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Synthesis and inotropic evaluation of 1-substituted-*N*-(4,5-dihydro-1-methyl-[1,2,4]triazolo[4,3-*a*]quinolin-7-yl)piperidine-4-carboxamides

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ABSTRACT

A series of 1-substituted-*N*-(4,5-dihydro-1-methyl-[1,2,4]triazolo[4,3-*a*]quinolin-7-yl) piperidine-4-carboxamides has been synthesized and evaluated for positive inotropic activity by measuring left atrium stroke volume in isolated rabbit-heart preparations. Some of these derivatives exhibited favorable activity compared with the standard drug, milrinone, among which 1-(2-fluorobenzyl)-*N*-(4,5-dihydro-1-methyl-[1,2,4]triazolo[4,3-*a*]quinolin-7-yl)piperidine-4-carboxamide **6a** was the most potent, increasing stroke volume by 11.92 ± 0.35% (milrinone: 6.36 ± 0.13%) at 1×10^{-4} M.

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Cardiac glycosides like digoxin are some of the most frequently prescribed cardiotonics used for the treatment of congestive heart failure (CHF). Unlike other CHF drugs, they do not increase mortality, but their high toxicity and narrow therapeutic window still limit their clinical use as positive inotropic agents.[1] The discovery of amrinone led to the synthesis of a number of agents with promise for the treatment of CHF as non-sympathomimetic, non-glycoside agents.[2] The phosphodiesterase (PDE)-inhibiting agent, milrinone (Fig. 1), has both vasodilator and inotropic properties and was approved for the treatment of CHF more than a decade ago. Nevertheless, the significant ventricular arrhythmias and tachycardia associated with elevated cAMP levels also limit the clinical use of milrinone,[3] as well as a newer agent, vesnarinone.[4,5] Therefore, newer positive inotropic agents with fewer side effects are still needed.

For many years, we have been searching for compounds with favorable positive inotropic activities with fewer side effects in a series of 3,4-dihydro-2(1*H*)-quinolinone derivatives. At the outset of our studies, a series of *N*-(4,5-dihydro-[1,2,4]triazolo[4,3-*a*]quinolin-7-yl)-2-(piperazine-1-yl)acetamide derivatives was synthesized and tested for their biological activity, among which the compound *N*-(1-benzyl-4,5-dihydro-[1,2,4]triazolo[4,3-*a*]quinolin-7-yl)-2-(4-benzylpiperazin-1-yl)acetamide **1** showed the most potent positive inotropic activity.[6] Compound **1** is inotropic through phosphodiesterase III inhibition (unpublished data). To further optimize compound **1**, we replaced the piperazine ring with a piperidine ring, changed the acetamide at the 7-position of 4,5-dihydro-[1,2,4]triazolo[4,3-*a*]quinoline to a carboxamide, and changed the substituents on the corresponding phenyl rings at the 1-position of piperidine-4-carboxamide, and then measured the effects on inotropic activity. The compounds were characterized by IR, ^{1}H NMR, MS, and elemental analysis, and inotropic activities were evaluated by measuring changes in left atrium stroke volume in isolated rabbit-heart preparations.

Compounds **6a–p**, **7a–o** were synthesized as outlined in Scheme 1. Compound **2** was synthesized through sulfurization and cyclization reactions according to previously described methods with commercially available 6-amino-3,4-dihydroquinolin-2(1*H*)-one as a starting material.[7] Isonipecotic acid **3** was used to prepare *tert*-butyl 4-(chlorocarbonyl)piperidine-1-carboxylate **4** as reported.[8,9] Thus **3** was reacted with $(Boc)_2O$ in dioxame to afford 1-(*tert*-butoxycarbonyl)piperidine-4-carboxylic acid, followed by halogenation with thionyl chloride in dichloromethane at 40–50 °C to provide **4** in high yield. Compound **2** was acylated with **4** in dichloromethane at room temperature to afford the corresponding amide, followed by deprotection with dry HCl gas to give **5** as a hydrochloride salt.[10] The nucleophilic substitution reaction of **5** with substituted benzyl chlorides or 4-substituted benzyloxy-3-methoxy benzyl chlorides in refluxing acetone in the presence of potassium carbonate and triethylamine afforded compounds **6a–p** and **7a–o**.[11]

The method of measuring left atrium stroke volume was described previously.[12,13] The features of CHF are cardiac dilatation,

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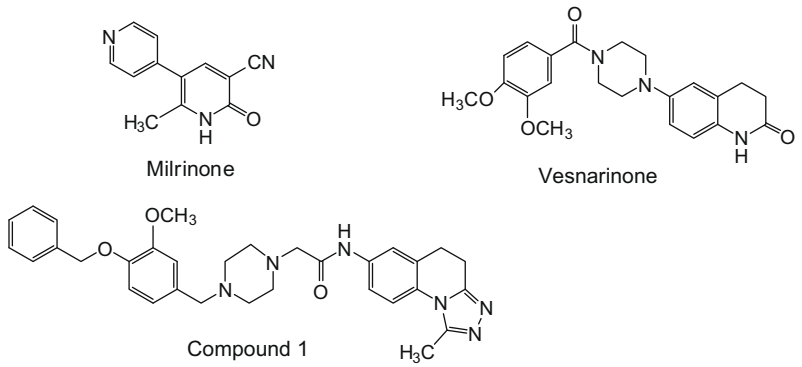
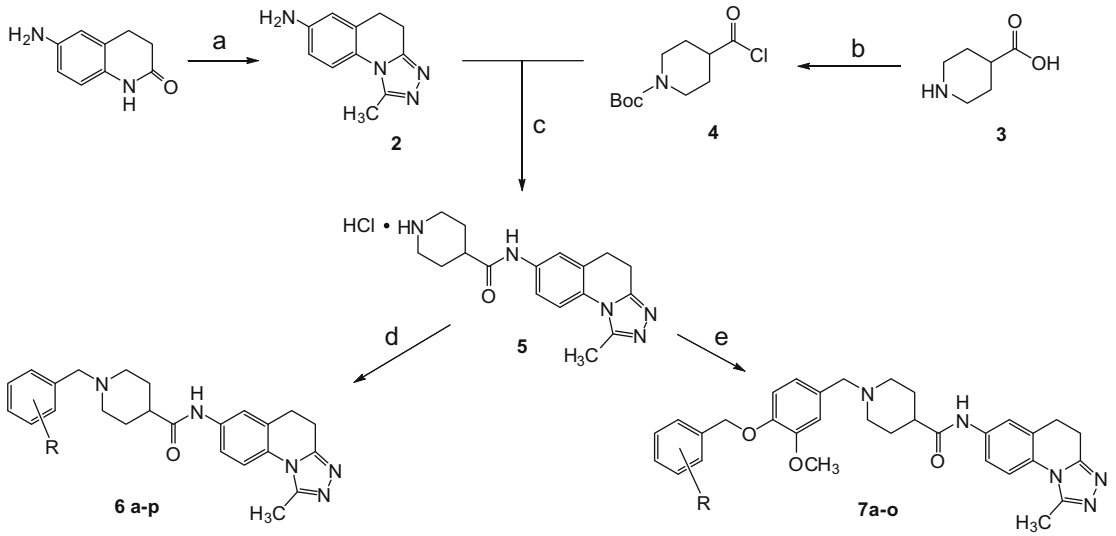


Figure 1. Cardiotonic agents used for CHF treatment and lead compound **1**.

poor contractility of cardiac muscle, decreased ejection fraction, and depression of left ventricular maximum pressure. Therefore, the macroscopic measurement of the variance of left atrium stroke volume can be used to estimate the positive inotropic effects of the compounds synthesized. Milrinone (Shuzhou Unite Pharmaceutical Co., Dongwu Road, Shuzhou) and DMSO (Sigma–Aldrich Chemical Co., St. Louis, MO) were used; all other reagents were of analytical grade. Atria were obtained from New Zealand white rabbits, and the mean atrial weight was 182.5 ± 6.8 mg. Briefly, hearts were removed from rabbits and the left atria were dissected free. A calibrated transparent atrial cannula containing two small catheters was inserted into the left atrium. The cannulated atrium was transferred to an organ chamber and perfused immediately with *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) buffer solution with a peristaltic pump (1.25 mL/min) at 34 °C. The composition of the buffer was as follows (in mM): 118 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 25 $NaHCO_3$, 10.0 glucose, 10.0 HEPES (adjusted to pH 7.4 with 1 M NaOH) and 0.1% bovine serum albumin (BSA). Soon after the perfused atrium was set up, transmural electrical field stimulation with a luminal electrode was started at 1.5 Hz (duration, 0.3–0.5 ms, voltage 30 V). Changes in atrial stroke volume were monitored by reading the lowest level of the water column in the calibrated atrial cannula during the end diastole. The atria were perfused for 60 min to stabilize the stroke volume. The atrial beat rate was fixed at 1.5 Hz, the left atrium stroke volume was recorded at 2-min intervals, and test compound or milrinone was infused for 36 min after a control period of 12 min.

Compounds were tested at 1×10^{-4} M. Samples were dissolved in DMSO and diluted with HEPES buffer to 0.1% DMSO. Data is



Scheme 1. Reagents and conditions: (a) (i) P_2S_5, Et_3N, CH_3CN; (ii) $CH_3CONHNH_2$, *n*-butanol, reflux, N_2; (b) (i) $(Boc)_2O$, NaOH, dioxame; (ii) $SOCl_2$, TEA, CH_2Cl_2, 40–50 °C; (c) (i) TEA, CH_2Cl_2; (ii) dry HCl gas, CH_2Cl_2, 0–5 °C; (d) substituted benzyl chlorides, K_2CO_3, KI, Et_3N, acetone; (e) 4-substitutedbenzyloxy-3-methoxybenzyl chlorides, K_2CO_3, KI, Et_3N, acetone.

Table 1
Inotropic activity of compounds **6a–p**, **7a–o** in changing left atrium stroke volume in isolated rabbit-heart preparations

Compound	R	Increased stroke volume[a] (%)
6a	2-F	11.92 ± 0.35*
6b	H	—[b]
6c	4-F	—
6d	4-Cl	—
6e	2-Br	1.67 ± 0.18
6f	3-F	1.56 ± 0.09
6g	3-Cl	9.97 ± 0.23*
6h	2,6-Cl_2	—
6i	4-CH_3	—
6j	4-OCH_3	—
6k	3-NO_2	8.14 ± 0.23*
6l	3,4-OCH_2O–	2.96 ± 0.11
6m	2,4-Cl_2	7.83 ± 0.20*
6n	3,4-$(OCH_3)_2$	1.87 ± ± 0.08
6o	2-NO_2	—
6p	2-Cl	0.40 ± 0.03
7a	2-F	7.41 ± 0.15*
7b	H	5.19 ± 0.12
7c	4-F	—
7d	4-Cl	5.13 ± 0.22
7e	2-Br	—
7f	3-F	0.18 ± 0.14
7g	3-Cl	5.56 ± 0.21
7h	2,6-Cl_2	—
7i	4-CH_3	—
7j	3-NO_2	—
7k	2-Cl	—
7l	2,4-Cl_2	2.78 ± 1.63
7m	2-NO_2	—
7n	4-OCH_3	6.08 ± 0.17
7o	3,4-$(OCH_3)_2$	—
Milrinone		6.36 ± 0.13

[a] Test sample concentration 1×10^{-4} M.
[b] None or negative stroke volume increase.
* $p < 0.05$ versus milrinone.

expressed as the mean of increased stroke volume percentage (Table 1). Repeated measurements were compared by an ANOVA test followed by Bonferroni's multiple-comparison test. Statistical significance was defined as $P < 0.05$ and the data are presented as means ± SE.

Most (16) compounds out of 31 tested showed inotropic effects on isolated rabbit-heart preparations (Table 1). Compounds **6a**, **6g**, **6k**, **6m** and **7a** exhibited more efficacy than milrinone (6.36 ± 0.13%, 1×10^{-4} M), with **6a** showing the strongest activity, an 11.92 ± 0.35% increase in stroke volume. Previous *N*-(4,5-dihydro-[1,2,4]triazolo[4,3-*a*]quinolin-7-yl)-2-(piperazin-1-yl)acetamide derivatives, such as compound **1,** were more efficacious than milrinone.[6] Thus replacing the 2-(piperazin-1-yl)acetamide moiety with a piperidine-4-carboxamide removed a critical nitrogen atom required for high-affinity binding to PDE III. Substitutions (R) with electron-withdrawing groups on the phenyl ring of the benzyl group at the 4-position of the piperidine in **6a–p** showed good activity, whereas compounds unsubstituted or substituted by electron-donating groups did not. One exception was **6n**, which was active (1.87 ± 0.08%) but had electron-donating groups. Furthermore, 2-fluorinated and 3-chlorinated compounds (**6a**, **6g**) displayed better activity, with **6a** the most potent derivative in this study. The position of the substituent on the phenyl ring significantly influenced the inotropic activity, with an activity order of $o > m > p$ for fluoro-substituted compounds, and $m > o > p$ for chloro-substituted compounds. Thus the 2-fluorinated derivative **6a** (11.92 ± 0.35%) was 7.5-fold more potent than 3-fluorinated **6f** (1.56 ± 0.09%), and the 3-chlorinated derivative **6g** (9.97 ± 0.23%) was 20-fold more active than 2-chlorinated **6p** (0.40 ± 0.03%). Similarly for the two nitro-substituted derivatives (**6k**, **6o)** and two dichloro-substituted derivatives (**6h**, **6m**), only the 3-nitrated **6k** (8.14 ± 0.23%) and 2,4-dichlorated **6m** (7.83 ± 0.20%) affected stroke volume, but 2-nitrated **6o** and 2,6-dichlorated **6h** did not.

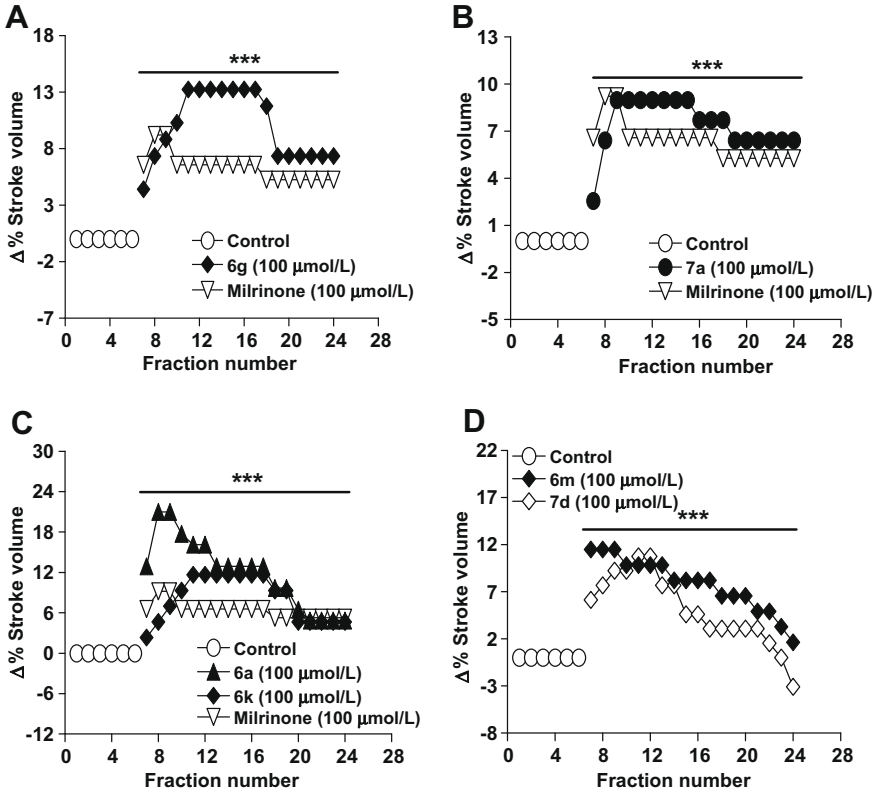


Figure 2. Effects of milrinone and compounds **6a**, **6g**, **6k**, **6m**, **7a** and **7d** on stroke volume in beating rabbit atria (1.5 Hz). The atrium stroke volume was recorded at 2-min intervals. Values are means ± SE. ***$P < 0.001$ versus control.

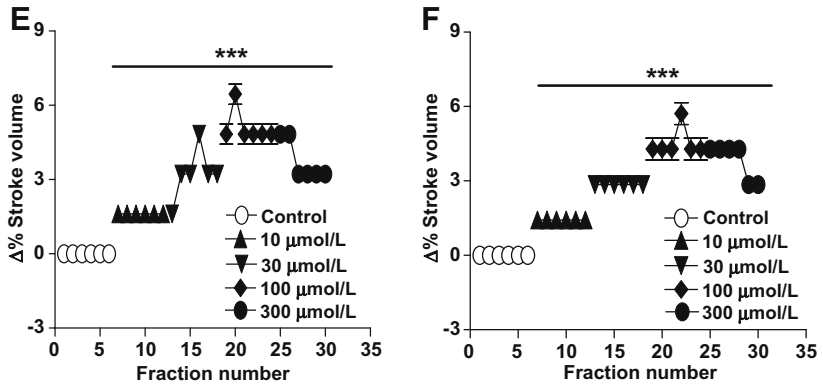


Figure 3. Concentration–response curves of compounds **6a** (E) and **7a** (F) on isolated rabbit-heart preparations. Values are means ± SE. ***$P < 0.001$ versus control.

For **7a–o**, which possesses 4-substituted phenylmethoxy-3-methoxybenzyl groups at the 4-position of the piperidine, there was no clear structure–activity relationship, and only one compound (2-fluorinated **7a,** 7.41 ± 0.15%) was more active than milrinone, with compounds **7b**, **7d**, **7g**, and **7n** showing slightly weaker activities. This series was generally weaker than **6a–p**, indicating that the length of the molecule seems to hinder PDE III binding.

Although **6m** and **7d** produced initial increases in stroke volume, longer treatment caused decreases in stroke volume (Fig. 2D), as did **6p**, **7g**, and **7n** (data not shown). Compounds **6g** and **7a** exhibited similar atrial dynamic profiles to milrinone, with increased stroke volume of 7.41 ± 0.15% and 9.97 ± 0.23%, respectively (Fig. 2A and B).

We next tested the dose-dependency of the most effective compounds, **6a** and **7a,** at 1×10^{-5} M, 3×10^{-5} M, 1×10^{-4} M, and 3×10^{-4} M. Both compounds showed maximal effects at 1×10^{-4} M, and less activity at the higher dose (3×10^{-4} M; Fig. 3E for **6a** and Fig. 3F for **7a**).

In conclusion, we designed and synthesized two series of 1-substituted-*N*-(4,5-dihydro-1-methyl-[1,2,4]triazolo[4,3-*a*]quinolin-7-yl)-piperidine-4-carboxamides based on compound **1** to identify compounds that improve cardiac contractility. Results show that compounds **6a**, **6g**, **6k**, **7a**, and **7n** exhibit promising cardiovascular profiles and better activity than milrinone at the concentration of 1×10^{-4} M, and further modification of compound **1** is in progress.

Acknowledgment

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11. *Preparation of* **6a**: A suspension of **5** (0.40 g, 1.15 mmol), 1-(chloromethyl)-2-fluorobenzene (0.33 g, 2.28 mmol), potassium carbonate (0.32 g, 2.32 mmol), KI (0.12 g, 0.72 mmol), triethylamine (0.78 mL, 3.45 mmol) in acetone (40 mL) was stirred under reflux for 10 h and filtered. The filtrate was evaporated in vacuo and the residue was dissolved in dichloromethane (50 mL). The solution was washed with water (2 × 15 mL) and brine (20 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (dichloromethane/methanol, 15:1 v/v) to afford **6a** (0.15 g, 31%) as a white solid. Mp 135–137 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.94–2.20 (6H, m), 2.40–2.44 (1H, m), 2.73 (3H, s), 2.94–3.06 (6H, m), 3.66 (2H, s), 7.01–7.73 (7H, m). MS *m/z* 420 (M+1). IR (KBr) cm^{-1}: 3529 (NH), 1682 (C=O). Anal. Calcd for $C_{24}H_{26}FN_5O$: C, 68.72; H, 6.25; N, 16.69. Found: C, 68.69; H, 6.36; N, 16.53.
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