Asian Journal of Chemistry; Vol. 29, No. 7 (2017), 1515-1521
ASIAN JOURNAL OF CHEMISTRY
https://doi.org/10.14233/ajchem.2017.20545

Synthesis and Antibacterial Activity of Thienopyrimidine Amide Derivatives

GIRI THARIKOPPULA[1], LAXMINARAYANA EPPAKAYALA[1,*], THIRUMALA CHARY MARINGANTI[2], CHIRANJEEVI KAMALAPURAM[1] and KARUNAKAR RAO KUDLE[3]

[1]Sreenidhi Institute of Science and Technology, Yamnampet, Ghatkesar, Hyderabad-501301 India
[2]Jawaharlal Nehru Technological University Hyderabad, College of Engineering, Hyderabad-505 327, India
[3]Department of Chemistry, Osmania University, Main Road, Hyderabad-500 007, India

*Corresponding author: E-mail: elxnkits@yahoo.co.in

Received: 27 January 2017; *Accepted*: 21 March 2017; *Published online*: 13 May 2017; AJC-18385

Thienopyrimidine amide derivatives are important class of organic compounds and show wide range of biological activity. Hence the researchers are paying more attention towards the synthesis of these compounds. A series of thienopyrimidine amide derivatives (**13a-m**) were synthesized. The newly synthesized amide derivatives (**13a-m**) were characterized by ^{1}H NMR, ^{13}C NMR, Mass and IR spectral data. Further these compounds were also evaluated for their antibacterial activity.

Keywords: Thienopyrimidine, Ampicilline, Antibacterial activity.

INTRODUCTION

Nearly two million cases of hospital associated infections occur annually within the U.S. The microorganism pathogens typically enter patients *via* canulation, urinary catheters and intravascular lines [1]. Treatments of microbic infections, that involve microorganism, is changing into tough as a result of everlasting downside of microbic resistance towards antibiotics [2]. Infections with drug resistant organisms stay a crucial downside in clinical observe that's tough to unravel. Several researchers are well productive within the recent years in reshaping the scaffolds of earlier antibiotics [3,4]. Heterocycles area unit a part of chemical science and plays a lead role within the biological activities.

Recent studies reveal that little heterocyclic frameworks, supply a plus in style of antibacterial drug agents as they *mimic* several biomolecules. Thienopyrimidines are nitrogen and sulfur atoms containing bicyclic heterocycle and it encompasses a thiophene ring unit with the pyrimidine moiety [5]. This scaffold shows a broad spectrum of biological activities. The thienopyrimidines were assessed as antitumour [6-9], medicine [10], enzyme [11-14], phosphodiesterase inhibitors [15], antioxidative [16,17], antimalarial drug [18,19], antibacterial drug [20], antiviral [21], antifungal [22,23], medication [24], antiplatelet [25] and medicament [26]. In view of these observations, the varied biological property of thienopyrimidine pharmacophore impelled to synthesize the title compounds. Consequently, with associate in nursing aim to develop promising antibacterial drug agents, a series of novel thienopyrimidine derivatives (**13a-m**) were designed and synthesized. Further, all target compounds were evaluated for antibacterial activity.

EXPERIMENTAL

The solvents were purified according to standard procedures prior to use and all commercial chemicals were as such used. For thin-layer chromatography (TLC) analysis, Merck pre-coated plates (silica gel 60 F254) were used and spots were visualized under UV light. Merck silica gel 60 (230-400 mesh) was used for flash column chromatography and the eluting solvents are indicated in the procedures. Melting point determinations were performed by using Mel-temp apparatus and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded in Varian MR-400 MHz instrument. The mass spectra were recorded on Agilent ion trap MS and infrared spectra were recorded on a Perkin Elmer FT-IR spectrometer.

Synthesis of thieno[3,2-d]pyrimidine-2,4-diol (2): To a solution of methyl 3-aminothiophene-2-carboxylate (**1**) (10 g, 63.69 mmol) in acetic acid (60 mL) and water (35 mL) was added slowly KOCN (10.3 g, 127.3 mmol) in water (35 mL) by drop wise at 0 °C over a period of 30 min and the reaction mixture was stirred at room temperature for 16 h. The resultant solid was filtered and treated with 50 % NaOH (40 mL) solution and stirred at room temperature for 4 h. After completion of the reaction, reaction mixture was cooled to 0 °C and

acidified with conc. HCl provided as off white solid **2**. Yield: 88 %; m.p: 166-170 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.55 (brs, 1H, OH), 11.20 (brs, 1H, OH), 8.05 (d, *J* = 5.2 Hz, 1H, ArH), 6.91 (d, *J* = 5.2 Hz, 1H, ArH); ESI-MS: *m/z*, 167.2 $(M-H)^-$.

Synthesis of 2,4-dichlorothieno[3,2-d]pyrimidine (3): To a suspention of thieno[3,2-d]pyrimidine-2,4-diol (**2**) (8.0 g, 47.61 mmol) in toluene was added N-methyl-2-pyrrolidone (NMP) (1.0 mL, catalytic), followed by the addition and $POCl_3$ (35 mL) at room temperature and heated to reflux for 16 h. After completion of reaction, excess $POCl_3$ was removed by distillation, crude residue was poured into ice cold water and filtered the formed precipitate compound **3** as off white solid. Yield: 7.7g, 80 %; m.p. 136-140 °C; ^{1}H NMR (300 MHz, DMSO-d_6): 8.70 (t, *J* = 4.4 Hz, 1H, ArH), 7.72 (d, *J* = 5.5 Hz, 1H, ArH), ESI-MS: *m/z*, 204.9 $(M+H)^+$.

Synthesis of N-(2-chlorothieno[3,2-d]pyrimidin-4-yl)-N-methylamine (5): To a solution of 2,4-dichlorothieno[3,2-d]pyrimidine (**3**) (7 g, 34.31 mmol) in THF (80 mL) was added 40 % methyl amine (**4**) aqueous solution (9 mL) and stirred at room temperature for 3 h. After completion, reaction mixture was concentrated and poured into ice water to afford solid of N-(2-chlorothieno[3,2-d]pyrimidin-4-yl)-N-methylamine (**5**) as off white solid. Yield: 88 %; m.p.: 294-303 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.34 (d, *J* = 4.7 Hz, 1H, ArH), 8.16 (d, *J* = 4.7 Hz, 1H, ArH), 7.32 (s, 1H, NH), 2.96 (d, *J* = 4.5 Hz, 3H); ESI-MS: *m/z*, 200.1 $(M+H)^+$.

Synthesis of *tert*-butyl-N-(2-chlorothieno[3,2-d]pyrimidin-4-yl)-N-methylcarbamate (6): To a solution of N-(2-chlorothieno[3,2-d]pyrimidin-4-yl)-N-methylamine (**5**) (6.0 g, 30.15 mmol) in DMF (42 mL) was added TEA (6 g, 60.30), $(Boc)_2O$ (7.9 g, 36.18 mmol) at room temperature for 4 h. After completion, reaction mixture was poured into ice cold water to get precipitate and dried the product under vacuum to afford pure compound **6** as off white solid. Yield: 82 %; m.p:126-130 °C; IR (KBr, ν_{max}, cm^{-1}) 3068, 2978, 1716, 1532, 1378, 1247, 1144, 886, 793; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.51 (d, *J* = 5.5 Hz, 1H, ArH), 7.54 (d, *J* = 5.5 Hz, 1H, ArH), 3.39 (s, 3H, CH_3), 1.50 (s, 9H, $C(CH_3)_3$); ESI-MS: *m/z*, 299.9 $(M+H)^+$.

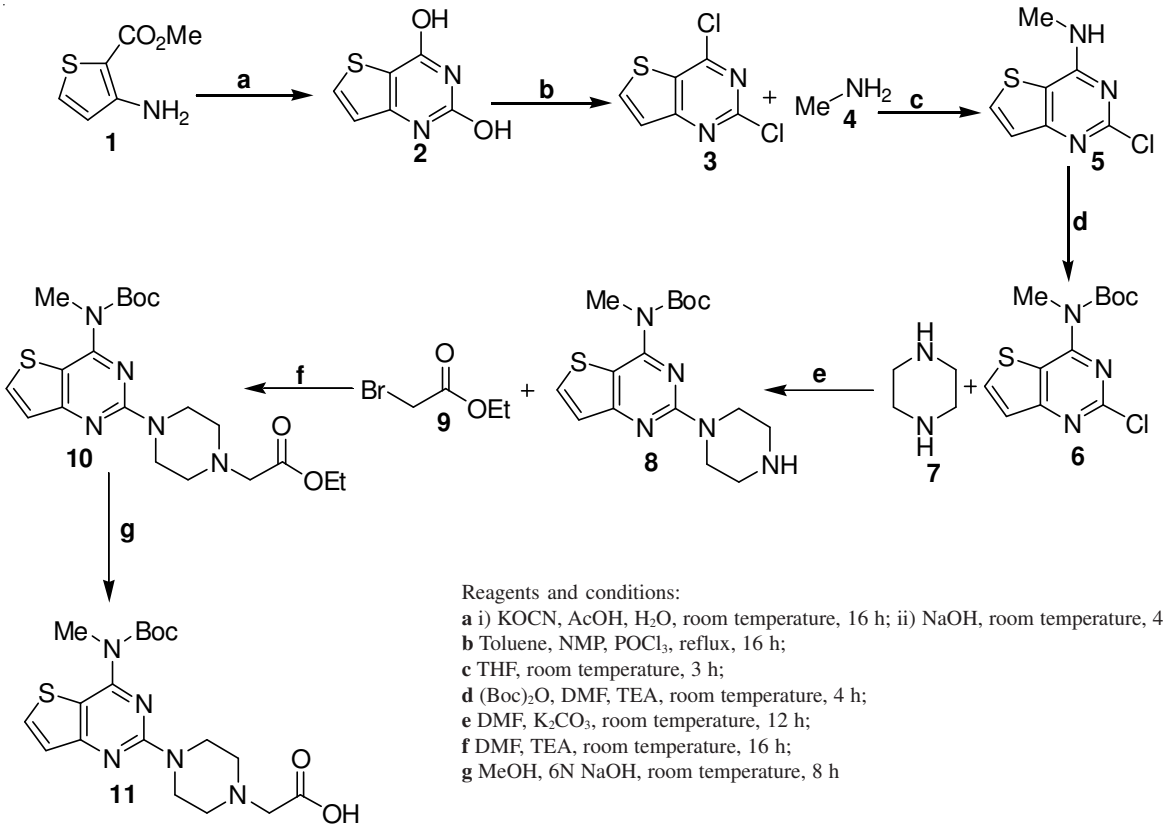
Synthesis of *tert*-butyl-N-methyl-N-(2-piperazino-thieno[3,2-d]pyrimidin-4-yl)carbamate (8): To a solution of piperazine (**7**) (3.45 g, 40.13 mmol) in DMF (30 mL) at 0 °C was added potassium carbonate (1.38 g, 10.03 mmol) followed by *tert*-butyl- N-(2-chlorothieno[3,2-d]pyrimidin-4-yl)-N-methylcarbamate (**6**) (3 g, 10.03 mmol) and stirred the reaction at room temperature for 12 h. After completion, reaction mixture was poured into ice cold water, filters the formed precipitate, washed the precipitate with water and dried the product under vacuum to afford **8** as off white solid. Yield: 2.6 g. m.p. 136-140 °C; IR (KBr, ν_{max}, cm^{-1}): 3452, 2932, 2862, 1711, 1668, 1567, 1389, 1370, 1269, 1151, 1101, 1001, 763, 750, 662; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.19-8.18 (d, *J* = 5.6 Hz, 1H, ArH), 7.19-7.18 (d, *J* = 5.6 Hz, 1H, ArH), 3.80 (brs, 4H, CH_2), 3.3 (s, 3H, CH_3), 2.93 (s, 4H, CH_2), 1.46 (s, 9H, $C(CH_3)_3$); ESI-MS: *m/z*, 359.3 $(M+H)^+$.

Synthesis of ethyl 2-(4-4-[(*tert*-butoxycarbonyl)(methyl)-amino]thieno[3,2-d]pyrimidin-2-ylpiperazino)acetate (10): To a solution of **8** (2.5 g, 7.1 mmol) in DMF (25 mL) was added TEA (1.44 g, 14.32 mmol) followed by ethyl bromo acetate (**9**) (1.31 g, 7.8 mmol) at 0 °C and stirred the reaction at room temperature for 16 h. After completion, reaction mixture was poured into ice cold water, extracted with ethyl acetate (25 mL × 3), combined extracts were washed with water, brine solution, dried the organic phase over anhy. Na_2SO_4 and evaporated the solvent to afford the cude product. The crude product was purified by column chromatography (elutent: 80 % EtOAc: pet ether) to afford pure compound **10** as off white solid. Yield: 89 %; m.p.: 245-249 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73-7.71 (d, *J* = 5.5 Hz, 1H, ArH), 7.13-7.12 (d, *J* = 5.5 Hz 1H, ArH), 4.22-4.20 (q, *J* = 7.5 Hz 2H, CH_2-ester), 3.92 (brs, 4H, CH_2- piperazine), 3.39 (s, 3H, CH_3), 3.27 (s, 2H, NCH_2CO), 2.67 (s, 4H, CH_2-piperazine), 1.52 (s, 9H, $C(CH_3)_3$) 1.30-1.26 (q, *J* = 8 Hz, 2H, OCH_2-ester); ESI-MS: *m/z*, 436.4 $(M+H)^+$.

Synthesis of 2-(4-4-[(*tert*-butoxycarbonyl)(methyl)-amino]thieno[3,2-d]pyrimidin-2-ylpiperazino)acetic acid (11): To a solution of compound **10** (2.5 g, 5.74 mmol) in methanol (25 mL) at 0 °C was added 6N NaOH (12 mL) and stirred the reaction at room temperature for 8 h. After completion, reaction mixture was poured into ice cold water, neutralized with aq. citric acid solution up to pH 5 and filtered the formed precipitate to afford **11** as off white solid (**Scheme-I**). Yield: 93 %; m.p.: 128-129 °C; IR (KBr, ν_{max}, cm^{-1}): 3435, 3004, 2979, 2940, 2869, 1698, 1638,1564, 1374, 1284, 1138, 983, 910, 794, 772; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.18-8.17 (d, *J* = 5.2 Hz, 1H, ArH), 7.19-7.17 (d, *J* = 5.2, 1H, ArH), 3.78 (brs, 4H, CH_2-piperazine), 3.30 (s, 3H, CH_3), 3.20 (s, 2H, NCH_2CO), 2.64 (s, 4H, CH_2-piperazine), 1.46 (s, 9H, $C(CH_3)_3$); ESI-MS: *m/z*, 408.3$(M+H)^+$.

General experimental procedure for synthesis of novel thienopyrimidine amide derivatives (13a-m): To a stirred solution of compound **11** (100 mg, 0.245 mmol) in DMF (4 mL) was added diisopropylethylamine (DiPEA) (95 mg, 0.735 mmol), 2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyl-uronium hexafluorophosphate (HATU) (112 mg, 0.294 mmol) followed by addition of aryl or aliphatic amines (**12a-m**) (0.279 mmol) and stirred at room temperature for 18 h. After completion reaction mixture was poured into ice cold water extracted with ethyl acetate (2 × 15 mL), combined extracts were washed with brine solution, dried over anhy. Na_2SO_4 and concentrated under reduced pressure to afford crude product. This crude compound was purified by column chromatography (elutent: 2 % methaol: CH_2Cl_2) to affords pure compounds **13a-m** as yields of the products varied between 80-94 %. By adapting this procedure the compounds **13a-13m** were synthesized (**Scheme-II**).

***tert*-Butyl-2-(4-((cyclohexylcarbamoyl)methyl)piperazin-1-yl)thieno[3,2-d]pyrimidin-4-ylmethylcarbamate (13a):** Yield: 81 %; m.p.: 148-152 °C; IR (KBr, ν_{max}, cm^{-1}): 3536, 2972, 2855, 1712,1642, 1448, 1333, 1294, 1135, 1094, 1004, 930, 854, 578; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.74-7.71 (d, *J* = 12, 1H, ArH), 7.14-7.13 (d, *J* = 4 Hz, 1H, ArH), 3.84 (brs, 4H, CH_2-piperazine), 3.80 (brs, 1H, CH-Cy.Hexyl), 3.39 (s, 3H, CH_3), 3.04 (s, 2H, NCH_2CO), 2.61 (brs, 4H, CH_2-piperazine), 1.92-1.89 (m, 2H. CH_2-Cy.Hexyl), 1.68-1.58 (m, 2H, CH_2-Cy.Hexyl), 1.52 (s, 9H, $C(CH_3)_3$), 1.50-1.42 (m, 2H, CH_2-Cy.Hexyl); 1.27-1.20 (m, 4H, CH_2-Cy.Hexyl); ^{13}C NMR



Reagents and conditions:
a i) KOCN, AcOH, H_2O, room temperature, 16 h; ii) NaOH, room temperature, 4 h;
b Toluene, NMP, $POCl_3$, reflux, 16 h;
c THF, room temperature, 3 h;
d $(Boc)_2O$, DMF, TEA, room temperature, 4 h;
e DMF, K_2CO_3, room temperature, 12 h;
f DMF, TEA, room temperature, 16 h;
g MeOH, 6N NaOH, room temperature, 8 h

Scheme-I

(400 MHz, $CDCl_3$): δ 22.7, 27.9, 29.6, 33.7, 36.3, 42.3, 43.6, 48.5, 54.42, 62.8, 81.4, 115.9, 123.6, 134.2, 154.2, 158.2, 160.2, 163.7, 168.6; ESI-MS: *m/z*, 489.4 $(M+H)^+$.

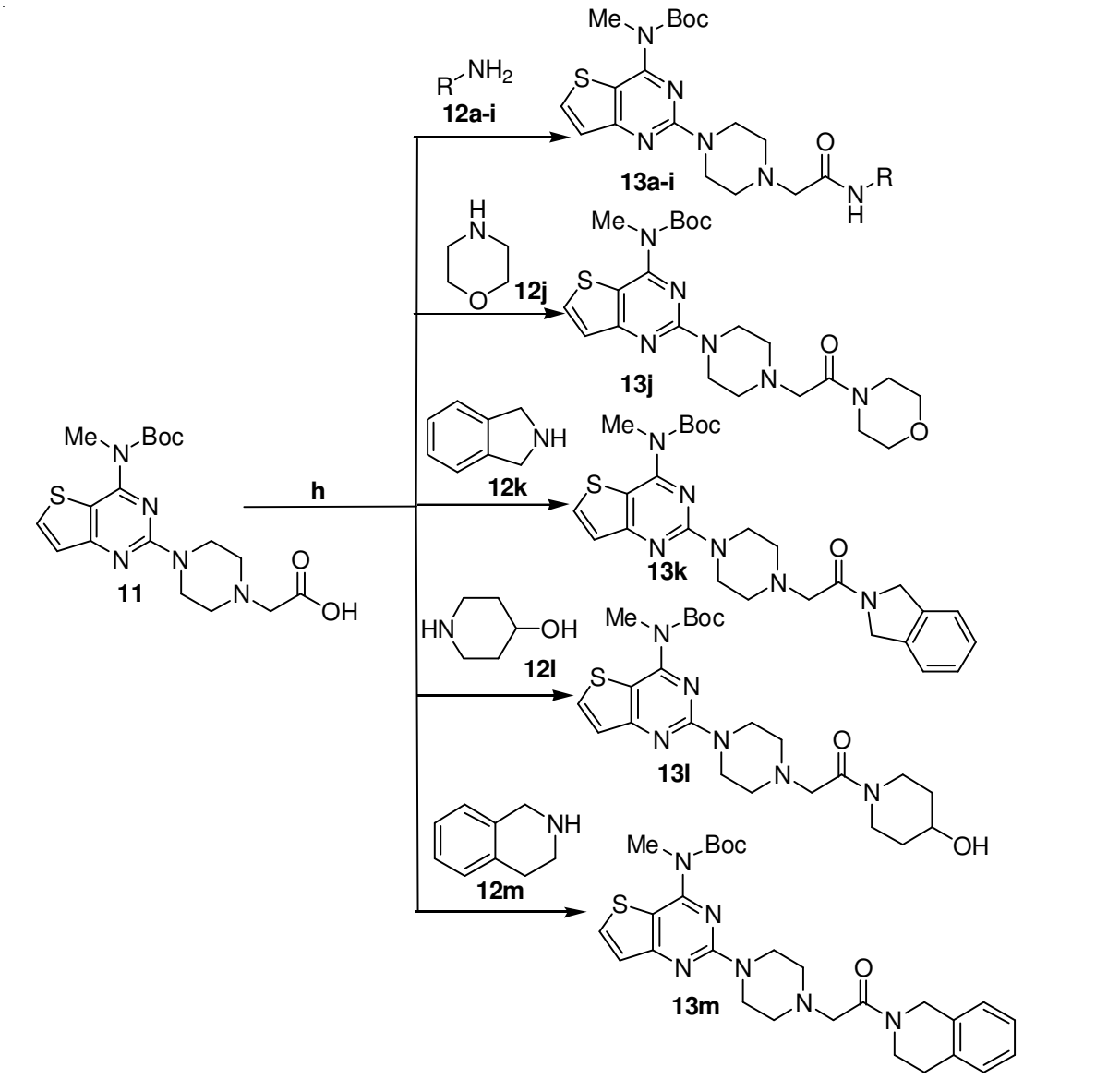
***tert*-Butyl-2-(4-((cycloheptylcarbamoyl)methyl)-piperazin-1-yl)thieno[3,2-d]pyrimidin-4-ylmethylcarbamate (13b):** Yield: 85 %; m.p.: 174-178 °C; IR (KBr, ν_{max}, cm^{-1}): 3555, 2928, 1714, 1673, 1572, 1504, 1371, 1266, 1146, 1003; ^1H NMR (400 MHz, $CDCl_3$): δ 7.75-7.73 (d, *J* = 8 Hz, 1H, ArH), 7.20-7.18 (d, *J* = 8 Hz, 1H, ArH), 4.03 (brs, 1H, CH-Cy.Heptyl), 3.87 (brs, 4H, CH_2-piperazine), 3.39 (s, 3H, CH_3), 3.03 (s, 2H, NCH_2CO), 2.60 (brs, 4H, CH_2-piperazine), 1.92-1.90 (m, 2H, CH_2-Cy. heptyl), 1.70 -1.60 (m, 10H, CH_2-Cy. Heptyl), 1.53 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (400 MHz, $CDCl_3$): δ 23.9, 28.1, 29.7, 34.9, 35.0, 44.4, 49.4, 44.4, 53.3, 61.7, 82.3, 114.9, 122.7, 135.3, 153.3, 157.4, 160.0, 164.6, 168.5; ESI-MS: *m/z*, 503.1 $(M+H)^+$.

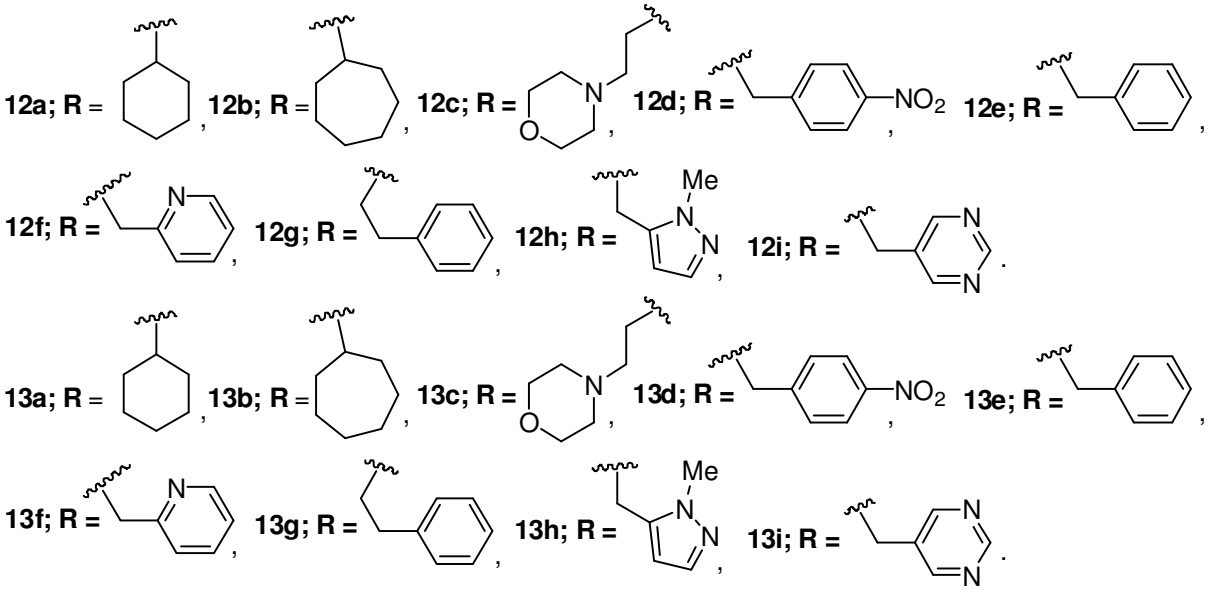
***tert*-Butyl-N-methyl-N-[2-(4-2-[(2-morpholinoethyl)-amino]-2-oxoethylpiperazino)thieno[3,2-d]pyrimidin-4-yl]carbamate (13c):** Yield: 92 %; m.p.: 106-110 °C; IR (KBr, ν_{max}, cm^{-1}): 3526, 3469, 3320, 3091, 2930, 2795, 2840, 2761, 1710, 1651, 1564, 1525,1450, 1372, 1334, 1269, 1151, 1116, 850,1002, 928, 861, 796, 762, 662, 584; ^1H NMR (400 MHz, $CDCl_3$): δ 7.75-7.7 (d, *J* = 8 Hz, 1H, ArH), 7.14-7.13 (d, *J* = 4, 1H, ArH), 3.89 (brs, 4H, OCH_2-Morpholine,), 3.71 (s, 4H, CH_2-piperazine), 3.44-3.39 (m, 2H, CH_2NHCO); 3.39 (s, 3H, CH_3), 3.07 (s, 2H, NCH_2CO), 2.62 (brs, 4H, CH_2-piperazine), 2.54-2.49 (m, 6H, NCH_2-Morpholine & NCH_2C), 1.52 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (400 MHz, $CDCl_3$): δ 28.1, 34.9, 35.1, 44.5, 53.3, 53.4, 57.1, 61.6, 67.1, 82.4, 114.9, 122.7, 135.3, 153.35, 157.4, 159.9, 164.6, 169.9; ESI-MS: *m/z*, 520.4 $(M+H)^+$.

***tert*-Butyl-N-methyl-N-[2-(4-2-[(4-nitrobenzyl)amino]-2-oxoethylpiperazino)thieno[3,2-d]pyrimidin-4-yl]carbamate (13d):** Yield: 80 %; m.p.: 94-97 °C ; IR (KBr, ν_{max}, cm^{-1}): 3528, 3466, 3320, 3080, 2976, 2931, 2842, 1711, 1649, 1550, 1525,1453, 1370, 1335, 1261, 1150, 1001, 1002, 850, 928, 854, 797, 765, 701, 581; ^1H NMR (400 MHz, $CDCl_3$): δ 8.22-8.20 (d, *J* = 8 Hz, 2H, Ar-H), 7.75-7.71 (d, *J* = 4 Hz, 1H,ArH), 7.47-7.45 (d, *J* = 8 Hz, 2H, Ar-H), 7.13-7.12 (d, *J* = 4 Hz, 1H, ArH), 4.62-4.61 (d, *J* = 4 Hz, 2H, NCH2Ar), 3.86 (brs, 4H, CH_2-piperazine), 3.40 (s, 3H, CH_3), 3.16 (s, 2H, NCH_2CO), 2.64 (brs, 4H, CH_2-piperazine), 1.52 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (400 MHz, $CDCl_3$): δ 28.1, 34.9, 42.2, 44.2, 46.2, 53.0, 61.3, 67.0, 82.2, 114.8, 122.7, 135.2, 153.3, 157.4, 160.0, 164.6, 168.0; ESI-MS: *m/z*, 542.40 $(M+H)^+$.

***tert*-Butyl-2-(4-((benzylcarbamoyl)methyl)piperazin-1-yl)thieno[3,2-d]pyrimidin-4-ylmethylcarbamate (13e):** Yield: 87 %; m.p.: 146-150 °C; IR (KBr, $\nu_{max,}$ cm^{-1}): 3531, 3468, 3101, 3080, 2971, 2838, 1713, 1665, 1556, 1520, 1371, 1328, 1266, 1152, 1091, 1001, 929, 799, 766, 622, 583; 1H NMR (400 MHz, $CDCl_3$): δ 7.76-7.74 (d, *J* = 6 Hz, 1H, ArH), 7.53 (brs, 1H, NH), 7.37-7.26 (m, 5H), 7.15-7.12 (d, *J* = 6 Hz, 1H, ArH), 4.52-4.51 (d, *J* = 4 Hz, 2H, NCH_2Ar-H), 3.88-3.82 (brs, 4H,, CH_2-piperazine), 3.38 (s, 3H, CH_3), 3.13 (s, 2H, NCH_2CO), 2.63 (brs, 4H,, CH_2-piperazine), 1.52 (s, 9H, $(CH_3)_3$); ^{13}C NMR (400 MHz, $CDCl_3$): δ 28.1, 34.9, 44.6, 53.5, 62.4, 82.4,115.1, 118.3, 119.7, 122.6, 124.9, 125.8, 125.9, 126.0,126.3,128.9, 132.1, 134.0, 135.5, 153.3, 157.5, 159.8, 164.5, 168.2; ESI-MS: *m/z*, 497.42 $(M+H)^+$.

***tert*-Butyl-2-(4-(((pyridin-2-yl)methylcarbamoyl)-methyl)piperazin-1-yl)thieno[3,2-d]pyrimidin-4-ylmethyl-carbamate (13f):** Yield: 83 %; m.p.: 142-146 °C; IR (KBr,





Reagents and conditions: **h** DMF, DIPEA, HATU, room temperature, 18 h

Scheme-II: Synthesis of thienopyrimidine amide derivatives **13a-m**

ν_{max}, cm^{-1}): 3343, 3101, 3043, 2967, 2924, 2855, 2837,1712, 1669, 1564, 1519.9, 1447,1373, 1332, 1303, 1269,1150,1001, 930, 798, 7667, 584; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.56-8.55 (d, *J* = 5 Hz, 1H, Py-H), 8.24 (brs, 1H, NH), 7.74-7.73 (d, *J* = 5.5 Hz, 1H, ArH) 7.68-7.71 (t, *J* = 1.5 Hz, 1H, Py-H), 7.29-7.27 (d, *J* = 8 Hz, 1H, Py-H), 7.21-7.20 (d, *J* = 5.5 Hz, 1H, ArH), 7.14-7.13 (d, *J* = 5.5 Hz, 1H, Py-H), 4.63-4.62 (d, *J* = 5 Hz, 2H, NCH2Ar), 3.90 (brs, 4H, CH_2-piperazine), 3.39 (s, 3H, CH_3), 3.14 (s, 2H, NCH_2CO), 2.64 (brs, 4H, CH_2-piperazine),1.52 (s, 9H, $(CH_3)_3$); ^{13}C NMR(400 MHz, $CDCl_3$): δ 28.1, 29.6, 34.9, 44.1, 44.3, 44.6, 53.4, 61.7, 82.4, 114.8, 121.9, 122.3, 122.7, 135.2, 136.7, 149.2, 153.3, 156.8, 157.4, 160.0, 164.6, 170.2; ESI-MS: *m/z,* 498.4 $(M+H)^+$.

***tert*-Butyl-2-(4-((phenethylcarbamoyl)methyl)piperazin-1-yl)thieno[3,2-d]pyrimidin-4-ylmethylcarbamate (13g):** Yield: 80 %; m.p.: 77-81 °C; IR (KBr, ν_{max}, cm^{-1}): 3530. 3469, 3326, 3082, 2975, 2842, 2763, 1710, 1649, 1566, 1453, 1372, 1332, 1263, 1151, 1002, 928, 854, 796, 758, 701,581; ^{1}H NMR (400 MHz, $CDCl_3$): 7.74-7.73 (d, *J* = 5.6 Hz, 1H, ArH), 7.32-7.21 (m, 6H, Ar-H), 7.14-7.12 (d, *J* = 5.6 Hz, 1H,, ArH), 3.73 (brs, 4H, CH_2-piperazine), 3.62-3.60 (q, *J* = 2.1 Hz, 2H, $NHCH_2C$), 3.39 (s, 3H, CH_3), 3.0 (s, 2H, NCH_2CO), 2.89-2.85 (t, *J* = 6.8 Hz, 2H,CCH_2Ar-H), 2.49-2.47 (s, 4H, CH_2-piperazine), 1.53 (s, 9H, $(CH_3)_3$); ^{13}C NMR (400 MHz, $CDCl_3$): δ 28.1, 29.6, 34.8, 35.5, 39.6, 44.2, 53.3, 61.6, 82.3, 114.8, 122.6, 126.6, 128.6,128.7, 135.3,1387, 153.3, 157.4, 159.8, 164.6, 169.9; ESI$(M+H)^+$. MS: *m/z,* 511.44 $(M+H)^+$.

***tert*-Butyl-2-(4-(((1-methyl-1*H*-pyrazol-4-yl)methylcarbamoyl)methyl)piperazin-1-yl)thieno[3,2-d]pyrimidin-4-ylmethylcarbamate (13h):** Yield: 86 %; m.p.: 168-172 °C; IR (KBr, ν_{max}, cm^{-1}): 3418, 3392, 3331, 2980, 2931, 2850, 2821, 1710, 1568, 1525, 1373, 1335, 1266, 1152, 1000, 931, 797, 763, 663, 582; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.74-7.73 (d, *J* = 6 Hz, 1H,ArH), 7.43 (s, 1H, Ar-H), 7.36 (s, 1H, CONH), 7.13-7.12 (d, *J* = 6 Hz 2H,ArH), 4.36-4.34 (d, *J* = 6 Hz, 2H), 3.88 (s, 3H, NCH_3), 3.84 (brs, 4H, CH_2-piperazine), 3.38 (s, 3H, CH_3), 3.07 (s, 2H, NCH_2CO), 2.59 (s, 4H, CH_2-piperazine), 1.52 (s, 9H, $(CH_3)_3$). ESI-MS: *m/z,* 501.1$(M+H)^+$.

***tert*-Butyl-2-(4-(((pyrimidin-5-yl)methylcarbamoyl)-methyl)piperazin-1-yl)thieno[3,2-d]pyrimidin-4-ylmethylcarbamate (13i):** Yield: 84 %; m.p.: 128-129 °C; IR (KBr, ν_{max}, cm^{-1}): 3452, 3007, 2983, 1708, 1659, 1566, 1386, 1258, 1147, 1003, 850, 728, 634; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.16 (s, 1H, Ar-H), 8.73 (s, 1H, Ar-H), 7.75-7.74 (m, 2H, ArH&Ar-H), 7.14-7.13 (d, *J* = 6 Hz, 1H,ArH), 4.8 (brs, 1H,CONH), 4.54-4.53 (d, *J* = 6.4 Hz, 2H. Ar-HCH2N), 3.86 (s, 4H, CH_2-piperazine), 3.38 (s, 3H, CH_3), 3.14 (s, 2H, NCH_2CO), 2.62 (s, 4H, CH_2-piperazine), 1.52 (s, 9H, $(CH_3)_3$); ESI-MS: *m/z,* 499. 4$(M+H)^+$.

***tert*-Butyl-methyl-2-(4-(2-morpholino-2-oxoethyl)-piperazin-1-yl)thieno[3,2-d]pyrimidin-4-ylcarbamate (13j):** Yield: 94 %; m.p.: 135-139 °C; IR (KBr, ν_{max}, cm^{-1}): 3434, 3085, 2978, 2919, 2849, 2795, 1710, 1641, 1571, 1523, 1461, 1372, 1264, 1155, 1111, 1001, 968, 932, 850, 798, 583; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.74-7.72 (d, *J* = 8 Hz, 1H, ArH), 7.15-7.12 (d, *J* = 8 Hz, 1H, ArH), 3.86 (brs, 4H, OCH_2-Morpholine), 3.74-3.64 (s, 4H, CH_2-piperazine), 3.40 (m, 7H, NCH_2-Morpholine), 3.24 (s, 2H, NCH_2CO), 2.61 (s, 4H, CH_2-piperazine), 1.52 (s, 9H, $(CH_3)_3$); ^{13}C NMR (400 MHz, $CDCl_3$): δ 28.3, 34.7, 45.4, 54.4, 54.5, 57.54, 62.6, 67.5, 82.4, 115.1, 123.7, 135.5, 154.4, 156.4, 159.9, 165.5, 168.8; ESI-MS: *m/z*, 477.1 $(M+H)^+$.

***tert*-Butyl-2-(4-(2-(isoindolin-2-yl)-2-oxoethyl)piperazin-1-yl)thieno[3,2-d]pyrimidin-4-ylmethylcarbamate (13k):** Yield: 93 %; m.p.: 91-95 °C; IR (KBr, ν_{max}, cm^{-1}): 3442, 2977, 924, 2849, 1710, 1646, 1567, 1527, 1453, 1371, 1332, 1263, 1151, 1002, 930, 846, 796, 758; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73-7.72 (d, *J* = 4 Hz, 1H,ArH), 7.31-7.26 (m, 4H, Ar-H), 7.14-7.13 (d, *J* = 4 Hz, 1H,ArH), 4.96 (s, 2H, $CONCH_2$), 4.85 (s, 2H, $CONCH_2$), 3.92 (brs, 4H, CH_2-piperazine), 3.39 (s, 3H, CH_3), 3.30 (s, 2H, NCH_2CO), 2.69 (s, 4H, CH_2-piperazine), 1.54 (s, 9H, $(CH_3)_3$); ^{13}C NMR (400 MHz, $CDCl_3$): δ 28.1, 34.9, 44.13, 44.27, 52.21, 52.37, 53.06, 53.40, 61.4, 82.3, 114.6, 122.5, 122.7, 122.9, 127.5, 127.7, 135.1,136.0, 136.3, 153.3, 157.3,160.0, 164.6, 168.3; ESI-MS: *m/z*, 509.39 $(M+H)^+$.

***tert*-Butyl-2-(4-(2-(4-hydroxypiperidin-1-yl)-2-oxoethyl)piperazin-1-yl)thieno[3,2-d]pyrimidin-4-ylmethylcarbamate (13l):** Yield: 93 %; m.p.: 119-123 °C; IR (KBr, ν_{max}, cm^{-1}): 545, 3321, 2928, 2855, 1714, 1673, 1572, 1504, 1333, 1266, 1147, 1094, 1050, 1003, 930, 854, 798, 708, 578; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73-7.72 (d, *J* = 4 Hz, 1H, ArH), 7.14-7.13 (d, *J* = 4 Hz, 1H, ArH), 4.10 (brs, 1H,C**H**-OH), 4.07-3.86 (m, 6H, NCH_2CO &CH_2-piperazine), 3.38 (s, 3H, CH_3), 3.2-3.18 (m, 4H, CH_2-piperdine), 2.60-2.59 (s, 4H, CH_2-piperazine) 1.90-1.85 (m, 5H, CH_2-piperdine& OH), 1.52 (s, 9H, $(CH_3)_3$); ESI-MS: *m/z,* 491.39 $(M+H)^+$.

***tert*-Butyl-2-(4-(2-(3,4-dihydroisoquinolin-2(1*H*)-yl)-2-oxoethyl)piperazin-1-yl)thieno[3,2-d]pyrimidin-4-ylmethylcarbamate (13m):** Yield: 91 %; m.p.: 120-124 °C; IR (KBr, ν_{max}, cm^{-1}): 33440, 3410, 3095, 2939, 2832, 1710, 1634, 1564, 1373, 1261, 1154, 1001, 929, 849, 797, 763, 579; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73-7.72 (d, *J* = 3 Hz, 1H), 7.26-7.11 (m, 5H), 4.81-4.75 (m, 2H,$CONCH_2$), 3.87-3.81 (m, 6H,CH_2), 3.37-3.32 (m, 5H, NCH_3, CH_2), 2.96-2.88 (m, 2H,CCH_2Ar-H), 2.61-2.60 (m, 4H, CH_2-piperazine),1.51 (s, 9H, $(CH_3)_3$); ^{13}C NMR (400 MHz, $CDCl_3$): δ 28.4, 29.6, 34.9, 40.1, 43.3, 44.1, 44.2, 44.4, 47.2, 53.1, 61.6, 61.8, 82.2, 122.7, 126.4, 126.6, 134.0, 153.3, 157.4, 160.0, 164.6, 168.2, 168.4; ESI-MS: *m/z,* 523.4 $(M+H)^+$.

RESULTS AND DISCUSSION

The target compounds **13a-m** was prepared as outlined in **Schemes I** and **II**. The compound methyl 3-aminothiophene-2-carboxylate (**1**) was reacted with KOCN in acetic acid and H_2O at room temperature for 16 h. The resulting solid was filtered and treated with 50 % NaOH and reaction mixture was stirred at room temperature for 4 h to afford pure thieno[3,2-*d*]pyrimidine-2,4-diol (**2**) in good yield. This diol intermediate (**2**) was reacted with $POCl_3$ in toluene and NMP at reflux for 16 h to afford pure 2,4-dichlorothieno[3,2-*d*]pyrimidine (**3**) and intermediate **3** was coupled with 40 % methylamine (**4**) in methanol and THF at room temperature for 3 h to afford *N*-(2-chlorothieno[3,2-*d*]pyrimidin-4-yl)-*N*-methylamine (**5**) in 91 % yield. The intermediate **5** undergoes to protection reacting with $(Boc)_2O$ in DMF and triethylamine at room temperature

for 4 h to afford protected compound **6**, it was coupled with piperizine (**7**) in DMF, K_2CO_3 at room temperature for 12 h to afford pure *tert*-butyl *N*-methyl-*N*-(2-piperazinothieno[3,2-*d*]pyrimidin-4-yl)carbamate (**8**) in 82 % yield. The piperizine intermediate **8** was reacted with ethylbromoacetate (**9**) in DMF, triethylamine at room temperature for 16 h to afford pure compound ethyl 2-(4-4-[(*tert*-butoxycarbonyl)(methyl)amino]-thieno[3,2-*d*]pyrimidin-2-ylpiperazino)acetate (**10**). This ethylester intermediate **10** was undergoes to basic hydrolysis with 6N NaOH in methanol at room temperature for 8 h to afford pure acid intermediate **11** in 82 % yield as shown in **Scheme-I**. The acid intermediate **11** was coupled with different substituted amines (**12a-m**) in DMF, DIPEA and HATU at room temperature for 18 h to afford pure amide compounds (**13a-m**) in excellent yield.

Biological assay: The synthesized thienopyrimidine amide derivatives **13a-m** were dissolved in dimethyl sulphoxide at 30 µg/µL concentration (standard antibacterial drug, ampicilline was used as the reference antibiotic) and tested against Gram-negative strains of (1) *Escherichia coli*, (2) *Klebsiella. pneumonia* and Gram-positive strains of (3) *Staphylococcus aureus* and (4) *Bacillus subtilis* using agar well diffusion method according to the literature protocol [12,13,27,28]. Activity was determined by zones showing complete inhibition (mm). Growth inhibition was calculated with reference to positive control. All the samples were taken in triplicates.

Antibacterial activity: These newly synthesized thienopyrimidine derivatives (**13a-m**) were evaluated for their antibacterial activity against two Gram-negative and two Gram-positive bacterial strains *viz.*, *Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus* and *Bacillus subtilis*. The results are summarized in Table-1 and ampicilline was used as positive control.

TABLE-1
in vitro ANTIBACTERIAL ACTIVITY OF COMPOUNDS **13a-m** (CONCENTRATION USED 30 µg/20 µL OF DMSO)

Compd.	Zones of inhibition of compounds **13a-m** in mm			
	Gram-negative		Gram-positive	
	E. coli	*K. pneumonia*	*S. aureus*	*B. subtilis*
13a	4	6	3	3
13b	0	3	4	0
13c	0	5	4	0
13d	4	3	5	6
13f	0	0	5	5
13g	5	7	2	0
13h	3	4	0	0
13i	8	5	4	4
13j	0	5	2	7
13k	12	13	17	15
13m	5	6	0	6
Ampicilline	16	14	17	16

It is observed that compounds **13g**, **13i, 13k** and **13m** revealed excellent antibacterial activity with zone of inhibition 30-33 mm against *E. coli* (Gram-negative bacteria) and *S. aureus* (Gram-positive bacteria) even in the case of *P. aeruginosa* (Gram-negative bacteria) and *S. pyogenes* (Gram-positive bacteria), compounds **13d, 13k** and **13f** displayed excellent anti-bacterial activity with zone of inhibition 22-25 mm (Table-1).

REFERENCES

1. R. Klevens, J. Edwards, C. Richards Jr., T. Horan, R. Gaynes, D. Pollock and D. Cardo, *Public Health Rep.*, **122**, 160 (2007); https://doi.org/10.1177/003335490712200205.
2. R. Kharb, P.C. Sharma and M.S. Yar, *J. Enzyme Inhib. Med. Chem.*, **26**, 1 (2011); https://doi.org/10.3109/14756360903524304.
3. R. Kharb, M. Shahar Yar and P. C. Sharma, *Curr. Med. Chem.*, **18**, 3265 (2011); https://doi.org/10.2174/092986711796391615.
4. R. Kharb, M. Shahar Yar and P.C. Sharma, *Mini Rev. Med. Chem.*, **11**, 84 (2011); https://doi.org/10.2174/138955711793564051.
5. V.S. Dinakaran, B. Bhargavi and K.K. Srinivasan, *Pharmachem*, **4**, 255 (2012).
6. M. Lindvall, C. McBride, M. McKenna, T.G. Gesner, A. Yabannavar, K. Wong, S. Lin, A. Walter and C.M. Shafer, *ACS Med. Chem. Lett.*, **2**, 720 (2011); https://doi.org/10.1021/ml200029w.
7. Y. Ni, A. Gopalsamy, D. Cole, Y. Hu, R. Denny, M. Ipek, J. Liu, J. Lee, J.P. Hall, M. Luong, J.-B. Telliez and L.-L. Lin, *Bioorg. Med. Chem. Lett.*, **21**, 5952 (2011); https://doi.org/10.1016/j.bmcl.2011.07.069.
8. T.P. Heffron, M. Berry, G. Castanedo, C. Chang, I. Chuckowree, J. Dotson, A. Folkes, J. Gunzner, J.D. Lesnick, C. Lewis, S. Mathieu, J. Nonomiya, A. Olivero, J. Pang, D. Peterson, L. Salphati, D. Sampath, S. Sideris, D.P. Sutherlin, V. Tsui, N.C. Wan, S. Wang, S. Wong and B. Zhu, *Bioorg. Med. Chem. Lett.*, **20**, 2408 (2010); https://doi.org/10.1016/j.bmcl.2010.03.046.
9. D.P. Sutherlin, D. Sampath, M. Berry, G. Castanedo, Z. Chang, I. Chuckowree, J. Dotson, A. Folkes, L. Friedman, R. Goldsmith, T. Heffron, L. Lee, J. Lesnick, C. Lewis, S. Mathieu, J. Nonomiya, A. Olivero, J. Pang, W.W. Prior, L. Salphati, S. Sideris, Q. Tian, V. Tsui, N.C. Wan, S. Wang, C. Wiesmann, S. Wong and B. Zhu, *J. Med. Chem.*, **53**, 1086 (2010); https://doi.org/10.1021/jm901284w.
10. A.B.A. El-gazzar, H.A.R. Hussein and H.N. Hafez, *Acta Pharm.*, **57**, 395 (2007); https://doi.org/10.2478/v10007-007-0032-6.
11. D.P. Sutherlin, L. Bao, M. Berry, G. Castanedo, I. Chuckowree, J. Dotson, A. Folks, L. Friedman, R. Goldsmith, J. Gunzner, T. Heffron, L. Lesnick, C. Lewis, S. Mathieu, J. Murray, J. Nonomiya, J. Pang, N. Pegg, W.W. Prior, L. Rouge, L. Salphati, D. Sampath, Q. Tian, N.C. Tsui, N.C. Wan, S. Wang, B.Q. Wei, C. Wiesmann, P. Wu, B.-Y. Zhu and A. Olivero, *J. Med. Chem.*, **54**, 7579 (2011); https://doi.org/10.1021/jm2009327.
12. J.J. Wallin, K.A. Edgar, J. Guan, M. Berry, W.W. Prior, L. Lee, J.D. Lesnick, C. Lewis, J. Nonomiya, J. Pang, L. Salphati, A.G. Olivero, D.P. Sutherlin, C. O'Brien, J.M. Spoerke, S. Patel, L. Lensun, R. Kassees, L. Ross, M.R. Lackner, D. Sampath, M. Belvin and L.S. Friedman, *Mol. Cancer Ther.*, **10**, 2426 (2011); https://doi.org/10.1158/1535-7163.MCT-11-0446.
13. S. Mathieu, S.N. Gradl, L. Ren, Z. Wen, I. Aliagas, J. Gunzner-Toste, W. Lee, R. Pulk, G. Zhao, B. Alicke, J.W. Boggs, A.J. Buckmelter, E.F. Choo, V. Dinkel, S.L. Gloor, S.E. Gould, J.D. Hansen, G. Hastings, G. Hatzivassiliou, E.R. Laird, D. Moreno, Y. Ran, W.C. Voegtli, S. Wenglowsky, J. Grina and J. Rudolph, *J. Med. Chem.*, **55**, 2869 (2012); https://doi.org/10.1021/jm300016v.
14. B.S. Safina, S. Baker, M. Baumgardner, P.M. Blaney, B.K. Chan, Y.-H. Chen, M.W. Cartwright, G. Castanedo, C. Chabot, A.J. Cheguillaume, P. Goldsmith, D.M. Goldstein, B. Goyal, T. Hancox, R.K. Handa, P.S. Iyer, J. Kaur, R. Kondru, J.R. Kenny, S.L. Krintel, J. Li, J. Lesnick, M.C. Lucas, C. Lewis, S. Mukadam, J. Murray, A.J. Nadin, J. Nonomiya, F. Padilla, W.S. Palmer, J. Pang, N. Pegg, S. Price, K. Reif, L. Salphati, P.A. Savy, E.M. Seward, S. Shuttleworth, S. Sohal, Z.K. Sweeney, S. Tay, P. Tivitmahaisoon, B. Waszkowycz, B. Wei, Q. Yue, C. Zhang and D.P. Sutherlin, *J. Med. Chem.*, **55**, 5887 (2012); https://doi.org/10.1021/jm3003747.
15. M.I. Crespo, L. Pages, A. Vega, V. Segarra, M. Lopez, T. Domenech, M. Miralpeix, J. Beleta, H. Ryder and J.M. Palacios, *J. Med. Chem.*, **41**, 4021 (1998); https://doi.org/10.1021/jm981012m.

16. Y. Kotaiah, N. Harikrishna, K. Nagaraju and C. Venkata Rao, *Eur. J. Med. Chem.*, **58**, 340 (2012); https://doi.org/10.1016/j.ejmech.2012.10.007.
17. H.A. Stefani, C.B. Oliveira, R.B. Almeida, C.M.P. Pereira, R.C. Braga, R. Cella, V.C. Borges, L. Savegnago and C.W. Nogueira, *Eur. J. Med. Chem.*, **41**, 513 (2006); https://doi.org/10.1016/j.ejmech.2006.01.007.
18. H. Kikuchi, K. Yamamoto, S. Horoiwa, S. Hirai, R. Kasahara, N. Hariguchi, M. Matsumoto and Y. Oshima, *J. Med. Chem.*, **49**, 4698 (2006); https://doi.org/10.1021/jm0601809.
19. B. Singh, A. Maheshwari, G. Dak, K. Sharma and G.L. Talesara, *Indian J. Pharm. Sci.*, **72**, 607 (2010); https://doi.org/10.4103/0250-474X.78529.
20. S. Rostamizadeh, M. Nojavan, R. Aryan, H. Sadeghian and M. Davoodnejad, *Chin. Chem. Lett.*, **24**, 629 (2013); https://doi.org/10.1016/j.cclet.2013.04.035.
21. H.N. Hafez, H.A. Hussein and A.R. El-Gazzar, *Eur. J. Med. Chem.*, **45**, 4026 (2010); https://doi.org/10.1016/j.ejmech.2010.05.060.
22. H.M. Aly, N.M. Saleh and H.A. Elhady, *Eur. J. Med. Chem.*, **46**, 4566 (2011); https://doi.org/10.1016/j.ejmech.2011.07.035.
23. B.V. Ashalatha, B. Narayana, K.K. Vijaya Raj and N.S. Kumari, *Eur. J. Med. Chem.*, **42**, 719 (2007); https://doi.org/10.1016/j.ejmech.2006.11.007.
24. T. George, C.L. Kaul, R.S. Grewal and R. Tahilramani, *J. Med. Chem.*, **14**, 913 (1971); https://doi.org/10.1021/jm00292a005.
25. O. Bruno, C. Brullo, A. Ranise, S. Schenone, F. Bondavalli, E. Barocelli, V. Ballabeni, M. Chiavarini, M. Tognolini and M. Impicciatore, *Bioorg. Med. Chem. Lett.*, **11**, 1397 (2001); https://doi.org/10.1016/S0960-894X(01)00221-9.
26. R.K. Russell, J.B. Press, R.A. Rampulla, J.J. McNally, R. Falotico, J.A. Keiser, D.A. Bright and A. Tobia, *J. Med. Chem.*, **31**, 1786 (1988); https://doi.org/10.1021/jm00117a019.
27. L.M. Lima, F.S. Frattani, J.L. Dos Santos, H.C. Castro, C.A.M. Fraga, R.B. Zingali and E.J. Barreiro, *Eur. J. Med. Chem.*, **43**, 348 (2008); https://doi.org/10.1016/j.ejmech.2007.03.032.
28. P.B. Nariya, N.R. Bhalodia, V.J. Shukla and M.B. Nariya, *Int. J. Pharm. Tech. Res.*, **2**, 2522 (2010).