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Non-Steroidal Pregnancy-Terminating Agents: Design, Synthesis and Structure–Activity Relationships of 2-Aryl-1,2,4-triazolo[1,5-*a*]pyridine

Tao Liu and Yongzhou Hu*

Zhejiang University, Department of Medicinal Chemistry, College of Pharmacy, Hangzhou 310031, PR China

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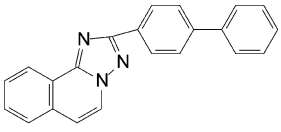
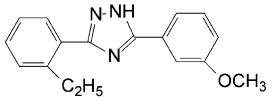
Abstract—The syntheses and the pregnancy-terminating activity relationships of compounds **5a**–**n** are reported. Compounds **5b** and **5l** are found to be more potent than DL-111—a known drug having effective pregnancy-terminating activity in vitro. Further research shows compounds **5b** and **5l** have the same activity as DL-111 in vivo. We also found an exciting result that they have excellent anti-implantation activity after oral administration.

In a search for new nonhormonal compounds with antifertility activity, a series of 2-aryl-1,2,4-triazolo-[5,1-*a*]isoquinoline 2-aryl-1,3-midazolo[2,1-*a*]isoquinoline were synthesized by Italian researchers during the 1980s. They found these compounds had some early pregnancy-terminating activity;[1–5] in particular, L14105 **1** and DL-111 **2** had stronger activity, with ED_{50} values of 0.016 and 0.04 mg/kg/day in hamster, respectively.[4] Research testifies that the mechanism of early pregnancy-terminating role of DL-111 is to induce apoptosis to cause a luteolytic effect in pregnant rats.[6] But their very sustained pharmacokinetic profiles and/or their low solubility, even in oily vehicles, hindered their use in clinical studies. In order to develop new triazole compounds that have high potency, reduced toxicity and good solubility, it is worth further investigation to modify the structure of the lead compound—L14105.



1 : L 14105



2 : DL-111

Research indicates that the ring of triazole is essential for the pregnancy-terminating activity through structural analysis and 2-phenyl ring can improve the activity.[4] However, the isoquinoline ring as a possible pharmacophore is still not well understood. In this paper, we describe the synthesis and biological properties of 2-aryl-1,2,4-triazolo[5,1-*a*]pyridines. The pattern of the isoquinoline ring was substituted by 2-methyl- or 4-methylpyridine to decrease the partition coefficient and the substituent of 2-phenyl was changed to increase the stability of metabolism.

The synthesis of target compounds is shown in Scheme 1. *N*-Amination of 2-methylpyridine or 4-methylpyridine **3** with *O*-mesitylenesulfonyl hydroxylamine (MSH) afforded *N*-amine-2-methylpyridinium mesitylenesulphonate or *N*-amine-4-methylpyridinium mesitylenesulphonate **4**. Subsequently, condensation of *N*-amine salts **4** with substituted benzonitrile in alkaline conditions gave the desired compounds **5a**–**n**, respectively (Table 1).

Newly synthesized compounds were tested for potency as early pregnancy-terminating agents. In vitro early pregnancy-terminating activity was tested to see whether they could induce apoptosis to cause a luteolytic effect. It revealed that one mechanism of early pregnancy-terminating activity of DL-111 was the action of luteolysis in the study of Bo Yang.[6] Corpora lutea which were excised from pseudopregnant rats were dissected out under a microscope and seeded at a density of $(1–2)\times10^5$ cells/well in 0.5 mL McCoy's 5A medium supplemented with 0.1% BSA for 24 h, then tested compounds were added. Cells were cultured at 37 °C in 5% CO_2 for 24 h. Cell viability was assessed by trypan

*Corresponding author. Tel.: +86-571-8721-7210; fax: +86-571-8721-7412; e-mail: huyz@zjuem.zju.edu.cn

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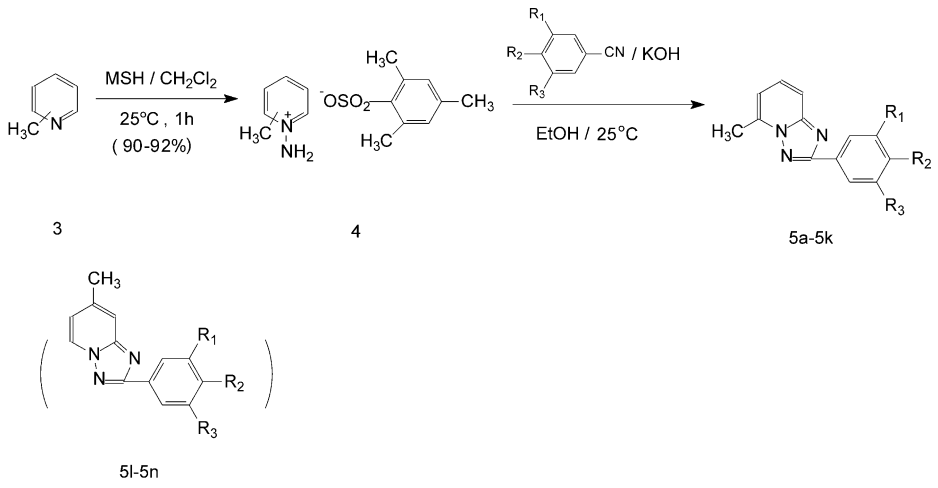

Scheme 1.

blue dye exclusion. The results are summarized in Table 2.

As shown in Table 2, all compounds have the activity of luteolysis except compound **5k**, which has no substituent on the benzene ring at the 2-position. We find the activity of luteolysis is influenced by the position of substituents as well as the type of substituents. In the trial, the activity of the *para*-position substituted compound (i.e., **5g**) is more potent than that of the *meta*-position substituted compound (i.e., **5h**) or the *meta*- and *para*-position substituted compounds (i.e., **5a**, **5d**). We also find that compounds having alkyloxy group (i.e., **5b**, **5g, 5l**) have more potent activity than those of other groups (i.e., **5e, 5i, 5j, 5m, 5n**). Especially, the ethyloxy group substituted compound has the most potent activity (i.e., **5b** versus **5c, 5g**).

Table 1. Physical data of **5a**–**5n**

	R_1	R_2	R_3	Mp (°C)	Yield (%)
5a	H	OMe	OMe	127–129	60
5b	H	OEt	H	133–134	59
5c	H	*n*-C_4H_9	H	105–106	67
5d	OMe	OMe	OMe	156–157	41
5e	H	NMe_2	H	149–151	38
5f	H	–O–CH_2–O–		159–161	42
5g	H	OMe	H	143–144	39
5h	H	H	OMe	105–108	53
5i	H	Ph	H	145–147	62
5j	H	Cl	H	142–144	50
5k	H	H	H	84–116	51
5l	H	OMe	H	169–171	52
5m	H	Ph	H	186–188	77
5n	H	Cl	H	192–194	66

Table 2. In vitro action of luteolysis (ED_{50} μM)

5a	571±6.1	**5b**	2±0.2	**5c**	25±1.9	**5d**	48±5.0
5e	28±2.1	**5f**	65±6.0	**5g**	21±2.0	**5h**	42±3.8
5i	32±3.0	**5j**	36±3.2	**5k**	—	**5l**	7±0.6
5m	38±3.7	**5n**	32±2.9	DL-111	28±2.8		

To testify their activity in vivo, compounds **5b** and **5l** were selected to test for their early pregnancy-terminating activity in mice. Compounds **5b** and **5l** were administered im and multiple treatments of them were given from day 4 to day 6 of gestation. The effects on pregnancy were examined on day 14. At autopsy, the numbers of live and dead embryos or implantation sites were counted to calculate the ED_{50} and ED_{95} of terminating pregnancy. ED_{50}s were calculated by Bliss method. The results are listed in Table 3.

From the data, it seems that the total dosage of compounds **5b** and **5l** in the trial of early pregnancy-terminating activity in mice is slightly lower than that of DL-111. Occasionally, compounds **5b** and **5l** were found having anti-implantation activity as oral administration. In particular, ED_{50} of oral anti-implantation is only 2.5 times the dosage of early pregnancy-termination by injection whereas ED_{50} of DL-111 by oral administration is higher than the parenteral one. Their ratio exceeds 50. Thus, oral activity of compounds **5b** and **5l** is more obviously excellent than that of the similar compound.[4]

In summary, newly pregnancy-terminating agents were prepared. Among 14 easily available 2-aryl-1,2,4-triazolo[1,5-*a*]pyridines, compounds **5b** and **5l** were more potent than DL-111 in vitro (14- and 4-fold, respec-

Table 3.

Compd	Early pregnancy-terminating activity in mice			Anti-implantation activity in mice		
	Route	Days of treatment	ED_{50} (mg/kg/day)	Route	Days of treatment	ED_{50} (mg/kg/day)
DL-111	sc	4–8	0.30 (0.12–0.51)	ig	1–3	>15.0
5b	im	4–6	0.64 (0.39–0.99)	ig	1–3	1.41 (0.68–2.92)
5l	im	4–6	0.45 (0.11–0.79)	ig	1–3	1.12 (0.32–2.30)

tively). Further research showed compounds **5b** and **5l** had the same early pregnancy-terminating activity as DL-111 in vivo and their oral anti-implantation effects were better than similar drugs.

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