SYNTHESIS OF 4-(5-NITRO-2-FURFURYLIDENE)AMINO-3-MERCAPTO-5-(SUBSTITUTED)-1,2,4-TRIAZOLES AS POSSIBLE ANTIBACTERIAL AGENTS

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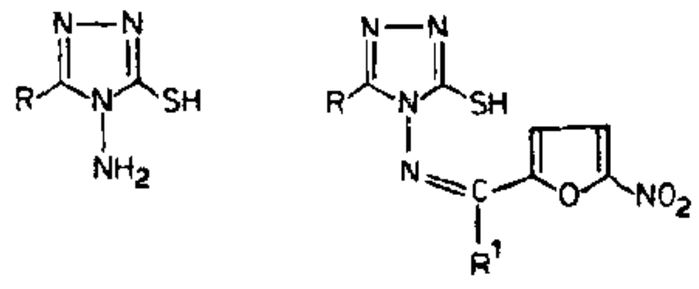
EVER since the introduction of Nitrofurazone (Furacin) as a topical antibacterial agent[1,2] other nitrofuran derivatives have been prepared so as to increase the activity. A large number of 5-nitro-2-furfuraldehyde hydrazones, semicarbazones and thiosemicarbazones have been synthesized and subjected to antibacterial screening by Nardi *et al*[3–5]. Most of the compounds so synthesized exhibited antibacterial activity *in vitro*, comparable to that of 5-nitro-2-furfuraldehyde semi-carbazone and thiosemicarbazone. Several nitrofurfuraldehyde thiosemicarbazones had *in vitro* antifungal activity against *Trichophyton mentagrophytes* and *Candia albicans*. Novinson *et al*[6] condensed nitrofurfuraldehyde with hydrazine derivatives of several pyrazolo [1,5-a] pyrimidines, pyrazolo [1,5-a]-1,3,5-triazines, s-triazolo [1,5-a] pyrimidines and imidazo [1,2-a] pyrimidines to obtain the corresponding nitrofurfural hydrazones carrying the respective heterocyclic rings. The compounds had *in vivo* activity against experimental infections of *T. cruzi* in laboratory mice.

Prompted by these findings and as a part of our general search[7,8] for pharmcologically active nitrofurans it was contemplated to synthesize and study the biological activities of a series of 4-(5-nitro-2-furfurylidene)amino-3-mercapto-1,2,4-triazoles (II) and related compounds (III) and the same is described in the present communication.

4-Amino-3-mercapto-5-alkyl or aryl-1,2,4-triazoles (I) were prepared according to the literature methods[9,10]. These triazoles, when condensed with 5-nitro-2-furfuraldehyde diacetate, in absolute alcohol or glacial acetic acid medium, employing concentrated sulphuric acid as catalyst, gave moderate to excellant yields of the title compounds (II). As a part of the structure-activity studies, triazoles (I) substituted at position 5 by alkyl, aryl and benzyl groups were taken for the present investigation. Instead of 5-nitro-2-furfuraldehyde diacetate, 5-nitro-2-acetylfuran was condensed with some triazoles (I) to give the compounds (III). The melting points of the new compounds were determined by capillary method and were uncorrected. The IR spectra were recorded in nujol mull and NMR spectra were recorded in DMSO-d_6 on a EM 390–90 MHz NMR spectrometer with TMS as an internal standard.

The structures of the title compounds were confirmed by elemental analyses, IR and NMR spectral data. The results of elemental analyses agree with theoretical values within the limits of experimental error. The characterization data of the title compounds (II) and (III) are listed in table 1. The IR spectra of all the title compounds showed a moderately strong band around 1600–1620 cm^{-1}, characteristic of the hydrazone moiety. The NMR spectrum of (II i) (R = *p*-chlorophenyl) showed a sharp singlet at δ, 10.05, characteristic of -N=CH-moiety. The doublet (J = 7.0 Hz) characteristic of the ortho-aromatic protons of the *p*-chlorophenyl moiety[11] was seen at δ, 7.50, while another doublet (J = 3.5 Hz) due to the nitrofuryl β-proton[12] appeared at δ, 7.35 in the NMR spectrum of (II i). The signal due to the other aromatic protons and the second β-proton of the nitrofuran moiety were found mingled and appeared as multiplet centred at δ, 7.15. The signal due to -SH proton, however, was not observed, presumably due to the fascile thiol-thione tautomerism of 3-mercapto-1,2,4-triazoles. The NMR spectra of few other title compounds (II) were also recorded and conformed with the assigned structures for these compounds.



(I) (II) (III)
R¹ = H R¹ = CH₃

Antibacterical activities of the title compounds were determined against *S. aureus*, *A. aerogenes*, *E. coli* and *B. subtilis* by disk-diffusion method as described earlier[8] and the results of the antibacterial testing are given in table 1. Most of the compounds showed moderate to good antibacterial activity against all the microorganisms tested at less than 5 μg/ml dilution. Compound (II k) with a *p*-methoxyphenyl substituent in the triazole moiety showed the highest activity against both gram-positive and

Table 1 *Characterization data and antibacterial activity of compounds (II) and (III)*

Compound No.	R	Yield (%) m.p. (°C)	IR (cm^{-1}) $\gamma_{C=N}$	IR (cm^{-1}) γ_{NO_2} asym sym	Antibacterial activity: Minimum inhibitory concentration* μg/disk (diameter of zone of inhibition in mm) *Bs*	*S.au*	*A.aer*	*Es*
IIa	Methyl	73 182–3[d]	1620	1560 1345	<5 (15.35)	5 (10.15)	5 (11.65)	5 (12.35)
IIb	H	84 192–3[a]	1620	1550 1340	5 (7.00)	5 (11.15)	<5 (12.35)	<5 (14.15)
IIc	Ethyl	75 156–7[b]	1620	1535 1360	5 (14.00)	5 (10.50)	<5 (13.20)	5 (10.40)
IId	Propyl	71 134–5[b]	1615	1540 1345	5 (10.55)	5 (11.35)	5 (11.00)	5 (9.15)
IIe	Phenyl	79 166–8[c]	1620	1550 1360	40 (13.15)	80 (10.90)	70 (11.60)	20 (13.00)
IIf	*o*-tolyl	54 251–2[b]	—	—	5 (7.65)	5 (11.15)	5 (8.40)	5 (9.15)
IIg	*p*-tolyl	73 251–2[b]	1620	1550 1350	5 (8.55)	5 (10.35)	5 (7.35)	5 (8.90)
IIh	*o*-chlorophenyl	63 146–7[c]	1615	1545 1350	<5 (12.25)	<5 (12.65)	<5 (13.25)	<5 (12.80)
IIi	*p*-chlorophenyl	86 187–8[a]	1615	1530 1350	<5 (11.95)	<5 (12.00)	<5 (13.60)	<5 (13.25)
IIj	*o*-hydroxyphenyl	45 241[a]	—	—	50 (11.30)	40 (13.20)	20 (8.65)	20 (8.70)
IIk	*p*-methoxyphenyl	69 195[a]	1615	1535 1345	<5 (15.95)	<5 (15.30)	<5 (13.35)	<5 (12.95)
III	Benzyl	79 161–2[b]	1620	1560 1350	5 (14.30)	<5 (11.25)	<5 (12.15)	5 (13.20)
IIIa	Methyl	67 227–8[b]	—	—	70 (14.85)	70 (9.15)	90 (8.30)	70 (9.75)
IIIb	Ethyl	60 73–4[b]	—	—	15 (11.55)	15 (9.20)	20 (10.90)	15 (12.25)
IIIc	Phenyl	60 177[d]	1625	1535 1360	60 (12.80)	70 (10.80)	50 (12.35)	40 (12.15)
Furacin (for comparison)		—	—	—	<5 (12.65)	<5 (14.45)	5 (10.20)	<5 (11.15)

Solvent of crystallization: (a) Dioxane; (b) Ethanol; (c) Acetic acid; (d) Benzene.
*Minimum inhibitory concentration is the lowest concentration of the compound that prevents visible growth after 24 hr of incubation.
Bs: Bacillus subtilis; S.au: Staphylococcus aureus; A.aer: Aerobacter aerogenes; Es: Escherichia coli.

gram-negative bacteria tested, while compounds II a, b, h, i and l showed higher antibacterial activity than Furacin only against gram-negative bacteria. The antibacterial activity of compounds III a, b and c, however, was not promising.

4-(5-Nitro-2-furfurylidene)amino-3-mercapto-5-substituted-1,2,4-triazoles (II a-l and III a-c): General procedure

An equimolecular mixture of 4-amino-3-mercapto-5-methyl-1,2,4-triazole (Ia, 1.30 g, 0.01 mol) and 5-

nitro-2-furfuraldehyde diacetate[13] (2.43 g, 0.01 mol) in absolute alchol (20 ml) was treated with concentrated sulphuric acid (0.5 ml) and was heated under reflux for 1–2 hr. On cooling the reaction mixture a yellow solid product separated out, which was crystallized from dioxane to give yellow micro needles of II a, m.p. 182–3°. Other triazoles (I) were similarly condensed with 5-nitro-2-furfuraldehyde diacetate and 5-nitro-2-acetylfuran[14] to give compounds (II) and (III) respectively. The characterization data of these compounds are reported in table 1.

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SPERM ABNORMALITIES IN A NATURAL POPULATION OF *POECILOCERUS PICTUS* (FABR)

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SPERMS are important indicators in reproductive toxicology as they can be used to assess the spermatogenic damage, the fertility effects and the heritable mutations induced either by physical or chemical mutagens or by the surrounding environment itself. The most commonly used system is the mouse sperm test. Chemical induction of sperm abnormalities has been reported in the grasshopper, *Poecilocerus pictus* (Fabr)[1,2]. The physical agent, viz constant high temperature[3], also induced sperm abnormalities in *P. pictus* (Fabr). In the present investigation, a natural population of *P. pictus* (Fabr) collected from Bangalore, was employed for sperm abnormality studies.

Testes were dissected out and fixed in a mixture of acetic acid and alcohol in the ratio 1:3. Heidenhain's iron haematoxylin squash preparations in 45% acetic acid were made. The sperms from uniformly well-spread areas were screened. Different types of morphologically abnormal sperms like shrunken, coiled, folded, zig-zag, broken and polyploid were noted and the percentage frequencies of individual anomalies were recorded (figure 1).

Polyploid spermatozoa have been reported in higher animals[4] like mouse, rabbit, bull and man[5]. In rabbit they form 0.03% of the total sperm, in the bull 0.01% to 0.17% and in man 1.02%. In *P. pictus* (Fabr), 4.21% of polyploid sperms were observed in the present investigation. Sperms were found to coil in bundles in different degrees and this observation resembled the coiling of the sperms of 'Sevin' treated grasshoppers[1]. A sudden bending at frequent intervals in opposite direction, giving a zig-zag appearence to the sperms was common with the extreme conditions resulting in the folded sperms. Many were seen broken either individually or in bundles at a single point or two or more points. Occasionally frequent coiling/coilings were seen in individual/bundles of sperms. The extreme case of folding and coiling followed by their streching might have resulted in the broken sperms. A few of the sperms were reduced in length with irregular bends and splits and were of shrunken nature. Such radical