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MICROWAVE INDUCED SYNTHESIS OF NOVEL BENZO [1,4] DIAZEPINES AS POTENTIAL ANTITUBERCULAR AND ANTITUMOR AGENTS

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A series of some benzodiazepine derivatives were prepared from chalcones and screened for *in vitro* anticancer and antitubercular activities. The conventional and microwave methods for the synthesis have been described in this paper. Antitumor activity was carried by tryphan blue dye exclusion method and antitubercular activity by microplate alamar blue assay. The structures of the compounds were characterized by IR, NMR and Mass spectral analysis.

Benzodiazepines and their derivatives are an important class of bioactive molecules and are widely used as anticonvulsant[1], antianxiety[2], antidepressant[3], analgesic[4], antiinflammatory[4], sedative[5], hypnotics[6], and antileishmanial[7] agents and some of them are known for their antitubercular activity[8].

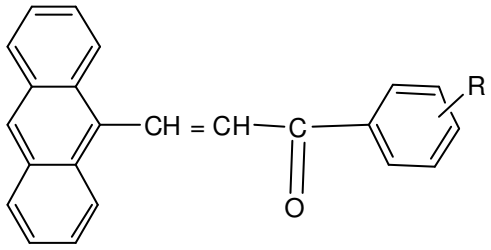
The adverse effects of first and second-line antituberculosis drugs, the increased occurrence of multi-drug resistant strains of *M. tuberculosis*, have led to renewed research in the hope of discovering new antitubercular leads[9]. Similarly benzodiazepines and their derivatives have been reported with anticancer activity. The naturally occurring pyrrolo [1,4] benzodiazepines (PBDs) have generated immense interest as potential anticancer and gene-targeting agents[10,11]. These have aroused a new interest in this field. Hence for the purpose of obtaining potent antitubercular and anticancer compounds we have synthesized and evaluated some benzodiazepine derivatives.

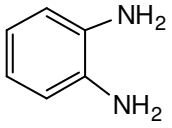
The condensation reaction of aromatic *o*-diamines with 1,3-dielectrophilic compounds such as α,β-unsaturated ketones (chalcones) corresponds to one of the most appropriate approaches among the different methods for the synthesis of 1,4-benzodiazepine derivatives[12].

On the other hand, microwave-assisted reaction have gained considerable importance because of the simplicity in operation, milder reaction conditions, increasing reaction rates and cleaner products formation.

Herein is reported the synthesis of benzo [1,4] diazepines by means of both conventional and microwave irradiation methods in an attempt to significantly improve biological spectrum of benzodiazepines. All the novel compounds were evaluated for their *in-vitro* antitubercular and anticancer activities.

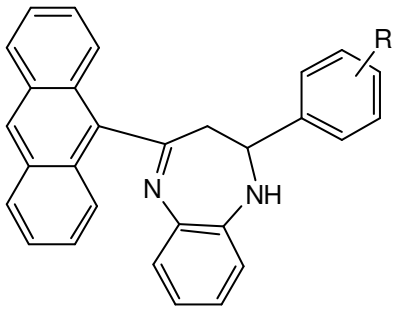
Structures of these products were established on the basis of spectral datas. In the IR spectrum of compound (BD2) the disappearance of characteristic absorption corresponding to carbonyl at 1694 cm^{-1} of unsaturated enones and appearance of bands at 3545 and 1650 correspond to N-H and C=N stretching respectively proved the formation of cyclic product. Further proof came from NMR spectrum which shows the singlet at 3.29 (s, 1H, NH) and 2.07 (s, 1H, -CH) for diazepines. The structure of the compound (BD2) was further ascertained through, mass spectrum for (M+2) at 478 which further confirm the formation of the compound. *In-vitro* anticancer studies for the synthesized benzodiazepines revealed that compounds BD1 and BD4 induced the greatest effect





o-phenylene diamine

Glacial acetic acid,
DMF/ Ethanol
Microwave irradiation (5-6 min. 800 W)/
Conventional synthesis (5-6 hr, Reflux)



R=Br,F,Cl, NH_2, NO_2

SCHEME-1

Table-1
Physical datas of synthesized benzodiazepines

Compd	R	Physical state	M.P. (^{0}C)	Yield (%)
BD1	4-Cl	Bright yellow crystals	154-156	72
BD2	4-Br	Yellow crystals	175-77	67
BD3	4-NO_2	Bright red crystals	200-202	72
BD4	4-F	Light yellow crystals	188-190	75
BD5	4-NH_2	Yellowish red crystals	189-192	69

on EAC cells. Among the compounds tested for antitubercular studies, BD1 and BD2 showed only moderate activity when compared with standard.

Antitumor activity by tryphan blue dye exclusion method[13,14]

The synthesized compounds were tested for their cytotoxicity *in-vitro*, in comparison with 5-fluorouracil as reference drug, against EAC cells. EAC cells ($1x10^6$) were incubated with synthesized compounds at various concentrations of 25, 50, 100, 200 μg/ml, in 1 ml phosphate buffered saline (incorporated with 10 μL DMSO) at 37^0 for 3 hr. Viable cells were counted in a haemocytometer using the tryphan blue dye exclusion method. Experiments were carried out in triplicate. The results are given in Table-3.

Antitubercular activity using microplate alamar blue assay[15]

The antimycobacterial activity of compounds was assessed against *M. tuberculosis* using microplate Alamar Blue Assay (MABA). The 96 well plates received 100 μl of the Middlebrook 7H9 broth containing *Mycobacterium tuberculosis* and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 0.2 to 100.0 μg/ml and standards used are Streptomycin and Pyrazinamide. Plates were covered and sealed with parafilm and incubated at 37^0 for seven days. After this time, 25 μl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hr. A blue color in the well was interpreted as of no bacterial growth, and pink color was scored as growth. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink. The MIC data is given in Table-4 and 4.1.

Experimental

Melting points were determined by capillary method and are uncorrected. The IR spectra were recorded on a SHIMADZU PERKIN ELMER 8201 PC IR spectrometer using a thin film on potassium bromide pellets techniques and frequencies are expressed in cm^{-1}.

The PMR spectra were recorded on a Bruker Avance II 400 NMR spectrometer. All spectra were obtained in $CDCl_3$ and DMSO. Chemical shift values are reported as values in ppm relative to TMS (δ=*0*) as internal standard. The FAB mass spectra were recorded on JEOL SX-102/DA-6000 Mass spectrometer using Argon/Xenon (6Kv, 10Ma) as the FAB gas. Final compounds have been analysed for elemental analysis, results were found to be within ± 0.4%.

Synthesis of benzodiazepines-conventional method : General methods

A mixture of 3-(anthracenyl-9-yl)-1-(aryl substituted) prop-2-en-1-one (0.01 mol) and

Table-2
Reaction time and yield of conventionally and microwave assisted synthesis of benzodiazepines

Compd	Conventional synthesis		Microwave assisted synthesis	
	Time (hr)	Yield (%)	Time (min)	Yield (%)
BD1-BD5	6	52-75	5-6	73-84

Table-3
Cytotoxicity activities of substituted benzodiazepines (BD1-BD5) by tryphan blue exclusion method

Compd	R	No. of dead cells (%) at different concentration µg/ml			
		20µ	50µ	100µ	200µ
Control	-	1	-	-	-
BD1	4-Cl	14	23	42	73
BD2	4-Br	18	28	44	64
BD3	4-NO_2	0	4	8	16
BD4	4-F	15	29	54	76
BD5	4-NH_2	3	7	22	35
Standard	5-Fluorouracil	35	50	90	98

orthophenylenediamine (0.01 mol) in ethanol (20 ml) were refluxed with catalytic amounts of gl acetic acid for 5-6 hr. The completion of reaction was monitored by TLC in chloroform and ethylacetate (3:1). The reaction mixture was cooled and poured into crushed ice with constant stirring. The solid mass thus obtained was filtered and washed with water and recrystallized from ethanol to get the compounds (BD1-BD5) Scheme-1.

Microwave irradiation method

A mixture of 3-(anthracenyl-9-yl)-1-(aryl substituted) prop-2-en-1-one (0.01 mol) and substituted orthophenylenediamine (0.01 mol) in DMF (15 ml) with catalytic amounts of gl acetic acid was taken in a conical flask and placed in a microwave and irradiated for 5-6 min. The completion of reaction was mnitored by TLC. The reaction mixture was cooled and poured into crushed ice with constant stirring. The solid mass thus obtained was filtered and washed with water and recrystallized from ethanol to get the compounds (BD1-BD5) Scheme-1.

Results are given in Table-1 and 2 respectively.

4-(Anthracenyl-9-yl)-2-(4-chlorophenyl)-2,3-dihydro-1*H*-benzo [*b*] [1,4] diazepine (BD1)

IR (KBr): 3390 (aromatic NH str), 3065 (aromatic CH str), 1519 (C=C str), 726 (C-Cl). ^{1}H NMR (400 MHz, DMSO): 3.29 (s, 1H, NH), 2.48-2.49 (d, 2H, -CH_2), 2.07 (s, 1H -CH), 7.57-8.69 (m, 17H, ArH). Mass (ESI): m/z (M+2) 434.

4-(Anthracenyl-9-yl)-2-(4-bromophenyl)-2,3-dihydro-1*H*-benzo [*b*] [1,4] diazepine (BD2)

IR (KBr): 3545 (aromatic NH str), 3149 (aromatic CH str), 1650 (C=N str), 1522 (C=C str), 635 (C-Br). ^{1}H NMR (400 MHz, DMSO): 3.29 (s, 1H, NH), 2.81-2.99 (d, 2H, -CH2), 2.07 (s, 1H, -CH), 7.55-8.69 (m, 17H, ArH). Mass (ESI): m/z (M+2) 478.

4-(Anthracenyl-9-yl)-2-(4-nitrophenyl)-2,3-dihydro-1*H*-benzo [*b*] [1,4] diazepine (BD3)

IR (KBr): 3539 (aromatic NH str), 3147 (aromatic CH str), 1516 (C=C str, C-NO_2). ^{1}H NMR (400 MHz, DMSO): 3.29 (s, 1H, NH), 2.48-2.49 (d, 2H, -CH_2), 2.07 (s, 1H, -CH), 7.56-8.71 (m, 17H, ArH). Mass (ESI): (M^+) 443.

4-(Anthracenyl-9-yl)-2-(4-fluorophenyl)-2,3-dihydro-1*H*-benzo [*b*] [1,4] diazepine (BD2)

IR (KBr): 3548 (aromatic NH str), 3112 (aromatic CH str), 1523 (C=C str), 1343 (C-F). ^{1}H NMR (400 MHz, DMSO): 3.09 (s, 1H, NH), 2.74-2.79 (d, 2H, -CH_2), 2.15 (s, 1H, -CH), 7.44-8.17 (m, 17H, ArH). Mass (ESI): m/z (M^+) 416.

Table-4
Antitubercular activity substituted benzodiazepines (BD1-BD5) by microplate alamar blue assay

Compd	100	50	25	12.5	6.25	3.125	1.6	0.8
BD1	S	S	S	**S**	R	R	R	R
BD2	S	S	S	**S**	R	R	R	R
BD3	S	**S**	R	R	R	R	R	R
BD4	S	**S**	R	R	R	R	R	R
BD5	S	S	S	R	R	R	R	R
Pyrazinamide	S	S	S	S	S	**S**	R	R
Streptomycin	S	S	**S**	S	S	S	R	R
S-Sensitive	S	S	S	S	**S**	R	R	R
R-Resistant								
S-MIC								

Table-4.1
MIC results of substituted benzodiazepines (BD1-BD5) for antitubercular activity

Compd	R	MIC in µg/ml
BD1	4-Cl	12.5
BD2	4-Br	12.5
BD3	4-NO_2	50
BD4	4-F	50
BD5	4-NH_2	25
Standard	Pyrazinamide	3.125
	Streptomycin	6.25

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References

1. B. Narayana, K.K.V. Raj, B.V. Ashalatha and N. Kumasi, *Eur. J. Med. Chem.*, **41(3)** (2006), 417.
2. J. Sepinwall and L. Cook, *Brain Research Bulletin,* **5 (2)** (1980), 839.
3. F. Petty, M.H. Trivedi, M. Fulton and A.J. Rush, *Biological Psychiatry*, **38(9)** (1995), 578.
4. M.D. Braccio, G. Roma, G.C. Grossi, M. Ghia and E. Mereto, *Eur. J. Med. Chem.,* **25(8)** (1990), 861.
5. S.G. Konda, B.M. Shaikh, S.A. Chavan and B.S. Dawane, *Chinese Chem. Lett.,* **22** (2011), 65.
6. N. Shvet, A. Terletskaya, A. Dimoglo and Y. Chumakov, *J. Mol. Structure Theochem.*, **463 (23)** (1999), 105.
7. R.L. Clark, K.C. Carter, A.B. Mullen, G.D. Coxon, G.O. Dapaah and E.M. McFarlane, *Bio. Med. Chem. Lett.,* **17** (2007), 624.

8. A. Kamal, K.L. Reddy, V. Devaiah, N. Shankaraiah, G.S.K. Reddy and S. Raghavan, *J. Comb. Chem.,* **9** (2007), 29.

9. A. Garcia, V.B. Garcia, J.P.P. Palma-Nicolas and G. Rivera, *Eur. J. Med. Chem.,* **49** (2012), 1.

10. A. Kamal, S. Prabhakar, M.J. Ramaiah, P.V. Reddy, C.R. Reddy and A. Mallareddy, *et al, Eur. J. Med. Chem.,* **46** (2011), 3820.

11. A. Kamal, Rajendera, D.R. Reddy, M.K. Reddy, G. Balakishan and T.B. Shaik, *Bioorg. Med. Chem.,* **17** (2009), 1557.

12. B. Insuasty, F. Orozco, J. Quiroga, R. Abonia, M. Nogueras and J. Cobo, *Eur. J. Med. Chem.,* **43** (2008), 1955.

13. P. M. Kumar, T.K. Ravi and G. Subbuchettiar, *Acta Pharm.,* **59** (2009), 159.

14. M.M. Ghorab, A.N. Osman and E. Noaman *et al., Phosphorus Sulfur Silicon and the Related Element,* **181** (2006), 1983.

15. L.A. Coollins and S.G. Franzblau, *Antimicrob. agents chemother.,* **41** (1997), 1004.

16. B. Insuasty, A. Garcia, J. Quiroga, R. Abonia, A. Ortiz and M. Nogueras, *Eur. J. Med. Chem.,* **46** (2011), 2436.

17. M.S. Bhatia, P.B. Choudhari, K.B. Ingale and B.E. Zarekar, *Orient. J. Chem.,* **24** (2008), 147.

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