ORIGINAL ARTICLE

INHIBITION OF AROMATIC AMINO ACID DECARBOXYLASE BY A GROUP OF NEW POTENTIAL NONSTEROIDAL ANTI-INFLAMMATORY DRUGS WITH ANTILEUKOTRIENIC EFFECTS

Jaroslav Držata[1], Miroslav Kuchař[2]

Charles University in Prague, Faculty of Pharmacy in Hradec Králové: Department of Biochemical Sciences and Research Centre LN00B125[1]; and Zentiva-VÚFB, Prague[2]

Summary: Possible *in vitro* inhibition of aromatic amino acid decarboxylase (AAD, EC 4.1.1.28) by a group of phenylsulfonylbenzoic acid derivatives VÚFB 19363, 19369, 19370, 19371, and 19760 as new potential anti-inflammatory compounds was studied using the substrate L-tyrosine. Enzyme inhibition by $2.7x10^{-5}$ M concentration of compound VÚFB 19363 (Quinlukast) was 17 %, AAD inhibition at 3.75×10^{-5} M concentration of compounds VÚFB 19369, 19370, 19371, and 19760 ranged between 9–23 %. There were distinct differences between individual compounds. Evaluation of inhibition kinetics suggested full reversibility with VÚFB 19369 and the uncompetitive type of inhibition in the case of compound VÚFB 19363. Considering the anti-inflammatory activity of the compounds studied, the weak AAD inhibition found is rather favourable for their prospective pharmacological effect.

Key words: *Aromatic amino acid decarboxylase; Antileukotrienic drugs; NSAIDs; Phenylsulfanylbenzoic acid derivatives; Quinlukast; Potential drugs; Influence of drugs*

Introduction

Derivatives of phenylsulfanylbenzoic and phenylsulfanylphenylacetic acids were synthetized within the framework of research of anti-inflammatory drugs with a potential antileukotrienic mechanism of action (7). In *in vitro* experiments, some of the compounds showed a combined antileukotrienic effect via the inhibition of binding of LTD_4 and LTB_4 on the receptors. In connection with the found mechanism of action, the pharmacological properties of compound VÚFB 19363 (Quinlukast) as a representative of the group were evaluated as a potential antihistaminic drug.

Studies of other effects, more or less specific, which may appear as enzyme inhibition or inactivation, belong to common investigations of structures synthesized as potential medicaments. We found such effects of drugs in the research of several groups of compounds prepared or already used in human therapy (8,9). On the other hand, in the case of phenylsulfanylbenzoic acid derivatives, a study with aminotransferases and glutamate decarboxylase showed very weak effects, which is favourable in view of their potential use as medicaments (5).

Compounds originally prepared for a certain activity sometimes show properties which may contribute to their pharmacologically desirable effect by another mechanism. This was the case of the influence of anti-inflammatory drugs on aromatic amino acid decarboxylase, the enzyme participating in the formation of serotonin and dopamine (1).

In the present study, a group of compounds with multiple antileukotrienic activities was examined for interaction with aromatic amino acid decarboxylase of the rat liver (AAD, EC 4.1.1.28).

Experimental

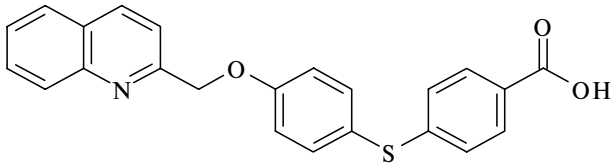
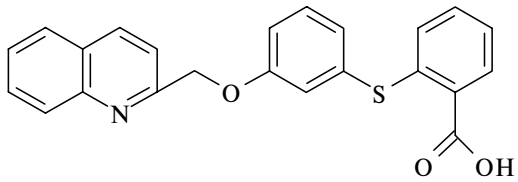
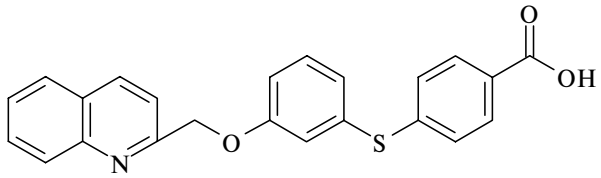
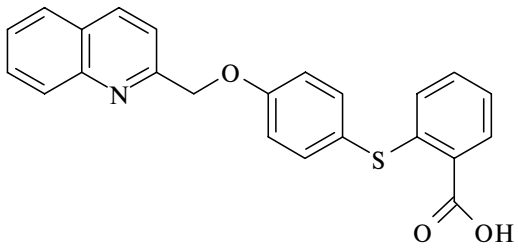
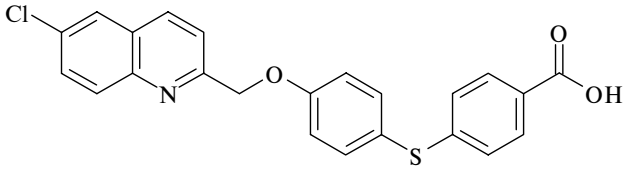
Compounds studied

Phenylsulfanylbenzoic acid derivatives with potential antileukotrienic effects (VÚFB 19363, 19369, 19370, 19371, and 19760) were synthesized as described in (4). Their structural formulae are given in Table 1. Compouds were dissolved in 0.05M solution of NaOH, solutions were neutralized by 0.05 M HCl and buffered with 0.1 M phosphate buffer pH 7.2.

AAD preparation

Supernatant 20 000xg of the homogenate of rat liver as an organ which is rich in AAD activity was used as the source of the enzyme. The enzyme preparation was obtained as described previously (1). All experiments with rats

Tab. 1: Inhibition of AAD by phenylsulfanylbenzoic acid derivatives. The influence on the enzyme is expressed in % of control activity. Each number represents the mean of three measurements in two independent experiments. Solutions of individual compounds were prepared in concentrations respecting their low solubility in water.

Concentration (in M)	Structure	AAD activity with substrate L-tyrosine in % of control
$2.7x10^{-5}$	 19363	83%, 83%
3.75×10^{-5}	 19369	67%, 64%
3.75×10^{-5}	 19370	78%, 76%
3.75×10^{-5}	 19371	89%, 87%
3.75×10^{-5}	 19760	93%, 89%

were approved by the Animal Use and Care Committee of Charles University, Faculty of Pharmacy in Hradec Králové, Czech Republic. Specific AAD activity of the preparation (3), measured by the radiometric method (5), was 66.5 nkat per mg of protein.

AAD assay and evaluation of enzyme inhibition by phenylsulfanylbenzoic acid derivatives

Chemicals used in AAD assay: L-Tyrosine-1-^{14}C (55mCi.mmol^{-1}, Amersham Pharmacia Biotech UK Ltd.), pyridoxal-5'-phosphate (Koch-Light Labs.), Bray's scintillation cocktail (Spolana, Neratovice). All chemicals used in AAD assay were at least of analytical grade.

Enzyme activity was determined radiometrically: The incubation mixture contained the AAD preparation, substrate 1-^{14}C-L-tyrosine (final concentrations $6x10^{-5}$-$6x10^{-4}$ M, specific activity 1.136 μCi.ml^{-1}), pyridoxal-5'-phosphate ($1x10^{-6}$ M in most experiments), the compounds under study and other components at different concentrations according to the aim of individual experiments, in 0.02 M Na-phosphate buffer, pH 7.2. The mixture was incubated at 37 °C for 30 min and the radioactivity of $^{14}CO_2$ (liberated from the mixture by means of sulfuric acid and absorbed in 0,1 ml 30 % KOH) was measured in the scintillation cocktail (1) using the 1219 Rackbeta scintillation counter LKB Wallac.

AAD activities in individual samples were expressed in dpm and calculated as relative activities. Control samples without compounds under study were considered as 100 %.

Estimation of inhibition kinetics

AAD inhibition by compounds VÚFB 19363 and 19369 was examined in experiments with constant concentrations of both AAD and the compound, and changing concentrations of the substrate. Reversibility of inhibition was evaluated graphically at changing concentrations of the enzyme. Type of inhibition was estimated using the Lineweaver and Burk ($1/v$ versus $1/s$) plot and the plot of enzyme concentration vs. velocity of reaction.

Results and discussion

Assessment of the influence of compounds under study on AAD activity in vitro

Table 1 summarizes AAD inhibition by individual compounds *in vitro*. Despite weak inhibition, two independent experiments with triplicate samples confirmed distinct differences between compounds. We tried to compare AAD inhibition with lipophilicity of compounds under study, using log P, obtained by means of HPLC, and with calculated distances of probable centers of interaction (values D). The results are summarized in Table 2. The results suggest a lack of correlation, which might be due to a low inhibitory effect, small differences in AAD inhibition, as well as due to using the equiconcentration condition instead of the equipotential one. The only exception in inhibition efficiency is compound 19369, which posesses lower lipophilicity and a shorter distance between the carboxy group and the hydrophobic part of the molecule.

If we consider the results in view of the position of AAD in the synthesis of the biogenic amines serotonin and dopamine, the enzyme inhibition might be considered as a certain contribution to the anti-inflammatory effect.

Tab. 2: Comparison of AAD inhibition with lipophilicity and distances of centres of probable interaction of phenylsulfanylbenzoic acid derivatives.

Compound	log P[a]	D (μm)[b]	AAD inhibition[c]
19363	6.11	14.42	17 %
19369	5.77	11.03	34 %
19370	6.11	14.32	23 %
19371	5.77	13.44	12 %
19760	6.75	12.73	9 %

[a]Lipophilicity
[b]Distance between centers of interaction
[c]The mean of two independent experiments with 3 samples each.

Inhibition kinetics

In the cases of compounds VÚFB 19363 (the compound in the centre of interest) and 19369 (the most effective inhibitor of AAD), an attempt to evaluate the inhibition kinetics was made (Fig. 1 and Fig. 2). The parallel lines of inhibited and control reaction in the Lineweaver-Burk plot of results (Fig. 3) suggested the uncompetitive type of inhibition by VÚFB 19363, while it was not possible to distinguish between competitive and non-competitive inhibition in the case of VÚFB 19369 (Fig. 4). Graphical evaluation of reversibility of inhibition suggested full reversibility for VÚFB 19369 (Fig. 5). In a similar experiment, we did not succeed in evaluating reversibility of inhibition by VÚFB 19363 because of a too low inhibitory effect.

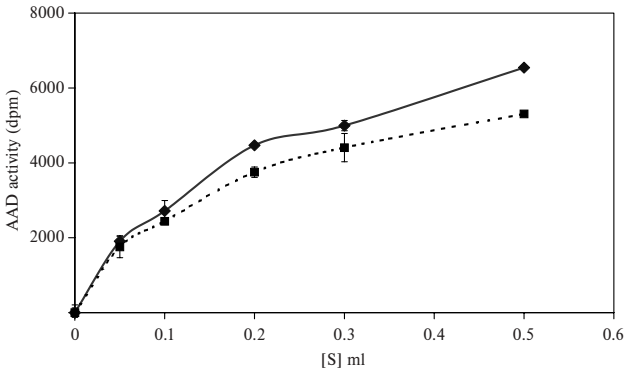


Fig. 1: Influence of compound VÚFB 19363 on rat liver AAD activity.
Michaelis-Menten plot. —◆— Control reaction. ---■--- $3.75x10^{-5}$M 13963.
Each point represents the mean of at least 3 samples.

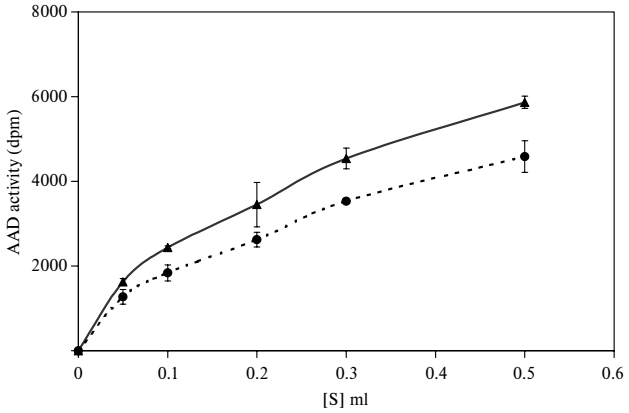


Fig. 2: Influence of compound VÚFB 19369 on rat liver AAD activity.
Michaelis-Menten plot. —▲— Control reaction. ---●--- $3.75x10^{-5}$M 13969
Each point represents the mean of at least 3 samples.

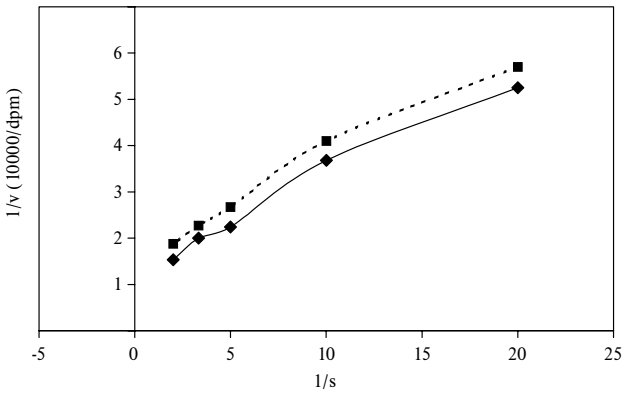


Fig. 3: Influence of compound VÚFB 19363 on rat liver AAD activity.
Lineweaver-Burk plot —♦— Control reaction. ---■--- $3.75x10^{-5}$M 13963.
The plot is based on recalculation of results presented in Fig. 1.

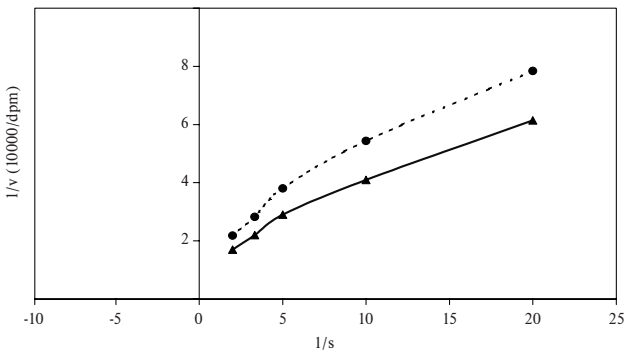


Fig. 4: Influence of the compound VÚFB 19369 on rat liver AAD activity.
Lineweaver-Burk plot. —▲— Control reaction. ---●--- $3.75x10^{-5}$M 13969.
The plot is based on recalculation of results presented in Fig. 3.

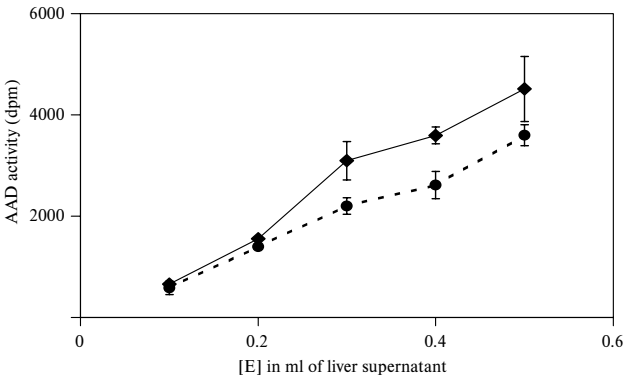


Fig. 5: Influence of enzyme concentration on AAD inhibition by VÚFB 19369. Concentration of compound VÚFB 19369. Each point represents the mean of at least 3 samples ± S.D.

Conclusions

1. *In vitro* inhibition of aromatic amino acid decarboxylase (AAD) by compounds VÚFB 19363, 19369, 19370, 19371, and 19760 was found.
2. Enzyme inhibition by $2.7x10^{-5}$ M concentration of the compound VÚFB 19363 was 17 %, AAD inhibition at 3.75×10^{-5} M concentration of compounds VÚFB 19369, 19370, 19371, and 19760 ranged between 9–23 %. There were distinct differences between individual compounds.
3. Evaluation of inhibition kinetics suggested full reversibility with VÚFB 19369 and uncompetitive type of inhibition in the case of compound VÚFB 19363.
4. Considering anti-inflammatory activity of the compounds studied, the weak AAD inhibition found is rather favorable for their prospective pharmacological effect.

Acknowledgments

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Prof. MUDr. Jaroslav Dršata, CSc.,
Charles University in Prague,
Faculty of Pharmacy in Hradec Králové,
Department of Biochemical Sciences,
Heyrovského 1203, 500 05 Hradec Králové,
Czech Republic.
e-mail: drsata@faf.cuni.cz