Arch. Pharm. Res.
DOI 10.1007/s12272-013-0192-5

Archives of Pharmacal Research
www.springer.com/12272

RESEARCH ARTICLE

Synthesis of thioether derivatives of quinazoline-4-one-2-thione and evaluation of their antiplatelet aggregation activity

Zahra Eskandariyan · Marjan Esfahani Zadeh · Kamaleddin Haj Mohammad Ebrahim Tehrani · Vida Mashayekhi · Farzad Kobarfard

Received: 4 February 2013 / Accepted: 16 June 2013

Abstract A series of 2-(arylmethylthio)-3-phenylquinazolin-4-one derivatives have been synthesized and their antiplatelet aggregation activities were assessed against ADP and arachidonic acid-induced platelet aggregation in human plasma. Among the tested thioethers, derivative **2**, **3**, **5** and **16** were the most potent compounds with satisfactory IC_{50} for inhibition of platelet aggregation induced by ADP. Analysis of global physicochemical parameters shows some correlations between activities and molecular volume and also surface area of the studied derivatives.

Keywords 2-(Arylmethylthio)-3-phenylquinazolin-4-one · Antiplatelet aggregation · Thioether · Structure–activity relationship · Adenosine diphosphate · Arachidonic acid

Introduction

Thromboembolic disorders are a major cause of death and disability in industrial countries and also worldwide (Mendis et al. 2011). Thrombosis that is caused secondary to disrupted atherosclerotic plaques is the initiator of most cardiovascular diseases including heart attacks and strokes. In the pathogenesis of atherothrombosis, platelets play a major role. Currently aspirin, which irreversibly inhibits cyclooxygenase I-mediated transformation of arachidonic acid (AA) to thromboxane A_2 (TXA_2), and the $P2Y_{12}$ antagonists clopidogrel and prasugrel, which selectively and irreversibly bind to the $P2Y_{12}$ ADP receptor are routinely used as antiplatelet agents (Maree and Fitzgerald 2007; Meadows and Bhatt 2007). However, there are still some serious limitations to these agents which include weak inhibition of platelet function (e.g., aspirin) (Patrono et al. 2004) slow onset of action (e.g., clopidogrel) (Bassand 2008), variable response to treatment among patients (e.g., clopidogrel and aspirin) (Bassand 2008; Patrono et al. 2004) and high incidence of bleeding events which is dose dependent in both aspirin and clopidogrel drug therapy (Guthrie 2011). Considering the current situation, development of novel antiplatelet agents which are safe and effective is an urgent need.

Some recent works have suggested that pyrimidine derivatives are potent antiplatelet agents (Liu et al. 2011; Bruno et al. 2001; Crepaldi et al. 2009; Dupin et al. 2002; Giridhar et al. 2012; Gryglewski et al. 2000). Pyrimidines play an essential role in several biological processes and have considerable chemical and pharmacological importance in terms that the pyrimidine ring can be found in nucleoside antibiotics, antibacterial and cardiovascular agents (Tozkoparan et al. 1999; Clark et al. 1993). Pyrimidine, especially its thioether derivatives have attracted much attention because of their quite high antiplatelet aggregation activity as inhibitors for P2 receptor family (Liu et al. 2011; Bruno et al. 2001; Crepaldi et al. 2009) (Fig. 1).

On the other hand, some new quinazolinone derivatives have also shown antiplatelet activity (Gravier et al. 1992; Ding et al. 1995; Chang et al. 2003). Quinazolinone derivatives are of special importance because of their

Z. Eskandariyan · M. Esfahani Zadeh · K. Haj Mohammad Ebrahim Tehrani · V. Mashayekhi · F. Kobarfard (✉)
Department of Medicinal Chemistry, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Vali Asr Avenue, Niayesh Junction, P.O. Box 14155-6153, Tehran, Iran
e-mail: kobarfard@sbmu.ac.ir

F. Kobarfard
Phytochemistry Research Center, Shahid Beheshti University of Medical Sciences, Vali Asr Avenue, Niayesh Junction, P.O. Box 14155-6153, Tehran, Iran

Published online: 16 July 2013

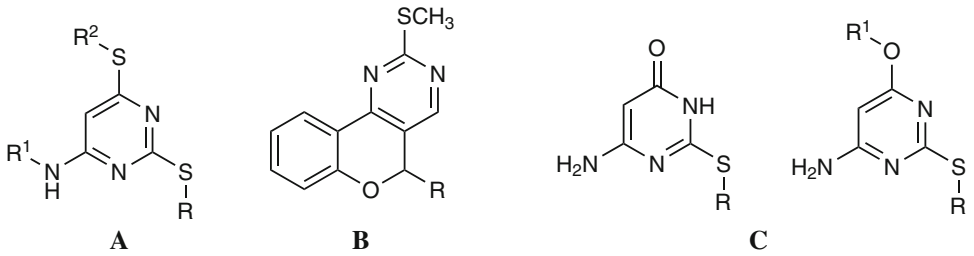


Fig. 1 Active antiplatelet pyrimidine derivatives. **A** 6-alkylamino-2,4-dialkyl(aryl)thiopyrimidines (Liu et al. 2011), R = Me, *n*-Pr, R^1 = *n*-Pr, *i*-Pr, Ph, Bn, R^2 = Bn, CH_2CH_2Ph; **B** tricyclic pyrimidines (Bruno et al. 2001), R = some amino substituents; **C** substituted 6-amino-2-mercaptopyrimidines (Crepaldi et al. 2009), R = CH_3, CH_2CH_2NHBOC, CH_2CH_2OH, CH_2CF_3, 2-chlorobenzyl, etc. R^1 = substituted arylsulfonyl moieties

biological and pharmacological activities, such as anti inflammatory (Laddha and Bhatnagar 2009) and antifungal (Giri and Nizamuddin 1978) agents etc. Wu and his coworkers in their published work investigated the antiplatelet activity of substituted 4-quinazolinone derivatives (Fig. 2) some of which inhibited platelet aggregation induced by AA and collagen (Chang et al. 2003).

Considering the structural elements of antiplatelet pyrimidine and 4-quinazolinone derivatives, in the present study a series of 2-(arylmethylthio)-3-phenylquinazolin-4-one derivatives have been synthesized and their antiplatelet activities were assessed against ADP and AA-induced aggregation in human plasma. The synthesized derivatives could be viewed as hybrid structures which contain both S-substituted mercaptopyrimidines and 4-quinazolinone structural backbones (Fig. 3). In order to study the structure–antiplatelet activity relationship of the derivatives, different aromatic rings with diverse physicochemical and electronic characters have been incorporated to the structure of the desired compounds.

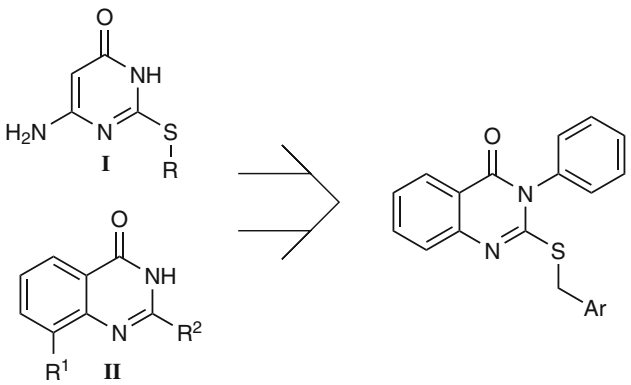


Fig. 3 General structure of 2-(arylmethylthio)-3-phenylquinazolin-4-one derivatives

Materials and methods

General

Reactions were monitored by thin-layer chromatography (TLC) on silica gel (precoated F_{254} Merck plates) using chloroform/methanol as mobile phase. Melting points were obtained by an Electrothermal 9100 apparatus and are uncorrected. The infrared spectra (IR) were measured on a Perkin-Elmer 843 spectrometer with KBr as diluent. Electron spray ionization mass spectra (ESI–MS) were obtained using Agilent 6410 Triple Quad. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded in DMSO-d_6 solution with a Bruker Avance DRX 400 MHz spectrometer. Peak positions have been reported in parts per million (δ) downfield from tetramethylsilane as internal standard, and *J* values have been given in hertz. The synthesized derivatives were analyzed for C, H, N and S on a Costech model 4010 and agreed with the proposed structures within ±0.4 % of the theoretical values. The physicochemical parameters including Clog *P* value, surface area, molecular volume, refractivity and polarizability were calculated by Hyperchem 8.0 software.

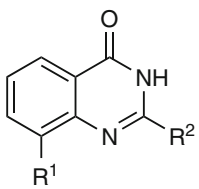


Fig. 2 Antiplatelet 4-quinazolinone derivatives. R^1 = H, OCH_3, R^2 = H, CH_3, CH_2CH_3, phenyl, 4-methoxyphenyl

Chemistry

General procedure for preparation of thioethers ***2–15***

To a solution of compound **1** (0.88 g, 3.49 mmol) in 35 mL of 0.1 M NaOH in CH_3OH, appropriate arylmethyl halides (3.49 mmol) were added and the solution was stirred at room temperature. After the completion of the reaction indicated by TLC, the developed precipitates were filtered off, washed with water (15 mL) and recrystallized from appropriate solvents. Compounds **2–15** were thus prepared.

*2-(Benzylthio)-3-phenylquinazolin-4-one (**2**)*

Yield (50 %); mp 171–173 °C. IR (KBr, cm^{-1}): 3055, 1683, 1543, 779, 690. ^1H NMR (DMSO-d_6): δ 8.10 (1H, d, J = 7.2 Hz, quinazolinone H-5), 7.87 (1H, t, J = 7.2 Hz, quinazolinone H-7), 7.72 (1H, d, J = 8.0 Hz, quinazolinone H-8), 7.43–7.56 (8H, m, H-2′B, 3′B, 4′B, 5′B, 6′B, 2′A, 6′A, quinazolinone H-6), 7.22–7.32 (3H, m, H-3′A, 4′A, 5′A), 4.42 (2H, s, methylene H). ESI–MS *m/z*: 345 ($M + H^+$). Anal. Calcd for $C_{21}H_{16}N_2OS$: C 73.23, H 4.68, N 8.13, S 9.31. Found: C 73.31, H 4.67, N 8.10, S 9.33.

*2-(2-Nitrobenzylthio)-3-phenylquinazolin-4-one (**3**) (Lakhan 1969)*

Yield (82 %); mp 201–204 °C. IR (KBr, cm^{-1}): 3124, 1723, 1581, 1556, 750. ^1H NMR (DMSO-d_6): δ 8.07 (1H, d, J = 7.6 Hz, quinazolinone H-5), 8.00 (1H, d, J = 8.4 Hz, H-3′B), 7.86–7.90 (2H, m, H-5′B, quinazolinone H-7), 7.69–7.73 (2H, m, H-4′B, 6′B), 7.43–7.58 (7H, m, H-2′A, 3′A, 4′A, 5′A, 6′A, quinazolinone H-6,8), 4.72 (2H, s, methylene H). ESI–MS *m/z*: 390 ($M + H^+$). Anal. Calcd for $C_{21}H_{15}N_3O_3S$: C 64.77, H 3.88, N 10.79, S 8.23. Found: C 64.83, H 3.88, N 10.78, S 8.25.

*2-(2-Chlorobenzylthio)-3-phenylquinazolin-4-one (**4**)*

Yield (76 %); mp 161–163 °C. IR (KBr, cm^{-1}): 3068, 1690, 1550, 744. ^1H NMR (DMSO-d_6): δ 8.10 (1H, d, J = 7.6 Hz, quinazolinone H-5), 7.88 (1H, t, J = 7.4, quinazolinone H-7), 7.75 (1H, d, J = 8.0 Hz, H-3′B), 7.71 (1H, dd, J_1 = 5.6 Hz, J_2 = 3.6 Hz, H-4′B), 7.55–7.58 (4H, m, H-3′A, 4′A, 5′A, quinazolinone H-8), 7.51 (1H, t, quinazolinone H-6), 7.44–7.47 (2H, m, H-2′A, 6′A), 7.28–7.33 (2H, m, H-5′B, 6′B), 4.53 (2H, s, methylene H). ESI–MS *m/z*: 379, 381 ($M + H^+$). Anal. Calcd for $C_{21}H_{15}ClN_2OS$: C 66.57, H 3.99, N 7.39, S 8.46. Found: C 66.73, H 4.01, N 7.37, S 8.48.

*2-(4-Nitrobenzylthio)-3-phenylquinazolin-4-one (**5**)*

Yield (55 %); mp 188–200 °C. IR (KBr, cm^{-1}): 1691, 1556, 1353, 785. ^1H NMR (DMSO-d_6): δ 8.16 (2H, d, J = 8.8 Hz, H-3′B, 5′B), 8.08 (1H, d, J = 7.2 Hz, quinazolinone H-5), 7.87 (1H, t, J = 7.4 Hz, quinazolinone H-7), 7.76 (2H, d, J = 8.4 Hz, H-2′B, 6′B), 7.73 (1H, d, J = 8.4 Hz, quinazolinone H-8), 7.56–7.60 (3H, m, H-3′A, 4′A, 5′A), 7.45–7.51 (3H, m, H-2′A, 6′A, quinazolinone H-6), 4.53 (2H, s, metylene H). ESI–MS *m/z*: 390 ($M + H^+$). Anal. Calcd for $C_{21}H_{15}N_3O_3S$: C 64.77, H 3.88, N 10.79, S 8.23. Found: C 64.59, H 3.89, N 11.82, S 8.25.

*2-(4-Fluorobenzylthio)-3-phenylquinazolin-4-one (**6**)*

Yield (66 %); mp 145–148 °C. IR (KBr, cm^{-1}): 3065, 1683, 1551, 770. ^1H NMR (DMSO-d_6): δ 8.10 (1H, d, J = 7.6 Hz, quinazolinone H-5), 7.87 (1H, t, J = 7.8 Hz, quinazolinone H-7), 7.72 (1H, d, J = 8.0 Hz, quinazolinone H-8), 7.55–7.59 (3H, m, H-3′A, 4′A, 5′A), 7.48–7.52 (3H, m, H-2′B, 6′B, quinazolinone H-6), 7.44–7.46 (2H, m, H-2′A, 6′A), 7.12 (2H, t, J = 9 Hz, H-3′B, 5′B), 4.41 (2H, s, methylene H). ESI–MS *m/z*: 363 ($M + H^+$). Anal. Calcd for $C_{21}H_{15}FN_2OS$: C 69.59, H 4.17, N 7.73, S 8.85. Found: C 69.68, H 4.16, N 7.73, S 8.87.

*2-(3-Fluorobenzylthio)-3-phenylquinazolin-4-one (**7**)*

Yield (74 %); mp 134–136 °C. IR (KBr, cm^{-1}): 3069, 1689, 1549, 783, 697. ^1H NMR (DMSO-d_6): δ 8.10 (1H, d, J = 7.6 Hz, quinazolinone H-5), 7.88 (1H, t, J = 7.4 Hz, quinazolinone H-7), 7.72 (1H, d, J = 8.0 Hz, quinazolinone H-8), 7.45–7.59 (6H, m, H-2′A, 6′A, quinazolinone H-6, H-3′A, 4′A, 5′A), 7.29–7.37 (3H, m, H-2′B, 4′B, 6′B), 7.07 (1H, t, J = 7.8 Hz, H-5′B), 4.44 (2H, s, methylene H). ESI–MS *m/z*: 363 ($M + H^+$). Anal. Calcd for $C_{21}H_{15}FN_2OS$: C 69.59, H 4.17, N 7.73, S 8.85. Found: 69.70, H 4.16, N 7.71, S 8.88.

*2-(2-Methylbenzylthio)-3-phenylquinazolin-4-one (**8**)*

Yield (77 %); mp 148–150 °C. IR (KBr, cm^{-1}): 1689, 1550, 781, 697. ^1H NMR (DMSO-d_6): δ 8.10 (1H, d, J = 7.6 Hz, quinazolinone H-5), 7.88 (1H, t, J = 7.6 Hz, quinazolinone H-7), 7.72 (1H, d, J = 8 Hz, H-8), 7.44–7.57 (6H, m, H-2′A, 6′A, quinazolinone H-6, H-3′A, 4′A, 5′A), 7.41 (1H, d, J = 6.8 Hz, H-6′B), 7.10–7.17 (3H, m, H-3′B, 4′B, 5′B), 4.45 (2H, s, methylene H), 2.32 (3H, s, methyl H). ESI–MS *m/z*: 359 ($M + H^+$). Anal. Calcd for $C_{22}H_{18}N_2OS$: C 73.71, H 5.06, N 7.82, S 8.95. Found: C 73.63, H 5.08, N 7.83, S 8.94.

*2-(4-Methylbenzylthio)-3-phenylquinazolin-4-one (**9**) (Bhargava and Srivastava 1968)*

Yield (83 %); mp 162–164 °C. IR (KBr, cm^{-1}): 1692, 1559, 774, 698. ^1H NMR (DMSO-d_6): δ 8.10 (1H, d, J = 6.8 Hz, quinazolinone H-5), 7.87 (1H, t, J = 7.8 Hz, quinazolinone H-7), 7.70 (1H, d, J = 8.0 Hz, quinazolinone H-8), 7.48–7.57 (4H, m, quinazolinone H-6, H-3′A, 4′A, 5′A), 7.43–7.45 (2H, m, H-2′A, 6′A), 7.31 (2H, d, J = 7.6 Hz, H-2′B, 6′B), 7.10 (2H, d, J = 7.6 Hz, H-3′B, 5′B), 4.37 (2H, s, methylene H), 2.25 (3H, s, methyl H). ESI–MS *m/z*: 359 ($M + H^+$). Anal. Calcd for $C_{22}H_{18}N_2OS$: C 73.71, H 5.06, N 7.82, S 8.95. Found: C 73.89, H 5.07, N 7.80, S 8.97.

*2-(3-Methoxybenzylthio)-3-phenylquinazolin-4-one (**10**)*

Yield (46 %); mp 123–125 °C. IR (KBr, cm^{-1}): 1694, 1553, 783, 698. ^{1}H NMR (DMSO-d_6): δ 8.10 (1H, d, $J = 8.0$ Hz, quinazolinone H-5), 7.88 (1H, t, $J = 7.8$ Hz, quinazolinone H-7), 7.72 (1H, d, $J = 8.4$ Hz, quinazolinone H-8), 7.55–7.58 (3H, m, H-3′A, 4′A, 5′A), 7.50 (1H, t, $J = 7.6$ Hz, quinazolinone H-6), 7.44–7.46 (2H, m, H-2′A, 6′A), 7.21 (1H, t, $J = 7.8$ Hz, H-5′B), 7.04 (1H, s, H-2′B), 7.00 (1H, d, $J = 7.6$ Hz, H-6′B), 6.80 (1H, d, $J = 7.6$ Hz, H-4′B), 4.40 (2H, s, methylene H), 3.72 (3H, s, methoxy H). ESI–MS *m/z*: 375 ($M + H^+$). Anal. Calcd for $C_{22}H_{18}N_2O_2S$: C 70.57, H 4.85, N 7.48, S 8.56. Found: C 70.41, H 4.86, N 7.47, S 8.57.

*2-(3-Methylbenzylthio)-3-phenylquinazolin-4-one (**11**)*

Yield (80 %); mp 138–140 °C. IR (KBr, cm^{-1}): 1687, 889, 779, 699. ^{1}H NMR (DMSO-d_6): δ 8.10 (1H, d, $J = 7.2$ Hz, quinazolinone H-5), 7.87 (1H, t, $J = 7.6$ Hz, quinazolinone H-7), 7.72 (1H, d, $J = 8$ Hz, quinazolinone H-8), 7.44–7.58 (6H, m, H-2′A, 6′A, quinazolinone H-6,H-3′A, 4′A, 5′A), 7.16–7.24 (3H, m, H-2′B, 5′B, 6′B), 7.05 (1H, d, $J = 7.2$ Hz, H-4′B), 4.38 (2H, s, methylene H), 2.26 (3H, s, methyl H). ESI–MS *m/z*: 359 ($M + H^+$). Anal. Calcd for $C_{22}H_{18}N_2OS$: C 73.71, H 5.06, N 7.82, S 8.95. Found: C 73.80, H 5.06, N 7.84, S 8.98.

*2-((Pyridin-3-yl)methylthio)-3-phenylquinazolin-4-one (**12**)*

Yield (49 %); mp 161–162.2 °C. IR (KBr, cm^{-1}): 1691, 1552, 695. ^{1}H NMR (DMSO-d_6): δ 8.70 (1H, s, H-2′B), 8.43 (1H, d, $J = 3.6$ Hz, H-4′B), 8.09 (1H, d, $J = 7.6$ Hz, quinazolinone H-5), 7.86–7.9 (2H, m, quinazolinone H-7, H-6′B), 7.74 (1H, d, $J = 8$ Hz, quinazolinone H-8), 7.45–7.57 (6H, m, H-2′A, 6′A, quinazolinone H-6, H-3′A, 4′A, 5′A), 7.33 (1H, dd, $J = 4.8$ Hz, H-5′B), 4.42 (2H, s, methylene H). ESI–MS *m/z*: 346 ($M + H^+$). Anal. Calcd for $C_{20}H_{15}N_3OS$: C 69.54, H 4.38, N 12.17, S 9.28. Found: C 69.44, H 4.39, N 12.21, S 9.26.

*2-((Pyridin-4-yl)methylthio)-3-phenylquinazolin-4-one (**13**)*

Yield (58 %); mp 182–184 °C. IR (KBr, cm^{-1}): 1691, 1557, 1466, 787. ^{1}H NMR (DMSO-d_6): δ 8.49 (2H, d, $J = 6$ Hz, H-3′B, 5′B), 8.09 (1H, d, $J = 8$ Hz, quinazolinone H-5), 7.86 (1H, t, $J = 7.4$ Hz, quinazolinone H-7), 7.70 (1H, d, $J = 8$ Hz, quinazolinone H-8), 7.56–7.58 (3H, m, H-3′A, 4′A, 5′A), 7.47–7.54 (5H, m, H-2′A, 6′A, quinazolinone H-6, H-6′B, 2′B), 4.41 (2H, s, methylene H). ESI–MS *m/z*: 346 ($M + H^+$), 368 ($M + Na^+$). Anal. Calcd for $C_{20}H_{15}N_3OS$: C 69.54, H 4.38, N 12.17, S 9.28. Found: C 69.61, H 4.38, N 12.16, S 9.29.

*2-(2-Cyanobenzylthio)-3-phenylquinazolin-4-one. (**14**)*

Yield (83 %); mp 172–174 °C. IR (KBr, cm^{-1}): 2240, 1694, 1555, 789. ^{1}H NMR (DMSO-d_6): δ 8.08 (1H, d, $J = 7.6$ Hz, quinazolinone H-5), 7.74–7.89 (4H, m, H-4′B, 5′B, quinazolinone H-7,8), 7.66 (1H, t, $J = 7.4$ Hz, H-3′B), 7.55–7.61 (3H, m, H-3′A, 4′A, 5′A), 7.43–7.51 (4H, m, H-2′B, 2′A, 6′A, quinazolinone H-6), 4.58 (2H, s, methylene H). ESI–MS *m/z*: 370 ($M + H^+$). Anal. Calcd for $C_{22}H_{15}N_3OS$: C 71.52, H 4.09, N 11.37, S 8.68. Found: C 71.64, H 4.10, N 11.39, S 8.65.

*2-(6-Chloro-3,4-methylenedioxybenzylthio)-3-phenylquinazolin-4-one (**15**)*

Yield (88 %); mp 193–195 °C. IR (KBr, cm^{-1}): 1681, 1556, 1045, 787. ^{1}H NMR (DMSO-d_6): δ 8.10 (1H, d, $J = 7.6$ Hz, quinazolinone H-5), 7.88 (1H, t, $J = 7.6$ Hz, quinazolinone H-7), 7.76 (1H, d, $J = 8.4$ Hz, quinazolinone H-8), 7.44–7.58 (6H, m, H-2′A, 6′A, quinazolinone H-6, H-3′A, 4′A, 5′A), 7.26 (1H, s, H-4′B), 7.08 (1H, s, H-2′B), 6.05 (2H, s, H-3′B), 4.43 (2H, s, methylene H). ESI–MS *m/z*: 423, 425 ($M + H^+$), 445 ($M + Na^+$). Anal. Calcd for $C_{22}H_{15}ClN_2O_3S$: C 62.48, H 3.58, N 6.62, S 7.58. Found: C 62.31, H 3.58, N 6.61, S 7.60.

*2-(2-Carbamoylbenzylthio)-3-phenylquinazolin-4-one (**16**)*

A solution of 0.5 g of compound **14** in 2 mL of concentrated sulphuric acid was warmed to 80–90 °C and allowed to stand for 5 min. Then the solution was cooled, poured into 20 mL of cold water and filtered. The solid was stirred with 10 mL of 5 % sodium hydroxide solution, then filtered and recrystallized from ethanol.

Yield (50 %); mp 231–233 °C. IR (KBr, cm^{-1}): 3390, 1665, 1548, 768. ^{1}H NMR (DMSO-d_6): δ 8.08 (1H, d, $J = 8$ Hz, quinazolinone H-5), 7.87 (1H, t, $J = 8.2$ Hz, quinazolinone H-7), 7.76 (1H, d, $J = 8$ Hz, quinazolinone H-8), 7.65 (1H, d, $J = 7.2$ Hz, H-5′B), 7.53–7.55 (3H, m, H-3′A, 4′A, 5′A), 7.38–7.52 (5H, m, H-2′B, 3′B, 2′A, 6′A, quinazolinone H-6), 7.29–7.33 (1H, m, H-4′B), 4.67 (2H, s, methylene H). ESI–MS *m/z*: 388 ($M + H^+$). Anal. Calcd for $C_{22}H_{17}N_3O_2S$: C 68.20, H 4.42, N 10.85, S 8.28. Found: C 68.13, H 4.41, N 10.88, S 8.29.

In vitro evaluation of anti platelet aggregation activity

The anti platelet aggregation activity of the derivatives were measured using human plasma. Fresh blood samples were obtained from non-smoker healthy volunteers with negative history of drug consumption up to 15 days prior to the test. Platelet-rich plasma (PRP) was obtained from whole blood collected in sodium citrate (9:1 by volume)

upon centrifugation at 1,000 rpm for 8 min. The remaining was centrifuged at 3,000 rpm for 15 min and PPP was collected from the above layer which was used as the test blank. The platelet count was adjusted to 250,000 plts/mL by diluting PRP with appropriate amount of PPP. To PRP samples, test compounds previously dissolved in ethylenglycol monomethyl ether were added and samples were incubated for 5 min at 37 °C. Then ADP (5 μM) or AA (1.25 mg/mL) was added and platelet shape change and aggregation were monitored for 5 min. Ethylenglycol monomethyl ether (0.5 % v/v) was used as negative control and indomethacin and aspirin as standard drugs. Platelet aggregation inhibition (%) was calculated by the following formula:

$$\text{Inhibition } \% = [1 - (D/S)] \times 100$$

where D = platelet aggregation in the presence of test compounds, and S = platelet aggregation in the presence of solvent.

Compounds were thus screened at the primary concentration of 100 μM and those that exhibited higher than 50 % inhibitory activity, were further diluted to calculate IC_{50}.

Results and discussion

Chemistry

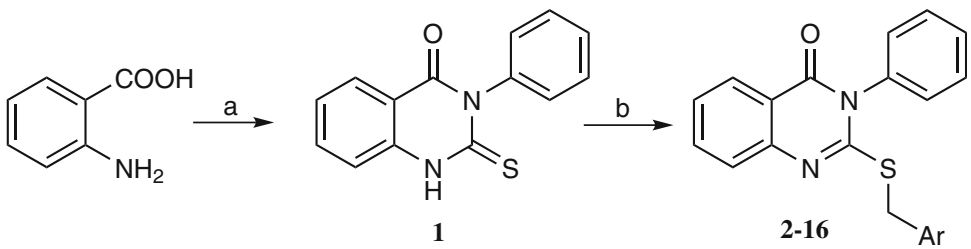
The synthetic pathway is disclosed in Scheme 1. Final desired derivatives were prepared by a two-step procedure. The first step was the formation of 2,3-dihydro-3-phenyl-2-thioxoquinazolin-4-one **1** synthesized by reacting anthranilic acid with phenylisothiocyanate according to the established procedure (Al-Rashood et al. 2006; Li et al. 2012), and in the second step compound **1** was reacted with different aryl methyl halides under basic condition (Crepaldi et al. 2009). Compounds **2**–**15** were thus prepared in good yields. The derivative **16** was prepared by hydrolyzing compound **14** in the presence of sulfuric acid (Furniss et al. 1989).

To obtain the title compounds with high purity, the crudes prepared by the above mentioned procedure were recrystallized from appropriate solvents and their structures were confirmed by different spectroscopic methods such as IR, MASS and ^{1}H NMR. In the IR spectra, a strong absorption band at 1,680–1,690 was assigned to the cyclic amide functionality of the quinazolinone ring which was present in IR spectra of all the derivatives. In the spectra of nitro-derivatives, strong absorptions at 1,375 and 1,581 (for derivative **3**) and 1,353 and 1,556 (for derivative **5**) were observed and assigned to N–O symmetric and asymmetric stretches. Characteristic of –CN functional group, a weak absorption band at 2,240 was observed in IR spectrum of compound **14**, which disappeared after its hydrolysis to the amide derivative **16**. In the ^{1}H NMR spectra, as a result of the existence of three aromatic rings, a complex spectrum of overlapping hydrogens was observed in the aromatic zone. However, at the upfield and downfield extremities, quinazoline H-5 (doublet at 8.1 ppm), H-7 (triplet at 7.9 ppm) and H-8 (doublet at 7.7 ppm) and also methylene hydrogens (singlet at 4.5 ppm) were weakly coupled and hence more readily distinguishable. Molecular mass of all the derivatives was detected by Electron-spray ionization mass spectrometry (ESI–MS) as M + 1 and/or M + 23 relating to hydrogen and sodium adducts of the intact molecules, respectively.

Antiplatelet aggregation activity

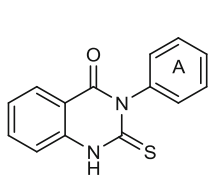
The in vitro antiplatelet activity of all the synthesized compounds was assayed on human PRP by using the Born's reported turbidimetric method (Born 1962) on an APACT 4004 aggregometer. ADP and AA were employed as inducers of platelet aggregation, and indomethacin and aspirin were used as the positive controls. IC_{50} was defined as the concentration of the test compounds that inhibits platelet aggregation by 50 %.

The antiplatelet aggregation activity of the derivatives is listed in Table 1. Data shows that the majority of the derivatives inhibited ADP-induced platelet aggregation more effectively than the aggregation induced by AA. Among the tested compounds, 2-(benzylthio)-3-phenylquinazolin-4-one **2** was the most potent compound with IC_{50} value of 9.5 μM against aggregation induced by ADP and compounds **16** and **5** showed satisfactory activity with IC_{50} values of 14.53 and 14.80 μM, respectively. Based on the activity data, it could be suggested that unsubstituted phenyl

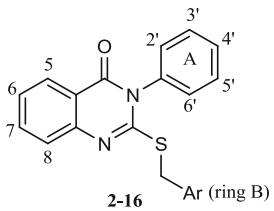


Scheme 1 The total synthetic pathway; reagents and conditions: (*a*) phenylisothiocyanate, triethylamine, ethanol, reflux. (*b*) NaOH in methanol, appropriate arylmethyl halides, r.t

Table 1 Antiplatelet aggregation activity of the synthesized derivatives



1



2-16

Derivative	Ar	ADP IC_{50} (μM)	AA IC_{50} (μM)
1	–	17.39	>100
2	Phenyl	9.58	>100
3	2-Nitrophenyl	15.70	>100
4	2-Chlorophenyl	34.18	>100
5	4-Nitrophenyl	14.80	>100
6	4-Fluorophenyl	28.91	>100
7	3-Fluorophenyl	>100	>100
8	2-Methylphenyl	>100	>100
9	4-Methylphenyl	24.80	51.99
10	3-Methoxyphenyl	66.60	>100
11	3-Methylphenyl	18.36	>100
12	Pyridin-3-yl	85.05	>100
13	Pyridin-4-yl	>100	28.7
14	2-Cyanophenyl	>100	36.39
15	(6-Chloro-3,4-metylenedioxy) phenyl	21.28	>100
16	2-Carbamoylphenyl	14.53	>100
Indomethacin		>100	3.0
Aspirin		>100	30.3

ring (as in compound **2**) is the best aromatic substituent on position 3 of quinazoline ring among other employed ones, since substitution on phenyl ring or replacing it by pyridine decreases the bioactivity to variable extents. However those with electron withdrawing substituents such as nitro and amide groups (**3**, **5** and **16**) showed less decreased activity. It should be added that the activity of the latter 3 derivatives is still acceptable and higher than their parent compound **1**. Interestingly, compounds **13** and **14** with 4-pyridyl and 2-cyano substituents were active against aggregation induced by AA. However, the other derivatives did not show a pronounced activity at 100 μM concentration when AA was used as inducer.

In order to obtain the relationship between the structural parameters of the investigated derivatives and their antiplatelet aggregation activity, quantitative structure–activity relationship (QSAR) analysis was performed with various molecular descriptors. The calculated octanol–water partition coefficient (Clog *P*) has been considered as descriptor for the hydrophobic effect. The steric effect has been described by means of the surface area (SA: approx and grid) and molecular volume (V). Refractivity (R) and polarizability (*P*) have been used as descriptors for both volume and electronic state (London dispersive forces) properties of the molecules. For each descriptor, the best multilinear regression equation was obtained.

Table 2 General molecular parameters of the synthesized derivatives

Derivative	Clog *P*	R[a]	*P*[b]	V[c]	SA[d]	
					Approx	Grid
1	2.63	74.46	29.02	707.77	325.77	438.50
2	5.36	102.23	39.86	992.04	466.33	598.38
3	5.02	109.56	41.70	1022.65	458.16	590.43
4	6.07	107.04	41.78	1027.09	489.99	607.39
5	5.1	109.56	41.70	1035.41	524.99	611.39
6	5.5	102.45	39.77	983.56	485.18	581.20
7	5.51	102.45	39.77	1000.5	475.15	596.98
8	5.81	107.27	41.69	1024.12	476.28	598.54
9	5.86	107.27	41.69	1046.01	508.11	625.93
10	5.28	108.69	42.33	1067.36	513.49	635.53
11	5.86	107.27	41.69	1027.45	513.82	604.77
12	3.86	100.01	39.15	945.96	401.81	552.53
13	3.86	100.01	39.15	962.14	463.33	571.96
14	4.94	107.97	41.71	1021.69	513.82	599.47
15	6.11	112.80	44.12	1047.65	447.41	590.58
16	3.54	110.81	43.13	1041.74	483.80	606.78

[a] Refractivity

[b] Polarizability

[c] Molecular volume

[d] Surface area

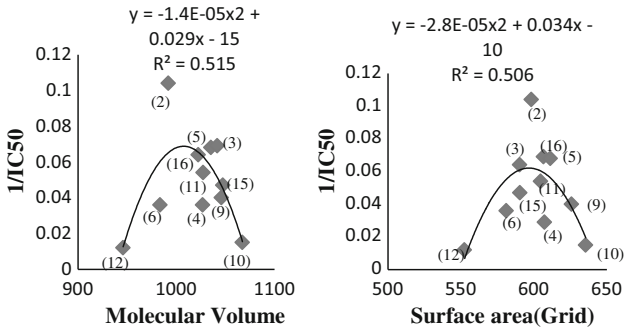


Fig. 4 IC_{50} values plotted against surface area and molecular volume

The calculated physicochemical parameters of the derivatives are listed in Table 2. Analysis of the physicochemical parameters showed a fairly parabolic correlation between the observed activities and surface area (grid) and also molecular volume of the compounds (Fig. 4). Although the correlation coefficient (R^2) values do not indicate a strong correlation (0.515 and 0.506 values, respectively) but if the data point number 2 is considered as an outlier, a fairly acceptable correlation will be obtained.

The correlation between molecular volume and/or surface area and different biological activities has been reported by independent researchers in many previously reported studies (Hemmateenejad et al. 2007; Srivastava and Gupta 2012; Kim et al. 2006). Attempts were failed to extract a correlation between activities and other parameters including Clog *P*, refractivity and polarizability. The surface area (grid) and molecular volume of the most potent compound (**2**) were calculated as 598.38 and 992.04 Å, respectively. According to Fig. 4, the most potent derivatives including **2**, **3**, **5** and **16** have an optimum surface area and molecular volume. In contrast, the derivatives that fall into the extremities of the plots (e.g. compounds **10** and **12**), exhibited weak bioactivity.

Surface area and molecular volume are two parameters which can be employed as measures of molecular similarity and help in understanding the steric requirements of possible receptors. The existence of a parabolic correlation with a maximum between the antiplatelet activity and surface area (or molecular volume) gives a clear indication that there may be some steric requirements on the receptor in the proximity of 607 for surface area and 1,036 for molecular volume which could be used in the future studies for the development of new biologically active compounds.

In conclusion, a series of 2-(arylmethylthio)-3-phenylquinazolin-4-one derivatives have been synthesized and their antiplatelet activities were assessed against ADP and AA-induced platelet aggregation in human plasma. The tested derivatives selectively inhibited platelet aggregation induced by ADP with satisfactory IC_{50} values. Among them, compound **2** with IC_{50} of 9.58 μM proved to be the most potent derivative of the series. By analyzing the physicochemical parameters it is suggested that there might be a correlation between bioactivity and parameters such as surface area and molecular volume. The findings of this study will be helpful for the development of new antiplatelet compounds providing us with some directions for conducting our new studies in the area of antiplatelet drug discovery.

References

Al-Rashood, S.T., I.A. Aboldahab, M.N. Nagi, L.A. Abouzeid, A.A.M. Abdel-Aziz, S.G. Abdel-hamide, K.M. Youssef, A.M. Al-Obaid, and H.I. El-Subbagh. 2006. Synthesis, dihydrofolate reductase inhibition, antitumor testing, and molecular modeling study of some new 4(3*H*)-quinazolinone analogs. *Bioorganic and Medicinal Chemistry* 14: 8608–8621.

Bassand, J.P. 2008. Unmet needs in antiplatelet therapy. *European Heart Journal of Supplements* 10: D3–D11.

Bhargava, P.N., and K.S.L. Srivastava. 1968. Antimalaria agent. Synthesis of S-substituted 2-thio-3-aryl(or alkyl)-4(3*H*)-quinazolinones. *Allgemeine und Praktische Chemie* 19: 424–425.

Born, G.V.R. 1962. Aggregation of blood platelets by adenosine diphosphate and its reversal. *Nature* 194: 927–929.

Bruno, O., S. Schenone, A. Ranise, F. Bondavalli, E. Barocelli, V. Ballabeni, M. Chiavarini, S. Bertoni, M. Tognolini, and M. Impicciatore. 2001. New polycyclic pyrimidine derivatives with anti platelet in vitro activity: synthesis and pharmacological screening. *Bioorganic and Medicinal Chemistry* 9: 629–636.

Chang, F.R., C.C. Wu, T.L. Hwang, R. Patnam, R.Y. Kuo, W.Y. Wang, Y.H. Lan, and Y.C. Wu. 2003. Effect of active synthetic 2-substituted quinazolinones on anti-platelet aggregation and the inhibition of superoxide anion generation by neutrophils. *Archives of Pharmacal Research* 26: 511–515.

Clark, J., M.S. Shahhet, D. Korakas, and G. Varvounis. 1993. Synthesis of thieno[2,3-D]pyrimidines from 4,6-dichloropyrimidine-5-carbaldehydes. *Journal of Heterocyclic Chemistry* 30: 1065–1072.

Crepaldi, P., B. Cacciari, M.C. Bonache, G. Spalluto, K. Varani, P.A. Borea, I. von Kügelgen, K. Hoffmann, M. Pugliano, C. Razzari, and M. Cattaneo. 2009. 6-Amino-2-mercapto-3H-pyrimidin-4-one derivatives as new candidates for the antagonism at the $P2Y_{12}$ receptors. *Bioorganic and Medicinal Chemistry* 17: 4612–4621.

Ding, Y.A., T.C. Chou, J.W. Chern, and M.H. Yen. 1995. Antiplatelet actions of 2-{4-[1-(2-chlorophenyl)piperazinyl]} methyl-2,3-dihydroimidazo[1,2-*c*]quinazolin-5(6*H*)-one compound. *Thrombosis Research* 77: 291–303.

Dupin, J.P., R.J. Gryglewski, D. Gravier, G. Hou, F. Casadebaig, J. Swies, and S. Chlopicki. 2002. Synthesis and thrombolytic activity of new thienopyrimidinone derivatives. *Journal of Physiology and Pharmacology* 53: 625–634.

Furniss, B.S., A.J. Hannaford, P.W.G. Smith, and A.R. Tatchell. 1989. *Vogel's textbook of practical organic chemistry*, 1230. London: Longman Scientific and Technical.

Giri, S., and Nizamuddin. 1978. Synthesis of some bis-(oxadiazolyl)sulphides, sulfones and 2-substituted-[1,3,4]-oxadiazolo-[2,3,*b*]quinazol-5-ones as potential fungicides. *Agricultural and Biological Chemistry* 42: 41–44.

Giridhar, R., R.S. Tamboli, R. Ramajayam, D.G. Prajapati, and M.R. Yadav. 2012. Assessment of antiplatelet activity of 2-aminopyrimidines. *European Journal of Medicinal Chemistry* 50: 428–432.

Gravier, D.J., F. Casadebaig, G. Hou, M. Boisseau, and H. Bernard. 1992. Synthesis and in vitro study of platelet antiaggregant activity of some 4-quinazolinone derivatives. *Pharmazie* 47: 91–94.

Gryglewski, R.J., J.P. Dupin, W. Uracz, J. Swies, J. Madej, G. Hou, D. Gravier, and F. Casadebaig. 2000. Thrombolysis by thienopyridines and their congeners. *Journal of Physiology and Pharmacology* 51: 683–693.

Guthrie, R. 2011. Review and management of side effects associated with antiplatelet therapy for prevention of recurrent cerebrovascular events. *Advances in Therapy* 28: 473–482.

Hemmateenejad, B., R. Miri, N. Edraki, M. Khoshneviszadeh, and A. Shafiee. 2007. Molecular modeling and QSAR analysis of some 4,5-dichloroimidazolyl-1,4-DHP-based calcium channel blockers. *Journal of the Iranian Chemical Society* 4: 182–193.

Kim, D., S.-I. Hong, and D.-S. Lee. 2006. The quantitative structure-mutagenicity relationship of polycylic aromatic hydrocarbon metabolites. *International Journal of Molecular Sciences* 7: 556–570.

Laddha, S.S., and P.S. Bhatnagar. 2009. A new therapeutic approach in Parkinson's disease: some novel quinazoline derivatives as dual selective phosphodiesterase 1 inhibitors and anti-inflammatory agents. *Bioorganic and Medicinal Chemistry* 17: 6796–6802.

Lakhan, R. 1969. Synthesis of mercaptoquinazolinone derivatives as potential antimalarials. *Chemical and Pharmaceutical Bulletin* 17: 2357–2361.

Li, H., J.P. Wang, F. Yang, T. Liu, W.W. Qiu, J.Y. Li, J. Li, and J. Tang. 2012. Design, synthesis and biological activity evaluation of 2-mercapto-4(3*H*)-quinazolinone derivatives as novel inhibitors of protein tyrosine phosphatase 1B. *Heterocycles* 85: 1897–1911.

Liu, G., J. Xu, K.C. Park, N. Chen, S. Zhang, Z. Ding, F. Wang, and H. Du. 2011. Novel synthesis approach and antiplatelet activity evaluation of 6-alkylamino-2,4-dialkyl(aryl)thiopyrimidines. *Tetrahedron* 67: 5156–5161.

Maree, A.O., and D.J. Fitzgerald. 2007. Variable platelet response to aspirin and clopidogrel in atherothrombotic disease. *Circulation* 115: 2196–2207.

Meadows, T.A., and D.L. Bhatt. 2007. Clinical aspects of platelet inhibitors and thrombus formation. *Circulation Research* 100: 1261–1275.

Mendis, S., P. Puska, and B. Norrving. 2011. *Global atlas on cardiovascular disease prevention and control*. Geneva: World Health Organization.

Patrono, C., B. Coller, G.A. FitzGerald, J. Hirsh, and G. Roth. 2004. Platelet-active drugs: the relationships among dose, effectiveness, and side effects—The Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. *Chest* 126(3): 234S–264S.

Srivastava, A.K., and R. Gupta. 2012. A QSAR approach for the prediction of anti-ulcer activity of *N*-acyl amino acids and imidazopyrazines/pyridines derivatives. *Journal of Chemical and Pharmaceutical Research* 4: 2228–2241.

Tozkoparan, B., M. Ertan, P. Kelicen, and R. Demirdamar. 1999. Synthesis and anti-inflammatory activities of some thiazolo[3,2-*a*]pyrimidine derivatives. *Il Farmaco* 54: 588–593.