Full Paper

Synthesis and Positive Inotropic Evaluation of 2-(4-(4-Substituted benzyloxy)-3-methoxybenzyl)-1,4-diazepan-1-yl)-*N*-(4,5-dihydro-1-methyl[1,2,4]triazolo[4,3-*a*]quinolin-7-yl)-acetamides

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In an attempt to search for more potent positive inotropic agents, a series of 2-(4-(4-substituted benzyloxy)-3-methoxybenzyl)-1,4-diazepan-1-yl)-*N*-(4,5-dihydro-1-methyl[1,2,4]triazolo[4,3-*a*]quinolin-7-yl)acetamides was synthesized and their positive inotropic activities were evaluated by measuring left atrium stroke volume on isolated rabbit-heart preparations. Several compounds showed favorable activity compared with the standard drug Milrinone among which 2-(4-(4-(2-chlorobenzyloxy)-3-methoxybenzyl)-1,4-diazepan-1-yl)-*N*-(4,5-dihydro-1-methyl-[1,2,4]triazolo[4,3-*a*]quinolin-7-yl)acetamide **6e** was found to have the most desirable potency with the 6.79 ± 0.18% increased stroke volume (Milrinone: 1.67 ± 0.64%) at a concentration of 1×10^{-5} M in our *in-vitro* study. The chronotropic effects of those compounds having inotropic effects were also evaluated in this work.

Keywords: 1,4-Diazepane / Positive inotropic activity / Stroke volume / [1,2,4]Triazolo[4,3-*a*]quinolines

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Introduction

For many years, cardiac glycosides like digoxin have consistently been among of the most frequently prescribed medications used for the treatment of congestive heart failure (CHF). Although they are the only medications used without increasing the CHF patient's mortality among those approved positive inotropic agents up to now, the high toxicity and narrow therapeutic window still limit their clinical application as positive inotropic agents [1]. The phosphodiesterase-inhibiting agent milrinone, having both vasodilator and inotropic properties, was approved for the treatment of CHF more than one decade ago. This agent, as an alternative synthetic replacement, has been proven quite useful for seriously ill patients with decompensated CHF. Nonetheless, the significant ventricular arrhythmias and tachycardia associated with the elevated cAMP level also limit the clinical use of milrinone [2]. Similar cases were found in the recently developed vesnarinone [3, 4] and toborinone [5]. These cardiotonic agents ultimately exert their inotropic effects via an increase in intracellular calcium and consequently possess strong arrhythmogenic effects that caused increased mortality when used in CHF patients. Because of overall deleterious effects on long-term survival in CHF, this class of drugs should now be considered most suitable for short-term use in acute episodes of decompensated heart failure [6]. Levosimendan, which exerts its positive inotropic effect by both increasing the sensitivity of the myofilament to intracellular calcium and inhibiting phosphodiesterase, could reduce mortal-

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Abbreviations: congestive heart failure (CHF)

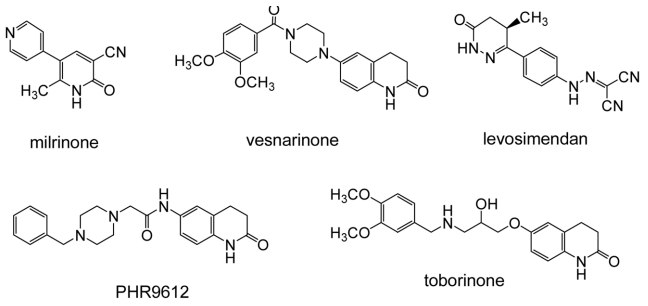


Figure 1. Cardiotonic agents used for the treatment of congestive heart failure (CHF).

ity in the short-term treatment of CHF, but it is uncertain whether levosimendan will reduce the mortality in the long-term treatment [7, 8]. Therefore, due to the lack of a desirable inotropic agent for the treatment of cardiac failure so far, the development of novel positive inotropic agents with approved therapeutic properties in the treatment of CHF, which not only improve the quality of life but also reduce the mortality of CHF patients, is still an important challenge for medicinal chemists [9]. Figure 1 shows cardiotonic agents used for the treatment of congestive heart failure (CHF).

In our previous work to search for more potent positive inotropic agents having fewer side effects, a series of 2-(4-substitutedpiperazin-1-yl)-*N*-(3,4-dihydro-2(1*H*)-quinolinon-6-yl)acetamides was synthesized and tested for their biological activity, among which the compound 2-(4-benzylpiperazin-1-yl)-*N*-(3,4-dihydro-2(1*H*)-quinolinon-6-yl)acetamide (**PHR9612**) showed moderate positive inotropic activity [10]. In our present study to further optimize the compound **PHR9612,** we incorporated a triazole ring to the 1,2-position of 3,4-dihydro-2(1*H*)-quinolinone, replaced the piperazine ring with a 1,4-diazepane ring that was substituted by the 4-benzyloxy-3-methoxybenzyl group at the 4-position, and changed the substituents on the benzene ring of the benzyloxy group simultaneously in order to preliminarily investigate the contribution of such a structural change to the biological activity. The compounds synthesized were characterized by IR, NMR, MS, and elemental analysis, and the positive inotropic activities were evaluated by measuring their effect on left atrium stroke volume in isolated rabbit-heart preparations.

Results and discussion

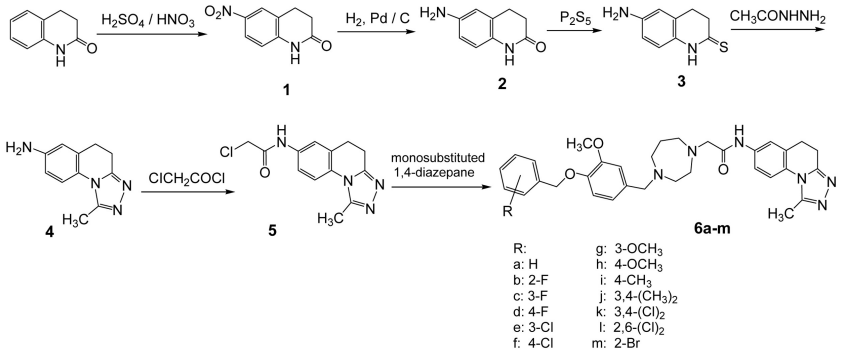
Synthesis

The synthesis of the compound **6a – m** is presented in Scheme 1. Compound **4** was synthesized through nitration, catalytic hydrogenation, sulfurization, and acylation reactions according to the previously described methods by using commercially available 3,4-dihydro-2(1*H*)-quinolinone as a starting material [11]. The amino group at the 7-position of **4** was acylated with 2-chloroacetyl chloride in dichloromethane at room temperature to provide corresponding amide **5** in excellent yield. Nucleophilic-substitution reaction of **5** with various monosubstituted 1,4-diazepanes in refluxing methanol in the presence of sodium carbonate afforded corresponding compounds **6a – m** in high yield.

Biological evaluation

The method of measuring left atrium stroke volume was adopted for the biological evaluation of the compounds **6a – m** in the present work. The features of congestive heart failure are cardiac dilatation, 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.

As shown in Table 1, ten compounds out of the 13 test compounds showed inotropic effects on isolated rabbit-



Scheme 1. Synthesis of compounds **6a – m**.

Table 1. Positive inotropic activity of the test compounds.

Compd	R	Increased stroke volume (%)[a)]
6a	H	1.68 ± 0.26
6b	2-F	6.05 ± 0.65*
6c	3-F	15.55 ± 1.54*
6d	4-F	6.05 ± 0.64*
6e	2-Cl	6.79 ± 0.18*
6f	4-Cl	2.56 ± 0.00*
6g	2-OCH_3	–[b)]
6h	4-OCH_3	–[b)]
6i	4-CH_3	6.95 ± 0.75*
6j	3,4-$(OCH_3)_2$	1.10 ± 0.22
6k	3,4-$(Cl)_2$	5.77 ± 1.04*
6l	2,6-$(Cl)_2$	4.43 ± 0.32*
6m	2-Br	–[b)]
Milrinone		1.67 ± 0.64

a) The concentration for the test sample is 1×10^{-5} M.
b) None or negative stroke volume increase.
* $P < 0.05$ *vs.* milrinone.

heart preparations. Compounds **6b**, **6c**, **6d**, **6e**, **6f**, **6i**, **6k**, and **6l** exhibited more potent effects, while **6a** showed the same potency, compared with milrinone (1.67 ± 0.64%, 1×10^{-5} M). Compound **6c** showed the highest potency with 15.55 ± 1.54% increased stroke volume. In contrast to the previously evaluated PHR9612 with a potency which was weaker compared with milrinone (no data), several compounds showed significantly increased inotropic activities through the structure modification of introducing a triazole ring to the 1,2-position of 3,4-dihydro-2(1*H*)-quinolinone and replacing the piperazine moiety with 1,4-diazepan. As for the relationship between inotropic activity and different substituents on the benzene ring of the benzyloxy group (R), most compounds having electron-withdrawing groups on the phenyl ring displayed enhanced effects except compound **6m**, while compounds **6g** and **6h**, having electron-donating substituents on the phenyl ring showed no or negative effects. However, reverse was found for compounds **6i** and **6j**, although both compounds possesed electron-donating groups. Interestingly, for the halogen-substituted derivatives, those chloro- and fluoro-substituted compounds showed good activity, but bromo-substituted **6m** showed no potency. These results seem to indicate that the contribution of the substituents at R to the biological effect might be different, and no clear regularity was found for the stucture-activity relationship (SAR).

On the other hand, we investigated the dynamics of the test compounds in perfused beating rabbit atria and found that compound **6c** did not show a desirable biological dynamic profile, in which the stroke volume of **6c** was markedly decreased as the time progressed, in spite of its significantly increased stroke volume, for which we

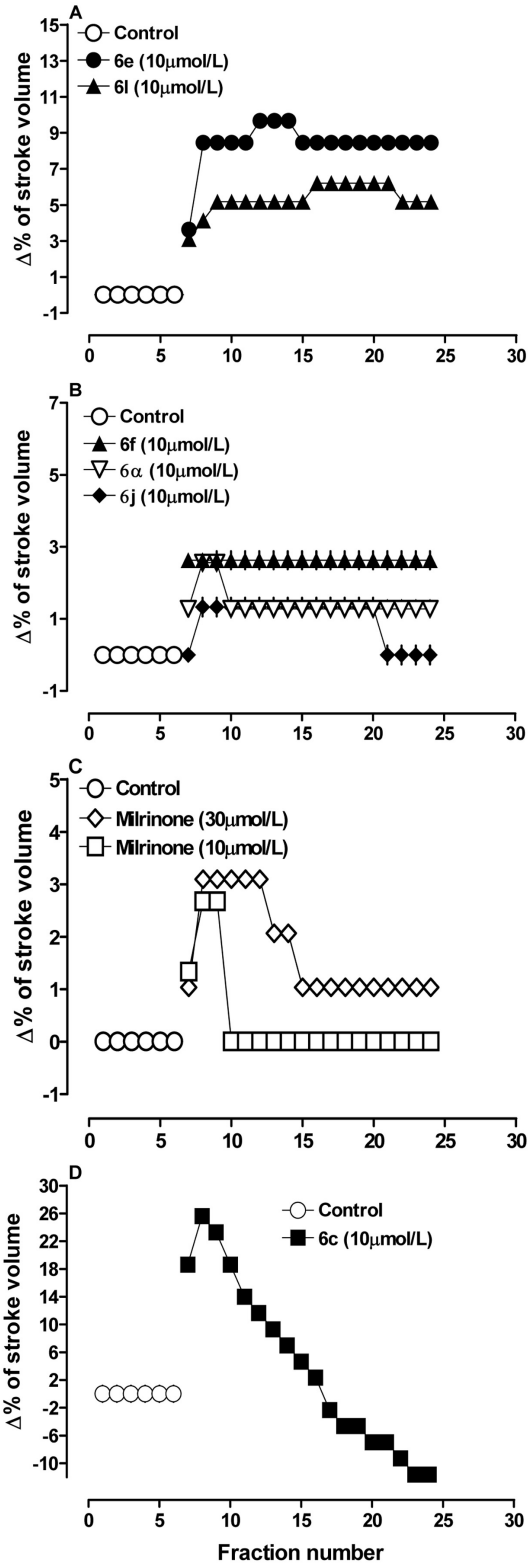


Figure 2. Effects of milrinone and compounds **6a**, **6b**, **6e**, **6f**, **6j**, and **6l** on stroke volume in beating rabbit atria (1.5 Hz). The atrium stroke volume was recorded at 2-min intervals. Values are means ± SE. $P < 0.001$ vs. control.

Table 2. Changes of heart rate caused by compounds in isolated rabbit heart preparations.

Compound	mean ± SE[a)]	mean ± SE[b)]
6a	104.50 ± 0.58	81.70 ± 0.58[c]
6e	128.90 ± 0.07	129.10 ± 0.33
6f	68.30 ± 0.13	66.86 ± 0.15
6l	137.60 ± 0.21	123.50 ± 0.51[c)]

[a)] Control.
[b)] Data after using the test samples.
[c)] $P < 0.01$ *vs.* control.

still could not afford a reasonable explanation (Fig. 2D). The same cases were also observed for compounds **6b**, **6c**, **6d**, **6i**, and **6k** (no figure afforded). Compounds **6e** and **6l** exhibited similar atrial dynamic profiles to milrinone with excellent increased stroke volume of 6.79 ± 0.18% and 4.43 ± 0.32%, respectively (Fig. 2A and C). Much more desirable atrial dynamic profiles were measured for the compounds **6a**, **6f**, and **6j** (Fig. 2B), although the lower potency was observed for **6j** (1.10 ± 0.22%). As shown in Table 2, compounds **6a**, **6e**, **6f**, and **6l** were also investigated for their chronotropic effects in a beating atria and no significantly increased heart rates ($P > 0.05$) were observed for compounds **6e** and **6f** at the same concentration. Compounds **6a** and **6l**, however, showed the changed heart rates unfortunately, for which an *in-vivo* study was required in order to further investigate their chronotropic effects.

Furthermore, based on our recent study of mechanism of action for similar compounds bearing a piperazine moiety instead of a 1,4-diazepan moiety, which has been revealed that they exert the inotropic effect through phosphodiesterase inhibition (unpublished), we presumed that the mechanism of the inotopic action for these compounds synthesized might involve PDE inhibition.

Conclusion

In conclusion, based on the structure of **PHR9612**, we synthesized 2-(4-(4-substitutedbenzyloxy) -3-methoxybenzyl)-1,4-diazepan-1-yl)-*N*-(4,5-dihydro-1-methyl[1,2,4]triazolo[4,3-*a*]quinolin-7-yl)acetamides and tried to find more potent compounds for cardiac contractility without increasing the heart rate. As a result, we obtained several compounds having enhanced inotropic effects and desirable biological profiles in our present study, in which compounds **6e** and **6f** exhibited more promising cardiovascular profiles. These compounds are now undergoing further biological tests including *in-vivo* evaluation, coronary vasodilating tests, and possible mechanism-of-action studies in order to be selected as candidates for further clinical trials.

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The authors have declared no conflict of interest.

Experimental

Chemistry

Melting points were determined in open capillary tubes and are uncorrected. Reaction courses were monitored by TLC on silica gel precoated F254 Merck plates (Merck, Germany) and developed plates were examined with UV lamps (254 nm). Column chromatographies were performed with Merck 200 mesh silica gel. IR spectra were recorded (in KBr) on a FT-IR1730. ^{1}H-NMR spectra were measured on Bruker AV-300 spectrometer (Bruker Bioscience, Billerica, MA, USA) using TMS as internal standard. Mass spectra were measured on an HP1100LC (Agilent Technologies, USA). Elemental analyses for C, H, and N were within ± 0.4% of the theoretical values and were performed on a 204Q CHN Rapid Analyzer (Perkin-Elmer, USA). The major chemicals were purchased from Aldrich (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) and Fluka (Buchs, Switzerland) Companies. Monosubstituted 1,4-diazepanes were synthesized by the method similar to prepare the monosubstituted piperazines [12].

*2-Chloro-N-(4,5-dihydro-1-methyl-[1,2,4]triazolo[4,3-a]quinolin-7-yl)acet-amide **5***

To a stirred solution of **4** in dichloromethane was added dropwise a solution of 2-chloroacetyl chloride (0.40 g, 0.002 mol) in 20 mL of dichloromethane (2 h). The resulting yellow solid was collected by filtration at the pump to afford **5** in 99% yield; m.p.: 306 – 308°C; ^{1}H-NMR ($CDCl_3$) δ: 2.67 (s, 3H, CH_3), 2.97 (m, 4H, CH_2CH_2), 4.10 (s, 2H, CH_2), 7.56 – 7.63 (m, 3H, Ar-H); IR (KBr) cm^{-1}: 3350 (NH), 1707 (C=O); ESI-MS m/z: 277 [M + 1]; Anal. Calcd. for $C_{13}H_{13}ClN_4O$: C, 56.42; H, 4.74; N, 20.25. Found: C, 56.32; H, 4.71; N, 20.45.

*General procedure for compounds **6a – m***

A mixture of **5** (0.28 g, 1.011 mmol), monosubstituted 1,4-diazepanes (2.022 mmol), and sodium carbonate anhydrous in refluxing methanol was stirred for 10 h. The solvent was evaporated under reduced pressure and the residue was dissolved in dichloromethane and washed with water and brine, dried over $MgSO_4$, and the solvent was removed under reduced pressure. The resulting residue was purified by silica gel column chromatography (dichloromethane / methanol 10 : 1). The Yield melting point, and spectra data of each compound are given below.

*2-(4-(4-Benzyloxy-3-methoxybenzyl)-1,4-diazepan-1-yl]-N-(4,5-dihydro-1-methyl-[1,2,4]triazolo[4,3-a]quinolin-7-yl)acetamide **6a***

Yield 71%; m.p.: 72 – 74°C; ^{1}H-NMR ($CDCl_3$) δ: 1.26 (m, 2H, CH_2), 2.74 (s, 3H, CH_3), 2.86 – 2.90 (m, 8H, CH_2), 3.02 (t, J = 7.4 Hz, 2H,

CH_2), 3.12 (t, *J* = 7.4 Hz, 2H, CH_2), 3.31 (s, 2H, CH_2), 3.65 (s, 2H, CH_2), 3.89 (s, 3H, CH_3), 5.14 (s, 2H, CH_2), 6.77 – 7.72 (m, 11H, Ar-H), 9.49 (s, 1H, NH); IR (KBr) cm^{-1}: 3445 (NH), 1695 (C=O); ESI-MS m/z: 567 [M + 1]; Anal. Calcd. for $C_{33}H_{38}N_6O_3$: C, 69.94; H, 6.76; N, 14.83. Found: C, 70.12; H, 6.78; N, 14.75.

2-(4-(4-(2-Fluorobenzyloxy)-3-methoxybenzyl)-1,4-diazepan-1-yl]-N-(4,5-dihydro-1-methyl-[1,2,4]triazolo[4,3-a]quinolin-7-yl)acetamide ***6b***

Yield 72%; m.p.: 56 – 58°C; ^{1}H-NMR ($CDCl_3$) δ: 1.26 (m, 2H, CH_2), 2.73 (s, 3H, CH_3), 2.85 – 2.90 (m, 8H, CH_2), 2.99 (t, *J* = 6.6 Hz, 2H, CH_2), 3.11 (t, *J* = 6.6 Hz, 2H, CH_2), 3.30 (s, 2H, CH_2), 3.64 (s, 2H, CH_2), 3.88 (s, 3H, CH_3), 5.19 (s, 2H, CH_2), 6.80 – 7.72 (m, 10H, Ar-H), 9.48 (s, 1H, NH); IR (KBr) cm^{-1}: 3443 (NH), 1655 (C=O); ESI-MS m/z: 585 [M + 1]; Anal. Calcd. for $C_{33}H_{37}FN_6O_3$: C, 67.79; H, 6.38; N, 14.37. Found: C, 67.92; H, 6.35; N, 14.46.

2-(4-(4-(3-Fluorobenzyloxy)-3-methoxybenzyl)-1,4-diazepan-1-yl]-N-(4,5-dihydro-1-methyl-[1,2,4]triazolo[4,3-a]quinolin-7-yl)acetamide ***6c***

Yield 75%; m.p.: 54 – 56°C; ^{1}H-NMR ($CDCl_3$) δ: 1.27 (m, 2H, CH_2), 2.68 (s, 3H, CH_3), 2.83 – 2.91 (m, 8H, CH_2), 3.00 (t, *J* = 7.8 Hz, 2H, CH_2), 3.09 (t, *J* = 7.8 Hz, 2H, CH_2), 3.30 (s, 2H, CH_2), 3.61(s, 2H, CH_2), 3.87 (s, 3H, CH_3), 5.10 (s, 2H, CH_2), 6.73 – 7.70 (m, 10H, Ar-H), 9.47 (s, 1H, NH); IR (KBr) cm^{-1}: 3350 (NH), 1697 (C=O); ESI-MS m/z: 585 [M + 1]; Anal. Calcd. for $C_{33}H_{37}FN_6O_3$: C, 67.79; H, 6.38; N, 14.37. Found: C, 67.86; H, 6.37; N, 14.26.

2-(4-(4-(4-Fluorobenzyloxy)-3-methoxybenzyl)-1,4-diazepan-1-yl]-N-(4,5-dihydro-1-methyl-[1,2,4]triazolo[4,3-a]quinolin-7-yl)acetamide ***6d***

Yield 78%; m.p.: 50 – 52°C; ^{1}H-NMR ($CDCl_3$) δ: 1.25 (m, 2H, CH_2), 2.74 (s, 3H, CH_3), 2.85 – 2.90 (m, 8H, CH_2), 2.99 (t, *J* = 8.0 Hz, 2H, CH_2), 3.11 (t, *J* = 8.0 Hz, 2H, CH_2), 3.31 (s, 2H, CH_2), 3.63 (s, 2H, CH_2), 3.88 (s, 3H, CH_3), 5.08 (s, 2H, CH_2), 6.78 – 7.72 (m, 10H, Ar-H), 9.48 (s, 1H, NH); IR (KBr) cm^{-1}: 3431 (NH), 1685 (C=O); ESI-MS m/z: 585 [M + 1]; Anal. Calcd. for $C_{33}H_{37}FN_6O_3$: C, 67.79; H, 6.38; N, 14.37. Found: C, 67.84; H, 6.36; N, 14.51.

2-(4-(4-(2-Chlorobenzyloxy)-3-methoxybenzyl)-1,4-diazepan-1-yl]-N-(4,5-dihydro-1-methyl-[1,2,4]triazolo[4,3-a]quinolin-7-yl)acetamide ***6e***

Yield 70%; m.p.: 53 – 55°C; ^{1}H-NMR ($CDCl_3$) δ: 1.23 (s, 2H, CH_2), 2.74 (s, 3H, CH_3), 2.83 – 2.90 (m, 8H, CH_2), 3.01 (t, *J* = 7.2 Hz, 2H, CH_2), 3.08 (t, *J* = 7.2 Hz, 2H, CH_2), 3.30 (s, 2H, CH_2), 3.65 (s, 2H, CH_2), 3.89 (s, 3H, CH_3), 5.29 (s, 2H, CH_2), 6.62 – 7.69 (m, 10H, Ar-H), 9.44 (s, 1H, NH); IR(KBr)cm^{-1}: 3451 (NH), 1695 (C=O); ESI-MS m/z: 601 [M + 1]; Anal. Calcd. for $C_{33}H_{37}ClN_6O_3$: C, 65.93; H, 6.20; N, 13.98. Found: C, 66.02; H, 6.23; N, 13.76.

2-(4-(4-(4-Chlorobenzyloxy)-3-methoxybenzyl)-1,4-diazepan-1-yl]-N-(4,5-dihydro-1-methyl-[1,2,4]triazolo[4,3-a]quinolin-7-yl)acetamide ***6f***

Yield 70%; m.p.: 51 – 53°C; ^{1}H-NMR ($CDCl_3$) δ: 1.26 (m, 2H, CH_2), 2.75 (s, 3H, CH_3), 2.89 – 2.90 (m, 8H, CH_2), 2.98 (t, *J* = 7.5 Hz, 2H, CH_2), 3.09 (t, *J* = 7.5 Hz, 2H, CH_2), 3.31 (s, 2H, CH_2), 3.60 (s, 2H, CH_2), 3.85 (s, 3H, CH_3), 5.08 (s, 2H, CH_2), 6.65 – 7.70 (m, 10H, Ar-H), 9.50 (s, 1H, NH); IR (KBr) cm^{-1}: 3446 (NH), 1686 (C=O); ESI-MS m/z: 601 [M + 1]; Anal. Calcd. for $C_{33}H_{37}ClN_6O_3$: C, 65.93; H, 6.20; N, 13.98. Found: C, 66.05; H, 6.21; N, 13.87.

2-(4-(4-(2-Methoxybenzyloxy)-3-methoxybenzyl)-1,4-diazepan-1-yl]-N-(4,5-dihydro-1-methyl-[1,2,4]triazolo[4,3-a]quinolin-7-yl)acetamide ***6g***

Yield 75%; m.p.: 53 – 55°C; ^{1}H-NMR ($CDCl_3$) δ: 1.25 (m, 2H, CH_2), 2.73 (s, 3H, CH_3), 2.83 – 2.90 (m, 8H, CH_2), 2.98 (t, *J* = 7.3 Hz, 2H, CH_2), 3.10 (t, *J* = 7.3 Hz, 2H, CH_2), 3.30 (s, 2H, CH_2), 3.66 (s, 2H, CH_2), 3.72 (s, 3H, CH_3), 3.73 (s, 3H, CH_3), 5.11 (s, 2H, CH_2), 6.66 – 7.71 (m, 10H, Ar-H), 9.48 (s, 1H, NH); IR (KBr) cm^{-1}: 3452 (NH), 1685 (C=O); ESI-MS m/z: 597 [M + 1]; Anal. Calcd. for $C_{34}H_{40}N_6O_4$: C, 68.43; H, 6.76; N, 14.08. Found: C, 68.45; H, 6.73; N, 14.26.

2-(4-(4-(4-Methoxybenzyloxy)-3-methoxybenzyl)-1,4-diazepan-1-yl]-N-(4,5-dihydro-1-methyl-[1,2,4]triazolo[4,3-a]quinolin-7-yl)acetamide ***6h***

Yield 72%; m.p.: 54 – 56°C; ^{1}H-NMR ($CDCl_3$) δ: 1.26 (m, 2H, CH_2), 2.69 (s, 3H, CH_3), 2.74 – 2.88 (m, 8H, CH_2), 3.00 (t, *J* = 7.1 Hz, 2H, CH_2), 3.12 (t, *J* = 7.1 Hz, 2H, CH_2), 3.30 (s, 2H, CH_2), 3.65 (s, 2H, CH_2), 3.79 (s, 3H, CH_3), 3.80 (s, 3H, CH_3), 5.30 (s, 2H, CH_2), 6.62 – 7.69 (m, 10H, Ar-H), 9.54 (s, 1H, NH); IR (KBr) cm^{-1}: 3428 (NH), 1687 (C=O); ESI-MS m/z: 597 [M + 1]; Anal. Calcd. for $C_{34}H_{40}N_6O_4$: C, 68.43; H, 6.76; N, 14.08. Found: C, 68.41; H, 6.78; N, 14.16.

2-(4-(4-(4-Methylbenzyloxy)-3-methoxybenzyl)-1,4-diazepan-1-yl]-N-(4,5-dihydro-1-methyl-[1,2,4]triazolo[4,3-a]quinolin-7-yl)acetamide ***6i***

iеld 75%; m.p.: 53 – 55°C; ^{1}H-NMR ($CDCl_3$) δ: 1.26 (m, 2H, CH_2), 2.34 (s, 3H, CH_3), 2.74 (s, 3H, CH_3), 2.84 – 2.90 (m, 8H, CH_2), 3.31 (t, *J* = 7.2 Hz, 2H, CH_2), 3.12(t, *J* = 7.2 Hz, 2H, CH_2), 3.31 (s, 2H, CH_2), 3.63 (s, 2H, CH_2), 3.87 (s, 3H, CH_3), 5.10 (s, 2H, CH_2), 6.77 – 7.72 (m, 10H, Ar-H), 9.49 (s, 1H, NH); IR (KBr) cm^{-1}: 3462 (NH), 1680 (C=O); ESI-MS m/z: 581 [M + 1]; Anal. Calcd. for $C_{34}H_{40}N_6O_3$: C, 70.32; H, 6.94; N, 14.47. Found: C, 70.36; H, 6.91; N, 14.28.

2-(4-(4-(3,4-Dimethoxybenzyloxy)-3-methoxybenzyl)-1,4-diazepan-1-yl]-N-(4,5-dihydro-1-methyl-[1,2,4]triazolo[4,3-a]quinolin-7-yl)acetamide ***6j***

Yield 78%; m.p.: 68 – 70°C; ^{1}H-NMR ($CDCl_3$) δ: 1.35 (m, 2H, CH_2), 2.84 (s, 3H, CH_3), 2.95 – 3.07 (m, 8H, CH_2), 3.07 (t, *J* = 7.8 Hz, 2H, CH_2), 3.23 (t, *J* = 7.8 Hz, 2H, CH_2), 3.36 (s, 2H, CH_2), 3.70 (s, 2H, CH_2), 3.78 (s, 3H, CH_3), 3.83 (s, 3H, CH_3), 4.00 (s, 3H, CH_3), 5.26 (s, 2H, CH_2), 6.75 – 7.71 (m, 9H, Ar-H), 9.54 (s, 1H, NH); IR (KBr) cm^{-1}: 3458 (NH), 1687 (C=O); ESI-MS m/z: 627 [M + 1]; Anal. Calcd. for $C_{35}H_{42}N_6O_5$: C, 67.07; H, 6.75; N, 13.41. Found: C, 67.12; H, 6.73; N, 13.28.

2-(4-(4-(3,4-Dichlorobenzyloxy)-3-methoxybenzyl)-1,4-diazepan-1-yl]-N-(4,5-dihydro-1-methyl-[1,2,4]triazolo[4,3-a]quinolin-7-yl)acetamide ***6k***

Yield 79%; m.p.: 63 – 64°C; ^{1}H-NMR ($CDCl_3$) δ: 1.26 (m, 2H, CH_2), 2.75 (s, 3H, CH_3), 2.84 – 2.92 (m, 8H, CH_2), 3.09 (t, *J* = 7.2 Hz, 2H, CH_2), 3.20 (t, *J* = 7.2 Hz, 2H, CH_2), 3.31 (s, 2H, CH_2), 3.62 (s, 2H, CH_2), 3.89 (s, 3H, CH_3), 5.07 (s, 2H, CH_2), 6.76 – 7.71 (m, 9H, Ar-H), 9.46 (s, 1H, NH); IR (KBr) cm^{-1}: 3446 (NH), 1687 (C=O); ESI-MS m/z: 635 (M+1); Anal. Calcd. for $C_{33}H_{36}Cl_2N_6O_3$: C, 62.36; H, 5.71; N, 13.22. Found: C, 62.37; H, 5.75; N, 13.32.

2-(4-(4-(2,6-Dichlorobenzyloxy)-3-methoxybenzyl)-1,4-diazepan-1-yl]-N-(4,5-dihydro-1-methyl-[1,2,4]triazolo[4,3-a]quinolin-7-yl)acetamide ***6l***

Yield 70%; m.p.: 60 – 62°C; ^{1}H-NMR ($CDCl_3$) δ: 1.26 (m, 2H, CH_2), 2.73 (s, 3H, CH_3), 2.85 – 3.00 (m, 8H, CH_2), 3.00 (t, *J* = 7.6 Hz, 2H, CH_2), 3.12 (t, *J* = 7.6 Hz, 2H, CH_2), 3.31 (s, 2H, CH_2), 3.63 (s, 2H, CH_2), 3.85 (s, 3H, CH_3), 5.30 (s, 2H, CH_2), 6.86 – 7.74 (m, 9H, Ar-H), 9.49 (s, 1H, NH); IR (KBr) cm^{-1}: 3452 (NH), 1686 (C=O); ESI-MS m/z: 635 [M + 1]; Anal. Calcd. for $C_{33}H_{36}Cl_2N_6O_3$: C, 62.36; H, 5.71; N, 13.22. Found: C, 62.34; H, 5.72; N, 13.12.

2-(4-(4-(2-Bromobenzyloxy)-3-methoxybenzyl)-1,4-diazepan-1-yl]-N-(4,5-dihydro-1-methyl-[1,2,4]triazolo[4,3-a]quinolin-7-yl)acetamide ***6m***

Yield 73%; m.p.: 65 – 67°C; ^{1}H-NMR ($CDCl_3$) δ: 1.26 (m, 2H, CH_2), 2.75 (s, 3H, CH_3), 2.86 – 2.91 (m, 8H, CH_2), 3.00 (t, *J* = 7.1 Hz, 2H, CH_2), 3.12 (t, *J* = 7.1 Hz, 2H, CH_2), 3.31 (s, 2H, CH_2), 3.64 (s, 2H, CH_2), 3.88 (s, 3H, CH_3), 5.20 (s, 2H, CH_2), 6.78 – 7.86 (m, 10H, Ar-H), 9.48 (s, 1H, NH); IR (KBr) cm^{-1}: 3455 (NH), 1688 (C=O); ESI-MS m/z: 645 [M + 1]; Anal. Calcd. for $C_{33}H_{37}BrN_6O_3$: C, 61.39; H, 5.78; N, 13.02. Found: C, 61.42; H, 5.75; N, 13.11.

Pharmacology

The following drugs and chemicals were used in this biological evaluation test: milrinone (Shuzhou Unite Pharmaceutical Co., Shuzhou, China), DMSO (Sigma-Aldrich Chemical Co., St. Louis, MO, USA). All other reagents were of analytical grade. Atria were obtained from New Zealand white rabbits and mean atrial weight was 193.1 ± 7.4 mg. The experiments were carried out in an isolated, perfused atrial preparation that was prepared by using the method described previously [13, 14]. 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 with *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) buffer solution by means of a peristaltic pump (1.25 mL/min) at 34°C [15]. 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). The 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 the stimulus effect of the sample was recorded after a circulation of the control group. The control period (12 min as an experimental cycle) was followed by infusion of the test compounds or milrinone for 36 min.

The compounds were investigated using the single dose technique at a concentration of 1×10^{-5} M. Samples were dissolved in DMSO and diluted with the HEPES buffer to a concentration of 0.1% of DMSO. The biological evaluation data for these compounds were expressed in means of increased stroke volume percentage as shown in Table 1. Heart-rate measurements for those selected compounds were carried out in isolated rabbit hearts by recording the electrocardiogram in the volume conduction model. In order to assess differences, repeated measurements were compared by means of an ANOVA test followed by the Bonferroni's multiple-comparison test. Statistical significance was defined as $P < 0.05$ and the data is presented as means ± SE.

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