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α-Cyano-*N*-(2,5-dibromophenyl)-β-hydroxybut-2-enamide

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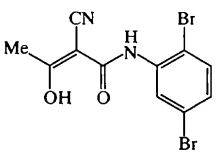
Abstract

The title compound, $C_{11}H_8Br_2N_2O_2$ (LFM-A13), is the first reported BTK-specific tyrosine kinase inhibitor and the first antileukemic agent targeting BTK (Bruton's tyrosine kinase). The crystal structure showed that the molecule adopts an approximately planar conformation aided by an intramolecular hydrogen bond formed between the hydroxyl group and the amide carbonyl group. The dihedral angle between the phenyl ring and the plane defined by the N—C—C=C—CH_3 group is 8.2 (6)°. The crystal packing shows an additional weak intermolecular hydrogen bond between the hydroxyl group and the amide carbonyl-O atom of the centrosymmetrically related molecule.

Comment

Selective inhibitors of the anti-apoptotic tyrosine kinase, BTK, hold promise as a new generation of antileukemic agents with apoptosis-promoting and chemosensitizing properties. As part of our on-going program in structure-based design of BTK inhibitors, we have designed and synthesized a novel leflunomide metabolite (LFM) analog, LFM-A13, targeting the ATP (adenosine triphosphate) binding site of BTK. The compound was designed based on a constructed three-dimensional



LFM-A13

homology model of the BTK kinase domain (Mahajan *et al.*, 1999). A model of the BTK binding pocket was used in combination with advanced docking procedures to predict the favorable placement of chemical groups with defined sizes at multiple modification sites on the parent compound, LFM. The title compound, LFM-A13, for which we estimated a binding constant (K_i) value of 1.4 μ*M*, subsequently inhibited human BTK *in vitro* with an inhibition (IC_{50}) value of 17.1±0.8 μ*M* and recombinant BTK expressed in a baculovirus expression vector system with an IC_{50} value of 2.5 μ*M*. Besides its remarkable potency in BTK kinase assays, LFM-A13 was also discovered to be a highly specific inhibitor of BTK. Even at concentrations as high as 100 μg/ml (~278 μ*M*) this novel inhibitor did not affect the enzymatic activity of other protein tyrosine kinases, including Janus kinase 1 (JAK1), Janus kinase 3 (JAK3), hematopoietic cell kinase (HCK), EGF receptor kinase (EGFR), and insulin-receptor kinase (IRK). The structural study of the title compound, LFM-A13, unequivocally established its connectivity and showed that its molecular conformation is very similar to its corresponding energy-minimized molecular coordinates which were generated and used for docking studies with BTK. To our knowledge, α-cyano-*N*-(2,5-dibromophenyl)-β-hydroxybut-2-enamide is the first reported BTK-specific tyrosine kinase inhibitor and the first antileukemic agent targeting BTK.

The crystallographic numbering scheme and the conformation adopted by the molecule are shown in Fig. 1. The structure is approximately planar and all bond distances and angles agree well with values for similar types of bonds reported in the Cambridge Structural Database (Allen & Kennard, 1993). The intramolecular hydrogen-bond distance between O7 and O9 is 2.553 (5) Å, and the intermolecular hydrogen-bond distance between O9 and O7($-x$, $1-y$, $-z$) is 2.872 (5) Å.

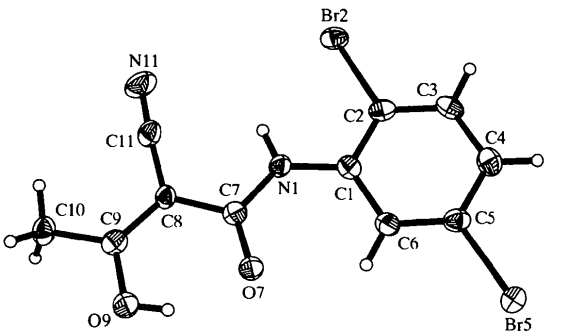


Fig. 1. The structure of the title compound showing 50% probability displacement ellipsoids and the atom-numbering scheme. H atoms are displayed as small circles of an arbitrary radius.

Experimental

1,3-Diisopropylcarbodiimide (6.3 ml, 40 mmol) was added to a solution of cyanoacetic acid (3.4 g, 40 mmol) and 2,5-dibromoaniline (11.0 g, 44 mmol) in dry tetrahydrofuran (80 ml). The mixture was stirred overnight at room temperature. The urea precipitate was removed by filtration and partitioned between ethyl acetate and 0.5 *N* HCl. The organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated by rotary evaporation. Recrystallization from ethyl alcohol gave 12.1 g of the crude product with 95% yield. Next, sodium hydride (3.1 g, 77 mmol) was added carefully to the solution of the crude compound (11.1 g, 35 mmol) in dry tetrahydrofuran (250 ml) at 273 K and the mixture was

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stirred for 1 h at room temperature. Acetyl chloride (3.0 ml, 42 mmol) was added slowly to the reaction mixture at 273 K. The reaction was continued at room temperature for 1 h and was quenched by adding acetic acid (5 ml). The mixture was poured into ice water (500 ml) containing hydrochloric acid (12.5 ml) to precipitate the crude product which was collected by filtration and washed with water. Recrystallization from alcohol gave 9.8 g of the final product with 78% yield. Single crystals of the compound were obtained by slow evaporation from acetonitrile.

Crystal data

$C_{11}H_8Br_2N_2O_2$
M_r = 360.01
Monoclinic
$P2_1/c$
a = 5.6134 (1) Å
b = 9.9847 (3) Å
c = 21.5896 (2) Å
β = 93.639 (1)°
V = 1207.62 (4) Å^3
Z = 4
D_x = 1.980 Mg m^{-3}
D_m not measured

Mo $K\alpha$ radiation
λ = 0.71073 Å
Cell parameters from 3648 reflections
θ = 1.89–25.01°
μ = 6.70 mm^{-1}
T = 173 (2) K
Needle
0.45 × 0.09 × 0.05 mm
Colorless

Data collection

Siemens SMART Platform CCD diffractometer
ω scans
Absorption correction: empirical (*SHELXTL-Plus*; Sheldrick, 1996)
T_{min} = 0.433, T_{max} = 0.715
5918 measured reflections

2107 independent reflections
1700 reflections with $I > 2\sigma(I)$
R_{int} = 0.040
θ_{max} = 25.01°
$h = -6 \rightarrow 6$
$k = -10 \rightarrow 11$
$l = -24 \rightarrow 25$

Refinement

Refinement on F^2
$R(F)$ = 0.040
$wR(F^2)$ = 0.10
S = 1.007
2107 reflections
155 parameters
H-atom parameters constrained
$w = 1/[\sigma^2(F_o^2) + (0.0573P)^2]$ where $P = (F_o^2 + 2F_c^2)/3$

$(\Delta/\sigma)_{max}$ = 0.001
$\Delta\rho_{max}$ = 0.76 e Å^{-3}
$\Delta\rho_{min}$ = −1.038 e Å^{-3} (0.86 Å from Br2)
Extinction correction: none
Scattering factors from *International Tables for Crystallography* (Vol. C)

Table 1. *Selected geometric parameters (Å, °)*

Br2—C2	1.899 (4)	N11—C11	1.146 (6)
Br5—C5	1.913 (5)	C7—C8	1.461 (6)
O7—C7	1.239 (5)	C8—C9	1.372 (7)
O9—C9	1.328 (6)	C8—C11	1.438 (6)
N1—C7	1.361 (6)	C9—C10	1.478 (7)
N1—C1	1.406 (6)		
C7—N1—C1	129.3 (4)	N1—C7—C8	116.0 (4)
C6—C1—N1	123.4 (4)	C9—C8—C11	118.2 (4)
C2—C1—N1	117.9 (4)	C9—C8—C7	121.3 (4)
C3—C2—Br2	118.1 (4)	C11—C8—C7	120.5 (4)
C1—C2—Br2	120.6 (4)	O9—C9—C8	121.5 (4)
C6—C5—Br5	119.3 (4)	O9—C9—C10	113.0 (4)
C4—C5—Br5	117.2 (4)	C8—C9—C10	125.5 (4)
O7—C7—N1	123.2 (4)	N11—C11—C8	175.8 (6)
O7—C7—C8	120.8 (4)		

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Data collection: *SMART* (Siemens, 1996). Cell refinement: *SAINT* (Siemens, 1996). Data reduction: *SAINT*. Program(s) used to solve structure: *SHELXTL-Plus* (Sheldrick, 1996). Program(s) used to refine structure: *SHELXTL-Plus*. Molecular graphics: *SHELXTL-Plus*. Software used to prepare material for publication: *SHELXTL-Plus*.

Supplementary data for this paper are available from the IUCr electronic archives (Reference: FR1196). Services for accessing these data are described at the back of the journal.

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Neotame, an alkylated dipeptide and high intensity sweetener

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Abstract

The title compound, *N*-[*N*-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl ester (neotame) hydrate, $C_{20}H_{30}N_2O_5 \cdot H_2O$, is an alkylated dipeptide. The zwitterionic structure, *i.e.* 3-(3,4-dimethylbutylammonio)-3-[*N*-(1-methoxycarbonyl-2-phenylethyl)aminocarbonyl]propanoate hydrate, in the solid state fits the 'L-shaped' topochemical structure proposed for the requirement of sweetness ability for aspartyl-based dipeptide compounds. The structure suggests that the extraordinary potency of neotame is due to the hydrophobic positioning of the alkyl and phenyl groups within the molecule. In this crystalline form, obtained from ethyl acetate/hexane, a single water molecule is also