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Synthesis, characterization and fungicidal activity of N-(5-oxo-3,7-diaryl-6,7-dihydro-*5H*-thiazolo [3,2,a] pyrimidin-6-yl) benzamide derivatives

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ABSTRACT

2-Amino-4-aryl thiazoles and their derivatives have long been used as precursors for the synthesis of biologically active molecules. Therefore new antifungal compounds ***IV(a-e)*** *have been synthesized incorporating 2-Amino-4-aryl thiazole. The title compounds were synthesized through isolable intermediates. All the synthesized compounds have been characterized by their elemental analysis,* ^{1}*HNMR and Mass spectra. The synthesized compounds were evaluated in vitro for their antifungal activity against the test fungi by poisoned food technique; at 1000, 100 and 10 ppm using Czapek's agar medium. A standard commercial fungicide Dithane M-45 was also tested under similar conditions for comparison. It was found that all the compounds were antifungal active but amongst them* ***IVd*** *displayed fungicidal action comparable with that of commercial fungicide Dithane M-45.*

Key words: 2-Amino-4-aryl thiazoles, Antifungal, Dithane M-45, Commercial fungicide.

INTRODUCTION

Many important biochemical compounds and drugs of natural origin contain heterocyclic rings. Heterocyclic compounds containing nitrogen and sulphur possess potential pharmacological activities [1, 4]. The structural and therapeutic diversity coupled with commercial viability of small heterocyclic molecules has fascinated organic and medicinal chemists. So, a great deal of research is carried out in the field of heterocycles containing sulphur and nitrogen, because of their immense biological importance [5]. Heterocycles like thiazole plays a vital role owing to its wide range of therapeutic activities, 2-amino thiazole derivatives are widely used as pharmaceuticals. For example, Talipexol [6] and Promipexole [7] with a 2-amino thiazole moiety are used as Antiparkinsonian drugs and dopamine agonists, Tigemonam [8] is an antibacterial drug and Amthamine [9] is known as antiasthamatic one. It is also known that heterocyclic compounds with free amino groups may exhibit teratogenic and mutagenic properties because of their ability to form non-covalent complexes with DNA [10, 11]. That is why 2-aminothiazole derivatives may be of interest as potentially less toxic drugs with a wide variety of pharmacological activities.

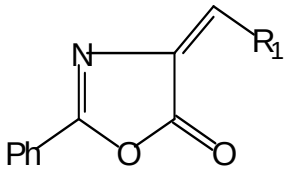
Hence, the objective of the present work is to synthesize and characterize the derivatives of new heterocyclic compounds and to evaluate their antifungal activity.

MATERIALS AND METHODS

With the hope of achieving new antifungal heterocyclic compounds and in view of the study of relationship between their chemical structure and fungitoxicity, new heterocyclic compounds have been synthesized. All the synthesized compounds have been characterized by their elemental analyses, ^{1}HNMR and mass spectra. ^{1}HNMR spectra were recorded on a Varian EM-360 (60MHz), Perkin-Elmer R-32 (90MHz) spectrophotometer in $CDCl_3$, DMSO-d_6, and $CDCl_3$ plus DMSO-d_6 using TMS as internal reference; chemical shifts are expressed in δ. Mass spectra were

recorded on a JEOL D-300 Mass spectrometer. Melting points were determined by open glass capillary methods and are uncorrected.

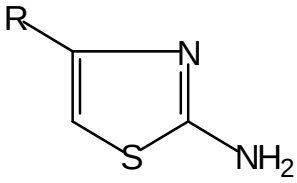
4-Arylidene-2-aryl-5-oxazolones:



Ia

A mixture of benzaldehyde 13.25g (0.125mol), hippuric acid 22.38g (0.125mol), acetic anhydride 35ml and anhydrous sodium acetate 10.25g (0.125mol) were heated in a 500ml conical flask on an electric hot plate with constant stirring. After the mixture became homogeneous, the flask was heated on a water bath for 2 hr. Then 50ml of alcohol was slowly added to the contents of the flask and it was allowed to stand overnight. The yellow crystalline product Ia thus formed was washed with cold and hot water respectively, then dried and recrytallised from ethanol, m.p. 166°C (reported mp 167°C), yield 80%. Similarly compounds I(b-c) were synthesized following the same procedure.

2-Amino-4-aryl thiazole:

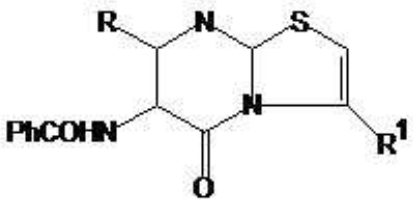


IIa

Thoroughly mixed aromatic ketone 8ml (100mmol), thiourea 10g (200mmol), iodine 17g (200 mmol) and a few drops of 1,4-dioxane and the mixture was heated for 8 hr at 80- 90°C. The mass obtained was triturated with ether for a period of 12 hr. Ether was then decanted off and the solid obtained was washed with a solution of sodium thiosulphate (3.0% w/v) and finally with cold water. The residue left was dissolved in boiling water, filtered, cooled and then treated with ammonia solution until basic. The resulting solid was washed and recrystallized from dimethyl sulfoxide-H_2O (1 : 1 v/v) and finally purified by silica gel column chromatography (benzene-MeOH, 8 : 2 v/v) yielding analytically pure compound IIa yield (70%), m.p.87-89°C. Similarly compounds II(d-e) were synthesized following same procedure.

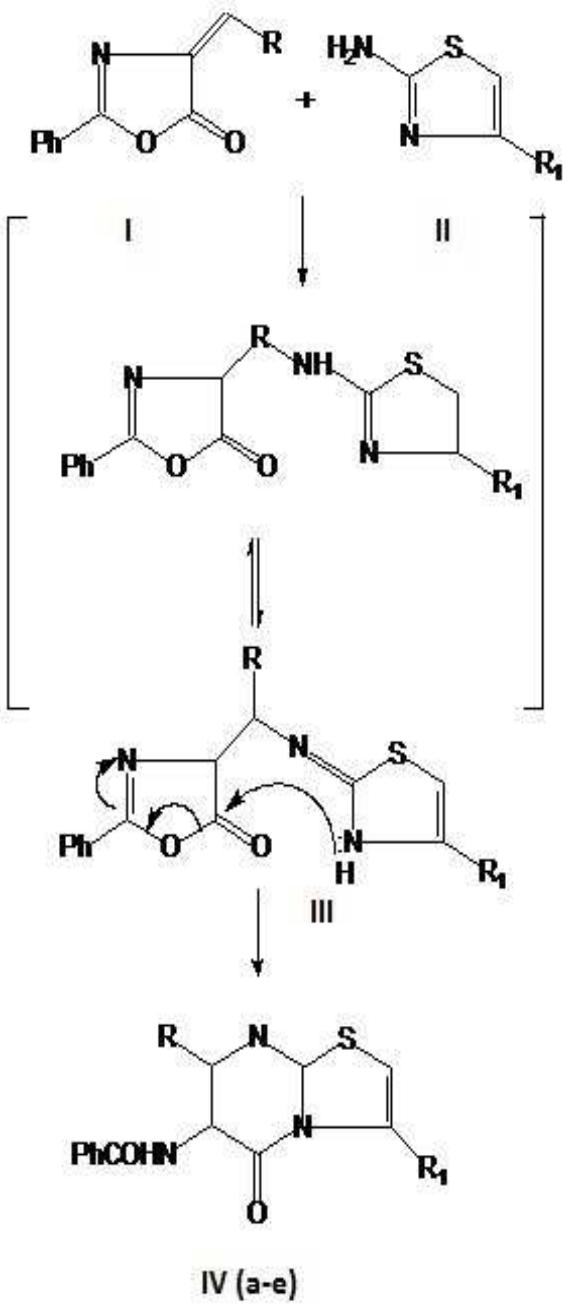
N-(5-oxo-3,7-diphenyl, 6,7-dihydro-5H-thiazolo [2,3-a] pyrimidin-6-yl) benzamide IV(a-e)

Ia (1 mol) and IIa (1 mol) were dissolved in ethyl alcohol and the mixture was refluxed on water bath for 7-8hrs to give Michael adduct (III a). This Michael adduct (III a) was dissolved in benzene and 10% H_2SO_4 was added, the mixture was then refluxed on water bath for 7-8 hrs to get the yellow solid which was recrystallised from ethanol. Similarly compounds IV(b-e) were synthesized following the same procedure.



IV (a-e)

Scheme –I



IV (a-e)

IVa. R = C_6H_5, R^1= C_6H_5
IVb. R = C_6H_5, R^1 = p-$CH_3OC_6H_4$
IVc. R = C_6H_5, R^1 = p-ClC_6H_4
IVd. R = p-$CH_3OC_6H_4$, R^1 = C_6H_5
IVe. R = p-ClC_6H_4, R^1 = C_6H_5
IV a- N-(5-oxo-3,7-diphenyl-6,7, dihydro-5H-thiazolo[3,2-a] pyrimidin-6-yl)-benzamide.
IV b- N-[3-(4-methoxy-phenyl)-5-oxo-7-phenyl-6,7-dihydro-5H-thiazolo[3,2-a] pyrimidin-6-yl]-benzamide
IV c- N-[3-(4-chloro-phenyl)-5-oxo-7-phenyl-6,7-phenyl-6,7-dihydro-5H-thiazolo [3,2-a] pyrimidin-6-yl]-benzamide
IV d- N-[7-(4-methoxy-phenyl)-5-oxo-3-phenyl-6,7-dihydro-5H-thiazolo[3,2-a] pyrimidin-6-yl]-benzamide
IV e- N-[7-(4-chloro-phenyl)-5-oxo-3-phenyl-6,7-dihydro-5H-thiazolo[3,2-a] pyrimidin-6-yl]-benzamide

Table No. 1. Elemental analysis, molecular formula, melting point and yield of the synthesized compounds IV(a-e)

Compound No.	Yield %	M.P. °C	Molecular Formula	Found (Calcd.)%		
				C	H	N
IV a	76	165-167	$C_{25}H_{19}N_3O_2S$	70.6 (70.8	4.5 4.6	9.8 9.9)
IV b	75	224-226	$C_{26}H_{21}N_3O_3S$	68.5 (68.6	4.6 4.6	9.2 9.4)
IV c	80	151-152	$C_{25}H_{18}ClN_3O_2S$	65.2 (65.4	3.9 4.0	9.1 9.3)
IV d	78	176-177	$C_{26}H_{21}N_3O_3S$	68.5 (68.7	4.6 4.8	9.2 9.4)
IV e	80	176-178	$C_{25}H_{18}Cl\ N_3O_2S$	65.2 (65.3	3.9 4.0	9.1 9.2)

Table No. 2. Spectral data of the synthesized compounds IV(a-e)

Compound No.	Molecular formula	MS m/z (M^+)	^{1}H NMR
IV a	$C_{25}H_{19}N_3O_2S$	425	7.21-7.95 (15H,m, ArH, aryl pyrimidine, aryl thiazole), 8.0 (1H, s, NH), 6.0 (1H, s, -CH-thiazole), 3.6 (1H, d, -CH-pyrimidine), 4.9 (1H, d, -CH-pyrimidine)
IV b	$C_{26}H_{21}N_3O_3S$	455	6.72-7.95 (14H,m, ArH, aryl pyrimidine, aryl thiazole), 8.0 (1H,s,NH), 6.0 (1H, s, -CH-thiazole), 3.6 (1H,d-CH-pyrimidine), 4.9(1H,d,-CH-pyrimidine), 3.73 (3H,s, OCH_3-thiazole)
IV c	$C_{25}H_{18}ClN_3O_2S$	459	7.12-7.95 (14H, m, ArH, aryl pyrimidine, aryl thiazole), 8.0 (1H,s, NH), 6.0 (1H,s,-CH-Thiazole), 3.6 (1H,d,-CH-pyrimidine), 4.9 (1H,d,-CH-pyrimidine).
IV d	$C_{26}H_{21}N_3O_3S$	455	6.72-7.95 (14H,m, ArH, aryl pyrimidine, aryl thiazole), 8.0 (1H, s, NH), 6.0 (1H, s, -CH-thiazole), 3.6 (1H, d, -CH- pyrimidine), 4.9 (1H,d,-CH-pyrimidine), 3.73 (3H,s, OCH_3-pyrimidine)
IV e	$C_{25}H_{18}Cl\ N_3O_2S$	459	7.06-7.95 (14H, m, ArH, aryl pyrimidine, aryl thiazole), 8.0 (1H, s, NH), 6.0 (1H, s, -CH-thiazole), 3.6 (1H, d, -CH- pyrimidine) 4.9 (1H, d, -CH- pyrimidine)

Evaluation of fungal toxicity

Organism: One weak old cultures of *Aspergillus niger* and *Fusarium oxysporum* were used.

Medium: The following synthetic Czapek's agar medium has been used:

Agar	-	15.00g
Sucrose	-	30.00g
Sodium nitrate	-	3.00g
Dipotassium hydrogen phosphate	-	1.00g
Potassium chloride	-	0.50g
Magnesium sulphate	-	0.500g
Ferrous sulphate	-	0.01g
Distilled water	-	1 litre

The antifungal activity of each compound was evaluated at three different concentrations, viz., 1000, 100 & 10ppm. The compounds were tested either as a solution or suspension in acetone-water (20:80, V/V) mixture.

Standard solutions or suspensions of different concentrations of each compound, viz., 10, 100 and 1000 ppm were prepared in acetone-water (20:80, V/V) mixture. One millilitre of each concentration of the test compound was added separately to presterilized Petri plates containing 9ml of the sterilized Czapek's agar medium to maintain the final concentrations of 1000, 100 and 10 ppm. The compound was thoroughly mixed with the medium by rotating the plates on table top, thus swirling the contents. A fungal disc of 5mm diameter, cut out with the help of a sterilized cork borer from the periphery of one week old culture of the test fungus already planted on the Czapek's medium, was inoculated in the centre of each petriplate in inverted position to bring the mycelia in direct contact with the medium. Petriplates containing 9ml of Czapek's medium and 1ml acetone-water (20:80, V/V) mixture served as controls. The number of replicate assays in each case was three, whereas six replications of the controls were provided. The plates were incubated at 28°C (± 1°C) for 96 h. No remarkable morphological change was observed in the developing fungi.

A commercial fungicide, **Dithane M-45** (Manganous ethylene bisdithiocarbamate with Zinc ions) was also tested under similar conditions for comparing the results.

For the highly active compounds, it was ascertained whether these were fungistatic or fungicidal. Thus, following the procedure of Garber and Houston [12], the compounds were added separately to Czapek's agar medium in

different petric dishes to maintain the final concentrations at their respective lethal doses. The test fungi were inoculated in the centre of these petri dishes and incubated at 28°C (± 1°C) for 96 hr, after which time the percent inhibition of mycelial growth compared with that in control dishes was recorded. Then the fungal discs were taken out from the treated and control dishes, washed with sterilized double - distilled water, and reinoculated in fresh petri dishes containing Czapek's agar medium only. The plates were incubated for 96 hr at 28°C (± 1°C), and the percent inhibition was recorded. The number of replicate assays in each case was three and six replicate controls were used. It was found that the fungicidal compounds caused complete inhibition of mycelial growth of the test fungi in treated as well as reinoculated dishes.

Expression of Inhibition

After 96 hr, four diameters of fungal colony intersecting one another at about 45°C, were measured by means of a millimeter scale. Inhibition in fungal growth was determined as the difference in growth between control plates and those treated with test compound. The percentage inhibition of mycelial growth was calculated by the following equation:

$$\% \text{ Inhibition} = \frac{(C - T) \times 100}{C}$$

Where, C = Average diameter of fungal colony (in mm) in control plates.
T = Average diameter of fungal colony (in mm) in treated plates.

Table No. 3. Fungicidal screening data of synthesized compounds IV(a-e)

Compound No.	Average % Inhibition against					
	A. niger at			*F. oxysporium at*		
	1000ppm	100ppm	10ppm	1000ppm	100ppm	10ppm
IV a	72	41	33	71	40	31
IV b	75	46	37	72	43	31
IV c	78	49	38	70	45	36
IV d	88	56	43	87	52	41
IV e	75	49	38	72	45	36
Dithane M-45 (commercial fungicide)	100	83	67	100	85	68

RESULTS AND DISCUSSION

The new heterocyclic compounds (**IVa-e**) were synthesized as outlined in scheme 1. The structure of the compounds was deduced on the basis of spectroscopic methods shown in Table No. 2. These compounds have been screened for their antifungal activity against two fungal species, viz., *Aspergillus niger* and *Fusarium oxysporum*. The screening results have been correlated with structural features of the tested compounds is shown in Table No. 3.

It was found that all the compounds were antifungal active but amongst them **IVd** displayed fungicidal action comparable with that of commercial fungicide Dithane M-45.

CONCLUSION

The antifungal action may not be numerical sum of several toxophoric group , perhaps in a congregation of such toxophoric functions, the role of only a few key factors is apparently important. All compounds are antifungal since they inhibit the growth of both the fungus viz; *Aspergillus niger* and *Fusarium oxysporum* in the range of 43-100% at 1000ppm concentration. It was also founded that phenyl nucleus was less toxic than substituted phenyl (i.e. bearing chloro and methoxy groups).

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REFERENCES

[1] A. R. Katrizky, Advances in Heterocyclic Chemistry Academic Press, London, **1985**, 135.
[2] G. Proto and R. H. Thomson, Endeavour **1976**, 35, 32.
[3] C. Faria, M. Pinza, A. Gabma and G. Piffen, *Eur. J. Med. Chem. Chim. Ther.*, **1979**, 14, 27.
[4] J. J. Roberts and G. P. Warwich, *Biochem. Pharmacol*., **1963**, 12, 135.
[5] G. K. Rao, S. Rajasekaran and M. Attimarad, *Ind. J. Pharm. Sci.*, **2000**, 475.

[6] C. Pifl, L. Pichler, W. Kobinger and O. Hornykiewicz, *Eur. J. Pharmacol.*, **1998**, 153, 33.
[7] C. S. Schmeider and J. Mierau, *J. Med. Chem.*, **1987**, 30, 494.
[8] J. M. Clark, S. J. Olsen, D. S. Weinberg, M. Dalvi, R. R. Whitney, D. P. Bonner and R. B. Sykes, *Antimicrobial Agents Chemother.*, **1987**, 31, 226.
[9] J. C. Eriks, H. Vandergoot and G. J. Sterk, H. Timmerman, *J. Med. Chem.*, **1992**, 35, 3239.
[10] C. E. Voogd, J. J. Vander Stel and H. W. Verharen, *Mutat Res.* **1983**, 118, 153.
[11] J. C. Greenaway, A. G. Fantel and M. R. Juchau, *Toxicol. Appl. Pharmacol*., **1986**, 82, 307.
[12] R. H. Garber and B. R. Houston, *Phytopathology*, **1959,** 49, 449.