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Synthesis and Docking Studies of Some 3-Amino-6-(thiophen-2-yl)thieno[2,3-b]pyridine Derivatives

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Some 3-amino-6-(thiophen-2-yl)thieno[2,3-b]pyridine derivatives (**4a-j**) were synthesized and characterized by ^{1}H NMR, ^{13}C NMR and elemental microanalyses based on the hit compound **DZ9202**, a nicotinate mononucleotide adenylyltransferase inhibitor against *Bacillus anthracis*. In the next step, molecular modeling and relative potency of these structures were studied.

Key Words: Thieno[2,3-b]pyridine, *Bacillus anthracis*, Molecular docking.

INTRODUCTION

Thieno[2,3-b]pyridines were found to exhibit, antimicrobial[1], antiallergic[2], antihepatocellular carcinoma[3] and potent antitumoragents activities[4]. Based on the previous report, 3-amino-*N*-(3-fluorophenyl)-6-(thiophen-2-yl)-thieno[2,3-b]pyridine-2-carboxamide (**DZ9202**) showed to be the most promising compound in a series of nicotinate mononucleotide adenylyltransferase inhibitors, a target from *Bacillus anthracis*[1]. Chemical structures of the compounds were showing bioisoteric substructures with a thieno[2,3-b]pyridine scaffold as the central ring. Based on these findings and inorder to find more knowledge about the structure activity relationship of these compounds, the synthesis and docking analysis of some 3-amino-thieno[2.3-b]pyridine derivatives have been carried out in the current study. Docking analysis showed that 3-amino-thieno[2.3-b]pyridine derivatives could exhibit antibacterial activity against *Bacillus anthracis* (Fig. 1).

EXPERIMENTAL

^{1}H NMR spectra were recorded on a 500 MHz Bruker spectrometer using $CDCl_3$ or DMSO-d_6 as solvent. ^{13}C NMR spectra were recorded on a 125 MHz Bruker spectrometer using $CDCl_3$ or DMSO-d_6 as solvent. Chemical shifts (δ) are reported in ppm relative to tetramethylsilane (TMS) as internal standard. Infrared spectra were acquired on a Nicolet Magna 550-FT spectrometer. IR spectra of solids were recorded in KBr and the absorption band was given in wave numbers ν in cm^{-1}. Elemental microanalyses were within ± 0.4 % of the theoretical values for C, H and N.

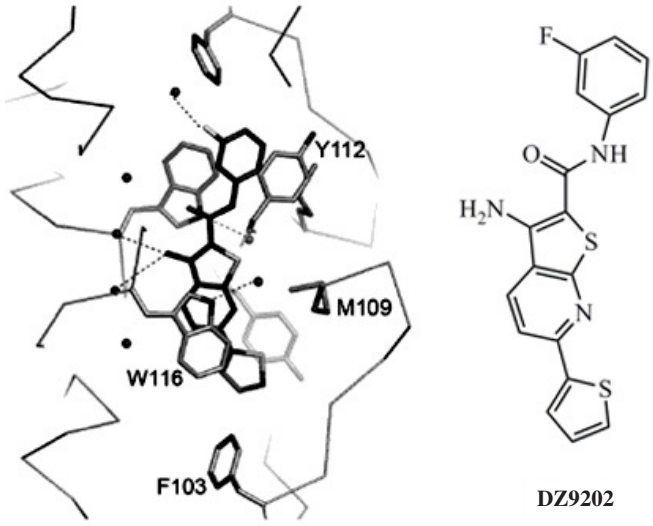


Fig. 1. Protein residues that interact with **DZ9202** are shown as sticks. Water molecules are shown as small spheres

(E)-3-(dimethylamino)-1-(thiophen-2-yl)prop-2-en-1-one (1): 0.0l mol 2-acetylthiophene was added to a solution of 0.01 dimethyl formamide dimethyl acetal in xylen (50 mL). The reaction mixture was refluxed for 12 h. The mixture was then concentrated under reduced pressure and the residue was purified by column chromatography on silica gel using ethyl acetate as the mobile phase.

Yield: 80 % m.p.: 148-150 ºC; IR (KBr, ν_{max}, cm^{-1}) : 1657 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.95 (s, 3H, CH_3), 3.17

(s, 3H, CH_3), 5.64 (d, *J* = 12.5 Hz, 1H, CH), 7.01 (dd, 1H, thienyl), 7.49 (d, *J* = 5Hz, 1H, thienyl), 7.64 (d, *J* = 5Hz, 1H, thienyl), 7.84 (d, *J* = 12.5 Hz, 1H, CH). Calcd. for $C_9H_{11}NOS$: C, 59.64; H, 6.12; N, 7.73. Found: C, 59.54; H, 6.39; N, 7.76.

6-Phenyl-2-thioxohydropyridine-3-carbonitrile (2): 0.5 mL of acetic acid and 10 mmol of cyanothioacetamide were added to a suspension of 10 mmol of compound **1** in 50 mL of ethanol. The mixture was heated to boiling and acidified with 0.2 mL of acetic acid. After 6 h the precipitate was filtered off and washed with ethanol and petroleum ether.

Yield: 69 %. m.p.: 234-236 ºC; IR (KBr, ν_{max}, cm^{-1}): 2218 (CN); ^{1}H NMR (500 MHz, DMSO-d_6): 7.25 (dd, 1H, thienyl), 7.80 (d, *J* = 5 Hz, 1H, thienyl), 7.94 (d, *J* = 8.5 Hz, 1H, CH), 8.04 (d, *J* = 3.5Hz, 1H, thienyl), 8.60 (d, *J* = 8.5 Hz, 1H, CH). 14.20 (bs, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6): δ 115.06, 119.10, 128.60, 129.32, 130.91, 135.15, 143.40, 152.45, 161.05, 172.60. Anal. calcd. for $C_{10}H_6N_2S_2$: C, 55.02; H, 2.77; N, 12.83. Found: C, 55.24; H, 2.79; N, 12.66.

Synthesis of compound (4a-j): 3 mL of aq KOH (2 M) was added to a suspension of 4 mmol compound **2** in 6 mL DMF. 5.2 g of the following the reagents; chloroacetonitrile (**3a**), chloroacetamide (**3b**), methyl bromoroacetate (**3c**), ethyl bromoacetate (**3d**), 2-bromoacetophenone (**3e**), 2,4'-dibromo-acetophenone (**3f**), 2-bromo-4'-methylacetophenone (**3g**), 2-bromo-4'-methylsulfonylacetophenone (**3h**), 2,2',4'-trichloro-acetophenone (**3i**) and 2-bromo-2'-acetonaphthone (**3j**) were added to the suspension at room temperature. The resulting mixture was stirred for 15 min at room temperature. Additional 3 mL of aq KOH 2 M was added to the reaction and re-stirred for 6 h at 85 ºC. Finally, the reaction mixture was allowed to cool to room temperature, the precipitate was collected by filtration and washed with ethanol.

3-Amino-6-(thiophen-2-yl)thieno[2,3-b]pyridine-2-carbonitrile (4a): Yield: 68 %. mp: 256-259 ºC; IR (KBr, ν_{max}, cm^{-1}) : 3464-3343 (NH_2), 2198 (CN); ^{1}H NMR (500 MHz, DMSO-d_6): 7.23 (dd, 1H, thienyl), 7.32 (bs, 2H, NH_2), 7.77 (d, *J* = 5 Hz, 1H, thienyl), 7.98 (d, *J* = 3.5 Hz, 1H, thienyl), 8.08 (d, *J* = 8.5 Hz, 1H, CH), 8.50 (d, *J* = 8.5 Hz, 1H, CH). Anal. calcd. for $C_{12}H_7N_3S_2$: C, 56.01; H, 2.74; N, 16.33. Found: C, 56.34; H, 2.66; N, 16.76.

3-Amino-6-(thiophen-2-yl)thieno[2,3-b]pyridine-2-carboxamide (4b): Yield: 64 %. m.p. 263-265 ºC; IR (KBr, ν_{max}, cm^{-1}) : 3409, 3355 (NH_2), 1635 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6): 7.00-7.30 (m, 5H), 7.73 (d, *J* = 5 Hz, 1H, thienyl), 7.94 (d, *J* = 3.5Hz, 1H, thienyl), 8.00 (d, *J* = 8.5 Hz, 1H, CH), 8.44 (d, *J* = 8.5 Hz, 1H, CH). Anal. calcd. for $C_{12}H_9N_3OS_2$: C, 52.34; H, 3.29; N, 15.26. Found: C, 52.24; H, 3.45; N, 15.42.

Methyl 3-amino-6-(thiophen-2-yl)thieno[2,3-b]pyridine-2-carboxylate (4c): Yield: 73 %. m.p. 232-235 ºC; IR (KBr, ν_{max}, cm^{-1}): 3421, 3308 (NH_2), 1677 (C=O); ^{1}H NMR (500 MHz, DMSO-*d*6): 3.80 (s, 3H, OCH_3), 7.22 (dd, 1H, thienyl), 7.32 (bs, 2H, NH_2), 7.76 (d, *J* = 5 Hz, 1H, thienyl), 7.97 (d, *J* = 3.5 Hz, 1H, thienyl), 8.01 (d, *J* = 8.5 Hz, 1H, CH), 8.55 (d, *J* = 8.5 Hz, 1H, CH). Anal. calcd. for $C_{13}H_{10}N_2O_2S_2$: C, 53.77; H, 3.47; N, 9.65. Found: C, 53.54; H, 3.79; N, 9.76.

Ethyl 3-amino-6-(thiophen-2-yl)thieno[2,3-b]pyridine-2-carboxylate (4d): Yield: 70 %. m.p. 230-232 ºC; IR (KBr, ν_{max}, cm^{-1}): 3417, 3304 (NH_2), 1669 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6): 1.30 (t, *J* = 7 Hz 3H, OCH_2CH_3), 4.28 (q, *J* = 7 Hz, 2H, OCH_2CH_3), 7.22 (dd, 1H, thienyl), 7.30 (bs, 2H, NH_2), 7.76 (d, *J* = 5 Hz, 1H, thienyl), 7.97 (d, *J* = 3.5 Hz, 1H, thienyl), 8.03 (d, *J* = 8.5 Hz, 1H, CH), 8.55 (d, *J* = 8.5 Hz, 1H, CH). Anal. calcd. for $C_{14}H_{12}N_2O_2S_2$: C, 55.24; H, 3.97; N, 9.20. Found: C, 55.34; H, 3.79; N, 9.16.

(3-Amino-6-(thiophen-2-yl)thieno[2,3-b]pyridin-2-yl)(phenyl)methanone (4e): Yield: 60 %. m.p. 180-182 ºC; IR (KBr, ν_{max}, cm^{-1}) : 3522, 3343 (NH_2), 1615 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6): 7.23 (dd, 1H, thienyl), 7.51-7.68 (m, 3H, phenyl), 7.77 (d, *J* = 5 Hz, 1H, thienyl), 7.81 (d, 2H, phenyl), 8.00 (d, *J* = 3.5 Hz, 1H, thienyl), 8.07 (d, *J* = 8.5 Hz, 1H, CH), 8.44 (bs, 2H, NH_2), 8.68 (d, *J* = 8.5 Hz, 1H, CH). Anal. calcd. for $C_{18}H_{12}N_2OS_2$: C, 64.26; H, 3.60; N, 8.33. Found: C, 64.34; H, 3.79; N, 8.46.

(3-Amino-6-(thiophen-2-yl)thieno[2,3-b]pyridin-2-yl)(4 bromophenyl)methanone (4f): Yield: 72 %. m.p. 227-230 ºC; IR (KBr, ν_{max}, cm^{-1}): 3452, 3289 (NH_2), 1596 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6): 7.23 (dd, 1H, thienyl), 7.76 (s, 4H, phenyl), 7.78 (d, *J* = 5 Hz, 1H, thienyl), 8.01 (d, *J* = 3.5Hz, 1H, thienyl), 8.08 (d, *J* = 8.5 Hz, 1H, CH), 8.48 (bs, 2H, NH_2), 8.68 (d, *J* = 8.5 Hz, 1H, CH). Anal. calcd. for $C_{18}H_{11}N_2OS_2Br$: C, 52.05; H, 2.67; N, 6.74. Found: C, 52.24; H, 2.79; N, 6.76.

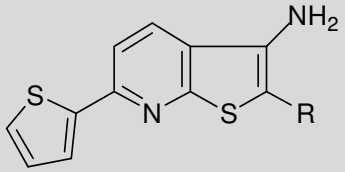
(3-Amino-6-(thiophen-2-yl)thieno[2,3-b]pyridin-2-yl)(*p*-tolyl)methanone (4g): Yield: 75 %. m.p. 248-250 ºC; IR (KBr, ν_{max}, cm^{-1}): 3444, 3390 (NH_2), 1592 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6): 2.40 (s, 3H, CH_3), 7.22 (dd, 1H, thienyl), 7.34 (d, *J* = 7.5Hz, 2H, phenyl), 7.72 (d, *J* = 7.5 Hz, 2H, phenyl), 7.76 (d, *J* = 5 Hz, 1H, thienyl), 7.99 (d, *J* = 3.5 Hz, 1H, thienyl), 8.05 (d, *J* = 8.5 Hz, 1H, CH), 8.39 (bs, 2H, NH_2), 8.66 (d, *J* = 8.5 Hz, 1H, CH); ^{13}C NMR (125 MHz, DMSO-d_6): δ 21.54, 102.86, 115.76, 124.06, 128.00, 129.27, 129.46, 130.71, 133.29, 138.53, 141.68, 143.96, 150.88, 154.24, 161.23, 188.72. Anal. calcd. for $C_{19}H_{14}N_2OS_2$: C, 65.12; H, 4.03; N, 7.90. Found: C, 65.34; H, 4.19; N, 7.76.

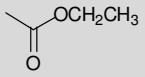
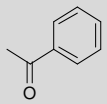
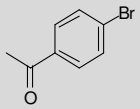
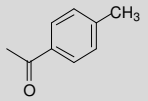
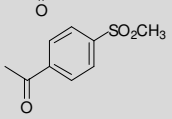
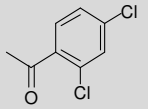
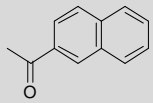
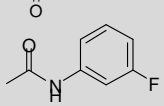
(3-Amino-6-(thiophen-2-yl)thieno[2,3-b]pyridin-2-yl)(4-(methylsulfonyl)phenyl)methanone (4h): Yield: 58 %. m.p. 185-187 ºC; IR (KBr, ν_{max}, cm^{-1}): 3433, 3304 (NH_2), 1592 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6): 3.33 (s, 3H, SO_2CH_3), 7.23 (dd, 1H, thienyl), 7.78 (d, *J* = 5 Hz, 1H, thienyl), 8.01 (d, *J* = 3.5Hz, 1H, thienyl), 8.03 (d, *J* = 8 Hz, 2H, phenyl), 8.08 (d, *J* = 8.5 Hz, 1H, CH), 8.10 (d, *J* = 8 Hz, 2H, phenyl), 8.57 (bs, 2H, NH_2), 8.70 (d, *J* = 8.5 Hz, 1H, CH). Anal. calcd. for $C_{19}H_{14}N_2O_3S_3$: C, 55.05; H, 3.40; N, 6.76. Found: C, 55.24; H, 3.49; N, 6.71.

(3-Amino-6-(thiophen-2-yl)thieno[2,3-b]pyridin-2-yl)(2,4-dichlorophenyl)methanone (4i): Yield: 68 %. m.p. 190-193 ºC; IR (KBr, ν_{max}, cm^{-1}): 3491, 3343 (NH_2), 1600 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6): 7.22 (dd, 1H, thienyl), 7.60 (m, 2H, phenyl), 7.76 (d, *J* = 5 Hz, 1H, thienyl), 7.80 (bs, 1H, phenyl), 7.99 (d, *J* = 3.5 Hz, 1H, thienyl), 8.06 (d, *J* = 8.5 Hz, 1H, CH), 8.44 (bs, 2H, NH_2), 8.68 (d, *J* = 8.5 Hz, 1H, CH). Anal. calcd. for $C_{18}H_{10}N_2OS_2Cl_2$: C, 53.34; H, 2.49; N, 6.91. Found: C, 53.51; H, 2.59; N, 6.76.

(3-Amino-6-(thiophen-2-yl)thieno[2,3-b]pyridin-2-yl)(naphthalen-2-yl)methanone (4j): Yield: 66 %. m.p. 215-218 ºC; IR (KBr, ν_{max}, cm^{-1}): 3433, 3312 (NH_2), 1615 (C=O);

TABLE-1
SYNTHESIS AND DOCKING STUDY OF COMPOUNDS **4a-j**



Compounds	R	Yield (%)	m.p. (ºC)	Ki	Free Energy of Binding
4a		68 %	256-259	404.75 nM	-8.72 kcal/mol
4b		64 %	263-265	393.39 nM	-8.74 kcal/mol
4c		73 %	232-235	190.11 nM	-9.17 kcal/mol
4d		70 %	230-232	344.50 nM	-8.82 kcal/mol
4e		60 %	180-182	9.51 nM	-10.94 kcal/mol
4f		72%	227-230	13.16 nM	-10.75 kcal/mol
4g		75%	248-250	7.01 nM	-11.12 kcal/mol
4h		58%	185-187	44.79 uM	-5.93 kcal/mol
4i		68%	190-193	22.12 nM	-10.44 kcal/mol
4j		66%	215-218	215.21 uM	-5.00 kcal/mol
DZ9202				10.40 nM	-6.80 kcal/mol

^{1}H NMR (500 MHz, DMSO-d_6): 7.23 (dd, 1H, thienyl), 7.66 (m, 2H, naphtyl), 7.77 (d, J = 5 Hz, 1H, thienyl), 7.87 (d, 1H, naphtyl), 8.01 (d, J = 3.5 Hz, 1H, thienyl), 8.04 (d, 1H, naphtyl), 8.06 (d, 1H, naphtyl), 8.07 (d, J = 8.5 Hz, 1H, CH), 8.14 (d, 1H, naphtyl), 8.47 (s, 1H, naphtyl), 8.48 (bs, 2H, NH_2), 8.70 (d, J = 8.5 Hz, 1H, CH). Anal. calcd. for $C_{22}H_{14}N_2OS_2$: C, 68.37; H, 3.65; N, 7.25. Found: C, 68.39; H, 3.79; N, 7.36.

Structure optimization: Structures **4a-j** were simulated in chem3D professional; Cambridge software; using MM2 method (RMS gradient = 0.05 kcal/mol)[5]. The output files were subsequently minimized by Semi-empirical AM1 method (Convergence limit = 0.01; Iteration limit = 50; RMS gradient = 0.05 kcal/mol; Fletcher-Reevesoptimizer algorithm) using HyperChem 7.5[6].

Crystal structure of *Bacillus anthracis* nicotinate mononucleotide adenylytransferase (nadD) in complex with DZ9202 inhibitor was retrieved from RCSB Protein Data Bank (PDB entry: 3HFJ).

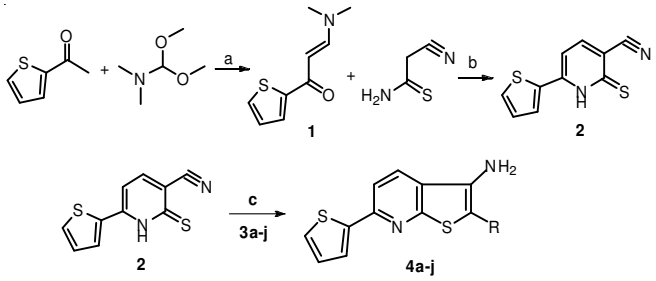
Molecular docking: Automated docking simulation was implemented with AutoDockTools 4.0 version 1.5.4[7] using **2a** Lamarckian genetic algorithm. The docking parameter files were generated using Genetic Algorithm and local search parameters with the number of generations set to 100. The docked complexes were clustered with a rootmean-square deviation tolerance of 0.5 Å. The results of docking processing are presented in (Table-1).

RESULTS AND DISCUSSION

Compound **1** was prepared by refluxing 2-acetylthiophene with dimethylformamide dimethylacetal. This enaminones proved to be in trans configuration since olefinic protons appeared as a doublet with J = 12.5 Hz. The key intermediate **2** was prepared by cyclization reaction of compound **1** and cyanothioacetamide in ethanol. The structure compound **2** was confirmed based on ^{1}H NMR, which revealed pyridyl hydrogen resonances as doublets with J = 8.5 Hz. Compound **1** and **2** are excellent precursors for the synthesis of some heterocyclic compounds[8-10].

Compounds **4** were prepared *via* the Thorpe-Ziegler cyclization[11,12] of compound **2** with chloroacetamide and

α-halo ketones. The structures of **4a-j** were fully characterized by ^{1}H NMR (**Scheme-I**).



Scheme-I: Reagents and conditions: (a) xylen, reflux, 12 h, (b) acetic acid, EtOH, reflux, 6 h, (c) KOH/DMF, rt, 15 min and then 85 ºC, 6 h

According to the docking studies, *N*-(3-fluorophenyl) amide group led to a dramatic loss of activity upon replacement with substituted, cyano (**4a**), amid (**4b**), methoxy (**4c**) and ethoxy (4d). After being verified as a potent substituent, the phenyl group was subjected to some modifications at position 4. As shown in Table-1, introduction of methyl substituent (**4g**) on the phenyl ring was well tolerated, whereas, methylsulfony (**4h**) and naphtyl groups (**4j**) substituted analog led to loss of activity. Neither were, the bromo (**4f**) and dichloro (**4i**) substituents tolerated.

Conclusion

The present study reveals the synthesis of some 3-aminothieno[2.3-b]pyridine derivatives. Furthermore, molecular modeling and relative potency of these structures were discussed.

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7. Auto Dock Tools (ADT), the Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037-1000, USA; (http://www.scripps.edu/pub/olson-web/ doc/autodock/); *Python, M.F.S. J. Mol. Graphics Mod.*, **17**, 57 (1999).
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