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2-Arylbenzoxazoles as CETP inhibitors: Raising HDL-C in cynoCETP transgenic mice

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ABSTRACT

We describe structure–activity studies leading to the discovery of 2-arylbenzoxazole **3**, the first in a series to raise serum high-density lipoprotein cholesterol levels in transgenic mice. Replacement of the 4-piperidinyloxy moiety with piperazinyl provided a more synthetically tractable lead, which upon optimization resulted in compound **4**, an excellent inhibitor of cholesteryl ester transfer protein function with good pharmacokinetic properties and in vivo efficacy.

It has been established that serum levels of high-density lipoprotein cholesterol (HDL-C) and the risk of cardiovascular disease (CVD) are inversely correlated. Niacin, the most effective HDL-C increasing agent currently available, suffers from lack of compliance. Thus, there is a need for better HDL raising therapies that combine efficacy with negligible side effects.[1,2] One approach to reducing CVD risk via HDL-C elevation is by inhibition of the cholesteryl ester transfer protein (CETP).[3]

CETP is a plasma glycoprotein that facilitates the reshaping of lipoprotein particles. It mediates the transfer of cholesteryl ester (CE) from CE-rich HDL particles to triglyceride (TG)-rich particles, primarily very low-density lipoproteins (VLDL), and the reciprocal movement of TG from VLDL to HDL. It is a process driven by the substrate concentration gradient between lipoproteins with a net effect of reducing serum HDL-C.[2] Clinical trials have confirmed that pharmacological inhibition of CETP causes elevation of serum HDL-C in humans.[4,5]

Recent reports by our labs[6,7] and others[8] have described the discovery and ongoing optimization of a series of 2-arylbenzoxazoles as CETP inhibitors. The potency was determined by measuring a compound's ability to inhibit the CETP-mediated transfer of fluorescently labeled CE. Aryloxyamides exemplified by **1**[6] showed good inhibition of CETP-mediated CE transfer and β-alkyloxyamine **2**[7] showed improved pharmacokinetic properties relative to the aryloxyamides. However, no in vivo efficacy was observed in a cynoCETP transgenic mouse pharmacodynamic (PD) model[9] with these or analogous compounds (Fig. 1).

This communication describes further structure–activity relationship studies and optimization of the α-aryloxyamide moiety of compound **1** that led to the discovery of **3**, the first CETP inhibitor in the 2-arylbenzoxazole class reported to show in vivo efficacy. Subsequent optimization efforts to improve potency and pharmacokinetic properties culminated in **4**, which was a potent inhibitor of CETP in vitro and raised HDL-C in the transgenic mouse PD assay.

We began by exploring cycloalkyloxy replacements of the aryloxy group (Table 1). Preparation of α-alkoxyamides **3** and **5–12** has been previously described.[7]

As shown in Table 1, the inhibitory potency of compounds increased with the steric bulk of the cycloalkyl group (**5**–**10**). However, the most potent compound in the series, the methylbicyclooctane analog **9**, elicited no effect in the in vivo PD assay. Initially, this lack of in vivo efficacy was attributed to the poor physicochemical properties of this series of molecules, in particular, their very low aqueous solubility, and the correspondingly low drug exposures seen in the pharmacokinetic analyses.

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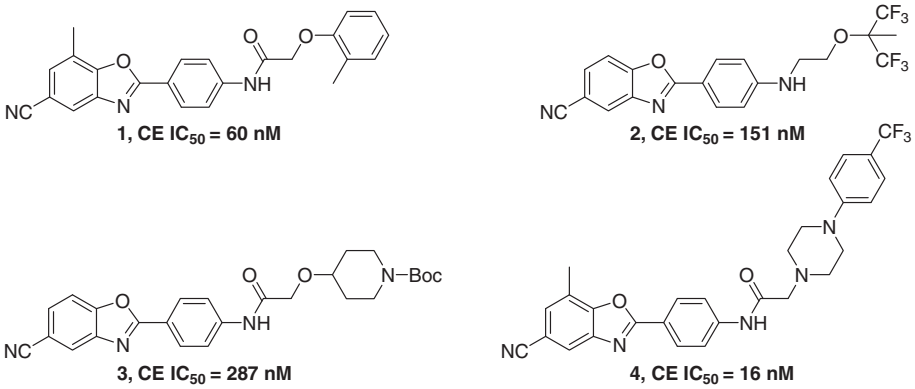
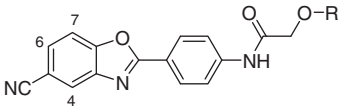
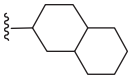
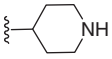
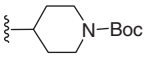


Figure 1. CETP inhibitors.

Table 1
Effect of cycloalkyloxy substituents on inhibition of CE transfer



Compd	R	CE IC_{50} (nM)[a]
5		474
6		280
7		146
8		92
9		72
10		115
11		1253
12		>30,000
3		492/287[b]

[a] Assay conditions have been described.[10]
[b] This data-point was generated by modifying the assay protocol to include a 1 h preincubation at 25 °C of inhibitor with CETP/serum premix. This change allowed for full development of time-dependent inhibition and resulted in better data reproducibility. Any data reported hereafter was obtained using these modified assay conditions.

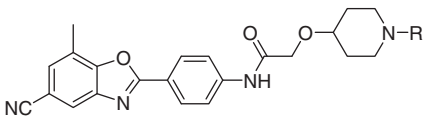
We also investigated introduction of heteroatoms (Table 1). This approach was generally detrimental to in vitro potency (**11**–**12**), although the Boc-protected piperidinyl **3** was a moderate inhibitor of CE transfer.

Over the course of the program, we evaluated selected compounds for their ability to inhibit the CETP-mediated transfer of fluorescently labeled triglyceride (TG).[10] We observed that, while other classes of compounds[3,11] inhibited both arms of CETP function and elicited a PD response, none of the 2-arylbenzoxazoles that had been tested inhibited TG transfer in this assay. A rescreen of the remaining benzoxazoles identified a single compound capable of inhibiting both arms of CETP-mediated neutral lipid transfer. Thus, Boc-piperidine **3** was a 287 nM inhibitor of CE and a 182 nM inhibitor of TG transfer. When tested in our PD assay at 20 mpk, compound **3** raised HDL-C by 13 mg/dL.

We then embarked on an effort to optimize this scaffold. Since we had previously established that methylation at the C_7 position of the benzoxazole is potency-enhancing,[6] we utilized that substitution in subsequent SAR exploration (Table 2). Piperidinyl analogs **16–30** were prepared following the procedure outlined in Scheme 1. Nitration and subsequent reduction of 4-bromo-2-methylphenol afforded the aminophenol **13**. Amide coupling of **13** with 4-nitrobenzoyl chloride was followed by acid-catalyzed cyclization to form the 4-nitrophenyl benzoxazole. Reduction of the nitro group and subsequent installation of the cyano group resulted in compound **14**. Acyl chloride **15** was prepared in a two-step fashion from *N*-Boc-4-hydroxypiperidine and coupled with aniline **14**. The resulting compound **16** was deprotected and treated with the appropriate electrophile in the presence of base to afford compounds **17–24** and **26–30**, while compound **25** was prepared by reductive alkylation with 3,3-dimethyl butyraldehyde.

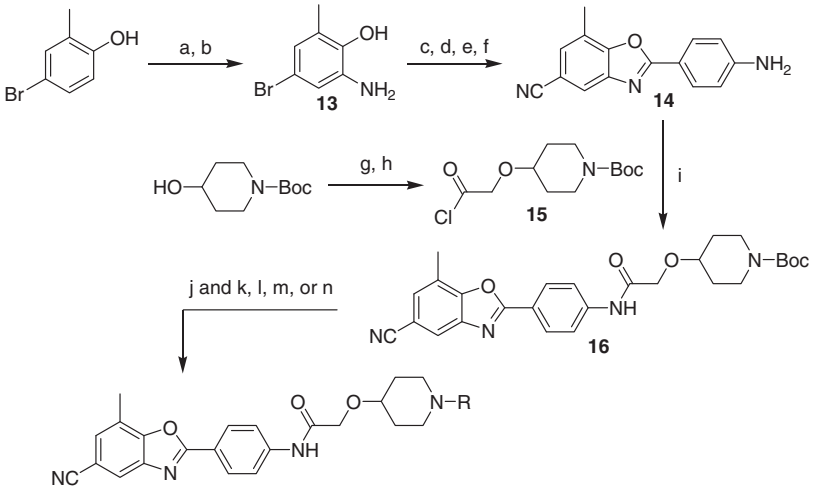
Compound **16**, the C_7-methylated analog of **3,** did not show an increased response in the PD assay, in spite of increased in vitro

Table 2
Effect of substituents on piperidine on HDL-C levels



Compd	R	CE IC_{50} (nM)[a]	TG IC_{50} (nM)[a]	ΔHDL-C (mg/dL)[b]
16	–C(O)–O*t*Bu	91	92	17[c]
17	–C(O)–OMe	382	nd	1[c]
18	–C(O)–OEt	254	nd	7[c]
19	–C(O)–O*i*Pr	160	99	3[d]
20	–C(O)–OCH_2-*t*Bu	111	130	4[c]
21	–C(O)–$OC(Me)_2$–Et	94	84	8[d]
22	–C(O)–OPh	221	524	4[d]
23	–C(O)–OBn	130	252	6[d]
24	–CH_2–C(O)–O*t*Bu	181	83	nd
25	–$(CH_2)_2$–*t*Bu	228	350	nd
26	–C(O)–CH_2-*t*Bu	370	256	nd
27	–C(O)–N*t*Bu	1301	625	4[c]
28	–$S(O)_2$–*n*Bu	207	nd	1[d]
29	–$S(O)_2$–Ph	336	nd	nd
30	–$S(O)_2$–Bn	169	31	nd

[a] Assay conditions have been described.[10]
[b] Assay conditions have been described.[9]
[c] Dosed at 20 mpk.
[d] Dosed at 10 mpk.



Scheme 1. Synthesis of 2-arylbenzoxazole α-alkyloxyamides **16**–**30**. Reagents and conditions: (a) HNO_3 (fuming), AcOH (48%); (b) $SnCl_2{\cdot}2H_2O$, HCl, MeOH, 0 °C–rt (95%); (c) 4-nitrobenzoyl chloride, 1,4-dioxane, reflux (quant.); (d) *p*-TsOH, toluene (97%); (e) 10% Pd/C, H_2, DCM (74%); (f) CuCN, NMP, 190 °C (86%); (g) bromoacetic acid, NaH, THF, 0 °C–rt (95%); (h) oxalyl chloride, DMF, DCM, 0 °C–rt (quant.); (i) LHMDS, THF, 0 °C–rt (66%); (j) TFA, DCM (quant.); (k) R–X, DIPEA, DCM (63–96%); (l) di-*t*-amyl dicarbonate, TEA, DCM (quant.); (m) 3,3-dimethyl butyraldehyde, $NaBH(OAc)_3$, DCE (81%); (n) *t*-butyl isocyanate, DIPEA, DCM (84%).

potency. However, this was attributed to a suboptimal pharmacokinetic profile.

Conservative modifications of the carbamate moiety of **16** did not increase potency (Table 2). Replacing the *t*-butyl substituent with other alkyl groups or phenyl (**17–23**), modifications including incorporation of a methylene spacer (**24**) and replacement of the carbamate with alkyl (**25**), amide (**26**), urea (**27**), and sulfonamide groups (**28–30**), all resulted in compounds of lesser or equivalent potency to **16**.

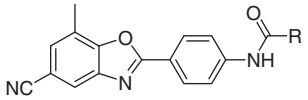
Unable to improve upon the Boc-piperidinyl moiety, we then made small modifications to the α-alkoxyamide 'linker' region of the molecule (Table 3). Compounds **31** and **32** were prepared by coupling aniline **14** with the corresponding acyl chloride, using an analogous procedure to the one used to prepare compound **16**.

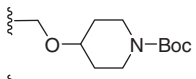
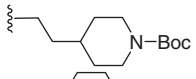
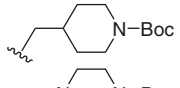
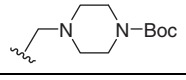
Replacing the oxygen in the linker with a methylene group caused a 12-fold decrease in CE transfer inhibition (**31**). However, when the linker was shortened by one carbon, the resulting compound (**32**) showed a modest increase in potency relative to **31**. Furthermore, replacing the piperidine with a piperazine, resulted in compound **33**, which was essentially equipotent to **16**.

A variety of N-substituted piperazine analogs were prepared as shown in Scheme 2. Compounds **33**, **35–44**, **46** and **4** were prepared by treating **14** with bromoacetyl bromide and displacing the resulting α-bromoacetamide with the appropriate N-substituted piperazine. Boc-deprotection of compound **33** afforded **34**. Acids **45** and **47** were prepared by treating the corresponding ester with aqueous LiOH in THF and the appropriate alcohol.

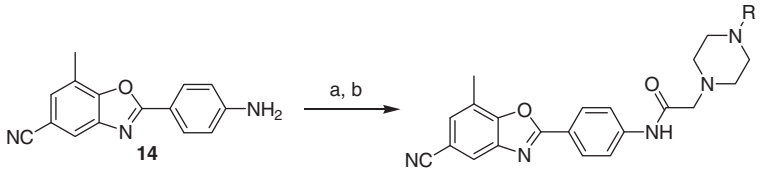
The SAR of the piperazine series is shown in Table 4. While the unsubstituted piperazine **34** did not inhibit CETP, the *N*-phenyl analog (**35**) had a comparable in vitro profile to compound **33**. A variety of substituents on the phenyl ring were then explored. No improvement over the phenyl (**35**) was observed with the regioisomeric methoxy analogs (**36–38**). When the substituent was chloro (**39–41**), the *meta* position was optimal. While the ester analogs **44** and **46** had similar potency to the *N*-phenyl analog (**35**), there was a drastic decrease in in vitro potency with the corresponding acids (**45** and **47**). In the case of the trifluoromethyl

Table 3
Effect of 'linker' modifications on HDL-C levels



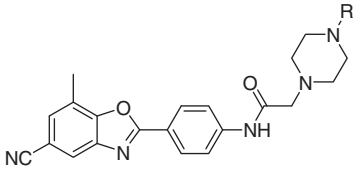
Compd	R	CE IC_{50} (nM)[a]	TG IC_{50} (nM)[a]	ΔHDL-C (mg/dL)[b]
16		91	92	17[c]
31		1094	951	nd
32		757	356	nd
33		64	83	8[d]

[a] Assay conditions have been described.[10]
[b] Assay conditions have been described.[9]
[c] Dosed at 20 mpk.
[d] Dosed at 10 mpk.



Scheme 2. Synthesis of N-substituted piperazin-1-yl acetamides **33**, **35–44**, **46** and **4**. Reagents and conditions: (a) bromoacetyl bromide, DIPEA, THF, 40 °C; (b) N-substituted piperazine, DIPEA, THF.

Table 4
Effect of substituents on piperazine on HDL-C levels



Compd	R	CE IC_{50} (nM)[a]	TG IC_{50} (nM)[a]	ΔHDL-C (mg/dL)[b]
34	H	>30,000	nd	nd
35	Ph	49	349	nd
36	2-OMe–Ph	111	nd	nd
37	3-OMe–Ph	58	123	nd
38	4-OMe–Ph	109	99	nd
39	2-Cl–Ph	69	3720	nd
40	3-Cl–Ph	19	29	11
41	4-Cl–Ph	53	135	nd
42	2-CF_3–Ph	65	3121	nd
43	3-CF_3–Ph	13	15	14
4	4-CF_3–Ph	16	18	24
44	3-C(O)OMe–Ph	64	51	nd
45	3-C(O)OH–Ph	>30,000	>30,000	nd
46	4-C(O)OEt–Ph	59	40	nd
47	4-C(O)OH–Ph	9164	nd	nd

[a] Assay conditions have been described.[10]
[b] Assay conditions have been described. Dosed at 10 mpk.[9]

Table 5
Comparison of mouse PK[12] profiles

Compd	$AUCN_{po}$[a] (μM h kg/mg)	Clearance (ml/min/kg)	Half-life (h)	Bioavailability (%)
43	0.19	49	2.2	26
4	0.72	25	4.1	56

[a] Normalized area under the curve.

substituent (**42–43** and **4**), both the *meta* and the *para* positions were potency-enhancing in vitro. However, the *para* analog **4** outperformed **43** in the transgenic mouse PD assay, raising HDL-C by 24 mg/dL (relative to 14 mg/dL for **43**). This was consistent with the observed pharmacokinetic profiles of the compounds in the mouse (Table 5). While the *meta* analog **43** had a clearance of 49 ml/min/kg and 26% oral bioavailability, the *para* analog **4** exhibited an improved pharmacokinetic profile with a clearance of 25 ml/min/kg and 56% oral bioavailability.

In summary, our efforts to optimize 2-arylbenzoxazoles as CETP inhibitors resulted in a series of α-cycloalkyloxyamides which were potent inhibitors of CE transfer; however, none of the compounds in this class that were tested in a transgenic mouse pharmacodynamic model proved efficacious.[6,7] A screen of our collection of benzoxazoles for inhibitors of CETP-promoted TG transfer allowed us to identify compound **3** as a 182 nM inhibitor. This 'dual' CE/TG transfer inhibitor raised HDL-C by 13 mg/dL in the transgenic mouse PD model. Further exploration of this series confirmed that those 2-arylbenzoxazole compounds which inhibited both arms (CE and TG) of CETP-promoted lipid transfer showed in vivo efficacy.

While the underlying cause for this biochemical difference of the benzoxazole series remains unclear, this observation guided our subsequent structure activity relationship studies around the piperidine moiety of compound **3**, which led us to compound **4**, a potent 'dual' CETP inhibitor with in vivo efficacy. We will report on the further optimization of this class of CETP inhibitors in due course.

Acknowledgements

F. Kallashi thanks P. J. Sinclair and J. A. Hunt for many useful discussions and their continuous support.

References and notes

1. Sinclair, P. J. *Annu. Rep. Med. Chem.* **2005**, *40*, 71.
2. Singh, I. M.; Shishehbor, M. H.; Ansell, B. J. *J. Am. Med. Assoc.* **2007**, *298*, 786.
3. Hunt, J. A.; Lu, Z. *Curr. Top. Med. Chem.* **2009**, *9*, 419.
4. Bloomfield, D.; Carlson, G. L.; Sapre, A.; Tribble, D.; McKenney, J. M.; Littlejohn, T. W.; Sisk, C. M.; Mitchel, Y.; Pasternak, R. C. *Am. Heart J.* **2009**, *157*, 352.
5. Barter, P. J.; Caulfield, M.; Eriksson, M.; Grundy, S. M.; Kastelein, J. J. P.; Komajda, M.; Lopez-Sendon, J.; Mosca, L.; Tardif, J.-C.; Waters, D. D.; Shear, C. L.; Revkin, J. H.; Buhr, K. A.; Fisher, M. R.; Tall, A. R.; Brewer, B. *N. Engl. J. Med.* **2007**, *357*, 2109.
6. Smith, C. J.; Ali, A.; Chen, L.; Hammond, M. L.; Anderson, M. A.; Chen, Y.; Eveland, S. S.; Guo, Q.; Hyland, S. A.; Milot, D. P.; Sparrow, C. P.; Wright, S. D.; Sinclair, P. J. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 346.
7. Hunt, J. A.; Gonzalez, S.; Kallashi, F.; Hammond, M. L.; Pivnichny, J. V.; Tong, X.; Xu, S. S.; Anderson, M. A.; Chen, Y.; Eveland, S. S.; Guo, Q.; Hyland, S. A.; Milot, D. P.; Sparrow, C. P.; Wright, S. D.; Sinclair, P. J. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1019.
8. Harikrishnan, L. S.; Kamau, M. G.; Herpin, T. F.; Morton, G. C.; Liu, Y.; Cooper, C. B.; Salvati, M. E.; Qiao, J. X.; Wang, T. C.; Adam, L. P.; Taylor, D. S.; Chen, A. Y. A.; Yin, X.; Seethala, R.; Peterson, T. L.; Nirschl, D. S.; Miller, A. V.; Weigelt, C. A.; Appiah, K. K.; O'Connell, J. C.; Lawrence, R. M. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2640.
9. *Transgenic mouse pharmacodynamic assay*: B6 male mice 12–16 weeks of age expressing cynomolgus monkey CETP were used (Marotti et al. *Arterioscler. Thromb. Vasc. Biol.* **1992**, *12*, 736.) Pre-dose blood samples were collected by retro-orbital bleed. Compounds were formulated in DMSO/cremophor/saline at a 4:4:92 ratio and screened at 10 or 20 mg/kg PO BID. Twenty-four hours after the first dose, the mice were euthanized and blood was collected by cardiac puncture. HDL-C was measured on the pre- and post-bleed samples using a HDL-E biochemical assay kit from Wako Chemicals USA (cat#431-52501), following manufacturer's instructions. The ΔHDL-C reported is relative to the baseline. A change in HDL-C of 7 mg/dl or less was generally considered not significant.
10. Eveland, S. S.; Milot, D. P.; Guo, Q.; Chen, Y.; Hyland, S. A.; Peterson, L. B.; Jezequel-Sur, S.; O'Donnell, G. T.; Zuck, P. D.; Ferrer, M.; Strulovici, B.; Wagner, J. A.; Tanaka, W. K.; Hilliard, D. A.; Laterza, O.; Wright, S. D.; Sparrow, C. P.; Anderson, M. S. *Anal. Biochem.* **2007**, *368*, 239. Human recombinant CETP was used in this assay.
11. Ali, A.; Napolitano, J. M.; Deng, Q.; Lu, Z.; Sinclair, P. J.; Taylor, G. E.; Thompson, C. F.; Quraishi, N.; Smith, C. J.; Hunt, J. A.; Dowst, A. A.; Chen, Y.; Li, H. U.S. Patent, US7652049 2010.
12. PK data was generated from wild-type mice.