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SOLID PHASE SYNTHESIS OF BENZO[D]THIAZOL-2-YLCARBAMODITHIOATES AND THEIR EVALUATION AS POTENTIAL ANTIFUNGALS

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ABSTRACT:

Benzo[d]thiazol-2-amines (**1** and **2**) were synthesized by bromocyclization of anilines and potassium thiocyanate. They were further treated with carbon disulphide and alkyl halide over basic aluminum to yield required benzo[d]thiazol-2-ylcarbamodithioates (**3-8**). The synthesized compounds were characterized on the basis of spectral analysis and screened for *in vitro* antifungal potential against *Pyricularia grisea, Drechslera oryzae, Fusarium moniliforme* and *Ustilaginoidea virens* by spore germination inhibition method. The results were presented in the terms of EC_{50} values. The compound **3** showed the high fungitoxicity with EC_{50} values comparable to standard fungicides against most of the test fungi.

KEYWORDS: *Benzo[d]thiazoles; Carbamodithioates; Antifungal activity*.

INTRODUCTION:

Organic dithiocarbamates have received devastating attention due to their interesting chemistry and wide utility as radical precursors in organic synthesis [3, 4]. It is one of the most widely used group of pesticides in terms of tonnage and have found

multiple applications especially, in agriculture due to their pronounced biological activity [1, 2, 8] , ease of preparation [17], easy biodegradability, multiple mode of action [10, 11] and non-systematic action. Due to the rapid development of fungal resistance to pesticide agents, it is vital to discover scaffold for the design and synthesis of the new fungicidal agents to help in the battle against fungal microorganisms, and thus the search for new agents is one of the most challenging tasks to the synthetic chemist. Thus, the functionalization of existing class of organic dithiocarbamate moiety offers great potential in the generation of large combinatorial libraries, which may constitute interesting medicinal and biological properties for rapid screening and drug design [9]. Commercially used dithiocarbamate are mostly analogues of straight chain and are non-systemic surface protectants. Derivatization of the known class of dithiocarbamates with heterocyclic ring enhances the systemic action.

Benzothiazoles belongs to aza-thio class of bioactive compounds that has received overwhelming response owing to its diversified molecular design. Benzothiazole nucleus is present in various compounds and responsible for its diverse biological activities *viz* antifungal [6], antibacterial [16], antitubercular [12] and anticancer [5]. Commercial fungicides containing this include TCMTB [2-(thiocyanomethylthio)benzothiazole], 2-(carboxymethylthio)benzothiazole and tricyclazol{5-methyl- 1,2,4-triazolo} [3,4-b] benzothiazole.

The concept of Lead hybridization [7, 15] that refers to the combination of two effective moieties in a single molecule may augment the fungitoxic profile of the individual molecules. Therefore, in this multicomponent bioactive regime, we planned to examine the fungitoxicity of the hybrid benzo[d]thiazol-2-ylcarbamodithioates that were expected to have broad spectrum antifungal potential.

MATERIALS AND METHODS:

All the reagents were procured from commercial sources (SD Fine Chemicls, India). Melting points were determined in scientific melting point apparatus and were uncorrected. The purity of compounds was checked by TLC using Merck silica gel 60 F254 and visualized by exposure to iodine vapors or UV light. IR spectra were recorded on a Perkin Elmer RX1 FTIR spectrophotometer, using potassium bromide pellets, the frequencies are expressed in cm^{-1}. The ^{1}H NMR was recorded with a Bruker Avance II 400 NMR spectrometer, using tetramethylsilane as the internal reference, with $CDCl_3$ and DMSO-d6 as solvents. The

chemical shifts are reported in parts per million (ppm). The mass spectra were recorded on Perkin Elmer Clarus 500 Mass Spectrometer. All spectral data were consistent with the proposed structure.

General procedure for the synthesis of benzo[d]thiazol-2-amines (1, 2)

Benzo[d]thiazol-2-amines (**1,2**) were synthesized by literature reported method [13, 14], by bromocyclization of 4-chloroaniline and 4-fluoroaniline with potassium thiocyanate in equimolar ratio. The products being solid were recrystallized from benzene. The products obtained were characterized on the basis of melting point analysis.

General procedure for the synthesis of benzo[d]thiazol-2-ylcarbamodithioates (3-10)

Benzo[d]thiazole-2-amines (**1,2**) (0.01 mol) in minimum quantity of ethyl acetate was mixed with aluminium oxide basic (10 mg) and the solvent was allowed to evaporate. Carbon disulfide (0.02 mol) dissolved in minimum quantity of ethyl acetate wad added to above, followed by stirring for 10 min. The progress of reaction was monitored by TLC. Then, alkyl halide (0.01 mol) was added dropwise to same which was stirred for another 10 minutes with glass rod. The completion of reaction was confirmed by disappearance of spot of alkyl halide, using dichloromethane solvent system. The product was eluted using ethyl acetate, and recrystallized to get pure benzo[d]thiazol-2-ylcarbamodithioates (**3-10**). The thin layer chromatography and spectral analysis confirmed the formation of products.

Butyl (6-chlorobenzo[d]thiazol-2-yl)carbamodithioate (3): Light yellow crystals, Yield (63%), mp 195^{o}C, TLC (dichloromethane/EtOAc 10:1). IR / cm^{-1} (KBr) λmax: 3430 (N-H str), 2956 (C-H str), 1595 (>C=N str), 1057 (>C=S str), 985 (C-S str) and 725 (C-Cl str). The ^{1}HNMR ($CDCl_3$) spectrum of this product showed signals: δ 7.2-7.8 (m, 3H, fused benzothiazole ring), δ 4.1 (1H, s, NH) and δ 0.88-3.0 (m, 9H, -CH_2) ppm. The mass spectrum contained molecular ion peaks at m/z 315.42 $[M]^+$ and 317.56 $[M+2]^+$.

Decyl (6-chlorobenzo[d]thiazol-2-yl)carbamodithioate (4): Yellow crystals, [Yield 63%]; mp 243-245^{o}C; TLC (dichloromethane/EtOAc 10:1); IR / cm^{-1} (KBr) λmax: 3435 (N-H str), 1355 (>C=N str), 2956 (C-H str), 1057 (>C=S str), 985 (C-S str), 740 (C-Cl str) . The ^{1}HNMR ($CDCl_3$) spectrum of this product showed signals: δ 7.3-7.8 (m, 3H, fused benzothiazole ring), δ 4.0 (s,1H, NH) and δ 0.90-3.30 (m, 21H, -CH_2) ppm.The mass spectrum contained molecular ion peaks at m/z 400.25 $[M]^+$ and 402.16 $[M+2]^+$.

Hexyl (6-chlorobenzo[d]thiazol-2-yl)carbamodithioate (5): Brown crystals; Yield (51%); mp 235-238^{o}C; TLC (dichloromethane/EtOAc 10:1); IR / cm^{-1} (KBr) λmax: 3432

(N-H str), 1353 (>C=N str), 2958 (C-H str), 1050 (>C=S str), 980 (C-S str), 715 (C-Cl str). The ^{1}HNMR ($CDCl_3$) spectrum of this product showed signals: δ 7.2-7.8 (m, 3H, fused benzothiazole ring), δ 4.1 (s, 1H, NH) and δ 0.88-3.10 (m, 13H, -CH_2) ppm. The mass spectrum contained molecular ion peak at m/z 344.12 $[M]^+$, 346.17 $[M+2]^+$.

Octyl (6-chlorobenzo[d]thiazol-2-yl)carbamodithioate (6): Yellow crystals; Yield (60 %); mp 180-185^{o}C; TLC (dichloromethane/EtOAc 10:1); IR / cm^{-1} (KBr) λmax: 3432 (N-H str), 1353 (>C=N str), 2958 (C-H str), 1050 (>C=S str), 980 (C-S str), 715 (C-Cl str). The ^{1}HNMR ($CDCl_3$) spectrum of this product showed signals: δ 7.2-7.9 (m, 3H, fused benzothiazole ring), δ 4.1 (s, 1H, NH) and δ 0.88-3.30 (m, 13H, -CH_2) ppm. The mass spectrum contained molecular ion peaks at m/z 372.42 $[M]^+$ and 374.37 $[M+2]^+$.

Butyl (6-fluorobenzo[d]thiazol-2-yl)carbamodithioate (7): Yellow crystals; Yield (68 %); mp 110-115^{o}C; TLC (dichloromethane/EtOAc 10:1); IR / cm^{-1} (KBr) λmax: 3435 (N-H str), 1350 (>C=N str), 2956 (C-H str), 1057 (>C=S str), 985 (C-S str), 1195 (C-F str). The ^{1}HNMR ($CDCl_3$) spectrum of this product showed signals: δ 7.4-8.0 (m, 3H, fused benzothiazole ring), δ 4.1 (s, 1H, NH) and δ 0.88-3.3 (m, 9H, -CH_2) ppm. The mass spectrum contained molecular ion peaks at m/z 300.16 $[M]^+$.

Decyl (6-fluorobenzo[d]thiazol-2-yl)carbamodithioate (8): Brown crystals; Yield (76 %); mp 282-285^{o}C; TLC (dichloromethane/EtOAc 10:1); IR / cm^{-1} (KBr) λmax: 3450 (N-H str), 1350 (>C=N str), 2960 (C-H str), 1060 (>C=S str), 990 (C-S str), 1215 (C-F str). The ^{1}HNMR ($CDCl_3$) spectrum of this product showed signals: δ 7.4-8.2 (m, 3H, fused benzothiazole ring), δ 4.5 (s, 1H, NH) and δ 0.90-3.4 (m, 21H, -CH_2) ppm. The mass spectrum contained molecular ion peaks at m/z 384.16 $[M]^+$.

Hexyl (6-fluorobenzo[d]thiazol-2-yl)carbamodithioate (9): Orange crystals; Yield (64 %); mp 178-180^{o}C; TLC (dichloromethane/EtOAc 10:1); IR / cm^{-1} (KBr) λmax: 3450 (N-H str), 1350 (>C=N str), 2960 (C-H str), 1060 (>C=S str), 990 (C-S str), 1215 (C-F str). The ^{1}HNMR ($CDCl_3$) spectrum of this product showed signals: δ 7.4-8.4 (m, 3H, fused benzothiazole ring), δ 4.3 (s, 1H, NH) and δ 0.85-3.6 (m, 21H, -CH_2) ppm**.** The mass spectrum contained molecular ion peaks at m/z 328.16 $[M]^+$.

Octyl (6-fluorobenzo[d]thiazol-2-yl)carbamodithioate (10): Light Yellow crystals; Yield (45 %); mp 218-220^{o}C; TLC (dichloromethane/EtOAc 10:1); IR / cm^{-1} (KBr) λmax: 3450 (N-H str), 1350 (>C=N str), 2960 (C-H str), 1060 (>C=S str), 990 (C-S str), 1215 (C-F str). The ^{1}HNMR ($CDCl_3$) spectrum of this product showed signals: δ 7.4-8.0 (m, 3H, fused

benzothiazole ring), δ 4.1 (s, 1H, NH) and δ 0.85-3.6 (m, 21H, -CH_2) ppm. The mass spectrum contained molecular ion peaks at m/z 356.19 $[M]^+$.

Antifungal Evaluation

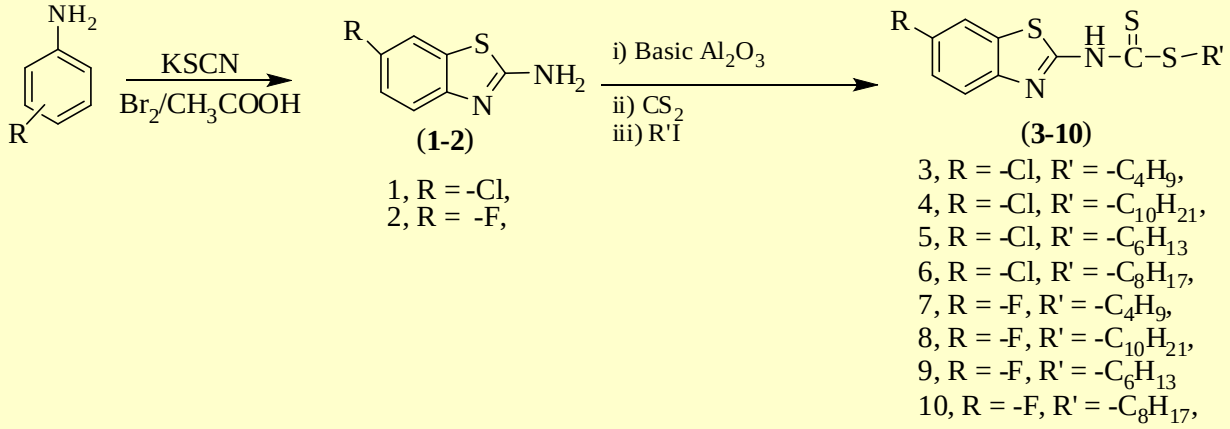
Fungicidal evaluation of the series of title compounds was performed against various phytopathogenic fungi by spore germination inhibition technique representing their results in terms of EC_{50} values. The four fungi used in fungicidal bioassay are *Pyricularia grisea, Drechslera oryzae, Fusarium moniliforme and Ustilaginoidea virens*. Commercial agricultural fungicide Bavistin 50 WP (methyl-2-benzimidazole-2-ylcarbamate) against *Fusarium moniliforme* and Tilt (1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1H1,2,4-triazole) against rest of the fungi were used as standards. For the sake of comparison of fungitoxicity of the molecules with standard fungicides, the results were appropriately expressed in terms of µmoles/ml. Spore suspension was made by adding sterilized distilled water to the fresh spores of respective fungi. Suspension was filtered through three layers of sterilized cheese cloth in order to remove mycelial particles under aseptic conditions. Haemocytometer was used to form standardized spore suspension (1 x 10^6spores/ml). Small droplets (0.02 ml) of test solution and spore suspension in equal amount were seeded in the cavity of the cavity slides. These slides were placed in Petri plates lined with moist filter paper and were incubated. The numbers of spores germinated were counted and per cent spore germination inhibition was calculated.

Investigation of antifungal screening listed in **Table 1** clearly revealed that most of lower alkyl chain substituted benzo[d]thiazol-2ylcarbamodithioates showed significant inhibition of germination against all the test fungi. This series were found to be most active against *D. oryzae*. Three compounds out of eight had inflicted antifungal potential comparable to the standard compound Tilt (EC_{50} 0.15 µmoles/ml). Compound **3** was the best with EC_{50} 0.13 µmoles/ml which was much favourable than the standard. Against *P. grisea* most of the compound showed moderate fungitoxicity with EC_{50} values less than 1.08 µmoles/ml. Against *U. virens*, compounds **3**, **5** and **7** have shown better antifungal potential. In case of *F. moniliforme* compounds **3** and **4** were the most effective compound with EC_{50} values 0.32 and 0.58 µmoles/ml respectively, which were less than standard fungicide Bavistin (EC_{50} values 0.78 µmoles/ml). In this case, chloro substituted benzo[d]thiazol-2-ylcarbamodithioates derivatives were found to be more effective than their flouro analogues. On the basis of alkylation, the butyl and hexyl substituted derivatives gave the better results in comparison to the molecules with longer alkyl chains.

RESULTS AND DISCUSSION:

Chemistry

The Benzo[d]thiazol-2-amines (**1,2)** were first prepared by bromo-cyclisation of substituted anilines according to the method given in the literature[16,17]. The route of the synthesis is outlined in **Scheme 1**. Further, the desired benzo[d]thiazol-2-ylcarbamodithioates were prepared by solid phase synthesis by the reaction of benzo[d]thiazol-2-amines with carbon disulfide and alkyl halide on basic aluminium oxide. All the synthesized carbamodithioates were characterized by ^{1}H NMR, IR and Mass spectral studies.



Scheme 1. Structures of the investigated compound

Bioactivity

The *in vitro* antifungal activity of all the title compounds were evaluated against four phytopathogenic fungi *viz. P. grisea, D. oryzae, F. moniliforme and U. virens* which are often encountered and compared with the standard fungicides Tilt (1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3dioxolan-2-yl]methyl]-1H-1,2,4-triazole) and and Bavistin 50 WP (methyl-2-benzimidazole-2-ylcarbamate). The isolates of phytopathogenic fungi were provided by the Plant Pathology Department of the Punjab Agricultural University and the standards Tilt and Bavistin, served as the positive control, were obtained from their respective manufacturers. 4 µmoles per millilitre of each of the test compounds and standards used, was made by dissolving the appropriate amount of compound in 1.0 ml of Tween and making the final volume 10 ml. The stock solutions of 4 µmol/ml of each compound, thus prepared on active ingredient basis were kept in refrigerator till further use. The required dilutions of 2, 1, 0.5, 0.25, 0.1 and 0.05 µmol/ml were subsequently made from the stock solution by adding distilled water as and when required.

The *in vitro* effective concentrations of the compounds were determined by Spore germination inhibition technique. The EC_{50} refers to the concentration which induces a response halfway. For assays, 0.02 ml of the title compounds to be tested and 0.02 ml of spore suspension were seeded in the cavity of the cavity slide and incubated at 15±2°C. Growth was determined at 24 h for all the phytopathogenic fungi. The results of assays in terms of EC_{50} are summarized in Table 2 The data points are the mean of triplicates.

CONCLUSION

In the present work, the synthesized novel benzo[d]thiazol-2-ylcarbamodithioates exhibited good antifungal activity against the phytopathogenic fungi. Compound **3** and **5** had shown the best antifungal potential against *D. oryzae* with EC_{50} value of 0.13 and 0.20 µmol/ml, respectively. Compound **3** and **4** had shown promising fungitoxity against *F. moniliforme* with EC_{50} value of 0.58 µmol/ml that is comparable to standards. Hence, the chlorobenzo[d]thiazol-2-ylcarbamodithioates containing lower groups on S-side of carbamodithioate can be explored as substrates for synthesis of better fungicidal agent used against *D. oryzae*

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Table 1. Antifungal potential of alkyl (benzo[d]thiazol-2-yl)carbamodithioates (3-8)

	EC_{50} values (µmol/ml)			
Compound No.	***P. grisea***	***D. oryzae***	***F. moniliforme***	***U. virens***
3	**0.76**	**0.13**	**0.32**	**0.58**
4	**0.69**	**1.45**	**0.58**	**1.73**
5	**0.67**	**0.20**	**0.90**	**0.69**
6	**0.82**	**2.40**	**1.40**	**1.32**
7	**0.38**	**0.23**	**1.13**	**0.65**
8	**1.08**	**1.00**	**1.16**	**1.48**
9	**0.52**	**2.11**	**1.12**	**0.74**
10	**0.78**	**2.56**	**1.17**	**1.31**
Bavistin*	**-**	**-**	**0.78**	**-**
Tilt**	**0.20**	**0.15**	**-**	**0.18**

Standard fungicide against,*F. moniliforme

**** Standard fungicide against *P. grisea, U.virens* and *D. oryzae.***