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Synthesis and characterization of 4-((5-bromo-1*H*-pyrazolo [3,4-*b*]pyridin-3-yl)amino)-*N*-(substituted)benzenesulfonamide as Antibacterial, and Antioxidant Candidates

Hiren H. Variya[a*], Vikram Panchal[a] and Ganpat.R.Patel[a]

[a]*Department of Chemistry, Sheth M. N. Science College, NGES campus, Patan, India*

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A B S T R A C T

A series of novel 5-Bromo-3-iodo-1H-pyrazolo[3,4-b]pyridine linked various sulfonamide derivatives **8a-8j** poly functionalized were designed and synthesized in moderate to good yield. A starting with iodination of 5-Bromo-1H-pyrazolo[3,4-b]pyridine **5** with iodine produced intermediate 5-Bromo-3-iodo-1H-pyrazolo[3,4-b]pyridine **6** with the reaction of various sulfonamide derivatives **7a-7j** via copper catalyzed coupling reaction produced targeted compounds**8a-8j**. The isolated compounds were accepted by spectral and elemental analysis. The compounds **8a,8c,8d**, and **8i** were excellent active against Gram-positive and gram-negative bacterial strain compare to streptomycin standard drug. All synthesized compounds showed moderate to good antioxidant properties with used DPPH and Superoxide radical scavenging assay, Compounds **8c**, **8g**, and **8i** exerted significant antioxidant scavenging activity for the DPPH radical.

1. Introduction

Mainly medicinal scientist has drawn the awareness to discover and rapid development of N, S and O containing versatile heterocyclic entities because of their natural and synthetic occurrence, efficacious activities and biological evolution.[1-2] Due to the first drug used as a preventive and chemotherapeutic agent and also the wide range of pharmacological applicability of sulfonamide (sulfa drugs), the researcher has been widely studied RSO_2NH_2 functionality containing compounds.[3-,4] This functionality has an extensive verity of biological activities as Antibacterial and Antifungal activity[5-6], Carbonic anhydrase inhibitors (CAIs)[7-8], Anti cancer[9] Anti HIV[10-11], Cyclooxygenase-2 (COX-2) inhibitors[12], Anti malarial[13], type-II diabetes[14], treating male erectile dysfunction[15], etc.

Another hand, The fused small synthesized heterocycles such as pyrazole work as potent pharmacophores such as celecoxib **1** sildenafil citrate **2** in **figure-1** having sub structural popular drug.[16-17] In addition, hetreobiaryle pyrazolopyridine (1*H*-pyrazolo[3,4-b]pyridine) considerable important in the group of fused heterocycles which has shown the most powerful therapeutic activity. Pyrazolo[3,4-b]pyridine show large numbers of significant biological properties such as antimicrobial[18], antiviral[19], antitumer[18], analgesic[20], anti inflammatory[21], cyclooxygenase-(COX) inhibitors[21], selective c-Met inhibitors[22], selective Raf inhibitors[23], antioxidant activities[24], etc.

* Corresponding author. Tel.: +91 9898968774
E-mail address: hirenvariya9@yahoo.com (H. H. Variya)

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It is a well-known literature study of Pyrazolo[3,4-b]pyridine combine with sulfonamides has been found an exhibit diverse range of therapeutic activities. Compound **3** reported by chandak *et al.* showed good antibacterial and antifungal activities[25], compound **4** reported by Yingjun Li *et al*. identified selective Raf inhibitors with potency B-Raf^{V600E} with IC_{50} low nanomolar values[23].(**figure-1**)

During our ongoing interest exploring new functionalized derivatives from the Motivation of reported compound **3,4** we developed some fused new series of 4-((5-bromo-1*H*-pyrazolo [3,4-*b*]pyridin-3-yl)amino)-*N*-(substituted)benzenesulfonamide **8a-8j** by consequent procedure were characterized by elemental analysis, spectral data and examine by well-recognized pharmacophore with different range of activity such as antibacterial activity against gram +ve and gram -ve strains with minimum inhibition concentration (MIC) and also antioxidant assay with DPPH radical scavenging activity assay and Superoxide radical scavenging assay.

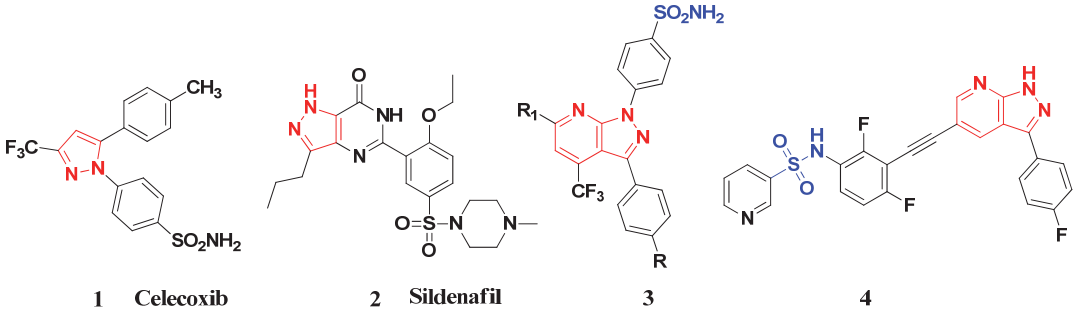
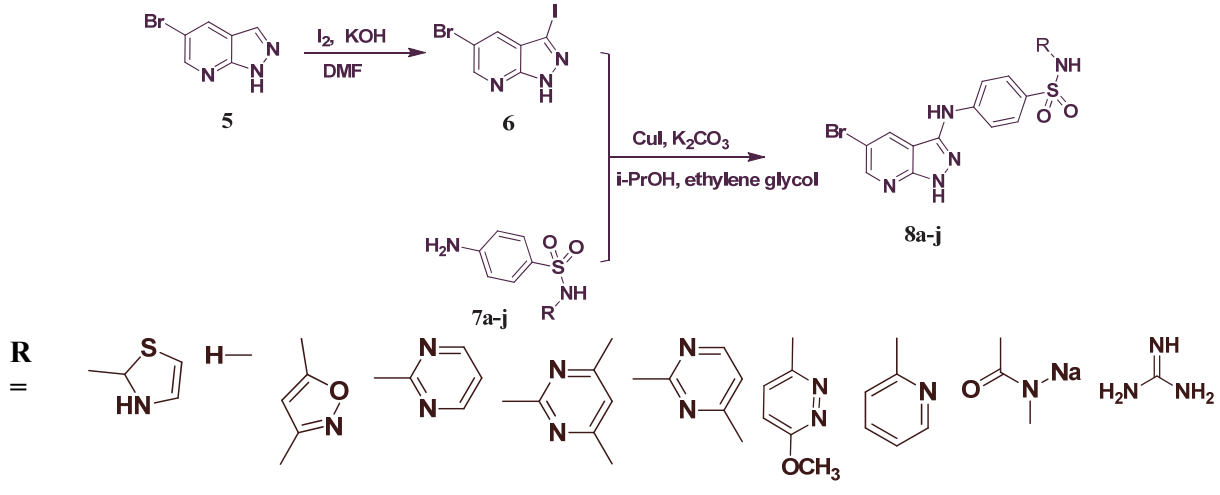


Fig. 1. Some of reported pyarazolo, pyrazolo [3,4-*b*]pyridin, sulfonamide structures

2. Results and Discussion

2.1 Chemistry

Our initial endeavour in this work was developed well potent chemotherapeutic agent. The key intermediate 5-Bromo-3-iodo-1H-pyrazolo[3,4-b]pyridine **6** were prepared by iodination with iodine of 5-Bromo-3-iodo-1H-pyrazolo[3,4-b]pyridine **5** with good yield (**Scheme-1**). Further, the compound **6** was effectively coupling with a various sulfonamide (Sulfa drugs) **7a-7j** using copper iodide and potassium carbonate as a catalyst in the presence of isopropyl alcohol produced targeted compounds **8a-8j** with good yield. Identification of structural 4-((5-bromo-1*H*-pyrazolo [3,4-*b*]pyridin-3-yl)amino)-*N*-(various substitution)benzenesulfonamide **8a-8j** were characterized by IR, ^{1}H NMR, ^{13}C NMR, ESI-MS and CHNS elemental analysis.



Scheme. 1. Synthetic route for compounds 8a-8j

In ^{1}H NMR (400 MHz, DMSO) of intermediate **6** were showed singlet for two pyridine ring proton (Py-H) at δ 8.21, 8.65 ppm and the signal of -NH display at 14.32 ppm. For **8a-8j** the phenyl ring hydrogen (Ar-H) of sulfonamide peak appear doublet in the region δ 6.8 ppm to 8.1 ppm. As expected, the singlet of -NH hydrogen for sulfonamide shows different range ~11 ppm while, singlet of pyrazole appear in the range ~14 ppm.

The infrared spectrum of all compounds showed stretching band ~3400 cm^{-1} and ~3200 cm^{-1} for sulfa -NH and pyrazolo-NH. The absence of symmetric and asymmetric band of -NH_2 indicate that sulfa drugs and pyrazolo [3,4-*b*]pyridin condensed in 1: 1-mole ratio. All compounds showed first strong asymmetric starching vibrations band for (O=S=O) within the range 1340-1387 cm^{-1} and second symmetric starching vibrations within the range of 1123-1188 cm^{-1}. The measure ^{13}C NMR spectrum for **8a-8j** was recorded in DMSO-d_6. The chemical shift for fused quaternary carbon allied to the pyrazolo nitrogen atom was appeared at ~158-162 ppm, whereas signal at ~137-140 ppm showed (O=S=O) linked carbon in phenyl ring. The signals due to -Br linked carbon recorded at ~108-111 ppm, while the signal display at ~149-152 ppm linked to -NH of the phenyl ring of sulfa drugs.

2.2 Biological evaluations

2.2.1 Antibacterial activity

$$\textbf{Activity index}(A.I) = \frac{\textbf{mean of the zone of inhibition of derivatives}}{\textbf{zone of inhibition obtained for standard antibiotic drug}}$$

This activity is done by in vitro agar well diffusion method[26]. Plates inoculated with the bacteria (two Gram-negative and two Gram-positive) (MTCC No.8558 Enterobacter aerogens, Escherichia coli MTCC No.1610, Micrococcus luteus MTCC No.11948 and Bacillus cereus MTCC No.8558). The inhibitions zone was measured were the microorganism inhibited after the incubation was done and were compared with standard streptomycin (1000µg/ml). shown in **Table 1.**

The significant results shown for all synthesized new series of **8a-8j** were excellent, good and average active against Gram-positive and gram-negative bacteria. On the bases of this results, we bring to a close that zone inhibition of the antibacterial activity of some synthesized compounds could be increased such as **8a**, **8c**, **8d** and **8i**, while the other compounds were decreased antibacterial compared to standard, shown in **Table 1.**

Table 1. Antibacterial activity of **8a-8j** compounds

Derivatives	*E. aerogens*		*E. coli*		*Micrococcus luteus*		*Bacillus cereus*	
	Mean value for Zone of Inhibition (mm)	Activity Index (A.I.)	Mean value for Zone of Inhibition (mm)	Activity Index (A.I.)	Mean value for Zone of Inhibition (mm)	Activity Index (A.I.)	Mean value for Zone of Inhibition (mm)	Activity Index (A.I.)
8a	26	1.083	24	1.000	30	1.250	27	1.125
8b	20	0.833	19	0.791	22	0.917	19	0.791
8c	30	1.250	27	1.125	30	1.250	24	1.000
8d	30	1.250	20	0.833	16	0.667	27	1.125
8e	20	0.833	22	0.917	19	1.125	22	0.917
8f	21	0.875	20	0.833	20	0.833	19	0.791
8g	20	0.833	27	1.125	15	0.625	19	0.791
8h	17	0.708	27	1.125	15	0.625	19	0.791
8i	29	1.208	20	0.833	19	0.791	27	1.125
8j	21	0.875	17	0.708	29	1.208	19	0.791
Std	24	-	24	-	24	-	24	-

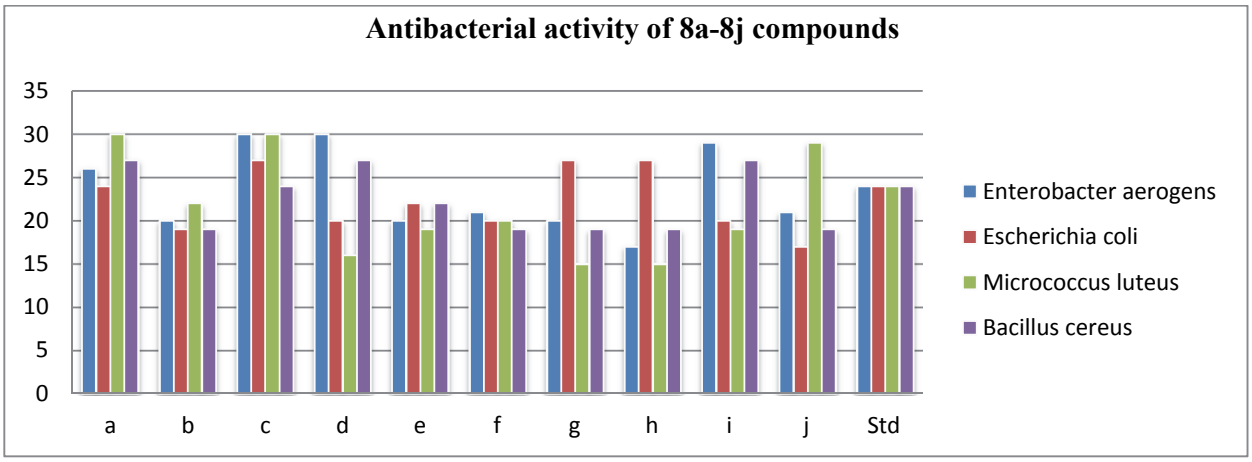


Fig. 2. Zone inhibition antibacterial activity of compounds **8a-8j**

The MIC values of these **8a-8j** series showed significant results For all synthesized compounds the **8a**, **8c**, **8d, 8h** and **8i** scaffold showed very good MIC values near to streptomycin shown in **table-2** and another compound has shown average MIC values. However, the compound **8a** and **8c** showed very good zone inhibition activity as well as in MIC for all bacterial strains.

Table 2. MIC results of ***8a-8j*** compounds

Derivatives	Enterobacter aerogens MTCC No. 8558	Escherichia coli MTCC No. 1610	Micrococcus luteus MTCC No. 11948	Bacillus cereus MTCC No. 8558
	MIC(µg/ml)	MIC(µg/ml)	MIC(µg/ml)	MIC(µg/ml)
I	12.5	25	50	100
II	400	200	200	400
III	50	50	25	12.5
IV	100	100	50	25
V	200	100	200	200
VI	200	25	50	50
VII	200	100	100	100
VIII	50	50	100	200
IX	50	25	50	25
X	200	200	100	100
Std	6.25	6.25	3.125	6.25

2.2.2 Antioxidant activity

2,2'-diphenyl-1-picrylhydrazyl (DPPH) assay used for in vitro free radical scavenging activity of all the synthesized compounds **8a-8j,** As reported method[26] shown in **table-3.** All synthesized compounds showed antioxidant properties which calculated by percentage (%) Inhibition and also dependent on scavenging radical.

The higher value of concentration indicated the increased value of the scavenging activity of the DPPH radical. **8a, 8c, 8g, 8i** and **8j** all compounds have shown very good radical scavenging activity. However, scaffold **8i** (38.10-81.15 µg/ml) displayed more efficient scavenging activity in all three concentration range while compounds **8c** showed steady activity and compound **8g** (82.15 µg/ml**)** showed excellent actively at higher concentration (600µg/ml). And the other derivatives showed an average reduction of DPPH scavenging activity.

Table 3. %DPPH radical scavenging activity assay of compound **8a-8j**

% DPPH radical scavenging activity assay at various concentration Mean ± S.E				
Derivatives	**0.00 µg/ml**	**200 µg/ml**	**400 µg/ml**	**600 µg/ml**
a	0.00	31.00±1.10	60.12±2.32	76.56±3.02
b	0.00	24.12±1.13	58.21±2.10	64.02±3.12
c	0.00	38.34±1.32	69.45±2.13	80.20±3.01
d	0.00	28.13±1.21	58.15±2.30	68.22±3.36
e	0.00	31.15±1.32	54.40±2.10	69.43±3.11
f	0.00	25.16±1.12	52.25±2.15	59.25±3.17
g	0.00	30.21±1.14	55.24±2.01	82.55±3.04
h	0.00	29.14±1.21	56.22±2.11	67.65±3.21
i	0.00	38.10±1.22	74.20±2.03	81.15±3.12
j	0.00	30.31±1.10	63.33±2.06	77.54±3.10

According to reported method.[26-27] The free radical scavenging activity of all the synthesized compounds **8a-8j** were screened by Superoxide anion system. phenezine methosulfate - nicotinamide adenine dinucleotide (PMS-NADH) system was used for evolved superoxide anion which tested by the reduction of nitroblue tetrazolium (NBT). Superoxide anion scavenging was assayed at different concentration 0.00 µg/ml, 200 µg/ml, 400 µg/ml and 600 µg/ml and calculated for IC_{50} value for all compounds which mentioned in **Table-4**. All compounds showed considerable results, however, the values correspond to **8i** showed excellent in both DPPH radical scavenging (38.10-81.15 µg/ml) and Superoxide anion scavenging (37.43-83.24 µg/ml) antioxidant activity. The other compounds relatively displayed average superoxide anion scavenging activity.

Table 4. Superoxide anion scavenging activity assay of compounds **8a-8j**

% Superoxide anion scavenging assay *at various concentration Mean ± S.E*				
Derivatives	**0.00 µg/ml**	**200 µg/ml**	**400 µg/ml**	**600 µg/ml**
a	0.00	30.23±0.81	64.11±1.53	76.02±1.20
b	0.00	28.11±0.86	56.60±1.47	65.62±1.21
c	0.00	34.32±0.68	62.15±1.50	79.34±1.27
d	0.00	23.32±0.62	57.58±1.51	68.75±1.24
e	0.00	30.21±0.17	57.22±1.53	66.35±1.26
f	0.00	28.66±0.82	59.23±1.52	74.73±1.21
g	0.00	36.12±0.74	61.33±1.51	79.52±1.24
h	0.00	29.65±0.82	57.22±1.53	67.25±1.23
i	0.00	37.43±0.73	65.44±1.56	83.24±1.22
j	0.00	31.55±0.68	62.33±1.53	75.63±1.28

3. Conclusions

In this present work we explain the synthesis and characterization of 4-((5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)amino)-N-(substituted)benzenesulfonamide **8a-8j** and evaluated for their in vitro antibacterial against two Gram-positive and two gram-negative strains, for the tested results **8a**, **8c**, **8d** and **8i**, derivatives found to have most potent antibacterial. Moreover, the derivatives of **8a, 8c, 8g, 8i** and **8j** appeared as good inhibition for DPPH radical scavenging antioxidant activity but compound **8i** being a most powerful antioxidant in both strain DPPH radical scavenging (38.10-81.15 µg/ml) and Superoxide anion scavenging (37.43-83.24 µg/ml).

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4. Experimental

4.1. Materials and Methods

Chemical and reagents were used all sulfa drugs and 5-Bromo-1H-pyrazolo[3,4-b]pyridine were acquired from commercial sources (Sigma-Aldrich). and iodine (I_2), Potassium hydroxide (KOH), dimethylformamide, (DMF), Isopropyl alcohol, Cuprous iodide (CuI), and Ethylene glycol from Merck (Germany). Pre-coated aluminium sheets (silica gel 60 $F_{254,}$ Merck) were used for thin-layer chromatography (TLC) and spots were visualized under ultraviolet light. Melting point (M.P) were measured by using a Mel-temp instrument, and results are uncorrected. Infra-red spectra were recorded on Shimadzu spectrophotometer in the frequency range 4000-400 cm^{-1} using KBr pallet disc, ^1H NMR and ^{13}C NMR spectra were recorded on Bruker at 400 MHz and 100 MHz in DMSO solution and chemical shifts were recorded in parts per million (ppm) with TMS at the internal reference. Advion expression CMS, USA were used for recorded mass spectra. The compound was analyzed for Carbon, Hydrogen, Nitrogen oxygen and Sulpher was estimated on CHNS analyzer serial NO. : 15084053

4.2. General procedure

4.2.1 synthesis of 5-Bromo-3-iodo-1H-pyrazolo[3,4-b]pyridine **6** Prepared by earlier reported method by Na Liu *et al.*[28] Dissolve 5-Bromo-1H-pyrazolo[3,4-b]pyridine **5** (2.0 g, 10.1 mmol) in DMF (25 mL) then add potassium hydroxide (KOH) (1.2 g 21.4 mmol) at 25 ºC with 10 min starring, then add iodine (I_2) (2.8 g 11.1 mmol) in two portions and stirred for 4h at 25 ºC. The product was diluted with water and add EtOH (20 mL × 3) extracted organic layer and then washed with $Na_2S_2O_3$ and brine (30 mL × 3) dried with using $MgSO_4$ and concentrated to give 5-Bromo-3-iodo-1H-pyrazolo[3,4-b]pyridine **6** a brown solid (2.7 g 82.5% yield)

4.2.2 synthesis of 4-((5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)amino)-N-(substituted)benzenesulfonamide **8a-8j** Dissolve compound 5-Bromo-3-iodo-1H-pyrazolo[3,4-b]pyridine **6** (3.6g 8.1 mmol) in i-PrOH (isopropanol, 50 mL) and added different sulfa drugs **7a-7j** (1.9 g 9.7 mmol) , potassium carbonate (K_2CO_3,16.2 mmol), Cuprous iodide (CuI 8.1 mmol) and 16.2 mmol ethylene glycol then starred for 6 hours at 100 ºC under inert atmosphere with N_2 at then cooled the mixture filtrated and washed with EtOH (80 mL) extracted organic layer and washed with ammonia water and brine (50 mL × 2). the layer of organic was dried over with using $MgSO_4$ and concentrated to give Pyrazolo sulfonamides derivatives **8a-8j** white to yellow solid. (70% yielded)

4.3 Physical and Spectral Data

4.3.1 4-((5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)amino)-N-(2,3-dihydrothiazol-2-yl)benzenesulfonamide **8a** White solid in 76.1% yield, mp 230-232ºC; Anal. Calcd for $C_{15}H_{13}BrN_6O_2S_2$: C, 39.74; H, 2.89; N, 18.54; O, 7..06; S, 14.15%; found C, 39.70; H, 2.82; N, 18.60; O, 7.07, S, 14.17%; IR (KBr) ($\upsilon_{max,}$ cm^{-1}); 3395 (sulfa-NH), 3218 (pyrazolo-NH) 3032 (C-H_{str} saturated hydrocarbon), 1620 (C=N_{str}) 1342 (C-N_{str}), 1550 (aromatic ring), 1382 Asy., 1123 Syn., (O=S=O), 1511 (thiazole ring); ^1H NMR (400 MHz, DMSO) δ 6.89-6.91 (d, aromatic Protons), 7.65-7.68 (d, aromatic Protons), 7.90, 8.06 (d 1H and d $1H_{thiazole}$), 6.62 (s 1H Ar-H Pyridine), 7.51 (s 1H Ar-H Pyridine), 10.44 (s, 1H -NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.36, 154.75, 149.93, 149.39, 146.83, 137.21, 135.41, 130.89, 130.32, 119.42, 114.21, 108.34; ESI-MS: *m/z* calculated 451.97, found $[M + H]^+$ 452.9.

4.3.2 4-((5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)aminobenzene-sulfonamide **8b** Light yellow solid in 77.3% yield, mp ~201ºC; Anal. Calcd for $C_{12}H_{10}BrN_5O_2S$: C, 39.14; H, 2.74; N, 19.02; O, 8..69; S, 8.71%; found C, 39.10; H, 2.72; N, 19.20; O, 8.67, S, 8.7 7%; IR (KBr)($\upsilon_{max,}$ cm^{-1}); 3479 (Asy- NH of SO_2NH_2), 3419 (Sym-NH of SO_2NH_2), 3238 (pyrazolo-NH) 3025 (C-H_{str} saturated hydrocarbon), 1625 (C=N_{str}) 1344 (C-N_{str}), 1460 (aromatic ring), 1342 Asy., 1157 Syn., (O=S=O), 1513 (thiazole ring); 1560 (N-H bend). ^{1}H NMR (400 MHz, DMSO) δ 6.8 (s, SO_2NH) 7.17-7.20 (d, aromatic Protons), 7.85-7.87 (d,aromatic Protons), 8.21 (s 1H Ar-H Pyridine), 8.59 (s 1H Ar-H Pyridine), 12.15 (s, 1H -NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.61, 151.7, 149.62, 145.34, 134.83, 132.99, 130.14, 128.72, 114.42, 114.32, 109.21, 92.34; ESI-MS: *m/z* calculated 366.97, found $[M + H]^+$ 367.8.

4.3.3 4-((5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)amino)-N-(5-methylisoxazole-3-yl)benzenesulfonamide **8c** White solid in 72.1% yield, mp 238-241ºC; Anal. Calcd for $C_{16}H_{13}BrN_6O_3S$: C, 42.77; H, 2.92; N, 18.71; O, 10..68; S, 7.14%; found C, 42.70; H, 2.82; N, 18.80; O, 10.17, S, 7.17%; IR (KBr) ($\upsilon_{max,}$ cm^{-1}); 3450 (NH), 3171 (pyrazolo-NH) 3037 (C-H_{str} saturated hydrocarbon), 1635 (C=N_{str}) 1352 (C-N_{str}), 1502 (aromatic ring), 1387 Asy., 1188 Syn., (O=S=O). ^{1}H NMR (400 MHz, DMSO) δ 2.02 (s, -CH_3) 7.17-7.19 (d, aromatic Protons), 7.90-7.92 (d, aromatic Protons), 7.35 (s 1$H_{methoxazole}$), 8.23 (s 1H Ar-H Pyridine), 8.62 (s 1H Ar-H Pyridine), 12.10 (s, 1H -NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.01, 159.17, 155.75, 150.01, 149.99, 146.83, 142.62, 132.84, 132.72, 120.22, 120.02, 109.41,109.12, 98.44, 13.25; ESI-MS: *m/z* calculated 448.00, found $[M + H]^+$ 449.02.

4.3.4 4-((5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)amino)-N-(pyrimidin-2-yl)benzenesulfonamide **8d** Light yellow solid in 75.9% yield, mp ~232ºC; Anal. Calcd for $C_{16}H_{12}BrN_7O_2S$: C, 43.06; H, 2.71; N, 21.97; O, 7..17; S, 7.18%; found C, 43.05; H, 2.80; N, 21.85; O, 7.17, S, 7.20%; IR (KBr) ($\upsilon_{max,}$ cm^{-1}); 3378 (NH), 3249 (pyrazolo-NH) 3047 (C-H_{str} saturated hydrocarbon), 1640 (C=N_{str}) 1351 (C-N_{str}), 1521 (aromatic ring), 1380 Asy., 1174 Syn., (O=S=O). ^{1}H NMR (400 MHz, DMSO) δ 7.22-7.24 (d, aromatic Protons), 7.66-7.69 (d, aromatic Protons), 6.73 (m, 1$H_{pyrimidin}$), 7.39-7.45 (d, 2$H_{pyrimidin}$), 7.55 (s 1H Ar-H Pyridine), 7.88 (s 1H Ar-H Pyridine), 12.71 (s, 1H -NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.73, 154.75, 154.34, 149.93, 149.39, 146.83, 137.21, 130.89, 130.02, 121.51, 119.52, 108.78; ESI-MS: *m/z* calculated 445.00, found $[M + H]^+$ 446.1.

4.3.5 4-((5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)amino)-N-(4,6-dimethylpyrimidin-2-yl)benzenesulfonamide **8e** Light yellow solid in 69.8% yield, mp ~243ºC; Anal. Calcd for $C_{18}H_{16}BrN_7O_2S$: C, 45.58; H, 3.40; N, 20.67; O, 6.75; S, 6.76%; found C, 45.55; H, 3.42; N, 20.65; O, 6.77, S, 6.75%; IR (KBr) ($\upsilon_{max,}$ cm^{-1}); 3380 (NH), 3189 (pyrazolo-NH) 3057 (C-H_{str} saturated hydrocarbon), 1639 (C=N_{str}) 1352 (C-N_{str}), 1501 (aromatic ring), 1387 Asy., 1170 Syn., (O=S=O). ^{1}H NMR (400 MHz, DMSO) δ 2.17 (s, 3H CH_3) 2.20 (s, 3H CH_3), 7.19-7.21 (d, aromatic Protons), 7.90-7.92 (d, aromatic Protons), 6.87 (s, 1$H_{pyrimidin}$), 8.24 (s 1H Ar-H Pyridine), 8.67 (s 1H Ar-H Pyridine), 12.24 (s, 1H -NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.23, 166.11, 160.17, 160.05, 149.98, 149.89, 146.89, 138.72, 131.83, 131.62, 121.41, 120.89, 108.86, 108.79, 24.44, 24.25; ESI-MS: *m/z* calculated 473.03, found $[M + H]^+$ 474.05

4.3.6 4-((5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)amino)-N-(4-methylpyrimidin-2-yl)benzenesulfonamide **8f** Light yellow solid in 67.8% yield, mp >237ºC; Anal. Calcd for $C_{17}H_{14}BrN_7O_2S$: C, 44.36; H, 3.07; N, 21.30; O, 6.95; S, 6.97%; found C, 44.35; H, 3.02; N, 21.45; O, 6.97, S, 6.95%; IR (KBr) ($\upsilon_{max,}$ cm^{-1}); 3385 (NH), 3179 (pyrazolo-NH) 3062 (C-H_{str} saturated hydrocarbon), 1642 (C=N_{str}) 1354 (C-N_{str}), 1505 (aromatic ring), 1377 Asy., 1172 Syn., (O=S=O). ^{1}H NMR (400 MHz, DMSO) δ 2.11 (s, 3H CH_3), 7.14-7.16 (d, aromatic Protons), 7.95-7.97 (d, aromatic Protons), 6.80 (d, 1$H_{pyrimidin}$), 8.35 (d, 1$H_{pyrimidin}$), 8.21 (s 1H Ar-H Pyridine), 8.62 (s 1H Ar-H Pyridine), 12.33 (s, 1H -NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.49, 163.05, 162.25, 161.20, 150.51, 149.89, 146.93, 138.15, 131.09, 130.84, 121.88, 119.72, 109.12, 108.92, 25.02; ESI-MS: *m/z* calculated 459.01, found $[M + H]^+$ 460.02

4.3.7 4-((5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)amino)-N-(4-methoxypyridazin-2-yl)benzenesulfonamide **8g** Light yellow solid in 72.2% yield, mp >239ºC; Anal. Calcd for $C_{17}H_{14}BrN_7O_3S$: C, 42.87; H, 2.96; N, 20.58; O, 10.08; S, 6.73%; found C, 42.85; H, 2.98; N, 20.55; O, 10.07, S, 6.75%; IR (KBr) ($\upsilon_{max,}$ cm^{-1}); 3388 (NH), 3159 (pyrazolo-NH) 3054 (C-H_{str} saturated hydrocarbon), 1635 (C=N_{str}) 1358 (C-N_{str}), 1506 (aromatic ring), 1348 Asy., 1128 Syn., (O=S=O). ^{1}H NMR (400 MHz, DMSO) δ 3.88 (s, 3H CH_3), 7.20-7.22 (d, aromatic Protons), 7.85-7.87 (d, aromatic Protons), 7.02 (d, 1$H_{pyrimidin}$), 7.05 (d, 1$H_{pyrimidin}$), 8.24 (s 1H Ar-H Pyridine), 8.60 (s 1H Ar-H Pyridine), 12.13 (s, 1H -NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.49, 161.17, 150.75, 149.99, 146.69, 146.75, 138.89, 131.04, 130.88, 124.72, 120.12, 116.32,108.11, 108.02, 53.52; ESI-MS: *m/z* calculated 475.01, found $[M + H]^+$ 476.02

4.3.8 4-((5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)amino)-N-(pyridin-2-yl)benzenesulfonamide **8h** Yellow solid in 74.6% yield, mp 227-230ºC; Anal. Calcd for $C_{17}H_{13}BrN_6O_2S$: C, 45.85; H, 2.94; N, 18.87; O, 7.19; S, 7.20%; found C, 45.85; H, 2.98; N, 18.85; O, 7.17, S, 7.25%; IR (KBr) ($\upsilon_{max,}$ cm^{-1}); 3355 (NH), 3169 (pyrazolo-NH) 3084 (C-H_{str} saturated hydrocarbon), 1648 (C=N_{str}) 1382 (C-N_{str}), 1498 (aromatic ring), 1352 Asy., 1135 Syn., (O=S=O). ^{1}H NMR (400 MHz, DMSO) δ, 7.17-7.20 (d, aromatic Protons), 7.92-7.94 (d, aromatic Protons), 6.88-8.55 (m, 6$H_{pyridine}$), 12.13 (s, 1H -NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.45, 161.36, 150.75, 149.96, 148.89, 146.93, 130.97, 130.52, 119.85, 118.31, 115.34, 109.02, 108.86; ESI-MS: *m/z* calculated 430.97, found $[M + H]^+$ 432.00

4.3.9 sodium acetyl((4-((5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)amino)phenyl)sulfonyl)amide **8i** Light yellow solid in 77.8% yield, mp ~217ºC; Anal. Calcd for $C_{14}H_{11}BrN_5NaO_3S$: C, 38.90; H, 2.57; N, 16.20; O, 11.10; S, 7.42%; found C, 38.85; H, 2.58; N, 16.15; O, 11.27, S, 7.45%; IR (KBr) ($\upsilon_{max,}$ cm^{-1}); 3169 (pyrazolo-NH) 3087 (C-H_{str} saturated hydrocarbon), 1705 (C=O) 1644 (C=N_{str}) 1336 (C-N_{str}), 1503 (aromatic ring), 1344 Asy., 1137 Syn., (O=S=O).). ^{1}H NMR (400 MHz, DMSO) δ 1.99 (s, 3H CH_3), 7.18-7.20 (d, aromatic Protons), 7.81-7.83 (d, aromatic Protons), 8.21 (s 1H Ar-H Pyridine), 8.64 (s 1H Ar-H Pyridine), 12.21 (s, 1H -NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.23, 161.14, 150.01, 149.93, 148.90, 135.83, 131.21, 130.91, 119.82, 109.21, 108.94, 21.85; ESI-MS: *m/z* calculated 430.97, found $[M + H]^+$ 431.89

4.3.10 4-((5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)amino)-N-(diaminomethylene)benzenesulfonamide **8j** Light yellow solid in 73.5% yield, mp ~208ºC; Anal. Calcd for $C_{13}H_{12}BrN_7O_2S$: C, 38.06; H, 2.95; N, 23.90; O, 7.80; S, 7.82%; found C, 38.05; H, 2.98; N, 23.95; O, 7.77, S, 7.85%; IR (KBr) ($\upsilon_{max,}$ cm^{-1}); 3474 (Asy-NH), 3420 (Sym-NH), 3165 (pyrazolo-NH) 3048 (C-H_{str} saturated hydrocarbon), 1631 (C=N_{str}) 1344 (C-N_{str}), 1501 (aromatic ring), 1387 Asy., 1145 Syn., (O=S=O). ^{1}H NMR (400 MHz, DMSO) δ 7.14-7.18 (d, aromatic Protons), 7.91-7.93 (d, aromatic Protons),6.75 (m, br, 1$H_{guanidine}$), 8.22 (s 1H Ar-H Pyridine), 8.65 (s 1H Ar-H Pyridine), 12.24 (s, 1H -NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.23, 161.36, 150.75, 149.94, 149.40, 138.10, 130.11, 130.01, 118.89, 118.32, 109.12, 108.91; ESI-MS: *m/z* calculated 409.00, found $[M + H]^+$ 410.0

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