was heated under reflux for 6 hrs. On completion of the reaction (monitored by TLC), water was poured in reaction mixture. A solid separated out, which was filtered, washed with water and crystallized in (8 mL) ethanol. The TLC and ^{1}H NMR spectrum of the product showed the formation of a single product.

Similarly, compounds **3b-d** were synthesized using the same procedure by the reaction of 3-chloro-6-hydrazinopyridazine with 3-oxo-2-phenylbutanenitrile, 3-oxo-2-phenylpentanenitrile and 4,4,4-trifluoro-3-oxo-2-phenylbutanenitrile respectively.

1-(6-chloropyridazin-3-yl)-4-phenyl-1*H*-pyrazol-5-amine (3a)

Yield 78 %; m.p. 210 ºC; IR (KBr, cm^{-1}): 3302 (symm.) and 3394 (asymm.) due to NH2; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.24 (bs, 2H, $-NH_2$); 7.25-7.28 (m, 1H, 4"-H); 7.41-7.46 (m, 4H, 2", 3", 5", 6"-H); 7.60-7.63 (d, 1H, J= 9.28 Hz, , 5'-H); 7.69 (s, 1H, 3-H); 8.25-8.27 (d, 1H, J= 9.32 Hz, 4'-H); Ms: m/z $[M+1]^+$ 272.1/274.1 (3:1). Anal. Calcd. For $C_{13}H_{10}ClN_5$: C, 57.4; H, 3.7; N, 25.7. Found: C, 58.6, H, 4.4; N, 24.6.

1-(6-chloropyridazin-3-yl)-3-methyl-4-phenyl-1*H*-pyrazol-5-amine (3b)

Yield 75%; m.p. 146 ºC; IR (KBr, cm^{-1}): 3340 (symm.) and 3456 (asymm.) due to NH_2; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.27 (s, 3H, 3-CH_3); 6.03 (bs, 2H, $-NH_2$); 7.25-7.29 (m, 1H, 4"-H); 7.36-7.37 (m, 2H, 3", 5"-H); 7.43-7.47 (m, 2H, 2", 6"-H); 7.56-7.59 (d, 1H, J= 9.28 Hz, 5'-H); 8.21-8.24 (d, 1H, J= 9.36 Hz, 4'-H); Ms: m/z $[M+1]^+$ 286.1/288.1 (3:1). Anal. Calcd. For $C_{14}H_{12}ClN_5$: C, 58.85; H, 4.23; N, 24.51. Found: C, 58.80; H, 4.20; N, 24.58.

1-(6-chloropyridazin-3-yl)-3-ethyl-4-phenyl-1*H*-pyrazol-5-amine (3c)

Yield 73%; m.p. 112 ºC; IR (KBr, cm^{-1}): 3333 (symm.) and 3441 (asymm.) due to NH_2; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.16-1.18 (t, 3H, J= 7.52 Hz, 3-CH_3); 2.63-2.64 (q, 2H, J= 7.52 Hz, 3-CH_2); 5.98 (bs, 2H, $-NH_2$); 7.25-7.31 (m, 1H, 4"-H); 7.35-7.37 (m, 2H, 3", 5"-H); 7.42-7.45 (m, 2H, 2", 6"-H); 7.55-7.57 (d, 1H, J= 9.32 Hz, 5'-H); 8.24-8.26 (d, 1H, 9.32 Hz, 4'-H); Ms: m/z $[M+1]^+$ 300.1/302.1 (3:1). Anal. Calcd. For $C_{15}H_{14}ClN_5$: C, 60.10; H, 4.71; N, 23.36. Found: C, 60.14; H, 4.73; N, 23.40.

1-(6-chloropyridazin-3-yl)-3-(trifluoromethyl)-4-phenyl-1*H*-pyrazol-5-amine (3d)

Yield 83%; m.p. 128 ºC; IR (KBr, cm^{-1}): 3348 (symm.) and 3456 (asymm.) due to NH_2; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.09 (bs, 2H, $-NH_2$); 7.35-7.41 (m, 3H, 3", 4", 5"-H); 7.44-7.48 (m, 2H, 2", 6"-H); 7.67-7.69 (d, 1H, J= 9.24 Hz, 5'-H); 8.30-8.33 (d, 1H, J= 9.32 Hz, 4'-H); Ms: m/z $[M+1]^+$ 340.1/342.1 (3:1). Anal. Calcd. For $C_{14}H_{19}ClF_3N_5$: C, 49.50; H, 2.67; N, 20.62. Found: C, 49.58; H, 2.75; N, 20.55.

Synthesis of 4-phenyl-1-(6-(pyrrolidin-1-yl)pyridazin-3-yl)-1*H*-pyrazol-5-amine (4a)

To a solution of **3a** (0.27g, 1 mmol) in ethanol (10 mL) pyrrolidine (0.07g, 1 mmol) was added and the reaction mixture was heated under reflux for 4 hrs. After completion of reaction (monitored by TLC) water was poured in reaction mixture. A solid separated out, which was filtered, washed with water and crystallized in ethanol. The TLC and ^{1}H NMR spectrum of the product showed the formation of a single product.

Compound **4b**, **4c** and **4d** were prepared by the same procedure through reaction of **3b-d** with pyrrolidine.

4-phenyl-1-(6-(pyrrolidin-1-yl)pyridazin-3-yl)-1*H*-pyrazol-5-amine (4a)

Yield 75%; m.p. above 300 ºC; IR (KBr, cm^{-1}) 3279, (symm.) and 3364 (asymm.) due to NH_2; ^1H NMR (300 MHz, $CDCl_3$) δ: 2.04-2.11 (q, 4H, J=3.60 Hz, 3''', 4'''-H); 3.55-3.60 (t, 4H, J= 6.60 Hz, 2''', 5'''-H); 6.16 (bs, 2H, -NH_2); 6.86-6.89 (d, 1H, J= 9.60 Hz, 5'-H); 7.39-7.53 (m, 5H, 2", 3", 4", 5", 6"-H); 7.63 (s, 1H, 3-H); 8.01-8.04 (d, 1H, J= 9.60 Hz, 4'-H); Ms: m/z $[M+1]^+$ 308.1. Anal. Calcd. For $C_{17}H_{18}N_6$: C, 66.65; H, 5.92; N, 27.43; Found: C, 66.54; H, 5.49; N, 27.46.

3-methyl-4-phenyl-1-(6-(pyrrolidin-1-yl)pyridazin-3-yl)-1*H*-pyrazol-5-amine (4b)

Yield 75%; m.p. 194.5 ºC; IR (KBr, cm^{-1}) 3286, (symm.) and 3371 (asymm.) due to NH_2; ^1H NMR (400 MHz, $CDCl_3$) δ: 2.06-2.09 (q, 4H, J= 3.57 Hz, 3''', 4'''-H); 2.29 (s, 3H, 3-CH_3); 3.54-3.58 (t, 4H, J= 6.56 Hz, 2''', 5'''-H); 5.95 (bs, 2H, -NH_2); 6.84- 6.86 (d, 1H, J= 9.70 Hz, 5'-H); 7.24-7.28 (m, 1H, 4"-H); 7.37-7.38 (m, 2H, 3", 5"-H); 7.41-7.45 (m, 2H, 2", 6"-H); 7.99-8.01 (d, 1H, J= 9.70 Hz, 4'-H); Ms: m/z $[M+1]^+$ 322.1. Anal. Calcd. For $C_{18}H_{20}N_6$: C, 67.48; H, 6.29; N, 26.23; Found: C, 68.90, H, 6.55; N, 26.57.

3-ethyl-4-phenyl-1-(6-(pyrrolidin-1-yl)pyridazin-3-yl)-1*H*-pyrazol-5-amine (4c)

Yield 75%; m.p. 210 ºC; IR (KBr, cm^{-1}) 3302, (symm.) and 3394 (asymm.) due to NH_2; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.17-1.21 (t, 3H, J= 7.52 Hz, 3-CH_3); 2.06-2.09 (q, 4H, J= 3.56 Hz, 3''', 4'''-H); 2.65-2.71 (q, 2H, J= 7.52 Hz, 3-CH_2); 3.54-3.58 (t, 4H, J= 6.64 Hz, 2''', 5'''-H); 5.89 (bs, 2H, -NH_2); 6.83-6.86 (d, 1H, 9.70 Hz, 5'-H); 7.24-7.28 (m, 1H, 4"-H); 7.37-7.44 (m, 4H, 3", 5"-H); 8.01-8.04 (d, 1H, 9.70 Hz, 4'-H); Ms: m/z $[M+1]^+$ 336.1. Anal. Calcd. For $C_{19}H_{22}N_6$: C, 68.24; H, 6.63; N, 25.13; Found: C, 68.67; H, 6.45; N, 25.23.

3-(trifluoromethyl)-4-phenyl-1-(6-(pyrrolidin-1-yl)pyridazin-3-yl)-1*H*-pyrazol-5amine (4d)

Yield 75%; m.p. 180 ºC; IR (KBr, cm^{-1}) 3263, (symm.) and 3364 (asymm.) due to NH_2; ^1H NMR (400 MHz, $CDCl_3$) δ: 2.08-2.11 (q, 4H, J= 3.46 Hz, 3''', 4'''-H); 3.56-3.59 (t, 4H, J= 6.44 Hz, 2''', 5'''-H); 5.98 (bs, 2H, -NH_2); 6.86-6.89 (d, 1H, J= 9.70 Hz, 5'-H); 7.32-7.35 (m, 1H, 4"-H); 7.407.46 (m, 4H, 2", 3", 5", 6"-H); 8.03-8.0 (d, 1H, J= 9.70 Hz, 4'-H); Ms: m/z $[M+1]^+$ 376.1. Anal. Calcd. For $C_{18}H_{17}F_3N_6$: C, 57.75; H, 4.58; N, 22.45; Found: C, 57.78; H, 4.34; N, 22.54.

Synthesis of 4-phenyl-1-(6-(piperidin-1-yl)pyridazine-3-yl)-1*H*-pyrazol-5-amine (5a)

To a solution of **3a** (0.27g, 1 mmol) in ethanol (10 mL) piperidine (0.08g, 1 mmol) was added and the reaction mixture was heated under refluxed for 4 hrs. After completion of reaction (monitored by TLC) water was poured in reaction mixture. A solid separated out, which was filtered, washed with water and crystallized in ethanol. The TLC and ^1H NMR spectrum of the product showed the formation of a single product.

Similarly compound **5b**, **5c** and **5d** were obtained by the reaction of **3b-d** with piperidine through same procedure.

4-phenyl-1-(6-(piperidin-1-yl)pyridazin-3-yl)-1*H*-pyrazol-5-amine (5a)

Yield 75%; m.p. above 300 ºC; IR (KBr, cm^{-1}) 3279, (symm.) and 3418 (asymm.) due to NH_2; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.59 (s, 6H, 3''', 4''', 5'''-H); 3.63 (s, 4H, 2''', 6'''-H); 6.16 (bs, 2H, -NH_2); 7.14-7.16 (d, 1H, J= 9.92 Hz, 5'-H); 7.24-7.26 (m, 1H, 4"-H); 7.43-7.47 (m, 4H, 2", 3", 5", 6"-H); 7.63 (s, 1H, 3-H); 8.00-8.02 (d, 1H, J= 9.84 Hz, 4'-H); Ms: m/z $[M+1]^+$ 322.1. Anal. Calcd. For $C_{18}H_{20}N_6$: C, 67.48; H, 6.29; N, 26.23. Found: C, 68.01, H, 6.25; N, 26.40.

3-methyl-4-phenyl-1-(6-(piperidin-1-yl)pyridazin-3-yl)-1*H*-pyrazol-5-amine (5b)

Yield 75%; m.p. above 300 ºC; IR (KBr, cm^{-1}) 3279, (symm.) and 3418 (asymm.) due to NH_2; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.63 (s, 6H, 3''', 4''', 5'''-H); 2.20 (s, 3H, 3-CH_3); 3.54 (s, 4H, 2''', 6'''-H); 5.89 (bs, 2H, -NH_2); 7.05-7.08 (d, 1H, J= 9.88 Hz, 5'-H); 7.30-7.32 (m, 1H, 4"-H); 7.35-7.38 (m, 4H, 2", 3", 5", 6"-H); 7.91-7.94 (d, 1H, J= 9.84 Hz, 4'-H); Ms: m/z $[M+1]^+$ 336.1. Anal. Calcd. For $C_{19}H_{22}N_6$: C, 68.24; H, 6.63; N, 25.13. Found: C, 68.50, H, 6.60, N, 25.10.

3-ethyl-4-phenyl-1-(6-(piperidin-1-yl)pyridazin-3-yl)-1*H*-pyrazol-5-amine (5c)

Yield 75%; m.p. 132 ºC; IR (KBr, cm^{-1}) 3286, (symm.) and 3387 (asymm.) due to NH_2; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.17-1.21 (t, 3H, J= 7.56 Hz, 3-CH_3); 1.70 (s, 6H, 3''', 4''', 5'''-H); 2.65-2.70 (q, 2H, J= 7.52 Hz, 3-CH_2); 3.61 (s, 4H, 2''', 6'''-H); 5.90 (bs, 2H, -NH_2); 7.12-7.14 (d, 1H, J= 9.80 Hz, 5'-H); 7.25-7.29 (m, 1H, 4"-H); 7.37-7.39 (m, 2H, 3", 5"-H); 7.43-7.45 (m, 2H, 2", 6"-H); 8.01-8.03 (d, 1H, J= 9.80 Hz, 4'-H); Ms: m/z $[M+1]^+$ 350.1. Anal. Calcd. For $C_{20}H_{24}N_6$: C, 68.94; H, 6.94; N, 24.12. Found: C, 68.90; H, 6.92; N, 24.32.

3-(trifluoromethyl)-4-phenyl-1-(6-(piperidin-1-yl)pyridazin-3-yl)-1*H*-pyrazol-5-amine (5d)

Yield 75%; m.p. above 300 ºC; IR (KBr, cm^{-1}) 3564, (symm.) and 3610 (asymm.) due to NH_2; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.71 (s, 6H, 3''', 4''', 5'''-H); 3.65 (s, 4H, 2''', 6'''-H); 5.98 (bs, 2H, -NH_2); 7.14-7.17 (d, 1H, J= 9.90 Hz, 5'-H); 7.32-7.36 (m, 1H, 4"-H); 7.40-7.46 (m, 4H, 2", 3", 5", 6"-H); 8.02-8.04 (d, 1H, J= 9.90 Hz, 4'-H); Ms: m/z $[M+1]^+$ 389.1. Anal. Calcd. For $C_{19}H_{19}F_3N_6$: C, 58.76; H, 4.93; N,

21.64. Found: C, 58.79; H, 4.95; N, 21.67.

Synthesis of 1-(6-(morpholin-4-yl)pyridazin-3-yl)-4-phenyl-1*H*-pyrazol-5-amine (6a)

To a solution of **3a** (0.27g, 1 mmol) in ethanol (10 mL) morpholine (0.08g, 1 mmol) was added and the reaction mixture was heated under reflux for 4 hrs. After completion of reaction (monitored by TLC) water was poured in reaction mixture. A solid separated out, which was filtered, washed with water and was crystallized in ethanol. The TLC and ^{1}H NMR spectra showed the formation of a single product.

Similarly, compound **6b**, **6c** and **6d** were prepared by the same procedure on reaction of **3b-d** with morpholine.

1-(6-morpholin-4-yl)pyridazin-3-yl)-4-phenyl-1*H*-pyrazol-5-amine (6a)

Yield 73%; m.p. 188 ºC; IR (KBr, cm^{-1}) 3263, (symm.) and 3340 (asymm.) due to NH_2; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.65-3.84 (t, 4H, J= 4.50 Hz, 2''', 6'''-H); 3.88-3.91 (t, 4H, J = 4.80 Hz, 3''', 5'''-H); 6.18 (bs, 2H, -NH_2); 7.15-7.19 (d, 1H, J= 9.90 Hz, 5'-H); 7.54-7.59 (m, 5H, 2", 3", 4", 5", 6"-H); 7.68 (s, 1H, 3-H); 8.10-8.13 (d, 1H, J= 9.90 Hz, 4'-H); Ms: m/z [M+1]$^+$ 323.1. Anal. Calcd. For $C_{17}H_{17}F_3N_6O$: C, 63.34; H, 5.63; N, 26.07; O, 4.96. Found: C, 58.33; H, 4.80; N, 23.10; O, 7.26.

3-methyl-1-(6-morpholin-4yl)pyridazin-3-yl)-4-phenyl-1*H*-pyrazol-5-amine (6b)

Yield 75%; m.p. 178 ºC; IR (KBr, cm^{-1}) 3271, (symm.) and 3379 (asymm.) due to NH_2; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.22 (s, 3H, 3-CH_3); 3.51-3.54 (t, 4H, J= 5.08 Hz, 2''', 6'''-H); 3.79-3.81 (t, 4H, J= 5.04 Hz, 3''', 5'''-H); 5.89 (bs, 2H, -NH_2); 7.04-7.07 (d, 1H, J= 9.80 Hz, 5'-H); 7.18-7.22 (m, 1H, 4"-H); 7.30-7.32 (m, 2H, 3", 5"-H); 7.35-7.39 (m, 2H, 2", 6"-H); 7.99-8.02 (d, 1H, J= 9.80 Hz, 4'-H); Ms: m/z [M+1]$^+$ 338.1. Anal. Calcd. For $C_{18}H_{20}N_6O$: C, 64.27; H, 5.99; N, 24.98; O, 4.76. Found: C, 64.56; H, 5.40; N24.50; O, 4.47.

3-ethyl-1-(6-morpholin-4-yl)pyridazin-3-yl)-4-phenyl-1*H*-pyrazol-5-amine (6c)

Yield 93%; m.p. 150 ºC; IR (KBr, cm^{-1}) 3256, (symm.) and 3364 (asymm.) due to NH_2; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.17-1.19 (t, 3H, J= 7.52 Hz, 3-CH_3); 2.65-2.67 (q, 2H, J= 7.52 Hz, 3-CH_2); 3.58-3.61 (t, 4H, J= 5.04 Hz, 2''', 6'''-H); 3.86-3.88 (t, 4H, J= 5.04 Hz, 3''', 5'''-H); 5.90 (bs, 2H, -NH_2); 7.11-7.13 (d, 1H, J= 9.80 Hz, 5'-H); 7.26-7.29 (m, 1H, 4"-H); 7.36-7.38 (m, 2H, 3", 6"-H); 7.41-7.45 (m, 2H, 2", 6"-H); 8.09-8.11 (d, 1H, J= 9.80 Hz, 4'-H); Ms: m/z [M+1]$^+$ 352.1. Anal. Calcd. For $C_{19}H_{22}N_6O$: C, 65.12; H, 6.33; N, 23.98; O, 4.57. Found: C, 65.46; H, 6.45; N, 23.87; O, 4.78.

3-(trifluoromethyl)-1-(6-morpholin-4-yl)pyridazin-3-yl)-4-phenyl-1*H*-pyrazol-5-amine (6d)

Yield 75%; m.p. 158 ºC; IR (KBr, cm^{-1}) 3271, (symm.) and 3379 (asymm.) due to NH_2; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.63-3.65 (t, 4H, J= 5.08 Hz, 2''', 6'''-H); 3.87-3.89 (t, 4H, J= 5.12 Hz, 3''', 5'''-H); 5.99 (bs, 2H, -NH_2); 7.14-7.17 (d, 1H, J= 9.80 Hz, 5'-H); 7.35-7.37 (m, 1H, 4"-H); 7.40-7.47 (m, 4H, 2", 3", 5", 6"-H); 8.10-8.12 (d, 1H, J= 9.80 Hz, 4'-H); Ms: m/z [M+1]$^+$ 392.1. Anal. Calcd. For $C_{18}H_{17}F_3N_6O$: C, 55.38; H, 4.39; N, 21.53; O, 4.10. Found: C, 55.45; H, 4.46; N, 21.60; O, 4.32.

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