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Note

Microwave and conventional techniques for the synthesis of a series of pyrazolo [5,4-*d*]-pyrimidine derivatives and their antimicrobial screening

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1-{4′-[(4″-6″-diaryl-(1″,3″,5″-triazin-2″-yl))amino]phenyl}-3-methyl-4-(substituted phenyl)-4,5-dihydropyrazolo[5,4-*d*]pyrimidin-6-ol **4$_{I\text{-}XXX}$** and 1-{4′-[(4″-6″-diaryl-(1″,3″,5″-triazin-2″-yl))-amino]phenyl}-3-methyl-4-(substituted phenyl)-4,5-dihydropyrazolo[5,4-*d*]pyrimidin-6-thiol **5 $_{I\text{-}XXX}$** have been synthesized by the reaction of 1-{4′-[(4″,6″-diaryl-(1″,3″,5″-triazin-2″-yl))amino]-phenyl}-3-methyl-2-pyrazolin-5-one **3**, with urea and various substituted aldehyde and with thiourea and various substituted aldehyde respectively. Both the reactions have been carried out by both the conventional and microwave techniques. It is noteworthy that the reaction which requires 6 hr in conventional method is completed within 3-4 min by microwave irradiation technique. The compounds have been screened for their antimicrobial activity against different micro-organisms. All the compounds show moderate to good activity against different micro-organisms at 256 µg/mL. The structures of novel synthesized compounds have been established on the basis of elemental analysis, IR, ^{1}H NMR and mass spectral data.

Keywords: *s*-Triazine, pyrazolo[5,4-*d*]pyrimidine, antimicrobial activity, microwave techniques, spectral data

It's important to note here that triazine and its derivatives are known for their useful properties, ranging from pharmacological to biological activities. *s*-Triazines are known to possess antibacterial[1], antiviral[2], antimalarial and anti-inflammatory activities[3,4]. Their anticancer, anti-leukemia and anti-HIV activities have also been evaluated and found to show promising properties in some instances[5,6].

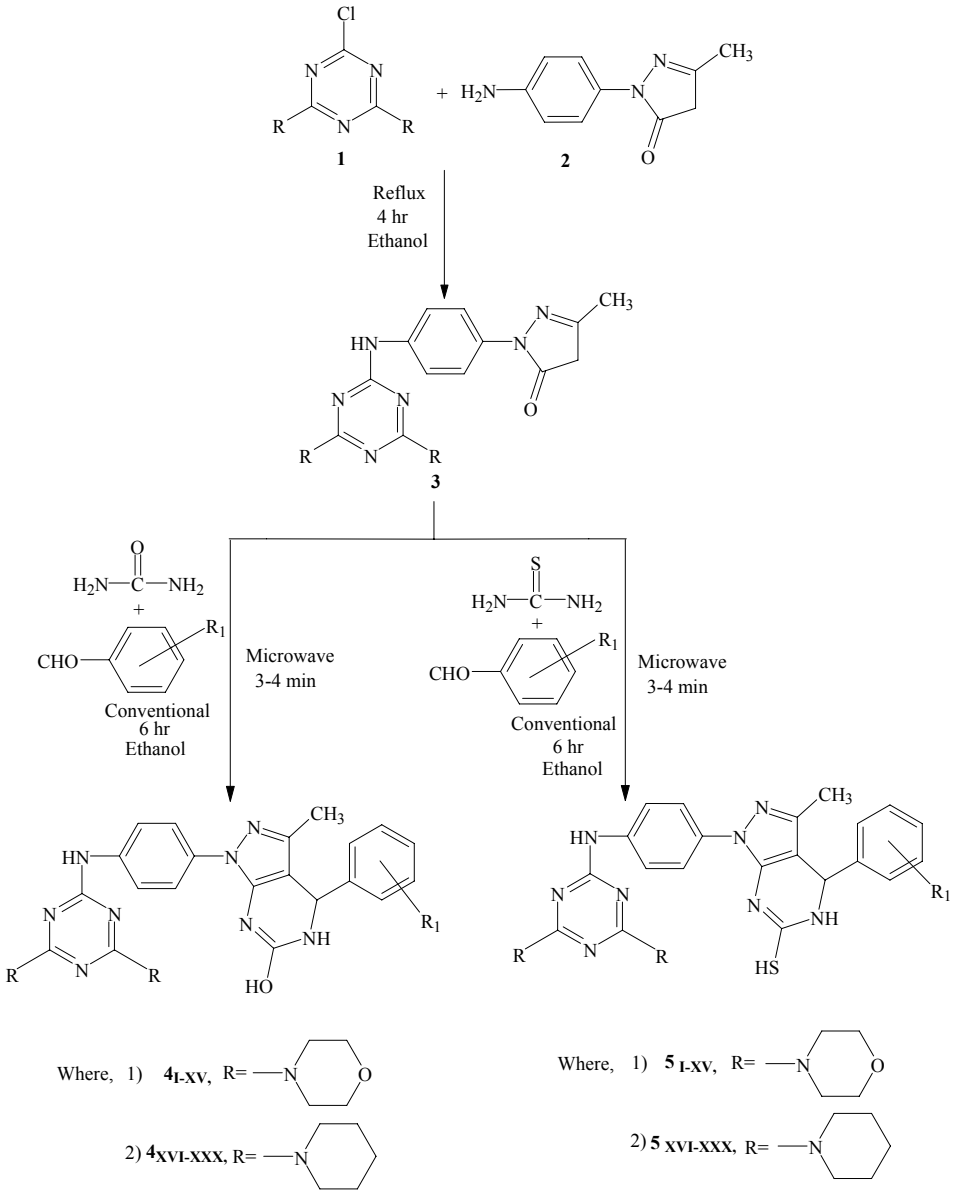
Pyrazolo-pyrimidine derivatives have received a great deal of attention due to their pharmacological activity[7-10], such as allopurinol[11], which is still the drug of choice for the treatment of hyperurecemia and gouty arthritis[12-14]. Pyrazolo-pyrimidines are purine analogues and as such they have useful properties as antimetabolites in purine biochemical reactions[15,16]. Moreover, these compounds also display marked antitumor and antileukemic activity[17]. Pyrazolo-pyrimidine derivatives have demonstrated promising antimicrobial activity against Gram-positive bacteria[18].

The application of microwave (MW) irradiation is used for carrying out chemical transformations which are pollution free and eco-friendly[19,20]. It provides chemical processes with special attributes, such as enhanced reaction rates, higher yields[21,22] of pure products, better selectivity, rapid optimization of reactions in parallel, and several eco-friendly advantages[23-28]. The application of MW irradiation to provide enhanced reaction rates and improved product yields in chemical synthesis has been extended to modern drug discovery processes[29,30].

Results and Discussion

The starting compound 1-{4′-[(4″,6″-diaryl-(1″,3″, 5″-triazin-2″-yl))amino]phenyl}-3-methyl-2-pyrazolin-5-one **3** have been synthesized by refluxing 6-chloro-2,4-diaryl-1,3,5-triazine **1** and 1-(4′-aminophenyl)-3-methyl-2-pyrazolin-5-one **2**. The reaction of **3** with urea and various substituted aldehydes was carried out by both conventional and microwave methods to give 1-{4′-[(4″,6″-diaryl-(1″,3″,5″-triazin-2″-yl))amino]phenyl}-3-methyl-4-(substituted phenyl)-4, 5- dihydro pyrazolo [5,4-*d*]pyrimidin-6-ol **4$_{I\text{-}XXX}$**. The reaction was completed in 6 hr in conventional method, while under MWI it was completed in 3-4 min. In conventional method the reaction of **3** with thiourea and various substituted aldehyde using ethanol as a solvent yielded 1-{4′-[(4″,6″-diaryl-(1″,3″,5″-triazin-2″-yl))amino]phenyl}-3-methyl-4-(substituted phenyl)-4,5-dihydro pyrazolo [5,4-*d*] pyrimidin-6-thiol **5$_{I\text{-}XXX}$** (**Scheme I**).

The structure of pyrazolo [5, 4-*d*] pyrimidines were established through IR, ^{1}H NMR and mass spectral data. In IR spectrum of **4$_I$**, significant bands appeared at 3502 (-OH) and 3427 (-NH) cm^{-1}. In their ^{1}H NMR spectra the same structure was evidenced by the appearance of signals at δ 9.82 and 4.70 due to -NH and -OH respectively. IR spectrum of pyrazolo [5,4-*d*] pyrimidines **5$_I$** compounds revealed significant bands at 3422 (-NH), 2508 (-SH) and 664 (C-S) cm^{-1}. In the ^{1}H NMR spectrum of **5$_I$**, signals were observed at δ 9.12 and 1.19 due to -NH and -SH respectively. In



Scheme I

mass spectra of pyrazolo [5,4-*d*]pyrimidines $\mathbf{4_{III}}$ and $\mathbf{4_{XXV}}$ the molecular ion peak appeared at 568 (M^+) and 607 (M^+) respectively. In $\mathbf{5_{III}}$ and $\mathbf{5_{XVIII}}$ the molecular ion peak appeared at 584 (M^+) and 580 (M^+) respectively, confirming the formation of pyrazolo [5,4-*d*] pyrimidine ring.

All the reactions were completed in 6 hr under conventional method, while under MWI they were completed in 3-4 min. A comparative study in terms of yield and reaction period is shown in **Table I** and **Table II**.

Experimental Section

All the melting points were determined in open capillaries and are uncorrected. The homogeneity of compounds was checked by TLC on silica gel 'G' coated glass plates. IR spectra were recorded in KBr on Shimadzu FT-IR 8300 spectrometer and ^{1}H NMR spectra were recorded in Varian 400 MHz and Bruker Avance II instruments in $CDCl_3$ by using TMS as internal standard. Mass spectra of the synthesized compounds have been recorded on a Jeol SX 102/DA-6000 spectrometer. Microwave assisted reactions

Table I — Characterization data of compounds $4_{I\text{-}XXX}$

Compd	-R_1	m.p. (°C)	Microwave Method		Conventional Method	
			Yield (%)	Time (min)	Yield (%)	Time (hr)
4_I	3- OCH_3	122	90	3-4	66	6
4_{II}	4- OCH_3	252(d)	88	3-4	71	6
4_{III}	-H	185	89	3-4	74	6
4_{IV}	2-Cl	171	85	3-4	63	6
4_V	3-Cl	129	88	3-4	69	6
4_{VI}	4-Cl	154	90	3-4	72	6
4_{VII}	4-F	167	90	3-4	62	6
4_{VIII}	3-NO_2	183	87	3-4	62	6
4_{IX}	4-NO_2	148	90	3-4	67	6
4_X	4-$N(CH_3)_2$	>300	90	3-4	70	6
4_{XI}	3-OC_6H_5	122	85	3-4	59	6
4_{XII}	2-OH	178	88	3-4	70	6
4_{XIII}	4-COOH	178	86	3-4	68	6
4_{XIV}	3-OCH_3, 4-OH	172	90	3-4	75	6
4_{XV}	3-OCH_3, 4- OCH_3	186	87	3-4	72	6
4_{XVI}	3- OCH_3	137	85	3-4	78	6
4_{XVII}	4- OCH_3	105	76	3-4	59	6
4_{XVIII}	-H	100	88	3-4	75	6
4_{XIX}	2-Cl	109	80	3-4	69	6
4_{XX}	3-Cl	110	77	3-4	65	6
4_{XXI}	4-Cl	102	83	3-4	70	6
4_{XXII}	4-F	124	78	3-4	59	6
4_{XXIII}	3-NO_2	129	81	3-4	67	6
4_{XXIV}	4-NO_2	146	86	3-4	73	6
4_{XXV}	4-$N(CH_3)_2$	202	90	3-4	75	6
4_{XXVI}	3-OC_6H_5	98	76	3-4	58	6
4_{XXVII}	2-OH	166	79	3-4	68	6
4_{XXVIII}	4-COOH	145	88	3-4	72	6
4_{XXIX}	3-OCH_3, 4-OH	116	89	3-4	72	6
4_{XXX}	3-OCH_3, 4- OCH_3	96	87	3-4	74	6

Note: The range of found error of %N is 0.01-0.06 of the calculated %N.

were carried out in "Q-Pro-M Modified Microwave system". The elemental analysis (%N) was carried out by total Kjeldahl method at Atul Limited, Valsad.

Synthesis of 1-{4′-[(4″,6″-diaryl-(1″,3″,5″-triazin-2″-yl))amino] phenyl}-3-methyl-2-pyrazolin-5-one, 3

Microwave method: The mixture of 6- chloro-2,4-diaryl-1,3,5- triazine **1** (0.01 mole) and 1-(4′-aminophenyl)-3-methyl-2-pyrazolin-5-one **2** (0.01 mole) in ethanol was stirred for 0.5 hr. Then the reaction mixture was subjected to microwave irradiation (350 W) for 2.5 min. Evolved HCl was neutralized with sodium bicarbonate during the reaction. The clear solution thus obtained was treated with crushed ice to form the solid product which was filtered and dried. The crude product was purified by recrystallization from absolute alcohol.

Conventional method: The mixture of **1** (0.01 mole) and **2** (0.01 mole) in ethanol was stirred for 0.5 hr. Then the reaction mixture was refluxed for 4 hr in water bath. Evolved HCl was neutralized with sodium bicarbonate during the reaction. The clear solution

Table II — Characterization data of compounds $5_{I\text{-}XXX}$

Compd	-R_1	m.p. (°C)	Microwave Method		Conventional Method	
			Yield (%)	Time (min)	Yield (%)	Time (hr)
5_I	3- OCH_3	169	88	3-4	71	6
5_{II}	4- OCH_3	167	87	3-4	67	6
5_{III}	-H	186	90	3-4	72	6
5_{IV}	2-Cl	189	85	3-4	63	6
5_V	3-Cl	173	89	3-4	69	6
5_{VI}	4-Cl	185	89	3-4	72	6
5_{VII}	4-F	120	87	3-4	62	6
5_{VIII}	3-NO_2	182	88	3-4	62	6
5_{IX}	4-NO_2	188	86	3-4	67	6
5_X	4-$N(CH_3)_2$	290(d)	85	3-4	70	6
5_{XI}	3-OC_6H_5	129	87	3-4	60	6
5_{XII}	2-OH	185(d)	89	3-4	76	6
5_{XIII}	4-COOH	150	86	3-4	72	6
5_{XIV}	3-OCH_3, 4-OH	171	90	3-4	72	6
5_{XV}	3-OCH_3,4- OCH_3	174	88	3-4	68	6
5_{XVI}	3- OCH_3	153	78	3-4	63	6
5_{XVII}	4- OCH_3	138	79	3-4	65	6
5_{XVIII}	-H	178	85	3-4	74	6
5_{XIX}	2-Cl	124	81	3-4	70	6
5_{XX}	3-Cl	143	84	3-4	74	6
5_{XXI}	4-Cl	134	79	3-4	66	6
5_{XXII}	4-F	151	76	3-4	63	6
5_{XXIII}	3-NO_2	162	80	3-4	69	6
5_{XXIV}	4-NO_2	137	77	3-4	65	6
5_{XXV}	4-$N(CH_3)_2$	161(d)	85	3-4	71	6
5_{XXVI}	3-OC_6H_5	164	75	3-4	60	6
5_{XXVII}	2-OH	192	84	3-4	72	6
5_{XXVIII}	4-COOH	150	88	3-4	73	6
5_{XXIX}	3-OCH_3, 4-OH	131	85	3-4	74	6
5_{XXX}	3-OCH_3, 4- OCH_3	157	82	3-4	70	6

Note: The range of found error of %N is 0.02-0.07 of the calculated %N.

thus obtained was treated with crushed ice to form the solid product which was filtered and dried. The crude product was purified by recrystallization from absolute alcohol. m.p. 175°C, Yield 71%.

Synthesis of 1-{4′-[(4″,6″-diaryl-(1″,3″,5″-triazin-2″-yl))amino]phenyl}-3-methyl-4-(substituted phenyl)-4,5-dihydropyrazolo[5,4-*d*]pyrimidin-6-ol, $4_{I\text{-}XXX}$

Microwave method: A mixture of **3** (0.01 mole) in ethanol was taken in a flat bottom flask, and urea (0.01 mole) with various substituted aldehyde (0.01 mole) were added to it. The mixture was irradiated in a microwave for about 3-4 min. It was then treated with crushed ice to give a product which was filtered, dried and purified by recrystallization from ethanol.

Conventional method: The mixture of **3** (0.01 mole) and urea (0.01 mole) with various substituted aldehyde (0.01 mole) in ethanol was refluxed for 6 hr in water bath. The clear solution thus obtained was treated with crushed ice to give the solid product which was filtered and dried. The crude product was purified by recrystallization from absolute alcohol.

Table III — Antimicrobial activity data of compounds $\mathbf{4_{I\text{-}XXX}}$

Compd	*E. coli*		*P. aeruginosa*		*S. aureus*		*Bacillus sp.*		*C. albicans*	
	128 µg/mL	256 µg/mL	128 µg/mL	256 µg/mL	128 µg/mL	256 µg/mL	128 µg/mL	256 µg/mL	128 µg/mL	256 µg/mL
4_{I}	-	++	-	++	-	++	-	++	-	-
4_{II}	-	++	-	++	-	++	-	++	-	-
4_{III}	-	++	-	++	-	++	-	++	-	-
4_{IV}	-	+++	-	+++	-	+++	-	+++	-	-
4_{V}	-	+++	-	+++	-	+++	-	+++	-	-
4_{VI}	-	+++	-	+++	-	+++	-	+++	-	-
4_{VII}	-	+++	-	+++	-	+++	-	+++	-	-
4_{VIII}	-	++	-	++	-	++	-	++	-	-
4_{IX}	-	++	-	++	-	++	-	++	-	-
4_{X}	-	++	-	++	-	++	-	++	-	-
4_{XI}	-	+++	-	+++	-	++	-	++	-	-
4_{XII}	-	+++	-	+++	-	+++	-	+++	-	-
4_{XIII}	-	+++	-	+++	-	+++	-	+++	-	-
4_{XIV}	-	+++	-	+++	-	+++	-	+++	-	-
4_{XV}	-	++	-	++	-	++	-	++	-	-
4_{XVI}	-	+++	-	+++	-	+++	-	+++	-	-
4_{XVII}	-	+++	-	+++	-	+++	-	+++	-	-
4_{XVIII}	-	+++	-	+++	-	+++	-	+++	-	-
4_{XIX}	-	+++	-	+++	-	+++	-	+++	-	-
4_{XX}	-	+++	-	+++	-	+++	-	+++	-	-
4_{XXI}	-	+++	-	+++	-	+++	-	+++	-	-
4_{XXII}	-	++	-	++	-	++	-	++	-	-
4_{XXIII}	-	++	-	++	-	++	-	++	-	-
4_{XXIV}	-	++	-	++	-	++	-	++	-	-
4_{XXV}	-	++	-	++	-	++	-	++	-	-
4_{XXVI}	-	+++	-	+++	-	+++	-	+++	-	-
4_{XXVII}	-	+++	-	+++	-	+++	-	+++	-	-
4_{XXVIII}	-	++	-	++	-	++	-	++	-	-
4_{XXIX}	-	+++	-	++	-	++	-	++	-	-
4_{XXX}	-	++	-	+++	-	++	-	++	-	-
Amikacin	-	++++	-	++++	-	++++	-	++++	-	-
Greseofulvin	-	-	-	-	-	-	-	-	-	++++

(-)< 6mm, (+) =7 - 10 mm, (++) =11 - 15mm, (+++) = 16 - 21mm, (++++) = 22 - 28mm

Spectral data of compound $\mathbf{4_I}$: IR (KBr): 3502 (-OH), 3427 (-NH), 3002 (-CH str, aromatic), 1630 (C=N str, pyrazolo[5,4-*d*]pyrimidine), 1576 (C=C str, pyrazolo-[5,4-*d*]pyrimidine), 1222 cm^{-1} (OCH_3); H^1 NMR ($CDCl_3$): δ 9.82 (s, 1H, -NH), 4.70 (s, 1H,-OH), 3.82 (s, 3H, $-OCH_3$), 3.41-3.78 (m, 8H, $-CH_2-O-CH_2-$), 2.0-2.70 (m, 8H, $-CH_2-N-CH_2-$), 2.50 (s, 3H, $-CH_3$), 6.73-8.04 (m, 8H, Ar-H); MS: *m/z* 598 (M^+). Compound $\mathbf{4_{III}}$: IR (KBr): 3506 (-OH), 3422 (-NH), 3005(-CH str, aromatic), 1632 (C=N str, pyrazolo[5,4-*d*]pyrimidine), 1572 cm^{-1} (C=C str, pyrazolo[5,4-*d*]pyrimidine); H^1 NMR ($CDCl_3$): δ 8.50 (s, 1H, -NH), 4.87 (s, 1H , -OH), 3.70-3.74 (m, 8H, $-CH_2-O-CH_2-$), 2.14-2.35 (m, 8H, $-CH_2-N-CH_2-$), 2.46 (s, 3H, $-CH_3$), 7.17-7.90 (m, 9H, Ar-H); MS: *m/z* 568 (M^+). Compound $\mathbf{4_{XVII}}$: IR (KBr): 3504 (-OH), 3421 (-NH), 3001 (-CH str, aromatic), 1635 (C=N str, pyrazolo[5,4-*d*]pyrimidine), 1575 cm^{-1} (C=C str, pyrazolo[5,4-*d*]pyrimidine); H^1 NMR (DMSO-d_6): δ 9.07 (s, 1H, -NH), 4.86 (s, 1H, -OH), 3.70 (s, 3H, -

Table IV — Antimicrobial activity data of compounds $\mathbf{5_{I\text{-}XXX}}$

Compd	*E. coli*		*P. aeruginosa*		*S. aureus*		*Bacillus sp.*		*C. albicans*	
	128 µg/mL	256 µg/mL	128 µg/mL	256 µg/mL	128 µg/mL	256 µg/mL	128 µg/mL	256 µg/mL	128 µg/mL	256 µg/mL
5_{I}	-	++	-	++	-	++	-	++	-	-
5_{II}	-	++	-	++	-	++	-	++	-	-
5_{III}	-	++	-	++	-	++	-	++	-	-
5_{IV}	-	+++	-	+++	-	+++	-	+++	-	-
5_{V}	-	+++	-	+++	-	+++	-	+++	-	-
5_{VI}	-	+++	-	+++	-	+++	-	+++	-	-
5_{VII}	-	+++	-	+++	-	+++	-	+++	-	-
5_{VIII}	-	++	-	++	-	++	-	++	-	-
5_{IX}	-	++	-	++	-	++	-	++	-	-
5_{X}	-	++	-	++	-	++	-	++	-	-
5_{XI}	-	+++	-	+++	-	+++	-	+++	-	-
5_{XII}	-	+++	-	+++	-	+++	-	+++	-	-
5_{XIII}	-	+++	-	+++	-	+++	-	+++	-	-
5_{XIV}	-	+++	-	+++	-	+++	-	+++	-	-
5_{XV}	-	++	-	++	-	++	-	++	-	-
5_{XVI}	-	+++	-	+++	-	+++	-	+++	-	-
5_{XVII}	-	++	-	++	-	+++	-	+++	-	-
5_{XVIII}	-	+++	-	+++	-	+++	-	+++	-	-
5_{XIX}	-	+++	-	+++	-	+++	-	+++	-	-
5_{XX}	-	+++	-	+++	-	+++	-	+++	-	-
5_{XXI}	-	+++	-	+++	-	+++	-	+++	-	-
5_{XXII}	-	+++	-	+++	-	+++	-	+++	-	-
5_{XXIII}	-	++	-	++	-	++	-	++	-	-
5_{XXIV}	-	++	-	++	-	++	-	++	-	-
5_{XXV}	-	++	-	++	-	++	-	++	-	-
5_{XXVI}	-	+++	-	+++	-	+++	-	+++	-	-
5_{XXVII}	-	+++	-	+++	-	+++	-	+++	-	-
5_{XXVIII}	-	+++	-	+++	-	+++	-	+++	-	-
5_{XXIX}	-	+++	-	+++	-	+++	-	+++	-	-
5_{XXX}	-	+++	-	+++	-	+++	-	+++	-	-
Amikacin	-	++++	-	++++	-	++++	-	++++	-	-
Greseofulvin	-	-	-	-	-	-	-	-	-	++++

(-)< 6mm, (+) =7 - 10 mm, (++) =11 - 15mm, (+++) = 16 - 21mm, (++++) = 22 - 28mm

OCH_3), 2.0-2.4 (m, 20H, $-CH_2-$), 2.46 (s, 3H, $-CH_3$), 6.85-8.70 (m, 8H, Ar-H); MS: *m/z* 594 (M^+). Compound $\mathbf{4_{XXV}}$: IR (KBr): 3503 (-OH), 3425 (-NH), 3002 (-CH str, aromatic), 1637 (C=N str, pyrazolo[5,4-*d*]pyrimidine), 1580 cm^{-1} (C=C str, pyrazolo[5,4-*d*]pyrimidine); H^1 NMR (DMSO-d_6): δ 9.06 (s, 1H, -NH), 5.14 (s, 1H, -OH), 1.3-1.8 (m, 20H, $-CH_2-$), 2.5 (s, 3H, $-CH_3$), 2.2 (t, 6H, $-N-(CH_3)_2$), 6.60-7.80 (m, 8H, Ar-H); MS: *m/z* 607 (M^+).

Synthesis of 1-{4′-[(4″, 6″-diaryl-(1″,3″,5″-triazin-2″-yl))amino]phenyl}-3-methyl-4-(substituted phenyl)-4, 5-dihydropyrazolo [5,4-*d*] pyrimidin-6-thiol, $\mathbf{5_{I\text{-}XXX}}$

Microwave method: A mixture of **3** (0.01 mole) in ethanol was taken in a flat bottom flask, and thiourea (0.01 mole) with various substituted aldehyde (0.01 mole) were added to it. The mixture was irradiated with microwave for about 3-4 min. It was then treated

with crushed ice to give a product which was filtered, dried and purified by recrystallization from ethanol.

Conventional method: The mixture of **3** (0.01 mole) and thiourea (0.01 mole) with various substituted aldehyde (0.01 mole) in ethanol was refluxed for 6 hr in water bath. The clear solution thus obtained was treated with crushed ice to give the solid product which was filtered and dried. The crude product was purified by recrystallization from absolute alcohol. Spectral data of compound $\mathbf{5_I}$; IR (KBr): 2550 (-SH), 3422 (-NH), 3001(-CH str, aromatic), 1633 (C=N str, pyrazolo[5,4-*d*]pyrimidine), 1572 (C=C str, pyrazolo[5,4-*d*]pyrimidine), 664 cm^{-1} (C-S); H^1 NMR ($CDCl_3$): δ 9.12 (s, 1H, -NH), 1.19 (s, 1H, -SH), 3.37-3.78 (m, 8H, $-CH_2-O-CH_2-$), 2.0-2.70 (m, 8H, $-CH_2-N-CH_2-$), 3.82 (s, 3H, $-OCH_3$), 2.50 (s, 3H, $-CH_3$), 6.73-8.04 (m, 8H, Ar-H); MS: *m/z* 614 (M^+). Compound $\mathbf{5_{III}}$: IR (KBr): 2558 (-SH), 3425 (-NH), 3005 (-CH str, aromatic), 1637 (C=N str, pyrazolo[5,4-*d*] pyrimidine), 1574 (C=C str, pyrazolo[5,4-*d*]pyrimidine), 660 cm^{-1} (C-S); H^1 NMR ($CDCl_3$): δ 8.50 (s, 1H, -NH), 1.25 (s, 1H, -SH), 3.72-3.74 (m, 8H, $-CH_2-O-CH_2-$), 2.17-2.32 (m, 8H, $-CH_2-N-CH_2-$), 2.35 (s, 3H, $-CH_3$), 7.16-7.91 (m, 9H, Ar-H); MS: *m/z* 584 (M^+). Compound $\mathbf{5_{XVIII}}$: IR (KBr): 2502 (-SH), 3427 (-NH), 3006 (-CH str, aromatic), 1638 (C=N str, pyrazolo[5,4-*d*] pyrimidine), 1572 (C=C str, pyrazolo[5,4-*d*]pyrimidine), 656 cm^{-1} (C-S); H^1 NMR ($CDCl_3$): δ 9.2 (s, 1H, -NH), 1.23 (s, 1H, -SH), 1.3-1.8 (m, 20H, $-CH_2-$), 2.52 (s, 3H, $-CH_3$), 7.2-8.15 (m, 9H, Ar-H); MS: *m/z* 580 (M^+). Compound $\mathbf{5_{XXX}}$: IR (KBr): 2508 (-SH), 3424 (-NH), 3005 (-CH str, aromatic), 1634 (C=N str, pyrazole [5,4-*d*]pyrimidine), 1576 (C=C str, pyrazolo[5,4-*d*] pyrimidine), 659 cm^{-1} (C-S); H^1 NMR ($CDCl_3$): δ 9.48 (s, 1H, -NH), 1.20 (s, 1H, -SH), 1.49-2.51 (m, 20H, $-CH_2-$), 3.68 (s, 3H, $-OCH_3$), 2.68 (s, 3H, $-CH_3$), 6.0-8.76 (m, 7H, Ar-H); MS: *m/z* 640 (M^+).

Antimicrobial activity

The synthesized compounds $\mathbf{4_{I\text{-}XXX}}$ and $\mathbf{5_{I\text{-}XXX}}$ were screened for their antibacterial activity against *E. coli*, *P. aeruginosa*, *S. aureus*, *Bacillus sp.*, and *C. albicans* by filter paper disc technique. The minimum inhibitory concentration (MIC) was determined by using tube dilution method according to the standard procedure[31]. The screening results exhibited the MIC against the micro-organisms in the range 128-256µg/mL. Results are presented in **Table III** and **Table IV**.

It is evident from the screening data that compounds $\mathbf{4_{IV}}$, $\mathbf{4_V}$, $\mathbf{4_{VI}}$, $\mathbf{4_{VII}}$, $\mathbf{4_{XII}}$, $\mathbf{4_{XIII}}$, $\mathbf{4_{XIV}}$, $\mathbf{4_{XVI}}$, $\mathbf{4_{XVII}}$, $\mathbf{4_{XVIII}}$, $\mathbf{4_{XIX}}$, $\mathbf{4_{XX}}$, $\mathbf{4_{XXI}}$, $\mathbf{4_{XXVI}}$, $\mathbf{4_{XXVII}}$, $\mathbf{4_{XXIX}}$, $\mathbf{5_{IV}}$, $\mathbf{5_V}$, $\mathbf{5_{VI}}$, $\mathbf{5_{VII}}$, $\mathbf{5_{XI}}$, $\mathbf{5_{XII}}$, $\mathbf{5_{XIII}}$, $\mathbf{5_{XIV}}$, $\mathbf{5_{XVI}}$, $\mathbf{5_{XVIII}}$, $\mathbf{5_{XIX}}$, $\mathbf{5_{XX}}$, $\mathbf{5_{XXI}}$, $\mathbf{5_{XXII}}$, $\mathbf{5_{XXIV}}$, $\mathbf{5_{XXVI}}$, $\mathbf{5_{XXVII}}$, $\mathbf{5_{XXVIII}}$, $\mathbf{5_{XXIX}}$ and $\mathbf{5_{XXX}}$ showed highest degree of inhibition only against *E. coli*, *P. aeruginosa*, *S. aureus* and *Bacillus sp.* at 256 µg/mL. While compounds $\mathbf{4_I}$, $\mathbf{4_{II}}$, $\mathbf{4_{III}}$, $\mathbf{4_{VIII}}$, $\mathbf{4_{IX}}$, $\mathbf{4_X}$, $\mathbf{4_{XV}}$, $\mathbf{4_{XXII}}$, $\mathbf{4_{XXIV}}$, $\mathbf{4_{XXV}}$, $\mathbf{5_I}$, $\mathbf{5_{II}}$, $\mathbf{5_{III}}$, $\mathbf{5_{VIII}}$, $\mathbf{5_{IX}}$, $\mathbf{5_X}$, $\mathbf{5_{XV}}$, $\mathbf{5_{XXIII}}$, $\mathbf{5_{XXIV}}$ and $\mathbf{5_{XXV}}$ have been found to show good activity against all the bacterial sp. at 256 µg/mL, no significant activity was found at 128µg/mL concentration.

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References

1 Chohan Z H, Humayun P A, Khan K M, Maharvi G M & Supuran C T, *J Enzyme inhibition and Med Chem*, 19(2), **2004**, 161.
2 Bozenna G, Piot J, Satoru I, Jan B & Eric De C, *J Med Chem*, 38, **1995**, 3558.
3 Davidson W M & Boykin W D, *J Pharm Sci*, 65, **1978**, 737.
4 Hielman P W, Gilman R D, Scozzie A J, Wayner R J, Gollo M J & Ariyan S Z, *J Pharm Sci*, 69, **1980**, 282.
5 El-Gendy Z & Abdel-Rahman R M, *J Heterocycl Chem*, 4, **1995**, 293.
6 Labouta I N, Eshba N H & Salma H M, *J Pharm Sci*, 16, **1988**, 29.
7 Xia Y, Chackalamannil S, Czarniecki M, Vaccaro H & Cleven R, *J Med Chem*, 40, **1997**, 4372.
8 Ahn H S, Bercovici A, Boykow G & Bronnenkant A, *J Med Chem*, 40, **1997**, 2196.
9 Traxler P, Bold G, Frei J, Lang M, Lydon N & Mett H, *J Med Chem*, 40, **1997**, 2217.
10 Zacharie B, Connoly T P, Rej R, Attardo G & Penney C L, *Tetrahedron*, 52, **1996**, 2271.
11 Robins R K, *J Am Chem Soc*, 78, **1956**, 784.
12 Kobayashi S, *Chem Pharm Bull*, 21, **1973**, 941.
13 Robins R K, Revankar G R, Springer R H, Novinson T & Albert A, *J Heterocycl Chem*, 22, **1985**, 601.
14 Satherland E W, Robinson G A & Butcher R W, *Circulation*, 37, **1968**, 279.
15 Earl, Pugmire R A, Revanker R J & Townsend G R, *J Org Chem*, 40, **1975**, 1822.
16 Hildick B G & Shaw G, *J Chem Soc* (C), **1971**, 1610.
17 Donker, Gangjee I O & Duah A, *J Heterocycl Chem*, 27, **1990**, 765.
18 Ali A, Tailor G E, Ellsworth K, Harris G, Painter R, Silver L L & Young K, *J Med Chem,* 46, **2003,** 1824.

19 Kidwai M, *Chem Edu Rev*, 15(4), **2000**, 34.
20 Srivastava K P, *Res J Chem Environ*, 5(2), **2001**, 77.
21 Michael D, *Chem Soc Rev*, 20(1), **1991**, 1.
22 Mogilaiah K, Reddy N V & Reddy P R, *J Heterocycl Chem*, 10, **2001**, 267.
23 Caddick S, *Tetrahedron*, 51, **1995**, 10403.
24 Varma R S, *Green Chem*, 1, **1999**, 43.
25 Lidstrom P, Tierney J, Wathey B & Westman J, *Tetrahedron*, 57, **2001**, 9225.
26 Perreux L & Loupy A, *Tetrahedron*, 57, **2001**, 9199.
27 Chisem J, Chisem I C, Rafelt J S, Macquarrie D J & Clark J H, *Chem Commun*, **1997**, 2203.
28 Meshram H M, Shekhar K C, Ganesh Y S & Yadav J S, *Synlett*, **2000**, 1273.
29 Strohmeier G A & Kappe C O, *J Comb Chem*, 4, **2004**, 154.
30 Alexandre F R, Domon L, Frere S, Testard A, Thiery V & Besson T, *Mol Diversity*, 7, **2003**, 273.
31 Andrews J M, *J Antimicrob Chemother*, 48, **2001**, 5.