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Journal of Molecular Structure 743 (2005) 103–115
Journal of MOLECULAR STRUCTURE
www.elsevier.com/locate/molstruc

Structural influence on the intermolecular/intramolecular hydrogen bonding in solid state of substituted leflunomides: evidence by X-ray crystal structure

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Received 15 November 2004; revised 1 February 2005; accepted 14 February 2005

Abstract

We report the results of an X-ray crystal structure study of nine substituted leflunomide metabolite analogs (LFM). Comparison of the hydrogen bonding characteristics exhibited by these structurally distinct LFM analogs was especially informative about the inter- and intra-molecular hydrogen bonding patterns that exist in the crystal structure of individual compounds. All compounds had the strong intramolecular hydrogen bonds. In addition, with the exception of the 2,5-difluorophenyl substituted LFM analog, all other compounds formed inter- or intra-molecular hydrogen bonds with the halogen atom and the NH group. However, we found that the presence of a fluorine atom at the 2-position on the phenyl ring of the 2,5-difluoro and 2-fluoro derivatives resulted in only one intramolecular hydrogen bond in the structural framework. Conversely, the 3,5-difluoro substituted LFM analog had an intramolecular hydrogen bond common to the other halide substituted derivatives. The anomaly exhibited by the 2,5-difluoro and the 2-fluoro substituted compounds may be owing to the smaller size of fluorine atom in comparison with the chlorine and bromine atoms in the structures of the other analogs. The presence of a fluorine at the 2-position of the phenyl ring may disrupt the intermolecular hydrogen bonding that was observed for the other derivatives due to differences in the crystal packing for these molecules.

Keywords: Leflunomides; Crystal structures; Fluorine atom; Hydrogen bonding

1. Introduction

Apoptosis is a common mode of eukaryotic cell death that is triggered by an inducible cascade of biochemical events leading to the activation of endonucleases that cleave the nuclear DNA into oligonucleosome length fragments [1–4]. Several biochemical events contribute to apoptotic cell death. An improved understanding of the molecular basis of apoptosis and the pro-apoptotic versus anti-apoptotic regulatory signals may provide further insight into the pathogenesis of human lymphoid malignancies. Selective inhibitors of the anti-apoptotic tyrosine kinase, BTK hold promise as a new generation of anti-leukemic agents with apoptosis promoting and chemosensitizing properties. The epidermal growth factor receptor (EGFR) is a membrane-associated tyrosine kinase that serves as an endogenous negative regulator of apoptosis in breast cancer cells [5]. Consequently, the development of new potent anti-breast cancer agents has emerged as an important focal point for translational research in the treatment of breast cancer [6]. Recently, compound A77 1726, which is the primary metabolite of the isoxazole leflunomide [*N*-(4-trifluoromethylphenyl)-5-methylisoxazol-4-carboxamide), was shown to possess anti-inflammatory activity with pleiotropic effects [7–10]. Mattar et al. [11] also provided experimental evidence that the above compound inhibited EGFR kinase in micromolar concentrations.

In a systematic search for potent anti-cancer agents, we have identified several structurally distinct LFM analogs as potent inhibitors of tyrosine kinases. In the previous studies, we have examined the X-ray crystal structures of a few LFM analogs [12–16]. In this paper we report a detailed study on

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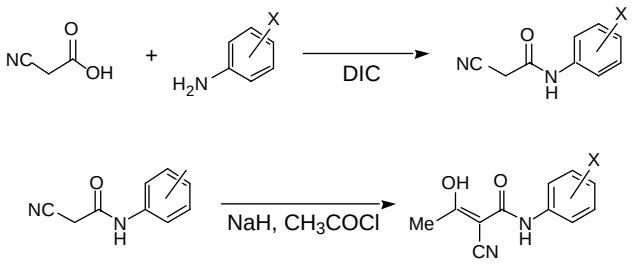
doi:10.1016/j.molstruc.2005.02.024



Scheme 1. General synthetic scheme for leflunomide derivatives.

the X-ray crystal structures of several of these LFM analogs and the influence of their structural differences on the anisotropy in intermolecular hydrogen bonding characteristics.

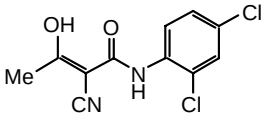
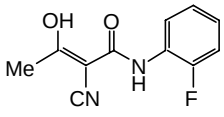
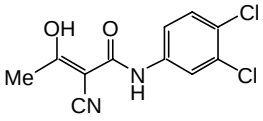
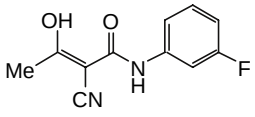
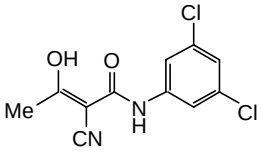
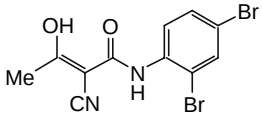
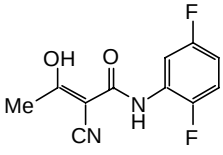
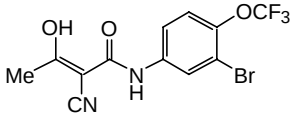
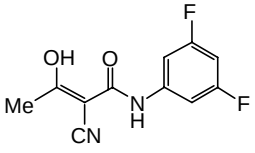
2. Experimental

All chemicals were purchased from Aldrich (Milwaukee, WI) and were used without further purification. Unless otherwise noted, each reaction vessel was secured with a rubber septum, and the reaction was performed under a nitrogen atmosphere. The leflunomide derivatives were prepared using previously published data [13]. The prepared compounds were purified using column chromatography on silica gel, followed by recrystallization. Samples were identified by NMR, IR and UV spectroscopy, mass spectrometry, and analytical HPLC. The ^{1}H and ^{13}C NMR spectra were obtained on a Varian Mercury 300 instrument at ambient temperature in DMSO-d_6. FT-IR spectra were recorded on a Nicolet Protégé 460 spectrometer between 600 and 4000 cm^{-1} as KBr pellets. UV spectra were recorded from a Beckmann DU 7400 UV/Vis spectrometer using a cell path length of 1 cm in methanol. Mass spectra were performed on a Hewlett Packard MALDI-TOF spectrometer (Model G2025A LD-TOF) using dithranol as the supporting matrix. Melting points were determined using a Melt John's apparatus and are uncorrected. HPLC data was collected using a Hewlett Packard 1100 series instrument consisting of an automatic sampler, an electronic degasser, a thermostatic control unit, and a diode array detector in conjunction with a Chemstation software assembly. The column was an analytical RP-18 Lichrospher column (4.6×250 mm^2, 5 μm particle size) and eluent was H_2O (0.1% ACOH):acetonitrile (35:65). The flow rate was maintained at 1.0 ml/min and the detection wavelength was set at 275 nm. The column was maintained at room temperature throughout the analysis.

A detailed procedure for the synthesis of **8** is provided below, and should be considered representative for the synthesis of the lefunomide derivatives presented in this paper.

Synthesis of (2*Z*)-*N*-(2,4-dibromophenyl)-2-cyano-3-hydroxybut-2-enamide (**8**). In a 250 ml RB flask, under a nitrogen atmosphere, cyanoacetic acid (3.4 g, 40 mmol) and 2,4-dibromoaniline (11.0 g, 44 mmol) were fully dissolved in anhydrous tetrahydrofuran (80 ml). To this

Table 1
Structures of LEF compounds

Compound	Structure	Compound	Structure
1		**6**	
2		**7**	
3		**8**	
4		**9**	
5		**10**