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IN VITRO AND *EX VIVO* ANTI-CLOTTING ACTIVITY OF FEW 1,3,4-OXADIZOLE DERIVATIVES

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ABSTRACT

Oxadiazole is thought to be produced from furan by substituting two pyridine type nitrogen (-N=) groups for the methane (-CH=) group. Compounds with the 1,3,4-oxadizole nucleus have been tested for anti-inflammatory, analgesic, antithrombotic, anticancer, anticonvulsant, antioxidant, and herbicidal properties. These include factors V, VII, VIII, XI, and XIII. Both the extrinsic and intrinsic pathways converge at this point. The prothrombin time is a clinical indicator of the extrinsic pathway. The liver is one of the organs involved in the coagulation process. Factors I, II, V, VII, VIII, IX, X, XI, and protein C and S are all produced in the liver. Fresh frozen plasma is used to treat coagulopathy in patients with liver disease. 500ul of streptokinase (30000IU) was used as a positive thrombolytic, and 100ul of PBS as a negative control. The order of the in vitro clot lysis effect of all investigated compounds is 3i>SK>3e>3a>3h>3g>3b>1>3c, which is consistent with the results of the previous studies.

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INTRODUCTION

Hetero compounds with a five-membered oxadiazole nucleus have a wide range of applications. It depicts a wide range of biological activity [1]. Oxadiazole is thought to be produced from furan by substituting two pyridine type nitrogen (-N=) groups for the methane (-CH=) group. Oxadiazoles are five-membered cyclic molecules containing one oxygen and two nitrogen atoms [2-3].

Compounds having the 1,3,4-oxadizole nucleus have been tested for anti-inflammatory, analgesic, antithrombotic, anticancer, anticonvulsant, anthelmintic, anticonvulsant, antithrombotic, antioxidant, and herbicidal properties [4-5].

Vitamin C is often obtained from nicotinic acid. Nicotinic acid is also used to treat niacin deficiency and to enhance the immune system (pellagra).

The coagulation route is a series of processes that leads to the formation of hemostasias. Rapid healing and the prevention of spontaneous bleeding are made possible by the complicated pathway. Intrinsic and extrinsic pathways diverge but converge at a precise point to result in fibrin activation. The goal is to use a fibrin mesh to stabilize the platelet plug at the end [6].

The coagulation pathway's job is to maintain hemostasis, or the stopping of bleeding or haemorrhaging. Platelets clump together and

form a plug at the injured site of exposed endothelial cells in primary hemostasis. The two primary coagulation pathways, intrinsic and extrinsic, intersect at a site to produce the common pathway in secondary hemostasis. Fibrinogen is activated into fibrin by the common route. These fibrin subunits have a strong affinity for one another and join together to form fibrin strands that bind platelets together and stabilise the platelet clog.

The process of coagulation that allows for hemostasis is a complex one that involves a number of clotting components. Factors I, II, IX, X, XI, and XII make up the intrinsic route. Fibrinogen, prothrombin, Christmas factor, Stuart-Prower factor, plasma thromboplastin, and Hageman factor are the names given to each of them. Factors I, II, VII, and X make up the extrinsic route. Factor VII is referred to as a steady factor. Factors I, II, V, VIII, and X make up the common route. The factors are activated into serine proteases as they circulate through the bloodstream as zymogens. These serine proteases operate as a catalyst, cleaving the next zymogen into more serine proteases and activating fibrinogen in the process [7].

Intrinsic Pathway

This is the more complicated secondary hemostasis pathway. After contact to endothelial collagen, Factor XII (a zymogen, inactivated serine protease) becomes Factor XIIA (activated serine protease). When endothelial damage occurs, endothelial collagen is revealed. Factor XIIA is a catalyst that converts factor XI into Factor XIA. After that, factor XIA activates factor IX to factor IXA. Factor IXA then acts as a catalyst in the conversion of factor X to factor Xa.

When either the intrinsic or extrinsic pathways activate factor II, it can strengthen the intrinsic pathway by providing positive feedback to factors V, VII, VIII, XI, and XIII. This makes factor XII less important; patients can clot normally without it. The partial thromboplastin time is a clinical marker of the intrinsic pathway (PTT)[8].

Extrinsic Route

The extrinsic pathway of secondary hemostasis is the quickest. After the vessel has been damaged, endothelial cells release tissue factor, which activates factor VII to factor VIIa. Factor VIIa then activates factor X, transforming it into factor Xa. Both the extrinsic and intrinsic pathways converge at this point. The prothrombin time is a clinical indicator of the extrinsic route (PT) [9].

Common Pathway

This process starts with factor X, which then becomes factor Xa. The activation of factor Xa is a difficult process. The complex that cleaves factor X into factor Xa is called tenase. Extrinsic tenase contains factor VII, factor III (tissue factor), and Ca2+, whereas intrinsic tenase contains cofactor factor VIII, factor IXA, a phospholipid, and Ca2+.It converts factor II (prothrombin) into factor IIa once activated to factor Xa (thrombin). In order for factor Xa to cleave prothrombin into thrombin, it needs factor V as a cofactor. Factor IIa (thrombin) then activates fibrinogen, converting it to fibrin. Thrombin also activates the intrinsic pathway's other factors (factor XI), as well as cofactors V and VIII and factor XIII. Factor XIII operates on fibrin strands to produce a fibrin mesh, and fibrin subunits come together to make fibrin strands. This mesh aids in the stabilisation of the platelet plug.

Negative Feedback

There are systems that maintain the coagulation cascade in control to prevent over-coagulation, which causes widespread thrombosis. Because thrombin is a procoagulant, it creates a negative feedback loop by converting plasminogen to plasmin and increasing antithrombin synthesis (AT). Plasmin breaks down the fibrin mesh by acting directly on it. AT reduces the quantity of activated factor X and reduces the synthesis of thrombin from prothrombin. Protein C and S also work to inhibit coagulation by inactivating factors V and VIII, respectively.

Organs That Are Involved in coagulation

The liver is one of the organs involved in the coagulation process. Factors I, II, V, VII, VIII, IX, X, XI, XIII, and protein C and S are all produced in the liver. The vascular endothelium produces factor VII.

Hepatic pathology can result in a deficiency of coagulation factors, which can lead to haemorrhage. A drop in coagulation factors usually indicates serious liver injury. Because Factor VII has the shortest half-life, it is the first to cause high PT in liver illness. The INR can be higher than 6.5. (Normal is close to 1.0). Fresh frozen plasma is used to treat coagulopathy in patients with liver disease.

MATERIAL AND METHODS

A method for making an oxadiazole deri vative from nicotinic acid is described below. M any of the compounds are supplied by a Mumba i-based research lab.

The data are uncorrected and the melting point i sdetermined using the open tube capillary techni que. TLC was carried out on silica G plates and observed in a UV or iodine chamber.

Synthesis of substituted carbohydrazide

Sulphuric acid (38ml) was progressively added t o nicotinic acid (0.1mol) in 100 percent ethanol while stirring (36ml). It was heated on a steam bath for 7hours, then cold on ice and poured over crushed ice while stirring.

The mixture was extracted with 25ml diethyl eth er after the pH was adjusted to 7 with 10% NaH CO3.By drying the mixed ethereal extracts on a nhydrous MgSO4, the solvent was removed. In 100% ethanol, solution (1) was combined with 9 8 percent hydrazine hydrate (7.5gm) (40ml). All ow it to cool after refluxing for 7 hours. After th at, enough icecold water was added to dilute the concoction. Filtered, rinsed with ice cold water, dried, and recrystallized with ethanol, a white pr ecipitate was formed [10].

Synthesis of 5- substituted 1,3,4-oxadiazole-2-thione

In 47mL of 1,4 dioxane, the mixture (2) was dissolved. The mixture was then added to 1. 25 grammes of carbon disulphide and refluxed u ntil S2H gas escaped at room temperature. The white substance was diluted with water and filte red before being recrystallized with ethanol.

Synthesis of N-Substituted{[5-(pyridine-3-yl)-1,3,4-oxadiazole-2-yl] sulfanyl} (3a-3i).

To the resulting mixture, equimolar amo unts of aniline were added(0.005mole). Dissolve in ethanol (0.025mole). Reflux for 4-5 hours in a cooled-to-room temperature water bath before neutralising with ice cold water. It should be filtered, washed in cold water, and re-crystallized in ethanol.

BIOLOGICAL EVALUATION

Clot lysis study:

A sample of blood was taken from a healthy goa tslaughterhouse (Caprahircus). As per protocol, t he process was carried out at room temperature within 3 hours of the sample collection. Prepare phosphate buffer saline (PBS) with a 7.4 pH cap sule as a reference, and test chemicals at 6.25u M, 12.5uM, and 25uM conc. Remove 30000 IU streptokinase equivalent as a standard to verify a ctivity from lyophilised streptokinase (150000 I U) produced with PBS and blended properly. 50 0ul blood was put to each eppendorf tube, which had already been weighed and labelled, and inc ubated at 37°C for 45 minutes for clotting.

After the clot had formed, the serum was withdrawn and each tube weighed again to calc ulate the clot weight. Fill designated tubes with clot after diluting chemicals with 100ul and with drawing around 6.25, 12.5, and 25 uM for assess ment. As a positive thrombolytic, 100ul of strept okinase (30000IU) was used, while 100ul of PB S was used as a negative thrombolytic control. All tubes were reincubated at 37°C for at least 9 0 minutes, after which the fluid was discarded a nd each tube was weighed separately before and after clot lysis and expressed as a percentage of clot lysis [11].

The Institutional Animal Ethics Committee examined and approved all animal experimental procedures and protocols used in the study. The experimental methodologies and protocols followed the recommendations established by the CPCSEA, Ministry of Forests and Environment, Government of India. The animals were procured from the Shriji Farms

Bhandara, India, and were housed in colony cages at a temperature of 25 2°C and a relative humidity of 45-55 percent, with a 12-hour light/dark cycle. They were fed conventional animal feed. All animals were acclimated for a week prior to use. Animals were fasted for 12 hours previous to the experiment and given only water ad libitum. Acute toxicity tests were conducted to determine the median lethal dose (LD50) of the synthesized compounds 3a-3q in accordance with OECD recommendations (TG 420), and the testing dose for the newly synthesized compounds was fixed for ex vivo anticoagulant activity. The LD50 values for the 1, 3, 4-oxadiazoles were calculated using the approach described before.

The first group received 0.5 percent carboxy methyl cellulose orally as a control. The second and third groups served as positive controls, with the second group receiving 1 mg kg The LD50 values for the 1, 3, 4-oxadiazoles were calculated using the approach described before. -1 Acenocoumarol was administered orally as a reference standard anticoagulant as a positive control for an increase in PT. The third group received 500 IU kg-1 unfractionated heparin intraperitoneally as a positive control for an increase in aPTT. The test chemicals were suspended in 0.5 percent carboxy methyl cellulose (CMC) and orally administered to rats at a concentration of 25 mg kg-1. After six hours of administration of the test compounds and reference standard, the animals were anaesthetized with 60 mg kg-1 Thiopental sodium (Intraval sodium® Piramal Healthcare), the caudal caval vein was exposed with a mid-line incision, and 1.8 mL blood was collected into a plastic syringe containing 0.2 mL 100 mM citrate buffer pH 4.5. The blood sample was immediately agitated and centrifuged at 1500 g for 10 minutes in a polypropylene tube. The plasma was then transferred to another plastic tube and the coagulation assays for PT determinations were done within 3 hours. The citrated plasma was coagulated with the addition of a coagulating agent, and the time required to form a clot was assessed using a coagulometer, which highlights the time in seconds required to coagulate the treated and untreated plasma samples [12].

The mean increase in PT values for reference medications acenocoumarol (1 mg kg-1) and unfractionated heparin (500 IU kg-1), as well as the tested substances at 25 mg kg-1 concentrations, were compared to control using repeated measures ANOVA with Dunnet's test. Calculations of the mean, standard error of the mean (SEM), and ANOVA were performed using the "GraphPad Prism version 4.0" software. The acquired data is expressed as mean standard error of the mean.

RESULT

When compared to SK, the results showed that compound 3i has significant clot lysis activity (46 percent), While compound 3c exhibited less clot lysis activity (10 percent), the order of the in vitro clot lysis effect of all investigated compounds is 3i>SK>3e>3a>3d=3f>3h>3g>3b>1>3c, which is consistent with the results of the previous studies.

Table 1: In vitro clot lysis effect of synthetic compounds on human blood.

Sr. No	Compounds	Clotlysis (%)
1.	**1**	20.6
2.	**3a**	28
3.	**3b**	20.9
4.	**3c**	13
5.	**3d**	26
6.	**3e**	34
7.	**3f**	26
8.	**3g**	20.7

9.	**3h**	21
10.	**3i**	46
11.	Distilled water	4
12.	Streptokinase (SK)	37.33

Table 2: In vivo study of synthetic compounds

Sr. No	**Compounds**	**PT (s)**
1.	**1**	12.33 ± 0.20
2.	**3a**	12.67 ± 0.21
3.	**3b**	12.38± 0.22
4.	**3c**	12.07 ± 0.21
5.	**3d**	12.27 ± 0.17
6.	**3e**	22.50 ± 0.27*
7.	**3f**	12.17 ± 0.10
8.	**3g**	12.02 ± 0.47
9.	**3h**	13.01 ± 0.33
10.	**3i**	36.50 ± 0.37***
11.	Acenocoumarol	48.01 ± 0.49***

DISCUSSION

For clot lysis, researchers employed conventional streptokinase. It was discovered that compound 3c had stronger anticoagulant activity than the others. In an aseptic environment, antithrombotic activity is investigated. Clot lysis test was used to carry out the activity. The formation of clots was determined by weighing the formation of clots with each prepared randomly marked concentration of the substances as shown in Table 1 and 2. The chemical 3c has stronger action than the other compounds at all concentrations. Other compounds are less successful at dissolving clots.

CONCLUSION

The standard approach is used to prepare freshly synthesized 1,3,4-oxadiazole derivatives. The structures of these compounds can be verified using spectroscopy. As a result, that derivative offers antithrombotic properties. According to the Antithrombotic study, a number of drugs had similar effects as standard therapy.

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