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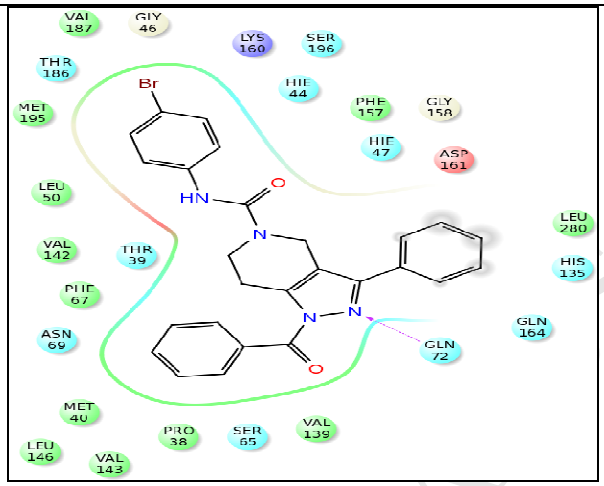

Compound 1-benzoyl-N-(4-nitrophenyl)-3-phenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide(**3**) was found to be the most active compound with IC_{50} of 21.8±0.8 µM against MTB PS, inhibited MTB with MIC of 26.7 µM and it was non-cytotoxic at 50 µM.

Development of 3-phenyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridine derivatives as novel *Mycobacterium tuberculosis* pantothenate synthetase inhibitors

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Abstract

Forty 3-phenyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridine derivatives were synthesized from piperidin-4-one by five step synthesis and evaluated for *Mycobacterium tuberculosis* (MTB) pantothenate synthetase (PS) inhibition study, *in vitro* activities against MTB, cytotoxicity against RAW 264.7 cell line. Among the compounds, 1-benzoyl-N-(4-nitrophenyl)-3-phenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide (**6ac**) was found to be the most active compound with IC_{50} of 21.8±0.8 µM against MTB PS, inhibited MTB with MIC of 26.7 µM and it was non-cytotoxic at 50 µM.

Key words: Tuberculosis, Pantothenate synthetase, Cytotoxicity

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1. Introduction

Tuberculosis (TB) is becoming a global health threat due to the emergence of multi-drug resistant strains of *Mycobacterium tuberculosis* (MTB) and the deadly synergy of TB with HIV [1]. This point to the critical need to develop new drugs against novel targets of MTB. Pantothenate synthetase (PS; EC 6.3.2.1), encoded by the *panC* gene, catalyzes the essential adenosine triphosphate (ATP)–dependent condensation of D-pantoate and ß-alanine to form pantothenate in bacteria [2]. Pantothenate is a key precursor of coenzyme A and acyl carrier protein, essential for many intracellular processes including fatty acid metabolism, cell signaling, synthesis of polyketides and non-ribosomal peptides. Both microorganisms and plants must synthesize pantothenate, while mammals must obtain it from their diet, and its biosynthetic pathway is not present. A *PanC* gene knockout (KO) of PS in MTB results in a highly attenuated phenotype in immunocompromised SCID mice and in immunocompetent BALB/c mice [3], whereas the Δ*lysA* Δ*pan CD*KO mutant exhibits substantially reduced replication and persistence [4]. This observation indicates that a functional pantothenate biosynthetic pathway is essential for persistent growth and virulence of MTB and PS is an appropriate target for developing new therapeutics to treat TB [5]. Till date some of the MTB PS inhibitors reported were (**Fig 1**) 5-*tert*-butyl-*N*-pyrazol-4-yl-4,5,6,7-tetrahydrobenzo[*d*]isoxazole-3-carboxamide derivatives (**A**) [6], actinomycin D (IC_{50} of 250.72 µM) [6], indole derivative (**B**) [7], nafronyl oxalate [8], and benzofuran derivatives (**C**) [9]. In this work, we discovered novel 3-phenyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridine derivatives as a novel scaffold as MTB PS inhibitors.

2. Results and discussion

2.1. Design and Chemistry

In a protein data bank (PDB) crystal structure of a PS from MTB in complex with a reaction intermediate, pantoyl adenylate was reported (PDB ID: 1N2H) with 2.0 Å resolution. The crystal structure of the PS protein in complex with the reaction intermediate shows that the reaction intermediate, pantoyl adenylate, has strong interactions with the active site atoms. This tight binding is necessary to stabilize this highly reactive compound. It can be expected that a nonreactive analog of pantoyl adenylate will be a good inhibitor [5]. A nonreactive analog of pantoyl adenylate is expected to be very specific to PS as well. Because there is no pantothenate biosynthesis in humans, this analog will be unlikely to inhibit any human enzyme. A high-throughput virtual screening of our in-house (BITS-Pilani) database was carried out using Glide XP (extra precision) docking and we identified 1-benzoyl-N-(4-bromophenyl)-3-phenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide (Compound **6aa**) as an inhibitor in the pantoyl adenylate site with docking score of -6.92, while the pantoyl adenylate docking score was -9.8 (**Fig 2**). Compound **6aa** showed a percentage inhibition of 60.6 at 100 μM when tested for MTB PS inhibitory activity. To investigate the potential of this lead, we synthesized a series of 3-phenyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridine derivatives to screen against MTB PS enzyme activity for establishing structure-activity relationship (SAR), and to evaluate antimycobacterial activity and cytotoxicity.

The target molecules were synthesized in five steps (**Scheme 1**), starting with commercially available, less expensive, 4-piperidone hydrochloride salt (**1**). Initially the amine was protected by *t*-butyloxycarbonyl (Boc) protecting group to get 4-*N*-Boc-piperidone (**2**), and then it was subjected to stark-enamine reaction conditions using morpholine, *p*-toluenesulfonic acid (catalytic) and benzoyl chloride to produce 1, 3-dicarbonyl intermediate; which is then treated *in situ* with hydrazine hydrate to get Pyrazole ring (**3**) according to procedure reported by X. M. Ye et al [10]. Compound **3** was then deprotected using trifluoroacetic acid to get compound **4**. Under

mild conditions the more nucleophilic amine of aliphatic ring was reacted selectively with various substituted isocyanates, isothiocyanates and arylsulfonyl halides using DIPEA as base and DMF as solvent at room temperature to yield corresponding urea, thiourea and sulphonamides (**5** and **8**). The free amino group of pyrazole ring was treated with benzoyl/cyclohexanecarbonyl chloride using DIPEA as base to get target compounds.

2.2 Pantothenate synthetase enzyme inhibition studies

Synthesized compounds were assayed for MTB PS inhibition study, that couples the AMP produced in the condensation of β-alanine and pantoate with the reduction of NADH to NAD+ through myokinase, pyruvate kinase and lactate dehydrogenase [2]. The NAD+ produced can be monitored spectrophotometrically at 340 nm. Initial screening at 100µM, compounds showed inhibition ranged from 9.0-95.7%. Seventeen compounds showed >50% inhibition against MTB PS and compounds which showed % inhibition of >60 were further selected for IC_{50} calculation. Among the five compounds (**6aa, 6ac, 6ag, 9aa**, and **9ac**) tested, showed IC_{50} ranged from 21.8±0.8 to 82.1±2.3 µM. Compound **6ac** (1-benzoyl-N-(4-nitrophenyl)-3-phenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide) was found to be the most potent compound with IC_{50} of 21.8 ± 0.8µM (**Fig 3**) and it was found to be four times more potent than our initial compound **6aa**. With respect to structure activity relationship, we have prepared twenty benzoyl and twenty cyclohexanecarbonyl derivatives at 1^{st} position of 4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridine scaffold (**R^2**). Benzoyl group substituted compounds (6aa-6ai, 7aa-7af and 9aa-9ae) showed better activity than cyclohexanecarbonyl derivatives (6ba-6bi, 7ba-7bf and 9ba-9be) against MTB PS. We have also done modification at 5^{th} position by preparing urea (**6aa-6ai, 6ba-6bi**), thiourea (**7aa-7af, 7ba-7bf**) and sulfonamides (**9aa-9ae, 9ba-9be**). In general the order of activity as follows urea (**6aa-6ai**) > sulfonamides (**9aa-9ae**) > thiourea (**7aa-7af**). Among the urea derivatives (**6aa-6ai**), we have prepared (sub) phenyl, naphthyl, benzyl and

alkyl derivatives. Electron withdrawing substituents in the phenyl ring (**6aa-6ac**) favors activity than electron donating substituents (**6ae, 6af**).

2.3 In-vitro MTB screening

All the new 3-phenyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridine derivatives were screened for their *in vitro* anti-tubercular activity against *Mycobacterium tuberculosis* H37Rv (ATCC27294) using an agar dilution method using drug concentration from 50 µg/mL to 0.78µg/mL in duplicates. The minimum inhibitory concentration (MIC) was determined for each compound. The MIC is defined as the minimum concentration of compound required to completely inhibit the bacterial growth. Isoniazid and Ethambutol were used as a reference compound for comparison. The MIC values of the synthesized compounds along with the standard drug for comparison were reported in **Table 1**. Among the forty compounds screened seventeen compounds showed activity against MTB with MIC ≤ 60 µM. Two compounds (**6ac,** and **9ab**) inhibited MTB with MIC of < 30 µM. Compound **9ab** ((5-(4-nitrophenylsulfonyl)-3-phenyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)(phenyl)methanone) was found to be the most active compound *in vitro* with MIC of 25.58 ìM against log-phase culture of MTB. Structural changes at N-1 position do not affect activity appreciably, whereas sulfonamides derivatives at N-5 position showed better activity in general.

2.4. In-vitro cytotoxicity study

All the compounds were also tested for in-vitro cytotoxicity against RAW 264.7 cells at 50 µM concentration by (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Percentage inhibition of cells was reported in Table 1. The most promising anti-TB compound **6ac** was devoid of cytotoxicity at 50µM [0% inhibition].

3. Conclusion

We identified 1-benzoyl-N-(4-bromophenyl)-3-phenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide (Compound **6aa**) as an inhibitor in the pantoyl adenylate site of MTB PS by virtual screening. To study the SAR we synthesized forty compounds and compound **6ac** was found to be the most potent compound with IC_{50} of 21.8±0.8 µM against MTB PS. Compound **6ac** also inhibit the growth of MTB with MIC of 26.7 µM and non-cytotoxic to RAW 264.7 cells till 50 µM.

4. Experimental

4.1. Chemistry

4.1.1 Preparation of tert-butyl 4-oxopiperidine-1-carboxylate (2)

Et_3N (26.45 mL, 185.17 mmol) was added drop wise to a stirred solution of compound **1** (10.0 g, 74.07 mmol) in CH_2Cl_2 (100 mL) and MeOH (10 mL) at 0 °C, then $(Boc)_2O$ (19.37 mL, 88.88 mmol) was added drop wise to the reaction mixture at same temperature and allowed to stir at room temperature for 16 h. The reaction mixture was diluted with H_2O (100 mL) and extracted with CH_2Cl_2 (3 X 200 mL). The separated organic layer was concentrated under reduced pressure and the crude residue was washed with hexanes to get compound **2** (11.5 g, 78%) as an off-white solid. ESI-MS found 200 $[M+H]^+$ and carried to next step.

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

In a 100 mL round-bottom flask equipped with a Dean-stark trap, a reflux condenser and an internal thermocouple, Compound **2** (1.0 g, 4.63 mmol), toluene (10 mL), morpholine (0.42 mL, 4.63 mmol), and *p*-toluenesulfonic acid (catalytic) were added sequentially. The reaction solution was refluxed under N_2 for 16 h. The solvent was evaporated and the crude was dissolved in CH_2Cl_2 (20 mL) and then triethylamine (1.07 mL, 7.53 mmol) was added at 0 °C, under N_2, a solution of benzoyl chloride (0.58mL, 5.02 mmol) in CH_2Cl_2 (10 mL) was added over 10 min,

the ice bath was then removed and the reaction solution was stirred at room temperature for 4 h. All the volatile solvents were removed in vacuo and the residue was dissolved in ethanol, at 0 °C, and hydrazine hydrate (0.25mL, 7.53 mmol) was then added over 5 min (exothermic reaction), the reaction solution was stirred at r.t. for 16h and the reaction mixture was concentrated under reduced pressure and the crude residue was purified by column chromatography to get compound **3** (1.34 g, 90%) as an off-white solid. ESI-MS found 300 $[M+H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.45 (s, 1H), 7.5–7.2 (m, 5H), 4.80 (s, 2H), 3.90 (t, *J* = 7.8 Hz, 2H), 2.81 (t, *J* = 7.8 Hz, 2H), 1.40 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.5, 147.6, 143.5, 136.6(2C), 132.7, 129,3(2C), 127.3, 117.5, 81.7, 43.9, 36.9, 31.5(3C), 27.7; 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%

***4.1.3 Preparation of 3-phenyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridine (4*)**

CF_3COOH (0.65 mL, 8.36 mmol) was added drop wise to a stirred solution of compound **3** (0.6 g, 1.67 mmol) in CH_2Cl_2 (10 mL) at 0 °C, and allowed to stir at room temperature for 4 h. The reaction mixture was concentrated under reduced pressure and the crude residue was washed with hexanes and diethyl ether to get compound **4** (0.45 g, 91%) as an off-white solid. ESI-MS showed 200 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6): 7.38–7.10 (m, 5H), 4.22 (s, 2H), 3.45 (t, *J* = 7.2 Hz, 2H), 2.61 (t, *J* = 7.8 Hz, 2H).

4.1.4 Preparation of 5

Substituted arylisocyanate/arylisothiocyanate (1.20 equiv.), was added to the stirred solution of Compound **4** (1.0 equiv) and Et_3N (2.5 equiv) in DMF at 0 °C under N_2 atm, and allowed to stir at room temperature for 6 h. The reaction mixture was diluted with EtoAc and washed with Brine solution, the separated organic layer was concentrated under reduced pressure and the crude residue was purified by column chromatography (EtOAc/Hexanes) to get compounds **5**.

4.1.5 Preparation of 8

Substituted arylsulphonyl halide (1.20 equiv), was added to the stirred solution of Compound **4** (1.0 equiv) and Et_3N (2.5 equiv) in DMF at 0 °C under N_2 atm, and allowed to stir at room temperature for 6 h. The reaction mixture was diluted with EtOAc and washed with Water, the separated organic layer was concentrated under reduced pressure and the crude residue was purified by column chromatography (EtOAc/Hexanes) to get compounds **8**.

4.1.6a. Preparation of 6aa-6ai, 7aa-7af, and 9aa-9ae:

Benzoylchloride (1.20 equiv), was added to the stirred solution of Compound **5/**Compound **8** (1.00 equiv) and DIPEA (2.20 equiv) in DMF at 0 °C under N_2 atm, and allowed to stir at room temperature for 12 h. The reaction mixture was quenched with water, extracted with EtOAc and washed the EtOAc layer with Brine solution, the separated organic layer was concentrated under reduced pressure and the crude residue was purified by column chromatography (EtOAc/Hexanes as eluent) to get compounds ***6aa-6ai, 7aa-7af, and 9aa-9ae.***

4.1.6b. Preparation of 6ba-6bi, 7ba-7bf, and 9ba-9be:

Cyclohexanecarbonylchloride (1.20 equiv), was added to the stirred solution of Compound **5/**Compound **8** (1.00 equiv) and DIPEA (2.20 equiv) in DMF at 0 °C under N_2 atm, and allowed to stir at room temperature for 12 h. The reaction mixture was quenched with water, extracted with EtOAc and washed the EtOAc layer with Brine solution, the separated organic layer was concentrated under reduced pressure and the crude residue was purified by column chromatography (EtOAc/Hexanes as eluent) to get compounds ***6ba-6bi, 7ba-7bf, and 9ba-9be.***

4.1.7. 1-Benzoyl-N-(4-bromophenyl)-3-phenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide (6aa): Yield: 78%, m.p. 167–169 °C; MS(ESI) m/z 501 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (s, 1H), 7.89 (d, *J* = 7.6 Hz, 2H), 7.50–7.25 (m, 12H), 4.70 (s, 2H), 3.82 (t, *J* = 8.0 Hz, 2H), 3.10 (t, *J* = 7.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.2, 159.4, 145.6,

144.6(2C), 134.9, 134.3, 133.7, 132.2, 131.4, 129.2, 128.3(2C), 127.1, 126.6(2C), 125.0, 124.2, 124.0, 123.4(2C), 122.7, 118.9, 54.7, 48.4, 29.6. Anal. calcd for $C_{26}H_{21}BrN_4O_2$: C, 62.28; H, 4.22; N, 11.17% Found C, 62.31; H, 4.26; N, 11.21%

4.1.8. 1-Benzoyl-N-(4-chlorophenyl)-3-phenyl-6, 7-dihydro-1H-pyrazolo [4,3-c]pyridine-5(4H)-carboxamide (6ab): Yield: 85%, m.p. 160–162 °C; MS(ESI) m/z 457 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.90 (s, 1H), 7.90 (d, *J* = 8.0 Hz, 2H), 7.78 (d, *J* = 8.4 Hz, 2H), 7.60–7.24 (m, 10H), 4.75 (s, 2H), 3.85 (t, *J* = 8.0 Hz, 2H), 3.21 (t, *J* = 8.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.2, 160.0, 145.2, 144.9, 141.3, 135.4(2C), 132.6, 131.9, 130.6, 130.1, 129.6(2C), 128.4, 128.5, 127.9, 127.4, 126.4, 126.0, 125.3(2C), 123.9, 119.6, 54.9, 48.6, 29.9. Anal. calcd for $C_{26}H_{21}ClN_4O_2$: C, 68.34; H, 4.63; N, 12.26% Found C, 68.36; H, 4.69; N, 12.34%

4.1.9. 1-Benzoyl-N-(4-nitrophenyl)-3-phenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide (6ac): Yield: 75%, m.p. 172–175 °C; MS(ESI) m/z 468 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.45 (s, 1H), 8.16 (d, *J* = 12.0 Hz, 2H), 8.03 (d, *J* = 10.0 Hz, 2H), 7.74–7.46 (m, 10H), 4.82 (s, 2H), 3.87 (t, *J* = 6.8 Hz, 2H), 3.26 (t, *J* = 7.2 Hz, 2H); ^{13} C NMR (75 MHz, DMSO-d_6) δ 167.2, 154.4, 150.2, 147.2, 142.6, 141.1, 132.8, 132.3, 131.4, 131.1, 129.2(2C), 129.0(2C), 128.0(2C), 127.1(2C), 124.6(2C), 118.6(2C), 116.6, 41.3, 40.8, 25.2. Anal. calcd for $C_{26}H_{21}N_5O_4$: C, 66.80; H, 4.53; N, 14.98% Found C, 66.88; H, 4.58; N, 14.91%

4.1.10. N-(4-Acetylphenyl)-1-benzoyl-3-phenyl-6, 7-dihydro-1H-pyrazolo [4, 3-c] pyridine-5(4H)-carboxamide (6ad): Yield: 64%, m.p. 166–168 °C; MS(ESI) m/z 465 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (bs, 1H), 7.63 (d, *J* = 6.8 Hz, 2H), 7.42–7.15 (m, 12H), 4.28 (s, 2H), 3.45 (t, *J* = 7.6 Hz, 2H), 3.05 (t, *J* = 7.6 Hz, 2H), 2.46 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 187.6, 172.4, 164.4, 143.6, 142.6, 141.9, 136.7, 136.4, 135.7, 134.2(2C), 132.9, 129.1(2C),

128.6, 128.3, 127.6(2C), 127.1, 126.9, 126.4, 124.7, 122.1, 120.3, 56.9, 45.7, 29.1, 25.9: Anal. calcd for $C_{28}H_{24}N_4O_3$: C, 72.40; H, 5.21; N, 12.06% Found C, 72.45, H, 5.24, N, 12.09%

4.1.11. 1-Benzoyl-3-phenyl-N-p-tolyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5 (4H)-carboxamide (6ae): Yield: 66%, m.p. 163–165 °C; MS(ESI) m/z 437 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (bs, 1H), 7.89–7.21 (m, 14H) ,4.92 (s, 2H), 3.45 (t, *J* = 8.0 Hz, 2H), 3.15 (t, *J* = 7.6 Hz, 2H), 2.40 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.6, 162.4, 139.8, 139.7, 138.6, 137.6, 137.8, 136.8, 134.8, 134.6(2C), 133.8, 133.0, 131.8, 130.8, 127.8, 126.7(2C), 125.1, 124.8(2C), 124.1, 119.9, 56.8, 44.7, 31.7, 25.2; Anal. calcd for $C_{27}H_{24}N_4O_2$: C, 74.29; H, 5.54; N, 12.84% Found C, 74.34; H, 5.59; N, 12.88%.

4.1.12. 1-Benzoyl-N-(4-ethoxyphenyl)-3-phenyl-6, 7-dihydro-1H-pyrazolo [4, 3-c] pyridine-5(4H)-carboxamide(6af): Yield: 68%, m.p. 155–158 °C; MS(ESI) m/z 467 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65–7.30 (m, 15H), 4.70 (s, 2H), 4.21–4.18 (m, 2H), 3.45 (t, *J* = 8.0 Hz, 2H), 2.95 (t, *J* = 7.2 Hz, 2H), 1.42 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 167.4, 158.3, 153.9, 144.6, 144.2, 142.6, 137.9, 136.6, 135.6, 133.4, 132.6(2C), 130.3(2C), 131.1, 128.9, 128.2, 126.7, 125.6, 124.3(2C), 121.9, 116.3, 66.3, 56.3, 47.3, 27.8, 16.9. Anal. calcd for $C_{28}H_{26}N_4O_3$: C, 72.09; H, 5.62; N, 12.01% Found C, 72.12; H, 5.66; N, 12.18%

4.1.13. 1-Benzoyl-N-(naphthalen-1-yl)-3-phenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide (6ag): Yield: 85%, m.p. 185–187 °C; MS(ESI) m/z 473 $[M+H]^+$; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.92 (s, 1H), 8.06 (t, *J* = 7.5 Hz, 2H), 7.93–7.39 (m, 15H), 4.78 (s, 2H), 3.95 (t, *J* = 7.6 Hz, 2H), 3.12 (t, *J* = 8.0 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.3, 156.3, 150.2, 142.8, 135.3, 133.7, 132.8, 132.4, 131.5, 131.1, 129.6, 129.1(2C), 128.9(2C), 128.0(2C), 127.9(2C), 127.0(2C), 125.8, 125.5, 125.2, 123.5, 123.3, 117.0, 41.3, 40.6, 25.3. Anal. Calcd. for $C_{30}H_{24}N_4O_2$: C, 76.25; H, 5.12; N, 11.86 Found C, 76.29; H, 5.16; N, 11.90%

4.1.14. 1-Benzoyl-N-benzyl-3-phenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide (6ah): Yield: 64%, m.p. 176–178 °C; MS(ESI) m/z 437 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (t, 1H), 7.65–7.18 (m, 15H), 4.92 (s, 2H), 4.65 (d, *J* = 6.8 Hz, 2H), 3.75 (t, *J* = 8.0 Hz, 2H), 2.96 (t, *J* = 7.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.4, 159.5, 138.5, 138.0, 137.4, 136.3, 135.9, 133.8, 133.0, 132.3(2C), 131.8, 129.0, 127.5, 126.4, 123.8, 122.4(2C), 121.6, 121.0(2C), 120.6, 120.0, 51.8, 43.7, 39.3, 22.5. Anal. calcd for $C_{27}H_{24}N_4O_2$: C, 74.29; H, 5.54; N, 12.84% Found C, 74.36; H, 5.52; N, 12.92%.

4.1.15. 1-Benzoyl-N-isopropyl-3-phenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide (ai): Yield: 60%, m.p. 144–146 °C; MS(ESI) m/z 389 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86–7.58 (m, 10H), 4.42 (s, 2H), 4.23 (m, 1H), 3.65 (t, *J* = 7.2 Hz, 2H), 3.06 (t, *J* = 7.6 Hz, 2H); 1.38 (d, *J* = 9.6 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.4, 158.3, 142.7, 142.2, 141.7, 140.1, 133.5, 133.1, 131.7, 130.9(2C), 130.3, 130.0, 127.5, 126.9, 126.6, 125.1, 51.3, 43.9, 40.5, 29.7, 21.6(2C). Anal. calcd for $C_{23}H_{24}N_4O_2$: C, 71.11; H, 6.23; N, 14.42% Found C, 71.21; H, 6.24; N, 14.52%.

4.1.16. 1-Benzoyl-N-(4-chlorophenyl)-3-phenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carbothioamide (7aa): Yield: 75%, m.p. 176–178 °C; MS(ESI) m/z 473 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.28 (bs, 1H), 8.05–7.40 (m, 14H), 4.95 (s, 2H), 3.82 (t, *J* = 7.6 Hz, 2H), 3.12 (t, *J* = 7.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.3, 163.9, 144.3, 144.0, 143.5, 141.8, 138.9, 137.8, 136.5, 135.9, 135.5, 134.9, 134.2, 133.9, 132.9, 129.6(2C), 129.1, 128.6, 127.6(2C), 126.7, 125.8, 59.6, 49.9, 27.3. Anal. calcd for $C_{26}H_{21}ClN_4OS$: C, 66.02; H, 4.48; N, 11.85% Found C, 66.12; H, 4.54; N, 11.89%.

4.1.17. 1-Benzoyl-N-(4-fluorophenyl)-3-phenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carbothioamide (7ab): Yield: 81%, m.p. 176–177 °C; MS(ESI) m/z 473 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (s, 1H), 7.92 (d, *J* = 8.0 Hz, 2H), 7.80 (d, *J* = 8.4 Hz, 2H), 7.62–7.35 (m,

10H), 4.85 (s, 2H), 3.92 (t, *J* = 7.2 Hz, 2H), 3.06 (t, *J* = 7.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.7, 159.6, 153.7, 144.3, 144.1, 143.7, 142.5, 139.9, 138.6, 135.9, 134.4, 133.8, 132.9, 132.3, 131.9, 130.7(2C), 129.6, 129.0, 128.5(2C), 126.3, 124.8, 61.6, 52.7, 26.9. Anal. calcd for $C_{26}H_{21}FN_4OS$: C, 68.40; H, 4.64; N, 12.27% Found C, 68.54; H, 4.68; N,12.31%

4.1.18 1-Benzoyl-3-phenyl-N-p-tolyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carbothioamide (7ac): Yield: 69%, m.p. 189–190 °C; MS(ESI) m/z 453 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85–7.21 (m, 13H), 7.18 (d, *J* = 7.6 Hz, 2H), 4.85 (s, 2H), 3.58 (t, *J* = 7.6 Hz, 2H), 2.95 (t, *J* = 7.6 Hz, 2H), 2.52 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.4, 165.7, 138.9, 137.4, 137.0, 136.5, 136.0, 134.9, 134.3, 134.1(2C), 131.8, 130.0, 129.8, 127.4, 127.0, 125.9(2C), 123.8(2C), 123.4, 122.1, 118.8, 58.9, 46.8, 29.5, 22.8. Anal. calcd for $C_{27}H_{24}N_4OS$: C, 71.65; H, 5.35; N, 12.38% Found C, 71.73; H, 5.40; N, 12.45%.

4.1.19. 1-Benzoyl-N-(4-methoxyphenyl)-3-phenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carbothioamide (7ad): Yield: 73%, m.p. 169–172 °C; MS(ESI) m/z 469 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.08 (s, 1H), 7.72–6.98 (m, 14H), 4.75 (s, 2H), 4.05 (s, 3H) 3.82 (t, *J* = 7.6 Hz, 2H), 2.89 (t, *J* = 7.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.6, 163.7, 156.8, 142.6, 142.2, 141.6, 141.0, 139.2, 138.4, 137.8, 133.9, 132.6, 129.3(2C), 129.1, 128.6, 127.0, 126.5, 124.9, 122.9(2C), 120.9, 119.4, 60.9, 52.7, 44.1, 23.4. Anal. calcd for $C_{27}H_{24}N_4O_2S$: C, 69.21; H, 5.16; N, 11.96% Found C, 69.24; H, 5.19; N, 12.04%.

4.1.20. 1-Benzoyl-N-benzyl-3-phenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carbothioamide (7ae): Yield: 61%, m.p. 180–181 °C; MS(ESI) m/z 453 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.15 (t, *J* = 7.6 Hz, 1H), 7.69–7.28 (m, 15H), 4.89 (s, 2H), 4.45 (d, *J* = 6.8 Hz, 2H), 3.85 (t, *J* = 7.6 Hz, 2H), 2.88 (t, *J* = 7.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.4, 155.5, 139.5, 136.9, 135.4, 134.9, 134.0, 133.8, 133.2, 132.8(2C), 1320, 128.9, 127.8, 127.3,

125.9, 125.4(2C), 124.4, 121.8(2C), 120.8, 119.5, 55.8, 43.5, 39.9, 20.9. Anal. calcd for $C_{27}H_{24}N_4OS$: C, 71.65; H, 5.35; N, 12.38% Found C, 71.69; H, 5.39; N, 12.41%

4.1.21. N-Allyl-1-benzoyl-3-phenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carbothioamide (7af): Yield: 84%, m.p. 184–186 °C; MS(ESI) m/z 403 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99–7.16 (m, 11H), 6.02 (m, 1H), 5.23 (m, 2H), 4.79 (s, 2H), 4.38 (m, 2H), 3.96 (t, *J* = 8.0 Hz, 2H), 2.95 (t, *J* = 7.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.5, 165.7, 140.7, 140.2, 133.5, 132.9, 131.9, 130.5(2C), 130.3, 129.5, 127.5, 126.5(2C), 126.2, 125.6, 124.5, 119.1, 114.3, 51.3, 45.9, 43.2, 21.6. Anal. calcd for $C_{23}H_{22}N_4OS$: C, 68.63; H, 5.51; N, 13.92% Found C, 68.69; H, 5.56; N, 13.99%.

4.1.22. (5-(4-fluorophenylsulfonyl)-3-phenyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)(phenyl)methanone (9aa): Yield: 71%, m.p. 193–195 °C; MS(ESI) m/z 462 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.02–7.91 (m, 4H), 7.7–7.42 (m, 10H), 4.45 (s, 2H), 3.55 (t, *J* = 7.6 Hz, 2H), 3.20 (t, *J* = 7.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.8, 167.1, 163.7, 150.8, 141.7, 133.5, 133.0, 132.3, 131.7(2C), 130.3, 130.2, 129.3, 129.0(2C), 128.0(2C), 127.2(2C), 116.8, 116.5, 115.0, 43.1, 42.9, 25.5. Anal. calcd for $C_{25}H_{20}FN_3O_3S$: C, 65.06; H, 4.37; N, 9.10% Found C, 65.12; H, 4.41; N, 9.16%

4.1.23. (5-(4-nitrophenylsulfonyl)-3-phenyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)(phenyl)methanone (9ab): Yield: 64%, m.p. 187–189 °C; MS(ESI) m/z 489 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (d, *J* = 9.2 Hz, 2H), 7.78 (d, *J* = 8.4 Hz, 2H), 7.72–7.39 (m, 10H), 4.86 (s, 2H), 3.95 (t, *J* = 8.0 Hz, 2H), 3.28 (t, *J* = 8.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.3, 144.8, 143.6, 143.1(2C), 139.8, 133.5, 132.2, 131.7, 130.6, 129.5, 129.2, 128.7(2C), 127.6, 127.0, 125.8, 124.9, 122.8(2C), 120.4, 120.8, 55.9, 42.8, 23.4. Anal. calcd for $C_{25}H_{20}N_4O_5S$: C, 61.47; H, 4.13; N, 11.47% Found C, 61.54; H, 4.19; N, 11.54%.

4.1.24. 1-(4-(1-Benzoyl-3-phenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridin-5(4H)-ylsulfonyl)phenyl)ethanone (9ac): Yield: 72%, m.p. 176–179 °C; MS(ESI) m/z 486 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83–6.96 (m, 14H), 4.68 (s, 2H), 3.65 (t, *J* = 7.6 Hz, 2H), 3.05 (t, *J* = 8.0 Hz, 2H), 2.52 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.9, 168.5, 143.6, 142.9, 142.2, 139.9, 138.4, 137.9, 136.7, 134.7, 133.9, 132.6(2C), 131.6, 129.6, 127.6(2C), 126.6, 125.2, 124.6, 124.0, 123.1, 122.3, 57.9, 46.3, 29.5, 22.5. Anal. calcd for $C_{27}H_{23}N_3O_4S$: C, 66.79; H, 4.77; N, 8.65% Found C, 66.84; H, 4.79; N, 8.69%

4.1.25. Phenyl(3-phenyl-5-tosyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methanone (9ad): Yield: 81%, m.p. 120–123 °C; MS(ESI) m/z 458 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99–7.21 (m, 14H), 4.62 (s, 2H), 3.65 (t, *J* = 7.6 Hz, 2H), 3.09 (t, *J* = 8.0 Hz, 2H), 2.42 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.4, 137.9, 137.4, 136.8, 136.5, 136.0, 133.9, 133.1, 131.4(2C), 129.8, 129.2, 128.8, 127.5, 126.7, 125.5(2C), 124.6(2C), 122.5, 121.5, 119.9, 59.5, 48.8, 31.5, 23.4. Anal. calcd for $C_{26}H_{23}N_3O_3S$: C, 68.25; H, 5.07; N, 9.18% Found C, 68.30; H, 5.11; N, 9.21%.

4.1.26. Phenyl(3-phenyl-5-(thiophen-2-ylsulfonyl)-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methanone (9ae): Yield: 75%, m.p. 221–223 °C; MS(ESI) m/z 450 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79–7.01 (m, 13H), 4.22 (s, 2H), 3.25 (t, *J* = 7.6 Hz, 2H), 2.86 (t, *J* = 7.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.2, 146.9, 145.3, 139.3, 138.7, 137.4, 136.7, 131.8, 131.5, 130.5, 128.4, 126.6, 125.7, 124.3, 122.8, 121.9, 120.4, 119.6, 119.2, 118.6, 56.7, 45.9, 23.6. Anal. Calcd. for $C_{23}H_{19}N_3O_3S_2$: C, 61.45; H, 4.26; N, 9.35% Found C, 61.50; H, 4.30; N, 9.40%.

4.1.27. N-(4-Bromophenyl)-1-(cyclohexanecarbonyl)-3-phenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide (6ba): Yield: 84%, m.p. 173–176 °C; MS(ESI) m/z 507 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (d, *J* = 9.6 Hz, 2H), 7.76 (d, *J* = 9.6 Hz, 2H), 7.62–7.48 (m,

6H), 4.84 (s, 2 H), 3.88 (t, *J* = 7.6 Hz, 2H), 3.52 (t, *J* = 8.0 Hz, 2H), 2.36–1.25 (m, 11H): ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5, 165.6, 144.7, 142.9, 132.3, 131.3, 128.9(2C), 127.5, 126.4(2C), 122.5(2C), 121.9(2C), 120.2, 119.4, 60.1, 49.4, 28.6(2C), 25.8(2C), 23.8, 23.4, 22.5. Anal. Calcd. for $C_{26}H_{27}BrN_4O$: C, 61.54; H, 5.36; N, 11.04% Found C, 61.60; H, 5.40; N, 11.12%.

4.1.28. N-(4-Chlorophenyl)-1-(cyclohexanecarbonyl)-3-phenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide (6bb): Yield: 92%, m.p. 160–162 °C; MS(ESI) m/z 463 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, *J* = 10.4 Hz, 2H), 7.86 (d, *J* = 9.6 Hz, 2H), 7.68–7.46 (m, 5H), 6.52 (s, 1H) 4.74 (s, 2H), 3.82 (t, *J* = 7.6 Hz, 2H), 3.42 (t, *J* = 8.0 Hz, 2H), 2.25–1.25 (m, 11H): ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.6, 151.7, 145.6, 143.7, 131.6, 130.8, 129.4(2C), 125.7, 121.6(2C), 121.0(2C), 119.9(2C), 119.2, 117.9, 61.5, 48.6, 29.3(2C), 26.2(2C), 24.9, 24.4, 23.4. Anal. calcd for $C_{26}H_{27}ClN_4O_2$: C, 67.45; H, 5.88; N, 12.10% Found C, 67.54; H, 5.93; N, 12.23%.

4.1.29. 1-(Cyclohexanecarbonyl)-N-(4-nitrophenyl)-3-phenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide (6bc): Yield: 62%, m.p. 157–159 °C; MS(ESI) m/z 474 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98–7.42 (m, 10H), 4.84 (s, 2H), 3.95 (t, *J* = 7.2 Hz, 2H), 3.08 (t, *J* = 7.6 Hz, 2H), 2.30 (s, 1H), 2.05–1.25 (m, 10H): ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.4, 166.6, 154.7, 153.7, 143.6, 136.8, 132.5(2C), 124.8, 123.7(2C), 122.6(2C), 121.2(2C), 119.2, 117.9, 59.6, 38.8, 26.3(2C), 25.8, 26.9(2C), 23.9, 22.1. Anal. calcd for $C_{26}H_{27}N_5O_4$: C, 65.95; H, 5.75; N, 14.79% Found C, 65.99; H, 5.83; N, 14.88%.

4.1.30. N-(4-Acetylphenyl)-1-(cyclohexanecarbonyl)-3-phenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide (6bd): Yield: 78%, m.p. 169–171 °C; MS(ESI) m/z 471 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, *J* = 9.6 Hz, 2H), 7.72 (d, *J* = 9.2 Hz, 2H), 7.56–7.44 (m, 5H), 6.75 (s, 1H), 4.79 (s, 2H), 3.82 (t, *J* = 7.6 Hz, 2H), 3.32 (t, *J* = 7.6 Hz, 2H), 2.55 (s, 3H),

2.16–1.21 (m, 11H): ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.3, 164.3, 154.3, 148.6, 145.6, 136.5, 133.5(2C), 128.4, 125.7(2C), 125.4, 124.8(2C), 122.7(2C), 121.6, 120.4, 58.5, 46.5, 37.6, 35.2, 28.4, 26.5(2C), 24.8, 24.3, 21.6. Anal. calcd for $C_{28}H_{30}N_4O_3$: C, 71.47; H, 6.43; N, 11.91% Found C, 71.52; H, 6.48; N, 11.85%.

4.1.31. 1-(Cyclohexanecarbonyl)-3-phenyl-N-p-tolyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide (6be): Yield: 88%, m.p. 171–172 °C; MS(ESI) m/z 443 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (d, *J* = 10.8 Hz, 2H), 7.72 (d, *J* = 9.6 Hz, 2H), 7.78–7.52 (m, 5H), 6.78 (s, 1H) 4.59 (s, 2H), 3.82 (t, *J* = 7.6 Hz, 2H), 3.18 (t, *J* = 8.0 Hz, 2H), 2.39 (s, 3H), 2.26 (s, 1H), 2.05–1.25 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.6, 161.3, 155.9, 151.6, 141.5, 139.5, 133.7(2C), 125.6, 124.6(2C), 124.4, 122.6, 121.9(2C), 118.5, 118.0, 58.9, 39.5, 34.9, 27.6(2C), 28.8, 26.5(2C), 24.3, 23.4. Anal. calcd for $C_{27}H_{30}N_4O_2$: C, 73.28; H, 6.83; N, 12.66% Found C, 73.32; H, 6.92; N, 12.69%.

4.1.32. 1-(Cyclohexanecarbonyl)-N-(4-methoxyphenyl)-3-phenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide (6bf): Yield: 76%, m.p. 147–149 °C; MS(ESI) m/z 459 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, *J* = 11.2 Hz, 2H), 7.76 (d, *J* = 9.6 Hz, 2H), 7.75–7.49 (m, 5H), 6.75 (s, 1H) 4.76 (s, 2H), 3.95 (t, *J* = 8.0 Hz, 2H), 3.90 (s, 3H), 3.38 (t, *J* = 6.8 Hz, 2H), 2.05–1.25 (m, 11H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.5, 160.3, 156.3, 152.2, 146.1, 141.5, 135.6(2C), 129.4, 128.6(2C), 125.4, 124.6, 123.2(2C), 119.5, 117.9, 57.6, 49.9, 38.4, 34.0, 29.4, 27.9(2C), 25.9, 24.3, 23.4. Anal. calcd for $C_{27}H_{30}N_4O_3$: C, 70.72; H, 6.59; N, 12.22% Found C, 70.76; H, 6.66; N, 12.25%.

4.1.33. 1-(Cyclohexanecarbonyl)-N-(4-ethoxyphenyl)-3-phenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide (6bg): Yield: 64%, m.p. 177–178 °C; MS(ESI) m/z 473 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05–7.56 (m, 9H), 4.69 (s, 2H), 4.11–4.03 (m, 2H), 3.68 (t, *J* = 8.4 Hz, 2H), 3.12 (t, *J* = 8.0 Hz, 2H), 2.41 (s, 1H), 2.45–1.25 (m, 10H), 1.39 (t, *J* = 8.0 Hz, 3H):

^{13}C NMR (100 MHz, $CDCl_3$) δ 176.9, 164.7, 155.4, 149.6, 147.5, 140.1, 134.2(2C), 125.2, 124.7(2C), 123.6, 123.0, 121.4(2C), 118.2, 115.7, 60.5, 54.9, 48.9, 39.6, 33.8, 27.7, 26.3(2C), 24.9(2C), 21.9. Anal. calcd for $C_{28}H_{32}N_4O_3$: C, 71.16; H, 6.83; N, 11.86% Found C, 71.21; H, 6.91; N, 11.92%.

4.1.34. 1-(Cyclohexanecarbonyl)-N-(naphthalen-1-yl)-3-phenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide (6bh): Yield: 74%, m.p. 184–186 °C; MS(ESI) m/z 478 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05–7.15 (m, 13H), 4.66 (s, 2H), 3.72 (t, *J* = 7.6 Hz, 2H), 3.03 (t, *J* = 8.0 Hz, 2H), 2.19–1.30 (m, 11H): ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.8, 166.6, 145.6, 141.7, 138.7, 136.6, 135.2(2C), 134.3(2C), 131.6, 126.3, 126.0, 125.8, 124.6(2C), 123.4, 122.7(2C), 120.6, 119.5, 60.6, 47.2, 29.4(2C), 28.6(2C), 26.3, 24.6, 24.1. Anal. calcd for $C_{30}H_{30}N_4O_2$: C, 75.29; H, 6.32; N, 11.71% Found C, 75.32; H, 6.29; N, 11.82%.

4.1.35. N-Benzyl-1-(cyclohexanecarbonyl)-3-phenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carboxamide (6bi): Yield: 69%, m.p. 176–178 °C; MS(ESI) m/z 443 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98–7.28 (m, 10H), 6.78 (bs, 1H), 4.82 (s, 2H), 4.64 (d, 2H, *J* = 8.8 Hz), 3.66 (t, *J* = 7.6 Hz, 2H), 3.21 (t, *J* = 8.0 Hz, 2H), 2.34 (m, 1H), 2.19–1.30 (m, 10H): ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.6, 162.9, 149.3, 144.5, 139.5, 138.6, 137.6, 136.4(2C), 124.9, 124.2, 123.6, 121.9, 120.4(2C), 119.6, 118.4, 59.5, 55.4, 34.5, 28.5(2C), 28.4(2C), 27.4, 23.6, 22.5. Anal. Calcd. for $C_{27}H_{30}N_4O_2$: C, 73.28; H, 6.83; N, 12.66% Found C, 73.43; H, 6.92; N, 12.75%.

4.1.36. N-(4-Chlorophenyl)-1-(cyclohexanecarbonyl)-3-phenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carbothioamide (7ba): Yield: 67%, m.p. 192–195 °C; MS(ESI) m/z 479 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.05 (s, 1H), 8.10 (d, *J* = 7.2 Hz, 2H), 7.92 (d, *J* = 7.2 Hz, 2H), 8.12–7.45 (m, 5H), 4.95 (s, 2H), 3.88 (t, *J* = 7.6 Hz, 2H), 3.02 (t, *J* = 7.2 Hz, 2H); 2.18–1.28 (m, 11H): ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.5, 172.6, 152.6, 143.7, 143.4, 141.6, 137.5, 137.2, 136.3, 132.7, 131.9, 128.5, 124.3(2C), 123.7, 119.3, 116.7, 59.2, 54.6, 31.6, 26.6(2C),

26.1(2C), 24.3, 22.5. Anal. calcd for $C_{26}H_{27}ClN_4OS$: C, 65.19; H, 5.68; N, 11.70% Found C, 65.27; H, 5.76; N, 11.79%.

4.1.37. 1-(Cyclohexanecarbonyl)-N-(4-fluorophenyl)-3-phenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carbothioamide (7bb): Yield: 62%, m.p. 180–182 °C; MS(ESI) m/z 463 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (s, 1H), 7.92 (d, *J* = 8.4 Hz, 2H), 7.85 (d, *J* = 8.4 Hz, 2H), 7.62–7.35 (m, 5H), 4.75 (s, 2H), 3.85 (t, *J* = 7.6 Hz, 2H), 3.06 (t, *J* = 6.8 Hz, 2H), 2.31(m, 1H), 2.11–1.28 (m, 10H): ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.7, 179.7, 151.8, 145.6, 144.6, 142.2, 140.9, 139.4, 136.4, 133.3, 131.6, 127.3, 124.7(2C), 123.4, 121.3(2C), 59.8, 58.5, 32.5, 27.8(2C), 26.5(2C), 25.6, 23.8. Anal. calcd for $C_{26}H_{27}FN_4OS$: C, 67.51; H, 5.88; F, 4.11; N, 12.11% Found C, 67.60; H, 5.93; N, 12.22%.

4.1.38. 1-(Cyclohexanecarbonyl)-N-(4-nitrophenyl)-3-phenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carbothioamide (7bc): Yield: 74%, m.p. 178–180 °C; MS(ESI) m/z 490 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98–7.42 (m, 10H), 4.84 (s, 2H), 3.95 (t, *J* = 7.2 Hz, 2H), 3.08 (t, *J* = 7.6 Hz, 2H), 2.30 (s, 1H), 2.05–1.25 (m, 10H): ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.4, 176.3, 153.4, 142.8, 141.6, 140.4, 139.4, 138.3, 136.5, 135.3, 129.9, 127.5, 123.9(2C), 122.5, 120.3, 119.5, 61.5, 49.6, 32.3, 27.4(2C), 25.7(2C), 25.6, 22.9. Anal. calcd for $C_{26}H_{27}N_5O_3S$: C, 63.78; H, 5.56; N, 14.30%. Found C, 63.83; H, 5.62; N, 14.41%.

4.1.39. 1-(Cyclohexanecarbonyl)-3-phenyl-N-p-tolyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carbothioamide (7bd): Yield: 75%, m.p. 170–174 °C; MS(ESI) m/z 459 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.92–7.35 (m, 9H), 7.20 (s, 1H), 4.93 (s, 2H), 3.71 (t, *J* = 6.8 Hz, 2H), 2.86 (t, *J* = 7.2 Hz, 2H), 2.49 (s, 3H), 2.24–1.35 (m, 11H): ^{13}C NMR (100 MHz, DMSO-d_6) δ 186.7, 166.7, 148.4, 143.9, 142.4, 139.9, 137.2, 136.9, 134.7, 133.9, 132.3, 132.0, 128.9, 125.7(2C), 123.4, 120.7(2C), 56.2, 45.0, 33.3, 27.7(2C), 25.0(2C), 24.3, 22.7. Anal. calcd for $C_{27}H_{30}N_4OS$: C, 70.71; H, 6.59; N, 12.22% Found C, 70.78; H, 6.69; N, 12.26%.

4.1.40. 1-(Cyclohexanecarbonyl)-N-(4-methoxyphenyl)-3-phenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carbothioamide (7be): Yield: 69%, m.p. 185–188 °C; MS(ESI) m/z 475 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.12 (s, 1H), 7.92–7.38 (m, 9H), 4.85 (s, 2H), 4.18 (s, 3H) 3.69 (t, *J* = 7.6 Hz, 2H), 2.95 (t, *J* = 8.0 Hz, 2H), 2.35 (m, 1H), 2.05–1.25 (m, 10H): ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.2, 163.3, 143.9, 141.4, 139.3, 138.6, 137.4, 136.5, 133.5(2C), 132.7, 131.6, 130.7, 129.6(2C), 124.1(2C), 123.7, 122.7(2C), 62.1, 57.9, 36.1, 31.3, 27.6(2C), 25.9(2C), 24.5, 24.3. Anal. calcd for $C_{27}H_{30}N_4O_2S$: C, 68.33; H, 6.37; N, 11.80%. Found C, 68.45; H, 6.46; N, 11.92%.

4.1.41. N-Benzyl-1-(cyclohexanecarbonyl)-3-phenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridine-5(4H)-carbothioamide (7bf): Yield: 61%, m.p. 189–192 °C; MS(ESI) m/z 459 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.15 (bs, 1H), 7.81–7.42 (m, 10H), 4.79 (s, 2H), 4.39 (d, *J* = 7.2 Hz, 2H), 3.99 (t, *J* = 7.6 Hz, 2H), 3.08 (t, *J* = 8.0 Hz, 2H), 2.25–1.35 (m, 11H): ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.8, 162.7, 142.9, 140.7, 138.7, 137.2, 135.3, 134.7, 133.9, 132.6, 131.4, 129.7, 124.3(2C), 123.7, 121.4(2C), 58.7, 56.1, 36.6, 34.6, 27.4(2C), 26.1(2C), 25.2, 23.2. Anal. calcd for $C_{27}H_{30}N_4OS$: C, 70.71; H, 6.59; N, 12.22%. Found C, 71.01; H, 6.68; N, 12.54%.

4.1.42. Cyclohexyl(5-(4-fluorophenylsulfonyl)-3-phenyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methanone (9ba): Yield: 62%, m.p. 186–188 °C; MS(ESI) m/z 468 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.02–7.38 (m, 9H), 4.56 (s, 2H), 3.65 (t, *J* = 8.4 Hz, 2H), 3.08 (t, *J* = 8.0 Hz, 2H), 2.19–1.16 (m, 11H): ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.8, 148.1, 140.6, 138.8, 137.5, 136.4(2C), 135.8, 134.8, 133.7, 130.7, 126.6, 123.2(2C), 119.5, 118.9, 62.5, 39.2, 34.5, 29.2(2C), 25.7(2C), 24.4, 22.3. Anal. calcd for $C_{25}H_{26}FN_3O3S$: C, 64.22; H, 5.60; N, 8.99%. Found 64.32; H, 5.67; N, 9.20%.

4.1.43. Cyclohexyl(5-(4-nitrophenylsulfonyl)-3-phenyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methanone (9bb): Yield: 81%, m.p.192–193 °C; MS(ESI) m/z 495 $[M+H]^+$; ^{1}H

NMR (400 MHz, $CDCl_3$) δ 7.93–7.29 (m, 9H), 4.76 (s, 2H), 3.88 (t, *J* = 7.6 Hz, 2H), 3.31 (t, *J* = 8.0 Hz, 2H), 2.25–1.30 (m, 11H): ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.6, 142.6, 139.6, 138.4, 136.3, 134.6(2C), 133.5, 132.7, 132.2, 129.6, 127.8, 121.6(2C), 120.7, 119.7, 59.6, 45.2, 33.6, 28.6(2C), 26.9(2C), 25.7, 24.3. Anal. calcd for $C_{25}H_{26}N_4O_5S$: C, 60.71; H, 5.30; N, 11.33%. Found C. 60.83; H, 5.36; N, 11.39%.

4.1.44. 1-(4-(1-(Cyclohexanecarbonyl)-3-phenyl-6,7-dihydro-1H-pyrazolo[4,3-c]pyridin-5(4H)-ylsulfonyl)phenyl)ethanone (9bc): Yield: 84%, m.p. 206–208 °C; MS(ESI) m/z 492 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 7.78–6.92 (m, 9H), 4.72 (s, 2H), 3.75 (t, *J* = 8.0 Hz, 2H), 3.16 (t, *J* = 7.6 Hz, 2H), 2.49 (s, 3H) 2.21–1.28 (m, 11H): ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.4, 171.5, 148.5, 139.6, 138.3, 137.5, 136.7(2C), 135.7, 134.6, 131.4, 127.5, 126.2, 122.5, 120.1, 119.8, 118.4, 63.4, 51.6, 49.6, 32.7, 28.3(2C), 27.4(2C), 24.8, 21.9. Anal. calcd for $C_{27}H_{29}N_3O_4S$: C, 65.97; H, 5.95; N, 8.55%. Found C, 66.02; H, 5.99; N, 8.64%

4.1.45. Cyclohexyl(3-phenyl-5-tosyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methanone (9bd): Yield: 69%, m.p. 194–196 °C; MS(ESI) m/z 464 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 7.86–7.34 (m, 9H), 4.85 (s, 2H), 3.75 (t, *J* = 8.0 Hz, 2H), 3.12 (t, *J* = 7.6 Hz, 2H), 2.56 (s, 3H), 2.25–1.32 (m, 11H): ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.9, 146.5, 139.4, 136.9, 133.4, 132.3(2C), 130.3, 129.7(2C), 127.8, 126.3(2C), 122.7(2C), 121.7, 119.5, 64.1, 38.8, 35.7, 30.3(2C), 26.8(2C), 25.8, 23.6. Anal. calcd for $C_{26}H_{29}N_3O_3S$: C, 67.36; H, 6.31; N, 9.06%. Found C, 67.43; H, 6.39; N, 9.16%.

4.1.46. Cyclohexyl(3-phenyl-5-(thiophen-2-ylsulfonyl)-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridin-1-yl)methanone (9be): Yield: 75%, m.p. 210–214 °C; MS(ESI) m/z 456 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 7.69–6.98 (m, 8H), 4.42 (s, 2H), 3.32 (t, *J* = 7.6 Hz, 2H), 2.98 (t, *J* = 8.0 Hz, 2H), 2.30 (m, 1H), 2.22–1.31 (m, 10H): ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.6, 145.6, 138.3, 136.7, 131.8, 129.6, 125.7, 124.7, 123.6, 122.6, 121.4, 120.4, 119.2, 118.6, 56.9, 46.9,

31.3(2C), 27.8(2C), 26.7, 24.5, 23.6. Anal. calcd for $C_{23}H_{25}N_3O_3S_2$: C, 60.63; H, 5.53; N, 9.22%. Found C, 60.73; H, 5.59; N, 9.31%.

4.2. Biological activity

4.2.1. Protein expression and MTB PS screening

The *M. tuberculosis panC* gene (Rv3602c) encoding the pantothenate synthetase was cloned and transformed into BL21 (DE3) cells and the expression of the protein was performed as reported in literature [2]. For the assay, in a 96-well plate, 60 μL of PS reagent mix containing NADH, pantoic acid, β-alanine, ATP, phosphoenolpyruvate, $MgCl_2$, myokinase, pyruvate kinase, and lactate dehydrogenase in buffer was added. Compounds were then added to plates in 1-μL volumes. The reaction was initiated with the addition of 39 μL of PS, diluted in buffer. The test plate was immediately transferred to a microplate reader, and absorbance was measured at 340 nm every 12 sec for 120 sec [2]. Percentage inhibition was calculated using following formula,

$$\%\text{Inhibition} = 100 \text{ X } \frac{1\text{- compound rate-background rate}}{\text{full reaction rate-background rate}}$$

4.2.2. In-vitro MTB screening

Two-fold serial dilutions of each test compound/drug were prepared and incorporated into Middle- brook 7H11 agar medium with oleic acid, albumin, dextrose, and catalase (OADC) growth supplement to get final concentrations of 50, 25, 12.5, 6.25, 3.13, 1.56, and 0.78 μg/mL. Inoculum of *M. tuberculosis* H37Rv ATCC 27294 was prepared from fresh Middlebrook 7H11 agar slants with OADC (Difco) growth supplement adjusted to 1 mg/ mL (wet weight) in Tween

80 (0.05%) saline diluted to 10^{-2} to give a concentration of $\sim 10^7$cfu/mL. Five microliters of this

bacterial suspension was spotted onto 7H11 agar tubes containing different concentrations of the drug as discussed above. The tubes were incubated at 37 °C, and final readings (as MIC in μg/mL) were determined after 28 days. The MIC is defined as the minimum concentration of compound required to give complete inhibition of bacterial growth. This method is similar to that recommended by the National Committee for Clinical Laboratory Standards for the determination of MIC in triplicate [11].

4.2.3. In vitro cytotoxicity screening

Some compounds were further examined for toxicity in a RAW 264.7 cell line at the concentration of 50 μM. After 72 h of exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega Cell Titer 96 non-radioactive cell proliferation assay [12].

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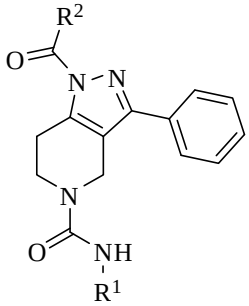
Figure 1: MTB PS inhibitors [6-9]

Figure 2: Compound (**6aa**) identified from our database by virtual High Throughput Screening (vHTS)

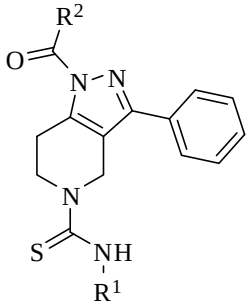
Figure 3: Dose response curve for compound **6ac**.

Scheme 1: Synthetic protocol of titled compounds

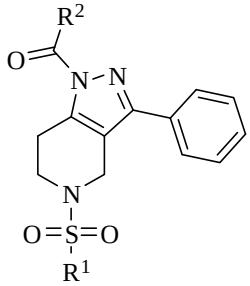
Table 1: Biological activities of synthesized compounds



6aa - 6ai
6ba - 6bi



7aa - 7af
7ba - 7bf



9aa - 9ae
9ba - 9be

Compd	R^1	R^2	% inhibition at 100 µM (IC_{50}) against MTB PS	MTB MIC in µM	Cytotoxicity at 50 µM (RAW 264.7 cells) % inhibition
6aa	4-Bromophenyl	Phenyl	60.6 (82.1±2.3)	99.7	42.0
6ab	4-Chlorophenyl	Phenyl	40.2	109.43	4.9
6ac	4-Nitrophenyl	Phenyl	95.7 (21.8±0.8)	26.7	0
6ad	4-Acetylphenyl	Phenyl	43.7	>107.6	12.8
6ae	p-Tolyl	Phenyl	49.4	114.1	57.1
6af	4-Ethoxyphenyl	Phenyl	47.2	>107.1	0
6ag	1-Naphthyl	Phenyl	89.8 (38.2±1.7)	48.8	4.7
6ah	Benzyl	Phenyl	46.2	114.1	0.7
6ai	Isopropyl	Phenyl	40.9	128.7	10.6
7aa	4-Chlorphenyl	Phenyl	37.7	105.7	0
7ab	4-Fluorophenyl	Phenyl	43.5	109.5	0.5
7ac	p-Tolyl	Phenyl	23.9	>110.4	0
7ad	4-Methoxyphenyl	Phenyl	10.2	106.7	0
7ae	Benzyl	Phenyl	52.1	110.4	0
7af	Allyl	Phenyl	41.7	>124.22	6
9aa	4-Fluorophenyl	Phenyl	77.0 (38.6±0.9)	54.17	0
9ab	4-Nitrophenyl	Phenyl	39.7	25.58	18.6
9ac	4-Acetylphenyl	Phenyl	78.5 (39.1±2.2)	51.49	5.3
9ad	p-Tolyl	Phenyl	9.0	109.31	0
9ae	Thiophen-2-yl	Phenyl	48.7	55.61	6.8
6ba	4-Bromophenyl	Cyclohexyl	50.5	98.54	0
6bb	4-Chlorphenyl	Cyclohexyl	54.7	108.0	4.0
6bc	4-Nitrophenyl	Cyclohexyl	50.8	52.79	14.9
6bd	4-Acetylphenyl	Cyclohexyl	51.4	108.1	22.5
6be	p-Tolyl	Cyclohexyl	40.8	56.49	15.1
6bf	4-Methoxyphenyl	Cyclohexyl	38.7	109.0	52.5
6bg	4-Ethoxyphenyl	Cyclohexyl	48.5	105.8	4.9
6bh	1-Naphthyl	Cyclohexyl	50.9	52.19	4.1
6bi	Benzyl	Cyclohexyl	49.1	54.51	35.7
7ba	4-Chlorphenyl	Cyclohexyl	56.3	109.0	51.1
7bb	4-Fluorophenyl	Cyclohexyl	49.2	105.3	58.4
7bc	4-Nitrophenyl	Cyclohexyl	47.3	53.5	0
7bd	*p*-Tolyl	Cyclohexyl	50.5	51.07	27.8
7be	4-Methoxyphenyl	Cyclohexyl	50.6	101.7	9.5
7bf	Benzyl	Cyclohexyl	51.4	108.0	20.6
9ba	4-Fluorophenyl	Cyclohexyl	46.8	53.5	1.4
9bb	4-Nitrophenyl	Cyclohexyl	50.2	50.5	8.3
9bc	4-Acetylphenyl	Cyclohexyl	39.2	50.8	11.5
9bd	p-Tolyl	Cyclohexyl	41.6	53.9	0

9be	Thiophen-2-yl	Cyclohexyl	52.0	54.9	8.1
	Isoniazid		0	0.72	-
	Ethambutol		0	15.28	-

Figure 1

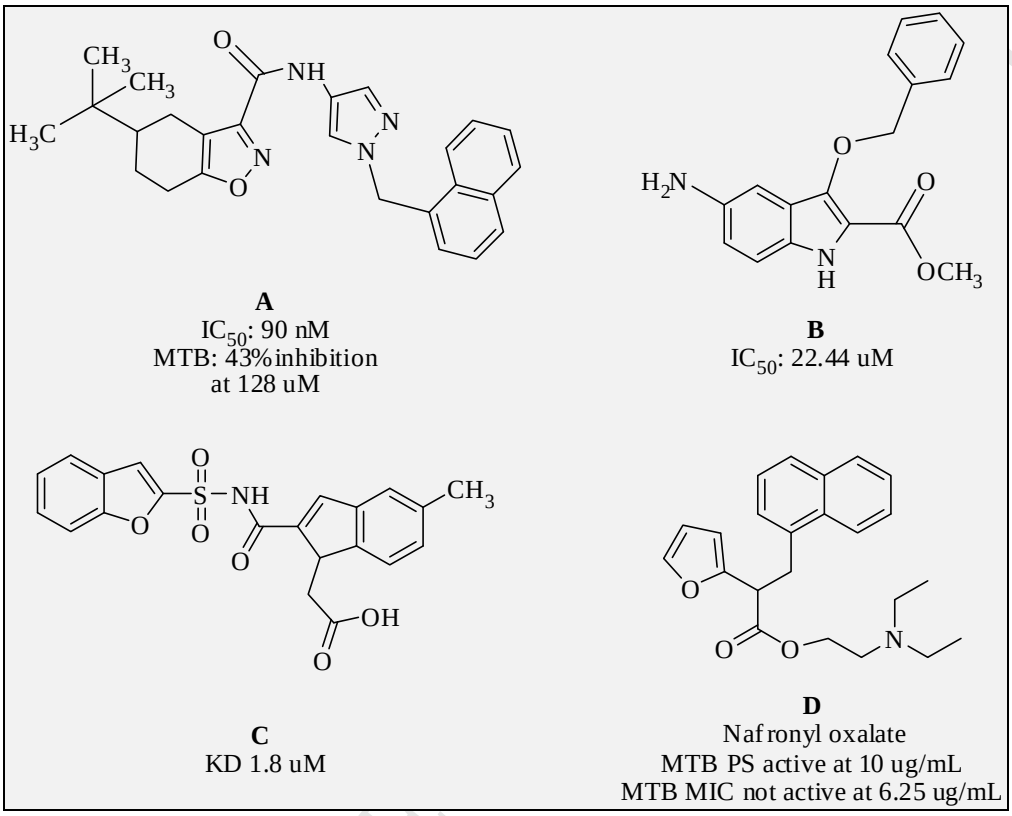


Figure 2

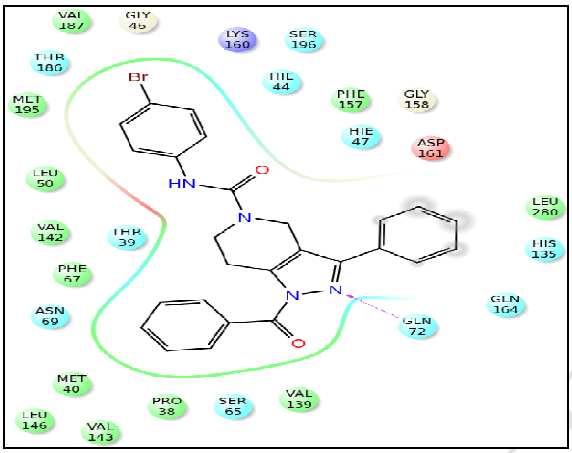
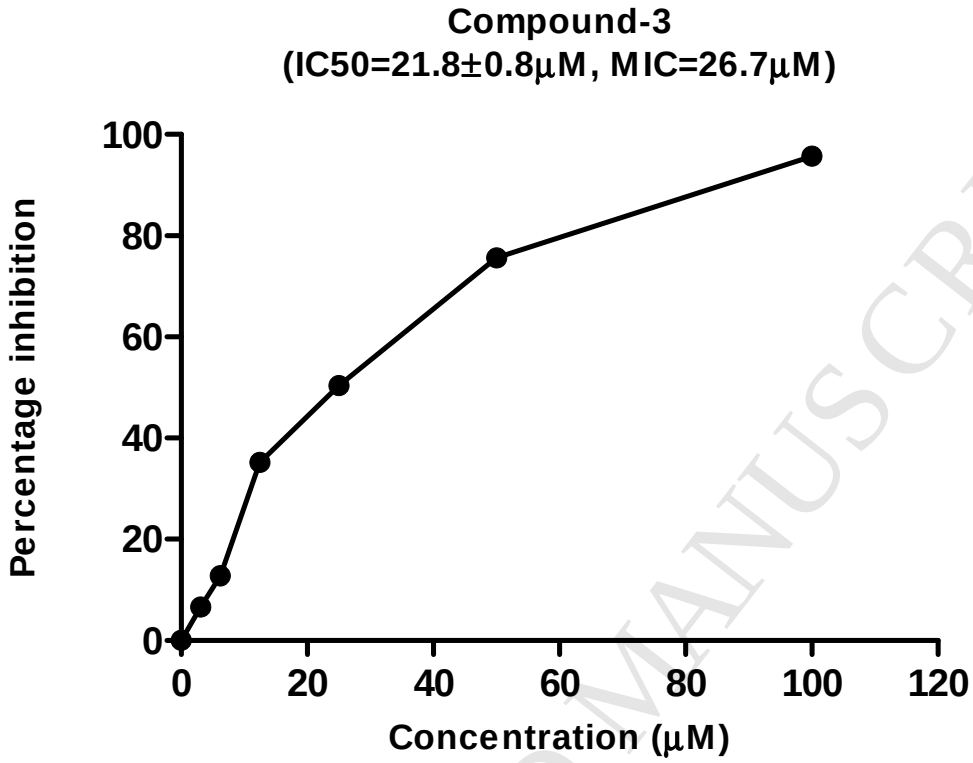
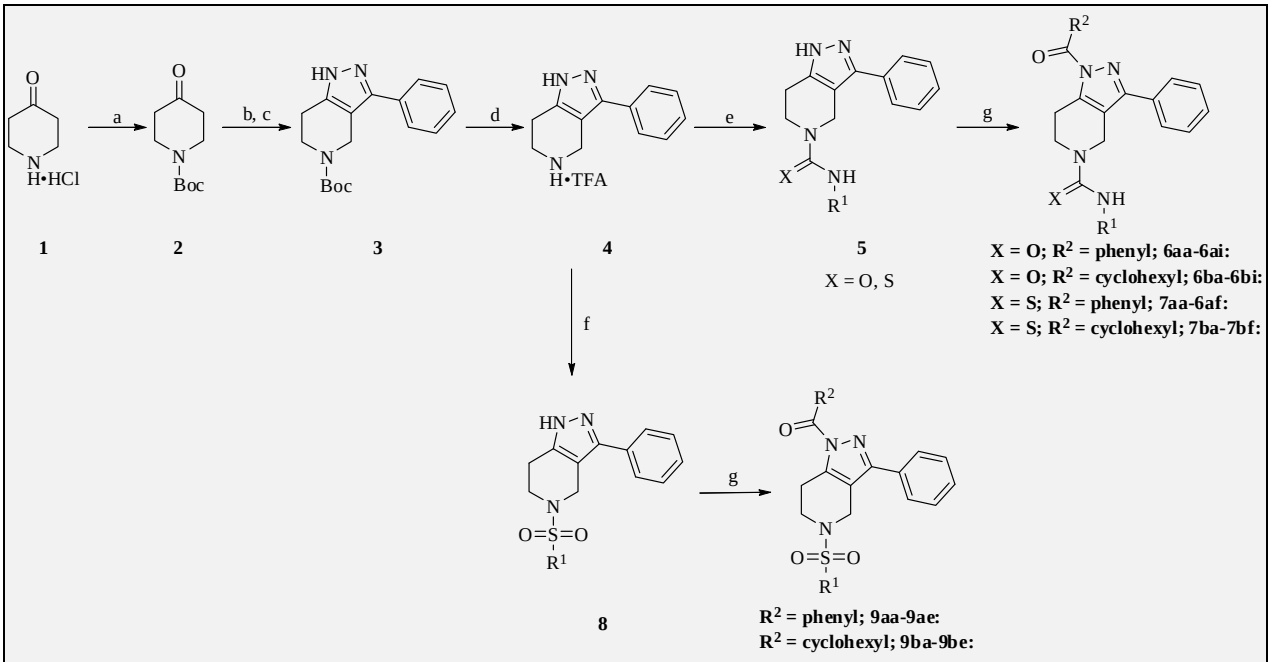


Figure 3



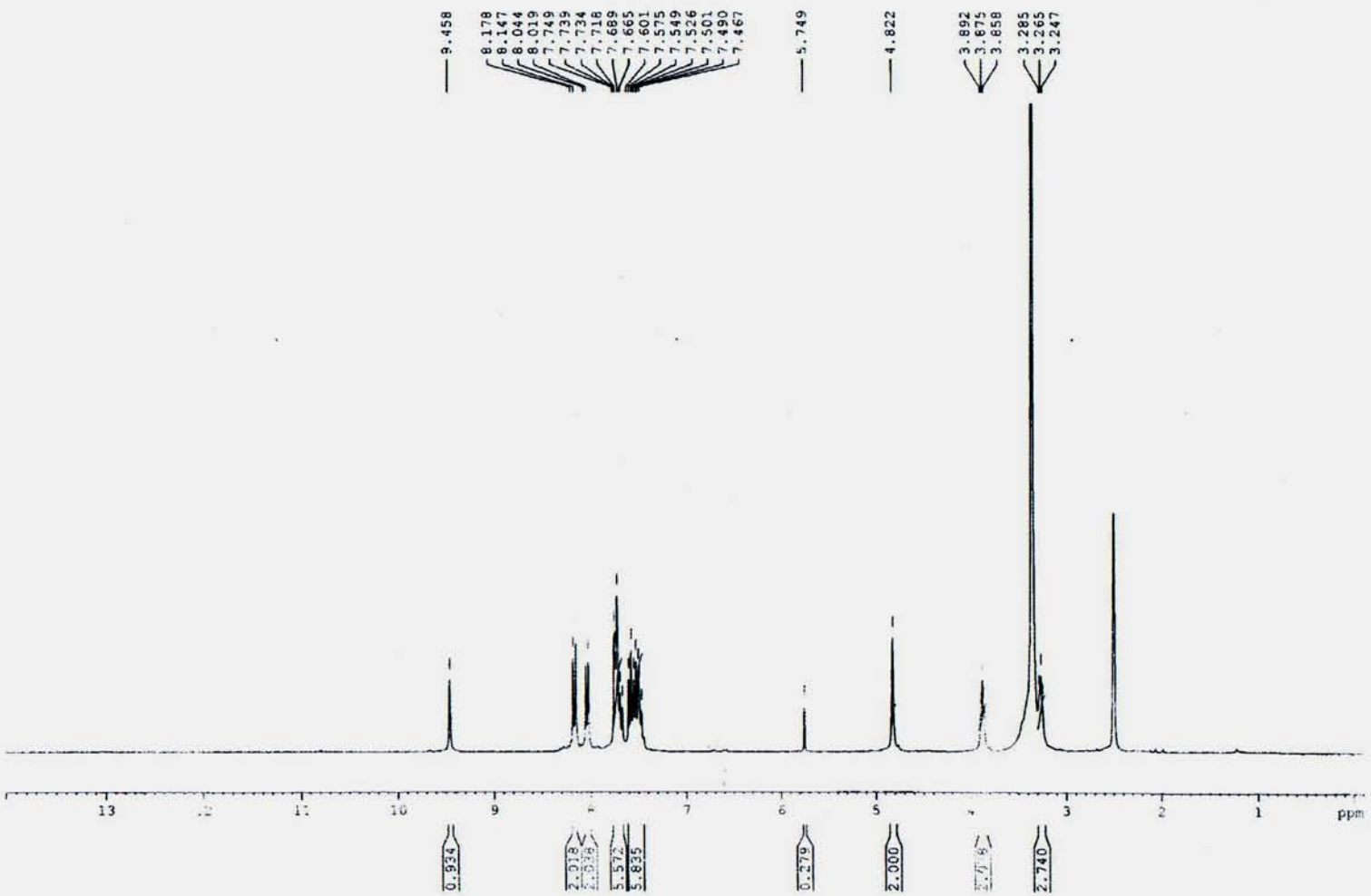
Scheme 1



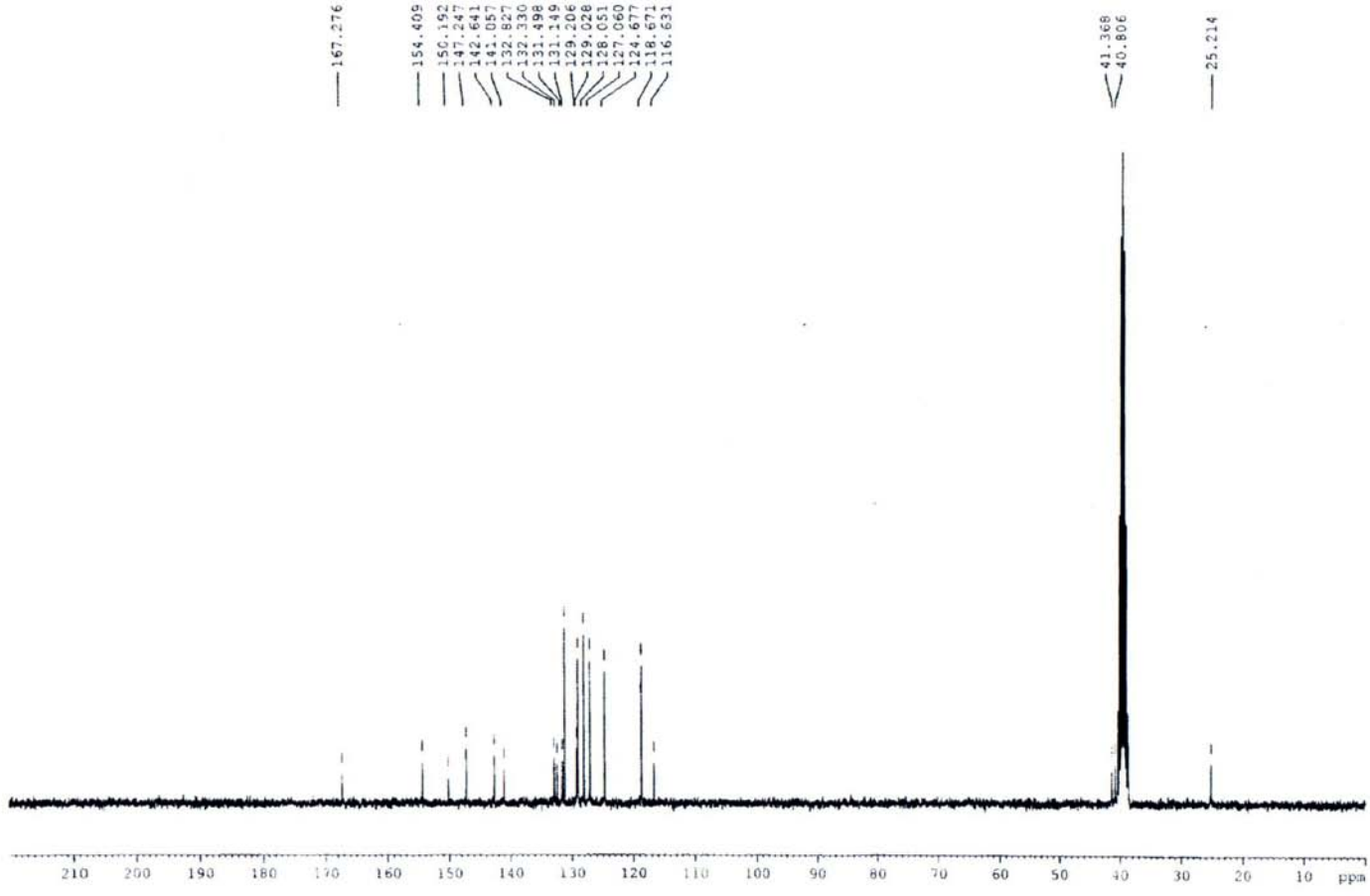
Reagents and Conditions: a. $(Boc)_2O$, Et_3N, CH_2Cl_2/MeOH, rt, 12h; b. Morpholine, PTSA, Benzoyl Chloride, toulene, reflux, 16h; c. N_2H_4, EtOH, rt, 7h; d . CF_3COOH, CH_2Cl_2, rt, 2h; e. R^1NCO/R^1NCS, Et_3N, DMF, rt, 6h; f. R^1SO_2Cl, Et_3N, DMF, rt, 6h; g. R^2COCl, DIPEA, DMF;

- A high-throughput virtual screening of in-house database
- Identified pyrazolopyridine scaffold as hit compound with good MTB PS inhibition.
- Synthesized 3-phenyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridine derivatives for SAR.
- Compound 3 identified as most effective one with no cytotoxicity.

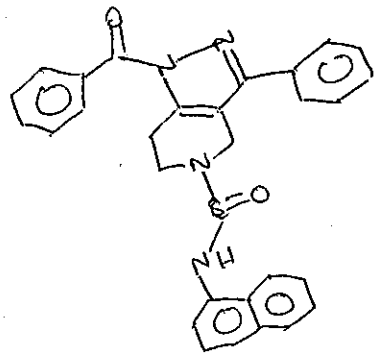
Compound-3,1H-DMSO-d6
120613018

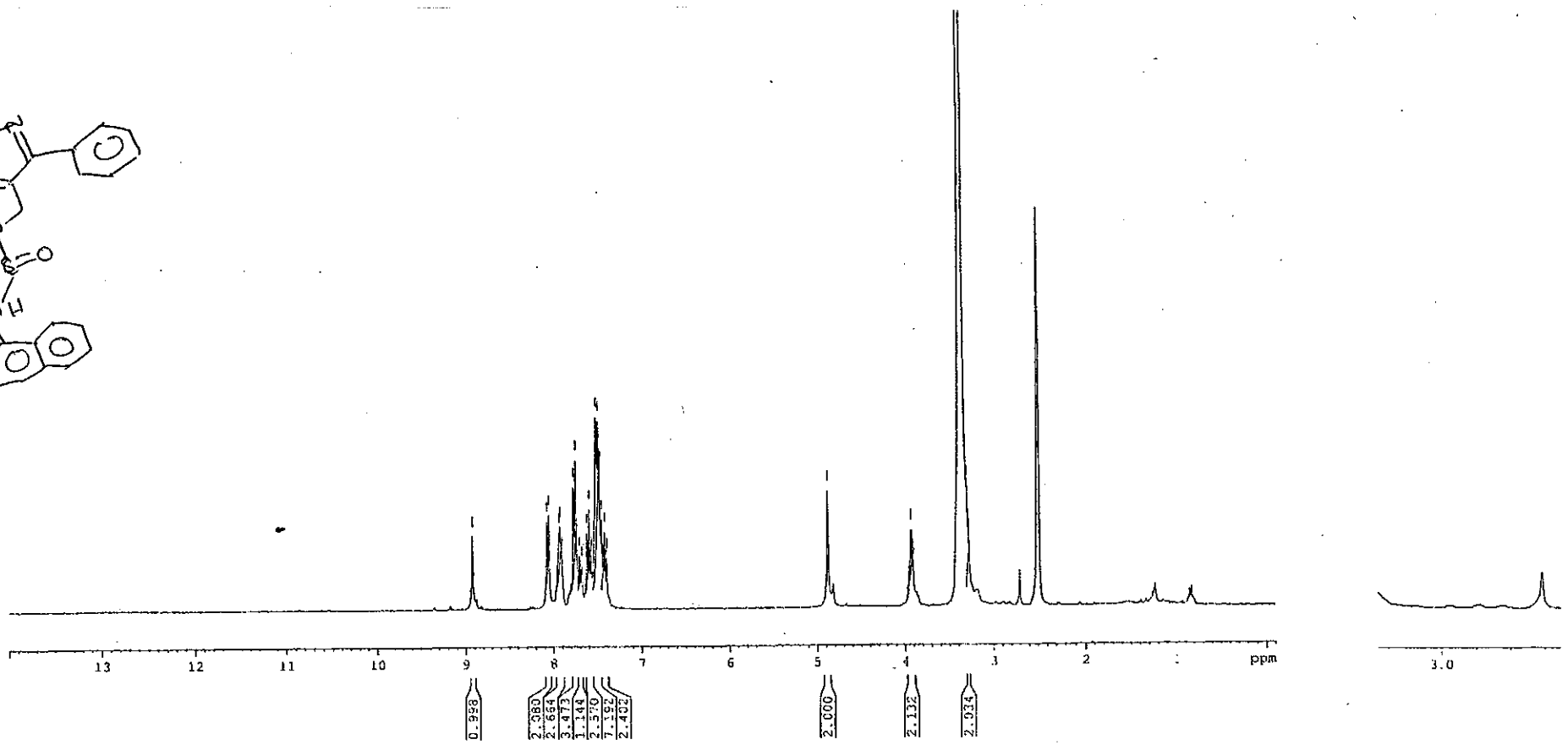


Compound-3,13C-DMSO-d6
120613013



Compound-7,1H-DMSO-d6
080813018





Compound-7,13C-DMSO-d6
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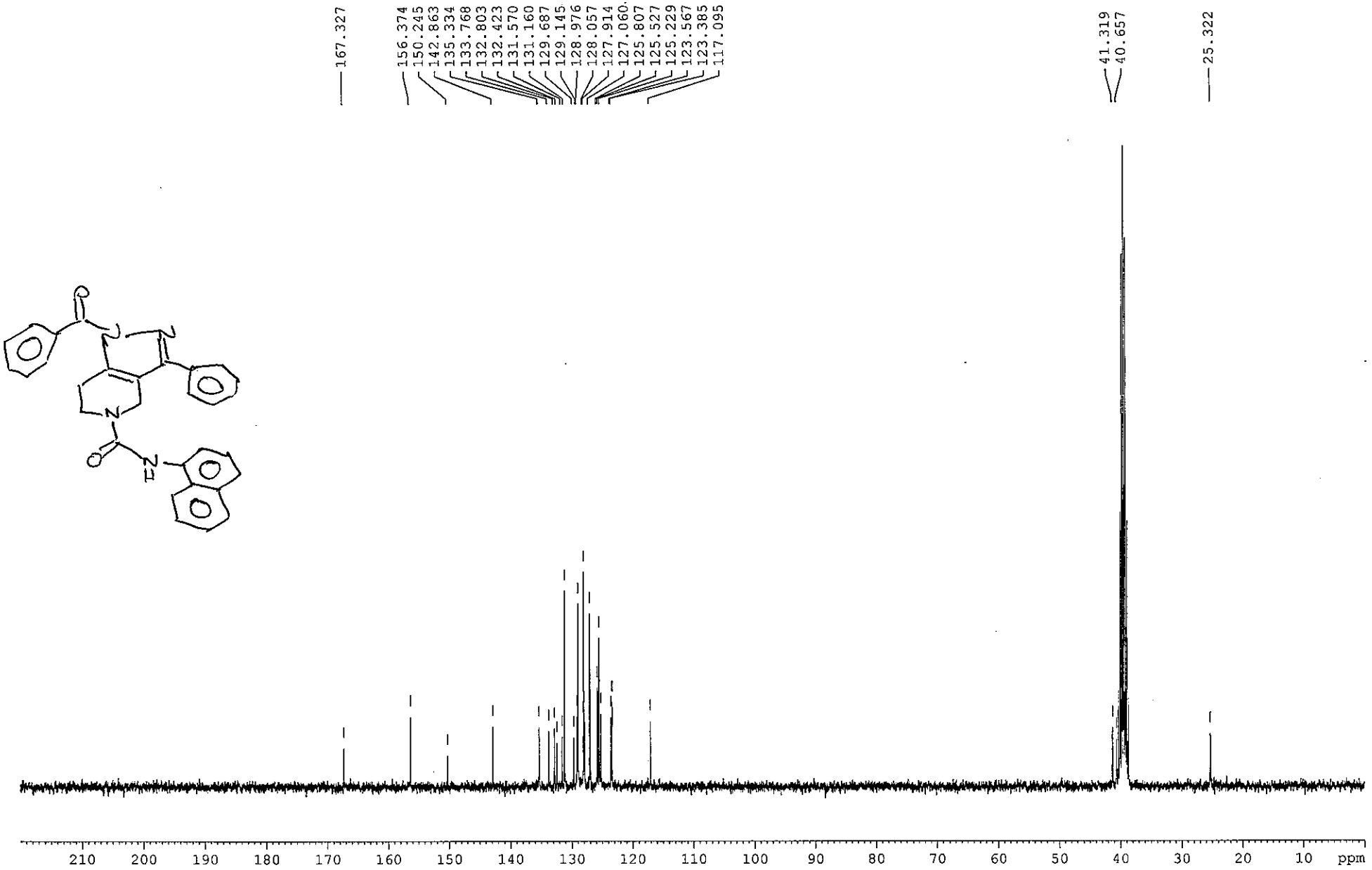
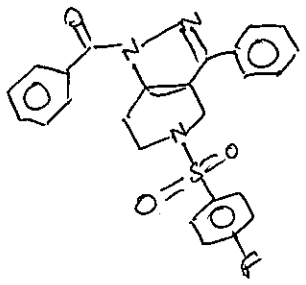


Compound - 16

DMSO-d_6



Compound-16,13C-CDCl3
080813020