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ORIGINAL RESEARCH

Synthesis, antiplatelet aggregation activity, and molecular modeling study of novel substituted-piperazine analogues

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Abstract New carbamoylpyridine and carbamoylpiperidine analogues containing nipecotic acid scaffold were designed, synthesized, and evaluated for their platelet aggregation inhibitory activity. Molecular modeling investigation was performed and the impact of lipophilicity on activity was also discussed. Structure activity relationship among this series was obtained. N^1-[1-(4-bromobenzyl)-3-piperidinocarbonyl]-N^4-(2-chlorophenyl)-piperazine hydrobromide (**20**), and 1,4-bis-[3-[N^4-(2-chlorophenyl)-N^1-(piperazinocarbonyl)]-piperidin-1-yl-methyl]-benzene dibromide (**30**) are the most active antiplatelet aggregating compounds in this study, both at concentration of 0.06 μM.

Keywords Synthesis · Piperazine analogues · Antiplatelet aggregation · Molecular modeling studies

K. M. Youssef (✉)
Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences & Pharmaceutical Industries, Future University, 12311 Cairo, Egypt
e-mail: khairia_m@yahoo.com

M. A. Al-Omar · H. I. El-Subbagh · N. G. Haress
Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia

L. A. Abou-zeid
Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, University of Mansoura, Mansoura 35516, Egypt

A.-G. M. Abdel-Gader · A. S. Al-Tuwaijri
Department of Physiology, College of Medicine, King Saud University, Riyadh 11451, Saudi Arabia

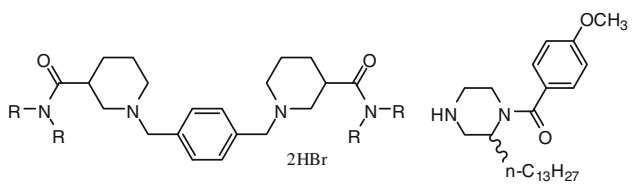
Introduction

Platelets are involved in the pathogenesis of many cardiovascular and thromboembolic diseases (Chatelain and Massingham, 1997; Fuster *et al*., 1992). Their hyperactivity increases the risk of various vaso-occlusive diseases, such as unstable angina, acute myocardial infarction, transient ischemic attacks, and complications following percutaneous coronary intervention (Fuster *et al*., 1992). Platelets can be activated by a number of agonists, such as adenosine 5(-diphosphate (ADP), thrombin, platelet-activating factor (PAF), serotonin, thromboxane A_2 (TxA_2), collagen, and catecholamines (Majerus and Tollesfsen, 2001). ADP plays a relevant role in platelet function. It can trigger platelet activation, which is mediated by at least three purinergic receptors (P_2Y_1, P_2Y_{12}, and P_2X) showing distinct specificity; ADP is also responsible for the secondary wave of platelet aggregation, followed by ADP release from dense granules which potentiates the aggregation response induced by other agents (Weksler, 2000; Mills, 1996).

Antiplatelet aggregation agents, including aspirin and thromboxane modulators, e.g., ridogrel; ADP antagonists, like the thienopyridine derivatives ticlopidine and clopidogrel; phosphodiesterase inhibitors, e.g., dipyridamole and cilostazol; and platelet glycoprotein IIb/IIIa antagonists, such as tirofiban and sibrafiban; are useful in the prophylaxis and treatment of thromboembolic diseases (Kunapuli, 1998; Gachet, 2001; Van De Graaff and Steinhubl, 2000; Patrono *et al*., 2001; Dogné *et al*., 2001). Nevertheless, currently available, orally administered antiplatelet drugs have limitations, especially with regard to side effects and broad clinical utility, as well as interference with physiological platelet function in hemostasis (Antithrombotic Trialists' Collaboration, 2002). Despite their efficacy, the

administration of thienopyridines is associated with some undesired side effects, such as neutropenia, thrombocytopenia, aplastic anemia, and thrombotic thrombocytopenic purpura (Storey, 2001). Moreover, most probably due to selective inhibition of some pathways of platelet activation and recruitment by aspirin and thienopyridines, a number of patients have been shown to be resistant to these drugs (Berger, 1999; McKee *et al*., 2002). These limitations are among the reasons stimulating the search for new antiplatelet drugs.

The interest in antiplatelet agents stemmed from the finding that some bis-3-carbamoyl-piperidine derivatives, namely α,α′-bis[3-(*N*,*N*-diethyl-carbamoyl)-piperidino]-*p*-xylenes (**1**, Chart 1), inhibit in vitro human platelet aggregation, and are effective in thrombosis models in vivo (Tucker *et al*., 1997; Youssef and Al-Shafie, 1998; De Marco *et al*., 2004; De Candia *et al*., 2005; Papadopoulou *et al*., 2005; Leoncini *et al*., 1997, 2004; Cody *et al*., 1999; Binisti *et al*., 2001; Heath *et al*., 2004). Due to their lipophilicity and surface activity, bis-nipecotamides can penetrate the platelet membranes and interact with anionic phospholipids (mainly phosphatidylinositol, PI, and phosphatidylserine, PS), thus rendering them resistant to hydrolysis catalyzed by phospholipase-C to the second messengers inositol 1,4,5-triphosphate (IP_3) and *s*,*n*-1,2-diacylglycerol (DAG). They also inhibit phosphoinositide turnover. As a consequence, the levels of IP_3 and of cytosolic Ca^{2+} concentrations, necessary for myosin phosphorylation and subsequent platelet activation, are reduced (Youssef and Al-Shafie, 1998; Heath *et al*., 2004; Feng *et al*., 1992; Dillingham *et al*., 1989). Phospholipases A_2 (PLA_2s) catalyze the hydrolysis of glycerol-phospholipids at the *sn*-2 position and generate free fatty acids and lysophospholipids (Slotboom *et al*., 1982). This can provide, in some cases, substrates for the biosynthesis of prostaglandins, thromboxanes, leukotrienes, and other oxygenated metabolites of arachidonic acid (AA), i.e., eicosanoids, as well as PAF (Binisti *et al*., 2001; Sallem *et al*., 2006) well-known mediators of inflammatory processes and tissue injury. It was reported that the piperazine derivative PMS 832 (**2**, Chart 1) showed remarkable inhibitory potency of PLA_2 activity with IC_{50} values in the micromolar range.



1 PMS 832 (**2**)

Chart 1 Literature cited antiplatelet aggregation agents

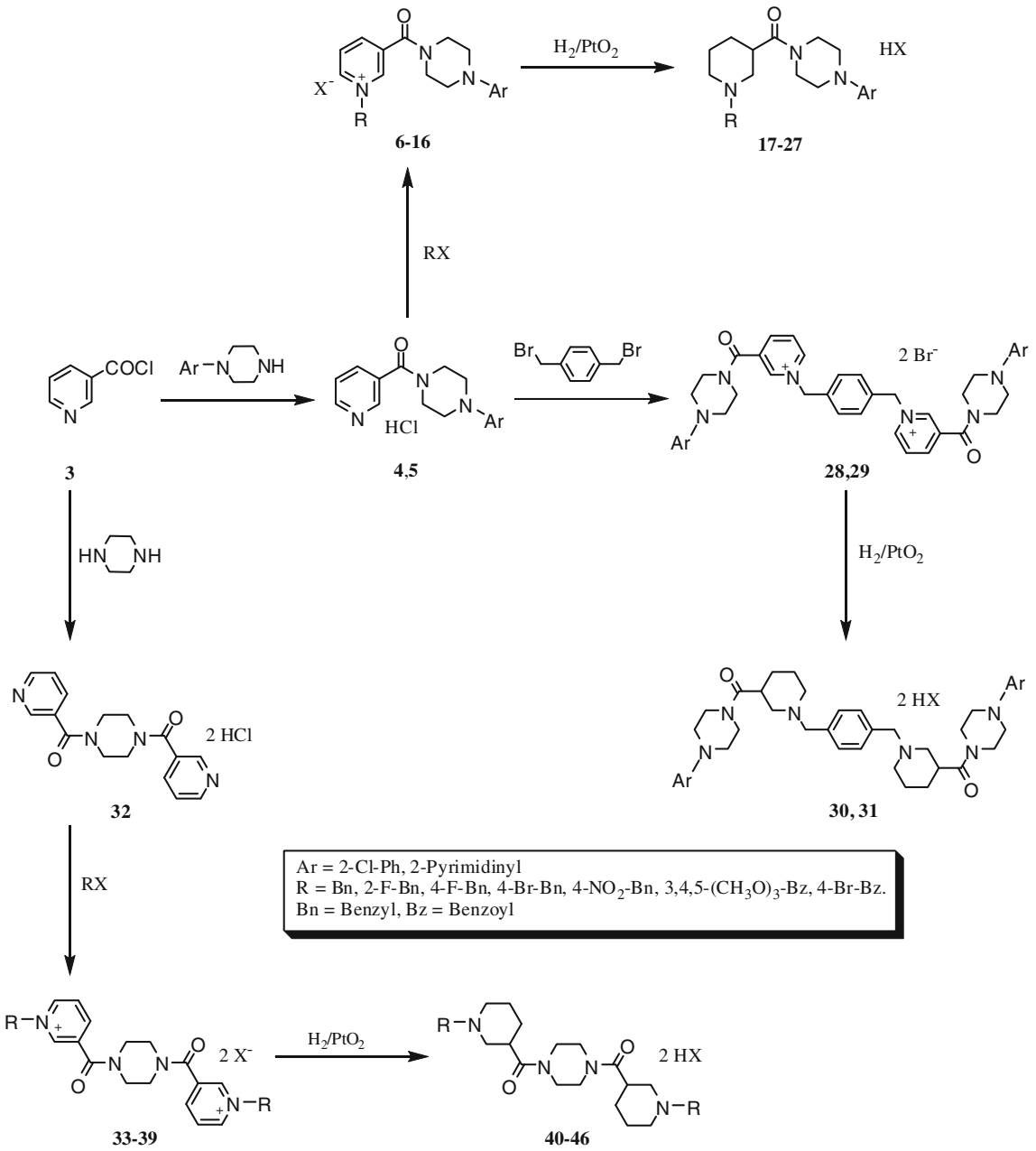
In this study, a series of new modified carbamoyl-pyridine and carbamoyl-piperidine analogues were designed and synthesized which related to the C-terminal γ-chain of fibrinogen, containing a nipecotic acid scaffold. The molecular modeling features of the designed compounds and their recognition profiles with the active site of thrombin receptor were investigated.

Chemistry

The synthesis of the target compounds is depicted in Scheme 1. Nicotinoyl chloride (**3**) was reacted with different 1-aryl and 1-aroyl-piperazines to furnish the carbamoyl-pyridines N^1-(3-pyridino-carbonyl)-N^4-aryl-piperazine hydrochlorides (**4**, **5**). Quaternization of **4** and **5** was carried out in acetone solutions of different alkyl or aroyl-halides in the presence of K_2CO_3 to afford the corresponding quaternary ammonium salts N^1-(1-aralkyl- or 1-aroyl-pyridinium-3-yl-carbonyl)-N^4-aryl-piperazine halides (**6**–**16**). Catalytic hydrogenation of the obtained pyridinium quaternary ammonium salts, using platinum oxide afforded the target compounds N^1-(1-aralkyl or 1-aroyl-3-piperidino-carbonyl)-N^4-aryl-piperazine hydrohalides (**17**–**27**) (Table 1). Two molar equivalents of N^1-(3-pyridino-carbonyl)-N^4-aryl-piperazine hydro-chlorides (**4**, **5**) were allowed to react with one mole equivalent of α,α′-dibromo-*p*-xylene to give the quaternary ammonium salts 1,4-bis-[3-(N^4-aryl-N^1-piperazino-carbonyl)-pyridinium-1-yl-methyl]-benzene dibromides (**28**, **29**), which were then subjected to catalytic hydrogenation using platinum oxide to afford the target compounds 1,4-bis-[3-(N^4-aryl-N^1-piperazino-carbonyl)-piperidin-1-yl-methyl]-benzene dibromides (**30**, **31**) (Table 2). Two molar equivalents of nicotinoyl chloride (**3**) were reacted with anhydrous piperazine to furnish the corresponding N^1,N^4-bis-[3-pyridino-carbonyl]-piperazine dihydrochloride (**32**). The acetone solution of the starting amide **32** was reacted with different alkyl or aroyl-halides in the presence of K_2CO_3 to afford the corresponding quaternary ammonium salts N^1,N^4-bis-(1-aralkyl- or 1-aroyl-pyridinium-3-yl-carbonyl)-piperazine dihalides (**33**–**39**), which were then subjected to catalytic hydrogenation using platinum oxide to afford the target compounds N^1,N^4-bis-(1-aralkyl- or 1-aroyl-piperidino-3-yl-carbonyl)-piperazine dihydrohalides (**40**–**46**) (Table 3).

Results and discussion

Platelet aggregation involves the cohesion of platelets to each other and this process is basic to form the hemostatic platelet plug. In vitro it can be induced by a number of agonists

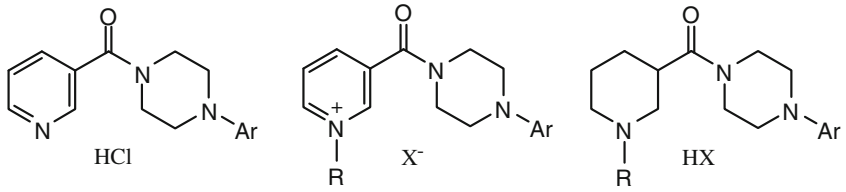


Scheme 1 Synthesis of the target compounds **4–46**

including adenosine-5′-diphosphate (ADP), adrenaline, collagen, thrombin, and other non-physiological compounds such as ristocetin. In this study, platelet aggregation was measured in response to ADP (20.0, 2.0, and 1.0 μmol/l), adrenaline (100 μmol/l), collagen (0.19 g/l), arachidonic acid (AA, 1.64 mmol/l), and ristocetin (1.5, 1.2, and 1.0 g/l) as reported. All these concentrations of agonists represent final concentrations obtained by adding 20 μl of the aggregating agent to 180 μl of platelet-rich plasma (PRP).

The antiaggregating effect of compounds N^1-(3-pyridino-carbonyl)-N^4-aryl-piperazine hydrochlorides (**4**, **5**) and N^1-(1-aralkyl- or 1-aroyl-pyridinium-3-yl-carbonyl)-N^4-aryl-piperazine halides (**6–9**, **11**, and **13**) showed low antiaggregating effects. Compound **4** showed antiaggregating effect for ADP, AA, adrenaline, and collagen at concentration of 0.4 μM. Compound **5** inhibited the aggregating effects of all agonists at concentration of 3.0 μM. Compound **6** showed antiaggregating effect for ristocetin only at concentration of 2.5 μM. Compounds **7**, **8**, **9**, **11**, and **13** inhibited the aggregating effect of ADP, AA, adrenaline, and collagen at concentration of 2.0, 10.0, 1.0, 0.5, and 10.0 μM, respectively. Compounds **7**, **11**,

Table 1 Physicochemical properties of the new synthesized compounds **4–27**



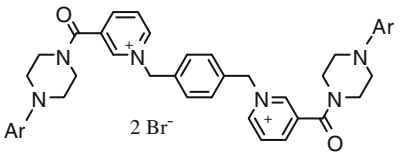
4, **5** **6-16** **17-27**

Compd	Ar	R	Yield (%)	MP (°C)	Molecular formulae	log *P*
4	2-Cl-Ph	–	62	225–227	$C_{16}H_{17}Cl_2N_3O$	2.46
5	2-Pyrimidinyl	–	–	–	Youssef and Al-Shafie (1998)	0.48
6	2-Cl-Ph	Bn	55	>300	$C_{23}H_{23}Cl_2N_3O$	–
7	2-Cl-Ph	2-F-Bn	57	102–104	$C_{23}H_{22}Cl_2FN_3O$	–
8	2-Cl-Ph	4-F-Bn	60	246–248	$C_{23}H_{22}Cl_2FN_3O$	–
9	2-Cl-Ph	4-Br-Bn	65	195–197	$C_{23}H_{22}Br_2ClN_3O$	–
10	2-Cl-Ph	4-NO_2-Bn	58	172–174	$C_{23}H_{22}BrClN_4O_3$	–
11	2-Cl-Ph	3,4,5-$(CH_3O)_3$-Bz	41	204–206	$C_{26}H_{27}Cl_2N_3O_5$	–
12	2-Cl-Ph	4-Br-Bz	61	170–172	$C_{23}H_{20}BrCl_2N_3O_2$	–
13	2-Pyrimidinyl	4-Br-Bn	78	218–220	$C_{21}H_{21}Br_2N_5O$	–
14	2-Pyrimidinyl	4-NO_2-Bn	81	>300	$C_{21}H_{21}BrN_6O_3$	–
15	2-pyrimidinyl	3,4,5-$(CH_3O)_3$-Bz	51	220–222	$C_{24}H_{26}ClN_5O_5$	–
16	2-Pyrimidinyl	4-Br-Bz	59	240–242	$C_{21}H_{19}BrClN_5O_2$	–
17	2-Cl-Ph	Bn	53	189–191	$C_{23}H_{29}Cl_2N_3O$	4.22
18	2-Cl-Ph	2-F-Bn	56	160–162	$C_{23}H_{28}Cl_2FN_3O$	4.38
19	2-Cl-Ph	4-F-Bn	59	195–197	$C_{23}H_{28}Cl_2FN_3O$	4.38
20	2-Cl-Ph	4-Br-Bn	64	191–193	$C_{23}H_{28}Br_2ClN_3O$	5.05
21	2-Cl-Ph	4-NO_2-Bn	57	135–137	$C_{23}H_{28}BrClN_4O_3$	3.22
22	2-Cl-Ph	3,4,5-$(CH_3O)_3$-Bz	42	170–172	$C_{26}H_{33}Cl_2N_3O_5$	3.28
23	2-Cl-Ph	4-Br-Bz	60	176–178	$C_{23}H_{26}BrCl_2N_3O_2$	4.48
24	2-Pyrimidinyl	4-Br-Bn	77	118–120	$C_{21}H_{27}Br_2N_5O$	3.07
25	2-Pyrimidinyl	4-NO_2-Bn	80	197–199	$C_{21}H_{27}BrN_6O_3$	0.84
26	2-Pyrimidinyl	3,4,5-$(CH_3O)_3$-Bz	60	102–104	$C_{24}H_{32}ClN_5O_5$	1.29
27	2-Pyrimidinyl	4-Br-Bz	58	196–198	$C_{21}H_{25}BrClN_5O_2$	2.50

and **13** inhibited the aggregating effect of ristocetin at concentration of 5.0, 1.0, and 20.0 μM, respectively (Table 4).

Compounds N^1-(1-aralkyl- or 1-aroyl-3-piperidinocarbonyl)-N^4-aryl-piperazine hydrohalides showed high antiaggregating effect. Compound **17** showed aggregation inhibition for all agonists at concentration of 5.0 μM. It inhibited the aggregating effect of ADP, AA, adrenaline, and collagen at concentration of 0.25 μM. Similarly, compounds **18** and **19** showed the same potency at concentration of 0.25 μM. Ristocetin-induced aggregation response was inhibited by compounds **18** and **19** at 18 and 68%, respectively at the same concentration. Compound **20** elicited maximum inhibition of aggregation against ADP, AA, adrenaline, and collagen at a concentration of 0.125 μM. Ristocetin-induced aggregation response was inhibited at 53% at this concentration. At a concentration of 0.06 μM compound **20** showed 20% disaggregation against ADP, and total inhibition of the aggregating activity of AA, adrenaline, and collagen, but ristocetin-induced activity was inhibited by only 62%. When the concentration of compound **20** was decreased to 0.03 μM, it elicited 55 and 56% inhibition of aggregation for ADP and AA, respectively, a total aggregation inhibition for adrenaline, collagen and 67% inhibition for ristocetin. Compounds **21**, **23**, **24**, and **26** inhibited the aggregating effect of ADP, AA, adrenaline, and collagen at concentration of 0.5, 0.5, 1.0, and 0.8 μM, respectively. Compound **23** completely inhibited ristocetin-induced aggregation at concentration of 2.5 μM (Table 5).

Table 2 Physicochemical properties of the new synthesized compounds **28–31**



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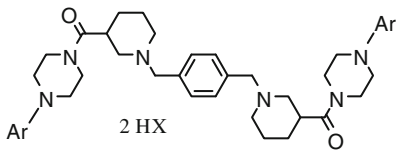
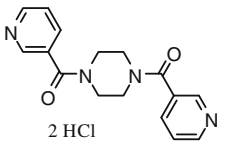


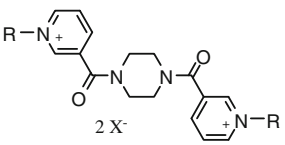
30,31

Compd	Ar	Yield (%)	MP (°C)	Molecular formulae	log *P*
28	2-Cl-Ph	72	238–240	$C_{40}H_{40}Br_2Cl_2N_6O_2$	–
29	2-Pyrimidinyl	78	240–242	$C_{36}H_{38}Br_2N_{10}O_2$	–
30	2-Cl-Ph	70	185–187	$C_{40}H_{52}Br_2Cl_2N_6O_2$	6.15
31	2-Pyrimidinyl	75	212–214	$C_{36}H_{50}\ Cl_2N_{10}O_2$	2.44

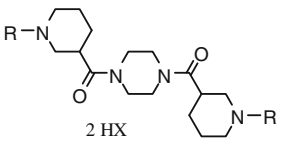
Table 3 Physicochemical properties of the new synthesized compounds **32–46**



32



33-39



40-46

Compd	R	Yield (%)	MP (°C)	Molecular formulae	log *P*
32	–	60	195–197	$C_{16}H_{18}Cl_2N_4O_2$	–
33	Bn	84	220–222	$C_{30}H_{30}Cl_2N_4O_2$	–
34	2-F-Bn	81	230–232	$C_{30}H_{28}\ Cl_2F_2N_4O_2$	–
35	4-F-Bn	78	>300	$C_{30}H_{28}\ Cl_2F_2N_4O_2$	–
36	4-Br-Bn	79	240–242	$C_{30}H_{28}Br_4N_4O_2$	–
37	4-NO_2-Bn	72	188–190	$C_{30}H_{28}Cl_2N_6O_6$	–
38	3,4,5-$(CH_3O)_3$-Bz	61	150–152	$C_{36}H_{38}Cl_2N_4O_{10}$	–
39	4-Br-Bz	69	297–299	$C_{30}H_{24}\ Br_2Cl_2\ N_4O_4$	–
40	Bn	59	173–175	$C_{30}H_{42}Cl_2N_4O_2$	3.17
41	2-F-Bn	58	135–137	$C_{30}H_{40}Cl_2F_2N_4O_2$	3.49
42	4-F-Bn	61	136–138	$C_{30}H_{40}\ Cl_2F_2N_4O_2$	3.49
43	4-Br-Bn	64	270–272	$C_{30}H_{40}Br_4N_4O_2$	4.83
44	4-NO_2-Bn	57	173–175	$C_{30}H_{40}Cl_2N_6O_6$	1.95
45	3,4,5-$(CH_3O)_3$-Bz	54	182–184	$C_{36}H_{50}Cl_2N_4O_{10}$	1.29
46	4-Br-Bz	68	178–180	$C_{30}H_{36}\ Br_2Cl_2N_4O_4$	3.70

The antiaggregating effect of 1,4-bis-[3-(N^4-aryl-N^1-piperazino-carbonyl)-pyridinium-1-yl-methyl]-benzene dibromides (**28** and **29**) showed the lowest antiaggregating activity in this study. Compounds **28** and **29** inhibited the aggregating effect of ADP, AA, adrenaline, and collagen at concentration of 5.0 and 50 μM, respectively. They inhibited the aggregating effect of ristocetin at 51 and 73%, respectively, at the same concentrations. While compounds 1,4-bis-[3-(N^4-aryl-N^1-piperazino-carbonyl)-piperidin-1-yl-methyl]-benzene dibromides (**30** and **31**) inhibited the aggregating effect of ADP, AA, adrenaline, and collagen at concentration of 0.125 and 1.0 μM, respectively. They inhibited the aggregating effect of ristocetin at 31 and 58%, respectively, at the same concentrations. The

Table 4 The antiaggregating activity of the new synthesized compounds **4–9**, **11**, and **13**

Compd	Conc.(μM)	ADP	AA	Adrenaline	Collagen	Ristocetin
4	*N*	70	69	65	81	84
	1.0	#	#	#	#	23*
	0.5	#	#	#	#	71
	0.4	#	#	#	#	77
5	*N*	88	90	81	98	99
	3.0	#	#	#	#	#
	1.0	13	#	#	72	74
	0.6	40	20*	#	58	76
6	*N*	83	81	71	74	82
	2.5	83	53	12	82	#
	1.0	83	67	75	80	89
7	*N*	73	86	74	86	88
	5.0	#	#	#	#	#
	2.0	#	#	#	#	66
	1.0	55*	80	#	#	85
	0.5	71	71	#	90	89
8	*N*	71	77	70	75	79
	10.0	#	#	#	#	24
	5.0	40*	#	#	#	87
	4.0	60*	#	#	#	88
9	*N*	74	77	78	79	85
	1.0	#	#	#	#	69
	0.5	52*	#	#	#	78
	0.25	74	#	#	77	84
	0.125	84	68	#	67	84
11	*N*	71	77	78	75	85
	1.0	#	#	#	#	#
	0.5	#	#	#	#	20
	0.25	#	#	#	#	79
13	*N*	58	57	56	50	65
	20.0	#	#	#	#	#
	10.0	#	#	#	#	27
	5.0	64	#	#	#	43
	1.0	60	52	#	64	60

Results expressed as maximum aggregation %

N neat, *ADP* adenosine 5′-diphosphate, *AA* arachidonic acid

Total aggregation inhibition; * disaggregation

antiaggregating effects of N^1,N^4-bis-(1-aralkyl- or 1-aroyl-pyridinium-3-yl-carbonyl)-piperazine dihalides, showed low antiaggregating activity. Compound **33** inhibited the aggregating effect of ADP, AA, adrenaline, collagen, and ristocetin at concentration of 2.0 μM. In the same manner, compounds N^1,N^4-bis-(1-aralkyl- or 1-aroyl-piperidino-3-yl-carbonyl)-piperazine dihydrohalides (**40** and **41**) showed complete antiaggregating effect against ADP, AA, adrenaline, and collagen at a concentration of 2.0 and 1.0 μM, respectively, while ristocetin-induced aggregation responses were inhibited at 9 and 64%, respectively, at the same concentrations (Table 6).

Table 5 The antiaggregating activity of the new synthesized compounds **17–21**, **23**, **24,** and **26**

Compd	Conc.(μM)	ADP	AA	Adrenaline	Collagen	Ristocetin
17	*N*	75	72	67	79	76
	5.0	#	#	#	#	#
	1.0	#	#	#	#	22
	0.5	#	#	#	#	26
	0.25	#	#	#	#	30
	0.1	86	23	#	100	90
18	*N*	81	77	71	68	87
	1.0	#	#	#	#	50
	0.5	#	#	#	#	73
	0.25	38*	#	#	#	18*
	0.1	74	59	#	#	50
19	*N*	89	100	100	89	96
	1.0	#	#	#	#	40
	0.5	#	#	#	#	67
	0.25	#	#	#	#	68
	0.125	80*	#	#	72	100
20	*N*	79	70	70	66	94
	1.0	#	#	#	#	39
	0.125	#	#	#	#	53
	0.06	20*	#	#	#	62
	0.03	55	56	#	#	67
21	*N*	59	55	63	65	68
	1.0	#	#	#	#	23
	0.5	#	#	#	#	50*
	0.25	28*	#	#	#	58
	0.1	57	#	#	58	66
23	*N*	79	69	72	85	83
	2.5	#	#	#	#	#
	0.5	#	#	#	#	75
	0.25	47*	#	#	#	81
24	*N*	67	71	#	72	77
	1.0	#	#	#	#	24
	0.5	18	28*	#	#	63
	0.25	65	22*	#	64	81
	0.1	76	72	#	67	91
26	*N*	64	74	68	70	69
	2.0	#	#	#	#	#
	1.0	#	#	#	#	75
	0.8	#	#	#	#	55
	0.5	50	54	#	78	68
	0.1	72	76	#	67	77

Results expressed as maximum aggregation %

N neat, *ADP* adenosine 5′-diphosphate, *AA* arachidonic acid

Total aggregation inhibition, * disaggregation

Table 6 The antiaggregating activity of the new synthesized compounds **28–31**, **33**, **40**, and **41**

Compd	Conc.(μM)	ADP	AA	Adrenaline	Collagen	Ristocetin
28	*N*	77	68	73	97	89
	5.0	13*	#	#	#	51
	2.0	79	16*	21	26	65
	1.0	91	40	22	84	78
29	*N*	72	76	70	79	82
	50.0	#	#	#	#	73
	10.0	71	71	28	82	82
	1.0	70	70	73	73	78
30	*N*	75	66	66	76	71
	1.0	#	#	#	#	30
	0.5	#	#	#	#	35
	0.125	#	#	#	#	31
	0.06	27*	#	#	#	61
	0.03	84	58	#	76	78
31	*N*	69	74	70	72	82
	1.0	#	#	#	#	58
	0.5	#	#	#	23	92
33	*N*	54	75	69	82	88
	2.0	#	#	#	#	#
	1.0	25*	#	#	#	73
	0.25	28	71	66	79	80
40	*N*	69	80	70	77	89
	2.0	#	#	#	#	9
41	*N*	63	62	63	59	72
	2.5	#	#	#	#	#
	1.0	#	#	#	#	64

Results expressed as maximum aggregation %

N neat, *ADP* adenosine 5′-diphosphate, *AA* arachidonic acid

Total aggregation inhibition; * disaggregation

Structure activity correlation

The obtained results revealed that N^1-(3-pyridino-carbonyl)-N^4-aryl-piperazine hydrochlorides (**4** and **5**) showed moderate activities as antiaggregating agents. Alkylation of the pyridine ring to give the corresponding quaternary ammonium salts namely, N^1-(1-aralkyl- or 1-aroyl-pyridinium-3-yl-carbonyl)-N^4-aryl-piperazine halides (**6–9**, **11**, and **13**), showed a decrease in the antiaggregating activity. Comparing the activities within this series revealed that N^1-[1-(4-bromo-benzyl)-pyridinium-3-yl-carbonyl)-N^4-(2-chlorophenyl)-piperazine (**9**) is more active than N^1-[1-(4-bromo-benzyl)-pyridinium-3-yl-carbonyl)-N^4-(2-pyrimidinyl)-piperazine (**13**), at a concentrations of 1.0 and 5.0 μM, respectively. Reduction of those quaternary ammonium salts afforded N^1-(1-aralkyl- or 1-aroyl-3-piperidino-carbonyl)-N^4-aryl-piperazine hydrohalides (**17–21**, **23**, **24**, and **26**) with increased antiaggregating activity. N^1-(1-benzyl-3-piperidino-carbonyl)-N^4-(2-chlorophenyl)-piperazine hydrohalides (**17**) proved to be active at a concentration of 0.25 μM. Replacement of the 2-chlorophenyl group of **17** by 2-pyrimidinyl moiety as in **24** resulted in a decrease in the antiaggregating effect (1.0 μM). N^1-(1-(1-(4-fluorobenzyl)-3-piperidino-carbonyl)-N^4-(2-chloro-phenyl)-piperazine (**19**) proved to be more active than N^1-(1-(1-(4-fluorobenzyl)-3-piperidino-carbonyl)-N^4-(2-pyrimidinyl)-piperazine (**26**) at concentrations of 0.25 and 0.8 μM, respectively. With respect to substitutions at the 1-aralkyl or the 1-aroyl moieties, it was found that 1-(4-fluorobenzyl)- (**19**) suites activity rather than 1-(2-fluorobenzyl)- (**18**) (0.25 vs. 0.5 μM, respectively). Replacement of the 1-benzyl group by a 1-benzoyl group resulted in an increase in the magnitude of the antiaggregating activity. Compound N^1-(1-(1-(4-bromobenzyl)-3-piperidino-carbonyl)-N^4-(2-chlorophenyl)-piperazine (**20**) showed a remarkable activity at 0.06 μM; while, its counterpart N^1-(1-(1-(4-bromobenzoyl)- (**23**) showed activity at 0.5 μM. The antiaggregating effect of 1,4-bis-[3-(N^4-aryl-N^1-piperazino-carbonyl)-pyridinium-1-yl-methyl]-benzene dibromides showed an obvious decrease in activity. Compounds **28** and **29** showed their potency at concentrations of 5.0 and 50.0 μM, respectively. Reduction of **28** and **29** afforded 1,4-bis-[3-(N^4-aryl-N^1-piperazino-carbonyl)-piperidin-1-yl-methyl]-benzene dibromides (**30** and **31**) with a remarkable activity. Compound **30**, with its N^4-(2-chlorophenyl)- and **31** with its N^4-(2-pyrimidinyl)-showed antiaggregating activity at concentrations of 0.06, 0.5 μM, respectively, which empathized the contribution of the 2-chlorophenyl moiety to activity rather than the 2-pyrimidinyl analogues. The carbamoylpyridines, quaternary ammonium salt series N^1-(1-aralkyl- or 1-aroyl-pyridinium-3-yl-carbonyl)-N^4-aryl-piperazine halides (**6–16**), proved to be the least active antiaggregating agents. This low activity may be attributed to their diminished lipophylic character. On the contrary, the carbamoyl-piperidines N^1-(1-aralkyl- or 1-aroyl-3-piperidino-carbonyl)-N^4-aryl-piperazine hydro-halides (**17–27**) and 1,4-bis-[3-(N^4-aryl-N^1-piperazino-carbonyl)-piperidin-1-yl-methyl]-benzene dibromides (**30**, **31**) proved to be the most active members in this study. This activity could be attributed to their lipophilic characters. The antiaggregating effect of N^1,N^4-bis-(1-aralkyl- or 1-aroyl-pyridinium-3-yl-carbonyl)-piperazine dihalides showed a decrease in activity as represented by **33** (2.0 μM). Their reduction products, N^1,N^4-bis-(1-benzyl-piperidino-3-yl-carbonyl)-piperazine dihydrohalides (**40**) and N^1,N^4-bis-[1-(2-fluoro-benzyl)-piperidino-3-yl-carbonyl]-piperazine dihydrohalides (**41**), showed a more or less the same potency at of 2.0 and 1.0 μM, respectively.

Molecular modeling study

Computational studies have been performed to the designed compounds (**4**–**46**) to examine their degree of recognition at the binding active site with the conserved amino acids of the thrombin receptor. Studying the conformational recognition of the test compounds indicated that, in general, the oxygen atom of the carbonyl group performed bifurcated H-bonds with two crucial amino acids; His57 and Ser195. The N^1 atom of the piperazine ring favorably oriented and exhibited bifurcated H-bonds with Ser195 and Ser214. Replacing the N^4-2-chlorophenyl moiety with 2-pyrimidinyl group improved the recognition where the nitrogen atom of the pyrimidine ring extended two H-bonds with two glycine amino acids, namely: Gly216 and Gly219.

Compounds possessing benzyl substituent showed free rotation around its methylene group, this conformational twist allowed the phenyl group to lie at the proper angle in the active site without causing any improper deviation to the neighboring residues. The introduction of 4-nitro group at those benzyl functions improved the binding interaction by forming an extra H-bond with the residue Gly216. Compounds bearing benzoyl group substitution behaved uniquely by rotation around the carbonyl group to generate the distinctive conformation that allowed the oxygen atom of the benzoyl group to be hooked between two bifurcated H-bonds with N^2-His57 and Ser214. Also, allowed the benzene ring geometrically oriented parallel to the phenyl fused ring of Trp215 performing favorable lipophilic interaction. The introduction of trimethoxybenzyl substituent was the reason behind the decreased antiaggregating activity due to their bulkiness which shifts the molecule out from binding to the key amino acids in the receptor site.

To gain a better insight into the molecular structures of the most active compounds **20** and **30** and the most inactive compound **29** the binding performance was studied (Fig. 1). Compound **20** indicated that the presence of the piperidino-carbonyl-piperazine function is responsible for the proper recognition of the compound with the conserved amino acid residues Ser195 and Gly219 at the active site that are essential for expressing high thrombin inhibition activity. In addition, the existence of the same group in bis-form, as in case of compounds **29** and **30** changes the conformational form making a turn-over-like shape, to be

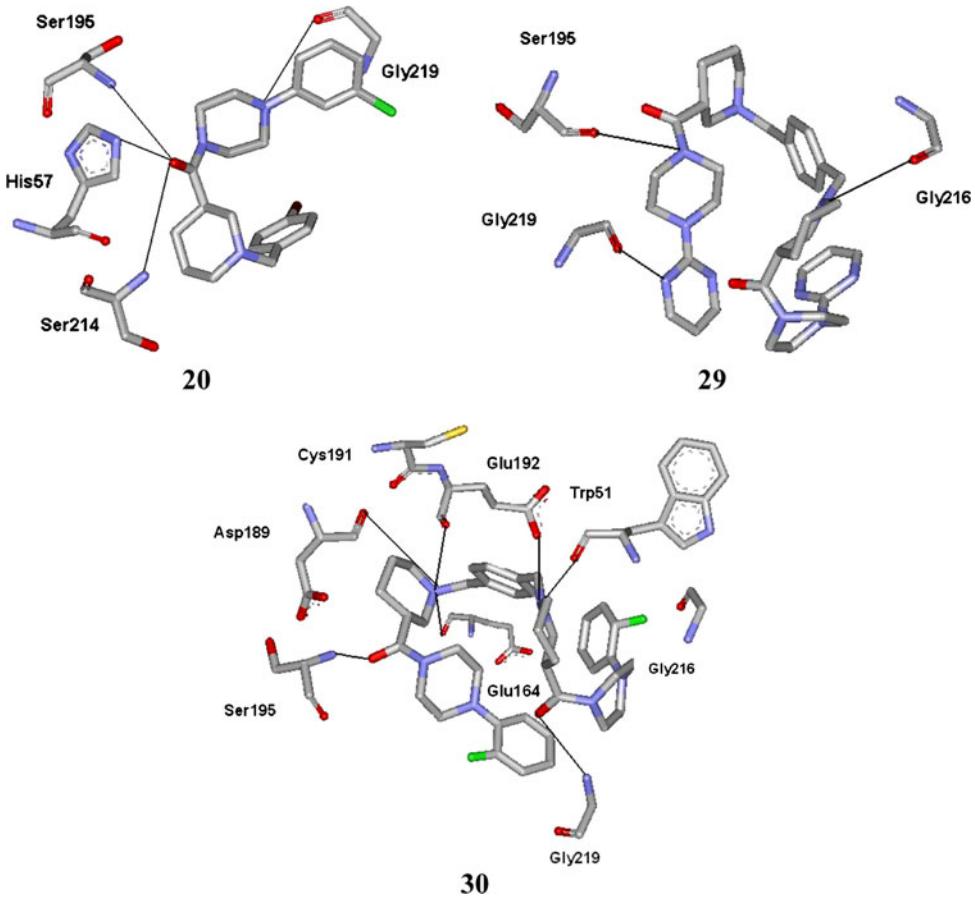


Fig. 1 Binding mode for compounds **20**, **29**, and **30** docked and minimized in the thrombin receptor binding pocket, showing residues involved in its recognition

accommodated within the enzyme cavity. In case of **29**, the two carbonyl function groups were recognized with Ser195, Gly216, and Gly219, the three conserved amino acids; however, the bis-pyridine and the bis-pyrimidine parts represent source of high hydrophobic interaction that reduce the stability and divert the proper interaction with the pocket amino acids (Fig. 1). In compound **30**, the significance of the oxygen of the carbonyl groups in the recognition with Ser195 and Gly219, where the 2-chlorophenyl moiety improves the ligand receptor interaction at the active site by interaction with both Gly216 and Gly219, the two conserved residues at the binding active site. In addition, most of the conserved amino acids at the binding site expressed proper recognition for the bis-piperidine groups including Trp60 and Asp189. In other words, comparing the conformational behavior of **29** and **30** indicated that the aromatic areas of pyridine and pyrimidine rings (**29**) severely decline the binding interaction and magnify the clash interaction. The carbocyclic areas of piperidine and piperazine rings (**30**) provide a hydrophobic interaction without conflict with the overall electrostatic interaction (Fig. 1). The data of molecular docking of the tested compounds indicated that the piperidino-carbonyl-piperazine functional group was recognized by the key amino acid residues Ser195 and Gly219 and are essential for expressing high thrombin inhibition activity.

Conclusion

Carbamoylpyridine and carbamoylpiperidine analogues containing nipecotic acid scaffold were designed, synthesized, and evaluated for their platelet aggregation inhibitory activity. Molecular modeling investigation was performed and the impact of lipophilicity on activity was also discussed. Structure activity relationship among this series was obtained. Figure 2 shows compounds N^1-[1-(4-bromobenzyl)-3-piperidino-carbonyl]-N^4-(2-chlorophenyl)-piperazine hydrobromide (**20**) and 1,4-bis-[3-[N^4-(2-chlorophenyl)-N^1-(piperazino-carbonyl)]-piperidin-1-yl-methyl]-benzene dibromide (**30**), which proved to be the most active antiplatelet aggregating compounds in this study, both at concentration of 0.06 μM. Those new carbamoylpiperidine analogues could be used as template for future development to get more active derivatives.

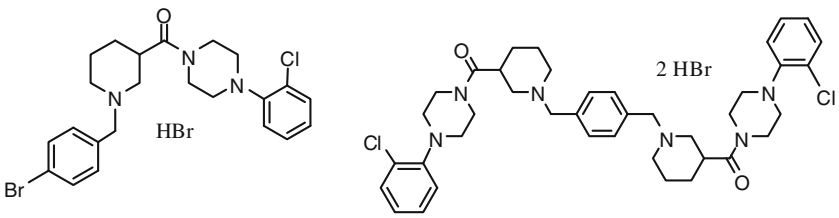
Experimental

Synthesis

Melting points (°C) were determined on Mettler FP 80 melting point apparatus and are uncorrected. Microanalyses were performed on a Perkin Elmer 240 elemental analyzer at the Central Research Laboratory, College of Pharmacy, King Saud University. All the new compounds were analyzed for C, H, and N and agreed with the proposed structures within ±0.4% of the theoretical values. ^{1}H and ^{13}C-NMR spectra were recorded on a Brucker XL 500 MHz FT spectrometer in DMSO-d_6; chemical shifts are expressed in δ ppm in reference to TMS. Mass spectral (MS) data were obtained on a Shimadzu GC/MS QP 5000 apparatus. Thin layer chromatography was performed on precoated (0.25 nm) silica gel GF_{254} plates (E. Merck, Germany), compounds were detected with 254 nm UV lamp. Silica gel (60–230 mesh) was employed for routine column chromatography separations. Compounds were reduced using Shaker Type Hydrogenation Apparatus Series 3911/3916. ADP, adrenaline, collagen, arachidonic acid, and ristocetin are purchased from Bio/Data, Corp. USA, and used for the biological evaluation. Compound **5** was previously reported (Youssef and Al-Shafie, 1998). Molecular modeling study of the newly synthesized compounds was conducted using Hyperchem: Molecular Modeling System, Hypercube, Inc., Release 6, Florida, USA, 1999. log *P* was calculated for each compound using ChemDraw Ultra 10.0.

*N^1-(3-Pyridino-carbonyl)-N^4-aryl-piperazine hydrochlorides (**4**, **5**)*

Thionyl chloride (35.7 g, 0.3 mol) was added dropwise to a cold stirred mixture of 36.9 g (0.3 mol) nicotinic acid in pyridine (50 ml) and toluene (15 ml). The reaction mixture was gradually heated to and maintained at 90°C for 1 h. The appropriate 1-substituted piperazine hydrochloride (0.3 mol) in toluene (50 ml) was added gradually into the reaction mixture. The stirred mixture was maintained at 60°C for 3 h and at 90°C for 1 h, after which the toluene layer containing the product was separated and washed with 1-N HCl (4 × 250 ml). The pH of the combined



20, 0.06 μM **30**, 0.06 μM

Fig. 2 Structures of the most active antiplatelet aggregating agents, compounds **20** and **30**

aqueous acidic solution was adjusted to pH 9.0 with 30% aqueous Na_2CO_3, and the product was extracted with toluene (4 × 250 ml). The extract was dried ($MgSO_4$), filtered, and concentrated to give the free base as an oil. A solution of the free base **4** and **5** in 100 ml of anhydrous ether was acidified to pH 5.0 with a saturated solution of dry HCl gas in diethyl ether at 0°C. The formed precipitate was recrystallized from ethanol to give **4**, **5** as hydrochlorides (Table 1). **4**: ^{1}H-NMR; δ 3.21 (s, 4H, piperazine-H), 3.51 (s, 4H, piperazine-H), 7.06–8.00 (m, 4H, Ar), 8.65–9.77 (m, 5H, pyridine-H & exchangeable-H). ^{13}C-NMR; δ 43.5, 48.1, 121.5, 116.7, 124.9, 126.3, 128.1, 145.1, 134.5, 142.0, 145.1, 148.4, 149.1, 165.1. MS; *m/z* 301 (1%). **5**: ^{1}H-NMR; δ 3.13 (s, 4H, piperazine-H), 3.97 (s, 4H, piperazine-H), 6.72–9.07 (m, 7H, ArH), 10.61 (s, 1H, exchangeable-H). ^{13}C-NMR; δ 40.6, 42.7, 111.6, 124.2, 127.3, 137.4, 150.7, 153.6, 158.5, 161.3, 166.7.

*N^1-(1-Aralkyl- or 1-aroyl-pyridinium-3-yl-carbonyl)-N^4-aryl-piperazine halides (**6–16**)*

To a stirred solution of **4** or **5** (0.02 mol) in absolute ethanol (50 ml), 0.02 mol of the appropriate aralkyl- or aroyl halide in acetone (50 ml) was added. The reaction mixture was heated under reflux for 9 h, the separated solids were filtered, dried, and recrystallized from ethanol to afford **6–16** (Table 1). **6**: ^{1}H-NMR; δ 3.20 (s, 4H, piperazine-H), 3.50 (s, 4H, piperazine-H), 6.07 (s, 2H, CH_2Ph), 7.08–8.33 (m, 9H, ArH), 8.98–9.81 (m, 4H, pyridine-H). ^{13}C-NMR; δ 43.6, 48.1, 63.6, 121.7, 125.2, 126.9, 128.2, 128.6, 128.7, 129.2, 129.4, 129.6, 129.9, 130.8, 134.4, 136.2, 145.6, 146.2, 148.1, 148.8, 164.9. MS; *m/z* 430 (0.5%) M + 2**.** **7**: ^{1}H-NMR; δ 3.06 (s, 4H, piperazine-H), 3.79 (s, 4H, piperazine-H), 6.18 (s, 2H, CH_2Ph), 7.04–8.05 (m, 8H, ArH), 8.30–9.55 (m, 4H, pyridine-H). ^{13}C-NMR; δ 43.4, 48.1, 58.6, 116.4, 116.5, 124.9, 126.9, 128.2, 128.7, 129.3, 129.7, 130.9, 132.2, 132.6, 132.7, 136.2, 144.9, 146.5, 148.4, 148.8, 162.1. MS; *m/z* 447 (0.6%) M + 1**.** **8**: ^{1}H-NMR; δ 3.21 (s, 4H, piperazine-H), 3.54 (s, 4H, piperazine-H), 6.07 (s, 2H, CH_2Ph), 7.06–8.32 (m, 8H, ArH), 8.71–9.64 (m, 4H, pyridine-H). ^{13}C-NMR; δ 43.6, 48.1, 62.8, 116.6, 121.7, 124.9, 125.2, 126.6, 128.2, 128.6, 128.7, 130.7, 130.8, 132.2, 132.4, 129.2, 142.8, 144.6, 148.0, 148.9, 163.4. MS; *m/z* 447 (0.9%) M + 1**.** **9**: ^{1}H-NMR; δ 3.21 (s, 4H, piperazine-H), 3.43 (s, 4H, piperazine-H), 6.03 (s, 2H, CH_2Ph), 7.21–8.31 (m, 8H, ArH), 8.98–9.57 (m, 4H, pyridine-H). ^{13}C-NMR; δ 43.8, 48.3, 63.2, 116.5, 116.8, 121.6, 125.4, 128.8, 128.9, 131.0, 132.2, 132.3, 129.4, 131.0, 146.1, 146.5, 148.4, 162.14. MS; *m/z* 551 (0.6%). **10**: ^{1}H-NMR; δ 3.18 (s, 4H, piperazine-H), 3.43 (s, 4H, piperazine-H), 6.25 (s, 2H, CH_2Ph), 6.91–8.28 (m, 8H, ArH), 8.78–9.31 (m, 4H, pyridine-H). ^{13}C-NMR; δ 42.9, 45.4, 63.3, 114.8, 115.9, 116.7, 117.5, 119.7, 124.6, 127.2, 129.5, 131.2, 134.3, 141.5, 144.2, 147.0, 148.5, 151.7, 164.71. MS; *m/z* 518 (0.5%). **11**: ^{1}H-NMR; δ 3.16 (s, 4H, piperazine-H), 3.34 (s, 4H, piperazine-H), 3.76 (s, 3H, OCH_3), 3.81 (s, 6H, OCH_3), 6.84–7.22 (m, 4H, ArH), 7.24 (s, 2H, ArH), 8.61–9.58 (m, 4H, pyridine-H). ^{13}C-NMR; δ 42.8, 45.4, 56.5, 106.8, 114.8, 115.9, 119.7, 125.9, 128.8, 131.2, 134.5, 151.8, 142.0, 147.0, 149.6, 153.2, 157.8, 165.4, 184.4. MS; *m/z* 532 (0.8%). **12**: ^{1}H-NMR; δ 2.50–3.79 (m, 8H, piperazine-H), 6.86–7.25 (m, 4H, ArH), 7.97–7.99 (m, 4H, ArH), 8.72–9.61 (m, 4H, pyridine-H). ^{13}C-NMR; δ 42.8, 45.4, 114.8, 115.9, 119.7, 126.8, 129.5, 131.2, 131.9, 132.3, 134.5, 131.9, 143.3, 145.9, 148.4, 151.8, 164.9, 167.1. MS; *m/z* 520 (18%) M − 1. **13**: ^{1}H-NMR; δ 3.13 (s, 4H, piperazine-H), 3.98 (s, 4H, piperazine-H), 6.53 (s, 2H, CH_2Ph), 6.78–8.48 (m, 7H, pyrimidine-H and ArH), 8.92–9.57 (m, 4H, pyridine-H). ^{13}C-NMR; δ 40.8, 42.8, 61.6, 111.7, 126.9, 132.0, 134.4, 129.8, 142.1, 145.5, 158.5, 158.6, 160.6, 160.9, 165.3. MS; *m/z* 520 (1%) M + 1. **14**: ^{1}H-NMR; δ 3.13 (s, 4H, piperazine-H), 4.02 (s, 4H, piperazine-H), 6.25 (s, 2H, CH_2Ph), 6.80–8.47 (m, 7H, pyrimidine-H and ArH), 8.82–9.91 (m, 4H, pyridine-H). ^{13}C-NMR; δ 41.0, 42.5, 60.0, 111.4, 127.3, 128.8, 130.0, 144.4, 144.6, 147.0, 158.3, 159.7, 164.4. MS; *m/z* 485 (30%). **15**: ^{1}H-NMR; δ 3.12 (s, 4H, piperazine-H), 3.49 (s, 4H, piperazine-H), 3.81 (s, 9H, OCH_3), 6.73–8.48 (m, 3H, pyrimidine-H), 7.22 (s, 2H, ArH), 8.89–9.65 (m, 4H, pyridine-H). ^{13}C-NMR; δ 40.7, 42.8, 56.5, 106.9, 111.7, 125.6, 128.3, 140.4, 148.3, 151.1, 158.6, 161.1, 165.9. MS; *m/z* 500 (5%). **16**: ^{1}H-NMR; δ 3.13 (s, 4H, piperazine-H), 3.43 (s, 4H, piperazine-H), 6.77–8.03 (m, 7H, pyrimidine-H and ArH), 8.44–9.73 (m, 4H, pyridine-H). ^{13}C-NMR; δ 40.3, 42.3, 111.6, 127.0, 127.4, 129.6, 129.9, 132.0, 143.7, 145.6, 148.0, 158.3, 160.0, 164.8, 168.8. MS; *m/z* 488 (9%).

*N^1-(1-Aralkyl- or 1-aroyl-3-piperidino-carbonyl)-N^4-aryl-piperazine hydrohalides (**17–27**)*

N^1-(1-Aralkyl- or 1-aroyl-pyridinium-3-yl-carbonyl)-N^4-aryl-piperazine halides (**6–16**, 0.05 mol) were dissolved in absolute ethanol (20 ml), and hydrogenated using 0.2 g of PtO_2 at a maximum pressure of 43 p.s.i. When hydrogen absorption was ceased, the catalyst was filtered, and the solvent was removed under reduced pressure. The obtained products were recrystallized from absolute ethanol to give **17–27** (Table 1). **17**: ^{1}H-NMR; δ 1.72–3.58 (m, 17H, piperazine-H and piperidine-H), 4.33–4.37 (m, 2H, CH_2Ph), 6.95–7.03 (m, 5H, ArH), 7.23–7.26 (m, 4H, ArH), 9.14 (brs, 1H, exchangeable-H). MS; *m/z* 398 (2%). **18**: ^{1}H-NMR; δ 1.18–3.59 (m, 17H, piperazine-H and piperidine-H), 5.3 (s, 2H, CH_2Ph), 7.20–7.31 (m, 4H, ArH), 7.43–7.45 (m, 4H, ArH), 9.57 (brs, 1H, exchangeable-H). MS; *m/z* 416 (6%). **19**: ^{1}H-NMR; δ 1.71–2.51 (m, 9H, piperidine-

H), 2.89 (s, 4H, piperazine-H), 3.89 (s, 4H, piperazine-H), 3.65 (s, 2H, CH_2Ph), 7.07–7.45 (m, 8H, ArH), 9.17 (s, 1H, exchangeable-H). MS; *m/z* 416 (12%). **20**: ^{1}H-NMR; δ 1.14–1.89 (m, 9H, piperidine-H), 3.35 (s, 4H, piperazine-H), 3.64 (s, 4H, piperazine-H), 5.17 (s, 2H, CH_2Ph), 6.71–7.48 (m, 8H, ArH), 9.48 (s, 1H, exchangeable-H). ^{13}C-NMR; δ 18.5, 21.0, 22.0, 23.9, 26.0, 43.4, 46.3, 58.2, 115.0, 116.9, 121.2, 130.5, 131.8, 132.5, 133.0, 135.1, 151.1, 170.8. MS; *m/z* 477 (5%). **21**: ^{1}H-NMR; δ 1.50–1.94 (m, 9H, piperidine-H), 3.17 (s, 4H, piperazine-H), 3.40 (s, 4H, piperazine-H), 4.50 (s, 2H, CH_2Ph), 7.12–8.18 (m, 8H, ArH), 9.48 (s, 1H, exchangeable-H). MS; *m/z* 443 (10%). **22**: ^{1}H-NMR; δ 1.58–2.50 (m, 9H, piperidine-H), 2.93–3.65 (m, 8H, piperazine-H), 3.71 (s, 3H, OCH_3), 3.79 (s, 6H, OCH_3), 7.07–7.45 (m, 6H, ArH), 9.17 (s, 1H, exchangeable-H). ^{13}C-NMR; δ 21.5, 24.4, 28.1, 38.3, 41.8, 44.9, 48.2, 56.5, 60.5, 105.1, 114.5, 115.5, 119.1, 125.7, 129.3, 132.1, 134.4, 139.0, 153.3, 169.4, 171.4. MS; *m/z* 502 (6%). **23**: ^{1}H-NMR; δ 1.72–1.95 (m, 9H, piperidine-H), 2.88 (s, 4H, piperazine-H), 3.20 (s, 4H, piperazine-H), 7.09–7.46 (m, 8H, ArH), 10.17 (s, 1H, exchangeable-H). ^{13}C-NMR; δ 21.6, 25.5, 35.2, 42.8, 43.3, 45.4, 48.9, 114.9, 115.8, 115.9, 119.5, 119.7, 131.1, 134.4, 152.1, 170.8, 173.8. MS; *m/z* 490 (2%) M − 1. **24**: ^{1}H-NMR; δ 1.74–2.76 (m, 9H, piperidine-H), 3.13 (m, 8H, piperazine-H), 4.33 (s, 2H, CH_2Ph), 6.72–8.44 (m, 7H, ArH), 9.43 (s, 1H, exchangeable-H). ^{13}C-NMR; δ 38.2, 39.2, 39.5, 39.8, 40.1, 40.3, 42.7, 56.4, 111.6, 125.8, 128.8, 129.5, 158.6, 160.7, 173.8. MS; *m/z* 444 (1%). **25**: ^{1}H-NMR; δ 2.49–3.97 (m, 17H, piperazine-H and piperidine-H), 6.00 (s, 2H, CH_2Ph), 6.76–6.78 (m, 3H, pyrimidine-H), 8.44–8.46 (m, 4H, ArH), 9.52 (s, 1H, exchangeable-H). MS; *m/z* 410 (5%). **26**: ^{1}H-NMR; δ 1.02–3.78 (m, 17H, piperazine-H and piperidine-H), 3.82 (s, 9H, OCH_3), 6.54–6.67 (m, 3H, pyrimidine-H), 7.42–7.45 (m, 2H, ArH), 9.68 (s, 1H, exchangeable-H). ^{13}C-NMR; δ 40.7, 42.8, 56.5, 106.9, 111.7, 125.9, 128.3, 140.4, 148.3, 151.1, 158.6, 161.2, 165.9. MS; *m/z* 469 (7%). **27**: ^{1}H-NMR; δ 2.49–2.81 (m, 9H, piperidine-H), 2.82–3.98 (m, 8H, piperazine-H) 6.75–8.46 (m, 7H, ArH),), 9.57 (s, 1H, exchangeable-H). MS; *m/z* 458 (14%).

*1,4-bis-[3-(N^4-Aryl-N^1-piperazino-carbonyl)-pyridinium-1-yl-methyl]-benzene dibromides (**28**, **29**)*

To a stirred solution of N^1-(3-pyridino-carbonyl)-N^4-aryl-piperazine hydro-chlorides (**4** or **5**, 0.02 mol) in absolute ethanol (50 ml), α,α'-dibromo-*p*-xylene (2.6 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 9 h. The separated product was filtered, dried, and recrystallized from ethanol to give **28** or **29** as dibromides (Table 2). **28**: ^{1}H-NMR; δ 3.17 (s, 8H, piperazine-H), 3.45 (s, 8H, piperazine-H), 5.41 (s, 4H, CH_2Ph), 6.06–7.92 (m, 12H, ArH), 8.64–9.23 (m, 8H, pyridine-H). ^{13}C-NMR; δ 42.7, 45.3, 63.1, 114.7, 115.8, 117.5, 119.6, 126.0, 128.8, 130.2, 131.2, 134.4, 141.5, 147.2, 149.8, 151.7, 165.3. **29**: ^{1}H-NMR; δ 3.13 (s, 8H, piperazine-H), 3.99 (s, 8H, piperazine-H), 5.52 (s, 4H, CH_2Ph), 6.77–8.46 (m, 10H, ArH), 9.03–9.69 (m, 8H, pyridine-H). ^{13}C-NMR; δ 40.9, 42.7, 63.3, 111.6, 127.1, 129.8, 158.5, 160.3, 129.8, 144.0, 145.3, 147.7, 148.8, 164.7.

*1,4-bis-[3-(N^4-aryl-N^1-piperazino-carbonyl)-piperidin-1-yl-methyl]-benzene dibromides (**30**, **31**)*

Compounds 1,4-bis-[3-(N^4-aryl-N^1-piperazino-carbonyl)-pyridinium-1-yl-meth-yl]-benzene dibromides (**28** or **29**, 0.05 mol) were dissolved in ethanol (20 ml), and hydrogenated using 0.2 g of PtO_2 at a maximum pressure of 43 p.s.i. When hydrogen absorption ceased, the catalyst was filtered, and the solvent was removed under reduced pressure. The obtained products were recrystallized from ethanol to afford **30** or **31** (Table 2). **30**: ^{1}H-NMR; δ 1.72–3.58 (m, 34H, piperazine-H, and piperidine-H), 4.30 (s, 2H, CH_2Ph), 4.32 (s, 2H, CH_2Ph), 6.95–7.03 (m, 4H, ArH), 7.23–8.26 (m, 8H, ArH), 9.14 (s, 1H, exchangeable-H), 9.25 (s, 1H, exchangeable-H). **31**: ^{1}H-NMR; δ 1.74–2.29 (m, 18H, piperidine-H), 3.14 (s, 8H, piperazine-H), 3.97 (s, 8H, piperazine-H), 4.32 (s, 4H, CH_2Ph), 6.74–9.17 (m, 10H, ArH), 9.45 (s, 2H, exchangeable-H).

*N^1,N^4-bis-[3-Pyridino-carbonyl]-piperazine dihydrochloride (**32**)*

Thionyl chloride (35.7 g, 0.3 mol) was added dropwise to a cold stirred mixture of 36.9 g (0.3 mol) nicotinic acid, in pyridine (50 ml) and toluene (15 ml). The reaction mixture was gradually heated to and maintained at 90°C for 1 h. piperazine (0.15 mol) in toluene (50 ml) was added gradually into the reaction mixture. The stirred mixture was maintained at 60°C for 3 h and at 90°C for 1 h, after which the toluene layer containing the product was separated and washed with 1-N HCl (4 × 250 ml). The pH of the combined aqueous acidic solution was adjusted to pH 9.0 with 30% aqueous Na_2CO_3, and the product was extracted with toluene (4 × 250 ml). The extract was dried ($MgSO_4$), filtered, and concentrated to give the free base as an oil. A solution of the free base in 500 ml of anhydrous ether was acidified to pH 5.0 with a saturated solution of dry HCl gas in diethyl ether at 0°C, and the precipitate was recrystallized from absolute ethanol to give **32** (Table 3). ^{1}H-NMR; δ 3.87 (s, 8H, piperazine-H), 7.54–7.56 (m, 2H, pyridine-H), 8.43–8.45 (m, 2H, pyridine-H), 8.79–8.80 (m, 2H,

pyridine-H), 9.07 (s, 2H, pyridine-H), 9.35 (s, 2H, exchangeable-H). ^{13}C-NMR; δ 39.5, 40.0, 40.3, 45.7, 124.1, 124.3, 127.0, 131.9, 135.4, 137.4, 148.7, 156.7, 151.0, 153.8, 166.7, 167.6.

*N^1,N^4-bis-(1-Aralkyl- or 1-aroyl-pyridinium-3-yl-carbonyl)-piperazine dihalides (**33–39**)*

To a stirred solution of N^1,N^4-bis-[3-pyridinocarbonyl]-piperazine dihydro-chloride (**32**, 0.02 mol) in absolute ethanol (50 ml), 0.02 mol of the appropriate aralkyl- or aroyl halide in acetone (50 ml) was added. The reaction mixture was heated under reflux for 9 h, the separated solids were filtered, dried, and recrystallized from ethanol to afford **33–39** (Table 3). **33**: ^{1}H-NMR; δ 3.21 (s, 4H, piperazine-H), 3.78 (s, 4H, piperazine-H), 5.51 (s, 4H, CH_2Ph), 7.05–7.42 (m, 10H, ArH), 7.66–9.17 (m, 8H, pyridine-H). **34**: ^{1}H-NMR; δ 5.48 (s, 4H, CH_2Ph), 3.13 (s, 4H, piperazine-H), 3.99 (s, 4H, piperazine-H), 6.77–8.46 (m, 8H, ArH), 9.03–9.69 (m, 8H, pyridine-H). ^{13}C-NMR; δ 40.9, 42.7, 63.3, 111.6, 127.1, 129.8, 158.5, 160.3, 129.8, 145.3, 147.7, 148.8, 164.7. **35**: ^{1}H-NMR; δ 3.48 (s, 4H, piperazine-H), 3.78 (s, 4H, piperazine-H), 5.52 (s, 4H, CH_2Ph), 7.05–7.42 (m, 8H, ArH), 7.66–9.17 (m, 8H, pyridine-H). **36**: δ ^{1}H-NMR; 5.56 (s, 4H, CH_2Ph), 3.72 (s, 8H, piperazine-H), 6.74–7.77 (m, 8H, ArH), 8.42–9.71 (m, 8H, pyridine-H). **37**: ^{1}H-NMR; δ 3.50 (s, 8H, piperazine-H), 5.70 (s, 4H, CH_2Ph), 6.20–8.33 (m, 8H, ArH), 8.81–9.67 (m, 8H, pyridine-H). ^{13}C-NMR; δ 48.0, 62.6, 124.1, 126.7, 130.8, 133.5, 135.7, 141.2, 144.4, 146.5, 148.7, 163.5. **38**: ^{1}H-NMR; δ 3.42 (s, 8H, piperazine-H), 3.79 (s, 18H, OCH3), 6.75 (s, 4H, ArH), 7.21–9.44 (m, 8H, pyridine-H). ^{13}C-NMR; δ 49.8, 60.5, 107.1, 124.1, 128.3, 131.4, 135.5, 131.1, 135.5, 146.7, 148.2, 149.9, 153.3, 167.60. **39**: ^{1}H-NMR; δ 3.21 (s, 4H, piperazine-H), 3.78 (s, 4H, piperazine-H), 7.05–7.42 (m, 8H, ArH), 7.66–9.17 (m, 8H, pyridine-H).

*N^1,N^4-bis-(1-Aralkyl- or 1-aroyl-piperidino-3-yl-carbonyl)-piperazine dihydro-halides (**40–46**)*

N^1,N^4-bis-(1-aralkyl- or 1-aroyl-pyridinium-3-yl-carbonyl)-piperazine dihalides (**33–39**, 0.05 mol) were dissolved in ethanol (20 ml), and hydrogenated using 0.2 g of PtO_2 at a maximum pressure of 43 p.s.i. When hydrogen absorption ceased, the catalyst was filtered, and the solvent was removed under reduced pressure. The obtained products were recrystallized from ethanol to afford **40–46** (Table 3). **40**: ^{1}H-NMR; δ 2.30 3.45(m, 26H, piperazine-H, and piperidine-H), 3.94–4.33 (m, 4H, 4 CH_2Ph), 7.16–8.21 (m, 10H, ArH), 9.26 (brs, 1H, exchangeable-H), 9.50 (brs, 1H, exchangeable-H). **41**: ^{1}H-NMR; δ 1.14–2.49 (m, 18H, piperidine-H), 3.17 (s, 4H, piperazine-H), 3.22 (s, 4H, piperazine-H), 3.68 (s, 4H, CH_2Ph), 7.16–7.23 (m, 8H, ArH), 9.80 (brs, 2H, exchangeable-H). **42**: ^{1}H-NMR; δ 1.74–2.94 (m, 18H, piperidine-H), 2.94–3.32 (m, 8H, piperazine-H) 3.45 (s, 4H, CH_2Ph), 7.18–7.64 (m, 8H, ArH), 9.36 (brs, 1H, exchangeable-H), 9.40 (brs, 1H, exchangeable-H). **43**: ^{1}H-NMR; δ 1.14–2.87 (s, 18H, piperidine-H), 2.91 (m, 4H, piperazine-H), 3.31 (m, 4H, piperazine-H), 3.45 (s, 4H, CH_2Ph), 7.18–7.64 (m, 8H, ArH), 9.20 (brs, 1H, exchangeable-H), 9.42 (brs, 1H, exchangeable-H). **44**: ^{1}H-NMR; δ 1.74-2.80 (m, 18H, piperidine-H), 2.90 (s, 4H, piperazine-H), 3.13 (s, 4H, piperazine-H), 3.17 (s, 4H, CH_2Ph), 7.15–8.22 (m, 8H, ArH), 9.38 (s, 2H, exchangeable-H). ^{13}C-NMR; δ 21.6, 25.5, 38.5, 39.2, 40.9, 42.0, 43.4, 60.4, 124.0, 125.9, 128.8, 129.4, 173.9. **45**: ^{1}H-NMR; δ 2.29–3.44 (m, 26H, piperazine-H, and piperidine-H), 3.89 (s, 18H, OCH_3), 7.96–7.99 (m, 4H, ArH), 9.14 (s, 1H, exchangeable-H), 9.99 (s, 1H, exchangeable-H). **46**: ^{1}H-NMR; δ 1.47–2.74 (m, 18H, piperidine-H), 3.68 (s, 8H, piperazine-H), 7.42–7.80 (m, 8H, ArH), 9.25 (s, 1H, exchangeable-H), 9.46 (s, 1H, exchangeable-H). ^{13}C-NMR; δ 21.5, 27.9, 29.1, 39.4, 40.5, 44.8, 48.2, 129.5, 130.5, 132.0, 140.5, 168.9, 176.9.

The antiaggregating effect of the new synthesized compounds

Reagents, methodology, and the turbidimetric procedure employed in the aggregometric determinations were reported previously (Gader *et al*., 1988). Certain laboratory conditions were carefully standardized and these include the number of platelets in PRP (200–400 $\times$ 10^9/l); temperature (37°C) and rate of stirring (1000 rpm), time interval between venepuncture and the measurement of platelet aggregation (<2 h). Aggregation was recorded in an Aggregation Profiler (PAP4, Bio/Data, USA), which registers the slopes (S) of the aggregation curves. The minimal concentration of adenosine diphosphate (ADP) and arachidonic acid (AA) eliciting full biphasic aggregation (8.16 $\pm$ 0.19 μM for 64 plasma samples) was determined using platelet-rich plasma (PRP) from each donor immediately prior to the determination of aggregation inhibition induced by the test compounds. The platelet count of PRP was adjusted to 250,000–300,000 platelet/mm^3 using autologous PPP. Test compounds were reconstituted to appropriate concentrations in redistilled 95% ethanol. Solubility permitting, 95% ethanol–water (1:1, 1:2, or 1:4) will be used in the aggregation system. Total ethanol concentration up to 0.190% was reported to be without effect on platelet aggregation. Control cuvettes receive equal volumes of the appropriate vehicle without test compound. Test compounds (50 μg) was added to PRP after 15 s of stirring (1100 rpm) in a Payton Associate Dual Chanel Aggregometer equipped with a Fisher

Dual Chanel OmniScribe recorder and transferred back to the Masters constant temperature block 15 s after the addition of compound. After 1 min and 45 s, the cuvette was returned to the aggregometer and the agonist (ADP and AA, 50 µl) was added after 4 min incubation with compound. Changes in light transmission associated with platelet change and aggregation were recorded for an additional 5.5 min. The (control) cuvette containing the vehicle-treated PRP will be initiated 1 min after the (treated) cuvette containing plasma treated with the appropriate test compound and follow the same sequence of events. Inhibition of aggregation (%) was expressed as the difference in maximum responses of the paired treated and control cuvettes as a percent of the control response.

Molecular modeling calculations

Molecular modeling studies of the new analogues in complex with 1DWD have been done to delineate features that differentiate their mode of interaction. Starting coordinate of the X-ray crystal structure of the thrombin 1DWD pdb receptor in complex is obtained from the RCSB Protein Data Bank of Brookhaven National Laboratory (Banner and Hadvary 1991). All the hydrogen were added and thrombin structure was subjected to a refinement protocol in which the constraints on the 1DWD were gradually removed and minimized until the RMS gradient was 0.01 kcal/mol Å. The energy minimization was carried out using the molecular mechanics force field "AMBER." The energy-minimized structure was used for molecular dynamics studies. The new analogues were constructed from fragment libraries in the Hyperchem Program (1999). The partial atomic charges for each analog were assigned with the semiempirical mechanical calculation method "AM1" implemented in Hyperchem 6.0. Conformational search was performed around all the rotatable bonds with an increment of 10° using conformational search module as implemented in HyperChem 6.0. All the conformers were minimized until the RMS deviation was 0.01 kcal/mol Å. For each of the chosen analogs, energy minimizations (EM) were performed using 1,000 steps of steepest descent, followed by conjugate gradient minimization to a RMS energy gradient of 0.01 kcal/mol Å. The docking was carried out on using a flexible fitting module in the program. Distance between the hydrogen bond donor and the acceptor atoms within the pocket surrounding the ligand and the ligand itself were defined and restrained. Each inhibitor was geometrically optimized in the enzyme-binding pocket (Budin *et al*., 2001).

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References

Antithrombotic Trialists' Collaboration (2002) Collaborative meta-analysis of randomized trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. Br Med J 324:71–86

Banner DW, Hadvary P (1991) Crystallographic analysis at 3.0-A resolution of the binding to human thrombin of four active site-directed inhibitors. J Biol Chem v266:20085–20093

Berger PB (1999) The thienopyridines in coronary artery disease. Curr Cardiol Rep 1:192–198

Binisti C, Assogba L, Touboul E, Mounier C, Huet J, Ombetta J-E, Dong CZ, Redeuilh C, Heymans F, Godfroid J-J (2001) Structure–activity relationships in platelet-activating factor (PAF). 11-From PAF-antagonism to phospholipase A2 inhibition: syntheses and structure–activity relationships in 1-arylsulfamido-2-alkylpiperazines. Eur J Med Chem 36:809–828

Budin N, Majeux N, Caflisch A (2001) Fragment-based flexible ligand docking by evolutionary optimization. Biol Chem 382:1365–1372

Chatelain P, Massingham R (1997) Cardiac ischaemia: possibilities for future drug therapy. Eur J Med Chem 32:687–707

Cody WL, Cai C, Doherty AM, Edmunds JJ, He JX, Narasimhan LS, Plummer JS, Rapundalo ST, Rubin JR, Van Huis CA, St-Denis Y, Winocour PD, Siddiqui MA (1999) The design of potent and selective inhibitors of thrombin utilizing a piperazinedione template: part 1. Bioorg Med Chem Lett 9:2497–2502

De Candia M, Fossa P, Cellamare S, Mosti L, Carotti A, Altomare C (2005) Insights into structure-activity relationships from lipophilicity profiles of pyridine-2(1*H*)-one analogs of the cardiotonic agent milrinone. Eur J Pharm Sci 26:78–86

De Marco A, de Candia M, Carroti A, Cellamare S, De Candia E, Altomare C (2004) Lipophilicity-related inhibition of blood platelet aggregation by nipecotic acid anilides. Eur J Pharm Sci 22:153–164

Dillingham EO, Lasslo A, Carter-Burks G, Bond SE, Gollamudi R (1989) Relationships between chemical structure and inhibition of ADP-stimulated human thrombocyte release of serotonin and platelet factor 4. Biochim Biophys Acta 990:128–132

Dogné J-M, de Leval X, Benoit P, Delarge J, Masereel B, David J-L (2001) Recent advances in antiplatelet agents. Curr Med Chem 9:577–589

Feng Z, Gollamudi R, Dillingham EO, Bond SE, Lyman BA, Purcell WP, Hill RJ, Korfmacher WA (1992) Molecular determinants of the platelet aggregation inhibitory activity of carbamoylpiperidines. J Med Chem 35:2952–2958

Fuster V, Badimon L, Badimon JJ, Chesebro JH (1992) The pathogenesis of coronary artery disease and the acute coronary syndromes. N Engl J Med 326:242–250

Gachet C (2001) ADP receptors of platelets and their inhibition. Thromb Haemost 86:222–232

Gader AMA, Bahakim HM, Jabbar FA, Lambourne AL, Gaafar TH, Edrees YB (1988) Dose-response aggregometry in maternal/neonatal platelets. Thromb Haemostat 60:314–318

Heath JA, Mehrotra MM, Chi S, Yu J-C, Hutchaleelaha A, Hollenbach SJ, Giese NA, Scarborough RM, Pandey A (2004) Corrigendum to "Identification of 4-piperazin-1-yl-quinazoline template based aryl and benzyl thioureas as potent, selective, and orally bioavailable inhibitors of platelet-derived growth factor (PDGF) receptor. Bioorg Med Chem Lett 14:6133–6142

Hyperchem (1999) Molecular Modeling System, Hypercube, Inc., Release 6, Florida, USA

Kunapuli SP (1998) Multiple P2 receptor subtypes on platelets: a new interpretation of their function. Trends Pharmacol Sci 19: 391–394

Leoncini G, Signorello MG, Roma G, Di Braccio M (1997) Effect of 2(1-piperazinyl)-4H-pyrido[1, 2-a]pyrimidin-4-one (AP155) on human platelets in vitro. Biochem Pharmacol 53:1667–1672

Leoncini G, Signorello MG, Bruzzese D, Di Braccio M, Grossi GC, Roma G (2004) Mechanisms involved in the antiplatelet activity of 8-methyl-4-(1-piperazinyl)-7-(3-pyridinylmethoxy)-2H–1-benzopyran-2-one (RC414). Biochem Pharmacol 67:911–918

Majerus W, Tollesfsen DM (2001) Anticoagulant, thrombolytic, and antiplatelet drugs. In: Goodman LS, Gilman AG, Limbird LE (eds) The Goodman Gilman's pharmacological basis of therapeutics. McGraw-Hill, New York, pp 1519–1537

McKee SA, Sane DC, Deliargyris EN (2002) Aspirin resistance in cardiovascular disease: a review of prevalence, mechanisms, and clinical significance. Thromb Haemost 88:711–715

Mills DC (1996) ADP receptors on platelets. Thromb Haemost 76:835–856

Papadopoulou C, Geronikaki A, Hadjipavlou-Litina D (2005) Synthesis and biological evaluation of new thiazolyl/benzothiazolyl-amides, derivatives of 4-phenylpiperazine. Farmaco 60:969–973

Patrono C, Coller B, Dalen JE, FitzGerald GA, Fuster V, Gent M, Hirsh J, Roth G (2001) Platelet-active drugs: the relationships among dose, effectiveness, and side effects. Chest 119:39S–63S

Sallem W, Serradji N, Dereuddre-Bosquet N, Dive G, Clayette P, Heymans F (2006) Structure–activity relationships in platelet-activating factor. Part 14: synthesis and biological evaluation of piperazine derivatives with dual anti-PAF and anti-HIV-1 activity. Bioorg Med Chem Lett 14:7999–8013

Slotboom AJ, Verheij HM, De Haas GH (1982) Specific transformations at the N-terminal region of phospholipase A2. In: Ansell GB (ed) Phospholipids. Elsevier, Amsterdam, pp 359–434

Storey F (2001) The P2Y12 receptor as a therapeutic target in cardiovascular disease. Platelets 12:197–209

Tucker TJ, Lumma WC, Mulichak AM, Naylor-Olsen AM, Lewis SD, Lucas R, Freidinger RM, Kuo LC (1997) Design of highly potent noncovalent thrombin inhibitors that utilize a novel lipophilic binding pocket in the thrombin active Site. J Med Chem 40:830–832

Van De Graaff E, Steinhubl SR (2000) Antiplatelet medications and their indications in preventing and treating coronary thrombosis. Ann Med 32:561–571

Weksler BB (2000) Antiplatelet agents in stroke prevention. Combination therapy: present and future. Cerebrovasc Dis 10:41–48

Youssef KM, Al-Shafie FS (1998) ADP-and arachidonic acid (AA)-induced platelet aggregation inhibitory activity of carbamoylpyridines and carbamoylpiperidines. Al-Azhar Bull Sci 9:569–582