Acta Crystallographica Section C
Crystal Structure Communications
ISSN 0108-2701

Three leflunomide metabolite analogs

Sutapa Ghosh,[a]* Jason D. Jennissen,[a] Yaguo Zheng[b] and Fatih M. Uckun[c]

[a]Department of Structural Biology (Drug Discovery Program), Parker Hughes Institute, 2665 Long Lake Road, Suite 330, St Paul, MN 55113, USA, [b]Department of Chemistry, Parker Hughes Institute, 2665 Long Lake Road, Suite 330, St Paul, MN 55113, USA, and [c]Parker Hughes Cancer Center (Drug Discovery Program), Parker Hughes Institute, 2665 Long Lake Road, Suite 330, St Paul, MN 55113, USA
Correspondence e-mail: sghosh@ih.org

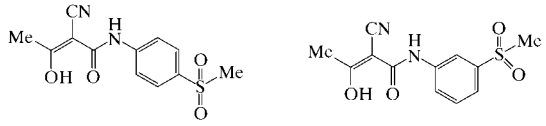
Received 5 April 2000
Accepted 13 July 2000

The title compounds, 1-cyano-2-hydroxy-*N*-[4-(methylsulfonyl)phenyl]but-2-enamide, $C_{12}H_{12}N_2O_4S$, PHI492, 1-cyano-2-hydroxy-*N*-[3-(methylsulfonyl)phenyl]but-2-enamide, $C_{12}H_{12}N_2O_4S$, PHI493, and *N*-[3-bromo-4-(trifluoromethoxy)-phenyl]-1-cyano-2-hydroxybut-2-enamide, $C_{12}H_8BrF_3N_2O_3$, PHI495, are potent inhibitors of Bruton's tyrosine kinase (BTK). The molecular structures of these compounds are similar and they display similar hydrogen-bonding networks and crystal packing. Examination of the crystal-packing interaction in the three compounds reveals an alternating direction of adjacent molecules in the crystalline lattice due to intermolecular cyano–amide hydrogen bonding. PHI492, a positional isomer of PHI493, does not form intermolecular O—H···O hydrogen bonds between molecules and crystallizes in a space group different from that of PHI493 and PHI495. The aromatic ring and the amide group of each molecule form a conjugated π-system which ensures planarity, with further stabilization gained from intramolecular O—H···O hydrogen bonds.

Comment

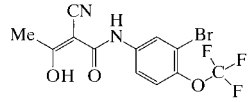
The title compounds were designed and synthesized as part of our ongoing program on the development of tyrosine kinase inhibitors as anticancer agents. The development of inhibitors of the PTK signaling pathway is an active area of translational cancer research. The identification of the compounds was guided by structure-based drug design methods, which included the construction of the kinase homology model of BTK (Mahajan *et al.*, 1999) and advanced docking procedures to generate novel molecules which are complementary in shape and electrostatics to the kinase domain topography. Based on these modeling studies, several leflunomide metabolite (LFM) analogs were synthesized and tested for their kinase inhibitory activity on BTK. The three title compounds, PHI492, PHI493 and PHI495, were subsequently found to have micromolar potency towards BTK.

The X-ray crystal structures of PHI492, PHI493 and PHI495 (Figs. 1–3) show that all three molecules contain an intramolecular O—H···O hydrogen bond that locks the compounds in a planar conformation. The presence of this intramolecular hydrogen bond is consistent with the results from docking studies of LFM analogs at the catalytic sites of several protein



PHI492

PHI493



PHI495

tyrosine kinases (Ghosh *et al.*, 1998; Ghosh, Narla *et al.*, 1999; Mahajan *et al.*, 1999). These studies reveal that the planar conformation of these inhibitors resulting from the intramolecular hydrogen bond would allow the molecules to fit snugly into the shallow catalytic sites of EGFR (epidermal growth factor receptor) and BTK. This binding mode is such that the title compounds can maintain close contact with the hinge region of the receptor on the edge of the inhibitor, and the aromatic rings of the inhibitors are sandwiched between hydrophobic residues at the catalytic site of the receptor. The electronegative SO_2 group of PHI492 and PHI493 is involved in hydrogen bonding with Lys430 and Arg525, respectively, on BTK, while in PHI495 the CF_3 group is oriented towards Val416.

In the crystal lattice PHI492 packs as discrete hydrogen-bonded dimers about inversion centers, whereas in PHI493 and PHI495, the hydrogen-bonded dimers form infinite chains through additional hydrogen bonding with neighboring molecules. Compound PHI492 does not display O—H···O intermolecular hydrogen bonds and the intramolecular O9···O7 distance is 2.534 (2) Å. The compound forms a centrosymmetrically related dimer *via* an N1—H1···N11^{i} intermolecular hydrogen bond [N1···N11($1-x, 4-y, -z$) = 3.132 (2) Å]. In compound PHI493, the intramolecular O9···O7 hydrogen-bond distance is 2.510 (2) Å and the dimers are linked by an N1···N11($-1-x, 2-y, 1-z$) intermolecular hydrogen bond [3.060 (2) Å] and an O7···O9($-x, 3-y, 1-z$) intermolecular contact

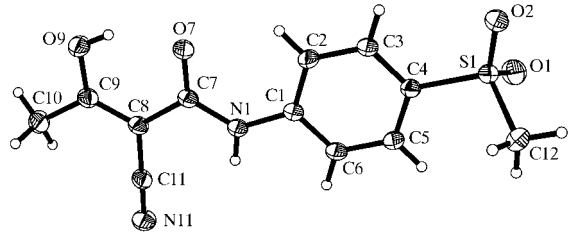


Figure 1
The structure of PHI492 showing the atomic numbering scheme. Displacement ellipsoids are drawn at the 30% probability level and H atoms are displayed as small circles of arbitrary radii.

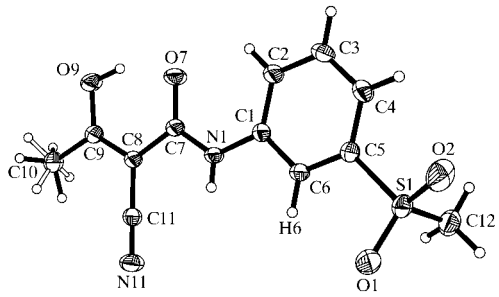


Figure 2
The structure of PHI493 showing the atomic numbering scheme. Displacement ellipsoids are drawn at the 30% probability level and H atoms are displayed as small circles of arbitrary radii.

[3.046 (2) Å]. In PHI495, the intramolecular O9···O7 distance is 2.508 (4) Å and the dimers are linked by an N1···N11($2-x$, $1-y$, $-z$) intermolecular hydrogen bond [3.218 (5) Å] and an O9···O7($1-x$, $-y$, $-z$) intermolecular contact [3.027 (4) Å]. The discrete hydrogen-bonded dimer pattern found in the crystal lattice of PHI492, has also been observed in the crystal structures of the leflunomide metabolite analogs LFM-A1, LFM-A10, LFM-A11 (Ghosh, Zheng & Uckun, 1999) and LFM-A13 (Ghosh & Uckun, 1999) reported earlier; whereas the chain pattern found in the crystal lattice of PHI493 and PHI495 has also been observed in the LFM-A9 crystal lattice (Ghosh, Zheng & Uckun, 1999).

The dihedral angle of the plane of the phenyl ring with the plane formed by atoms N1, C7, C8, C9 and C10 is 2.3 (3) for PHI492, 2.4 (3) for PHI493 and 2.5 (7)° for PHI495. The planarity of the molecules is extended to the hydroxyl, cyano and methyl groups *via* the intramolecular O9—H9···O7 hydrogen bond. The mean-plane deviation for each of the molecules as a whole is 0.0422 for PHI492, 0.0368 for PHI493 and 0.0372 Å for PHI495.

There is no significant difference in the corresponding bond lengths and angles in the three structures. However, the C8—C11 (Csp^2—Csp) bond distances are 1.424 (3) for PHI492, 1.424 (2) for PHI493 and 1.427 (6) Å for PHI495, and are slightly longer than the expected Csp^2—Csp bond length of 1.416 Å. The C11≡N11 bond distances, on the other hand, are significantly shorter than the expected C≡N bond length of 1.165 Å; they are 1.138 (2) for PHI492, 1.136 (2) for PHI493 and 1.134 (5) Å for PHI495. A similar lengthening of the C8—C11 bond and shortening of the C≡N bond has been observed in the molecular structures of the six LFM analogs reported earlier (Ghosh, Zheng & Uckun, 1999; Ghosh & Uckun, 1999).

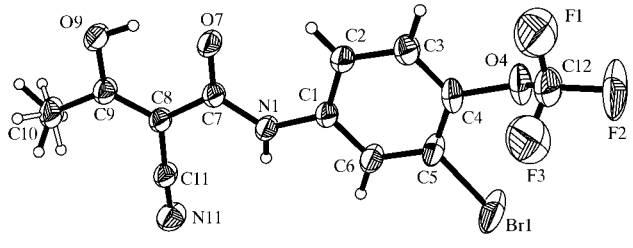


Figure 3
The structure of PHI495 showing the atomic numbering scheme. Displacement ellipsoids are drawn at the 30% probability level and H atoms are displayed as small circles of arbitrary radii.

Experimental

The title compounds were synthesized according to the methods of Sjogren *et al.* (1991) and Kuo *et al.* (1996). Cyanoacetic acid was coupled with a desired substituted aniline in the presence of 1,3-diisopropylcarbodiimide (DIC) to form a cyanoacetamide. Treatment of the amide with NaH followed by acylation with acetyl chloride provided an α-cyano-β-hydroxy-β-methylpropenamide. Methylsulfonyl was obtained by oxidation of methylthio in the presence of peracetic acid. PHI492 was crystallized through slow evaporation from an ethanol/dichloromethane/dimethylformamide solution (3:2:1 *v*/*v*/*v*); colorless prisms, suitable for diffraction, were obtained after 27 d. PHI493 was crystallized as colorless needles through slow evaporation from tetrahydrofuran. PHI495 was dissolved in acetonitrile and crystallized through vapor diffusion of diethyl ether at 277 K; colorless prisms, suitable for diffraction, had grown after 18 d.

Compound PHI492

Crystal data

$C_{12}H_{12}N_2O_4S$	D_x = 1.492 Mg m^{-3}
M_r = 280.30	Mo $K\alpha$ radiation
Monoclinic, $P2_1/n$	Cell parameters from 5320 reflections
a = 8.8002 (10) Å	θ = 1.59–26.40°
b = 5.5336 (6) Å	μ = 0.271 mm^{-1}
c = 25.852 (3) Å	T = 295 (2) K
β = 97.6440 (10)°	Prism, colorless
V = 1247.7 (2) Å^3	0.34 × 0.31 × 0.28 mm
Z = 4	

Data collection

Bruker SMART CCD area-detector diffractometer	2503 independent reflections
	2157 reflections with $I > 2\sigma(I)$
ω scans	R_{int} = 0.030
Absorption correction: empirical (*SADABS*; Sheldrick, 1996)	θ_{max} = 26.40°
	$h = -11 \rightarrow 10$
T_{min} = 0.913, T_{max} = 0.928	$k = -6 \rightarrow 6$
10 595 measured reflections	$l = -32 \rightarrow 31$

Table 1
Selected geometric parameters (Å, °) for PHI492.

S1—O2	1.4336 (15)	N1—C1	1.412 (2)
S1—O1	1.4339 (16)	N11—C11	1.138 (2)
S1—C4	1.7596 (19)	C7—C8	1.472 (3)
S1—C12	1.760 (2)	C8—C9	1.366 (3)
O7—C7	1.239 (2)	C8—C11	1.424 (3)
O9—C9	1.313 (2)	C9—C10	1.486 (3)
N1—C7	1.353 (3)		
O2—S1—O1	117.86 (10)	O7—C7—N1	123.37 (17)
O2—S1—C4	108.26 (9)	O7—C7—C8	119.91 (17)
O1—S1—C4	109.02 (9)	N1—C7—C8	116.72 (16)
O2—S1—C12	108.63 (11)	C9—C8—C11	117.63 (17)
O1—S1—C12	108.03 (11)	C9—C8—C7	120.73 (17)
C4—S1—C12	104.20 (10)	C11—C8—C7	121.64 (17)
C7—N1—C1	128.78 (16)	O9—C9—C8	122.25 (18)
C6—C1—N1	116.07 (16)	O9—C9—C10	114.07 (18)
C2—C1—N1	124.71 (17)	C8—C9—C10	123.69 (18)
C3—C4—S1	120.80 (14)	N11—C11—C8	178.9 (2)
C5—C4—S1	119.07 (14)		

Refinement

Refinement on F^2
$R(F) = 0.044$
$wR(F^2) = 0.113$
$S = 1.07$
2503 reflections
182 parameters
2 restraints
H atoms treated by a mixture of independent and constrained refinement
$w = 1/[\sigma^2(F_o^2) + (0.0570P)^2 + 0.5101P]$ where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max} = 0.003$
$\Delta\rho_{max} = 0.23$ e Å^{-3}
$\Delta\rho_{min} = -0.45$ e Å^{-3}

Table 2
Hydrogen-bonding geometry (Å, °) for PHI492.

D—H···A	D—H	H···A	D···A	D—H···A
O9—H9···O7	0.819 (10)	1.800 (18)	2.534 (2)	148 (3)
N1—H1···N11^{i}	0.851 (10)	2.307 (11)	3.132 (2)	164 (2)

Symmetry code: (i) $1-x, 4-y, -z$.

Compound PHI493

Crystal data

$C_{12}H_{12}N_2O_4S$
$M_r = 280.30$
Triclinic, $P\overline{1}$
$a = 5.4734$ (3) Å
$b = 11.4048$ (6) Å
$c = 11.5200$ (6) Å
$\alpha = 115.4780$ (10)°
$\beta = 95.9410$ (10)°
$\gamma = 93.0350$ (10)°
$V = 641.88$ (6) Å^3
$Z = 2$
$D_x = 1.450$ Mg m^{-3}
Mo $K\alpha$ radiation
Cell parameters from 4558 reflections
$\theta = 1.98$–25.68°
$\mu = 0.264$ mm^{-1}
$T = 295$ (2) K
Needle, colorless
0.55 × 0.35 × 0.17 mm

Data collection

Bruker SMART CCD area-detector diffractometer
ω scans
Absorption correction: empirical (*SADABS*; Sheldrick, 1996)
$T_{min} = 0.869$, $T_{max} = 0.957$
6510 measured reflections
2428 independent reflections
2152 reflections with $I > 2\sigma(I)$
$R_{int} = 0.022$
$\theta_{max} = 25.68$°
$h = -6 \rightarrow 6$
$k = -13 \rightarrow 13$
$l = -14 \rightarrow 14$

Table 3
Selected geometric parameters (Å, °) for PHI493.

S1—O2	1.4312 (14)	O7—C7	1.2472 (19)
S1—O1	1.4360 (14)	O9—C9	1.316 (2)
S1—C12	1.750 (2)	C7—C8	1.463 (2)
S1—C5	1.7768 (16)	C8—C9	1.374 (2)
N1—C7	1.354 (2)	C8—C11	1.425 (2)
N1—C1	1.412 (2)	C9—C10	1.483 (2)
N11—C11	1.136 (2)		
O2—S1—O1	118.71 (10)	O7—C7—N1	122.34 (14)
O2—S1—C12	108.16 (10)	O7—C7—C8	119.39 (14)
O1—S1—C12	107.80 (10)	N1—C7—C8	118.27 (13)
O2—S1—C5	107.75 (8)	C9—C8—C11	117.83 (14)
O1—S1—C5	108.60 (8)	C9—C8—C7	120.80 (14)
C12—S1—C5	105.01 (9)	C11—C8—C7	121.35 (14)
C7—N1—C1	127.91 (13)	O9—C9—C8	121.60 (15)
C2—C1—N1	124.02 (14)	O9—C9—C10	114.07 (14)
C6—C1—N1	116.97 (13)	C8—C9—C10	124.33 (15)
C6—C5—S1	119.22 (12)	N11—C11—C8	179.7 (2)
C4—C5—S1	118.73 (12)		

Refinement

Refinement on F^2
$R(F) = 0.034$
$wR(F^2) = 0.102$
$S = 1.05$
2428 reflections
182 parameters
2 restraints
H atoms treated by a mixture of independent and constrained refinement
$w = 1/[\sigma^2(F_o^2) + (0.0546P)^2 + 0.1902P]$ where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max} = 0.002$
$\Delta\rho_{max} = 0.22$ e Å^{-3}
$\Delta\rho_{min} = -0.28$ e Å^{-3}

Table 4
Hydrogen-bonding geometry (Å, °) for PHI493.

D—H···A	D—H	H···A	D···A	D—H···A
N1—H1···N11^{i}	0.844 (9)	2.239 (11)	3.060 (2)	164.2 (19)
O9—H9···O7	0.833 (10)	1.749 (15)	2.5102 (17)	151 (2)
O9—H9···O7^{ii}	0.833 (10)	2.57 (2)	3.0464 (18)	117.5 (19)

Symmetry codes: (i) $-1-x, 2-y, 1-z$; (ii) $-x, 3-y, 1-z$.

Compound PHI495

Crystal data

$C_{12}H_8BrF_3N_2O_3$
$M_r = 365.11$
Triclinic, $P\overline{1}$
$a = 5.2834$ (4) Å
$b = 10.8265$ (8) Å
$c = 13.1634$ (10) Å
$\alpha = 69.0090$ (10)°
$\beta = 81.0850$ (10)°
$\gamma = 83.2380$ (10)°
$V = 692.91$ (9) Å^3
$Z = 2$
$D_x = 1.750$ Mg m^{-3}
$D_m = 1.76$ (2) Mg m^{-3}
D_m measured by flotation in phenyl iodide and hexane
Mo $K\alpha$ radiation
Cell parameters from 1906 reflections
$\theta = 1.67$–25.74°
$\mu = 3.012$ mm^{-1}
$T = 295$ (2) K
Needle, colorless
0.45 × 0.11 × 0.10 mm

Data collection

Bruker SMART CCD area-detector diffractometer
ω scans
Absorption correction: empirical (*SADABS*; Sheldrick, 1996)
$T_{min} = 0.344$, $T_{max} = 0.753$
7235 measured reflections
2643 independent reflections
1364 reflections with $I > 2\sigma(I)$
$R_{int} = 0.037$
$\theta_{max} = 25.74$°
$h = -6 \rightarrow 6$
$k = -13 \rightarrow 13$
$l = -16 \rightarrow 16$
Intensity decay: 0.05%

Table 5
Selected geometric parameters (Å, °) for PHI495.

Br1—C5	1.884 (4)	N1—C7	1.349 (5)
F1—C12	1.288 (7)	N1—C1	1.415 (5)
F2—C12	1.305 (6)	N11—C11	1.134 (5)
F3—C12	1.286 (7)	C7—C8	1.469 (5)
O4—C12	1.319 (7)	C8—C9	1.364 (5)
O4—C4	1.418 (5)	C8—C11	1.427 (6)
O7—C7	1.241 (5)	C9—C10	1.479 (6)
O9—C9	1.320 (5)		
C12—O4—C4	117.6 (4)	C9—C8—C7	121.1 (4)
C7—N1—C1	128.8 (4)	C11—C8—C7	120.2 (3)
C6—C1—N1	116.4 (4)	O9—C9—C8	121.0 (4)
C2—C1—N1	124.1 (4)	O9—C9—C10	114.0 (4)
C3—C4—O4	121.6 (4)	C8—C9—C10	125.1 (4)
C5—C4—O4	118.2 (4)	N11—C11—C8	177.9 (4)
C4—C5—Br1	121.4 (3)	F3—C12—F1	105.1 (7)
C6—C5—Br1	118.8 (3)	F3—C12—F2	108.6 (6)
O7—C7—N1	122.7 (4)	F1—C12—F2	106.6 (5)
O7—C7—C8	119.7 (3)	F3—C12—O4	114.2 (5)
N1—C7—C8	117.5 (4)	F1—C12—O4	113.7 (6)
C9—C8—C11	118.7 (3)	F2—C12—O4	108.2 (6)

Refinement

Refinement on F^2	$w = 1/[\sigma^2(F_o^2) + (0.0639P)^2]$ where $P = (F_o^2 + 2F_c^2)/3$
$R(F) = 0.045$	$(\Delta/\sigma)_{max} = 0.004$
$wR(F^2) = 0.131$	$\Delta\rho_{max} = 0.61$ e Å^{-3}
$S = 1.01$	$\Delta\rho_{min} = -0.51$ e Å^{-3}
2643 reflections	
200 parameters	
H atoms treated by a mixture of independent and constrained refinement	

Table 6
Hydrogen-bonding geometry (Å, °) for PHI495.

D—H···A	D—H	H···A	D···A	D—H···A
O9—H9···O7	0.89 (6)	1.70 (6)	2.508 (4)	150 (5)
O9—H9···O7^{i}	0.89 (6)	2.52 (5)	3.027 (4)	117 (4)
N1—H1···N11^{ii}	0.82 (3)	2.44 (4)	3.218 (5)	161 (3)

Symmetry codes: (i) $1-x, -y, -z$; (ii) $2-x, 1-y, -z$.

The H atoms attached to the N and O atoms for all three compounds appeared well resolved in the difference Fourier maps but, with the exception of PHI495, proved difficult to refine freely. The positions of the hydroxyl and amide protons in PHI492 and PHI493 were refined by restrained methods using the *DFIX* command in *SHELXL*97 (Sheldrick, 1997), where the distances were restrained to 0.82 and 0.86 Å, respectively, and their U_{eq} values were allowed to refine freely. All H atoms attached to C atoms were placed in ideal positions and refined using a riding model with aromatic C—H = 0.96 Å and methyl C—H = 0.98 Å, and with fixed isotropic displacement parameters equal to 1.2 (1.5 for methyl-H atoms) times the equivalent isotropic displacement parameter of the atom to which they were attached. The methyl groups were allowed to rotate about their local threefold axis during refinement. The H atoms in the C10 methyl group of PHI493 and PHI495 were found to be rotationally disordered, in a 0.6:0.4 ratio in PHI493 and in a 0.3:0.7 ratio in PHI495. The major features in the final difference map of PHI495 were $\leq$1.1 Å from the Br atom.

For all compounds, data collection: *SMART* (Bruker, 1998); cell refinement: *SAINT* (Bruker, 1998); data reduction: *SAINT*; program(s) used to solve structure: *SHELXS*97 (Sheldrick, 1990); program(s) used to refine structure: *SHELXL*97 (Sheldrick, 1997); molecular graphics: *SHELXTL* (Bruker, 1997); software used to prepare material for publication: *SHELXTL*.

Supplementary data for this paper are available from the IUCr electronic archives (Reference: BK1544). Services for accessing these data are described at the back of the journal.

References

Bruker (1997). *SHELXTL*. Version 5.1. Bruker AXS Inc., Madison, Wisconsin, USA.
Bruker (1998). *SMART* and *SAINT*. Bruker AXS Inc., Madison, Wisconsin, USA.
Ghosh, S., Narla, R. K., Zheng, Y., Liu, X.-P., Jun, X., Mao, C., Sudbeck, E. A. & Uckun, F. M. (1999). *Anti-Cancer Drug Des.* **14**, 403–410.
Ghosh, S. & Uckun, F. M. (1999). *Acta Cryst.* C**55**, 1364–1365.
Ghosh, S., Zheng, Y., Jun, X., Narla, R. K., Mahajan, S., Navara, C., Mao, C., Sudbeck, E. A. & Uckun, F. M. (1998). *Clin. Cancer Res.* **4**, 2657–2668.
Ghosh, S., Zheng, Y. & Uckun, F. M. (1999). *Acta Cryst.* C**55**, 2117–2122.
Kuo, E. A., Hambleton, P. T., Kay, D. P., Evans, P. L., Matharu, S. S., Little, E., McDowall, N., Jones, C. B., Hedgecock, C. J., Yea, C. M., Chan, A. W., Hairsine, P. W., Ager, I. R., Tully, W. R., Williamson, R. A. & Westwood, R. (1996). *J. Med. Chem.* **39**, 4608–4621.
Mahajan, S., Ghosh, S., Sudbeck, E. A., Zheng, Y., Downs, S., Hupke, M. & Uckun, F. M. (1999). *J. Biol. Chem.* **274**, 9587–9599.
Sheldrick, G. M. (1990). *Acta Cryst.* A**46**, 467–473.
Sheldrick, G. M. (1996). *SADABS*. University of Göttingen, Germany.
Sheldrick, G. M. (1997). *SHELXL*97. University of Göttingen, Germany.
Sjogren, E. B., Rider, M. A., Nelson, P. H. Jr, Bingham, S., Poulton, A. L., Emanuel, M. A. & Komuniecki, R. (1991). *J. Med. Chem.* **34**, 3295–3301.