Bioorganic & Medicinal Chemistry Letters 24 (2014) 2444–2447

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Highly-selective 4-(1,2,3-triazole)-based P450c17a 17,20-lyase inhibitors

Stephen W. Rafferty, Joel R. Eisner, William R. Moore, Robert J. Schotzinger, William J. Hoekstra *

Viamet Pharmaceuticals, Inc., Durham, NC 27703, USA

ARTICLE INFO

Article history:
Received 13 March 2014
Revised 3 April 2014
Accepted 7 April 2014
Available online 16 April 2014

Keywords:
CYP17 lyase
Prostate cancer

ABSTRACT

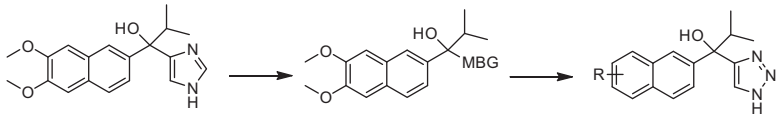
The orally-active CYP17A1 inhibitor abiraterone acetate (AA) decreases adrenal and intratumoral androgen biosynthesis and is an effective agent for the treatment of prostate cancer. Abiraterone potently inhibits both reactions catalyzed by CYP17, the 17α-hydroxylase (hydroxylase) reaction as well as the 17,20-lyase (lyase) transformation. CYP17 hydroxylase inhibition prevents the synthesis of adrenal glucocorticoids and causes an accumulation of circulating mineralocorticoids. As a consequence of potent CYP17 hydroxylase inhibition (i.e., lack of lyase selectivity), AA must be co-administered with the cortisol replacement prednisone and patients may experience the effects of mineralocorticoid excess syndrome (MES). Herein, we describe rationally-designed, CYP17 lyase-selective inhibitors that could prove safer and more effective than abiraterone. Using proprietary methodology, the high-affinity pyridine or imidazole metal-binding group found in current clinical CYP17 inhibitors was replaced with novel, less avid, metal-binding groups in concert with potency-enhancing molecular scaffold modifications. This process produced a unique series of CYP17 lyase-selective inhibitors that included the oral agent **6** (VT-464), now in Phase 2 prostate cancer clinical trials. The chemical methodology described is potentially applicable to the design of new and more effective metalloenzyme inhibitor treatments for a broad array of diseases.

Cytochrome P-450 enzymes (CYPs), due to high homology inclusive of a common heme–iron motif, present a major challenge to the discovery of target-selective inhibitors. The potent CYP17 inhibitor, abiraterone acetate, inhibits cortisol biosynthesis and produces side-effects associated with MES at effective doses.[1] Our metalloenzyme inhibitor design strategy, which focuses on the metal-binding group (MBG), has produced some of the most selective CYP17[2,3] and fungal CYP51A1 (lanosterol 14α-demethylase)[4] inhibitors reported for the potential treatment of prostate cancer and fungal infections, respectively. This iterative technology approach synergistically combines the application of inorganic chemistry with classical medicinal chemistry (i.e., in silico-derived MBGs are affixed to progressable chemical scaffolds). The process is exemplified in Scheme 1 wherein starting point **1** is a reported potent inhibitor of rat CYP17 lyase (IC_{50} = 0.029 μM) and hydroxylase (IC_{50} = 0.14 μM) as well as the human enzyme (IC_{50}s are 0.05 and 0.22 μM for lyase and hydroxylase activities, respectively).[5] Given its potency, lyase selectivity, and physicochemical properties, imidazole **1** was chosen as a non-steroidal starting point to more lyase-selective CYP17 inhibitors. A survey of potential alternative MBGs[2] [e.g., less basic 4-(1,2,3-triazole)] was followed by further scaffold optimization of the naphthyl-triazolyl-butanol inhibitor series **3** as described below.

Many metalloenzyme inhibitors consist of two chemical components: the MBG, the portion of the inhibitor designed to bind to the metal, and the scaffold, the portion of the inhibitor recognized by the amino acid residues that form the substrate-binding site of the metalloenzyme. The MGB is often a major contributor to the overall potency of the inhibitor (though it is acknowledged that many examples of metalloenzyme inhibitors have been reported that do not utilize a MBG). The attraction of the MBG to the metal ion is governed by electronic factors. The metal ion is generally electron-deficient, while the MBG is nucleophilic. The magnitude of the MBG's interaction with the metal, and therefore inhibitor potency, can be 'tuned' by modulating its electronic character. If the metal/MBG interaction is strong the inhibitor will certainly be effective against the intended target but may also inhibit unintended related metalloenzymes. Our approach has been to attenuate the magnitude of the MGB/metal interaction in order to initially improve target selectivity while retaining residual potency. Inhibitor potency and selectivity are then further improved through modifications to the scaffold that increase the magnitude of interactions within the substrate binding pocket.

* Corresponding author. Tel.: +1 919 467 8539x303.
E-mail address: whoekstra@viamet.com (W. J. Hoekstra).

http://dx.doi.org/10.1016/j.bmcl.2014.04.024
0960-894X/



Scheme 1.

MBGs were initially selected using an in silico method that rank ordered them by affinity for heme–iron. Emphasis was placed on MBGs with lower affinity than the current high-potency imidazoles and pyridines that appear in inhibitors that respectively show modest (TAK-700) or no selectivity (abiraterone) for CYP17 lyase compared to hydroxylase. Due to lack of lyase selectivity, these inhibitors must be administered with prednisone to address MES-associated side-effects (hyperkalemia, hypertension).[1]

CYP17, a steroidogenic enzyme that is required for androgen biosynthesis, catalyzes two sequential chemical steps. CYP17 hydroxylase catalyzes the conversion of progesterone and pregnenolone to their 17-hydroxy analogs and is required for the synthesis of glucocorticoids (Fig. 1).[6] CYP17 lyase breaks the covalent bond between C_{17} and C_{20} of 17-hydroxypregnenolone, forming the androgen, dehydroepiandrosterone (DHEA), and thus represents the first committed step in sex steroid biosynthesis. Though inhibition of either CYP17 hydroxylase or lyase should result in decreased androgen biosynthesis, lyase is the preferred point of intervention since hydroxylase inhibition also prevents glucocorticoid synthesis and causes the accumulation of mineralocorticoids. Since cortisol is the most abundant steroid synthesized and secreted by the adrenal gland, mineralocorticoids get pulled by mass action into the glucocorticoid pathway and will unlikely accumulate as a result of CYP17 lyase-selective inhibition.[7]

A key challenge to the design of lyase-selective inhibitors is that the single-chain CYP17 protein uses the same active site to catalyze both the lyase and hydroxylase reactions.[8] Both reactions require heme–iron and oxidoreductase cofactors, as well as NADPH and molecular oxygen as co-substrates. However, CYP17 has an additional cofactor, cytochrome b5, that enhances lyase activity.[9] The crystal structure of the CYP17 hydroxylase conformation has been solved but not the CYP17-cytochrome b5 complex.[10] Thus, there is little structural information to guide the design of CYP17 lyase-selective inhibitors and their optimization has been empirical to date. Not surprisingly, given the dearth of structural information and protein target similarity constraints, all CYP17 inhibitors reported to date affect both the hydroxylase and lyase enzyme functions to some extent. Our design and discovery strategy focused on the investigation of alternative, lower-affinity MBGs on the premise that lyase-selective CYP17 inhibitors may provide safer and more effective clinical agents. Herein, we disclose a unique series of inhibitors, including the Phase 2 clinical agent **6** (VT-464), which exhibit selective potency for CYP17 lyase.

Compounds **2a**–**2i** are representatives of an array of MBG targets (**2**, Scheme 1) related to imidazole standard **1**[5] that were synthesized and initially tested in a rat CYP17 lyase screening assay (Table 1).[11] The inhibitors were synthesized as racemates by coupling of 6,7-dimethoxy-naphthalene-2-isopropyl ketone with the requisite lithiated heterocycle in modest yields (15–65%; Scheme 2).[12,13] Heteroaromatic MBGs were selected that encompassed a wide range of basicities though emphasis was placed on low-affinity moieties that might demonstrate selectivity for rat CYP17 lyase versus the metabolic human enzyme CYP3A4.[14] As shown in Table 1, the oxazole **2f** and 4-pyridine **2h** furnished adequate lyase potency (IC_{50}s of 86 and 60 nM) but both inhibited CYP3A4 as well (IC_{50}s <10 μM). An MBG that exhibited threshold potency (IC_{50} = 0.18 μM) and markedly enhanced selectivity over CYP3A4 (317-fold) was the 4-(1,2,3-triazole) **2c**. This promising MBG was selected for further optimization within the naphthalene chemical series.

Triazole **2c** exhibited equal potency for rat and human CYP17 lyase (IC_{50} = 0.18 μM) and was selected for further optimization through scaffold modifications to achieve the following thresholds: h-CYP17 lyase potency IC_{50} <0.2 μM, h-CYP17 lyase/hydroxylase selectivity >10-fold, and selectivity >50-fold for h-CYP17 lyase versus h-CYP3A4. Representative 4-(1,2,3-triazoles) **3a**–**f** containing a variety of substituents (*R*) at the 6- and 7-positions were synthesized and tested for human lyase, human hydroxylase, and CYP3A4 potency in comparison to imidazole **1** (Table 2).[12] Several molecules in this chemical series (**2c**, **3b**, **3c**) exhibited improved lyase selectivity versus hydroxylase and CYP3A4 (H/L and 3A4/L

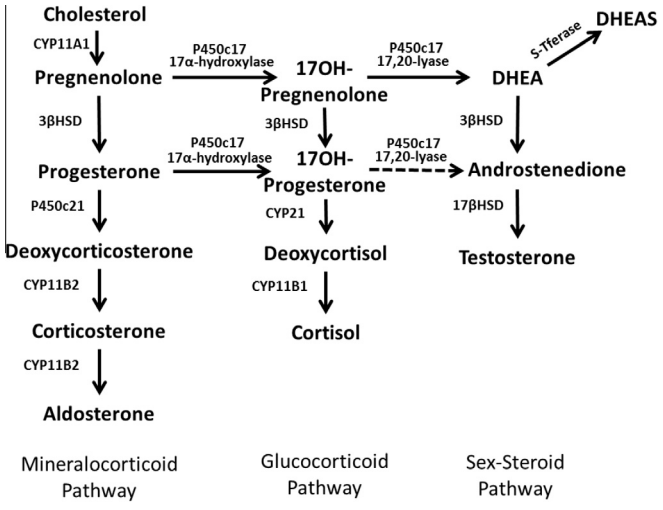
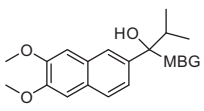


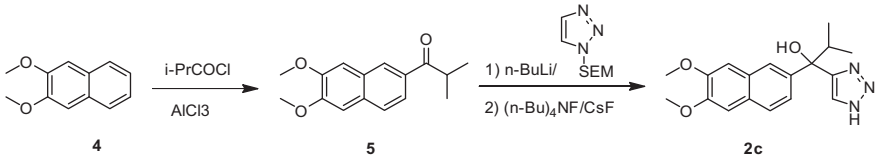
Figure 1. Human adrenal steroid biosynthetic pathway.

Table 1
In vitro activity of naphthalene-based CYP17 inhibitors



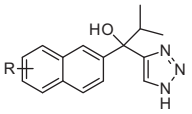
No.	MBG	Rat CYP17 lyase IC_{50}[a,11]	h-CYP3A4 IC_{50}[b,11]
2a	3-Pyrazole	1.6	1.8
2b	4-Pyrazole	0.43	27
2c	4-(1,2,3-Triazole)	0.18	57
2d	3-(1,2,4-Triazole)	7.1	100
2e	5-Thiazole	0.29	0.77
2f	5-Oxazole	0.086	6.5
2g	3-Pyridine	4.2	9.4
2h	4-Pyridine	0.060	4.5
2i	4-Pyrimidine	0.44	18
1[5]	4-Imidazole	0.029	1.6

[a] IC_{50} (μM) measured in a rat testicular microsome preparation.
[b] IC_{50} (μM) measured in a pooled human hepatocyte microsome preparation.



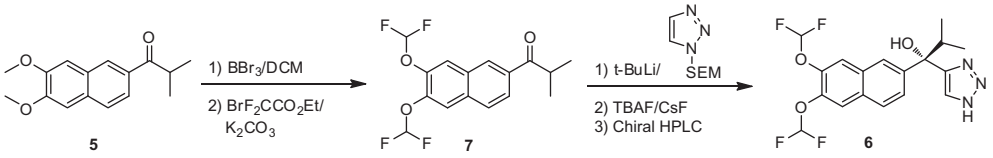
Scheme 2.

Table 2
In vitro CYP potency and selectivity of triazolyl naphthalene-based CYP17 inhibitors



No.	R	h-Lyase IC_{50}[a]	h-Hydroxylase IC_{50}[a,11]	h-H/L ratio[b]	h-CYP3A4 IC_{50}[c]	3A4/L ratio
2c	6,7-Di-OMe	0.18	2.3	13	57	320
3a	6,7-Di-OCHF2	0.17	0.69	3.9	23	140
3b	6,7-Di-OCH_2CF_3	0.31	3.7	12	25	80
3c	6-(5-oxazole)	0.94	13	14	102	110
3d	6-$OCHF_2$	0.33	0.074	0.22	14	42
3e	6-OCH_2CF_3	0.57	1.0	1.8	⩾195	⩾340
3f	6-(2-Thiophene)	>4	12	<3.0	81	<20
1	—	0.05	0.22	4.4	1.6	32

[a] IC_{50} (μM) measured in a recombinant human enzyme yeast microsome system.
[b] Human IC_{50} ratio as an indicator of lyase selectivity; H = hydroxylase, L = lyase.
[c] Assay described in Table 1.

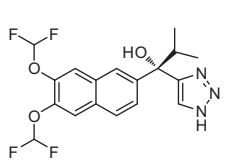


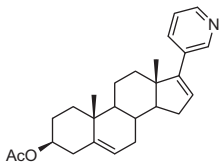
Scheme 3.

ratios, Table 2) compared to the literature 1-imidazole (**1**). While **2c** exhibited good lyase potency and promising selectivity it was not evaluated further due to poor human liver microsome stability (29% parent **2c** remaining after 60 min compared to 54% remaining for **3a**).

Racemic compound **3a**, an analogue of **2c**, furnished superior lyase potency and selectivity. Though the active enantiomer of **3b** also exhibited excellent selectivity, it furnished inferior lyase potency compared to racemic **3a** (**3b** enantiomer IC_{50} data not shown). The enantiomers of **3a** were synthesized and isolated

Table 3
In vitro potency, selectivity, and oral activity of 3 classes of CYP17 inhibitors





No.	h-Lyase IC_{50}[a]	h-Hydroxylase IC_{50}[a]	h-H/L ratio	Mean testosterone ± SE[b] (ng/mL)	Mean progesterone ± SE[b] (ng/mL)
6	0.069	0.670	9.7	0.37 ± 0.02*	1.2 ± 0.1
1[c]	0.05	0.22	4.4	0.30 ± 0.0[d,*]	3.9 ± 0.9*
AA	0.015	0.0025	0.17	0.30 ± 0.0[d,*]	2.1 ± 0.3[d,*]

[a] Method described in Table 2.
[b] Plasma steroid values measured by LC/MS/MS 2 h post-dose.
[c] Racemate **1** was used for h-lyase and hydroxylase IC_{50} determinations and administration to hamsters.
[d] All values at or below the LLOQ of 0.3 ng/mL.
* $p \leqslant 0.05$ versus 2 h vehicle control values of 1.98 ± 0.057 ng/mL for testosterone and 1.02 ± 0.05 ng/mL for progesterone.

according to Scheme 3.[12] Briefly, 6,7-dimethoxy-naphthyl-isopropyl ketone **5** was demethylated under strongly-acidic conditions (boron tribromide) and the resultant *o*-dihydroxy-naphthalene intermediate then capped with two difluoromethyl groups using standard base-mediated α-bromo-difluoroacetate conditions to furnish intermediate **7**. Ketone **7** was then converted to **3a** by sequential addition of lithiated 1-*N*-SEM-triazole and subsequent SEM removal with fluoride. Resolution of the antipodes of **3a** using chiral HPLC provided a potent CYP17 lyase inhibitor **6**[15] that demonstrated both exceptional in vitro lyase/hydroxylase selectivity (~10-fold)[2,3] and oral activity in a hamster model of androgen biosynthesis inhibition (Table 3).[16,17]

Inhibitor **6** and the comparator compounds abiraterone acetate (AA), an approved CYP17 inhibitor,[1] and the active imidazole **1**,[5] were evaluated for their effects on steroid biosynthesis in hamster following a single 50 mg/kg oral dose.[16,17] All three CYP17 inhibitors significantly decreased plasma testosterone concentrations to the lower limit of quantitation (LLOQ) 2 h post-dose (Table 3). However, administration of the 3-pyridine, AA, and the imidazole **1** produced a statistically-significant increase in plasma progesterone, a marker for CYP17 hydroxylase inhibition. In contrast, triazole **6** administration produced only a modest increase in progesterone consistent with its superior in vitro CYP17 lyase/hydroylase selectivity. Similar in vivo effects on testosterone and progesterone following oral administration of **6** and AA were observed in castrate monkeys.[3]

In summary, we have described the design, synthesis, and discovery of novel 4-(1,2,3-triazole)-based CYP17 lyase-selective inhibitors. An orally active representative from this chemical series, VT-464 (**6**), is in clinical development for the treatment of patients with castration-refractory prostate cancer. The MBG-based design process described herein was used to produce the most lyase-selective CYP17 inhibitors reported to date, including the potent oral clinical agent **6**. The process is generalizable and broadly applicable to the design and evaluation of new and improved chemical entities within the metalloenzyme inhibitor class.

Acknowledgments

We thank Dr. Richard Auchus for kindly providing recombinant human CYP17 and cytochrome b5 preparations, and Drs. Barry Sharpless and Manuel Navia for their insightful inhibitor design guidance.

References and notes

1. (a) Dreicer, R.; MacLean, D.; Suri, A. et al. *Clin. Cancer Res.* **2014**, in press (epub doi:http://dx.doi.org/10.1158/1078-0432.CCR-13-2436).; (b) de Bono, J. S.; Logothetis, C. J.; Molina, A., et al *N. Engl. J. Med.* **2011**, *364*, 1995.
2. Abbott, D. H.; Eisner, J. R.; Bird, I. M.; Rafferty, S. W.; Moore, W. R.; Schotzinger, R. J. *Endocr. Rev.* **2012**, *33*. SAT-256.
3. Eisner, J. R.; Abbott, D. H.; Bird, I. M.; Rafferty, S. W.; Moore, W. R.; Schotzinger, R. J. *Endocr. Rev.* **2012**, *33*. SAT-266.
4. Schotzinger, R.J.; Degenhardt, T.P.; Wargin, W.A.; Still, J.G.; Gutierrez, M.J. 'A Phase 1 Multiple-Ascending-Dose Trial of VT-1161, a Highly Potent and Selective Oral Antifungal Agent for the Treatment of Superficial Fungal Infections'. Abstract #7516, Am. Acad. Dermatology Summer Academy Meeting, 2013.
5. Matsunaga, N.; Kaku, T.; Ojida, A.; Tanaka, T.; Hara, T.; Yamaoka, M.; Kusaka, M.; Tasaka, A. *Bioorg. Med. Chem.* **2004**, *12*, 4313.
6. Conley, A. J.; Bird, I. M. *Biol. Reprod.* **1997**, *56*, 789.
7. Geller, D. H.; Auchus, R. J.; Miller, W. L. *Mol. Endocrinol.* **1999**, *13*, 167.
8. Miller, W. L. *J. Clin. Endocrinol. Metab.* **2012**, *97*, 59.
9. Auchus, R. J.; Lee, T. C.; Miller, W. L. *J. Biol. Chem.* **1998**, *273*, 3158.
10. DeVore, N. M.; Scott, E. E. *Nature* **2012**, *482*, 116.
11. IC_{50} values for inhibition of rat or human CYP enzymes were determined using microsomal enzyme preparations, an NADPH-regenerating system (Sigma–Aldrich, St. Louis, MO), and substrate concentrations of 1 μM 17α-hydroxypregnenolone for lyase and 150 μM testosterone (experimental Km) for CYP3A4. Reactions were analyzed for product using HPLC/MS/MS methods, and IC_{50} values (in μM) were determined from a 4-parameter logistical fit to the dose response data (K. Lewis, OpAns, LLC, Durham, NC).
12. Hoekstra, W.J.; Schotzinger, R.J.; Rafferty, S.W. U.S. Patent 8,389,543, 2013.
13. Matsunaga, N.; Kaku, T.; Ojida, A.; Tasaka, A. *Tetrahederon: Asymmetry.* **2004**, *15*, 2021.
14. Using spartan 2006 program package, Me-metal binding group (Me-MBG) ligands were minimized using the MMFF-94 force field and optimized with the semi-empirical PM3 method. The CYP-51 Fe-porphyrin construct (Podust, *PNAS*, **2001**, *98*, 3068) was minimized (MMFF94) and then optimized using the PM3 semi-empirical method to obtain unligated structure. Me-MBGs were introduced and the energy was determined by a single point calculation. The Fe-porphyrin and Me-MBGs were complexed with only the Me-MBG ligand free to move during optimization. Next, Me-MBGs were submitted for geometry optimization and enthalpies measured (K. Page and W. Mascarella, Research Triangle Institute).
15. *Compound **6** was synthesized from **5** as follows:* To a stirred solution of **5** (18 g, 69 mmol) in DCM (180 mL) was added BBr_3 (87.2 g, 348 mmol) dropwise at −40 °C. After completion of addition, stirring was continued for 1 h at −40 °C and 1 h at RT. The reaction mixture was poured into ice-cold water and the aqueous layer then extracted with DCM (2 × 200 mL). The combined organic extracts were washed with water (100 mL), brine (100 mL), and dried (Na_2SO_4). After filtration and solvent evaporation in vacuo, the crude material was purified by column chromatography (SiO_2, 100–200 mesh) to afford the catechol intermediate (9.0 g, 39 mmol, 56%) as a brown solid. To a stirred solution of this intermediate (5.0 g, 21.7 mmol) in DMF (50 mL) were added ethyl bromodifluoroacetate (17.6 g, 86.6 mmol) and K_2CO_3 (18 g, 130 mmol), and the mixture was stirred at 110 °C for 48 h. The reaction mixture was poured into cold water and the aqueous layer then extracted with DCM (2 × 100 mL). Combined organic extracts were washed with water (50 mL), brine (50 mL), and dried (Na_2SO_4). After filtration and evaporation of solvent, the crude material was purified by column chromatography (SiO_2, 100–200 mesh) to afford solid **7** (2.3 g, 4.3 mmol, 32%). To a stirred solution of 1-*N*-SEM-1,2,3-triazole (2.25 g, 11.8 mmol) in dry Et_2O (25 mL) was added *t*-BuLi (0.69 g, 10.7 mmol) dropwise at −78 °C under inert atmosphere. After stirring for 1 h at −78 °C, **7** (1.5 g, 2.83 mmol) in dry ether (25 mL) was added to reaction mixture and stirring continued for 1 h at −78 °C. The reaction mixture was quenched with sat'd NH_4Cl solution and extracted with EtOAc (2 × 50 mL). Combined organic phases were washed with brine, dried (Na_2SO_4), and concentrated to afford the SEM-protected precursor of **3a** (2.0 g) as a syrup. Crude material was taken to the next step without further purification. To a stirred solution of this material (3.0 g, 5.6 mmol) in THF (30 mL) were added TBAF (1.48 g, 5.67 mmol, 1 M in THF) and CsF (2.58 g, 16.8 mmol) at RT. The reaction mixture was stirred at 80 °C for 4 h. The mixture was concentrated in vacuo; the obtained residue was partitioned between water and DCM. The organic phase was separated and the aqueous layer was extracted with DCM (2 × 25 mL); the combined organic phases were washed with brine, dried (Na_2SO_4) and concentrated to give crude material. The crude material was purified by column chromatography (SiO_2, 100–200 mesh) to afford **3a** (2.2 g, 5.5 mmol, 61%) as a white solid. ^1H NMR (500 MHz, $CDCl_3$): δ 11.4 (br s, 1H), 8.03 (s, 1 H), 7.76–7.61 (m, 5H), 6.60 (t, $J_{F,H}$ = 74 Hz, 2H). 2.88 (br s, 1H), 2.86–2.80 (m, 1H), 0.97 (d, *J* = 7.0 Hz, 3H), 0.80 (d, *J* = 7.0 Hz, 3 H). HPLC: 96%. MS (ESI): *m*/*z* 398 $[M+H]^+$. Compound **6** was isolated from racemate **3a** using the following conditions: Column: Chiralpak IC, 250 × 4.6 mm, 5-micron; mobile phase: Isocratic *n*-hexane/IPA (95:5); Flow rate: 1.00 mL/min; HPLC: 99.5% (13 mg isolated as a white powder); optical rotation $[\alpha]_D$: −54° (*c* = 0.5 % in MeOH).
16. Hamsters were chosen as a model species because they, unlike most rodents, have adrenal CYP17 similar to human Conley, A. J.; Bird, I. M. *Biol. Reprod.* **1997**, *56*, 789.
17. Gonadally-intact, experimentally-naïve male hamsters (6 per group) were administered a CYP17 inhibitor (50 mg/kg by oral gavage in 20% cremaphor) at the same morning time and then blood samples were collected for the determination of plasma steroid concentrations 2 h later.