Prediction of Physicochemical Properties, Bioactivity, Pharmacokinetics, Drug-Likeness and Synthetic Accessibility of Phenyl and Isoxazole Azo Dyes

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ABSTRACT

The prediction of physicochemical properties, bioactivity score, pharmacokinetics, drug-likeness and synthetic accessibility of computationally designed phenyl and isoxazole azo dyes was carried out using Molinspiration Cheminformatics and SwissADME. All the compounds were identified as drug-like molecules based on assessment of physicochemical properties and ADME. Among all, 2-hydroxy-5-(phenyldiazenyl)benzoic acid was predicted as enzyme inhibitor. Two new isoxazole azo compounds, 2-hydroxy-5-((5-methylisoxazol-3-yl)diazenyl)benzoic acid and 2-hydroxy-5-((3-methylisoxazol-5-yl)diazenyl)benzoic acid, showed better physicochemical properties, pharmacokinetics and synthetic accessibility comparable to the standard drug Balsalazide.

KEY WORDS: AZO DYES, AMINOISOXAZOLE, BALSALAZIDE, DRUG-LIKENESS.

INTRODUCTION

Azo dyes are characterized by the presence of azo group ($-N=N-$) in their structure, conjugated with two distinct or identical, mono/polycyclic aromatic or heteroaromatic system. They have a wide range of applications in pharmaceutical, cosmetic, food and textile industry (Ali et al., 2018). Various medicinally important drugs containing azo moiety are Prontosil, Phenazopyridine, Sulfasalazine, Olsalazine and Balsalazide. Prontosil was the first azo dye to treat bacterial infections successfully, which contain an additional sulphonamide functional group. Phenazopyridine was used to relieve the symptoms caused by irritation of urinary tract. Sulfasalazine, Olsalazine and Balsalazide were used for the treatment of inflammatory bowel disease, consists of salicylic acid functional group in their structures. Upon oral administration, these drugs are delivered intact to colon where they cleaved by bacterial azoreductase into an active anti-inflammatory agent 5-aminosalicylic acid.

Isoxazole is a five membered heterocycle containing oxygen and nitrogen atoms at the adjacent position. Isoxazole compounds possess broad biological activities such as antibacterial, antifungal, antiviral, anticancer and anti-inflammatory activities. Several therapeutically active drugs consist of isoxazole ring, which includes Sulfamethoxazole, Sulfisoxazole, Oxacillin, Cloxacillin, Dicloxacillin, Flucloxacillin, Leflunomide, Valdecixib, Parecoxib, Muscimol and Ibotenic acid. In the literature, synthesis of few isoxazole azo dyes were reported from 5-amino-3-methylisoxazole or 3-methyl-4H-isoxazol-5-one and phenyl diazonium salts (Hamama et al., 2017; Banpurkar et al., 2018). It was reported that 3-aminoisoxazole undergo diazotization using strong hydrochloric acid and the resulting diazonium salt couples with activated aromatic molecules (Duff 1981; Quilico 1962).

In silico study represent relatively a new method of research investigation. To reduce time and chemical consumption in drug discovery and development, a large variety of *In silico* or computational methods were developed with the objective of predicting physicochemical properties, biological activity, drug-likeness and also the ease of synthesis from the chemical structure. Hence, the present study is aimed to design computationally different phenyl and isoxazole azo dyes containing phenol, salicylic acid, o-cresol and resorcinol moieties.

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Further, it is aimed to predict the physicochemical properties, bioactivity score, pharmacokinetics, drug-likeness and synthetic accessibility of computationally

designed compounds and the standard drug Balsalazide using free online software.

Table 1. Structure, nomenclature and SMILES notation of compounds (1-13)

S. No	Structure and nomenclature	SMILES notation
1	(E)-4-(phenyldiazenyl)phenol	<chem>OC1=CC=C(/N=N/C2=CC=CC=C2)C=C1</chem>
2	(E)-2-hydroxy-5-(phenyldiazenyl)benzoic acid	<chem>OC1=CC=C(/N=N/C2=CC=CC=C2)C=C1C(=O)O</chem>
3	(E)-2-methyl-4-(phenyldiazenyl)phenol	<chem>OC1=CC=C(/N=N/C2=CC=CC=C2)C=C1C</chem>
4	(E)-4-(phenyldiazenyl)benzene-1,3-diol	<chem>OC1=CC=C(/N=N/C2=CC=CC=C2)C(O)=C1</chem>
5	4-((5-methylisoxazol-3-yl)diazenyl)phenol	<chem>OC(C=C1)=CC=C1N=NC2=NO(C)C=C2</chem>
6	2-hydroxy-5-((5-methylisoxazol-3-yl)diazenyl)benzoic acid	<chem>OC(C=C1)=C(C(O)=O)C=C1N=NC2=NO(C)C=C2</chem>
7	2-methyl-4-((5-methylisoxazol-3-yl)diazenyl)phenol	<chem>OC(C=C1)=C(C)C=C1N=NC2=NO(C)C=C2</chem>
8	4-((5-methylisoxazol-3-yl)diazenyl)benzene-1,3-diol	<chem>OC(C=C1)=CC(O)=C1N=NC2=NO(C)C=C2</chem>
9	4-((3-methylisoxazol-5-yl)diazenyl)phenol	<chem>OC(C=C1)=CC=C1N=NC2=CC(C)=NO2</chem>
10	2-hydroxy-5-((3-methylisoxazol-5-yl)diazenyl)benzoic acid	<chem>OC(C=C1)=C(C(O)=O)C=C1N=NC2=CC(C)=NO2</chem>
11	2-methyl-4-((3-methylisoxazol-5-yl)diazenyl)phenol	<chem>OC(C=C1)=C(C)C=C1N=NC2=CC(C)=NO2</chem>
12	4-((3-methylisoxazol-5-yl)diazenyl)benzene-1,3-diol	<chem>OC(C=C1)=CC(O)=C1N=NC2=CC(C)=NO2</chem>

MATERIAL AND METHODS

The software used for the *in silico* studies of phenyl and isoxazole azo dyes as well as standard drug Balsalazide include:

- ChemDraw Ultra 12.0
- Molinspiration Cheminformatics
- SwissADME

ChemDraw Ultra 12.0 used for the generation of structure, nomenclature and SMILES notations. Molinspiration Cheminformatics (<https://www.molinspiration.com>) was used to calculate physicochemical properties like miLogP, molecular weight, number of rotatable bonds, hydrogen bond acceptors, hydrogen bond donor, and TPSA of title compounds. The percentage of absorption estimated using the equation $\%ABS = 109 - (0.345 \times TPSA)$ (da Silva et al., 2015). Molinspiration Cheminformatics

was also used to predict the bioactivity score of title compounds as GPCR ligands, ion channel modulators, kinase inhibitors, nuclear receptor ligands, protease inhibitors, and enzyme inhibitors. SwissADME (<http://www.swissadme.ch>) was used to evaluate individual ADME behavior and drug-likeness of the compounds under investigation. SwissADME web tool also used to predict medicinal chemistry friendliness properties, particularly synthetic accessibility (Daina et al., 2017).

Table 2. Predicted physicochemical properties of the compounds (1-13) by Molinspiration Cheminformatics

S.No	miLogP	MW	HBA	HBD	Volume	n Violation	nroth	TPSA	%ABS
1	3.65	198.22	3	1	182.57	0	2	44.96	93.48
2	4.04	242.23	5	2	209.57	0	3	82.25	80.62
3	4.49	212.25	3	1	199.13	0	2	44.96	93.48
4	3.36	214.22	4	2	190.59	0	2	65.18	86.51
5	2.48	203.20	5	1	176.55	0	2	70.99	84.51
6	2.87	247.21	7	2	203.55	0	3	108.29	71.64
7	3.32	217.23	5	1	193.11	0	2	70.99	84.51
8	2.19	219.20	6	2	184.56	0	2	91.22	77.53
9	2.48	203.20	5	1	176.55	0	2	70.99	84.51
10	2.87	247.21	7	2	203.55	0	3	108.29	71.64
11	3.32	217.23	5	1	193.11	0	2	70.99	84.51
12	2.19	219.20	6	2	184.56	0	2	91.22	77.53
13	2.75	357.32	9	4	301.57	0	8	148.65	57.72

(miLogP: n-octanol-water partition coefficient, MW: Molecular weight, HBA: Hydrogen bond acceptor, HBD: Hydrogen bond donor, n Violations: Number of violations, nroth: Number of rotatable bonds, TPSA: Topological polar surface area, %ABS: Percentage of absorption)

Table 3. Prediction of bioactivity score of the compounds (1-13)

S.No	GPCRL	ICM	KI	NRL	PI	EI
1	-0.42	-0.01	-0.31	-0.85	-0.60	-0.12
2	-0.21	-0.04	-0.16	-0.43	-0.30	0.03
3	-0.48	-0.18	-0.29	-0.75	-0.62	-0.21
4	-0.33	-0.27	-0.23	-0.79	-0.67	-0.05
5	-0.60	-0.13	-0.35	-1.30	-0.91	-0.39
6	-0.35	-0.14	-0.19	-0.81	-0.56	-0.18
7	-0.63	-0.26	-0.31	-1.17	-0.88	-0.42
8	-0.48	-0.39	-0.31	-1.07	-0.95	-0.31
9	-0.43	-0.35	-0.27	-0.94	-1.04	-0.26
10	-0.21	-0.32	-0.13	-0.56	-0.66	-0.08
11	-0.46	-0.46	-0.24	-0.83	-1.00	-0.31
12	-0.32	-0.59	-0.23	-0.73	-1.07	-0.19
13	0.13	-0.01	-0.01	-0.18	0.15	0.12

(GPCRL: GPCR Ligand, ICM: Ion channel modulator, KI: Kinase inhibitor, NRL: Nuclear receptor ligand, PI: Protease inhibitor, EI: Enzyme inhibitor)

RESULTS AND DISCUSSION

The 2D structures of phenyl and isoxazole azo dyes along with standard drug Balsalazide (compounds 1-13) were drawn using ChemDraw Ultra 12.0. Their nomenclature and SMILES notations were generated and presented in Table-1.

Molinspiration Cheminformatics tool kit was used for the calculation of physicochemical properties of phenyl and isoxazole azo dyes as well as standard drug Balsalazide (compounds 1-13). The data presented in Table-2. The results revealed that all the compounds obeyed the Lipinski's rule of five ($\text{miLogP} < 5$, molecular weight < 500 , the number of hydrogen bond acceptors < 10 and hydrogen bond donors < 5), thus indicating good bioavailability on drug administration. TPSA and %ABS predict the transport properties like intestinal absorption and blood brain barrier (BBB) penetration. The estimated TPSA for isoxazole azo compounds (5-12) was greater than 60 indicating good GI absorption but not BBB penetration. The phenyl azo compounds 1 and 3 have low TPSA (< 60) indicating the BBB penetration. The data revealed that the standards drug Balsalazide (13) have high TPSA and low %ABS value. This indicates very large amount of the drug accumulate in the colon after oral administration and then cleaved by bacterial

azoreductase into an active drug 5-aminosalicylic acid. Among the series of phenyl and isoxazole azo dyes, compounds 6 and 10 have high TPSA values and low %ABS values. Therefore, large amount of isoxazole azo compounds 6 and 10 may enter colon after oral administration and cleaved to 5-aminosalicylic acid.

The bioactivity score of phenyl and isoxazole azo dyes along with standard drug Balsalazide (compounds 1-13) was predicted by Molinspiration Cheminformatics. The values were listed in Table-3. A molecule having bioactivity score more than 0.00 expected to be active, while values between -0.50 and 0.00 are expected to be moderately active and if the score is less than -0.50 considered as inactive. Except compound 2, all the phenyl and isoxazole azo dyes (compounds 1, 3-12) were moderately active as enzyme inhibitors. The compound 2 and standard drug Balsalazide were bioactive as enzyme inhibitors. All the phenyl and isoxazole azo dyes were moderately active as GPCR ligands, ion channel modulators (except compound 12) and kinase inhibitors. All the phenyl and isoxazole azo dyes except compound 2 were inactive as nuclear receptor ligands and protease inhibitors. The standard drug Balsalazide was predicted as moderately active ion channel modulator, kinase inhibitor, nuclear receptor ligand and bioactive GPCR ligand and protease inhibitor.

Table 4. Prediction of Pharmacokinetics, Drug-likeness and Synthetic Accessibility of the compounds (1-13) by SwissADME

S.No	Pharmacokinetics				Drug-likeness		Synthetic Accessibility
	GI absorption	BBB permeant	P-gp substrate	Log K _o (cm/s)	Lipinski	Bioavailability Score	
1	High	Yes	No	-4.84	Yes	0.55	2.24
2	High	No	No	-5.03	Yes	0.56	2.13
3	High	Yes	No	-4.68	Yes	0.55	2.14
4	High	Yes	No	-5.48	Yes	0.55	2.52
5	High	Yes	No	-5.57	Yes	0.55	2.87
6	High	No	No	-5.96	Yes	0.56	2.75
7	High	yes	No	-5.57	Yes	0.55	2.86
8	High	No	No	-6.10	Yes	0.55	2.98
9	High	Yes	No	-5.75	Yes	0.55	2.94
10	High	No	No	-5.96	Yes	0.56	2.78
11	High	Yes	No	-5.57	Yes	0.55	2.93
12	High	No	No	-6.10	Yes	0.55	3.05
13	Low	No	No	-6.92	Yes	0.56	2.47

SwissADME web tool was used to estimate the physicochemical properties, pharmacokinetics, drug-likeness and medicinal chemistry friendliness especially synthetic accessibility. The results revealed that the predicted physicochemical properties were found similar to the results obtained with Molinspiration Cheminformatics. The results of pharmacokinetics, drug-likeness and synthetic accessibility were presented in Table-4. The data indicated that the phenyl and

isoxazole azo dyes have good GI absorption, whereas the standard drug Balsalazide has low GI absorption. The compounds 2, 6, 8, 10, 12 and Balsalazide do not have BBB penetration, as evidenced by an intuitive graphical classification model BOILED-Egg.

All the compounds including Balsalazide were predicted as non-substrates for the permeability glycoprotein (P-gp). The estimated Log K_p (with K_p in cm/s) values

of all the compounds were highly negative indicating less skin permeation. The compounds 2, 6, 8, 10, 12 and Balsalazide were identified as non-inhibitors of the most important cytochrome P450 isoenzymes (CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4). All the compounds obeyed Lipinski's rule and have the calculated bioavailability score between 0.55 and 0.56 indicating the drug-likeness. The SwissADME synthetic accessibility score of the compounds was comparable with the standard drug Balsalazide and lies between 2.13 and 3.05. This indicates synthesis of all the compounds was not difficult.

CONCLUSION

Computational tools have become increasingly important in drug screening and design. In the present study, eight new isoxazole azo dyes and four phenyl azo dyes containing phenol, salicylic acid, o-cresol and resorcinol moieties were designed computationally. The physicochemical properties, bioactivity score, pharmacokinetics, drug-likeness and medicinal chemistry friendliness of the designed compounds were predicted using Molinspiration Cheminformatics and SwissADME. All the designed compounds were identified as drug-like molecules. Among the series, phenyl azo compound (2) was active as enzyme inhibitors. The newly designed isoxazole azo compounds (6 and 10) having salicylic acid moiety showed better pharmacokinetics, drug-likeness and synthetic accessibility similar to the standard drug Balsalazide. Therefore, these compounds can be synthesized by a retrosynthetic approach and evaluated for the biological activity using suitable *in vitro* and *in vivo* models to develop them as drug candidates for the

treatment of bacterial, fungal and viral infections as well as cancer and inflammation.

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