IDENTIFICATION OF THE INHIBITORY ACTIVITY OF CARBAZOMYCINS B AND C AGAINST 5-LIPOXYGENASE, A NEW ACTIVITY FOR THESE COMPOUNDS

Sir:

In the course of our screening program for substances that inhibit the activity of 5-lipoxygenase (5-LPO) and potential as anti-inflammatory agents, we have identified a new biological activity for carbazomycins B and C.

The inhibitory effect of fermentation broth extracts was determined by measuring the amount of 5-hydroxy-eicosatetraenoic acid (5-HETE) produced in a cell free extract of RBL-1 cells (ATCC CRL 1378), in the presence of fermentation broth extracts, arachidonic acid, ATP, calcium chloride and glutathione. Quantitation of the 5-HETE produced was by liquid chromatography (Rainin Dynamax C18, 5 cm × 0.46 cm) with an HPLC solvent (85% methanol - 29.2 mM lithium acetate buffer pH 6.3) at 1 ml/minute. Detection was at 230 nm with a Waters 481 detector and a Hewlett Packard 3396A integrator.

A fermentation broth extract from a microorganism, tentatively identified as a *Streptoverticillium* species, showed significant 5-LPO inhibitory activity. For identification of the active species, fermentation broth was extracted with butanol. After removal of the solvent, the residue was reconstituted in acetonitrile - water (50 : 50) and subjected to HPLC and spectrophotometric detection as described previously[1]. The effluent from the HPLC was collected in two 96 well microtiter plates as 200 μl fractions. These were then evaporated to dryness, reconstituted in 10% DMSO-DULBECCO's phosphate buffered saline (PBS) and tested for 5-LPO inhibitory activity. Biological activity peaked at an elution time of 14.6 minutes, and the UV-visible spectrum obtained by diode array spectrophotometry was very similar, if not identical to that of carbazomycin B[2,3]. The NMR spectrum of a semi-purified sample of the active compound was consistent with the carbazomycin B structure. The identity of the material as carbazomycin B was confirmed by both HPLC-thermospray MS of the sample and Tandem FAB-MS-MS with a $(M+H)^+$ ion at *m/z* 242. The fragmentation pattern on Tandem MS/MS was identical with an authentic sample of carbazomycin B obtained from M. KONISHI, Bristol-Myers Squibb Research Institute, Tokyo, Japan (*m/z* ions at 242, 227, 210, 182 and 167). A minor component of the fermentation was also noted at *m/z* 272, with a UV-spectrum also suggestive of a carbazole chromophore. Large scale fermentation of the organism, isolation of the active minor component and Tandem MS/MS indicated that this was carbazomycin C with a $(M+H)^+$ ion at *m/z* 272, which also showed 5-LPO inhibitory activity. The IC_{50} values for carbazomycin B, carbazomycin C and a number of other 5-LPO inhibitors are shown in Table 1.

Table 1. Inhibition of 5-LPO.

Compound	IC_{50} for inhibition of 5-LPO[a] (μM)
Carbazomycin B	1.5
Carbazomycin C	1.9
Butylated-hydroxytoluene	31.8
Hydroquinone	201.8
Carbazole	≫354.5

[a] RBL-1 cells were grown in DULBECCO's modified minimal essential medium with 20% heat-inactivated calf serum for 5 days at 37°C and 5% CO_2. They were harvested by centrifugation and washed twice with DULBECCO's PBS containing 1 mM EDTA. The cells were resuspended in the same buffer and sonicated. The suspension was centrifuged at 13,000 × *g* to remove cell debris and the supernatant stored at −70°C. Enzyme assays were conducted by diluting the extract to the desired specific activity with DULBECCO's PBS containing 28.5 mM phosphate, 1 mM EDTA, 0.9 mM ATP and 0.9 mM glutathione. 110 μl of the extract solution was preincubated at 37°C for 5 minutes, followed by the addition of the test substance in 20 μl of 10% DMSO-PBS. The reaction was started by the addition of 5 μl of substrate (2 mM arachidonic acid plus 25 mM calcium chloride in ethanol - water (3 : 1)). After incubation for 5 minutes the reaction was terminated by the addition of 110 μl of ethanol and the solution centrifuged to remove precipitated protein. The supernatant was analyzed for 5-HETE by liquid chromatography as described.

The 5-LPO inhibitory activity of these compounds is probably due to radical scavenger activity which is consistent with the recent report of carazostatin as a free radical scavenger[4]. However, tests on carbazole itself and other compounds with a carbazole nucleus, but without a free phenolic hydroxyl group, gave poor 5-LPO inhibition. These results, to be published later†, suggest that the

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presence of such a free phenolic group may be essential for radical scavenging activity.

DEREK J. HOOK
JOSEPH J. YACOBUCCI
SEAN O'CONNOR
MIKE LEE[a]
ED KERNS[a]
BALA KRISHNAN[a]
JIM MATSON[b]
GRACE HESLER[b]

Bristol-Myers Squibb Research Institute,
Department of Screening and
Biochemical Research,
[a]Department of Analytical Research,
[b]Department of Antitumor Chemistry,
5 Research Parkway,
Wallingford, CT 06492, U.S.A.

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