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# Novel Inhibitors of Neural Nitric Oxide Synthase Based on Inula Ssp. Compounds

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***Abstract*:-Nitric oxide is a signaling molecule. Known as endothelium derived relaxing factor is biosynthesized from l-arginine, oxygen, and nicotinamide adenine dinucleotide phosphate by nitric oxide synthase enzymes. Inhibitors of nitric oxide synthase are thought as neuroprotective agents in traumatic brain injuries. Second derivative data from literature on a series of Inula ssp. compounds with inhibitory activity on nitric oxide production were retrieved. Data were used for further research of nitric oxide synthase inhibitors. Computational strategies were used in order to bring together a hypothesis, further used in screening for pharmacologically active compounds with potential NO production inhibitory ability. A series of compounds resulted classified after docking energies and some drug like pharmacological filters. rnNOS ligands interactions are described. Acceptor groups and carbonyl groups seems to be crucial in inhibiting nNOS.**

*Keywords:- Nitric oxide inhibition, nitric oxide synthase, blood brain barrier.*

## I. INTRODUCTION

Nitric oxide (NO) is involved in several pathologies, particularly in shock excessive NO production [1]. Inhibiting NO in such conditions was recognized improving the outcome[2] . Inhibiting NO production results in an increase in vascular pressure, with diminishing the inflammation. NO is involved in migraine, Parkinson disease and neural acute trauma[3]. In oncology, nitric oxide promotes tumor progression and metastasis[4]. Nitric oxide synthase is the main factor implied in NO production; it is an L-arginine based enzyme. Crystal structure of nitric oxid synthase (NOS) isoforms were consecutively elucidated: endothelial NOS (eNOS), inducible NOS (iNOS), and lastly in 2002 neuronal NOS (nNOS)[5]. NOS isoforms were validated as targets for new drugs, soon after their X-ray crystallography was available. Based on these, the design of effective and selective inhibitors has become an important approach in modern drug discovery involving NO biochemical pathways, related to dysfunctions of the human organism[6] .The objective of this study was to develop novel nNOS inhibitors starting from Inula ssp. compounds witch were tested on RAW264.7. These are macrophage-like cells derived from Balbc mice. They keep many of the properties of macrophages including NO production, phagocytosis (beads, other), extreme sensitivity to TLR agonists and motility. They are susceptible to genetic drift so freezer stocks must be made from early passage number cells[7].

## II. METHODS

To identify new compound with nNOS inhibitory ability, a structural-activity relationship hypothesis was first developed. In this respect, 52 compounds with IC50 between 0.01 – 10.5 µM with inhibitory activity against NO production in LPS-simulated RAW264.7 cells were first considered. Two computational strategies were side by side used:(i) Propose a pharmacophore hypothesis, able to explain majority of activity; this hypothesis was then used for a virtual screening.(ii) Develop a QSAR (Quantitative Structure-Activity Relationship) in order to predict IC50 and further explore structural relations between ligands and their bioactivity. A model using neural network regression was computed. It resulted a model with r=0.966, r2=0.991, p(Speareman rank correlation)=0.990, MSD (mean square deviation)=0.149, RMSD (root mean square deviation)=0.38646, q2(cross validated square)=0.991. Model regression equation is y=0.987IC50 observed+0.090 (point 20.8 = 20.7735 was not detected as an outlier).

Regarding (i), to obtain a robust pharmacophore, other 42 Inula spp. compounds, without NO inhibitory properties, were considered. The concluding pharmacophore was derived using 42 inactive molecules and 52 active (IC50=0.01-10.5 µM) ones. Pharmacophore features considered include hydrophobic centroids, aromatic rings, hydrogen bond acceptors and donors. Screening was performed using a commercially available data base[8].

Pharmacophore hypothesis, generated using 52 active compounds (Table 1) and 42 inactive compounds (Table 2) is shown in Figure 2.

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| Table1 Active compounds; IC50 -half maximal inhibitory concentration(μM); S index-similarity index; arranged in ascending order of S index. | | | |
|---|---|---|---|
| Compounds | | IC50 | S index |
| 1 | OC(C1)C2C(=C)C(=O)OC2C3OC3(C)CCC=C1C | 0.01 | 0 |
| 2 | O=C1OC2CC3(C)C(O)CC(O)C(C)C3CC2C1=C | 0.11 | 12.0513 |
| 3 | O=C1OC2C=C(C)CCC=C(C)C(O)C(O)C2C1=C | 5.6 | 12.4969 |
| 4 | O=C1OC2CC3(C)CCC(=O)C(C)=C3C(O)C2C1=C | 7.9 | 19.4699 |
| 5 | O=C(C1(=C))OC(CC2(C))C1CC=C2CCC(=O)C | 6.9 | 22.3426 |
| 6 | O=C1OC2CC(C)C3C(O)CC(=O)C3(C)CC2C1=C | 9.5 | 25.5101 |
| 7 | O=C(C)CCC1C2(C)CC1CC3CC(=O)OC3C2 | 5.3 | 27.5709 |
| 8 | O=C1OC2CC(=C)C3CCC(O)(C)C3C(O)C2C1=C | 0.11 | 29.0629 |
| 9 | O=C1OC2C=C(C)C3CCC(O)(C)C3C(O)C2C1=C | 0.13 | 29.0802 |
| 10 | O=C1OC2CC3(C)CCCC(C)(O)C3C(O)C2C1=C | 7.9 | 31.3892 |
| 11 | O=C1OC2CC(C)C3C=CC(=O)C3(C)C(O)C2C1=C | 1.1 | 39.3352 |
| 12 | OC1C2C(=C)C(=O)OC2CC(C)C3CCC(=O)C31C | 8.4 | 40.1091 |
| 13 | O=C1OC2CC(C)C3C=CC(=O)C3(C)C(O)C2C1C | 10.5 | 40.6126 |
| 14 | O=C1OC2CC(C)C3CCC4(C)OC34C(O)C2C1=C | 0.11 | 42.1873 |
| 15 | O=C1C=CC2C1(C)C3OC(=O)C(=C)C3C(O)CC2C | 3.5 | 47.3375 |
| 16 | OC1CC2C(=C)C(=O)OC2CC(C)=CCCC1=C | 6.35 | 61.6194 |
| 17 | OC(C1)C2C(=C)C(=O)OC2C=C(C)CCC=C1C | 3.5 | 66.1759 |
| 18 | O=C1OC2C3OC3(C)CCC=C(C)CCC2C1=C | 2.45 | 72.7035 |
| 19 | O=C1OC2CC(C)C(CCC(=O)C)=CC(O)C2C1=C | 7 | 76.7985 |
| 20 | O=C1OC2CC(C)C(CCC(=O)C)=CC(O)C2C1=C | 7.2 | 76.817 |
| 21 | O=C1OC2CC3(C)CCCC(C)C3=CC2=C1CO | 3.2 | 77.8637 |
| 22 | O=C1OC2CC3(C)CC(O)CC(C)=C3CC2C1=C | 5.39 | 81.9833 |
| 23 | O=C1OC2CC(=C)C3CCC(O)(C)C3CC2C1=C | 0.6 | 92.5224 |
| 24 | O=C1OC2C=C(C)C3CCC(O)(C)C3CC2C1=C | 6.3 | 92.7287 |
| 25 | O=C1OC2CC(=C)C3CCC(O)(C)C3CC2C1=C | 8 | 92.8649 |
| 26 | O=C1OC2CC3(C)CCC=C(C)C3C(O)C2C1=C | 7.9 | 101.613 |
| 27 | OCC(C1)=CCCC2(C)OC2C3OC(=O)C(=C)C3C1O | 0.013 | 101.803 |
| 28 | O=C1OC2CC(C)C3CCC(O)C3(C)CC2C1=C | 0.1 | 102.129 |
| 29 | O=C1OC2CC(C)C3CCC(=O)C3(C)CC2C1=C | 9.2 | 103.314 |
| 30 | O=C1OC2CC(C)C3CCC(=O)C3(C)CC2C1=C | 9.8 | 103.37 |
| 31 | O=C1OC2CC(C)C3C=CC(=O)C3(C)CC2C1=C | 8.2 | 103.601 |
| 32 | O=C1OC2CC(C)C3C(OC)CC(=O)C3(C)C(O)C2C1=C | 5.1 | 150.325 |
| 33 | O=C1OC2CC3(C)CCCC(C)C3=CC2C1=C | 7.3 | 162.822 |
| 34 | O=C(O1)C(=C)C2C1CC(C)C3C=CC(=O)C3(C)C2OC(=O)C | 0.46 | 249.564 |
| 35 | O=C(O1)C(=C)C2C1CC(C)C3CCC(=O)C3(C)C2OC(=O)C | 3.9 | 249.983 |
| 36 | O=C1OC2CC(C)C3C(OCC)CC(=O)C3(C)C(O)C2C1=C | 3.8 | 274.82 |
| 37 | O=C1OC2CC(C)C3C(OC(C)=O)CC(O)C3(C)CC2C1=C | 0.25 | 313.36 |
| 38 | O=C1OC2CC(C)C3C(OC(C)=O)CC(O)C3(C)CC2C1=C | 3.5 | 313.38 |
| 39 | O=C1OC2CC(C)=C3C(OC(C)=O)CC(O)(C)C3CC2C1=C | 2.2 | 322.92 |
| 40 | O=C1OC2CC(=C)C3C(OC(C)=O)CC(O)(C)C3CC2C1=C | 9.9 | 323.056 |
| 41 | O=C(C1(=C))OC(C2)C1CC3(C)C(=O)CCC3C2COC(=O)C | 6.6 | 333.059 |
| 42 | O=C(O1)C(=C)C2C1CC(C)C(CCC(=O)C)=CC2OC(C)=O | 6.4 | 396.632 |
| 43 | O=C(O1)C(=C)C2C1CC(C)C3C(OC)CC(=O)C3(C)C2OC(=O)C | 1.5 | 480.777 |
| 44 | O=C(O1)C(=C)C2C1CC(C)C3C(OC(C)=O)CC(O)C3(C)C2OC(=O)C | 0.11 | 783.961 |
| 45 | O=C(C)OC(C1)C2C(C)CC3OC(=O)C(=C)C3C(O)C2(C)C1OC(=O)C | 2.2 | 807.76 |
| 46 | O=C1OC2C=C(C)CCC=C(C)CCC2C1(C3)CC45C(C)CC6OC(=O)C(=C)C6CC5=C(C)C3C4OC(C)=O | 1.59 | 3565.76 |
| 47 | OCCCC(C)C(C(=C)C1)=CC2C1OC(=O)C23CC45C(C)CC6OC(=O)C(=C)C6CC5=C(C)C3C4OC(=O)C | 1.52 | 3696.54 |
| 48 | OC(C1(C2)(C))CCC(C)C1(O)CC3C2OC(=O)C34CC56C(C)CC7OC(=O)C(=C)C7CC5=C(C)C4C6OC(=O)C | 4.1 | 3764.74 |
| 49 | OC(C1(C2)(C))CCC(C)C1(OO)CC3C2OC(=O)C34CC56C(C)CC7OC(=O)C(=C)C7CC5=C(C)C4C6OC(=O)C | 20.8 | 4040.21 |
| 50 | O=C1OC2CC(C)=C(C(C)CCC(O)C)CC2C1(C3)CCC4CC5C(=C)C(=O)OC5CC(C)C34C(C)OC(=O)C | 12 | 4191.84 |
| 51 | O=C1OC2(C)C=3CC4C(=C)C(=O)OC4CC(C)C=3C(OC(C)=O)C2CC1C(=C5)C=CC(CO)=C5CCCCOC(=O)C | 4.5 | 5498.53 |
| 52 | O=C(C(C)C)OC(C1)(C)C(CCCCO)=CC2C1OC(=O)C23CC45C(C)CC6OC(=O)C(=C)C6CC4=C(C)C3C5COC(=O)C | 9.6 | 5860.64 |

| Table2 Inactive compounds. MW- molecular weight . S index-similarity index. Arrange in ascending order in respect to similarity index. | | | |
|---|---|---|---|
| Compounds | | MW | S index |
| 1 | O=C1OC2CC3(C)CCCC(C)=C3CC2C1=C | 232.318 | 0 |
| 2 | O=C1OC2C=C(C)CCCC(C)=CCC2C1=C | 232.318 | 30.15 |
| 3 | O=C(O)C(=C)C1CCC2(C)CCCC(=C)C2C1 | 234.334 | 38.2493 |
| 4 | O=C1OC2CC3(C)CCCC(C)C3CC2C1=C | 234.334 | 4.48171 |
| 5 | O=C1OC2CC3(C)CCCC(C)C3=CC2C1C | 234.334 | 4.59192 |
| 6 | OC1C2C(=C)C(=O)OC2CC(C)=C1C(C)CO | 238.28 | 24.2974 |
| 7 | O=C1OC2CC(=C)C3CCC(O)(C)C3CC2C1=C | 248.317 | 71.4268 |
| 8 | O=C1OC2CC3(C)CCCC4(C)OC43CC2C1=C | 248.317 | 56.6423 |
| 9 | O=C1CCC(C)CC2OC(=O)C(=C)C2CC=C1C | 248.317 | 109.454 |
| 10 | OC12CC3C(=C)C(=O)OC3CC2(C)C(=C)CCC1 | 248.317 | 62.4100 |
| 11 | O=C1OC2CC3(C)CCCC(C)C34OC4C2C1=C | 248.317 | 52.7517 |
| 12 | O=C1OC2CC3(C)CCCC(C)C3=CC2=C1CO | 248.317 | 85.4745 |
| 13 | O=C(C1(=C))OC(C2)C1CC=C2C(C)CCC(C)O | 250.333 | 194.314 |
| 14 | O=C1OC2CC(C)C(CCC(O)C)=CCC2C1=C | 250.333 | 164.608 |
| 15 | O=C1CC2CC3OC3(C)CCC4OC4(C)CC2O1 | 252.306 | 86.7258 |
| 16 | O=C(C)OCC(C)C1=C(C)CCCC1OC(=O)C | 254.322 | 176.044 |
| 17 | OC1C2C(=C)C(=O)OC2CC(C)=C1C(C)COC(=O)C | 280.316 | 340.867 |
| 18 | O=C(C=1)C2=C(O)C=C(O)C=C2OC=1C=3C=C(O)C(O)=CC=3 | 286.236 | 450.48 |
| 19 | O=C(C1)C=2C(O)=CC(OC)=CC=2OC1C=3C=CC(O)=CC=3 | 286.279 | 462.272 |
| 20 | O=C(O1)C(=C)C2C1C=C(C)CCC=C(C)CC2OC(=O)C | 290.354 | 388.531 |
| 21 | OC=1C=C(C)C=CC=1C(C2)(C)OC=3C=C(C)C=CC=3C2(C)C | 296.403 | 502.604 |
| 22 | O=C(C1(O))C=2C(O)=CC(OC)=CC=2OC1C=3C=CC(O)=CC=3 | 302.279 | 555.264 |
| 23 | O=C1OC2CC(=C)C3C(OC(C)=O)CC(O)(C)C3CC2C1=C | 306.354 | 485.076 |
| 24 | O=C1OC2C=C(C)C3C(OC(C)=O)CC(C)(O)C3CC2C1=C | 306.354 | 485.084 |
| 25 | O=C(C1(=C))OC(C=2)C1CC3C(C)(O)CCC3C=2COC(=O)C | 306.354 | 508.831 |
| 26 | O=C(C=1)C2=C(O)C(OC)=C(O)C=C2OC=1C=3C=C(O)C(O)=CC=3 | 316.262 | 709.786 |
| 27 | O=C(C=1(O))C2=C(O)C=C(O)C=C2OC=1C(C=3)=CC=C(O)C=3OC | 316.262 | 678.025 |
| 28 | OC(C1)(C)C23OC3C4C(=C)C(=O)OC4CC(C)C2C1OC(=O)C | 322.353 | 561.653 |
| 30 | O=C(C=1(OC))C2=C(O)C=C(O)C=C2OC=1C(=C3)C=CC(O)=C3OC | 330.289 | 798.709 |
| 31 | O=C(C(C)C)OCC(COC(C(C)C)=O)(O)C1=CC=C(C)C=C1O | 338.395 | 916.648 |
| 32 | O=C(C1(OC(=O)C))C=2C(O)=CC(OC)=CC=2OC1C=3C=CC(O)=CC=3 | 344.315 | 947.263 |
| 33 | O=C(C=1(O))C2=C(O)C(OC)=C(O)C=C2OC=1C(C=3)=CC=C(O)C=3OC | 346.288 | 973.308 |
| 34 | O=C(O1)C(=C)C2C1CC(C)C3C(OCC)CC(=O)C3(C)C2OC(=O)C | 350.406 | 793.172 |
| 35 | O=C(O1)C(=C)C(C2)C1C3OC3(C)CCC=C(C)C2OCCC4(C)OC4C | 362.46 | 1183.6 |
| 36 | O=C(O1)C(=C)C(C2)C1C3OC3(C)CCC=C(C)C2OC(=O)C(C)C(C)O | 364.433 | 1112.36 |
| 37 | OC1C(C)=CC2OC(=O)OC2CC(OC(=O)C(C)=CC)C(C)CC1OC(C)=O | 396.432 | 1463.61 |
| 38 | OC1CCC2C3CCC4C5C(C(=C)C)CCC5(C)CCC4(C)C3CCC2C1(C)C | 398.664 | 1523.09 |
| 39 | OC(C=1)=C(O)C=CC=1C=CC(=O)OC2CC(O)C3(C)CC(O)C(C(C)C)CC3C2=C | 416.507 | 2196.38 |
| 40 | O=C1C2=C(O)C=C(O)C=C2OC(C=3C=CC(O)=C(O)C=3)=C1OC4OC(C)C(O)C(O)C4O | 448.377 | 2237.92 |
| 41 | OC1(O2)C(C)CC2CC(C)(O)C(OC(C(C)CC)=O)C3OC(=O)C(=C)C3C1OC(=O)C(C)=CC | 480.548 | 2468.05 |
| 42 | O=C(O)C(C1)(OC(C=CC=2C=CC(O)=C(O)C=2)=O)CC(O)C(O)C1OC(=O)C=CC(=C3)C=CC(O)=C3O | 516.451 | 4499.49 |

Furthermore, a molecular dynamic statistical study[9] was performed on pharmacophore hypothesis[10] by considering each hypothetical atom as a Carbon atom and representing the particular hypothesis as a C-atom chain. Tinker software package[11] was used in this respect. Molecular dynamics parameters were as follows: step interval 2.0 fs, frame interval 10fs, 10000 steps termination, heat cooling rate 1.000kcal/atom/ps, target temperature 301.15K. Model equation was used to predict IC50 for compounds remaining after the screening using the best suited pharmacophore hypothesis. Descriptors used in the model building are: H -atoms number, O-atoms number, Csp3(3???)- number of sp3 hybridized C-atom, carbonyl- number of carbonyl groups, Et –number of ether groups, Ha-Ha-min – minimal distance between 2 hydrogen acceptor groups, HA-HA-mean- average distance between two hydrogen acceptor groups. Descriptors composing the model were analyzed by Tolerance and Value of Inflation. A tolerance <0.20[12] and a VIF>10[13].Hydrogen acceptor groups and carbonyl groups seems to be crucial in inhibiting nNOS.

| Table 3 Descriptors composing model Tolerance and Value of inflation (VIF) | | | | |
|---|---|---|---|---|
| Nr | Descriptor | r2 | Tolerance(1-r2) | VIF 1/(1-r2) |
| 1 | 2HA min | 0.255814 | 0.744186 | 1.34375 |
| 2 | CO | 0.72975 | 0.27025 | 3.700278 |
| 3 | 2Ha average | 0.769236 | 0.230764 | 4.333432 |
| 4 | Et | 0.894086 | 0.105914 | 9.441622 |
| 5 | O | 0.916796 | 0.083204 | 12.01865 |
| 6 | Csp3 | 0.959513 | 0.040487 | 24.69929 |
| 7 | H | 0.964551 | 0.035449 | 28.20954 |

Several hypotheses were analyzed: a three future hypothesis AAH explains 90% of activity. Pharmacophore is shown in Figure 2;a four future pharmacophore AAHH explains 80% of activity; a five future pharmacophore AAAHH explains 60% of activity.

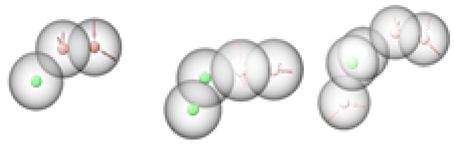

Fig 2:- AAH : H x0.45 y0.16 z 0.11 Ax -0.45 y -0.21 z 2.90 A x -2.06 y -1.50 z 3.89 AAHH : H x -1.06 y 1.44 z -0.80 H x -0.22 y -0.37 z 0.27 A x -1.12 y -0.75 z 3.06 A x -2.73 y -2.03 z 4.06; AAAHH A x -1.11 y 0.97 z -3.32 H x -0.35 y 2.08 z -0.55H x 0.53y 0.31 y 0.26 A x -0.39 y -0.13 z 3.02 A x -1.97 y -1.46 z 3.99; (green-hydrophobic, red – acceptor);

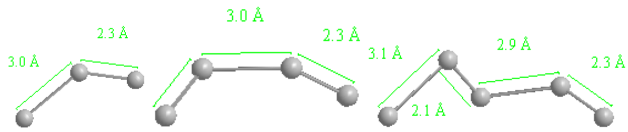


Fig 3:- Pharmacophore hypothesis drawn using Cartesian coordinates: (from a to c) AAH, AAHH and AAAHH hypothesis respectively. AAH distance atoms (2)-(3) 2.288Å,(1)-(2)2.955Å; angle (3)-(2)-(1) 133.899Å. AAHH distance atoms (3)-(4)2.287Å, (2)-(3)2.956Å, (1)-(2)2.264Å; angle(4)-(3)-(2)134.343Å, (3)-(2)-(1)115.838Å; dihedral angle (1)-(2)-(3)-(4)97.880Å. AAAHH distance atoms (4)-(5)2.282Å, (3)-(4)2.942Å, (2)-(3)2.136Å, (1)-(2)3.079Å; angle(5)-(4)-(3)134.624Å, (4)-(3)-(2)110.535Å, (3)-(2)-(1)98.284Å; dihedral angle (2)-(3)-(4)-(5)99.718Å, (1)-(2)-(3)-(4)-135.606Å.

Concerning (ii), a similarity score based selection was performed. Only active compounds were considered. Data set was divided into a training set and a test set. Molecules were

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ordered by the similarity score using as similarity template OC(C1)C2C(=C)C(=O)OC2C3OC3(C)CCC=C1 having the lowest $IC_{50}$( 0.01 μM );the first 26 results were taken, in $IC_{50}$ ascending order. Model was built using multiple linear regression[12] (MLR). Descriptors used to build the model were: H, C, N, O,P,S atoms, molecular weight (MW), total number of atoms, number of heavy atoms, number of rotational bounds, number of hydrogen donor groups (HD), number of hydrogen accepting groups (HA), number of rings, minimal distance between two hydrogen donor groups, maximal distance between two hydrogen donor groups, minimal distance between two hydrogen accepting groups, maximal distance between two hydrogen accepting groups, aromaticity (Aro). In order to correlate model data with nNOS, docking was performed; the binding energy (kcal/mol) nNOS-ligand was introduced in the QSAR model. A further selection was done to keep the proper descriptors. Model was internally validated by the leave-one-out technique and externally, using the test set data. The descriptors used in the model were finally selected by evaluating their variance, tolerance and interrelationship.

The result on $IC_{50}$% explanation by MD pharmacophore AAHH hypothesis was used for screening (over 11.000 hits) in a data base applying the filters discussed in Methods section. Resulted screening compounds were classified after docking energies (see Table 5) as discussed in the Methods section. The best 42 compounds binding energies were discussed (the remaining data are shown in Supplementary materials).

A second screening was performed applying the pharmacological filters: molecular weight (390-420g/mol), partition coefficient xlogP (1-3), number of rotatable bounds (4-6), hydrogen bound donors (2-4), hydrogen bond acceptors (4-6). Filters characterizing solvent interaction were also used: apolar desolvation (0-10 kcal/mol) and polar desolvation(-40-0 kcal/mol). Membrane permeation was also considered: polar surface area was limited at 60-80 Å2 and the net charge of a molecule was set zero. The $IC_{50}$ of remaining 21 molecules was then predicted by a QSAR model.

## III. RESULTS

Pharmacophore hypothesis, generated using 52 active compounds (Table 1) and 42 inactive compounds (Table 2) is shown in Figure 2.

Table1 Active compounds; IC50 -half maximal inhibitory concentration(μM); S index-similarity index; arranged in ascending order of S index.

| Compounds | | **IC50** | **S** index |
|---|---|---|---|
| 1 | OC(C1)C2C(=C)C(=O)OC2C3OC3(C)CCC=C1C | 0.01 | 0 |
| 2 | O=C1OC2CC3(C)C(O)CC(O)C(C)C3CC2C1=C | 0.11 | 12.0513 |
| 3 | O=C1OC2C=C(C)CCC=C(C)C(O)C(O)C2C1=C | 5.6 | 12.4969 |
| 4 | O=C1OC2CC3(C)CCC(=O)C(C)=C3C(O)C2C1=C | 7.9 | 19.4699 |
| 5 | O=C(C1(=C))OC(CC2(C))C1CC=C2CCC(=O)C | 6.9 | 22.3426 |
| 6 | O=C1OC2CC(C)C3C(O)CC(=O)C3(C)CC2C1=C | 9.5 | 25.5101 |
| 7 | O=C(C)CCC1C2(C)CC1CC3CC(=O)OC3C2 | 5.3 | 27.5709 |
| 8 | O=C1OC2CC(=C)C3CCC(O)(C)C3C(O)C2C1=C | 0.11 | 29.0629 |
| 9 | O=C1OC2C=C(C)C3CCC(O)(C)C3C(O)C2C1=C | 0.13 | 29.0802 |
| 10 | O=C1OC2CC3(C)CCCC(C)(O)C3C(O)C2C1=C | 7.9 | 31.3892 |
| 11 | O=C1OC2CC(C)C3C=CC(=O)C3(C)C(O)C2C1=C | 1.1 | 39.3352 |
| 12 | OC1C2C(=C)C(=O)OC2CC(C)C3CCC(=O)C31C | 8.4 | 40.1091 |
| 13 | O=C1OC2CC(C)C3C=CC(=O)C3(C)C(O)C2C1C | 10.5 | 40.6126 |
| 14 | O=C1OC2CC(C)C3CCC4(C)OC34C(O)C2C1=C | 0.11 | 42.1873 |
| 15 | O=C1C=CC2C1(C)C3OC(=O)C(=C)C3C(O)CC2C | 3.5 | 47.3375 |
| 16 | OC1CC2C(=C)C(=O)OC2CC(C)=CCCC1=C | 6.35 | 61.6194 |
| 17 | OC(C1)C2C(=C)C(=O)OC2C=C(C)CCC=C1C | 3.5 | 66.1759 |
| 18 | O=C1OC2C3OC3(C)CCC=C(C)CCC2C1=C | 2.45 | 72.7035 |
| 19 | O=C1OC2CC(C)C(CCC(=O)C)=CC(O)C2C1=C | 7 | 76.7985 |
| 20 | O=C1OC2CC(C)C(CCC(=O)C)=CC(O)C2C1=C | 7.2 | 76.817 |
| 21 | O=C1OC2CC3(C)CCCC(C)C3=CC2=C1CO | 3.2 | 77.8637 |
| 22 | O=C1OC2CC3(C)CC(O)CC(C)=C3CC2C1=C | 5.39 | 81.9833 |
| 23 | O=C1OC2CC(=C)C3CCC(O)(C)C3CC2C1=C | 0.6 | 92.5224 |
| 24 | O=C1OC2C=C(C)C3CCC(O)(C)C3CC2C1=C | 6.3 | 92.7287 |
| 25 | O=C1OC2CC(=C)C3CCC(O)(C)C3CC2C1=C | 8 | 92.8649 |
| 26 | O=C1OC2CC3(C)CCC=C(C)C3C(O)C2C1=C | 7.9 | 101.613 |
| 27 | OCC(C1)=CCCC2(C)OC2C3OC(=O)C(=C)C3C1O | 0.013 | 101.803 |
| 28 | O=C1OC2CC(C)C3CCC(O)C3(C)CC2C1=C | 0.1 | 102.129 |
| 29 | O=C1OC2CC(C)C3CCC(=O)C3(C)CC2C1=C | 9.2 | 103.314 |
| 30 | O=C1OC2CC(C)C3CCC(=O)C3(C)CC2C1=C | 9.8 | 103.37 |
| 31 | O=C1OC2CC(C)C3C=CC(=O)C3(C)CC2C1=C | 8.2 | 103.601 |
| 32 | O=C1OC2CC(C)C3C(OC)CC(=O)C3(C)C(O)C2C1=C | 5.1 | 150.325 |
| 33 | O=C1OC2CC3(C)CCCC(C)C3=CC2C1=C | 7.3 | 162.822 |
| 34 | O=C(O1)C(=C)C2C1CC(C)C3C=CC(=O)C3(C)C2OC(=O)C | 0.46 | 249.564 |
| 35 | O=C(O1)C(=C)C2C1CC(C)C3CCC(=O)C3(C)C2OC(=O)C | 3.9 | 249.983 |
| 36 | O=C1OC2CC(C)C3C(OCC)CC(=O)C3(C)C(O)C2C1=C | 3.8 | 274.82 |
| 37 | O=C1OC2CC(C)C3C(OC(C)=O)CC(O)C3(C)CC2C1=C | 0.25 | 313.36 |
| 38 | O=C1OC2CC(C)C3C(OC(C)=O)CC(O)C3(C)CC2C1=C | 3.5 | 313.38 |
| 39 | O=C1OC2CC(C)=C3C(OC(C)=O)CC(O)(C)C3CC2C1=C | 2.2 | 322.92 |
| 40 | O=C1OC2CC(=C)C3C(OC(C)=O)CC(O)(C)C3CC2C1=C | 9.9 | 323.056 |
| 41 | O=C(C1(=C))OC(C2)C1CC3(C)C(=O)CCC3C2COC(=O)C | 6.6 | 333.059 |
| 42 | O=C(O1)C(=C)C2C1CC(C)C(CCC(=O)C)=CC2OC(C)=O | 6.4 | 396.632 |
| 43 | O=C(O1)C(=C)C2C1CC(C)C3C(OC)CC(=O)C3(C)C2OC(=O)C | 1.5 | 480.777 |
| 44 | O=C(O1)C(=C)C2C1CC(C)C3C(OC(C)=O)CC(O)C3(C)C2OC(=O)C | 0.11 | 783.961 |
| 45 | O=C(C)OC(C1)C2C(C)CC3OC(=O)C(=C)C3C(O)C2(C)C1OC(=O)C | 2.2 | 807.76 |
| 46 | O=C1OC2C=C(C)CCC=C(C)CCC2C1(C3)CC45C(C)CC6OC(=O)C(=C)C6CC5=C(C)C3C4OC(C)=O | 1.59 | 3565.76 |
| 47 | OCCCC(C)C(C(=C)C1)=CC2C1OC(=O)C23CC45C(C)CC6OC(=O)C(=C)C6CC5=C(C)C3C4OC(=O)C | 1.52 | 3696.54 |
| 48 | OC(C1(C2)(C))CCC(C)C1(O)CC3C2OC(=O)C34CC56C(C)CC7OC(=O)C(=C)C7CC5=C(C)C4C6OC(=O)C | 4.1 | 3764.74 |
| 49 | OC(C1(C2)(C))CCC(C)C1(OO)CC3C2OC(=O)C34CC56C(C)CC7OC(=O)C(=C)C7CC5=C(C)C4C6OC(=O)C | 20.8 | 4040.21 |
| 50 | O=C1OC2CC(C)=C(C(C)CCC(O)C)CC2C1(C3)CCC4CC5C(=C)C(=O)OC5CC(C)C34C(C)OC(=O)C | 12 | 4191.84 |
| 51 | O=C1OC2(C)C=3CC4C(=C)C(=O)OC4CC(C)C=3C(OC(C)=O)C2CC1C(=C5)C=CC(CO)=C5CCCCOC(=O)C | 4.5 | 5498.53 |
| 52 | O=C(C(C)C)OC(C1)(C)C(CCCCO)=CC2C1OC(=O)C23CC45C(C)CC6OC(=O)C(=C)C6CC4=C(C)C3C5COC(=O)C | 9.6 | 5860.64 |

Table2 Inactive compounds. MW- molecular weight . S index-similarity index. Arrange in ascending order in respect to similarity index.

| Compounds | | MW | S index |
|---|---|---|---|
| 1 | O=C1OC2CC3(C)CCCC(C)=C3CC2C1=C | 232.318 | 0 |
| 2 | O=C1OC2C=C(C)CCCC(C)=CCC2C1=C | 232.318 | 30.15 |
| 3 | O=C(O)C(=C)C1CCC2(C)CCCC(=C)C2C1 | 234.334 | 38.2493 |
| 4 | O=C1OC2CC3(C)CCCC(C)C3CC2C1=C | 234.334 | 4.48171 |
| 5 | O=C1OC2CC3(C)CCCC(C)C3=CC2C1C | 234.334 | 4.59192 |
| 6 | OC1C2C(=C)C(=O)OC2CC(C)=C1C(C)CO | 238.28 | 24.2974 |
| 7 | O=C1OC2CC(=C)C3CCC(O)(C)C3CC2C1=C | 248.317 | 71.4268 |
| 8 | O=C1OC2CC3(C)CCCC4(C)OC43CC2C1=C | 248.317 | 56.6423 |
| 9 | O=C1CCC(C)CC2OC(=O)C(=C)C2CC=C1C | 248.317 | 109.454 |
| 10 | OC12CC3C(=C)C(=O)OC3CC2(C)C(=C)CCC1 | 248.317 | 62.4100 |
| 11 | O=C1OC2CC3(C)CCCC(C)C34OC4C2C1=C | 248.317 | 52.7517 |
| 12 | O=C1OC2CC3(C)CCCC(C)C3=CC2=C1CO | 248.317 | 85.4745 |
| 13 | O=C(C1(=C))OC(C2)C1CC=C2C(C)CCC(C)O | 250.333 | 194.314 |
| 14 | O=C1OC2CC(C)C(CCC(O)C)=CCC2C1=C | 250.333 | 164.608 |
| 15 | O=C1CC2CC3OC3(C)CCC4OC4(C)CC2O1 | 252.306 | 86.7258 |
| 16 | O=C(C)OCC(C)C1=C(C)CCCC1OC(=O)C | 254.322 | 176.044 |
| 17 | OC1C2C(=C)C(=O)OC2CC(C)=C1C(C)COC(=O)C | 280.316 | 340.867 |
| 18 | O=C(C=1)C2=C(O)C=C(O)C=C2OC=1C=3C=C(O)C(O)=CC=3 | 286.236 | 450.48 |
| 19 | O=C(C1)C=2C(O)=CC(OC)=CC=2OC1C=3C=CC(O)=CC=3 | 286.279 | 462.272 |
| 20 | O=C(O1)C(=C)C2C1C=C(C)CCC=C(C)CC2OC(=O)C | 290.354 | 388.531 |
| 21 | OC=1C=C(C)C=CC=1C(C2)(C)OC=3C=C(C)C=CC=3C2(C)C | 296.403 | 502.604 |
| 22 | O=C(C1(O))C=2C(O)=CC(OC)=CC=2OC1C=3C=CC(O)=CC=3 | 302.279 | 555.264 |
| 23 | O=C1OC2CC(=C)C3C(OC(C)=O)CC(O)(C)C3CC2C1=C | 306.354 | 485.076 |
| 24 | O=C1OC2C=C(C)C3C(OC(C)=O)CC(C)(O)C3CC2C1=C | 306.354 | 485.084 |
| 25 | O=C(C1(=C))OC(C=2)C1CC3C(C)(O)CCC3C=2COC(=O)C | 306.354 | 508.831 |
| 26 | O=C(C=1)C2=C(O)C(OC)=C(O)C=C2OC=1C=3C=C(O)C(O)=CC=3 | 316.262 | 709.786 |
| 27 | O=C(C=1(O))C2=C(O)C=C(O)C=C2OC=1C(C=3)=CC=C(O)C=3OC | 316.262 | 678.025 |
| 28 | OC(C1)(C)C23OC3C4C(=C)C(=O)OC4CC(C)C2C1OC(=O)C | 322.353 | 561.653 |
| 30 | O=C(C=1(OC))C2=C(O)C=C(O)C=C2OC=1C(=C3)C=CC(O)=C3OC | 330.289 | 798.709 |
| 31 | O=C(C(C)C)OCC(COC(C(C)C)=O)(O)C1=CC=C(C)C=C1O | 338.395 | 916.648 |
| 32 | O=C(C1(OC(=O)C))C=2C(O)=CC(OC)=CC=2OC1C=3C=CC(O)=CC=3 | 344.315 | 947.263 |
| 33 | O=C(C=1(O))C2=C(O)C(OC)=C(O)C=C2OC=1C(C=3)=CC=C(O)C=3OC | 346.288 | 973.308 |
| 34 | O=C(O1)C(=C)C2C1CC(C)C3C(OCC)CC(=O)C3(C)C2OC(=O)C | 350.406 | 793.172 |
| 35 | O=C(O1)C(=C)C(C2)C1C3OC3(C)CCC=C(C)C2OCCC4(C)OC4C | 362.46 | 1183.6 |
| 36 | O=C(O1)C(=C)C(C2)C1C3OC3(C)CCC=C(C)C2OC(=O)C(C)C(C)O | 364.433 | 1112.36 |
| 37 | OC1C(C)=CC2OC(=O)OC2CC(OC(=O)C(C)=CC)C(C)CC1OC(C)=O | 396.432 | 1463.61 |
| 38 | OC1CCC2C3CCC4C5C(C(=C)C)CCC5(C)CCC4(C)C3CCC2C1(C)C | 398.664 | 1523.09 |
| 39 | OC(C=1)=C(O)C=CC=1C=CC(=O)OC2CC(O)C3(C)CC(O)C(C(C)C)CC3C2=C | 416.507 | 2196.38 |
| 40 | O=C1C2=C(O)C=C(O)C=C2OC(C=3C=CC(O)=C(O)C=3)=C1OC4OC(C)C(O)C(O)C4O | 448.377 | 2237.92 |
| 41 | OC1(O2)C(C)CC2CC(C)(O)C(OC(C(C)CC)=O)C3OC(=O)C(=C)C3C1OC(=O)C(C)=CC | 480.548 | 2468.05 |
| 42 | O=C(O)C(C1)(OC(C=CC=2C=CC(O)=C(O)C=2)=O)CC(O)C(O)C1OC(=O)C=CC(=C3)C=CC(O)=C3O | 516.451 | 4499.49 |

QSAR model was computed. Descriptors used for building the model were discussed in the Method section. In order to improve the model, principal components were computed for each descriptor. A model using neural network regression was computed. It resulted a model with r=0.966, $r^2$=0.991, p(Speareman rank correlation)=0.990, MSD (mean square deviation)=0.149, RMSD ( root mean square

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deviation)=0.38646, $q^2$(cross validated square)=0.991. Model regression equation is y=0.987$IC_{50}$ observed+0.090 (point 20.8 = 20.7735 was not detected as an outlier).

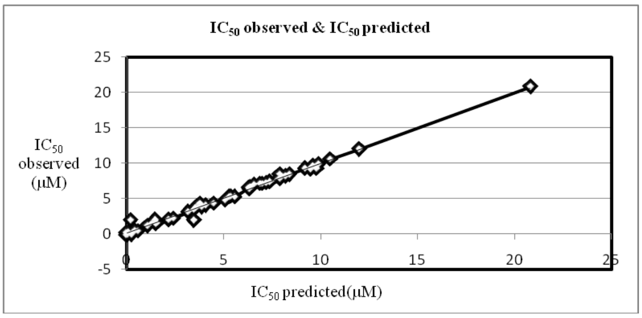



Fig 4:- Plot representing correlation between observed and predicted $IC_{50}$ values.

Model equation was used to predict $IC_{50}$ for compounds remaining after the screening using the best suited pharmacophore hypothesis. Descriptors used in the model building are: H -atoms number, O-atoms number, Csp3-number of $sp^3$ hybridized C-atom, carbonyl- number of carbonyl groups, Et –number of ether groups, Ha-Ha-min – minimal distance between 2 hydrogen acceptor groups, HA-HA-mean- average distance between two hydrogen acceptor groups. Descriptors composing the model were analyzed by Tolerance and Value of Inflation. A tolerance <0.20[12] and a VIF>10[13].Hydrogen acceptor groups and carbonyl groups seems to be crucial in inhibiting nNOS.

Table 3 Descriptors composing model Tolerance and Value of inflation (VIF)

| Nr | Descriptor | r2 | Tolerance(1-r2) | VIF 1/(1-r2) |
|---|---|---|---|---|
| 1 | 2HA min | 0.255814 | 0.744186 | 1.34375 |
| 2 | CO | 0.72975 | 0.27025 | 3.700278 |
| 3 | 2Ha average | 0.769236 | 0.230764 | 4.333432 |
| 4 | Et | 0.894086 | 0.105914 | 9.441622 |
| 5 | O | 0.916796 | 0.083204 | 12.01865 |
| 6 | Csp3 | 0.959513 | 0.040487 | 24.69929 |
| 7 | H | 0.964551 | 0.035449 | 28.20954 |

Several hypotheses were analyzed: a three future hypothesis AAH explains 90% of activity. Pharmacophore is shown in Figure 2;a four future pharmacophore AAHH explains 80% of activity; a five future pharmacophore AAAHH explains 60% of activity.

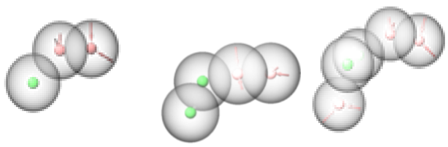


Fig 2:- AAH : H x0.45 y0.16 z 0.11 Ax -0.45 y -0.21 z 2.90 A x -2.06 y -1.50 z 3.89 AAHH : H x -1.06 y 1.44 z -0.80 H x -0.22 y -0.37 z 0.27 A x -1.12 y -0.75 z 3.06 A x -2.73 y -2.03 z 4.06; AAAHH A x -1.11 y 0.97 z -3.32 H x -0.35 y 2.08 z -0.55H x 0.53y 0.31 y 0.26 A x -0.39 y -0.13 z 3.02 A x -1.97 y -1.46 z 3.99; (green-hydrophobic, red – acceptor);

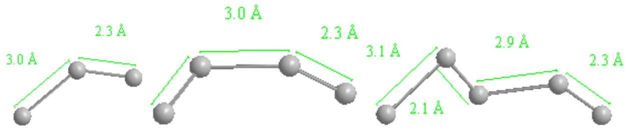



Fig 3:-Pharmacophore hypothesis drawn using Cartesian coordinates: (from a to c) AAH, AAHH and AAAHH hypothesis respectively. AAH distance atoms (2)-(3)2.288Å,(1)-(2)2.955Å; angle (3)-(2)-(1) 133.899Å. AAHH distance atoms (3)-(4)2.287Å, (2)-(3)2.956Å, (1)-(2)2.264Å; angle(4)-(3)-(2)134.343Å, (3)-(2)-(1)115.838Å; dihedral angle (1)-(2)-(3)-(4)97.880Å. AAAHH distance atoms (4)-(5)2.282Å, (3)-(4)2.942Å, (2)-(3)2.136Å, (1)-(2)3.079Å;angle(5)-(4)-(3)134.624Å,(4)-(3)-(2)110.535Å,(3)-(2)-(1)98.284Å; dihedral angle (2)-(3)-(4)-(5)99.718Å, (1)-(2)-(3)-(4)-135.606Å.

MD analysis[14] of all the three pharmacophores showed that hypothesis AAHH has the smallest SD values for three points (Table 3: AAH 5.444, AAHH 5.053, AAAHH 5.106). Lowest SD correlates with low variability i.e low spatial geometric variability. Low geometric variability results in pharmacophore futures-properties conservation.

Table 4Pharmacophore hypothesis

| Pharmacophore | Measurement | min | max | average | SD |
|---|---|---|---|---|---|
| AAH | C(2)-C(3) | 1.426 | 1.678 | 1.542 | 0.037 |
| | C(1)-C(2) | 1.428 | 1.680 | 1.542 | 0.037 |
| | C(1)-C(2)-C(3) | 99.222 | 127.143 | 112.520 | 5.444 |
| AAHH | C(3)-C(4) | 1.434 | 1.681 | 1.543 | 0.037 |
| | C(3)-C(2) | 1.424 | 1.700 | 1.547 | 0.040 |
| | C(2)-C(1) | 1.429 | 1.706 | 1.542 | 0.037 |
| | C(2)-C(3)-C(4) | 98.481 | 127.474 | 113.720 | 4.935 |
| | C(3)-C(2)-C(1) | 99.455 | 128.729 | 113.675 | 5.053 |
| | C(4)-C(3)-C(2)-C(1) | -179.665 | 179.992 | 58.176 | 62.629 |
| AAAHH | C(5)-C(4) | 1.441 | 1.667 | 1.542 | 0.033 |
| | C(4)-C(3) | 1.440 | 1.696 | 1.546 | 0.035 |
| | C(2)-C(3) | 1.449 | 1.677 | 1.546 | 0.034 |
| | C(2)-C(1) | 1.438 | 1.672 | 1.542 | 0.035 |
| | C(5)-C(4)-C(3) | 99.454 | 130.096 | 114.005 | 5.403 |
| | C(2)-C(3)-C(4) | 98.784 | 130.474 | 115.209 | 5.106 |
| | C(3)-C(2)-C(1) | 97.764 | 130.462 | 113.657 | 5.176 |

Docking site for 5VUX is represented in Figure 4.Two symmetrical potential binding sites corresponding to chain A and B were detected (binding site bs-1, with a volume of 662.016 $Å^3$and surface of 1342.72$Å^2$ and bs-2, with a volume of 171.520 $Å^3$ and a surface of 1176.32$Å^2$, respectively). The Cartesian coordinates are: for bs-1, a cube with origin at x =127.56 Å; y=250.39 Å and z=356.59 Å; cube side=40Å; for bs-2, a cube centered at x=107.00Å; y= 245.40Å; z=327.61Å; cube side=40Å. Coordinates are discussed for 5VUX chain A.

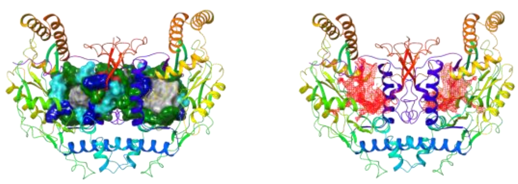


Fig 4:-Binding site of 5VUX corresponding to A and B chains: left- binding site colored by potential energy (chain B is represented as ball and stick); right- same binding site without protein chains showing only H2o atoms trapped in the binding surface.

Binding site 1 was considered for docking due to its larger size and correspondence with 7-(((4-(Dimethylamino)benzyl)amino)methyl)quinolin-2-amine binding site.

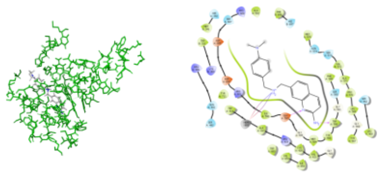


Fig 5:-(a) Human neuronal nitric synthase (nNOS) represented asball and sticks, in element colors, in the complex with 7-(((4-(Dimethylamino) benzyl) amino) methyl) quinolin-2-aminerepresented as space filling,in element colors; (b) 7-(((4-(Dimethylamino) benzyl) amino) methyl) quinolin-2-amine interaction with amino acids at nNOS binding site- (two hydrogen bounds are represented: GLI 597 and N atom; TRP 502 and $NH_2$ group; a salt bridge is also detected between $NH^{+}_{2}$ and HEM 801.

12 best screened complexes ligand-5VUX are discussed below in figure 6

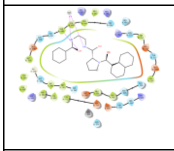
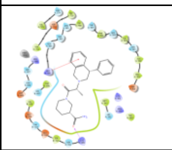
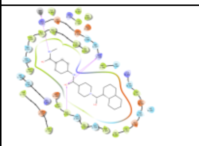
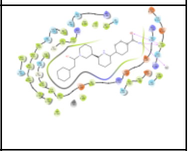
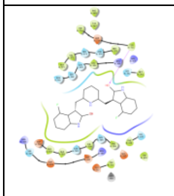
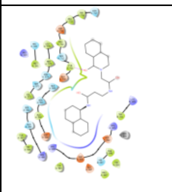
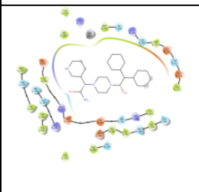
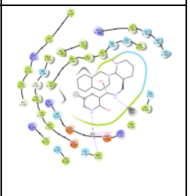
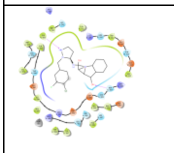
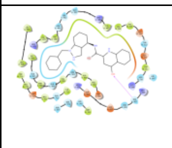
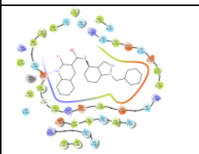
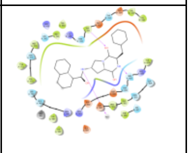
| | | | |
|---|---|---|---|
|  |  |  |  |
| 1. | 2. | 3. | 4. |
|  |  |  |  |
| 5. | 6. | 7. | 8. |
|  |  |  |  |
| 9. | 10. | 11. | 12. |

Fig 6:-First 12 compounds are shown docked at 5VUX binding site;hydrogen bounds are shown in pink;a 4Å off was used;1 H bond between Phe 696 and OH group, 2 H bound between Arg 486 and aromatic ring, H bonding Lys 309 and O; 3 H bound between Hem 801 and Nh group, Arg 608 and OH group, Arg 608 and NH, Ala 502 and OH ; 4 H bound between Trp 311 and OH, Trp 311 and NH2; 5 H bound between Tyr 711 and OH; 6 H bound between Tyr 711 and OH group; 7 no H bound detected computationally; 8 H bound between Hem 801 and NH, Val 572 and NH; 9 H bound between Trp 683 and NH, Ser 607 and OH; 10 H bound between Ser 607 and OH; 11 H bound between Hem 801 and NH; 12 H bound between Ser 607 and NH, Asp 500 and NH, Trp 311 and OH.

**Table 5 Screening results of the compounds Ba-computed binding affinity, IC50 predicted –predicted values for IC50 by the QSAR model.**

| Compounds | | Ba | IC50 predicted |
|---|---|---|---|
| 1 | c1ccc(cc1)C(=O)NCCNC(=O)[C@H]2CCCN2C(=O)c3cccc4c3cccc4 | -12.952 | -1.47503 |
| 2 | C[C@H](C(=O)N1CCC[C@H](C1)C(=O)N)N2Cc3ccccc3[C@@H](C2)c4ccccc4 | -12.655 | -1.47563 |
| 3 | CNC(=O)c1ccc(cc1)NC(=O)C2CCN(CC2)C(=O)c3cccc4c3cccc4 | -12.448 | -1.47583 |
| 4 | c1ccc(cc1)CC(=O)N2CCC[C@H](C2)c3cccc(n3)c4ccc(cc4)C(=O)N | -12.355 | -1.47603 |
| 5 | c1cc(nc(c1)C[C@H]2c3c(cccc3F)NC2=O)C[C@@H]4c5c(cccc5F)NC4=O | -12.336 | -1.47623 |
| 6 | c1ccc2c(c1)ccn(c2=O)CC(=O)NCCC(=O)N[C@@H]3CCCc4c3cccc4 | -12.287 | -1.47629 |
| 7 | c1ccc(cc1)C(c2ccccc2)C(=O)N3CCN(CC3)[C@@H](c4cccnc4)C(=O)N | -12.235 | -1.47689 |
| 8 | c1ccc2c(c1)CCN(C2)c3c(cccn3)CNC(=O)c4cc(c[nH]c4=O)Cl | -12.183 | -1.47745 |
| 9 | c1ccc2c(c1)c(=O)cc([nH]2)C(=O)Nc3ccnn3Cc4ccc(c(c4)F)Cl | -12.172 | -1.47802 |
| 10 | c1ccc(cc1)Cn2c3c(cn2)[C@@H](CCC3)NC(=O)c4cc(=O)c5ccccc5[nH]4 | -12.153 | -1.47587 |
| 11 | c1ccc(cc1)Cn2c3c(cn2)[C@@H](CCC3)NC(=O)c4cc5ccccc5[nH]c4=O | -12.15 | -0.36312 |
| 12 | c1ccc(cc1)C[C@@H]2C(=O)N3C[C@H](C[C@H]3C(=O)N2)NC(=O)c4cccc5c4cccc5 | -12.074 | -0.3632 |
| 13 | 5 | -12.067 | -0.36357 |
| 14 | c1ccc(cc1)CC(=O)N2CCC[C@H](C2)c3cccc(n3)c4cccc(c4)C(=O)N | -12.06 | -0.3639 |
| 15 | c1c(cc(c2c1C[C@H](O2)CNC(=O)[C@@H]3CCC(=O)N3)C4=CC5=CC=N[C@H]5C=C4)Cl | -12.057 | -0.36402 |
| 16 | Cc1cccc(c1C)NC(=O)CN2CCN(CC2)C(=O)c3cc4cc(ccc4[nH]3)F | -12.045 | -0.36438 |
| 17 | c1ccc(cc1)C(c2ccccc2)N3CCN(CC3)C(=O)c4ccc(cc4)C(=O)N | -12.044 | -0.36539 |
| 18 | c1cc(cc(c1)C(F)(F)F)[C@@H]2C[C@H]2NC(=O)N[C@@H]3CCCc4c3ccc(=O)[nH]4 | -11.992 | -0.36589 |
| 19 | C[C@@H](C(=O)N1CCC[C@H](C1)C(=O)N)N2Cc3ccccc3[C@@H](C2)c4ccccc4 | -11.968 | -0.36609 |
| 20 | c1ccc2c(c1)c(=O)c(c[nH]2)C(=O)Nc3ccnn3Cc4ccc(c(c4)F)Cl | -11.96 | 18.4646 |
| 21 | Cc1cc(c2ccccc2n1)C(=O)NCc3ccc(c(c3)F)N4CCC(CC4)O | -11.949 | 18.4683 |
| 22 | CC(=O)Nc1cccc(c1)NC(=O)[C@@H]2CCCN(C2)C(=O)c3cccc4c3cccc4 | -11.948 | 18.4697 |
| 23 | Cc1ccc(cc1)[C@H](CNC(=O)c2cc(=O)c3ccccc3[nH]2)N4CCOCC4 | -11.943 | 18.46702 |
| 24 | c1ccc2c(c1)c(c[nH]2)[C@@H]3c4ccccc4C(=O)N3CCC(=O)Nc5cccnc5 | -11.938 | 18.46734 |
| 25 | Cc1cccc(c1)c2c(cc3cc4c(cc3n2)CCC4)CN5CCO[C@H](C5)C(=O)N | -11.931 | 18.46756 |
| 26 | c1c(cc(c2c1C[C@H](O2)CNC(=O)[C@@H]3CCC(=O)N3)C4=CC5=CC=N[C@@H]5C=C4)Cl | -11.928 | 18.46789 |
| 27 | c1ccc(cc1)c2ccc3c(c[nH]c3n2)C(=O)N4CC[C@@H](C4)NC(=O)c5ccccc5 | -11.913 | 18.46812 |
| 28 | c1ccc2c(c1)cc(c(n2)[C@H]3CCCN(C3)C(=O)Cc4cccc(c4)F)C(=O)N | -11.91 | 18.46833 |
| 29 | Cc1cccc(c1)c2c(cc3cc4c(cc3n2)CCC4)CN5CCO[C@@H](C5)C(=O)N | -11.889 | 18.46853 |
| 30 | c1ccc(c(c1)CC(=O)N2CCN(CC2)c3cc(c4ccccc4n3)C(=O)N)F | -11.888 | 18.46879 |
| 31 | c1cc(cc(c1)c2cccnc2)C[C@]3(CCCN(C3)C(=O)c4cccc(c4)F)C(=O)N | -11.885 | 18.4689 |
| 32 | c1ccc(cc1)c2c3c(c([nH]2)C(=O)Nc4ccc(cc4)C(=O)N5CCOCC5)CCC3 | -11.874 | 18.46902 |
| 33 | c1ccc2c(c1)[C@@H](CCO2)NC(=O)c3cc4n(n3)[C@@H](C[C@@H](N4)C5CC5)C(F)(F)F | -11.859 | 20.7806 |
| 34 | c1ccc2c(c1)c(c[nH]2)CCNC(=O)C3CCN(CC3)C(=O)c4ccc(cc4F)F | -11.852 | 20.7816 |
| 35 | C[C@@H](C(=O)N1CCC[C@@H](C1)C(=O)N)N2Cc3ccccc3[C@@H](C2)c4ccccc4 | -11.842 | 20.7823 |
| 36 | CC(=O)Nc1ccc(c(c1)Cl)NC(=O)[C@H]2CCCN2C(=O)Cc3ccccc3 | -11.839 | 20.7837 |
| 37 | c1ccc(cc1)C(=O)NCCNC(=O)[C@H]2CCCN2C(=O)c3ccc4ccccc4c3 | -11.838 | 20.7856 |
| 38 | c1ccc(cc1)[C@H](CNC(=O)N[C@@H]2CCc3c2cccc3)C(=O)N4CCOCC4 | -11.836 | 20.7889 |
| 39 | Cc1ccc(cc1)[C@H](CNC(=O)c2cc3c([nH]c2=O)CCCC3)N4CCOCC4 | -11.822 | 20.7902 |
| 40 | c1ccc(cc1)C[C@@H]2C(=O)N3C[C@H](C[C@H]3C(=O)N2)NC(=O)c4cc(c(cc4F)F)F | -11.82 | 20.7946 |
| 41 | c1ccc(cc1)CC(=O)N2CCC[C@@H](C2)c3cccc(n3)c4cccc(c4)C(=O)N | -11.82 | 20.797 |
| 42 | c1cc2c3c(c1)[C@@H](C[C@H]3CCC2)NC(=O)Cc4csc(n4)N5CCCNC5=O | -11.818 | 20.7976 |

Compounds similarity score for active inactive and screening resulted compounds is represented in figure 7. At „ efficent'' $IC_{50}$ it is notice that inactive and active compounds have similar structures.

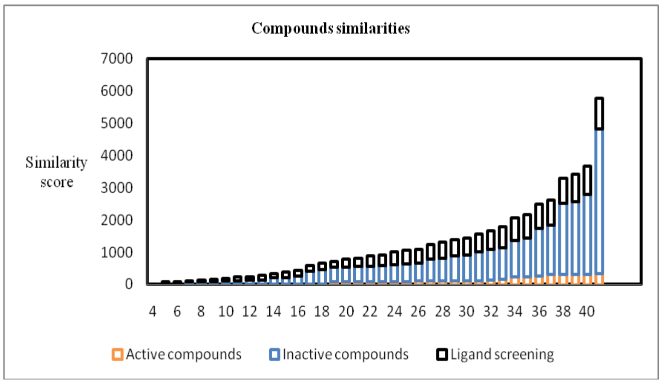



Fig 7:- Similarity scatter plot for active, inactive and screening resulted compounds.

## IV. CONCLUSION

Based on *Inula* ssp. compounds, a valid pharmacophore hypothesis was computed. Novel compounds with potential inhibitory properties on Human neuronal nitric synthase were found. Neural network regression based on *Inula* ssp. compounds predicted $IC_{50}$ of the new screening resulted compounds. Hydrogen acceptor groups and carbonyl groups seems to be crucial in inhibiting nNOS. Presence of two hydrogen accepting group is also needed.

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