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The Design and Synthesis of Novel Hydroxyproline Inhibitors of HIV-1 Proteinase

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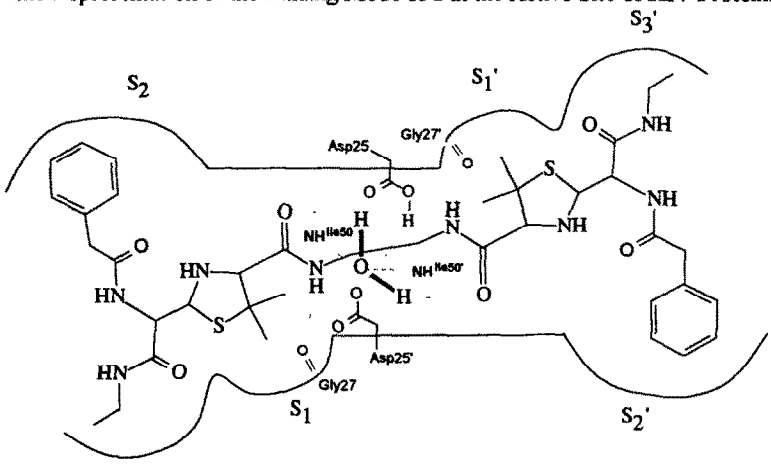
Abstract. The synthesis and activity of novel hydroxyproline inhibitors of HIV-1 proteinase is described. Compound (R)-**4** was a potent inhibitor, IC_{50} = 0.07 μM, however it is likely that the thiazolidine moiety rather than the novel hydroxyproline group is the primary determinant of binding.

Human immunodeficiency virus (HIV) is the causative agent of acquired immunodeficiency syndrome (AIDS)[1]. Inhibition of the virally encoded proteinase is recognised as an important therapeutic target for the treatment of AIDS[2]. The enzyme is a member of the aspartyl proteinase family[3] and has been shown to be a C_2 symmetric homodimer[4].

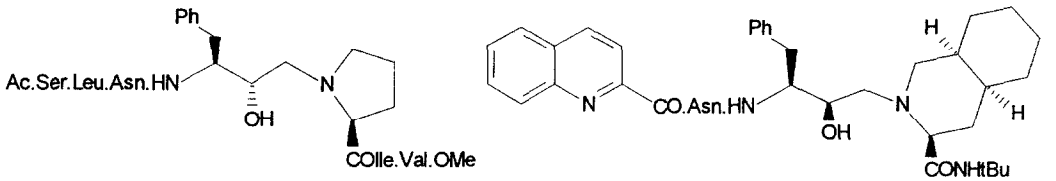
Identification, through random screening, of a penicillin derived inhibitor of HIV proteinase, led to a series of potent inhibitors based on compound **1**[5], Figure 1. Determination of the structure of a co-crystal of a HIV proteinase-inhibitor complex confirmed the symmetric binding mode of these inhibitors[5], Figure 1. This paper describes the design and synthesis of a novel alternative to one of the thiazolidine units in **1**, thus introducing groups to interact with the catalytic Asps and both the lipophilic pockets S_1' and S_2'.

Figure 1: Schematic Representation of the Binding Mode of **1** at the Active Site of HIV Proteinase



Several groups have reported hydroxyethylamine based inhibitors of HIV proteinase[6,7], however different series are characterised by having different stereochemical preferences at the carbon atom bearing the hydroxyl group that interacts with the catalytic aspartates. JG365[6] (**2**), Figure 2, is typical of the class of compounds that have S stereochemistry while the Roche compound Ro31-8959[7] (**3**) exemplifies the R series. The reason for the different preference appears to be due to the relative size of the Ile.Val dipeptide in **2** *verses* the smaller *tert*-butyl group in **3**. With the availability of the X-ray structure of **2** bound to HIV-proteinase[8] and model structures of **3** bound to HIV-proteinase it was possible to investigate this further, Figure 3. The binding mode of **3** relative to **2** has been proposed by Rich *et al*[9]. and later confirmed through X-ray crystallography[10].

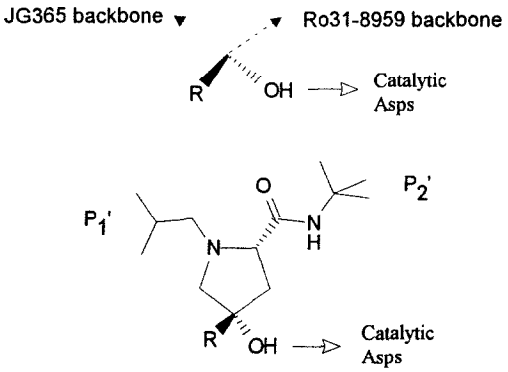
Figure 2: JG365 (**2**) and Ro31-8959 (**3**)



2 **3**

The hydroxyl group is similarly bound in both compounds by the catalytic Asp groups of the active site, however the different stereochemistry of the two inhibitors causes the backbones to diverge at this point as illustrated, Figure 4. As the enzyme seems able to bind both forms, the possibility of combining both "backbone paths" into one compound was investigated. The type of compound envisaged would branch both left and right (Figure 4) from the carbon bearing the hydroxyl group. The general structure, Figure 4, illustrates this concept with the proposed mode of binding of each group; the tertiary amine should be protonated at physiological pH and thus could assist in the interactions with the Asps or to Gly27/Gly27'. The amide carbonyl would be expected to interact with water 301. When the JG365 (**2**)/Ro31-8959 (**3**) overlay was combined with that of the protein bound X-ray structure of the penicillin dimer, **1**, a novel combined inhibitor, compound **4**, was conceived, Scheme I.

Figure 4: The Binding Mode of JG365 and Ro31-8959



N-(*tert*-Butoxycarbonyl)-(2S)-hydroxyproline (**5**) was coupled to *tert*-butylamine using 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU)[11] and diisopropylethylamine (DIPEA) in DMF to give compound **6**[12], Scheme I. The protecting group was then removed under acidic conditions to afford the amine hydrochloride salt which underwent reductive alkylation[13] with 2-methylpropionaldehyde to afford *tert*-amine (**7**) in 80% yield. Swern oxidation[14] followed by reaction with a sulphoxonium ylide[15] at room temperature afforded a 2:1 diastereomeric mixture of epoxide **8**. This mixture was subsequently opened with ammonia to afford amines **9** which were separated by flash silica column chromatography. The major diastereoisomer was determined from n.O.e experiments to have 4(S) stereochemistry. Finally each amine was coupled to the known benzylamine opened penicillin moiety (**10**)[16] to afford the target compounds (**4**) Both diastereoisomers of **4** were found to be potent inhibitors of HIV-1 proteinase[17] (4(R)-**4** IC_{50}= 0.07 μM, 4(S)-**4** IC_{50}= 0.03 μM).

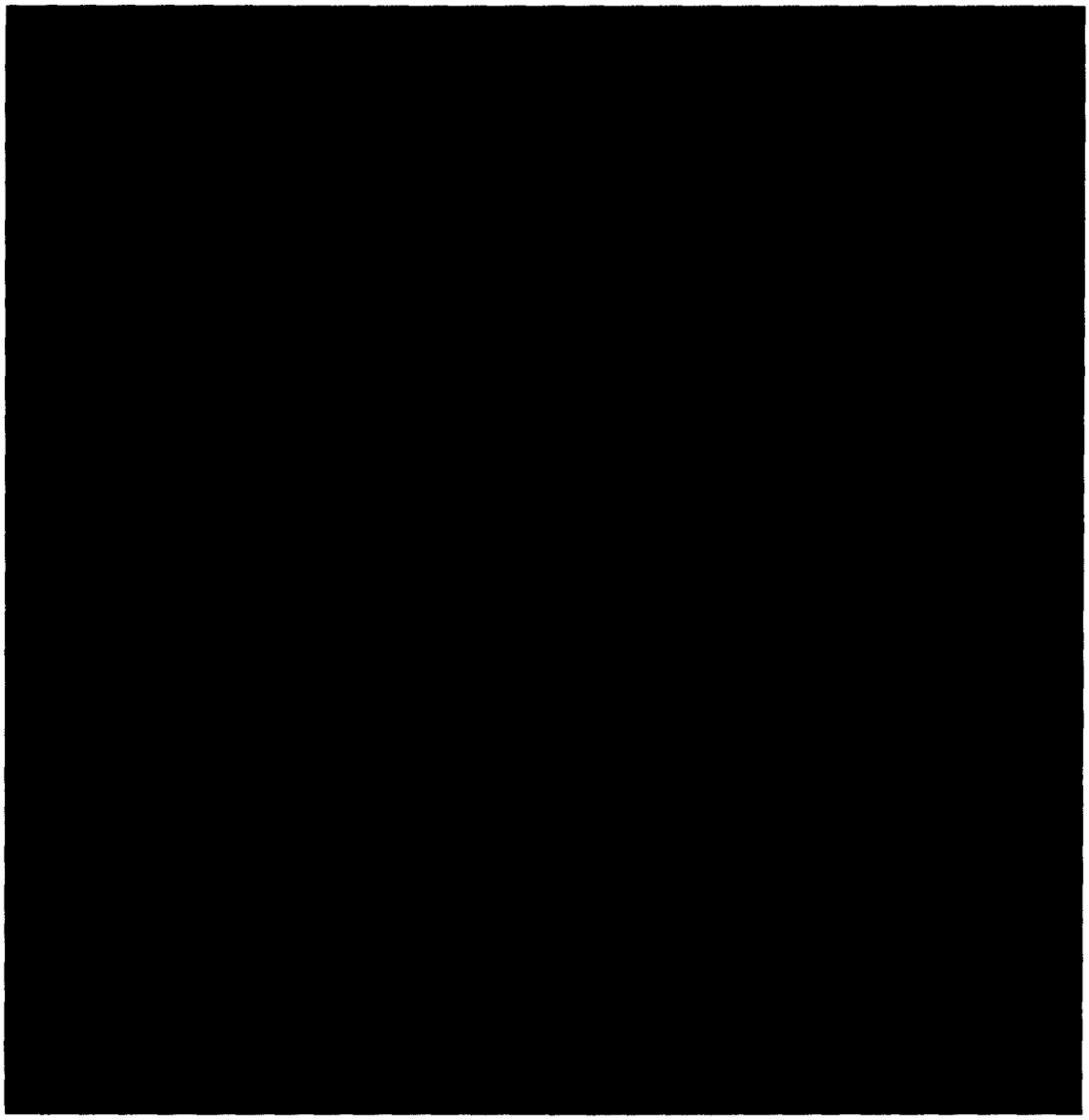
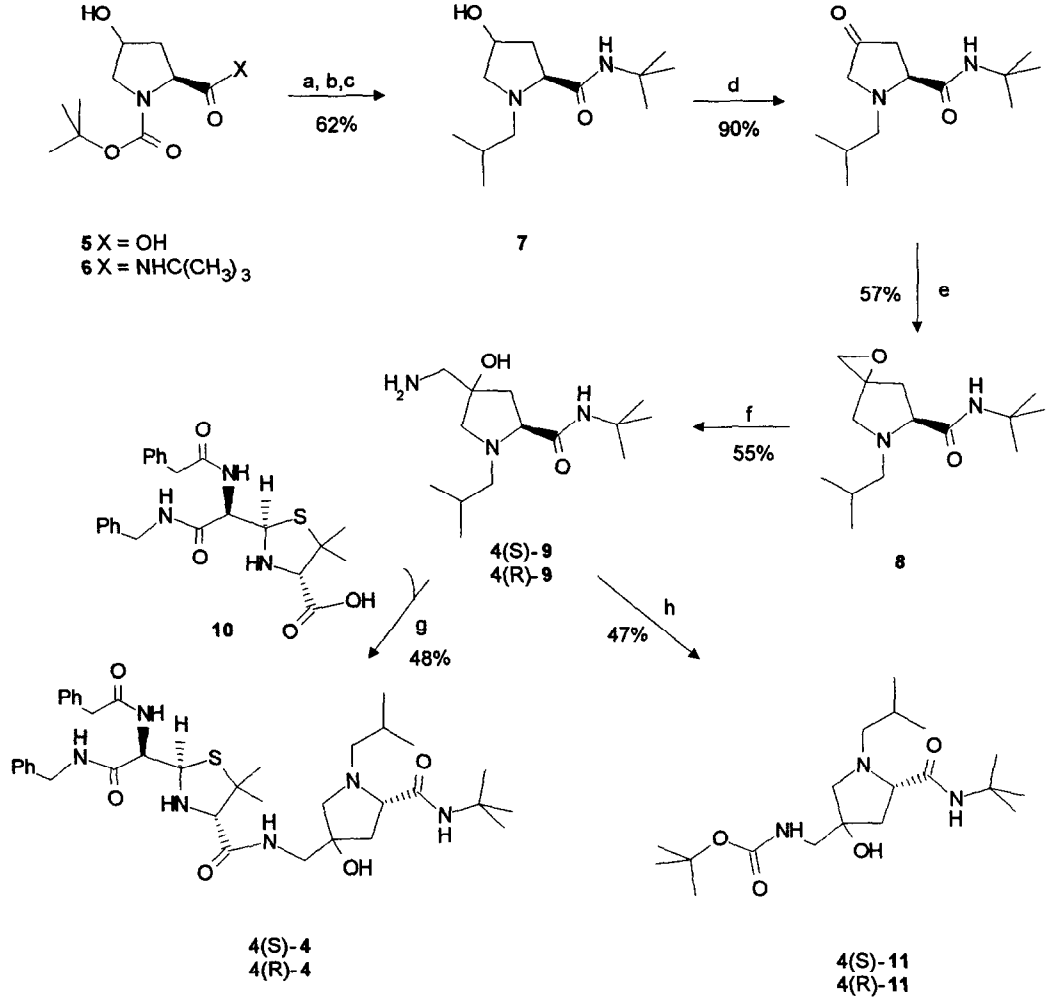


Figure 3: X-ray structure of HIV proteinase bound JG365[8] superimposed on a modelled structure of Ro31-8959 built using the InsightII modelling programme on a SG4D/35.

Scheme I[a]



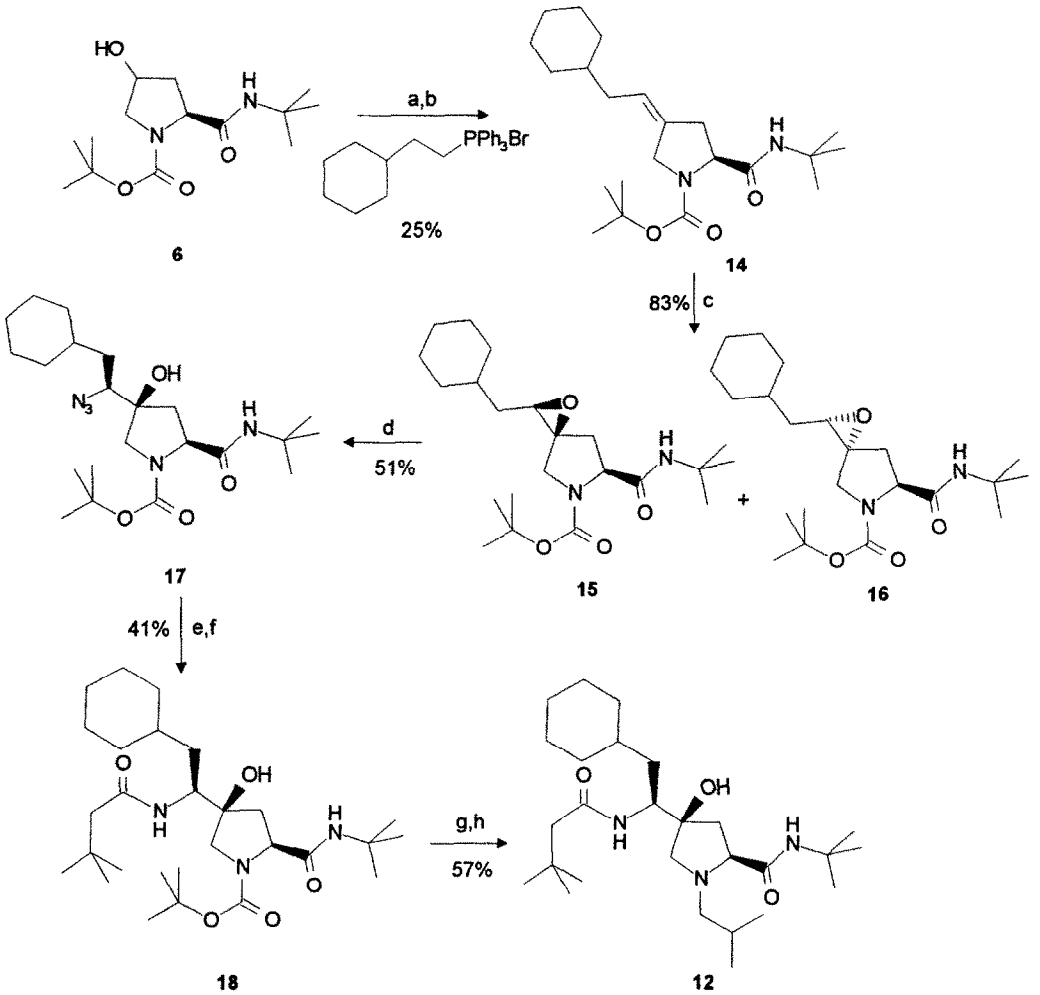
[a]a) $(CH_3)_3CNH_2$, TBTU, DIPEA, DMF; b) HCl, dioxan; c) $(CH_3)_2CHCHO$, H_2, Pd/C; d) $(COCl)_2$, DMSO, -60°C, DIPEA; e) $(CH_3)_3SOI$, NaH, DMSO; f) NH_3, *i*PrOH; g) TBTU, DIPEA, DMF; h) BOC_2O, Et_3N, dioxan.

The activity of 4(R)-4 (hydroxyproline numbering is used throughout) is consistent with the binding interactions proposed from the modelling experiments. It is of interest to note, however, that 4(S)-4 also shows potent activity. The similar level of activity associated with both diastereoisomers may be explained by the weak interaction of the tertiary hydroxyl group with the catalytic aspartates. Alternatively, 4(S)-4 may bind at the active site with the *iso*-butyl group in S_2' and the *tert*-butyl group in S_1' (i.e., in the opposite fashion compared to 4(R)-4).

From the initial modelling and crystallographic studies it was noted that the central linker for the thiazolidine dimer (1) was not directly coincident on the JG365/Ro31-8959 type linker. Therefore due to the nature of the hybrid molecule there was some ambiguity as to whether the new hydroxyproline moiety or the thiazolidine moiety would be the primary determinant of binding. Further hydroxyproline analogues in which the thiazolidine moiety was replaced were therefore investigated.

Introduction of a N-(*tert*-butoxycarbonyl) group gave compounds 4(R)-**11** and 4(S)-**11**, neither of which showed activity in the HIV-1 proteinase assay (IC_{50}> 90 μM). In both **2** and **3** a lipophilic group α to the hydroxyl group of the linker interacts with the S_1 pocket. We therefore incorporated such a group into our hydroxyproline moiety in an attempt to recover some of the binding interactions that were lost with the removal of the thiazolidine moiety. From molecular modelling studies, compound **12** was proposed with the absolute stereochemistry as shown, Scheme II.

Scheme II[a]



[a]a) $(COCl)_2$, DMSO, -60°C, Et_3N; b) $KOC(CH_3)_3$; c) *m*CPBA, CH_2Cl_2; d) NaN_3; e) H_2, Pd/C; f) $(CH_3)_3CCH_2CO_2H$, TBTU, DIPEA; g) HCl, dioxan; h) $(CH_3)_2CHCHO$, H_2, Pd/C

Swern oxidation of intermediate **6** gave ketone **13** which underwent Wittig coupling[18] with 2-cyclohexylethyltriphenylphosphonium bromide to afford **14** with Z : E ratio of 6:1. Reaction with *m*CPBA afforded epoxides **15** and **16** in ratio 3:2 which were separated by flash silica column chromatography. The desired epoxide (**15**) was opened with azide to give **17** and this was hydrogenated to the amine followed by

TBTU coupling to pivalic acid to afford **18**. The protecting group was then removed under acidic conditions and the resulting hydrochloride salt underwent reductive alkylation with 2-methylpropionaldehyde to afford target compound **12** in 57 % yield.

Compound **12** showed activity in the HIV-1 proteinase assay (IC_{50}= 9 μM) thus confirming the importance of the lipophilic group which had been proposed from the modelling studies to interact with the S_1 pocket. Despite introduction of the P_1 group, **12** was significantly less potent than the thiazolidine hybrid, **4**. The relatively low activity of **12** may have been due to the choice of the P_1 group, however, this result does support the argument that it is the thiazolidine moiety of **4** which is the primary determinant for binding rather than the hydroxyproline group.

Through molecular modelling, with known inhibitors of HIV proteinase, a novel hydroxyproline analogue, **4**, was conceived. Compound **4** showed potent activity as an inhibitor of HIV-1 proteinase, however in the light of subsequent results, it is likely that the thiazolidine moiety of this combined inhibitor is the primary determinant for binding.

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References and Notes

1. (a) Barre-Sinoussi, F.; Chermann, J.C.; Nugeyre, M.T.; Chamoret, S.; Gruest, J.; Daugnet, C.; Axler-Blin, C.; Vezinet-Brun, F.; Rouzioux, C.; Rozenbaum, W.; Montagnier, L. *Science*, 1983, **220**, 868.
(b) Gallo, R.C.; Salahuddin, S.Z.; Popovic, M.; Shearer, G.M.; Kaplan, M.; Haynes, B.F.; Palker, T.J.; Redfield, R.; Oleske, J.; Safai, B.; White, G.; Foster, P.; Markham, P.D. *Science*, 1984, **224**, 500.
2. Huff. J.R. *J. Med. Chem.*, 1991, **34**, 2305.
3. Seelmeier, S.; Schmidt, H.; Turk, V.; von der Helm, K. *Proc. Natl. Acad. Sci., USA*, 1988, **85**, 6612.
4. Wlodawer, A.; Miller, M.; Jaskolski, M.; Sathyanarayana, B.K.; Baldwin, E.; Weber, I.T.; Selk, L.M.; Clawson, L.; Schneider, J.; Kent, S.B.H. *Science*, 1989, **245**, 616.
5. Humber, D.C.; Cammack, N.; Coates, J.A.V.; Cobley, K.N.; Orr, D.C.; Storer, R.; Weingarten, G.G.; Weir, M.P. *J. Med. Chem.*, 1992, **35**, 3080.
6. Rich, D.H.; Green, J.; Toth, M.V.; Marshall, G.R. ; Kent, S.B.H. *J. Med. Chem.*,1990, **33**, 1285.
7. Roberts, N.A.; Martin, J.A.; Kinchington, D.; Broadhurst, A.V.; Craig, J.C.; Duncan, I.B.; Galpin, S.A.; Handa, B.K.; Kay, J.; Krohn, A.; Lambert, R.W.; Merrett, J.H.; Mills, J.S.; Parkes, K.E.B.; Redshaw, S.; Ritchie, A.J.; Taylor, D.L.; Thomas, G.J.; Machin, P.J. *Science*, **1990**, *248*, 358.
8. Swain, A.L.; Miller, M.M.; Green, J.; Rich, D H.; Schneider, J.; Kent, S.B.H.; Wlodawer, A. *Proc. Natl. Acad. Sci.*, 1990, **87**, 8805.
9. Rich, D.H.; Sun, C.-Q.; Prasad, J.V.N.V.; Pathiasseril, A.; Toth, M.V.; Marshall, G.R.; Clare, M.; Mueller, R.A.; Houseman, K. *J. Med. Chem.*, **1991**, *34*, 1225.
10. Krohn, A.; Redshaw, S.; Ritchie, J.C.; Graves, B.J.; Hatada, M.H. *J. Med. Chem.* **1991**, *34*, 3340.
11. Knorr, R.; Trzeciak, A.; Bannwarth, W.; Gillessen, D. *20th European Peptide Symposium*, Tübingen, Germany, 1988.
12. All new compounds gave satisfactory combustion analysis or accurate mass values for $(M+H)^+$, ^1H NMR data and were homogeneous by TLC and/or HPLC.
13. Nordmann, R.; Petcher, T.J. *J. Med. Chem.*, 1985, **28**, 367.
14. Omura, K.; Swern, D. *Tetrahedron*, 1978, **34**, 1651.
15. Corey, E.J.; Chaykovsky, M. *J. Am. Chem. Soc.*, 1965, **87**, 1353.
16. Herak, J.J.; Kovocevic, M.; Gaspert, B. *Croat. Chem. Acta.*, 1978, **49**, 265.
17. Assays were performed as for ref. 5. IC_{50} determinations were performed in duplicate at each concentration with mean values used for data analysis. Results were standardised relative to the initial IC_{50} value obtained for compound **1** which was used as a control in all assays.
18. Fitjer, L.; Quabeck, U. *Syn. Commun.*, 1985, **15**, 855.