Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 253–256

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

1-[2-(Diphenylmethoxy)ethyl]-2-methyl-5-nitroimidazole: a Potent Lead for the Design of Novel NNRTIs

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Received 3 May 1999; accepted 23 November 1999

Abstract—A novel family of non-nucleoside reverse transcriptase inhibitors (NNRTIs) active at submicromolar concentrations was discovered. The new derivatives are 1-[2-(diarylmethoxy)ethyl]imidazoles bearing substituents both at benzene and imidazole rings. The most potent derivatives were those having nitro and methyl groups as substituents in the imidazole ring. Among 10 test derivatives compound **6d** was found to be as potent as nevirapine and was selected as a lead for further studies.

The HIV-1 non-nucleoside reverse transcriptase inhibitors (NNRTIs) are a structurally diverse set of compounds that inhibit the enzyme by an allosteric mechanism involving binding to a site that is closely associated with, but distinct from, the NRTI binding site. Since the time of their discovery NNRTI inhibitors have been particularly attractive targets due to their low cytotoxicity and high selectivity. However, the rapid emergence of resistant mutants allowed only a few compounds to reach the stage of clinical trials.[1]

Recently, NNRTIs have come up again for their potential in combination therapy. In fact, a new generation of NNRTIs is proving to possess broader spectrum of activity against clinically relevant resistant mutants and the ability to select resistant mutants with multiple mutations.[2–4] For these reasons RT remains a central target in the development of anti-HIV-1 drugs and new classes of NNRTIs having high potency as well as the properties reported above, are pursued.

Molecular modeling and X-ray structure investigations have led Schäfer et al.[5] to derive, from comparison of Nevirapine (**1**), TIBO (**2**) and **3**, a three-dimensional model with some structural elements that are common to these molecules: two π systems (generally a benzene ring and an extended π system) arranged in a 'butterfly-like' orientation, an additional lipophilic region between them, a carbonyl (thiocarbonyl) group near the benzene ring and a methyl group in the extended π system (Fig. 1). Several other active inhibitors, such as PBTD (**4**),[6] TBZ (**5**)[7] and DABO,[8–13] were also found to assume the 'butterfly-like' conformation of compounds **1**–**3** and attempts to design molecules that fit into the above 'butterfly-like' model could lead to novel lead compounds useful for developing new chemical classes of potential RT inhibitors.

On this basis, we decided to start a search for 'butterfly-like'-based anti-HIV-1 RT agents which presumably would interact with RT as well as nevirapine (**1**) and other conformationally related inhibitors (**2**–**5**).

First of all, we designed 1-[2-(diarylmethoxy)ethyl]-imidazoles **6** as potential candidates for anti-HIV-1 assays in MT-4 cells. Compounds **6** are open-analogues derived from **3** by disruption of two nitrogen linkages of thiazolidine ring. Imidazole was chosen as a terminus ring because of its presence in the tricyclic TBZ (**5**) and TIBO (**2**) active structures.

The synthesis of the diarylmethoxyethylimidazoles **6a**–**e** was performed by reaction of the proper diarylbromomethane with 1-(2-hydroxyethyl)-1*H*-imidazole

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0960-894X/00/$ - see front matter
PII: S0960-894X(99)00664-2

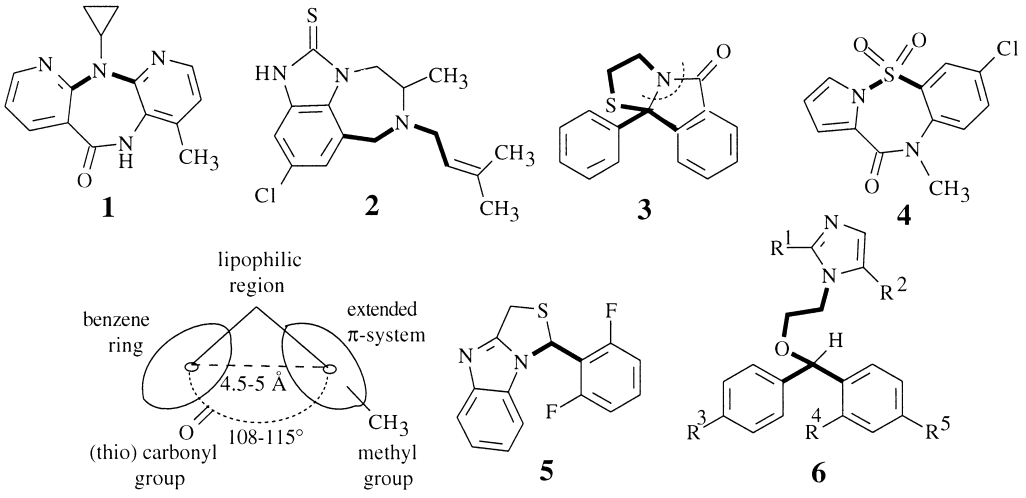


Figure 1. HIV-1 RT inhibitors arranged in space according to the Schäfer's 'butterfly-like' model.

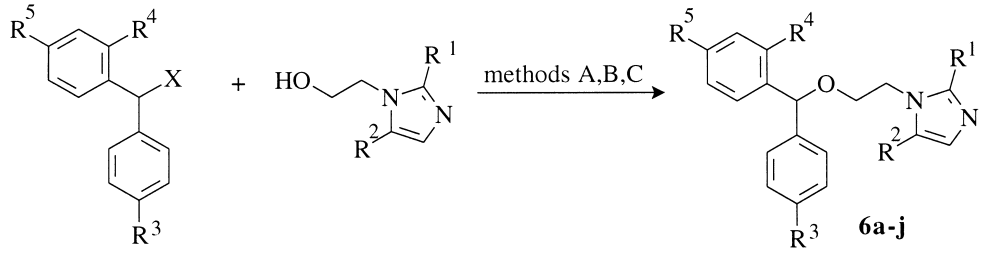
in the presence of KOH-DMSO (derivatives **6a**–**c**) or with 1-(2-hydroxyethyl)-2-methyl-5-nitro-1*H*-imidazole in the presence of K_2CO_3 (derivatives **6d**,**e**). Compounds **6f**–**j** were prepared by refluxing the proper diarylcarbinol with 1-(2-hydroxyethyl)-2-methyl-5-nitro-1*H*-imidazole in benzene in the presence of PTSA with azeotropic removal of water (Scheme 1).

Transformation of (phenyl)(2,4-dichlorophenyl)methanone and (4-substituted phenyl)(2,4-dichlorophenyl)-methanone into the related carbinols and then into bromides was performed by standard procedures.

The newly synthesized derivatives **6a**–**j**[14] are diarylmethoxyethylimidazoles bearing substituents at both phenyl and imidazole rings. The parent structure **6a** ($R^{1-5}=H$) is devoid of cytotoxicity but also of anti-HIV-1 activity ($CC_{50}=100\,\mu M$ and $EC_{50}>100\,\mu M$) (Table 1). The introduction of chlorine atoms in the benzene rings of **6a** with formation of **6b** ($R^{1-3}=H$; $R^4=R^5=Cl$) and **6c** (R^1, $R^2=H$; $R^{3-5}=Cl$) resulted in higher cytotoxicity but no changing in activity (**6b**, $CC_{50}=31\,\mu M$, $EC_{50}>31\,\mu M$; **6c**, $CC_{50}=26\,\mu M$, $EC_{50}>26\,\mu M$).

The appearance of anti-HIV-1 activity was obtained with the introduction of nitro and methyl substituents on the imidazole ring to give 1-[2-(diarylmethoxy)ethyl]-2-methyl-5-nitroimidazoles **6d**–**j** (DAMNI). The EC_{50} of these derivatives was always lower than 17 μM and in the majority of cases the CC_{50} was higher than 200 μM. Compound **6d** ($EC_{50}=0.2\,\mu M$) was the most potent and due to the lack of cytotoxicity ($CC_{50}>200\,\mu M$) it also showed the highest selectivity index (SI >1000). It is worth noting that this compound lacks substituents on the benzene rings. The introduction of electron-withdrawing or electron-donating groups led to less potent compounds. Among monosubstituted derivatives the inhibitory potency was close to that of the lead compound only when a fluorine atom was present (compound **6h**, $EC_{50}=0.9\,\mu M$). Replacement of fluorine with a chloro atom led to a 10-fold less potent derivative (**6i**), while the introduction of a second chlorine (**6j**) restored the anti-HIV-1 activity but with a concomitant increase of cytotoxicity. Introduction of a methyl group at position 4 of the benzydryl moiety led to a 7-fold decrease in potency (**6e**) and a further abatement of activity was observed when bulkier substituents like *t*-butyl (**6f**) and phenyl (**6g**) replaced methyl.

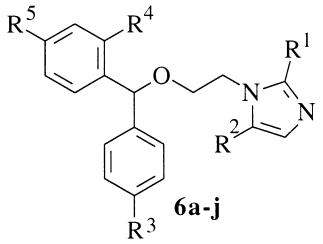
SAR studies suggest that the introduction of nitro and methyl groups on the imidazole ring is determinant for the anti-HIV-1 activity, whereas the presence of different substituents on the benzydryl moiety modulates both potency and cytotoxicity. In general, the effect of electron-withdrawing groups on the anti-HIV-1 activity



R^1 = H,CH_3; R^2 = H, NO_2; R^3 = H, CH_3, $C(CH_3)_3$, C_6H_5, Cl, F; R^4 = H, Cl; R^5 = H, Cl

Scheme 1. Methods. A: (X = Br, $R^1=R^2=H$) KOH-DMSO, rt, overnight. B: (X = Br, $R^1=CH_3$, $R^2=NO_2$) K_2CO_3, acetone, reflux, 7 days. C: (X = OH, $R^1=CH_3$, $R^2=NO_2$) PTSA, benzene, reflux, 4 h, Dean–Stark trap.

Table 1. Diarylmethoxyethylmethylnitroimidazoles (DAMNI): chemical and physical data cytotoxicity, in vitro anti-HIV-1 activities and enzymatic assays against HIV-1 rRT[a]



6a-j

Compd	R^1	R^2	R^3	R^4	R^5	m.p. (°C)	Method	Yield (%)	Crystallized from [b]	Chromatographic system [c]	CC_{50}[d] (μM)	EC_{50}[e] (μM)	S.I.[f]	IC_{50}[g] (μM)
6a[h]	H	H	H	H	H	111–112	A	45	a	a	100	>100	—	ND[i]
6b[h]	H	H	H	Cl	Cl	172–175	A	45	b	a	31	>31	—	ND
6c[h]	H	H	Cl	Cl	Cl	142–145	A	20	b	a	26	>26	—	ND
6d[h]	CH_3	NO_2	H	H	H	138–141	B (C)	45 (80)	b	b	>200	0.2	>1000	0.05
6e[h]	CH_3	NO_2	CH_3	H	H	93–95	B (C)	37 (50)	b	b	86	1.4	61	2.1
6f	CH_3	NO_2	*t*-Bu	H	H	Thick oil	C	50	—	b	44	4	11	1.5
6g	CH_3	NO_2	Ph	H	H	Thick oil	C	90	—	b	>200	17	12	ND
6h	CH_3	NO_2	F	H	H	136–138	C	75	c	b	>200	0.9	222	2.3
6i	CH_3	NO_2	Cl	H	H	114–116	C	70	c	b	>200	12.5	>16	ND
6j	CH_3	NO_2	H	Cl	Cl	Thick oil	C	15	—	b	54	1.5	36	1.4
Nevirapine											>300	0.3	>1000	

[a]Data represent mean values of at least two separate experiments.
[b]a: *i*-propanol; b: *i*-propanol/*i*-propyl ether; c: toluene/ligroin.
[c]a: silica gel/chloroform:ethanol 95:5; b: silica gel/chloroform.
[d]Compound dose required to reduce the viability of mock-infected cells by 50%, as determined by the MTT method.
[e]Compound dose required to achieve 50% protection of MT-4 cells from HIV-1 induced cytopathicity, as determined by the MTT method.
[f]Selectivity index, CC_{50}:EC_{50} ratio.
[g]Compound dose required to inhibit the HIV-1 rRT activity by 50%.
[h]Mononitrate.
[i]ND, not determined.

of DAMNIs is more determinant than that produced by electron-donating groups.

Since compounds **1**, **2**, **4** and **5** targeted the HIV-1 RT, the most potent DAMNI derivatives were also tested in enzyme assays against highly purified recombinant HIV-1 RT using homopolymeric template primers. Their inhibitory activity was expressed as IC_{50}. An excellent correlation was found between the EC_{50} and IC_{50} values indicating that the in vitro anti-HIV-1 activity does entirely reflect the capability of test compounds to inhibit the enzyme. The conclusion that DAMNI are a new class of NNRTIs is also supported by the fact that, besides being specific inhibitors of the HIV-1 acute infection, they do not inhibit the multiplication of HIV-2 and of HIV-1 in cronically infected $H9/III_B$ cells (results not shown).

Antiviral assays of compounds **6a**–**j** were performed as previously reported.[15,16] Data of cytotoxicity (CC_{50}), in vitro anti-HIV-1 activities (EC_{50}) and enzymatic assays against HIV-1 rRT (IC_{50}) are shown in Table 1. Compounds **6b**, **c**, **e**–**j** were tested as racemates.

Acknowledgements

We thank the Italian Ministero della Sanità, Istituto Superiore di Sanità, XI Progetto AIDS 1997 (grants nos. 40A.0.06 and 40A.0.55) for financial support. We also acknowledge the support of the Italian MURST (40%) and the Regione Autonoma della Sardegna (Progetto Biotecnologie).

References and Notes

1. De Clercq, E. *Antiviral Research* **1998**, *38*, 153.
2. Fujiwara, T.; Sato, A.; El-Farrash, M.; Miki, S.; Abe, K.; Isaka, Y.; Kodama, M.; Wu, Y.; Chen, L. B.; Harada, H.; Sugimoto, H.; Hatanaka, M.; Hinuma, Y. *Antimicrob. Agents Chemother*. **1998**, *42*, 1340.
3. Cywin, C. L.; Klunder, J. M.; Hoermann, M. A.; Brickwood, J. R.; David, E.; Grob, P. M.; Schwartz, R.; Pauletti, D.; Barringer, K. J.; Shih, C.-K.; Sorge, C. L.; Erickson, D. A.; Joseph, D. P.; Hattox, S. E. *J. Med. Chem*. **1998**, *41*, 2972.
4. Klunder, J. M.; Hoermann, M. A.; Cywin, C. L.; David, E.; Brickwood, J. R.; Schwartz, R.; Barringer, K. J.; Pauletti, D.; Shih, C.-K.; Erickson, D. A.; Sorge, C. L.; Joseph, D. P.; Hattox, S. E.; Adams, J.; Grob, P. M. *J. Med. Chem*. **1998**, *41*, 2960.
5. Schäfer, W.; Friebe, W.-G.; Leinert, H.; Mertens, A.; Poll, T.; von der Saal, W.; Zilch, H.; Nuber, B.; Ziegler, M. L. *J. Med. Chem*. **1993**, *36*, 726.
6. Artico, M.; Silvestri, R.; Pagnozzi, E.; Massa, S.; Loi, A. G.; Putzolu, M.; Corrias, S.; Spiga, M. G.; La Colla, P. *Bioorg. Med. Chem*. **1996**, *6*, 837.
7. Chimirri, A.; Grasso, S.; Monforte, A.-M.; Monforte, P.; Zappalà, M. *Farmaco* **1991**, *46*, 817.
8. Artico, M.; Massa, S.; Mai, A.; Marongiu, M. E.; Piras, G.; Tramontano, E.; La Colla, P. *Antiviral Chem. Chemother*. **1993**, *4*, 361.

9. Tramontano, E.; Marongiu, M. E.; De Montis, A.; Loi, A. G.; Artico, M.; Massa, S.; Mai, A.; La Colla, P. *Microbiologica* **1994**, *17*, 269.
10. Massa, S.; Mai, A.; Artico, M.; Sbardella, G.; Tramontano, E.; Loi, A. G.; Scano, P.; La Colla, P. *Antiviral Chem. Chemother*. **1995**, *6*, 1.
11. Mai, A.; Artico, M.; Sbardella, G.; Massa, S.; Loi, A. G.; Tramontano, E.; Scano, P.; La Colla, P. *J. Med. Chem*. **1995**, *38*, 3258.
12. Mai, A.; Artico, M.; Sbardella, G.; Quartarone, S.; Massa, S.; Loi, A. G.; De Montis, A.; Scintu, F.; Putzolu, M.; La Colla, P. *J. Med. Chem*. **1997**, *40*, 1447.
13. Ettorre, A.; Mai, A.; Artico, M.; Massa, S.; De Montis, A.; La Colla, P. *Acta Cryst*. **1996**, *C52*, 2115.
14. ^{1}H NMR data for test DAMNI derivatives were — **6a**: δ 3.67 (t, J = 5.0 Hz, 2H), 4.13 (t, J = 5.0 Hz, 2H), 5.30 (s, 1H), 6.97 (s, 1H), 7.07 (s, 1H), 7.15–7.36 (m, 10H), 7.55 ppm (s, 1H); **6b**: δ 3.49 (m, 2H), 4.14 (t, J = 5.0 Hz, 2H), 5.71 (s, 1H), 6.94 (s, 1H), 7.08 (s, 1H), 7.16–7.37 (m, 8 H), 7.54 ppm (s, 1H); **6c**: δ 3.68 (t, J = 4.9 Hz, 2H), 4.16 (t, J = 4.9 Hz, 2H), 5.68 (s, 1H), 6.94 (s, 1H), 7.05–7.35 (2H), 5.25 (s, 1H), 7.03–7.35 (m, 10H), 7.96 ppm (s, 1H); **6e**: δ 2.29 (s, 3H), 2.55 (s, 3H), 3.76 (t, J = 4.9 Hz, 2H), 4.53 (t, J = 4.9 Hz, 2H), 5.22 (s, 1H), 6.93–7.36 (m, 9H), 7.95 ppm (s, 1H). **6f**: δ 1.27 (s, 9H), 2.55 (s, 3H), 3.76 (t, J = 4.9 Hz, 2H), 4.52 (t, J = 4.9 Hz, 2H), 5.22 (s, 1H), 6.96–7.40 (m, 9H), 7.92 ppm (s, 1H); **6g**: δ 2.56 (s, 3H), 3.80 (t, J = 4.9 Hz, 2H), 4.55 (t, J = 4.9 Hz, 2H), 5.29 (s, 1H), 7.08–7.60 (m, 14H), 7.94 ppm (s, 1H); **6h**: δ 2.55 (s, 3H), 3.75 (t, J = 5.0 Hz, 2H), 4.53 (t, J = 5.0 Hz, 2H), 5.23 (s, 1H), 6.85–7.03 (m, 2H), 7.03–7.16 (m, 4H), 7.19–7.34 (m, 3H), 7.94 ppm (s, 1H); **6i**: δ 2.53 (s, 3H), 3.76 (t, J = 4.9 Hz, 2H), 4.53 (t, J = 4.9 Hz, 2H), 5.22 (s, 1H), 6.98–7.13 (m, 4H), 7.18–7.33 (m, 5H), 7.94 ppm (s, 1H); **6j**: δ 2.50 (s, 3H), 3.75 (m, 2H), 4.54 (t, J = 4.9 Hz, 2H), 5.64 (s, 1H), 7.02–7.35 (m, 8H), 7.94 ppm (s, 1H).
15. Pauwels, R.; Balzarini, J.; Baba, M.; Snoeck, R.; Schols, D.; Herdewijn, P.; Desmyster, J.; De Clercq, E. *J. Virol. Methods* **1988**, *20*, 309.
16. Mai, A.; Artico, M.; Sbardella, G.; Massa, S.; Novellino, E.; Greco, G.; Loi, A. G.; Tramontano, E.; Marongiu, M. E.; La Colla, P. *J. Med. Chem*. **1999**, *42*, 619.