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Original Article

Synthesis and Antiplatelet Aggregation Activity Evaluation of some 2-Aminopyrimidine and 2-Substituted-4,6-diaminopyrimidine Derivatives

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Abstract

A series of novel 2-aminopyrimidine and 2-Substituted-4,6-diaminopyrimidine derivatives have been synthesized and their antiplatelet aggregation activities were assessed against ADP and arachidonic acid-induced platelet aggregation in human plasma using light transmission aggregometry. Among the tested derivatives, compounds Ia, I_b, I_B and II_{16} exhibited the highest antiplatelet aggregation activity (36.75, 72.4, 62.5 and 80 μM). None of the compounds showed satisfactory activity against the aggregation induced by ADP but acceptable activities were observed against the aggregation induced by arachidonic acid. 2- aminopyrimidines were more active than 4,6- diaminopyrimidines in this respect.

Keywords: 2-aminopyrimidines; 2-Substituted-4,6-diaminopyrimidines; Antiplatelet aggregation.

Introduction

Platelets play an important role in maintaining cardiovascular integrity and in regulating the bleeding process by blood-clot formation (1). However, uncontrolled platelet aggregation is dangerous in arterial blockage and may lead to life threatening disorders (2). Antiplatelet agents are therefore considered as a significant tool in the treatment and/or prevention of cardiovascular thrombotic disease (3-5). Antiplatelet agents such as aspirin (acetylsalicylic acid), clopidogrel or ticlopidine and anticoagulants such as warfarin are currently two predominant groups of orally consumable drugs in standard therapeutic protocols for prophylaxis and treatment of venous thrombosis and reducing the risk of recurrent myocardial infarction (6-8).

Currently aspirin, which irreversibly inhibits cyclooxygenase I-mediated transformation of arachidonic acid to thromboxane A_2 (TXA_2), and the P_2Y_{12} antagonists clopidogrel and prasugrel, which selectively and irreversibly bind to theP_2Y_{12} ADP receptor are routinely used as antiplatelet agents (9, 10).

However there are still some serious limitations to these agents which include weak inhibition of platelet function (*eg*. aspirin) (11), slow onset of action (*eg*. clopidogrel) (12), variable response to treatment among the patients (*eg*. clopidogrel and aspirin) (11, 12) and high incidence of bleeding events which occur in both aspirin and clopidogrel drug therapy (13, 14).

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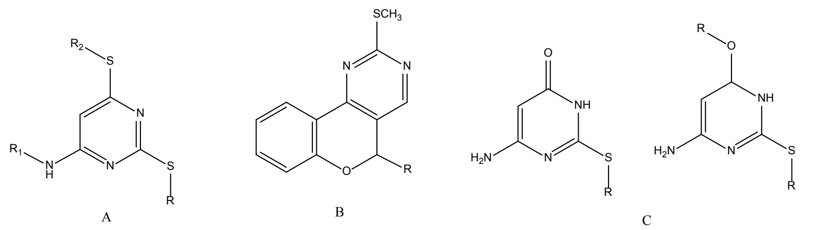


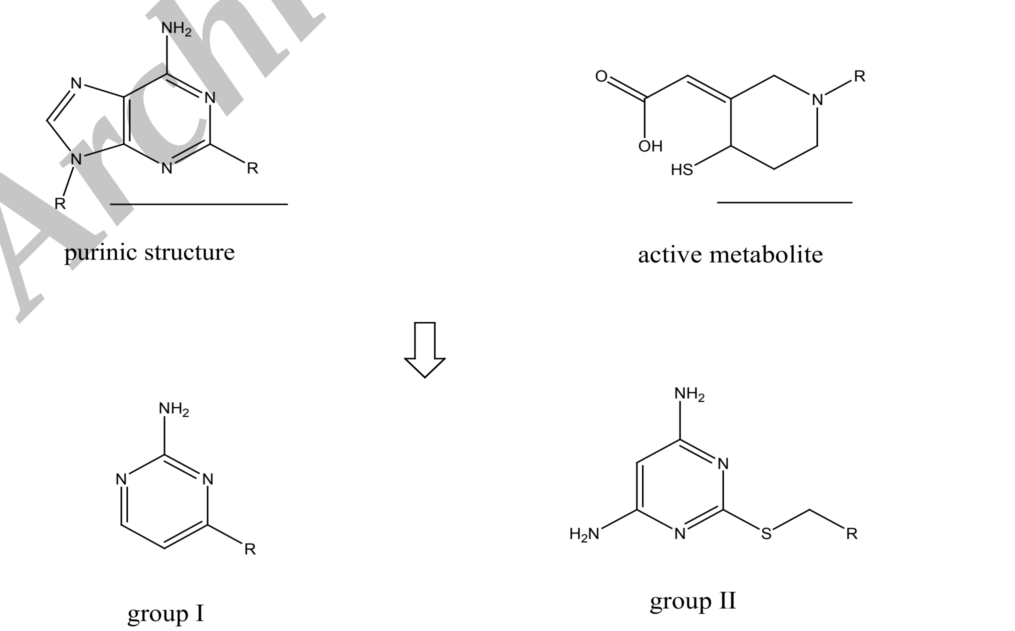
Figure 1. Active antiplatelet pyrimidine derivatives. A. 6-alkylamino-2,4-dialkyl(aryl)thiopyrimidines [7], R = Me, *n*-Pr, R1 = *n*-Pr, *i*-Pr, Ph, Bn, R2 = Bn, CH_2CH_2Ph; **B.**Tricyclic pyrimidines [8], R = some amino substituents; c. Substituted 6-amino-2-mercaptopyrimidines [9], R = CH_3, CH_2CH_2NHBOC, CH_2CH_2OH, CH_2CF_3, 2-Chlorobenzyl, *etc*. R1 = substituted arylsulfonyl moieties.

Considering the current situation, development of novel antiplatelet agents which are safe and effective is an urgent need (15).

Aminopyrimidine derivatives are an interesting group of compounds with various reported biological properties. Pyrimidine ring can be found in the structures of many important drugs such as nucleoside antibiotics, antibacterials and cardiovascular agents (16, 17).

Based on the hypothesis suggested by Cattaneo *et al*. (18), amino pyrimidine ring could be considered as a simplified form of the active metabolites of the thienopyridines and ATP derivatives. The active metabolites of thienopyridines have a simple monocyclic structure which implies that the presence of a bicyclic structure like that of a purine ring is not an absolute requirement for the affinity for the ADP receptor or so on platelet membrane.

A group of amino pyrimidine derivative with thioether substituents has been synthesized and evaluated by Cattaneo *et al*. The compound showed satisfactory anti platelet aggregation effects when ADP had been used for aggregation induction (18). Based on the mentioned reports and in order to investigate the capability of amino pyrimidine derivatives in inhibition of platelet aggregation pathways, we synthesized two groups of amino pyrimidines with the following structures:



4-substituted-2-aminopyrimidines 4,6-diaminopyrimidines carrying alkyl/aralkylthiosubdtituent at position 2

Figure 2. Comparing the structures of aminopyrimidines with the structures of purine and the active metabolite of clopidogrel.

group I group II

Figure 3. Chemical structure of the synthesized compounds.

Comparing the activity of these compounds in inhibition of platelet aggregation induced by ADP and arachidonic acid will provide some insights into the structure activity relationship of these compounds.

Chemistry

The synthetic procedures for groups I and II are illustrated in Figures 4 and 5.

Group I: Methyl ketones (1a-h) were allowed to react with dimethylformamide-dimethylacetate (DMF-DMA) to produce 3-(dimethylamino)-1-aryl-2en-1-ones (2a-h). These intermediates could be then condensed with guanidine HCl to obtain the corresponding amino pyrimidine ring systems (19).

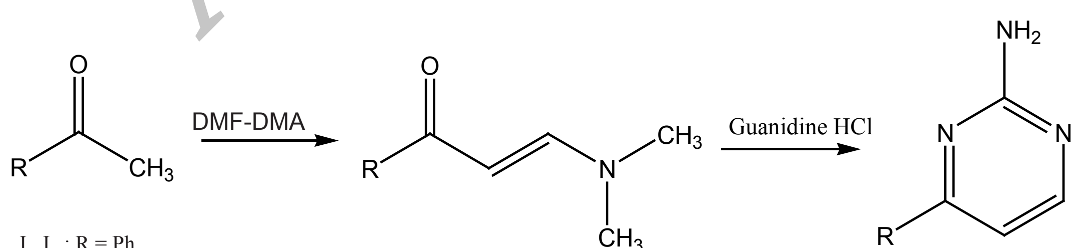
Group II: As it is shown in Figure 5 the intermediate II_3 (4,6-diaminopyrimidine-2-thiol) was obtained by the reaction of thiourea and malononitrile in absolute ethanol as the solvent. Subsequent reaction of compound $II_3$with various alkylhalides at room temperature afforded compounds II_{4-25} in good yields.

Structure confirmation of the synthesized intermediates and the final products was performed using IR, NMR and Mass spectrometry (20).

Experimental

General

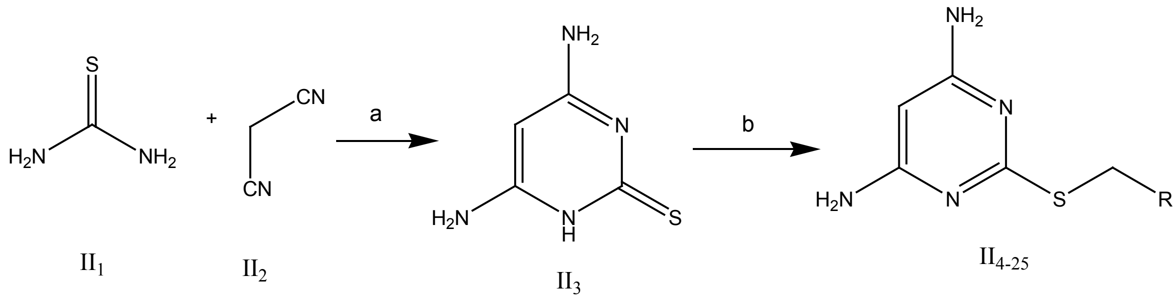
All evaporations were carried out in vacuo with a rotary evaporator. Melting points (˚C) were determined by capillary method on an electrothermal melting point apparatus. Infrared spectra were recorded as thin films of KBr plates with U_{max} in inverse centimeters. Nuclear magnetic resonance spectra for proton ([1]H NMR) were recorded on a Bruker DRX-Avance (500 MHz) spectrometer. Chemical shift values are expressed in ppm (parts per million) relative to tetramethylsilane (TMS) as internal standard; s: singlet, d: doublet, dd: double doublet, t: triplet, q: quartet, m: multiplet, br s: broad singlet. Thin layer chromatography (TLC) was performed on Whatman SilG/UV_{254} silica gel plates with fluorescent indicator and the



I_a, I_A : R = Ph
I_b, I_B : R = 4-F-Ph
I_c, I_C : R = 3-thienyl
I_d, I_D : R = 4-(CF3)-Ph
I_e, I_E : R = 4-(Ph)-Ph
I_f, I_F : R = 4-pyridyl
I_g, I_G : R = 3-pyridyl
I_h, I_H : R = 2-pyridyl

I_a-h I_A-H

Figure 4. Compounds (I_{a-h}) and (I_{A-H}) synthesis scheme. Reagent and conditions: (a) DMF, reflux, 24 h; (b) $NaOCH_3$, Isopropanol, reflux, 48 h.



II_4: R = H
II_5: R = methyl
II_6: R = ethyl
II_7: R = propyl
II_8: R = butyl
II_9: R = pentyl
II_{10}: R = cyclobutyl
II_{11}: R = morpholinomethyl
II_{12}: R = 2-methyl phenyl
II_{13}: R = 3-methyl phenyl
II_{14}: R = 2-nitrophenyl
II_{15}: R = 2-chlorophenyl
II_{16}: R = 2-fluorophenyl
II_{17}: R = 3-fluorophenyl
II_{18}: R = 4-fluorophenyl
II_{19}: R = 3-methoxyphenyl
II_{20}: R = 4-cyanophenyl
II_{21}: R = 3-(trifluoromethyl)phenyl
II_{22}: R = benzyl
II_{23}: R = 2-phenethyl
II_{24}: R = 1-naphtalenyl
II_{25}: R = 1-(1-methylpiperidin-2-yl)methyl

Figure 5. Compounds (II_{4-25}) synthesis scheme. Reagents and conditions: (a) EtONa, reflux, 3 h; (b) NaOH 0.1 M, CH_3OH, R-CH_2-X (X = Cl, Br), rt, 18 h10).

spots were visualized under 254 and 366 nm illumination. Mass analyses were performed on an Agilent 6400 series equipped with an electrospray (ESI) ionization interface (drying gas adjusted at 300 ˚C, nebulizing gas flow at 12 L/min). All the compounds 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.

General procedure for the preparation of compounds (I_{a-h})

The detailed description of the methods used for preparation of compounds I_a to I_h and compounds I_A to I_H is reported in reference 19. Briefly to a solution of acetophenone (84 mmol) in DMF (16 mL), was added DMF-DMA (84 mmol) and the solution was heated under reflux for 24 h. Brine (25 mL) was added to the reaction mixture after cooling and the reaction mixture was then extracted with CH_2Cl_2 (3 × 25 mL). The combined organic fractions were dried over anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure. The residue was dissolved in EtOAc (12 mL), followed by addition of n-hexane (100 mL). The precipitate thus obtained was filtered and dried to give the pure product I_a as a yellow solid (19).

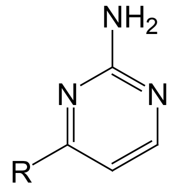
General procedure for the preparation of compounds (I_{A-H})

To a solution of compounds I_a (2.68 mmol) in isopropanol (13.5 mL) were added sodium methoxide (10.7 mmol) and guanidine hydrochloride (4.02 mmol) and the solution was heated under reflux for 48h. Distilled water (25 mL) was added to the reaction mixture after cooling and the mixture was then extracted with EtOAc (3 × 15 mL). The combined organic fractions were dried over anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure. The residue was dissolved in EtOAc (2 mL), followed by addition of *n*-hexane (25 mL). The precipitate thus obtained was filtered and dried to give I_{A-H}.

Representative procedure for the preparation of compounds (II_{4-25})

The detailed description of the methods used for preparation of compounds II_4 to $II_{25}$is reported in reference 20. Briefly, to a solution of 4,6-diaminopyrimidine-2-thiol (II_3, 1.4 mmol) in methanol, was added alkyl halide (3.5 mmol) under basic conditions (NaOH 0.1 M, 14 mL). The mixture was then stirred for 18h at

Table 1. Antiplatelet activity of group I derivatives.



group I

Compound	**R**	**A.A IC_{50} MM**	**ADP %inhibition**
Ia (1.25 mM)	ph	36.75	45.2
I_A (0.75 mM)	ph	544	29
Ib (1 mM)	4-F- ph	72.4	28.6
I_B (2.5 mM)	4-F- ph	62.5	53
I_c (1 mM)	3-thienyl	1000	35.5
I_C (1.6 mM)	3-thienyl	>1000	57.3
I_d (1.3 mM)	4-(CF_3)- ph	>1000	24.3
I_D (0.5 mM)	4-(CF_3)- ph	340	42.5
Ie (1.3 mM)	4-(ph)- ph	>1000	24.3
I_E (1 mM)	4-(ph)- ph	>1000	50
If (1 mM)	4-pyridyl	>1000	1
I_F (1 mM)	4-pyridyl	ND	ND
Ig (2.5 mM)	3-pyridyl	1000	35
I_G (0.5 mM)	3-pyridyl	192	39.5
I_h (1.25 mM)	2-pyridyl	752	27.1
I_H (1.25 mM)	2-pyridyl	>1000	44
Indomethacin		3.0	
Aspirin		30.3	

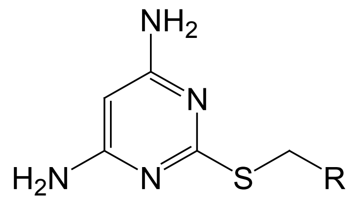
room temperature. After removing the solvent under reduced pressure, the residue was washed by water and the precipitate was collected as a solid. All compounds were obtained in acceptable purity and no further purification was needed (20).

Results and Discussion

All the synthesized compounds were screened for their effects on human platelet aggregation induced by arachidonic acid and ADP using light transmission aggregometry. IC_{50} was determined as the concentration of the test compounds that exhibit platelet aggregation by 50%. The *in-vitro* antiplatelet activity of the synthesized derivatives is listed in Tables 1 and 2.

Comparing the activities of the two aminopyrimidine groups indicates that none of the compounds showed satisfactory activity against the aggregation induced by ADP. Therefore it could be concluded that the compounds do not interfere with ADP receptors on platelet membrane. However, acceptable activities were observed in both groups against the aggregation induced by arachidonic acid. This is not in agreement with the report by Cattaneo *et al.* who introduced a group of aminopyrimidines as active platelet aggregation

Table 2. Antiplatelet activity of group II derivatives.



group II

Compound (In 1 mM)	R	A.A IC_{50} MM	ADP % inhibition
II_4	H	>1000	10
II_5	Methyl	>1000	33.8
II_6	Ethyl	500	42
II_7	Propyl	ND	ND
II_8	Butyl	500	42
II_9	Pentyl	>1000	53
II_{10}	Cyclobutyl	>1000	49
II_{11}	Morpholinomethyl	>1000	ND
II_{12}	2-methylphenyl	213	38.3
II_{13}	3-methylphenyl	220	10
II_{14}	2-nitrophenyl	209	20
II_{15}	2-chlorophenyl	>1000	14.7
II_{16}	2-fluorophenyl	**80**	23
II_{17}	3-fluorophenyl	700	49
II_{18}	4-fluorophenyl	214	24.3
II_{19}	3-methoxyphenyl	>1000	8
II_{20}	4-cyanophenyl	>1000	28
II_{21}	3-(trifluoromethyl)phenyl	>1000	50
II_{22}	Benzyl	ND	ND
II_{23}	2-phenethyl	760	70
II_{24}	1-naphthalenyl	1000	51.9
II_{25}	1-(1-methylpiperidin-2-yl)methyl	>1000	95
Indomethacin		3.0	
aspirin		30.3	

inhibitors which interfere with ADP receptors.

Comparing the overall results obtained for aminopyrimidines I and II indicates that 2-aminopyrimidines (I) were more active than 4,6-diaminopyrimidines (II). Only compound 16 in group II showed satisfactory IC_{50} (80 μM).

Among the 2-aminopyrimidines group (I), on the other hand, a few compounds (I_a, I_b, I_B and I_G) showed good activities (36.75, 72.4, 62.5 and 192 μM). Interestingly, compounds with fluorine substituent on phenyl ring (I_b, I_B) were among the most active compounds.

Global physicochemical properties for compounds I_{a-h}, I_{A-H} and II_{4-25} were calculated using Chemdraw Ultra, Chem3D Ultra version 8.0 and Hyper Chem professional and the results are presented in Tables 3 and 4.

Efforts to find a relationship between these

Table 3. Global physicochemical properties for compounds group I.

Compound	ClogP[A]	P [B]	V [C]	SA[D]	D [E]
Ia	2.2974	20.85	606.6	388.66	2.669
I_A	1.774	20.03	549.5	259.2	1.319
Ib	2.516	24.43	632.5	398.5	3.181
I_B	1.926	22.06	553.45	357.2	1.122
I_c	2.0739	20.38	581.23	379.5	2.56
I_C	1.4697	19.55	521.3	342.54	0.778
I_d	3.3127	22.42	683.67	430.8	3.844
I_D	2.6730	21.59	620.86	395.87	2.208
Ie	4.185	30.5	811.04	495.06	2.708
I_E	3.66	29.69	747.46	458.7	1.548
If	1.1964	20.14	592.2	382.9	3.2
I_F	0.409	19.32	529.56	344.89	1.194
Ig	1.1964	20.14	591.97	383.02	1.283
I_G	0.1997	19.32	529.45	345.85	1.944
I_h	1.5964	22.95	611.69	389.11	1.914
I_H	0.409	19.32	535.7	351.3	1.3

[A]ClogP were calculated by using Chem Draw Ultra version 8.0. [B]Polarizability values were calculated by using Hyper Chem Professional. [C]Molecular volume values were calculated by using Hyper Chem Professional. [D] Surface area values were calculated by using Hyper Chem Professional. [e]Dipole (debye) values were calculated by using Chem3D Ultra version 8.0.

Table 4. Global physicochemical properties for compounds group II.

Compound	ClogP[A]	P[B]	V[C]	SA[D]	D[E]
II_4	0.866	16.55	487.68	331.52	3.1
II_5	1.395	18.39	542.6	362.6	2.926
II_6	1.924	20.22	596.38	394.4	2.933
II_7	2.453	22.06	650.7	423.37	2.895
II_8	2.982	23.89	703.83	455.17	2.9047
II_9	3.511	25.73	749.73	477.11	2.884
II_{10}	2.398	23.12	653.14	414.09	2.898
II_{11}	0.8381	26.94	745.2	461.24	3.168
II_{12}	2.883	28.05	744.66	467.25	3.084
II_{13}	2.933	28.05	754.32	472.14	2.902
II_{14}	2.097	32.61	767.72	477.94	4.137
II_{15}	3.147	28.14	729.07	460.65	41.994
II_{16}	2.577	26.12	706.53	448.67	1.818
II_{17}	2.577	26.12	711.03	452.9	3.835
II_{18}	2.577	26.12	711.7	453.5	4.995
II_{19}	2.353	28.68	771.85	485.4	1.774
II_{20}	1.867	28.06	760.45	479.2	6.326
II_{21}	3.317	27.77	779.18	489.7	4.5
II_{22}	2.963	28.05	754.7	477.364	3.067
II_{23}	3.342	29.88	809.23	507.6	3.145
II_{24}	3.608	33.48	826.6	509.25	2.885
II_{25}	2.397	29.47	799.19	477.84	1.924

[A]ClogP were calculated by using Chem Draw Ultra version 8.0. [B]Polarizability values were calculated by using Hyper Chem Professional. [C]Molecular volume values were calculated by using Hyper Chem Professional. [D]Surface area values were calculated by using Hyper Chem Professional. [e]Dipole (debye) values were calculated by using Chem3D Ultra version 8.0.

physicochemical parameters and anti platelet aggregation activity of the compounds did not result in a clear correlation. This could be due to the fact that the compounds are different in their access to their targets in the platelet aggregation pathway induced by arachidonic acid.

Further mechanistic studies are needed to clarify the mechanism of antiplatelet activity of these compounds.

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