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SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SOME NEW 3,5-DISUBSTITUTED PYRAZOLINES CONTAINING 1,2,4-TRIAZINE MOIETY

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Several new 3,5-disubstituted pyrazolines containing 5,6-diphenyl-1,2,4- triazin-3-yl moiety were prepared by condensation of 3-hydrazino-5,6-diphenyl- 1,2,4-triazine with benzoyl acetanilide derivatives, chalcons, ethyl cyanoacetate/acetic anhydride. These compounds were characterized by IR, PMR and UV spectral studies and also tested for their antimicrobial activity. Promising results were obtained which have been discussed.

Key words: Substituted pyrazoles, 1,2,4-Triazine, Antimicrobial.

Introduction

Recently, we reported [1-10] the synthesis and biological activity of a large number of 1,2,4-triazine derivatives which containing the indole, thiazolidinone, triazole and pyrazole ring systems as the heteroaromatic moiety. It was found that some pyrazole derivatives in this series exhibited antibacterial, antifungal, antimicrobial, anticancer and anti HIV activities. In continuation of our ongoing synthetic work on these biologically interesting heterocycle pyrazoles [4-6], we sought to prepare some new substituted pyrazoles containing the 1,2,4-triazine moiety and to evaluate their antimicrobial activity.

Experimental

Melting points reported are uncorrected. UV spectra were recorded in pure ethanol on a Perkin Elmer (Type 550 S) UV spectrophotometer (λ_{max} in nm), IR spectra were obtained as KBr discs on a Pye Unicam 1100 spectrophoto-meter (υ_{max} in cm^{-1}) and 60 MHz PMR spectra were obtained as DMSO-d_6 solution on an EM 360 NMR spectrometer using TMS as internal standard solvent (chemical shifts in δppm). Compounds II and IV were prepared accor-ding to a previously described methods [11].

Alkylation of benzoyl acetanilides Ia-d with 3-chloro-5,6-diphenyl-1,2,4-triazine (II): Formation of compounds IIIa-d. A mixture of Ia-d (0.01 mol) and 3-chloro-5,6-diphenyl-1,2,4-triazine (II) (0.01 mol) in DMF (50 ml) was heated under reflux for 1 hr, cooled, poured into ice and the solid obtained by filteration was crystallized from dil. DMF to give products IIIa-d (Table 1). UV(IIIa):300, 275, 255 and 215. IR(IIIa): 3155-3100 (NH,NH), 2600(OH enolic R-CH=CH(OH) NH), 1720(C=O, PhCO), 1665(C=O, R-CONH), 1440 (def. CH_2) and 1000, 985, 850 (Phenyl groups). PMR (IIIa): 3.5 (s, 2H, CH_2), 3.8 (s, 1H, = CH), 5.8-6(s, 1H,OH enolic), 7.0-7.8 (m, 19H, aromatic protons), 8.2 and 9.0 (each s, 1H, NH).

Cyclocondensation of 3-hydrazino-5,6-diphenyl-1,2,4-triazine (IV) with benzoyl acetanilides IIIa-d : Formation of 1-(5',6'-diphenyl-1',2',4'-triazin-3'-yl)-3-arylamino-5-phenyl-pyrazoles (Va-d) . A mixture of IIIa-d(0.01 mol) and IV(0.01 mol) in abs. ethanol (100 ml) were refluxed for 5 hrs, left overnight and diluted with cold water. The obtained solids were crystallized from the appropriate solvent to give products Va-d (Table 1). UV (Va): 285, 255 and 200. IR(Va):3200-3100 (b, NH), 1610-1580 (C=N) and 990, 890, 850 (aryl and phenyl groups). PMR (Va):5.0 (s,1H, = CH), 6.9-8.5 (m, 29H, aromatic protons) 11.1(s, 1H, NH) and 11.5 (s,1H,NH).

Reaction of 3-hydrazino-5,6-diphenyl-1,2,4-triazine (IV) with chalcones (VIa-d); Formation of 1-(5',6'-diphenyl-1',2',4'-triazin-3'-yl)-3-aryl-4-dihydro-5(H) arylpyrazolines (VIIa-d). A mixture of chalcones (VIa-d) (0.01 mol) and IV (0.01 mol) in abs. ethanol (100 ml), piperidine (one drop) were refluxed for 8-10 hrs, cooled and poured into ice-HCl. The obtained solids were recrystallized from the appropriate solvent to give products VIIa- d (Table 1). IR(VIId):3040 (aromatic CH), 2960, 2840) (aliphatic CH), 1610 (C=N), 1550(asy. NO_2), 1500, 1440-1410 (def. CH), 1350 (sy. NO_2), 1020, 960, 870 (aryl groups), 750 and 700 (C-Cl). PMR (VIId) 2.8 (s, 2H, CH_2), 3.9 (s, 1H, = CH Ar at position-5 of pyrazole ring) and at 7.1-7.7 (m, 17H, aromatic protons).

Oxidation of compounds VIIa-d: Formation of 1,3,5-trisubstituted pyrazolines (VIIIa-d). A suspension of VIIa-d (0.01 mol) in ethanol (100 ml) and aq. $FeCl_3$ (10%, 20 ml), was heated under reflux for 4 hrs, cooled and diluted with cold water. The resultant solid was collected filteration and recrystallized from the appropriate solvent to give VIIIa-d (Table 1). IR(VIIId):3060 (aromatic CH), 2940, 2840 (aliphatic CH), 1660 (C=C, endocyclic pyrazole), 1610 (C=N), 1550 (asy. NO_2), 1440 (def. CH), 1350 (sy. NO_2), 1020, 960, 920 (aryl groups), 760 and 700-690 (C-Cl). PMR (VIIId):3.9 (s, 1H, = CH of position-4 of pyrazole) and 7-7.8 (m, 17H, aromatic protons).

Reaction of compound IV with ethyl cyanoacetate/acetic anhydride: Formation of IX. A mixture of IV (0.01 mol) and

ethyl cyanoacetate (0.01 mol) in acetic anhydride (20 ml) was refluxed for 4 hrs, cooled and poured into ice. The obtained solid was recrystallized from the appropriate solvent to product IX (Table 1). IR: 3300-3100 (b, NH), 3060 (aromatic CH), 2940, 2860 (aliphatic CH), 2220 (C=N), 1750-1670 (b,C=O), 1610, 1580 (C=N), 1440 (def. CH), 1070 (C-O-C) and 1020, 920 (aryl groups).

Reaction of compound IX with piperidine : Formation of compound X. A mixture of IX (0.01 mol) and piperidine (1 ml) was refluxed for 2 hrs., cooled, the solid collected by filteration, washed with ether and crystallised from ethanol to give the pyrazolone derivative X (Table 1). IR: 3200 (NH), 3060 (aromatic CH), 2900, 2860 (aliphatic CH), 1710-1680 (C=O), 1570-1550 (C=N), 1440 (def. CH), 1080-1060 (C-O-C), 1020, 970 and 900 (aryl groups).

Biological screening . The *invitro* antibacterial and yeast activities of the selected compounds were tested by the diffusion method using the solid glycerine peptone medium against the gram +ve bacteria and the gram -ve bacteria as well as the yeast. The test organisms were subcultured on nutrient agar slants incubated at 37° for 24 hrs. One loopful of each test organism was suspended in 5 ml sterile distilled water. The melted Nutrient agar flasks (45°) were inoculated by the spore suspension (1 ml/100 ml), shaken well and then poured into plates. After solidification of the medium and four holes 0.8cm diameter were made in each plate and 0.2 ml of each tested compound (10 mg ml) was inserted in each hole incunat and then kept at 37° for 24 hrs in case of bacteria and 28° for 48 hrs in case of yeast. The average of inhibition clear zones diameter were calculated for each compound and recorded.

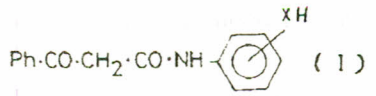
TABLE 1. CHARACTERIZATION DATA OF THE VARIOUS PREPARED COMPOUNDS.

Compd. No.	Solvent	m.p. (°C)	Yield (%)	Mol. formula*
IIIa	Dil. DMF	130-132	70	$C_{30}H_{23}N_5O_2$
IIIb	Dil. DMF	148-149	65	$C_{30}H_{23}N_5O_2$
IIIc	Dil. DMF	135-136	70	$C_{30}H_{23}N_5O_2$
IIId	Dil. DMF	121-122	70	$C_{30}H_{22}N_4S^{+}O_2$
Va	Dil. EtOH	145-146	60	$C_{45}H_{32}N_{10}$
Vb	Dil. EtOH	119-120	55	$C_{45}H_{32}N_{10}$
Vc	Dil. EtOH	198-200	60	$C_{45}H_{32}N_{10}$
Vd	DMF	109-110	70	$C_{45}H_{31}N_9S^{\ddagger}$
VIIa	EtOH	160-162	55	$C_{30}H_{20}N_5Cl_3$
VIIb	EtOH	245-247	67	$C_{30}H_{21}N_5Cl_2O$
VIIc	AcOH	198-200	60	$C_{31}H_{23}N_5Cl_2O$
VIId	AcOH	222-224	56	$C_{30}H_{20}N_6Cl_2O$
VIIIa	DMF	122-124	65	$C_{30}H_{18}N_5Cl_3$
VIIIb	EtOH	140-142	50	$C_{30}H_{19}N_5Cl_2O$
VIIIc	EtOH	158-160	60	$C_{31}H_{21}N_5Cl_2O$
VIIId	MeOH	130-132	45	$C_{30}H_{18}N_6Cl_2O_2$
IX	Dil.EtOH	198-200	57	$C_{22}H9_{19}N_5O_3$
X	EtOH	234-237	60	$C_{35}H_{24}N_{10}O$

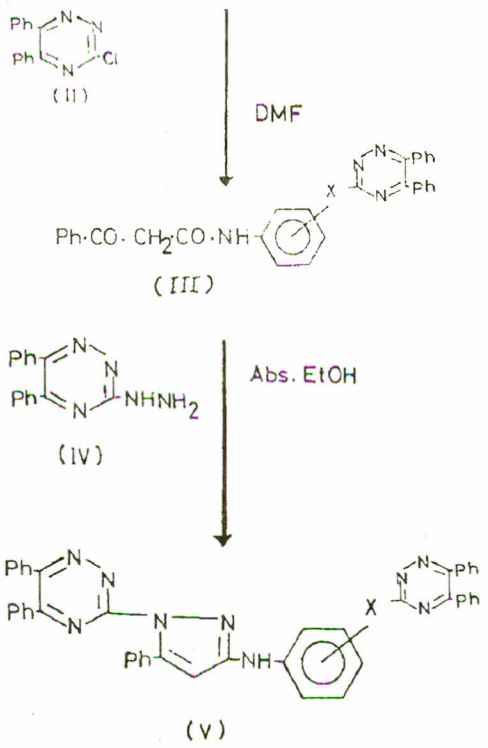
*All the compounds gave satisfactory CH, N and Cl analysis.
*Found: S, 15.55. Calc: S, 15.68 %
‡ Found: S, 22.38. Calc: S, 22.78 %

Results and Discussion

In this study, the treatment of benzoyl acetanilides Ia-d [12] with 3-chloro-5,6-diphenyl-1,2,4-triazine (II) in the presence of DMF gave products of the type IIIa-d. The aim of the present work was to synthesize the pyrazole derivatives containing 5,6-diphenyl-1,2,4-triazin-3-yl. Thus, by refluxing 3-hydrazino- 5,6-diphenyl-1,2,4-triazine (IV) with 1,3-bicarbonyl compounds (IIIa-d) in abs. ethanol, the cyclized product 1-(5',6'-diphenyl- 1',2',4'-triazin-3'-yl)-3-arylamino-5-phenyl-pyrazolines (Va-d) was obtained in one step (Scheme 1). Structure of V was proved by the basis of UV, IR and PMR spectra. The UV spectra showed absorption bands at 285(n=π*), 255 (n=σ) and 200 nm, and the IR spectra revealed that



X : o, m and p- NH, p- S.

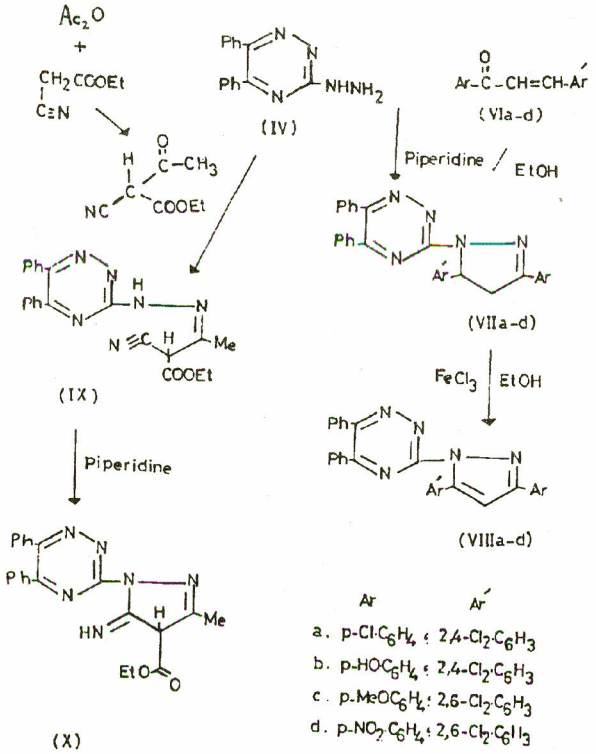


Scheme 1

presence of absorption bands characteristic for NH at 3200-3100, C=N at 1610-1580 and aryl groups at 990, 890, 850cm^{-1}. On the other hand, the band assigned to the C=O group was entirely absent. The PMR spectrum showed singlets at δ5 corresponding to =CH proton at position-4 of the pyrazoline ring and the resonance at δ6.9 - 8.5 corresponds to the aromatic protons. In addition, the resonances at 11.1 and 11.5 were assigned to bonded NH protons. This data indicates that V is present in the cyclic form rather than the acyclic structure. The formation of these pyrazolines was also confirmed by their positive Knorr's colour test [13].

Although several methods for the synthesis of pyrazoles are reported in the literature, however the synthesis of pyrazoles from chalcones is not reported yet. Thus, it was of interest to investigate the behaviour of chalcones towards hydrazine derivatives. The treatment of chalcones VIa-d under reflux in ethanol/piperidine with an equimolar quantity of 3-hydrazino-5, 6- diphenyl-1,2,4-triazine (IV) afforded 1-(5',6'-diphenyl-1',2',4'- triazin-3'-yl)-3-aryl-4-dihydro-5(H) arylpyrazoles (VIIa-d) (Scheme 2). The assigned structures for VII were inferred from their IR and PMR spectra.

In a similar manner, this work was extended to the synthesis of pyrazoles. Thus, when compounds VIIa-d were treated with aq. $FeCl_3$ in the presence of ethanol [15], 1,3,5-trisubstituted pyrazoles VIIIa-d were respectively obtained. The structures of all the new compounds were based on their method of synthesis, elemental analyses, IR and PMR spectra.



Scheme 2

The reaction of compound IV with ethyl cyanoacetate in the presence of acetic anhydride afforded N^1-(5',6'-diphenyl-1',2',4'- triazin-3'-yl)-N^2-(1-methyl-2(H) ethylcarboxy-2-cyano) hydrazone (IX) which on heating with piperidine gave the corresponding trisubstituted iminopyrazolone derivative (X) [Scheme 2]. Compounds IX and X being characterised by elemental analysis and IR spectra.

The biological activity of representative compounds III-X were tested and the results showed that, compounds IIId, Vd, VIId, VIIId and IX were the most active against *Candida albicans* and they have a lethal activity against *Bacillus cereus* and *Staphylococcus aureus*. On the other hand, the other tested compounds were found inactive against *Kelebsiella aerogenes*. The biological screening results of the present study also indicate that there is little improvement in the biological activity profile of these 1,2,4-triazines and pyrazoles by incorporation of nitro groups and thioether residues. None of the new compounds exhibited any significant activity against the yeast.

Conclusion

In conclusion, the preliminary screening carried out chiefly on the synthesized compounds III-X showed that only derivatives endowed with high molecular complexity and rigidity such as substituted aminopyrazolotriazines V and pyrazolotriazines VIII exhibited a strong platelet antibacterial activity *invitro* and that compounds III with a free pyrazole pattern were less active. This remarkable activity found in a number of substituted aminopyrazolotriazines, an effect not found until now in other pyrazolotriazines, gives us the opportunity to better investigate this class of compounds, and work is in progress on this.

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