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# Pharmacophore Design, Synthesis, Characterization, Inhibition of Renin and Anti microbial Activity of some 3-phenyl Indole derivatives

**S. Muthu Kumar*[1, 2], D. Selva Kumar[3], Shiv Prakash Pandey[2], Rajpati Yadav[2], Sanjay Kumar Gupta[2]**

[1]Karpagam University, Coimbatore, Tamilnadu.
[2]Kamla Nehru Institute of Management and Technology, Sultanpur (U.P).
[3]School of pharmacy, Taylor's University, Subang Jaya - 47500. Malaysia.

**ABSTRACT**

The aim of present study was to develop renin inhibitor as anti-hypertensive drug. Selective inhibition of the renin has gained attraction as an interesting approach to control hypertension and associated cardiovascular risk factors given its unique position in the renin–angiotensin system. Using a best validated HypoGen model consists of four pharmacophore features such as two hydrogen bond acceptors, one hydrogen bond donor and one hydrophobic. Identification of common pharmacophore features responsible for inhibiting activity Renin using Hip Hop module of catalyst software 4.11 from Accelrys. Development and Validation of quantitative pharmacophore hypothesis for series of Renin Receptors using HypoGen/Hypo Refine module of catalyst software 4.11 from Accelrys. Generation of 10,000 molecules from the drug using Scaffold Hoping technique. Prediction of activity for designed molecules using the Hypo Refine model and to identify novel and potent Renin inhibitors using Lipinski Rule of Five. The Pharmacophore developed, identified and optimized a novel series of potent and 3 substituted phenyl -2- (hydrazinocarbonyl)-1H indole -5- sulfonamide with remarkable potency for renin. A series of 3-phenyl Indole derivatives have been synthesized by the interaction of ethyl 5-(amino sulfonyl)-3-substituted phenyl-1H-indole -2- carboxylates with hydrazine hydrate and ethanol. The newly synthesized compounds were tested for antibacterial and anti-fungal activity also.

**Keywords**: Renin inhibitors, Anti-hypertensive, Pharmacophore model, HypoGen, Docking, Hypo Refine module.

## INTRODUCTION

The history of medicine is related to antiquity and to the very creator of this universe. In order to gain immortality to creator (BRAHAMA) gave the science of ayurveda (ayu mean life) which was propagated through his descendents mainly Daksa Prajapati. The modern drug development is now being done on a more rational basis. In this regard more and more information is being obtained in cell biochemistry and cell biology at the molecular level. The rational approach envisages a physiological basis of disease. Over the past decade advances in biotechnology have ushered in a new approach to drug discovery termed "structure based drug design" (Tornroos *et al.*, 1987).

Corresponding Author

**S.Muthu Kumar**
Email id:- smuthupharma81@gmail.com

The approach to the practice of medicinal chemistry has developed from an empirical one involving organic synthesis of new compounds based on largely on modification of structure of known activity by a more logical approach. Medicinal chemistry is chemistry based discipline involving aspect of biological, medical and pharma, discovery, design, identification and preparation of biological active compound. The main objective of medicinal chemistry is the design and the production of compound that can be used as medicine for prevention, treatment, and cure of human and animal disease (Scheiper B *et al.*, 2010; Diana Frechilla *et al.*, 1992).

A pharmacophore is a molecular framework that carries (phoros) the essential features responsible for a drug's (pharmacon's) biological activity. It is a set of structural features in a molecule that is recognized at a receptor site and is responsible for that molecule's biological activity. It is alternatively described as an

ensemble of interactive functional groups with defined geometry. CATALYST, an Accelrys developed software is used for pharmacophore generation. Catalyst treats molecular structures as templates consisting of chemical functions positioned in space that will bind effectively with complementary functions on the respective binding proteins (Ramadevi Sanam *et al.,* 2009; Rambabu *et al.,* 2009; Frangulian RR, 2010).

The chemical functions used in the CATALYST include hydrogen-bonding acceptors and donors, and hydrophobic interactions. CATALYST generates pharmacophore hypotheses in terms of the 3D arrangement of chemical functions explaining the activity variations of compounds. The total energy cost of each generated hypothesis can be calculated from the difference between the observed activity value and the activity value estimated by the hypothesis based on the pharmacophore features. The hypothesis having the lowest energy cost is considered the best one. Pharmacophore model is used to discover novel structural templates that may interact with your target, helping to overcome the challenges of *in vivo* drug delivery (Ramadevi Sanam *et al.,* 2009).

Hypertension is one of the major risk factors for cardiovascular diseases, which are the leading cause of mortality in the Western world. Lowering blood-pressure can considerably reduce the risk of myocardial infarction, stroke, heart failure and end-stage kidney disease. However, despite available therapies, approximately 70% of patients with hypertension do not reach their target blood pressure levels. Some of them yet do not respond fully to a combination of treatments. Consequently, opportunities remain for designing and developing well-tolerated effective medicines to control hypertension and associated cardiovascular diseases (Marvin Moseret *et al.,* 2007; Antonio Monge *et al.,* 2005).

The renin–angiotensin system (RAS) is well-established as an endocrine system involved in regulation of blood pressure and fluid electrolytes. Activation of the RAS is initiated by several signals including lowering of blood pressure, decrease in circulating volume or decrease in plasma-sodium concentration. These signals stimulate the release of the aspartyl protease renin, which cleaves angiotensinogen to produce angiotensin I. Since renin forms the rate-limiting step in this cascade and angiotensinogen is its only known substrate, inhibition of this step would be a very effective antihypertensive strategy (Luca Mascitelli and Francesca Pezzetta, 2007).

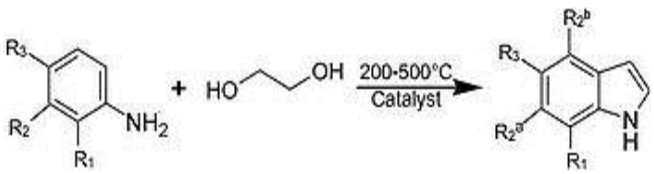
Any appropriate medication affecting the RAS might also result in optimal end-organ protection, in particular for heart and kidney as shown in animal models. This might also be accompanied by a diminished potential for cough side effects, affecting 5–35% of patients treated with ACE inhibitors. Accordingly, substantial efforts were reported over the last decades to discover renin inhibitors for clinical use, for example, those structures shown in and to overcome issues like low oral bioavailability. Aliskiren (SPP100), an orally active renin inhibitor with four chiral centers is currently the only compound, which has reached the market. Consequently, several research groups have reported novel renin inhibitors on diverse scaffolds with different renin active-site binding topologies (Yamazato Y *et al.,* 2009).

Indole is an aromatic heterocyclic organic compound. It has a bicyclic structure, consisting of a six-membered benzene ring fused to a five-membered nitrogen-containing pyrrole ring. Indole is a popular component of fragrances and the precursor to many pharmaceuticals. Compounds that contain an indole ring are called indoles. Indole undergoes electrophilic substitution, mainly at position 3. we report here the synthesis and inhibitory properties against all the series of 2-(hydrazinocarbonyl)-3-substituted-phenyl-1H-indole-5-substitutedphenyl) propionates, by literature procedures. Diazotization of sulfanilamide 4 led to the diazonium salt which has been coupled with the key intermediates 3 allowing the preparation of the hydrazones, which were cyclized in the presence of concentrated acid (HCl) to the indoles. The last step consisted in conversion of the ester moieties of 7 to the corresponding hydrazides by treatment with hydrazine hydrate at reflux, leading thus to the desired series of compounds (Biradar JS *et al.,* 2010).

**1. a Synthesis of indole**

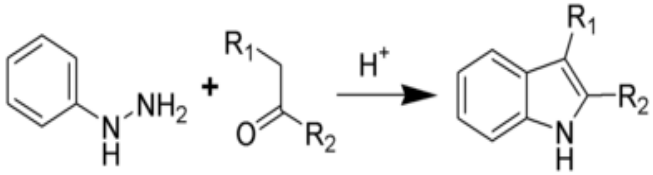
Indole is a major constituent of coal-tar, and the 220-260 °C distillation fractions is the main industrial source of the material. Indole and its derivatives can also be synthesized by a variety of methods. The main industrial routes start from aniline.

Illustrative of such large-scale syntheses, indole (and substituted derivatives) forms via vapor-phase reaction of aniline with ethylene glycol in the presence of catalysts:




Reactions are generally conducted between 200 and 500 °C. Yields can be as high as 60%. Other precursors to indole include formyltoluidine, 2-ethylaniline, and 2-(2-nitrophenyl) ethanol, all of which undergo cyclizations. Many other methods have been developed that are applicable.

**1. b Fischer indole synthesis**



One-pot microwave-assisted synthesis of indole from phenyl hydrazine and pyruvic acid.

One of the oldest and most reliable methods for synthesizing substituted indoles is the Fischer indole synthesis developed in 1883 by Emil Fischer. Although the synthesis of indole itself is problematic using the Fischer indole synthesis, it is often used to generate indoles substituted in the 2- and/or 3-positions. Indole can still be synthesized however using the Fischer indole synthesis by reacting phenyl hydrazine with pyruvic acid followed by decarboxylation of the formed indole-2-carboxylic acid. This has also been accomplished in a one-pot synthesis using microwave irradiation.

**1. c Renin-angiotensin system**

Renin activates the renin-angiotensin system by cleaving angiotensinogen, produced by the liver, to yield angiotensin I, which is further converted into angiotensin II by ACE, the angiotensin-converting enzyme primarily within the capillaries of the lungs. Angiotensin II then constricts blood vessels, increases the secretion of ADH and aldosterone, and stimulates the hypothalamus to activate the thirst reflex, each leading to an increase in blood pressure. Renin is secreted from juxtaglomerular cells (of the afferent arterioles), which are activated via signaling (the release of prostaglandins) from the macula densa, which respond to the rate of fluid flow through the distal tubule, by decreases in renal perfusion pressure (through stretch receptors in the vascular wall), and by nervous stimulation, mainly through beta-1 receptor activation. A drop in the rate of flow past the macula densa implies a drop in renal filtration pressure. Renin's primary function is therefore to eventually cause an increase in blood pressure, leading to restoration of perfusion pressure in the kidneys.

## 2. RESULTS AND DISCUSSION

### 2. a. Pharmacophore model

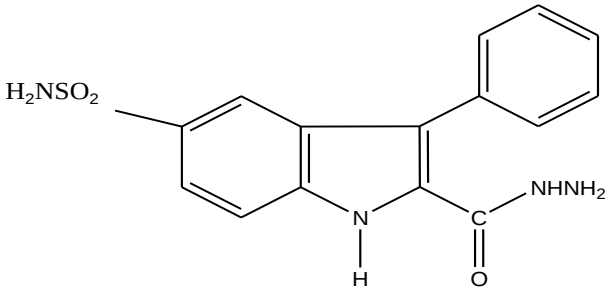
**Renin inhibitors:**

The number of molecules in the database is 169. Of these, 213 are highly active, 54 are moderately active and 35 are low active compounds. While the False positives and negatives, 16 and 12 respectively, are minimal, enrichment factor of 1.31 against a maximum value of 3.0 is a very good indication on the high efficiency of the screening. Of the 213 highly active molecules, 15 were predicted as moderately active and 4 were predicted as least active. In the 54 moderately active, 6 were predicted as low active and 3 as highly active. The model also predicted 3 of the low active molecules as moderately active and 2 more molecules from the same set as highly active.

The steric and other interaction effects might have a subtle, yet crucial role on the predicted activity. While these additional groups may not prevent in identifying many low energy conformers or add any penalty for the total cost, but could be detrimental to fit these conformers in the active site. Thus the features of Hyporefine 1 are relatively well optimized. However, in the case of highly active molecules, there are bulky groups present which may decrease the abitlity of the hyporefine to select the most highly active molecules. They are reported in Table No 1.

The most common inhibitor Aliskiren (FDA approved drug) of renin was taken and 10,000 structures were generated from it based on the knowledge of features obtained in the pharmacophore using Scaffold Hoping. The $IC_{50}$ values were predicted for the designed 10,000 molecules according to pharmacophore using score hypothesis in catalyst software.

**Common inhibitor of renin**




In these 10,000 structures, the best 25 structures were selected based on the predictions and Lipinski rule. The following table will show the best 25 molecules with the parameters of lipinski's rule.

**2. a Chemistry**

Sulfonamide I was previously reported by Salman's group, being easily prepared from sulfanilamide as starting material. Diazotization of sulfanilamide followed by condensation of the diazonium salt with ethyl 2-benzylacetoacetate led to an intermediate which was cyclized in acidic medium with formation of the ethyl ester derivative of L, which was then converted to the lead compound by treatment with hydrazine. We used a similar approach for the preparation of the series of congeners of L bearing different moieties in position 4 of the indole ring (Scheme). Condensation of ring-substituted benzyl bromides with ethyl acetoacetate gave the key intermediates, ethyl 2-acetyl-3-(substitutedphenyl) propionates (1a), by literature procedures. Diazotization of sulfanilamide led to the diazonium salt which has been coupled with the key intermediates (1a) allowing the preparation of the hydrazones (2a), which were cyclized in the presence of concentrated acid (HCl) to the indoles (3a). The last step consisted in conversion of the ester moieties of (3a) to the corresponding hydrazides by treatment with hydrazine hydrate at reflux, leading thus to the desired series of compounds (Spa-h). We have chosen the various substituents of the 3-phenyl group of indoles ar $4^{th}$ position (Spa-h) by considering both the limited available space within the hydrophobic pocket of the enzyme active site, as discussed above, as well

as general medicinal chemistry considerations, for example, moieties that may increase lipo-or hydro solubility of the new compounds, and eventually also interacting in a positive manner with amino acid residues present in the active site region where this moiety of the inhibitors. Thus, we have incorporated 4-substituted phenyl groups possessing methyl-, halogeno- and methoxy- functionalities ensued by the presence of the additional functionality in the 3-phenyl ring may lead to diverse interactions of compounds 8 with amino acid residues within the various isoforms active sites cavity. The main interest in this class of compounds is that of detecting derivatives with a more isoform-selective profile as compared to the clinically used sulfonamides.

## 2. b MATERIALS AND METHODS

### Pharmacophore model

The best HipHop pharmacophore model indicated the importance of hydrogen bond acceptors and hydrophobic features which were further, confirmed in the Hyporefine generated models. This study also resulted in very good Cost values, Correlation (r) and Root mean square deviations (RMSD). The best hypothesis is characterized by the highest cost difference (98.468 bits), lowest RMS error (0.68 bits) and with correlation 0.954. The fixed cost, pharmacophore (total) cost, error and null cost are 92.768, 70.63 and 123.712 bits respectively. It is evident that as error, weight and configuration component are very low and not deterministic to the model; the total pharmacophore cost is also low and close to the fixed cost. Also, as total cost is less than the null cost, this model accounts for all the pharmacophore features and has a good predictability power. Its represents the Hyporefine aligned with the most active and inactive molecules (14 and 115) IC50 values are 0.03 and 2(value to check) nM respectively. They are reported in Table No 3.

**a** + Indicates that the Predicted $IC_{50}$ is higher than the Experimental $IC_{50}$; - indicates that the Predicted $IC_{50}$ is lower than the Experimental $IC_{50}$; a value of 1 indicates that the predicted $IC_{50}$ is equal to the Experimental $IC_{50}$

**b** Fit value[12] indicates how well the features in the Pharmacophore overlap the chemical features in the molecule. Fit = weight*[max (0, 1-SSE)] where SSE = $(D/T)^2$, D = displacement of the feature from the center of the location constraint and T = the radius of the location constraint sphere for the feature (tolerance)

**c** Activity scale - $IC_{50}$ < 15 nM = +++ (Highly active) - $IC_{50}$ 15 – 100 nM = ++ (Moderately active) - $IC_{50}$ > 100 nM = + (Low active).

All starting materials were from different manufactured company like (sd.fine chemicals, Merck, Lobachem etc.) And all the materials used without further purification all reactions were monitored by thin- layer-chromatography using TLC sheet coated with silica gel GF254 spots were visualized with UV light. And Their physico chemical properties are reported in Table No 4.

## 3. Experimental protocols

### 3.a.Pharmacophore model

The features mapped by this pharmacophore are two hydrogen bond acceptors, and three hydrophobic. In the case of **14** (most active), it is clearly seen that all the features of the pharmacophore are very well fitted to the molecule On the other hand, for molecule (least active), one hydrogen bond acceptor is not at all mapped and the hydrophobic feature does not fit well.

On considering the results, out of **9** highly active molecules, **4** was as moderately active and the rest were predicted as highly active. In the **4** moderately active molecules, one was predicted as inactive and the other one was predicted as highly active, the rest were correctly predicted. There were 8 low active molecules and all the eight were correctly predicted as inactive. The error value for the pharmacophore is less, this is evident from Table and the correlation values along with the predictions above, make the pharmacophore suitable to predict molecular properties well. The activity of the test set molecules were also scored using the best hypothesis generated and the results gave a correlation value of 0.8324. This indicates that the pharmacophore model generated is capable of predicting the activity of the unknown molecules with reasonable accuracy. The plot showing the correlation between the actual and predicted activity for the test set and the training set molecules is given in Figure 3.

### Model Validation and Knowledge based Screening

The purpose of the pharmacophore hypothesis generation is not just to predict the activity of the training set compounds accurately but also to verify whether the pharmacophore models are capable of predicting the activities of compounds of the test set series and classifying them correctly as active or inactive. The best pharmacophore hypothesis was used initially to screen the Renin inhibitors. All queries were performed using the Best Flexible search databases/Spreadsheet method.

Hyporefine 1 was used to screen the known high, medium and low active inhibitors of the test set. Database mining was performed in Catalyst software using the BEST flexible searching technique. A number of parameters such as hit list (Ht), number of active percent of yields (%Y), percent ratio of actives in the hit list (%A), enrichment factor of (E), False negatives, False positives and Goodness of hit score (GH) are calculated (Table 16) while carrying out the pharmacophore model and Virtual screening of test set molecules (Vadivelan S *et al.*, 2007).

### 3. b. Chemistry

Buffers and chemicals were from sd.fine chemicals, Merck, Lobachem of highest purity available, and were used without further purification. All the synthesized 3 phenyl indole derivatives produced

and purified in laboratory as described earlier. Melting points are recorded in open capillary one ended tubes and are uncorrected. The IR spectra (KBr) were recorded on a SHIMADZU FTIR-8300, spectrophotometer. The 1H-NMR spectra were recorded on a Bruker Advance-400 MHz spectrometer.

**3. b. a. Ethyl 2-acetyl-3-(substitutedphenyl) propionate (1 a)**

An ethanolic solution of sodium ethoxide was prepared by the addition of sodium (1 g, 44 mmol) to dry ethanol (40 mL). Ethyl acetoacetate (20.8 g, 160.0 mmol) was added to the reaction mixture, and the solution was stirred for 10 min. at room temperature. Substituted benzyl bromide (10 g, 40 mmol) was added, and the reaction mixture was heated under reflux for 15 h. The mixture was concentrated under reduced pressure and the residue was taken up in ether (200 mL). The ether solution was washed with water (100 mL) and was dried. The residue after removal of solvent under reduced pressure was purified by fractional distillation.

**3. b.b. 2-Substitutedbenzyl-2-[N-(4-sulfonamidophenyl) hydrazono] ethanoates (2 a)**

To a solution of 0.01 mol sulfanilamide in 8 ml of 37% HCl, 10 ml of 7% NaNO2 aqueous solution was added dropwise at 0 _C. This solution, containing diazonium salt, was poured into an ice-cold mixture of 4.6 g (a little excess of 0.02 mol) ethyl 2-substitutedbenzylacetoacetate, 20 ml of EtOH, 40 ml of H2O and 5.4 g of KOH. The mixture was kept cold overnight. The hydrazone produced as an oil was separated, dissolved in Et2O, washed with H2O and dried over anhydrous Na2SO4. Et2 O was distilled; the oily residue was treated with 10 ml of 37% HCl and set aside for 5 h at room temperature. The resulting solid substance was recrystallized from EtOH.

**3. b.c. Ethyl 5-(aminosulfonyl)-3-substitutedphenyl-1H-indole- 2-carboxylates (3 a)**

A mixture of 0.02 mol ethyl 2-substitutedbenzyl-2-[N-(4-sulfonamidophenyl) hydrazono] ethanoate (2a) and about 20 ml of 37% HCl was heated on a water bath for 4 h, cooled and poured into 200 ml of H2O, the crude product was filtered, washed with H2O, and recrystallized from EtOH.

**3. b.d. 2-(Hydrazinocarbonyl)-3-substitutedphenyl-1H-indole-5-sulfonamides (Sp a-h)**

Ethyl 5-(aminosulfonyl)-3-substitutedphenyl-1H-indole-2-carboxylate Spa (0.01 mol, 6.9 g) was dissolved in 40 ml of EtOH, 8ml of H2NNH2.H2O was added and refluxed for 6 h, cooled andkept cold overnight. The resulting crystals were filtered off, washed with Et2O and recrystallized from EtOH/DMF.

**Sp a. 2-(Hydrazinocarbonyl)-3-phenyl-1H-indole-5-sulfonamide sulfonamide**

Yield 70%; mp 273–5_C; IR(KBr) (t, cm_1),1640 (CO) ; 1H NMR (DMSO-d6, 500 MHz) d (ppm): 4.50 (2H, s,NHNH2), 7.13 (2H, s, SO2NH2), 7.48 (2H, d, J = 8.79 Hz, Ar-H),7.53 (2H, d, J = 8.30 Hz, Ar-H), 7.58 (1H, d, J = 8.30 Hz, indole C7–H), 7.68 (1H, dd, J = 8.78,1.46 Hz, indole C6–H), 8.01 (1H, d,J = 0.98 Hz, indole C4–H), 9.14 (1H, s, CONH), 12.15 (1H, br s, indole NH).

**Sp b. 3-(4-Chlorophenyl)-2-(hydrazinocarbonyl)-1H-indole- 5-sulfonamide**

Yield 70%; mp 273–5_C; IR(KBr) (t, cm_1),1640 (CO) ,712 (C-Cl); 1H NMR (DMSO-d6, 500 MHz) d (ppm): 4.50 (2H, s,NHNH2), 7.13 (2H, s, SO2NH2), 7.48 (2H, d, J = 8.79 Hz, Ar-H),7.53 (2H, d, J = 8.30 Hz, Ar-H), 7.58 (1H, d, J = 8.30 Hz, indole C7–H), 7.68 (1H, dd, J = 8.78,1.46 Hz, indole C6–H), 8.01 (1H, d,J = 0.98 Hz, indole C4–H), 9.14 (1H, s, CONH), 12.15 (1H, br s, indole NH).

**Sp c. 3-(4-Hydroxyphenyl)-2-(hydrazinocarbonyl)-1H-indole- 5-sulfonamide**

Yield 70%; mp 273–5_C; IR(KBr) (t, cm_1),1640 (CO) ,1536 (C-OH); 1H NMR (DMSO-d6, 500 MHz) d (ppm): 4.50 (2H, s,NHNH2), 7.13 (2H, s, SO2NH2), 7.48 (2H, d, J = 8.79 Hz, Ar-H),7.53 (2H, d, J = 8.30 Hz, Ar-H), 7.58 (1H, d, J = 8.30 Hz, indole C7–H), 7.68 (1H, dd, J = 8.78,1.46 Hz, indole C6–H), 8.01 (1H, d,J = 0.98 Hz, indole C4–H), 9.14 (1H, s, CONH), 12.15 (1H, br s, indole NH).

**Sp d. 3-(4-Nitrophenyl)-2-(hydrazinocarbonyl)-1H-indole- 5-sulfonamide**

Yield 70%; mp 273–5_C; IR(KBr) (t, cm_1),1640 (CO) ,1342 (C-$NO_2$); 1H NMR (DMSO-d6, 500 MHz) d (ppm): 4.50 (2H, s,NHNH2), 7.13 (2H, s, SO2NH2), 7.48 (2H, d, J = 8.79 Hz, Ar-H),7.53 (2H, d, J = 8.30 Hz, Ar-H), 7.58 (1H, d, J = 8.30 Hz, indole C7–H), 7.68 (1H, dd, J = 8.78,1.46 Hz, indole C6–H), 8.01 (1H, d,J = 0.98 Hz, indole C4–H), 9.14 (1H, s, CONH), 12.15 (1H, br s, indole NH).

**Sp e. 3-(4-Aminophenyl)-2-(hydrazinocarbonyl)-1H-indole- 5-sulfonamide**

Yield 70%; mp 273–5_C; IR(KBr) (t, cm_1),1640 (CO) ,1572 (C-$NH_2$); 1H NMR (DMSO-d6, 500 MHz) d (ppm): 4.50 (2H, s,NHNH2), 7.13 (2H, s, SO2NH2), 7.48 (2H, d, J = 8.79 Hz, Ar-H),7.53 (2H, d, J = 8.30 Hz, Ar-H), 7.58 (1H, d, J = 8.30 Hz, indole C7–H), 7.68 (1H, dd, J = 8.78,1.46 Hz, indole C6–H), 8.01 (1H, d,J = 0.98 Hz, indole C4–H), 9.14 (1H, s, CONH), 12.15 (1H, br s, indole NH).

**Sp f. 3-(4-bromophenyl)-2-(hydrazinocarbonyl)-1H-indole- 5-sulfonamide**

Yield 70%; mp 273–5_C; IR(KBr) (t, cm_1),1640 (CO) ,581 (C-Br); 1H NMR (DMSO-d6, 500 MHz) d (ppm): 4.50 (2H, s,NHNH2), 7.13 (2H, s, SO2NH2), 7.48 (2H, d, J = 8.79 Hz, Ar-H),7.53 (2H, d, J = 8.30 Hz, Ar-H), 7.58 (1H, d, J = 8.30 Hz, indole C7–H), 7.68 (1H, dd, J = 8.78,1.46 Hz, indole C6–H), 8.01 (1H, d,J = 0.98 Hz, indole C4–H), 9.14 (1H, s, CONH), 12.15 (1H, br s, indole NH).

**Sp g. 3-(4-Methylphenyl)-2-(hydrazinocarbonyl)-1H-indole- 5-sulfonamide**

Yield 70%; mp 273–5_C; IR(KBr) (t, cm_1),1640 (CO) ,3072 (C-$CH_3$); 1H NMR (DMSO-d6, 500 MHz) d (ppm): 4.50 (2H, s,NHNH2), 7.13 (2H, s, SO2NH2), 7.48 (2H, d, J = 8.79 Hz, Ar-H),7.53 (2H, d, J = 8.30 Hz, Ar-H), 7.58 (1H, d, J = 8.30 Hz, indole C7–H), 7.68 (1H, dd, J = 8.78,1.46 Hz, indole C6–H), 8.01 (1H, d,J = 0.98 Hz, indole C4–H), 9.14 (1H, s, CONH), 12.15 (1H, br s, indole NH).

**Sp h. 3-(4-Flurophenyl)-2-(hydrazinocarbonyl)-1H-indole- 5-sulfonamide**

Yield 70%; mp 273–5_C; IR(KBr) (t, cm_1),1640 (CO) ,1218 (C-F); 1H NMR (DMSO-d6, 500 MHz) d (ppm): 4.50 (2H, s,NHNH2), 7.13 (2H, s, SO2NH2), 7.48 (2H, d, J = 8.79 Hz, Ar-H),7.53 (2H, d, J = 8.30 Hz, Ar-H), 7.58 (1H, d, J = 8.30 Hz, indole C7–H), 7.68 (1H, dd, J = 8.78,1.46 Hz, indole C6–H), 8.01 (1H, d,J = 0.98 Hz, indole C4–H), 9.14 (1H, s, CONH), 12.15 (1H, br s, indole NH).

**Table 1: Statistical parameters from screening test set molecules.**

| S. No | Parameter | Renin |
|---|---|---|
| 1 | Total molecules in database (D) | 169 |
| 2 | Total Number of actives in database (A) | 71 |
| 3 | Total Hits (Ht) | 70 |
| 4 | Active Hits (Ha) | 66 |
| 5 | % Yield of actives [(Ha/Ht)*100] | 94.29 |
| 6 | % Ratio of actives [(Ha/A)*100] | 92.96 |
| 7 | Enrichment factor (E) [(Ha*D)/(Ht*A)] | 2.26 |
| 8 | False Negatives [A - Ha] | 5 |
| 9 | False Positives [Ht - Ha] | 4 |
| 10 | Goodness of Hit Score$ | 0.82-0.87 |

$ [(Ha/4HtA) (3A+Ht))*(1-((Ht-Ha)/ (D-A))]; GH Score of 0.7 – 0.9 indicates a very good model

**Table 2: Various parameters of lipinski's rule**

| S.no | Molecule | Mol.Wt g/mol | AlogP | Hydrogen bond acceptors | Hydrogen bond donors | Rotatable bonds |
|---|---|---|---|---|---|---|
| 1 | 12 | 546.7 | 3.93 | 10 | 0 | 20 |
| 2 | 124 | 486.65 | 4.97 | 7 | 1 | 16 |
| 3 | 125 | 330.68 | 4.7 | 8 | 1 | 17 |
| 4 | 128 | 523.61 | 4.83 | 8 | 0 | 16 |
| 5 | 141 | 488.62 | 3.41 | 9 | 1 | 17 |
| 6 | 146 | 581.71 | 2.87 | 10 | 2 | 20 |
| 7 | 20 | 523.67 | 4.83 | 8 | 0 | 16 |
| 8 | 23 | 585.74 | 4.34 | 10 | 0 | 20 |
| 9 | 246 | 541.68 | 4.84 | 9 | 0 | 17 |
| 10 | 25 | 494.61 | 4.46 | 7 | 0 | 14 |
| 11 | 27 | 417.55 | 3.37 | 7 | 0 | 14 |
| 12 | 29 | 526.68 | 4.33 | 8 | 0 | 15 |
| 13 | 309 | 522.64 | 4.32 | 9 | 0 | 16 |
| 14 | 31 | 383.55 | 2.87 | 6 | 1 | 14 |
| 15 | 314 | 488.58 | 2.15 | 9 | 3 | 17 |
| 16 | 318 | 475.55 | 3.28 | 9 | 1 | 17 |
| 17 | 322 | 516.67 | 4.24 | 8 | 0 | 17 |
| 18 | 323 | 530.7 | 4.16 | 9 | 0 | 18 |
| 19 | 356 | 326.63 | 2.40 | 9 | 2 | 18 |
| 20 | 52 | 580.03 | 4.25 | 10 | 0 | 20 |
| 21 | 235 | 339.62 | 4.41 | 7 | 2 | 15 |
| 22 | 239 | 486.68 | 4.97 | 7 | 1 | 16 |
| 23 | 240 | 398.68 | 4.7 | 8 | 1 | 17 |
| 24 | 259 | 530.46 | 4.35 | 8 | 2 | 19 |
| 25 | 262 | 531.69 | 4.85 | 9 | 1 | 20 |

**Table 3: Experimental $IC_{50}$ and Predicted $IC_{50}$ data of 24 training set molecules.**

| Molecule | Exp. $IC_{50,}$ nM | Predicted $IC_{50,}$ nM | Error a | Fit value b | Experimental Scale c | Predicted Scale c |
|---|---|---|---|---|---|---|
| 1 | 0.02 | 8.40E-03 | -2.4 | 13.93 | +++ | +++ |
| 2 | 0.04 | 0.45 | 11 | 12.2 | +++ | +++ |
| 3 | 0.05 | 0.079 | 1.6 | 12.95 | +++ | +++ |
| 4 | 0.09 | 0.37 | 4.2 | 12.28 | +++ | +++ |
| 5 | 0.1 | 0.29 | 2.9 | 12.39 | +++ | +++ |
| 6 | 0.22 | 0.34 | 1.5 | 13.32 | +++ | +++ |
| 7 | 0.63 | 2.9 | 4.6 | 11.39 | +++ | +++ |
| 8 | 1.5 | 1.1 | -1.4 | 11.83 | +++ | +++ |
| 9 | 3.8 | 8.6 | 2.3 | 10.92 | +++ | +++ |
| 10 | 6 | 14 | 2.3 | 10.7 | +++ | +++ |
| 11 | 7.8 | 11 | 1.4 | 10.81 | +++ | +++ |
| 12 | 18 | 12 | -1.5 | 10.76 | +++ | +++ |
| 13 | 25 | 14 | -1.8 | 10.7 | ++ | +++ |
| 14 | 30 | 7.9 | -3.8 | 10.95 | ++ | +++ |
| 15 | 45 | 38 | -1.2 | 10.27 | ++ | ++ |
| 16 | 56 | 130 | 2.3 | 9.75 | ++ | + |
| 17 | 70 | 17 | -4.2 | 10.63 | ++ | ++ |
| 18 | 100 | 150 | 1.5 | 9.67 | ++ | + |
| 19 | 120 | 160 | 1.3 | 9.65 | + | + |
| 20 | 210 | 140 | -1.5 | 9.7 | + | + |
| 21 | 320 | 170 | -1.8 | 9.61 | + | + |
| 22 | 570 | 110 | -5.4 | 9.83 | + | + |
| 23 | 600 | 140 | -4.2 | 9.7 | + | + |
| 24 | 910 | 170 | -5.3 | 9.62 | + | + |

**Table no 4. Physico chemical properties of different 3-phenyl indole derivatives**

| S.No | Compound | R | Mol.Formula | M.Wt | Yield (%) | M.P | Rf value |
|---|---|---|---|---|---|---|---|
| 1 | Sp a | -H | $C_{15}H_{14}SO_3N_4$ | 330 | 65.8% | $207^0$C-$209^0$ C | 0.721 |
| 2 | Sp b | -Cl | $C_{15}H_{13}SO_3N_4Cl$ | 365 | 55.2% | $209^0$C-$210^0$C | 0.734 |
| 3 | Sp c | -OH | $C_{15}H_{14}SO_4N_4$ | 346 | 69.5% | $211^0$C-$213^0$C | 0.754 |
| 4 | Sp d | $-NO_2$ | $C_{15}H_{13}SO_5N_5$ | 375 | 68.3% | $206^0$C-$208^0$C | 0.643 |
| 5 | Sp e | $-NH_2$ | $C_{15}H_{15}SO_3N_5$ | 345 | 59.9% | $217^0$C-$218^0$C | 0.654 |
| 6 | Sp f | -Br | $C_{15}H_{13}SO_3N_4Br$ | 409 | 58.1% | $210^0$C-$212^0$C | 0.739 |
| 7 | Sp g | $-CH_3$ | $C_{16}H_{16}SO_3N_4$ | 344 | 59.0% | $205^0$C-$206^0$C | 0.689 |
| 8 | Sp h | -F | $C_{15}H_{13}SO_3N_4F$ | 348 | 60.7% | $204^0$C-$206^0$C | 0.651 |

**Table no 5. Cost analysis of pharmacophore future**

| HypoNo. | Total cost | Cost difference[$] | Error Cost | RMS deviation | Training set (r) | Features[#] |
|---|---|---|---|---|---|---|
| 1 | 121.379 | 29.1885 | 92.1905 | 0.977411 | 0.954052 | AHHHR |
| 2 | 130.732 | 25.56 | 105.172 | 1.42729 | 0.885766 | HHHRR |
| 3 | 132.757 | 28.369 | 104.388 | 1.4042 | 0.89394 | HHHRR |
| 4 | 134.539 | 29.853 | 104.686 | 1.41301 | 0.895244 | AHHHR |
| 5 | 135.164 | 29.631 | 105.533 | 1.43778 | 0.890618 | AHHHR |
| 6 | 135.965 | 25.021 | 110.944 | 1.58687 | 0.855166 | AHHHR |
| 7 | 136.352 | 26.704 | 109.648 | 1.55246 | 0.864453 | AHHHR |
| 8 | 136.442 | 24.427 | 112.015 | 1.61473 | 0.848713 | AHHHR |
| 9 | 139.657 | 24.649 | 115.008 | 1.69019 | 0.832947 | AHHHR |
| 10 | 141.824 | 28.17 | 113.654 | 1.65649 | 0.846376 | AHHHR |

**Renin inhibitor activity as compared to aliskiren.**

**Table no 6. IC $_{50}$ value of different derivatives of 3-phenyl indole**

| Compounds | $IC_{50}$Value |
|---|---|
| Sp a | 86 |
| Sp b | $5.4x10^2$ |
| Sp c | 50 |
| Sp d | 45 |
| Sp e | 63 |
| Sp f | $2.2x10^2$ |
| Sp g | $1.6x10^2$ |
| Sp h | 1.5 |

**Table no 7. Biological activities of the compound SPa-SPh (500 µg/ml )**

| Compounds | R | Zone of inhibition (mm) | | | | |
|---|---|---|---|---|---|---|
| | | Antimicrobial activity (500 µg/ml ) | | | Anti fungal activity(500 µg/ml ) | |
| | | *S.aureus* | *B.subtilis* | *E.coli* | *C.albicans* | *A.niger* |
| Spa | Cl | 25 | 23 | 24 | 15 | NS |
| Spb | H | 23 | 25 | 20 | 21 | NS |
| Spc | OH | 34 | 22 | 25 | 21 | 27 |
| Spd | NO2 | 27 | 20 | 22 | 15 | NS |
| Spe | NH2 | 29 | 26 | 20 | 22 | NS |
| Spf | Br | 35 | 23 | 22 | 20 | 27 |
| Spg | CH3 | 12 | 20 | 26 | 23 | NS |
| Sph | F | 20 | 22 | 24 | 28 | 26 |

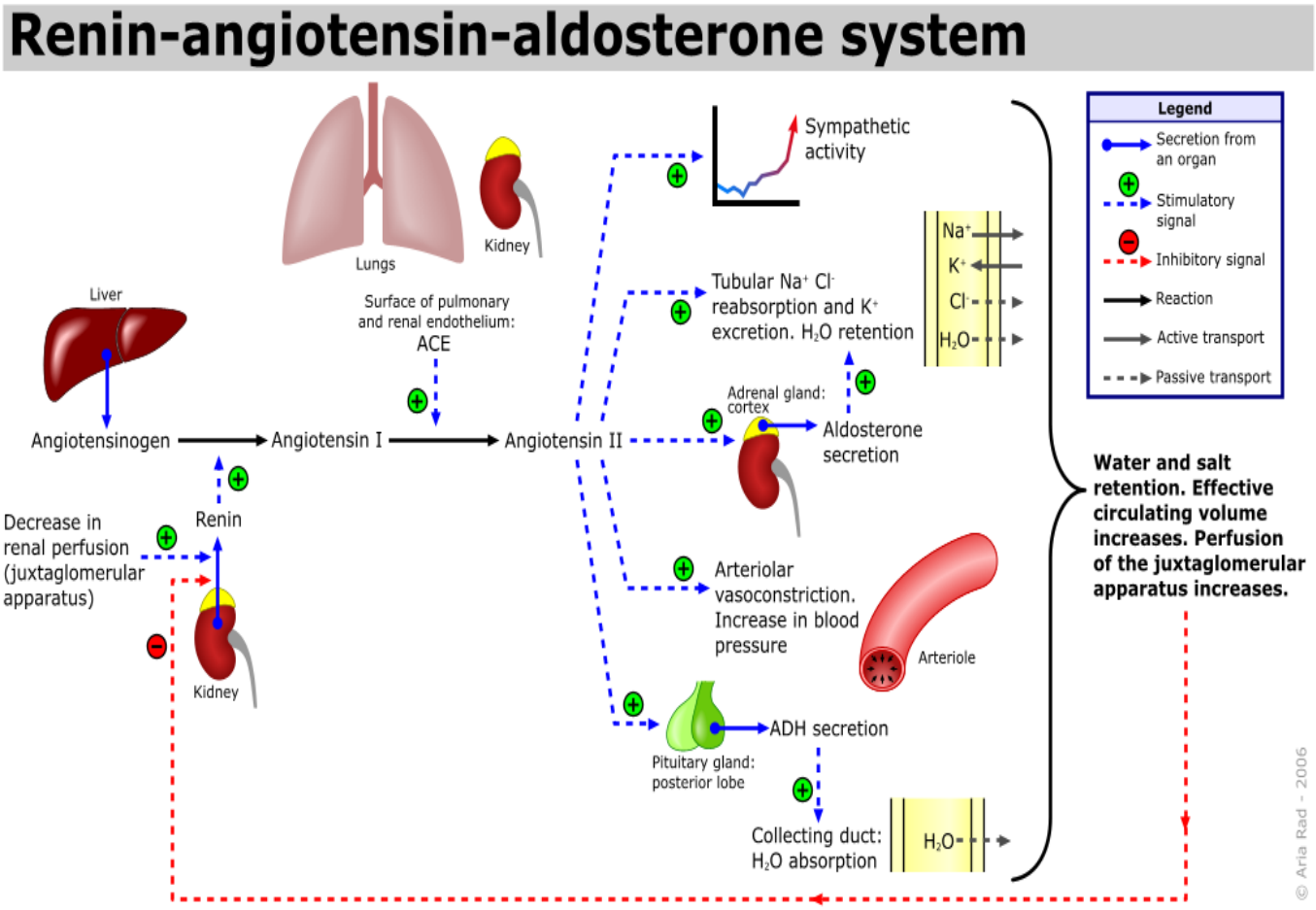
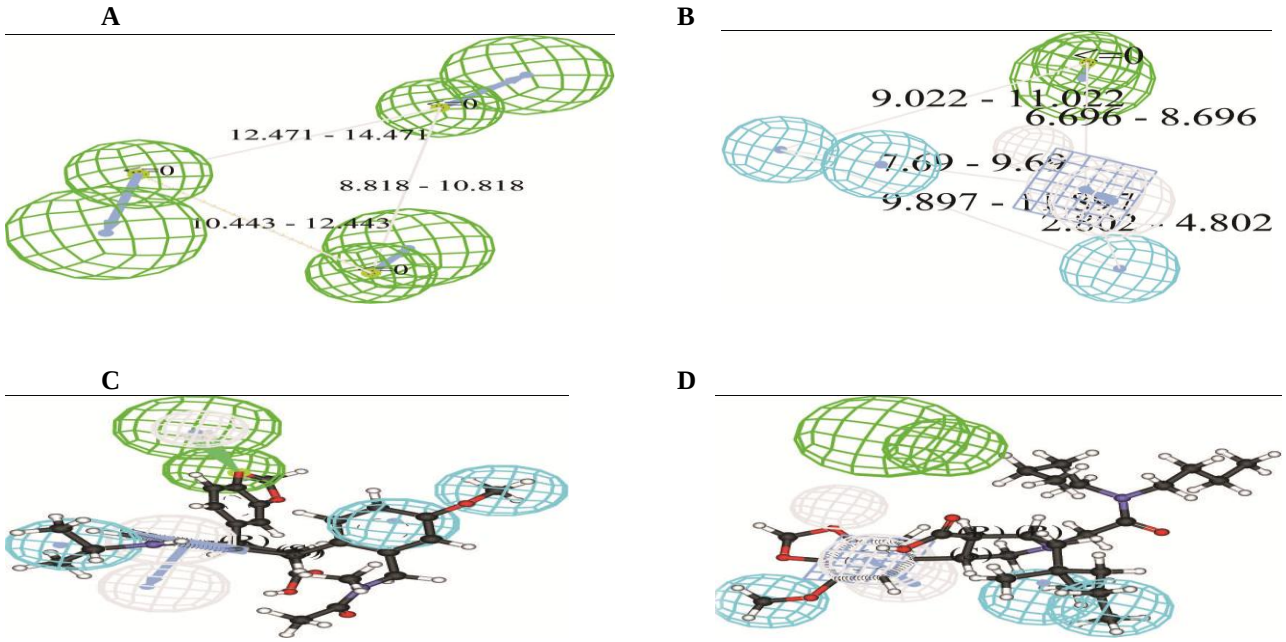
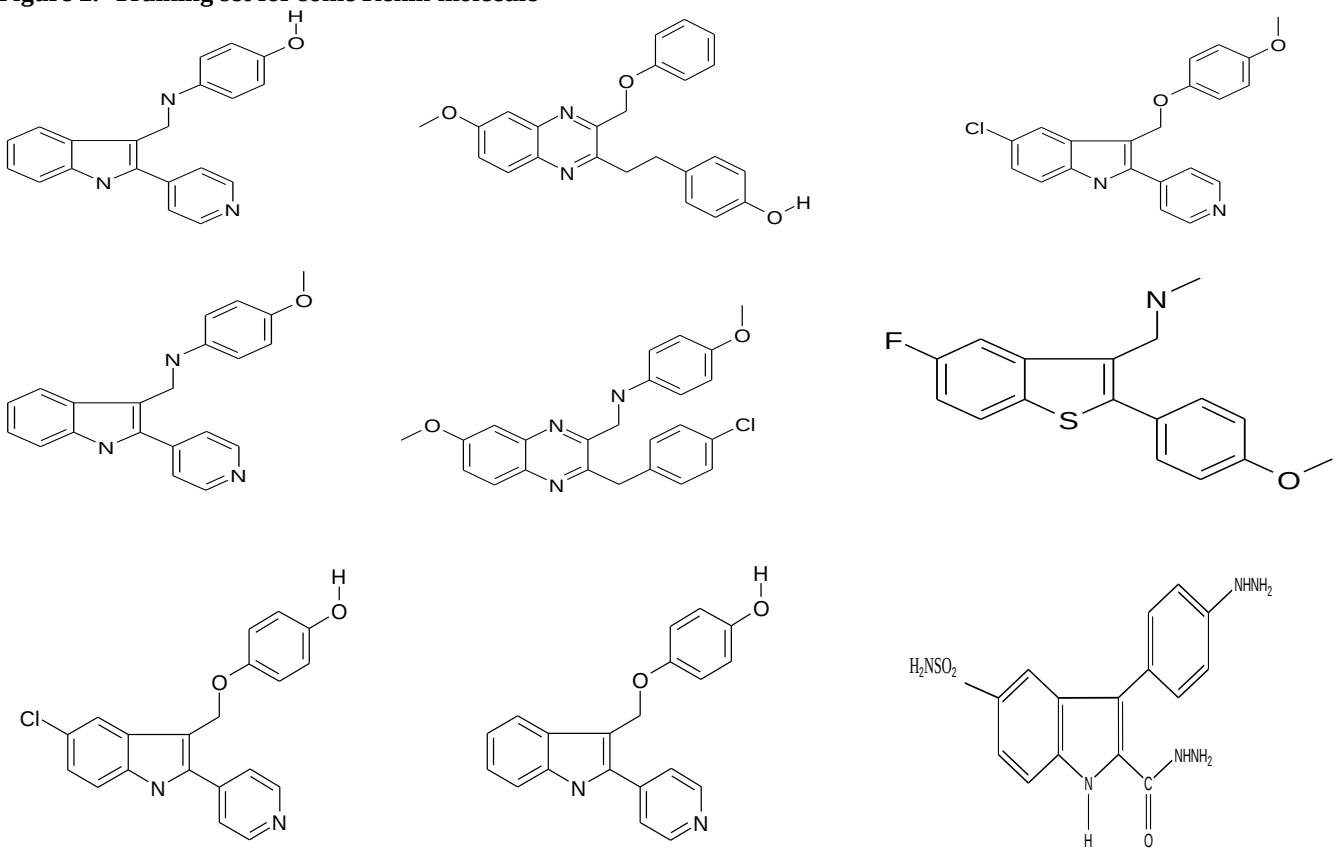


**Figure 1**. **Pharmacophore model for Renin inhibitors**



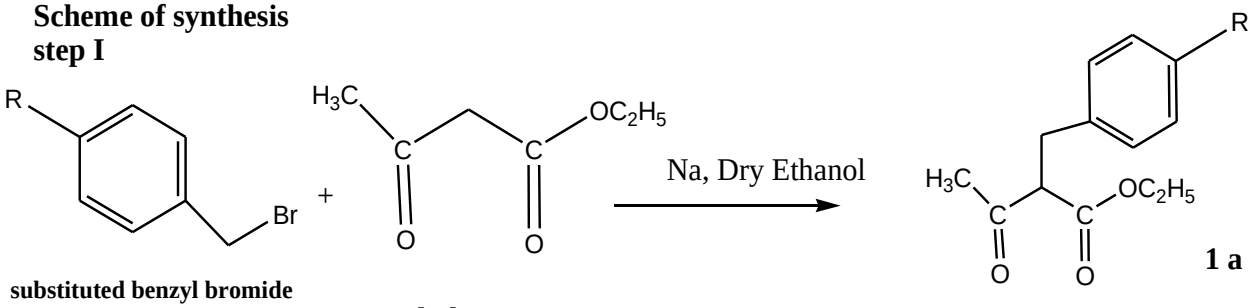

A. Hip Hop model displayed with the distance between the features.
B. Hypogen features with distance.
C. Pharmacophore mapped to the most active molecule.
D. Pharmacophore mapped to the least active molecule.

**Figure 2. Training set for some Renin molecule**



**Scheme of synthesis**

**step I**



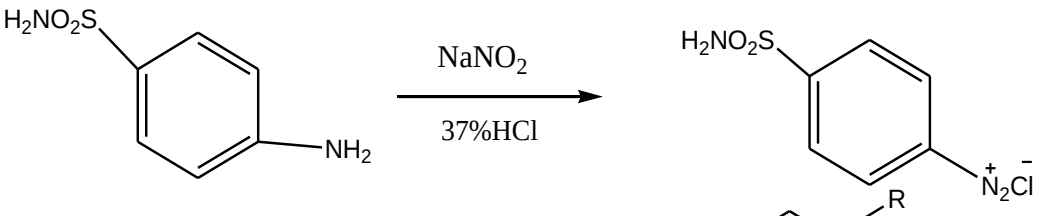

**substituted benzyl bromide**

**Ethyl acetoacetate**

**1 a**

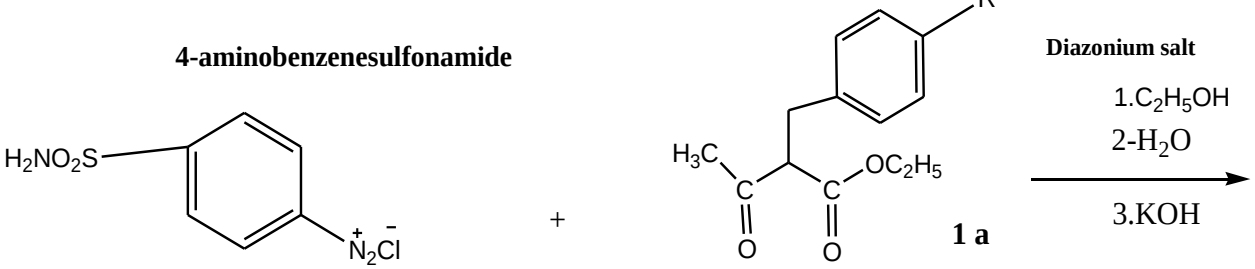
**Ethyl-2-acetyl-3-(substituted phenyl)propionate**

**step II**




**4-aminobenzenesulfonamide**

**Diazonium salt**

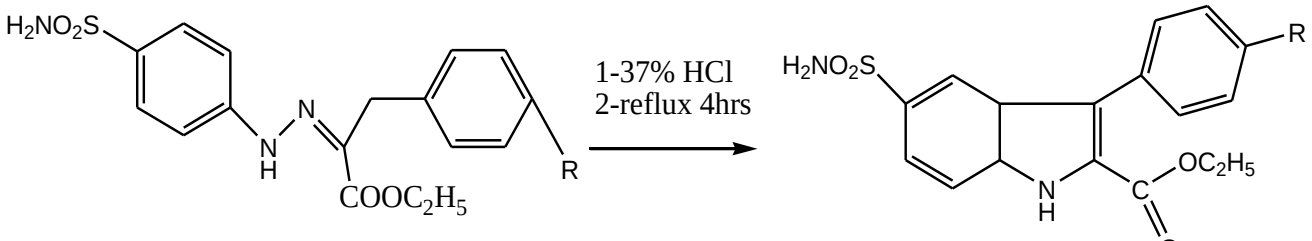



**Diazonium salt**

**1 a**

**Ethyl-2-acetyl-3-(substituted phenyl)propionate**

**step III**



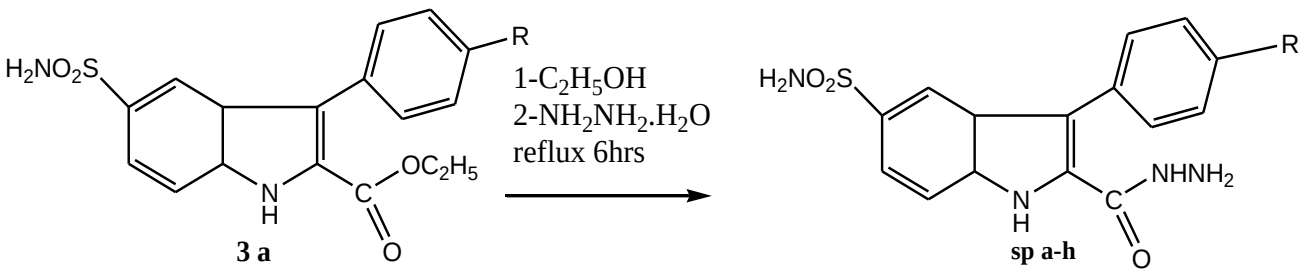

**2 a**

**2-substituted benzyl-2-[N-(4-sulfonamido phenyl) hydrazone]ethanoate**

**3 a**

**Ethyl5-(amino sulfonyl)-3substituted phenyl-1H-indole2-corboxylate**

**step IV**



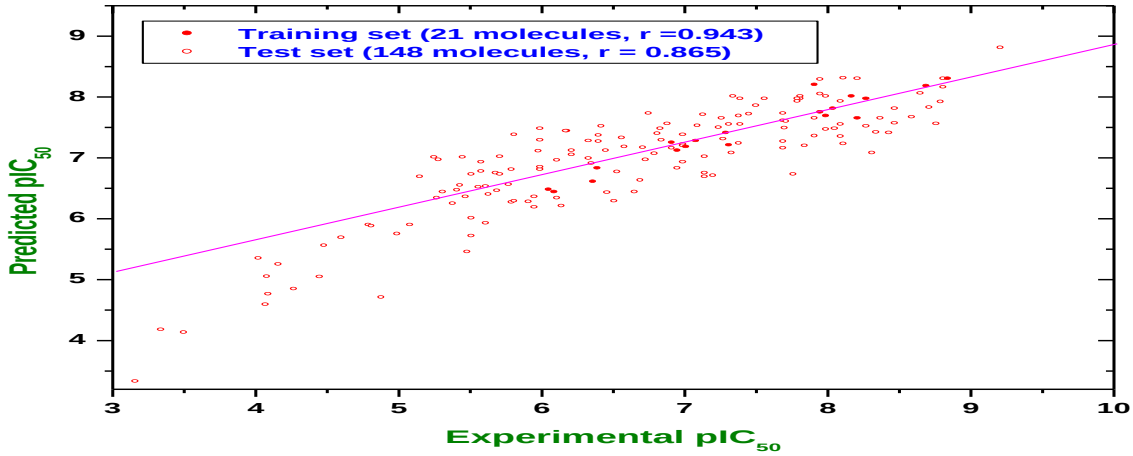

**3 a**

**Ethyl5-(amino sulfonyl)-3substituted phenyl-1H-indole2-corboxylate**

**sp a-h**

**2-(hydrazino corbonyl)-3-substituted phenyl-1H-indole 5-sulfonamides**

**Figure.3** Graph showing the Correlation (r) between experimental and predicted activities for the 169 test set molecules against Hypo-1 model along with 21 training set molecules for Renin inhibitors




## CONCLUSION

The work presented in this study shows how chemical features of a set of compounds along with their activities ranging over several orders of magnitudes can be used to generate pharmacophore hypotheses that can successfully predict the activity. The models were capable of predicting the activities over a wide variety of scaffolds and showed distinct chemical features that may be responsible for the activity of the inhibitors. This knowledge can be used to identify and design inhibitors with greater selectivity.

Thus the pharmacophores generated from the Renin inhibitors can be used as a three-dimensional query in database searches to identify compounds with diverse structures that can function as potent inhibitors to evaluate how well any newly designed compound maps on the pharmacophore before undertaking any further study including synthesis. Both these applications may help in identifying or designing compounds for further biological evaluation and optimization. We have synthesized some 3 phenyl indole derivatives (Sp a-h) and evaluated these ompounds for their inhibition of renin activities. Most of them demonstrated a broad spectrum of Anti microbial activities. The simple 3 phenyl indole derivatives Sp b, Sp f and Sp g were concluded as most potent derivatives in all the cases.

This pharmacophore model was further used to screen the generated structure. The activities of those molecules were predicted using the developed Pharmacophore model and sorted out using the lipinski's rule of five by accelrys cerius2 software.

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