INTRODUCTION

The protection of carbonyl groups plays an important role in drug design chemistry. The most common method for the preparation of Mannich bases are the reaction of aldehydes with active hydrogen containing amide moieties such as semicarbazide, thiosemicarbazide and 4,4-difluorocyclohexane carboxylic acid hydrazide in the presence of acid or base as catalyst [1-5]. The examples of clinically useful Mannich bases which consist of amino alkyl chain are cocaine, fluoxetine, atropine, ethacrynic acid, procyclidine and so forth. Mannich bases also known to play a vital role in the development of synthetic pharmaceutical chemistry. Mannich reactions are widely used for the construction of nitrogen containing compounds. These bases have important biological applications such as antifungal [6], antimicrobial [7-13], anticonvulsant [14,15], antitumor [16], antiepileptic activity [17], anti-inflammatory, analgesic [18], antimalarial [19] and antioxidant activity [20].

EXPERIMENTAL

All the compounds used in the synthesis are of analytical grade. The melting points of the compounds were determined in open head capillary in paraffin bath and are uncorrected. The IR spectra of the compounds were recorded in the region of 4000-400 cm⁻¹ by FT-IR Perkin spectrophotometer. ¹H NMR spectra were recorded on Bruker FT NMR (400Hz) spectrophotometer in DMSO- d₆. The values of the chemical shift are expressed in δ ppm as a unit. Mass spectral data were obtained by using a network mass selective detector (Agilent). All the compounds were checked for purity by thin layer chromatography (TLC).

in vitro Antibacterial screening: The compounds (4a-4m) were evaluated for their in vitro antibacterial activity against Escherichia coli (MTCC No. 42), Pseudomonas aeruginosa (MTCC No. 1034), Staphylococcus aureus (MTCC No. 3160), Bacillus megaterium (MTCC No. 6544) by disc diffusion method [21,22]. It was performed using a LBS agar medium. Each compound and standard were used at a concentration of 100 µg/mL in DMSO. The zone of inhibition was measured after 24 h incubation at 37 °C.

in vitro Antifungal screening: The compounds 4a-4m were evaluated for their in vitro antifungal activity against Candida albicans (MTCC No: 3017) and Rizopus microsporus var. oligosporus: (MTCC No: 2785) using disc diffusion method with culture media, which provides all essential nutrients for
the growth of microorganisms. Potassium dextrose agar (PDA) medium is used for fungal strains. Each compound and standard were used at a concentration of 100 μg/mL in DMSO. The zone of inhibition was measured after 24 h incubation at 37 °C.

**General procedure for the synthesis of N-[[3-(3-isopropyl-5-methyl)-[1,2,4]triazol-4-yl]-8-azabicyclo[3.2.1]oct-8-yl]-substituted phenyl methyl]acid hydrazides (4a-m):** To an aqueous solution of active hydrogen containing amide, few drops of aqueous ammonia solution (1 eq.) and secondary amine (1 eq.) were added in drops in an ice-cold solution under constant stirring for dissolution. Aromatic aldehydes dissolved in methanol, added dropwise to the above mixture and stirring was continued for 2 h. The formation of compounds were observed within 30 min. Reaction was monitored by TLC, after completion of reaction, the product was filtered and washed with distilled water and dried at 45-50 °C (Scheme I).

**Spectral data**

\(N^2\)-(2-Chloro-phenyl)-[3-(3-isopropyl-5-methyl-[1,2,4]-triazol-4-yl)-8-azabicyclo[3.2.1]oct-8-yl]methyl)hydrazine carbothiamide (4a):** White solid, yield: 78.5 %, m.p. 230-232 °C. IR (KBr, \nu max, cm\(^{-1}\)): 3458, 3344, 3068, 2951, 1722, 1654, 1591, 1510, 1415, 1344, 1278, 1219, 1157, 1093, 1051, 1033, 981, 852, 736, 627. \(^1\)H NMR (DMSO-d\(_6\), 400 MHz): \(\delta 10.49\) (s, 2H, -NH\(_2\)), 7.389 (s, 3H, -CH\(_3\)), 3.46 (m, 1H, Ha), 3.31-3.36 (1H, m, J = 5.27 Hz), 1.88-1.97 (m, 4H, J = 2.008 Hz), 2.668-2.677 (m, 2H, J = 1.757 Hz), 2.326-2.335 (m, 2H, J = 1.757 Hz), 1.88-1.97 (m, 4H, J = 2.008 Hz), 1.235-1.236 (m, 6H, 2 × CH\(_2\), J = 7.027 Hz); \(^13\)C NMR: 81.1 (CH\(_3\)), 24.4 (2 × CH\(_2\)), 28.4 (2 × CH\(_2\)), 26.3 (1CH)-isopropyl, 31.8 (1CH), 36.6 (2 × CH\(_2\)), 52.7 (2 × CH\(_2\)), 65.2 (1CH), 123.6, 123.8, 128.6, 130.4, 136.7, 137.3 (6C-arom. ring), 158.8 (O=C-amide), 163.7 (2C, imine (C=N)). Mass (m/z): 462 (M+2), 439 (M-NH\(_2\)), 412 (M-isopropyl), 395.29 (M-CSNH\(_2\)).

\(N^2\)-(2-Chloro-quinolin-3-yl)-[3,3-isopropyl-5-methyl)-[1,2,4]-triazol-4-yl)]-8-azabicyclo[3.2.1]oct-8-yl]-methyl}hydrazine carbothiamide (4d):** Pale brown solid, yield: 82.21 %, m.p. 208-210 °C. IR (KBr, \nu max, cm\(^{-1}\)): 3456, 3344, 3140, 3080, 2980, 1710, 1670, 1645, 1589, 1512, 1421, 1295, 1218, 1157, 1139, 974, 740, 684. \(^1\)H NMR (DMSO-d\(_6\), 400 MHz): \(\delta 10.207\) (s, 2H, -NH\(_2\)), 7.674-7.694 (d, 1H, chiral proton, J = 0.83 Hz), 7.519-7.537 (d, 2H, Ar-H, J = 7.278 Hz), 7.352-7.389 (t, 2H, Ar-H, J = 7.278 Hz, 7.529 Hz), 7.24-7.310 (t, 1H, Ar-H, J = 7.278 Hz), 6.864-6.88 (d, 2H, CH=CH, J = 6.274 Hz), 6.294 (s, 2H, 2 × NH), 3.783-3.723 (m, 1H, -CH\(_2\)-CH\(_3\)), 3.48-3.47 (m, 1H, Ha), 3.36-3.34 (m, 1H, Hb), 2.672-2.676 (m, 2H, J = 1.757 Hz), 2.325-2.334 (m, 1H, J = 1.757 Hz), 1.728-1.742 (m, 2H, J = 2.008 Hz, J = 1.25Hz), 1.534-1.537 (m, 2H, J = 1.25 Hz), \(^13\)C NMR: 8.1 (CH\(_3\)), 24.4 (2 × CH\(_2\)), 28.5 (2 × CH\(_2\)), 26.3 (1CH)-isopropyl, 31.8 (1CH), 36.7 (2 × CH\(_2\)), 52.7 (2 × CH\(_2\)), 65.2 (1CH, adjacent to arom. ring), 126.3, 128.4, 128.6, 130.4, 136.7, 137.3 (6C-arom. ring), 158.8 (O=C-amide), 163.7 (2C, imine (C=N)). Mass (m/z): 440.25 (M+1), 396.20 (M-isopropyl), 379.52 (M-CSNH\(_2\)).
Scheme-I: Synthetic route of compounds 4(a-m)
157.9 (9C-quinolinel ring), 163.7 (2C, Imine (C=N)), 186 (-C= S). Mass (m/z): 499, 461.8 (M-Cl), 437.8 (M-CSNH$_2$), 279.2.

N'-(3-Nitro phenyl)-[3-(3-isopropyl-5-methyl)[1,2,4]-triazol-4-yl]-8-azabicyclo[3.2.1]oct-8-yl)methyl]hydrazidine carbothiamide (4f): Cream solid, yield: 69.0 %, m.p. 232-234°C. IR (KBr, $\mu_{\text{vmax}}$, cm$^{-1}$): 3392, 3226, 3153, 3030, 2980, 1600, 1525, 1471, 1346, 1294, 1226, 1163, 1105, 1068, 937, 887, 842, 815, 732, 705, 673. $^1$H NMR (DMSO-d$_6$, 400 MHz): $\delta$ 11.704 (s, 1H, -NH), 11.513 (s, 1H, -NH) D$_2$O-exchangeable protons. 8.518-8.509 (t, 1H, Ar-H, $J = 1.757$ Hz, 2.008 Hz), 8.457-8.447 (t, 1H, Ar-H, $J = 1.757$ Hz, 2.008 Hz), 8.16-8.18 (d, 1H, Ar-H, $J = 3.811$ Hz), 8.311-8.32 (d, 1H, chiral proton $J = 3.514$ Hz), 7.706-7.755 (dt, 1H, Ar-H, $J = 8.03$ Hz, 4.015 Hz, 124.1 (2 × CH$_3$), 28.4 (2 × CH$_2$), 26.3 (1CH)-isopropyl, 31.8 (1CH)-hydrazine, 36.6 (2 × CH$_2$), 52.7 (2 × CH), 74 (1CH, adjacent to arom. ring), 122.1, 124.1, 129.1, 135.1, 137.2, 148.1 (6C-arom. ring), 163.7 (2C, imine (C=Im), 186 (-C= S). Mass (m/z): 459.22 (M+1), 415.49 (M-isopropyl), 414.8 (M-[2×-NO2]). 398.25 (M-CSNH$_2$).

N'-(3-Methoxyphenyl)-[3-(3-isopropyl-5-methyl)[1,2,4][triazol-4-yl]-8-azabicyclo[3.2.1]oct-8-yl)methyl]-hydrazidine carbothiamide (4g): White shiny solid, yield: 79.65 %, m.p. 158-160°C. IR (KBr, $\mu_{\text{vmax}}$, cm$^{-1}$): 3392, 3157, 3034, 2980, 1595, 1541, 1508, 1465, 1444, 1415, 1377, 1294, 1249, 1161, 1101, 1051, 924, 927, 883, 819, 690. $^1$H NMR (DMSO-d$_6$, 400 MHz): $\delta$ 10.2 (s, 2H, -NH), 7.804 (s, 1H, -chiral proton), 7.632-7.611 (d, 2H, Ar-H, $J = 8.228$ Hz, $J = 1.219$-1.20 (d, 2H, Ar-H, $J = 0.572$ Hz, 7.777-7.778 (s, 1H, Ar-H, 1H, $J = 2.048$ Hz), 6.54-6.56 (dd, 1H, Ar-H, $J = 6.776$ Hz). $^{13}$C NMR: $\delta$ 8.1 (CH$_3$), 24.2 (2 × CH$_3$), 28.4 (2 × CH$_2$), 26.3 (1CH)-isopropyl, 31.8 (1CH)-hydrazine, 36.6 (2 × CH$_2$), 52.7 (2 × CH), 74 (1CH, adjacent to arom. ring), 113.8, 128.6, 130, 160.5 (4C-arom. ring), 163.7 (2C, imine (C=Im)), 186 (-C= S). Mass (m/z): 546.2 (M+1), 503.5 (M-isopropyl), 500.63 (M-[NO$_2$]).

4,4-Difluoro-cyclohexanecarboxylic acid N'-(4-hydroxy-3,5-dimethoxy-phenyl)methyl]-[3,3-isopropyl-5-methyl][1,2,4][triazol-4-yl]-8-azabicyclo[3.2.1]oct-8-yl)methyl]hydrazide (4j): Off white solid, yield: 78.24 %, m.p. 193-195°C. IR (KBr, $\mu_{\text{vmax}}$, cm$^{-1}$): 3448, 3340, 3257, 3120, 2972, 1685, 1589, 1527, 1504, 1450, 1421, 1365, 1319, 1269, 1207, 1166, 1109, 1024, 939, 813, 738. $^1$H NMR (DMSO-d$_6$, 400 MHz): $\delta$ 10.53 (s, 2H, 2 × NH), 8.665 (s, 2H, -NH), 8.065 (s, 1H, chiral proton), 7.77-7.778 (s, 1H, Ar-H, 1H, $J = 6.869$ Hz), 6.54-6.56 (dd, 1H, $J = 6.4$ Hz), 5.1 (s, 1H, -OH), 3.85 (s, 6H, -OCH$_3$), 3.73 (s, 1H, $J = 1.978$ Hz), 3.23-3.28 (m, 2H, -CH$_2$(CH$_3$)$_3$), 2.74 (s, 3H, -CH$_3$), 2.35 (m, 2H, 1.59-2.17 (m, 5H), 1.29 (d, 6H, -2 × CH$_3$, $J = 6.776$ Hz). $^{13}$C NMR: $\delta$ 8.1 (CH$_3$), 24.2 (2 × CH$_3$), 28.4 (2 × CH$_2$), 26.3 (1CH)-isopropyl, 31.8 (1CH)-hydrazine, 36.6 (2 × CH$_2$), 52.7 (2 × CH), 74 (1CH, adjacent to arom. ring), 99.4, 106.1, 161.5, 163.5 (4C-arom. ring), 163.7 (2C, imine (C=Im)), 186 (-C= S). Mass (m/z): 474.26 (M+1), 431.5 (M-isopropyl), 443.61 (M-OCH$_3$), 441.65 (M-N$_2$H$_2$).
isopropyl), 31.8 (1CH), 34.4 (2 × CH2), 36.6 (2 × CH2), 42 (1CH-difluoro cyclohexyl ring), 52.7 (2 × CH), 74.8 (1CH, adjacent to arom. ring). 108.3 (1C-geminal fluoro carbon), 126, 128.7, 133.5, 147.2 (4C-arom. ring), 163.7 (2C, imine (C=N)), 177.9 (-C=O). Mass (m/z): 543.7 (M+1), 500.63 (M-isopropyl), 424.27, 396.30.

4,4-Difluoro-cyclohexanecarboxylic acid N′-{[(2-chloro-quinolin-3-yl)-[3-(3-isopropyl-5-methyl)[1,2,4]triazol-4-yl]-8-azabicyclo[3.2.1]oct-8-yl-methyl]}hydrazide (4m): Pale yellow solid, yield: 76.25 %, m.p. 240-242 ºC. IR (KBr, v_max, cm⁻¹): 3448, 3342, 3219, 2951, 2868, 1658, 1620, 1575, 1418, 1384, 1306, 1265, 1224, 1111, 1051, 950, 804, 738, 781, 754. 1H NMR (DMSO-d6, 400 MHz): δ 8.54 (s, 2H, NH) D2O-exchangeable protons, 8.52-8.50 (d, 1H, chiral proton), 8.023-7.959 (dd, 2H, 1H, J = 7.278 Hz, 2 × CH3). 13C NMR: δ 8.1 (CH), 14.2 (2 × CH2), 24.4 (2 × CH2), 28.4 (2 × CH2), 26.3 (1CH-isopropyl), 31.8 (1CH), 34.4 (2 × CH2), 36.6 (2 × CH2), 42 (1CH-difluoro cyclohexyl ring), 52.7 (2 × CH), 66.1 (1CH, adjacent to arom. ring), 108.3 (1C-geminal fluoro carbon), 126, 128.7, 133.5, 147.2 (4C-arom. ring), 163.7 (2C, imine (C=N)), 177.9 (-C=O). Mass (m/e): 586.5 (M+1), 588.5 (M+3), 543.2 (M-isopropyl), 439.23.

RESULTS AND DISCUSSION

8-Aza-bicyclo[3.2.1]octyl hydrazide derivatives 4(a-m) were synthesized by using modified Mannich reaction and characterized by IR, NMR, and mass spectral data. In general, IR absorption band of 1º amine and 2º amines of N-H absorption bands observed in the region 3400-3130 cm⁻¹. 1º amine N-H stretching absorption frequency appeared as doublet at 3460 and 3342 cm⁻¹ for asymmetric and symmetric stretching frequencies. For 2º amine, the absorption band appears as singlet at 3200-3143 cm⁻¹. The peaks at 3080-3030 cm⁻¹ appeared for aromatic -C-H stretching. At 2980-2850 cm⁻¹ methyl C-H stretching absorption was observed. The peak at 1691-1659 cm⁻¹ caused primarily by imine (C=N), it is conjugation with C=C in aromatic ring so it shifts to higher absorption frequency.

The out of plane N-H wagging is responsible for broad band in the region 844-813 cm⁻¹. The bands at 1597.06-1525 cm⁻¹ are due to the absorption frequency of C=C and N-H bending vibrations and at 1600-1597 cm⁻¹ absorption is due to C=O. The peaks at 1274 and 1128-1105 cm⁻¹ for C-N and C-O stretching frequencies, respectively. The band at 1354 cm⁻¹ is attributed for N-N absorption band while at 1045 cm⁻¹ for C=O and 758.02 cm⁻¹ for C-Cl stretching absorption band.

1H NMR spectra of compounds 4(a-m) revealed that the chemical shift of chiral proton de-shielding to down field in the region at δ 7.5-8.5 ppm as doublet due to the chiral proton attached to aromatic ring and electronegativity of nitrogen atom on both sides. So it de-shields to higher δ value than expected and for NH protons appeared as singlet in the region δ 12.063-11.654 ppm, i.e. one NH proton at δ 12.063, two hydrogens of NH2 at δ 11.83 and for one NH proton at δ 11.654 ppm, these protons are D2O exchangeable. The two protons of aromatic ring appeared as doublet of doublet at δ 7.59 ppm, it’s J1 = 8.03 Hz and J2 = 8.28 Hz. One aromatic proton appeared as triplet at δ 7.84-7.87 ppm and its coupling constant J3 = 7.278 Hz. The other peak at δ 7.732-7.695 appeared as triplet and it’s coupling constant is J3 = 7.278 Hz. The axial proton at azabicyclo[3.2.1]octane ring appeared as multiplet at δ 4.136-4.175 ppm having coupling constant J1 = 7.278 Hz. At 3.48 ppm, three hydrogens of methyl group appeared as singlet and at δ 3.18 appeared as multiplet. The peaks at δ 2.353 appeared as multiplet and at δ 1.868 and 1.446 appeared as multiplet of 4 × CH2 groups of azabicyclic ring. The six protons of 2 × CH3 groups of isopropyl appeared as doublet with lower intensity with coupling constant J = 6.776 Hz. IR absorption band of 1º amine and 2º amines of N-H absorption was observed. The peak at 1691-1659 cm⁻¹ caused primarily by imine (C=N), it is conjugation with C=C in aromatic ring so it shifts to higher absorption frequency.

Acknowledgements

One of the authors, T. Lakshmi Viveka is thankful to Osmania University, Hyderabad, India for providing the research facilities. The authors also acknowledge to Dr. Y. Rambabu, Indian Institute of Chemical Technology, Hyderabad, India for providing the analytical facilities.
CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES