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NOVEL PENTA- SUBSTITUTED PYRIDINE NUCLEUS WITH PYRAZOLE ANALOGUES: MICROWAVE ASSISTED SYNTHESIS, DOCKING AND BIOLOGICAL SCREENING

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Abstract:
*A novel series of 5-(4-formyl substituted phenoxy)-3-methyl-1-phenyl-1H-pyrazole-4-carbonitrile **(1a-b)** based penta-substituted pyridine derivatives **4(a–n)** was synthesized by piperidine catalyzed cyclocondensation reaction through microwave. The newly synthesized compounds were characterized by spectral studies and also by C, H and N analyses. The synthesized compounds were tested for their in vitro tuberculosis activity against H37Rv strains using rifampicin, isoniazide and ethambutol as the standard drugs. All novel synthesized compounds were tested for their in vitro antimalarial activity against P. falciparum strains using quinine and chloroquine as the standard drugs. Molecular docking and pharmacokinetic study were carried out for all the targeted compounds.*

Keywords: *- antimalarial activity, antituberculosis, docking, microwave assisted, pyridine*

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INTRODUCTION:

Malaria, a mosquito-borne disease, is the most critical health evils in the world at the current time [1]. Due to malaria approximate 500 million people get affected per annum and about 3 million deaths happen due to malaria [2-4]. Amongst the four Plasmodium parasites, Plasmodium falciparum is considered responsible for 95% of deaths [5]. The swell of multidrug-resistant Plasmodium falciparum has highlighted the urgent need of discovering new antimalarial drugs.

In the current scenario *tuberculosis* (TB) is a serious global health problem [6]. Approximately 32 % of the overall mankind is infected with TB and every year around 2 million people die and 8 million new people are detected tuberculosis active [7]. The increase of multidrug-resistant TB (MDR-TB) and the emergence of extensively drug-resistant TB (XDR-TB) pose new challenge for the preclusion [8]. Therefore, there is the urgency for the need of novel and effective novel drug to treat TB in a shorter duration with less toxicity and fewer side effects.

Microwave assisted synthesis is a branch of green chemistry that has attained considerable attention in recent years. Chemical transformations involved *via* this technique are eco-friendly, pollution free and offer high yields together with simplicity in processing and handling [9, 10]. The main advantages of microwave-assisted synthesis are rate accelerations, high selectivity, improved yields, less bye products, shorter reaction times, and easier work-up [11, 12].

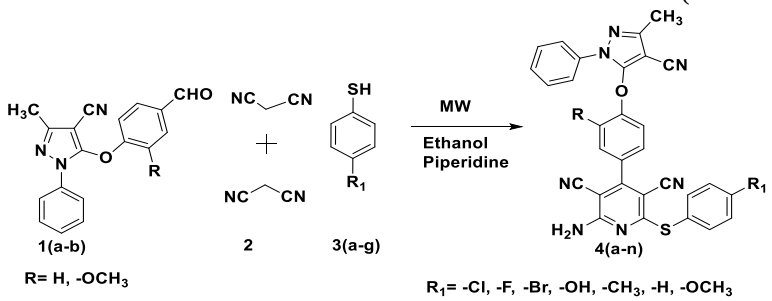
In modern period pyridine derivatives are a very important class of compounds which have good biological activity due to their pharmacological properties. The biologically active cyano pyridine moiety is proving to show potent antifungal [13], antibacterial [14], anticancer [15] and antimicrobial activity [16]. In addition to that substituted 2-amino-3-cyanopyridine derivatives are known for various biological activities, such as anti-microbial [17], anti inflammatory [18], potent inhibitor of HIV-1 integrase [19], anti-inflammatory [20] and antimalarial activity [21].

In continuation of our on-going research on various biologically active heterocyclic derivatives [22, 23] and potent biological screening, microwave assisted synthesis of novel 2-amino-6-((4-substituted phenyl)thio)-4-(4-((4-cyano-3-methyl-1-phenyl-1H-pyrazol-5yl)oxy) substituted phenyl)pyridine-3,5-dicarbonitrile **(4a-n)** has been contemplated with the view of getting biologically active compounds for pharmaceutical applications.

RESULT AND DISCUSSION:

Chemistry

The starting material 5-(4-formyl substituted phenoxy)-3-methyl-1-phenyl-1H-pyrazole-4-carbonitrile **(1a-b)** was prepared in three step. In the first step Vilsmeier–Haack reaction [24]**)** of 3-methyl-1-phenyl-1H-pyrazol-5(4*H*)-one gives 5-Chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde. In second step 5-Chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde containing aldehyde group is converted to nitirile group [25]. The final aldehyde 5-(4-formyl substituted phenoxy)-3-methyl-1-phenyl-1*H*-pyrazole-4-carbonitrile **(1a-b)** were prepared by nucleophilic displacement of chloro group at C_5 in 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbonitrile **(1a-b)** with substituted 4-hydroxy benzaldehyde by refluxing in DMF using anhydrous K_2CO_3 as a base. The final compounds 2-amino-6-((4-substituted phenyl)thio)-4-(4-((4-cyano-3-methyl-1-phenyl-1*H*-pyrazol-5 yl) oxy) substituted phenyl)pyridine-3,5-dicarbonitrile**(4a-n)** were prepared in good yield (75-90 %) under by microwave irradiation by reacting the mixture of substituted 5-(4-formyl substituted phenoxy)-3-methyl-1-phenyl-1*H*-pyrazole-4-carbonitrile **(1a-b),** malononitrile (**2**), substituted thiols **(3a-g)** and catalytic amount of piperidine in absolute alcohol for 8-15min at 350 W. (**Table 1**).



Scheme 1. Synthesis of 2-amino-6-((4-substituted phenyl)thio)-4-(4-((4-cyano-3-methyl-1-phenyl-1H-pyrazol-5 yl) oxy) substituted phenyl)pyridine-3,5-dicarbonitrile derivatives **(4a-n)**

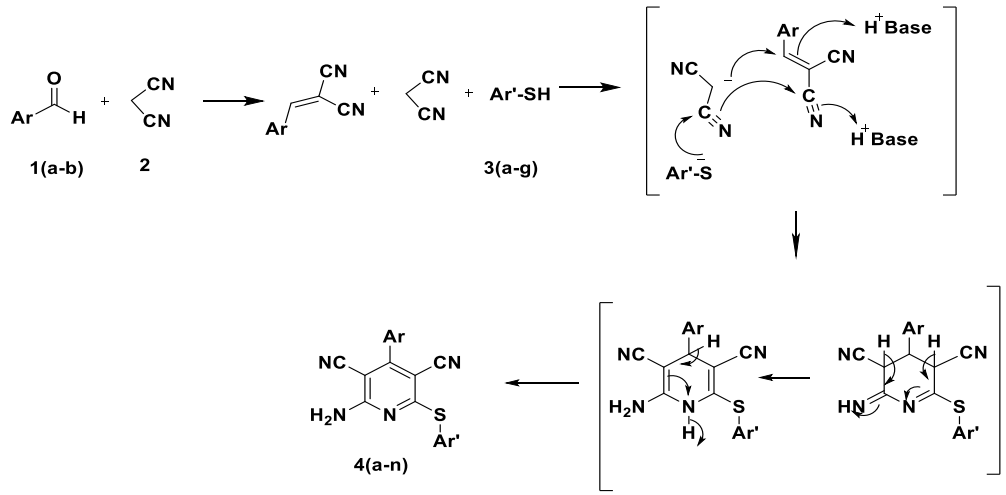
The agreement of target compounds **4(a–n)** may progress via the initial formation of an intermediate afforded by Knoevenagel condensation of a substituted pyrazole aldehyde with malononitrile by loss of water molecule, which would undergo intermolecular cyclisation of another molecule of malononitrile driven through the nucleophilic attack of thiophenols in presence of piperidine basic reaction medium (**Scheme 2**).

Table 1: Preliminary characterization of synthesized compounds 4a-n.

			conventional method		Microwave method	
Entry	R	R_1	Time (h)	Yield[a] (%)	Time (min)	Yield[a] (%)
4a	-H	-Cl	1.5	78	09	89
4b	- OCH_3	-Cl	2	76	10	87
4c	-H	-CH_3	1.5	81	12	90
4d	- OCH_3	-CH_3	2	75	14	88
4e	-H	-Br	1.5	69	12	81
4f	- OCH_3	-Br	2	68	13	78
4g	-H	-F	1.5	74	09	85
4h	- OCH_3	-F	2	68	10	81
4i	-H	-OH	1.5	66	11	75
4j	- OCH_3	-OH	2	60	13	73
4k	-H	- OCH_3	1.5	63	12	78
4l	- OCH_3	- OCH_3	2	64	12	75
4m	-H	-H	1.5	67	14	88
4n	- OCH_3	-H	2	69	14	83

[a]Yields of isolated products

Reaction mechanism



Ar = 5-(4-formyl-2-(un) substituted phenoxy)-3-methyl-1-phenyl-1H-pyrazole-4-carbonitrile
Ar' = 4-(un)-sustituted thiophenol

Scheme 2. Plausible mechanistic pathway for the synthesis of pyridine derivatives **4(a–n)**

RESULTS AND DISCUSSION:

^{1}H NMR, FT-IR, ^{13}C NMR, elemental analysis and mass spectrometry techniques were used for confirmation of newly synthesised compounds. The ^{1}H NMR spectra of compounds **4a-n** showed the presence of –CH_3 protons (pyrazole ring) as a sharp singlet in the range between δ 2.34-2.45 ppm. The broad singlet at δ 5.45-5.65 ppm was observed due to –NH_2 proton. In the range between δ 7.15-7.78 ppm the signals (multiplets) appeared for aromatic protons. The IR spectrum of compounds **4a-n** exhibited characteristic absorption band in the range

730-771 cm^{-1} which can be ascribed to the presence of thioether linkage. While absorption band in the range of 1212-1231 cm^{-1} was observed for ether linkage. The absorption band in the range 3410-3311 cm^{-1} may be attributed to asymmetric & symmetric stretching of $-NH_2$.The absorption band was observed in the range of 1369-1376 cm^{-1} may be due to presence of $-CH_3$ group. The absorption band in the range of 2180-2270 cm^{-1} was observed due to -C≡N stretching. The mass spectrum of all the compounds showed molecular ion peak (M^+) corresponding to their respective molecular weights, which confirmed the chemical structures.

Biological result

***In vitro* antituberculosis activity**

Evaluation of all newly synthesized compounds was performed for antituberculosis activity against *Mycobacterium tuberculosis H37Rv* strain. Screening of all the synthesized compounds was conducted at 250 $mg.mL^{-1}$ by using Lowenstein–Jensen medium (conventional method) as described by Rattan (**22**). The observed results are presented in **Table 2** in the form of %inhibition. The standard drugs rifampicin, ethambutol and isoniazid were used for comparison.

Table 2: In vitro antituberculosis activity (% inhibition) of compounds (4a-o) against *M. tuberculosis H37Rv* (at concentration 250 mg/mL).

Entry	% inhibition	Entry	% inhibition
4a	**97**	4j	40
4b	**95**	4k	81
4c	79	4l	72
4d	64	**4m**	**94**
4e	57	4n	42
4f	52	**Rifampicin**	**98**
4g	**98**	**Ethambutol**	**99**
4h	46	**Isoniazide**	**99**
4i	70		

Antituberculosis activity of the all compounds **4a–n** was conducted at 250 µg/mL concentrations against *Mycobacterium tuberculosis* H37Rv strain. Compounds **4a** (R = 3-H, R_1 = 4-Cl), **4b** (R = 3-CH_3, R_1 = 4-Cl), **4g** (R = 3-H, R_1 = 4-F) and **4m** (R = 3-H, R_1 = 4-H) were found to have brilliant activity (i.e. **97%, 95%, 98%** and **94%** at 250 µg/mL) against M. tuberculosis H37Rv. Remaining all other compounds showed medium inhibition against *M. tuberculosis* H37Rv.

***In vitro* antimalarial activity**

In vitro antimalarial activity of the all novel synthesized compounds **7a-n** aligned with *P. falciparum* strain was tested using quinine and chloroquine as the reference drugs. The consequences of the antimalarial screening are communicated as the drug concentration resulting in 50% inhibition (IC_{50}) of parasite growth and are listed in **Table 3**.

Table 3: *In vitro* antimalarial activity of derivatives 4a-n

Entry	IC_{50}(µg/mL)	Entry	IC_{50}(µg/mL)
4a	**0.023**	4i	0.20
4b	0.18	4j	0.59
4c	0.54	**4k**	**0.042**
4d	1.84	4l	0.98
4e	0.37	4m	1.58
4f	0.75	4n	1.65
4g	**0.057**	**Quinine**	**0.268**
4h	1.61	**Chloroquine**	**0.020**

The compounds **4a** (R = -H, R_1 = 2,4-di Cl), **4g** (R = -H, R_1 = 2-CH3), **4k**(R = -H, R_1 = 2-OCH_3), were found to have IC_{50} 0.023, 0.057 and 0.042 respectively. In this antimalarial screening only three compounds were found to be are more active against *P. falciparum.*

5. Docking study of compounds

***In silico* pharmacokinetic evaluation**

The different pharmacokinetic parameters *viz.* hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), rotatable bonds (Rot B), logP, polar surface area (PSA) and binding energy s for compounds **4a-n** are listed in **Table-4**. These data were evaluated by molecule docking server software. The synthesized compounds have a high molecular weight (≤ 500) as compared to the standard drugs. The compounds **4j and 4l** showed elevated hydrogen bond acceptor (HBA) value while **4i** and **4j** have a high hydrogen bond donor value. The values of rotational bond of all synthesized compounds **(4a-n)** are in between **6-9** which is very near to ethambutol. All compounds showed higher than logP (≤ 5) values and polar surface area then isoniazide and ethambutol.

***In silico* molecular docking study**

Molecular interaction for all the ligands was studied using molecule docking server software. The 3D diagrams **Figs 1A-4A** show the binding sites of all the ligands within the receptor (2B35). **Figs. 1B-4B** show 2D interaction diagram between amino acids and compounds that interacted to the active sites (amino acids) of receptor (2B35) through like a hydrogen bond. We performed molecular docking studies of all compounds (**4a-n**) and 3D presentation of the complex between receptor (PDB ID: 2B35). Ethambutol and isoniazide were used as the standard drugs. The binding energy docking scores are presented in **Table-3**. It is remarkable that all the compounds (**4a-n**) showed binding energy in between **-6.39** to **-8.26** kcal/mol and formed stronger complexes with receptor as compared to the standard drugs. Compounds **4a**, **4b**, **4g** and **4m** showed very poor binding energy **(-8.22, -8.26, -8.02**, and **-8.03 kcal/mol**. respectively) which are potently active against tuberculosis (**Table-2**).

Table 4: Pharmacokinetic parameters

Compound ID(s)	Mol.wt[a]	HBA[b]	HBD[c]	RotB[d]	logp[e]	PSA[f]	Binding energy[g]
4a	**560.02**	**8**	**1**	**6**	**7.61**	**162.63**	**-8.22**
4b	**590.05**	**9**	**1**	**7**	**7.62**	**171.86**	**-8.26**
4c	539.61	8	1	6	7.27	162.63	-7.68
4d	569.63	9	1	7	7.28	171.86	-6.55
4e	604.48	8	1	6	7.72	162.63	-7.32
4f	634.50	9	1	7	7.73	171.86	-7.79
4g	**543.57**	**8**	**1**	**6**	**7.10**	**162.63**	**-8.02**
4h	573.60	9	1	7	7.11	171.86	-7.12
4i	541.58	9	2	6	6.67	182.86	-7.58
4j	571.60	10	2	7	6.67	192.09	-6.39
4k	555.61	9	1	7	6.97	171.86	-6.83
4l	585.63	10	1	8	6.98	181.09	-6.78
4m	**525.58**	**8**	**1**	**6**	**6.96**	**162.63**	**-8.03**
4n	555.61	9	1	7	6.91	171.86	-7.63
Isoniazide	**137.13**	**4**	**2**	**2**	**0.77**	**68.01**	**-5.1**
Ethambutol	**204.30**	**4**	**4**	**9**	**0.48**	**64.52**	**-4.5**

[a] Molecular Weight ≤ 500 (gm/mol) [26], [b] Hydrogen Bond Acceptor ≤ 10 [27], [c] Hydrogen Bond Donor ≤ 5 [27], [d] Rotatable Bonds ≤ 10 [27] , [e] logP ≤ 5 [26], [f]Polar Surface Area ≤140 A^2 [27], [g]Binding energy

Experimental section

Starting materials were obtained from Spectrochem and Sigma Aldrich. The progress of the reactions was checked by TLC on aluminum plates coated with silica gel 60 F254, 0.25 mm thickness (Merck). The developed chromatograms were visualized under UV light. FT-IR spectra were recorded in KBr on a Perkin -Elmer Spectrum GX FT-IR Spectrophotometer (Perkin-Elmer, USA). The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 400. Tetramethylsilane (TMS) was used as the internal standard and $CDCl_3$ as the solvent. Chemical shifts are reported in parts per million (ppm). Melting points are uncorrected and were determined using µThermoCal10 melting point apparatus (Analab Scientific Pvt. Ltd, India). The elemental analysis was performed on Perkin- Elmer 2400 series-II elemental analyzer (Perkin- Elmer, USA) at (SICART), Vallabh Vidyanagar, India .Mass spectra were recorded on Shimadzu LCMS 2010 spectrometer (Shimadzu, Tokyo, Japan) at Sardar Patel University (PURSE programme of DST), Vallabh Vidyanagar.

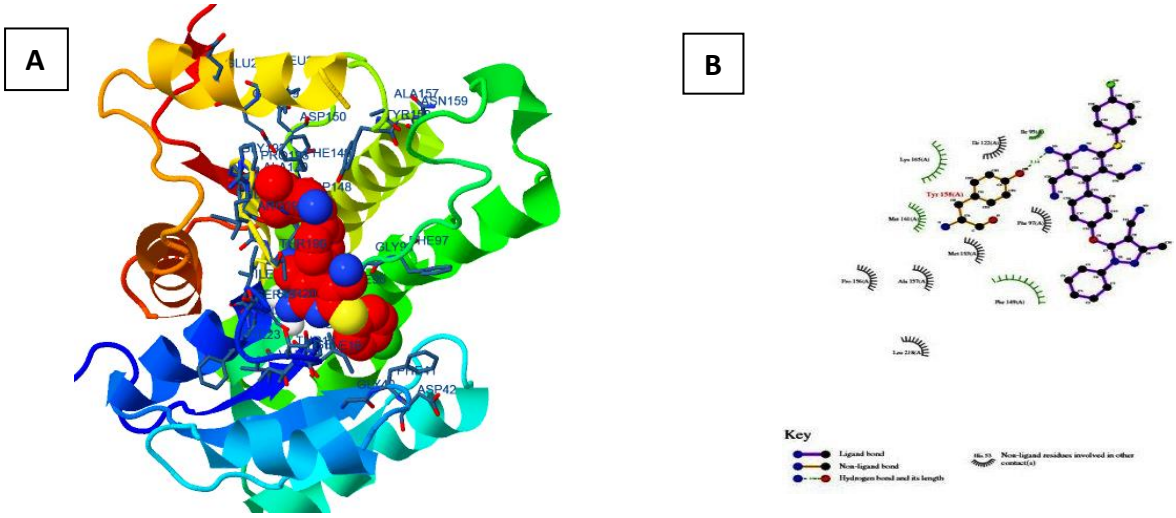


Fig.1 (A) 3D presentation of the complex between receptor (**2B35**) and **4a.**
(**B**) Schematic 2D diagram of interaction between active site residues and **4a.**

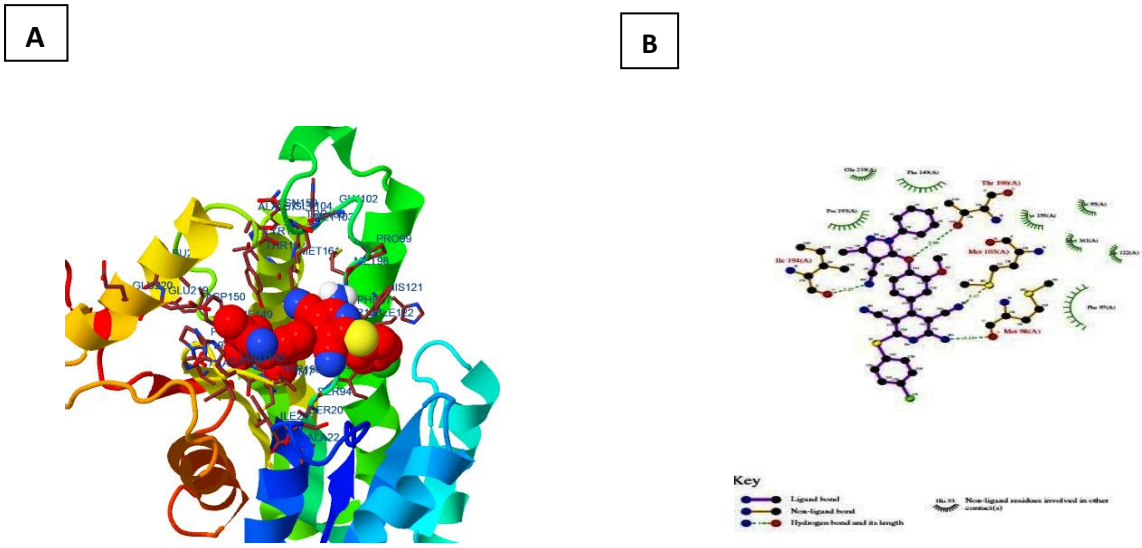


Fig.2 (A) 3D presentation of the complex between receptor (**2B35**) and **4b**.
(B) Schematic 2D diagram of interaction between active site residues and **4b.**

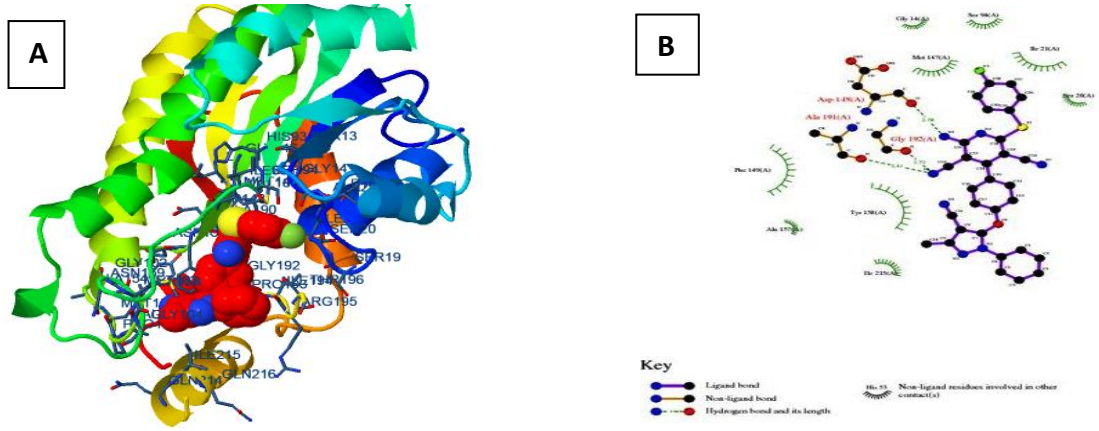


Fig.3 (A) 3D presentation of the complex between receptor (**2B35**) and **4g.**
(**B**) Schematic 2D diagram of interaction between active site residues and **4g.**

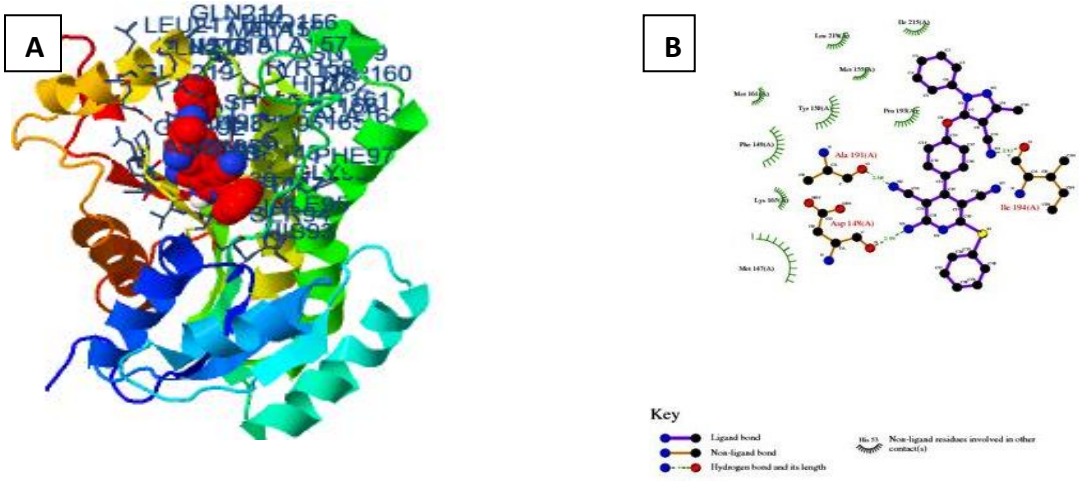


Fig.4 (A) 3D presentation of the complex between receptor (**2B35**) and **4m**.
(B) Schematic 2D diagram of interaction between active site residues and **4m.**

General procedure for the microwave promoted novel penta- substituted pyridine nucleus with pyrazole

Substituted pyrazole aldehyde (**1(a-b), 1 mmol**), malononitrile (**2, 2 mmol**), substituted thiophenols (**3(a-g), 1 mmol**) and catalytic amount of piperidine were mixed carefully in the ethanol. The reaction mixture was heated in microwave oven for 9-14 min at 350 W. After cooling to room temperature the final products (**4a-n**) were filtered and crystallized from ethanol.

2-amino-6-((4-chlorophenyl)thio)-4-(4-((4-cyano-3-methyl-1-phenyl-1H-pyrazol-5-yl)oxy)-phenyl)pyridine-3,5-dicarbonitrile (4a)

White solid; yield: 78%; mp 180-183 ºC; IR (KBr, *v*max, cm^{-1}) = 3430 & 3352 (asym. & sym. stretching of $–NH_2$); 2209 (-C≡N stretching), 1374 ($-CH_3$ str.), 1231 (C-O-C str.), 761 (C-S-C str.), 3010 (Ar-C-H); ^{1}H NMR (400 MHz, DMSO-d_6) δ = 2.39 (s, 3H,Pyrazole $-CH_3$), 5.58 (s, 2H, $–NH_2$), 7.15-7.73 (m, 13H, Ar-H); ^{13}C NMR (100 MHz DMSO-d6) δ: 13.46, 78.88, 88.60, 96.60, 112.35, 113.49, 115.04, 121.40, 121.96, 124.05, 125.55, 128.15, 130.15, 130.64, 133.30, 136.56, 138.09, 139.01, 145.20, 151.23, 152.59, 157.90, 159.30, 168.60, LC-MS: 560.03 (M)+; Anal.Calc. For $C_{30}H_{18}ClN_7OS$: Elemental Analysis: C, 64.34; H, 3.24; N, 17.51; Observed, C, 64.40; H, 3.64; N, 17.93 %.

2-amino-6-((4-chlorophenyl)thio)-4-(4-((4-cyano-3-methyl-1-phenyl-1H-pyrazol-5-yl)oxy)-3-methoxyphenyl)pyridine-3,5-dicarbonitrile (4b)

White solid; yield: 80%; mp 175-177 ºC; IR (KBr, *v*max, cm^{-1}) = 3454 & 3331 (asym. & sym. stretching of $–NH_2$); 2199 (-C≡N stretching), 1370 ($-CH_3$ str.),

1214 (C-O-C str.), 730 (C-S-C str.), 3018 (Ar-C-H); ^{1}H NMR (400 MHz, DMSO-d_6) δ = 2.37 (s, 3H,Pyrazole -CH_3), 3.93(s, 3H, -OCH_3) 5.65 (s, 2H, –NH_2), 7.17-7.75 (m, 13H, Ar-H); ^{13}C NMR (100 MHz DMSO-d_6) δ: 13.43, 56.41, 78.83, 87.53, 95.65, 111.45, 113.49, 114.53, 114.84, 121.60, 121.99, 123.03, 125.43, 128.05, 129.25, 129.65, 132.31, 136.56, 136.99, 137.11, 144.49, 151.23, 151.55, 153.60, 156.91, 159.28, 168.67, LC-MS: 590.06 (M)+ ; Anal.Calc. For $C_{31}H_{20}ClN_7O_2S$; Elemental Analysis: C, 63.10; H, 3.42; N, 16.62; Observed, C, 62.80; H, 3.23; N, 16.14 %.

2-amino-4-(4-((4-cyano-3-methyl-1-phenyl-1H-pyrazol-5-yl)oxy)phenyl)-6-(p-tolylthio)pyri- dine-3,5-dicarbonitrile (4c)

White solid; yield: 84; mp 165-167 ºC IR (KBr, *ν*max, cm^{-1}) = 3419 & 3315 (asym. & sym. stretching of –NH_2); 2180 (-C≡N stretching), 1369 (-CH_3 str.), 1226 (C-O-C str.), 769 (C-S-C str.), 3015 (Ar-C-H); ^{1}H NMR (400 MHz, DMSO-d_6) δ = 2.35 (s, 3H,Pyrazole -CH_3), 2.36 (s, 3H, -CH_3), 5.45 (s, 2H, –NH_2), 7.18-7.56 (m, 13H, Ar-H); ^{13}C NMR (100 MHz DMSO-d_6) δ: 13.55, 21.45, 81.63, 87.04, 95.47, 111.46, 114.71, 114.98, 115.04, 118.28, 122.92, 123.40, 128.50, 129.47, 130.20, 130.46, 130.95, 135.67, 136.59, 140.39, 151.80, 152.07, 156.59, 159.32,169.73; LC-MS: 539.62 (M)+; Anal.Calc. For $C_{31}H_{21}N_7OS$; Elemental Analysis: C, 69.00; H, 3.92; N, 18.17; Observed, C, 68.67; H, 3.85; N, 17.97 %.

2-amino-4-(4-((4-cyano-3-methyl-1-phenyl-1H-pyrazol-5-yl)oxy)-3-methoxy- phenyl)-6-(p-tolylthio)pyridine-3,5-dicarbonitrile (4d)

White solid; yield: 83; mp 156-160 ºC; IR (KBr, *ν*max, cm^{-1}) = 3445 & 3335 (asym. & sym. stretching of –NH_2); 2194 (-C≡N stretching), 1375 (-CH_3 str.), 1219 (C-O-C str.), 752 (C-S-C str.), 3012 (Ar-C-H); ^{1}H NMR (400 MHz, DMSO-d_6) δ = 2.34(s, 3H, CH_3), 2.36 (s, 3H,Pyrazole -CH_3), 3.91(s, 3H, -OCH_3), 5.48 (s, 2H, –NH_2), 7.17-7.74 (m, 13H, Ar-H); ^{13}C NMR (100 MHz DMSO-d_6) δ: 13.58, 21.47, 56.45, 82.03, 86.59, 97.14, 112.40, 113.91, 114.90, 115.14, 119.08, 122.45, 123.85, 127.10, 129.49, 130.27, 131.01, 132.25, 134.43, 135.50, 140.19 140.397, 143.81, 151.40, 152.47, 157.54, 160.02, 168.99 ; LC-MS: 569.64 (M)+ ; Anal.Calc. For $C_{32}H_{23}N_7O_2S$; Elemental Analysis: C, 67.47; H, 4.07; N, 17.21; Observed, C, 67.30; H, 3.88; N, 17.04 %.

2-amino-6-((4-bromophenyl)thio)-4-(4-((4-cyano-3-methyl-1-phenyl-1H-pyra-zol-5-yl)oxy)-phenyl)pyridine-3,5-dicarbonitrile (4e)

White solid; yield: 89; mp 190-194 ºC; IR (KBr, *ν*max, cm^{-1}) = 3418 & 3333 (asym. & sym. stretching of –NH_2); 2295 (-C≡N stretching), 1372 (-CH_3 str.), 1220 (C-O-C str.), 742 (C-S-C str.), 2990 (Ar-C-H); ^{1}H NMR (400 MHz, DMSO-d_6) δ = 2.35 (s, 3H,Pyrazole -CH_3), 5.46 (s, 2H, –NH_2), 7.19-7.78 (m, 13H, Ar-H); ^{13}C NMR (100 MHz DMSO-d_6) δ: 12.96, 76.70, 85.10, 95.59, 111.15, 114.41, 115.04, 120.10, 121.6, 125.15, 126.50, 129.11, 130.05, 131.45, 132.24, 137.58, 139.15, 140.52, 146.15, 15.03, 152.64, 157.55, 159.54, 169.02; LC-MS: 604.49 (M)+; Anal.Calc. For $C_{30}H_{18}BrN_7OS$; Elemental Analysis: C, 59.61; H, 3.00; N, 16.22; Observed, C, 59.29; H, 2.88; N, 15.98 %.

2-amino-6-((4-bromophenyl)thio)-4-(4-((4-cyano-3-methyl-1-phenyl-1H-pyra-zol-5-yl)oxy)-3-methoxyphenyl)pyridine-3,5-dicarbonitrile (4f)

White solid; yield: 79; mp 169-173 ºC; IR (KBr, *ν*max, cm^{-1}) = 3442 & 3351 (asym. & sym. stretching of –NH2); 2215 (-C≡N stretching), 1374 (-CH_3 str.), 1222 (C-O-C str.), 764 (C-S-C str.), 3005 (Ar-C-H); ^{1}H NMR (400 MHz, DMSO-d_6) δ = 2.39 (s, 3H,Pyrazole -CH_3), 3.94(s, 3H, -OCH_3) 5.67 (s, 2H, –NH_2), 7.15-7.72 (m, 13H, Ar-H); ^{13}C NMR (100 MHz DMSO-d_6) δ: 13.43, 56.41, 78.82, 87.54, 95.63, 111.45, 113.50, 114.52, 114.83, 121.59, 121.98, 123.03, 124.84, 126.09, 128.05, 129.25, 132.31, 132.60, 136.99, 137.30, 144.49, 151.23, 151.55, 153.61, 156.9, 159.30, 168.48; LC-MS: 634.51 (M)+ ; Anal.Calc. For $C_{31}H_{20}BrN_7O_2S$; Elemental Analysis: C, 58.68; H, 3.18; N, 15.45; Observed, C, 58.54; H, 2.97; N, 15.19 %.

2-amino-4-(4-((4-cyano-3-methyl-1-phenyl-1H-pyrazol-5-yl)oxy)phenyl)-6-((4-fluoro-phen -yl) thio)pyridine-3,5-dicarbonitrile (4g)

White solid; yield: 85; mp 192-195 ºC IR (KBr, *ν*max, cm^{-1}) = 3458 & 3342 (asym. & sym. stretching of –NH_2); 2211 (-C≡N stretching), 1375 (-CH_3 str.), 1213 (C-O-C str.), 738 (C-S-C str.), 3011 (Ar-C-H); ^{1}H NMR (400 MHz, DMSO-d_6) δ = 2.45 (s, 3H,Pyrazole -CH_3), 5.55 (s, 2H, –NH_2), 7.17-7.62 (m, 13H, Ar-H);^{13}C NMR (100 MHz DMSO-d_6) δ: 13.56, 81.63, 87.04, 95.47, 111.46, 114.71, 115.04, 118.28, 122.92, 123.40, 128.50, 129.29, 129.47, 129.63, 133.33, 136.91, 139.11, 147.49, 151.28, 151.55, 153.60, 156.91, 160.28, 167.47; LC-MS: 543.58 (M)+ ; Anal.Calc. For $C_{30}H_{18}FN_7OS$; Elemental Analysis: C, 66.29; H, 3.34; N, 18.04; Observed, C, 66.01; H, 3.21; N, 17.85 %.

2-amino-4-(4-((4-cyano-3-methyl-1-phenyl-1H-pyrazol-5-yl)oxy)-3-methoxy- phenyl)-6-((4-fluorophenyl) thio) pyridine-3,5-dicarbonitrile (4h)

White solid; yield: 84; mp 210-213 ºC; IR (KBr, *ν*max, cm^{-1}) = 3420 & 3347 (asym. & sym. stretching of –NH_2); 2208 (-C≡N stretching), 1376(-CH_3 str.), 1215 (C-O-C str.), 752 (C-S-C str.), 2979 (Ar-C-H); ^{1}H NMR (400 MHz, DMSO-d_6) δ = 2.34 (s, 3H,Pyrazole -CH_3), 3.90 (s, 3H, -OCH_3) 5.60 (s, 2H, –NH_2), 7.19-7.77 (m, 13H, Ar-H); ^{13}C NMR (100 MHz DMSO-d_6) δ: 14.03, 57.01, 78.93, 89.25, 98.05, 112.41, 113.99, 114.03, 115.74, 120.28, 121.09,

123.03, 124.83, 127.45, 129.25, 131.45, 132.81, 135.91, 136.54, 137.82, 143.67, 151.57, 152.51, 154.29, 156.82, 160.21, 168.60; LC-MS: 534.64 (M)+ ; Anal.Calc. For $C_{31}H_{20}FN_7O_2S$; Elemental Analysis: C, 64.91; H, 3.51; N, 17.09; Observed, C, 64.79; H, 3.47; N, 16.86 %.

2-amino-4-(4-((4-cyano-3-methyl-1-phenyl-1H-pyrazol-5-yl)oxy)phenyl)-6-((4-hydroxy phenyl)thio) pyridine-3,5-dicarbonitrile (4i)

White solid; yield: 81; mp 209-211 ºC; IR (KBr, *v*max, cm^{-1}) = 3438 & 3311 (asym. & sym. stretching of $–NH_2$); 2299 (-C≡N stretching), 1372 ($-CH_3$ str.), 1223 (C-O-C str.), 771 (C-S-C str.), 3019 (Ar-C-H); ^{1}H NMR (400 MHz, DMSO-d_6) δ = 2.40 (s, 3H, Pyrazole $-CH_3$), 5.66 (s, 2H, $–NH_2$), 7.16-7.72 (m, 13H, Ar-H), 9.24 (s, 1H, -OH); ^{13}C NMR (100 MHz DMSO-d_6) δ: 14.04, 82.61, 87.14, 94.97, 112.41, 114.76, 115.54, 119.59, 122.49, 125.41, 129.80, 129.99, 130.05, 130.48, 132.30, 137.51, 139.11, 148.19, 151.28, 152.05, 153.40, 157.51, 161.18, 167.59; LC-MS: 541.59 (M)+; Anal.Calc.: $C_{30}H_{19}N_7O_2S$ For; Elemental Analysis: C, 66.53; H, 3.54; N, 18.10; Observed, C, 66.45; H, 3.19; N, 17.94 %.

2-amino-4-(4-((4-cyano-3-methyl-1-phenyl-1H-pyrazol-5-yl)oxy)-3-methoxy- phenyl)-6-((4-hydroxyphenyl) thio)pyridine-3,5-dicarbonitrile (4j)

White solid; yield: 90 mp 222-225 ºC; IR (KBr, *v*max, cm^{-1}) = 3452 & 3337 (asym. & sym. stretching of $–NH_2$); 2208 (-C≡N stretching), 1371 ($-CH_3$ str.), 1224 (C-O-C str.), 765 (C-S-C str.), 2983 (Ar-C-H); ^{1}H NMR (400 MHz, DMSO-d_6) δ = 2.38 (s, 3H,Pyrazole $-CH_3$), 3.92 (s, 3H, $-OCH_3$) 5.64 (s, 2H, $–NH_2$), 7.18-7.73 (m, 13H, Ar-H), 9.25 (s, 1H, -OH); ^{13}C NMR (100 MHz DMSO-d_6) δ: 14.07, 56.49, 78.88, 87.40, 95.40, 112.45, 113.94, 114.54, 115.58, 120.19, 121.49, 123.53, 126.17, 129.13, 129.95, 130.48, 131.58, 135.43, 136.90, 137.01, 140.19, 152.53, 153.50, 153.80, 156.82, 159.58, 168.98; LC-MS: 571.62 (M)+ ; Anal.Calc. $C_{31}H_{21}N_7O_3S$: For; Elemental Analysis: C, 65.14; H, 3.70; N, 17.15; Observed, C, 64.99; H, 4.06; N, 17.01 %.

2-amino-4-(4-((4-cyano-3-methyl-1-phenyl-1H-pyrazol-5-yl)oxy)phenyl)-6-((4-methoxy phenyl)thio)pyridine-3,5-dicarbonitrile (4k)

White solid; yield: 83; mp 218-221 ºC; IR (KBr, *v*max, cm^{-1}) = 3421 & 3330 (asym. & sym. stretching of $–NH_2$); 2270 (-C≡N stretching), 1375 ($-CH_3$ str.), 1220 (C-O-C str.), 747 (C-S-C str.), 3005 (Ar-C-H); ^{1}H NMR (400 MHz, DMSO-d_6) δ = 2.35 (s, 3H,Pyrazole $-CH_3$), 3.91 (s, 3H, $–OCH_3$), 5.59 (s, 2H, $–NH_2$), 7.20-7.72 (m, 13H, Ar-H); ^{13}C NMR (100 MHz DMSO-d_6) δ: 13.58, 21.46, 81.59, 88.05, 969.21, 112.40, 113.54, 114.90, 115.54, 117.24, 121.42, 123.40, 128.80, 129.07, 130.46, 130.46, 131.64, 134.15, 136.9, 144.51, 152.59, 158.07, 155.49, 160.12,169.75; LC-MS: 555.62 (M)+ ; Anal.Calc. $C_{31}H_{21}N_7O_2S$: For; Elemental Analysis: C, 67.01; H, 3.81; N, 17.65; Observed, C, 66.91; H, 3.57; N, 17.43 %.

2-amino-4-(4-((4-cyano-3-methyl-1-phenyl-1H-pyrazol-5-yl)oxy)-3-methoxy- phenyl)-6-((4-methoxyphenyl) thio)pyridine-3,5-dicarbonitrile (4l)

White solid; yield: 79; mp 220-223 ºC; IR (KBr, *v*max, cm^{-1}) = 3410 & 3312 (asym. & sym. stretching of $–NH_2$); 2189 (-C≡N stretching), 1370 ($-CH_3$ str.), 1222 (C-O-C str.), 721 (C-S-C str.), 2999 (Ar-C-H); ^{1}H NMR (400 MHz, DMSO-d_6) δ = 2.34 (s, 3H,Pyrazole $-CH_3$), 3.88 (s, 3H, $–OCH_3$), 3.93 (s, 3H, $–OCH_3$), 5.57 (s, 2H, $–NH_2$), 7.19-7.70 (m, 13H, Ar-H); ^{13}C NMR (100 MHz DMSO-d6) δ: 14.05, 22.14, 57.25, 83.17, 86.50, 97.84, 111.10, 113.91, 114.95, 114.54, 118.88, 122.49, 125.05, 128.84, 129.79, 130.47, 131.91, 132.05, 133.43, 138.00, 141.51 142.97, 143.01, 151.40, 153.94, 159.04, 160.09, 167.48 ; LC-MS: 585.64 (M)+ ; Anal.Calc. $C_{32}H_{23}N_7O_3S$: For Elemental Analysis: C, 65.63; H, 3.96; N, 16.74; Observed, C, 65.48; H, 3.76; N, 16.57 %.

2-amino-4-(4-((4-cyano-3-methyl-1-phenyl-1H-pyrazol-5-yl)oxy)phenyl)-6- (phenyl thio) pyridine-3,5-dicarbonitrile (4m)

White solid; yield: 80; mp 219-222 ºC; IR (KBr, *v*max, cm^{-1}) = 3441 & 3324 (asym. & sym. stretching of $–NH_2$); 2212 (-C≡N stretching), 1375 ($-CH_3$ str.), 1212 (C-O-C str.), 749 (C-S-C str.), 3007 (Ar-C-H); ^{1}H NMR (400 MHz, DMSO-d_6) δ = 2.37 (s, 3H, Pyrazole $-CH_3$), 5.58 (s, 2H, $-NH_2$), 7.17-7.71 (m, 9H, -Ar-H), ^{13}C NMR (100 MHz DMSO-d6) δ: 13.60, 81.85, 87.15, 99.69, 112.58, 113.91, 116.81, 119.18, 122.92, 124.50, 129.45, 129.99, 130.47, 130.69, 132.13, 138.51, 139.01, 149.45, 150.27, 151.55, 154.40, 157.61, 162.08, 168.57; LC-MS: 525.59 (M)+ ; Anal.Calc. $C_{30}H_{19}N_7OS$: For; Elemental Analysis: C, 68.56; H, 3.64; N, 18.66; Observed, C, 65.48; H, 3.47; N, 18.48 %.

2-amino-4-(4-((4-cyano-3-methyl-1-phenyl-1H-pyrazol-5-yl)oxy)-3-methoxy- phenyl)-6-(phenylthio)pyridine-3,5-dicarbonitrile (4n)

White solid; yield: 90; mp 227-230 ºC; IR (KBr, *v*max, cm^{-1}) = 3430 & 3320 (asym. & sym. stretching of $–NH_2$); 2182 (C≡N stretching), 1374 ($-CH_3$ str.), 1219 (C-O-C str.), 751 (C-S-C str.), 2987 (Ar-C-H); ^{1}H NMR (400 MHz, DMSO-d_6) δ = 2.35 (s, 3H,Pyrazole $-CH_3$), 3.90 (s, 3H, $–OCH_3$), 5.61 (s, 2H, $–NH_2$), 7.15-7.73 (m, 13H, Ar-H); ^{13}C NMR (100 MHz DMSO-d_6) δ: 13.45, 56.42, 78.83, 87.26, 95.52, 111.46, 113.52, 114.71, 114.97, 121.61, 121.95, 123.03, 127.02, 125.06, 129.26, 129.39, 130.02, 132.46, 135.79, 136.98, 144.41, 151.19, 151.55,

153.63, 156.83, 159.31, 169.26,; LC-MS: 555.62 (M)+; Anal.Calc. $C_{31}H_{21}N_7O_2S$: For; Elemental Analysis: C, 67.01; H, 3.81; N, 17.65; Observed, C, 66.66; H, 3.64; N, 17.48 %.

CONCLUSION:

A highly sustainable and efficient multicomponent synthesis of novel penta- substituted pyridine bearing a pyraozle scaffolds under microwave irradiation is reported. The notable features of this methodology are eco-friendly reaction conditions, no side product, the avoidance of toxic catalysts and easy purification. Amongst the all novel tested compounds **4a**, **4g and 4k** showed more potent antimalarial activities against *P. falciparum* Compounds **4a**, **4b**, **4g and 4m** have shown brilliant anti tuberculosis activity against *Mycobacterium tuberculosis* H37Rv *strains* as well as very poor binding energy in molecule docking study as compared to isoniazide and ethambutol. Compounds 4a and 4g are more potent against both activity *viz.* antimalarial and antituberculosis.

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