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Structures and anti-inflammatory properties of 4-halogenated -mofebutazones

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ABSTRACT

The crystal structures of the 4-halogenated (hal: F, Cl, Br)-4-butyl-1-phenyl-1,3-pyrolidine-dione (mofebutazone) are determined, and compared with their solution structures. The racemic 4-halogenated mofebutazone approximants crystallize in a monoclinic space group with four molecules in the unit cell. The 4-hal-mofebutazone molecules reveal strong hydrogen bonding between the hydrogen atom located at the N-2 nitrogen atom and a carbonyl oxygen atom of an adjacent 4-hal-mofebutazone molecule. The hydrogen bond angle for 4-Br-mifebutazone N (2)—H (1)···O (1) is 173(3) °, so that the hydrogen bond is essentially linear indicating an infinite chain hydrogen bond network. The *3d* and *2d* structures are stabilized by π-π and σ-π interactions, short intermolecular distances, and apolar forces between adjacently stacked phenyl rings. Small-angle-X-ray scattering (SAXS) experiments and osmometric measurements reveal the presence of dimers for the 4-hal-mofebutazone molecules. Molecular simulations indicate similar solution structure factors for the 4-hal-mofebutazones solutions, S(Q), and in the solid state. There is a strong indication that the [1,1,0], [1,0,0], and [1,0,0] periodicities of the 4-Br—, 4-Cl— and 4-F-mofebutazone in the crystalline solid state were also present in the solution phase. The biochemical and cellular activities of the different 4-hal-mofebutazones were monitored by the magnitude of their inhibition of the PGE_2 biosynthesis through the cyclo-oxygenase (COX-1) in macrophages, and on the inhibition of LTD_4 (5-lipoxygenase) in polymorphonuclear leukocytes.

1. Introduction

The pyrazolon derivatives have been in clinical use for long years due to their strong ant-inflammatory, antipyretic and analgesic effects. This is particularly true for the 4-butyl-1,2-diphenyl-3,5-pyrazolidine-dione (phenylbutazone) exhibiting strong analgesic and anti-inflammatory activities. The corresponding 4-butyl-1-phenyl-3,5-pyrazolidine-dione (mofebutazone) reveals strong anti-inflammatory activities but is less analgesic than the diphenyl derivative (Fig. 1A) [1–4]. However, the noncyclic metabolite of mofebutazone, the enantiomeric 2-(S) -(-)-*n*-butyl-(1-phenyl-hydrazinocarbonyl)-hexanoic acid (Fig. 1 B), exerts its inhibitory activities on the biosynthesis of leukotrienes LTA_4, LTD_5 and LTB_4, and possesses a higher therapeutic index *in vitro* than the parent compounds mofebutazone and phenylbutazone [5]. The dissimilarity of the monophenyl- and the diphenyl-3,5-pyrazolidine-dione in their biological activities has been demonstrated by *in-vitro* studies on the cyclo-oxygenase (COX-1) - and the 5-lipoxygenase systems [6]. Furthermore, structural differences between phenylbutazone and mofebutazone in their solid-state structures have been previously elucidated [7].

There are additional metabolic differences between mofebutazone and phenylbutazone: ii) mofebutazone is not hydroxylated in the phenyl ring and not in the n-butyl group; ii) the non-cyclic form, the 2-*n*-butyl-2-(1-phenylhydrazino-carbonyl)-hexanoic acid (Fig. 1B) is not metabolized back into the cyclic form; iii) but is excreted whereas the diphenyl derivative remains in the blood stream. Particularly, the chiral stereochemistry at the carbon C-4 in the pyrazolidine-3,5-dione and the involvement of a halogen atom instead of a hydrogen atom is challenging and amendable to oxidation by O_2/OH^- [8].

The insertion of a halogen atom, particularly a fluorine atom at the chiral carbon atom (C-4) could lead to a range of potentiation of

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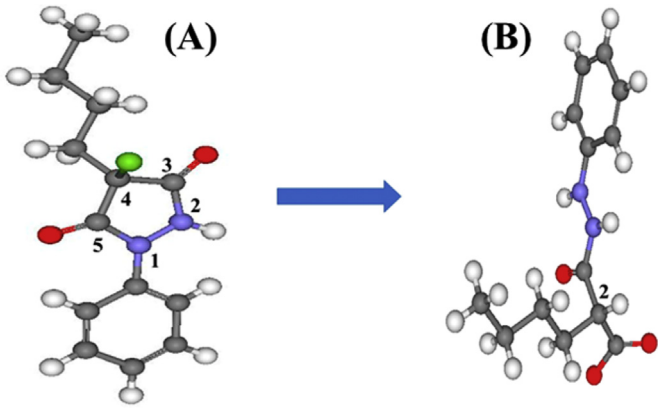

Fig. 1. (A) Chemical structure of the halogenated 4-*n*-butyl-4-(●) hal)-1-phenyl-3,5-pyrazolidine-dione (hal = F, Cl, Br, 4-hal-mofebutazone). **(B)** 2-(S) -(-)-*n*-butyl-(1-phenyl-hydrazinocarbonyl)-hexanoic acid [5].

the biological and cellular activities of mofebutazone. This applies even more considering the active enantiomeric 2-(S)-2-hal-*n*-butyl-(1-phenyl-hydrazinocarbonyl)-hexanoic acid approximants and the enantiomers of the 4-hal-mofebutazones. The introduction of a fluorine atom in an aminoglycoside neomycin and its future biological potential has recently demonstrated by Hanessian et al. [9]. It is expected that the introduction of a halogen atom modulates non-covalent interactions, e.g. hydrogen bonds, π … π interactions and other agnostic interaction directionality [10]. The various kinds of halogen atoms in the racemic 4-hal-mofebutazone derivatives affect the intermolecular and intramolecular interactions through the coexistence of halogen bond contacts and hydrogen bond contacts and have an impact on crystal stability, polymorphism, liquid crystal formation, ordering in solution and rheological behaviour (thixotropy).

This contribution presents the crystal structures of the racemic 4-fluoro-, 4-chloro- and 4-bromo-mofebutazone, their structures in solutions e.g. in 75% (v/v) water, 20% (v/v) ethanol and 5% (v/v) glycerol. It was possible to collect small-angle X-ray scattering (SAXS) and wide-angle X-ray scattering (WAXS) data for analysis due to i) the favourable solubility of the 4-hal-mofebutazones (20.0 mg/mL), ii) the chemical stability and no hydrolysis in solution at ambient temperature to compare the crystal structures with the solution structures and iii) any changes in the solid-state structure and in solution was possible to explain with the aid of molecular simulation methods. The SAXS and osmometric measurements corroborate the existence of dimers of the 4-hal-mofebutazone in solution rather than monomeric structures. The 4-F-mofebutazone dimer, however, accommodates up to six water molecules whereas the 4-Cl-and the 4-Br-mofebutazone only two water molecules. Finally, the basic anti-inflammatory activities with respect to inhibition of the PGE_2 biosynthesis and LTD_4 (5-lipoxygenase) inhibition in polymorphonuclear leukocytes are presented.

2. Experimental section

Mofebutazone was received from Medice Ltd (Iserlohn, Germany). The halogenated 4-butyl-4-(hal)-1-phenyl-3,5-pyrazolidine-dione (hal-mofebutazone, hal: F, Cl, Br) were prepared by adopting established routes using halogenated diethyl n-butyl-malonate ester [11], or by contacting mofebutazone with fluoro -, chloro- and bromo sulfonic acid (−10 °C), or applying specific modifications for inserting the halogens [12]. The cyclic 4-halogenated mofebutazone derivatives were isolated and purified by HPLC column chromatography. IR spectra were recorded with a JASCO FT/IR 6100 FT-IR spectrometer equipped with a DLATGS detector. All hal-mofebutazones exhibit strong ν(C=O) bands at 1753–11744 cm^{-1} and 1724–1715 cm^{-1}, which are coupled modes, and the asymmetric band at lower frequency was twice as intense as the symmetric ones a strong indication for the existence of the dioxo form ($CHCl_3$, CH_3 OH). A complete analysis of the hal-mofebutazone derivatives with respect to impurities were performed using MALDI-TOF-MS (GSG, Karlsruhe, Germany). H^1, C^{13}, F^{19} and Cl^{35} NMR (600 MHz, Bruker, Karlsruhe, Germany) were measured at 298 K in $CDCl_3$ (CD_3OD) (2:1, v/v).

Osmotic pressure measurements on 4-hal-mofebutazone solutions were made with a Knauer Membrane Osmometer (Hamburg, Germany) applying a 3200 MW membrane (Visking, UK) 1. Surface tension experiments were carried out with Digital Tensiometer (K12 version 2.0, Krüss, Hamburg, Germany) at 20 °C using the Du Noüy (Pt–Ir) ring method.

2.1. Crystallization

Racemic hal-mofebutazone crystals were prepared from 10% (w/w) hal-mofebutazone dispersed in 70% (w/w) ethanol and 30% (w/w) acetonitrile by slowly evaporation at 15 °C, by lowering the temperature of the solution from 25 °C to 15 °C, in small Conway-like dishes stored in a desiccator over a controlled water atmosphere humidity (< 5%) [13]. Once nucleated, the crystal growth occurs within 3–4 hs at any temperature between 0 °C and 25 °C, but the crystals redissolve above 45 °C. Similarly, crystals can be grown from solutions containing 65% (w/w) ethyl acetate, 30% (w/w) acetonitrile and 5% (v/v) water (20 °C). Some of these crystals are prism or rhombic in shape whereas the ones grown from ethanol solutions are mostly plates.

2.2. Crystallography

The 4-hal-mofebutazone-crystals were mounted on a glass fiber in a moisture-free N_2 (99.9%) atmosphere at 5 °C. The X-ray measurements were made on a Rigaku AFC5R Diffractometer with graphite monochromated CuK_α radiation and a 12 KW rotating anode generator. The cell constants and an orientation matrix for data collection were obtained from a least-squares refinement using setting angles of 25 carefully centered reflections in the range $51.86 < 2\theta < 78.85°$. The data were collected at a temperature of 15 °C using the ω-2θ scan technique to a maximum 2θ value of 120.1°. To maintain the 4-hal-mofebutazone crystal quality and improve high-angle data quality it is essential to keep the crystals at 10–15° omega scans of several intense reflections, made prior to data collection, had an average width at half-height of 0.58°, with a take-off angle of 6.0°. Scans of $(1.37 + 0.30 \tan\theta)°$ were made at a speed of 32.0°/min (in omega). The weak reflections ($I < 10.0\ \sigma(I)$) were rescanned (maximum of two rescans) and the counts were accumulated to ensure good counting statistics. Stationary background counts were recorded on each side of the reflection. The ratio of peak counting time to background counting time was 2:1. The diameter of the incident beam collimator was 0.5 mm, the crystal-to-detector distance was 400.0 mm. The structure was solved by direct methods [14–18]. The non-hydrogen atoms were refined either anisotropically or isotropically. Hydrogen atom positions were located from difference Fourier maps and a riding model with fixed thermal parameters ($u_{ij} = ½\,[U_{ij}(eq)]$ was applied. Hydrogen atoms were included in the structure-factor calculation and located in idealized positions with $d_{C\text{-}H} = 1.09(5)$ Å. The atoms were assigned isotropic thermal parameters that were 15 times greater than the B_{eq} value of the atom to which they were bonded. To compute the structure factors from the model, an isotropic