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Synthesis of 2-(4-substituted benzyl-1,4-diazepan-1-yl)-*N*-(3,4-dihydro-3-oxo-2*H*-benzo[*b*][1,4]oxazin-7-yl)acetamides and their positive inotropic evaluation

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ABSTRACT

Herein we describe the discovery of compound **3g**, a potent positive inotropic agent compared with the standard drug, milrinone. Compound **3g** was developed from a series of 2-(4-substitutedbenzyl-1,4-diazepan-1-yl)-*N*-(3,4-dihydro-3-oxo-2*H*-benzo[*b*][1,4]oxazin-7-yl) acetamides found in an evaluation of inotropic activity by measuring left atrium stroke volume on isolated rabbit heart preparations. Several compounds showed favorable activities, but **3g** was the most potent, with 7.68 ± 0.14% increased stroke volume (milrinone 2.38 ± 0.05%) at 1×10^{-5} M in our in vitro study. The chronotropic effects of compounds having significant inotropic effects were also evaluated.

The development of innovative positive inotropic agents with approved therapeutic properties for the treatment of congestive heart failure (CHF) remains a great challenge in medicinal chemistry.[1] During our previous studies that aimed to discover more potent positive inotropic agents with fewer side effects, a series of 2-(4-substitutedpiperazin-1-yl)-*N*-(3,4-dihydro-2(1*H*)-quinolinone-6-yl)acetamides, as a vesnarinone[2] analog, were synthesized and tested for their biological activity. Among these, 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] Replacement of the carbon atom at the 4-position of PHR9612 with an oxygen atom yielded 2-(4-(3-methoxybenzyl)piperazin-1-yl)-*N*-(3,4-dihydro-3-oxo-2*H*-benzo[*b*][1,4]oxazin-7-yl)acetamide (PHRL0010), which possessed significant inotropic effects,[5] and which is now undergoing further biological tests (Fig. 1).

In the present study, to further optimize compound PHRL0010, we replaced the piperazine ring with a 1,4-diazepane ring. We also varied the substituents on the phenyl ring of the benzyl group to preliminarily investigate the contribution of such a structural change on biological activity. The compounds synthesized were characterized by IR, NMR, MS, and elemental analysis. The positive inotropic activity was evaluated by measuring the left atrium stroke volume on isolated rabbit heart preparations.

The synthesis of compounds **3a**–**j** and **4a**–**j** is presented in Scheme 1. Compound **1** was synthesized through cyclization and reduction according to previously described methods by using commercially available 2-amino-5-nitrophenol as a starting material.[6] A catalytic hydrogenation method was used to reduce the nitro group instead of using iron powder in hydrochloric acid, and the yield was increased from 75% to 98%. The resulting amino group of compound **1** was acylated with 2-chloroacetyl chloride in dichloromethane to provide amide **2** in excellent yield. A nucleophilic-substitution reaction of **2** with various monosubstituted 1,4-diazepanes[7] in refluxing acetone in the presence of potassium carbonate afforded the corresponding compounds **3a**–**j** and **4a**–**j** in moderate yields.[8]

The method for measuring left atrium stroke volume was described previously.[9,10] The features of CHF are cardiac dilatation, poor contractility of cardiac muscle, decreased ejection fraction, and depression of left ventricular maximum pressure. Therefore, macroscopic measurement of the variance in left atrium stroke volume can be used to estimate the positive inotropic effects of the compounds synthesized. Milrinone (Suzou Unite Pharmaceutical Company, Suzou, China) and DMSO (Sigma–Aldrich Chemical Company, St. Louis, MO, USA) 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. In brief, hearts were removed from rabbits and the left atria 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

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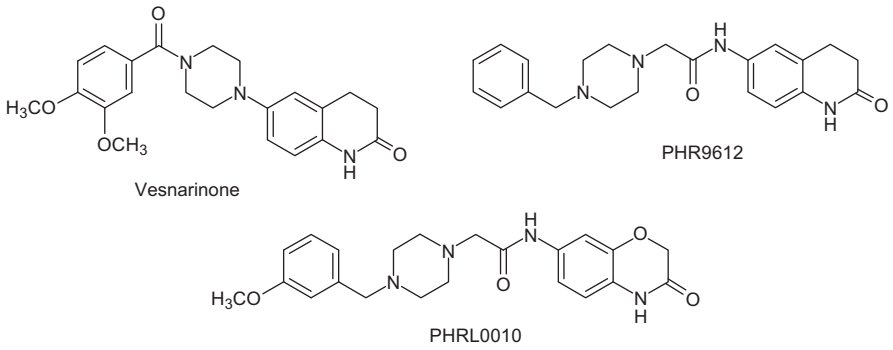
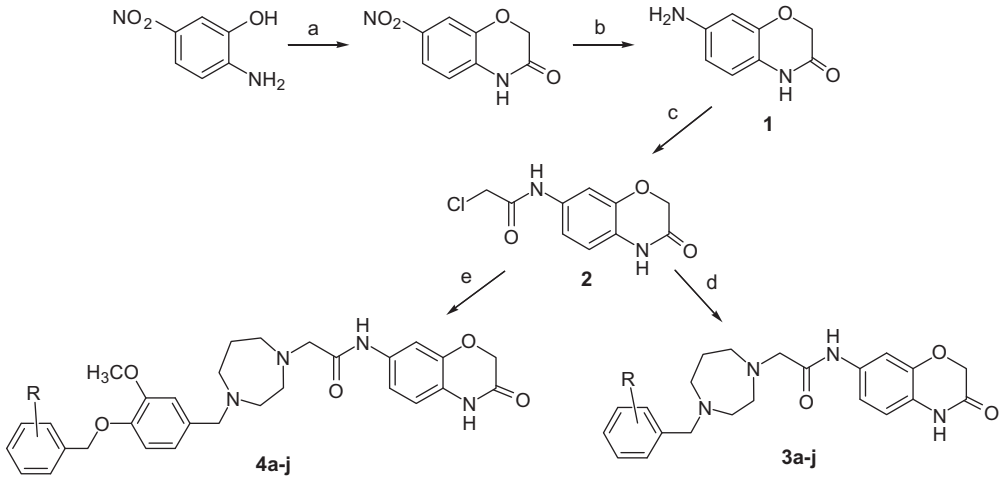


Figure 1. Cardiotonic agents used for the treatment of CHF and previous lead compounds.



Scheme 1. Synthetic scheme for the synthesis of compounds **3a–j** and **4a–j**. Reagents: (a) $ClCH_2COCl$, acetonitrile, TEBA, Na_2CO_3; (b) H_2/Pd, MeOH; (c) $ClCH_2COCl$, CH_2Cl_2; (d) 1-substitutedbenzyl-1,4-diazepanes, KI, K_2CO_3, acetone; (e) 1-(4-(substitutedbenzyloxy)-3-methoxybenzyl)-1,4-diazepanes, KI, K_2CO_3, acetone.

solution with a peristaltic pump (1.25 mL/min) at 34 °C.[11] 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 end diastole. 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 infused for 36 min after a control period of 12 min.

Compounds were tested at 1×10^{-5} M. Samples were dissolved in DMSO and diluted with HEPES buffer to 0.1% DMSO. Data are 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 data are presented as means ± SE.

Table 1 shows the inotropic activity data for the 20 test compounds. Four compounds (**3c**, **3g**, **3h**, and **4d**) showed significantly increased inotropic effects on isolated rabbit heart preparations compared with milrinone (2.38 ± 0.05%, 1×10^{-5} M). Compounds **3g** and **3h** clearly exhibited more potent effects compared with our lead PHRL0010 and milrinone; as 2-Br and 2-F derivatives, **3g** was 3.2-fold and **3h** was 2.6-fold more potent (7.68 ± 0. 14% and 6.05 ± 0.11%, 1×10^{-5} M) than milrinone. Compared with the activities of the same substituted derivatives in the piperazine series, 2-Br derivative was 0.9-fold and 2-F derivative was 2.6-fold more potent than milrinone at the same concentrations, respec-

Table 1
Positive inotropic activity of the test compounds

Compound	R	Increased stroke volume[a] (%)
3a	4-CH_3	—[b]
3b	H	—
3c	4-OCH_3	3.22 ± 0.06
3d	4-Cl	—
3e	2-Cl	—
3f	3-OCH_3	—
3g	2-Br	7.68 ± 0.14
3h	2-F	6.05 ± 0.11
3i	2,6-$(Cl)_2$	—
3j	3,4-$(OCH_3)_2$	—
4a	2-Cl	—
4b	H	—
4c	4-OCH_3	—
4d	4-Cl	2.87 ± 0.08
4e	4-CH_3	—
4f	3-OCH_3	—
4g	2-Br	—
4h	2-F	—
4i	2,6-$(Cl)_2$	—
4j	3,4-$(OCH_3)_2$	—
PHRL0010		3.96 ± 0.15
Milrinone		2.38 ± 0.05

[a] Concentration for the test sample is 1×10^{-5} M.
[b] None or negative stroke volume increases.

tively.[5] This indicated that replacing the piperazine ring with the 1,4-diazepane did not significantly affect the activity. Most compounds from the series **4a–j** were not active (except **4d**), so a suitable length of the molecule may be required for the activity. As for the relationship between inotropic activity and different substitutions on the phenyl ring of the benzyl group for compounds **3a–j**, we found that, among halo-substituted compounds, bromo and fluoro derivatives **3g** and **3h** had significant activity, but not the chloro or dichloro analogs (**3d**, **3e**, and **3i**). Among the methoxy-substituted derivatives, only the 4-methoxy derivative (**3c**) had activity (3-methoxy or 3,4-dimethoxy derivatives (**3f** and **3j**) were inactive). Thus, the substituents on the phenyl ring seemed to significantly affect the biological activity. A clear pattern was not observed for the relationship between the inotropic activity and the different substituents for these derivatives.

We also investigated the dynamics of the test compounds (**3c**, **3g, 3h**, and **4d**) in the perfused beating atria of rabbits and found that compound **3g** showed a similar atrial dynamic profile to that of milrinone (Fig. 2B). Much more desirable atrial dynamic profiles were measured for compounds **3c** and **3h** (Fig. 2A and C). We also investigated the preliminary dose-dependency for **3g** and **3h** at 3×10^{-6}, 1×10^{-5}, and 3×10^{-5} M, and for milrinone at 1×10^{-6}, 3×10^{-6}, 1×10^{-5}, 3×10^{-5}, and 1×10^{-4} M (Fig. 2D for **3h**, Fig. 2E for **3g**, and Fig. 2F for milrinone). Compound **3g** displayed stronger potency than milrinone at all tested concentrations with a maximal effect at 3×10^{-5} M, and **3h** showed maximal effect at 1×10^{-5} M. Compounds **3c**, **3g**, **3h**, and **4d** were also investigated for their chronotropic effects in beating atria, and no significant increase in heart rates ($P > 0.05$) was observed for **3c**, **3g**, and **3h** at the same concentration (Table 2). However, compound **4d** unfortunately resulted in changed heart rates. Further in vivo studies are therefore required for these compounds to further investigate their chronotropic effects.

In conclusion, based on the structure of compound PHRL0010, we designed and synthesized two series of 2-(4-substitutedbemzyl-1,4-diazepan-1-yl)-*N*-(3,4-dihydro-3-oxo-2*H*-benzo[*b*][1,4]oxazin-7-yl)acetamides with the aim of identifying more potent compounds that increase cardiac contractility without increasing heart rate. Compounds **3g** and **3h** exhibited the most promising cardiovascular profiles compared with the lead compound PHRL0010 and milrinone (3.2-fold more potent at 1×10^{-5} M for **3g**). Further modification of compound **3g** is in progress.

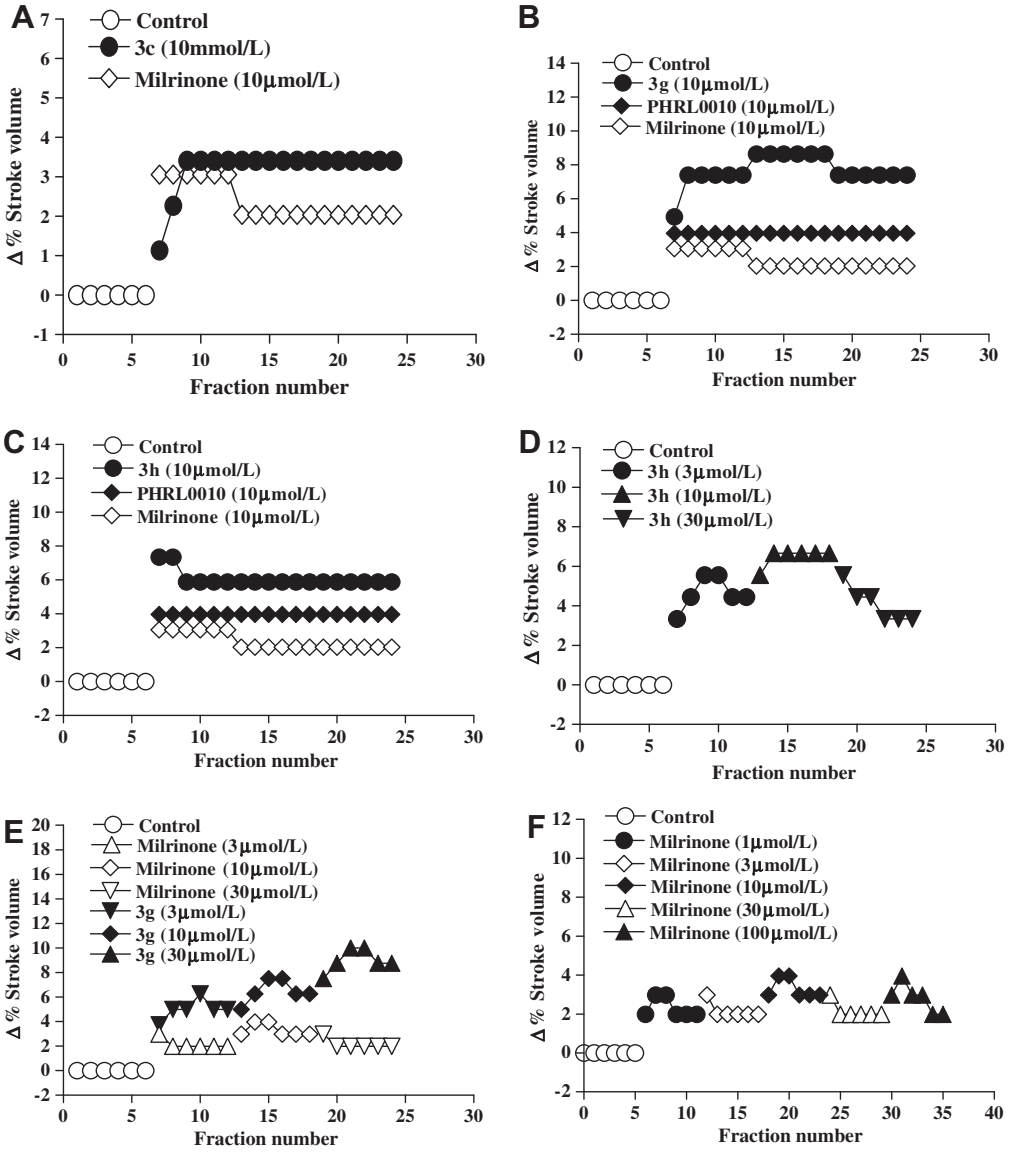


Figure 2. Effects of milrinone and compounds **3c** (A), **3g** (B), and **3h** (C) on stroke volume in beating rabbit atria (1.5 Hz). Concentration–response curves of **3g** (E) and **3h** (D) on isolated rabbit heart preparations. Values are means ± SE.

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

Compound	Mean ± SE[a]	Mean ± SE[b]
3c	92.52 ± 0.17	89.37 ± 0.08
3g	102.31 ± 0.07	98.64 ± 0.24
3h	105.33 ± 0.12	102.88 ± 0.19
4d	89.51 ± 0.09	80.02 ± 0.15[c]

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

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8. Preparation of **3g**: A mixture of **2** (0.24 g, 1.0 mmol), 1-(2-bromobenzyl)-1,4-diazepane (0.54 g, 2.0 mmol), and KI/K_2CO_3 in refluxing acetone was stirred for 12 h. The solvent was evaporated in vacuo and the residue was dissolved in dichloromethane, 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, 30:1 v/v) to afford **3g** (0.26 g, 56%). Mp 185–186 °C. ^1H NMR ($CDCl_3$, 300 MHz): δ 1.85–1.91 (m, 2H), 2.78–2.92 (m, 8H), 3.28 (s, 2H), 3.77 (s, 2H), 4.62 (s, 2H), 6.76–7.56 (m, 7H), 8.61 (s, 1H), 9.30 (s, 1H). MS *m/z* 473 (M+1). IR (KBr) cm^{-1}: 3462 (NH), 1691 (C=O), 1683 (C=O). Anal. Calcd for $C_{22}H_{25}BrN_4O_3$: C, 55.82; H, 5.32; N, 11.88. Found: C, 55.78; H, 5.34; N, 11.74.
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