RESEARCH ARTICLE

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Synthesis and in Vitro Biological Activity Study of Novel Phenol Mannich Base Analogs Containing Spiroheterocycle as Core Compound

Kumar G. Gamit and Navin B. Patel

ABSTRACT

The condensation reactions between Gabapentin lactam and phenols have been carried out in the presence of formaldehyde for the synthesis of various mannich base series. To confirm the formation of these series of newly prepared mannich bases thus synthesized, various characterization techniques have been used, such as, GC-MS, 1H-NMR, 13C-NMR and FT-IR. Further their antibacterial and antifungal activities against pathogenic bacteria have been evaluated. These compounds show promising activities against the pathogenic bacteria, hence are useful as antibacterial and antifungal active compounds.

Keywords: Antibacterial Activity, Antifungal Activity, Formaldehyde, Gabapentin, Lactam, Phenolic Mannich Bases.

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I. Introduction

The mannich base formation by the condensation reaction between the aldehyde, active hydrogen compounds, and amine, leading to produce Mannich bases. There are four different types of classification of Mannich bases, which includes c-type, S-type, N-type, and P-type mannich bases. Mannich bases are getting industrial importance due to their vast applicability in the various industrial synthesis processes, such as in paints, cosmetics, synthetic polymer products, water treatment chemicals, petroleum additives, paper and leather industries and in textiles industries [1]. Besides these, the most important application of the Mannich base is in the field of medicinal chemistry, it can be used to improve the solubility and distribution of the drug molecules into the human body. The hydrophobic properties of the drugs could be increased by inserting the polar group into the drug molecules through the Mannich base reaction. In addition to that, the lipophilic properties of the drug molecules could be enhanced, if the suitable amine containing reagent is utilized in the Mannich base reaction [2]. The phenolic Mannich bases are classified as the C-Mannich bases which tends to forms C-C bonds and are considered as a very important class of the compounds due to their biological activities such as antibacterial activities [3],[4], antimycobacterial activities [5],[6], anticancer and cytotoxic activities [7]-[9], anti-inflammatory activities [10],[11], antihepatitis-B activities [12,13], antiviral activities [14]-[16], and antifungal activities [17],[18], analgesic activities [19], anticonvulsant activities [20],[21], enzyme inhibition activities [22], antioxidant properties [23],[24], blood pressure regulation [25],[26], and platelet aggregation inhibition activities [27].

Gabapentin lactam is one of the gabapentin derivatives and it is considered to be useful as the neuroprotective agent in the retinal ischemia. It is also considered as the spiro compound as it contains two rings connected with single carbon atom, such spiro compounds are of great interest in the field of medicinal chemistry, the spiro carbon available in the structure gives the adjustment along the precise vectors and allows structure rigidity because of the unique conformational properties and complexity in the structure [28]-[32].

According to the growing demand and in a continuation of our work in the field of synthesis of chemically useful and pharmacologically active compounds, it seems to be of great idea to synthesize various heterocyclic spiro compounds having gabapentin lactam moieties as a core compound and evaluate their biological activities. This research work comprising the synthesis methodology of the novel spiroheterocyclic compounds containing the gabapentin lactam as a backbone of the compound, through the reactions between various derivatives of phenols, formal dehydes and gabapentin lactam.

II. Experimental Section

A. Material and Apparatus

The chemicals utilized in this research work were mostly obtained from Fisher Scientific Ltd. and Sigma-Aldrich. The compounds thus received were further purified using thin layer chromatography (TLC) plates and column chromatography (silica gelG). The melting points of the compounds were determined using the open tube capillary method, and the results are unmodified. Fourier transform infrared spectroscopy (FT-IR) was used to generate IR spectrums using KBr pellets and an Agilent resolution Pro FT-IR spectrometer and a Perkin Elmer RZX infrared spectrophotometer, frequencies are provided in cm^{-1}. The ^{1}H-NMR and ^{13}C-NMRspectral data of the compounds are obtained through the Bruker Advance II 400 spectrometer (500 MHz FT-NMR) by dissolving in dimethyl sulfoxide (DMSO-d6) and tetramethylsilane is used as the internal standard (chemical shifts in ppm). Mass spectra were collected (electrospray ionization-MS). Merck coloumn chromatography (0.043-0.06 mm) was performed on silica gel 60. Antibacterial experiments are carried out in vitro against *E.coli, B cereus and B.substilis* and *C. albicans* are tested for antifungal activity. Ciprofloxacine and fluconazole are used as the standard drugs for the in vitro antibacterial and antifungal activity evaluation, respectively.

B. Chemical Synthesis

1) General Procedure for Synthesis of 2-(3, 5-2-hydroxybenzyl)-2-azaspiro [4.5] decan-3-one analogs 5a-5l

The reaction is carried out between the ethanolic solution of phenol (45 mL) and the mixture of gabapentin lactam and aqueous formaldehyde at 45°C. The reaction mixture was stirred gently at 45°C and then heated gently at 30°C for an hour. The completion of the reaction is checked using the TLC plate. After completion of the reaction as indicated by the TLC, the solvent is then evaporated through the vacuum and the products were obtained through the crystallization.

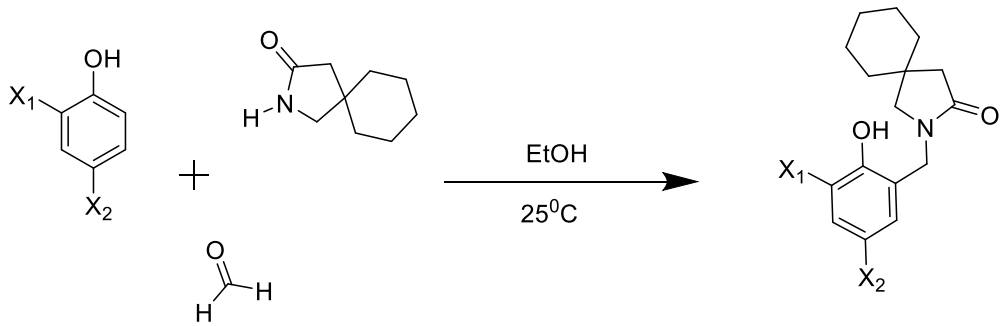

Fig. 1. General Reaction.

III. Results and Discussion

A. Chemistry

A new series of substituted 2-(5-bromo-2-hydroxybenzyl)-2-azaspiro [4.5] decan-3-one analogs and some spiro derivatives systems have been obtained through the synthetic route shown in Fig. 1. All compounds were obtained with good to moderate yields.

B. Characterization

The FT IR, ^{1}H NMR, ^{13}CNMR and Mass spectral data obtained for all the newly synthesized compounds are found in well agreement with the proposed structures, which further confirms the formation of the synthesized spiro derivatives compounds.

In the IR spectrum of intermediate sharp str. bands around at cm^{-1} - 2900 (sp3 -CH), 1000-1100 (C-C), 1600 (benzene), 3200-3500 (-OH),1200-1300 (C-N), 1695 (Amide), 1480(CH2-).

^{1}H-NMR (ppm) =9 S (1H, OH) ,7.45 S (1H,J= 7.6 Hz),7.27 d (1H, J= 7.6 Hz), 6.73 d (1H, J= 7.6 Hz), 4.21 S (2H),3.37 S (2H),2.01 S (2H),1.61-1.65 t (4H,J=6.9),1.42-1.64 m (6H, J=6.9).

^{13}CNMR δ ppm =172 (-amide), 154 (-benzyl C), 132,132,126,116,115 (-aromatic carbon), 55, 46, 43(-CH2-), 37(4^{0}C), 36, 26, 27 (Cyclohexane). The molecular ion peak at m/z [M^{+}] -337 also further confirms the formation of spiro compound 2-(5-bromo-2-hydroxybenzyl)-2-azaspiro [4.5] decan-3-one.

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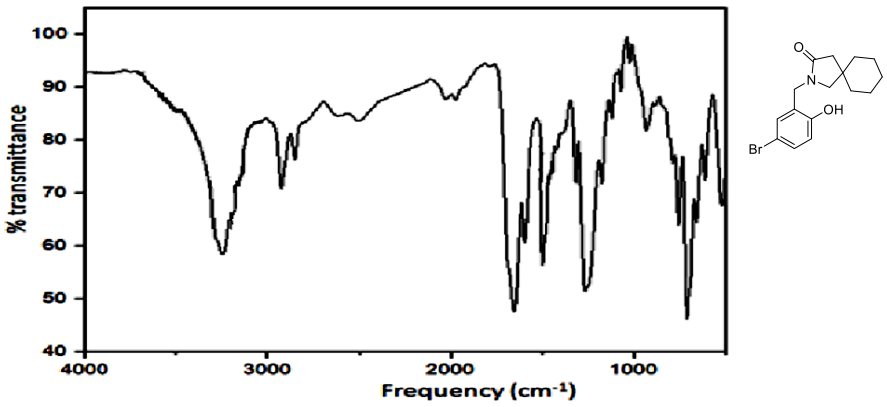


Fig. 2. FT-IR spectrum of 2-(5-bromo-2-hydroxybenzyl)-2-azaspiro [4.5] decan-3-one.

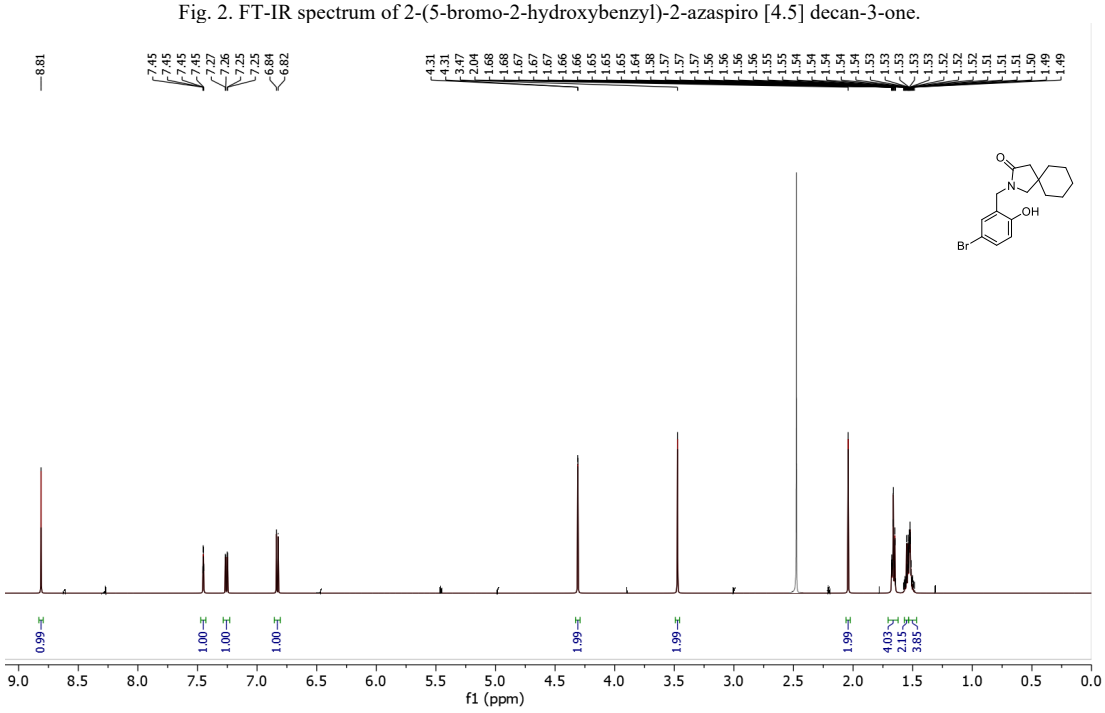


Fig. 3. ^{1}H-NMR spectrum of 2-(5-bromo-2-hydroxybenzyl)-2-azaspiro [4.5] decan-3-one.

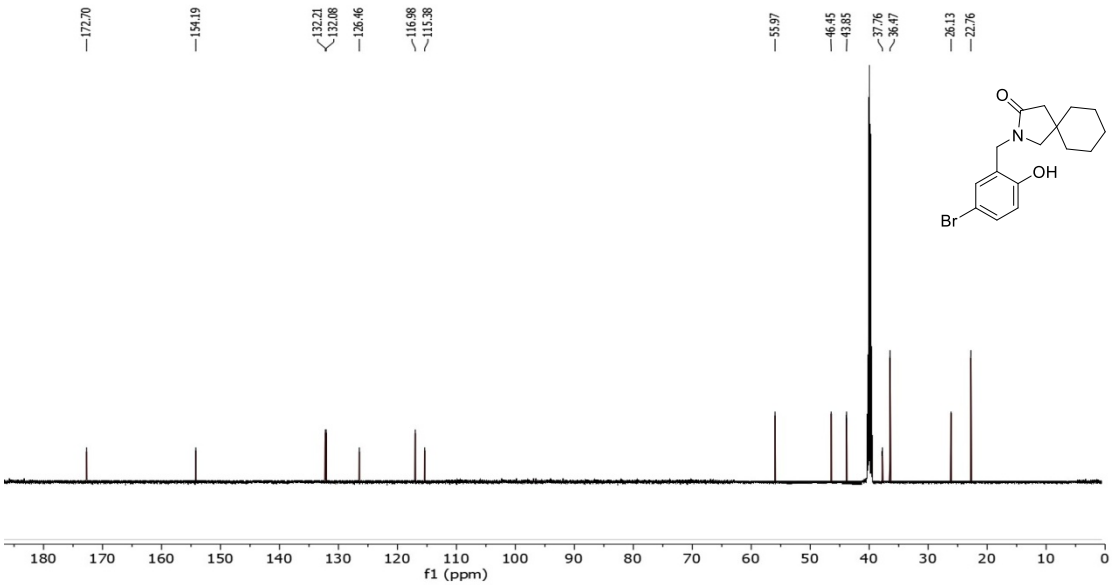
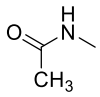
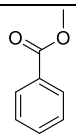
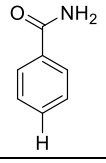
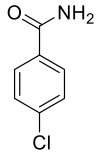
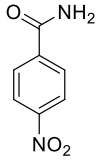
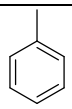
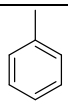
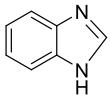


Fig. 4. ^{13}CNMR spectrum of 2-(5-bromo-2-hydroxybenzyl)-2-azaspiro [4.5] decan-3-one. & Mass m/z: [M^{+}] 337.

TABLE I: PHYSICAL DATA OF THE COMPOUNDS

Compounds	X1	X2	%Yields	M.P (±5) ^{0}C	M.F
5a	H	t-C4H9	62	178	$C_{20}H_{29}NO$
5b	H	Neo-C5H11	55	185	$C_{21}H_{31}NO_2$
5c	H		60	185	$C_{18}H_{24}N_2O_3$
5d	H		68	189	$C_{23}H_{25}NO_4$
5e	H		66	182	$C_{23}H_{26}N_2O_3$
5f	H	Br	78	151	$C_{16}H_{20}BrNO_2$
5g	H		70	110	$C_{23}H_{25}ClN_2O_3$
5h	H		55	115	$C_{23}H_{25}N_3O_5$
5i	H		50	93	$C_{22}H_{25}NO_2$
5j		H	65	66	$C_{22}H_{25}NO_2$
5k		H	70	180	$C_{23}H_{25}N_3O_2$
5l		H	61	140	$C_{19}H_{25}NO_2$

C. Biological Activities

The activity of the synthetic compounds against the vulnerable bacteria Ecoli*, B.cereus, B.suhstilisas* well as species of fungi as *C. albicans*, was evaluated by the Agar-well diffusion method. The agar-well diffusion method [33]-[36] which is adapted earlier was used as the simple susceptibility screening test. The microorganisms as stated above were suspended in the broth namely Mueller Hinton (MH) (Difco, Detroit, MI), which was further diluted approximately to 106 colony forming unit (CFU)/mL. Then flood-inoculation of above diluted suspensions were carried out on the surface of the MH agar and the Sabouraud Dextrose Agar (SDA) (Difco, Detroit, MI) and then dried. The SDA was also utilized for the species of fungi, C. albicans and C. tropicalis. Then, the wells with 5mm diameter were cutting down from the agar using a well sterile cork-borer and the extract substances (50 mL) were delivered into the wells. Further, the plates were placed into the incubator at the temperature of 35 °C for the duration of 18h. The antimicrobial activity was then evaluated by the analysis of the inhibition zone against the test organisms. Ciprofloxacine (5 mg) and fluconazoIe (7 mg) were utilized as the standard drugs. Ethanol was utilized as the solvent control.

TABLE II: ANTIBACTERIAL SCREENINGS OF THE COMPOUNDS

Zone of inhibition for minimal bactericidal and fungicidal concentration					
Antibacterial Activity				Antifungal Activity	
Compounds	Inhibition zone (mm) Gram negative		Gram positive	Compounds	Inhibition zone (mm)
	E.coli	***B.cereus***	***B.substilis***		***C. albicans***
5a	9	11	-	5a	-
5b	9	11	8	5b	-
5c	11	9	-	5c	6
5d	11	9	8	5d	8
5e	12	8	10	5e	14
5f	19	20	19	5f	15
5g	18	19	18	5g	15
5h	14	16	15	5h	8
5i	15	14	14	5i	10
5j	11	9	8	5j	12
5k	21	23	22	5k	16
5l	8	10	9	5l	-
Ciprofloxacin	22	25	24	-	-
fluconazole	-	-	-	-	20

IV. CONCLUSION

We have synthesized mannich base type derivatives bearing phenol, formaldehyde and gabapantine lactam cores. All the newly synthesized compounds showed different levels of activity towards bacteria and fungi. Compounds5h, 5I, 5J good activity and 5f, 5g 5k showed the good active against *E.coli, B.cereus, B.substilis, and C. albicans* respectively with inhibition zone 9 to 23 mm. The results suggest that these phenol mannich base derivatives are a promising class of molecular entities for the development of new anti-microbial leads.

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CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest.

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