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4*H*-Pyrimido[2,1-*b*]benzothiazole-3-Carboxamide Derivatives; Design, Synthesis, Biological Evaluation and Docking Studies

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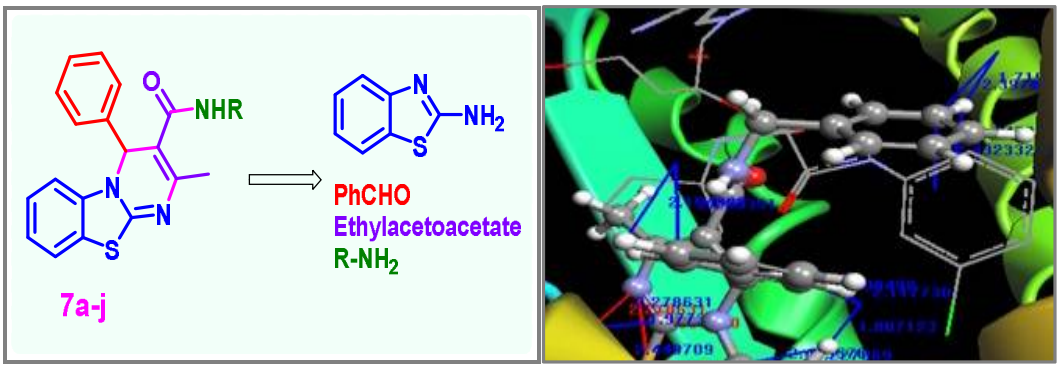
ABSTRACT

*Design and synthesis of a series of novel substituted 4H-Pyrimido[2,1-b]benzothiazole-3-carboxamides (**7a-j**) starting from commercially available and inexpensive starting materials (benzaldehyde, ethyl acetoacetate and 2-aminobenzothiazole) were generated and fully characterized using ^{1}H ^{13}C NMR, IR and mass spectral analysis. Furthermore, the synthesized compounds were tested for their in vitro antibacterial and antifungal activities, which indicated that the majority of 4H-Pyrimido[2,1-b]benzothiazole-3-carboxamides exhibit good to moderate activity compared to the standard drugs, streptomycin, penicillin and amphotericin-B. In particular, compounds **7b**, **7d**, **7j** have shown superior antibacterial activity against selected bacterial strains with 9.37μg mL^{-1}. Compound **7e** has shown excellent antifungal activity against A.niger with ZOI 48mm. The findings of biological activities are further supported by molecular docking studies. Experimental biological activities are exactly correlated with the docking scores.*

Graphical Abstract



Keywords: 2-Aminobenzothiazole, 4*H*-Pyrimido[2,1-*b*]benzothiazole, Molecular docking studies, Antimicrobial activity.

INTRODUCTION

The past decades have witnessed significant development in multicomponent reactions as a beneficial approach in the construction of complex molecules from structurally simple chemical entities. The highly atom- and step-economic transformations in multicomponent reactions have attracted increasing attention in utilizing simple starting materials. Therefore, multicomponent reaction is one of the most powerful and straightforward method to construct complex molecules, which has great potential application in synthetic organic and medicinal chemistry [**1-9**]. Among them, Biginelli reaction have become an efficient strategy for the synthesis of structurally complex heterocyclic molecules in organic synthesis, which has been successfully applied in the preparation of substituted pyrimidine derivatives and many other heterocyclic compounds.

Heterocyclic nuclei are present in many natural products, drugs and biologically active compounds. They are also important structural motifs in synthetic pharmaceuticals and agrochemicals. Some of the heterocyclic compounds display photochromic, solvatochromic and biochemical-luminescence properties. Heterocyclic compounds are also used in materials science such as fluorescent sensors, dyestuffs, plastics, brightening agents, and analytical reagents. Among them, pyrimido [2,1-*b*]benzothiazoles are one of the prevalent nitrogen-containing heterocycles in natural and synthetic drug molecules. Such well known compounds include ocinaplon, lamivudine, raltegravir, imatinib, linagliptinand rosuvastatin. Additionally, some pyrimidine derivatives show unique biological activities, such as anticonvulsant, antitumor, anti-inflammatory and antitubercular activities. Pyrimidine derivatives are also reported to possess antibacterial, antimicrobial, antifungal, anticancer and anticonvulsant activities [**10-17**]. Herein, we report the synthesis of pyrimido[2,1-*b*]benzothiazoles using Biginelli type reaction in an efficient manner starting from simple units such as aldehyde, *α*-ketoester and 2-aminobenzothiazole and subsequent functional group modification to give 4*H*-Pyrimido[2,1-*b*]benzothiazole-3-carboxamies. Furthermore, we have studied their *in vitro* antimicrobial activities and molecular docking studies.

MATERIALS AND METHODS

General experimental: All the procured chemicals are standard commercial reagents and are used as received. Purity of the products in each step is determined by TLC analysis using pre-coated aluminium plates (Merck), coated with silica gel 60 F_{254}. Melting points of the compounds were determined using open capillaries and are uncorrected. ^{1}H-NMR and ^{13}C-NMR-spectra were recorded with an Avance Brucker-300 (300MHz) spectrometer by using chloroform-*d* as solvent and tetramethyl silane (TMS) as internal standard. Chemical shift values are given in parts per million (δ) and coupling constants (*J*) in Hz. Mass spectrum is recorded on VG Micromass 7070H (ESI-MS) and given in mass units (m/z).

General method for the preparation of Ethyl-2-methyl-4-phenyl-4H-pyrimido[2,1-b] benzothiazole-3-carboxylate (4): In a 20 mL RB flask, equipped with a magnetic stir bar was taken 2-aminobenzothiazole(1) (0.15 g, 1 mmol), benzaldehyde (2) (0.212 g, 2 mmol), ethyl aceto acetate (3) (0.26 g, 2 mmol), ethylene glycol (5mL) and TBAHS (30 mol %) at room temperature. The resulting mixture was heated at 120°C for 3 h and completion of the reaction was monitored by TLC (ethyl acetate: hexane 4:7). After completion of the reaction, the mixture was allowed to cool, diluted with water and extracted with ethyl acetate (2x15 mL). The combined organic layer was washed with water (25 mL), dried over anhydrous sodium sulphate and filtered. The filtrate was concentrated in vacuo, and purified by flash chromatography (20:1 hexane: ethyl acetate) to give **4** as an yellow solid. m.p.173-175^{o}C; IR (KBr) υ:2924, 1664, 1588 1056,746 cm^{-1}; ^{1}H NMR (300 MHZ, CDCl3): δ 1.30 (t, 3H), 4.19 (m, 2H), 4.59 (s, 1H), 6.42 (s, 1H), 7.01-7.46 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.6, 23.4, 57.5, 59.9, 103.0, 111.8, 122.2, 123.8, 124.0, 126.7, 127.1, 128.4, 128.6, 137.8, 141.1, 154.1, 163.2, 166.3; ESI-MS: *m/z* 351 $[M+H]^{+}$.

General method for the preparation of 2-methyl-4-phenyl-4H-pyrimido[2,1-b]benzothiazole-3-carboxylic acid (5): Compound (**4**) (1 g, 2.85 mmol, 1 equiv.) in 20 mL of methanol were placed in 100 mL round-bottomed flask equipped with magnetic stirrer. KOH (0.8 g, 14.28 mmol, 5 equiv.) was added and the reaction mixture was refluxed for 12 h and completion of the reaction was monitored by TLC (ethyl acetate: hexane 4: 7). After completion of the reaction, the mixture was evaporated under vacuo, then diluted with water (30 mL) and extracted with ethyl acetate (30 mL). The aqueous layer was acidified to pH=2 with 6N HCl and extracted with ethyl acetated (30 mLx3). The combined ethyl acetate extracts were washed with water (50 mL), dried over anhydrous sodium sulphate and concentrated under reduced pressure. The crude carboxylic acid (**5**) was obtained in 70% yield as an orange brown solid after drying under vacuum. m.p. 138-140°C; IR (KBr) υ : 3426, 2923, 1622, 1593, 1513, 1461 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.71 (S, 3h), 4.59 (S, 1H), 6.43 (s, 1H), 7.12-7.46 (m, 8H), 11.0 (s, 1H); 13C NMR (75 MHz, CDCl3): δ 16.6, 50.8, 112.6, 116.3, 117.2, 122.2, 126.1, 126.5, 127.0, 127.1, 128.3, 130.0, 142.4, 144.2, 148.8, 163.0, 170.0; ESI-MS: *m/z* 323 $[M+H]^+$.

General method for the preparation of 4H-pyrimido[2,1-b]benzothiazole derivatives (7a-b): The crude carboxylic acid 5 (0.33 g, 1 mmol, 1 uquiv.) in 25 mL of DMF, 1-ethyl-3-(5-dimethylamino-propyl) carbo diimine hydrochloride (EDCI) was added and the reaction mixture was stirred for 20 min until the color of the contents change to orange. Then, N,N-diisopropyl ethyl amine (DIPEA) and primary amine (**6a-j**, 1 equiv.) were added and the reaction mixture was stirred at room temperature for 8-12 h. Completion of the reaction was monitored by TLC (ethyl acetate: hexane 4:6). After completion of the reaction, the mixture was allowed to cool, diluted with water (30 mL) and extracted with ethyl acetated (30 mLx3). The combined ethyl acetate layers were washed with water (50 mL) and dried over anhydrous sodium sulphate, concentrated and then it was purified by column chromatography to afford the corresponding amide (**7a-j**).

N-(Phenyl)-2-methyl-4-phenyl-4H-pyrimido[2,1-b]benzothiazole-3-carboxamide (7a): Yellow solid; 65%; m.p. 205-207°C; IR (KBr) υ: 3078, 2924, 1678, 1545, 1496, 1443 CM^{-1}; ^1H NMR (300 MHz; $CDCl_3$): δ 1.71 (s, 3H), 4.19 (s, 1H), 6.43 (s, 1H), 7.01-7.70 (m, 12H), 8.01 (s, 1H); ESI-MS: *m/z* 398 $[M+H]^+$.

N-(p-nitrophenyl)-2-methyl-4-phenyl-4H-pyrimido[2,1-b]benzothiazole-3-carboxamie(7b): Yellow solid; 60%; m.p. 222-224°C; IR (KBr) υ: 3061, 2924, 1713, 1599, 1462, 772 cm^{-1}; ^1H NMR (300MHz, $CDCl_3$): δ 1.71 (s, 3H), 4.19 (s, 1H), 6.43 (s, 1H), 7.01-7.7. (m, 8H), 7.60-8.30 (m, 4H), 8.10 (S, 1H); ESI-MS: *m/z* 443 $[M+H]^+$.

N-(p-chlorophenyl)-2-methyl-4-phenyl-4H-pyrimido[2,1-b]benzothiazole-3-carboxamide(7c): Yellow solid; 55%; m.p. 230-232°C; IR (KBr) υ: 3287, 3064, 2925, 1606, 1534, 1469, 752 cm^{-1}; ^1H NMR (300MHz, $CDCl_3$): δ 1.71 (s, 3H), 4.19 (s, 1H), 6.43 (s, 1H), 7.01-7.70 (m, 12H), 8.10 (s, 1H); ESI-MS: m/z 433 $[M+H]^+$.

N-(p-bromophenyl)-2-methyl-4-phenyl-4H-pyrimido[2,1-b]benzothiazole-3-carboxamide(7d): Yellow solid; 60%; m.p. 244-246°C; IR (KBr) υ: 3063, 2924, 1646, 1543, 1465, 739 cm^{-1}; 1H NMR (300MHz, $CDCl_3$): δ 1.71 (s, 3H), 4.20 (s, 1H), 6.40 (s, 1H), 7.01-7.70 (m, 12H), 8.10 (s, 1H); ESI-MS: m/z 476 $[M+H]^+$.

N-(p-methoxyphenyl)-2-methyl-4-phenyl-4H-pyrimido[2,1-b]benzothiazole-3-carboxamide(7e): Yellow solid; 55%; m.p. 224-226°C; IR (KBr) υ: 3088, 2938, 1639, 1590, 1063, 753 cm^{-1}; 1H NMR (300MHz, $CDCl_3$): δ 1.71 (s, 3H), 3.73 (s, 1H), 4.59 (s, 1H), 6.40 (s, 1H), 7.01-7.70 (m, 12H), 8.10 (s, 1H); ESI-MS: m/z 428 $[M+H]^+$.

N-(p-methylphenyl)-2-methyl-4-phenyl-4H-pyrimido[2,1-b]benzothiazole-3-carboxamide(7f): Yellow solid; 60%; m.p. 233-235^{0}C; IR (KBr) υ: 3072, 2946, 1653, 1598, 750 cm^{-1}; ^1H NMR 300MHz, $CDCl_3$): δ 1.30 (s, 1H), 1.70 (s, 3H), 4.59 (s, 1H), 6.40 (s, 1H), 7.01-7.46 (m, 12H), 8.11 (s, 1H); ESI-MS: m/z 412 $[M+H]^+$.

N-(o,m-dimethoxyphenyl)-2-methyl-4-phenyl-4H-pyrimido[2,1-b]benzothiazole-3-carboxamide (7g): Yellow solid; 55%; m.p. 222-224°C; IR (KBr) υ: 3095, 2945, 1627, 1588, 1069, 749 cm^{-1}; ^1H NMR (300MHz, $CDCl_3$): δ 1.71 (s, 3H), 3.73 (m, 6H), 4.59 (s, 1H), 6.43 (s, 1H), 7.01-7.46 (m, 11H), 8.08 (s, 1H); ESI-MS: m/z 458 $[M+H]^+$.

N-(o-chlorophenyl)-2-methyl-4-phenyl-4H-pyrimido[2,1-b]benzothiazole-3-carboxamide(7h): Yellow solid; 55%; m.p. 242-244°C; IR (KBr) υ: 3089, 2928, 1612, 1551, 749 cm^{-1}; ^1H NMR (300MHz, $CDCl_3$): δ 1.71 (s, 3H), 4.19 (s, 1H), 6.45 (s, 1H), 7.01-7.70 (m, 12H), 8.12 (s, 1H); ESI-MS: m/z 433 $[M+H]^+$.

N-(m-nitrophenyl)-2-methyl-4-phenyl-4H-pyrimido[2,1-b]benzothiazole-3-carboxamide(7i): Yellow solid; 65%; m.p. 248-250°C; IR (KBr) υ: 3058, 2924, 1721, 1592, 1469, 773 cm^{-1}; ^1H NMR (300MHz, $CDCl_3$): δ 1.70 (s, 3H), 4.20 (s, 1H), 6.45 (s, 1H), 7.01-8.20 (m, 12H), 8.11 (s, 1H); ESI-MS: m/z 443 $[M+H]^+$.

N-benzyl-2-methyl-4-phenyl-4H-pyrimido[2,1-b]benzothiazole-3-carboxamide (7j): Yellow solid; 65%; m.p. 212-214°C; IR (KBr) υ: 3049, 2941, 2924, 1661, 1602 cm^{-1}; ^1H NMR (300MHz, $CDCl_3$): δ 1.70 (s, 3H), 3.81 (s, 2H), 4.38 (s, 1H), 6.42 (s, 1H), 7.01-7.46 (m, 12H), 8.11 (s, 1H); ESI-MS: m/z 412 $[M+H]^+$.

***In vitro* antimicrobial assays:**

Minimum inhibitory concentration (MIC) measurement: Minimum inhibitory concentration (MIC) values for synthesised compounds were deterimined using microorganism's susceptibility tests in netrient and potato dextrose broths were used. Stock solutions in DMSO were prepared for test compounds, Penicillin and Streptomycin at concentration of 150 µg mL^{-1}, which are diluted at different concentrations upto 1.17 µg mL^{-1}. These different concentrations of test compounds and controls were inoculated in the selected microorganism suspensions, incubated at 37°C for 1-3 days to determine MIC [**18-28**] . Minimum inhibitory concentration (MIC) is the lowest concentration of the test comopund which prevents the appearance of the turbidity, where the compound is considered bacteriostatic.

Antifungal activity: Fungal strains *Saccharomyces cerevisiae*, *Aspergillus niger*, *Rhizopus oryzae*, *Candida albicans* and *Aspergillus flavus* are used for the screening of antifungal activity of the test compounds. These fungal strains are inoculated in tubes containing sterilized medium at 25°C for 48 h and then kept in refrigerator at 4°C. In a test tube, the inoculum is prepared with 5mL of potato dextrose broth, is added a loop of stock culture and incubated at 25°C for 48 h before use. 10 mg of each test compound is dissolved in dimethyl sulphoxide (DMSO, 10 mL) to prepare the stock solutions and are further diluted to prepare 100 µg mL^{-1}. Amphotericin-B in 1 mL of dimethyl suphoxide (DMSO) is used as reference standard solution.

In an autoclave, potato dextrose agar (PDA) medium is sterilized at 121°C (15 lb sq. $inches^{-1}$) for 15 min and petri-plates are sterilized in hot air oven at 160°C for 1 h. 27 mL of molten PDA medium is taken each sterilized petri-plate, spread with 100 µL of 48 h of old culture. Cups of 6 mm diameter are taken in each petri-plate with sterile borer. Accurately 100 µg concentration of test compound solutions along with reference standard Amphotericin-B are transferred to the respective petri-plates aseptically with appropriate labeling. After incubating the plate for 48 h at 25°C, the diameter of zone of inhibition is measured using an antibiotic zone reader. The average value of zone of inhibition in mm is obtained by performing the experiment in triplicate for each test compound.

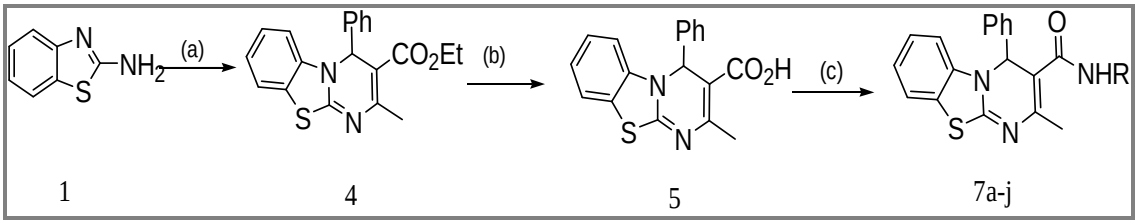
Molecular docking studies: Binding affinities of the compounds (**7a-j**) with autodockvina 4.0 Insilco molecular modeling: From PDB Bank RSCB (http://www.rcsb.org/pdb), the 3D structure of *E. Coli* (**2H7M**) selected protein with an X-ray resolution in the range of 1.62 A° was taken. CS ChemDraw

Ultra 8.0 was used to prepare the 3D structures of the test compounds in pdb form. Using standard default settings, all the molecular docking runs were carried out with 100 populations, a selection pressure of 1.1, up to a maximum of 100000 operations of 5 islands with niche size of 2 and a mutation and cross over rate of 95. In these molecular dockings, protein-test compound complexes were prepared by adding hydrogen atoms and by removing water molecules and refined by using the Deep View/Swiss PdbViewer 3.7 (SP5) to calculate partial atomic charges [**27, 29**]. For these docking studies, Version 2.0 of the GOLD (Genetic optimization for ligand docking) docking program was used. This GOLD program uses a genetic algorithm (GA) to evaluate the ligand flexibility and the rotational flexibility of selected protein [**27, 30-32**].

Virtual screening studies were performed using Autodockvina 4.0 software with a grid box set of 40, 40 and 40 A^o (x, y and z) with a spacing of 0.375A°. Conformer with lowest binding energy (in k.cal mol^{-1}) was obtained using the Lamarckian generic algorithm (LGA). Binding orientation of the synthesized compounds (**7a-j**) within the active site of (**2H7M**) protein is visualized using ArgusLab 4.0.1 was used [**27**]. These studies showed good correlation between Autodock binding free energy values obtained from docking studies and MIC values of antimicrobial screening experiments.

RESULTS AND DISCUSSION

Chemistry: The reaction of benzaldehyde (**1**) with ethyl acetoacetate (**2**) and 2-aminobenzothiazole (**3**) in ethylene glycol as a solvent and TBAHS as a promoter at 120°C for 4 h, afforded Ethyl 4*H*-pyrimido[2,1-*b*]benzothiazole-3-carboxylate(4), which on hydrolysis with KOH in methanol for 12 h at reflux to give 4*H*-pyrimido[2,1-*b*]benzothiazole-3-carboxylic acid (5). Carboxylic acid (**5**) on reacting with different aromatic amines in presence of EDCI and DIPEA in DMF solvent furnished the corresponding *N*-aryl-4*H*-pyrimido[2,1-*b*]benzothiazole-3-carboxamides (**7a-j**). Structures of the products (**7a-j**) were confirmed using ^1H and ^{13}C NMR, IR, and mass spectroscopic data. ^1H NMR spectra of the compounds (4) showed the formation of pyrimidine ring for benzylic proton with a single peak at 4.59ppm. Amide formation of the final products (**7a-j**) is confirmed by a single peak at 8.10 ppm for amide-*N*H proton. The mass spectra of these compounds displayed molecular ion peaks at appropriate m/z values confirmed the products (**7a-j**) formation.



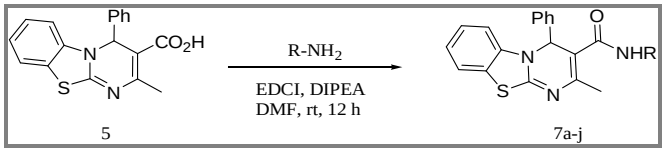
Reagents and conditions: (a) benzaldehyde (**2**), ethyl acetoacetate (**3**), tetrabutylammonium hydrogen sulphate(TBHAS), ethylene glycol, 120°C, 4 h. (b) KOH, methanol, reflux, 12h. (c) R-NH_2 (**6**), 1-ethyl-3-(5-dimethylaminopropyl) carbodiimine (EDCI),*N*,*N*-diisopropylethylamine (DIPEA), DMF,rt, 12 h.

Scheme 1. synthesis of 4*H*-pyrimido[2,1-*b*]benzothiazole-3-carboxamide derivatives (**7a-j**).

Biology

Antimicrobial evaluation: In order to investigate the antimicrobial activity of the synthesized compounds (**7a-j**), we were pleased to evaluate the *in vitro* antimicrobial activity against three Gram-positive bacteria *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC25923) and *Staphylococcus epidermidis* (ATCC12228) and three Gram-negative bacteria *Pseudomonas aeruginosa* (ATCC 27853), Escherichia coli (ATCC 25922) and *Klebsiella pheumoniae* (ATCC10031) by serial dilution method. Moreover, we also evaluated for their *in vitro* antifungal activity against five fungal organisms *Saccharomyces cerevisiae* (ATCC 204508), *Aspergillus niger* (ATCC 9029), *Rhizopus oryzae* (ATCC 2809), *Candida albicance*(ATCC 76615) and *Aspergillus flavus*(NRRL3357) by agar diffusion (cup plate) method.

Table 1. Synthesis of 4*H*-pyrimindo[2,1-*b*]benzothiazole-3-carboxamide derivatives **(7a-j)**[a].



Entry	Proudct (7a-j)[a] R	Reaction Time	Yield[b] (%)
1	7a; R=C_6H_5-	10	74
2	7b; R=4-NO_2-C_6H_4-	12	68
3	7cb; R=Cl-C_6H_4-	12	70
4	7d; R=4Br-C_6H_4-	11	71
5	7e; R=4-MeO-C_6H_4-	9	79
6	7f; R=4-Me-C_6H_4-	10	75
7	7g; R=2,3-di-MeO-C_6H_3-	8	76
8	7h; R=2-Cl-C_6H_4-	12	55
9	7i, R=3-NO_2-C_6H_4-	12	65
10	7J; R=$C_6H_5CH_2$-	8	76

[a]Reaction Conditions: Carboxylic acid 5 (1 mmol), substituted amine 6(1.1 mmol), EDCI (1 mmol) and DIPEA (1.5 mol), 120°C. All the products were characterized by1H, 13CNMR, IR and MS. [b]isolated yields.

Antibacterial activity: The antibacterial activities of the set of heterocyclic compounds (**7a-j**) are tested by two fold serial dilution method taking drug at a concentration of 150 µg mL^{-1} [**18-28, 33**]. The MIC (µg mL^{-1}) values are presented in table 2 and compared to that of the standard drug i.e. penicillin and streptomycin. All the compounds **7a-j** exhibited significant activity against Gram-positive and Gram-negative bacteria. Compound **7j** having N-benzyl amide derivative possess significant activity against both *S. aureus* and *E. coli* with MIC value of 9.37 µg mL^{-1}. Similarly, the heterocyclic amide derivatives **7b** and **7d** having 4-nitro-phenyland 4-bromo-phenyl *N*-substitutions, respectively have displayed remarkable activity against *B. subtillis* and *S. epidermidis*, with MIC value of 9.37 µg mL^{-1}. It is noteworthy to mention that all the compounds were also exhibit moderate

Table 2. Antibacterial activity of heterocyclic amide derivatives (**7a-j**)

Compound	Minimum inhibitory Concentration (µg mL^{-1})					
	Gram-positive organism			Gram-negative organism		
	B. subtillis	*S. aureus*	*S. epidermidis*	*E. coli*	*P. aeroginosa*	*K. pnemoniae*
7a	18.75	75.00	150.0	18.75	18.75	18.75
7b	09.37	18.75	75.00	75.00	18.75	150.0
7c	18.75	37.50	75.00	18.75	75.00	75.00
7d	18.75	18.75	09.37	18.75	75.00	37.50
7e	18.60	16.75	150.0	150.0	150.0	150.0
7f	37.50	75.00	18.75	75.00	75.00	150.0
7g	75.00	150.0	150.0	150.0	75.00	150.0
7h	37.50	75.00	75.00	37.50	150.0	150.0
7i	18.75	37.50	37.50	18.75	75.00	37.50
7j	18.75	09.37	18.75	09.37	18.75	37.50
STD Streptomycin	09.37	01.17	01.17	04.68	04.68	04.68
STD Penicillin	01.17	09.37	04.68	09.37	09.37	09.37

to good antibacterial activities with the specific stain. Compounds **7a**, **7c**, **7d**, **7e**, **7i** and **7j** have shown moderate activity against *B. subtillis* with MIC value 18.75µg mL^{-1}. Next, the tricyclic compounds **7a**, **7c**, **7d** and **7i** were display moderate activity against *E. coli* with MIC value was 18.75 µg mL^{-1}. Similarly, heteroaromatic amides derived from aniline, 4-notroaniline and benzyl amine (**7a**,

7b and **7j)** were also shown moderate activity against *P. aeroginosa* with MIC value is 18.75 µg mL^{-1}. Substituted heteroaromatic amide derivatives **7b** and **7d** were effective with *S. aureus* with the moderate activity and MIC value was 18.75 µg mL^{-1}. Amide derivatives **7f** and **7j** have shown moderate activity against *S. epidermidis* with MIC value was 18.75 µg mL^{-1}. Finally, aniline derivative of heterocyclic amide (**7a**) showed moderate activity against *K. pnenmoniae* with MIC value was 18.75 µg mL^{-1}.

Anti-fungal activity: The antifungal activity of the synthesized compounds is determined by agar diffusion method (cup and plate method) [19-28] taking drug at a concentration of 100 µg mL^{-1} against the five fungal strains. The zones of inhibition (ZOI) value of the compounds were compared to that of the standard drug i.e. amphotericin-B and the results are presented in table 3. Compound **7e** having 4-methoxy substitution on amide-has shown maximum activity against *A.niger* with maximum ZOI (48 mm). Next, the heteroaromatic amide derivatives such as **7f**, **7i** and**7j** having 4-methyl-, 3-nitro-and benzyl substituted amide derivative, respectively have shown moderate activity on *A.niger* with ZOI 40-45 mm. Furthermore, amide with chloro substitution (**7c**) and amide derived from benzyl amine (**7j**) were shown to be active and possess moderate activity against *C. albicans* and *R .oryzae*, respectively with ZOI 41 mm. Similarly, the tricyclic amides with electron-donating groups on aromatic ring of amide (**7e** and **7f**) were shown moderate activity on *S. cerevisiae* with ZOI 31-32 mm.

Table 3. Antifungal activity of heterocyclic amides (**7a-j) at 100** µg mL^{-1}

Compound (100 µg mL^{-1})	Zone of Inhibition (mm)				
	R.oryzae	*A.niger*	*A.flavus*	*C.albicans*	*S.cerevisiae*
7a	--	38	--	--	--
7b	19	--	--	32	--
7c	32	18	--	41	19
7d	28	18	28	15	-
7e	12	48	-	14	32
7f	28	44	22	22	31
7g	16	--	13	--	10
7h	19	22	25	20	--
7i	38	41	35	--	29
7j	41	45	21	28	26
Amphotericin-B	24	25	24	24	22

The results of *in vitro* antimicrobial studies revealed that the biological activity of the tested compounds will give structure activity relationships. The studies suggested that the heterocyclic amide containing electron withdrawing groups like 4-nitro, 4-chloro and 4-bromo phenyl substitution have shown good antibacterial activities than electron releasing groups like methoxy and methyl substitution on phenyl ring. In antifungal activity, amide derivatives having 4-methoxy substitution on aromatic ring exhibit excellent activity against *A.niger* and *S.cerevisiae* fungal strains. Similarly, the amide derived from benzyl amine (**7j**) has shown good antibacterial activity against all selected Gram-positive and Gram-negative bacteria and also fungal strains.

Molecular Docking Studies: In addition to the synthesis of novel heterocyclic amide derivatives in a facile and multicomponent manner, and evaluation of their biological activities, an exploration of the molecular docking studies were carried out to better perceive and explain the observed variance in their biological activities of 4H-pyrimido[2,1-b]benzothiazole derivatives. These docking studies are useful to predict the best drug compound with its substituent's and configuration for the optimum receptor pit, of which can be able to exhibit the best pharmacophore activity. In our present docking studies, Version 2.0 of the GOLD (Genetic Optimization for Ligand Docking) docking program was used. The interaction of the synthesized compounds with the receptor in the modeled complex was investigated and observed the ability of different inhibitors to fit into oxidoreductase protein.

The 3D structures of selected protein [*E. Coli* (**2H7M**)] was screened for molecular modeling studies using the synthesized heterocyclic amide inhibitors (**7a-j**). Based on the fitness score generated for their corresponding Gold score, Chemscore values and RMSD have been indicated in the table 4 and 5.

Table 4. GOLD score of 4H-Pyrimido[2,1-*b*]benzothiazole derivatives (**7a-j**)

Compound	Fitness	S (hb_ext)	S (vdw_ext)	S (hb_ext)	S (vdw_ext)
7a	44.32	0.00	37.14	0.00	-6.75
7b	45.18	0.00	37.76	0.00	-6.74
7c	49.51	1.79	40.01	0.00	-7.29
7d	51.76	0.29	42.96	0.00	-7.60
7e	41.48	0.00	36.73	0.00	-9.02
7f	39.23	0.00	38.76	0.00	-14.07
7g	27.07	1.81	34.17	0.00	-21.73
7h	47.86	2.00	37.28	0.00	-5.39
7i	48.31	2.21	37.60	0.00	-5.60
7j	54.23	0.55	40.82	0.00	-2.44

$Gold_{fitnesss}$=(Fitness)=S(hb_ext)+1.3750*S(vdw_ext)+S(hb_int)+1.0000*S(hb_ext)

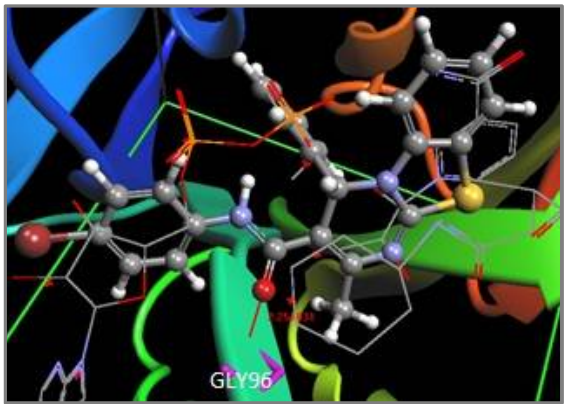
Table 5. Chem score of 4H-Pyrimido[2,1-*b*]benzothiazole derivatives (**7a-j**)

Compound	Score	DG	S (H-bond)	S (Meta)	S (Lipo)	DE (Clash)	DE (Int)
7a	33.98	-36.98	0.96	0.00	263.59	0.94	2.06
7b	27.97	-29.90	0.00	0.00	238.60	0.02	1.91
7c	31.77	-34.88	0.00	0.00	273.13	0.89	2.22
7d	32.07	-35.96	0.00	0.00	282.39	1.71	2.18
7e	31.42	-33.87	0.00	0.00	267.49	0.39	2.05
7f	28.82	-31.74	0.95	0.00	219.14	0.94	1.99
7g	29.72	-37.15	0.00	0.00	299.25	0.82	6.61
7h	33.28	-37.25	1.00	0.00	264.85	1.74	2.23
7i	35.67	-40.04	1.98	0.00	268.70	0.40	3.96
7j	34.28	-36.62	0.99	0.00	263.74	0.68	1.66

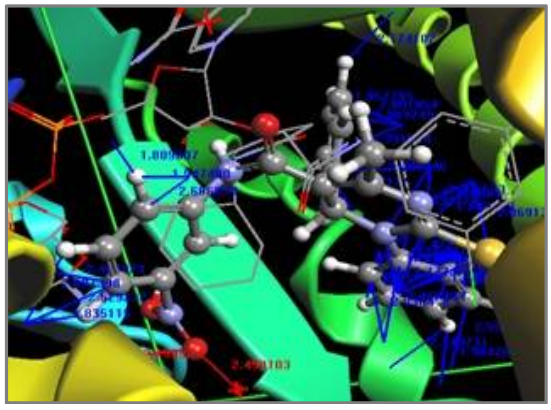
$ChemScore = \Delta G_{binding} + P_{clash} + C_{internal}P_{internal} + (C_{covalent}P_{covalent} + p_{constraint})$

Score = -(DG + DE(clash) + DE(int))

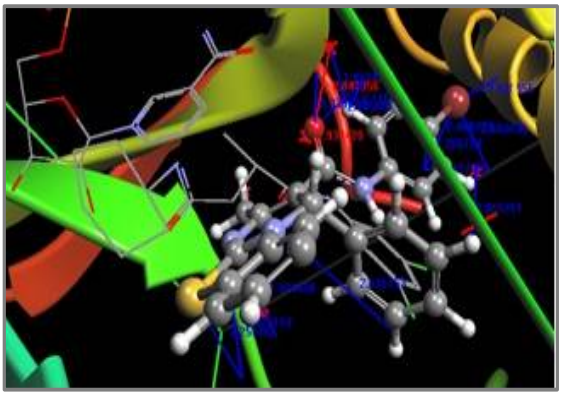
The binding conformations of the synthesized compounds (**7a-j**) within the active site of (**2H7M**) have been visualized using ArgusLab 4.0.1 and the details are displayed in figure 1. In the active site region (15Å) of (**2H7M**) proteinIle194, Lys165, Tyr158, Ser20, Ile95, Asp64, Ile21, Phe149, Gly96, Arg195, Thr196, Phe41, Asp14, Val 65 amino acids can play important role and are shown in figure 2. The synthesized compounds which fit effectively into the active site of the target protein (**2H7M**) will have optimum lipophilicity and maximum hydrogen bonding with low clash requirements.



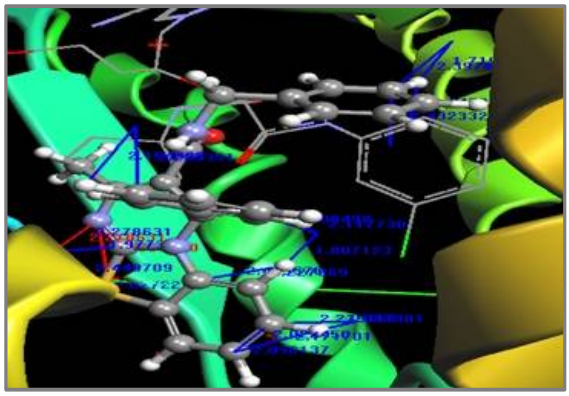
Binding orientation of compound 7c, Binding Energy = -11.3194Kcal mol^{-1}



Binding orientation of compound 7i, Binding Energy = -13.2094 Kcal mol^{-1}



Binding orientation of compound 7d Binding Energy = -12.2653Kcal mol^{-1}



Binding orientation of compound 7j Binding Energy = -13.2308Kcal mol^{-1}

Figure 1. Docking of molecules **7c**, **7d**, **7i** and **7j** with active site of 2H7M: Binding orientations of compounds **7c, 7d, 7i** and **7j** with the crystallographic conformation of active site (**2H7M**) which are obtained using ball-cylinder model with amino acid residues, where the hydrogen bonding is indicated with red colored dotted lines.

Figure 2. Crystallographic protein of 2H7M showing the active site of amino acids.

The docking score of the synthesized compounds calculated by Gold software is in good correlation with the experimental activity (MIC) values. Molecular modeling studies revealed that all the synthesized compounds (**7a-j**) have good docking scores with comparable binding energy values with the target protein (**2H7M**). Among (**7a-j**),**7c, 7d, 7i** and **7j** showed the best docking scores with binding energy values also exactly correlated with the experimental antimicrobial activity on *E.coli* bacterial strain. Binding orientations of compounds **7c, 7d, 7i** and **7j** with the crystallographic conformation of active site (**2H7M**) have been obtained using ball cylinder model with amino acid residues, where the hydrogen bonding is indicated with red colored dotted lines. Using Argus Lab 4.0.1, the binding energy values of the analogues (**7a-j**) have been estimated and presented in table 6.

Table 6. binding energy values of 4*H*-Pyrimido[2,1-*b*]benzothiazoles (**7a-j**)

Compound	Binding energy (K. Cal mol^{-1})	Compound	Binding energy (K. Cal mol^{-1})
7a	-10.8288	7f	-10.5541
7b	-11.4515	7g	-11.3317
7c	-11.3194	7h	-11.4423
7d	-12.2653	7i	-13.2094
7e	-9.07546	7j	-13.2308

APPLICATION

The observed biological activity results of the synthesized analogues (7a-j) will be useful for the future efforts to discover and develop new synthetic analogues with further improved antimicrobial activity, eventually to better the treatment of the pathogenic infectious diseases.

CONCLUSION

In the present study, some novel*N*-(aryl)-2-methyl-4-phenyl-4*H*-pyrimido[2,1-*b*]benzothiazole-3-carboxamides (**7a-j**) have been synthesized. Compounds (**7a-j**) were tested for their *in vitro* antibacterial and antifungal activities and found to have good activities against selected Gram-positive, Gram-negative and fungal strains. The result indicates that the synthesized compounds bearing electron withdrawing groups like 4-nitro, 4-chloro and 4-bromo substituent's have good antibacterial activities than having electron releasing groups like methoxy and methyl substitution on amide-N-bonded aromatic ring. In antifungal activity, electron releasing groups like methoxy and methyl substitution on amide-N-bonded aromatic ring have shown moderate activity.

To support the observed variation in antibacterial activity of the tested 4*H*-pyrimido[2,1-*b*]benzothiazole carboxamides (**7a-j**) against *E. coli* bacterial strain, an exploration of molecular docking studies have been conducted. In these docking studies, the docking scores obtained by using Gold software were found to have a good correlation with the experimental antimicrobial activity (MIC values) on *E. coli* bacterial strain. The observed results may be useful in guiding future efforts to discover new compounds with improved antimicrobial activity.

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