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Synthesis and positive inotropic activity of *N*-(4,5-dihydro-[1,2,4]triazolo[4,3-*a*]quinolin-7-yl)-2-(piperazin-1-yl)acetamide derivatives

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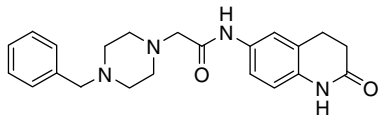
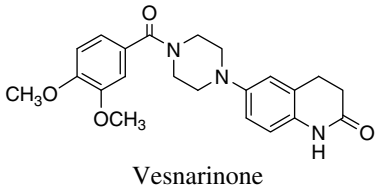
ABSTRACT

A series of *N*-(4,5-dihydro-[1,2,4]triazolo[4,3-*a*]quinolin-7-yl)-2-(piperazin-1-yl)acetamide derivatives were synthesized and their positive inotropic activity was 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 *N*-(1-benzyl-4,5-dihydro-[1,2,4]triazolo[4,3-*a*]quinolin-7-yl)-2-(4-benzylpiperazin-1-yl)acetamide **6j** was found to be the most potent with the 13.2% increased stroke volume (milrinone 4.7%) at concentration of 3×10^{-5} M in our in vitro study. The chronotropic effects of those compounds having inotropic effects were also evaluated in this work.

The development of innovative positive inotropic agents with approved therapeutic properties in the treatment of congestive heart failure (CHF)[1] is still a great challenge for the medicinal chemists. In our previous work to search for more potent positive inotropic agents having less side effects, a series of 2-(4-substituted piperazin-1-yl)-*N*-(3,4-dihydro-2(1*H*)-quinolinone-6-yl)acetamides, as a vesnarinone analog,[2] were synthesized and tested for their biological activity, among which the compound 2-(4-benzylpiperazin-1-yl)-*N*-(3,4-dihydro-2(1*H*)-quinolinone-6-yl)acetamide PHR9612 showed moderate positive inotropic activity.[3,4]



PHR9612



Vesnarinone

In our present study to further optimize the compound PHR9612, we incorporated triazole ring to the 1,2-position of 3.4-dihydro-2(1*H*)-quinolinone and changed the substituents on the 4-position of piperazine ring simultaneously to investigate the contribution of such a structure change to the biological activity. Only two kinds of substituents, methyl and benzyl groups, were considered to be introduced to the 1-position in order to preliminarily investigate their effects on the inotropic activity at this position. The compounds synthesized were characterized by IR, NMR, MS, and elemental analysis. The positive inotropic activity was evaluated by the test of measuring left atrium stroke volume on isolated rabbit heart preparations.

The synthesis of the compounds **6a**–**s** is presented in Scheme 1, and their structures are displayed in Table 1. Commercially available 3,4-dihydro-2(1*H*)-quinolinone was used as a starting material, which was treated with a mixture of concentrated sulfuric acid and nitric acid (4:1) under ice cooling to give compound **1**. The nitro group at the 6-position of **1** was reduced with palladium on activated carbon under a hydrogen atmosphere to afford corresponding amino compound **2** in high yield. Sulfurization of the resultant amino compound **2** with phosphorous pentasulfide in refluxing acetonitrile in the presence of triethylamine gave the corresponding thione **3**. Cyclization of **3** with acetyl hydrazide or phenylacetyl hydrazide in refluxing cyclohexanol under nitrogen atmosphere afforded the desired triazole compound **4** in moderate yield,[5] followed by acylation of the amino group with 2-chloroacetyl chloride in dichloromethane at room temperature to provide corresponding amide **5**. Nucleophilic-substitution reaction of **5** with various monosubstituted piperazines in refluxing methanol in the presence of sodium carbonate afforded compounds **6a**–**s** in high yields.[6]

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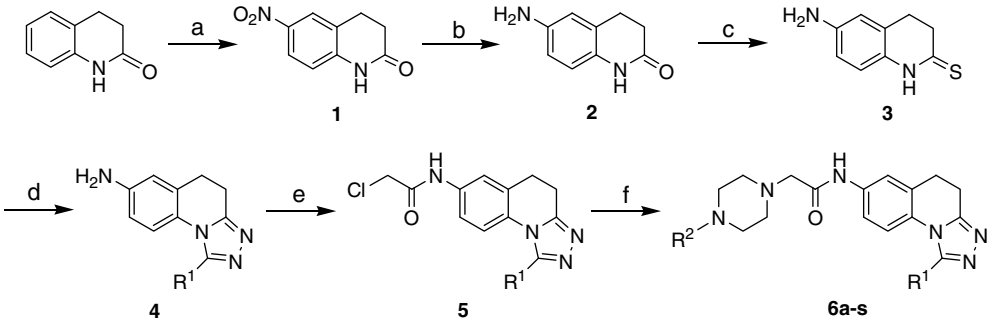


Scheme 1. Synthetic scheme for the synthesis of compounds **6a**–**s**. Reagents and conditions: (a) H_2SO_4, HNO_3 (95%); (b) H_2, Pd/C, MeOH (85%); (c) P_2S_5, Et_3N, CH_3CN (70%); (d) $R^1CONHNH_2$, cyclohexanol, reflux, N_2, 4 h (55%); (e) $ClCH_2COCl$, CH_2Cl_2, rt (98%); (f) 4-substitued piperazine, Na_2CO_3, MeOH, reflux, 10 h (83–93%).

The method of the measuring left atrial stroke volume was adopted to evaluate positive inotropic activity of the compounds synthesized above. An isolated, perfused atrial preparation was prepared by using the method described previously.[7,8] Thus, the atrium was perfused with *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) buffer solution by means of a peristaltic pump (1.25 mL/min).[9] 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 setup, 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 the 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 interval, and the stimulus effect of the sample was recorded after a circulation of the control group. Every circulation was 12 min.

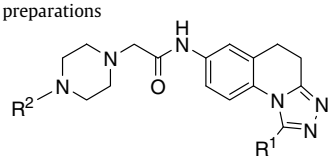
The compounds were investigated using the single dose technique at concentration of 3×10^{-5} M. Samples were dissolved in DMSO and diluted with the HEPES buffer to an appropriate volume. The biological evaluation data for these compounds was expressed in means of increased stroke volume percentage as 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. The statistical significance was defined as $P < 0.05$ and the data are presented as means ± SE.

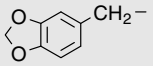
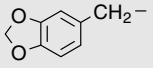
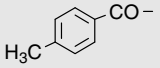
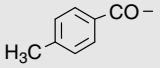
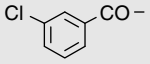
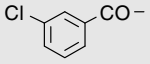
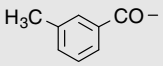
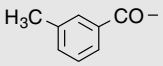
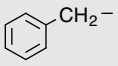
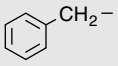
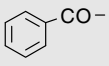
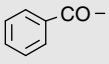
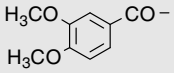
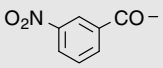
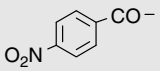
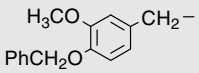
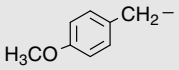
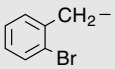
As shown in Table 1, 11 compounds out of the 19 tested compounds showed inotropic effects on isolated rabbit heart preparations. Compounds **6a**, **6b, 6g**, **6j**, **6m**, **6q**, and **6r** exhibited more potent effects compared to milrinone (4.7 ± 0.3%, 3×10^{-5} M), among which the compound **6j** showed the most potent activity with 13.2 ± 1.3% increased stroke volume. As for the relationship between inotropic activity and different substituents on the triazole ring and benzene ring attached to piperazine (R^1, R^2), those compounds only having electron-donating groups on the phenyl ring displayed enhanced effects for the 1-methyl substituted derivatives, but such a phenomenon was not found so obvious for those derivatives possessing 1-phenylmethyl substituents on the triazole ring. These results indicate that the contribution of the substituent R^1 on triazole ring to the biological effect might be more important than that of the substituent R^2 on phenyl ring. Interestingly, in contrast to the previously evaluated PHR9612 (no data), **6i** showed no efficiency in such a case of structure modification to introduce triazole ring to the 1,2-position of 3,4-dihydro-2(1*H*)-quinolinone, but for the compound **6j,** a significant inotropic effect was found, in which the only difference is that the methyl group is replaced by the phenylmethyl group. A similar case was also observed between **6e** and **6f** (2.4 ± 0.0%), and the results seem to support the indication mentioned above. The adverse results, however, were also found between **6a** (5.1 ± 0.2%) and **6b** (5.2 ± 0.3%), **6c** (1.3 ± 0.3%) and **6d** (0.7 ± 0.1%), and **6g** (8.8 ± 0.5%) and **6h** (0.5 ± 0.2%), respectively. In latter cases, the electron-donating effect of the substituent R^2 seems still to exert more dominating influence on the inotropic activity. As for the influence of the substituents at 4-position of piperazine on the inotropic effects, we found that there were no significant differences between acyl groups and benzyl groups for the biological efficiency. Nevertheless, among those compounds having more potent effects compared to milrinone, five compounds (**6a**, **6b**, **6j**, **6q**, and **6r**) possess substituted benzyl groups and only two compounds (**6g** and **6m**) have substituted aromatic acyl groups at this position.

On the other hand, we investigated the dynamics of the tested compounds in perfused beating rabbit atria and found that compound **6g** did not show a desirable biological dynamic profile, in which the stroke volume of **6g** was changed markedly decreasing as the time progressed, in spite of its significant increased stroke volume (no figure afforded). Compounds **6a** and **6r** exhibited similar atrial dynamic profiles to milrinone with good increased stroke volume (Fig. 1A). Much more desirable atrial dynamic profiles were measured for the compounds **6b**, **6j**, **6m**, and **6q** (Fig. 1B and C), in which the compound **6j** displayed excellent inotropic effects with the highest increased stroke volume (Fig. 1B). As shown in Table 2, compounds **6a**, **6b**, **6j**, **6m**, **6q**, and **6r** were also investigated for their chronotropic effects in a beating atrium, and no significant increased heart rates ($P > 0.05$) were observed for compounds **6a**, **6b**, **6m**, and **6q** at the same concentration. Compounds **6j** and **6r**, however, showed the changed heart rates unfortunately, for which further in vivo study was required in order to investigate their chronotropic effects.

In conclusion, based on the structure of PHR9612, we synthesized 4,5-dihydro-[1,2,4]triazolo[4,3-*a*]quinoline derivatives and tried to find more potent compounds for cardiac contractility without increasing heart rate. As a result, we obtained several compounds having enhanced inotropic effects and desirable biological profile in our present study, in which the compounds **6a**, **6b**, **6m**, and **6q** exhibited more promising cardiovascular profiles. These compounds are now undergoing other biological tests including in vivo evaluation, coronary vasodilating tests, and possible action mechanism study in order to be selected to the new candidates for further clinical trials.

Table 1
Positive inotropic activity of compounds **6a–s** in left atrium stroke volume test on isolated rabbit heart preparations



Compound	R^1	R^2	Increased stroke volume[a] (%)
6a	CH_3–		5.1 ± 0.2
6b	$PhCH_2$–		5.2 ± 0.3
6c	CH_3–		1.3 ± 0.3
6d	$PhCH_2$–		0.7 ± 0.1
6e	CH_3–		–[b]
6f	$PhCH_2$–		2.4 ± 0.0
6g	CH_3–		8.8 ± 0.5
6h	$PhCH_2$–		0.5 ± 0.2
6i	CH_3–		–
6j	$PhCH_2$–		13.2 ± 1.3
6k	CH_3–		–
6l	$PhCH_2$–		–
6m	CH_3–		5.3 ± 0.3
6n	CH_3–		–
6o	CH_3–		–
6p	CH_3–	H_3CO–	–
6q	CH_3–		9.0 ± 0.3
6r	CH_3–		10.1 ± 0.5
6s	CH_3–		–
		Milrinone	4.7 ± 0.3

[a] The concentration for the test sample is 3×10^{-5} M.
[b] None or negative stroke volume increase.

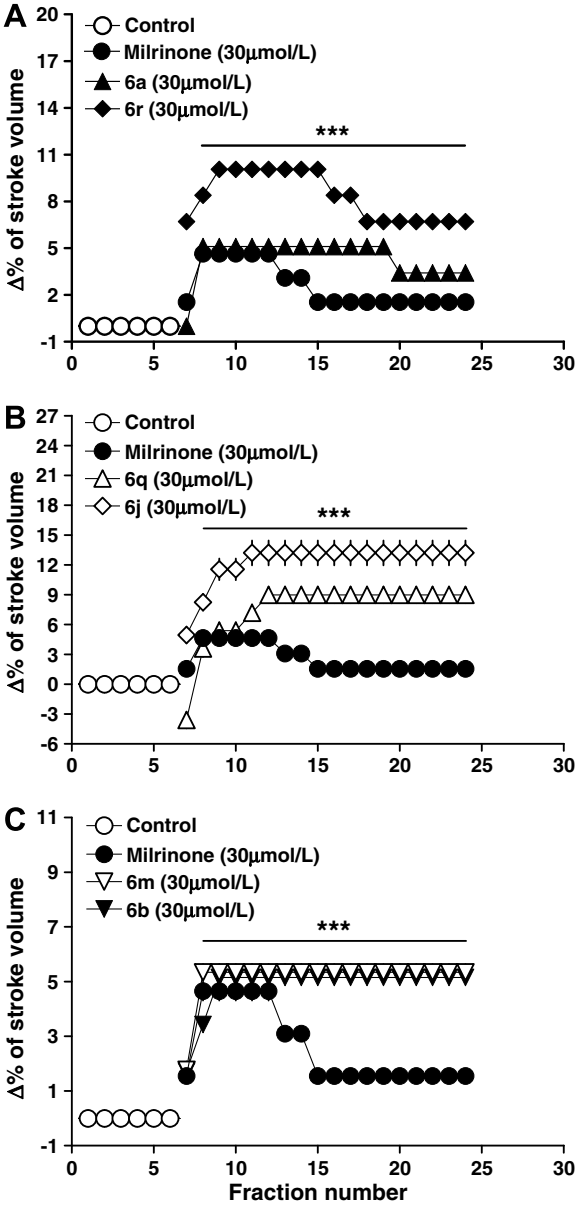


Figure 1. Effects of milrinone and compounds **6a**, **6g**, **6h**, **6j**, **6q**, and **6r** on stroke volume in beating rabbit atria (1.5 Hz). Values are means ± SE. $^{***}P < 0.001$ versus control.

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

Compound	Mean ± SE[a]	Mean ± SE[b]
6a	132.9 ± 0.0	136.9 ± 0.1
6b	90.6 ± 0.5	89.2 ± 0.1
6j	117.3 ± 0.2	109.3 ± 0.1**
6m	128.4 ± 0.2	129.2 ± 0.3
6q	111.3 ± 2.2	111.5 ± 0.2
6r	115.3 ± 0.1	105.6 ± 0.5**

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

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6. Preparation of **6a**: A mixture of **5** ($R^1 = CH_3$, 276 mg, 1.01 mmol), 1-((benzo[*d*][1,3]dioxol-5-yl)methyl)piperazine (223 mg, 1.01 mmol), Na_2CO_3 (54 mg, 0.5 mmol) in methanol (10 mL) was stirred under reflux for 10 h and concentrated under reduced pressure. The resulting residue was purified by chromatography (CH_2Cl_2/CH_3OH 9:1) to afford **6a** (414 mg, 90%) as white solid. Mp 130–132 °C. ^1H NMR ($CDCl_3$, 300 MHz) δ 2.56–2.63 (4H, m), 2.73 (3H, s), 3.02 (2H, t, $J = 7.0$ Hz), 3.05 (2H, t, $J = 7.0$ Hz), 3.19 (2H, s), 3.46 (2H, s), 5.90 (2H, s), 6.75–6.84 (3H, m), 7.60–7.70 (3H, m), 8.35 (1H, br s). MS *m*/*z* 461 (M+1). Anal. Calcd for $C_{25}H_{28}N_6O_3$: C, 65.20; H, 6.13; N, 18.25. Found: C, 65.00; H, 6.23; N, 18.52.
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