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Synthesis of sulpha drug based hydroxytriazene derivatives: Anti-diabetic, antioxidant, anti-inflammatory activity and their molecular docking studies

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ABSTRACT

Herein, we report synthesis, characterization, anti-diabetic, anti-inflammatory and anti-oxidant activities of hydroxytriazenes derived from sulpha drugs, namely sulphanilamide, sulphadiazine, sulphapyridine and sulphamethazine. Before biological screening of the compounds, theoretical prediction using PASS was done which indicates probable activities ranging from Pa (probable activity) values 65–98% for anti-inflammatory activity. As per the predication, experimental validation of some of the predicted activities particularly anti-diabetic, anti-inflammatory and anti-oxidant was done. Anti-diabetic activities have been screened using two methods namely α-amylase and α-glucosidase inhibition method and IC_{50} values were ranging from 66 to 260 and 148 to 401 μg/mL, while for standard drug acarbose the values were 12 μg/mL and 70 μg/mL, respectively. Docking studies have also been done for antidiabetic target pancreatic alpha amylase. The molecular docking studies in α-amylase enzyme reveal that the middle phenyl ring of all the compounds mainly occupies in the small hydrophobic pocket formed by the Ala198, Trp58, Leu162, Leu165 and Ile235 residues and sulphonamide moiety establish H-bond interaction by two water molecules. Further, anti-inflammatory activity has been evaluated using carrageenan induced paw-edema method and results indicate excellent anti-inflammatory activity by hydroxytriazenes (71 to 97%) and standard drug diclofenac 94% after 4 h of treatment. Moreover, antioxidant effect of the compounds was tested using DPPH and ABTS methods. All the compounds displayed good results (24–488 μg/mL) against ABTS radical and many compounds are more active than ascorbic acid (69 μg/mL) while all other compounds showed moderate activity against DPPH radical (292–774 μg/mL) and ascorbic acid (29 μg/mL). Thus, the studies reveal potential of sulfa drug based hydroxytriazenes as candidates for anti-diabetic, anti-inflammatory and antioxidant activities which have been experimentally validated.

1. Introduction

Nowadays diabetes is considered as most common chronic diseases and leading cause of death and disability worldwide [1,2]. Diabetes mellitus is a group of metabolic disorders in which increased oxidative stress plays an important role in long term damages, dysfunctions and failure of different organs such as kidneys, eyes nerves, heart and blood vessels [3–5]. Oxidative stress is mainly due to imbalance between free radicals generation and scavenging systems. Some experimental studies have reported that increased production of free radicals and defeat antioxidant protection of free radicals involved in pathogenesis of diabetes [6]. Another source of oxidative stress in diabetic patient might be auto-oxidation of glucose, redox imbalance, decreased concentration of low molecular weight antioxidant and metabolic abnormalities [7,8]. This can lead to increased lipid peroxidation, development of insulin resistance and damages of cellular organelles and enzymes.

Further, the antioxidant therapy provide a therapeutic strategy to defend the β-cells against oxidative stress and prevent related diabetic vascular complication. An antioxidant diminishes free radicals, enhance scavenging of free radicals and antioxidant defense mechanism. Although, previous studies demonstrated benefit of antioxidants with diabetic drugs in prevention of diabetic complications [9]. Antioxidant may inhibit formation of reactive oxygen species (ROS) or scavenge free radicals or enhance capabilities of enzymes which prevent oxidative stress. It is also reported that administration or dietary vitamin E as

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supplement significantly decreases blood glucose by inhibiting the sequence of oxidative stress [10,11].

Computer aided drug designing (CADD) has attracted attention of chemists and its application has been gaining importance of late. PASS is an online freely accessible web resource (http://www.way2drug.com/passonline), designed for the prediction of the biological activity spectra of organic compounds based on their structural formulas which predicts more than 4000 types of biological activities [12]. The prediction is mainly based on an examination of the structure activity relationships with the training set containing information on the structure and biological activity of more than 300,000 organic compounds. In our study, the PASS for 26 compounds with hydroxytriazene moiety have shown Pa values for the validated activities around ≥40%. Further, not only theoretical prediction has been done but also molecular docking studies with particular targets for each of the representative series compound has been done. The docking studies describe the DNA microgroove binding throwing light on the drug action of these compounds.

The inflammation is caused in animals as a protective response to a cell injury. The clinical signs such as edema, erythema, hyperalgesia, pain and loss of function at microscopic level are exhibited in this response. The non-steroidal anti-inflammatory drug (NSAID) normally acts at the periphery and not at CNS. These drugs block the synthesis of ecosanoids at the site of injury which results in blocking the cytokines and cyclooxygenase pathway (COX) and thereby reducing the production of prostaglandins [13] and inhabiting release of histamine [14].

Hydroxytriazenes have occupied an important role in analytical and medicinal chemistry due to their easy synthesis, good yield and atom economy. However, biological activities of hydroxytriazenes have hardly been attempted and only few reports on anti-microbial [15–17], wound-healing [18], anti-inflammatory [19,20], antidiabetic [21], antioxidant [21,22], anti-dyslipidemic [22] and insecticidal [23,24] activities are reported in literature. Thus, in the present study four series of sulfa drug based hydroxytriazenes have been synthesized, characterized and evaluated for antidiabetic, anti-inflammatory and antioxidant activities.

2. Result and discussion

2.1. Chemistry

Hydroxytriazenes incorporated sulpha drugs namely sulphanilamide (SFN-1 to 8), sulphadiazine (SFD-1 to 7), sulphapyridine (SFP-1 to 5) and sulphamethazine (SMT-1 to 6) were synthesized by using the method reported by Elkins and Hunter [25] and further modified by Sogani and Bhattacharya [26,27]. This method involves diazocoupling reaction of alkyl or aryl hydroxylamine with diazonium salt obtained from sulfa drugs at 0–5 °C in acetate buffer medium of pH 5–6. All the synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR and EI MS techniques (Scheme 1 and Supporting data, S2). The structures of hydroxytriazenes were confirmed by appearance of a singlet for *N*-OH proton around 12 ppm in ^{1}H NMR spectra followed by the presence of peak around 3400 cm^{-1}for (*N*-OH) and 1500 cm^{-1}for (N=N), in the IR spectrum.

2.2. Antidiabetic study

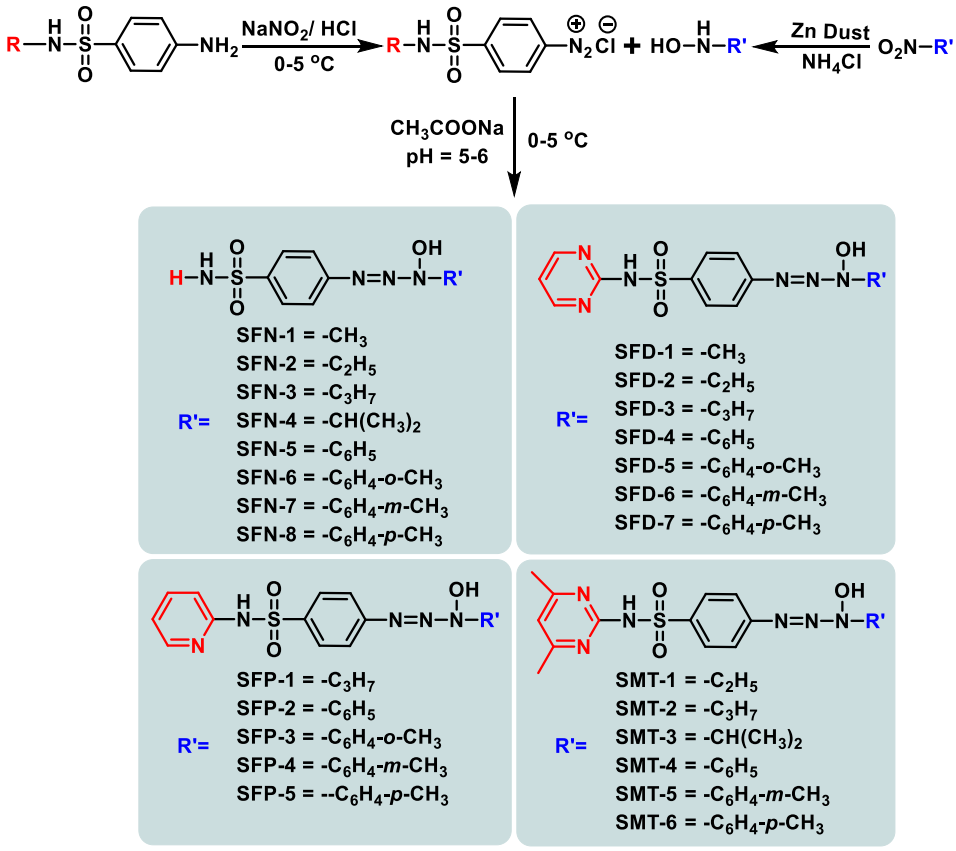
In this investigation all the synthesized compounds were evaluated for their antidiabetic action via two in-vitro assays namely, α-amylase and α-glucosidase inhibition method and results were compared with acarbose standard reference in both α-glucosidase (viz 12.21 μg/mL, $y = 0.226x + 47.24$, $R^2 = 0.939$) and α-amylase (69.74 μg/mL, $y = 0.435x + 19.66$, $R^2 = 0.995$) methods. The α-glucosidase and α-amylase activity of four different series of hydroxytriazenes (SFN, SFD, SFP and SMT series) are present in Table 1, and results are expressed as IC_{50} values. As revealed from Table 1, in case of SFN series, IC_{50} values for α-glucosidase and α-amylase inhibitory activity varies from 148 to 361 and 31–214 μg/mL respectively, which can be concluded as moderate to excellent activity. It is interesting to note that out of this series, SFN-2 (66 μg/mL), SFN-3 (31 μg/mL) SFN-4 (52 μg/mL) and SFN-7 (72 μg/mL) have shown excellent α-amylase activity as compared to standard acarbose (69.74 μg/mL) which warrants future investigation on these compounds. Remaining all series namely SFD, SFP and SMT have shown moderate to good α-glucosidase (IC_{50}, 175–401 μg/mL) and α-amylase (IC_{50}, 149–260 μg/mL) inhibition activity. Looking at the results of all the four series, it can be concluded that these compounds inhibit α-amylase enzyme better than α-glucosidase enzyme.

The investigation of *Structure-activity relationship (SAR)* was based on the two ending substituents placed on amino group of 4-sulphonamidophenyl ring and another one at nitrogen atom of the hydroxy triazene moiety. The amplification of cyclic and/or long chain bulky substitutions at both ends as in compounds SFN-2 – SFN-8, SFD-2 – SFD-7, SFP-1 – SFP-5 and SMT-1 to SMT-6 significantly reduce the α-glucosidase inhibitory activity. The presence of small steric substituent like methyl at the end of the triazene moiety made the molecules moderate potent (SFN-1 & SFD-1; IC_{50} = 148.19 & 175.64 μg/ml, respectively). Hence, concurrently occurrence of two steric substituents (pyridyl, pyrimidinyl, alkyl & aralkyl) at both ends is not significant for inhibiting the α-glucosidase enzyme. In the SFN series, four compounds (SFN-2, SFN-3, SFN-4 and SFN-7) possess good α-amylase enzyme inhibition activity. Among the four compounds, the increase of non-polar alkyl carbons from ethyl to isopropyl substituents in compounds SFN-2 (IC_{50} = 66. μg/mL), SFN-3 (IC_{50} = 31.71 μg/mL), SFN-4 (IC_{50} = 51.77 μg/mL) enhance the potency of the α-amylase inhibition. It suggests that the α-amylase enzyme inhibition is related with lipophilicity attachment on the nitrogen atom of the hydroxy triazene moiety. The presence of aromatic/alkyl group in the compounds SFN-5, SFN-6 and SFN-8 showed moderate α-amylase enzyme inhibitory activity except the compound SFN-7 which bearing *m*-methylphenyl substituent. The insertion of pyrimidine (in SFD series), pyridine (in SFP series) and dimethylpyrimidine (in SMT series) at the amino group of 4-sulphonamidophenyl exhibited moderate biological potency. Therefore, heteroaromatic incorporation is not crucial for improving the α-amylase enzyme inhibition activity. The SAR analysis suggests that, only hydrophobic modification tolerable at hydroxy triazene portion in all series would be a promising lead for the further development of antidiabetic agents.

It is known that treatments of type 2 diabetes mellitus (T2DM) include improvement of insulin sensitivity or reducing the rate of carbohydrate absorption from the gastrointestinal tract. α-Amylase and α-glucosidase are the key enzymes taking part in carbohydrate metabolism. Both enzymes are the key players in the postprandial hyperglycemia noticed in case of non-insulin dependent diabetes mellitus (NIDDM; T2DM). So, inhibition of α-amylase and α-glucosidase enzymes can act as potential targets that can serve as a powerful strategy in the treatment of NIDDM [28,29].

2.3. Antiradical activity

Antiradical activity of sulpha drug based hydroxytriazenes by DPPH and ABTS methods are depicted in Table 1. The results were expressed by IC_{50} values and compared with standard reference ascorbic acid (29.12 μg/mL $Y = 0.1371 X + 46.017$, $R^2 = 0.9609$ for DPPH method and 69.133 μg/ml $Y = 0.599x + 8.589$ $R^2 = 0.991$ for ABTS method). In case of SFN series, the IC_{50} values vary from 292 to 523 μg/mL against DPPH free radical, which can be considered as poor to moderate antiradical activity. The IC_{50} values for this series vary from 24 to 359 μg/mL for ABTS free radical, which can be considered as moderate to excellent activity. The compounds SFN-5,6,7,8 exhibited excellent antiradical activity with IC_{50} values 24, 31, 27 and 28 μg/mL respectively, which are better than standard reference ascorbic acid (69, μg/mL). The SFD, SFP and SMT series showed moderate to poor anti-



Scheme 1. Synthesis of sulpha drug based hydroxytriazene derivatives.

Table 1
Antidiabetic and antioxidant activities sulpha drug based hydroxytriazene derivatives.

S.No.	Compound	α-Glucosidase Inhibition IC_{50} (μg/mL)	α-Amylase Inhibition IC_{50} (μg/mL)	DPPH Inhibition IC_{50} (μg/mL)	ABTS Inhibition IC_{50} (μg/mL)
1.	SFN-1	148.19	111.42	305	265
2	SFN-2	190.83	**66**	337	277
3	SFN-3	231.5	**31.28**	337	359
4	SFN-4	204.71	**51.71**	348	316
5	SFN-5	264.98	113.28	523	**24.18**
6	SFN-6	361.26	122	292	**31.08**
7	SFN-7	278.82	**71.77**	298	**27.52**
8	SFN-8	319.75	214.39	457	**28.24**
9	SFD-1	175.64	154.86	641	488.25
10	SFD-2	238.26	149.52	774	291.76
11	SFD-3	282.65	184.67	365	363.25
12	SFD-4	321.33	168.16	302	402.16
13	SFD-5	353	213.86	406	**42.65**
14	SFD-6	325.2	170.78	367	**34.53**
15	SFD-7	205	201.86	341.83	78.51
16	SFP-1	256.22	125.67	370	249.07
17	SFP-2	366.43	223.19	357.5	**38.04**
18	SFP-3	357.97	218.45	355.57	**36.85**
29	SFP-4	393.68	260.13	383.8	**44.95**
20	SFP-5	328.16	214.53	345.04	**43.13**
21	SMT-1	230.47	148.3	331	277
22	SMT-2	183.93	157.54	425	218.71
23	SMT-3	231.8	171.93	344	279.02
24	SMT-4	344.22	161.4	265.75	**33.06**
25	SMT-5	401.28	253	440	**33.53**
26	SMT-6	320	222.5	562	131.08
27	Standard drug	12.21 (Acarbose)	69.74 (Acarbose)	29.12 (Ascorbic acid)	69.13 (Ascorbic acid)

radical activity by DPPH method with IC_{50} values 302–774 μg/mL. In the SFD series, compound SFD-5 (42.65 μg/mL), SFD-6 (34.53 μg/mL) and SFD-7 (78.51 μg/mL) exhibited excellent activity in ABTS method while all the remaining compounds from this series showed moderate activity as compare to standard reference ascorbic acid (69 μg/mL). The compound SMT-4 (33.06 μg/mL) and SMT-5 (33.53 μg/mL) from SMT series showed excellent activity by ABTS method, which is also better than standard reference ascorbic acid (69 μg/mL) and remaining compounds from this series showed moderate activity. Only compound SMT-4 (IC_{50} 265 μg/mL) showed moderate DPPH radical scavenging activity among all the reported compounds and standard reference with IC_{50} value 29 μg/mL.

The measurement of radical scavenging activity of any antioxidant is frequently associated with using of DPPH and ABTS methods just because their reliable, quick and reproducible quality to search the *in vitro* general antioxidant activity of compounds [30]. These methods depend on the reduction of the purple DPPH radical and blue ABTS radical by accepting electron or radical hydrogen from antioxidant (A–H) to a stable diamagnetic molecule as described in the following reactions:

$(DPPH)^{\cdot} + A\text{-}H \rightarrow DPPH\text{-}H + (A)^{\cdot}$ (Purple to yellow)

$(ABTS)^{\cdot} + A\text{-}H \rightarrow ABTS\text{-}H + (A)^{\cdot}$ (Blue to colorless)

The intensity of color change from purple to yellow and blue to colorless at variable concentrations are measured spectrophotometrically at 517 nm and 734 nm, respectively. The degree of discoloration shows the scavenging potential of the antioxidant compounds in the term of hydrogen donating ability.

Structure-activity relationship: In ABTS radical scavenging activity, most of the compounds exhibited potent activities with IC_{50} range from 24 to 45 μg/mL (Examples: compound SFN-5 to 8, SFD-5, 6, SFP-2 to 5 and SMT-4,5) in comparison with reference compound, ascorbic acid

(IC_{50} = 69.13 μg/mL). The ABTS radical scavenging properties depend on the incorporation of phenyl and o/m-alkyl substituted phenyl groups at the hydroxytriazene moiety. Inclusion of only 2–3 alkyl groups significantly reduces the ABTS radical scavenging activity as in compounds SFN-1, SFD-2, SFP-1, SMT-1 etc (IC_{50} = > 218 μg/mL). The addition of pyridine & pyrimidine at -SO_2-NH- portion did not influence the scavenging activity in all series of most active compounds. Hence the heteroaromatic substituents are not vital for the ABTS radical scavenging activity.

The compound SMT-4 revealed moderate DPPH radical scavenging activity (IC_{50} = 265 μg/mL) due the presence of presence of unsubstituted phenyl at the basic skeleton of sulfonamide linked triazene. The drastic reduction of DPPH radical scavenging activity appears in remaining compounds by means of existence of the -SO_2-NH-substituted heteroaromatic rings like pyridine & pyrimidine along with triazene *N*-alkyl or alkyl substituted phenyl ring at either side of the basic skeleton of the compounds, respectively.

2.4. Anti-inflammatory study

Biological activity spectra of all the synthesized compounds were calculated using PASS web tool. PASS tool will interpret the biological active spectra using 2D structure of molecules. Interpretation of results of PASS as described in supporting information reveled that all the hydroxytriazenes displayed good anti-inflammatory probable activity upto 96%. The probable anti-inflammatory activity of the compounds has been experimentally validated by carrageenan induced hind paw edema method.

Results of anti-inflammatory activity are presented as % inhibition of paw-edema after 2 and 4 h. As revealed from Table 2, all the compound showed good to excellent anti-inflammatory activity in terms of percent reduction of paw edema volume which were 32–62% after 2 h and 71–97% after 4 h. This is explicitly clear from the value that all the compounds screened for anti-inflammatory activity have shown ≈97% inhibition of paw-edema after 4 h which are comparable to or better than the standard drug (diclofenac). It has been observed that presence of alkyl group attached to triazene moiety may enhance the anti-inflammatory activity. If we compare results of anti-inflammatory activity of all the three series reported, sulphamethazine containing hydroxytriazenes (SMT series) has exhibited best results since it has two additional methyl group attached to the molecule in addition to the other functional group. The tentative mechanism of action exhibited by hydroxytriazenes is proposed to be NSAID like as described under introduction.

Table 2
Anti-inflammatory activity of sulpha drug based hydroxytriazene derivatives.

S. No.	Code	% Inhibition of paw edema after 2 h	% Inhibition of paw edema after 4 h
1	SFN-1	32.50	71.42
2	SFN-2	45.00	85.71
3	SFN-3	47.51	**91.42**
4	SFN-4	57.53	88.57
5	SFN-5	55.00	88.71
6	SFN-6	62.52	85.71
7	SFN-7	50.00	88.57
8	SFP-1	55.00	80.0
9	SFP-2	52.53	**94.28**
10	SFP-3	55.00	77.14
11	SFP-4	55.00	**91.42**
12	SFP-5	47.51	71.42
13	SMT-1	55.00	88.57
14	SMT-2	55.00	82.85
15	SMT-3	50.00	**94.28**
16	SMT-4	62.52	**94.28**
17	SMT-5	55.00	**94.28**
18	SMT-6	50.00	**97.14**
19	Diclofenac	57.50	94.28

2.4.1. Correlation of antidiabetic, antioxidant and anti-inflammatory activities in type 2 diabetes mellitus

Current studies have demonstrated that oxidative stress plays an important role in the pathogenesis of type 2 diabetes [31,32] and may diminish the antioxidative defense system of the body. This can lead to damage of cellular organelles and enzymes, increased lipid peroxidation and development of insulin resistance and after damage has occurred, preventing further cell degeneration [33]. Type 2 diabetes is also associated with endothelial dysfunction, which may develop into macro and microvascular diseases such as retinopathy, nephropathy, lower extremity amputations, coronary artery and cardiovascular diseases [34,35].

Many studies have demonstrated that inhibition of intracellular free radicals generation would offer an alternate way to prevent oxidative stress and related diabetic complications. Source of oxidative stress in diabetic patient is related to leaking of ROS from mitochondria, autoxidation of glucose, redox imbalance etc. Bajaj et al. [36] reviewed the effect of antioxidant therapy in management of diabetes. Supplementation with antioxidants may potentially improve endothelial dysfunction in T2DM by recoupling of RNS, mitochondrial function as well as macro/microvascular complications. The non-enzymatic antioxidant such as biogenic and synthetic substance and natural cellular defense mechanism may work by neutralizing free radicals by producing glutathione, ubichinol and uric acid. The enzymatic antioxidants particularly dismutase, glutathione peroxidase, glutathione reductase and catalase may remove ROS directly or sequentially preventing their excessive accumulation and consequent adverse effect. Rajendiran et al. [11] studied that vitamin C and E, alpha-lipoic acid, selenium play important role in controlling in hyperglycemia. These antioxidants may work by significantly increasing SOD and GSH-Px activity and inhibiting sequence of oxidative stress. The mechanism of inhibition of oxidative stress is not very clear but the plasma glucose level decreased by increasing glucose metabolism in peripheral tissue. Aforementioned findings suggest that intake of antioxidant with hypoglycemic drugs may have cumulative/synergistic antioxidant effects alleviating the progression of conditions caused by oxidative stress. More studies are warranted to better understand the biological mechanisms of simultaneous intake of hypoglycaemic drug with antioxidants.

The association between hyperglycemia, inflammation and vascular complications in diabetes is now well established [37]. In diabetes, hyperglycemia and elevated free fatty acids may promote inflammation by stimulating glucose utilization. Glucotoxicity and lipotoxicity might also exert oxidative and endoplasmic reticulum stress, which in turn elicits an inflammatory response. Metabolic stress (hyperglycemia) and elevated free fatty acid levels, may initiate insulin dysfunction [38]. Multiple mechanisms are thought to contribute to β-cell dysfunction, insulin resistance and vascular complications of diabetes. Thus, the regulation of inflammation play a major role in the management of diabetic mellitus complications. Furthermore, under the condition of oxidative stress by generation of ROS and RNS species also play crucial role in activation of signaling pathway which affect intra and extra cellular pathway. At the site of inflammation accumulation of ROS occurs because mast cells and leukocytes are recruited, which leads to a 'respiratory burst' due to an increased uptake of oxygen. This sustained inflammatory/oxidative environment leads to a vicious circle, which can damage healthy neighboring epithelial and stromal cells and over a long period of time may lead to carcinogenesis [39,40].

Xie et al. described the multifunctional role of hydroxytyrosol-fenofibrate with antidiabetic, antihyperlipidemic, antioxidant and anti-inflammatory activities towards the defense of pancreatic β cells from oxidative stress damage, preserve liver function and death induced by sustained oxidative stress in type 2 diabetes mellitus disorder (T2DM) complications [41]. Similarly, our synthesized compounds also possess the triple functional biological activities (antidiabetic, antioxidant and anti-inflammatory) as shown in Tables 1 and 2. Such a combination of activities present in the sulpha drug based hydroxytriazene derivatives

could suppress the progress of T2DM by inhibiting the damage of pancreatic β cells and liver tissues through their antioxidant and anti-inflammatory biological properties.

2.5. Docking study

A molecular operating environment (MOE 2015.1001) software package [42,43] was used for docking study. This was attempted to bring better understanding of mechanism of action of sulpha drug based hydroxytriazene compounds (SFN-2, SFD-1, SFP-2 & SMT-2) toward the human pancreatic alpha-amylase enzyme. Analysis was done for induced-fit docking in to the active site of co-crystal structure of PDB: 2QV4. The results showed dissimilarity in binding sites of hydroxytriazenes and reference acarbose due to the different geometries with short length and/or greater hindrance of the compounds. The binding energies have been predicted within the range of 6.97–7.29 Kcal/mol (S score) compared to standard drug of acarbose (−9.95 Kcal/mol). The middle phenyl ring of all compounds occupies in the small hydrophobic pocket formed by the Ala198, Trp58, Leu162, Leu165 and Ile235 residues. The sulphonamide moiety of SFN-2 compound forms three hydrogen bond interactions: with the NH− of His201 residue, Ile235 and Lys200 residues through two water molecules. The negative charges of hydroxytriaz-1-enyl of all compounds interact electrostatically with the acidic residues of Asp197, Glu233 and Asp300. The ethyl end of SFN-2 makes hydrophobic contact with the Trp58 and Trp59 residues. The hydroxytriazene moiety of SFD-1 and SFP-2 compounds forms H-bonding interactions with the His201 residue. In compound SFD-1, −NH and −S═O of sulphonamide groups make strong double H-bonding interactions with the Asp300, His299 & Arg195 residues. The position of the pyridine and pyrimidine (SFD-1, SFP-2 & SMT-2) moieties is stabilized by π-stacking interactions with aromatic side chains of Trp58, Trp59 and Tyr62. The alkyl fragments of isopropyl and propyl in SFP-2 and SMT-2 make a small hydrophobic contact with Tyr151 and Ile235 (Figs. 1 & 2). Molecular docking results indicated that double hydrogen bonding contacts of sulphonamide moiety and the middle phenyl hydrophobic interactions play roles in the binding of these compounds within the binding site of alpha-amylase enzyme. It gives clue to optimize the derivatives with strong potency in further lead development.

3. Conclusion

It is apparent from the present studies that hydroxytriazenes based on sulpha drugs have shown excellent anti-diabetic, anti-oxidants as well as anti-inflammatory activity. Compounds SFN-2, SFN-3, SFN-4 and SFN-7 were found to be most potent α-amylase inhibitors among all the tested compounds with IC_{50} value of 66 μg/mL, 31.28 μg/mL 51.71 μg/mL and 71.77 μg/mL respectively, comparable to standard acarbose (69.74 μg/mL). Many compounds from all series were displayed excellent antioxidant activities by ABTS method, even better than standard ascorbic acid. All the compounds were also exhibited 71–97% reduction in inflammation while standard drug diclophenac reduce by 94% after 4 h of treatment. However, the SAR as well as prediction studies indicated that, particularly anti-inflammatory activity can be explored further with more such compounds. Docking results indicated that the best binding was with middle phenyl ring of all compounds with small hydrophobic pocket formed by the Ala198, Trp58, Leu162, Leu165 and Ile235 residues and hydrogen bonding with hydroxyl group responsible for inhibition of α-amylase enzyme. Thus, this study explores, theoretical prediction, antidiabetic, antioxidant, anti-inflammatory activities and docking studies of hydroxytriazenes for development of potential multifunctional medicinal candidates.

4. Experimental section

4.1. Material and methods

Commercially available Sulpha drugs of Sigma-Aldrich have been used as it is, and other chemicals and solvents of analytical reagent grade have been used. Melting point was measured in open glass capillaries using SONAR apparatus. NMR spectra of sulpha drug based hydroxytriazene derivatives which had been synthesized were collected using Bruker Avance II 400 NMR spectrometer operating at 400 MHz in deuterated DMSO-D6 solvent at SAIF Chandigarh. XEVO-G2SQTOF-EI-MS was used to determine mass at MNIT, Jaipur. IR spectra were collecting using Bruker Optik GmbH Alpha Sample Compartment RT-DLaTGS ZnSe HR 0.8 IR instrument. Thin layer chromatography was used to confirm single spot of pure compound using precoated silica gel plate and visualized using UV lamp at 254 nm.

4.2. General procedure for synthesis of compounds

Sulpha drug based hydroxytriazene derivatives were obtained in three steps. First one is reduction, in which 0.02 mol of nitro compounds (aromatic as well as aliphatic) reduced with 0.02 mol NH_4Cl and 0.04 gm of zinc dust at 50–60 °C to get hydroxylamine. Then second one is diazotization of various sulpha drugs taken as an amine group through diazotization procedure with HNO_2/HCl at 0–5 °C. Third one is coupling, in which products of above two steps were coupled at 0–5 °C and pH 5–6. Upon completion of coupling, product was obtained as precipitation which was filtered and crystallized using in appropriate solvent to afford pure product.

4.2.1. 4-(3-hydroxy-3-methyltriaz-1-en-1-yl)benzenesulfonamide (SFN-1)

4-(3-hydroxy-3-methyltriaz-1-en-1-yl)benzenesulfonamide (SFN-1) m.p. 198–200 °C; Crystallization: methanol; IR (KBr): V_{O-H} (3559 cm^{-1}), V_{NH2} (3322, 3185 cm^{-1}), $V_{N=N}$ (1588 cm^{-1}), V_{C-N} (1222 cm^{-1}), $V_{S=O}$ (1145 cm^{-1}); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.96 (3H, S, H_1), 11.59 (1H, S, H_2), 7.17–7.38 (2H, d, Ar−$H_{4,5}$, J = 6.92 Hz), 7.70–7.73 (2H, d, Ar−H_{6-7}, J = 6.92 Hz), 7.174 (2H, S,

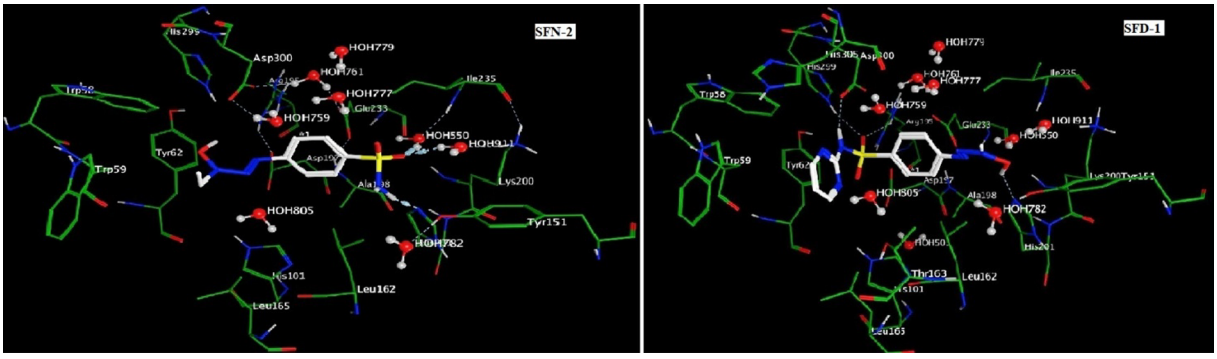


Fig. 1. Predicted binding modes of **SFN-2 and SFD-1**with the human pancreatic alpha-amylase active site.

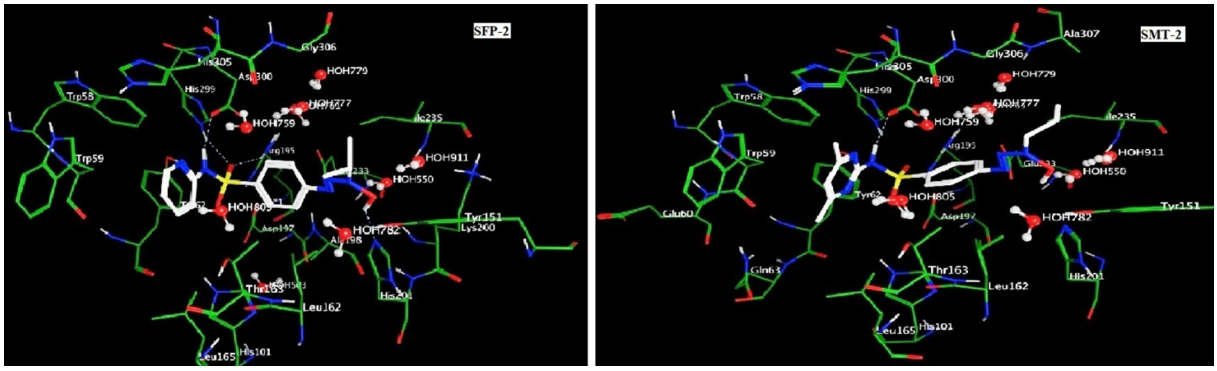


Fig. 2. Predicted binding modes of **SFP-2 & SMT-2**with the human pancreatic α-amylase active site.

H_9); ^{13}C NMR (400 MHz, DMSO-d_6): δ 50.68 (1C, C_1), 143.62 (1C, C_3), 113.49 (2C, $C_{4,5}$), 127.22 (2C, C_{6-7}), 136.43 (1C, C_8); ESI-HRMS for $C_7H_{10}N_4O_3S$, calcd. 230.244, found 231.2469.

4.2.2. 4-(3-ethyl-3-hydroxytriaz-1-en-1-yl)benzenesulfonamide (SFN-2)

4-(3-ethyl-3-hydroxytriaz-1-en-1-yl)benzenesulfonamide (SFN-2) m.p. 194–196 °C; Crystallization: methanol; IR (KBr): V_{O-H} (3410 cm^{-1}), V_{NH2} (3301, 3226 cm^{-1}), $V_{N=N}$ (1595 cm^{-1}), V_{C-N} (1217 cm^{-1}), $V_{S=O}$ (1149 cm^{-1}); ^1H NMR (400 MHz, DMSO-d_6): δ 1.44 (3H, t, H_1, J = 7.28 Hz), 4.18 (2H, q, H_2, J = 7.28 Hz), 11.53 (1H, S, H_3), 7.39–7.73 (2H, d, Ar—$H_{5,8}$), 7.71–7.73 (2H, d, Ar—$H_{6,7}$, J = 8.8 Hz), 7.174 (2H, S, H_{10}); ^{13}C NMR (400 MHz, DMSO-d_6): δ 12.37 (1C, C_1), 58.31 (1C, C_2), 143.73 (1C, C_3), 113.51 (2C, $C_{4,8}$), 127.21 (2C, $C_{5,7}$), 136.39 (1C, C_6); ESI-HRMS for $C_8H_{12}N_4O_3S$, calcd. 244.2709, found 245.2770.

4.2.3. 4-(3-hydroxy-3-propyltriaz-1-en-1-yl)benzenesulfonamide (SFN-3)

4-(3-hydroxy-3-propyltriaz-1-en-1-yl)benzenesulfonamide (SFN-3) m.p. 169–171 °C; Crystallization: methanol; IR (KBr): V_{O-H} (3410 cm^{-1}), V_{NH2} (3324, 3214 cm^{-1}), $V_{N=N}$ (1592 cm^{-1}), V_{C-N} (1215 cm^{-1}), $V_{S=O}$ (1149 cm^{-1}); ^1H NMR (400 MHz, DMSO-d_6): δ 0.916 (3H, t, H_1, J = 7.2 Hz), 1.9202 (2H, m, H_2, J = 7.2 Hz, 7.5 Hz), 4.12 (2H, t, H_3, J = 7.5 Hz), 11.55 (1H, S, H_4), 7.36–7.39 (2H, d, Ar—$H_{6,10}$, J = 8.84 Hz), 7.71–7.74 (2H, dd, Ar—$H_{7,9}$, J = 8.84 Hz), 7.178 (2H, S, H_{11}); ^{13}C NMR (400 MHz, DMSO-d_6): δ 10.64 (1C, C_1), 20.21 (1C, C_2), 64.59 (1C, C_3), 143.71 (1C, Ar—C_5), 113.53 (2C, Ar—$C_{6,10}$), 127.22 (2C, Ar—$C_{7,9}$), 136.41 (1C, Ar—C_8); ESI-HRMS for $C_9H_{14}N_4O_3S$, calcd. 258.2975, found 259.2971.

4.2.4. 4-[3-hydroxy-3-(propan-2-yl)triaz-1-en-1-yl] benzenesulfonamide (SFN-4)

4-[3-hydroxy-3-(propan-2-yl)triaz-1-en-1-yl] benzenesulfonamide (SFN-4) m.p. 190 °C; Crystallization: methanol; IR (KBr): V_{O-H} (3344 cm^{-1}), V_{NH2} (3289, 3209 cm^{-1}), $V_{N=N}$ (1590 cm^{-1}), V_{C-N} (1208 cm^{-1}), $V_{S=O}$ (1150 cm^{-1}); ^1H NMR (400 MHz, DMSO-d_6): δ 1.146 (6H, d, $H_{1,2}$, J = 6.8 Hz), 4.53 (1H, m, H_3, J = 6.8 Hz), 11.29 (1H, S, H_4), 7.18–7.358 (2H, m, Ar—$H_{6,10}$), 7.70–7.731 (2H, m, Ar—$H_{7,9}$), 7.185 (2H, S, H_{11}); ^{13}C NMR (400 MHz, DMSO-d_6): δ 20.12 (2C, $C_{1,2}$), 65.20 (1C, C_3), 143.90 (1C Ar—C_5), 113.50 (2C, Ar—$C_{6,10}$), 127.19 (2C, Ar—$C_{7,9}$), 136.31 (1C, Ar—C_8); ESI-HRMS for $C_9H_{14}N_4O_3S$, calcd. 258.2975, found 259.2971.

4.2.5. 4-(3-hydroxy-3-phenyltriaz-1-en-1-yl) benzene sulfonamide (SFN-5)

4-(3-hydroxy-3-phenyltriaz-1-en-1-yl) benzene sulfonamide (SFN-5) m.p.170 °C; Crystallization: ethylalcohol; IR (KBr): V_{O-H} (3356 cm^{-1}), V_{NH2} (3260, 3199 cm^{-1}), $V_{N=N}$ (1597 cm^{-1}), V_{C-N} (1252 cm^{-1}), $V_{S=O}$ (1150 cm^{-1}); ^1H NMR (400 MHz, DMSO-d_6): δ 7.58–7.65 (5H, m, H_{2-6}), 12.29 (1H, S, H_7), 7.79–7.82 (2H, d, Ar—$H_{9,13}$, J = 8.84 Hz), 8.12–8.15 (2H, d, Ar—$H_{10,12}$, J = 8.84 Hz), 7.26 (2H, S, H_{14}); ^{13}C NMR (400 MHz, DMSO-d_6): δ142.93 (1C, C_1), 119.92 (2C, Ar—$C_{2,6}$), 130.20 (2C, Ar—$C_{3,5}$), 129.27 (1C, Ar—C_4), 142.20 (1C Ar—C_8), 114.49 (2C, Ar—$C_{9,13}$), 127.24 (2C, Ar—$C_{10,12}$), 137.47 (1C, Ar—C_{11}); ESI-HRMS for $C_{12}H_{12}N_4O_3S$, calcd. 292.3137, found 292.3223.

4.2.6. 4-[3-hydroxy-3-(2-methylphenyl)triaz-1-en-1-yl] benzenesulfonamide (SFN-6)

4-[3-hydroxy-3-(2-methylphenyl)triaz-1-en-1-yl]benzenesulfonamide (SFN-6) m.p.189–191 °C; Crystallization: ethylalcohol; IR (KBr): V_{O-H} (3391 cm^{-1}), V_{NH2} (3313, 3263 cm^{-1}), $V_{N=N}$ (1583 cm^{-1}), V_{C-N} (1301 cm^{-1}), $V_{S=O}$ (1147 cm^{-1}); ^1H NMR (400 MHz, DMSO-d_6): δ 2.42 (3H, S, H_1), 7.39–7.50 (4H, m, Ar—H_{3-6}), 12.18 (1H, S, H_8), 7.65–7.67 (2H, d, Ar—$H_{10,14}$, J = 7.84 Hz), 7.75–7.78 (2H, d, Ar—$H_{11,13}$, J = 7.84 Hz), 7.22 (2H, S, H_{15}); ^{13}C NMR (400 MHz, DMSO-d_6):δ 17.99 (1C, C_1), 131.40 (1C, Ar—C_2), 131.68 (1C, Ar—C_3), 126.83 (1C, Ar—C_4), 129.94 (1C, Ar—C_5), 123.87 (1C, Ar—C_6), 143. 63 (1C, Ar—C_7), 143.68 (1C, Ar—C_9), 114.22 (2C, Ar—$C_{10,14}$), 127.28 (2C, Ar—$C_{11,13}$), 137.21 (1C, Ar—C_{12}), ESI-HRMS for $C_{13}H_{14}N_4O_3S$, calcd. 306.3403, found 307.3417.

4.2.7. 4-[3-hydroxy-3-(3-methylphenyl)triaz-1-en-1-yl] benzenesulfonamide (SFN-7)

4-[3-hydroxy-3-(3-methylphenyl)triaz-1-en-1-yl]benzenesulfonamide (SFN-7) m.p.178–180 °C; Crystallization: ethylalcohol; IR (KBr): V_{O-H} (3418 cm^{-1}), V_{NH2} (3335, 3275 cm^{-1}), $V_{N=N}$ (1598 cm^{-1}), V_{C-N} (1296 cm^{-1}), $V_{S=O}$ (1148 cm^{-1}); ^1H NMR (400 MHz, DMSO-d_6): 2.44 (3H, S, H_1), 7.61–7.64 (2H, d, Ar—$H_{10,14}$, J = 8.84 Hz), 7.77–7.80 (2H, d, Ar—$H_{11,13}$, J = 8.84 Hz), 7.94 (1H, d, Ar—H_5, J = 8.12 Hz), 7.90 (1H, m, Ar—H_4), 7.48 (1H, d, Ar—H_3, J = 7.8 Hz), 7.36 (1H, d, Ar—H_7, J = 7.6 Hz); ^{13}C NMR (400 MHz, DMSO-d_6):δ 20.88 (1C, C_1), 129.01 (1C, C_3), 139.08 (1C, C_2), 130.82 (1C, C_4), 117.13 (1C, C_5), 120.18 (1C, C_7) 142.95 (1C, C_6), 114.57 (2C, $C_{10,14}$), 127.23 (2C, $C_{11,13}$), 143.22 (1C, C_9), 137.42 (1C, C_{12}); ESI-HRMS for $C_{13}H_{14}N_4O_3S$, calcd. 306.3403, found 307.3470.

4.2.8. 4-[3-hydroxy-3-(4-methylphenyl)triaz-1-en-1-yl] benzenesulfonamide (SFN-8)

4-[3-hydroxy-3-(4-methylphenyl)triaz-1-en-1-yl]benzenesulfonamide (SFN-8) m.p. 112 °C; Crystallization: ethylalcohol; IR (KBr) V_{O-H} (3444 cm^{-1}), V_{NH2} (3361, 3257 cm^{-1}), $V_{N=N}$ (1595 cm^{-1}), V_{C-N} (1237 cm^{-1}), $V_{S=O}$ (1156 cm^{-1}); ^1H NMR (400 MHz, DMSO-d_6): δ 2.32 (3H, S, H_1), 7.30–7.32 (2H, d, Ar—$H_{3,7}$, J = 8.24 Hz), 7.54–7.52 (2H, dd, Ar—$H_{4,6}$, J = 8.24 Hz), 12.12 (1H, S, H_8), 7.69–7.71 (2H, d, Ar—$H_{10,14}$, J = 8.84 Hz), 7.91–7.93 (2H, d, Ar—$H_{11,13}$, J = 8.84 Hz), 7.16 (2H, S, H_{15}); ^{13}C NMR (400 MHz, DMSO-d_6):δ 20.67 (1C, C_1), 129.64 (2C, $C_{3,7}$), 119.76 (2C, $C_{4,6}$), 140.110 (1C, C_2), 140.78 (1C, C_5), 114.48 (2C, $C_{10,14}$), 127.23 (2C, $C_{11,13}$), 143.26 (1C, C_9), 137.31 (1C, C_{12}); ESI-HRMS for $C_{13}H_{14}N_4O_3S$, calcd. 306.3403, found 307. 3474.

4.2.9. 2-{[4-(3-hydroxy-3-methyltriaz-1-en-1-yl)phenyl]sulfonyl} pyrimidine (SFD-1)

2-{[4-(3-hydroxy-3-methyltriaz-1-en-1-yl)phenyl]sulfonyl}pyrimidine (SFD-1) m.p. 238–240 °C; Crystallization: methanol; IR (KBr): V_{O-H} (3421 cm^{-1}), V_{N-H} (3252 cm^{-1}), $V_{N=N}$ (1579 cm^{-1}), V_{C-N} (1259 cm^{-1}), $V_{S=O}$ (1152 cm^{-1}); ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.14 (3H, S, H_1),11.56 (1H, S, H_2), 7.20–7.41 (2H, d, Ar—$H_{4,8}$, J = 8.24 Hz), 7.71–7.92 (2H, d, Ar—$H_{5,7}$, J = 8.24 Hz), 10.03 (1H, S, H_{10}), 8.65 (2H, d, Ar—$H_{12,14}$), 6.99 (1H, d, Ar—H_{13}); ^{13}C NMR (400 MHz, DMSO-d_6): δ 50.68 (1C, C_1), 143.62 (1C, C_3), 113.49 (2C, $C_{4,8}$), 127.47 (2$C_{5,7}$), 137.43 (1C, C_6), 157.62 (1C, C_{10}), 149.2 (2C, $C_{11,13}$), 112.80 (1C, C_{12}); ESI-HRMS for $C_{11}H_{12}N_6O_3S$, calcd. 308.3164, found 309.3172.

4.2.10. 2-{[4-(3-ethyl-3-hydroxytriaz-1-en-1-yl)phenyl]sulfonyl} pyrimidine (SFD-2)

2-{[4-(3-ethyl-3-hydroxytriaz-1-en-1-yl)phenyl]sulfonyl}pyrimidine (SFD-2) m.p. 160–162 °C; Crystallization: methanol; IR (KBr): V_{O-H} (3464 cm^{-1}), V_{N-H} (3227 cm^{-1}), $V_{N=N}$ (1583 cm^{-1}), V_{C-N} (1224 cm^{-1}), $V_{S=O}$ (1153 cm^{-1}); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.58 (3H, t, H_1, J = 6.66 Hz), 4.19 (2H, q, H_2, J = 6.66 Hz),11.06 (1H, s, H_3), 7.15–7.18 (2H, d, Ar—$H_{5,9}$, J = 8.1 Hz), 7.88–7.90 (2H, d, Ar—$H_{6,8}$, J = 8.1 Hz), 10.26 (1H, S, H_{10}), 8.60–8.62 (2H, d, Ar—$H_{12,14}$, J = 5.5 Hz), 8.05–8.08 (1H, d, Ar—H_{13}, J = 5.5 Hz); ^{13}C NMR (400 MHz, DMSO-d_6): δ 12.92 (1C, C_1), 58.96 (1C, C_2), 145.14 (1C, C_4), 113.59 (2C, $C_{5,9}$), 130.67 (2C, $C_{6,8}$), 136.76 (1C, C_7), 158.63 (1C, C_{11}), 142.13 (2C, $C_{12,14}$), 117.91 (1C, C_{13}); ESI-HRMS for $C_{12}H_{14}N_6O_3S$, calcd. 322.3430, found 323.3471.

4.2.11. 2-{[4-(3-hydroxy-3-propyltriaz-1-en-1-yl)phenyl]sulfonyl} pyrimidine (SFD-3)

2-{[4-(3-hydroxy-3-propyltriaz-1-en-1-yl)phenyl]sulfonyl}pyrimidine (SFD-3) m.p. 175–177 °C; Crystallization: methanol; IR (KBr): V_{O-H} (3465 cm^{-1}), V_{N-H} (3258 cm^{-1}), $V_{N=N}$ (1585 cm^{-1}), V_{C-N} (1223 cm^{-1}), $V_{S=O}$ (1150 cm^{-1}); ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.9566 (3H, t, H_1, J = 7.42 Hz), 2.01 (2H, m, H_2, J = 7.42 Hz, J = 7.5 Hz), 4.12 (2H, t, H_3, J = 7.5 Hz), 7.24–7.54 (2H, d, Ar—$H_{6,10}$, J = 8.0 Hz), 7.90–8.061 (2H, d, Ar—$H_{7,9}$, J = 8.0 Hz), 10.25 (1H, S, H_{11}), 8.59–8.61 (2H, d, Ar—$H_{13,15}$, J = 5.5 Hz), 7.643 (1H, d, Ar—H_{14}, J = 5.5 Hz); ^{13}C NMR (400 MHz, DMSO-d_6): δ10.64 (1C, C_1), 20.21 (1C, C_2), 64.60 (1C, C_3), 143.71 (1C, C_5), 113.53 (2C, $C_{6,10}$), 128.22 (2C, $C_{7,9}$), 136.41 (1C, C_8), 157.68 (1C, C_{12}), 152.77 (2C, $C_{13,15}$), 115.10 (1C, C_{14}); ESI-HRMS for $C_{13}H_{16}N_6O_3S$, calcd. 336.3695, found 337.3670.

4.2.12. 2-{[4-(3-hydroxy-3-phenyltriaz-1-en-1-yl)phenyl]sulfonyl} pyrimidine (SFD-4)

2-{[4-(3-hydroxy-3-phenyltriaz-1-en-1-yl)phenyl]sulfonyl}pyrimidine (SFD-4) m.p. 170–172 °C; Crystallization: methanol; IR (KBr): V_{O-H} (3394 cm^{-1}), V_{N-H} (3212 cm^{-1}), $V_{N=N}$ (1579 cm^{-1}), V_{C-N} (1226 cm^{-1}), $V_{S=O}$ (1163 cm^{-1}); ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.036 (1H, S, H_7),6.96–6.987 (1H, m, Ar—H_4), 7.33–7.35 (2H, d, Ar—$H_{3,5}$, J = 7.99 Hz) 7.491–7.51 (2H, d, Ar—$H_{9,13}$, J = 8.1 Hz), 8.07–8.15 (2H, d, Ar—$H_{10,12}$, J = 8.1 Hz), 10.39 (1H, S, H_{18}), 8.59–8.61 (2H, d, Ar—$H_{15,17}$, J = 6.1 Hz), 7.00–7.26 (1H, d, Ar—H_{16}, J = 6.1 Hz); ^{13}C NMR (400 MHz, DMSO-d_6): δ 120.16 (2C, $C_{2,6}$), 130.54 (2C, $C_{3,5}$), 124.72 (1C, C_4), 139.92 (1C, C_1), 114.00 (2C, $C_{9,13}$), 128.25 (2C, $C_{10,12}$), 144.11 (1C, C_8), 136.42 (1C, C_{11}), 150.76 (1C, C_{14}), 146.11 (2C, $C_{15,17}$), 115.98 (1C, C_{16}); ESI-HRMS for $C_{16}H_{14}N_6O_3S$ calcd. 370.3858, found 371.4857.

4.2.13. 2-({4-[3-hydroxy-3-(2-methylphenyl)triaz-1-en-1-yl]phenyl} sulfonyl)pyrimidine (SFD-5)

2-({4-[3-hydroxy-3-(2-methylphenyl)triaz-1-en-1-yl]phenyl}sulfonyl)pyrimidine (SFD-5) m.p. 148–150 °C; Crystallization: ethylalcohol; IR (KBr): V_{O-H} (3392 cm^{-1}), V_{N-H} (3216 cm^{-1}), $V_{N=N}$ (1578 cm^{-1}), V_{C-N} (1223 cm^{-1}), $V_{S=O}$ (1157 cm^{-1}); ^{1}H NMR (400 MHz, DMSO-d_6): δ.2.47 (3H, S, H_1), 7.26–7.41 (4H, m, Ar—H_{3-6}), 7.58–7.62 (2H, d, Ar—$H_{10,14}$, J = 8.1 Hz), 8.09–8.11 (2H, d, Ar—$H_{11,13}$, J = 8.1 Hz), 11.54 (1H, S, H_8), 8.63–8.65 (2H, d, Ar—$H_{16,18}$), 7.26 (1H, m, Ar—H_{17}); ^{13}C NMR (400 MHz, DMSO-d_6): 18.80 (1C, C_1), 130.89 (1C, C_2), 131.94 (1C, C_3), 126.73 (1C, C_4), 130.26 (1C, C_5), 123.94 (1C, C_6), 142.3 (1C, C_7), 113.99 (2C, $C_{10,14}$), 127.40 (2C, $C_{10,13}$), 132.89 (1C, C_{12}), 143.53 (1C, C_9), 158.60 (1C, C_{15}), 156.78 (2C, $C_{16,18}$), 112.40 (1C, C_{17}); ESI-HRMS for $C_{17}H_{16}N_6O_3S$ calcd. 384.4123, found 385.4145

4.2.14. 2-({4-[3-hydroxy-3-(3-methylphenyl)triaz-1-en-1-yl]phenyl} sulfonyl)pyrimidine (SFD-6)

2-({4-[3-hydroxy-3-(3-methylphenyl)triaz-1-en-1-yl]phenyl}sulfonyl)pyrimidine (SFD-6) m.p. 165–167 °C; Crystallization: ethylalcohol; IR (KBr): V_{O-H} (3392 cm^{-1}), V_{N-H} (3201 cm^{-1}), $V_{N=N}$ (1579 cm^{-1}), V_{C-N} (1224 cm^{-1}), $V_{S=O}$ (1157 cm^{-1}); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.46 (3H, S, H_1), 7.26–7.39 (3H, m, Ar—$H_{4,6}$), 7.21 (1H, d, Ar—H_3, J = 3.2 Hz), 11.31 (1H, S, H_8), 7.86–7.88 (2H, d, Ar—$H_{10,14}$, J = 7.99 Hz), 8.12–8.14 (2H, d, Ar—$H_{11,13}$, J = 7.99 Hz), 10.25 (1H, S, H_{19}), 8.61–8.63 (2H, d, Ar—$H_{16,18}$), 6.97–6.99 (1H, d, Ar—H_{17}); ^{13}C NMR (400 MHz, DMSO-d_6): δ 20.66 (1C, C_1), 120.54 (1C, C_3), 139.66 (1C, C_2), 129.28 (1C, C_4), 130.22 (1C, C_5), 117.13 (1C, C_7), 113.99 (2C, $C_{10,14}$), 127.20 (2C, $C_{11,13}$), 149.34 (1C, C_9), 132.81 (1C, C_{12}), 158.05 (1C, C_{15}), 155.15 (2C, $C_{16,18}$), 115.63 (1C, C_{17}); ESI-HRMS for $C_{17}H_{16}N_6O_3S$ calcd. 384.4123, found 385.4125.

4.2.15. 2-({4-[3-hydroxy-3-(4-methylphenyl)triaz-1-en-1-yl]phenyl} sulfonyl)pyrimidine (SFD-7)

2-({4-[3-hydroxy-3-(4-methylphenyl)triaz-1-en-1-yl]phenyl}sulfonyl)pyrimidine (SFD-7) m.p. 102–104 °C; Crystallization: acetone; IR (KBr): V_{O-H} (3390 cm^{-1}), V_{N-H} (3218 cm^{-1}), $V_{N=N}$ (1578 cm^{-1}), V_{C-N} (1215 cm^{-1}), $V_{S=O}$ (1154 cm^{-1}); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.433 (3H, S, H_1), 7.26–7.29 (2H, d, Ar—$H_{2,5}$), 7.31–7.33 (2H, d, Ar—$H_{3,6}$), 11.46 (1H, S, H_8), 7.94–7.96 (2H, d, Ar—$H_{10,14}$, J = 8.1 Hz), 8.11–8.14 (2H, d, Ar—$H_{11,13}$, J = 8.1 Hz), 10.82 (1H, S, H_{19}), 8.61–8.635 (2H, d, Ar—$H_{16,18}$, J = 6.01 Hz), 6.96–6.99 (1H, d, Ar—H_{17}, J = 6.01 Hz); ^{13}C NMR (400 MHz, DMSO-d_6): 21.22 (1C, C_1), 138.90 (1C, C_4), 130.49 (2C, $C_{2,5}$), 118.81 (2C, $C_{3,6}$), 143. 75 (1C, C_7), 113.64 (2C, $C_{10,14}$), 129.51 (2C, $C_{11,13}$), 146.70 (1C, C_9), 135.29 (1C, C_{12}), 158.31 (1C, C_{15}), 154.13 (2C, $C_{16,18}$), 112.78 (1C, C_{17}); ESI-HRMS for $C_{17}H_{16}N_6O_3S$ calcd. 384.4123, found 385.4178.

4.2.16. 2-{[4-(3-hydroxy-3-propyltriaz-1-en-1-yl)phenyl]sulfonyl}pyridine 2-{[4-(3-hydroxy-3-propyltriaz-1-en-1-yl)phenyl]sulfonyl}pyridine (SFP-1) (SFP-1)

2-{[4-(3-hydroxy-3-propyltriaz-1-en-1-yl)phenyl]sulfonyl}pyridine 2-{[4-(3-hydroxy-3-propyltriaz-1-en-1-yl)phenyl]sulfonyl}pyridine (SFP-1)(SFP-1) m.p. 138–140 °C; Crystallization: acetone; IR (KBr): V_{O-H} (3334 cm^{-1}), V_{N-H} (3244 cm^{-1}), $V_{N=N}$ (1595 cm^{-1}), V_{C-N} (1222 cm^{-1}), $V_{S=O}$ (1136 cm^{-1}); ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.898 (3H, t, H_1, J = 7.16 Hz), 1.88 (2H m, H_2), 4.08 (2H, t, H_3, J = 7.57 Hz), 11.36 (1H, S, H_4), 7.32–7.57 (2H, d, Ar—$H_{6,10}$, J = 7.99 Hz), 7.84–7.88 (2H, d, Ar—$H_{7,9}$, J = 7.99 Hz), 10.30 (1H, S, H_{11}), 8.35–8.38 (1H, m, Ar—H_{16}), 8.02–8.04 (1H, m, Ar—H_{15}), 7.62–7.66 (1H, m, Ar—H_{14}), 8.12–8.16 (1H, m, Ar—H_{13}); ^{13}C NMR (400 MHz, DMSO-d_6): δ 11.25 (1C, C_1), 20.39 (1C, C_2), 67.59 (1C, C_3), 113.39 (2H, $C_{6,10}$), 127.29 (2C, $C_{7,9}$), 143.21 (1C, C_5), 136.12 (1C, C_8), 156.41 (1C, C_{12}), 146.31 (1C, C_{16}), 116.89 (1C, C_{15}), 135.20 (1C, C_{14}), 114.50 (1C, C_{13}); ESI-HRMS for $C_{14}H_{17}N_5O_3S$, calcd. 335.3815, found 336.3871.

4.2.17. 2-{[4-(3-hydroxy-3-phenyltriaz-1-en-1-yl)phenyl]sulfonyl} pyridine (SFP-2)

2-{[4-(3-hydroxy-3-phenyltriaz-1-en-1-yl)phenyl]sulfonyl}pyridine (SFP-2) m.p. 118–120 °C; Crystallization: acetone; IR (KBr): V_{O-H} (3444 cm^{-1}), V_{N-H} (3311 cm^{-1}), $V_{N=N}$ (1595 cm^{-1}), V_{C-N}

(1256 cm^{-1}), $V_{S=O}$ (1134 cm^{-1}); ^1H NMR (400 MHz, DMSO-d_6): δ 7.26–7.32 (1H, m, Ar−H_4), 7.39–7.49 (1H, m, Ar−$H_{5,3}$), 7.52–7.57 (1H, m, Ar−$H_{2,6}$), 11.32 (1H, S, H_7), 7.66–7.70 (2H, d, Ar−$H_{9,13}$, J = 8.01 Hz), 7.90–7.92 (2H, d, Ar−$H_{10,12}$, J = 8.01 Hz), 8.31–8.33 (1H, d, Ar−H_{19}, J = 5.53 Hz), 10.86 (1H, S, H_{15}), 7.99–8.01 (1H, m, Ar−H_{18}), 8.02–8.06 (1H, m, Ar−H_{17}), 8.16–8.18 (1H, m, Ar−H_{16}); ^{13}C NMR (400 MHz, DMSO-d_6): δ 118.18 (2C, $C_{2,6}$), 130.81 (2C, $C_{3,5}$), 129.19 (1C, C_4), 114.27 (2C, $C_{9,13}$), 127.24 (2C, $C_{10,12}$), 143.46 (1C, C_8), 136.34 (1C, C_{11}), 155.43 (1C, C_{15}), 147.95 (1C, C_{19}), 120.06 (1C, C_{18}), 139.56 (1C, C_{17}), 112.28 (1C, C_{16}); ESI-HRMS for $C_{17}H_{15}N_5O_3S$, calcd. 369.3977, found 370.3974.

4.2.18. 2-({4-[3-hydroxy-3-(2-methylphenyl)triaz-1-en-1-yl]phenyl} sulfonyl)pyridine (SFP-3)

2-({4-[3-hydroxy-3-(2-methylphenyl)triaz-1-en-1-yl]phenyl}sulfonyl)pyridine (SFP-3) m.p. 109–111 °C; Crystallization: acetone; IR (KBr): V_{O-H} (3432 cm^{-1}), V_{N-H} (3229 cm^{-1}), $V_{N=N}$ (1594 cm^{-1}), V_{C-N} (1232 cm^{-1}), $V_{S=O}$ (1137 cm^{-1}) ^1H NMR (400 MHz, DMSO-d_6): δ 2.47 (3H, S, H_1), 6.79–7.38 (4H, m, Ar−$H_{3–6}$), 11.30 (1H, S, H_8), 7.45–7.51 (2H, d, Ar−$H_{10,14}$, J = 8.24 Hz), 7.51–7.60 (2H, d, Ar−$H_{11,13}$, J = 8.24 Hz), 10.80 (1H, S, H_{15}), 8.31–8.33 (1H, m, Ar−H_{20}), 7.91–7.93 (1H, m, Ar−H_{19}), 7.67–7.69 (1H, m, Ar−H_{18}), 7.88–7.90 (1H, m, Ar−H_{17}); ^{13}C NMR (400 MHz, DMSO-d_6): δ 18.83 (1C, C_1), 138.37 (1C, C_2), 130.18 (1C, C_3), 126.33 (1C, C_4), 123.94 (1C, C_5), 131.92 (1C, C_6), 142.14 (1C, C_7), 114.43 (2C, $C_{10,14}$), 128.81 (2C, $C_{11,13}$), 146.31 (1C, C_9), 137.58 (1C, C_{12}), 154.75 (1C, C_{16}), 146.31 (1C, C_{20}), 115.18 (1C, C_{19}), 138.87 (1C, C_{18}), 112.03 (1C, C_{17}); ESI-HRMS for $C_{18}H_{17}N_5O_3S$, calcd. 383.4243, found 384.4282.

4.2.19. 2-({4-[3-hydroxy-3-(3-methylphenyl)triaz-1-en-1-yl]phenyl} sulfonyl)pyridine (SFP-4)

2-({4-[3-hydroxy-3-(3-methylphenyl)triaz-1-en-1-yl]phenyl}sulfonyl)pyridine (SFP-4) m.p. 108–110 °C; Crystallization: acetone; IR (KBr): V_{O-H} (3423 cm^{-1}), V_{N-H} (3192 cm^{-1}), $V_{N=N}$ (1594 cm^{-1}), V_{C-N} (1275 cm^{-1}), $V_{S=O}$ (1137 cm^{-1}); ^1H NMR (400 MHz, DMSO-d_6): δ 2.45 (3H, S, H_1), 7.60–7.68 (1H, m, Ar−H_3), 7.79–6.82 (2H, m, Ar−H_7), 7.30–7.34 (1H, m, Ar−H_6), 7.38–7.44 (1H, m, Ar−H_5), 11.45 (1H, S, H_8), 7.50–7.54 (2H, d, Ar−$H_{10,14}$, J = 8.01 Hz), 7.80–7.90 (2H, d, Ar−$H_{11,13}$, J = 8.01 Hz), 10.87 (1H, S, H_{15}), 8.02–8.06 (3H, m, Ar−H_{17}), 8.31–8.33 (1H, m, Ar−H_{20}), ^{13}C NMR (400 MHz, DMSO-d_6): δ 21.45 (1C, C_1), 138.94 (1C, C_2), 129.49 (1C, C_7), 131.07 (1C, C_6), 120.46 (1C, C_5), 118.95 (1C, C_3), 142.81 (1C, C_4), 114.67 (2C, $C_{10,14}$), 128.75 (2C, $C_{11,13}$), 144.0 (1C, C_9), 135.30 (1C, C_{12}), 153.82 (1C, C_{16}), 146.98 (1C, C_{20}), 118.95 (1C, C_{19}), 139.46 (1C, C_{18}), 111.92 (1C, C_{17}); ESI-HRMS for $C_{18}H_{17}N_5O_3S$, calcd. 383.4243, found 384.4262.

4.2.20. 2-({4-[3-hydroxy-3-(4-methylphenyl)triaz-1-en-1-yl]phenyl} sulfonyl)pyridine (SFP-5)

2-({4-[3-hydroxy-3-(4-methylphenyl)triaz-1-en-1-yl]phenyl}sulfonyl)pyridine (SFP-5) m.p. 111–113 °C; Crystallization: acetone; IR (KBr): V_{O-H} (3392 cm^{-1}), V_{N-H} (3192 cm^{-1}), $V_{N=N}$ (1594 cm^{-1}), V_{C-N} (1274 cm^{-1}), $V_{S=O}$ (1134 cm^{-1}); ^1H NMR (400 MHz, DMSO-d_6): δ2.42 (3H, S, H_1), 7.42–7.48 (2H, m, Ar−$H_{4,6}$), 7.26–7.30 (2H, m, Ar−$H_{3,7}$), 11.45 (1H, S, H_8), 7.66–7.70 (2H, d, Ar−$H_{10,14}$, J = 7.89 Hz), 7.870–7.90 (2H, d, Ar−$H_{11,13}$, J = 7.89 Hz), 10.82 (1H, S, H_{15}), 8.32–8.34 (1H, m, Ar−H_{20}), 7.26 (1H, dd, Ar−H_{19}), 7.92–7.95 (2H, m, Ar−H_{17}); ^{13}C NMR (400 MHz, DMSO-d_6): δ 46.09 (1C, C_1), 138.91 (1C, C_2), 129.61 (2C, $C_{3,7}$), 119.88 (2C, $C_{4,6}$), 140.81 (1C, C_5), 114.58 (2C, $C_{10,14}$), 127.18 (2C, $C_{11,13}$), 146.32 (1C, C_9), 137.97 (1C, C_{12}), 155.46 (1C, C_{16}), 143.11 (1C, C_{20}), 117.27 (1C, C_{19}), 134.32 (1C, C_{18}), 112.32 (1C, C_{17}); ESI-HRMS for $C_{18}H_{17}N_5O_3S$, calcd. 383.4243, found 384.4274.

4.2.21. 2-{[4-(3-ethyl-3-hydroxytriaz-1-en-1-yl)phenyl]sulfonyl}-4,6-dimethylpyrimidine (SMT-1)

2-{[4-(3-ethyl-3-hydroxytriaz-1-en-1-yl)phenyl]sulfonyl}-4,6-dimethylpyrimidine (SMT-1) m.p. 102–104 °C; Crystallization: acetone; IR (KBr): V_{O-H} (3458 cm^{-1}), V_{N-H} (3228 cm^{-1}), $V_{N=N}$ (1597 cm^{-1}), V_{C-N} (1218 cm^{-1}), $V_{S=O}$ (1152 cm^{-1}); ^1H NMR (400 MHz, DMSO-d_6): δ 1.41 (3H, t, H_1, J = 7.28 Hz), 4.17 (2H, q, H_2, J = 7.28 Hz), 11.60 (1H, S, H_3), 7.34–7.41 (2H, m, Ar−$H_{5,9}$), 7.87–7.90 (2H, m, Ar−$H_{6,8}$), 10.31 (1H, S, H_{10}); 2.51 (6H, s, $H_{11,15}$), 6.74 (1H, s, Ar−H_{13}); ^{13}C NMR (400 MHz, DMSO-d_6): 11.73 (1C, C_1), 58.38 (1C, C_2), 144.42 (1C, C_4), 113.60 (2C, $C_{5,9}$), 127.86 (2C, $C_{6,8}$), 132.30 (1C, C_7), 167.29 (1C, C_{16}), 156.29 (2C, $_{12,14}$), 112.97 (1C, C_{13}), 22.91 (2C, $C_{11,15}$); ESI-HRMS for $C_{14}H_{18}N_6O_3S$, calcd. 350.3961, found 351.3971.

4.2.22. 2-{[4-(3-hydroxy-3-propyltriaz-1-en-1-yl)phenyl]sulfonyl}-4,6-dimethylpyrimidine (SMT-2)

2-{[4-(3-hydroxy-3-propyltriaz-1-en-1-yl)phenyl]sulfonyl}-4,6-dimethylpyrimidine (SMT-2) m.p. 102–104 °C; Crystallization: acetone; IR (KBr): V_{O-H} (3432 cm^{-1}), V_{N-H} (3225 cm^{-1}), $V_{N=N}$ (1598 cm^{-1}), V_{C-N} (1218 cm^{-1}), $V_{S=O}$ (1153 cm^{-1}); ^1H NMR (400 MHz, DMSO-d_6): δ 0.89 (3H, t, H_1, J = 7.42 Hz), 1.85–1.90 (2H, m, H_2, J = 7.42 Hz, J = 7.57 Hz), 4.10 (2H, t, H_3, J = 7.57 Hz), 11.58 (1H, S, H_4), 7.36–7.34 (2H, d, Ar−$H_{6,10}$, J = 8.84 Hz), 7.90–7.88 (2H, d, Ar−$H_{7,9}$, J = 8.84 Hz), 10.20 (2H, S, H_{11}), 2.26 (6H, S, $H_{14,17}$), 6.75 (1H, s, Ar−H_{15}); ^{13}C NMR (400 MHz, DMSO-d_6): δ 10.61 (1C, C_1), 22.92 (1C, C_2), 64.65 (1C, C_3), 144.40 (1C, C_5), 113.84 (2C, $C_{6,10}$), 129.83 (2C, $C_{7,9}$), 136.40 (1C, C_8), 168.29 (1C, C_{12}), 156.29 (2C, $C_{13,16}$), 112.98 (1C, C_{15}), 20.20 (2C, $C_{14,17}$); ESI-HRMS for $C_{15}H_{20}N_6O_3S$, calcd. 364.4227, found 365.4325.

4.2.23. 2-({4-[3-hydroxy-3-(propan-2-yl)triaz-1-en-1-yl]phenyl} sulfonyl)-4,6-dimethylpyrimidine (SMT-3)

2-({4-[3-hydroxy-3-(propan-2-yl)triaz-1-en-1-yl]phenyl}sulfonyl)-4,6-dimethylpyrimidine (SMT-3) m.p. 164–166 °C; Crystallization: acetone; IR (KBr): V_{O-H} (3432 cm^{-1}), V_{N-H} (3223 cm^{-1}), $V_{N=N}$ (1598 cm^{-1}), V_{C-N} (1217 cm^{-1}), $V_{S=O}$ (1154 cm^{-1}); ^1H NMR (400 MHz, DMSO-d_6): δ 1.05 (6H, d, $H_{1,2}$, J = 6.43 Hz), 2.97 (1H, m, H_3, J = 6.43 Hz), 7.36–7.34 (2H, m, $ArH_{6,10}$), 7.83–7.94 (2H, m, Ar−$H_{7,9}$), 11.58 (1H, S, H_4), 10.30 (1H, S, H_{11}), 2.18 (6H, S, $H_{14,17}$), 6.73 (1H, s, Ar−H_{15}); ^{13}C NMR (400 MHz, DMSO-d_6): δ 20.09 (2C, $C1,2_2$), 65.30 (1C, C_3), 145.20 (1C, C_5), 112.97 (2C, $C_{6,10}$), 127.87 (2C, $C_{7,9}$), 136.21 (1C, C_8), 165.13 (1C, C_{12}), 151.50 (2C, $C_{13,16}$), 110.9 (1C, C_{15}), 22.93 (2C, $C_{14,17}$); ESI-HRMS for $C_{15}H_{20}N_6O_3S$, calcd. 364.4227, found 365.4375.

4.2.24. 2-{[4-(3-hydroxy-3-phenyltriaz-1-en-1-yl)phenyl]sulfonyl}-4,6-dimethylpyrimidine (SMT-4)

2-{[4-(3-hydroxy-3-phenyltriaz-1-en-1-yl)phenyl]sulfonyl}-4,6-dimethylpyrimidine (SMT-4) m.p. 168–170 °C; Crystallization: acetone; IR (KBr): V_{O-H} (3446 cm^{-1}), V_{N-H} (3214 cm^{-1}), $V_{N=N}$ (1595 cm^{-1}), V_{C-N} (1224 cm^{-1}), $V_{S=O}$ (1148 cm^{-1}); ^1H NMR (400 MHz, DMSO-d_6): δ 7.36–7.47 (5H, m, $H_{2–6}$), 12.26 (1H, S, H_7), 7.59–7.61 (2H, m, Ar−$H_{9,13}$), 7.89–7.96 (2H, m, Ar−$H_{10,12}$), 11.45 (1H, S, H_{14}), 2.24 (6H, S, $H_{17,20}$), 6.71 (1H, dd, Ar−H_{18}); ^{13}C NMR (400 MHz, DMSO-d_6): δ 119.23 (2C, $C_{2,6}$), 124.93 (2C, $C_{3,5}$), 123.2 (1C, C_4), 113.96 (2C, $C_{9,13}$), 128.93 (2C, $C_{10,12}$), 142.92 (1C, C_8), 132.20 (1C, C_{11}), 166.87 (1C, C_{15}), 165.01 (2C, $C_{16,19}$), 110.2 (1C, C_{18}), 22.94 (2C, $C_{17,20}$); ESI-HRMS for $C_{18}H_{18}N_6O_3S$, calcd. 398.4389, found 399.4326.

4.2.25. 2-({4-[3-hydroxy-3-(3-methylphenyl)triaz-1-en-1-yl]phenyl} sulfonyl)-4,6-dimethylpyrimidine (SMT-5)

2-({4-[3-hydroxy-3-(3-methylphenyl)triaz-1-en-1-yl]phenyl}sulfonyl)-4,6-dimethylpyrimidine (SMT-5) m.p. 181–183 °C; Crystallization: acetone; IR (KBr): V_{O-H} (3486 cm^{-1}), V_{N-H} (3187 cm^{-1}), $V_{N=N}$ (1593 cm^{-1}), V_{C-N} (1222 cm^{-1}), $V_{S=O}$ (1143 cm^{-1}); ^1H NMR (400 MHz, DMSO-d_6): δ 2.43 (3H, S, H_1),

7.36–7.47 (3H, m, Ar−H_{3-5}), 7.59 (1H, m, Ar−H_7), 7.59–7.61 (2H, m, Ar−$H_{10,14}$), 7.89–7.96 (2H, m, Ar−$H_{11,13}$), 12.26 (1H, S, H_8), 11.59 (1H, S, H_{15}), 6.76 (1H, S, Ar−H_{19}) 2.26 (); ^{13}C NMR (400 MHz, DMSO-d_6): δ 22.93 (1C, C_1), 139.08 (1C, C_2), 143. 87 (1C, C_6), 120.22 (1C, C_5), 130.89 (1C, C_4), 129.05 (1C, C_3), 114.09 (2C, $C_{10,14}$), 127.05 (2C, $C_{11,13}$), 136.8 (1C, C_{12}), 156.28 (1C, C_{16}), 146.22 (2C, $C_{17,20}$), 20.87 (2C, $C_{18,21}$), 110.23 (1C, C_{19}); ESI-HRMS for $C_{19}H_{20}N_6O_3S$, calcd. 412.4655, found 413.4689.

4.2.26. 2-({4-[3-hydroxy-3-(4-methylphenyl)triaz-1-en-1-yl]phenyl} sulfonyl)-4,6-dimethylpyrimidine (SMT-6)

2-({4-[3-hydroxy-3-(4-methylphenyl)triaz-1-en-1-yl]phenyl}sulfonyl)-4,6-dimethylpyrimidine (SMT-6) m.p. 186–188 °C; Crystallization: acetone; IR (KBr): V_{O-H} (3400 cm^{-1}), V_{N-H} (3208 cm^{-1}), $V_{N=N}$ (1597 cm^{-1}), V_{C-N} (1230 cm^{-1}), $V_{S=O}$ (1152 cm^{-1}); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.40–7.58 (2H, m, Ar−$H_{4,6}$), 7.34–7.38 (2H, dd, Ar−$H_{3,7}$), 2.43 (3H, S, H_1), 7.92–7.99 (2H, m, Ar−$H_{10,14}$), 78.08–8.14 (2H, m, Ar−$H_{11,13}$), 12.30 (1H, S, H_4), 11.20 (1H, S, H_{15}), 2.24 (6H, S, $H_{18,21}$), 6.72 (1H, S, Ar−H_{19}); ^{13}C NMR (400 MHz, DMSO-d_6): δ20.77 (1C, C_1), 138.48 (1C, C_2), 119.77 (2C, $C_{4,6}$), 129.37 (2C, $C_{3,7}$), 141.25 (1C, C_5), 142.22 (1C, C_9), 113.86 (2C, $C_{10,14}$), 127.23 (2C, $C_{11,13}$), 139.89 (1C, C_{12}), 165.50 (1C, C_{16}), 152 22 (2C, $C_{17,20}$), 109.01 (1C, C_{19}), 21.04 (2C, $C_{18,21}$); ESI-HRMS for $C_{19}H_{20}N_6O_3S$, calcd. 412.4655, found 413.4687.

4.3. α-Glucosidase inhibition assay

In this method inhibition of α-glucosidase enzyme was determined spectrophotmetrically in a 96-well microtiterplate which is based on p-nitrophenyl-α-D-glucopyranoside (PNPG) as a substrate.

Tripathi and his co-worker's protocol was followed in this study [44]. In that, rat intestinal powder was dissolved in 100 mL of saline water and properly sonicated at 4 °C, then the suspension was centrifuged (3000 rpm, at 4 °C for 30 min) and the resulting supernatant was used as an enzyme solution. Total reaction mixture was set at 250 μL, containing 50 μL of phosphate buffer (50 mM, pH 6.8), 75 μL of α-glucosidase solution and 50 μL of test samples of different concentrations (viz. 20, 60, 120, 180, 240 and 300 μL/mL) which have been prepared in DMSO. Reaction mixture was pre incubated for 5 min at 37 °C, and then added 75 μL of 3 mM PNPG as substrate, then again reaction mixture was incubated for 30 min at 37 °C. On completion of 30 min, enzymatic activity was quantified by measuring the absorbance at 405 nm. Inhibition efficiency of compounds was determined by comparison with solution containing 50 μL of phosphate buffer, 75 μL of α-glucosidase solution, 50 μL DMSO and 75 μL of PNPG treated control absorbance. Acarbose was taken as standard in this assay. Following formula was used for calculation of enzyme inhibition activity. Experiments were done in triplicates.

$$\%\ \text{Inhibition} = \frac{(\text{Absorbance control} - \text{Absorbance sample})}{\text{Absorbance control}} \times 100$$

4.4. α-Amylase inhibition assay

The protocol followed in this study was previously reported [44]. Total reaction volume was set at 350 μL. 50 μL of various concentration (20, 60, 120, 180, 240 and 300 μL/mL in DMSO) of test sample and 50 μL of α-amylase solution (prepared by dissolving 0.005gm α-amylase in 5 mL sodium phosphate buffer of 0.02 M concentration, pH 6.8) were taken and incubate for 10 min at 25 °C. After incubation for 10 min, added 50 μL of 1% starch (1 gm of starch dissolved in 100 mL of sodium-phosphate buffer) and reaction mixture was again incubated for 10 min at 25 °C. Then added 100 μL of DNS solution (1 gm of DNS dissolved in 50 mL of water) and again incubated in boiling water for 5 min. Upon completion of incubation time, added 100 μL of distilled water and enzymatic activity was quantified by measuring the absorbance at 540 nm in multimode reader. To afford control absorbance 350 μL reaction mixture was prepared with same procedure without containing test sample. Following formula was used for calculation of enzyme inhibition activity. Experiments were done in triplicates.

$$\%\ \text{Inhibition} = \frac{(\text{Absorbance control} - \text{Absorbance sample})}{\text{Absorbance control}} \times 100$$

4.5. DPPH inhibition assay

The DPPH radical scavenging activity was performed using reported method [45]. The method was performed by taking 125 μL of 0.004% DPPH solution which was prepared in methanol 30 min before use, and 125 μL of various dilution (viz. 20, 60, 120, 180, 240 and 300 μL/mL, prepared in DMSO) and 50 μL of tris-HCl buffer in 96-well micro plate. Total reaction mixture was set at 300 μL and was incubated at 37 °C for half an hour. Final absorbance was recorded on BioTek Synergy H4 hybrid multimode micro plate reader (BioTek instruments, Inc Winooski, VT, USA) at 517 nm. Radical quenching efficiency of compounds was determined by comparison with 125 μL DMSO, 50 μL of tris–HCl buffer and 125 μL of DPPH solution treated control absorbance. Ascorbic acid was taken as standard in this assay. Following formula was used for calculation of radical scavenging activity. Experiments were done in triplicates.

$$\text{DPPHscavengingeffect}(\%) = \frac{(\text{Absorbancecontrol} - \text{Absorbancesample})}{\text{Absorbancecontrol}} \times 100$$

The concentration of sample required to scavenge 50% of DPPH• (IC_{50}) were determined. Decreasing of the DPPH• solution absorbance indicates an increase of the DPPH radical scavenging activity.

4.6. ABTS inhibition assay

The ability of the synthesized compound to scavenge $ABTS^{\cdot+}$ radical was reported in the literature [46]. Activated ABTS (7 mM concentration) solution through ammoniumpersulphate was used and further diluted to get required absorbance at 734 nm. Reaction volume was set on 300 μL, for that took 100 μL of test samples of various concentration (viz. 20, 60, 120, 180, 240 and 300 μL/mL, prepared in DMSO) and 200 μL of $ABTS^{\cdot+}$ solution. For control absorbance, solvent was used in place of test sample. Absorbance was recorded after 30 min incubation in dark at 734 nm on multimode micro plate reader. Ascorbic acid was used as positive antioxidant and percentage scavenging was calculated from the formula-

$$\text{ABTSscavengingeffect}(\%) = \frac{(\text{Absorbance control} - \text{Absorbance sample})}{\text{Absorbance control}} \times 100$$

4.7. Carrageenan induced paw edema assay for Anti-inflammatory activity

In the present study carrageenan induced acute inflammation model has been used and the results have been compared with standard drug diclofenac sodium.

Six rats of either sex in each group were taken and starved overnight with free access to water. The group I which served as control was given water (1 mL/kg) orally, while group II which served as carrageenan was given only carrageen. Group III were given diclofenac sodium (12.5 mg/kg) orally as a standard drug. The remaining groups were treated with test samples (100 mg/kg) orally. Just above the tibio-torsal joint of the hind paw was marked to ensure uniform dipping every time. Diclofenac sodium and test samples were administered 1 h prior to carrageenan injection. Edema was produced according to the method

described by Winter et al. [47] by sub planter injection of 0.1 mL of 1% freshly prepared suspension of carrageenan. Immediately after injection, paw volume was measured plethysmographically (by mercury displacement) to note initial volume. Similar procedure was repeated at intervals of 1 h, 2 h, 3 h and 4 h. The difference between the initial and subsequent reading at a given time was the actual edema volume. Thus edema volume in control (Vc) and in-group treated with drugs (Vt) were calculated. Mean increase in paw volume was measured. % inhibition was calculated by this formula

$$\text{Anti} - \text{inflammatory Activity } (\%) = \frac{[(\text{Vt} - \text{V0}) \text{ carrageenan} - (\text{Vt} - \text{V0}) \text{ treated}]}{(\text{Vt} - \text{V0}) \text{ carrageenan}} \times 100$$

4.8. Docking studies

Docking studies have been done from Medicinal and Pharmaceutical Chemistry Department, Sree Vidyanikethan College of Pharmacy, Tirupathi, Andhra Pradesh, India.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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Appendix A. Supplementary material

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