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Synthesis,Characterization and Cytotoxic Activity of Noveldithiocarbamates

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Abstract: The present work emphasizes the synthesis, characterization of Arylsulfonyldithiocarbamates. The six synthesized dithiocarbamate compounds were purified by recrystallisation or thin layer chromatographic techniques. All the synthesized compounds were characterised by physical and spectral methods (^{1}H-NMR and ^{13}C-NMR).The present study also aimed to evaluate the cytotoxic activity of arylsulfonyldithiocarbamates attached to different heterocyclic amines by standard assay using Bengalgram seed radical model were compared with the standard drug cyclophospomide and control.
Key words : Arylsulfonyldithiocarbamates, characterisation, cytotoxic activity, radical model, cyclophospomide, control.

1. Introduction:

Cancer is a prevalent cause of death worldwide. Every year, several natural and synthetic compounds are tested for various anticancer activities. Dithiocarbamates are a group of organosulfur compounds that have extensively been used asantimmitotic agents.Compoundshaving sulphur containing functional groups along with other functional groups are of massive interest due to more than one pharmacophore within the molecule [1].In recent years several reports have been published based on thedithiocarbamates, in which they have been utilized to generate newer chemical scaffolds meant for promising biological activity.

Some Pyrrolidinedithiocarbamates were reported that they show inhibitory ability againstmurine colon adenocarcinoma bearing mice through the inhibition of nuclear factor kB in the tumor tissue [2-3].The antimycobacterial activity of Pyrrolidinedithiocarbamates and dialkyldithiocarbamate derivatives have been demonstrated [4-7].A novel class of benzimidazoledithiocarbamate and chalconedithiocarbamate derivatives has also been reported in literature and these molecules can be considered as potent antimitoticagents [8].The thiocarbamateclass of compounds was active against dermatophytes and is used clinically as a topical treatment for the fungal infection[9].LalitKumar*etal.*[10]reported some spermicidal agents likepiperidine containing dithiocarbamatehybridsof2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethane as 2-(2-Methyl-5-nitro-1H-imidazol-1-yl) ethyl-4-substituted piperidine-1- carbodithioates.Fabrizio Carta*et al.* [11] have reported *in vivo* antiglaucoma action of synthesized DTCs due to their carbonic anhydrase (CA) inhibitory potential.

In view of the importance of potent biological activities and structural diversity of dithiocarbamates, the author was proposed to synthesize new arylsulfonyldithioccarbamates by adopting appropriate synthetic methodology.

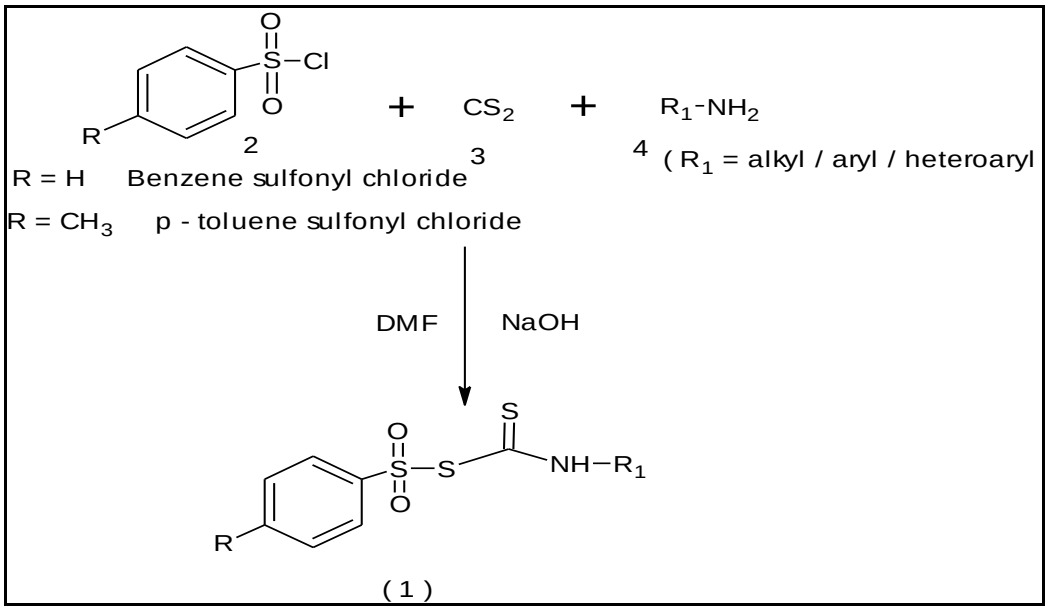
2. Experimental:

2.1. Chemicals andInstrumentation:

All solvents and reagents were purifiedby standard techniques. All evaporationof solvents was carried outunder reduced pressureon rotaryevaporator below 45°C.All chemicals(sigma Aldrich and E-Merck) used in the present work are LR grade chemicals.

Proton Nuclear Magnetic Resonanceand[13] C-NMR spectra are recorded on varian**Gemini-200, varianunity-200 and advance-400MHZ Bruker UX-NMR** instrument. The samples are made in chloroform-d (1:1) or/and DMSO-d6 using tetramethylsilane (Me_4Si) as the internal standard and are given in the d scale. Thin-layer Chromatography (TLC) is performed on pre coated **silica-gel-60F.**Visualisation of the sports on TLC plates is achieved either to iodine vapour or UV light.

2.2. **Proposed scheme:**



Scheme 1

2.3.Experimental procedure for the preparation of heterocyclic amines:

2.3.1 Synthesis of 2-amino benzothiazole (I):

Step I: Synthesis of Phenylthiourea:

To aniline (25mL), conc. Hydrochloric acid (25mL) was added and the solution was warmed. A saturated solution of ammonium thiocyanate in water (30g in 60mL) was added slowly in above solution. The mixture was boiled until the solution got turbid. The turbid solution was poured in cold water. The separated precipitate as phenylthiourea was filtered and crystallized from aqueous ethanol (80%) so as to obtain the pure compound.

Step II: Synthesis of 2-amino benzothiazole:

The phenylthiourea (15g, 0.098mol) in chloroform (75mL) was brominated by using bromine solution in chloroform (5%) till the orange – yellow color appeared. The slurry is kept aside overnight and filter. The precipitate is washed with chloroform and dissolved in rectified spirit. The solution is basified with ammonia to get the crude product which is filtered and recrystallized with aqueous ethanol to get I.

2.3.2.Synthesis of 2-phenyl-3-amino-quinazolin-4-one (II):

Step-1: Method of synthesis of 2-phenyl-3, 1-benzoxazin-4-one:

Anthranilic acid (0.05mol) was dissolved in 30 mL pyridine and benzoylchloride(0.075mol) was added drop wise. The reaction mixture was stirred for 2 h at room temperature until the reaction was completed by monitoring with TLC. The reaction mixture was neutralized with saturated sodium bicarbonate solution and the solid separated in each case was filtered and dried. The crude compounds thus obtained were purified by recrystallisation from ethanol to get 2-phenyl-3H-benzoxazin-4-one.

Step-2:Synthesis of 3-amino-2-phenylquinazolin-4(3H)-one:

Tothe solution of benzoxazin-4-one (0.05mol) in 50mL of ethanol, hydrazine hydrate (0.15mol) was added and the mixture was stirred and refluxed for 1 h. The reaction mixture was cooled to room temperature. The solid formed was filtered and dried. The crude compounds thus obtained were purified by recrystallisation from ethanol to get compound II.

2.3.3. Synthesis of 2- amino-benzoxazole- 5-carboxylate (III):

Step I:Synthesis of 4-carbomethoxy-2-nitrophenol:

To a solution of aluminium nitrate (40grms) in acetic acid- acetic anhydride (1:1) mixture (160mL), an appropriate phenol (40grms) in small portions was added and cooled by shaking occasionally. The reaction mixture was left at room temperature for 1.5 hours while shaking the contents intermittently to complete the nitration. The resulting brown solution was diluted to complete the nitration. The resulting brown solution was diluted with ice-cold water and acidified with concentrated Nitric acid to get a bulky, yellow precipitate. It was filtered washed with small quantity of methanol and purified by recrystallisation from alcohol to get a yellow crystalline solid (44g, 85%), melting point 73^{O}C.

Step II:Synthesis of 4-carbomethoxy-2-aminophenol:

4-Carbomethoxy-2-nitrophenol (10 grams) was dissolved in boiling alcohol (50%, 100mL) and sodium dithionite was added to this boiling alcohol solution until it becomes almost colourless. Then the alcohol was reduced to one-third of its volume by distillation and the residual liquid was triturated with crushed ice. The resulting colourless, shiny product was filtered, washed with cold water and dried in the air. Its purification was effected by recrystallisation from benzene to get colourless, shiny scales (5.1 g; 60%) melting point 143^{O}C.

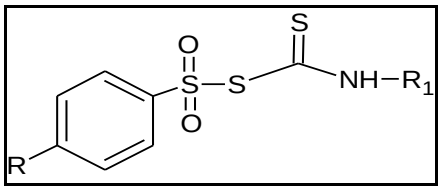
Step III:Synthesis of Methyl-2-aminobenzoxazole-5-carboxylate:

4-Carbomethoxy-2-aminophenol (1.3 moles) was dissolved in 1lit. Methyl alcohol and cooled the solution to 5^{O}C by adding chopped ice. A cold suspension of cyanogenbromide (1.5moles) in 1lit.of water was added over a period of 5min with rapid stirring. The reaction mixture was stirred for 0.75hrs at room temperature, solid sodium bicarbonate (1.3moles) in small portions over a period of 1.5 hrs was added to bring the p^{H} 6.5 -7.0. Stirring was continued for another 1hour. The solid was separated by filtration, washed with cold water and on recrystallisation from ethyl alcohol has resulted white solid (III), yield 70% and melting point is 238^{O}C.

2.4. Procedure for the synthesis of Arylsulfonyldithiocarbamates (Ia,b-IIIa,b):

Hetero cyclic amine prepared (1Eq)(I/II/III) is dissolved in dimethyl formamide (DMF) and Carbon disulphide (1 Eq). NaOH (20M) is added to the mixture and stirred at r. t for about ½ hr. After 30min.Sulfonyl chloride (2 Eq) is added and stirred at room temperature for 2-3 hours to obtain the product.

The author has prepared successfully various titled compounds such as Ia,b to IIIa,b using heterocyclic amines prepared (I to III) and aryl sulfonyl chloride by adopting the above procedure.



R = -H (a) R = -CH_3 (b) R_1 = Heterocyclic amine prepared

2.5. Spectral characterisation of Arylsulfonyldithiocarbamates (Ia,b to IIIa,b):

Compound Ia: ^{1}H-NMR(500MHz,$CDCl_3$+DMSO):δ7.54(m, J=7.2 Hz,J=7.4 Hz, 2H),7.93(m, J=1.9 Hz, J=7.4 Hz, 2H),7.34(s, J=7.2Hz, J=1.9Hz, 1H), 4.03 (bs,1H),8.24(s, 1H), 7.56(s, 1H), 7.55(s, 1H), 8.12(s, 1H) ; ^{13}C-NMR (125MHz, $CDCl_3$+DMSO-*d*6): δ129.8, 133.8, 128.4, 138.6, 196.2, 174.7, 124.6, 149.0, 121.8, 121.9, 125.3, 125.9.

Compound Ib: ^{1}H-NMR(500MHz,$CDCl_3$+DMSO):δ7.34(m, 2H, J=7.0 Hz, J=1.1 Hz),7.83(m, 2H, J= 7.0Hz, J= 1.1 Hz), 2.35 (s, 3H), 4.03(bs, 1H),8.12(s, 1H), 7.56(s, 1H),7.55(s, 1H), 8.24(s, 1H); ^{13}C-NMR (125MHz, $CDCl_3$+DMSO-*d*6): δ24.3, 143.4, 130.1, 128.2, 135.6, 196.2, 174.7, 124.6, 149.0, 121.8, 121.9, 125.3, 125.9.

Compound IIa: ^{1}H-NMR(500MHz,$CDCl_3$+DMSO): δ7.54(m, J=7.2 Hz,J=7.4 Hz, 2H),7.93(m, J=1.9 Hz, J=7.4 Hz, 2H),7.34(s, J=7.2Hz, J=1.9Hz,1H), 4.03(bs,1H,),7.92(s, 1H), 7.56(s, 1H), 7.41(s, 2H),7.26 (s, 5H);^{13}C-NMR (125MHz, $CDCl_3$+DMSO-*d*6): δ 133.8, 129.8, 128.3, 138.6, 200.7, 160.8, 147.1, 127.5, 120.8, 122.5, 128.8, 127.4, 133.5, 125.0, 127.5, 127.4, 127.6.

Compound IIb: ^{1}H-NMR(500MHz,$CDCl_3$+DMSO): δ 7.34(m, 2H, J=7.0 Hz, J=1.1 Hz),7.83(m, 2H, J= 7.0Hz, J= 1.1 Hz), 2.35 (s, 3H), 4.03(bs, 1H),7.92(s, 1H), 7.43(s, 2H),7.55(s,1H),7.28(m, 5H);^{13}C-NMR (125MHz, $CDCl_3$+DMSO-*d*6): δ 24.3, 143.4, 130.1, 128.2, 135.5, 200.6, 160.7, 127.6, 120.9, 122.4, 128.9, 127.4, 133.9, 125.2, 127.4, 126.2, 126.8.

Compound IIIa: ^{1}H-NMR(500MHz,$CDCl_3$+DMSO): δ7.54(m, J=7.2 Hz,J=7.4 Hz, 2H),7.93(m, J=1.9 Hz, J=7.4 Hz, 2H),7.34(s, J=7.2Hz, J=1.9Hz, 1H), 4.03(bs, 1H),7.95(s,1H), 7.37(s,1H), 7.86(m,2H), 3.58(s, 3H); ^{13}C-NMR (125MHz, $CDCl_3$+DMSO-*d*6): δ133.8, 129.8,128.3, 200.1, 153.7, 141.4, 120.6, 110.6, 124.6, 134.3, 199.8, 69.4.

Compound IIIb: ^{1}H-NMR(500MHz,$CDCl_3$+DMSO):δ7.34(m, 2H, J=7.0 Hz, J=1.1 Hz),7.83(m, 2H, J= 7.0Hz, J= 1.1 Hz), 2.35 (s, 3H), 4.03(bs, 1H),7.95(s, 1H), 7.37(s,1H), 7.86(s, 2H), 3.58(s, 3H); ^{13}C-NMR (125MHz, $CDCl_3$+DMSO-*d*6): δ24.3, 143.4, 130.2, 128.3, 200.1, 153.7, 141.4, 120.6, 110.6, 124.6, 134.3, 199.8, 69.4.

2.6. Biological activity study (Cytotoxic activity):

The present study is aimed to evaluate the cytotoxic activity of arylsulfonyldithiocarbamates attached to different heterocyclic amines by standard assay using Bengalgram seed radical model.

Bengal gram seeds (Ciceraurantinum) (locally available in the market),Petridishes (HI media, Mumbai, India), Dimethyl sulfoxide (DMSO) (E.Merk, Mumbai, India), and Drug concentrations (10µg to 100µg/mL) were used in the present study.

Bengal gram seeds of a good quality were taken and soaked overnight with water to hasten the germination process.The next day the seeds were distributed in a group of ten each in petridishes on moistened filter paper.Solutions of synthesized compounds (**$I_{a,\ b}$ to $III_{a,\ b}$**) were prepared in 10% methanol at concentrations ranging from 10µg/mL, 20µg/mL to 50µg/mL and added to the filter paper in the petridishes.

Commercially available cyclophosphamide was taken as a standard drug and 10 µg/mL, 20µg/mL concentrations of standard drug were used.Then after germination of seeds, a group of 10 seeds were distributed in each petri dish.For every 24hrs, 1mL of 10, 20, 50µg/mL of test compounds **$I_{a,b}$ to$III_{a,\ b}$** and 10, 20µg/mL of the standard drug were added to the petridishes and labeled accordingly. 2mL of 10% methanolic solution was added to the seeds in one petri dish which served as control.The treatment with test compounds and standard

was continued for two days.The length of the radicals was measured in cm at the end of the second day.The length of the radicals in the germinated seeds, treated with test compounds was compared with that of standard and control. The cytotoxic results of titled compounds at various concentrations such as10µg/mL, 20µg/mL and 50µg/mL against length of germinating roots of Bengal gram were shown in **Table-1.** Percentage inhibition values were calculated.

Table 1:Inhibition of germination of the root in Bengalgram seeds.

S.No	Compounds	Length of the germinating roots in (cm)		
		Concentration 1 (10µg/mL)	**Concentration 2 (20µg/mL)**	**Concentration 3 (50µg/mL)**
1	Ia	2.6±0.32	2.4±0.43	2.1±0.32
2	Ib	2.5±.021	2.3±0.33	2.0±0.31
3	IIa	2.7±0.22	2.5±0.14	2.2±0.21
4	IIb	2.6±0.10	2.4±0.11	2.3±0.19
5	IIIa	2.9±0.30	2.7±0.34	2.5±0.32
6	IIIb	2.9±0.09	2.6±0.12	2.3±0.12
7	cyclophospomide	2.0±0.24	2.5±0.12	0
8	control	Length of germinating roots in cm is 5.0±0.32		

3. Results and Discussion:

New arylsulfonyldithiocarbamates were synthesized as mentioned in the scheme 1, and all compounds synthesized were obtained in good yields and were purified by recrystallisation. Titled compounds were analysed by ^{1}H-NMR and carbon-13 NMR spectroscopy.The synthesized derivatives were evaluated for their potential cytotoxic activity using Bengalgram seeds. The synthesized compounds were found to inhibit the growth of the germinating roots of the seeds.The various heterocyclic amines were found to exhibit the activity which is equal to the half of the activity exhibited by the standard drug cyclophospomide.

4. Conclusions:

Dithiocarbamates are valuable synthetic intermediates found in a variety of biologically active compounds, which may constitute interesting medicinal and biological properties. The author in the present investigation synthesized six new arylsulfonyldithiocarbamates using Bergillini reaction. All the titled compounds were characterized by ^{1}H-NMR and^{13}C-NMR data. Cytotoxic activity of arylsulfonyldithiocarbamates attached to different heterocyclic amines was evaluated by standard assay using Bengalgram seed radical model. The synthesized compounds were found to inhibit the growth of the germinating roots of the Bengal gram seeds. The various heterocyclic amines were found to exhibit the activity which is equal to the half of the activity exhibited by the standard drug cyclophospomide. Very recentDTCapplications reported includeitspotentialforuseasanti-HIVmicrobicidalvaginalcontraceptive. Thus,afurtherresearchonDTCmightleadtonovelchemicalscaffoldswithdiversebiologicalpotential.

5. References:

1. WHO, Dithiocarbamate Pesticides, Ethylenethiourea and Propylenethiourea: A General Introduction, WHO, Geneva,1988.
2. Wang, D., Lippard,SJ., Nat. Rev. Drug Discov, 2005, 4, 307-320.
3. Jung, Y.,Lippard, SJ.,Chem. Rev, 2007, 107, 1387-1407.
4. Cvek, B.,Milacic,V.,Taraba,J.,Dou,QP., J. Med. Chem,2008, 51, 6256-6258.
5. Elgemeie,GH,Sayed,SH., Synthesis, 2001,1747-1771.
6. Scozzafava, A., Mastrolorenzo,A.,Supuran, CT., Bioorg. Med. Chem. Lett., 2000, 10, 1887-1891.
7. Gaspari, P.,Banerjee,T.,Malachowski,WP., Muller,AJ., Prendergast,GC., Duhadaway, J., Bennett, S.,Donovana, AM., J. Med. Chem, 2006, 49, 684-692.
8. Bodenner,DL.,Dedon, PC.,Keng, PC.,Borch, R.,Cancer Res,1986, 46, 2745-2750.

9. Workshop on HIV/AIDS and adult mortality in developing countries: UN/POP/MORT/2003/13.
10. Kumar, L., Jain, A.,Lal, N.,Sarswat, A.,Jangir, S., Kumar, L., Singh, V., Shah, P., Jain, S.K.,Maikhuri, J.P.,Siddiqi, M.I., Gupta, G., Sharma, VL., Potentiating Metronidazole Scaffold against Resistant Trichomonas:Design,Synthesis,Biologyand3D-QSARAnalysis, J.Med.Chem,2012,3,83-87.
11. Carta, F.,Aggarwal, M.,Maresca, A.,Scozzafava, A., McKenna, R.,Masini, E.,Supuran, CT., Dithiocarbamates strongly inhibit carbonic anhydrases and show antiglaucoma action in vivo,J. Med. Chem, 2012,*55*, 1721-1730.

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