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RESEARCH ARTICLE

SOLVENT-FREE SYNTHESIS OF MANNICH PRODUCTS CATALYZED BY ETHYL AMMONIUM NITRATE AS REUSABLE IONIC LIQUID AND THEIR IN VITRO MICROBIAL STUDIES

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ABSTRACT

A solvent-free, simple one-pot reaction has been used for synthesis of mannich products (HA_1-HA_6) in excellent yields via three-component condensation of heterocyclic aldehyde, phenol as u.v. absorbing material and various amide derivatives using ethylammonium nitrate (EAN) as reusable ionic liquid as catalyst under 80 °C temperature. All compounds were characterized by 1H-NMR, ^{13}C NMR and infrared spectra were recorded in KBr pellets on a Perkin–Elmer Spectrum GX FT-IR model. All compounds also screened for their in vitro antibacterial activity by using the agar dilution technique. Specially two compounds shows very good microbial activity as compared to standard drugs, HA_3 and HA4 compounds showed potential activity especially against P. Aeruginosa MTCC 1688(MIC, 40-50 µg/mL), and S. Aureus MTCC 96 (MIC, 45-50 µg/mL). HA_3 compound shows good Antifungal activity between 100-200 µg/mL similarly HA_4shows 100-150 µg/mL.Antituberculosis activity of HA_3 and HA^4 compounds shows moderate activity 5-7 µg/mL using L. J. conventional method.

INTRODUCTION

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], antianginal [6], antihypertensive[7], antirheumatics [8] and HIV protease inhibitors [9]. The bradycardiac 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 K [10], PtSA [12], iodine [13], $Fe(HSO_4)3$ [14], $K5CoW_{12}O_{40}•3H_2O$ [15], $HClO_4$-SiO_2 [16], cationexchange resins [17], silica sulfuric acid [18], thiamine hydrochloride[19], zwitter-ionic salts [20]and supported acid catalyst [21,22], and ionic liquids [23-27].

However, almost all these methods reported so far lack general applicability and suffer from 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. Therefore, the development of more general and cost effective multi component reactions (MCRs) protocol for the synthesis of γ-methyl amino-β-hydroxycarbonyl compounds is still challenging and an active research area. 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. However, the developed MCRs protocol for the synthesis γ-methyl amino-β-hydroxycarbonyl compounds using ionic liquids reported at higher temperature is not compatible with sensitive functional groups and hence limits their application for the commercial exploitation as catalysts and/or reaction media to achieve high yield of the products. A cost effective construction of its structural unit γ-methyl amino-β-hydroxycarbonyl compounds 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 are used as ultraviolet absorber in such reactions [28-45] and halo derivatives of heterocyclic compounds show very good biological activities [46-55].

Looking to the above synthetic applications and their medicinal importance in earlier work [56] 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,we have

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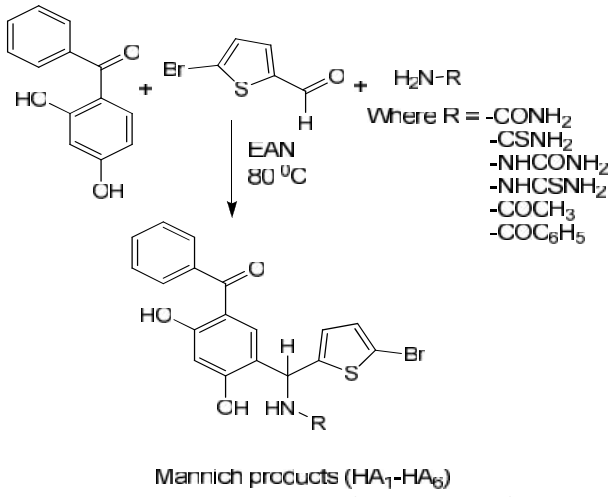
planned to carry out one pot multi componentmannichreaction of 5-bromothiophene-2-carboxaldehyde,various amides derivatives and 2,4-dihydroxybenzophenonen as ultraviolet absorbing material under neat reaction conditions for the preparation of various new heterocyclic compounds i.e.(HA_1-HA_6) shown in Scheme1. We report herein a highly efficient, cost effective, general and much milder MCRs protocol for the synthesis of (HA_1-HA_6) in good to excellent yields via one-pot three-component condensation.



Scheme 1. EAN catalyzed synthesis of γ-methyl amino-β-hydroxy carbonyl compounds (HA_1-A_6) from Heterocyclic aldehyde, phenol and various amides.

Synthesis

The Mannich reaction is the amino alkylation reaction, involving the condensation of an enolizable carbonyl compound (acidic compound) with a non-enolizable aldehyde and amides to furnish a (HA_1-HA_6) also known as Mannich products. 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 produce new mannich products (HA^1-HA_6).

General procedure for Mannich products (HA_1-HA_6)

A mixture of 2,4-dihydroxybenzophenone 2.14 g (0.01 M), 5-bromothiophene-2-carboxaldehyde 2 mL (0.01 M), various amides 0.6 g (0.01M) and EAN 30 mL (1 M) was stirred at 80 °C temperature. The completion of reaction was monitored by TLC by using chloroform:methanol mixture (70:30, *v*:*v*). 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 recrystallization from ethanol or ethanol: acetone (3:2, v:v) to obtain pure mannich products (HA_1-HA_6). Synthetic Route for one pot multi component mannich reaction is shown in Scheme 1.All the isolated

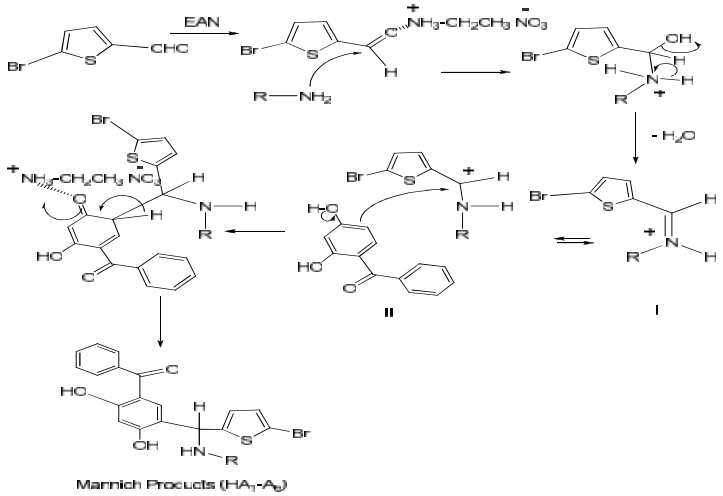


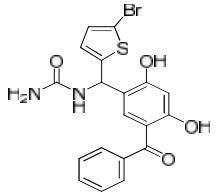
Figure 1 Mechanism of mannich reaction.

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 Asear Chemical Co. and solvents were used without further purification. Analytical thin-layer chromatography was performed with E. Merck silica gel 60F glass plates. Visualization of the developed chromatogram was per-formed by UV light. Melting points were determined with Shimadzu DS-50 thermal analyzer. $_1$HNMR spectra were recorded on Bruker Advance II (400 MHz) in DMSO-d6 using TMS as internal standard.^{13}C NMR spectra were recorded on Bruker Advance II (100 MHz) in DMSO-d6using TMS as internal standard. FT-IR spectra were obtained in KBr pellets on a Perkin–Elmer Spectrum GX FT-IR model. Elemental analysis was measured by means of Perkin Elmer 2400 CHNS elemental analyzer.

reaction products were characterized and confirmed by IR, ^{1}H-NMR, ^{13}C-NMR, Mass spectra and elemental analysis.

1-((5-benzoyl-2,4-dihydroxyphenyl)(5-bromothiophen-2-yl)methyl)urea (HA_1)



Chemical Formula: $C_{19}H_{15}BrN_2O_4S$
Exact Mass: 445.99
Molecular Weight: 447.30
m/z: 447.99 (100.0%), 445.99 (98.1%), 448.99 (22.0%), 447.00 (20.5%), 449.99 (4.7%), 448.00 (3.0%), 450.00 (2.9%), 446.99 (1.5%)
Elemental Analysis: C, 51.02, H, 3.38, Br, 17.86, N, 6.26, O, 14.31, S, 7.17

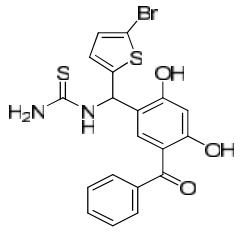
Color: Yellowish green.Yield: 86%.M.p.: 240-245 °C (Dec.).
FT-IR (KBr, v, cm^{-1}): 3463 (OH)(phenol), 3072 (CH)(aromatic),1628 (C=O)(diaryl), 1481 (C-C)(aromatic), 1333 (C–NH), 1101 (C–OH), 732, 584(for substituted benzene).

^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 5.35 (s, 1H, Ar-OH), 6.3 (s, 1H,-NH), 6.16 (d, 1H, aliphatic-N-CH), 6.56-6.67 (m, 2H, -CH for thiophene) 6.48-7.78 (m, 7H, Ar–H), 7.84 (s, 1H, Ar-OH, Intra), 7.98 (s, 2H, NH_2).

^{13}C NMR (100 MHz, DMSO–d6, δ, ppm): 199.7 (1C, C=O, diaryl), 162.7 (1C, C=O, $NHCONH_2$), 163.9 (1C, C-OH, phenolic), 159.1 (1C, C-OH, phenolic), 142.0 (1C, Ar-C), 138.4(1C, Ar-C), 132.4 (1C, Ar-C), 131.2 (1C, Ar-C), 130.3(2C, Ar-C), 128.6(1C, Ar-C), 128.4(2C, Ar-C), 128.2(1C, Ar-C), 111.7 (1C, Ar-C), 111.2 (1C, Ar-C), 109.7(1C, Ar-C), 103.1 (1C, Ar-C), 54.5 (1C,CH, aliphatic).
MS(EI, m/z (%)): 447,(M^+,100).

Anal.calc. for $C_{19}H_{15}BrN_2O_4S$: C, 51.02; H, 3.38; N, 6.26; S, 7.17.Found: C, 51.02; H, 3.33; N, 6.22; S, 7.12 %.

1-((5-benzoyl-2,4-dihydroxyphenyl)(5-bromothiophen-2-yl)methyl)thiourea(HA_2)



Chemical Formula: $C_{19}H_{15}BrN_2O_3S_2$
Exact Mass: 461.97
Molecular Weight: 463.37
m/z: 463.97 (100.0%), 461.97 (93.1%), 464.97 (22.8%), 462.97 (21.4%), 465.96 (8.4%), 463.98 (1.9%), 465.98 (1.9%), 466.97 (1.8%), 465.97 (1.3%)
Elemental Analysis: C, 49.25; H, 3.26; Br, 17.24; N, 6.05; O, 10.36; S, 13.84

Color: Yellowish green.
Yield: 83%.
M.P.: decomposition temperature 248–252 ^{0}C.
FT-IR (KBr, v, cm^{-1}): 3450 (OH) (phenol), 3060 (CH) (aromatic), 1625(C=O) (diaryl), 1478 (C-C) (aromatic), 1330 (C–NH), 1100 (C–OH), 730, 581 (for substituted benzene).
^{1}H–NMR (400 MHz, DMSO–d6, δ, ppm): 5.83 (d, 1H, aliphatic-N-CH), 5.35 (s, 1H, Ar-OH), 6.3 (s, 1H,-NH), 6.56-6.67 (m, 2H, -CH for thiophene), 6.48-7.78 (m, 7H, Ar–H) 7.87 (s, 1H, Ar-OH, Intra), 8.56 (s, 2H, NH_2).

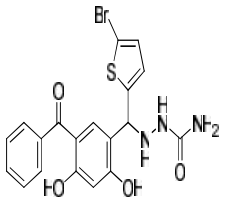
^{13}C NMR (100 MHz, DMSO–d6, δ, ppm): 199.7 (1C, C=O, diaryl), 182.5 (1C, C=S, $NHCSNH_2$), 163.9 (1C, C-OH, phenolic), 159.1 (1C, C-OH, phenolic), 142.0 (1C, Ar-C), 138.4 (1C, Ar-C), 132.4 (1C, Ar-C), 131.2 (1C, Ar-C), 130.3 (2C, Ar-C), 128.6 (1C, Ar-C), 128.4 (2C, Ar-C), 128.2 (1C, Ar-C), 111.7 (1C, Ar-C), 111.2 (1C, Ar-C), 109.7 (1C, Ar-C), 103.1 (1C, Ar-C), 59.1 (1C, CH, aliphatic).
MS (EI, m/z (%)): 463, (M^+, 100).

Anal.calc. for$C_{19}H_{15}BrN_2O_3S_2$ (%): C, 49.25; H, 3.26; N, 6.05; S, 13.84. Found: C, 49.21; H, 3.23; N, 6.00; S, 13.81 %.

2-((5-benzoyl-2,4-dihydroxyphenyl)(5-bromothiophen-2-yl)methyl)hydrazine carboxamide(HA_3)

Color: Yellowish green.
Yield: 79%.
M.P.: decomposition temperature 260–264 ^{0}C.

FT-IR (KBr, v, cm^{-1}): 3453 (OH) (phenol), 3071 (CH) (aromatic), 1624(C=O) (diaryl), 1476 (C-C) (aromatic), 1329 (C–NH), 1102 (C–OH), 728, 574 (for substituted benzene).
^{1}H–NMR (400 MHz, DMSO–d6, δ, ppm): 5.35 (s, 1H, Ar-OH), 6.31 (d, 1H, aliphatic-N-CH), 6.56-6.67 (m, 2H, -CH for thiophene), 6.48-7.78 (m, 7H, Ar–H), 6.76 (d, 2H,-NHNH), 7.83 (s, 1H, Ar-OH, Intra), 8.16 (s, 2H, NH_2).

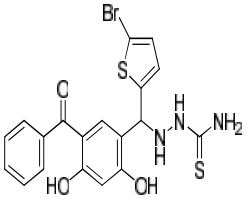


Chemical Formula: $C_{19}H_{16}BrN_3O_4S$
Exact Mass: 461.00
Molecular Weight: 462.32
m/z: 463.00 (100.0%), 461.00 (98.0%), 462.01 (20.5%), 464.01 (20.3%), 465.00 (4.6%), 465.01 (3.0%), 463.01 (3.0%), 464.00 (2.8%), 462.00 (1.9%)
Elemental Analysis: C, 49.36; H, 3.49; Br, 17.28; N, 9.09; O, 13.84; S, 6.94

^{13}C NMR (100 MHz, DMSO–d6, δ, ppm): 199.7 (1C, C=O, diaryl), 157.4 (1C, C=O, $NHNHCONH_2$), 163.9 (1C, C-OH, phenolic), 159.1 (1C, C-OH, phenolic), 142.0 (1C, Ar-C), 138.4 (1C, Ar-C), 132.4 (1C, Ar-C), 131.2 (1C, Ar-C), 130.3 (2C, Ar-C), 128.6 (1C, Ar-C), 128.4 (2C, Ar-C), 128.2 (1C, Ar-C), 111.7 (1C, Ar-C), 111.2 (1C, Ar-C), 109.7 (1C, Ar-C), 103.1 (1C, Ar-C), 59.5 (1C, CH, aliphatic).
MS (EI, m/z (%)): 462,(M^+,100).

Anal.calc. for$C_{19}H_{16}BrN_3O_4S$ (%): C, 49.36; H, 3.49; N, 9.09; S, 6.94.Found: C, 49.33; H, 3.46; N, 9.07; S, 6.91 %.

2-((5-benzoyl-2,4-dihydroxyphenyl)(5-bromothiophen-2-yl)methyl)hydrazine carbothioamide (HA_4)



Chemical Formula: $C_{19}H_{16}BrN_3O_3S_2$
Exact Mass: 476.98
Molecular Weight: 478.38
m/z: 478.98 (100.0%), 476.98 (93.5%), 479.98 (23.1%), 477.99 (19.5%), 480.98 (9.6%), 477.98 (2.5%), 478.99 (2.5%), 480.99 (1.9%), 481.98 (1.8%)
Elemental Analysis: C, 47.70; H, 3.37; Br, 16.70; N, 8.78; O, 10.03; S, 13.41

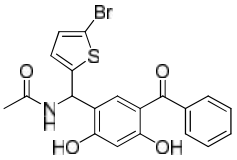
Color: Yellow.
Yield: 84%.
M.P.: decomposition temperature 258–262 ^{0}C.
FT-IR (KBr, v, cm^{-1}): 3465 (OH) (phenol), 3069 (CH) (aromatic), 1630(C=O) (diaryl), 1483 (C-C) (aromatic), 1323(C–NH),, 1106(C–OH), 736, 589, (for substituted benzene).

^{1}H–NMR (400 MHz, DMSO–d6, δ, ppm): 5.19 (d, 1H, aliphatic-N-CH), 6.35 (s, 1H, Ar-OH), 6.56-6.67 (m, 2H, -CH for thiophene), 6.67 (d, 2H,-NHNH), 6.48-7.78 (m, 7H, Ar–H), 7.82 (s, 1H, Ar-OH, Intra), 8.11 (s, 2H, NH_2).

^{13}C NMR (100 MHz, DMSO–d6, δ, ppm):199.7 (1C, C=O, diaryl), 182.5 (1C, C=O, NHNHCSNH$_2$), 163.9 (1C, C-OH, phenolic), 159.1 (1C, C-OH, phenolic), 142.0 (1C, Ar-C), 138.4 (1C, Ar-C), 132.4 (1C, Ar-C), 131.2 (1C, Ar-C), 130.3 (2C, Ar-C), 128.6 (1C, Ar-C), 128.4 (2C, Ar-C), 128.2 (1C, Ar-C), 111.7 (1C, Ar-C), 111.2 (1C, Ar-C), 109.7 (1C, Ar-C), 103.1 (1C, Ar-C), 60.2 (1C, CH, aliphatic).
MS (EI, m/z (%)): 478 (M$^+$, 100).

Anal.calc. for$C_{19}H_{16}BrN_3O_3S_2$ (%): C, 47.70; H, 3.37; N, 8.78; S, 13.41.Found: C, 47.68; H, 3.32; N, 8.75; S, 13.38 %.

N-((5-benzoyl-2,4-dihydroxyphenyl)(5-bromothiophen-2-yl)methyl)acetamide(HA_5)



Chemical Formula: $C_{20}H_{16}BrNO_4S$
Exact Mass: 445.00
Molecular Weight: 446.31
m/z: 445.00 (100.0%), 447.00 (98.4%), 448.00 (23.2%), 446.00 (23.1%), 446.99 (4.5%), 448.99 (4.4%), 449.00 (3.4%), 447.01 (2.3%), 450.00 (1.2%)
Elemental Analysis: C, 53.82; H, 3.61; Br, 17.90; N, 3.14; O, 14.34; S, 7.18

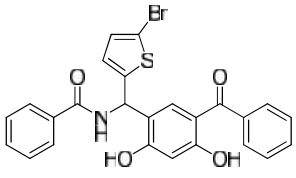
Color: Yellowish green.
Yield: 88%.
M.P.: decomposition temperature 235–239 ^{0}C.
FT-IR (KBr, v, cm^{-1}): 3462 (OH) (phenol), 3066 (CH) (aromatic), 1626(C=O) (diaryl), 1486 (C-C) (aromatic), 1334(C–NH),, 1103(C–OH), 738, 580 (for substituted benzene).

^{1}H–NMR (400 MHz, DMSO–d6, δ, ppm): 2.01 (t, 3H, -CH3), 6.35 (s, 1H, Ar-OH), 6.47 (d, 1H, aliphatic-N-CH), 6.56-6.67 (m, 2H, -CH for thiophene), 6.48-7.78 (m, 7H, Ar–H), 7.87 (s, 1H, Ar-OH, Intra), 8.85 (s, 1H, NH).

^{13}C NMR (100 MHz, DMSO–d6, δ, ppm): 199.7 (1C, C=O, diaryl), 169.0 (1C, C=O, NHCOCH$_3$), 163.9 (1C, C-OH, phenolic), 159.1 (1C, C-OH, phenolic), 142.0 (1C, Ar-C), 138.4 (1C, Ar-C), 132.4 (1C, Ar-C), 131.2 (1C, Ar-C), 130.3 (2C, Ar-C), 128.6 (1C, Ar-C), 128.4 (2C, Ar-C), 128.2 (1C, Ar-C), 111.7 (1C, Ar-C), 111.2 (1C, Ar-C), 109.7 (1C, Ar-C), 103.1 (1C, Ar-C), 59.5 (1C, CH, aliphatic), 23.6 (1C, CH$_3$).
MS (EI, m/z (%)): 446, (M$^+$, 100).

Anal.calc. for$C_{20}H_{16}BrNO_4S$ (%): C, 53.82; H, 3.61; N, 3.14; S, 7.18.Found: C, 53.72; H, 3.60; N, 3.11; S, 7.14 %.

N-((5-benzoyl-2,4-dihydroxyphenyl)(5-bromothiophen-2-yl)methyl)benzamide (HA_6)



Chemical Formula: $C_{25}H_{18}BrNO_4S$
Exact Mass: 507.01
Molecular Weight: 508.38
m/z: 509.01 (100.0%), 507.01 (98.1%), 508.02 (26.9%), 510.02 (26.7%), 511.01 (4.7%), 509.02 (4.6%), 511.02 (4.4%), 510.01 (2.3%), 512.01 (1.2%), 508.01 (1.1%)
Elemental Analysis: C, 59.06; H, 3.57; Br, 15.72; N, 2.76; O, 12.59; S, 6.31

Color: Yellowish green.
Yield: 87%.
M.P.: decomposition temperature 190–196 ^{0}C.
FT-IR (KBr, v, cm^{-1}): 3463 (OH) (phenol), 3072 (CH) (aromatic), 1628 (C=O) (diaryl), 1481 (C-C) (aromatic), 1333 (C–NH), 1101 (C–OH), 732, 584, 481 (for substituted benzene).

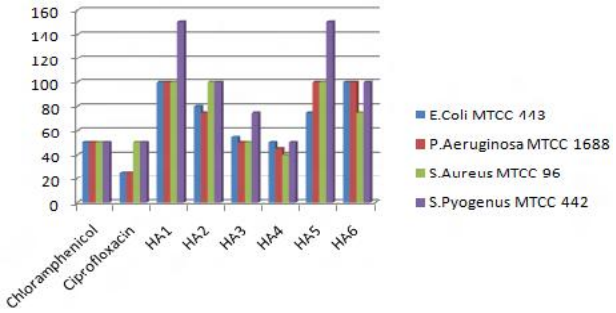


Figure 2 Diagram for antibacterial activity.

^{1}H–NMR (400 MHz, DMSO–d6, δ, ppm): 6.35 (s, 1H, Ar-OH), 6.46 (d, 1H, aliphatic-N-CH), 6.56-6.67 (m, 2H, -CH for thiophene), 6.48-8.03 (m, 12H, Ar–H), 7.87 (s, 1H, Ar-OH, Intra), 8.85 (s, 1H, NH).

^{13}C NMR (100 MHz, DMSO–d6, δ, ppm): 199.7 (1C, C=O, diaryl), 166.1 (1C, C=O, NHCOPh), 163.9 (1C, C-OH, phenolic), 159.1 (1C, C-OH, phenolic), 142.0 (1C, Ar-C), 138.4 (1C, Ar-C), 132.4 (1C, Ar-C), 132.1, (1C, Ar-C), 131.2 (1C, Ar-C), 131.1 (1C, Ar-C), 130.3 (2C, Ar-C), 128.8 (2C, Ar-C), 128.6 (1C, Ar-C), 128.4 (2C, Ar-C), 128.2 (1C, Ar-C), 127.5 (2C, Ar-C), 111.7 (1C, Ar-C), 111.2 (1C, Ar-C), 109.7 (1C, Ar-C), 103.1 (1C, Ar-C), 52.7 (1C, CH, aliphatic),
MS (EI, m/z (%)): 508, (M$^+$, 100).
Anal.calc. for$C_{25}H_{18}BrNO_4S$ (%): C, 59.06; H, 3.57; N, 2.76; S, 6.31.Found: 59.01; H, 3.53; N, 2.75; S, 6.25 %.

Antibacterial and antifungal activity of Mannichproducts (HA_1-HA_6)

The antimicrobial activity of the synthesized compounds has been evaluated by filter paper disc method [57-61]. The synthesized compounds have been tested for their antibacterial activity against *E. coliMTCC 443*, *P. AeruginosaMTCC 1688*, *S. AureusMTCC 96*, and *S. PyogenusMTCC 442* and antifungal activity against *Candida albicans* MTCC 227, *Aspergillusniger*, *MTCC 282* and *A. ClavatusMTCC 1323* at a concentration of 500µg/mL in DMF. Nutrient agar and potato dextrose agars were used to culture the bacteria and fungi, respectively. The plates were inculcated 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.

Ciprofloxacin and chloramphenicol, at a concentration 500µg/mL, was used as standard against bacteria and Greseofulvin is used as standard drug for fungi, respectively.
Test results are shown in Table I and Figure 2 for antibacterial and Table II and Figure 3 for antifungal activity from the data, it is clear that compounds **HA_3**and

HA_4 possess high activity, while compounds HA_1, HA_5 and HA_6 possess poor activity.

Table 1 Antibacterial Activity

Sr. no	Code No.	Minimum inhibition concentration			
		E.ColiMTCC 443	P.AeruginosaMTCC 1688	S.AureusMTCC 96	S.PyogenusMTCC 442
1	HA1	100	100	100	150
2	HA2	80	75	100	100
3	HA3	55	50	50	75
4	HA4	50	45	40	50
5	HA5	75	100	100	150
6	HA6	100	100	75	100
	Chloramphenicol	50	50	50	50
	Ciprofloxacin	25	25	50	50

Table 2 Antifungal activity

Sr. no	Code No.	Minimum fungicidal concentration		
		C. Albicans MTCC 227	A. Niger MTCC 282	A. Clavatus MTCC 1323
1	HA1	700	300	200
2	HA2	500	500	1000
3	HA3	100	200	100
4	HA4	100	150	100
5	HA5	200	500	200
6	HA6	500	500	100
	Nystatin	100	100	100
	Greseofulvin	500	100	100

Table 3 Anti tuberculosis activity

Concentrations	Anti Tuberculosis Activity *1000µg/ml, 500µg/ml, 250µg/ml, 100 µg/ml, 62.5 µg/ml, 50 µg/ml,25 µg/ml, 12.5µg/ml, 6.25 µg/ml, 3.25 µg/ml	
Sr. No.	Code No.	MIC µg/ml
1	HA_2	25
2	HA_3	5
3	HA_4	7
4	HA_5	35
	Isoniazide	0.20

Method: L. J. Medium [conventional method].
Bacteria: H37RV
*Different concentrations was used for four samples

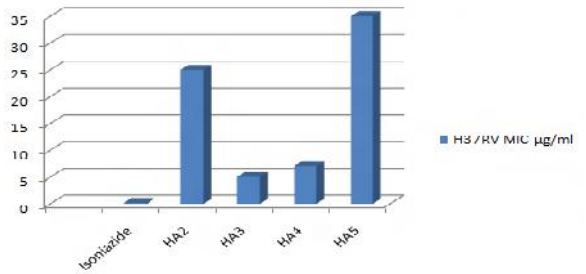


Figure 4 diagram for antituberculosis activity having 300 DPI Resolutions

RESULTS & DISCUSSION

Mannich products and their spectral characteristics

The promising results on 5-bromothiophene-2-carboxaldehyde, 2,4-dihydroxybenzophenone 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 bromosubstituted aldehydes, amides/carbamates/urea and 2,4-dihydroxybenzo phenonefor the synthesis of (HA_1-HA_6).

A bromo derivative of hetrocyclic aldehydes, amides/carbamates/urea possessing various electron donating and electron withdrawing functional groups reacted smoothly with 2,4-dihydroxybenzophenone under neat reaction conditions to give desired products in excellent 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/hetrocyclic aldehydes and hence solvent-free MCRs protocol is highly effective, promising and general for the synthesis of (HA_1-HA_6). The substituted aromatic aldehydes with electron withdrawing group reacted with 2,4-dihydroxybenzophenone and different amides provided desired products in excellent yields.

The recovery and recyclability of EAN was still investigated for the synthesis of (HA_1-HA_6)by one-pot three component condensations of above said aldehyde, phenol and differentamide as model substrates in the presence of EAN is under progress. The high yield of (HA_1-HA_6) 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 (HA1-HA6)were purified by re-crystallization with suitable solvents and characterized by spectral FT-IR, ^{1}HNMR, ^{13}CNMR and elemental analysis. The results of elemental analyses of each new mannich products (HA_1-HA_6) 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 δ 6.16 ppmis confirmed obtained mannich 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 2,4-dihydroxybenzophenone, chloro derivatives of quinoline and amide group show important signals at their respective positions, confirming the structures of (HA_1-HA_6), as shown in scheme 1. Methyl group protons gave a singlet between 2.01 δ ppm in compounds HA_5, and hydroxy groups in all compounds shows singlet between δ 5.35 to 6.67 ppm and δ 7.87 to 8.48 ppm for hydroxy group having intramolecular hydrogen bonding whereas -NH of amide group shows singlet between δ 8.03 to 8.85 ppm. At δ 5.19 to 6.16 ppm occurs doublet for aliphatic (–CH-N), which clearly indicated that obtained new products are correct one.

The IR spectra of all compound (HA_1-HA_6)showed absorption band at around 3463, 3198, 3072, 2974, 741, 1705, 1628,1605, 1519, 1481, 1212-1024, 1101, 732 and 584 cm-1 regions, conforming the groups presence in each compounds are retained.

^{13}C NMR spectra of all compounds showed characteristic signals appearing for aliphatic –CH is δ54.8 ppmwhereas δ149.7 (C-Cl), 157.2 (C-OCH3), 163.9 (2C, Ar–OH), 182.5 (-NHCSNH2), 199.7 (Carbonyl) and 55.8 ppm for (-OCH3). The results of spectral analysis indicated that the compounds are pure.

Antimicrobial activity of the Mannich products

All synthesized compounds were screened for their 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. HA_3 and HA_4 compounds showed potential activity especially against P. Aeruginosa MTCC 1688 (MIC=40-50 µg/mL), and S. AureusMTCC 96 (MIC=45-50 µg/mL). HA_3 compound shows good Antifungal activity between 100-200 µg/mL similarly HA4shows 100-150 µg/mL.Antituberculosis activity of HA^3 and HA^4 compounds shows moderate activity 5-7 µg/mL using L. J. medium conventional method.

Structure activity relationship (SAR)

In this study a new series of mannich products (HA_2-HA_5) were synthesized and evaluated against M. tuberculosis, as part of the (TAACF) TB screening program. From the six compounds only (HA_2-HA_5)is tested and HA_3, HA_4 displayed significant inhibition effects HA_1 and HA_2 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 bromo substituent at the 5th position of thiophene 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 5thposition of thiophene ring and due to different amide substituent in mannich product exhibited the significant anti-tubercular activity Among the electron withdrawing groups, bromosubstituent at fifth position of thiophene and hydrazinecarboxamide inHA_3, hydrazine thiocarboxamide group in HA4shows an enhanced anti-tubercular activity with MIC of 100 mg/mL.

CONCLUSION

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 mannich products (HA_1-HA_6)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 mannich products (HA_1-HA_6). EAN was recovered and recycled several times without loss of catalytic activity.

All synthesized compounds were screened for their in vitro antibacterial activity by using the agar dilution technique. Only HA_3 and HA_4 compounds shows potential activity against Gram-positive bacteria (minimal inhibitory concentration [MIC] = 40-50 µg/mL). All compounds shows antifungal activity between 250-1000 µg/mL only HA_3, HA_4 shows [MIC] = 100-200 µg/mL.Antituberculosis activity of four compounds shows poor activity but only HA_3 and HA_4shows moderate activity [MIC] = 5-7 µg/mL using L. J. medium conventional method.

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