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Original article

Design, synthesis and neuroprotective effects of Fenazinel derivatives

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Graphical Abstract

Design, synthesis and neuroprotective effects of Fenazinel derivatives

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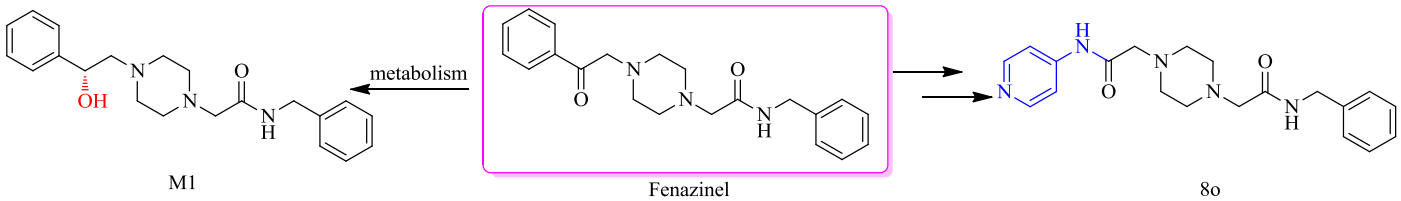

M1
hERG IC_{50}=0.43 μ mol/L

Fenazinel
hERG IC_{50}=8.64 μ mol/L
OGD PC12 cells:
survival rate, 90.6% (10 μ mol/L)
Mice hypoxia tolerance model:
survival time, 2761.1 s (20 mg/kg)

8o
hERG IC_{50}>30 μ mol/L
OGD PC12 cells:
survival rate, 94.8% (10 μ mol/L)
Mice hypoxia tolerance model:
survival time, 4565.0 s (20 mg/kg)

In search of novel neuroprotective agents with higher potency and lower hERG liability, a series of novel Fenazinel derivatives were designed and synthesized.

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ABSTRACT

In search of novel neuroprotective agents with higher potency and lower hERG liability, a series of novel Fenazinel derivatives were designed and synthesized, among which compounds **8m~o** containing amide moiety exhibited good neuroprotective effects *in vitro* and *in vivo*. Especially, the representative compound **8o** showed lower activity in a patch clamp hERG K^+ ion channel screen and could be considered as a lead compound for further development. These findings provided an alternative approach to the development of drugs more potent than Fenazinel for the intervention of ischemic stroke.

1. Introduction

Stroke is a major cause of morbidity and mortality, with a high case fatality worldwide. In China, stroke is the second-leading cause for mortality in all diseases[1, 2]. Particularly, ischemic strokes account for 60%~80% of these strokes[3, 4]. For nearly two decades, only tissue plasminogen activator (tPA) [5, 6] is an FDA-approved drug treatment, and it is used in less than 5% of stroke patients because it is associated with an increased risk of intracranial hemorrhage (ICH)[7-9]. Edaravone (Fig. 1)[10-12], also known as MCI-186, is a recently developed neuroprotective drug that has been successfully used for treating acute stroke caused by cerebral thrombosis and embolism, while its potency is limited unless administered with other pharmacological agents[13-15]. Hence, there is an urgent need for other effective neuroprotective agents to treat ischemic strokes. In previous work, we designed and synthesized several dicarbonylalkyl piperazine derivatives to explore their neuroprotective properties [16,17]. Especially the compound **2a**(Fenazinel, Fig. 1) [18-22] and compound **7o** [23]exhibited good neuroprotection *in vitro* PC12 cells and *in vivo* rat focal cerebral ischemic animal model, and Fenazinel was developed into clinical trials as a novel neuroprotective agent.

However, side effects with use of Fenazinel were found in phase I clinical trials: two cases had reactions that activity of serum creatine phosphokinase (CPK) increased, and one case suffered potentially atrial premature.

The overall ancillary pharmacology profile of Fenazinel was evaluated to establish if there is any significant off-target activity or other metabolic liabilities associated with the compound. Subsequent studies found that Fenazinel had weak activity in the hERG patch clamp K^+ channel binding assay with a IC_{50}=8.64 μmol/L, while the hERG IC_{50} for its main metabolite M1 was 0.43 μmol/L in the same assay, which predicts a potential liability for M1 to cause drug-induced QT prolongation or other drug-related cardiac toxicity.

With an increased awareness by regulatory agencies on the liabilities associated with drug-induced QT prolongation, we felt that it is necessary to minimize the hERG activity for compounds within this chemical series [24]. We detail below our efforts to identify a novel compound with the same promising biological profile as Fenazinel but with reduced hERG liability.

2. Results and discussion

Based on the previous study, the metabolizing of the carbonyl moiety into hydroxyl on Fenazinel was believed to play a very important role in drug-related cardiac toxicity.

To further discover potential neuroprotectant with less hERG activity, we designed and synthesized a novel series of piperazine derivatives bearing benzenesulfonic acyloxy or the benzoheterocycle-one groups replacing the acetophenone moiety of Fenazinel. Furthermore, we introduced substituent groups at the *β*-site of carbonyl moiety and replaced carbonyl group with amide-containing group to find new chemical entities with better neuroprotective activity and weaker hERG liability (Fig. 2). Herein, a total of 15 target compounds were designed and synthesized.

In order to study the potential neuroprotective activities of the title compounds, a preliminary screening was performed investigating neuroprotection on impairment induced by oxygen–glucose deprivation (OGD) in PC12 cells, as evaluated by MTT assay. The results are showed in Table 1.

Unexpectedly, compounds **8a~j** showed potent cytotoxicity against PC12 cells for all three test concentrations (0.1, 1.0, 10 μmol/L), indicating that the neuroprotective activities disappeared after introduction of the benzenesulfonic acyloxy or the benzoheterocycle--one groups connecting bridge into structures. It was surprising that the potency and toxicity were highly sensitive to structural variations, though the mechanism still need to be further explored.

Meanwhile, the target compounds **8k~o** showed moderate to good neuroprotective effect at three levels of concentrations against OGD-induced neurotoxicity in PC12 cells, with a dose-dependent survival rate from 63.5% to 95.7%.

Among the derivatives, compounds **8k** and **8l** with *β*-substituent groups in carbonyl moiety displayed similar survival rate as Fenazinel, revealing that the *β*-substituted group seems to be little difference for the neuroprotective inhibitory activity. Also, it was found that the amide moiety addition of the compounds **8m~o** caused concentration-dependent neuroprotective effects *in vitro* PC12 cells with the maximal effect observed at 10 μmol/L (cell protection: 92.9%, 95.7% and 94.8%, respectively), even stronger than that of their precursor compound Fenazinel (90.6% viable rate at 10 μmol/L), clearly indicating that the introduction of amide moiety could significantly increase their neuroprotective activity.

Based upon the finding that the amide moiety derivatives are the most potent compounds among the different position analogues, compound **8m~o** was further evaluated in hERG binding assay and in hypoxia tolerance model in mice (Table 2). Compounds **8m~o** were inactive in our hERG binding assay with a $IC_{50} > 30$ μmol/L, which does not predict a liability for the compounds to cause drug-induced QT prolongation. Hypoxia tolerance assay *in vivo* showed that compounds **8m~o** could prolong the survival time of mice under hypoxic condition at dose of 6 mg/kg and 20 mg/kg than the control group, and were comparable with with Fenazinel group. Especially, the analog **8o** exhibited a highly potent neuroprotective activity *in vivo* of prolonging the survival time of mice compared to Fenazinel at 6mg/kg (4565 s *vs* 2829 s), therefore, it can be considered as a new lead compound for further development in specific tests for a potential neuroprotective agent.

3. Conclusion

In conclusion, a novel class of Fenazinel derivatives were synthesized and evaluated on their neuroprotective activity based on our previous studies. The result obtained indicated that the analog **8m~o** with amide moiety exhibited neuroprotective activity in OGD test. Particularly, compound **8o**, which was inactive in hERG binding assay and showed prolonged life time of mice in hypoxia tolerance model, may be a promising candidate for further intensive study. Further investigation on *in vitro*/*in vivo* assay of compound **8o** is in progress and will be reported in due course.

4. Experimental

The synthesis of the compounds **8a~o** was shown in Scheme 1. Step " a" was commenced with 2-chloroacetyl chloride with benzylamine under ice-water bath for 6 h to give **5**. Intermediate **7** was obtained by alkylation of **5** with piperazine hydrochloride in EtOH via refluxing in 81% yield. **7** and HCHO were dissolved in CH_3CH_2OH and the solution was stirred at room temperature for 20 min. To the solution benzenesulfonic acid or benzoheterocycle-one was added. The resulting mixture was stirred at room temperature for 15 h, followed by acidification with HCl/EA to give the targeted compounds **8a~j** in 21%~42% yield. The reaction of **7** with various commercially available aryl carbamic chloride, followed by acidification with HCl/EA, afforded compounds **8k~o** in 40%~55% yield.

The structures of new compounds **8a~o** are illustrated in Table 1. All the compounds were evaluated on their neuroprotective activity via *in vitro* (oxygen–glucose deprivation test in PC12 cells). Several potential analogs were further evaluated *in vivo* assays (hypoxia tolerance model in mice) and hERG patch clamp K^+ channel binding assay .

The detailed experimental procedures, characterization data and ^1H NMR and MS spectra of target compounds, *in vitro* and *in vivo* test methods are available in Supporting information.

Acknowledgment

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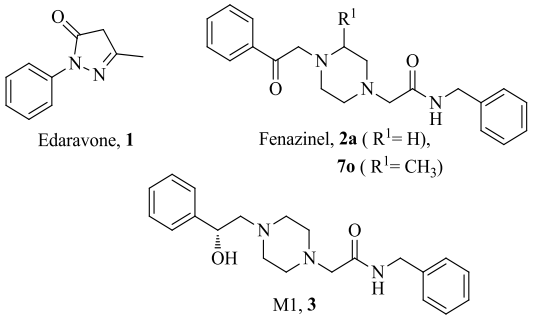


Fig. 1. Edaravone, Fenazinel, **7o** and the main metabolite of Fenazinel (M1)

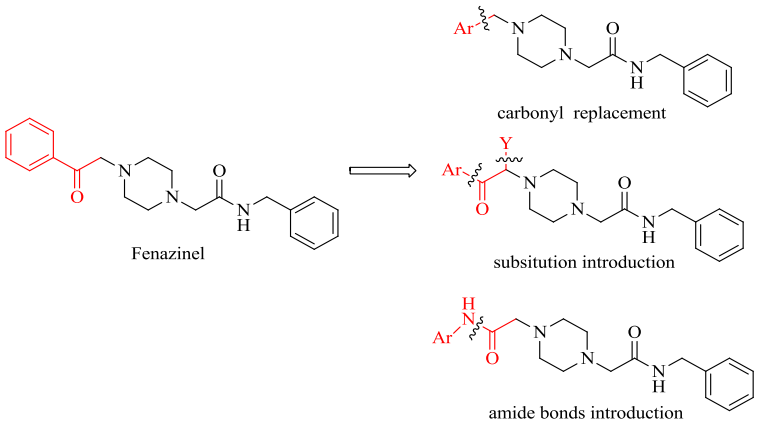
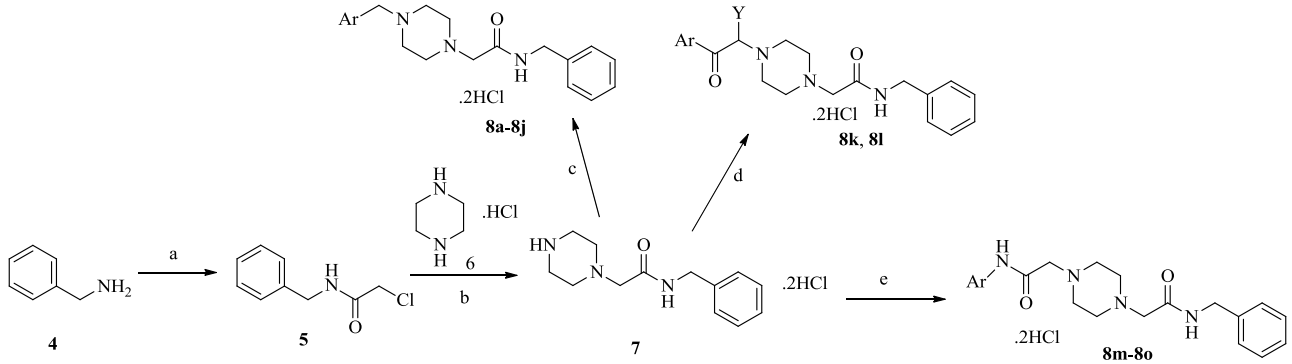
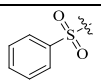
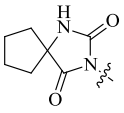
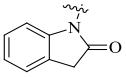
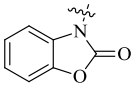
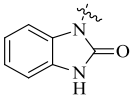
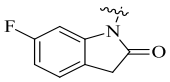
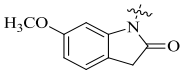
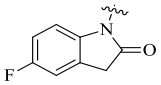
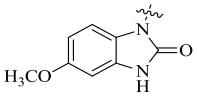
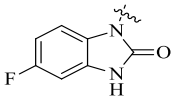
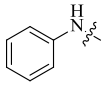
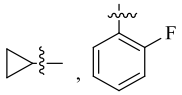
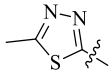


Fig. 2. Design novel derivatives based on Fenazinel



Scheme 1. Synthetic route of the designed compounds. Reagents and conditions: (a) 2-chloroacetyl chloride, TEA, CH_3CN, 0-15 ℃, 6 h; (b) EtOH, reflux, 1.5 h; (c) benzenesulfonic acid or benzoheterocycle-one, HCHO, CH_3CH_2OH, r.t., 15 h; HCl/EA, r.t., 1 h; (d) 2-chloro-*N*-phenylpropanamide or 2-chloro-1-cyclopropyl-2-(2-fluorophenyl)ethanone,CH_3COCH_3, K_2CO_3, KI, 40 ℃, 8h; HCl/EA, r.t., 1 h; (e) corresponding aromatic-containing carbamic chloride,CH_3COCH_3, K_2CO_3, KI, 40 ℃, 8 h; HCl/EA, r.t., 1 h.

Table 1. *In vitro* neuroprotective activity of the targeted compounds of **8a~o**.

Compd.	Ar, Y	OGD test[a] Survival rate (%)		
		0.1 μmol/L	1 μmol/L	10 μmol/L
8a		69.8±0.39	69.4±4.1	53.7±7.3
8b		65.3±6.9	54.3±0.87	51.0±2.9
8c		69.1±4.9	53.6±4.4	42.4±9.1
8d		72.3±11.7	64.7±13.0	48.0±10.3
8e		65.6±1.6	58.6±8.2	44.2±2.2
8f		70.5±4.9	73.6±1.6	77.6±1.6
8g		74.1±11.0	69.4±16.3	70.4±9.0
8h		69.5±4.3	62.6±2.9	44.5±2.3
8i		64.8±5.0	53.9±2.8	51.9±2.7
8j		67.7±9.1	55.5±10.3	49.9±3.4
8k		69.1±1.5	77.2±6.2	78.6±3.4
8l		69.3±0.3	77.1±3.3	84.8±4.1
8m		63.5±8.0	79.8±8.2	92.9±8.9
8n		75.3±2.6	81.3±8.5	95.7±0.28
8o		69.3±0.3	87.1±3.3	94.8±4.1
Fenazinel		66.6±4.6	80.0±14.9	90.6±3.2

[a] Oxygen–glucose deprivation (OGD) test as described in Ref [23].

Table 2. *In vitro* and *in vivo* data for selected compounds

Compd.	hERG IC_{50}[a] (μmol/L)	Hypoxia tolerance assay[b] Survival time (s)	
		6 mg/kg	20 mg/kg
8m	>30	2829.1±129.8	3025.2±199.9
8n	>30	2484.7±390.4	3474.2±567.0
8o	>30	3130.0±239.4	4565.0±260.7
Fenazinel	8.64	——	2761.1±409.6
Control（2% DMSO)	——	2355.6±356.7	

[a] hERG Patch clamp screen as described in Ref [25]. IC_{50} values represent the concentration to inhibit 50% of hERG current (IKr). Numbers represent IC_{50} values generated from 3-point concentration response relationships in duplicate.

[b] Hypoxia tolerance assay in mice as described in Ref [23].