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Journal Name

ARTICLE

Identification and development of 1-((1-(substituted)-1*H*-1,2,3-triazol-4-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carboxamides as *Mycobacterium tuberculosis* Pantothenate synthetase inhibitors

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The purpose of this study was to prepare various 1-((1-(substituted)-1*H*-1,2,3-triazol-4-yl)methyl)-*N*,3-diphenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carboxamides using click chemistry. The synthesized compounds were characterized using various analytical techniques like NMR, Mass, Elemental analysis and screened for *in vitro* antitubercular activity against *Mycobacterium tuberculosis* (MTB) *H37Rv* strain and two 'wild' strains *Spec. 210* and *Spec. 192* using a classical test-tube method of successive dilution in Youmans' modification of the Proskauer and Beck liquid medium and were evaluated for MTB pantothenate synthetase (PS) inhibition study as well. The final analogues exhibited minimum inhibitory concentration ranging from 24.72 - >200 µM. Among the compounds, 1-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methyl)-N,3-diphenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carboxamide (**7d**), was found to be the most active compound with IC_{50} 1.01±0.32 µM against MTB PS; it inhibited MTB with MIC 24.72 µM and it was non-cytotoxic at 50 µM in RAW 267 cell line. Further, **7d** was docked to crystal structure of MTB PS to know its binding interactionpattern.

Introduction

Tuberculosis (TB) is contagious and airborne. It has major impact on the society due to the emergence of multi-drug resistant strains of *Mycobacterium tuberculosis* (MTB). In addition, multidrug-resistant *Mycobacterium tuberculosis* (MDR-TB) and especially, extensively drug resistant TB (XDR-TB) are now increasing in incidence in many areas around the world.[1] Around 9.6 million people fell ill with TB in 2014, including 1.2 million cases among people living with HIV. Globally in 2014, an estimated 0.48 million developed MDR-TB and there were an estimated 0.19 million deaths from MDR-TB.[1] Therefore, the development of new therapeutic drugs targeting MDR-TB and XDR-TB is important due to lack of effective drug treatments.[2]

The treatment of TB infections is generally carried out through the use of a combination of drugs including isoniazid (INH), rifampicin (RMP), pyrazinamide, ethambutol (ETB) and streptomycin.[3-6]

In order to overcome the issue of resistance there is a challenging need to develop new drugs using novel targets of MTB.

The biosynthetic pathway of pantothenate involves four steps catalyzed by *panB, panC, panD,* and *panE* genes.[7] The last step of pantothenate biosynthesis *viz.,* the ATP-dependent condensation of *D*-pantoate and *β*-alanine to form pantothenate is catalyzed by *PanC.* Pantothenate is essential for several processes such as cell signaling, fatty acid metabolism, and synthesis of non-ribosomal peptides and polyketides.[8] The biosynthesis of pantothenate is essential for the growth of MTB. The pantothenate biosynthesis pathway is a latent drug target and hence is important for determined growth and virulence of MTB and PS is very good target for developing new therapeutics to treat TB.[9, 10]

Till date many MTB PS inhibitors are reported (**Figure 1**), which include *5-tert*-butyl-*N*-pyrazol-4-yl-4,5,6,7-tetrahydrobenzo[*d*]isoxazole-3-carboxamide derivatives (**a**),[8] 3-phenyl-4,5,6,7-tetrahydro-1*H*-pyrazolo[4,3-*c*]pyridine derivatives (**b**),[11] 2,6-disubstituted 4,5,6,7-tetrahdrothieno[2,3-*c*]pyridine-3-carboxamide derivatives (**c**),[12] nafronyl oxalate (**d**),[13] *N*'-(1-naphthoyl)-2-methylimidazo[1,2-*a*]pyridine-3-carbohydrazide (**e**),[14] and imidazo[2,1-*b*]thiazole and benzo[*d*]imidazo[2,1-*b*]thiazole derivatives (**f**).[15]

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[d] † Electronic Supplementary Information (ESI) available: photophysical data, copies of NMR spectra for products and Single Crystal X-ray data See DOI: 10.1039/x0xx00000x

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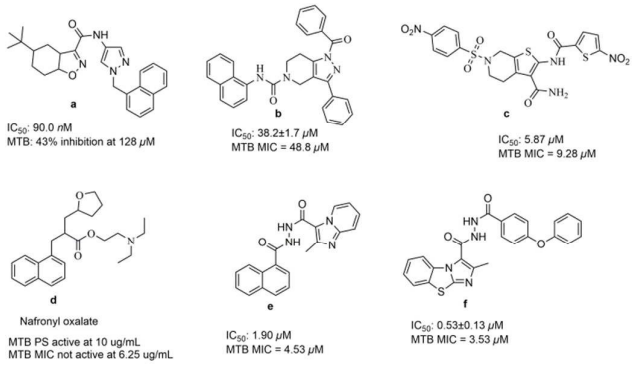

Figure 1 Structures of literature reported MTB PS inhibitors.

Pyrazole derivatives are known to contain broad spectrum of pharmacological properties such as antifungal,[16] antidiabetic,[17] antitumor,[18] antibacterial.[19, 20] Pyrazoles play an essential role in biologically active compounds and therefore signify an interesting template for medicinal chemistry.[21] Mamolo *et al.,* reported 5-aryl-1-isonicotinoyl-3-(pyridin-2-yl)-4,5-dihydro-1*H*-pyrazole derivatives which exhibited an interesting *in vitro* antimycobacterial activity against MTB, with minimum inhibitory concentration (MIC) values ranging from 8 to 16 μg/mL.[22] Ravindra *et al.,* reported 3-(4-chlorophenyl)-4-substituted pyrazole derivatives with MIC values ranging from 0.35 to 3.15 μg/mL against MTB *H37Rv*.[23] Chovatia *et al.,* reported 1-acetyl-3,5-diphenyl-4,5-dihydro-(1*H*)-pyrazole derivatives which were screened against MTB *H37Rv*.[24] Series of *N*-phenyl-3-(4-fluorophenyl)-4-substituted pyrazole derivatives exhibited significant antimycobacterial activity with IC_{50} values ranging from 0.47 to 118.0 μM against MTB *H37Rv*.[25]

Triazoles have several medicinal applications.[26] It is quite obvious that, the favorable properties of 1,2,3-triazole ring like moderate dipole character, hydrogen bonding capability, rigidity and stability under *in vivo* conditions are responsible for their enhanced biological activities.[27, 28] For instance, a variety of 1*H*-1,2,3-triazole compounds have been known to exhibit antitubercular activity (**g-j**) (**Figure 2**).[29-33]

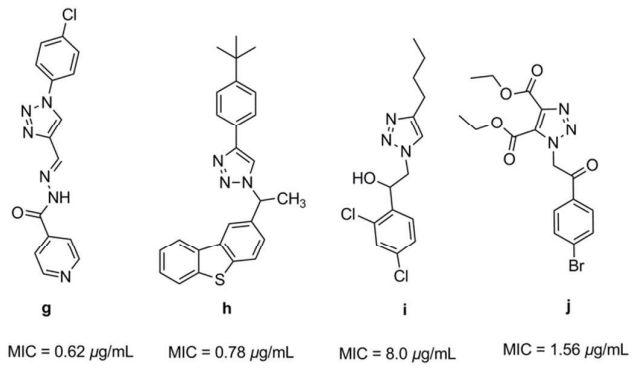

Figure 2 Heterocyclic hybrids of [1,2,3] triazole with antitubercular activity.

In the present work, we designed and developed novel MTB PS inhibitors by molecular hybridization approach using the two known antimycobacterial agents.

Design and chemistry

The molecular hybridization approach involves rational design of new ligands or the recognition of pharmacophoric sub-units in the molecular structure of two or more known bioactive derivatives which, through the adequate combination of these subunits, lead to the design of new hybrid architectures that maintain preselected characteristics of the original templates. In this work, we designed novel MTB inhibitors by hybridizing reported MTB PS inhibitor (1-benzoyl-*N*-(4-nitrophenyl)-3-phenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carboxamide) (**AA**)[12] and 3-(4-((1-(4-bromo-3-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)benzo[*d*]isoxazole (**BB**),[34] anticipating a new lead in the development of novel MTB PS inhibitors with potential MTB MIC (**Figure 3**). **BB** is our most active compound from our previous work and when screened for MTB PS it exhibited *panC* IC_{50} 58.4 μM.

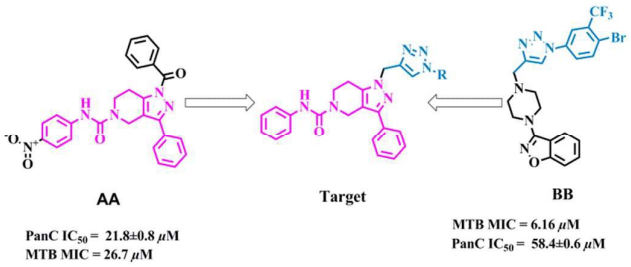

Figure 3 Design strategy of the title compounds.

Results and Discussion

The designed molecules were synthesized in six steps (**Scheme 1**). Initially we prepared 1,3-dicarbonyl intermediate (**2**) using 1-Boc-4-piperidone (**1**), morpholine, *p*-toluenesulfonic acid (catalytic) and benzoyl chloride (stark-enamine reaction conditions). Treatment of **2** with hydrazine hydrate yielded pyrazole ring (**3**).[35] Compound **3** on reacting with propargyl bromide in the presence of CS_2CO_3 formed *N*-alkyl product (**4**). Compound **4** was then deprotected using trifluoroacetic acid to yield compound (**5**). With weak base TEA, more nucleophilic amine of aliphatic ring reacted with phenylisocyanate yielding urea derivative (**6**). The free acetylene group was converted to various 1*H*-1,2,3-triazoles using different aromatic azides via click chemistry method.[33] The purity of compounds synthesized was checked by LC-MS and elemental analyses. Structures of the compounds were confirmed by spectral data. In ^{1}H NMR and ^{13}C NMR, the signals of the respective protons and carbons were verified on the basis of their chemical shifts, multiplicities, and coupling constants. The results of elemental analysis were within ± 0.05 of the theoretical values.

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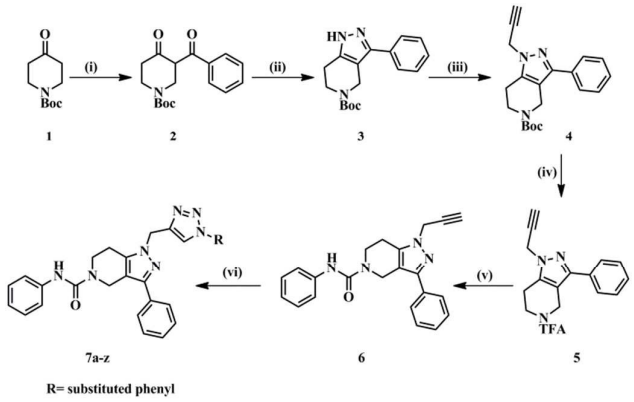

Scheme 1 Synthetic protocol of titled compounds.

Reagents and conditions: (i) (a) Morpholine (1.0 eq), PTSA (0.01 eq), toluene, 100 °C, 16 h; (b) Benzoyl Chloride (1.1 eq), TEA (2.0 eq), DCM, 0 °C - rt, 4 h. (ii) N_2H_4 (1.0 eq), EtOH, 0 °C - rt, 4 h (iii) Propargyl bromide (80% in toluene) (1.2 eq), Cs_2CO_3 (1.5 eq), DMF, rt, 16 h. (iv) CF_3COOH, DCM, rt, 16 h. (v) Phenyl isocyanate (1.3 eq), TEA (3.0 eq), DMF, rt, 4 h. (vi) Substituted aromatic azides, $CuSO_4.5H_2O$ (10 mol %), Sodium ascorbate (10 mol %), H_2O:t-BuOH (1:2), rt, 4 h.

In-vitro MTB screening

All the synthesized compounds were tested for their capacity to inhibit the growth of MTB. In assay three different *M. tuberculosis* strains were used. One of them was reference strain *M. tuberculosis H37Rv ATTC 25618* and the others were 'wild' strains isolated from tuberculosis patients.[34, 36] MTB strain *spec. 210* was resistant to *p*-aminosalicylic acid (PAS), INH, ETB and RMP and another (*Spec. 192*) fully sensitive to the administrated tuberculostatics.[37] In this study three different strains were used for screening as we wanted to know the kind of activity synthesized compounds showed against the reference strain as well as against the strains isolated from TB patients. In this study the influence of the compound on the growth of mycobacteria at a certain concentration: 3.1, 6.2, 12.5, 25, 50 and 100 µg/mL were evaluated INH was used as reference drug. The *in vitro* antimycobacterial results of title compounds are arranged in **Table 1** as MIC (µM) and the activity ranged from 24.72 - >200 µM.

Table 1 Result of Antimycobacterial screening of title compounds

Entry	R	MIC (µM) against MTB *H37Rv*	MIC (µM) against MTB *Spec.* 192	MIC (µM) against MTB *Spec.* 210
7a	Phenyl	105.14	105.14	105.14
7b	4-Methylphenyl	25.53	25.53	51.06
7c	4-Ethylphenyl	49.64	49.64	49.64
7d	4-Methoxyphenyl	24.72	24.72	24.72
7e	4-Fluorophenyl	>200	>200	>200
7f	4-Chlorophenyl	196.08	196.08	196.08
7g	4-Bromophenyl	90.18	90.18	90.18
7h	4-Iodophenyl	166.26	166.26	166.26
7i	4-Nitrophenyl	192.10	192.10	192.10
7j	4-Trifluoromethylphenyl	91.98	91.98	91.98
7k	2-Chlorophenyl	196.08	196.08	196.08
7l	2-Fluorophenyl	>200	>200	>200
7m	2-Bromophenyl	180.36	180.36	180.36
7n	2-Iodophenyl	166.26	166.26	166.26
7o	2-Nitrophenyl	192.10	192.10	192.10
7p	3-Chlorophenyl	98.04	98.04	98.04
7q	3-Methoxyphenyl	98.89	98.89	98.89
7r	2,4-dichlorophenyl	183.67	183.67	183.67
7s	3,4-dichlorophenyl	183.67	183.67	183.67
7t	3,5-dichlorophenyl	91.83	91.83	91.83
7u	3-Chloro-4-Fluorophenyl	189.40	189.40	189.40
7v	3,4-difluorophenyl	195.49	195.49	195.49
7w	3,4-dimethoxyphenyl	186.70	186.70	186.70
7x	4-bromo-3 trifluoromethylphenyl	160.65	160.65	160.65
7y	3,4-dimethylphenyl	198.57	198.57	198.57
7z	benzo[*d*][1,3]dioxole	192.47	192.47	192.47
INH	-	≤22.60	≤22.60	≤91.14

Among the twenty six compounds screened, eight compounds (**7b, 7c, 7d, 7g, 7j, 7p, 7q** and **7t**) showed activity against MTB with MIC <100 µM. Three compounds (**7b**, **7c,** and **7d**) inhibited MTB with MIC <50 µM. Compound **7d**, (1-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methyl)-*N*,3-diphenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)carboxamide) was found to be the most active compound with *in vitro* MIC 24.72 µM. In addition, compounds **7b**, **7c** and **7d** with MIC <50 µM were further subjected to toxicity studies on normal cell line to analyze the selectivity profile.

Amongst synthesized derivatives, electron donating group containing substituent play major impact in exhibiting anti-TB activity. Structural activity relationship studies are explained based

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on activity of compound **7a**. Structural changes at 4th position alter the activity. Compound **7a** was inhibiting 99% growth of MTB *H37Rv* strain at 105.14 µM. In this series, introduction of electron donating groups at 4th position on phenyl ring increased the activity. Introduction of electron donating ethyl group (**7c**) increased the activity by two fold with MIC 49.64 µM. Presence of electron donating methyl group (**7b**) increased the activity by four folds with MIC 25.53 µM. With introduction of methoxy group (**7d**) activity increased by four folds (MIC 24.72 µM), with same methoxy group at *meta* position on phenyl ring (**7q**, MIC 98.89 µM) activity remained unaltered compared to compound **7a**. Presence of electron withdrawing groups *viz.,* **F, Cl, Br** and **I** resulted in decrease in activity compared to compounds **7b, 7c,** and **7d**. Even, presence of electron withdrawing groups at *ortho* or *meta* position decreased the activity. Introduction of electron donating methoxy group at *meta* and *para* position (**7w**, MIC 186.70 µM) yielded decrease in activity by two folds compared to compound **7a**. In conclusion, presence of electron donating methoxy group at *para* position on phenyl ring enhanced the activity. Amongst the synthesized derivatives, **7d** emerged to be the most active compound.

Pantothenate synthetase enzyme inhibition studies

PS enzyme inhibition studies are carried on synthesized compounds by estimating the amount of NAD^+ produced.[7, 15] The NAD^+ produced can be examined spectrophotometrically at 340 nm. In the initial screening at 50 µM, all compounds exhibited more than 50% inhibition against MTB PS and their IC_{50}'s were further determined. Most of the compounds showed good IC_{50} ranging from 0.91±0.32 to 8.97±0.05 µM (**Table 2**). Seven compounds (**7b, 7d, 7h, 7p, 7r, 7s** and **7v**) inhibited MTB PS with IC_{50} <2.00 µM. Compounds **7d** and **7s** emerged as the most active compounds with IC_{50} 1.01±0.32 and 0.91±0.32 µM respectively.

Table 2 Docking scores and MTB PS assay

Compound	*In-silico*		*In-vitro*
Entry	XP GScore	Glide energy	MTB *PanC* IC_{50} µM
Co-LIGAND	-8.32	-78.66	--
7a	-7.19	-71.33	2.13±0.12
7b	-5.69	-58.61	1.54±0.22
7c	-6.44	-64.89	2.56±0.04
7d	-8.19	-66.25	1.01±0.32
7e	-6.61	-67.18	3.41±0.08
7f	-3.25	-69.57	6.36±0.12
7g	-5.24	-65.40	2.46±0.07
7h	-7.61	-73.33	1.04±0.55
7i	-3.80	-62.50	8.97±0.05
7j	-4.70	-58.79	5.04±0.54
7k	-4.62	-65.89	2.78±0.07
7l	-4.46	-61.60	5.93±0.64
7m	-6.31	-71.76	3.54±0.55
7n	-5.24	-60.42	3.92±0.19
7o	-6.33	-72.18	4.94±0.03
7p	-7.69	-70.79	1.14±0.19
7q	-4.25	-65.23	7.78±0.56
7r	-6.07	-67.82	1.29±0.14
7s	-7.83	-69.26	0.91±0.32
7t	-5.99	-63.35	4.76±0.02
7u	-5.37	-63.08	6.43±0.12
7v	-7.37	-64.55	1.56±0.11
7w	-4.96	-64.16	8.21±0.03
7x	-4.44	-57.95	6.54±0.21
7y	-6.60	-56.87	6.91±0.07
7z	-4.84	-73.86	8.39±0.14

Docking study

All the final compounds were docked into the crystal structure of MTB PS protein (PDB ID: 3IUB) to know the exact binding pattern with the receptor. Validation of docking protocol revealed that, the value of RMSD obtained between experimental binding mode of co-crystallized ligand (as in X-ray) and its re-docked pose (**Figure 4**) was found to be 0.76, which suggested that, docking procedure could be relied on for further docking studies.

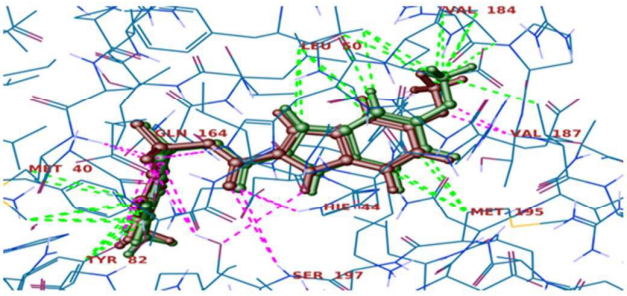

Figure 4 Superimposed view of co-crystallized ligand (green) with its X-ray pose (red) in 3IUB

Further, in the docking studies, molecules exhibited good binding energy in the range of -3.25 to -8.19 kcal/mol and exhibited good fitness with the MTB PS protein. Several compounds displayed hydrogen bonding interaction with HIE47, HIE44, Met40, Ser196, Tyr82 and Ser197, amino acid residues. One of the most active ligand, 1-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methyl)-*N*,3-diphenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)carboxamide (**7d**) with IC_{50} 1.01±0.32 µM showed docking score of -8.19 kcal/mol. The active site in the hydrophobic pocket is within the vicinity of Leu146, Val187, Val142, Met195, Ala42, Leu50, Pro38, Ile168 and Met40 some polar amino acid residues HIE47, Ser196, Ser197 and Thr82 respectively. The ligand also exhibited hydrogen bonding interaction with Met40, Ser197, Val187 residues.[15] The binding pattern of **7d** with MTB PS is shown in **Figure 5**.

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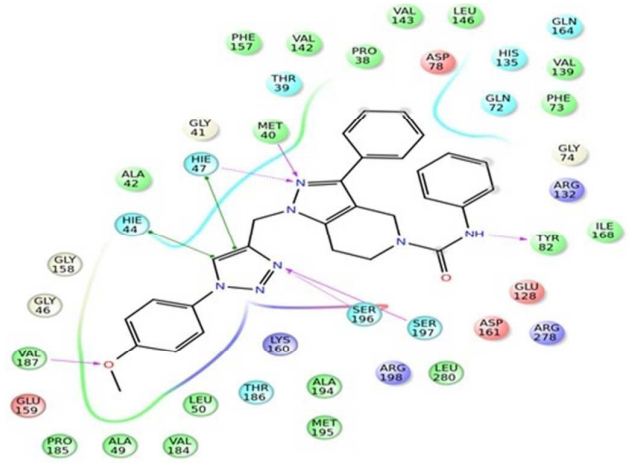

Figure 5 Docked pose of compound **7d** inside the 3IUB, showing two-dimensional interactive diagram

In vitro cytotoxicity studies

The active compounds **7b, 7c** and **7d** were evaluated for Promega Cell Titer 96 non-radioactive cell proliferation assay to analyze the selectivity profile against mouse macrophage (RAW264.7) cell lines.[38] The IC_{50} values and selectivity index (SI) values are tabulated in **Table 3**. The most active compound (**7d**) showed SI value 13.76. The results imply that the compounds are suitable for further investigation in TB.

Table 3 Cytotoxicity results of the active compounds

Entry	MIC (μM) in MTB *H37Rv*	*IC_{50} approximation	[a]SI values IC_{50}/MIC
7b	25.53	335.19	13.12
7c	49.64	370.12	7.45
7d	24.72	340.15	13.76

[a] Selectivity index; * units in μM

Single Crystal X-ray Crystallographic Structure of Compound 7g:

The suitable crystals of the compound **7g** for X-ray crystallographic study were grown from methanol solution. The single crystal X-ray diffraction measurement of the molecule ($C_{28}H_{24}N_7OBr.CH_3OH$) was on Rigaku XtaLAB P200 diffract meter using graphite monochromated Mo-Kα radiation (λ = 0.71073 Å) on 0.1mm x 0.1mm x 0.1mm pale yellow crystal. Data were collected and processed using CrysAlisPro (Rigaku Oxford Diffraction). The data were collected at a temperature of -180 ± 1 °C to a maximum 2θ value of 58.2°. Of the 19673 reflections collected, 6164 were unique (Rint = 0.0791) and equivalent reflections were merged. The diffraction data were refined and structure was solved using Crystal Structure 4.2.2 software program. The structure was solved by direct methods and expanded using Fourier techniques. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were refined using the riding model. The compound crystallized into a triclinic crystal system with P-1 space group. In a single unit cell four partially occupying molecules along with two methanol solvent of crystallization are observed with Z=2. The basic crystallographic data are shown in **Table 4**. The molecular structure of the compound with methanol solvent of crystallization is given as an ORTEP diagram in **Figure 6**. The part of the molecule containing *p*-bromophenyl ring is directly attached to triazole ring nitrogen. These two rings are not coplanar; the phenyl ring plane is deviated from the triazole plane by around 30°. The torsional angle between these two rings with selected bonds C5-C4-N1-N2 = 30.90 and C3-C4-N1-C7 = 31.71 degrees. The deviation is comparatively less in the other part of the molecule. Pyrazole ring plane with respect to the attached phenyl ring plane deviated by around 7.5°, the corresponding dihedral angle for the selected four bonds are N5-C12-C13-C18 = 7.54, C11-C12-C13-C14 = 7.34 degrees. Crystallographic data for the compound **7g** is deposited to the Cambridge Crystallographic Data Center and corresponding deposition number is **CCDC 1500428.**

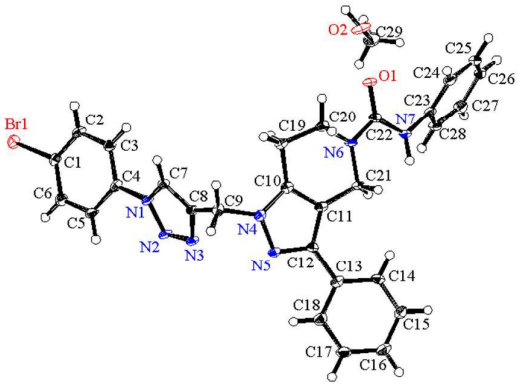

Figure 6 ORTEP diagram showing the X-ray crystal structure of the compound **7g** with a methanol solvent of crystallization.

Table 4 Crystal data and structure refinement for **7g**

Empirical Formula	$C_{28}H_{24}BrN_7O.\ CH_3OH$
Formula Weight	586.49
Crystal Color, Habit	Light yellow
Crystal Dimensions	0.1 mm x 0.1 mm x 0.1 mm
Crystal System	Triclinic
Lattice Type	Primitive
Lattice Parameters	a = 7.8845(3) A°
	b = 11.9643(4) A°
	c = 14.9423(4) A°
	α = 102.268(2) A°
	β = 101.916(2) A°
	γ = 95.003(3) A°
	δ = 1334.95(8) A°^{3}
Space Group	P-1 (#2)
Z value	2
D_{calc}	1.459 g/cm^3
F_{000}	604.00
μ(MoKα)	15.855 cm^{-1}
Radiation	Mo-Kα(λ = 0.71073 A°)
Radiation monochromator	Graphite
Voltage, Current	50kV, 40mA

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Temperature	-180.0 °C
Maximum 2θ	58.2°
Number of measured reflections	19673°
Number of Unique reflections	6164 (R_{int} = 0.0791)
Number of parameters	380
Goodness-of-fit on F^2	1.00
$\Delta\rho_{max,mix}(e^-/A^{\circ 3})$	1.89, -0.78
Reflection/Parameter Ratio	16.22
Residuals: R1 (I>2.00σ(I))	0.0479
Residuals: R (All reflections)	0.0538
Residuals: wR2 (All reflections)	0.1931
Crystal refinement	CrystalStructure 4.2.2

Conclusions

In this work, we designed novel 1-((1-(substituted)-1*H*-1,2,3-triazol-4-yl)methyl)-*N*,3-diphenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carboxamide derivatives by molecular hybridization approach using reported MTB PS inhibitor and substituted 1*H*-1,2,3-triazole antitubercular compounds. Twenty six compounds were synthesized and well characterized. One of the compound **7d** showed better MTB PS inhibition and MTB MIC than one of the lead compound **AA**. Thus, this 1-((1-(substituted)-1*H*-1,2,3-triazole scaffold could be further optimized to develop MTB PS specific agents. In conclusion, it has been shown that, the potency and low cytotoxicity of the title compounds make them suitable leads for synthesizing new compounds with better anti-tubercular activity.

Experimental

Materials and methods

Chemicals and solvents were procured from commercial source. The solvents and reagents were of LR grade and if necessary purified before use. Thin-layer chromatography (TLC) was carried out on aluminium-supported silica gel plates (Merck 60 F254) with visualization of components by UV light (254 nm). Column chromatography was carried out on silica gel (Merck 100-200 mesh). ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 MHz and 101 MHz respectively using a Bruker AV 400 spectrometer (Bruker CO., Switzerland) in $CDCl_3$ and DMSO-d_6 solution with tetramethylsilane as the internal standard and chemical shift values (δ) were given in ppm. Melting points were determined on an electro thermal melting point apparatus (Stuart-SMP30) in open capillary tubes and are uncorrected. Elemental analyses were performed by Elementar Analysensysteme GmbH vario MICRO cube CHN Analyzer. Mass spectra (ESI-MS) were recorded on Schimadzu MS/ESI mass spectrometer. Purity of all tested compounds were determined by LC-MS/MS on Schimadzu and was greater than 95%.

Chemistry

tert-butyl 3-benzoyl-4-oxopiperidine-1-carboxylate (2)

In a two neck 100 mL round-bottom flask equipped with a Dean-stark trap, a reflux condenser and an internal thermocouple, compound **1** (5.0 g, 23.15 mmol), toluene (50 mL), morpholine (2.1 mL, 23.15 mmol), and *p*-toluenesulfonic acid (catalytic) were added sequentially. The reaction mixture was refluxed under N_2 atmosphere for 16 h. The solvent was evaporated and the crude reaction mixture was dissolved in DCM (40 mL) and then triethylamine (5.35 mL, 37.65 mmol) was added at 0 °C, under N_2, benzoyl chloride (2.9 mL, 25.1 mmol) was added over 10 min, the ice bath was then removed and the reaction solution was stirred at room temperature for 4 h. Once completion of the reaction, as indicated by TLC, the reaction was quenched with $NaHCO_3$ solution and extracted with DCM. The organic layers were collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The resultant crude product was purified by column chromatography [ethyl acetate / hexane (10 - 15%)] to get the 1,3-dicarbonyl compound **2** (7.0 g, 92%) as a colorless liquid. ESI-MS found 304 $(M+H)^+$.

tert-butyl 3-phenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxylate (3)

A stirred solution of *tert*-butyl 3-benzoyl-4-oxopiperidine-1-carboxylate (**2**) (7.6 g, 25.05 mmol) in ethanol was cooled to 0 °C and hydrazine hydrate (0.8 mL, 25.05 mmol) was added and stirred for 4h. Once completion of the reaction, as indicated by TLC the reaction mixture was concentrated under reduced pressure and the crude residue was purified by column chromatography to get compound **3** (6.9 g, 92%) as an off-white solid. ESI-MS found 300 $(M+H)^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.48 (s, 1H), 7.58-7.19 (m, 5H), 4.82 (s, 2H), 3.91 (t, *J* = 7.6 Hz, 2H), 2.83 (t, *J* = 7.8 Hz, 2H), 1.34 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 163.54, 146.99, 143.56, 136.64, 132.75, 129.31, 127.39, 117.45, 81.67, 43.91, 36.89, 31.49, 27.79; Anal. calcd for $C_{17}H_{21}N_3O_2$: (%) C, 68.20; H, 7.07; N, 14.04, Found: C, 68.24; H, 7.09; N, 14.13.

tert-butyl-3-phenyl-1-(prop-2-yn-1-yl)-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxylate (4)

A solution of *tert*-butyl 3-phenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carboxylate (**3**) (5.0 g, 16.70 mmol) in DMF was cooled to 0 °C and Cs_2CO_3 (8.16 g, 25.05 mmol) and propargyl bromide (80% in toluene) (1.64 mL, 21.17 mmol) were added and allowed to reach room temperature and stirred for 16 h. Once completion of the reaction, as indicated by TLC, the reaction was quenched with cold water and extracted with diethyl ether. The organic layers were collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude product was purified by column chromatography [ethyl acetate / hexane (20 - 30%)] to get the compound **4** (4.6 g, 83%) as a semisolid. ESI-MS found 338.15 $(M+H)^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60-7.23 (m, 5H), 4.80 (s, 2H), 4.56 (s, 2H), 3.92 (t, *J* = 7.4 Hz, 2H), 2.81 (t, *J* = 7.6 Hz, 2H), 2.72 (s, 2H), 1.31 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 163.57, 147.64, 143.65, 136.76, 132.87, 129.38, 127.40, 117.51, 81.7, 77.96, 69.05, 43.91, 42.07, 36.77, 31.59, 27.90; Anal. calcd for $C_{20}H_{23}N_3O_2$: (%) C, 69.79; H, 6.96; N, 13.20, Found: C, 69.80; H, 7.01; N, 13.23.

3-phenyl-1-(prop-2-yn-1-yl)-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridine (5)

A solution of *tert*-butyl 3-phenyl-1-(prop-2-yn-1-yl)-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carboxylate (**4**) (4.0 g, 11.85 mmol) in CH_2Cl_2 was cooled to 0 °C and CF_3COOH (4.5 mL, 59.27 mmol) was

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added drop wise and stirred at room temperature for 16 h. The reaction mixture was concentrated under reduced pressure and the crude residue was washed with hexane and diethyl ether to get compound **5** (2.6 g, 92%) as an off-white solid. ESI-MS showed 238.10 $(M+H)^+$.

N,3-diphenyl-1-(prop-2-yn-1-yl)-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide (6)

Phenylisocyanate (1.3 mL, 10.95 mmol), was added to the stirred solution of Compound **5** (2.0 g, 8.42 mmol) and Et_3N (3.5 mL, 25.26 mmol) in DMF at 0 °C under N_2 atm, and stirred at room temperature for 4 h. Once completion of the reaction, as indicated by TLC, the reaction was quenched with cold water then solid was filtrated and washed with water and hexane to get the key intermediate **6** (2.7 g, 90%) as an off-white solid. ESI-MS showed 357.15 $(M+H)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.74 (s, 1H), 7.88 (d, J = 8.6 Hz, 2H), 7.78 (d, J = 8.8 Hz, 2H), 7.67 (d, J = 7.6 Hz, 2H), 7.72 – 7.64 (m, 2H), 7.28 – 7.18 (m, 2H), 5.47 (s, 2H), 4.71 (s, 2H), 3.86 (t, J = 7.6 Hz, 2H), 2.90 (t, J = 7.5 Hz, 2H), 2.69 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) 164.58, 155.57, 143.57, 139.51, 136.69, 132.85, 129.38, 128.92, 128.12, 127.40, 121.16, 117.52, 81.73, 77.94, 69.0, 44.01, 42.12, 23.94; Anal. calcd for $C_{22}H_{20}N_4O$: (%) C, 74.14; H, 5.66; N, 15.72, Found: C, 74.16; H, 5.67; N, 15.73.

N,3-diphenyl-1-((1-phenyl-1H-1,2,3-triazol-4-yl)methyl)-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide (7a-z)

A solution of *N*,3-diphenyl-1-(prop-2-yn-1-yl)-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carboxamide (**6**) (0.20 g, 1.0 equiv.) is reacted with substituted phenyl azides (1.2 equiv.) in the presence of sodium ascorbate (0.01 equiv.), $CuSO_4.5H_2O$ (0.02 equiv.) and *t*-BuOH: H_2O (2:1), at rt for 4 h. Once completion of the reaction, as indicated by TLC, the reaction was quenched with cold water and extracted with DCM. The DCM layers were collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude product was purified by column chromatography [MeOH / DCM (1 -3%)] to yield the title compounds **7a-z**.

***N,3-diphenyl-1-((1-phenyl-1H-1,2,3-triazol-4-yl)methyl)-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide* (7a)**

Off white solid (83%); m.p. 221-223 °C; IR (KBr) υ_{max} / cm^{-1} 3490, 3021, 2843, 1650, 1410, 1340, 1060. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.88 (s, 1H), 8.75 (s, 1H), 7.94 – 7.86 (m, 2H), 7.69 – 7.66 (m, 2H), 7.58 (dd, J = 8.6, 7.1 Hz, 2H), 7.50 – 7.42 (m, 5H), 7.38 – 7.28 (m, 1H), 7.28 – 7.15 (m, 2H), 6.94 (t, J = 7.2, 1.2 Hz, 1H), 5.49 (s, 2H), 4.72 (s, 2H), 3.83 (t, J = 5.8 Hz, 2.98 (t, J = 5.8 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 155.67, 145.55, 144.46, 140.85, 139.15, 136.98, 133.97, 130.32, 129.20, 129.14, 128.74, 127.78, 126.51, 122.38, 122.39, 120.61, 120.48, 112.00, 44.40, 42.17, 41.16, 40.59, 22.33. EI-MS m/z 476.20 $(M+H)^+$; Anal. calcd for $C_{28}H_{25}N_7O$: (%) C, 70.72; H, 5.30; N, 20.62; Found: C, 70.74; H, 5.31; N, 20.63.

***N,3-diphenyl-1-((1-(p-tolyl)-1H-1,2,3-triazol-4-yl)methyl)-1,4,6,7-tetrahydro-5H-pyrazolo[4,3-c]pyridine-5-carboxamide* (7b)**

Light yellow solid (87%); m.p. 204-206 °C; (KBr) υ_{max} / cm^{-1} 3455, 3025, 2867, 1645, 1420, 1348, 1062. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.81 (s, 1H), 8.75 (s, 1H), 7.77 (d, J = 8.5 Hz, 2H), 7.67 (d, J = 7.2 Hz, 2H), 7.48 – 7.42 (m, 4H), 7.40 – 7.30 (m, 3H), 7.26 – 7.20 (m, 2H), 6.94 (t, J = 6.8 Hz, 1H), 5.47 (s, 2H), 4.71 (s, 2H), 3.83 (t, J = 5.6 Hz, 2H), 2.97 (t, J = 5.3 Hz, 2H), 2.36 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 155.68, 145.54, 144.42, 140.91, 139.16, 138.77, 134.74, 133.95, 130.71, 129.21, 128.82, 127.78, 126.48, 122.38, 122.24, 120.49, 120.47, 111.99, 44.46, 42.18, 41.66, 22.29, 21.06. EI-MS m/z 490.23 $(M+H)^+$; Anal. calcd for $C_{28}H_{25}N_7O$: (%) C, 71.15; H, 5.56; N, 20.03; Found: C, 71.16; H, 5.58; N, 20.06.

***1-((1-(4-ethylphenyl)-1H-1,2,3-triazol-4-yl)methyl)-N,3-diphenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide* (7c)**

Off white solid (89%); m.p. 128-130 °C; IR (KBr) υ_{max} / cm^{-1} 3478, 3031, 2913, 1635, 1422, 1341, 1056. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.82 (s, 1H), 8.75 (s, 1H), 7.78 (d, J = 8.4 Hz, 2H), 7.68 (d, J = 7.3 Hz, 2H), 7.48 – 7.42 (m, 4H), 7.40 – 7.30 (m, 3H), 7.26 – 7.20 (m, 2H), 6.94 (t, J = 6.8 Hz, 1H), 5.47 (s, 2H), 4.71 (s, 2H), 3.83 (t, J = 5.6 Hz, 2H), 2.97 (t, J = 5.4 Hz, 2H), 2.76 (m, 2H), 1.36 (t, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 155.17, 145.09, 144.67, 144.21, 143.62, 140.38, 138.66, 133.47, 129.93, 128.66, 128.22, 127.25, 126.04, 121.89, 121.84, 121.77, 119.95, 114.78, 48.12, 44.19, 41.67, 28.23, 21.85, 14.45. EI-MS m/z 504.25 $(M+H)^+$; Anal. calcd for $C_{30}H_{29}N_7O$: (%) C, 71.55; H, 5.80; N, 19.47; Found: C, 71.56; H, 5.82; N, 19.48.

***1-((1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)-N,3-diphenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide* (7d)**

Brown solid (90%); m.p. 115-117 °C; IR (KBr) υ_{max} / cm^{-1} 3442, 3027, 2832, 1645, 1424, 1365, 1034, 1020. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.76 (s, 1H), 8.73 (s, 1H), 7.81 – 7.78 (m, 2H), 7.68 – 7.64 (m, 2H), 7.47 – 7.41 (m, 4H), 7.35 – 7.31 (m, 1H), 7.25 – 7.21 (m, 2H), 7.11 (d, J = 9.1 Hz, 2H), 6.95 (d, J = 7.4 Hz, 1H), 5.46 (s, 2H), 4.70 (s, 2H), 3.83 (s, 3H), 3.82 (t, J = 7.1 Hz, 2H), 2.97 (t, J = 5.4 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 156.47, 153.18, 145.02, 143.72, 140.36, 138.63, 133.49, 129.91, 128.64, 128.24, 127.27, 126.01, 121.88, 121.86, 121.77, 119.98, 114.78, 111.48, 55.51, 48.12, 44.93, 41.67, 22.13. EI-MS m/z 506.20 $(M+H)^+$; Anal. calcd for $C_{29}H_{27}N_7O_2$: (%) C, 68.89; H, 5.38; N, 19.39; Found: C, 68.91; H, 5.39; N, 19.41.

***1-((1-(4-fluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-N,3-diphenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide* (7e)**

Off white solid (82%); m.p. 158-160 °C; IR (KBr) υ_{max} / cm^{-1} 3490, 3021, 2843, 1655, 1410, 1340, 1120, 1060. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.85 (s, 1H), 8.74 (s, 1H), 8.00 – 7.90 (m, 2H), 7.71 – 7.63 (m, 2H), 7.50 – 7.29 (m, 7H), 7.28 – 7.17 (m, 2H), 6.94 (tt, J = 7.3, 1.2 Hz, 1H), 5.48 (s, 2H), 4.71 (s, 2H), 3.82 (t, J = 5.7 Hz, 2H), 2.97 (t, J = 5.4 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.01, 155.21, 145.02, 143.72, 140.36, 138.63, 133.49, 129.91, 128.64, 128.24, 127.27, 126.01, 121.88, 121.86, 121.77, 119.98, 114.78, 111.48, 50.19, 48.93, 41.87, 24.23. EI-MS m/z 494.20 $(M+H)^+$; Anal. calcd for $C_{28}H_{24}FN_7O$: (%) C, 68.14; H, 4.90; N, 19.87; Found: C, 68.16; H, 4.91; N, 19.89.

***1-((1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-N,3-diphenyl-1,4,6,7-tetrahydro-5H-pyrazolo[4,3-c]pyridine-5-carboxamide* (7f)**

White solid (89%); m.p. 145-147 °C; (KBr) υ_{max} / cm^{-1} 3428, 3027, 2834, 1647, 1412, 1343, 1043, 615. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.82 (s, 1H), 8.73 (s, 1H), 7.80 (d, J = 9.2 Hz, 2H), 7.66 (d, J = 8.5 Hz,

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2H), 7.43 (t, *J* = 7.6 Hz, 4H), 7.37 (t, *J* = 7.5 Hz, 1H), 7.27 – 7.21 (m, 2H), 7.18 (d, *J* = 9.5 Hz, 2H), 6.91 (t, *J* = 7.8 Hz, 1H), 5.46 (s, 2H), 4.74 (s, 2H), 3.83 (t, *J* = 5.7 Hz, 2H), 2.96 (t, *J* = 5.2 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 155.28, 145.12, 143.92, 141.36, 138.63, 134.12, 133.39, 129.91, 128.64, 128.24, 127.27, 126.11, 121.88, 121.89, 121.77, 119.88, 114.78, 111.48, 52.19, 48.83, 41.67, 24.83. EI-MS *m/z* 510.20 $(M+H)^+$; Anal. calcd for $C_{28}H_{24}ClN_7O$: (%) C, 65.94; H, 4.74; N, 19.23; Found: C, 65.16; H, 4.75; N, 19.89.

***1-((1-(4-bromophenyl)-1H-1,2,3-triazol-4-yl)methyl)-N,3-diphenyl-6,7-dihydro-1H-pyrazolo[4,3-c] pyridine-5(4H)-carboxamide* (7g)**
Pale yellow solid (87%); m.p. 150-152 °C; IR (KBr) υ_{max} / cm^{-1} 3492, 3022, 2913, 1657, 1422, 1332, 1055, 680. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.90 (s, 1H), 8.75 (s, 1H), 7.88 (d, *J* = 8.6 Hz, 2H), 7.78 (d, *J* = 8.8 Hz, 2H), 7.67 (d, *J* = 7.6 Hz, 2H), 7.45 (dt, *J* = 7.8, 3.6 Hz, 4H), 7.33 (t, *J* = 7.4 Hz, 1H), 7.23 (t, *J* = 7.8 Hz, 2H), 6.94 (t, *J* = 7.4 Hz, 1H), 5.48 (s, 2H), 4.71 (s, 2H), 3.82 (t, *J* = 5.6 Hz, 2H), 2.96 (t, *J* = 6.0 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 155.66, 145.58, 144.65, 140.84, 139.15, 136.17, 133.95, 133.19, 129.14, 128.74, 127.79, 126.45, 122.51, 122.43, 122.38, 121.92, 120.42, 112.05, 44.33, 42.24, 41.24, 22.22. EI-MS *m/z* 554.12$(M+H)^+$; 556.10 $(M+H)^{+2}$; Anal. Calcd for $C_{28}H_{24}BrN_7O$: (%) C, 60.66; H, 4.36; N, 17.68; Found: C, 60.68; H, 4.37; N, 17.69.

***1-((1-(4-iodophenyl)-1H-1,2,3-triazol-4-yl)methyl)-N,3-diphenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide* (7h)**
Light brown solid (80%); m.p.147-149 °C; ; IR (KBr) υ_{max} / cm^{-1} 3485, 30311, 2905, 1658, 1402, 1043, 560. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.85 (s, 1H), 8.76 (s, 1H), 7.99 (d, *J* = 9.2 Hz, 2H), 7.76 (d, *J* = 8.5 Hz, 2H), 7.46 (t, *J* = 7.8 Hz, 4H), 7.35 (t, *J* = 7.5 Hz, 1H), 7.27 – 7.21 (m, 2H), 7.12 (d, *J* = 9.3 Hz, 2H), 6.92 (t, *J* = 7.8 Hz, 1H), 5.47 (s, 2H), 4.69 (s, 2H), 3.83 (t, *J* = 5.8 Hz, 2H), 2.97 (t, *J* = 5.4 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 155.48, 145.02, 144.23, 143.72, 140.36, 138.63, 129.91, 128.64, 128.24, 127.27, 126.01, 121.88, 121.86, 121.77, 119.98, 116.78, 115.48, 95.15, 45.23, 42.13, 41.67, 23.93. EI-MS *m/z* 602.12 $(M+H)^+$; Anal. Calcd for $C_{28}H_{24}IN_7O$: (%) C, 55.92; H, 4.03; N, 16.30; Found: C, 55.94; H, 4.31; N, 16.32.

***1-((1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl)-N,3-diphenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide* (7i)**
Light yellow solid (81%); m.p. 144-146 °C; IR (KBr) υ_{max} / cm^{-1} 3505, 3031, 2912, 1675, 1532, 1372, 1027. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.08 (s, 1H), 8.74 (s, 1H), 8.48 – 8.40 (m, 2H), 8.28 – 8.19 (m, 2H), 7.71 – 7.63 (m, 2H), 7.50 – 7.39 (m, 4H), 7.40 – 7.27 (m, 1H), 7.28 – 7.18 (m, 2H), 6.94 (tt, *J* = 7.3, 1.2 Hz, 1H), 5.51 (s, 2H), 4.71 (s, 2H), 3.83 (t, *J* = 5.7 Hz, 2H), 2.97 (t, *J* = 5.4 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 155.65, 147.17, 145.65, 145.10, 141.20, 140.83, 139.19, 133.92, 129.13, 128.74, 127.81, 126.50, 125.96, 122.89, 122.38, 121.14, 120.47, 112.03, 44.29, 42.15, 41.13, 22.28. EI-MS *m/z* 521.19 $(M+H)^+$; Anal. Calcd for $C_{28}H_{24}N_8O_3$: (%) C, 64.61; H, 4.65; N, 21.53; Found: C, 64.63; H, 4.66; N, 21.55.

***N,3-diphenyl-1-((1-(4-(trifluoromethyl)phenyl)-1H-1,2,3-triazol-4-yl)methyl)-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide* (7j)**
White solid (76%); m.p. 246-248 °C; IR (KBr) υ_{max} / cm^{-1} 3505, 3029, 2903, 1650, 1402, 1357, 1279, 1045. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.03 (s, 1H), 8.75 (s, 1H), 8.17 (d, *J* = 8.4 Hz, 2H), 7.97 (d, *J* = 8.5 Hz, 2H), 7.69 – 7.64 (m, 2H), 7.47 – 7.42 (m, 4H), 7.37 – 7.29 (m, 1H), 7.27 – 7.20 (m, 2H), 6.96 – 6.92 (m, 1H), 5.51 (s, 2H), 4.72 (s, 2H), 3.83 (t, *J* = 5.7 Hz, 2H), 2.97 (t, *J* = 6.7 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 155.66, 145.62, 144.86, 140.84, 139.75, 139.18, 133.94, 129.14, 128.73, 127.80, 127.60, 127.60, 126.51, 122.91, 122.71, 122.38, 121.06, 120.47, 112.02, 44.33, 42.16, 41.14, 22.30. EI-MS *m/z* 544.19 $(M+H)^+$; Anal. Calcd for $C_{29}H_{24}F_3N_7O_3$: (%) C, 64.08; H, 4.45; N, 18.04; Found: C, 64.10; H, 4.46; N, 18.05.

***1-((1-(2-chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-N,3-diphenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide* (7k)**
Off white solid (88%); m.p. 137-139 °C; IR (KBr) υ_{max} / cm^{-1} 3523, 3032, 2923, 1675, 1445, , 1052, 602. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.87 (s, 1H), 8.75 (s, 1H), 7.81 (d, *J* = 9.2 Hz, 2H), 7.69 (d, *J* = 8.5 Hz, 2H), 7.40 (t, *J* = 7.6 Hz, 5H), 7.37 (t, *J* = 7.5 Hz, 1H), 7.27 – 7.21 (m, 1H), 7.19 (d, *J* = 9.5 Hz, 2H), 6.99 (t, *J* = 7.8 Hz, 1H), 5.42 (s, 2H), 4.64 (s, 2H), 3.84 (t, *J* = 5.7 Hz, 2H), 2.96 (t, *J* = 5.2 Hz, 2H). ^{13}C NMR (101MHz, DMSO-d_6) δ 155.28, 145.22, 142.92, 141.36, 138.53, 136.23, 134.12, 133.39, 129.91, 128.64, 128.24, 127.27, 126.11, 121.88, 121.89, 121.77, 120.21, 119.88, 114.78, 111.48, 52.19 48.83, 41.67, 24.83. EI-MS *m/z* 510.15 $(M+H)^+$; EI-MS *m/z* 510.15 $(M+H)^+$; Anal. calcd for $C_{28}H_{24}ClN_7O$: (%) C, 65.94; H, 4.74; N, 19.23; Found: C, 65.16; H, 4.75; N, 19.89.

***1-((1-(2-fluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-N,3-diphenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide* (7l)**
White solid (91%); m.p. 156-158 °C; IR (KBr) υ_{max} / cm^{-1} 3576, 3031, 2925, 1665, 1421, 1330, 1050. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.76 (s, 1H), 8.68 (s, 1H), 7.83 (t, *J* = 7.8 Hz, 1H), 7.68 (d, *J* = 7.6 Hz, 2H), 7.64 – 7.51 (m, 2H), 7.51 – 7.38 (m, 5H), 7.33 (t, *J* = 7.4 Hz, 1H), 7.24 (t, *J* = 7.7 Hz, 2H), 6.95 (t, *J* = 7.4 Hz, 1H), 5.51 (s, 2H), 4.72 (s, 2H), 3.83 (t, *J* = 6.1 Hz, 2H), 2.98 (t, *J* = 6.3 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 155.67, 153.06, 145.56, 143.95, 140.85, 139.19, 133.96, 131.82, 129.16, 128.75, 127.80, 126.51, 125.99, 125.74, 125.13, 122.38, 120.48, 117.66, 117.47, 112.00, 44.20, 42.16, 41.16, 22.34. EI-MS *m/z* 494.20 $(M+H)^+$; Anal. calcd for $C_{28}H_{24}FN_7O$: (%) C, 68.14; H, 4.90; N, 19.87; Found: C, 68.16; H, 4.91; N, 19.89.

***1-((1-(2-bromophenyl)-1H-1,2,3-triazol-4-yl)methyl)-N,3-diphenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide* (7m)**
White solid (90%); m.p. 119-121 °C; IR (KBr) υ_{max} / cm^{-1} 3490, 3021, 2843, 1650, 1410, 1340, 570. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.75 (s, 1H), 8.71 (s, 1H), 7.81(d, *J* = 9.1 Hz, 2H), 7.67 (d, *J* = 8.4 Hz, 2H), 7.44 (t, *J* = 7.9 Hz, 5H), 7.33 (t, *J* = 7.4 Hz, 1H), 7.26 – 7.20 (m, 1H), 7.11 (d, *J* = 9.1 Hz, 2H), 6.94 (t, *J* = 7.8 Hz, 1H), 5.46 (s, 2H), 4.73 (s, 2H), 3.82 (t, *J* = 5.6 Hz, 2H), 2.97 (t, *J* = 5.4 Hz, 2H). ^{13}C NMR (101MHz, DMSO-d_6) δ 155.21, 145.02, 144.23, 143.72, 140.36, 139.21, 138.63, 134.24, 133.49, 133.67, 129.91, 128.64, 128.24, 127.27, 126.01, 121.88, 121.86, 121.77, 120.08, 118.12, 48.12, 44.73, 41.67, 22.83 EI-MS *m/z* 554.12$(M+H)^+$, 556.10 $(M+H)^{+2}$; Anal. Calcd for $C_{28}H_{24}BrN_7O$: (%) C, 60.66; H, 4.36; N, 17.68; Found: C, 60.68; H, 4.37; N, 17.69.

***1-((1-(2-iodophenyl)-1H-1,2,3-triazol-4-yl)methyl)-N,3-diphenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide* (7n)**
White solid (87%); m.p. 188-120 °C; (KBr) υ_{max} / cm^{-1} 3512, 3025,

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2843, 1650, 1408, 1344, 500. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.79 (s, 1H), 8.72 (s, 1H), 7.99 (d, J = 9.2 Hz, 2H), 7.76 (d, J = 8.5 Hz, 2H), 7.46 (t, J = 7.8 Hz, 5H), 7.35 (t, J = 7.5 Hz, 2H), 7.19 (d, J = 9.3 Hz, 2H), 6.98(t, J = 7.8 Hz, 1H), 5.47 (s, 2H), 4.70 (s, 2H) , 3.82 (t, J = 5.6 Hz, 2H), 2.97 (t, J = 5.4 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 155.46, 145.02, 144.23, 143.72, 140.36, 138.63, 130.23, 129.91, 128.64, 128.24, 127.61, 127.27, 126.01, 121.88, 121.86, 121.77, 119.98, 116.78, 115.48, 95.15, 48.23, 44.83, 41.67, 22.93. EI-MS m/z 602.12 $(M+H)^+$; Anal. Calcd for $C_{28}H_{24}IN_7O$: (%) C, 55.92; H, 4.03; N, 16.30; Found: C, 55.94; H, 4.31; N, 16.32.

***1-((1-(2-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl)-N,3-diphenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide* (7o)**

Off white solid (86%); m.p. 118-120 °C; IR (KBr) υ_{max} / cm^{-1} 3505, 3027, 2846, 1655, 1525, 1410, 1360, 1025. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.07 (s, 1H), 8.75 (s, 1H), 8.49 – 8.41 (m, 2H), 8.28 – 8.20 (m, 2H), 7.73 – 7.62 (m, 2H), 7.50 – 7.39 (m, 4H), 7.40 – 7.27 (m, 1H), 7.28 – 7.18 (m, 2H), 6.94 (tt, J = 7.3, 1.2 Hz, 1H), 5.47 (s, 2H), 4.69 (s, 2H), 3.82 (t, J = 5.6 Hz, 2H), 2.97 (t, J = 5.4 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 155.18, 147.87, 147.15, 145.12, 143.72, 141.36, 138.63, 135.23, 134.56, 133.49, 129.91, 128.64, 128.24, 127.27, 126.01, 121.88, 121.86, 121.77, 120.98, 112.03, 44.29, 42.15, 41.13, 22.28. EI-MS m/z 521.19 $(M+H)^+$; Anal. Calcd for $C_{28}H_{24}N_8O_3$: (%) C, 64.61; H, 4.65; N, 21.53; Found: C, 64.63; H, 4.66; N, 21.55.

***1-((1-(3-chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-N,3-diphenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide* (7p)**

White solid (86%); m.p. 147-149 °C; IR (KBr) υ_{max} / cm^{-1} 3497, 3029, 2847, 1650, 1444, 1306, 1032, 753. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.80 (s, 1H), 8.75 (s, 1H), 7.89 (s, 1H), 7.81 (d, J = 9.2 Hz, 2H), 7.69 (d, J = 8.5 Hz, 2H), 7.40 (t, J = 7.6 Hz, 4H), 7.37 (t, J = 7.5 Hz, 1H), 7.27 – 7.21 (m, 1H), 7.19 (d, J = 9.5 Hz, 2H), 6.93 (t, J = 7.8 Hz, 1H), 5.42 (s, 2H), 4.64 (s, 2H), 3.84 (t, J = 5.7 Hz, 2H), 2.96 (t, J = 5.2 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 155.38, 145.42, 142.82, 141.39, 138.55, 136.23, 134.32, 133.30, 129.96, 128.74, 128.29, 127.20, 126.51, 121.98, 121.80, 121.75, 121.34, 120.31, 119.78, 118.78, 45.29 42.80, 41.66, 22.73. EI-MS m/z 510.15 $(M+H)^+$; Anal. calcd for $C_{28}H_{24}ClN_7O$: (%) C, 65.94; H, 4.74; N, 19.23; Found: C, 65.95; H, 4.76; N, 19.24.

***1-((1-(3-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)-N,3-diphenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide* (7q)**

Brown solid (79%); m.p. 135-136 °C; IR (KBr) υ_{max} / cm^{-1} 3502, 3023, 2875, 1657, 1454, 1350, 1090. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.78 (s, 1H), 8.75 (s, 1H), 7.80 (d, J = 9.1 Hz, 2H), 7.67 (d, J = 8.4 Hz, 2H), 7.47 – 7.41 (m, 4H), 7.34 (t, J = 7.4 Hz, 1H), 7.26 – 7.20 (m, 2H), 7.11 (d, J = 9.1 Hz, 2H), 6.94 (t, J = 7.8 Hz, 1H), 5.48 (s, 2H), 4.71 (s, 2H), 3.83 (t, J = 5.7 Hz, 2H), 2.96 (t, J = 5.5 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 156.25, 154.08, 145.22, 143.87, 141.36, 138.63, 133.49, 129.91, 128.64, 128.24, 127.27, 126.01, 125.32, 121.88, 121.76, 121.77, 120.14, 119.98, 114.78, 111.42, 53.51, 46.12, 44.73, 41.87, 22.21. EI-MS m/z 506.20 $(M+H)^+$; Anal. calcd for $C_{29}H_{27}N_7O_2$: (%) C, 68.89; H, 5.38; N, 19.39; Found: C, 68.91; H, 5.39; N, 19.41.

***1-((1-(2,4-dichlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-N,3-diphenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide* (7r)**

White solid (82%); m.p. 139-141 °C; IR (KBr) υ_{max} / cm^{-1} 3505, 3029, 2840, 1654, 1415, 1342, 1070, 740. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.77 (s, 1H), 8.73 (s, 1H), 7.80 (d, J = 9.2 Hz, 2H), 7.66 (d, J = 8.5 Hz, 2H), 7.69 (s, 1H), 7.62 (d, J = 9.5 Hz, 2H), 7.50 (t, J = 7.6 Hz, 2H), 7.41 (t, J = 7.5 Hz, 3H), 6.91 (t, J = 7.8 Hz, 1H), 5.45 (s, 2H), 4.69 (s, 2H), 3.84 (t, J = 5.7 Hz, 2H), 2.96 (t, J = 5.2 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 155.31, 145.53, 144.16, 142.72, 141.48, 139.56, 136.29, 135.74, 133.40, 129.99, 129.24, 128.44, 127.25, 126.21, 123.58, 123.49, 121.57, 120.21, 119.88, 114.78, 46.21 44.63, 41.67, 22.23. EI-MS m/z 544.13 $(M+H)^+$; Anal. calcd for $C_{28}H_{23}Cl_2N_7O$: (%) C, 61.77; H, 4.26; N, 18.01; Found: C, 61.79; H, 4.27; N, 18.03.

***1-((1-(3,4-dichlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-N,3-diphenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide* (7s)**

Off white solid (83%); m.p. 228-230 °C; IR (KBr) υ_{max} / cm^{-1} 3503, 3029, 2842, 1654, 1410, 1345, 1066, 745. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.78 (s, 1H), 8.74 (s, 1H), 7.81 (d, J = 9.2 Hz, 2H), 7.77 (s, 1H), 7.66 (d, J = 8.6 Hz, 2H), 7.62 (d, J = 9.4 Hz, 2H), 7.54 (t, J = 7.5 Hz, 2H), 7.44 (t, J = 7.4 Hz, 3H), 7.01 (t, J = 7.9 Hz, 1H), 5.43 (s, 2H), 4.63 (s, 2H), 3.82 (t, J = 5.7 Hz, 2H), 2.98 (t, J = 5.4 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 155.18, 146.41, 143.80, 141.43, 138.26, 136.49, 134.31, 133.41, 129.90, 128.52, 128.41, 127.43, 125.32, 121.78, 121.59, 121.47, 120.20, 119.82, 115.78, 111.78, 46.65, 46.24, 40.97, 22.73. EI-MS m/z 544.20 $(M+H)^+$; Anal. calcd for $C_{28}H_{23}Cl_2N_7O$: (%) C, 61.77; H, 4.26; N, 18.01; Found: C, 61.79; H, 4.27; N, 18.03.

***1-((1-(3,5-dichlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-N,3-diphenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide* (7t)**

White solid (85%); m.p. 139-141 °C; IR (KBr) υ_{max} / cm^{-1} 3505, 3027, 2846, 1650, 1411, 1345, 1030, 752. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.98 (s, 1H), 8.74 (s, 1H), 8.08 (d, J = 1.8 Hz, 2H), 7.78 – 7.64 (m, 3H), 7.45 (t, J = 7.4 Hz, 4H), 7.38 – 7.16 (m, 3H), 6.94 (t, J = 7.3 Hz, 1H), 5.49 (s, 2H), 4.71 (s, 2H), 3.83 (t, J = 5.8 Hz, 2H), 2.95 (t, J = 5.9 Hz, 2H^{13}C NMR (101 MHz, DMSO-d_6) δ 155.64, 145.60, 144.79, 140.84, 139.15, 138.57, 135.63, 133.93, 129.13, 128.73, 127.80, 126.50, 122.83, 122.38, 120.47, 119.25, 112.02, 44.35, 42.16, 41.12, 22.26. EI-MS m/z 544.13 $(M+H)^+$; Anal. calcd for $C_{28}H_{23}Cl_2N_7O$: (%) C, 61.77; H, 4.26; N, 18.01; Found: C, 61.79; H, 4.27; N, 18.03.

***1-((1-(3-chloro-4-fluorophenyl)-1H-1,2,3-triazol-4-yl) methyl)-N,3-diphenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide* (7u)**

White solid (77%); m.p. 240-242 °C; IR (KBr) υ_{max} / cm^{-1} 3505, 3022, 2840, 1654, 1410, 1345, 1178, 1020, 780. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.90 (s, 1H), 8.73 (s, 1H), 8.23 (dd, J = 6.4, 2.7 Hz, 1H), 8.01 – 7.92 (m, 1H), 7.71 – 7.62 (m, 3H), 7.49 – 7.39 (m, 4H), 7.38 – 7.28 (m, 1H), 7.28 – 7.18 (m, 2H), 6.98 – 6.89 (m, 1H), 5.48 (s, 2H), 4.70 (s, 2H), 3.82 (t, J = 5.8 Hz, 2H), 2.95 (t, J = 5.8 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 155.28, 145.09, 144.14, 140.34, 138.63, 133.46, 128.62, 128.22, 127.27, 126.00, 122.44, 122.30, 121.87, 120.95, 120.77, 120.58, 119.99, 118.13, 117.90, 111.51, 43.88, 41.67, 40.64,

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21.79. EI-MS *m/z* 528.17 $(M+H)^+$; Anal. calcd for $C_{28}H_{23}ClFN_7O$: (%) C, 63.70; H, 4.39; N, 18.57; Found: C, 63.72; H, 4.40; N, 18.59.

***1-((1-(3,4-difluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-N,3-diphenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide* (7v)**

Off white solid (80%); m.p. 226-228 °C; IR (KBr) υ_{max} / cm^{-1} 3501, 3021, 2846, 1659, 1412, 1335, 1130, 1060. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.91 (s, 1H), 8.73 (s, 1H), 8.24 (dd, *J* = 6.5, 2.6 Hz, 1H), 8.01 – 7.91 (m, 1H), 7.70 – 7.62 (m, 3H), 7.46 – 7.40 (m, 4H), 7.35 (t, *J* = 7.4 Hz, 1H), 7.20 (t, *J* = 7.9 Hz, 2H), 7.04 (t, *J* = 7.3 Hz, 1H), 5.48 (s, 2H), 4.70 (s, 2H),), 3.82 (t, *J* = 5.6 Hz, 2H), 2.95 (t, *J* = 5.4 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 155.21, 149.84, 149.21, 145.02, 143.72, 141.78, 140.36, 138.63, 133.49, 130.12, 129.91, 128.64, 128.24, 127.27, 126.01, 124.12, 121.88, 121.86, 121.77, 119.98, 45.22, 42.13, 40.81, 23.53. EI-MS *m/z* 528.17 $(M+H)^+$; Anal. calcd for $C_{28}H_{23}F_2N_7O$: (%) C, 65.74; H, 4.53; N, 19.17; Found: C, 65.76; H, 4.54; N, 19.19.

***1-((1-(3,4-dimethoxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)-N,3-diphenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide* (7w)**

Brown solid (88%); m.p. 207-209 °C; IR (KBr) υ_{max} / cm^{-1} 3500, 3020, 2845, 1654, 1410, 1345, 1080. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.78 (s, 1H), 8.72 (s, 1H), 7.82 (d, *J* = 9.1 Hz, 2H), 7.67 (d, *J* = 8.4 Hz, 2H), 7.44 (t, *J* = 7.9 Hz, 4H), 7.33 (t, *J* = 7.4 Hz, 1H), 7.26 (m, 1H), 7.11 (d, *J* = 9.1 Hz, 2H), 6.94 (s, 1H), 5.46 (s, 2H), 4.72 (s, 2H), 3.84 (s, 6H), 3.82 (d, *J* = 7.2 Hz, 2H), 2.97 (t, *J* = 5.4 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 156.31, 153.21, 148.74, 139.76, 136.99, 135.44, 129.89, 129.54, 128.44, 127.45, 126.81, 123.98, 123.69, 121.77, 120.81, 119.88, 118.78, 114.25, 109.13, 101.21, 55.86, 46.90, 44.63, 40.32, 22.09. EI-MS *m/z* 536.20 $(M+H)^+$; Anal. calcd for $C_{30}H_{29}N_7O_3$: (%) C, 67.27; H, 5.47; N, 18.31; Found: C, 67.29; H, 5.48; N, 18.33.

***1-((1-(4-bromo-3-(trifluoromethyl)phenyl)-1H-1,2,3-triazol-4-yl)methyl)-N,3-diphenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide* (7x)**

White solid (79%); m.p. 221-223 °C; IR (KBr) υ_{max} / cm^{-1} 3500, 3022, 2854, 1660, 1405, 1343, 1119, 1070. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.91 (s, 1H), 8.73 (s, 1H), 8.24 (dd, *J* = 6.5, 2.6 Hz, 1H), 8.01 – 7.82 (m, 1H), 7.69 – 7.53 (m, 3H), 7.46 – 7.41 (m, 4H), 7.36 (t, *J* = 7.4 Hz, 1H), 7.21 (t, *J* = 7.9 Hz, 2H), 7.04 (t, *J* = 7.3 Hz, 1H), 5.48 (s, 2H), 4.70 (s, 2H),), 3.82 (t, *J* = 5.6 Hz, 2H), 2.95 (t, *J* = 5.4 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 155.18, 145.02, 143.72, 140.36, 139.10, 138.63, 136.89, 136.12, 133.49, 130.23, 129.91, 128.64, 128.24, 127.27, 126.01, 121.88, 121.86, 121.77, 120.98, 120.01, 114.78, 48.01, 46.32, 44.83, 22.19. EI-MS *m/z* 622.20, 624.15 $(M+1, M+2)^+$; Anal. calcd for $C_{30}H_{23}BrF_3N_7O$: (%) C, 55.96; H, 3.72; N, 15.75; Found: C, 55.98; H, 3.74; N, 15.76.

***1-((1-(3,4-dimethylphenyl)-1H-1,2,3-triazol-4-yl)methyl)-N,3-diphenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide* (7y)**

Off white solid (84%); m.p. 221-223 °C; IR (KBr) υ_{max} / cm^{-1} 3505, 3022, 2844, 1658, 1412, 1345, 1076. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.82 (s, 1H), 8.79 (s, 1H), 7.78 (d, *J* = 8.6 Hz, 2H), 7.66 (d, *J* = 7.3 Hz, 2H), 7.47 – 7.42 (m, 3H), 7.44 (s, 1H), 7.41 – 7.31 (m, 2H), 7.27 – 7.20 (m, 2H), 6.93 (t, *J* = 6.9 Hz, 1H), 5.47 (s, 2H), 4.72 (s, 2H), 3.82 (t, *J* = 5.7 Hz, 2H), 2.97 (t, *J* = 5.5 Hz, 2H), 2.36 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 159.25, 155.18, 145.02, 143.72, 140.36, 138.63, 136.89, 136.12 133.49, 129.91, 128.64, 128.24, 127.27, 126.01, 121.88, 121.86, 121.77, 119.98, 114.78, 111.48, 45.51, 44.02, 41.57, 22.23, 20.92. EI-MS *m/z* 504.25 $(M+H)^+$; Anal. calcd for $C_{30}H_{29}N_7O$: (%) C, 71.55; H, 5.80; N, 19.47; Found: C, 71.56; H, 5.82; N, 19.49.

***1-((1-(benzo[d][1,3]dioxol-5-yl)-1H-1,2,3-triazol-4-yl)methyl)-N,3-diphenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide* (7z)**

Brown solid (78%); m.p. 168-170 °C; IR (KBr) υ_{max} / cm^{-1} 3504, 3025, 2840, 1655, 1416, 1345, 1080. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.77 (s, 1H), 8.72 (s, 1H), 7.81 (d, *J* = 9.1 Hz, 2H), 7.67 (d, *J* = 8.4 Hz, 2H), 7.44 (t, *J* = 7.9 Hz, 4H), 7.33 (t, *J* = 7.4 Hz, 1H), 7.26 (m, 1H), 7.11 (d, *J* = 9.1 Hz, 2H), 6.94 (s, 1H), 5.94 (s, 2H), 5.46 (s, 2H), 4.70 (s, 2H), 3.82 (d, *J* = 7.1 Hz, 2H), 2.97 (t, *J* = 5.4 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 156.31, 148.74, 147.53, 139.76, 136.99, 135.44, 133.49, 129.89, 129.54, 128.44, 127.45, 126.81, 123.98, 123.69, 121.77, 120.81, 119.88, 114.78, 109.98, 101.21, 52.21, 48.63, 41.67, 24.73. EI-MS *m/z* 520.20 $(M+H)^+$; Anal. calcd for $C_{29}H_{25}N_7O_3$: (%) C, 67.05; H, 4.85; N, 18.87; Found: C, 67.06; H, 4.87; N, 18.89.

Biological activity

MTB PS screening

The MTB *panC* gene (Rv3602c) encoding the pantothenate synthetase was cloned and changed into BL21 (DE3) cells and the protien was expressed.[7, 15] For the assay, to each well of a 96-well plate, 60 µL of PS reaction mixture containing 0.4 mM NADH, 10 mM $MgCl_2$, 5 mM *β*-alanine, 5 mM pantoic acid, 1 mM potassium phosphoenolpyruvate,10 mM ATP, and 20 µL of enzyme mixture consisting of 18 units/mL each of chicken muscle myokinase, rabbit muscle lactate dehydrogenase and rabbit muscle pyruvate kinase, diluted in 100 mM HEPES buffer were added. The reaction mixture and enzyme mixture were added to the plate to a final volume of 100 µL with 100 mM HEPES buffer (pH 7.8). Concentration of enzyme was determined based on the range finding experiments by varying the concentration of enzymes. Compound solutions were then added to the plates (from 50 µM to lower concentration) and the reaction began with the addition of 10 µL of 4.32 pM of MTB PS, diluted in buffer. The test plate was immediately moved to a microplate reader and the reduction of NADH was quantified at 340 nm. The reaction elements except MTB PS were mixed in the well and the background reaction was calculated; MTB PS was then added and the reaction kinetics was monitored. Reactions were carried out at 37 °C in a heat controlled Perkin Elmer Victor X3 Spectrophotometer. % inhibitions were calculated using following formula: 100 x [(1 - compound rate - background rate) / (full reaction rate - background rate)].[7, 15]

In vitro MTB screening

The antimycobacterial activities of the compounds **7a-z** were evaluated against MTB *H37Rv* strain and two "wild" strains extracted from tuberculosis patients: one strain is *Spec. 210* resistant to PAS, INH, ETB and RMP and the other strain is *Spec. 192* fully sensitive to the administrated anti-TB agents. *In vitro* anti-TB activity is performed by a classical test-tube method of successive

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dilution in Youmans' modification of the Proskauer and Beck liquid medium containing 10% of bovine serum.[36, 37] Bacterial respites was prepared from 14 days old cultures of gradually growing strains. Solutions of compounds in DMSO were tested. Stock solutions contained 10 mg of compounds in 1 mL. Dilutions (in geometric progression) were prepared in Youmans' medium.[37] The medium is without compounds and containing INH as reference drug was used for comparison. Incubation was performed at 37 °C. The MIC values were determined as MIC inhibiting the growth of tested TB strains in relation to the probe with no tested compound. The influence of the compound on the growth of bacteria at concentrations of 3.12, 6.25, 12.5, 25, 50 and 100 µg/mL was evaluated**.**

In vitro cytotoxicity screening

Compounds **7b, 7c** and **7d** were further tested for toxicity in a RAW 264.7 cell line at the concentration of 50 µM. After 72 h of exposure, viability was evaluated on the basis of cellular translation of MTT into a formazan product by the Promega Cell Titer 96 nonradioactive cell proliferation assay.[38, 39]

Docking Study

Docking studies of the title compounds (**7a-z**) was performed using Glide 5.9 (Extra Precision) running on maestro version 9.4, in order to investigate their *in-silico* biding affinity as well as their binding pattern with enzyme Pantothenate synthetase.[40] Enzyme used for the docking study was retrieved from RCSB Protein Data Bank (PDB ID: 3IUB) in complex with co-crystallised ligand (indole-2-carboxamide derivative). Selected protein consist of two chains asymmetric units (A and B), both consist of co-crystallized ligand, in the current study unit A was separated and used further for docking studies. Protein preparation wizard of Schrödinger suite was used for preparation of selected protein. Protein was pre-processed separately by deleting the substrate co-factor as well as the crystallographically observed water molecules (water without H bonds), followed by optimization of hydrogen bonds. After assigning charge and protonation position, finally energy was minimized with root mean square deviation (RMSD) value of 0.30 Å using optimized potentials for liquid simulations-2005 (OPLS-2005) force field.[41] Finally energy minimized protein and co-crystallized ligand was used to build energy grids using the default value of protein atom scaling (1.0 Å) within a cubic box of 14 Å dimensions, centered on the centroid of the X-ray ligand pose.[42] The structures of **7a-z** were drawn using ChemSketch and converted to 3D structure with the help of 3D optimization tool. Using LigPrep 2.6 module, the drawn ligands were geometry optimized; partial atomic charges were computed using OPLS-2005 force field.[43] Finally, prepared ligands were docked with prepared protein using Glide 5.9 module, in extra precision mode (XP). The leading docked pose (with lowest Glide score value) found from Glide was analyzed. RMSD value was calculated between the experimental binding mode of co-crystallized ligand as in X-ray and re-docked pose to ensure accuracy and reliability of the docking procedure.

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Graphical Text

Novel series of pyrazolo[4,3-c]pyridine carboxamides was synthesized and characterized using various spectroscopic techniques. The synthesized compounds were evaluated for their antitubercular activity, pantothenate synthetase enzyme inhibition study and were docked to 3IUB crystal structure of MTB PS.