Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Suppl 1, 2013

Research Article

# 3D-QSAR ANALYSIS OF SERIES 3-GUANIDINOPROPIONIC ACIDS AS ANTIDIABETC AGENTS

**SATISH SAHU*, YOGESH POUNIKAR, LOPAMUDRA BANERJEE AND D V KOHLI**

**Pharmaceutical Chemistry and Drug Design Research Laboratory, Dept. of Pharm. Sciences, Dr. H. S. Gour University, Sagar (M.P.), India-469002. Email: satishbu@yahoo.co.in**

*Received: 14 Aug 2012, Revised and Accepted: 30 Sep 2012*

**ABSTRACT**

The structures of series of 3-guanidinopropionic acids were submitted to molecular modeling software and after energy minimization and conformational analysis of the structures; a number of electronic, spatial and thermodynamic descriptors were calculated. Several statistical regression expressions were obtained using multiple regression analysis. Amongst them, one model was found to be best on various statistical criteria, involving the descriptor viz. steric parameters (molar refractivity) and hydrophobic parameters (hydrophobicity) with significant correlation coefficient.

## INTRODUCTION

Non-insulin dependent diabetes mellitus (NIDDM) is a complex, chronic metabolic disorder characterized by a resistance of the peripheral target tissues to fully respond to the binding of insulin and insufficient insulin secretion by the pancreas to overcome this reduced response[1,2]. The result of these two pathologies is impaired glucose uptake and metabolism, leading to fasting hyperglycemia. The etiology of NIDDM is complex but is now generally accepted to entail the initial development of insulin resistance in the prediabetic state that leads to compensatory hyperinsulinemia. Eventually the β-cells of the pancreas can no longer maintain the hyperinsulinemic state, and the ensuing insulin deficiency leads to chronic hyperglycemia. Untreated NIDDM leads to several chronic diseases such as neuropathy, nephropathy and cardiovascular diseases[3]. The later lead to increase in mortality. At present, therapy for type II diabetes relies mainly on several approaches intended to reduce hyperglycemia itself: sulfonylureas, biaguanides, thiazolidinediones, α-glucosidase inhibitors, insulin sensitizer and insulin secretagogues.

Meglasson *et. al.* reported that 3-guanidino-propionic acid possess both antihyperglycemic and antiobesity activity in KKA$^{\gamma}$ mouse, a rodent model of NIDDM.[4,5]

Although the antidiabetic potential of lipophilic guanidine derivatives has been recognized by Bailey[6,7]. Survey of literature showed that biguanides have potential hypoglycemic agents. Though some research has been done on this molecule, off and on for the past century, the development of guanidine derivative (3-Guanidinopropionic acid) as a novel antidiabetic agent is yet to emerge. The extreme hydrophobicity of 3-guanidinopropionic acid (GPA) may offer an advantage over more lipophilic guanidine antidiabetic agents which have historically been associated with lactic acidosis, a potentially fatal overproduction of lactic acid resulting from inhibition of mitochondrial oxidative phosphorylation[8,9]. The higher observed incidence of lactic acidosis in patients receiving phenformin relative to that observed in patients treated with the closely related but markedly less lipophilic drug, metformin, tends strong clinical support the hypothesis that lipophilicity and toxicity are positively correlated.[9,10].

We therefore decided to study quantitative structure activity relationship (QSAR) of 3-guanidinopropionic acid analogues as antidiabetic agents[11].

## MATERIALS AND METHODS

The *in-vitro* transativation activity data of 3-guanidinopropionic acid analogues were taken from reported work of Larsen *et al* [11] (Table 1). The biological activity was converted to negative logarithm for QSAR analysis. For the present 3D-QSAR analysis Apex-3D expert system on a silicon graphics INDY-4000 was used. All molecular modeling and 3D-QSAR studies were performed on a silicon graphics INDY-4000 workstation employing molecular simulation software.

A series of 55 compounds were taken as a training set. The molecular structure of all compounds were constructed in 2D using the sketch program in the builder module of INSIGHT-II software and then converted to 3D for optimization of their geometry (net charge 0.0) by selecting the forcefield potential action and charge action as fixed. The molecules structures were finally minimized using the steepest descent, conjugate gradients and Newton Raphson's algorithm followed by Quasi-Newton-Raphson. Optimization techniques implemented in Discover module (version 2.9) by energy tolerance value of 0.001 Kcal/mol and maximum number of iteration set at 1000. A total of 1193 conformers were generated for total molecules and lowest energy conformer of each cluster was selected by conformation clustering methodology. These conformations were subjected to different computational chemistry program including MOPAC 6.0 version (MNDO Hamiltonian) for the calculations of physicochemical parameters (π-population, atomic charges, electron donor and acceptor indexes, HOMO and LUMO coefficient and hydrophobicity and molar refractivity based on atomic contributions) and quantum chemical parameters.

The data was used by Apex-3D program for automated identification of biophores, superimposition of compounds and quantitative model building. Compounds present in the test have been predicted to check the validity of model. In addition to it "Leave One Out (LOO)" cross validation was also performed in which the objects were left out randomly but only once. On the basis of chance value, RMSA, RMSP, *R* and size, models have been selected which can be considered to be most robust model for the series.

## RESULT AND DISCUSSION

Pharmacophore models with different size and arrangements were generated for the training set given in Table 2.

Among several 3D biophoric models for all the molecules of training set, Model No. 50 was selected based on criterion and is given in Table 3.

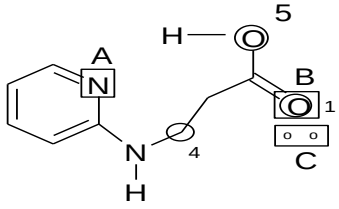
1. R (Correlation coefficient) > 0.70

2. The difference between RMSA and RMSP < 0.02

3. Chance < 3

4. Number of variables < 5

5. Number of compounds as maximum as possible (n=49)




□ = Biophoric site ❍ = Secondary site

**Representative Example of Most Active Compound (36) in Model No. 50**

In figure, site A, B and C represent the three biophoric sites corresponding to the nitrogen of pyridine ring, carbonyl oxygen of -COOH group and its oxygen lone pair. Site A and B are electron rich sites capable of donating electrons by the nitrogen and oxygen atom respectively. So, we can say both the sites (mainly site B) may be involved in ionic bonding. Site C which is an electronic cloud on oxygen atom is necessary for hydrogen bonding. All these properties of the biophoric sites are given in Table 4.

The mean interatomic distances between the biophoric sites A-B, B-C, and C-A are 8.626, 5.976 and 3.000 $A^0$ respectively. All these properties, distances and spatial arrangement of the biophoric sites are important for their interaction with the receptor to show Antidiabetic activity.

3D-QSAR equation for Model No. 50 is

-log (HA) = 0.644(CHARGE) (±0.164) - 0.036 (TOTAL HYDROPHOBICITY) (±0.016) - 0.098 (H-DONOR) (±0.037) + 0.023 (REFRACTIVITY) (±0.012) - 1.729.

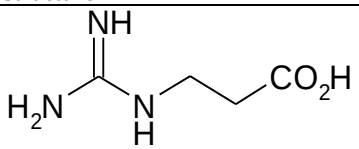
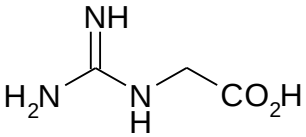
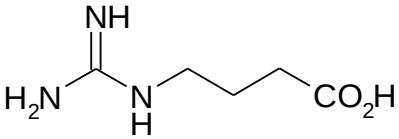
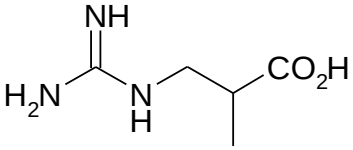
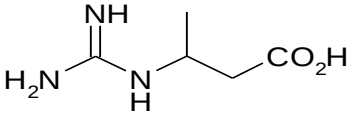
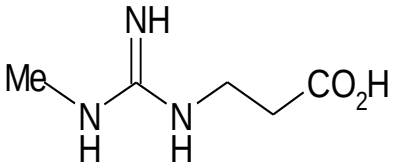
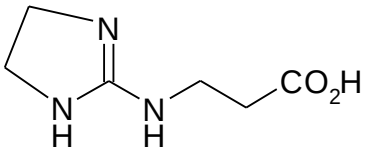
n = 49, $R^2$=0.59, R= 0.77, F(6,42) = 7.588, S = 7.588

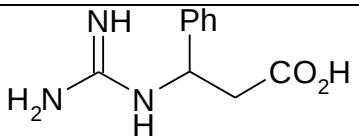
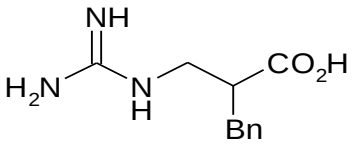
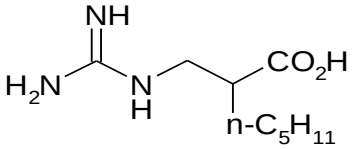
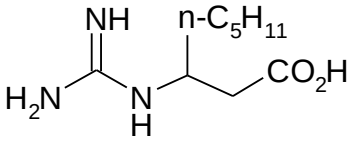
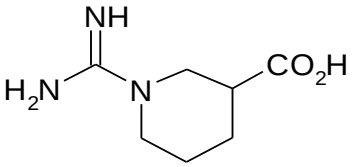
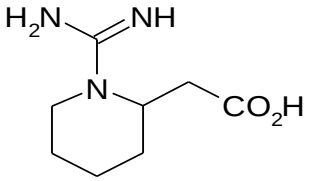
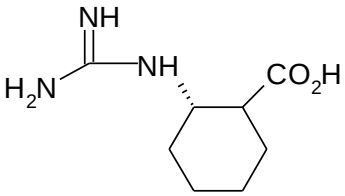
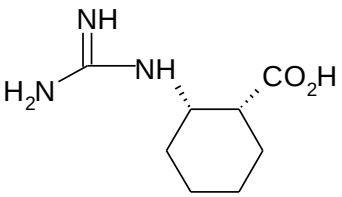
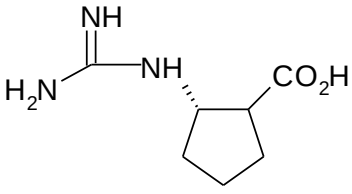
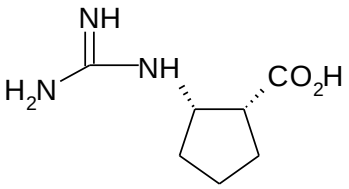
It was derived using these biophore as a template for superimposition.

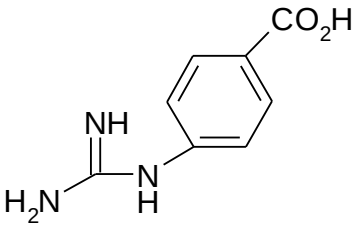
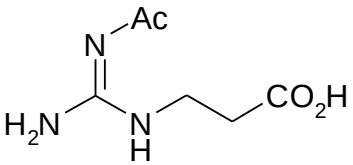
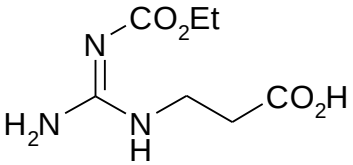
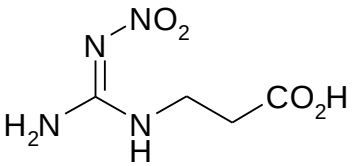
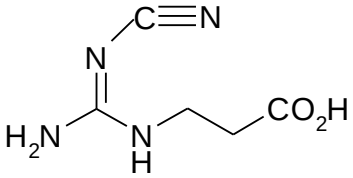
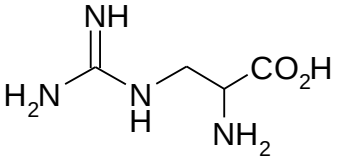
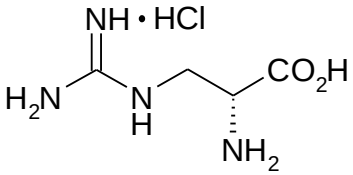
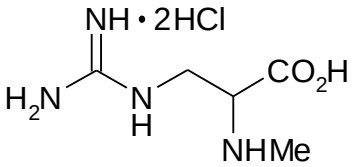
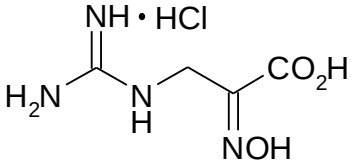
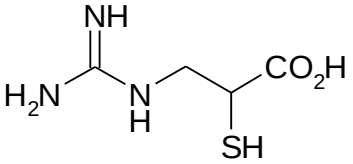
In addition to the biophoric sites, secondary sites are also important for a compound to show Antidiabetic activity. Total hydrophobicity which is a global property for a molecule has negative contribution for the Antidiabetic activity.

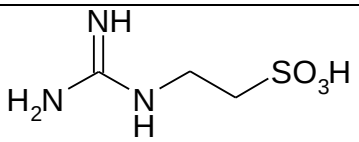
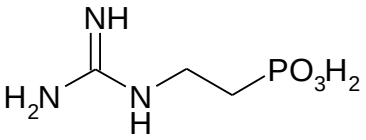
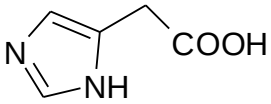
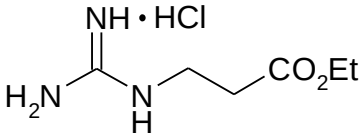
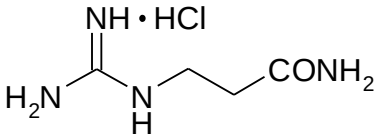
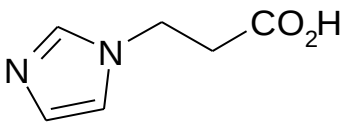
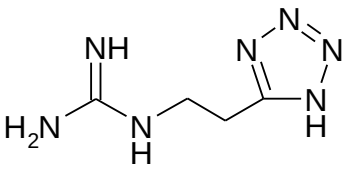
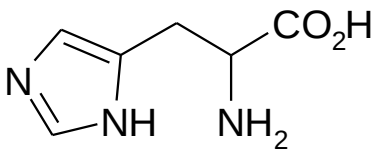
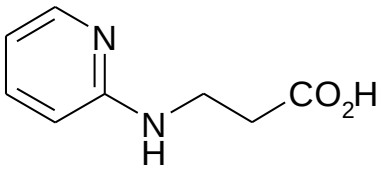
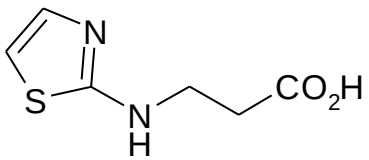
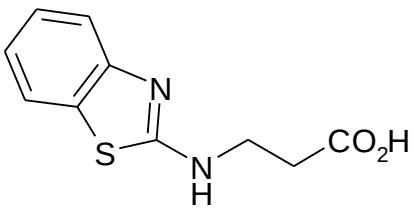
The variation in the binding affinity with the receptor site is best described by three parameters. One being the charge at the biophoric centre B corresponding to oxygen atom, second is hydrophobicity at the methylene group near to -NH group and third is refractivity at the terminal oxygen. Apart from acting as electron donor or nucleophilic centre the biophoric site B corresponding to oxygen atom contributes positively for activity. The second parameter explaining the variation in activity and contributing negatively as secondary site described as hydrophobicity near to -NH group. So substitution of hydrophilic group at this site is favourable for activity. The third parameter is refractivity, which contributes positively for activity. Therefore substitution of large bulky group at this site is favourable for activity. Experimental, predicted & calculated activity data are given in Table 5 and Fig 1

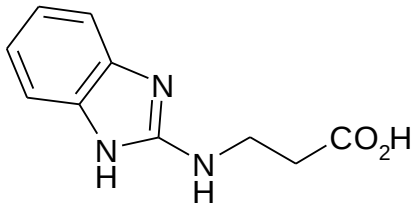
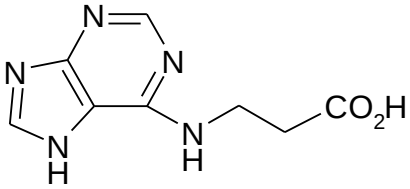
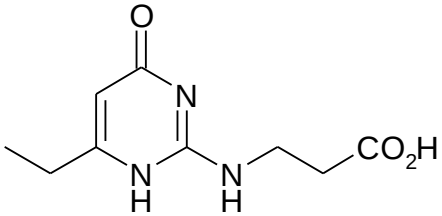
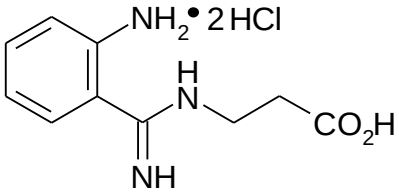
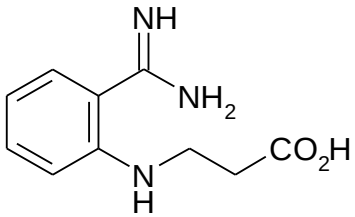
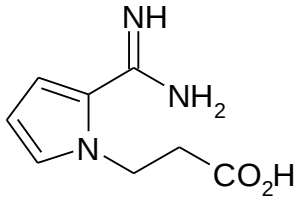
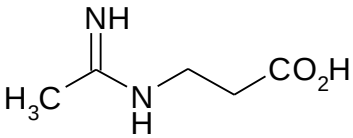
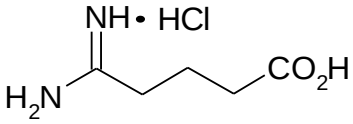
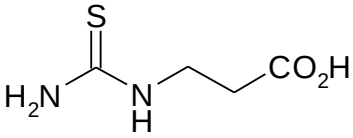
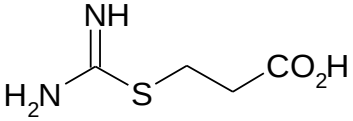
**Table 1: *In vitro* Activity Data (HA) Of Series 3-Guanidinopropionic Acid**

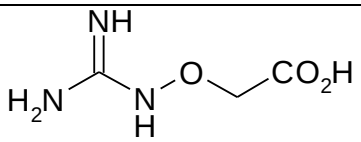
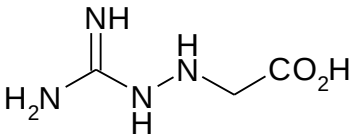
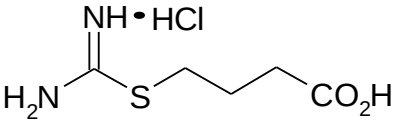
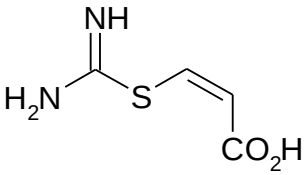
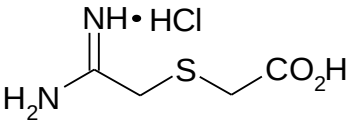
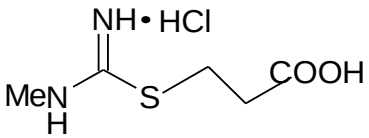
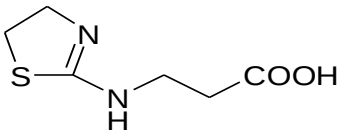
| Compound No. | Structure | MISS T/C | -log (HA) |
|---|---|---|---|
| 1. |  | 52 | -1.7160 |
| 2. |  | 62 | -1.7924 |
| 3. |  | 80 | -1.9031 |
| 4. |  | 86 | -1.9345 |
| 5. |  | 77 | -1.8865 |
| 6. |  | 102 | -2.009 |
| 7. |  | 97 | -1.9868 |

| | | | |
|---|---|---|---|
| 8. |  | 95 | -1.9777 |
| 9. |  | 93 | -1.9685 |
| 10. |  | 79 | -1.8976 |
| 11. |  | 88 | -1.9445 |
| 12. |  | 100 | -2.000 |
| 13. |  | 91 | -1.9590 |
| 14. |  | 93 | -1.9685 |
| 15. |  | 92 | -1.9638 |
| 16. |  | 90 | -1.9542 |
| 17. |  | 97 | -1.9868 |

| | | | |
|---|---|---|---|
| 18. |  | 80 | -1.9031 |
| 19. |  | 88 | -1.9445 |
| 20. |  | 88 | -1.9445 |
| 21. |  | 84 | -1.9243 |
| 22. |  | 92 | -1.9638 |
| 23. |  | 35 | -1.5441 |
| 24. |  | 92 | -1.9638 |
| 25. |  | 96 | -1.9823 |
| 26. |  | 97 | -1.9868 |
| 27. |  | 83 | -1.9191 |

| | | | |
|---|---|---|---|
| 28. |  | 91 | -1.9590 |
| 29. |  | 81 | -1.9085 |
| 30. |  | 68 | -1.8325 |
| 31. |  | 87 | -1.9395 |
| 32. |  | 96 | -1.9823 |
| 33. |  | 81 | -1.9085 |
| 34. |  | 94 | -1.9731 |
| 35. |  | 80 | -1.9031 |
| 36. |  | 20 | -1.3010 |
| 37. |  | 99 | -1.9956 |
| 38. |  | 87 | -1.9395 |

| | | | |
|---|---|---|---|
| 39. |  | 95 | -1.9777 |
| 40. |  | 85 | -1.9294 |
| 41. |  | 96 | -1.9823 |
| 42. |  | 92 | -1.9638 |
| 43. |  | 73 | -1.8633 |
| 44. |  | 93 | -1.9685 |
| 45. |  | 77 | -1.8865 |
| 46. |  | 93 | -1.9685 |
| 47. |  | 99 | -1.9956 |
| 48. |  | 53 | -1.7243 |

| | | | |
|---|---|---|---|
| 49. |  | 83 | -1.9191 |
| 50. |  | 61 | -1.7853 |
| 51 |  | 87 | -1.9395 |
| 52 |  | 68 | -1.8325 |
| 53 |  | 92 | -1.9638 |
| 54 |  | 59 | -1.7709 |
| 55 |  | 85 | -1.9294 |

MISS (T/ C) = Mouse Insulin Sensitizing Screen Nonfasting Blood Glucose Level (Test / Control)

HA = Hypoglycaemic Activity

**Table 2: 3D - Pharmacophore Models For Series 3- Guanidinopropionic Acids.**

| Model No. | RMSA | RMSP | $R^2$ | Chance | Size | Match | Variable | No. of Compounds |
|---|---|---|---|---|---|---|---|---|
| 525 | 0.08 | 0.09 | 0.25 | 1.00 | 3 | 0.37 | 2 | 46 |
| 50 | 0.09 | 0.11 | 0.59 | 0.21 | 3 | 0.34 | 4 | 49 |
| 393 | 0.10 | 0.12 | 0.34 | 0.85 | 3 | 0.23 | 4 | 45 |
| 190 | 0.11 | 0.12 | 0.24 | 1.00 | 3 | 0.34 | 3 | 55 |
| 476 | 0.10 | 0.15 | 0.40 | 0.44 | 3 | 0.42 | 3 | 45 |

**Table 3: 3D-QSAR Model Describing Correlation and Statistical Reliability for Series =3-Guanidinopropionic Acids**

| Model No. | RMSA | RMSP | $R^2$ | Chance | Size | Match | Variable | No. of Compounds |
|---|---|---|---|---|---|---|---|---|
| 50 | 0.09 | 0.11 | 0.59 | 0.21 | 3 | 0.34 | 4 | 49 |

Where,

RMSA - Root mean squared error of activity approximation.

RMSP - Root mean squared error of activity prediction

R - Correlation coefficient between experimental and approximated activity.

Chance - Probability of chance correlation.

Size - Number of descriptor centres in biophore.

Match - Quality of match for molecules having common biophores.

Variable - Number of variables in 3D-QSAR model.

**Table 4: Property Matrix for Model No. 50**

| Compound No. | Sites | DON-01 | H-site | Compound No. | sites | DON-01 | H-site |
|---|---|---|---|---|---|---|---|
| | A | 8.000 | - | | A | 7.483 | - |
| 1 | B | 8.315 | - | 3 | B | 8.434 | - |
| | C | - | 1.000 | | C | - | 1.000 |
| | A | 7.997 | - | | A | 7.927 | - |
| 4 | B | 8.310 | - | 5 | B | 8.339 | - |
| | C | - | 1.000 | | C | - | 1.000 |
| | A | 7.980 | - | | A | 8.334 | - |
| 6 | B | 8.344 | - | 7 | B | 8.376 | - |
| | C | - | 1.000 | | C | - | 1.000 |
| | A | 8.000 | - | | A | 7.988 | - |
| 8 | B | 8.363 | - | 9 | B | 8.309 | - |
| | C | - | 1.000 | | C | - | 1.000 |
| | A | 9.990 | - | | A | 8.006 | - |
| 10 | B | 8.311 | - | 11 | B | 8.339 | - |
| | C | - | 1.000 | | C | - | 1.000 |
| | A | 7.970 | - | | A | 7.997 | - |
| 12 | B | 8.387 | - | 13 | B | 8.440 | - |
| | C | - | 1.000 | | C | - | 1.000 |
| | A | 7997 | - | | A | 7.996 | - |
| 14 | B | 8.440 | - | 15 | B | 8.374 | - |
| | C | - | 1.000 | | C | - | 1.000 |
| | A | 7.985 | - | | A | 7.993 | - |
| 16 | B | 8.376 | - | 17 | B | 8.290 | - |
| | C | - | 1.000 | | C | - | 1.000 |
| | A | 7.782 | - | | A | 7.772 | - |
| 19 | B | 8.559 | - | 20 | B | 8.572 | - |
| | C | - | 1.000 | | C | - | 1.000 |
| | A | 7.856 | - | | A | 7.935 | - |
| 21 | B | 8.533 | - | 23 | B | 8.224 | - |
| | C | - | 1.000 | | C | - | 1.000 |
| | A | 7.917 | - | | A | 7.993 | - |
| 24 | B | 8.332 | - | 25 | B | 8.430 | - |
| | C | - | 1.000 | | C | - | 1.000 |
| | A | 7.923 | - | | A | 7.936 | - |
| 26 | B | 8.332 | - | 27 | B | 8.103 | - |
| | C | - | 1.000 | | C | - | 1.000 |
| | A | 7.946 | - | | A | 7.934 | - |
| 28 | B | 7.598 | - | 29 | B | 6.952 | - |
| | C | - | 1.000 | | C | - | 1.000 |
| | A | 8.471 | - | | A | 8.319 | - |
| 31 | B | 7.980 | - | 32 | B | 7.952 | - |
| | C | - | 1.000 | | C | - | 1.000 |

| Compound No. | Sites | DON-01 | H-site | Compound No. | Sites | DON-01 | H-site |
|---|---|---|---|---|---|---|---|
| | A | 8.709 | - | | A | 7.892 | - |
| 33 | B | 8.406 | - | 34 | B | 9.209 | - |
| | C | - | 1.000 | | C | - | 1.000 |
| | A | 8.737 | - | | A | 8.045 | - |
| 35 | B | 8.437 | - | 36 | B | 8.358 | - |
| | C | - | 1.000 | | C | - | 1.000 |
| | A | 7.829 | - | | A | 6.233 | - |
| 37 | B | 8.327 | - | 38 | B | 8.327 | - |
| | C | - | 1.000 | | C | - | 1.000 |
| | A | 8.665 | - | | A | 8.208 | - |
| 39 | B | 8.358 | - | 40 | B | 8.550 | - |
| | C | - | 1.000 | | C | - | 1.000 |
| | A | 8.083 | - | | A | 8.003 | - |
| 41 | B | 8.381 | - | 42 | B | 8.284 | - |
| | C | - | 1.000 | | C | - | 1.000 |
| | A | 7.968 | - | | A | 8.064 | - |
| 44 | B | 8.522 | - | 45 | B | 8.430 | - |
| | C | - | 1.000 | | C | - | 1.000 |
| | A | 8.087 | - | | A | 6.148 | - |
| 46 | B | 8.305 | - | 47 | B | 8.371 | - |
| | C | - | 1.000 | | C | - | 1.000 |
| | A | 8.141 | - | | A | 8.042 | - |
| 48 | B | 8.349 | - | 49 | B | 8.361 | - |
| | C | - | 1.000 | | C | - | 1.000 |
| | A | 8.003 | - | | A | 6.545 | - |
| 50 | B | 8.356 | - | 51 | B | 8.437 | - |
| | C | - | 1.000 | | C | - | 1.000 |
| | A | 8.119 | - | | A | 8.163 | - |
| 53 | B | 8.271 | - | 54 | B | 8.418 | - |
| | C | - | 1.000 | | C | - | 1.000 |
| | A | 6.461 | - | | | | |
| 55 | B | 8.304 | - | | | | |
| | C | - | 1.000 | | | | |

**Table 5: Structure - Activity Data for Model No. 50**

| Compound No. | Experimental values | Calculated values | Calculated Error | Predicted values | Predicted Error |
|---|---|---|---|---|---|
| 1 | -1.72 | -1.80 | 0.09 | -1.82 | 0.10 |
| 3 | -1.90 | -1.95 | 0.04 | -1.95 | 0.05 |
| 4 | -1.93 | -1.80 | -0.13 | -1.79 | -0.14 |
| 5 | -1.89 | -1.95 | 0.07 | -1.96 | 0.07 |
| 6 | -2.01 | -1.95 | -0.06 | -1.94 | -0.07 |
| 7 | -1.99 | -1.98 | -0.01 | -1.98 | -0.01 |
| 8 | -1.98 | -1.97 | -0.01 | -1.97 | -0.01 |
| 9 | -2.00 | -1.94 | -0.05 | -1.93 | -0.07 |
| 10 | -1.90 | -1.94 | 0.04 | -1.95 | 0.06 |
| 11 | -1.94 | -1.98 | 0.03 | -1.98 | 0.04 |
| 12 | -2.00 | -2.04 | 0.04 | -2.05 | 0.05 |
| 13 | -1.96 | -1.94 | -0.02 | -1.94 | -0.02 |
| 14 | -1.97 | -1.93 | -0.04 | -1.92 | -0.05 |
| 15 | -1.96 | -1.99 | 0.03 | -1.99 | 0.03 |
| 16 | -1.95 | -1.91 | -0.05 | -1.90 | -0.05 |
| 17 | -1.99 | -1.91 | -0.08 | -1.90 | -0.09 |
| 19 | -1.94 | -1.98 | 0.03 | -1.98 | 0.04 |
| 20 | -1.94 | -2.00 | 0.06 | -2.01 | 0.07 |
| 21 | -1.92 | -1.93 | 0.00 | -1.93 | 0.00 |
| 23 | -1.54 | -1.86 | 0.32 | -1.89 | 0.35 |
| 24 | -1.96 | -1.92 | -0.04 | -1.91 | -0.05 |
| 25 | -1.98 | -1.87 | -0.11 | -1.86 | -0.12 |
| 26 | -1.99 | -1.95 | -0.04 | -1.94 | -0.05 |
| 27 | -1.92 | -1.89 | -0.03 | -1.88 | -0.04 |
| 28 | -1.96 | -1.94 | -0.02 | -1.92 | -0.04 |
| 29 | -1.91 | -1.90 | -0.01 | -1.89 | -0.01 |
| 31 | -1.94 | -1.96 | 0.02 | -1.96 | 0.02 |
| 32 | -1.98 | -1.91 | -0.07 | -1.90 | -0.08 |
| 33 | -1.91 | -1.97 | 0.06 | -1.98 | 0.07 |
| 34 | -1.97 | -1.90 | -0.07 | -1.85 | -0.12 |
| 35 | -1.90 | -1.92 | 0.02 | -1.93 | 0.03 |
| 36 | -1.30 | -1.55 | 0.25 | -1.72 | 0.42 |

| Compound No. | Experimental values | Calculated values | Calculated Error | Predicted values | Predicted Error |
|---|---|---|---|---|---|
| 37 | -2.00 | -1.95 | -0.05 | -1.94 | -0.05 |
| 38 | -1.94 | -1.99 | 0.05 | -1.99 | 0.05 |
| 39 | -1.98 | -2.05 | 0.07 | -2.06 | 0.09 |
| 40 | -1.93 | -1.94 | 0.01 | -1.94 | 0.01 |
| 41 | -1.98 | -2.02 | 0.04 | -2.03 | 0.05 |
| 42 | -1.96 | -1.98 | 0.01 | -1.98 | 0.01 |
| 44 | -1.97 | -1.94 | -0.03 | -1.94 | -0.03 |
| 45 | -1.89 | -1.74 | -0.15 | -1.70 | -0.19 |
| 46 | -1.97 | -1.86 | -0.11 | -1.85 | -0.12 |
| 47 | -2.00 | -1.82 | -0.17 | -1.79 | -0.20 |
| 48 | -1.72 | -1.72 | 0.00 | -1.72 | 0.00 |
| 49 | -1.92 | -1.89 | -0.02 | -1.89 | -0.03 |
| 50 | -1.79 | -1.91 | 0.12 | -1.91 | 0.12 |
| 51 | -1.94 | -1.98 | 0.04 | -1.99 | 0.05 |
| 53 | -1.96 | -1.92 | -0.05 | -1.91 | -0.05 |
| 54 | -1.77 | -1.73 | -0.04 | -1.73 | -0.05 |
| 55 | -1.93 | -1.95 | 0.02 | -1.95 | 0.02 |

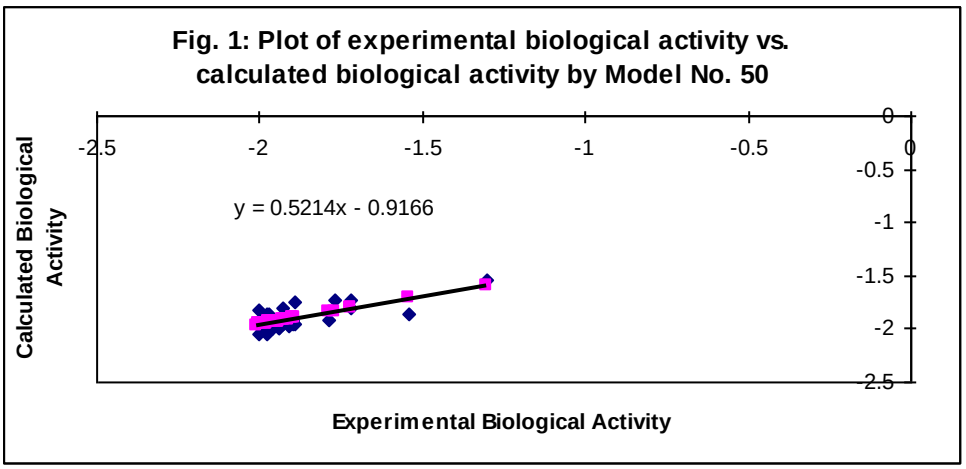



**Fig. 1: Plot of experimental biological activity vs. calculated biological activity by Model No. 50**

**CONCLUSION**

This study has resulted in the development of statistically significant and predictive QSAR equations for 3-guanidino propionic acid analogues using pharmacophoric mapping technique. Bearing the above biophoric patterns and the related properties in mind, several molecules can be designed and developed. The field is further open for the study of these compounds with respect to other indirect drug design techniques as receptor surface model generation, molecular shape analysis and comparative molecular field analysis.

**ACKNOWLEDGEMENT**

Authors are thankful to Director, Central Drug Research Institute, Lucknow for providing facilities for this work. The first author is also grateful to UGC, New Delhi, for Providing Junior Research Fellow.

**REFERENCES**

1. Reaven GM. Role of insulin resistance in human disease. Diabetes 1998; 37: 1595-1607.
2. Reaven GM. Role of insulin resistance in the pathophysiology of non-insulin-dependent diabetes mellitus. Diabetes 1993; 9: 5S-12S.
3. Porte D, Schwartz MW. Diabetes complications: Why is glucose is potentially toxic?.Science 1996; 27: 699-700.
4. Meglasson MD, Wilson JM, Yu JH, Robinson DD, Wyse BM, Desouza CJ. Antihypertensive action of guanidino alkanoic acids: 3-Guanidino alkanoic acid ameliorates hyperglycemia in diabetic KKA$^{y}$ and C57BL6J ob/ob mice and increases glucose disappearance in Rhesus monkeys. Journal of Pharmacology and Experimental Therapeutics 1993; 266: 1454-62.
5. Meglasson, MD. Use of 3-Guanidino propionic acid in the treatment of excess adiposity. U.S. Patent 5 1992; 7: 134 & 164.
6. Bailey CJ. Biaguanides and NIDDM. Diabetes care 1992; 15: 755-772.
7. Greenhill JV. Amidines and guanidines in medicinal chemistry. Progress in Medicinal Chemistry 1993; 30: 203-317.
8. Schafer G. On the mechanism of action hypoglycemia producing biguanides. A reevaluation and a molecular theory. Biochemical Pharmacology 1976; 25: 2005-2014.
9. Schafer G. Guanidine and biguanides. In inhibitors of mitochondrial function. Erecinska M, Wilson DF, Eds.; Pergamon Press: Newyork, 1981. p. 165-185.
10. Bergman U, Boman G, Bengt-Eric W. Epidemiology of adverse drug reactions to phenformin and metformin. British Medical Journal 1978; 2: 464-466.
11. Larsen SD, Connell MA, Cudaby MM, Evans BR, May PD, Meglasson MD O'sallivan JJ, Schosstarez HJ, Sih JC, Stevens FC, Tanis SP, Tegley CM, Tucker JA, Vaillancourt VA,Vidmar TJ, Watt W, Yu JH. Synthesis and biological activity of analogues of the antidiabetic/antiobesity agent 3-guanidino propionic acid: Discovery of a novel aminoguaninoacetic acid antidiabetic agent Journal Medicinal Chemistry 2001; 44: 1213-1230.