World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

Thrombolytic activity of 1,3,4-oxadizole derivatives

B. Vishwanathan* and B.M. Gurupadayya

Department of Pharmaceutical Chemistry, JSS College of Pharmacy, JSS University, Sri Shivarathreeshwara Nagar, Mysore, Karnataka-570 015, India

Received: 25-07-2015 / Revised: 04-09-2015 / Accepted: 19-09-2015

ABSTRACT

The currently available medication for management of arterial thromboembolism (ATE) disorders by antithrombotic therapy highlights its lacunae due to recurrent ATE episodes and indicates the need for better thrombolytic agents with clinical advantage. In the present study, a series of 1,3,4-oxadiazole derivatives (3a-3q) derived from benzofuran were evaluated for *in vitro* clot lysis study for thrombolytic activity. The thrombolytic evaluation was performed for decrease in solid clot weight by the clot lysis process at a concentration of 6.25, 12.5 and 25 µM strength. The results of *in vitro* clot lysis for thrombolytic evaluation revealed that the tested compounds 3a-3q exhibited significant clot lysis with respect to streptokinase (30,000 IU) employed as reference drug. Among all the tested compounds, compound 3o, 3n and 3m exhibited potent thrombolytic activity with ED_{50} value of 18.8, 20.4 and 20.9 µM, respectively. The thrombolytic efficacy investigation highlights that the synthesized compound 3o could be considered for further clinical studies to ascertain its possible hit as thrombolytic agents.

Keywords: 1,3,4-Oxadizole; Antithrombotic; Arterial thromboembolism; Benzofuran; Clot lysis; Thrombolytic.

INTRODUCTION

Myocardial infarction due to arterial thromboembolism (ATE) is currently the leading causes of death under cardiovascular diseases (CVDs) in developed countries. The American Heart Disease foundation estimates more than 30% of all deaths in the world are from CVDs, thus the study highlights that a person has greater chance of dying from heart disease than cancer, AIDS, diabetes and accidents combined. [1]

Indicating that ATE as the leading cause of morbidity and mortality world-wide. ATE typically forms under high shear conditions of blood flow and consists of platelets bound by small amounts of fibrin. ATE is the most common cause of cardioembolic events which includes myocardial infarction, ischemic stroke, and limb gangrene. [2]
The treatment of acute myocardial infarction has changed during the past decade as newer approaches have become accessible, as prevention of complications has been the cornerstones for treatment. The management of ischemic heart diseases is now flanked by newer, more aggressive forms of therapy, which includes the early administration of thrombolytic drugs, highlighting clinical advantage of thrombolytic therapy for its ability to produce clot lysis, which directly restores nutritive myocardial perfusion. [3]

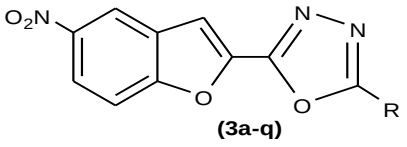
Thrombolytic drugs like tissue plasminogen activator (t-PA), urokinase, streptokinase etc. play a crucial role in the management of ATE. The t-PA like streptokinase and urokinase which are widely used as thrombolytic drugs have marked clinical drawback; these agents have a narrow therapeutic index and require continuous monitoring. Also, these agents have significant risk of haemorrhage, and produce anaphylactic reaction and lacks specificity. These entire therapeutic shortcomings of presently available streptokinase and urokinase and other t-PA indicate the need for better thrombolytic agents with clinical advantage. [4]

In our previous study, we had reported the synthesis, characterization and evaluation of antioxidant and anti-inflammatory activities of 1,3,4-oxadiaozle derivatives derived from benzofuran 3a-3q (Figure 1), from ethyl 5-nitrobenzofuran-2-carboxylate [5]. Wherein, 5-(5-nitrobenzofuran-2-yl)-2-substituted-1,3,4-

***Corresponding Author Address: B. Vishwanathan,** Department of Pharmaceutical Chemistry, JSS College of Pharmacy, JSS University, Sri Shivarathreeshwara Nagar, Mysore, Karnataka-570 015, India; E-mail:: vishwanathan_b@yahoo.co.in*

oxadiazole; 3a-3p (Table 1) were prepared from nucleophilic addition of aryl/heteroaryl/aliphatic carboxylic acids with 5-nitrobenzofuran-2-carbohydrazide in presence of phosphorous oxychloride. The acetohydrazide derivative was prepared by condensation of ethyl 5-nitrobenzofuran-2-carboxylate with hydrazine monohydrate. The ester derivative was prepared by condensation and followed by cyclization of 2-hydroxy-5-nitrobenzaldehyde with ethyl 2-chloroacetate in presence of anhydrous potassium carbonate. The compound 5-(5-nitrobenzofuran-2-yl)-1,3,4-oxadiazole-2-thiol (3q) was prepared by condensation of the acetohydrazide derivative with carbon disulphide and potassium hydroxide. The synthesized compounds were evaluated for *in vitro* free radical scavenging activity by 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical assay method using ascorbic acid as standard and *in vivo* anti-inflammatory activity at a dose of 50 mg kg^{-1} by carrageenan induced paw edema method using indomethacin as standard. The compounds 3a-3q exhibited significant antioxidant efficacy ranging from 34 to 86% and significant anti-inflammatory efficacy with edema reduction ranging from 9.1 to 72.5%. The results of anti-inflammatory evaluation revealed that compounds 3c, 3e and 3d exhibited potent anti-inflammatory activity of 72, 68 and 65% respectively.

In continuation of the work, in our present investigation, we herein report the *in vitro* clot lysis efficacy of these seventeen 1,3,4-oxadiazole derivatives 3a-3q.



(3a-q)

5-(5-nitrobenzofuran-2-yl)-2-substituted-1,3,4-oxadiazole

R =H (3a)
=CH3 (3b)
=C2H5 (3c)
=C3H7 (3d)
=CH(OH)CH3 (3e)
=CH2NH2 (3f)
=C_6H_5(3g)
=2-NO_2-C_6H_4(3h)
=3,5-$(NO_2)_2$-C_6H_3(3i)
=4-NO_2-C_6H_4(3j)
R =CH=CH-C_6H_4(3k)
=2-NH_2-C_6H_4(3l)
=4-NH_2-C_6H_4(3m)
=2-OH-C_6H_4(3n)
=4-OH-C_6H_4(3o)
=3-C_5H_4N(3p)
=SH(3q)

Figure 1: Synthesized 1,3,4-oxadiazole derivative from benzofuran 3a-3q

Table 1: Synthesized 1,3,4-oxadiazole derivatives from benzofuran 3a-3q

Sl. No.	Compound
1.	2-(5-nitrobenzofuran-2-yl)-1,3,4-oxadiazole(3a)
2.	2-methyl-5-(5-nitrobenzofuran-2-yl)-1,3,4-oxadiazole(3b)
3.	2-ethyl-5-(5-nitrobenzofuran-2-yl)-1,3,4-oxadiazole (3c)
4.	5-(5-nitrobenzofuran-2-yl)-2-propyl-1,3,4-oxadiazole (3d)
5.	1-[5-(5-nitrobenzofuran-2-yl)-1,3,4-oxadiazol-2-yl]ethanol (3e)
6.	[5-(5-nitrobenzofuran-2-yl)-1,3,4-oxadiazol-2-yl]methanamine (3f)
7.	5-(5-nitrobenzofuran-2-yl)-2-phenyl-1,3,4-oxadiazole (3g)
8.	5-(5-nitrobenzofuran-2-yl)-2-(2-nitrophenyl)-1,3,4-oxadiazole (3h)
9.	2-(3,5-dinitrophenyl)-5-(5-nitrobenzofuran-2-yl)-1,3,4-oxadiazole (3i)
10.	5-(5-nitrobenzofuran-2-yl)-2-(4-nitrophenyl)-1,3,4-oxadiazole (3j)
11.	5-(5-nitrobenzofuran-2-yl)-2-styryl-1,3,4-oxadiazole (3k)
12.	2-(2-aminophenyl)-5-(5-nitrobenzofuran-2-yl)-1,3,4-oxadiazole (3l)
13.	2-(4-aminophenyl)-5-(5-nitrobenzofuran-2-yl)-1,3,4-oxadiazole (3m)
14.	2-(2-hydroxyphenyl)-5-(5-nitrobenzofuran-2-yl)-1,3,4-oxadiazole(3n)
15.	2-(4-hydroxyphenyl)-5-(5-nitrobenzofuran-2-yl)-1,3,4-oxadiazole (3o)
16.	5-(5-nitrobenzofuran-2-yl)-2-(pyridin-3-yl)-1,3,4-oxadiazole (3p)
17.	5-(5-nitrobenzofuran-2-yl)-1,3,4-oxadiazole-2-thiol (3q)

EXPERIMENTAL

Pharmacology: The chemical were procured from Sigma Aldrich and were used without further purification. All the animal experimental procedures and protocols adapted in the study were reviewed and approved by the Institutional Animal Ethics Committee. The experimental procedures and protocols were in accordance with the guidelines of the CPCSEA, Ministry of Forests and Environment, Govt. of India.

In vitro* thrombolytic evaluation*: *In vitro* test adapted for screening of test compounds for clot lysis study was performed on whole blood as per the reported method [7]. The blood samples were collected from butcher house of healthy sheep (*Ovis aries*).The entire procedure was carried out at room temperature and studies was need to be completed within 3 h after blood withdrawal as per the protocol. Phosphate buffered saline (PBS) was used as control and test compounds were screened at 6.25, 12.5 and 25 µM concentrations, respectively. The commercially available lyophilized streptokinase vial (1,500,000 I.U.) was diluted with PBS and mixed properly. From which streptokinase equivalent to 30,000 I.U was used as a standard to observe the thrombolytic activity.

Whole blood was collected and 500µl of blood was transferred to each of the previously weighed micro centrifuge tubes and incubated at 37 °C for 45 minutes for clot formation. After clot formation, serum was completely removed (aspirated out without disturbing the clot formed) and each tube with the clot was again weighed to determine the clot weight. 100µL of diluted test compounds (6.25, 12.5 and 25 µM) for screening were added to the labeled micro centrifuge tube containing clot. 100 µL of streptokinase (30,000 IU) was added as the positive thrombolytic control and 100 µL of PBS as the negative thrombolytic control was employed. All the tubes were then again incubated at 37 °C for 90 minutes and observed for clot lysis. After incubation, fluid obtained was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis. The percent clot lysis was calculated according to the following formula (Equation 1).

$$\%\ Clot\ lysis = \left[\frac{Intial\ clot\ weight - Final\ clot\ weight}{Intial\ clot\ weight}\right] \times 100$$

Equation 1: Calculation of percentage clot lysis

The mean clot lysis percentage of test compounds in different concentrations was compared with the standard streptokinase and phosphate buffered saline using the repeated measures ANOVA with Dunnet's test. Mean, standard error of mean (SEM) calculations and ANOVA test were performed using ''GraphPad Prism version 4.0'' software. The data obtained is expressed as mean ± SEM. And the concentration which produced clot lysis half maximally (ED_{50}) was also determined graphically from the percentage of clot lysis at various concentrations of the test compound. The ED_{50} value and the percentage clot lysis exhibited by the test compounds at dose strength 6.25, 12.5 and 25.0 µM are expressed in mean ± SEM in Table 2.

Table 2: The *in vitro* thrombolytic activity results of the test compounds 3a-3q

Sl. No.	Compound	Percentage Clot Lysis[a,b]			ED50 Value (µM)
		6.25 µM	12.5 µM	25 µM	
	3a*	27.74 ± 0.66	34.55± 0.69	42.18 ± 0.25	34.87 ± 0.46
	3b*	12.07 ± 0.39	20.88 ± 0.28	29.28 ± 0.43	47.93 ± 1.97
	3c*	19.63 ± 0.85	23.72 ± 0.40	33.63 ± 0.92	47.01 ± 1.58
	3d**	14.44 ± 0.26	20.51 ± 0.80	30.92 ± 0.08	46.74 ± 0.21
	3e***	25.32 ± 0.61	34.16 ± 0.41	51.40 ± 0.56	23.98 ± 0.08
	3f*	25.23 ± 1.08	34.51 ± 0.69	47.01 ± 0.52	27.31 ± 0.65
	3g*	28.52 ± 0.65	31.35 ± 0.35	39.72 ± 0.27	42.47 ± 1.93
	3h*	21.67 ± 0.80	30.89 ± 0.77	44.97 ± 0.56	28.85 ± 0.31
	3i*	29.28 ± 0.54	38.16 ± 0.16	45.01 ± 0.89	30.45 ± 1.17
	3j**	22.48 ± 0.66	34.54 ± 0.04	40.18 ± 0.35	34.91 ± 1.35

3k*	20.77 ± 1.45	24.10 ± 0.16	28.92 ± 0.78	74.15 ± 1.37
3l***	26.13 ± 0.47	40.11 ± 0.86	53.40 ± 0.28	21.85 ± 0.44
3m***	38.94 ± 0.25	46.97 ± 0.43	51.83 ± 1.21	20.96 ± 0.24
3n***	44.60 ± 0.47	47.82 ± 0.47	51.40 ± 0.31	20.44 ± 0.30
3o***	43.73 ± 0.74	47.00 ± 0.30	53.00 ± 0.30	18.81 ± 0.32
3p***	32.98 ± 0.82	40.11 ± 0.86	51.02 ± 0.22	23.69 ± 0.18
3q**	10.82 ± 0.36	18.89 ± 0.26	22.08 ± 0.52	74.14 ± 2.41
NS	01.97 ± 0.17	02.58 ± 0.65	02.63 ± 0.17	-------
Streptokinase (30000 IU)	46.57 ± 0.38			

[a] Results are expressed as the mean values from three parallel experiments ± S.E.M.
[b] Data was analyzed by Dunnet's test. n = 3; (***) equals $P<0.001$, (**) equals $P<0.01$, (*) equals $P<0.05$.

RESULTS AND DISCUSSIONS

The *in-vitro* thrombolytic activity of the 1,3,4-oxadizole derivatives were determined by clot lysis study. The activity of the compounds was determined by comparison with the thrombolytic activity of Streptokinase. The test compounds were measured for the decrease in clot weight at different concentrations 6.25, 12.5 and 25 µM, respectively, streptokinase (30,000 IU) was employed as positive control and PBS as negative control. The results were plotted conc. vs percentage clot lysis and ED50 values were obtained by regression analysis.

The results of the *in vitro* thrombolytic activity were encouraging and the tested compounds exhibited substantial aggregation inhibition. All the seveenteen tested 1,3,4-oxadizole derivative exhibited substantial clot lysis, with percentage value ranging from 20.7 to 53.0 % in comparison to 46 % clot lysis exhibited by the reference standard streptokinase (30,000 IU). The result of thrombolytic evaluation highlighted that compounds with electron donating substituent on the phenyl moiety at position C2 of the 1,3,4-oxadiazole series exhibited significant activity. Compound 3l (2-amino), 3o (4-hydroxy), 3m (4-amino), 3n (2-hydroxy), 3e (ethan-1-ol) 3p (pyridin-3-yl), and 3f (methanamine), exhibited substantial clot lysis efficacy than that of the standard streptokinase (46.6%) at the test dose strength of 25 µM with an percentage clot lysis of 53.4, 53.0, 51.8, 51.5, 51.4, 51.0 and 47.0 %, respectively in comparison to the other set of oxadiazole derivatives.

Whilst, compounds with electron withdrawing substituents on the phenyl moiety at position C2 of the oxadiazole series namely; 3i (3,5-dinitro), 3h (2-nitro) and 3j (4-nitro) exhibited moderate thrombolytic activity at the test dose strength of 25 µM with an percentage clot lysis of 45.1, 45.0 and 40.2 % respectively. While, compound 3g (phenyl) and 3k (styryl) exhibited moderate thrombolytic activity at the test dose strength of 25 µM with a percentage clot lysis of 39.7 and 28.9 % respectively.

Whereas, compounds with aliphatic substituents at position C2 of the oxadiazole series, 3b (methyl), 3c (ethyl) and 3d (propyl) exhibited moderate thrombolytic activity at the test dose strength of 25 µM with a percentage clot lysis of 29.3, 33.6 and 30.9 % respectively in comparison to 3e and 3f. Compound 3q (mercapto) proved to be the major exception of all the tested compounds with clot lysis of only 22.1%.

The statistical analysis of the compounds thrombolytic activity by Dunnet's test highlighted that the compounds 3e, 3l, 3m, 3n, 3o, 3p exhibited a significance value of $P<0.001$ highlighting the confidence interval of 99.9 % with respect to control. Compound 3d, 3j and 3q exhibited a significance value of $P<0.01$ highlighting the confidence interval of only 99.0 % with respect to control. Whilst, compound 3a, 3b, 3c, 3f, 3g, 3h, 3i and 3k exhibited a significance value of $P<0.05$ highlighting the confidence interval of only 95.0 % with respect to control.

CONCLUSION

A series of benzofuran encompassing 1,3,4-oxadiazole derivatives were evaluated for *in vitro* thrombolytic activity by measuring percentage clot lysis. Result of present study highlights that the tested 1,3,4-oxadiazole derivatives exhibited significant clot lysis by producing decrease in the solid clot weight when compared to standard drug streptokinase. Compound 3o exhibited potent thrombolytic activity with ED_{50} value of 18.8 µM to produce clot lysis.

ACKNOWLEDGEMENT

We express our gratitude to the JSS College of Pharmacy, Mysuru and JSS University for providing us all necessary facilities.

REFERENCES

1. Murray CJ, Lopez AD. Global mortality, disability, and the contribution of risk factors: Global Burden of Disease Study. Lancet 1997; 349: 1436–42.
2. Meschia JF et al. Thrombolytic treatment of acute ischemic stroke. Mayo Clin Proc 2002; 77: 542-51.
3. Godfrey EM et al. Don't be a clot: A radiologist's guide to haemostasis including novel antiplatelet and anticoagulant therapy. Clin Rad 2011; 66: 693-700.
4. Sobel BE, Braunwald E. *The management of acute myocardial infarction. In: Braunwald E (ed) Heart disease: A textbook of cardiovascular medicine*, Braunwald E, 2nd ed. Philadelphia: WB Saunders, 1984, pp 1301-33.
5. Vishwanathan B et al. Design, synthesis, in vitro antioxidant and in vivo anti-inflammatory activities of novel oxadiazole derivatives. Int J Pharm Pharm Sci 2014; 6: 514-20.
6. Ertl P et al. Fast Calculation of Molecular Polar Surface Area as a Sum of Fragment-Based Contributions and Its Application to the Prediction of Drug Transport Properties. J Med Chem 2000; 43: 3714-17
7. Prasad S et al. Development of an *in vitro* model to study clot lysis activity of thrombolytic drugs. Thrombosis 2006; 4:1-4.