Synthesis and Study of the Antimicrobial Activity of Novel Tricyclic 2H-pyrimido[2,1-*b*]benzothiazoles

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Abstract: Efficient synthesis of 4*H*-pyrimido[2,1-*b*]benzothiazoles by novel one-pot three-component reaction of an aldehyde, β-ketoester and 2-aminobenzothiazole using sulphamic acid as a catalyst is described. All synthesized compounds were evaluated for *in vitro* antibacterial activity using Gram-positive bacteria and Gram-negative bacteria (*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa , Bacillus subtilis, Proteus Valgaris*). *In vitro* antifungal activity was also determined against the five fungal species (*Aspergillus flavus, Candida albicans Aspergillus fumigatus, Penicillium mareneffei, Chrysosporium tropicum*). Structure of the synthesized compounds was established by elemental analysis and spectral data.

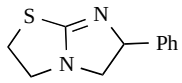
Keywords: Multicomponent condensation, 2-Aminobenzothiazole, 4H-Pyrimido[2,1-b]benzothiazoles, Sulphamic acid, Antimicrobial activity, synthesis.

1. INTRODUCTION

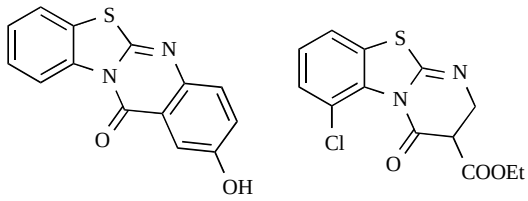
2-Aminobenzothiazole demonstrated interesting pharmacological activities including anticonvulsant [1], analgesic [2], anti-tumor [3, 4], antibacterial [5, 6], antimicrobial [7, 8] and muscle relaxant agents [9]. Particularly, these are highly reactive compounds and extensively utilized as reactants or reaction intermediates since the NH_2 and endocyclic *N* functions are suitably situated to enable reactions with common *bis* electrophilic reagents to form a variety of fused heterocyclic compounds [10]. In recent years, several attempts were made for modifying the benzothiazole nucleus to improve their biological activities. Modifications on the benzothiazole nucleus have resulted in a large number of compounds having diverse pharmacological activities. Among them, substituted benzothiazole compounds have been shown to be fungicidal agents [11], while tetrahydro derivatives were tested in cancer chemotherapy [12].

Thiazole and pyrimidine heterocyclic ring systems are considered to be the typical privileged structures in pharmaceutical research as both the heterocyclic ring systems are the active core of various bioactive molecules [13-18]. Pyrimidobenzothiazoles have been reported to exhibit a wide spectrum of pharmacological activities such as antiviral, antitumor, anti-inflammatory, analgesic, anticonvulsant, muscle relaxant, sedative etc. [19-22].

Moreover, pyrimido[2,1-*b*]benzothiazoles were evaluated for their affinity at the central benzodiazepine receptor [23]. Trapani and co-workers [24-26] have explored the widespread activities of these compounds displaying anxiolytic, anticonvulsant, muscle relaxant and sedative properties**.** Hence in continuation of our efforts on the design of novel antibacterial agents [27,28] and keeping in mind the medicinal importance of pyrimidobenzothiazolone moiety, it was thought worthwhile to synthesize certain novel derivatives of 4*H*-Pyrimido[2,1-*b*]benzothiazoles and screen them for their biological activities particularly for their antibacterial and antifungal activity. Representatives of some biologically important pyrimidobenzothiazolone compounds have been illustrated in Fig. (**1**).



Tetramisole
Anthelmintic and as
levamisole clinically used
anti-cancer agent



Pyrimido[2,1-b]benzothiazoles
Cytotoxic Agonist for benzodiazepine receptor

Fig. (1). Chemical structure of pyrimidobenzoyhiazole derivatives.

Methods for preparation of 4*H*-Pyrimido[2,1-*b*]benzothiazoles derivatives are scarce in literature. Earlier, Shaabani *et al.* [29], have reported a multicomponent approach for their preparation by one pot condensation of an aldehyde **1**, β-ketoester **2** and 2-aminobenzothiazole **3**, using ionic liquid 1,1,3,3-tetramethylguanidinium trifluoroacetate as reaction medium to obtain 4*H*-Pyrimido[2,1-*b*]benzothiazoles **4** in 75-85 % yield. This method has become expensive due to ionic liquids. Here we report a simple and economical method for this condensation producing derivatives 4 in improved yields (80-89 %) using sulphamic acid as a catalyst (Scheme **1**).

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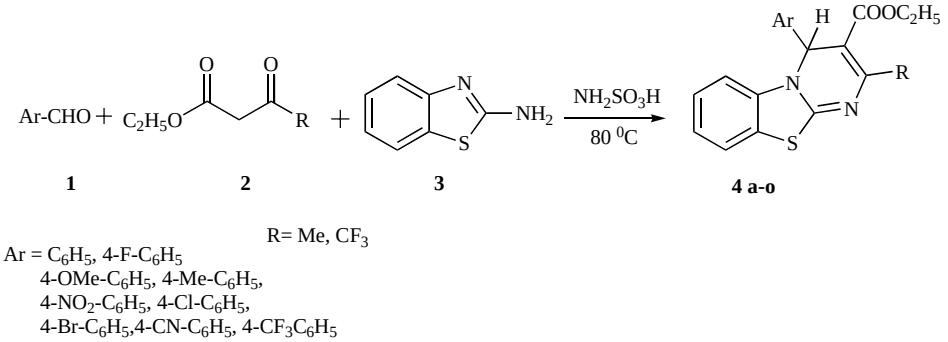


Scheme 1.

2. RESULTS AND DISCUSSIONS

Chemistry

In our preliminary experiment we have studied preparation of 4*H*-Pyrimido [2,1-*b*]benzothiazoles by multi-component condensation using a variety of aryl aldehydes with a 1,3-diketo-ester and 2-aminobenzothiazole by refluxing in ethanol using sulphamic acid as a catalyst and obtained the products in the range in 80-89% yield. The electron- withdrawing or electron-releasing functional groups on benzaldehydes did not have any influence on the reaction (Table **1**).

The products (entry **4a- 4o**) were characterized based on their IR, ^{13}C NMR, ^{1}H NMR and elemental analysis. The mass spectra of these compounds displayed molecular ion peaks at appropriate m/z values. This synthetic strategy is more attractive than earlier methods due to easily recoverable and reusable catalyst, easy workup and higher yields of the product. The plausible reaction mechanism involves condensation of aldehyde with β-ketoester by Knoevenagel reaction produces 3-arylidine-2,4-pentanedione **5**, which reacts with 2-aminobenzothiazole *in situ via* intermediate **6**, followed by cyclization through Michael addition to afford 4*H*-pyrimido[2,1-*b*] benzothiazole (Scheme **2**) .

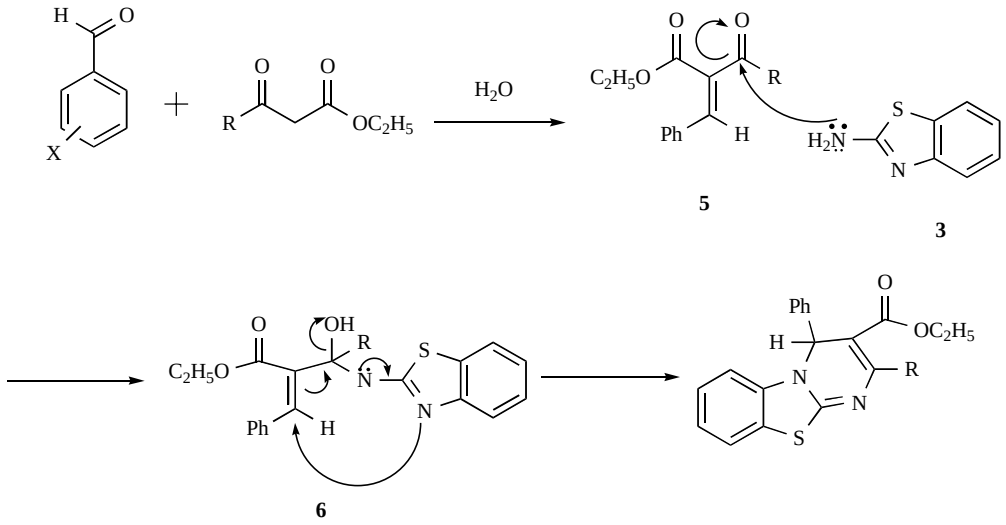
3. MICROBIOLOGY

Newly synthesized target compounds **4a-4o** were evaluated for their *in vitro* anti-bacterial activity against various pathogenic bacterial strains (Gram-negative and Gram-positive) viz., *Escherichia coli* (ATCC–25922), *Staphylococcus aureus* (ATCC–25923), *Pseudomonas aeruginosa* (ATCC–27853), *Bacillus subtilis* (ATCC–6633), *Proteus Valgaris* (ATCC–13315). Anti-fungal activity of the above compounds **4a–o** was evaluated against fungal strains viz. *Aspergillus flavus* (NCIM No.524), *Candida albicans* (NCIM No.3100), *Aspergillus fumigatus* (NCIM No.902), *Penicillium mareneffei* (recultured) and *Chrysosporium tropicum* (MTCC 2821). The anti-bacterial and antifungal activities were evaluated by agar disc diffusion method as per the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS, 1997) [30]. The solvent,

Table 1. Sulphamic Acid Catalyzed Synthesis of Tricyclic 4H-pyrimido[2,1-b] benzothiazoles[a] (4a-o)

Compounds	Ar	R[b]	Time (min)	Yield(%)[c]
4a	C_6H_5	CH_3	50	86
4b	4-OMe- C_6H_5	CH_3	55	90
4c	4-Me-C_6H_5	CH_3	45	83
4d	4-NO_2-C_6H_5	CH_3	60	88
4e	4-F- C_6H_5	CH_3	45	84
4f	4-Cl- C_6H_5	CH_3	70	90
4g	C_6H_5	CF_3	60	85
4h	4-OMe- C_6H_5	CF_3	50	80
4i	4-Me-C_6H_5	CF_3	45	83
4j	4-F- C_6H_5	CF_3	70	84
4k	4-Cl- C_6H_5	CF_3	55	84
4l	4-Br- C_6H_5	CF_3	60	82
4m	4- NO_2- C_6H_5	CF_3	55	89
4n	4- CF_3 - C_6H_5	CF_3	45	82
4o	4-CN- C_6H_5	CF_3	55	87

[a]Reaction was performed at 1mmol scale. [b]Products were characterized by NMR,IR and Mass spectroscopy. [c]Yield refers to pure products after column chromatography.



Scheme 2.

DMSO used for the preparation of compounds did not show inhibition against the tested organisms.

3.1. Anti-bacterial Activity

The results of anti-bacterial screening of all the newly synthesized compounds are presented in Table **2**. Some of them showed moderate to good activity with MIC value in the range of 6.25–50 μg/ml in DMSO. Particularly, compound Ethyl-2-trifluoromethyl-4-(4-chlorophenyl)-4H-pyrimido[2,1-b][1,3] benzothiazole-3-carboxylate **4k** showed good activity (zone of inhibition 20 and 22 mm) against *S. aureus* and *P. aeruginosa*. Compounds **4h, 4i, 4l** showed good activity against *p. aeruginosa* and *B. subtilis*, while compounds **4d**, **4j**, and **4o** showed moderate activity against few bacterial strains.

3.2. Anti-Fungal Activity

The results of anti-fungal screening of all the newly synthesized compounds are presented in Table **3**. Some of them showed good to excellent activity with MIC value in the range of 6.25–25 μg/ml in DMSO. Compounds ethyl-2-trifluoromethyl-4-(4-fluorophenyl)-4H-pyrimido[2,1-b][1,3] benzothiazole-3-carboxylate **4j** and Ethyl-2-trifluoromethyl-4-(4-trifluorophenyl)-4H-pyrimido[2,1-b][1,3]benzothiazole-

Table 2. Antibacterial Activity of Compounds 4a-4o

Compounds	*E. coil*	*S.aureus*	*P.aeruginosa*	*B. subtilis*	*P.valgaris*
4a	14 (6.25)	12 (6.25)	14 (12.5)	16 (6.25)	16 (12.5)
4b	14 (12.5)	14 (50)	14 (50)	16 (12.5)	14 (6.25)
4c	12 (6.25)	12 (25)	14 (12.5)	12 (25)	16 (6.25)
4d	21 (12.5)	19 (6.25)	22 (12.5)	19 (12.5)	16 (6.25)
4e	15 (6.25)	17 (12.25)	18 (6.25)	19 (6.25)	17 (6.25)
4f	16 (6.25)	14 (12.5)	14 (6.25)	17 (12.5)	16 (12.5)
4g	14 (12.5)	14 (25)	16 (12.5)	16 (12.5)	16 (6.25)
4h	20 (6.25)	17 (6.25)	20 (6.25)	19 (6.25)	17 (6.25)
4i	23 (6.25)	18 (6.25)	21 (6.25)	18 (6.25)	16 (6.25)
4j	17 (12.5)	20 (12.5)	17 (6.25)	18 (6.25)	16 (12.5)
4k	20 (12.5)	20 (6.25)	22 (6.25)	17 (6.25)	18 (12.5)
4l	20 (6.25)	18 (6.25)	20 (6.25)	18 (6.25)	16 (12.5)
4m	16 (12.5)	19 (12.5)	16 (12.5)	18 (6.25)	18 (12.5)
4n	18 (12.5)	16 (6.25)	17 (12.5)	18 (12.5)	16 (12.5)
4o	17 (6.25)	18 (12.5)	19 (6.25)	19 (12.5)	14 (12.5)
Ciprofloxacin	27 (6.25)	24 (6.25)	28 (6.25)	24 (6.25)	22 (6.25)

MIC values are given in bracket. MIC (μg/ml) = Minimum inhibitory concentration, i.e. the lowest concentration of drug which completely inhibit bacterial growth. Ciprofloxacin was used as standard for anti-bacterial activity. Diameter of inhibition zone was measured in mm.

Table 3. Antifungal Activity of Compounds 4a-4o

Compounds	*A.flavus*	*C.albicans*	*A.fumigatus*	*P.mareneffei*	*C.tropicum*
4a	12 (12.5)	15 (6.25)	14 (12.5)	14 (6.25)	12 (12.5)
4b	12 (6.25)	14 (25)	18 (25)	16 (6.25)	14 (12.5)
4c	14 (6.25)	14 (25)	18 (25)	18 (6.25)	16 (12.5)
4d	14 (6.25)	15 (6.25)	18 (12.5)	14 (6.25)	12 (12.5)
4e	18 (6.25)	20 (6.25)	16 (6.25)	19 (6.25)	17 (6.25)
4f	17 (6.25)	14 (12.5)	14 (6.25)	18 (12.5)	16 (12.5)
4g	17 (6.25)	17 (12.5)	18 (6.25)	14 (6.25)	14 (25)
4h	17 (6.25)	18 (6.25)	17 (6.25)	15 (6.25)	19 (6.25)
4i	19 (12.5)	18 (6.25)	21 (6.25)	20 (6.25)	14 (12.5)
4j	15 (12.5)	22 (6.25)	21 (6.25)	20 (6.25)	16 (6.25)
4k	20 (6.25)	20 (6.25)	21 (6.25)	14 (25)	19 (12.5)
4l	18 (6.25)	16 (6.25)	19 (6.25)	18 (12.5)	15 (12.5)
4m	18 (6.25)	18 (12.5)	18 (12.5)	18 (6.25)	18 (12.5)
4n	18 (12.5)	20 (6.25)	22 (6.25)	18 (6.25)	19 (6.25)
4o	17 (6.25)	18 (6.25)	20 (6.25)	19 (12.5)	18 (6.25)
Fluconazole	27 (6.25)	24 (6.25)	28 (6.25)	24 (6.25)	22 (6.25)

MIC values are given in bracket. MIC (µg/ml) = Minimum inhibitory concentration, i.e. the lowest concentration of drug which completely inhibit fungal growth. Fluconazole was used as standard for anti-fungal activity. Diameter of inhibition zone was measured in mm.

3-carboxylate **4n**, exhibited good activity against the four fungal strains viz. *C. albicans, A.fumigatus, p.mareneffei* and *C.tropicum*. Compound **4k** showed excellent activity against *A.flavus, C. albicans and A. fumigates* but no activity against *P.mareneffei* . Compound **4i** showed good activity against *C. albicans, A.fumigatus, P.mareneffei* but no activity against *C.tropicum*. Compounds **4g, 4l** showed moderate activity against *A.flavus* and A.fumigatus but no activity against *C.tropicum*. Compounds **4e**, **4o** showed good activity against *A. flavus, C. albicans, A. fumigates and C.tropicum*.

4. EXPERIMENTAL SECTION

All the chemicals and solvents used for this work were obtained from Merck (Germany), Himedia (Mumbai) and Aldrich chemical company (U.S.A.). The chemicals purchased were of analytical reagent grade or were purified by standard methods prior to use. Melting points of the synthesized compounds were determined in open-glass capillaries on Veego VMP-AM melting point apparatus and are uncorrected. IR absorption spectra were recorded on Shimadzu FTIR-8400s using KBr pellets in the range of 4000–400cm^{-1}; ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian Gemini 300 MHz spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported in parts per million (ppm) down field from tetra methyl silane. Spin multiplicities are described as s (singlet), d (doublet), and m (multiplet). EI mass spectra were recorded on a VG-7070H Micro mass spectrometer. IR, ^{1}H NMR, ^{13}C NMR and Mass spectra were consistent with the assigned structures. Elemental analyses (C, H, N) were done on a CHN rapid analyzer. All the new compounds gave C, H and N analysis within ± 0.03% of the theoretical values. Purity of the compounds was checked by thin layer chromatography (TLC) on Merck silica gel 60 F254 precoated sheets in n-hexane/ ethyl acetate mixture and spots were developed using iodine vapor/ultraviolet light as visualizing agent.

4.1. The General Procedure for the Preparation of Compounds 4a-o

A mixture of β-ketoester (1 mmol), substituted aryl aldehyde (1 mmol), 2-aminobenzothiazole (1 mmol) and sulphamic acid (40mg, 8mol%) in ethanol (10 ml) was refluxed and the progress of the reaction was followed by TLC. After completion of the reaction, water (5 ml) was added to reaction mixture and extracted with ethyl acetate (2x15 ml). The organic layer was separated and dried over Na_2SO_4, and solvent was removed under reduced pressure. The crude product were purified by column chromatography (60-120 mesh) using (ethyl acetate: n-hexane, 30:70) and obtained **4a-o**.

Ethyl-2-methyl-4-(phenyl)-4H-pyrimido [2, 1-b] [1, 3] benzothiazole-3- carboxylate (4a)

Pale yellow solid, M.P. 174-176 ^{0}C; IR (KBr): 3212, 3041, 1738, 1582, 1453, 1230, 740 cm^{-1}; ^{1}H NMR (300MHz, $CDCl_3$ + DMSO): δ 1.28 (t, *J* = 6.58 Hz, 3H), 2.35 (s, 3H), 4.10-4.16 (q, 2H), 6.38 (s, 1H), 7.10-7.24 (m, 6H), 7.40-7.51 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$+DMSO): δ 166.37, 163.28, 141.14, 137.85, 128.70, 128.45, 127.20, 126.68, 123.10, 123.91, 122.23, 120.90, 111.90, 103.11, 60.17, 57.83, 23.45, 14.35; EI-MS: m/z 351(M+1); Anal. Calc. for ($C_{20}H_{18}N_2O_2S$) C, 68.55; H, 5.18; N, 7.99; found; C, 68.56; H, 5.16; N, 7.99.

Ethyl-2-methyl-4-(4-methoxyphenyl)-4H-pyrimido[2,1-b][1,3]benzothiazole-3- carboxylate (4b)

Yellow Powder; M.P. 139-141^{0}C; IR (KBr): 3207, 1742, 1508, 1242, 746 cm^{-1}; ^{1}H NMR (300MHz, $CDCl_3$ + DMSO): δ 1.27 (t, J=7.34 Hz, 3H), 2.35 (s, 3H), 3.70 (s, 3H), 4.08-4.17 (q, 2H), 6.35 (s, 1H), 6.74 (d, J= 8.81 Hz, 2H), 7.05-7.14 (m, 5H), 7.43-7.51 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$+DMSO): δ 164.55, 161.13, 157.39, 152.15, 135.96, 131.68, 126.50, 124.61, 121.93, 121.82, 120.15, 111.89, 109.83, 101.27, 58.03, 55.21, 53.17, 21.55, 12.38; EI-MS: m/z 381(M+1); Anal. Calc. for ($C_{21}H_{20}N_2O_3S$) C, 66.29; H, 5.30; N, 7.36;found; C, 66.30; H, 5.27; N, 7.37.

Ethyl-2-methyl-4-(4-methylphenyl)-4H-pyrimido[2,1-b][1,3]benzothiazole-3- carboxylate (4c)

Yellow Powder; M.P. 143-145 ^{0}C; IR (KBr): 3208, 2973, 1742, 1603, 1465, 1335, 1235, 1105, 1075, 836, 695 cm^{-1}; ^{1}H NMR (300MHz, $CDCl_3$ + DMSO): δ 1.28 (t, J=7.35 Hz, 3H), 2.26 (s, 3H), 3.10 (s, 3H), 4.08-4.15 (q, 2H), 6.31 (s, 1H), 7.00-7.20 (m, 6H), 7.40-7.46 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$+DMSO): δ 164.60, 162.14, 157.45, 153.10, 134.98, 132.08, 126.30, 123.63, 121.98, 121.79, 120.45, 111.66, 109.63, 101.37, 58.13, 55.19, 53.27, 15.45, 12.38; EI-MS: m/z 365 (M+1); Anal. Calc. for ($C_{21}H_{20}N_2O_2S$) C, 69.20; H, 5.53; N, 7.69; found; C, 69.21; H, 5.51; N, 7.69.

Ethyl-2-methyl-4-(4-nitrophenyl)-4H-pyrimido[2,1-b][1,3]benzothiazole-3- carboxylate (4d)

Pale yellow Powder; M.P.149-151^{0}C; IR (KBr): 3215, 3110, 1740, 1503, 1335, 1230, 750 cm^{-1}; ^{1}H NMR (300MHz, $CDCl_3$ + DMSO): δ 1.30 (t, J=7.34 Hz, 3H), 2.38 (s, 3H), 4.10-4.16 (q, 2H), 6.55 (s, 1H), 7.10-7.20 (m, 3H), 7.50-7.71 (m, 3H), 8.13 (d, J= 8.81Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$+DMSO): δ 165.95, 163.23, 147.79, 147.35, 137.06, 128.10, 127.23, 124.96, 124.13, 124.09, 122.75, 111.69, 102.33, 60.73, 57.29, 23.05, 14.38; EI-MS: m/z 396 (M+1); Anal. Calc. for ($C_{20}H_{17}N_3O_4S$) C, 60.75; H, 4.33; N, 10.63; found; C, 60.79; H, 4.31; N, 10.60.

Ethyl-2-methyl-4-(4-fluorophenyl)-4H-pyrimido[2,1-b][1,3]benzothiazole-3- carboxylate (4e)

Yellow Powder; M.P. 137-139^{0}C; IR (KBr): 2925, 2854, 1744, 1694, 1585, 1505, 1372, 1241, 1201, 1079, 1011, 846, 739 cm^{-1}; ^{1}H NMR (300MHz, $CDCl_3$ + DMSO): δ 1.27 (t, J=7.34 Hz, 3H), 2.35 (s, 3H), 4.10-4.16 (q, 2H), 6.40 (s, 1H), 6.95 (d, J=8.81 Hz, 2H), 7.15-7.28 (m, 4H), 7.53 (d, J=8.81 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$+DMSO): δ 166.23, 163.15, 139.99, 137.55, 131.92, 128.96, 127.11, 124.65, 123.99, 122.60, 122.48, 111.89, 102.86, 60.39, 57.38, 23.22, 14.40; EI-MS: m/z 369 (M+1); Anal. Calc. for ($C_{20}H_{17}FN_2O_2S$) C, 65.20; H, 4.65; N, 7.60; found; C, 65.21; H, 4.68; N, 7.58.

Ethyl-2-methyl-4-(4-chlorophenyl)-4H-pyrimido[2,1-b][1,3]benzothiazole-3- carboxylate (4f)

Pale yellow Powder; M.P. 133-135^{0}C; IR (KBr): 3195, 2981, 1739, 1600, 1472, 1340, 1228, 1177, 1088, 1015, 841, 750 cm^{-1}; ^{1}H NMR (300MHz, $CDCl_3$ + DMSO): δ 1.27 (t, J=6.98 Hz, 3H), 2.37 (s, 3H), 4.09-4.15 (q, 2H), 6.38 (s, 1H), 7.10-7.20 (m, 4H), 7.35-7.42 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$+DMSO): δ 166.21, 163.13, 139.97, 137.52, 131.90, 128.82, 126.91, 124.51, 123.98, 122.57, 122.45, 111.87, 102.83, 60.35, 57.37, 23.20, 14.37; EI-MS: m/z 385 (M+1); Anal. Calc. for ($C_{20}H_{17}ClN_2O_2S$) C, 62.41; H, 4.45; N, 7.28; found; C, 62.49; H, 4.43; N, 7.29.

Ethyl-2-trifluoromethyl-4-(phenyl)-4H-pyrimido[2,1-b][1,3]benzothiazole-3- carboxylate (4g)

Yellow Powder; M.P.165-167 ^{0}C; IR (KBr): 3202, 2978, 1737, 1520, 1446, 1335, 1170, 1084, 1080, 830, 746 cm^{-1}; ^{1}H NMR (300MHz, $CDCl_3$ + DMSO): δ 1.00 (t, J=7.18 Hz, 3H), 4.05-4.12 (q, 2H), 6.43 (s, 1H), 6.79-6.89 (m, 2H), 6.94 (t, J=7.55 Hz, 1H), 7.18-7.40 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$+DMSO): δ 167.93, 162.54, 137.61, 135.22, 131.78, 128.76, 128.28, 127.70, 126.70, 125.62, 124.59, 121.69, 120.98, 112.29, 109.96, 60.24, 56.51, 12.88; EI-MS: m/z 405 (M+1); Anal. Calc. for ($C_{20}H_{15}F_3N_2O_2S$) C, 59.40; H, 3.74; N, 6.93; found; C, 59.39; H, 3.73; N, 6.93.

Ethyl-2-trifluoromethyl-4-(4-methoxyphenyl)-4H-pyrimido[2,1-b][1,3] benzothiazole-3-carboxylate (4h)

Yellow Powder; M.P. 170-172^{0}C; IR (KBr): 3198, 2976, 1743, 1518, 1440, 1332, 1169, 1082, 1078, 834, 743 cm^{-1}; ^{1}H NMR (300MHz, $CDCl_3$ + DMSO): δ 1.04 (t, J=7.17 Hz, 3H), 3.79 (s, 3H), 3.95-4.05 (q, 2H), 6.15 (s, 1H), 6.70-6.98 (m, 5H), 7.14 (d, J=7.74 Hz, 2H), 7.34 (d, J=7.74 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$+DMSO): δ 167.65, 162.48, 147.10, 138.18, 132.12, 128.95, 128.30, 126.54, 125.90, 124.85, 122.18, 121.45, 120.90, 112.60, 110.12, 60.40, 56.80, 55.12, 12.90; EI-MS: m/z 435 (M+1); Anal. Calc. for ($C_{21}H_{17}F_3N_2O_3S$) C, 58.06; H, 3.94; N, 6.45; found; C, 58.05; H, 3.93; N, 6.44.

Ethyl-2-trifluoromethyl-4-(4methylphenyl)-4H-pyrimido[2,1- b][1,3] benzothiazole-3- carboxylate (4i)

Yellow Powder; M.P. 181-183^{0}C; IR (KBr): 3196, 2974, 1740, 1520, 1442, 1330, 1168, 1083, 1076, 834, 740 cm^{-1}; ^{1}H NMR (300MHz, $CDCl_3$ + DMSO): δ 1.02 (t, J=7.17 Hz, 3H), 2.40 (s, 3H), 3.95-4.14 (q, 2H), 6.19 (s, 1H), 6.80-7.00 (m, 6H), 7.35 (d, J=7.55 Hz, 2H); ^{13}C NMR (75MHz, $CDCl_3$ + DMSO): δ 167.62, 162.15, 146.62, 137.51, 131.93, 128.70, 128.14, 126.30, 125.32, 124.41, 121.40, 121.19, 120.73, 112.14, 109.30 59.92, 56.02, 20.07, 12.67; EI-MS: m/z 419 (M+1); Anal. Calc. for ($C_{21}H_{17}F_3N_2O_2S$) C, 60.28; H, 4.10; N, 6.69; found;C,60.27; H, 4.11; N, 6.70.

Ethyl-2-trifluoromethyl-4-(4-fluorophenyl)-4H-pyrimido[2,1-b][1,3] benzothiazole-3-carboxylate (4j)

Yellow Powder; M.P.174-176 ^{0}C; IR (KBr): 3207, 2982, 2927, 1738, 1579, 1510, 1471, 1340, 1227, 1176, 1085, 1018, 850, 751 cm^{-1}; ^{1}H NMR (300MHz, $CDCl_3$ + DMSO): δ 1.04 (t, J=6.98 Hz, 3H), 3.96-4.12 (q, 2H), 6.45 (s, 1H), 6.82-7.12 (m, 6H), 7.36 (d, J=7.74 Hz, 1H), 7.65-7.72 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$+DMSO): δ 167.98, 163.07, 138.59, 137.54, 131.29, 131.27, 127.64, 127.56, 124.76, 121.89, 121.64, 121.17, 115.56, 112.35, 109.94, 60.46, 55.93, 13.00; EI-MS: m/z 423 (M+1); Anal. Calc. for ($C_{20}H_{14}F_4N_2O_2S$) C, 56.87; H, 3.34; N, 6.63; found; C, 56.86; H, 3.33; N, 6.65.

Ethyl-2-trifluoromethyl-4-(4-chlorophenyl)-4H-pyrimido[2,1-b][1,3] benzothiazole-3-carboxylate (4k)

Yellow Powder; M.P. 167-169^{0}C; IR (KBr): 3187, 2984, 1741, 1522, 1454, 1323, 1175, 1086, 1079, 838, 754 cm^{-1};

^{1}H NMR (300MHz, $CDCl_3$ + DMSO): δ 1.06 (t, J=6.61 Hz, 3H), 3.98-4.05 (q, 2H), 6.39 (s, 1H), 6.79-6.98 (m, 4H), 7.17-7.36 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$+DMSO): δ 167.80, 162.45, 138.51, 137.40, 135.76, 134.10, 132.45, 128.53, 127.84, 124.77, 121.89, 121.55, 121.18, 112.21, 109.76, 60.48, 55.87, 12.95; EI-MS: m/z 439(M+1); Anal. Calc. for ($C_{20}H_{14}ClF_3N_2O_2S$) C, 54.74; H, 3.22; N, 6.38; found; C, 54.78; H, 3.20; N, 6.39.

Ethyl-4-trifluoromethyl-2-(4-bromophenyl)-4H-pyrimido[2,1-b][1,3]benzothiazole-3- carboxylate (4l)

Pale yellowish Powder; M.P.168-170^{0}C; IR (KBr): 3186, 2982, 1740, 1520, 1456, 1324, 1176, 1085, 1078, 836, 756 cm^{-1}; ^{1}H NMR (300MHz, $CDCl_3$ + DMSO): δ 1.06 (t, J=7.18 Hz, 3H), 3.98-4.13 (q, 2H), 6.45 (s, 1H), 6.95-7.05 (m, 2H), 7.18-7.29 (m, 4H), 7.41 (d, J=8.31 Hz, 2H); ^{13}C NMR (75MHz, $CDCl_3$ + DMSO): δ 167.85, 160.67, 136.35, 134.69, 131.52, 130.82, 128.52, 127.59, 125.47, 124.80, 121.92, 121.60, 121.22, 112.23, 109.70, 60.53, 55.97, 12.97; EI-MS: 484 m/z (M+1); Anal. Calc. for ($C_{20}H_{14}BrF_3N_2O_2S$) C, 49.70; H, 2.92; N, 5.80 found; C, 49.71; H, 2.89; 5.79.

Ethyl-2-trifluoromethyl-4-(4-nitrophenyl)-4H-pyrimido[2,1-b][1,3] benzothiazole-3-carboxylate (4m)

Yellow Powder; M.P.124-126^{0}C; ^{1}H NMR (300MHz, $CDCl_3$ + DMSO) *δ*: 1.08 (t, *J*=6.98 Hz, 3H), 3.90-4.10 (q, 2H), 6.48 (s, 1H), 6.82-7.15 (m, 6H), 7.36 (d, *J*=7.74 Hz, 1H), 7.60-7.72 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$+DMSO) *δ*: 168.98, 164.07, 136.59, 137.54, 131.29, 131.27, 127.64, 127.56, 124.76, 121.89, 121.64, 121.17, 115.56, 112.35, 109.94, 60.46, 55.93, 13.00; IR (KBr) *ν*: 3200, 2982, 2937, 1735, 1579, 1514, 1471, 1340, 1280, 1236, 1085, 1018, 850, 751 cm^{-1}; EI-MS *m/z* 450 (M+1); Anal. Calc. for ($C_{20}H_{14}F_3N_3O_4S$) C, 53.45; H, 3.14; N, 9.35; found; C, 53.46; H, 3.10; N, 9.33.

Ethyl-2-trifluoromethyl-4-(4-trifluorophenyl)-4H-pyrimido[2,1-b][1,3]benzothiazole- 3-carboxylate (4n)

Yellow Powder; M.P. 169-1172^{0}C; ^{1}H NMR (300MHz, $CDCl_3$ + DMSO) *δ*: 1.08 (t, *J*=6.61 Hz, 3H), 4.00-4.08 (q, 2H), 6.39 (s, 1H), 6.79-6.98 (m, 4H), 7.20-7.36 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$+DMSO) *δ*: 167.80, 162.45, 138.51, 137.40, 135.76, 134.10, 132.45, 128.53, 127.84, 125.45, 124.77, 121.89, 121.55, 121.18, 112.21, 109.76, 60.48, 55.87, 12.95; IR (KBr) *ν*: 3180, 2984, 1741, 1522, 1404, 1323, 1240, 1086, 1079, 838, 754 cm^{-1}; EI-MS *m/z* 473 (M+1); Anal. Calc. for ($C_{21}H_{14}F_6N_2O_2S$) C, 53.39; H, 2.99; N, 5.93; found; C, 53.40; H, 2.96; N, 5.90.

Ethyl-2-trifluoromethyl-4-(4-cynophenyl)-4H-pyrimido[2,1-b][1,3]benzothiazole-3-carboxylate (4o)

Pale yellowish Powder; M.P.132-136^{0}C; ^{1}H NMR (300MHz, $CDCl_3$ + DMSO) *δ*: 1.10 (t, *J*=7.18 Hz, 3H), 3.96-4.12 (q, 2H), 6.45 (s, 1H), 6.95-7.05 (m, 2H), 7.24-7.38 (m, 4H), 7.46 (d, *J*=8.31 Hz, 2H); ^{13}C NMR (75MHz, $CDCl_3$ + DMSO) *δ*: 167.85, 160.67, 136.35, 134.69, 131.52, 130.82, 128.52, 127.59, 125.47, 124.80, 121.92, 121.60, 121.22, 120.40, 112.23, 109.70, 60.53, 55.97, 12.97; IR (KBr) *ν*: 3179, 2980, 1740, 1520, 1456, 1326, 1176, 1074, 1076, cm^{-1}; EI-MS *m/z* 430 (M+1); Anal. Calc. for ($C_{21}H_{14}F_3N_3O_2S$) C, 58.74; H, 3.29; N, 9.79 found; C, 58.71; H, 3.28; 9.76.

4.1. Experimental Procedure for Antimicrobial Activity

4.1.1. Disc Diffusion Method

The antimicrobial activity of newly synthesized compounds was evaluated according to the guidelines of National Committee for Clinical Laboratory Standards (NCCLS, 1997) using the agar disc diffusion method [30]. Briefly, a 24/48 h-old culture of selected Bacteria/fungi was mixed with sterile physiological saline (0.85%) and the turbidity was adjusted to the standard inoculum of Mac- Farland scale 0.5 [10^6 colony forming units (CFU) per milliliter]. Petri plates containing 20 mL of Mueller Hinton Agar (MHA, Hi- Media) were used for all the bacteria tested. Fungi were cultured in Sabouraud's dextrose agar (SDA)/potato dextrose agar (PDA) (Hi- Media) and were purified by single spore isolation technique [31]. The inoculum was spread on the surface of the solidified media and Whatman no. 1 filter paper discs (6 mm in diameter) impregnated with the test compound (20 µl/disc) were placed on the plates. Ciprofloxacin (5 µg/disc, Hi-Media) was used as positive control for bacteria. Fluconazole (10 µg/disc, Hi-Media), was used as positive control for fungi. A paper disc impregnated with dimethylsulfoxide (DMSO) was used as negative control. Plates inoculated with the bacteria were incubated for 24 h at 37 ^{0}C and the fungal culture was incubated for 72 h at 25^{0}C. The inhibition zone diameters were measured in millimeters. All the tests were performed in triplicate and the average was taken as final reading.

4.1.2. Determination of MIC

Minimum inhibitory concentration (MIC) of any compound is defined as the lowest concentration which completely inhibits visible growth (turbidity on liquid media). MIC values were determined by testing performed according to the guidelines of NCCLS document M27-A [30]. Solutions of the test compounds, ciprofloxacin and fluconazole were prepared in DMSO at a concentration of 100 µg/ml. From this stock solution, serial dilutions of the compounds (50, 25… 3.12 µg/ml) were prepared to determine the MIC. All determinations were done in triplicates and the average was taken as final reading. The standard antibiotic, ciprofloxacin (100 µg/ml) for bacteria and Fluconazole (100 µg/ml) for fungi were used as positive controls and 100 µl of DMSO used as a negative control. At the end of the incubation period, the MIC values were determined.

5. CONCLUSION

In conclusion, we have developed a novel and efficient three-components condensation reaction of an aldehyde, β-ketoester and 2-aminobenzothiazole producing 4*H*-pyrimido[2,1-*b*]benzothiazoles ring systems. The results of anti-bacterial screening reveal that among all the compounds screened, compounds **4d, 4j,4k,4l** showed good inhibition toward all the bacteria tested. The structure activity relationship studies (SAR) reveal that the compounds with CF_3 substitution (**4j,4k,4l**) are very much active. The presence of NO_2 substituted 4*H*-pyrimido[2,1-*b*]benzothiazole derivative **4d** also showed very good antibacterial activity against *S.aureus*. Since almost all the compounds are effective.

In case of Antifungal activities *C. albicans*, *A. fumigates*, and *C. tropicum* the compounds showing significant to excellent activity are **4j**,**4n** and **4k**. In the case of *P. mareneffei*, all the compounds posses moderate to good inhibition with reference to control. The structure activity relationship studied (SAR) revealed that 2-CF_3 group of 4*H*-pyrimido[2,1-*b*]benzothiazole **4g,4j,4k,4l,4n,4n** at 2-CF_3 group of 4*H*-pyrimido[2,1-*b*]benzothiazole very much effectiveness against these fungi. CF_3 substituted 4*H*-pyrimido [2, 1-*b*] benzothiazoles **4g**−**4o** (Table **1**) have shown better antibacterial and antifungal activity than corresponding non-fluoro derivatives. In general, the antifungal activity of compounds was better than their antibacterial activity.

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