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RESEARCH ARTICLE

Synthesis and biological evaluation of some dibenzofuran-piperazine derivatives

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Abstract

In the present paper, a novel series of dibenzofuran-piperazine derivatives were synthesized via the treatment of *N*-(2-methoxy-3-dibenzofuranyl)-2-chloroacetamide with substituted piperazine derivatives. The chemical structures of the compounds were elucidated by ^{1}H NMR, ^{13}C NMR, mass spectral data; elemental analysis and HPLC analysis. Each derivative was evaluated for antiplatelet activity and anticholinesterase activity. Compound **2 m** with 2-furoyl moiety exhibited high percentage inhibition as much as standard drug aspirin on arachidonic acid (AA)-induced platelet aggregation. None of the compounds presented significant inhibitor effect on collagen-induced platelet aggregation. Furthermore, the anticholinesterase activity of the compounds was determined and they did not show promising inhibitor activity compared with standard drug donepezil.

Keywords

Anticholinesterase activity, antiplatelet activity, dibenzofuran, piperazine

History

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Introduction

Platelets, also called trombocytes, play an essential role in hemostasis and pathological thrombosis[1–3]. Platelet activation and aggregation are important necessities in the pathogenesis of athero thrombotic events characteristic of the acute coronary syndromes (ACSs) or due to mechanical disruption of plaque by percutaneous coronary interventions (PCIs). Hyperactivity of platelets increase the risk of various vaso-occlusive diseases, such as unstable angina, acute myocardial infarction and transient ischemic attacks[4,5]. Antiplatelet therapy is a basic tool in the prevention and treatment of thromboembolic diseases. In injured vessels, blood aggregation is generated as a physiological defense reaction by releasing biologically active compound, such as adenosine 5′-diphosphate (ADP), thrombin and prostaglandine endoperoxide. Agonists such as thrombin, arachidonic acid (AA), thromboxane A2 (TxA2), platelet-activating factor (PAF) and collagen are able to induce platelet aggregation. Among these agonists, AA is one of the most powerful agonists for platelet activation[6,7]. Currently, essential antiplatelet drugs used for the prophylaxis and treatment of thromboembolic diseases are aspirin, ridogrel, ticlopidine, clopidogrel, dipyridamole, cilostazol, tirofiban and sibrafiban[8–12]. Nevertheless, these orally administered antiplatelet drugs have certain disadvantages, such as gastric erosion, agranulocytosis, neutropenia, thrombocytopenia, aplastic anemia and thrombotic thrombocytopenic purpura along with inefficient therapy[13–16]. Moreover, development of resistance to these drugs is another handicap for therapy[17,18]. These limitations are among the reasons stimulating the search for new antiplatelet drugs.

Beraprost sodium (Figure 1) is a stable synthetic analaogue of prostaglandin I2 (PGI2) which have both potent antiplatelet and peripheral vasodilating actions. It possesses benzofurane ring in its structure[19,20]. The drug which is in clinical trial in Europe and USA presently, inhibits platelet aggregation induced by ADP, collagen and arachidonic acid[21]. Aglafolin (Figure 1) is another benzofuran including molecule, isolated from the stems of *Aglaia elliptifolia*, was notified with effective inhibitor activity of platelet aggregation induced by PAF both *in vitro* and *in vivo*[22]. Recently, in a series of study Sidhu et al.[23–27] and Thalji et al.[28] have also declared different benzofuran-bearing compounds with significant antiplatelet activity. Moreover, benzofuran ring analog dibenzofuran-bearing compounds have been reported to exhibit a great variety of biological some effects, including thrombosis[29,30] and anticholinesterase activity[31–33] distinct from the mentioned above. However, piperazine ring plays an important role for antiplatelet activity[34]. By the effect of the findings about antiplatelet and anticholinesterase activities of dibenzofurans and piperazines, it was thought that it would be worthwhile to synthesize on a combination system that bears these vital moieties on the same chemical skeleton. Thus our research group synthesize dibezofuran-piperazine compounds so as to investigate probable antiplatelet and anticholinesterase activity.

Experimental

Chemistry

All chemicals were purchased from Sigma-Aldrich Chemical Co (St. Louis, MO) and Merck Chemicals (Merck KGaA, Darmstadt, Germany). All melting points (m.p.) were determined by Electrothermal 9100 digital melting point apparatus

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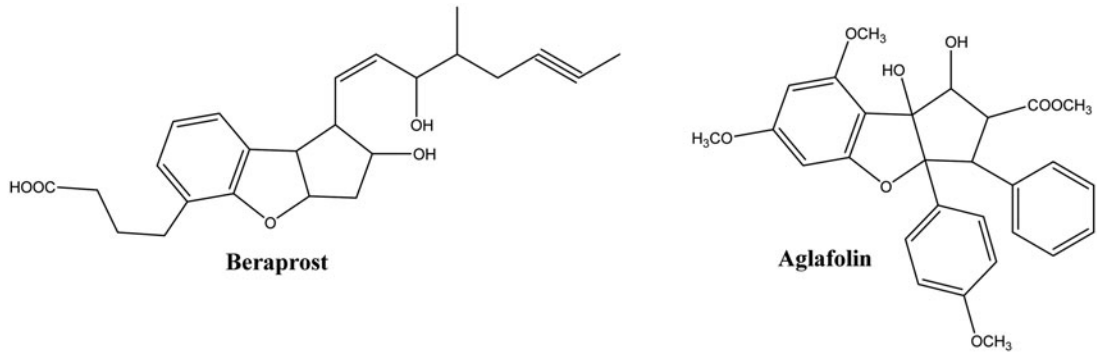


Figure 1. The chemical structures of beraprost and aglafolin.

(Electrothermal, Essex, UK) and are uncorrected. All the reactions were monitored by thin-layer chromatography (TLC) using Silica Gel 60 F254 TLC plates (Merck KGaA, Darmstadt, Germany). Spectroscopic data were recorded with the following instruments: IR, Shimadzu 8400S spectrophotometer (Shimadzu, Tokyo, Japan); NMR, Bruker DPX 500 NMR spectrometer (Bruker Bioscience, Billerica, MA, USA), in DMSO-d_6, using TMS as internal standard; M + 1 peaks were determined by AB Sciex-3200 Q-TRAP LC/MS/MS system (AB Applied Biosystems Co., MA). Purity of synthesized compounds was checked by Shimadzu LC-20 A Prominence HPLC system, equipped with a Shimadzu DGU-14 A degasser, LC-20 A dual piston pump, CTO-10 ASVP column oven and SPD-MI20A PDA detector. Reodyne 7725i injection valve and a stainless steel GL Science Inertsil ODS-3 (4.6 × 250 mm) column. Solvents for the separation compounds **2a**, **2i**, **2 l** and **2 m** were: solvent A, acetonitrile (95%); solvent B, water (5%) at a flow rate of 1.0 ml/min and a sample injection volume of 20 µl. Solvents for the separation of all other compounds were: solvent A, acetonitrile (70%); solvent B, water (30%) at a flow rate of 1.2 ml/min and a sample injection volume of 20 µl. Elemental analyses were also performed on a Leco TruSpec Micro CHN/CHNS elemental analyzer (Leco, St. Joseph, MI).

Synthesis of the compounds

*N-(2-Methoxy-3-dibenzofuranyl)-2-chloroacetamide **(1)***

Chloroacetyl chloride (0.2 mol, 16 ml) was added drop wise over 15 min to a magnetically stirring solution of 2-methoxy-3-aminodibenzofuran (0.2 mol) and triethylamine (0.2 mol, 28 ml) in dry THF (200 ml) at 0–5 °C and the reaction mixture was stirred for one hour at room temperature. After controlled the reaction ending by TLC, it completed and the solvent was evaporated under reduced pressure and then water was added to wash the resulting solid and the mixture was filtered, dried and recrystallized from ethanol to give compound **1**.

*General synthesis procedure for N-(2-methoxy-3-dibenzofuranyl)-2-(4-substituted piperazin-1-yl)acetamide derivatives **(2a-m)***

Compound **1** (10 mmol) was refluxed with 1-substituted piperazine derivatives (10 mmol) and potassium carbonate (10 mmol) in acetone for 5 h (200 °C). After TLC screening, the solvent was evaporated and then water was added to wash the resulting solid and the mixture was filtered and the obtained crude product was dried and then crystallized from ethanol.

*N-(2-Methoxy-3-dibenzofuranyl)-2-(4-methylpiperazin-1-yl) acetamide **(2a)***

Yield 72–75%, m.p. 166–168 °C. HPLC: 95.2% purity. IR (KBr, cm^{-1}): ν_{max} 3346 (amide N–H), 1671 (C = O), 1541–1316 (C = C), 1267–926 (C–O, C–N). ^{1}H NMR (500 MHz, DMSO-d_6, ppm): δ 2.23 (s, 3H, N–CH_3), 2.42 (brs, 4H, piperazine–H), 2.64 (brs, 4H, piperazine–H), 3.19 (s, 2H, –CO–CH_2), 4.04 (s, 3H, O–CH_3), 7.37 (t, 1H, *J*: 7.5 Hz, Ar–H), 7.45 (t, 1H, *J*: 7.5 Hz, Ar–H), 7.65 (d, 1H, *J*: 8 Hz, Ar–H), 7.85 (s, 1H, Ar–H), 8.08 (d, 1H, *J*: 7.5 Hz, Ar–H), 8.58 (s, 1H, Ar–H), 10.09 (s, 1H, N–H). ^{13}C NMR (125 MHz, DMSO-d_6, ppm): δ 26.27, 53.16, 55.59, 57.23, 61.67, 101.93, 103.20, 111.96, 118.24, 120.24, 123.33, 124.66, 126.93, 127.76, 145.54, 150.20, 156.32, 168.77. For $C_{20}H_{23}N_3O_3$, calculated: 67.97% C, 6.56% H, 11.89% N; found: 67.84% C, 6.54% H, 11.81% N. MS $[M + 1]^+$: *m/z* 354.

*N-(2-Methoxy-3-dibenzofuranyl)-2-(4-fenilpiperazin-1-yl) acetamide **(2b)***

Yield 74%, m.p. 200–203 °C. HPLC: >99.9% purity. IR (KBr, cm^{-1}): ν_{max} 3356 (amide N–H), 1674 (C = O), 1534–1313 (C = C), 1269–926 (C–O, C–N). ^{1}H NMR (500 MHz, DMSO-d_6, ppm): δ 2.23 (s, 3H, N–CH_3), 2.75 (brs, 4H, piperazine–H), 3.25 (brs, 4H, piperazine–H), 3.28 (s, 2H, –CO–CH_2), 3.99 (s, 3H, O–CH_3), 6.82 (t, 1H, *J*: 7.0 Hz, Ar–H), 6.99 (d, 1H, *J*: 7.5 Hz, Ar–H), 7.25 (t, 1H, *J*: 8.5 Hz, Ar–H), 7.37 (t, 1H, *J*: 7.5 Hz, Ar–H), 7.45 (t, 1H, *J*: 8.0 Hz, Ar–H), 7.65 (d, 1H, *J*: 8 Hz, Ar–H), 7.85 (s, 1H, Ar–H), 8.08 (1H, d, *J*: 7 Hz, Ar–H), 10.08 (s, 1H, N–H). ^{13}C NMR (125 MHz, DMSO-d_6, ppm): δ 49.17, 53.23, 57.26, 61.76, 102.07, 103.20, 111.97, 116.02, 118.31, 119.50, 120.97, 123.34, 124.65, 126.95, 127.71, 129.46, 145.58, 150.18, 151.37, 156.32, 168.65. For $C_{25}H_{25}N_3O_3$, calculated: 72.27% C, 6.06% H, 10.11% N; found: 72.26% C, 6.09% H, 10.21% N. MS $[M + 1]^+$: *m/z* 416.

*N-(2-Methoxy-3-dibenzofuranyl)-2-[4-(2-methylphenyl)piperazin-1-yl]acetamide **(2c)***

Yield 70–73%, m.p. 168–170 °C. HPLC: >99.9% purity. IR (KBr, cm^{-1}): ν_{max} 3329 (amide N–H), 1670 (C = O), 1538–1325 (C = C), 1276–9265 (C–O, C–N). ^{1}H NMR (500 MHz, DMSO-d_6, ppm): δ 2.25 (s, 3H, C–CH_3), 2.77 (brs, 4H, piperazine–H), 2.96 (brs, 4H, piperazine–H), 3.29 (s, 2H, –CO–CH_2), 4.05 (s, 3H, O–CH_3), 6.99 (t, 1H, *J*: 6.5 Hz, Ar–H), 7.08 (d, 1H, *J*: 9.0 Hz, Ar–H), 7.17–7.21 (m, 3H, Ar–H), 7.37 (t, 1H, *J*: 7.5 Hz, Ar–H), 7.47 (t, 1H, *J*: 8.0 Hz, Ar–H), 7.67 (d, 1H, *J*: 8.5 Hz, Ar–H), 7.87 (s, 1H, Ar–H), 8.09 (1H, d, *J*: 7.5 Hz, Ar–H), 10.11 (s, 1H, N–H). ^{13}C NMR (125 MHz, DMSO-d_6, ppm): δ 18.04, 52.28, 53.75, 57.32, 61.76, 102.04, 103.23, 111.97, 118.30, 119.23, 120.97, 123.34, 123.47, 124.66, 126.95, 127.11, 127.75, 131.36, 132.33, 145.60, 150.20, 151.60, 156.32, 168.76. For $C_{26}H_{27}N_3O_3$, calculated: 72.71% C, 6.34% H, 9.78% N; found: 72.76% C, 6.36% H, 9.71% N. MS $[M + 1]^+$: *m/z* 430.

N-(2-Methoxy-3-dibenzofuranyl)-2-[4-(3-methylphenyl)piperazin-1-yl]acetamide ***(2d)***

Yield 78–81%, m.p. 77–82 °C. HPLC: 99.1% purity. IR (KBr, cm^{-1}): ν_{max} 3338 (amide N–H), 1673 (C = O), 1562–1311 (C = C), 1249–926 (C–O, C–N). ^{1}H NMR (500 MHz, DMSO-d_6, ppm): δ 2.25 (s, 3H, C–CH_3), 2.73 (brs, 4H, piperazine–H), 3.23 (brs, 4H, piperazine–H), 3.28 (s, 2H, –CO–CH_2), 4.05 (s, 3H, O–CH_3), 6.63 (d, 1H, *J*: 7.5 Hz, Ar–H), 6.77 (d, 1H, *J*: 8.0 Hz, Ar–H), 6.81 (s, 1H, Ar–H), 7.12 (t, 1H, *J*: 8.0 Hz, Ar–H), 7.37 (t, 1H, *J*: 7.5 Hz, Ar–H), 7.43 (t, 1H, *J*: 8.0 Hz, Ar–H), 7.87 (s, 1H, Ar–H), 7.65 (2H, d, *J*: 8.0 Hz, Ar–H), 8.08 (1H, d, *J*: 7.5 Hz, Ar–H), 10.07 (s, 1H, N–H). ^{13}C NMR (125 MHz, DMSO-d_6, ppm): δ 21.89, 49.21, 53.26, 57.26, 61.78, 102.07, 103.18, 111.96, 113.17, 116.70, 118.31, 120.33, 120.95, 123.32, 124.66, 126.94, 127.72, 129.27, 138.51, 145.58, 150.19, 151.39, 156.33, 168.63. For $C_{26}H_{27}N_3O_3$, calculated: 72.71% C, 6.34% H, 9.78% N; found: 72.82% C, 6.21% H, 9.73% N. MS $[M+1]^+$: *m/z* 430.

N-(2-Methoxy-3-dibenzofuranyl)-2-[4-(4-methylphenyl)piperazin-1-yl]acetamide ***(2e)***

Yield 74–78%, m.p. 192–195 °C. HPLC: >99.9% purity. IR (KBr, cm^{-1}): ν_{max} 3258 (amide N–H), 1675 (C = O), 1589–1302 (C = C), 1242–925 (C–O, C–N). ^{1}H NMR (500 MHz, DMSO-d_6, ppm): δ 2.20 (s, 3H, C–CH_3), 2.74 (brs, 4H, piperazine–H), 3.19 (brs, 4H, piperazine–H), 3.27 (s, 2H, –CO–CH_2), 4.05 (s, 3H, O–CH_3), 6.89 (d, 1H, *J*: 8.5 Hz, Ar–H), 7.05 (d, 1H, *J*: 8.5 Hz, Ar–H), 7.38 (t, 1H, *J*: 7.5 Hz, Ar–H), 7.47 (t, 1H, *J*: 7.5 Hz, Ar–H), 7.65 (d, 2H, *J*: 8.0 Hz, Ar–H), 7.85 (s, 1H, Ar–H), 8.08 (2H, d, *J*: 8.0 Hz, Ar–H), 8.60 (1H, s, Ar–H), 10.08 (s, 1H, N–H). ^{13}C NMR (125 MHz, DMSO-d_6, ppm): δ 20.51, 49.67, 53.25, 57.25, 61.75, 102.06, 103.20, 111.97, 116.27, 118.30, 120.96, 123.33, 124.65, 126.95, 127.72, 128.33, 129.89, 145.58, 149.31, 150.19, 156.32, 168.66. For $C_{26}H_{27}N_3O_3$, calculated: 72.71% C, 6.34% H, 9.78% N; found: 72.85% C, 6.36% H, 9.78% N. MS $[M+1]^+$: *m/z* 430.

N-(2-Methoxy-3-dibenzofuranyl)-2-[4-(2-methoxyphenyl)piperazin-1-yl]acetamide ***(2f)***

Yield 75%, m.p. 133–136 °C. HPLC: >99.9% purity. IR (KBr, cm^{-1}): ν_{max} 3358 (amide N–H), 1671 (C = O), 1510–1339 (C = C), 1285–926 (C–O, C–N). ^{1}H NMR (500 MHz, DMSO-d_6, ppm): δ 2.75 (brs, 4H, piperazine–H), 3.08 (brs, 4H, piperazine–H), 3.26 (s, 2H, –CO–CH_2), 3.80 (s, 3H, O–CH_3), 4.0 (s, 3H, O–CH_3), 6.91–7.0 (m, 4H, Ar–H), 7.37 (t, 1H, *J*: 7.5 Hz, Ar–H), 7.38 (t, 1H, *J*: 7.5 Hz, Ar–H), 7.45 (t, 1H, *J*: 7.5 Hz, Ar–H), 7.65 (d, 1H, *J*: 8.0 Hz, Ar–H), 7.86 (s, 1H, Ar–H), 8.09 (1H, d, *J*: 7.5 Hz, Ar–H), 8.60 (1H, s, Ar–H), 10.13 (s, 1H, N–H). ^{13}C NMR (125 MHz, DMSO-d_6, ppm): δ 50.99, 53.54, 55.85, 57.28, 61.81, 102.04, 103.21, 111.96, 112.49, 118.29, 118.53, 120.95, 121.39, 123.08, 123.33, 124.66, 126.94, 127.75, 141.53, 145.59, 150.20, 152.54, 156.32, 168.72. For $C_{26}H_{27}N_3O_4$, calculated: 70.09% C, 6.11% H, 9.43% N; found: 70.04% C, 6.14% H, 9.48% N. MS $[M+1]^+$: *m/z* 446.

N-(2-Methoxy-3-dibenzofuranyl)-2-[4-(3-methoxyphenyl)piperazin-1-yl]acetamide ***(2 g)***

Yield 68–71%, m.p. 130–132 °C. HPLC: 99.3% purity. IR (KBr, cm^{-1}): ν_{max} 3352 (amide N–H), 1667 (C = O), 1516–1315 (C = C), 1276–926 (C–O, C–N). ^{1}H NMR (500 MHz, DMSO-d_6, ppm): δ 2.73 (brs, 4H, piperazine–H), 3.25 (brs, 4H, piperazine–H), 3.27 (s, 2H, –CO–CH_2), 3.72 (s, 3H, O–CH_3), 3.99 (s, 3H, O–CH_3), 6.40 (d, 1H, *J*: 8.5 Hz, Ar–H), 6.51 (s, 1H, Ar–H), 6.75 (d, 1H, *J*: 8.5 Hz, Ar–H), 7.14 (t, 1H, *J*: 8.0 Hz, Ar–H), 7.37 (t, 1H, *J*: 7.5 Hz, Ar–H), 7.45 (t, 1H, *J*: 8 Hz, Ar–H), 7.65 (d, 1H, *J*: 8.0 Hz, Ar–H), 7.85 (s, 1H, Ar–H), 8.08 (d, 1H, *J*: 8.0 Hz, Ar–H), 8.60 (s, 1H, Ar–H), 10.07 (s, 1H, N–H). ^{13}C NMR (125 MHz, DMSO-d_6, ppm): δ 49.12, 53.20, 55.37, 57.26, 61.75, 102.08, 102.15, 103.18, 104.82, 108.63, 111.96, 118.31, 120.96, 123.33, 124.66, 126.95, 127.71, 130.15, 145.59, 150.18, 152.72, 156.32, 160.73, 168.63. For $C_{26}H_{27}N_3O_4$, calculated: 70.09% C, 6.11% H, 9.43% N; found: 70.07% C, 6.18% H, 9.51% N. MS $[M+1]^+$: *m/z* 446.

N-(2-Methoxy-3-dibenzofuranyl)-2-[4-(4-methoxyphenyl)piperazin-1-yl]acetamide ***(2 h)***

Yield 69–73%, m.p. 181–184 °C. HPLC: 99.1% purity. IR (KBr, cm^{-1}): ν_{max} 3349 (amide N–H), 1673 (C = O), 1519–1298 (C = C), 1275–927 (C–O, C–N). ^{1}H NMR (500 MHz, DMSO-d_6, ppm): δ 2.74 (brs, 4H, piperazine–H), 3.13 (brs, 4H, piperazine–H), 3.27 (s, 2H, –CO–CH_2), 3.70 (s, 3H, O–CH_3), 4.0 (s, 3H, O–CH_3), 6.84 (d, 2H, *J*: 8.0 Hz, Ar–H), 7.38 (t, 1H, *J*: 7.5 Hz, Ar–H), 7.45 (t, 1H, *J*: 7.5 Hz, Ar–H), 7.65 (d, 2H, *J*: 8.5 Hz, Ar–H), 7.85 (s, 1H, Ar–H), 8.08 (2H, d, *J*: 7.5 Hz, Ar–H), 8.60 (1H, s, Ar–H), 10.09 (s, 1H, N–H). ^{13}C NMR (125 MHz, DMSO-d_6, ppm): δ 50.57, 53.33, 55.68, 57.25, 61.74, 102.05, 103.19, 111.96, 114.80, 117.97, 118.30, 120.95, 123.33, 124.66, 126.95, 127.73, 145.58, 145.73, 150.19, 153.54, 156.32, 168.66. For $C_{26}H_{27}N_3O_4$, calculated: 70.09% C, 6.11% H, 9.43% N; found: 70.01% C, 6.15% H, 9.50% N. MS $[M+1]^+$: *m/z* 446.

N-(2-Methoxy-3-dibenzofuranyl)-2-[4-(4-nitrophenyl)piperazin-1-yl]acetamide ***(2i)***

Yield 72–73%, m.p. 285–290 °C. HPLC: 99.2% purity. IR (KBr, cm^{-1}): ν_{max} 3354 (amide N–H), 1676 (C = O), 1512–1319 (C = C), 1213–927 (C–O, C–N). ^{1}H NMR (500 MHz, DMSO-d_6, ppm): δ 2.74 (brs, 4H, piperazine–H), 3.59 (brs, 4H, piperazine–H), 3.30 (s, 2H, –CO–CH_2), 4.39 (s, 3H, O–CH_3), 7.12 (d, 2H, *J*: 8.0 Hz, Ar–H), 7.35 (t, 1H, *J*: 7.5 Hz, Ar–H), 7.45 (t, 1H, *J*: 7.5 Hz, Ar–H), 7.65 (d, 2H, *J*: 8.0 Hz, Ar–H), 7.80 (s, 1H, Ar–H), 8.05 (2H, d, *J*: 8.5 Hz, Ar–H), 8.59 (1H, s, Ar–H), 10.03 (s, 1H, N–H). ^{13}C NMR (125 MHz, DMSO-d_6, ppm): δ 50.72, 54.33, 57.25, 62.67, 103.09, 104.43, 113.61, 118.21, 121.59, 123.33, 124.66, 125.20, 126.95, 127.31, 128.82, 138.54, 146.84, 150.08, 153.73, 156.32, 168.81. For $C_{25}H_{24}N_4O_5$, calculated: 65.21% C, 5.25% H, 12.17% N; found: 65.25% C, 5.15% H, 12.20% N. MS $[M+1]^+$: *m/z* 461.

N-(2-Methoxy-3-dibenzofuranyl)-2-[4-(2-pyridyl)piperazin-1-yl]acetamide ***(2j)***

Yield 74%, m.p. 177–179 °C. HPLC: >99.9% purity. IR (KBr, cm^{-1}): ν_{max} 3334 (amide N–H), 1678 (C = O), 1512–1303 (C = C), 1280–925 (C–O, C–N). ^{1}H NMR (500 MHz, DMSO-d_6, ppm): δ 2.63 (brs, 4H, piperazine–H), 3.25 (s, 2H, –CO–CH_2), 3.79 (brs, 4H, piperazine–H), 4.0 (s, 3H, O–CH_3), 6.65–6.69 (2H, m, Ar–H), 7.38 (d, 1H, *J*: 8.0 Hz, Ar–H), 7.42–7.45 (m, 2H, Ar–H), 7.54 (t, 1H, *J*: 7.5 Hz, Ar–H), 8.08 (1H, d, *J*: 8.5 Hz, Ar–H), 8.47 (2H, d, *J*: 7.5 Hz, Ar–H), 10.03 (s, 1H, N–H). ^{13}C NMR (125 MHz, DMSO-d_6, ppm): δ 45.53, 53.05, 57.27, 61.86, 102.11, 103.19, 107.68, 111.97, 113.65, 118.32, 120.96, 123.33, 124.66, 126.95, 127.72, 138.05, 145.62, 148.06, 150.18, 156.33, 159.40, 168.64. For $C_{23}H_{23}N_5O_3$, calculated: 66.17% C, 5.55% H, 16.78% N; found: 66.24% C, 5.45% H, 16.80% N. MS $[M+1]^+$: *m/z* 417.

N-(2-Methoxy-3-dibenzofuranyl)-2-[4-(2-pyrimidinyl)piperazin-1-yl]acetamide ***(2 k)***

Yield 69–71%, m.p. 183–186 °C. HPLC: 99.4% purity. IR (KBr, cm^{-1}): 3358 (amide N–H), 1669 (C = O), 1542–1316 (C = C), 1288–926 (C–O, C–N). ^{1}H NMR (500 MHz, DMSO-d_6, ppm): δ 2.65 (brs, 4H, piperazine–H), 3.27 (s, 2H, –CO–CH_2), 3.86 (brs,

4H, piperazine–H), 4.04 (s, 3H, O–CH_3), 6.67 (t, 1H, *J*: 4.5 Hz, Ar–H), 7.38 (t, 1H, *J*: 7.5 Hz, Ar–H), 7.46 (t, 1H, *J*: 7.5 Hz, Ar–H), 7.66 (d, 1H, *J*: 8.0 Hz, Ar–H), 7.86 (s, 1H, Ar–H), 8.08 (2H, d, *J*: 8.5 Hz, Ar–H), 8.39 (2H, d, *J*: 5 Hz, Ar–H), 8.60 (s, 1H, Ar–H), 10.03 (s, 1H, N–H). ^{13}C NMR (125 MHz, DMSO-d_6, ppm): δ 44.16, 53.03, 57.27, 61.85, 102.12, 103.18, 110.79, 111.97, 118.33, 120.96, 123.33, 124.66, 126.95, 127.71, 145.65, 150.18, 156.33, 158.44, 161.66, 168.61. For $C_{23}H_{23}N_5O_3$, calculated: 66.17% C, 5.55% H, 16.78% N; found: 66.24% C, 5.45% H, 16.80% N. MS $[M+1]^+$: *m/z* 418.

N-(2-Methoxy-3-dibenzofuranyl)-2-(4-benzylpiperazin-1-yl) acetamide ***(2l)***

Yield 70–73%, m.p. 102–105 °C. HPLC: 98.8% purity. IR (KBr, cm^{-1}): ν_{max} 3348 (amide N–H), 1673 (C = O), 1536–1312 (C = C), 1284–927 (C–O, C–N). ^{1}H NMR (500 MHz, DMSO-d_6, ppm): δ 2.62 (brs, 4H, piperazine–H), 3.20 (s, 2H, –CO–CH_2), 3.60 (brs, 2H, C–CH_2), 3.86 (brs, 4H, piperazine–H), 3.99 (s, 3H, O–CH_3), 7.31–7.38 (m, 5H, Ar–H), 7.38 (t, 1H, *J*: 8.5 Hz, Ar–H), 7.64 (d, 1H, *J*: 8.0 Hz, Ar–H), 7.85 (s, 1H, Ar–H), 7.08 (d, 1H, *J*: 7.5 Hz, Ar–H), 8.08 (d, 1H, *J*: 8.5 Hz, Ar–H), 8.57 (s, 1H, Ar–H), 10.06 (s, 1H, N–H). ^{13}C NMR (125 MHz, DMSO-d_6, ppm): δ 53.14, 57.20, 61.59, 102.00, 103.20, 111.96, 118.27, 120.94, 123.33, 124.65, 126.94, 127.51, 127.72, 128.70, 129.55, 145.57, 150.18, 156.31, 168.66. For $C_{26}H_{27}N_3O_3$, calculated: 72.71% C, 6.34% H, 9.78% N; found: 72.74% C, 6.45% H, 9.82% N. MS $[M+1]^+$: *m/z* 430.

N-(2-Methoxy-3-dibenzofuranyl)-2-[4-(2-furoyl)piperazin-1-yl]acetamide ***(2 m)***

Yield 75%, m.p. 150–154 °C. HPLC: 95.9% purity. IR (KBr, cm^{-1}): ν_{max} 3334 (amide N–H), 1678 (C = O), 1512–1303 (C = C), 1280–925 (C–O, C–N). ^{1}H NMR (500 MHz, DMSO-d_6, ppm): δ 2.65 (brs, 4H, piperazine–H), 3.28 (s, 2H, –CO–CH_2), 3.78 (brs, 4H, piperazine–H), 4.05 (s, 3H, O–CH_3), 6.64–6.65 (m, 2H, Ar–H), 7.04 (d, 1H, *J*: 3.5 Hz, Ar–H), 7.36 (t, 1H, *J*: 8.0 Hz, Ar–H), 7.42 (t, 1H, *J*: 7.5 Hz, Ar–H), 7.65 (d, 1H, *J*: 8.5 Hz, Ar–H), 7.86 (s, 1H, Ar–H), 8.08 (d, 1H, *J*: 7.5 Hz, Ar–H), 8.58 (s, 1H, Ar–H), 10.03 (s, 1H, N–H). ^{13}C NMR (125 MHz, DMSO-d_6, ppm): δ 43.97, 53.26, 57.28, 61.55, 102.24, 103.17, 111.79, 111.97, 116.17, 118.40, 120.97, 123.33, 124.64, 126.97, 127.65, 145.24, 145.71, 147.41, 150.15, 156.34, 158.56, 168.44. For $C_{24}H_{23}N_3O_5$, calculated: 66.50% C, 5.35% H, 9.69% N; found: 66.54% C, 5.32% H, 9.72% N. MS $[M+1]^+$: *m/z* 434.

Antiplatelet activity

Platelet aggregation was measured with the APACT aggregometer with software APACT professional version 1.1. (Automated Platelet Aggregation and Coagulation Tracer, Biochemica GmbH, Flacht, Germany) according to the turbidimetric method described by Born et al.[35]. Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were obtained at the Blood Center of the Gazi University Hospital, Ankara. For collagen-induced aggregation studies, PRP and PPP were prepared from the venous blood from healthy volunteers who had not taken any drugs with antiplatelet activity for 15 d. In brief, freshly drawn venous human citrated blood (3.2% sodium citrate, 9:1) was centrifuged at 800 rpm and then at 3500 rpm for 10 min to obtain PRP and PPP, respectively. Platelet numbers were determined with a cell counter (Swelab Alfa, × Boule Medical AB, Stockholm, Sweden) and adjusted to 3.6×10^8 platelets/ml with PPP. The test compound (or the reference inhibitor) is dissolved in dimethylsulfoxide (DMSO). To eliminate solvent effects, the final concentration of DMSO was fixed at <1%. 199 µl sample of PRP was placed in the cuvette of aggregometer and incubated for 5 min at 37 °C with 0.5 µl of test compound (or reference inhibitor, or DMSO) before addition of 10 µl inducer (arachidonic acid, AA, 350 µM collagen, 5 µg/ml final concentration). The percentage of aggregation is determined as the ratio of heights of the aggregation curves with and without the test compound. Each curve is corrected automatically for the light absorption of PPP of the same donor. All experiments were performed in triplicate. Inhibition of platelet aggregation was expressed as percentage of inhibition using the following equation:

$$\%\,\text{Inhibition} = \left(1 - \frac{\text{maximum aggregation of compound-treated PRP}}{\text{maximum aggregation of DMSO-treated PRP}}\right) \times 100$$

AChE inhibition

All compounds were subjected to a slightly modified method of Ellman's test[36] in order to evaluate their potency to inhibit the AChE. The spectrophotometric method is based on the reaction of released thiocholine to give a colored product with a chromogenic reagent 5,5-dithio-bis(2-nitrobenzoic)acid (DTNB). AChE (E.C.3.1.1.7 from Electric Eel, 500 units) and donepezil hydrochloride were purchased from Sigma Aldrich (Steinheim, Germany). Potassium dihydrogen phosphate, DTNB, potassium hydroxide, sodium hydrogen carbonate, gelatine and acetylthiocholine iodide (ATC) were obtained from Fluka (Buchs, Switzerland). Spectrophotometric measurements were performed on a 1700 Shimadzu UV-1700 UV–Vis spectrophotometer. Cholinesterase activity of the compounds (**1–30**) was measured in 100 mM phosphate buffer (pH 8.0) at 25 °C, using ATC as substrates, respectively. DTNB (10 mM) was used in order to observe absorbance changes at 412 nm. Donepezil hydrochloride was used as a positive control[37]. Enzyme solutions were prepared in gelatin solution (1%), at a concentration of 2.5 units/ml. AChE and compound solution (50 µL) which is prepared in 2% DMSO at a concentration range of 0.001–100 µM were added to 3.0 ml phosphate buffer (pH 8 ± 0.1) and incubated at 25 °C for 5 min. The reaction was started by adding DTNB (50 µL) and ATC (10 µL) to the enzyme–inhibitor mixture. The production of the yellow anion was recorded for 10 min at 412 nm. As a control, an identical solution of the enzyme without the inhibitor is processed following the same protocol. The blank reading contained 3.0 ml buffer, 50 µL 2% DMSO, 50 µL DTNB and 10 µL substrate. All processes were assayed in triplicate. The inhibition rate (%) was calculated by the following equation:

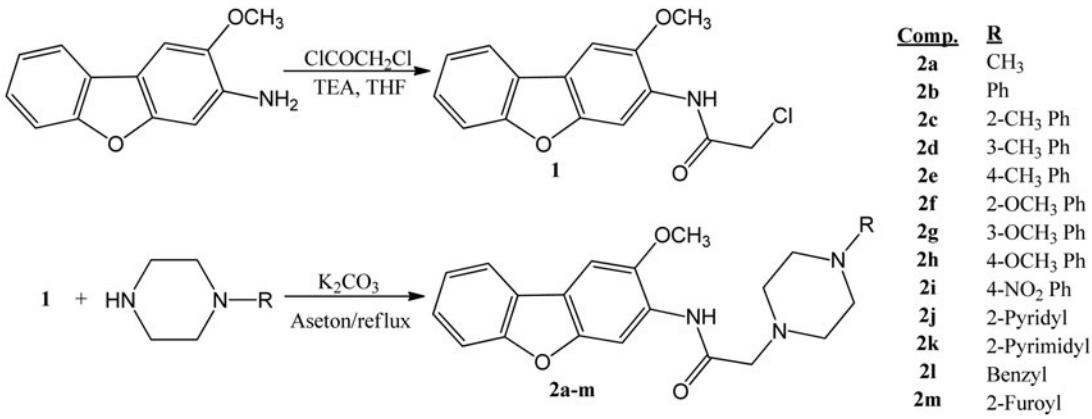
$$\text{Inhibition } \% = \frac{A_C - A_I}{A_C} \times 100$$

where A_I is the absorbance in the presence of the inhibitor, A_C is the absorbance of the control and AB is the absorbance of blank reading. Both of the values are corrected with blank-reading value. SPSS for Windows 15.0 was used for statistical analysis. Data were expressed as mean ± SD.

Results and discussion

Chemistry

The synthesis of the *N*-(2-methoxy-3-dibenzofuranyl)-2-(4-substituted piperazin-1-yl)acetamide derivatives (**2a-m**) were carried out as shown in Scheme 1. The starting material 2-methoxy-3-aminodibenzofuran was first acetylated with chloroacetyl chloride in tetrahidrofuran and trietilamin. The obtained intermediate product *N*-(2-methoxy-3-dibenzofuranyl)-2-chloroacetamide (**1**)



Comp.	R
2a	CH_3
2b	Ph
2c	2-CH_3 Ph
2d	3-CH_3 Ph
2e	4-CH_3 Ph
2f	2-OCH_3 Ph
2g	3-OCH_3 Ph
2h	4-OCH_3 Ph
2i	4-NO_2 Ph
2j	2-Pyridyl
2k	2-Pyrimidyl
2l	Benzyl
2m	2-Furoyl

Scheme 1. Synthesis of the compounds (**2a-m**).

was then refluxed with piperazine derivatives in acetone with the presence of potassium carbonate to give final compounds (**2a-m**). The structures of the obtained compounds were elucidated using spectral data. In the IR spectra, the characteristic N–H bands and amide carbonyl functions were observed at 3329–3358 cm^{-1}. In the NMR spectra, protons of piperazine ring were seen as broad singlets at about 2.42–3.86 ppm range, changing different substituents bonded to the ring. For all compounds, peaks at about 3.19–3.30 ppm, 3.99–4.39 ppm and 10.03–10.13 ppm were seen belonging to CH_2CO, OCH_3 and NH protons, respectively. M + 1 peaks in MS spectra were in agreement with the calculated molecular weight of the title compounds (**2a-m**) and elemental analysis results for C, H and N elements were satisfactory with calculated values of the compounds. According to HPLC analysis, purity ratio was found greater than 95% for all compounds. Peak purity index of all compounds were also checked and no impurity was determined. Some of the results were given as supplementary material.

Biology

The antiplatelet activity of the *N*-(2-methoxy-3-dibenzofuranyl)-2-(4-substituted piperazin-1-yl)acetamide derivatives **(2a-m)** was studied according to Born test. The arachidonic acid or collagen were used as inducer of platelet aggregation on citrated platelet rich human plasma and aspirin was used as standard drug. The synthesized 13 compounds were studied at 100 μM concentration as initial dose and the inhibition percentages were shown in Table 1. Compound **2m** bearing 2-furoyl moiety exhibited the highest inhibition percentage of 100% which is nearly about standard drug's inhibition value (100%). The other compounds (**2a-l**) displayed inhibition values below 10%. IC_{50} was calculated only for compound **2 m** as 26.1 μM, whereas the IC_{50} was calculated as 3.9 μM for standard drug aspirin (Table 2). The inhibitor effect of the compounds on the collagen-induced aggregation was observed insuffiecient which was found less than 8.9%. The final compounds possess an acetamide bearing 4-substituted piperazine skeleton which is also present in drug named ranolazine that is used to treat a cardiovascular disease, chronic angina. In a recent study, to develop the activity potential, diazabicyclic ranolazine analogs were also investigated and they have been determined with higher activity[38]. Besides the final compounds differ from each other with the aromatic structure bonded to the fourth position piperazine ring. The high activity of compound **2 m** shows that heterocyclic aromatic structure connected to piperazine is important for activity of the *N*-(2-methoxy-3-dibenzofuranyl)-2-(4-substituted piperazin-1-yl)acetamide derivatives. But same high activity was not seen for the

Table 1. Inhibitory data (100 μM) for compounds **2a-m** against arachidonic acid (AA, 350 μM) and collogen (5 μg/ml) induced platelet aggregation.

	% Inhibition	
Compounds	AA (350 μM)	Collagen (5 μg/ml)
2a	4.7 ± 6.7	3.08 ± 4.34
2b	10.2 ± 7.1	7.93 ± 0.96
2c	5.8 ± 8.2	4.41 ± 4.63
2d	1.1 ± 1.6	NI
2e	2.8 ± 3.9	8.59 ± 5.40
2f	1.6 ± 1.2	3.60 ± 3.20
2g	11.0 ± 3.4	4.49 ± 4.27
2h	7.38 ± 8.9	1.79 ± 1.71
2i	7.6 ± 10.8	1.19 ± 2.06
2j	3.6 ± 3.6	1.66 ± 1.50
2k	9.5 ± 3.6	3.56 ± 3.22
2l	0.3 ± 0.3	2.20 ± 3.25
2m	100	1.87 ± 2.70
Aspirin	100	–

NI: no inhibition.

compounds **2j** with 2-pyridyl, **2k** with 2-pyrimidyl and **2 l** with benzyl substitutions. At this point, it can be claimed that distance with a carboyl function or any other groups between piperazine and heterocyclic or any aromatic ring is important for the antiplatelet activity of the studied compounds. This is an interesting finding for further studies.

The anticholinesterase activities were also determined by slightly modified Ellman's assay. All final compounds (**2a-m**) were tested for their inhibition potency against AChE (Table 3). Among these compounds, compound **2a** with 4-methyl substitution at the fourth position of piperazine ring and compound **2i** with 4-nitro phenyl substitution were found as the most active compounds. The inhibition percentages were calculated 42.0 and 41.12% at 1 and 0.1 mM concentrations for compound **2a** and the inhibition percentages were calculated 41.35 and 30.22% at 1 and 0.1 mM concentrations for compound **2i**. The IC_{50} values could not be defined none of all compounds. The inhibition percentages were not determined for compounds **2 h** and **2m** and these compounds were evaluated as inactive at two tested concentrations. Compounds **2e** bearing 4-methyl phenyl moiety and compound **2k** bearing 2-pyrimidyl moiety exhibited anticholinesterase activity with nearly 40% inhibition value at 1 mM concentration, but both of them did not exhibited similar inhibition value at 0.1 mM which were found below 4%. The other compounds showed relatively weak activity and the

Table 2. IC_{50} (μM) of the compound **2m** on 350 μM AA-induced platelet aggregation.

Compound	IC_{50} (μM)
2m	26.1
Aspirin	3.9

Table 3. % AChE inhibition of the compounds and IC_{50} values.

	AChE inhibition (%)		
Compounds	1 mM	0.1 mM	IC_{50} (mM)*
2a	42.0 ± 3.44	41.12 ± 1.34	>1
2b	27.37 ± 2.11	19.17 ± 4.45	>1
2c	20.23 ± 4.24	10.81 ± 2.49	>1
2d	31.27 ± 2.45	11.26 ± 3.14	>1
2e	46.64 ± 3.46	3.63 ± 1.17	>1
2f	15.89 ± 2.35	4.39 ± 2.40	>1
2g	12.89 ± 2.54	1.92 ± 3.48	>1
2h	ND	ND	ND
2i	41.35 ± 1.75	30.22 ± 2.19	>1
2j	5.79 ± 2.54	1.78 ± 3.45	>1
2k	41.24 ± 3.41	3.92 ± 4.12	>1
2l	12.80 ± 2.21	0.23 ± 2.43	>1
2 m	ND	ND	ND
Donepezil	99.01 ± 4.89	95.52 ± 5.01	0.054 ± 0.002 (μM)

ND: not determined.
*IC_{50}: 50% inhibitory concentration (means ± SD of three independent experiments) of AChE.

inhibition values were found less than 27.37%. Standard drug Donepezil was studied at lower concentrations for the purpose of finding IC_{50} value and it was determined as 0.054 μM. None of the compounds showed comparable activity with Donepezil and significant anticholinesterase activity contrary to expectations.

Conclusion

Thirteen new *N*-(2-methoxy-3-dibenzofuranyl)-2-(4-substituted piperazin-1-yl)acetamide derivatives were synthesized and evaluated for their antiplatelet and anticholinesterase activity. Compound **2m** bearing furoyl moiety was determined as a strong antiplatelet active molecule as inhibitors of AA-induced platelet aggregation. Inversely, none of the compounds showed sufficient acetylcholinesterase inhibitory activity.

Declaration of interest

The authors report no conflicts of interest.

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Supplementary material available online