Design, synthesis and biological evaluation of new Mannich products using ethyl ammonium nitrate as reusable ionic liquid

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Abstract: Solvent free and simple one-pot synthesis for three-component protocol has been used for efficient synthesis of new mannich products (HB$_2$B$_{12}$) in excellent yields from heterocyclic aldehyde, phenol as uv absorbing material and various amide derivatives with using Ethyl ammonium nitrate (reusable ionic liquid) as catalyst under 80$^\circ$C temperature. All synthesized compounds were characterized by $^1$H-NMR, $^{13}$C NMR spectra and screened for their in vitro antibacterial activity by using the agar dilution technique. Specially two compounds shows very good microbial activity as Shown by standard drugs, HB$_8$ and HB$_{10}$ compounds showed potential activity especially against E. Coli MTCC 443 (MIC = 45-50 µg/mL) P. Aeruginosa MTCC 1688 (MIC = 45-50 µg/mL) and S. Aureus MTCC 96 (MIC=45–50 µg/mL). All compounds HB$_{9,12}$ shows very good activity but HB$_7$ and HB$_{16}$ compound shows excellent antifungal activity between 75-100 µg/mL which is most active rather than Greoselufvin. Antituberculosis activity of HB$_8$ and HB$_{16}$ compounds shows good activity 1 µg/mL using L. J. medium conventional Method.

Keywords: Solvent-free, One-pot synthesis, Reusable ionic liquid, Uv absorbing materials, Mannich products.

1. Introduction

In the field organic synthesis, Mannich reactions are well reported since 1912. In which there is a reaction between compounds containing at least one active hydrogen atom condense with aldehyde and primary or secondary amines [1-2]. It is also reports as a variety of natural products containing 1,3-amino-oxygenated functional groups act as potential drugs, as antibiotic [3], antitumor [4], antimalarial [5], antiangiogenic [6], antihypertensive [7], antirheumatics [8] and HIV protease inhibitors [9]. The bradycardic effects of these motifs have also been reported [10]. Owing to the biological and medicinal as well pharmacological importance of 1-amidoalkyl-2-naphthols derivatives, efforts have been made by the various researchers in developing multi-component coupling reactions for the synthesis of 1-amidoalkyl-2-naphthols from aldehydes, phenols and amides/ carbamates under thermal and/or heating or sonication conditions using various catalysts such as montmorillonite K10 [11], Ptsa [12], iodine [13], Fe(HSO4)3 [14], K$_5$CoW$_{12}$O$_{40}$·3H$_2$O [15], HClO$_4$·SiO$_2$ [16], cation exchange resins [17], silica sulfuric acid [18], thiamine hydrochloride [19], zwitter-ionic salts [20] and supported acid catalyst [21] and ionic liquids [22].

All these methods reported so far lack general applicability and having one or other limitations such as high reaction temperature, longer reaction time, and lower yield of the desire product, tedious work-up and use of toxic reagents. Recently, use of ionic liquids in organic synthesis has become the center of interest due to their dual role as catalyst and media along with their unique properties such as hydrophobicities/ hydrophilicities, good solvating capability, easy recoverability, reusability, high thermal stability and non-flammability with almost no vapour pressure. Due to novel properties of ionic liquids, their use in MCRs for name reactions such as Kabachnik-Field reaction, Biginelli reaction, Ugi reaction, and Mannich reaction have been well documented. A cost effective construction of its structural unit of new mannich products (HB$_2$B$_{12}$) using MCRs protocol under much more efficient, environment friendly conditions using recyclable, ecofriendly ionic liquid as green catalyst is still a possibility to explore. As compared to other ionic liquids, ethylammonium nitrate (EAN) with acidic properties (pH=5) is cheap, easily recoverable and reusable at room temperature. Literature survey also reveals that derivatives of hydroxybenzophenone and derivatives of dihydrobenzofuran/benzoferan are used as u. v. absorber in such reactions [23-29] and halo derivatives of heterocyclic compounds show very good biological activities [30-35].

Looking to the above synthetic applications and their medicinal importance and as part of continuous efforts to explore the application of ethylammonium nitrate as a reusable ionic liquid catalyst and/or reaction media for organic transformation, I have planned to carry out one pot multi component manich reaction of 5-bromothiophene-2-carboxaldehyde, various amides derivatives and 2-hydroxy-4-methoxybenzophenone as u. v. absorbing material under neat reaction conditions for the preparation of various new mannich products (HB$_7$, B$_{12}$) shown in (Scheme 1). I report herein a highly efficient, cost effective, general and much milder MCRs protocol for the synthesis of new mannich products (HB$_2$B$_{12}$) in good to excellent yields via one-pot three-component condensation.
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Scheme 1: EAN catalyzed synthesis of new mannich products (HB₁₂ to HB₁₂) from Halo derivatives of Heterocyclic aldehyde, phenol and various amides.

Mechanism of mannich reaction is shown in (Figure 1). It involves two steps: Initially an iminium ion I and II is formed due to nucleophilic addition of amide to aldehyde and subsequent loss of water molecule. In the second step the enolizable carbonyl compound is converted to enol form, which attacks the iminium ion at positively charged carbon adjacent to nitrogen to give new mannich products (HB₁₂ to HB₁₂).

Figure 1: Mechanism of Mannich reaction
II. Experimental

2.1 Materials and methods

All reactions were performed at 80°C with high speed stirring was carried out with magnetic force. All chemicals were purchased from Alfa Aesar Chemical Co. and solvents were used without further purification. Analytical thin-layer chromatography was performed with E. Merck silica gel 60F2 glass plates. Visualization of the developed chromatogram was performed by UV light. Melting points were determined with Shimadzu DS-50 thermal analyzer. \(^1^H\)-NMR spectra were recorded on Bruker Advance II (400 MHz) in DMSO using TMS as internal standard. \(^1^3^C\) NMR spectra were recorded on Bruker Advance II (400 MHz) in DMSO using TMS as internal standard. FT-IR spectra were obtained as KBr discs on Shimadzu spectrometer. Elemental analysis was measured by means of Perkin Elmer 2400 CHN's elemental analyzer.

2.2. General procedure for synthesis of new Mannich products (HB\(_7\)/HB\(_{12}\))

A mixture of 2-hydroxy-4-methoxybenzophenone 2.17 gm (0.01M), 2-chloro-6-methoxyquinoline 3-carboxaldehyde 1.106 gm (0.01 M), various amides 0.6 gm (0.01M) and EAN 60 ml (2M) was stirred at 80°C temperature. The completion of reaction was monitored by TLC using (chloroform/methanol, 80:20). On completion of reaction, the reaction mixture was extracted thrice with 20 ml ethyl acetate. The extract was dried over anhydrous sodium sulfate, evaporated under vacuum and the residue was purified via recrystallisation from ethanol or ethanol/acetonitrile (v/v = 3:2) to obtain pure new mannich products (HB\(_7\), B\(_{12}\)). Synthetic Route for one pot multi component mannich reaction is shown in scheme 1:

2.2.1. 1-((5-benzoyl-4-hydroxy-2-methoxyphenyl)(2-chloro-6-methoxyquinolin-3-yl)methyl)urea (HB\(_7\))

Yield: 88%; decomposition temperature 210-214 °C; color: yellow; IR (KBr,cm\(^{-1}\)): 3463, (Ar–OH), 3198 (-NH), 3072 (CH, aromatic), 2974, 741 (CH aliphatic), 1705,1628 (C=O, diaryl), 1605 (C=N), 1519 (C–N), 1481 (C–C, aromatic),1212-1024 (C=O–C), 1101 (C–O), 732, 584 (for substituted benzene); \(^1^H\)-NMR (400 MHz, DMSO-d6): \(\delta\) 3.83 (s, 3H, OMe), 5.35 (s, 2H, Ar–OH), 6.0 (s, 3H, NHCONH\(_2\)), 6.16 (1H, aliphatic-CH), 6.47-7.83 (m, 7H,Ar–H), 7.14-8.20 (4H, -CH for substituted quinoline); \(^1^3^C\) NMR (400 MHz, DMSO-d6): \(\delta\) 47.9 (aliphatic-CH), 55.8 (-OCH\(_3\)), 105, 122.3, 128.8, 131.4, 135.0, 137.0, 141.1, 149.7 (C=Cl),157.2 (C=OCH\(_3\)), (9CH aromatic), 103.1, 111.2, 111.7, 128.4, 128.4, 130.3, 130.3, 131.2, 132.4, 138.4, 159.1, 163.9 (2C,Ar–OH), (12CH aromatic), 162.7 (amide), 199.7 (Carbonyl); ESI-MS m/z: 491(M+1)\(^+\); Anal. Calc. for C\(_{29}\)H\(_{22}\)Cl\(_5\)N\(_5\)O\(_3\): C, 63.48; H, 4.51; Cl, 7.21; N, 8.54; O, 16.26% found: C, 63.42; H, 4.50; Cl, 7.20; N, 8.51; O, 16.22%;

2.2.2. 1-((5-benzoyl-4-hydroxy-2-methoxyphenyl)(2-chloro-6-methoxyquinolin-3-yl) methyl) thiourea (HB\(_{12}\))

Yield: 90%; decomposition temperature 226-230 °C; color: pale yellow; IR (KBr,cm\(^{-1}\)): 3463, (Ar–OH), 3198 (-NH), 3072 (CH, aromatic), 2974, 741 (CH aliphatic), 1705,1630 (C=O, diaryl), 1605 (C=N), 1519 (C–N), 1481 (C–C, aromatic),1329 (C=S), 1212-1024 (C=O–C), 1101 (C–O), 732, 584 (for substituted benzene), 691 (C=S); \(^1^H\)-NMR (400 MHz, DMSO-d6): \(\delta\) 2.0 (s, 1H, -NH), 3.83 (s, 3H, OMe), 5.19 (1H, aliphatic-CH), 5.35 (s, 2H, Ar–OH), 6.48-7.78 (m, 7H, Ar–H), 7.14-8.20 (4H, -CH for substituted quinoline), 8.56 (s, 2H, NH\(_2\)); \(^1^3^C\) NMR (400 MHz, DMSO-d6): \(\delta\) 53.0 (aliphatic-CH), 55.8 (-OCH\(_3\)), 105, 122.3, 128.8, 131.4, 135.0, 137.0, 141.1, 149.7 (C=Cl),157.2 (C=OCH\(_3\)), (9CH aromatic), 103.1, 111.2, 111.7, 128.4, 128.4, 130.3, 130.3, 131.2, 132.4, 138.4, 159.1, 163.9 (2C,Ar–OH), (12CH aromatic), 182.5 (amide), 199.7 (Carbonyl); ESI-MS m/z: 507(M+1)\(^+\); Anal. Calc. for C\(_{29}\)H\(_{22}\)Cl\(_5\)N\(_5\)O\(_3\): C, 61.47; H, 4.37; Cl, 6.98; N, 8.27; O, 12.60; S, 6.31%; found: C, 61.43; H, 4.32; Cl, 6.94; N, 8.22; O, 12.58; S, 6.28%;

2.2.3. 2-((5-benzoyl-4-hydroxy-2-methoxyphenyl)(2-chloro-6-methoxyquinolin-3-yl) methyl)hydrazine carbamidox (HB\(_{13}\))

Yield: 92%; decomposition temperature 242-245 °C; color: yellowish green; IR (KBr,cm\(^{-1}\)): 3463, (Ar–OH), 3198 (-NH), 3072 (CH, aromatic), 2974, 741 (CH aliphatic), 1705,1629 (C=O, diaryl), 1605 (C=N), 1519 (C–N), 1481 (C–C, aromatic),1212-1024 (C=O–C), 1101 (C–O), 732, 584 (for substituted benzene); \(^1^H\)-NMR(400 MHz, DMSO-d6): \(\delta\) 2.0 (s, 1H, -NH), 3.83 (s, 3H, OMe), 5.19 (1H, aliphatic-CH), 5.35 (s, 2H, Ar–OH), 6.0 (m, 3H, NHCONH\(_2\)), 6.48-7.78 (m, 7H,Ar–H), 7.14-8.20 (4H, -CH for substituted quinoline); \(^1^3^C\) NMR (400 MHz, DMSO-d6): \(\delta\) 54.8 (aliphatic-CH), 55.8 (-OCH\(_3\)), 105, 122.3, 128.8, 131.4, 135.0, 137.0, 141.1, 149.7 (C=Cl),157.2 (C=OCH\(_3\)), (9CH aromatic), 103.1, 111.2, 111.7, 128.4, 128.4, 130.3, 130.3, 131.2, 132.4, 138.4, 159.1, 163.9 (2C,Ar–OH), (12CH aromatic), 157.4 (-NHCONH\(_2\)), 199.7 (Carbonyl); ESI-MS m/z: 506(M+1)\(^+\); Anal. Calc. for C\(_{29}\)H\(_{22}\)Cl\(_5\)N\(_5\)O\(_3\): C, 61.60; H, 4.57; Cl, 6.99; N, 11.05; O, 15.78%; found: C, 61.56; H, 4.52; Cl, 6.91; N, 11.01; O, 15.73 %;
2.2.4. 2-((5-benzoyl-4-hydroxy-2-methoxyphenyl)(2-chloro-6-methoxyquinolin-3-yl) methyl) hydrazinecarboxamide (HB₉)  
Yield: 91%; decomposition temperature 260–264 °C; color: pale yellow; IR (KBr, cm⁻¹): 3463, (Ar–OH), 3198 (-NH), 3072 (CH, aromatic), 2974, 741 (CH aliphatic), 1705, 1633 (C=O, diaryl), 1605 (C=N), 1519 (C–N), 1481 (C–C, aromatic), 1329 (C=S), 1212-1024 (C=O-C), 1101 (C–O), 732, 584 (for substituted benzene), 690 (C=S); ¹H-NMR(400 MHz, DMSO-d₆): δ 2.0 (d, 2H, -NH), 3.83 (s, 3H, OMe), 5.19 (1H, aliphatic-CH), 5.35 (s, 2H, Ar–OH), 6.48-7.78 (m, 7H, Ar–H), 7.14-8.20 (4H, -CH for substituted quinoline), 8.56 (s, 2H, NH₃); ¹³C NMR (400 MHz, DMSO-d₆): δ 55.5 (aliphatic-CH), 55.8 (-OCH₃), 105, 122.3, 128.8, 131.4, 135.0, 137.0, 141.1, 149.7 (C-Cl), 157.2 (C–OCH₃), (9CH aromatic), 103.1, 111.2, 111.7, 128.4, 128.4, 130.3, 130.3, 131.2, 132.4, 133.8, 159.1, 163.9 (2C, Ar–OH), (12CH aromatic), 182.5 (-NHCSNH₂), 199.7 (Carbonyl); ESI-MS m/z: 522 (M+1)⁺; Anal. Calc. for C₂₇H₂₃ClN₄O₈S (%): C, 59.71; H, 4.43; Cl, 6.78; N, 10.71; O, 12.24; S, 6.13%; found: C, 59.67; H, 4.40; Cl, 6.71; N, 10.65; O, 12.21; S, 6.10%.

2.2.5. N-((5-benzoyl-4-hydroxy-2-methoxyphenyl)(2-chloro-6-methoxyquinolin-3-yl) methyl) acetamide (HB₁₀)  
Yield: 90%; decomposition temperature 188-192 °C; color: yellowish green; IR (KBr, cm⁻¹): 3463, (Ar–OH), 3198 (-NH), 3072 (CH, aromatic), 2974, 741, 650 (CH aliphatic), 1705, 1628 (C=O, diaryl), 1605 (C=N), 1519 (C–N), 1481 (C–C, aromatic), 1212-1024 (C=O-C), 1101 (C–O), 732, 584 (for substituted benzene); ¹H-NMR(400 MHz, DMSO-d₆): δ 1.84 (t, 3H, -CH₃), 3.83 (s, 3H, OMe), 5.35 (s, 2H, Ar–OH), 6.16 (1H, aliphatic-CH), 6.48-7.78 (m, 7H, Ar–H), 7.14-8.20 (4H, -CH for substituted quinoline), 8.03 (s, 1H, NH₃); ¹³C NMR (400 MHz, DMSO-d₆): δ 23.6 (CH₃), 45.8 (aliphatic-CH), 55.8 (-OCH₃), 105, 122.3, 128.8, 131.4, 135.0, 137.0, 141.1, 149.7 (C-Cl), 157.2 (C–OCH₃), (9CH aromatic), 103.1, 111.2, 111.7, 128.4, 128.4, 130.3, 130.3, 131.2, 132.4, 133.8, 159.1, 163.9 (2C, Ar–OH), (12CH aromatic), 169.0 (amide), 199.7 (Carbonyl); ESI-MS m/z: 590 (M+1)⁺; Anal. Calc. for C₂₇H₂₃ClN₄O₇S (%): C, 66.06; H, 4.72; Cl, 7.22; N, 5.71; O, 16.29%; found: C, 66.01; H, 4.66; Cl, 7.20; N, 5.67; O, 16.21%.

2.2.6. N-((5-benzoyl-4-hydroxy-2-methoxyphenyl)(2-chloro-6-methoxyquinolin-3-yl) methyl) benzamide (HB₁₁)  
Yield: 92%; decomposition temperature 212–216 °C; color: yellowish green; IR (KBr, cm⁻¹): 3463, (Ar–OH), 3198 (-NH), 3072 (CH, aromatic), 2974, 741 (CH aliphatic), 1705, 1627 (C=O, diaryl), 1605 (C=N), 1519 (C–N), 1481 (C–C, aromatic), 1212-1024 (C=O-C), 1101 (C–O), 732, 584 (for substituted benzene); ¹H-NMR (400 MHz, DMSO-d₆): δ 3.83 (s, 3H, OMe), 5.35 (s, 2H, Ar–OH), 6.16 (1H, aliphatic-CH), 6.48-8.03 (m, 12H, Ar–H), 7.14-8.20 (4H, -CH for substituted quinoline), 8.13 (s, 1H, NH₃); ¹³C NMR (400 MHz, DMSO-d₆): δ 46.6 (aliphatic-CH), 55.8 (-OCH₃), 105, 122.3, 128.8, 131.4, 135.0, 137.0, 141.1, 149.7 (C-Cl), 157.2 (C–OCH₃), (9CH aromatic), 103.1, 111.2, 111.7, 128.4, 128.4, 130.3, 130.3, 131.2, 132.4, 133.8, 159.1, 163.9 (2C, Ar–OH), (12CH aromatic), 166.1 (amide), 199.7 (Carbonyl); ESI-MS m/z: 552 (M+1)⁺; Anal. Calc. for C₂₇H₂₃ClN₄O₆ (%): C, 69.50; H, 4.56; Cl, 6.41; N, 5.07; O, 14.47%; found: C, 69.47; H, 4.51; Cl, 6.37; N, 5.01; O, 14.42%.

2.3. Antimicrobial Activity

2.3.1. Antibacterial and antifungal activity

The antimicrobial activity of the synthesized compounds has been evaluated by filter paper disc method [36-41]. The synthesized compounds have been tested for their antibacterial activity against E. coli MTCC 443, P. Aeruginosa MTCC 1688, S. Aureus MTCC 96, and S. Pyogenus MTCC 442 and antifungal activity against Candida albicans MTCC 227, Aspergillus niger, MTCC 282 and A. Clavatus MTCC 1323 at a concentration of 500 μg/mL in DMF. Nutrient agar and potato dextrose agar were used to culture the bacteria and fungi, respectively. The plates were inoculated by the bacteria or fungi and incubated for 24 h at 37 °C for bacteria and for 72 h at 27°C for fungi and then the inhibition zones of microbial growth surrounding the filter paper disc (5 mm) were measured in millimeters. Ampicillin and Chloramphenicol, at a concentration 500 μg/mL, were used as standard against bacteria and Greseofulvin is used as standered drug for fungi, respectively. Test results are shown in (Table 1, Figure 2) for antibacterial and (Table 2 & Figure 3) for antifungal activity from the data, it is clear that compounds HB₉, HB₁₀ possess excellent activity, while compounds HB₁₀, HB₁₁ and HB₁₂ possess good activity.

2.3.2. Anti-mycobacterial activity

Only four compounds were evaluated for in vitro anti tuberculosis Activity [42-43] against M. tuberculosis, as part of the TAAFC TB screening program under direction of the Micro Care Laboratory. Isoniazide was used as a reference drug. MIC of compounds (HB₁₁) was determined against M. tuberculosis.
H₃⁷Rv strain by using L.J. Medium conventional method and only HB₉ and HB₁₀ shows moderate activity as compared to isoniazide. The MIC is defined as the lowest concentration affecting a reduction in fluorescence of 90 % relative to controls. The activity data of compounds (HB₈-₁₁) are given in (Table 3, Figure 4).

2.3.3. Structure activity relationship (SAR)
In this study a new series of new mannich products (HB₇-₁₂) were synthesized and evaluated against ant tuberculosis, as part of the TAACF TB screening program. From the six compounds only (HB₈-₁₁) is tested and HB₆, HB₁₀ displayed significant inhibition effects HB₁₂ and HB₅ shows poor effect in the primary screening against M. tuberculosis H₃₇Rv in the BACTEC 12B medium. Compounds demonstrating at least 90% inhibition in the primary screening were re-tested in order to determine the actual minimum inhibitory concentration (MIC) against M. tuberculosis. A brief investigation of the structural activity reveals that the activity is considerably moderate affected by chloro substituent at the 2nd position of quinoline nucleus and different amide substituents in all compounds. It has been observed that among the series, most of the compounds having electron withdrawing group at the 2nd position of quinoline ring and due to different amide substituent in manich product exhibited the significant anti-tubercular activity Among the electron withdrawing groups, chlorosubstituent at 2nd position of quinoline nucleus and hydrazinecarboxamide in HB₆, hydrazinethiocarboxamide group in HB₁₀ shows an enhanced anti-tubercular activity with MIC of 100 mg mL⁻¹.

Table 1 Antibacterial activity

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Table 2 Antifungal activity

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Fig 2 Diagram for antibacterial activity
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Fig 3 Diagram for antifungal activity

Fig 4 Diagram for anti tuberculosis activity

Table 3 Anti tuberculosis activity

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III. Result & DISCUSSION

The promising results obtained on 2-chloro-6-methoxyquinoline-3-carboxaldehyde, 2-hydroxy-4-methoxybenzophenone and various amides using 1 M EAN as catalyst at the 80°C temperature encouraged us to investigate the feasibility of solvent-free MCRs protocol to a wide range of chloro substituted aldehydes, amides/ carbamates/urea and 2-hydroxy-4-methoxybenzophenone for the synthesis of new mannich products (HB₈-HB₁₁).

A chloroderivative of heterocyclic aldehydes, amides/ carbamates/urea possessing various electron donating and electron withdrawing functional groups reacted smoothly with 2-hydroxy-4-methoxy benzophenone under neat reaction conditions to give desired products in good yields over EAN at 80°C temperature. The results illustrate that the one-pot three component condensation reactions show excellent performance irrespective of the presence of electron withdrawing or electron donating groups on aromatic/heterocyclic aldehydes and hence solvent-free MCRs protocol is highly effective, promising and general for the
synthesis of new manich products (HB$_5$,B$_{12}$). The substituted aromatic aldehydes with electron withdrawing group reacted with 2-hydroxy-4-methoxybenzophenone and different amides provided desired products in excellent yields.

The recovery and recyclability of EAN was still investigated for the synthesis of new manich products (HB$_5$,B$_{12}$) by one-pot three component condensations of above said aldehyde, phenol and different amide as model substrates in the presence of EAN is under progress. The high yield of new manich products (HB$_5$,B$_{12}$) using EAN at milder reaction condition compared to other ionic liquid can be rationalized due to high acidity associated with it (pH=5) along with its capacity to absorb water formed during course of the reaction.

All the synthesized compounds (HB$_5$,B$_{12}$) were purified by re-crystallization with suitable solvents and characterized by spectral FT-IR, $^1$H-NMR, $^{13}$C-NMR and elemental analysis. The results of elemental analyses of each new manich products (HB$_5$-B$_{12}$) were consistent with the predicted structure, as shown in scheme 1. The presence of aliphatic (–CH group) in each compound shows $^1$H-NMR spectra 6.16 δ is confirmed obtained manich reaction. The IR spectrum of each compounds comprised the important features of aromatic, methoxy, hydroxyl, keto and chloro and different amide groups. The $^1$H-NMR spectra of all the new compounds based on 2-hydroxy-4-methoxybenzo phenone, chlorodervatives of quinoline and amide group show important signals at their respective positions, confirming the structures of (HB$_5$-B$_{12}$), as shown in scheme 1. Methyl group protons gave a singlet between 1.84 δ ppm in compounds HB$_{11}$, methoxy and hydroxy groups in all compounds shows 3.83 to 3.90 δ and 5.35 δ where as –NH of amide group shows 6.0 δ.

The IR spectra of all compound (HB$_5$,B$_{12}$) showed absorption band at around 3463, 3198, 3072, 2974, 741, 1705, 1628,1605, 1519, 1481, 1212-1024, 1101, 732, 584 cm$^{-1}$ regions, confirming the groups presence in each compounds are retained.$^{13}$C-NMR spectra of all compounds showed characteristic signals appearing for aliphatic –CH is 54.8 δ where as 149.7 (C-Cl), 157.2 (C-OCH$_3$), 163.9 (2C, Ar–OH)$_2$, 182.5 (-NICS$\text{SNH}_3$), 199.7 (Carbonyl)and 55.8 δ for (-OCH$_3$) respectively. The results of spectral analysis indicated that the compounds are pure.

All synthesized compounds were screened in vitro antibacterial activity by using the agar dilution technique. Out of six compounds only two compounds shows very good microbial activity as compared to standard drugs. HB$_5$ and HB$_{10}$ compounds showed very good activity especially against P. aeruginosa MTCC 1688 (MIC=45 µg/mL), E. coli MTCC 443 (MIC = 45–50 µg/mL) and S. Aureus MTCC 96 (MIC=45–50 µg/mL). HB$_5$ and HB$_{10}$ compounds shows excellent antifungal activity against C. Albicans MTCC 227 and A. Niger MTCC 282 (MIC=75 µg/mL) where as other four compounds also shows good activity between (MIC=100–150 µg/mL). Similarly antituberculosis activity of HB$_5$ and HB$_{10}$ compounds shows good activity 1 µg/mL using L. J. medium conventional Method.

IV. Conclusions

In summary, we have developed an environmentally friendly, high yield and mild condition protocol for the three-component Mannich-type reactions, which is a rapid and convenient procedure for the synthesis of new manich products (HB$_5$-B$_{12}$) via multi-component Mannich reaction catalyzed by ionic liquid EAN under high speed stirring. This method offers several advantages, compared to those reported in the literature, i.e., (a) mild, highly efficient catalyst activity, (b) ease of handling and cost efficiency of the catalyst, (c) avoidance of the troublesome preparation of enol derivatives and pre-formed imines, (d) wide substrate scope and generality especially for substituted amides, and (e) effective reusability of catalyst, making it a useful and attractive strategy. The present method is convenient and applicable to a wide variety of aldehydes, phenols and amides or urea or carbamates for the synthesis of corresponding of new manich products (HB$_5$,B$_{12}$). EAN was recovered and recycled several times without loss of catalytic activity.

All synthesized compounds were characterized and screened for their in vitro antibacterial activity by using the agar dilution technique. Only HB$_5$ and HB$_{10}$ compounds shows potential activity against Gram-positive bacteria (minimal inhibitory concentration [MIC] = 45-50 µg/mL). All compounds shows Antifungal activity between 75-150 µg/mL only HB$_5$, HB$_{10}$ shows [MIC] = 75 µg/mL against C. Albicans MTCC 227 and A. Niger MTCC 282. Antituberculosis activity of four compounds shows moderate activity but only HB$_5$ and HB$_{10}$ shows moderate activity [MIC] = 1 µg/mL using L. J. medium conventional Method.

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