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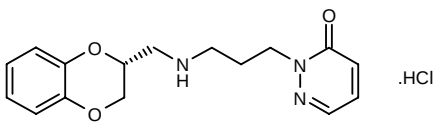
GYKI-16084

Treatment of BPH
α_1/α_2-Adrenoceptor Antagonist

IDR-16084

(+)-(*R*)-2-[3-(Benzo-1,4-dioxan-2-ylmethylamino)propyl]pyridazin-3(2*H*)-one hydrochloride



$C_{16}H_{19}N_3O_3$.HCl Mol wt: 337.8050

CAS: 185739-21-3 (as free base)

EN: 241391

Synthesis

The synthesis of GYKI-16084 has been achieved by coupling of 3(2*H*)-pyridazinone (XII) with (*R*)-2-(3-chloropropylamino)methylbenzo[1,4]dioxane (VIII) (1). These intermediates were obtained in independent ways.

The enantiomerically pure benzodioxane derivative (VIII) was obtained in a straightforward route including five steps starting from pyrocatechin (I). Racemic (VI) was then resolved by means of crystallization with (–)-*O*,*O*'-dibenzoyl-L-tartaric acid (Scheme 1). The pyridazinone intermediate (XII) was prepared from dihydroxypiridazine (IX) in three synthetic steps (Scheme 2). Finally, *N*-alkylation of the potassium salt of (XII) with the base form of (VIII) was performed in dimethyl sulfoxide to afford GYKI-16084 (Scheme 3).

Description

White crystalline subtance, m.p. 155-6 °C; [α] +49° (c 2, EtOH).

Introduction

Benign prostatic hyperplasia (BPH), a frequently occurring phenomenon in the older male population, is the cellular proliferation of the prostatic tissue. It may cause irritative and obstructive urinary symptoms which unfavorably influence both the quality of life and health status of patients (2-4).

Due to recent developments in the pathology and pharmacology of BPH, and to the well-recognized risks and high costs of conventional surgical interventions, it is now widely expected that drug therapy will represent the first choice in the management of BPH (5).

There are currently two main medical therapies for the treatment of BPH. Formation of dihydrotestoserone, which is a permissive and not a real causative factor of enlargement of the prostate (the static component of the disease), is inhibited by steroid 5α-reductase enzyme inhibitors. Finasteride, the prototype of competitive inhibitors of the 5α-reductase, has been shown to reduce the prostate size and more or less some symptoms in patients with BPH. However, it has not proved to be uniformly effective in all patient groups (2, 4), and, in some cases, it has been found to produce serious side effects, most frequently decreased libido, impotence and ejaculatory disorders (2, 5). Therefore, finasteride and 5α-reductase inhibitors in general seem to have their limitations in the treatment of BPH.

The smooth muscle of the prostate has a sympathetic innervation mediated by α-adrenoceptors. It has been widely accepted that the dynamic component of BPH is primarily determined by α_1-adrenoceptors, as dominant α-adrenoceptor subtype in the prostate. Therefore, α_1-receptor has been a major target for pharmacologic therapy. Several α_1-blockers, such as prazosin, terazosin, doxazosin, alfuzosin and tamsulosin, recently marketed for symptomatic treatment of BPH have been found to have a marked effect on the symptoms of BPH (6, 7). However, the threshold doses for hypotension of these agents, which were originally developed for antihypertensive therapy, are close to the effective doses for intraurethral pressure-lowering effects. This may, therefore, represent a risk for cardiovascular side effects. Therefore, uroselectivity has become a major issue of the α-adrenergic treatment option of BPH.

Many articles have more recently suggested that different subtypes of α_1-adrenoceptors might mediate

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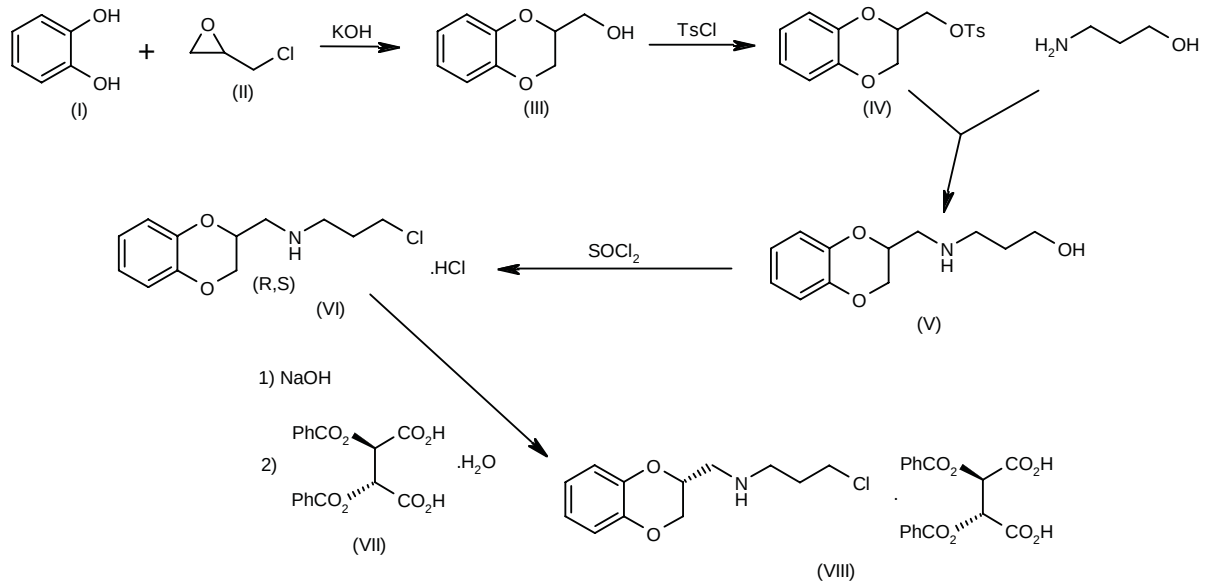
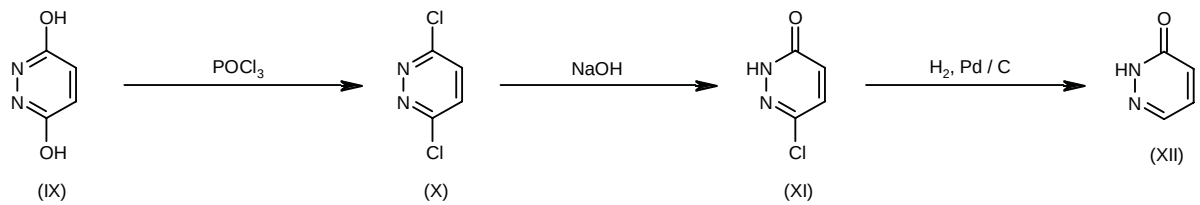
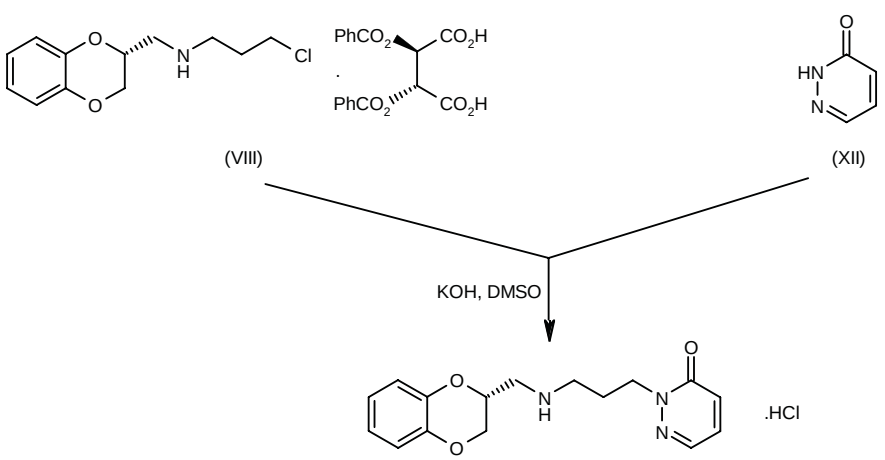
Scheme 1: Synthesis of Intermediate (VIII)



Scheme 2: Synthesis of Intermediate (XII)



Scheme 3: Synthesis of GYKI-16084



hypotensive and intraurethral pressure-lowering (α_{1A}) effects. Therefore, it has been proposed that side effects could be minimized by using subtype selective agents (6, 8-10). Although this sound hypothesis has not been unanimously proven (6, 11), and, on the contrary, results suggest that selectivity for urogenital α_1-adrenoceptor subtype does not neccesarily translate into the ability to significantly improve symptoms of patients with BPH (6, 12), current research has focused on the development of α_{1A}-adrenoceptor selective antagonists (13, 14). Interestingly, α_2-adrenoceptors as possible targets for treatment of BPH have attracted less attention, although they may also be involved in overactivity of sympathetic control in the prostate (15, 16). Moreover, α_2-antagonists are also able to effectively reduce hormonally induced prostatic stiffness and gross weight (17).

Therefore, we thought that a combined α_1- and α_2-adrenoceptor blockade would have significant therapeutic benefits over α_1-blockade, thereby providing a rational and particularly effective approach to treat BPH. GYKI-12743, the racemic form of GYKI-16084, was developed as an antihypertensive agent which successfully underwent phase Ia clinical trials with no significant side effects and was well tolerated (18, 19). GYKI-16084 has now been found to be a promising drug candidate for the treatment of BPH. Its uroselectivity and efficacy have been demonstrated *in vitro* and in various animal models of BPH (20, 21).

Pharmacological Actions

The α_1-adrenoceptor antagonistic effect of GYKI-16084 was determined against phenylephrine on human hyperplasic prostatic strip and rat small mesenteric artery. The data demonstrated that GYKI-16084 exerted α_1-adrenoceptor antagonism with a significant prostate selectivity (Table I).

The effects of GYKI-16084 on pre- and postsynaptic α_2-adrenoceptors were investigated in rat vas deferens and dog vena saphena models by using xylazine and UK-14304 as selective agonists, respectively. Results of this study indicated that GYKI-16084 possessed a strong postsynaptic α_2-adrenoceptor antagonism and had no significant effect on presynaptic α_2-adrenoceptors. The racemic compound GYKI-12743 and the reference compound yohimbine showed modest selectivity (Table II).

Table I: Evaluation of α_1 antagonism of GYKI-16084 in isolated tissues.

Compound	pA_2[a] A. mesenth. (rat)	pA_2[a] Prostate (human, hyperplasic)	Selectivity[b]
GYKI-16084	6.56 ± 0.16	7.20 ± 0.08	4.4
GYKI-12743	7.16 ± 0.09	7.51 ± 0.18	2.2
Terazosin	8.45 ± 0.09	8.39 ± 0.13	0.9
Alfuzosin	8.60 ± 0.13	8.01 ± 0.09	0.3

[a]pA_2 is the negative logarithmic value of the concentration of antagonist shifting the agonist response curve by a factor of 2; [b]antilogarithmic ratio of pA_2 values measured on arteria mesentherica and human prostate strips.

Table II: Evaluation of pre- and postsynatic α_2 antagonism of GYKI-16084.

Compound	pA_2[a] Presynaptic (rat vas def.)	pK_b Postsynaptic (dog v. saph.)	Selectivity[a]
GYKI-16084	5.81 ± 0.03	7.87 ± 0.23	115.0
GYKI-12743	6.29 ± 0.04	7.57 ± 0.25	19.0
Yohimbine	6.93 ± 0.06	8.05 ± 0.24	13.2

[a]Antilogarithmic ratio of pA_2 and pK_b values measured on pre- and postynaptic α_2-receptors.

GYKI-16084 was also shown to have beneficial effects on voiding parameters measured in various *in vivo* assays.

The effect of GYKI-16084 on voiding parameters of rats pretreated with testosterone (3 mg/kg/day s.c. for 2 weeks) was determined. Administration of testosterone caused voiding disturbances as indicated by the increased values of expulsion time, pressure threshold and intercontraction interval. During the experiments, mean arterial blood pressure and heart rate were also monitored. Upon treatment with GYKI-16084, prazosin or alfuzosin (all at a dose of 100 μg/kg i.v.), the voiding parameters were alleviated or even fully normalized. At the same time, the mean arterial blood pressure was significantly reduced by prazosin and alfuzosin, whereas a slight hypotension was detected with GYKI-16084. The results are shown in Table III.

In another *in vivo* experiment, the duration of effect of GYKI-16084 was also measured. The effect of GYKI-16084 on the voiding cycle lasted for at least 5 h, while its slight hypotensive effect was abolished completely within 1 h.

To study the role of α_2-adrenoceptor antagonism in the *in vivo* effects of GYKI-16084, rats were pretreated with the selective α_2-agonist clonidine (5 μg/kg i.v.), followed by administration of GYKI-16084 (100 μg/kg i.v.) and alfuzosin (100 μg/kg i.v.). The effects on voiding reflex, blood pressure and heart rate were recorded and are shown in Table IV. In this experiment, clonidine induced significant changes in voiding parameters by stimulation of postsynaptic α_2-receptors of the lower urogenital tract. In addition, it decreased mean arterial blood pressure by its well-characterized central action. While the worsening effect of clonidine on micturition was reversed by GYKI-16084, the degree of hypotension was the same as that obtained by treatment with the α_2-receptor agonist alone. Alfuzosin, on the other hand, was not able to counterbalance the voiding disturbances caused by clonidine. Moreover, it induced an additional decrease in blood pressure due to its nonselective α_1-adrenoceptor antagonistic effect.

Table III: Effect of GYKI-16084 on voiding reflex, blood pressure and heart rate in rats pretreated with testosterone.

	ET (sec)		PT (mmHg)		AP (mmHg		HR (min^{-1})	
Compound	Control	Treated	Control	Treated	Control	Treated	Control	Treated
GYKI-16084	21.96	11.60*	4.50	2.94*	116.6	106.2*	364.0	350.0
Prazosin	31.47	17.40*	5.43	3.77*	110.0	72.3*	340.0	231.7*
Alfuzosin	27.23	16.97*	6.43	3.98*	112.0	89.8*	353.3	325.0

ET = Expulsion time; PT = pressure threshold; AP = mean arterial pressure; HR = heart rate. *$p < 0.05$.

Table IV: Effect of GYKI-16084 and alfuzosin on voiding reflex, blood pressure and heart rate in rats pretreated with clonidine (values are mean ± SE).

	ET (sec)	RP (mmHg)	PT (mmHg)	ICI (sec)	AP (mmHg)	HR (min^{-1})
Control	16.54	2.33	3.35	87.00	110.25	300.00
	2.35	0.17	0.26	5.20	5.96	21.13
Clonidine	22.90*	2.96*	4.90*	105.62*	90.56*	252.50
	2.21	0.25	0.57	6.13	5.85	18.68
GYKI-16084	12.26**	1.48**	2.60**	77.12**	86.00	251.25
	1.42	0.16	0.29	5.23	6.13	16.74
Control	16.18	2.14	3.73	84.67	128.11	286.67
	1.70	0.38	0.37	5.14	6.73	21.68
Clonidine	23.51*	3.72*	4.87*	104.67*	88.56**	227.78*
	0.83	0.39	0.31	6.17	3.28	15.70
Alfuzosin	19.38	2.89	3.97	73.78**	67.78**	205.56
	1.67	0.29	0.21	4.67	1.71	13.45

ET = Expulsion time; RP = resting pressure; PT = pressure threshold; ICI = intercontraction interval; AP = arterial pressure; HR = heart rate. *$p < 0.05$; **.

The stiffness of rat prostate was examined in various settings. Testosterone is known to increase stiffness of the prostate, which was thought to be associated with the tension developed by a streching effect (characterized by the length of extension). Results were accordingly expressed in g/cm values. In the assay, one group of animals was treated with testosterone (3 mg/kg s.c.) and GYKI-16084 (10 or 20 mg/kg), while control groups received testosterone (3 mg/kg s.c.) and/or vehicle only. As shown in Table V, testosterone alone induced a significant reduction in the stiffness of prostate, and this effect was inhibited by coadministration of GYKI-16084.

Table V: Effect of GYKI-16084 on stiffness of rat prostate.

Treatment	Dose (mg/kg)	Response on stretching (g/cm)
Vehicle	0	2.06 ± 0.24
Testosterone + Vehicle	3 s.c. 0	1.16 ± 0.14[a]
GYKI-16084	20 p.o.	2.18 ± 0.14
Testosterone + GYKI-16084	3 s.c. 10 p.o.	2.03 ± 0.16[b]
Testosterone + GYKI-16084	3 s.c. 20 p.o.	1.99 ± 0.32[b]

[a]$p < 0.05$ Student's *t* test *vs.* vehicle control: [b]$p < 0.05$ Student's *t* test *vs.* testosterone control.

Table VI: Effect of GYKI-16084 on phenylephrine-induced increases in urethral and mean arterial pressure in anesthetized cats.

Compound	ED_{50}UP (μg/kg)	ED_{50}AP (μg/kg)	Uroselectivity (ED_{50}AP/ED_{50}UP)
GYKI-16084	13.9	54.3	3.9
Prazosin	18.7	15.2	0.8
Alfuzosin	9.5	15.6	1.6

The *in vivo* uroselectivity of GYKI-16084 was studied in anesthetized cats. This experiment was based on the evaluation of the drug's effect on the increase in urethral and mean arterial pressure induced by continuous infusion of phenylephrine (1 mg/kg/h). Results are expressed as ED_{50}UP and ED_{50}AP values, which correspond to the doses required to inhibit by 50% the phenylephrine-induced increases in urethral and mean arterial pressure, respectively. In this experiment, threshold doses of GYKI-16084 and alfuzosin required for reduction of the mean arterial pressure to below the baseline of control were 388 μg/kg and 88 μg/kg, respectively. Of the compounds studied, GYKI-16084 exerted the highest uroselectivity (Table VI).

Table VII: Effect of GYKI-16084 on norepinephrine-induced increase in urethral pressure and its diastolic blood pressure-lowering effect in anesthetized dogs.

Compound	ED_{50}UP (μg/kg i.v.)	ED_{25}DBP (μg/kg i.v.)	Uroselectivity (ED_{25}DBP/ED_{50}UP)
GYKI-16084	112	273	2.44
Prazosin	3.6	6.6	1.83
Terazosin	21	61	2.90

The uroselectivity was also determined in anesthetized dogs. In this study, the inhibitory effects of GYKI-16084, prazosin and alfuzosin on elevation of urethral pressure induced by norepinephrine (0.5-1 μg/kg into the iliac artery) were compared to their effects on diastolic blood pressure. Dose response curves of the compounds were taken, and ED_{50}UP (dose inducing 50% inhibition of the increase in urethral pressure) and ED_{25}DBP (dose inducing 25% decrease in diastolic blood pressure) values were calculated by linear regression analysis. Although GYKI-16084 exerted a modest antagonistic effect in this experiment, its selectivity for the lower urinary tract as compared to the vascular bed was higher than that of prazosin and terazosin (Table VII).

Taken together, the above data form a good pharmacological basis for considering GYKI-16084 as a potent, prostate-specific and safe drug candidate, with possible benefits over existing drugs for the treatment of BPH.

Pharmacokinetics

Preliminary analysis in rats indicates a preferential distribution of GYKI-16084 in the prostate as compared to serum; the AUC value of the prostate was significantly higher after a single oral dose and the compound could be detected in the prostate for more than 6 h.

Toxicology

Acute oral LD_{50} values of GYKI-16084 in mice and rats were found to be 420 and 357 mg/kg, respectively. No mutagenic potential was detected in Ames and micronucleus assays. In a 3-month toxicity study, beagle dogs were treated with 1.3 and 10 mg/kg repeated oral doses of GYKI-16084. Except for mild symptoms related to the pharmacodynamic effect of the compound (*i.e.*, transient hyperemia) in the 10 mg/kg dose group, no signs of toxicity were recorded.

Possible unwanted side effects of GYKI-16084 (5 mg/kg p.o.) were also examined in various experiments performed in rodents. The compound's effects were evaluated by standard assays and methods such as general behavior in mice, hexobarbital sodium-induced narcosis in mice, electroshock-induced seizures in mice, spontaneous motility in rats, body temperature in rats, gastrointestinal motility in mice, ulcerogenic effect in rats, diuretic effect in rats with determination of electrolyte content, respiratory effects in anesthetized rats and analgesic effect in mice.

GYKI-16084 had no effect on central nervous system or body temperature and did not evoke vegetative signs. It had no anticonvulsive or narcosis-potentiating effects and did not influence gastrointestinal motility. The compound showed no respiratory or ulcerogenic activity, but did exert a slight diuretic and saluretic effect.

In a dose range of 1.25-10 mg/kg i.v., GYKI-16084 had no significant effects on arterial pressure, heart rate, left ventricular end-systolic and end-diastolic pressure, left ventricular contractility, oxygen consumption or ECG in anesthetized dogs.

Clinical Studies

A phase Ia study of GYKI-16084 has recently been completed.

Manufacturer

Institute for Drug Research Ltd. (HU).

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