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BIOLOGICAL EVALUATION OF SOME SYNTHESIZED BENZOTHIAZOLE DERIVATIVES AND THEIR CHARACTERIZATION

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ABSTRACT

Substituted 1, 3-benzothiazole derivatives are an important class of heterocyclic compounds. The benzothiazole nucleus containing compounds in this work is been treated with hydrazine hydrate to form hydrazone derivative then it is treated with acetophenone further cyclisation is done by Vilsmeier-Haack reaction using DMF/$POCl_3$ finally the intermediate is condensed by using substituted thiourea to form the desired products. All the synthesized compounds was confirmed by spectral analysis such as FT-IR, NMR Mass Spectrophotometer for structure confirmation and synthesized compounds were screened for various *in-vitro* biological activities such as anti-oxidant, anti-inflammatory, anti-microbial activity It was found that the synthesized compounds showed enhancing biological activity.

KEYWORDS: Benzothiazole, Vilsmeier-Haack reaction, Anti-Oxidant, Anti-Inflammatory Anti-microbial etc.

INTRODUCTION

Benzothiazole is a heterocyclic compound, weak base, having varied biological activities and still of great scientific interest now a days. They are widely found in bio-organic and medicinal chemistry with application in drug discovery. Benzothiazole moieties are part of compounds showing numerous biological activities such as antimicrobial [1] anticancer [2],

anthelmintic,[3] anti-diabetic[4] activities. They have also found application in industry as anti-oxidants, vulcanization accelerators. Various benzothiazoles such as 2-aryl benzothiazole received much attention due to unique structure and its uses as radioactive amyloidal imagining agents[5] and anticancer agents.[6] Benzothiazoles are bicyclic ring system with multiple applications. In the 1950s, a number of 2-aminobenzothiazoles were intensively studied, as the 2-amino benzothiazole scaffold is one of privileged structure in medicinal chemistry[7] and reported cytotoxic on cancer cells.[8] It must be emphasized that combination of 2-aminobenzothiazoles with other heterocyclic is a well known approach to design new drug like molecules, which allows achieving new pharmacological profile action, toxicity lowering compounds.

MATERIAL AND METHODS

All commercial solvents used in the experimental work were redistilled and dried before use. Melting points were recorded on a Thermonik Melting point Apparatus (Campbell Electronics, Mumbai, India) and are uncorrected. IR spectra were recorded on a Hartmann Braun, MB-104 Series (Bomem, Quebec, Canada) in KBr Pellets. ^{1}H-NMR spectra have been recorded on a JEOL AL-300 FT-NMR spectrometer (400 MHz, JEOL Ltd., Tokyo, Japan), using TMS as internal standard in solvent DMSO. Elemental analysis has been carried out on a C, H, and N Elemental Analyzer (Thermo-Finnigan Flash EA 1112, Italy). GC-MS done through (GC7890-MS200 Agilent).

EXPERIMENTAL

Synthesis of 2-hydrazinyl-6-methoxy-1,3-benzothiazole (Compound2)

Conc. HCl was added drop wise with stirring to hydrazine hydrate at 0-5^{0}C temperature. 6-methoxy-2- amino benzothiazole (0.01mol) was dissolved in ethylene glycol reflux it few mins, to that mixture add previously prepared mixture of HCl and Hydrazine hydrate (0.01mol). Reflux the reaction mixture for 6 hrs. Cooled to room temperature and keep the reaction mixture overnight. Needle shaped crystals appears, filter wash with water. Reaction was monitored by TLC. Recrystallized the product with Ethyl alcohol.

Yield 85%; colourless solid; mp;143^{0}C. ^{1}H NMR (400 MHz, DMSO-δ6) δ (ppm) 4.89 (s, 3H), 5.67(s, 1H), 5.21 (s, 2H), 7.28-8.26 (m, 3H, Ar-H) Anal. calcd for $C_{13}H_{15}O_3S_2N$:C, 49.21; H, 4.65; N, 21.52 Found: C, 49.37; H,4.16; N 21.21; IR (KBr) cm^{-1}: 3245(-NH), 1235(C-O). MS (m/z): 195[M^{+}] ($C_8H_9N_3SO$), 165 ($C_7H_6N_3SO$), 135 (C_7H_5NS).

Synthesis of 6-methoxy-2-[(2E)-2-(1-phenylethylidene) hydrazinyl]-1,3-Benzothiazole (Compound 3)

Crystallized compound 2 (0.005mol) was dissolved in alcohol. Add 1-2 drops of glacial acetic acid. To this solution add acetophenone (0.05mol) in alcohol. Reflux the reaction mixture for 6-8 hrs, completion of reaction was monitored by TLC. After completion of reaction, cooled the reaction mixture to room temperature. Poured the reaction mixture into ice-cold water, solid product appears. The crude product was filtered, dried & recrystallized. Yield 66%; pinkish colour solid; mp;236^{0}C; ^{1}H NMR(400 MHz, DMSO-δ6) δ (ppm) 4.2 (s, 3H), 5.6 (s, 1H), 4.6 (s, 3H), 7.12-8.16 (m, 8H, Ar-H) Anal. calcd for $C_{16}H_{15}N_3OS$:C, 64.62; H, 5.08; N, 14.13Found: C, 64.12; H, 5.52; N, 14.73. IR (KBr) cm^{-1}: 1645, 2923, 1710, 3442, 3398 MS (m/z): 297[M^+] ($C_{16}H_{15}N_3OS$), 267 ($C_{15}H_{13}N_3S$), 135 (C_7H_5NS).

Synthesis of Synthesis of 1-(6-methoxy-1,3-benzothiazole-2-yl)-3-phenyl-1H-pyrazole-4-carbaldehyde (compound 4)

Crystallized compound 3 (0.007mol) was dissolved in Dimethyl formamide and kept in ice cold condition. To this solution, Vilsmeier Haack reagent [(1.5ml) of $POCl_3$ was added drop by drop with stirring in (6ml) of DMF] was added with constant stirring at room temperature for 4 hrs then content was poured into crushed ice (previously neutralized with $NaHCO_3$ or Liq ammonia) solid separates out which was filter washed with water, dried and recrystallized from ethanol.

Yield 44%;reddish yellow colour solid; mp;185^{0}C;^{1}H NMR(400 MHz, DMSO-δ6) δ (ppm) 7.25-8.15 (m, 10H Ar-H), 10.0 (s, 1H),4.6 (s, 3H) Anal. calcd for $C_{18}H_{13}O_2N_3S$: C, 64.46; H, 3.91; N, 12.53. Found: C, 64.37; H, 3.91; N, 12.53 IR (KBr) n: 1615 (C-O), 3456 (-N=). MS (m/z): 335[M^+] ($C_{18}H_{13}O_2N_3S$), 305 ($C_{17}H_{11}N_3SO$), 229 ($C_{11}H_7N_3OS$), 135 (C_7H_5NS).

Synthesis of 1-{(E)-[1-(1,3-benzothiazol-2-yl) -33phenyl-1H-pyrazol-4-yl] methylidene} thiourea (Compound 5a)

Crystallized compound 4 (0.001mol) was dissolved in alcohol reflux the reaction mixture till it forms clear solution. To this clear solution phenyl thiourea (0.001 mol) was added and refluxes the reaction mixture for 8-10 hrs. Completion of reaction was monitoring by TLC. The reaction mixture was poured into the ice-cold water. Product was filtered wash with water. Recrystallized the product with Ethyl alcohol.

Yield 63%; grey colour solid; mp;206^{0}C;^{1}H NMR(400 MHz, DMSO-δ6) δ (ppm) 7.15-8.15 (m, 14H Ar-H), 4.2 (s,3H),5.35 (s, 1H), 2.24 (s, 1H). Anal. calcd for $C_{25}H_{19}N_5OS_2$: C, 63.60; H,4.08; N, 14.91. Found: C, 63.94; H, 4.61; N, 14.40 IR (KBr) cm^{-1}: 1325 (C-O), 2923 (C=C), 3434 (-NH) MS (m/z): 469 [M$^+$] ($C_{25}H_{19}N_5OS_2^+$), 439 ($C_{22}H_{16}N_5S_2$), 277 ($C_{16}H_{11}N_3S$), 201 ($C_{10}H_7N_3S$), 135 (C_7H_5NS).

Characterization of 4-ethoxy{(E)-[1-(1,3-benzothiazol-2-yl)-3,3phenyl-1H-pyrazol-4-yl] methylidene}thiourea (Compound 5b)

Yield 60%; brown colour solid; mp;212^{0}C;^{1}H NMR(400 MHz, DMSO-δ6) δ (ppm) 7.62-8.78 (m, 13H Ar-H), 4.4 (s,3H),5.15 (s, 1H), 2.64 (s, 1H), 5.38 (t, 3H), 5.69 (q, 2H Anal. calcd for $C_{27}H_{23}N_5O_2S_2$: C, 63.14; H,4.51; N, 13.63. Found: C, 63.94; H, 4.51; N, 13.40 IR (KBr) cm^{-1}: 1365 (C-O), 2984 (C=C), 3487 (-NH) MS (m/z): 513 [M$^+$] ($C_{27}H_{23}N_5O_2S_2^+$), 469 $C_{25}H_{19}N_5OS_2^+$), 439 ($C_{22}H_{16}N_5S_2$), 277 ($C_{16}H_{11}N_3S$), 201 ($C_{10}H_7N_3S$), 135 (C_7H_5NS).

Characterization of 3,5-dimethoxy{(E)-[1-(1,3-benzothiazol-2-yl)-3,3phenyl-1H-pyrazol-4-yl] methylidene}thiourea (Compound 5c)

Yield 74%; yellow colour solid; mp; 237^{0}C ^{1}H NMR(400 MHz, DMSO-δ6) δ (ppm) 7.62-8.78 (m, 13H Ar-H), 4.4 (s,3H),5.15 (s, 1H), 2.64 (s, 1H), 2.38 (s, 3H), 2.69 (s, 2H) Anal. calcd for $C_{27}H_{23}N_5O_3S_2$:C, 61.23; H, 4.38, N, 13.22; Found: C, 61.23; H,4.45. N, 13.32. IR(KBr) n: 1322 (-CO), 2930(C=C), 3546(-NH). MS (m/z): 529 [M$^+$] ($C_{27}H_{23}N_5O_3S_2^+$), 469 $C_{25}H_{19}N_5OS_2^+$), 439 ($C_{22}H_{16}N_5S_2$), 277 ($C_{16}H_{11}N_3S$), 201 ($C_{10}H_7N_3S$), 135 (C_7H_5NS).

Characterization of 4-hydroxy{(E)-[1-(1,3-benzothiazol-2-yl)-3,3phenyl-1H-pyrazol-4-yl] methylidene}thiourea (Compound 5d)

Yield 76%; brown colour solid; mp;242^{0}C ^{1}H NMR(400 MHz, DMSO-δ6) δ (ppm) 7.23-8.14 (m, 13H Ar-H),10.0 (s, 1H) 4.7 (s,3H),5.45 (s, 1H), 2.36 (s, 1H). Anal. calcd for $C_{25}H_{19}N_5O_2S_2$:C, 61.84; H, 3.94, N, 14.42; Found: C, 61.01; H, 3.16. N, 14.32. IR(KBr) cm^{-1}: 3590(-N=), 3348 (-OH),1300 (C-O). MS (m/z): 485[M$^+$] ($C_{25}H_{19}N_5O_2S_2^+$), 469 $C_{25}H_{19}N_5OS_2^+$), 439 ($C_{22}H_{16}N_5S_2$), 277 ($C_{16}H_{11}N_3S$), 201 ($C_{10}H_7N_3S$), 135 (C_7H_5NS).

Characterization of 4-Chloro{(E)-[1-(1,3-benzothiazol-2-yl)-3,3phenyl-1H-pyrazol-4-yl] methylidene}thiourea (Compound 5e)

Yield 66%; buff colour solid; mp; 232^{0}C ^{1}H NMR(400 MHz, DMSO-d6) d (ppm) 7.28-8.17 (m, 13H Ar-H), 4.7 (s,3H),5.15 (s, 1H), 2.84 (s, 1H).. Anal. calcd for $C_{25}H_{18}ClN_5OS_2$:C, 59.57; H, 3.60, N, 13.89; Found: C, 59.01; H, 3.16. N, 13.32. IR(KBr) cm^{-1}: 1298(C-O),

3452(-N=) MS (m/z): 504 [M^+] ($C_{25}H_{18}ClN_5OS_2^+$), 469 $C_{25}H_{19}N_5OS_2^+$), 439 ($C_{22}H_{16}N_5S_2$), 277 ($C_{16}H_{11}N_3S$), 201 ($C_{10}H_7N_3S$), 135 (C_7H_5NS).

Biological Activity

Antimicrobial activity

The benzothiazole derivatives were screened for in vitro for their antimicrobial activity against a panel of selected Bacteria and fungi and the minimal inhibitory concentrations that inhibited the growth of the tested microorganisms (MIC) were detected. In order to elucidate the kind of the exhibited antimicrobial activity, when MIC values were lower than 100 µg/mL, the minimal bactericidal concentrations (MBCs) and the minimal fungicidal concentrations (MFCs) were determined. The results of antimicrobial testing are reported in Table 1 and are compared with those of standards ampicillin, Trimethoprim and Miconazol.

Table No. I: Anti-Microbial activity of synthesized compounds

Sr. No	Code	R	Anti-Microbial Activity (µ/ml) [MIC]						
			Bacterial strains					Fungal strains	
			E. coli	*S. typhi*	*P. aeroginosa*	*Kleb pneumoniae*	*Vibrio chlorae*	*C. Albican*	*A. niger*
1	5a	-H	NA	100	200	NA	200	100	50
2	5b	4-OC_2H_5	200	200	200	200	100	200	100
3	5c	3,5-diOCH_3	200	200	200	200	25	200	200
4	5d	4-OH	200	200	200	200	25	200	200
5	5e	4-Cl	100	200	200	100	25	NA	200

1. Ampicillin (MIC-0.04 µg/ml) used as standard against ***E. coli***., ***Kleb pneumonia,***
2. Trimethoprim (MIC 0.01 µg/ml) used as standard against ***P. aeroginosa***, ***S. typhi, V. chlorae***
3. Miconazole (MIC 6.25 µg/ml) as standard against *C. albicans* and *A. niger*.

Anti-Oxidant Activity &Anti Inflammatory Activity

Anti-oxidant is done by using Free radical Scavenging method[9] and readings were taken on 517nm of UV-Visible Spectrophotometer.

Anti-inflammatory is done by using HRBC-Membrane Stabilization method[10] and readings were taken on 560nm of UV-Spectrophotometer.

Table No. II: Antioxidant and Anti-Inflammatory activity of synthesized compounds

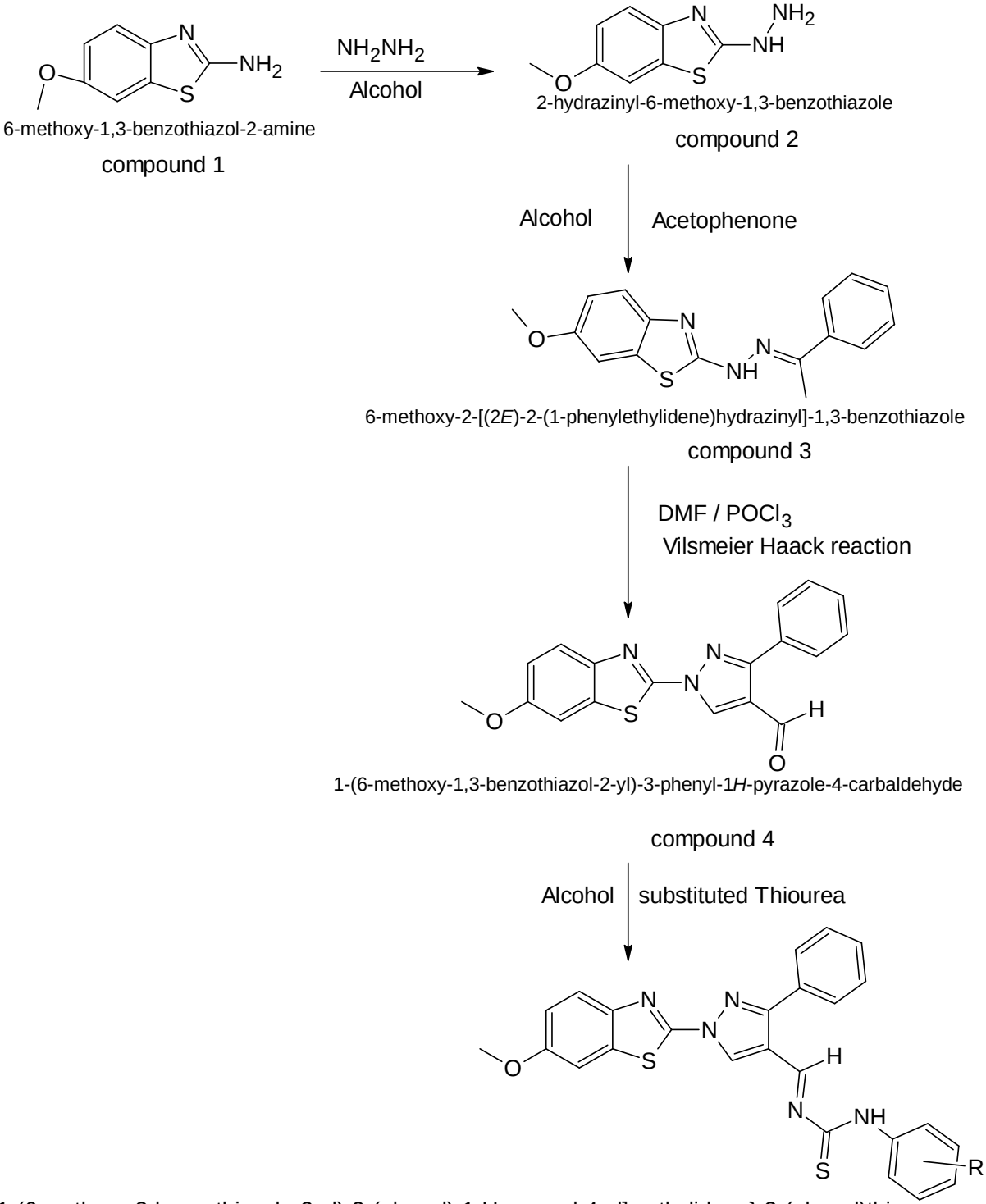
Sr. no.	Code	R	Anti-Oxidant Activity	Anti-Inflammatory Activity
			IC_{50}±SD	IC_{50}±SD
1	5a	-H	89.32±1.77	72.19±0.94
2	5b	4-OC_2H_5	11.90±3.48	38.32±0.65
3	5c	3,5-diOCH_3	78.57±4.21	29.23±1.00
4	5d	4-OH	97.00±2.64	87.64±2.36
5	5e	4-Cl	86.34±1.99	36.21±1.00
6	Std		8.25±0.325	11.36±0.214

1. Standard Anti-oxidant BHT (Butylated Hydrogen Toulene)
2. Standard Ant-Inflammatory Dichlofenac Sodium

RESULT AND CONCLUSION

The synthesized compounds were tested for their anti-microbial activity by using tube dilution method, The synthesized compounds 5a-5e were checked against microorganisms such as *E. coli, S. typhi, P. aeroginosa, Kleb pneumoniae, Vibrio chlorae C. Albican, A.niger* in order to establish their bioactivities. The results obtained clearly show the efficiency of some of the new compounds, The results indicated that some of the synthesized compounds have moderate activity. We found that the activity of the synthesized compounds depends on their concentration and the strain of tested bacteria Gram positive bacteria were more susceptible to the synthesized compounds than Gram negative ones. This effect can be attributed in part to the great complexity of the double membrane-containing cell envelope in Gram negative bacteria, compared to the single membrane structure of positive ones. Among the derivatives synthesized 5c,5d,5e derivative have found more potent compound for anti microbial activities while the compound that has been synthesized without substituents showed mild activity.

Schematic Representation



Antioxidant activity of the test compounds was determined by diphenylpicrylhydrazyl (DPPH) radical scavenging method. BHT was taken as the positive control. The test compounds such as 5b showed the IC_{50} 11.90µg/ml which is comparatively good result when

compared to standard however, the remaining test compounds showed the IC_{50} values above 50mg/ml as compared to standard which shows IC50 at 8.25mg.

The activity of Benzothiazole derivatives was studied for *in vitro* anti-inflammatory Activity by HRBC membrane stabilization method. Among all the compounds, a compound 5c. 5b, 5e has shown moderate result as compared to standard with IC50 of 8.25µg/mL.

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