SYNTHESIS OF NOVEL ANTI-INFLAMMATORY USNIC ACID-BASED IMIDAZOLIUM SALTS

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Ten novel usnic acid based imidazolium salts were synthesized by employing a two-step protocol. The anti-inflammatory potential of the newly synthesised compounds was evaluated *in vitro* against cytokine proteins TNF- α and IL-1 β secreted from U937 cells. Some of the imidazolium salts exhibited promising anti-inflammatory activity against the TNF-α and IL-1β with IC_{50} values ranging between 5.3 μM - 7.5 μM, which are many folds lower than that of the parent compound (>100 μM). Most significantly, substitution with electronegative groups in imidazolium salts of usnic acid found to be more potent and exhibiting enhanced anti-inflammatory activity.

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INTRODUCTION

Usnic acid, a natural dibenzofuran lichen metabolite, has attracted the attention as 'Hot Natural Scaffold' in view of its structural diversity and therapeutic potential. Usnic acid is reported to exhibit a wide range of biological activities such as anti-inflammatory, antioxidant as well as pro-oxidant, antimicrobial, antiviral, antibiotic, and antitumor activities etc.[1] It was isolated from different genera of lichens such as Usnea (Usneaceae), Lecanora (Lecanoraceae) and Cladonia (Cladoniaceae) in large quantity (up to 26%).[2] It plays a crucial role in restricting prostaglandin and it acts like an anti-inflammatory drug.[3] The anti-inflammatory property of usnic acid[4] has been shown by down-regulating the expression of TNF- α, MIP-2, IL-8, IL-6. Huang et al. have explored the mechanism of usnic acid against inflammation by encouraging the lipopolysaccharide from the RAW264.7 cell line. It acts in a dose dependent manner towards pro-inflammatory mediators and cytokines that leads to attenuate the IL-6, IL-1β, iNOS, to COX-2 through dwindling the NFkB factor.[5] Usnic acid possesses a wide range of functional groups and associated with intramolecular hydrogen bonding between the functional groups. D. N. Sokolov et al. have analysed some usnic acid- amine hybrids of both enantiomers, against influenza virus A (H1N1)pdm09. Interestingly, (+)-usnic acid enamine derivatives explored with good antiviral activity than (-)-usnic acid –amine hybrids.[6] Usnic acid consists of three rings such as A, B & C rings. Out of these, A and C rings are amenable for a wide range of chemical transformations by introducing pharmacophore moieties such as chalcones, thiazoles, aurones, enamines, coumarins, and flavones. Among these analogues, enamines have shown the best biological profiles.[7] Enamines such as usenamines were identified as chemically and biologically diversified structures in lichens and exhibited a broad spectrum of biological activities.[2] Bruno et al. explored the wound repairing mechanism of usnic acid enamine hybrids by employing *in vitro* and *in vivo* assays.[8] Imidazole is a five-membered N-heterocyclic ring contains two nitrogen atoms and is an important pharmacophore in medicinal chemistry.[9] Imidazolium salts are obtained by the alkylation of Imidazole nitrogen atoms.[10] Zheng et.al reported two naturally occurring imidazolium salts such as lepidiline A & lepidiline B from Lepidium meyenii.[11] These imidazolium salts and their hybrids possess multiple biological activities such as antimicrobial[12], antitumour[13], antioxidants, anti-fibrotic, HIV-integrase inhibition etc.,.[14] Yang et.al has reported the synthesis of a series of imidazolium salts bearing different functional groups by refluxing alkyl halides and imidazole in toluene. The resultant salts were tested against various cancer cell lines.[15] With this background in view, the chemical transformation of usnic acid has now been done by incorporating pharmacophore moiety of imidazolium salts through the key bioactive enamine linkage to afford the desired usnic acid-based imidazolium salts and evaluation of the resultant products for their anti-inflammatory potential.

EXPERIMENTAL

Isolation of (+)- usnic acid (1)

(+)-Usnic acid was isolated from the lichen *Usnea longissima* and *Usnea orientalis* as per our reported procedure.[2] The lichen material was collected from NBRI, Lucknow, India. The lichen material (250+200 g) was air-dried, powdered and extracted with n-hexane (3 L) using a Soxhlet extractor for 12 h. Concentration of the n-hexane extract under vacuum gave the residue (8.8 g), which was chromatographed over silica gel column to afford (+)-usnic acid (1) as pale yellow shining crystals, (8.6 g, mp: 204 ºC, $[\alpha]_D$ = +490º ($CHCl_3$) in 1.91 % yield. IR (KBr) ν_{max} : 2650-

3254, 1692, 1632, 1541, 1454, 1374, 1357, 1288, 1190 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.76 (3H, s, H-10), 2.11 (3H, s, H-15), 2.66 (3H, s, H-12), 2.68 (3H, s, H-14), 5.98 (1H, s, H-4), 11.03 (1H, s, 9-OH), 13.34 (1H, s, 7-OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 7.47, 27.80, 31.19, 32.07, 59.01, 98.25, 101.45, 103.89, 105.17, 109.22, 155.13, 157.42, 163.80, 179.27, 191.63, 197.98, 200.23, 201.68; ESI-HRMS (m/z) $[M+H]^+$ cacld for $C_{18}H_{17}O_7$ 345.0968, found 345.0970.

Isolation of methyl barbatate (2)

The molecule **2** has been achieved with successive treatment of *Usnea longissima* and *Usnea orientalis* with ethyl acetate solvent. Concentration of the ethyl acetate extract under vacuum gave the residue (1.3 g, 0.28 %), which was chromatographed over silica gel column to afford methyl barbatate (2) as colourless crystals, (1.3 g, mp: 187-192 ^{0}C in 0.28 % yield. IR (KBr) ν_{max}: 2551-3452, 1738, 1637, 1572, 1493, 1462, 1398, 1314, 1259, and 1228; ^1H NMR (400 MHz, $CDCl_3$): 2.16 (3H,S), 2.18 (3H,S), 2.47 (3H,S), 2.61 (3H,S), 3.85 (3H,S), 3.86(3H,S), 6.54 (1H,S), 6.62 (1H,S), 11.75 (1H,S); ^{13}C NMR (75 MHz, $CDCl_3$): 8.86, 9.09, 20.18, 24.10, 55.69, 62.08, 108.11, 116.66, 117.38, 117.75, 119.55, 135.45, 140.85, 154.10, 157.21, 160.05, 163.81, 166.19, 174.85. ESI-HRMS (m/z) $[M+H]^+$ calcd. for $C_{20}H_{23}O_7$ 375.1444, found 375.1444.

Synthesis of compound 3

A solution of compound **1**(1.5 g**,** 4.360 mmol) and 3-(1H-imidazol-1-yl)propan-1-amine (4.360 mmol) in methanol (12 mL) was stood at 60 ˛°C and refluxed for four hours under stirring conditions. The reaction mixture was then concentrated under reduced pressure and the resulting residue was chromatographed over silica gel column using n-hexane: ethyl acetate mixture as eluent to afford pure compound 3.

(R,E)-6-Acetyl-2-[1-(3-(1H-imidazol-1-yl)propylamino)ethylidene]-7,9-dihydroxy-8,9b-dimethyl dibenzofuran-1,3(2H,9bH)-dione (3).

Pale yellow solid (0.157 g, 48%); mp: 80-82 °C; IR (KBr ν_{max}): 2650-3444, 1701, 1626, 1555, 1467, 1368, 1283, 1186 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$+DMSO-d_6): δ 1.71 (3H, s, H-10), 2.06 (3H, s, H-15), 2.24 (2H, m, H-17), 2.59 (3H, s, H-12), 2.68 (3H, s, H-14), 3.46 (2H, m, H-16), 4.17 (2H, t, J = 6.6 Hz, H-18), 5.79 (1H, s, H-4), 7.01 (1H, S, H-4'), 7.07 (1H, S, H-5'), 7.55 (1H, S, H-2'), 11.90 (1H, s, 9-OH), 13.36 (1H, s, 7-OH), 13.51 (1H, brs, NH);^{13}C NMR (75 MHz, $CDCl_3$+DMSO-d_6): δ 6.9 (C-15), 17.6 (C-12), 29.6 (C-17), 30.6 (C-14), 31.4 (C-10), 39.9 (C-16), 43.2 (C-18), 56.5 (C-9b), 100.6 (C-6), 101.7 (C-4), 101.8 (C-2), 104.4 (C-9a), 107.1 (C-8), 118.1 (C-5'), 129.3 (C-4'), 136.5 (C-2'), 155.2 (C-5a), 157.6 (C-9), 162.7 (C-7), 173.4 (C-11), 174.7 (C-4a), 189.5 (C-3), 197.6 (C-1), 200.1 (C-13); ESI-HRMS (m/z) $[M+H]^+$ calcd. for $C_{24}H_{26}N_3O_6$ 452.1816, found 452.1798.

General procedure for the synthesis of salts

To a solution of compound 3 (0.1 g, 0.221 mmol) in dry toluene (5 mL) was added appropriate phenacyl bromide (0.221 mmol) and the resulting mixture were refluxed at 80 °C for 4-12 hours under stirring. Colourless precipitate was formed in the round-bottom flask after reaction was completed. This precipitate was filtered and washed with toluene (3×10 mL), to achieve the imidazolium salts (**4-13**) in 72-93% yield.

(E)-1-(3-((1-(6-Acetyl-7,9-dihydroxy-8-methyl-1,3-dioxo-1,9b-dihydrodibenzo[*b,d*]furan-2(3H)-yliden e)ethyl)amino)propyl)-3-(2-oxo-2-phenylethyl)-1H-imidazol-3-ium (4).

Colourless solid (0.122 g, 85%); mp: 148-150 °C; IR (KBr.ν_{max}): 2926-3411, 1700, 1623, 1555, 1460, 1366, 1281, 1231, 1181 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$+DMSO-d_6): 1.68 (3H, S) , 2.03 (3H, s), 2.34 (3H, s), 2.42 (2H, t, J= 6.877), 2.65 (3H, s), 2.67 (3H, s), 3.74 (2H, broad peak), 4.62 (2H, t, J= 6.877) , 5.74 (1H, s) , 6.18 (2H, s), 7.55 (2H,t, J=6.602) , 7.68 (1H, t, J= 6.877) , 7.74 (1H, s), 7.90 (1H, s), 8.04 (2H, d, J= 6.602), 9.72 (1H, s), 11.96 (1H, s), 13.34 (2H, broad peak). ^{13}C NMR (75 MHz, $CDCl_3$+DMSO-d_6): 6.72, 17.80, 28.92, 30.41, 31.14, 46.52, 54.96, 56.18, 100.41, 101.59, 104.30, 106.74, 121.22, 123.78, 124.46, 127.59, 128.31, 131.82, 132.79, 133.86, 137.13, 155.05, 157.37,162.47,173.00, 174.82, 189.09, 189.80, 197.32, 199.84. ESI-HRMS (m/z) $[M+H]^+$ calcd. for $C_{32}H_{32}N_3O_7$ 570.2240, found 570.2241.

(E)-1-(3-((1-(6-Acetyl-7,9-dihydroxy-8-methyl-1,3-dioxo-1,9b-dihydrodibenzo[*b,d*]furan-2(3H)-ylidene)ethyl)amino)propyl)-3-(2-(4-chlorophenyl)-2-oxo ethyl)-1H-imidazol-3-ium (5).

Pale yellow solid (0.115 g, 76%); mp: 137-139 °C; IR (KBr) ν_{max}: 2925-3414, 1700, 1625, 1554, 1465, 1372, 1283, 1232, 1187 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$+DMSO-d_6):1.67 (3H, s) , 2.02 (3H, s) , 2.33 (3H, s) , 2.41 (2H, t, J= 6.877), 2.64 (3H, s), 2.66 (3H, s), 3.25 (2H, broad peak), 3.69-3.78 (2H, m), 4.61 (2H,t, J=6.602), 5.74 (1H, s), 6.21(2H, s), 7.52 (2H, d, J=8.253), 7.78 (1H, S), 7.93 (1H, s), 8.03 (2H, d, J= 8.52), 9.69 (1H, s), 11.97 (1H, S), 13.34 (2H, broad peak). ^{13}C NMR (75 MHz, $CDCl_3$+DMSO-d_6): 6.49, 17.49, 20.35, 28.65, 30.16, 30.87, 46.19,54.61, 55.86, 100.12, 101.25, 101.35, 104.02, 106.35, 120.98, 123.49, 124.22, 127.12, 127.90, 128.28, 128.90, 131.05, 136.83, 139.68, 154.78, 157.08, 162.17, 172.63, 174.48, 188.69, 196.99, 199.54. ESI-HRMS (m/z) $[M+H]^+$ calcd for $C_{32}H_{31}N_3O_7Cl$ 570.2240, found 570.2241.

(E)-1-(3-((1-(6-Acetyl-7,9-dihydroxy-8-methyl-1,3-dioxo-1,9b-dihydrodibenzo[*b,d*]furan-2(3H)-ylidene)ethyl)amino)propyl)-3-(2-(4-nitrophenyl)-2-oxoethyl)-1H-imidazol-3-ium (6).

Pale yellow solid (0.134 g, 87%); mp: 136-138 ºC; IR (KBr) ν_{max} : 2927-3410, 1705, 1623, 1556, 1465, 1351, 1282, 1184 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$+DMSO-d_6): δ 1.68 (3H, s) , 2.00 (3H, S), 2.36-2.45 (2H, m), 2.59 (2H, broad peak), 2.65 (3H, s), 2.67(3H, s), 3.70-3.79 (2H, m), 4.58 (2H, broad peak), 5.76 (1H, s), 6.27 (2H,S), 7.79 (1H, s), 7.94 (1H, s), 8.27-8.42 (4H, m), 9.51 (1H, s), 12.02 (3H, s) , 13.27 (3H, s) , 13.33 (3H, S). ^{13}C NMR (75 MHz, $CDCl_3$+DMSO-d_6): δ 6.29, 17.24, 29.93, 30.65, 45.99, 54.85, 55.60, 100.01, 101.14, 103.83, 106.03, 120.91, 123.29, 128.58, 136.53, 137.10,149.49, 154.58, 156.85, 161.89, 172.37, 174.27, 188.41, 188.95, 196.71, 199.35. ESI-HRMS (m/z) $[M+H]^+$ calcd. for $C_{32}H_{31}N_4O_9$ 615.2091, found 615.2090.

(E)-1-(3-((1-(6-Acetyl-7,9-dihydroxy-8-methyl-1,3-dioxo-1,9b-dihydrodibenzo[*b,d*]furan-2(3H)-ylidene)ethyl)amino)propyl)-3-(2-(3-methoxyphenyl)-2-oxoethyl)-1H-imidazol-3-ium (7)

Pale yellow solid (0.108 g, 72%); mp: 219-221 ºC; IR (KBr) ν_{max}: 2927-3420, 1698, 1627, 1551, 1467, 1367, 1286, 1190 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$+DMSO-d_6): δ 1.69 (3H, s), 2.03 (3H, s), 2.38-2.45 (2H, m), 2.66 (3H, s) , 2.67 (3H, s), 3.71-3.78 (2H, m), 3.88 (3H, S) , 4.60 (2H, t, J= 6.602), 7.47 (1H, t, J= 7.825), 7.53 (1H, bp) , 7.62-.65 (2H, m), 7.71 (1H, s), 7.86 (1H, S), 9.65 (1H, s), 11.96 (1H, s), 13.32 (1H, s), 13.34 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$+DMSO-d_6): δ 6.74, 17.79, 28.91, 30.43, 31.14, 46.52, 54.89, 54.89, 56.20, 100.44, 101.62, 104.31, 106.78, 112.2, 120.02, 121.17, 123.76, 129.43, 134.07, 137.18, 155.07, 157.37, 159.20, 162.50, 173.06, 174.84, 189.12,189.64, 19.36, 199.86. ESI-HRMS (m/z) $[M+H]^+$ calcd. for $C_{33}H_{34}N_3O_8$ 600.2346, found 600.2346.

(E)-1-(3-((1-(6-Acetyl-7,9-dihydroxy-8-methyl-1,3-dioxo-1,9b-dihydrodibenzo[*b,d*]furan-2(3H)-ylidene)ethyl)amino)propyl)-3-(2-(3-carbamoyl-4-hydroxyphenyl)-2-oxo-ethyl)-1H-imidazol-3-ium (8)

Colourless solid (0.132 g, 84%); mp: 196-198 °C; IR (KBr) ν_{max}: 2925-3416, 1663, 1625, 1554, 1465, 1368, 1315, 1283, 1242, 1189 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$+DMSO-d_6): δ 1.69 (3H, s), 2.03 (3H, s), 2.34 (2H, s), 2.37-2.47 (2H,m), 2.66 (3H, s), 2.67 (3H, s), 3.68-3.76 (2H, m), 4.56 (2H, t, J= 6.602) , 5.77 (1H, s) , 6.13 (2H, s) , 7.00 (1H, d, J=8.803), 7.61 (1H, s) , 7.73 (2H, S), 7.84 (1H, s), 8.01 (1H, d, J= 8.803), 8.61(1H, s), 9.53 (1H, S) , 11.97 (1H, s), 13.35 (2H, m).^{13}C NMR (75 MHz, $CDCl_3$+DMSO-d_6): δ 6.58, 17.55,3.26, 46.28, 54.56, 55.99, 64.37, 100.26, 101.46, 104.12, 106.51, 113.08, 117.94, 120.96, 123.51, 127.23, 129.60, 132.79, 136.86, 154.89, 157.18, 162.29, 166.22, 170.92, 172.85, 174.63, 187.81, 188.88, 197.12, 199.68. ESI-HRMS (m/z) $[M+H]^+$ calcd. for $C_{33}H_{33}N_4O_9$ 629.2248, found 629.2240.

(E)-1-(3-((1-(6-Acetyl-7,9-dihydroxy-8-methyl-1,3-dioxo-1,9b-dihydrodibenzo[*b,d*]furan-2(3H)-ylidene)ethyl)amino)propyl)-3-(2-(3,4-dihydro-2H-benzo[*b*][1,4]dioxepin-7-yl)-2-oxoethyl)-1H-imidazol-3-ium (9)

Colourless solid (0.145 g, 91%); mp: 204-206 °C; IR (KBr) ν_{max}: 2927-3423, 1694, 1625, 1556, 1465, 1366, 1271, 1191 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$+DMSO-d_6): δ 1.69 (3H, s) , 2.04 (3H, s) , 2.22-2.30 (2H, m) , 2.38-2.47 (2H, m), 2.66 (3H, s), 2.67 (3H, s) , 3.71-3.78 (2H, m), 4.28-4.37 (4H, m), 4.62 (2H, t, J=5.77), 5.75 (1H, s), 6.05 (2H, s), 7.03 (1H, d, J= 7.978), 7.61 (1H, s), 7.65 (2H, s), 7.83 (1H, S), 9.79 (1H, s), 11.94 (1H, S), 13.35 (2H, bp). ^{13}C NMR (75 MHz, $CDCl_3$+DMSO-d_6): δ 5.95,16.77, 27.83, 29.06, 29.54, 30.24, 45.49, 53.59, 55.10, 68.76, 68.89, 99.45, 100.79, 103.43, 105.44, 120.30, 122.34, 122.87, 127.10,136.23, 149.06, 154.19, 154.79, 156.42, 161.46, 171.84, 173.76, 187.38, 187.77, 196.16, 198.87. ESI-HRMS (m/z) $[M+H]^+$ calcd. for $C_{35}H_{36}N_3O_9$ 642.2452, found 642.2458

(E)-1-(3-((1-(6-Acetyl-7,9-dihydroxy-8-methyl-1,3-dioxo-1,9b-dihydrodibenzo[*b,d*]furan-2(3H)-ylidene)ethyl) amino)propyl)-3-(2-(4-fluorophenyl)-2-oxoethyl)-1H-imidazol-3-ium (10)

Colourless solid (0.115 g, 78%); mp: 118-120 °C; IR (KBr) ν_{max}: 2927-3415, 1699, 1623, 1555, 1465, 1368, 1282, 1231, 1188 cm-1; 1H NMR (300 MHz, $CDCl_3$+DMSO-d_6): δ 1.67 (3H, s), 2.04 (3H, s), 2.39-2.48 (2H, bp), 2.65 (6H, s), 3.74 (2H, bp), 4.62 (2H, bp) , 5.73 (1H, s), 6.21 (2H, s), 7.18 (2H,bp), 7.68 (1H, s), 7.79 (1H, s), 8.11 (2H, bp), 9.83 (1H, s), 11.90 (1H, s), 13.34 (2H, s). ^{13}C NMR (100 MHz, $CDCl_3$+DMSO-d_6): δ 7.38, 18.11, 28.82, 30.91, 31.55, 46.67, 55.17, 56.22, 100.75, 101.70, 102.22, 104.96, 106.25, 116.00, 116.22, 121.97, 124.12, 130.33, 131.12, 131.22, 137.42, 155.60, 157.52, 162.42, 172.92, 175.14, 188.77, 189.77, 197.17, 200.71. ESI-HRMS (m/z) $[M+H]^+$ cacld for $C_{32}H_{31}N_3O_7F$ 588.2146, found 588.2153.

(E)-1-(3-((1-(6-Acetyl-7,9-dihydroxy-8-methyl-1,3-dioxo-1,9b-dihydrodibenzo[*b,d*]furan-2(3H)-ylidene)ethyl)amino)propyl)-3-(2-oxo-2-(p-tolyl)ethyl)-1H-imidazol-3-ium (11)

Colourless solid (0.120 g, 82%); mp: 98-100 °C; IR (KBr) ν_{max}: 2924-3414, 1697, 1623, 1555, 1464, 1368, 1282, 1236, 1183 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$+DMSO-d_6): δ 1.69 (3H, s) , 2.03 (3H, s), 2.34 (2H, s), 2.42 (6H, s), 3.75 (2H, bp), 4.63 (2H, t, J=6.327), 5.73 (1H, s), 6.12 (2H, s), 7.31 (2H, d, J=7.427), 7.67 (1H, s), 7.85 (1H, s), 7.91 (2H,d,J=7.427), 9.72 (1H, s), 11.94 (1H, s), 13.30 (1H, bp) , 13.34 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$+DMSO-d_6): δ 6.88, 17.98, 21.18, 29.11, 30.57, 31.30, 46.68, 54.97, 56.37, 100.59, 101.73, 104.45, 106.96, 121.36, 123.87, 127.85, 129.13, 130.38, 137.28, 145.23, 155.20, 157.52, 162.52, 162.66, 173.20, 175.00, 189.31, 189.40, 197.51, 200.02. ESI-HRMS (m/z) $[M+H]^+$ calcd. for $C_{32}H_{34}N_3O_7$ 584.2397, found 584.2399.

(E)-1-(3-((1-(6-Acetyl-7,9-dihydroxy-8-methyl-1,3-di-oxo-1,9b-dihydrodibenzo[b,d]furan-2(3H)-ylidene)eth-yl)amino)propyl)-3-(2-(naphthalen-2-yl)-2-oxoethyl)-1H-imidazol-3-ium bromide (12)

Colourless solid (0.144 g, 93%); mp: 224-226 °C; IR (KBr) ν_{max} : 2927-3414, 1693, 1625, 1554, 1467, 1365, 1282, 1180 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$+DMSO-d_6): δ 1.65 (3H, s), 2.03 (3H, s), 2.34 (2H, s), 2.38-2.46 (2H,m), 2.62 (3H, s), 2.66 (3H, s), 3.70-3.79 (2H,m), 4.62 (2H, t, J=5.777), 5.72 (1H, s), 6.30 (2H, s), 7.57-7.66 (3H,m), 7.75 (1H, s), 7.88 (1H, s), 7.91-8.06 (4H,m), 8.70 (1H,S), 9.76 (1H, s), 11.94 (1H, s), 13.30 (1H,s), 13.33 (1H,S).^{13}C NMR (75 MHz, $CDCl_3$+DMSO-d_6): δ 6.88, 17.96, 29.05, 30.55, 31.28, 46.70, 55.13, 56.13, 100.57, 101.71, 104.43, 106.94, 121.35, 122.54, 123.92, 126.63, 127.14, 128.27, 128.76, 129.25, 130.12, 130.33, 131.64, 135.36, 137.34, 155.16, 157.49, 162.63, 173.20, 174.99, 189.30, 189.90, 197.49, 199.99. ESI-HRMS (m/z) $[M+H]^+$ calcd. for $C_{36}H_{34}N_3O_7$ 620.2397, found 620.2396.

(E)-1-(3-((1-(6-Acetyl-7,9-dihydroxy-8-methyl-1,3-dioxo-1,9b-dihydrodibenzo[*b,d*]furan-2(3H)-ylide ne)ethyl) amino)propyl)-3-(2-(4-bromophenyl)-2-oxoethyl)-1H-imidazol-3-ium bromide (13)

Colourless solid (0.127g, 79%); mp: 97-99 °C; IR (KBr) ν_{max}: 2926-3417, 1698, 1622, 1556, 1462, 1369, 1281,1231, 1179 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$+DMSO-d_6): δ 1.68 (3H, s), 2.04 (3H, S), 2.39-2.49 (2H,m), 2.59 (2H, s), 2.660 (3H, s), 2.668 (3H, s), 4.15-4.22 (2H, m), 4.61 (2H, t, J=6.327), 5.75 (1H, s), 6.20 (2H, s), 7.68 (2H, d, J=7.978), 7.73 (1H, s), 7.86 (1H, s), 7.94 (2H, d, J=7.703), 9.71 (1H, s), 11.45 (1H, s), 11.95 (1H, s) , 13.35 (2H, bp). ^{13}C NMR (75 MHz, $CDCl_3$+DMSO-d_6): δ 6.37, 17.31, 17.33, 20.21, 28.42, 29.00, 30.02, 30.73, 46.05, 54.42, 55.70, 99.99, 101.24, 103.93, 106.17, 120.89, 123.38, 124.09, 127.00, 127.77, 128.86, 131.12, 136.63, 154.68, 156.96, 162.01, 172.51, 174.11, 174.37, 188.90, 188.54, 196.82, 199.42. ESI-HRMS (m/z) $[M+H]^+$ calcd. for $C_{32}H_{31}N_3O_7$ 648.1345, found 648.1354.

RESULTS AND DISCUSSION

Isolation of (+) - usnic acid and methyl barbatate from the fruticose lichen species *Usnea longissima* and *Usnea orientalis.*

Usnic acid was isolated as the major metabolite from two fruticose Usnea lichen species viz. *Usnea longissima* (250 g) and *Usnea orientalis* (200 g) by soxhlet extraction using n-hexane as solvent at 60 °C for 12 hours. Usnic acid was separated during soxhlet extraction as an insoluble substance. The insoluble substance was purified by column chromatography followed by recrystallisation from chloroform - hexane solvent mixture to afford (+)-usnic acid **(1)** in 2.0and 1.8 % yields respectively (Chart 1) mp: 204 °C.

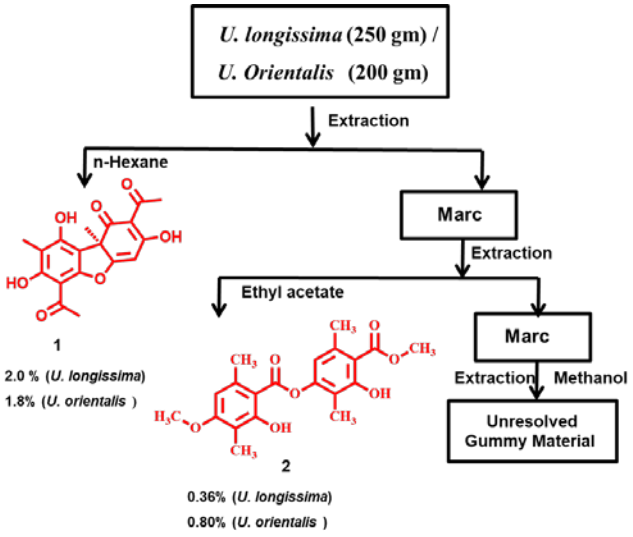


Chart 1. Extraction of Usnic acid from *U. longissima* and *U. orientalis*

The IR spectrum, of usnic acid showed three peaks at 1692, 1632 and 1630 cm^{-1} corresponding to C1, C6 and C2 groups respectively. In its ^1H NMR spectrum, five singlet's appeared at δ 1.76, 2.11, 2.66, 2.68 and 5.98 confirms 9b-CH_3, 8-CH_3, 2-$COCH_3$, 6-$COCH_3$ and 4-CH protons respectively. In its ^{13}C NMR spectrum, three signals appeared at δ 201.68, 200.23 and 197.98 ppm corresponding to C2-carbonyl, C6-carbonyl and C1-carbonyl groups respectively. Further, its structure was confirmed by its HRMS spectrum, which showed the protonated molecular ion $[M+H]^+$ at 345.0970 corresponding to the molecular formula $C_{18}H_{17}O_7$. In addition to usnic acid, methyl barbatate **(2)** was also isolated from the fruticose lichens of *usnea longisimma*(0.36%) and *usnea orientalis*(0.8%)from the ethyl acetate extract (Figure 1).

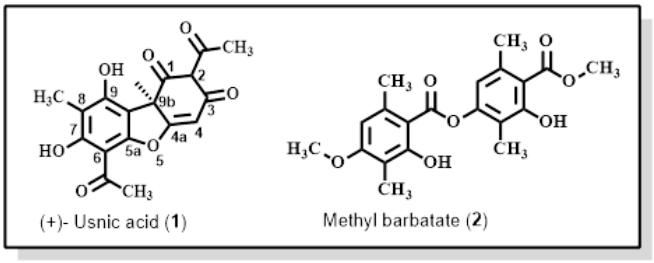
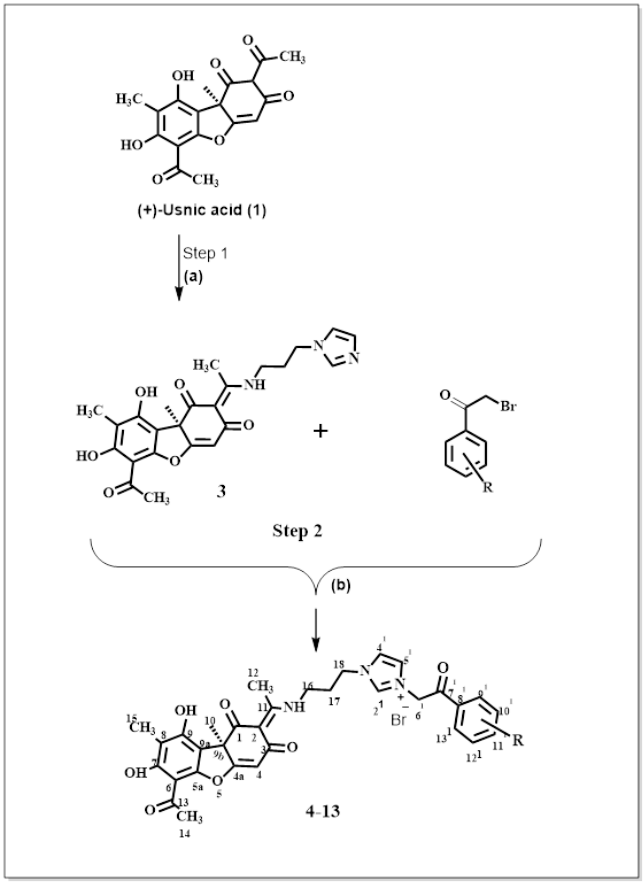


Figure 1. Natural products isolated from *Usnea longissima* and *Usnea orientalis*.

The IR spectrum of methyl barbatate showed peaks at 3452, 1738 and 1637 cm^{-1} corresponding to hydroxyl and carbonyl functionalities. The ^1H NMR spectrum of the compound showed six singlets at δ 2.16, 2.18, 2.47, 2.61, 3.85, and 3.86. The first four values representing aromatic methyl groups and the preceding values corresponding to two methoxy groups. It also showed the signals corresponding to two aromatic protons at δ 6.54, and 6.62 ppm. The ^{13}C NMR spectrum of the compound showed peak δ 174.85 ppm corresponding to the ester carbonyl carbon and the peaks appeared between δ 8.86-62.08 ppm corresponding to aromatic methyl and methoxy carbons. Further, the structure was confirmed as methyl barbatate by its HRMS spectrum, which showed the protonated molecular ion $[M+H]^+$ at 375.1444 corresponding to the molecular formula $C_{20}H_{23}O_7$.

Synthesis of novel (+)-usnic acid based imidazolium salts

The protocols adopted for the synthesis of novel (+)-usnic acid based imidazolium salt hybrids are presented in Scheme 1. In the first step (+)- usnic acid **(1)** was treated with 3-(1H-imidazol-1-yl)propan-1-amine in methanol at 60 °C to synthesise the corresponding enamine **(3)** in 48% yield as per our earlier reported procedure.[16] Compound **3** was thoroughly characterised by its spectroscopic data. In the ^1H NMR spectrum of compound **3,** three peaks appeared at δ 7.01, δ 7.07 and δ 7.55 corresponding to imidazole moiety. The ^{13}C NMR spectrum while confirming these observations exhibited the characteristic carbon signals of imidazole moiety at δ 118.1, δ 129.3 and δ 136.5 ppm. In the second step, compound 3 was refluxed with various phenacyl bromides in toluene at 90 °C (Table 1) to afford the corresponding imidazolium salt derivatives **(4-13)** in very good to excellent (72-93%) yields (Scheme 1). Compounds **4-13** were thoroughly characterised by their spectral data (^1H and ^{13}C NMR, IR and HRMS). The IR spectra of compounds **4-13** showed the characteristic ester functionality between 1663-1705 cm^{-1}.



(a) 3-(1H-imidazol-1-yl)propan-1-amine, methanol, reflux at 60 °C, 48 %; (b) phenacyl bromide, toluene, heat at 90 °C, 72-93%

Scheme 1. Synthesis of imidazolium salts from usnic acid (**1**)

The ^{1}H NMR spectra of the compounds showed the characteristic phenacyl methylene protons between δ 6.05-6.30. The ^{13}C NMR spectra of the compounds while confirming their structures exhibited the characteristic phenacyl methylene and carbonyl carbons between δ 55.10-56.37 and δ 187.77-189.90 respectively.

Table 1. Synthesis of compounds from **4** to **13**.

Compounds	R	Yield, %
4	$C_6H_5COCH_2$-	85
5	4-$ClC_6H_4COCH_2$-	76
6	4-$O2NC_6H_4COCH_2$-	87
7	3-$MeOC_6H_4COCH_2$-	72
8	3-(H_2NCO)-4-$HOC_6H_3COCH_2$-	84
9	3,4-[$CH_2(CH_2O)_2$]$C_6H_3COCH_2$-	91
10	4-$FC_6H_4COCH_2$-	78
11	4-$CH_3C_6H_4COCH_2$-	82
12	β-$C_{10}H_7COCH_2$-	93
13	4-$BrC_6H_4COCH_2$-	79

The anti-inflammatory activity of the synthesized compounds **4-13** was tested *in vitro* by estimating the amount of cytokines, TNF-α and IL-1β, secreted in the LPS challenged U937 cell lines on treatment with the test compounds. Though the parent compound **1** displayed weak anti-inflammatory activity, the majority of the synthesised hybrids showed promising anti-inflammatory activity against TNF- α at a concentration of 10 μM. As shown in Table 2, compound **5** (80.10%), exhibited good inhibitory potential against TNF-α and the compounds **6** (90.4%) and **13** (85.5%) exhibited potential inhibitory activities against IL-1β. However, it is seen from the study that most of the compounds failed to show inhibition activity against IL-1β.

Table 2. Anti-inflammatory activity of synthesised compounds in percentage of inhibition.

Compound	Inhibition, %	
	TNF-α	**IL-1β**
1	4.84	-1.24
3	-8.1	25.5
4	50.9	16.7
5	80.1	25.4
6	17.3	90.4
7	-3.6	44.0
8	24.5	-24.6
9	7.0	21.8
10	-24.5	-3.5
11	21.4	2.5
12	31.9	24.9
13	4.7	85.5
Dexamethasone	81.4	80.5

Table 3.IC_{50} values of synthesized compounds.

Compound	IC_{50}, μM	
	TNF-α	**IL-1β**
1	>100	>100
5	5.3±0.005	>100
6	>100	7.5±0.1
13	>100	6.8±0.5
Dexamethasone	1.5±0.04	2.9±0.05

Compounds with good TNF-α inhibitory activity were further screened to identify their IC_{50} values. As shown in Table 3, the above compounds proved to possess good anti-inflammatory activity with IC_{50} values 5.3±0.005 μM (**5**), 7.5±0.1 μM (**6**), and 6.8±0.5 μM (**13**), when compared to the parent compound **1** (>100 μM).

The anti-inflammatory activities of the synthetic analogues were also found to be comparable with that of the standard dexamethasone [IC_{50}: 4.18±0.1 (TNF-α); 2.9±0.05 (IL-1β)]. From the close analysis of the results (Table 2 and 3), it is evident that the introduction of enamine functionality at the C-2 position of (+)- usnic acid (**5, 6** and **13**) increases the TNF- α & IL-1β inhibitory activity many folds (IC_{50}: 5.3 μM - 7.5 μM). Interestingly, the synthesized imidazolium salts with electron-withdrawing groups on phenacyl moiety such as chloro, nitro and bromo groups (**5, 6** and **13**) showed potent activity (IC_{50} : 5.3 μM - 7.5 μM) against TNF-α and IL-1β , whereas imidazolium salts with an aromatic or heteroaromatic substituent's (**4** and **8-12**) showed weak activity. Most significantly, imidazoles **5** (IC_{50}: 5.3±0.005 μM), **6**(IC_{50}: 7.5±0.1 μM) and **13** (IC_{50}: 6.8±0.5 μM) found to be many folds more active than the parent compound (**1**). Because of significant activities exhibited by compounds **5**, **6** and **13**, they can be considered as lead compounds for further development to synthesise highly potent anti-inflammatory agent.

CONCLUSION

In total, ten novel hybrids of (+)-usnic acid based imidazolium salts were synthesized and evaluated for their anti-inflammatory potential against the cytokine proteins TNF-α and IL-1β secreted from U937 cells. The imidazolium salts (**5**, **6**, and **13**) exhibited promising anti-inflammatory activity against the TNF-α and IL-1β with IC_{50} values ranging between 5.3 μM - 7.5 μM, which are many folds lower than that of the parent compound (>100 μM). Most significantly, imidazolium salts with electronegative groups (**5**, **6** and **13**) found to be more potent and exhibiting enhanced anti-inflammatory activity. Hence, these compounds can be considered as lead molecules for further fine tuning to make highly potent anti-inflammatory therapeutic agents.

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