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Synthesis of new derivatives of 2-Substituted 1,5-Benzodiazepine and Evaluation of their Anti-microbial activities

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ABSTRACT

In order for our synthetic plan to succeed to give the above 2-subsituted 1, 5 benzodiazepine derivatives, we required a good synthesis of 2-thiomethyl ether substituted analogue of face 'c' cyclohexano annulated 1, 5- benzodiazepine. An innovative protocol to the synthesis of this material emerged on exploring the potential of the ketene dithioacetal derivative of cyclohexanone, on its reaction with o-phenylenediamine. Nucleophilic development of 2-iminothiomethylether function with the vital fragments of etravirine such as p-aminobenzonitrile, 2, 6-dichloro-4-amino pyrimidine, 6-(p-cyanophenylamino)-2-chloro-4-amino pyrimidine, p-acetyl, dimethylamino metyleneketone, pyrazole and isoxazole derivatives etc afforded the corresponding 2-substituted 1, 5-benzodiazepine analogues, in acceptable yields. The purity of the compounds was checked by TLC and their structures were established on the basis of their spectral data.

Keywords: 1,5-benzodiazepine, o-phenylenediamine(OPD), Antibacterial, Antifungal activities.

INTRODUCTION

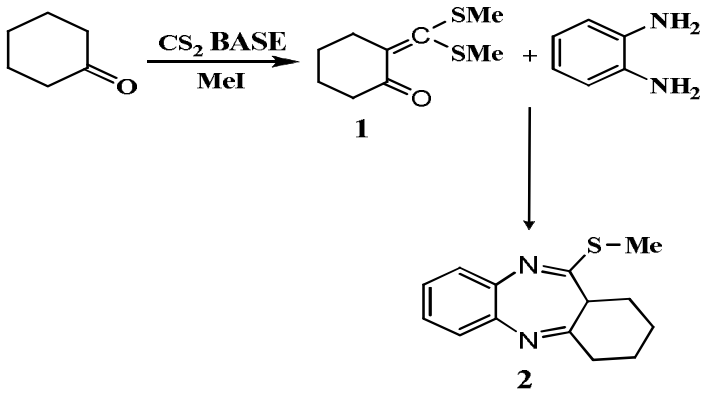
Much attention has paid to the synthesis of nitrogen containing heterocyclic compounds like benzodiazepines, mainly due to their broad spectrum biological and pharmacological activities. Benzodiazepine derivatives are important compounds family with various biological properties. They have attributed to many pharmacological activities among which are tranquilisant, antiviral, anti-inflammatory, analgesic, antipyretic and anticonvulsant [1-12].

Benzodiazepine adducts are also considered as important precursors in the synthesis of benzimidazole, pyrazole, isoxazole and quinoxaline derivatives [13-18]. Additionally, they are valuable synthons for the synthesis of various fused ring benzodiazepine derivatives[19-21] Although, the first benzodiazepine was introduced as a drug nearly 35 years ago, the research in this area is still very active and is directed towards the synthesis of compounds with enhanced pharmacological activity. The discovery of diazepam followed by many other psychotropic agents sharing a 1,4-benzodiazepines skeleton has also promoted the studies on the isomeric 1,5-benzodiazepine ring system[22] along with the synthetic approaches to mono and diannelated 1,5-benzodiazepines[23] due to their accessibility, easily functional and potential pharmacological properties, mainly1,5-benzodiazepines and 1,5-benzodiazepinone derivatives have received significant attention. The use of 1, 5-benzodiazepines have been extended to several diseases such as cancer, viral infection and cardiovascular disorders [24-27].

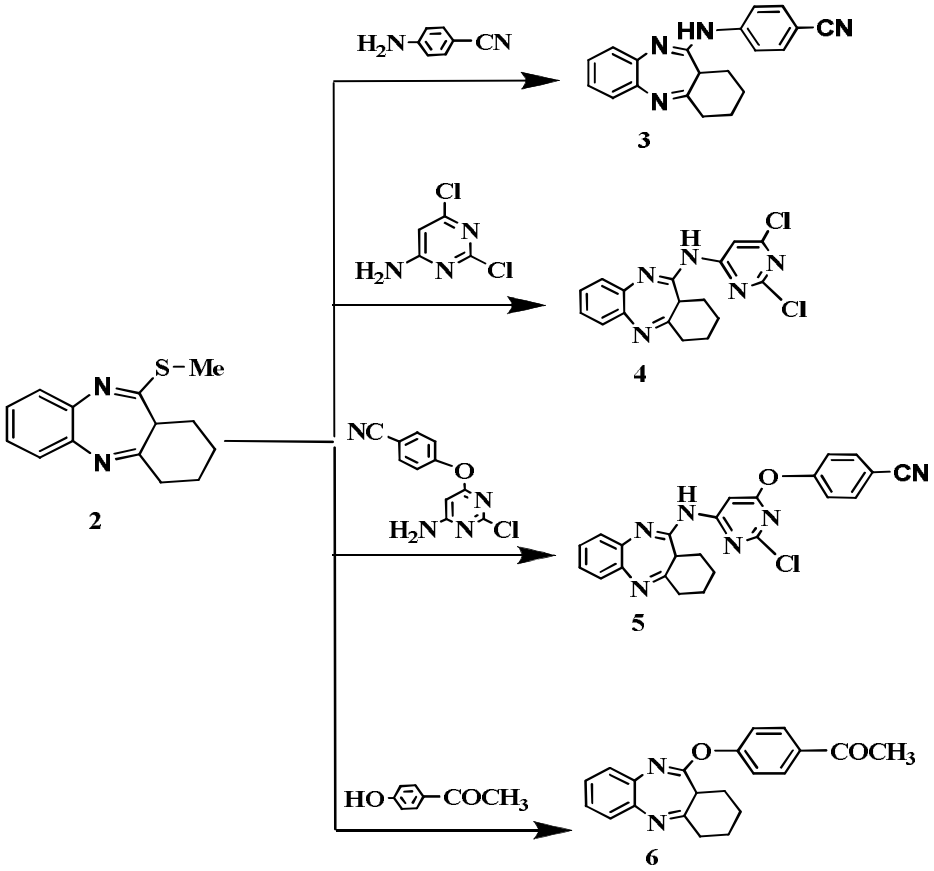
MATERIALS AND METHODS

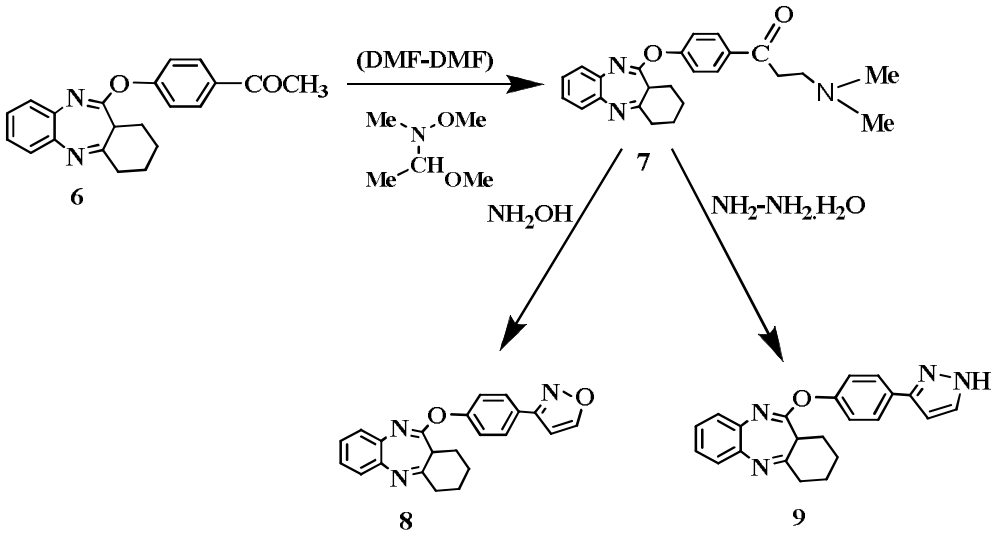
Experimental : Melting points were determined in an open capillary and are uncorrected. The IR spectra were recorded on Schimadzu FTIR-8400S. ^{1}H-NMR spectra were recorded in $CDCl_3$ on Bruker DRX300 MHz. spectrometer using TMS as internal reference with their values expressed in d ppm. Purity of all the synthesized compounds were routinely checked by TLC on silica gel G in the solvent system (9:1, benzene: methanol).

General procedure and spectral data of the compounds



Scheme-1





Scheme-2

Preparation of 2-(bis(metylthio)methylene)cyclohexanone,1 : A mixture of cylohexanone **1** (2.04g,0.006mole) and **CS_2** 1.0ml, (0 .006 moles) was added to a well stirred and cold suspension of t-Buok ,1.34g(0.012moles) in dry benzene (4.0ml) and DMF (3.0ml) and the reaction mixture was allowed to stand at room temperature for 4 hours. Methyl iodide (2.0ml, 0.012moles) was gradually added with stirring and external cooling (exothermic reaction) and the reaction was allowed to stand for 4 hours at room temperature with occasional shaking and then refluxed on a water bath for 3 hours. The aqueous portion was extracted with benzene and the combined extracts were washed with water, dried over anhydrous sodium sulphate and the solvent was removed by distillation. The product thus obtained was recrystallized ethanol, to give compound **1,**

The yield: 75%; m.p-30^{0}C. IR (KBR) 3010[C-H str. ArH], 1580[C=C str. ArH], 1550[C=N str.], 1HNMR 7.62-8.08[m, 4H, ArH], 1.41-1.78[m, 5H, cyclohexane]; m/z: 202.05 (100.0%), 203.05 (11.5%), 204.04 (9.0%).

Preparation of 11-(methylthio)-2,3,4,11a-tetrahydro-1H-dibenzo[b,e][1,4]diazepine,2:

To a mixture of oxyketenedithio acetal,1.29g (0.01mmol) in 20ml DMF and o-phenylenediamine 1.08g (0.01mol), the mixture was heated under reflux for 4-5h and then 1 ml of AcOH was added. The refluxing was continued for 1-2h. The solvent was distilled under reduced pressure and the residue was quenched in crushed ice. It was extracted with chloroform, washed with water and dried over anhydrous sodium sulphate to give **2.** The yield is 75%; m.p 130-132^{0}C. IR (KBR) 3280[N-H str.], 3020 [C-H str. ArH], 2210[CN str.], 1650[C=C str. ArH], 1580 [C=N str.] 1HNMR 9.89 [s, H, NH], 7.60-8.12[m, 4H, ArH], 7.39[d, 2H, phenoxyl], 6.81[d, 2H, phenoxyl],1.29-1.81[m,4H, cyclohexane]; m/z: 244.10 (100.0%), 245.11 (15.3%), 246.10 (4.6%), 245.10 (1.5%), 246.11 (1.2%).

Preparation of 4-(1,2,3,10a-tetrahydrobenzo[b]cyclopentane[e][1,4]diazepin-10-ylamino) benzonitrile,3 : 11-(methylthio)-2,3,4,11a-tetrahydro-1H-dibenzo[b,e][1,4]diazepine ,8 g (0.024 mol) and p-amino benzonitrile 3.808 g (0.025 mol) in 15 ml DMF was slowly added potassium-tert-butoxide (5.36 g, 0.048) in an ice-water bath, then stirred at room temperature for 6-7 h until reaction was completed. The mixture was poured into ice water and pH was adjusted to 6 with 5% aqueous HCl and the mixture extracted with EtOAc three times. After removal of organic solvent in vacuo, crude product was purified by TLC or a silica column (eluent: petroleum ether/EtOAc) to give **3,** The yield is 68% ; m.p. 165-167°C.IR(KBr) 3025[C-H str. ArH], 1530[C=C str.ArH], 1165[C-N str.],3380[NH str], 1690[C=N str], 1HNMR 7.33-7.45[m, 4H, ArH], 1.95 [S, 1H CH], 4.0[1S, C-NH], 6.81 [d, 2H, phenoxyl], 7.39 [d, 2H

phenoxyl], 1.28-1.92[m, 4H, cylohexane], m/z: 300.14 (100.0%), 301.14 (20.7%), 302.14 (2.3%), 301.13 (1.5%).

Preparation of N-(2,6-dichloropyrimidin-4-yl)-2,3,4,11a-tetrahydro-1H-dibenzo[b,e] [1,4] diazepin-11-amine,4 : 11-(methylthio)-2,3,4,11a-tetrahydro-1H-dibenzo[b,e][1,4]diazepine 8 g,(0.024 mol) and 2,6-dichloropyrimidin-4-amine 3.808 g, (0.025 mol) in 15 ml DMF was slowly added potassium-tert-butoxide 5.36 g, (0.048) in an ice-water bath, then stirred at room temperature for 6-7 h until reaction was completed. The mixture was poured into ice water and pH was adjusted to 6 with 5% aqueous HCl and the mixture extracted with EtOAc three times. After removal of organic solvent in vacuo, crude product was purified by TLC or a silica column (eluent: petroleum ether/EtOAc) to give **4.** The yield is 65% yield; m.p. 180-182°C. IR(KBr) 3025[C-H str. ArH], 1530[C=C str.ArH], 1165[C-N str.],3380[NH str], 1690[C=N str], 1HNMR 7.33-7.45[m, 4H, ArH], 6.95 [S, 1H CH], 6.496 [S, 1H CH], 4.0[1S, C-NH], 1.28-1.92[m, 4H, cylohexane], m/z: 359.07 (100.0%), 361.07 (64.3%), 360.07 (20.2%), 362.07 (11.9%), 363.06 (10.2%), 364.07 (1.9%), 361.08 (1.6%), 363.07 (1.2%), 362.06 (1.2%)

Preparation of 4-(2-chloro-6-(2,3,4,11a-tetrahydro-1H-dibenzo[b,e][1,4]diazepin-11-ylamino) pyrimidin-4-yloxy)benzonitrile 5 : 11-(methylthio)-2,3,4,11a-tetrahydro-1H-dibenzo[b,e][1,4]diazepine (8 g, 0.024 mol) and 1-(4-hydroxyphenyl)ethanone (3.808 g, 0.025 mol) in DMF (15 ml) was slowly added potassium-tert-butoxide (5.36 g, 0.048) in an ice-water bath, then stirred at room temperature for 6-7 h until reaction was completed. The mixture was poured into ice water and pH was adjusted to 6 with 5% aqueous HCl and the mixture extracted with EtOAc three times. After removal of organic solvent in vacuo, crude product was purified by TLC or a silica column (eluent: petroleum ether/EtOAc) to give **5.** 6 The yield is 68%; m.p-197-200°C. IR(KBr) 3025[C-H str. ArH], 1530[C=C str.ArH], 1165[C-N str.],1085[C-O str.], 1HNMR 7.33-7.45[m, 4H, ArH], 6.97 [d, 2H, phenoxyl], 7.71 [d, 2H phenoxyl], 1.95 [S, 1H CH], 5.69 [S, CH], 4.0[1S, C-NH], 1.28-1.92[m, 4H, cylohexane], m/z: 442.13 (100.0%), 444.13 (32.5%), 443.13 (28.2%), 445.13 (8.5%), 444.14 (3.5%), 446.13 (1.3%).

Preparation of 1-(4-(2,3,4,11a-tetrahydro-1H-dibenzo[b,e][1,4]diazepin-11-yloxy) phenyl) ethanone 6 : To a solution , 0.024 mol (8g) of **5** and 1-(4-hydroxyphenyl)ethanone 3.808 g, (0.025 mol) in 15 ml DMF was slowly added potassium-tert-butoxide 5.36 g,(0.048) in an ice-water bath, then stirred at room temperature for 6-7 h until reaction was completed. The mixture was poured into ice water and pH was adjusted to 6 with 5% aqueous HCl and the mixture extracted with EtOAc three times. After removal of organic solvent in vacuo, crude product was purified by TLC or a silica column (eluent: petroleum ether/EtOAc) to give **6.** The yield is 70% ; m.p-210-212°C. IR(KBr) 3025[C-H str. ArH], 1530[C=C str.ArH], 1569[C=N str.],1165[C-N str.],1085[C-O str.], 1HNMR 7.33-7.45[m, 4H, ArH], 6.81 [d, 2H, phenoxyl], 7.49[d, 2H phenoxyl], 1.95 [S, 1H CH], 2.50 [S, CH_3], 1.28-1.92[m, 4H, cylohexane], m/z: 332.15 (100.0%), 333.16 (23.0%), 334.16 (2.9%).

Preparation of 3-(dimethylamino)-1-(4-(2,3,4,11a-tetrahydro-1H-dibenzo[b,e] [1,4] diazepin -11-yloxy)phenyl)propan-1-one,7:1-(4-(2,3,4,11a-tetrahydro-1H-dibenzo[b,e][1,4]diazepin-11-yloxy)phenyl)ethanone 2.9g(15.7 mmol) was dissolved in *N, N*-dimethylformamide dimethyl acetal (15 ml), and the solution was heated under refluxed for 4 hours and concentrated. The residue was triturated with hexane, filtered, and washed with hexane to give as a powder to give **7.** The yield is70%, m. p-240-245°C. IR(KBr) 3038[C-H str. ArH], 2999[C-H str.], 1710[C=Ostr.] 1610[C=C str.ArH], 1569[C=N str.], 1499[C=C str. ArH], 1290[C-N str.], 1090[C-O str.], 1HNMR 8.06[d, 2H, phenoxyl], 7.53-8.12[m,4H, phenoxyl], 6.61[d, H, CH], 5.98[d, H, CH],3.04 [s, 6H, $N(CH_3)_2$], 1.29-1.79[m, 4H, cylohexane], m/z: 389.21 (100.0%), 390.21 (27.1%), 391.22 (3.3%).

Preparation of 11-(4-(1H-pyrazol-3-yl)phenoxy)-2,3,4,11a-tetrahydro-1H-dibenzo [b,e] [1,4]diazepine,8 : A mixture 3-(dimethylamino)-1-(4-(2,3,4,11a-tetrahydro-1H-dibenzo[b,e][1,4]diazepin-11-yloxy)phenyl)propan-1-one 1.97g(5mmol) in methanol was added hydrazine hydrate (0.50ml, 5mmol),

and the solution was heated under reflux for 1 h and concentrated . The residue was purified by column chromatography and eluted with CHCL3:2-propanol (10:1) to give brown powder to give **8.** The yield is 88%, m.p-180-182^{0}C. IR(KBr) 3025[C-H str. ArH], 1530[C=C str.ArH], 1569[C=N str.],1165[C-N str.],1085[C-O str.], 1585[C=N-O], 1HNMR 7.33-7.45[m, 4H, ArH], 6.81 [d, 2H, phenoxyl], 7.49[d, 2H phenoxyl], 1.95 [S, 1H CH], 6.75[S 1H isoxazole], 8.74[S 1H isoxazole], 1.28-1.92[m, 4H, cylohexane], m/z: 357.15 (100.0%), 358.15 (24.1%), 359.15 (3.4%), 358.14 (1.1%).

Preparation of 3-(4-(2,3,4,11a-tetrahydro-1H-dibenzo[b,e][1,4]diazepin-11-yloxy henyl) isoxazole 9 : Hydroxyl amine hydrochloride (2.78g, .04mol) was added to sodium methoxide 3.24g (0. 06mol) in 30 ml absolute methanol and stirred for 10 minutes. 3-(dimethylamino)-1-(4-(2,3,4,11a-tetrahydro-1H-dibenzo[b,e][1,4]diazepin-11- loxy)phenyl)propan-1-one ,1.77g (4mmol) was added and the mixture was refluxed for 5h. Most of the methanol was evaporated under reduced pressure and the mixture was poured in to ice-cold water. The solid separated was filtered, washed with diethyl ether and dried. Recrystallization from ethanol give **9**. The yield is 70% ; m.p. 105-107°C. IR(KBr) 3025[C-H str. ArH], 1530[C=C str.ArH], 1569[C=N str.],1165[C-N str.],1085[C-O str.], 3380[NH str], 1HNMR 7.33-7.45[m, 4H, ArH], 6.81 [d, 2H, phenoxyl], 7.49[d, 2H phenoxyl], 1.95 [S, 1H CH], 6.53[S 1H pyrazole], 7.52[S 1H pyrazole], 12.62[S, 1H, NH] 1.28-1.92[m, 4H, cylohexane], m/z: 356.16 (100.0%), 357.17 (24.1%), 358.17 (3.0%), 357.16 (1.5%).

RESULTS AND DISCUSSION

The results of the analytical data are given in table 1.

Tabel-1 Physical and Analytical data of the compounds (1-9).

Comp. No	Molecular formula	Molecular Weight	Melting Point °C	Yield (%)	Elemental analysis					
					C	H	N	S	O	Cl
1	$C_9H_{14}OS_2$	202.34	30	75	53.42	6.97	-	31.69	7.91	-
2	$C_{14}H_{16}N_2S$	244.36	130-132	75	68.81	6.6	11.46	13.12	-	-
3	$C_{20}H_{18}N_4$	314.15	165-167	68	76.41	5.77	17.82	-	-	-
4	$C_{17}H_{15}Cl_2N_5$	359.07	180-182	65	56.68	4.2	19.44	-	-	19.68
5	$C_{24}H_{19}ClN_6O$	442.13	197-200	68	65.08	4.32	18.97	-	3.61	8
6	$C_{21}H_{2O}N_2O_2$	332.4	210-212	70	75.88	6.06	8.43	-	9.63	-
7	$C_{24}H_{27}N_3O_2$	389.49	240-245	70	74.01	6.99	10.79	8.22	-	-
8	$C_{22}H_{19}N_3O_2$	357.41	180-182	88	73.93	5.36	11.76	-	8.95	-
9	$C_{22}H_{20}N_2O$	336.47	105-107	70	74.14	5.66	15.72	-	4.49	-

Antimicrobial activity of compounds (3-9) : The newly synthesized benzodiazepine compounds have been screened for antimicrobial activities against bacterial species (E.coli and B. subtilis) and fungal species (A.niger) by Agar-well diffusion method against the standard drugs (Streptomycin / Ampiciilin) for bacteria and fluconazole for fungi). In antibacterial studies, the stock solution of standard and test compounds were prepared in Methanol and subsequent dilutions were made with the same solvent.

All the synthesized compounds gave satisfactory results of C, N and S analysis. IR and ^{1}H-NMR Spectral data were found to be consistent to the assigned structures. The physical and analytical Data of the compounds are presented in Table-1. The antibacterial activity was evaluated against two pathogenic

strains (*E. coli* and *B. subtilis*). The zone of inhibition and activity index were determined by comparison with the standard drugs Flucanazole, Streptomycin / Ampiciilin. The outcome of this study is presented in table-2**.** The antibacterial screening against B.subtilis compound **(9)** displayed highest activity in µg/ml. The compound **(6)** showed minimum activity in ug/ml, in all compounds. The remaining compounds showed only moderate activity. Contrary to this observation, compound **(5)** showed highest activity in ug/ml in screened for this activity against E.coli.

Table 2. Results of Screening antibacterial activity of products 3-9 of 1, 5-benzodiazepine in µg ml $^{-1}$.

Comp No.	Con in E .coli/ B.septalus 1mg ml $^{-1}$	Zone of inhibition in Streptomycin / Ampiciilin Diameter(mm)		Standard Fluconazole 1mg ml $^{-1}$	Zone of inhibition in mg/ml A. Niger Diameter(mm)
3	13	17	_	12	16
4	16	21	_	15	18
5	18	25	_	12	17
6	15	_	11	13	21
7	12	_	25	14	20
8	15	_	25	16	19
9	15	_	28	15	24

The antifungal activity was evaluated against pathogenic strains (A. Niger). Compound (7) exhibited highest activity compare than standard drug and compound **(6)** showed lowest activity and **(9)** displayed highest activity in ug/ml amongst all the compounds. The remaining compounds showed only moderate activity.

APPLICATIONS

The synthesized compounds have better potential antibacterial and antifungal activities than the commercially available cyclohexanone, o-phenyldiamine .

CONCLUSIONS

In conclusion, we have synthesized various derivative of 1,5-diazepines with good yields. The main advantage of this method is that reactions were found clean and had operational simplicity. In conclusion, several derivatives of 1,5-benzodiazepine (3-9) displaying potential antibacterial and antifungal activities were synthesized from the commercially available cylohexanone, o-phenyldiamine by the condensation reaction.

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