



Green Synthesis Approaches of 2-Bromo-4-methyl thiazole-5-carboxamide Derivatives as Potent Microbial Agents

JEEVANREDDY MIRYALA, MAHESH MORTHAD and SATYANARAYANA BATTU*

Department of Chemistry, Osmania University, Hyderabad-500007, India

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

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As a part of our ongoing research in the development of new, selective and environmental friendly methodologies, herein we report a new series of 2-bromo-4-methylthiazole-5-carboxamide derivatives using polyethylene glycol-400 (PEG-400) as a solvent. All the newly synthesized compounds were screened to their *in vitro* antimicrobial activities and all the compounds exhibited remarkable growth inhibition of wide spectrum of Gram-positive bacteria and Gram-negative bacteria. Molecular docking was done performed computational software GOLD (Genetic algorithm based, flexible ligand, partial flexibility for protein).

Keywords: Thiazole, PEG-400, Antimicrobial activity, Docking.

INTRODUCTION

Thiazoles have been discovered as key components of novel and structurally diverse natural products possessing a wide range of biological and pharmaceutical activities and their usefulness as medicines are established. Thiazoles play an important role in drug development for the treatment of bacterial, allergies and inflammation due to presence of (S-C=N) unit [1-3]. Thiazoles have been reported to have significant antileukemic activity on various human cells [4].

Thiazoles occurs in vitamin B₁ or thiamine the most widely recognised naturally occurring derivative in the form of its thiazolium salt [5], aeruginosic acid [6], a living organism also contains a 1,3-thiazole ring and luciferin is a light-emitting natural product found in firefly also contain Δ^2 -thiazole ring [7]. They are also among the most important building blocks in today's drug discovery and are found in biologically active compounds across a number of different therapeutic areas such as antifungal [8], antibacterial [9], antiviral [10], antimalarial [11], anticancer [2], hypertension [12], inflammation [13], schizophrenia [14], HIV infections [15], hypnotics [16] and more recently for the treatment of pain [17], as fibrinogen receptor antagonists with antithrombotic activity [18], and as new inhibitors of bacterial DNA gyrase B [19].

Polyethylene glycol (PEG) is widely used as a solvent for the synthesis of fine chemicals and biologically important compounds [20-22] due to its inherent advantages over toxic solvents. Furthermore, PEG is inexpensive, thermally stable, non-toxic, easy to handle and recyclable. To the best of our knowledge, this is the first protocol for amidation of derivatives of thiazole using PEG-400 as a reaction medium. Thiazolyl carboxamides, amides of 2-aminothiazoles are very important structural motifs used by drug discovery chemists and found in many important disease-intervening substances [23]. Therefore, in this paper we report the green synthesis of 2-bromo-4-methylthiazole-5-carboxamide derivatives and their biological evaluation against a series of Gram-positive bacteria, Gram-negative bacteria and fungi.

EXPERIMENTAL

The starting materials and solvents were obtained from Sigma-Aldrich Chemie GmbH (Munich, Germany), alfa aesar (Karlsruhe, Germany) and Avra chemicals (Hyderabad, India) and used without further purification. The progress of the reaction was monitored by thin layer chromatography (TLC). The NMR spectra were recorded on a Bruker BioSpin GmbH (400 MHz). Chemical shifts are expressed as δ ppm relative to TMS in CDCl₃ or DMSO-*d*₆. The J constants are given in Hz.

ESI-MS data were recorded on a Micromass Quattro LC instrument using ESI+ software with a capillary voltage of 3.98 kV and an ESI mode positive ion trap detector. Melting points were determined with open capillary tubes and are uncorrected. TLC was performed using Silica gel (Kieselgel 60 F254) plates.

Synthesis of ethyl-2-amino-4-methylthiazole-5-carboxylate (2): To a mixture of ethyl acetoacetate (**1**) (0.05 mol) in water (60 mL) at 5 °C was added N-bromosuccinimide (1.2 eq, 0.06 mol). The reaction mixture was stirred at room temperature for 2 h and the reaction progress was monitored by thin layer chromatography. Thiourea (3.80 g, 0.05 mol) was added and the reaction mixture was heated to 60 °C for 3 h. After cooling to room temperature, liquor NH₃ (6 mL) was added to the reaction mixture. The resultant precipitate was filtered and the filtrate was washed with water (50 mL × 4) and recrystallized with ethyl acetate to get target compound **2** with 80 % yield (yellow solid). ¹H NMR (CDCl₃) δ 5.60 (br, NH), 4.27 (q, 2H), 2.52 (s, 3H), 1.33 (t, 3H); ESI-MS *m/z* 187 (M+1), 100 %.

Synthesis of ethyl-2-bromo-4-methylthiazole-5-carboxylate (3): Ethyl-2-amino-4-methylthiazole-5-carboxylate (**2**) (0.05 mol) was dissolved in phosphoric acid (25 mL) and after cooling the solution to 5 °C and conc. sulphuric acid (35 mL) was slowly run in and stirred well. When the temperature of the mixture was 0 to 5 °C, it was diazotized with a solution of sodium nitrite (4.20 g, 1.2 eq). After addition of total nitrite, stirring was continued for 45 min more and then diazonium solution was added to pre-cooled aqueous solution of cupric bromide (13.38 g, 0.06 mol) in 500 mL round bottom flask and maintained the temperature below 0-5 °C. The evolution of nitrogen was immediately formed and ceased in about 10-15 min. The mixture was allowed to stir at room temperature for about 1 h. After completion of reaction, the reaction mixture was diluted with water and extracted desired compound using dichloromethane. The organic layer was collected and washed with Na₂CO₃ solution, dried over Na₂SO₄. The organic fraction was concentrated by evaporating solvent under reduced pressure with rotary evaporator to obtain pure compound **3** with 65 % yield (Off white solid). ¹H NMR (CDCl₃) δ 5.60 (br, NH), 4.27 (q, 2H), 2.52 (s, 3H), 1.33 (t, 3H); ESI-MS *m/z* 251 (M+1), 51 %, 253 (M+3) 49 %.

Synthesis of 2-bromo-4-methylthiazole-5-carboxylic acid (4): Ethyl-2-bromo-4-methylthiazole-5-carboxylate (**3**) (0.05 mol) in water (50 mL) taken in a 100 mL round bottom flask was added 5 % NaOH and stirred for 10-12 h. The reaction progress was monitored by TLC until the compound **3** was completely disappeared. After completion of the reaction, the mixture was diluted with water and extracted desired compound using ethyl acetate. The organic fraction was concentrated by evaporating solvent under reduced pressure to obtain pure compound **4** with 70 % yield (Off white solid). ¹H NMR (CDCl₃) δ 10.63 (br, OH), 2.52 (s, 3H), 1.33 (t, 3H); ESI-MS *m/z* 222 (M+1).

General synthetic procedure for 2-bromo-4-methylthiazole-5-carboxamide derivatives (5a-o). To the solution of compound **4** (1.0 eq) in PEG-400 (5 mL) was added O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluroniumhexafluorophosphate (HATU) (1.2 eq) and stirred for 15 min and then added amine (1.0 eq) and N,N-diisopropylethylamine

(DIPEA) (3.0 eq) and continued the reaction for 10-12 h. The reaction progress was monitored by TLC until the starting compounds were completely disappeared. After completion reaction mixture was diluted with water and extracted desired compound using ethyl acetate. The organic fraction was concentrated by evaporating solvent under reduced pressure to obtain pure compound **5** with 60-85 % yield.

2-Bromo-4-methyl-N-(*p*-tolyl) thiazole-5-carboxamide (5a): Off- white crystalline solid (Yield: 85 %); m.p. 130-132 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.39 (d, *J* = 8.4 Hz, 2H), 7.17 (d, *J* = 8.2 Hz, 2H), 2.72 (thiazole-CH₃), (s, 3H) 2.34 (aromatic-CH₃), (s, 3H); ¹³C NMR (100.1 MHz, CDCl₃) δ 158.56, 155.20, 136.87, 135.17, 134.37, 129.74, 120.62, 20.96, 17.34; ESI-MS *m/z* 311 [M+1], 313 [M+3], 333 [M+Na], 335 [M+Na+2], 365 [M+Na+MeOH], 367 [M+Na+MeOH+2]. Anal. Calcd. (found) % for C₁₂H₁₁N₂OSBr: C 46.28 (46.31); H 3.40 (3.56); N 8.91 (9.00); S 10.21 (10.30).

2-Bromo-N-(4-methoxyphenyl)-4-methylthiazole-5-carboxamide (5b): Off- white crystalline solid (Yield: 83 %); m.p. 135-137 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.44 (d, *J* = 8.5 Hz, 2H) 6.90 (d, *J* = 8.5 Hz, 2H) 3.81 (s, 3H), 2.71 (s, 3H); ¹³C NMR (100.1 MHz, CDCl₃) δ 158.67, 157.23, 155.22, 136.82, 129.90, 122.59, 114.36, 55.54, 17.34; ESI-MS *m/z* 327 [M+1], 329 [M+3]. Anal. Calcd. (found) % for C₁₂H₁₁N₂O₂SBr: C 44.01 (44.05); H 3.30 (3.39); N 8.49 (8.56); S 9.56 (9.80).

2-Bromo-N-(4-butylphenyl)-4-methylthiazole-5-carboxamide (5c): Off white crystalline solid (Yield: 85 %); m.p. 95-97 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.43 (d, *J* = 8.43 Hz, 2H), 7.18 (d, *J* = 8.34 Hz, 2H), 2.59 (t, 2H), 1.58 (m, 2H), 1.35 (m, 2H), 0.92 (t, 3H); ¹³C NMR (100.1 MHz, CDCl₃) δ 158.59, 155.18, 140.27, 136.89, 134.52, 130.62, 129.11, 120.64, 35.11, 33.62, 22.29, 17.33, 13.96; ESI-MS *m/z* 353 [M+1], 355 [M+3], 375 [M+Na], 377 [M+Na+2]. Anal. Calcd. (found) % for C₁₅H₁₇N₂OSBr: C 49.86 (51.00); H 4.81 (4.85); N 7.86 (7.93); S 8.89 (9.08).

2-Bromo-N-(4-chlorophenyl)-4-methylthiazole-5-carboxamide (5d): Off white crystalline solid (Yield: 75 %); m.p. 128-130 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.58 (br, NH), 7.48 (d, *J* = 8.80 Hz, 2H), 7.33 (d, *J* = 8.68 Hz, 2H), 2.71 (s, 3H); ¹³C NMR (100.1 MHz, CDCl₃) δ 158.59, 155.79, 137.17, 135.56, 130.43, 129.28, 121.75, 17.40; ESI-MS *s* 331 [M+1], 333 [M+3], 353 [M+Na], 355 [M+Na+2]. Anal. Calcd. (found) % for C₁₁H₈N₂OSBrCl: C 40.10 (39.84); H 2.35 (2.43); N 8.39 (8.45); S 9.56 (9.67).

2-Bromo-N-(4-bromophenyl)-4-methylthiazole-5-carboxamide (5e): Off white crystalline solid (Yield: 70 %); m.p. 137-139 °C; ¹H NMR (400 MHz, CDCl₃) δ 8.03 (d, *J* = 8.7 Hz, 2H), 7.04 (d, *J* = 8.7 Hz, 2H), 2.61 (s, 3H); ¹³C NMR (100.1 MHz, CDCl₃) δ 158.59, 155.79, 137.17, 135.56, 130.43, 129.28, 121.75, 17.40; ESI-MS *m/z* 375 [M+1], 377 [M+3]. Anal. Calcd. (found) % for C₁₁H₈N₂OSBr₂: C 35.04 (35.13); H 2.08 (2.14); N 7.38 (7.45); S 8.48 (8.53).

2-Bromo-4-methyl-N-(1-phenylethyl)thiazole-5-carboxamide (5f): White solid (Yield: 75%); m.p. 110-113 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.36-7.35 (5H, Ar), 5.23 (m, 1H) 2.63 (s, 3H), 1.53 (d, 3H); ¹³C NMR (100.1 MHz, CDCl₃) δ 159.67, 154.56, 142.43, 136.50, 128.92, 127.79, 126.16, 49.82, 21.77, 17.28; ESI-MS *m/z* 325 [M+1], 327 [M+3], 379 [M+Na+]

MeOH], 381 [M+Na+MeOH+2]. Anal. Calcd. (found) % for C₁₃H₁₃N₂OSBr: C 48.10 (48.01); H 3.95 (4.03); N 8.55 (8.61); S 9.78 (9.86).

2-Bromo-N-(2,4-dimethylphenyl)-4-methylthiazole-5-carboxamide (5g): Off white crystalline solid (Yield: 72 %); m.p. 150-152 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.63 (br, NH), 7.22 (s, 1H), 7.05(d, 2H), 2.74(s, 3H), 2.32 (s, 3H), 2.26 (s, 3H); ¹³C NMR (100.1 MHz, CDCl₃) δ 158.69, 155.53, 151.45, 143.84, 137.06, 132.27, 131.42, 127.55, 20.94, 17.85, 17.44; ESI-MS *m/z* 325 [M+1], 327 [M+3], 347 [M+Na], 349 [M+Na+2], 379 [M+Na+MeOH], 381 [M+Na+MeOH+2]. Anal. Calcd. (found) % for C₁₃H₁₃N₂OSBr: C 48.15 (48.01); H 3.90 (4.03); N 8.58 (8.61); S 9.80 (9.86).

2-Bromo-N-(3,5-dimethylphenyl)-4-methylthiazole-5-carboxamide (5h): White solid (Yield: 77 %); m.p. 143-147 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.39 (br, NH), 7.16 (s, 2H), 6.82 (s, 1H), 2.72 (3H), 2.31 (6H); ¹³C NMR (100.1 MHz, CDCl₃) δ 159.53, 158.54, 155.19, 139.02, 136.88, 136.78, 130.68, 127.07, 118.21, 21.36, 17.31; ESI-MS *m/z* 325 [M+1], 327 [M+3], 347 [M+Na], 349 [M+Na+2]. Anal. Calcd. (found) % for C₁₃H₁₃N₂OSBr: C 47.92 (48.01); H 4.10 (4.03); N 8.50 (8.61); S 9.78 (9.86).

2-Bromo-N-(4-methoxybenzyl)-4-methylthiazole-5-carboxamide (5i): Off white crystalline solid (Yield: 80 %); m.p. 118-121 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.27 (d, *J* = 9.28 Hz, 2H), 6.90 (d, *J* = 8.79 Hz, 2H), 4.50 (d, *J* = 5.52 Hz), 3.81 (s, 3H), 2.64 (s, 3H); ¹³C NMR (100.1 MHz, CDCl₃) δ 160.31, 159.34, 154.71, 136.57, 130.38, 129.43, 129.31, 114.31, 55.35, 43.84, 17.31; ESI-MS *m/z* 341 [M+1], 343 [M+3], 363 [M+Na], 365 [M+Na+2]. Anal. Calcd. (found) % for C₁₃H₁₃N₂O₂SBr: C 45.55 (45.76); H 3.60 (3.84); N 8.11 (8.21); S 9.32 (9.40).

2-Bromo-N-cyclohexyl-4-methylthiazol-5-carboxamide (5j): Orange to red crystalline solid (Yield: 75 %); m.p. 123-125 °C; ¹H NMR (400 MHz, CDCl₃) δ 5.56 (br, 1NH), 3.91 (m, 1H), 2.64 (s, 3H), 2.01 (m, 4H), 1.76-1.63 (m, 4H), 1.27-1.16 (m, 4H); ¹³C NMR (100.1 MHz, CDCl₃) δ 159.58, 153.97, 136.26, 49.12, 33.07, 25.42, 24.74, 17.19; ESI-MS *m/z* 303 [M+1], 305 [M+3], 325 [M+Na], 327 [M+Na+2]. Anal. Calcd. (found) % for C₁₁H₁₅N₂OSBr: C 43.21 (43.57); H 5.01 (4.99); N 9.10 (9.24); S 10.46 (10.57).

2-Bromo-N-cyclopropyl-4-methylthiazol-5-carboxamide (5k): White solid (Yield: 80 %); m.p. 95-98 °C; ¹H NMR (400 MHz, CDCl₃) δ 5.92 (br, NH), 2.83 (m, 1H), 2.64 (s, 3H), 0.88 (dd, 19.44 Hz), 0.61 (dd, 16.14 Hz, 2H); ¹³C NMR (100.1 MHz, CDCl₃) δ 161.93, 154.93, 136.40, 23.36, 17.21, 6.95; ESI-MS *m/z* 261 [M+1], 263 [M+3], 283 [M+Na], 285 [M+Na+2]. Anal. Calcd. (found) % for C₈H₉N₂OSBr: C 36.55 (36.79); H 3.38 (3.47); N 10.85 (10.73); S 12.30 (12.28).

N-(Benzo[d]thiazol-2-yl)-2-bromo-4-methylthiazole-5-carboxamide (5l): Off white amorphous solid (Yield: 60 %); m.p. 190-192 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.75-7.55 (m, 4H), 2.79 (s, 3H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 167.98, 155.09, 152.32, 136.52, 135.17, 129.01, 122.72, 111.35, 16.54; ESI-MS *m/z* 354 [M+1], 355 [M+3], 376 [M+Na], 378 [M+Na+2]. Anal. Calcd. (found) % for C₁₂H₈N₃O₂SBr: C 40.55 (40.69); H 2.30 (2.28); N 11.80 (11.86); S 18.01 (18.10).

N-(1*H*-benzo[d]imidazol-2-yl)-2-bromo-4-methylthiazole-5-carboxamide (5m): Off white amorphous solid (Yield:

65 %); m.p. 215-217 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.40-7.38 (m, 7.39 Hz, 2H), 7.21-7.19 (m, 7.20 Hz, 2H), 2.73 (s, 3H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 167.98, 155.09, 152.32, 136.52, 135.17, 129.01, 122.72, 111.35, 16.54; ESI-MS *m/z* 337 [M+1], 339 [M+3]. Anal. Calcd. (found) % for C₁₂H₉N₄OSBr: C 42.50 (42.74); H 2.48 (2.69); N 16.42 (16.62); S 9.48 (9.51).

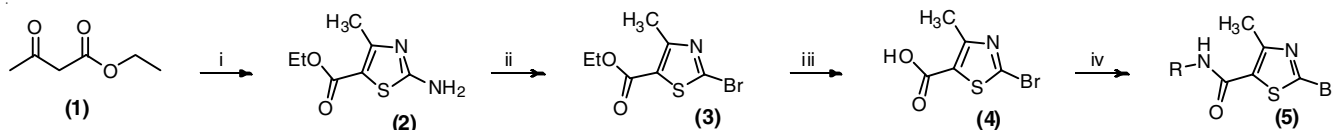
2-Bromo-N-(furan-2-ylmethyl)-4-methylthiazole-5-carboxamide (5n): Off white amorphous solid (Yield: 60 %); m.p. 176-178 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.38 (d, 1H), 6.34 (dd, 1H), 6.30 (d, 1H), 4.57 (d, 5.50 Hz, 2H), 2.66 (s, 3H); ¹³C NMR (100.1 MHz, CDCl₃) δ 160.25, 154.99, 150.32, 142.57, 136.76, 110.62, 108.08, 37.09, 17.27; ESI-MS *m/z* 301 [M+1], 303 [M+3], 323 [M+Na], 325 [M+Na+2]. Anal. Calcd. (found) % for C₁₀H₉N₂O₂SBr: C 40.02 (39.88); H 2.89 (3.01); N 9.11 (9.30); S 10.49 (10.65).

2-Bromo-N-(*tert*-butyl)-4-methylthiazole-5-carboxamide (5o): Yellow-white amorphous solid (Yield: 85 %); m.p. 140-143 °C; ¹H NMR (400 MHz, CDCl₃) δ 5.27 (br, NH), 2.62 (s, 3H), 1.44 (s, 9H); ¹³C NMR (100.1 MHz, CDCl₃) δ 159.82, 153.41, 136.00, 132.17, 52.61, 28.88, 17.13; ESI-MS *m/z* 277 [M+1], 279 [M+3]. Anal. Calcd. (found) % for C₉H₁₃N₂OSBr: C 38.85 (39.00); H 4.55 (4.73); N 10.05 (10.11); S 11.60 (11.57).

Antimicrobial evaluation: Antibacterial evaluation was tested using agar well diffusion method. The activity of tested samples was tested against *Staphylococcus aureus* (NCIM 2079) and *Bacillus subtilis* (NCIM 2063), as Gram positive bacteria and *Pseudomonas aeruginosa* (ATCC 10145), *Escherichia coli* (ATCC 11775), as Gram negative bacteria and *Aspergillus niger* (ATCC 6275) were used to test antifungal activity. The solution of 10 mg/mL of each compound in DMSO was used for testing against bacteria. Known antibiotics like norfloxacin, ciprofloxacin (reference antibacterial drugs) and itraconazol (reference antifungal drug) were used for comparison. The antibacterial activity of compounds was evaluated by diffusion method on a nutrient medium (Luria-Bertani Broth agar for bacteria and potato dextrose agar for fungi). The required incubation periods were 12-16 h at 37 °C for bacteria and 48-72 h at 28-30 °C for fungi. The control disk contained norfloxacin and ciprofloxacin in the appropriate wells. The tested compounds were dissolved in DMSO applying the necessary concentration. The exact volume of solution of compounds is brought into a nutrient medium. 100 μL of tested samples (10 mg/mL) were loaded into the wells of plates. The results were estimated according to the presence or absence of microorganism growth. The activity was determined by measuring the diameter of the inhibition zone (in mm).

RESULTS AND DISCUSSION

The amidation is a highly useful synthetic strategy used by nature as exemplified by proteins, peptides and many other naturally occurring substances. The reaction also occupies a prominent position in many fields of chemical industry including material science and drug development. Numerous amidation protocols have been extensively explored and developed and in principle, most amides are synthesized by the reaction between amines with activated carboxylic acid derivatives [24]. Herein, we report an efficient method for the synthesis of title compounds according to the procedure outlined in **Scheme-I**.



Scheme-I: Synthesis of 2-bromo-4-methylthiazole-5-carboxamide derivatives (**5a-o**) (i) NBS, thiourea, H₂O, r.t. (ii) a. conc H₃PO₄, conc. H₂SO₄, aq. NaNO₂, 0-5 °C, b. aq. CuBr₂ 0-5 °C (iii) 5 % NaOH, r.t. (iv) HATU, RNH₂, DIPEA, PEG-400, 0 °C → r.t.

Briefly, 2-bromo-4-methylthiazole-5-carboxylic acid (**4**) was coupled to a variety of amines to afford compound **5**. Nucleophilic displacement of activated carboxylic group by converting the -OH of acid into a good leaving group prior to treatment with amine was accomplished at room temperature to afford analogs **5a-o** (Table-1). In general, most of the amide coupling reactions is run in polar aprotic solvents like DMF or DCM, *etc.* Instead of these organic solvents, we used eco-friendly PEG-400 as reaction medium. 1-[bis(Dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxidehexafluorophosphate (HATU) and Hunig's base (N,N-diisopropylethylamine, DIPEA) were used as coupling reagent and base, respectively.

TABLE-1
SYNTHESIS OF 2-BROMO-4-METHYLTHIAZOLE-5-CARBOXAMIDE DERIVATIVES USING PEG-400

Comp.	RNH ₂	Time (h)	Yield (%)	m.p. (°C)
5a	<i>p</i> -Toluidine	10	85	130-132
5b	4-Methoxyaniline	10	83	135-137
5c	4-Butylaniline	10	85	95-97
5d	4-Chloroaniline	10	75	128-130
5e	4-Bromoaniline	10	70	137-139
5f	1-Phenylethanamine	10	75	110-113
5g	2,4-Dimethylaniline	10	72	150-152
5h	3,5-Dimethylaniline	10	77	143-146
5i	(4-Methoxyphenyl)methanamine	10	80	118-121
5j	Cyclohexanamine	10	75	123-125
5k	Cyclopropanamine	10	80	95-98
5l	Benzo[<i>d</i>]thiazol-2-amine	10	60	195-198
5m	1 <i>H</i> -benzo[<i>d</i>]imidazol-2-amine	10	65	215-217
5n	Furan-2-ylmethanamine	10	60	133-136
5o	2-Methylpropan-2-amine	10	85	142-144

Optimization of base: Initially, we investigated the reaction of 2-bromo-4-methyl thiazole-5-carboxylic acid (**4**) with *p*-toluidine in PEG-400 at room temperature to afford the corresponding amide product (**5a**) with 85 % yield. In order to optimize the reaction conditions, Optimization focused on the above reaction with different amine bases, like triethylamine, DIPEA, N-methylmorpholine and 2,6-lutidine with varying equivalents in PEG-400 were screened. Eventually, amide product (**5a**) (Table-1), could be formed in 85 % yield after 10 h (Table-2, entry 2) using DIPEA as a base in PEG-400.

Biological evaluation: The *in vitro* antibacterial activity was performed against a series of Gram-positive bacteria *S. aureus* (NCIM 2079), *Bacillus subtilis* (NCIM 2063) and Gram-negative bacteria *Escherichia coli* (ATCC 11775), *Pseudomonas aeruginosa* (ATCC 10145) and fungi including *Aspergillus niger* (ATCC 1015) were used to test antifungal activity. Known antibiotics like norfloxacin, ciprofloxacin (reference anti bacterial drugs) and itraconazole (reference antifungal drug)

TABLE 2
OPTIMIZATION OF BASE USING 2-BROMO-4-METHYLTHIAZOLE-5-CARBOXYLIC ACID (**4**) WITH *p*-TOLUIDINE

Entry	Base (e.q)	Time (h)	Yield of (5a) (%) ^a
1	Triethylamine (3 e.q)	10	60
2	DIPEA (3 e.q)	10	85
3	N-methylmorpholine (3 e.q)	10	40
4	2,6-Lutidine (3 e.q)	10	45
5	DIPEA (2 e.q)	10	63

^aYields are calculated after column chromatography and ¹H NMR spectrometry.

were used for comparison. Compounds **5a**, **5b**, **5j** and **5n** showed a broad spectrum of activity against bacteria and other compounds showed varying antimicrobial activity. Whereas all the compounds exhibited poor antifungal activity. The activity was determined by measuring the diameter of inhibition zone (in mm) (Table-3). The control disk contained norfloxacin and ciprofloxacin in the appropriate wells. The results were estimated according to the presence or absence of microorganism growth.

Molecular docking studies: The antimicrobial potency of all the compounds were subjected for further docking studies to explore their potential binding mode at DHFR protein of *S. aureus* [25]. PDB (ID 3SRW) with 1.70 Å resolution was retrieved from Protein Data Bank. Protein preparation and finding of active site receptors was done using discovery studio, residues including Leu6, Val 7, Ala 8, Leu 21, Asp 28, Leu 29, Val 32, Thr 47, Ile 51 and Phe 93 were found to be in the active site of receptor, responsible for interactions. Conformational search of ligands were investigated *via* Gold docking program with extensive genetic algorithm. In this study, ten conformers were generated for each ligand using default parameters. Docking of all the newly synthesized inhibitors showed hydrogen bond interactions with residues. One of the most active newly synthesized compounds as an antimicrobial agent (**5m**) showed potent inhibitory activity because of involving in hydrogen bonding interaction of H24-N10 of thiazole moiety at 2.643 Å with oxygen of Phe 93. While the hydroxyl group of Ala 8 showed strong hydrogen bond interaction with nitrogen of thiazole ring (N5) at a bond distance of 3.009 Å. Hydrophobic interaction also observed between Thr 47 and imidazole ring moiety (Fig. 1). The binding affinity in terms of dock fitness score in Kcal/mol given in Table-4.

Conclusion

A simple, efficient and green method for the synthesis of thiazole derivatives using PEG-400 as a green reaction medium is developed. The present procedure has an advantage of reduced reaction times, high yields and mild reaction conditions. In addition, all the newly synthesized compounds (**5a-o**) exhibited remarkable growth inhibition of wide spectrum of Gram-positive bacteria and Gram-negative bacteria. While antifungal activity

TABLE-3
ANTIBACTERIAL ACTIVITY DATA IN mm (mg/mL)

Entry	Compound	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>A. niger</i>
1	5a	10	04	07	04	+ ^d
2	5b	11	05	09	05	+
3	5c	08	– ^b	08	04	+
4	5d	08	–	09	–	+
5	5e	08	06	08	–	+
6	5f	07	–	09	04	+
7	5g	08	–	09	–	+
8	5h	07	–	07	05	+
9	5i	07	04	08	05	+
10	5j	10	–	11	–	+
11	5k	09	–	09	–	+
12	5l	08	07	10	–	+
13	5m	12	–	07	–	+
14	5n	08	08	08	06	+
15	5o	07	–	09	–	+
Standard	Norfloxacin	11	11	NT ^c	NT	NT
Standard	Ciprofloxacin	NT	NT	13	13	NT
Standard	Itraconazol	NT	NT	NT	NT	20

^bno activity; ^cnot tested; ^dpoor activity

TABLE-4
DOCK FITNESS SCORE (Kcal/mol)

Entry	Fitness	S (hb_ext)	S (vdw_ext)	S (hb_int)	S (int)	Compound
1	46.55	0.00	38.57	0.00	-6.48	5a
2	45.51	0.80	39.87	0.00	-10.11	5b
3	54.26	1.02	45.27	0.00	-9.01	5c
4	45.92	0.00	36.71	0.00	-4.56	5d
5	45.79	0.00	35.84	0.00	-3.49	5e
6	46.81	0.00	36.90	0.00	-3.93	5f
7	46.35	0.00	38.76	0.00	-6.95	5g
8	48.60	0.00	39.69	0.00	-5.97	5h
9	53.03	1.62	41.39	0.00	-5.51	5i
10	47.09	0.00	36.74	0.00	-3.43	5j
11	39.57	1.93	28.93	0.00	-2.13	5k
12	51.37	0.00	39.15	0.00	-2.46	5l
13	51.81	0.12	38.98	0.00	-1.90	5m
14	47.09	2.00	35.24	0.00	-3.37	5n
15	39.75	1.74	30.26	0.00	-3.60	5o

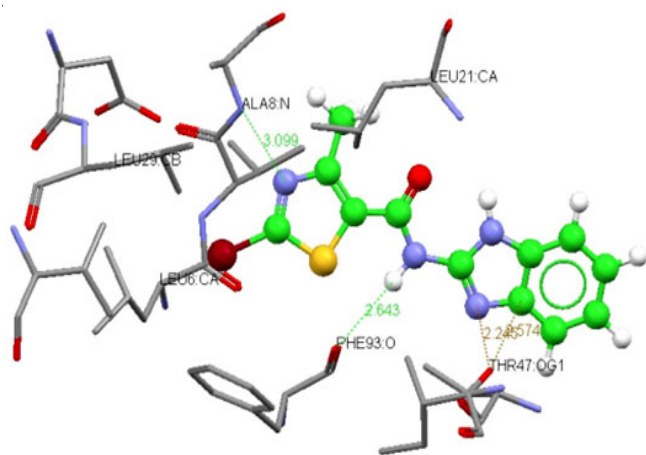


Fig. 1. Proposed binding interactions of thiazole (**5m**) (ball and stick mode) with the DHFR protein of *S. aureus*

was less significant compared to standard drug itraconazol. The most sensitive bacterial species to the tested compounds were *S. aureus*. The promising properties of this class of new synthesized antibacterial compounds deserve further investi-

gation in order to clarify the mode of action at the molecular level responsible for the activity observed.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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