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AMINO AZOLES AND AZOLO-AZINES AS POTENTIAL HEPATOPROTECTANTS+: PART III

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Abstract: The synthesis and hepatoprotective activity of 3,5-diaminopyrazoles **(2)**, isoxazoles **(4)**, 5-aryl-3-(2,4-diamino-1,3,5-triazin-6-yl)methyl-1H-pyrazoles **(7)** and 5-(2-amino-1,3,4-thiadiazol-5-yl)methyl-3-(4-methylphenyl)-1-phenylpyrazole **(9)** are delineated.

Introduction: Liver is an organ of paramount importance and is vital for metabolism and excretion. Continuous exposure of liver to hepatotoxins such as halogenated hydrocarbons, heavy metals and excessive use of therapeutic agents damage the endoplasmic reticulum and other membranes of hepatocytes which makes the treatment more problematic. Except herbal preparations, no single drug has so far been designed to treat hepatic ailments. The synthetic and semisynthetic drugs in clinical use such as carticosteroids, immunosuppressants and antiviral agents which provide only symptomatic relief[1] with chances of relapse and severe side effects. Hence search for safe and suitable synthetic hepatoprotectants is inevitable.

The cytotoxic effects of high concentrations of highly reactive species such as superoxide (O_2^-), hydrogen peroxide and hydroxy radicals produce mutations of diverse nature causing ageing, cataract, cancer and hepatitis[1]. Hence the importance of radical quencher[2] as therapeutic agents was recognised. The property of nitrogen and sulphur compounds as radical scavenger[2-4] aroused considerable interest to design and synthesize aminopyrazoles **(2)**, isoxazoles **(4)**, 1,3,4-thiadiazoles **(9)** and 1,3,5-triazines **(7)** as potential hepatoprotectants. All the synthesized compounds were tested for hepatoprotective activity and three of them **2a,b** and **9** displayed high order of efficacy and gave better % protection in various levels of serum enzymes and bilirubin. Compounds **2b** and **9** were found almost equipotent and superior to silymarin, a standard drug used.

Synthesis: 2,4-Diaminopyrazoles **(2)** and isoxazoles **(4)** were prepared[5,6] by the reaction of benzylmalononitrile with hydrazine hydrate and hydroxylamine hydrochloride respectively. The monoacetyl derivative **(3)** was obtained by boiling **2** in acetic acid (Scheme 1).

5-Aryl-3-(2,4-diamino-1,3,5-triazin-6-yl)methyl-1H-pyrazoles **(7)** were prepared in two steps from 6-aryl-3-cyano-4-methylthio-2H-pyran-2-ones **(5)**. The first step involved the transformation[7] of **5** to 5-aryl-3-cyanomethyl-1H-pyrazoles **(6)** by reaction with hydrazine hydrate. The intermediate **6** on base catalysed condensation cyclization[8] with dicyandiamide lead to the formation of **7**. Similarly **8** was obtained from the interaction of **5** with phenylhydrazine which on acid catalysed reaction with

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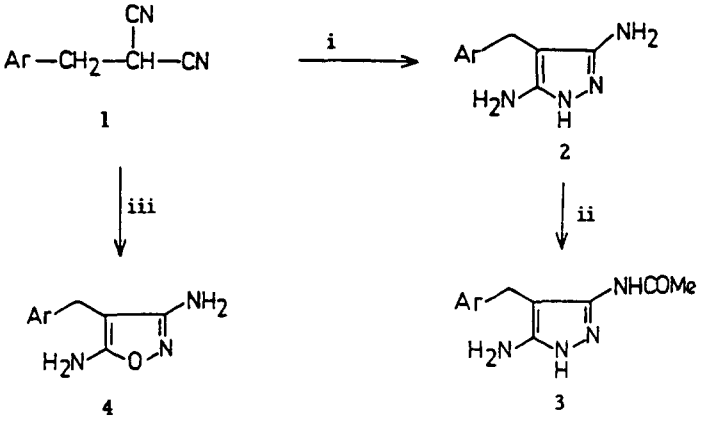
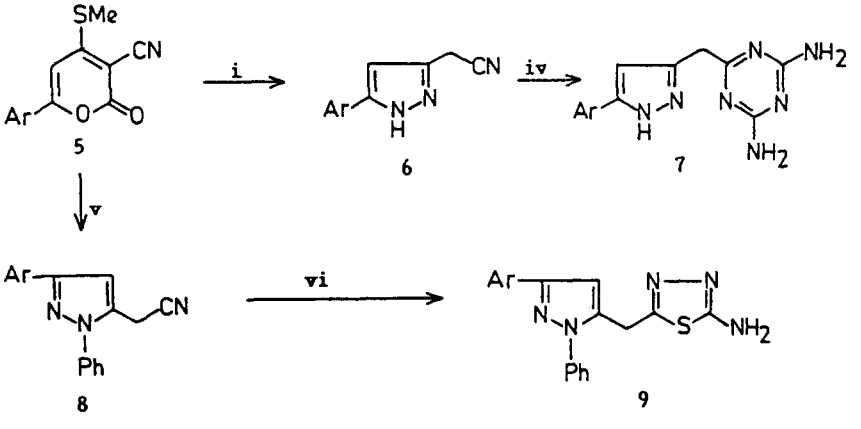
thiosemicarbazide yielded[9] 5-(2-amino-1,3,4-thiadiazol-5-yl)methyl-3-(4-methylphenyl)-1-phenylpyrazole (9) (Scheme 2).

Biological Activity: All the synthesized compounds listed in Table 1 were screened for their hepatoprotective activity against thioacetamide induced hepatitis in rats according to the procedure reported earlier[10]. The activity of test chemicals were assessed on the basis of % protection afforded in various levels of serum enzymes such as glutamate pyruvate transaminase (GPT), glutamate oxaloacetate transaminase (GOT), alkaline phosphatase (ALP) and bilirubin. The screening results are presented in Table 1.

Scheme-1



Scheme-2



Reagents/Conditions: i) N_2H_4/EtOH/80°C, ii) CH_3COOH/130°C, iii) $NH_2OH.HCl$/MeOH/Et_3N, iv) $NH_2-\overset{\overset{NH}{\|}}{C}-NHCN$/$(CH_3)_2CHOH$/KOH/100°C, v) $C_6H_5NHNH_2$/EtOH/80°C, vi) $H_2NNH-\overset{\overset{S}{\|}}{C}-NH_2$/TFA/65°C.

Table 1: Hepatoprotective activity of **2-4**, **7** and **9** against thioacetamide (200 mg/kg s.c.) induced toxicity in rats at 6 mg/kg dose (P.O. x 7 days).

Group/ Compd. No.	Ar	GOT (U/L)	GPT (U/L)	ALP (U/L)	Bilirubin (mg %)
Normal (I)	--	41.3±1.77	40.4±1.88	176.7±3.83	0.482±0.029
TAA treated (II)	--	128.0±2.86**	171.5±5.28**	218.9±4.96**	0.829±0.013**
Compd. treated (III)	-	-	-	-	-
2a	4-$CH_3OC_6H_4$	70.4±2.89**	85.5±3.83**	187.0±4.27**	0.619±0.04**
2b	4-$(CH_3)_2NC_6H_4$	67.1±4.29**	93.8±4.53**	187.1±3.65**	0.568±0.03**
2c	3,4-$CH_2O_2C_6H_3$	75.6±3.82**	99.2±9.27**	196.9±4.68**	0.758±0.01*
2d	4-ClC_6H_4	97.5±5.96*	136.2±3.46*	205.3±2.64*	0.757±.03*
3	4-ClC_6H_4	96.7±7.56*	146.0±7.26*	204.0±3.25*	0.693±0.01*
4a	4-$CH_3OC_6H_4$	99.9±9.64*	87.4±5.68**	215.9±4.83	0.745±0.03*
4b	4-$(CH_3)_2NC_6H_4$	114.0±5.28	146.2±6.46	206.6±5.81*	0.786±0.01
4c	3,4-$CH_2O_2C_6H_3$	101.5±3.69*	109.3±5.40**	205.8±3.47*	0.690±0.05**
4d	4-ClC_6H_4	106.0±7.36*	112.5±4.24**	201.0±2.50**	0.735±0.02*
7a	4-ClC_6H_4	119.0±6.87	148.5±3.24	190.5±3.68**	0.748±0.04*
7b	4-FC_6H_4	121.6±6.88	129.0±4.35*	203.2±7.43*	0.807±0.01
9	4-$CH_3C_6H_4$	65.2±2.67**	87.5±2.68**	196.0±2.86**	0.575±0.05**
	Silymarin (Standard Drug)	84.5±5.98**	109.5±4.69**	199.0±2.96**	0.617±0.04**

Values are mean ± S.D. of six rats in each group. Group II compared with group I (** $P < 0.001$), group III compared with group II (**$P < 0.001$, *$P < 0.01$). Analysis of variamu (ANOVA) was done for significance. Individual comparision within the group was carried out by Student's 't' test.

A critical structure-activity analyses of 3,5-diaminopyrazoles and isoxazoles revealed that the substitution in benzyl substituent at position 4 in **2** and **4** plays a pivotal role in expressing hepatoprotective activity. Compounds with 4-methoxybenzyl, 4-dimethylaminobenzyl and 3,4-methylenedioxybenzyl substituents in **2** were found most potent. The order of activity of **2a** and **2b** in all the serum enzymes parameters and bilirubin was almost comparable, while **2c** was found inferior to them. A change of hetero atom from N **(2)** to O **(4)** demonstrated loss in hepatoprotective activity.

Presence of (2,4-diamino-1,3,5-triazino)methyl substituent at position 3 in **7** did not contribute to the activity while presence of (2-amino-1,3,4-thiadiazolo)methyl moiety with phenyl substituent at position 1 displayed significant activity and gave better protection than silymarin in all the serum enzymes parameters.

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b) Male Sprague-Dawley rats (100-125 g) were caged separately in groups of 5 animals each. Group I consisted of normal animals. Group II animals were administered thioacetamide (200 mg/kg, P.O. x 1). Group III animals were fed the test compound daily at a dose level of 6 mg/kg (P.O. x 7 days). Thioacetamide was administered to them on day 7.
Animals of all the groups were sacrificed 24 h after administration of the toxin and their blood collected. Serum enzyme parameters described in Table 1 were analysed according to standard procedures and the percent protection was calculated using the formula:

$$\frac{\text{(Toxin treated) - (Toxin + test compound treated)}}{\text{(Toxin treated) - (Normal)}} \times 100$$

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