Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 619–622

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Biological Activities of Potential Metabolites of the Non-Nucleoside Reverse Transcriptase Inhibitor Efavirenz

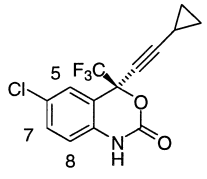
Jay A. Markwalder,* David D. Christ Abdul Mutlib, Beverly C. Cordova, Ronald M. Klabe and Steven P. Seitz

DuPont Pharmaceuticals Company, Experimental Station, PO Box 80500, Wilmington, DE 19880-0500, USA

Received 20 October 2000; accepted 12 December 2000

Abstract—Studies on the biotransformation of the clinically important non-nucleoside reverse transcriptase inhibitor efavirenz have shown that oxidation and secondary conjugation are important components of the processing of this molecule in vivo. We have synthesized metabolites of efavirenz to confirm their structure and to evaluate their activity as antivirals. © 2001 DuPont Pharmaceuticals Company. Published by Elsevier Science Ltd. All rights reserved.

The chemotherapy of HIV infection is most effective when mechanistically distinct agents are used in combination. The ability to suppress viral replication to very low levels and the necessity to accrue multiple mutations to circumvent agents acting at different sites presumably contribute to the effectiveness of this approach. There are currently three classes of agents used in the chemotherapy of HIV infection: nucleoside competitive reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and HIV protease inhibitors. The non-nucleoside reverse transcriptase inhibitor efavirenz **1** (Sustiva™) was approved by the FDA in 1998 and has become an important component of combination treatment.



1 Efavirenz

In the course of safety assessment studies on efavirenz, a number of metabolites were observed in rat urine.[1] Mass spectral analysis suggested that many of these compounds were oxidized species, which had been in some cases subsequently conjugated. The major species observed in rat urine was a glucuronide. In order to confirm structural assignments and obtain sufficient quantities for biological evaluation, we endeavored to synthesize metabolites of efavirenz.

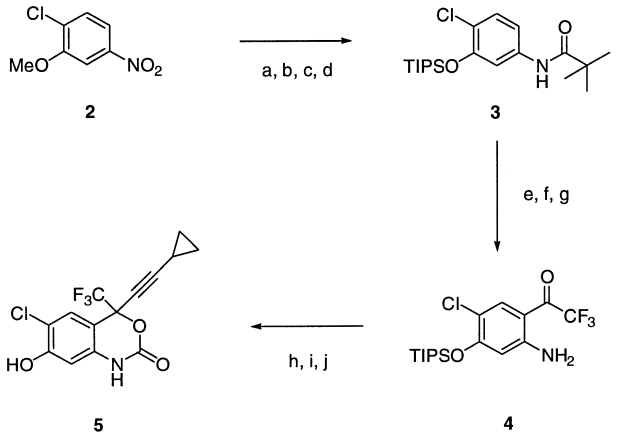
The synthetic routes used to prepare the metabolites parallel the methodology used to prepare efavirenz.[2] This chemistry employs directed *ortho*-metallation of an anilide derivative to introduce a trifluoromethyl ketone. Addition of acetylides to the trifluoromethyl ketone then gives derivatives suitable for further elaboration into the benzoxazinone ring system.

The synthesis of the 7-hydroxy compound is shown in Scheme 1. 2-Chloro-5-nitroanisole **2** was demethylated and converted to a tri-isopropylsilyl ether. The nitro group was then reduced to the aniline, which was then derivatized as the pivalamide. Lithiation of **3** occurred smoothly, adjacent to the directing amide group. The organometallic generated in this fashion was quenched with ethyl trifluoroacetate to provide the required trifluoromethyl ketone. The activating pivalamide was then hydrolyzed and the phenol reprotected to give **4**. The presence of the TIPS protecting group was crucial for the success of this synthetic sequence. Metallation of the methoxy version of **3** gave the organolithium between the amide and methoxy. The bulky silyl ether effectively suppresses this favorable process. The remainder of the synthesis was completed by addition of lithium cylopropylacetylide to the trifluoromethyl ketone followed by cyclization with phosgene and deprotection to give the target molecule **5**.

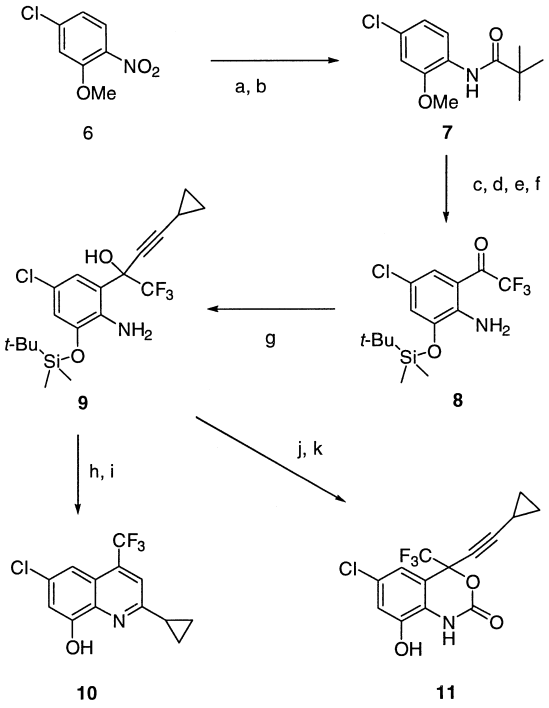
*Corresponding author. Tel.: +1-302-695-1882; e-mail: jay.a.markwalder@dupontpharma.com

PII: S0960-894X(01)00012-9

The synthesis of the 8-hydroxy isomer is described in Scheme 2. 3-Chloro-6-nitroanisole was converted to a pivalamide derivative **7**, which was metallated as before. In this example the strong amide directing group effectively controls the lithiation to give the desired trifluoromethylketone. The methyl ether was then converted to the more labile silyl ether. This exchange was performed prior to the introduction of the acetylene



Scheme 1. Synthesis of 7-hydroxy metabolite. Reagents and conditions: (a) BBr_3, CH_2Cl_2; (b) TIPSOTf, imidazole, DMF; (c) H_2, Pd/C, EtOAc; (d) $(CH_3)_3CCOCl$, Et_3N, CH_2Cl_2 (60% for 4 steps); (e) *s*-BuLi, ether, 0 °C to rt, CF_3CO_2Et; (f) HCl, DME, H_2O; (g) TIPSOTf, imidazole, DMF (19% for 3 steps); (h) lithium cyclopropylacetylide, THF–hexanes, −20 °C; (i) $COCl_2$, *i*-Pr_2NEt, toluene, −20 °C; (j) *n*-Bu_4NF, THF (56% for 3 steps).

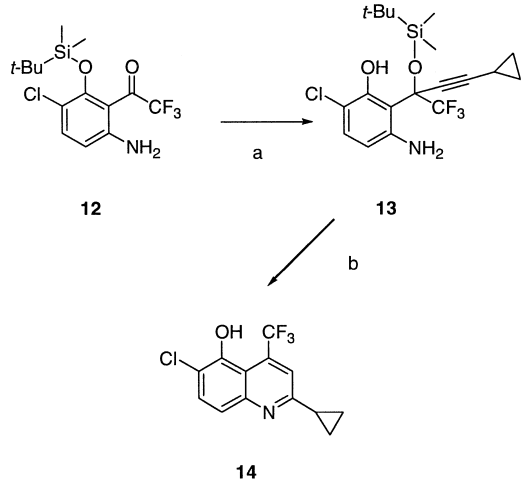


Scheme 2. Synthesis of the 8-hydroxy metabolite. Reagents and conditions: (a) $SnCl_2$, EtOH, reflux; (b) $(CH_3)_3CCOCl$, NEt_3, CH_2Cl_2 (97% for 2 steps); (c) *s*-BuLi, THF, −20 °C to 0 °C, CF_3CO_2Et; (d) HCl, DME, H_2O, reflux; (e) BBr_3, CH_2Cl_2; (f) TBDMSOTf, DMF, imidazole, 0 °C (60% for 4 steps); (g) lithium cyclopropylacetylide, THF–hexanes, −20 °C (76%); (h) carbonyldiimidazole, CH_2Cl_2, 0 °C to rt; (i) *n*-Bu_4NF, THF (53% for 2 steps); (j) $COCl_2$, *i*-Pr_2NEt, toluene, −25 °C to rt; (k) *n*-Bu_4NF, THF (66% for 2 steps).

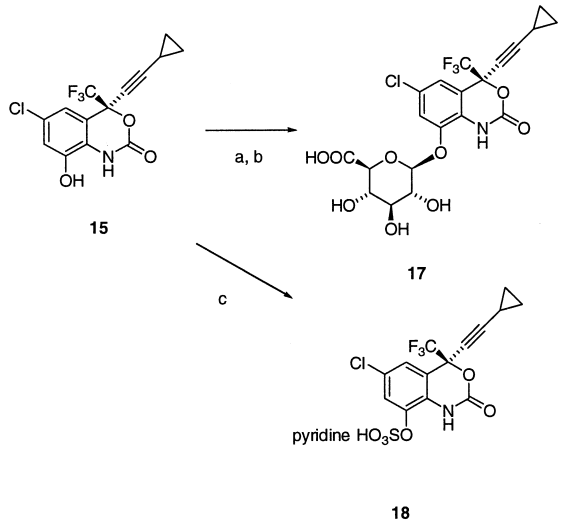
functionality to avoid exposing advanced intermediates to strongly Lewis acidic conditions necessary for the methyl group cleavage. Addition of lithium cyclopropylacetylide to **8** gave the desired carbinol in high yield. The cyclization of **9** proved to be somewhat more challenging than anticipated. Treatment of **9** with carbonyldiimidazole followed by removal of the silyl ether gave quinoline **10** as the major product. This reaction is analogous to results recently published by chemists at Merck.[3] In that work, the reaction was proposed to proceed through the formation of a highly strained cyclic allene intermediate. The formation of quinoline products can be suppressed by modification of the cyclization conditions. Thus, treatment of **9** with phosgene and Hunig's base followed by silyl ether removal gave the target molecule **11** in good overall yield.

The 5-position is the last site of benzene ring hydroxylation. The synthetic approach to this compound parallels that used for the 7- and 8-isomers. We thus prepared the trifluoromethyl ketone **12** (Scheme 3). Upon addition of lithium cyclopropylacetylide, a silyl migration occurred to give the phenol **13**. Attempts to deprotect **13** with fluoride gave the quinoline product **14**. The formation of quinoline products has thus far frustrated efforts to prepare the 5-hydroxylated analogue.

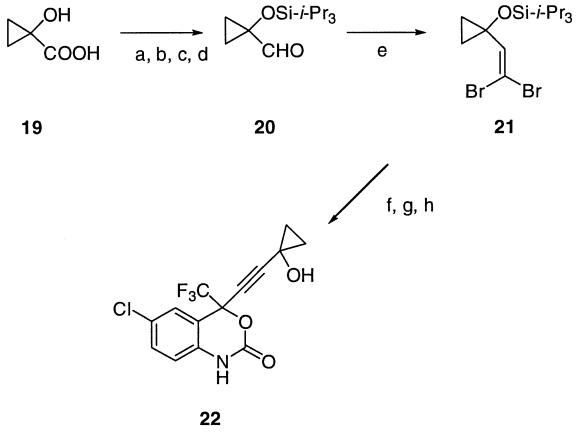
Many of the metabolites observed in vivo have undergone secondary processing to form glucuronides or sulfates. The synthesis of two of these secondary metabolites is shown in Scheme 4. Compound **11** was first resolved by chiral phase SCFC.[4] The two antipodes were isolated and characterized ($[\alpha]_D^{25}$ −29.3° (*c* 0.35, MeOH) and $[\alpha]_D^{25}$ +30.7° (*c* 0.16, MeOH)). The levorotatory isomer was shown to possess the efavirenz stereochemistry in two ways. First, a small sample of glucuronide from rat urine was treated with β-glucuronidase to give an aglycone that was identical to the levo-isomer by chiral SCFC. Second, the two isomers were evaluated for their ability to inhibit the reverse transcriptase enzyme (vide infra). The residual inhibitory activity resided with the levo-isomer, consistent



Scheme 3. Attempted synthesis of the 5-hydroxy metabolite. Reagents and conditions: (a) lithium cyclopropylacetylide, THF–hexanes, −20 °C; (b) *n*-Bu_4NF, THF.



Scheme 4. Synthesis of conjugated metabolites. Reagents and conditions: (a) methyl 2,3,4-tri-*O*-acetyl-D-glucuronate, Ph_3P, DEAD, THF–CH_2Cl_2; (b) LiOH, THF–H_2O; (c) sulfur trioxide–pyridine complex, pyridine.



Scheme 5. Synthesis of oxidized side-chain analogues. Reagents and conditions: (a) MeOH, $SOCl_2$; (b) *i*-Pr_3SiOTf, 2,6-lutidine; (c) Dibal, −78 °C; (d) oxalyl chloride, DMSO, NEt_3, CH_2Cl_2; (e) CBr_4, Ph_3P, NEt_3; (f) *n*-BuLi, −78 °C; (g) 4,6-dichloro-4-trifluoromethyl-1,4-dihydro-2*H*-3,1-benzoxazin-2-one, −60 °C; (h) *n*-Bu_4NF, THF.

with the earlier results.[5] These results suggest that the levo-isomer **15** has the (*S*) and the dextro-isomer **16** has the (*R*) configuration.

The carbohydrate was attached as shown in Scheme 4. Compound **15** was allowed to react with methyl 2,3,4-tri-*O*-acetyl-D-glucuronate[6] under Mitsunobu conditions[7] to give the protected glucuronide as a mixture of anomers. Saponification of the esters was accomplished with lithium hydroxide. The mixture of glycosidic linkage isomers was separated by HPLC and the β-anomer identified by its characteristic vicinal coupling constant ($J_{1,2}=7.3$ Hz). Sulfation was accomplished by exposure of **15** to sulfur trioxide–pyridine complex[8] to give the pyridine salt **18**.

The cyclopropylacetylene group is also a site of potential hydroxylation. As the cyclopropyl methine is the most activated site, we initiated the synthesis of this hydroxylated analogue. The requisite acetylene was prepared as shown in Scheme 5. The commercial 1-hydroxycyclopropane carboxylic acid **19** was converted in a four-step sequence to the protected aldehyde **20**. The aldehyde was converted to dibromoolefin **21** using a modified Corey procedure.[9] The lithium acetylide was then generated by exposure of **21** to 2 equiv of *n*-butyllithium. This species reacted smoothly with 4,6-dichloro-4-trifluoromethyl-1,4-dihydro-2*H*-3,1-benzoxazin-2-one[10] to provide, after deprotection with tetra-*n*-butylammonium fluoride, carbinol **22**.

Table 1. Biological data for efavirenz and its metabolites[11]

Compound	RT IC_{50} (nM)	RNA IC_{90} (nM)
1	47(±25)	1.7(±0.5)
15	2341	63
16	>12,500	>1507
5	2450	422
17	9489	>985
18	>12,500	1014
22	1983	33

The availability of the compounds described in this paper allowed the unambiguous identification of many of the metabolites seen in animals dosed with efavirenz. Compounds **15**, **17**, and **18** were shown to be identical to compounds seen in rats, guinea pigs, hamsters and cynomolgus monkeys. Compound **5** was only observed in guinea pigs, cynomolgus monkeys and humans. The carbinol **22** was not detected in any species. Metabolites were found that had the cyclopropyl methine oxidized; however, these all had additional oxidation on the benzene ring. Thus, it is likely that methine oxidation requires prior oxidation of the benzene ring.

We were also interested in determining the activity of these metabolites as antivirals. As shown in Table 1, all of the compounds disclosed are significantly weaker inhibitors of reverse transcriptase and poorer antivirals than efavirenz. The most potent antiviral **22** has not been observed in animals. Of the metabolites evaluated to date, none is of comparable potency to efavirenz. It is unlikely that a significant portion of the antiviral activity observed with efavirenz in vivo is attributable to the metabolites characterized thus far.

Acknowledgements

The authors would like to thank Al Mical for his expert assistance on chiral chromatography.

References and Notes

1. Mutlib, A. E.; Chen, H.; Nemeth, G. A.; Markwalder, J. A.; Seitz, S. P.; Gan, L. A.; Christ, D. D. *Drug Met. Disp.* **1999**, *27*, 1319.
2. Pierce, M. E.; Parsons, R. L., Jr.; Radesca, L. A.; Young, Y. S.; Silverman, S.; Moore, J. R.; Islam, Q.; Choudhury, A.;

Fortunak, J. M. D.; Nguyen, D.; Luo, C.; Morgan, S. J.; Davis, W. P.; Confalone, P. N. *J. Org. Chem.* **1998**, *63*, 8536.
3. Frey, L. F.; Tillyer, R. D.; Ouellet, S. G.; Reamer, R. A.; Grabowski, E. J. J.; Reider, P. J. *J. Am. Chem. Soc.* **2000**, *122*, 1215.
4. Column: Chiralpak AD® (Chiral Technologies Inc.) Solvent: 25% methanol, 75% CO_2. Pressure: 150 atm. The (*S*)-isomer (**15**) elutes prior to (*R*)-isomer (**16**).
5. 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.
6. Herzig, J.; Nudelman, A.; Gottlieb, H. E. *Carbohydr. Res.* **1988**, *177*, 21.
7. Budman, G. T.; Green, D. V. S.; Voyle, M. *J. Organomet. Chem.* **1990**, *338*, 117.
8. Bernauer, K.; Borgulya, J. *Synthesis* **1983**, 29.
9. Corey, E. J.; Fuchs, P. L. *Tetrahedron Lett.* **1972**, *36*, 3769.
10. Christ, D. D.; Cocuzza, A. J.; Ko, S. S.; Markwalder, J. A.; Mutlib, A. E.; Parsons, R. L., Jr.; Patel, M.; Seitz, S. P.; U.S. Patent 5 874 430, 1999; *Chem. Abstr.* **1999**, *130*, 196659.
11. Assays were performed as described in: Corbett, J. W.; Ko, S. S.; Rodgers, J. D.; Jeffrey, S.; Bacheler, L. T.; Klabe, R. M.; Diamond, S.; Lai, C.-M.; Rabel, S. R.; Saye, J. A.; Adams, S. P.; Trainor, G. L.; Anderson, P. S.; Erickson-Viitanen, S. K. *Antimicrob. Agents Chemother.* **1999**, *43*, 2893.