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SYNTHESIS OF SOME MERCAPTO BENZOTHIAZOLE DERIVATIVE WITH THEIR CHARACTERIZATION AND BIOLOGICAL EVALUATION

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ABSTRACT

In present study we synthesized some 2-amino mercapto benzothiazole and Acid Chloride derivatives and screen synthesized compounds for their anti-microbial activity. 2-amino mercapto benzothiazole on reacting with ethyl chloro acetate forms ester derivative of benzothiazole which on further treatment of hydrazine hydrate formed hydrazino derivatives of mercaptobenzothiazole, It was then treated with carbon disulphide to form oxadiazole derivative of mercapto benzothiazole and finally it was condensed with different substituted Acid chloride to form final compound. Synthesized compounds have been confirmed on the basis of spectral studies by using FT-IR, NMR, Mass Spectrophotometer. All the compounds were screened for their in *vitro* anti-inflammatory, i*n vitro* anti oxidant and i*n vitro* antibacterial activity against *E. coli, S. typhi, P. aeroginosa, Kleb pneumoniae, Vibrio chlorae.* Antifungal strain *Candida albicans* and *Aspergillus niger* using tube dilution method shows promising activity.

KEYWORDS: 2-amino mercapto benzothiazole, anti-Inflammatory activity, anti-oxidant activity, and anti-microbial activity

INTRODUCTION

Heterocyclic compounds containing nitrogen and sulphur possess potential pharmacological activities. Benzothiazoles are bicyclic ring systems which have been the subject of great interest because of their biological activities. Benzothiazole moiety possesses diverse type of biological activities. The various mercapto benzothiazole and their derivatives are important biological and pharmacological activities. In particular they are used as anti-inflammatory, analgesics, antibacterial, antifungal, antiviral, anti-diabetics and anti-tubercular agents.[1-6] Benzothiazole are bicyclic ring with multiple application, 2-mercapto benzothiazole have been studied extensively and found to have diverse chemical reactivity and broad spectrum of activity, like antimicrobial, antitumor, anthelmintic, antileishmanial, anticonvulsant, anti-inflammatory activity.[7-13]

The derivatives of benzothiazole have been studied extensively and have been reported to exhibit antitumor1, vasodilator,[14] antitubercular,[15] antifungal,[16] anti-inflammatory[17] and antidiabetic[18]. In the present work we are reporting the synthesis of some derivatives of 2-mercapto benzothiazole to obtain pharmacological more potent derivatives.

MATERIALS AND METHODS

All raw materials used in the synthesis have been obtained from M/S Fluka AG (Bachs, Switzerland) and M/S Sigma-Aldrich chemicals and Co. Inc. (Milwoukee, WI,USA). Melting points were recorded on a Thermonik Melting point Apparatus (Campbell Electronics, Mumbai, India) and are uncorrected. IR spectra were recorded on a IR-Affinity, Shimadzu using DRS system. ^{1}H-NMR spectra have been recorded on a JEOL AL-300 FT-NMR spectrometer (300 MHz, JEOL Ltd., Tokyo, Japan), using TMS as internal standard in solvent DMSO. Mass data have been recorded on Agilent GC-MS Elemental analysis has been carried out on a C, H, and N Elemental Analyzer (Thermo-Finnigan Flash EA 1112, Italy) GC-MS is done through (GC7890-MS200 Agilent).

Experimental

Synthesis of ethyl [(6-ethoxy-1, 3-benzothiazol-2-yl)sulfanyl]acetate (Compound 1)

6-ethoxy2-mercapto benzothiazole (0.98 mol) was dissolved in acetone stirred for 30 mins and K_2CO_3 (0.98mol), was added and reflux the reaction mixture leading to formation of potassium salt of mercapto benzothiazole. After salt formation (0.98mol) ethyl chloroacetate was added over the period of 15 mins. Reflux the reaction mixture till completion of reaction. Reaction was monitoring was done by TLC. Crude product was isolated. Clear solution was

extracted with diethyl ether for three times to extract product from aqueous layer. Recrystallized the product with Ethyl alcohol.

Yield 74%; Buff colour solid; mp;95^{0}C. ^{1}H NMR(400 MHz, DMSO-δ6) δ (ppm) 2.20 (t, 3H), 3.65 (q, 2H),4.17 (s, 2H), 7.01-8.02 (m, 3H, Ar-H) Anal. calcd for $C_{13}H_{15}NO_3S_2$:C, 52.50; H, 5.08; N, 4.71. Found: C, 52.37; H, 5.16; N, 4.71. IR (KBr) cm^{-1}: 2943(-CH_3), 1214 (C-O). MS (m/z): 297[M^+] ($C_{13}H_{15}NO_3S_2^+$), 209($C_9H_7NOS_2$), 181($C_8H_7NS_2$), 135(C_7H_5NS).

Synthesis of 2-[(6-ethoxy-1, 3-benzothiazol-2-yl)sulfanyl] acetohydrazide (Compound 2)

Crystallized Compound 1 (0.56mol) product was dissolved in alcohol, reflux the reaction mixture till it forms clear solution. To this clear solution of (0.58 mol) Hydrazine hydrate was added and refluxes the reaction mixture for 8-10 hrs leading to formation of product. Reaction was monitored by TLC. After completion of reaction, keep the reaction mixture on standing for overnight, needle shaped crystal was formed. Filter the product and wash with water till pH 7-8. Recrystallized the product with Ethyl alcohol.

Yield 62%; white colour solid; mp;185^{0}C. ^{1}H NMR(400 MHz, DMSO-δ6) δ (ppm) 2.35 (t, 3H), 3.08 (q, 2H),4.23 (s, 2H), 6.12 (s, 1H), 6.34 (s, 1H), 7.18-8.26 (m, 3H, Ar-H) Anal. calcd for $C_{11}H_{13}N_3O_2S_2$:C, 46.62; H, 4.62; N, 14.83 Found: C, 46.32; H, 4.26; N, 14.32.IR (KBr) cm^{-1}:3345(-NH_2), 3378 (-NH), 1214 (C-O) . MS (m/z): 283[M^+] ($C_{11}H_{13}N_3O_2S_2^+$),239 ($C_9H_9N_3OS_2$), 209($C_9H_7NOS_2$), 181($C_8H_7NS_2$), 135(C_7H_5NS).

Synthesis of 5-{[(6-ethoxy-1,3-benzothiazol-2-yl)sulfanyl]methyl}-1,3,4-oxadiazole-2-thiol (Compound 3)

Compound 2 (0.79 mol) was dissolved in alcohol to this KOH was added to form potassium salt of Compound 2. To this mixture carbon disulfide was added. Reflux the reaction mixture, as reaction progress H_2S gas was evolved. After 8-10 hrs libration of H_2S gas stopped. Further completion of reaction was monitored by TLC. The product was drown in cold water, mixture was highly basic in nature, neutralized by using dil. HCl to pH 7. Product was filtered, wash with water to remove traces of impurities. Recrystallized the product with Ethyl alcohol.

Yield 48%; brown colour solid; mp;95^{0}C. ^{1}H NMR(400 MHz, DMSO-δ6) δ (ppm) 2.19 (t,3H), 3.28 (q, 2H), 4.27 (s, 2H), 5.11 (s, 1H), 7.18-8.26 (m, 3H, Ar-H) Anal. calcd For

$C_{12}H_{11}N_3O_3S_2$:C, 44.29; H, 3.41; N, 12.91. Found: C, 44.12; H, 3.46, N, 13.01 IR (KBr) cm^{-1}:2988(-CH_3), 1201 (C-O). MS (m/z): 325[M^+] ($C_{12}H_{11}N_3O_3S_2^+$), 209($C_9H_7NOS_2$), 181($C_8H_7NS_2$), 135(C_7H_5NS).

Synthesis of *S*-(5-{ [(6-ethoxy-1,3-benzothiazol-2-yl) sulfanyl]methyl}- 1,3,4- oxadiazol-2 yl) benzenecarbothioate (Compound 4a)

Compound 3 (1.2 mol) was dissolved in pyridine to this (1.25mol) benzoyl chloride was added drop wise at room temperature, as it is exothermic reaction addition of benzoyl chloride was done slowly over the period of 30 mins., then stirred the reaction mixture at same temperature for next 30 mins slowly raised the temperature to reflux the reaction mixture. Completion of reaction was monitored by TLC. The product was drown in cold water, solid appeared. Product was filtered, wash with water. Recrystallized the product with Ethyl alcohol. Similarly other derivatives were prepared.

Yield 54%; Yellow colour solid; mp155^{0}C. ^{1}H NMR(400 MHz, DMSO-δ6) δ (ppm) 3.30 (t, 3H), 3.18 (q,2H),4.13 (s, 2H), 7.18-8.26 (m, 8H, Ar-H) Anal. calcd for $C_{19}H_{15}N_3O_3S_3$:C, 53.13; H, 3.52;N, 9.78,Found: C,53.37; H, 3.16, N, 9.35..IR (KBr) cm^{-1}:1119(-O-), 987(=C-H), 1258 (-N=). MS (m/z): 429[M^+] ($C_{19}H_{15}N_3O_3S_3^+$), 385 ($C_{19}H_{11}N_3O_2S_3$), 167($C_7H_5NS_2$),

Characterization of S-(5-{[(6-ethoxy -1, 3-benzothiazoll -2-yl)sulfanyl] methyl}-1,3,4 oxadiazol-2-yl)4- chloro benzenecarbothioate (Compound 4b)

Yield 58%; Brown colour solid; mp;140^{0}C. ^{1}H NMR(400 MHz, DMSO-δ6) δ (ppm) 2.16 (t,3H), 3.25 (q,2H),4.93 (s, 2H), 7.12-8.12 (m, 7H, Ar-H) Anal. calcd for $C_{19}H_{14}ClN_3O_3S_3$: C,49.18; H, 3.04;N, 9.06 Found: C,49.32; H, 3.16, N, 9.34.IR (KBr)cm^{-1}:1041(-O-), 1258 (C-O), 937 (=C-H), 713 (-Cl). MS (m/z): 463[M^+]($C_{19}H_{14}ClN_3O_3S_3^+$),419 ($C_{17}H_{10}ClN_3O_2S_3$), 385 ($C_{19}H_{11}N_3O_2S_3$), 249 ($C_{10}H_7N_3OS$),167($C_7H_5NS_2$).

Characterization of S-(5-{[(6-ethoxy-1,3-benzothiazol-2-yl)sulfanyl]methyl}-1,3,4-oxadiazol-2-yl) 4-hydroxy benzenecarbothioate (Compound 4c)

Yield 62%; Pale yellow colour solid; mp;154^{0}C. ^{1}H NMR(400 MHz, DMSO-δ6) δ (ppm) 2.66 (t, 3H), 3.12 (q,2H), 4.03 (s, 2H), 5.13 (s, 1H), 7.01-8.06 (m, 7H, Ar-H) Anal. calcd for $C_{19}H_{15}N_3O_4S_3$:C, 51.22; H, 3.39; N, 9.43; Found: C, 51.12;H, 3.09; N, 9.63. .IR (KBr) cm^{-1}:1039(-O-), 1259(C-O), 939 (=C-H), 3419 (-OH). MS (m/z): 445[M^+] ($C_{19}H_{15}N_3O_4S_3^+$), 401($C_{17}H_{11}N_3O_3S_3$), 385 ($C_{19}H_{11}N_3O_2S_3$), 249 ($C_{10}H_7N_3OS$), 167($C_7H_5NS_2$).

Characterization of S-(5-{[(6-ethoxy-1,3-benzothiazol-2-yl)sulfanyl]methyl}-1,3,4-oxadiazol-2-yl) 4-methoxy benzenecarbothioate Compound 4d)

Yield 66%; Brown colour solid; mp;206^{0}C. ^{1}H NMR(400 MHz, DMSO-δ6) δ (ppm) 2.13 (t, 3H), 3.27 (q,2H),4.63 (s, 2H), 5.22 (s, 3H), 7.18-8.26 (m, 7H, Ar-H) Anal. calcd for $C_{20}H_{17}N_3O_2S_3$:C, 52.27; H, 3.39; N, 9.43 Found: C, 52.37; H, 3.11; N, 9.62.IR (KBr) cm^{-1}:1091(-O-), 1286(C-O), 929 (=C-H), 1236 (C-S) . MS (m/z): 459[M^+] ($C_{20}H_{17}N_3O_2S_3^+$), 385 ($C_{19}H_{11}N_3O_2S_3$), 249 ($C_{10}H_7N_3OS$), 167($C_7H_5NS_2$),

Characterization of S-(5-{[(6-ethoxy-1,3-benzothiazol-2-yl)sulfanyl]methyl}-1,3,4-oxadiazol-2yl) 4-ethoxy benzenecarbothioate (Compound 4e)

Yield 52%; Light brown colour solid; mp;212^{0}C. ^{1}H NMR(400 MHz, DMSO-δ6) δ (ppm) 2.33 (t, 3H), 3.28,(q, 2H),4.23 (s, 2H), 7.28-8.26 (m, 7H, Ar-H) Anal. calcd for $C_{21}H_{19}N_3O_4S_3$:C, 53.26; H, 4.04; N, 8.87 Found: C, 53.33; H, 4.06, N, 8.74.IR (KBr) cm^{-1}:1064(-O-), 1253(C-O), 937 (=C-H), 1226(C-S) . MS (m/z): 473[M^+] ($C_{21}H_{19}N_3O_4S_3^+$), 385 ($C_{19}H_{11}N_3O_2S_3$), 249 ($C_{10}H_7N_3OS$), 167($C_7H_5NS_2$).

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 I and are compared with those of standards Ampicillin, Trimethoprim and Miconazole.

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

Sr. No	Code	R	Anti-Microbial Activity (µg/ml) [MIC]						
			Bacterial strains					Fungal strains	
			E. coli	*S. typhi*	*P. aeroginosa*	*Kleb pneumoniae*	*Vibrio chlorae*	*C. albican*	*A. niger*
11	4a	-H	200	200	200	200	50	100	100
12	4b	4-Cl	200	200	200	200	200	100	100
13	4c	4-OH	200	200	100	100	50	100	200
14	4d	4-OCH_3	100	100	100	100	25	200	100
15	4e	4-OC_2H_5	200	200	200	200	25	200	200

1. Ampicillin (MIC-0.04 µg/ml) used as standard against *S. aureus*, *E.coli*,*P.aeroginosa*

2. Trimethoprim (MIC 0.01 µg/ml) used as standard against *S. typhi, K.pneumonia*
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[19] and readings were taken on 517nm of UV-Visible Spectrophotometer Anti-inflammatory is done by using HRBC-Membrane Stabilization method[20] and readings were taken on 560nm of UV-Spectrophotometer.

Table no II:- Anti-oxidant and Anti-Inflammatory activity of Synthesized Compounds

Sr. no.	Code	R	Anti-oxidant	Anti-inflammatory
			IC_{50}±SD	IC_{50}±SD
1	4a	-H	58.23±2.364	61.02±1.123
2	4b	4-Cl	54.00±2.136	61.32±1.109
3	4c	4-OH	36.00±2.196	38.13±0.38
4	4d	4-OCH_3	52.32±1.330	66.19±0.23
5	4e	4-OC_2H_5	50.13±2.036	68.94±0.54
6	Std		8.25±0.336	11.70±0.987

1. Standard Anti-Oxidant Butyrate hydrogen Toluene
2. Standard Anti-Inflammatory Sodium Dichlofenac.

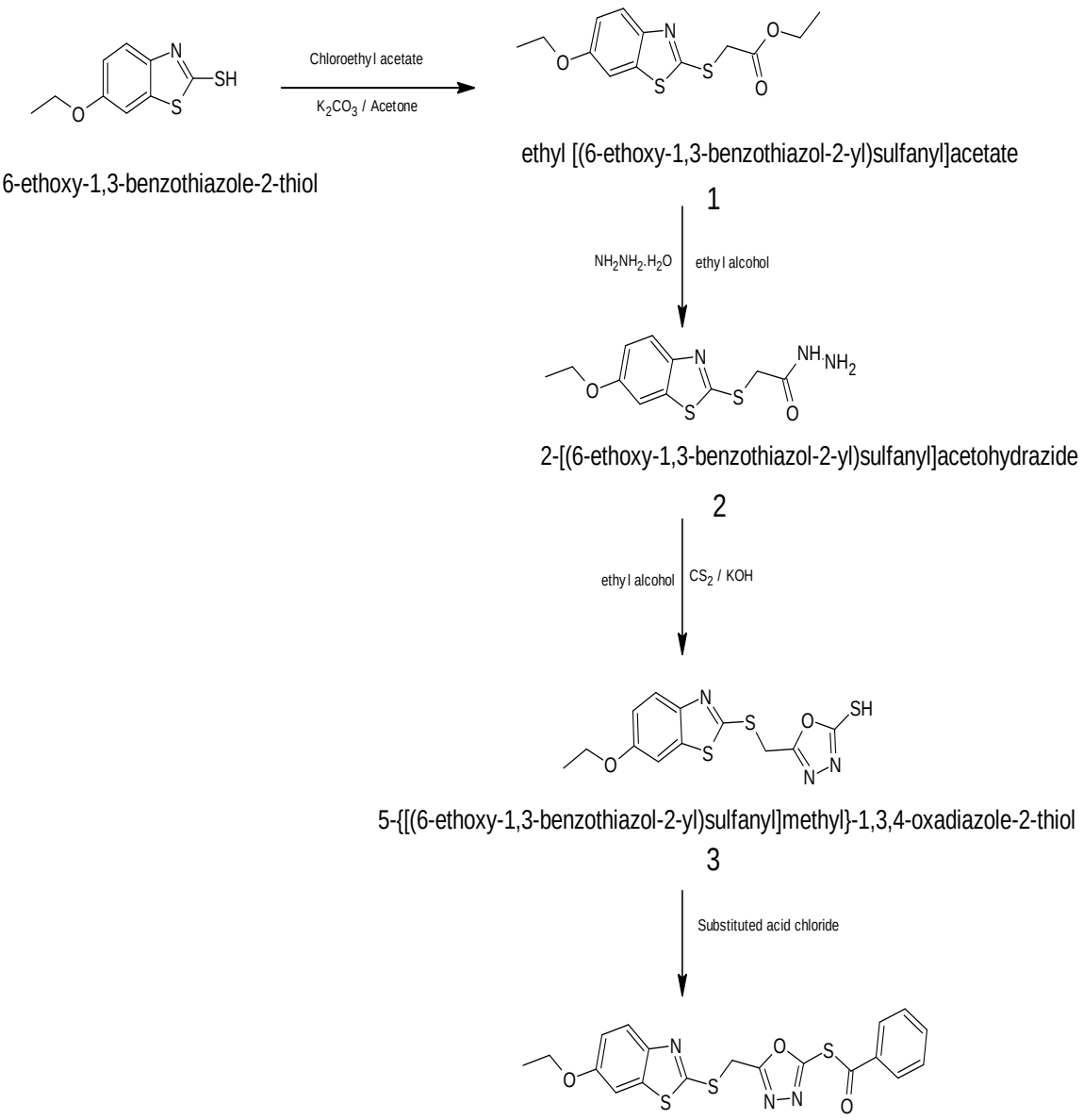
RESULT AND DISCUSSION

Synthesized compound were screened for anti-microbial activity by tube dilution method. The compound was tested on Bacterial stains *E. coli, S. typhi, P. aeroginosa, Kleb Pneumoniae, Vibrio chlorae,* Fungal Stains *C. Albicans, A.niger.* Each test compound was tested against each strain. The activity was then monitored for 24-48 hours and the data is presented in the Table I. A comparative study of the 5 compounds synthesized reveals the following information: the compounds showed mild to moderate anti-microbial activity. Compound 4d, 4e has show*n* themaximum activity among electron donating substitution for*Vibrio chlorae* (25µg/ml) but the compound 4d, 4e has not shown activity for bacterial or fungal stains.

In Radical scavenging activity done by DPPH method, standard drug BHT shows IC_{50} at 8.25µg/mL All Synthesized compounds compared with standard, we observed that, compound 4c shows remarkable activity at 36µg/mL while others shows moderate activity. In Anti-inflammatory activity done by Human Red Blood Cell (HRBC) membrane stabilization method, standard drug shows IC_{50} at 11.70µg/mL ,Compound 4c shows IC_{50} 38.13 µg/mL

which when compared with the other synthesized compound showed maximum activity but moderately as compared with standard.

Schematic Representation



CONCLUSION

The series of derivatives of 2-mercaptobenzothiazole were synthesized and evaluated for anti-microbial activity. Among halogen substitution Chloro was not active substituent against any bacterial and fungal stains, where as substituent like methoxy and ethoxy have shown moderate result. In anti oxidant and in Anti Inflammatory only hydroxy substituent was found to be exclusively active while other has shown moderate activity.

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