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Design of a series of bicyclic HIV-1 integrase inhibitors. Part 1: Selection of the scaffold

Eric D. Jones [a], Nick Vandegraaff [a], Giang Le [a], Neil Choi [a], William Issa [a], Katherine Macfarlane [a], Neeranat Thienthong [a], Lisa J. Winfield [a], Jonathan A. V. Coates [a], Long Lu [b], Xinming Li [b], Xiao Feng [b], Changjiang Yu [b], David I. Rhodes [a], 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

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ABSTRACT

HIV integrase inhibitors based on a novel bicyclic pyrimidinone core is presented. Nine variations of the core scaffold are evaluated leading to optimization of the 6:6 core giving compound **48** with an EC_{50} of 3 nM against wild type HIV infected T-cells.

The Human Immunodeficiency Virus (HIV) is the causative agent of acquired immunodeficiency syndrome (AIDS), a disease for which there is presently no cure. Therapy currently consists of a combination of three drugs targeting two or more critical targets within the viral life cycle in what has proved to be an extremely successful treatment regimen known as highly active antiretroviral therapy (HAART). Raltegravir (Isentress™),[1] a drug approved by the FDA in October 2007 for the treatment of HIV infection, targets the viral integrase (IN) protein that is responsible for recombining newly synthesized HIV-1 DNA into the host cellular chromosome. IN achieves this via a two-step enzymic process in which the 3′ terminal two nucleotides on each newly made viral DNA strand are trimmed (3′-processing [3′] reaction) prior to insertion into the host cellular DNA (strand transfer [ST] reaction). Assays that recapitulate these two reactions in vitro using recombinant IN rely on the presence of divalent metal ions (such as Mg^{2+} or Mn^{2+}) that are known to be coordinated by a trio of conserved basic residues (D64, D116, and E152) within the active site. Raltegravir and related integration strand transfer inhibitors (INSTIs Fig. 1) are proposed[2] to interrupt viral DNA binding of integrase by coordinating these metal ions via a hydrogen bond acceptor–donor–acceptor motif.

While no crystal structure of HIV integrase with viral or host DNA and its inhibitors is yet available, the foamy virus integrase structure with viral DNA bound confirms this interaction.[3] Since 6-carboxamido-5,4-hydroxypyrimidines were known as a metal coordinating motif,[4] we chose the para-fluorobenzyl 6-carboxam-

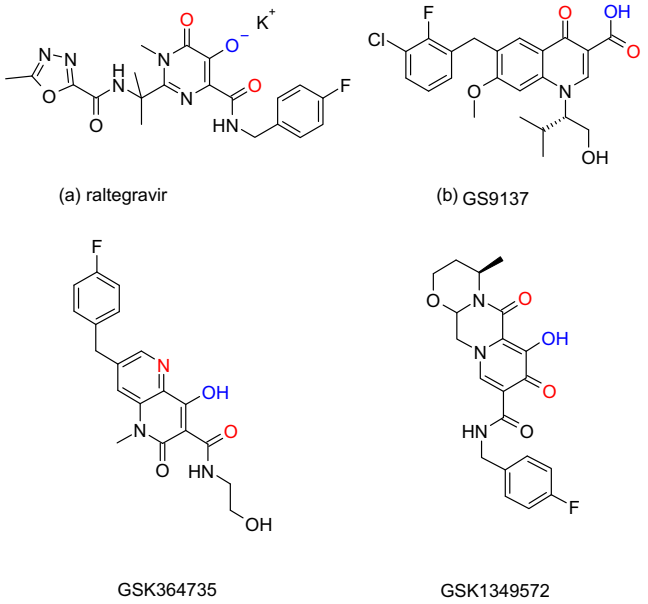

Figure 1. Reported INSTIs.

* Corresponding author. Tel.: +61 408538488.
E-mail address: jjdeadman@bigpond.com (J.J. Deadman).

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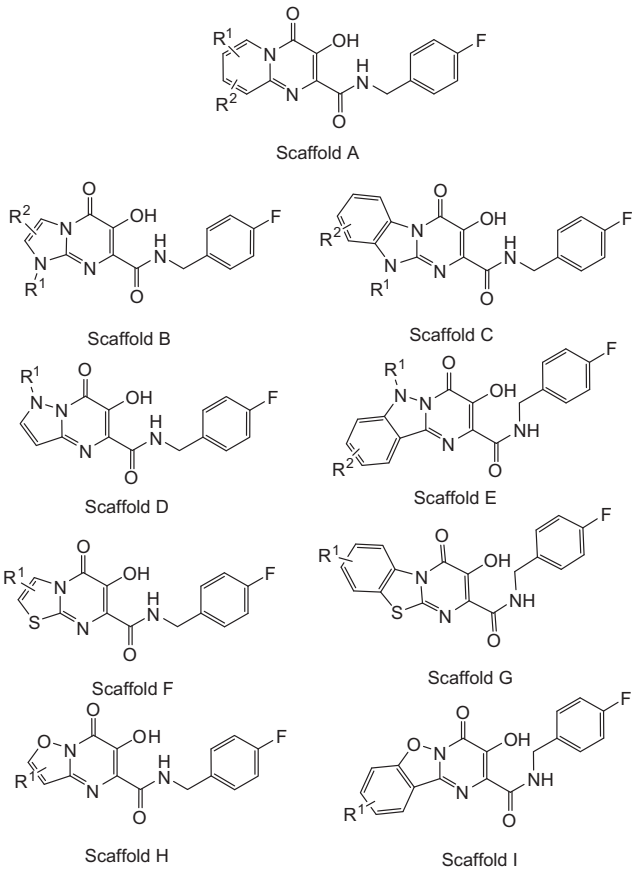


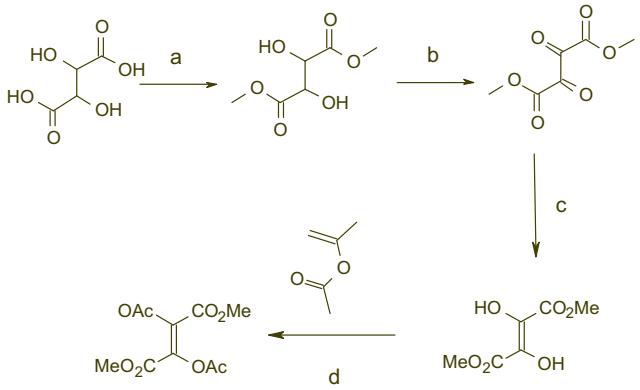
Figure 2. Target scaffolds A–I.

ide side chain on a 4-keto-5-hydroxy pyrimidine to mimic the acceptor–donor–acceptor motif. We reasoned that incorporation of a bicyclic system could firstly modulate the electronics of the coordinating motif and optimize the binding to the catalytic metal and secondly provide a scaffold to introduce functionality to enhance the interaction with the integrase enzyme. Accordingly a series of bi- and tricyclic novel compounds were prepared (Fig. 2).

Previously a single report[5] had described a route to unsubstituted 5:6 and 6:6 bicyclic pyrimidinones **3**, from the reaction of diacetoxy fumerate (DAF) **2** with the appropriate 2-amino heterocycle **1** (Scheme 1). From **3** introduction of the required benzyl side chain was achieved by reaction with excess amine.

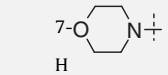
In our hands the procedure of Tisler was found to be low yielding (5–40%) and attempts to optimize the yield by varying reaction time, temperature, and catalyst used were not successful. In order to access larger quantities of core materials, it was necessary to prepare kilogram quantities of DAF as described in Scheme 2.

With the synthetic route on hand the examples of the target scaffolds in Figure 2 were prepared and assayed and the results are presented in Table 1.

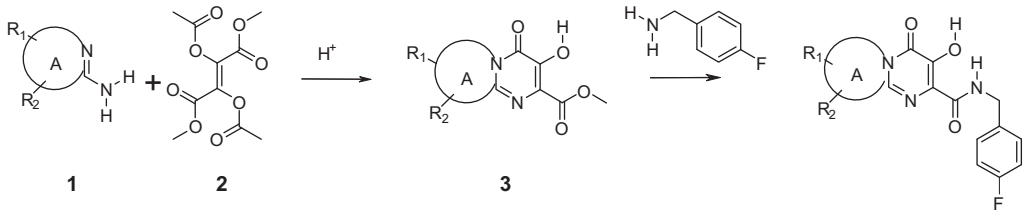


Scheme 2. Reagents and conditions: (a) $SOCl_2$, MeOH, 0 °C, 1 h then reflux 3 h, 85%; (b) NBS, CCl_4, reflux, 4 h; (c) $Na_2S_2O_4$, H_2O, rt, 2 h, 30% overall b and c; (d) pTsOH, reflux, 24 h, 72%.

Table 1
IC_{50} achieved with scaffold selection

Compound	Scaffold	R1	R2	IC_{50} (μM)
4	A	7-Br	H	0.445
5	A	7- 	H	0.080
6	A	H	H	0.587
7	B	CH_3	H	0.0768
8	C	H	H	>100
9	C	CH_3	H	20.25
10	D	H	7-Ph	>100
11	D	H	8-CN	13.65
12	D	CH_3	H	>30
13	E	H	H	>10
14	E	CH_3	H	0.626
15	F	H	H	33.25
16	F	7-CH_3	H	4.25
17	F	7-Morph	H	1.13
18	G	9-OCH_3	H	>50
19	G	H	H	>30
20	H	7-CH_3	H	5.1
21	I	H	H	>10
22	I	7-Morph	H	>10

Activity of the compounds were assessed in an isolated protein assay using full length integrase whereby a model 5′-biotin labeled DNA substrate already in the pre-processed form with a 3′-CA sequence is inserted into an acceptor 3′-digoxin DNA strand as previously described.[6] This strand transfer assay format was selected over the combined 3′-ST assay to allow optimization for the second strand transfer step of integrase function. It was encouraging to find that a range of scaffolds were active against the enzyme with sub micromolar IC_{50}s as shown by **4**–**7** and **14**, and that some preliminary SAR could be established. For scaffold E, unsubstituted nitrogen in the ring was not tolerated (**13** vs **14**) and extension



Scheme 1.

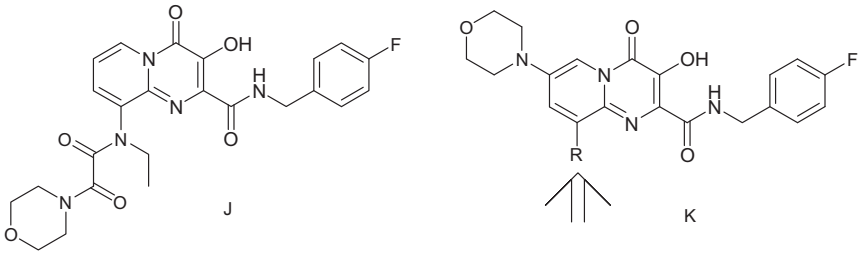
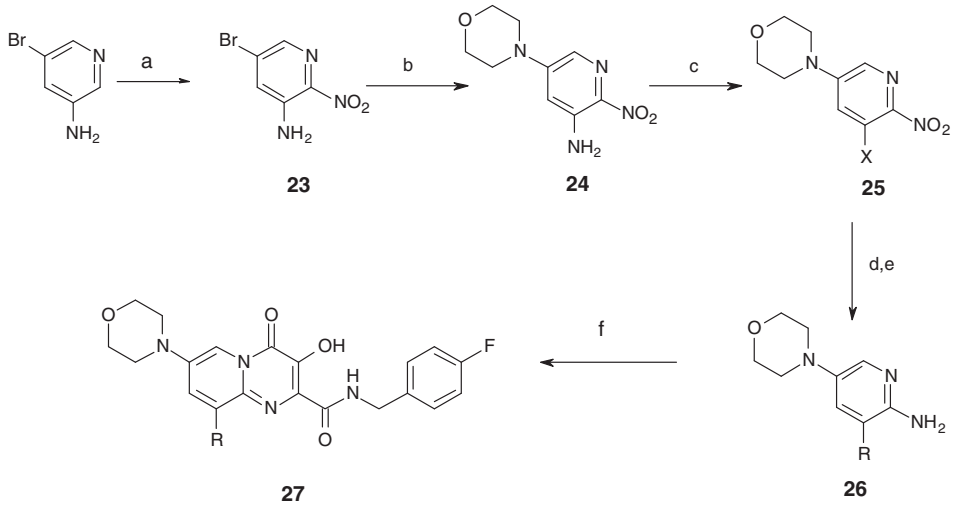


Figure 3. Bi-functionalized pyrimido-pyrimidine.



Scheme 3. Reagents and conditions: (a) (i) ClCOOEt, Py 93%; (ii) HNO_3, H_2SO_4, 30 °C, 20 h, 68%; (iii) KOH/EtOH/H_2O, 75%; (b) morpholine, 140 °C, 10 h, 85%; (c) $NaNO_2$, H_2SO_4, H_2O, 0 °C, 2 h then 80 °C 2 h, 75% for X = OH or CuBr, X = Br 80%; (d) X = OH then DIAD, Ph_3P, THF, ROH, 0 °C, 2 h, 75% or from X = Br with isothiazolidine-1,1-dioxide [entry **43** and ROH, **44**], DMEDA, CuI/K_2CO_3, toluene, 80 °C, 5 h, 43.1%; (e) $SnCl_2$, EtOH/H_2O, 80 °C, 5 h, 70–90%; (f) (i) HOAc, DAF, sealed tube 90°, 20 h C; (ii) 4-*F*-benzyl amine, MeOH, reflux, 3 h for **38** 15.2% overall.

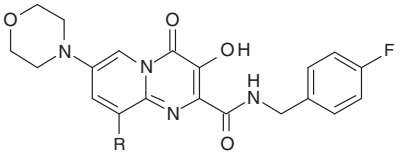
to the tricyclic systems was not tolerated for scaffolds C and I (**7** vs **9**).

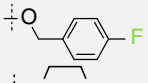
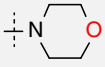
Compounds that showed IC_{50}s of ~1 μM were assessed for anti-HIV-1 activity in Hut78 cells infected with HIV (NL4-3 strain). Of note were examples of scaffolds A, **4** and **5**, B compound **7**, and F compound **17** showing sub micromolar inhibition in (EC_{50}'s 0.65, 0.028, 0.113, and 0.48 μM, respectively) while showing no cytotoxicity up to 10 μM. These three classes represent potentially novel inhibitors of HIV and herein we report the optimization of Scaffold A. Optimization of scaffolds B and F will be the subject of future publications.

In the course of this work, researchers at Merck reported[7] their discovery of the pyrimido-pyrimidine core as a new class of HIV-1 integrase inhibitors. This program focused on derivatives at position 9, (structure J in Fig. 3) achieving low nM potencies against HIV. We decided to exploit our different route of synthesis by directing our efforts into investigating the effect of a substitution at the C9 position of **5** (K in Fig. 3).

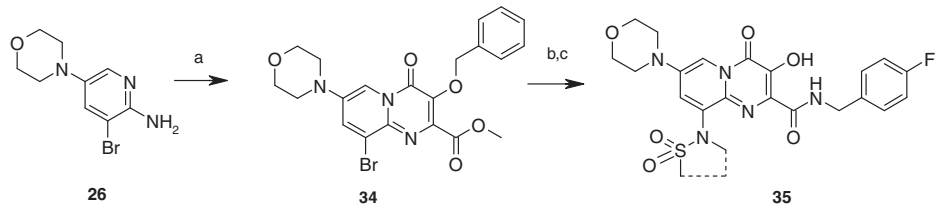
In order to assess the effect of substitution at the C9 of **5**, the route as in Scheme 3 was devised. Starting from 3-amino-5-bromopyridine, regioselective nitration allowed SnAr conditions to introduce the required morpholine **24**. Diazotization allowed the introduction of bromine (**25**) or Mitsunobu reaction to give the aryl ether and subsequent reduction afforded the amino pyridine **26**. Core formation by reaction with DAF and aminolysis provided the desired targets **27**.

Table 2
Effect on activity by introduction of an EDG at C9 of **5**

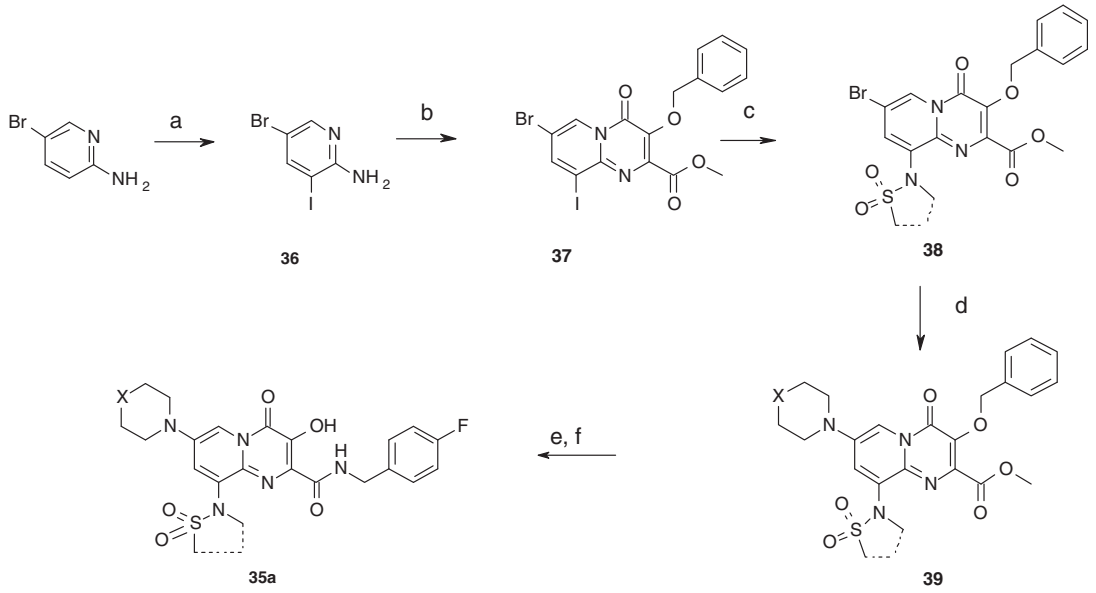


Compound	R	IC_{50} (nM)	EC_{50} (nM)	
			10% FBS	50% NHS
5	H	80	215	ND
28	OMe	170	9.5	1400
29	O*i*Pr	245	5	ND
30		429	12	590
31		275	80	ND
32		755	730	1550

Initially we investigated the effects of introducing an electron withdrawing substituent at C9 of **5**. Compounds were again assayed in the isolated enzyme system and subjected to single-round



Scheme 4. Reagents and conditions: (a) (i) DAF, HOAc, MeOH, reflux, 24 h; (ii) BnBr, K_2CO_3, DMF, 80 °C, 6 h, 25% two-steps; (b) $Pd_2(dba)_3$, xanthphos, Cs_2CO_3, dioxane, 100 °C, 4 h, 67%; (c) (i) TFA, 70 °C, 5 h; (ii) pF-benzylamine, MeOH, reflux, 15 h, 50% overall two-steps.



Scheme 5. Reagents and conditions: (a) I_2, $HIO_4{\cdot}H_2O$, AcOH, $H_2SO_4{\cdot}H_2O$, 75 °C, 7 h, 70%; (b) (i) DAF, pTSOH, 100 °C, 30 h; (ii) BnBr, K_2CO_3, DMF, 70 °C, 16 h, 16% two-steps; (c) $Pd_2(dba)_3$, xanthphos, Cs_2CO_3, dioxane, 2-methyl-[1,2,5]thiadiazolidine-1,1-dioxide, 80 °C, 6 h, 61% or $MeSO_2NHMe$, 50 °C, 16 h, 85%; (d) $Pd_2(dba)_3$, xanthphos, Cs_2CO_3, dioxane, 90 °C, 15 h, 40%; (e) TFA, 70 °C, 5 h, 90%; (f) pF-benzylamine, MeOH, reflux, 15 h, 39%.

HIV-1-based infectivity assays, which allowed the determination of EC_{50} values in low serum (10% FBS) and the more therapeutically relevant high serum level (50% NHS). Table 2 summarizes the results.

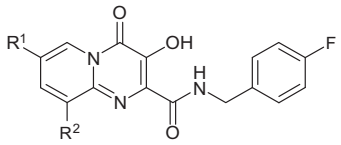
Improvements in potency against the enzyme by incorporation of an electron donating group, EDG, into the base compound **5** as shown by **28**–**32** was not obtained. In contrast, potency in the anti-HIV assay was greatly enhanced by the inclusion of alkoxy groups (**28**–**31**). The anti-HIV result for **29** compared to the C9-smaller **28** and larger **30** and **31** suggests a finite aliphatic binding pocket in the region of C9, and the discordance between the isolated integrase enzyme and cell-based data indicates that the enzyme may adopt a slightly different conformation in the whole cell system. Although low nanomolar potency has been achieved in **28** the >100-fold shift of inhibitory activity in cell-based assays conducted in a high serum environment indicate the detrimental effect of plasma protein binding. In order to explore this, a series of analogues containing EDG at C9 were prepared by modification of the chemistry described in Scheme 3. As Scheme 4 shows, direct core formation of the intermediate **26** from Scheme 3, followed by benzyl protection of the 2-OH afforded the C9-halogenated scaffold **33** which was primed for further extension at C9 by a series of metal mediated aminations.

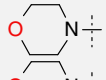
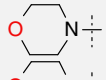
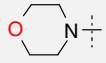
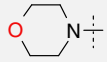
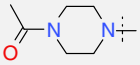
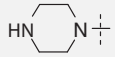
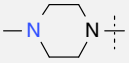
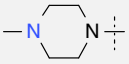
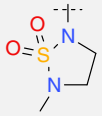
In order to explore the R1 group at position C7 in structures based on **35** beyond morpholine, the route as in Scheme 5 was devised where the di-halo intermediate **36** was formed under standard core formation conditions followed by protection of the C3-OH gave the intermediate **37**. The increased reactivity of the C9-iodo to Buchwald conditions was exploited to introduce polar substituents as C9 followed by a second amination to functionalize at C7.

The introduction of an electron withdrawing group, EWG, at R1 as in Scheme 4 continued to provide low nanomolar potency against HIV (**40**–**42**). Increasing the polarity by introduction of a sultam, made by the literature method,[8] at C9 in **43** was also tolerated, and a reduced shift of less than twofold in 50% NHS (Table 3). Solubility needed to be balanced, and although the dichlorobenzyl amine compound **44** was potent, it showed a drastic shift in the presence of serum compared to **43**. Introduction of a piperazine functionality R1 at C7 to improve solubility was also tolerated in **45**, although the *N*-des-methyl version **46** was not. Scheme 5 allowed the introduction of a basic piperazine at R1 with a linear sulfonamide in **47**, which was potent (EC_{50} 19.8 nM) and showed a threefold shift in serum. However, the more basic *N*-methyl thiadiazolidine, made following the literature method[9], when introduced into position C9 in **48** with an EC_{50} 3 nM gave a minimal fold change in the presence of 50% serum.

In summary, the work presented here has defined a range of novel scaffolds that form the basis of HIV integrase inhibitors. Optimization of the 6:6 bicyclic scaffold has lead to significant improvements in antiviral activity superior to that of the launched drug raltegravir. Further optimization of this series will be presented in due course.

Table 3
Effect on activity EWG at C9 of scaffold A



Compound	R^1	R^2	IC_{50} (nM)	EC_{50} (nM)	
				10% FBS	50% NHS
40		I	85	6	50
41		Br	130	7	70
42		Cl	135	11	158
43			195	10.3	18
44[a]			130	0.2–1.2	43
45		Br	110	8	40
46			ND	280	43
47			ND	19.8	61.5
48			ND	3	4
Raltegravir			130	9.4	7.1

[a] 3,5-Dichlorobenzylamine analogue of **43**.

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(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′-ACTGCTAGAGATTTTCCACACTGACTAA-AAGGGTC-DIG-3′) or biotin (5′-Bio-GACCCTTTTAGTCAGTGT-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 ℃, 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.
(c) *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%.
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