SYNTHESIS AND BIOLOGICAL ACTIVITY OF 3-ARYL-1-(3-METHYL-5-STYRYL-4-ISOXAZOLYL)-2-OXO-(1H,3H,5H)-PYRIMIDINE-4,6-DIONES

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Abstract: A Series of 3-aryl-1-(3-methyl-5-styryl-4-isoxazolyl)-2-oxo-(1*H*,3*H*,5*H*)-pyrimidine-4,6 diones **3** have been prepared from 1-aryl-3-(-3-methyl-5-styryl-4-isoxazolyl)ureas **2** by heating with malonic acid in acetyl chloride. Compounds **2** are obtained by interaction of 4-amino-3-methyl-5 styrylisoxazole **1** with aryl isocyanates. The analytical and spectral data agree well with the structuresof **2** and **3**. Antibacterial and antifungal activity of compounds **2** and **3** have been studied.

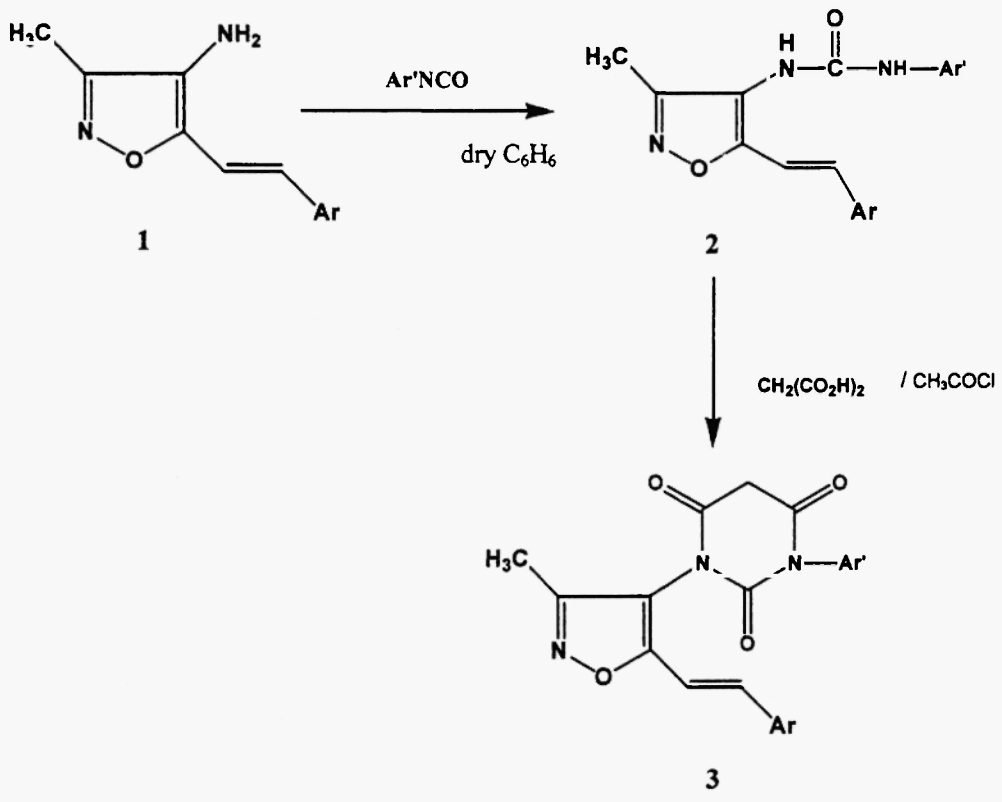
Introduction

Heterocycles are widely utilised compounds in both pharmaceutical and agricultural fields[1]. Consequently the development of methodologies useful for the assembly of molecules containing heterocyclic templates continues to attract the attention of both the academic and industrial communities. Among aromatic heterocycles, the isoxazole unit constitutes an easly accessible nucleus that is present in a number of natural and pharmacological compounds[2].Similarly, the pyrimidine ring is also associated with wide ranging physiological activity[3]. Literature survey revealed that when one biodynamic heterocyclic system was coupled with another,a molecule with enhanced biological activity [4,5] was produced. The chemistry of these linked biheterocycles has been the fascinating field of investigation in medicinal chemistry as they have been found to exhibit enhanced biological profile[6]. Prompted by these observations and in continuation of our interest in the synthesis of substituted biheterocycles[7], it was thought worthwhile to synthesize and investigate the activity of the compounds in which isoxazole moiety has been linked with pyrimidine nucleus. We report in this paper, the synthesis and antimicrobial activity of isoxazolyl pyrimidinediones.

Results and Disussion

The reaction of 4-amino-3-methyl-5-styrylisoxazole[8] **1** obtained from the corresponding nitroisoxazole, with aryl isocyanates in dry benzene led to the formation of 1-aryl-3-(3-methyl-5-styryl-4-isoxazolyl)-ureas **2** in 60-80% yields. IR spectrum of **2a** showed absorption peaks at 1660 and 3280 cm^{-1} due to carbonyl and amide groups. ^1H NMR spectrum of **2** supported the formation of urea derivatives by showing two broad singlets at δ 7.8 and 8.5 due to NH ptotons which are D_2O exchangeable. Mass spectrum of **2a** showed molecular ion peak at m/z 319.The cyclocondensation of malonic acid with **2** has been carried out in acetyl chloride for 4hr. at 40°c. The resulting products have been identified as 3-aryl-1-(-3-methyl-5-styryl-4-isoxazolyl)-2-oxo-(1*H*,3*H*,5*H*)-pyrimidine-4,6-diones**3.** RSpectrum of **3a** showed absorption peaks at 1720 and 1660 cm^{-1} for two >C=O functional groups. ^1H NMR of **3a** in contrast to that of **2a,** showed the absence of broad signals at δ 7.8 nd 8.5. This suggested that the expected cyclocondensation has taken place between the urea-hydrogens and malonic acid to yield **3a.** Methyl protons of isoxazole appeared as a singlet at δ2.4, where as methylene protons of pyrimidine ring showed up at δ3.8 as

a singlet. The aromatic protons appeared as a complex multiplet between δ7.0-7.8. The mass spectrum of **3a** showed the molecular ion at m/z 387(M^+).



2&3 Ar = C_6H_5, C_6H_4-CH_3(p), $C_6H_3Cl_2$(2,4),C_6H_4-OCH_3(p), C_6H_4-OCH_3(o). Ar' = C_6H_5, C_6H_4- OCH_3(p), C_6H_4-Cl(p), $C_6H_5CH_2$.

Scheme-1

Antibacterial activity

The antibacterial activity of isoxazolyl ureas **2** as well as isoxazolyl-2-oxo-pyrimidine-4, 6-diones **3** has been evaluated following filter paper disc technique of Vincent and Vincent[9]. Four bacteria, namely *Escherichia coli* and *Proteus Vulgaris* (gram-negative) and *Bacillus subtilis*, *Staphylococcus aureus* (gram- positive) have been used as test organisms. In general, appreciable activity is exhibited by isoxazolyl pyrimidine-4,6-diones **3** compared to isoxazolyl ureas **2**. In isoxazolyl pyrimidine-4,6-diones, remarkable activity is exhibited by phenyl (**3a**) and p-methoxyphenyl (**3d**) substituted compounds, which inhibited growth of all the bacteria to a significant extent and other compounds in this series are moderately active. In isoxazolyl ureas, most of the compounds exhibited moderate activity. The antibacterial activity of the compounds **2** and **3** were compared with known standard drug *streptomycin*.

Antifungal activity

The antifungal activity of isoxazolyl ureas **2** and isoxazolyl pyrimidine-4,6- diones **3** was evaluated by glass slide humid chamber techinique[10]. *Fusarium oxysporum* schl. and *Curvularia lunata* (walker) Boed. were used as test organisms. Among isoxazolyl pyrimidine- 4,6- diones, p-tolyl (**3b**) and p-methoxy (**3d**) compounds exhibited strong

fungicidal activity, which was lethal even at low concentration (480 μg/mL) and other compounds also inhibited the spore germination to a significant level. In isoxazolyl ureas **2,** most of the compounds inhibit the spore germination of both the fungi to a reasonable extent. The antifungal activity of the compounds **2** and **3** were compared with known drug *Carbendazim.*

Conclusion

In conclusion ,isoxazolyl pyrimidine-4,6- diones **3a** and **3d** exhibited maximum activity by inhibiting growth of all the four bacteria to a greter extent in comparison with standard drug *Streptomycin.* Compounds **3b** and **3d** are highly toxic towords both the fungi under investigation and are lethal even at low concentration in comparison with standard carbendazim at the same concentration . Hence compounds **3a,3b** and **3d** may be exploited for formulation of bacterocide and may be exploited for control of wilt diseases of different crops as fungicides.

Experimental

Melting points were determined on a Cintex melting point apparatus and are uncorrected. The purity of the compounds was checeked by TLC. IR spectra was recorded in KBr (cm^{-1}) on a Perkin Elmer spectrum BX series FT-IR spectrometer, ^1H NMR spectra on a varian Gemini 200MHz spectrometer using tetramethyl silane as internal standard in $CDCl_3$ on δ scale and mass spectra on a Jeol JMC D-300 spectrometer. C, H and N analyses were carried out on a Carlo Erba 106 and Perkin Elmer model 240 analysers.

Preparation of 1-aryl-3-(-3-methyl-5-styryl-4-isoxazolyl) urea 2a-h:

4-amino-3-methyl-5-styrylisoxazole **1** (0.01mol) and phenyl isocyanate (0.01mol) were refluxed in dry benzene for 3hr. The reaction mixture was cooled, filtered, washed thoroughly with benzene and dried. The colorless solid thus obtained was purified by crystallization from benzene. Compounds **2a-h** were obtained in 70-85% yields and the analytical data of these compounds is included in **Table 1**.

Preparation of 3-aryl-1-(-3-methyl -5-styryl-4-isoxazolyl)-2-oxo-(1*H*,3*H*,5*H*)-pyrimidine-4,6-dione 3a-h:

A mixture of **2** (0.01mol) and malonic acid (0.01mol) was refluxed in acetyl chloride (20ml) for 4hr. at 40^0c. The reaction mixture was cooled and poured in to ice cold water. The solid that separated was collected and recrystallized from benzene-ethyl acetate. Compounds **3a-h** were obtained in 60-80% yields and the analytical data of these compounds is included in **Table 1**.

Spectra of representative compounds:

1-Phenyl-3-(-3-methyl-5-styryl-4-isoxazolyl)-urea 2a:

IR (KBr): 1640 (C=O), 3280 (NH) cm^{-1}; ^1H NMR (200MHz, $CDCl_3$) : δ 2.3(s,3H,CH_3) , 6.8 (d,1H,*J*=12Hz,CH=CH), 7.0(d,1H,*J*=12HZ,CH=CH), 7.1-7.6(m,10H ,Ar-H), 7.8(bs,1H,NH, D_2O exchangeable), 8.4(bs,1H,NH, D_2O exchangeable) ; MS: m/z 319(M^+).

1-Phenyl-3-(3-methyl-5-p- methylstyryl -4-isoxazolyl)-urea 2b:

IR(KBr) : 1640 (C=O) , 3280 (NH) cm^{-1}, ^1H NMR (200MHz, $CDCl_3$): δ2.2 (s,3H,CH_3), 2.4(s,3H,CH_3),6.8 (d,1H,*J*=12Hz,CH=CH) 7.0 (d,1H,*J*=12Hz,CH=CH),7.2-7.5 (m,9H,Ar-H),7.6(bs,1H,NH, D_2O exchangeable),8.1(bs,1H,NH, D_2O exchangeable) MS: m/z 333 (M^+).

1-Phenyl-3-(3-methyl-5-(2,4 dichloro styryl-)-4-isoxazolyl urea 2c:
IR (KBr): 1645 (C=O), 3275(NH) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 2.2 (s, 3H, CH_3), 6.8 (d, 1H, *J*=12Hz, CH=CH) 7.0(d,1H, *J*= 12Hz, CH=CH), 7.2-7.5 (m,8H,Ar-H), 7.8 (bs,1H,NH, D_2O exchangeable), 8.1(bs,1H,NH, D_2O exchangeable), MS m/z 387 (M^+).

1-p-Chlorophenyl-3-(3-methyl-5-(p-methyl styryl-4-isoxazolyl)-Urea 2e:
IR (KBr): 1650 (C=O), 3300(NH) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 2.2 (s, 3H, CH_3), 2.5 (s,3H,CH_3) 6.8 (d, 1H, *J*=12Hz, CH=CH) 7.0(d,1H, *J*= 12Hz, CH=CH), 7.3-7.6 (m,8H,Ar-H), 7.8 (bs,1H,NH, D_2O exchangeable), 8.6(bs,1H,NH, D_2O exchangeable), MS m/z 367 (M^+).

3-Phenyl-1-(-3-methyl-5-styryl-4-isoxazolyl)-2-oxo-(1*H*,3*H*,5*H*)-pyrimidine-4,6-dione)3a:
IR(KBr): 1715,1680(C=O) cm^{-1};^1H NMR (200MHz, $CDCl_3$); δ2.3(s,3H,CH_3), 3.8(s,2H,CH_2), 6.8(d,1H, *J*=12Hz, CH=CH) 7.0 (d,1H,*J*=12Hz, CH=CH),7.1-7.8(m,10H,Ar-H);Ms:m/z 387 (M^+).

3-Phenyl-1-(-3-methyl-5-p-methylstyryl-4-isoxazolyl)-2-oxo-(1*H*,3*H*,5*H*)-pyrimidine-4,6-dione)3b:
IR(KBr) 1720, 1700(C=O)cm^{-1};^1H NMR (200MHz, $CDCl_3$): δ2.2(S,3H,CH_3), 2.5 (s,3H, CH_3), 3.9 (s,2H,CH_2) 6.7(d,1H,*J*=12Hz, CH=CH), 6.9(d,1H,*J*=12Hz, CH=CH), 7.0-7.7 (m,9H,Ar-H),MS: m/z 401 (M^+).

3-Phenyl-1-(-3-methyl-5-(2,4-dichlorostyryl)-4-isoxazolyl)-2-oxo-(1*H*,3*H*,5*H*) pyrimidine-4,6-dione)3c:
IR(KBr): 1710, 1695(C=O)cm^{-1};^1H NMR(200MHz, $CDCl_3$):δ2.3(s,3H,CH_3),4.0(s,2H,CH_2), 6.8(d,1H,*J*=12Hz,CH=CH), 7.0(d,1H,*J*=12Hz,CH=CH),7.1-7.8 (m,8H,Ar-H); MS: m/z 455 (M^+).

3-p-Chlorophenyl-1-(3-methyl-5-(p-methylstyryl-4-isoxazolyl)- 2-oxo-(1*H*,3*H*,5*H*) pyrimidine-4,6-dione)3e:
IR(KBr): 1715, 1680(C=O)cm^{-1};^1H NMR(200MHz, $CDCl_3$):δ2.3(s,3H,CH_3), 2.5(s,3H,CH_3), 6.8(d,1H,*J*=12Hz,CH=CH), 6.9(d,1H,*J*=12Hz,CH=CH),7.0-7.6 (m,8H,Ar-H); MS: m/z 435 (M^+).

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Table 1 :Physical and analytical data of 1-Aryl-3-(-3-methyl-5-styryl-4-isoxazolyl urea) **(2a-h)**, and 3-aryl-1-(3-methyl-5-styryl-4-isoxazolyl)-2-oxo-(*1H,3H,5H)* -pyrimidine-4,6-dione) **(3a-h)**.

Compo-und	Ar	Ar'	m.p (°c)	Mol. Formula	Found (%) (Calcd).		
					C	H	N
2a	C_6H_5	C_6H_5	190	$C_{19}H_{17}N_3O_2$	71.42 (71.47	5.27 5.32	13.12 13.16)
2b	C_6H_4-$CH_{3(p)}$	C_6H_5	173	$C_{20}H_{19}N_3O_2$	72.04 (72.70	5.65 5.70	12.57 12.61)
2c	C_6H_3-Cl_2(2,4)	C_6H_5	210	$C_{19}H_{15}N_3O_2Cl_2$	58.87 (58.91	3.83 3.87	11.03 11.08)
2d	C_6H_4-OCH_3(p)	C_6H_4-OCH_3(p)	187	$C_{21}H_{21}N_3O_4$	66.53 (66.49	5.49 5.54	11.10 11.08)
2e	C_6H_4-$CH_{3(P)}$	C_6H_4-Cl(p)	198	$C_{20}H_{18}N_3O_2Cl$	65.33 (65.39	4.84 4.90	11.38 11.44)
2f	C_6H_4-$CH_{3(P)}$	C_6H_4-OCH_3(p)	165	$C_{21}H_{21}N_3O_3$	69.37 (69.42	5.71 5.78	11.52 11.57)
2g	C_6H_4-OCH_3(O)	$C_6H_5CH_2$	150	$C_{21}H_{21}N_3O_3$	69.35 (69.42	5.73 5.78	11.55 11.57)
2h	C_6H_3-Cl_2(2,4)	C_6H_4-Cl(p)	225	$C_{19}H_{14}N_3O_2Cl_3$	54.11 (54.51	3.27 3.32	9.91 9.97)
3a	C_6H_5	C_6H_5	215	$C_{22}H_{17}N_3O_4$	68.17 (68.21	4.31 4.39	10.81 10.85)
3b	C_6H_4-$CH_{3(P)}$	C_6H_5	180	$C_{23}H_{19}N_3O_4$	68.77 (68.82	4.68 4.73	10.42 10.47)
3c	C_6H_3-Cl_2(2,4)	C_6H_5	230	$C_{22}H_{15}N_3O_4Cl_2$	57.82 (57.89	3.22 3.28	9.16 9.21)
3d	C_6H_4-OCH_3(p)	C_6H_4-OCH_3(p)	195	$C_{24}H_{20}N_3O_6$	64.52 (64.57	4.43 4.48	9.38 9.41)
3e	C_6H_4-$CH_{3(P)}$	C_6H_4-Cl(p)	200	$C_{23}H_{18}N_3O_4Cl$	63.32 (63.37	4.08 4.13	9.60 9.64)
3f	C_6H_4-$CH_{3(P)}$	C_6H_4-OCH_3(p)	173	$C_{24}H_{20}N_3O_5$	66.94 (66.97	4.62 4.65	9.68 9.76)
3g	C_6H_4-OCH_3(O)	$C_6H_5CH_2$	165	$C_{24}H_{20}N_3O_5$	66.91 (66.97	4.60 4.65	9.72 9.76)
3h	C_6H_3-Cl_2(2,4)	C_6H_4-Cl(p)	263	$C_{22}H_{14}N_3O_4Cl_3$	53.92 (53.98	2.83 2.86	8.53 8.50)

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