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The homodimer approach to the design of a new long-acting depot prodrug of abiraterone

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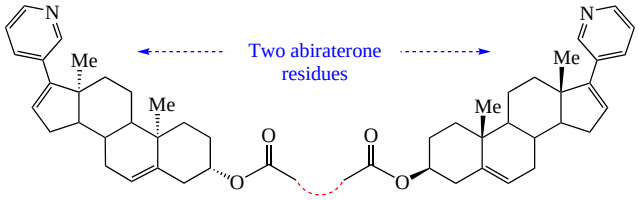
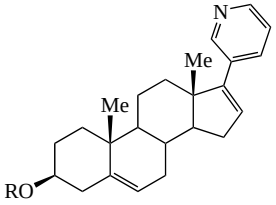
The synthesis of homodimeric bis-esters of abiraterone (an inhibitor of androgens synthesis used for treatment of prostate cancer) as a novel depot form of the drug intended for parenteral administration has been undertaken. The conjugate with succinic linker has been synthesized, while only mono-esters of malic and citric acid were obtained. The target 'twin' demonstrated excellent characteristics in preliminary biotests, proving the concept of long-lasting depot-prodrug.



Keywords: steroid derivatives, succinic acid, pimelic acid, citric acid, abiraterone prodrugs, depot formulations, twin drugs, cytochrome P450 17A1 inhibitors, plasma hydrolysis, prostate cancer.

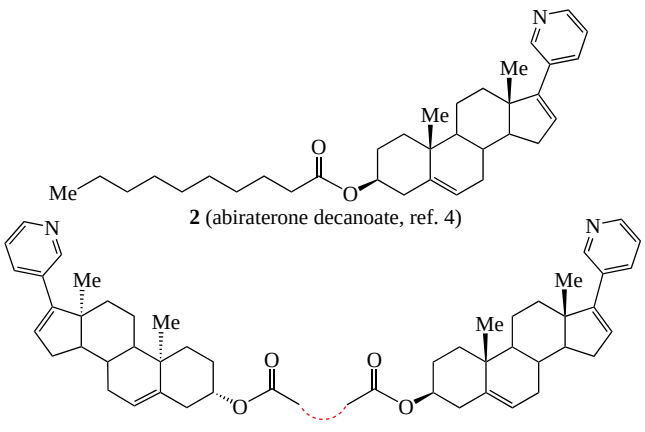
The growth and metastasis of hormone-dependent tumors is regulated by hormones that interact with membrane receptors in tumor cells. Reducing the levels of these hormones by inhibiting the enzymes involved in their synthesis is used in the therapy of the corresponding diseases. Thus, abiraterone **1a** (Figure 1), which interferes with the synthesis of androgens, is widely used for treatment of prostate cancer.[1,2]

Abiraterone inhibits cytochrome P450 17A1 (CYP17A1) 17α-hydroxylase and 17,20-lyase activities and is required for the production of androgenic steroids. Due to poor solubility in the gastrointestinal fluids, abiraterone has low intestinal permeability and is used in clinics as its orally administered acetate prodrug **1b** (see Figure 1). The latter itself is also poorly absorbed, but can be hydrolyzed to the parent molecule in the intraluminal environment leading to abiraterone supersaturation, which forces its absorption.[3] The major disadvantages of currently approved oral dosage form of abiraterone acetate are high daily doses (about 1 g per day) and strong dependence of blood concentration on food fat content. Attempts to create both better oral dosage forms and new compounds intended for parenteral administration have been undertaken. The latter were obtained within the classical approach to long-acting depot prodrugs represented mainly by highly lipophilic esters of abiraterone with fatty acids, *e.g.* abiraterone decanoate **2** (Figure 2).[4,5] Parenteral injection of such compounds in acceptable oil creates an oily depot, penetrating into lymphatics and/or fatty tissue, from which the prodrug molecules are slowly released into the blood plasma, where they are hydrolyzed by esterases to the active molecules.[6,7] Intravenous or intramuscular administration of abiraterone decanoate **2**, which eventually hydrolyzes to abiraterone **1a** and a non-toxic degradable fatty



1a R = H (abiraterone)
1b R = Ac (Zytiga®)

Figure 1 Structure of abiraterone **1a**, an active substance of its acetate prodrug Zytiga® **1b**, which is used for the treatment of prostate cancer as an orally administered medicine.



bis-Abi, this work

Figure 2 Long-acting depot prodrugs: abiraterone decanoate **2** and bis-Abi (schematic representation of homodimeric abiraterone conjugates).

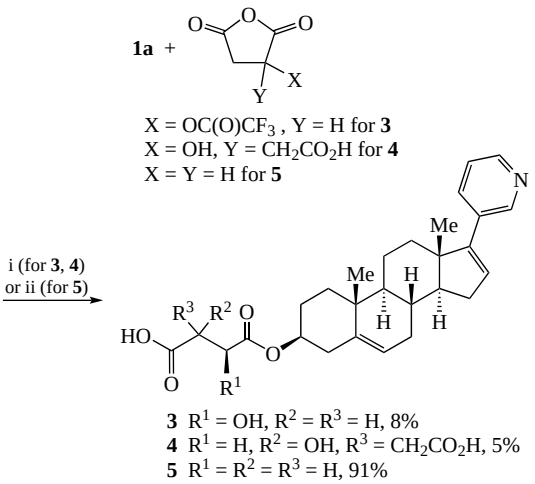
acid, allows the dose (as shown in tests on rats, dogs and primates) to be reduced by at least an order of magnitude compared to the oral dose of compound **1b**.[4]

In addition to fatty acids, other classes of lipids such as steroids, glycerides and phospholipids could be selected for the sustained-release of a drug. Such lipid moieties have been exploited for various drugs,[8,9] but to the best of our knowledge, steroid-containing long-lasting abiraterone prodrugs have not yet been studied. Owing to the steroidal structure of abiraterone itself a simple and straightforward approach to prodrug design seemed to be a formation of homodimer ('twin') bis-ester which (in contrast to decanoate **2**) upon hydrolysis should give two molecules of abiraterone. In the present work, we aimed to examine such homodimers (bis-Abi) with two parent molecules connected by a short bis-ester linker (see Figure 2).

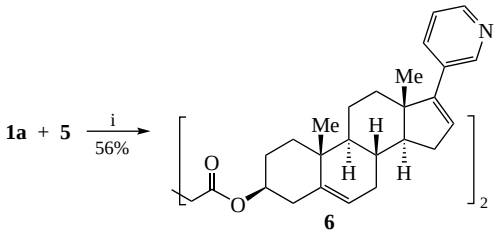
When choosing a linker fragment of bis-Abi, we considered both the need for non-toxicity of the diacid removed during its hydrolysis and the desire to obtain a structure with a lipophilicity not exceeding the lipophilicity of compound **2** (calc. log*P* ~ 8, SwissADME software[10]). Therefore, the possibility of synthesizing conjugates with malic, succinic and citric acids was studied (Scheme 1). The standard esterification of abiraterone **1a** with the appropriate acids in the presence of DCC/DMAP or with acid chlorides was ineffective either due to the formation of a complex mixture of products or due to extremely low conversion (according to liquid chromatography mass spectrometry data in no case a dual conjugate was formed). Therefore, the reaction was carried out sequentially using acid anhydrides.[11–13]

Mono-esters of malic and citric acids **3** and **4**, respectively, were obtained in very low yields due to poor conversion and the formation of complex mixtures of products, from which the isolation of the mono-esters with 83–95% purity was achieved only after repeated column and thin-layer chromatography (for details, see Online Supplementary Materials). Considering the very low yield of mono-esters **3** and **4**, their further modification was not carried out. Succinic acid mono ester **5** (described earlier[13]) was obtained by our optimized preparative method in high yield and was further introduced into the next esterification to give the target bis-conjugate **6** (Scheme 2).

The primary biotesting of compound **6** was intended to confirm the depot-prodrug concept that is poorly active towards the molecular target but is able to release slowly the active metabolite (abiraterone, **1a**) into the blood plasma. The low probability of interaction of bis-conjugate **6** with CYP17A1 follows from the visual inspection of the abiraterone binding site and adjacent regions in the enzyme (PDB ID: 3RUK[14]) and



Scheme 1 *Reagents and conditions*: i, DMAP, CH_2Cl_2, room temperature, 4 days; ii, THF, reflux, 12 h.



Scheme 2 *Reagents and conditions*: i, EDCI, DMAP, CH_2Cl_2, room temperature, 12 h.

molecular modeling data (carried out as earlier described,[15–18] see Online Supplementary Materials). Besides, the binding of compound **6** to the active site of recombinant truncated CYP17A1 (Δ2–19 variant[14]) was studied by *in vitro* spectrophotometric titration. UV-visible absorption difference spectra of homodimer **6** [Figure 3(*a*)] and abiraterone [Figure 3(*b*)] were measured to compare their binding affinities. According to the binding analysis, compound **6** has significantly low affinity to CYP17A1 compared to abiraterone, as expected (for details, see Online Supplementary Materials).

Next, hydrolysis of bis-conjugate **6** in different media was studied. Incubation of compound **6** in PBS (pH 7.4) at 37 °C for 72 h and subsequent analysis of the mixture by HPLC demonstrated that during this time the conjugate was not hydrolyzed at any ester group and confirmed the expected stability of both ester groups with respect to purely chemical hydrolysis. The hydrolysis of bis-ester prodrug **6** in rat plasma was standardly evaluated by measuring its plasma half-life, that

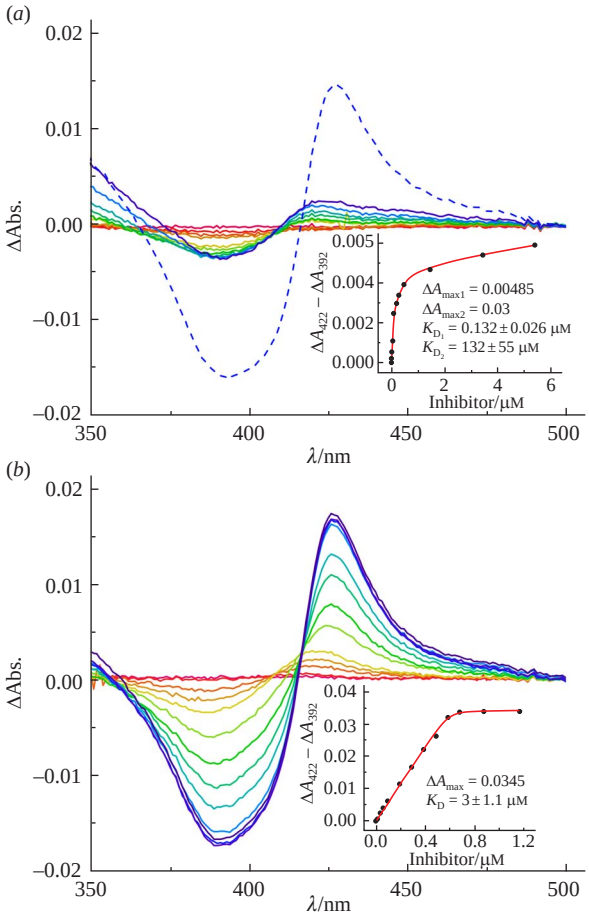


Figure 3 UV-visible difference spectra measured during the titration of CYP17A1 (1 μM) with (*a*) compound **6** and (*b*) abiraterone **1a**. Insets correspond to the data curve fitting for the determination of the apparent equilibrium dissociation constant (K_D). The dashed line in graph *a* corresponds to the extra addition of abiraterone (1 μM) to CYP17A1 titrated with compound **6**.

Table 1 Absolute amounts of compounds at different time points during the incubation in blood plasma.

Compound	Analyte area (cps[a]), mean values at the indicated time		
	0 h	3 h	22 h
6	31521.5	17295.0	10072.0
5	0	0	0
1a	345.5 (1.1%)[b]	923.0 (5.3%)[b]	1176.0 (11.7%)[b]
Blank	0	0	0

[a] Unit 'cps' is 'counts per second'. [b] In parentheses: the percentage of **1a** relative to the amount of **6**.

Table 2 Half-times for prodrug ester cleavage in blood plasma.

Compound	Remain (%), mean values at the indicated time			$t_{1/2}$/h
	0 h	3 h	22 h	
6	100.0	54.9	32.0	15.9
Propranolol	100.0	74.4	64.7	45.2

is the conversion of prodrug **6** to mono-ester **5** and active drug **1a** (non-productive metabolism was not considered).[19,20] In brief, the procedure was based on the incubation of the test (or reference propranolol) compound in rat plasma at 37 °C during various time intervals. At the end of incubation, the reaction was stopped by deproteinization of blood plasma (precipitation of proteins) with ice-cold acetonitrile. Quantitative determination of the content of the compounds **6**, **5** and **1a** was carried out by liquid chromatography tandem mass spectrometry (HPLC-MS/MS) method using selected reaction monitoring (SRM) mode to monitor precursor → product ion transitions[19,20] (for details, see Online Supplementary Materials). The experiment was carried out in duplicate, the basic results are presented in Tables 1 and 2.

The data on the absolute amounts of compounds at different time points (see Table 1) show that monoester **5** (MW = 449 Da) was not detected in any sample, containing compound **6**. However, the active drug **1a** (abiraterone, MW = 349 Da) was detected in sufficient concentration for quantitative determination in all samples with homodimer **6** (and was not detected only in samples not containing conjugate **6**, referred as Blank in Table 1). These data and the calculated half-life ($t_{1/2}$, see Table 2) of the test compound **6** indicate that in rat plasma bis-ester **6** undergoes long-lasting enzymatic cleavage of both masking units.

In conclusion, the first synthesis of homodimeric bis-ester of abiraterone was carried out. Since initial biotesting confirms that it hydrolyzes slowly and continuously to parent molecule in rat blood plasma, this compound is a good candidate for testing in *in vivo* models as a novel depot-form of abiraterone.

Design, synthesis and molecular modeling were carried out in Moscow State University under financial support of the Russian Science Foundation (grant no. 22-63-00016). Hydrolysis in rat plasma was studied in FRC for Innovator and Emerging Biomedical and Pharmaceutical Technologies. Assessment of inhibitory activity to CYP17A1 was performed in the Institute of Bioorganic Chemistry (Belarus). The authors are very grateful to Prof. Tatiana A. Gudasheva for her assistance.

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2024.06.010.

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