SYNTHESIS OF 3-METHYL-4-[(2,4-DIHYDRO-4-SUBSTITUTED-3H-1,2,4-TRIAZOLE-3-THIONE-5-YL)PHENYLHYDRAZONO]-5-ISOXAZOLONE AND EVALUATION OF THEIR ANTIMICROBIAL ACTIVITIES

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SUMMARY

3-Methyl-4-[(2,4-dihydro-4-substituted-3H-1,2,4-triazole-3-thione-5-yl)phenylhydrazono]-5-isoxazolone derivatives have been synthesised. The structure of these compounds was determined using spectral data and elemental analyses. These compounds were tested for antimicrobial activity.

KEY WORDS

drazoxolon, 5-isoxazolone, antimicrobial activity, agar diffusion method

INTRODUCTION

Drazoxolon, 3-methyl-4-(o-chlorophenylhydrazono)-5-isoxazolone is known to possess fungicidal activity /1/. Rollas and co-workers have previously synthesised some 5-(4-aminophenyl)-4-substituted-2,4-dihydro-3H-1,2,4-triazole-3-thione derivatives which have been found as active as miconazole against *Candida albicans* ATCC 10231 and *Candida tropicalis* K 1022 /2/. Ergenç and co-workers have prepared some compounds carrying the isoxazole skeleton which have been found active against *B. subtilis* with MIC values of 62.5 μg/ml /3/. These observations led us to synthesise some new compounds and to

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investigate their possible antimicrobial activities. 3-Methyl-4-[(2,4-dihydro-4-substituted-1,2,4-triazole-3-thione-5-yl)phenylhydrazono]-5-isoxazolone derivatives were synthesised (Fig. 1). The structures of the compounds were determined using their spectral data and elemental analyses. These compounds were evaluated for antimicrobial activity.

MATERIALS AND METHODS

Chemicals

Methyl isothiocyanate (Fluka), ethyl isothiocyanate, phenyl isothiocyanate and ethyl acetoacetate were purchased from Sigma. All other chemicals were purchased from Merck. All melting points were recorded on a Buchi 530 melting point apparatus and uncorrected. UV spectra were recorded on a Shimadzu UV-2100S spectrophotometer (1 mg/100 ml in ethanol). IR spectra were run on a Perkin Elmer 1600 FTIR spectrophotometer (1 mg/100 mg in KBr). MS spectra were obtained on a Kratos MS-9/50 spectrometer in the electron impact mode (EI, 70 eV). Elemental analyses were performed on a Carlo Erba 1106 instrument.

Preparation of 5-(4-aminophenyl)-4-substituted-2,4-dihydro-3H-1,2,4-triazole-3-thiones [1a-d]

Ethyl p-(benzoylamino)benzoate was prepared from benzoyl-chloride (0.03 mol in diethylether) and ethyl p-aminobenzoate (0.03 mol in diethylether). The product was filtered, washed with water and recrystallized from ethanol. Ethyl p-(benzoylamino)benzoate (0.011 mol) and hydrazine-hydrate (0.185 mol, 80%) were heated at 110-130°C for 45 min.

Ethanol (10 ml) was added to this mixture and refluxed for 1 h. The separated solid was filtered, washed with water and dried. *p*-(Benzoylamino)benzoic acid hydrazide (0.01 mol) in ethanol (120 ml) was heated for 15 min. The appropriate isothiocyanate (0.01 mol) was added and the mixture was refluxed for 2 h. The crystalline product was filtered and recrystallized from ethanol /4/. 1-Aroyl-4-alkyl/aryl thiosemicarbazides (0.01 mol) were refluxed in 15 ml 2N NaOH for 4 h. On cooling, the sodium salt precipitated. The precipitate was

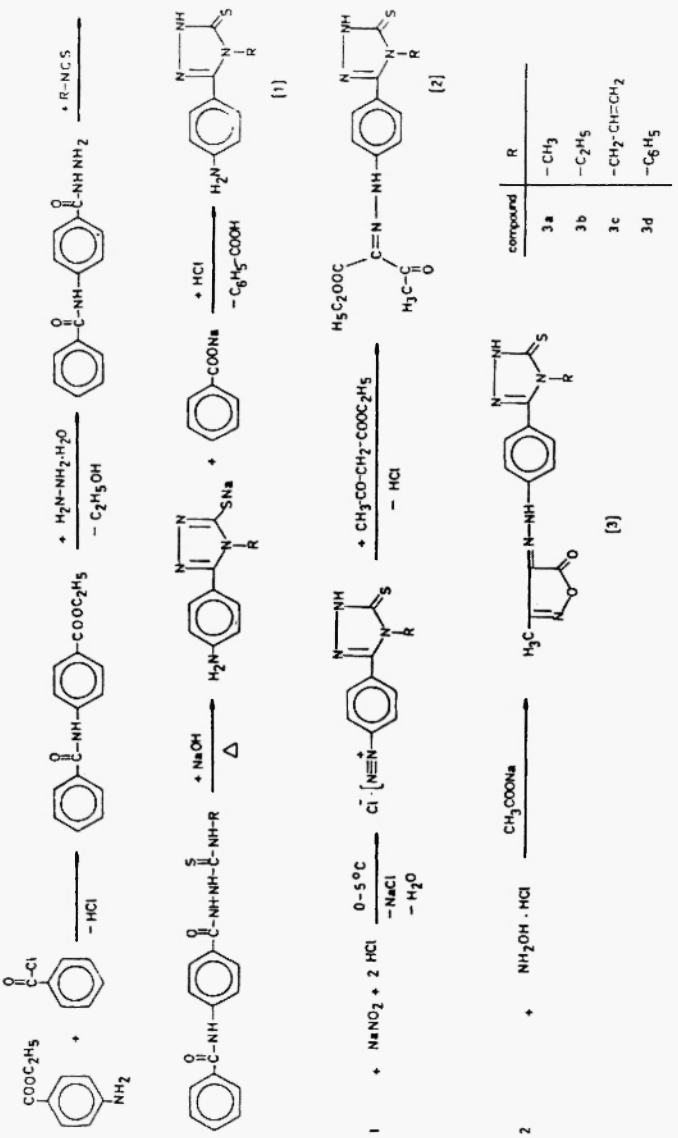

Fig. 1: Synthesis of 3-methyl-4-[(2,4-dihydro-4-substituted-3H-1,2,4-triazole-3-thione-5-yl)phenylhydrazono]-5-isoxazolone.

dissolved in water and acidified with hydrochloric acid (37%). The product was washed with water and recrystallized from ethanol /2/.

Synthesis of the coupling products obtained from diazonium salts of 5-(4-aminophenyl)-4-substituted-2,4-dihyro-3H-1,2,4-triazole-3-thiones and ethylacetoacetate [2a-d]

An ice-cold solution of 10 ml 10% sodium nitrite was added to a cooled solution of compounds 5-(4-aminophenyl) 4-substituted-2,4-dihydro-3H-1,2,4-triazole-3-thione **[1a-d]** (0.01 mol) in 3-5 ml of hydrochloric acid (37%). The reaction mixture was poured into an ice-cold mixture of 1 ml ethylacetoacetate, 50 g sodium acetate, 50 ml 50% ethanol and allowed to stand in a refrigerator for 24 h. The product was isolated by filtration, washed with water, dried and recrystallized from ethanol.

Synthesis of 3-methyl-4-[(2,4-dihydro-4-substituted-3H-1,2,4-triazole-3-thione-5-yl)phenylhydrazono]-5-isoxazolones [3a-d]

The compounds **2a-d** (0.002 mol) were refluxed with hydroxylamine hydrochloride and excess sodium acetate in boiling aqueous ethanol for 3 h. The product was washed with water and ethanol.

3-Methyl-4-[(2,4-dihydro-4-methyl-3H-1,2,4-triazole-3-thione-5-yl)phenylhydrazono]-5-isoxazolone **[3a]** (74%): M.p. 200-210°C; UV 365, 269 nm; IR 3104, 1711 cm^{-1}; Analysis calculated for $C_{13}H_{12}N_6O_2S.1/2H_2O$ 48.00 (C), 4.00 (H); found 48.20 (C), 3.99 (H).

3-Methyl-4-[(2,4-dihydro-4-ethyl-3H-1,2,4-triazole-3-thione-5-yl) phenylhydrazono]-5-isoxazolone **[3b]** (74%): M.p. 220°C; UV 367, 271 nm; IR 3424, 1718 cm^{-1}; Analysis calculated for $C_{14}H_{14}N_6O_2S.$ $1/2H_2O$ 49.56 (C), 4.42 (H), 24.78 (N); found 50.15 (C), 4.25 (H), 24.55 (N).

3-Methyl-4-[(2,4-dihydro-4-allyl-3H-1,2,4-triazole-3-thione-5-yl) phenylhydrazono]-5-isoxazolone **[3c]** (70%): M.p. 212°C; UV 363, 270 nm; IR 3384, 1701 cm^{-1}; Analysis calculated for $C_{15}H_{14}N_6O_2S.$ $1/2H_2O$ 51.28 (C), 4.27 (H); found 50.68 (C), 4.18 (H); MS (EI) m/e 342 (M^+), m/e 44 (100%, base peak).

3-Methyl-4-[(2,4-dihydro-4-phenyl-3H-1,2,4-triazole-3-thione-5-yl) phenylhydrazono]-5-isoxazolone **[3d]** (76%): M.p. 170-180°C; UV 384, 269 nm; IR 3422, 1701 cm^{-1}; Analysis calculated for $C_{18}H_{14}N_6O_2S$ 57.14 (C), 3.73 (H); found 57.22 (C), 3.87 (H).

Testing for antimicrobiological activity

The *in vitro* antimicrobial activity of the synthesised compounds was tested using an agar diffusion method /11/. Saboraud dextrose agar (DIFCO), Saboraud dextrose broth (DIFCO), Mueller-Hinton broth (DIFCO) and Mueller-Hinton agar (DIFCO) were used. The microorganisms used in these experiments were as follows: *P. mirabilis* ATCC 14153, *S. aureus* ATCC 6538, *P. aeruginosa* ATCC 1539, *K. pneumoniae* ATCC 4352, *E. coli* ATCC 11229, *C. krusei* KUEN 1001, *C. tropicalis* KUEN 1021, *C. pseudotropicalis* KUEN 1012, *C. utilis* KUEN 1031, *C. albicans* ATCC 10231. Overnight fresh cultures of 10^7 microorganisms/ml of strain dilutions were inoculated in these media. The synthesised compounds were dissolved in DMSO (4 mg/ml) and placed in the indicated agar media (100 μl). The incubation time was 24 h for bacteria and 48 h for *Candida* species at 37°C. Fluconazole and ampicillin were used as standards to compare with the test compounds for fungal strains and bacteria respectively. Measurements of the inhibition zones (mm) were compared with the diameter of the DMSO zone. The results were obtained by subtracting the values of control experiments (with only DMSO) from the values of test experiments.

RESULTS AND DISCUSSION

5-(4-Aminophenyl)-4-substituted-2,4-dihydro-3H-1,2,4-triazole-3-thiones were diazotised and coupled with ethylacetoacetate. The coupling products were treated with hydroxylamine hydrochloride and an excess of sodium acetate in boiling aqueous ethanol. 3-Methyl-4-[(2,4-dihydro-4-substituted-3H-1,2,4-triazole-3-thione-5-yl) phenyl-hydrazono]-5-isoxazolone derivatives were synthesised by methods described previously /3,5-10/. The structures of these compounds were elucidated by their UV, IR, MS spectral data and elemental analyses. In the UV spectra of compounds **3a-d** -N=N- bond absorption (at about 400 nm) was not seen. The UV spectra of these compounds exhibited a hydrazone (-HN-N=C-) group at 362-366, 384 nm /12/. For this reason, the structure of these compounds has been given in the form of the hydrazone. Compound **3d** which contains a phenyl group showed a bathochromic shift. IR spectra of compounds **3a-d** showed the characteristic absorption of hydrazone (N-H, C=O), triazoline (N-H, C=N), lacton (C=O), thiolactam (C=S) and aromatic (C=C) bonds.

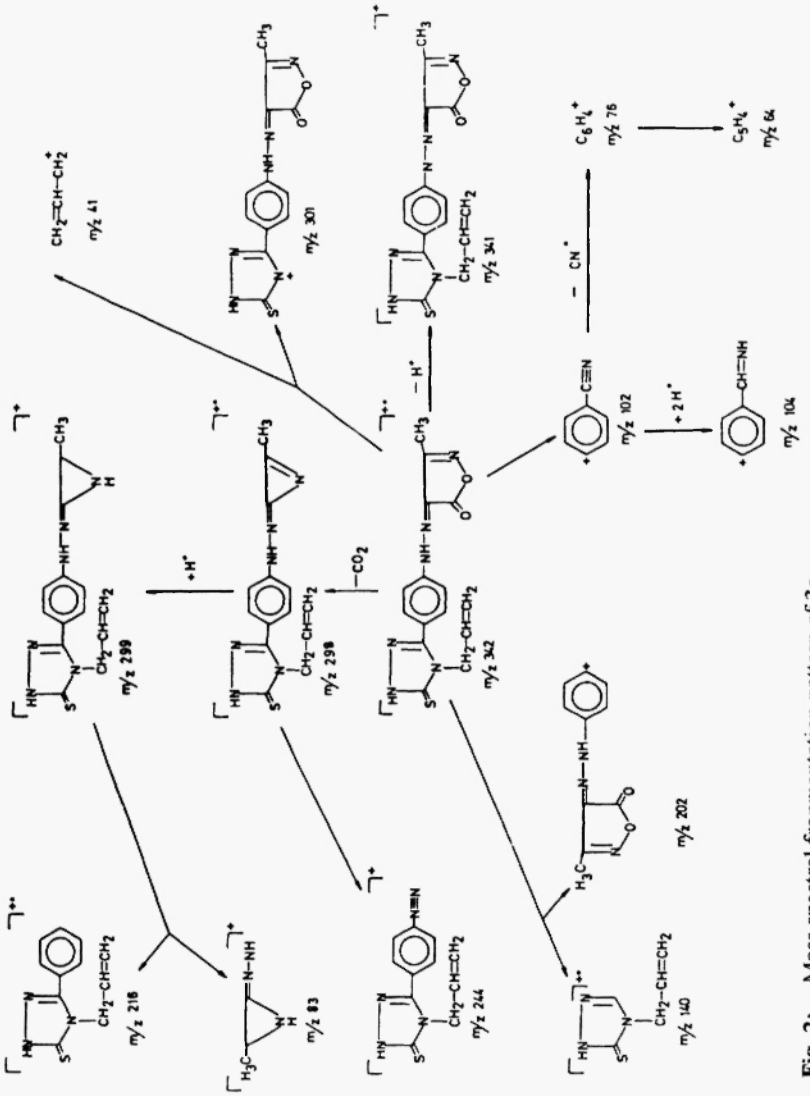

Fig. 2: Mass spectral fragmentation pattern of 3c

TABLE 1

Antimicrobial activity of **2a-d** and **3a-d**

	2a	3a	2b	3b	2c	3c	2d	3d	Ampicillin	Fluconazole
P.mirabilis ATCC 14153	–	–	–	1	–	–	–	1	30	
S.aureus ATCC 6538	–	–	–	2	–	–	–	7	28	
P.aeruginosa ATCC 1539	–	1	6	8	–	1	–	3	29	
K.pneumoniae ATCC 4352	–	–	–	–	–	–	–	–	5	
E coli ATCC 11229	–	–	–	–	–	–	–	–	30	
C.krusei KUEN 1001	6	18	6	10	–	–	–	–		7
C.tropicalis KUEN 1021	7	11	8	10	2	6	6	9	.....	26
C pseudotropicalis KUEN 1012	1	5	2	8	–	4	1	5		15
C.utilis KUEN 1031	2	13	1	3	2	7	–	–		–
C.albicans ATCC 10231	2	4	18	21	–	16	4	10		10

(Diameter of inhibition zones [mm])

These are in line with values given in the literature /2,3,7,13,14/. The m/z 342 peak in the MS spectrum confirmed the molecular weight of **3c** (Fig. 2).

An agar diffusion method /11/ was employed for the *in vitro* study of antifungal and antibacterial effects against the fungal and bacterial strains mentioned above. The antibacterial and antifungal effects of compounds **2a-d** and **3a-d** are shown in Table 1. The antimicrobial activity of compounds **3a-d** was stronger than that of compounds **2a-d**.

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