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Synthesis and identification of novel 11β-aryl-4′, 5′-dihydrospiro[estra-4,9-diene-17β,4′-oxazole] analogs with dissociated antiprogesterone activities

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Abstract—A series of novel 11β-aryl-4′,5′-dihydrospiro[estra-4,9-diene-17β,4′-oxazole] analogs have been evaluated for their antagonist hormonal properties using the T47D cell-based alkaline phosphatase assay and the A549 cell-based functional assay. Some of the compounds showed highly potent, and more selective antiprogestational activity against antiglucocorticoid activity than mifepristone (RU 486).

Progesterone, mediated by interaction with progesterone receptor (PR), plays a critical role in the regulation of female reproductive events associated with the establishment and maintenance of pregnancy.[1] It also has less clearly defined functions in the brain, the immune system, and the vascular endothelial system. The last two decades have witnessed the development of synthetic compounds that range from full PR antagonists to compounds with mixed agonist/antagonist actions, the latter also known as selective progesterone receptor modulators (SPRMs).[2] Many such compounds have numerous clinically proven and potential therapeutic applications such as for abortion, contraception, and in the treatment of uterine leiomyomata, endometriosis, and breast cancer.[3]

The first competitive PR antagonist, mifepristone (RU 486, **1**, Fig. 1), was introduced by the Roussel-Uclaf group.[4] Mifepristone is currently used as an abortifacient in the first 2 months of pregnancy and in smaller doses as an emergency contraceptive. However, due to its glucocorticoid receptor (GR) antagonism, mifepristone has compromised clinical applications (e.g., as a long-term contraceptive agent).[5] Since the discovery of mifepristone, much effort has been made to optimize

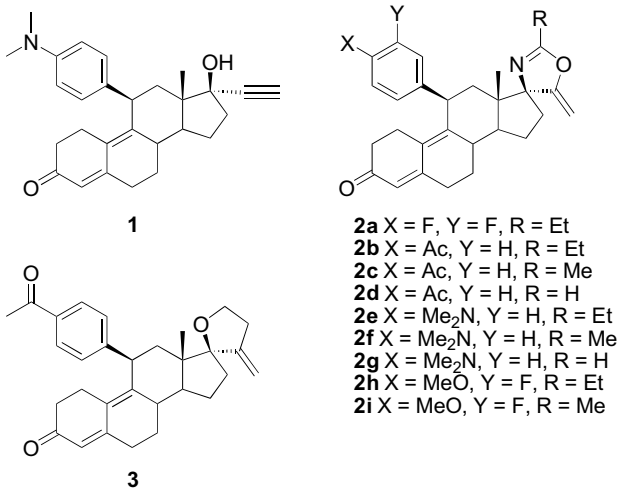

Figure 1. Structures of mifepristone (**1**), steroidal spiro-oxazoles (**2**), and ORG 33628 (**3**).

Keywords: Steroidal spiro-oxazole; Progesterone receptor; Glucocorticoid receptor; Antagonist; Selective progesterone receptor modulators.
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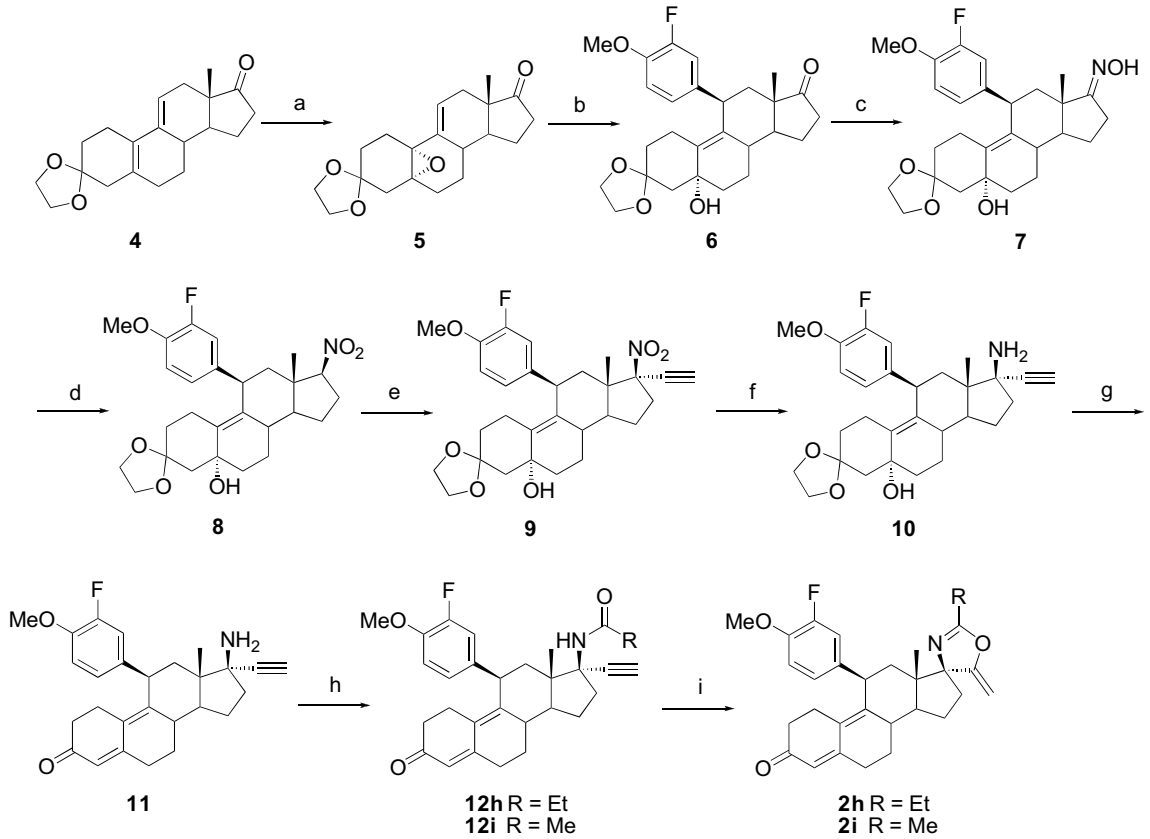
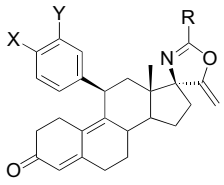
the antiprogestational effect with regard to the reduction of antiglucocorticoid activity.[6] At present few clinically successful selective PR antagonists are available. We recently developed a convenient synthesis of novel 11β-aryl-4′,5′-dihydrospiro[estra-4,9-diene-17β,4′-oxzole]s (e.g., **2a**; X = F; Y = F; R = Et) via copper(I)-catalyzed cyclization of the corresponding acylaminoacetylenes[7] as part of a research program to discover highly potent antiprogestins with reduced endocrine side effects. These novel spiro-oxazoles are structurally similar to the reported 17,17-spirocyclic steroids (e.g., ORG 33628, **3**), which have been claimed to have enhanced antiprogestational effects, in some cases, with considerably reduced antiglucocorticoid activities.[8] Herein we report a series of new 11β-aryl-4′,5′-dihydrospiro[estra-4,9-diene-17β,4′-oxazole] analogs (**2b**–**i**, Fig. 1) and their antagonist hormonal properties.

The synthesis of 11β-aryl-4′,5′-dihydrospiro[estra-4,9-diene-17β,4′-oxazole] analogs **2b**–**g** was accomplished following the previously reported procedures.[7,9] Compounds **2h** and **2i** were prepared using the same synthetic approach, outlined in Scheme 1, with some modifications. Briefly, the regio-/stereo-selective epoxidation of commercially available 3,3-[1,2-ethanediylbis(oxy)]estra-5(10),9(11)-dien-17-one (**4**) using hexafluoroacetone trihydrate and hydrogen peroxide gave epoxide **5** in 53% yield. Copper-catalyzed 1,4-addition of **5** with the Grignard reagent, generated from 4-bromo-2-fluoroanisole, led to the corresponding 11β-aryl substituted compound **6** in 87% yield.[10] Oxime formation with hydroxylamine hydrochloride in pyridine provided **7**. Crude **7**, used without purification, was treated with NBS to afford the 17-bromo-17-nitro compound, which was readily reduced by $NaBH_4$ to give **8** in 78% yield.[11] The introduction of the 17α-ethynyl substituent in the C17-position was achieved stereoselectively by treatment of the anion of **8** in DMSO with ethynyllead(IV) triacetate to give a 66% yield of **9**.[12,13] Reduction of **9** using zinc at 0 °C gave the corresponding hydroxylamine, which upon treatment with 2-mercaptoethyl ether and sodium tetraborate produced amine **10** in 70% yield.[14] This two-step reduction procedure is superior to the zinc reduction at 70 °C, in which the ethynyl group was also reduced to the ethene and **10** was obtained in low yield. Deketalization and simultaneous dehydration of **10** with TFA provided dienone **11**. Finally, acylation with propionyl chloride or acetyl chloride followed by cyclization[7] using 10 mol % of CuI in 1:1 benzene/Et_3N at 90 °C furnished spiro-oxazoles **2h** and **2i** in 80% and 42% yield, respectively. All synthesized spiro-oxazoles **2b**–**i** were ⩾98% pure as determined by HPLC analyses. The ^1H NMR spectra of the compounds were in agreement with the assigned structures.[7,15]



Scheme 1. Synthesis of **2h** and **2i**. Reagents and conditions: (a) CF_3COCF_3, Na_2HPO_4, H_2O_2, 0 °C; (b) 4-bromo-2-fluoroanisole/Mg, THF, CuI, 0 °C; (c) $HONH_2$·HCl, pyridine, rt; (d) NBS, $KHCO_3$, dioxane, H_2O, rt, then $NaBH_4$, rt; (e) Bu_3SnCCH, $Pb(OAc)_4$, rt, then **8**/KO*t*-Bu/DMSO, rt; (f) Zn, NH_4Cl, 0 °C, then 2-mercaptoethyl ether, $FeSO_4(NH_4)_2SO_4$, $Na_2B_4O_7$, 0.1 N HCl, reflux; (g) TFA, CH_2Cl_2, H_2O, 0 °C; (h) propionyl chloride or acetyl chloride, Et_3N, 0 °C; (i) 10 mol % CuI, 1:1 benzene/Et_3N, 90 °C.

Table 1. Antihormonal properties of spiro-oxazoles **2b–i**



Compound	Structure			T47D (PR) and A549 (GR) activity		
	X	Y	R	T47D IC_{50} (nM)	A549 IC_{50} (nM)	Ratio (GR/PR)
Mifepristone				0.054	6	110
2b	Ac	H	Et	0.34	210	620
2c	Ac	H	Me	0.59	460	780
2d	Ac	H	H	82	14,000	171
2e	Me_2N	H	Et	0.98	23	24
2f	Me_2N	H	Me	0.63	82	130
2g	Me_2N	H	H	18	540	30
2h	MeO	F	Et	1.0	>10,000	>10,000
2i	MeO	F	Me	4.6	660	140

Compounds **2b–i** were evaluated for PR antagonist activity based on the ability to block promegestone (R5020) induction of alkaline phosphatase activity in the human breast cancer cell line T47D.[6e] Their GR antagonist activity was also tested on the ability to inhibit corticoid-induced transcription from a glucocorticoid response element (GRE)-linked luciferase reporter gene in the human lung carcinoma cell line A549.[6e] The IC_{50} values of the tested compounds from the T47D and A549 assays are listed in Table 1. The ratio of the A549 IC_{50} to the T47D IC_{50} was also calculated as a measure of the separation of PR and GR antagonism. The commercial drug mifepristone was tested as a control.

In the T47D assay, all the tested compounds are potent progesterone receptor antagonists, with nanomolar IC_{50} values (**2d** and **2g–i**) or subnanomolar IC_{50} values (**2b**, **2c**, **2e**, and **2f**). However, none of the compounds is as potent as mifepristone (IC_{50} 0.054 nM). The 11β-(4-acetophenyl) substituted compounds (**2b** and **2c**) are more potent than the compounds (**2e** and **2f**) with a 11β-(4-*N*,*N*-dimethylaminophenyl) substitution, which is consistent with the findings that replacement of the 11β-(4-*N*,*N*-dimethylaminophenyl) substituent in the mifepristone derivatives with the 11β-(4-acetophenyl) moiety increases the relative binding affinity for the PR.[16] For the same 11-substitution, the compounds with R = Et and Me have higher potency than the R = H compound. The most potent compound in the series was achieved when R = Et with the 11β-(4-acetophenyl) substituent (**2b**). It is interesting to note that both of **2d** and **2g** (R = H) have the highest affinity for PR in the competitive binding assay (data not shown). The low potency of **2d** and **2g** in the T47D assay, as well as in the A549 assay, is presumably due to the instability of the spiro-oxazole moiety of these compounds.

In the A549 assay, all compounds are less potent than mifepristone, with IC_{50} values greater than 23 nM. Marked reduction in the potency was observed by substitution of an 11β-(4-acetophenyl) group for 4-*N*,*N*-dimethylaminophenyl. In terms of selectivity, all 11β-(4-acetophenyl) substituted compounds (**2b–d**) show greater separation of antiprogestational and antiglucocorticoid activity compared to mifepristone, while the 11β-(4-*N*,*N*-dimethylaminophenyl) compounds (**2e–g**) have similar or less selectivity than mifepristone. Compounds **2b** and **2c** are 6- and 7-fold, respectively, more selective than mifepristone with subnanomolar potencies for PR. The best selectivity in differentiating PR from GR was realized when R = Et with the 3-fluoro-4-methoxyphenyl group at the 11-position (**2h**). This compound has nanomolar potency (IC_{50} 1.0 nM) as a PR antagonist and shows no activity in the A549 assay for GR agonism or antagonism at the tested concentrations.

In conclusion, a series of novel 11β-aryl-4′,5′-dihydrospiro[estra-4,9-diene-17β,4′-oxazole] analogs have been synthesized in a 10-step sequence from commercially available 3,3-[1,2-ethanediylbis(oxy)]-estra-5(10),9(11)-dien-17-one (**4**). Some of the compounds (**2b**, **2c**, and **2h**) demonstrated high potency and a better selectivity profile in the separation of antiprogestational and antiglucocorticoid activity than mifepristone. These novel steroidal spiro-oxazoles are promising leads for further investigation of highly potent and selective antiprogestins.

Acknowledgment

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