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Research Article

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Evaluation of *in vitro* antioxidant and thrombolytic studies of some *N*-(2,3-dihydro-1,4-benzodioxan-6-yl)-4-methylbenzenesulfonamide-*N*-acetamide derivatives

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ABSTRACT

In current perspective of time there is a need to develop new compounds having great therapeutic potential. In the present research work different antioxidant and thrombolytic activities of sulfonamide derivative synthetic compounds were screened out. Sulfonamides are enthusiast to a biologically very important category of compounds and are under consideration of many pharmacologists and synthetic chemists. By keeping in view the prominence of sulfonamide derivative compounds were tested for their antioxidant properties through DPPH free radical scavenging abilities and oxidative DNA damage protection assays. It was observed that all the tested compounds showed good to moderate antioxidant potential. Similarly, the thrombolytic effect was screened out through in vitro clot lysis activity which indicated that such synthetic derivatives have biological potential and could be integrated in drug formation for achievement of therapeutic goals.

Key words: Sulfonamide, Antioxidant, DNA damage protection, Thrombolytic activity, DPPH.

INTRODUCTION

Various antioxidants are reported from natural, semisynthetic and synthetic sources for example quercitin from plants, by derivation from natural sources like gallic acid esters and purely synthetic antioxidants like Butylated hydroxyl toluene (BHT) which is a derivative of phenol respectively. All of these antioxidant compounds are frequently used in pharmaceuticals and food industries. New compounds having antioxidant properties as well as other therapeutic potential are of great importance. There is a need to develop new pharmacologically active compounds with multiple therapeutic goals having antioxidant properties and low toxicities. Although synthetic drugs have many adverse effects but pharmaceutical research is going on to minimize such adverse effects or to remodel the existing synthetic medicine in a way to prevent their adverse reactions in body (Abbasi *et al*., 2014).

The sulphonamides play central role in pharmaceutical industry and work as anticonvulsant, antiviral, antifungal agents as well as enzyme inhibitors. Aryl sulfonamides are used in opposition to tumor cell lines. Clinically, sulfonamides are typically used to treat various types of gastrointestinal, and urinary, infections. They take action as anticancer agents and inhibitors of carbonic anhydrase, which is the origin for cancer. The dynamic force in the creation of sulfonamides is the empathy of nitrogen atom of amine for the sulfonyl group of sulfonyl halide and therefore is the most generally in use method for their synthesis (Abbasi *et al*., 2015).

EXPERIMENTAL SECTION

Antioxidant assay by DPPH Scavenging Activity
DPPH assay was done by using 1-diphenyl-2-picrylyhdrazylreagent and BHT was used as standard. In 1mL of (0.0004% DPPH solution in methanol), add 10 µL of test compound solution and incubated for half hour in dark after incubation take spectrophotometric reading at 517 nm. DPPH solution without test compounds taken as blank.

$I\ \% = (A_{blank} - A_{sample} / A_{blank}) \times 100$

Percentage DPPH radical scavenging activity provided by above mentioned formula (Bozin*et al*., 2006).

Oxidative DNA damage protection assay
CT-DNA (Calf thymus) 0.5 µg/µL was taken and it was diluted up to two folds as 0.5 µg/3µL) with 50 mM phosphate buffer with pH 7.4. 20 µL of diluted ctDNA was transferred to the microeppendorf tube followed by 20 µL of stock solution of the selected synthetic compounds in the final reaction mixture. 3 µL of TAE buffer, 4 µL of 30% $H_2O_2$and 4 µL of 0.2 M $FeSO_4$were added successively. Negative control of 20 µL of diluted ctDNA and positive control without any protectant were used in assay.

First 1X TAE buffer was prepared by dissolving 10 mL 50 X buffer in 490 mL of distilled water. Then 1% solution of agarose gel in 1X TAE buffer was prepared by dissolving 1 g agarose in 100 mL of 1x TAE buffer. Then it was heated in oven for 1 min so that a homogenous mixture was formed. This was slightly cooled to bearable temperature. Then 20 µL of staining ethedium bromide dye was added to agarose gel solution. This was shaked and poured in gel tray of gel electrophoresis system. This was allowed to solidify for 30 min. After solidification the 1X TAE (gel running buffer) was poured to such level that both of electrodes were dipped in buffer. After incubation 3µL of loading dye (Bromophenol blue) was added to each reaction mixture. Then these samples were loaded in to the wells made with the help of comb on 1% agarose gel containing TAE buffer and ethedium bromide. Each reaction mixture with column was run horizontally in TAE buffer at 100 volts for 45 minutes in gel electrophoresis system.

The gel was photographed under UV light using gel document system (SynGene, England). For each run, a molecular marker, a negative control and positive control were loaded, as well as various antioxidant treatments (Noor*et al.*, 2014).

Thrombolytic assay:
The solution of synthetic compounds (10 mg/mL) in DMSO and added to the micro ependroff tube containing the clot to check thrombolytic activity. The streptokinase was used as a positive control for this study (Parsad et al., 2006).

RESULTS AND DISCUSSION

Novel sulfonamide derivative which are made from bioactive benzodioxane, acetamide and chlorobenzyle sulfonamide moieties with side chain benzene ring methyl, ethyl, ethoxy and methoxy group substitutions on benzene ring different orientations. All of the functional groups attached to the parent molecule incorporated new properties to derivative molecule which was elaborated by antioxidant and thrombolytic properties. Results of antioxidant and thrombolytic activities are recorded in Table 1. The aim of this study was to synthesize pharmacologically more potent compounds and to study the effect of substitutions of functional groups at different orientations. In the present piece of research following series of twelve derivatives of N-(2, 3-Dihydro-1, 4-Benzodioxan-6-yl)-4-chlorobenzene sulfonamide-N-acetamide synthetic compounds were evaluated by *in vitro* assays.

Antioxidant studies of synthetic compounds by DPPH assay
Antioxidant activity of synthetic compound series was checked by DPPH free radical scavenging capacity of the test compound. 1, 1-Diphenyl-2-picryl-hydrazyl is a stable free radical which is soluble in methanol and in its radical form it existed in violet color while on reduction it turned its color to yellow coloration. Capacity of antioxidants to reduce the DPPH radical is directly related to its antioxidant activity (Brand-Williams *et al*., 1999).

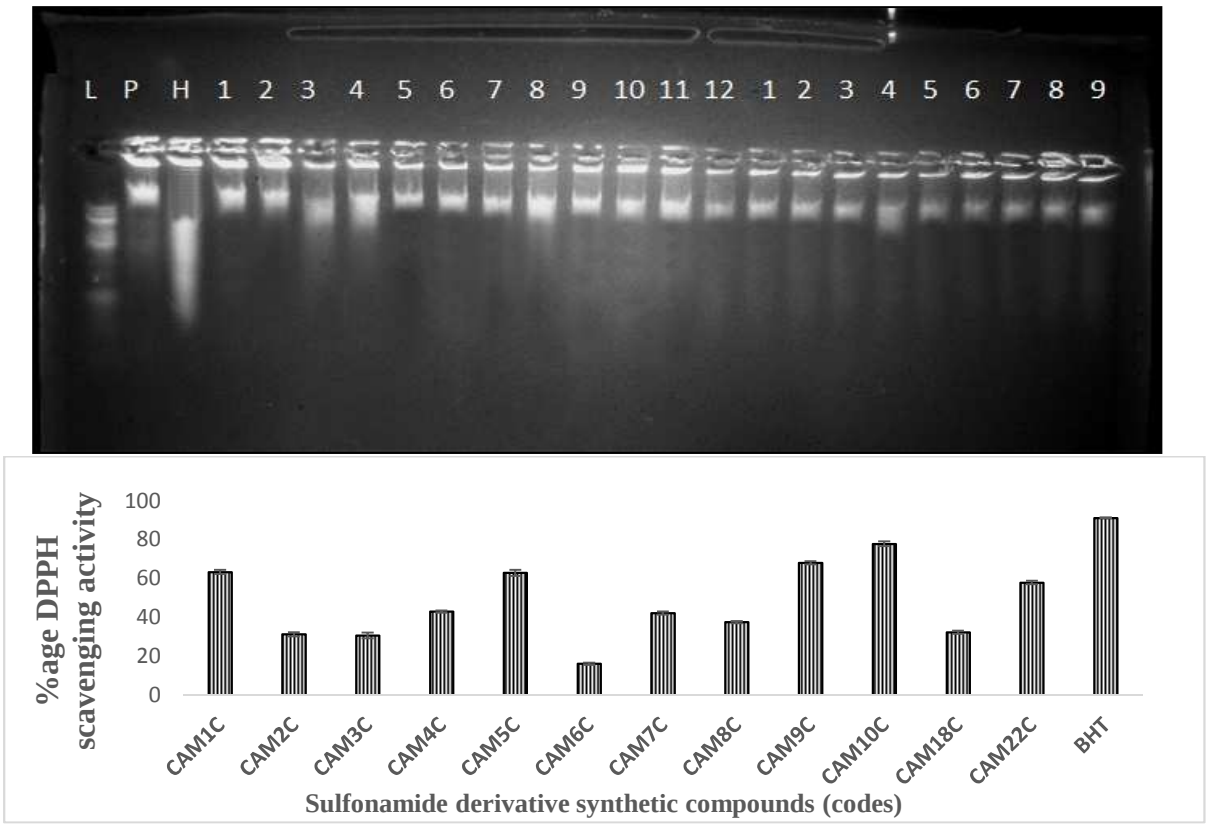


Figure 1: Percentage DPPH scavenging activity of selected sulfonamide derivative synthetic compounds

Antioxidant studies of Sulfonamide derivative compounds showed that all the compounds of sulfonamide series showed good antioxidant activities as shown in Table 1 and graphically in Figure 1. Among these compound CAM10 showed maximum antioxidant activity that was 77.85%. The antioxidant activity of these compounds are might be due to proton donating ability. In case of CAM10 molecule one methyl group is present at *Para* position away from electronegative nitrogen. Inductive effect may be responsible for ease of departure of proton from methyl group which is attacked by DPPH free radical having a singlet electron. On reduction of DPPH free radical, it changed its color from violet to yellow and this color change was analyzed spectrophotometrically at wave length of 517nm. Results were calculated as percentage DPPH scavenging or Percentage antioxidant activity. Concentration of test compound was 10 mg/mL of DMSO. While the concentration of DPPH was 0.004% in methanol (Villano*et al*., 2007).

DNA damage protection ability of synthetic compounds

This study was intended to evaluate antioxidant and oxidative DNA damage protection properties of sulfonamide of synthetic compounds *in vitro* using appropriate models to offer scientific basis and to justify their folkloric use as medicines. Hydrogen peroxide H_2O_2 is a n oxidizing agent produced inside the living bodies which inactivate some body enzymes directly by oxidation of amino acid thiol (-SH) groups, present in backbone of enzyme(Loft and Moller, 2006). Oxidative alteration in enzymatic bioactive functional thiol group resulted in enzyme inactivation. H_2O_2 can easily cross cell membranes upon entry into a cell it reacts with iron (Fe^{+3}) and copper (Cu^{+2}) and yield hydroxyl free radicals which further attacked on biomolecules like proteins, lipids and DNA (Colognato *et al*., 2006). Thus, removing of H_2O_2 is very important for protection of biomolecules(Silva *et al*., 2008). Our synthesized series of compounds were checked by *in vitro* oxidative DNA damage protection assay as follows

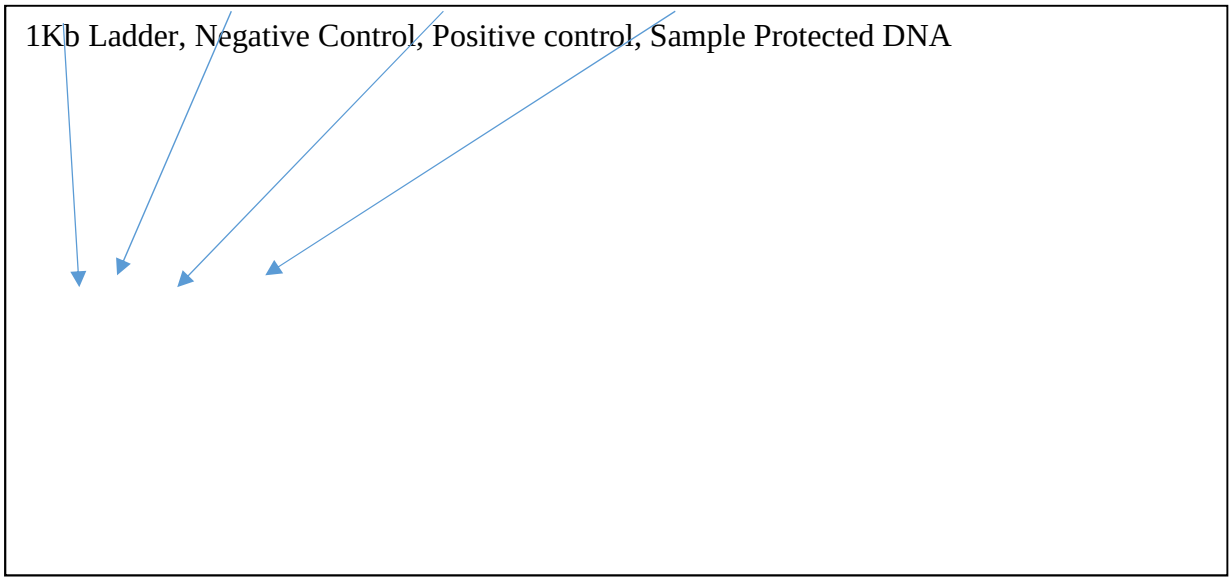


Figure 2. DNA damage protection assay of Sulfonamide series synthetic compounds. L= 1kb ladder, **P**= pure ctDNA, **H**= ctDNA+ H_2O_2+$FeSO_4$, **1**= CAM 1 + ctDNA+ H_2O_2+$FeSO_4$,**2**= CAM 2 + ctDNA+ H_2O_2+$FeSO_4$, **3**= CAM 3 + ctDNA+ H_2O_2+$FeSO_4$, **4**= CAM 4 + ctDNA+ H_2O_2+$FeSO_4$, **5**= CAM 5 + ctDNA+ H_2O_2+$FeSO_4$, **6**= CAM 6 + ctDNA+ H_2O_2+$FeSO_4$, **7**= CAM 7 + ctDNA+ H_2O_2+$FeSO_4$, **8**= CAM 8 + ctDNA+ H_2O_2+$FeSO_4$, **9**= CAM 9 + ctDNA+ H_2O_2+$FeSO_4$, **10**= CAM 10 + ctDNA+ H_2O_2+$FeSO_4$, **11**= CAM 18 + ctDNA+ H_2O_2+$FeSO_4$, **12**= CAM 22 + ctDNA+ H_2O_2+$FeSO_4$,

Pure ctDNA formed an intact band in lane P which was considered as negative control and in lane H ctDNA was subjected to Fenton reaction in which production of hydroxyl free radicals attacked on DNA and fragmentation of ctDNA was occurred. This destruction of ctDNA can be visualized by smear formation of fragmented DNA in lane H. In the presence of Fenton reagents and ctDNA in lane 1 to lane 12 showed no smear formation or slight smear formation in lane 3 and 4. This protection of ctDNA in the presence of Fenton reagents in reaction mixture showed the oxidative DNA damage protection potential of our test compounds of sulfonamide series. This activity is possibly due to presence of methyl groups which may offer their hydrogen to hydroxyl free radicals and neutralize them to render ctDNA intact from hydroxyl free radical oxidative attack. Another possible reason for this activity is due to presence of electron withdrawing groups like sulfonamide moiety, halide group like chloride and amide group present in all members of sulfonamide series of synthetic compound (Silva *et al*., 2006).

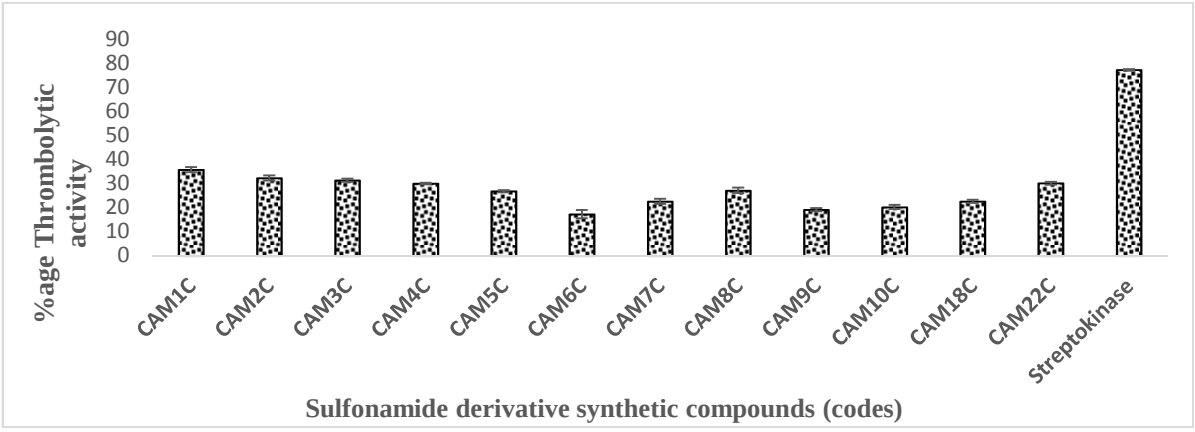


Figure 3: Percentage Thrombolytic activity of Sulfonamide derivative synthetic compounds

In vitro thrombolytic ability of synthetic compounds

In vitro clot lysis assay is performed to assess the test compound potential to lyse a blood clot. In this method fresh blood drawn from vein of healthy volunteer and poured 1 milliliter in Eppendorf. Blood was allowed to stand for an hour to form a complete clot and blood serum was squeeze out. Serum was pipetted out and weight of clot was determined by subtracting the weight of the clot containing Eppendorf tube and an empty Eppendorf tube. After that sample to be analyzed for its thrombolytic potential was poured on blood clot and incubated for three hours at 37ºC. Test sample may interact with clot fibrin thread to dissolve it and slashing of clot occurred. Liquefaction of clot after

lysis was aspired off by the help of pipette. Difference in weight of clot before and after lysis gives the results of thrombolytic activity of respective test sample. Results are shown in Table 1. and graphically demonstrated in Figure 3.

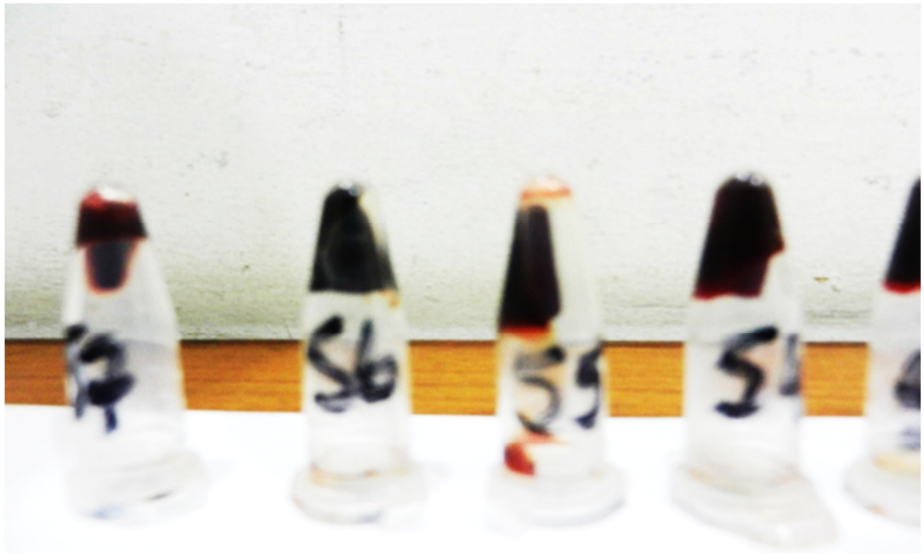
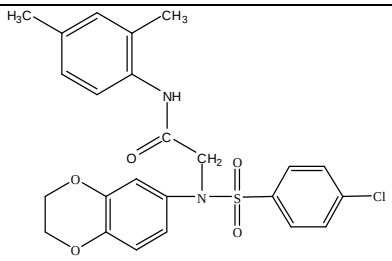
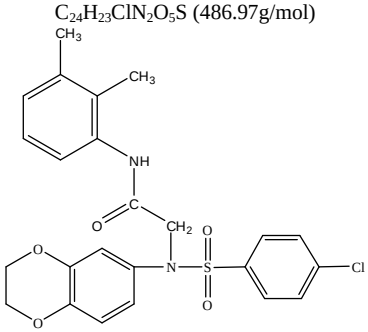
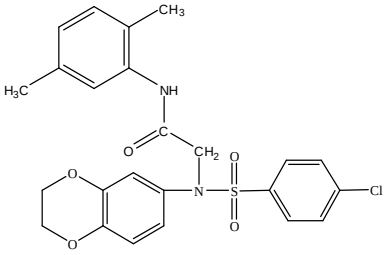
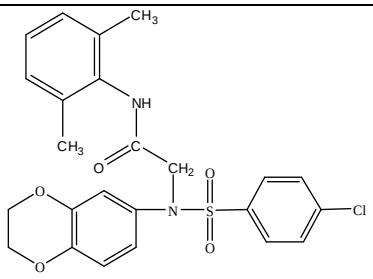
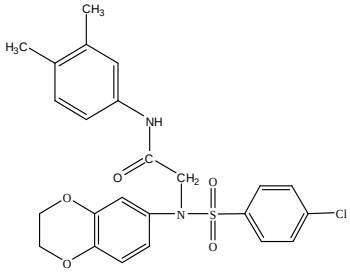
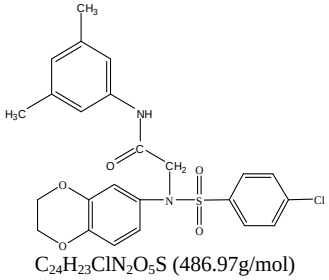
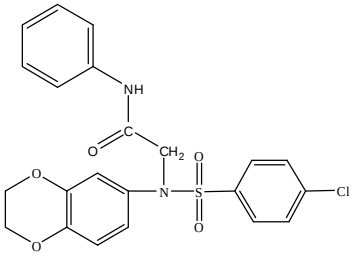
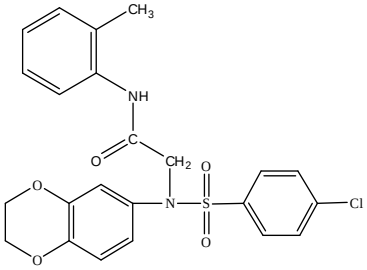
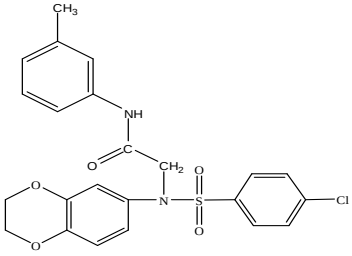
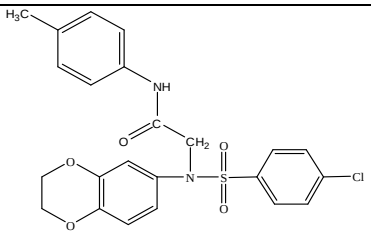
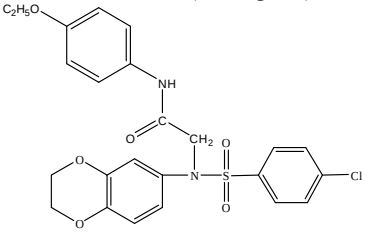
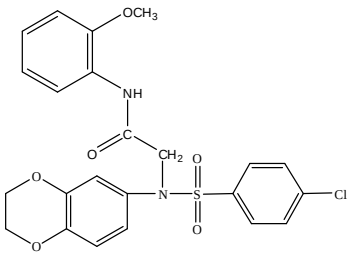


Figure 4. Thrombolytic activity of Sulfonamide derivatives

Table 1. Antioxidant and Thrombolytic potential of selected sulfonamide derivatives

Sr.no.	Compounds Code	Structure, Chemical formula & molecular weight	Percentage DPPH free radical scavenging Mean ± S.D	Percentage Thrombolytic activity Mean ± S.D
1	CAM1	 $C_{24}H_{23}ClN_2O_5S$ (486.97g/mol)	63.33^{c} ± 1.04	35.78^{a} ± 1.06
2	CAM 2	 $C_{24}H_{23}ClN_2O_5S$ (486.97g/mol)	31.22^{g} ± 1.02	32.33^{b} ± 1.03
3	CAM3	 $C_{24}H_{23}ClN_2O_5S$ (486.97g/mol)	30.60^{g}± 1.44	31.40^{bc} ± 0.70

4	CAM4	 $C_{24}H_{23}ClN_2O_5S$ (486.97g/mol)	42.91^{e} ± 0.57	30.03^{c} ± 0.33
5	CAM5	 $C_{24}H_{23}ClN_2O_5S$ (486.97g/mol)	62.91^{c} ± 1.55	26.86^{d} ± 0.46
6	CAM6	 $C_{24}H_{23}ClN_2O_5S$ (486.97g/mol)	16.01^{h} ± 0.59	17.32^{g} ± 1.72
7	CAM7	 $C_{22}H_{19}ClN_2O_5S$ (458.91g/mol)	42.12^{e} ± 0.79	22.63^{e} ± 1.07
8	CAM8	 $C_{23}H_{21}ClN_2O_5S$ (472.94g/mol)	37.53^{f} ± 0.58	27.21^{d} ± 1.15
9	CAM9	 $C_{23}H_{21}ClN_2O_5S$ (472.94g/mol)	68.09^{b} ± 0.88	19.18^{f} ± 0.71

10	CAM10	 $C_{23}H_{21}ClN_2O_5S$ (472.94g/mol)	77.85[a] ± 1.21	20.25[f] ± 0.86
11	CAM18	 $C_{24}H_{23}ClN_2O_6S$ (502.97g/mol)	32.26[g] ± 0.80	22.59[e] ± 0.66
12	CAM22	 $C_{23}H_{21}ClN_2O_6S$ (488.94g/mol)	57.89[d] ± 0.91	30.17[c] ± 0.57
13	Butylated Hydroxy Toluene (BHT)		91.21 ± 0.29	***
14	Streptokinase		***	75.30 ± 0.32

***S.D**= Standard Deviation, *Means sharing similar alphabet letters are statistically non-significant (P>0.05).*

CONCLUSION

Sulfonamide derivative test series of compounds showed satisfactory antioxidant properties along with mild thrombolytic activity. Biological activities were screened out by *in vitro* assay procedures. Up till now our research findings suggested that these chemicals are good antioxidant agents and by doing some modifications in their structure upon incorporation of active functional groups these compounds may exhibited increase in their thrombolytic potential. These findings could helpful for the research field of medicinal chemistry.

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