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Synthesis and *in-vitro* Anti-platelet aggregation activity of some New substituted Thiophenes

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ABSTRACT

***Plan**: To synthesize some novel 2-amino thiophenes with various substitutions at 2-amino position for anti-platelet aggregation activity.*

***Preface**: Various substituted and condensed thiophenes are reported to possess a wide variety of biological and pharmacological activities such as antibacterial, antifungal, anti-inflammatory, anti-platelet aggregation activity, antipyretic, antitumor and so on. Thus a series of new thiophenes have synthesized with various substituents at 2-amino position and screened for anti-platelet aggregation activity.*

***Methodology**: The starting material (JMS-2) was prepared by the application of versatile Gewald reaction. It was then derivatized to various Schiff bases JMS-2(a-m) by reacting with various substituted aromatic aldehydes. The synthesized new compounds were characterized by MP, TLC, IR, NMR and Mass spectra and were screened for their In-vitro anti-platelet aggregation activity by GVR Born method using Heparin as the standard.*

***Outcome**: Compound JMS-2a, JMS-2b, JMS-2d and JMS-2i showed good % inhibition and were found to be more significant.*

***Keywords**: 2-amino thiophene, Gewald reaction, anti-platelet aggregation.*

INTRODUCTION

Pharmaceutical chemistry is one of the front line field of chemical sciences in the modern world as scientists all over engage in keen research to find out better drugs to combat diseases of mankind. The approach to practice medicinal chemistry has developed from an empirical one, which involves organic synthesis of new compounds, largely based on modification of structures of known activity.

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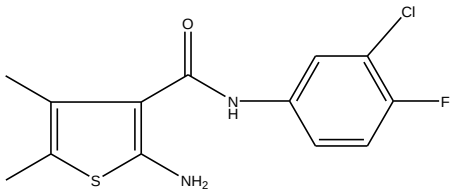
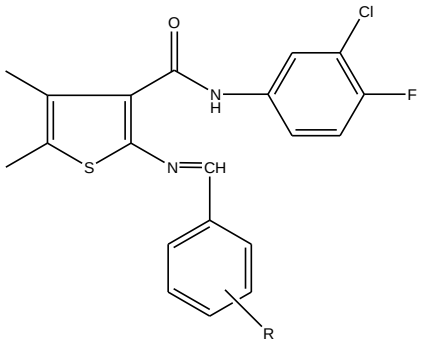
Generally many drugs are obtained from plant and animals, but most drugs used in modern medicine are products of advances in synthetic organic chemistry and biotechnology.

Thiophene containing organic compounds forms a significant group of drugs which exhibit an array of biological activities ranging from anti-platelet aggregation activity[1], anti-inflammatory[2-4], antioxidant[5], analgesic[6], antibacterial[7], antifungal[8], anti-neoplastic, local anaesthetic, antiarthritic, antitussive and so on.The starting material 2-amino-3-(3-chloro-4-fluorophenyl carboxamido)-4,5 dimethyl thiophene (JMS-2) was prepared by the application of the versatile Gewald reaction[9-11]. Treatment of starting material with various substituted aromatic aldehydes gave the title compounds JMS-2(a-m).

All the synthesized compounds were characterized by their physical and spectral data. The IR spectra of compound JMS-2 showed an intense sharp NH_2 absorption peak at 3422.37 cm^{-2}. The formation of Schiff's bases JMS-2 (a-m) was confirmed by the presence of an imine (HC=N) peak at 1670.55-1634.59 cm^{-1} and the absence of NH_2 peak which was present in the IR spectra of JMS-2. The ^{1}NMR spectra of compound JMS-2a, JMS-2f, JMS-2h exhibited all the expected protons. Mass spectra of compound JMS-2l exhibited M^+ ion peak at 476.95 indicating that this molecule is rather unstable at 70eV and undergo fragmentation to form daughter ions. Appearance of M^+ ion and their characteristic daughter ions confirm the structure proposed for the compounds.



(JMS-2)



JMS-2(a-m)

EXPERIMENTAL

Drugs and Chemicals

Ethylcyanoacetate (Sisco Research Laboratories Pvt. Ltd., India), n-butanone (Sisco Research Laboratories Pvt. Ltd., India), Sulphur (SD Fine Chem, India). The Adenosine 5'-diphosphate, Standard Heparin, solvents and other chemical used for the study were of analytical grade and purchased from local firms.

Procedure

Step 1: Synthesis of 3-Chloro-4-fluoro cyanoacetanilide (JMS-1).

A mixture of 3-chloro-4-fluoro aniline (0.5M, 36.35gm) and Ethyl cyanoacetate (0.5M, 26.60ml) was heated at 160^0-170^0c for 6 hours. The reaction mixture was left at room temperature over night. The solid obtained was washed with ethanol, dried and then recrystallized from suitable solvent, from acetone water mixture (5:1). Yield: 55.70 %. M.P. 172 ºC.

Step 2: Synthesis of 2-Cyano-2-(isobutylidene)-3-chloro-4-fluorocarboxanilide.

A mixture of JMS-1 (8.48 gm, 0.04M), n-butanone (3.59ml, 0.04M), ammonium acetate (2 gm) and glacial acetic acid (2 ml) in benzene (150 ml) was refluxed for 8 hrs using Dean stark apparatus with an arrangement for continuous separation of water. After 8 hrs, the reaction mixture was cooled, diluted with 20 ml of benzene and washed 3 times with sodium carbonate solution (10 % w/v in water) and water successively. The solvent was removed under vacuum and the intermediate crude product obtained was immediately processed for the next step.

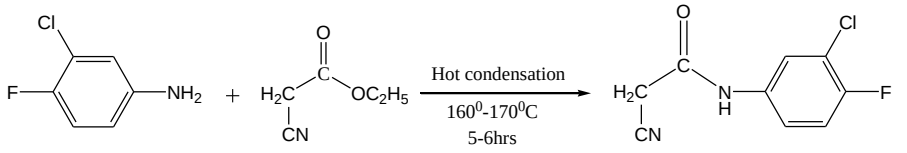
Step 3- Synthesis of 2-amino-3-(3-chloro-4-fluorophenyl carboxamido)-4,5-dimethyl thiophene

(JMS-2): A mixture of 2-Cyano-2-(isobutylidene)-3-chloro-4-fluorocarboxanilide, Sulphur (1.28gm, 0.04 Mol) and Ethanol (30 ml) was taken in conical flask. The above mixture was stirred at 45-50ºC. Once the temperature was attained, Diethyl amine (4ml) was added drop wise until Sulphur completely went in. The solid obtained was filtered, washed with ethanol and recrystallized from benzene. Yield: 74.6 %, M.P. 138 ºC.

Step-4: General method for the syntheses of 2-substituted benzylidene imino-3-carboxamido-4,5-dimethyl thiophenes JMS-2(a-m):

A mixture of the starting compound (JMS-2) (0.005 Mol) and the required aryl aldehydes (a-m) (0.005 Mol) in isopropyl alcohol (10 ml) and catalytic amount of glacial acetic acid (2 ml) was subjected to Microwave irradiation for 2-4 minutes. Then the reaction mixture was cooled to room temperature. The solid separated was filtered, washed with isopropyl alcohol and recrystallized with DMF, Ethanol mixture (6:1).

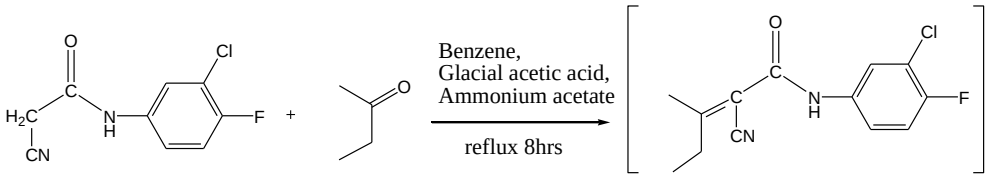
Scheme: Step-1



3-Chloro-4-Fluoro aniline Ethyl cyano acetate 3-Chloro-4-Fluoro cyanoacetanilide

JMS-1

Step 2:

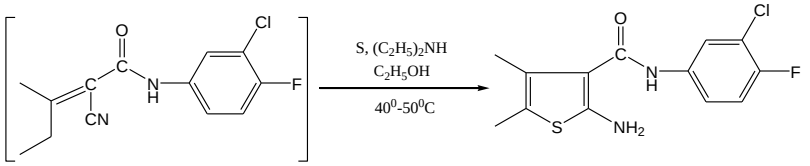


3-Chloro-4-Fluoro cyanoacetanilide JMS-1

n-Butanone

2-Cyano-2-(isobutylidene)-3-chloro-4-fluorocarboxanilide

Step 3:

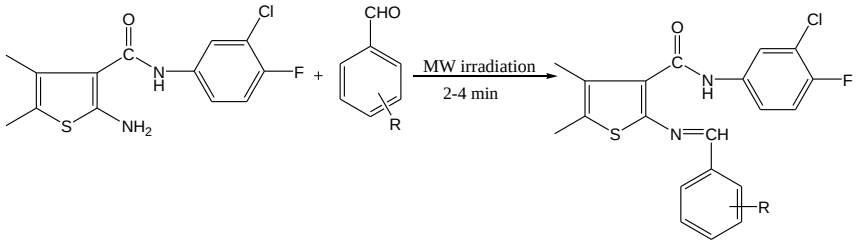


2-Cyano-2-(isobutylidene)-3-chloro-4-fluorocarboxanilide

2-amino-3-(3-chloro-4-fluorophenyl carboxamido)-4,5-dimethyl thiophene

JMS-2

Step-4:



JMS-2
2-amino-3(3-chloro-4-fluorophenyl carboxamido)-4,5-dimethyl thiophene

a-m
various substitued aromatic aldehydes

JMS-2(a-m)

	R		R		R		R
a	2-chloro	e	4-methyl	h	4-dimethyl amino	k	3,4-dimethoxy
b	4-chloro	f	4-methoxy	i	4-hydroxy	l	3,4,5-trimethoxy
c	2-nitro	g	4-hydroxy-3 methoxy	j	2-hydroxy	m	H
d	3-nitro	-	-	-	-	-	-

Biological activity:

In-vitro anti-platelet aggregation activity[12-14]

The synthesized compounds were screened for *in-vitro* anti-platelet aggregation activity by GVR Born method[12], measuring the ADP-induced platelet aggregation inhibitory activity on human blood platelets by ELISA plate reader. The % inhibition of platelets and IC_{50} of the synthesized compounds were measured and compared with the standard reference drug Heparin.

Preparation of Platelet Rich Plasma (PRP):

Blood was collected from the cubital vein of healthy male volunteers into a plastic syringe containing 3.8% sodium citrate (9:1). The citrated blood was centrifuged at 800 rpm for 10 min to obtain platelet-rich plasma (PRP).

Preparation of ADP:

Adenosine 5'-diphosphate was dissolved in DMSO to get a concentration of 2.5μM/5μl.

Preparation of test solutions:

Each test compound was dissolved in DMSO to get a concentration of 30, 50, 80 &100 μg/ml. This concentration was used for testing antiplatelet aggregation activity.

Preparation of standard solution:

Heparin was dissolved in DMSO to get a concentration of 30, 80 &100 μg/ml. This concentration was used for testing antiplatelet aggregation activity.

Procedure:

The Platelet-Rich Plasma (PRP) was obtained from citrated blood. 250 μL of Platelet-Rich Plasma (PRP) were distributed in the test cuvettes and inserted in incubation chamber at 37^0C for 2 min. Platelet aggregation was measured using ELISA plate reader at 520nm by 2.5 μM ADP according to Born. The test compounds were dissolved in DMSO (at 0.01% final concentration) and added to the PRP, 2 min before activation with ADP. The extent of aggregation was quantified by determining the maximum height of the curve, when compared with standard as heparin. The platelet aggregation inhibitory activity was expressed as percent inhibition by comparison with that measured in presence of vehicle (DMSO) alone. The platelet aggregation inhibitory activity of test compounds was expressed as IC_{50} values.

Procedure for determining the IC_{50} value:

The percent inhibition values of platelet aggregation were plotted against concentration and linear regression equation was obtained. IC_{50} values were obtained from the linear regression equation. By definition, IC_{50} is the concentration of the test compounds required which produces 50% inhibition of ADP-induced platelet aggregation:

Percentage inhibition was calculated by using the formula,

$$\% \text{ inhibition} = \frac{A - B}{B} \times 100$$

Where, A= maximal aggregation of the control.
B = maximal aggregation of the PRP-treated sample.
The IC_{50} value was calculated by using the formula,

$$y = mx+c.$$

RESULTS AND DISCUSSION

Physical data

Melting points were determined in open capillaries and are uncorrected. Purity of the compounds was checked by TLC on silica gel plates. The solvent system used to carry out the TLC is Benzene: Chloroform at a ratio of 7:3. The physical data are reported in Table-1.

Table-1: Physical data of the compounds prepared

Compound	*Molecular formula*	*M.W. (gm)*	*M.P. (^oC)*	*R_f Value*	*Yield (%)*
JMS-2	$C_{13}H_{12}N_2OSClF$	298	138	0.63	74.6
JMS-2a	$C_{20}H_{15}N_2OSCl_2F$	421	164	0.76	64.47
JMS-2b	$C_{20}H_{15}N_2OSCl_2F$	421	152	0.84	58.57
JMS-2c	$C_{20}H_{15}N_3O_3SClF$	431	186	0.80	67.42
JMS-2d	$C_{20}H_{15}N_3O_3SClF$	431	178	0.86	62.28
JMS-2e	$C_{21}H_{18}N_2OSClF$	400	194	0.76	55.34
JMS-2f	$C_{21}H_{18}N_2O_2SClF$	416	159	0.78	72.56
JMS-2g	$C_{21}H_{15}N_2OSCl_2F$	432	203	0.93	54.48
JMS-2h	$C_{22}H_{21}N_3OSClF$	429	183	0.94	68.75
JMS-2i	$C_{20}H_{16}N_2O_2SClF$	402	215	0.88	53.68
JMS-2j	$C_{20}H_{16}N_2O_2SClF$	402	197	0.74	67.24
JMS-2k	$C_{22}H_{20}N_2O_3SClF$	446	189	0.94	59.42
JMS-2l	$C_{23}H_{22}N_2O_4SClF$	476	148	0.88	70.82
JMS-2m	$C_{20}H_{16}N_2OSClF$	386	172	0.74	68.34

Spectral data

IR spectra (cm^{-1}) were recorded in KBr on a Shimadzu FTIR-8700 spectrometer.^{1}H NMR (ppm) in $CDCl_3$ using TMS as reference on Bruker 400 AMX. Mass spectra of the compound coded JMS-2-l was carried out.

IR (KBr) cm^{-1}:

JMS-2: 3422.37 (-NH_2); 3309.98 (NH); 3069.82 (Ar-CH); 2918.78 (Ali-CH); 1653.73 (C=O); 1638.33 (-NH bend); 1522.47 (Ar-C=C); 1216.34 (C-F).
JMS-2a: 3246.62(-NH-st); 3068.48 (Ar-CH); 2919.99 (Ali-CH); 1681.82 (C=O); 1654.22 (C=N); 1636.44 (-NH bend); 1582.57 (Ar C=C); 1218.63(C-F); 845.57 (C-N); 729.63 (C-Cl); 753.71 (C-S).
JMS-2b: 3270.14(-NH-st); 3067.97 (Ar-CH); 2918.70 (Ali-CH); 1679.14(C=O); 1661.94 (C=N); 1623.67 (-NH bend); 1577.88 (Ar C=C); 1214.86 (C-F); 818.77 (C-N); 719.61(C-Cl); 769.31 (C-S).
JMS-2c: 3265.70 (-NH-st); 3081.97 (Ar-CH); 2915.01 (Ali-CH); 1669.86(C=O); 1648.09 (C=N); 1618.13 (-NH bend); 1538.40 (Ar C=C); 1217.13 (C-F); 818.05 (C-N); 720.06(C-Cl); 744.17 (C-S); 1457.99 (N=O of NO_2).
JMS-2d: 3310.64(-NH-st); 3080.23 (Ar-CH); 2940.79 (Ali-CH); 1674.37 (C=O); 1652.22 (C=N); 1624.87 (-NH bend); 1537.91 (Ar C=C); 1219.52 (C-F); 819.99 (C-N); 731.70(C-Cl); 750.07 (C-S); 1467.09 (N=O of NO_2).
JMS-2e: 3325.78(-NH-st); 3078.74 (Ar-CH); 2930.29 (Ali-CH); 1669.98 (C=O); 1652.09 (C=N); 1621.98 (-NH bend); 1531.72 (Ar C=C); 1219.03 (C-F); 818.62 (C-N); 697.88 (C-Cl); 759.86 (C-S).
JMS-2f: 3226.87(-NH-st); 3056.23 (Ar-CH); 2927.85 (Ali-CH); 1674.96 (C=O); 1653.42 (C=N); 1635.93 (-NH bend); 1558.77 (Ar C=C); 1218.11(C-F); 826.03 (C-N); 682.15 (C-Cl); 753.31 (C-S); 1259.48 (Ar-C-O of Ar-OCH_3).
JMS-2g: 3413.39(-OH-); 3287.05 (-NH-st); 3085.04(Ar-CH); 2946.32 (Ali-CH);1666.30 (C=O); 1642.18 (C=N); 1624.05 (-NH bend);1577.95 (Ar C=C); 1216.52 (C-F); 818.24(C-N); 725.19 (C-Cl); 756.50 (C-S); 1260.53(Ar-C-O of Ar-OCH_3).
JMS-2i: 3185.82(-NH-st); 3065.79(Ar-CH); 2916.36 (Ali-CH); 1677.49 (C=O); 1670.55 (C=N); 1635.89 (-NH bend); 1578.35 (Ar C=C); 1212.49(C-F); 816.50(C-N); 718.65(C-Cl); 750.33 (C-S); 2853.22 (CH of CH_3).
JMS-2j: 3453.17(-OH-); 3226.98 (-NH-st); 3085.94(Ar-CH); 2926.12 (Ali-CH); 1668.53 (C=O); 1635.33 (C=N); 1621.42 (-NH bend); 1570.70 (C=C); 1213.45(C-F); 832.86 (C-N); 713.13 (C-Cl); 765.81 (C-S).
JMS-2k: 3282.95(-NH-st); 3010.16(Ar-CH); 2930.29 (Ali-CH); 1672.05 (C=O); 1652.68 (C=N); 1625.09 (-NH bend); 1572.39 (Ar C=C); 1218.82 (C-F); 823.85(C-N); 716.53 (C-Cl); 754.83 (C-S); 1256.46 (Ar-c-o of Ar OCH_3).
JMS-2l: 3332.99(-NH-st); 3051.69(Ar-CH); 2939.57 (Ali-CH); 1676.59 (C=O); 1659.72 (C=N); 1620.92 (-NH bend); 1577.82 (Ar C=C); 1211.71(C-F); 818.84(C-N); 720.17(C-Cl); 768.57 (C-S); 1265.24 (Ar-c-o of Ar-OCH_3).**JMS-2m:** 3284.13(-NH-st); 3067.66(Ar-CH); 2921.81 (Ali-CH); 1674.05(C=O); 1660.45 (C=N); 1621.94 (-NH bend); 1563.40 (Ar C=C); 1216.52 (C-F); 818.24(C-N); 725.19 (C-Cl); 756.50 (C-S).

^{1}NMR ($CDCl_3$) δ (ppm)

Compound JMS-2a: 2.39 (s, 3H, CH_3); 2.44 (s, 3H, CH_3); 7.45 (d, 1H, Ar-CH); 7.49 (d, 1H, Ar-CH); 7.50 (d, 1H, Ar-CH); 7.86 (d, 1H, Ar-CH); 7.87 (d, 1H, Ar-CH); 7.87 (t, 1H, Ar-CH); 7.88 (d, 1H, Ar-CH); 8.09 (s, 1H, N=CH); 9.84 (s, 1H, NH).
Compound JMS-2f: 2.34 (s, 3H, CH_3); 2.46 (s, 3H, CH_3); 3.72(s, 3H, OCH_3); 7.42 (d, 1H, Ar-CH); 7.49 (d, 1H, Ar-CH); 7.50 (d, 1H, Ar-CH); 7.50 (d, 1H, Ar-CH); 7.85 (d, 1H, Ar-CH); 7.86 (d, 1H, Ar-CH); 7.87 (d, 1H, Ar-CH); 8.07 (s, 1H, N=CH); 8.98 (s, 1H, NH).
Compound SPJ-1-i: 2.14 (s, 3H, CH_3); 2.16 (s, 3H, CH_3); 2.88 (s, 3H, CH_3); 2.90(s, 3H, CH_3); 7.37 (d, 1H, Ar-CH); 7.37 (d, 1H, Ar-CH); 7.39 (d, 1H, Ar-CH); 7.42 (d, 1H, Ar-CH); 7.43 (d, 1H, Ar-CH); 7.44 (d, 1H, Ar-CH); 7.45 (d, 1H, Ar-CH); 8.04 (s, 1H, N=CH); 9.85 (s, 1H, NH).

In vitro anti-platelet aggregation activity data

Anti-platelet aggregation activity of all the synthesized compounds was carried out by GVR Born method at a concentration of 30, 50, 80, 100µg/ml using DMSO as solvent. The % inhibition IC_{50} was measured, and reported in the Table-2, Fig.1, Fig.2 and Fig.3.

Table-2: *In vitro* anti-platelet aggregation activity data

Comp. Code.	*% inhibition*					*$IC_{50} \pm SEM$*
	30µg	*50µg*	*80µg*	*100µg*	*Mean*	
JMS-2a	50.24905	52.6003	55.92222	57.39471	54.04157	25.92843±1.614***
JMS-2b	48.82615	53.4178	55.21509	56.05615	53.3788	29.98965±1.532***
JMS-2c	37.62086	39.58395	42.72245	44.5968	46.00917	101.3636±1.739**
JMS-2d	50.13185	51.73848	54.50796	55.01143	52.84743	26.30137±1.156***
JMS-2e	41.95722	44.33878	47.17148	52.04048	46.37699	91.83824±2.168**
JMS-2f	35.68708	39.64339	41.75015	42.76853	39.96229	169.8958±1.567*
JMS-2g	35.21828	37.29569	39.95286	40.25465	38.18037	225.2703±1.190*
JMS-2h	37.62086	39.58395	42.72245	44.5968	41.13102	154±1.561*
JMS-2i	47.99297	50.34175	53.77136	55.50114	51.90181	47.68519±1.687***
JMS-2j	41.69352	43.32838	47.58397	49.49396	45.52496	103.7069±1.814**
JMS-2k	36.71257	37.62259	41.72068	44.85798	40.22846	147.395±1.889*
JMS-2l	36.39027	38.24666	42.6046	43.2256	40.11678	159.2381±1.664*
JMS-2m	37.76736	40.87313	43.0312	46.11078	41.94562	136.9526±1.759*
Heparin	56.51919	66.53789	79.11019	87.43062	72.39947	13.87126±1.103***

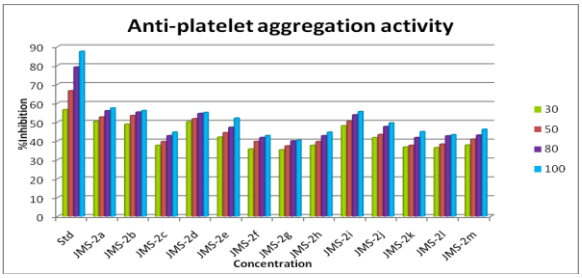


Fig. 1: Graphical representation of in-vitro anti-platelet aggregation activity data

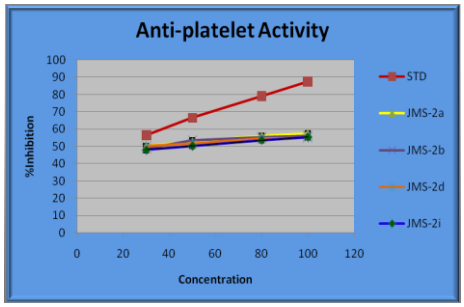


Fig:2

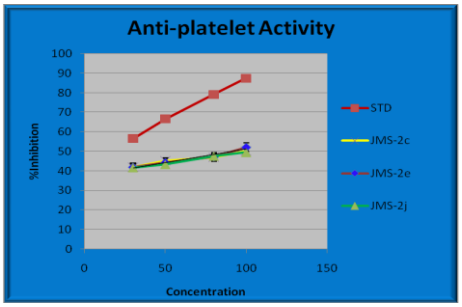


Fig:3

Fig.2: Antiplatelet Activity of JMS-2a, JMS-2b, JMS-2d and JMS-2i by GVR Bron method, which showing good activity when compare to std. Data represented as IC_{50}±SEM.

Fig.3: Antiplatelet Activity of JMS-2c, JMS-2e and JMS-2j by GVR Bron method, which showing moderate activity when compare to std. Data represented as IC_{50}±SEM.

The purpose of the present work was to synthesize a series of desired title compounds (JMS 2-a-m) from 2-amino-3-(3-chloro-4-fluorophenyl carboxamido)-4,5-dimethyl thiophene (JMS-2) by reacting with various substituted aromatic aldehydes (a-m). The syntheses were carried out in accordance with the literature as in the Scheme.

As discussed earlier, thiophenes are a class of heterocyclic compounds that shows an array of biological activities which include antiplatelet aggregation, anti-inflammatory, anti-bacterial, anti-fungal, anti-tubercular, anti-convulsant, anti-cancer, and local anesthetic activity.

The presence of fluorine on a bioactive molecule enhances cell penetration and protein binding. Thus it was felt worthwhile to take up the present investigation to synthesize some novel thiophenes and test their effect on *in-vitro* antiplatelet aggregation activity.

CONCLUSION

In conclusion from the anti-platelet activity results, it was observed that both the electron donating groups and the electron withdrawing groups on the aldehydic phenyl ring of the compounds influenced the activity. The anti-platelet screening results suggest that the test compounds JMS-2a, JMS-2b, JMS-2d and JMS-2i with 2'-cholro, 4'-chloro, 3'-nitro and 4'-hydroxy respectively showed more significant activity. Compounds JMS-2c, JMS-2e, JMS-2j with 2'-nitro, 4'-methyl, 2'-hydroxy respectively showed significant activity. Remaining compounds JMS-2m, JMS-2h, JMS-2l, JMS-2k, JMS-2g and JMS-2f with H, 4'-dimethylamino, 4'-hydroxy,3'-methoxy, 4'-methoxy, 3',4',5',-trimethoxy, 3',4'-dimethoxy, respectively showed mild activity compared to the standard Heparin.

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