Heterocyclic Letters
Vol. 12| No.4|725-735|Aug-Oct|2022
ISSN : (print) 2231–3087 / (online) 2230-9632
CODEN: HLEEAI
http://heteroletters.org

DESIGN AND *IN SILICO* EVALUATION OF NEW AZO BARBITURIC ACID ANALOGS AS POSSIBLE ANTICANCER AGENTS

J. Shaikh[a,b], A. Pangal[a,b], I. Tamboli[a], G. Pote[a] and K. Ahmed[a,b]*

[a]*Post-Graduate Department of Chemistry and Research Centre, Abeda Inamdar Senior College of Arts, Science and Commerce (Autonomous), Pune – 411001, India.*
[b]*Advanced Scientific Research Laboratory, Azam Campus, Pune – 411001, India.*
**Corresponding author Email: khursheedahmed@azamcampus.org*

ABSTRACT:
The barbituric acid nucleus is one of the most privileged scaffolds for the designing of pharmacodynamic compounds. There were several molecules originated from natural as well as synthetic sources. Molecules with barbituric acid nucleus have been found not only as potential anticancer agents but also for treating other diseases. In the present study, the designed compounds were subjected to *in silico* screening. Initially, compounds were screened for ADMET properties, drug-likeness and toxicity studies using pKCSM, SwissADME, ProTox-II web servers and followed by molecular docking with caspase-3 protein. During the preliminary screening, the compounds have shown good ADMET properties, drug-likeness and devoid of any mutagenicity and immunotoxicity. It has been found that all the compounds interact with caspase-3 through molecular docking studies. All the results indicate that these scaffolds could be synthesized and tested for their anticancer efficacy.

KEYWORDS: Barbituric acid, Caspase-3, Drug-likeness, Molecular docking, Anticancer

INTRODUCTION:

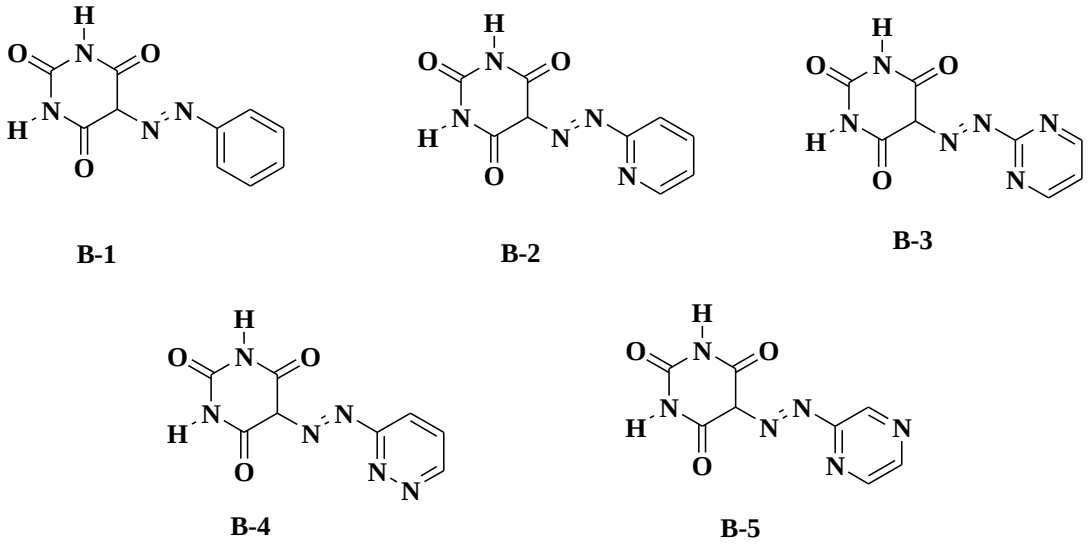
Barbituric acids have been extensively studied by the pharmaceutical and medicinal scientist for over 100 years due to their therapeutic value[i]. Barbituric acid and its derivatives have exhibited biological activities such as in antibacterial, hypotensive, and antisclerotics[ii], sedative, hypnotic, antispasmodic, anticonvulsant, and local anesthetic drugs[iii] as well as in anticancer[iv], anti-inflammatory[v] and matrix metalloproteinase inhibitors[vi]. Recent investigations suggest barbituric acids to have applications in antibacterial, anti-viral, as well as anti-cancer treatments. In addition, recent literature reports suggest that barbituric acid derivatives may also act as immunomodulators. The presence of the pyrimidinetrione ring and the nature of the C-5 substituent determine the biological activity of the barbituric acid

derivative. Therefore, before synthesizing a new derivative, it is necessary to determine qualitative and quantitative dependencies between its structure, properties, and activity[vii].

Azo compounds are the organic molecules with an azo linkage (−N=N−) in its molecular structure are widely used in textile, fiber, cosmetic, leather, paint and printing industries. In addition to their characteristic coloring function, azo compounds have been described as antibacterial, antiviral, antifungal, and cytotoxic agents[viii].

Heterocyclic compounds play a role in most scientific fields such as medicinal chemistry and biochemistry, as well as in other scientific fields. Most of the drugs available contain heterocycles, and at the crossroads of chemistry and biology. So many new scientific knowledge, discoveries and applications are being made and heterocyclic compounds are infiltrating. Compounds derived from heterocycles in pharmacies, medicine, agriculture, plastics, polymers and other fields. The most active heterocycles showing important biological effects such as antifungal, anti-inflammatory, antibacterial, antispasmodic, antiallergic, herbicidal and anticancer [x-xi]. There is always a strong need for new and efficient methods for synthesizing new heterocycles.

The synthesis of hybrid structures from different classes of compounds is one of the most common strategies for developing drug candidates with increased activity and increased specificity[xii]. To be effective as a drug, a potent molecule must reach its target in the body in sufficient concentration and stay there in a bioactive form long enough for the expected biological events to occur[xiii]. To study this aspect, the methods of Computer Aided Drug Design (CADD) are useful to identify and develop a potential lead. Similarly, Absorption-Distribution-Metabolism-Excretion (ADME) studies are utilized in the drug development process to examine numerous factors which influence on drug activity and viability. In recent years, molecular hybridization strategy has emerged as a novel approach that involves joining of two or more pharmacophores in one molecular scaffold to develop hybrid multifunctional molecules[xiv]. Bearing this in mind, we became interested to design new barbituric acid derivatives by combining with heterocyclic compounds to generate a new hybrid class of molecules with better pharmacological properties. Hence, in order to obtain a good pharamcophore with better activities and higher selectivity, the target compounds were developed by checking their ADMET, pharmacokinetic parameters, other physicochemical properties and drug-likeness. New analogs were designed by joining the barbituric acid with different hetero aromatic amines like aniline, 2-aminopyridine, 2-aminopyrimidine, 3-aminopyridazine and 2-aminopyrazine through the dizotisation reaction as shown in the following figure1.



B-1 **B-2** **B-3**

B-4 **B-5**

Figure 1. Designed azo barbituric acid analogs

EXPERIMENITAL:

Finding possible medicinal compounds is a major issue for many researchers because there are many drugs fail to arrive in clinical trials owing to their unsuitable drug likeness and poor ADMET (absorption, distribution, metabolism, elimination and toxicity) properties[xv]. For this great importance and to avoid any further problems associated to the suggested molecules, we subjected the new azo barbituric acid analogs **(B-1 to B-5)** to an in silico study for drug-likeness and ADMET parameters using the pkCSM[xvi], SwissADME[xiii] and ProTox-II[xvii] as online tools.

pKCSM: Designed molecules in the SMILES format are submitted the pKCSM web server. The pkCSM provided the information with respect to some parameters such as gastrointestinal (GI) absorption, blood-brain barrier (BBB) permeant, CYP2D6 and CYP3A4 inhibitors, human skin permeability coefficients (log Kp), Caco-2 permeability, volume of distribution at steady state (VDss), central nervous system (CNS) permeability, total clearance, AMES toxicity, maximum recommended tolerated dose (MRTD) human, oral rat acute toxicity (LD50) and chronic toxicity-lowest observed adverse effect (LOAEL), hepatotoxicity, skin sensitization etc.

Swiss ADME: Using this web server, molecules can be estimated for, drug-likeness, pharmacokinetics and medicinal chemistry friendliness properties. By applying the web server, molecular and physicochemical descriptors like molecular weight (MW), molecular refractivity (MR), molecular formula, number of heavy atoms, number of aromatic heavy atoms, number of rotatable bonds, number of H-bond acceptors, number of H-bond donors, molar refractivity, count of specific atom types and polar surface area (PSA) have been computed. Similarly, the drug likeness was established based on the physicochemical properties to find oral drug candidates by verifying five different rule-based filters like Lipinski, Ghose, Veber, Egan and Muegge. The medicinal chemistry properties like Brenk and pan assay interference compounds (PAINS) structural alerts have been used in to predict unstable, reactive, toxic fragments present in the structure.

Toxicity studies: ProTox-II, virtual lab software, is used for the prediction of toxicities of the designed molecules. ProTox-II contained computer-based models trained on real data (*in vitro* or *in vivo*) to predict the toxic potential of the existing and virtual compounds. ProTox-

II incorporates molecular similarity, pharmacophores, fragment propensities, and machine-learning models for the prediction of acute toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, immunotoxicity, adverse outcome pathways (Tox21) and toxicity targets. The predictive models are built on data from both *in vitro* assays (e.g., Tox21 assays, Ames bacterial mutation assays, hepG2 cytotoxicity assays, immunotoxicity assays) and *in vivo* cases (e.g. carcinogenicity, hepatotoxicity). The models have been validated on independent external sets and have shown strong performance. This web server predicts toxicity and uses a two-dimensional chemical structure as input and the possible toxicity profile of the chemical for 33 models with confidence scores and an overall toxicity radar chart along with the three most similar compounds with known acute toxicity.

Molecular Docking: In the field of molecular modeling, molecular docking is a technique that widely employed to investigate in-depth the interactions between a ligand and receptor and to determine the favored orientation of this ligand to a target receptor[xviii]. mcule.com is an online, integrated drug discovery platform that offers molecular modeling tools for hit identification, hit expansion and lead optimization. The modeling tools are integrated with the mcule database containing 4.5M commercially available compounds[xix]. In order to understand the probable binding affinities of the designed derivatives, molecular docking studies were carried out at active site of Caspase-3, a lysosomal enzyme involved in the apoptotic pathway (PDB ID: 1nmq) using online drug discovery platform mcule.com. The docking results were saved as PDB file and the PDB was processed in Discovery Studio for observing the interactions and for the creation of images.

RESULTS AND DISCUSSION:

ADMET Properties and Drug Likeliness

A better pharmacokinetic profile with better bioactivity and lesser toxicities are essential for a compound which should be investigated in the drug discovery to reduce the wastage of time and resources[xx]. Therefore, when the ADMET properties of a druglike compound are of sufficiently high quality, and when the target has been validated, the compound could be developed into new medication. The pharmacokinetic profile and druglike properties of the designed analogs (B-1 to B-5) were predicted by using pkCSM and SwissADME servers to elaborate their drug potential. These web servers were selected because they are freely accessible and provide strongly built computational methods to estimate a global appraisal of the pharmacokinetics and toxicity of small molecules. The predicted results are shown in **Table 1.**

Table 1: ADMET properties of new azo barbituric acid analogs calculated from pkCSM

Property	**Model Name**	**B-1**	**B-2**	**B-3**	**B-4**	**B-5**
Absorption	Water solubility[a]	-2.405	-2.504	-2.689	-3.226	-2.723
	Caco-2 permeability[b]	-0.031	-0.233	-0.097	-0.262	-0.16
	Intestinal absorption (human)[c]	70.36	70.396	66.392	66.746	71.531
	Skin Permeability[d]	-2.945	-3.685	-3.246	-3.334	-3.377
Distribution	VDss (human)[e]	-0.896	-1.069	-1.213	-1.272	-1.269

	Fraction unbound (human)	0.47	0.691	0.666	0.703	0.676
	BBB permeability[f]	-0.846	-1.124	-1.279	-1.458	-1.279
	CNS permeability[g]	-3.047	-4.033	-4.117	-4.103	-4.121
Metabolism	CYP2D6 inhibitor[h]	No	No	No	No	No
	CYP3A4 inhibitor[h]	No	No	No	No	No
Excretion	Total Clearance[i]	0.007	0.051	0.569	-0.27	0.455
Toxicity	Max. tolerated dose (human)	0.329	0.939	1.149	0.979	1.111
	Oral Rat Acute Toxicity (LD50)	2.459	2.703	2.826	2.97	2.728
	Oral Rat Chronic Toxicity (LOAEL)	1.772	1.397	2.309	1.34	2.316
	Minnow toxicity	2.671	3.021	3.08	3.008	3.396
	Hepatotoxicity[h]	No	No	Yes	Yes	Yes
	Skin Sensitization[h]	No	No	No	No	No
	AMES toxicity	No	No	No	No	No

[a](log mol/L), [b](log Papp in 10-6 cm/s), [c](% Absorbed), [d](log Kp), [e](log L/kg), [f](Fu), [g](log PS), [h](Yes/No), [i](log ml/min/kg), [j](LD50 in mol/kg), [k](LOAEL in log mg/kg_bw/day)

The molecules have a great solubility potential, both in water as well as $CaCO_2$ permeability. The evaluated parameters signify the molecules to have high potential for solubility in water. Log S (S in mol/L) is a parameter used to evaluate aqueous solubility and all of the analogs were found to show good solubility values ranging from -2.405 to -3.226 mol/L. All the analogs were found to have good intestinal absorption and exhibited, low skin permeability and during the prediction all analogs showed low skin permeability as the value of log Kp < -2.5. Drugs can bind extensively to proteins in the plasma. The designed molecules showed the unbound fraction in the range of 0.47 to 0.703 and the analog B-4 may show good effect as it showed highest unbounded fraction of the drug. A logBB < -1 suggests the compounds may not easily cross the blood–brain barrier. The only analog B-1 can cross the blood–brain barrier while other analogs were found to be not able to cross membrane as the logBB is less than -1. The penetration of drug through Central Nervous System (CNS) is measured by parameter log PS, for all the analogs is less than -3 which indicates inability of these analogs to penetrate the CNS. The results also showed the molecules were unable to inhibit these two enzymes like CYP2D6 and CYP3A4 and all these hybrids will not be able to metabolize the xenobiotics in the body. Total clearance is an important pharmacokinetic parameter and it influences both the half life and bioavailability altogether with the volume of distribution and oral absorption which will decide how often and how much of a drug can be given to patients. Its prediction helps us to determine the feasibility of clinical dosing and provides a framework for the starting dose for first in human studies. The molecular weight and hydrophilicity of the compound are important for the removal of drug ingredients from the body. The prediction results show that the total clearance of B-3 is highest followed by B-5 and other analogs B-1, B-2 and B-4 will not be eliminated from the body which may be associated with certain types of toxicities. All analogs have LD_{50} values above 0.5 mM and are non-toxic as per the predictions. For a compound, the predicted LOAEL is expressed in log(mg/kg_bw/day) and all the derivatives shows LOAEL in the range of 1.309 to 1.772. The predicted results show that the analogs B-1 and B-2 are non-hepatotoxic while all these analogs may not have skin sensitization. The lethal concentration values (LC50) below 0.5

mM (log LC50 < -0.3) are regarded as high acute toxicity in Minnow toxicity test. The designed analogs depicted LC50 values greater than 0.5 mM and are non toxic. Furthermore, all these analogs act as non-carcinogenic which is depicted from negative AMES toxicity test. These overall results of ADMET studies disclosed that the compounds have got good pharmacokinetic properties.

The physicochemical properties give a global description of the structures of analogs such as molecular weight, molecular refractivity, topological polar surface area, number of rotatable bonds, heavy atoms, and hydrogen bond acceptors and donors. We predicted the physicochemical properties and drug likeness of the designed analogs(B1 to B-5) by using the SwissADME. The results are presented in **Table 2**. The bioavailability predictions of the compounds displayed a rapid evaluation of drug likeness. The bioavailability properties exhibited by the analogs are within the range except the lipophilicity that indicates they are excellent drug candidates. Hence form these physicochemical properties, we can be conclude that these compounds have excellent pharmacological properties and are orally bioavailable.

Table 2: Physicochemical properties of the new azo barbituric acid analogs

Properties		**B-1**	**B-2**	**B-3**	**B-4**	**B-5**
Molecular weight (g/mol)		232.20	233.18	234.17	234.17	234.17
No. of Heavy atoms		17	17	17	17	17
No. of Arom. heavy atoms		6	6	6	6	6
No. of Rotatable bonds		2	2	2	2	2
No. of H-Bond acceptors		4	5	6	6	6
No. of H-Bond donors		3	3	3	3	3
Molar Refractivity		66.16	63.96	61.75	61.75	61.75
Total polar surface area Å^2		99.66	112.55	125.44	125.44	125.44
Solubility	Log S (ESOL)	-2.13	-1.67	-1.26	-1.04	-1.00
	Log S (Ali)	-2.83	-2.33	-1.93	-1.55	-1.49
	Log S (SILICOS-IT)	-3.37	-3.00	-2.62	-2.62	-2.62
Lipophilicity	MLOGP	-0.64	-1.32	-2.01	-1.60	-2.42
	WLOGP	-1.13	-1.74	-2.34	-2.34	-2.34
	XLOGP3	1.14	0.40	-0.25	-0.61	-0.67

The drug likeness was established based on the physicochemical properties to find oral drug candidates. There are five different rule-based filters[xxi] which are used to predict whether the chemical compounds can act as drug. The result of drug likeness evaluation of analogs is shown in **Table 3** –

Table 3: Drug Likeness evaluation of the new azo barbituric acid analogs

Rule-based filters	**B-1**	**B-2**	**B-3**	**B-4**	**B-5**
Lipinski violations	0	0	0	0	0
Ghose violations	1 (WLOGP < -0.4)	1 (WLOGP < -0.4)	1 (WLOGP < -0.4)	1 (WLOGP < -0.4)	1 (WLOGP < -0.4)
Veber violations	0	0	0	0	0
Egan violations	0	0	0	0	0
Muegge violations	0	0	0	0	0
Bioavailability Score	0.55	0.55	0.55	0.55	0.55
PAINS No. of Alerts[#]	1	1	1	1	1

	(imine)	(imine)	(imine)	(imine)	(imine)
Brenk No. of Alerts[#]	1 (imine)	1 (imine)	1 (imine)	1 (imine)	1 (imine)
Lead likeness No. of Violations	1 (MW<250)	1 (MW<250)	1 (MW<250)	1 (MW<250)	1 (MW<250)
Synthetic accessibility	2.32	2.74	2.65	2.75	2.90
[#]- due to tautomeric imine form of designed azo barbituric acid analogs					

The predictions revealed that all test compounds have good drug similarity and can be good drug candidates for further study. They have zero violation druglikeness according to the criteria defined by Lipinski, Veber, Egan and Muegge, while these hybrids showed one violation according to Ghose. According to the Five Laws, a molecule can be orally active / absorbent only if it does not violate two or more of the above rules. The Brenk and pan assay interference compounds (PAINS) structural alerts have been used in medicinal chemistry to predict unstable, reactive, toxic fragments present in the structure. Brenk considered compounds that are smaller and less hydrophobic and not those defined by "Lipinski's rule of 5" to widen opportunities for lead optimization. The PAINS referred to compounds which would form aggregates, react with proteins or interfere in screening assays, leading to false positive values during the assay[xxii]. Among the compounds examined, all molecules resist Brenk's rule due to an imine fragments. All analogs contain imine bond, which is responsible for one alert in PAINS. However, all the compounds showed one violations in Lead likeness due to smaller molecular weight (MW<250). Thus, these preliminary results provide the lead for the design of more potent drug.

Pro Tox-II: The new azo barbituric acid analogs were subjected to toxicity prediction by ProTox-II and for measuring their LD_{50} and classification. The compounds were found in class V toxicity and if swallowed they may be harmful and toxic to human being at a concentration of ($2000 < LD50 \leq 5000$) mg/kg body weight. The lethal dose ranges from 2250 to 5000 mg/kg weight for all the compounds and the average similarity of the designed compounds ranged between 36.42 to 46.21%. The prediction accuracy was found to be in between 23 to 55. The ProTox-II platform is divided into five different classification steps: (1) acute toxicity (2) organ toxicity (3) toxicological endpoints (4) toxicological pathways and (5) toxicity targets. The probability score for hepatotoxicity of the designed azo barbituric acid analogs was varied between 0.56 to 0.58 and the probability score for carcinogenicity of the analogs was 0.63 as minimum and 0.61as maximum. While immunotoxicity differed between 0.98 to 0.99 and the mutagenicity of the designed analogs had been stretched from 0.50 to 0.55. The cytotoxicity probability has extended up to 0.78 to 0.80 for the designed analogs. Certainly, the compounds possess hepatotoxicity and carcinogenicity, but these need to be experimentally verified through *in vivo* experiments. For Tox21-nuclear receptor signaling pathways, several parameters such as AhR, AR, AR-LBD, Aromatase, ER, ER-LBD, and PPAR-gamma were predicted for the designed compounds and for all the protein pathways the compounds have shown inactive probability. These results suggest that these compounds exhibit not only weak estrogenic, but also antiestrogenic, antiandrogenic, and anti-TH activities via different pathways. For Tox21-stress response pathways, parameters like nrf2/ARE, HSE, MMP, p53, and ATAD5 have been studied. All the compounds displayed inactive probability for all types of stress response pathways.

Molecular docking: Caspase-3 acts as a major executioner protein in proteolytic degradation during apoptosis. Caspase-3 can regulate cell death and apoptosis, which are processes involved in human malignancies, such as breast cancer, oral cancer, colorectal cancer and hepatocellular carcinoma[xxiii]. Caspase-3 down-regulation in tumors usually results in resistance to cancer therapy. In order to understand the probable binding affinities of the designed derivatives, molecular docking studies were carried out at active site of Caspase-3 (PDB ID: 1nmq) using 1-Click Docking tool. The best docking score of -7.1 was achieved by compounds B-2 and B-4. However, other analogs B-1, B-3 and B-5 showed a docking score of -7.0, -6.9 and -6.7 respectively. Most of the compounds established H-bond with different amino acid residues **(Figure 2)**. B-4 had the best binding orientation since it established six H-bonds throughout the whole extended molecule, and such binding mode should have made the molecule well-fixed in the protein active site: pyrimidinetrione ringed H-bonded with three amino acid residues SER-170, SER-210 and TRP-167, azo group interacted with PHE-208 and pyridazine ring had two H-bonds with GLU-207 and ASP-143. B-1 and B-5 also seem to have rigid poses, and each of them establishes five H-bonds with ASP-143, GLU-207, GLU-209, PHE-208, TRP-167 and SER-170, PHE-208, GLU-207, SER-210, TRP-167 respectively. However, the analogs B-2 and B-3 showed four H-bonding each with PHE-208, SER-210, TRP-167, ARG-168 and PHE-208, PHE-211, TRP-167, ARG-169 respectively. From this, it can be concluded that the best among the five compounds employed in this study, the analog B-4 binds more strongly in the protein pockets and showed good interactions. Similarly, the remaining compounds also exhibited reasonably good interactions with the binding site amino acid residues with a good binding energy.

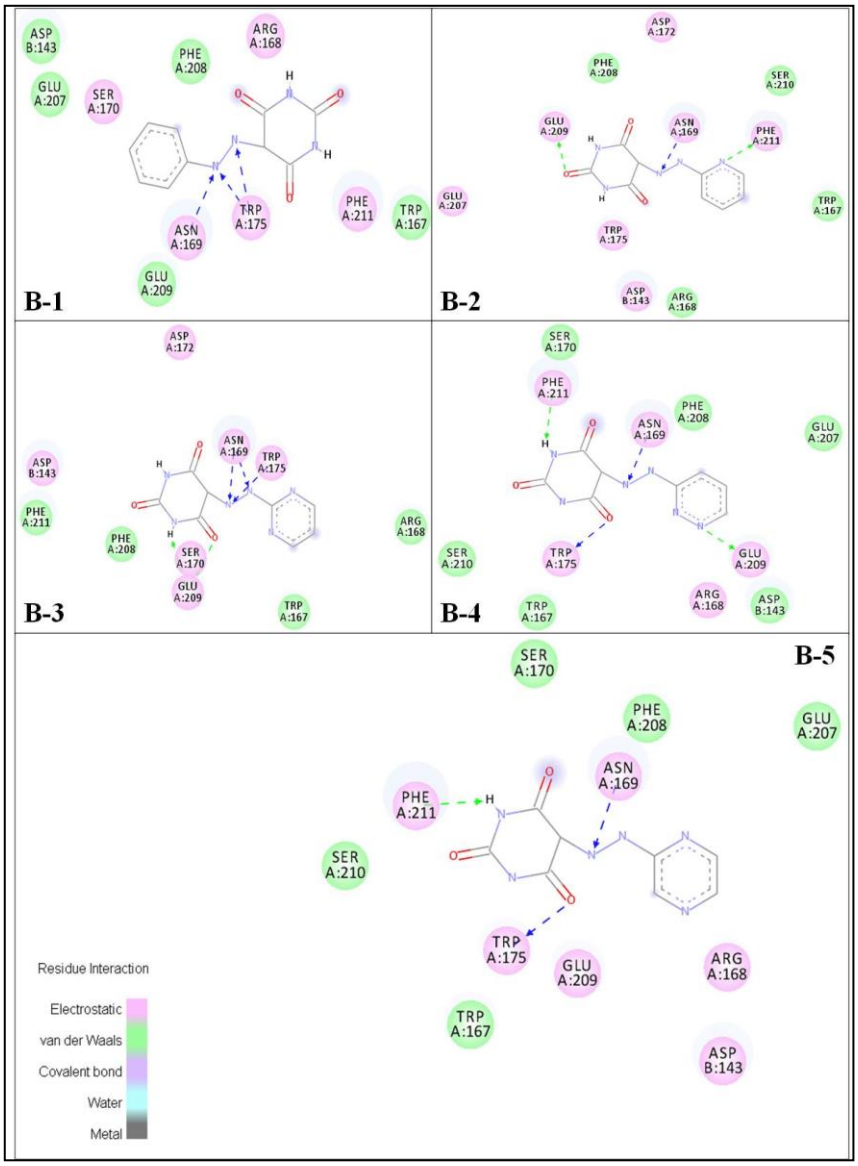


Figure 2: Molecular docking results of new bioactive azo barbituric acid analogs

Synthetic accessibility of the designed azo barbituric acid analogs:

The target compounds designed in this study can be synthesized according to **Figure 3** for further examination. For this purpose, firstly, the heteroaryl diazonium salt **(1)** can be prepared from a cold solution of sodium nitrite (3 mmol) and different substituted hetero aromatic amines (3 mmol) in conc. HCl (8–9 mmol) and water (5 ml) in an ice bath. Secondly, ice-cold solution of barbituric acid (3 mmol) in 10 ml of 2N NaOH reacts with **(1)** to get the corresponding compound. Finally, the target compounds can be achieved by purification either by recrystallisation or colum chromatography.

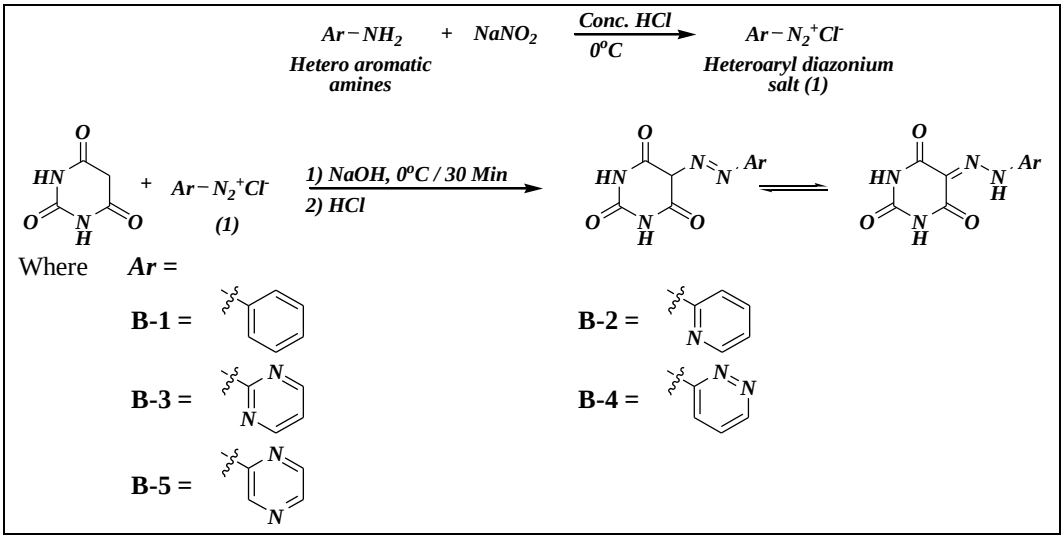


Figure 2: Possible synthetic scheme for new bioactive azo barbituric acid analogs

CONCLUSION:

The new azo barbituric acid analogs have been designed in the present study by considering the privileged medicinal scaffold barbituric acid and with the incorporation of hetero aromatic amines groups through molecular hybridization and in silico computational method. The designed analogs were subjected to *in silico* screening followed by molecular docking analysis. Most of the compounds have been found to interact with caspase-3 with H-bonding interactions. It is hoped that these compounds interact with caspase-3 protein and display anticancer properties. The preliminary screening results of show that these analogs have good ADMET properties, drug-likeness and are devoid of toxic properties such as immunotoxicity, tumorogenicity, mutagenicity. The above results are quite encouraging and these azo barbituric acid analogs may be considered as potential antiproliferative agents in future and these scaffolds could be further established as possible anticancer agents.

ACKNOWLEDGEMENT:

We are thankful to the Department of Science and Technology, Govt. of India for providing financial assistance under DST-FIST (0 Level) to Abeda Inamdar Senior College, Pune vide letter number SR/FST/COLLEGE-277/2018 dated 20th December 2018. We are grateful to Dr. P. A. Inamdar, the President of MCE Society, Pune for providing the lab facilities.

REFERENCES:

i. Khan K.M.; Ali M.; Farooqui T.A.; Khan M.; Taha M.; Perveen S.; Journal of the Chemical Society of Pakistan; 2009, **31**, 823.
ii. Levina R.Y.; Velichko F.K.; Advances in the chemistry of barbituric acids; Russian Chemical Reviews; 1960, **29**, 437.
iii. Sing P.; Paul K.; A Simple Synthesis of 5-Spirobarbituric Acids and Transformations of Spirocyclopropanobarbiturates to 5-Substituted Barbiturates; Indian Journal of Chemistry; 2006 **45B**, 247.
iv. Sokmen B.;, Ugras S.; Sarikaya H.Y.; Ugras H.I.; Yanardag R.; Antibacterial, antiurease, and antioxidant activities of some arylidene barbiturates; Appl Biochem Biotechnol.; 2013, **171(8)**, 2030.
v. Orzalesi G.P.; Gratteri P., Selleri S.; Synthesis of new 1,3-dicyclohexyl barbituric acid derivatives with anti-inflammatory potential activity; European Journal of Medicinal Chemistry; 1990, **25**, 197.

vi. Puerta D.T.; Cohen S.M.; A Bioinorganic Perspective on Matrix Metalloproteinase; Inhibition Current Topics in Medicinal Chemistry; 2004, **4**, 1551.
vii. Suzana A.; Đenđi V.; Borko M.; Gorana M.; Jelena N.; Study of the biological activity descriptors of the barbituric acid derivatives; Contemporary Materials; 2020, **XI–2**, 77-84.
viii. Ali Y.; Hamid A.S.; Rashid U.; Biomedical Applications of Aromatic Azo Compounds; Mini-Reviews in Medicinal Chemistry; 2018, **18(18)**, 1548.
ix. Kerru N.; Gummidi L.; Maddila S.; Gangu K.K.; Jonnalagadda S.B.; A Review on Recent Advances in Nitrogen-Containing Molecules and Their Biological Applications; Molecules; 2020, **25(8)**, 1909.
x. Arora P.; Arora V.; Lamba H.S.; Wadhwa D.; Importance of Heterocyclic Chemistry: A Review; Int J Pharm Res Sci; 2012, **3(9)**, 2947.
xi. Abbas A.; A Review: Biological Importance of Heterocyclic Compounds; Der Pharma Chemica; 2017, **9(13)**,141.
xii. Pangal A.; Shaikh J.; Mulani M.; Ahmed K.; Synthesis, anticancer activities and in silico screening of 3-acetylcoumarin hydrazone scaffolds; Journal of Advanced Scientific Research; 2021, **12(04 Suppl 1)**, 225-233.
xiii. Daina A.; Michielin O.; Zoete V.; SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules; Sci Rep; 2017,**7**, 42717.
xiv. Szumilak M.; Wiktorowska-Owczarek A.; Stanczak A.; Hybrid Drugs - A Strategy for Overcoming Anticancer Drug Resistance?; Molecules; 2021, **26(9)**, 2601.
xv. Guan L.; Yang H.; Cai Y. et al.; ADMET- score - a comprehensive scoring function for evaluation of chemical drug-likeness; Med chem comm.; 2018, **10(1)**, 148.
xvi. Pires D.E.V.; Blundell T.L.; Ascher D.B.; pkCSM: Predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures; J. Med. Chem.; 2015, **58(9)**, 4066.
xvii. Banerjee P.; Eckert A.O.; Schrey A.K.; Preissner R.; ProTox-II: a webserver for the prediction of toxicity of chemicals; Nucleic Acids Res.; 2018, **46(W1)**, W257.
xviii. Meng X.Y.; Zhang H.X.; Mezei M.; Cui M.; Molecular docking: a powerful approach for structure-based drug discovery; Curr Comput Aided Drug Des.; 2011, **7(2)**, 146.
xix. Kiss R.; Sandor M.; Szalai F.A.; http://Mcule.com: a public web service for drug discovery; J Chem inform; 2012, **4**, P17.
xx. Yusuf I.; Adamu U.; Sani U.; Computational studies of a series of 2-substituted phenyl-2-oxo-, 2-hydroxyl- and 2-acylloxyethylsulfonamides as potent anti-fungal agents; Heliyon; 2020, **6(4)**, e03724.
xxi. Daina A.; Michielin O.; Zoete V.; SwissADME, a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Scientific reports; 2017, **7**, 42717.
xxii. Pereyra C.E.; Dantas R.F.; Ferreira S.B. et al.; The diverse mechanisms and anticancer potential of naphthoquinones; Cancer Cell Int.; 2019, **19,** 207.
xxiii. Huang K.; Fang W.; Li A.F.; Liang P.; Wu C. et al.; Caspase-3, a key apoptotic protein, as a prognostic marker in gastric cancer after curative surgery; International Journal of Surgery; 2018, **52**,258.

Received on July 7, 2022.