Bioorganic & Medicinal Chemistry Letters 20 (2010) 5013–5018

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of potent HIV integrase inhibitors active against raltegravir resistant viruses

Giang Le [a,*], Nick Vandegraaff [a], David I. Rhodes [a], Eric D. Jones [a], Jonathan A. V. Coates [a], Long Lu [b], Xinming Li [b], Changjiang Yu [b], Xiao Feng [b], John J. Deadman [a,*]

[a] Avexa Ltd, 576 Swan Street, Richmond, Victoria 3121, Australia
[b] Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, 345 Lingling Lu, Shanghai 200032, China

ARTICLE INFO

Article history:
Received 20 June 2010
Revised 9 July 2010
Accepted 12 July 2010
Available online 16 July 2010

Keywords:
Integrase
Inhibitor
Synthesis
HIV
QHGS
NHEQ
Mutant virus

ABSTRACT

A series of novel HIV integrase inhibitors active against rategravir resistant strains are reported. Initial SAR studies revealed that activities against wild-type virus were successfully maintained at single digit nanomolar level with a wide range of substitutions. However, inclusion of nitrogen-based cyclic substitutions was crucial for achieving potency against mutant viruses. Several compounds with excellent activities against wild-type virus as well as against the viruses with the mutations Q148H/G140S or N155H/E92Q were reported.

Recently, a new inhibitor of the human immuno-deficiency virus (HIV) targeting the viral integrase enzyme was approved for clinical use by the FDA. Raltegravir (RAL, marketed as Isentress™ by Merck and Co.) disrupts the critical viral process of integration in which newly made viral DNA is inserted into the host cell chromosomal DNA.[1] RAL inhibits integrase by binding within the active site of a pre-assembled integrase–viral DNA complex, and is able to co-ordinate two divalent metal (Mg) ions known to be indispensable for enzyme catalysis.[2] The core scaffold of RAL comprises a phenolic hydroxyl group and one exocyclic and one ring amide functionality arranged in such a way as to present as a hydrogen bond acceptor–donor–acceptor motif responsible for metal co-ordination.[3]

As with other classes of HIV drugs, under the highly selective evolutionary pressures associated with exposure to integration inhibitors, viral resistance emerges. To date, around 40 mutations within the viral integrase gene have been associated with resistance to either RAL and/or another integration inhibitor currently in clinical trials, elvitegravir (EVR, Gilead Sciences).[4] However, two main pathways bestowing viral resistance to RAL have been identified in the clinic and these involve mutations at either positions Gln-148 (Q148K/R/H) or Asn-155 (N155S/H) in the IN central core domain.[5] Mutations of these key amino acids have also been associated with significant resistance to EVR. A third less common pathway involving mutation at Tyr-143 (Y143C/R) has also been associated with resistance to RAL.[6] Additionally, specific secondary mutations are also commonly associated with each primary mutation and serve to significantly enhance resistance and/or the replicative capacity of these viruses. A recent Letter indicates that the Q148R natural polymorphism in HIV IN is present in a significant proportion of therapy naïve as well as treatment experienced patients studied as a minor quasi-species.[7] Thus, there is a clear need to identify scaffolds that allow the development of integration inhibitors that are active against RAL-resistant (and EVR-resistant) HIV strains.

We have previously reported a series of bicyclic pyrimidinone integrase inhibitors displaying excellent antiviral activity against wild-type (WT) HIV in cell culture.[8] Here we present evidence that compounds active against viruses resistant to RAL, as well as wild-type (WT) virus, can be derived from scaffolds containing a thiazole replacement of the exocyclic amide. Furthermore, modification at position 7 and/or 9 of the bicyclic pyrimidinone core can also significantly influence the activity of compounds against drug resistant viruses.

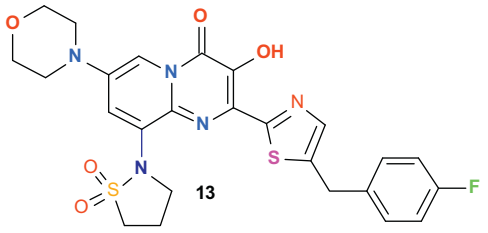
We have developed a convenient route for the formation of thiazole ring.[8] The starting acid **1** was activated with 1,1′-carbonyldiimidazole in the presence of NaSH, followed by substitution with

* Corresponding authors. Tel.: +61 408538488 (J.J.D.).
E-mail addresses: jjdeadman@hotmail.com, jdeadman@avexa.com.au (J.J. Deadman).

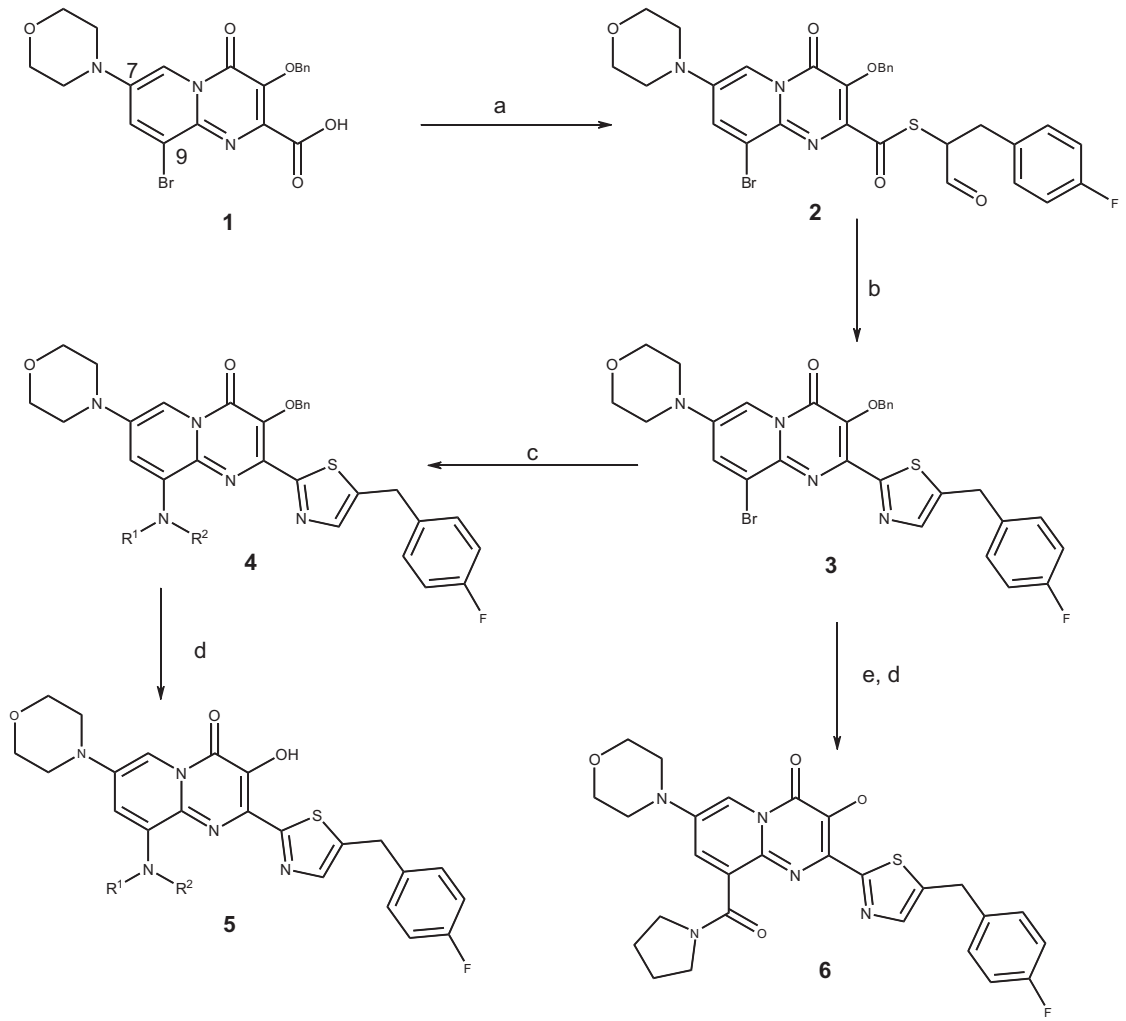
0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.07.041

alpha bromoaldehyde to furnish thioester **2**. Upon treating with an ammonia source, such as ammonium acetate at elevated temperature, the aldehyde–thioester **2** (Scheme 1) cyclized to result in thiazole **3** in reasonable overall yield. Intermediate **3** was further derivatized in position 9 via Buchwald coupling of the versatile bromo handle.[9] Deprotection of the *O*-Bn group with TFA cleanly gave the desired final products, of which the numerous examples are exemplified by the general structure **5**. Bromo precursor **3** was also utilized for other metal mediated reactions, such as carbamoylation to give the amide derivative **6**.[10]

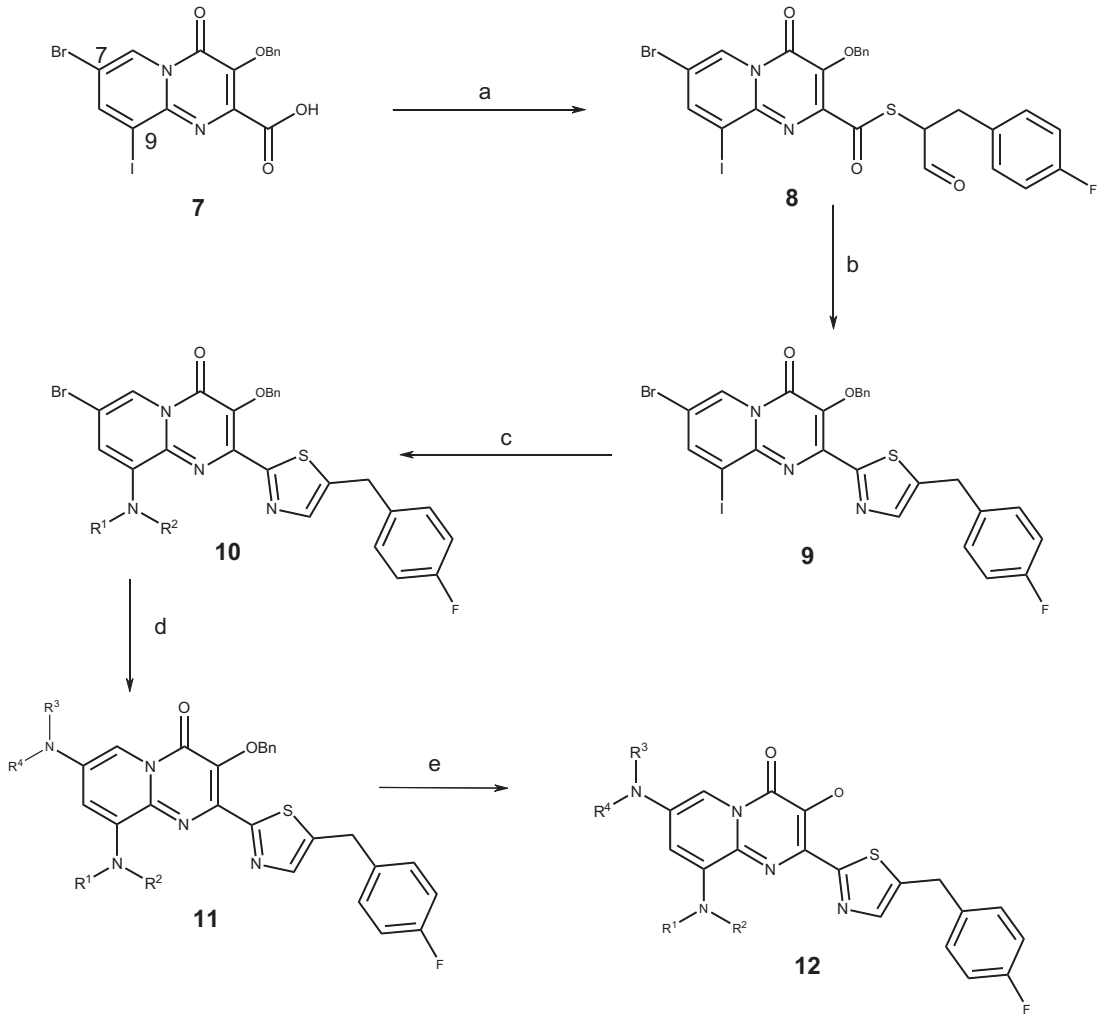
To provide a key intermediate to facilitate analog generation, the synthetic strategy shown in Scheme 2 was developed further to allow diversification of both R7 and R9 substitutions of the fused bicyclic core at a late stage. The versatile 7-Br-9-I thiazole precursor **9** was prepared from acid **7** in two steps as described in Scheme 2. The more active 9-I substituted carbon underwent coupling with various nitrogen-based substitutions under Buchwald[9] conditions using toluene as the solvent. The less active 7-Br group was then coerced to undergo the second coupling reaction at higher temperatures in dioxane. These two coupling steps could be executed sequentially in one-pot fashion or as separate reactions. The final deprotection step of the O-Bn group was achieved by heating in TFA as before, and all products were purified on preparative HPLC to purity of greater than 95%.



From preliminary SAR studies we identified thiazole derivative **13** as a potent HIV integrase inhibitor, displaying an EC_{50} of 6.4 nM in a cell-based assay.[11] As this compound represents a novel scaffold, we were interested to investigate its inhibitory potency against RAL-resistant HIV mutants. To this end we have adapted the single round infectivity assay for a large panel of mutant viruses, including the clinically most important Q148H/G140S and N155H/E92Q double mutations.[12] Upon assaying we found that compound **13** exhibited a reasonable level of potency against QHGS and NHEQ mutants, with an EC_{50} of 280 nM and 49.5 nM, respectively. The encouraging activity levels prompted us to investigate the SAR of compound **13**. Initially we focused on the effect of substitutions of the position C-9 of the bicyclic system. As reported



Scheme 1. Reagents and conditions: (a) (i) CDI, NaSH and (ii) 2-bromo-3-(4-fluorophenyl)propanal, proton sponge, CH_2Cl_2, reflux; (b) ammonium acetate, AcOH, reflux, 20–27% overall yield for (a) and (b); (c) NHR^1R^2, xantphos, $Pd_2(dba)_3$, Cs_2CO_3, toluene, 80 °C, 40–60%; (d) TFA, 80 °C, 3 h, 15–80%; (e) (i) $Mo(CO)_6$, pyrrolidine, $Pd(OAc)_2$, DBU, dioxane, 80 °C, 5%.



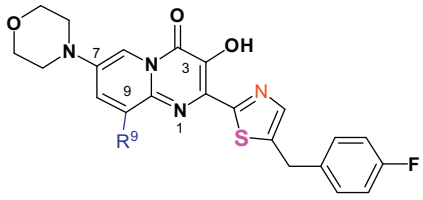
Scheme 2. Reagents and conditions: (a) (i) CDI, NaSH and (ii) 2-bromo-3-(4-fluorophenyl)propanal, proton sponge, CH_2Cl_2, reflux; (b) ammonium acetate, AcOH, reflux, 20–27% overall yield for (a) and (b); (c) NHR^1R^2, xantphos, $Pd_2(dba)_3$, Cs_2CO_3, toluene, 80 °C, 40–60%; d) NHR^3R^4, xantphos, $Pd_2(dba)_3$, Cs_2CO_3, dioxane, 100 °C, 30–70%; TFA, 80 °C, 3 h, 15–80%.

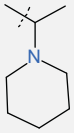
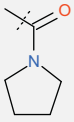
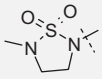
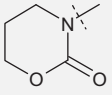
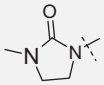
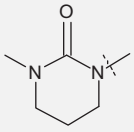
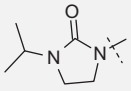
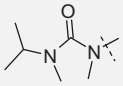
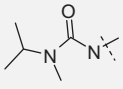
previously, unsubstituted derivative **14** or halogenated **15** exhibited comparable WT activity,[8] however, these compounds inhibited QHGS infectivity with only modest potency at 2–3 μM. Likewise, methoxy (**16**) or carbon-based functionalities such as acetyl (**17**), 2-hydroxyethane (**18**), tertiaryalkylamine (**19**), or amide (**6**) in this position resulted in compounds with micromolar or high nanomolar activities against QHGS mutant. The acetyl group of compound **17** was introduced via Heck coupling into the ester derivative of acid **1**,[8] from which the thiazole ring was prepared as described in Scheme 1. Hydroxyethane congener **18** was obtained from **17** via a simple reduction step with $NaBH_4$, while **19** was formed through reductive amination (Table 1).

Introduction of a cyclic nitrogen-based substitution in this position restored the mutant activities in contrast to compounds **14**–**19**. For example, sultam derivative **20** was shown to be twice as active against the QHGS or NHEQ mutants as the lead compound **13**, while **21** with the lactam substitution maintained the same level potency as **13** with an EC_{50} of 335 nM. The five-membered cyclic carbamate (**22**) was beneficial, exhibiting an EC_{50} of 108 nM against QHGS virus. Enlarging the carbamate moiety to a six-membered-ring resulted in the most significant improvement of potency. Compound **23** displayed a WT EC_{50} of 4.3 nM, a value which is about twice as active as RAL in our assays, and **23** had an excellent activity of 16.5 nM and 10.5 nM against QHGS or NHEQ double mutant viruses, respectively. This represents the most potent integrase inhibitor for QHGS reported in the peer-reviewed literature to date. We also examined cyclic ureas as potential substitutions for position 9. The five-membered ring urea **24** had comparable activity to carbamate **22**, however, its six-membered congener **25** improved the QHGS activity by only two-fold, resulting in an EC_{50} of 34 nM. Substitution on the second nitrogen of the urea proved more beneficial. Replacement of the methyl group with isopropyl furnished compound **26** which is four times more active than **24** against the QHGS mutant virus. Interestingly, opening up the cyclic urea ring (**27**) led to a significant loss of QHGS activity, even though compound **27** was only slightly less active than **25** with regard to WT virus. Removal of the *N*-Me substitution caused an even more profound effect. Derivative **28** was not capable of inhibiting QHGS infectivity up to a concentration of 10 μM, despite having an EC_{50} of 22 nM against WT virus (Table 2).

Having identified that cyclic amide analogs are essential for good level of QHGS potency we set to investigate the effect of substitutions in position 7. For this purpose we chose cyclic urea **24** for comparison. It was found that the bulky morpholine group could be replaced with a smaller dimethyl amine group with negligible loss of WT and QHGS potency. The NHEQ activity decreased by threefold, however. Insertion of a methylene linker between the morpholine and the bicyclic ring furnished compound **30** which

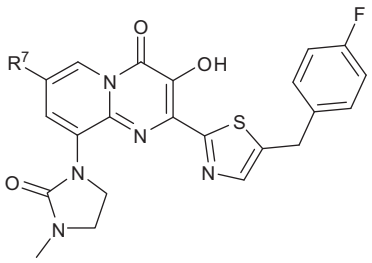
Table 1
SAR of R^9 substitutions

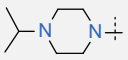


	R^9	IC_{50}[13] (nM)	WT EC_{50}[11] (nM)	QHGS EC_{50} Luc[12] (nM)	NHEQ EC_{50} Luc[12] (nM)
13		48	6.4	280	49.5
14	H	20	32	3000	74.5
15	Br	42	14	2500	19
16	MeO-	na	100	5900	na
17		15	9.5	885	na
18		na	10.5	800	na
19		na	110	5500	na
6		70	81	1175	200
20		38	8.5	112	18.5
21		na	21	335	na
22		26	7.1	108	25
23		na	4.3	16.5	10.5
24		47	24	71.0	10
25		na	11.5	34	21.5
26		19	7.5	18	9.7
27		na	16.5	120	na
28		na	22	>10000	na

is seven times more potent than **24** against WT virus, but surprisingly its QHGS activity dropped threefold. Other aminomethylene substitutions, for example, **31** displayed the same trend of SAR, exhibiting a similar level to **30** of QHGS potency of 200–300 nM.

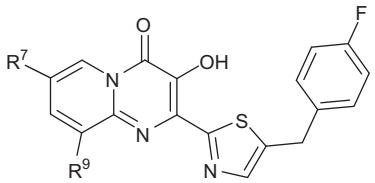
Table 2
SAR of R^7 substitutions

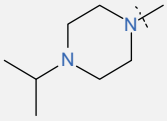
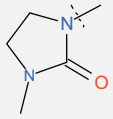
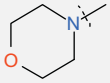
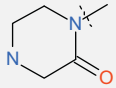
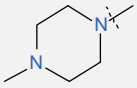
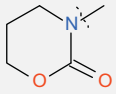
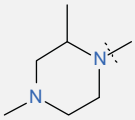
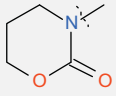


AVX	R^7	IC_{50}[13] (nM)	WT EC_{50} Luc[11] (nM)	QHGS EC_{50} Luc[12] (nM)	NHEQ EC_{50} Luc[12] (nM)
24		47	24	71.0	10
29		90	19.5	92.5	32.5
30		25	3.5	243	14.5
31		na	11.5	268	na
32		na	27	1100	na
33		19	6.8	21	8.9

Amide moieties, as exemplified by **32** retained the WT potency but caused significant decrease of QHGS inhibitory effect, having an EC_{50} of 1.1 μM. In contrast, cyclic amine derivative **33** exhibited excellent potency against both WT and resistant viruses, with EC_{50} in the range of 6.8–21 nM. The results suggested that unlike position 9, cyclic amines are preferable as R7 substitutions. However, in both cases the steric effects are not the dominant factors, instead electronic properties proved to be far more important in increasing QHGS potency. With the SAR knowledge in hand, we prepared a large number of compounds following the synthetic route depicted in Scheme 2. Our investigations have resulted in the identification of numerous potent inhibitors of HIV integrase (Table 3). Several examples displayed excellent inhibitory activity against WT virus in a high serum environment. For example, compound **33** shows less than a twofold shift in EC_{50} from 6.8 nM to 12.5 nM in the presence of 50% normal human serum (NHS). In addition, these compounds also demonstrated outstanding potency against all major RAL-resistant strains, such as QHGS and NHEQ. The specificity of the inhibitors for the inhibition of integration during virus infection of a cell was monitored during the development of this series of compounds by quantitative PCR techniques,[14,15] using RAL as positive control. Both earlier compounds of the series and examples of the most recent compounds containing the thiazole moiety as reported here were demonstrated to specifically inhibit integration events in the cell and had no effect on entry or reverse transcription events occurring prior to integration. These results taken together with the potency of the compounds against the isolated enzyme demonstrate these compounds to be bona fide integrase inhibitors. Initial PK studies indicated that these compounds had relatively short half life in both in vitro microsomal metabolism and in vivo pharmacokinetic experiments. The results suggested that oxidative metabolism was the major pathway. Compound **33** was treated with microsomes at high protein concentration in presence of NADPH and UDPGA co-factors. Analysis

Table 3
Activities and PK characteristics of selected examples



	R^7	R^9	WT EC_{50} (nM) 10% NHS[11]	WT EC_{50} (nM) 50% NHS	QHGS EC_{50} (nM) 10% FBS[12]	NHEQ EC_{50} (nM) 10% FBS[12]	In vitro $t_{1/2}$ (min)	In vivo $t_{1/2}$ (min)	%F
33			6.8	12.5	21	8.9	17.7	18	3
34			4	6.1	34.5	6.7	52	21.6	0.5
35			3.5	6.6	29.5	4.9	67.8	39	5
36			4.2	5.5	40.5	5.2	18	44	5.7

of the reaction mixture showed that the substituted piperazine in position 7 was metabolized extensively. The benzyl-thiazole moiety also underwent oxidative metabolism, although the analysis did not allow elucidation of the exact metabolic sites. In addition, glucuronidation of the phenolic 3-OH was also identified as a potential clearance pathway. Glucuronidation of a similar hydroxyl group was also reported to be the major mechanism of clearance for RAL.[16]

In summary we have disclosed a series of potent HIV integrase inhibitors based on a novel scaffold consisting of a hydroxypyridinone and a thiazole ring. The reported compounds are the first inhibitors that exhibit low nanomolar potency against all major clinically relevant RAL-resistant HIV strains, including double mutants Q148HG140S and N155HE92Q. Initial SAR studies suggested that electronic effects were dominant and important for resistant activities, with nitrogen-based substitutions preferred in both positions of the bicyclic ring system. We also demonstrated that cyclic substitutions were crucial for maintaining mutant activities while a range of diverse functionalities exhibited similarly potent level of inhibition against WT virus. The data presented herein validated thiazole-pyridinone systems as a potential platform for developing second-generation integrase inhibitors to treat RAL-resistant viruses. A comprehensive lead optimization of these promising leads to optimize PK characteristics is underway and the results will be reported in due course.

Acknowledgments

We are grateful to the Centre for drug candidate optimization, Faculty of Pharmacy and Pharmaceutical Sciences, Monash University, Melbourne, Australia, 3052 for the in-vitro metabolism studies.

References and notes

1. Summa, V.; Petrocchi, A.; Bonelli, F.; Crescenzi, B.; Donghi, M.; Ferrara, M.; Fiore, F.; Gardelli, C.; Gonzalez Paz, O.; Hazuda, J. D.; Jones, P.; Kinzel, O.; Laufer, R.; Monteagudo, E.; Muraglia, E.; Nizi, E.; Orvieto, F.; Pace, P.; Pescatore, G.; Scarpelli, R.; Stillmock, K.; Witmer, V. M.; Rowley, M. *J. Med. Chem.* **2008**, *51*, 5843.
2. (a) Sunderland, J.; Botta, M.; Aime, S.; Raymond, K. N. *Inorg. Chem.* **2001**, *40*, 6746; (b) Hare, S.; Gupta, S. S.; Valkov, E.; Engelman, A.; Cherepanov, P. *Nature* **2010**, *464*, 232.
3. Vandegraaff, N.; Engelman, A. *Expert Rev. Mol. Med.* **2007**, *9*, 1.
4. Ceccherini-Silberstein, F.; Malet, I.; D'Arrigo, R.; Antinori, A.; Marcelin, A.; Perno, C. *AIDS Rev.* **2009**, *11*, 17.
5. Hatano, H.; Lampiris, H.; Fransen, S.; Gupta, S.; Huang, W.; Hoh, R.; Martin, J. N.; Lalezari, J.; Bangsberg, D.; Petropoulos, C.; Deeks, S. G. *J. Acquir. Immune Defic. Syndr.* **2010**, *54*, 389.
6. Reigadas, S.; Anies, G.; Masquelier, B.; Calmels, C.; Stuyver, L. J.; Parissi, V.; Fleury, H.; Andreola, M. L. *PLoS One* **2010**, *5*, 10311.
7. Charpentier, C.; Laureillard, D.; Piketty, C.; Tisserand, P.; Batisse, D.; Karmochkine, M.; Si-Mohamed, A.; Weiss, L. *AIDS* **2010**, *24*, 867.
8. (Part 1) Jones, E.; Vandegraff, N.; Le, G.; Choi, N.; Issa, W.; Macfarlane, K.; Thienthong, N.; Winfield, L.; Coates, J.; Lu, L.; Li, X.; Feng, X.; Yu, C.; Rhodes, D.; Deadman, *J. Bioorg. Med. Chem.* Lett., accepted for publication; (part 2) Le, G.; Vandegraff, N.; Rhodes, D.; Jones, E.; Thienthong, N.; Winfield, L.; Coates, J.; Lu, L.; Li, X.; Feng, X.; Yu, C.; Deadman, J. *Bioorg. Med. Chem. Lett.*, accepted for publication.
9. Buchwald, S. L.; Yin, J. *J. Am. Chem. Soc.* **2002**, *124*, 6043.
10. (a) Ren, W.; Yamane, M. *J. Org. Chem.* **2010**, *75*, 3017; (b) Wu, X.; Rönn, R.; Gossas, T.; Larhed, M. *J. Org. Chem.* **2005**, *70*, 3094.
11. *Inhibition of HIV replication*: Cells are seeded into 96 well microtitre plates at 50,000 cells per 50 μl per well in RF-10 containing 2 μg/ml polybrene (RF-10/2). Compounds are prepared to 4× final concentration in RF-10/2, and 30 μl added to cells. Virus (40 μl in RF-10/2 containing 1600 pfu) is added to each well or 40 μl RF-10/2 for negative controls and for assaying compound cytotoxicity. After 24 h, an additional 90 μl of media or media containing 1× compound is added to each well. At 4 days post-infection, 100 μl of media is removed from each well and replaced with 100 μl of fresh media with or without compound. Forty eight hours later supernatants are harvested and levels of extracellular p24 determined. Supernatants are diluted 1 in 10,000 and p24 levels assayed using the Vironostika p24 assay kit. EC_{50} is calculated as the concentration required to inhibit HIV p24 production to 50%.
12. Chang, T. L.-Y.; Francois, F.; Mosiam, M.; Klotman, M. E. *J. Virol.* **2003**, *77*, 6777. Infectivity assays used virus stocks generated by transfecting a plasmid encoding a full-length HIV-1 genome which had the envelope gene deleted and contained a reporter luciferase gene cloned into the *nef* region (pHIV env-Luc) together with an expression plasmid producing the vesicular stomatitis virus envelope glycoprotein (VSV-G) into 293T cells. Culture supernatants containing VSV-G pseudotyped virions were harvested 64 h post-transfection, clarified by centrifugation to remove cell debris, and stocks stored at −70 °C. HIV integrase mutations known to confer resistance to published integrase inhibitors were prepared by site directed mutagenesis of the integrase gene in a shuttle vector which contained the majority of the HIV-1 *gag* and *pol* sequence. The mutated integrase coding region was then sequence verified and exchanged for the

wild-type coding sequence in the pHIV env-Luc reporter virus plasmid. *Assay method* 293T cells were seeded in 96 well plates 16 h prior to compound addition. Compounds were preincubated with cells for 4 h at 37 °C followed by the addition of sufficient virus to generate approximately 10,000 Luciferase light units in the absence of compound. Luciferase readout was measured 48 h post-infection using the Bright-Glo™ reagent (Promega) according to the manufacturer's instructions.

13. (a) Ovenden, S. P.; Yu, J.; Wan, S. S.; Sberna, G.; Tait, R. M.; Rhodes, D.; Cox, S.; Coates, J.; Walsh, N. G.; Meurer-Grimes, B. M. *Phytochemistry* **2004**, *65*, 3255; (b) *Combined strand transfer assay*: Briefly, for a 96 well format, 400 ng IN is incubated with 30 nM pre-processed substrate DNA, consisting of annealed U5 LTR sequence oligonucleotides tagged with Digoxigenin (DIG; 5′-ACTGCTAGAG ATTTTCCACACTGACTAA-AAGGGTC-DIG-3′) or biotin (5′-Bio-GACCCTTTTAGT CAGTGT-GGAAAATCTCTAGCA-3′) so that each substrate has either a DIG or Bio tag on opposite strands. Reactions are carried out for 2 h at 37 °C, products generated as a result of 3′ processing and strand transfer activity are bound to streptavidin plates and detected using anti-DIG-alkaline phosphatase conjugate and *p*-nitro phenyl phosphate substrate.
14. Engelman, A.; Oztop, I.; Vandegraaff, N.; Raghavendra, N. K. *Methods* **2009**, *47*, 283.
15. Butler, S. L.; Hnasen, M. S.; Bushman, F. D. *Nat. Med.* **2001**, *7*, 631.
16. Kassahun, K.; McIntosh, I.; Cui, D.; Hreniuk, D.; Merschman, S.; Lasseter, K.; Azrolan, N.; Iwamoto, M.; Wagner, J. A.; Wenning, L. A. *Drug Metab. Dispos.* **2007**, *35*, 1657.