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Virtual Screening of New azo Coumarin Derivatives as Possible Alkaline Phosphatase Inhibitors

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Abstract

To develop new alkaline phosphatase inhibitors, a series of new azo coumarin derivatives were designed by using computer aided drug designing and virtually assessed using online platforms. At first, the compounds were screened for ADMET, physicochemical properties, drug-likeness, toxicity studies and target prediction using pKCSM, SwissADME, SwissTargetPrediction and ProTox-II tools. The predictions were supported by *in silico* molecular docking with alkaline phosphatase enzyme using CB-Dock2 molecular docking tool. The compounds possessed good ADMET and physicochemical properties, drug-likeness and devoid of any immunotoxicity and cytotoxicity. The evaluated binding energy values reveal that all compounds fit favorably into the alkaline phosphatase active site displaying hydrogen bonding with different amino acid residues of the target protein and could be good scaffolds for designing new alkaline phosphatase inhibitors. These results collectively framed the way for the development of new azo coumarin derivatives as possible alkaline phosphates inhibitors.

Keywords: Alkaline phosphatase, coumarin, ADMET, Drug-likeness, Molecular docking.

Introduction

In recent years, the strategy of making hybrids of two or more than two biologically active motifs has emerged as a popular approach that involves conglomeration of two or more pharmacophores in one molecular scaffold to develop multifunctional biological agents (1-2). These hybrid structures are expected to exhibit multiple biological activities, modified selectivity profile, different or dual modes of action without reduced or no undesired side effects due to mixing of different pharmacophores in a single molecule (3-4). The cheminformatic tools, have played a major role in the development of therapeutically important small molecules or drugs including the Prediction of Activity Spectra for Substances (PASS), Lipinski's rule of five, predictions of absorption, distribution, metabolism, excretion, and toxicity (ADMET) are useful applications for the optimization and well-targeting of chemical synthesis, biological testing, and drug discovery (5). ADMET Predictor is a designed program of the computer for estimating pharmacokinetic parameters or properties of drug-like compounds from their molecular structures (6). Being highly bioactive and low toxic by a drug/drug-like compound are not good enough criteria to qualify the compound as a good can-

didate. A better profile of pharmacokinetic is exclusively important for a novel compound and it is very significant to evaluate the ADMET profile of new compounds earlier to avoid waste of time and resources (7).

Alkaline phosphatase is a hydrolase enzyme found in many organisms from bacteria to human and has been known to play a crucial role in various biological functions (8). It is used as tumor marker in various types of cancers like seminomas and ovarian cancer. Elevated levels of alkaline phosphatase have been found in several multifactorial disorders viz. bones diseases, thyrotoxicosis, hepatic or liver diseases, intestinal disease, cancerous growth (tumors) and rheumatoid arthritis etc. indicating it to be an interesting target for drug discovery (9).

On the other hand, coumarin or 2-oxo-2H-chromene derivatives are also known as therapeutic agents with broad and diverse biological activities (10) such as antioxidant (11), antimicrobial (12-13), antiviral (14), anti-inflammatory (15), antidepressant (16), anti-asthmatic (17), anti-HIV (18), anti-tubercular (19) and antitumor (20-21) are only few of them. The role of coumarin moiety in medicinal chemistry as a good therapeutic agent is well established and a number of different natural and synthetic coumarin based molecules have been reported as a potent alkaline phosphatase inhibitor (22-23).

Moreover, heterocyclic compounds are the exceptional targets for anticancer research and drug discovery (24). Among them, nitrogen containing compounds have shown excellent effects than non-nitrogen containing compounds (25). Further, it is reported that the combination of distinct pharmacophores produces compounds with improved activity (26). At the same time synthesis of hybrid structures from different class of compounds is one of the popular strategies for the development of drug candidates with increased activity and improved specificity (27-28).

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 (29). In the design and development of new biological agents, molecular hybridization is a useful strategy and is based on the combination of two or more pharmacophoric units in the same molecule (30). In order to obtain a good pharamcophore with better activities and higher selectivity, we designed the target compounds by virtual screening using the computational tools like ADMET, pharmacokinetic, physicochemical and drug-likeness properties and molecular docking. The new coumarin analogs were designed by joining 4-hydroxycoumarin with different aromatic amines like aniline, 2-aminopyridine, 2-aminopyrimidine, 3-aminopyridazine and 2-aminopyrazine through the diazotization reaction as shown in the following Figure 1.

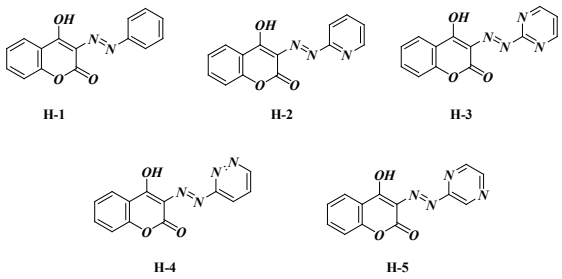

Figure 1. Designed azo coumarin derivatives

Materials and methods

The designed new azo coumarin derivatives (H-1 to H-5) were subjected to an in silico screening for ADMET, pharmacokinetic, physicochemical and drug-likeness properties, target prediction, toxicity prediction and molecular docking using the computational tools the pkCSM, SwissADME, ProTox-II and CB-Dock2 as online tools (31-32). These web servers were selected because they are freely accessible and provide strongly built computational methods to estimate a universal judgment of the pharmacokinetics and toxicity of small molecules.

ADMET prediction

The ADMET studies are significant to estimate the pharmacodynamics of the designed compounds, which could be a candidate agent in drug design and discovery studies. SMILES format of the designed molecules are uploaded on the pKCSM web server (https://biosig.lab.uq.edu.au/pkcsm/prediction). The pkCSM web server provide the information with respect ADMET parameters like gastrointestinal (GI) absorption, blood-brain barrier (BBB) permeability, CYP2D6 and CYP3A4 substrates and inhibitors, human skin permeability coefficients (log Kp), Caco-2 permeability, volume of distribution at steady state (VDss), CNS permeability, total clearance, AMES toxicity, maximum recommended tolerated dose (MRTD) human, oral rat acute toxicity (LD50) and hepatotoxicity, skin sensitization etc.

Pharmacokinetic, physicochemical properties and drug-likeness prediction

The designed molecules were estimated for pharmacokinetic, physicochemical properties and drug-likeness properties using SwissADME (http://www.swissadme.ch/). SwissADME is a web-based platform that lets users upload or draw their target compounds with structure or SMILES code. This tool supplies many parameters like lipophilicity (iLOGP, XLOGP3, WLOGP, MLOGP, SILICOS-IT, Log Po/w), water solubility – Log S (ESOL, Ali, SILICOS-IT), drug-likeness rules (Lipinski, Ghose, Veber, Egan, and Muegge) and Medicinal Chemistry (PAINS, Brenk, Leadlikeness, Synthetic accessibility) methods.

Target prediction

SMILES codes of the designed compounds were uploaded to the SwissTargetPrediction website (https://www.swisstargetprediction.ch) to analyze their putative off-targets in the human organism.

Toxicity prediction

Toxicological predictions for bioorganic compounds are essential to estimate the amount of tolerability of the lead compounds before *in vitro*, *in vivo,* and clinical studies. ProTox-II is virtual lab software used for the prediction of toxicities of the designed molecules. The SMILES codes of the designed molecules were uploaded to the The ProTox-II website (https://tox-new.charite.de/protox_II/) to analyze their acute toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, immunotoxicity, adverse outcome pathways (Tox21) and toxicity targets.

Molecular docking

CB-Dock2 is an improved version of the protein-ligand blind docking tool that inherits the curvature-based cavity detection procedure and the AutoDock Vina-based molecular docking procedures. In order to understand the probable binding affinities of the designed azo coumarin derivatives, molecular docking studies were carried out at active site of human alkaline phosphatase (PDB ID: 1EW2) using online molecular docking platform CB-Dock2 (https://cadd.labshare.cn/cb-dock2). The docking results were saved as PDB file and the PDB were processed for observing the interactions according to the literature.

Results and Discussion

ADMET prediction

A compound with better bioactivity and lesser toxicities should be investigated in the drug discovery to reduce the wastage of time and resources (33). These compounds could be developed into new medication when the target has been validated with sufficiently high quality ADMET properties. The ADMET properties of the designed analogs (H-1 to H-5) were predicted by using pkCSM web server and their drug potential was elaborated. The predicted results are shown in Table 1.

Table 1: ADMET properties of new azo coumarin derivatives calculated from pkCSM

Property	Model Name	H-1	H-2	H-3	H-4	H-5
Absorption	Water solubility[a]	-3.931	-3.494	-3.306	-3.2	-3.136
	Caco-2 permeability[b]	0.819	0.926	1.299	1.152	1.247
	Intestinal absorption (human)[c]	91.113	93.421	95.887	97.861	100
	Skin Permeability[d]	-2.837	-2.915	-2.851	-2.904	-2.872
Distribution	VDss (human)[e]	-0.13	-0.271	-0.275	-0.39	-0.334
	Fraction unbound (human)	0.118	0.248	0.199	0.228	0.221
	BBB permeability[f]	-0.222	-0.474	-0.68	-0.68	-0.68
	CNS permeability[g]	-1.697	-2.774	-2.897	-2.682	-2.896
Metabolism	CYP2D6 inhibitor[h]	No	No	No	No	No
	CYP3A4 inhibitor[h]	No	No	No	Yes	No
Excretion	Total Clearance[i]	0.199	0.251	0.333	0.063	0.248
Toxicity	Max. tolerated dose (human)	0.328	-0.261	0.067	-0.005	-0.004
	Oral Rat Acute Toxicity (LD50)	2.158	2.535	2.554	2.514	2.532
	Oral Rat Chronic Toxicity (LOAEL)	1.382	1.314	0.989	1.097	0.976
	Minnow toxicity	0.255	0.379	-0.079	-0.388	0.01
	Hepatotoxicity[h]	No	No	Yes	Yes	Yes
	Skin Sensitization[h]	No	No	No	No	No
	AMES toxicity	Yes	Yes	Yes	Yes	Yes

Note: [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)

Log S (S in mol/L) is a parameter used to evaluate aqueous solubility. All of the derivatives were found to show good solubility values ranging from -3.136 to -3.931 mol/L. The analog H-1 is more soluble in water than all other analogs. More than 0.90 value of the Papp coefficient and more than 30% intestinal absorption indicates the compound has high Caco-2 permeability and good gastrointestinal absorption. All the derivatives showed poor gastrointestinal absorption and compound H-1 possessed the highest value of intestinal absorption among. The distribution of the compound or the drug in the body is characterized by Volume of distribution (VDss), blood-brain barrier permeability (logBB), and CNS permeability. Drugs can bind extensively to proteins in the plasma. The free or unbound fraction of a drug is usually the portion that is responsible for a pharmacologic effect. The designed molecules showed the unbound fraction in the range of 0.118 to 0.248 and the analog H-2 may show good pharmacological effect as it showed highest unbounded fraction of the drug. All the hybrids were poorly distributed within the tissues. 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. Cytochrome P450s is an important enzyme system for drug metabolism in liver and the CYP2D6 and CYP3A4 are the two main subtypes of cytochrome P450. The molecules were unable to inhibit these enzymes and restrain the metabolism of the xenobiotics in the body. The prediction of total clearance helps to determine the feasibility of clinical dosing for the starting dose during clinical trial studies and it depends on

the molecular weight and hydrophilicity of the compound. The prediction showed that the total clearance of H-3 is highest followed by H-2 and other analogs H-5, H-4 and H-1 will not be eliminated from the body which may be associated with certain types of toxicities. The maximum recommended tolerated dose (MTD) provides an estimate of the toxic dose threshold of drugs in humans. From the values for MTD it is clear that the high dose is required for toxicity. All analogs have LD50 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 0.976 to 1.382. The predicted results show that the analogs H-1 and H-2 are non-hepatotoxic while all these analogs may not have skin sensitization. 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.

Furthermore, the BOILED-Egg profile enables the perceptive consideration of passive gastrointestinal absorption (HIA) and brain penetration (BBB) in the function of the position of the molecules in the WLOGP-vs-TPSA referential was screened for the selected five compounds (34). In this model, the white area corresponds to a high probability of passive absorption in the GIT, while the yellow area is for a high probability of brain penetration. Also, the marks are colored in blue if predicted as actively effluxes by P-gp (PGP+) and in red if estimated as non-substrate of P-gp (PGP−). All designed compounds were estimated to be well-absorbed but not accessing the brain, and all compounds were not subject to active efflux (red dot) (Figure 2).

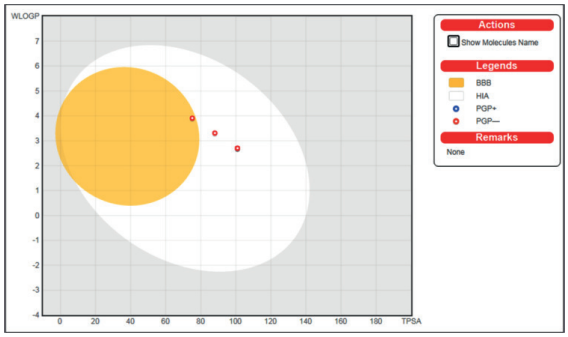

Figure 2. BOILED-Egg presentation of the compounds

Table 2. Physicochemical properties of the new azo coumarin derivatives

Properties		H-1	H-2	H-3	H-4	H-5
Molecular weight (g/mol)		266.25	267.24	268.23	268.23	268.23
No. of Heavy atoms		20	20	20	20	20
No. of Arom. heavy atoms		16	16	16	16	16
No. of Rotatable bonds		2	2	2	2	2
No. of H-Bond acceptors		5	6	7	7	7
No. of H-Bond donors		1	1	1	1	1
Molar Refractivity		75.13	72.92	70.72	70.72	70.72
Total polar surface area Å²		75.16	88.05	100.94	100.94	100.94
Solubility	Log S (ESOL)	-4.00	-3.54	-3.14	-2.91	-2.88
	Log S (Ali)	-4.51	-4.02	-3.61	-3.24	-3.18
	Log S (SILICOS-IT)	-5.65	-5.28	-4.91	-4.91	-4.91
Lipophilicity	MLOGP	2.07	1.81	1.16	1.56	0.75
	WLOGP	3.91	3.31	2.70	2.70	2.70
	XLOGP3	3.26	2.52	1.87	1.51	1.45

Physicochemical properties and drug-likeness prediction

The physicochemical properties give a comprehensive depiction of the structures of derivatives such as molecular weight (MW), molar refractivity (MR), topological polar surface area (TPSA), number of rotatable bonds, heavy atoms and hydrogen bond acceptors and donors. The physicochemical properties of the designed azo coumarin derivatives (H-1 to H-5) were predicted by using the SwissADME and the results are presented in Table 2. 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 concluded that these compounds have excellent pharmacological properties and are orally bioavailable.

The bioavailability predictions of the compounds displayed a rapid evaluation of drug likeness. The drug likeness was evaluated based on the physicochemical properties to find oral drug candidates. There are five different rule-based filters (35) 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 coumarin derivatives

Rule-based filters	H-1	H-2	H-3	H-4	H-5
Lipinski violations	0 violation	0 violation	0 violation	0 violation	0 violation
Ghose violations	0 violation	0 violation	0 violation	0 violation	0 violation
Veber violations	0 violation	0 violation	0 violation	0 violation	0 violation
Egan violations	0 violation	0 violation	0 violation	0 violation	0 violation
Muegge violations	0 violation	0 violation	0 violation	0 violation	0 violation
Bioavailability Score	0.55	0.55	0.55	0.55	0.55
PAINS No. of Alerts	1 alert azo	1 alert azo	1 alert azo	1 alert azo	1alert azo
Brenk No. of Alerts	2 alerts: coumarin, diazo group	2 alerts: coumarin, diazo group	2 alerts: coumarin, diazo group	2 alerts: coumarin, diazo group	2 alerts: coumarin, diazo group
Lead likeness No. of Violations	0 violation	0 violation	0 violation	0 violation	0 violation
Synthetic accessibility	3.35	3.29	3.14	3.33	3.38

All the test compounds showed good drug similarity and can be a good drug candidates. They have zero violation for druglikeness according to the five laws framed by Lipinski, Ghose, Veber, Egan and Muegge. The Brenk and Pan Assay INterference compoundS (PAINS) structural alerts used in medicinal chemistry for the identifying unstable, reactive, toxic fragments present in the structure. Among the compounds examined, all molecules resist Brenk's rule due to coumarin and diazo groups. All analogs contain azo bond responsible for one alert in PAINS. However, all the compounds showed no violations in Lead likeness due to smaller molecular weight. Thus, these preliminary results provide the lead for the design of more potent drug. Furthermore, the bioavailability radar of the compounds is given in Figure 3, and the colored zone depicts suitable physicochemical space for oral bioavailability. The Bioavailability

Radar gives a first glance at the drug-likeness of the compounds. The pink area represents the optimal range for each property (lipophilicity: XLOGP3 between −0.7 and +5.0, size: MW between 150 and 500 g/mol, polarity: TPSA between 20 and 130 Å2, solubility: log S not higher than 6, saturation: fraction of carbons in the sp3 hybridization not less than 0.25, and flexibility: no more than 9 rotatable bonds.

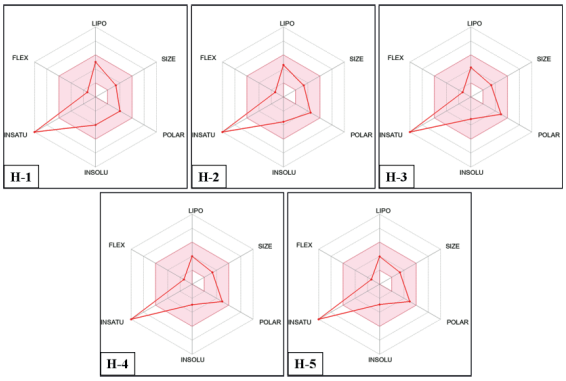

Figure 3. The Bioavailability Radar for drug-likeness of the new azo coumarin derivatives.

Target Prediction

The target prediction of all compounds was performed using the SwissTargetPrediction platform (36) and the results are depicted as a pie-chart (Figure 4). The H-1, containing phenyl ring linked to coumarin through diazo bond was predicted as 33.3% oxidoreductase. All other derivatives H-2 to H-5, containing 1 to 2 nitrogen atoms in the phenyl ring were predicted as enzymes inhibitors with varying percentages, as given in Figure 4.

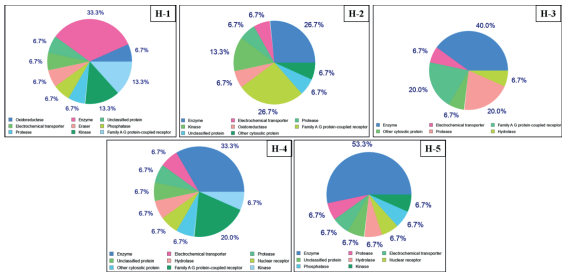

Figure 4. SwissTargetPrediction of the designed compounds.

Toxicity Prediction

The new azo coumarin derivatives were evaluated for toxicity, LD50 and classification by ProTox-II. The compound H-1 was found in class V toxicity while H-2 to H-5 were in class IV, which indicates if swallowed they may be harmful and toxic to human being at a concentration of (2000 < LD50 ≤ 5000) mg/kg body weight. The lethal dose varies from 2000 to 3200 mg/kg weight for all the compounds. The average similarity of the designed compounds ranged between 39.79 to 53.28% with the prediction accuracy of 23 to 67.38%. 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 compounds exhibited slight activities for hepatotoxicity, carcinogenicity and mutagenicity while the compounds were inactive for immunotoxicity and cytotoxicity which needs 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 except Mitochondrial Membrane Potential (MMP) stress response pathway.

Molecular docking

Understanding how to predict interactions between proteins and small molecules is essential for identifying various biological processes, advancing drug development, and comprehending protein functions. A powerful approach for this purpose is protein-ligand blind docking, which identifies protein binding re-

gions, and foretelling a molecule's binding pose (32, 37-38). Alkaline phosphatase is an enzyme that is increased by a number of hepatobiliary disorders. Its increase is typically assumed to signify bile stasis. According to available reports, alkaline phosphatase is a significant prognostic factor for a number of malignancies, including colon, lung, and stomach cancer. Alkaline phosphatase increase is frequently interpreted as bile stasis brought on by liver metastases (39). To predict the best conformational position within the active region of target protein, all the designed azo coumarin derivatives (H-1 to H-5) were docked against alkaline phosphatase. The derivatives were docked into the active sites of human alkaline phosphatase (PDB ID: 1EW2). All the generated docked complexes were analyzed on the basis of minimum energy values expressed as binding energy (kcal/mol) and hydrogen/hydrophobic interactions. Docking results justified that all of designed compounds exhibited excellent binding affinities with the target protein. It was found that the molecules interact with different residues inside the active site of the alkaline phosphatase. The free binding energy for these derivatives was found to be in the range of -7.3 to -7.8 kcal/mol displaying good binding of these inhibitors inside the active site pocket of the protein. The 3D and 2D diagrams of interactions with the amino acid side chains of the target protein is shown in Figure 5 and Figure 6.

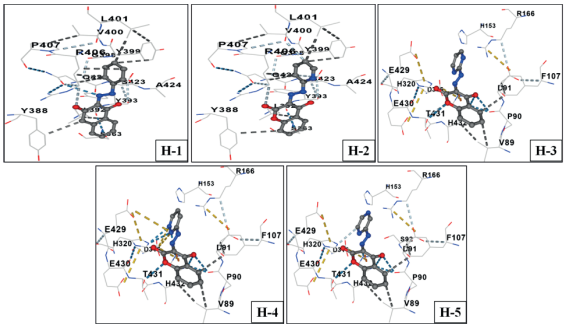

Figure 5. 3D-Binding modes of new azo coumarin derivatives (H-1 to H-5) in the active binding site of alkaline phosphatase

The basic chemical nuclei of all the designed compounds were the same and therefore most derivatives exhibited a good binding affinity with the target protein. The best docking score of -7.8 was shown by derivatives H-4 and H-5. However, other analogs showed a docking score of -7.3, -7.4 and -7.6 respectively. All the designed compounds showed well established H-bonding with different amino acid residues. The binding free energy and other details from docking studies are presented in Table 4.

The compounds H-4 and H-5 displayed three pi-cation interactions with ARG166, HIS432 and LYS87. The nitrogen of azo group, the lactone ring oxygen and the –OH on C-4 of coumarin moiety displayed hydrogen bond with THR431 and HIS432. Along with these, all molecules showed electrostatic and van der Waals interactions with other amino acid residues as shown in Figure 6. Such binding mode should have made these molecules well-fixed in the protein active site of the alkaline phosphatase.

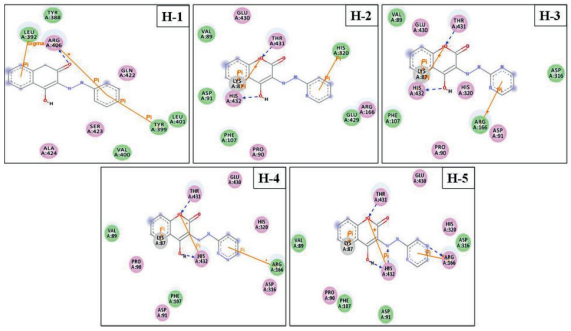

Figure 6. 2D-Interaction analysis of new azo coumarin derivatives (H-1 to H-5) in the active binding site of alkaline phosphatase

However, the other compounds also exhibited reasonably good interactions with the binding site amino acid residues and showed a good binding energy. The evaluated binding energy values reveal that all compounds fit favorably into the alkaline phosphatase active site displaying hydrogen bonding with different amino acid residues of the target protein. Our predicted results are in well agreement with the predicted in silico results. Therefore, these new

Table 4. Molecular docking results of the new azo coumarin derivatives

Sr. No.	Azo coumarin derivative	Binding Energy Kcal/Mol	Binding amino acid residues
1	H-1	-7.3	SER363, TYR388, LEU392, TYR393, GLY398, TYR399, VAL400, LEU40,1 ARG406, PRO407, GLN422, SER423, ALA424
2	H-2	-7.4	VAL89, PRO90, ASP91, SER92, PHE107, HIS153, ARG166, ASP316, HIS320, GLU429, GLU430, THR431, HIS432
3	H-3	-7.6	VAL89, PRO90, ASP91, PHE107, HIS153, ARG166, ASP316, HIS320, GLU429, GLU430, THR431, HIS432
4	H-4	-7.8	VAL89, PRO90, ASP91, PHE107, HIS153, ARG166, ASP316, HIS320, GLU429, GLU430, THR431, HIS432
5	H-5	-7.8	VAL89, PRO90, ASP91, SER92, PHE107, HIS153, ARG166, ASP316, HIS320, GLU429, GLU430, THR431, HIS432

derivatives containing a coumarin ring and heterocyclic amines joined through diazo group could be good scaffolds for designing new alkaline phosphatase inhibitors. These molecules also showed good ADMET, physicochemical properties and drug-likeness. These results collectively framed the way for the development of new azo coumarin derivatives as alkaline phosphates inhibitors.

Synthetic accessibility of the designed azo coumarin derivatives

The target compounds designed in this study can be synthesized according to Figure 7 for further examination. A cold solution of sodium nitrite (0.207 g, 3 mmol) was added dropwise into the solution of aromatic amines (3 mmol) dissolved in a mixture of conc. HCl and water (5 ml) in an ice bath. The temperature of the reaction was maintained below 5 °C. After the complete addition, the solution was kept for 15-30 min with occasional stirring to complete the diazotization. After completion of reaction as monitored by TLC, it was dropwise added into an ice-cold solution of 4-hydroxy coumarin (0.543 g, 3 mmol) in 10 ml of 2N NaOH. Then resultant mixtures were stirred at room temperature for 30 min to 1 hour. The colour products obtained were filtered and washed with water. Finally, the obtained products were dried and recrystallised by ethanol.

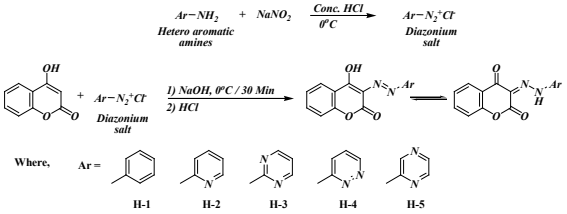

Figure 7. Possible synthetic scheme for new azo coumarin derivatives

Conclusion

In the present study, we designed the new azo coumarin derivatives by considering the advantages of important medicinal scaffold 4-hydroxycoumarin through molecular hybridization and computational methods. All the designed derivatives were virtually screened for their ADMET, physicochemical, drug-like ness and toxicity studies. All the compounds showed good pharmacodynamics, no violations in drug-likeness and devoid of any immunotoxicity and cytotoxicity. These compounds were further subjected for possible target prediction and the compounds were found to target enzymes. The predicted target was confirmed by molecular docking studies using alkaline phosphatase enzyme. All compounds exhibited reasonably good interactions with amino acid residues and showed a good binding energy. The evaluated binding energy values indicated that all com-

pounds fit favorably into the alkaline phosphatase active site displaying hydrogen bonding with different amino acid residues of the target protein. These results fit well with the predicted *in silico* results though these have to be experimentally verified. Therefore, these new derivatives containing a coumarin ring and heterocyclic amines joined through diazo group can become good drug candidate for designing new alkaline phosphatase inhibitors. These molecules also showed good ADMET, physicochemical properties and drug-likeness. These results collectively make the way for the development of new azo coumarin derivatives as possible alkaline phosphates inhibitors.

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