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Design and synthesis of novel HIV-1 protease inhibitors incorporating oxyindoles as the P_2'-ligands

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Abstract—A series of novel oxyindole-derived HIV-1 protease inhibitors were designed and synthesized based upon our X-ray crystal structure of inhibitor **2** (TMC-114) bound to HIV-1 protease. The effects of substituents, spirocyclic rings, and ring sizes have been investigated. A number of inhibitors exhibited low nanomolar inhibitory potencies against HIV protease.

The AIDS epidemic has grown into one of the most pressing medical concerns of our time.[1] The advent of highly active antiretroviral therapy (HAART) with HIV protease inhibitors and reverse transcriptase inhibitors has resulted in an improved quality of life, enhanced HIV management, and halted the progression of AIDS.[2] However, drug side effects and the emergence of drug-resistance are making these therapies ineffective.[3] In our continuing effort to develop new inhibitors that maintain their potencies against mutant strains of HIV, we have recently reported the design and synthesis of a novel inhibitor (**2**, now known as TMC-114 or Darunavir, Fig. 1) which is currently undergoing phase III clinical trials.[4,5] This inhibitor is exceedingly potent against wild-type ($K_i = 15 \pm 1$ pM, $n = 4$ and $ID_{50} = 1.4 \pm 0.25$ nM, $n = 5$) as well as resistant viruses.[4]

Subsequently, to gain molecular insight into the ligand-binding site interaction, we determined a high resolution X-ray crystal structure of this inhibitor bound to HIV-1 protease.[6] An intriguing feature of this structure is the presence of a tetracoordinated critical water molecule that donates its hydrogen bonds to the urethane carbonyl and one of the sulfonamide oxygens of the inhibitor and accepts two hydrogen bonds from the N–H of Ile 50 and Ile 50' amides of the HIV protease. This tight bound water molecule is also present in saquinavir-bound HIV-1 protease as well as other protein–ligand complexes.[7] Based on this key interaction, we postulated that an oxyindole derivative could be designed to interact with this critical water molecule as well as to fill the S_2' region of the enzyme active site effectively. Such inhibitor with a basic amine

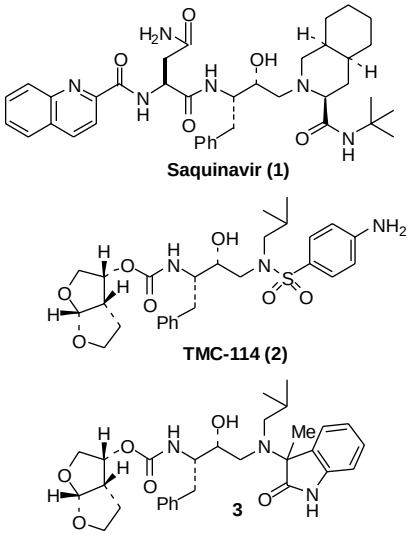

Figure 1. HIV protease inhibitors.

Keywords: HIV proptease; Inhibitors; Oxyindole; TMC-114; Design; Synthesis.
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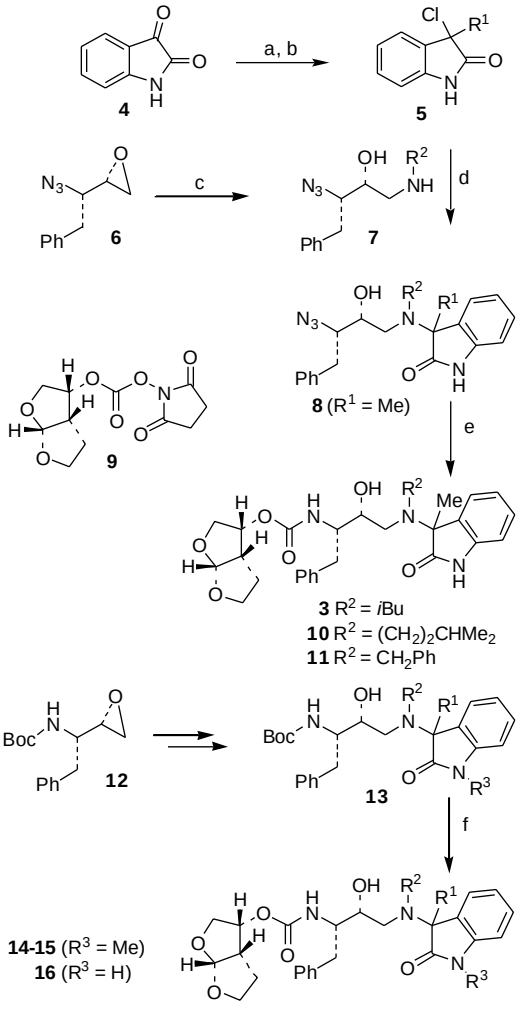
functionality may improve absorption profiles. Oxyindoles have been previously utilized in several FDA-approved drugs.[8] Herein, we report our preliminary results of these investigations in which an oxyindole ring has been incorporated in the P'_2 position of inhibitor **2**. This has resulted in a series of inhibitors with subnanomolar enzyme inhibitory potencies. We have also examined the feasibility of spirocyclic oxyindole derivatives as P'_2-ligands. However, acyclic inhibitors were more potent than their cyclic counterparts.

The general synthesis of various oxyindole-derived inhibitors is outlined in Scheme 1. As shown, commercially available isatin was reacted with 2.2 equiv of the appropriate alkyl Grignard reagent at 0 °C to provide the corresponding tertiary alcohol in 57–72% yield.[9] Chlorination of the resulting alcohol using thionyl chloride and triethylamine in CH_2Cl_2 produced chloride **5** in good overall yield (57–76%).[10] Reaction of optically active azido epoxide **6**[11] with the appropriate amine in isopropanol at reflux gave the corresponding secondary amine **7** in essentially quantitative yield. Reaction of the respective amine **7** with chloride **5** (R^1=Me) and triethylamine in acetonitrile smoothly provided oxyindole derivative **8** as a mixture (1:1 ratio by ^1H NMR analysis) of diastereomers in excellent yields (81–94%). Both diastereomers for inhibitor **3** were separated by silica gel chromatography. Catalytic hydrogenation of various azides **8** with optically active bis-tetrahydrofuranyl



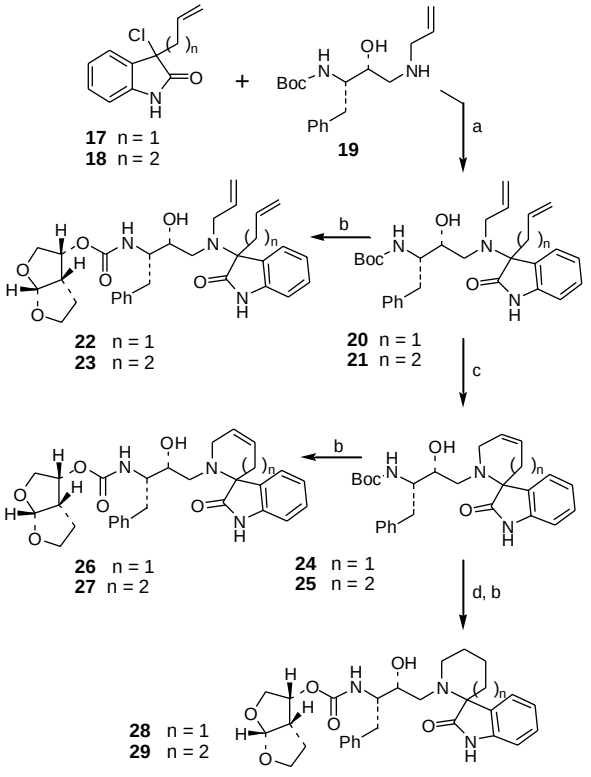
Scheme 1. Reagents and condition: (a) R^1MgBr, THF, 0 °C; (b) $SOCl_2$, TEA, CH_2Cl_2; (c) R^2NH_2, *i*-PrOH; (d) CH_3CN, TEA; (e) **9**, H_2, Pd/C, THF; (f) i—TFA, CH_2Cl_2; ii—**9**, TEA, CH_2Cl_2.

Table 1. Inhibitory activity of oxyindole derivatives

Entry	Compound	K_i (nM)
1	**3a** (isomer A)	6 ± 0.6
2	**3b** (isomer B)	3 ± 0.3
3	**10**	7 ± 0.05
4	**11**	26 ± 2.5
5	**14a** (isomer A)	2 ± 0.3
6	**14b** (isomer B)	7 ± 0.7
7	**15a** (isomer A)	102 ± 4.9
8	**15b** (isomer B)	130 ± 12.5
9	**16a** (isomer A)	42 ± 3.2
10	**16b** (isomer B)	60 ± 8

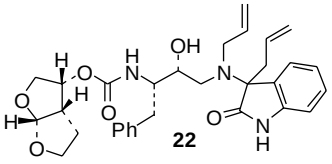
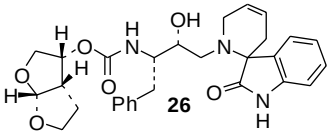
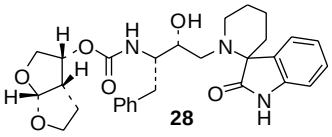
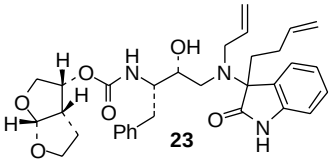
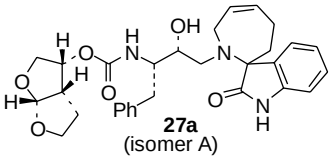
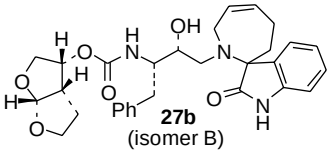
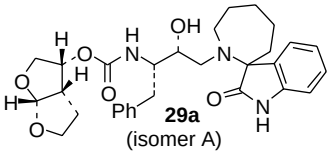
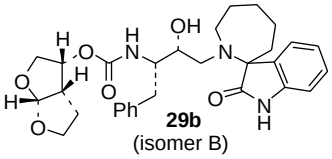
carbonate **9** in THF in the presence of triethylamine afforded optically pure inhibitors **3a** and **3b** as well as diastereomeric mixture of **10** and **11** in good yields (60–75%). Preparation of inhibitors **14**–**16** was carried out with commercially available Boc-protected epoxide **12** as starting material. Epoxide opening followed by reaction with chloride **5** provided the corresponding Boc derivatives **13**. Diastereomers were separated by silica gel chromatography using 25% ethyl acetate in hexane as the eluent. Removal of the Boc group by exposure to TFA followed by the reaction of the resulting amine with mixed carbonate **9**[12] in the presence of triethylamine in CH_2Cl_2 furnished the final inhibitors **14**–**16** in good yield (47–65%). Thus, the corresponding oxyindole diastereomers for **14**–**16** were prepared in an optically active form. Stereochemical identity of the oxyindole ring chiral center was not determined for our preliminary studies.

The inhibitory potencies of various oxyindole-derived inhibitors are shown in Table 1. The assay protocol of Toth and Marshall[13] was utilized and the values denote mean values from two determinations. As can be seen, both oxyindole diastereomers of the *N*-isobutyl analogs **3a** and **3b** have shown potencies of 6 and 3 nM, respectively. It appears that S_2'-enzyme active site has only a slight preference for one diastereomer over the other. Either *R* or *S* absolute configuration of the oxyindole chiral center in **3** seems to bind into HIV protease S_2'-active site effectively. Nevertheless, our attempts to assign stereochemical identity of the oxyindole ring were unsuccessful. Diastereomeric mixtures of *N*-isoamyl (**10**) and *N*-benzyl (**11**) derivatives also showed good activity, with the larger benzyl analog being less potent (26 vs 7 nM). Introduction of an allyl group at the oxyindole C-3 center (**14a** and **14b**) resulted in a separable mixture of diastereomers which showed comparable potency (2 and 7 nM, respectively) with that of the methyl analogs (**3a** and **3b**). In an effort to interact with the residues in the active site, we have incorporated a 5-methoxy substituent on the oxyindole aromatic ring. Thus, 5-methoxyisatin was converted to inhibitors **15a** and **15b** as a diastereomeric mixture (1:1 ratio by ^1H NMR analysis)



Scheme 2. Reagents and conditions: (a) CH_3CN, TEA, reflux; (b) i—TFA, CH_2Cl_2; ii—**9**, TEA, CH_2Cl_2; (c) Grubbs' 1st generation catalyst, CH_2Cl_2, 42 °C; (d) H_2, 10% Pd/C, MeOH.

Table 2. Inhibitory activity of spirocyclic derivatives

Entry	Compound	K_i (nM)
1	 **22**	5 ± 0.5
2	 **26**	126 ± 0.5
3	 **28**	>1000
4	 **23**	47 ± 1.1
5	 **27a** (isomer A)	>1000
6	 **27b** (isomer B)	>1000
7	 **29a** (isomer A)	>1000
8	 **29b** (isomer B)	>1000

and the mixture was separated. However, these inhibitors have shown significantly lower inhibitory activity compared to unsubstituted inhibitors **3**. The *N*-methyl oxyindole derivative was also synthesized and the individual diastereomers (**16a** and **16b**) displayed K_i values of 42 and 60 nM, respectively. The fact that the potency displayed an approximately 10-fold decrease (as compared to compounds **3a** and **3b**) suggests that the oxyindole N–H may be participating in hydrogen bonding with the enzyme active site.

We have also examined the feasibility of spirocyclic oxyindole derivatives as the P_2'-ligand. It has been shown by us and others that constrained rings in the HIV protease active site significantly improved enzyme inhibitory activity.[14,15] Our preliminary molecular modeling suggested that such spirocycles can make effective interaction in the active site. Scheme 2 shows the synthesis of six- to seven-membered spirocyclic oxyindole-derived inhibitors. Opening epoxide **12** with allylamine in *i*-PrOH provided quantitative yield of secondary amine **19**. The olefinic chlorooxyindoles (**17**, 48%) and (**18**, 52%) were prepared following the same 2-step sequence as described in Scheme 1. Reactions of these chlorooxyindoles with amine **19** afforded tertiary amines **20** and **21** as 1:1 mixtures of diastereomers (by ^{1}H NMR) in 68–82% yield. These diastereomers could be separated and the mixture was utilized in subsequent reactions. The dienes were then subjected to ring closing metathesis using Grubbs' first generation[16] catalyst in refluxing CH_2Cl_2 to provide six and seven-membered spirocycles **24** and **25**, respectively, in excellent yield (80–85%). The seven-membered ring diastereomers were separated at this point by silica gel chromatography, while the six-membered ring was used as a 1:1 mixture of diastereomers. The unsaturated rings were converted into urethane derivatives **26** and **27** containing P_2-bis-tetrahydrofuranyl ligand following the standard protocol described in Scheme 1. Saturated inhibitors **28** and **29** were prepared by removal of Boc from **24** and **25** and reaction of the resulting amines with carbonate **9** in the presence of triethylamine in CH_2Cl_2 followed by catalytic hydrogenation of the resulting olefins (60–65% yield).

The spirocyclic oxyindole derivatives were assayed and their potencies are displayed in Table 2. Acyclic compounds **22** and **23** showed good activity (5 and 47 nM, respectively) and were generally consistent with those observed for similar compounds shown in Table 1. Interestingly, there is a significant reduction in inhibitory potency for the corresponding six and seven-membered unsaturated and saturated spirocyclic inhibitors. As shown, inhibitor with a cyclohexene ring has shown a K_i value of 126 nM. However, saturation of the double bond provided compound **28** with very little activity (K_i value > 1 μM). Also, all spirocyclic derivatives with a seven-membered ring have displayed no significant enzyme inhibitory activity. A closer inspection of the preliminary model structure reveals that the oxyindole carbonyls of the spirocyclic derivatives do not overlap with the sulfone oxygen of **2** that effectively interacts with the tight-bound water molecule in the active site. Further structural modifications of the oxyindole derivatives are necessary for effective binding in the active site.

We have determined the antiviral activity of **3a** and **3b** against HIV-1_{IIIb} in MT-2 cells. The results are summarized in Table 3. The IC_{50} values shown were determined based on the inhibition of HIV-induced cytopathogenicity in MT-2 cells. All assays were conducted in duplicate, and the values with standard deviation denote mean values from two or three. As can be seen, the antiviral activity of these compounds was substantially limited compared to saquinavir.[17] To improve antiviral potency, further modifications of functionalities are in progress. To gain molecular insight, an energy minimized model of 3(*R*′)-configuration of oxyindole derivative **3** was created (Fig. 2). The structure was built based on our published crystal structure of **2**-complexed with

Table 3. Antiviral activity of **3a** and **3b**

Inhibitor	IC_{50} (μM)	CC_{50} (μM)
3a	0.30 ± 0.071	>10
3b	0.48 ± 0.38	>10
Saquinavir	0.005 ± 0.002	>10

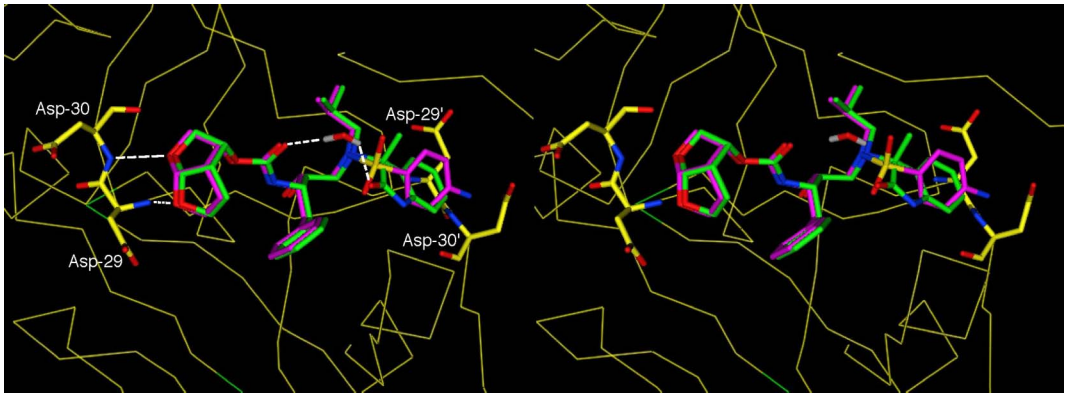


Figure 2. (3′*R*)-Oxyindole isomer of compound **3** modeled into the active site of HIV-1 protease. The inhibitor (green), superimposed upon the crystal structure of TMC-114 (magenta).

HIV-1 protease.[6] The conformation of **3** was optimized using the MMFF94x force field.[18]

In summary, a series of novel HIV protease inhibitors incorporating oxyindole-derived P_2'-ligand has been designed, synthesized, and evaluated. The oxyindole derivatives can be readily prepared from isatin. The oxyindole derivatives incorporate a basic amine functionality. Various 3-alkyl substitutents on the oxyindole rings resulted in inhibitors with low nanomolar potency. In general, acyclic inhibitors are considerably more potent than their cyclic counterparts. Preliminary structure–activity studies have shown that the lactam N–H is critical to enhanced potency. We have also investigated the feasibility of spiro oxyindoles as the P2′-ligands. However, spirocyclic inhibitors have shown significantly reduced potencies compared to their acyclic counterparts. Further design and optimization of these inhibitors are currently underway.

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References and notes

1. United Nations. 2004 Report on the Global HIV/AIDS Epidemic: 4th Global Report. New York, USA, 2004.
2. (a) Flexner, C. *N. Engl. J. Med.* **1998**, *338*, 1281; (b) Cihlar, T.; Bischofberger, N. *Annu. Rep. Med. Chem.* **2000**, *35*, 177.
3. Tamalet, C.; Pasquier, C.; Yahi, N.; Colson, P.; Poizot-Martin, I.; Lepeu, G. *J. Med. Virol.* **2000**, *61*, 181.
4. (a) Ghosh, A. K.; Shin, D. W.; Swanson, L.; Krishnan, K.; Cho, H.; Hussain, K. A.; Walters, D. E.; Holland, L.; Buthod, J. *Il Farmaco* **2001**, *56*, 29; (b) Ghosh, A. K.; Kincaid, J. F.; Cho, W.; Walters, D. E.; Krishnan, K.; Hussain, K. A.; Koo, Y.; Cho, H.; Rudall, C.; Holland, L.; Buthod, J. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 687; (c) Koh, Y.; Nakata, H.; Maeda, K.; Ogata, H.; Bilcer, G.; Devasamudram, T.; Kincaid, J. F.; Boross, P.; Wang, Y.-F.; Tie, Y.; Volarath, P.; Gaddis, L.; Harrison, R. W.Weber, I. T.; Ghosh, A. K.; Mitsuya, H. *Antimicrob. Agents Chemother.* **2003**, *47*, 3123; (d) Yoshimura, K.; Kato, R.; Kavlick, M. F.; Nguyen, A.; Maroun, V.; Maeda, K.; Hussain, K. A.; Ghosh, A. K.; Gulnik, S. V.; Erickson, J. W.; Mitsuya, H. *J. Virol.* **2002**, *76*, 1349.
5. (a) De Meyer, S.; Azijn, H.; Surleraux, D.; Jochmans, D.; Tahri, A.; Pauwels, R.; Wigerinck, P.; de Bethune, M.-P. *Antimicrob. Agents Chemother.* **2005**, *49*, 2314; (b) De Meyers, S.; Peeters, M. Conference on Retroviruses and opportunistic Infections (11th CROI), February 8–11, 2004, San Francisco, CA. Abstracts 533 and 620.
6. Tie, Y.; Boross, P. I.; Wang, Y.-F.; Gaddis, L.; Hussain, A. K.; Leshchenko, S.; Ghosh, A. K.; Louis, J. M.; Harrison, R. W.; Weber, I. T. *J. Mol. Biol.* **2004**, *338*, 341.
7. Swain, A. L.; Miller, M. M.; Green, J.; Rich, D. H.; Schneider, J.; Kent, S. B. H.; Wlodawer, A. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 8805.
8. (a) Gunasekara, N. S.; Spencer, C. M.; Keating, G. M. *Drugs* **2002**, *62*, 1217; (b) Gallagher, G., Jr.; Lavanchy, P. G.; Wilson, J. W.; Hieble, J. P.; DeMarinis, R. M. *J. Med. Chem.* **1985**, *28*, 1533; (c) Haynes, J.; Obiako, B.; Babal, P.; Stevens, T. *Am. J. Physiol. Heart Circ. Physiol.* **1999**, *276*, H1877; (d) Liu, Y.; Liu, D.; Printzenhoff, D.; Coghlan, M. J.; Harris, R.; Kraft, D. S. *Eur. J. Pharmacol.* **2002**, *435*, 153.
9. (a) Alvarez, R. G.; Hunter, I. S.; Suckling, C. J.; Thomas, M.; Vitinius, U. *Tetrahedron* **2001**, *57*, 8581; (b) Sharma, V. M.; Prasanna, P.; Adi Seshu, K. V.; Renuka, B.; Laxman Rao, C. V.; Sunil Kumar, G.; Prasad Narasimhulu, P.; Aravind Babu, P.; Puranik, R. C.; Subramanyam, D. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2303.
10. Powers, J. C. *J. Org. Chem.* **1966**, *31*, 2627.
11. Ghosh, A. K.; Thompson, W. J.; Holloway, M. K.; McKee, S. P.; Duong, T. T.; Lee, H. Y.; Munson, P. M.; Smith, A. M.; Wai, J. M.; Darke, P. L.; Zugay, J. A.; Emini, E. A.; Schleif, W. A.; Huff, J. R.; Anderson, P. S. *J. Med. Chem.* **1993**, *36*, 2300.
12. (a) Ghosh, A. K.; Thompson, W. J.; Fitzgerald, P. M. D.; Culberson, J. C.; Axel, M. G.; McKee, S. P.; Huff, J. R.; Anderson, P. S. *J. Med. Chem.* **1994**, *37*, 2506; (b) Ghosh, A. K.; Leshchenko, S.; Noetzel, M. *J. Org. Chem.* **2004**, *69*, 7822; (c) Ghosh, A. K.; Chen, Y. *Tetrahedron Lett.* **1995**, *36*, 505.
13. Toth, M. V.; Marshall, G. R. *Int. J. Pept. Protein Res.* **1990**, *36*, 544.
14. (a) Ghosh, A. K.; Swanson, L.; Liu, C.; Cho, H.; Hussain, A.; Walters, D. E.; Holland, L.; Buthod, J. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1993; (b) Ghosh, A. K.; Swanson, L. M.; Cho, H.; Leshchenko, S.; Hussain, K. A.; Kay, S.; Walters, D. E.; Koh, Y.; Mitsuya, H. *J. Med. Chem.* **2005**, *48*, 3576.
15. (a) Fairlie, D. P.; Tyndall, J. D. A.; Reid, R. C.; Wong, A. K.; Abbenante, G.; Scanlon, M. J.; March, D. R.; Bergman, D. A.; Chai, C. L. L.; Burkett, B. A. *J. Am. Chem. Soc.* **1996**, *118*, 8511; (b) Abbenante, G.; Bergman, D. A.; Brinkworth, R. I.; March, D. R.; Reid, R. C.; Hunt, P. A.; James, I. W.; Dancer, R. J.; Garnham, B.; Stoermer, M. L.; Fairlie, D. P. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2531; (c) Reid, R. C.; Pattenden, L. K.; Tyndall, J. D. A.; Martin, J. L.; Walsh, T.; Fairlie, D. P. *J. Med. Chem.* **2004**, *47*, 1641; (d) Glenn, M. P.; Pattenden, L. K.; Reid, R. C.; Tyssen, D. P.; Tyndall, J. D. A.; Birch, C. J.; Fairlie, D. P. *J. Med. Chem.* **2002**, *45*, 371.
16. Grubbs, R. H.; Chang, S. *Tetrahedron* **1998**, *54*, 4413.
17. 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. *Science* **1990**, *248*, 358.
18. Halgren, T. A. *J. Comput. Chem.* **1999**, *20*, 720.