International Journal of Pharmaceutics, 9 (1981) 7–16
Elsevier/North-Holland Biomedical Press

Hydrolysis of N-Mannich bases and its consequences for the biological testing of such agents

Hans Bundgaard * and Marianne Johansen **

*The Royal Danish School of Pharmacy, * Department of Pharmaceutical Chemistry AD and ** Department of Pharmaceutics, 2 Universitetsparken, DK-2100 Copenhagen (Denmark)*

(Received April 13th, 1981)
(Accepted April 27th, 1981)

Summary

Although N-Mannich bases of amides, imides, hydantoins and various other NH-acidic compounds have been known for a long time, and several drug substances and other compounds bearing an NH-acidic group have been modified by N-aminomethylation and tested as potential medicinal agents, the facile decomposition of several N-Mannich bases in aqueous solution has not been generally recognized. In this paper recent kinetic studies by the authors on the stability of a great number of N-Mannich bases are summarized and used to show that several previous reports on biological testing of various N-Mannich bases may be unrealistic because of very rapid decomposition of the derivatives in aqueous medium. The structural factors influencing the decomposition rate of N-Mannich bases are discussed and structure–reactivity relationships are given which may be useful for the prediction of stability of new N-Mannich bases as well as for retrospective evaluations.

Introduction

N-Mannich bases are formed by reacting an NH-acidic compound with formaldehyde, or in rare cases, other aldehydes and a primary or secondary aliphatic or aromatic amine. The process can be considered as an N-aminomethylation or N-amidomethylation (in the case of the NH-acidic compound being an amide, Scheme 1).

$$R\text{–}CONH_2 + CH_2O + R_1R_2NH \rightleftarrows R\text{–}CONH_2\text{–}CH_2\text{–}NR_1R_2 + H_2O$$

Scheme 1

Although N-Mannich bases of amides, imides and various other NH-acidic

compounds have been known for a long time (for reviews, see Hellman and Opitz, 1960; Tramontini, 1973) information on the stability and reactivity, in a quantitative manner, of such derivatives in aqueous solution has only recently become available (Bundgaard and Johansen, 1980a, b and c, 1981b; Johansen and Bundgaard, 1980b, 1981). In these studies, a great number of N-Mannich bases of various carboxamides, thioamides, sulphonamides, imides, urea derivatives and other NH-acidic compounds were shown to decompose quantitatively to the parent compounds (amide-type compound, amine and formaldehyde (Scheme 1)) in aqueous solution with rates highly dependent on pH and on various structural factors. The stability of the derivatives studied varied widely, the half-lives of decomposition at 37°C ranging from several hours to less than a second. Along with other studies (Bundgaard and Johansen, 1981a; Johansen and Bundgaard, 1980a) these investigations aimed to explore the concept of N-aminomethylation or N-amido-methylation as a potentially useful means of obtaining-drug forms of various NH-acidic compounds and amines.

Several drug substances and other compounds bearing an NH-acidic group have been modified by N-aminomethylation and tested for various biological and

TABLE 1

SOME NH-ACIDIC COMPOUNDS OF WHICH DIFFERENT N-MANNICH BASES HAVE BEEN TESTED FOR VARIOUS BIOLOGICAL OR PHARMACOLOGICAL EFFECTS [a]

Parent NH-acidic compound	Reference
Trichloroacetamide	Schönenberger et al. (1973)
Acetazolamide	Siegel et al. (1971)
Methazolamide	Sieger et al. (1971)
2-Benzoxazolinones	Varma and Nobles (1968)
Phthalimide	Chiavarelli et al. (1963); Schönenberger et al. (1973)
Succinimides	Chiavarelli et al. (1963); Eckstein et al. (1967); Lucka-Sobstel and Zejc (1971); Schönenberger and Lippert (1972); Schönenberger et al. (1973); Magarian et al. (1973); Lange et al. (1977a and b); Lapszewicz et al. (1978)
Glutarimides	Abou-Gharbia et al. (1978)
Hydantoins	Winstead et al. (1965); Malec (1966); Zejc (1967, 1968); Lucka-Sobstel and Zejc (1970); Zjec and Pawlowski (1980)
2-Thiohydantoins	Malec (1966); Lucka-Sobstel and Zejc (1970)
2,4-Dithiohydantoins	Zejc (1968)
Oxazolidine-2,4-diones	Malec (1966); Eckstein and Pazdro (1966)
Barbituric acids	Eckstein et al. (1966); Sladowska (1979)
Isatins	Varma and Nobles (1967b, 1975); Lucka-Sobstel et al. (1974); Movrin and Medic-Saric (1978); Kupinic et al. (1979); Maysinger et al. (1980a and b)
Imidazolidine-2-thione	Sawlewicz et al. (1975)
3-Arylimino-2-indolinones	Varma and Khan (1978); Movrin and Maysinger (1979)
Benzimidazoles	Orth et al. (1968); Bahadur et al. (1976); Varma (1977); Varma et al. (1980)
Benzotriazole	Orth et al. (1968)
Theophylline	Buckhalter and Dill (1959)

[a] Apparently with no recognition of the high lability of the compounds tested.

pharmacological effects. In Table 1 are listed a selected number of studies in which various N-Mannich bases have been subjected to biological testing. However, the facile decomposition of these N-Mannich bases in aqueous solution does not appear to have been recognized. Since N-Mannich bases continue to receive interest as potential medicinal agents, as reflected in recent literature describing studies on synthesis and pharmacological screening of such compounds, it seems highly appropriate to point out that such studies (and those referred to in Table 1) may be meaningless or become invalidated or misinterpreted, simply due to the fact that the derivatives are decomposed to the parent compounds immediately upon dissolution in an aqueous medium. The purpose of this paper is to address this warning on the basis of our recent kinetic studies on the stability of various N-Mannich bases and to point out some structure–reactivity relationships which may be useful for the prediction of the stability of N-Mannich bases. The stability data given for some N-Mannich bases have not been reported before.

Discussion

Kinetics and mechanism of decomposition of N-Mannich bases

The previous kinetic studies (Bundgaard and Johansen, 1980a and b, 1981b) showed that at constant pH and temperature, the decomposition rates of the N-Mannich bases of various amides and imides followed strict first-order kinetics and all reactions went to completion. No general acid–base catalysis by the buffers used was apparent. The pH–rate profiles for most compounds have a sigmoidal shape as seen in Fig. 1. These pH dependences of the observed apparent first-order rate constant, k_{obs}, could be accounted for by assuming spontaneous decomposition of the free Mannich bases (B) and their conjugate acids (BH^+); the expression for k_{obs} is:

$$k_{obs} = \frac{k_1 K_a}{a_H + K_a} + \frac{k_2 a_H}{a_H + K_a} \quad (1)$$

where K_a is the apparent ionization constant of the protonated N-Mannich bases, a_H is the hydrogen ion activity, and k_1 and k_2 are the apparent first-order rate constants for the spontaneous degradation of B and BH^+, respectively.

The reaction mechanism proposed (Bundgaard and Johansen, 1980b) for the decomposition involves as rate-determining step an unimolecular N–C bond cleavage with formation of an amide (or imide) anion and an immonium cation. In subsequent fast steps, a solvent molecule transfers a proton to the anion and a hydroxide ion to the immonium ion, giving methylolamine, which rapidly dissociates to formaldehyde and amine.

Structural effects on decomposition rate

The structural effects on the decomposition rate of N-Mannich bases derived from carboxamides, thioamides, sulphonamides or imides and aliphatic or aromatic amines were shown to involve steric effects and basicity of the amine component and acidity of the amide-type component (Bundgaard and Johansen, 1980b, 1981b).

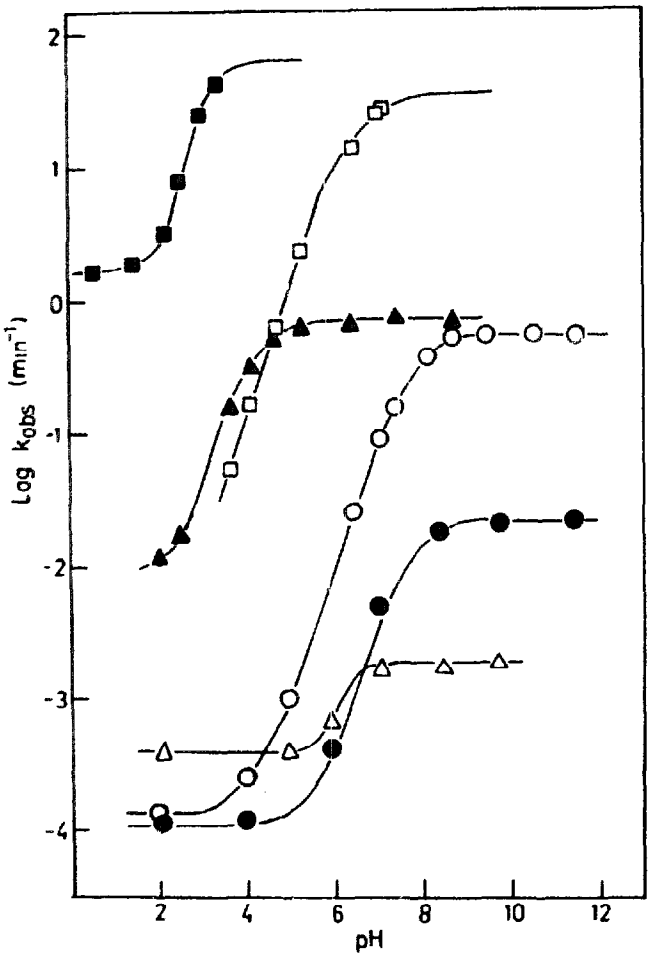

Fig. 1. The pH–rate profiles for the decomposition of various N-Mannich bases in aqueous solution at 37°C. Key: ■, N-(morpholinomethyl)-*p*-toluenesulphonamide; □, N-(piperidinomethyl)trichloroacetamide; ▲, N-(morpholinomethyl)trichloroacetamide; ○, N-(diethylaminomethyl)benzamide; ●, N-(isobutylaminomethyl)benzamide; and △, N-(benzylaminomethyl)benzamide (from Bundgaard and Johansen (1980b)).

These factors are most pronounced with respect to the rate constant k_1 and, accordingly, to the decomposition rate in weakly acidic to basic aqueous solutions. The rates of the hydrolysis of unprotonated Mannich bases are accelerated strongly by: (a) increasing steric effects within the amine substituent; (b) increasing basicity of the amine component; and (c) increasing acidity of the parent amide-type compound.

For some N-Mannich bases of benzamide and various amines the rate constant, k_1, could be expressed by the following expression (Bundgaard and Johansen, 1980b):

$$\log k_1 = 2.30\nu - 3.50 \quad (k_1 \text{ in } min^{-1};\ 37°C) \tag{2}$$

where ν is Charton's steric substituent parameter for alkylamino groups (Charton, 1978). The marked influence of the steric effect on k_1 can be exemplified by

comparing the k_1-values for the benzamide Mannich bases of diethylamine (0.52 min^{-1}) and ethylamine (0.0084 min^{-1}).

For amines with the same steric properties but differing in basicity, the rate constants, k_1, for the decomposition of the respective N-Mannich bases were shown to increase almost 10-fold with an increase of unity of the pK_a of the amines. Thus, for various N-(arylaminomethyl)succinimide derivatives the following relationship was derived (Bundgaard and Johansen, 1981b):

$$\log k_1 = 0.93\, pK_a - 4.81 \quad (k_1 \text{ in } min^{-1};\ 37°C) \tag{3}$$

The structural effect of the amide-type component in the Mannich bases on the decomposition rate was delineated from rate data obtained for several Mannich bases with either piperidine or morpholine (Bundgaard and Johansen, 1980b). The reactivity was shown to increase strongly with increasing acidity of the parent amide-type compound. For the Mannich bases with piperidine the following relationship was derived:

$$\log k_1 = -1.42\, pK_a + 19.3 \quad (k_1 \text{ in } min^{-1};\ 37°C) \tag{4}$$

For morpholine derivatives Eqn. 5 was obtained:

$$\log k_1 = -1.15\, pK_a + 13.9 \quad (k_1 \text{ in } min^{-1};\ 37°C) \tag{5}$$

Eqns. 4 and 5 in which pK_a refers to the ionization constant for the parent amide-type compounds (at 20–25°C) cover both aromatic and aliphatic carboxamides as well as a thioamide and a sulphonamide. N-Mannich bases of urea, thiourea and N-acyl thiourea derivatives were found to deviate from these relationships showing a *greater* reactivity than expected on the basis of their pK_a values. A positive deviation has also been observed with N-Mannich bases of salicylamide (Johansen and Bundgaard, 1980b).

Some representative rate data for the decomposition of various N-Mannich bases are given in Table 2.

Stability predictions

The correlations given by Eqns. 4 and 5 may be highly useful for the prediction of the stability of new N-Mannich bases as well as for a retrospective evaluation of already described Mannich bases since piperidine and morpholine have been the most commonly used amines in the synthesis of N-Mannich bases. Thus, the Mannich bases referred to in Table 1 have almost all contained piperidine or morpholine as the amine component. Furthermore, in the sense of serving purpose of prediction, piperidine and morpholine represent amines with relative greatly different basicity, the pK_a being 11.1 and 8.3, respectively.

Since the rate constant, k_1, accounts for the overall rate constant for decomposition in weakly acidic to basic solutions the half-life for the hydrolysis under these conditions may be expressed by:

$$t_{1/2} = \frac{0.693(a_H + K_a)}{k_1 K_a} \tag{6}$$

TABLE 2

OBSERVED RATE DATA FOR THE DECOMPOSITION OF VARIOUS N-MANNICH BASES IN AQUEOUS SOLUTION AT 37°C [a]

Compound	k_1 (min^{-1})	$t_{1/2}$ (min) [b]
N-(Piperidinomethyl)benzamide	0.051	47
N-(Piperidinomethyl)-4-nitrobenzamide	0.17	8
N-(Piperidinomethyl)acetamide	0.0055	400
N-(Piperidinomethyl)dichloroacetamide	2.48	0.4
N-(Piperidinomethyl)trichloroacetamide	35	0.02
N-(Piperidinomethyl)thiobenzamide	13	0.06
N-(Morpholinomethyl)benzamide	0.0005	1400
N-(Morpholinomethyl)thiobenzamide	0.52	1.3
N-(Morpholinomethyl)-*p*-toluenesulphonamide	60	0.01
N-(Methylaminomethyl)benzamide	0.0026	600
N-(Ethylaminomethyl)benzamide	0.0084	190
N-(Diethylaminomethyl)benzamide	0.52	4.0
N-(Benzylaminomethyl)benzamide	0.0020	380
N-(Morpholinomethyl)-N'-acetylthiourea	0.91	0.8
N-(Piperidinomethyl)-N'-methylurea	–	5.0
N-(Piperidinomethyl)salicylamide [c]	–	14
N-(Morpholinomethyl)salicylamide [c]	–	41
N-(Anilinomethyl)succinimide [d]	0.36	1.9
N-(*p*-Toluidinomethyl)succinimide [d]	0.76	0.9

[a] From Bundgaard and Johansen (1980b) if not otherwise indicated.
[b] At pH 7.40.
[c] From Johansen and Bundgaard (1980b).
[d] From Bundgaard and Johansen (1981b).

where k_a is the ionization constant of the Mannich base. In neutral and basic solutions the Mannich bases occur mostly or entirely in the free base form (Bundgaard and Johansen, 1980b; 1981b) and therefore, Eqn. 6 may be simplified to:

$$t_{1/2} = \frac{0.693}{k_1} \tag{7}$$

Combining Eqn. 7 and Eqns. 4 and 5 affords the following equations:

$$\log t_{1/2} = 1.42\, pK_a - 19.5 \tag{8}$$

for piperidine derivatives and

$$\log t_{1/2} = 1.15\, pK_a - 14.1 \tag{9}$$

for morpholine derivatives.

In the equations, $t_{1/2}$ is given in min at 37°C. These equations readily allow a predictive estimation of the stability of piperidine or morpholine Mannich bases; some calculations are given in Table 3.

N-Mannich bases with morpholine and piperidine of succinimide, phthalimide, phenytoin, 5,5-diethylbarbituric acid, 5-chloro-2-benzoxazolinone, acetazolamide, allopurinol, chlorothiazide and hydrochlorothiazide were previously shown to be so

TABLE 3

PREDICTED HALF-LIVES OF DECOMPOSITION OF N-MANNICH BASES OF MORPHOLINE OR PIPERIDINE IN NEUTRAL TO BASIC AQUEOUS SOLUTIONS (37°C) AS A FUNCTION OF THE pK_a OF PARENT NH-ACIDIC COMPOUND

pK_a of parent NH-acidic compound	$t_{1/2}$	
	morpholine N-Mannich base	piperidine N-Mannich base
15	23.3 h	63 min
14	1.7 h	2.4 min
13	7.1 min	5.4 s
12	0.5 min	0.2 s
10	0.2 s	3×10^{-4} s

unstable in aqueous solution at 20°C that no quantitative rate data could be obtained in the pH range 0–12 (Bundgaard and Johansen, 1980b and c; 1981a). The upper limit for the half-lives which could be determined was about 10 sec when using direct UV-spectrophotometry. In the spectrophotometric assay for formaldehyde (i.e. incubation of the compounds for 30 min at 20°C in an acetate buffer solution of pH 4 containing 3-methyl-benzothiazol-2-one hydrazone hydrochloride (Johansen and Bundgaard, 1979)) the compounds showed a quantitative release of formaldehyde. This great lability of the compounds are in accord with the predictions made from Eqns. 8 and 9 since the parent amide-type compounds are all characterized by a relatively high acidity, the pK_as being less than 10.

All the parent compounds referred to in Table 1 possess NH-acidic moieties with pK_as below about 11. In addition to the compounds mentioned above the following N-Mannich bases were prepared (refs. stated in parenthesis) and tested for decomposition in the present study: benzimidazole with piperidine (Bachman and Heisey, 1946) and aniline (Bahadur et al., 1976), phenytoin with aniline (Winstead et al., 1965), isatin with piperidine (Varma and Nobles, 1967a) and theophylline with morpholine and piperidine (Burckhalter and Dill, 1959). Using similar experimental procedures as referred to above these N-Mannich bases proved so unstable in aqueous solution (pH 0–12) that no quantitative rate data could be obtained. Again, this result is in accordance with the structure–reactivity relationships described above.

It should be added that some qualitative rate data for the decomposition of various N-Mannich bases have been described several years ago (Weitzel et al., 1964). However, the experimental procedures used appear to be poorly controlled and the rate data differ considerably from those mentioned above.

In conclusion, these results along with those summarized from the previous kinetic studies show that it is very important to recognize the lability characteristics of N-Mannich bases when performing biological or pharmacological testing of such agents. For future studies with new N-Mannich bases as well as for retrospective evaluations the structure–reactivity relationships pointed out should be useful for

the prediction of the stability of an N-Mannich base. For morpholine and piperidine Mannich bases the only information required is the pK_a of the parent NH-acidic compound. For N-Mannich bases with other amines the pK_a and the steric properties of the amine component should also be taken into account as has been described.

References

Abou-Gharbia, M., Su, T.T. and Joullie, M.M., Synthesis of spirofluorenes of biological interest. J. Pharm. Sci., 67 (1978) 935–956.

Bachman, G.B. and Heisey, L.V., The condensation of aldehydes and amines with nitrogenous five-atom ring systems. J. Am. Chem. Soc., 68 (1946) 2496–2499.

Bahadur, S., Goel, A.K. and Varma, R.S., Synthesis of 2-alkyl-1-substituted anilinomethylbenzimidazoles. J. Indian Chem. Soc., 53 (1976) 1163–1165.

Bundgaard, H. and Johansen, M., Pro-drugs as drug delivery systems. IV. N-Mannich bases as potential novel pro-drugs for amides, ureides, amines, and other NH-acidic compounds. J. Pharm. Sci., 69 (1980a) 44–46.

Bundgaard, H. and Johansen, M., Pro-drugs as drug delivery systems. X. N-Mannich bases as novel pro-drug candidates for amides, imides, urea derivatives, amines and other NH-acidic compounds. Kinetics and mechanisms of decomposition and structure–reactivity relationships. Arch. Pharm. Chem., Sci. Edn., 8 (1980b) 29–52.

Bundgaard, H. and Johansen, M., Pro-drugs as drug delivery systems. XV. Bioreversible derivatization of phenytoin, acetazolamide, chlorzoxazone and various other NH-acidic compounds by N-aminomethylation to effect enhanced dissolution rates. Int. J. Pharm., 7 (1980c) 129–136.

Bundgaard, H. and Johansen, M., Pro-drugs as drug delivery systems. XVIII. Bioreversible derivatization of allopurinol by N-aminomethylation to effect enhanced dissolution rates. Acta Pharm. Suec., 18 (1981a) 129–134.

Bundgaard, H. and Johansen, M., Pro-drugs as drug delivery systems. XIX. Bioreversible derivatization of aromatic amines by formation of N-Mannich bases with succinimide. Int. J. Pharm., (1981b) in press.

Burckhalter, J.H. and Dill, D.R., Theophylline in the Mannich reaction. J. Org. Chem., 24 (1959) 562–564.

Charton, M., Steric effects. 13. Composition of the steric parameter as a function of alkyl branching. J. Org. Chem., 43 (1978) 3995–4001.

Chiavarelli, S., Mazzeo, P. and Russo, A.M., Su alcuni N-amminometilderivati della ftalimide e della succinimide. Il Farmaco, Ed. Sci., 18 (1963) 750–755.

Eckstein, M. and Pazdro, H., Mannich base derivatives of pharmacologically active compounds. Part IV. 3-Alkylaminomethyl derivatives of oxazolidine-2,4-dione and 2-thiooxazolidine-4. Diss. Pharm. Pharmacol., 18 (1966) 139–146.

Eckstein, M., Zejc, A. and Klusek, A., Mannich base derivatives of physiologically active compounds. Part V. N-alkylaminomethyl derivatives of imides of arylsuccinic acids. Diss. Pharm. Pharmacol., 19 (1967) 263–269.

Eckstein, M., Zejc, A. and Sulko, J., Mannich base derivatives of pharmacologically active compounds Part III. 3-Alkylaminomethyl derivatives of 5,5-dialkylbarbituric acids. Diss. Pharm. Pharmacol., 18 (1966) 131–137.

Hellmann, H. and Opitz, G., α-Aminoalkylierung, Verlag Chemie, Weinheim, West Germany, 1960.

Johansen, M. and Bundgaard, H., Pro-drugs as drug delivery systems. VI. Kinetics and mechanism of the decomposition of N-hydroxymethylated amides and imides in aqueous solution and assessment of their suitability as possible pro-drugs. Arch. Pharm. Chem., Sci. Edn., 7 (1979) 175–192.

Johansen, M. and Bundgaard, H., Pro-drugs as drug delivery systems. XII. Solubility, dissolution and partitioning behaviour of N-Mannich bases and N-hydroxymethyl derivatives. Arch. Pharm. Chem., Sci. Edn., 8 (1980a) 141–151.

Johansen, M. and Bundgaard, H., Pro-drugs as drug delivery systems. XIII. Kinetics of decomposition of N-Mannich bases of salicylamide and assessment of their suitability as possible pro-drugs for amines. Int. J. Pharm., 7 (1980b) 119–127.

Johansen, M. and Bundgaard, H., Decomposition of rolitetracycline and other N-Mannich bases and of N-hydroxymethyl derivatives in the presence of plasma. Arch. Pharm. Chem., Sci. Edn., 9 (1981) 40–42.

Kupinic, M., Medic-Saric, M., Movrin, M. and Maysinger, D., Antibacterial and antifungal activities of isatin N-Mannich bases. J. Pharm. Sci., 68 (1979) 459–462.

Lange, J., Rump, S., Galecka, E., Ilczuk, I., Lechowska-Postek, M., Rabsztyn, T., Szymanska, T. and Walczyna, K., Synthesis and properties of new cyclic derivatives of succinic acid with anticonvulsant activity. Pharmazie, 32 (1977a) 82–84.

Lange, J., Rump, S., Ilczuk, I., Lapszewicz, J., Rabsztyn, T. and Walczyna, K., Synthesis and properties of cyclic derivatives of succinic acid with anticonvulsant activity. Part 2. Pharmazie, 32 (1977b) 579–581.

Lapszewicz, J., Lange, J., Rump, S. and Walczyna, K., Quantitative structure–activity relationship in anticonvulsants. I. QSAR analysis of new phenylsuccinimide derivatives. Eur. J. Med. Chem., 13 (1978) 465–468.

Lucka-Sobstel, B. and Zejc, A., Mannich base derivatives of pharmacologically active compounds. Part XII. 2-Methyl and cis-2,5-dimethylpiperazine in the reaction of aminoalkylation of hydantoin and thiohydantoin derivatives. Diss. Pharm. Pharmacol., 22 (1970) 13–19.

Lucka-Sobstel, B. and Zejc, A., Mannich base derivatives of pharmacologically active compounds. Part XIV. 2-Methyl and cis- or trans-2,5-dimethylpiperazine in the reaction of aminoalkylation of alkyl- and arylsuccinimides. Diss. Pharm. Pharmacol., 23 (1971) 135–140.

Lucka-Sobstel, B., Zejc, A., Borysiewicz, J. and Potec, Z., In-vitro antiviral activity of Mannich bases derived from 5-bromoisatin and its β-thiosemicarbazone. Acta Pharm. Jugoslav., 24 (1974) 95–100.

Magarian, E.O., Becker, G.W. and Diamond, L., Anticonvulsant properties of Mannich base derivatives of 2-phenylsuccinimide. J. Pharm. Sci., 62 (1973) 325–327.

Malec, D., Pharmacologic properties of new derivatives in the group of anticonvulsant drugs. Part I. Screening studies of derivatives of hydantoin, thiohydantoin and oxazolidinedione. Diss. Pharm. Pharmacol., 18 (1966) 337–343

Maysinger, D., Ban, J. and Movrin, M., Effects of isatin N-Mannich bases on HeLa cells. Arzneim.-Forsch., 30 (1980b) 932–935.

Maysinger, D., Movrin, M.and Saric, M.M., Structural analogues of isatin and their antimicrobial activity. Pharmazie, 35 (1980a) 14–16.

Movrin, M. and Maysinger, D., Biologisch aktive N-Mannich-basen von Isatin-3-(phenyl)iminen. Pharmazie, 34 (1979) 535–536.

Movrin, M. and Medic-Saric, M., Biologically active Mannich bases derived from isatin and 5-nitroisatin. Eur. J. Med. Chem., 13 (1978) 309–312.

Orth, R.E., Bennett, J.W., Ma, O.-H. and Young, L., Centrally acting isosteric Mannich bases. J. Pharm. Sci., 57 (1968) 1814–1817.

Sawlewicz, J., Wisterowicz, K. and Vogel, S., Synthesis of Mannich bases of imidazolidine-2-thione. Acta Pol. Pharm., 32 (1975) 435–443.

Schönenberger, H., Bindl, L. and Adam, A., Synthese und tumorhemmende Wirkung von N-[Bis(2-chloräthyl)aminomethyl]-amiden. 21. Mitt.: Über cytostatica. Arch. Pharm., 306 (1973) 64–75.

Schönenberger, H. and Lippert, H., Untersuchungen zur tumorhemmenden und antimikrobiellen Wirkung acylierter N-Halbacetale, Aminale und Aminomethylester der Dithiocarbamidsäure. 18. Mitt.: Über Cytostatica. Arch. Pharm. 305 (1972) 427–441.

Sieger, G.M., Barringer, W.C. and Krueger, J.E., Mannich derivatives of medicinals. 2. Derivatives of some carbonic anhydrase inhibitors. J. Med. Chem., 14 (1971) 458–460.

Sladowska, H., Aminomethyl derivatives of 1-cyclohexyl-5-alkyl- and 1-cyclohexyl-5,5-dialkylbarbituric acids as potential anti-inflammatory agents. Il Farmaco, Ed. Sci., 34 (1979) 979–986.

Tramontini, M., Advances in the chemistry of Mannich bases. Synthesis (1973) 703–775.

Varma, R.S., Substituted 1-arylaminomethyl-5,6-dimethylbenzimidazoles andrelated products. J. Indian Chem. Soc., 54 (1977) 727–728.

Varma, R.S., Kapoor, A. and Ellis, G.P., Potential biologically active agents. XXV. 1-Arylaminomethyl-5- and -6-chlorobenzimidazoles [1]. Eur. J. Med. Chem., 15 (1980) 536–538.

Varma, R.S. and Khan, I.A., Synthesis of 5-chloro-3-arylimino-, 1-methyl-5-chloro-3-arylimino and

1-morpholino(piperidino)methyl-5-chloro-3-arylimino-2-indolinones. Indian J. Chem., 16B (1978) 1113–1114.

Varma, R.S. and Nobles, W.L., Substituted N-aminomethylisatins. J. Med. Chem., 10 (1967a) 510.

Varma, R.S. and Nobles, W.L., Synthesis and antiviral and antibacterial activity of certain N-dialkylaminomethylisatin β-thiosemicarbazones. J. Med. Chem., 10 (1967b) 972–974.

Varma, R.S. and Nobles, W.L., Synthesis and antibacterial activity of certain 3-substituted benzoxazolinones. J. Pharm. Sci., 57 (1968) 39–44.

Varma, R.S. and Nobles, W.L., Antiviral, antibacterial, and antifungal activities of isatin N-Mannich bases. J. Pharm. Sci., 64 (1975) 881–882.

Weitzel, G., Schneider, F.and Finger, H., Weitere tumorhemmende Verbindungsklassen. II. Cytostatische Effekte von N-C-N-Verbindungen. Z. Physiol. Chem., 336 (1964) 107–126.

Winstead, M.B., Barr, D.E., Hamel, C.R., Renn, J., Parker, H.I. and Neumann, M., Substitution in the hydantoin ring. I. N-3-Aminomethyl derivatives. J. med. Chem., 8 (1965) 117–120.

Zejc, A., Mannich base derivatives of pharmacologically active compounds. Part VI. 3-Alkylamino methyl derivatives of some 5,5-spirohydantoins. Diss. Pharm. Pharmacol., 19 (1967) 173–180.

Zejc, A., Mannich base derivatives of pharmacologically active compounds. Part VIII. 3-Alkyl- or arylpiperazino-methyl derivatives of 5,5-disubstituted hydantoin, 2-thiohydantoin, and 2,4-dithiohydantoin and their chemical transformations. Diss. Pharm. Pharmacol., 20 (1968) 507–524.

Zejc, A. and Pawlowski, M., 3-Alkyl(aryl)piperazinoalkyl derivatives of 5,5-diphenylhydantoin. Pol. J. Pharmacol. Pharm., 32 (1980) 173–178.