SYNTHESIS AND ANTIMICROBIAL ACTIVITIES OF SOME NEW 4-ARYLAMINO ISOXAZOLO [5,4-d] PYRIMIDINE DERIVATIVES

V. A. ADHIKARI, V. P. SAVALGI and V. V. BADIGER
Department of Chemistry, Karnatak University, Dharwad 580 003, India.

ABSTRACT

Twentyone new substituted arylamino isoxazolo [5,4-d] pyrimidines have been synthesized from (un)substituted benzaldehydes. The structures of these compounds have been established by elemental and spectral analyses. The compounds were subjected to *in vitro* antibacterial screening against pathogenic strains and antifungal testing against fungi. Some of the compounds exhibit promising results.

INTRODUCTION

Isoxazolo [5,4-d] pyrimidine derivatives have been reported to exhibit antibacterial[1,2], miticidal[3] and analgesic[4] activities. As anilino group is an important pharmacophore in many antimalarials[5], antimycobacterial[6], antibacterial[7–9] and antifungal[10] agents, a series of new 4-arylamino-3-substituted isoxazolo [5,4-d] pyrimidines have been synthesized. It is hoped the presence of arylamino moiety in this class of compounds might give improved therapeutic results. The new compounds were evaluated for antibacterial and antifungal activity using standard methods.

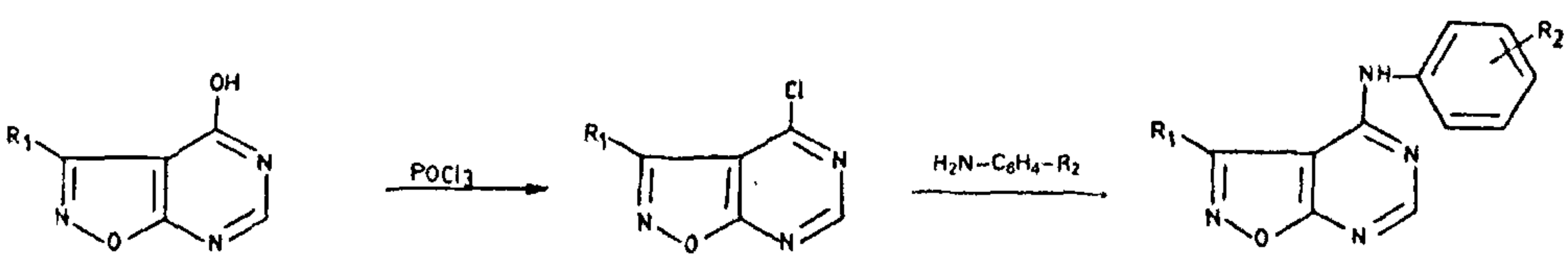
MATERIALS AND METHODS

The compounds were synthesized as shown in scheme 1.

The required 4-hydoxy-3-substituted isoxazolo [5,4-d]-pyrimidines (I) were synthesized from (un)-substituted benzaldehydes by the reported procedures[11–13] and converted to 4-chloro-3-substituted isoxazolo [5,4-d] pyrimidines (II) by refluxing them in excess of phosphorus oxychloride. The compounds were synthesized by heating a suspension of II and arylamine in ethanol under reflux temperature for 3–4 *h*. Their characterization data are summarized in table 1. The structures of new compounds have been authentically established by their correct elemental analyses and spectral studies. All the compounds were evaluated for *in vitro* antibacterial activity against five pathogenic microorganisms viz. *Escherichia coli, Proteus vulgaris, Proteus aurigasa, Klebsiella pneumoniae* (all gram-negative) and *Staphylococcus aureus* (gram-positive) by the disc diffusion method[14,15]. They were also subjected to antifungal screening against three fungi viz. *Aspergillus niger, Candida albicans* and *Aspergillus flavus* by turbidity method[16]. The results, subjectively graded, are presented in table 2 along with results obtained for standards i.e. sulphanylamide and phenol in antibacterial screening and salicylic acid in antifungal testing.

RESULTS AND DISCUSSION

The formation of 4-(*p*-acetylphenylamino)-3-*p*-chlorophenylisoxazolo [5,4-d] pyrimidine (III m) from the corresponding 4-chloro deriavative was



Ia. R_1 = p–CH_3 C_6H_4
Ib. R_1 = p–Cl C_6H_4
Ic R_1 = C_6H_5

IIa. R_1 = p–CH_3 C_6H_4
IIb. R_1 = p–Cl C_6H_4
IIc. R_1 = C_6H_5

IIId–u

(Table 1)

Scheme 1.

Table 1 *Characterization data of 4-arylamino-3-substituted isoxazolo [5,4-d] pyrimidines (IIIa-u)*

Compd No.	R_1	R_2	Yield (%)	M. P. °C Cryst. Solv.	Mol. formula (Mol. weight)	Calculated (Found) C	H	N	IR data in cm^{-1}
IIIa	*p*–$CH_3C_6H_4$	H	67	203–204 Benzene	$C_{18}H_{14}N_4O$ (302.18)	71.54 (71.38)	4.63 (4.82)	18.53 (18.77)	3400 (N–H)
IIIb	*p*–$CH_3C_6H_4$	*p*–Br	71	183–184 Ethanol	$C_{18}H_{13}BrN_4O$ (381.09)	56.72 (56.91)	3.41 (3.62)	14.69 (14.52)	3410 (N–H)
IIIc	*p*–$CH_3C_6H_4$	*p*–Cl	70	197–198 Ethanol	$C_{18}H_{13}ClN_4O$ (336.63)	64.21 (64.45)	3.86 (3.75)	16.63 (16.97)	3415 (N–H)
IIId	*p*–$CH_3C_6H_4$	*p*–C_2H_5O	72	165–166 Methanol	$C_{20}H_{18}N_4O_2$ (346.2)	69.38 (69.19)	5.19 (5.34)	16.17 (16.02)	3430 (N–H)
IIIe	*p*–$CH_3C_6H_4$	*p*–OH	62	278–279 Dioxan + ethanol	$C_{18}H_{14}N_4O_2$ (318.18)	67.94 (67.78)	4.40 (4.59)	17.60 (17.45)	3330 (N–H) 3100 (O–H)
IIIf	*p*–$CH_3C_6H_4$	*p*–CH_3CO	65	216–217 Benzene	$C_{20}H_{16}N_4O_2$ (344.21)	69.78 (69.89)	4.64 (4.76)	16.26 (16.54)	–
IIIg	*p*–$CH_3C_6H_4$	*o*–OH	60	260–261 Ethanol	$C_{18}H_{14}N_4O_2$ (318.80)	67.94 (67.72)	4.40 (4.28)	17.6 (17.83)	–
IIIh	*p*–ClC_6H_4	*H*	65	167–168 Ethanol	$C_{17}H_{11}ClN_4O$ (322.62)	63.28 (63.52)	3.40 (3.27)	17.35 (17.58)	3380 (N–H)
IIIi	*p*–ClC_6H_4	*p*–Br	70	184–185 Ethanol	$C_{17}H_{10}BrClN_4O$ (401.53)	50.84 (50.62)	2.49 (2.38)	13.94 (13.82)	–
IIIj	*p*–ClC_6H_4	*p*–Cl	73	191–192 Methanol	$C_{17}H_{10}Cl_2N_4O$ (357.07)	57.17 (56.23)	2.80 (3.02)	15.68 (15.78)	3448 (N–H) 840 (C–Cl)
IIIk	*p*–ClC_6H_4	*p*–C_2H_5O	72	156–157 Ethanol	$C_{19}H_{15}ClN_4O_2$ (366.64)	62.23 (62.45)	4.09 (3.89)	15.27 (15.42)	3440 (N–H)
IIIl	*p*–ClC_6H_4	*p*–OH	64	249–250 Benzene	$C_{17}H_{11}ClN_4O_2$ (338.62)	60.29 (60.43)	3.24 (3.12)	16.53 (16.69)	–
IIIm	*p*–ClC_6H_4	*p*–CH_3CO	72	205–206 Benzene	$C_{19}H_{13}ClN_4O_2$ (364.65)	62.52 (62.38)	3.56 (3.74)	15.35 (15.42)	3510 (N–H), 1695 (C=O) 800 (C–Cl)
IIIn	*p*–ClC_6H_4	*o*–OH	62	254–255 Ethanol	$C_{17}H_{11}ClN_4O_2$ (338.62)	60.29 (60.47)	3.24 (3.38)	16.53 (16.39)	3390 (N–H), 3077 (O–H) 776 (C–Cl)
IIIo	C_6H_5	H	67	160–161 Benzene	$C_{17}H_{12}N_4O$ (288.32)	70.82 (70.98)	4.20 (4.03)	19.43 (19.62)	–
IIIp	C_6H_5	*p*–Br	76	173–174 Ethanol	$C_{17}H_{11}BrN_4O$ (367.08)	55.62 (55.84)	2.99 (3.14)	15.25 (15.11)	–
IIIq	C_6H_5	*p*–Cl	70	186–187 Ethanol	$C_{17}H_{11}ClN_4O$ (322.76)	63.26 (63.47)	3.43 (3.59)	17.36 (17.18)	3410 (N–H)
IIIr	C_6H_5	*p*–C_2H_5O	65	162–163 Ethanol	$C_{19}H_{16}N_4O_2$ (332.19)	68.69 (68.51)	4.81 (4.98)	16.85 (17.03)	–
IIIs	C_6H_5	*p*–OH	67	249–250 Benzene	$C_{17}H_{17}N_4O_2$ (304.17)	67.12 (67.27)	3.94 (4.12)	18.41 (18.28)	3448 (N–H) 3130 (O–H)
IIIt	C_6H_5	*p*–CH_3CO	71	208–209 Benzene	$C_{19}H_{14}N_4O_2$ (330.19)	68.10 (68.29)	4.23 (4.45)	16.95 (17.09)	–
IIIu	C_6H_5	*o*–OH	63	250–251 Ethanol	$C_{17}H_{12}N_4O_2$ (304.32)	67.12 (67.25)	3.94 (4.09)	18.41 (18.32)	–

Table 2 *In vitro antimicrobial activities of compounds IIIa-u*

Compd. No.	Antibacterial activity* Zone of inhibition after 24 h					Antifungal activity** % transmission after 48 h		
	E. C.	P. V.	P. A.	K. P.	S. A.	A. N.	C. A.	A. F.
IIIa	+	+	+	+	+	+	+	+
IIIb	+	+	+	+	+	+	+	+
IIIc	+	+	+	+	+	+	+	−
IIId	+	+	+	+	+	+	+	+
IIIe	+	−	+	−	+	+	+	+
IIIf	+	+	+	−	+	+	+	+
IIIg	+	+	−	+	+	+	+	+
IIIh	−	+	+	−	+	+	+	−
IIIi	+	+	+	+	+	+	+	+
IIIj	−	+	+	+	+	−	+	−
IIIk	−	−	+	+	+	+	+	−
IIIl	+	+	−	−	+	+	+	−
IIIm	+	+	+	+	+	+	+	−
IIIn	+	+	+	−	+	−	−	−
IIIo	+	+	+	+	+	+	+	−
IIIp	+	+	+	+	+	−	−	−
IIIq	−	+	+	+	+	−	−	−
IIIr	+	+	−	+	+	+	−	−
IIIs	+	+	+	+	+	+	+	+
IIIt	+	+	+	+	+	+	+	+
IIIu	+	+	+	+	+	+	−	−
S-1	+	+	+	+	+			
S-2	+	+	+	−	+			
S-3						+	+	+

*Antibacterial activity: E. C. = *E. coli*; P. V. = *P. vulgaris*; P. A. = *P. aurigasa*; K. P. = *K. pneumoniae*; S. A. = *S. aureus*; S-1 = Sulfanilamide; S-2 = Phenol; S-3 = Salicylic acid; Zone of inhibition in mm: 14–16 = − no activity; 17–28 = + Positive activity.

**Antifungal activity: A. N. = *A. niger*; C. A. = *C. albicans*; A. F. = *A. flavus*; % transmission: 1–25 = − no activity; 26–100 = + Positive activity. Drug concentration: 200 μg; Solvent = DMF.

evidenced by its IR and ^{1}H NMR spectral studies. This was confirmed by its mass spectrum which shows a molecular ion ($M^{\cdot+}$) peak at m/e, 364, constituting the base peak. Spectral features of III m are reported below.

The results of antibacterial screening of test compounds (table 2) show that most of the compounds showed significant activity against all the five strains. Amongst them, the compounds IIIb, IIIc, IIIf, IIIi, IIIj, IIIm, IIIp, IIIq, IIIs and IIIt were very effective when compared to standard sulphanilamide and phenol. A majority of the compounds showed maximum activity with respect to *P. aurigasa*, of which IIIf, IIIn, IIIq, IIIs and IIIu were highly potent. From the point of view of structure activity relationship it is seen that substitution by *p*–Cl, *p*–CH_3CO and *o*–OH for H in the phenyl nucleus enhanced the activity whereas non-substitution in phenyl ring at postition-3 of isoxazolo [5,4-d] pyrimidine increases the activity in most cases.

The antifungal activity shows that only a few compounds demonstrate moderate activity against *A. niger* and *C. albicans* and none of them had any activity against *A. flavus*. Only IIId, IIIe and IIIf exhibit some amount of antifungal activity compared to the standard salicylic acid.

EXPERIMENTAL

The melting points are determined in open capillaries and are uncorrected. IR spectra were recorded on spectrophotometers (Perkin-Elmer 221 and 1378)

using Nujol Mull. ^{1}H NMR spectra were run in spectrometer (Varian T-60A) using TMS as the internal reference (chemical shift in δ, ppm). Mass spectrum was recorded on mass spectrometer (Finnigan 4121, GC) at 70eV (low resolution). All the new compounds gave satisfactory C, H and N analyses.

4-chloro-3-substituted isoxazolo [5,4-d] pyrimidines (IIa-c)

A mixture of 4-hydroxy-3-substituted isoxazolo [5,4-d]-pyrimidine (Ia-c, 0.03 mol) and redistilled phosphorus oxychloride (83 ml) was refluxed for 2.5 h and evaporated to dryness under reduced pressure. The reaction mixture was worked out to get IIa-c in good yield. The melting points of IIa-c agreed with the reported values[11–13].

4-substituted arylamino-3-substituted isoxazolo [5,4-d] pyrimidines (IIIa-u)

A suspension of 4-chloro-3-substituted isoxazolo [5,4-d]-pyrimidine (IIa-c, 0.01 mol) in 50 ml of dry ethanol was added with agitation to a solution of 0.01 mol of substituted aniline in 30 ml of ethanol, which was then refluxed for 3–4 h. It was concentrated, cooled, filtered and finally recrystallized to get IIIa-u. The characterization data of compounds are presented in table 1. The compound IIIm gave the following spectral data.

IIIm; IR (Nujol): 3510 (N-H), 1695 (C=O), 1613 (pyrimidine), 1600, 1563 (aromatic), 800 cm^{-1} (C–Cl).

^{1}H NMR (δ, DMSO-d_6): 9.35 (H, *s*, N-H), 8.65 (H, *s*, C_6–H), 8.0–7.5 (8H, *m*, aromatic), 2.5 ppm (3H, *s*, CH_3). MS: 364 ($M^{\cdot+}$), 349 ($M-CH_3^+$), 321 ($M-COCH_3^+$), 252 ($M-ClC_6H_4^+$), 230 ($M-NHC_6H_4COCH_3^+$), 111 ($ClC_6H_4^+$).

Evaluation of antibacterial activity by disc diffusion method

The sterilized nutrient agar (25 ml, pH = 7.2), while hot, was poured into the sterilized petri dishes and allowed to cool to room temperature. The agar plates were inoculated with 18 to 24-hour-old test culture by spreading uniformly with sterile swabs. Sterilized (10 mm) discs, punched from Whatman No.1 filter paper were impregnated with 0.1 ml (concentration: 200μg) of drug solution (prepared by dissolving 10 mg of the compound in 5 ml of DMF) and were placed in the petri dishes with sterile fine-pointed forceps. The dishes were then incubated for 24 h at 37° and the inhibition zone was measured. In control, the filter paper discs saturated with pure DMF were used simultaneously. Sulphanilamide and phenol were used as standards.

Evaluation of antifungal activity by turbidity method

0.1 ml of the test compound (200μg in DMF) in 5 ml of sterilized fungi medium was treated with 3–4 drops of 48-hour-old culture in a sterilized test tube. The test tubes after thorough shaking were incubated for 48 h at 37°. The extent of inhibition was determined by measuring the decrease in turbidity in terms of percentage of transmission at 660 mμ. Salicylic acid (5%) was used as standard and DMF as solvent control.

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NEWS

ANAEROBES FOR IMPROVED SEWAGE TREATMENT

..."Agricultural engineers at Cornell University have devised a new system for treating sewage that can produce reservoir-quality water at little or no cost, according to the researchers [in Ithaca, New York]. By using an unusual bacterial technique to filter out heavy pollutants and then growing plants on the partially cleansed waste water, the Cornell system produces such commercial products as natural gas and nursery plants and trees while it cleans the water. Head researcher William J. Jewell devised a first-stage biological treatment system that removes 'as much sludge, soluble organics and suspended solids as possible' before using the water to grow plants. In the process, which has been patented by Cornell, anaerobic bacteria are attached to small particles of pulverized corncobs, which are then suspended in the waste water. These bacteria, which grow in the absence of oxygen, are very slow-growing and thus do not accumulate in the system. However, they rapidly convert soluble organic materials in the waste water into methane gas. 'Currently,' Jewell said, 'most sewage treatment systems use aerobic bacteria and a large amount of energy for first-stage treatment and end up with a lot of sludge.' He said it costs the nation about half a billion dollars a year just to aerate sewage so that aerobic bacteria, which depend upon oxygen, can remove some of the organic material. In the Cornell system, the initial treatment gets rid of most of the suspended solids and some of the toxic materials in waste water, which is then ready for hydroponic agriculture. The hydroponically grown plants remove nutrients and most of the remaining pollutants in the water. To produce drinking water, a third step would be needed to remove remaining pollutants." [Jane E. Brody in *New York Times*, 3 Nov. 87, p. C1; C4. Reproduced with permission from Press Digest, *Current Contents*®, No. 21, May 23, 1988, p. 13. (Published by the Institute for Scientific Information®, Philadelphia, USA.)]

POLISH ACADEMY OF SCIENCES

Prof. C. N. R. Rao, Chairman of the Science Advisory Council to the Prime Minister, and Director, Indian Institute of Science, Bangalore, has been elected a foreign member of the Polish Academy of Sciences. Prof. Rao is the first Indian to be elected to the Polish Academy of Sciences. Prof. C. N. R. Rao, is the President of the Current Science Association and Editor of Publications of the Indian Academy of Sciences, Bangalore.