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# Synthesis, *In-Silico* ADMET Properties Predictions and Biological Activity of AZO Derivatives

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**ABSTRACT:** Azo compounds are involved in a number of biological reactions such as inhibition of DNA, RNA and protein synthesis, carcinogenesis and biological activity against bacteria and fungi. Molecular absorption, distribution, metabolism and excretion (ADME) play primary role in drug discovery and development. Predicted molecular properties using Molsoft, Molinspiration, Osiris, Swiss ADME, PkCSM Soft wares. All the compounds followed the Lipinski 'Rule of five' and showing good oral bioavailability. we synthesized cheaply several azo compounds and characterized by IR, NMR and Mass spectral analysis. The diazo group confers interesting chemical and biological properties to the amino derivatives. Hence, the consideration on synthesized compounds is to screen for anti-microbial activity against *Escherichia coli* and *Bacillus subtilis*. Compound 3(a),3(b),(3e) showed good anti-bacterial activity and compound 3c,3d,3f,3g 6a-b shown moderate activity and Antioxidant activity values of all the compounds are less than the standard value.

**KEY WORDS:** Azo dye, Molsoft, Swiss ADME, anti-bacterial activity, anti-oxidant activity

## INTRODUCTION

Azo dyes are important class of organic compounds consisting of at least one conjugated azo(-N=N-) groups as chromophore[1]. Azo compounds are also involved in a number of biological reactions such as inhibition of DNA, RNA and protein synthesis, carcinogenesis and biological activity against bacteria and fungi. They show herbicidal,[2]anti-inflammatory, anti-microbial or anti-parasitic, anti-ulcer, anti-fungal, anti-bacterial, anti-tubercular, anti-diabetics, antiseptics, antibiotic and other chemotherapeutic activities[3].They can also be synthesized cheaply because the starting materials are readily available, inexpensive compounds.The diazo group confers interesting chemical and biological properties to the amino derivatives. In this view, the reactivity of some aromatic amines has been explored towards diazotization and further coupling with a variety of coupling components like benzimidazole, phenols [4-5], coumarins and antipyrin Benzimidazole or its different derivatives have been successfully used as drugs in different fields, like: Anti-cancer and Anti-diabetic agents. It has also different biological activities, such as: Antiviral, Antifungal, Anthelmintic, Antibacterial, Antagonist and selective inhibitor [6] anti-tuberculosis, anticancer, cytotoxic, antitumor, antioxidant, anti-HIV, analgesic and anti-inflammatory activity.[7-13].In the development of drugs intended for oral use, good drug absorption and appropriate drug delivery are very important. These properties mainly hydrophobicity, molecular size, flexibility and presence of various pharmacophoric features influence the

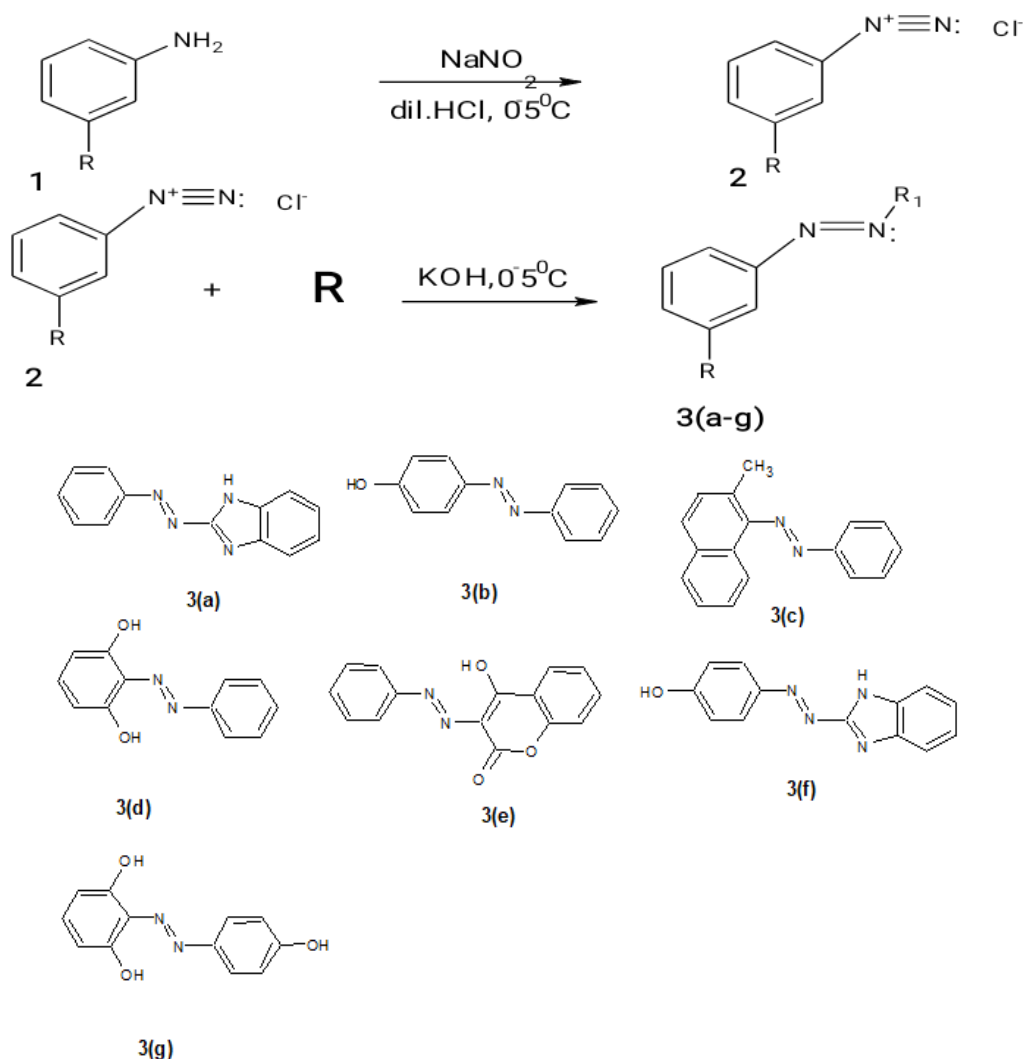
behavior of molecules in a living organism, including oral bioavailability. [14] Molinspiration, Molsoft, Swiss ADME, PkCSM, are applications of modern cheminformatics techniques. Calculation of molecular physicochemical properties relevant to drug design including log P, molecular polar surface area (PSA), and the Lipinski's Rule of 5 descriptors.[15-16]

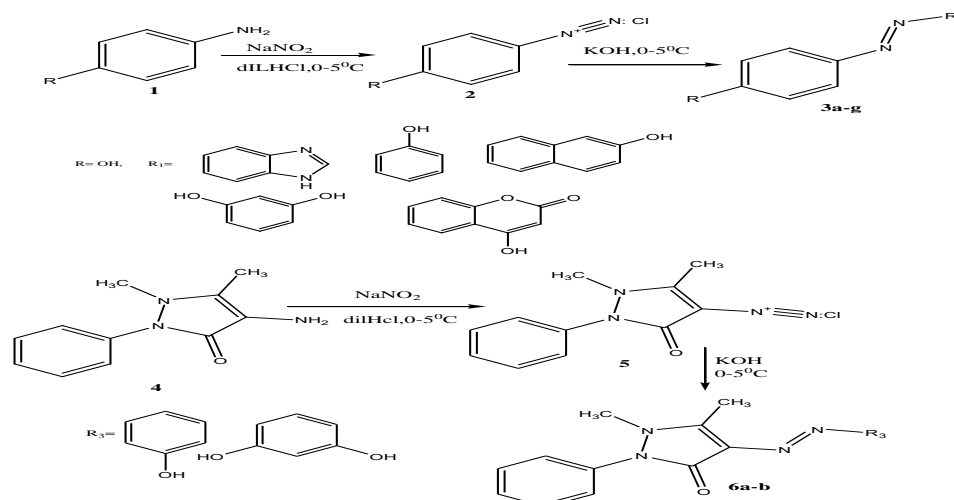
## II. MATERIAL AND METHODS

Melting points were recorded in open glass capillaries using a melting point apparatus and are unrecorded. Infrared spectra recorded on BRUKER Infrared spectrometer in KBr pellet and values are given  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR spectra were recorded at 400MHz on BRUKER AVANCE  $^1\text{H}$  NMR in  $\text{CDCl}_3$  spectrometer. MASS spectrum is recorded on V G MICROMASS-7070H (ESI and EI). Were given mass unit (m/z). All the chemicals are purchased from M/s. Sigma Aldrich, Sd fine chemicals, Molechem. and Merck. All the solvents are purchased from molechemchemicals. Thin layer chromatography was performed on pre-coated silica gel F<sub>254</sub>(merck).

### EXPERIMENTAL PROCEDURE:

#### Scheme-A:



**Scheme-B****SYNTHETIC PROCEDURES:**

**General Procedure:- Step-1** Aniline, [0.01 mol 1.5ML] mixed with 4N HCL (0.01 mol, 0.37 g, 0.73 ml) and (0.01 mol, 0.69g) of cold aqueous solution of NaNO<sub>2</sub> was added drop wise with vigorous stirring. The temperature of the solution was maintained between 0° and 5°C, Which was again stirred for 5-10 min. the diazonium salt used for the coupling reaction. **Step-2;** To the diazonium salt solution prepared in step 1 was added to aromatic or heterocyclic ring systems with NaOH /KOH solution with constant stirring for 10-20minutes. The precipitation was kept between 0° and 5°C. Colored products obtained were separated by filtration, washed the precipitate with water. For synthesized crude compound TLC was performed. Pure compounds were separated from crude synthesized compounds by column chromatographic method and eluted as benzene, acetonitrile, and methanol solvent containing compounds. Methanol solvent eluted product was isolated after evaporation of solvent as solids [17-19].

**ANTIBACTERIALACTIVITY**

The antibacterial activity of synthesized compounds was screened by using diffusion method. In this method, the drug will diffuse into the agar media and prevent the growth of microbes and produce a clear zone of inhibition.

**ANTI-OXIDANT ACTIVITY**

Synthesized compounds evaluated for antioxidant activity, we have used stable free radicals 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), at the concentration of 0.3mM in methanol. To 0.2 ml of the test compound at concentration 1.5ml methanol and 0.1ml DPPH solution were added, mixed thoroughly and absorbance was read at 517nm against the blank. The percentage reduction of the free radical concentration with different concentration of test compound were calculated and was compared with standard ascorbic acid. The result was expressed as IC<sub>50</sub> values (the concentration of test required to scavenge 50% free radicals) the anti-oxidant DPPH free radicals scavenge activity of all the synthesized compound performed using DPPH method. [16]

**III. RESULTS AND DISCUSSION**

Compound	Name of the Compound	Physical data of Synthesized Compounds 3(a-g),6(a-b)
(3a)	Synthesis of (2-[(E)-phenyl diazenyl] - 2,3-dihydro-1H-benzimidazole).	Mol. for . C <sub>13</sub> H <sub>12</sub> N <sub>4</sub> , <sup>1</sup> H NMR : δ (ppm) 6.98-7.26 (m, H of benzimidazole), 7.35-7.43 (m, H, phenol ring), 7.63-7.69 (m, H), 12.44 (s, -NH). Mass: (EI-MS) : 223 [M <sup>+</sup> ] Mol. Wt. 222, M.P 127-130 °C, % Yield 89%
(3b)	Synthesis of 4-[(E)-phenyl diazenyl] phenol	Mol. for : C <sub>12</sub> H <sub>10</sub> N <sub>2</sub> O, Mobile phase : benzene, Acetonitrile, and Methanol, IR : 3318 (OH), 1475 (N=N stretching), 1218 (C-N), 1593 (C=C aromatic), <sup>1</sup> H NMR δ (ppm) 8.68 (m, phenol), 10.2 (s, OH), Mass : (EI-MS): 199 [M <sup>+</sup> ] Mol. Wt : 198, M.P: 103-105 °C, Yield : 82%.



(3c)	Synthesis of 1-[(E)-phenyl diazenyl] naphthalen-2-ol	Mol.For.C <sub>16</sub> H <sub>10</sub> N <sub>2</sub> O IR : 3331cm <sup>-1</sup> (OH), 1492cm <sup>-1</sup> (N=N), <sup>1</sup> H NMR:δ (ppm) 8.56-6.97 (m,phenyl and aryl), 11.02-11.35 (s,OH), Mass : (EI-MS): 249 [M <sup>+</sup> ] Mol.Wt.-248, M.P 116-118 °C, % Yield 86%
(3d)	Synthesis of 2-[(E)-phenyl diazenyl] benzene-1,3-diol:	Mol.For C <sub>12</sub> H <sub>10</sub> N <sub>2</sub> O <sub>2</sub> IR:3244cm <sup>-1</sup> (OH), 1510cm <sup>-1</sup> (N=N), <sup>1</sup> H NMR : δ(ppm)7.9-.2(d,H,Phe), 8.5(s,H,Res),10.8(s,OH),Mass : EI-MS) : 215[M <sup>+</sup> ] Mol.Wt. 214, M.P 90-92 °C, % Yield 78%
(3e)	Synthesis of 4-hydroxy-3-[(E)-phenyldiazenyl]-2H-chromen-2-one	Mol.For C <sub>15</sub> H <sub>10</sub> N <sub>2</sub> O <sub>3</sub> IR : 3190cm <sup>-1</sup> (OH), 1575cm <sup>-1</sup> (N=N), <sup>1</sup> H NMR:δ(ppm)8.0-7.89(d), 7.45 (m, coumarin ), 7.15-7.86(m,5H,aromatic ring),16.02(s,OH).Mass : (EI-MS):267[M <sup>+</sup> ] Mol.Wt 266 M.P.118-120 °C, Yield 75%
(3f)	Synthesis of 4-[(E)-1H-benzimidazol-2-yl]diazenyl]phenol	Mol.For C <sub>13</sub> H <sub>10</sub> N <sub>4</sub> O, IR:3340cm <sup>-1</sup> (OH),3500cm <sup>-1</sup> (NH), 1476cm <sup>-1</sup> (N=N),1570cm <sup>-1</sup> (-C=N). <sup>1</sup> H NMR:δ (ppm)6.98-7.26 (q), 7.4-7.6(d, benzimidazole), 7.15-7.47(m, phenol ring). Mass : (E I-MS):239[M <sup>+</sup> ], Mol.Wt 238, Yield 89%
(3g)	Synthesis of 2-[(E)-(4-hydroxy phenyl) diazenyl] benzene-1,3-diol	Mol.For C <sub>12</sub> H <sub>10</sub> N <sub>2</sub> O <sub>3</sub> IR :3318cm <sup>-1</sup> (OH of resorcinol), 1593cm <sup>-1</sup> (N=N), <sup>1</sup> H NMR: δ(ppm)7.86-6.69 (m,Ar ),10.12-10.23 (m, phenyl and aryl),MS :231[M <sup>+</sup> ], Yield 86%, M.P.100-102 °C
(6a)	Synthesis of 4-[(E)-2-(4-hydroxy phenyl) ethenyl]-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one	Mol.For C <sub>17</sub> H <sub>18</sub> N <sub>4</sub> O <sub>2</sub> IR:3341cm <sup>-1</sup> (OH), 1586 (N=N), <sup>1</sup> H NMR 6.69-7.87(m,phenyl,aryl) 10.12(s,OH),Mass : (EI-MS) :311[M <sup>+</sup> ]Mol.Wt.310, M.P 110-112 °C Yield 87%,
(6b)	Synthesis of 4-[(E)-2-(3,5-dihydroxyphenyl)ethenyl]-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one	Mol.For IR:3173cm <sup>-1</sup> (OH),1687cm <sup>-1</sup> (C=O),1493cm <sup>-1</sup> (N=N)(ppm)2.49,3.39(2s,methyl,n-methyl), 6.43, 6.40-7.43 (1s,d, resorcinol),7.41-7.59 (m,H,aryl), 10.14 (s,OH), Mass:(EI-MS): 325 [M <sup>+</sup> ], Mol.Wt. 324, M.P 92-94 °C, Yield 85%,

### Molecular Properties Prediction

In the present investigation several azo compounds containing benzimidazole moiety, coumarin, caffeine and phenols are subjected to molecular properties prediction utilizing mol inspiration, Mol soft software's. A good bioavailability can be achieved with an appropriate balance between solubility and partitioning properties. All the designed compounds fulfill the solubility requirements and these are the most useful program to predict lipophilicity and aqueous solubility of molecules.

### Results and discussion of molecular property prediction

Comp	Log P	TPSA	n Atoms	M. Wt	Non	nOHNH	DLMS	n-rotb	Volume
3(a)	3.94	53.41	17	222.25	4	1	-1.03	2	199
3(b)	3.46	73.64	18	238.25	5	2	-1.51	2	207.40
3(c)	3.65	44.96	15	198.22	3	1	-1.42	2	182.57
3(d)	3.60	65.18	16	214.22	4	2	0.36	2	190.59
3(e)	5.02	44.96	19	248.28	3	1	-1.03	2	226.57

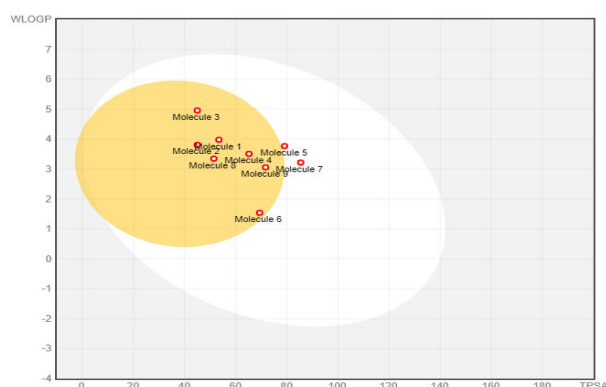
3(f)	3.52	51.67	22	292.34	5	0	-0.66	3	268.80
3(g)	2.58	86.56	22	298.31	8	0	-1.85	2	258.14
6(a)	3.21	50.75	15	197.24	3	2	-1.32	2	185.84
6(b)	3.12	85.41	17	230.22	5	3	-1.23	2	198.61

MW – Molecular Weight, N On - No. Of Hydrogen Acceptors, Tpsa - Total Polar Surface Area, N Ohnh - No. Of Hydrogen donors, Nroth - No. Of Rotatable bonds. DLMS - drug likeness score

#### ADMET calculations of compounds:

Comp	Pharmacokinetics						Drug-Likeness	
	GI Absorption	BBB Permeability	P-Gp	Cyp1a2 Inhibitor	Cyp2d6 Inhibitor	Log <sub>p</sub> (Skin Permeation)C m/S	Lipinski	Bioavailability
3(a)	High	Yes	No	Yes	No	-5.26cm/S	Yes	0.55
3(b)	High	Yes	No	No	No	-5.40	Yes	0.55
3(c)	High	Yes	No	No	No	-4.75	Yes	0.55
3(d)	Low	Yes	No	Yes	No	-4.75	Yes	0.15
3(e)	High	Yes	No	Yes	No	-5.08	Yes	0.55
3(f)	High	No	No	No	No	-7.28	Yes	0.55
3(g)	Low	No	Yes	No	No	-5.36	Yes	0.17
6(a)	High	Yes	No	No	No	-5.40	Yes	0.55
6(b)	High	No	No	Yes	No	-5.45	Yes	0.55

#### Boiled Egg:



#### ANTIBACTERIAL ACTIVITY:

The diazo group confers interesting chemical and biological properties to the amino derivatives. Hence, the consideration on synthesized compounds is to screening for anti-microbial activity against *Escherichia coli* and *Bacillus subtilis*, compound 3(a), 3(b), (3e) showed good anti-bacterial activity, 3c, 3d, 3f, 3g, and 6a-bS shown moderate activity. [20-22]

Compound	Zone of Inhibition(in mm)	
	<i>Escherichia coli</i>	<i>Bacillus subtilis</i>
3(a)	11	<b>20</b>
3(b)	10	14
3(c)	9	10
3(d)	8	10
3(e)	<b>15</b>	<b>20</b>
3(f)	11	15
3(g)	12	9
6(a)	9	12
6(b)	<b>15</b>	<b>18</b>

All values are expressed as zone of inhibition mm, bore size=6mm; compounds showed maximum activity against respective bacteria; zone size 9-11=poor activity; zone size 12-18=moderate activity, concentration of test compounds is 50µg/ml.

#### ANTIOXIDANT ACTIVITY:

Compound	IC50	Compound	IC50
3(a)	0.50	3(e)	0.52
3(b)	0.50	3(f)	0.50
3(c)	0.49	3(g)	0.52
3(d)	0.50	6(a)	0.50

All the title compounds were screened for antioxidant activity using DPPH method. Compounds were evaluated for antioxidant activity against the standard ascorbic acid. All compounds IC50 values are less than the standard that is 3.41. All the compounds not showed potent compared to standard.

#### IV. CONCLUSION

Diazonium compounds are involved in a number of biological reactions and biological activity against bacteria and fungi, herbicidal, anti-inflammatory, anti-microbial or antiparasitic, antiulcer, anti-fungal, anti-bacterial, anti-tubercular, anti-diabetics, antiseptics, antibiotic and other chemotherapeutic activities. Molecular absorption, distribution, metabolism and excretion (ADME) play primary role in drug discovery and development. A large number of *In silico* models are hence developed for prediction of admet properties, as a result enabling the reduction of time, costs and animal experiments. So the objective of this study was to obtain drug likeness of azo derivatives. Calculated molecular properties of azo compounds using Molsoft and Molinspiration, Osiris, Swiss ADMET, and PkCSM soft wares. According to the pharmacokinetic properties, all compound showed high Gastro intestinal absorption. Compounds Resorcinol derivatives has no BBB permeability however, most of them showed inhibition to Cytochrome P450 isomers (CYP1A2 and CYP2D6). Screening process with Lipinski Rule of Five showed that all compounds meet the criteria of druglikeness. The success of azo colorant is due to the simplicity of their synthesis by diazotization and azo coupling. There compounds which are showing good oral bioavailability score were synthesized cheaply because the starting materials are readily available and inexpensive. Compounds which are synthesized are characterized by IR, NMR and Mass spectral analysis. The diazo group confers interesting chemical and biological properties to the amino derivatives. Hence, the consideration on synthesized compounds is to screening for anti-microbial activity against *Escherichia coli* and *Bacillus subtilis*, compound 3(a), 3(b), (3e) showed good anti-bacterial activity, 3c, 3d, 3f, 3g, and 6a-bS shown moderate activity. Antioxidant activity using DPPH method, IC50 values are less than the standard that is 3.41.

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