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6-IMINO-1,3-OXAZINES: A NEW CLASS OF HEPATOPROTECTANTS+

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Abstract: A convenient synthesis of polysubstituted 6-imino-1,3-oxazines (3,5,9) from corresponding ketene dithioacetals has been described and their conversion to pyrimidin-6-one (6) and pyrazolo[3,4-*d*]pyrimidine (8) studied. The oxazines synthesized, have shown a significant hepatoprotective activity.

Perpetual exposure of liver to xenobiotics and therapeutic agents lead to toxic manifestations of complex and diverse nature. Except for some natural products[1] claimed to be effective, no safe synthetic product is yet available for management of hepatic disorders.

The biological activity of a drug resides in a distinctive structural feature of the molecule and simulation of such identity in a molecular makeup, produces desired responses. Recently, 3-cyano-4-methoxy-1H-pyridin-2-one (ricinine), isolated from *Ricinus communis* has been found effective at 6 mg/kg/P.O. dose with LD_{50}, 80 mg/kg/P.O. in rats in various models of liver injuries[2]. Conferring some of the structural features of ricinine to 1,3-oxazine, this new class of compounds emerged to explore their efficacy as hepatoprotectants.

After Barker's synthesis[3], numerous procedures have been reported for the synthesis of 1,3-oxazine derivatives which include intramolecular cyclization of N-acyl-β-aminocrotonates[4] by pyrolysis, oxidative ring expansion of isoxazolones[5] and pyrroles[6]. Alternate syntheses from the reaction of isoxazolones with benzonitrile oxide[7] and cyclo-addition between N-iminopyridinium ylides and diphenylcyclopropanone[8] are also reported. Yamamoto *et al.*[9] have recently reported the one pot synthesis of 1,3-oxazine-6-ones from the reaction of imidate hydrochloride with Meldrum's acid.

Our approach to synthesize functionalised 6-imino-1,3-oxazines (3) from ketene dithioacetals (1) is a deviation from the classical approaches. Ethyl 2-cyano-3,3-dimethylthioacrylate (1a) employed as precursor, on amination followed by acetylation yielded intermediate 2. The intermediate 4 was also directly obtained from 1a by base catalysed[10] substitution reaction with benzamide. Both 2 and 4 on thermal cyclization yielded 3. Attempts to isolate the intermediate corresponding to 4 from the reaction of 2-cyano-3,3-dimethylthioacrylonitrile (1b) with benzamide failed and a mixture of 5-cyano-6-imino-4-methylthio-2-phenyl-1,3-oxazine (5) and 5-cyano-1,6-dihydro-4-methylthio-2-phenylpyrimidin-6-one (6) was isolated. The latter product was also obtained by thermal rearrangement of 5 at 200°C or refluxing it in methanol in presence of a trace of KOH. Reaction of 6 with neat hydrazine hydrate yielded

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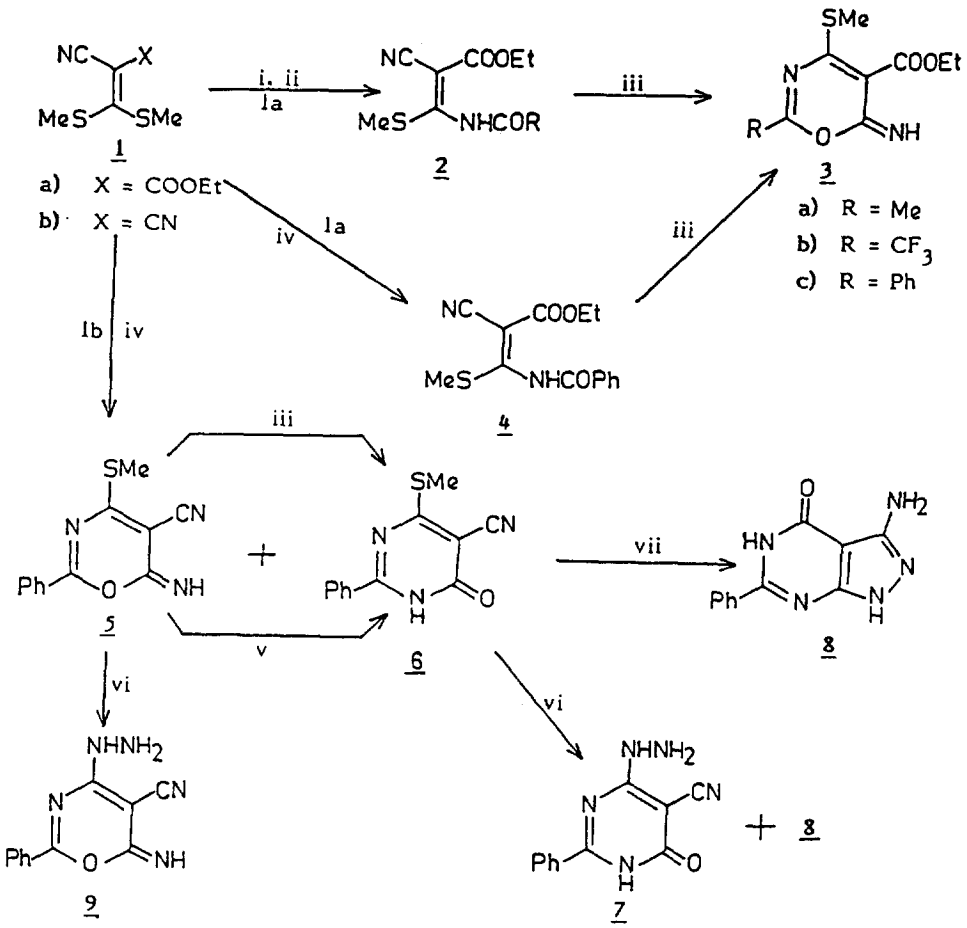
3-amino-6-phenyl pyrazolo[3,4-*d*]pyrimidin-4-one **(8)** but in presence of ethanol as solvent provided a mixture of 4-hydrazinopyrimidin-6-one **(7)** and pyrazolo[3,4-*d*]pyrimidine **(8)**. Similar reaction of **5** with hydrazine hydrate in ethanol yielded 6-imino-4-hydrazino-1,3-oxazine **(9)** (Scheme 1). All the synthesized 1,3-oxazine derivatives were characterized by elemental and spectroscopic analyses[11] and evaluated for hepatoprotective activity against thioacetamide-induced hepatic damage in rats according to the procedure reported earlier[12,13]. The activity of the compounds was assessed on the basis of % protection afforded in various levels of serum enzyme parameters 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



Reagents/Conditions: (i) Methanolic NH_3/80°C, (ii) $(RCO)_2O$/R.T., (iii) Heat/200°C, (iv) $C_6H_5CONH_2$/NaH/$(CH_3)_2NCOCH_3$/C_6H_6, (v) CH_3OH/KOH/80°C, (vi) N_2H_4/EtOH/80°C, (vii) N_2H_4/100°C.

Table 1: Hepatoprotective activity of 6-imino-1,3-oxazines **(3a-c,5,9)** against thioacetamide-induced toxicity in rats at 6 mg/kg dose (P.O. x 7 days). Values are the % protection afforded by the compounds in serum enzyme parameters.

Compound	GOT	GPT	ALP	Bilirubin
3a	26	29	36	11
3b	66*	54*	28	14
3c	33	39*	41*	33
5	26	19	30	19
9	17	19	12	0
Silymarin (standard drug)	50.14*	47.25*	47*	61*

(*$P < 0.05$) as compared to toxin treated group.

A comparative study on the serum enzyme levels obtained by dosing the 6-imino-1, 3-oxazine derivatives or the standard drug silymarin revealed that among all the screened compounds only **3b** demonstrated better efficacy for GOT and GPT while **3c** displayed consistent efficacy in all the serum enzyme parameters and bilirubin. This study provided a new prototype structure displaying significant hepatoprotective activity and opens a new avenue for further exploration.

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11. All the compounds were characterized spectroscopically. Selected data for the compounds: **3a**; m.p. 198°C; MS m/z 228 (M^+); IR(KBr) 1680 (CO), 3320 (NH) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.28 (3H, t), 2.34 (3H, s), 2.40 (3H, s), 4.35 (2H, q). **3b**; m.p. 125°C; MS m/z 282 (M^+); IR (KBr) 1690 (CO), 3300 (NH) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.52 (3H, t), 2.59 (3H, s), 4.56 (2H, q), 6.53 (1H, s). **3c**; m.p. 205°C; MS m/z 290 (M^+); IR (KBr) 1680 (CO), 3320 (NH) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.42 (3H, t), 2.61 (3H, s), 4.46 (2H, q), 7.35-7.64 (3H, m), 8.24-8.58 (2H, m). **5**; m.p. 140°C; MS m/z 243 (M^+); IR (KBr) 2200 (CN), 3340 (NH) cm^{-1}; ^1H NMR ($CDCl_3$) δ2.64 (3H, s), 6.08 (1H, brs), 7.36-7.62 (3H, m), 7.76-8.02 (2H, m). **6**; m.p. >280°C; MS m/z 243 (M^+); IR (KBr) 1650 (CO), 2220 (CN) cm^{-1}; ^1H NMR (DMSO-d_6) δ2.71 (3H, s), 7.58 (2H, t, J=7.3Hz), 7.66 (1H, t, J=7.5Hz), 8.22 (2H, d, J=9Hz). **7**; m.p. 200°C; MS m/z 227 (M^+); IR (KBr) 1630 (CO), 2190 (CN), 3220 (NH), 3400 (NH_2) cm^{-1}. **8**; m.p. >270°C; MS m/z 227 (M^+); IR (KBr) 1635 (CO), 3390 (NH_2) cm^{-1}. **9**; m.p. 256°C; MS m/z 227 (M^+); IR (KBr) 2180 (CN), 3240 (NH), 3340 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 5.42 (1H, s), 7.5-7.62 (2H, m), 7.66 (1H, s), 7.75 (2H, s), 7.95-8.08 (3H, m).
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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.x1). 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:

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

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