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2-Heteroaryl 2-Substituted Phenylketone Derivatives and Their Inhibitory Activity on Platelet Aggregation

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Abstract

R 68070 and CV-4151 are two compounds possessing both thromboxane synthetase inhibitory activity and thromboxane receptor antagonist properties. 2-Heteroaryl 2-substituted phenylketone derivatives with a partial structural similarity to R 68070 and CV-4151, i.e. possessing a phenyl and a heteroaryl moiety, have been prepared and found to have antiplatelet activity. The compound 2-thienyl 2'-hydroxyphenyl ketone (4) was shown to completely inhibit platelet aggregation induced by arachidonic acid at a concentration of 5·0 μM. Structure-activity analysis indicated that the presence of a ketone group is an important requirement for this inhibitory activity. An *o*-hydroxyl substitution on the phenyl ring, and a 2-thienyl of heteroaryl ring might increase inhibitory activity.

Thromboxane A_2 (TXA_2), an unstable metabolite of arachidonic acid, induces platelet aggregation and contraction of vascular smooth muscle (Hamberg et al 1975; Bhagwat et al 1985 a, b). An enhanced production of TXA_2 is associated with the pathogenesis of various thrombotic and ischaemic disorders (Catella et al 1987; Fitzgerald et al 1987). Therefore drugs that suppress thromboxane A_2 formation should be effective in preventing and in curing ischaemic disease. For example, ozagrel and dazoxiben, two thromboxane synthase inhibitors which suppress the formation of TXA_2 and the compound GR32191 a thromboxane receptor antagonist which blocks the activity of TXA_2 have been developed for the prevention of the action of TXA_2 (Cross & Dickinson 1987; Fiddler & Lumley 1990). Furthermore studies on combination therapy with thromboxane synthase inhibitors and thromboxane receptor antagonists in animals (Fitzgerald et al 1988; Shebuski et al 1988), and in normal volunteers (Gresele et al 1987) have demonstrated that the two agents have greater therapeutic benefit when given in combination than when given alone. Very recently, two compounds, R 68070 (De Clerck et al 1989) and CV-4151 (Imura et al 1988) possessing both thromboxane synthetase-inhibitory activity and thromboxane receptor-antagonist properties in a single chemical entity, have been described. The common structural features of R 68070 and CV-4151 are a phenyl and a heteroaryl moiety at one end and an extended carboxylic acid side chain at the other end of a double bond of the molecule (Fig. 1).

In the search of new compounds which have potential inhibitory activity on the arachidonic acid cascade, we have undertaken the synthesis of new compounds with similar structural features to R 68070 and CV-4151 but having the 3-pyridynyl ring replaced with a 2-furyl or 2-thienyl ring. Surprisingly, in the process of preparation of these compounds an important intermediate showed significant antiplatelet activity. Therefore, we concentrated our efforts on the modification of the carbonyl function group of the ketone intermediates. These compounds contain a phenyl and a heteroaryl moiety, but are devoid of the extended carboxylic acid side chain (Fig. 1). Here we report their synthesis. The products were synthesized via dehydration of furan, thiophene and *o*-methoxybenzoic acid with phosphorous pentoxide, followed by modification of the ketone group (Fig. 2). The 14 final compounds obtained were tested for antiplatelet activity and for their effect on thromoboxane B_2 formation.

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Materials and Methods

Synthetic methods

Melting points are not corrected. For chromatography, silica gel 60 (70–230 mesh) was used (E. Merck, Darmstadt, Germany). Thin-layer chromatography (TLC) was performed on pre-scored DC-Alufolien Kieselgel $60F_{254}$ (E. Merck). Compounds were visualized under UV light (254 nm). All evaporations were carried out using a rotary evaporator connected to a water-aspirator pump. The water-bath temperature was maintained between 40° and 50°C unless otherwise stated. Proton nuclear magnetic resonance (^1H NMR) spectra were obtained using an Am-300 WB FT-NMR spectrometer operating in the FT mode at 300 MHz . IR spectra were recorded on a Perkin-Elmer 938G spectrophotometer. Elemental analyses were obtained in a Perkin-Elmer 2400.

Preparation of 2-furyl 2'-methoxyphenyl ketone (1)

A mixture of *o*-methoxybenzoic acid (54·82 g, 0·36 mol), phosphorous pentoxide (54·82 g, 0·36 mol) in benzene (300 mL) was heated under reflux for 2 h. When the mixture was cooled to room temperature (25°C), furan (30 mL, 0·51 mol) was added. The mixture was heated again at 60°C under reflux for 7 h and passed through a filter. The filtrate was concentrated in-vacuo. The residue was

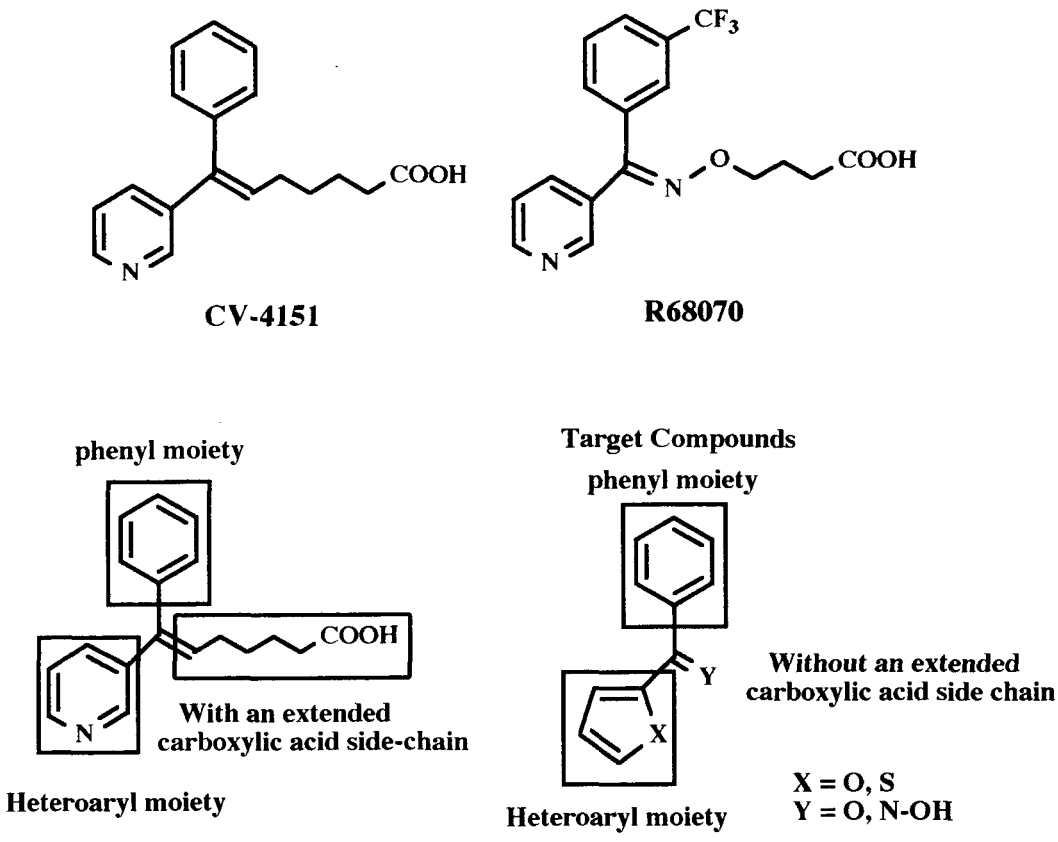

FIG. 1. Chemical structures.

chromatographed on silica gel with *n*-hexane/ethyl acetate (5/1). The desired fractions were concentrated in-vacuo to give 47·68 g, 65% of the pure **1**. M.p. 50–51°C. R_f: 0·68 (*n*-hexane/ethyl acetate = 2/1); IR (KBr): 3119, 2964, 2836, 1639, 1485, 1017, 755 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 7·61–6·49 (m, 7 H, Ar), 3·78 (s, 3 H, OCH_3) Anal. Calcd. for $C_{12}H_{10}O_3$ (202·2): C, 71·27; H, 4·99. Found: C, 70·93; H, 5·22.

Preparation of 2-thienyl 2'-methoxyphenyl ketone (2)
Compound **2** was prepared in a manner similar to the preparation used to synthesize compound **1**. Reagent: *o*-methoxybenzoic acid (1·99 g, 13 mmol), a mixture of phosphorous pentoxide (2·05 g, 14 mmol), thiopene (1·8 mL, 22 mmol) and benzene (10 mL). Purification of **2** was accomplished by chromatography on silica gel with *n*-hexane/ethyl acetate (5/1). The desired fractions were concentrated in-vacuo to give 1·7 g, 60% of pure **2**. R_f: 0·42 (*n*-hexane/ethyl acetate = 2/1); IR (neat): 1640, 1596, 1410, 1302, 1262, 844, 756 cm^{-1}; Mass spectrum, m/z (relative intensity): 218 (M^+, 66), 135 (M^+–thiophene, 100); (^{1}H-NMR (DMSO-d_6): δ 8·0–6·6 (m, 7 H, Ar), 3·72 (s, 3 H, OCH_3). Anal. Calcd. for $C_{12}H_{10}O_2S$ (218·26): C, 66·03; H, 4·62. Found: C, 65·69; H, 4·65.

Preparation of 2-furyl 2'-hydroxyphenyl ketone (3)
BCl_3 (11·5 mL, 11 mmol) was added to a mixture of compound **1** (1·5 g, 7·4 mmol) in 15 mL dichloromethane at 0°C. The mixture was stirred for 2 h, and then the temperature was raised to room temperature. The solution was neutralized with sodium bicarbonate (powder) to pH 7 and stirred for an additional 30 min. The organic layer was separated and concentrated in-vacuo. The residue was chromatographed on silica gel with *n*-hexane/ethyl acetate (15/1). The desired fractions were concentrated in-vacuo to give 1·2 g, 86% of pure **3**. R_f: 0·36 (*n*-hexane/ethyl acetate = 95/5); IR (neat): 3435, 3149, 1624, 1589, 1459, 1333, 1307, 1251, 884, 756 cm^{-1}; Mass spectrum, m/z (relative intensity): 188 (M^+, 88), 171 (M^+–OH, 8), 120 (M^+–furan, 100). ^{1}H-NMR (DMSO-d_6): δ 10·56 (s, 1 H, OH), 8·05-6·72 (m, 7 H, Ar). Anal. Calcd. for $C_{11}H_8O_3$ (188·17): C. 70·2; H, 4·28. Found: C, 69·83; H, 4·04.

Preparation of 2-thienyl 2'-hydroxyphenyl ketone (4)
A mixture of 47% hydrogen bromide (272 mL, 2·4 mol) and compound **2** (34·34 g, 0·16 mmol) was heated under reflux for 5 h. After cooling to room temperature, the mixture was added to ice water (300 mL) and extracted with chloroform. The organic layer was separated and concentrated in-vacuo. The residue was chromatographed on silica gel with *n*-hexane/ethyl acetate (90/1). The desired fractions were concentrated in-vacuo to give 27·18 g, 85% of pure (**4**). R_f: 0·19 (*n*-hexane/ethyl acetate = 90/1): IR (neat): 3103, 1618, 1585, 1508, 1480, 1412, 1354, 1327, 1305, 1248, 1214, 856, 802, 759, 723 cm^{-1}; Mass spectrum, m/z (rel. intensity): 203 (M^+, 58), 120 (M^+–thiophene, 100). ^{1}H-NMR (DMSO-d_6): δ 10·26 (s, 1 H, OH), 8·03–6·89 (m, 7 H, Ar), 3·72 (s, 3 H,

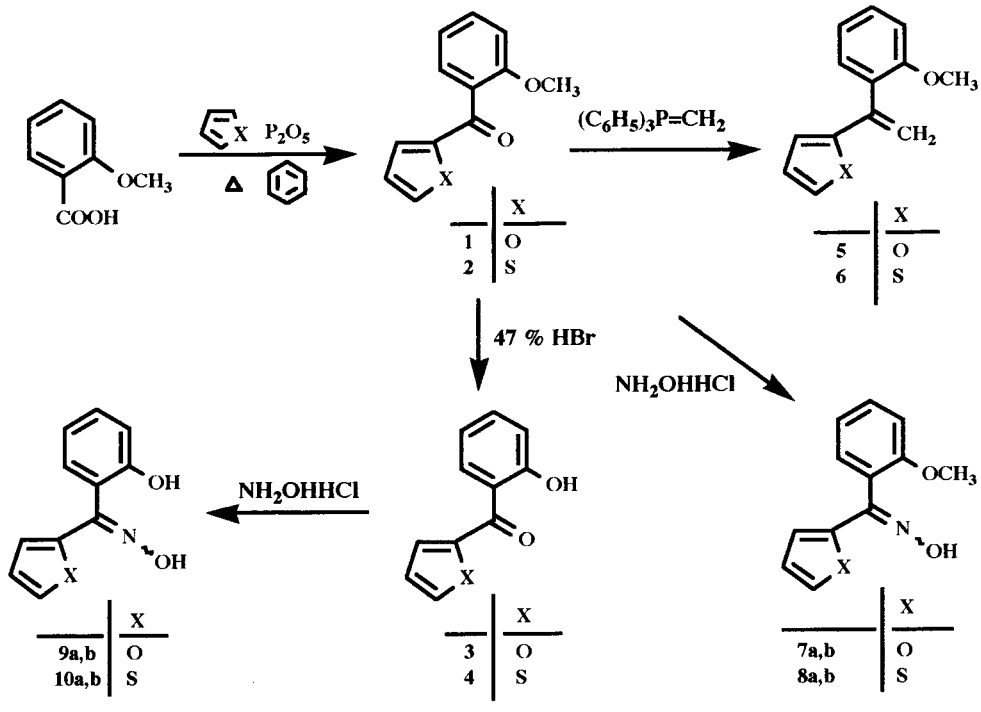

FIG. 2. Synthetic scheme of target compounds.

OCH_3. Anal. Calcd. for $C_{11}H_8O_2S$ (202·2): C, 64·68; H, 3·95. Found: C, 64·63; , 3·97.

Preparation of 1-(2-furyl)-1-(2'-methoxyphenyl) ethylene (5)
n-Butyl lithium (6·3 mL, 10 mmol) was added to a mixture of methyltriphenyl phosphonium, bromide (3·57 g, 10 mmol) in ether (50 mL) under nitrogen. After stirring (28°; 4 h), compound **1** (2·5 mL, 12 mmol) was added and stirring continued for another 12 h after which the mixture was filtered, concentrated in-vacuo and chromatographed on silica gel with *n*-hexane/ethyl acetate (15/1). 1·44 g compound **5** were obtained with an 80% purity after concentration of the desired fractions. R_f: 0·78 (*n*-hexane/ethyl acetate = 40/1); Mass spectrum, m/z (relative intensity): 200 (M^+, 52), 185 (M^+-CH_3, 100). ^{1}H-NMR ($CDCl_3$): d 7·3–5·93 (m, 7 H, Ar), 5·86, 5·14 (d, 1 H each, J = 1·6 Hz, $=CH_2$), 3·74 (s, 3 H, OCH_3). Anal. Calcd. for $C_{13}H_{12}O_2 \cdot H_2O$ (218·23): C, 71·54; H, 6·47. Found: C, 71·89; H, 6·08.

Preparation of 1-(2-thienyl)-1-(2'-methoxyphenyl)ethylene (6)
Compound **6** was prepared in a manner similar to the preparation used to synthesize compound **5** using *n*-butyl lithium (2·5 mL, 4 mmol), a mixture of methyltriphenyl phosphonium bromide (1·01 g, 2·8 mmol) in ether (30 mL) and compound **2** (1 mL, 2·8 mmol). Purification of **6** was accomplished by chromatography on silica gel with *n*-hexane/ethyl acetate (40/1). The desired fractions were concentrated in-vacuo to give 0·26 g, 43% of pure **6**. R_f: 0·38 (*n*-hexane/ethyl acetate = 40/1); IR (neat): 3434, 2925, 1590, 1484, 1454, 1428, 1240, 1024, 753 cm^{-1}; Mass spectrum, m/z (rel. intensity): 216 (M^+, 78), 201 (M^+-CH_3, 100). ^{1}H-NMR (DMSO-d_6): d 7·4–6·73 (m, 7 H, Ar), 5·67, 5·02 (s, 1 H each, $=CH_2$), 3·65 (s, 3 H, OCH_3). Anal. Calcd. for $C_{13}H_{12}OS$ (202·2): C, 72·18; H, 5·59. Found: C, 71·96; H, 5·95.

General procedure of reactions of ketone derivatives with hydroxyamine hydrochloride. 2-Furyl 2'-methoxyphenyl ketone oxime (7a, b)
A mixture of hydroxyamine hydrochloride (5 g, 72 mmol), compound **1** (3 g, 14·8 mmol), and pyridine (10 mL) was heated under reflux for 8 h. The mixture was concentrated and then extracted with a solvent mixture of ethyl ether and 5% aqueous hydrogen chloride. The ethyl ether layer was concentrated and the residue was chromatographed on silica gel with *n*-hexane/ethyl acetate (3/1). The fractions (Rf = 0·43) and fractions (Rf = 0·32) were concentrated to give two isomers, **7a** and **7b** respectively.

7a: 1·1 g, 34%; m.p. 132–134°C; R_f: 0·43 (*n*-hexane/ethyl acetate = 3/1); IR (KBr): 3203, 1590, 1496, 1473, 1451, 1433, 1248, 1024, 1008 cm^{-1}; UV λ_{max}nm (logϵ): methanol: 271 (3·98); mass spectrum (70eV) m/z (rel. intensity): 217 (M^+, 58), 200 (M^+-OH, 18), 185 (M^+-NOH, 75); ^{1}H-NMR (300 MHz, DMSO-d_6); δ 11·78 (s, 1 H, N—OH), 7·58–6·60 (m, 7 H, Ar), 3·65 (s, 3 H, OCH_3). Anal. Calcd. for $C_{12}H_{11}NO_3$ (217·217): C, 66·35; H 5·11; N, 6·45. Found: C, 65·93; H, 5·15; N, 6·54.

7b: 0·7 g, 22%; m.p. 150–153°C; R_f: 0·43 (*n*-hexane/ethyl acetate = 3/1); IR (KBr): 3267, 1596, 1494, 1250, 990, 960, 883, 873, 758 cm^{-1}; UV λ_{max} nm (logϵ): methanol: 272 (4·11); MS (70eV) m/z (rel intensity): 217 (M^+, 80), 200 (M^+-OH, 17), 185 (M^+-NOH, 100); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 11·11 (s, 1H, N—OH), 7·67–6·10 (m, 7 H, Ar), 3·68 (s, 3 H, OCH_3). Anal. Calcd. for $C_{12}H_{11}NO_3$ (217·217): C,

Table 1. Inhibitory effect (%) of 2-heteroaryl 2-substituted phenylketone derivatives in arachidonic acid-(10 μg mL^{-1}) induced platelet aggregation.

Compounds	Concentration (μM) 50	5·0	2·5	1·0	0·5
2	100	99 ± 1·7	93 ± 6·2	25 ± 3	4 ± 1
3	100	99 ± 2·3	85 ± 15·3	29 ± 13	25 ± 11
4	100	100 ± 1·6	95 ± 5·2	98 ± 3·5	38 ± 11

Mean ± s.e. (n = 4). Control aggregation amplitude 57·7 ± 4·5 was set as 100%.

66.35; H, 5·11; N. 6·45. Found: C, 66·00; H, 5·10; N, 6·56.

The following compounds were prepared from the indicated starting materials by using the general procedure described above.

2-Thienyl 2′-methoxyphenyl ketone oxime (***8a, b***) was prepared from 2-thienyl 2′-methoxyphenyl ketone (3 g, 13·74 mmol) and hydroxyamine hydrochloride (5 g, 72 mmol).

8a: 0·97 g, 30%; m.p. 140–142°C; R_f: 0·25 (*n*-hexane/ethyl acetate = 4/1); IR (KBr): 3232, 3101, 1594, 1581, 1489, 1457, 1424, 1232, 992, 892, 730 cm^{-1}; UV λ_{max} nm (logϵ): methanol: 283 (3·93); mass spectrum (70eV) m/z (rel. intensity): 233 (M^+, 80); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 12·00 (s, 1 H, N—OH), 7·71–6·58 (m, 7 H, Ar), 3·69 (s, 3 H, OCH_3). Anal. Calcd. for $C_{12}H_{11}NO_2S$ (217·217): C, 61·78; H, 4·75; N, 6·00. Found: C, 61·85; H, 4·70; N, 5·79.

8b: 2·06 g, 64%; m.p. 162–165°C; R_f: 0·14 (*n*-hexane/ethyl acetate = 4/1); IR (KBr): 3267, 3082, 1598, 1581, 1457, 1414, 1274, 965, 906, 753 cm^{-1}; UV λ_{max} nm (logϵ): methanol: 280 (4·03); mass spectrum (70eV) m/z (rel. intensity): 233 (M^+, 72); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 11·32 (s, 1 H, N—OH), 7·67–6·10 (m, 7 H, Ar), 3·68 (s, 3 H, OCH_3). Anal. Calcd. for $C_{12}H_{11}NO_2S$ (217·217): C, 61·78; H, 4·75; N, 6·00. Found: C, 61·12; H, 4·82; N, 5·83.

2-Furyl 2′-hydroxyphenyl ketone oxime (***9a, b***) was prepared from 2-furyl 2′-hydroxyphenyl ketone (3 g, 16 mmol) and hydroxyamine hydrochloride (5 g, 72 mmol).

9a: 0·832 g, 26%; m.p. 122–125°C; R_f: 0·45 (*n*-hexane/ethyl acetate = 3/1); IR (KBr): 3393, 3044, 2886, 1307, 1257, 1097, 1086, 1031, 1004, 917, 895 cm^{-1}; UV λ_{max} nm (logϵ): methanol: 281 (3·90); mass spectrum (70eV) m/z (rel. intensity): 203 (M^+, 60), 1·86 ($M^+ - OH - 1$, 100); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 11·37 (s, 1 H, N—OH), 9·49 (s, 1 H, ArOH), 7·62–6·60 (m, 7 H, ArH). Anal. Calcd. for $C_{11}H_9NO_3$ (203·182): C, 65·02; H 4·46; N, 6·89. Found: C, 64·43; H, 4·55; N, 6·64.

9b: 1·987 g, 61%; m.p. 115–117°C; R_f: 0·1 (*n*-hexane/ethyl acetate = 3/1); IR (KBr): 3352, 3097, 1586, 1444, 1409, 1371, 1287, 1228, 1100, 990, 839, 758 cm^{-1}; UV λ_{max} nm (logϵ): methanol: 285 (4·0); mass spectrum (70eV) m/z (rel. intensity): 203 (M^+, 45), 186 ($M^+ - OH - 1$, 100), 185 (M^+–NOH, 100); ^{1}H-NMR (300 MHz, DMSO-d_6); δ 11·05 (s, 1 H, N—OH), 9·42 (s, 1 H, ArOH), 7·45–6·62 (m, 7 H, ArH). Anal. Calcd. for $C_{11}H_9NO_3$ (203·182): C, 65·02; H, 4·46; N, 6·89. Found: C, 65·44; H, 4·16; N, 6·60.

2-Thienyl 2′-hydroxyphenyl ketone oxime (***Xa, b***) was prepared from 2-thienyl 2′-hydroxyphenyl ketone (1·3 g, 6·4 mmol) and hydroxyamine hydrochloride (0·48 g, 7 mmol).

10a: 0·34 g, 24%; m.p. 121–122°C; R_f: 0·4 (*n*-hexane/ethyl acetate = 3/1); IR (KBr): 3423, 1605, 1449, 1411, 900, 726 cm^{-1}; UV λ_{max} nm (logϵ): methanol: 281 (3·9); mass spectrum (70eV) m/z (rel. intensity): 219 (M^+, 100); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 11·94 (s, 1 H, N—OH), 9·51 (s, 1 H, ArOH), 7·70–6·82 (m, 7 H, Ar). Anal. Calcd. for $C_{11}H_9NO_2S.1/2H_2O$ (228·253): C, 57·88; H, 4·14; N, 6·14. Found: C, 57·79; H, 4·38; N, 6·04.

10b: 0·12 g, 9%; m.p. 155–157°C; R_f: 0·3 (*n*-hexane/AcOEt = 3/1): IR (KBr): 3351, 3098, 1809, 1612, 1499, 1316, 1036, 942, 681, 640 cm^{-1}; UV λ_{max} nm (logϵ): methanol: 285 (4·0); mass spectrum (70eV) m/z (rel. intensity): 219 (M^+, 100); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 11·05 (s, 1 H, N—OH), 9·42 (s, 1 H, ArOH), 7·45–6·62 (m, 7 H, Ar). Anal. Calcd. for $C_{11}H_9NO_2S.1/2H_2O$ (228·253): C, 57·88; H, 4·14; N, 6·14. Found: C, 57·61; H, 4·18; N, 6·31.

Materials

Collagen (type 1, equine tendon) was obtained from Chrono-Log Co. and stored (1 mg mL^{-1}) at −70°C. U46619 (9,11-dideoxy-9α,11α-methanoepoxy-PGF$_{2\alpha}$) was obtained from Biomol Co. and dissolved in dimethylsulphoxide. Thrombin (bovine) was purchased from Sigma Chemical Co. and dissolved in 50% (v/v) glycerol to give a stock solution of 100 units mL^{-1}. Platelet-activating factor (PAF) was purchased from Sigma Chemical Co., and dissolved in chloroform. Arachidonic acid, bovine serum albumin (BSA), indomethacin, EDTA (disodium salt), sodium citrate, luciferase-luciferin and Quin-2/AM were purchased from Sigma Chemical Co. Cyclic-AMP RIA kits and thromboxane B_2 RIA kits were purchased from Amersham, UK.

Table 2. Inhibitory effect (%) of 2-heteroaryl 2-substituted phenylketone derivatives in collagen-(1 μM) induced platelet aggregation.

Compounds	Concentration (μM) 100	50	25	12·5
2	55 ± 32	58 ± 35	47 ± 45	—
3	63 ± 26	54 ± 26	41 ± 27	—
4	72 ± 19	59 ± 20	53 ± 27	23 ± 10

Mean ± s.e. (n = 4).

Table 3. Inhibitory effect (%) of 2-heteroaryl 2-substituted phenylketone derivatives in U46619-(1 μM) and PAF-(2 ng mL^{-1}) induced platelet aggregation.

Concn (μM)	U46619 (1 μM)			PAF (2 ng mL^{-1})		
	2	**3**	**4**	**2**	**3**	**4**
300	27 ± 10	18 ± 3	64 ± 8	77 ± 18	60 ± 19	71 ± 15
100	16 ± 5	—	34 ± 15	32 ± 10	57 ± 20	35 ± 10
50	7 ± 1	—	16 ± 7	—	—	29 ± 8
25	—	—	—	—	—	24 ± 9

Mean ± s.e. (n = 4).

Preparation of rabbit washed platelets

Whole blood anticoagulated with EDTA (100 mM, 14 : 1 v/v) was withdrawn from rabbit marginal ear vein. Platelet-rich plasma (PRP) was obtained from the supernatant after centrifugation at 160 *g* at 25°C for 10 min. Platelet suspension was prepared from EDTA-anticoagulated PRP according to the washing procedures described by Teng et al (1987). Briefly, the PRP was centrifuged at 500 *g* for 10 min and the platelet pellets were suspended in Tyrode solution; the platelet suspension was centrifuged at 500 *g* for 10 min. The platelet pellets were finally suspended in Tyrode solution of the following composition (mM): $CaCl_2$ 1, NaCl 136·8, KCl 2·7, $NaHCO_3$ 11·9, $MgCl_2$ 2·1, NaH_2PO_4 0·4, and glucose 10 containing BSA (0·35%). Platelet concentration was counted (Coulter Counter, model ZM) and adjusted to $3{\cdot}0 \times 10^8$) platelets mL^{-1}.

Platelet aggregation

Aggregation was measured by the turbidimetric method as described by O'Brien (1962), with the absorbance of Tyrode solution taken as 100% aggregation and the absorbance of platelet suspension taken as 0% aggregation. The aggregation was measured using a Lumi-aggregometer (Model 560, Chrono-Log Co., USA.) connected to two dual-channel recorders. The platelet preparations were stirred at 1200 rev min^{-1} at 37°C. To eliminate the effect of the solvent on the aggregation and release reaction of platelets, the final concentration of dimethylsulphoxide was fixed at 0·5%.

Thromboxane B_2 assay

Thromboxane B_2 (TXB_2) concentration was measured to reflect the amount of TXA_2, because TXB_2 is a stable metabolite of TXA_2. Six minutes after the challenge of platelets with the aggregation inducer, EDTA (2 mM) and indomethacin (50 μM) were added. Following the procedure described by the manufacturer, the quantitative formation of TXB_2 was measured after centrifugation in a Sigma centrifuge (model 2K15) for 5 min at 10 000 *g*, using the radioimmunoassay kit.

Results and Discussion

The inhibitory effects of compounds **2**, **3** and **4** on platelet aggregation induced by arachidonic acid, collagen, U46619 and PAF are presented in Tables 1–3. Table 4 lists the TXB_2 formation induced by collagen, arachidonic acid and thrombin. The other derivatives of 2-heteroaryl 2-substituted phenylketone described in this paper did not show significant inhibitory effect on platelet aggregation or TXB_2 formation at a concentration up to 200 μM.

Table 1 shows that compounds **2**, **3** and **4** at concentrations of 5·0 μM were able to completely inhibit platelet aggregation induced by arachidonic acid. When the concentration was reduced to 0·5 μM, compounds **3** and **4** were still able to inhibit platelet aggregation by 25 and 38%, respectively. Of the three compounds tested, compound **4** was the most potent.

Compounds **2**, **3** and **4** were not able to completely inhibit

Table 4. Effect of 2-heteroaryl 2-substituted phenylketone derivatives on the TXB_2 formation of rabbit washed platelets, induced by collagen, arachidonic acid or thrombin.

Treatment	μM	Thromboxane B_2 (ng mL^{-1}) formation induced by		
		Collagen	Arachidonic acid	Thrombin
		(10 μg mL^{-1})	(100 μM)	(0·1 units mL^{-1})
Control		52 ± 10	170 ± 16	53 ± 8
2	100	0·4 ± 0·2***	10·4 ± 4·2***	0·7 ± 0·4***
	50	1·7 ± 0·7***	94 ± 35*	—
3	100	1·1 ± 0·6***	3·3 ± 1·0***	0·7 ± 0·3***
	50	2·0 ± 1·0***	37 ± 5***	—
4	100	0·4 ± 0·1***	0·6 ± 0·1***	1·3 ± 0·5***
	50	0·9 ± 0.3***	2·7 ± 0·6***	—

Compounds or dimethylsulphoxide (0·5%, control) was preincubated with platelets at 37°C for 1 min, then inducer was added. Aggregation and TXB_2 formation were terminated by EDTA (2 μM) and indomethacin (50 μM) 6 min after the addition of inducer. Values are presented as mean ± s.e. (n = 4). *$P < 0{\cdot}05$, ***$P < 0{\cdot}001$ compared with the control value.

platelet aggregation induced by collagen (Table 2) even when the concentration was increased to 100 μM. However, a moderate inhibitory activity was obtained at a dose of 25 μM with values of 47, 41 and 38% respectively.

The inhibitory effect on platelet aggregation induced by U46619 and PAF is shown in Table 3. None of the new compounds synthesized were able to inhibit even 50% of the U46619-induced platelet aggregation at a concentration as high as 100 μM. At the same concentration of 100 μM, only compound **3** showed a 57% inhibition against PAF-induced platelet aggregation.

TXB_2 formation in platelets was measured 6 min after the addition of inducers. As shown in Table 4, at 100 μM, all three compounds inhibited markedly TXB_2 formation caused by the indicated inducers. When the concentration was reduced to 50 μM, compounds **3** and **4** still showed significant decrease on TXB_2 formation induced by collagen and arachidonic acid.

In the present investigation, modified 2-heteroaryl 2-substituted-phenylketone derivatives, including oximes (compounds **7**, **8**, **9** and **10**) and olefins (compounds **5** and **6**), did not show any inhibitory activity on platelet aggregation. Only ketone derivatives (compounds **2**, **3** and **4**) were shown to possess good inhibitory activity on arachidonic- and collagen-induced aggregation of platelets in rabbit platelet-rich plasma. Of the 2-heteroaryl 2-substituted phenylketone derivatives, compounds **3** and **4** with a 2′-hydroxyl substitution on the phenyl ring had greater antiplatelet activity on arachidonic-induced platelet aggregation than compound **3** with a 2′-methoxy substitute on the phenyl ring. The activity of 2-heteroaryl 2-substituted-phenylketone derivatives were also affected by the heteroaryl group. For arachidonic- and collagen-induced platelet aggregation and TXB_2 formation, compound **4** possessing a thienyl group appeared to be a more potent antiplatelet reagent than compound **3** which possesses a furyl group instead.

Fourteen compounds of 2-heteroaryl 2-substituted phenylketone derivatives were prepared and evaluated for their antiplatelet aggregation ability. The results suggest that the presence of a ketone group is an important requirement for inhibition of TXA_2 synthesis. An *o*-hydroxyl substitution on the phenyl ring, and a 2-thienyl of heteroaryl ring might increase their potency.

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