Part – A
(Part – VII)

Studies on Mannich Base Derivatives
INTRODUCTION

Mannich bases containing bridged N-atom exhibit pronounced biological activities. The study of manich reaction attracted a great deal of attention to the chemists because it plays a vital role due to their wide range of biological and industrial applications. Mannich bases are also employed as intermediate in chemical synthesis.¹⁻³

Much interest has been focused on the synthesis of mannich bases due to its wide variety of pharmacological activities. Mostly, they are found to be antineoplastic, analgesic and antibiotic drugs. Several therapeutic important molecules prepared through manich reaction have received more attention in recent years.⁴⁻⁶ Mannich bases have gained important because of their technological application in polymer chemistry.⁷ especially as paints and surface active agent and it also exhibits complexation characteristic with many transition metal ions.

SYNTHETIC ASPECT

Different methods have been cited to synthesize some new mannich bases by using various interesting substrates.

1. Gabriela Laura Almajan et al.⁸ have synthesized mannich bases (I) from some triazole moiety.

2. Y. Sumalatha et al.⁹ have synthesized mannich base (II) of imidazo[1,2-a]pyridine moiety.
3. Katsifis et al.\textsuperscript{10} have prepared mannich bases (III) of imidazo[1,2-\(a\)]pyridine.

4. Several new aminobenzylated mannich bases (IV) have been prepared by condensing reaction between heterocyclic secondary amines, aldehydes and acetamide, urea and thiourea.\textsuperscript{11}

5. A. A. Jameel et al.\textsuperscript{12} have synthesized mannich bases derivatives (V) from benzhydrazide.

6. C. S. Sharma et al.\textsuperscript{13} have synthesized novel mannich bases of Benzimidazole derivatives.(VI)

Over the years there has been much controversy about the mechanism of the mannich reaction. Studies of the reaction kinetics have led to the following mechanistic proposals.
REACTION MECHANISM

The mechanism of the Mannich reaction starts with the formation of an iminium ion from the amine and the formaldehyde.

Because the reaction takes place under acidic conditions, the compound with the carbonyl functional group (in this case a ketone) can tautomerize to the enol form, after which it can attack the iminium ion.
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THERAPEUTIC IMPORTANCE

A wide variety of pharmacological properties and industrial applications have been encountered with several mannich bases such as,

1. Antimicrobial14 7. Antiproliferation20
2. Antitumor15 8. Antiparasitic21
3. Antiinflammatory16 9. Antituberculare22
4. Cytotoxic Activity17 10. Antifungal23
5. AntiHIV18 11. Anticancer24
6. Antimalarial19

Several therapeutic important molecules containing heterocyclic secondary amines are well known. For example, Manafflazine is a famous antiarthritic agent and Minorine is used as antidepressant.

S. K. Sridhar25 have synthesized some new mannich bases and screened for antiinflammatory activity. A. R. Bhat et al.26 have reported mannich bases of quinoxaline and evaluated for their antibacterial, antifungal and antituberculare activities.

P. Y. Shirodkar and M. M. Vartak27 have studied the antituberculare activity of mannich bases of 6-nitro-3-N-arylaminomethyl-1,2,3,4-tetrahydro-4-oxo-2-thioquinoxalines (VII).

Craig J. Roxburgh et al.28 have reported mannich bases and tested them for local anesthetic activity (VIII).
Christina Reichwald et al.\textsuperscript{29} have synthesized mannich bases of 9-tert-butyl-2-phenylethynylpau llone (IX) from 1,3-diarylpropenones using aromatic aldehydes and acetophenone derivatives as starting materials and studied their antileishmanial activity.

Yan Huang, et al.\textsuperscript{30} have synthesized some mannich bases and reported as anticancer agent. Ya. L. Garazd et al.\textsuperscript{31} have prepared mannich bases of hydroxylcoumarines (X) which posses various biological activities. A. R. Bendale et al.\textsuperscript{32} have synthesized some Ciprofloxacin (XI) mannich base gives antimicrobial activity.

Thus with an effort to capitalize the biological potential of the heterocyclic system and to provide more interesting compounds for biological screening, we have undertaken the synthesis of several mannich bases which has been described as under.

SECTION-I: SYNTHESIS AND BIOLOGICAL EVALUATION OF (E)-N'-{((DIALKYLAMINE)METHYL)-1H-IMIDAZOL-4-YL) METHYLENE)-2-(3-(TRIFLUOROMETHYL)PHENYLAMINO) BENZOHYDRAZIDES
Part - A

(Part - VII)(Section-i)

Synthesis and biological evaluation of (E)-N\(^\prime\)-((1-((dialkylamine)methyl)-1H-imidazol-4-yl)methylene)-2-(3-(trifluoromethyl)phenylamino) benzohydrazides
SECTION-I

SYNTHESIS AND BIOLOGICAL EVALUATION OF \((E)-N'-(1-((DIALKYLAMINE)METHYL)-1H-IMIDAZOL-4-YL)METHYLENE)-2-(3-(TRIFLUOROMETHYL)PHENYLAMINO)BENZOHYDRAZIDES\)

Encouraged by earlier positive result in respect of mannich bases compounds have gained lot of interest in last several year due to their biological, physiological and industrial importance, based on extensive studies of pharmacological properties, it was thought of interest to synthesize some new mannich bases by condensation of \((E)-N'-(1H-imidazol-4-yl)methylene)-2-(3-(trifluoromethyl)phenylamino) benzo hydrazide with different secondary amines and formaldehydes in the presence of acid catalyst.

REACTION SCHEME

The constitution of all the synthesized compounds have been characterized by using elemental analysis, FT-IR, \(^1\)H NMR, \(^{13}\)C NMR spectroscopy and further supported by mass spectroscopy. Purity of all the compounds has been checked on thin layer chromatographic plate and HPLC technique.

All the synthesized compounds were tested for their antibacterial and antifungal activity (MIC) \textit{in vitro} by broth dilution method with two Gram-positive bacteria, two Gram-negative bacteria and three fungal strains. The biological activities of the synthesized compounds have been compared with standard drugs.
**EXPERIMENTAL SECTION**

Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine and UV-254nm light. IR spectra were recorded on Bruker FT-IR-instrument using KBr pellet method. Mass spectra were recorded on Applied Biosystems API 2000 model using direct inlet probe technique. $^1$H NMR and $^{13}$C NMR were determined in DMSO-$_d_6$ solution on a Bruker 400 MHz spectrometer. Purity of the synthesized compounds was checked by Waters ACQUITY UPLC H-CLASS Elemental analysis of the all the synthesized compounds was carried out on Euro EA 3000 elemental analyzer and the results are in agreements with the structures assigned.

**[A] Synthesis of 2-(3-(Trifluoromethyl)phenylamino)benzohydrazide**

See PART-A, part-I, section-I

**[B] Synthesis of (E)-N'-((1H-Imidazol-4-yl)methylene)-2-(3-(trifluoromethyl)phenylamino)benzohydrazide**

To a mixture of 2-(3-(trifluoromethyl)phenylamino)benzohydrazide (2.95 gm, 0.01 mol) in methanol (20 mL) was stirred for 5 minutes, then add 1H-imidazole-4-carbaldehyde (0.96 gm, 0.01 mol) and glacial acetic acid (0.3 mL) stirred for 30 minutes. Then heated to reflux for 2-3 hours. Solvent was distilled out under vacuo to get solid. Crystallized from methanol.

**[C] General procedure for the preparation of (E)-N'-((1-(dialkylamine)methyl)-1H-imidazol-4-yl)methylene)-2-(3-(trifluoromethyl)phenylamino)benzohydrazides**

To a solution of (E)-N'-((1H-imidazol-4-yl)methylene)-2-(3-(trifluoromethyl)phenyamino)benzohydrazide (3.73 gm, 0.01 mol), formaldehyde (0.3 gm, 0.01 mol) and different secondary amine (0.01 mol) in methanol (20 mL) was added. The reaction mixture was refluxed for 5-6 hours with stirring in the presence of 1-2 drop concentrated HCl. After completion of reaction (monitoring by TLC). The reaction mass was cooled in ice cold water and extracted with ethyl acetate. The organic layer
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was washed with water (2 x 10 mL) and dried with Na₂SO₄, solvent was removed in vacuo and the resulting crude product was purified by column chromatography to give the pure compound. The physical constants of the products are recorded in Table-8a.

[D] Biological evaluation of (E)-N⁰-((1-((dialkylamine)methyl)-1H-imidazol-4-yl)methylene)-2-(3-(trifluoromethyl)phenylamino)benzohydrazides

Antimicrobial testing was carried out as described in Part-A, Part-1, Section-I, antimicrobial activity. The MIC values of the test compounds are recorded in Table-8b.
Table-8a: Physical constant of \((E)-N'-(1-((\text{dialkylamine})\text{methyl})-1H-\text{imidazol-4-yl})\text{methylene})-2-(3-\text{(trifluoromethyl)phenylamino})\text{benzohydrazides}\)

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<td>(-\text{CH}_3)- (-\text{CH}_3)</td>
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TLC solvent system: Ethyl acetate: Hexane = 6 : 4
(E)-N'-(1-(piperidin-1-ylmethyl)-1H-imidazol-4-yl)methylene)-2-(3-(trifluoromethyl)phenylamino)benzohydrazide (8a). mp 198-199°C; Purity by UPLC: 99.13%; IR (KBr): 3423 (N-H str), 3251 (N-H str), 3112 (Ar, C-H str), 2932 (C-H str), 2855 (C-H str), 1641 (C=O str), 1591 (C=N str), 1519 (C=C str), 1332 (CF3, C-F str), 1275 (C-N str), 1036 (alkyl, C-N str), 831 (C-H O,P, bending) cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆): δ ppm 2.73-2.75 (t, 4H, CH2-N-CH2), 1.47 (s, 6H, CH2), 5.01 (s, 2H, N-CH2-N), 7.04-7.08 (t, J=7.2 Hz, 1H, ArH), 7.19-7.21 (d, J=7.2 Hz, 1H, ArH), 7.37-7.48 (m, 6H, ArH), 7.71-7.44 (m, 2H, ArH), 7.89 (s, 1H, ArH), 9.38 (s, 1H, NH), 12.81 (s, 1H, NH); ¹³C NMR (100 MHz, DMSO-d₆): δ ppm 24.93, 26.93, 50.09, 70.80, 114.64, 114.68, 117.42, 118.43, 121.27, 121.90, 122.02, 123.25, 125.96, 129.02, 130.36, 130.81, 132.83, 136.01, 136.78, 143.15, 143.83, 164.73; MS: m/z = 470.7 [M⁺]; Anal. Calcd for C24H25F3N6O: C, 61.27; H, 5.36; N, 17.86. Found: C, 61.01; H, 5.13; N, 17.66%.

(Mannich base derivatives...)
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(E)-N'-((1-(morpholinomethyl)-1H-imidazol-4-yl)methylene)-2-(3-(trifluoromethyl)phenylamino)benzohydrazide (8d). mp 183-185°C; IR (KBr): 3398, 3260, 3076, 2961, 2863, 1648, 1589, 1542, 1332, 1275, 1037, 832 cm⁻¹; MS: m/z = 471.01 [M]+; Anal. Calcd for C₂₃H₂₃F₃N₆O₂: C, 61.27; H, 5.36; N, 17.86% Found: C, 61.01; H, 5.10; N, 17.61%.

(E)-N'-((1-((diisopropylamino)methyl)-1H-imidazol-4-yl)methylene)-2-(3-(trifluoromethyl)phenylamino)benzohydrazide (8e). mp 124-126°C; IR (KBr): 3405, 3268, 3084, 2967, 2857, 1645, 1559, 1462, 1331, 1277, 1054, 845 cm⁻¹; MS: m/z = 486.99 [M]+; Anal. Calcd for C₂₅H₂₉F₃N₆O: C, 61.72; H, 6.01; N, 17.27% Found: C, 61.45; H, 5.85; N, 17.03%.

(E)-N'-((1-((4-ethylpiperazin-1-yl)methyl)-1H-imidazol-4-yl)methylene)-2-(3-(trifluoromethyl)phenylamino)benzohydrazide (8f). mp 223-224°C; IR (KBr): 3389, 3268, 3078, 2959, 2886, 1642, 1551, 1487, 1332, 1056, 848 cm⁻¹; MS: m/z = 500.1 [M]+; Anal. Calcd for C₂₅H₂₈F₃N₇O: C, 60.11; H, 5.65; N, 19.63% Found: C, 60.01; H, 5.50; N, 19.40%.

(E)-N'-((1-((4-methylpiperazin-1-yl)methyl)-1H-imidazol-4-yl)methylene)-2-(3-(trifluoromethyl)phenylamino)benzohydrazide (8g). mp 173-175°C; IR (KBr): 3420, 3260, 3097, 2956, 2872, 1649, 1534, 1456, 1332, 1034, 864 cm⁻¹; MS: m/z = 486.1 [M]+; Anal. Calcd for C₂₄H₂₆F₃N₇O: C, 59.37; H, 5.40; N, 20.19% Found: C, 59.10; H, 5.10; N, 19.91%.

(E)-N'-((1-((pyrrolidin-1-yl)methyl)-1H-imidazol-4-yl)methylene)-2-(3-(trifluoromethyl)phenylamino)benzohydrazide (8h). mp 142-145°C; IR (KBr): 3410, 3252, 3082, 2954, 2868, 1648, 1332, 1043, 870 cm⁻¹; MS: m/z = 457.1 [M]+; Anal. Calcd for C₂₃H₂₃F₃N₆O: C, 60.52; H, 5.08; N, 18.41% Found: C, 60.36; H, 4.89; N, 18.08%.

(E)-N'-((1-((2-methylpiperidin-1-yl)methyl)-1H-imidazol-4-yl)methylene)-2-(3-(trifluoromethyl)phenylamino)benzohydrazide (8i). mp 153-155°C; IR (KBr): 3413, 3248, 3046, 2964, 2834, 1649, 1590, 1541, 1333, 1126, 1048, 837 cm⁻¹; MS: m/z = 485.1 [M]+; Anal. Calcd for C₂₅H₂₇F₃N₆O: C, 61.87; H, 5.40; N, 17.10%.

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(E)-N'-(1-((4-methylpiperidin-1-yl)methyl)-1H-imidazol-4-yl)methylene)-2-(3-(trifluoromethyl)phenylamino)benzohydrazide (8j). mp 134-136°C; IR (KBr): 3480, 3242, 3089, 2956, 2885, 1645, 1545, 1332, 1146, 1023, 842 cm⁻¹; MS: m/z = 485.1 [M]⁺; Anal. Calcd for C₂₅H₂₇F₃N₆O: C, 61.97; H, 5.62; N, 17.35% Found: C, 61.75; H, 5.45; N, 17.09%.
SPECTRAL STUDY OF SYNTHESIZED COMPOUNDS

IR spectra of compound 8a.

IR spectra of compound 8b.
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Mass spectrum of compound 8a.

Mass spectrum of compound 8b.

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UPLC spectrum of compound 8a.

**SAMPLE INFORMATION**

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**Peak Results**

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**Channel: SQ 1: MS Scan**

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**Mannich base derivatives...**
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$^1$H NMR spectrum of compound 8a.

Exapanded spectrum of compound 8a.
Studies on heterocyclic...

$^1$H NMR spectrum of compound 8b.

[Diagram of the NMR spectrum]

Expanded spectrum of compound 8b.

[Diagram of the expanded spectrum]

Mannich base derivatives...
Studies on heterocyclic...

$^{13}$C NMR spectrum of compound 8a.

$^{13}$C NMR spectrum of compound 8b.

Mannich base derivatives...
Studies on heterocyclic...

Table-8b: Antimicrobial activity of (E)-N'-((1-((diarylamine)methyl)-1H-imidazol-4-yl)methylene)-2-(3-(trifluoromethyl)pheny lamino) benzohydrazides

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MINIMAL INHIBITION CONCENTRATION

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### REFERENCES


Studies on heterocyclic... 3226 (2010).