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Naphthyridinone (NTD) integrase inhibitors 4. Investigating *N*1 acetamide substituent effects with C3 amide groups

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Abstract— A series of *N*1 acetamide substituted naphthyridinone HIV-1 integrase inhibitors have been explored to understand structure-activity relationships (SAR) with various C3 amide groups. Investigations were evaluated using integrase enzyme inhibition, antiviral activity and protein binding effects to optimize the sub-structures. Lipophilicity was also incorporated to understand ligand lipophilic efficiency as a function of the structural modifications. Three representative analogs were further examined in a peripheral blood mononuclear cell (PBMC) antiviral assay as well as *in vitro* and *in vivo* drug metabolism and pharmacokinetic studies.

We have previously reported on 7-benzyl substituted naphthyridinone (NTD) HIV-1 integrase inhibitors.[1,2,3] This hetereocyclic template contains the requisite two-metal chelation elements necessary to bind to the essential enzyme active site Mg^{2+} metals and has been shown to be intrinsically potent as an integrase strand-transfer inhibitor. This provided a strong baseline to justify further study, however in order to be of use as a potential medicine, numerous other considerations required careful optimization. Our strategy was to explore the impact of changes to the *N*1 and C3 positions of the NTD template which were convenient chemical handles for tractable modifications, while not altering the basic metal-binding pharmacophore or 7-benzyl elements. A methodical evaluation of *N*1 protio and methyl substituents[1] resulted in the discovery of GSK364735[4,5] (**1**) which was progressed into early stage clinical trials. During these structure-activity relationship (SAR) studies, we became interested in exploring additional functionality at the *N*1 and C3 positions; namely how these combinations would impact both the potency of inhibiting the viral enzyme target and also the drug properties of the molecules including the pharmacokinetic profiles, as well as ligand efficiency measurements.[6] The focus of this report is *N*1 acetamide substitutions (e.g. **2**) that were envisioned to provide improved solubility and an overall reduction in the hydrophobicity of the NTD drug molecules (Figure 1).

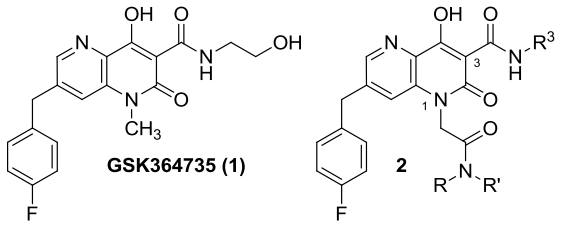

Figure 1 Naphthyridinone (NTD) HIV-1 integrase strand-transfer inhibitors.

The synthetic methods used to prepare the title compounds in Tables 1-3 began with the substituted 3-amino-2-pyridinecarboxylate ester **3**[2,7] (Scheme 1). Formation of the Schiff base with glyoxal followed by a one pot reduction using $NaBH_3CN$ provided the acetic acid adduct **4**. Amide formation using HATU coupling conditions smoothly provided amide **5** which was acylated using chloro ethyl malonate under thermal conditions in the absence of a base. The intermediate mixed malonate ester amide was subjected to NaOEt in ethanol to facilitate a Dieckmann cyclization to give the naphthyridinone ring

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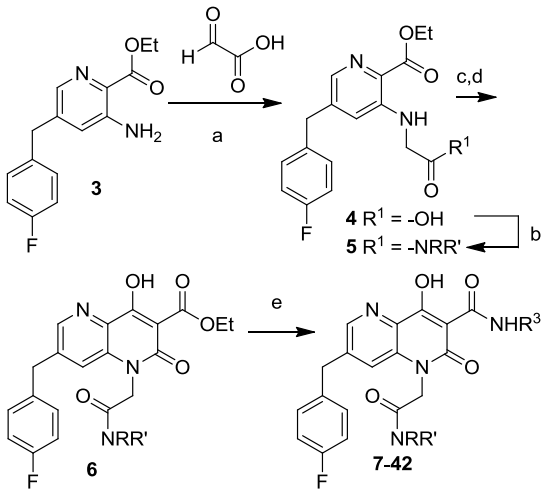
system **6**. This was converted to the C3 amide through direct transamination using a primary amine and either oil bath or microwave heating. In some cases the amines were used neat or ethanol was used as the solvent to afford the final neutral free acid NTD target molecules **7-42**.

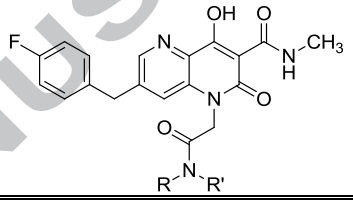


Scheme 1. Reagents and conditions: (a) EtOH, Δ; $NaBH_3CN$, r.t. (b) HNRR', HATU, Et_3N, DMF (c) $ClC(O)CH_2CO_2Et$; (d) DCE, Δ; (d) NaOEt, EtOH; (e) R^3NH_2, EtOH, Δ or μW.

Initially we performed a survey of *N*1 acetamide groups while maintaining an N-methyl amide at the 3 position of the NTD ring system (Table 1). We elected to maintain the 7-(4-fluoro)benzyl moiety consistently throughout the study as this group was believed to be a suitably optimal group from our previous naphthyridinone studies as well as related systems[8,9]. This also minimized the number of variables to contend with as we explored substituent effects at the *N*1 and C3 positions. The assays used to evaluate new compounds consisted of a biochemical enzymatic integrase strand-transfer assay ($^{ST}IC_{50}$)[10] and a single-round pseudotyped antiviral assay utilizing a luciferase reporter ($^{pHIV}IC_{50}$).[11] Additionally, the pHIV assay provided a convenient means to estimate protein binding effects (i.e. fold shift) on the antiviral activity through the addition of purified human serum albumin (HSA), giving rise to a protein-adjusted IC_{50} ($^{pHIV}PAIC_{50}$).[12] All compounds in the series had a substantial therapeutic index when comparing antiviral potency versus cellular toxicity (data not shown). We have also used ligand lipophilicity efficiency (LLE = pIC_{50}-clogP)[13,14] which is a key measure of true potency improvement and reduced toxicity risk. In general, it is desirable to have the LLE >5.

We began by systematically examining the simplest primary, secondary and tertiary amides (**7-9**). All three variations delivered potent enzyme activity. Likewise the antiviral activities were within 2-fold amongst the three amides, however the protein-shift values varied from 28-fold for the primary amide to only 3-fold for the tertiary amide. The less lipophilic primary amide also had the highest LLE as a result of a decreased clogP value. Additional simple alkyl secondary and tertiary amide substitutions (**10-14**, **16**) provided similar antiviral potencies and protein-adjusted activities. The lack of improvement as a result of the added lipophilicity was detrimental to the ligand efficiency estimates making these analogs less attractive. The *N*-cyclopropyl analog **15** showed a lower protein shift and increased LLE, however the trend did not continue with the homologated example **17**, which had a 4-fold lower $PAIC_{50}$. The electron withdrawing effects of the trifluoroethyl analog **18** did not impact either activity or lipophilicity substantially compared to the ethyl derivative **10**. Finally the addition of neutral polar groups in example **19** and **21** resulted in good activity while decreasing the clogP values and an increase in the LLE assessment. However, the basic tail in the dimethylaminoethyl analog **20** had a negative impact on the enzymatic and antiviral potencies but did reduce the protein shift to 2-fold.

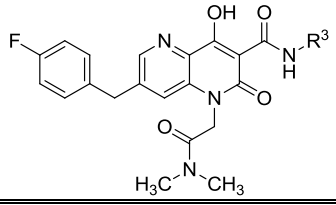
Table 1. *N*1 acetamide/C3 methyl amide SAR.



Compd	R, R'	(nM) $^{ST}IC_{50}$	$^{pHIV}IC_{50}$	$^{pHIV}PAIC_{50}$	LLE
1[3]	-	8.1	1.7	20	5.8
7	H, H	7.6	2.2	56	6.6
8	Me, H	9.3	3.3	22	5.6
9	Me, Me	9.3	5.9	19	5.3
10	Et, H	8.2	2.9	11	5.2
11	Et, Et	4.4	9.2	17	4.5
12	cyclopentyl	13	4.9	16	4.8
13	cyclopropyl, H	9.2	5.5	14	4.6
14	isopropyl, H	7.8	3.2	12	4.9
15	cyclopropyl, H	4.4	2.6	7.6	5.4
16	t-Bu, H	6.9	4.8	16	4.6
17	cyclopropylmethyl, H	8.0	5.9	33	4.7
18	CF_3, H	7.1	1.5	15	5.0
19	OMe, H	6.8	3.7	91	5.3
20	NMe_2, H	22	42	83	4.6
21	OMe, H	5.7	3.3	13	5.6

This initial assessment suggested it would be feasible to replace the *N*1-Me of the original analog **1** with more polar functionality and retain similar or improved potency. These effects were further probed through modifications of the C3 amide moiety. The dimethyl amide group in **9** appeared to have a good balance of potency and lipophilicity as indicated in the LLE value and was chosen as the basis for the amide survey.

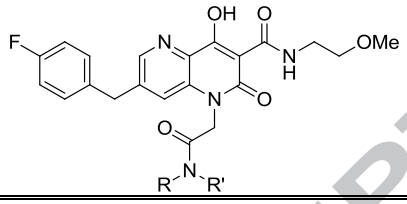
Table 2. *N*,*N*-dimethylacetamide/C3 amide SAR.



Compd	R^3	(nM) $^{ST}IC_{50}$	$^{pHIV}IC_{50}$	$^{pHIV}PAIC_{50}$	LLE
9	Me	9.3	5.9	19	5.3
22	cyclopropyl	3.0	9.3	193	5.1
23	CH2CH2OH	5.7	17	35	6.0
24	CH2CH2OMe	4.0	3.1	9.0	5.4
25	CH2CH(OH)CH3	8.6	31	40	5.6
26	CH(CH3)CH2OH	7.1	17	24	5.6
27	C(CH3)2CH2OH	6.5	3.6	82	5.2
28	1-hydroxycyclopentyl	23	7.2	24	3.9
29	CH2CH(OH)CH3	9.2	11	70	5.5
30	CH(CH3)CH2OH	4.1	7.1	15	5.8
31	CH2CH2-morpholino	8.6	19	25	4.9

The *N*-cyclopropyl group again had an influence on the protein-binding effects, although this time a near 20-fold reduction in potency was observed for compound **22** (Table 2). The more polar 2-hydroxyethyl amide similar to that of **1** was installed to give **23**. The overall activity was very similar with a protein-adjusted activity of 35 nM (vs. 20 nM for GSK735), however the lower clogP value for the methyl to acetamide change resulted in an increase in ligand lipophilicity efficiency. Capping the hydroxyl group as the 2-methoxyethyl amide resulted in an improvement in the protein-adjusted potency for **24**. Other hydroxyethyl derivatives (**25-26**, **28-30**) showed lower activity or did not improve over the protein-adjusted activity of **24**. The geminal-dimethyl analog **27** was similarly potent to the methoxymethyl analog **24**. The basic amine **31** had a slightly lower antiviral activity, but the lack of any shift in the presence of added serum albumin was noteworthy.

Table 3. NTD acetamide/R^3 methoxyethyl amide SAR .



Compd	R^3	(nM) $^{ST}IC_{50}$	$^{pHIV}IC_{50}$	$^{pHIV}PAIC_{50}$	LLE
24	NMe2	4.0	3.1	9.0	5.4
32	NH2	7.7	4.3	22	5.9
33	NHMe	9.0	3.0	14	5.5
34	cyclopropylamino	7.3	3.0	18	5.0
35	cyclobutylamino	8.5	3.3	39	4.5
36	isopropylamino	6.2	3.2	57	4.8
37	CH2CF3	11	2.4	20	4.6
38	CH2CH2NMe2	45	27	124	4.1
39	pyrrolidin-1-yl	42	3.8	11	4.3
40	piperidin-1-yl	4.7	3.6	12	4.4
41	morpholin-4-yl	7.6	3.5	6.7	5.0
42	4-methylpiperazin-1-yl	28	5.3	13	4.0

The C3 2-methoxymethyl amide **24** was of particular interest due to a low (3-fold) protein shift and a $PAIC_{50}$ under 10 nM. We decided to re-examine the impact of the acetamide substitution in the context of the 2-methoxyethyl amide as shown in Table 3. The rank ordering of potency was consistent with the *N*-methyl amides in Table 1 wherein the primary amide (**32**) had the least potent $PAIC_{50}$ followed by the secondary amide **33** and then the tertiary amide **24** with the most potent $PAIC_{50}$. The SAR findings largely followed the results in Table 1 wherein additional secondary acetamides did not provide any substantial changes in protein-adjusted potency while the basic amine group (e.g. **20** and **38**) again led to the largest loss in potency. A group of cyclic tertiary amides were also studied resulting in similar potency results compared to **24** but offering little discernible advantage in terms of ligand lipophilicity efficiency measures.

Three analogs (**9**, **24**, and **33**) were selected for further antiviral evaluation as well as *in vitro* and *in vivo* pharmacokinetic studies. Table 4 shows the antiviral

activity in a 7-day multi-round peripheral blood mononuclear cell (PBMC) assay. All three analogs had slightly improved antiviral IC_{50} values in the PBL assay when compared to the screening pHIV system. Applying the fold-shift from the pHIV conditions provided a predicted $^{PBL}PAIC_{50}$. Additionally, a 4-fold multiplier was applied to empirically translate the IC_{50} to IC_{90} and thus predict a clinical trough concentration target $PAIC_{90}$.

Table 4. Peripheral blood mononuclear cell (PBMC) antiviral activity and determination of protein adjusted IC_{90} clinical trough concentration targets.

Compd	$^{PBL}IC_{50}$ (nM)	Fold Shift[a]	$^{PBL}PAIC_{50}$ (nM)[b]	$^{PBL}PAIC_{90}$ (nM)[c]
9	4.2	3.3	14	55
24	4.3	2.9	13	50
33	2.3	4.5	10	41

[a] Fold shift determined from pHIV assay.
[b] $^{PBL}PAIC_{50}$ = $^{PBL}IC_{50}$ x fold shift
[c] $^{PBL}PAIC_{90}$ = 4 x $^{PBL}PAIC_{50}$

Compounds **9** and **24** showed no inhibition of CYP450's up to 33μM while **33** inhibited CYP1A2 at a level of 4.7μM. All three had good metabolic stability across rat, dog, cyno and human S9 preparations. Each of the three compounds were dosed in rats, beagle dogs and cynomolgus monkeys via IV (1mg/kg) and oral (5 mg/kg) routes of administration (Table 5). Oral dosing was performed using a 10/10/80 DMSO/solutol/0.05M meglumine vehicle solution formulation. All three compounds had low clearance in rats while in dogs they had slightly higher clearance ranging from 19% to 35% of hepatic blood flow. Oral bioavailability values for **9** were the most consistent across the three species suggesting no permeability or solubility limitations in a therapeutic dose range. However, the two methoxyethyl amide analogs **24** and **33** had a much larger range of the fraction absorbed across the species with no clear pattern. We had a keen interest in the concentration of drug at 24 hours post dose (C24) relative to the $^{PBL}PAIC_{90}$. This serves as a crude estimation of the potential inhibitory quotient (IQ)[15] and ultimately a property that would be expected to be a key driver of efficacy. For the compounds studied, these values were encouraging in rodent studies, especially in the case for **33**. However in higher species, the half-life of each of these was simply not sufficient to support robust coverage of the potency target suggesting these molecules would likely require a twice-daily dose.

Table 5. Rat in vivo pharmacokinetic screening results[16]

Compd	Species	Clp (mL/min/kg)	IV $T_{1/2}$ (hr)	%F	C24/$^{PBL}PAIC_{90}$
9	rat	0.7	13.8	69%	2.4
9	dog	10.9	1.3	75%	0.1
9	cyno	6.3	5.0	60%	0.7
24	rat	3.6	12.5	59%	0.5
24	dog	19.5	1.8	7%	ND
24	cyno	9.2	5.4	31%	0.6
33	rat	0.5	3.7	51%	56
33	dog	13.8	1.2	111%	0.1
33	cyno	2.0	2.3	19%	0.8

ND = no drug detected at 24h

The results created further interest in exploring other functionality at the *N*1 position. Further SAR studies to optimize the potency, protein binding, ligand efficiencies, and pharmacokinetics of this series and achieve a potential for once-daily dosing will be the focus of subsequent reports.

References and Notes

† Current address for EPG is Viamet Pharmaceuticals, Inc., Durham, NC; and current address for SAF is Chimerix, Inc., Durham, NC.

[1] For the previous account in this series see: Johns, B. A.; Kawasuji, T.; Weatherhead, J. G.; Boros, E. E.; Thompson, J. B.; Koble, C. K.; Garvey, E. G.; Foster, S. A.; Jeffrey, J. L.; Fujiwara, T. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 422.
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[14] LLE is also referred to as LipE, see Ryckmans, R.; Edwards, M. P.; Horne, V. A>; Correia, A. M.; Owen, D. R.; Thompson, L. R.; Tran, I.; Tutt, M. F.; Young, T. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4406.

[15] Inhibitory quotient (IQ) is defined as ratio of the drug trough concentration in plasma and a measurement of viral susceptibility. For the purposes of this study we have chosen to define viral susceptibility as the $PAIC_{90}$ (An IQ of 1 would be a trough drug concentration equal to the $PAIC_{90}$).

[16] Oral doses of 5 mg/kg were delivered as a DMSO/solutol/water solution formulation. IV data was from a 1 mg/kg bolus injection. Studies were performed in fasted animals.

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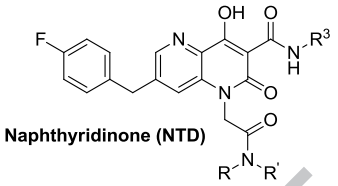

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