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Naphthyridinone (NTD) integrase inhibitors: *N*1 Protio and methyl combination substituent effects with C3 amide groups

Brian A. Johns [a,*], Takashi Kawasuji [b], Jason G. Weatherhead [a], Eric E. Boros [a], James B. Thompson [a], Cecilia S. Koble [a], Edward P. Garvey [a,†], Scott A. Foster [a,‡], Jerry L. Jeffrey [a], Tamio Fujiwara [b]

[a] GlaxoSmithKline Research & Development, Infectious Diseases Therapy Area Unit, Five Moore Drive, Research Triangle Park, NC 27709, USA
[b] GlaxoSmithKline Research & Development, Infectious Diseases Therapy Area Unit, Shionogi Pharmaceutical Research Center, Shionogi & Co., Ltd, 3-1-1, Futaba-cho, Toyonaka-shi, Osaka 561-0825, Japan

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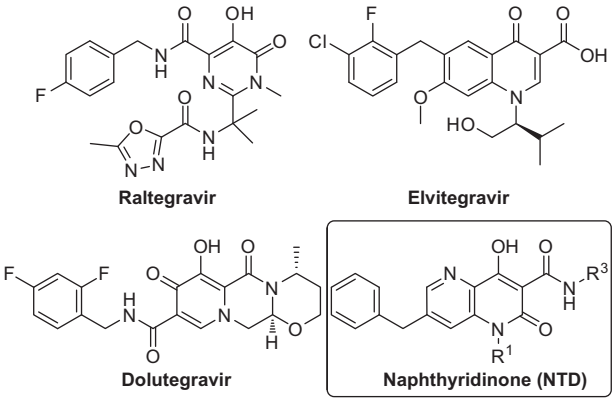
ABSTRACT

Substituent effects of a series of *N*1 protio and methyl naphthyridinone HIV-1 integrase strand-transfer inhibitors has been explored. The effects of combinations of the *N*1 substituent and C3 amide groups was extensively studied to compare enzyme inhibition, antiviral activity and protein binding effects on potency. The impact of substitution on ligand efficiency was considered and several compounds were advanced into in vivo pharmacokinetic studies ultimately leading to the clinical candidate GSK364735.

HIV therapeutics has been one of the most successful endeavors in medicine. However as with most infectious diseases, the virus continues to challenge those therapies which are further complicated by tolerability and adherence problems resulting in suboptimal viral suppression over periods of chronic therapy. As a result of the opposing pressures of drug therapy and viral persistence it would be remiss for the scientific community to think that HIV is a solved problem.[1] Viral evolution, including the transmission of resistant virus, is of concern and currently a topic of intense debate.[2] Additionally the long term tolerability and safety of many of the available therapeutic successes are not optimal.[3] Of particular interest are finding regimens that allow delay of the use of nucleoside analogs (nuc sparing) until later lines of therapy.[4] A yet more interesting rationale for continued introduction of antiretroviral agents ironically comes from the curative approaches now becoming an area of exploration. It has been argued that intensification of current regimens will be required for complete suppression during transcriptional activation envisaged during attempts to purge latent reservoirs.[5] As such, there continues to be a need for new drugs from either new classes or from further study of existing targets.

The inclusion of an HIV-1 integrase inhibitor as part of highly active antiretroviral therapy (HAART) regimens has become increasingly attractive since the 2007 registration of raltegravir.[6] Integrase inhibitors have been well tolerated and are among the most potent antiretrovirals discovered to date. Raltegravir (RAL) is a twice daily therapy (400 mg BID) and is saddled with high PK variability and well established resistance pathways suggesting that while an excellent drug, there is substantial room for further study and improvement of the class.[7]



Two additional integrase inhibitors appear to partially or substantially address the raltegravir limitations. Elvitegravir (EVG) is a once daily alternative that recently achieved registration in the US but

* Corresponding author.
E-mail address: brian.a.johns@gsk.com (B.A. Johns).
† Current address: Viamet Pharmaceuticals, Inc., Morrisville, NC, USA.
‡ Current address: Chimerix, Inc., Durham, NC, USA.

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requires a P450 inhibitor to boost exposures and is highly cross-resistant to the raltegravir signature mutations.[8] Slightly further behind in development is the carbamoyl pyridone derivative dolutegravir (DTG) which is a true once daily low dose drug with no need for PK enhancers and has a superior resistance profile compared to both RAL and EVG.[9]

We recently reported the naphthyridinone (NTD) scaffold containing a 7-benzyl moiety to be inhibitors of the HIV integrase strand transfer step resulting in a potent antiretroviral thus establishing the core as an attractive platform for further optimization.[10] The NTD scaffold consists of the chelating motif that has served to establish a two-metal binding pharmacophore synonymous with integrase strand transfer inhibitors.[11,12] The emergence of resistance to raltegravir[13] suggests a continued need for additional agents in this class of ARV's as well as the need for further understandings of the scope and limitations of the two-metal binder class of inhibitor.[7] To that end we report herein results of our continued exploration of structure–activity relationships (SAR) of the NTD integrase inhibitor series by elaborating on the effects of *N*1 protio and methyl substitutions (R^1) in combination with an array of C3 amido groups (R^3) on biochemical and antiviral activity along with select pharmacokinetic properties. This work led to the discovery of the clinical candidate GSK364735.[14]

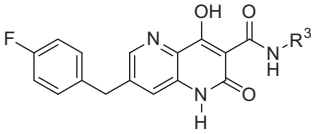
During the investigations reported herein, we elected to maintain the 7-benzyl moiety consistently substituted with a 4-fluoro group. It has been shown by us and others that the haloaryl substitution consistently delivers improved potency and may also improve pharmacokinetic profiles. Our assay systems involved an integrase biochemical strand transfer assay ($^{ST}IC_{50}$)[15] and a single round pseudotyped antiviral assay utilizing a luciferase reporter readout ($^{pHIV}IC_{50}$).[16] The pHIV assay also allowed for the addition of a relevant amount of purified human serum albumin (HSA) to be used as an estimate of serum protein binding effects on compound potency giving rise to a protein adjusted IC_{50} ($^{pHIV}PAIC_{50}$).[17] All compounds in the series had a substantial therapeutic index when comparing antiviral potency versus cellular toxicity (data not shown). An additional measure is included to compare ligand lipophilicity efficiency (LLE, pIC_{50}-*c*log*P*) which is gaining acceptance as a key measure of true potency improvement and reduce toxicity risks.[18] In general, it is desirable to have the LLE >5.

The synthetic methods used to prepare the title compounds in Tables 1–3 began with the substituted 3-amino-2-pyridinecarboxylate ester **1a**[19] (Scheme 1). This material could be carried forward directly for the *N*1 protio analogs or could be methylated using an intermediate trifluoroacetamide which was alkylated with methyl iodide and removed with MeOH and potassium carbonate to give **1b**. Acylation with the mixed malonate **2** in dichloroethylene without a base resulted in the compounds **3**. Dieckmann cyclization to form the naphthyridinone ring system proceeded smoothly with sodium ethoxide. At this point, the *N*1 protio material **4a** could be alkylated to give the *N*1 methyl intermediate **4b** which matched the material made from the early methylation method using **1b**. Amide formation with a variety of amines was used typically in neat conditions with heating to give the target drug molecules. In some cases when the amines were not amenable to use neat, the condensation was performed in ethanol, DMF or NMP as a solvent.

We initially sought to explore a variety of groups at the C3 amide position while maintaining the ring nitrogen of the naphthyridinone system unsubstituted (*N*1=H). The completely unsubstituted primary amide **5** was relatively potent in the biochemical assay but had a significant loss of potency in the presence of HSA (Table 1). Increasing the size and lipophilicity of the amide group had little impact on the enzymatic or antiviral activity but did increase the protein shift. Alkoxyalkyl groups improved $^{pHIV}PAIC_{50}$'s (**8**–**10**) but at the cost of ligand efficiency with little gain in intrinsic potency with the added lipophilicity and molecular weight. Similar amides in the form of hydroxyalkyl substituents gave high potency in both the strand transfer and pHIV assays and also had a very modest protein shift (**11**–**12**). The SAR was further developed with a representative series of basic amines **13**, **14**, **16** and **17** and clear improvements in $^{pHIV}PAIC_{50}$ were observed with increased polarity of the amine group. LLE values also improved as a result of both c log*P* decreased and pIC_{50} increases in **16** and **17** versus **14** and **15**. Several sulfur containing analogs were also examined with sulfone **20** showing the largest LLE. However this example also demonstrates that, at least for HIV antiretroviral agents, the situation can be complicated with a significant protein shift leading to a loss of activity.

From the data set acquired thus far, the most promising analogs appeared to be alcohols **11** and **12** when LLE and $^{pHIV}PAIC_{50}$ both were taken into consideration. As such, a focused set of amino alcohols were reacted with **4b** to form the corresponding amides shown in Table 2. Ligand efficiencies are uniformly high with some differentiation in the HSA-shifted antiviral data. Further substitution of the hydroxyethyl system did help potency (~2.5 fold

Table 1
SAR of *N*1 protio NTD analogs



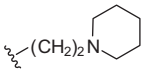
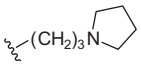
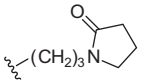
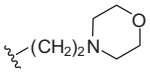
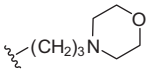
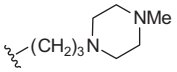
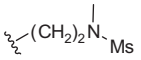
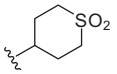
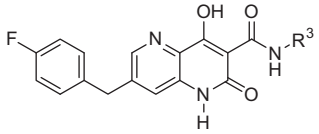
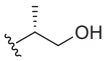
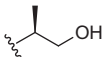
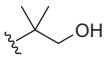
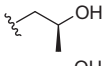
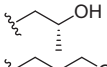
Compd	R^3	(μM)			LLE
		$^{ST}IC_{50}$	$^{pHIV}IC_{50}$	$^{pHIV}PAIC_{50}$	
5	H	0.045	0.066	0.66	4.8
6	CH_3	0.006	0.003	0.12	5.3
7	$(CH_2)_2iPr$	0.005	0.008	0.23	3.4
8	$(CH_2)_2OMe$	0.005	0.005	0.035	5.2
9	$(CH_2)_2OEt$	0.009	0.004	0.065	4.5
10	$(CH_2)_3OiPr$	0.009	0.008	0.19	3.9
11	$(CH_2)_2OH$	0.005	0.006	0.051	6.1
12	$(CH_2)_3OH$	0.011	0.004	0.041	5.3
13		0.014	0.038	0.172	3.3
14		0.023	0.039	0.109	3.7
15		0.004	0.005	0.031	5.4
16		0.007	0.006	0.042	4.8
17		0.008	0.006	0.037	4.6
18		0.011	0.028	0.11	5.3
19		0.004	0.009	0.049	5.5
20		0.009	0.048	0.17	6.9
21	$(CH_2)_2SMe$	0.005	0.005	0.256	4.6

Table 2
SAR of *N*1 protio C3 hydroxyalkyl amide NTD analogs



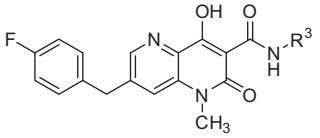
Compd	R^3	(μM)			LLE
		$^{ST}IC_{50}$	$^{pHIV}IC_{50}$	$^{pHIV}PAIC_{50}$	
22		0.005	0.007	0.033	5.7
23		0.004	0.006	0.019	5.7
24		0.003	0.002	0.019	5.5
25		0.003	0.007	0.078	5.9
26		0.006	0.007	0.282	5.6
27		0.004	0.004	0.041	4.9

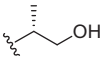
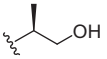
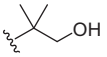
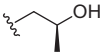
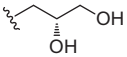
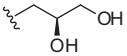
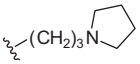
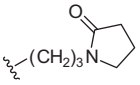
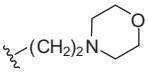
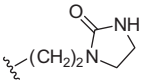
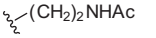
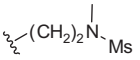
improvement **22**–**24** vs **11**) however again at the expense of ligand efficiency and thus were not considered significant improvements in the absence of further data.

Encouraged by the initial NTD SAR, we turned our attention to improving the scaffold properties. The next logical step was to substitute at *N*1 and revisit some of the amide SAR. The unfunctionalized amides **28** and **29** performed in a similar manner to the protio counterparts **6** and **7** whereby potent antiviral activity was observed only to be lost in the presence of HSA. This was further demonstrated with the difluoroethyl analog **30** resulting in a 2900 fold shift. However, revisiting the methoxy and hydroxyethyl substitution retained activity with only a modest protein shift with the alcohol-containing derivative showing an approximately twofold improvement in activity over the methyl ether. Further extension of the chain to a C3 alcohol (**33**) resulted in a higher protein shifted value. Slight increases in potency were noticed with the methyl substituted amide chains in **34** and **35** however at the cost of slightly lowered LLE due to increased lipophilicity. Increased branching of the hydroxyethyl chain substitution SAR did not show improvements in either LLE or $^{pHIV}PAIC_{50}$ measures (**36**–**38**). However, improvements in LLE as a result of a lowered clog*P* were observed for diol enantiomers **39** and **40** through translation into greater antiviral activity was not observed. Basic amine containing tails **41** and **43** while potent did not deliver any improvements in activity and suffered some loss of LLE. Similarly, the pyrrolidione analog **42** had excellent potency as a result of an attenuated protein shift value. The addition of a nitrogen to give urea **44** provided a slight improvement in potency along with LLE. Similar potency levels were attained with the acetamide **45** and *N*-methyl-*N*-methylsulfonamide **46**.

A select group of compounds from the protio and methyl series were assayed in an MT4 cell multicycle full replication assay (Table 4). Some differences in activity were observed such as comparing compound **11** and **32** which differ only in the *N*1 protio versus methyl substituent respectively. The methyl analog **32** is threefold more potent in the pseudotyped assay but over 16-fold better in the MT4 assay. The related analog pairing of **22** and **34** whereby a methyl branch is added to the amide group results in a fivefold differential favoring the *N*1 methyl analog **34**. Conversely, the more polar basic amine pair **16** and **43** while both less potent, have an only twofold activity difference favoring in this case the *N*1 protio

Table 3
SAR of *N*1 methyl NTD analogs

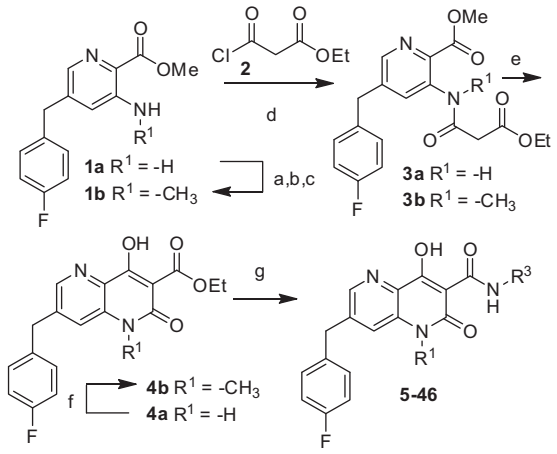


Compd	R^3	(μM)			LLE
		$^{ST}IC_{50}$	$^{pHIV}IC_{50}$	$^{pHIV}PAIC_{50}$	
28		0.004	0.001	0.055	5.3
29		0.004	0.002	0.65	4.8
30		0.005	0.001	2.9	4.7
31		0.004	0.002	0.048	5.2
32		0.008	0.002	0.020	5.8
33		0.005	0.001	0.040	5.5
34		0.008	0.002	0.011	5.3
35		0.004	0.001	0.015	5.7
36		0.006	0.003	0.028	5.0
37		0.007	0.003	0.18	4.0
38		0.002	0.002	0.11	5.6
39		0.003	0.002	0.015	6.1
40		0.004	0.003	0.018	6.1
41		0.006	0.009	0.034	3.9
42		0.016	0.002	0.015	4.6
43		0.013	0.004	0.023	4.4
44		0.005	0.002	0.009	5.8
45		0.005	0.002	0.012	5.8
46		0.005	0.001	0.007	5.4

derivative. The urea, acetamide and sulfonamide analogs **44**, **45**, **46** that were highly potent in the pHIV assay fell off slightly in activity in the MT4 system.

The pharmacokinetic (PK) properties of the two sets of analogs were evaluated using male CD rats as a screening species. Initially select compounds from those with the highest potency were examined using oral dosing with AUC(0–24 h) and the ratio of the concentration at 24 h divided by the $PAEC_{50}$ as key measures to decide on further progression. Three compounds in Table 5 showed significantly elevated oral AUC levels (**32**, **35**, and **39**).

Based on the rat data, the three best compounds based on oral AUC's were taken into a dog study (Table 6). Unfortunately, the methyl substituted hydroxyethyl amide **35** and diol **39** had only modest exposure from oral dosing. However, the hydroxylethyl



Scheme 1. Reagents and conditions: (a) TFAA, DCE; (b) MeI, CH_3CN, K_2CO_3, Δ; (c) K_2CO_3, MeOH; (d) DCE, Δ; (e) NaOEt, EtOH; (f) LiHMDS, MeI, DMF; (g) amine (neat), Δ.

Table 4
MT4 antiviral activity

Compd	$^{MT4}IC_{50}$ (μM)	T.I.[22]
11	0.080	>250
16	0.032	>250
22	0.020	>400
32	0.005	>2000
34	0.004	>12,500
43	0.064	165
44	0.011	1616
45	0.022	807
46	0.007	>7200

Table 5
Rat in vivo pharmacokinetic screening results[23]

Compd	Clp (mL/min/kg)	$T_{1/2}$ (h)	po AUC (ng h/mL)	C24/$^{pHIV}PAIC_{50}$
8	ND	ND	1649	0.1
11	ND	ND	3060	0.8
22	ND	ND	6374	0.0
31	11.8	4.0	1850	0.1
32	3.2	1.5	22,806	0.3
35	ND	ND	22,552	2.7
39	ND	ND	12,570	1.8
44	ND	ND	3548	0.0
46	ND	ND	5813	1.7

Table 6
Dog and cynomolgus monkey in vivo pharmacokinetic data[23]

Compd	Species	Clp (mL/min/kg)	$T_{1/2}$ (h)	po AUC (ng h/mL)	C24/$^{pHIV}PAIC_{50}$
28	Dog	8.6	1.6	13,230	0.8
28	Cyno	2.0	3.9	38,600	9.2
31	Dog	ND	ND	1241	0.0
35	Dog	ND	ND	1123	0.0

amide **32** again had a robust AUC exposure with evidence for drug levels at 24 h after dosing. Compound **32** was then progressed into a cynomolgus monkey low dose iv/po pk study and showed excellent exposures. The compound demonstrated low clearance and high oral bioavailability (97%F). This combined with a highly potent protein adjusted EC_{50} justified further progression of **32** into safety assessment evaluation and ultimately it was chosen for clinical evaluation. Compound **32** is also known as GSK364735.[14,20,21]

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