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Short communication

Synthesis of 2-(4-substitutedmethylpiperazin-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

In an attempt to search for more potent positive inotropic agents, a series of 2-(4-substitutedmethylpiperazin-1-yl)-*N*-(3,4-dihydro-3-oxo-2*H*-benzo[*b*][1,4]oxazin-7-yl)acetamides were synthesized and their positive inotropic activities were evaluated by measuring left atrium stroke volume on isolated rabbit heart preparations. Several compounds showed favorable activities compared with the standard drug, milrinone, among which 2-(4-(4-methylbenzyl)piperazin-1-yl)-*N*-(3,4-dihydro-3-oxo-2*H*-benzo[*b*][1,4]oxazin-7-yl) acetamide **4e** showed the most potent activity with the $5.09 \pm 0.00\%$ increased stroke volume (milrinone $1.67 \pm 0.64\%$) at a concentration of 1×10^{-5} M in our *in vitro* study. The chronotropic effects of those compounds having significant inotropic effects were also evaluated in this work.

1. Introduction

Cardiac glycosides, like digoxin, have consistently been one of the most frequently prescribed cardiotonics used for the treatment of congestive heart failure (CHF) for many years and are the only medications used without increasing the CHF patient's mortality up to now, but their high toxicity and narrow therapeutic window still limit their clinical application as positive inotropic agents [1]. Other alternative synthetic replacements, like the phosphodiesterase-inhibiting agent milrinone [2], recently developed vesnarinone [3,4] and toborinone [5], ultimately exert their inotropic effects via an increase in intracellular calcium and consequently result in strong arrhythmogenic effects that caused increased mortality when used to 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 mortality in the short-term treatment of CHF, but it is uncertain whether it will reduce the mortality in 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].

In our previous work to search for more potent positive inotropic agents having less side effects, a series of 2-(4-substitutedpiperazin-1-yl)-*N*-(3,4-dihydro-2(1*H*)-quinolinone-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*)-quinolinone-6-yl)acetamide PHR9612 showed moderate positive inotropic activity [10]. In our present continual research to further optimize the compound PHR9612, we replaced the 3,4-dihydro-2(1*H*)quinolinone moiety with the 3,4-dihydro-3-oxo-2*H*-benzo[*b*][1,4]oxazine and changed the substituents on the piperazine ring at the 4-positon, simultaneously, in which either substituted benzyl groups or (2-substituted naphthalen-6-yl)methyl groups were introduced, 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 their positive inotropic activities were evaluated by measuring their effects on left atrium stroke volume in isolated rabbit heart preparations using milrinone, a marked drug, as a reference compound for a comparison.

2. Results and discussion

2.1. Chemistry

The synthesis of compounds **3a–c** and **4a–k** is presented in Scheme 1. Compound **1** was synthesized through cyclization and

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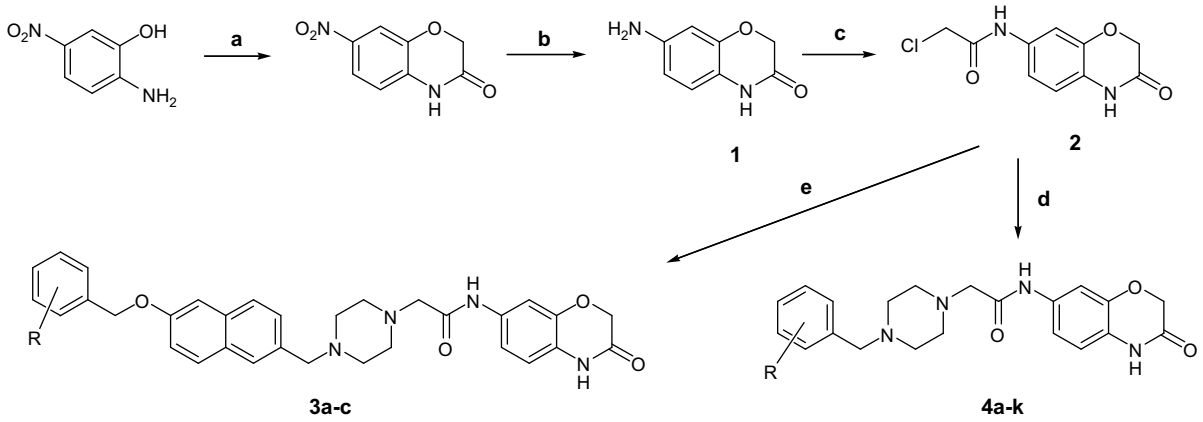

Scheme 1. Synthetic scheme for the synthesis of compounds **3a–d** and **4a–k**. Reagents and conditions: (a) $ClCH_2COCl$/acetonitrile/TEBA/Na_2CO_3, (b) H_2/Pd/C/MeOH, (c) $ClCH_2COCl$/Cl_2CH_2, (d) monosubstituted piperazines/Na_2CO_3/MeOH, (e) 1-((2-substitutedbenzyloxy)naphthalen-6-yl)methylpiperazines/Na_2CO_3/MeOH.

reduction according to the previously described methods by using commercially available 2-amino-5-nitrophenol as a starting material. Catalytic hydrogenation method was used to reduce the nitro group instead of the method of using iron powder in hydrochloride, and the yield was increased from 75% to 98% [11]. The resulting amino group at the 7-position of **1** was acylated with 2-chloroacetyl chloride according to the reported method in dichloromethane to provide corresponding amide **2** in excellent yield [10]. Nucleophilic-substitution reaction of **2** with various monosubstituted piperazines in refluxing methanol in the presence of sodium carbonate afforded corresponding compounds **3a–c** and **4a–k** in moderate yield.

2.2. Biological evaluation

As shown in Table 1, 4 compounds out of the 15 test compounds showed inotropic effects on isolated rabbit heart preparations. Compounds **4c**, **4e**, and **4f** exhibited more potent effects while **4j** showed slightly lower potency compared to milrinone ($1.67 \pm 0.64\%, 1 \times 10^{-5}$ M), among which compound **4e** showed the most potency with $5.09 \pm 0.00\%$ increased stroke volume. In contrast to the previously evaluated PHR9612 with a potency which was weaker compared with milrinone (no data), three compounds showed significantly increased inotropic activities through the structure modification of replacing 3,4-dihydro-2(1*H*)-quinolinone moiety with 3,4-dihydro-3-oxo-2*H*-benzo[*b*][1,4]oxazine, but none of those compounds possessing (2-substituted naphthalen-6-yl) methyl groups at 4-position of the piperazine ring showed any inotropic activities. It seems that a suitable length of molecule is required for the activity, while for the compounds **3a–c** an extremely extended molecular length was caused by inleting a naphthalene ring that was further connected with substituted benzyloxy group at 2-position of the naphthalene. As for the relationship between inotropic activity and the different substituents (R) on the phenyl ring of the benzyl group at the 4-position of the piperazine for **4a–k**, we couldn't find any clear regularity for the contribution of the substituents R to the biological effect, but those compounds having electron-donating substituents on phenyl ring appeared to have more potent effects although **4d** and **4g** didn't show any potency. Interestingly, for the halogen substituted derivatives, bromo- and fluoro-substituted compounds (**4c**, **4j**) showed good activities except **4b**, but those chloro-substituted compounds (**4h**, **4i,** and **4k**) showed no potency. In addition, the effects of the substituent's position on the benzene ring to the efficancy also showed no regularity, for which more compounds need to be designed and synthesized for further investigation.

On the other hand, we investigated the dynamics of the test compounds in perfused beating rabbit atria and found that four compounds possessing inotropic effects (**4c**, **4e**, **4f** and **4j**) showed desirable biological dynamic profiles compared with that of milrinone, although the lower potency was observed for **6j** ($1.10 \pm 0.22\%$, Fig. 1C). It was found that the stroke volumes of **4c** and **4e** were gradually increased as the time progressed (Fig. 1A), but more desirable dynamic property was observed for **4f** whose stroke volume was kept unchanged after rising to a certain extent (Fig. 1B).

As shown in Table 2, compounds **4c**, **4e**, and **4f** were also investigated for their chronotropic effects in a beating atria and no significant increased heart rate ($P > 0.05$) was observed for compound **4f** at the same concentration. Compounds **4c** and **4e**, however, showed the changed heart rates unfortunately, for which *in vivo* study was required in order to further investigate their chronotropic effects. Compound **4f**, due to its more promising cardiovascular property, is now undergoing further biological tests including *in vivo* evaluation, coronary vasodilating test, and possible mechanism of action study in order to be selected as a candidate for further clinical trials.

3. Experimental section

3.1. Chemistry

Melting points were determined in open capillary tubes and are uncorrected. Reaction courses were monitored by TLC on silica gel

Table 1
Positive inotropic activity of the test compounds

Compound	R	Increased stroke volume (%)[a]
3a	H	–[b]
3b	4-OCH_3	–
3c	4-CH_3	–
3d	3-Cl	–
4a	H	–
4b	4-F	–
4c	2-F	4.29 ± 0.00
4d	3,4-OCH_2O-	–
4e	4-CH_3	5.09 ± 0.00
4f	3-OCH_3	3.23 ± 0.00
4g	4-OCH_3	–
4h	4-Cl	–
4i	3-Cl	–
4j	2-Br	1.43 ± 0.00
4k	2,6-$(Cl)_2$	–
Milrinone		1.67 ± 0.64

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

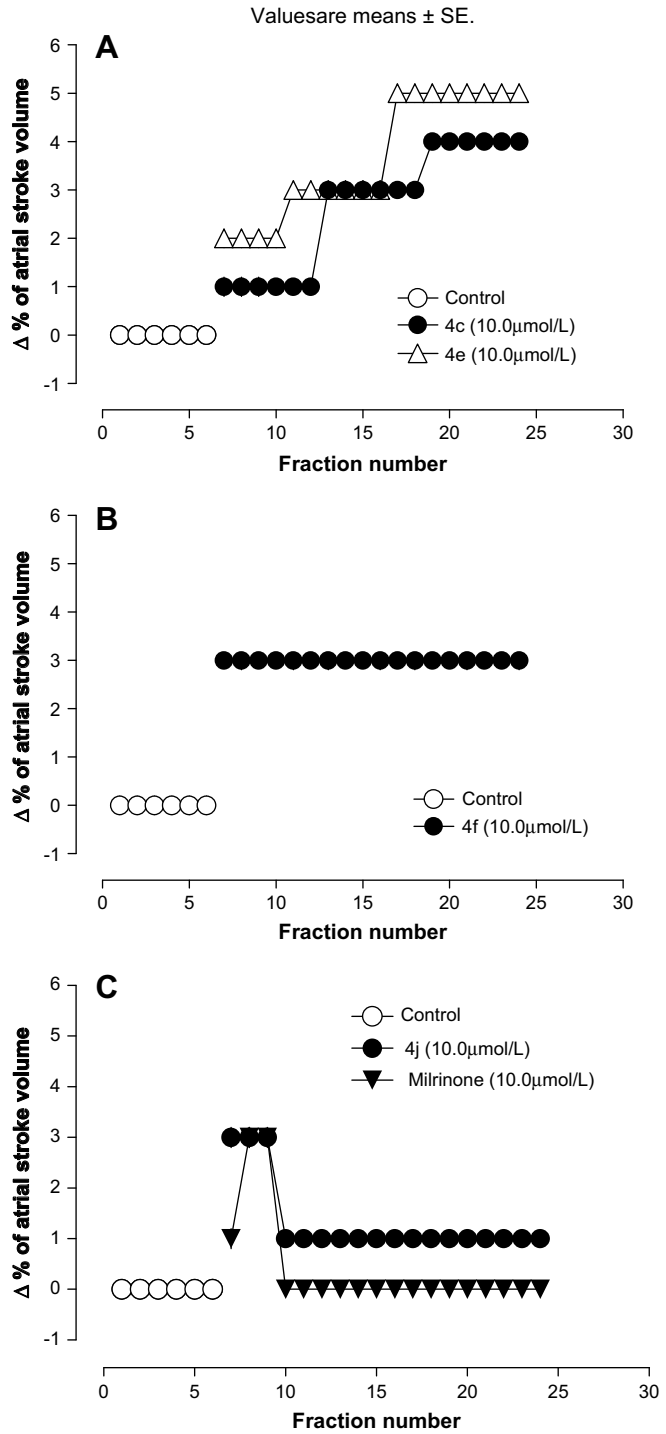

Fig. 1. Effects of milrinone and compounds **4c**, **4e**, **4f**, and **4j** on stroke volume in beating rabbit atria (1.5 Hz). Values are mean ± SE.

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

Compound	Mean ± SE[a]	Mean ± SE[b]
4c	146.80 ± 0.09	139.10 ± 0.77[c]
4e	152.20 ± 0.42	142.20 ± 0.37[c]
6f	134.30 ± 0.24	134.80 ± 0.23

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

precoated F254 Merck plates and developed plates were examined with UV lamps (254 nm). IR spectra were recorded (in KBr) on a FT-IR1730. ^{1}H NMR spectra were measured on Bruker AV-300 spectrometer, 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 and Fluka Companies. Monosubstituted piperazines were synthesized by the method reported [12].

*3.1.1. 7-Amino-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one (**1**)*

A suspension of 7-nitro-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one (4.0 g, 0.021 mol), 5% palladium on charcoal (0.4 g), and absolute ethanol (50 mL) was stirred at room temperature under atmospheric pressure of hydrogen until the absorption of hydrogen ceased. The catalyst was filtered off and the solvent was evaporated to afford **1** in 98% yield; mp 211–213 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 4.41 (s, 2H, –$COCH_2$), 4.88 (s, 2H, –NH_2), 6.17–6.57 (m, 3H, Ar–H), 10.27 (s, 1H, –CONH).

*3.1.2. 2-Chloro-*N*-(3,4-dihydro-3-oxo-2*H*-benzo[*b*][1,4]oxazin-7-yl)acetamide (**2**)*

To a stirred solution of **1** (2.0 g, 0.012 mol) in dichloromethane was added dropwise a solution of 2-chloroacetyl chloride (1.49 g, 0.013 mol) in 20 mL of dichloromethane (2 h). The resulting yellow solid was collected by filtration at the pump to afford **2** in 99% yield; m.p. 306–308 °C. IR (KBr) cm^{-1}: 3350 (NH), 1707 (C=O), 1695 (C=O). ^{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); MS *m*/*z* 241 (M + 1). Anal. Calcd for $C_{10}H_9ClN_2O_3$: C, 49.91; H, 3.77; N, 11.64. Found: C, 50.02; H, 3.73; N, 11.82.

*3.1.3. General procedure for compounds **3a–d** and **4a–k***

A mixture of **2** (0.24 g, 0.001 mol), monosubstituted piperazine (0.002 mmol), and sodium carbonate anhydrous in refluxing methanol was stirred for 24 h. The solvent was evaporated under reduced pressure 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, 40:1). The yield, melting point and spectral data of each compound are given below.

*3.1.4. 2-(4-(2-Benzyloxynaphthalen-6-yl)piperazin-1-yl)-*N*-(3,4-dihydro-3-oxo-2*H*-benzo[b][1,4]oxazin-7-yl)acetamide (**3a**)*

Yield 49%; m.p. 189–190 °C. IR (KBr) cm^{-1}: 3451 (NH), 1691 (C=O), 1686 (C=O). ^{1}H NMR ($CDCl_3$) δ 2.51–2.56 (m, 8H, –CH_2), 3.51 (s, 2H, –CH_2), 4.01 (s, 2H, –CH_2), 4.57 (s, 2H, –CH_2), 5.18 (s, 2H, –CH_2), 6.32–7.66 (m, 14H, Ar–H), 8.19 (s, 1H, –NH), 8.86 (s, 1H, –NH). MS *m*/*z* 536 (M + 1). Anal. Calcd for $C_{32}H_{32}N_4O_4$: C, 71.62; H, 6.01; N, 10.44. Found: C, 71.62; H, 6.03; N, 10.58.

*3.1.5. 2-(4-(2-(4-Methoxybenzyloxy)naphthalen-6-yl)piperazin-1-yl)-*N*-(3,4-dihydro-3-oxo-2*H*-benzo[b][1,4]oxazin-7-yl) acetamide (**3b**)*

Yield 46%; m.p. 190–191 °C. IR (KBr) cm^{-1}: 3456 (NH), 1691 (C=O), 1687 (C=O). ^{1}H NMR ($CDCl_3$) δ 2.58–2.63 (m, 8H, –CH_2), 3.12 (s, 3H, –CH_3), 3.50 (s, 2H, –CH_2), 3.73 (s, 2H, –CH_2), 4.38 (s, 2H, –CH_2), 4.60 (s, 2H, –CH_2), 6.74–7.89 (m, 16H, Ar–H), 8.77 (s, 1H, –NH), 9.11 (s, 1H, –NH). MS *m*/*z* 567 (M + 1). Anal. Calcd for $C_{33}H_{34}N_4O_5$: C, 69.95; H, 6.05; N, 9.89. Found: C, 70.01; H, 6.03; N, 9.92.

*3.1.6. 2-(4-(2-(4-Methylbenzyloxy)naphthalen-6-yl)piperazin-1-yl) -*N*-(3,4-dihydro-3-oxo-2*H*-benzo[b][1,4]oxazin-7-yl)acetamide (**3c**)*

Yield 43%; m.p. 195–196 °C. IR (KBr) cm^{-1}: 3452 (NH), 1692 (C=O), 1681 (C=O). ^{1}H NMR ($CDCl_3$) δ 2.38 (s, 3H, –CH_3), 2.55–2.61

(m, 8H, $-CH_2$), 3.50 (s, 2H, $-CH_2$), 3.67 (s, 2H, $-CH_2$), 4.57 (s, 2H, $-CH_2$), 5.14 (s, 2H, $-CH_2$), 6.28–7.74 (m, 16H, Ar–H), 8.36 (s, 1H, –NH), 8.62 (S, 1H, –NH). MS *m*/*z* 551 (M + 1). Anal. Calcd for $C_{33}H_{34}N_4O_4$: C, 71.98; H, 6.22; N, 10.17. Found: C, 71.81; H, 6.23; N, 10.22.

3.1.7. 2-(4-(2-(3-Chlorobenzyloxy)naphthalen-6-yl)piperazin-1-yl)-N-*(3,4-dihydro-3-oxo-2*H-*benzo[b][1,4]oxazin-7-yl) acetamide (**3d**)*

Yield 47%; m.p. 203–204 °C. IR (KBr) cm^{-1}: 3458 (NH), 1692 (C═O), 1687 (C═O). ^1H NMR ($CDCl_3$) δ 2.56–2.61 (m, 8H, $-CH_2$), 3.13 (s, 2H, $-CH_2$), 3.67 (s, 2H, $-CH_2$), 4.51 (s, 2H, $-CH_2$), 5.14 (s, 2H, $-CH_2$), 7.31–7.84 (m, 13H, Ar–H), 8.36 (s, 1H, –NH), 8.84 (s, 1H, –NH). MS *m*/*z* 571 (M + 1). Anal. Calcd for $C_{32}H_{31}ClN_4O_4$: C, 67.30; H, 5.47; N, 9.81. Found: C, 67.43; H, 5.58; N, 9.98.

3.1.8. 2-(4-Benzylpiperazin-1-yl)-N-*(3,4-dihydro-3-oxo-2*H-*benzo[b][1,4]oxazin-7-yl)acetamide (**4a**)*

Yield 50%; m.p. 203–204 °C. IR (KBr) cm^{-1}: 3467 (NH), 1690 (C═O), 1682 (C═O). ^1H NMR ($CDCl_3$) δ 2.43–2.48 (m, 8H, $-CH_2$), 3.47 (s, 2H, $-CH_2$), 3.80 (s, 2H, $-CH_2$), 4.56 (s, 2H, $-CH_2$), 6.28–7.27 (m, 8H, Ar–H), 7.84 (s, 1H, –NH), 8.16 (s, 1H, –NH). MS *m*/*z* 381 (M + 1). Anal. Calcd for $C_{21}H_{24}N_4O_3$: C, 66.30; H, 6.36; N, 14.73. Found: C, 66.29; H, 6.38; N, 14.42.

3.1.9. 2-(4-(4-Fluorobenzyl)piperazin-1-yl)-N-*(3,4-dihydro-3-oxo-2*H-*benzo[b][1,4]oxazin-7-yl)acetamide (**4b**)*

Yield 51%; m.p. 202–203 °C. IR (KBr) cm^{-1}: 3462 (NH), 1696 (C═O), 1682 (C═O). ^1H NMR ($CDCl_3$) δ 2.42–2.47 (m, 8H, $-CH_2$), 2.08 (s, 2H, $-CH_2$), 3.58 (s, 2H, $-CH_2$), 4.56 (s, 2H, $-CH_2$), 6.29–7.35 (m, 7H, Ar–H), 8.27 (s, 1H, –NH), 8.76 (s, 1H, –NH). MS *m*/*z* 399 (M + 1). Anal. Calcd for $C_{21}H_{23}FN_4O_3$: C, 63.30; H, 5.82; N, 14.06. Found: C, 63.29; H, 5.82; N, 14.31.

3.1.10. 2-(4-(2-Fluorobenzyl)piperazin-1-yl)-N-*(3,4-dihydro-3-oxo-2*H-*benzo[b][1,4]oxazin-7-yl)acetamide (**4c**)*

Yield 52%; m.p. 185–186 °C. IR (KBr) cm^{-1}: 3462 (NH), 1695 (C═O), 1684 (C═O). ^1H NMR ($CDCl_3$) δ 2.59–2.65 (m, 8H, $-CH_2$), 3.56 (s, 2H, $-CH_2$), 3.77 (s, 2H, $-CH_2$), 4.60 (s, 2H, $-CH_2$), 7.08–7.66 (m, 7H, Ar–H), 8.76 (s, 1H, –NH), 9.11 (s, 1H, –NH). MS *m*/*z* 399 (M + 1). Anal. Calcd for $C_{21}H_{23}FN_4O_3$: C, 63.30; H, 5.82; N, 14.06. Found: C, 63.28; H, 5.81; N, 14.35.

*3.1.11. 2-(4-(Benzo[*d*][1,3]dioxol-5-yl-methyl)piperazin-1-yl)*-N-*(3,4-dihydro-3-oxo-2*H-*benzo[b][1,4]oxazin-7-yl)acetamide (**4d**)*

Yield 48%; m.p. 179–180 °C. IR (KBr) cm^{-1}: 3462 (NH), 1693 (C═O), 1680 (C═O). ^1H NMR ($CDCl_3$) δ 2.39–2.44 (m, 8H, $-CH_2$), 3.51 (s, 2H, $-CH_2$), 4.01 (s, 2H, $-CH_2$), 4.62 (s, 2H, $-CH_2$), 5.95 (s, 2H, $-CH_2$), 6.29–8.20 (m, 6H, Ar–H), 8.57 (s, 1H, –NH), 8.85 (s, 1H, –NH). MS *m*/*z* 425 (M + 1). Anal. Calcd for $C_{22}H_{24}N_4O_5$: C, 62.25; H, 5.70; N, 13.20. Found: C, 62.10; H, 5.71; N, 13.47.

3.1.12. 2-(4-(4-Methylbenzyl)piperazin-1-yl)-N-*(3,4-dihydro-3-oxo-2*H-*benzo[b][1,4]oxazin-7-yl)acetamide (**4e**)*

Yield 47%; m.p. 193–194 °C. IR (KBr) cm^{-1}: 3468 (NH), 1690 (C═O), 1685 (C═O). ^1H NMR ($CDCl_3$) δ 2.31–2.37 (m, 8H, $-CH_2$), 2.95 (s, 2H, $-CH_2$), 3.37 (s, 2H, $-CH_2$), 3.88 (s, 3H, $-CH_3$), 4.37 (s, 2H, $-CH_2$), 6.15–7.47 (m, 7H, Ar–H), 8.79 (s, 1H, –NH), 9.27 (s, 1H, –NH). MS *m*/*z* 395 (M + 1). Anal. Calcd for $C_{22}H_{26}N_4O_3$: C, 66.99; H, 6.64; N, 14.20. Found: C, 66.92; H, 6.66; N, 14.46.

3.1.13. 2-(4-(3-Methoxybenzyl)piperazin-1-yl)-N-*(3,4-dihydro-3-oxo-2*H-*benzo[b][1,4]oxazin-7-yl)acetamide (**4f**)*

Yield 51%; m.p. 182–183 °C. IR (KBr) cm^{-1}: 3463 (NH), 1695 (C═O), 1687 (C═O). ^1H NMR ($CDCl_3$) δ 2.61–2.67 (m, 8H, $-CH_2$), 3.19 (s, 2H, $-CH_2$), 3.55 (s, 2H, $-CH_2$), 3.95 (s, 3H, $-CH_3$), 4.62 (s, 2H, $-CH_2$), 6.35–7.58 (m, 7H, Ar–H), 8.85 (s, 1H, –NH), 9.11 (s, 1H, –NH). MS *m*/*z* 411 (M + 1). Anal. Calcd for $C_{22}H_{26}N_4O_4$: C, 64.37; H, 6.38; N, 13.65. Found: C, 64.42; H, 6.36; N, 13.45.

3.1.14. 2-(4-(4-Methoxybenzyl)piperazin-1-yl)-N-*(3,4-dihydro-3-oxo-2*H-*benzo[b][1,4]oxazin-7-yl)acetamide (**4g**)*

Yield 48%; m.p. 175–176 °C. IR (KBr) cm^{-1}: 3465 (NH), 1694 (C═O), 1688 (C═O). ^1H NMR ($CDCl_3$) δ 2.29–2.35 (m, 8H, $-CH_2$), 2.93 (s, 2H, $-CH_2$), 3.35 (s, 2H, $-CH_2$), 3.93 (s, 3H, $-CH_3$), 4.36 (s, 2H, $-CH_2$), 6.14–7.53 (m, 7H, Ar–H), 9.05 (s, 1H, –NH), 9.99 (s, 1H, –NH). MS *m*/*z* 411 (M + 1). Anal. Calcd for $C_{22}H_{26}N_4O_4$: C, 64.37; H, 6.38; N, 13.65. Found: C, 64.52; H, 6.31; N, 13.85.

3.1.15. 2-(4-(4-Chlorobenzyl)piperazin-1-yl)-N-*(3,4-dihydro-3-oxo-2*H-*benzo[b][1,4]oxazin-7-yl)acetamide (**4h**)*

Yield 47%; m.p. 174–176 °C. IR (KBr) cm^{-1}: 3466 (NH), 1698 (C═O), 1690 (C═O). ^1H NMR ($CDCl_3$) δ 2.31–2.36 (m, 8H, $-CH_2$), 3.39 (s, 2H, $-CH_2$), 3.93 (s, 2H, $-CH_2$), 5.19 (s, 2H, $-CH_2$), 6.14–7.56 (m, 7H, Ar–H), 8.76 (s, 1H, –NH), 8.89 (s, 1H, –NH). MS *m*/*z* 415 (M + 1). Anal. Calcd for $C_{21}H_{23}ClN_4O_3$: C, 60.79; H, 5.59; N, 13.50. Found: C, 60.92; H, 5.62; N, 13.75.

3.1.16. 2-(4-(3-Chlorobenzyl)piperazin-1-yl)-N-*(3,4-dihydro-3-oxo-2*H-*benzo[b][1,4]oxazin-7-yl)acetamide (**4i**)*

Yield 52%; m.p. 185–187 °C. IR (KBr) cm^{-1}: 3469 (NH), 1697 (C═O), 1691 (C═O). ^1H NMR ($CDCl_3$) δ 2.69–2.74 (m, 8H, $-CH_2$), 3.50 (s, 2H, $-CH_2$), 3.94 (s, 2H, $-CH_2$), 4.36 (s, 2H, $-CH_2$), 7.08–7.74 (m, 7H, Ar–H), 9.48 (s, 1H, –NH), 10.01 (s, 1H, –NH). MS *m*/*z* 415 (M + 1). Anal. Calcd for $C_{21}H_{23}ClN_4O_3$: C, 60.79; H, 5.59; N, 13.50. Found: C, 60.82; H, 5.64; N, 13.85.

3.1.17. 2-(4-(2-Bromobenzyl)piperazin-1-yl)-N-*(3,4-dihydro-3-oxo-2*H-*benzo[b][1,4]oxazin-7-yl)acetamide (**4j**)*

Yield 51%; m.p. 164–166 °C. IR (KBr) cm^{-1}: 3464 (NH), 1696 (C═O), 1687 (C═O). ^1H NMR ($CDCl_3$) δ 2.48–2.53 (s, 8H, $-CH_2$), 3.11 (s, 2H, $-CH_2$), 3.54 (s, 2H, $-CH_2$), 4.42 (s, 2H, $-CH_2$), 6.14–7.59 (m, 7H, Ar–H), 8.38 (s, 1H, –NH), 9.03 (s, 1H, –NH). MS *m*/*z* 459 (M + 1). Anal. Calcd for $C_{21}H_{23}BrN_4O_3$: C, 54.91; H, 5.05; N, 12.20. Found: C, 55.16; H, 5.04; N, 12.45.

3.1.18. 2-(4-(2,6-Dichlorobenzyl)piperazin-1-yl)-N-*(3,4-dihydro-3-oxo-2*H-*benzo[b][1,4]oxazin-7-yl)acetamide (**4k**)*

Yield 45%; m.p. 182–184 °C. IR (KBr) cm^{-1}: 3463 (NH), 1696 (C═O), 1688 (C═O). ^1H NMR ($CDCl_3$) δ 2.45–2.50 (s, 8H, $-CH_2$), 2.99 (s, 2H, $-CH_2$), 3.67 (s, 2H, $-CH_2$), 4.58 (s, 2H, $-CH_2$), 6.57–7.78 (m, 6H, Ar–H), 8.65 (s, 1H, –NH), 9.12 (s, 1H, –NH). MS *m*/*z* 449 (M + 1). Anal. Calcd for $C_{21}H_{23}Cl_2N_4O_3$: C, 56.13; H, 4.94; N, 12.47. Found: C, 56.22; H, 4.91; N, 12.65.

3.2. Evaluation of positive inotropic effects in vitro

The method of measuring left atrium stroke volume was adopted for the biological evaluation of the compounds synthesized 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 pressure maximum alleosis. 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.

3.2.1. Drugs and chemicals

The following drugs and chemicals were used in this biological evaluation test: milrinone (Shuzhou Unite Pharmaceutical Co., Dongwu Road, Shuzhou), DMSO (Sigma–Aldrich Chemical Co., St. Louis, MO). All other reagents were of analytical grade.

3.2.2. Methods

The experiments were carried out in an isolated, perfused atrial preparation that was prepared by using the method described previously [13,14]. Thus, the atrium was perfused with *N*-2-hydroxyethyl piperazine-*N*′-2-ethanesulfonic acid (HEPES) buffer solution by means of a peristaltic pump (1.25 mL/min) [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 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 intervals, 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 a concentration of 1×10^{-5} M. Samples were dissolved in DMSO and diluted with the HEPES buffer to an appropriate volume. The biological evaluation data for these compounds were expressed as 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 mean ± SE.

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