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Selective inhibition of nicotinamide adenine dinucleotide dependent oxidations by piperazinothioureas*

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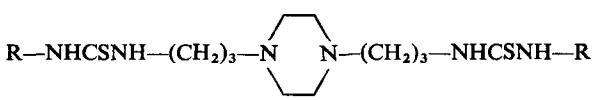
INHIBITION of potassium stimulated oxidation of glucose[1] and selective inhibition of NAD-dependent oxidation have provided evidence for the mechanism of the anticonvulsant[2] and hypnotic[3] properties of 2-methyl-3-*o*-tolyl-4-quinazolone. These observations prompted us to investigate structure–activity relationship of piperazinothioureas, reported earlier to exhibit anticonvulsant activity,[4] with respect to their ability to inhibit NAD-dependent oxidations by rat brain homogenate.

Male albino rats kept fed *ad lib.* were decapitated and the brains were immediately taken out and homogenized in ice cold 0·25 M sucrose (1:9, w/v) in a Potter–Elvehjem homogenizer. All incubations were carried out at 37° and the oxygen uptake was measured every 10 min by conventional Warburg manometric technique with air as the gas phase. In the present study, oxidation of sodium pyruvate, citrate, DL-isocitrate, α-ketoglutarate, β-hydroxybutyrate, NADH and succinate[5] was investigated. The reaction mixture, in a total volume of 3·0 ml, contained 6·7 mM $MgSO_4$, 20 mM Na_2HPO_4 in a buffer solution of pH 7·4, 1 mM AMP, 33 mM KCl, 500 μg of cytochrome C and rat brain homogenate equivalent to 100 mg of wet wt of tissue. It was presumed that endogenous NAD was sufficient for these oxidative processes. The central well contained 0·2 ml of 20% KOH. The piperazinothioureas were dissolved in propylene glycol (100 per cent) and used at a final concentration of 1 mM. An equal volume of the solvent was added to the control vessels. Different substrates and NADH were used at a final concentration of 10 mM and 0·5 mM, respectively.

All piperazinothioureas were found to inhibit selectivity the NAD-dependent oxidation of pyruvate,

TABLE 1. INHIBITORY EFFECTS OF PIPERAZINOTHIOUREAS†



R	% Inhibition of substrate oxidation‡						
	Pyruvate	Citrate	DL-Iso-citrate	α-Keto-glutarate	β-Hydroxy butyrate	NADH	Succinate
C_6H_5	45·2 ± 1·2	38·7 ± 1·1	42·1 ± 0·9	42·8 ± 1·8	42·6 ± 1·2	21·9 ± 1·3	Nil
2-$CH_3C_6H_4$	39·4 ± 2·1	34·8 ± 1·7	44·9 ± 1·8	39·0 ± 1·3	38·3 ± 2·0	17·6 ± 1·3	Nil
3-$CH_3C_6H_4$	69·7 ± 1·9	44·5 ± 1·0	59·6 ± 1·0	55·8 ± 1·8	59·7 ± 1·0	37·0 ± 0·9	Nil
4-$CH_3C_6H_4$	67·6 ± 1·0	49·0 ± 1·8	55·8 ± 1·8	49·0 ± 0·8	61·5 ± 1·4	30·2 ± 1·7	Nil
3,4-$(CH_3)_2C_6H_3$	84·4 ± 2·9	50·4 ± 1·7	64·9 ± 1·3	62·0 ± 1·7	63·5 ± 0·8	51·9 ± 1·0	Nil
2-$OCH_3C_6H_4$	10·7 ± 2·3	21·8 ± 1·3	46·0 ± 1·0	43·0 ± 1·3	37·2 ± 1·3	9·4 ± 1·7	Nil
4-$OCH_3C_6H_4$	49·6 ± 1·3	37·0 ± 0·9	54·6 ± 1·7	56·8 ± 2·0	52·2 ± 3·0	30·9 ± 1·3	Nil
4-ClC_6H_4	84·0 ± 3·0	45·0 ± 1·2	46·4 ± 1·2	73·1 ± 1·8	62·1 ± 1·0	46·3 ± 1·4	Nil
CH_2—CH=CH_2	18·3 ± 1·5	11·6 ± 0·8	21·9 ± 0·5	14·6 ± 2·3	13·6 ± 1·0	9·0 ± 1·3	Nil
C_2H_5	Nil	9·0 ± 0·7	20·9 ± 0·7	14·6 ± 2·0	13·2 ± 0·8	Nil	Nil

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† Previously synthesized and reported earlier.[4]

‡ Mean ± S.E. All values are the mean of three duplicate experiments. The percentage inhibition and the standard error are calculated from the decrease in the oxygen uptake $hour^{-1}$ 100 mg^{-1} of wet wt of tissue. The final concentration of piperazinothioureas was 1 mM. Different substrates and NADH were used at a final concentration of 10 mM and 0·5 mM, respectively. The oxygen uptake (in μl) in control experiments during the oxidation of pyruvate, citrate, DL-isocitrate, α-ketoglutarate, β-hydroxybutyrate, NADH and succinate was 84·0 ± 2·3, 67·7 ± 2·1, 142·9 ± 1·5, 111·5 ± 3·5, 75·1 ± 2·8 and 208·2 ± 2·2, respectively.

citrate, DL-isocitrate, α-ketoglutarate and β-hydroxybutyrate as well as the oxidation of NADH while the oxidation of sodium succinate which is not NAD dependent remained unaltered (Table 1). Maximum inhibition was observed with compounds having the 3,4-dimethylphenyl group at position R in the nucleus while *N,N'*-bis[3(3'-ethylurea)propyl] piperazine [R = C_2H_5] had minimal inhibitory effect. Piperazinothiourea possessing *meta* or *para* substituent in the benzene ring in general had greater inhibitory activity than the corresponding *ortho* substituted derivatives. Introduction of substituents at both *meta* and *para* position in the benzene ring caused a significant increase in the degree of inhibition. It is interesting to note that introduction of aliphatic groups, i.e. R = CH_2—CH= CH_2 or C_2H_5 in general significantly decreased the inhibitory power of the piperazinothioureas. These results do not show a clear-cut structure activity relationship but have indicated that the aromatic nucleus of the piperazinothioureas plays an important role in their ability to exhibit selective inhibition of NAD-dependent oxidations.

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