Received Date : 08-Jun-2016

Revised Date : 15-Jul-2016

Accepted Date : 21-Jul-2016

Article type : Research Article

Design, synthesis, and negative inotropic evaluation of 4-phenyl-1*H*-1,2,4-triazol-5(4*H*)-one derivatives containing triazole or piperazine moieties

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/cbdd.12828

Abstract

In the present study, four novel series of 4-phenyl-1*H*-1,2,4-triazol-5(4*H*)-one derivatives containing triazole or piperazine moieties were designed, synthesized and evaluated for negative inotropic activity by measuring the left atrium stroke volume in isolated rabbit heart preparations. Almost all of the compounds showed an ability to moderate the cardiac workload by decreasing the heart rate and contractility. Among them, **7h** was found to be the most potent with a change in stroke volume of −48.22 ± 0.36% at a concentration of 3×10^{-5} mol/L (metoprolol: −9.74 ± 0.14%). The cytotoxicity of these compounds was evaluated using the human cervical cancer cell line HeLa, the liver cancer cell line Hep3B and the human normal hepatic cell line LO2. A preliminary study of the mechanism of action for the compound **7h** on the regulation of atrial dynamics with ATP-sensitive K^+ channel and L-type Ca^{2+} channel blockers glibenclamide and nifedipine was performed in the isolated perfused beating rabbit atria.

Keywords: [1,2,4]triazol-5(4*H*)-one; triazole; piperazine; negative inotropic activity; stroke volume.

Congestive heart failure (CHF) is a major human health problem and a leading cause of death worldwide (1). Inotropic drugs alter the force or strength of the heart's muscular contractions and can be divided into two different types: negative and positive. Negative inotropic drugs make the heart beat less strongly and positive inotropic drugs make the heart beat more strongly. Both types of drug are used in the management of various conditions of congestive heart failure (2,3). Cardiac glycosides, such as digoxin, have been traditionally used as positive inotropic drugs (4,5). However, there are serious problems with digoxin use because of the frequency and severity of digitalis intoxication (6,7). The clinical use of the phosphodiesterase-inhibiting agent milrinone is also limited because of significant ventricular arrhythmias and tachycardia associated with elevated cAMP levels

caused by milrinone (8). Similar issues have been identified with the recently developed drugs, vesnarinone and toborinone (9,10). In the past decade, the treatment of heart failure (HF) has undergone a fundamental shift, from treatment using short-term, hemodynamic pharmacological measures to a long-term, corrective maintenance strategy aimed at changing the biological properties of the diseased heart. The negative inotropic drug metoprolol, once used as contraindication in patients of heart failure due to their negative inotropic activity, has become a conventional treatment for HF, based on the biological effects of long-term treatment (11-13). Therefore, the development of innovative positive and negative inotropic agents with improved therapeutic properties and fewer side effects for the treatment of CHF is still a great challenge for medicinal chemists (14).

In our previous work searching for lead compounds with inotropic activities, we found that compounds **A** and **B** (Figure 1) showed weak negative inotropic activity (compound **A**: −24.70%) (15,16). In a summary of our previous work (17,18), we hypothesized that the triazole and piperazine rings were essential to the observed higher levels of affinity for the receptor and consequently enhanced the inotropic activity. Considering that triazoles and their derivatives have attracted significant attention for their various biological activities, including anti-inflammatory, analgesic, hypotensive, and their effect on diastolic blood vessels (19-21), and several medicines used in the treatment of HF contain a piperazine moiety (22,23). In an attempt to search for more potent inotropic agents, we kept benzylpiperazine moiety unchanged and used 4-phenyl-1*H*-1,2,4-triazol-5(4*H*)-one moiety to take the place of 4,5-dihydro-[1,2,4]triazolo[4,3-a]quinolin-1(2*H*)-one. Then introduced a triazole ring to replace the piperazine ring with 4-phenyl-1*H*-1,2,4-triazol-5(4*H*)-one moiety unchanged to further optimize lead compound **A**. Thus, we designed and synthesized four novel series of 4-phenyl-1*H*-1,2,4-triazol-5(4*H*)-one derivatives containing triazole and piperazine moieties (Figure 1). In addition, we simultaneously changed the substituents on the phenyl ring to investigate the contribution of such a structural change on biological activity. The negative inotropic activities of the synthesized compounds were evaluated by measuring the left atrium stroke volume in isolated rabbit heart preparations. Furthermore,

to preliminary investigate the mechanism of action on the regulation of atrial dynamics, another series experiments were performed with ATP-sensitive K^+ channel and L-type Ca^{2+} channel blockers glibenclamide and nifedipine in the isolated perfused beating rabbit atria. What's more, compounds were also evaluated for their cytotoxicity against the human cervical cancer cell line HeLa and liver cancer cell line Hep3B, as indicated by the half inhibitory concentration (IC_{50}) values of the tested compounds according to the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-*H*-tetrazolium bromide (MTT) assay.

Experimental Section

Material and methods

All commercially available reagents were obtained from Sigma–Aldrich (Saint Louis, MO, USA) and Fluka (Milwaukee, WI, USA). Melting points were determined in open capillary tubes and are uncorrected. Reaction courses were monitored by TLC on silica gel precoated F254 Merck plates and the developed plates were examined under UV lamps (254 nm). IR spectra were recorded (in KBr) on a FT-IR1730. Column chromatography was undertaken with 200-mesh silica gel (Merck). ^{1}H NMR and ^{13}C NMR spectra were measured on Bruker AV-300 spectrometer at 300 MHz and 75 MHz respectively used tetramethylsilane (TMS) as an internal standard. Chemical shifts were expressed in δ, ppm. Mass spectra were measured on an HP1100LC (Agilent Technologies, USA). High resolution mass spectroscopy (HRMS) was measured on a Bruker ultrafleXtreme MALDI-TOF/TOF.

General procedure for synthesis of compounds 4I-4III

A mixture of aniline **1I** (0.93g, 10 mmol) or **1II** (10 mmol) or **1III** (10 mmol), triethyl orthoformate **2** (2.22g, 15 mmol) and methyl hydrazinecarboxylate **3** (1.35g, 15 mmol) in refluxing ethanol (50mL) at 78 °C was stirred for 24 h. sodium methoxide (0.81g, 15 mmol) was added to the mixture and the resulting mixture was stirred for another 24 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 to afford intermediates **4I**-**III** in 85%-90% yields.

General procedure for synthesis of compounds 5I-5III

The key intermediates **5I**-**5III** were synthesized by reacting intermediates **4I** (1.61g, 10 mmol) or **4II** (10 mmol) or **4III** (10 mmol) with 1,3-dibromopropane (2.02g, 10 mmol) in the presence of potassium carbonate and potassium iodide in refluxing acetone (50mL) at 57 °C for 6 h. The solvent was evaporated with reduced pressure and the residue was dissolved in dichloromethane, washed with water and brine, and dried by $MgSO_4$. A white solid was collected through filtration and dried in a vacuum to give the compounds **5I**-**III** in 80%-85% yields.

General procedure for synthesis of compounds 6a-i and 7a-i

Just as outlined in Scheme 1, nucleophilic substitution of **5I** (10 mmol) or **5II** (10 mmol) with various monosubstituted piperazines (10 mmol) in refluxing acetone (20mL) at 57 °C in the presence of KI/K_2CO_3 afforded **6a**-**i** and **7a**-**i** in 40%-60% yields. The solution was evaporated to dryness under reduced pressure, and the residue was purified by silica gel column chromatography with dichloromethane: methanol (200:1). The yield, melting point and spectra data of each compound are given below.

1-(3-(4-Benzylpiperazin-1-yl)propyl)-4-phenyl-1H-1,2,4-triazol-5(4H)-one (6a)

Yield: 42%; m.p. 124–126°C. IR (KBr) cm^{-1}: 1685 (C=O). ^1H NMR ($CDCl_3$, 300 MHz, ppm): δ 7.70 (s, 1H, N=CH), 7.57-7.46 (m, 4H, Ar-H), 7.40-7.25 (m, 6H, Ar-H), 3.92 (t, J = 6.9 Hz, 2H, CH_2), 3.51 (s, 2H, CH_2), 2.68-2.32 (m, 10H, $(CH_2)_5$), 2.01 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 151.75, 138.17, 134.01, 133.45, 129.58, 129.58, 129.19, 129.19, 128.18,

128.18, 127.45, 126.99, 121.83, 121.83, 63.06, 55.53, 53.17, 53.17, 53.12, 53.12, 44.05, 26.04. HRMS (MALDI) calcd for $C_{22}H_{28}N_5O$ $(M + H)^+$: 378.2288, found: 378.2289.

1-(3-(4-(2-Chlorobenzyl)piperazin-1-yl)propyl)-4-phenyl-1H-1,2,4-triazol-5(4H)-one (6b)

Yield: 58%; m.p. 120–122°C. IR (KBr) cm^{-1}: 1689 (C=O). ^1H NMR ($CDCl_3$, 300 MHz, ppm): δ 7.70 (s, 1H, N=CH), 7.61-7.48 (m, 5H, Ar-H), 7.41-7.14 (m, 4H, Ar-H), 3.93 (t, J = 6.9 Hz, 2H, CH_2), 3.62 (s, 2H, CH_2), 2.54-2.44 (m, 10H, $(CH_2)_5$), 2.08-1.94 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 151.68, 146.53, 136.75, 134.72, 132.76, 130.49, 128.98, 128.98, 128.68, 128.34, 128.31, 128.31, 127.96, 126.87, 58.27, 58.27, 57.46, 55.30, 55.30, 51.24, 40.89, 22.19. HRMS (MALDI) calcd for $C_{22}H_{27}ClN_5O$ $(M + H)^+$: 412.1899, found: 412.1906.

1-(3-(4-(3-Chlorobenzyl)piperazin-1-yl)propyl)-4-phenyl-1H-1,2,4-triazol-5(4H)-one (6c)

Yield: 47%; m.p. 116–118°C. IR (KBr) cm^{-1}: 1685 (C=O). ^1H NMR ($CDCl_3$, 300 MHz, ppm): δ 7.70 (s, 1H, N=CH), 7.59-7.47 (m, 4H, Ar-H), 7.40-7.20 (m, 5H, Ar-H), 3.91 (t, J = 6.9 Hz, 2H, CH_2), 3.44 (s, 2H, CH_2), 2.68-2.29 (m, 10H, $(CH_2)_5$), 2.04-1.92 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 150.70, 146.62, 136.87, 134.83, 132.64, 132.52, 128.45, 128.45, 127.90, 127.90, 127.72, 126.96, 126.88, 126.06, 65.26, 58.21, 58.21, 55.01, 55.01, 51.40, 40.84, 22.59. HRMS (MALDI) calcd for $C_{22}H_{27}ClN_5O$ $(M + H)^+$: 412.1899, found: 412.1892.

1-(3-(4-(4-Chlorobenzyl)piperazin-1-yl)propyl)-4-phenyl-1H-1,2,4-triazol-5(4H)-one (6d)

Yield: 52%; m.p. 159–161°C. IR (KBr) cm^{-1}: 1674 (C=O). ^1H NMR ($CDCl_3$, 300 MHz, ppm): δ 7.69 (s, 1H, N=CH), 7.55-7.47 (m, 4H, Ar-H), 7.36-7.21 (m, 5H, Ar-H), 3.91 (t, J = 6.9 Hz, 2H, CH_2), 3.45 (s, 2H, CH_2), 2.66-2.17 (m, 10H, $(CH_2)_5$), 2.05-1.91 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 150.68, 146.53, 136.75, 132.72, 132.49, 130.48, 130.48, 128.94, 128.94, 128.31, 128.31, 128.10, 128.10, 127.72, 65.34, 58.27, 58.27, 55.30, 55.30, 51.24, 40.89, 22.19. HRMS (MALDI) calcd for $C_{22}H_{27}ClN_5O$ $(M + H)^+$: 412.1899, found: 412.1901.

2-((4-(3-(5-Oxo-4-phenyl-4,5-dihydro-1H-1,2,4-triazol-1-yl)propyl)piperazin-1-yl)methyl)benzonitrile (6e)

Yield: 50%; m.p. 118–120°C. IR (KBr) cm^{-1}: 1683 (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): *δ* 7.70 (s, 1H, N=CH), 7.65 (m, 1H, Ar-H), 7.62-7.38 (m, 8H, Ar-H), 3.93 (t, *J* = 6.8 Hz, 2H, CH_2), 3.70 (s, 2H, CH_2), 2.53-2.45 (m, 10H, $(CH_2)_5$), 2.12-1.92 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): *δ* 151.76, 142.42, 133.98, 133.45, 132.90, 132.47, 129.99, 129.59, 129.59, 127.48, 127.46, 121.84, 121.84, 117.79, 112.96, 60.39, 55.47, 53.04, 53.04, 52.95, 52.95, 44.04, 25.97. HRMS (MALDI) calcd for $C_{23}H_{27}N_6O$ $(M + H)^+$: 403.2241, found: 403.2247 $(M + H)^+$.

1-(3-(4-(3-Fluorobenzyl)piperazin-1-yl)propyl)-4-phenyl-1H-1,2,4-triazol-5(4H)-one (6f)

Yield: 57%; m.p. 114–116°C. IR (KBr) cm^{-1}: 1682 (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): *δ* 7.69 (s, 1H, N=CH), 7.64-7.46 (m, 4H, Ar-H), 7.44-7.33 (m, 1H, Ar-H), 7.27 (m, 1H, Ar-H), 7.09 (m, 2H, Ar-H), 6.95 (m, 1H, Ar-H), 3.93 (t, *J* = 6.9 Hz, 2H, CH_2), 3.50 (s, 2H, CH_2), 2.51-2.21 (m, 10H, $(CH_2)_5$), 2.12-1.95 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): *δ* 162.69, 150.96, 146.52, 138.77, 132.73, 128.96, 128.96, 128.42, 128.42, 128.15, 126.30, 124.70, 119.61, 114.02, 65.36, 58.20, 58.20, 55.31, 55.31, 51.20, 40.87, 22.09. HRMS (MALDI) calcd for $C_{22}H_{27}FN_5O$ $(M + H)^+$: 396.2194, found: 396.2195.

1-(3-(4-(4-Fluorobenzyl)piperazin-1-yl)propyl)-4-phenyl-1H-1,2,4-triazol-5(4H)-one (6g)

Yield: 51%; m.p. 150–152°C. IR (KBr) cm^{-1}: 1677 (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): *δ* 7.70 (s, 1H, N=CH), 7.57 (m, 2H, Ar-H), 7.53-7.45 (m, 2H, Ar-H), 7.38 (m, 1H, Ar-H), 7.28 (m, 2H, Ar-H), 7.06-6.96 (m, 2H, Ar-H), 3.92 (t, *J* = 7.0 Hz, 2H, CH_2), 3.48 (s, 2H, CH_2), 2.69-2.30 (m, 10H, $(CH_2)_5$), 2.08-1.93 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): *δ* 161.68, 150.63, 146.53, 134.75, 132.72, 128.49, 128.49, 128.04, 128.04, 127.92, 115.65, 115.65, 65.34, 58.27, 58.27, 55.30, 55.30, 51.24, 40.89, 22.19. HRMS (MALDI) calcd for $C_{22}H_{27}FN_5O$ $(M + H)^+$: 396.2194, found: 396.2198.

1-(3-(4-(4-Methoxybenzyl)piperazin-1-yl)propyl)-4-phenyl-1H-1,2,4-triazol-5(4H)-one (6h)

Yield: 43%; m.p. 158–160°C. IR (KBr) cm^{-1}: 1675 (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): δ 7.70 (s, 1H, N=CH), 7.54 (m, 4H, Ar-H), 7.39-7.21 (m, 3H, Ar-H), 6.86 (m, 2H, Ar-H), 3.93 (t, J = 7.0 Hz, 2H, CH_2), 3.81 (s, 3H, CH_2), 3.46 (s, 2H, CH_2), 2.76-2.17 (m, 10H, $(CH_2)_5$), 2.07-1.94 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 158.69, 151.59, 135.01, 134.64, 130.49, 130.43, 130.43, 129.77, 129.77, 127.36, 121.77, 121.77, 113.95, 113.95, 61.96, 55.42, 53.21, 53.21, 52.98, 52.98, 49.06, 43.69, 25.91. HRMS (MALDI) calcd for $C_{22}H_{30}N_5O_2$ $(M + H)^+$: 408.2394, found: 408.2388.

1-(3-(4-(2,4-Dichlorobenzyl)piperazin-1-yl)propyl)-4-phenyl-1H-1,2,4-triazol-5(4H)-one (6i)

Yield: 49%; m.p. 157–159°C. IR (KBr) cm^{-1}: 1684 (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): δ 7.70 (s, 1H, N=CH), 7.59-7.52 (m, 2H, Ar-H), 7.48 (m, 2H, Ar-H), 7.38 (m, 3H, Ar-H), 7.20 (m, 1H, Ar-H), 3.92 (t, J = 6.4 Hz, 2H, CH_2), 3.55 (s, 2H, CH_3), 2.70-2.29 (m, 10H, $(CH_2)_5$), 2.06-1.93 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 150.69, 146.52, 135.77, 134.73, 134.46, 132.42, 130.35, 128.95, 128.95, 128.16, 128.16, 128.07, 126.70, 58.20, 58.20, 57.45, 55.31, 55.31, 51.20, 40.87, 22.09. HRMS (MALDI) calcd for $C_{22}H_{26}Cl_2N_5O$ $(M + H)^+$: 446.1509, found: 446.1494.

1-(3-(4-Benzylpiperazin-1-yl)propyl)-4-(4-nitrophenyl)-1H-1,2,4-triazol-5(4H)-one (7a)

Yield: 56%; m.p. 134–136°C. IR (KBr) cm^{-1}: 1680 (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): δ 8.38 (d, J = 9.2 Hz, 2H, Ar-H), 7.88 (d, J = 9.2 Hz, 2H, Ar-H), 7.83 (s, 1H, N=CH), 7.30 (m, 5H, Ar-H), 3.96 (t, J = 7.0 Hz, 2H, CH_2), 3.52 (s, 2H, CH_2), 2.69-2.31 (m, 10H, $(CH_2)_5$), 2.09-1.97 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 150.49, 146.22, 143.58, 138.86, 138.67, 129.23, 129.23, 128.82, 128.82, 128.45, 128.45, 127.22, 124.41, 65.16, 58.45, 58.45, 55.27, 55.27, 51.32, 40.57, 22.72. HRMS (MALDI) calcd for $C_{22}H_{27}N_6O_3$ $(M + H)^+$: 423.2139, found: 423.2138.

1-(3-(4-(2-Chlorobenzyl)piperazin-1-yl)propyl)-4-(4-nitrophenyl)-1H-1,2,4-triazol-5(4H)-one (7b)

Yield: 56%; m.p. 118–120°C. IR (KBr) cm^{-1}: 1677 (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): δ 8.38 (d, J = 9.0 Hz, 2H, Ar-H), 7.89 (d, J = 9.0 Hz, 2H, Ar-H), 7.84 (s, 1H, N=CH), 7.46 (d, J = 6.1 Hz, 1H, Ar-H), 7.35 (m, 1H, Ar-H), 7.22 (m, 2H, Ar-H), 3.95 (t, J = 6.7 Hz, 2H, CH_2), 3.63 (s, 2H, CH_2), 2.54-2.25 (m, 10H, $(CH_2)_5$), 2.03 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 150.67, 146.55, 143.72, 138.70, 135.48, 134.45, 134.38, 131.32, 130.74, 129.63, 129.63, 126.85, 124.63, 124.63, 58.24, 58.24, 57.45, 55.11, 55.11, 51.27, 40.85, 22.39. HRMS (MALDI) calcd for $C_{22}H_{26}ClN_6O_3$ $(M + H)^+$: 457.1749, found: 457.1752.

1-(3-(4-(3-Chlorobenzyl)piperazin-1-yl)propyl)-4-(4-nitrophenyl)-1H-1,2,4-triazol-5(4H)-one (7c)

Yield: 54%; m.p. 84–86°C. IR (KBr) cm^{-1}: 1675 (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): δ 8.38 (d, J = 9.1 Hz, 2H, Ar-H), 7.88 (d, J = 9.1 Hz, 2H, Ar-H), 7.84 (s, 1H, N=CH), 7.33 (s, 1H, Ar-H), 7.24 (m, 2H, Ar-H), 7.21 (m, 1H, Ar-H), 3.95 (t, J = 6.8 Hz, 2H, CH_2), 3.47 (s, 2H, CH_2), 2.49-2.41 (m, 10H, $(CH_2)_5$), 2.09-1.95 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 150.79, 146.12, 143.57, 138.37, 135.23, 134.65, 134.52, 131.35, 130.31, 129.92, 129.92, 126.22, 124.86, 124.86, 65.16, 58.45, 58.45, 55.27, 55.27, 51.38, 40.17, 22.09. HRMS (MALDI) calcd for $C_{22}H_{26}ClN_6O_3$ $(M + H)^+$: 457.1749, found: 457.1746.

1-(3-(4-(4-Chlorobenzyl)piperazin-1-yl)propyl)-4-(4-nitrophenyl)-1H-1,2,4-triazol-5(4H)-one (7d)

Yield: 55%; m.p. 138–140°C. IR (KBr) cm^{-1}: 1673 (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): δ 8.38 (d, J = 9.0 Hz, 2H, Ar-H), 7.88 (d, J = 9.0 Hz, 2H, Ar-H), 7.83 (s, 1H, N=CH), 7.28 (dd, J = 7.2, 5.6 Hz, 4H, Ar-H), 3.94 (t, J = 6.9 Hz, 2H, CH_2), 3.50 (s, 2H, CH_2), 2.49-2.31 (m, 10H, $(CH_2)_5$), 2.10-1.94 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 151.12, 145.92, 139.28, 136.71, 132.67, 132.01, 130.40, 130.40, 128.32, 128.32, 125.28, 125.28, 120.88, 120.88,

62.20, 55.42, 53.12, 53.12, 53.03, 53.03, 44.23, 25.83. HRMS (MALDI) calcd for $C_{22}H_{25}ClN_6O_3$ $(M + H)^+$: 457.1749, found: 457.1751.

2-((4-(3-(4-(4-Nitrophenyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)propyl)piperazin-1-yl)methyl)benzonitrile (7e)

Yield: 51%; m.p. 114–116°C. IR (KBr) cm^{-1}: 1671 (C=O). ^1H NMR ($CDCl_3$, 300 MHz, ppm): δ 8.38 (d, J = 8.9 Hz, 2H, Ar-H), 7.89 (d, J = 8.9 Hz, 2H, Ar-H), 7.85 (s, 1H, N=CH), 7.65 (m, 1H, Ar-H), 7.53 (m, 2H, Ar-H), 7.43-7.33 (m, 1H, Ar-H), 3.95 (t, J = 6.7 Hz, 2H, CH_2), 3.68 (s, 2H, CH_2), 2.92-2.32 (m, 10H, $(CH_2)_5$), 2.07-1.94 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 150.49, 146.22, 143.50, 139.78, 138.57, 132.53, 131.76, 129.52, 129.35, 129.35, 127.92, 124.11, 124.11, 115.89, 113.15, 59.45, 58.45, 58.45, 55.27, 55.27, 51.32, 40.57, 22.72. HRMS (MALDI) calcd for $C_{23}H_{26}N_7O_3$ $(M + H)^+$: 448.2092, found: 448.2078.

1-(3-(4-(3-Fluorobenzyl) piperazin-1-yl) propyl)-4-(4-nitrophenyl)-1H-1,2,4-triazol -5(4H)-one (7f)

Yield: 47%; m.p. 80–82°C. IR (KBr) cm^{-1}: 1677 (C=O). ^1H NMR ($CDCl_3$, 300 MHz, ppm): δ 8.38 (d, J = 9.1 Hz, 2H, Ar-H), 7.88 (d, J = 9.1 Hz, 2H, Ar-H), 7.84 (s, 1H, N=CH), 7.27 (m, 1H, Ar-H), 7.07 (m, 2H, Ar-H), 6.95 (t, J = 8.2 Hz, 1H, Ar-H), 3.95 (t, J = 6.8 Hz, 2H, CH_2), 3.50 (s, 2H, CH_2), 2.49-2.23 (m, 10H, $(CH_2)_5$), 2.08-1.97 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 150.72, 146.34, 143.89, 138.74, 135.25, 134.68, 134.42, 131.55, 130.01, 129.81, 129.81, 126.22, 124.67, 124.67, 65.18, 58.48, 58.48, 55.22, 55.22, 51.38, 40.27, 21.76. HRMS (MALDI) calcd for $C_{22}H_{26}FN_6O_3$ $(M + H)^+$: 441.2045, found: 441.2039.

1-(3-(4-(4-Fluorobenzyl)piperazin-1-yl)propyl)-4-(4-nitrophenyl)-1H-1,2,4-triazol-5(4H)-one (7g)

Yield: 56%; m.p. 136–138°C. IR (KBr) cm^{-1}: 1687 (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): *δ* 8.38 (d, *J* = 9.0 Hz, 2H, Ar-H), 7.88 (d, *J* = 9.2 Hz, 2H, Ar-H), 7.83 (s, 1H, N=CH), 7.56-7.03 (m, 4H, Ar-H), 4.31 (s, 2H, CH_2), 3.97-3.95 (t, *J* = 6.3 Hz, 2H, CH_2), 2.54-2.58 (m, 10H, $(CH_2)_5$), 1.34-1.37 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): *δ* 161.55, 151.11, 145.88, 139.28, 133.82, 132.03, 130.64, 130.53, 125.26, 125.26, 120.86, 120.86, 115.08, 114.80, 62.16, 55.41, 53.11, 53.11, 52.97, 52.97, 44.22, 25.82. HRMS (MALDI) calcd for $C_{22}H_{26}FN_6O_3$ $(M + H)^+$: 441.2045, found: 441.2039.

1-(3-(4-(4-Methoxybenzyl)piperazin-1-yl)propyl)-4-(4-nitrophenyl)-1H-1,2,4-triazol-5(4H)-one (7h)

Yield: 53%; m.p. 134–136°C. IR (KBr) cm^{-1}: 1684 (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): *δ* 8.37 (d, *J* = 9.1 Hz, 2H, Ar-H), 7.88 (d, *J* = 9.1 Hz, 2H, Ar-H), 7.83 (s, 1H, N=CH), 7.22 (d, *J* = 8.4 Hz, 2H, Ar-H), 6.85 (d, *J* = 8.5 Hz, 2H, Ar-H), 3.94 (t, *J* = 6.9 Hz, 2H, CH_2), 3.81 (s, 3H, OCH_3), 3.49 (s, 2H, CH_2), 2.49-2.35 (m, 10H, $(CH_2)_5$), 2.01 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): *δ* 158.71, 151.14, 145.91, 139.1028, 132.02, 130.39, 130.39, 129.93, 125.27, 125.27, 120.88, 120.88, 113.56, 113.56, 62.39, 55.45, 55.23, 53.11, 53.11, 52.92, 52.92, 44.25, 25.82. HRMS (MALDI) calcd for $C_{23}H_{29}N_6O_4$ $(M + H)^+$: 453.2245, found: 453.2248.

1-(3-(4-(2,4-Dichlorobenzyl)piperazin-1-yl)propyl)-4-(4-nitrophenyl)-1H-1,2,4-triazol-5(4H)-one (7i)

Yield: 51%; m.p. 130–132°C. IR (KBr) cm^{-1}: 1677 (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): *δδ* 8.38 (d, *J* = 9.1 Hz, 2H, Ar-H), 7.89 (d, *J* = 9.1 Hz, 2H, Ar-H), 7.84 (s, 1H, N=CH), 7.38 (m, 2H, Ar-H), 7.21 (m, 1H, Ar-H), 3.95 (t, *J* = 6.9 Hz, 2H, CH_2), 3.56 (s, 2H, CH_2), 2.68-2.36 (m, 10H, $(CH_2)_5$), 2.07-1.94 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): *δ* 150.49, 146.22, 143.87, 138.57, 135.53, 134.76, 134.52, 131.35, 130.31, 129.92, 129.92, 126.22, 124.86, 124.86,

65.16, 58.45, 58.45, 55.27, 55.27, 51.32, 40.57, 22.72. HRMS (MALDI) calcd for $C_{22}H_{25}Cl_2N_6O_3$ $(M + H)^+$: 491.1360, found: 491.1354.

General procedure for synthesis of compounds 13a-j and 14a-j

Synthesis of compound **13a**-**j** and **14a**-**j** was depicted in Scheme 2. Esterification of substituted benzoic acid **8a**-**j** (10 mmol) with methanol (20mL) in the presence of sulfuric acid afforded **9a**-**j**, which were reacted with hydrazine hydrate (10 mmol) to give compounds **10a**-**j**. Compounds **12a**-**j** was prepared by the reaction of **10a**-**j (**8 mmol**)** with potassium thiocyanate (8 mmol) in refluxing hydrochloric acid and sodium hydroxide. Nucleophilic substitution of **5I** (10 mmol) or **5III** (10 mmol) with **12a**-**j** (10 mmol) in refluxing acetone (20 mL) in the presence of KI/K_2CO_3 at 57 °C for 6 h afforded **13a**-**j** and **14a**-**j** in high yields 70%–90%. The solution was evaporated to dryness under reduced pressure, and the residue was purified by silica gel column chromatography with dichloromethane: methanol (200:1). The yield, melting point and spectra data of each compound are given below.

4-Phenyl-1-(3-((5-phenyl-4H-1,2,4-triazol-3-yl)thio)propyl)-1H-1,2,4-triazol-5(4H)-one (13a)

Yield: 77%; m.p. 190–191°C. IR (KBr) cm^{-1}: 3186 (NH), 1685 (C=O). ^1H NMR ($CDCl_3$, 300 MHz, ppm): δ 8.32 (s, 1H, NH), 7.97 (d, J = 7.6 Hz, 2H, Ar-H), 7.67 (d, J = 8.6 Hz, 2H, Ar-H), 7.56-7.28 (m, 7H, Ar-H and N=CH), 3.90 (t, J = 6.5 Hz, 2H, CH_2), 3.23 (t, J = 5.5 Hz, 2H, CH_2), 2.23-2.08 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 158.86, 157.65, 151.02, 137.73, 137.73, 132.75, 131.40, 129.23, 129.23, 128.91, 128.91, 128.05, 127.46, 127.46, 119.43, 119.43, 45.64, 34.21, 24.60. HRMS (MALDI) calcd for $C_{19}H_{19}N_6OS$ $(M + H)^+$: 379.1336, found: 379.1339.

1-(3-((5-(2-Chlorophenyl)-4H-1,2,4-triazol-3-yl)thio)propyl)-4-phenyl-1H-1,2,4-triazol-5(4H)-one (13b)

Yield: 83%; m.p. 120–122°C. IR (KBr) cm^{-1}: 3165 (NH), 1678 (C=O). ^1H NMR ($CDCl_3$, 300 MHz, ppm): δ 8.49 (s, 1H, NH), 7.77-7.34 (m, 10H, Ar-H and N=CH), 3.90 (t, J = 6.4 Hz, 2H, CH_2), 3.22 (t, J = 6.4 Hz, 2H, CH_2), 2.25-2.07 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 158.86, 157.62, 151.22, 138.75, 137.71, 137.71, 132.74, 130.01, 129.85, 128.94, 128.94, 128.94, 128.02, 127.47, 119.41, 119.41, 45.65, 35.12, 26.63. HRMS (MALDI) calcd for $C_{19}H_{18}ClN_6OS$ $(M + H)^+$: 413.0946, found: 413.0944.

1-(3-((5-(3-Chlorophenyl)-4H-1,2,4-triazol-3-yl)thio)propyl)-4-phenyl-1H-1,2,4-triazol-5(4H)-one (13c)

Yield: 88%; m.p. 154–156°C. IR (KBr) cm^{-1}: 3172 (NH), 1679 (C=O). ^1H NMR ($CDCl_3$, 300 MHz, ppm): δ 8.07 (s, 1H, NH), 7.94 (s, 1H, Ar-H), 7.77 (s, 1H, Ar-H), 7.55-7.27 (m, 8H, Ar-H and N=CH), 4.13 (t, J = 5.9 Hz, 2H, CH_2), 3.26 (t, J = 6.4 Hz, 2H, CH_2), 2.27 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 158.86, 157.69, 150.26, 146.77, 134.83, 132.77, 132.07, 129.53, 128.91, 128.91, 128.80, 128.18, 128.18, 127.98, 126.43, 125.65, 45.20, 34.53, 27.07. HRMS (MALDI) calcd for $C_{19}H_{18}ClN_6OS$ $(M + H)^+$: 413.0946, found: 413.0939.

1-(3-((5-(4-Chlorophenyl)-4H-1,2,4-triazol-3-yl)thio)propyl)-4-phenyl-1H-1,2,4-triazol-5(4H)-one (13d)

Yield: 93%; m.p. 158–160°C. IR (KBr) cm^{-1}: 3178 (NH), 1684 (C=O). ^1H NMR ($CDCl_3$, 300 MHz, ppm): δ 7.99 (s, 1H, NH), 7.76-7.56 (m, 2H, Ar-H), 7.55-7.23 (m, 8H, Ar-H and N=CH), 4.15 (t, J = 6.5 Hz, 2H, CH_2), 3.26 (t, J = 5.6 Hz, 2H, CH_2), 2.28-2.25 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 158.86, 157.65, 150.22, 146.90, 134.75, 132.40, 130.63, 129.31, 129.31, 128.95, 128.95, 128.95, 128.95, 128.12, 128.12, 128.06, 45.64, 34.21, 26.59. HRMS (MALDI) calcd for $C_{19}H_{18}ClN_6OS$ $(M + H)^+$: 413.0946, found: 413.0934.

4-Phenyl-1-(3-((5-(o-tolyl)-4H-1,2,4-triazol-3-yl)thio)propyl)-1H-1,2,4-triazol-5(4H)-one (13e)

Yield: 92%; m.p. 120–122°C. IR (KBr) cm^{-1}: 3179 (NH), 1679 (C=O). ^1H NMR ($CDCl_3$, 300 MHz, ppm): δ 7.71 (s, 1H, NH), 7.67-7.18 (m, 10H, Ar-H and N=CH), 4.09 (t, J = 6.4 Hz, 2H, CH_2), 3.26 (t, J = 6.8 Hz, 2H, CH_2), 2.55 (s, 3H, CH_3), 2.45-2.21 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 158.50, 157.68, 150.09, 146.95, 138.58, 136.78, 132.69, 130.08, 129.54, 129.46, 128.91, 128.91, 128.65, 128.01, 128.01, 127.96, 42.68, 35.42, 24.69, 19.12. HRMS (MALDI) calcd for $C_{20}H_{21}N_6OS$ $(M + H)^+$: 393.1492, found: 393.1495.

4-Phenyl-1-(3-((5-(m-tolyl)-4H-1,2,4-triazol-3-yl)thio)propyl)-1H-1,2,4-triazol-5(4H)-one (13f)

Yield: 80%; m.p. 125–127°C. IR (KBr) cm^{-1}: 3180 (NH), 1684 (C=O). ^1H NMR ($CDCl_3$, 300 MHz, ppm): δ 7.85 (s, 1H, NH), 7.82-7.19 (m, 10H, Ar-H and N=CH), 4.11 (t, J = 5.5 Hz, 2H, CH_2), 3.26 (t, J = 5.4 Hz, 2H, CH_2), 2.36 (s, 3H, CH_3), 2.33-2.17 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 158.56, 157.32, 151.22, 137.75, 137.75, 137.31, 131.25, 130.61, 129.14, 129.01, 128.95, 128.92, 128.92, 128.01, 124.56, 119.52, 119.52, 45.89, 34.51, 27.54, 21.83. HRMS (MALDI) calcd for $C_{20}H_{21}N_6OS$ $(M + H)^+$: 393.1492, found: 393.1493.

4-Phenyl-1-(3-((5-(p-tolyl)-4H-1,2,4-triazol-3-yl)thio)propyl)-1H-1,2,4-triazol-5(4H)-one (13g)

Yield: 86%; m.p. 160–162°C. IR (KBr) cm^{-1}: 3174 (NH), 1676 (C=O). ^1H NMR ($CDCl_3$, 300 MHz, ppm): δ 8.48 (s, 1H, NH), 7.83 (d, J = 7.5 Hz, 2H, Ar-H), 7.69 (d, J = 7.9 Hz, 2H, Ar-H), 7.67-7.14 (m, 6H, Ar-H and N=CH), 3.90 (t, J = 6.4 Hz, 2H, CH_2), 3.17 (t, J = 5.4 Hz, 2H, CH_2), 2.35 (s, 3H, CH_3), 2.27-2.05 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 158.82, 157.73, 151.72, 137.75, 137.75, 131.79, 129.54, 129.54, 129.54, 128.93, 128.93, 128.01, 125.72, 125.72, 119.45, 119.45, 45.89, 34.36, 26.98, 21.55. HRMS (MALDI) calcd for $C_{20}H_{21}N_6OS$ $(M + H)^+$: 393.1492, found: 393.1490.

1-(3-((5-(2-Bromophenyl)-4H-1,2,4-triazol-3-yl)thio)propyl)-4-phenyl-1H-1,2,4-triazol-5(4H)-one (13h)

Yield: 71%; m.p. 110–112°C. IR (KBr) cm^{-1}: 3167 (NH), 1676 (C=O). ^1H NMR ($CDCl_3$, 300 MHz, ppm): δ 7.95 (s, 1H, NH), 7.81-7.24 (m, 10H, Ar-H and N=CH), 4.08 (t, J = 6.3 Hz, 2H, CH_2), 3.28 (t, J = 6.5 Hz, 2H, CH_2), 2.31-2.29 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 158.75, 157.67, 151.52, 139.75, 137.71, 137.71, 132.14, 130.91, 129.75, 128.90, 128.90, 128.24, 128.12, 119.39, 119.39, 46.65, 34.12, 26.93. HRMS (MALDI) calcd for $C_{19}H_{18}BrN_6OS$ $(M + H)^+$: 457.0441, found: 457.0445.

1-(3-((5-(3-Bromophenyl)-4H-1,2,4-triazol-3-yl)thio)propyl)-4-phenyl-1H-1,2,4-triazol-5(4H)-one (13i)

Yield: 75%; m.p. 144–146°C. IR (KBr) cm^{-1}: 3172 (NH), 1680 (C=O). ^1H NMR ($CDCl_3$, 300 MHz, ppm): δ 8.25 (s, 1H, NH), 8.01-7.25 (m, 10H, Ar-H and N=CH), 4.15 (t, J = 6.2 Hz, 2H, CH_2), 3.25 (t, J = 5.9 Hz, 2H, CH_2), 2.28-2.26 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 158.86, 157.65, 151.06, 137.77, 137.77, 132.83, 131.76, 131.43, 128.291, 128.91, 128.10, 128.02, 126.10, 122.03, 119.48, 119.48, 45.20, 37.53, 27.57. HRMS (MALDI) calcd for $C_{19}H_{18}BrN_6OS$ $(M + H)^+$: 457.0441, found: 457.0446.

1-(3-((5-(4-Methoxyphenyl)-4H-1,2,4-triazol-3-yl)thio)propyl)-4-phenyl-1H-1,2,4-triazol-5(4H)-one (13j)

Yield: 87%; m.p. 149–151°C. IR (KBr) cm^{-1}: 3173 (NH), 1681 (C=O). ^1H NMR ($CDCl_3$, 300 MHz, ppm): δ 8.48 (s, 1H, NH), 7.83-7.36 (m, 10H, Ar-H and N=CH), 3.90 (t, J = 6.4 Hz, 2H, CH_2), 3.17 (t, J = 5.6 Hz, 2H, CH_2), 2.35 (s, 3H, OCH_3), 2.27-2.05 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 161.07, 158.89, 157.65, 151.65, 145.32, 132.58, 130.76, 130.76, 128.92, 128.92, 128.25, 128.25, 127.98, 124.91, 114.77, 114.77, 55.72, 44.09, 32.35, 29.19. HRMS (MALDI) calcd for $C_{20}H_{21}N_6O_2S$ $(M + H)^+$: 409.1441, found: 409.1449.

4-(3-Chlorophenyl)-1-(3-((5-phenyl-4H-1,2,4-triazol-3-yl)thio)propyl)-1H-1,2,4-triazol-5(4H)-one (14a)

Yield: 84%; m.p. 138–140°C. IR (KBr) cm^{-1}: 3167 (NH), 1678 (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): δ 8.03 (s, 1H, NH), 7.91-7.03 (m, 10H, Ar-H and N=CH), 4.11 (t, J = 6.5 Hz, 2H, CH_2), 3.26 (t, J = 6.9 Hz, 2H, CH_2), 2.44-2.22 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 158.91, 156.71, 150.17, 146.52, 134.63, 134.43, 133.50, 132.50, 131.91, 131.02, 128.94, 128.94, 127.22, 127.03, 127.03, 48.93, 34.11, 33.67. HRMS (MALDI) calcd for $C_{19}H_{18}ClN_6OS$ $(M + H)^+$: 413.0946, found: 413.0951.

4-(3-Chlorophenyl)-1-(3-((5-(2-chlorophenyl)-4H-1,2,4-triazol-3-yl)thio)propyl)-1H-1,2,4-triazol-5(4H)-one (14b)

Yield: 87%; m.p. 141–143°C. IR (KBr) cm^{-1}: 3176 (NH), 1687 (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): δ 8.22 (s, 1H, NH), 7.73-7.27 (m, 9H, Ar-H and N=CH), 4.09 (t, J = 6.4 Hz, 2H, CH_2), 3.29 (t, J = 6.5 Hz, 2H, CH_2), 2.33 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 158.87, 151.39, 135.87, 135.03, 134.05, 131.99, 131.78, 131.75, 131.70, 131.41, 130.83, 127.89, 127.18, 121.35, 120.04, 44.01, 34.46, 29.03. HRMS (MALDI) calcd for $C_{19}H_{17}Cl_2N_6OS$ $(M + H)^+$: 447.0556, found: 447.0558.

4-(3-Chlorophenyl)-1-(3-((5-(3-chlorophenyl)-4H-1,2,4-triazol-3-yl)thio)propyl)-1H-1,2,4-triazol-5(4H)-one (14c)

Yield: 82%; m.p. 148-149°C. IR (KBr) cm^{-1}: 3165 (NH), 1678 (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): δ 8.07 (s, 1H, NH), 7.94-7.92 (m, 1H, Ar-H), 7.77-7.73 (m, 2H, Ar-H), 7.61-7.28 (m, 6H, Ar-H and N=CH), 4.14 (t, J = 6.4 Hz, 2H, CH_2), 3.27 (t, J = 6.8 Hz, 2H, CH_2), 2.38-2.18 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 158.94, 156.16, 150.11, 149.47, 134.99, 134.56, 134.04, 133.29, 132.11, 130.87, 129.23, 128.17, 127.99, 126.54, 126.23, 125.71, 48.81, 34.19, 33.62. HRMS (MALDI) calcd for $C_{19}H_{17}Cl_2N_6OS$ $(M + H)^+$: 447.0556, found: 447.0555.

4-(3-Chlorophenyl)-1-(3-((5-(4-chlorophenyl)-4H-1,2,4-triazol-3-yl)thio)propyl)-1H-1,2,4-triazol-5(4H)-one (14d)

Yield: 78%; m.p. 180–182°C. IR (KBr) cm^{-1}: 3172 (NH), 1681 (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): δ 8.01 (s, 1H, NH), 7.76-7.72 (m, 3H, Ar-H), 7.62-7.28 (m, 6H, Ar-H and N=CH), 4.14 (t, J = 6.5 Hz, 2H, CH_2), 3.26 (t, J = 6.7 Hz, 2H, CH_2), 2.39-2.20 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 158.06, 157.69, 150.06, 146.77, 134.51, 134.21, 133.23, 132.77, 129.93, 129.93, 128.21, 128.21, 128.10, 127.28, 127.10, 127.03, 45.20, 37.53, 27.57. HRMS (MALDI) calcd for $C_{19}H_{17}Cl_2N_6OS$ $(M + H)^+$: 447.0556, found: 447.0554.

4-(3-Chlorophenyl)-1-(3-((5-(o-tolyl)-4H-1,2,4-triazol-3-yl)thio)propyl)-1H-1,2,4-triazol-5(4H)-one (14e)

Yield: 88%; m.p. 101–103°C. IR (KBr) cm^{-1}: 3174 (NH), 1676 (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): δ 7.82 (s, 1H, NH), 7.71-7.18 (m, 9H, Ar-H and N=CH), 4.08 (t, J = 6.1 Hz, 2H, CH_2), 3.27 (t, J = 6.6 Hz, 2H, CH_2), 2.53 (s, 3H, CH_3), 2.30-2.27 (m, 2H, CH_2). ^{13}C NMR (CDCl3, 75 MHz, ppm): δ 158.06, 157.69, 150.06, 146.77, 134.86, 134.57, 133.23, 132.77, 130.05, 129.93, 128.21, 128.10, 127.28, 127.10, 127.03, 125.64, 44.09, 32.35, 26.78, 19.75. HRMS (MALDI) calcd for $C_{20}H_{20}ClN_6OS$ $(M + H)^+$: 427.1102, found: 427.1112.

4-(3-Chlorophenyl)-1-(3-((5-(m-tolyl)-4H-1,2,4-triazol-3-yl)thio)propyl)-1H-1,2,4-triazol-5(4H)-one (14f)

Yield: 75%; m.p. 158–160°C. IR (KBr) cm^{-1}: 3168 (NH), 1683 (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): δ 7.83 (s, 1H, NH), 7.79-7.73 (m, 2H, Ar-H), 7.61-7.21 (m, 7H, Ar-H and N=CH), 4.09 (t, J = 6.6 Hz, 2H, CH_2), 3.25 (t, J = 6.5 Hz, 2H, CH_2), 2.38 (s, 3H, CH_3), 2.35-2.20 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 158.56, 157.32, 150.22, 146.75, 134.56, 132.71, 132.47, 129.74, 128.91, 128.85, 128.64, 128.53, 128.43, 128.22, 127.47, 125.41, 43.65, 38.12, 24.63, 19.65. HRMS (MALDI) calcd for $C_{20}H_{20}ClN_6OS$ $(M + H)^+$: 427.1102, found: 427.1100.

4-(3-Chlorophenyl)-1-(3-((5-(p-tolyl)-4H-1,2,4-triazol-3-yl)thio)propyl)-1H-1,2,4-triazol-5(4H)-one (14g)

Yield: 85%; m.p. 168–170°C. IR (KBr) cm^{-1}: 3168 (NH), 1680 (C=O). ^1H NMR ($CDCl_3$, 300 MHz, ppm): δ 7.89 (s, 1H, NH), 7.87-7.22 (m, 7H, Ar-H and N=CH), 4.10 (t, J = 6.5 Hz, 2H, CH_2), 3.26 (t, J = 6.4 Hz, 2H, CH_2), 2.37 (s, 3H, CH_3), 2.30-2.27 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 158.42, 157.73, 150.72, 146.35, 132.70, 132.48, 129.55, 129.55, 129.55, 128.52, 128.52, 127.67, 126.55, 126.41, 126.20, 125.45, 43.62, 38.23, 24.54, 19.78. HRMS (MALDI) calcd for $C_{20}H_{20}ClN_6OS$ $(M + H)^+$: 427.1102, found: 427.1095.

1-(3-((5-(2-Bromophenyl)-4H-1,2,4-triazol-3-yl)thio)propyl)-4-(3-chlorophenyl)-1H-1,2,4-triazol-5(4H)-one (14h)

Yield: 87%; m.p. 140–142°C. IR (KBr) cm^{-1}: 3176 (NH), 1682 (C=O). ^1H NMR ($CDCl_3$, 300 MHz, ppm): δ 7.99 (s, 1H, NH), 7.73-7.25 (m, 9H, Ar-H and N=CH), 4.07 (t, J = 6.0 Hz, 2H, CH_2), 3.27 (t, J = 6.6 Hz, 2H, CH_2), 2.42-2.22 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 158.56, 157.32, 150.22, 146.75, 134.71, 134.47, 134.34, 134.05, 132.91, 130.85, 129.64, 128.53, 127.43, 126.22, 126.07, 125.41, 43.65, 38.12, 26.83. HRMS (MALDI) calcd for $C_{19}H_{17}BrClN_6OS$ $(M + H)^+$: 491.0051, found: 491.0053.

1-(3-((5-(3-Bromophenyl)-4H-1,2,4-triazol-3-yl)thio)propyl)-4-(3-chlorophenyl)-1H-1,2,4-triazol-5(4H)-one (14i)

Yield: 72%; m.p. 142–144°C. IR (KBr) cm^{-1}: 3167 (NH), 1680 (C=O). ^1H NMR ($CDCl_3$, 300 MHz, ppm): δ 8.21 (s, 1H, NH), 7.97 (m, 1H, Ar-H), 7.77 (s, 1H, Ar-H), 7.60-7.27 (m, 7H, Ar-H and CH), 4.13 (t, J = 5.6 Hz, 2H, CH_2), 3.27 (t, J = 6.5 Hz, 2H, CH_2), 2.29-2.26 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 158.06, 157.69, 150.06, 146.77, 134.89, 134.56, 134.23, 134.02, 133.23, 132.77, 129.93, 128.21, 127.90, 127.78, 127.10, 125.03, 45.20, 37.53, 27.57. HRMS (MALDI) calcd for $C_{19}H_{17}BrClN_6OS$ $(M + H)^+$: 491.0051, found: 491.0041.

4-(3-Chlorophenyl)-1-(3-((5-(4-methoxyphenyl)-4H-1,2,4-triazol-3-yl)thio)propyl)-1H-1,2,4-triazol-5(4H)-one (14j)

Yield: 82%; m.p. 158–160°C. IR (KBr) cm^{-1}: 3167 (NH), 1680 (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): δ 8.01-7.18 (m, 10H, Ar-H, CH), 4.10 (t, J = 6.5 Hz, 2H, CH_2), 3.26 (t, J = 6.4 Hz, 2H, CH_2), 2.37 (s, 3H, OCH_3), 2.30-2.27 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 161.07, 158.56, 157.63, 150.02, 146.36, 135.32, 134.58, 130.43, 130.43, 130.43, 129.76, 128.01, 127.43, 124.91, 114.75, 114.75, 55.70, 44.10, 32.36, 29.18. HRMS (MALDI) calcd for $C_{20}H_{20}ClN_6O_2S$ $(M + H)^+$: 443.1051, found: 443.1053.

Evaluation of negative inotropic effects in vitro

The means of measuring left atrium stroke volume was represented previously (24). The characteristic of CHF are cardiac dilatation, reduced ejection fraction and poor contractility of cardiac muscle, as well as depression of left ventricular pressure maximum alleosis. As a result, the macroscopic measurement of the variance atrium stroke volume can be applied to evaluate the negative inotropic effects of the compounds synthesized. Almost all reagents were of analytical grade. Metoprolol was used as reference drug as negative inotropic drug clinically used for the treatment of congestive heart failure. The tested left atrium was perfused with N-2-hydroxyethyl piperazine-N-2-ethanesulfonic acid (HEPES) buffer solution use a peristaltic pump (1.25 mL/min) at 34 ℃ (25). The constituent of the buffer was as follows (in mmol): 4.7 KCl, 118 NaCl, 1.2 $MgCl_2$, 2.5 $CaCl_2$, 25 $NaHCO_3$, 10.0 glucose, 10.0 HEPES, (adjusted to pH 7.4 with 1 mol NaOH) and 0.1% bovine serum albumin (BSA). After the perfused atrium was build, transmural electrical field stimulation using a luminal electrode was started at 1.5 Hz (voltage 30 V, 0.3-0.5 ms, duration). The changes in the atrial stroke volume were monitored through reading the lowest level of the water column in the calibrated atrial cannula during the end diastole. The tested atria were perfused for 60 minutes to stabilize the atrial stroke volume. The tested atrial beat rate was fixed at 1.5 Hz, the atrium stroke volume was recorded at 2 minutes intervals and the stimulus effect of the tested sample was recorded after a circulation of the control group.

Every circulation was 12 minutes (26). The tested compounds were studied using the single dose technique at a concentration of 3×10^{-5} mol/L. The tested samples were dissolved in DMSO, diluted with the HEPES buffer to an appropriate volume. The biological evaluation data of the tested compounds were presented with increased stroke volume percentage as shown in Table 1 and 2. In the aim of assess differences, repeated measurements were compared with an ANOVA test followed by a Bonferroni's multiple-comparison test. Statistical significance was defined as $P<0.05$ and the data is presented as means ±SE.

Evaluation of cytotoxicity on human cancer cells

The cytotoxicity test of the selected compounds with potent inotropic activity was measured through the colorimetric MTT assay. Human cervical cancer cells (HeLa), liver cancer cells (Hep3B) and human normal hepatic cells (LO2) suspension in DMEM medium supplemented with 10% FBS and antimycotic was added in 96-well microplates at 1.8×10^4 cells/well. A variety of concentrations of the test compounds (200, 100, 50, 25, 12.5, 6.25, 3.125, 1.625 μmol/L) dissolved by distilled 10% DMSO was added to each well. After incubation for 24h at 37℃ under 5% CO_2, 2.5mg/mL of MTT solution was added to each well. The plate was incubated for another 4h. Then, the incubation medium was aspirated, added DMSO (100 μL) to solubilize the MTT formazan product. The absorbance at 570 and 630 nm were measured after mixing. The difference between absorbance of 570 and 630 nm was used as an index of the cell viability. The morphology of the cells was observed by Giemsa stain under Phase contrast microscope. The cells with test compounds were used as positive control, whereas untreated cells were used as negative controls. The concentration of the test compound that killed 50% of the cancer cells (IC_{50}) was calculated by GraphPad Prism 4 software (27). The results were summarized in Table 3. All experiments were performed in triplicate.

Results and discussion

Chemistry

The synthesis of the compounds **6a**-**i**, **7a**-**i**, **13a**-**j**, and **14a**-**j** is outlined in Scheme 1 and 2 and structures are shown in Figure 1. The key intermediates **5I**-**III** were synthesized by reacting aniline **1I**-**III** with methyl hydrazinecarboxylate in refluxing ethanol in the presence of triethyl orthoformate and sodium methoxide (28,29), followed by reaction with 1,3-dibromopropane in the presence of potassium carbonate and potassium iodide in refluxing acetone. Nucleophilic substitution of **5I** or **5II** with various monosubstituted piperazines in refluxing acetone in the presence of KI/K_2CO_3 afforded **6a**-**i** and **7a**-**i** in 40%-60% yields. Esterification of substituted benzoic acid **8a**-**j** with methanol in the presence of sulfuric acid afforded **9a**-**j**, which was reacted with hydrazine hydrate to give compounds **10a**-**j**. Compounds **12a**-**j** was prepared by the reaction of **10a**-**j** with potassium thiocyanate in refluxing hydrochloric acid and sodium hydroxide (30). Nucleophilic substitution of **5I** or **5III** with **12a**-**j** in refluxing acetone in the presence of KI/K_2CO_3 afforded **13a**-**j** and **14a**-**j** in 70%-90% yields. The structures of the target compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, HRMS and mass spectrometry. Data are shown in the Experimental section.

Biological activity

As shown in Tables 1 and 2, most of the tested compounds showed negative inotropic effects in isolated rabbit heart preparations. Among them, 28 compounds exhibited more potent effects compared with the reference drug (metoprolol: −9.74 ± 0.14% at 3×10^{-5} M). Compounds **6e** and **7h** showed significant negative inotropic activity with stroke volume changes of −41.56 ± 0.55% and −48.22 ± 0.36%, respectively, which are 4-fold more potent than that of the standard drug. Some preliminary conclusions on structure–activity relationship can be drawn from the results of bioactivities. The presence of different substituents on the phenyl ring of the benzyl group at the 4-position of the piperazine ring

was found to exert a different influence on the observed negative inotropic activity, but no clear pattern could be found for the SAR. As for the halo substituted derivatives (**6b**, **6c**, **6d**, **6f**, **6g**, **6i**), an activity order of *o* > *m* > 2,4-Cl_2 > *p* was observed for the chlorinated compounds, *m* > *p* for the fluorinated compounds, the unsubstituted **6a** exhibited slightly weaker activity with -4.55 ± 0.24% increased stroke volume and para-methoxy substituted derivative **6h** exhibited more potent activity than the reference drug metoprolol (-27.65 ± 0.61%). For the series of **7**, compound **7h** with *para*-methoxy substituted showed the most potent activity with -48.22 ± 0.36% changed stroke volume. For the halo substituted derivatives (**7b**, **7c**, **7d**, **7f**, **7g**, **7i**), an activity order of *p* > *o* > 2,4-Cl_2 > *m* was observed for the chlorinated compounds, *p* > *m* for the fluorinated compounds. The cyano substituted **7e** exhibited weak activity (-4.76 ± 0.07%). Unsubstituted derivatives **7a** (-15.39 ± 0.39%) exhibited less potent negative inotropic activity than the reference drug. For the compounds in series **6** and **7**, the nitro group at the 4-position of the phenyl ring on the triazole in series **7** significantly affected the activity compared with series **6**, resulting in increased activities for most compounds except for the *m*-Cl and *o*-CN substituted derivatives **7c**, and **7e**.

For the compounds in series **13** and **14**, there appeared to be no significant differences in activity between the two series of compounds. The position of the methyl-substituent on the benzene ring was found to exert a significant influence on the activity, and an activity order of *o* > *m* > *p* was found for both series. While methoxy substituted derivative **13j** exhibited slightly weaker activity with -1.83 ± 0.20% increased stroke volume and **14j** showed no activity. For halogen substituents (Cl, Br) on the phenyl ring, an activity order of *m* > *p* > *o* was found for chlorinated compounds in series of **13**, whereas an activity order of *m* > *o* > *p* was observed for **14** series. For brominated compounds, the activity order of *o* > *m* was obtained in both **13** and **14** series. The aforementioned results also suggest that there are no remarkable differences for the activity between the triazole ring and the piperazine moiety, and no clear regularity could be found for the effects of the substituents R_1' position and physicochemical properties to the activity, indicating that the electronic effect of the substituent on the benzene ring is not critical.

In addition, we investigated the dynamics of the compounds in perfused beating rabbit atria and found that 18 compounds possessed inotropic effects (**6b**, **6e**, **6h**, **7b**, **7f**, **7g**, **7h**, **13c**, **13d**, **13e**, **13f**, **14a**, **14b**, **14c**, **14e**, **14h**, **14i**, and **14f**) and presented desirable biological dynamic profiles compared with that of the standard drug nifedipine (Figure 2). It was found that the stroke volumes of the test compounds (**6b**, **6e**, **6h**, **7b**, **7f**, **7g**, **7h**, **13c**, **13d**, **13e**, **13f**, **14a**, **14b**, **14c** and **14e**) were gradually decreased as the time progressed and kept unchanged after falling to a certain extent (Figure 2A, 2B, 2C, 2D and 2E), but less desirable dynamic property was observed for **14h**, **14i**, and **14f** whose stroke volume was kept changed (Figure 2F). On the other hand, the dose–activity relationships of selected compounds **6e** and **7h** were also tested at concentrations of 1×10^{-5} mol/L, 3×10^{-5} mol/L, and 1×10^{-4} mol/L, respectively. Both compounds showed a maximal effect at 3×10^{-5} mol/L, whereas less activity was observed at the lower 1×10^{-6} mol/L and higher 1×10^{-4} mol/L doses (Figure 3). Fortunately, none of the test compounds showed any significant effect on heart rates.

Investigation of the mechanism of action of compound 7h

A preliminary investigation of the mechanism of action of compound **7h** on the regulation of atrial dynamics was performed (Figure 4). It is well known that decrease of the influx of Ca^{2+} ions or increase of the outflow of K^+ ion generates a negative inotropic effect on the myocardium. Therefore, another series of experiments were performed with ATP-sensitive K^+ channel and L-type Ca^{2+} channel blockers glibenclamide and nifedipine in the isolated perfused beating rabbit atria. As shown in Figure 4A, there was a slight decrease of the trend line, but was not obvious after treated with K^+ ion channel blocker glibenclamide. However, the trend line decreased rapidly with the addition of **7h**. It is obvious that the negative inotropic effect of **7h** was not directly related to the blocking of K^+ ion channel. On the contrary, the myocardial cell stroke was significantly decreased after treated with L-type Ca^{2+} channel blocker nifedipine and no further decrease was found with the addition of **7h** (Figure 4C). As shown in Figure 4D, the similar trends of L-type Ca^{2+} channel blocker

nifedipine (Figure 4B) and nifedipine + **7h** (Figure 4C) indicated that the negative inotropic effects of **7h** on the rabbit atrium might be blocked by the L-type Ca^{2+} channel blocker nifedipine. As shown in Figure 4, glibenclamide failed to modulate the negative inotropic effect of compound **7h** on atrial dynamics. However, nifedipine completely abolished the negative regulatory effect of compound **7h** in the beating rabbit atria. The results demonstrate that the negative inotropic effect of these synthesized compounds is likely associated with the blockade of the L-type calcium channel.

Cytotoxicity

Several compounds with significant inotropic activities were evaluated for their toxicity against human cervical cancer cells HeLa, liver cancer cells Hep3B and human normal hepatic cells LO2 with the half inhibitory concentration (IC_{50}) value of the tested compounds measured by the MTT assay. IC_{50} values are the average of three independent experiments running in triplicate. As shown in Table 3, these compounds were tested at multiple doses and did not show any significant cytotoxic activity ($IC_{50} \geq 100$ μmol/L) against two human cancer cell lines and one human normal cell lines.

Conclusion

Thirty-eight 4-phenyl-1*H*-1,2,4-triazol-5(4*H*)-one derivatives containing triazole or piperazine moieties were synthesized and evaluated for negative inotropic activity for the first time. Most of the synthesized compounds showed potential negative inotropic activity. In particular, compound **7h** exhibited promising cardiovascular properties and potent negative inotropic activity, being 4-fold more potent compared with the standard drug metoprolol. The results of the cytotoxic tests indicated that these series of compounds did not exhibit any remarkable cytotoxic activity. Furthermore, a preliminary study of the mechanism of action of **7h** on the atrial dynamics was also conducted. The result demonstrates that the negative inotropic property of these compounds is likely associated with the blockade of the

L-type calcium channels. The work might provide an experimental basis for the development of new candidates with potent negative inotropic activities for the clinical treatment of chronic congestive heart failure.

Acknowledgement

The work was supported by the National Science Foundation of China (No. 81160381).

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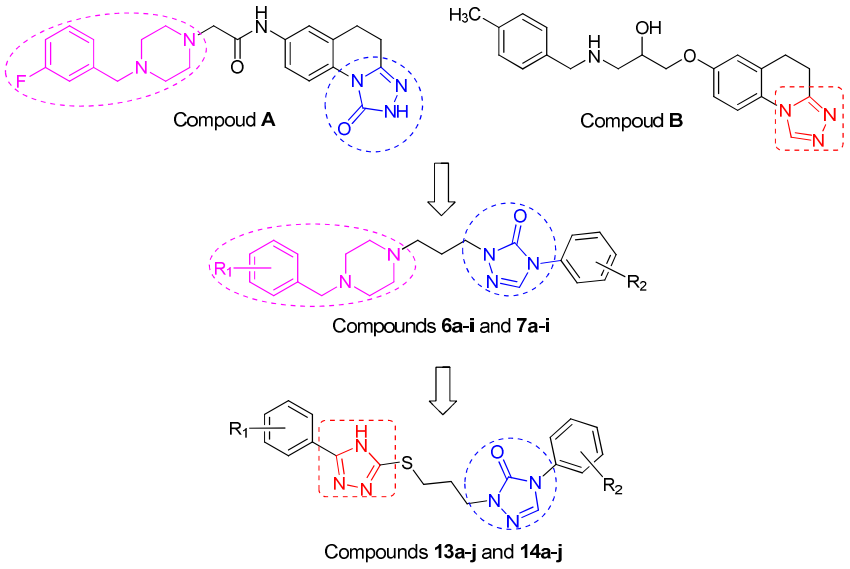


Figure 1. Structures of the previously reported compound **A**, **B** and target compounds.

Figure 2. Effects of the test compounds **6b**, **6e**, **6h**, **7b**, **7f**, **7g**, **7h**, **13c**, **13d**, **13e**, **13f**, **14a**, **14b**, **14c**, **14e**, **14h**, **14i**, **14f** and metoprolol on stroke volume in beating rabbit atria (1.5 Hz). Values are means ± SE. $^{***}P < 0.001$ vs. control.

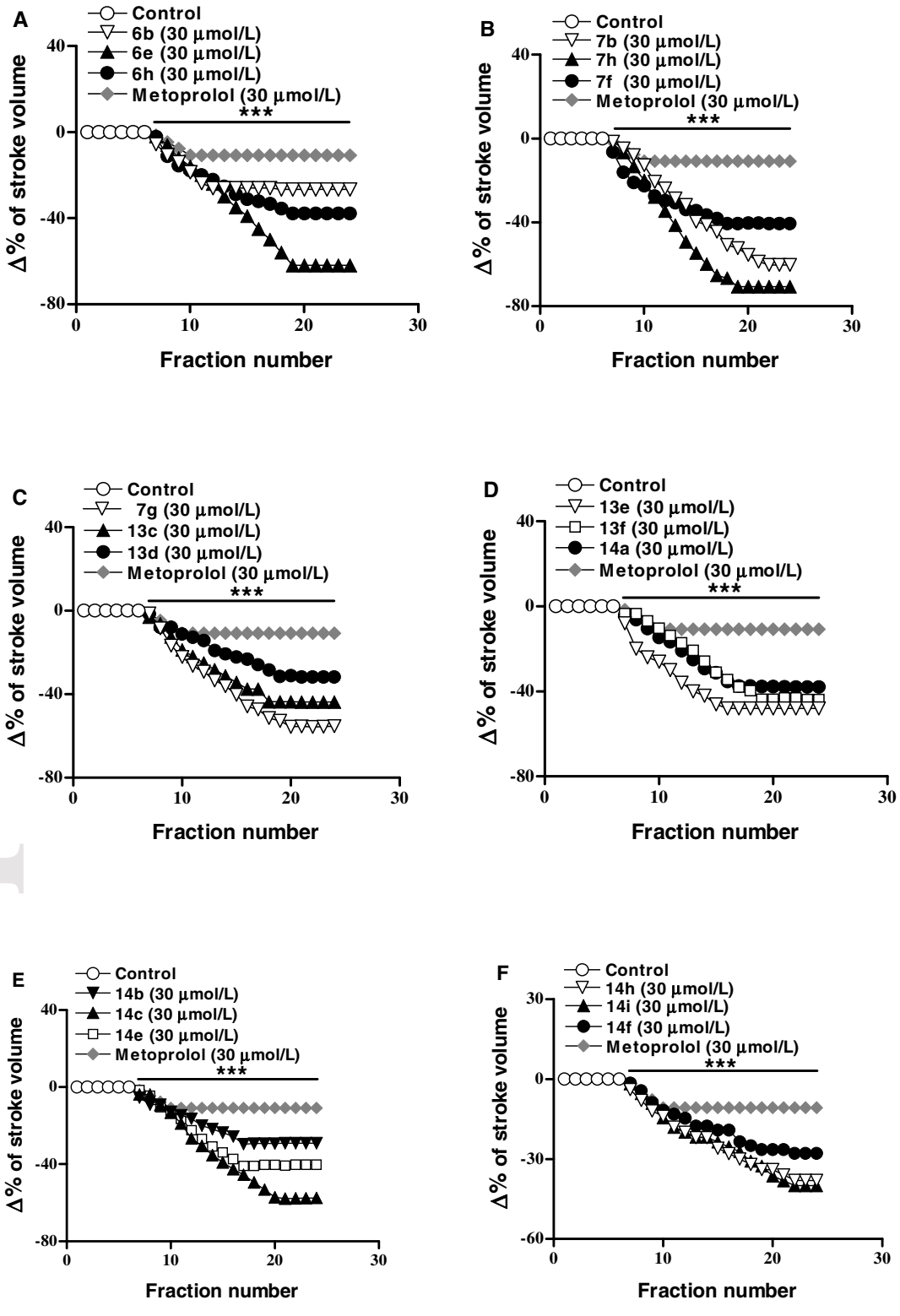


Figure 3. Concentration-response curves of compounds **6e**, **7h** and metoprolol on stroke volume in beating rabbit atria (1.5 Hz). Values are means ± SE. $^{***}P < 0.001$ versus control.

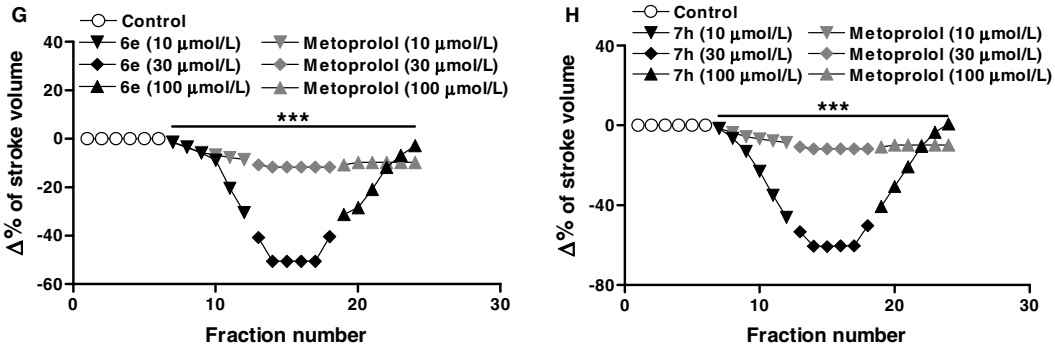
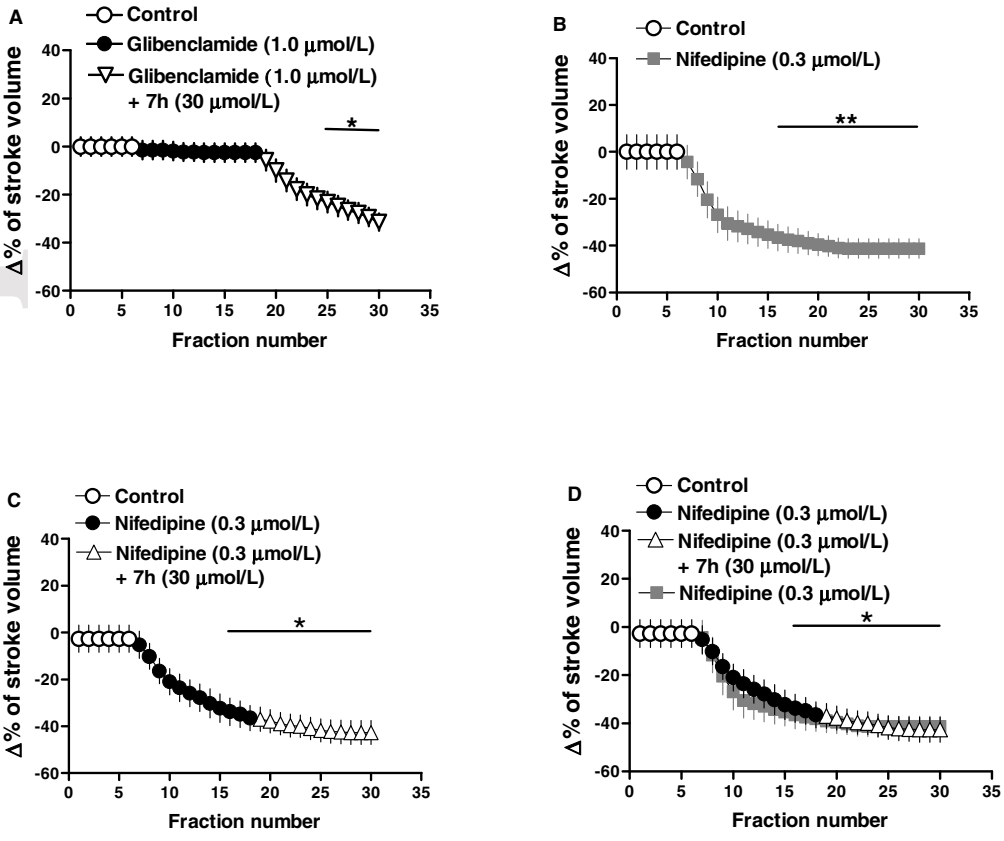
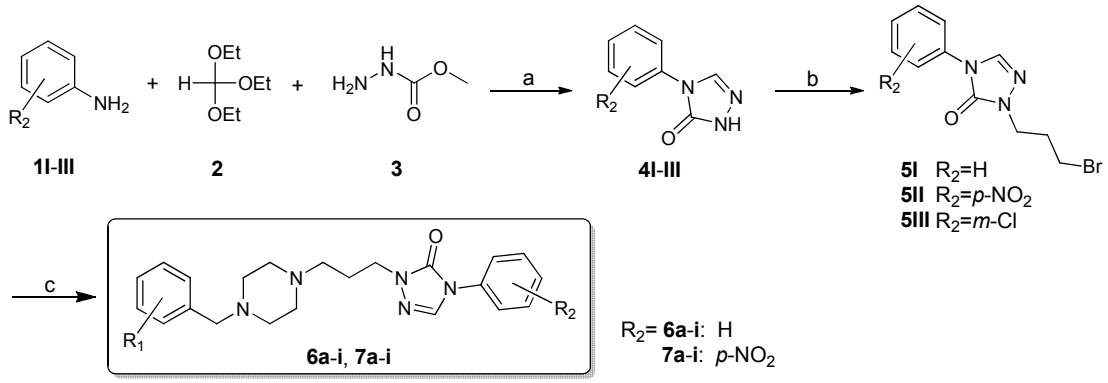
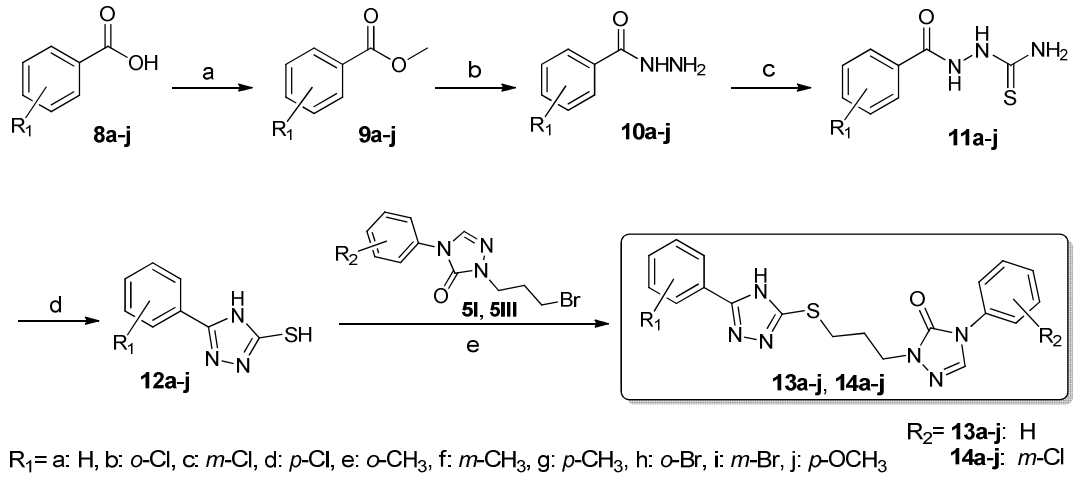


Figure 4. Effects of L-type Ca^{2+} channel inhibitor nifedipine and K^{+} channel inhibitor glybenclamide on compound **7h** induced decrease in atrial pulse pressure and stroke volume. Date are the mean ± SE (n = 6). $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the control period.



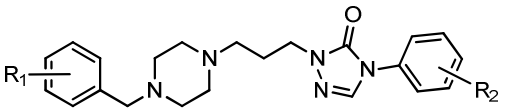


Scheme 1. Synthetic scheme for the synthesis of compounds **6a**-**i** and **7a**-**i**. Reagents and conditions: (a) EtOH, MeONa, reflux, 48h; (b) $Br(CH_2)_3Br$, K_2CO_3, KI, acetone, reflux, 6 h; (c) monosubstituted piperazines, K_2CO_3, KI, acetone, reflux, 10 h.



Scheme 2. Synthetic scheme for the synthesis of compounds **13a**-**j** and **14a**-**j**. Reagents and conditions: (a) MeOH, H_2SO_4, reflux, 5h; (b) $NH_2NH_2.H_2O$, EtOH, reflux, 10h; (c) KSCN, HCl, reflux, 3h; (d) 2N NaOH, reflux, 3h; (e) K_2CO_3, KI, acetone, reflux, 10 h.

Table1. Negative inotropic activity of compounds **6a**-**i** and **7a**-**i** in the left atrium stroke volume test in isolated rabbit heart preparations.



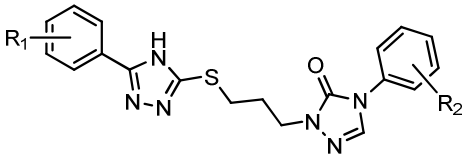
R_2= **6a-i**: H
7a-i: *p*-NO_2

Compound	R_1	Change in stroke volume (%)[a]
6a	H	−4.55±0.24
6b	*o*-Cl	−26.30±0.24
6c	*m*-Cl	−16.67±0.21
6d	*p*-Cl	−0.79±0.14
6e	*o*-CN	−41.56±0.55
6f	*m*-F	−19.83±0.43
6g	*p*-F	−8.60±0.35
6h	*p*-OCH_3	−27.65±0.61
6i	*2,4*-$(Cl)_2$	−11.18±0.30
7a	H	−15.39±0.39
7b	*o*-Cl	−36.42±0.14
7c	*m*-Cl	−11.85±0.29
7d	*p*-Cl	−39.06±0.68
7e	*o*-CN	−4.76±0.07
7f	*m*- F	−32.53±0.57
7g	*p*-F	−39.58±0.06
7h	*p*-OCH_3	−48.22±0.36
7i	*2,4*-$(Cl)_2$	−14.47±0.40
Metopeolol[b]		−9.74±0.14

[a] The concentration of the test samples was 3×10^{-5}mol/L.

[b] Metoprolol was used as positive control.

Table2. Negative inotropic activity of compounds **13a-j** and **14a-j** in the left atrium stroke volume test in isolated rabbit heart preparations.



R_2= **13a-j**: H
14a-j: *m*-Cl

Compound	R_1	Change in stroke volume (%)[a]
13a	H	−3.93±0.09
13b	*o*-Cl	−13.74±0.83
13c	*m*-Cl	−30.73±0.74
13d	*p*-Cl	−20.81±0.53
13e	*o*-CH_3	−38.11±0.65
13f	*m*-CH_3	−27.78±0.83
13g	*p*-CH_3	−9.54±0.24
13h	*o*-Br	−17.62±0.48
13i	*m*-Br	−11.17±0.28
13j	*p*-OCH_3	−1.83±0.20
14a	H	−30.21±0.83
14b	*o*-Cl	−21.01±0.44
14c	*m*-Cl	−32.81±0.09
14d	*p*-Cl	−8.17±0.26
14e	*o*-CH_3	−27.20±0.71
14f	*m*-CH_3	−18.87±0.47

14g	*p*-CH_3	−2.56±0.18
14h	*o*-Br	−25.22±0.60
14i	*m*-Br	−24.85±0.67
14j	*p*-OCH_3	–[c]
Metopeolol[b]		−9.74±0.14

[a] The concentration of the test samples was 3×10^{-5} mol/L.

[b] Metoprolol was used as positive control.

[c] No effect.

Table3. Cytotoxicity data (IC_{50}, μmol/L) of compounds in HeLa, Hep3B and LO2 cells.

Compound	**IC_{50} (μmol/L)** [a]		
	HeLa[b]	**Hep3B**[c]	**LO2**[d]
6e	>200	200	>200
7d	>200	>200	>200
7h	100	>200	>200
13e	>200	>200	135
14c	>200	>200	189

[a] Values are the average of three independent experiments running in triplicate. Variation was generally between 5–10%.

[b] Human cervical cancer cells.

[c] Human liver cancer cells.

[d] Human normal hepatic cells.

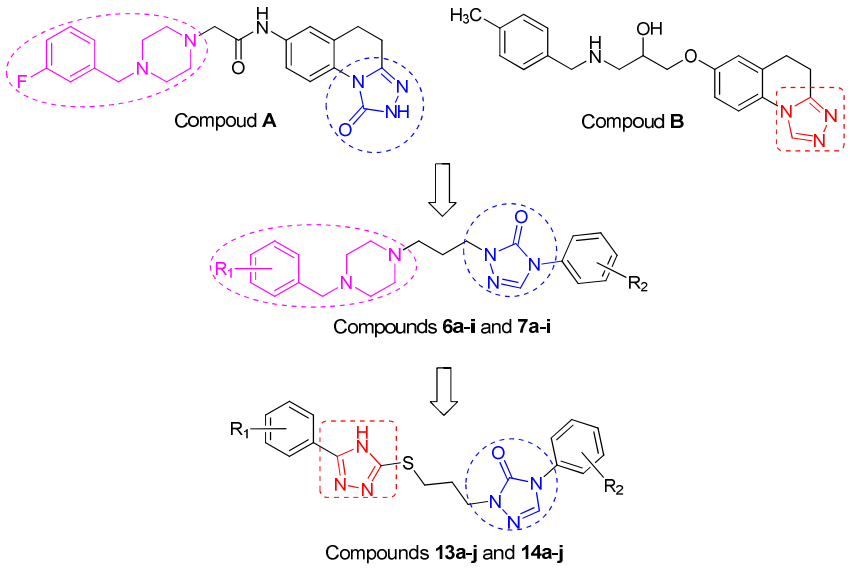
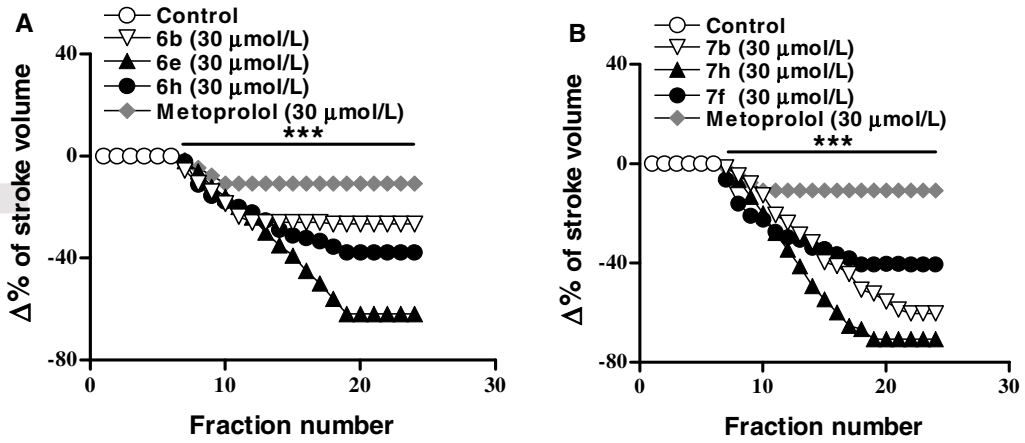


Figure 1. Structures of the previously reported compound **A**, **B** and target compounds.

Figure 2. Effects of the test compounds **6b**, **6e**, **6h**, **7b**, **7f**, **7g**, **7h**, **13c**, **13d**, **13e**, **13f**, **14a**, **14b**, **14c**, **14e**, **14h**, **14i**, **14f** and metoprolol on stroke volume in beating rabbit atria (1.5 Hz). Values are means ± SE. $^{***}P < 0.001$ vs. control.



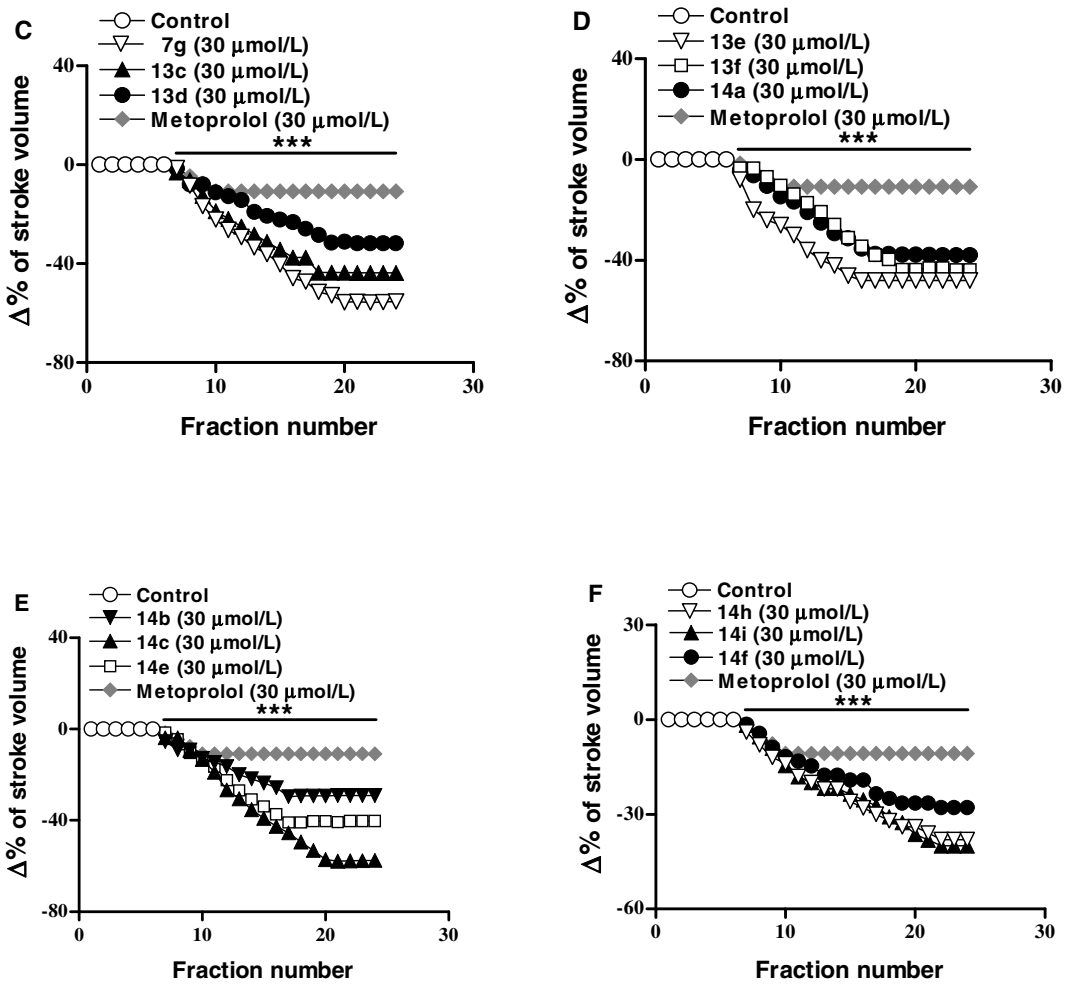


Figure 3. Concentration-response curves of compounds **6e**, **7h** and metoprolol on stroke volume in beating rabbit atria (1.5 Hz). Values are means ± SE. $^{***}P < 0.001$ versus control.

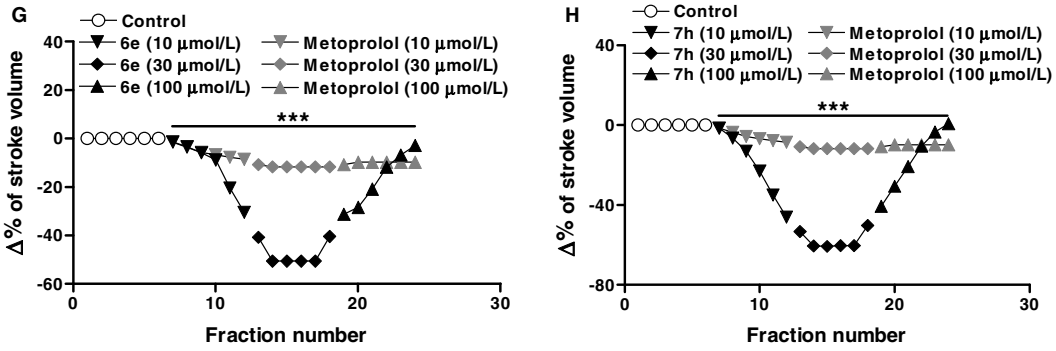
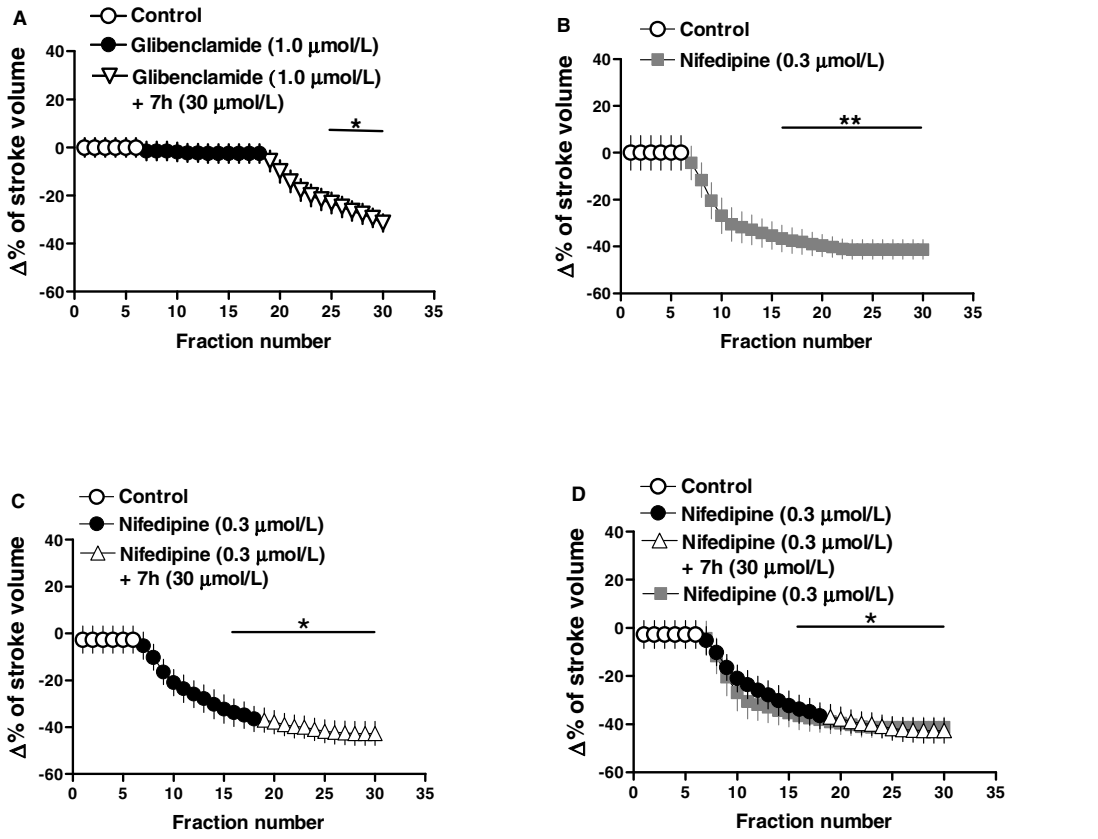
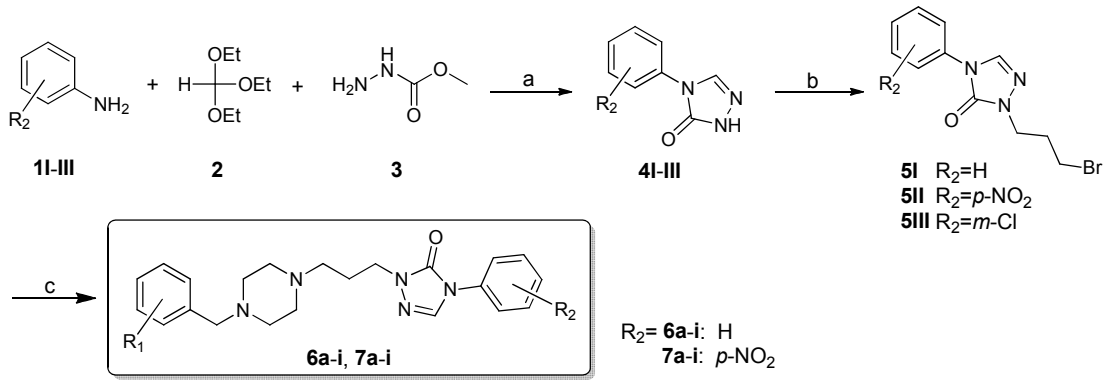


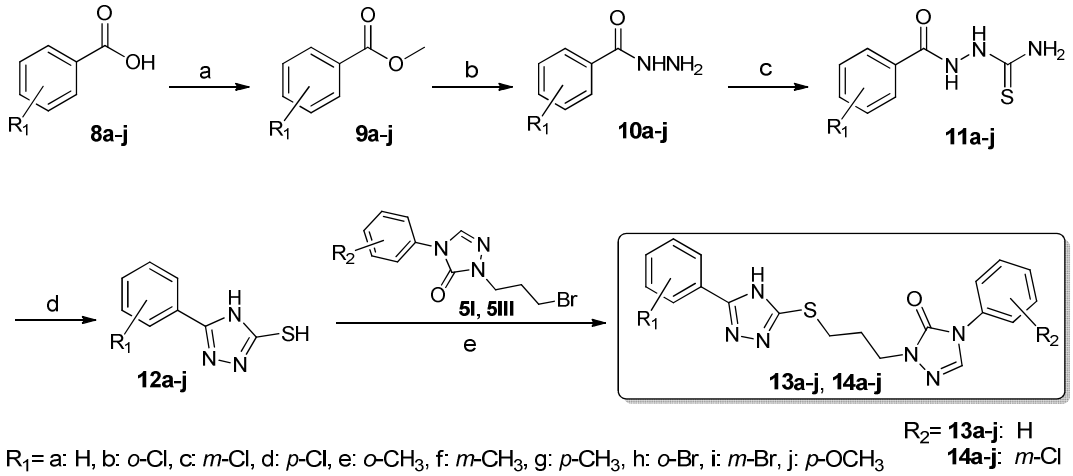
Figure 4. Effects of L-type Ca^{2+} channel inhibitor nifedipine and K^+ channel inhibitor glybenclamide on compound **7h** induced decrease in atrial pulse pressure and stroke volume. Date are the mean ± SE (n = 6). $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the control period.





R_1= a: H, b: *o*-Cl, c: *m*-Cl, d: *p*-Cl, e: *o*-CN, f: *m*-F, g: *p*-F, h: *p*-OCH_3, i: 2,4-Cl_2

Scheme 1. Synthetic scheme for the synthesis of compounds **6a**-**i** and **7a**-**i**. Reagents and conditions: (a) EtOH, MeONa, reflux, 48h; (b) $Br(CH_2)_3Br$, K_2CO_3, KI, acetone, reflux, 6 h; (c) monosubstituted piperazines, K_2CO_3, KI, acetone, reflux, 10 h.



Scheme 2. Synthetic scheme for the synthesis of compounds **13a**-**j** and **14a**-**j**. Reagents and conditions: (a) MeOH, H_2SO_4, reflux, 5h; (b) $NH_2NH_2.H_2O$, EtOH, reflux, 10h; (c) KSCN, HCl, reflux, 3h; (d) 2N NaOH, reflux, 3h; (e) K_2CO_3, KI, acetone, reflux, 10 h.