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Synthesis, characterization and thrombolytic activity of n-benzyl piperidin 4-one phenyl hydrazone

K. Sundaresan, V. Priyadarshini, K. Tharini*

Dept of Chemistry, GOVT Arts College, Trichy-22, Tamil Nadu, India

ABSTRACT

The aim of this study was to synthesize, characterization and thrombolytic activity of N-Benzyl piperidin 4-one phenyl hydrazone derivative. Check the purity of all the synthesized compounds using thin layer chromatography. The synthesized compound was characterized by IR, ^{13}C and ^{1}H NMR spectral studies. The synthesized compound was subjected to thrombolytic activity. The thrombolytic activity was observed in 2 different concentrations of N-Benzyl piperidin 4-one phenyl hydrazone. Our findings support the reported therapeutic use of this compound as a thrombolytic agent in the Indian system of medicine.

Keywords: N-Benzyl piperidin 4-one phenyl hydrazone, thrombolytic activity, streptokinase.

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***Address for Correspondence:**

K. Tharini, Dept of Chemistry, GOVT Arts College, Trichy-22, Tamil Nadu, India

INTRODUCTION

Aromatic hetero cyclic chemistry is an enormous and complex subject of great industrial and academic significance. A number of molecules of life are derived from aromatic heterocycles and many pharmaceutical and agrochemical compounds are based on aromatic Heterocycles. A cyclic aromatic compound containing all C-atoms in ring formation is referred to as a carboxylic compound. If at least one atom other than carbon form a part of ring system then it is designated as a heterocyclic compound. Organic chemistry and medicinal chemistry are becoming very vital chemistry. The primary objective of an organic chemist is to work towards isolation, characterization and synthesis of new compounds that are suitable for use as drugs. Medicinal or pharmaceutical chemistry is a discipline at the intersection of chemistry and pharmacology involved with designing, synthesizing and developing pharmaceutical drugs. However their derivatives having N-C linkage have been used in the fields of medicinal and pharmaceutical chemistry and reported to exhibit a variety of biological activities[1-4]. Hydrazones and their derivatives constitute an important class of compounds that has found wide utility in organic synthesis. The chemistry of carbon-nitrogen double bond of hydrazone is becoming the backbone of condensation reaction in benzo-fused N-heterocyclics also it constitutes an important class of compounds for new drug development.

Thrombolytic drugs are widely used for the management of cerebral venous sinus thrombosis patients. Several in vitro models have been developed to study clot lytic activity of thrombolytic drugs, but all of these have certain limitations. There is need of an appropriate model to check the clot lytic efficacy of thrombolytic drugs. In the present study, an attempt has been made to design and develop a new model system to study clot lysis in a simplified and easy way using a thrombolytic drug, streptokinase.[5]

MATERIALS AND METHODS

All the chemicals (solvents and reagents) were purchased from foreign companies (Hi-media and Sigma/Aldrich) and were used as such with no further purification and distillation. Local chemical has not been used in the research work. The purity of these chemicals was 98-99.9%. The hydroxyl amine hydrochloride (Hi-media sigma/Aldrich) was used as received. The other reagents used were sodium acetate and sodium hydroxide (Merck). Analytical grade solvents like ethanol, methanol, ethyl acetate, chloroform ($CHCl_3$) and N-hexane were used as such without further distillation. The synthesized compounds were scaled for yield and purified by recrystallization with suitable solvent system.

IR spectra were recorded in AVATAR-330 FT-IR spectrophotometer (Thermo Nicolet) and only noteworthy absorption levels (reciprocal centimeters) are listed. ^{1}H NMR spectra were recorded on BRUKER AMX 300 MHz and 300

MHz NMR recorded on a BRUKER AMX 300 MHz NMR spectrometer operating at 100 MHz. For recording ^{1}H NMR spectrum of compound solution were prepared by dissolving about 10mg of the compound in 0.5 ml of $CDCl_3$ was used as solvent while for ^{13}C NMR spectra, about 50 mg of the compound was dissolved in the same volume of the respective solvents. TMS (Tetra methyl silane) was used as a internal standard.

Preparation of N-benzyl piperidin-4-one phenyl hydrazone

To the boiling solution of N-benzyl piperdine-4-one (0.01mol) in methanol (45ml) and few drops of concentrated hydrochloric acid, the methanolic solution of phenyl hydrazine hydrochloride (0.01mol) was added dropwise by stirring.The reaction was refluxed for 4 hrs on a water both. After cooling the solid product was filtered and recrystallized methanol to get the corresponding phenyl hydrazone.

***In vitro* thrombolytic activity**

Thrombolytic activity determined by the method of Fatema Tabassum et al(2017). [6-7]

Preparation of streptokinase (SK)

About 5 ml sterile distilled water was added to the commercially available lyophilized SK vial of 15,00,000 I.U. and mixed properly. This suspension was used as a stock from which 100 µl (30,000 I.U) was used for *in vitro* thrombolysis study.

Collection of blood

Whole blood was drawn from healthy human volunteers without a history of oral contraceptives or anticoagulant therapy and 1 ml of blood was transferred to the previously weighted sterile eppendorf tubes and was allowed to form clots.

Procedure

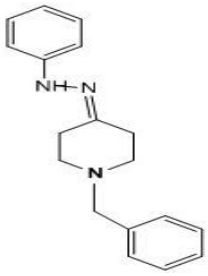
3ml venous blood drawn from own blood was distributed in four different pre- weighted eppendorf tube and incubated at 37^0 c for 45minutes. After clot formation, serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight **(clot weight = weight of clot containing tube-weight of tube alone).** To each eppendorf tube containing pre-weighted clot, 100µl (100µg/ml) of sample was added and another eppendorf tube containing pre-weighted clot, 200µl (200µg/ml) of sample was added. As a negative control, 100µl of distilled water was added to the control tube. For positive control, 100µl of streptokinase (SK) was added. All the tubes were then incubated at 37^0C for 90minutes and observed for clotlysis. After incubation, fluid released was removed and tubes were again weighted to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clotlysis. The equation for calculating weight of clot is given below.

Clot weight = Weight of clot filled tube –Weight of empty tube

$$\% \text{ of clot lysis} = \frac{\text{Weight of clot after lysis}}{\text{Weight of clot before lysis)}} \text{x } 100$$

RESULTS AND DISCUSSION

Table 1: Analytical and spectral data of N-benzyl piperidin-4-one phenyl hydrazone

MF: $C_{18}H_{21}N_3$	**M.Pt.**163-165^{0}C	**Yield** (%):86	
IR (cm^{-1}): 3195,3113 (N-H stretching); 2915-2689 (C-H stretching); 1597 (C=N stretching); 1495 (C=C stretching-phenyl); 1328, 1239 (aromatic amine C-N); 1166.			
^{1}H NMR (δ ppm) : 4.47 (t, 2H, H-2); 3.17 (t, 2H, H-6); 2.72 (t, 2H, H-3); 2.00 (t, 2H, H-5); 4.36 (s, 2H, -N-CH_2-Ph); 4.29 (s, 1H, NH proton) 7.48-7.43 (m, 10H, aryl protons).			
^{13}C NMR (δ ppm) : 48.99 (C-2); 47.84 (C-6); 23.09 (C-3); 19.94 (C-5); 136.41 (C=N); 58.22 (N-CH_2-Ph); 131.30-124.66 (aromatic carbons).			

***In vitro* thrombolytic activity of compound N-benzyl piperidin-4-one phenyl hydrazone (BPPH)**

Thrombolytic therapy, also known as clot busting drug, is a breakthrough treatment which has saved untold lives. It has been used in the clinical area to treat venous and arterial thromboembolic complaints which are a foremost cause of death.

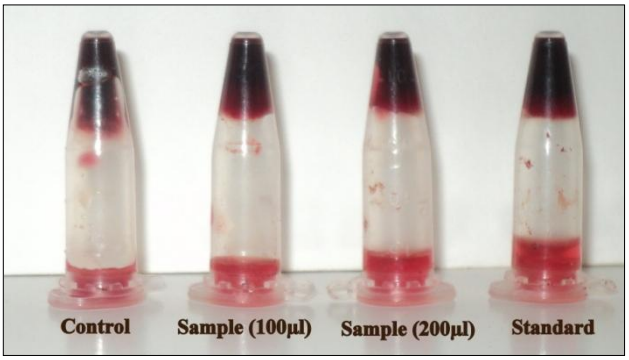
The *in-vitro* thrombolytic activity of the BPPH was determined by clot lysis study. The activity of the compounds was determined by comparison with the thrombolytic activity of Streptokinase. The test compound was measured for the decrease in clot weight at different concentrations.

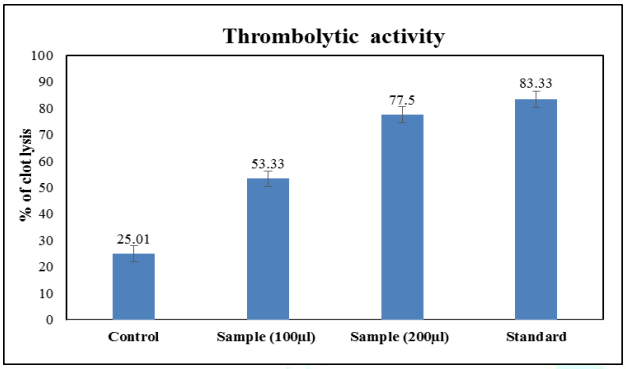
Table 2: Thrombolytic activity of BPPH

S.No.	Sample	% of clot lysis
	Control	25.01±1.75
	Sample (100µl)	53.33±3.71
	Sample (200µl)	77.50±5.39
	Standard (Streptokinase)	83.33±5.81

In the present study, thrombolytic activity analysis of sample extract (100µl and 200µl) showed removal of clot by 53.33% and 77.50%, respectively, with that of positive Control streptokinase (SK) of 83.33% and negative control of 25.01% clot lysis.

The clot lysis at 100 µl 200 µl of compound was 53.33%, 77.50% in 37^{0}c at 45 min respectively while standard shows 83.33%. The highest dose as 200 µl of compound has significant activity and near to the standard.





Thrombolytic activity of BPPH

CONCLUSION

The structures of the synthesized compound are established on the basis of their analytical and spectral data (IR, ^{1}H NMR, ^{13}C NMR). The result from the study showed that the N-benzyl piperidin-4-one phenyl hydrazone had excellent thrombolytic activity that was comparable to the activity of Streptokinase. As from the research findings of the under taken in vitro clotlysis study, we demonstrated that the compound showed mainly moderate thrombolytic activity. Our findings support the reported therapeutic use of this compound as clot lysis or thrombolytic agent in the Indian system of medicine.

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REFERENCES

1. Hasan MU, Arab M, Pandiarajan K, Sekar R, Marko D, Magn. Reson. Chem, 1985, 23:292.
2. Pandiarajan K, Sabapathy Mohan RT, Krishnakumar R, Indian J. Chem, 1987; 26B:624.
3. Pandiarajan K, Sekar R, Anantharaman R, Ramalingam V, Indian J. Chem,1991; 30B:490.
4. Pandiarajan K, Tamilselvi P, Gurumani V, Indian J. Chem, 1994; 33B:971.
5. Prasad S et al, Development of an in vitro model to study clot lysis activity of thrombolytic drugs, Thrombosis, 2006; 4:1-4.
6. Fathima SN, Ahmad SV, Kumar BR. Evaluation of In Vitro Thrombolytic Activity of Ethanolic Extract of CURCUMA CAESIA Rhizomes, International Journal of Pharma Research & Review, 2015; 4(11):50-54.
7. Fatema Tabassum, Somaia Haque Chandi, Kamrun Nahar Mou, KM Imrul Hasif, Tareque Ahamed , Mahbuba Akter, Invitro thrombolytic activity and phytochemical evaluation of leaf extracts of four medicinal plants of Asteraceae family, Journal of Pharmacognosy and Phytochemistry, 2017; 6(4):1166-1169.