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Synthesis And Biological Evaluation Of *N*-(Benzo[D]Thiazol-2-Yl)-6-Methoxy-5-(Phenylamino)Picolinamide Derivatives As Antimicrobial Agents

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ABSTRACT

*A new series of novel 2-methoxy-N-phenyl-6-(5-phenyl-1, 3, 4-oxadiazol-2-yl) pyridin-3-amine (**6a-i**) have been synthesized. All the newly synthesized compounds were screened for their in vitro antibacterial activity, against various Gram-positive and Gram-negative strains of bacteria and fungal strains. Amongst these compounds **6c** and **6e** were found to be the most potent against bacterial strains. Compounds **6h** and **6i**, with fluoro atoms on aniline ring exhibited selective inhibition against E. coli. Further, these compounds were exhibited mild to moderate antifungal activity in comparison to the standard drugs.*

Keywords: 2-Amino benzothiazoles, Antibacterial activity, Antifungal activity.

INTRODUCTION

The developing countries are mostly affected by health problems due to microbial infections. These infections are highly contagious and increasing with course of time round the world due to the emergence of new multidrug resistance microbial organisms. Thus it is becoming challenging to develop and deliver new antiinfective agents with high efficacy. Therefore, there is an overwhelming need to design and develop new antibacterials with better activity profile.

Molecules with benzothiazole moiety have been attractive targets since they often exhibit diverse and important biological properties [1-3]. Benzothiazoles are known for different biological properties like antimicrobial [4], anticancer [5, 6], antitubercular [7], anticonvulsant [8], antihelmintic [9], analgesic [10] and anti-inflammatory [11].

These results as well as our earlier efforts prompted us in this direction to design and synthesize a new series of *N*-(benzo[d]thiazol-2-yl)-6-methoxy-5-(phenylamino)picolinamide as antimicrobial agents. In the present work, it is intended to describe the synthesis and biological evaluation of benzothiazole derivatives (**6a-i**).

MATERIALS AND METHODS

All reagents and solvents were used as purchased without further purification. Crude products were purified by column chromatography on silica gel of 60–120 mesh. NMR spectra were recorded on a Varian 400 MHz & 500 MHz spectrometer for ^{1}H NMR. Melting points were determined on a Fisher–Johns melting point apparatus and are uncorrected. The chemical shifts were reported as ppm down field using TMS as an internal standard. A mass spectrum was recorded on a VG-Micro mass 7070H spectrometer operating at 70 eV.

Synthesis of 2-amino benzothiazole (2): To a mixture of aniline **1** (2.0 g, 0.021 mol), ammonium thiocyanate (6.5 g, 0.086 mol), bromine (2.2 mL, 0.43 mol) in DCM (20 mL), stirred for 16 h at room temperature. After completion of the reaction, water was added to the reaction mixture and the product was extracted in DCM (3x50 mL). The solvent was evaporated under vacuum to afford the crude product and purified by column chromatography using a gradient of hexane–EtOAc (3:7-4:6) to give compounds **2**. 2-Amino-benzothiazole (2) is Off-white solid; yield : 75%, mp: 126-128 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.98-7.00 (m, 1H, Ar-H), 7.18-7.20 (m, 1H, Ar-H), 7.32-7.34 (m, 1H, Ar-H), 7.40-7.42 (s, 2H, NH_2),8.02-8.21 (m, 1H, Ar-H); MS: m/z (%) 150.8 [M^+], UPLC purity (99.89%).

Synthesis of 5-Bromo-6-methoxy-pyridine-2-carboxylic acid benzothiazol-2-ylamide (4): To a solution of compound **2** (1.05 g, 0.007 mol) in DCM (28 mL), cooled to 0 ^{o}C, was added compound **3** (1.785 g, 0.0077 mol), HATU (3.99 g, 0.0105 mol), DIPEA (2.50 mL, 0.0140 mol) and the mixture was stirred for 16 h at ambient temperature. Water was added and extracted with ethyl acetate, dried and purified by column chromatography using a gradient of hexane–EtOAc (8:2–5:5) to give compound **4**. Off-white solid; yield: 66%, mp: 190-192 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.20 (s, 3H, OCH_3),7.37-7.39 (m, 1H, Ar-H), 7.42-7.44 (m, 1H, Ar-H), 7.80-7.84 (m, 3H, Ar-H), 8.06-8.08 (m, 1H, Ar-H),10.76-10.78 (s, 1H, NH); MS: m/z (%) 363.8 [M^+], UPLC purity (98.61%).

Synthesis of 6-Methoxy-5-phenylamino-pyridine-2-carboxylic acid benzothiazol-2-ylamide (6):
To a solution of **int-4** (50 mg,0.00015mol), aniline **5**(0.021ml,0.00018mol), $Pd_2(dba)_3$ (14mg, 0.000 015mol), Xantphos (17.4mg,0.000030mol), Cs_2CO_3 (98 mg, 0.00030 mol) in Dioxane(1.0 mL), and the mixture was stirred for 12 h at 120^{o}C, cooled to room temperature, water was added, extracted with EtOAc,dried with Na_2SO_4,filtered and concentrated under reduced pressure, purified by column chromatography (2-8:3-7) to give pure compound **6**.

6-Methoxy-5-phenylamino-pyridine-2-carboxylic acid benzothiazol-2-ylamide (**6a):** Off-white solid; yield : 70%, mp: 158-160 °C; ^{1}H NMR (500 MHz, DMSO- d_6) δ: 4.22 (s, 3H, OCH_3), 7.04 (s, 1H, Ar-H), 7.28-7.40 (m, 5H, Ar-H), 7.45(m, 2H, Ar-H), 7.76-7.81 (m, 2H, Ar-H),8.02 (d, 1H, Ar-H), 8.38 (s, 1H, NH), 11.82 (s, 1H, NH), ^{1}H NMR (500 MHz, D_2O exchange) δ: 4.22 (s, 3H, OCH_3), 7.04 (s, 1H, Ar-H), 7.28-7.40 (m, 5H, Ar-H), 7.45(m, 2H, Ar-H), 7.76-7.81 (m, 2H, Ar-H),8.02 (d, 1H, Ar-H),MS: m/z 376.9 [M^+], HPLC purity (97.02%), Anal. Calcd. for $C_{20}H_{16}N_4O_2S$: C, 63.81; H, 4.28; N, 14.88. Found C, 63.78; H, 4.26; N, 14.85.

6-Methoxy-5-o-tolylamino-pyridine-2-carboxylic acid benzothiazol-2-ylamide (6b): Off-white solid; yield : 68%, mp: 184-186 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 2.32(s,3H,CH3), 4.06 (s, 3H, OCH_3), 6.86 (m, 1H, Ar-H), 7.02-7.04 (m, 1H, Ar-H),7.12-7.14 (m, 3H, Ar-H),7.42(m, 2H, Ar-H), 7.80 (m, 2H, Ar-H),8.01 (d, 1H, Ar-H), 8.36 (s, 1H, NH), 11.82(s, 1H, NH), ^{1}H NMR (500 MHz, D_2O exchange) δ:2.32(s,3H,CH3), 4.06 (s, 3H, OCH_3), 6.86 (m, 1H, Ar-H), 7.02-7.04 (m, 1H, Ar-H),7.12-7.14 (m, 1H, Ar-H,7.42(m, 2H, Ar-H), 7.80 (m, 2H, Ar-H),8.01 (d, 1H, Ar-H), MS: m/z 390.9 [M^+], HPLC purity (98.42%),Anal. Calcd. for $C_{21}H_{18}N_4O_2S$: C, 64.60; H, 4.65; N, 14.35. Found C, 64.55; H, 4.64; N, 14.31.

6-Methoxy-5-p-tolylamino-pyridine-2-carboxylic acid benzothiazol-2-ylamide 6(c): Off-white solid; yield : 62%, mp: 145-147 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 2.32(s,3H,CH3), 4.06 (s, 3H, OCH_3), 7.00-7.04 (m, 2H, Ar-H),7.10-7.14 (m, 3H, Ar-H), 7.36(m, 2H, Ar-H), 7.76(m, 2H, Ar-H),8.00 (d, 1H, Ar-H), 8.32 (s, 1H, NH), 11.80(s, 1H, NH), ^{1}H NMR (500 MHz, D_2O exchange) δ: 2.32(s,3H,CH3), 4.06 (s, 3H, OCH_3), 6.84 (m, 1H, Ar-H), 7.00-7.04 (m, 2H, Ar-H),7.10-7.14 (m, 3H, Ar-H), 7.36(m, 2H, Ar-H), 7.76(m, 2H, Ar-H),8.00 (d, 1H, Ar-H),MS: m/z 390.9 [M^+], HPLC purity (97.92%), Anal. Calcd. For $C_{21}H_{18}N_4O_2S$: C, 64.60; H, 4.65; N, 14.35. Found C, 64.58; H, 4.63; N, 14.33.

6-Methoxy-5-(2-methoxy-phenylamino)-pyridine-2-carboxylic acid benzothiazol-2-ylamide 6(d): Off-white solid; yield : 65%, mp: 165-167 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 3.8(s,3H,OCH_3) 4.18 (s, 3H, OCH_3), 6.84 (m, 1H, Ar-H), 7.02 (m, 2H, Ar-H),7.14-7.18 (m, 2H, Ar-H), 7.42(m, 2H, Ar-H), 7.76(m, 2H, Ar-H),8.02 (d, 1H, Ar-H), 8.32 (s, 1H, NH), 11.84(s, 1H, NH), ^{1}H NMR (500 MHz, D_2O exchange) δ: 3.8(s,3H,OCH_3) 4.18 (s, 3H, OCH_3), 6.84 (m, 1H, Ar-H), 7.02 (m, 2H, Ar-H),7.14-7.18 (m, 2H, Ar-H), 7.42(m, 2H, Ar-H), 7.76(m, 2H, Ar-H),8.02 (d, 1H, Ar-H),, MS: m/z 406.9 [M^+], HPLC purity (97.12%), Anal. Calcd. For $C_{21}H_{18}N_4O_3S$: C, 62.05; H, 4.46; N, 13.78. Found C, 62.01; H, 4.45; N, 13.74.

6-Methoxy-5-(4-methoxy-phenylamino)-pyridine-2-carboxylic acid benzothiazol-2-ylamide 6(e): Off-white solid; yield : **6e,** 60%, mp: 158-160 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 3.8(s,3H,OCH_3) 4.18 (s, 3H, OCH_3), 7.00 (m, 2H, Ar-H),7.10-7.18 (m, 3H, Ar-H), 7.40 (m, 2H, Ar-H), 7.76(m, 2H, Ar-H),8.01 (d, 1H, Ar-H), 8.30 (s, 1H, NH), 11.82(s, 1H, NH), ^{1}H NMR (500 MHz, D_2O exchange) δ: 3.8(s,3H,OCH_3) 4.18 (s, 3H, OCH_3), 7.00 (m, 2H, Ar-H),7.10-7.18 (m, 3H, Ar-H), 7.40 (m, 2H, Ar-H), 7.76(m, 2H, Ar-H),8.01 (d, 1H, Ar-H),MS: m/z 406.9 [M^+], HPLC purity (98.92%), Anal. Calcd. For $C_{21}H_{18}N_4O_3S$: C, 62.05; H, 4.46; N, 13.78. Found C, 62.00; H, 4.47; N, 13.76.

6-Methoxy-5-(2-trifluoromethyl-phenylamino)-pyridine-2-carboxylic acid benzothiazol-2-ylamide (6f): Off-white solid; yield : 70%, mp: 179-181 °C; ^{1}H NMR (500 MHz, DMSO- d_6) δ: 4.18 (s, 3H, OCH_3), 6.82 (m, 1H, Ar-H), 6.92 (m, 1H, Ar-H),7.02 (m, 2H, Ar-H), 7.18 (m, 1H, Ar-H), 7.42(m, 2H, Ar-H), 7.76-7.80 (m, 2H, Ar-H),8.02 (d, 1H, Ar-H), 8.36 (s, 1H, NH), 11.82 (s, 1H, NH), ^{1}H NMR (500 MHz, D_2O exchange) δ: 4.08 (s, 3H, OCH_3), 6.82 (m, 1H, Ar-H), 6.92 (m, 1H, Ar-H),7.02 (m, 2H, Ar-H), 7.18 (m, 1H, Ar-H), 7.42(m, 2H, Ar-H), 7.76-7.80 (m, 2H, Ar-H),8.02 (d, 1H, Ar-H),MS: m/z 443.9 [M^+], HPLC purity (99.20%), Anal. Calcd. For $C_{21}H_{15}F_3N_4O_2S$: C, 56.75; H, 3.40; N, 12.61.Found: C, 56.71; H, 3.38; N, 12.60.

6-Methoxy-5-(4-trifluoromethyl-phenylamino)-pyridine-2-carboxylic acid benzothiazol-2-ylamide (6g): Off-white solid; yield : 70%, mp: 159-161 °C; ^{1}H NMR (500 MHz, DMSO- d_6) δ: 4.18 (s, 3H, OCH_3), 6.90 (m, 2H, Ar-H),7.02 (m, 2H, Ar-H), 7.12-7.14 (m, 1H, Ar-H), 7.42-7.44(m, 2H, Ar-H), 7.76-7.81 (m, 2H, Ar-H),8.02 (d, 1H, Ar-H), 8.38 (s, 1H, NH), 11.82 (s, 1H, NH), ^{1}H NMR (500 MHz, D_2O exchange) δ: 4.08 (s, 3H, OCH_3), 6.90 (m, 2H, Ar-H),7.02 (m, 2H, Ar-H), 7.12-7.14 (m, 1H, Ar-H), 7.42-7.44(m, 2H, Ar-H), 7.76-7.81 (m, 2H, Ar-H),8.02 (d, 1H, Ar-H),MS: m/z 443.9 [M^+], HPLC purity (97.42%). Anal. Calcd. for $C_{21}H_{15}F_3N_4O_2S$: C, 56.75; H, 3.40; N, 12.61.Found: C, 56.70; H, 3.41; N, 12.63.

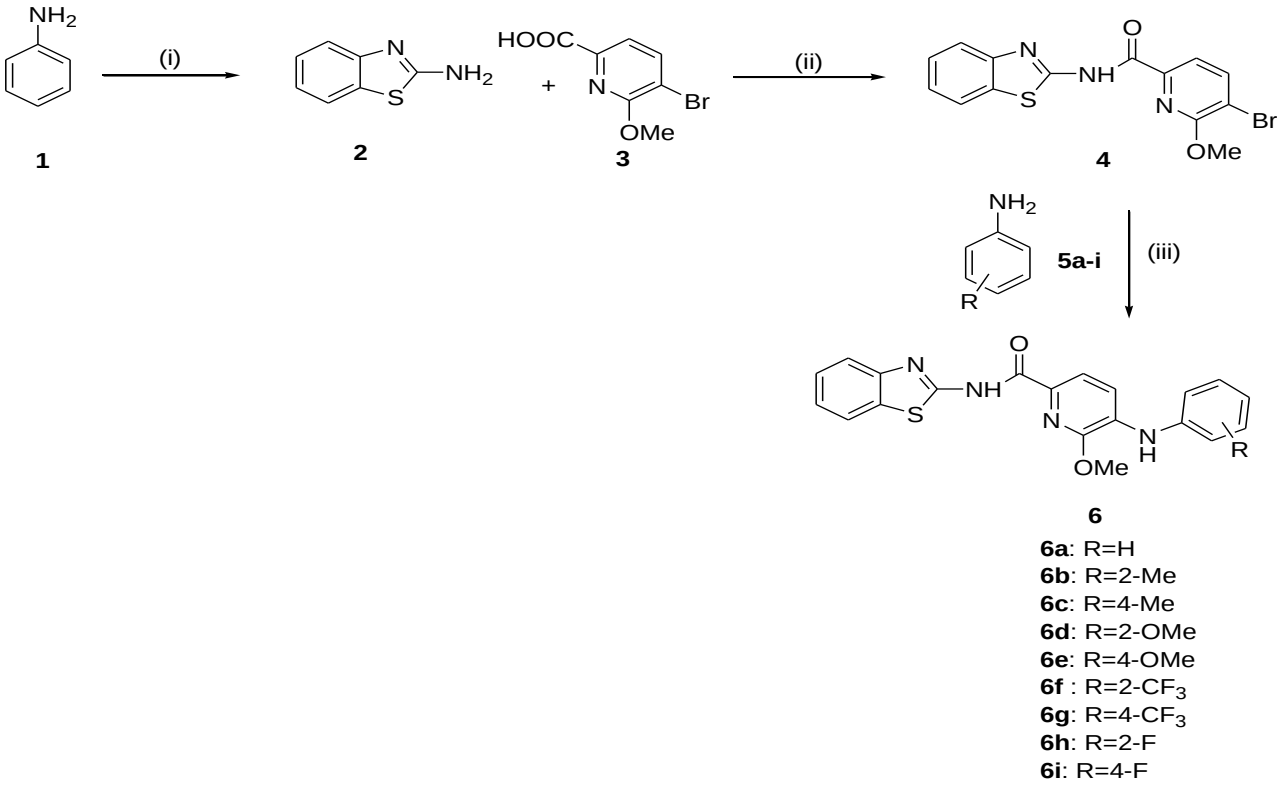
5-(2-Fluoro-phenylamino)-6-methoxy-pyridine-2-carboxylic acid benzothiazol-2-ylamide (6h): Off-white solid; yield : 70%, mp: 174-176 °C; ^{1}H NMR (500 MHz, DMSO- d_6) δ: 4.18 (s, 3H, OCH_3), 6.86 (m, 1H, Ar-H), 6.98 (m, 1H, Ar-H),7.02-7.04 (m, 3H, Ar-H), 7.48(m, 2H, Ar-H), 7.76-7.80 (m, 2H, Ar-H),8.02 (d, 1H, Ar-H), 8.34 (s, 1H, NH), 11.80 (s, 1H, NH), ^{1}H NMR (500 MHz, D_2O exchange) δ: 4.08 (s, 3H, OCH_3), 6.86 (m, 1H, Ar-H), 6.98 (m, 1H, Ar-H),7.02-7.04 (m, 3H, Ar-H), 7.48(m, 2H, Ar-H), 7.76-7.80 (m, 2H, Ar-H),8.02 (d, 1H, Ar-H),MS: m/z 394.9 [M^+], HPLC purity (97.02%). Anal. Calcd. for $C_{20}H_{15}FN_4O_2S$: C, 60.90; H, 3.83; N, 14.20. Found: C, 60.86; H, 3.81; N, 14.18.

5-(4-Fluoro-phenylamino)-6-methoxy-pyridine-2-carboxylic acid benzothiazol-2-ylamide (**6i):** Off-white solid; yield : 70%, mp: 171-173 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 4.18 (s, 3H, OCH_3), 6.96 (m, 2H, Ar-H),7.02-7.04 (m, 3H, Ar-H), 7.46(m, 2H, Ar-H), 7.74-7.76 (m, 2H, Ar-H),8.02 (d, 1H, Ar-H), 8.32(s, 1H, NH), 11.80 (s, 1H, NH), ^{1}H NMR (500 MHz, D_2O exchange) δ: 4.18 (s, 3H, OCH_3), 6.96 (m, 2H, Ar-H),7.02-7.04 (m, 3H, Ar-H), 7.46(m, 2H, Ar-H), 7.74-7.76 (m, 2H, Ar-H),8.02 (d, 1H, Ar-H), 8.32(s, 1H, NH), 11.80 (s, 1H, NH), MS: m/z 394.9 [M^+], HPLC purity (96.12%). Anal. Calcd. for $C_{20}H_{15}FN_4O_2S$: C, 60.90; H, 3.83; N, 14.20. Found: C, 60.84; H, 3.80; N, 14.15.

RESULTS AND DISCUSSION

The synthesis of 2-amino benzothiazoles (**2**) was carried out by the reaction of ammonium thiocyanate and bromine on aniline (**1**) to give 2-aminobenzothiazoles (**2**) as reported in the literature (Scheme 1) [12]. Then coupling of **2** with picolinic acid **3** using HATU and DIPEA to furnished **4.** Finally, compound **4** was treated with various anilines (**5a-i**) under Buchwald coupling furnished the final targets **6a-i.**

Scheme 1



1 **2** **3** **4**

6

6a: R=H
6b: R=2-Me
6c: R=4-Me
6d: R=2-OMe
6e: R=4-OMe
6f : R=2-CF_3
6g: R=4-CF_3
6h: R=2-F
6i: R=4-F

Reactions and condition: (i) Ammoniumthiocyanate, Br_2, DCM, RT, 16h; (ii) HATU, DIPEA, RT, 16h; (iii) $Pd_2(dba)_3$, Xantphos, Cs_2CO_3, Dioxane, 16h, 120 ^{o}C. R = (i) H, (ii) 2-Me, (iii) 4-Me,(iv) 2-OMe, (v) 4-OMe,(vi)2-CF_3,(vii)4-CF_3, (viii)2-F, (ix) 4-F.

Scheme 1

Antibacterial Activity: For evaluating the antibacterial activity ampicillin was used as the standard drug. All the synthesized compounds **6a-i** were assayed for their antibacterial activity against three Gram-positive bacteria *Bacillus subtilis* MTCC 2415, *Staphylococcus aureu*s MTCC 9886 and *Micrococcus luteus* MTCC 1538 and Gram-negative bacteria *Escherichia coli* MTCC 448. The minimum inhibitory concentrations (MIC) of the synthesized compounds were determined using broth dilution method

according to the protocols of National Committee for Clinical Laboratory Standards (NCCLS) [13]. The observed minimum inhibitory concentrations (MICs) are given in table 1. In all determinations, tests were performed in duplicate and results were reported as mean of at least three determinations. In general, all the compounds exerted a modest to good antibacterial activity in vitro against the tested organisms. The data from Table 1 shows that all the compounds possessing methoxy groups on the *ortho* and *para* positions on aniline rings in compounds **6d** and **6e** have shown more potent inhibition against most of the compounds in comparison with the standard. Compound **6a** which lacks the substituents on the aniline ring showed poor inhibitory activity. Compound **6c** having 4-methyl group on aniline ring was active against most of the organisms tested and exhibited MIC value of 100 µg mL^{-1} against only one organism *M. luteus*. Compounds **6h** and **6i** possessing fluoro groups on the second and fourth positions of the aniline ring showed highest activity against *E. coli* with MIC value of 50 µg mL^{-1}, whereas these compounds displayed moderate activity against other organisms.

Table 1 Antibacterial activity (MIC in µg/mL) of **6a-i**

Compd	Antibacterial strains			
	M. luteus[a]	*B. subtilis*[a]	*S. aureus*[a]	*E. coli*[b]
6a	-[c]	100	200	200
6b	200	50	-[c]	100
6c	100	-[c]	-[c]	-[c]
6d	50	50	100	50
6e	25	50	100	100
6f	-[c]	200	50	100
6g	100	-[c]	200	50
6h	100	200	100	25
6i	50	50	-[c]	25
Ampicillin	25	12.5	50	50

[a]Gram-positive bacteria: *Bacillus subtilis* MTCC 2415, *Staphylococcus aureus* MTCC 9886 and *Micrococcus luteus* MTCC 1538; [b]Gram negative bacteria: *Escherichia coli* MTCC 448. [c] Not active.

Antifungal Activity: For evaluating the antifungal activity amphotericin-B was used as the standard drug. The investigation of antifungal screening data from Table 2 revealed that all tested compounds showed mild to moderate fungal inhibition. The compound (**6c** and **6d**) possessing methoxy and methyl groups in *para* position of the aniline derivatives exhibited better inhibitory activity against all the four fungal strains than the corresponding other derivatives, whereas, compound **6b** and **6d** with the same groups at *orhto* positions were mild active only against *A. fumigatus* and *T. mentagropytes.* Further compound **6a** without any substituents on the aniline ring was found to be inactive against all the fungal strains tested.

Table 2 Antifungal activity (Zone of inhibition in mm at 100 µg/mL) of **6a-i**

Compd	*C. albicans*	*T. rubrum*	*A. fumigatus*	*T. mentagropytes*
6a	2	-	-	-
6b	-	3	2	3
6c	4	10	10	12
6d	3	5	2	3
6e	15	10	6	4
6f	-		2	-
6g	5	3	-	5
6h	4	-	5	4
6i	5	4	2	-
Amphotericin-B	5	8	6	10

-means inactive at the concentration used.

APPLICATIONS

In the present study the derivatives which we have synthesized were screened for their antibacterial activity, which are promising as active pharmacophore. Further studies are undergoing to explore the scope of the various biological activities.

CONCLUSIONS

In conclusion, we have described a simple and efficient protocol for the synthesis of novel *N*-(benzo[d]thiazol-2-yl)-6-methoxy-5-(phenylamino)picolinamide derivatives (**6a-i**) with good yields. All the compounds were evaluated for their antibacterial and antifungal activities. It is evident that newly synthesized compounds have displayed excellent antibacterial and antifungal activities. Compounds **6c** and **6e** were found to be more active against bacterial strains and compounds **6b** and **6d** exhibited better inhibitory activity against fungal strains. Compounds **6h** and **6i** possessing fluoro groups on the aniline ring found to be selective inhibitory agents against *E. coli*. This novel class of new benzothiazole derivatives reported to have a probability to emerge as a valuable lead series with great potential to be used as antibacterial and antifungal agents, and as promising candidates for further efficacy evaluation.

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