International Journal of Research in Engineering and Applied Sciences(IJREAS)
Available online at http://euroasiapub.org/journals.php
Vol. 6 Issue 8, August 2016,
ISSN(O): 2249-3905, ISSN(P) : 2349-6525 | Impact Factor: 6.573 | Thomson Reuters ID: L-5236-2015

Eco-friendly Synthesis: A greener route preparation of new 1, 5-benzodiazepines and its antimicrobial activity.

Santosh S.Chobe[1]* Bapu S.Jagdale[1], Resham Bhalla[2]

Corresponding author : ***dr.resham.bhalla@gmail.com***

Santosh S.Chobe[1]*
Bapu S.Jagdale[1],
Resham Bhalla [2]

Keywords:

o-Phenylenediamine;
PEG-400;
1,5-Benzodiazepines
Antimicrobial activity.

Abstract

A simple, efficient, single step and environmentally benigr synthesis of a new series of pyrazole containing 1 5-benzodiazepines was described here by the condensation o pyrazolone with *o*-phenylenediamine usingg piperidine ir polyethylene glycol (PEG-400) as a reaction solvent. The advantages of this protocol are mild reaction condition, easy work-up, excellent yield, and avoidance of volatile organic solvent. Furthermore, these newly synthesized compounds were analyzed by spectrum and screened for their antimicrobial activity

[1]Organic research Laboratory, P.G.Department of Chemistry.
L V H Arts, Science and commerce College, Nashik (MS) India.
[2]P.G.Department of Zoology, L V H Arts, Science and commerce College, Nashik.

Introduction: The discovery of new, anxiolytic drugs that are fast-acting and free from the unwanted side effects associated with traditional benzodiazepines (BZD) continues to be an important scientific concern. Many neuroactive drugs, including benzodiazepines, interact with the GABAA receptor, the major inhibitory ion-channel in the mammalian central nervous system.[1-6]. On the other hand organic compound containing pyrazole nucleus has wide applications in medicinal chemistry as well as considerable interest in the chemotherapeutic activity. Pyrazole and its synthetic analogues have been found to exhibit industrial, agricultural and some biological applications [7,8]. The most reported method of 1,5 benzodiazepines are α-β unsaturated ketone reacted with o-phenylenediamine in presence of variety of catalyst such as PPA-SiO_2 [9], MgO–$POCl_3$ [10], $Yb(OTf)_3$ [11], HO

Ac-microwave [12], SO_4−2–ZrO_2 [13], $InBr_3$ [14], $Fe(ClO4)_3$ [15] Preyssler heteropolyacid [16]. However, many of these procedures have one or more disadvantages such as use of expensive catalyst, long reaction time, high catalyst loading, low selectivity, requirements of special apparatus, and side reaction. In recent years replacement of hazardous-solvents with environmentally benign solvents is one of the major focus areas of green chemistry. The utility of alternative reaction solvents such as water [17], ionic liquid [18], flourous [19], supercritical media [20] and polyethylene glycol (PEG) [21] is rapidly growing. Liquid polymers or low melting polymers have emerged as alternative green reaction media. Polyethylene glycol (PEG-400) prompted reactions [22–25] have attracted the attention of organic chemists due to their solvating ability and aptitude to act as a phase transfer catalyst, negligible vapor pressure, easy recyclability, ease of work-up, eco-friendly nature and economical cost. PEG is nontoxic, non-halogenated, inexpensive potentially recyclable and water soluble which facilitate its removal from reaction product.

Material and methods:

Melting points were uncorrected and determined in an open capillary tube. IR spectra were recorded on FTIR Shimadzu spectrometer. ^{1}HNMR spectra were recorded in DMSO-d_6 on Avance 300 MHz spectrometer using TMS as an internal standard. The mass spectra were recorded on EI-Shimadzu-GC-MS spectrometer. Elemental analyses were performed on a Carlo Erba 106 Perkin-Elmer model 240 analyzer.

General procedure for the synthesis of pyrazole containing 1, 5-benzodiazepines derivatives 3(a-l)

A mixture of substituted pyrazol-5-one **1** (1 mmol), *o*-phenylenediamine (1.5 mmol) and piperidine (1 mL) in polyethylene glycol (PEG-400) (15 mL) was heated at 60^0C for the period as shown in Table-1. After completion of reaction (TLC), the reaction mixture was extracted with ethyl acetate (2×20 mL). The combined organic layers were dried over anhydrous Na_2SO_4, and the solvent was evaporated under reduced pressure. The obtained product was recrystallized by aqueous acetic acid to give pure product. PEG-400 was recovered and further used for next run.

Spectral data of selected compounds:

4-(4-chlorophenyl)(3-methyl-1-phenyl-1,3a,4,5-tetrahydrobenzo[b]pyrazolo(3,4-e)[1,4]diazepin-4yl-phenol. (3a).

IR (KBr): 1636, 2954 cm^{-1} ^{1}H NMR (DMSO-d_6): δ 1.18 (s, 3H, CH_3), δ3.32 (dd, 1H, Ha), δ 4.19 (dd, 1H, Hb), δ 7.15-8.24 (m, 13H, Ar-H), δ 8.50 (s, 1H, NH), ppm; M.S. (m/z): 386 (M^+), Anal. Calcd for $C_{23}H_{19}ClN_4$: C, 71.38; H, 4.96; N, 14.48%. Found: C, 71.28; H, 5.03; N, 14.52%.

4-(2,4-dichlorophenyl)3-methyl-1-phenyl-4-(p-tolyl)1,3a,4,5-tetrahydrobenzo[b]-pyrazolo (3,4-e)[1,4] diazepine. (3b).

IR (KBr): 1634, 3145 cm^{-1} ^1H NMR (DMSO-d_6): δ 1.10 (s, 3H, CH_3), δ3.30(dd, 1H, Ha), δ 4.53 (dd, 1H, Hb), δ 7.25-8.24 (m, 13H, Ar-H), δ 8.76 (s, 1H, NH), ppm; M.S. (m/z): 420(M^+), Anal. Calcd for $C_{23}H_{18}Cl_2N_4$: C, 65.57; H, 4.30; N, 13.30%. Found: C, 65.36; H, 4.16; N, 13.16%.

3-methyl-1-phenyl-4-(p-tolyl)1,3a,4,5-tetrahydrobenzo[b]pyrazolo(3,4-e)-[1,4] diazepine . (3e).

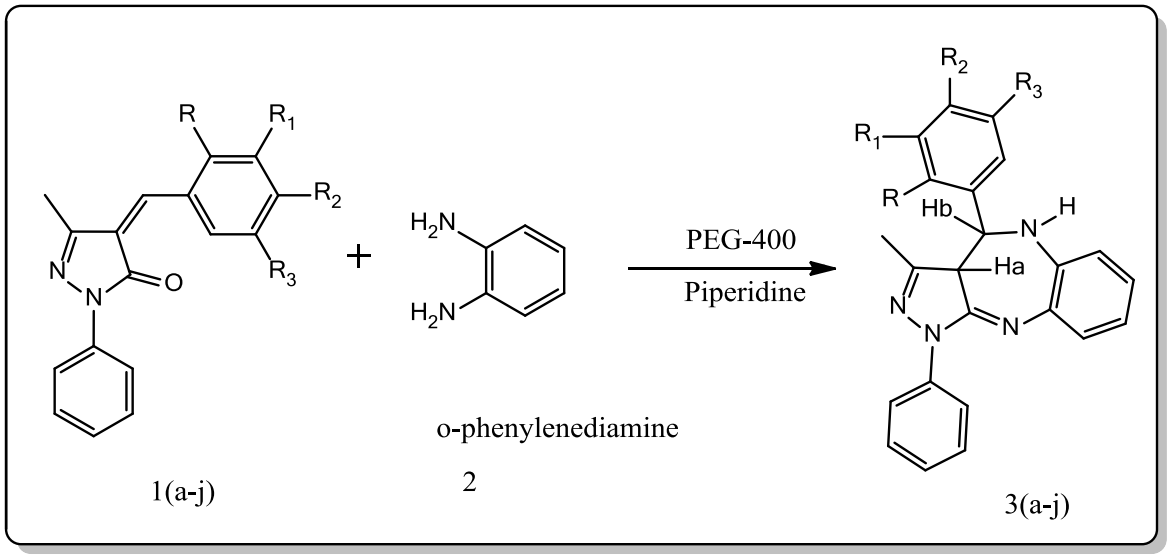
IR (KBr): 1620, 3126 cm^{-1} ^1H NMR (DMSO-d_6): δ 0.98 (s, 3H, CH_3), δ 2.12 (s, 3H, CH_3), δ3.36(dd, 1H, Ha), δ 4.23 (dd, 1H, Hb), δ 7.10-8.24 (m, 13H, Ar-H), δ 8.56 (s, 1H, NH), ppm; M.S. (m/z): 366(M^+), Anal. Calcd for $C_{23}H_{19}FN_4$: C, 78.68; H, 6.05; N, 15.29%. Found: C, 78.56; H, 5.93; N, 15.23%.

N,N-dimethyl-4-(4-chlorophenyl)(3-methyl-1-phenyl-1,3a,4,5-tetrahydrobenzo[b]-pyrazolo (3,4-e)[1,4] diazepin-4yl-aniline. (3i).

IR (KBr): 1645, 2950 cm^{-1} ^1H NMR (DMSO-d_6): δ 0.96 (s, 3H, CH_3), δ3.43 (dd, 1H, Ha), δ 4.54 (dd, 1H, Hb), δ 7.36-8.43 (m, 13H, Ar-H), δ 8.68 (s, 1H, NH), ppm; M.S. (m/z): 395 (M^+), Anal. Calcd for $C_{25}H_{25}N_5$: C, 75.92; H, 6.37; N, 17.71%. Found: C, 75.78; H, 6.24; N, 17.65%.

4-(3-methyl-1-phenyl-1,3a,4,5-tetrahydrobenzo[b]pyrazolo(3,4-e)[1,4]diazepin-4yl-phenol. (3h).

IR (KBr): 1620, 3160 cm^{-1} ^1H NMR (DMSO-d_6): δ 1.2 (s, 3H, CH_3), δ3.28 (dd, 1H, Ha), δ 4.12 (dd, 1H, Hb), δ 7.10-8.30 (m, 13H, Ar-H), δ 8.56 (s, 1H, NH), δ 10.9(s, 1H, OH), ppm; M.S. (m/z): 368 (M^+), Anal. Calcd for $C_{23}H_{20}N_4O$: C, 74.96; H, 5.46; N, 15.19%. Found: C, 74.87; H, 5.53; N, 15.22%.



Scheme 1: synthesis route of 1, 5-benzodiazepine

Table 1: Physical-chemical data of 1, 5-benzodizepine derivatives

Entry	Product	R	R_1	R_2	R_3	M.P. (°C)	Yield %	Time(min)
1	**3a**	H	H	Cl	H	148	88	45
2	**3b**	H	Cl	H	Cl	152	92	48
3	**3c**	H	H	F	H	138	90	54
4	**3d**	H	H	Br	H	140	84	47
5	**3e**	H	H	CH_3	H	128	82	50
6	**3f**	H	F	H	F	156	81	52
7	**3g**	H	H	OCH_3	H	162	76	48
8	**3h**	H	H	OH	H	138	88	46
9	**3i**	H	H	$N(CH_3)_2$	H	140	81	56
10	**3j**	H	OH	H	OH	158	89	53

Table2:
4-(4-chlorophenyl)(3-methyl-1-phenyl-1,3a,4,5-tetrahydrobenzo[b]pyrazolo(3,4-e)[1,4] Diazepin - 4yl-phenol.

Entry	Solvent	Time (h)	Yield (%)
1	EtOH	5	69
2	THF	4	74
3	Dioxane	3	70
4	Acetonitrile	4	65
5	PEG-400	45(min)	93

Result and Discussion:

In continuation of our work on the synthesis of some new bioactive heterocyclic compounds [26, 27], herein we report new series of 1, 5-benzodiazepines by the condensation of pyrazolone with *o*-phenylenediamine using piperidine in polyethylene glycol (PEG-400) as a solvent**.** We attempted the condensation of substituted pyrazol-5-one with *o*-phenylenediamine by using piperidine in polyethylene glycol (PEG-400) as reaction solvent. The reaction went to completion within 45 minutes and a series of corresponding product was obtained in 93% yield. In order to optimize the reaction conditions, we carried

out the above reaction in different solvents such as ethanol, tetrahydrofuran, dioxane, acetonitrile and polyethylene glycol-400 **(Table 2).** We found that polyethylene glycol-400 as an efficient reaction medium in terms of reaction time as well as yields (93%). Encouraged by the results, we turned our attention to variety of substituted pyrazole-5-ones. In all cases, the reaction proceeded efficiently in high yields at 60-65 °C using PEG-400 as an alternative reaction solvent. These newly synthesized compounds were screened for their antimicrobial activity.

The antimicrobial activities of the synthesized compounds were determined by agar well diffusion method [28]. The compounds were evaluated for antibacterial activity against *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhi* and *Staphylococcus aureus*. The antifungal activity was evaluated against *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans* and *Penicillium chrysogenum*. were procured from Institute of Microbial technology (IMTech), Chandigarh, India. The antibiotic penicillin (25 µg/mL) was used as reference drug for both antibacterial and antifungal activities. Dimethyl sulphoxide (1%, DMSO) was used a control without compound. The culture strains of bacteria were maintained on nutrient agar slant at 37±0.5 °C for 24 hrs. The antibacterial activity was evaluated using nutrient agar plate seeded with 0.1 mL of respective bacterial culture strain suspension prepared in sterile saline (0.85%) of 10^5 CFU/mL dilutions. The wells of 6 mm diameter were filled with 0.1 mL of compound solution at fixed concentration 25 µg/mL separately for each bacterial strain. All the plates were incubated at 37±0.5 °C for 24 hrs. Zone of inhibition of compounds in mm were noted.

For antifungal activity, all the culture strains of fungi maintained on potato dextrose agar (PDA) slant at 27±0.2 °C for 24-48 hrs, till sporulation. Spore of strains were transferred in to 5 mL of sterile distilled water containing 1% Tween-80 (to suspend the spore properly). The spores were counted by haemocytometer (10^6 CFU/mL). Sterile PDA plate was prepared containing 2% agar; 0.1 mL of each fungal spore suspension was spread on each plate and incubated at 27±0.2 °C for 12 hrs. After incubation well prepared using sterile cork borer and each agar well was filled with 0.1 mL of compound solution at fixed concentration 25 µg/mL. The plates were kept in refrigerator for 20 minutes for diffusion and then incubated at 27±0.2 °C for 24-28 hrs. After incubation, zone of inhibition of compounds were measured in mm along with standard. The results of antibacterial and antifungal activity were summarized in Table-3. In comparison with Penicillin as bacterial and nystatin as antifungal, compounds **3b, 3c and 3f** showed good activity against *E. coli* and *B. subtillis*. And the compound **3e** and **3g** showed more potent activity at *S. aurues*. Compounds **3e 3f, 3j** were showed comparable activity against *S. typhi*.

When antifungal activities of these compounds were compared with Nystatin, most of the compounds were displayed significant activity. Compounds **3b, 3d, 3h** showed potent against A. *niger* strains. Compounds **3b, 3g, 3i** were showed no activity than reference compounds

against A. *flavus* and the more potent active compound is **3d** againt A. *flavus*. Compounds **3c, 3g** and **3j** showed lower activity and compound **3b, 3d** showed moderate to good activity compared to standard against *P. Chrysogenium*. Only the compounds **3b, 3j** showed no activity against *C. albicans*. Where a compound **3f, 3h** shows highest activity against *C. albicans*. Considering these results from antibacterial and antifungal activity, it is noteworthy to mention that compounds were more active towards the fungal than bacterial. Carrying substitution at R_1, R_2 and R_3 in 1, 5-benzodiazepine emerged as active in both antimicrobial and antifungal screening. The substitution of halogens as well dihalo compounds and *o*-hydroxy may increase the activity against various pathogens.

Table 3. Antimicrobial activity of synthesized 1,5-benzodiazepines (3a-j)

Product	Bacteria				Fungi			
	Ec	**St**	**Sa**	**Bs**	**An**	**Af**	**Pc**	**Ca**
3a	16	--	13	14	15	17	14	13
3b	18	12	18	17	16	--	16	--
3c	19	14	17	19	12	11	12	10
3d	10	12	--	9	16	19	18	16
3e	10	18	18	16	14	11	13	13
3f	21	20	12	18	14	14	16	20
3g	16	10	18	16	10	--	12	12
3h	14	--	13	10	16	12	14	19
3i	12	10	08	14	11	--	13	09
3j	13	18	10	08	--	14	12	--
Penicillin	22	22	24	24	NA	NA	NA	NA
Nystatin	NA	NA	NA	NA	20	22	24	24

Zone of inhibition is expressed in mm.

Ec- Escherichia coli,
An-Aspergillus niger,
St- Salmonella typhi,
Af-Aspergillus flavus,
Sa- Staphylococcus aureus *Ca-*
Candida albicans
Bs- Bacillus subtillis, *Pc-Penicillium*
chrysogenum,

-- *No activity, NA-Not Applicable*

Summary:

In summary, we have designed and synthesized some new pyrazole containing 1, 5-benzodiazepines is described here by the condensation of pyrazole-5-ones with *o*-phenylenediamine using piperidine in polyethylene glycol (PEG-400) as a solvent. The preliminary *in vitro* antimicrobial screening of this series revealed that, compounds showed potent activity. Therefore, the present study is useful drugs in medicinal investigation against bacterial and fungal diseases.

Acknowledgment:

Authors are thankful to Dr. Apoorva Hiray, Coordinator, M. G. Vidyamandir, Nashik and the Principal, LVH College, Nashik for providing laboratory facilities and IICT Hyderabad for spectral analysis.

References:

[1] **Sigel, E., Buhr, A.,** The benzodiazepine binding site of GABAA receptors. Trends Pharm. Sci. 184 (1997) 25;

[2] **Togashi, H., Mori, K., Kojima, T., Matsumoto, M., Ohashi, S.,** Different effects of anxiolytic agents, diazepam and 5-HT1A agonist tandospirone, on hippocampal long-term potentiation in vivo, Pharmacol. Biochem. Behav.69, (2001) 367;

[3] **Mohler, H., Okada, T.,** Benzodiazepine receptor: demonstration in the central nervous system Science. 198 (1977) 849;

[4] **Mombereau, C., Kaupmann, K., van der Putten, H., Cryan, J.,** Altered response to benzo- diazepine anxiolytics in mice lacking GABA B receptors, European Journal of Pharmacology. 497 (2004) 119;

[5] **Basile, A., Lippa, A. S., Skolnick, P.,** Anxioselective anxiolytics: can less be more, European Journal of Pharmacology. 500 (2004) 441;

[6] **Berezhnoy, D., Nyfeler, Y., Gonthier, A., Schwob, H., Goeldner, M., Sigel, E.,** On the benzodiazepine binding pocket in GABA A receptors, J. Biological Chemistry. 279 (2004) 3160;

[7] **H El-Kashef, T El-Emary, M Gasquet, P Timon-David, J Maldonado and P Vanelle.** Pharmazie 55 (2000) 572;

[8] **M Taha, O Moukha-chafiq, H Lazrek, J Vasseur and J Imbach.** Nucleosides Nucleotides Nucleic Acids. 20(2001) 955;

[9] **D.I. Jung, T.W. Choi, Y.Y. Kim, et al**. Synth. Commun. 29(1999) 1941;

[10] **M.S. Balakrishna, B. Kaboudin,** Tetrahedron Lett. 42 (2001) 1127;

[11] **M. Curini, F. Epifano, M.C. Marcotullio, O. Rosati,** Tetrahedron Lett. 42 (2001) 3193;

[12] **M. Pozarentzi, J.S. Stephanatou, C.A. Tsoleridis,** Tetrahedron Lett.43 (2000) 1755;

[13] **B.M. Reddy, P.M. Sreekanth,** Tetrahedron Lett. 44 (2003) 4447;

[14] **B.P. Bandgar, S.V. Bettigeri, N.S. Joshi**, Synth. Commun. 34 (2004) 1447;

[15] **M Majid. Heravi, Z.Vahideh, K Farahnaz. Behbahani, A Hossien. Oskooie** Journal of Molecular Catalysis A: Chemical 259 (2006)201;

[16] **M Majid.Heravi, D Fatemeh, R Leila, F Fatemeh.** Bamoharram Journal of Molecular Catalysis A: Chemical. 261 (2007) 156;

[17] **(a) J. Chen, S.K. Spear, J.G. Huddieston, et al.** Green Chemistry. 7 (2005) 64;

(b) Z.H. Zhang, L. Yin, Y.M. Wang, et al. Green Chemistry. 6 (2004) 563;

(c) R. Kumar, P. Chudhary, S. Nimesh, R. Chandra, Green Chemistry. 8 (2006) 356.

[18] **D. Heldebrant, P.G. Jessop,** J. Am. Chem. Soc. 125 (2003) 5600;

[19] **S. Chandrasekhar, Ch. Narsihmulu, S.S. Sultana, et al.** Org. Lett. 4 (2002) 4399;

[20] **R. Jiang, Y.Q. Kuang, X.L. Sun, et al.** Tetrahedron Asymmetry. 15 (2004) 743;

[21] **V.V. Namboodiri, R.S. Varma,** Green Chem. 3 (2001) 146;

[22] **N. Suryakiran, D. Ramesh, Y. Vemkateswarulu,** Green Chem. Lett. 1 (2007) 73;

[23] **A. Kamal, D.R. Reddy, Rajendar,** Tetrahedron Lett. 46 (2005) 7951.

[24] **V.T. Kamble, B.S. Dawane, S.A. Chavan, R.B. Bhosale,** Aust. J. Chem. 60 (2007) 302;

[25] **B. Das, M. Krishnaiah, P. Balasybramanyam, et al.** Tetrahedron Lett. 49 (2008) 2225;

[26] **B.S. Dawane, B. M. Shaikh, N. T. Khandare, S. S. Chobe and Shankaraiah G. Konda,** Green Chemistry Letters and Reviews. 3(3) (2010) 205-208;

[27] **B. S. Dawane, S. G. Konda, R. G. Bodade, Baseer M Shaikh, Namdev T. Khandare, Santosh S. Chobe, Vishwas D. Joshi,** Organic Communcations. 3 (2010) 22-29;

[28] **D. Shrinivasan, N. Sangeetha, T. Suresh, P. Lakshmanaperumalsamy,** J. Ethnophrmacol. 74 (2001) 217;