Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 2225–2228

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Evolution of Anti-HIV Drug Candidates. Part 1: From α-Anilinophenylacetamide (α-APA) to Imidoyl Thiourea (ITU)

Donald W. Ludovici,[a] Michael J. Kukla,[a,*] Philip G. Grous,[a] Suma Krishnan,[a] Koen Andries,[b] Marie-Pierre de Béthune,[c,†] Hilde Azijn,[c,†] Rudi Pauwels,[c,†] Erik De Clercq,[c] Edward Arnold[d] and Paul A. J. Janssen[e]

[a]*Janssen Research Foundation, Welsh and McKean Roads, Spring House, PA 19477, USA*
[b]*Janssen Research Foundation, Turnhoutseweg 30, B-2340 Beerse, Belgium*
[c]*Rega Institute for Medical Research, K.U.Leuven, B-3000 Leuven, Belgium*
[d]*Center for Advanced Biotechnology and Medicine and Rutgers University Chemistry Department, 679 Hoes Lane, Piscataway, NJ 08854, USA*
[e]*Janssen Research Foundation, Center for Molecular Design, Antwerpsesteenweg 37, B-2350 Vosselaar, Belgium*

Received 5 September 2000; accepted 9 May 2001

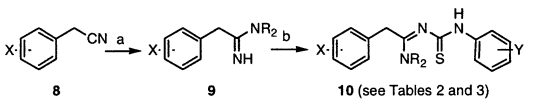
Abstract—Stemming from work on a previous clinical candidate, loviride, and other α-APA derivatives, a new series of potent non-nucleoside reverse transcriptase inhibitors (NNRTIs) has been synthesized. The ITU analogues, which contain a unique diarylated imidoyl thiourea, are very active in inhibiting both wild-type and clinically important mutant strains of HIV-1.

As part of an anti-AIDS program initiated in the late 1980s at Janssen Pharmaceutica, representative compounds from the corporate library were screened for their ability to inhibit HIV replication. As a consequence, the Janssen group was the first to discover unique inhibitors of the key multifunctional HIV-1 enzyme, reverse transcriptase. Compounds of the tetrahydroimidazo[4,5,1-*jk*][1,4]-benzodiazepin-2(1*H*)-one (TIBO) series were found to be noncompetitive inhibitors. Crystallographic studies later showed that they inhibited the enzyme's action by binding to a hydrophobic pocket close to, but distinct from, the active site.[1–3] Subsequently, many new structural classes of non-nucleoside reverse transcriptase inhibitors (NNRTIs) were found to specifically inhibit the reverse transcriptase of HIV-1 by binding at this site.[4] Although less likely to cause deleterious side effects than nucleoside inhibitors, NNRTIs were found to be more vulnerable to HIV's high mutation rate, leading to rapid selection of strains that are resistant to inhibition.[5] In spite of this shortcoming, nevirapine from Boehringer Ingelheim and delavirdine from Upjohn were developed and approved for sale as important adjuncts to the accepted multidrug therapies used to treat HIV-positive patients. Recently a third NNRTI, efavirenz from DuPont-Merck, has been approved and can be considered a true second generation NNRTI because of its ability to inhibit some of the mutant strains resistant to its predecessors.

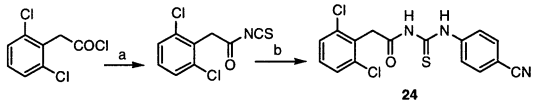
Nearly simultaneous with the development of the TIBO series, a second structural class of NNRTIs was discovered in our laboratories. The α-anilinophenylacetamide (α-APA) series was potent versus the wild-type HIV-1 (LAI strain) and had a high selectivity index (inhibition of HIV-1 versus cell toxicity).[6] Indeed, the simplicity of the structures and the relative ease of synthesis made them an extremely attractive target for optimization. Ultimately, a clinical candidate, loviride (**1**), was chosen and pursued through Phase II clinical trials. Its development was discontinued when it became apparent that it was not going to offer any significant advantage over the NNRTI therapies already approved at the time (vide supra). As described in this and the two following communications, continued research in this

*Corresponding author. Tel.: +1-215-542-9405; e-mail: m-rkukla@erols.com
†Present address: TIBOTEC, Generaal De Wittelaan L 11 B3, B-2800 Mechelen, Belgium.

0960-894X/01/$ - see front matter
PII: S0960-894X(01)00410-3

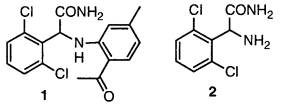


Scheme 1. (a) HNR_2·HCl, Me_3Al, toluene; (b) ArNCS, 3 N NaOH, MeOH.



Scheme 2. (a) $SnCl_4$, KSCN, 69%; (b) *p*-cyanoaniline, 50%.

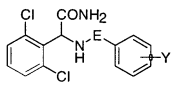
area has led to the discovery of several new families of potent RT inhibitors from which second and possibly third generation development candidates are being chosen.



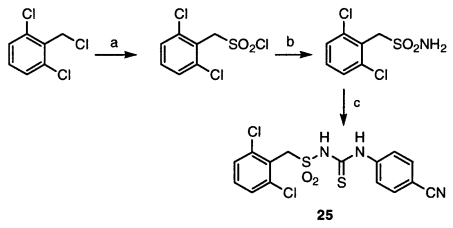
In structure–activity relationship (SAR) studies in the α-APA series, intermediate **2** was allowed to react with a number of electrophilic reagents to make analogues related to **3a** and **3b**. Table 1 shows that extending the *ortho* substituted inhibitor **3a** by a methylene (**4**), a carbonyl (**5**), an amide (**6**), or thioamide (**7a**) yielded only inactive compounds. However, activity was enhanced when the aromatic substituent was moved to the *meta* (**7b**) or *para* (**7c**) position of the thiourea. This was contrary to the SAR that had evolved in the α-APA series in which *ortho* substituents (cf. **3a** and **3b**) were highly favored over *para* (**3c**). Thus, we realized that we were potentially exploring a SAR different from the α-APA series.

A variety of aromatic substitutions on **7**, as well as variations to the basic structure, showed no significant enhancement of anti-HIV-1 activity (data not shown) until the imidoyl thiourea (ITU) **10d** was prepared (see Scheme 1 and Table 2). This compound was extremely active when compared to the α-APA leads. Thus, we embarked on an optimization program that is the subject of this communication.

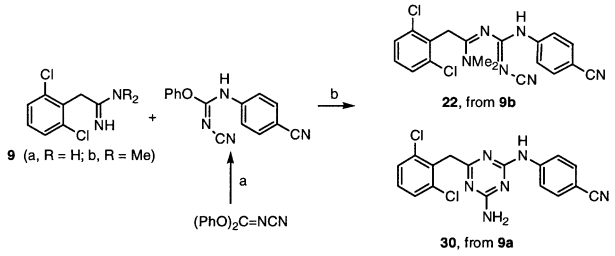
Table 1. Activity (IC_{50}, μM) versus HIV-1[7]



Compd	E	Y	IC_{50} (μM)
3a	—	*o*-NO_2	0.1
3b	—	*o*-Cl	0.4
3c	—	*p*-Cl	>160
4	CH_2	*o*-NO_2	>250
5	CO	*o*-NO_2	>79
6	CONH	*o*-NO_2	>320
7a	CSNH	*o*-Cl	>60
7b	CSNH	*m*-Cl	5.2
7c	CSNH	*p*-Cl	0.16



Scheme 3. (a) 1. Na_2SO_3; 2. $POCl_3$; (b) NH_4OH, MeOH, 54% (c) 1. *p*-cyanophenyl isothiocyanate, NaH, DMF; 2. K_2CO_3; 3. HCl, 41%.

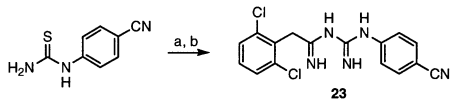


Scheme 4. (a) *p*-Cyanoaniline, DMF, 30%; (b) DMF.

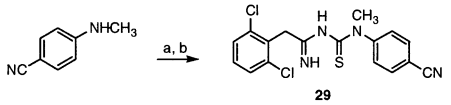
A series of ITUs was prepared using the synthetic route illustrated in Scheme 1 that allowed for variation of the substitution pattern of both aromatic rings (**10a**–**10p**, Table 2) and the functional group backbone of the linker (**13**–**21**, Table 3). Isothiocyanates were allowed to react with benzyl amidines under basic conditions, in order to free the latter, which were added as acid addition salts. Many of the amidines (**9**) used in this study were generated by addition of trimethyl aluminum amide reagents generated in situ to the corresponding nitriles (**8**).[8]

A number of conclusions were reached from this group of analogues (Table 2). Substitutions on the anilino ring (Y, **10a**–**10g**) demonstrated a positional preference for *para* substitution (**10g**≫**10f**≫**10e**). Di- or tri-substitution significantly decreased activity as compared to the corresponding mono-*para* substitution (**10d**≫**10b** or **10c**). The order of activity (IC_{50}, μM) of the synthesized *para* substituents (data not shown in Table 2) was: CN (0.003), NO_2 (0.003) > Br (0.013), Cl (0.013) > OBzl (0.020) > CF_3 (0.048), I (0.059), *n*-Bu (0.065), Ac (0.066), Et (0.074), F (0.102), CO_2Et (0.112), H (0.123) > SMe (0.407), OMe (0.417). There was some ambiguity about the relative importance of electronic effects and steric bulk. In general, electron withdrawing substituents were favored. The fact that compounds with alkyl substituents were as active as those with fluorine indicated that some bulk was preferable and size could play as important a role as electronic effects.

Keeping the *p*-CN anilino motif (Y) as the optimum subunit, a number of benzyl aromatic substitutions (X, **10h**–**10p**) were compared. Each substitution enhanced activity. Indeed, unsubstituted compound **10k** was the least active analogue. There seemed to be little difference in electronic effects with monosubstitution. Thus, the chloro, methyl, and methoxy substituted molecules were basically equipotent when positional (*ortho*) isomers were compared (**10l**, **10o**, and **10p**). In two of three possible direct comparisons, we found *ortho* > *meta* > *para*



Scheme 5. (a) Mel, acetone, NH_4OH, 84%; (b) **9a**, CH_3CN, 42%.



Scheme 6. (a) Im_2CS, THF, 38%; (b) **9a**, DMF, 63%.

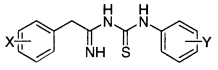
substitution (**10l** > **10m** > **10n**). Clearly multiple substitution, especially *ortho* disubstitution, led to the highest potency (**10g** and **10j**).

We then turned our attention to variations of the lead structure **10g** to explore the importance of each portion of the imidoyl thiourea backbone. Schemes 2–6 illustrate the preparation of compounds **22**–**25**, **29**, and **30**. In general, the basic strategy was the same as was used to make the lead structures; that is, the combination of aromatic electrophilic and aromatic nucleophilic moieties corresponding to the target analogues. Variations in the imidoyl portion of the lead involved initial replacement of the nitrogen by a carbonyl group. In Scheme 2, treatment of the acid chloride with potassium thiocyanate in the presence of tin chloride yielded the electrophilic acyl thioisocyanide, which was allowed to react with *p*-cyanoaniline to readily yield the acyl thiourea analogue **24**.[9] The corresponding sulfone analogue **25** (Scheme 3) was generated by treatment of *p*-cyanophenyl isothiocyanate with the anion of the dichlorobenyl sulfonamide, itself obtained by a three step sequence. The *o*,*o*-dichlorobenzyl chloride was treated with sodium sulfite, followed by phosphorous oxychloride, to give the sulfonyl chloride that, after treatment with ammonium hydroxide, gave the sulfonamide.[10]

To modify the thiourea portion of the imidoyl thiourea (Scheme 4), diphenyl cyanocarbonimidate was allowed to react sequentially with *p*-cyanoaniline and with dimethylamidine **9b** to yield the cyanoguanidine analogue **22**. When the reaction was carried out with the unsubstituted amidine **9a**, the analogous product was not isolated. Instead a cyclization occurred with the nitrile of the cyanoguanidine to yield the triazine product **30**. The biguanide analogue **23** was synthesized by methylating *N*-*p*-cyanophenyl thiourea and displacing methylmercaptan with **9a** (Scheme 5). Methylated analogue **29** was prepared by allowing *N*-methyl *p*-cyanoaniline to react with diimidazolethiocarbonyl followed by displacement of the second imidazole with amidine **9a** (Scheme 6).

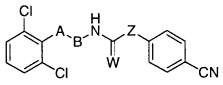
Table 3 summarizes the biological activity of a series of analogues of **10g** after optimization of the aromatic substituents. Substitution (**14** and **15**), elongation (**13** and **17**), or shortening (**16**) of the methylene (A–B) from the lead all led to significant decreases in activity. When the imidoyl nitrogen was substituted with a methyl group (**18**), potency was nearly the same as **10g**. However, larger groups such as ethyl (**20**) and phenyl (**21**) gave diminished potency. A dimethylated analogue (**19**) was nearly 2000× less active. Replacement of the imidoyl with carbonyl (**24**) or sulfonyl (**25**) led to complete loss of activity. Conversion of the thiourea in **10g** to a urea gave a compound (**11**) that was 6-fold less active. When this change was combined with methylation of the imidoyl nitrogen (**12**), the resultant analogue was nearly 100× less active. Other replacements of the sulfur, such as the biguanide (**23**) or the bioisosteric cyanoguanidine (**22**) were nearly inactive. Unfortunately, the direct cyanoguanidine analogue of **10g** could not be prepared since, as mentioned previously, it spontaneously cyclizes. Similarly, elongation at the cyanoaniline end

Table 2. Activity (IC_{50}, μM) versus HIV-1[7]



Compd	X	Y	IC_{50} (μM)
10a	*o*,*o*-diCl	H	0.123
10b	*o*,*o*-diCl	*o*,*p*-diCl	0.347
10c	*o*,*o*-diCl	*o*,*o*,*p*-triCl	42.7
10d	*o*,*o*-diCl	*p*-Cl	0.013
10e	*o*,*o*-diCl	*o*-CN	631
10f	*o*,*o*-diCl	*m*-CN	0.126
10g	*o*,*o*-diCl	*p*-CN	0.003
10h	*o*,*p*-diCl	*p*-CN	0.007
10i	*o*,*o*,*m*-triCl	*p*-CN	0.005
10j	*o*,*o*-diF	*p*-CN	0.006
10k	H	*p*-CN	0.380
10l	*o*-Cl	*p*-CN	0.014
10m	*m*-Cl	*p*-CN	0.068
10n	*p*-Cl	*p*-CN	0.148
10o	*o*-Me	*p*-CN	0.026
10p	*o*-OMe	*p*-CN	0.030

Table 3. Activity (IC_{50}, μM) versus HIV-1[7]



Compd[a]	A	B	W	Z	IC_{50} (μM)
10g	CH_2	C=NH	S	NH	0.0025
11			O		0.015
12		C=N–Me	O		0.214
13	CH=CH (E)		O		2.00
14	1,1-CycloPr				1.48
15	$CHCH_3$				0.158
16	Nothing				22.9
17	CH_2CH_2				0.525
18		C=N–Me			0.0039
19		C–N–Me_2			5.13
20		C=N–Et			0.158
21		C=N–Ph			1.32
22		C–NMe_2	N–CN		12.9
23			NH		7.94
24		C=O			631
25		SO_2			100
26[b]				NH	0.013
27[b]				$NHCH_2$	0.692
28[b]				$NHCH_2CH_2$	1.62
29				N–CH_3	58.9

[a]Unless noted, all variables (A, B, W, Z) for listed compounds (**11**–**29**) are the same as **10g**.
[b]In this analogue, the *p*-cyano was replaced by *p*-Cl.

Table 4. Activity (IC_{50}, μM) of **10g** and reference NNRTI versus mutant strains of HIV-1[7]

	LAI	100I	101E	103N	106A	181C	188L	190A
Delavirdine	0.063	2.51	0.158	2.51	1.59	2.00	1.26	0.063
Loviride	0.050	0.050	0.063	1.26	1.00	15.8	50.1	2.51
Nevirapine	0.032	0.316	0.316	6.31	5.01	10.0	>100.0	7.94
10g	0.003	0.513	0.019	0.589	0.382	0.511	0.318	0.002

by one (**27**) or two (**28**) carbons decreased activity significantly. Thus, the length of the molecule was critical to good potency. From the options explored, there seems to be little tolerance for changes to **10g**. Virtually all modifications cause a significant decrease in potency except the simple methylation of the imidoyl nitrogen (**18**); even extension to the homologous ethyl substituent (**20**) causes a drastic drop in activity. Replacement of the thiourea in **10g** by urea (**11**) was tolerated but led to 6-fold loss of activity.

One of the biggest challenges in finding improved generations of NNRTIs has been to find compounds that maintain activity against the common mutations. We tested the lead structure against a number of the most commonly reported drug-resistant mutant strains of HIV-1. As indicated in Table 4, **10g** has superior activity as compared to our original clinical candidate, loviride, as well as the FDA-approved agents delavirdine and nevirapine. Of the 24 possible comparisons (3 agents×8 HIV strains), **10g** is the more potent in 22 cases.

In summary, through a SAR program beginning with the clinical candidate loviride, **10g** was discovered to be a potent, noncytotoxic compound with activity against a wide variety of HIV-1 mutant strains. Furthermore, it could be synthesized in one step from commercially available starting materials and it contained no optical centers! Consequently, **10g** was considered for clinical development. Unfortunately, the hydrolytic instability of the imidoyl thiourea functionality became an issue during formulation studies. An obvious solution to this problem was to synthesize the well known cyanoguanidine bioisostere. However, as mentioned earlier in the SAR discussion, this compound was not available since it immediately cyclized to triazine **30**. Evaluation of this unexpected product indicated that it was nearly as active as **10g**; this led to a renewed optimization program that is the subject of the accompanying communication.[11]

Acknowledgements

Many people have made important contributions to this work. Thanks go to Paul Lewi and our colleagues in the JRF-CMD for many stimulating discussions and suggestions;[12] to co-workers in Eddy Arnold's group at CABM and Rutgers University for X-ray analysis of key compounds with the reverse transcriptase;[12] to Diane Gautier and Greg Leo for help with NMR spectral analyses; and to Theo Thielemans for continuously updating extremely well organized data presentations.

References and Notes

1. Pauwels, R.; Andries, K.; Desmyter, J.; Schols, D.; Kukla, M. J.; Breslin, H. J.; Raeymaeckers, A.; Van Gelder, J.; Woestenborghs, R.; Heykants, J.; Schellenkens, K.; Janssen, M. A. C.; De Clercq, E.; Janssen, P. A. J. *Nature* **1990**, 470.
2. Kukla, M. J.; Breslin, H. J.; Pauwels, R.; Fedde, C.; Miranda, M.; Scott, M. K.; Sherrill, R. G.; Raeymaekers, A.; Van Gelder, J.; Andries, K.; Janssen, M. A. C.; De Clercq, E.; Janssen, P. A. J. *J. Med. Chem.* **1991**, *34*, 746.
3. (a) Kohlstaedt, L. A.; Wang, J.; Friedman, J. M.; Rice, P. A.; Steitz, T. A. *Science* **1992**, *256*, 1783. (b) Ding, J.; Das, K.; Moereels, H.; Koymans, L.; Andries, K.; Janssen, P. A. J.; Hughes, S. H.; Arnold, E. *Nat. Struct. Biol.* **1995**, *2*, 407. (c) Mager, P. P. *Med. Res. Rev.* **1997**, *17*, 235.
4. (a) Swindells, S. *Emerging Drugs* **1997**, *2*, 155. (b) Pedersen, O. S.; Pedersen, E. B. *Antiviral Chem. Chemother.* **1999**, *10*, 285. (c) De Clercq, E. *Il Farmaco* **1999**, *54*, 26.
5. (a) Richman, D.; Shih, C. K.; Lowy, I.; Rose, J.; Prodanovich, P.; Goff, S.; Griffin, J. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 11241. (b) Richman, D. *Annu. Rev. Pharmacol. Toxicol.* **1993**, *132*, 149.
6. Pauwels, R.; Andries, K.; Debyser, Z.; Van Daele, P.; Schols, D.; Stoffels, P.; De Vreese, K.; Woestenborghs, R.; Vandamme, A.-M.; Janssen, C. G. M.; Anne, J.; Cauwenbergh, G.; Desmyter, J.; Heykants, J.; Janssen, M. A. C.; De Clercq, E.; Janssen, P. A. J. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 1711.
7. All compounds were tested for potency (IC_{50}, μM) to achieve 50% protection of MT-4 cells from HIV-1 cytopathicity as determined by the MTT method (Pauwels, R.; Balzarini, J.; Baba, M.; Snoek, R.; Schols, D.; Herdewijn, P.; Desmyter, J.; De Clercq, E. *J. Virol. Methods* **1988**, *20*, 309). Unless noted otherwise, the LAI strain of HIV-1 was the infecting virus. Other infecting viral strains with mutations in reverse transcriptase are characterized in the tables by the mutated amino acid position and the one letter codes. For instance, 181C refers to replacement of tyrosine at position 181 with cysteine. Each determination is the result of multiple tests. Although the data are not reported, in the same experiment mock-infected cells were tested with compound to determine the dose to reduce cells to 50% viability (CC_{50}). Thus a selectivity index (CC_{50}/IC_{50}) could be determined.
8. Garigipati, R. S. *Tetrahedron Lett.* **1990**, *31*, 1972.
9. Cambie, R. C.; Davis, P. F.; Rutledge, P. S.; Woodgate, P. D. *Aust. J. Chem.* **1984**, *37*, 2073.
10. Hamer, M.; Batzer, O. F.; Moats, M. J.; Wu, C. C.; Lira, E. P. *J. Pharm. Sci.* **1975**, *64*, 1961.
11. Ludovici, D. W.; Kavash, R. W.; Kukla, M. J.; Ho, C. Y.; Ye, H.; De Corte, B. L.; Andries, K.; de Béthune, M.-P.; Azijn, H.; Pauwels, R.; Moereels, H. E. L.; Heeres, J.; Koymans, L. M. H.; de Jonge, M. R.; Van Aken, K. J. A.; Daeyaert, F. F. D.; Lewi, P. J.; Das, K.; Arnold, E.; Janssen, P. A. J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2229 (following paper in series).
12. Detailed results will be part of future publications.