Chinese Chemical Letters Vol. 14, No. 12, pp 1223 – 1226, 2003
http://www.imm.ac.cn/journal/ccl.html

Synthesis and Bronchodilatory Activities of New Pyrazolo[4,3-d]pyrimidin-7-ones

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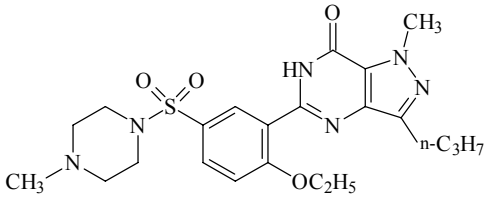
Abstract: Twelve novel pyrazolo [4,3-d] pyrimidin-7-ones were synthesized and their structures were confirmed by IR, ^{1}H NMR and MS. Their *in vitro* bronchodilatory activities were tested in guinea-pigs. The pharmacological results show that compound **11c** has more potent activity than aminophylline.

Keywords: Pyrazolo [4,3-d] pyrimidin-7-ones, synthesis, bronchodilatory activity.

In recent years there has been a renewed interest in the potential utility of isoenzyme--selective phosphodiesterase (PDE) inhibitors. The inhibition of PDE activity increases cellular levels of the key second messengers, cyclic AMP and cyclic GMP, thereby activating specific protein phosphorylation cascade that elicit a variety of functional responses[1]. Cyclic GMP PDE (PDE Ⅴ) inhibition is a particularly attractive target because cGMP mediates the vasorelaxant action of endothelium derived relaxing factor (NO) as well as the natriuretic and diuretic effect of atrial natriuretic factor (ANF) through activation of cGMP dependent protein kinase. This potent and selective inhibitor could display vasodilating, relaxant, and diuretic effects and be useful in treatment of hypertension and congestive heart failure.

Figure 1 The structure of sildenafil



There have been several reports of pyrazolo[4,3-d]pyrimidin-7-ones as potent and selective inhibitors of PDE Ⅴ[2,3]. Kapui[4] *et al.* reported that isolated histamine--precontracted guinea-pig trachea was relaxed by PDEⅤ inhibitor. Sildenafil has been reported as a potent PDE Ⅴ inhibitor and used in clinical now. Therefore we took

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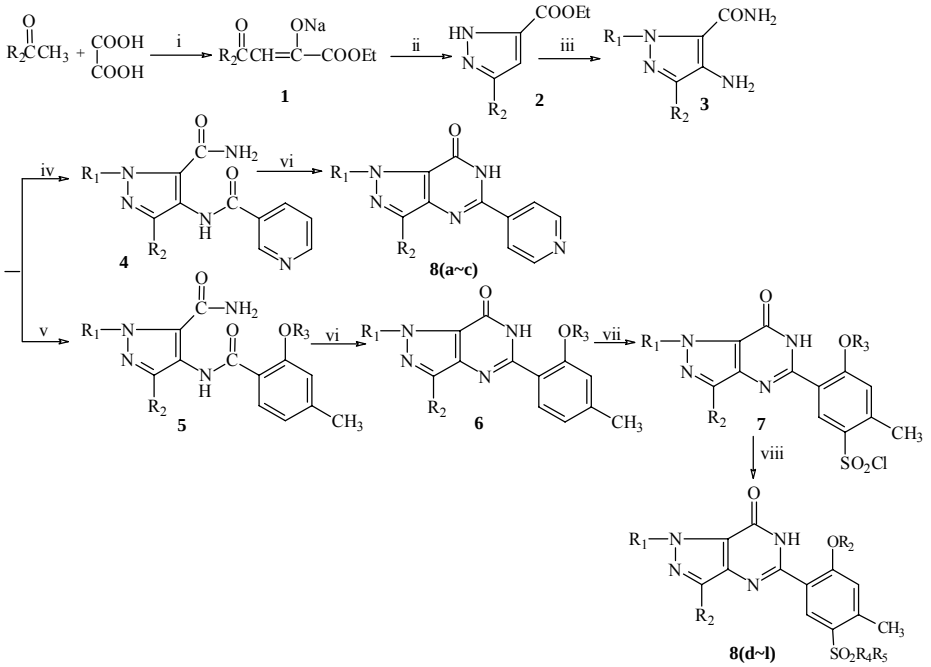
sildenafil as a leading compound. We designed and synthesized a series of pyrazolo-[4,3-d]pyrimidin-7-ones by substitution the benzyl of sildenafil to the pyridyl or changing the benzyl-substituted groups, and tested their bronchodilatory effect on isolated guinea-pig trachea in order to find new potent PDE Ⅴ inhibitors.

We describe here the synthesis of twelve novel pyrazolo [4,3-d] pyrimidine-7-ones (**Scheme 1**). Their structures were confirmed by MS and ^{1}H NMR. The structures and physical data of these new compounds were listed in **Table 1**.

Scheme 1 The synthetic route of pyrazolo [4,3-d] pyrimidin-7-ones



Reagents and conditions: i. $NaOC_2H_5$, C_2H_5OH, rt 12 h; ii. $H_2NNH_2 \cdot H_2O$, EtOH, reflux 10 h, 60~72% (for i, ii steps); iii. Compoud 3 was prepared according to the literature[2]; iv. Nicotinyl chloride, pyridine, dimethylamino- pyridine, CH_2Cl_2, reflux 4 h, 67~75%; v. 2-alkyloxy-4-methyl benzoyl chloride, pyridine, dimethylaminopyridine, CH_2Cl_2, reflux 4 h, 67~75%; vi. NaOH, H_2O_2, EtOH, reflux 6 h, 70~77%; vii. $ClSO_3H$, rt 24 h, 90~95%; viii. HNR_4R_5, CH_3OH, rt 24 h, 69~80%.

General procedures for the preparation of compounds **8** were as follows: the substituted ketone (0.1 mol) and diethyl oxalate (0.1 mol) were added to a solution (50 mL) of sodium ethoxide (0.1mol) in ethanol at 0~5℃, and the resulted mixture was stirred at r.t. for 10 h. The mixture was diluted with water, and then acidified to pH 2~3 by dilute sulfuric acid. 85% hydrazine hydrate (0.12 mol) was added and the resulted mixture was heated under reflux for 10 h. After evaporation of ethanol, the residue was extracted with CH_2Cl_2. The organic extract was washed with water, dried, and concentrated to give **2** (60~72%) which can be used directly without further purification.

Table 1 The structures and physical data of compound **8**

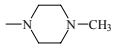
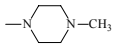
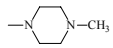
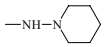
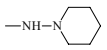
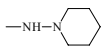
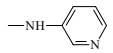
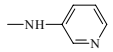
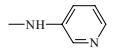
Compd.	R_1	R_2	R_3	NR_4R_5	mp (℃)	Yields(%)
8a • HCl	Et	*iso-Pr*			206~208	17.2
8b • HCl	Et	*n-Pr*			240~242	14.5
8c • HCl[6]	Me	*n-Pr*			242~244	11.6
8d • HCl	Me	*iso-Pr*	Et		228~230	8.0
8e	Me	*iso-Bu*	Me		151~153	7.1
8f	Me	*iso-Bu*	Et		104~106	10.6
8g	Me	*iso-Pr*	Et		90~92	9.7
8h	Me	*iso-Bu*	Me		170~172	11.6
8i	Et	*iso-Pr*	Et		146~148	12.4
8j	Et	*iso-Pr*	Et		180~182	6.8
8k	Et	*n-Pr*	Et		230~232	7.2
8l	Et	*iso-Pr*	Me		270~272	8.3

Table 2 The bronchodilatory effect of compounds **8** on the isolated guinea-pig trachea (n=4)

Compd.	Bronchodilatory effect (%) ($\bar{x}\pm s$)	Compd.	Bronchodilatory effect (%) ($\bar{x}\pm s$)
aminophylline	38.8±11.9		
8a • HCl	7.3±2.1	**8g**	0
8b • HCl	26.7±6.3	**8h**	5.0±1.4
8c • HCl	43.0±9.5	**8i**	0
8d • HCl	26.7±4.2	**8j**	9.7±2.1
8e	4.7±2.1	**8k**	2.7±2.1
8f	12.0±5.1	**8l**	19.3±3.7

Compound **8(a~l)** were prepared by the method of literature[2] from compound **2**.

The bronchodilatory effect of compound **8** on isolated histamine-precontracted guinea-pig trachea were investigated according to the method described by Xu Shuyun[5].

The control was aminophylline and the results were summarized in **Table 2**.

Compounds **8c** showed more potent bronchodilatory activity on the isolated guinea-pig trachea than amionphylline, and its further investigation is underway. The pharmacological tests shown that pyridyl substituted pyrazolo[4,3-d]pyrimidin-7-ones have potent bronchodilatory activity.

References and Notes

1. M. E. David, *Biochem. Pharmac.*, **1999**, *57*, 965.

2. A. S. Bell, D. Brown, N. K. Terrett, *EP 463,756*, **1991**.
3. A. S. Bell, N. K. Terrett, *EP 526,004*, **1992**.
4. Z. Kapui, P. Schaeffer, E. G. Mikus, *et al*., *Arzneim-Forsch./Drug Re*s., **1999**, *49,* 685.
5. S. Y. Xu, R. L. Bian, X. Chen., *Pharmacological experimental methods,* People health press. Beijing, **1991**, 1172.
6. Melting points were determined with capillary tube method, and the themometer was uncorrected. Mass spectra were obtained with a Finnigan LCQ HPLC-MS instrument. ^{1}H NMR spectrum were run on a Bruker ARX-300 instrument. Mass spectra data for compound 8(a~l): [MH^+] (*m/z*) **8a** •HCl 284, **8b** •HCl 284, **8c** •HCl 270, **8d** •HCl 489, **8e** 489, **8f** 503, **8g** 489, **8h** 489, **8i** 503, **8j** 497, **8k** 520, **8l** 483, which were all consistent with calculated data.Data for compound **8c**·HCl:
8c·HCl: ^{1}H NMR (DMSO-d_6): δ ppm: 0.96 (t, 3 H, *J*=7.3 Hz, -CH_2CH_2**CH_3**); 1.78 (m, 2 H, -CH_2**CH_2**CH_3), 2.84 (t, 2 H, *J*=7.5 Hz, **-CH_2**CH_2CH_3), 4.18 (s, 3 H, 1-**CH_3**), 8.43 (d, 2 H, *J*=5.6 Hz, pyridine-2,6-2**H**), 8.97 (d, 2 H, *J*=6.0 Hz, pyridine-3,5-2**H**).The strctures of other compounds were also confirmed by MS and ^{1}H NMR.

Received November 18, 2002