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PRELIMINARY COMMUNICATION

3-N-SUBSTITUTED-AMINO-1-[3-(TRIFLUOROMETHYL)PHENYL]-2-PYRAZOLINES HAVE ENHANCED ACTIVITY AGAINST ARACHIDONATE 5-LIPOXYGENASE AND CYCLOOXYGENASE

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The potent *in vitro* inhibition of arachidonate 5-lipoxygenase (LO) and cyclooxygenase (CO) by 3-amino-1-[3-(trifluoromethyl)phenyl]-2-pyrazoline (BW 755C) (Fig. 1; R = H) is well known, as are the effects of this drug on leukocyte migration and prostaglandin (PG) production *in vivo* [1-3]. The present work extends the earlier findings, and shows that the *in vitro* activity of BW 755C against the enzymes LO and CO is promoted by alkylation of the exocylic nitrogen atom (Fig. 1; R = alkyl etc.).

MATERIALS AND METHODS

The new pyrazolines (Fig. 1) were prepared by standard procedures (eg reaction of BW 755C with the appropriate aldehyde, and $NaBH_4$ reduction) [4] and their structures and enzyme inhibitory activities are listed in Table 1.

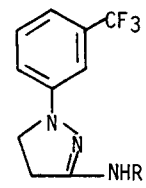

Fig. 1

Intact peritoneal leukocytes were obtained from the peritoneal cavities of male New Zealand white rabbits, and were shown to metabolize arachidonic acid (AA) to afford a pattern of products similar to those produced by human leukocytes [5]. These include various PGs and leukotrienes and their breakdown products.

Drugs were pre-incubated for 5 min at 37° with a suspension of the cells in Krebs-Henseleit buffer (1 ml) prior to the addition of substrate (5μg AA; 0.2μCi ^{14}C-AA) and Ca-ionophore A23187 (0.03μg). Incubations were continued (shaking) for 15 min before the addition of acetone (2 ml) containing 1% v/v 5N-HCl. Blank samples were boiled for 5 min before incubation with substrate. Extraction with $CHCl_3$, chromatography on silica gel TLC plates (elution with 60:40:1 Et_2O:hexane:HOAc), location of radioactive zones, and quantitation of metabolites were by standard procedures. 5-HETE and 5,12-di-HETE levels were determined as a measure of LO activity; total PG and 12-hydroxyheptadecatrienoic acid (HHT) levels were similarly determined to establish CO activity. For each drug treatment the IC_{50} values for the individual products of a pathway did not differ significantly. Table 1 therefore presents a single, mean IC_{50} value for each drug against each pathway.

RESULTS

The pyrazolines each caused a concentration-dependent inhibition of both LO and CO. It is particularly interesting that although the non-steroidal antiinflammatory agent indomethacin has much greater activity against CO than LO, the new N-alkyl-pyrazolines appear to inhibit both pathways. Monoalkylation clearly results in an increase in activity over BW 755C; thus the N-propyl congener (compound 4) is at least 10 times more active as an inhibitor of LO and CO than the parent drug. Surprisingly, the N-methyl compound (2) (BW 540C) is significantly more effective against LO than CO ($p < 0.01$).

The N-alkylated phenylpyrazolines have also been shown to be active in a number of experimental models of inflammation following topical or systemic administration. Thus they reduce oedema, pyrexia, hyperaemia, hyperalgesia and leukocyte migration in inflammation,

and they also cause dose-dependent reductions in the concentrations of PGs and leukotrienes in inflammatory exudates [6].

Compound (2) (BW 540C) has been selected for further evaluation as a potential anti-inflammatory agent, particularly in psoriasis, a condition frequently associated with high local LO activity [7].

TABLE 1. *In vitro* inhibitory effects of 3-amino-1-[3-(trifluoromethyl)phenyl]-2-pyrazolines (as HCl salts) against LO and CO from rabbit leukocytes

Compound No.	Fig. 1; R	IC_{50} (μM)[a] LO	CO
1	H(BW 755C)	2.9 (1.5-4.0; n=18)	3.1 (1.8-4.4; n=18)
2	Me(BW 540C)	0.4 (0.1-0.7; n=10)	1.2 (0.7-3.7; n=10)
3	Et	0.6 (0.3-0.8; n=4)	0.9 (0.4-1.6; n=3)
4	n-Pr	0.04(0.03-0.05; n=2)	0.3 (0.1-0.5; n=4)
5	n-Bu	0.08(0.05-0.1; n=2)	0.2 (0.1-0.3; n=4)
6	$PhCH_2$	0.1 (0.03-0.13; n=2)	0.1 (0.09-0.13; n=2)
7	2-butenyl	0.18(0.16-0.2; n=4)	0.23(0.17-0.31; n=4)
indomethacin	-	116 (73-184; n=5)	1.3 (1.0-1.9; n=5)

[a]Ranges are given, with the no (n) of experiments (each effected in duplicate).

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