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SYNTHESIS AND EVALUATION OF ANALOGS OF EFAVIRENZ (SUSTIVA™) AS HIV-1 REVERSE TRANSCRIPTASE INHIBITORS

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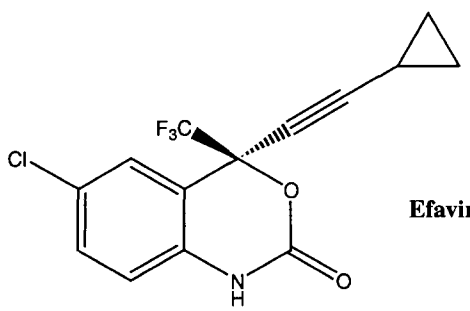
Abstract: Efavirenz (SUSTIVA™) is a potent non-nucleoside reverse transcriptase inhibitor. Due to the observation of breakthrough mutations of the reverse transcriptase enzyme during Efavirenz therapy, we sought to develop an optimized second generation series. To that end, SAR of the substituents on the aromatic ring was undertaken and the results are summarized here. The 5,6-difluoro (**4f**) and the 6-methoxy (**4m**) substituted benzoxazinones were determined to be equipotent, and as a result such substitution patterns will be incorporated in second generation scaffolds.

Effective treatment regimens for the human immunodeficiency virus (HIV-1) infection have included both HIV protease and reverse transcriptase inhibitors. The initial series of nucleoside reverse transcriptase inhibitors (NRTI) include AZT, 3TC, ddI, and ddC. These compounds although synergistic with protease inhibitors were considerably. toxic.[1] Nevirapine,[2] Delavirdine,[3] and Efavirenz[4] are the only non-nucleoside reverse transcriptase inhibitors (NNRTI) that have received regulatory approval, with several NNRTIs (MKC442, Trovirdine, S-1153/AG1549, PNU142721, ACT, HBY1293/GW420867X) currently undergoing clinical trials. Efavirenz, a potent reverse transcriptase inhibitor was discovered at Merck Research Laboratories.[4] Efavirenz is the first anti-HIV drug to be approved by the FDA for once a day dosing when used in a combination regimen in both adult and pediatric patients.[5] Studies have shown that Efavirenz penetrates into the cerebrospinal fluid, a common viral sanctuary.[5] Mutations of the reverse transcriptase enzyme associated with Efavirenz therapy include K103N, L100I, and Y188L.[4] In pursuit of a second generation series to address such breakthrough mutants, we sought to first define the SAR of the substituents on the aromatic ring in terms of enzyme inhibition and whole cell activity. The results of such a survey are summarized here.



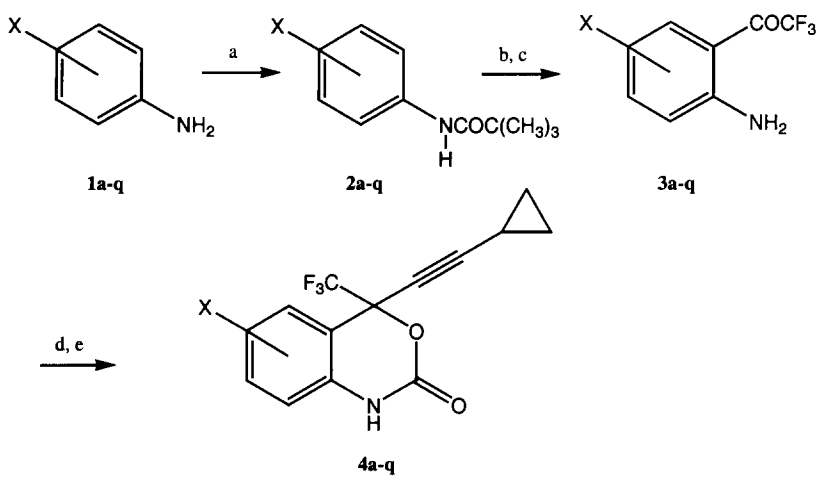
Efavirenz (Sustiva™)

0960-894X/99/$ - see front matter
PII: S0960-894X(99)00486-2

Chemistry

The analogs reported herein were prepared using the methodology developed for Efavirenz as shown in Scheme I.[4] The preparation of the desired compounds **4a–q** involved directed metallation to introduce the trifluoromethyl ketone functionality. Commercially available anilines **1a–q** were treated with pivaloyl chloride and triethylamine in dichloromethane to provide **2a–q** in excellent yields. The ortho directing effect of the pivalolyl group allows for the introduction of the trifluoromethyl ketone functionality using *n*-butyl lithium and ethyl trifluoroacetate in moderate to good yields. The intermediate pivaloyl protected compounds were deprotected without purification using 6 *N* hydrochloric acid in refluxing dimethoxyethane.

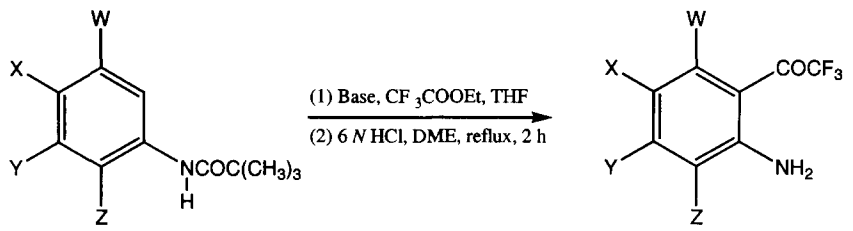
Scheme I



Reagents and conditions: (a) $(CH_3)_3COCl$, TEA, DCM, 0–25 °C, 3 h, 83–94%; (b) *n*-BuLi, CF_3COOEt, THF, -78 to 0 °C, 1.5 h; (c) 6 *N* HCl, DME, reflux, 2 h, 19–88% over 2 steps; (d) *n*-BuLi, cyclopropylacetylene, THF, 0 °C, 1 h; (e) Phosgene, Toluene, DIPEA, 0 °C, 1 h, 2.5–34% over 2 steps.

Addition of lithium cyclopropylacetylide to ketones **3a–q** were carried out at 0 °C followed by ring closure using phosgene in toluene to give the benzoxazinones **4a–q.** The metallation conditions varied with the substituent(s) on the ring and have been summarized in Table I. In the case of **4d**, the addition of TMEDA prior to the addition of *s*-BuLi to the reaction mixture was critical to the success of the reaction.

Table I



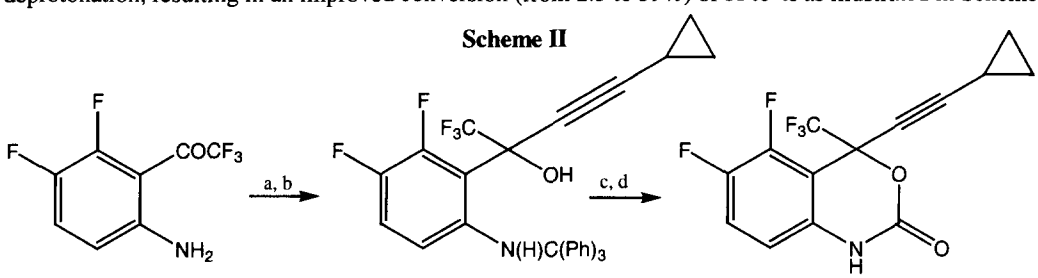
2 3

Compound	W	X	Y	Z	Base	Temp (°C)	Time (min)	Yield (%)*
3c	H	*i*Pr	H	H	*t*-BuLi	0	60	56
3d	H	$N(CH_3)_2$	H	H	*s*-BuLi/TMEDA	0	60	23
3i	F	F	F	H	*n*-BuLi	-40	60	40
3k	H	Cl	H	OCH_3	*s*-BuLi	-20	40	63
3l	H	Cl	H	Cl	*t*-BuLi	-50	40	63
3n	H	Cl	H	F	*t*-BuLi	-50	5	39
3o	H	OCH_3	H	H	*t*-BuLi	-30	40	75
3p	H	Ph	H	H	*n*-BuLi	-40	60	nd

* Yield determined over 2 steps
nd = not determined

In the case of the di and trifluoro substituted compounds **3f–j** the deprotonation of the anilino hydrogen led to inactivation of the aromatic ring toward nucleophilic attack by lithium acetylide. Thus, the nitrogen of the 5,6-difluoro compound **3f** was protected with a trityl group to provide steric bulk and thereby reduce deprotonation, resulting in an improved conversion (from 2.5 to 39%) of **3f** to **4f** as illustrated in Scheme II.[6]

Scheme II



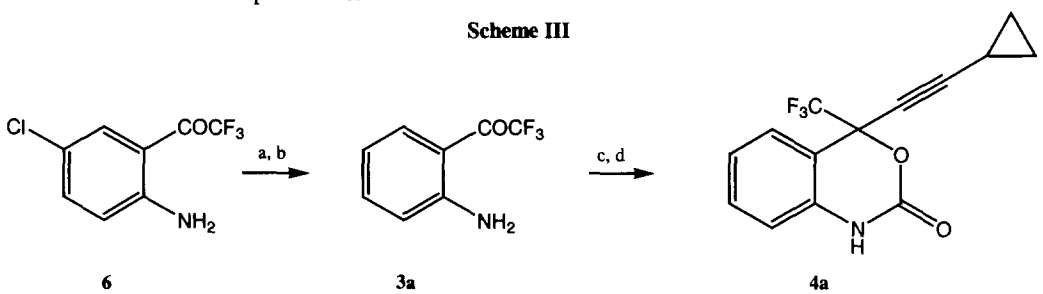
3f 5 4f

Reagents and conditions: (a) Ph_3COH, cat. *p*-TsOH, toluene, reflux, Dean–Stark trap, 3 h, 69%; (b) *n*-BuLi, cyclopropylacetylene, THF, 0 °C, 1 h, 39%; (c) conc. HCl, MeOH, 25 °C, 0.3 h, 65%; (d) Phosgene, DIPEA, toluene, 0 °C, 0.5 h, 79%.

For the preparation of the deschloro compound **4a** the synthetic sequence described in Scheme III[6] was carried out to provide the desired 1-(2-amino-5-chloro-phenyl)-2,2,2-trifluoro-ethanone. Readily available 4-chloro-2-trifluoroacetylaniline **6** (an intermediate in the synthesis of Efavirenz)[4] was subjected to hydrogenation conditions to provide the deschloro secondary alcohol in 98% yield. MnO_2 oxidation of the compound was

carried out in dichloromethane to give **3a** in quantitative yield. Due to the unstable nature of the compound, it was used without further purification.

Scheme III

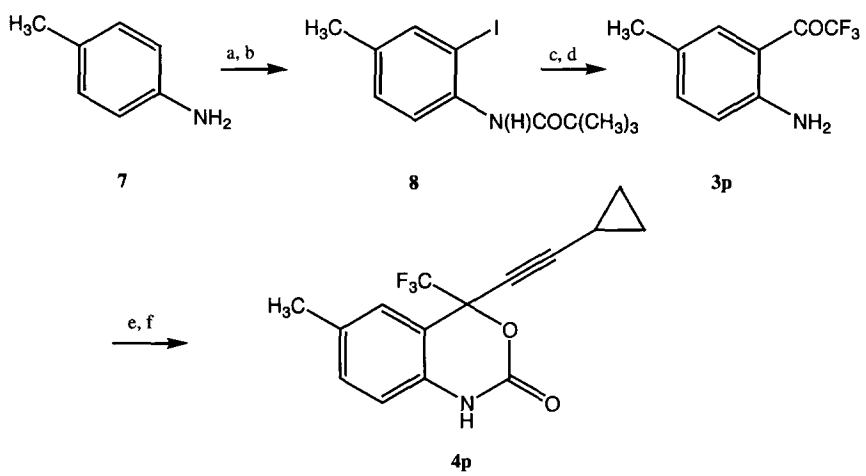


6 **3a** **4a**

Reagents and conditions: (a) Pd/C, H_2, MeOH, 25 °C, 2 h, 98%; (b) MnO_2, DCM, 25 °C, 0.5 h, 55%; (c) *n*-BuLi, cyclopropylacetylene, THF, 0 °C, 1 h; (d) Phosgene, DIPEA, Toluene, 0 °C, 1 h, 13% over 2 steps.

For the preparation of compound **4p**, the synthetic sequence described in Scheme IV[6] was carried out to provide key intermediate **3p**. Iodinated compound **8** was subjected to halogen-metal exchange conditions followed by an ethyl trifluoroacetate quench to provide **3p**, a key intermediate in the synthesis.

Scheme IV



7 **8** **3p**

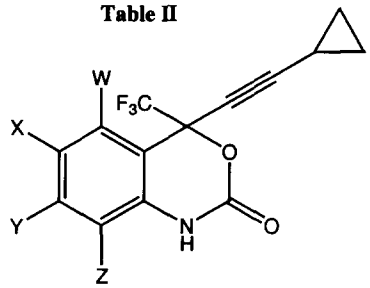
4p

Reagents and conditions: (a) I_2, $NaHCO_3$, DCM:H_2O, 25 °C, 14 h, 97%; (b) $(CH_3)_3CCOCl$, TEA, DCM, 25 °C, 3 h, 96%; (c) *n*-BuLi, CF_3COOEt, THF, -78 °C, 0.5 h, 13%; (d) 6 *N* HCl, DME, reflux, 3 h, 93%. (e) *n*-BuLi, cyclopropylacetylene, THF, 0 °C, 1 h; (f) Phosgene, DIPEA, toluene, 0 °C, 1 h, 7% over 2 steps.

Results and Discussion

The results of the enzyme inhibition and antiinfectivity assays have been summarized in Table II. We initiated the SAR with the introduction of hydrogen followed by nitrogen, oxygen, carbon and fluorine in place of the 6-chloro substituent in Efavirenz. Accordingly, we prepared the deschloro compound **4a**, and discovered we lost an order of magnitude in enzyme inhibition with a marginal loss in the whole cell assay.

Table II



Compound	W	X	Y	Z	IC_{50} (nM)[7a]	IC_{90} (nM)[7b]
Efavirenz[8]	H	Cl	H	H	48	2.03
4a	H	H	H	H	478	10.31
4b	H	F	H	H	190	7.35
4c	H	iPr	H	H	1958	27.84
4d	H	$N(CH_3)_2$	H	H	816	8.33
4e	H	OCF_3	H	H	1249	18.89
4f	F	F	H	H	84	3.15
4g	F	H	H	F	796	17.97
4h	F	F	H	F	800	14.02
4i	F	F	F	H	442	19.6
4j	H	F	F	F	>2000	417.65
4k	H	Cl	H	OCH_3	----	122
4l	H	Cl	H	Cl	>2000	28.6
4m	H	OCH_3	H	H	131	2.0
4n	H	Cl	H	F	----	7.19
4o	H	Ph	H	H	>2000	249.09
4p	H	CH_3	H	H	133	7.11
4q	H	-CH=CH-CH=CH-		H	1909	26.86

The dimethylamino substituted compound **4d** showed a similar loss in both assays. The oxygen substituted compounds prepared were **4m** (6-OCH_3) and **4e** (6-OCF_3). Clearly, compound **4m** bearing the 6-methoxy substituent was a potent compound and could be an effective alternate to the 6-chloro substituent in Efavirenz. The 6-trifluoromethoxy substituted compound (**4e**) showed a two log order decrease in enzyme inhibition capability. The alkyl substituted compounds are represented by compounds **4c** and **4p**. Athough **4p** (6-CH_3) showed good activity, **4c** (6-*i*Pr) showed a substantial loss in activity in both IC_{50} and IC_{90} determinations as compared to Efavirenz. Compounds **4o** (6-Ph) and naphthyl derivative **4q** (-CH=CH-CH=CH-) were attempts to probe steric constraints of that region of the reverse transcriptase enzyme. These compounds were relatively inactive in both the enzyme inhibition and whole cell antiinfectivity assays. The substitution of fluorine for chlorine in the C-6 position (**4b**) does not appear to have a detrimental effect on the antiviral properties of the compound. Next, we decided to prepare a series of di and trifluoro substituted compounds (**4f–j**) to determine the effect of the halogens on the overall activity of the benzoxazinones. Compound **4f** (5,6-difluoro) appears to

be comparable to both Efavirenz and compound **4m** (6-OCH_3) and could be another alternative to the 6-Cl substituent in Efavirenz, whereas compound **4g** appears to have moderate activity. Three trifluoro substituted compounds **4h**, **4i** and **4j** were prepared. Compounds **4h** and **4i** show similar activity in the biological assays, both being a log order lower in the enzyme assay with a 3 to 4 fold loss in the whole cell based assay as well. Compound **4j** bearing the 6,7,8-trifluoro substitution pattern was inactive, leading us to conclude that a 7,8 disubstitution pattern may not be favorable for good activity. Studies conducted by the metabolism group had determined one of the metabolites of Efavirenz to be the 8-hydroxylation product, with an IC_{90} value of 50nM.[9] In an effort to block this metabolic site and thereby affect the pharmacokinetic profile, compounds **4k**, **4l** and **4n** were prepared. However, compounds **4k** (6-Cl, 8-OCH_3) and **4l** (6,8-dichloro) lost substantially in whole cell based assays, whereas compound **4n** (6-Cl, 8-F) showed good activity. The order of activity of benzoxazinones bearing both C-6 and C-8 substituents is 6-Cl, 8-H > 6-Cl, 8-F > 6-Cl, 8-Cl > 6-Cl, 8-OH > 6-Cl, 8-OCH_3, leading us to believe that only small substituents would be tolerated at the C-8 position.

Conclusions

We have discovered two substitution patterns on the aromatic ring that give rise to benzoxazinones that are equipotent to Efavirenz, they are the 6-methoxy (**4m**) and the 5,6-difluoro (**4f**) substituted compounds. The potential metabolic liability of the methoxy group precluded further consideration and as a result only the 5,6-difluoro substitution pattern will be incorporated in the second generation series.

References and Notes

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5. METEOR, Press Release, DuPont Pharmaceuticals Company, 18th September, 1998.
6. All compounds provided satisfactory spectral data (^1H NMR, ^{19}F NMR, CIMS/ESIMS, and HRMS/peak match) and were homogeneous by TLC.
7. (a) All compounds were assayed for enzyme inhibitory activity IC_{50} according to the protocol described in: Sardana, V. V.; Emini, E. A.; Gotlib, L.; Graham, D. J. Lineberger, D. W.; Long, W. J.; Schlabach, A. J.; Wolfgang, J. A.; Condra, J. H. *J. Biol. Chem.* **1992**, *267*, 17526 using a template primer poly (rA) oligo $(dT)_{12-18}$. (b) All compounds were assayed for whole cell based antiviral activity IC_{90} according to the protocol described in: Bacheler, L. T.; Paul, M.; Jadhav, P. K.; Otto, M.; Miller, J. *Antiviral Chem. Chemother.* **1994**, *5*, 111.
8. The data presented for Efavirenz reflects values determined for a single enantiomer, whereas the data shown for **4a–q** are that of racemic mixtures. The biological evaluation of each enantiomer of Efavirenz and other benzoxazinones has determined that only the S enantiomer is active.
9. Markwalder, J. A.; Seitz, S. P.; Christ, D. D.; Mutlib, A. Presented at the 26th National Medicinal Chemistry Symposium, Richmond, VA, June 14-18, 1998; Abstract A-23.