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Novel Steroid Inhibitors of Glucose 6-Phosphate Dehydrogenase

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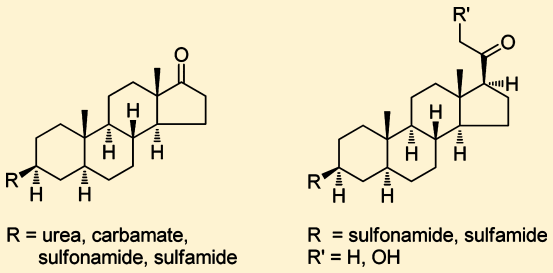
Supporting Information

ABSTRACT: Novel derivatives of the steroid DHEA **1**, a known uncompetitive inhibitor of G6PD, were designed, synthesized, and tested for their ability to inhibit this dehydrogenase enzyme. Several compounds with approximately 10-fold improved potency in an enzyme assay were identified, and this improved activity translated to efficacy in a cellular assay. The SAR for steroid inhibition of G6PD has been substantially developed; the 3β-alcohol can be replaced with 3β-H-bond donors such as sulfamide, sulfonamide, urea, and carbamate. Improved potency was achieved by replacing the androstane nucleus with a pregnane nucleus, provided a ketone at C-20 is present. For pregnan-20-ones incorporation of a 21-hydroxyl group is often beneficial. The novel compounds generally have good physicochemical properties and satisfactory in vitro DMPK parameters. These derivatives may be useful for examining the role of G6PD inhibition in cells and will assist the future design of more potent steroid inhibitors with potential therapeutic utility.



INTRODUCTION

In recent years there has been a resurgence of interest in the role of metabolism in the development and maintenance of cancer. Over 80 years ago Warburg discovered that tumor cells prefer to use glycolysis as a source of adenosine triphosphate (ATP) even when the oxygen levels present would allow oxidative phosphorylation in the mitochondrial tricarboxylic acid cycle (TCA) to be used.[1] This aerobic glycolysis, although rapid, generates only two molecules of ATP compared to 32 molecules from the TCA cycle. To redress the energetic balance, tumor cells can increase the flux of glucose into the cell. This increased rate of glucose entering tumor cells has enabled highly glycolytic tumors to be assessed noninvasively in the clinic using fluorescent glucose analogues.[2]

It has since become clear that there are many metabolic reactions that become altered in tumor cells not only to allow a rapid production of ATP but also to feed the requirement for new lipids and nucleotides. This enhanced supply of metabolites allows tumors to both continue their unrestricted proliferation and adapt to unfamiliar environments.

A key pathway involved in metabolite production is the pentose phosphate pathway (PPP) (Figure 1). The PPP contains an initial oxidative arm that diverges from glycolysis at the point of glucose 6-phosphate (G6P). This substrate is used to generate a supply of reduced nicotinamide adenine dinucleotide phosphate (NADPH) and ribulose 5-phosphate (Ru5-P). NADPH is a cofactor for many enzymes that are used in macromolecular biosynthesis, while Ru5-P is used for nucleotide synthesis.

The rate-limiting enzyme for the PPP is G6PD, which catalyzes the conversion of G6P to 6-phosphogluconolactone (6PGL) and results in the concomitant production of NADPH. In addition to its role in synthesis of new molecules, NADPH is used in the protection of cells against reactive oxygen species (ROS) which are generated in rapidly proliferating cells and can

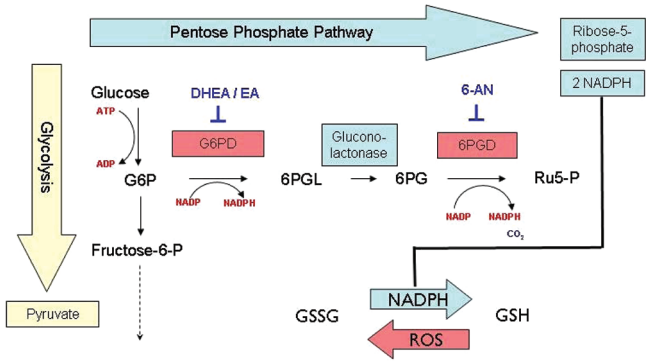


Figure 1. PPP. Oxidative metabolism of glucose diverges from the glycolysis pathway to pyruvate at G6P. Generation of NADPH maintains glutathione (GSH) in its reduced state. GSH is oxidized to GSSG with ROS. Production of Ru5-P and subsequent isomerization to ribose 5-phosphate enable nucleotide synthesis. As glucose 6-phosphate dehydrogenase (G6PD) is rate-limiting for 6-phosphogluconate (6PG) production, inhibition of G6PD with steroids such as DHEA and EA will decrease the flux through the PPP and decrease the accumulation of 6PG. Metabolism of 6PG by 6-phosphogluconate dehydrogenase (6PGD) is blocked with the inhibitor 6-aminonicotinamide (6-AN).

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damage many macromolecules. ROS are dealt with by an antioxidant defense that uses glutathione and thioredoxin, both of which rely on NADPH to maintain their activity.

The importance of G6PD in cancer has only become appreciated in the past decade with the development of mouse models and tumor cell lines that either carry mutations or are null for G6PD. Cell lines that overexpress G6PD are able to form tumors in nude mice, and these cells have an altered morphology.[3] In contrast, tumor cells lacking G6PD grow more slowly and display enhanced apoptosis.[4,5] Furthermore, Chinese hamster ovary cells that are devoid of G6PD display increased sensitivity to radiation[6] and human fibroblasts deficient in G6PD display increased ROS and radiation sensitivity.[7] Clinically, there is some evidence from studies in larynx and gastric cancer that tumors increase G6PD activity,[8,9] and this correlates to a poor clinical outcome.[9] These data prompted us to initiate a program to develop a potent inhibitor of G6PD. It is envisaged that such an inhibitor might reduce the ability of a cell to manage ROS and when combined with radiotherapy result in increased tumor cell death.[10]

Currently, there are no drugs that selectively inhibit G6PD, although a few small molecules with micromolar activity have been reported.[11,12] It has been known since 1960 that steroids including dehydroepiandrosterone **1** (DHEA) and epiandrosterone **2** (EA) uncompetitively inhibit enzyme function.[13] Following this discovery several publications describing the SAR of DHEA and other steroids appeared,[14] and more recently there have been some attempts to improve activity through synthetic modification[15−21] and analysis of electrostatic potential maps.[22] Although a 16α-bromo substituent increased the G6PD inhibitory activity of DHEA **1** and EA **2**,[15,20,21] there remains considerable scope for further improvement.

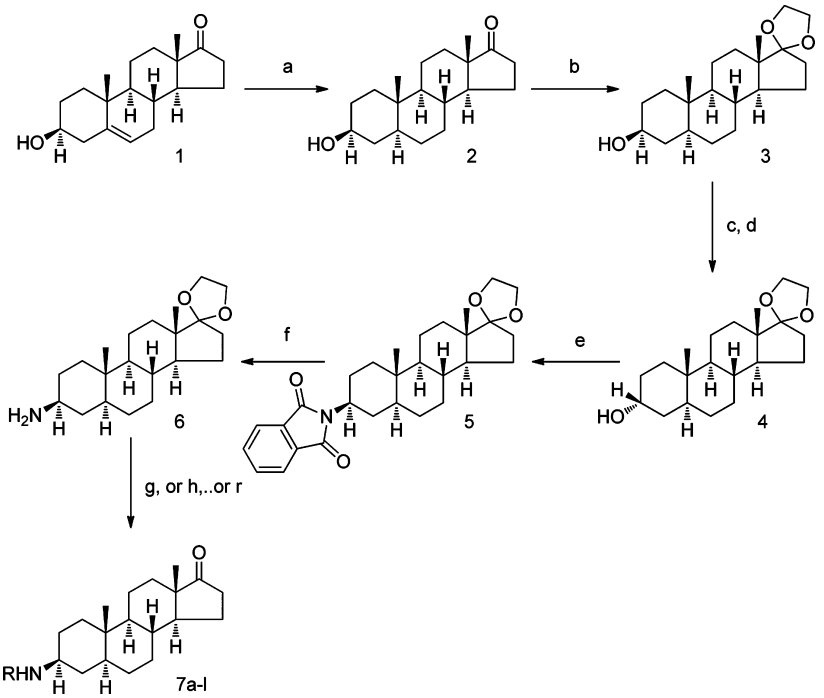
DHEA is an abundant steroid in human circulating blood and exhibits chemopreventive and antiproliferative effects that have been attributed to its inhibitory G6PD activity,[23−25] although other mechanisms associated with DHEA or its metabolites have also been invoked to explain these effects.[26−28] These studies highlight the potential for off-target enzyme activity when using DHEA and the need to identify a more potent and selective G6PD inhibitor that is less metabolically labile.

In this study we aimed to identify a novel and potent steroid inhibitor of G6PD that would be more amenable than DHEA for examining the role of G6PD modulation in cells. A more potent and selective compound would help establish whether inhibition of G6PD does indeed enhance tumor cell sensitivity to oxidative stress and whether such an inhibitor may also find therapeutic utility as a treatment for cancer.

CHEMISTRY

The synthesis of target compounds is summarized in Schemes 1−8. Scheme 1 illustrates the synthesis of novel androstane derivatives bearing a 3β-amino substituent. Hydrogenation of DHEA **1** over palladium/carbon cleanly afforded the desired 5α-isomer EA **2** in good yield. Following protection of the 17-ketone as the cyclic ketal **3**, the 3β-alcohol was inverted via the benzoate ester using benzoic acid, diisopropyl

Scheme 1[a]



[a]Reagents: (a) H_2, Pd/C, EtOH (96%); (b) ethylene glycol, PTSA, $HC(OMe)_3$, CH_2Cl_2 (84%); (c) benzoic acid, DIAD, TTP, THF (81%); (d) NaOMe/MeOH, DCM (72%); (e) phthalimide, TPP, DIAD, THF (68%); (f) hydrazine hydrate, EtOH, reflux (97%); (g) for **7a**, 2 M HCl, THF, acetone (89%); (h) for **7b**, AcCl, Et_3N, DCM, then 2 M HCl, THF, acetone (80%); (i) for **7c**, (4-nitrophenyl)-*N*-benzylcarbamate, Et_3N, DCM, then 2 M HCl, acetone and then H_2, Pd/C, AcOH (22%); (j) for **7d**, (4-nitrophenyl)-*N*-methylcarbamate, Et_3N, DCM, then HCl, acetone (53%); (k) for **7e**, EtNCO, DCM, then 2 M HCl, THF, acetone (77%); (l) for **7f**, *N*-butyl isocyanate, DCM, then 2 M HCl, THF, acetone (58%); (m) for **7g**, Me_2NCOCl, Et_3N, DCM, then 2 M HCl, THF, acetone (75%); (n) for **7h**, MeOCOCl, Et_3N, DCM, then 2 M HCl, THF, acetone, DCM (80%); (o) for **7i**, $MeSO_2Cl$, Et_3N, DCM, then aq $MeSO_3H$, MeOH (46%); (p) for **7j**, $EtSO_2Cl$, Et_3N, DCM, then 2 M HCl, THF, acetone (75%); (q) for **7k**, $PhSO_2Cl$, Et_3N, DCM, then 2 M HCl, THF, acetone (58%); (r) for **7l**, sulfamide, dioxane, reflux, then aq $MeSO_3H$, MeOH (62%).

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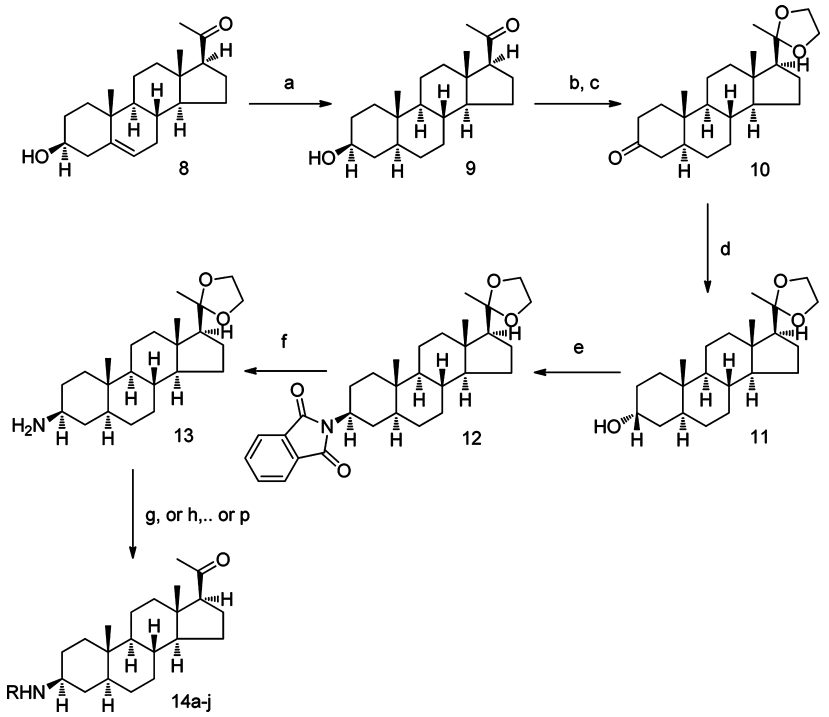
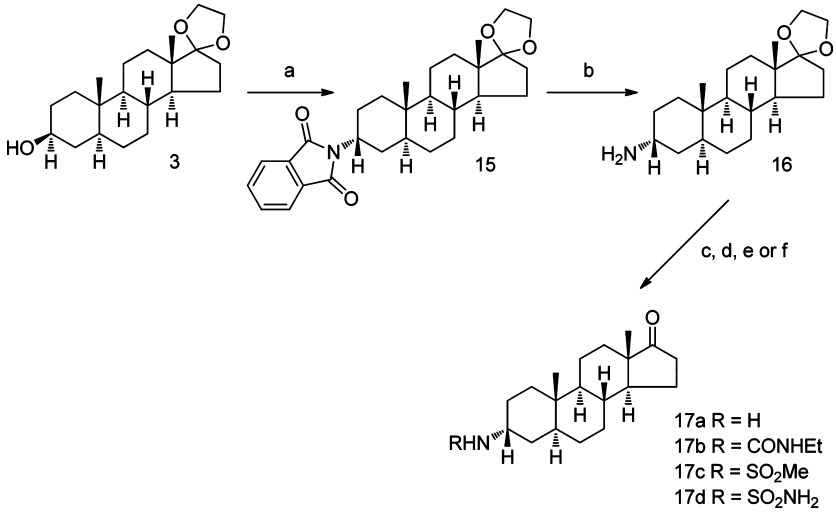
Scheme 2[a]



[a]Reagents: (a) H_2, Pd/C, EtOH (97%); (b) ethylene glycol, PTSA, $HC(OMe)_3$, DCM (100%); (c) Dess–Martin periodinane, DCM (45%); (d) K-Selectride, THF, −78 °C (91%); (e) phthalimide, TPP, DIAD, THF (70%); (f) hydrazine hydrate, EtOH, reflux (100%); (g) for **14a**, aq $MeSO_3H$, MeOH, DCM (22%); (h) for **14b**, AcCl, Et_3N, DCM, then aq $MeSO_3H$, MeOH (89%); (i) for **14c**, (4-nitrophenyl)-*N*-benzylcarbamate, Et_3N, DCM, then 2 M HCl, acetone and then H_2, Pd/C, AcOH (14%); (j) for **14d**, Me_2NCOCl, Et_3N, DCM, then aq $MeSO_3H$, MeOH (85%); (k) for **14e**, MeOCOCl, Et_3N, DCM, then 2 M HCl, THF, acetone, DCM (50%); (l) for **14f**, $MeSO_2Cl$, Et_3N, DCM, then 2 M HCl, THF, acetone, DCM (60%); (m) for **14g**, $EtSO_2Cl$, Et_3N, DCM, then 2 M HCl, THF, acetone, DCM (63%); (n) for **14h**, $PhSO_2Cl$, Et_3N, DCM, then 2 M HCl, THF, acetone, DCM (86%); (o) for **14i**, sulfamide, dioxane, reflux, then aq $MeSO_3H$, MeOH (39%); (p) for **14j**, $Me_2NSO_2NH_2$, DIPEA, DCM, then aq $MeSO_3H$, MeOH (15%).

Scheme 3[a]



[a]Reagents: (a) phthalimide, TPP, DIAD, THF (74%); (b) hydrazine hydrate, EtOH, reflux (95%); (c) for **17a**, 2 M HCl, THF, acetone (87%); (d) for **17b**, EtNCO, DCM, then 2 M HCl, THF, acetone, DCM (37%); (e) for **17c**, $MeSO_2Cl$, Et_3N, DCM, then 2 M HCl, THF, acetone, DCM (73%); (f) for **17d**, sulfamide, dioxane, reflux, then 2 M HCl, THF, acetone (50%).

azodicarboxylate (DIAD), and triphenylphosphine (TPP) in THF, and the ester was hydrolyzed with sodium methoxide in methanol to give the 3α-ol **4**. This alcohol was converted to the 3β-phthalimide **5** using phthalimide, DIAD, and TPP in THF and then converted in good yield to the 3β-amine **6** through treatment with hydrazine hydrate in ethanol. The cyclic ketal of amine **6** was cleaved with acid to yield the desired aminoketone **7a**. This method of inversion of the stereochemistry from the 3α-ol **4** represented a substantial advantage over other literature methods as the 3β-amine **7a** was isolated as a single

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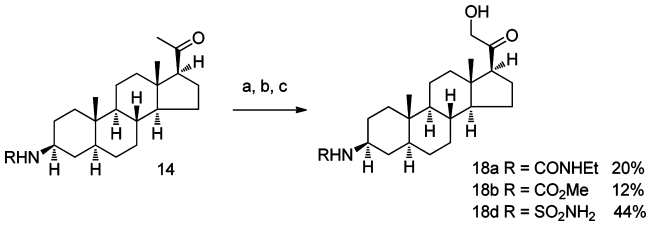
diastereoisomer in good yield without any need for chromatographic purification of the intermediates.[29] The amine **6** was also converted to a range of target compounds **7b**–**l** that included an amide **7b**, ureas **7c**–**g**, carbamate **7h**, sulfonamides **7i**–**k**, and sulfamide **7l**.

Scheme 2 shows the synthesis of novel pregnane derivatives with a 3β-amino substituent. Similar chemistry as described for the above androstanes was employed to convert pregnenolone **8** to a range of target compounds **14**, although initially the inversion of the 3β-alcohol **9** was effected via oxidation with Dess–Martin periodinane, followed by reduction of the 3-ketone **10** with K-Selectride in THF. By use of standard conditions, the amine **13** was converted to the target compounds **14a**–**j** that included an amine **14a**, amide **14b**, ureas **14c**,**d**, carbamate **14e**, sulfonamides **14f**–**h**, and sulfamides **14i**,**j**.

The phthalimide-based Mitsunobu inversion chemistry described above was used to prepare additional target androstanes with a 3α-substituted amine (Scheme 3). Thus, the 3β-alcohol **3** afforded the 3α-amine **16** in two steps, and the ketal was cleaved to give amine **17a**. The 3α-amine **16** was also functionalized using ethyl isocyanate, methanesulfonyl chloride, and sulfamide and following ketal deprotection afforded urea **17b**, sulfonamide **17c**, and sulfamide **17d**, respectively.

Two routes were employed to convert pregnan-20-ones to 21-hydroxypregnan-20-ones. Oxidation of the silyl enol ether of ketone **14** and treatment with acid gave the desired urea **18a**, carbamate **18b**, and sulfamide **18d** (Scheme 4). Alternatively

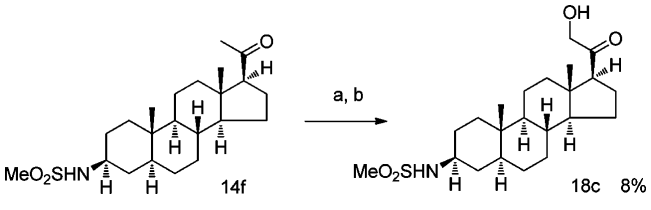
Scheme 4[a]



[a]Reagents: (a) *tert*-butyldimethyl trifluoromethanesulfonate, triethylamine, DCM; (b) *m*-CPBA, −10 °C; (c) 5:1 THF/2 M HCl.

bromination of ketone **14f** at the 21-position in low yield using bromine in acidified methanol and then rapid treatment with aqueous sodium hydroxide in DMF gave sulfonamide **18c** (Scheme 5). This latter route was less successful, as purification of the 21-bromo intermediate was protracted.

Scheme 5[a]



[a]Reagents: (a) Br_2, HBr, MeOH (13%); (b) NaOH, aq DMF (59%).

Three additional novel compounds bearing a 3β-alcohol were also synthesized (Schemes 6–8). For synthesis of the homologated ketone **23** (Scheme 6), the 3β-alcohol of epiandrosterone **2** was first protected as its TBDMS ether and the 17-ketone then reacted with triethyl phosphonoacetate and sodium ethoxide to give the known α,β-unsaturated ester **19**. Hydrogenation over palladium/carbon gave the 17β-alkyl ester **20** which was hydrolyzed to the acid **21** using aqueous lithium hydroxide and then converted to the Weinreb amide **22**. Reaction of amide **22** with methylmagnesium bromide in THF and then acidic deprotection of the TBDMS ether afforded the desired ketone **23**.

The oxetane **25** (Scheme 7) was prepared in low yield by heating the known epoxide **24** with trimethylsulfoxonium iodide and potassium *tert*-butoxide in *tert*-butanol at reflux, while the methyl ether **26** (Scheme 8) was obtained by reacting 3-hydroxypregnan-20-one **9** with copper(II) bromide in methanol at reflux.

BIOLOGY

The enzyme G6PD converts its substrate, G6P, to 6PGL with the concomitant reductive conversion of the cofactor $NADP^+$ to NADPH. Rather than measurement of the intrinsic fluorescence of NADPH, it was decided to quantify its production via an Amplite fluorimetric NADPH assay kit through enzymatic coupling and reductive conversion of a nonfluorescent NADPH sensor substrate to a product detectable by excitation/emission at 540/590 nm. The addition of the coupled Amplite step and subsequent measurement of the assay signal in the red visible range significantly reduces the potential for compound interference effects in comparison to directly measuring the intrinsic fluorescence of NADPH. This method generated a robust assay with $Z' > 0.7$. This G6PD in vitro enzyme assay was used to test the inhibitory activity of the target compounds, and the results are reported in Tables 1 and 2.

Following screening in the enzyme assay, the most active compounds were then tested in a cell assay. Measurement of flux through the oxidative PPP in living cells has previously been achieved by using glucose molecules labeled on their first carbon atom.[30] Metabolism of 1-^{14}C-glucose by the PPP results in ^{14}C-labeled CO_2 release by the cells, and this release can be quantified. However, this method measures the combined activity of both G6PD and 6PGD and was not suited to the higher specificity required for our experiments. We therefore decided to use mass spectrometry to quantify production of the metabolite 6PG (Figure 1). Initial experiments showed that the absolute levels of 6PG varied little after treatment of cells with DHEA **1**. We speculated that the enzyme 6PGD rapidly metabolized 6PG and therefore masked any change in the flux accomplished by inhibiting G6PD. We hypothesized that inhibition of 6PGD should lead to an accumulation of 6PG, and this was tested using a commercially available inhibitor of 6PGD, 6-AN, which has been reported to induce 6PG accumulation in breast cancer cells.[31] The addition of 200 μM 6-AN for 4–6 h led to a substantial accumulation of 6PG. As G6PD is rate-limiting for 6PG production, it is expected that inhibition of G6PD would decrease the flux through the PPP and decrease the observed accumulation of 6PG. This was confirmed by inhibition of G6PD using either DHEA or siRNA (data not shown) in the presence of 6-AN, which led to a decrease in the accumulation of 6PG. The ability of selected test compounds to inhibit G6PD activity in HEK293T cells is reported in Table 3.

SAR AND COMPOUND DESIGN

The activity of DHEA as an inhibitor of G6PD was first reported by Marks and Banks in 1960.[13] In general the SAR from this and subsequent studies[14−22] indicated that G6PD

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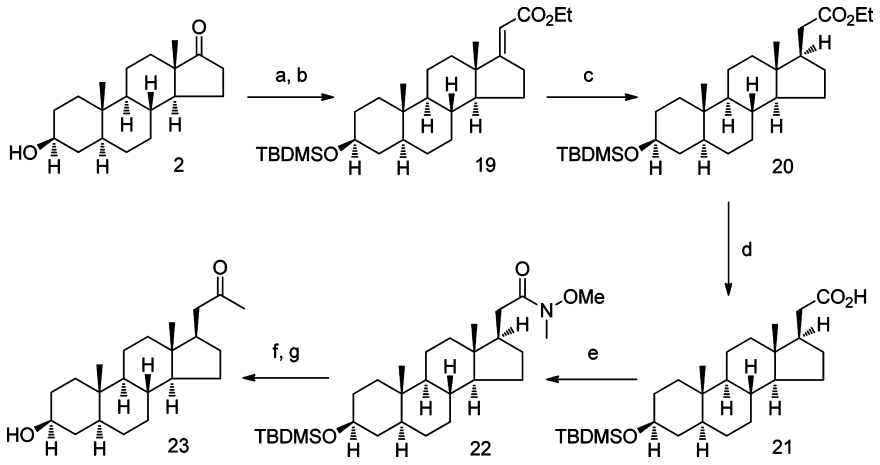
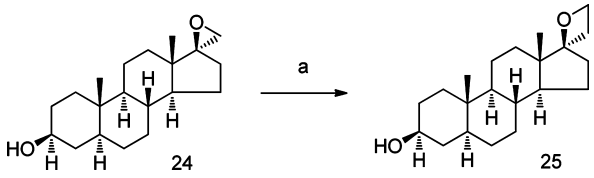
Scheme 6[a]



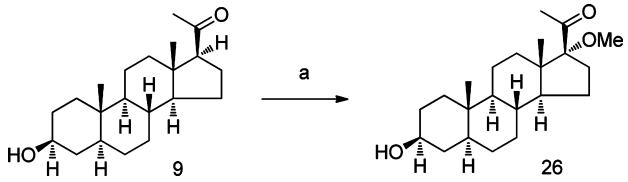
[a]Reagents: (a) TBDMS-Tf, pyridine (90%); (b) triethyl phosphonoacetate, NaOEt (100%); (c) H_2, Pd/C, ethyl acetate (92%); (d) LiOH, aq THF (88%); (e) 1,1′-carbonyldiimidazole, *N*,*O*-dimethylhydroxylamine HCl, Et_3N, DMF, DCM (92%); (f) MeMgBr, THF, then (g) 2 N HCl, acetone (24%).

Scheme 7[a]



[a]Reagents: (a) Me_3SOI, KO^{t}Bu, ^{t}BuOH, reflux (6.5%).

Scheme 8[a]



[a]Reagents: (a) $CuBr_2$, MeOH, reflux (24%).

inhibitory activity required an androst-5-ene or pregn-5-ene nucleus bearing a 3β-alcohol and either a 17-ketone (for androstenes) or 20-ketone (for pregnenes). Saturation of the steroid nucleus was well tolerated only when the resulting stereochemistry was 5α (allo configuration). Oxidation or inversion of the 3β-alcohol reduced activity. Introduction of other polar (alcohol or ketone) groups at the 7 or 11 position was detrimental, while alcohols at the 16α or 21 position were tolerated. Relative to DHEA **1** or EA **2** the only improvement was incorporation of a 16α-bromo substituent.[15,20,21] These SARs are summarized in Figure 2.

For new G6PD inhibitors we chose to retain the preferred steroid nuclei and explore the replacement of the 3β-alcohol with other H-bond donors and the 17- or 20-ketone with other H-bond acceptors. We were keen to incorporate these features that we felt were likely to preclude metabolism to potent androgens (or progestins), a problem exhibited by both DHEA **1** and EA **2**, which makes interpretation of biological activity more speculative. Both **1** and **2** have relatively poor aqueous solubility[32,33] which we hoped to improve by increasing polarity with new 3β-, 17-, or 20-substituents and, if possible, incorporating additional polar groups at synthetically accessible positions. To better understand the activity of our novel steroids, several literature compounds were purchased or prepared for comparison in the enzyme assay, including the aforementioned 16α-bromo derivatives. Although the 16α-bromo compounds are reported to be more potent G6PD inhibitors than either **1** or **2**, we wished to avoid reactive functional groups in newly designed compounds to minimize potential off-target activity and toxicity.

RESULTS AND DISCUSSION

We focused on saturated steroids, as the literature compound EA **2** was as potent as the Δ5-analogue DHEA **1**, and allopregnanolone **9** was more potent than pregnenolone **8**. The removal of the double bond in the B-ring should also help prevent formation of potent androgens bearing a Δ4-3-one that are formed by oxidative metabolism from 3β-hydroxy-Δ5-steroids such as DHEA **1**.

The compounds were tested in a G6PD enzyme assay and the results are shown in Tables 1 and 2. For the steroids shown in Table 1, androstane derivatives are labeled **7** and **17** while pregnane derivatives are labeled **14** and **18**. Direct replacement of the 3β-alcohol with a 3β-amine abolished activity in both the androstane and pregnane series (compounds **7a** and **14a**, respectively). The lack of activity for the parent 3β-amines may be due to the large desolvation penalty that would need to be overcome, as protonation of the amines will occur at the pH of the assay. Within the androstane series, improved activity relative to EA **2** was realized upon conversion of the 3β-amine **7a** to the 3β-ureas **7c**, **7e**, and **7f**, 3β-carbamate **7h**, 3β-sulfonamides **7i** and **7k**, and 3β-sulfamide **7l**. This important finding demonstrated that G6PD inhibition was not wholly dependent on retention of a 3β-alcohol and that improved activity was possible with alternative H-bond donors. Within the pregnane series, improved activity relative to allopregnanolone **9** was also found with sulfonamide **14f** and sulfamide **14i**, although the trend did not extend to either of the ureas **14c** or **14d** or the carbamate **14e**, suggesting binding modes (or perhaps binding sites) are dependent on the specific steroid nucleus.

Closer examination of the 3β-ureas, 3β-sulfonamides, and 3β-sulfamides revealed that inhibitory activity remains good in the androstane series with small substituents and tends to fall as

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Table 1. Inhibition of Glucose 6-Phosphate Dehydrogenase by Steroids[a]

compd	3-substituent	IC_{50} (μM)[b]	n[c]	compd	3-substituent	IC_{50} (μM)[b]	n[c]
1	β-OH	11.3 ± 2.2	4	**14b**	β-NHAc	>200	2
2	β-OH	9.4 ± 1.0	2	**14c**	β-$NHCONH_2$	>100	2
7a	β-NH_2	>200	5	**14d**	β-$NHCONMe_2$	>200	2
7b	β-NHAc	128 ± 18	3	**14e**	β-$NHCO_2Me$	>200	8
7c	β-$NHCONH_2$	5.0 ± 0.4	2	**14f**	β-$NHSO_2Me$	3.4 ± 0.7	4
7d	β-NHCONHMe	12.2 ± 0.3	2	**14g**	β-$NHSO_2Et$	11.0 ± 7.6	4
7e	β-NHCONHEt	1.0 ± 0.1	2	**14h**	β-$NHSO_2Ph$	20.3 ± 6.5	4
7f	β-$NHCONH^nBu$	1.7 ± 0.8	2	**14i**	β-$NHSO_2NH_2$	2.0 ± 0.4	2
7g	β-$NHCONMe_2$	>200	2	**14j**	β-$NHSO_2NMe_2$	37.8 ± 20.4	2
7h	β-$NHCO_2Me$	1.5 ± 1.2	6	**17a**[d]	α-NH_2	>200	2
7i	β-$NHSO_2Me$	6.6 ± 1.2	2	**17b**	α-NHCONHEt	>200	2
7j	β-$NHSO_2Et$	70.5 ± 58.6	6	**17c**	α-$NHSO_2Me$	>200	2
7k	β-$NHSO_2Ph$	9.9 ± 3.5	2	**17d**	α-$NHSO_2NH_2$	>200	2
7l	β-$NHSO_2NH_2$	1.2 ± 0.1	2	**18a**[e]	β-NHCONHEt	34.9 ± 3.7	2
8	β-OH	81.4 ± 19.5	2	**18b**	β-$NHCO_2Me$	41.6 ± 10.2	2
9	β-OH	4.3 ± 1.3	4	**18c**	β-$NHSO_2Me$	3.8 ± 0.5	2
14a	β-NH_2	>200	2	**18d**	β-$NHSO_2NH_2$	1.2 ± 0.2	2

[a]Inhibition of G6PD was determined by measuring the NADPH generated during the assay using an Amplite fluorimetric NADPH assay kit. [b]IC_{50} ± SD determined from 10 point concentration/effect experiments. [c]Geometric mean of at least two independent duplicate experimental determinations. [d]**17a** tested as hydrochloride salt. [e]**18a** tested as 1:1 mixture of 3α- and 3β-isomers.

Table 2. Inhibition of Glucose 6-Phosphate Dehydrogenase by Steroids Shown in Chart 1[a]

compd	IC_{50} (μM)[b]	n[c]	compd	IC_{50} (μM)[b]	n[c]
1	11.3 ± 2.2	4	**34**	0.9 ± 0.5	8
2	9.4 ± 1.0	2	**35**	>200	2
8	81.4 ± 19.5	2	**36**	9.3 ± 1.4	2
9	4.3 ± 1.3	4	**37**	2.5 ± 1.0	2
23	>200	2	**38**	>200	2
24	>200	2	**39**	>200	2
25	>200	2	**40**	>200	2
26	>200	2	**41**	66.0 ± 29.3	4
27	48.0 ± 2.6	2	**42**	31.6 ± 6.1	2
28	15.8 ± 3.3	2	**43**	15.8 ± 1.9	2
29	20.8 ± 4.4	2	**44**	6.3 ± 0.4	2
30	>200	2	**45**	>200	2
31	>200	2	**46**	5.2 ± 1.3	2
32	>200	2	**47**	47.0 ± 1.3	2
33	5.3 ± 1.3	2			

[a]Inhibition of G6PD was determined by measuring the NADPH generated during the assay using an Amplite fluorimetric NADPH assay kit. [b]IC_{50} ± SD determined from 10 point concentration/effect experiments. [c]Geometric mean of at least two independent duplicate experimental determinations.

substituent size increases. Thus, the small monoalkylated ureas **7c–f** are more potent than the dimethylurea **7g**, with the ethylurea **7e** most potent. Similarly the methyl carbamate **7h** retains good activity, and the methanesulfonamide **7i** is more potent than the ethanesulfonamide **7j**. However, the benzenesulfonamide **7k** does not fit this trend, being more active than the ethanesulfonamide **7j**, although the reason is not obviously apparent. Comparison of androstane sulfamide **7l** with the pregnane sulfamide **14i** and dimethylsulfamide **14j** shows that, like the ureas, dialkylation of the sulfamide nitrogen further from the steroid is deleterious. For the pregnane series there appears to be a more stringent steric effect as the analogous ureas **14c** and **14d** and methyl carbamate **14e** are all inactive. Good activity is only retained with the methanesulfonamide **14f** and the sulfamide **14i**, with a substantial reduction in potency for the larger ethanesulfonamide **14g**, benzenesulfonamide **14h**, and dimethylsulfamide **14j**.

Literature results have previously demonstrated that inversion of the 3β-alcohol abolishes activity.[14d,g] To confirm whether this extended to alternative H-bond donors, the 3α-amine **17a**, 3α-urea **17b**, 3α-sulfonamide **17c**, and 3α-sulfamide **17d** were prepared and tested. All of these 3α-isomers failed to inhibit G6PD activity in the enzyme assay, confirming previous observations.

Novel 3β-substituents were combined with a 21-hydroxypregan-20-one scaffold to yield compounds **18a–d**. As with 3β-alcohols (discussed below), the incorporation of a 21-hydroxyl group improved the activity of the 3β-carbamate **18b** and 3β-sulfamide **18d** relative to the unsubstituted analogues **14e** and **14i**, respectively. The activity of the 21-hydroxy-3β-sulfonamide **18c** was similar to the unsubstituted analogue **14f**. The ethylurea **18a** was more active than the primary urea **14c** but substantially less active than the androstrane ethylurea **7e**.

We then turned our attention to the opposite end of the steroid to establish whether the ketone could be replaced with other H-bond acceptors (Chart 1). The strategy was to explore SAR predominantly with literature compounds retaining a 3β-alcohol and then to combine any improvements with the novel 3β-H-bond donors to investigate if SARs are additive. Enzyme assay results for compounds depicted in Chart 1 are shown in Table 2.

In contrast to the literature reporting improved inhibition of glucose 6-phosphate dehydrogenase from trypanosomal parasites with the incorporation of a 16α-bromo group,[20,21] androstanes **27** and **29** did not display improved potency in the enzyme assay relative to DHEA **1** or EA **2**, respectively. The 16α-hydroxyandrostane **28** retained activity but was slightly less active than DHEA **1**.

The lack of activity with compounds **24**, **25**, and **30–32** demonstrated that replacement of the 17-ketone with an oxime, epoxides, or oxetanes is detrimental and suggested that for compounds retaining an androstane nucleus, SAR in this region is tight. Synthetic ketal intermediates were also inactive in the enzyme assay (results not shown).

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Table 3. Steroid Inhibition of Glucose 6-Phosphate Dehydrogenase in HEK293T Cells and Aqueous Solubility of the Steroids[a]

compd	3β-substituent	IC_{50} (μM)[b]	n[c]	(cell IC_{50})/(enzyme IC_{50})	solubility[d] (μM)
1	OH	24.9 ± 13.2	5	2.2	>100
7e	NHCONHEt	9.5 ± 4.7	3	9.5	30−100
7h	$NHCO_2Me$	7.8 ± 1.6	3	5.2	>100
7l	$NHSO_2NH_2$	17.4 ± 8.3	2	14.5	30−100
14f	$NHSO_2Me$	4.8 ± 1.2	3	1.4	3−10
14i	$NHSO_2NH_2$	9.2 ± 1.7	3	4.6	10−65
18c	$NHSO_2Me$	35.2 ± 1.8	3	9.2	10−30
18d	$NHSO_2NH_2$	14.1	1	11.8	>100
34	OH	12.3 ± 2.5	3	13.7	>100

[a]Inhibition of G6PD was determined by measuring the accumulation of 6PG and blocking its metabolism using 200 μM 6-AN. [b]IC_{50} ± SD determined from 10 point concentration/effect experiments. [c]Geometric mean of at least two independent experimental determinations (except for compound **18d**). [d]Turbidimetric aqueous solubility, 1% DMSO in PBS.

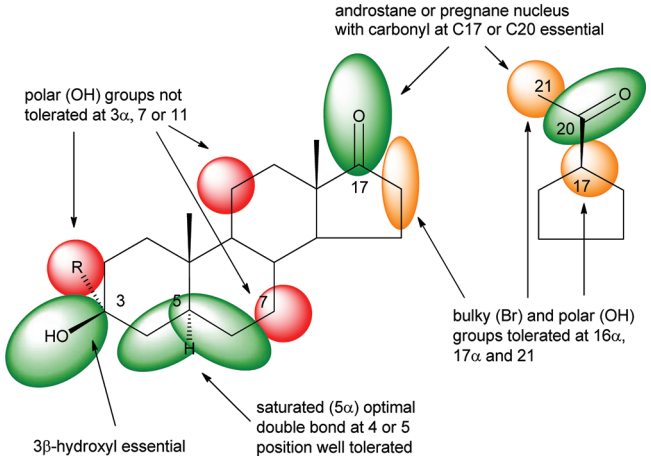

Figure 2. Established SAR for steroid inhibition of G6PD. Functional groups shown in green areas are essential for good inhibitory activity. Areas in amber tolerate some modification, and substituents in red areas typically reduce or abolish activity.

To investigate whether pregnanes offered greater scope for further elaboration in this region, compounds **33**−**38** were examined. Relative to pregnenolone **8** or pregnanolone **9**, substantial improvements in activity were realized with the 21-hydroxyl derivatives **33** and **34**, respectively, while 21-halo derivatives **36** and **37** retained activity. However, the 21-acetate **35** had no activity. As in the androstane series, conversion of the ketone to the oxime **38** abolished activity and the novel homologated ketone **23** also lacked activity.

As anticipated from prior art,[14d,g] the 3α-alcohols **39**, **41**, and **42** were either inactive or substantially less active than the corresponding 3β-alcohols, but in contrast to a literature report,[14d] 5α-androstan-17-one **40** (which lacks the 3β-ol) in our hands is also inactive. Pregnanes **43** and **44**, which possess an 11-ketone, retain moderate activity but are less potent than the corresponding unsubstituted steroids **9** and **34**, respectively. The 17α-methoxypregnane **26** and 16,17-epoxypregnane **45** both lack activity. Moderate activity is retained with the 17α-hydroxypregnane **46** which also has the beneficial 21-alcohol, while the 17α-hydroxypregnane **47**, which lacks the 21-alcohol and also has a deleterious 11-ketone, is almost devoid of activity.

From this assessment it was apparent that most modifications to the 17- or 20-ketones reduce or abolish activity, but a 21-hydroxyl group markedly improved activity.

To summarize the enzyme assay results, four derivatives with a potency increase of approximately 10-fold relative to DHEA **1** and EA **2** were identified: compounds **7e**, **7l**, **18d**, and **34**. The 3β-alcohol of DHEA can be replaced with alternative 3β-H-bond donors such as sulfamide, sulfonamide, urea, and carbamate. Good potency is retained with either an androstane or pregnane nucleus, provided a carbonyl H-bond acceptor at C-17 (androstane) or C-20 (pregnane) is present. A 21-hydroxyl group is well tolerated and may improve activity. The activity of the series plateaus at about 1 μM in the enzyme assay, although the assay is capable of detecting substantially more potent compounds. The results also demonstrate that potency can be improved with a reduction in lipophilicity, best illustrated by the sulfamides **7l** (XlogP = 2.78) and **18d** (XlogP = 2.27) compared with DHEA **1** (XlogP = 3.62) or EA **2** (XlogP = 3.78). In addition the alternative H-bond donors are expected to have better metabolic stability than the 3β-alcohol of **1** and **2**, with reduced propensity to form androgenic metabolites or phase II conjugates.

On the basis of these considerations, a number of the compounds were selected and tested in a cellular assay. The aqueous solubility of these steroids was also determined, and the combined results are shown in Table 3.

The improved activity of the novel compounds over DHEA **1** in the enzyme assay, with the exception of the sulfonamide **18c**, was repeated in the cell assay. There was, however, no clear correlation between the inhibitory activity of the compounds in the two assays, and the reduction in activity on translation from enzyme to cell assay varied from 1.4-fold (sulfonamide **14f**) to 14.5-fold (sulfamide **7l**). Five of the novel compounds in Table 3 have moderate−good aqueous solubility. The reduction in cellular activity does not correlate with poor aqueous solubility. This is best exemplified by the water-soluble sulfamides **7l** and **18d**, which are among the least potent compounds in cells.

To establish whether permeability adversely affects cell activity, a small number of compounds were tested in vitro in a Caco-2 permeability assay (Table 4). The stability of some compounds in the presence of P450 (phase 1) metabolic enzymes was tested in vitro using human liver microsomes, and the results are shown in Table 4. With the exception of sulfamide **14i**, the Caco-2 permeability was moderate−good for the steroids tested, and there was no evidence of efflux. The good permeability of urea **7e** and sulfamide **7l** suggests that permeability is unlikely to be the main reason for the reduction in activity on translation from the enzyme assay to the cell assay. No clear rationale for the discrepancy between the enzyme and cell assays was established; the discrepancy may be due to a combination of factors including solubility, permeability, and biological or assay variability.

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Chart 1

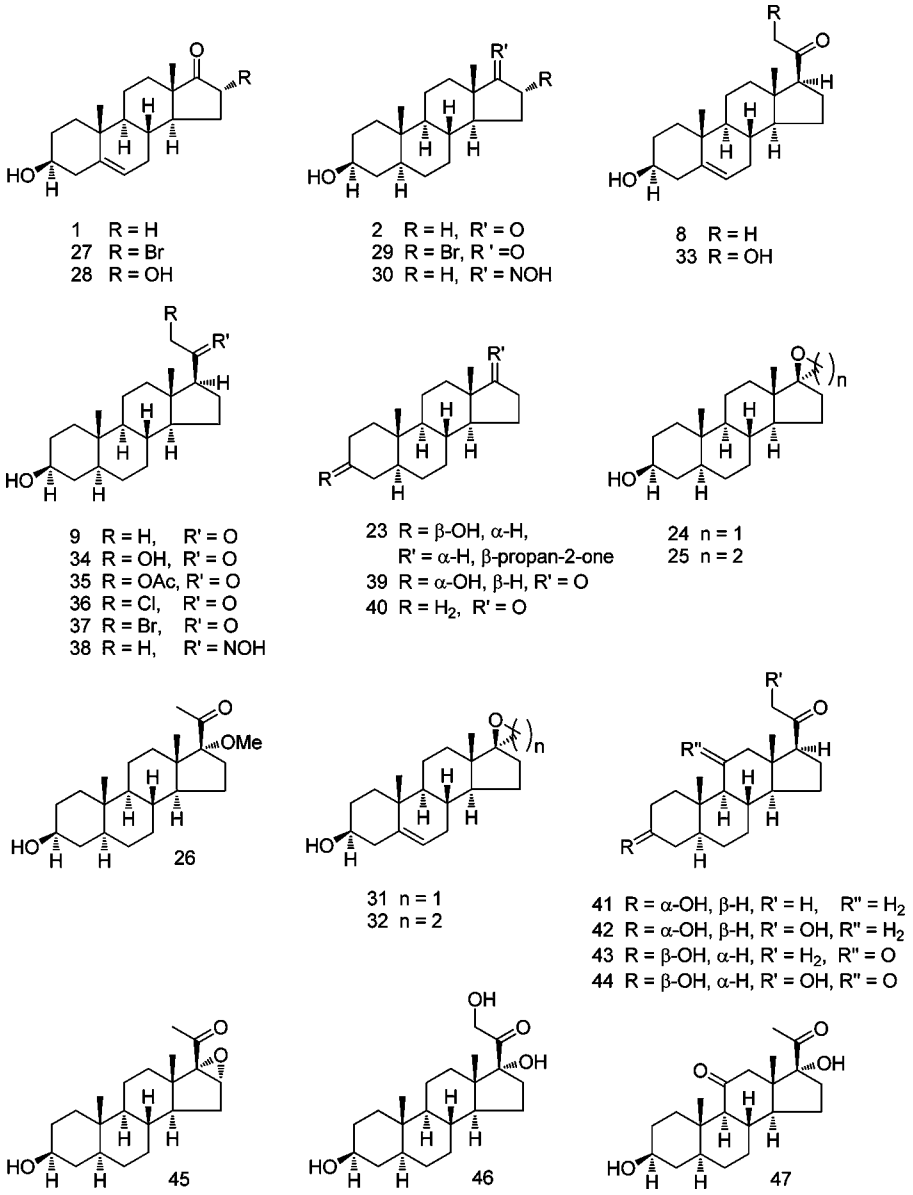

Table 4. In Vitro DMPK Parameters of Selected Steroids

compd	3β-substituent	$P_{a\to b}$ [a] (10^{-6} cm s^{-1})	$P_{b\to a}/P_{a\to b}$ [a]	Cl_{int} [b]	$T_{1/2}$ [c]
7e	NHCONHEt	31.6 ± 3.1	0.54	56.8	24.4
7l	$NHSO_2NH_2$	31.8 ± 5.4	0.66	ND[d]	ND[d]
14f	$NHSO_2Me$	ND[d]	ND[d]	10.3	135
14i	$NHSO_2NH_2$	2.1 ± 0.1	0.53	ND[d]	ND[d]
18c	$NHSO_2Me$	17.4 ± 4.2	0.30	ND[d]	ND[d]
18d	$NHSO_2NH_2$	13.1 ± 0.01	0.49	28.3	48.9

[a] $P_{a\to b}$ is the permeability when applied to the apical side, while $P_{b\to a}$ is the permeability when applied to the basolateral side in a Caco-2 membrane assay (10^{-6} cm s^{-1}). The ratio of these two values is a measure of Pgp efflux, while the $P_{a\to b}$ value is a measure of permeability. [b] Metabolic stability in human liver microsomes: intrinsic clearance Cl_{int} in (μL/min)/(mg protein). [c] Half-life ($T_{1/2}$) in min. [d] ND = not determined.

In addition to enzyme and cell potency compounds **7e** and **18d** also showed moderate in vitro metabolic stability against human liver microsomes. Sulfonamide **14f** appeared to show excellent in vitro metabolic stability against human liver microsomes, although the result should be treated with caution owing to the low solubility of this compound. Although none of the compounds in Table 3 have been tested against a broad range of receptors, preliminary results demonstrate that none of them have appreciable activity against two related dehydrogenase enzymes (≪50% inhibition at 200 μM) and the alcohol **34** is devoid of CYP450 inhibition against Cyp 1A, 2C19, 2C9, 2D6, 3A4 (>25 μM for all five isoforms tested). The improved potency of the urea **7e**, sulfamide **18d**, and alcohol **34** relative to DHEA **1** and EA **2**, coupled with their apparent selectivity and their good physicochemical and in vitro DMPK parameters, suggests that these compounds may be useful pharmacological tools to further elucidate the role of G6PD in cells.

CONCLUSION

By use of DHEA **1** and EA **2** as literature leads, the SAR for steroid inhibition of G6PD has been substantially developed and a number of derivatives with a potency increase of

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approximately 10-fold were identified. In particular this study has established that the 3β-alcohol can be replaced with alternative 3β-H-bond donors such as sulfamide, sulfonamide, urea, and carbamate. Good potency can also be retained by replacing the androstane nucleus with a pregnane nucleus, provided a carbonyl H-bond acceptor at C-17 (androstane) or C-20 (pregnane) is retained. For pregnan-20-ones a 21-hydroxyl group may improve activity and in combination with a 3β-alcohol, 3β-sulfamide, or 3β-sulfonamide leads to compounds with good potency. The urea **7e**, sulfamide **18d**, and alcohol **34** offer substantial benefits over DHEA **1**, EA **2**, and their 16α-bromo derivatives for examining the role of inhibiting G6PD in cells and will assist the future design of more potent steroid inhibitors with potential therapeutic utility.

EXPERIMENTAL SECTION

General Methods. All reagents obtained from commercial sources were used without further purification. Anhydrous solvents were obtained from the Sigma-Aldrich Chemical Co. Ltd. or Fisher Chemicals Ltd. and used without further drying. Solutions containing products were either passed through a hydrophobic frit or dried over anhydrous $MgSO_4$ or Na_2SO_4 and filtered, prior to evaporation of the solvent. Solvents were evaporated under reduced pressure. Thin layer chromatography (TLC) was conducted with 5 cm × 10 cm plates coated with Merck type 60 F_{254} silica gel to a thickness of 0.25 mm. As the steroids typically lack a UV chromophore, TLC was often used to monitor reactions and initially assess compound purity; elution was usually with isohexane/ethyl acetate mixtures. TLC plates were then sprayed with a 4% H_2SO_4/methanol solution and heated on a hot plate to develop. Chromatography was performed on Biotage SNAP HP-Sil cartridges using a CombiFlash Companion machine.

Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on a 300 MHz Bruker spectrometer at ambient temperature. Solutions were typically prepared in either deuterochloroform ($CDCl_3$) or deuterated dimethylsulfoxide (DMSO-d_6) with chemical shifts referenced to deuterated solvent as an internal standard. ^{1}H NMR data are reported indicating the chemical shift (δ), the multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad; dd, doublet of doublets, etc.), the coupling constant (J) in Hz, and the integration (e.g., 1H). Deuterated solvents were obtained from the Sigma-Aldrich Chemical Co., Goss or Fluorochem.

HRMS results were obtained on a Waters QTof Micro instrument, using positive and negative polarity electrospray with sodium formate as standard calibrant.

LC−MS spectra with evaporative light scattering detection (ELSD) were recorded on a Waters Acquity UPLC. This analytical method was used to determine compound purity which is ≥95% unless otherwise stated. Mass spectrometry was performed on a Waters Acquity SQD quadrupolar spectrometer running in dual ES^+ and ES^- mode. High pH runs were conducted at pH 10 and low pH runs were conducted at pH 3, with a run time of 2 mins. The column temperature was 40 °C, and the flow rate was 0.6 mL/min. Further details including solvent gradients are given in the Supporting Information. Details of the preparative HPLC instrument and the solvent gradient used to purify two compounds are also given in the Supporting Information

DHEA **1**, (3α,5α)-3-hydroxypregnan-20-one **41**, (3α,5α)-3,21-dihydroxypregnan-20-one **42**, (5α)-3-hydroxypregnane-11,20-dione **43**, (3β,5α)-3,21-dihydroxypregnane-11,20-dione **44**, and (3β,5α)-3,17-dihydroxypregnane-11,20-dione **47** were purchased from Sigma-Aldrich. EA **2** and (3β)-3-hydroxypregn-5-en-20-one **8** were purchased from Acros Organics. (5α)-Androstan-17-one **40** was purchased from Interbioscreen. (3β,5α)-3,17,21-Trihydroxypregnan-20-one **46** was purchased from Steraloids/Makaira.

Preparative methods and spectroscopic data for compounds **1**−**4**, **8**−**11**, **14a**, **14b**, **16**, **17a**, **19**, **24**, and **27**−**47** and ^{1}H NMR spectra of target compounds are included in the Supporting Information.

2-[(3β,5α)-17-Oxoandrostan-3-yl, 17-cyclic 1,2-ethanediyl acetal]-1*H*-isoindole-1,3(2*H*)-dione 5. (3α,5α)-3-Hydroxyandrostan-17-one, cyclic 1,2-ethanediyl acetal **4** (8.17 g, 24.4 mmol), phthalimide (3.59 g, 24.4 mmol), and TPP (7.08 g, 25.6 mmol) were dissolved in THF (175 mL), and the solution was cooled to 5 °C using an ice bath. DIAD (5.3 mL, 26.8 mmol) was added slowly maintaining the temperature below 5 °C. A white precipitate formed during the reaction. The mixture was stirred and warmed to ambient temperature overnight under a nitrogen atmosphere. All volatiles were evaporated. Then methanol (100 mL) was added and the mixture stirred for 30 min. The precipitate was filtered off and washed with methanol. The solid was dried under vacuum to give the product **5** as a white solid (7.67 g, 68%). ^{1}H NMR ($CDCl_3$): δ 0.87 (s, 3H), 0.98 (s, 3H), 1.95−2.05 (m, 1H), 2.22−2.49 (m, 2H), 3.85−3.97 (m, 4H), 4.13−4.24 (m, 1H), 7.68−7.74 (m, 2H), 7.79−7.85 (m, 2H).

(3β,5α)-3-Aminoandrostan-17-one, Cyclic 1,2-Ethanediyl Acetal 6.[29] 2-[(3β,5α)-17-Oxoandrostan-3-yl, 17-cyclic 1,2-ethanediyl acetal]-1*H*-isoindole-1,3(2*H*)-dione **5** (7.67 g, 15.6 mmol) was mixed with ethanol (100 mL) and hydrazine hydrate (11 mL, 226 mmol) and refluxed overnight under a nitrogen atmosphere. The reaction mixture was cooled, and the solid was filtered off. The filtrate was evaporated to dryness. The residue was partitioned between DCM and water, and the solution passed through a hydrophobic frit. The DCM phase was evaporated to dryness to give the product **6** (5.34 g, 97%). ^{1}H NMR ($CDCl_3$): δ 0.79 (s, 3H), 0.84 (s, 3H), 1.92−2.04 (m, 1H), 2.61−2.73 (m, 1H), 3.82−3.98 (m, 4H). ^{13}C NMR ($CDCl_3$): δ 11.2, 13.3, 19.4, 21.5, 27.5, 29.6, 30.3, 31.4, 33.1, 34.5, 34.7, 36.6, 38.2, 44.4, 44.9, 49.3, 50.0, 53.2, 63.4, 64.0, 118.4.

(3β,5α)-3-Aminoandrostan-17-one 7a.[29] (3β,5α)-3-Aminoandrostan-17-one, cyclic 1,2-ethanediyl acetal **6** (100 mg, 0.30 mmol) was dissolved in acetone (1 mL), DCM (1 mL) and THF (4 mL). Then 2 M HCl (0.5 mL, 1 mmol) was added and the mixture stirred overnight. The reaction mixture was basified with 1 M NaOH. DCM was added and the mixture stirred before passing through a hydrophobic frit. The organic phase was concentrated and chromatographed on silica (10 g SNAP cartridge, DCM/MeOH gradient elution) to give the product **7a** as a white solid (10 mg, 12%). ^{1}H NMR ($CDCl_3$): δ 0.84 (s, 3H), 0.88 (s, 3H), 2.40−2.52 (m, 1H), 2.70−2.82 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 11.2, 12.7, 19.3, 20.7, 27.3, 29.8, 30.4, 31.0, 34.0, 34.6, 34.7, 34.8, 36.4, 37.6, 44.4, 46.7, 49.9, 50.4, 220.3. HRMS (ESI) m/z $[M + H]^+$ calcd for $C_{19}H_{32}NO$: 290.2479. Found: 290.2480.

***N*-[(3β,5α)-17-Oxoandrostan-3-yl]acetamide 7b.** To a stirred solution of (3β,5α)-3-aminoandrostan-17-one, cyclic 1,2-ethanediyl acetal **6** (200 mg, 0.600 mmol) and triethylamine (121 mg, 1.20 mmol) in DCM (5 mL) at 20 °C under nitrogen was added acetyl chloride (49 mg, 0.63 mmol), and the resulting mixture was stirred overnight. Water (4 mL) was added, and stirring continued for 15 min. The reaction mixture was filtered through a hydrophobic filter and the organic phase evaporated to dryness. The 17-ketal was deprotected in a similar manner to compound **7a**, affording the product **7b** as a white foam (160 mg, 80%). ^{1}H NMR ($CDCl_3$): δ 0.82 (s, 3H), 0.86 (s, 3H), 1.96 (s, 3H), 2.38−2.50 (m, 1H), 3.68−3.84 (m, 1H), 5.43 (br d, J = 7.9 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ 12.2, 13.8, 20.4, 21.8, 23.5, 28.2, 28.7, 30.8, 31.5, 35.1, 35.3, 35.6, 35.9, 37.3, 45.3, 47.8, 48.9, 51.4, 54.3, 169.3, 221.3.

***N*-[(3β,5α)-17-Oxoandrostan-3-yl]urea 7c.** To a stirred solution of (3β,5α)-3-aminoandrostan-17-one, cyclic 1,2-ethanediyl acetal **6** (100 mg, 0.30 mmol) in DCM (1 mL) were added (4-nitrophenyl)-*N*-benzylcarbamate (89.8 mg, 0.33 mmol) and triethylamine (0.05 mL, 0.33 mmol). The mixture was heated at 40 °C for 6 h and then stirred at ambient temperature overnight. The reaction mixture was flash chromatographed on silica (25 g SNAP cartridge, ethyl acetate/isohexane gradient elution) to give the benzylurea intermediate (148 mg) as a viscous oil. To a stirred solution of the benzylurea intermediate (148 mg, 0.32 mmol) in acetone (4 mL) was added 2 M HCl (1 mL) and the mixture stirred at ambient temperature for 2 h. The mixture was evaporated. Acetone (10 mL) was added and the solution evaporated. The residue was dried at 40 °C under vacuum for 12 h to give a gum which was purified by flash chromatography (isohexane/ethyl acetate gradient elution), eluting to give the ketone intermediate (113 mg, 0.27 mmol, 84%) as a white foam. To a stirred solution of the ketone benzylurea intermediate (93 mg, 0.22 mmol) in

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acetic acid (3 mL) under nitrogen was added 10% Pd/C, and the mixture was stirred vigorously under hydrogen for 3 h. The catalyst was removed by filtration through Celite and the solvent evaporated. The residue was purified by flash chromatography (ethyl acetate elution) and then by preparative HPLC to give the product **7c** (13.6 mg, 22%). ^{1}H NMR ($CDCl_3$): δ 0.81 (s, 3H), 0.85 (s, 3H), 2.10 (t, J = 9.0 Hz, 1H), 2.44 (dd, J = 19.4 and 9.0 Hz, 1H), 3.48 (m, 1H), 4.29 (s, 2H), 4.37 (d, J = 9.2 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ 12.3, 13.8, 20.4, 21.8, 28.3, 29.3, 30.8, 31.5, 35.1, 35.6, 35.86, 35.93, 37.4, 45.4, 47.8, 51.4, 54.4, 157.9, 221.3. LC−MS m/z 333.1 $[M + 1]^+$, 100% purity; m/z 331.1 $[M - 1]^-$, 100% purity.

***N*-Methyl-*N*′-[(3*β*,5*α*)-17-oxoandrostan-3-yl]urea 7d.** To a stirred solution of (3*β*,5*α*)-3-aminoandrostan-17-one, cyclic 1,2-ethanediyl acetal **6** (100 mg, 0.30 mmol) in DCM (1 mL) was added (4-nitrophenyl)-*N*-methylcarbamate (59 mg, 0.30 mmol), and the mixture was stirred at ambient temperature for 2 h. Triethylamine (0.04 mL, 0.30 mmol) was added and the mixture warmed to 40 °C for 6 h and then stirred at ambient temperature overnight. The reaction mixture was chromatographed on silica (25 g SNAP cartridge, ethyl acetate elution). Evaporation of the solvent afforded the 17-ketal intermediate as a white solid (72 mg). To a stirred solution of this intermediate (72 mg, 0.18 mmol) in acetone (4 mL) was added 2 M HCl (1 mL), and the mixture was stirred at ambient temperature for 2 h. The mixture was evaporated, acetone (10 mL) added, and the solution evaporated. Repeating this process caused precipitation of the product **7d** as a crystalline white solid (55.5 mg, 53%). ^{1}H NMR ($CDCl_3$): δ 0.84 (s, 3H), 0.86 (s, 3H), 2.05 (dd, J = 19.0 and 8.7 Hz, 1H), 2.45 (dd, J = 19.0 and 8.7 Hz, 1H), 2.90 (s, 3H), 3.51 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 11.2, 12.7, 19.3, 20.6, 26.5, 27.1, 27.8, 29.7, 30.4, 33.9, 34.3, 34.5, 34.5, 36.1, 44.3, 49.8, 50.3, 53.2, 158.1, 220.1. LC−MS m/z 347.2 $[M + 1]^+$, 100% purity; m/z 345.2 $[M - 1]^-$, 100% purity.

***N*-Ethyl-*N*′-[(3*β*,5*α*)-17-oxoandrostan-3-yl]urea 7e.** To a stirred solution of (3*β*,5*α*)-3-aminoandrostan-17-one, cyclic 1,2-ethanediyl acetal **6** (350 mg, 1.05 mmol) in DCM (5 mL) at 20 °C under nitrogen was added ethyl isocyanate (112 mg, 1.57 mmol). The mixture was stirred overnight, and then DCM and water were added before the mixture was passed through a hydrophobic frit. The DCM phase was concentrated to give the intermediate ketal as a white solid (472 mg). The 17-ketal intermediate (472 mg) was deprotected in a similar manner to compound **7a** and the crude product chromatographed on silica (25 g SNAP, DCM/ethyl acetate gradient elution), affording the product **7e** as a white solid (293 mg, 77%). ^{1}H NMR ($CDCl_3$): δ 0.82 (s, 3H), 0.86 (s, 3H), 1.14 (t, J = 7.3 Hz, 3H), 2.38−2.50 (m, 1H), 3.20 (q, J = 7.2 Hz, 2H), 3.46−3.59 (m, 1H), 4.35 (br s, 1H). ^{1}H NMR (DMSO-d_6): δ 0.78 (s, 6H), 0.96 (t, J = 7.2 Hz, 3H), 2.32−2.44 (m, 1H), 2.92−3.03 (m, 2H), 3.22−3.37 (m, 1H), 5.58−5.65 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 12.3, 13.8, 15.5, 20.4, 21.8, 28.3, 29.6, 30.8, 31.5, 35.1, 35.3, 35.6, 35.9, 36.2, 37.5, 45.5, 47.8, 49.7, 51.4, 54.4, 157.6, 221.4. LC−MS m/z 361.2 $[M + 1]^+$, 100% purity; m/z 359.2 $[M - 1]^-$, 100% purity. HRMS (ESI) m/z $[M + H]^+$ m/z calcd for $C_{22}H_{37}N_2O_2$: 361.2850. Found: 361.2851.

***N*-Butyl-*N*′-[(3*β*,5*α*)-17-oxoandrostan-3-yl]urea 7f.** **7f** was prepared and purified in a similar manner to compound **7e**, using (3*β*,5*α*)-3-aminoandrostan-17-one, cyclic 1,2-ethanediyl acetal **6** (100 mg, 0.30 mmol) and *N*-butyl isocyanate (44.5 mg, 0.45 mmol). Evaporation from ethyl acetate/isohexane afforded the product **7f** as a white crystalline solid (67 mg, 58%). ^{1}H NMR (DMSO-d_6): δ 0.77 (s, 6H), 0.86 (t, J = 6.0 Hz, 3H), 2.32−2.44 (m, 1H), 2.90−2.99 (br q, J = 6.2 Hz, 2H), 3.22−3.36 (m, 1H), 5.56−5.67 (m, 2H). ^{13}C NMR (DMSO-d_6): δ 11.3, 12.8, 13.1, 18.9, 19.3, 20.7, 27.4, 28.4, 29.8, 30.7, 31.6, 33.9, 34.60, 34.64, 35.2, 36.5, 38.2, 44.5, 46.4, 47.9, 50.0, 53.2, 156.7, 219.1. LC−MS m/z 389.2 $[M + 1]^+$, 100% purity; m/z 387.2 $[M - 1]^-$, 100% purity.

***N*,*N*-Dimethyl-*N*′-[(3*β*,5*α*)-17-oxoandrostan-3-yl]urea 7g.** (3*β*,5*α*)-3-Aminoandrostan-17-one, cyclic 1,2-ethanediyl acetal **6** (100 mg, 0.30 mmol) was dissolved in DCM (5 mL), and triethylamine (0.14 mL, 1 mmol) was added. The mixture was cooled in an ice bath, and dimethylcarbamyl chloride (0.03 mL, 0.33 mmol) was added. The mixture was stirred for 3 days. DCM and 2 M HCl were added, and the mixture was stirred before passing through a hydrophobic frit. The DCM was evaporated, and the resulting 17-ketal intermediate was deprotected and purified in a similar manner to compound **7e**. Evaporation from ethyl acetate/isohexane afforded the product **7g** as a white crystalline solid (81 mg, 75%). ^{1}H NMR (DMSO-d_6): δ 0.78 (s, 3H), 0.80 (s, 3H), 2.32−2.43 (m, 1H), 2.75 (s, 6H), 3.30−3.47 (m, 1H), 5.88 (br d, J = 8.2 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ 11.9, 13.4, 20.0, 21.3, 28.1, 28.6, 30.4, 31.3, 34.5, 35.3 (×2), 35.4, 35.8 (×2), 37.3, 45.2, 47.1, 49.3, 50.6, 53.7, 157.5, 219.8. LC−MS m/z 361.5 $[M + 1]^+$, 100% purity.

[(3*β*,5*α*)-17-Oxoandrostan-3-yl]carbamic Acid, Methyl Ester 7h. To a stirred solution of methyl chloroformate (95 mg, 1.0 mmol) and (3*β*,5*α*)-3-aminoandrostan-17-one, cyclic 1,2-ethanediyl acetal **6** (350 mg, 1.05 mmol) in DCM (5 mL) at 20 °C under nitrogen was added triethylamine (121 mg, 1.2 mmol); the mixture became very warm. Stirring was continued overnight under nitrogen. Then DCM and water were added and the mixture was passed through a hydrophobic frit. The DCM was evaporated to give a white solid (432 mg) that was dissolved in THF (8 mL), DCM (1 mL), and acetone (1 mL). Then 2 M HCl (0.5 mL) was added and the mixture stirred for 3 days. DCM and water were added, and the mixture was passed through a hydrophobic frit. The DCM was evaporated to give a white solid (312 mg) that was chromatographed on silica (25 g SNAP cartridge, DCM/ethyl acetate gradient elution). Evaporation of the solvent afforded the product **7h** as a white crystalline solid (293 mg, 80%). ^{1}H NMR (DMSO-d_6): δ 0.77 (s, 6H), 2.32−2.43 (m, 1H), 3.17−3.33 (m, 1H), 3.49 (s, 3H), 6.98 (d, J = 8.1 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ 11.9, 13.4, 19.9, 21.3, 27.9, 28.2, 30.4, 31.3, 34.5, 34.9, 35.2, 35.3, 36.9, 44.9, 47.0, 49.8, 50.6, 50.9, 53.6, 155.7, 219.7. LC−MS m/z 348.5 $[M + 1]^+$, 100% purity. HRMS (ESI) m/z $[M + H]^+$ calcd for $C_{21}H_{34}NO_3$: 348.2534. Found: 348.2539.

***N*-[(3*β*,5*α*)-17-Oxoandrostan-3-yl]methanesulfonamide 7i.** To a stirred solution of (3*β*,5*α*)-3-aminoandrostan-17-one, cyclic 1,2-ethanediyl acetal **6** (200 mg, 0.60 mmol) and triethylamine (0.17 mL, 1.2 mmol) in DCM (5 mL) at 20 °C under nitrogen was added methanesulfonyl chloride (0.05 mL, 0.60 mmol). The resulting mixture was stirred at ambient temperature for 24 h.The reaction mixture was treated with water (2 mL), MeOH (3 mL), and $MeSO_3H$ (0.4 mL) and stirred vigorously for 15 min. The reaction mixture was basified with saturated $NaHCO_3$ (10 mL), extracted with DCM, and passed through a hydrophobic frit. Evaporation of the solvent afforded a residue that was chromatographed on silica (isohexane/ethyl acetate gradient elution) and recrystallized from ethyl acetate to give the product **7i** as a white crystalline solid (101 mg, 46%). ^{1}H NMR ($CDCl_3$): δ 0.84 (s, 3H), 0.88 (s, 3H), 2.40−2.52 (m, 1H), 3.00 (s, 3H), 3.22−3.40 (m, 1H), 4.09 (d, J = 7.5 Hz, 1H). ^{1}H NMR (DMSO-d_6): δ 0.77 (s, 3H), 0.78 (s, 3H), 2.32−2.43 (m, 1H), 2.89 (s, 3H), 3.00−3.16 (m, 1H), 6.95 (d, J = 7.5 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ 11.8, 13.4, 19.9, 21.3, 27.9, 29.4, 30.4, 31.3, 34.4, 35.0, 35.3, 35.9, 36.9, 41.2, 44.9, 47.0, 50.6, 52.3, 53.6, 219.7. LC−MS m/z 366.1 $[M - 1]^-$, 100% purity.

***N*-[(3*β*,5*α*)-17-Oxoandrostan-3-yl]ethanesulfonamide 7j.** (3*β*,5*α*)-3-Aminoandrostan-17-one, cyclic 1,2-ethanediyl acetal **6** (100 mg, 0.30 mmol) was dissolved in DCM (5 mL), and triethylamine (0.14 mL, 1 mmol) was added. The mixture was cooled in an ice bath, and ethanesulfonyl chloride (0.04 mL, 0.45 mmol) was added. The mixture was stirred for 3 days under a nitrogen atmosphere. THF (5 mL), acetone (2 mL), and 2 M HCl (0.5 mL) were added, and stirring continued overnight. DCM and water were added, and the mixture was passed through a hydrophobic frit. Evaporation of the solvent afforded a residue that was chromatographed on silica (10 g SNAP cartridge, isohexane/ethyl acetate gradient elution). Evaporation of the solvent afforded the product **7j** as a white crystalline solid (86 mg, 75%). ^{1}H NMR ($CDCl_3$): δ 0.83 (s, 3H), 0.87 (s, 3H), 1.38 (t, J = 7.4 Hz, 3H), 2.39−2.51 (m, 1H), 3.05 (q, J = 7.4 Hz, 2H), 3.20−3.35 (m, 1H), 4.11 (d, J = 7.9 Hz, 1H). ^{1}H NMR (DMSO-d_6): δ 0.76 (s, 3H), 0.77 (s, 3H), 1.18 (t, J = 7.3 Hz, 3H), 2.32−2.44 (m, 1H), 2.96 (q, J = 7.3 Hz, 2H), 2.90−3.10 (m, 1H), 6.98 (d, J = 7.9 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ 7.4, 11.1, 12.7, 19.3, 20.6, 27.1, 29.3, 29.6, 30.4, 33.9, 34.3, 34.7, 35.9, 36.3, 44.4, 46.6, 47.2, 50.2, 52.2, 53.2, 220.1. LC−MS m/z 380.0 $[M - 1]^-$, 100% purity.

***N*-[(3β,5α)-17-Oxoandrostan-3-yl]benzenesulfonamide 7k.** To a stirred solution of (3β,5α)-3-aminoandrostan-17-one, cyclic 1,2-ethanediyl acetal **6** (200 mg, 0.60 mmol) and triethylamine (121 mg, 1.20 mmol) in DCM (5 mL) at 20 °C under nitrogen was added benzenesulfonyl chloride (111 mg, 0.63 mmol). The mixture was stirred overnight. Water (4 mL) was added. Stirring was continued for 15 min and the mixture filtered through a hydrophobic frit. The organic phase was evaporated to dryness. The residue was dissolved in THF (5 mL) and acetone (1 mL), and 1 N HCl solution (1 mL) was added. This mixture was stirred overnight. Water (4 mL) and DCM (10 mL) were added, and the mixture was filtered through a hydrophobic frit. Evaporation of the solvent afforded the product **7k** as a white foam (150 mg, 58%). ^{1}H NMR ($CDCl_3$): δ 0.76 (s, 3H), 0.84 (s, 3H), 2.37−2.50 (m, 1H), 3.05−3.20 (m, 1H), 4.68 (br s, 1H), 7.48−7.61 (m, 3H), 7.87−7.94 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 11.0, 12.7, 19.2, 20.6, 27.0, 28.6, 29.6, 30.4, 33.8, 34.3, 34.7, 35.2, 36.2, 44.4, 46.6, 50.2, 52.2, 53.2, 125.8 (×2), 127.9 (×2), 131.3, 140.3, 220.1. LC−MS *m*/*z* 428.1 $[M - 1]^-$, 100% purity (combined).

***N*-[(3β,5α)-17-Oxoandrostan-3-yl]sulfamide 7l.** A stirred solution of sulfamide (481 mg, 5.01 mmol) and (3β,5α)-3-aminoandrostan-17-one, cyclic 1,2-ethanediyl acetal **6** (167 mg, 0.50 mmol) in 1,4-dioxane (5 mL) was heated at reflux for 48 h. The reaction mixture was diluted with water and extracted with ethyl acetate. The combined extracts were washed with saturated $NaHCO_3$ solution and water. The organic phase was dried and evaporated to leave a residue that was treated with water (2 mL), MeOH (3 mL), and $MeSO_3H$ (0.4 mL). The mixture was stirred for 15 min and then basified with saturated $NaHCO_3$ solution (∼10 mL) and passed through a hydrophobic frit. The aqueous phase was washed with DCM. The organic phase was evaporated affording a residue (220 mg) that was chromatographed on silica (isohexane/ethyl acetate gradient elution) to give the product **7l** as a white solid (115 mg, 62%). ^{1}H NMR ($CDCl_3$): δ 0.84 (s, 3H), 0.88 (s, 3H), 2.40−2.52 (m, 1H), 3.26−3.39 (m, 1H), 4.48 (br s, 2H). ^{1}H NMR (DMSO-d_6): δ 0.76 (s, 3H), 0.77 (s, 3H), 2.31−2.44 (m, 1H), 2.95−3.10 (m, 1H), 6.40 (br s, 2H), 6.42 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 11.9, 13.4, 20.0, 21.3, 28.0, 28.9, 30.4, 31.3, 34.5, 35.1, 35.3, 35.5, 37.1, 45.2, 47.1, 50.6, 52.2, 53.7, 219.7. LC−MS *m*/*z* 367.1 $[M - 1]^-$, 100% purity. HRMS (ESI) *m*/*z* $[M - H]^-$ calcd for $C_{19}H_{31}N_2O_3S$: 367.2060. Found: 367.2050.

2-[(3β,5α)-20-Oxopregnan-3-yl, 20-cyclic 1,2-ethanediyl acetal]-1*H*-isoindole-1,3(2*H*)-dione 12. To a stirred solution of (3α,5α)-3-hydroxypregnan-20-one, cyclic 1,2-ethanediyl acetal **11** (2.03 g, 5.6 mmol) and TPP (1.63 g, 5.88 mmol) in THF (45 mL) at 20 °C under nitrogen was added phthalimide (0.87 g, 5.88 mmol). The resulting suspension was cooled to 5 °C and stirred for 5 min. A solution of DIAD (1.24 mL, 6.27 mmol) in THF (50 mL) was then slowly added to the reaction mixture, maintaining the temperature below 9 °C. The mixture was stirred at ambient temperature for 16 h. Methanol (100 mL) was added to the reaction mixture, and the resulting slurry was stirred for 30 min. The solid was filtered off and washed with methanol to give the product **12** as a white solid (1.93 g, 70%). ^{1}H NMR ($CDCl_3$): δ 0.79 (s, 3H), 0.98 (s, 3H), 1.32 (s, 3H), 2.22−2.49 (m, 2H), 3.87−4.06 (m, 4H), 4.13−4.24 (m, 1H), 7.69−7.73 (m, 2H), 7.79−7.84 (m, 2H).

(3β,5α)-3-Aminopregnan-20-one, Cyclic 1,2-Ethanediyl Acetal 13. 2-[(3β,5α)-20-Oxopregnan-3-yl, 20-cyclic 1,2-ethanediyl acetal]-1*H*-isoindole-1,3(2*H*)-dione **12** (3.61 g, 7.35 mmol) was treated with ethanol (60 mL) and hydrazine hydrate (5 mL, 103 mmol). The mixture was heated at reflux overnight under a nitrogen atmosphere. The mixture was allowed to cool, and the solid was filtered off. The solid was washed with chloroform and the combined filtrates were evaporated to give the product **13** as a white solid (2.79 g, 100%). ^{1}H NMR ($CDCl_3$): δ 0.76 (s, 3H), 0.80 (s, 3H), 1.30 (s, 3H), 1.98−2.07 (m, 1H), 2.61−2.74 (m, 1H), 3.83−4.05 (m, 4H). ^{13}C NMR ($CDCl_3$): δ 12.4, 13.1, 21.0, 22.9, 23.8, 24.6, 28.8, 32.0, 32.4, 35.0, 35.6, 37.6, 39.1, 39.7, 42.0, 45.6, 51.2, 54.5, 56.4, 58.4, 63.2, 65.2, 112.0.

***N*-[(3β,5α)-20-Oxopregnan-3-yl]urea 14c.** **14c** was prepared and purified in a similar manner to compound **7c**, using (3β,5α)-3-aminopregnan-20-one, 20-cyclic 1,2-ethanediyl acetal **13** (100 mg, 0.28 mmol) and (4-nitrophenyl)-*N*-benzylcarbamate (82.8 mg, 0.30 mmol). Evaporation from ethyl acetate and then purification by preparative HPLC afforded the product **7f** as a white solid (13.8 mg, 14%). ^{1}H NMR ($CDCl_3$): δ 0.60 (s, 3H), 0.78 (s, 3H), 1.57 (s, 3H), 2.52 (t, *J* = 8.8 Hz, 1H), 3.49 (m, 1H), 4.22 (s, 2H), 4.27 (d, *J* = 8.2 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ 12.3, 13.5, 21.1, 22.8, 24.4, 28.4, 29.4, 31.6, 31.9, 35.5, 35.9, 37.4, 39.0, 44.2, 45.4, 50.3, 54.2, 56.6, 63.8, 84.8, 157.8, 209.7. LC−MS *m*/*z* 361.2 $[M + 1]^+$, 100% purity; *m*/*z* 359.0 $[M - 1]^-$, 100% purity.

***N*,*N*-Dimethyl-*N*′-[(3β,5α)-20-oxopregnan-3-yl]urea 14d.** **14d** was prepared and purified in a similar manner to compound **14b**, using (3β,5α)-3-aminopregnan-20-one, 20-cyclic 1,2-ethanediyl acetal **13** (100 mg, 0.28 mmol) and dimethylcarbamyl chloride (0.04 mL, 0.40 mmol). Crystallization from diethyl ether gave product **14d** as a white solid (93 mg, 85%). ^{1}H NMR ($CDCl_3$): δ 0.60 (s, 3H), 0.80 (s, 3H), 2.12 (s, 3H), 2.50−2.58 (m, 1H), 2.89 (s, 6H), 3.56−3.69 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 12.3, 13.5, 21.1, 22.8, 24.4, 28.4, 29.7, 31.6, 31.9, 34.8, 35.5 (×2), 36.2, 36.3, 37.6, 39.0, 44.3, 45.4, 50.2, 54.1, 56.6, 63.8, 157.8, 209.7. LC−MS *m*/*z* 389.2 $[M + 1]^+$, 100% purity; *m*/*z* 387.2 $[M - 1]^-$, 100% purity.

[(3β,5α)-20-Oxopregnan-3-yl]carbamic Acid, Methyl Ester 14e. **14e** was prepared and purified in a similar manner to compound **7h**, using (3β,5α)-3-aminopregnan-20-one, 20-cyclic 1,2-ethanediyl acetal **13** (350 mg, 0.97 mmol) and methyl chloroformate (0.11 mL, 1.45 mmol). Evaporation of the solvent afforded the product **14e** as a white crystalline solid (182 mg, 50%). ^{1}H NMR (DMSO-d_6): δ 0.51 (s, 3H), 0.74 (s, 3H), 2.05 (s, 3H), 2.56 (m, 1H), 3.17−3.33 (m, 1H), 3.49 (s, 3H), 6.98 (d, *J* = 8.0 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ 13.9, 15.1, 22.6, 24.1, 25.9, 28.4, 30.1, 30.2, 33.1, 33.5, 36.9, 37.0, 38.9, 40.1, 45.5, 46.9, 51.8, 52.9, 55.4, 57.8, 64.6, 157.8, 210.5. LC−MS *m*/*z* 376.5 $[M + 1]^+$, 100% purity.

***N*-[(3β,5α)-20-Oxopregnan-3-yl]methanesulfonamide 14f.** **14f** was prepared and purified in a similar manner to compound **7h**, using (3β,5α)-3-aminopregnan-20-one, 20-cyclic 1,2-ethanediyl acetal **13** (390 mg, 1.08 mmol) and methanesulfonyl chloride (0.13 mL, 1.62 mmol). Evaporation of the solvent afforded the product **14f** as a white solid (255 mg, 60%). ^{1}H NMR ($CDCl_3$): δ 0.62 (s, 3H), 0.81 (s, 3H), 2.13 (s, 3H), 2.50−2.58 (m, 1H), 3.00 (s, 3H), 3.25−3.38 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 12.2, 13.5, 21.2, 22.8, 24.4, 28.4, 30.3, 31.6, 31.8, 35.3, 35.4, 36.7, 37.4, 39.0, 42.3, 44.2, 45.5, 53.5, 54.1, 56.6, 63.8, 209.7. LC−MS *m*/*z* 394.1 $[M - 1]^-$, 100% purity. HRMS (ESI) *m*/*z* $[M + Cl]^-$ calcd for $C_{22}H_{37}ClNO_3S$: 430.2188. Found: 430.2201.

***N*-[(3β,5α)-20-Oxopregnan-3-yl]ethanesulfonamide 14g.** **14g** was prepared and purified in a similar manner to compound **7h**, using (3β,5α)-3-aminopregnan-20-one, 20-cyclic 1,2-ethanediyl acetal **13** (100 mg, 0.28 mmol) and ethanesulfonyl chloride (0.04 mL, 0.41 mmol). Evaporation of the solvent afforded the product **14g** as a white solid (72 mg, 63%). ^{1}H NMR ($CDCl_3$): δ 0.61 (s, 3H), 0.80 (s, 3H), 1.39 and 1.41 (2 × d, *J* = 7.4 and 7.2 Hz, 3H), 2.50−2.58 (m, 1H), 3.05 and 3.16 (2 × q, *J* = 7.4 and 7.2 Hz, 2H), 3.21−3.34 (m, 1H). ^{13}C NMR ($CDCl_3$): δ (8.5, 8.7), 12.2, 13.5, 21.1, 22.8, 24.4, 28.4, 30.5, 31.6, 31.8, 35.3, 35.4, 37.0, 37.5, 39.0, (44.2, 45.5), 46.1, 48.4, 53.4, 54.1, 56.6, 63.8, 209.7. LC−MS no peak.

***N*-[(3β,5α)-20-Oxopregnan-3-yl]benzenesulfonamide 14h.** **14h** was prepared and purified in a similar manner to compound **7h**, using (3β,5α)-3-aminopregnan-20-one, 20-cyclic 1,2-ethanediyl acetal **13** (100 mg, 0.28 mmol) and benzenesulfonyl chloride (51 mg, 0.29 mmol). Evaporation of the solvent afforded the product **14g** as a white solid (110 mg, 86%). ^{1}H NMR ($CDCl_3$): δ 0.51 (s, 3H), 0.66 (s, 3H), 2.03 (s, 3H), 2.38−2.48 (m, 1H), 2.99−3.14 (m, 1H), 4.35 (d, *J* = 7.3 Hz, 1H), 7.40−7.54 (m, 3H), 7.79−7.84 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 12.1, 13.4, 21.1, 22.8, 24.4, 28.3, 29.9, 31.5, 31.8, 35.3, 35.4, 36.4, 37.4, 39.0, 44.2, 45.5, 53.4, 54.0, 56.6, 63.8, 126.9 (×2), 129.0 (×2), 132.4, 141.4, 209.6. LC−MS *m*/*z* 456.2 $[M - 1]^-$, 100% purity.

***N*-[(3β,5α)-20-Oxopregnan-3-yl]sulfamide 14i.** **14i** was prepared in a similar manner to compound **7l**, using (3β,5α)-3-aminopregnan-20-one, 20-cyclic 1,2-ethanediyl acetal **13** (100 mg, 0.28 mmol) and sulfamide (242 mg, 2.52 mmol). Trituration of the crude product (111 mg) with diethyl ether gave the product **14i** as a white solid (43 mg, 39%). ^{1}H NMR ($CDCl_3$): δ 0.62 (s, 3H),

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0.81 (s, 3H), 2.13 (s, 3H), 2.50−2.58 (m, 1H), 3.25−3.38 (m, 1H), 4.54 (br s, 2H). ^{13}C NMR ($CDCl_3$): δ 12.2, 13.5, 21.1, 22.8, 24.4, 28.4, 29.7, 31.6, 31.9, 35.3, 35.4, 36.2, 37.5, 39.0, 44.2, 45.5, 53.9, 54.1, 56.6, 63.8, 209.7. LC−MS m/z 95.1 $[M - 1]^-$, 100% purity. HRMS (ESI) m/z $[M - H]^-$ calcd for $C_{21}H_{35}N_2O_3S$: 395.2373. Found: 395.2373.

***N*,*N*-Dimethyl-*N*′-[(3*β*,5*α*)-20-oxopregnan-3-yl]sulfamide 14j.** To a stirred solution of (3*β*,5*α*)-3-aminopregnan-20-one, 20-cyclic 1,2-ethanediyl acetal **13** (100 mg, 0.266 mmol) and dimethylsulfamoyl chloride (42 mg, 0.29 mmol) in DCM (5 mL) at 20 °C under nitrogen was added *N*,*N*-diisopropylethylamine (69 mg, 0.53 mmol), and the resulting mixture was stirred overnight. The reaction mixture was evaporated to dryness and the residue dissolved in MeOH/water (11 mL, 10/1). A few drops of $MeSO_3H$ were added until the solution was acidic. After 10 min the reaction mixture was diluted with water (50 mL), basified with aqueous $NaHCO_3$, and extracted with DCM. Evaporation of the solvent afforded the product **14j** as a white solid (18 mg, 15%). ^{1}H NMR ($CDCl_3$): δ 0.53 (s, 3H), 0.71 (s, 3H), 2.04 (s, 3H), 2.41−2.49 (m, 1H), 2.72 (s, 6H), 3.05−3.18 (m, 1H), 3.77 (br s, 1H). ^{13}C NMR ($CDCl_3$): δ 12.2, 13.5, 21.1, 22.8, 24.4, 28.4, 30.1, 31.5, 31.9, 35.3, 35.4, 36.6, 37.5, 38.1 (×2), 39.0, 44.2, 45.5, 53.8, 54.1, 56.6, 63.8, 209.7. LC−MS m/z 425.1 $[M + 1]^+$, 100% purity; m/z 423.1 $[M - 1]^-$, 100% purity.

2-[(3*α*,5*α*)-17-Oxoandrostan-3-yl, 17-cyclic 1,2-ethanediyl acetal]-1*H*-isoindole-1,3(2*H*)-dione 15. (3*β*,5*α*)-3-Hydroxyandrostan-17-one, cyclic 1,2-ethanediyl acetal (5.83 g, 17.4 mmol), phthalimide (2.56 g, 17.4 mmol), and TPP (5.06 g, 18.3 mmol) were dissolved in THF (140 mL) and cooled to 5 °C using an ice bath. DIAD (3.8 mL, 19.2 mmol) was added slowly, maintaining the temperature below 7 °C. The yellow color was allowed to disappear between additions. The mixture was allowed to warm to ambient temperature overnight under a nitrogen atmosphere. The solvent was evaporated and methanol was added to the oil, forming a white precipitate. The suspension was stirred for 30 min. The precipitate was filtered off and washed with methanol (100 mL), affording the product **15** as a white solid (5.93 g, 74%). ^{1}H NMR ($CDCl_3$): δ 0.77 (s, 3H), 0.78 (s, 3H), 3.75−3.86 (m, 4H), 4.41−4.42 (m, 1H), 7.60−7.65 (m, 2H), 7.71−7.77 (m, 2H).

***N*-Ethyl-*N*′-[(3*α*,5*α*)-17-oxoandrostan-3-yl]urea 17b.** **17b** was prepared and purified in a similar manner to compound **7h**, using (3*α*,5*α*)-3-aminoandrostan-17-one, cyclic 1,2-ethanediyl acetal **16** (100 mg, 0.30 mmol) and ethyl isocyanate (0.05 mL, 0.60 mmol). Evaporation of the solvent afforded the product **17b** as a white crystalline solid (40 mg, 37%). ^{1}H NMR (DMSO-d_6): δ 0.78 (s, 6H), 0.97 (t, J = 7.2 Hz, 3H), 2.32−2.46 (m, 1H), 2.93−3.04 (m, 2H), 3.71−3.79 (m, 1H), 5.62 (br t, J = 5.5 Hz, 1H), 6.01 (d, J = 7.9 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ 11.2, 13.4, 15.7, 19.6, 21.3, 26.5, 27.9, 30.6, 31.4, 32.4, 33.4, 33.8, 34.4, 35.2, 35.7, 43.7, 47.1, 50.8, 54.1, 157.2, 219.7. LC−MS m/z 361.1 $[M + 1]^+$, 100% purity; m/z 359.1 $[M - 1]^-$, 100% purity.

***N*-[(3*α*,5*α*)-17-Oxoandrostan-3-yl]methanesulfonamide 17c.** **17c** was prepared and purified in a similar manner to compound **7h**, using (3*α*,5*α*)-3-aminoandrostan-17-one, cyclic 1,2-ethanediyl acetal **16** (100 mg, 0.30 mmol) and methanesulfonyl chloride (0.03 mL, 0.45 mmol). Evaporation of the solvent afforded the product **17b** as a white crystalline solid (81 mg, 73%). ^{1}H NMR (DMSO-d_6): δ 0.77 (s, 3H), 0.78 (s, 3H), 2.32−2.44 (m, 1H), 2.87 (s, 3H), 3.49−3.56 (m, 1H), 6.91 (br d, J = 6.0 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ 10.7, 12.8, 19.0, 20.7, 26.1, 27.1, 29.8, 30.7, 31.2, 33.0, 33.8, 34.6, 34.9 (×2), 38.2, 46.4, 48.0, 50.1, 53.1, 219.1. LC−MS m/z 366.1 $[M - 1]^-$, 100% purity.

***N*-[(3*α*,5*α*)-17-Oxoandrostan-3-yl]sulfamide 17d.** **17d** was prepared in a similar manner to compound **7l**, using (3*α*,5*α*)-3-aminoandrostan-17-one, cyclic 1,2-ethanediyl acetal **16** (214 mg, 0.64 mmol) and sulfamide (685 mg, 7.13 mmol). The 17-ketal was deprotected in a similar manner to compound **7a**. The residue was chromatographed on silica (10 g SNAP, DCM/MeOH gradient elution). Evaporation of the solvent afforded the product **17d** as a white solid (119 mg, 50%). ^{1}H NMR (DMSO-d_6): δ 0.77 (s, 3H), 0.78 (s, 3H), 2.32−2.44 (m, 1H), 3.42−3.51 (m, 1H), 6.36 (br d, J = 5.9 Hz, 1H), 6.40 (br s, 2H). ^{13}C NMR (DMSO-d_6): δ 11.4, 13.4, 19.6, 21.3, 26.2, 27.8, 30.5, 31.4, 31.9, 33.1, 34.4, 35.2, 35.4, 38.7, 47.1, 48.2, 50.8, 53.6, 219.8. LC−MS m/z 367.1 $[M - 1]^-$, 100% purity.

***N*-Ethyl-*N*′-[(5*α*)-21-hydroxy-20-oxopregnan-3-yl]urea 18a.** *N*-Ethyl-*N*′-[(5*α*)-20-oxopregnan-3-yl]urea was prepared from (5*α*)-3-aminopregnan-20-one, 20-cyclic 1,2-ethanediyl acetal **13** in a similar manner to urea **7e**. To a stirred solution of *N*-ethyl-*N*′-[(5*α*)-20-oxopregnan-3-yl]urea (100 mg, 0.26 mmol) and triethylamine (1.07 mL, 7.72 mmol) in DCM (1 mL) at 20 °C under nitrogen was added *tert*-butyldimethylsilyl trifluoromethanesulfonate (0.59 mL, 2.57 mmol), and the resulting mixture was stirred at ambient temperature overnight. The mixture was diluted with DCM (10 mL) and washed with saturated $NaHCO_3$ solution (2 × 10 mL). Evaporation afforded the silyl enol ether intermediate which was redissolved in DCM (5 mL). A suspension of *m*-CPBA (95 mg, 0.41 mmol) in isohexane (10 mL) was cooled to −10 °C, and a solution of the silyl enol ether in DCM was added slowly over 5 min. The mixture was stirred at −10 °C for 15 min and then stirred at ambient temperature for 60 min. The solvent was evaporated and the residue chromatographed on silica (10 g SNAP cartridge, DCM/MeOH gradient elution). Evaporation of the solvent from different fractions afforded the TBDMS ether (80 mg) and the product **18a** as a white foam (20 mg, 19%). ^{1}H NMR (DMSO-d_6): δ 0.50 (s, 3H, 3*α* and 3*β*), 0.72 (s, 3H, 3*α* and 3*β*), 2.88−3.01 (m, 2H, 3*α* and 3*β*), 3.67−3.77 (m, 1H, 3*β*-H of 3*α* urea), 4.00 (br d, J = 5.3 Hz, 2H, 3*α* and 3*β*), 4.82−4.90 (m, 1H, 3*α* and 3*β*), 5.53−5.64 (m, 2H, 3*α* and 3*β*), 5.93−6.03 (m, 1H, N−H of 3*α* urea). LC−MS m/z 405.1 $[M + 1]^+$, 100% purity; m/z 403.1 $[M - 1]^-$, 82% purity.

[(3*β*,5*α*)-21-Hydroxy-20-oxopregnan-3-yl]carbamic Acid, Methyl Ester 18b. **18b** was prepared in a similar manner to compound **18a**, using [(3*β*,5*α*)-20-oxopregnan-3-yl]carbamic acid, methyl ester **14e** (270 mg, 0.72 mmol), except the intermediate TBDMS ether was treated with THF (5 mL) and 2 M HCl (1 mL). The mixture was stirred overnight. The solvent was evaporated and the residue partitioned between water and DCM. The mixture was passed through a hydrophobic frit and the DCM evaporated. The residue was triturated with diethyl ether to give the product **18b** as an off-white solid (33 mg, 12%). ^{1}H NMR ($CDCl_3$): δ 0.54 (s, 3H), 0.70 (s, 3H), 2.33−2.41 (m, 1H), 3.30−3.45 (m, 1H), 3.57 (s, 3H), 4.05, 4.13 (ABq, J_{AB} = 18.9 Hz, 2H), 4.36−4.48 (br s, 1H). ^{1}H NMR (DMSO-d_6): δ 0.49 (s, 3H), 0.71 (s, 3H), 2.50−2.58 (m, 1H), 3.13−3.30 (m, 1H), 3.46 (s, 3H), 4.00 (br d, J = 5.9 Hz, 2H), 4.87 (t, J = 5.9 Hz, 2H), 6.95 (br d, J = 7.8 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ 11.9, 13.3, 20.6, 22.3, 24.1, 28.1, 28.2, 31.5, 34.9, 35.0, 37.0, 38.0, 43.9, 44.9, 45.5, 50.9, 53.4, 56.0, 57.7, 68.7, 160.3, 210.4. LC−MS m/z 392.3 $[M + 1]^+$, 100% purity; m/z 390.3 $[M - 1]^-$, 100% purity.

***N*-[(3*β*,5*α*)-21-Hydroxy-20-oxopregnan-3-yl]methanesulfonamide 18c.** To a stirred solution of *N*-[(3*β*,5*α*)-20-oxopregnan-3-yl]methanesulfonamide **14f** (500 mg, 1.26 mmol) in methanol (30 mL) at ambient temperature was added aqueous HBr (3 drops), and then a solution of bromine (0.13 mL, 2.53 mmol) in methanol (10 mL) was added slowly over 10 min. The resulting mixture was stirred for 2.5 h. (Unlike bromination of the corresponding 3-alcohols the solution did not decolorize upon the slow addition of bromine.) The mixture was diluted with water, extracted with ethyl acetate, and evaporated to leave a residue (640 mg). This residue was chromatographed on silica (isohexane/acetone gradient elution) and the crude product crystallized from diethyl ether and then ethyl acetate to give the 21-bromo intermediate (85 mg) in adequate purity for the next step. To a stirred solution of the 21-bromo intermediate (80 mg, 0.17 mmol) in DMF (10 mL) and water (5 mL) was added sodium hydroxide (8 mg, 0.20 mmol), and the mixture was stirred at ambient temperature for 30 min. The mixture was diluted with water and extracted with ethyl acetate. The extracts were washed with water, and the solvent was evaporated. The residue was chromatographed on silica (isohexane/ethyl acetate gradient elution) and the residue recrystallized from diethyl ether to give the product **18c** as a white crystalline solid (41 mg, 8%). ^{1}H NMR (DMSO-d_6): δ 0.52 (s, 3H), 0.74 (s, 3H), 2.52−2.61 (m, 1H), 2.88 (s, 3H), 3.00−3.15 (m, 1H), 3.97−4.07 (m, 2H), 4.89 (t, J = 5.7 Hz, 1H), 6.94 (d, J = 7.6 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ 11.8, 13.3, 20.6, 22.3, 24.1, 28.1, 29.4, 31.5, 34.8, 34.9,

35.9, 37.0, 38.0, 41.2, 43.9, 44.9, 52.3, 53.4, 56.0, 57.7, 68.7, 210.4. LC−MS m/z 410.1 $[M - 1]^-$, 88% purity. HRMS (ESI) m/z $[M - H]^-$ calcd for $C_{22}H_{36}NO_4S$: 410.2370. Found: 410.2387.

***N*-[(3β,5α)-21-Hydroxy-20-oxopregnan-3-yl]sulfamide 18d.** **18d** was prepared in a similar manner to compound **18c**, using *N*-[(3β,5α)-20-oxopregnan-3-yl]sulfamide **14i** (120 mg, 0.30 mmol). The residue was triturated with diethyl ether to give the product **18d** as a white solid (55 mg, 44%). ^{1}H NMR ($CDCl_3$): δ 0.56 (s, 3H), 0.72 (s, 3H), 2.34−2.42 (m, 1H), 3.23 (m, 1H), 4.07, 4.15 (ABq, J_{AB} = 19.0 Hz, 2H), 4.38 (br s, 2H). ^{13}C NMR (DMSO-d_6): δ 11.3, 12.7, 20.0, 21.7, 23.5, 27.6, 28.3, 31.0, 34.3 (×2), 34.9, 36.6, 37.4, 43.3, 44.5, 51.6, 52.9, 55.3, 57.1, 68.1, 209.8. LC−MS m/z 411.1 $[M - 1]^-$, 95% purity. HRMS (ESI) m/z $[M - H]^-$ calcd for $C_{21}H_{35}N_2O_4S$: 411.2323. Found: 411.2327.

1-[(3β,5α)-3-Hydroxyandrostan-17-yl]-2-propanone 23. To a stirred solution of (3β,5α,17*E*)-3-[[(1,1-dimethylethyl)dimethylsilyl]-oxy]pregn-17(20)-en-21-oic acid, ethyl ester **19** (1. g, 2.11 mmol) in ethyl acetate (30 mL) was added Pd/C 10% (85 mg) as a suspension in ethanol (2 mL) under nitrogen. The mixture was then hydrogenated at ambient temperature for 16 h. The mixture was filtered through Celite, washed with ethyl acetate and the combined filtrates were evaporated to give (3β,5α)-3-[[(1,1-dimethylethyl)dimethylsilyl]-oxy]pregnan-21-oic acid, ethyl ester, **20** (920 mg, 1.93 mmol, 92%). ^{1}H NMR ($CDCl_3$): δ 0.00 (s, 6H), 0.53 (s, 3H), 0.75 (s, 3H), 0.83 (s, 9H), 1.13 (t, J = 7.1 Hz, 3H), 1.99−2.10 (m, 1H), 2.25−2.34 (m, 1H), 3.43−3.56 (m, 1H), 4.06 (q, J = 7.1 Hz, 2H).

To a stirred solution of (3β,5α)-3-[[(1,1-dimethylethyl)dimethylsilyl]-oxy]pregnan-21-oic acid, ethyl ester, **20** (835 mg, 1.75 mmol) in THF (10 mL) was added a solution of lithium hydroxide (84 mg, 3.5 mmol) in water (4 mL). The mixture was stirred for 4 h, heated at 50 °C for 3 h, and then left to cool for 72 h. The solvent was evaporated and the residue treated with 2 N HCl and then extracted with DCM. The organic liquors were washed with water, dried, and evaporated to give the crude product (648 mg). Crystallization from diethyl ether/isohexane gave (3β,5α)-3-[[(1,1-dimethylethyl)dimethylsilyl]oxy]pregnan-21-oic acid **21** as a white solid (690 mg, 1.54 mmol, 88%). ^{1}H NMR ($CDCl_3$): δ 0.00 (s, 6H), 0.54 (s, 3H), 0.75 (s, 3H), 0.83 (s, 9H), 2.03−2.14 (m, 1H), 2.31−2.40 (m, 1H), 3.43−3.56 (m, 1H).

To a stirred solution of (3β,5α)-3-[[(1,1-dimethylethyl)dimethylsilyl]-oxy]pregnan-21-oic acid **21** (367 mg, 0.82 mmol) in a mixture of DMF (3.9 mL) and DCM (3.9 mL) was added 1,1′-carbonyldiimidazole (159 mg, 0.98 mmol), and the mixture was stirred for 2 h. *N*,*O*-Dimethylhydroxylamine hydrochloride (239 mg, 2.45 mmol) and triethylamine (0.34 mL, 2.45 mmol) were added, and the mixture was stirred overnight. The DCM was evaporated and water (30 mL) added. The precipitated solid was filtered off and washed with water. The crude product was purified by flash chromatography (isohexane/ethyl acetate gradient elution) to give (3β,5α)-3-[[(1,1-dimethylethyl)dimethylsilyl]-oxy]-*N*-methoxy-*N*-methyl-pregnan-21-amide **22** (370 mg, 0.75 mmol, 92%). ^{1}H NMR ($CDCl_3$): δ 0.00 (s, 6H), 0.56 (s, 3H), 0.75 (s, 3H), 0.83 (s, 9H), 2.12−2.23 (m, 1H), 2.37−2.46 (m, 1H), 3.12 (s, 3H), 3.44−3.56 (m, 1H), 3.63 (s, 3H).

To a stirred solution of (3β,5α)-3-[[(1,1-dimethylethyl)dimethylsilyl]-oxy]-*N*-methoxy-*N*-methylpregnan-21-amide **22** (98 mg, 0.20 mmol) in THF (2 mL) at 0 °C was added methylmagnesium bromide (0.71 mL, 0.99 mmol). The mixture was stirred for 2 h at ambient temperature. More methylmagnesium bromide (0.71 mL, 0.99 mmol) was added, and stirring continued for a further 3 h. Then 2 N HCl (10 mL) was added cautiously and the mixture was stirred for 10 min and then extracted with DCM. The organic liquors were washed with saturated sodium bicarbonate, dried, and evaporated. The residue was dissolved in acetone (5 mL), 2 N HCl (1 mL) added, and the mixture stirred for 2 h. The mixture was evaporated and the residue dissolved in DCM. This solution was passed through a hydrophobic frit and purified by flash chromatography (isohexane/ethyl acetate gradient elution) to give product **23** (16 mg, 0.048 mmol, 24%). ^{1}H NMR ($CDCl_3$): δ 0.57 (s, 3H), 0.81 (s, 3H), 2.13 (s, 3H), 2.22 (dd, J = 6.7 and 4.0 Hz, 1H), 2.47 (dd, J = 15.5 and 10.1 Hz, 1H), 3.59 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 12.6, 13.1, 21.2, 24.9, 28.6, 28.9, 30.6, 31.8, 32.4, 35.9, 37.3, 37.8, 38.5, 42.5, 45.1, 45.2, 46.3, 54.9, 55.5, 71.6, 84.9, 209.9. LC−MS no peak.

(3β,5α,17α)-17,21-Epoxypregnan-3-ol 25. To a stirred suspension of trimethylsulfoxonium iodide (439 mg, 1.99 mmol) in *tert*-butanol (5 mL) at 50 °C was added a solution of potasium *tert*-butoxide (224 mg, 1.99 mmol) in *tert*-butanol (5 mL). After 30 min a solution of (3β,5α,17β)-spiro[androstane-17,27′-oxiran]-3-ol **24** (304 mg, 0.99 mmol) in *tert*-butanol (5 mL) was added and the mixture heated at reflux for 72 h. The reaction mixture was concentrated to low volume and diluted with DCM. This solution was washed with water and the solvent evaporated. The residue (324 mg) was flash chromatographed on silica (isohexane/ethyl acetate gradient elution) and the residue (133 mg) recrystallized from diethyl ether to give product **25** as a white crystalline solid (41 mg, 6.5%). ^{1}H NMR ($CDCl_3$): δ 0.76 (s, 3H), 0.83 (s, 3H), 2.72−2.85 (m 1H), 3.54−3.67 (m 1H), 4.27−4.36 (m 1H), 4.39−4.48 (m 1H). ^{13}C NMR ($CDCl_3$): δ 12.0, 12.4, 18.6, 20.8, 23.1, 28.6, 30.9, 31.5, 32.2, 35.6, 36.1, 37.1, 37.8, 38.2, 44.6, 44.9, 48.3, 54.2, 64.9, 71.3, 96.7. LC−MS no peak.

(3β,5α)-3-Hydroxy-17-methoxypregnan-20-one 26. To a stirred solution of (3β,5α)-3-hydroxypregnan-20-one **9** (318 mg, 0.99 mmol) in methanol (30 mL) was added aqueous copper(II) bromide (669 mg, 3.0 mmol), and the resulting mixture was stirred at reflux for 24 h.[34] The mixture was diluted with water and extracted with ethyl acetate. The extracts were evaporated and the residue (450 mg) was chromatographed on silica (isohexane/ethyl acetate gradient elution). Recrystallization of the residue from diethyl ether gave product **26** as a white crystalline solid (83 mg, 24%). ^{1}H NMR ($CDCl_3$): δ 0.58 (s, 3H), 0.82 (s, 3H), 2.15 (s, 3H), 2.37−2.49 (m, 1H), 3.15 (s, 3H), 3.54−3.68 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 12.3, 15.0, 21.0, 23.1, 23.8, 26.6, 28.6, 30.9, 31.5, 32.0, 35.4, 35.6, 37.0, 38.1, 44.8, 47.6, 51.2, 52.3, 53.7, 71.4, 97.4, 211.4. LC−MS no peak.

Biological Experimental Methods. Protein Production. Recombinant human G6PD (NM_000402.3) was cloned into a pHis expression vector (University of Manchester, U.K.) to generate a N-terminal His tagged construct and the protein expressed in *E. coli* JM109(DE3) cells. Purification was performed via a single step nickel affinity column (Qiagen) followed by imidazole elution to afford protein with >70% purity. The determined kinetic parameters of the recombinant enzyme (G6P K_m = 47 μM, $NADP^+$ K_m = 5.5 μM) were in accordance with previously published values for this enzyme.[35,36]

Enzyme Assay. The assay was performed in a 25 μL final volume in 384-well black plates (Corning, 3575) with a reaction buffer composed of 20 mM Tris-HCl, pH 8.0, 10 mM $MgCl_2$, 0.03% BSA, 0.5 mM glucose 6-phosphate, 1% DMSO-d_6 plus or minus inhibitory compound, and 1 ng of G6PD. The reaction was initiated by the addition of 50 μM NADP, and the assay was allowed to proceed at 25 °C for 30 min before stopping by the addition of 0.05% SDS. The NADPH generated during the assay was then quantified via an Amplite fluorimetric NADPH assay kit (AAT Bioquest) in accordance with the manufacturer's instructions, and the assay plate was read on a BioTek Synergy 2 multimode microplate reader with excitation and emission at 540 and 590 nm, respectively.

Cell (6PG) Assay. HEK293T cells (1 mL) were plated at 16×10^4 mL^{-1} in each well of a 24-well poly-D-lysine coated plate. After 18 h, compounds at 100 mM in DMSO-d_6 were further diluted in DMSO-d_6 (to generate a dose response from 1 to 100 μM final concentration), and 1 μL of compound solution was added to each well. At 30 min after compound addition, 200 mM 6-AN was diluted 1:10 in serum-free medium and 10 μL added to all wells. After 5 h of incubation, cells were rinsed with cold PBS and 500 μL of −80 °C MeOH/H_2O (80:20) was added directly to the monolayer to lyse the cells. Cell extracts were transferred to Eppendorf tubes and centrifuged at 20000*g* for 10 min at 4 °C. The supernatant was removed to a fresh tube and stored at −80 °C prior to analysis by mass spectrometry. Relative concentrations of 6-PG in each supernatant sample were measured using LC−MS/MS. Liquid chromatography was performed using a Macherey-Nagel, Nucleosil 100-5C18 Nautilus column, particle size 5 μm, 70 mm × 3.0 mm (catalog no. 721134.30), heated to 35 °C. An isocratic mobile phase of 100% 2 mM ammonium acetate in water, flow rate of 700 μL/min with a run time of 2.5 min, was used. Quantitative detection of 6PG was undertaken using a Sciex API4000

Q-Trap turbospray operating in negative ion mode. Ions monitored in multiple reaction monitoring (MRM) were m/z 275−177.

■ ASSOCIATED CONTENT

Supporting Information

Summary of purity data; LC−MS methods and solvent gradients; preparative HPLC instrument and solvent gradients; preparative methods and spectroscopic data for compounds **1**−**4**, **8**−**11**, **14a**, **14b**, **16**, **17a**, **19**, **24**, and **27**−**47**; ^{1}H NMR spectra of target compounds; additional references. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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JChem for Excel was used for structure property prediction and calculation, and general data handling (JChem for Excel, version 5.4.0.411, 2008−2009, ChemAxon (http://www.chemaxon.com)).

■ ABBREVIATIONS USED

DHEA, dehydroepiandrosterone; EA, epiandrosterone; G6PD, glucose 6-phosphate dehydrogenase; PPP, pentose phosphate pathway; NADP$^+$, nicotinamide-adenine dinucleotide phosphate; NADPH, reduced nicotinamide adenine dinucleotide phosphate; 6PGD, 6-phosphogluconate dehydrogenase; SAR, structure−activity relationship

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