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# 4-(Alkylthio)- and 4-(arylthio)-benzonitrile derivatives as androgen receptor antagonists for the topical suppression of sebum production

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ABSTRACT

The first examples of thioether-substituted benzonitriles as potential soft-drug androgen receptor antagonists are reported. A number of 4-(alkylthio)- and of 4-(arylthio)-benzonitrile analogs were evaluated in human androgen receptor binding and cellular functional assays. Analogs with potent in vitro binding and cellular activities were evaluated for topical in vivo efficacy in the Golden Syrian hamster ear model. Analogs from both the 4-(alkylthio)- and of 4-(arylthio)-benzonitrile series showed moderate reduction of wax esters in vivo.



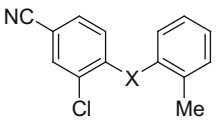
The androgen receptor (AR) is responsible for the activation of genes involved in the pathogenesis of acne and alopecia.[1] The onset of acne vulgaris infections in the pilosebaceous unit is associated with excess sebum production. The production of sebum is regulated by the androgens testosterone and 5α-dihydrotestosterone (5α-DHT). Androgen receptor antagonists, such as cyproterone acetate[2,3] and RU-58841[4] have been shown to suppress sebum production when applied topically.

Mining of data from our AR agonist program identified a number of compounds that bound to the androgen receptor but did not possess agonist activity. A reevaluation showed that some of these were full antagonists (for example diphenyl ethers such as **1**), and we have used them as starting points for our program to identify AR antagonists for the topical suppression of sebum production.[5]

Part of our design strategy to access compounds devoid of systemic antiandrogen effects and suitable for topical delivery centered on introducing metabolic lability into our target compounds. In the current work we investigated replacement of the ether linkage with a thioether as a potential soft-drug approach.[6]

We found that the oxygen linker of diphenyl ether **1** could be replaced with sulfur (**2**) with retention of AR binding (ARB)[7] and

**Table 1**
Human androgen binding and cellular activities for oxygen, sulfur, and sulfoxide-linked analogs[a]




| Compound | X | ARB (nM) | ARCELL (nM) |
|---|---|---|---|
| **1** | O | 64 | 46 |
| **2** | S | 43 | 78 |
| **3** | S=O | >10000 | – |

[a] Values ($IC_{50}$) are given as an average of ⩾2 experiments; –, not tested; **ARB**, human androgen receptor binding assay; **ARCELL**, human androgen receptor cellular functional assay.

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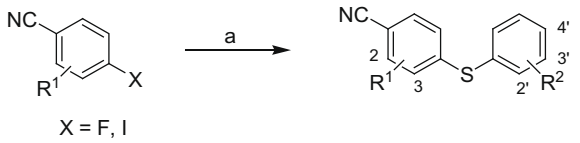



**Scheme 1.** Reagents and conditions: (a) ArSH, $Cs_2CO_3$ (10–90%).

full antagonist activity in a cellular assay (ARCELL)[8] (Table 1). The readily prepared sulfoxide of this (**3**) was inactive in the AR binding assay suggesting that thioether linked compounds could have potential as soft-drugs.[9]

The diphenyl thioethers were synthesized by displacement of fluorine or iodine from the appropriate 4-halobenzonitrile with arylthiols under basic conditions (Scheme 1).[10]

We chose not to synthesize further chlorobenzonitriles since some of them exhibited photoinstability, which is not desirable in a topically applied agent. The 2-trifluoro-methyl-benzonitrile was selected as an alternative, based on known AR binders.[11] Starting materials for 2- or 3-methoxy derivatives were also readily available and gave targets with an alternative metabolic soft-spot, although the chemistry proved not to be as general (the displacement reactions were lower yielding and had more synthetic failures for the electron-rich methoxy templates than the trifluoromethyl template).

We initially explored a series of tolyl ethers based on hit **1** because they presented an additional metabolizable group. Perhaps surprisingly, diphenyl ethers with a 2-methoxy-benzonitrile (**4a–c**) were found to have better AR binding activity than either their 3-methoxy- (**5a–c**) or 2-trifluoromethyl-(**6a–c**) analogs. For a particular benzonitrile, potency of $R^2$ was found to follow *ortho* > *meta* >> *para* (Table 2).

A variety of *ortho*-aryl substitution was then explored (Table 3). In the 2-trifluorobenzonitrile template the unsubstituted aryl analog **7a** had similar potency in the AR binding assay as the *ortho*-tolyl analog **6a**. Replacement of the *ortho*-methyl with fluorine (**8b**) or methoxy (**7c**) was tolerated in terms of AR binding and both these compounds had functional activity less than 100 nM. *Ortho*-disubstituted analogs, such as **7d** and **7e**, did not offer improvement in binding activity, in the case of **7d**, or were less active, in the case of **7e**. By comparison in the 2-methoxybenzonitrile template the *ortho*-methyl (**4a**) *ortho*-methoxy (**8a**), dimethyl (**8b**), and mesityl (**8c**) analogs all possessed good AR binding and functional activity.

Although our previous AR agonist program had found that ethers of benzonitriles with small alkyl groups had no AR activity, we were interested in investigating the alkyl thioether case. Since many of the desired alkyl thiols were not commercially available, we prepared 4-thiobenzonitrile templates by Newman–Kwart rearrangement[12] and alkylated them with commercially available alkyl halides (Scheme 2).[13]

Intriguingly, even small alkyl thioethers such as isopropyl and propyl were active in the binding assay and shown to be full antagonists. By contrast, the direct ether analogs of **10a** and **10c** had ARB $IC_{50}$'s >1000 nM.

In contrast to the diphenyl thioethers, alkyl thioethers from the 2-trifluoromethyl-benzonitrile series (**10a–f**) had similar AR binding activity to those from the 2-methoxy-benzonitrile series (**9a–10f**) (Table 4).

Selected compounds with good in vitro profiles were tested in vivo in Golden Syrian hamsters for their ability to reduce wax and cholesterol esters. Wax and cholesterol esters constitute 28% of total human sebum[14] and it has been shown that there is a direct correlation between reduction in wax esters and reduction in total sebum production in a clinical trial with oral cyproterone acetate.[15] The hamster ear model is a widely used animal model to test drug effects on sebaceous glands (Table 5).[16]

All compounds tested in the hamster ear model showed some activity but all were only moderately active compared to the positive control, RU-58841, which gave a 95% reduction in wax esters.

**Table 2**
SAR of tolyl analogs[a]

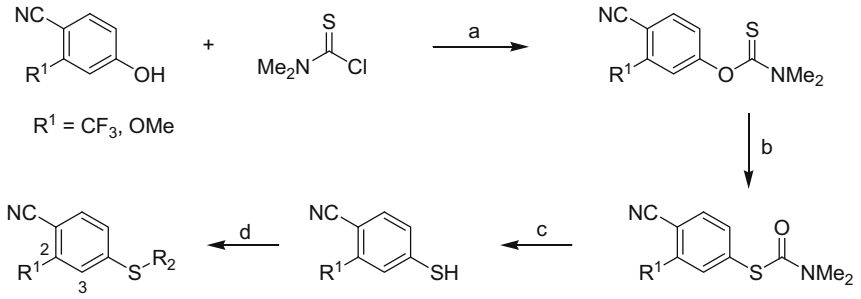
| Compound | $R^1$ | $R^2$ | ARB (nM) | ARCELL (nM) |
|---|---|---|---|---|
| **4a** | 2-OMe | 2′-Me | 24 | 81 |
| **4b** | 2-OMe | 3′-Me | 57 | 197 |
| **4c** | 2-OMe | 4′-Me | 200 | 209 |
| **5a** | 3-OMe | 2′-Me | 115 | 48 |
| **5b** | 3-OMe | 3′-Me | 353 | – |
| **5c** | 3-OMe | 4′-Me | 5210 | – |
| **6a** | 2-$CF_3$ | 2′-Me | 535 | – |
| **6b** | 2-$CF_3$ | 3′-Me | 1680 | – |
| **6c** | 2-$CF_3$ | 4′-Me | 5330 | – |

[a] Values ($IC_{50}$) are given as an average of ⩾2 experiments; –, not tested; ARB, human androgen receptor binding assay; ARCELL, human androgen receptor cellular functional assay.

**Table 3**
SAR of other diphenyl thioethers[a]

| Compound | $R^1$ | $R^2$ | ARB (nM) | ARCELL (nM) |
|---|---|---|---|---|
| **7a** | 2-$CF_3$ | H | 225 | 123 |
| **7b** | 2-$CF_3$ | 2′-F | 184 | 54 |
| **7c** | 2-$CF_3$ | 2′-OMe | 56 | 37 |
| **7d** | 2-$CF_3$ | 2′,6′-diMe | 411 | – |
| **7e** | 2-$CF_3$ | 2′,4′,6′-triMe | 1340 | – |
| **8a** | 2-OMe | 2′-OMe | 20 | 35 |
| **8b** | 2-OMe | 2′,6′-diMe | 43 | 30 |
| **8c** | 2-OMe | 2′,4′,6′-triMe | 39 | 69 |

[a] Values ($IC_{50}$) are given as an average of ⩾2 experiments; –, not tested; ARB, human androgen receptor binding assay; ARCELL, human androgen receptor cellular functional assay.



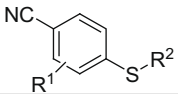

**Scheme 2.** Reagents and conditions:, (a) DABCO, DMF, 65 °C (89–93%); (b) 200–240 °C, neat (92–100%); (c) NaOH, MeOH, thf (63–78%); (d) $R_2Br$, $Cs_2CO_3$, DMF, r.t (10–90%).

**Table 4**
SAR of alkyl thioether analogs[a]

| Compound | $R^1$ | $R^2$ | ARB (nM) | ARCELL (nM) |
|---|---|---|---|---|
| **9a** | 2-OMe | –Pr | 40 | >1000 |
| **9b** | 2-OMe | –Bu | 84 | 66 |
| **9c** | 2-OMe | –$^i$Pr | 29 | 10 |
| **9d** | 2-OMe | –$CH_2{}^c$Pr | 169 | 55 |
| **9e** | 2-OMe | –$CH_2{}^c$Bu | 441 | – |
| **9f** | 2-OMe | –$CH_2CH_2{}^i$Pr | 1730 | – |
| **10a** | 2-$CF_3$ | –Pr | 23 | 11 |
| **10b** | 2-$CF_3$ | –Bu | 49 | 838 |
| **10c** | 2-$CF_3$ | –$^i$Pr | 20 | 89 |
| **10d** | 2-$CF_3$ | –$CH_2{}^c$Pr | 35 | 133 |
| **10e** | 2-$CF_3$ | –$CH_2{}^c$Bu | 63 | 702 |
| **10f** | 2-$CF_3$ | –$CH_2CH_2{}^i$Pr | 519 | – |
| **10g** | 2-$CF_3$ | –$^t$Bu | 315 | – |
| **10h** | 2-$CF_3$ | –$^c$Bu | 194 | 167 |

[a] Values ($IC_{50}$) are given as an average of $\geqslant 2$ experiments; –, not tested; **ARB**, human androgen receptor binding assay; **ARCELL**, human androgen receptor cellular functional assay.

**Table 5**
In vivo efficacy in Golden Syrian hamster model of thioether linked analogs[a]




| Compound | $R^1$ | $R^2$ | % WE reduction | % CE reduction |
|---|---|---|---|---|
| **8b** | 2-OMe | 2,6-diMe-$C_6H_3$ | 66 | 45 |
| **7b** | 2-$CF_3$ | 2-F-$C_6H_4$ | 64 | 50 |
| **9c** | 2-OMe | –$^i$Pr | 59 | 46 |
| **10c** | 2-$CF_3$ | –$^i$Pr | 46 | 40 |
| **10d** | 2-$CF_3$ | –$CH_2{}^i$Pr | 43 | 35 |

[a] All compounds were tested at dose of 1% in a polyethylene glycol/transcutol/ethanol 20/20/60 v/v/v% formulation.

In summary, we have prepared series of 4-(alkylthio)- and 4-(arylthio)-benzonitriles as androgen receptor antagonists with potential as soft-drugs and demonstrated that compounds from these series exhibit moderate reduction in sebum when applied topically in a validated animal model.

## References and notes


1. Redmond, G. P.; Bergfeld, W. F. *Cleveland Clinic J. Med.* **1990**, *57*, 428.
2. Bingham, K. D.; Low, M.; Wyatt, E. H. *Lancet* **1979**, *314*, 304.
3. Gruber, D. M.; Sator, M. O.; Joura, E. A.; Kokoschka, E. M.; Heinza, G.; Huber, J. C. *Arch. Dermatol.* **1998**, *134*, 459.
4. Gao, W.; Bohl, C. E.; Dalton, J. T. *Chem. Rev.* **2005**, *105*, 3352.
5. (a) Hu, L. Y.; Du, D.; Hoffman, J.; Smith, Y.; Fedij, V.; Kostlan, C.; Johnson, T. R.; Huang, Y.; Kesten, S.; Harter, W.; Yue, W. S.; Li, J. J.; Barvian, N.; Mitchell, L. H.; Lei, H. J.; Lefker, B.; Carroll, M.; Dettling, D.; Krieger-Burke, T.; Samas, B.; Yalamanchili, R.; Lapham, K.; Pocalyko, D.; Sliskovic, D.; Ciotti, S.; Stoller, B.; Hena, M. A.; Ding, Q.; Maiti, Samarendra N.; Stier, M.; Welgus, H. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5983; (b) Van Camp, J. A.; Hu, L.-Y.; Kostlan, C.; Lefker, B.; Li, J. J.; Mitchell, L. H.; Wang, Z.; Yue, W.-S.; Carroll, M.; Dettling, D.; Du, D.; Pocalyko, D.; Wade, K. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5529; (c) Hu, L.-Y.; Lei, H. J.; Du, D.; Johnson, T. R.; Fedij, V.; Kostlan, C.; Yue, W.-S.; Lovdahl, M.; Li, J. J.; Carroll, M.; Dettling, D.; Asbill, J.; Fan, C.; Wade, K.; Pocalyko, D.; Lapham, K.; Yalamanchili, R.; Samas, B.; Vrieze, D.; Ciotti, S.; Krieger-Burke, T.; Sliskovic, D.; Welgus, H. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5693.
6. Bodor, N.; Buchwald, P. *Mol. Biotechnol.* **2004**, *26*, 123.
7. The androgen receptor binding assay run was a modification of that described in Liao, S.; White, D.; Schilling, K.; Chang, C. *J. Steroid Biochem.* **1984**, *1*, 11. The human AR cDNA cloned in baculovirus was expressed in Sf9 cells. Cell lysates from transfected Sf9 cells were isolated and used as the source of human AR in the radio–ligand binding assay. Different concentrations of test compounds (10,000, 1000, 200, 40, 8, 1.6, and 0.16 nM) were incubated in the presence of human AR extract, hydroxylapatite, and 1 nM $^3$H-DHT for one hour at 4 °C with gentle rocking. After incubation, plates were placed on a filter apparatus and the reaction mixture was removed under 15 psi vacuum pressure, then washed three times with cold buffer. The plates were then dried at room temperature overnight. The next day, scintillation fluid was added to each well and the plates were counted using a Microbeta Trillux. To determine non-specific binding, 1000-fold concentration of cold DHT was added to each well. Triplicate wells were tested for each concentration. The experimental results were expressed as the concentration of compound at which 50% of maximum binding was inhibited ($IC_{50}$). Compound RU-58841 was run as a standard revealing an inter-run variability within 2-fold.
8. The androgen receptor cellular functional assay was conducted in a human breast tumor cell line expressing androgen receptor (MDA-MB453-MMTV clone 54–19). The cell line is a stably transfected cell line with MDA-MB453 cell background. A MMTV minimal promoter containing Androgen Response Element (ARE) was first cloned in front of a firefly luciferase receptor gene. Then the cascade was cloned into a transfection vector pUV120-puro. Electroporation was used for transfecting MDAMB-453 cells and a puromycin resistant stable cell line was selected. See Chang, C.; Wang, C.; DeLuca, H. F.; Ross, T. F.; Shih, C.C.-Y. *Proc. Natl. Acad. Sci. USA.* **1992**, *89*, 5946.
9. Collection of metabolic stability data failed using our high through-put procedure as these thioether compounds did not give mass ion under the standard conditions. Alternative studies were not conducted due to the moderate level of in vivo efficacy in this series.
10. Generalized procedures for the synthesis of diphenylthioethers from commercially available thiols; *Method A*: a mixture of 4-fluoro-2-trifluoromethyl-benzonitrile (or 4-fluoro-2-methoxy-benzonitrile), the thiol (1.05 equiv), and cesium carbonate (2 equiv) in dimethylformamide was stirred at room temperature or at 50–70 °C for 1 h–2 days or until reaction was complete. The reaction mixture was poured into ice and if the product precipitated out it was collected by filtration. Otherwise the reaction mixture was extracted twice with ethyl acetate, the combined extracts were dried with brine and then magnesium sulfate and concentrated on the rotovap. If necessary the crude product was purified by column chromatography on silica. *Array method A*: a mixture of 4-fluoro-2-trifluoromethyl-benzonitrile, the thiol (1 equiv), and cesium carbonate (1.5 equiv) in acetonitrile was heated at 60 °C overnight. The reaction mixture was then diluted with acetonitrile and an excess of silica supported maleimide was added. The reaction mixture was heated at 40 °C for 2 h and then filtered. The solvent was evaporated. The product purified by HPLC using an XTerra RP18, 30 × 100 mm column eluted with an acetonitrile, water, formic acid solution. *Array method B:* a mixture of 4-iodo-2-methoxy-benzonitrile, the thiol (1 equiv), and cesium carbonate (1.5 equiv) in dimethylformamide was heated at 65 °C for 2 days. The reaction mixture was then diluted with acetonitrile and an excess of silica supported maleimide was added. The reaction mixture was heated at 40 °C for 2 h and then filtered. The solvent was evaporated. The product was purified by HPLC using a Sunfire 19 × 100 mm Prep C18 OBD 5 micron column with an acetonitrile, water, formic acid solution.
11. (a) Koch, H. *Drugs Today* **1984**, *20*, 561; (b) Fradet, Y. *Exp. Rev. Anticancer Ther.* **2004**, *4*, 37.
12. Lloyd–Jones, G. C.; Moseley, J. D.; Renny, J. S. *Synthesis* **2008**, 661.
13. *Synthesis of 4-mercapto-2-trifluoromethyl-benzonitrile*. Step 1. Preparation of dimethyl-thiocarbamic acid O-(4-cyano-3-trifluoromethyl-phenyl) ester: To a 250 mL round bottom flask were added 4-hydroxy-2-trifluoromethyl-benzonitrile (5.0 g, 26.7 mmol), DABCO (7.49 g, 66.8 mmol), and 40 mL DMF. The mixture was stirred until all solids dissolved. Dimethylthiocarbamoyl chloride (4.13 g, 33.4 mmol) was then added, and the mixture was heated at 65 °C for 4 h. the mixture was then cooled to room temperature and quenched into ice water. 1 N HCl was used to adjust the pH to 3. Ethyl acetate was added to the mixture. The layers were separated. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with water and brine, dried (MgSO4), filtered and concentrated in vacuo. The crude product was purified by Biotage Horizon System (10–50% EA/Hexane) to afford a light yellow solid (6.51 g, 88.8%), MS(AP+) = 275.0, $^1$H NMR (400 MHz, *CHLOROFORM-d*) δppm 1.55 (1 H, br s), 3.38 (2 H, s), 3.47 (3 H, s), 7.42 (1 H, dd), 7.53 (1 H, d), 7.87 (1 H, d). Step 2: preparation of dimethyl-thiocarbamic acid S-(4-cyano-3-trifluoromethyl-phenyl) ester: dimethyl-thiocarbamic acid O-(4-cyano-3-trifluoromethyl-phenyl) ester (6.51 g, 23.7 mmol) was heated at 200 °C under nitrogen for 3 hr. On cooling, the product solidified (6.45 g, 92.1%), $^1$H NMR (400 MHz, *CHLOROFORM-d*) δppm 3.10 (6 H, d, *J* = 19.3 Hz), 7.83 (2 H, s), 7.94 (1 H, s). Step 3: Preparation of 4-Mercapto-2-trifluoromethyl-benzonitrile: To a 250 mL round bottom flask were added dimethyl-thiocarbamic acid S-(4-cyano-3-trifluoromethyl-phenyl) ester (6.32 g, 23.0 mmol), 30 mL methanol, and 46 mL 1 M NaOH solution. The mixture was stirred at room temperature. Tetrahydrofuran was added to make all solids into the solution. After 5 h, 1 N HCl solution was added to adjust pH to 4. The mixture was concentrated. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with water and brine, dried (MgSO4), filtered and concentrated in vacuo. The crude product was purified by Biotage Horizon System (0–40% EA/hexane) to afford an off white solid. $^1$H (400 MHz, *CHLOROFORM-d*) δppm 3.86 (1 H, s), 7.51 (1 H, dd, *J* = 8.2, 1.9 Hz), 7.68 (1 H, d, *J* = 8.2 Hz), 7.63 (1 H, d, *J* = 1.6 Hz). MS(AP-) = 202.0; IR 2539 cm$^{-1}$ (S-H). 4-mercapto-2-trifluoromethyl-benzonitrile was made by the same method using 4-hydroxy-2-methoxy-benzonitrile as the starting material. $^1$H NMR (400 MHz, *CHLOROFORM-d*) δppm 3.69 (1 H, s), 3.92 (3 H, s), 6.86 (1 H, d), 6.82 (1 H, d), 7.39 (1 H, d).
14. Thiboutot, D. *J. Invest. Dermatol.* **2004**, *123*, 1.
15. Miller, C. G.; Wojnarowska, F. T.; Dowd, P. M.; Ashton, R. E.; O'Brien, T. J.; Griffiths, W. A.; Jacobs, H. S. *Br. J. Dermatol.* **1986**, *114*, 705.
16. Male Syrian hamsters aged 9–10 weeks were introduced into the laboratory environment and acclimated for 2 weeks prior to use in each study. Each

experimental group consisted of five animals. Vehicle control and a reference AR antagonist (RU-58841) were included in each experiment. Animals were topically dosed twice daily (BID) for 2 weeks, 5 days a week (Monday to Friday). Each dose consisted of 25 μL of vehicle control or formulated test article, which was evenly applied to ~3 $cm^2$ of the ventral surfaces of both the right and left ears. Animals were sacrificed approximately 18–24 h after the final dose. The ears were collected from each animal for sebum analysis. The ear samples were prepared for sebum analysis as follows. One 8 mm distal biopsy punch was taken just above the anatomical "V" mark in the aural cartilage to normalize sample area. The punch was then split into ventral and dorsal layers. The ventral layer, where the topical dose was applied, was retained for sebum analysis. Each ventral side sample was placed in a 1-dram glass vial, flushed with nitrogen gas ($N_2$), sealed, and stored at −80 °C until for sample lipid extraction and HPLC lipid analysis. See Plewig, G.; Luderschmidt, C. *J. invest. Dermatol.* **1977**, *68*, 171.