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Research article

Synthesis, antidiabetic, antioxidant and anti-inflammatory activities of novel hydroxytriazenes based on sulpha drugs

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ABSTRACT

The present study is aimed to investigate the anti-inflammatory, antioxidant and antidiabetic activities of three series of hydroxytriazenes based on sulfa drugs viz; Sulphathiazole (ST), Sulfisoxazole (SF) and Sulphamethoxazole (SM). Antidiabetic activities of the synthesized hydroxytriazenes were investigated by α-glucosidase and α-amylase inhibition method and IC_{50} values were recorded. The compounds presented significant α-glucosidase and α-amylase inhibition effect with IC_{50} values ranging from 122 to 341 μg/mL. Anti-inflammatory activity was also investigated by carrageenan-induced paw edema (CPE) method, where % inhibition was up to 89% after 4 h of treatment and antioxidant properties of the similar compounds were assessed by DPPH and ABTS radical scavenging assays. Antioxidant capacity of all the hydroxytriazenes detected by ABTS assay, was significantly higher as compared to DPPH assay. The hydroxytriazenes having highest antioxidant capacity presented IC_{50} values for compound ST-1 and ST-6 are 488 μg/mL for DPPH, 54.12 μg/mL for ABTS and 858.5 μg/mL for DPPH, 48.0 μg/mL for ABTS, respectively. These results suggested that ABTS assay may be more useful than DPPH assay for synthetic antioxidants. The findings from the molecular docking experiments may also expand the formation of new potent sulpha drugs based hydroxytriazenes targeting towards the subunit of C-terminal of human maltase-glucoamylase for the treatment of diabetes metabolic disorder. Overall, highlight the multifunctional role of hydroxytriazenes as antidiabetic, antioxidant and anti-inflammatory agents.

1. Introduction

Diabetes mellitus is a chronic metabolic disorder (Golbidi et al., 2012) characterized by means of abnormally high plasma glucose, give rise to diabetic complications which include diabetic neuropathy (Ziegler et al., 1995), retinopathy (Hammes et al., 2003), nephropathy (Lim, 2014) and cardiovascular diseases (Levine et al., 2006). Diabetes is a fast increasing life style related disease (Taslimi et al., 2020; Bouguerra et al., 2007) that requires the significant persisted research, therefore, there is an urgent need for novel approaches to prevent and deal with this pandemic.

Although, obesity and physical inertness are regarded to be primary causes associated with type-2 diabetes, however, few latest researches suggest that oxidative stress may make contributions to the pathogenesis of diabetes through enhancing insulin resistance or by impairing secretion of insulin (Montonen et al., 2004).

Diabetes can be effectively managed by decreasing postprandial hyperglycaemia (DeFronzo, 2000) by delaying the glucose absorption through inhibiting the enzymes particularly *α*-glucosidase and *α*-amylase which hydrolyze carbohydrate (Chiasson et al., 2002). *α*-Glucosidase enzyme catalyse the digestion of carbohydrates and *α*-amylase splits the *α*-1,4-glycosidic linkage to produce maltose and glucose. Thus, these inhibitors inhibit the release of D-glucose from dietary carbohydrates and delay the absorption of glucose from intestine, leading to diminished postprandial hyperglycaemia (Hanefeld et al., 2004; Bösenberg and Van Zyl, 2008).

Although, treatment of diabetes is mostly centred on the management of hyperglycemia, the approaches targeting reduction of oxidative stress perceived to be an effective way of treatment of diabetes and related complications. Oxidative stress is the accretion of reactive oxygen species (ROS) and reactive nitrogen species (RNS) that cannot be coerced by the

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endogenous move of free radical neutralizing agents and antioxidants (Türkan et al., 2020). ROS and RNS are produced through regular cellular metabolism and have harmful effect to living systems (Valko et al., 2006). The antioxidant therapy would provide a therapeutic strategy to defend the β-cells against oxidative stress and prevent related diabetic vascular complication (Golbidi et al., 2011).

Antioxidants are enzymes or synthetic or natural substances, that significantly decreases the adverse effects of reactive species, such as reactive oxygen and nitrogen species, on normal physiological function in humans (Huang et al., 2005). An antioxidant diminishes free radicals, enhance scavenging of free radicals and antioxidant defence mechanism. The measurement of total antioxidant capacity of a substance using one method seems to be somewhat impracticable and not easy. However, there are several methods reported claiming to measure total antioxidant capacity *in vitro*. Due to the unavailability of standard assays, it is very difficult to select reliable method to measure antioxidant capacity of synthetic, natural and biological samples. Therefore, it is planned to examine antioxidant capacity of hydroxytriazenes by two methods DPPH and ABTS radical scavenging assays for the possible beneficial effects in management of diabetes (Baragob et al., 2014; Rajendiran et al., 2018).

Oxidative stress and inflammation are largely related, many studies indicated that vascular inflammation facilitating arterial diseases. Under the condition of oxidative stress by generation of ROS and RNS species also play crucial role in activation of signalling pathway which affect intra and extra cellular pathway. At the site of inflammation, accumulation of ROS occurs because mast cells and leukocytes are produced, which leads to a 'respiratory burst' due to an increased uptake of oxygen (Pollack et al., 2016; Donath, 2013).

Hydroxytriazene is a bidentate ligand which has alpha hydroxyl group relative to azo group and diazo group. This class of compounds have analytical applications as they from chelate complex with most of transition metals (Naulakha et al., 2009; Panwar et al., 2014; Singh et al., 2008) and they also have broad spectrum of biological activities such as lipid lowering, antioxidant, antidiabetic, anti-inflammatory, antifungal, antibacterial, insecticidal and analgesic agents (Sharma et al., 2020; Regar et al., 2016; Agarwal et al., 2016; Baroliya et al., 2014; Jain et al., 2020; Hura et al., 2003; Chauhan et al., 2007, 2010; Khanam et al., 2014; Goswami et al., 2012; Rezaie et al., 1997; Goswami, 2002).

In view of versatile applications of hydroxytriazenes, an attempt has been made to study the anti-diabetic activity of hydroxytriazenes by *α*-glucosidase and *α*-amylase enzymes inhibition method and molecular docking studies were carried out to correlate structure-activity relationship. Moreover, anti-inflammatory and antioxidant effects of hydroxytriazenes were also investigated by CPE and radical scavenging methods, respectively.

2. Experimental

2.1. Material and methods

Ascorbic acid, acarbose, porcine pancreatic α-amylase and α-glucosidase, p-nitrophenyl-α-D-glucopyranoside (PNPG), 3,5_dinitrosalicylic acid (DNSA) were purchased from SRL, India. While rat intestinal acetone powder was procured from Sigma Aldrich and 2,2-Diphenyl-1-picryl-hydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) was brought from Alfa-Aesar. All the other chemicals and reagents used in synthesis, of AR grade were purchesed from Sigma-Aldrich, India and used without further refinement.

Weighing measurements were carried out on Sartorious electronic balance model BSA224S_CW. Melting points were recorded by using SONAR electro thermal apparatus (Biotechno lab, India) and are uncorrected. All the Infrared (IR) spectrum have been recorded in the frequency range of 400–4000 cm^{-1} by the KBR method using Bruker Alfa-T FTIR spectrometer (Bruker Instrument, Germany) at department of chemistry, MLSU, Udaipur. The spectra of ^{1}H NMR and ^{13}C NMR spectra were recorded by using DMSO_d6 as the solvent in a 400 MHz in BRUKER AVANCE II NMR spectrometer (Bruker Instrument, Switzerland) at SAIF, Panjab University, Chandigarh. Molecular weights of the compounds were determined using XEVO_G2SQTOF_ MS ES^{+} spectrometry (Waters Instrument, USA) at MRC MNIT, Jaipur. The antioxidant activity was studied using Synergy H4 Hybrid Multimode Reader (Bio-TEK Instrument, Inc Winooski, VT, USA) at department of environmental sciences, MGGCV, Chitrakoot, Satna.

2.2. Synthesis of hydroxytriazenes

Hydroxytriazenes based on sulpha drugs have been synthesized as per reported method (Elkins and Hunter, 1938; Sogani and Bhattacharya, 1956). This method involves reduction of nitro compounds using Zn dust in neutral medium to get corresponding hydroxylamines and its coupling with diazonium salt resulted from diazotization of sulpha drugs [Sulphathiazole (ST), Sulfisoxazole (SF) and Sulphamethoxazole (SM)] at 0–5 °C, in pH 5–6 (Figure 1). Synthesized compounds were recrystallized many times to ascertain purity by means of methanol/acetone/DMF as a solvent.

2.3. Structural characterization

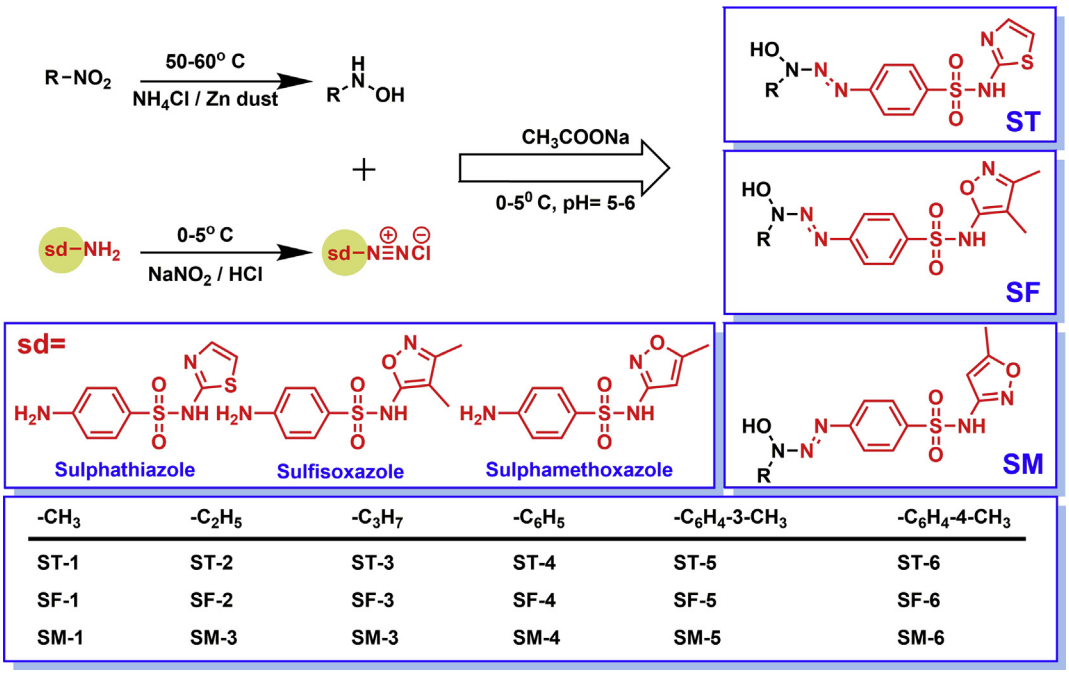
Synthesized compounds were characterized using standard spectroscopic techniques. Detailed data and structures of all the compounds are given in supporting information (S6).

3_hydroxy_3_methyl_4)_1_(N_(thiazol_2_yl)sulfamoyl)phenyl)triazene (ST-1): Reddish brown shining powder (methanol); mp 153 °C; FTIR (KBr): V_{max} 3472, 3208, 1529, 1286, 1138 cm^{-1}); ^{1}H NMR (DMSO_d6, 400 MHz): δ 3.95 (3H, s, H_1), 11.62 (1H, s, H_2), 7.69 (2H, d, J = 8.7 Hz, $H_{4,8}$), 7.36 (2H, d, J = 8.8 Hz, $H_{5,7}$), 12.8 (1H, s, H_9), 7.22 (1H, d, J = 7.6 Hz, H_{11}), 6.81 (1H, d, J = 7.6 Hz, H_{12}); ^{13}C NMR (DMSO_d6, 100 MHz): δ 50.6 (1C, C_1), 144.4 (1C, C_3), 113.7 (2C, $C_{4,8}$), 134.4 (2C, $C_{5,7}$), 128.8 (1C, C_6), 168.9 (1C, C_{10}), 141.0 (1C, C_{11}), 107.9 (1C, C_{12}) ; HRMS_ESI (*m/z*) calc. for $C_{10}H_{11}N_5O_3S_2$ $[M+1]^+$ 314.03, found 314.3633.

3_hydroxy_3_ethyl_4)_1_(N_(thiazol_2_yl)sulfamoyl)phenyl)triazene (ST-2): Light brown powder (methanol); mp 184 °C; FTIR (KBr): V_{max} 3453, 3223, 1526, 1299, 1137, cm^{1} ;^{1}H NMR (DMSO_d6, 400 MHz): δ 1.40 (3H, s, H_1), 4.14 (2H, q, J = 7.32 Hz, H_2), 11.55 (1H, s, H_3), 7.70 (2H, d, J = 8.80 Hz, $H_{5,9}$), 7.37 (2H, d, J = 8.84 Hz, Ar_$H_{6,8}$), 12.64 (1H, s, H_{10}), 7.22 (1H, d, J = 4.4 Hz, H_{12}), 6.80 (1H, d, J = 4.4 Hz, H_{13}); ^{13}C NMR (DMSO_d6, 100 MHz): δ 58.32 (1C, C_1), 12.37 (1C, C_2), 143.9 (1C, C_4), 113.5 (2C, $C_{5,9}$), 127.6 (2C, $C_{6,8}$), 124.3 (1C, C_7), 168.5 (1C, C_{11}), 134.3 (1C, C_{12}), 107.9 (1C, C_{13}); HRMS_ESI (m/z) calc. for $C_{11}H_{13}N_5O_3S_2$ $[M+1]^+$ 327.05, found 328.3878.

3_hydroxy_3_propyl_4)_1_(N_(thiazol_2_yl)sulfamoyl)phenyl)triazene (ST-3): Buff color powder (methanol); mp 160161_ °C; FTIR (KBr): V_{max} 3455, 3248, 1531, 1297, 1137 cm^{1} ;^{1}H NMR (DMSO_d6, 400 MHz): δ 0.89 (3H, s, H_1), 1.86 (2H, m, H_2), 4.08 (2H, t, J = 6.76 Hz, H_3), 11.57 (1H, s, H_4), 7.70 (2H, d, J = 8.76 Hz, $H_{6,10}$), 7.37 (2H, d, J = 8.76 Hz, $H_{7,9}$), 12.64 (1H, s, H_{11}), 7.22 (1H, d, J = 4.52 Hz, H_{13}), 6.80 (1H, d, J = 4.56 Hz, H_{14}); ^{13}C NMR (DMSO_d6, 100 MHz): δ 64.5 (1C, C_1), 20.2 (1C, C_2), 10.6 (1C, C_3), 143.9 (1C, C_5), 113.5 (2C, $C_{6,10}$), 124.3 (2C, $C_{7,9}$), 127.4 (1C, C_8), 168.51 (1C, C_{12}), 134.3 (1C, C_{13}), 107.9 (1C, C_{14}); HRMS_ESI (m/z) calc. for $C_{12}H_{15}N_5O_3S_2$, $[M+1]^+$ 342.07, found 341.5714.

3_hydroxy_3_phenyl_4)_1_(N_(thiazol_2_yl)sulfamoyl)phenyl)triazene (ST-4): Light skin color powder (DMF); mp 179 °C; FTIR (KBr): V_{max} 3446, 3210, 1524, 1276, 1137 cm^{1} ;^{1}H NMR (DMSO_d6, 400 MHz): δ 7.86 (2H, d, J = 8.18 Hz, $H_{2,6}$), 7.37 (2H, d, J = 7.92 Hz, $H_{3,5}$), 7.29 (1H, t, J = 7.6 Hz, H_4), 12.21 (1H, s, H_7), 7.71 (2H, d, J = 8.8 Hz, $H_{9,13}$), 7.55 (2H, d, J = 8.8 Hz, $H_{10,12}$), 12.64 (1H, s, H_{14}), 7.18 (1H, d, J = 4.8 Hz, H_{16}), 6.76 (1H, d, J = 4.32 Hz, H_{17}); ^{13}C NMR (DMSO_d6, 100 MHz): δ 143.4 (1C, C_1), 120.1 (2C, $C_{2,6}$), 130.8 (2C, $C_{3,5}$), 124.3 (1C, C_4), 142.9 (1C, C_8), 114.5 (2C, $C_{9,13}$), 135.4 (2C, $C_{10,12}$), 127.4 (1C, C_{11}), 168.6 (1C,



-CH_3	-C_2H_5	-C_3H_7	-C_6H_5	-C_6H_4-3-CH_3	-C_6H_4-4-CH_3
ST-1	ST-2	ST-3	ST-4	ST-5	ST-6
SF-1	SF-2	SF-3	SF-4	SF-5	SF-6
SM-1	SM-3	SM-3	SM-4	SM-5	SM-6

Figure 1. Synthesis of sulpha drugs based hydroxytriazenes.

C_{15}), 139.0 (1C, C_{16}); HRMS_ESI (m/z) calc. for $C_{10}H_{11}N_5O_3S_2$, $[M+1]^+$ 376.05, found 376.4989.

3_hydroxy_3)_3_methylphenyl)_4)_1_(N_(thiazol_2_yl)sulfamoyl) phenyl)triazene (ST-5): Light buff color powder (DMF); mp 194 °C; FTIR (KBr): V_{max} 3346, 3200, 1531, 1296, 1140 cm^1 ;^{1}H NMR (DMSO_d6, 400 MHz): δ 7.70 (1H, m, J = 2.2 Hz, H_2), 7.37 (1H, m, J = 8.0 Hz, H_4), 7.54 (1H, m, J = 5.4 Hz, H_5), 7.29 (1H, m, J = 7.6 Hz, H_6), 12.20 (1H, s, H_7), 7.82 (4H, m, J = 8.8, H_91) 12.61 ,($_{13}$H, s, H_{14}), 7.17 (1H, d, J = 4.4 Hz, H_{16}), 6.76 (1H, d, J = 4.4 Hz, H_{17}), 2.35 (3H, s, H_{18}); ^{13}C NMR (DMSO_d6, 100 MHz): δ 143.4 (1C, C_1), 120.1 (2C, $C_{2,6}$), 130.8 (2C, $C_{3,5}$), 124.3 (1C, C_4), 142.9 (1C, C_8), 114.5 (2C, $C_{9,13}$), 135.4 (2C, $C_{10,12}$), 127.4 (1C, C_{11}), 168.6 (1C, C_{15}), 139.0 (1C, C_{16}), 108.0 (1C, C_{17}); HRMS_ESI (m/z) calc. for $C_{16}H_{15}N_5O_3S_2$, $[M+1]^+$ 390.07, found 390.4573.

3_hydroxy_4)_3_methylphenyl)_4)_1_(N_(thiazol_2_yl)sulfamoyl) phenyl)triazene (ST-6): Light buff shining powder (DMF); mp 230 °C; FTIR (KBr): V_{max} 3447, 3147, 1528, 1287, 1139 cm^1 ;^{1}H NMR (DMSO_d6, 400 MHz): δ 7.57 (2H, d, J = 8.8 Hz, $H_{2,6}$), 7.59 (2H, d, J = 9.8 Hz, Ar_$H_{3,5}$), 12.22 (1H, s, H_7), 7.96 (2H, m, J = 8.4 Hz, $H_{9,13}$), 7.37 (2H, d, J = 8.4 Hz, $H_{10,12}$), 12.68 (1H, s, H_{14}), 7.24 (1H, d, J = 4.4 Hz, H_{16}), 6.82 (1H, d, J = 4.4 Hz, H_{17}), 2.38 (3H, s, H_{18}); ^{13}C NMR (DMSO_d6, 100 MHz): δ 140.1 (1C, C_1), 119.7 (2C, $C_{2,6}$), 129.6 (2C, $C_{3,5}$), 124.3 (1C, C_4), 140.7 (1C, C_8), 114.5 (2C, $C_{9,13}$), 135.2 (2C, $C_{10,12}$), 127.4 (1C, C_{11}), 168.6 (1C, C_{15}), 143.5 (1C, C_{16}), 107.9 (1C, C_{17}), 20.6 (1C, C_{18}); HRMS_ESI (m/z) calc. for $C_{16}H_{15}N_5O_3S_2$, $[M+1]^+$ 389.06, found 390.4573.

3_hydroxy_3_methyl_4)_1_(N_3,4)_dimethylisoxazol_5_yl)sulfamoyl)phenyl)triazene (SF-1): Light yellow color powder (methanol); mp 195 °C; FTIR (KBr): V_{max} 3437, 3097, 1592, 1385, 1184 cm^1 ;^{1}H NMR (DMSO_d6, 400 MHz): δ 3.98 (3H, s, H_1), 11.20 (1H, s, H_2), 7.45 (2H, m, J = 8.6 Hz, $H_{4,8}$), 7.95 (2H, m, J = 7.28 Hz, $H_{5,7}$), 11.80 (1H, s, H_9), 2.2 (3H, s, H_{13}), 1.85 (3H, s, H_{14}); ^{13}C NMR (DMSO_d6, 100 MHz): δ 30.8 (1C, C_1), 156.2 (1C, C_3), 112.6 (2C, $C_{4,8}$), 124.4 (2C, $C_{5,7}$), 128.6 (1C, C_6), 161.1 (1C, C_{10}), 104.2 (1C, C_{11}), 153.2 (1C, C_{12}), 10.2 (1C, C_{13}), 5.7 (1C, C_{14}); HRMS_ESI (m/z) calc. for $C_{12}H_{15}N_5O_4S$, $[M+1]^+$ 326.09, found 324.3581.

3_hydroxy_3_ethyl_4)_1_(N_3,4)_dimethylisoxazol_5_yl)sulfamoyl) phenyl)triazene (SF-2): Dark yellow shining powder (methanol); mp 149 °C. FTIR (KBr): V_{max} 3482, 3379, 1594, 1335, 1161 cm^1 ;^{1}H NMR (DMSO_d6, 400 MHz): δ 1.62 (3H, m, H_1), 4.01 (2H, q, J = 6.50 Hz, H_2), 10.48 (1H, s, H_3), 6.61 (2H, d, J = 5.28 Hz, $H_{5,9}$), 7.36 (2H, d, J = 5.28 Hz, $H_{6,8}$), 11.20 (1H, s, H_{10}), 2.50 (3H, s, H_{14}), 2.07 (3H, s, H_{15}); ^{13}C NMR (DMSO_d6, 100 MHz): δ 30.8 (1C; C_1), 5.7 (1C, C_2), 156.2 (1C, C_4), 112.6 (2C, $C_{5,9}$), 124.4 (2C, $C_{6,8}$), 128.6 (1C, C_7), 161.1 (1C, C_{11}), 104.2 (1C, C_{12}), 153.2 (1C, C_{13}), 10.2 (1C, C_{14}), 5.7 (1C, C_{15}); HRMS_ESI (m/z) calc. for $C_{13}H_{17}N_5O_4S$, $[M+1]^+$ 340.11, found 340.3773.

3_hydroxy_3_propyl_4)_1_(N_3,4)_dimethylisoxazol_5_yl)sulfamoyl)phenyl)triazene (SF-3): Dark yellow color powder (acetone); mp 162 °C. FTIR (KBr): V_{max} 3484, 3379, 1594, 1336, 1162 cm^1 ;^{1}H NMR (DMSO_d6, 400 MHz): δ 1.62 (3H, t, J = 7.32 Hz, H_1), 2.07 (2H, m, H_2), 4.0 (2H, t, J = 7.2 Hz, H_3), 10.49 (1H, s, H_4), 6.62 (2H, d, J = 8.72 Hz, $H_{6,10}$), 7.36 (2H, d, J = 8.72 Hz, $H_{7,9}$), 11.01 (1H, s, N_{11}), 3.18 (3H, s, H_{15}), 2.51 (3H, s, H_{16}); ^{13}C NMR (DMSO_d6, 100 MHz): δ 30.8 (1C, C_1), 15.7 (1C, C_2), 10.2 (1C, C_3), 156.2 (1C, C_5), 112.6 (2C, $C_{6,10}$), 124.4 (2C, $C_{7,9}$), 128.6 (1C, C_8), 161.1 (1C, C_{12}), 104.3 (1C, C_{13}), 153.2 (1C, C_{14}), 12.2 (1C, C_{15}), 5.7 (1C, C_{16}); HRMS_ESI (m/z) calc. for $C_{14}H_{19}N_5O_4S$, $[M+1]^+$ 354.12, found 354.4033.

3_hydroxy_3_phenyl_4)_1_(N_3,4)_dimethylisoxazol_5_yl)sulfamoyl)phenyl)triazene (SF-4): Light yellow color powder (methanol); mp 109110_ °C. FTIR (KBr): V_{max} 3474, 3380, 1595, 1339, 1159 cm^1 ;^{1}H NMR (DMSO_d6, 400 MHz): δ 7.67 (2H, d, J = 9.2 Hz, $H_{2,6}$), 7.58 (2H, m, J = 10.4 Hz, $H_{3,5}$), 7.50 (1H, m, H_4), 10.96 (1H, s, H_7), 7.71 (2H, d, J = 8.8 Hz, $H_{9,13}$), 8.11 (2H, dd, J = 2.4, 1.44 Hz, $H_{10,12}$), 12.41 (1H, s, H_{14}), 2.09 (3H, s, H_{18}), 1.65 (3H, s, H_{19}); ^{13}C NMR (DMSO_d6, 100 MHz): δ 144.4 (1C, C_1), 114.8 (2C, $C_{2,6}$), 129.2 (2C, $C_{3,5}$), 120.0 (1C, C_4), 155.7 (1C, C_8), 122.0 (2C, $C_{9,13}$), 124.9 (2C, $C_{10,12}$), 132.6 (1C, C_{11}), 161.3 (1C, C_{15}), 104.8 (1C, C_{16}), 142.9 (1C, C_{17}), 10.2 (1C, C_{18}), 5.8 (1C, C_{19}); HRMS_ESI (m/z) calc. for $C_{17}H_{17}N_5O_4S$, $[M+1]^+$ 388.11, found 388.4193.

3_hydroxy_3)_3_methylphenyl) _4)_1_(N_3,4)_dimethylisoxazol_5_yl)sulfamoyl)phenyl)triazene (SF-5): Orange brown color powder (methanol); mp 8082_ °C. FTIR (KBr): V_{max} 3483, 3379, 1597, 1336, 1159 cm^1 ;^{1}H NMR (DMSO_d6, 400 MHz): δ 7.37 (2H, d, J = 8.76 Hz, $H_{2,6}$), 7.72 (1H, m, H_3), 7.66 (1H, m, H_4), 6.61 (2H, m, H_6), 10.54 (1H, s, H_7), 8.07 (2H, d, J = 7.96 Hz, $H_{10,12}$), 7.91 (2H, m, J = 8.4 Hz, $H_{9,13}$), 11.98 (1H, s, H_{14}), 2.43 (3H, s, H_{18}), 2.08 (3H,

s, H_{19}), 2.07 (3H, s, H_{20}); ^{13}C NMR (DMSO_d6, 100 MHz): δ 153.2 (1C, C_1), 114.8 (2C, $C_{2,6}$), 124.5 (1C, C_3), 121.8 (1C, C_4), 130.3 (1C, C_5), 156.2 (1C, C_8), 120.2 (2C, $C_{9,13}$), 125.4 (2C, $C_{10,12}$), 161.1 (1C, C_{15}), 104.3 (1C, C_{16}), 144.5 (1C, C_{17}), 10.2 (1C, C_{18}), 5.8 (1C, C_{19}), 20.8 (1C, C_{20}); HRMS_ESI (m/z) calc. for $C_{18}H_{19}N_5O_4S$, [M+1]+ 402.12, found 396.4172.

3_hydroxy_4)_3_methylphenyl) _4)_1_(N_3,4)_dimethylisoxazol_5_yl)sulfamoyl)phenyl)triazene (SF-6): Light yellow color powder (methanol); mp 161 °C. FTIR (KBr): V_{max} 3483, 3379, 1597, 1336, 1164 cm^1 ;^{1}-H NMR (DMSO_d6, 400 MHz): δ 7.96 (2H, d, J = 8.4 Hz, $H_{2,6}$), 7.45 (2H, d, J = 8.4 Hz, $H_{3,5}$), 10.48 (1H, s, H_7), 6.14 (2H, m, J = 9.2 Hz, $H_{9,13}$), 6.26 (2H, d, J = 10.2 Hz, $H_{10,12}$), 12.26 (1H, s, H_{14}), 2.38 (3H, s, H_{18}), 2.07 (3H, s, H_{19}), 1.61 (3H, s, H_{20}); ^{13}C NMR (DMSO_d6, 100 MHz): δ 142.2 (1C, C_1), 112.6 (2C, $C_{2,6}$), 128.6 (2C, $C_{3,5}$), 139.6 (1C, C_4), 156.3 (1C, C_8), 119.5 (2C, $C_{9,13}$), 125.1 (2C, $C_{10,12}$), 140.6 (1C, C_{11}), 161.1 (1C, C_{15}), 104.2 (1C, C_{16}), 153.3 (1C, C_{17}), 10.2 (1C, C_{18}), 5.7 (1C, C_{19}), 20.6 (1C, C_{20}); HRMS_ESI (m/z) calc. for $C_{18}H_{19}N_5O_4S$, [M+1]$^+$ 401.43, found 402.446.

3_hydroxy_3_methyl_4)_1_(N_5)_methylisoxazol_3_yl)sulfamoyl) phenyl)triazene (SM-1): Dark orange color powder (methanol); mp 208 °C. FTIR (KBr): V_{max} 3429, 3269, 1603, 1314, 1157 cm^1 ;^{1}-H NMR (DMSO_d6, 400 MHz): δ 3.96 (3H, s, H_1), 10.68 (1H, s, H_2), 7.39 (2H, m, J = 8.88 Hz, $H_{4,8}$), 7.73 (2H, d, J = 8.84 Hz, $H_{5,7}$), 11.63 (1H, s, H_9), 2.29 (3H, s, H_{13}), 6.12 (1H, s, H_{14}); ^{13}C NMR (DMSO_d6, 100 MHz): δ 50.8 (1C, C_1), 157.6 (1C, C_3), 113.7 (2C, $C_{4,8}$), 128.5 (2C, $C_{5,7}$), 131.0 (1C, C_6), 144.8 (1C, C_{10}), 95.3 (1C, C_{11}), 107.1 (1C, C_{12}), 12.0 (1C, C_{13}); HRMS_ESI (m/z) calc. for $C_{11}H_{13}N_5O_4S$, [M+1]$^+$ 312.08, found 312.3243.

3_hydroxy_3_ethyl_4)_1_(N_5)_methylisoxazol_3_yl)sulfamoyl) phenyl)triazene (SM-2): Pale yellow color powder (methanol); mp 158 °C. FTIR (KBr): V_{max} 3436, 3298, 1605, 1322, 1160 cm^1 ;^{1}-H NMR (DMSO_d6, 400 MHz): δ 2.08 (3H, t, J = 6.36 Hz, H_1), 2.48 (2H, q, J = 6.36 Hz, H_2), 10.70 (1H, s, H_3), 7.80 (2H, d, J = 8.84 Hz, $H_{5,9}$), 7.43 (2H, m, J = 8.84 Hz, $H_{6,8}$), 11.69 (1H, s, H_{10}), 2.28 (3H, s, H_{14}), 6.11 (1H, s, H_{15}); ^{13}C NMR (DMSO_d6, 100 MHz): δ 32.8 (1C, C_1), 11.7 (1C, C_2), 158.1 (1C, C_4), 114.1 (2C, $C_{5,9}$), 128.6 (2C, $C_{6,8}$), 132.3 (1C, C_7), 147.0 (1C, C_{11}), 95.4 (1C, C_{12}), 169.8 (1C, C_{13}), 12.0 (1C, C_{14}); HRMS_ESI (m/z) calc. for $C_{12}H_{15}N_5O_4S$, [M+1]$^+$ 326.09, found 325.3503.

3_hydroxy_3_propyl_4)_1_(N_5)_methylisoxazol_3_yl)sulfamoyl) phenyl)triazene (SM-3): Light brown color shiny powder (acetone); mp 272 °C. FTIR (KBr): V_{max} 3460, 3210, 1603, 1388, 1159 cm^1 ;^{1}-H NMR (DMSO_d6, 400 MHz): δ 0.88 (3H, s, J = 7.4 Hz, H_1), 1.87 (2H, m, J = 7.08 Hz, H_2), 4.08 (2H, t, J = 6.8 Hz, H_3), 10.70 (1H, s, H_4), 7.73 (2H, d, J = 2.24 Hz, $H_{6,10}$), 7.38 (2H, m, J = 2.2 Hz, $H_{7,9}$), 11.63 (1H, s, H_{11}), 2.26 (3H, s, H_{15}), 6.80 (1H, s, H_{16}); ^{13}C NMR (DMSO_d6, 100 MHz): δ 64.6 (1C, C_1), 20.1 (1C, C_2), 10.5 (1C, C_3), 158.8 (1C, C_5), 113.6 (2C, $C_{6,10}$), 128.4 (2C, $C_{7,9}$), 132.3 (1C, C_8), 169.4 (1C, C_{12}), 95.5 (1C, C_{13}), 144.4 (1C, C_{14}), 12.1 (1C, C_{15}); HRMS_ESI (m/z) calc. for $C_{13}H_{17}N_5O_4S$, [M+1]$^+$ 340.11, found 335.3568.

3_hydroxy_3_phenyl_4)_1_(N_5)_methylisoxazol_3_yl)sulfamoyl) phenyl)triazene (SM-4): Light pale yellow powder (acetone); mp 170 °C. FTIR (KBr): V_{max} 3415, 3178, 1596, 1342, 1165 cm^1 ;^{1}-H NMR (DMSO_d6, 400 MHz): δ 7.66 (2H, m, J = 9.2 Hz, $H_{2,6}$), 7.59 (2H, m, J = 7.56 Hz, $H_{3,5}$), 11.31 (1H, s, H_7), 7.81 (2H, d, J = 8.8 Hz, $H_{9,13}$), 8.10 (2H, d, 9.76 $H_{10,12}$), 12.38 (1H, s, H_{14}), 2.30 (3H, s, H_{18}), 6.16 (1H, d, H_{19}); ^{13}C NMR (DMSO_d6, 100 MHz): δ 142.9 (1C, C_1), 114.8 (2C, C_2-C_6), 129.2 (2C, $C_{3,5}$), 128.5 (1C, C_4), 157.6 (1C, C_8), 119.9 (2C, $C_{9,13}$), 130.3 (2C, $C_{10,12}$), 132.0 (1C, C_{11}), 144.4 (1C, C_{15}), 95.3 (1C, C_{16}), 170.2 (1C, C_{17}), 12.0 (1C, C_{18}); HRMS_ESI (m/z) calc. for $C_{16}H_{15}N_5O_4S$, [M+1]$^+$ 374.09, found 374.3863.

3_hydroxy_3)_3_methylphenyl)_4)_1_(N_5)_methylisoxazol_3_yl) sulfamoyl)phenyl)triazene (SM-5): Light yellow color powder (acetone); mp 182 °C. FTIR (KBr): V_{max} 3436, 3210, 1604, 1335, 1164 cm^1 ;^{1}-H NMR (DMSO_d6, 400 MHz): δ 7.44 (1H, d, J = 7.6 Hz, H_2), 7.80 (1H, d, J = 8.4, H_3), 7.37 (1H, d, J = 7.6 Hz, H_4), 7.93 (1H, m, J = 2.94 Hz, H_6), 11.32 (1H, s, H_7), 7.81 (2H, m, J = 9.2 Hz, $H_{9,13}$), 7.65 (2H, d, J = 8.8 Hz, $H_{10,12}$), 12.35 (1H, s, H_{14}), 2.42 (3H, s, H_{18}), 6.16 (1H, m, H_{19}), 2.30 (3H, s, H_{20}); ^{13}C NMR (DMSO_d6, 100 MHz): δ 142.9 (1C, C_1), 114.8 (1C, C_2), 130.9 (1C, C_3), 128.5 (1C, C_4), 132.0 (1C, C_5), 117.1 (1C, C_6), 157.6 (1C, C_8), 120.2 (2C, $C_{9,13}$), 129.0 (2C, $C_{10,12}$), 139.0 (1C, C_{11}), 144.5 (1C, C_{15}), 95.3 (1C, C_{16}), 170.2 (1C, C_{17}), 12.1 (1C, C_{18}), 20.8 (1C, C_{19}); HRMS_ESI (m/z) calc. for $C_{17}H_{17}N_5O_4S$, [M+1]$^+$ 388.11, found 388.4193.

3_hydroxy_4)_3_methylphenyl)_4)_1_(N_5)_methylisoxazol_3_yl) sulfamoyl)phenyl)triazene (SM-6): light lemon yellow color powder (acetone); mp 192 °C. FTIR (KBr): V_{max} 3409, 3188, 1601, 1341, 1164 cm^1 ;^{1}-H NMR (DMSO_d6, 400 MHz): δ 7.28 (2H, d, J = 8.8 Hz, Ar_$H_{2,6}$), 7.28 (2H, d, J = 8.4 Hz, Ar_$H_{3,5}$), 11.29 (1H, s, H_7), 7.79 (2H, d, J = 7.2 Hz, $H_{9,13}$), 7.63 (2H, d, J = 7.2 Hz, $H_{10,12}$), 12.30 (1H, s, H_{14}), 2.39 (3H, s, H_{18}), 6.15 (1H, m, H_{19}), 2.30 (3H, s, H_{20}); ^{13}C NMR (DMSO_d6, 100 MHz): δ 140.7 (1C, C_1), 114.7 (2C, $C_{2,6}$), 129.6 (2C, $C_{3,5}$), 131.9 (1C, C_4), 157.6 (1C, C_8), 119.8 (2C, $C_{9,13}$), 128.5 (2C, $C_{10,12}$), 140.3 (1C, C_{11}), 144.5 (1C, C_{15}), 95.3 (1C, C_{16}), 170.2 (1C, C_{17}), 12.0 (1C, C_{18}), 20.6 (1C, C_{19}); HRMS_ESI (m/z) calc. for $C_{17}H_{17}N_5O_4S$, [M+1]$^+$ 388.11, found 388.4192.

2.4. Biological activity

2.4.1. Anti-diabetic

To evaluate anti-diabetic activity of synthesized hydroxytriazenes α-glucosidase and α-amylase inhibition methods (Tripathi et al., 2014) were used. Several dilutions of primary solution (5 μg/mL, DMSO) were made and assayed accordingly to obtain concentration of the test sample required to inhibit 50% activity of the enzyme (IC_{50}). Quantification was performed with respect to the standard curve of acarbose (Y = 0.2262x + 47.244, R^2 = 0.9397) for α-glucosidase and standard curve of acarbose (Y = 0.4357x + 19.663, R^2 = 0.9951) for α-amylase and results were expressed as standard acarbose in 10, 30, 60, 90, 120, 150 μg/mL.

2.4.1.1. Assay for α-glucosidase inhibition method. In this method different concentrations viz 300, 240, 180, 120, 60 and 20 μg/mL of each test compounds were prepared. Rat-intestinal powder was dissolved in 100 mL of saline water and sonicated properly at 4 °C. After sonication, the suspension was centrifuged (1008 xg, 4 °C, 30 min) and the resulting supernatant was used for assay. A reaction mixture containing 50 μL of phosphate buffer (50 mM, pH 6.8), 75 μL of rat α-glucosidase and 50 μL sample of varying concentrations (20–300 μg/mL) was pre-incubated for 5 min at 37 °C, and then 75 μL of 3 mM p-nitrophenyl-α-D-glucopyranoside (PNPG) was added to the mixture as a substrate. After incubation at 37 °C for 30 min, enzymatic activity was quantified by measuring the absorbance at 405 nm in Multi-Mode Reader. Acarbose was used as a positive control, which is the standard drug and water as a negative control. Experiments were done in triplicate, detailed results are shown in supporting information (S1) and IC_{50} values are given in Table 1. The % inhibition of α-glucosidase activity was calculated using the following formula:

$$\%\ inhibition = \frac{OD_{control} - OD_{sample}}{OD_{control}} X100$$

2.4.1.2. Assay for α-amylase inhibitory method. Six different concentrations 300, 240, 180, 120, 60 and 20 μg/mL for each of the test compounds were prepared in test tubes and six more solutions (10, 30, 60, 90, 120, 150 μg/mL) of standard drug acarbose were also prepared for comparison. A control test tube was prepared which did not contain any sample or drug. To each of the test compounds, 50 μL of the α-amylase solution (0.5 μg/mL in 0.02 M sodium phosphate buffer solution, pH 6.8) was added and allowed to incubate for 10 min at room temperature. After this, 50 μL starch solution (1% starch in 0.02 M phosphate buffer solution) was added to all the test tubes and kept for incubation at 25 °C for 10 min. Then, 100 μL 3,5-dinitrosalicylic acid (DNSA) was added to each of the test tube and incubated again in boiling water for 5 min to stop the

Table 1. Result of anti-diabetic, antioxidant and anti-inflammatory activities of hydroxytriazenes.

Compound	α-Glucosidase inhibition method IC_{50} value (μg/mL)	α-Amylase inhibition method IC_{50} value (μg/mL)	DPPH Assay IC_{50} value (μg/mL)	ABTS Assay IC_{50} value (μg/mL)	Inhibition of paw edema after 2 h (%)	Inhibition of paw edema after 4 h (%)
ST-1	238	175.02	488.06	**54.131**	44.85	60.57
ST-2	219	231.35	532.35	232.393	30.28	71.71
ST-3	252	200.17	662.79	160.145	35.14	82.28
ST-4	280.03	206.55	565.88	54.217	17.42	79.71
ST-5	341	217.31	472.08	92.075	37.14	74.28
ST-6	314	326.89	858.54	**48.055**	25.71	65.71
SF-1	270	172.65	556.65	89.032	30.28	77.42
SF-2	248.64	212.11	614.17	229.83	22.28	77.14
SF-3	235.55	148.08	490.82	218.94	24.00	68.57
SF-4	233	175.14	578.76	88.14	39.71	77.57
SF-5	292	219.96	423.91	164.34	29.42	68.28
SF-6	329	245.55	587.28	70.62	45.42	76.85
SM-1	**160.7**	217.22	426.75	252.96	**54.00**	80.28
SM-2	201.99	**122.17**	**367.17**	401.72	41.14	**88.85**
SM-3	222.91	211.69	624	282.32	**50.28**	81.71
SM-4	249	175.14	583.31	104.13	57.14	82.57
SM-5	314.43	200.94	660.39	81.50	45.14	71.42
SM-6	262.74	164.62	550.32	250.26	**56.87**	**88.57**
Standard drug	12.21 (acarbose)	69.74 (acarbose)	29.12 (ascorbic acid)	69.13 (ascorbic acid)	52.00 (diclofenac sodium	94.28 (diclofenac sodium)

Bold data shows best results in the respective activity.

reaction. The test tubes were diluted to 350 μL by adding distilled water. Their absorbance was recorded using the Multi-Mode Reader at 540 nm. The % inhibition of α-amylase activity was calculated using same formula used in α-glucosidase inhibition method. The results, % inhibition as mean ± SD and IC_{50} values are shown in supporting information (S2) and Table 1 respectively.

2.4.2. Antioxidant activity

Antioxidant activities of all the hydroxytriazenes were screened by DPPH and ABTS radical scavenging method (Tripathi et al., 2013). The quantification of radicals was done using Multimode Reader with ascorbic acid ($Y = 0.1371x + 46.017$, $R^2 = 0.9609$) for DPPH and ascorbic acid ($Y = 0.5996x + 8.5897$, $R^2 = 0.9918$) for ABTS as a positive control whose concentration was 10, 30, 60, 90, 120, 150 μg/mL in this experiment.

2.4.2.1. DPPH free radical scavenging activity. Briefly, 125 μL solution of each synthesized compound with different concentrations (20–300 μg/mL) was added to 125 μL DPPH solution. After shaking vigorously, the mixture was incubated at room temperature in the dark place for 30 min, the absorbance was measured at 517 nm. The % DPPH scavenging effects were calculated using following formula. The detailed results and IC_{50} values are depicted in supporting information (S3) and Table 1, respectively.

$$\%\ scavenging = \frac{OD_{control} - OD_{sample}}{OD_{control}} X100$$

2.4.2.2. ABTS radical scavenging assay. The working solution was prepared by mixing 7 mM ABTS and 2.4 mM ammonium persulfate solution in equal ratio and allowed to react in dark for 18–24 h at room temperature. Different concentrations of the synthesized compounds (20–300 μg/mL) were added to ABTS solution. ABTS scavenging capacity of the test compounds along with standard drug were evaluated by measuring absorbance at 734 nm by Multi-Mode Reader. The percentage inhibition of ABTS radical scavenging activity (supporting information (S4) and Table-1) was calculated using same formula as used in DPPH scavenging activity.

2.4.3. Anti-inflammatory

Anti-inflammatory activity of the compounds was determined by carrageenan induced paw edema inhibition method in rats as reported in literature (Winter et al., 1962). In this method, Male or female Wistar albino rats with a body weight between 150 to 200 g were used. The animals have been starved overnight with free access of water. Thirty minutes after the oral administration of the compounds, the thickness of right hind paw was measured by a plethysmograph. Carrageenan (0.1 mL of 1%) was injected subcutaneously into the plantar surface of the right hind paw. The paw was marked with ink at the level of the lateral malleolus and immersed in mercury up to this mark. One hour later the volume of the edema was measured again and the antiedematous effects of the drugs were estimated in terms of percent inhibition and procedure was repeated at 2, 3 and 4 h after carrageenan injection.

Wister albino rats (150–200 g) were obtained from the animal house B.N. College of Pharmacy, Udaipur (Registration No. 870/ac/08/CPCSEA) and Protocol No. is 52/ACR/BNCP-10/IAEC. They were housed at temperature of 25 ± 2 °C for 12 h, light-dark cycle at 40–60 % humidity, in polypropylene cages and feed a standard rodent diet with water and libitum. Animal were deprived of food but not water 4 h before experiment.

The rats were divided into 21 groups (n = 6) total 126 animals were used, each receiving distilled water (control), diclofenac 12.5 mg/kg p.o. (reference standard) and test compounds groups (ST-1 to 6, SF-1 to 6 and SM-1 to 6) at a dose of 100 mg/kg, bw, p.o. A 1% solution of carrageenan (0.1 mL/kg, p.o.) was injected into subplantar tissue of the right hind paw of each rate. The paw volume at different time intervals was recorded using plethysmometer and compared with control and the percentage inhibition was calculated by this formula.

$$Antiinflammatory\ \ activity(\%) = \frac{\left[(V_t - V_0)_{carrageenan} - (V_t - V_0)_{treated}\right]}{(V_t - V_0)_{carrageenan}} X100$$

V_0 and V_t indicate volume of hind paw edema of at 0 and 1, 2, 3, 4 h after injection of carrageenan respectively. Comprehensive data of study (supporting information (S5)) and results as % inhibition are described in Table 1 and Figures 3, 4,5.

2.5. Statistical analysis

The data were recorded as mean ± standard error mean (S.E.M). The significance of variance of the groups was calculated using one way and multiple way analyses of variance (ANOVA). The test followed by Dunnett's test and P values less than 0.01 were noted as significance (Mahajan 1989).

3. Results and discussion

3.1. Anti-diabetic activity

One therapeutic approach for treating diabetes is to decrease postprandial hyperglycemia. This is done by hindering the absorption of glucose through inhibition of the carbohydrate hydrolysing enzymes particularly α-amylase and α-glucosidase in the digestive tract. Results of the activity were expressed as IC_{50} values. Acarbose has been used as reference drug, which have IC_{50} values 12.21 and 69.74 μg/mL for α-glucosidase and α-amylase enzymes, respectively.

In α-glucosidase activity the most active hydroxytriazene was SM-1 (IC_{50} = 160.7 μg/mL) followed by SM-2 (IC_{50} = 201.99 μg/mL) and ST-2 (IC_{50} = 219 μg/mL), SM-3 (IC_{50} = 222.91 μg/mL). Compounds ST-6 (IC_{50} = 314 μg/mL), SM-5 (IC_{50} = 314.43 μg/mL) and SF-6 (IC_{50} = 329 μg/mL), ST-5 (IC_{50} = 341 μg/mL) were showed the lowest activity. Other samples displayed medium range of α-glucosidase activity. In α-amylase activity the most active hydroxytriazene was SM-2 (IC_{50} = 122.17 μg/mL) followed by SF-3 (IC_{50} = 148.08 μg/mL) and SM-6 (IC_{50} = 164.62 μg/mL), SF-1 (IC_{50} = 172.65 μg/mL), SF-5 (IC_{50} = 219 μg/mL), ST-2 (IC_{50} = 231.35 μg/mL) and SF-6 (IC_{50} = 245.55 μg/mL), ST-6 (IC_{50} = 326.89 μg/mL) showed the lowest activity. Other samples displayed medium range of α-amylase activity.

3.1.1. SAR studies

To look at the structure–activity relationship (SAR) of the novel hydroxytriazenes incorporated sulfonamide drugs moiety, the variations were determined on one end of hydroxytriazene moiety with alkyl/aryl alkyl groups. On the other hand sulphonamide group with five membered heterocyclic of methyl substituted thiazole and isoxazole rings were substituted. Among the three series, compound SM-1 seems to be more favourable inhibitor towards the α-glucosidase enzyme due to the presence of small methyl substituents at the nitrogen atom of the hydroxytriazene and isoxazole ring. The increment of alkyl carbons from methyl to ethyl & propyl (compounds ST-3,4, SF-3,4 & SM-3,4) on the hydroxytriazene led to decrease the α-glucosidase enzyme inhibition activity by moderate level. The potency of α-glucosidase enzyme inhibition drastically reduced while increasing the bulky groups from methyl to phenyl ring at the hydroxytriazene moiety (compounds ST-5,6, SF-5,6 & SM-5,6). This indicated that only smaller alkyl substituents are tolerable at this region. Concerning the enzyme inhibition towards the α-amylase, compounds with mono and dimethyl substituents of isoxazole moiety at sulfonamide group showed better enhancement of activity (compounds SF-3,4, SM-2,4 & 6) than the thiazole nucleus placed at the same position. The hydroxytriazenes integrated sulfonamide series exhibited moderate inhibition against the both α-glucosidase and α-amylase enzymes when compared with standard acarbose drug. This preliminary SAR investigation gave a structural requirement guidance to further lead optimisation of the series against these enzymes.

Recent advances have developed the newer strategies in management of diabetes by hindering the activity of intestinal enzymes particularly α-amylase and α-glucosidase, both play important role in carbohydrate digestion by degrading starch and oligosaccharides to monosaccharides before they can be absorbed and glucose absorption. Suppression of the activity of such digestive enzymes would delay the degradation of starch and oligosaccharides, which would in turn cause a decrease in the absorption of glucose and consequently the reduction of postprandial blood glucose level elevation (Kim et al., 2005). Alpha-glucosidase and α-amylase inhibitors inhibit the digestion of carbohydrates and slows down the absorption. Acarbose, voglibose, miglitol etc. are well known drug used in the treatment of diabetes mellitus by inhibiting the digestive enzymes in intestines (Derosa and Maffioli, 2012). However, this drug has some unwanted effects such as abdominal or stomach pain, diarrhea and flatulence (Agu et al., 2019). On the other hand, synthetic compounds possessing antioxidant properties have much less side effects and are thus being preferred. Thus, there is need to explore compounds having antioxidant properties for the control and treatment of diabetes.

In our present study, we have examined the *in-vitro* antidiabetic activities of the 18 hydroxytriazenes by α-amylase and α-glucosidase enzyme inhabitation methods and comparing them with the standard drug acarbose. From Table 1, we can see that all the compounds have shown significant inhibition of α-amylase and α-glucosidase enzymes. Comparison of results of both the activities revealed that hydroxytriazenes are stronger inhibitor for α-amylase than α-glucosidase enzyme. The mechanisms involved in inhibition of the enzymes by hydroxytriazenes might be due to interaction with proteins of enzymes.

3.2. Antioxidant activity

Antioxidant tests using free radical traps are relatively straightforward to perform. Among free radical scavenging methods available, the DPPH and ABTS methods are rapid, simple, highly reproducible and inexpensive in comparison to other models (Gulcin, 2020). Both methods are based on the reduction of DPPH and ABTS radical solution in the presence of hydrogen donating antioxidants, due to the formation of the non-radical form DPPH-H (Figure 2) and ABTS-H.

The synthesized compounds were able to reduce the stable radical DPPH (purple to yellow) and ABTS (blue-green to colourless), in a concentration-dependent manner. Results were expressed as IC_{50} (Table-1) indicating all the synthesized compounds showed a fair DPPH and ABTS scavenging activity, as compare to ascorbic acid which was used as standard.

In this study two methods DPPH and ABTS assay are used, both methods are technically simple. It has been observed that IC_{50} values are lower in DPPH assay in comparison to ABTS assay. This may be attributed to difference in redox potential and slow reaction of DPPH radicals because it is a long lived nitrogen radical, which is not reactive like peroxyl radicals involved in lipid peroxidation. This would result in result in low readings for antioxidant capacity of samples (Bondet et al., 1997). The ABTS assay measures the relative ability of antioxidants to scavenge the ABTS generated in aqueous phase, as compared with a standard. The method is rapid and can be used over a wide range of pH values, in both aqueous and organic solvent systems. It also has good repeatability and is simple to perform; hence, it is widely reported.

In DPPH scavenging activity the most active hydroxytriazene was SM-2 (IC_{50} = 367.17 μg/mL) followed by SF-5 (IC_{50} = 423 μg/mL) and SM-1 (IC_{50} = 426 μg/mL). ST-3 (IC_{50} = 662 μg/mL), SM-5 (IC_{50} = 660 μg/mL) and ST-6 (IC_{50} = 858 μg/mL) showed the lowest activity. Other samples displayed medium range of DPPH scavenging activity. In ABTS scavenging activity the most active hydroxytriazenes were ST-6 (IC_{50} = 48.055 μg/mL), ST-4 (IC_{50} = 54.217 μg/mL) and ST-1 (IC_{50} = 54.131 μg/mL) followed by SF-6 (IC_{50} = 70 μg/mL), SM-5 (IC_{50} = 81.50 μg/mL), SF-4 (IC_{50} = 88.14 μg/mL) and SF-1 (IC_{50} = 89.032 μg/mL). SM-2 (IC_{50} = 401.72 μg/mL), SM-3 (IC_{50} = 282.32 μg/mL), SM-6 (IC_{50} = 250.26 μg/mL), SM-1 (IC_{50} = 252.96 μg/mL) and ST-2 (IC_{50} = 232.39 μg/mL) showed the lowest activity. Other samples displayed medium range of ABTS scavenging activity.

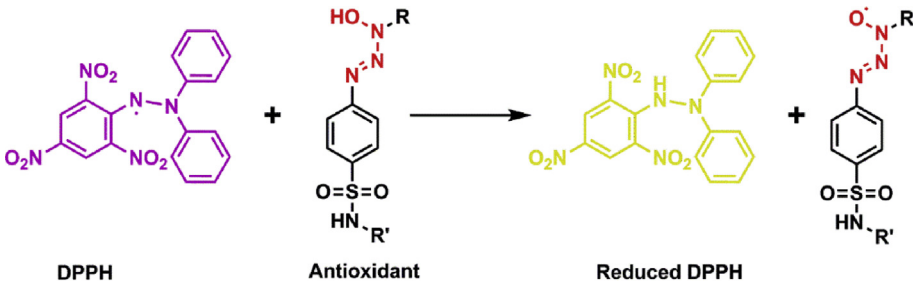


Figure 2. Conversion of DPPH• (purple) to its corresponding hydrazine form (yellow) by the addition of synthesized compounds to DPPH• due to proton transfer.

3.2.1. SAR studies

In particular, DPPH scavenging activity, only SM-2 demonstrated the moderate activity among the series. The activity fully based upon the presence of ethyl and methyl isoxazole moieties at the both end of the hydroxytriazenes integrated sulfonamide scaffold. The other substituents like phenyl, tolyl, propyl and methyl made the compounds less active. Therefore, analogue SM-2 could be considered as a lead compound for further development for the DPPH scavenging activity. But in the ABTS scavenging activity, thiazole containing three compounds ST-1, ST-4 and ST-6 exhibited the best activity than the isoxazole containing compounds, when compared with ascorbic acid drug. In the thiazole installed series, compounds with more hydrophobic aryl/alkyl groups at triazenyl (compound ST-6) displayed high ABTS scavenging activity. In the dimethyl isoxazole substituted derivatives, three compounds such as SF-1, SF-4 & SF-7 showed equipotent activity with the standard ascorbic acid drug. Except compound SM-5, the remaining compounds in the monomethyl isoxazole substituted derivatives showed lesser activity possibly due to reducing the nonpolar methyl at the isoxazole ring. Therefore, the thiazole fragment in the ST series important for enhancement of ABTS scavenging activity as well as for further structural optimization.

3.3. Anti-inflammatory activity

Synthesized hydroxytriazenes and marketed formulation of diclofenac sodium produced dose-dependent inhibition of carrageenan-induced paw edema as compared to the control and the values are reported in terms of % inhibition in Table 1 and Figures 3, 4 and 5. These results indicated that all hydroxytriazenes with a dose of 100 mg/kg, bw, p.o. shows a significant anti-inflammatory activity as compared to the reference drug diclofenac sodium.

The inflammation in animals is a protective response to a cell injury. Edema, erythema, hyperalgesia, pain and loss of function at microscopic level are the clinical signal of injury. The non-steroidal anti-inflammatory drug (NSAID) normally works at the periphery and not at CNS. NASID block the production of cytokines and cyclooxygenase pathway (COX) by hindering synthesis of ecosanoids at injured tissue and thereby reducing the production of prostaglandins (Vane and Botting, 1998) and inhabiting release of histamine (Han et al., 2012). Thus the tentative mechanism of action demonstrated by hydroxytriazenes is proposed to be NSAID like.

3.3.1. SAR studies

Hydroxytriazenes SM-1, SM-3, SM-4 and SM-6 particularly from SM series showed excellent activity comparable to standard diclofenac (52%) after 2 h of treatment. Compounds having alkyl chain substituted derivatives particularly ST-3(82.28%), SF-1(77.42%), SM-2(88.85%) and SM-6(88.57%) showed excellent anti-inflammatory activity after 4 h of treatment compared with their phenyl or substituted phenyl analogues. It has been observed that presence of alkyl substituent linked to triazene moiety may enhance the anti-inflammatory activity. Compounds having monomethyl isoxazole unit at sulfonamide nucleus showed superior potency among the screened compounds. In the case of SM series, the percentage of inhibition of inflammation values were ranged from 80.28 % to 88.85 % except the compound SM-5. The other series of (ST-1 to 6 & SF-1 to 6) having di-methyl isoxazole and thiazole substituents at the sulfonamide nucleus resulted in moderate anti-inflammatory activity. The percentage inflammation reducing activity ranging from 60.57 to 77.57 %. Hence molecules having monomethyl isoxazole unit represent promising leads for the development of novel class of potent anti-inflammatory agents. The significant high anti-inflammatory activity of hydroxytriazenes may be due to the inhibition of the mediators of inflammation such as histamine, serotonin and prostaglandin.

3.4. Molecular docking

To investigate the binding mode of the sulpha drug based hydroxytriazene derivatives further, *Insilco* molecular docking was performed for

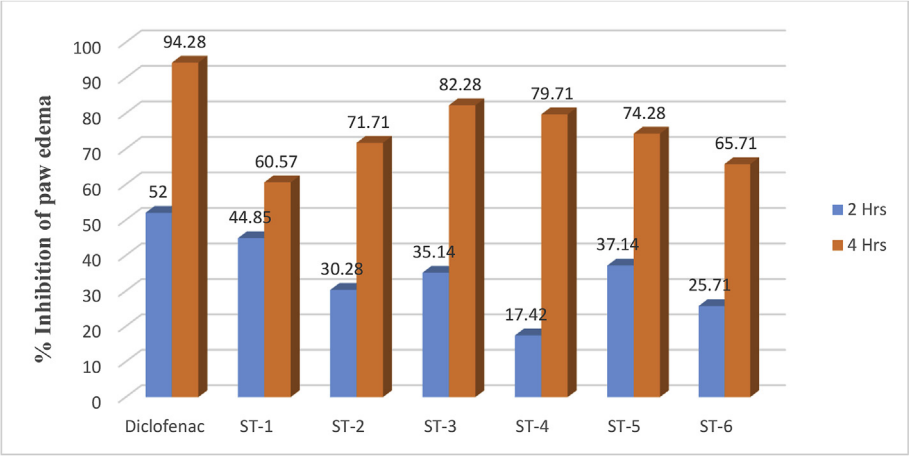


Figure 3. Anti-inflammatory activity of ST-series of hydroxytriazenes with % Inhibition of paw edema.

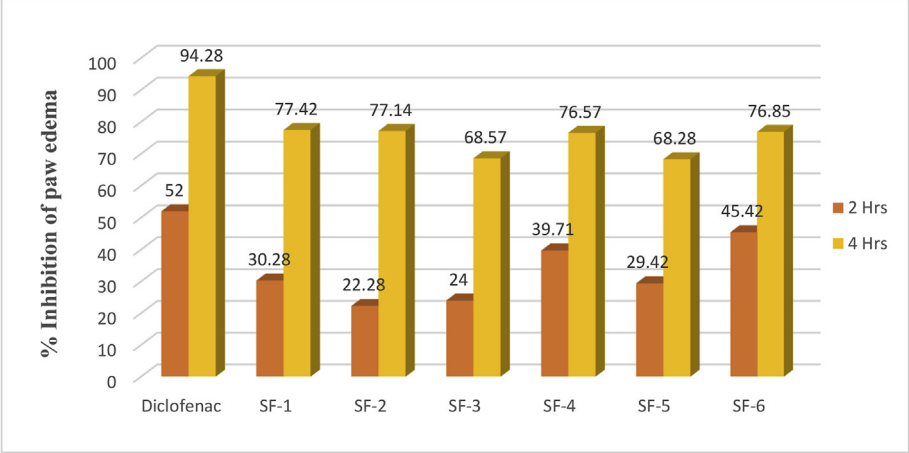


Figure 4. Anti-inflammatory activity of SF-series of hydroxytriazenes with % Inhibition of paw edema.

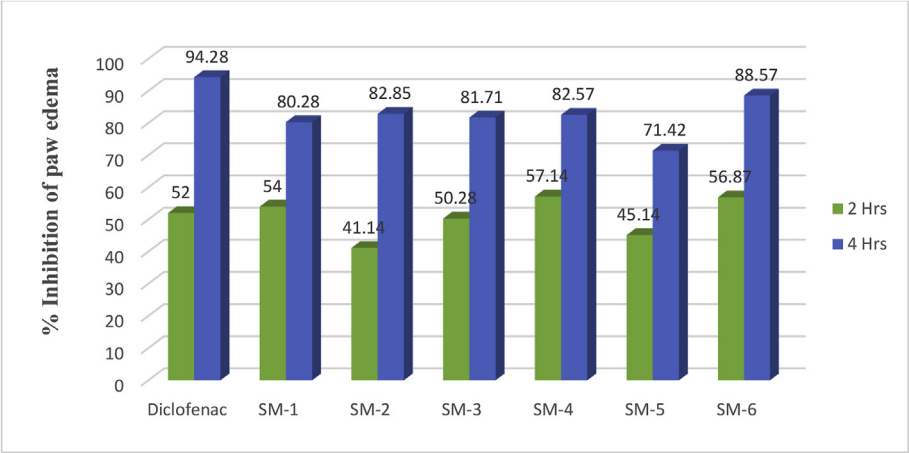


Figure 5. Anti-inflammatory activity of SM-series of hydroxytriazenes with % Inhibition of paw edema.

active compounds SF-1 and SM-1 to determine their binding affinity in to the C_terminal subunit (PDB: 3TOP) of human maltase_glucoamylase of the α_glucosidase enzymes (Ren et al., 2011). The enzymes X-ray crystal structure coordinates and the docking method were estimated before docking the SF-1 and SM-1 by replicating the bound conformation of acarbose in to the corresponding enzymes active site. Docking simulations of these molecules was accomplished by using the Molecular Operating Environment (MOE 2015.1001) software package (Chemical Computing Group Inc. 2016). The triangle matcher placement technique followed by rigid receptor protocol was selected to search and generate the good fit conformers in the active site of the enzymes. We used GBVI/WSA dG scoring function to determine the binding energy values

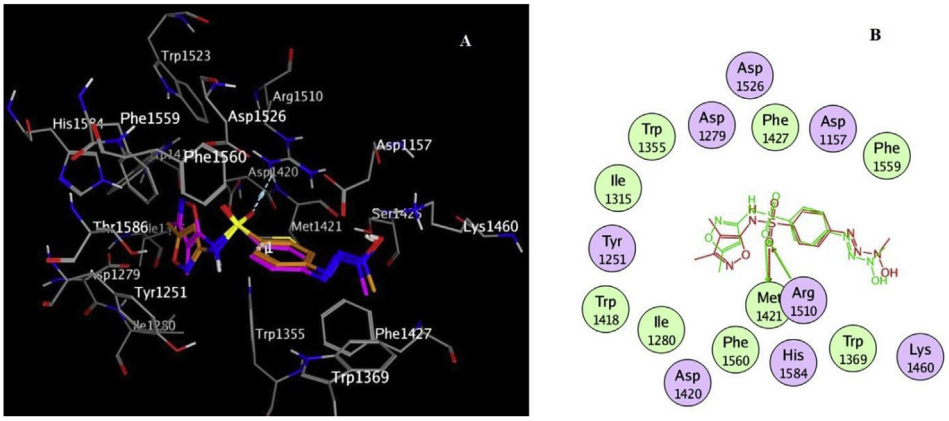


Figure 6. 3D (Panel A) and 2D (Panel B) structural representations of binding mode of compounds SF-1 (pink colour) and SM-1 (brown colour). Both molecules are shown as a ball_and_stick model in the active site of C_terminal subunit of human maltase_glucoamylase of α_glucosidase.

of the best protein_ligand complex (pose). Molecular modelling showed that, SF-1 and SM-1 molecules has privileged binding affinity scores of 6.61_ and 6.68_ kcal/mol towards the active site of C_terminal of maltase_glucoamylase, respectively. As shown in Figure 6, both docked compounds showed similar binding manner inside the pocket of the enzyme. Theoretically, the hydroxyl group which is attached at the triazene moiety have entirely projected towards the solvent interface of the enzyme. It revealed that restriction for lengthening at this triazene position is essential. The sulphonamide oxygen make a hydrogen bonding interaction with the Arg1510 residue by the amino group of guanidine. The phenyl ring in SF-1 and SM-1 molecules primarily formed an arene-H contact with the Trp1355 amino acid and also forms a lipophilic interaction with the aromatic amino acids such as Phe1427, Phe1560 and Trp1369 residues. The methyl substituted isoxazole and oxazole rings interact with hydrophobic region which is occupied by Trp1355, Ile1315, Trp1418, Ile1280, Phe1560 & Met1421 residues. The heterocyclic atoms of oxygen and nitrogen of the compounds involve in polar contacts with the aspartic acid amino acids of Asp 1279, Asp 1526 and Asp 1420. Whilst compared to the control acarbose binding interaction points (too many H_bond interactions), these derivatives only have one H_bond interaction is accessible to inhibit the α_glucosidase enzymes. Due to these reasons, sulpha drug based hydroxytriazene compounds somewhat lesser active and binding energies lesser than the standard acarbose drugs. Therefore, more polar functionalities like –OH, _NH_ & _COOH etc groups are needed around the heterocyclic rings to enhance the better inhibition potency against the α_glucosidase. These explorations might direct to promote the development of potent sulpha drug based hydroxytriazene compounds against the diabetes disorder by targeting against the C_terminal of human maltase_glucoamylase of the α_glucosidase.

4. Conclusion

In the present investigation, eighteen hydroxytriazenes (**ST 1-6, SF 1-6, SM 1-6**) were synthesized and characterized using various spectral analysis techniques. The synthesized compounds were evaluated for their anti-diabetic, antioxidant and anti-inflammatory activities. These hydroxytriazenes are promising anti-diabetic compounds that delay absorption of glucose by inhibiting α_glucosidase and α_amylase enzymes. They also demonstrated good anti-inflammatory and antioxidant activity. Results of antioxidant assays revealed that the compounds were showed good radical scavenging capacity against ABTS radical as compare to DPPH free radical. This may be attributed to high stability of DPPH free radical. The findings from the molecular docking experiments may also expand the formation of new potent sulpha drugs based hydroxytriazene derivatives targeting towards the subunit of C_terminal of human maltase_glucoamylase for the treatment of diabetes metabolic disorder. The compounds can be potential multi_targeted drugs if explored further.

Declarations

Author contribution statement

VARSHA DAYMA, Amit Bhargava: Performed the experiments; Analyzed and interpreted the data.

Jaishri Chopra, Aparna Dwivwdi: Performed the experiments.

Poonam Sharma: Analyzed and interpreted the data.

Indra P Tripathi: Conceived and designed the experiments.

Vanangamudi Murugesan: Contributed reagents, materials, analysis tools or data.

Ajay K Goswami, PRABHAT KUMAR BAROLIYA: Conceived and designed the experiments; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

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References

Agarwal, S., Baroliya, P.K., Bhargava, A., Tripathi, I.P., Goswami, A.K., 2016. Synthesis, characterization, theoretical prediction of activities and evaluation of biological activities of some sulfacetamide based hydroxytriazenes. Bioorg. Med. Chem. Lett 26 (12), 2870–2873, 15.

Agu, K.C., Eluehike, N., Ofeimun, R.O., Abile, D., Ideho, G., Ogedengbe, M.O., Onose, P.O., Elekofehinti, O.O., 2019. Possible anti-diabetic potentials of Annona muricata (soursop): inhibition of α-amylase and α-glucosidase activities. Clinical Phytoscience 5 (1), 21, 1.

Baragob, A.E., AlMalki, W.H., Alla, H.E., Ibrahim, A., Muhammed, S.K., Abdella, S., 2014. Investigate evaluation of oxidative stress and lipid profile in STZ-induced rats treated with antioxidant vitamin. Pharmacol. Pharm. 4, 2014.

Baroliya, P.K., Regar, M., Chauhan, R.S., Goswami, A.K., 2014. Synthesis, characterization and antimicrobial activities of hydroxytriazenes and their Co (II) complexes. Afinidad 31 (568), 71.

Bondet, V., Brand-Williams, W., Berset, C.L., 1997. Kinetics and mechanisms of antioxidant activity using the DPPH* free radical method. Food Sci. Techn. Zurich 30, 609–615, 1.

Bösenberg, L.H., Van Zyl, D.G., 2008. The mechanism of action of oral antidiabetic drugs: a review of recent literature. J. Endocrinol. Metabol. Diabetes S. Afr. 13 (3), 80–88, 1.

Bouguerra, R., Alberti, H., Salem, L.B., Rayana, C.B., Atti, J.E., Gaigi, S., Slama, C.B., Zouari, B., Alberti, K.G., 2007. The global diabetes pandemic: the Tunisian experience. Eur. J. Clin. Nutr. 61 (2), 160–165.

Chauhan, L.S., Jain, C.P., Chauhan, R.S., Goswami, A.K., 2007. Synthesis and analgesic activity of hydroxytriazenes. Asian J. Chem. 19 (6), 4684, 20.

Chauhan, L.S., Jain, C.P., Chauhan, R.S., Goswami, A.K., 2010. Anti-inflammatory activity of some substituted hydroxytriazenes. J. Glob. Phrama Techn. 2 (3), 107–111.

Chemical Computing Group Inc., 2016. Molecular Operating Environment. 2015.10.1010; Sherbrook St. West, Suite #910, Montreal, QC, Canada, H3A 2R7.

Chiasson, J.L., Josse, R.G., Gomis, R., Hanefeld, M., Karasik, A., Laakso, M., STOP-NIDDM Trial Research Group, 2002. Acarbose for prevention of type 2 diabetes mellitus: the STOP-NIDDM randomised trial. Lancet 359 (9323), 2072–2077, 15.

DeFronzo, R.A., 2000. Pharmacologic therapy for type 2 diabetes mellitus. Ann. Intern. Med. 133 (1), 73–74, 4.

Derosa, G., Maffioli, P., 2012. α-Glucosidase inhibitors and their use in clinical practice. Arch. Med. Sci.: AMS 8 (5), 899, 9.

Donath, M.Y., 2013. Targeting inflammation in the treatment of type 2 diabetes. Diabetes Obes. Metabol. 15 (s3), 193–196.

Elkins, M., Hunter, L., 1938. 252. The azo-group as a chelating group. Part III. Metallic derivatives of hydroxytriazens. J. Chem. Soc. 1346–1350.

Golbidi, S., Badran, M., Laher, I., 2012. Antioxidant and anti-inflammatory effects of exercise in diabetic patients. Exp. Diabetes Res. 2012.

Golbidi, S., Alireza Ebadi, S., Laher, I., 2011. Antioxidants in the treatment of diabetes. Curr. Diabetes Rev. 7 (2), 106–125, 1.

Goswami, A.K., Chauhan, R.S., Sharma, D., 2012. Hydroxytriazenes and their copper (II) complexes: a new class of antibiotic compounds. J. Pharm. Res. 5 (7), 3661–3664.

Goswami, A.K., 2002. Synthesis and insecticidal activity of some hydroxytriazenes and their vanadium complexes. Pestic. Res. J. 14 (2), 213–215.

Gulcin, İ., 2020. Antioxidants and antioxidant methods: an updated overview. Arch. Toxicol. 16, 1–65.

Hammes, H.P., Du, X., Edelstein, D., Taguchi, T., Matsumura, T., Ju, Q., Lin, J., Bierhaus, A., Nawroth, P., Hannak, D., Neumaier, M., 2003. Benfotiamine blocks

three major pathways of hyperglycemic damage and prevents experimental diabetic retinopathy. Nat. Med. 9 (3), 294–299.
Han, Y.S., Kim, M.S., Hwang, J.K., 2012. Macelignan inhibits histamine release and inflammatory mediator production in activated rat basophilic leukemia mast cells. Inflammation 35 (5), 1723–1731, 1.
Hanefeld, M., Cagatay, M., Petrowitsch, T., Neuser, D., Petzinna, D., Rupp, M., 2004. Acarbose reduces the risk for myocardial infarction in type 2 diabetic patients: meta-analysis of seven long-term studies. Eur. Heart J. 25 (1), 10–16, 1.
Huang, D., Ou, B., Prior, R.L., 2005. The chemistry behind antioxidant capacity assays. J. Agric. Food Chem. 53 (6), 1841–1856, 23.
Hura, I.S., Naulakha, N., Goswami, A.K., Shrivastava, M.K., 2003. Biological activity of some substituted hydroxytriazenes. Indian J. Microbiol. 43 (4), 275–276.
Jain, S., Dayma, V., Sharma, P., Bhargava, A., Baroliya, P.K., Goswami, A.K., 2020. Synthesis of some new hydroxytriazenes and their antimicrobial and anti-inflammatory activities. Anti-Inflammatory Anti-Allergy Agents Med. Chem. 19 (1), 50–60, 1.
Khanam, R., Jodha, J.S., Dashora, R., Goswami, A.K., 2014. Antifungal activity of hydroxytriazenes and their ternary complexes with vanadium (V) and thiourea. J. Glob. Pharma Technol. 6, 1–3.
Kim, Y.M., Jeong, Y.K., Wang, M.H., Lee, W.Y., Rhee, H.I., 2005. Inhibitory effect of pine extract on α-glucosidase activity and postprandial hyperglycemia. Nutrition 21 (6), 756–761, 1.
Levine, T.B., Levine, A.B., Levine, T.B., 2006. The endothelium and nitric oxide. Metabolic syndrome and cardiovascular disease 173–210.
Lim, A.K., 2014. Diabetic nephropathy–complications and treatment. Int. J. Nephrol. Renovascular Dis. 7, 361.
Mahajan, B.K., 1989. Significance of Difference in Mean. Methods in Biostatistics, fifth ed. Medical Publishers (P) Ltd, pp. 129–153.
Montonen, J, Knekt, P, Järvinen, R, Reunanen, A, 2004. Dietary antioxidant intake and risk of type 2 diabetes. Diabetes Care 27 (2), 362–366. In this issue.
Naulakha, N., Meenakshi, G., Jodha, J.S., Neelam, P., Pooja, J., Chauhan, R.S., Goswami, A.K., 2009. Antifungal activity of hydroxytriazenes and their Cu (II) complexes. Pestology 33 (2), 46–48.
Panwar, A.K., Choubisa, N., Garg, M., Dashora, R., Chauhan, R.S., Goswami, A.K., 2014. Binary complex formation of Cu (II) with HMCPT and their activity prediction by PASS. Asian J. Res. Chem. 7 (5), 487–491.
Pollack, R.M., Donath, M.Y., LeRoith, D., Leibowitz, G., 2016. Anti-inflammatory agents in the treatment of diabetes and its vascular complications. Diabetes Care 39 (Supplement 2), S244–S252, 1.
Rajendiran, D.E., Packirisamy, S.U., Gunasekaran, K.R., 2018. A review on role of antioxidants in diabetes. Asian J. Pharmaceut. Clin. Res. 11 (2), 48–53.
Regar, M., Baroliya, P.K., Patidar, A., Dashora, R., Mehta, A., Chauhan, R.S., Goswami, A.K., 2016. Antidyslipidemic and antioxidant effects of novel hydroxytriazenes. Pharmaceut. Chem. J. 50 (5), 310–314, 1.
Ren, L., Qin, X., Cao, X., Wang, L., Bai, F., Bai, G., Shen, Y., 2011. Structural insight into substrate specificity of human intestinal maltase-glucoamylase. Protein & cell 2 (10), 827–836, 1.
Rezaie, B., Ressalan, S., Chauhan, R.S., Goswami, A.K., Purohit, D.N., 1997. Synthesis and insecticidal studies of hydroxytriazenes. Asian J. Chem. 9 (4), 891, 1.
Sharma, P., Dayma, V., Dwivedi, A., Baroliya, P.K., Tripathi, I.P., Vanangamudi, M., Chauhan, R.S., Goswami, A.K., 2020. Synthesis of sulpha drug based hydroxytriazene derivatives: anti-diabetic, antioxidant, anti-inflammatory activity and their molecular docking studies. Bioorg. Chem. 1 (96), 103642.
Singh, K., Patel, P., Goswami, A.K., 2008. Anti-inflammatory activity of Hydroxytriazenes and their vanadium complexes. E-Journal of Chemistry 1, 5.
Sogani, N.C., Bhattacharya, S.C., 1956. 3-Hydroxyl-1, 3-diphenyltriazine as reagent or palladium. Anal. Chem. 28 (1), 81–83, 1.
Taslimi, P., Köksal, E., Gören, A.C., Bursal, E., Aras, A., Kılıç, Ö., Alwasel, S., Gülçin, İ., 2020. Anti-Alzheimer, antidiabetic and antioxidant potential of Satureja cuneifolia and analysis of its phenolic contents by LC-MS/MS. Arabian Journal of Chemistry 13 (3), 4528–4537, 1.
Tripathi, I.P., Aarti, K., Kumar, M.M., Atul, D., Ruchita, T., Chinmayi, M., Laxmikant, S., 2014. Synthesis, spectral, electrochemical analysis and screening for α-glucosidase inhibition of some complexes of copper (II) with amino acids. Indian J. Appl. Res. 4 (5), 66–69.
Tripathi, I.P., Mishra, M.K., Mishra, C., Tripathi, R., Kamal, A., Tripathi, P., Shukla, V.P., Gangele, R., Pandey, K.B., 2013. Assessment of antioxidant and total polyphenolic content of some plants of euphorbiaceae family. Indian J. Appl. Res. 3 (10), 1–4.
Türkan, F., Atalar, M.N., Aras, A., Gülçin, İ., Bursal, E., 2020. ICP-MS and HPLC analyses, enzyme inhibition and antioxidant potential of Achillea schischkinii Sosn. Bioorg. Chem. 1 (94), 103333.
Valko, M., Rhodes, C., Moncol, J., Izakovic, M.M., Mazur, M., 2006. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem. Biol. Interact. 160 (1), 1–40, 10.
Vane, J.R., Botting, R.M., 1998. Mechanism of action of nonsteroidal anti-inflammatory drugs. Am. J. Med. 104 (3S1), 2S–8S, 30.
Winter, C.A., Risley, E.A., Nuss, G.W., 1962. Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. Exp. Biol. Med. 111 (3), 544–547.
Ziegler, D., Hanefeld, M., Ruhnau, K.J., Mei, H.P., Lobisch, M., Schütte, K., Gries, F.A., ALADIN Study Group, 1995. Treatment of symptomatic diabetic peripheral neuropathy with the anti-oxidant α-lipoic acid. Diabetologia 38 (12), 1425–1433, 1.