

**Effects of Fluorine Substitution on Substrate Conversion by
Cytochromes P450 17A1 and 21A2**

Journal:	<i>Organic & Biomolecular Chemistry</i>
Manuscript ID	OB-ART-06-2021-001178.R2
Article Type:	Paper
Date Submitted by the Author:	12-Aug-2021
Complete List of Authors:	Vogt, Caleb; University of North Carolina at Chapel Hill, Chemical Biology and Medicinal Chemistry Bart, Aaron; University of Michigan, Department of Medicinal Chemistry Yadav, Rahul; Mississippi State University, Department of Chemistry Scott, Emily; University of Michigan Aubé, Jeffrey; University of North Carolina at Chapel Hill, Chemical Biology and Medicinal Chemistry

ARTICLE

Effects of Fluorine Substitution on Substrate Conversion by Cytochromes P450 17A1 and 21A2

Caleb D. Vogt,^a Aaron G. Bart,^b Rahul Yadav,^b Emily E. Scott,^{b, c} and Jeffrey Aubé^a

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

Cytochromes P450 17A1 (CYP7A1) and 21A2 (CYP21A2) catalyze key reactions in the production of steroid hormones, including mineralocorticoids, glucocorticoids, and androgens. With the ultimate goal of designing probes that are selectively metabolized to each of these steroid types, fluorinated derivatives of the endogenous substrates, pregnenolone and progesterone, were prepared to study the effects on CYP17A1 and CYP21A2 activity. In the functional assays, the hydroxylase reactions catalysed by each of these enzymes were blocked when fluorine was introduced at the site of metabolism (positions 17 and 21 of the steroid core, respectively). CYP17A1, furthermore, performed the 17,20-lyase reaction on substrates with a fluorine installed at the 21-position. Importantly, none of the substitutions examined herein prevented compound entry into the active sites of either CYP17A1 or CYP21A2 as demonstrated by spectral binding assays. Taken together, the results suggest that fluorine might be used to redirect the metabolic pathways of pregnenolone and progesterone to specific types of steroids.

Introduction

Endogenous steroid hormones are produced by a series of metabolic pathways that stem from a common ancestor, cholesterol. At the core of these enzymatic reactions are the heme-containing cytochromes P450 17A1 (CYP17A1) and 21A2 (CYP21A2), which differentiate steroids into one of three distinct classes (i.e., mineralocorticoids, glucocorticoids, or androgens) early in their biosynthesis (Figure 1).^{1, 2} CYP17A1 performs two unique reactions: C17 hydroxylation of pregnenolone and progesterone, followed by C17–C20 bond cleavage to give dehydroepiandrosterone and androstenedione, respectively. The initial 17 α -hydroxylase reaction catalysed by CYP17A1 produces precursors to glucocorticoids, which regulate glucose metabolism, and androgens, the male sex hormones. The latter 17,20-lyase activity is additionally required to complete androgen biosynthesis. Thus, CYP17A1 is a target for hormone-dependent prostate cancer.^{3, 4} CYP21A2 also catalyses a key hydroxylation reaction, but at the 21-position of progesterone and its 17 α -hydroxylase product. This reaction is essential in the production of mineralocorticoids (which influence electrolyte and fluid balance) in addition to glucocorticoids.

Given that CYP17A1 and CYP21A2 operate at key points in steroidogenesis, selectively blocking the sites of metabolism on pregnenolone and progesterone is expected to dramatically influence the metabolic pathways of these feedstock steroids. For example, a substrate for CYP17A1 but not CYP21A2 would only be channelled toward androgens. Conversely, mineralocorticoids would be produced from precursors that are not metabolized by CYP17A1. Thus, specific classes of steroids could be enriched, which would be useful for studying the biosynthesis and activities of these hormones.

Incorporation of fluorine into probe molecules can modify biological activity in mechanistically informative ways.^{5, 6} In this case, introducing a fluorine atom at C17 or C21 is expected to direct substrates toward CYP21A2 or CYP17A1 metabolism, respectively. To this end, Yoshimoto et al. previously described several different halogenated compounds that were used to study the activities of CYP17A1 and CYP21A2 in yeast microsomes.⁷ Along with structural biological approaches,^{8, 9} this work has helped profile how changes near C17 of substrates affects enzymatic function. In the current study, we sought to expand this approach by focusing on how enzyme kinetics and binding respond to C17 and C21 fluorination.

To accomplish this objective, we prepared a series of pregnenolone and progesterone substrates along with their predicted metabolites (i.e., product standards for identification and quantification). CYP17A1 and CYP21A2 are known to display low promiscuity compared to other cytochrome P450 enzymes, and so the scope of our study was limited to monofluorinated substrates to minimize confounding effects, such as steric hindrance. Notably, the Van der Waals radii of fluorine (1.47 Å) is closer to oxygen than hydrogen (1.52 and 1.20 Å, respectively). We then evaluated the synthetic substrates for conversion by CYP17A1 and CYP21A2 and

^a Division of Chemical Biology and Medicinal Chemistry, UNC Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, North Carolina 27599, United States.

^b Department of Medicinal Chemistry, University of Michigan, Ann Arbor, Michigan 48109, United States.

^c Department of Pharmacology, University of Michigan, Ann Arbor, Michigan 48109, United States.

Electronic Supplementary Information (ESI) available: Additional schemes, synthetic procedures, experimental details for functional and spectral binding assays, as well as copies of ¹H, ¹³C, and ¹⁹F NMR spectra. See DOI: 10.1039/x0xx00000x

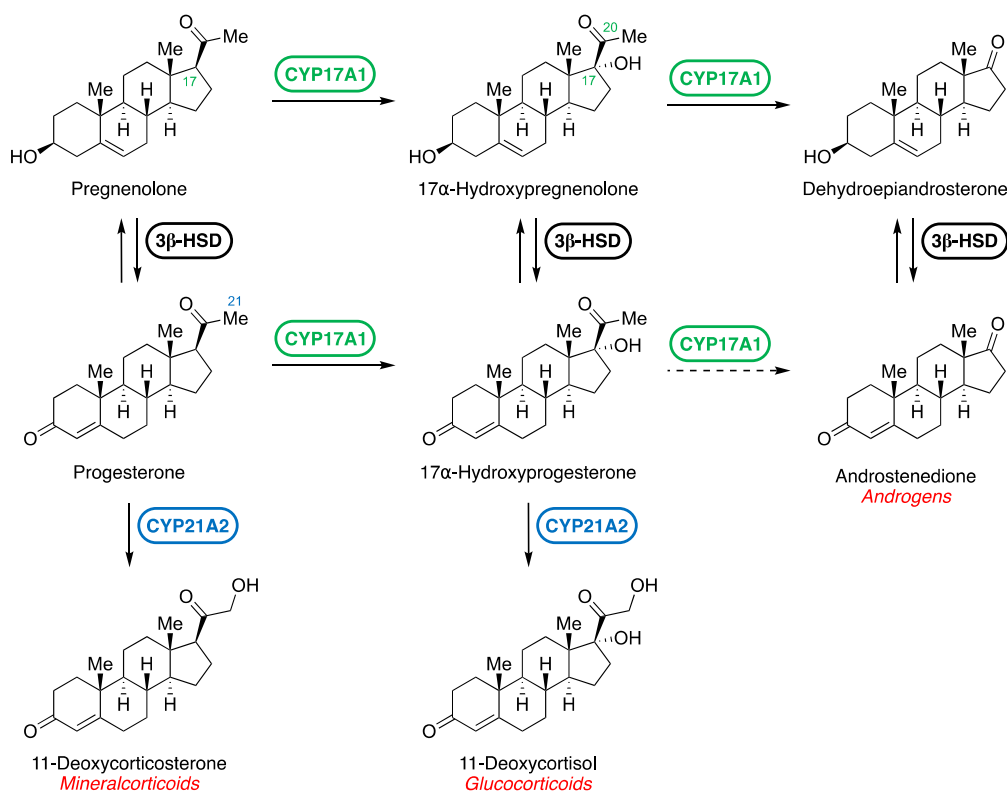


Figure 1 CYP17A1 and CYP21A2 channel precursors to either mineralocorticoids, glucocorticoids, or androgens (red text) in steroid metabolism. 3 β -Hydroxysteroid dehydrogenase (3 β -HSD) interconverts Δ^5 -3 β -hydroxy- and Δ^4 -3-oxosteroids. Lower conversion of 17 α -hydroxyprogesterone by CYP17A1 is indicated by the dashed arrow.

quantified the effects fluorine substitutions had on their binding affinity to each enzyme.

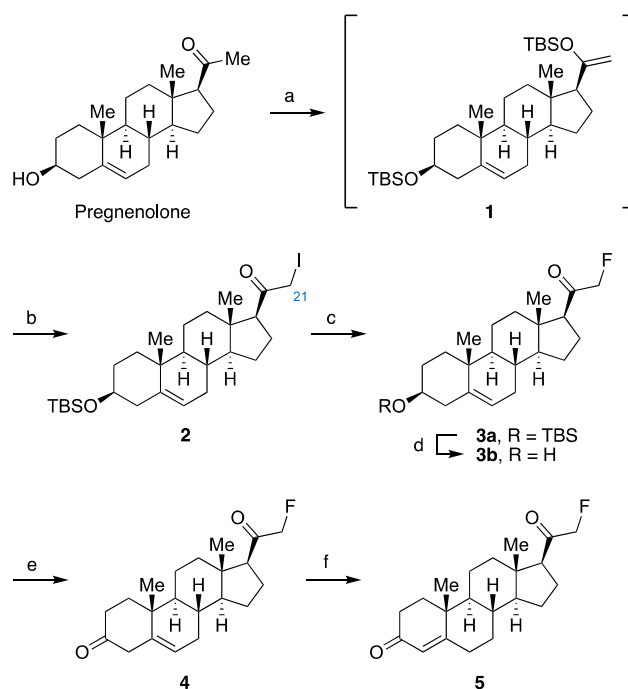
Following work published by Djerassi et al., 16-dehydropregnenolone acetate (16-DPA) was transformed into enol acetate **6**, which was reacted with NIS to afford the α -iodo

Results and Discussion

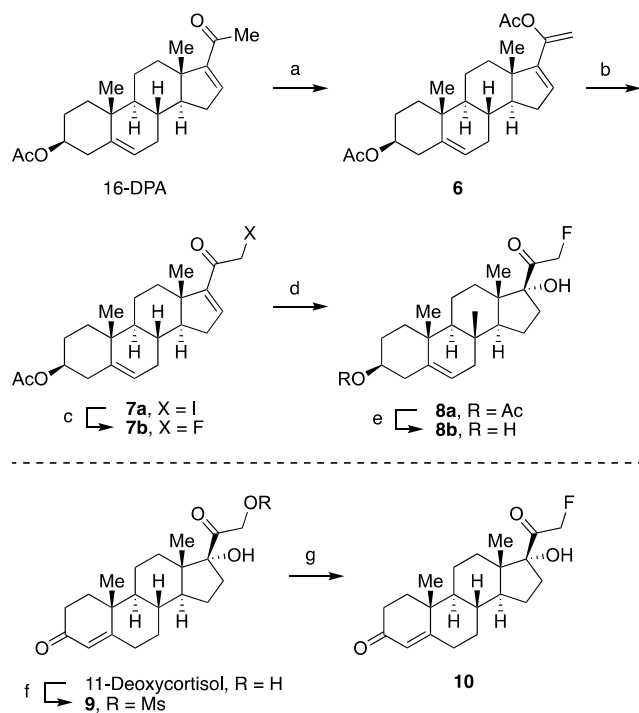
Synthesis of Fluorinated Substrates and Product Standards

For the first set of compounds, we introduced a single fluorine atom at the 21-position of the steroid core (Scheme 1). Starting from pregnenolone itself, silyl enol ether **1** was prepared by adapting known reaction conditions.¹⁰ This intermediate was then treated with *N*-iodosuccinimide (NIS) to install an iodine at C21 (**2**) in 87% yield over two steps. Nucleophilic substitution using tetrabutylammonium difluorotriphenylsilicate (TBAT) then delivered the fluorinated **3a**, which was subsequently deprotected with HF \cdot pyridine (**3b**). With this material in hand, we prepared the corresponding progesterone analogue by pyridinium chlorochromate (PCC) oxidation followed by acid-catalysed isomerization (**4** then **5**).

Since both of the 21-fluorosteroids (**3b** and **5**) were predicted to act as substrates of CYP17A1, we also synthesized standards of their 17 α -hydroxylase products. Initially, a variation of the previous route was planned, starting from commercially available 17 α -hydroxypregnenolone. While troubleshooting preliminary reactions however, we discovered that this material was sold in ca. 80% purity (determined by ¹H NMR), which could not be improved by chromatography or recrystallization. As a result, alternative strategies were devised and executed to access these compounds (Scheme 2).



Scheme 1 Introduction of a fluorine at position 21 of pregnenolone and progesterone. Reagents and conditions: (a) TBSOTf, NEt₃, CH₂Cl₂, -78 °C, 1 h; (b) NIS, CH₂Cl₂, rt, 2 h, 87% over 2 steps; (c) TBAT, MeCN, reflux, 19 h, 74%; (d) HF \cdot pyridine, CH₂Cl₂, 0 °C, 1 h, 86%; (e) PCC, CH₂Cl₂, rt, 5 h, 56%; (f) oxalic acid dihydrate, MeOH, 80 °C, 2 h, 85%.

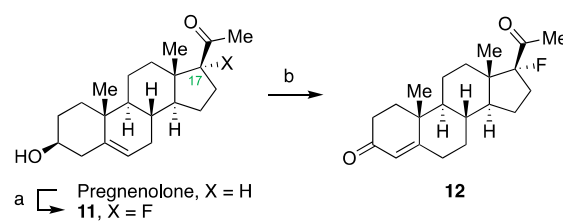


Scheme 2 Routes to the 17 α -hydroxylase products of 21-fluoropregnenolone and -progesterone. Reagents and conditions: (a) isopropenyl acetate, *p*TsOH \cdot H₂O, reflux, 4 h, 77%; (b) NIS, CH₂Cl₂, rt, 2 h, 68%; (c) TBAT, MeCN, reflux, 24 h, 75%; (d) Mn(dpm)₃, O₂, PhSiH₃, *i*-PrOH, 0 °C, 4 h, then P(OEt)₃, 0 °C, 2 h, 66%; (e) K₂CO₃, MeOH, rt, 18 h, 89%; (f) MsCl, NEt₃, THF, 0 °C to rt, 1.5 h, 91%; (g) TBAT, MeCN, reflux, 24 h, 44%.

ketone **7a**.¹¹ As before, nucleophilic substitution with TBAT provided the C21 fluorinated version of 16-DPA (**7b**). We then converted this intermediate to its α -hydroxy ketone (**8a**) in 66% yield using a variation of the Mukaiyama hydration developed by Magnus et al.¹² Acetate deprotection ultimately afforded the target compound **8b**, which was isolated directly from the reaction mixture by vacuum filtration. We also used the last two steps of this route to access authentic samples of 17 α -hydroxypregnenolone starting from 16-DPA (see Supplementary Information, Scheme S1).

The 17 α -hydroxylase product of **5**, by comparison, was much more straightforward to prepare. Adopting a strategy by Pomper et al.,¹³ we trapped the more reactive 21-hydroxy group of 11-deoxycortisol as its mesylate (**9**). This leaving group was later displaced with TBAT to deliver **10** in 40% overall yield.

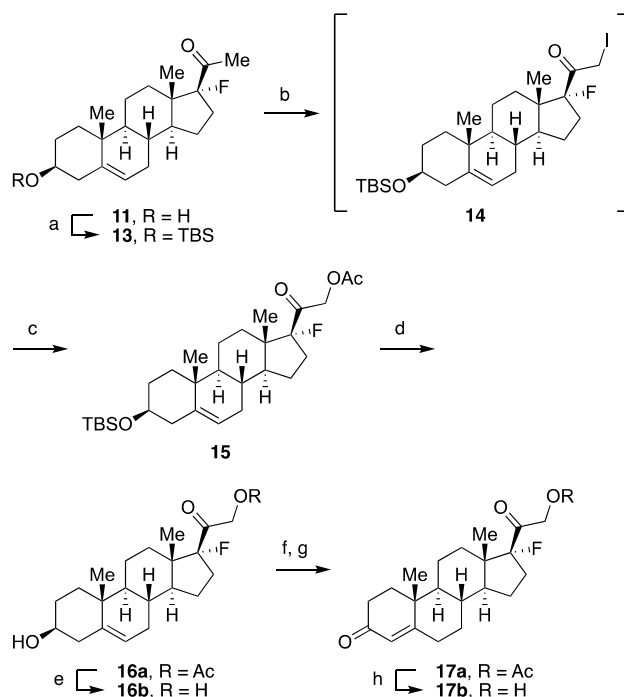
For the second series, we replaced the hydrogen atom at position 17 of pregnenolone and progesterone with a fluorine. While routes to both compounds are known, our efforts to reproduce earlier work were either unsuccessful or avoided altogether because hazardous reagents, like ClO₃F, were used.^{7, 14-16} Consequently, we devised a new approach that took inspiration from work published by Stavber et al.¹⁷ (Scheme 3). Specifically, pregnenolone was treated with *N*-fluorobenzenesulfonimide (NFSI) to directly produce the α -fluoro ketone **11** in 24% yield (4.0 g scale). This material was then converted into the related progesterone analogue (**12**) by an Oppenauer oxidation in just two steps, a notable improvement over the previous route (five steps).⁷



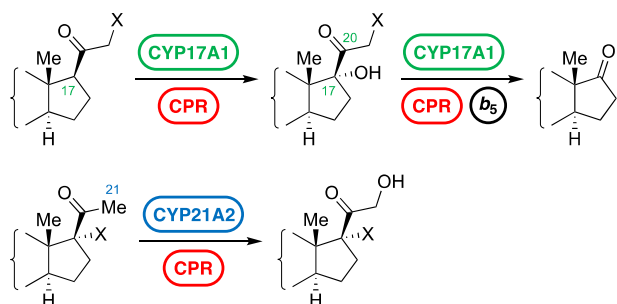
Scheme 3 Introduction of a fluorine at position 17 of pregnenolone and progesterone. Reagents and conditions: (a) NFSI, MeOH, reflux, 5 h, 24%; (b) Al(O-*i*-Pr)₃, cyclohexanone, toluene, reflux, 3 h, 48%.

Similar to before, we did not expect that the 17 α -fluoro group of **11** and **12** would block catalysis by CYP21A2. Accordingly, methods to prepare the 21-hydroxylase products of these fluorinated steroids were also pursued (Scheme 4). Initially, we developed a pilot route for the synthesis of 21-hydroxypregnenolone (see Supplementary Information, Scheme S2) using a previous intermediate (**2** from Scheme 1). However, this strategy relied on functionalizing C21 via a silyl enol ether, whose 17 α -fluorinated counterpart could not be prepared from **11**. So instead, we installed the hydroxyl group by way of a radical halogenation.¹⁸ Thus, TBS-protected **13** was treated with molecular iodine in the presence of an initiator (AIBN) to afford crude **14**. KOAc was then used to displace the intermediate iodine, giving **15** in 61% yield over two steps.

Removal of the TBS group on **15** gave **16a**, but attempts to deprotect the acetate using standard conditions (K₂CO₃ in MeOH) led to a complex mixture of products. Alternatively, treatment of **16a** with Sc(OTf)₃, which was previously reported



Scheme 4 Routes to the 21-hydroxylase products of 17 α -fluoropregnenolone and -progesterone. Reagents and conditions: (a) TBSCl, DMAP, imidazole, CH₂Cl₂, rt, 16 h, 95%; (b) I₂, AIBN, CaO, THF, MeOH, rt, 3 h; (c) KOAc, acetone, reflux, 18 h, 61% over 2 steps; (d) HF \cdot pyridine, CH₂Cl₂, 0 °C, 1 h, 93%; (e) Sc(OTf)₃, MeOH, H₂O, MW, 80 °C, 4 h, 36%; (f) PCC, CH₂Cl₂, rt, 5 h, 63%; (g) oxalic acid dihydrate, MeOH, 80 °C, 2 h, 90%; (h) Sc(OTf)₃, MeOH, H₂O, MW, 80 °C, 4 h, 57%.

Table 1 Effects of fluorine substitution on the kinetic parameters of CYP17A1 and CYP21A2.

Products were not detected by LCMS for the following substrates:

CYP17A117 α -Fluoroprogesterone (**12**)**CYP21A2**

Pregnenolone

17 α -Hydroxypregnenolone**CYP17A1 and CYP21A2**17 α -Fluoropregnenolone (**11**)21-Fluoropregnenolone (**3b**)21-Fluoroprogesterone (**5**)

Activity	Entry	Substrate	X	Product ^a	k_{cat} (min ⁻¹) ^b	K_m (μ M) ^b
17 α -Hydroxylase	1	Pregnenolone	H	17 α -Hydroxypregnenolone	1.2 [1.2, 1.3]	1.0 [0.8, 1.3]
	2	21-Fluoropregnenolone (3b)	F	21-Fluoro-17 α -hydroxypregnenolone (8b)	0.88 [0.84, 0.93]	0.42 [0.30, 0.58]
	3	Progesterone	H	17 α -Hydroxyprogesterone	2.3 [2.0, 2.6]	5.0 [3.0, 8.1]
	4	21-Fluoroprogesterone (5)	F	21-Fluoro-17 α -hydroxyprogesterone (10)	2.3 [2.2, 2.4]	6.3 [5.4, 7.4]
17,20-Lyase	5	17 α -Hydroxypregnenolone	H	Dehydroepiandrosterone	0.23 [0.20, 0.25]	0.20 [0.12, 0.32]
	6	21-Fluoro-17 α -hydroxypregnenolone (8b)	F		0.17 [0.16, 0.18]	0.60 [0.48, 0.74]
	7	17 α -Hydroxyprogesterone	H	Androstenedione	0.059 [0.052, 0.067]	4.3 [2.8, 6.6]
	8	21-Fluoro-17 α -hydroxyprogesterone (10)	F		0.031 [0.027, 0.036]	3.4 [2.1, 5.4]
21-Hydroxylase	9	Progesterone	H	11-Deoxycorticosterone	0.22 [0.20, 0.23]	0.12 [0.087, 0.16]
	10	17 α -Hydroxyprogesterone	OH	11-Deoxycortisol	3.7 [3.5, 4.0]	1.00 [0.79, 1.3]
	11	17 α -Fluoroprogesterone (12)	F	17 α -Fluoro-21-hydroxyprogesterone (17b)	0.064 [0.059, 0.070]	0.071 [0.047, 0.11]

^aDetection limits for the observed and unobserved products are presented in Table S2 (see Supplementary Information).

^bThe 95% confidence intervals are reported in brackets (n = 2).

to selectively cleave acetates adjacent to a carbonyl group,¹⁹ afforded the predicted 21-hydroxylase product (**16b**) in 36% yield. For the corresponding 17 α -fluoroprogesterone analogue, oxidation followed by isomerization yielded **17a**, which was deprotected with Sc(OTf)₃ to give **17b**.

Functional Evaluation of CYP17A1 and CYP21A2 against Fluorinated Substrates

We then set out to quantify how different sites of fluorination affected the kinetics of CYP17A1 and CYP21A2 action on our collection of substrates. Briefly, compounds were incubated with either enzyme along with NADPH-cytochrome P450 reductase (CPR), which is essential for the catalytic activities of CYP17A1 and CYP21A2. Substrate conversion was followed by LCMS, and products were confirmed using commercially available standards or those synthesized as above (see Supplementary Information for details).

Initially, we investigated the 17 α -hydroxylase reaction catalysed by CYP17A1 on the native substrates, pregnenolone

and progesterone (Table 1, entries 1 and 3). While hydroxylation was observed for these control substrates, no products were detected by LCMS for either of the 17 α -fluorosteroids (**11** and **12**), which agrees with previous work by Yoshimoto et al.⁷ Nonetheless, this result is somewhat surprising given that CYP17A1 can install a hydroxy group at alternative positions on the steroid core generating minor products, such as 16 α -hydroxyprogesterone.²⁰⁻²² By comparison, CYP17A1 did perform the 17 α -hydroxylase reaction on substrates bearing a fluorine atom at the 21-position. Interestingly, this substitution lowered the K_m value of **3b** by 2.4-fold compared to pregnenolone (entries 1 and 2). Nearly identical kinetic parameters, however, were observed between progesterone and its 21-fluorinated analogue, **5** (entries 3 and 4).

To study the 17,20-lyase reaction, cytochrome b_5 (b_5) was added to the previous enzyme system (i.e., CYP17A1 and CPR) to augment C17–C20 bond cleavage. In agreement with previous studies,²³ 17 α -hydroxypregnenolone was a better

substrate in the 17,20-lyase reaction than its progesterone counterpart (Table 1, entries 5 and 7). This trend was also observed for the 21-fluorosteroids (entries 6 and 8); although, the K_m for **8b** was 3-fold greater than 17 α -hydroxypregnenolone (entries 5 vs. 6), while the k_{cat} for **10** was 1.9-fold smaller than 17-hydroxyprogesterone (entries 7 vs. 8). In addition, no products were formed in the 17,20-lyase reaction when a fluorine was substituted for the 17 α -hydroxy group (i.e., **11** and **12**).

Switching to the 21-hydroxylase activity of CYP21A2, none of the predicted products were detected for pregnenolone or any of its derivatives by LCMS. This result was expected seeing as CYP21A2 demonstrates high specificity for its native substrates, progesterone and its 17 α -hydroxylase product.²⁴ Thus, 11-deoxycorticosterone and 11-deoxycortisol were both observed in these assays (Table 1, entries 9 and 10). Similar to CYP17A1, product formation was undetectable when a fluorine was introduced at the site of normally hydroxylated, in this case C21 on progesterone (**5**). Consequently, the only fluorinated analogue of progesterone that did act as a substrate for the 21-hydroxylase reaction placed a fluorine at the 17-position of the steroid core (entry 11). However, this substitution dramatically lowered catalysis by 3.4-fold compared to its parent substrate (entries 9 vs. 11), once again highlighting the specificity of CYP21A2.

Spectral Binding of Compounds to CYP17A1 and CYP21A2

Based on the functional assays alone, it was unclear whether fluorine blocked catalysis directly or disrupted binding in the enzyme active site such that catalysis could not occur. To help distinguish between these two possibilities, we performed a series of spectral binding assays using CYP17A1 or CYP21A2 to characterize the interaction between ligand and enzyme. Notably, all of the monofluorinated pregnenes prepared in this study displayed a shift in the UV–Vis difference spectra from ca. 420 to ca. 395 nm (see Supplementary Information, Figure S2). This so-called type I binding is consistent with each ligand displacing the water molecule coordinated to the heme iron. Such binding was also observed for most of the endogenous steroids. The only interaction that showed no significant change in the UV–Vis difference spectrum was between 17 α -hydroxypregnenolone and CYP21A2. However, the absence of a spectral shift cannot be used to rule out ligand binding, as some compounds can occupy the cytochrome P450 active site in such a way that water remains bound to the iron.²⁵ Regardless, these experiments demonstrated that a single fluorine atom at either C17 or C21 does not prevent ligand entry into the CYP17A1 or CYP21A2 active sites.

Dissociation constants between enzyme and compound were also measured from these assays using a single binding site model (see Supporting Information for details). For CYP17A1, we observed that pregnenolone (Table 2, entry 1) and progesterone (entry 6) generally had lower binding affinities (larger K_d values) than their corresponding fluorinated analogues (entries 2, 3, and 8). The exception to this trend was the 21-fluorosteroid **5**, whose dissociation constant was similar to progesterone (entries 6 vs. 7). Interestingly, a single fluorine

Table 2 Effects of fluorine substitution on binding affinity to CYP17A1 and CYP21A2.

Entry	Ligand	K_d (μM) ^a	
		CYP17A1	CYP21A2
1	Pregnenolone	<0.1 ^b	1.6 [0.55, 4.6]
2	21-Fluoropregnenolone (3b)	0.053 [0.045, 0.062]	1.8 [1.5, 2.1]
3	17 α -Fluoropregnenolone (11)	<0.022 ^c	1.6 [1.2, 2.1]
4	17 α -Hydroxypregnenolone	0.21 ^b	-
5	21-Fluoro-17 α -hydroxypregnenolone (8b)	0.14 [0.13, 0.16]	2.7 [2.1, 3.5]
6	Progesterone	0.23 ^b	0.23 [0.14, 0.38]
7	21-Fluoroprogestrone (5)	0.18 [0.15, 0.22]	1.6 [1.4, 2.0]
8	17 α -Fluoroprogestrone (12)	0.032 [0.026, 0.039]	0.51 [0.44, 0.59]
9	17 α -Hydroxyprogesterone	0.33 ^b	4.6 [2.9, 7.2]
10	21-Fluoro-17 α -hydroxyprogesterone (10)	0.59 [0.47, 0.74]	14 [10, 19]

^aThe 95% confidence intervals are reported in brackets ($n = 1$).

^bLiterature values.^{8, 26}

^cThis ligand is a very tight binder, which precludes definition of a dissociation constant with similar accuracy to the other experiments.

atom at the 21-position of 17 α -hydroxypregnenolone resulted in greater binding affinity for CYP17A1 compared to the parent ligand (entries 4 vs. 5), while the opposite was found for 17 α -hydroxyprogesterone and its counterpart (entries 9 vs. 10). Even with a 1.8-fold larger K_d value, 21-fluoro-17 α -hydroxyprogesterone was still turned over in the 17,20-lyase reaction (*vide supra*).

In contrast to CYP17A1, the introduction of a fluorine into the structure of pregnenolone at either position had little effect on its dissociation constant for CYP21A2 (Table 2, entries 1–3). Progesterone, on the other hand, exhibited a higher sensitivity to substitution, with particularly decreased affinity upon fluorination at the 21 position (entries 6 vs. 7 and 8). Unexpectedly, the K_d values for pregnenolone and its analogues (entries 1–3) were ca. 3-fold lower than 17 α -hydroxyprogesterone (entry 9), a native substrate.

While the structural basis for the trends observed in Table 2 is beyond the scope of this work, we have used techniques in computational molecular modelling to dock the fluorinated analogues of the endogenous substrates into the activity sites of CYP17A1 and CYP21A2 (data not included). These studies, however, provided ambiguous results and did not suggest any key differences in the binding interactions.

Conclusions

To summarize this work, we report syntheses of a collection of fluorinated substrates and product standards that were evaluated for catalysis by CYP17A1 and CYP21A2. In the

functional assays, hydroxylation by CYP17A1 and CYP21A2 was not observed by LCMS when fluorine was introduced at C17 or C21, respectively. Moreover, the 17,20-lyase reaction catalysed by CYP17A1 still took place on substrates that incorporated fluorine at position 21. While different sites of fluorination could have prevented ligand entry into the CYP17A1 and CYP21A1 active sites, spectral binding assays revealed that such substitutions were instead blocking catalysis directly. In certain cases, a single fluorine atom unexpectedly resulted in greater binding affinity between ligand and enzyme (e.g., **11** and **12** for CYP17A1). Looking to the future, the results presented herein provide valuable insight as to how fluorine might be used to redirect the metabolic pathways of pregnenolone and progesterone.

Author Contributions

C.D.V. synthesized and characterized all compounds. A.G.B. and R.Y. recombinantly expressed and purified the enzymes used in this study. Preliminary functional assays were performed by R.Y., and A.G.B. determined the final kinetic parameters. In addition, A.G.B. undertook the spectral binding assays and obtained dissociation constants for each compound. E.E.S. and J.A. conceived the study and assisted the other authors in data analysis. The initial draft of this article was written by C.D.V., and all authors participated in its revision.

Conflicts of interest

The authors declare no competing financial interest.

Acknowledgements

This work was supported in part by the National Cancer Institute (F31CA239322), the National Science Foundation (CHE-1726291), the National Institute of General Medical Sciences (R01GM102505), the Eshelman Institute for Innovation, the University of North Carolina at Chapel Hill, and the University of Michigan. We also thank Brandie M. Ehrmann and Diane Wallace, who assisted in mass spectrometry data analysis; Patrick O'Day, who optimized the LC-MS/MS methods used to detect and quantify steroid concentrations; Richard Auchus, who provided advice on MS analysis and access to LC-MS/MS instrumentation; as well as Michael C. Gregory, Ilia G. Denisov, and Stephen G. Sligar, who ran preliminary functional and spectral binding assays.

Notes and references

1. W. L. Miller and R. J. Auchus, *Endocr. Rev.*, 2011, **32**, 81–151.
2. L. Schiffer, L. Barnard, E. S. Baranowski, L. C. Gilligan, A. E. Taylor, W. Arlt, C. H. L. Shackleton and K.-H. Storbeck, *J. Steroid Biochem. Mol. Biol.*, 2019, **194**, 105439.
3. T. A. Yap, C. P. Carden, G. Attard and J. S. de Bono, *Curr. Opin. Pharmacol.*, 2008, **8**, 449–457.
4. D. Porubek, *Curr. Top Med. Chem.*, 2013, **13**, 1364–1384.
5. E. P. Gillis, K. J. Eastman, M. D. Hill, D. J. Donnelly and N. A. Meanwell, *J. Med. Chem.*, 2015, **58**, 8315–8359.
6. P. Shah and A. D. Westwell, *J. Enzyme Inhib. Med. Chem.*, 2007, **22**, 527–540.
7. F. K. Yoshimoto, M. C. Desilets and R. J. Auchus, *J. Steroid Biochem. Mol. Biol.*, 2012, **128**, 38–50.
8. N. M. DeVore and E. E. Scott, *Nature*, 2012, **482**, 116–119.
9. P. S. Pallan, C. Wang, L. Lei, F. K. Yoshimoto, R. J. Auchus, M. R. Waterman, F. P. Guengerich and M. Egli, *J. Biol. Chem.*, 2015, **290**, 13128–13143.
10. A. Marek, B. Klepetářová and T. Elbert, *Collect. Czech. Chem. Commun.*, 2011, **76**, 443–456.
11. C. Djerassi and C. T. Lenk, *J. Am. Chem. Soc.*, 1953, **75**, 3493–3495.
12. P. Magnus, A. H. Payne, M. J. Waring, D. A. Scott and V. Lynch, *Tetrahedron Lett.*, 2000, **41**, 9725–9730.
13. M. G. Pomper, M. J. Kochanny, A. M. Thieme, K. E. Carlson, H. F. Vanbrocklin, C. J. Mathias, M. J. Welch and J. A. Katzenellenbogen, *Nucl. Med. Biol.*, 1992, **19**, 461–480.
14. J. Pataki and E. V. Jensen, *Steroids*, 1976, **28**, 437–447.
15. R. Deghenghi and R. Gaudry, *Can. J. Chem.*, 1961, **39**, 1553–1557.
16. R. R. Eng, L. A. Spitznagle and W. F. Trager, *J. Labelled Compd. Radiopharm.*, 1983, **20**, 63–72.
17. S. Stavber, M. Jereb and M. Zupan, *Synthesis*, 2002, **2002**, 2609–2615.
18. E. Batres, T. Cardenas, J. A. Edwards, G. Monroy, O. Mancera, C. Djerassi and H. J. Ringold, *J. Org. Chem.*, 2002, **26**, 871–878.
19. H. Kajiro, S. Mitamura, A. Mori and T. Hiyama, *Tetrahedron Lett.*, 1999, **40**, 1689–1692.
20. F. K. Yoshimoto, Y. Zhou, H.-M. Peng, D. Stidd, J. A. Yoshimoto, K. K. Sharma, S. Matthew and R. J. Auchus, *Biochemistry*, 2012, **51**, 7064–7077.
21. F. K. Yoshimoto and R. J. Auchus, *J. Steroid Biochem. Mol. Biol.*, 2015, **151**, 52–65.
22. F. K. Yoshimoto, E. Gonzalez, R. J. Auchus and F. P. Guengerich, *J. Biol. Chem.*, 2016, **291**, 17143–17164.
23. E. Gonzalez and F. P. Guengerich, *J. Biol. Chem.*, 2017, **292**, 13168–13185.
24. D. Mizrachi, Z. Wang, K. K. Sharma, M. K. Gupta, K. Xu, C. R. Dwyer and R. J. Auchus, *Biochemistry*, 2011, **50**, 3968–3974.
25. J. H. Dawson, L. A. Andersson and M. Sono, *J. Biol. Chem.*, 1982, **257**, 3606–3617.
26. E. M. Petrunak, N. M. DeVore, P. R. Porubsky and E. E. Scott, *J. Biol. Chem.*, 2014, **289**, 32952–32964.