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Identification of a novel series of tetrahydrodibenzazocines as inhibitors of 17β-hydroxysteroid dehydrogenase type 3

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Abstract—A novel series of 17β-hydroxysteroid dehydrogenase type 3 (17β-HSD3) inhibitors has been identified. These inhibitors, based on a dibenzazocine core, exhibited picomolar to low nanomolar inhibition of 17β-HSD3 in cell-free enzymatic as well as in cell-based transcriptional reporter assays.

Regulation of androgen biosynthesis or its action on the androgen receptor (AR) is central to the management of prostate cancer (PCa).[1] The production of androgens is controlled at two levels: within the central nervous system (CNS), but also locally in peripheral target tissues of hormone action. In these non-gonadal target tissues, the active androgens, testosterone and dihydrotestosterone (DHT), can be synthesized directly through the conversion of inactive precursors. These inactive precursors, dihydroepiandrostenedione (DHEA) and androstenedione (ADT), are present at high levels in the circulation through production by the adrenal gland.[2] It has been estimated that the levels of DHEA and ADT are in great excess (e.g., 100- to 500-fold) relative to that of testosterone, representing a large reservoir of inactive precursor for potential conversion to active hormone within target tissues which are the site of hormone action.[3] The 17β-hydroxysteroid dehydrogenases (17β-HSDs) are a family of short-chain alcohol dehydrogenases which have been shown to mediate the last steps in the conversion of active sex steroids in peripheral target tissues such as the prostate, ovary, and seminal vesicles.[4] Studies analyzing the effects of surgical castration on serum testosterone levels revealed a >90% decrease in serum levels of the hormone, but levels of testosterone within key target tissues such as the prostate were only decreased by ~50%.[5] Such observations indicate that significant locally active hormone synthesis occurs in the absence of a gonadal source and this local tissue synthesis of active hormone could also contribute to the pathobiology of prostate cancer progression. 17β-HSD3 is a candidate enzyme for mediating this non-gonadal production of active hormone and has been characterized for its ability to mediate the conversion of androstenedione to testosterone.[6] 17β-HSD3 is expressed at high levels in the testis and seminal vesicles but has also been shown to be present in prostate tissue, suggesting its potential involvement in both gonadal and non-gonadal testosterone biosyntheses.[7,8] The role of 17β-HSD3 in testosterone biosynthesis makes this enzyme an attractive molecular target for the identification of small molecule inhibitors for the treatment of prostate cancer.

Initial lead identification was accomplished through high-throughput screening of a compound library (~200,000 compounds) using an enzymatic scintillation proximity assay (SPA) measuring the conversion of [^{3}H]ADT to

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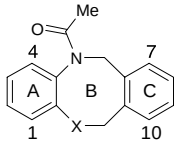
[^{3}H]testosterone. Cellular effects were evaluated using a secreted alkaline phosphatase reporter assay (SEAP), as previously described.[8] The dibenzothiazocine **1**[9] represented an attractive starting point for medicinal chemistry, based on its potency in the biochemical and cell-based assays. A brief SAR generated from the high-throughput screen surrounding this chemotype is described in Table 1. While both sulfur and carbon were tolerated at the 12-position (**1** and **2**), substitution with oxygen or nitrogen led to a substantially reduced potency in the in vitro assays.

Our initial focus, therefore, centered around the dibenzothiazocine (DBT, **1**) and tetrahydrodibenzazocine (THB, **2**) series. Compounds in the DBT and THB series were synthesized by procedures outlined in Schemes 1 and 2, respectively.

The C-ring portion of the dibenzothiazocines **12** was prepared from appropriately substituted 2-methyl benzoates, which were subjected to benzylic bromination by treatment with NBS in dichloromethane to afford the corresponding bromides **6**. The A-ring component was prepared from commercially available 2-aminobenzothiazoles **7**. Treatment with KOH in *i*PrOH at reflux afforded the dithianes **8** in quantitative yield. Reduction of **8** with dithiothreitol (DTT) in DMF and subsequent reaction with the benzyl bromide **6** afforded the thioether **9** in one pot with fair to excellent yields. The ester **9** was reduced to the corresponding alcohol **10** with lithium borohydride. Treatment of the alcohol with triphenylphosphine dibromide generated the corresponding bromide that underwent an intramolecular cyclization to provide the dibenzoazocines **11** in moderate yield. N-acylation with acetic anhydride in refluxing toluene yielded the desired dibenzothiazocines **12** in good yield.

Table 1. Novel 17β-HSD3 inhibitors identified from high-throughput screen[a]

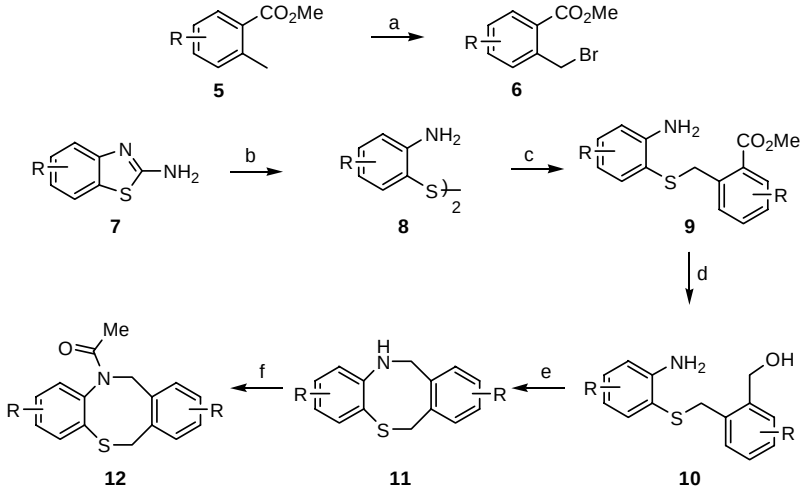


Compound	X	IC_{50}[b] (μM)	
		Enzyme	Cellular
1	S	0.40	0.32
2	CH_2	0.09	0.22
3	O	2.97	NA
4	NH	4.74	>10

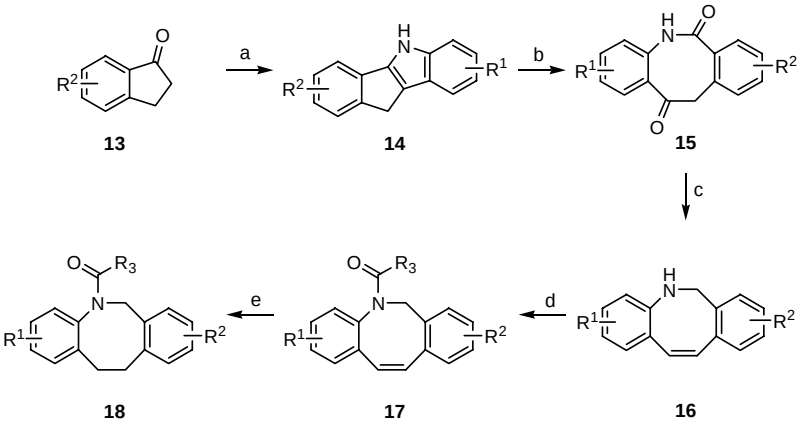
[a] IC_{50} values are representative of multiple determinations (N = 2–3).
[b] See Ref. 8 for assay conditions.

The synthesis of the dibenzazocines followed the route of Jung and is shown in Scheme 2.[10] Readily available indanones **13** were condensed with arylhydrazines under acid catalysis to form the corresponding hydrazones. The intermediate hydrazones underwent indole cyclization in refluxing toluene to afford **14** in moderate yield. Treatment of **14** with an oxidant such as *m*CPBA or ozone led to ring opening and resulted in keto-amides **15** with a central 8-membered ring. Concurrent reduction of the amide and ketone functionalities with $BH_3{\cdot}SMe_2$ afforded a C-12 benzyl alcohol intermediate which readily eliminated water under acidic conditions to provide the dihydrodibenzazocines **16**. The desired *N*-acyl dihydrodibenzazocines (DHB) **17** were prepared by treatment of **16** with the corresponding acid chlorides. The olefin could be reduced under standard Pd/C conditions; however, 5% Rh/C was preferred as the hydrogenation catalyst since it selectively reduced the olefin in the presence of other sensitive functional groups, such as a halogen.

Structure–activity relationships in the DBT and THB series are presented in Table 2. Within the DBT series, incorporation of a halogen at either the 2- or 3-position (**12a**–**12d**) resulted in a 4- to 10-fold improvement in potency in the biochemical as well as cell-based assays.

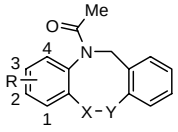


Scheme 1. Reagents and conditions: (a) NBS, $(C_6H_5CO)O_2$, CH_2Cl_2, 54–75%; (b) KOH, reflux, 100%; (c) (1) DTT, DMF; (2) **6**, DMF, rt, 20–100%; (d) $LiBH_4$, THF, reflux, 60-80%; (e) $PPh_3{\cdot}Br_2$, imidazole, CH_2Cl_2, 50 °C, 33–85%; (f) Ac_2O, DMAP, toluene, reflux, 80–100%.



Scheme 2. Reagents and conditions: (a) (1) arylhydrazine, H_2SO_4, EtOH, reflux; (2) AcOH, H_2SO_4, reflux, 65–80%; (b) *m*CPBA, CH_2Cl_2, 50–70%; (c) (1) $BH_3{\cdot}Me_2S$; (2) HCl, EtOH, reflux, 60–85%; (d) $R_3C(O)Cl$, CH_2Cl_2, 90–100%; (e) 5% Rh/C, H_2, MeOH, 50–80%.

Table 2. A-ring SAR[a]



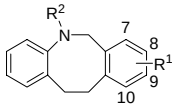
Compound	X–Y	R	IC_{50}[b] (μM)	
			Enzyme	Cellular
12a	S-CH_2	2-Cl	0.10	0.16
12b	S-CH_2	3-Cl	0.03	0.17
12c	S-CH_2	2-F	0.09	0.29
12d	S-CH_2	2-Br	0.25	0.12
12e	S-CH_2	2,3-diCl	0.01	0.13
12f	S-CH_2	4-Cl	11.5	NA
12g	S-CH_2	1,2-diCl	0.76	1.3
12h	S-CH_2	3-CF_3	2.81	NA
12i	S-CH_2	2-OMe	2.06	>10
12j	S-CH_2	2-OH	4.86	NA
12k	S-CH_2	2-SO_2Me	>10	NA
12l	S-CH_2	2-Me	1.0	0.87
17a	CH=CH	2-Cl	0.24	0.50
18a	CH_2-CH_2	2-Cl	0.07	0.63
18b	CH_2-CH_2	3-Cl	0.05	0.13

[a] IC_{50} values are representative of multiple determinations (N = 2–3).
[b] See Ref. 8 for assay conditions.

Chlorine was the optimum substitution, as activity decreased with both fluorine and bromine. The 2,3-dichloro analog **12e** was the most potent in the enzymatic assay; however, this did not result in a commensurate increase in potency in the cell-based assay. Halogen substitution was not tolerated at the 1- or 4-position (**12f**–**12g**), suggesting that the enzyme makes close contacts with these regions of the DBT nucleus. Indeed, bulkier substitutions on the A-ring (e.g., **12h**–**12l**) were not well tolerated. The drop in activity of the 2-hydroxyl (**12j**) and the 2-methyl (**12l**) analogs highlights the electronic requirements for A-ring substitution. Similar halogen substitution within the DHB (**17a**) and THB (**18a**–**18b**) series, while tolerated, did not improve biochemical or cellular activity. Overall, analogs prepared within the DHB series were several fold less active than their THB counterparts. Although good activity was observed in the DBT series, the aryl sulfur atom in these compounds represents a potential metabolic 'soft spot'. Therefore, the carbocyclic THB series was pursued for further development of SAR.

While halogen substitution of the A-ring in the carbocyclic systems did not improve activity, bromine substitution on the C-ring at either the 8- (**18c**) or 9-position (**18d**) dramatically improved both biochemical and cellular activity (Table 3). The acetamide functionality at the 5-position of the THB represented an optimal substitution for 17β-HSD3-inhibitory potency. Reduction of the carbonyl (**18f**) or replacement of the methyl with an amino group (**18e**) resulted in a precipitous drop in enzymatic activity. Even a small increase in size (**18g**) was not tolerated. Other substitutions at the 8- or 9-positions were also explored. A primary amine (**18h**) or a primary carboxamide (**18i**) resulted in a loss of activity; however, activity could be recovered by incorporating more hydrophobic character. For example, benzylamine **18j** retained activity relative to **18c**. Similarly, the more lipophilic phenyl amide (**18k**) and

Table 3. B and C-ring SAR[a]



Compound	R^1	R^2	IC_{50}[b] (μM)	
			Enzyme	Cellular
18c	8-Br	C(O)Me	0.009	0.032
18d	9-Br	C(O)Me	0.007	0.036
18e	9-Br	C(O)NH_2	0.28	0.93
18f	9-Br	Et	>1.0	2.2
18g	9-Br	C(O)c-Prop	0.32	1.75
18h	9-NH_2	C(O)Me	0.49	0.49
18i	9-C(O)NH_2	C(O)Me	>1.0	2.98
18j	9-NHBn	C(O)Me	0.008	0.004
18k	9-NHC(O)Ph	C(O)Me	0.16	0.26
18l	C(O)NH*n*Bu	C(O)Me	0.035	0.052
18m	8-Ph	C(O)Me	0.00002	0.004

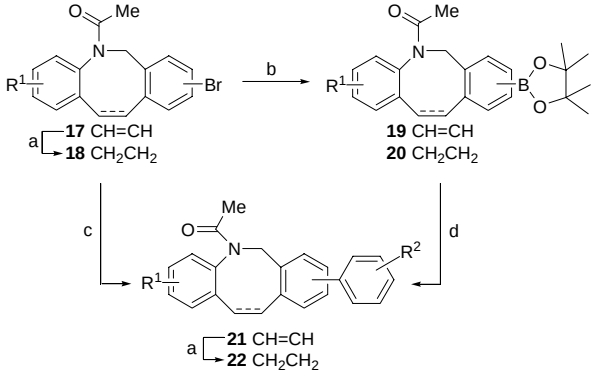
[a] IC_{50} values are representative of multiple determinations (N = 2–3).
[b] See Ref. 8 for assay conditions.

butyl amide (**18l**) exhibited an improved potency versus the primary amide. Taken together, these data suggest that this region of the molecule extends into a hydrophobic pocket. This hypothesis was further substantiated by the observation that the introduction of a phenyl group at C-8 (**18m**) resulted in a significant improvement in potency in the biochemical as well as the cell-based assays.

Further exploration of SAR at the 8- and 9-positions was facilitated by the use of the Suzuki reaction for the rapid preparation of analogs as described in Scheme 3.

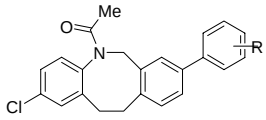
Either bromo-substituted DHB (**17**) or THB (**18**) could be directly coupled with an appropriately substituted arylboronic acid to afford the corresponding coupled products **21** or **22**, respectively. Alternatively, given the greater variety of aryl halides than boronic acids, **17** and **18** could be converted to the corresponding aryl boronate ester **19** or **20** and then subjected to Suzuki coupling to afford **21** or **22**. At any point, conversion of the DHB to the corresponding THB analog could be accomplished by hydrogenation of the B-ring olefin catalyzed by 5% Rh/C.

The SAR developed for the 8-aryl-substituted THB series is described in Table 4. Introduction of electron-donating groups around the aryl ring was well tolerated in both the enzyme and cellular assays. A preference was observed for *ortho*-substitution, particularly in the cellular assay (compare **22a** with **22b** and **22c**). Electron-withdrawing groups were also well tolerated in the enzyme assay (**22d**–**22f**) although there was an overall decrease in cellular activity compared to electron-donating substituents. The same preference for substitution at the *ortho*-position continued for sulfonamides **22g** and **22h**, with **22g** being 100-fold more potent. Introduction of basic functionality as in benzyl amine **22i** or basic heterocycles such as pyridines **22j** or **22k** generally resulted in reduced activity. Interestingly, when a carbonyl group was incorporated at the *ortho*-position, a dramatic increase in potency was observed.



Scheme 3. Reagents and conditions: (a) 5% Rh/C, H_2, MeOH, 80%; (b) $PdCl_2$(dppf), dppf, KOAc, bis(pinacolato)borane, 80%; (c) arylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, 50–100%; (d) arylbromide, $Pd(PPh_3)_4$, Na_2CO_3, 30–70%.

Table 4. SAR surrounding C-Ring 8-position[a]



Compound	R	IC_{50}[b] (μM)	
		Enzyme	Cellular
18o	H	0.004	0.018
22a	*o*-OMe	0.002	0.005
22b	*m*-OMe	0.008	0.053
22c	*p*-OMe	0.003	0.048
22d	*o*-CN	0.005	0.062
22e	*m*-CN	0.003	0.015
22f	*p*-CN	0.003	0.019
22g	*o*-$NHSO_2Me$	0.002	0.009
22h	*m*-$NHSO_2Me$	0.14	0.19
22i	*m*-CH_2NH_2	0.29	0.18
22j	3′-pyridyl	0.04	0.02
22k	4′-pyridyl	0.04	0.04
22l	*o*-CO_2Me	0.00002	0.0005
22m	*m*-CO_2Me	0.0014	0.002
22n	*o*-C(O)Me	0.00002	0.0005
22o	*o*-CH_2OH	0.0009	0.002
22p	*o*-CH_2NH_2	0.239	0.182
22q	*o*-C(O)NHMe	0.025	0.025
21a (DHB)	*o*-CO_2Me	0.0007	0.001

[a] IC_{50} values are representative of multiple determinations ($N = 2$–3).
[b] See Ref. 8 for assay conditions.

Methyl ester **22l** showed picomolar activity, more than 100-fold greater than that of the corresponding *meta*-substituted analog (**22m**). The methyl ketone **22n** was equipotent, as were trifluoromethyl and aldehyde analogs (data not shown); however, the benzyl alcohol **22o** was less active by an order of magnitude, indicating a possible requirement for a strong hydrogen bond acceptor at this position. This was further substantiated by the loss in activity upon incorporation of an aminomethyl group at this position (**22p**). Replacement of the ester with a methyl amide isostere resulted in a 1000-fold loss in activity, indicating a subtle stereoelectronic requirement (**22q**).

In keeping with the increase in activity seen within the THB series when incorporating the ester group, the DHB series also showed marked improvement in both enzymatic and cellular activity. While **21a** lost an order of magnitude relative to **22l**, activity remained in the low or sub-nanomolar range. This unsaturated series historically showed improved metabolic stability relative to the THB series. For example, incubation of **22l** and **21a** in human liver microsomes at 10 μM for 10 min provided metabolic rates of 0.9 and 0.4 nmol/min/mg, respectively. The improvement in metabolic stability bodes well for improved drug exposure in vivo.

In order to rationalize the favorable impact of the carbonyl group of **22l** on the biochemical potency, a homology model of human 17β-hydroxysteroid-dehydrogenase type 3 (17β-HSD-3) was built from the crystal structure of 17β-HSD-1 complexed with estradiol and NADP+[11] using Schrodinger's Prime software.[12] A

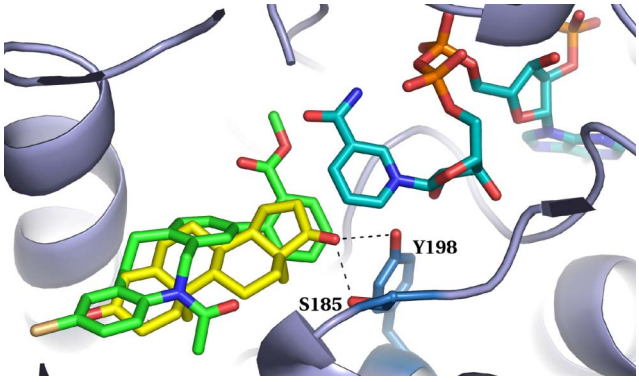


Figure 1. Model showing Δ4-dione (carbon atoms in yellow), **22l** (carbon atoms in green), and NADP+ (carbon atoms in cyan) in the homology model of 17β-HSD-3 (blue ribbon representation). Potential hydrogen bonds of Δ4-dione to S185 and Y198 are shown with dotted lines.

model of 4-androstene-3,17-dione (Δ4-dione) was manually placed in the 17β-HSD-3 model in the corresponding binding pocket of estradiol. The ligand and residues of 17β-HSD-3 were allowed to relax during 1000 cycles of conjugate gradient energy minimization with the CFF force field.[13] It was observed in the model that the carbonyl oxygen at C-17 of Δ4-dione is within hydrogen bonding of the conserved residues S185 and Y198, interactions that most likely assist in the reduction of the carbonyl. In addition, the C-17 carbon is within 4 Å of the carbon bearing the transferring hydride of the co-factor NADP+. A low-energy conformation of **22l** could be manually docked into the homology model in a number of ways guided by the shape similarity between the inhibitor and Δ4-dione, mainly the overlap of the corresponding hydrocarbon skeletons. Figure 1 shows one possible binding mode in which the amide methyl group occupies the pocket normally bound by the C-19 methyl group of Δ4-dione. Support for this orientation is derived from the very narrow SAR at this position, as only a specifically positioned methyl group was tolerated. The electronegative Cl atom occupies the same area as does the C-3 carbonyl oxygen of Δ4-dione. The methyl benzoate group of **22l** is positioned near the nicotinamide ring suggesting an aromatic ring–ring type of interaction. In a second orientation (not pictured), rotation of the methyl benzoate group relative to the core reveals another possible binding mode which would place the ester carbonyl within hydrogen bonding distance of S185 and Y198 and the ester methyl group in the Δ4-dione C-18 pocket. Further refinements of this model will have to await future crystallographic and kinetic data.

The search for novel non-steroidal inhibitors of 17β-HSD3 has led to the identification of a potent series of compounds based on the dibenzazocine scaffold. These inhibitors demonstrated low nanomolar to picomolar activity in enzymatic as well as cellular assays. Compounds targeting 17β-HSD3[14] may provide useful tools for examining the role of the enzyme in both endocrine-sensitive and -resistant prostate tumor models and may provide an alternative approach for the disruption of testosterone biosynthesis in the treatment of prostate cancer.

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