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PREPARATION, CHARACTERIZATION AND ANTI EPILEPTIC ACTIVITY EVALUATION OF SIMPLE BENZOTHIAZEPINE DERIVED -MANNICH BASES

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Abstract:

Mannich bases are the end products of Mannich reaction and are known as beta-amino ketone carrying compounds. Mannich reaction is a carbon-carbon bond forming nucleophilic addition reaction and is a key step in synthesis of a wide variety of natural products, pharmaceuticals, and so forth. Mannich reaction is important for the construction of nitrogen containing compounds. There is a number of aminoalkyl chain bearing Mannich bases like fluoxetine, atropine, ethacrynic acid, trihexyphenidyl, and so forth with high curative value. The present research, conjugation of moieties like 1,5-benzoxazepines and 1,5-benzothiazepines with secondary amines like piperazine, methyl piperazine and morpholine was carried out in a Mannich base with an anticipation of good anticonvulsant activity. The present study has majorly focused on the above mentioned issue and thus the distinctive feature of this series is the substitution by the different moieties at second and third position of benzothiazepine ring and benzoxazepine ring. The overall results reflect an improved anticonvulsant activity with 2-chloro and 3-aminomethylene substituted benzothiazepine ring.

Key Words: *Mannich base, Benthothiazepine, antiepileptic activity*

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INTRODUCTION:

Mannich reaction is a three-component condensation in which a compound containing an active H atom (substrate) is allowed to react with an aldehyde or ketone and a secondary amine. The first Mannich reaction took place accidentally in 1912 when Carl Mannich was making a pharmaceutical preparation based on salicyl-antipyrine and hexamethylenetetramine [1-3]. Mannich realized the synthetic relevance of the reaction that it allowed the linkage of two different chemical moieties in one-step by means of a methylene bridge [4,5]. Mannich bases are known to be an important pharmacophore or bioactive leads in the synthesis of various potential agents that have a variety of therapeutic activities like anticancer, antipsychotic, anticonvulsant, and antimalarial, anti-inflammatory, antibacterial and so forth. Anticonvulsant activity of hydrazones of Mannich bases of isatin by maximal electro shock (MES) and metrazole induced convulsions (MET) at 30, 100 and 300 mg/kg dose levels were studied by Sridhar et al. Neurotoxicity of these compounds also has been evaluated. Niharika et al"' have prepared several appropriately substituted 4-(dialkyl-aminoalkyl)stryl-alkyl ketones and subjected them to the Mannich reaction. Spermicidal activity of the derivatives has been evaluated [6-9]. He then studied the reaction in depth assisted by a number of collaborators, and demonstrated its general applicability as a method for obtaining aminomethylated products [10-12]. The formation of both carbon-carbon and carbon-nitrogen bonds in this aminomethylation process makes the Mannich reaction an extremely useful synthetic transformation.

MATERIALS AND METHOD:

The chemicals and reagents were procured from S. D. Fine Chemicals, Mumbai and were used as such for the reactions. Melting ranges were determined using melting point apparatus and are uncorrected. Progress of the reaction was monitored by thin layer chromatography on pre-coated aluminum silica gel G plates, using iodine vapors and UV chamber as visualizing agents. The synthesized compounds were subjected to physical, chemical and spectral analysis. Partition coefficient (log P) of the synthesized derivatives was determined by "Shake Flask Method". UV spectra were recorded on a PC-based double beam spectrophotometer (2202 Systronics). Infrared spectra were recorded on Shimadzu 8400S and Perkin-Elmer AX-1 spectrometers and the values are expressed in cm-1. Mass spectra were recorded on a Jeol-Accu TOF, JMS-T100LC spectrometer. Proton Nuclear Magnetic resonance spectra were recorded on Bruker DRX-300 (at 300 MHz) spectrophotometer and 13C NMR data were recorded on Advance-400 MHz, Bruker (Switzerland) spectrometer. Chemical shift (delta values) values are reported in parts per million (ppm), taking TMS as a reference standard. All the spectral studies were performed at IICT, Hyderabad (TS), India.

Experimental:

General Procedure for the Syntheses of Substituted 4 Hydroxy chalconyl- benzenes (1a-1b):

A mixture of 4-hydroxyacetophenone (0.01 mol), appropriate aldehyde (0.01 mol) in ethanol (40 ml) and aqueous potassium hydroxide (60%, 10 ml) was stirred and kept at room temperature for 12 h. The mixture was poured on to crushed ice and acidified with dil. HCl. The precipitate obtained was filtered, washed thoroughly with distilled water and recrystallized from ethanol.

General Procedure for the Syntheses of 4-(4'-hydroxy Phenyl)-2 (Substituted Phenyl)-2,3-dihydro-1,5-Benzothiazepines (2a-2b):

The methanolic solution (50 ml) of substituted 4-hydroxychalconylbenzenes 1a-1b (0.01 mol) was added to 2- aminothiophenol (0.01 mol), with a few drops of glacial acetic acid, and refluxed for 16 - 18 h. After refluxing, the solvent was distilled off under reduced pressure and the crude product was washed and recrystallized from ethanol.

General Procedure for the Syntheses of 4-(4'-hydroxy Phenyl)-2-(Substituted Phenyl)-2,3-dihydro-1,5-benzoxazepines (2a'-2b'):

The methanolic solution (50 ml) of substituted 4-hydroxychalconylbenzenes 1a-1b (0.01 mol) was added to 2-aminophenol (0.01 mol), with a few drops of glacial acetic acid, and refluxed for 16 - 18 h. After refluxing, the solvent was distilled off under reduced pressure and the crude product was washed and re-crystallized from ethanol.

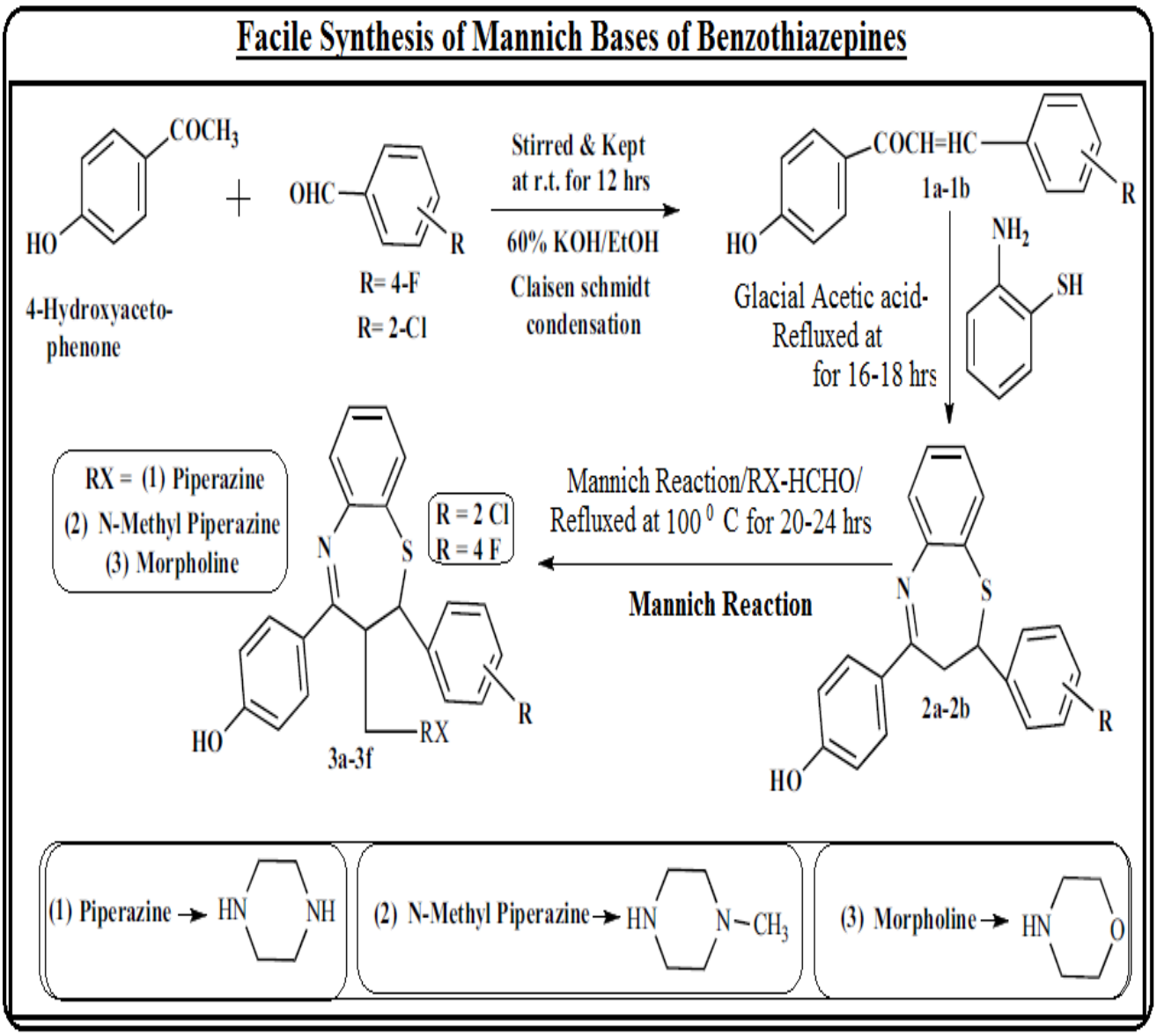

Figure-1: Synthesis of Mannich base derivative of Benzothiazepines

Spectral interpretation of Synthesized Compounds

Compound 1a Light orange color, solid, mp: 150-160°C, yield: 71.80%, IR (KBr, v cm-1) OH (3423.41), C-Cl (756.04), C=O (1650.95), C=C (612.38); MS: m/z M+ 259.05.

Compound 1b Light orange color, solid, mp: 170-176°C, yield: 56.73%, IR (KBr, vmax) OH (3419.56), C-F (1164.92), C=O (1650.95), C=C (1612.38); MS: m/z M++1 243.09.

Compound 2a Creamy white color, solid, mp: 210-220°C, yield: 53.80%. IR (KBr, vmax) OH (3434.72), C-Cl (771.71), C-S-C (669.11), C=C (1595.52); MS: m/z M+366.11.

Compound 2b Creamy white color, solid, mp: 180-190°C, yield: 66.70%. IR (KBr, vmax) OH (3438.35), C-F (1162.34), C-S-C (670.80), C=C (1606.52); MS: m/z M++1 350.16.

Compound 2a' Brownish black color, solid, mp: 100-102°C, yield: 49.50%. IR (KBr, vmax) OH (3436.01), C-Cl (771.93), C-O-C (1038.11), C=C (1607.23); MS: m/z M+ 350.13.

Compound 2b' Brownish black color, solid, mp: 138-140°C, yield: 61.37%. IR (KBr, vmax) OH (3375.98), C-F (1167.72), C-O-C (1032.03), C=C (1600.23); MS: m/z M++1 334.18.

Determination of Partition Coefficient of the Synthesized Compounds:

The conventional and most reliable method, *i.e.* "Shake Flask Method", was used to determine the partition coefficient (log P) of the title compounds.

Initially, 2 mg of the synthesized compounds were dissolved in 100 ml of the solvent (50 mL water + 50 mL n-octanol). It was then shaken for 30 min and kept aside for 24 h to attain equilibrium. Two layers were then allowed to separate completely, and the separated layers were diluted using equal volume of n-octanol. The diluted layers were analyzed using UV visible spectrophotometer. Finally, the observed absorbance (Abs.) values were extrapolated over the standard curve so as to obtain the concentrations of the organic phase and the aqueous phase. The logP value of the title compounds was then determined from the concentrations obtained, of the n-octanol phase and the aqueous phase (Table **1**). Similar procedure was adopted for all the synthesized compounds.

In vitro Hydrolysis of the Synthesized Compounds:

Stability in gastric juice is of prime importance for the drugs intended for oral administration. It is observed that many drugs have low bioavailability as they are degraded in the stomach due to low pH (1-2). Simulated gastric fluid (SGF) mimics the gastric fluid in term of acidity and molarity, and simulated intestinal fluid (SIF) mimics the intestinal fluid in terms of basicity. These fluids are the perfect media to determine the stability of drug candidate's in-vitro. In the present study, the synthesized compounds were tested for invitro hydrolysis using SGF and SIF.

Preparation of Simulated Gastric Fluid:

Sodium chloride (2.0 g) and purified pepsin (3.2 g) were dissolved in 7.0 mL of hydrochloric acid (HCl). Sufficient water was added to this solution to make the volume up to 1000 mL, and the pH was adjusted to 1.2.

Preparation of Simulated Intestinal Fluid:

Monobasic potassium phosphate (6.8 g) was dissolved in 250 ml of distilled water and mixed. To this solution, 77 mL of 0.2N sodium hydroxide and 500 ml of distilled water were added. 10.0 g of pancreatin was then added and mixed. The pH of the resulting solution was maintained either with 0.2 N sodium hydroxide or 0.2 N hydrochloric acid to a range of 6.8±0.1. The resulting solution was diluted with water to make the volume up to 1000 mL.

Procedure of in vitro Hydrolysis:

Initially, (10 mg) of the synthesized compound was dispersed/ dissolved in 100 mL of simulated gastric fluid/simulated intestinal fluid. The resulting mixture was shaken in an orbital shaker at a temperature of 37±2 °C for 2 h. Samples were withdrawn at regular intervals of 0, 15, 30, 45, 60, 75, 90, 105, 120 min, and the volume was replaced. Samples were suitably diluted (twice), filtered and analyzed spectro-photometrically at the designated absorption maxima of each compound. Percent hydrolysis was calculated by subtracting the percent remaining from 100% (Table **2**)

% Hydrolyzed in SGF = [(Abs at 0 min. - Abs at 90 min.) /(Abs at 0 min.)] x 100

% Hydrolyzed in SIF= [(Abs at 0 min.- Abs at 120 min.) /(Abs at 0 min.)] x 100

Anticonvulsant Activity:

The anticonvulsant activity studies were performed using Maximal electroshock seizures (MES) model and Isoniazid (INH) induced convulsions model in mice. The MES model is associated with the electrical induction of the seizure, whereas INH model involves a chemical induction to generate convulsions. MES seizures were elicited using the apparatus with corneal electrodes [Medicraft Electro-Convulsiometer]. The synthesized compounds of both the series were administered to animals (male albino mice, weighing 25-35 g) intraperitoneally (i. p.) at 0.5mL/100g, suspended in 0.5% aqueous carboxymethylcellulose (CMC) in both the models and at 1mL/100gm body weight by oral route (p.o.) in INH model. Phenytoin (25mg/kg body weight) was used as the standard drug in MES model whereas Diazepam (4 mg/kg body weight) was used in INH model. Five animals for each dose level were used for the study. Animals were procured from the local authorized dealer and the animals were housed in polypropylene cages with steel net, in temperature controlled room under standard living conditions of 25±5 °C and relative humidity of 55±5 with regular 12 h light and dark cycles respectively and were given standard free access to food and water. All the animals were treated humanely in accordance with the guidelines laid down by the Institutional Animal Ethics Committee. The project proposal was approved by the Institutional Animal Ethics Committee (IAEC).

Maximal Electroshock (MES) Seizure Test:

Maximal electroshock seizures are elicited with a 50 Hz alternating current of 30 mA (5-7 times that is required to generate minimal electroshock seizures) delivered for 0.2 seconds *via* corneal electrodes. A drop of 0.9% saline was instilled in the eye prior to application of the electrodes in order to prevent the death of the animal This intensity was enough to produce a characteristic extensor tonus phase. Animals were observed approximately for 5-10 minutes and the characteristic phases like flexion, extensor, clonus, stupor and recovery or death were recorded. Finally, the percentage inhibition of seizures relative to the control/standard was calculated at various doses of the synthesized derivatives.

Isoniazid (INH) Induced Convulsion Model:

Isoniazid (300 mg/kg body weight) was used as the inducing agent for the precipitation of seizures.

Isoniazid was administered at 0.5mL/100g body weight by intra peritoneal route (i.p.) and at 1mL/100g body weight by oral route. The animals were observed after 30 min, and during the next 120 min, the occurrence of tonic-clonic seizures and death were recorded. Finally, the percentage protection from seizures, relative to control/standard, was calculated for various doses of the synthesized derivatives

Statistical Analysis:

All the values of the experiment are expressed as mean% inhibition ± SEM, and statistical significance between the groups was calculated by one-way analysis of variance (ANOVA) followed by Dennett's multiple comparison test; $P<0.01$ was considered statistically significant. Statistical analysis was carried out using Graph Pad Instat 3.0 (Graph-Pad Software, San Diego, CA).

RESULTS AND DISCUSSION

Benzothiazepine analogs reflect a very good example of "scaffold hopping" (bioisosteric replacement of the initial molecular scaffold by a different one, keeping the same biological activity) [43]. Many polar active molecules, selected through *in vitro* screening tests, are unable to cross the biomembranes and their bioavailability is particularly low. Thus, attaching a lipophilic moiety can sometimes help to overcome this drawback. The present study has majorly focused on the above mentioned issue and thus the distinctive feature of this series is the substitution by the different moieties at second and third position of benzothiazepine. All the synthesized derivatives were characterized by physical, chemical and spectral analysis (Melting range, solubility, partition coefficient, *in vitro* hydrolysis, IR, mass and NMR). All the spectral studies were performed and the observed values were found in agreement with the calculated values. The literature available for the seven membered fused anticonvulsant drugs proposes a great number of possibilities of variations to the present central ring so as to increase its lipophilicity and bioavailability. These compounds were screened for their anticonvulsant activity against maximal electroshock induced seizure (MES) tested at 30mg kg-1 i.p., showed activity in the range of 66.84 - 95.74%, where as isonizid induced convulsions (INH) at 30mg kg-1 i.p., showed activity in the range of 19.50-71.80%. MES is the animal model of human generalized tonic-clonic seizure, characterized by flexion, extensor, clonus, stupor and recovery/ death. INH model mainly induces petitmal seizure. Isosteric replacement of nitrogen with sulphur and oxygen in the benzodiazepine ring has proven to be the most useful tool in CNS acting drugs, since it improves the lipophilicity and possesses diverse bioactivities. The basic requirement involves aproton accepting group on the 2nd position of the seven membered fused rings for ligand binding to the GABA receptor. So the classical isosteric series of halogen substitution falls in agreement with, this statement. Thus, the reduced anticonvulsant activity of flourine substituted derivatives attributes to steric as well as electronic aspects. The amino-methylene substitution presents at the 3rd position of thiazepine ring accounts for the increased anticonvulsant activity, in the order of N-methylpiperazine>morpholine>piperazine. The extended profile of anticonvulsant activity due to the change in heterocylic ring at 3rdposition could be because of the basicity of nitrogen and methylation, which drastically modifies lipophilicity and thus its efficacy. The common group, *i.e*. phenolic hydroxy, substituted at 4th position of thiazepine ring may account for the change in partition coefficient towards more hydrophilicity and renders the molecule more water soluble. All the synthesized derivatives of present synthesis are showing a very interesting pattern of mechanism. Benzothiazepines are the analogs of benzodiazepines, as a consequence of isosteric replacement of N with O and S. Thus, the actual mode of action is expected to be similar to benzodiazepines, *i.e*. by modulating the effect of GABA binding to GABA-A chloride channel, and thus inhibiting neuronal excitability. It appears convincing that the synthesized derivatives should follow the same pattern of mechanism as the benzodiazepines. Since the synthesized derivatives were found to be more active in MES model against phenytoin than INH model against diazepam, accordingly the proposed mode of action seems to be similar to phenytoin, *i.e*. interaction with voltage dependent sodium channels and generation of action potential leading to the rapid depolarization of cell network. Thus, blocking ion influx and thereby causing the channels to inactivate to a greater degree and with smaller depolarization than normal, by stabilizing and prolonging this inactive state, prevents the rapid repetitive neuronal firing. The overall results reflect an improved anticonvulsant activity with 2-chloro and 3-aminomethylene substituted benzothiazepine ring. It is worth saying that there is a wide scope of revision/modification in the structure, using different secondary amine substitutions at third position of the benzothiazepine ring and third position of the benzoxazepine ring. In future, results of the above studies can provide a basis for the development of interesting chemical entities with improved pharmacological profiles. Therefore, this class of compounds may be used as templates to generate better drugs to suppress convulsions.

CONCLUSION:

The synthesized derivatives were found to be more active in the MES model than INH model, with phenytoin and diazepam being the standards respectively. Accordingly, the mode of action of the synthesized compounds may be similar to phenytoin. The methyl piperazine containing compound, at a dose of 30 mg/kg., was found to be the most active and promising compound in the series. In conclusion, a new series of 4-(4'-hydroxy phenyl)-2 (substituted phenyl)-3-[(substituted amino methylene)]-2,3-dihydro-1, 5 benzothiazepines were synthesized and evaluated for anticonvulsant activity against maximal electroshock induced seizure (MES) test and isoniazide induced convulsion (INH) test. The results of the present study ascertain the following statements: The Presence of benzothiazepine moiety showed better anticonvulsant activity and SAR studies confirmed that the 2-chlorophenyl substitution at second position of benzothiazepine rings showed more potent activity than the 4-fluorophenyl substituted benzothiazepines. Overall, in conclusion synthesized derivatives were found to be more active in MES model against phenytoin than INH model against diazepam, accordingly the proposed mode of action seems to be similar to phenytoin.

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