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EFFECT OF CATALYST ON SYNTHESIS OF 1, 5- BENZODIAZEPINE DERIVATIVES AND EVALUATION OF THEIR PHARMACOLOGICAL ACTIVITY

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ABSTRACT

1,5-Benzodiazepines are psychoactive drugs Benzene ring and Diazepine ring fusion, widely used as hypnotics, sedatives and in several Central Nervous System disorders. 1,5-Benzodiazepines are regularly used because of their nature of exhibiting pharmacological effects with minimal effect on patient performance. Derivatives of 1, 5- benzodiazepines were synthesized using the catalysts like Formic Acid and Glacial Acetic Acid, characterized by spectral studies using ^{1}H-NMR Spectroscopy, Mass Spectroscopy, and FT-IR Spectroscopy. The results showed that the percentage yield of the reaction involving Formic acid as a catalyst is higher when compared to that of the Glacial Acetic Acid as catalyst. This indicates that the acidic nature of formic acid is enhancing the cyclization process thus producing higher percentage yield. Hence the use of stronger acid as a catalyst is enhancing the cyclization. The pharmacological activity of synthesized compounds are screened using animal models for muscle relaxant property and catatonic activity and are showing significant effect when compared with the standard drug diazepam. When compared the compound- A which is a derivative of acetone and 1, 2- diaminobenzene is showing more activity than the rest of synthesized compounds. Thus the study shows that the use of strong acid as a catalyst is increasing the yield of the 1,5-Benzodiazepines in synthesis of pharmacologically active derivatives.

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INTRODUCTION

Benzodiazepines are an important class of pharmacologically active organic compounds. The benzodiazepine nucleus is a well-studied traditional pharmacophoric scaffold that has emerged as a core structural unit of various sedative, Hypnotic, muscle relaxant, Anxiolytic, Antihistamine and anti convulsant agents [1]. These benzodiazepine derivatives also serve as key intermediates for the formation of Triazolo and Oxadiazolo benzodiazepines [2]. The fusion of heterocyclic system to the benzodiazepine ring appears quite promising for the synthesis of derivatives with greater activity and specificity[4]. Although antidepressants with anxiolytic properties have been introduced, and there is increasing awareness of the adverse effects of benzodiazepines, prescriptions for short-term anxiety relief have not significantly dropped. Here we report the effect of catalyst on the synthesis of substituted 1, 5 benzodiazepine derivatives from ketones then evaluate skeletal muscle relaxant and anticatatonic activity of Benzodiazepine.

MATERIALS AND METHODS

All Chemicals were obtained from S.D. Fine Chem. Limited, Mumbai. All glassware is of Borosilicate grade. Melting Points were determined in open capillaries and are uncorrected.

Synthesis:

a. 2,2,4-Trimethyl 1,5 Benzodiazepines (compound-BZD A_1):

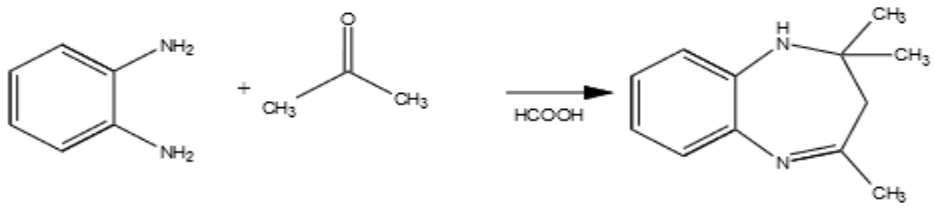

FIGURE- I.

To 1, 2-diaminobenzene (20 mmol) in Formic Acid (0.1ml), Acetone (40 mmol) was added while stirring, and kept stirred at 25^0C for 4hrs. The reaction mixture is stirred until it turns to thick and kept for overnight drying at 25^0C (The reaction monitored by TLC). The dried mixture is recrystallized with 95% ethanol and decolourised using charcoal and filtered. The orange yellow coloured filtrate thus obtained was kept in refrigerator for 4-5hrs and the yellowish brown crystals are collected.

IR Spectral data for 2,2,4-Trimethyl 1,5 Benzodiazepines -CH_3 (1380-1460 cm^{-1}), -CH_2-(1442-1455$cm^{-1)}$, NH-(3300-3500 cm^{-1}) N-H bend (1550-1640 cm^{-1}), -C-H-Ar(690-900 cm^{-1}), -C-H-Ar(3050-3150). H' NMR Spectral Data of Synthesized Compound – A_1: -CH_3 (1.7(s)), -CH_2- (2.8, 3.1(d)), -NH- (3.6(s)), Ar-H (7-7.6(m)). Mass Spectral data of compound is molecular ion peak (m^+) 189.1 fragmentation ions peak 251.1, 309.2, 349.1, etc.,

b. Preparation of 2,2,4-Trimethyl 1,5 Benzodiazepines (compound-BZD A_2):

To 1, 2-diaminobenzene (20 mmol) in Glacial Acetic Acid (0.12ml), Acetone (40 mmol) was added while stirring, and kept stirred at 25^0C for 4hrs. The reaction mixture is stirred until it turns to thick and kept for overnight drying at 25^0C (The reaction monitored by TLC). The dried mixture is recrystallized with 95% ethanol and decolourised using charcoal and filtered. The orange yellow coloured filtrate thus obtained was kept in refrigerator for 4-5hrs and the yellowish brown crystals are collected.

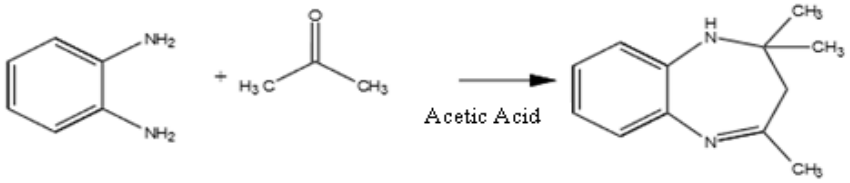

FIGURE-II.

IR Spectral data for 2,2,4-Trimethyl 1,5 Benzodiazepines -CH_3 (1380-1460 cm^{-1}), -CH_2-(1442-1455$cm^{-1)}$, NH-(3300-3500 cm^{-1}) N-H bend (1550-1640 cm^{-1}), -C-H-Ar(690-900 cm^{-1}), -C-H-Ar(3050-3150). H' NMR Spectral Data of Synthesized Compound – A_2: -CH_3 (1.7(s)), -CH_2- (2.8, 3.1(d)), -NH- (3.6(s)), Ar-H (7-7.6(m)). Mass Spectral data of compound is molecular ion peak (m^+) 189.8 fragmentation ions peak 213.1, 290, 393, etc.,

c. Preparation of 2,4 Diphenyl,2-methyl 1,5 Benzodiazepines (compound-BZD B_1):

To 1, 2-diaminobenzene (20 mmol) in Formic Acid (0.1ml), Acetopheone (40 mmol) was added while stirring, and kept stirred at 25^0C for 4hrs. The reaction mixture is stirred until it turns to thick and kept for overnight drying at 25^0C (The reaction monitored by TLC). The dried mixture is recrystallized with 95% ethanol and decolourised using charcoal and filtered. The orange yellow coloured filtrate thus obtained was kept in refrigerator for 4-5hrs and the yellowish brown crystals are collected.

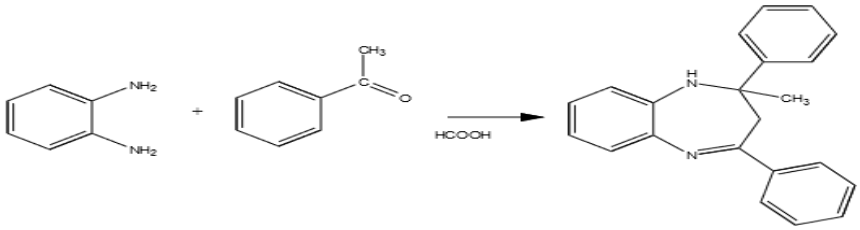

FIGURE-III.

IR Spectral data for 2,4 Diphenyl,2-methyl 1,5 Benzodiazepines -CH_3 (1380-1460 cm^{-1}), -CH_2-(1442-1455$cm^{-1)}$, NH-(3300-3500 cm^{-1}) N-H bend (1550-1640 cm^{-1}), -C-H-Ar(3050-3150). H' NMR Spectral Data of Synthesized Compound – B_1: -CH_3 (1.7(s)), -CH_2- (2.8, 3.1(d)), -NH- (3.6(s)), Ar-H (7-7.6(m)). Mass Spectral data of compound is molecular ion peak (m^+) 313.2, fragmentation ions peak 242.3, 337.2, etc.,

d. Preparation of 2,4 Diphenyl,2-methyl 1,5 Benzodiazepines (compound-BZD B_2):

To 1, 2-diaminobenzene (20 mmol) in Glacial Acetic Acid (0.12ml), Acetopheone (40 mmol) was added while stirring, and kept stirred at 25^{0}C for 4hrs. The reaction mixture is stirred until it turns to thick and kept for overnight drying at 25^{0}C (The reaction monitored by TLC). The dried mixture is recrystallized with 95% ethanol and decolourised using charcoal and filtered. The orange yellow coloured filtrate thus obtained was kept in refrigerator for 4-5hrs and the yellowish brown crystals are collected.

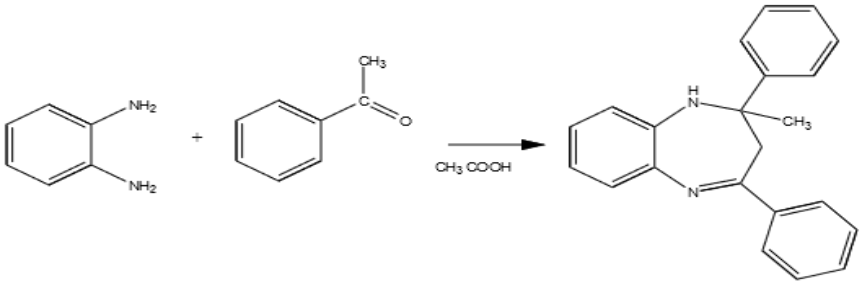

FIGURE-IV.

IR Spectral data for 2,4 Diphenyl,2-methyl 1,5 Benzodiazepines -CH_3 (1380-1460 cm^{-1}), -CH_2-(1442-1455$cm^{-1)}$, NH-(3300-3500 cm^{-1}) N-H bend (1550-1640 cm^{-1}), -C-H-Ar(3050-3150). H' NMR Spectral Data of Synthesized Compound – B_2: -CH_3 (1.7(s)), -CH_2- (2.8, 3.1(d)), -NH- (3.6(s)), Ar-H (7-7.6(m)). Mass Spectral data of compound is molecular ion peak (m^+) 313.2, fragmentation ions peak 242.3, 337.2, etc.,

e. Preparation of 10– spirocyclohexane - 2,3,4,10,11,11a hexahydro – 1 H – dibenzo (b,e) (1,4) diazepine (compound-BZD C_1):

To 1, 2-diaminobenzene (20 mmol) in Formic Acid (0.1ml), Cyclohexaone (40 mmol) was added while stirring, and kept stirred at 25^{0}C for 4hrs. The reaction mixture is stirred until it turns to thick and kept for overnight drying at 25^{0}C (The reaction monitored by TLC). The dried mixture is recrystallized with 95% ethanol and decolourised using charcoal and filtered. The orange yellow coloured filtrate thus obtained was kept in refrigerator for 4-5hrs and the yellowish brown crystals are collected.

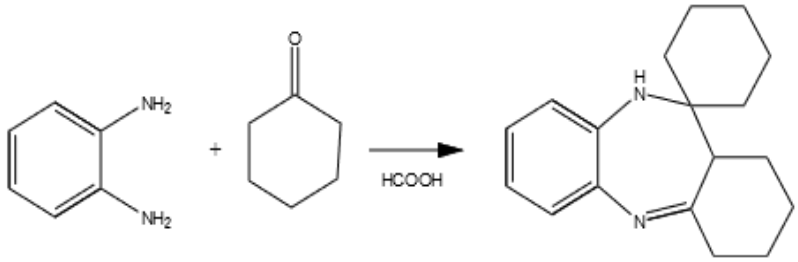

FIGURE-V.

IR Spectral data for 0-spirocyclohexane-2,3,4,10,11,11a hexahydro-1H-dibenzo(b,e) (1,4)diazepine -CH_2-(1442-1455$cm^{-1)}$, NH-(3300-3500 cm^{-1}) N-H bend (1550-1640 cm^{-1}), -C-H-Ar(690-900 cm^{-1}), -C-H-Ar(3050-3150). H' NMR Spectral Data of Synthesized Compound – C_1: -CH_2- (3.0-4.5(m)), -NH- (3.5(s)), Ar-H (6.2-7.4(m)). Mass Spectral data of compound is molecular ion peak (m^+) 271.2, fragmentation ions peak 187.1, 242.1, etc.,

f. Preparation of 10-spirocyclohexane-2,3,4,10,11,11a hexahydro-1H-dibenzo(b,e) (1,4)diazepine (compound-BZD C_2):

To 1, 2-diaminobenzene (20 mmol) in Glacial Acetic Acid (0.12ml), Cyclohexaone (40 mmol) was added while stirring, and kept stirred at 25^0C for 4hrs. The reaction mixture is stirred until it turns to thick and kept for overnight drying at 25^0C (The reaction monitored by TLC). The dried mixture is recrystallized with 95% ethanol and decolourised using charcoal and filtered. The orange yellow coloured filtrate thus obtained was kept in refrigerator for 4-5hrs and the yellowish brown crystals are collected.

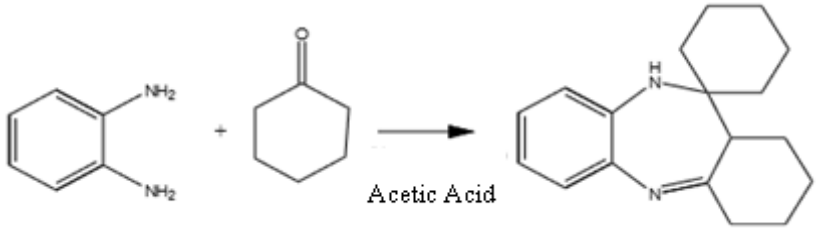

FIGURE-VI.

IR Spectral data for 0-spirocyclohexane-2,3,4,10,11,11a hexahydro-1H-dibenzo(b,e) (1,4)diazepine -CH_2-(1442-1455$cm^{-1)}$, NH-(3300-3500 cm^{-1}) N-H bend (1550-1640 cm^{-1}), -C-H-Ar(690-900 cm^{-1}), -C-H-Ar(3050-3150). H' NMR Spectral Data of Synthesized Compound – C_2: -CH_2- (3.0-4.5(m)), -NH- (3.5(s)), Ar-H (6.2-7.4(m)). Mass Spectral data of compound is molecular ion peak (m^+) 271.2, fragmentation ions peak 187.1, 242.1, etc.,

Spectral Studies:

Spectra analysis was done for the synthesized compounds. Mass spectroscopy NMR spectroscopy and IR spectroscopy were employed for the spectral study.

Physical data of Synthesis:

The below Table - I indicates the percentage yield, melting point of derivative

Table- I: Percentage Yeild, Melting Point of 1,5-Benzodiazepine Deivatives.

Compound	% Yeild	Melting Point in ^{0}C	M. Wt. in g/mol
A_1	95.5%	70-72	188
A_2	87.5%	70-75	188
B_1	91.37%	72-76	312.41
B_2	70.68%	72-76	312.41
C_1	92.74%	74-78	268
C_2	88.70 %	76-80	268

Table Toxicity Studies:

Acute Toxicity Studies[3]:

Acute oral toxicity studies were conducted by following the OECD Guidelines 420 method which has been designed and employed to evaluate the substances at a fixed dose and provides information both for hazard assessment as well as for the classification purposes. The compounds Benzodiazepine- A, Benzodiazepine – B and Benzodiazepine- C were administered at a starting dose of 5mg/kg b.w. in 5% acacia+ water for injection and are observed for 14 days for acute toxicity in the form of mortality. Observations were made regularly so as to check the general signs of toxicity as well as the CNS, ANS, motor acitivities among many others. It was observed that no sign of toxicity was being shown at 5mg/kg b.w. which in turn proved the non toxic nature of the compounds selected.

Skeletal Muscle Relaxant Activity using Rotarod Apparatus:

The rota rod apparatus consists of a metal rod (3cm) coated with rubber attached to a motor with the speed adjusted to 30 rotations per min. The rod is 75cm in length and divided into 3 sections, in the height of 50 cm above the table top. Mice underwent pretest on the apparatus. Only those animals that demonstrated their ability to remain on the revolving rod (30rpm for min) were used for the test[7].

Mice were divided into 5 groups consisting of six animals each. Group I served as control which received acacia suspension (5%). Group II received standard drug diazepam at a dose of 5 mg/kg body weight. Group III received test (compound-BZD A) at dose of 5 mg/kg body weight. Group IV received test (compound-BZD B) at dose of 5 mg/kg body weight. Group V received test (compound-BZD C) at dose of 5 mg/kg body weight. The animals were placed on the rotating rod and fall off time was recorded. The data obtained was subjected to statistical analysis. All results are expressed as Mean $\pm$ SEM (Standard error of mean) six animals of each group all statistical comparision were made by ANOVA.

Table – II: Pharmacological response of 1,5-Benzodiazepines when screened for Skeletal Muscle relaxant Activity.

	Time spent on revolving rod in Rotarod apparatus (in seconds)	
Groups	5 minutes before administration	30 minutes after administration
Diazepam(standard)	139.7±1.9	54± 0.37**
BZD A	135.33 ± 1.06	36.6 ± 0.04**
BZD B	131.66 ± 1.55	45 ± 0.67*
BZD C	137 ± 1.6	56.7 ± 1.03*

Values are expressed in Mean ± SD, n=6,** p<0.001,* P<0.01.

Catatonic Activity:

Catatonia is a state of neurogenic motor immobility and behavioral abnormality manifested by stupor. In this study the capacity of the drug to induce catatonia is observed. All animal were grouped accordingly and injected with respective drugs.

Mice were divided into 5 groups consisting of six animals each. Group I served as control which received acacia suspension (5%). Group II received standard drug diazepam at a dose of 5 mg/kg body weight. Group III received test (compound-BZD A) at dose of 5 mg/kg body weight. Group IV received test (compound-BZD B) at dose of 5 mg/kg body weight. Group V received test (compound-BZD C) at dose of 5 mg/kg body weight. After 30 min. of administration each animal is tested for induced catatonia. An animal cage is taken is lined up to 2 inches with bedding maintained and a wooden board of 2-3 cm height is placed on it. Each animal was placed on the board such that one front and consecutive hind paw are on the board. The paw retrieval time is noted and compared. The more time a mice takes to retrieve the paw the more potent the catatonic activity of the drug.

Table- III: Screening of Catatonic Activity of 1,5-Benzodiazepine Derivatives.

S.No	Group	Mean ± SEM
		30 Minutes after drug administration
1.	Standard	18.7±2.6
2.	BZDA	11.4±0.21
3.	BZDB	10±0.73
4.	BZDC	10±0.73
5.	BLANK	3.7±0.33

RESULTS AND DISCUSSION

The effect of catalyst (formic acid, Glacial Acetic Acid) in the reaction between the o-phenylene diamine and various ketones was studied. The HCOOH as a catalyst is enhancing the rate of reaction when compare to Glacial Acetic Acid. CH_3COOH does not effect the rate of reaction since pk_a value of Glacial Acetic Acid is 4.75. Hence the strong acid increases the rate of reaction by enhancing cyclization and thereby produces higher yield when compared to weak acid.

Synthesized compounds when evaluated for acute toxicity are found to be safe for animal studies at concentrations less than 5mg/kg. Further they were evaluated for Muscle relaxant property and catatonic activity. All three compounds are found to be producing moderately significant activity when compared to the standard. The compound A was found to be more active among all three synthesized compounds.

CONCLUSION

Thus developed synthetic compounds should be further study in order to establish a strong pharmacological and toxicological database to be generated for the long term as well as short term effects of compounds. Discovery of such synthetic drugs will play a major role in the ever demanding field of medicine either it be for human treatment or in veterinary medicine.

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