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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 *

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SUMMARY

A number of N-Mannich bases of various NH-acidic compounds (benzamide, phthalimide, chlorzoxazone, phenytoin, barbital, *p*-toluenesulfonamide, acetazolamide, chlorothiazide and hydrochlorothiazide) with morpholine or piperidine as the amine component were evaluated as potential pro-drugs with the purpose of enhancing the aqueous solubility and dissolution rate.

The N-Mannich bases were shown to decompose very rapidly in aqueous solution with formation of formaldehyde, amine and parent compound in stoichiometric amounts. Dissolution rate studies showed that the derivatives, especially as hydrochloride salts, possessed markedly greater intrinsic dissolution rates in 0.1 M hydrochloric acid in comparison with the parent compounds, the largest rate acceleration observed being a factor of 2,000. It is concluded that N-Mannich bases of various NH-acidic drug substances of poor aqueous solubility may be of potential usefulness as pro-drugs with the aim of increasing the dissolution rate in an effort to improve the oral bioavailability.

INTRODUCTION

Several drugs show poor and variable oral absorption characteristics as a result of insufficient aqueous solubility and absorption becomes dissolution rate-limited (Mattok et al., 1977; Poole, 1977). The procedures commonly used for improving the rate of dissolution of such drugs involve modification of the physical or physicochemical characteristics of

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the drugs, e.g. reduction of the particle size, and use of various formulation techniques (Lippold, 1977). An alternative method of accelerating the dissolution and release of drug substances is to use the pro-drug approach. The use of such a procedure for this purpose implies derivatization of the drugs to produce hydrophilic and more soluble compounds which revert to the parent drugs after dissolution or after their absorption from the gastrointestinal tract. Up to now, there are only few examples of this pro-drug approach being used to increase the aqueous solubility and dissolution behaviour of poorly soluble drugs in an effort to improve the oral absorptivity of the drugs (Stella, 1975, 1977).

In previous studies (Bundgaard and Johansen, 1980a and b; Johansen and Bundgaard, 1980a and b), the concept of N-aminomethylation of various carboxamides, thioamides, sulphonamides, imides, urea derivatives and various other NH-acidic compounds as a potentially useful means of obtaining pro-drug forms of such not easily derivatizable chemical entities was explored. Such N-Mannich bases were shown to decompose quantitatively to the parent compounds (for an amide Mannich base, see Scheme 1) in aqueous solution at rates determined by pH of the medium and by various structural factors.

SCHEME 1

$$R\text{–}CONHCH_2NR_1R_2 + H_2O \rightarrow R\text{–}CONH_2 + CH_2O + R_1R_2NH$$

Transformation of an amide-type compound into an N-Mannich base introduces a readily ionizable amine moiety which may allow the preparation of derivatives with improved solubility characteristics. For a number of N-Mannich bases of benzamide it was recently shown that large modifications in aqueous solubility, intrinsic dissolution rate and lipophilicity for the parent amide can be achieved by the appropriate selection of the amine component and by the proper choice of salt form (Johansen and Bundgaard, 1980a).

To explore further the possibilities of N-aminomethylation in making pro-drugs with improved solubility and dissolution characteristics this study has been extended to include a number of other NH-acidic compounds and various poorly water-soluble drugs amenable to N-aminomethylation. It is shown in this paper that a marked improvement in the dissolution rate for various compounds can be achieved by the pro-drug approach involving N-Mannich bases and salts thereof.

MATERIALS AND METHODS

Apparatus

Ultraviolet and visible spectral measurements were performed with a Zeiss PMQ II spectrophotometer and a Perkin-Elmer 124 recording spectrophotometer, using 1-cm cuvettes. Infrared spectra were recorded using the potassium chloride disc technique on a Unicam SP 200 spectrophotometer. Readings of pH were carried out on a Radiometer Type PHM 26 meter at the temperature of study. Melting points were taken on a capillary melting-point apparatus and are uncorrected.

Compounds

N-Mannich bases of various NH-acidic compounds with morpholine or piperidine were prepared by heating formaldehyde (as 37% aqueous solution), the amine and the amide-

type compound in water, ethanol or methanol according to previously described procedures: N-(piperidinomethyl)phthalimide (Heine et al., 1956), N-(morpholinomethyl)-chlorzoxazone (Varma and Nobles, 1968), N-(piperidinomethyl)-*p*-toluenesulfonamide (Sekiya and Ito, 1966), N-(piperidinomethyl)acetazolamide and N-(morpholinomethyl)-acetazolamide (Sieger et al., 1971). The melting points of these derivatives were in agreement with those reported in the literature given above. The N-Mannich bases of benzamide, barbital, phenytoin as well as N-(morpholinomethyl)-*p*-toluenesulfonamide were from a previous study (Bundgaard and Johansen, 1980b). N-(piperidinomethyl)chlorothiazide was prepared by refluxing a solution of 5 mmol each of chlorothiazide, piperidine and formaldehyde in 35 ml of methanol for 15 min, evaporation of the methanol under reduced pressure and recrystallization of the residue from methanol, m.p. >265°C. N-(Morpholinomethyl)hydrochlorothiazide was prepared in a similar manner, dec. 192–195°C. The structures of these compounds (Fig. 1) were confirmed by elemental and IR analyses as well as by molecular weight determination by measuring the amount of for-

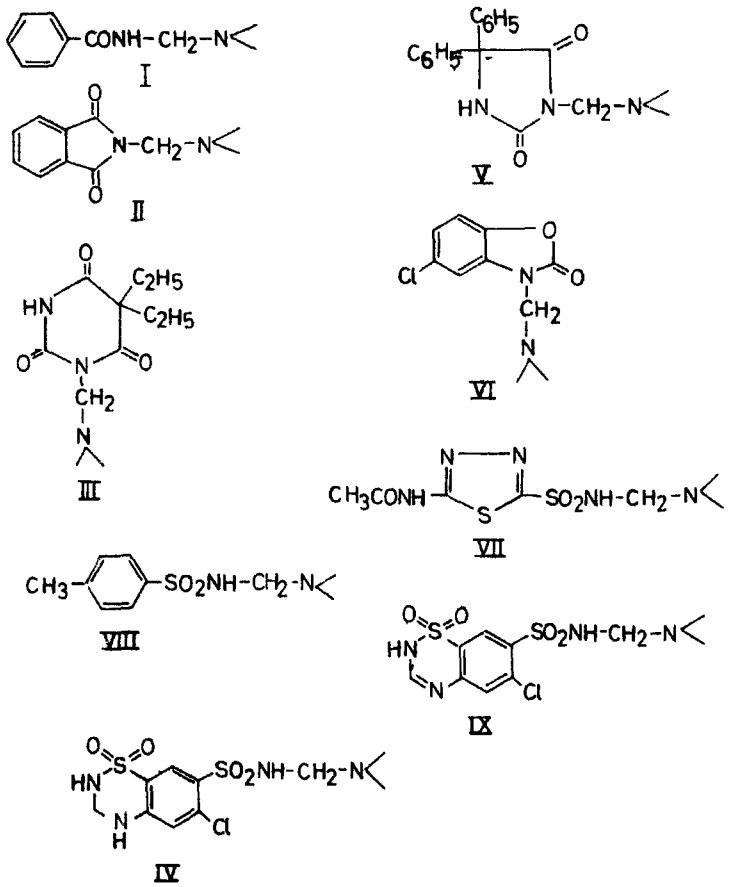

Fig. 1. Chemical structures of tested N-Mannich bases derived from the following parent compounds: I, benzamide; II, phthalimide; III, barbital; IV, hydrochlorothiazide; V, phenytoin; VI, chlorzoxazone; VII, acetazolamide; VIII, *p*-toluenesulfonamide; and IX, chlorothiazide.

maldehyde released upon hydrolysis (Johansen and Bundgaard, 1979, 1980a). Hydrochloride salts of the N-Mannich bases were generally prepared by mixing a solution of the base in methanol and a 2.6 M methanolic solution of HCl in stoichiometric amounts. Upon addition of ether until turbidity and standing for several hours at 0°C the salt precipitated out and was recrystallized from ethanol or methanol–ether. The salts were characterized by IR and UV analyses and by molecular weight determination by measuring the amount of formaldehyde released upon hydrolysis as referred to above. For all salts the molecular weights thus determined agreed within ±3% with the calculated values.

Measurements of dissolution rates

Intrinsic dissolution rates were measured using the rotating disc method of Nogami et al. (1966). Discs of 100 mg and 11.3 mm diameter were prepared by compression of the compounds at about 4000 kg cm^{-2} in a compression punch-die assembly. The dissolution medium was 500 ml of 0.1 M hydrochloric acid, the temperature 22°C, and the rotation velocity of the disc holder 200 rpm. At appropriate intervals, samples of 5 ml were taken and were replaced by fresh dissolution medium. Analysis of the samples withdrawn was carried out UV-spectrophotometrically at an appropriate wavelength and by reference to standard curves.

RESULTS AND DISCUSSION

Decomposition of the N-Mannich bases

It was previously found (Bundgaard and Johansen, 1980a and b; Johansen and Bundgaard, 1980b) that N-Mannich bases of amides and various other NH-acidic compounds readily decompose in aqueous solution to yield the parent compounds in stoichiometric amounts. The rates of decomposition increased with pH in a sigmoid fashion and the pH–rate profiles were generally accounted for by assuming spontaneous decomposition of both free and protonated Mannich base. These reactions were accelerated strongly by increasing acidity of the parent amide-type compound and also exhibited a marked dependency on amine basicity and steric effects of the amine substituent. The compounds tested in the present study are all characterized by a relatively high acidity, the pK_as being less than 10.5. With the exception of the *p*-toluenesulfonamide (pK_a 10.2 (Willi, 1956)) derivatives, the N-Mannich bases were so unstable, in accord with the high acidity of the parent compounds, that no quantitative rate data could be obtained in the pH range 0–12, i.e. the upper limit for the half-lives being about 10 sec (cf. Bundgaard and Johansen, 1980b). In the assay for formaldehyde (i.e. incubation of the compounds for 30 min at 20–25°C in an acetate buffer solution of pH 4.0 containing 3-methyl-benzothiazol-2-one hydrazone hydrochloride (Johansen and Bundgaard, 1979)) the compounds showed a quantitative release of formaldehyde. For N-(morpholinomethyl)-*p*-toluenesulfonamide the half-time of decomposition has previously been determined to be 0.4 min at pH < 1.2 and 37°C and to decrease with increasing pH above this range (Bundgaard and Johansen, 1980b). Using a similar experimental procedure as described in this previous paper the following rate data were presently obtained for N-(piperidinomethyl)-*p*-toluenesulfonamide at 37°C: $t_{1/2}$ = 9.8 min at pH 1.14, $t_{1/2}$ = 1.2 min at pH 2.14 and $t_{1/2}$ = 8 sec at pH 3.00.

Dissolution rates

The initial dissolution rates of the N-Mannich bases and their parent compounds in 0.1 M hydrochloric acid followed apparent zero-order kinetics in accord with the experimental conditions of constant dissolution surface area and apparent sink conditions. Representative plots of various compounds are shown in Fig. 2. The intrinsic dissolution rates were calculated from the slopes of plots as those in Fig. 2 divided by the surface area of the compressed disc and are listed in Table 1.

The results show that most of the N-Mannich bases and especially salts thereof possess greatly increased intrinsic dissolution rates as compared with the parent compounds. Some of the salts of the Mannich bases were so soluble that the compressed discs were dissolved after less than one minute. Accordingly, the dissolution rates given for these compounds are not of the same accuracy as the values for the other derivatives. It can be seen from Table 1 that rate enhancements (on a molar basis) amounting to a factor of up to 26 (benzamide), 256 (phthalimide), >295 (barbital), 465 (phenytoin), 1950 (chlorozoxazone), 67 (*p*-toluenesulfonamide), 31 (acetazolamide), 8 (chlorothiazide) and 6 (hydrochlorothiazide) can be achieved by transforming the parent NH-acidic compounds into transient N-Mannich base pro-drug forms.

In all cases the salts of the N-Mannich bases exhibited greater dissolution rates than the corresponding free base forms. This difference may be explained on basis of the diffusion layer model for dissolution processes as described in terms of the equation of Noyes and Whitney (1897):

$$\frac{dC}{dt} = kS(C_s - C) \qquad (1)$$

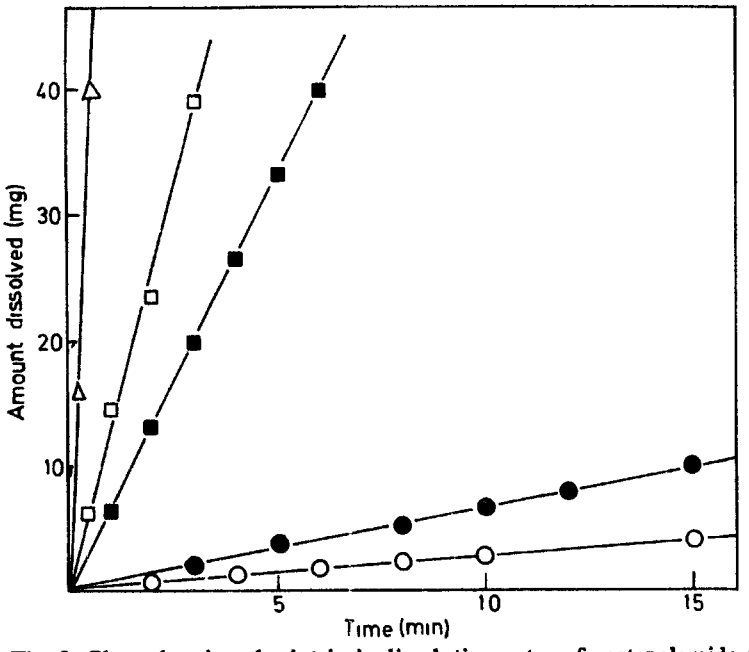

Fig. 2. Plots showing the intrinsic dissolution rates of acetazolamide (○), N-(piperidinomethyl)acetazolamide hydrochloride (□), *p*-toluenesulfonamide (●), N-(morpholinomethyl)-*p*-toluenesulfonamide (■) and N-(morpholinomethyl)-*p*-toluenesulfonamide hydrochloride (△).

TABLE 1

INTRINSIC DISSOLUTION RATES (D) FOR VARIOUS N-MANNICH BASES AND THEIR PARENT COMPOUNDS IN 0.1 M HYDROCHLORIC ACID AT 22°C

Compound	D (mg cm^{-2} min^{-1})	D (mol cm^{-2} min^{-1})
Benzamide *	1.6	1.3×10^{-5}
N-(Dimethylaminomethyl)benzamide hydrochloride *	73	3.4×10^{-4}
N-(Piperidinomethyl)benzamide hemitartrate *	60	1.6×10^{-4}
Phthalimide	0.063	4.3×10^{-7}
N-(Piperidinomethyl)phthalimide	7.1	2.9×10^{-5}
N-(Piperidinomethyl)phthalimide hydrochloride	31	1.1×10^{-4}
Barbital	0.80	4.4×10^{-6}
N-(Piperidinomethyl)barbital	2.0	7.0×10^{-6}
N-(Piperidinomethyl)barbital hydrochloride	>400	$>1.3 \times 10^{-3}$
N-(Morpholinomethyl)barbital	1.1	4.0×10^{-6}
N-(Morpholinomethyl)barbital hydrochloride	>400	$>1.3 \times 10^{-3}$
Phenytoin	0.0050	2.0×10^{-8}
N-(Morpholinomethyl)phenytoin	0.52	1.5×10^{-6}
N-(Piperidinomethyl)phenytoin	1.1	3.1×10^{-6}
N-(Piperidinomethyl)phenytoin hydrochloride	3.6	9.3×10^{-6}
Chlorzoxazone	0.034	2.0×10^{-7}
N-(Morpholinomethyl)chlorzoxazone	0.56	2.0×10^{-6}
N-(Morpholinomethyl)chlorzoxazone hydrochloride	~120	$\sim 3.9 \times 10^{-4}$
p-Toluenesulfonamide	0.67	3.9×10^{-6}
N-(Morpholinomethyl)-*p*-toluenesulfonamide	6.7	2.5×10^{-5}
N-(Morpholinomethyl)-*p*-toluenesulfonamide hydrochloride	81	2.6×10^{-4}
N-(Piperidinomethyl)-*p*-toluenesulfonamide	6.0	2.2×10^{-5}
N-(Piperidinomethyl)-*p*-toluenesulfonamide hydrochloride	39	1.3×10^{-4}
Acetazolamide	0.27	1.2×10^{-6}
N-(Piperidinomethyl)acetazolamide	0.27	8.5×10^{-7}
N-(Piperidinomethyl)acetazolamide hydrochloride	13.0	3.7×10^{-5}
N-(Morpholinomethyl)acetazolamide	0.24	7.5×10^{-7}
Chlorothiazide	0.062	2.1×10^{-7}
N-(Piperidinomethyl)chlorothiazide	0.61	1.6×10^{-6}
Hydrochlorothiazide	0.086	2.9×10^{-7}
N-(Morpholinomethyl)hydrochlorothiazide	0.65	1.6×10^{-6}

* The dissolution rates for these compounds are in water at 22°C and are from a previous study (Johansen and Bundgaard, 1980a).

where dC/dt is the dissolution rate, k is a constant, S is the surface area of the dissolving solid, C_s is the saturation solubility of the solute in the diffusion layer and C is the concentration of the solute in the bulk solution at time t. Under the experimental conditions $C_s >> C$ and Eqn. 1 is simplified to:

$$\frac{dC}{dt} = kSC_s \qquad (2)$$

According to this equation the dissolution is controlled by the solubility in the diffusion

layer which, in turn, is determined by the pH of that layer in case of acidic or basic compounds. Salts of benzamide N-Mannich bases with morpholine or piperidine as the amine components have been shown to be much more soluble in water than the free base forms (Johansen and Bundgaard, 1980a) and this may also apply to the other N-Mannich bases studied (for obvious stability reasons the equilibrium solubilities could not be determined). The free bases but not the salts may be assumed to increase the pH of the diffusion layer and, accordingly, possess a lower C_s than the salts, resulting in a lower dissolution rate. Such difference in the dissolution rate behaviour of basic compounds and salts thereof in an acidic medium is a generally recognized phenomenon as recently discussed by Berge et al. (1977).

Sieger et al. (1971) have previously reported on the saturation solubilities of some N-Mannich bases of acetazolamide and methazolamide in water. However, the great lability of the compounds appears not to have been recognized and accordingly, the solubility data given may not be realistic.

N-Mannich bases as potentially useful pro-drugs

The results of the present study show that N-Mannich bases (as salts) of various NH-acidic drug substances of poor aqueous solubility may be of potential usefulness as pro-drugs with the purpose of increasing the dissolution rate to effect an improved bioavailability upon oral administration. Once dissolved the compounds studied are cleaved very rapidly with the quantitative release of the parent compounds.

In designing N-Mannich base pro-drugs in order to increase the solubility and dissolution characteristics secondary amines should be preferred to primary ones, at least in the case of amides. It has been found that benzamide N-Mannich bases with primary amines possess a surprisingly low aqueous solubility even in salt form (Johansen and Bundgaard, 1980a). This behaviour was ascribed to the occurrence of intramolecular hydrogen bonding in these derivatives. In N-Mannich bases derived from secondary amines such an effect is not possible.

The concept of N-aminomethylation of NH-acidic compounds to yield more soluble and more rapidly dissolving pro-drugs may be extended to include aminomethylation of compounds containing a CH-acidic group. This has recently been demonstrated with phenylbutazone as a model substance (Bundgaard and Johansen, 1980c). The study showed that 4-(piperidinomethyl)phenylbutazone as the hydrochloride salt possessed a 250-fold greater intrinsic dissolution rate in 0.1 M hydrochloric acid in comparison with the parent drug and that phenylbutazone was released from the derivative immediately ($t_{1/2} < 3$ s) upon dissolution.

It should finally be mentioned that, while evaluating the potential application of N-Mannich bases as pro-drug forms, the toxicity of formaldehyde must be considered. However, this does not appear to represent a severe problem because various compounds which have been marketed as safe drugs, also release formaldehyde in the body upon oral administration, e.g. the pro-drug esters pivampicillin and pivmecillinam (cf. Johansen and Bundgaard, 1979).

REFERENCES

Berge, S.M., Bighley, L.D. and Monkhouse, D.C., Pharmaceutical salts. J. Pharm. Sci., 66 (1977) 1–19.

Bundgaard, H. and Johansen, M., Prodrugs 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. XIV. Bioreversible derivatization of phenylbutazone by C_4-aminomethylation to effect enhanced solubility and dissolution rate. Arch. Pharm. Chem., Sci. Edn., 8 (1980c) 207–214.

Heine, H.W., Winstead, M.B. and Blair, R.P., Identification of amines. II. Phthalimidomethyl derivatives of primary and secondary amines. J. Am. Chem. Soc., 78 (1956) 672–674.

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. (1980b) in press.

Lippold, B.C., Solid dosage forms: mechanism of drug release. In J. Polderman (Ed.), Formulation and Preparation of Dosage Forms, Elsevier/North-Holland Biomedical Press, Amsterdam, 1977, pp. 215–236.

Mattok, G.L., Lovering, E.G. and McGilveray, I.J., Bioavailability and drug dissolution. Progr. Drug Metabolism, 2 (1977) 259–308.

Nogami, H., Nagai, T. and Suzuki, A., Studies on powdered preparations. XVII. Dissolution rate of sulfonamides by rotating disk method. Chem. Pharm. Bull., 14 (1966) 329–338.

Noyes, A.A. and Whitney, W.R., The rate of solution of solid substances in their own solutions. J. Am. Chem. Soc., 19 (1897) 930–934.

Poole, J.W., Effects of formulation and dosage forms on drug bioavailability. In J. Blanchard, R.J. Sawchuk and B.B. Brodie (Eds.), Principles and Perspectives in Drug Bioavailability, S. Karger, Basel, 1979, pp. 59–89.

Sekiya, M. and Ito, K., Reaction of amide homologs. XIII. Catalytic hydrogenolysis of N-acylaminomethyl and N-arylsulfonamidomethyl compounds. Chem. Pharm. Bull., 14 (1966) 996–1006.

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.

Stella, V., Pro-drugs an overview and definition. In T. Higuchi, and V. Stella (Eds.), Pro-drugs as Novel Drug Delivery Systems, Am. Chem. Soc., Washington, 1975, pp. 1–115.

Stella, V., Drug substances in particular pro-drugs. Problems and methods of approach. In J. Polderman (Ed.), Formulation and Preparation of Dosage Forms, Elsevier/North-Holland Biomedical Press, Amsterdam, 1977, pp. 91–111.

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

Willi, A.V., 5. Die Aciditätskonstanten von Benzolsulfonamiden und ihre Beeinflussbarkeit durch Substitution. Helv. Chim. Acta, 39 (1956) 46–53.