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Synthesis of Piperazine Derivatives and Biological Evaluation of Its Inhibitory Activities on Vascular Smooth Muscle Cell Proliferation

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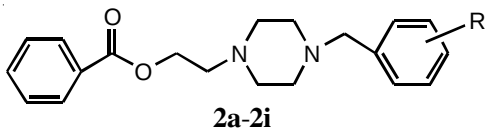
A series of new N-substituted benzyl piperazine derivatives were synthesized using the appropriate synthetic route. The new compounds were characterized by IR, ^{1}H NMR, MS and elemental analysis. All the compounds were screened for their inhibitory activities on vascular smooth muscle cell proliferation. Some of the compounds demonstrated moderate to good inhibition of vascular smooth muscle cell proliferation. The compounds (**2h**, **6h**) were approximately equivalent in activity to heparin as a potent inhibitor. The possible structure-activity relationship of the inhibitory activity of all compounds was also discussed.

Keywords: Piperazine, Vascular smooth muscle cell, Inhibitory activity.

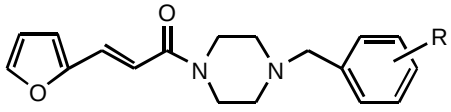
INTRODUCTION

Atherosclerosis (AS) is a syndrome affecting arterial blood vessels, a chronic inflammatory response in the walls of arteries[1]. There has been growing concern about the treatment of atherosclerosis, but many problems remain to be solved for most inhibitors available[2,3]. Several studies have demonstrated that vascular smooth muscle cell (VSMC) proliferation plays an important role in the initiation and aggravation of atherosclerosis[4-6]. Hence, new inhibitors for vascular smooth muscle cell proliferation are needed.

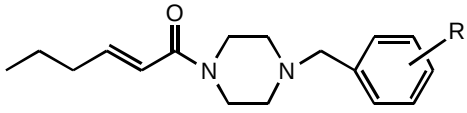
Piperazine is an important class of heterocyclic compounds and many piperazine derivatives have been reported to possess a high spectrum of biological activities, such as GBR12909, a potent inhibitor of dopamine uptake[7]; clozapine, an antipsychotic agent used for the treatment of schizophrenia[8]; ranolazine, an antianginal medication[9]; and quipazine, a moderately selective serotonin receptor agonist[10]. The piperazine core acts as a scaffold to generate biologically active molecules. In this paper, we chose N-substituted benzyl piperazines as key intermediates and incorporated them in a single molecule for synthesis of the target compounds (Fig 1). All structures were characterized by IR, ^{1}H NMR, MS and elemental analysis and tested for their inhibitory activities on vascular smooth muscle cell proliferation.



2a-2i



4a-4i



6a-6i

Fig. 1. General formulas of the prepared compounds

EXPERIMENTAL

Melting points were recorded on an electrothermal type 9100 melting point apparatus. The IR spectra were measured with a Bruker IR Prestige-21 instrument. For all compounds, ^{1}H NMR spectra were measured on a Bruker DPX 600 instrument. Chemical shifts were given in parts per million with TMS as the internal reference. Elemental analyses were performed by PE-2400II. The analytical results (C, H, N) were within ±0.3 % of the theoretical values. MS spectra were recorded

on a Shimadzu QP-1000EX. All reactions were monitored by thin layer chromatography, carried out on 0.2 mm silica gel 60-254 plates using UV light (254 and 366 nm) for detection.

General procedure for the synthesis of compounds 2a-2i: Potassium carbonate (0.02 mol) was added to a solution of 2-chloroethyl benzoate **1** (0.01 mol) and different N-substituted benzyl piperazines (0.01 mol) in DMF (20 mL). The mixture was refluxed for 4 h. After completion of the reaction, potassium carbonate was removed by filtration and the solvent was concentrated under reduced pressure to yield the crude product. The obtained residue was purified by recrystallized ethanol.

2-[4-(2-Chlorobenzyl)piperazin-1-yl]ethyl benzoate (2a): Yield: 91.1 %; m.p.: 223-225 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.292-8.063 (m, 9H, Ar-H), 4.483 (s, 2H, CH_2), 3.473 (s, 2H, CH_2), 2.764-2.825 (d, 2H, CH_2), 2.752 (s, 4H, pip), 2.253-2.587 (s, 4H, pip); IR (KBr, ν_{max}, cm^{-1}): 3420, 3156, 2985, 2643, 1720, 1618, 1403, 1284, 823; Anal. Calcd. for $C_{20}H_{23}N_2O_2Cl$: C, 66.94; H, 6.46; N, 7.81. Found: C, 70.06; H, 6.76; N, 7.62. MS (ESI) *m/z*: 358.86 $[M]^+$.

2-[4-(3-Chlorobenzyl)piperazin-1-yl]ethyl benzoate (2b): Yield: 94.7 %; m.p.: 237-239 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.303-8.057 (m, 9H, Ar-H), 4.491 (s, 2H, CH_2), 3.496 (s, 2H, CH_2), 2.862-3.012 (d, 2H, CH_2), 3.073 (s, 4H, pip), 1.680-2.212 (s, 4H, pip); IR (KBr, ν_{max}, cm^{-1}): 3420, 3111, 2985, 2681, 2594, 1724, 1618, 1405, 714; Anal. Calcd. for $C_{20}H_{23}N_2O_2Cl$: C, 66.94; H, 6.46; N, 7.81. Found: C, 66.81; H, 6.69; N, 7.51. MS (ESI) *m/z*: 358.86 $[M]^+$.

2-[4-(4-Chlorobenzyl)piperazin-1-yl]ethyl benzoate (2c): Yield: 96.4 %; m.p.: 214 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.281-8.057 (m, 9H, Ar-H), 4.471(s, 2H, CH_2), 3.481 (s, 2H, CH_2), 2.795-2.834 (d, 2H, CH_2), 2.628 (s, 4H, pip), 2.189-2.493 (s, 4H, pip); IR (KBr, ν_{max}, cm^{-1}): 3430, 3139, 2947, 2816, 1719, 1580, 1400, 1284, 801; Anal. Calcd. for $C_{20}H_{23}N_2O_2Cl$: C, 66.94; H, 6.46; N, 7.81. Found: C, 66.92; H, 6.58; N, 7.84. MS (ESI) *m/z*: 358.86 $[M]^+$.

2-[4-(2-Methylbenzyl)piperazin-1-yl]ethyl benzoate (2d): Yield: 93.8 %; m.p.: 225-227 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.196-8.008 (m, 9H, Ar-H), 4.457 (s, 2H, CH_2), 3.651 (s, 2H, CH_2), 2. 573 (s, 2H, CH_2), 2.932 (s, 4H, pip), 2.864 (s, 4H, pip), 1.548 (s, 3H, CH_3); IR (KBr, ν_{max}, cm^{-1}): 3421, 2853, 2649, 2261, 1716, 1692, 1453, 1295, 1024; Anal. Calcd. for $C_{21}H_{26}N_2O_2$: C, 74.52; H, 7.74; N, 8.28. Found: C, 74.48; H, 7.50; N, 8.37. MS (ESI) *m/z*: 338.44 $[M]^+$.

2-[4-(3-Methylbenzyl)piperazin-1-yl]ethyl benzoate (2e): Yield: 94.5 %; m.p.: 216-218 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.197-8.002 (m, 9H, Ar-H), 4.492 (s, 2H, CH_2), 3.914 (s, 2H, CH_2), 2.358 (s, 2H, CH_2), 3.015 (s, 4H, pip), 2.924 (s, 4H, pip), 1.385-1.434 (s, 3H, CH_3); IR (KBr, ν_{max}, cm^{-1}): 3420, 3135, 2922, 2603, 1729, 1410, 1279, 1033; Anal. Calcd. for $C_{21}H_{26}N_2O_2$: C, 74.52; H, 7.74; N, 8.28. Found: C, 74.87; H, 7.52; N, 8.34. MS (ESI) *m/z*: 338.44 $[M]^+$.

2-[4-(4-Methylbenzyl)piperazin-1-yl]ethyl benzoate (2f): Yield: 97.6 %; m.p.: 240-241 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.101-8.030 (m, 9H, Ar-H), 4.426 (s, 2H, CH_2), 3.461 (s, 2H, CH_2), 2.787 (s, 2H, CH_2), 2.601 (s, 4H, pip), 2.325-2.485 (s, 4H, pip), 1.813 (s, 3H, CH_3); IR (KBr, ν_{max}, cm^{-1}): 3425, 2947, 2811, 1719, 1603, 1458, 1284, 1130, 1009; Anal. Calcd. for $C_{21}H_{26}N_2O_2$: C, 74.52; H, 7.74; N, 8.28. Found: C, 74.69; H, 7.63; N, 8.48. MS (ESI) *m/z*: 338.44 $[M]^+$.

2-[4-(2-Fluorobenzyl)piperazin-1-yl]ethyl benzoate (2g): Yield: 95.2 %; m.p.: 209-211 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.043-8.001 (m, 9H, Ar-H), 4.632 (s, 2H, CH_2), 3.862 (s, 2H, CH_2), 2.764-2.804 (d, 2H, CH_2), 3.062 (s, 8H, pip); IR (KBr, ν_{max}, cm^{-1}): 3437, 2857, 2635, 1658, 1459, 1276, 1148, 1024, 861; Anal. Calcd. for $C_{20}H_{23}N_2O_2F$: C, 70.15; H, 6.77; N, 8.18. Found: C, 70.26; H, 6.68; N, 8.32. MS (ESI) *m/z*: 342.43 $[M]^+$.

2-[4-(3-Fluorobenzyl)piperazin-1-yl]ethyl benzoate (2h): Yield: 96.8 %; m.p.: 233-235 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.275-7.621 (m, 9H, Ar-H), 4.634 (s, 2H, CH_2), 3.827 (s, 2H, CH_2), 2.821-2.954 (d, 2H, CH_2), 2.785 (s, 4H, pip), 2.251-2.463 (d, 4H, pip); IR (KBr, ν_{max}, cm^{-1}): 3425, 2927, 2599, 1719, 1603, 1405, 1284, 1120, 714; Anal. Calcd. for $C_{20}H_{23}N_2O_2F$: C, 70.15; H, 6.77; N, 8.18. Found: C, 70.32; H, 6.91; N, 8.28. MS (ESI) *m/z*: 342.43 $[M]^+$.

2-[4-(4-Fluorobenzyl)piperazin-1-yl]ethyl benzoate (2i): Yield: 98.4 %; m.p.: 228-230 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 6.974-8.050 (m, 9H, Ar-H), 4.465 (s, 2H, CH_2), 3.479 (s, 2H, CH_2), 2.789-2.829 (d, 2H, CH_2), 2.629 (s, 4H, pip), 2.185-2.494 (d, 4H, pip); IR (KBr, ν_{max}, cm^{-1}): 3425, 3135, 1719, 1603, 1516, 1405, 1284, 1125, 825; Anal. Calcd. for $C_{20}H_{23}N_2O_2F$: C, 70.15; H, 6.77; N, 8.18. Found: C, 70.34; H, 6.86; N, 8.29. MS (ESI) *m/z*: 342.43 $[M]^+$.

General procedure for the synthesis of compounds 4a-4i: To a stirred solution of 2-furylacrylic acid **3** (0.01 mol) and different N-substituted benzyl piperazine (0.01 mol) in CH_2Cl_2 (20 mL) were added ethyldiisopropylamine (0.02 mol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.04 mol) at room temperature. After stirred overnight, the reaction mixture was partitioned between water and CH_2Cl_2. The organic layer was washed with 10 % aqueous hydrochloric acid, aqueous $NaHCO_3$ and brine and then dried over anhydrous $MgSO_4$. The solvent was evaporated to get the crude product. The obtained residue was purified by recrystallized ethanol.

(*E*)-1-[4-(2-Chlorobenzyl)piperazin-1-yl]-3-(furan-2-yl)prop-2-en-1-one (4a): Yield: 97.8 %; m.p.: 132-135 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 6.56 (d, 1H, furan-H), 6.44 (d, 1H, =CH), 6.82 (d, 1H, =CH), 7.55 (s, 2H, furan-H), 7.67-7.69 (m, 2H, Ar-H), 7.40-7.47 (m, 2H, Ar-H), 3.46 (s, 2H, CH_2), 4.16 (s, 4H, pip), 2.78-3.24 (d, 4H, pip); IR (KBr, ν_{max}, cm^{-1}): 3436, 2924, 2580, 2454, 1657, 1615, 1423, 1255, 951; Anal. Calcd. for $C_{18}H_{19}N_2O_2Cl$: C, 65.36; H, 5.79; N, 8.47. Found: C, 65.52; H, 5.86; N, 8.61. MS (ESI) *m/z*: 330.84 $[M]^+$.

(*E*)-1-[4-(3-Chlorobenzyl)piperazin-1-yl]-3-(furan-2-yl)prop-2-en-1-one (4b): Yield: 96.4 %; m.p.: 211-213 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 6.54 (d, 1H, furan-H), 6.42 (d, 1H, =CH), 6.81 (d, 1H, =CH), 7.54 (s, 2H, furan-H), 7.49-7.64 (m, 2H, Ar-H), 7.27-7.49 (m, 2H, Ar-H), 3.42 (s, 2H, CH_2), 4.13 (d, 4H, pip), 2.74-2.95 (d, 4H, pip); IR (KBr, ν_{max}, cm^{-1}): 3436, 2924, 2580, 2454, 1657, 1615, 1423, 1225, 951; Anal. Calcd. for $C_{18}H_{19}N_2O_2Cl$: C, 65.36; H, 5.79; N, 8.47. Found: C, 65.49; H, 5.91; N, 8.72. MS(ESI)*m/z*: 330.84 $[M]^+$.

(*E*)-1-[4-(4-Chlorobenzyl)piperazin-1-yl]-3-(furan-2-yl)prop-2-en-1-one (4c): Yield: 98.2 %; m.p.: 109-110 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 6.57(d, 1H, furan-H), 6.42 (d, 1H, =CH), 6.80 (d, 1H, =CH), 7.54 (s, 2H, furan-H), 7.51-7.64 (m, 2H, Ar-H), 7.35-7.47 (m, 2H, Ar-H), 3.49 (s, 2H, CH_2), 3.64 (s, 2H, pip), 3.74 (s, 2H, pip), 2.45 (s, 4H, pip); IR (KBr,

ν_{max}, cm^{-1}): 3444, 3042, 2940, 2814, 1649, 1599, 1481, 1229, 1011; Anal. Calcd. for $C_{18}H_{19}N_2O_2Cl$: C, 65.36; H, 5.79; N, 8.47. Found: C, 65.30; H, 5.78; N, 8.43. MS (ESI) *m/z*: 330.84 $[M]^+$.

(*E*)-1-[4-(2-Methylbenzyl)piperazin-1-yl]-3-(furan-2-yl)prop-2-en-1-one (4d): Yield: 96.7 %; m.p.: 243-245 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 6.57 (d, 1H, furan-H), 6.44 (d, 1H, =CH), 6.81 (d, 1H, =CH), 7.55 (s, 2H, furan-H), 7.52-7.63 (m, 2H, Ar-H), 7.34-7.47 (m, 2H, Ar-H), 3.62 (s, 2H, CH_2), 3.82 (s, 4H, pip), 2.69-2.75 (s, 4H, pip), 2.39 (s, 3H, CH_3); IR (KBr, ν_{max}, cm^{-1}): 3426, 2924, 2522, 2432, 1645, 1605, 1423, 1229, 1007; Anal. Calcd. for $C_{19}H_{22}N_2O_2$: C, 73.52; H, 7.14; N, 9.03. Found: C, 73.46; H, 7.06; N, 8.97. MS (ESI) *m/z*: 310.39 $[M]^+$.

(*E*)-1-[4-(3-Methylbenzyl)piperazin-1-yl]-3-(furan-2-yl)prop-2-en-1-one (4e): Yield: 95.8 %; m.p.: 221-224 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 6.57 (d, 1H, furan-H), 6.45 (d, 1H, =CH), 6.81 (d, 1H, =CH), 7.57 (s, 2H, furan-H), 7.54-7.67 (m, 2H, Ar-H), 7.38-7.45 (m, 2H, Ar-H), 3.62 (s, 2H, CH_2), 4.08 (s, 4H, pip), 2.72-2.92 (s, 4H, pip), 2.37 (s, 3H, CH_3); IR (KBr, ν_{max}, cm^{-1}): 3412, 2974, 2522, 2404, 1715, 1477, 1371, 1271, 1111; Anal. Calcd. for $C_{19}H_{22}N_2O_2$: C, 73.52; H, 7.14; N, 9.03. Found: C, 73.62; H, 7.18; N, 9.16. MS (ESI) *m/z*: 310.39 $[M]^+$.

(*E*)-1-[4-(4-Methylbenzyl)piperazin-1-yl]-3-(furan-2-yl)prop-2-en-1-one (4f): Yield: 97.4 %; m.p.: 261-263 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 6.58 (d, 1H, furan-H), 6.44 (d, 1H, =CH), 6.81 (d, 1H, =CH), 7.57 (s, 2H, furan-H), 7.51-7.64 (m, 2H, Ar-H), 7.35-7.46 (m, 2H, Ar-H), 3.62 (s, 2H, CH_2), 4.14 (s, 4H, pip), 2.72 (s, 4H, pip), 2.35 (s, 3H, CH_3); IR (KBr, ν_{max}, cm^{-1}): 3428, 2924, 2522, 2446, 1649, 1607, 1423, 1229, 1011; Anal. Calcd. for $C_{19}H_{22}N_2O_2$: C, 73.52; H, 7.14; N, 9.03. Found: C, 73.59; H, 7.21; N, 9.11. MS (ESI) *m/z*: 310.39 $[M]^+$.

(*E*)-1-[4-(2-Fluorobenzyl)piperazin-1-yl]-3-(furan-2-yl)prop-2-en-1-one (4g): Yield: 96.3 %; m.p.: 134-137 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 6.58 (d, 1H, furan-H), 6.44 (d, 1H, =CH), 6.80 (d, 1H, =CH), 7.55 (s, 2H, furan-H), 7.51-7.64 (m, 2H, Ar-H), 7.25-7.46 (m, 2H, Ar-H), 3.42 (s, 2H, CH_2), 3.82-4.14 (d, 4H, pip), 2.46 (d, 4H, pip); IR (KBr, ν_{max}, cm^{-1}): 3436, 3024, 2937, 2801, 1649, 1601, 1423, 1229, 1011; Anal. Calcd. for $C_{18}H_{19}N_2O_2F$: C, 68.77; H, 6.09; N, 8.91. Found: C, 68.82; H, 6.15; N, 9.02. MS (ESI) *m/z*: 314.35 $[M]^+$.

(*E*)-1-[4-(3-Fluorobenzyl)piperazin-1-yl]-3-(furan-2-yl)prop-2-en-1-one (4h): Yield: 97.1 %; m.p.: 163-165 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 6.57 (d, 1H, furan-H), 6.44 (d, 1H, =CH), 6.81 (d, 1H, =CH), 7.57 (s, 2H, furan-H), 7.43-7.57 (m, 2H, Ar-H), 7.23-7.34 (m, 2H, Ar-H), 3.43 (s, 2H, CH_2), 3.81-4.12 (d, 4H, pip), 2.83 (d, 4H, pip); IR (KBr, ν_{max}, cm^{-1}): 3436, 3115, 2932, 2827, 1649, 1601, 1423, 1229, 1011; Anal. Calcd. for $C_{18}H_{19}N_2O_2F$: C, 68.77; H, 6.09; N, 8.91. Found: C, 68.86; H, 6.24; N, 8.99. MS (ESI) *m/z*: 314.35 $[M]^+$.

(*E*)-1-[4-(4-Fluorobenzyl)piperazin-1-yl]-3-(furan-2-yl)prop-2-en-1-one (4i): Yield: 98 %; m.p.: 96-98 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 6.58 (d, 1H, furan-H), 6.44 (d, 1H, =CH), 6.81 (d, 1H, =CH), 7.59 (s, 2H, furan-H), 7.22-7.48 (m, 2H, Ar-H), 7.01-7.15 (m, 2H, Ar-H), 3.47 (s, 2H, CH_2), 3.62-3.71 (d, 4H, pip), 2.43 (d, 4H, pip); IR (KBr, ν_{max}, cm^{-1}): 3436, 3126, 2932, 2814, 1649, 1599, 1423, 1229, 1011; Anal. Calcd. for $C_{18}H_{19}N_2O_2F$: C, 68.77; H, 6.09; N, 8.91. Found: C, 68.59; H, 6.10; N, 8.87. MS (ESI) *m/z*: 314.35 $[M]^+$.

General procedure for the synthesis of compounds (6a-6i): To a solution of (*E*) hex-2-enoic acid **5** (0.01 mol) and different N-substituted benzyl piperazine (0.01 mol) in CH_2Cl_2 (20 mL) was added ethyldiisopropylamine (0.02 mol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.04 mol). The mixture was stirred at room temperature overnight. The reaction mixture was partitioned between water and CH_2Cl_2. The organic layer was washed with 10 % aqueous hydrochloric acid, aqueous $NaHCO_3$ and brine and then dried over anhydrous $MgSO_4$. The solvent was evaporated to give the crude product. The obtained residue was purified by recrystallized ethanol.

(*E*)-1-[4-(2-Chlorobenzyl)piperazin-1-yl]hex-2-en-1-one (6a): Yield: 96.4 %; m.p.: 93-95 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.22-7.41 (m, 4H, Ar-H), 6.81-6.83 (d, 1H, =CH), 6.17-6.21 (d, 1H, =CH), 3.61 (s, 2H, CH_2), 3.56 (s, 2H, CH_2), 3.38 (s, 4H, pip), 2.46 (s, 4H, pip), 1.32-2.24 (d, 2H, CH_2), 0.92-1.16 (m, 3H, CH_3); IR (KBr, ν_{max}, cm^{-1}): 2897, 2736, 1752, 1615, 1394, 1068, 974; Anal. Calcd. for $C_{17}H_{23}N_2OCl$: C, 66.55; H, 7.56; N, 9.13. Found: C, 66.62; H, 7.51; N, 9.24. MS (ESI) *m/z*: 306.83 $[M]^+$.

(*E*)-1-[4-(3-Chlorobenzyl)piperazin-1-yl]hex-2-en-1-one (6b): Yield: 95.3 %; m.p.: 97-98 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.13-7.35 (m, 4H, Ar-H), 6.82-6.91 (d, 1H, =CH), 6.17-6.24 (d, 1H, =CH), 3.63 (s, 2H, CH_2), 3.51 (s, 2H, CH_2), 3.42 (s, 4H, pip), 2.43 (s, 4H, pip), 1.42-2.29 (d, 2H, CH_2), 1.03-1.21 (m, 3H, CH_3); IR (KBr, ν_{max}, cm^{-1}): 2907, 2851, 1748, 1614, 1421, 1057, 936; Anal. Calcd. for $C_{17}H_{23}N_2OCl$: C, 66.55; H, 7.56; N, 9.13. Found: C, 66.57; H, 7.61; N, 9.19. MS (ESI) *m/z*: 306.83 $[M]^+$.

(*E*)-1-[4-(4-Chlorobenzyl)piperazin-1-yl]hex-2-en-1-one (6c): Yield: 94.6 %; m.p.: 86-87 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.22-7.30 (m, 4H, Ar-H), 6.80-6.89 (d, 1H, =CH), 6.16-6.21 (d, 1H, =CH), 3.66 (s, 2H, CH_2), 3.52 (s, 2H, CH_2), 3.45 (s, 4H, pip), 2.40 (s, 4H, pip), 1.42-2.19 (d, 2H, CH_2), 0.88-0.93 (m, 3H, CH_3); IR (KBr, ν_{max}, cm^{-1}): 2915, 2795, 1763, 1684, 1433, 1152, 956; Anal. Calcd. for $C_{17}H_{23}N_2OCl$: C, 66.55; H, 7.56; N, 9.13. Found: C, 66.51; H, 7.49; N, 9.22. MS (ESI) *m/z*: 306.83 $[M]^+$.

(*E*)-1-[4-(2-Methylbenzyl)piperazin-1-yl]hex-2-en-1-one (6d): Yield: 92.4 %; m.p.: 85-87 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.19-7.26 (m, 4H, Ar-H), 6.80-6.90 (d, 1H, =CH), 6.19-6.23 (d, 1H, =CH), 3.68 (s, 2H, CH_2), 3.53 (s, 2H, CH_2), 3.49 (s, 4H, pip), 2.42 (s, 4H, pip), 2.41 (s, 3H, Ph-CH_3), 1.43-2.19 (d, 2H, CH_2), 0.89-0.94 (m, 3H, CH_3); IR (KBr, ν_{max}, cm^{-1}): 2879, 2716, 1746, 1631, 1408, 1034, 949; Anal. Calcd. for $C_{18}H_{26}N_2O$: C, 75.48; H, 9.15; N, 9.78. Found: C, 75.51; H, 9.19; N, 9.83. MS (ESI) *m/z*: 286.41 $[M]^+$.

(*E*)-1-[4-(3-Methylbenzyl)piperazin-1-yl]hex-2-en-1-one (6e): Yield: 93.1 %; m.p.: 79-81 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.16-7.28 (m, 4H, Ar-H), 6.80-6.91 (d, 1H, =CH), 6.19-6.24 (d, 1H, =CH), 3.67 (s, 2H, CH_2), 3.56 (s, 2H, CH_2), 3.49 (s, 4H, pip), 2.42 (s, 4H, pip), 2.59 (s, 3H, Ph-CH_3), 1.45-2.21 (d, 2H, CH_2), 0.81-0.93 (m, 3H, CH_3); IR (KBr, ν_{max}, cm^{-1}): 2913, 2707, 1774, 1682, 1396, 1146, 953; Anal. Calcd. for $C_{18}H_{26}N_2O$: C, 75.48; H, 9.15; N, 9.78. Found: C, 75.56; H, 9.23; N, 9.84. MS (ESI) *m/z*: 286.41 $[M]^+$.

(*E*)-1-[4-(4-Methylbenzyl)piperazin-1-yl]hex-2-en-1-one (6f): Yield: 93.8 %; m.p.: 90-91 °C; ^{1}H NMR ($CDCl_3$,

600 MHz) δ: 7.13-7.27 (d, 4H, Ar-H), 6.81-6.91 (d, 1H, =CH), 6.19-6.24 (d, 1H, =CH), 3.68 (s, 2H, CH_2), 3.54 (s, 2H, CH_2), 3.49 (s, 4H, pip), 2.43 (s, 4H, pip), 2.35 (s, 3H, Ph-CH_3), 1.45-2.19 (d, 2H, CH_2), 0.91-0.96 (m, 3H, CH_3); IR (KBr, ν_{max}, cm^{-1}): 2913, 2707, 1774, 1682, 1396, 1146, 953; Anal. Calcd. for $C_{18}H_{26}N_2O$: C, 75.48; H, 9.15; N, 9.78. Found: C, 75.53; H, 9.24; N, 9.69. MS (ESI) *m/z*: 286.41 $[M]^+$.

(*E*)-1-[4-(2-Fluorobenzyl)piperazin-1-yl]hex-2-en-1-one (6g): Yield: 91.6 %; m.p.: 65-68 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.11-7.34 (m, 4H, Ar-H), 6.81-6.91 (d, 1H, =CH), 6.18-6.23 (d, 1H, =CH), 3.68 (s, 2H, CH_2), 3.54 (s, 2H, CH_2), 3.48 (s, 4H, pip), 2.42 (s, 4H, pip), 1.47-2.43 (d, 2H, CH_2), 0.90-0.97 (m, 3H, CH_3); IR (KBr, ν_{max}, cm^{-1}): 2937, 2708, 1764, 1609, 1434, 1138, 1026; Anal. Calcd. for $C_{17}H_{23}N_2OF$: C, 70.32; H, 7.98; N, 9.65. Found: C, 70.39; H, 7.92; N, 9.69. MS (ESI) *m/z*: 290.37 $[M]^+$.

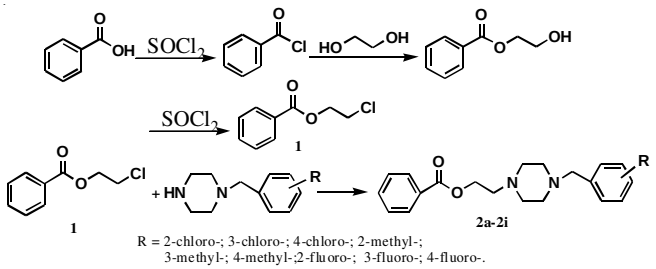
(*E*)-1-[4-(3-Fluorobenzyl)piperazin-1-yl]hex-2-en-1-one (6h): Yield: 92.7 %; m.p.: 69-71 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 6.98-7.32 (m, 4H, Ar-H), 6.81-6.91 (d, 1H, =CH), 6.18-6.25 (d, 1H, =CH), 3.68 (s, 2H, CH_2), 3.52 (s, 2H, CH_2), 3.47 (s, 4H, pip), 2.42 (s, 4H, pip), 1.45-2.40 (d, 2H, CH_2), 0.91-0.95 (m, 3H, CH_3); IR (KBr, ν_{max}, cm^{-1}): 2890, 2716, 1767, 1638, 1409, 1105, 983; Anal. Calcd. for $C_{17}H_{23}N_2OF$: C, 70.32; H, 7.98; N, 9.65. Found: C, 70.41; H, 8.02; N, 9.68. MS (ESI) *m/z*: 290.37 $[M]^+$.

(*E*)-1-[4-(4-Fluorobenzyl)piperazin-1-yl]hex-2-en-1-one (6i): Yield: 95.4 %; m.p.: 74-76 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 6.98-7.30 (m, 3H, Ar-H), 6.81-6.91 (d, 1H, =CH), 6.18-6.23 (d, 1H, =CH), 3.68 (s, 2H, CH_2), 3.54 (s, 2H, CH_2), 3.47 (s, 4H, pip), 2.42 (s, 4H, pip), 1.44-2.40 (d, 2H, CH_2), 0.90-0.95 (m, 3H, CH_3); IR (KBr, ν_{max}, cm^{-1}): 2943, 2731, 1752, 1639, 1408, 1031, 959; Anal. Calcd. for $C_{17}H_{23}N_2OF$: C, 70.32; H, 7.98; N, 9.65. Found: C, 70.37; H, 8.01; N, 9.69. MS (ESI) *m/z*: 290.37 $[M]^+$.

Pharmacology: Vascular smooth muscle cells were isolated from male rats as described by *Gunther et al.*[13]. Cells were grown in DMEM (Gibco, USA) supplemented with 0.125 % trypsin (Sigma, USA) and were seeded in white 96-well microplates for luminescence for 24 h. Then, the change of vascular smooth muscle cell was synchronized for 24 h in serum-free Eagle's DMEM to obtain quiescent cells (control group). The samples were pulsed with thymidine (sigma, USA) for the last 4 h and were subcultured with 10 % FBS (Hyclone, USA) for 24 h. Inhibitory activity was determined at controlled temperature (25 ± 2 °C). The inhibition of each test compound was calculated by MTT assay and IC_{50} values were determined by logistic regression analysis.

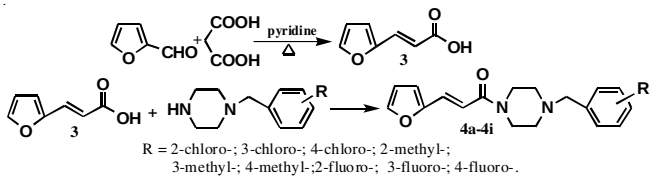
RESULTS AND DISCUSSION

The route 2-chloroethyl benzoate **1** was synthesized using the procedure indicated in the literature[11] and then coupled with different N-substituted benzyl piperazine derivatives in the presence of a base in DMF to obtain compounds **2a-2i**. This reaction was monitored by ^{1}H NMR to confirm the presence of -NH (δ1.58). Findings indicated the absence of -NH (δ1.58) of compounds **2a-2i** and the presence of -NH proton at 1.58 δ in the substituted benzyl piperazine derivatives (**Scheme-I**).



Scheme-I: Synthetic scheme for the synthesis of compounds **2a-2i**

Compounds **4a-4i** were obtained in two steps. In the first step, the preparation of 2-furylacrylic acid **3** was done as described in the literature[12] and then **3** with N-substituted benzyl piperazines afforded compounds **4a-4i** in the second step. This result was confirmed by ^{1}H NMR spectrum, similar to **2a-2i**. Findings showed the disappearance of -NH (δ1.58) and the -OH (δ11.23) of 2-furylacrylic acid (**3**) (**Scheme-II**).



Scheme-II: Synthetic scheme for the synthesis of compounds **4a-4i**

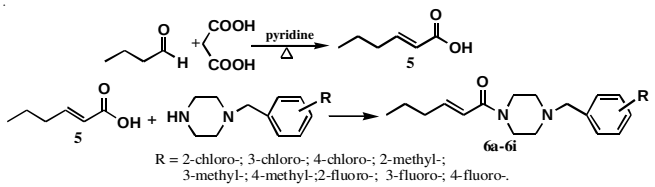
The synthesis of compounds **6a-6i** was achieved by previously synthesized (*E*) hex-2-enoic acid **5** with N-substituted benzyl piperazines (**Scheme-III**).

Biological evaluation: All compounds were tested for their efficacy as inhibitors of vascular smooth muscle cell proliferation using the MTT colourimetric assay. IC_{50} inhibition values were determined at least three times. The results of these studies are summarized in Table-1. All the target compounds

TABLE-1
INHIBITORY ACTIVITY OF THE TARGET COMPOUNDS

R	Series A	IC_{50} (μM)[a]	Series B	IC_{50} (μM)[a]	Series C	IC_{50} (μM)[a]
2-Chloro-	2a	6.72	4a	20.34	6a	5.82
3-Chloro-	2b	8.96	4b	51.36	6b	6.34
4-Chloro-	2c	1.35	4c	29.85	6c	1.21
2-Methyl-	2d	1.54	4d	26.43	6d	1.52
3-Methyl-	2e	2.36	4e	21.07	6e	1.93
4-Methyl-	2f	1.97	4f	21.18	6f	4.32
2-Fluoro-	2g	1.62	4g	34.01	6g	2.64
3-Fluoro-	2h	0.53	4h	16.84	6h	0.49
4-Fluoro-	2i	0.97	4i	25.11	6i	1.47
Heparin	-	0.79	-	-	-	-

[a]Mean ± standard deviation calculated based on three experiments



Scheme-III: Synthetic scheme for the synthesis of compounds **6a-6i**

exhibited inhibitory activity. Several compounds (**2h**, **6h**) showed significant activity with IC_{50} values less than that of heparin (0.79 μM). Further studies on compounds **2h** and **6h** should be carried out to further evaluate their inhibitory activity on vascular smooth muscle cell proliferation.

Generally, the inhibitory activity of N-substituted benzyl piperazine derivatives may be increased remarkably after the introduction of a substitute atom. The inhibitory activities of synthesized compounds vary depending on the N-substituted benzyl piperazines. Therefore, different substituted derivatives were designed and synthesized in this paper. The structure-activity relationship (SAR) studies revealed that the series of **2a-2i** and **6a-6i** showed relatively better inhibitory activities than compounds **4a-4i**. The characteristic of the type of furan ring seemed to have more influence on inhibitory activity. Comparison of different substituted derivatives indicated that the F-substituted compounds (**2g**, **2h**, **2i**, **6g**, **6h**, **6i**) of **2a-2i** and **6a-6i** exhibited moderate to good activity. By comparing the derivatives with different F-substitution positions on the benzyl piperazine, we found that the *meta*-F-substituted derivative (**2h**, **6h**) showed high activity. The electron-withdrawing groups seemed to contribute to inhibitory activity. The electronic effect of the substituent on the benzyl piperazine was an important factor on such activity.

Conclusion

In summary, a series of piperazine derivatives were synthesized and their inhibitory activities of vascular smooth muscle cell proliferation were evaluated. Some compounds presented good inhibitory activity. Several derivatives, including the compounds **2h** and **6h**, demonstrated potent inhibition against all the strains tested. These derivatives will be further refined in our future work.

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REFERENCES

1. A.V. Finn, M. Nakano, J. Narula, F.D. Kolodgie and R. Virmani, *Arterioscler. Thromb. Vasc. Biol*., **30**, 1282 (2010).
2. A. Mahmud and J. Feely, *Am. J. Hypertens*., **15**, 321 (2002).
3. M.V. Autieri, C.J. Carbone and H. Eisen, *J. Heart Lung Transplant*., **20**, 198 (2001).
4. T. Jacob, E. Ascher, D. Alapat, Y. Olevskaia and A. Hingorani, *Eur. J. Vasc. Endovasc. Surg*., **29**, 470 (2005).
5. A. Sanmartin, R. Foncea, F. Laurindo, R. Ebensperger, K. Griendling and F. Leighton, *Free Radic. Biol. Med*., **42**, 1671 (2007).
6. N. Fujita, Y. Furukawa, J. Du, N. Itabashi, G. Fujisawa, K. Okada, T. Saito and S. Ishibashi, *Mol. Cell. Endocrinol*., **192**, 75 (2002).
7. M. Kimura, T. Masuda, K. Yamada, M. Mitani, N. Kubota, N. Kawakatsu, K. Kishii, M. Inazu, Y. Kiuchi, K. Oguchi and T. Namiki, *Bioorg. Med. Chem*., **12**, 3069 (2004).
8. A. Alves-Rodrigues, R. Leurs, E. Willems and H. Timmerman, *Arch. Pharm*., **329**, 413 (1996).
9. M.P. Chandler, W.C. Stanley, H. Morita, G. Suzuki, O. Nass, B. Blackburn, A. Wolfe and H. Sabbah, *J. Mol. Cell. Cardiol*., **34**, 81 (2002).
10. Y. Rival, R. Hoffmann, B. Didier, V. Rybaltchenko, J.-J. Bourguignon and C.G. Wermuth, *J. Med. Chem*., **41**, 311 (1998).
11. A.H. Ford, *Org. Synth*., **30**, 84 (1963).
12. S. Rajagopalan and J.R. Johnson, *Org. Synth*., **3**, 425 (1955).
13. S. Gunther, R.W. Alexander, W.J. Atkinson and M.A. Gimbrone, *J. Cell Biol*., **92**, 289 (1982).