PART II

Pyrimidines as anti-Platelet agents

CHAPTER – II.1

Literature Survey

Pyrimdines as anti-platelet agents

Platelets have long been recognized to be of central importance in haemostasis, but their participation in pathological conditions such as thrombosis, atherosclerosis and inflammation is also well established. The platelet has therefore become a key target in therapies to combat cardiovascular disease. Anti-platelet therapies are used widely, but current approaches lack efficacy in a proportion of patients, and are associated with side effects like severe bleeding. In the last decade, substantial progress has been made in understanding the regulation of platelet function, including the characterization of new ligands, platelet-specific receptors and cell signalling pathways. It is anticipated this progress will have a positive impact on the future innovations towards more effective and safer anti-platelet agents.[211]

The thrombus formation process

Platelets are anucleate cells derived from megakaryocytes within the bone marrow that possess the ability to respond explosively at sites of injury. Blood vessel injury results in the exposure of subendothelial extracellular matrix components, mainly collagens which provide a surface on which platelets can adhere and stimulate platelet activation. Activation results in platelet aggregation to form a thrombus, to stem the loss of blood from the injury site. The conversion of platelets from their circulating quiescent form to a thrombus may be characterized in three distinct phases (**Fig. 16**): adhesion, activation and thrombus propagation.

Adhesion

Initial slowing or rolling of platelets over exposed collagens is mediated by transient and indirect binding via the glycoprotein (GP) complex GPIb–V–IX on the platelet surface. Under arterial flow conditions, GPIb–V–IX binds to plasma von Willebrand factor (vWF), which also binds to exposed collagen. These interactions are superseded by more stable adhesion to collagen via integrin $\alpha_2\beta_1$.[212] Stable adhesion enables collagen binding to GPVI, which is non-covalently associated with the Fc receptor (FcR) g-chain. Clustering of the receptor complex upon collagen binding results in the stimulation of signalling pathways that result in shape change, secretion and aggregation.[213-216] A

number of studies suggest that upon binding to the vWF–collagen complex, GPIb–V–IX also stimulates cell signalling that culminates in the intracellular mobilization of calcium and thereby contributes to platelet activation.[217-220]

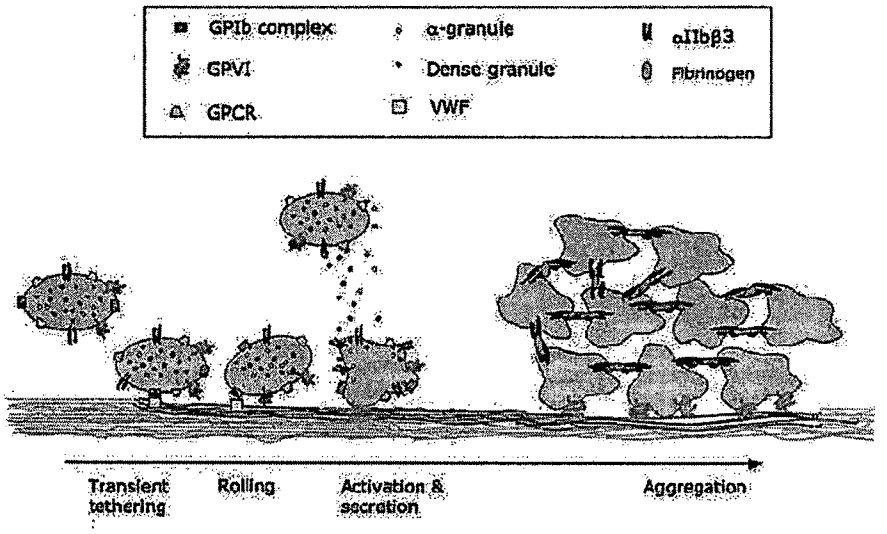


Fig. 16. Adhesion, activation and thrombus propagation.

Receptors for secondary agonists

The secretion reaction upon platelet activation results in local high concentrations of a number of pro-thrombotic factors such as ADP and serotonin that act via cognate receptors on the platelet surface to reinforce stimulation and trigger positive feedback regulation (and thereby contribute

to thrombus propagation).[221] Platelets possess two receptors for ADP: P2Y1 and P2Y12. Both are G protein-coupled receptors (GPCRs): P2Y1, which is essential for platelet activation, is coupled to Gq signalling; and P2Y12, which synergizes via Gi coupling.[222,223] During platelet activation, phospholipase A2 is activated, resulting in the liberation of arachidonic acid from membranes, and via the actions of COX and thromboxane synthase, results in the generation of thromboxane A2 (TXA2).[224] TXA2 binds to thromboxane-prostaglandin receptors, which through coupling to Gq also contribute to positive feedback activation.[225] Individually, these secreted or released factors are weak agonists, but through synergism make important contributions to platelet activation, and are targeted by a number of current anti-platelet drugs.

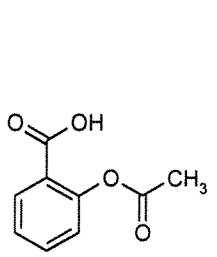
Integrin receptors

A process known as inside-out signalling results in an increase in integrin affinity for respective ligands following platelet activation. It is this 'switch' mechanism that enables platelet–platelet adhesion and thrombus formation.[226-228] this process, which is incompletely understood, is mediated through interactions with and between the cytoplasmic tails of the receptors, molecules on the external face of the membrane, and post-translational modifications such as phosphorylation. Of particular importance for thrombus formation is integrin $\alpha_{IIb}\beta_3$, which through bivalent fibrinogen interactions and also by binding to vWF supports platelet aggregation.[216] Integrin $\alpha_2\beta_1$ also contributes to thrombus formation through supporting platelet adhesion to collagen. There is extensive evidence that fibrinogen binding to its receptor results in the generation of 'outside-in' signalling, a second wave of signalling that further enhances thrombus stability.[229]

Platelets and coagulation

Injury leads to the activation of the coagulation pathways, which result in the generation of thrombin in the vicinity of a platelet thrombus. The platelet thrombus provides a surface for the assembly of the prothrombinase complex and therefore thrombin is generated and fibrin produced within the developing platelet thrombus. Furthermore, through stimulation of the protease-activated receptors (PAR)1 and PAR4, present on the platelet surface, thrombin also acts as a powerful platelet activator.[229]

The relevance of antiplatelet drugs has been firmly established by clinical trials and experience with drugs, such as aspirin (**78**), dipyridamole **(68)** and the thienopyridines **(79)**. These drugs are the only oral antiplatelet agents currently approved by the FDA (Food and Drug Administration) for use in patients.[230-232]



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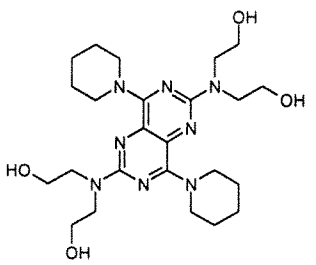

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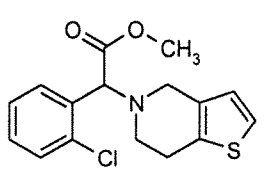
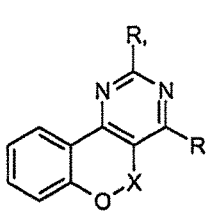


(79)

Pyrimidine containing compounds were also found to possess potent anti-platelet activity. Two types of pyrimidine compounds were reported fused pyrimidines **(69)** and vicinal diaryl pyrimidines **(70)**. Pyrimidines were reported with fused ring system like dipyridamole and benzopyran-2-one system and vicinal pyrimidines like 4,5- diaryl pyrimidines.[233-234]



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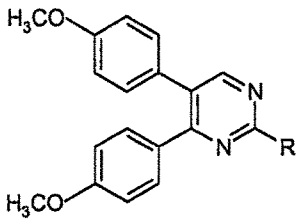


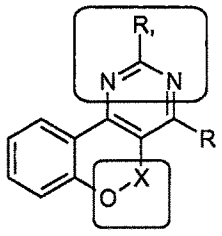
(70)

The large number of data obtained in the screening of fused pyrimidine and the benzopyran-2-one systems, well-known as the typical structure of anticoagulants drugs. The anticoagulant activity of coumarins mainly depends on blocking the prothrombin biosynthesis by inhibition of vitamin K-epoxide reductase, which is closely dependent on the presence of a hydroxy group and a highly lipophilic substituent in the benzopyrano like Warfarin.[233] In fact, several lines of evidence have confirmed that antiplatelet activity was dependent on concomitant contribution of both functions inserted in of the heterocyclic system. Also guanidine fragment was reported as crucial requirement for antiplatelet activity. It is a pharmacophoric requirement to act as GPIIa/IIIb antagonist.[233] The vicinal diarylpyrimidine ring system is found to be a potent platelet aggregation inhibitor *via* inhibition of cyclooxygenase. The vicinal diaryl system was found to be the crucial requirement for COX inhibition, specifically 4-methoxyphenyl substituted compounds. [234]

CHAPTER – II.2

Aims and Objectives

AIMS AND OBJECTIVES



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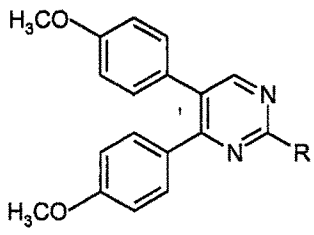
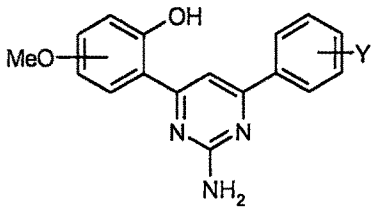


(70)

As discussed previously 4,6-diaryl-2-aminopyrimidines share the some common features of the anti platelet drugs and anti thrombotic drug as shown in structure **(69)** and **(70).** The anticoagulant activity of coumarins mainly depends on blocking the prothrombin biosynthesis by inhibition of vitamin K-epoxide reductase, which is closely dependent on the presence of a **hydroxy group** and a **highly lipophilic substituent** in the benzopyrano like Warfarin.[233] The vicinal diarylpyrimidine ring system is found to be a potent platelet aggregation inhibitor *via* inhibition of cyclooxygenase. The **vicinal diaryl system** was found to be the crucial requirement for COX inhibition, specifically **4-methoxyphenyl** substituted compounds. [234] With this background we synthesized the compounds with variation in the substitutions on both the aromatic rings (**27-44**),



(27-44)

CHAPTER – II.3

Results and Discussion

RESULTS AND DISCUSSION

The result and discussion is discussed under two heads:

II.3a. Chemical work

II.3b Biological studies

II.3a: Chemical work

The substituted 4,6-diaryl-2-aminopyrimidines were synthesized according to the route presented in **Scheme I.** Condensation of 4/5-methoxy-2-hydroxyacetophenone (**A**) with various substituted benzoic acids (**2**) in dry pyridine and $POCl_3$ furnished the esters (**C**). The esters were converted into 1,3-diketones (**D**) by the base catalyzed Baker-Venkatraman transformation reaction. The diketones so obtained, were cyclised to the flavone derivatives (**E**) in presence of sulphuric acid as the dehydrating agent. Treatment of flavones with a slight excess of guanidine hydrochloride in the alkaline medium afforded 4,6-diaryl-2-aminopyrimidines.

The synthesis carried out has been discussed under following heads:

II.3a.1: Synthesis of 4/5-Methoxy-2-hydroxyacetophenone

II.3a.2: Synthesis of 4/5-Methoxy-2-acetyl-1-(substituted benzoyloxy) benzene derivatives

II.3a.3: Synthesis of 1- (4/5-Methoxy-2-hydroxyphenyl)- 3-(substituted phenyl)-1,3-propanedione derivatives

II.3a.4: Synthesis of 6/7-Methoxy-2-(substituted phenyl)-4*H*-chromen-4-one derivatives

II.3a.5: Synthesis of 4-(4/5-Methoxy-2-hydroxyphenyl)-6-(substutited phenyl)-2-Aminopyrimidines

II.3a.1: Synthesis of 4/5-Methoxy-2-hydroxyacetophenone (A3, A4)

II.3a.1a. Synthesis of 4-Methoxy-2-hydroxyacetophenone (A3)

The synthesis of 4-methoxy-2-hydroxyactophenone (**A3**) was afforded by selective methylation of 2,4-dihydroxyacetophenone (**I**) (Scheme II). 2,4-dihydroxyacetophenone was procured commercially. The formation of compound was confirmed by melting point and IR (cm^{-1}).

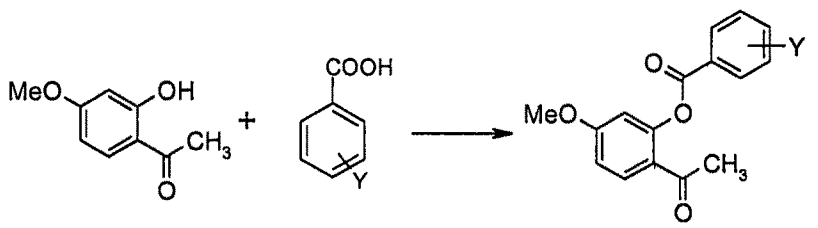
II.3a.1b. Synthesis of 5-Methoxy-2-hydroxyacetophenone (A4)

The 5-methoxy-2-hydroxyacetophenone (**A3**) was obtained by the selective methylation of 2,5-dihydroxyacetophenone (**IV**). 2,5-dihydroxyacetophenone was synthesized by Fries rearrangement of hydroquinone-1,4-diacetate (**III**) which was synthesized by acetylation of hydroquinone (**II**) in the presence of sulphuric acid. Compound (**A4**) displayed the absorption bands at 3066 (OH), 3000 & 999 cm^{-1} and 1640 cm^{-1} in its IR spectrum.

II.3a.2: Synthesis of 4/5-Methoxy-2-acetyl-1-(substituted benzoyloxy) benzene derivatives

II.3a.2a. Synthesis of 2-Acetyl-5-methoxyphenyl-substitutedbenzoate derivative

The phenoxybenzoate (**C27-C37**) esters were obtained by condensation of 4-methoxy-2-hydroxyacetophenones (**A3**) and various substituted aryl/heteroaryl carboxylic acids (**B**) in dry pyridine and phosphorus oxychloride. The melting points of the esters are given in **Table 14.**



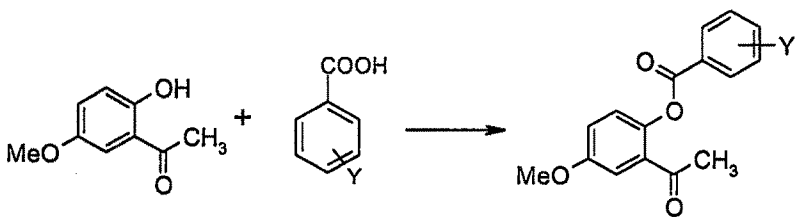
(A3) **(B)** **(C26-36)**

Table 14.

Compound No.	X	Y	M.P. (°C)
C27	5- OCH_3	H	liquid
C28	5- OCH_3	4-Cl	97-98
C29	5- OCH_3	2-Cl	80-82
C30	5- OCH_3	3-Cl	67-68
C31	5- OCH_3	2,4-Di chloro	79-80
C32	5- OCH_3	4-OCH_3	81-80
C33	5- OCH_3	3-OCH_3	71-73
C34	5- OCH_3	4-F	101-102
C35	5- OCH_3	4-CH_3	120-121
C36	5- OCH_3	3-CH_3	liquid
C37	5- OCH_3	4-NO_2	140-142

II.3a.2b. Synthesis of 4-Methoxy-2-acetyl-1-(substituted benzoyloxy) benzene derivatives

The phenoxybenzoate (**C38-C44**) esters were obtained by condensation of 5-methoxy-2-hydroxyacetophenones **(A4)** and various substituted aryl/heteroaryl carboxylic acids in dry pyridine and phosphorus oxychloride . The melting point of the esters are given in **Table 15.**



(A4) **(B)** **(C38-44)**

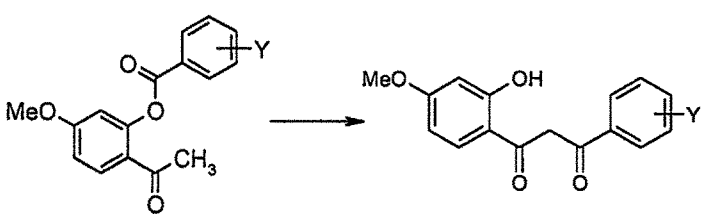
Table 15.

Compound No.	X	Y	M.P. (°C)
C38	4-OCH_3	4-OCH_3	95-96
C39	4-OCH_3	3- OCH_3	80-82
C40	4-OCH_3	2-Cl	92-94
C41	4-OCH_3	3-Cl	56-57
C42	4-OCH_3	2,4-Di chloro	107-109
C43	4-OCH_3	4-F	78-80
C44	4-OCH_3	Furan	77-78

II.3a.3: Synthesis of 1- (4/5-Methoxy-2-hydroxyphenyl)- 3-(substituted phenyl) 1,3-propanedione derivatives

II.3a.3a. Synthesis of 1-(4-Methoxy-2-hydroxyphenyl)-3-(substituted phenyl)-1,3-propanedione derivatives (D27-D37)

The synthesized esters **(C)** were subjected to Baker-Venkataraman rearrangement to afford yellow colored 1,3-propanediones **(D)**. The IR spectrum of 1,3-propanedione showed two absorption bands of carbonyl at the range of 1615-1625 cm^{-1} and 1685-1695 cm^{-1} as shown in **Table 16**. The yellow crystals of 1,3-propanediones were used for the next step.



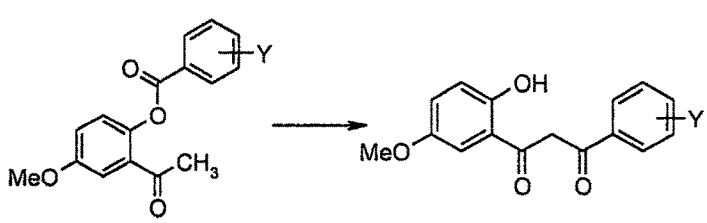
(C) **(D)**

Table 16.

Compound No.	X	Y	M.P. (°C)
D27	4- OCH_3	H	70-72
D28	4- OCH_3	4-Cl	116-117
D29	4- OCH_3	2-Cl	87-88
D30	4- OCH_3	3-Cl	NT
D31	4- OCH_3	2,4-Di chloro	123-125
D32	4- OCH_3	4-OCH_3	105-106
D33	4- OCH_3	3-OCH_3	93-94
D34	4- OCH_3	4-F	141-142
D35	4- OCH_3	4-CH_3	102-104
D36	4- OCH_3	3-CH_3	80-82
D37	4- OCH_3	4-NO_2	170-172

II.3a.3b. Synthesis of 1-(5-Methoxy-2-hydroxyphenyl)-3-(substituted phenyl)-1,3-propanedione derivatives

The synthesized esters **(C15-C26)** were subjected to Baker-Venkataraman rearrangement to afford yellow colored 1,3-propanediones **(D)**. The IR spectrum of 1,3-propanedione showed two absorption bands of carbonyl at the range of 1615-1625 cm^{-1} and 1685-1695 cm^{-1} as shown in **Table 17**. The yellow crystals of 1,3-propanediones were used for the next steps.



(C) **(D)**

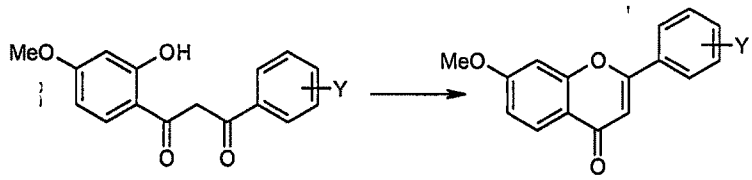
Table 17.

Compound No.	X	Y	M.P. (°C)
D38	5-OCH_3	4-OCH_3	171-173
D39	5-OCH_3	3- OCH_3	liquid
D40	5-OCH_3	2-Cl	101-103
D41	5-OCH_3	3-Cl	94-95
D42	5-OCH_3	2,4-Di chloro	148-149
D43	5-OCH_3	4-F	83-85
D44	5-OCH_3	Furan	85-86

II.3a.4: Synthesis of 6/7-Methoxy-2-substituted phenyl-4*H*-chromen-4-one derivatives

II.3a.4a. Synthesis of 7-Methoxy-2-substituted-phenyl-4*H*-chromen-4-one derivatives (E27-37)

The chromen derivatives were synthesized from 1,3-propanedione derivative **(D27-D37)** by the acid catalyzed cyclisation using glacial acetic acid as solvent. It produced light brown color crystals. The cyclised chromen derivatives **(E27-E37)** were confirmed by IR which gives specific stretching of conjugated C=C and C=O at 1675 cm^{-1} as shown in **Table 18.**



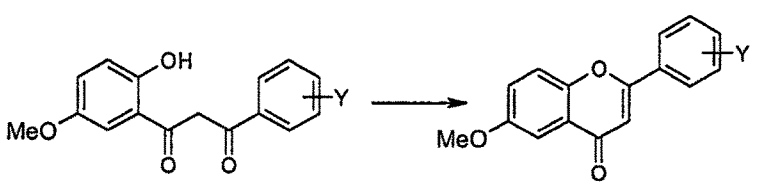
(C27-37) **(E27-37)**

Table 18.

Compound No.	X	Y	M.P. (°C)
E27	7-OCH_3	H	96-97
E28	7-OCH_3	4-Cl	139-141
E29	7-OCH_3	2-Cl	111-113
E30	7-OCH_3	3-Cl	94-95
E31	7-OCH_3	2,4-Di chloro	Liquid
E32	7-OCH_3	4-OCH_3	145-147
E33	7-OCH_3	3-OCH_3	118-120
E34	7-OCH_3	4-F	143-145
E35	7-OCH_3	4-CH_3	90-92
E36	7-OCH_3	3-CH_3	87-88
E37	7-OCH_3	4-NO_2	200-203

II.3a.4b. Synthesis of 6-Methoxy-2-substituted phenyl-4*H*-chromen-4-one derivative (E)

The chromen derivatives were synthesized from 1,3-propanedione derivative (**D**) by the acid catalyzed cyclisation using glacial acetic acid as solvent. It was produce light brown color crystals. The cyclised chromen derivatives **(E38-44)** were confirmed by IR which gives specific stretching of conjugated C=C and C=O at 1675 cm^{-1} as shown in **Table 19.**

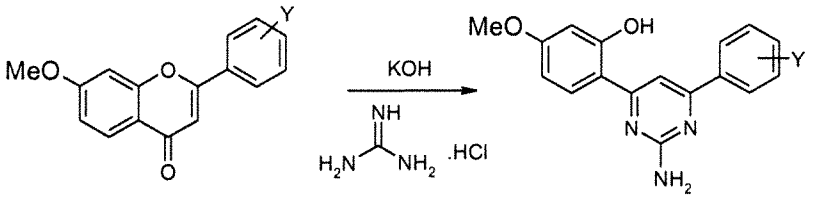


(D) **(E38-44)**

Table 19.

Compound No.	X	Y	M.P. (°C)
E38	6-OCH_3	4-OCH_3	191-193
E39	6-OCH_3	3- OCH_3	152-154
E40	6-OCH_3	2-Cl	98-100
E41	6-OCH_3	3-Cl	114-116
E42	6-OCH_3	2,4-Di chloro	152-153
E43	6-OCH_3	4-F	163-164
E44	6-OCH_3	Furan	171-173

II.3a.5: Synthesis of 4-(4/5-Methoxy-2-hydroxyphenyl)-6-(substutited phenyl)-2-aminopyrimidines

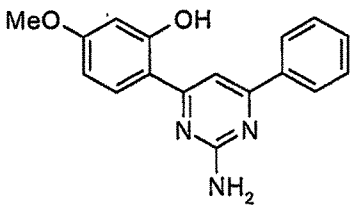


(E27-E37) **(27-37)**

The synthesis of 2-aminopyrimdines was carried out from the chromen derivatives, using guanidine hydrochloride and potassium hydroxide in methanol in reflux condition. The reaction was completed in 12-15 hrs which gives characteristic yellow spot on TLC. The compounds were confirmed by IR, NMR and MASS spectroscopy.

4-(4-Methoxy-2-hydroxyphenyl)-6-phenyl-2-aminopyrimidine **(27)** gives stretching of amino group at 3508 and 3354 cm^{-1} and broad peak of hydroxyl stretching at 3220 cm^{-1}. The C=N ring stretching observed at 1625 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet at δ 3.8 (S, 3H, -O***CH₃***) for methoxy protons. The amino protons at δ 5.1 (2H, -***NH₂***) and aromatic protons displayed at δ 7.37 (1H, Pyri***H***), 6.5-8.01 (8H,

Ar***H***). The aromatic hydroxyl proton was observed at δ 13.84 (1H, ***-OH***). The mass spectrum gives M+1 peak at 293.2 (m/z).



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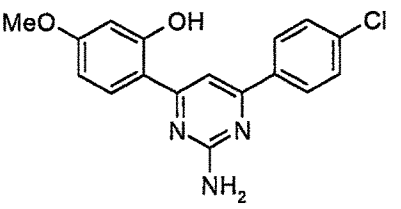
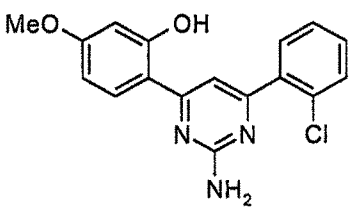


(28)

4-(4-Methoxy-2-hydroxyphenyl)-6-(4-chlorophenyl)-2-aminopyrimidine **(28)** gives stretching of amino group at 3494.8, and 3365.6 cm^{-1} and broad peak of hydroxyl stretching at 2999 cm^{-1}. The C=N ring stretching observed at 1618 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet at δ 3.8 (S, 3H, -O***CH₃***) for methoxy protons. The amino protons at δ 5.1 (2H, -***NH₂***) and aromatic protons displayed at δ 6.5-8.0 (7H, Ar***H***), 7.38 (1H, Pyri***H***). The aromatic hydroxyl proton observed at δ 13.00 (1H, ***-OH***). The mass spectrum gives M+1 peak at 327.2 (m/z).

4-(4-Methoxy-2-hydroxyphenyl)-6-(2-chlorophenyl)-2-aminopyrimidine **(29)** gives stretching of amino group at 3498 and 3281 cm^{-1} and broad peak of hydroxyl stretching at 3173 cm^{-1}. The C=N ring stretching observed at 1623 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet at δ 3.8 (S, 3H, -O***CH₃***) for methoxy protons. The amino protons at δ 5.1 (2H, -***NH₂***) and aromatic protons displayed at δ 6.9-7.6 (7H, Ar***H***), 7.39 (1H, Pyri***H***). The aromatic hydroxyl proton observed at δ 13.18 (1H, ***-OH***). The mass spectrum gives M+1 peak at 327.2 (m/z).



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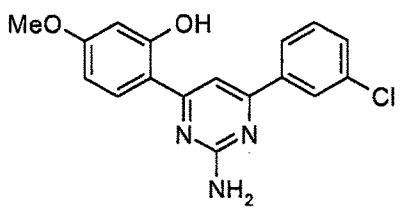
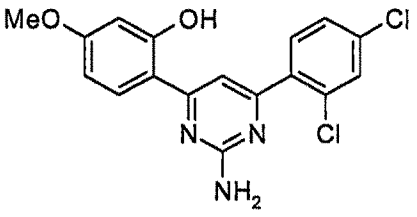


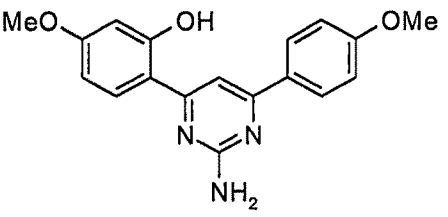
(30)

4-(4-Methoxy-2-hydroxyphenyl)-6-(3-chlorophenyl)-2-aminopyrimidine **(30)** gives stretching of amino group at 3425 and 3310 cm^{-1} and broad peak of hydroxyl stretching at 3190 cm^{-1}. The C=N ring stretching observed at 1630 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet at δ 3.8 (S, 3H, -O***CH₃***) for methoxy protons,. The amino protons at δ 5.1 (2H, -***NH₂***) and aromatic protons displayed at δ 7.38 (1H, Pyri***H***.), 6.4-7.69 (7H, Ar***H***). The aromatic hydroxyl proton observed at δ 13.84 (1H, ***OH***). The mass spectrum gives M+1 peak at 328.2 (m/z).

4-(4-Methoxy-2-hydroxyphenyl)-6-(2,4-dichlorophenyl)-2-aminopyrimidine **(31)** gives stretching of amino group at 3649 and 3575 cm^{-1} and broad peak of hydroxyl stretching at 3200 cm^{-1}. The C=N ring stretching observed at 1635 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet at δ 3.8 (S, 3H, -O***CH₃***) for methoxy protons,. The amino protons at δ 6.8 (2H, -***NH₂***) and aromatic protons displayed at δ 7.33 (1H, Pyri***H***.), 6.9-7.7 (6H, Ar***H***). The aromatic hydroxyl proton observed at δ 13.19 (1H, -***OH***). The mass spectrum gives M+1 peak at 362.2 (m/z).



(31)

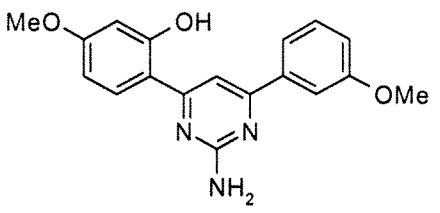


(32)

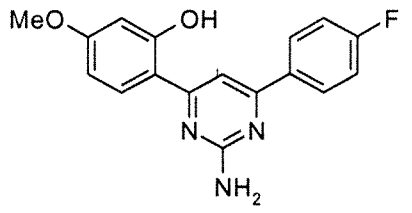
4-(4-Methoxy-2-hydroxyphenyl)-6-(4-methoxyphenyl)-2-aminopyrimidine **(32)** gives stretching of amino group at 3410 and 3300 cm^{-1} and broad peak of hydroxyl stretching at 3110 cm^{-1}. The C=N ring stretching observed at 1652 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet at δ 3.8 (S, 3H, -O***CH₃***) and 3.9 (s, 3H, -O***CH₃***) for both methoxy protons. The amino protons at δ 5.1 (2H, -***NH₂***) and aromatic protons displayed at δ 7.35 (1H, Pyri.***H***), 6.5-8.1 (7H, Ar***H***). The mass spectrum gives M+1 peak at 323.2 (m/z).

4-(4-Methoxy-2-hydroxyphenyl)-6-(3-methoxyphenyl)-2-aminopyrimidine **(33)** gives stretching of amino group at 3390 and 3260 cm^{-1} and broad peak of hydroxyl stretching

at 3115 cm^{-1}. The C=N ring stretching observed at 1635 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet at δ 3.8 (S, 3H, -O***CH₃***) and 3.9 (s, 3H, -O***CH₃***) for both methoxy protons. The amino protons at δ 5.1 (2H, -***NH₂***) and aromatic protons displayed at δ 7.35 (1H, Pyri.***H***), 6.0-8.1 (7H, Ar***H***). The aromatic hydroxyl proton observed at δ 14.08 (1H, -***OH***). The mass spectrum gives M+1 peak at 324.2 (m/z).



(33)



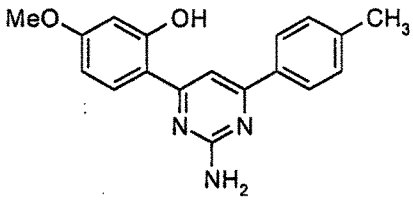
(34)

4-(4-Methoxy-2-hydroxyphenyl)-6-(4-fluorophenyl)-2-aminopyrimidine **(34)** gives stretching of amino group at 3500 and 3380 cm^{-1} and broad peak of hydroxyl stretching at 3120 cm^{-1}. The C=N ring stretching observed at 1620 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet at δ 3.8 (S, 3H, -O***CH₃***) for methoxy protons,. The amino protons at δ 5.1 (2H, -***NH₂***) and aromatic protons displayed at δ 6.9-7.6 (7H, Ar***H***), 7.39 (1H, Pyri***H***). The aromatic hydroxyl proton observed at δ 14.08 (1H, -***OH***). The mass spectrum gives M+1 peak at 312.2 (m/z).

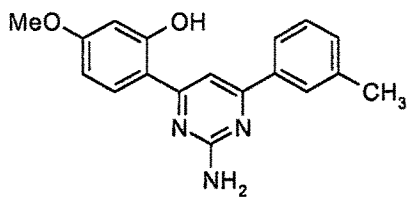
4-(4-Methoxy-2-hydroxyphenyl)-6-(4-methylphenyl)-2-aminopyrimidine **(35)** gives stretching of amino group at 3500 and 3380 cm^{-1} and broad peak of hydroxyl stretching at 3120 cm^{-1}. The C=N ring stretching observed at 1620 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet at δ 2.44 (S, 3H, -***CH₃***) for methyl proton and 3.8 (S, 3H, -O***CH₃***) for methoxy protons. The amino protons at δ 5.1 (2H, -***NH₂***) and aromatic protons displayed at δ 6.4-8.0 (m, 7H, Ar***H***), 7.34 (1H, Pyri.***H***). The aromatic hydroxyl proton observed at δ 13.96 (1H, -***OH***). The mass spectrum gives M+1 peak at 308.2 (m/z).

4-(4-Methoxy-2-hydroxyphenyl)-6-(3-methylphenyl)-2-aminopyrimidine **(36)** gives stretching of amino group at 3490 and 3395 cm^{-1} and broad peak of hydroxyl stretching at 3120 cm^{-1}. The C=N ring stretching observed at 1635 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet at δ 2.45 (S, 3H, -***CH₃***) for methyl proton and 3.7 (S, 3H, -

O***CH₃***) for methoxy protons. The amino protons at δ 5.94 (2H, -***NH₂***) and aromatic protons displayed at δ 6.4-7.8 (m, 7H, Ar***H***), 7.36 (1H, Pyri***H***.). The aromatic hydroxyl proton observed at δ 14.21 (1H, -***OH***). The mass spectrum gives M+1 peak at 308.2 (m/z).

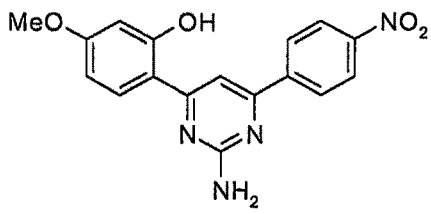


(35)



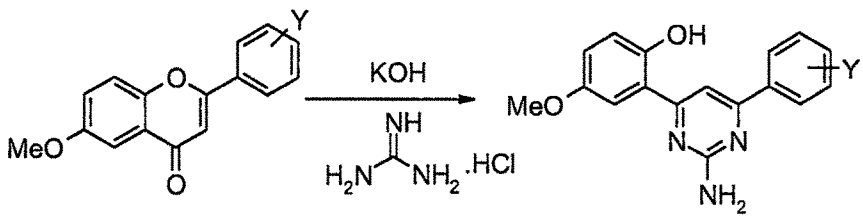
(36)

4-(4-Methoxy-2-hydroxyphenyl)-6-(4-nitrophenyl)-2-aminopyrimidine **(37)** gives stretching of amino group at 3490 and 3310 cm^{-1} and broad peak of hydroxyl stretching at 3120 cm^{-1}. The C=N ring stretching observed at 1635 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet at δ 3.7 (S, 3H, -O***CH₃***) for methoxy protons. The amino protons at δ 5.94 (2H, -***NH₂***) and aromatic protons displayed at δ 7.5 (1H, Pyri.***H***), 6.4-8.03 (7H, Ar***H***). The aromatic hydroxyl proton observed at δ 14.12 (1H, -***OH***). The mass spectrum gives M+1 peak at 384.2 (m/z).

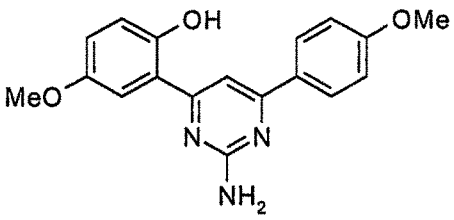


(37)

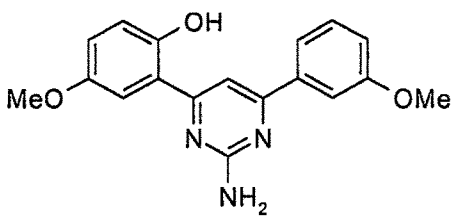
II.3a.5. Synthesis of 4-(5-Methoxy-2-hydroxyphenyl)-6-(substituted phenyl)-2-amino pyrimidine derivatives (38-44)



4-(5-Methoxy-2-hydroxyphenyl)-6-(4-methoxyphenyl)-2-aminopyrimidine (**38**) gives stretching of amino group at 3519 and 3367 cm^{-1} and broad peak of hydroxyl stretching at 3110 cm^{-1}. The C=N ring stretching observed at 1600 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet at δ 3.8 (S, 3H, -O***CH₃***) and 3.9 (s, 3H, -O***CH₃***) for both methoxy protons. The amino protons at δ 5.1 (2H, -***NH₂***) and aromatic protons displayed at δ 7.35 (1H, Pyri***H***), 6.5-8.1 (7H, Ar***H***). The mass spectrum gives M+1 peak at 324.2 (m/z).



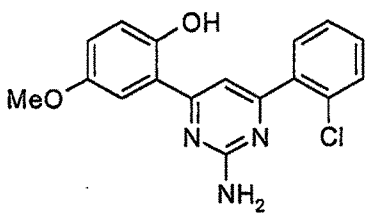
(**38**)



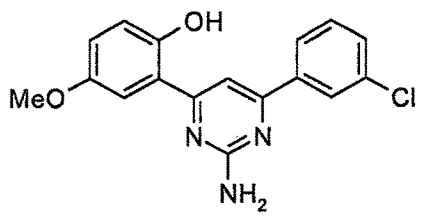
(**39**)

4-(5-Methoxy-2-hydroxyphenyl)-6-(3-methoxyphenyl)-2-aminopyrimidine (**39**) gives stretching of amino group at 3430 and 3320 cm^{-1} and broad peak of hydroxyl stretching at 3200 cm^{-1}. The C=N ring stretching observed at 1599 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet at δ 3.8 (S, 3H, -O***CH₃***) and 4.1 (s, 3H, -O***CH₃***) for both methoxy protons. The amino protons at δ 5.1 (2H, -***NH₂***) and aromatic protons displayed at δ 7.36 (1H, Pyri***H***.), 7.0-8.0 (7H, Ar***H***). The aromatic hydroxyl proton observed at δ 13.19 (1H, -***OH***). The mass spectrum gives M+1 peak at 324.2 (m/z).

4-(5-Methoxy-2-hydroxyphenyl)-6-(2-chlorophenyl)-2-aminopyrimidine (**40**) gives stretching of amino group at 3480 and 3310 cm^{-1} and broad peak of hydroxyl stretching at 3110 cm^{-1}. The C=N ring stretching observed at 1650 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet at δ 3.8 (S, 3H, -O***CH₃***) for methoxy protons. The amino protons at δ 5.2 (2H, -***NH₂***) and aromatic protons displayed at δ 7.39 (1H, Pyri***H***), 6.9-7.9 (7H, Ar***H***). The aromatic hydroxyl proton observed at δ 13.09 (1H, ***OH***). The mass spectrum gives M+1 peak at 328.2 (m/z).



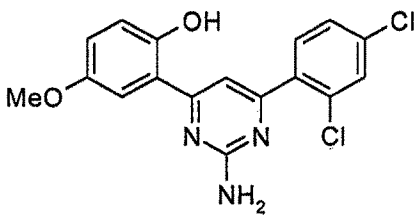
(40)



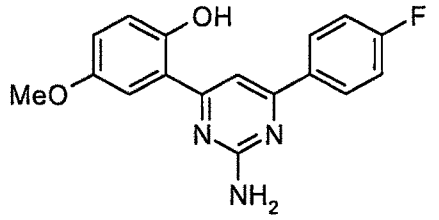
(41)

4-(5-Methoxy-2-hydroxyphenyl)-6-(3-chlorophenyl)-2-aminopyrimidine **(41)** gives stretching of amino group at 3456 and 3352 cm^{-1} and broad peak of hydroxyl stretching at 3110 cm^{-1}. The C=N ring stretching observed at 1639 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet at δ 3.8 (S, 3H, -O***CH₃***) for methoxy protons. The amino protons at δ 5.2 (2H, -***NH₂***) and aromatic protons displayed at δ 7.37 (1H, Pyri***H***), 7.0-7.8 (7H, Ar***H***). The aromatic hydroxyl proton observed at δ 13.84 (1H, ***OH***). The mass spectrum gives M+1 peak at 328.2 (m/z).

4-(5-Methoxy-2-hydroxyphenyl)-6-(2,4-dichlorophenyl)-2-aminopyrimidine **(42)** gives stretching of amino group at 3450 and 3300 cm^{-1} and broad peak of hydroxyl stretching at 3210 cm^{-1}. The C=N ring stretching observed at 1610 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet at δ 3.7 (S, 3H, -O***CH₃***) for methoxy protons. The amino protons at δ 6.8 (2H, -***NH₂***) and aromatic protons displayed at δ 7.33 (1H, Pyri***H***), 6.9-7.8 (7H, Ar***H***). The aromatic hydroxyl proton observed at δ 13.19 (1H, -***OH***). The mass spectrum gives M+1 peak at 362.2 (m/z).



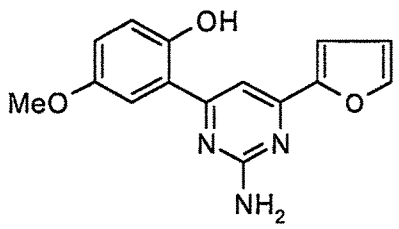
(42)



(43)

4-(5-Methoxy-2-hydroxyphenyl)-6-(4-fluorophenyl)-2-aminopyrimidine **(43)** gives stretching of amino group at 3460 and 3310 cm^{-1} and broad peak of hydroxyl stretching at 3220 cm^{-1}. The C=N ring stretching observed at 1635 cm^{-1} in IR spectrum. The PMR

spectrum displayed singlet at δ 3.8 (S, 3H, -O***CH₃***) for methoxy protons. The amino protons at δ 5.1 (2H, -***NH₂***) and aromatic protons displayed at δ 7.34 (1H, Pyri***H***.), 6.9-8.0 (7H, Ar***H***). The aromatic hydroxyl proton observed at δ 13.16 (1H, ***OH***). The mass spectrum gives M+1 peak at 312.2 (m/z).



(44)

4-(5-Methoxy-2-hydroxyphenyl)-6-furan-2-aminopyrimidine **(44)** gives stretching of amino group at 3410 and 3300 cm^{-1} and broad peak of hydroxyl stretching at 3220 cm^{-1}. The C=N ring stretching observed at 1652 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet at δ 3.8 (S, 3H, -O***CH₃***) for methoxy protons. The amino protons at δ 5.1 (2H, -***NH₂***) and aromatic protons displayed at δ 7.34 (1H, Pyri***H***), 6.5-7.4 (3H, ***furan***), 6.9-7.8 (3H, Ar***H***). The aromatic hydroxyl proton observed at δ 13.18 (1H, -***OH***). The mass spectrum gives M+1 peak at 284.2 (m/z).

II.3b Biological studies

Antiplatelet Studies

The newly synthesized compounds were studied for their *in-vitro* platelet aggregation inhibitory activity on whole human blood. This study was performed using Whole Blood Aggregometer, Chronolog Corporation, Haverton, PA, USA. The inhibitory activity of nineteen compounds was measured to compare with inhibition induced by standard drug Aspirin (ASA) (10 μg/ml). Each assay was performed three times, taking control and aspirin for comparative assay every time. Whole blood aggregometer measures inhibition of platelet aggregation induced by ADP (10 μL) in Ohms which is the resistance produce

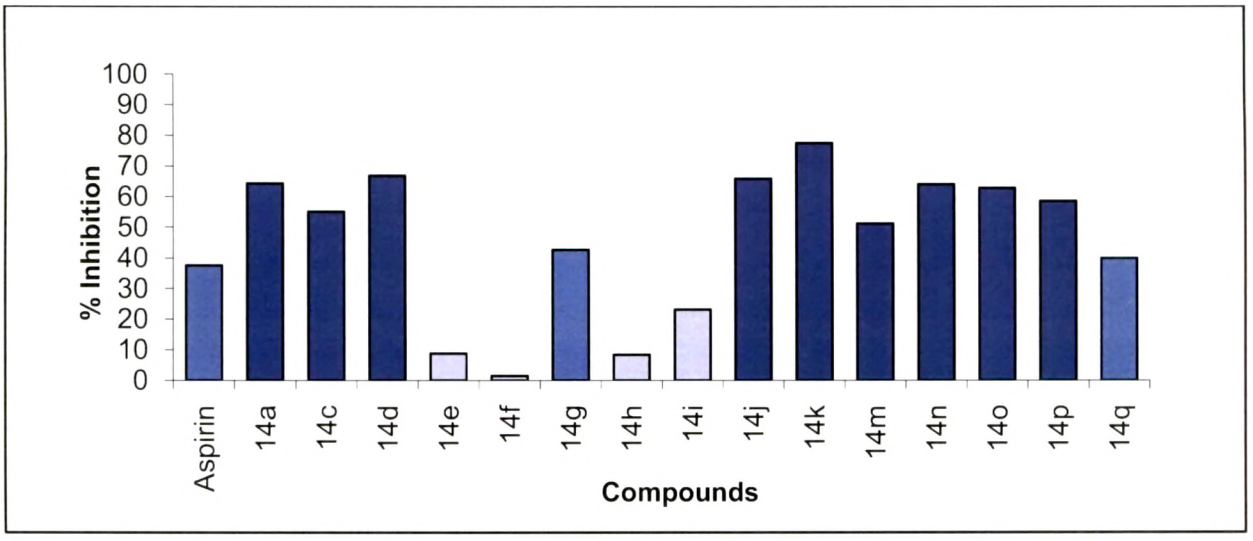
by accumulation of aggregates on the electrode. The control or normal platelet aggregation found 10.92±1.92 **Ω** and for aspirin (10 µg/ml) the inhibition of aggregation 6.82±1.21 **Ω (37 %)**. The standard range for inhibition of ADP (10 µL) induced aggregation for aspirin is 6-24 **Ω.** From the comparative study of test compounds (**27-43**) at same concentration of aspirin, eleven compounds found active as shown in **Table 20.** The results were shown in terms of % inhibition in ohms, which was calculated using the **equation 1**. From eleven compounds **31** is the most potent compound it is two time potent than aspirin. also **30, 34, 35, 37, 38, 41** produce more than 60 % inhibition and **27, 33, 40** found equipotent to aspirin. The graphical explorations of the results are shown in **Graph 1**.

Table 20. % inhibition of platelet aggregation.

Sr. No.	Compounds (10µ)	Mean±S.D. (Ω)	Mean±SEM (Ω)	% Inhibition of Platelet Aggregation
1	Control	10.92±1.92	10.92±0.47	–
2	Aspirin	6.82±1.21	6.82±0.29	37.55
3	27	6.26±0.15	6.26±0.09	**42.67**
4	28	10.0±0.20	10.0±0.11	8.42
5	29	8.4±0.10	8.4±0.06	23.08
6	30	3.73±1.41	3.73±0.82	**65.84**
7	31	2.46±0.25	2.46±0.14	**77.47**
8	32	11.56±0.06	11.56±0.03	Omitted
9	33	5.33±0.40	5.33±0.23	**51.19**
10	34	3.93±0.06	3.93±0.03	**64.01**
11	35	4.06±0.74	4.06±0.42	**62.82**
12	37	4.53±1.23	4.53±0.71	**58.52**
13	38	3.9±1.14	3.9±0.64	**64.28**

14	39	11.06±0.15	11.06±0.09	Omitted
15	40	4.9±1.19	4.9±1.01	**55.13**
16	41	2.9±0.90	2.9±0.52	**66.85**
17	42	9.96±1.55	9.96±0.89	8.79
18	43	10.76±1.95	10.76±1.13	1.46

Table 20 : The results are given as the % inhibition of the platelet aggrgation



Graph 1. Graphical illustration of the results in terms of % inhibition

eq.1

$$\% \text{ Inhibition of Platelet Aggregation} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

CHAPTER – II.4

Experimental Work

EXPERIMENTAL WORK

The melting points were taken in open capillaries, using the heating block type melting point apparatus and are uncorrected. Thin-Layer Chromatography (TLC) was carried out on precoated silica gel Merk plates. Compounds were visualized by illuminating with UV light (254 nm). Column chromatography was carried out using silica gel (100-200 mesh). The IR spectra were recorded on Shimadzu-8300 FT-IR instrument using KBR pellets. The PMR spectra were recorded in $CDCl_3$ and DMSO on a Brüker spectrometer (300 or 400MHz), using tetramithylsilane as an internal standard. Chemical shift data were reported in parts per million (δ in ppm) where s, d, t, m and bs designate singlet, doublet, triplet multiplet and broad singlet, respectively. Mass spectra were recorded on APISciEX mass spectrometer equipped with an electrospray ionization (ESI) interface. Most of the solvents and chemicals were obtained from S.D.Fine Chemicals, Spectrochem and Loba Chemie and purified using standard purification methods.

II.4a: Chemical work

II.4b: Biological work

II.4a: CHEMICAL WORK

The synthesis carried out has been discussed under following heads:

II.4a.1: Synthesis of 4/5-Methoxy-2-hydroxyacetophenone

II.4a.2: Synthesis of 4/5-Methoxy-2-Acetyl-1-(substituted benzoyloxy) benzene derivatives

II.4a.3: Synthesis of 1- (4/5-Methoxy-2-hydroxyphenyl)- 3-(substituted phenyl)-1,3-propanedione derivatives

II.4a.4: Synthesis of 6/7-Methoxy-2-substituted phenyl-4*H*-chromen-4-one derivatives

II.4a.5: Synthesis of 4-(4/5-Methoxy-2-hydroxyphenyl)-6-(substutited phenyl)-2-aminopyrimidines

II.4a.1: Synthesis of 4/5-Methoxy-2-hydroxyacetophenone (A3, A4)

II.4a.1a. Synthesis of 4-Methoxy-2-hydroxyacetophenone (A3)

Methyl iodide (4.6 ml) and anhydrous potassium carbonate (13.6 g) was added to a solution of 2,4-dihydroxyacetophenone (10 g) **(I)** in acetone (30 ml). The reaction mixture was stirred and refluxed for 8 hr at 60 °C and filtered to remove K_2CO_3. Acetone was recovered and brown liquid was obtained. It was dissolved in chloroform and washed with water three times to remove traces of base. After removal of solvent in vacuum, the resulting oily compound was subjected to silica gel column chromatography using chloroform as eluent. The yield is 9 g, 82 %.

Anal:

R_f : 0.80 (chloroform)

IR (KBr) : 3066, 1695, 850 and 769 cm^{-1}

II.4a.1b. Synthesis of 5-Methoxy-2-hydroxyacetophenone (A4)

Methyl iodide (4.6 ml) and anhydrous potassium carbonate (13.6 g) was added to a solution of 2, 5-dihydroxyacetophenone (10 g) **(IV)** in acetone (30 ml). The reaction mixture was stirred and refluxed for eight hour at 60 °C. Acetone was recovered and residues acidified with dil.H_2SO_4. the product was purified by distilled out using steam. The distillate was kept in cold temp. overnight to afford yellow crystals. Yield 6.5 g, 60 %.

Anal:

M.P. : 48-50 °C

R_f : 0.62 (chloroform)

IR (KBr) : 3066, 1695, 1220, 850 and 769 cm^{-1}

II.4a.2. Synthesis of 4/5-methoxy-2-Acetyl-1-(substituted benzoyloxy) benezene derivatives (C27-C37)

II.4a.2a Synthesis of 5-Methoxy-2-acetyl-1-(substituted benzoyloxy)-benzene derivatives

Table 21. Details of 5-Methoxy-2-acetyl-1-(substituted benzoyloxy)-benzene derivatives

No.	A3	B (substituted benzoic acid)	$POCl_3$	Method	R_f ($CHCl_3$)	Yield (%)	IR (cm^{-1})	M.P. (°C)
C27	3 gm 18.0 mmol	3.3 gm, 21.0 mmol	4.1 gm, 27 mmol	II	0.72	4.1 g, 86 %	1738, 1685, 1485, 849 and 769 cm^{-1}	NT
C28	3 gm, 18.0 mmol	3.1 gm, 19.0 mmol	4.1 gm, 27 mmol	I	0.62	4.7 g, 86 %	1738, 1685, 1485, 849 and 769	97-98
C29	3.6 g, 21.0 mmol	3.7 g, 23.0 mmol	4.81 gm, 31.0 mmol	I	0.82	5.6 g, 83 %	1736, 1685, 1485, 849 and 760	80-82
C30	2.37 g, 16 mmol	2.67 g, 17.0 mmol	3.7 g, 24 mmol	I	0.72	4.3 g, 73 %	1740,1690, 1480, 849 and 765	67-68
C31	4.28 g, 25.0 mmol	4.91 g, 25.0 mmol	5.7 g, 37 mmol	I	0.78	4.1 g, 47 %	1735, 1685, 1480, 849 and 760	79-80
C32	2.08 g, 12.0 mmol	2.09 g, 13.0 mmol	3 g, 18.0 mmol	I	0.58	2.9 g, 79 %	1732, 1685, 1485, 849 and 770	81-80

C33	3.15 g, 18 mmol	3.46 g, 22 mmol	4.1 g, 27 mmol	I	0.80	5.14 g, 90 %)	1735, 1680, 1485, 855 and 780	71-73
C34	5.48 g, 32.9 mmol	5.24 g, 39 mmol	5.53 g, 39.4 mmol	I	0.89	8 g, 84 %	1741, 1690, 1480, 849 and 760	101-102
C35	2 g, 12 mmol	1.79 g, 13 mmol	2.75 g, 18 mmol	I	0.86	1.9 g, 54 %	1734, 1682, 1485, 855 and 780	120-121
C36	2 g, 12 mmol	1.79 g, 13 mmol	2.75 g, 18 mmol	II	0.89	1.9 g, 54 %	1733, 1680, 1485, 855 and 780	NT
C37	3.6 g, 22 mmol	4.2 g, 25 mmol	4.9 g, 32 mmol	I	0.68	5.9 g, 86 %	1740, 1689, 1480, 849 and 769	140-142

II.4a.2b. Synthesis of 4-methoxy-2-acetyl-1-(substituted benzoyloxy)-benzene derivatives

(C38-C44)

Table 22. Details of 4-methoxy-2-acetyl-1-(substituted benzoyloxy)-benzene derivatives

No.	A4	B (substituted benzoic acid)	$POCl_3$	Method	R_f ($CHCl_3$)	Yield (%)	IR (cm^{-1})	M.P. (°C)
C38	2.5g, 1.5mmol	2.51g, 1.60 mmol	3.4g, 2.2mmol	I	0.56	2.7 g, 60 %	1720, 1685, 1490, 850 and 778	95-96
C39	3.3 g, 19.0	3.07 g, 20.0 mmol	4.36 g, 28 mmol	I	0.60	5 g, 82 %	1730, 1680, 1495, 850	80-82

	mmol						and 778	
C40	3.6 g, 21.0 mmol	3.7 g, 23.0 mmol	4.81 g, 31.0 mmol	I	0.82	5.6 g, 83 %	1725, 1690, 1495, 853 and 779	92-94
C41	5 g, 30.0mmol	5.65 g, 36.0 mmol	6.9 g, 45.0 mmol	I	0.72	9 g, 96 %	1735, 1695, 1490, 850 and 778	56-57
C42	2.28 g, 13.0 mmol	2.9 g, 15.0 mmol	2.9 g, 19 mmol	I	0.78	4.16 g, 89 %	1740, 1692, 1485, 850 and 778	107-109
C43	3.58 g, 21.0 mmol	3.32 g, 23.0 mmol	3.6 g, 23.0 mmol	I	0.77	4.4 g, 71 %	1735, 1688, 1490, 850 and 778	78-80
C44	1.8 g, 10.0 mmol	1.23 g, 11.0 mmol	2.48 g, 16.0 mmol	I	0.70	2.6 g, 92 %	1735, 1698, 1480, 850 and 767	77-78

II.4a.3: Synthesis of 1- (4/5-Methoxy-2-hydroxyphenyl)- 3-(substituted -phenyl)-1,3-propanedione derivatives

II.4a.3a. Synthesis of 1-(4-Methoxy-2-hydroxyphenyl)-3-(substituted phenyl)-1,3-propanedione derivatives (D27-D37)

Table 23. Details of propanedione derivatives.

No.	3a-k	KOH	Method	R_f ($CHCl_3$)	Yield (%)	IR (cm^{-1})	M.P. (°C)
D27	4 g, 1.4 mmol	2.2 g, 2.2 mmol	I	0.65	3.6 g, 90 %	1616, 1580, 1482, 1290, 1030 and 850	70-72

D28	4 g, 1.3 mmol	2.2 g, 2.2 mmol	I	0.68	3.4 g, 85 %	1615, 1590, 1480, 1295, 1035 and 845	116-117
D29	1.8 g, 5.9 mmol	2.2 g, 2.2 mmol	I	0.76	1.5 g, 83 %	1615, 1575, 1480, 1290, 1040 and 845	87-88
D30	3 g, 9.8 mmol	2.2 g, 2.2 mmol	II	0.68	2.4 g, 80 %	-------	NT
D31	2.6 g, 7.6 mmol	1 g, 1.7 mmol	I	0.77	1.9 g, 73 %	1611, 1589, 1488, 1295, 1045 and 840	123-125
D32	2.6 g, 8.6 mmol	0.75 g, 12.0 mmol	I	0.60	2.5 g, 96 %	1616, 1595, 1470, 1260, 1070 and 854	105-106
D33	3.67 g, 12 mmol	1 g, 1.7 mmol	I	0.78	2.5 g, 68 %	1612, 1580, 1490, 1265, 1070 and 840	93-94
D34	7.6 g, 26.3mmol	1 g, 1.7 mmol	I	0.69	7.1 g, 80 %	1610, 1575, 1470, 1290, 1055 and 840	141-142
D35	1.7 g, 5.9 mmol	1 g, 1.7 mmol	I	0.81	1.6 g, 94 %	1615, 1585, 1495, 1270, 1010 and 840	102-104
D36	1.7 g, 5.9 mmol	1 g, 1.7 mmol	I	0.77	1.4 g, 88 %	1600, 1575, 1490, 1275, 1060 and 880	80-82
D37	5 g, 15 mmol	1 g, 1.7 mmol	I	0.76	4.2 g, 84 %	1615, 1590, 1475, 1280, 1070 and 840	170-172

II.4a.3b. Synthesis of 1-(5-Methoxy-2-hydroxyphenyl)-3-(substituted phenyl)-1,3-propanedione derivatives (D)

Table 24. Details of propanedione derivatives

No.	C	KOH	Method	R_f ($CHCl_3$)	Yield (%)	IR (cm^{-1})	M.P. (°C)
D38	2.3g, 7.6 mmol	1 g, 1.7 mmol	I	0.60	2 g, 86 %	1600, 1485, 1275, 1055 and 878	171-173
D39	3.4 g, 11 mmol	1 g, 1.7 mmol	II	0.64	3.1 g, 91 %	------	NT
D40	3 g, 9.8 mmol	1 g, 1.7 mmol	I	0.70	2.7 g, 90 %	1620, 1599, 1490, 1275, 1050 and 880	101-103
D41	8.5 g, 33.0 mmol	1 g, 1.7 mmol	I	0.69	8.2 g, 96 %	1618, 1591, 1495, 1280, 1077 and 880	94-95
D42	4 g, 11.0 mmol	1 g, 1.7 mmol	I	0.79	3.7 g, 92 %	1618, 1592, 1480, 1225, 1050 and 878	148-149
D43	4 g, 13.0 mmol	1 g, 1.7 mmol	I	0.78	3.98 g, 99 %	1615, 1587, 1485, 1260, 1045 and 878	83-85
D44	2.54 g, 9.6 mmol	1 g, 1.7 mmol	I	0.73	2.3 g, 90 %	1620, 1590, 1480, 1280, 1055 and 878	85-86

II.4a.4: Synthesis of 6/7-Methoxy-2-substituted phenyl-4*H*-chromen-4-one derivatives (E)

II.4a.4a. Synthesis of 7-Methoxy-2-substituted phenyl-4*H*-chromen-4-one derivatives

Table 25. Details of 7-Methoxy-2-substituted phenyl-4*H*-chromen-4-one derivatives

No.	D	Method	R_f ($CHCl_3$)	Yield (%)	IR (cm^{-1})	M.P. (°C)
E27	3.2g, 1.18 mmol	I	0.42	1.5 g, 51 %	1659, 1615, 1590, 1480, 1265, 1079 and 850	96-97
E28	2.5g, 8.2 mmol	I	0.55	1.4 g, 60 %	1690, 1615, 1480, 1200, 1079 and 850	139-141
E29	1.3 g, 6.5 mmol	I	0.43	1 g, 74 %	1685, 1610, 1485, 1210, 1079 and 850	111-113
E30	1.7g, 5.5 mmol	I	0.45	1.2 g, 80 %	1690, 1610, 1490, 1240, 1080 and 850	94-95
E31	1.5 g, 4.48 mmol	II	0.40	1 g, 71 %	-------	NT
E32	2.1 g, 6.6 mmol	I	0.47	2.4g, 7.9 %	1675, 1608, 1495, 1255, 1070 and 850	145-147
E33	2 g, 6.6 mmol	I	0.38	1.2 g, 66 %	1680, 1620, 1470, 1225, 1075 and 850	118-120
E34	3 g, 10.40	I	0.52	2.1 g, 75 %	1690, 1620, 1470, 1225, 1075 and	143-145

	mmol				850	
E35	1.1 g, 3.8 mmol	I	0.45	0.8 g, 77 %	1675, 1611, 1470, 1225, 1075 and 850	90-92
E36	1 g, 3.2 mmol	I	0.47	0.6 g, 67 %	1680, 1625, 1485, 1230, 1075 and 850	87-88
E37	2.1 g, 6.6 mmol	I	0.51	1.6 g, 84 %	1699, 1640, 1480, 1240, 1075 and 850	200-203

II.4a.4b. Synthesis of 6-Methoxy-2-substituted phenyl-4*H*-chromen-4-one derivatives

Table 26. Details of 6-Methoxy-2-substituted phenyl-4*H*-chromen-4-one derivatives

No.	ID	Method	R_f ($CHCl_3$)	Yield (%)	IR (cm^{-1})	M.P. (°C)
E38	1.5 g,4.9 mmol	I	0.47	1.1g, 78%	1698, 1630, 1490, 1235, 1065 and 850	191-193
E39	2.2 g, 7.3 mmol	I	0.55	1.8 g, 87%	1688, 1627, 1498, 1230, 1065 and 850	152-154
E40	2.4g, 6.5.mmol	I	0.58	1.6 g, 77 %	1698, 1630, 1480, 1235, 1080 and 850	98-100
E41	5 g, 16.mmol	I	0.49	3.6 g, 78%	1705, 1650, 1480, 1235, 1080 and 850	114-116
E42	2.5 g,7.3 mmol	I	0.42	2 g, 84 %	1695, 1625, 1470, 1220,	152-153

					1075 and 850	
E43	3.6 g, 12.0 mmol	I	0.46	3.3 g, 80 %	1685, 1620, 1480, 1230, 1089 and 850	163-164
E44	2 g, 7.6 mmol	I	0.57	1.2 g, 66 %	1690, 1615, 1475, 1225, 1090 and 850	171-173

II.4a.5: Synthesis of 4-(4/5-substituted-2-hydroxyphenyl)-6-(substutited phenyl)-2-Aminopyrimidines

4-(4-Methoxy-2-hydroxyphenyl)-6-phenyl-2-aminopyrimidine (27)

A mixture of 7-methoxy-2-phenyl-4*H*-chromen-4-one **(E27)** (0.4 g, 1.5 mmol), guanidine hydrochloride (0.3 g, 3.1 mmol) and potassium hydroxide (0.2 g, 3.5 mmol) was refluxed in methanol (30 ml) for 12 hrs. After removal of excess methanol the reaction mixture was poured on crushed ice containing acetic acid. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(27)**, (0.11 g, 24 %) m.p. 186-189 °C

Anal:

TLC : 0.51 (benzene)

IR (KBr): 3508, 3354, 3205, 1625, 1230, 890 and 760 cm^{-1}

PMR : δ 3.8 (S, 3H, -O***CH***$_3$), 5.1 (2H, -***NH***$_2$), 7.37 (1H, Pyri***H***), 6.5-8.01 (8H, Ar***H***), 13.84 (1H, -***OH***)

Mass : 293.2 (m/z) (M+1)

4-(4-Methoxy-2-hydroxyphenyl)-6-(4-chlorophenyl)-2-aminopyrimidine (28)

A mixture of 7-methoxy-2-(4-chlorophenyl)-4*H*-chromen-4-one **(E28)** (0.5 g, 1.5 mmol), guanidine hydrochloride (0.4 g, 4.1 mmol) and potassium hydroxide (0.5 g, 4.1 mmol)

was refluxed in methanol (30 ml) for 12 hrs. After removal of excess methanol the reaction mixture was poured on crushed ice containing acetic acid. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(28)**, (0.21 g, 33 %), m.p. 215-217 °C

Anal:

TLC : 0.49 (benzene)

IR (KBr): 3499, 3348, 3200, 1618, 1230, 890 and 760 cm^{-1}

PMR : δ 3.8 (S, 3H, -O***CH₃***), 5.1 (2H, -***NH₂***), 7.39 (1H, Pyri***H***), 6.5-8.01 (8H, Ar***H***), 13.00 (1H, -***OH***)

Mass : 327.2 (m/z) (M+1)

4-(4-Methoxy-2-hydroxyphenyl)-6-(3-chlorophenyl)-2-aminopyrimidine (29)

A mixture of 7-methoxy-2-(3-chlorophenyl)-4*H*-chromen-4-one **(E29)** (0.8 g, 2.7 mmol), guanidine hydrochloride (0.6 g, 6.2 mmol) and potassium hydroxide (0.6 g, 10 mmol) was refluxed in methanol (30 ml) for 12 hrs. After removal of excess methanol the reaction mixture was poured on crushed ice containing acetic acid. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(29)**, (0.35 g, 35 %) m.p. 189-190 °C

Anal:

TLC : 0.54 (benzene)

IR (KBr): 3425, 3310, 3190, 1630, 1230, 890 and 760 cm^{-1}

PMR : δ 3.8 (S, 3H, -O***CH₃***), 5.1 (2H, -***NH₂***), 7.38 (1H, Pyri***H***.), 6.4-7.69 (7H, Ar***H***), 13.84 (1H, -***OH***)

Mass : 328.2 (m/z) (M+1)

4-(4-Methoxy-2-hydroxyphenyl)-6-(2-chlorophenyl)-2-aminopyrimidine (30)

A mixture of 7-methoxy-2-(2-chlorophenyl)-4*H*-chromen-4-one **(E30)** (0.3 g, 1 mmol), guanidine hydrochloride (0.2 g, 2.5 mmol) and potassium hydroxide (0.5 g, 1.5 mmol) was refluxed in methanol (30 ml) for 12 hrs. After removal of excess methanol the reaction mixture was poured on crushed ice containing acetic acid. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(30)**, (0.09 g, 26 %) m.p. 198-199 °C

Anal:

TLC : 0.51 (benzene)

IR (KBr): 3498, 3281, 3173, 1621, 1230, 890 and 760 cm^{-1}

PMR : δ 3.8 (S, 3H, -O***CH*$_3$**), 5.1 (2H, -***NH*$_2$**), 7.38 (1H, Pyri***H***.), 6.4-7.69 (7H, Ar***H***), 13.18 (1H, -***OH***)

Mass : 327.9 (m/z) (M+1)

4-(4-Methoxy-2-hydroxyphenyl)-6-(2,4-dichlorophenyl)-2-aminopyrimidine (31)

A mixture of 7-methoxy-2-(2,4-dichlorophenyl)-4*H*-chromen-4-one **(E31)** (0.5 g, 1.5 mmol), guanidine hydrochloride (0.4 g, 3.1 mmol) and potassium hydroxide (0.4 g, 4 mmol) was refluxed in methanol (30 ml) for 12 hrs. After removal of excess methanol the reaction mixture was poured on crushed ice containing acetic acid. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(31)**, (0.3 g, 54 %) m.p. 204-205 °C

Anal:

TLC : 0.52 (benzene)

IR (KBr): 3649, 3571, 3200, 1635, 1230, 890 and 760 cm^{-1}

PMR : δ 3.8 (S, 3H, -O***CH*$_3$**), 5.1 (2H, -***NH*$_2$**), 7.33 (1H, Pyri***H***.), 6.9-7.7 (6H, Ar***H***), 13.19 (1H, -***OH***)

Mass : 362.2 (m/z) (M+1)

4-(4-Methoxy-2-hydroxyphenyl)-6-(4-methoxyphenyl)-2-aminopyrimidine (32)

A mixture of 7-methoxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one **(E32)** (0.5 g, 1.7 mmol), guanidine hydrochloride (0.3 g, 3.2 mmol) and potassium hydroxide (0.5 g, 8.9 mmol) was refluxed in methanol (30 ml) for 12 hrs. After removal of excess methanol the reaction mixture was poured on crushed ice containing acetic acid. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(32)**, (0.2 g, 35 %) m.p. 223-25 °C

Anal:

TLC : 0.53 (benzene)

IR (KBr): 3410, 3300, 3110, 1652, 1230, 890 and 760 cm^{-1}

PMR : δ 3.8 (S, 3H, -O***CH₃***), 3.9 (S, 3H, -O***CH₃***), 5.1 (2H, -***NH₂***), 7.35 (1H, Pyri.***H***), 6.5-8.1 (7H, Ar***H***),

Mass : 323.2 (m/z) (M+1)

4-(4-Methoxy-2-hydroxyphenyl)-6-(3-methoxyphenyl)-2-aminopyrimidine (33)

A mixture of 7-methoxy-2-(3-methoxyphenyl)-4*H*-chromen-4-one **(E33)** (0.4 g, 1.4 mmol), guanidine hydrochloride (0.3 g, 3.2 mmol) and potassium hydroxide (0.2 g, 3.5 mmol) was refluxed in methanol (30 ml) for 12 hrs. After removal of excess methanol the reaction mixture was poured on crushed ice containing acetic acid. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(33)**, (0.1 g, 22 %) m.p. 186-87 °C

Anal:

TLC : 0.54 (benzene)

IR (KBr): 3390, 3260, 3115, 1635, 1230, 890 and 760 cm^{-1}

PMR : δ 3.8 (S, 3H, -O***CH₃***), 3.9 (S, 3H, -O***CH₃***), 5.1 (2H, -***NH₂***) 7.35 (1H,

Pyri.***H***), 6.0-8.1 (7H, Ar***H***), 14.08 (1H, -***OH***)

Mass : 323.2 (m/z) (M+1)

4-(4-Methoxy-2-hydroxyphenyl)-6-(4-fluorophenyl)-2-aminopyrimidine (34)

A mixture of 7-methoxy-2-(4-fluorohenyl)-4*H*-chromen-4-one **(E34)** (1 g, 3.7 mmol), guanidine hydrochloride (0.53 g, 5.5 mmol) and potassium hydroxide (0.24 g, 4.4 mmol) was refluxed in methanol (30 ml) for 12 hrs. After removal of excess methanol the reaction mixture was poured on crushed ice containing acetic acid. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(34)**, (0.25 g, 25 %) m.p. 205-07 °C

Anal:

TLC : 0.54 (benzene)

IR (KBr): 3500, 3180, 3120, 1620, 1230, 890 and 760 cm^{-1}

PMR : δ 3.8 (S, 3H, -O***CH₃***), 5.1 (2H, -***NH₂***), 6.9-7.6 (7H, Ar***H***), 7.39 (1H, Pyri***H***), 13.19 (1H, -***OH***)

Mass : 312.2 (m/z) (M+1)

4-(4-Methoxy-2-hydroxyphenyl)-6-(4-methylphenyl)-2-aminopyrimidine (35)

A mixture of 7-methoxy-2-(4-methylphenyl)-4*H*-chromen-4-one **(E35)** (0.5 g, 1.9 mmol), guanidine hydrochloride (0.4 g, 4.1 mmol) and potassium hydroxide (0.2 g, 3.5 mmol) was refluxed in methanol (30 ml) for 12 hrs. After removal of excess methanol the reaction mixture was poured on crushed ice containing acetic acid. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(35)**, (0.1 g, 16 %) m.p. 195-97 °C

Anal:

TLC : 0.54 (benzene)

IR (KBr): 3500, 3380, 3120, 1620, 1230, 890 and 760 cm^{-1}

PMR : δ 2.44 (S, 3H, -CH_3), 3.8 (S, 3H, -OCH_3), 5.1 (2H, -NH_2), 6.4-8.0 (m, 7H, Ar*H*), 7.34 (1H, Pyri.*H*), 13.96 (1H, -*OH*)

Mass : 308.2 (m/z) (M+1)

4-(4-Methoxy-2-hydroxyphenyl)-6-(3-methylphenyl)-2-aminopyrimidine (36)

A mixture of 7-methoxy-2-(3-methylphenyl)-4*H*-chromen-4-one **(E36)** (0.5 g, 1.9 mmol), guanidine hydrochloride (0.4 g, 4.1 mmol) and potassium hydroxide (0.2 g, 3.5 mmol) was refluxed in methanol (30 ml) for 12 hrs. After removal of excess methanol the reaction mixture was poured on crushed ice containing acetic acid. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(36)**, (0.13 g, 20 %) m.p. 180-82 °C

Anal:

TLC : 0.52 (benzene)

IR (KBr): 3490, 3395, 3120, 1630, 1230, 890 and 760 cm^{-1}

PMR : δ 2.45 (S, 3H, -CH_3), 3.7 (S, 3H, -OCH_3), 5.94 (2H, -NH_2)6.4-8.0 (m, 7H, Ar*H*), 7.34 (1H, Pyri.*H*), 14.21 (1H, -*OH*)

Mass : 308.2 (m/z) (M+1)

4-(4-Methoxy-2-hydroxyphenyl)-6-(4-nitrophenyl)-2-aminopyrimidine (37)

A mixture of 7-methoxy-2-(4-nitrophenyl)-4*H*-chromen-4-one **(E37)** (0.5 g, 1.6 mmol), guanidine hydrochloride (0.3 g, 3.2 mmol) and potassium hydroxide (0.2 g, 4 mmol) was refluxed in methanol (30 ml) for 12 hrs. After removal of excess methanol the reaction mixture was poured on crushed ice containing acetic acid. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(37)**, (0.2 g, 35 %) m.p. 300< °C

Anal:

TLC : 0.49 (benzene)

IR (KBr): 3490, 3310, 3120, 1635, 1230, 890 and 760 cm^{-1}

PMR : δ 3.7 (S, 3H, -O***CH₃***), 5.94 (2H, -***NH₂***), 7.5 (1H, Pyri.***H***), 6.4-8.03 (7H, Ar***H***), 14.12 (1H, -***OH***)

Mass : 384.2 (m/z) (M+1)

4-(5-Methoxy-2-hydroxyphenyl)-6-(4-methoxyphenyl)-2-aminopyrimidine (38)

A mixture of 6-methoxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one **(E38)** (0.5 g, 1.7 mmol), guanidine hydrochloride (0.3 g, 3.1 mmol) and potassium hydroxide (0.2 g, 1.5 mmol) was refluxed in methanol (30 ml) for 12 hrs. After removal of excess methanol the reaction mixture was poured on crushed ice containing acetic acid. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(38)**, (0.17 g, 29 %) m.p. 184-86 °C

Anal:

TLC : 0.56 (benzene)

IR (KBr): 3519, 3367, 3120, 1600, 1230, 890 and 760 cm^{-1}

PMR : δ 3.8 (S, 3H, -O***CH₃***), 3.9 (S, 3H, -O***CH₃***) 5.1 (2H, -***NH₂***), 7.35 (1H, Pyri***H***), 6.9-7.8 (7H, Ar***H***), 13.16 (1H, -***OH***)

Mass : 324.2 (m/z) (M+1)

4-(5-Methoxy-2-hydroxyphenyl)-6-(3-methoxyphenyl)-2-aminopyrimidine (39)

A mixture of 6-methoxy-2-(3-methoxyphenyl)-4*H*-chromen-4-one **(E39)** (1 g, 3.5 mmol), guanidine hydrochloride (0.8 g, 8.3 mmol) and potassium hydroxide (0.6 g, 1.5 mmol) was refluxed in methanol (30 ml) for 12 hrs. The work up as per described for **(27)**. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(39)**, (0.6 g, 54 %) m.p. 158-60 °C

Anal:

TLC : 0.49 (benzene)

IR (KBr): 3430, 3320, 3220, 1598, 1230, 890 and 760 cm^{-1}

PMR : δ 3.8 (S, 3H, -O***CH₃***), 4.1 (S, 3H, -O***CH₃***) 5.1 (2H, -***NH₂***), 7.36 (1H, Pyri***H***), 6.9-7.8 (7H, Ar***H***), 13.19 (1H, -***OH***)

Mass : 324.2 (m/z) (M+1)

4-(5-Methoxy-2-hydroxyphenyl)-6-(2-chlorophenyl)-2-aminopyrimidine (40)

A mixture of 6-methoxy-2-(2-chlorophenyl)-4*H*-chromen-4-one **(E40)** (1 g, 3.4 mmol), guanidine hydrochloride (0.7 g, 7.0 mmol) and potassium hydroxide (0.8 g, 1.4 mmol) was refluxed in methanol (30 ml) for 12 hrs. After removal of excess methanol the reaction mixture was poured on crushed ice containing acetic acid. The solid so obtained was filtered, washed with water and recrystallised from to yield the title product **(40)**, (0.6 g, 52 %) m.p. 163-65 °C

Anal:

TLC : 0.52 (benzene)

IR (KBr): 3480, 3310, 3110, 1650, 1230, 890 and 760 cm^{-1}

PMR : δ 3.8 (S, 3H, -O***CH₃***), 5.2 (2H, -***NH₂***), 7.39 (1H, Pyri***H***), 6.9-7.9 (7H, Ar***H***), 13.09 (1H, -***OH***)

Mass : 328.2 (m/z) (M+1)

4-(5-Methoxy-2-hydroxyphenyl)-6-(3-chlorophenyl)-2-aminopyrimidine (41)

A mixture of 6-methoxy-2-(3-chlorophenyl)-4*H*-chromen-4-one **(E41)** (1.5 g, 5.3 mmol), guanidine hydrochloride (1 g, 10 mmol) and potassium hydroxide (0.6 g, 10 mmol) was refluxed in methanol (30 ml) for 12 hrs. After removal of excess methanol the reaction mixture was poured on crushed ice containing acetic acid. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(41)**, (1.2 g, 70 %) m.p. 178-79 °C

Anal:

TLC : 0.54 (benzene)

IR (KBr): 3456, 3352, 3110, 1639, 1230, 890 and 760 cm^{-1}

PMR : δ 3.8 (S, 3H, -O***CH***$_3$), 5.2 (2H, -***NH***$_2$), 7.37 (1H, Pyri***H***), 7.0-7.8 (7H, Ar***H***), 13.84 (1H, -***OH***)

Mass : 328.2 (m/z) (M+1)

4-(5-Methoxy-2-hydroxyphenyl)-6-(2,4-dichlorophenyl)-2-aminopyrimidine (42)

A mixture of 6-methoxy-2-(2,4-dichlorophenyl)-4*H*-chromen-4-one **(E42)** (0.5 g, 1.5 mmol), guanidine hydrochloride (0.4 g, 3.1 mmol) and potassium hydroxide (0.4 g, 1.4 mmol) was refluxed in methanol (30 ml) for 12 hrs. After removal of excess methanol the reaction mixture was poured on crushed ice containing acetic acid. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(E42)**, (0.36 g, 64 %) m.p. 243-45 °C

Anal:

TLC : 0.52 (benzene)

IR (KBr): 3450, 3300, 3220, 1620, 1230, 890 and 760 cm^{-1}

PMR : δ 3.7 (S, 3H, -O***CH***$_3$), 6.8 (2H, -***NH***$_2$), 7.33 (1H, Pyri***H***), 6.9-7.8 (7H, Ar***H***), 13.19 (1H, -***OH***)

Mass : 362.2 (m/z) (M+1)

4-(5-Methoxy-2-hydroxyphenyl)-6-(4-fluorophenyl)-2-aminopyrimidine (43)

A mixture of 6-methoxy-2-(4-fluorophenyl)-4*H*-chromen-4-one **(E43)** (0.5 g, 1.8 mmol), guanidine hydrochloride (0.5 g, 4.1 mmol) and potassium hydroxide (0.6 g, 10 mmol) was refluxed in methanol (30 ml) for 12 hrs. After removal of excess methanol the reaction mixture was poured on crushed ice containing acetic acid. The solid so obtained was filtered, washed with water and recrystallised from to yield the title product **(43)**, (0.37 g, 56 %) m.p. 202-03 °C

Anal:

TLC : 0.45 (benzene)

IR (KBr): 3460, 3310, 3220, 1620, 1230, 890 and 760 cm^{-1}

PMR : δ 3.8 (S, 3H, -O***CH₃***), 5.1 (2H, -***NH₂***), 7.34 (1H, Pyri***H***), 6.9-7.8 (7H, Ar***H***), 13.16 (1H, -***OH***)

Mass : 312.2 (m/z) (M+1)

4-(5-Methoxy-2-hydroxyphenyl)-6-furan-2-aminopyrimidine (44)

A mixture of 6-methoxy-2-furan-4*H*-chromen-4-one **(E44)** (1 g, 4.1 mmol), guanidine hydrochloride (0.8 g, 8.3 mmol) and potassium hydroxide (0.6 g, 1.0 mmol) was refluxed in methanol (30 ml) for 12 hrs. The work up as per described for **(27)**. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(44)**, (0.5 g, 48 %) m.p. 216-217 °C

Anal:

TLC : 0.51 (benzene)

IR (KBr): 3410, 3300, 3220, 1652, 1230, 890 and 760 cm^{-1}

PMR : δ 3.8 (S, 3H, -O***CH₃***), 5.1 (2H, -***NH₂***), 7.34 (1H, Pyri***H***), 6.5-7.4 (3H, ***furan***), 6.9-7.8 (3H, Ar***H***).13.19 (1H, -***OH***)

Mass : 284.2 (m/z) (M+1)

5.2 Biological Work

Anti Platelet activity

The synthesized compounds were studied for their *ex-vivo* platelet aggregation inhibitory activity on whole human blood. This study was performed using Whole Blood Aggregometer, Chronolog Corporation, Haverton, PA, USA. The inhibitory activity of nineteen compounds was measured to compare with inhibition induced by standard drug Aspirin (ASA) (10 μg/ml). Each assay was performed three times, every time taking control and aspirin for comparative assay. Whole blood aggregometer measures inhibition of platelet aggregation induced by ADP (10 μL) in the ohms which is resistance produce by accumulation of agggrgates on the electrode.

General procedure for Antiplatlet studies

Blood was collected from healthy human volunteers and treated with 3.4% of trisodium citrate solution. The inhibition of ADP induced platelet aggregation was measured in ohms. Aspirin was taken as standard drug. In siliconised cuvette 0.5 ml saline and 0.5 ml blood was incubated for 5 min. Zero reading of aggregation was set, 10 μl of ADP was added as aggregation inducer and the impedance produced was measured. The inhibition produced by inhibitor was measured comparison with Aspirin at same dose. Test compound **(27-43)** was incubated at 37°C for 10 min before the aggregation inducer was added, and aggregation was recorded for 6 min. Maximum intensity of aggregation will be quantified as the maximum change in electronic impedance in samples without the drug or a given concentration of each drug for blank and test readings respectively. The results were described in the following **Table 27**.

Compound No.	Concentration (μg/ml)	Inhibition of platelet aggregation in Ohms	Compound No.	Concentration (μg/ml)	Inhibition of platelet aggregation in Ohms
Aspirin	10	3-4 (avg.4.2)	29	10	8.4
38	10	**3.7**	30	10	**2.2**

39	10	11.2	31	10	**1**
40	10	**2.4**	32	10	11.6
41	10	12.5	33	10	**0.6**
42	10	8.4	34	10	**1.6**
43	10	12.7	35	10	**4.9**
27	10	6.3	37	10	6.9
28	10	10.2			

Table 27. Inhibition of platelet aggregation in ohms.