Mannich reaction involves condensation of a carbonyl compound with formaldehyde and a secondary amine. It is a mild procedure for obtaining unsaturated ketones (usually -CO-C=CH₂). Mannich bases derived from chalcones and 2-dimethyl amino ethyl benzo suberone methiodide have shown promise as anticancer agents, l-Ethyl-6-fluoro-l,4-dihydro-4-oxo-7-[(N'-[5-chloro-3'-thiosemicarbazono-isatin-l-yl)] methyl]-N-piperazinyl]-3-quinoline carboxylic acid had been found to be more active than norfloxacin.

A reaction between a compound containing a reactive hydrogen atom, formaldehyde and a secondary amine became a general reaction, by the name of Mannich. An example is the reaction between acetophenone, formaldehyde and a secondary amine (Scheme 1). The Mannich reaction has been reviewed by Blicke, Karbe, Nobles, Reichert, Thompson and others and has been mentioned in many books. This reaction is useful in adding one carbon atom in a reaction in making amino ketones and many drug molecules. It can be useful in making enones. These enones possess exomethylene compounds and are very reactive. There are two important types of Mannich reactions, the C-type (Scheme 1) and the N-type. Scheme 2 illustrates a N-type reaction.

The steps involved in the Mannich reaction are, 1. formation of the imine salt, 2. the electrophilic salt can now add to the enol of the ketone to give the product an amine, called a Mannich base and 3. the Mannich base could be further alkylated to form an ammonium salt which on treatment with a base leads to the formation of enone. These enones can react with cellular thiol containing compound (1) which may account for their cytotoxic properties.

Scheme 1: C-Type Mannich reaction.
Mechanism of action of ethacrynic acid (2) by thiol adduct (Scheme 3): 

The anticancer properties of Mannich bases has recently been reviewed by Dimmock. However, these Mannich bases also show other properties like anticonvulsant, antimalarial, antiviral, antifungal, antibacterial, analgesic and antiinflammatory and other activities. Most recently, there has been an upsurge in exploring various activities of Mannich bases in our laboratory. Therefore, it was thought pertinent to review the newer biological activities of Mannich bases.

Anticancer activity:

The anticancer activity of Mannich bases is supposed to be due to the formation of enones, which add to the cellular thiol containing biomolecules. Mannich bases derived from chalcones (3) and related compounds were found to possess anticancer activity. The chloro series showed very good antitumour activity. Some Mannich bases have been patented for anticancer and antifungal activity. The compound (4) shows MIC of 6.25-25 µg/ml, against Aspergillus fumigatus.
compounds have been evaluated against Bacillus anthracis, Corynebacterium pyogenes. Some heterocyclic nuclei, especially, isatin has been explored for antimicrobial activities. Isatin can form both Schiff and Mannich bases. In the isatin molecule, the Schiff bases have been selected from wide ranging antibacterial agents like trimethoprim, sulphasemethoxazole and other potential amino containing heterocyclic compounds. The active -NH of isatin has been converted into Mannich bases with secondary amines and important quinolone antibacterials.

Schiff bases of isatin and 5-methylisatin with sulphadoxine and its Mannich bases were evaluated for antibacterial activity. Among them the compounds with piperidinomethyl group were found to be most active when compared to sulphadoxine with a MIC of 0.15 μg/ml against Staphylococcus aureus, Staphylococcus albus, Shigella flexneri, Shigella sonnei, Pseudomonas aeruginosa and Citrobacter ferndii.

Mannich bases of fluoroquinolones have shown better antibacterial activity than the parent molecule. Mannich bases of ciprofloxacin and lomefloxacin with isatin and its derivatives were synthesized by condensing the acidic NH group of isatin with formaldehyde and secondary amino group (piperazine moiety) of ciprofloxacin and lomefloxacin and screened for antibacterial activity. Mannich bases of ciprofloxacin were more potent than ciprofloxacin against Vibrio cholerae and equipotent against Escherichia coli and P. aeruginosa. Mannich bases of lomefloxacin were more potent than lomefloxacin against E. coli and were equipotent against Salmonella typhi, V. cholerae, Staphylococcus epidemicus and P. aeruginosa. Similarly, Mannich bases of norfloxacin were synthesized by reacting with formaldehyde and several isatin derivatives and evaluated for their antibacterial activity. All the compounds were more active than norfloxacin (MIC: 9.76 μg/ml) against Edwardsiella tarda, Salmonella paratyphi A (0.018-1.22 μg/ml), V. cholerae-0139 (≤0.018-0.078 μg/ml), Enterococcus faecalis 0.3-2.44 μg/ml), S. sonnei (0.018-2.44 μg/ml), Plesiomonas shigelloides and
Proteus rettgeri (0.018-0.6 μg/ml). The compounds were more active (0.075-0.3 μg/ml) than norfloxacin (1.22 μg/ml) against Bacillus subtilis. All the synthesized compounds were more active (≤0.018-0.037 μg/ml) than norfloxacin (0.3 μg/ml) against Aeromonas hydrophila. Some compounds were more active (≤0.018-0.075 μg/ml) than norfloxacin (1.22 μg/ml) against E. coli NCTC-10418 and more active (1.22-9.76 μg/ml) than norfloxacin (19.53 μg/ml) against P. aeruginosa. Among the compounds tested, 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7[N-‘(5-chloro-3-thiosemicarbazone)-isatin-1-yl]-methyl]-N’-piperazinyl]-3-quinoline carboxylic acid (6) showed most promising antibacterial activity.

Isatin, its 5-chloro and 5-bromo derivatives have been reacted with N-[4-(4’-chlorophenyl) thiazol-2-yl] thiosemicarbazide to form Schiff bases and the N-Mannich bases32 of these compounds were synthesized by reacting them with formaldehyde and secondary amines. These compounds were evaluated for their antibacterial activity. All compounds showed more activity (less MIC) than sulphamethoxazole except P. aeruginosa. When compared to trimethoprim, these compounds were more active against Salmonella typhimurium, S. aureus, E. faecalis, S. typhi, P. aeruginosa, Klebsiella pneumoniae, S. albus, A. hydrophila, V. cholerae-O1, B. subtilis and P. rettgeri. Among the compounds tested 1-N,N-dimethylaminomethyl]-5-bromo isatin-3-[1-N,N-dimethylaminomethyl]-thiazol-2-yl]-thiosemicarbazone (7) emerged as a potent antibacterial agent.

Schiff bases of isatin and its 5-chloro and 5-bromo derivatives with 3-amino-2-methyl mercapto quinazolin-4 (3H)-one were prepared and the N-Mannich bases33 of these compounds were synthesized. All the compounds showed higher activity than sulphamethoxazole except against P. aeruginosa. When compared to trimethoprim, the compounds were more active against S. typhimurium, S. aureus, P. aeruginosa, A. hydrophila, V. cholerae-O1, B. subtilis and P. rettgeri. Five compounds were more active against E. tarda, seven compounds against E. faecalis and six compounds were equivalent against Sh. flexneri. Among the compounds tested, 5-chloro-3-(3’,4’-dihydro-2-methyl-mercapto-4’-oxoquinazolin-3-yl)-l-morpholino methyl imino isatin (8) was the most active antibacterial agent.

Isatin and its derivatives have been reacted with 4-(4’-chlorophenyl)-6-(4’-methyl phenylD-2-amino pyrimidine to form Schiff bases which were converted into N-Mannich bases34. The compounds were more active than the standard drug sulphamethoxazole (MIC 5000 μg/ml). When the antibacterial activity of these compounds was compared with trimethoprim, the MIC of the compounds (series 9) was less than the standard against only S. typhimurium, S. aureus, E. faecalis, P. aeruginosa, Klebsiella pneumoniae, S. albus, Aeromonas hydrophila, Vibrio cholerae-O1, B. subtilis and P. rettgeri. None of the compounds were more active against V. cholerae non-O1. The compound (9) with 5-bromo and morpholino methyl group emerged as the most active antibacterial agent among them.

Schiff bases of isatin with N-(6-chloro benzthiazol-2-yl) thiosemicarbazide were prepared and the N-Mannich bases36 of the above Schiff bases were synthesized. It has been observed that all the compounds tested showed significant activity against tested bacteria. When compared to trimethoprim all the compounds were more active against S. typhimurium, E. faecalis, P. aeruginosa, A. hydrophila, V. cholerae-O1, B. subtilis and P. rettgeri. The compound, l-[N,N-dimethylaminomethyl]-isatin-3-[1’-(6-chlorobenzthiazol-2’-yl)] thiosemicarbazone (10) was more active than standard antibacterials sulphamethoxazole and trimethoprim.

Isatin and its 5-chloro and 5-bromo derivatives have been reacted with 3-(4’-pyridyl)-4-amino-5-mercapto-4(H)-1,2,4-triazole to form Schiff bases and the N-Mannich bases37 of these compounds were synthesized. The compounds were more active, when compared to trimethoprim against S. typhimurium, S. aureus, P. aeruginosa, K. pneumoniae, A. hydrophila, V. cholerae-1, B. subtilis and P. rettgeri and E. faecalis. When compared to norfloxacin all the compounds were more active against S. aureus and were equipotent (9.76 μg/ml) against E. tarda, E. faecalis, S. paratyphi B and S. sonnei. One compound which was more active (4.88 μg/ml) when compared to norfloxacin, had a MIC of 9.76 μg/ml against E. faecalis and Plesiomonas shigelloides. The compound, 1-(piperidinomethyl]-5-bromo-3-[3’-4’-pyridyl]-5’-mercapto-4’(H)]2,2’-triazol-4’-yl]-imino isatin (11) showed the most favourable antibacterial activity.

Mannich bases of substituted triazoles38 were synthesized and they were found to be highly active against Gram positive bacteria at 5 μg/ml concentration. N-Mannich bases of isatin39 showed antimicrobial activity against acid fast. Gram positive and Gram negative bacteria, yeast and fungi.

Antifungal Activity:

Mannich bases have also been explored in the area of antifungal drugs. Research is in progress to discover good antifungal agents that are effective against mycosis and dermatophyta infections. Conjugated styryl ketones40,41 were
converted to their Mannich bases and they exhibited good antifungal activity against *Candida albicans* strains B311 and 3153 A. Norfloxacin Mannich bases\(^{38}\) displayed significant antifungal activity. When compared to clotrimazole (MIC 2.44 \(\mu\text{g/ml}\)), 13 compounds were found to be active (0.6-1.22 \(\mu\text{g/ml}\)) against Cryptococcus neoformsans, 10 compounds were found to be active against Microsporum gypseum. The compounds were more active (1.22-2.44 \(\mu\text{g/ml}\)) against Histoplasma capsulatum. Some compounds were more active (0.6-1.22 \(\mu\text{g/ml}\)) against Microsporum audouini, when compared to clotrimazole (4.88 \(\mu\text{g/ml}\)). Among them the compound with 5-bromo and 3-[4′-amino-5′-3(3′,4′,5′-trimethoxy benzyl)-pyrimidin-2′-yl]-imino isatin (12) showed a promising antifungal activity.

Mannich bases derived from isatin derivatives and N-[4-(4′-chlorophenyl) thiazol-2-yl] thiosemicarbazide\(^{39}\) displayed good antifungal activity against *Epidermophyton floccosum*, *C. neoformsans*, *M. audoumi*, *M. gypseum* and *Trichophyton mentagrophytes* with MIC of less than 10 \(\mu\text{g/ml}\).

Schiff bases of isatin derivatives with 3-amino-methyl mercapto quinazolin-4(3H)-one\(^{34}\) were converted to their Mannich bases and were found to be active (2.44 \(\mu\text{g/ml}\)) against Microsporum audouini and equipotent (2.44 \(\mu\text{g/ml}\)) against *T. mentagrophytes*, *E. floccosum* and *M. gypseum*. Isatin and derivatives with pyrimidine\(^{36}\) were further converted to their Mannich bases and the compounds displayed promising activity against dermatophytes like *M. gypseum*, *M. audoumi* and *T. mentagrophytes* with a MIC of less than 10 \(\mu\text{g/ml}\). Mannich bases of isatin with N-[6-chlorobenzothiazol-2-yl]-thiosemicarbazide\(^{38}\) displayed good activity against *M. gypseum*, *E. floccosum*, *T. mentagrophytes* and *M. audoumi* with MIC of less than 10 \(\mu\text{g/ml}\).

Schiff bases of triazole\(^{37}\) with isatin and its derivatives were further transformed into Mannich bases. Among them one compound was more active against *H. capsulatum* when compared to clotrimazole (19.53 \(\mu\text{g/ml}\)). The most active compound in this series was 1-[(morpholinio methyl)-3-(3′-(4′-pyridyl)-5′-mercaptopo-4′-(H)-1,2′,4′-trizol-4′-yl]-imino isatin (13).

**Antiviral activity:**

Norfloxacin Mannich bases\(^{32}\) with 3-[4′-amino-5′-(3′,4′,5′-trimethoxy benzyl) pyrimidin-2-yl]-imino isatin and compounds with 5-chloro and 5-bromo groups displayed anti HIV activity against replication of HIV-I (III B) strain in MT\(_4\) cells, with EC\(_{50}\) values of 11.3 and 13.9 \(\mu\text{g/ml}\) respectively.

Mannich bases of 5-methyl isatin with trimethoprim\(^{43}\) were synthesized and among them the N,N-dimethyl amino and morpholino compounds gave maximum protection against HIV-I (III B) strain. Mannich bases of isatin derivatives\(^{44}\) displayed 40% protection against herpes simplex virus type-I (7135666) *in vivo*. Among the compounds synthesized, 3-(4′-methoxy phenyl)-imino isatin and 3-(thiazol-2′-yl)-imino isatin (14) were the most active compounds. Mannich bases of aryl imidazolines\(^{45}\) displayed significant activity against vaccinia viruses. Recently, Mannich bases of 1,1,3-trisubstituted ureas or thioureas\(^{46}\) have been reported to have antiviral activity.

**General structure activity relationship; Antibacterial activity:**

1. In any given series of compounds, the 5-bromo substituted isatins were found to be most active.
2. The Mannich bases with pyrrolidine and in some cases morpholine exhibited more sensitivity towards many bacterial strains.
3. The norfloxacin Mannich bases have shown more *in vitro* antibacterial activity than norfloxacin.
4. The Schiff bases of isatin with trimethoprim were found more potent than others like triazole, benzothiazole, semicarbazone or thiosemicarbazones.
5. The Mannich bases in selected cases exhibited more antibacterial activity *in vivo* than *in vitro*.

**Antifungal Activity:**

1. In any given series, the 5-chlorosubstituted isatin was found to be the most active derivative against various fungi especially against *Microsporum gypseum*.
2. The Mannich bases of isatin with pyrrolidine and dimethylamine were generally more active than others.
3. Amongst a variety of Schiff and Mannich bases, the most active compound was found to have trimethoprim as Schiff base and norfloxacin as Mannich base with 5-bromo isatin.

**Antitubercular Activity:**

1. In the triazole and pyrimidine Schiff base series, the compounds with 5-chlorosubstitution in isatin
nucleus and no Mannich base (free NH group of indole) exhibited more percentage inhibition of *M. tuberculosis* H37RV than standard drug rifampin (96%).

2. The quinazoline and benzothiazole series Schiff bases exhibited poor antitubercular activity.

3. Schiff bases of isoniazid with isatin and norflaxin Mannich bases exhibited highest percentage (100) inhibition and were better than rifampin (95%) against *M. tuberculosis* H37RV.

**AntihIV activity:**

1. All the Schiff bases derived from triazole, pyrimidine, quinazoline or benzothiazole were inactive against HIV-1 (IIIB).

2. Only two compounds, with chloro and bromo substitution in isatin nucleus and Schiff base with trimethoprim and Mannich base with norflaxin were having selectivity index (CC50 to EC50) of 5 and 4 and protection of 95% and 73%, respectively.

3. Isatin derivatives containing pyrimidine nucleus possessed remarkable effect on 60 cancer cell lines.

**Anticonvulsant Activity:**

Anticonvulsant activity was displayed by Mannich bases of 1-aryl-1-ethanones and related ketones. The compounds (15,16) were found to be the lead molecules with ED₅₀ values of 62 mg/kg which were comparable to mephentoin with ED₅₀ of 60.5 mg/kg. Mannich bases of conjugated arylidene ketones were evaluated for their anticonvulsant activity. Most of the compounds had activity equal to clinically used drug in maximal electroshock seizure screening.

**Antimalarial Activity:**

Amodiaquine (17), a Mannich base derivative is a clinically useful antimalarial drug. Antimalarial activity was exhibited by Mannich bases of amodiaquine and cycloquina type to yield 10-H-indolo quinoline-1 1-yl amines. The amodiaquine analogue (18) showed a comparable activity with chloroquine. 7-Chloro quinolines have been converted to their Mannich base derivatives and evaluated for antimalarial activity. The most active compound in this series was 2-[7-chloro-quinolin-4-yl-amino]-5-fluoro-4,6-bis[piperidin-1-yl-methyl]-phenol (19). Mannich bases of substituted quinoxalines (20) have been evaluated against rodent malarial parasite *Plasmodium yoelli*. Few compounds showed significant antimalarial activity.

**Miscellaneous Activities:**

Mannich bases of 2-(N-aryl aminomethyl)-5(E)-pentylidene cyclopentanone and their structural isomers displayed significant antiinflammatory activity. It was also reported that some Mannich bases of N-phenyl piperazine derivatives exhibited antidepressant activity along with muscle relaxant activity, 3-aryloxy-3-phenylpropanamines (21) also displayed antidepressant activity. Anticoagulant activity was also displayed by Mannich bases of naphthalamorpholino and C-methylated naphthala morpholino compounds.

**Synthetic Utilities of Mannich Bases:**

Mannich reactions have been used as a synthetic tool in the preparation of various therapeutic agents, fluoxetine an antidepressant agent (Scheme 4), ethacrynic acid a high ceiling loop diuretic (Scheme 5), benzoxacinamide an antiplasmodic agent (Scheme 6), Ranitidine a H₂-receptor antagonist (Scheme 7), Tripropidine H₁-receptor antagonist (Scheme 8) and trihexyphenidyl hydrochloride an antispasmodic (Scheme 9).

**Mannich bases as prodrugs:**

The reaction mechanism proposed for the decomposition of N-Mannich bases involves as rate-determining step an unimolecular N-C bond cleavage with formation of an amide (or imide) anion and an ammonium cation. In subsequent fast steps, a solvent molecule transfers a proton to the anion and a hydroxide ion to the ammonium ion, giving methylolamine, which rapidly dissociates to formaldehyde and amine (Scheme 10). The rate of decomposition increases strongly with increasing acidity of the parent amide type compound. By appropriate selection of the amine component, it is feasible to obtain prodrugs of a given amide-type drug with varying degrees of in vitro lability. The physicochemical properties such as aqueous solubility, dissolution rate and lipophilicity can be modified for the parent drugs. Transformation of an amide into an N-Mannich base intro-

**Scheme 4: Synthesis of fluoxetine.**
Scheme 5: Synthesis of ethacrynic acid (2).

Scheme 6: Synthesis of benzoquinamide.

duces a readily ionizable amino moiety which may allow the preparation of derivatives with greatly increased water solubilities at slightly acidic pH values.

Mannich bases, have been also explored, into the area of prodrugs. The hydrochloride salt of Mannich base of carbamazepine (anticonvulsant) with dipropylamine (22) is

Scheme 7: Synthesis of ranitidine.

Scheme 8: Synthesis of triprolidine.

Scheme 9: Synthesis of trihexylphenidyl hydrochloride.

R−CONH−CH₂−N⁺⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻安倍 R₁ R₂ Kₐ = R−CONH−CH₂−N⁺⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻安倍 R₁ R₂ + H⁺ → k₁

Scheme 10: Release of amide drug from N-Mannich base.
found to be more than $10^4$-fold soluble in water than the parent drug. Following intramuscular administration in rats, higher and more rapidly appearing carbamazepine plasma levels were observed from aqueous solutions of the N-Mannich base prodrug than from administering a suspension of the parent drug\textsuperscript{59}. Hetacillin (23), formed by condensation of ampicillin with acetone, is regarded as cyclic N-Mannich base. Sodium ampicillin in solution loses almost 10\% of its activity in 1 h at room temperature at a concentration of 250 mg/ml. Hetacillin, on the other hand, loses somewhat less than 10\% activity in 6 h under identical conditions\textsuperscript{60}. The pharmacokinetics of oral and intravenous doses of ampicillin and hetacillin have been compared. Bioavailability studies showed 32\% absorption from ampicillin capsules, while 42\% absorption values from hetacillin capsules in non-fasting subjects. The primary rationale for clinical use of ampicillin precursors, in general, is the improvement of the limited intestinal absorption of the antibiotic (increased lipophilicity of the prodrug) as well as increasing the stability of ampicillin in aqueous solution\textsuperscript{61}. Rolitetracycline, (24) a prodrug of
tetracyclines prepared by reacting pyrrolidine and formaldehyde is very soluble in water whereas the parent drug tetracycline has low solubility in water.

The concept of N-Mannich base prodrug may be useful for improving the dissolution behaviour of poorly soluble drugs in an effort to improve oral bioavailability of various NH-acidic compounds (e.g. chlozoxazine, phenytoin, barbital, acetazolamide, chlorothiazide and allopurinol). In a study, N-Mannich bases with morpholine or piperidine were found to possess markedly greater (up to a factor of 2,000) intrinsic dissolution rates in comparison with the parent compounds. Mannich bases of antibiotic norfloxacin (ED₅₀: 6 mg/kg) have been prepared. The Mannich bases (25,26) exhibited ED50: 1.25 and 1.62 mg/kg respectively in vivo against E. coli NCTC 10418. From the comparison of antibacterial activities of these compounds, (25) was 4-8 times
and (26) was 3.7 times more active than norfloxacin.

In conclusion, the Mannich bases have made rapid advances in the field of anticancer, antiviral and anticonvul-
sant activities. In the field of anticancer activity, the chalcone derivative (3) is in advanced stage of clinical trials. Thus Mannich bases have emerged as promising therapeuti-

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