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QSAR STUDY AND EVALUATION OF *IN VITRO* ANTI-INFLAMMATORY ACTIVITY FOR 1*H*-BENZOTRIAZOL-1-YL{2-HYDROXY-5- [(*E*) PHENYLDIAZENYL]PHENYL}METHANONE DERIVATIVES

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ABSTRACT

The purpose of present work is to study *in vitro* anti-inflammatory activity of 1*H*-benzotriazol-1-yl{2-hydroxy-5- [(*E*) phenyldiazenyl]phenyl}methanone derivatives (BZT). Anti-inflammatory activity was measured for each derivative to study the QSAR of the derivatives with Hansch and Free Wilson model. Egg albumin protein precipitation method was used to study *in vitro* anti-inflammatory activity. Diclofenac was used as standard and absorbance of samples, standard and control was measured spectrophotometrically at 660 nm. Five derivatives were screened for anti-inflammatory activity, out of which the compounds S2, S3 showed medium anti-inflammatory activity, S1, S5 showed better anti-inflammatory activity in comparison with standard and S4 showed more activity than standard. Contribution of different substituent on benzotriazole was reported. All the samples showed anti-inflammatory activity in comparison with Diclofenac.

KEYWORDS: Benzotriazole, QSAR, anti-inflammatory activities, physico-chemical parameters.

INTRODUCTION

Inflammation is immune reaction developed due to endo and exogenous stimuli in the presence of microbes or antigens or injury. This is protective in nature characterized by heat, redness, swelling and pain[1-3]. Anti-inflammatory activities for herbal extracts were performed[4-6]. The success of imidazole as an important moiety of number of medicinal

agents led to introduction of the triazoles[7]. The triazoles are said to be the isosters of imidazoles in which the carbon atom of imidazole is isosterically replaced by nitrogen. Benzotriazoles nucleus have been incorporated into a wide variety of therapeutically attractive drug candidates including anti inflammatory[8-9], Antimicrobial[10-14], antineoplastic[15], anticonvulsant[16], anti amoebic[17], anti viral[18], antioxidants[19], as corrosion inhibitors and as additives with a variety of other functions. The importance of triazole derivatives lies in the field that these have good position in heterocyclic chemistry, due to its various biological activities.

The benzotriazole derivatives (BZT) synthesized (Figure 2) with preplanned scheme and procedure[13] were screened for *in vitro* anti inflammatory activity following egg albumin protein precipitation method.

MATERIALS AND METHODS

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

Method: 0.2 mL of egg albumin (from fresh hen's egg) was transferred to 10 ml volumetric flask, 2.8 mL of phosphate buffered saline (PBS, pH 6.4) was added and 2 mL of varying concentrations of sample so that final concentrations to form 25, 50, 75, 100 µg/mL. For the control, the same volume of double-distilled water was used. Then the mixtures were incubated at (37±2) ^{o}C in a BOD incubator (Labline Technologies) for 15 min and then heated at 70 ^{o}C for 5 min. After cooling, their absorbance was measured at 660 nm (SHIMADZU, UV 1800) by using vehicle as blank and their viscosity was determined by using Ostwald viscometer. Diclofenac sodium at the final concentration of (78.125, 156.25, 312.5, 625, 1250, 2 500 µg/mL) was used as reference drug and treated similarly for determination of absorbance and viscosity.

The percentage inhibition of protein denaturation was calculated by using the following formula.

% inhibition = 100 X (Vt / Vc - 1)

Where, Vt = absorbance of test sample, Vc = absorbance of control.

The extract/drug concentration for 50% inhibition (IC50) was determined by plotting percentage inhibition with respect to control against treatment concentration.

QSAR analysis

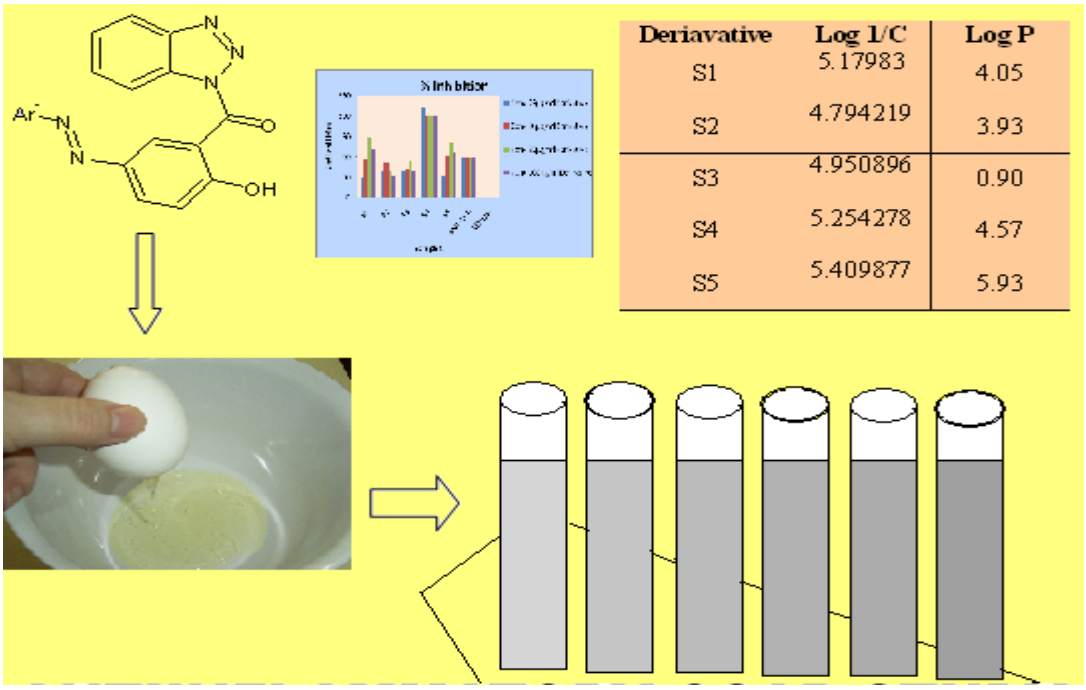
The QSAR analysis of compounds is based on use of dependant variables designated as v, w, x, y, z and B (log 1/P) for Molar refractivity, Parachor, Refractive index, Surface tension, polarizability and Lipophilicity respectively in Hansch equation(Table 3 and 4). The activities of the compounds A1, A2, A3, A4, and A5 are the dependent variables or descriptors that depend upon the innate property of the molecule such as functional groups like $-SO_2NH_2$, -Cl, $–NO_2$, Aromatic group, -COOH. The hypothesis can be formulated as given in Eqn. below, (Hansch approach)

$$log1/C = A1\ v + A2\ w + A3\ x + A4\ y + A5\ z + B$$

where log1/C implies that the lower the concentration higher calculated activity that depend upon independent variables or molecular structure.

RESULTS

The percentage of inhibition of protein denaturation (Figure 1) as screened by various BZT derivatives showed that the derivatives S2 and S3 exhibit good protein inhibition, S1 and S5 displayed moderate inhibition and S4 showed higher inhibition of denaturation than the standard at 25 µg/ml concentration(Table 1 and 2).



Deriavative	Log 1/C	Log P
S1	5.17983	4.05
S2	4.794219	3.93
S3	4.950896	0.90
S4	5.254278	4.57
S5	5.409877	5.93

QSAR Study was conducted for 1*H*-benzotriazol-1-yl{2-hydroxy-5- [(*E*) phenyldiazenyl]phenyl}methanone derivatives (BZT) with evaluation of *in vitro* Anti-inflammatory activity using eggs albumin. Quantitative Structural activity relationship with invitro anti-inflammatory activity determined for five derivatives of BZT.

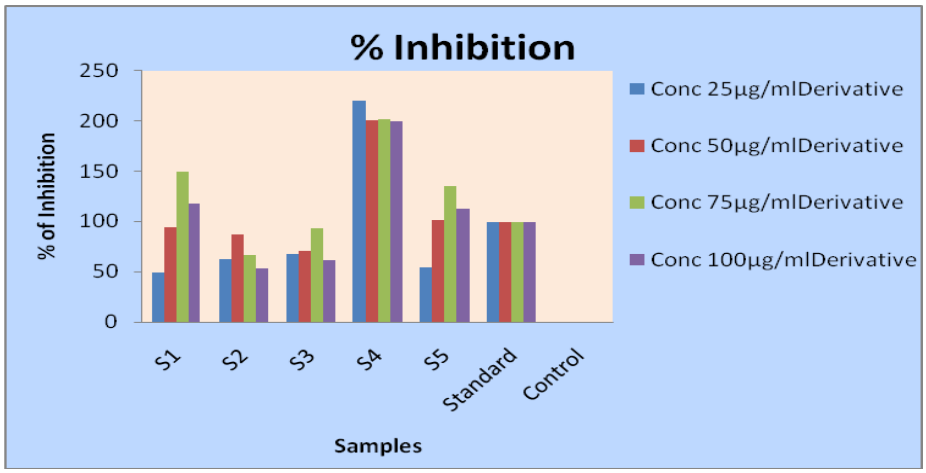


Figure 1: Graphical Representaion of Perecntage of Inhibition of protien denaturation

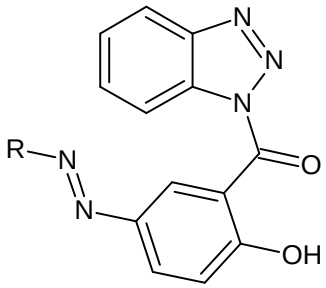


Figure 2: General Structure of Benzotriazole derivatives (BZT)

Table 1: Synthesized derivatives from S1 to S5.

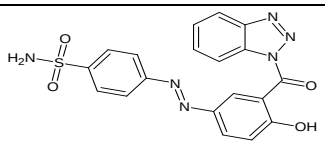
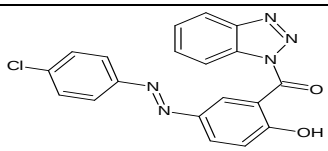
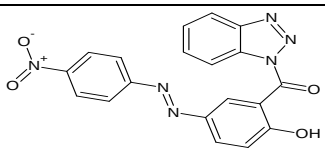
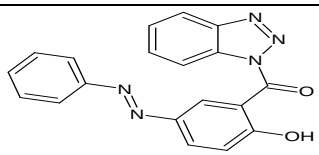
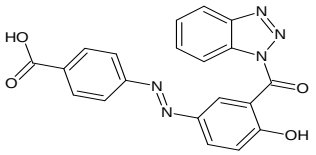
Derivative	*Name of Derivative*	*Structure*
S1	4-{(*E*)-[3-(1*H*-benzotriazol-1-ylcarbonyl)-4hydroxyphenyl]diazenyl}benzenesulfonam	
S2	1*H*-benzotriazol-1-yl{5-[(*E*)-(4-chlorophenyl)diazenyl]-2-hydroxyphenyl}methanone	
S3	1*H*-benzotriazol-1-yl{2-hydroxy-5-[(*E*)-(4-nitrophenyl)diazenyl] phenyl}methanone	
S4	1*H*-benzotriazol-1-yl{2-hydroxy-5-[(*E*)-phenyldiazenyl] phenyl}methanone	
S5	4-{(*E*)-[3-(1*H*-benzotriazol-1-ylcarbonyl)-4- hydroxyphenyl]diazenyl}benzoic acid	

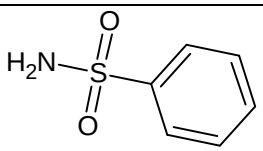
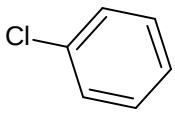
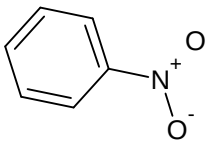
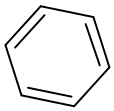
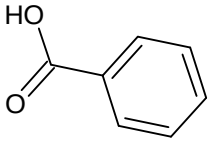
Table 2: Perecntage of Inhibition of protien denaturation

Samples/Concentration	S1	S2	S3	S4	S5	Standard	Control
Conc 25µg/ml	49.63	62.29	67.35	220.51	54.69	99	0
Conc 50µg/ml	93.89	86.59	70.53	200.45	101.18	99	0
Conc 75µg/ml	149.7	67.07	93.36	201.34	135.61	99	0
Conc 100µg/ml	118.33	53.19	61.34	200.27	112.23	99	0

Table 3: Physicochemical Properties of synthesized derivatives

Derivative	Molecular Formula	Formula Weight	Molar Refractivity	Parachor	Index of Refraction	Surface Tension	Polarizability
S1	$C_{19}H_{14}N_6O_4S$	422.4	109.4 ± 0.5 cm^3	781.5 ± 8.0 cm^3	1.762 ± 0.05	75.1 ± 7.0 dyne/cm	43.3 ± 0.5 $10^{-24}cm^3$
S2	$C_{19}H_{12}ClN_5O_2$	377.78	102.3 ± 0.5 cm^3	722.1 ± 8.0 cm^3	1.725 ± 0.05	61.5 ± 7.0 dyne/cm	40.5 ± 0.5 $10^{-24}cm^3$
S3	$C_{19}H_{12}N_6O_4$	388.33	103.4 ± 0.5 cm^3	738.7 ± 8.0 cm^3	1.750 ± 0.05	71.7 ± 7.0 dyne/cm	41.01 ± 0.5 $10^{-24}cm^3$
S4	$C_{19}H_{13}N_5O_2$	343.33	97.7 ± 0.5 cm^3	693.2 ± 8.0 cm^3	1.716 ± 0.05	60.5 ± 7.0 dyne/cm	38.76 ± 0.5 $10^{-24}cm^3$
S5	$C_{20}H_{13}N_5O_4$	387.34	104.1 ± 0.5 cm^3	743.2 ± 8.0 cm^3	1.736 ± 0.05	67.7 ± 7.0 dyne/cm	41.26 ± 0.5 $10^{-24}cm^3$

Table 4: QSAR Analysis values for synthesized derivatives

Derivative	R	Log 1/C	Log P
S1		**5.17983**	**4.05**
S2		**4.794219**	**3.93**
S3		**4.950896**	**0.90**
S4		**5.254278**	**4.57**
S5		**5.409877**	**5.93**

DISCUSSION

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

The derivatives of BZT, standard and control were treated with egg albumin and phosphate buffer. The mixture was subjected for heat-induced protein (albumin) denaturation at 70^{o}C. Inhibition of protein denaturation or stabilization of the BZT derivatives and reference drug diclofenac sodium was measured by UV-Visible spectroscopic absorbance. 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.

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