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SYNTHESIS, CHARACTERIZATION, *IN VITRO* ANTI INFLAMMATORY ACTIVITY AND QSAR EVALUATION OF BENZOTRIAZOLYL)-3-{5-(CARBOXYMETHYL) DIAZENYL] -2-HYDROXYPHENYL}PROP-2-ENOIC ACID DERIVATIVES

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ABSTRACT

Synthesis of Benzotriazol-1-yl-3-{5-[(*E*)-(carboxymethyl) diazenyl]-2-hydroxyphenyl}prop-2-enoic acid derivatives based on diazonium coupling reaction. The five derivatives from Ie to Ve obtained by diazonium coupling reaction with various amino acids through intermediate compound (2*E*)-2-(1*H*-benzotriazol-1-yl)-3-(2-hydroxyphenyl)prop-2-enoic acid. The synthesized derivatives were characterized on basis of physical, chemical properties, TLC, IR, NMR and Mass spectral data. All the derivatives evaluated for *in vitro* anti-inflammatory activity. Quantitative Structural Activity Relationship (QSAR) was analyzed using set of predictor variables like physicochemical parameters and response variables like in vitro anti-inflammatory activity data. The data obtained reveal the derivatives IIe showed remarkable % inhibitory activity of protein precipitation, IIIe, Ve showed good and Ie, IVe exhibited moderate percentage of inhibition in comparison with standard.

KEYWORDS: Benzotriazole, Diazonium Coupling Reaction, Condensation reaction, benzotriazole derivatives, anti-inflammatory activity.

INTRODUCTION

Inflammation is a body's attempt to protect and defensive mechanism that results in complex response to injury.[1] Inflammation is a primeval problem of mankind observed during injury and infection.[2] Non steroidal anti-inflammatory agents are prescribed for treatment of inflammation but each end with some drawback such as toxicity, adverse reaction and recur after the drug is excreted .[3] Hence drug discovery for anti inflammatory agents is continuous process. Benzotriazole heterocyclic is biologically very active molecule. Various activities of Benzotriazole heterocyclic structure are reported such as antimicrobial[4-8], antineoplastic[9], anticonvulsant [10], anti amoebic[11], anti viral[12], antioxidants[13], and anti inflammatory.[14-17] In continuation of our efforts to investigate effective anti inflammatory agents, five newer Benzotriazole derivatives (Ie to Ve) were synthesized following the scheme of reactions (Figure 1, 2 and Figure 3).

MATERIALS AND METHODS

Materials: All the chemical and reagents used in the method are of analytical grade.

Method for synthesis of Benzotriazole: 10.8 gm of *O*-phenylenediamine was added to mixture of 12g (11.5 ml) of glacial acetic acetic acid and 30 ml of water, which was cooled to 15°C, stir. Then solution of 7.5g of sodium nitrite in 15 ml water was added in portion. The temperature rises slowly to 85°C and then cooled slowly. When temperature was 45°C the mixture is chilled at ice bath for 30 min. Pale brown solid separated by the filtration. The recrystallization was done using benzene as solvent.

Method for synthesis of ethyl 1*H*-benzotriazol-1-ylacetate: A mixture of Benzotriazole (0.1M), ethyl chloroacetate (0.1M) and 0.3g of K_2CO_3 in 60 ml of acetone was stirred for 10 hrs. The solvent was removed under reduced pressure. A solid mass was produced and then needle shaped brown crystals were obtained after recrystallization from the mixture of chloroform and ether (8:2%V/V).The yield obtained was 60% and M.P. was 40°C.

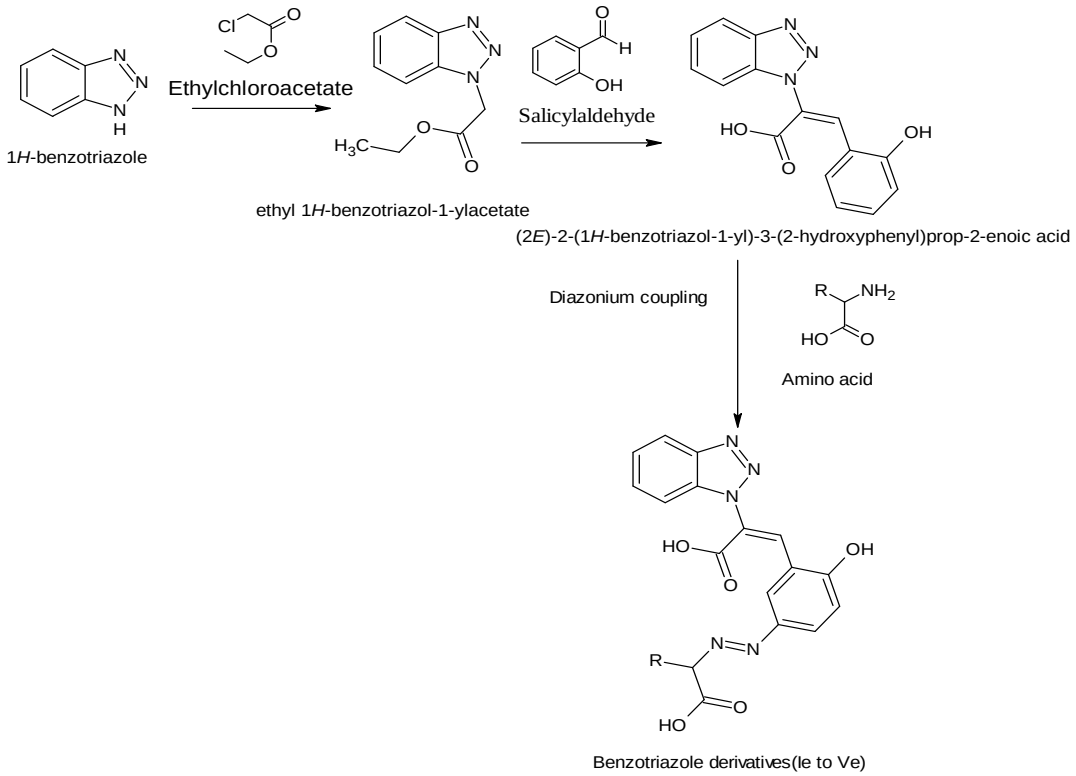


Figure 1: Synthetic Scheme of Benzotriazole derivatives (Ie to Ve)

Method for synthesis of (2*E*)-2-(1*H*-benzotriazol-1-yl)-3-(2-hydroxyphenyl)prop-2-enoic acid: In 250ml beaker, 50ml of ethanol and 25 ml of ammonia solution was transferred. In above 75ml mixture 5.95gm salicylaldehyde was added with stirring for 15 min and then 10 gm of ethyl 1.H.benztrizole acetate was added, stirred constantly to dissolve. The mixture was refluxed at 180^0 on oil bath and temperature of oil bath was maintained for 2hr then it was cooled. The pale brown compound precipitate was separated by filtration, washed and dried.

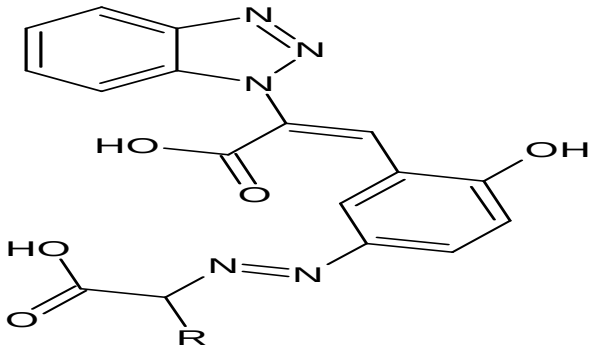


Figure 2: General Structure of Benzotriazole derivatives.

Method for synthesis of 2(*E*)-2-(1*H*-benzotriazol-1-yl)-3-{2-hydroxy-5-[(*E*)-phenyldiazenyl]phenyl}prop-2-enoic acid (Ie): Equimolar glycine was transferred to 100 ml flask and mixture of 15 ml of glacial acetic acid and 30 ml of water was added, cooled with occasional stirring, in other beaker prepared solution of 7.5gm of sodium nitrite in 15ml water cooled for 4^0c. The sodium nitrate solution was added slowly to the aniline mixture maintaining temperature of 4^oC with constant stirring. Then equivalent quantity of (2*E*)-2-(1*H*-benzotriazol-1-yl)-3-(2-hydroxyphenyl)prop-2-enoic acid was added. Diazotization takes place at low temperature. The product of brown precipitate was separated, filtered and dried. IR (cm^{-1}) 3024.65 (Ar C-H, stretch); 1283.31 (C-N, stretch), 3765.32(-OH, stretch), 1743.21 (C=O stretch); 1 H NMR (300 MHz) (DMSO Solvent, δ ppm): 7.9-7.8 (m, 7H, Ar-H), 5.0 (1H, s, C-OH), 8.18 (1H, -CH); MS: *m/z* 367.

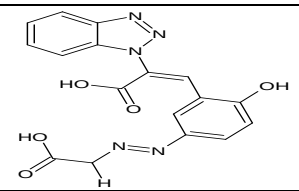
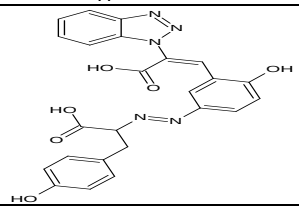
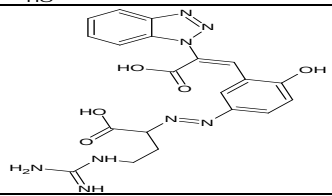
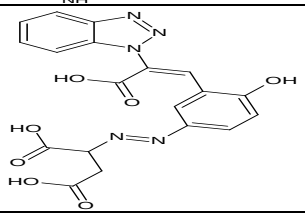
Method for synthesis of (2*E*)-2-(1*H*-benzotriazol-1-yl)-3-{2-hydroxy-5-[(*E*)-(4-nitrophenyl)diazenyl]phenyl}prop-2-enoic acid (IIe): Equimolar tyrosine was transferred to 100 ml flask and mixture of 15 ml of glacial acetic acid and 30 ml of water was added, cooled with occasional stirring, in other beaker prepare solution of 7.5gm of sodium nitrite in 15ml water cooled it for4^0c. The sodium nitrate solution was added slowly to the para nitro aniline mixture maintaining temperature of 4^oC with constant stirring. Then equivalent quantity of (2*E*)-2-(1*H*-benzotriazol-1-yl)-3-(2-hydroxyphenyl)prop-2-enoic acid was added. Diazotization takes place at low temperature. The product of yellowish brown precipitate was separated, filtered and dried. IR (cm^{-1}) 3031.56 (Ar C-H, stretch); 1241.28 (C-N, stretch), 3769.07(-OH, stretch), 1718.34 (C=O stretch); 1 H NMR (300 MHz) (DMSO Solvent, δ ppm): 7.9-7.8 (m, 11H, Ar-H), 5-5.3 (2H, s, C-OH), 1.82 (1H, s, -CH); MS: *m/z* 474.

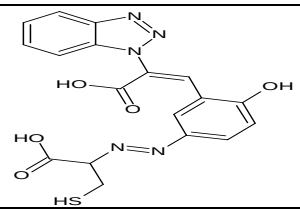
Method for synthesis of 4-[(*E*)-{3-[(*E*)-2-(1*H*-benzotriazol-1-yl)-2-carboxyethenyl]-4-hydroxyphenyl}diazenyl] benzoic acid (IIIe): Equimolar arginine was transferred to 100 ml flask and mixture of 15 ml of glacial acetic acid and 30 ml of water was added, cooled with occasional stirring, in other beaker prepare solution of 7.5gm of sodium nitrite in 15ml water cooled it for4^{0}c. The sodium nitrate solution was added slowly to the para amino benzoic acid mixture maintaining temperature of 4^{o}C with constant stirring. Then equivalent quantity of (2*E*)-2-(1*H*-benzotriazol-1-yl)-3-(2-hydroxyphenyl)prop-2-enoic acid was added. Diazotization takes place at low temperature. The product of yellowish brown precipitate was separated, filtered and dried. IR (cm^{-1}) 3028.41 (Ar C-H, stretch); 1275.31 (Aryl C-N, stretch), 3762.86(-OH, stretch), 1725.93 (C=O stretch); 1 H NMR (300 MHz) (DMSO Solvent, δ ppm): 7.8-7.7 (m, 7H, Ar-H), 5.0 (1H, s, C-OH), 8.18 (1H, s, =CH); MS: *m/z* 452.

Method for synthesis of (2*E*)-2-(1*H*-benzotriazol-1-yl)-3-{2-hydroxy-5-[(*E*)-(4-sulfophenyl)diazenyl]phenyl}prop-2-enoic acid (IVe): Equimolar aspartic acid was transferred to 100 ml flask and mixture of 15 ml of glacial acetic acid and 30 ml of water was added, cooled with occasional stirring, in other beaker prepare solution of 7.5gm of sodium nitrite in 15ml water cooled it for4^{0}c. The sodium nitrate solution was added slowly to the para amino sulphonic acid mixture maintaining temperature of 4^{o}C with constant stirring. Then equivalent quantity of (2*E*)-2-(1*H*-benzotriazol-1-yl)-3-(2-hydroxyphenyl)prop-2-enoic acid was added. Diazotization takes place at low temperature. The product of yellowish brown precipitate was separated, filtered and dried. IR (cm^{-1}) 3264.37 (Ar C-H, stretch); 1205.21 (C-N, stretch), 3760.07(-OH, stretch), 1708.35 (C=O stretch); 1 H NMR (300 MHz) (DMSO Solvent, δ ppm): 7.8-7.7 (m, 7H, Ar-H), 5.0 (1H, s, C-OH), 8.18 (1H, s, =CH); MS: *m/z* 425.

Method for synthesis of (2*E*)-2-(1*H*-benzotriazol-1-yl)-3-{5-[(*E*)-(4-chlorophenyl)diazenyl]-2-hydroxyphenyl} prop-2-enoic acid (Ve): Equimolar cystiene was transferred to 100 ml flask and mixture of 15 ml of glacial acetic acid and 30 ml of water was added, cooled with occasional stirring, in other beaker prepare solution of 7.5gm of sodium nitrite in 15ml water cooled it for4^{0}c. The sodium nitrate solution was added slowly to the para chloro aniline mixture maintaining temperature of 4^{o}C with constant stirring. Then equivalent quantity of (2*E*)-2-(1*H*-benzotriazol-1-yl)-3-(2-hydroxyphenyl)prop-2-enoic acid was added. Diazotization takes place at low temperature. The product of yellowish brown precipitate was separated, filtered and dried. IR (cm^{-1}) 3264.37 (Ar C-H, stretch); 1205.21 (C-N, stretch), 3635.07(-OH, stretch), 1675.15 (C=O stretch); 1 H NMR (300 MHz) (DMSO Solvent, δ ppm): 7.8-7.7 (m, 7H, Ar-H), 5.3 (1H, s, C-OH), 8.18 (1H, s, =CH); MS: *m/z* 413.

Table 1 : Structure of Synthesized derivatives(Ie to Ve)

Derivative	*Name of Derivative*	*Melting Point*	*Structure*
Ie	(2*E*)-2-(1*H*-benzotriazol-1-yl)-3-{5-[(*E*)-(carboxymethyl)diazenyl]-2-hydroxyphenyl}prop-2-enoic acid	80^{o}C	
IIe	(2*E*)-2-(1*H*-benzotriazol-1-yl)-3-{5-[(*E*)-(2-(4-hydroxyphenyl) carboxyethyl) diazenyl]-2-hydroxyphenyl}prop-2-enoic acid	259^{o}C	
IIIe	(2*E*)-2-(1*H*-benzotriazol-1-yl)-3-{5-[(*E*)-(2-(gaunidino) carboxyethyl) diazenyl]-2-hydroxyphenyl}prop-2-enoic acid	120^{o}C	
IVe	2-[(*E*)-{3-[(*E*)-2-(1*H*-benzotriazol-1-yl)-2-carboxyethenyl]-4-hydroxyphenyl} diazenyl]butanedioic acid	268^{o}C	

Ve	(2*E*)-2-(1*H*-benzotriazol-1-yl)-3-{5-[(*E*)-(1-carboxy-2-sulfanylethyl)diazenyl]-2-hydroxyphenyl}prop-2-enoic acid	210°C	

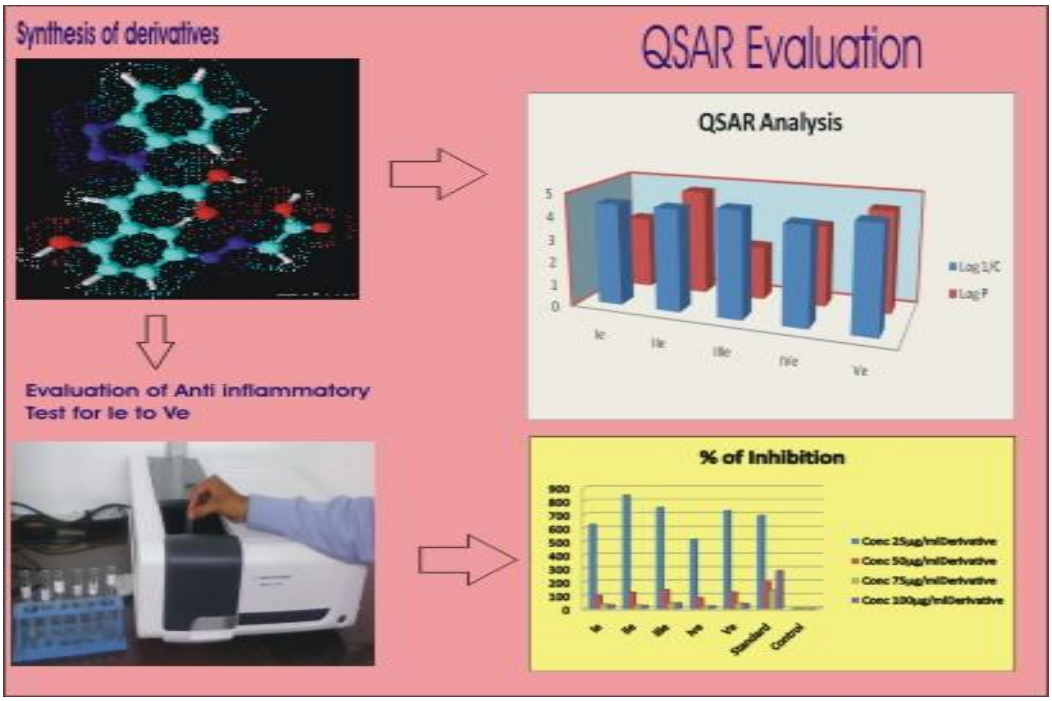


Figure 3: Schematic diagram for Benzotriazole derivatives synthesis, evaluation of anti-inflammatory activity and QSAR evaluation.

QSAR Evaluation

Benzotriazole derivatives synthesized were evaluated for QSAR on basis Hansch equation by use of dependant variables designated as v, w, x, y, z and B (log 1/P) for the parameters Molar refractivity, Parachor, Refractive index, Surface tension, Polarizability and Lipophilicity respectively (Table 3 and 4). The dependent variables are derived from computational calculation using Hyperchem8 software. The activities of the compounds A_1, A_2, A_3, A_4, and A_5 are the independent variables or descriptors that depend upon the innate property of the molecule such as functional groups like aromatic, –H, -Phenolic, -Guanidino, -CH_2COOH, and –CH_2SH. The hypothesis can be formulated as given in Eqn. below, (Hansch approach).

$$Log(1/C) = A_1 v + A_2 w + A_3 x + A_4 y + A_5 z + B$$

QSAR evaluation values for log(1/C) and log 1/P were determined for synthesized derivatives.

Table 2: Percentage of Inhibition of Protien denaturation

Concentration/ Sample	Ie	IIe	IIIe	IVe	Ve	Standard	Control
Conc 25µg/mlDerivative	625	843	751	514	727	690	0
Conc 50µg/mlDerivative	96.19	120.65	139.34	80.65	122.82	197.82	0
Conc 75µg/mlDerivative	34.11	27.44	43	19.77	41.88	136.66	0
Conc 100µg/mlDerivative	28.25	22.62	42.23	19.70	37.96	281.55	0

Table 3: Physico-chemical Properties of Benzotriazole derivatives

Derivative	Molecular Formula	Formula Weight	Molar Refractivity (cm3)	Parachor (cm3)	Index of Refraction	Surface Tension (dyne/cm)	Polarizability (cm3)
Ie	C17H13N5O5	367.31	93.67 ± 0.5	687.8 ± 8	1.72 ± 0.05	71.3 ± 7	37.13 ± 0.5
Iie	C24H19N5O6	473.43	124.24 ± 0.5	908.9 ± 8	1.71 ± 0.05	66.9 ± 7	49.25 ± 0.5
IIIe	C20H20N8O5	452.42	114.38 ± 0.5	837.7 ± 8	1.74 ± 0.05	76.8 ± 7	45.34 ± 0.5
Ive	C19H15N5O7	425.35	104.60 ± 0.5	776.4 ± 8	1.72 ± 0.05	75.6 ± 7	41.46 ± 0.5
Ve	C18H15N5O5S	413.4	105.61 ± 0.5	755.2 ± 8	1.74 ± 0.05	69.6 ± 7	41.86 ± 0.5

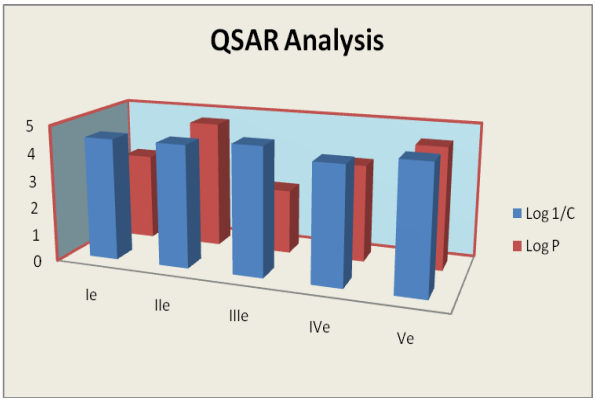


Figure 4: QSAR Evaluation Benzotriazole derivatives.

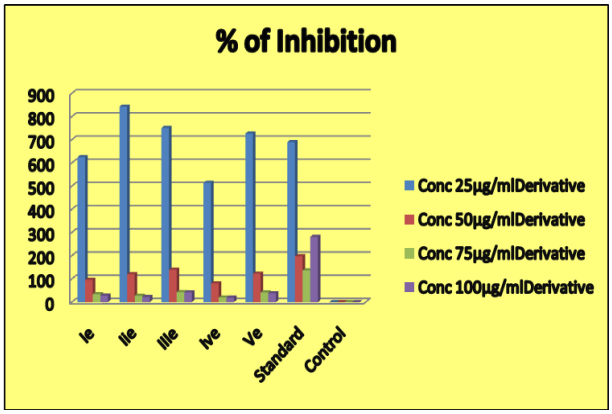
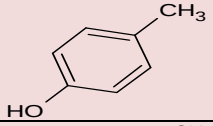
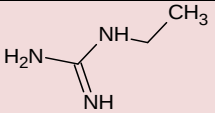
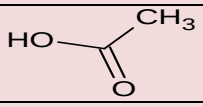
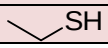


Figure 5: Percentage Inhibition of protein denaturation by derivatives and standard in various concentrations.

Table 4: QSAR Analysis values for synthesized derivatives

Derivative	R	Log 1/C	Log P
Ie	H	4.48	3.21
IIe		4.49	4.68
IIIe		4.67	2.37
IVe		4.29	3.57
Ve		4.61	4.47

RESULTS

The percentage of inhibition of protein denaturation (Figure 3) as screened by various Benzotriazole derivatives showed that the derivatives IIe showed excellent inhibition of protein denaturation, whereas derivatives IIIe, Ve showed good and Ie and IVe showed moderate inhibition of protein denaturation than standard at 25, 50, 75 and 100 µg/ml concentrations (Table 1 and 2). All the derivatives and standard showed inhibition of protein denaturation high at 25µg/ml concentration.

DISCUSSION

The Benzotriazole derivatives synthesized were screened for *in vitro* anti-inflammatory activity. The molecular structures of the compounds which vary with different functional groups influence differently for anti-inflammatory property. The evaluation of that is measured by the proteins denaturation of egg albumin.

The inhibitory activity was supported further by the determination of viscosities of the anti-denaturation. It was observed that increase in the protein denaturation decreases the viscosities of solutions. QSAR evaluation using various physicochemical properties and in vitro anti inflammatory activity revealed that the phenol substituted derivatives exhibit excellent activity, guanidine and thiol substituted derivatives showed higher Quantitative Structural Activity as reflected in increased percentage of inhibition (Table 1 and Figure 4). The derivatives with acidic functional groups and no substitutions on diazenyl group of derivatives showed less and moderate Quantitative Structural Activity

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Conflict of Interest: Authors hereby declare that do not have any conflict of interest.

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