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Bioisosteric Modification of PETT-HIV-1 RT-Inhibitors: Synthesis and Biological Evaluation

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Abstract—Bioisosteric substitution of the thiourea (**3**, **5**, **7**, **9**) and urea (**10**) moiety of PETT compounds with sulfamide (**1**), cyanoguanidine (**2**, **4**) and guanidine (**6**, **8**) functionalities, and replacement of the phenethyl group with benzoylethyl group (compounds **11**–**20**) have been studied. Synthesis and antiviral activities are described. © 2000 Elsevier Science Ltd. All rights reserved.

PETT (phenylethylthiazolethiourea) compounds[1] as well as nevirapine,[2–4] delavirdine,[5] efavirenz,[6] HBY-097,[7] loviridine[8] and tivirapine[9] belong to the family of non-nucleoside reverse transcriptase inhibitors (NNRTIs) which inhibit the viral reverse transcriptase by binding to it non-competitively.[9]

As earlier reported, thiourea[10,11] and urea[12,13] derivatives in the PETT series are potent HIV-1 RT inhibitors both at the enzyme level and in cell culture assays. As with other NNRTIs, they bind to an allosteric site of HIV-1 RT. The three-dimensional structure of complexes between HIV-1 RT and some PETT derivatives have been determined.[13] To explore the effect of a bioisosteric replacement for the thiourea and urea moieties, sulfamide, cyanoguanidine and guanidine functionalities (Table 1) were selected as synthetic targets. Additionally, a series of benzoylethyl analogues to the phenylethyl, pyridylethyl and phenylcyclopropyl moieties of PETT compounds, was synthesized (Table 2). The antiviral activity was determined both at the RT level and in cell culture against HIV-1 wild type virus.

Chemistry

The synthesis of the thiourea and urea compounds **3**, **5**, **7**, **9** and **10** is described in our previous papers.[10–12] The sulfamide derivative, compound **1**, was synthesized from phenethylamine hydrochloride[14] as shown in Scheme 1. Phenethylamine hydrochloride was treated with SO_2Cl_2 and a catalytic amount of $SbCl_5$ in acetonitrile at reflux to afford amidosulfonyl chloride. This was then coupled with an anion of 2-amino-5-chloropyridine which was generated by the reaction of 2-amino-5-chloropyridine with NaH at $-10\,^{\circ}C$ in THF to give compound **1**.

Two different methods to prepare cyanoguanidine derivatives are described in Scheme 2. (i) Thiourea derivative **5** can be directly converted to the corresponding cyanoguanidine by treatment with PbNCN in acetonitrile-DMF at reflux to afford compound **4**.[15] (ii) Compound **2** was prepared from 4-chloro-2-pyridylisothiocyanate[11] by the procedure of Atwal.[16] Isothiocyanate was treated with NaHNCN in EtOH at room temperature to give an intermediate thiourea compound which was reacted with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and phenethylamine in a one pot reaction to give compound **2**.

A direct conversion of a thiourea compound **7** into guanidine was achieved by reacting **7** with $AgSO_2CF_3$, in NH_3/CH_2Cl_2 at $-30\,^{\circ}C$ to give compound **6** as described in Scheme 3. Compound **8** was synthesized according to the same procedure.

The synthesis of the benzoylethyl derivative **11** is described in Scheme 4 and this general methodology was also used for the preparation of compounds **12–14, 16–20.** Compound **15** was synthesized from compound **12** by converting it to the urea analogue by a procedure described earlier.[12,13] Benzoylethylamine was prepared by a Mannich reaction where formaldehyde was condensed with ammonium chloride and acetophenone to give an amine which was coupled with an intermediate[11]

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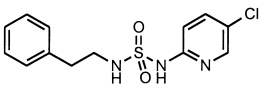
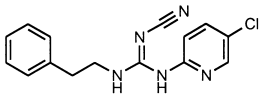
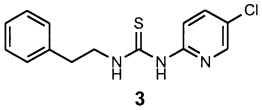
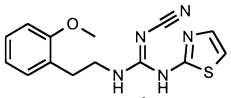
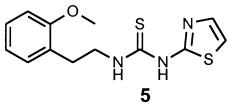
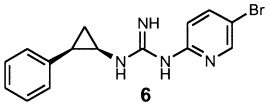
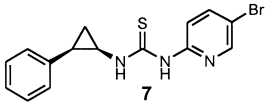
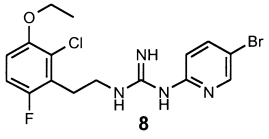
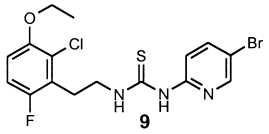
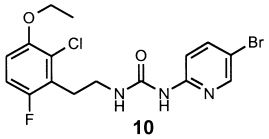
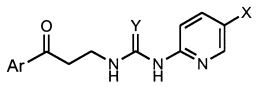
Table 1.

Compound	HIV-1 RT (rCdG), IC_{50}, μM[a]	HIV-1 MT-4 cells, ED_{50}, μM[b]
 **1**	>10	>100
 **2**	>10	>100
 **3**	0.003	0.03
 **4**	>10	>100
 **5**	0.04	0.4
 **6**	0.18	1.2
 **7**	0.008	0.02
 **8**	0.4	1.0
 **9**	0.012	0.007
 **10**	0.004	0.05

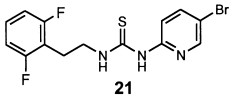
[a]The HIV-1 RT assay which used (poly)rC.(oligo)dG as the template/primer is described in ref 17.

[b]Anti HIV activity assay: MT4 cells (human T cell line) grown in RPMI 1640 medium supplemented with 10% fetal calf serum, penicillin and streptomycin were seeded into 96 well microplates (2×10^4 cells/well) and infected with 10–20 TCID50 of HIV-1, IIIb per well. Test compounds in different concentrations were added. The cultures were incubated at 37 °C in CO_2 atmosphere and the viability of cells was determined at day five or six with XTT vital dye.[18] The anti HIV-1 activity was measured as the reduction in cytopathic effect caused by the virus.

Table 2.



11-20

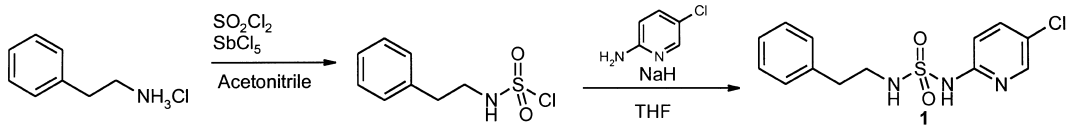
Compound	Ar	Y	X	HIV-1 RT (rCdG), IC_{50}, μM[a]	HIV-1 MT-4 cells, ED_{50}, μM[b]
11	Phenyl	S	Br	< 0.027	0.036
12	2-fluorophenyl	S	Br	0.004	0.009
13	2-chlorophenyl	S	Br	0.010	0.100
14	2-methoxyphenyl	S	Br	0.003	0.076
15	2-fluorophenyl	O	Br	0.054	0.201
16	3-methoxyphenyl	S	Br	0.080	0.911
17	4-fluorophenyl	S	Br	0.047	0.300
18	2,5-difluorophenyl	S	Br	< 0.025	0.067
19	2,6-difluorophenyl	S	Cl	0.006	0.028
20	2,6-difluorophenyl	S	Br	0.003	0.050
 **21**				0.001	0.010

[a,b]See footnote a and b, Table 1.

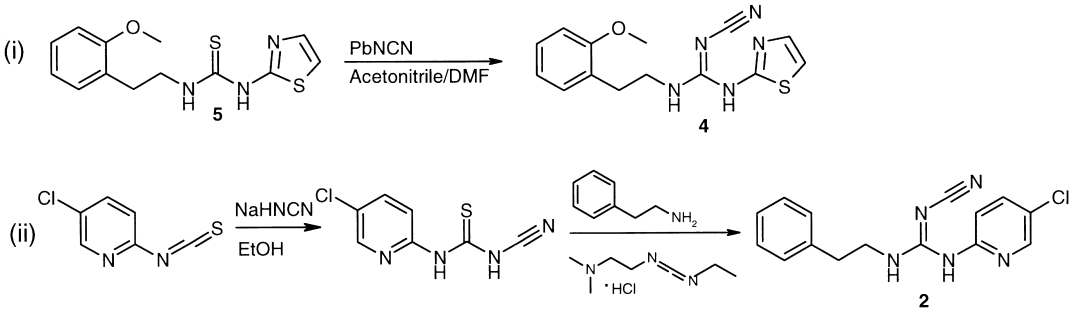
derived from 2-amino-5-bromopyridine and 1,1′-thiocarbonyldiimidazole to afford compound **11**.

Biological Results and Discussion

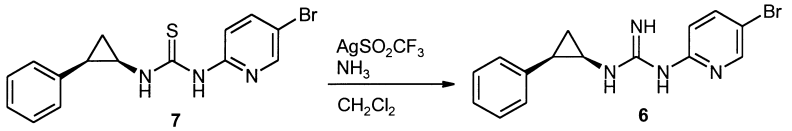
All compounds in this study were tested in an HIV-1 RT wild-type enzyme assay and in a cell culture assay using MT-4 cells and wild-type virus. The IC_{50} and ED_{50} values from these assays are presented in Tables 1 and 2. The replacement of the thiourea moiety of compound **3** with a sulfamide functionality, compound **1**, caused complete loss of activity as well as did the replacement of the thiourea moiety of compounds **3** and **5** with the cyanoguanidine moiety, compounds **2** and **4** (Table 1). The replacement of the thiourea moiety of compounds **7** and **9** or the urea moiety of compound **10** with a guanidine functionality, compounds **6** and **8**, caused a decrease in activity, but the compounds retained an IC_{50} of 0.18–0.4 μM and ED_{50} of 1.0–1.2 μM. The role of thiourea and urea moiety of PETT compounds thus appears essential for optimal anti-HIV activity. The size of guanidine moiety is close to that of thiourea and urea, whereas the sulfamide and cyanoguanidine moieties are somewhat larger which may explain their respective activities. Benzoylethyl derivatives **11**–**20** in Table 2 were quite potent inhibitors of wild-type HIV-1 RT and HIV-1 virus in cell culture. 2,6-Difluorobenzoyl compound **20** showed a three fold loss of activity at the HIV-1 RT level and a five fold loss in cell culture against HIV-1 wild type virus compared to the corresponding phenethyl compound **21**.[11] The structure–activity relationships followed the same substitution pattern as we have described earlier for phenethyl thiourea compounds.[11] The change of a fluoro substituent from the 2-position of the phenyl ring,



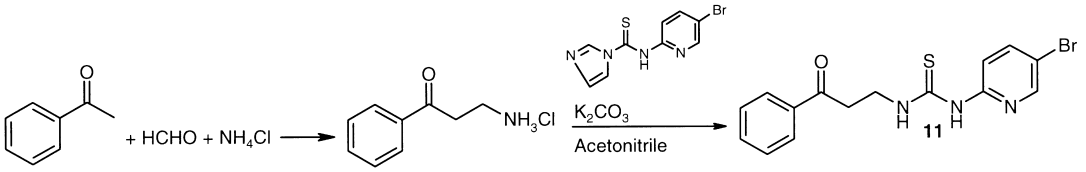
Scheme 1.



Scheme 2.



Scheme 3.



Scheme 4.

compound **12**, to 4-position of the phenyl ring, compound **17**, decreased the activity on wild type virus from ED_{50} of 0.009 μM to ED_{50} of 0.3 μM. When benzoylethyl compounds were tested on mutant HIV-1 RT and virus they differed from the phenethyl compounds by losing much more activity. The ED_{50} on mutant virus for benzoylethyl compound **20** was >260 μM compared to 5.7 μM for phenethyl compound **21**. Activities on mutant HIV-1 RT (Ile100) (IC_{50}:0.052 μM) and mutant HIV-1 RT (Cys181) (IC_{50}:0.013 μM) for phenethyl compound **21** were about ten times better than for compound **19** (IC_{50}: >0.3 μM and 0.14 μM, respectively). The benzoylethyl compounds have thus not warranted being investigated further. We have in our previous papers compared ethyl compounds to cyclopropyl analogues and speculated that the more flexible ethyl derivatives might have problems in adopting conformations that fit in the mutant enzymes compared to the more restricted and more potent cyclopropyl analogues.[12,13] We can hypothesize that the benzoylethyl compounds belong to the same category of PETT compounds as the ethyl derivatives.

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References and Notes

1. Ahgren, C.; Backro, K.; Bell, F. W.; Cantrell, A. S.; Clemens, M.; Colacino, J. M.; Deeter, J. B.; Engelhardt, J. A.; Hogberg, M.; Jaskunas, S. R.; Johansson, N. G.; Jordan, C. L.; Kasher, J. S.; Kinnick, M. D.; Lind, P.; Lopez, C.; Morin, Jr., J. M.; Muesing, M. A.; Noreen, R.; Oberg, B.; Paget, C. J.; Palkowitz, J. A.; Parrish, C. A.; Pranc, P.; Rippy, M. K.; Rydergard, C.; Sahlberg, C.; Swanson, S.; Ternansky, R. J.; Unge, T.; Vasileff, R. T.; Vrang, L.; West, S. J.; Zhang, H.; Zhou, X.-X. *Antimicrob. Agents Chemother.* **1995**, *39*, 1329.
2. Grob, P. M.; Wu, J. C.; Cohen, K. A.; Ingraham, R. H.; Shih, C. K.; Hargrave, K. D.; Mctague, T. L.; Merluzzi, V. J. *AIDS Res. Hum. Retroviruses* **1992**, *8*, 145.
3. Hargrave, K. D.; Proudfood, J. R.; Grozinger, K. G.; Cullen, E.; Kapadia, S. R.; Patel, U. R.; Fuchs, V. U.; Mauldin, S. C.; Vitous, J.; Behnke, M. L.; Klunder, J. M.; Pal, K.; Skiles,

J. W.; McNeil, D. W.; Rose, J. M.; Chow, G. C.; Skoog, M. T.; Wu, J. C.; Schmidt, G.; Engel, W. W.; Eberlein, W. G.; Saboe, T. D.; Campbell, S. J.; Rosenthal, A. S.; Adams, J. *J. Med. Chem.* **1991**, *34*, 2231.
4. Cheeseman, S. H.; Hattox, S. E.; McLaughlin, M. M.; Koup, R. A.; Andrews, C. A.; Bova, C. A.; Pav, J. W.; Roy, T.; Sullivan, J. L.; Keirns, J. J. *Antimicrob. Agents Chemother.* **1993**, *37*, 178.
5. Romero, D. L.; Morge, R. A.; Biles, C.; Berrios-Péna, N.; May, P. D.; Palmer, J. R.; Johnson, P. D.; Smith, H. W.; Busso, M.; Tan, C.-K.; Voorman, R. L.; Reusser, F.; Althaus, I. W.; Downey, K. M.; So, A. G.; Resnick, L.; Tarpley, W. G.; Aristoff, P. A. *J. Med. Chem.* **1994**, *37*, 999.
6. Young, S. D.; Britcher, S. F.; Tran, L. O.; Payne, L. S.; Lumma, W. C.; Lyle, T. A.; Huff, J. R.; Anderson, P. S.; Olsen, D. B.; Carroll, S. S.; Pettibone, D. J.; O'Brien, J. A.; Ball, R. G.; Balani, S. K.; Lin, J. H.; Chen, I.-W.; Schleif, W. A.; Sardana, V. V.; Long, W. J.; Byrnes, V. W.; Emini, E. A. *Antimicrob. Agents Chemother.* **1995**, *39*, 2602.
7. Kleim, J.-P.; Bender, R.; Kirsch, R.; Meichsner, C.; Paessens, A.; Rösner, M.; Rübsamen-Waigmann, H.; Kaiser, R.; Wichers, M.; Schneweis, K. E.; Winkler, I.; Riess, G. *Antimicrob. Agents Chemother.* **1995**, *39*, 2253.
8. 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. USA* **1993**, *90*, 1711.
9. Pauwels, R.; Andries, K.; Debyser, Z.; Kukla, M. J.; Schols, D.; Breslin, H. J.; Woestenborghs, R.; Desmyter, J.; Janssen, M. A. C.; De Clercq, E.; Janssen, P. A. *J. Antimicrob. Agents Chemother.* **1994**, *38*, 2863.
10. Bell, F. W.; Cantrell, A. S.; Högberg, M.; Jaskunas, S. R.; Johansson, N. G.; Jordan, C. L.; Kinnick, M. D.; Lind, P.; Morin, Jr., J. M.; Noréen, R.; Öberg, B.; Palkowitz, J. A.; Parrish, C. A.; Pranc, P.; Sahlberg, C.; Ternansky, R. J.; Vasileff, R. T.; Vrang, L.; West, S. J.; Zhang, H.; Zhou, X.-X. *J. Med. Chem.* **1995**, *38*, 4929.
11. Cantrell, A. S.; Engelhardt, P.; Högberg, M.; Jaskunas, S. R.; Johansson, N. G.; Jordan, C. L.; Kangasmetsä, J.; Kinnick, M. D.; Lind, P.; Morin, Jr., J. M.; Muesing, M. A.; Noréen, R.; Öberg, B.; Pranc, P.; Sahlberg, C.; Ternansky, R. J.; Vasileff, R. T.; Vrang, L.; West, S. J.; Zhang, H. *J. Med. Chem.* **1996**, *39*, 4261.
12. Sahlberg, C.; Noréen, R.; Engelhardt, P.; Högberg, M.; Kangasmetsä, J.; Vrang, L.; Zhang, H. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1511.
13. Högberg, M.; Sahlberg, C.; Engelhardt, P.; Noréen, R.; Kangasmetsä, J.; Johansson, N. G.; Öberg, B.; Vrang, L.; Zhang, H.; Sahlberg, B.-L. *J. Med. Chem.* **1999**, *42*, 4150.
14. Weiss, G.; Schulze, G. *Liebigs Ann. Chem.* **1969**, *729*, 40.
15. Durant, G. J.; Emmett, J. C.; Ganellin, C. R.; Miles, P. D.; Parsons, M. E.; Prain, H. D.; White, G. R. *J. Med. Chem.* **1977**, *20*, 901.
16. Atwal, K. A.; Ahmed, S. D.; O'Reilly, B. C. *Tetrahedron Lett.* **1989**, *30*, 7313.
17. Zhang, H.; Vrang, L.; Unge, T.; Öberg, B. *Antiviral Chem. Chemother.* **1993**, *4*, 301.
18. Weislow, O. S.; Kiser, R.; Fine, D. L.; Bader, J.; Shoemaker, R. H.; Boyd, M. R. *J. Nat. Cancer Inst.* **1989**, *81*, 577–586.