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Discovery of novel phosphorus-containing steroids as selective glucocorticoid receptor antagonist

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Abstract—Mifepristone is a non-selective antagonist of 3-oxosteroid receptors with both abortifacient and anti-diabetic activities. For glucocorticoid receptor (GR) program, we sought an unexplored, synthetically accessible phosphorus-containing steroidal mimetic of mifepristone, suitable for parallel synthesis of analogues. One compound **4a**, with high oral bioavailability (59%) in rat, exhibited functional antagonism of GR in oral glucose tolerance test (OGTT). Thus this series of compounds might be potentially useful for the treatment of type II diabetes.

Glucocorticoid receptors (GRs), members of the steroid–thyroid–retinoid superfamily, are soluble, intracellular receptor proteins acting as ligand-regulated transcription factors controlling specific gene expression in most mammalian cells.[1] Glucocorticoids bind to and activate GRs, and they play a crucial role in the normal development and maintenance of basal and stress-related homeostasis.[2] The steroid nucleus of typical glucocorticoids is associated with a rich variety of hormonal activities and has inspired the development of an assortment of therapeutic agents. Potent and selective GR antagonists could be used for the possible treatment of a variety of disorders, including diabetes,[3] obesity,[4] depression,[5] neurodegeneration,[6] glaucoma,[7] and Cushing's disease.[8]

The potent steroidal GR antagonist **1**, mifepristone (RU-486), is known for its abortifacient effects due to potent progesterone receptor (PR) antagonism (Fig. 1).[9]

The goal of our GR antagonist program is to identify a compound with similar potency and higher selectivity than mifepristone (RU-486) in terms of its progesterone receptor antagonism.

There have been literature reports on using phosphinic acids as bioisosteres of the carboxylic acid group.[10] We envisioned that dialkyl or dialkoxyl phosphonyl groups can serve as bioisosteres in steroids for either the carbonyl group on the 11β-aryl group of ZK112993 (**2**), Org-33628 (**3**) or *N*,*N*-dimethyl group in RU-486 (**1**) (Fig. 1). In the present article, we describe the synthesis and biological properties of phosphorus-containing steroidal derivatives **4** and **5**. According to X-ray crystal of RU-486 with GR,[11] as a replacement of *N*,*N*-dimethyl group in compound **1** or acetyl group in compounds **2** and **3**, phosphate or phosphinic mono-acid might provide additional functional groups in GR binding pocket to increase the interactions toward GR receptor. This might result in both increase in potency in GR antagonism and selectivity versus PR antagonism.[12]

The chemistry used for target compound **4** is shown in Scheme 1. Thus, commercially available ethylene deltanone **6** was functionalized at C17 position by the treatment of allyl, propynyl or phenyl Grignard reagent. Alcohol **7** was converted to epoxide **8** after exposed to *m*-CPBA. Cuperate 1,4-addition to epoxide **8** led to C-11 phenyl substituted compound **9**. Deprotection of THP functional group under acidic condition and reaction with Tf_2NPh under basic condition resulted in phenyl triflate **10**. Coupling reaction with phosphorus reagent under microwave irradiation condition furnished target

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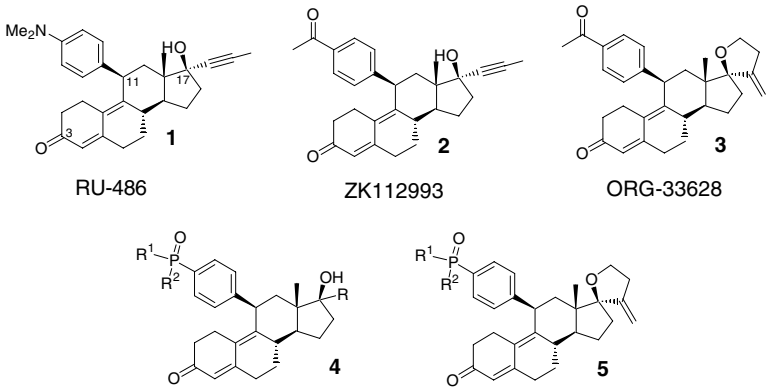
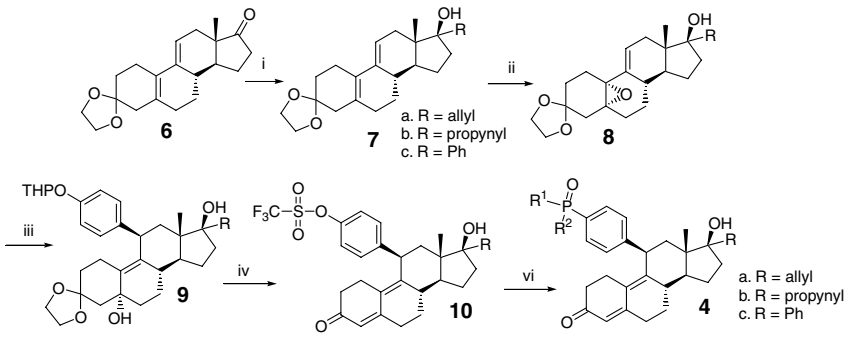


Figure 1. Structures of steroids and target compounds.



Scheme 1. Synthetic route for phosphorus-containing steroids. Reagents and condition: (i) RMgBr, THF, 85%; (ii) *m*-CPBA, DCM, 35%; (iii) 4-THPO-PhMgBr, CuCl, THF, 53%; (iv) a—oxalic acid or *p*-TSA, 75%; b—Tf_2NPh, NaH, 60%; (v) $Pd(OAc)_2$, dppb, *i*-Pr_2NEt, $HP(O)R^1R^2$, microwave, 20–70%.

compound **4**. The synthesis of compound **5** could be found in Ref. 13.

The compounds were tested for their glucocorticoid receptor (GR) antagonist activity based on their ability to inhibit corticoid-induced transcription from a glucocorticoid response element (GRE)-linked luciferase reporter gene in the human lung carcinoma cell line A549 (Table 1). The compounds were also evaluated for progesterone receptor (PR) antagonist activity based on their ability to block progesterone induction of alkaline phosphatase activity in the human breast cancer cell line T47D. The IC_{50} of the compounds from the A549 and T47D assays are listed in Table 1. The ratio of the T47D IC_{50} to the A549 IC_{50} was calculated and is listed in the column 'Ratio', as a measure of the separation of GR and PR antagonism. The commercial drug mifepristone **1** (RU-486) was tested as a control. Compounds **4a**–**4f** are potent GR antagonists, with **4c**, almost as potent as RU-486. Compounds **4a**–**4f** have similar or better selectivity (up to 127-fold for **4c**) than RU-486 (2.6-fold). The interesting observation is that esters **5a** and **5c** are active GR or PR antagonists, while both GR and PR activities are totally abolished for corresponding monoacids **5b** and **5d**.[14]

In order to assess the in vivo potential of this class of selective GR modulators, liver microsomal metabolism

Table 1. In vitro biological activities for compound **4a**–**4e** and **5a**–**5d**

Compound	R^1	R^2	R	A549 K_i[a] (nM)	T47[b] IC_{50} (nM)	Ratio
RU-486				1.0	2.6	2.60
4a	Ph	Ph	Allyl	10.0	763	76.3
4b	EtO	EtO	Allyl	10.0	308	30.8
4c	Ph	Ph	Propynyl	3.0	381	127
4d	EtO	EtO	Propynyl	9.0	141	15.7
4e	Me	Me	Propynyl	10.0[a]	101	10.1
4f	Ph	Ph	Ph	20.0	275	13.8
5a	EtO	EtO	—	56.3	9.9	0.17
5b	OH	OEt	—	>3000	>1000	—
5c	EtO	Me	—	59.8	1.6	0.027
5d	OH	Me	—	>3000	>1000	—

[a] This value is IC_{50} (nM).

Table 2. Pharmacokinetic data for compound **4a** compared with RU-486

Compound	Fomulation	Route	Dose (mg/kg)	C_{max} (μg/mL)	CL (mL/min/kg)	T_{max} (h)	$t_{1/2}$ (h)	AUC (mg/h/mL)	F (%)
4a	10% soluto	iv	2	5.290	7.98	0.080	0.77	4.173	—
	Seasame oil	po	10	1.847		2.00	2.20	12.407	59
RU-486	10% soluto	iv	2	2.797	115	0.080	4.00	1.371	—
	Seasame oil	po	10	0.030		1.00	2.80	1.863	31

and rat oral/iv pharmacokinetic data were obtained. Compound **4a** has good level of in vitro metabolic stability in microsome across different species, as shown in $t_{1/2}$: human, >30 min; rat, >30 min; mouse, 28 min; dog, 21 min; monkey, 8 min; rabbit, 30 min. In mice, the GR modulator **4a** had a short iv half-life (t = 0.77 h) and high iv clearance (7.98 mL/min/kg), consistent with rapid metabolism, observed for steroids.[15]

Compounds **4a** and **4b** have good CACO-2 permeability (2.6×10^{-6} cm/s and 14.5×10^{-6} cm/s, respectively). Compound **4a** has a bioavailability of 59% in mice while RU-486 was 31% tested side by side (Table 2). Compound **4a** did not show hERG binding activity (27.8% inhibition at 10 μM).

RU-486 is a non-selective GR antagonist, which showed potential utility as anti-diabetic agent.[3] Treatment of human or rodent models of diabetes, with RU-486 (**1**), lowers hepatic glucose production (HGP) by reducing the expression of the key gluconeogenic enzymes (PEPCK) and glucose-6-phosphatase (G6Pase).[16] Extrahepatic GR antagonism, however, has both desirable and undesirable consequences. Potential benefits include increased insulin sensitivity, fat redistribution, and effects on bone while detriments include activation of the hypothalamic pituitary adrenal (HPA) axis, diminution of immune response, and a decreased stress response.[17] Clinically, the side effects of RU-486 are significant. Experience with up to 12 months RU-486 treatment can lead to symptoms of adrenal insufficiency (nausea, vomiting, and exhaustion) and hypercortisolemia (activation of HPA axis, leading to increased cortisol secretion).[18] A liver-specific derivative of RU-486 would be expected to decrease hepatic glucose output (HGO) and improve glucose metabolism without the risk of these peripherally driven side effects.

Compounds **4a**, **4b**, **4c**, and **4d** were tested in the oral glucose tolerance test (OGTT). The compound is dosed to three ob/ob mice orally, once a day, at 10, 30, and 100 mg/kg, over a period of 15 days. Postprandial plasma glucose levels are measured everyday and are compared with vehicle. The results of OGTT suggest that both RU-486 and compound **4a** improved the ability of obese ob/ob mice to handle glucose at the same dose, while other three compounds **4b**, **4c**, and **4d** did not show significant glucose lowering effect compared to vehicle (data not shown). We hope the better selectivity of our compounds versus PR would significantly reduce the adverse effects associated with PR antagonism.

In summary, we have designed and synthesized a series of novel phosphorus-containing steroidal GR antagonists. The best compounds demonstrated comparable potency to RU-486 and better selectivity versus PR than RU-486. Selected compound showed good efficacy in mice in lowering plasma glucose level. With the potent and selective GR antagonists in hands, our future efforts will be focused on achieving liver selectivity.

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