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Platelet aggregation inhibition potential of some novel piperidino thiophenes analogs

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ABSTRACT

In an attempt to explore antiplatelet inhibitory potential of newly synthesized compounds, a series of fused thiophene containing Schiff base moiety were prepared via the reaction of ketone, 4-N-methyl piperidone with the isobutylcyanoacetamide using ammonium acetate/glacial acetic acid as an acidic catalyst followed by reaction of starting compounds with substituted aryl aldehydes under microwave radiation. The synthesized compounds were screened for in-vitro anti-platelet aggregation activity by GVR Born method. The results clearly revealed that all these piperidinothiophenes contains anti-platelet aggregation activity when compared with the standard Heparin.

Key words: thienopyridine, antiplatelet, adenosine diphosphate, fused thiophene.

INTRODUCTION

Clopidogrel and related drugs are an oral, thienopyridine-class antiplatelet agent used to inhibit blood clots in coronary artery disease, peripheral vascular disease, cerebrovascular disease, and to prevent myocardial infarction (heart attack) and stroke. The drug works by irreversibly inhibiting a receptor called $P2Y_{12}$, adenosine diphosphate (ADP) chemoreceptor on platelet cell membranes. These molecules are prodrugs, which must be transformed in the body into an active metabolite and reversibly or irreversibly inhibit the process involved in platelet activation resulting in decreased tendency of platelets to adhere to one another and to damaged blood vessels endothelium [1].

Thiophenes are a class of heterocyclic compounds that shows an array of biological activities which include antiplatelet aggregation [1], anti-inflammatory [2-10], anti-bacterial [2-10], anti-fungal [2-10], Antiarrhythmic [14], serotonin antagonist [14], and Intestinal Calcium-Activated Chloride Channel inhibitor [15] to name a few. To explore newly synthesized compounds antiplatelet aggregation potential and continuation of our work on fused thiophenes [2-10,16-17], we report the antiplatelet inhibitory activity of synthesized Schiff's bases of 2-amino-3-N-(isobutyl carboxamido)-6-N-methyl piperidino thiophenes [2].

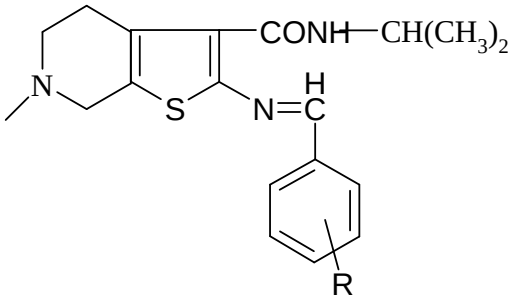

Table 1: Percentage inhibition with increase in concentration of test compounds

Code		Percentage Inhibition					IC_{50} ± SEM
	R	30 µg	50 µg	80 µg	100 µg	Mean	
Th 1	2- chloro	51.21450	53.06782	55.74563	57.25432	54.32057	24.68356± 1.252
Th 2	4- chloro	50.72543	53.76235	55.87620	57.78635	54.53758	26.62626± 1.327
Th 3	2- fluoro	50.75347	53.01726	55.67323	55.89562	53.83490	25.67288± 1.452
Th 4	3- fluoro	51.87643	53.52725	55.52725	56.52726	54.36455	26.52278± 1.428
Th 5	4- fluoro	52.62378	54.67328	56.71818	57.82627	55.46038	23.78261±1.0134
Th 6	H	44.72317	45.78788	46.78369	48.09767	46.34810	67.73828± 1.086
Th 7	2- methyl	45.62788	46.52672	48.05276	49.98622	47.54840	94.52762± 1.425
Th 8	4- methyl	44.78272	45.78326	47.19739	47.67257	46.35899	99.67268±1.562
Th 9	4- hydroxy	42.79863	44.82628	45.96375	46.87262	45.11532	101.78362± 1.23
Th10	4- methoxy	38.28273	39.72829	40.28729	41.62728	39.98140	104.56174±1.722
Th 11	3,4-dimethoxy	36.67780	37.97839	40.62828	40.83727	39.03044	134.67272±1.237
Th 12	3,4,5-trimethoxy	34.62782	35.78762	37.10232	38.86433	36.59552	154.63275±1.273
Heparin		56.8652	67.67368	78.63738	87.53272	72.67725	13.87224±1.102

Pharmacological Screening: *(In-vitro* anti-platelet aggregation activity)
The synthesized compounds were screened for in-vitro anti-platelet aggregation activity following GVR Born method, measuring the ADP-induced platelet aggregation inhibitory activity on human blood platelets by ELISA plate reader. The percentage 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% (w/v) sodium citrate (9:1) as an anticoagulant. Platelet-poor plasma (PPP) was obtained by centrifugation of the remaining blood at 1200 rpm for 15 min. The citrated blood was centrifuged at 800 rpm for 10 min to obtain platelet-rich plasma (PRP). Platelet counts in PRP were adjusted to $3X10^8$ platelets per ml by adding PPP.

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.

Standard solution: Heparin was dissolved in DMSO to get a concentration of 30, 50, 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 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 method. The test compounds were dissolved in DMSO (at 0.01% final concentration) and added to the platelet-rich plasma, 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 IC50 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,

$$\%\ Platelet\ aggregation\ inhibition = \frac{A-B}{B}\ X\ 100$$

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,

$$y = mx + c$$

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-1.

The presence of electron withdrawing elements/ group 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 fused thiophenes and investigate their effect on in-vitro antiplatelet aggregation activity.

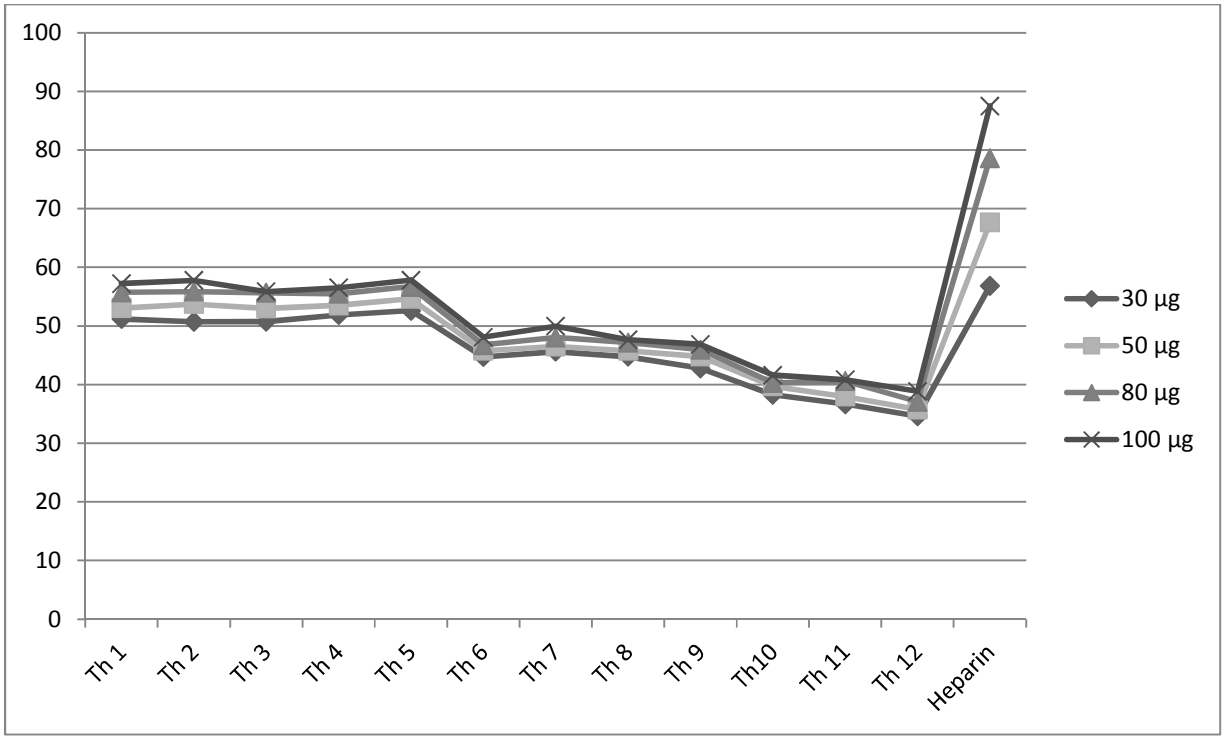

Fig.1- Graphical representation of percentage inhibition of anti-platelet aggregation activity

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 Schiff base phenyl ring of the compounds influenced the activity. The anti-platelet screening results suggest that the test compounds Th1, Th2, Th3, Th4 and Th5 with 2'-cholro, 4'-chloro, 2'-

fluoro, 3'- fluoro, 4'- fluoro groups on benzene ring shown significant activity. Compounds Th6, Th7, Th8 and Th9 with hydrogen, 2'- methyl, 4'- methyl and 4'- hydroxyl as a attachment (R) on benzene ring shown slight lesser activity than compounds with electron withdrawing groups. Remaining compounds Th10, Th11 and Th12 showed mild activity compared to the standard Heparin. All the compounds shown anti platelet aggregation activity that proves the functional groups on Schiff bases have significant effect on pharmacological activity and potency.

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REFERENCES

[1]. P. Savi, J. Combalbert, C. Gaich, MC Rouchon, JP Maffrand, Y. Berger, JM Herbert, *Thromb. Haemost*, **1994**, 72, 313–317.
[2]. B Das , S Srivastava , S. C. Dinda, Y. RajendraPrasad, *Der Pharma Chemica*, **2013**, 5(1):161-166.
[3]. B Das, S Srivastava, S. C. Dinda, Y. RajendraPrasad, *Der Pharma Chemica*, **2013**, 5(2):256-262.
[4]. S. Srivastava, B. Das, Y. RajendraPrasad, *Int. J. Chem. Sci*., **2012**, 10(4), 1999-2009.
[5]. S. Srivastava, B. Das, R. Shukla, Y. Rajendra Prasad, *Asian J. Pharm. Life Sci*., **2012**, 2(2), 234-251.
[6]. S. Srivastava, B. Das, Y. Rajendra Prasad, *Der. Pharm. Let*., **2012**, 4(4), 1214-1220.
[7]. S. Srivastava, B. Das, *Der. Pharm. Chem*., **2011**, 3(6), 103-111.
[8]. S. Srivastava, B. Das, *Int. J. Chem. Sci*., **2009**, 7(3), 1779-1783.
[9]. S. Srivastava, B. Das, M. Raghuprasad, S. Mohan, *Asian J Chem*, **2007**, 19(4), 2813-2817. [10]. B. Das, S. Srivastava, J. Sarvanan, S. Mohan, *Asian J. Chem*., **2007**, 19(5), 4118-20.
[11]. G V R Born, *Nature*, **1962**; 194, 927-9.
[12]. R. Amoroso, A Ammazzalorso, M. Baraldi, G. Bettoni, D. Braghiroli, *Euro J Org Chem*. **2005,** 40: 918–21.
[13]. V. Koleckar, E. Brojerova, Z Rehakova, K. Kubikova, F. Cervenka, K. Kuca, D. Jun, M. Hronek, V. Opletalova, L. Opletal, *Drug Chem Toxicol*. **2008**, 31(1), 27-35.
[14]. A.G.E. Amra, M.H. Sherif, M.G. Assy, M.A. Al-Omar, I. Ragab, *Eur. J. Med. Chem*., **2012**, 45, 5935- 42.
[15]. R.D.L. Fuente, W. Namkung, R. Mills, A.S.Verkman, *Mol. Pharm*., **2008**, 73, 758–68.
[16]. T. B. Hadda , S. Srivastava , B. Das , H. S. Zamora , *Medicinal Chemistry Research*, **2014**, 23 (2), 995-1003.
[17]. T. B. Hadda , M. Ahmad, , S. Sultana, U. Shaheen, , A. Bader, ,S. Srivastava, B. Das, *Medicinal Chemistry Research*, **2014**, 23(1), 16-24.