

SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF NATURAL PRODUCT ANALOGUE OF THIAZOLES

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ABSTRACT

A series of 5-bromo-pyridyl containing substituted 2-amino-thiazole derivatives were synthesized by the cyclization of 2-bromo-1-(5-bromopyridin-3-yl)ethanone with different thiourea moieties and they were characterized by means of ¹H-NMR, ¹³C-NMR, LCMS techniques. All the newly synthesized compounds were screened for anti-bacterial activity against *Staphylococcus aureus* (ATCC-9144), *Staphylococcus epidermidis* (ATCC-155), *Bacillus cereus* (ATCC-10987), *Salmonella typhi* (ATCC-700931), *Pseudomonas aeruginosa* (ATCC-2853), *Escherichia coli* (ATCC-25922) and antifungal activity against *Aspergillus niger* (ATCC-9029), *Aspergillus fumigatus* (ATCC-46645) by paper disc diffusion technique. The test compounds showed significant level of activity against the bacterial and fungal organisms tested and vast scope is there to explore these new compounds as drugs.

Key words: Thiazole, Pyridyl, Antibacterial, Antifungal.

INTRODUCTION

The discovery, development and clinical use of antibiotics thought to eliminate the scourge of all infectious diseases. However, due to the widespread use and misuse of antibiotics, bacterial resistance to antibiotics has become a serious public health problem. Some of these resistant strains, such as vancomycin-resistant enterococci (VRE) and multidrug resistant *Staphylococcus aureus* (MRSA), are capable of surviving the effects of most, if not all, antibiotics currently in use.¹⁻⁴ With the increase in resistance of bacteria to antibiotic treatment, attention has focused on developing novel approaches to antimicrobial therapy.⁵⁻⁸ The search of new antimicrobial agents with reduced toxicity and lower side effects is of continuous process. One of the most frequently encountered heterocycles in medicinal chemistry is thiazole and its derivatives were reported to possess diverse biological applications such as anti-tuberculous, antibiotic, antibacterial, antifungal, and anti-inflammatory.⁹⁻¹² Moreover, it has been found in the drug development program for the treatment of microbial and cancer agents.¹³⁻¹⁵ The literature survey revealed that the alkaloids camalexin and methoxycamalexin appear to be the first reported naturally occurring antifungal compounds contain a 2-substituted thiazole.¹⁶ These observations led to the conception that a series of some aryl thiazoles (7a-o) were synthesized. These compounds were screened for their anti-bacterial activity against *Staphylococcus aureus* (ATCC-9144), *Staphylococcus epidermidis* (ATCC-155), *Bacillus cereus* (ATCC-10987), *Salmonella typhi* (ATCC-700931), *Pseudomonas aeruginosa* (ATCC-2853), *Escherichia coli* (ATCC-25922) and antifungal activity against *Aspergillus niger* (ATCC-9029), *Aspergillus fumigatus* (ATCC-46645) by paper disc diffusion technique.

EXPERIMENTAL

Chemistry

Melting points were taken in Buchi -545 apparatus. Nuclear magnetic resonance (NMR) spectrum was recorded in DMSO-d₆ on a Bruker-DPX-400MHz spectrophotometer using TMS as internal standard. Liquid Chromatography-Mass Spectrum (LC-MS) were recorded on Agilent 1200(Liquid Chromatography). Thin Layer Chromatography was performed using Aluminum plates precoated with Silica gel 60F 254 [E-Merk], spots were visualized in the ultraviolet light chamber, iodine chamber, KMnO₄ solution and 2,2'-di nitro phenyl hydrazine solution. Column chromatography was performed by using silica gel (230-400 mesh, Merk) with the indicated solvent system.

General procedure for the Synthesis of 7a-o

The Round bottom flask containing the mixture of 2-bromo-1-(5-bromopyridin-3-yl)ethanone (1g, 3.59 mmol) and the N-substitued thiourea(6a-e) (3.59 mmol) in 15 ml of Ethanol was refluxed for 2 h. The completion of the reaction was checked by TLC. Then cooled to 0°C and added saturated NaHCO₃ solution and extracted with ethyl acetate and the organic phase washed with water and brine solution. Then organic phase was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. Then the resulting residue was purified by column chromatography (60-120 mesh silica gel, ethylacetate: hexane). Scheme -1 represents the synthesis of 2-amino-thiazole derivatives.

The following compounds (7a-o) were synthesized by this procedure and their characterization data as shown in Table-1

7a. N-(3-bromobenzyl)-4-(5-bromopyridin-3-yl)thiazol-2-amine

¹H NMR (DMSO-d₆): δ= 4.54 (s, 2H), 7.30-7.34 (t, 1H), 7.38-7.42 (t, 1H), 7.46-7.47 (m, 2H), 7.62(s, 1 H), 8.41-8.42 (t, 1H), 8.617-8.622 (d, 1H, J= 2 Hz), 9.027-9.031 (d, 1H, J= 1.6 Hz). ¹³C (DMSO-d₆): δ = 48.17, 103.04, 112.20, 126.78, 127.03, 127.20, 128.91, 130.70, 130.89, 131.13, 139.44, 141.14, 169.23. Mass Spectrum m/z: 426.0 (M⁺+1).

7b. N-(3-bromophenyl)-4-(5-bromopyridin-3-yl)thiazol-2-amine

¹H NMR (DMSO-d₆): δ= 7.15-7.17 (t, 1H), 7.30-7.34 (t, 1H), 7.61-7.63 (m, 2H), 7.78 (s, 1 H), 8.11-8.12 (t, 1H), 8.537-8.547 (t, 1H), 8.684-8.690 (d, 1H, J=2.4 Hz), 9.132-9.136 (d, 1H, J=1.6 Hz), 10.65 (s, 1H). ¹³C (DMSO-d₆): δ = 104.44, 116.11, 119.59, 122.37, 124.08, 126.67, 126.89, 127.19, 127.87, 131.38, 134.18, 138.89, 147.10, 163.08. Mass Spectrum m/z: 409.9 (M⁺+1).

7c. 4-(5-bromopyridin-3-yl)-N-(3-chlorophenyl)thiazol-2-amine

¹H NMR (DMSO-d₆): δ= 7.02-7.05 (m, 1H), 7.36-7.41 (t, 1H), 7.57-7.59 (m, 1H), 7.78 (s, 1 H), 7.95-7.96 (t, 1H), 8.53-8.54 (t, 1H), 8.680-8.686 (d, 1H, J=2.4 Hz), 9.132-9.136 (d, 1H, J=1.6 Hz), 10.66 (s, 1H). ¹³C (DMSO-d₆): δ = 104.46, 115.77, 116.71, 121.19, 126.67, 127.19, 131.07, 133.84, 133.95, 135.12, 139.08, 142.99, 150.10, 136.13.

Mass Spectrum m/z: 366.0 (M⁺+1).

7d. 4-(5-bromopyridin-3-yl)-N-(3-(trifluoromethyl)phenyl)thiazol-2-amine

¹H NMR (DMSO-d₆): δ= 7.31-7.33 (d, 1H, J=8 Hz), 7.57-7.61 (t, 1H), 7.81-7.83 (d, 2H, J= 6.8 Hz), 8.41(s, 1 H), 8.53 (s, 1H), 8.67-8.68 (d, 1H, J=2 Hz), 9.142-9.145 (d, 1H, J=1.2 Hz), 10.83 (s, 1H). ¹³C (DMSO-d₆): δ = 104.59, 113.23, 117.76, 120.75, 123.42, 126.13, 126.62, 127.62, 127.29, 130.09, 130.40, 133.96, 139.12, 142.12, 150.19, 163.10. Mass Spectrum m/z: 400.0 (M⁺-1).

7e. 4-(5-bromopyridin-3-yl)-N-(3-fluorophenyl)thiazol-2-amine

¹H NMR (DMSO-d₆): δ= 6.78-6.83 (m, 1H), 7.35-7.42 (m, 2H), 7.74-7.77 (t, 2H), 8.52-8.53 (t, 1 H), 8.67-8.68 (d, 1H, d, 1H, J=2.4 Hz), 9.134-9.139 (d, 1H, J=2 Hz), 10.67 (s, 1H). ¹³C (DMSO-d₆): δ = 104.03, 104.30, 107.85, 108.32, 113.40, 121.08, 131.15, 132.43, 135.99, 145.38, 145.83, 148.82, 161.85, 163.80. Mass Spectrum m/z: 350.0 (M⁺+1).

7f. N-(4-(5-bromopyridin-3-yl)thiazol-2-yl)acetamide

¹H NMR (DMSO-d₆): δ= 2.18 (s, 3H), 7.94(s, 1H), 8.49-8.50 (m, 1H), 8.66-8.67 (d, 1H, J=2.4 Hz), 9.106-9.107 (d, 1H, J=2 Hz), 12.33 (s, 1H). (M⁺+1). ¹³C (DMSO-d₆): δ = 22.95, 111.94, 121.14, 132.47, 136.43, 138.02, 144.37, 144.99, 148.63, 159.14, 169.32

Mass Spectrum m/z: 298.0 (M⁺+1).

7g. 4-(5-bromopyridin-3-yl)-N-propylthiazol-2-amine

¹H NMR (DMSO-d₆): δ= 0.92-0.96 (m, 6H), 1.24-1.28 (t, 2 H), 1.52-1.64 (m, 4H), 3.17-3.12 (m, 2H), 3.23-3.30 (m, 4H), 7.43 (s, 1H), 8.45 (s, 1H), 8.66-8.68 (m, 1H), 9.02(s, 1 H), 9.08-9.16 (br, s.1H). 9.54 (br, s, 1H). Mass Spectrum m/z: 298.0 (M⁺+1).

7h. 4-(5-bromopyridin-3-yl)-N-methylthiazol-2-amine

¹H NMR (DMSO-d₆): δ= 2.17(s, 1H), 7.94 (s, 1H), 8.50 (s, 1H), 8.66-8.68 (m, 1H), 9.094-9.098 (d, 1H, J=1.6 Hz), 12.32 (s, 1H). Mass Spectrum m/z: 298.0 (M⁺+1).

7i. methyl 3-(4-(5-bromopyridin-3-yl)thiazol-2-ylamino)benzoate

¹H NMR (DMSO-d₆): δ= 3.91 (s, 3H), 7.49-7.53 (m, 1H), 7.58-7.60(m, 1H), 7.77 (s, 1H), 7.81-7.84(m, 1H) 8.55(s, 1H), 8.67-8.70(m, 2H), 9.18 (s, 1H), 10.67 (s, 1H). ¹³C (DMSO-d₆): δ = 52.75, 107.65, 117.78, 121.11, 121.84, 122.49, 129.94, 130.85, 132.42, 135.88, 141.66, 145.47, 145.74, 148.86, 163.79, 166.70. Mass Spectrum m/z: 390.0 (M⁺+1).

7j. 4-(5-bromopyridin-3-yl)-N-p-tolylthiazol-2-amine

¹H NMR (DMSO-d₆): δ = 2.27 (s, 3H), 7.16-7.18 (d, 2H, J= 8.4 Hz), 7.56-7.58 (d, 2H, J=8.4 Hz), 7.66 (s, 1H), 8.50 (s, 1H) 8.65(s, 1H), 9.12 (s, 1H), 10.29 (s, 1H). ¹³C (DMSO-d₆): δ = 20.85, 107.03, 117.77, 188.11, 121.14, 129.94, 131.01, 132.73, 136.70, 137.90, 138.95, 144.99, 145.59, 148.25, 164.54. Mass Spectrum m/z: 346.0 (M⁺+1).

7k. 4-(5-bromopyridin-3-yl)-N-(3, 5-dichlorophenyl)thiazol-2-amine

¹H NMR (DMSO-d₆): δ = 7.18(s, 1H), 7.80-7.83 (m, 3H), 8.50-8.51 (d, 1H J = 2 Hz) 8.67-8.68 (d, 1H, J= 2 Hz), 9.11 (s, 1H), 10.85 (s, 1H). ¹³C (DMSO-d₆): δ = 108.63, 115.49, 115.78, 120.80, 121.13, 132.30, 134.74, 136.18, 143.35, 145.10, 145.69, 148.78, 163.24.

Mass Spectrum m/z: 401.7 (M⁺+1).

7l. 4-(5-bromopyridin-3-yl)-N-isopropylthiazol-2-amine

¹H NMR (DMSO-d₆): δ= 1.15-1.18 (m, 6H), 1.22-1.26 (m, 6 H), 3.93-4.04 (m, 2H), 7.50 (s, 1H), 8.55 (s, 1H), 8.75 (s, 1H), 9.01-9.02 (d, 1H, J= 2 Hz), 9.15 (s, 1H).

Mass Spectrum m/z: 298.0 (M⁺+1).

7m. 4-(5-bromopyridin-3-yl)thiazol-2-amine

¹H NMR (DMSO-d₆): δ= 7.43 (s, 1H), 8.41-8.42 (t, 1H J= 2 Hz), 8.66 (s, 1H), 8.98-8.99 (d, 1H, J= 1.6 Hz). Mass Spectrum m/z: 256.0 (M⁺+1).

7n. N-(4-(5-bromopyridin-3-yl)thiazol-2-yl)pyridin-4-amine

¹H NMR (DMSO-d₆): δ= 8.12 (s, 1H), 8.16(br s, 1H), 8.24-8.26 (d, 2H, J=7.2 Hz), 8.61-8.65 (m, 3H), 9.01(br s, 1H), 11.17 (s, 1H). ¹³C (DMSO-d₆): δ = 111.89, 114.63, 121.11, 131.61, 135.59, 142.06, 142.31, 146.18, 149.95, 153.21, 154.52, 161.47, 181.74.

Mass Spectrum m/z: 333.0 (M⁺+1).

7o. 3-(4-(5-bromopyridin-3-yl)thiazol-2-ylamino)benzoic acid

¹H NMR (DMSO-d₆): δ= 7.46-7.50 (m, 1H), 7.55-7.57 (d, 1H J=7.6 Hz), 7.77 (s, 1H), 7.87-7.90 (m, 1H) 8.53 (s, 1H), 8.57-8.58 (m, 1H), 8.68 (s, 1H), 9.17 (s, 1H), 10.65 (s, 1H). Mass Spectrum m/z: 376.0 (M⁺+1).

Biological activity

Antibacterial and antifungal activity was studied using paper disc diffusion technique. The sterilized (autoclaved at 120 °C for 30min.) medium (40-50 °C) was inoculated (1 mL/100 mL of medium) with the suspension (10⁵ cfu mL⁻¹) of the microorganism (matched to McFarland barium sulphate standard) and poured into a petridish to give a depth of 3-4 mm. The paper impregnated with the test compounds (µg mL⁻¹ in N,N-dimethyl formamide) was placed on the solidified medium. The plates were pre-incubated for 1 h at room temperature and incubated at 37 °C for 24 and 48 h for anti-bacterial and anti-fungal activities, respectively. Ciprofloxacin (50 µg/disc) and Ketoconazole (50 µg/disc) were used as standard for anti-bacterial and anti-fungal activity, respectively.

RESULTS AND DISCUSSION

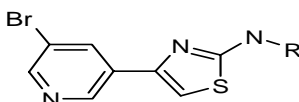
5-bromo-pyridyl containing substituted 2-amino-thiazole derivatives was synthesized using the procedure given above. Complete structural analysis was done using NMR and mass spectrum, which confirms the

absence of any other impurity compounds. These synthesized novel compounds were tested for the antibacterial and antifungal activity studies.

Antibacterial activity

The investigation of antibacterial screening (Table-2) revealed that all the newly synthesized compounds showed moderate to good inhibition at 25-100 µg/ml in DMSO. Compounds 7a, 7d, 7e, 7j, 7k, 7m and 7n exhibited comparatively good activity against the six tested bacterial strains. Compounds 7d, 7f, 7m, 7n showed good activity against gram negative *Escherichia coli* and *Pseudomonas aeruginosa*. Compounds 7d, 7e, 7j, 7m, and 7n showed good activity against gram positive *Staphylococcus aureus* and *Bacillus cereus*. Compounds 7c, 7d, 7f, 7j, 7l, 7m, and 7n showed good activity against *Salmonella typhi*.

Table-1: Characterization data of compounds 7a-o



Entry No	R	Mol. formula	M.wt	M.p(°C)	Yield (%)
7a	3Br-benzyl	C ₁₅ H ₁₁ Br ₂ N ₃ S	425.15	284.9	98.2
7b	3Br-phenyl	C ₁₄ H ₉ Br ₂ N ₃ S	411.12	295.7	90.3
7c	3Cl-phenyl	C ₁₄ H ₉ BrClN ₃ S	366.67	293.9	95.5
7d	3CF ₃ -phenyl	C ₁₅ H ₉ BrF ₃ N ₃ S	400.22	287.6	99.0
7e	3F-Phenyl	C ₁₄ H ₉ BrFN ₃ S	350.21	285.9	97.6
7f	Acetyl	C ₁₀ H ₈ BrN ₃ OS	298.16	293.9	97.2
7g	Propyl	C ₁₁ H ₁₂ BrN ₃ S	298.21	291.6	96.0
7h	Methyl	C ₉ H ₈ BrN ₃ S	270.15	269.5	92.8
7i	3-Me Benzoate	C ₁₆ H ₁₂ BrN ₃ O ₂ S	390.26	243.7	91.1
7j	4-Me-Phenyl	C ₁₅ H ₁₂ BrN ₃ S	346.25	302.7	90.5
7k	3,5-Di-Cl-Phenyl	C ₁₄ H ₈ BrCl ₂ N ₃ S	401.11	304.4	90.0
7l	IsoPropyl	C ₁₁ H ₁₂ BrN ₃ S	298.21	263.7	90.8
7m	H	C ₈ H ₆ BrN ₃ S	256.13	290.6	91.0
7n	4-Pyridyl	C ₁₃ H ₉ BrN ₄ S	333.21	301.6	90.2
7o	3-carboxylic acid	C ₁₅ H ₁₀ BrN ₃ O ₂ S	376.23	273.5	90.5

Antifungal activity

The investigation of antifungal screening (Table-2) revealed that all the newly synthesized compounds showed moderate to good inhibition at 25-100 mcg/ml in DMSO. Compounds 7d, 7e, 7m, and 7n exhibited good activity against all the two fungal strains. Compounds 7c, 7d, 7m and 7n showed good activity against *Aspergillus niger*. Compounds 7a, 7d, 7e, 7h, 7j, 7k, 7m, 7n and 7o showed good activity against *Aspergillus fumigatus*. The anti-fungal activities of the compounds were tested against two fungi namely *A. niger* and *A. fumigatus* using sabouraud dextrose agar medium (Hi-Media Laboratories, India).

CONCLUSION

Synthesized several thiazole derivatives containing 5-Bromo-pyridyl moiety and studied their antibacterial and antifungal properties. In particular unsubstituted (7m) compound showing potent antimicrobial activity, the compounds which are substituted with fluoro-phenyl(7d), carbon trifluoro-phenyl (7e), and pyridyl phenyl(7n) are also showing potent antimicrobial activity. The chlorophenyl (7c) is showing good antimicrobial activity than the fluoro substituted compound because it is less lipophilic than the fluoro substituted compound. The substitution of bromophenyl (7b) bromobenzyl(7a), acetyl(7f), methyl benzoate(7i), dichlorophenyl(7k) and carboxylic acid(7o) are showing the good antimicrobial activity. But substitution containing hydrophilic groups like propyl(7g), methyl(7h), methylphenyl(7j) and isopropyl(7l) are showing less antimicrobial activity than other compounds.

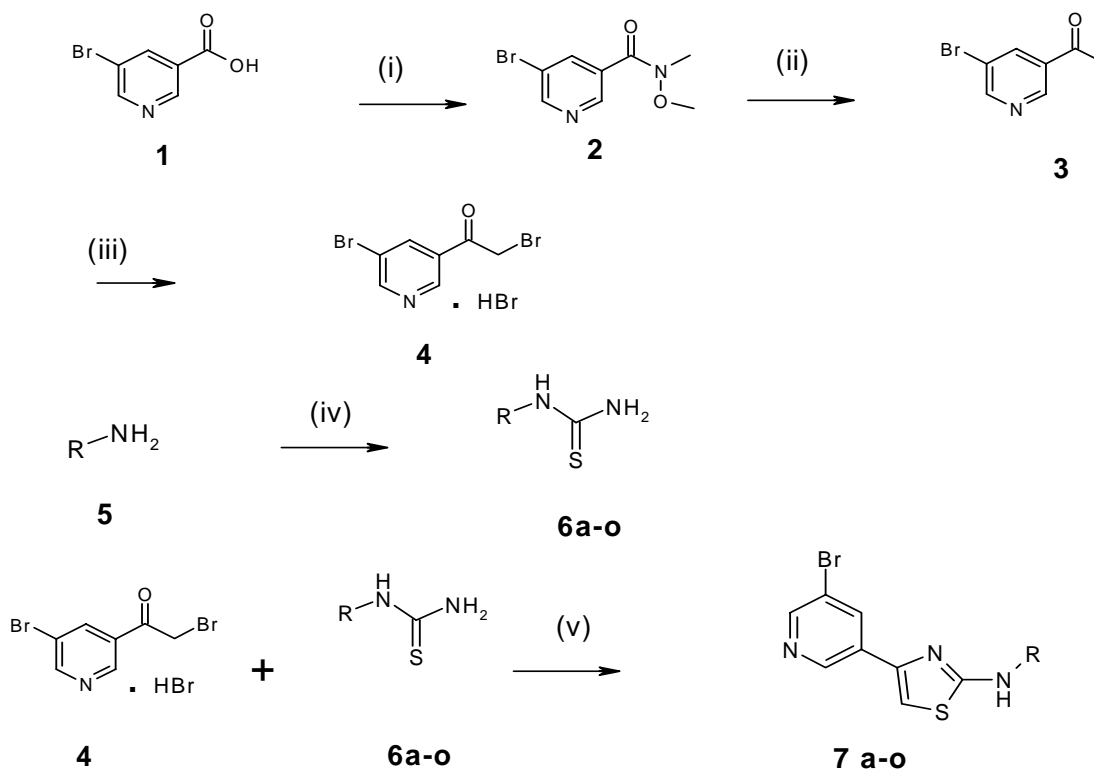
Table-2: Anti-microbial activities of compounds (Zone of inhibition (in mm))

Compd.	<i>S.aureus</i>	<i>S.epidermi dis</i>	Bacillus cereus	<i>Salmonella typhi</i>	<i>P.aeruginosa</i>	<i>E.coli</i>	<i>A.niger</i>	<i>A.fumicatus</i>
7a	16	14	14	10	11	12	13	15
7b	08	06	10	12	08	11	08	12
7c	10	14	12	16	14	14	16	10
7d	18	16	18	22	20	19	18	17
7e	20	17	16	14	18	10	15	18
7f	14	15	12	15	16	14	14	10
7g	12	10	11	10	08	08	08	07
7h	11	12	13	14	14	12	10	16
7i	08	05	08	07	12	10	11	10
7j	17	19	21	19	14	17	12	18
7k	15	17	14	10	16	14	13	15
7l	14	15	12	15	08	14	14	09
7m	18	19	21	19	18	17	16	18
7n	16	10	18	17	19	16	15	21
7o	14	16	16	14	18	12	14	18
Ciprofloxacin (100µg/disc)	29	28	31	33	29	31	-	-
Ketoconazole (100µg/disc)	-	-	-	-	-	-	26	24

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Scheme-1: (i) HOBT, EDC.HCl, DIPEA, N-methoxymethanamine.hydrochloride, THF 5 h. (ii) CH₃MgI, Dry THF 0°C to RT, 30-60 min. (iii) 40% HBr in AcOH, Br₂, 0 °C, 40 °C (2 h), 80 °C (2 h). (iv) DIPEA, Thiophosgene, THF, NH₃ (g). (v) Ethanol, Reflux, 2 h.

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