Bioorganic & Medicinal Chemistry Letters 22 (2012) 4229–4232

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and positive inotropic evaluation of *N*-(1-oxo-1,2,4,5-tetrahydro-[1,2,4]triazolo[4,3-*a*]quinolin-7-yl)acetamides bearing piperazine and 1,4-diazepane moieties

Yan Wu [a], Long-Xu Ma [a], Tian-Wei Niu [a], Xun Cui [b,*], Hu-Ri Piao [a,*]

[a] *Key Laboratory of Natural Resources and Functional Molecules of the Changbai Mountain, Affiliated Ministry of Education, Yanbian University College of Pharmacy, Yanji 133000, PR China*
[b] *Yanbian University College of Medicine, Yanji 133002, PR China*

ARTICLE INFO

Article history:
Received 21 February 2012
Revised 19 April 2012
Accepted 11 May 2012
Available online 18 May 2012

Keywords:
[1,2,4]Triazolo[4,3-*a*]quinolin-1(2*H*)-one
Positive inotropic activity
Stroke volume

ABSTRACT

Two series of *N*-(1-oxo-1,2,4,5-tetrahydro-[1,2,4]triazolo[4,3-*a*]quinolin-7-yl)acetamides bearing piperazine and 1,4-diazepane moieties were synthesized and screened for their positive inotropic activity by measuring left atrium stroke volume on isolated rabbit heart preparations. Most of the derivatives exhibited better in vitro positive inotropic activity than the existing drug, milrinone, among which 2-(4-(4-chlorobenzyl)-1,4-diazepan-1-yl)-*N*-(1-oxo-1,2,4,5-tetrahydro-[1,2,4]triazolo[4,3-*a*]quinolin-7-yl)acetamide **6c** proved to be the most potent with 15.48 ± 0.27% increased stroke volume (milrinone: 2.46 ± 0.07%) at a concentration of 3×10^{-5} M. The chronotropic effects of the compounds that exhibited inotropic effects were also evaluated.

Therapeutic treatment of cardiac failure (CHF) includes, among other measures, the use of cardiac glycosides such as digoxin or digitalis compounds.[1–3] The narrow safety margin of digitalis compounds has until now represented a serious problem due to the high frequency and severity of digitalis intoxication.[4,5] The discovery of amrinone led to the synthesis of a number of promising non-sympathomimetic and non-glycoside agents for the treatment of CHF.[6] The phosphodiesterase-inhibiting agent milrinone (Fig. 1), which has both vasodilator and inotropic properties, was approved for the treatment of CHF more than one decade ago. Nonetheless, the significant ventricular arrhythmias and tachycardia associated with the elevated cAMP level also limit its clinical use.[7] Similar issues have been recognized for the recently developed vesnarinone[8,9] and toborinone.[10,11] Therefore, the identification of novel positive inotropic agents, which not only improve the quality of life but also reduce the mortality of CHF patients, is still an important challenge for medicinal chemists.[12]

For several years, our laboratory has been pursuing intensive work to identify more potential positive inotropic agents with fewer side effects. A series of 3,4-dihydro-2(*1H*)-quinolinone derivatives were synthesized and tested for their biological activity. Among these, the compound 2-(4-(4-(benzyloxy)-3-methylbenzyl)piperazin-1-yl)-*N*-(1-methyl-4,5-dihydro-[1,2,4]triazolo[4,3-*a*] quinolin-7-yl)acetamide PHR0007 (Fig. 1) showed the most potent positive inotropic activity,[13] which it displays through phosphodiesterase III inhibition.[14] In this study, to further optimize PHR0007, we kept the [1,2,4]triazolo[4,3-*a*]quinoline moiety unchanged, replaced the methyl group at the 1-position of the triazole ring with a carbonyl group, and changed the substituents on the piperazine ring at the 4-position. Replacement of the piperazine ring with a 1,4-diazepan ring was also considered in order to investigate the contribution of such a structural change on biological activity. All newly synthesized compounds were characterized by IR, ^{1}H

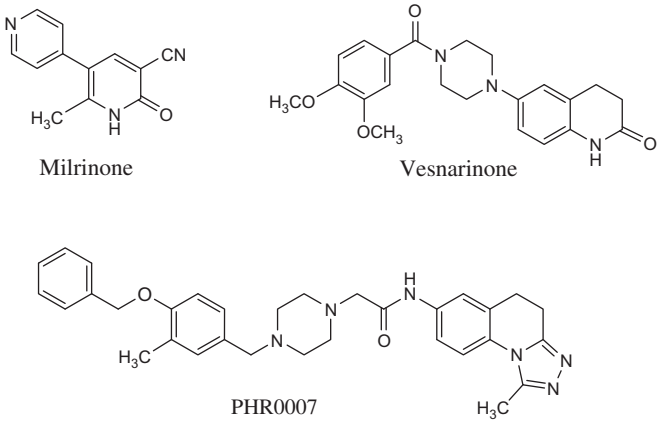
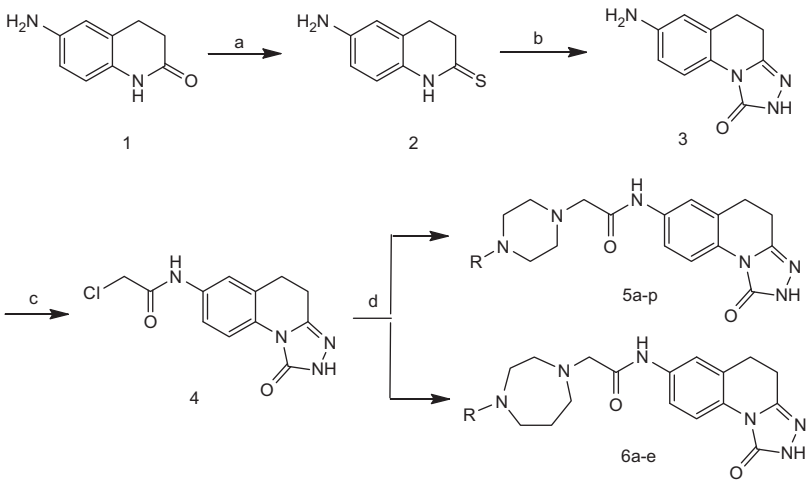


Figure 1. Cardiotonic agents used for the treatment of congestive heart failure (CHF) and the previously reported compound PHR0007.

* Corresponding authors. Tel.: +86 433 2435132; fax: + 86 433 2435004 (X.C.); tel.: +86 433 2435003; fax: + 86 433 2435004 (H.-R. P.).
E-mail addresses: cuixun@ybu.edu.cn (X. Cui), piaohuri@yahoo.com.cn (H.-R. Piao).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2012.05.049



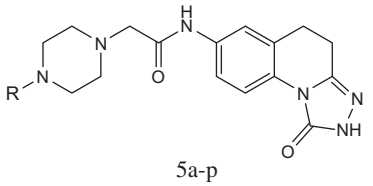
Scheme 1. Synthetic scheme for the synthesis of compound **5a–p** and **6a–e**. Reagents and conditions: (a) P_2S_5, Et_3N, CH_3CN; (b) Ethyl carbazate, cyclohexanol, reflux, N_2, 6 h; (c) $ClCH_2COCl$, CH_2Cl_2, rt; (d) monosubstituted piperazines or 1,4-diazepanes, K_2CO_3, acetone, reflux, 10 h.

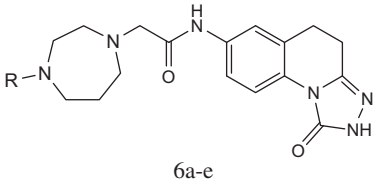
NMR, ^{13}C NMR, MS and elemental analysis, and their positive inotropic activities were evaluated by measuring their effects on left atrium stroke volume in isolated rabbit heart preparations.

Compounds **5a–p**, **6a–e** were synthesized by the route outlined in Scheme 1, and their structures and inotropic activities are displayed in Table 1.[15] Compound **1** was synthesized according to previously described methods.[16] Sulfurization of 6-amino-3,4-dihydroquinolin-2(1*H*)-one **1** with phosphorous pentasulfide in refluxing acetonitrile in the presence of triethylamine gave the corresponding thione **2**. Cyclization of **2** with ethyl carbazate in refluxing cyclohexanol under nitrogen atmosphere afforded the desired triazolone compound **3** in moderate yield,[17] followed by the acylation of the amino group with 2-chloroacetyl chloride in dichloromethane at room temperature to provide the corresponding amide **4**. Nucleophilic-substitution of **4** with various monosubstituted piperazines and 1, 4-diazepans in refluxing acetone in the presence of potassium carbonate afforded compounds **5a–p** and **6a–e** in high yields.[18]

As shown in Table 1, most (15) of the 21 compounds tested displayed inotropic effects on isolated rabbit heart preparations, of which 11 compounds exhibited more potent effects than milrinone ($2.46 \pm 0.07\%$, 3×10^{-5} M). For **5a–m**, chlorinated compounds **5d**, **5e**, and **5f** showed good activity, but the dichloro-substituted derivative **5k** showed no potency. Among the bromo- and fluoro-substituted compounds, only one compound (2-fluorinated **5a**,

Table 1
Positive inotropic activity of the test compounds


5a-p


6a-e

Compound	R	Increased stroke volume[a] (%)
5a	$-CH_2C_6H_4$(*o*-F)	1.82 ± 0.01
5b	$-CH_2C_6H_4$(*m*-F)	–[b]
5c	$-CH_2C_6H_4$(*p*-F)	–
5d	$-CH_2C_6H_4$(*o*-Cl)	4.01 ± 0.10
5e	$-CH_2C_6H_4$(*m*-Cl)	3.46 ± 0.03
5f	$-CH_2C_6H_4$(*p*-Cl)	3.09 ± 0.06
5g	$-CH_2C_6H_4$(*o*-Br)	–
5h	$-CH_2C_6H_4$(*m*-Br)	–
5i	$-CH_2C_6H_4$(*p*-Br)	–
5j	$-CH_2C_6H_5$	3.56 ± 0.05
5k	$-CH_2C_6H_3$(2,6-Cl_2)	–
5l	$-CH_2C_6H_4$(*p*-CH_3)	5.48 ± 0.07
5m	$-CH_2C_6H_4$(*p*-OCH_3)	1.46 ± 0.04
5n	$-CH_2CH{=}CHC_6H_5$	9.40 ± 0.12
5o	$-CH_2C_6H_3$(4-C_6H_4(4-Cl)CH_2–, 3-OCH_3)	2.22 ± 0.02
5p	$-CH_2C_6H_3$(4-$C_6H_5CH_2$-, 3-OCH_3)	2.26 ± 0.01
6a	$-CH_2C_6H_4$(*o*-Cl)	8.98 ± 0.07
6b	$-CH_2C_6H_4$(*m*-Cl)	6.41 ± 0.08
6c	$-CH_2C_6H_4$(*p*-Cl)	15.48 ± 0.27
6d	$-CH_2C_6H_5$	9.07 ± 0.16
6e	$-CH_2C_6H_4$(*p*-CH_3)	6.75 ± 0.07
Milrinone		2.46 ± 0.04

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

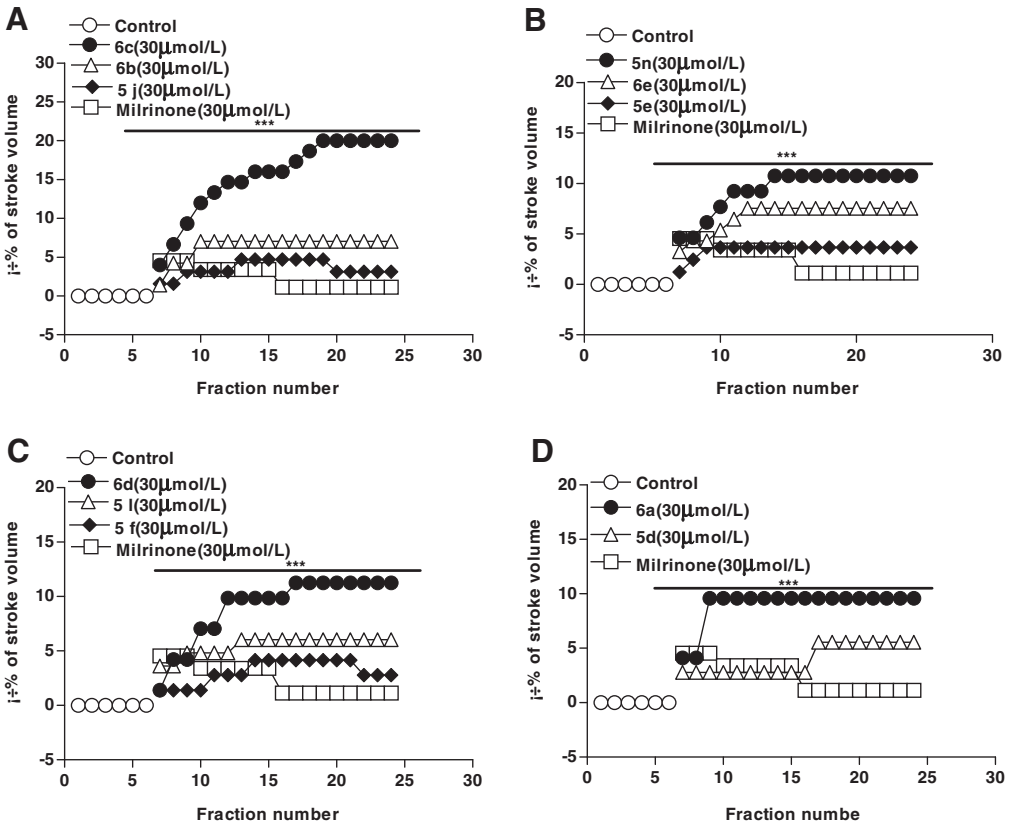


Figure 2. Effects of milrinone and compounds **5d**, **5e**, **5f**, **5j**, **5l**, **5n**, **6a**, **6b**, **6c 6d** and **6e** on stroke volume in beating rabbit atria (1.5 Hz). Values are means ± SE. ***P <0.001 versus control.

1.82 ± 0.01%) exhibited weak activity. Unsubstituted and *para*-methyl substituted derivatives (**5j**, 3.56 ± 0.05%; **5l**, 5.48 ± 0.07%) exhibited more potent activity than milrinone, while *para*-methoxy substituted derivative **5m** exhibited slightly weaker activity with 1.46 ± 0.04% increased stroke volume. The compounds **5o** and **5p** bearing a 4-benzyloxy-3-methoxybenzyl group at the 4-position of the piperazine ring displayed moderate activity, with 2.22 ± 0.02% and 2.26 ± 0.01% increased stroke volume, respectively. Furthermore, the cinnamyl substituted derivative **5n** (9.40 ± 0.12%) presented the most favorable activity in this series, which might be attributed to hydrophobic interactions of cinnamyl moiety with conjugate structure. The series of derivatives **6a–e**, bearing 4-substituted 1,4-diazepanes, displayed higher potency than the piperazine series. In particular, the 4-chlorinated derivative **6c** showed the most potent activity in this study by a 15.48 ± 0.27% increased stroke volume, which was 5-fold more potent than **5f** in the piperazine series. Similar results were obtained for the inotropic activity of **6a**, **6b**, **6d** and **6e**, in which 2-chlorinated **6a** (8.98 ± 0.07%) was 2.3-fold more potent, 3-chlorinated **6b** (6.41 ± 0.08%) was 1.8-fold more potent, unsubstituted **6d** (9.07 ± 0.16%) was 2.6-fold more potent, and *para*-methyl **6e** (6.75 ± 0.07%) was 1.2-fold more potent than the corresponding compounds in the piperazine series.

Based on these facts, some preliminary remarks on structure–activity relationship can be drawn from the results of bioactivities. For the series **5** and **6**, the latter showed much more stronger activity, generally. The results obtained indicated that the 1,4-diazepan ring was important for the activity of these compounds, for which further investigation should be considered. For the series **5**, it could be found that different substituent groups on the phenyl ring of the benzyl group at the 4-position of the piperazine have different effect on activity. Herein some halogen substituted derivatives (**5a–i** and

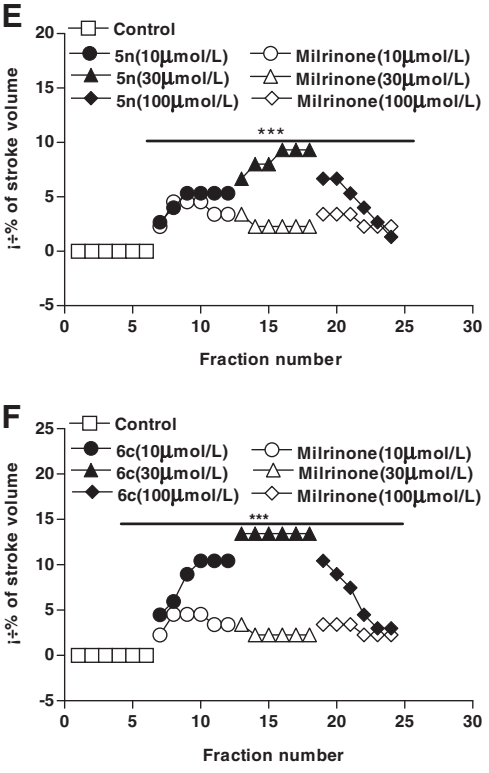


Figure 3. Concentration-response curves of compounds **5n**, **6c** and milrinone on stroke volume in beating rabbit artia (1.5 Hz). Values are means ± SE. ***P <0.001 versus control.

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

Compound	Mean ± SE[a]	Mean ± SE[b]
5n	99.97 ± 0.04	99.89 ± 0.05
6a	118.73 ± 0.07	118.36 ± 0.10
6b	109.86 ± 0.12	120.84 ± 0.08
6c	120.67 ± 0.12	121.68 ± 0.09
6d	99.52 ± 0.09	90.01 ± 0.16[c]
6e	111.36 ± 0.18	111.86 ± 0.16

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

5k) were designed and synthesized, different halogen atoms contributed to the activity in the order of Cl > F > Br, generally. Comparing the derivatives with different Cl-substitution positions on the benzyl ring, their activity order was *o*-Cl > *m*-Cl > *p*-Cl > *2, 6*-2Cl. The electron-donor derivatives were also designed and prepared, containing CH_3 and OCH_3. Their activity order was *p*-CH_3 > *p*-OCH_3. For the series **6**, comparing the derivatives with different substitutents on the benzyl ring, their activity order was *p*-Cl > H > *o*-Cl > *m*-Cl > *p*-CH_3. From these results, we can see that the effects of the substituent's position on the benzene ring to the biological activity showed no clear regularity, for which more this kind of compounds need to be designed and synthesized for further investigation.

The dynamics of the test compounds were also investigated in perfused beating rabbit atria. We found that compounds **5j** and **5f** (Fig. 2 A and C) did not show a desirable biological dynamic profile, because they decreased the stroke volume with time. Nine compounds possessing inotropic effects (**5d**, **5e**, **5l**, **5n**, **6a**, **6b**, **6c**, **6d**, and **6e**) showed desirable biological dynamic profiles compared to milrinone (Fig. 2 A, B, C and D), of which compound **6c** displayed excellent inotropic effects with the highest increased stroke volume (Fig. 2 A).

We next tested the dose–activity relationships of the most effective compounds, **5n** and **6c**, at concentrations of 1×10^{-5}, 3×10^{-5} and 1×10^{-4} M. Both compounds showed maximal effects at 3×10^{-5} M, and less activity at the higher dose of 1×10^{-4} M, as shown in Fig. 3 E and F. Compounds **5n**, **6a**, **6b**, **6c**, **6d**, and **6e** were also investigated for their chronotropic effects in beating atria. As shown in Table 2, no significantly increased heart rates (P >0.05) were observed for **5n**, **6a**, **6b**, **6c** and **6e** at the same concentration. Unfortunately, however, heart rate was changed by compound **6d**.

In summary, we report herein the design and synthesis of novel *N*-(1-oxo-1,2,4,5-tetrahydro-[1,2,4]triazolo[4,3-*a*]quinolin-7-yl)acetamides, and demonstrate their positive inotropic activities. The compounds were based on the structure of compound PHR0007, modified to bear 4-substituted piperazine and 1,4-diazepane moieties. Among these compounds, **5n** and **6c** in particular exhibited promising cardiovascular properties and potent activities compared with milrinone. The results suggested that their favourable activity was due to the 1,4-diazepane ring in the 4,5-dihydro-[1,2,4]triazolo[4,3-*a*]quinolin-1(2*H*)-one scaffold rather than the piperazine ring, and further development of such compounds may be of interest. Other biological tests, including in vivo evaluation, coronary vasodilating tests and studies on the possible mechanism of action, are currently ongoing in our laboratory.

Acknowledgment

This work was supported by the National Science Foundation of China (81160381).

References and notes

1. Thomas, R.; Gray, P.; Andrews, J. *Adv. Drug Res.* **1990**, *19*, 313.
2. Smith, J. R.; Gheorghiade, M.; Goldstein, S. *Coronary Artery Dis.* **1993**, *4*, 16.
3. Smith, T. W. N. *Engl. J. Med.* **1988**, *318*, 358.
4. Becker, D. E. *Anesth Prog.* **2007**, *54*, 178.
5. Hallberg, P.; Lindbäck, J.; Lindahl, B.; Stenestrand, U.; Melhus, H. *Eur. J. Clin. Pharmacol.* **2007**, *63*, 959.
6. Takafumi, F.; Shuji, T.; Toyoki, M.; Tetsumi, H.; Takumi, S.; Michiaki, T.; Youichi, Y. *J. Med. Chem.* **1992**, *35*, 3607.
7. Packer, M.; Carver, J. R.; Rodeheffer, R. J.; Ivanhoe, R. J.; DiBianco, R.; Zeldis, S. M.; Hendrix, G. H.; Bommer, W. J.; Elkayam, U.; Kukin, M. L. N. *Engl. J. Med.* **1991**, *325*, 1468.
8. Cohn, J. N.; Goldstein, S. O.; Greenberg, B. H.; Lorell, B. H.; Bourge, R. C.; Jaski, B. E.; Gottlieb, S. O.; McGrew, F.; DeMets, D. L.; White, B. G. *N Engl. J. Med.* **1810**, *1998*, 339.
9. Nikpour, M.; Sadeghian, H.; Saberi, M. R.; Nick, R. S.; Seyedi, S. M.; Hosseini, A.; Parsaee, H.; Bozorg, A. T. *Bioorg. Med. Chem.* **2010**, *18*, 855.
10. Fedman, M. D.; Pak, P. H.; Wu, C. C.; Haber, H. L.; Heesch, C. M.; Bergin, J. D.; Powers, E. R.; Cowart, T. D.; Ohnson, W.; Feldman, A. M.; Kass, D. A. *Circulation* **1996**, *93*, 474.
11. Macgowan, G. A. *Expert Opin. Invest. Drugs* **2000**, *9*, 1109.
12. Neubauer, S. N. *Eng. J. Med.* **2007**, *356*, 1140.
13. Zhang, C. B.; Cui, X.; Hong, L.; Quan, Z. S.; Piao, H. R. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4606.
14. Lan, Y.; Piao, H. R.; Cui, X. *Drug Disc. Ther.* **2009**, *6*, 272.
15. The method of measuring left atrium stroke volume was described previously.[19,20] The features of CHF are cardiac dilatation, poor contractility of cardiac muscle, decreased ejection fraction, and depression of left ventricular pressure maximum alleosis. 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 (Shuzhou Unite Pharmaceutical Co., Dongwu Road, Shuzhou), DMSO (Sigma–Aldrich Chemical Co., St. Louis, MO, USA) were used. All other reagents were of analytical gradused. Atria were obtained from New Zealand white rabbits, and the mean atrial weight was 183.6 ± 6.8 mg. 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-hydroxyethyl piperazine-*N*-2-ethanesulfonic acid (HEPES) buffer solution by means of a peristaltic pump (1.25 mL/min) at 34 °C.[21] 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 3×10^{-5} M. Samples were dissolved in DMSO and diluted with the HEPES buffer to 0.1% 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.
16. Yang, K.; Sun, L. P.; Liu, J. Y.; Cui, X.; Hong, L.; Piao, H. R. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4464.
17. Zhang, C. B.; Piao, H. R.; Quan, Z. S. *Chem. Res. Appl.* **2002**, *5*, 618.
18. *Preparation of* ***6c***: A mixture of 4 (224 mg, 1.0 mmol), 1-(4-chlorobenzyl)-1,4-diazepane (278 mg, 1.0 mmol), K_2CO_3 (54 mg, 0.5 mmol) in acetone (10 ml) was stirred under reflux for 6 h and concentrated under reduced pressure. The resulting residue was purified by chromatography (CH_2Cl_2/CH_3OH 30:1) to afford **6c** (428 mg, 92%) as white solid. Mp: 160–162 °C; IR (KBr) cm^{-1}: 3276 (NH), 1716, 1687 (C=O) ; ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): 1.77–1.81 (m, 2H, CH_2), 2.66–2.75 (m, 8H, CH_2), 2.79 (t, J = 6.5 Hz, 2H, CH_2), 2.83 (t, J = 6.5 Hz, 2H, CH_2), 3.22 (s, 2H, CH_2), 3.57 (s, 2H, CH_2), 7.17–8.25 (m, 7H, Ar-H), 9.30 (s, 1H, NH), 9.67 (s, 1H, NH). ^{13}C NMR ($CDCl_3$, 75 MHz , ppm): δ 168.00, 151.62, 143.18, 136.70, 133.99, 131.24, 128.72, 127.53, 127.07, 125.86, 118.17, 117.14, 116.33, 76.06, 75.64, 75.22, 60.83, 60.70, 54.89, 53.77, 53.62, 52.85, 26.67, 24.88, 20.30; ESI-MS (m/z): 467 $[M+H]^+$; Anal. Calcd for $C_{25}H_{27}ClN_6O_2$: C, 61.73; H, 5.83; N, 18.00. Found: C, 61.66; H, 5.93; N, 18.12.
19. Cho, K. W.; Kim, S. H.; Kim, C. H.; Seul, K. H. *Am. J. Physiol.* **1995**, *268*, 1129.
20. Lee, S. J.; Kim, S. Z.; Cui, X.; Kim, S. H.; Lee, K. S.; Chung, Y. J.; Cho, K. W. *Am. J. Physiol. Heart Circ. Physiol.* **2000**, *278*, H208.
21. Cui, X.; Wen, J. F.; Jin, J. Y.; Xu, W. X.; Kim, S. Z.; Kim, S. H.; Lee, H. S.; Cho, K. W. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2002**, *282*, R1477.