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Review Article

Recent advances in the structural modification of ligustrazine and cerebro/cardiovascular activity of ligustrazine derivatives

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CONTENTS

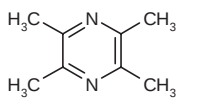
Abstract

Recent advances in the structural modification of ligustrazine and studies on the bioactivities of ligustrazine derivatives are reviewed in this article. Due to its rapid metabolism and short half-life, ligustrazine is not suitable for application in clinical practice. Derivatives of ligustrazine were therefore designed and synthesized, including ether, amine, amide, hydrocarbon and deuterated derivatives of ligustrazine and ester and alcohol derivatives of 2-hydroxymethyl-3,5,6-trimethylpyrazine, an active metabolite of ligustrazine. Some of these compounds present better cerebro- and cardiovascular activities and more favorable pharmacokinetic properties compared to the parent compound.

Introduction

Ligustrazine (tetramethylpyrazine, TMP; **1**) is a major active component of the Chinese traditional medicine Chuanxiong (*Ligusticum wallichii* Franchat), which was introduced on the market during the early 1970s and can now be synthesized chemically. Ligustrazine is widely used in China as a calcium channel antagonist for the treatment of coronary atherosclerotic disease and ischemic cerebrovascular disease. Ligustrazine has been reported to inhibit platelet aggregation, to cause negative chronotropic and inotropic responses in isolated atria, to inhibit vasoconstriction in isolated vascular strips, and to act as a vasodilator, a free radical scavenger and an antithrombotic and antihypertensive agent.

However, pharmacokinetic studies revealed that ligustrazine presented low bioavailability and was rapidly metabolized *in vivo* with a short half-life. Thus, cumulative toxicity often appeared in patients administered the drug frequently to maintain effective plasma concentrations (1). Therefore, it was necessary to develop new-generation drugs via molecular modification of ligustrazine. Recent advances in the structural modification of ligustrazine and studies on the cerebro- and cardiovascular activity of ligustrazine derivatives are reviewed in this article.



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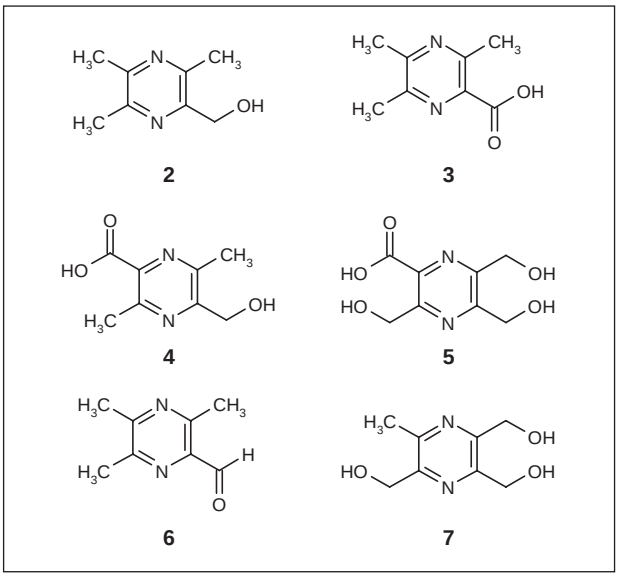
In vivo metabolism of ligustrazine

It has been reported (1) that ligustrazine has pharmacokinetic characteristics of rapid absorption, broad distribution and rapid elimination in the liver, presenting a short half-life due to metabolic transformation *in vivo*. Multiple metabolites of ligustrazine, including 2-hydroxymethyl-3,5,6-trimethylpyrazine (**2**), 3,5,6-trimethylpyrazine-2-carboxylic acid (**3**), 5-hydroxymethyl-3,6-dimethylpyrazine-2-carboxylic acid (**4**), 3,5,6-trihydroxymethylpyrazine-2-carboxylic acid (**5**), 3,5,6-trimethylpyrazine-2-formaldehyde (**6**) and 2,3,5-trihydroxymethyl-6-methylpyrazine (**7**), were identified in the urine of rabbits, rats and humans after administration of ligustrazine (2-4). Some of the metabolites present similar pharmacological activity and lower toxicity than ligustrazine, providing a basis for structural modification.



Structural modification of ligustrazine

Ligustrazine derivatives derived from metabolites

Ligustrazine was oxidized with 30% hydrogen peroxide in the presence of acetic acid and the resulting ligustrazine mono-*N*-oxide was refluxed with acetic anhydride to produce 2-acetoxymethyl-3,5,6-trimethylpyrazine. The latter was then saponified with sodium hydroxide to give 2-hydroxymethyl-3,5,6-trimethylpyrazine (**2**; Fig. 1). Rheological parameters were evaluated and no significant difference was observed compared to ligustrazine used as positive control (5). Further studies (6) found that **2** significantly prolonged cardiac action potential duration and blood coagulation time in mice. Compound **2** also significantly reduced arterial blood pressure but had no effect on heart rate in rats. It was further demonstrated that **2** improved the resistance to anoxemia and induced antihypertensive and anticoagulant effects. Compound **2** has lower toxicity and improved solubility compared to ligustrazine.

Based on the above research, Liu *et al*. (7, 8) introduced drug-like groups and pharmacophores, including nicotinoyl, acetylsalicyloyl, ferulolyl, 2-(4-chlorophenoxy)-2-methylpropionyl, cinnamoyl and 3,4,5-trimethoxybenzoyl, to the hydroxyl group of **2** in an attempt to obtain improved pharmacological activity and pharmacokinetic properties. Other acyl groups were also introduced to better understand the structure-activity relationships of ligustrazine derivatives. A series of novel ligustrazine ester derivatives (**8a-u**; Fig. 2, Tables I and II) were designed and synthesized. All compounds were tested for their ability to stimulate the proliferation of normal human umbilical vein endothelial cells (HUVECs) and protect against acute hyperoxic injury in comparison to ligustrazine. The viability of normal and injured HUVECs was assessed by the MTT assay. The preliminary results from these experiments demonstrated that most compounds were 1.5-4.5-fold more potent than ligustrazine in stimulating the proliferation of normal HUVECs and in protecting against acute hyperoxic injury. The most active was the 2-nicotinoyl ester (**8a**), which exhibited a maximum proliferation rate (P_{max}) of 88.57% at the concentration of 0.1 mmol/l in normal HUVECs and a P_{max} of 3.79% at the concentration of 1.5 mmol/l in injured cells. Screening for other cerebro- and cardiovascular activities is under way.

The structure-activity relationships of these compounds were examined. In the cinnamoyl-substituted series, compounds **8c**, **8h**-**j** showed decreasing activity with an increase in the number of methoxy group substitutions at the phenyl moiety. Replacement of the cinnamoyl group (**8h**) with a dihydrocinnamoyl group (**8g**) improved the activity, with P_{max} increasing from 77.32% to 80.54%. In the substituted or unsubstituted benzoic acid ester series (**8k-t**), compounds with methoxy substituents were moderately active and those with chloro substituents showed markedly reduced activity, except for the 4-chlorobenzoic acid ester (**8q**), which presented particular high potency (P_{max} = 82.55% at 0.1 mmol/l). The 2-iodobenzoic acid ester (**8t**) possessed better activity than the corresponding 2-chloro compound (**8o**). The bromobenzoic acid esters **(8r, 8s)** showed a complete loss of activity. As far as other analogues are concerned, the

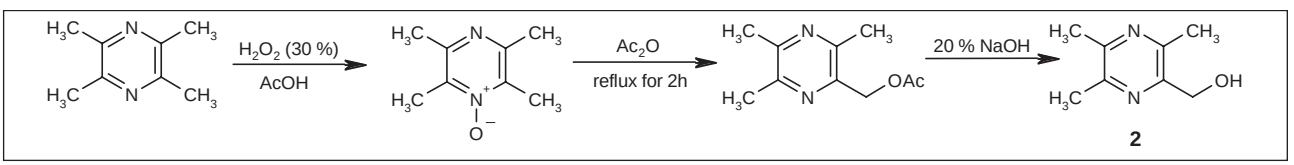


Fig. 1. Synthesis of 2-hydroxymethyl-3,5,6-trimethylpyrazine.

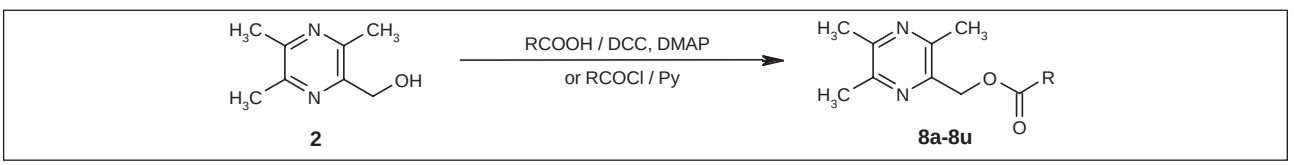


Fig. 2. Synthesis of ligustrazine ester derivatives.

*Table I: The structures and maximum stimulation of the proliferation (P_{max}) of normal human umbilical vein endothelial cells (HUVECs) of ligustrazine ester derivatives **8a-u**.*

Compound	RCO-	$A_{0\ (570\ nm)}$[a]	$A_{570\ nm}$[a]	P_{max} (%)[b]	Concentration (mmol/l)
8a	Nicotinoyl	0.831±0.05	1.56±0.03**	88.57	0.1
8b	Acetylsalicyloyl	0.831±0.05	1.420±0.03**	70.88	0.1
8c	Feruloyl	0.831±0.05	1.528±0.05**	83.87	0.1
8d	2-(4-Chlorophenoxy)-2-methylpropionyl	0.831±0.05	1.561±0.06**	87.85	0.1
8e	Benzoyl	0.629±0.02	0.859±0.05*	37.00	0.6
8f	Phenylacetyl	0.745±0.04	1.312±0.05**	76.11	0.1
8g	Dihydrocinnamoyl	0.745±0.04	1.345±0.02**	80.54	0.1
8h	Cinnamoyl	0.882±0.05	1.564±0.04**	77.32	0.6
8i	4-Methoxycinnamoyl	0.831±0.05	1.458±0.05**	75.45	0.1
8j	2,4-Dimethoxycinnamoyl	0.831±0.05	1.414±0.06**	70.16	0.1
8k	2-Methoxybenzoyl	0.745±0.04	1.281±0.05**	71.95	0.1
8l	4-Methoxybenzoyl	0.745±0.04	1.237±0.04**	66.04	0.1
8m	3,4-Dimethoxybenzoyl	0.882±0.05	1.349±0.03**	52.95	0.1
8n	3,4,5-Trimethoxybenzoyl	0.882±0.05	1.286±0.03**	45.80	0.1
8o	2-Chlorobenzoyl	0.629±0.02	0.831±0.07 *	32.10	0.6
8p	3-Chlorobenzoyl	0.745±0.04	1.187±0.03**	59.32	0.1
8q	4-Chlorobenzoyl	0.745±0.04	1.36±0.112**	82.55	0.1
8r	4-Bromobenzoyl	0.629±0.02	0.565±0.03	NA	0.1-1.5
8s	2,4-Dibromobenzoyl	0.629±0.02	0.561±0.01	NA	0.1-1.5
8t	2-Iodobenzoyl	0.629±0.02	0.858±0.08*	61.36	1.5
8u	2-Furoyl	0.882±0.05	1.494±0.04**	69.39	0.6
1		1.433±0.06	1.729±0.06*	20.66	0.6
2		1.433±0.06	1.835±0.06**	28.03	0.6

*$p < 0.01$, **$p < 0.05$ compared with corresponding control value. [a]The absorption values (mean ± SEM) at 570 nm were measured using an ELISA plate reader; $A_{0\ (570nm)}$: absorption values of the blank control, A_{570nm}: absorption values of compounds. [b]Results were expressed as percentage of absorption value in vehicle-treated control culture; $P = (A-A_0)/A_0$. NA: not active at concentrations of 0.1-1.5 mmol/l.

dihydrocinnamic acid ester (**8g**) was more active than the phenylacetic acid ester (**8f**), which might reflect the importance of the alkyl chain length of the aralkylcarboxylic acid. All the features described above should be considered in the design of novel ligustrazine derivatives.

3,5,6-Trimethylpyrazine-2-formaldehyde (**6**) was obtained by oxidation of 2-hydroxymethyl-3,5,6-trimethylpyrazine (**2**) with dimethylsulfoxide (DMSO) in the presence of dicyclohexylcarbodiimide (DCCI) and anhydrous phosphoric acid (9). Compound **6** was reacted with a Grignard reagent to give 1-(3,5,6-trimethylpyrazine)-1-alkylmethanol (**9a**-**c**; Fig. 3). The measurement of the activated partial thromboplastin time (APTT) showed that 1-(3,5,6-trimethylpyrazine)-1-butyl alcohol hydrochloride (**9b′**) presented stronger pharmacological activity than ligustrazine (Tables III and IV).

Table II: The protective effects of ligustrazine derivatives on injured HUVECs.

Compound	Concentration (mmol/l)					
	0[a]	0.1	0.3	0.6	1.2	1.5
8a	-49.8	-46.7	-36.4	-13.9	-12.0	3.79
8b	-45.0	-34.2	-46.4	-53.8	-63.4	ND
8c	-49.8	-43.2	-76.7	-79.7	-60.6	-15.6
8d	-49.8	-51.2	-40.4	-72.3	-83.4	-69.0
8f	-45.0	-29.1	-24.4	-12.2	6.01	4.43
8g	-45.0	-29.7	-23.5	-11.5	5.62	5.70
8i	-45.0	-37.6	-33.3	-21.2	-25.1	-28.5
8q	-45.0	-40.5	-28.6	-28.3	-2.46	-1.20
1	-49.8	-48.7	-47.5	-41.0	-44.2	ND
2	-49.8	-43.6	-40.5	-38.2	-36.4	-35.8

[a]Blank control: the proliferation rate (P%) of HUVECs injured by 75 μmol/l of hydrogen peroxide in the absence of compound; the proliferation rate of normal HUVECs was designated as 0.00%. ND: not determined.

3,5,6-Trimethylpyrazine-2-carboxylic acid (**3**) was synthesized by Liu *et al*. (10) by oxidization of ligustrazine with $KMnO_4$ (Fig. 4) and the antiatherosclerotic effect of **3** was studied in rabbits. The results showed that **3** could reduce serum cholesterol and low-density lipoprotein (LDL), indicating that it may be a biologically active compound warranting further research.

Ligustrazine ether derivatives

The important intermediate 2-bromomethyl-3,5,6-trimethylpyrazine (**10**) was synthesized from anhydrous ligustrazine and *N*-bromosuccinimide (NBS) (9) using benzoyl peroxide as catalyst. Compound **10** was then reacted with the corresponding alcoholized sodium to give ligustrazine ether derivatives (**11a-c**; Fig. 5). Ligustrazine isopropyl ether hydrochloride (**11a′**) possessed stronger pharmacological activity *in vitro* than ligustrazine (Tables III and IV).

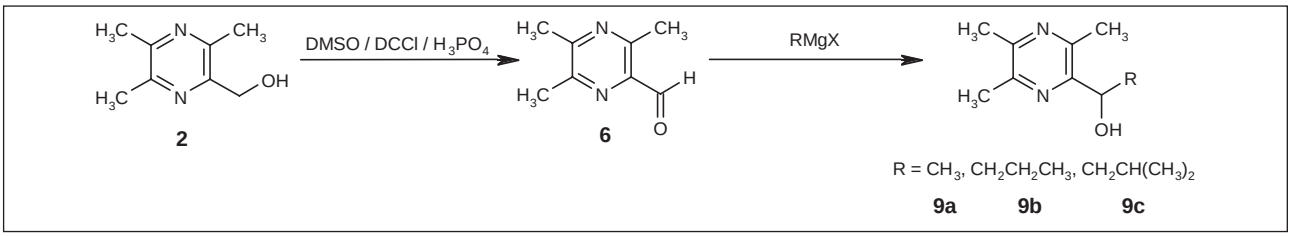


Fig. 3. Synthesis of 1-(3,5,6-trimethylpyrazine)-1-alkylmethanol.

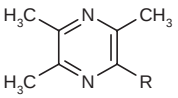


Table III: The relative activities (%) of ligustrazine derivatives compared to ligustrazine on the APTT of human blood in vitro.

Compound[a]	R	Concentration (μmol/ml)			
		14.45	28.90	43.35	57.80
9b´	$CH(OH)CH_2CH_2CH_3$	7.73 [b]	-2.58	44.47	152.80
11a´	$CH_2OCH(CH_3)_2$	-6.59	29.61	51.19	157.53
15´	$CH_2CH_2COCH_3$	0.24	6.14	46.32	101.59

Table IV: The relative activities (%) of ligustrazine derivatives compared to ligustrazine on the APTT of rabbit blood in vitro

Compound[a]	R	Concentration (μmol/ml)			
		14.45	28.90	43.35	57.80
9b´	$CH(OH)CH_2CH_2CH_3$	3.2 [b]	-2.53	73.75	115.22
11a´	$CH_2OCH(CH_3)_2$	9.64	68.13	317.40	—
15´	$CH_2CH_2COCH_3$	4.07	22.29	142.20	—

[a]All three compounds were in the form of the hydrochloride salt. [b] The relative activities (%) were obtained by calculation of A_{TP}-$A_T/A_T \times 100\%$ (A_{TP}: APTT of ligustrazine derivatives; A_T: APTT of ligustrazine); a negative sign means that the ligustrazine derivative was less active than ligustrazine.

Ligustrazine amine and amide derivatives

Based on the structural characteristics of calcium antagonists such as cinnarizine and flunarizine, ligustrazine was modified in our laboratory (11) by combining a piperazine and pharmacophores or drug-like groups, including nicotinoyl, acetylsalicyloyl, cinnamyl and diphenylmethyl, to form a novel class of ligustrazine

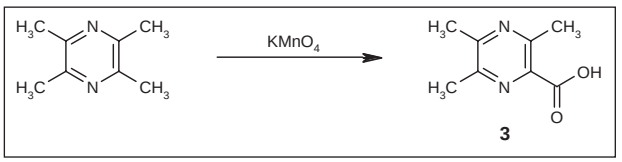


Fig. 4. Synthesis of 3,5,6-trimethylpyrazine-2-carboxylic acid.

amine derivatives (**12a**-**d**) in a further attempt to obtain improved pharmacological activity and pharmacokinetic properties. Preliminary results demonstrated that some compounds possessed better pharmacological activity than ligustrazine and are promising lead compounds for the development of a new generation of cerebro- and cardiovascular drugs.

2-Bromomethyl-3,5,6-trimethylpyrazine (**10**) was nucleophilically substituted by potassium phthalimide and then reacted with hydrazine to give 2-aminomethyl-3,5,6-trimethylpyrazine (**13**), which was then acylated with acyl chloride to give ligustrazine amide derivatives (**14a**-**c**; Fig. 6). Pharmacological testing for antiplatelet activity was performed with the ligustrazine amide derivatives. The results showed that some derivatives such as **14a**, **14b** and **14c** exhibited favorable antiplatelet activity (Table V) (12).

Ligustrazine hydrocarbon derivatives

2-Bromomethyl-3,5,6-trimethylpyrazine (**10**) was reacted with ethyl acetoacetate in a solution of sodium ethylate/ethanol to give ethyl acetoacetate-substituted ligustrazine. Ketonic decomposition of this intermediate provided 4-(3,5,6-trimethylpyrazin-2-yl)butan-2-one (**15**), and acid decomposition of this intermediate gave 3-(3,5,6-trimethylpyrazin-2-yl)propanoic acid (**16**), which was then esterified to afford ethyl 3-(3,5,6-trime-

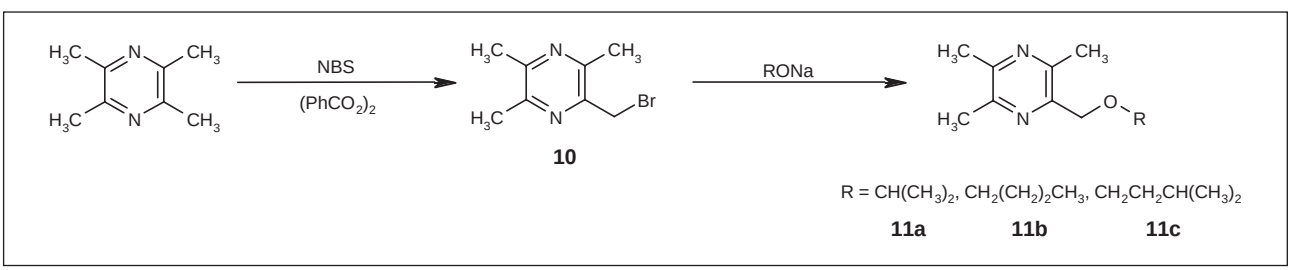


Fig. 5. Synthesis of ligustrazine ether derivatives.

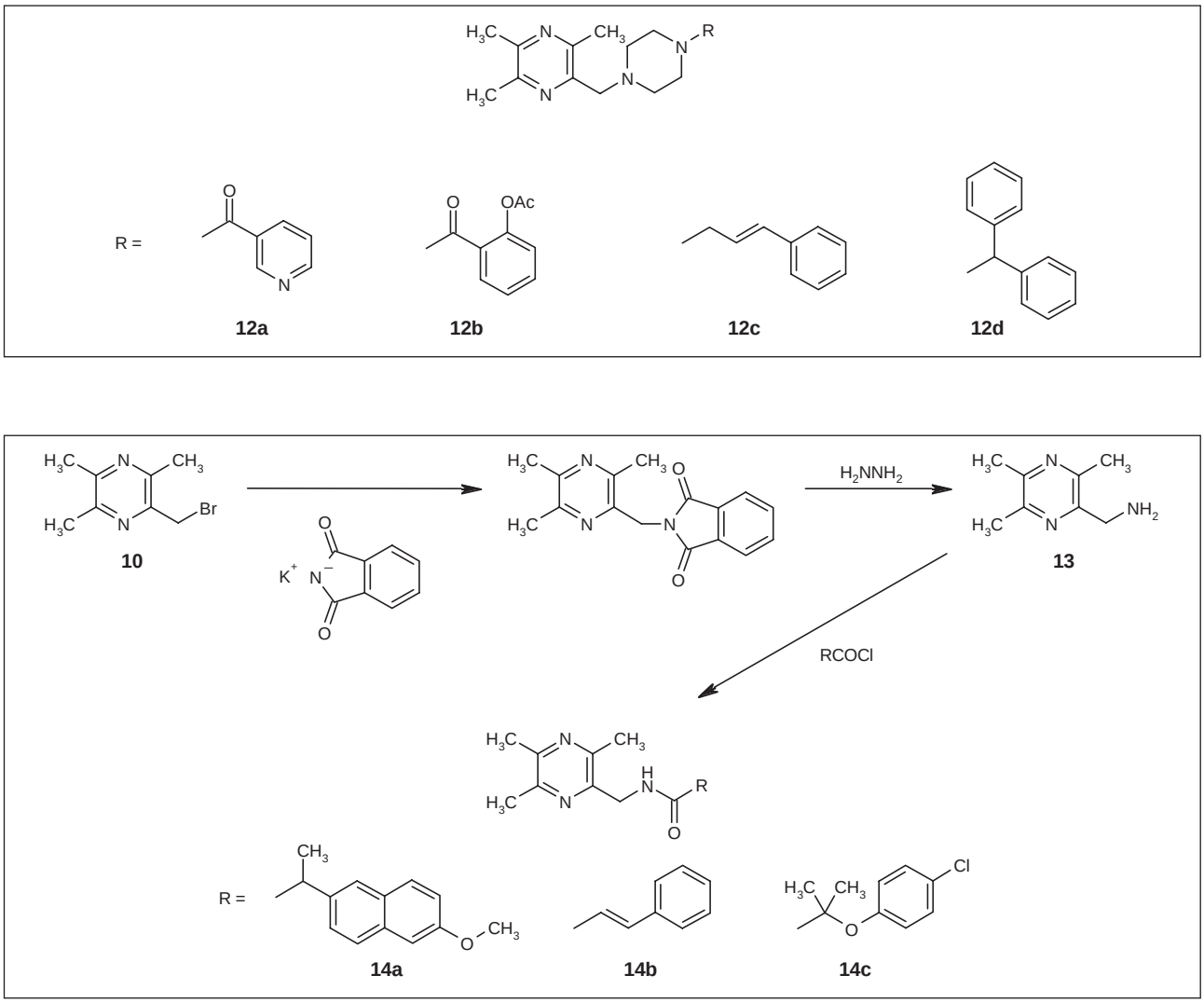


Fig. 6. Synthesis of ligustrazine amide derivatives.

Table V: The effect of ligustrazine amide derivatives on platelet aggregation in rats

Compound	Dose (mmol/kg)	Platelet aggregation (%)
Control	—	42.39±11.36
Ligustrazine phosphate	0.56	25.27±14.91*
2	0.56	26.84±10.78*
14a	0.56	30.05±6.68*
14b	0.56	32.58±8.93
14c	0.56	28.76±10.67*

*$p < 0.01$ compared with control group.

thylpyrazin-2-yl)propanoate (**17**) (Fig. 7). 4-(3,5,6-Trimethylpyrazin-2-yl)butan-2-one hydrochloride (**15′**) possessed stronger pharmacological activity than ligustrazine (see Tables III and IV) (9).

Other ligustrazine derivatives

Isotope-substituted drugs can present remarkable changes in metabolic pathways, a phenomenon known as metabolic switching. Various deuterated ligustrazine derivatives, such as 6D-ligustrazine (**18**) and 12D-ligustrazine (**19**) (6 or 12 atoms of hydrogen at the methyl groups of ligustrazine were replaced by deuterium) were synthesized by Jiang *et al*. (13) with the aim of blocking or slowing ligustrazine oxidation and increasing its pharmacological effects. Systematic pharmacological experiments were performed to investigate the effects of deuterated ligustrazine derivatives. *In vivo* and *in vitro* studies showed that both 12D-ligustrazine and 6D-ligustrazine exhibited significant antithrombotic effects on rabbit jugular vein thrombus labeled with ^{131}I and ^{125}I, where thrombosis and thrombolysis can be observed simultaneously by a quantitative and convenient method. 12D-Ligustrazine and 6D-ligustrazine caused a slightly greater

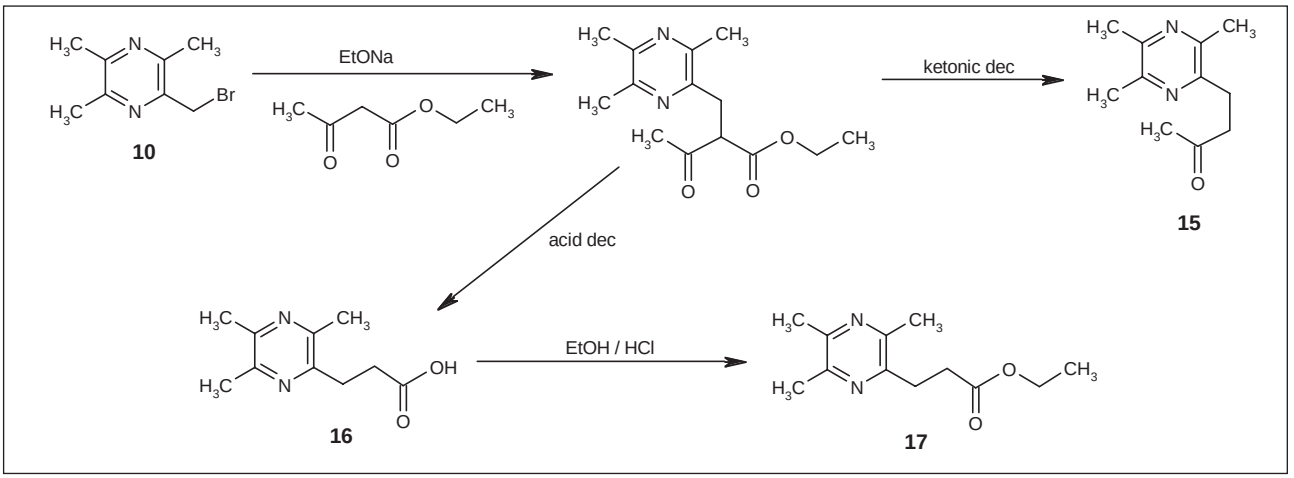
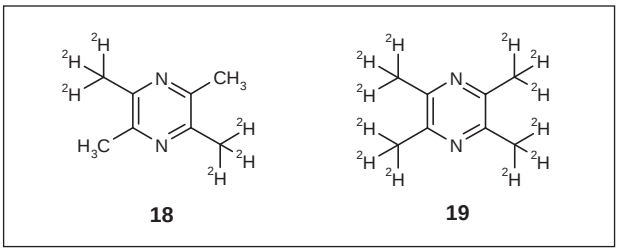


Fig. 7. Synthesis of ligustrazine hydrocarbon derivatives.

change in thrombolytic activity compared to ligustrazine in plasma and fibrinogen clots labeled with ^{125}I. The deuterated analogues significantly inhibited human and rabbit platelet aggregation induced by ADP, and the potency of 12D-ligustrazine and 6D-ligustrazine, as evaluated by IC_{50}, was about 2.67 and 1.27 times, respectively, that of ligustrazine. The results suggested that 12D-ligustrazine and 6D-ligustrazine possessed marked isotope effects, and that the pyrazine ring of ligustrazine may be responsible for its pharmacodynamics, while the substituted groups may primarily govern the pharmacokinetics and toxicity. The results also indicated that the study of the structure-activity relationships of ligustrazine and modification of its chemical structure have resulted in the development of analogues of high potency.



Ligustrazine ferulic acid salt (**20a**) was synthesized by Tang *et al.* (14) from ferulic acid and ligustrazine. Compound **20a** showed anticoagulant activity and antithrombotic effects more potent than ligustrazine.

Salt derivatives of ligustrazine (**20a**-**f**; see Fig. 8) were synthesized by Yang *et al*. (15) from ligustrazine and acids, such as sulfuric acid, malonic acid, *p*-aminobenzoic acid, 3,5-dinitrobenzoic acid, citric acid and ferulic acid. The free radical-scavenging effects of these derivatives of ligustrazine were studied by spectrophotometry, and the results showed that the effect of ligustrazine 3,5-dinitrobenzoic acid salt (**20e**) on O_2^- was superior to ligustrazine and the effects of all six derivatives on ·OH were also superior to ligustrazine.

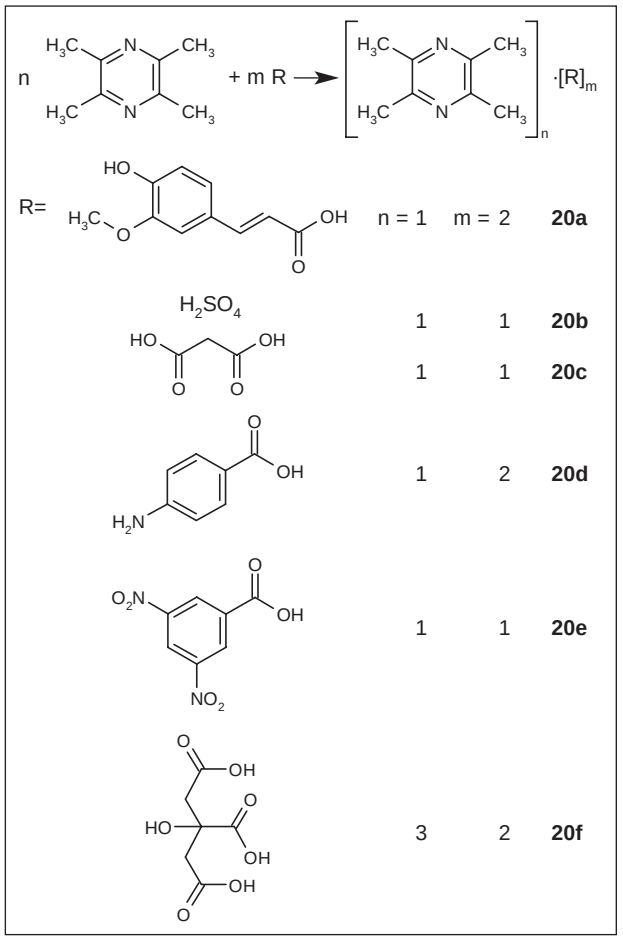


Fig. 8. Synthesis of ligustrazine salt derivatives.

Conclusions

In the search for new drugs, an important approach is to structurally modify natural product ingredients possessing biological activity with the aim of designing novel

drugs with high efficacy and low toxicity. Ligustrazine is an active component of a Chinese traditional medicine that has been widely and effectively used in the clinic. However, certain disadvantages, such as rapid metabolism and a short half-life, seriously restrict its application. Therefore, it is important to develop a new generation of cerebro- and cardiovascular drugs with higher potency, lower toxicity and superior curative effects based on the structural modification of ligustrazine.

Acknowledgements

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