



ORIGINAL ARTICLE

Design, synthesis, characterization, computational study and *in-vitro* antioxidant and anti-inflammatory activities of few novel 6-aryl substituted pyrimidine azo dyes

Aziz Unnisa^{a,*}, Amr S. Abouzieed^{a,b}, Anupama Baratam^c,
K.N.V. Chenchu Lakshmi^d, Talib Hussain^e, Rama Devi Kunduru^f, Humera Banu^g,
Syeda Bushra Fatima^g, Arshad Hussain^h, Kesavanarayanan Krishnan Selvarajan^e

^a Department of Pharmaceutical Chemistry, College of Pharmacy, University of Hail, Hail, Saudi Arabia

^b National Organization for Drug Control & Research, Cairo, Egypt

^c Department of Pharmaceutical Chemistry, KVSR Siddhartha College of Pharmaceutical Sciences, Andhra Pradesh, India

^d Department of Pharmaceutical Chemistry, QIS College of Pharmacy, Andhra Pradesh, India

^e Department of Pharmacology and Toxicology, College of Pharmacy, University of Hail, Hail, Saudi Arabia

^f Department of Biology, College of Sciences, University of Hail, Hail, Saudi Arabia

^g Department of Nutrition, College of Applied Medicine, University of Hail, Hail, Saudi Arabia

^h Department of Clinical Pharmacy, College of Pharmacy, University of Hail, Hail, Saudi Arabia

Received 24 July 2020; accepted 21 September 2020

Available online 7 October 2020

KEYWORDS

Pyrimidine azo dyes;
DPPH assay;
Heat hemolysis;
BOILED egg

Abstract A series of 6-aryl substituted pyrimidine azo dyes were synthesized by coupling of phenyl pyrimidine 2-amine with different aromatic amines. The synthetic compounds were screened for their *in-vitro* antioxidant and anti-inflammatory activities. The characterization of the synthesized compounds was carried out by IR, ¹H NMR, ¹³C NMR and Mass spectrophotometry. Computational study of designed compounds was done by OCHEM, Molinspiration cheminformatics, Datawarrior, and Swiss ADME. DPPH assay was used to determine the antioxidant activity and heat hemolysis method for anti-inflammatory activity.

© 2020 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

* Corresponding author.

E-mail address: khushiazeez@yahoo.co.in (A. Unnisa).

Peer review under responsibility of King Saud University.



Production and hosting by Elsevier

1. Introduction

Azo dyes constitute one of the largest, versatile, and important classes of organic compounds with a range of uses in science and technology (Hamid, 2007; Zollinger, 2003). Of the diverse classes of dyes, azo dyes are one of the key class constituting a

majority of the dyes used in the industry. The basic skeleton of Azo dyes is composed of Ar – N = N – Ar', where Ar represents the aromatic groups. The unit containing the N = N is designated as an 'azo group'. The color and polarity of the dyes depend on the nature of the aromatic substituents on the sides of the azo group.

Azo dyes are successfully employed as chemical sensors, LCD color filters, textile dyes, the chromophoric substrate for redox enzymes, optical polymers (Derkowska-Zielinska et al., 2019, 2020b), lasers, non-linear optics, and in many more specific applications, such as photovoltaic production (Derkowska-Zielinska et al., 2020a), as drug, cosmetic, food, and in organic synthesis.

Azo derivatives were effective biologic agents (Shridhar et al., 2012), possessing potent medicinal values. A Survey of the literature reveals their application as antidiabetics (Garg and Prakash, 1972), antitumor (Bradshaw et al., 2001; Kini et al., 2007; Racane et al., 2006), anti-inflammatory (Abadi et al., 2003; Venkatesh and N. Pandeya, 2009), anti-tuberculosis (Sah, 2010; Sah and Oneto, 2010), anti-neoplastic (Child et al., 1977; Yazdanbakhsh et al., 2012). Hence, studies have been conducted for the design, synthesis, purification, and use of azo dyes derived from different drug moieties to assess its antimicrobial properties (Koshti et al., 2008; Pathan and Borul, 2008; Rathod, 2011, 2010; Rathod and Thakre, 2013).

In the present study, we aim to design and synthesize novel azo compounds that can exhibit antioxidant and anti-inflammatory activities with lesser toxicity. The study will also be considering the influence of the three-dimensional nature of chemical structures on the ligand-receptor binding in the process of new drug development.

2. Materials and methods

The laboratory reagent grade chemicals were used without further purification. Melting points were determined in open glass capillaries using Tempo (600) melting point apparatus. IR spectra obtained from Bruker analyzers were established by Shimadzu FT-IR Spectrophotometer using the KBr pellets, Model No.8400S (Japan). ¹H and ¹³C NMR spectra were documented on Bruker 400 MHz NMR spectrometer (Switzerland) using DMSO as a solvent. Shimadzu 1800 double beam UV-Visible spectrophotometer with a wavelength accuracy of 0.5 nm and a pair of 1 cm matched quartz cell was used for the measurement of optical density values. Centrifuge (REMI-C854/8) was used for centrifugation. TLC was run on silica gel-G plates using n-hexane: ethyl acetate (5:5) as a mobile phase to assess the reaction progression and purity of synthesized compounds.

2.1. General procedure for the synthesis of substituted chalcones (Palleros, 2004)

Equimoles of acetophenone (0.01 mol) and different aromatic benzaldehyde (0.01 mol) were taken into a mortar, NaOH was added and ground by pestle at room temperature by employing the friction method. Then the mixture was moistened with water. The reaction progression was checked by TLC, and all

the reactions were observed to be completed in a time range of 15–45 min. The product was recrystallized from methanol and the purity was confirmed by melting point and TLC.

2.2. General procedure for the synthesis of substituted phenyl pyrimidine-2-amine derivatives (Kumar et al., 2017)

Equimoles of substituted Chalcones (0.01 mol) and Guanidine HCl (0.01 mmol) were refluxed for 4hrs in the presence of DMF by maintaining the temperature between 50 and 60 °C in a water bath. The mixture was moved into ice-cold water, the precipitate was filtered, dried, and recrystallized from methanol. The purity and progress of the reaction were confirmed by TLC and melting point.

2.3. General procedure for synthesis of azo compounds

Compounds 5a-5l (Table 1 & scheme 1) were prepared by the conventional method. The Phenyl pyrimidine-2-amine derivatives were dissolved in 2.5 ml conc. HCl and transferred into the beaker containing 2.5 ml (4 N) ice-cold solution of NaNO₂ with stirring until the fumes disappear. The temperature of the reaction was maintained up to 0–5 °C. Diazonium salt solution prepared above was added dropwise to the acidic solutions of different aromatic amines. The reaction mixture was stirred for 10 min by maintaining the temperature between 5 and 10 °C. The dye products obtained were filtered, washed, and recrystallized from ethanol.

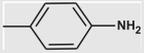
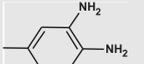
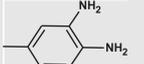
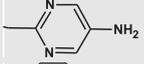
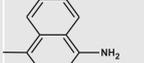
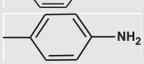
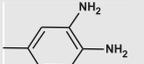
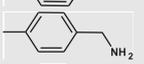
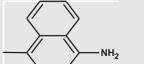
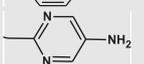
2.3.1. 4-{(E)-[4-(4-nitrophenyl)-6-phenylpyrimidin-2-yl]diazanyl}aniline 5a

Molecular formula: C₂₂H₁₆N₆O₂; **Composition:** C (66.66%) H (4.07%) N (21.20%) O (8.07%); **Yield:** 84.07%; **M.P:** 234°C ± 1; **¹H NMR** (400 MHz, DMSO d₆) δ 8.70 (s, 1H), 8.26 – 8.20 (m, 2H), 8.12 – 8.06 (m, 2H), 7.92 – 7.86 (m, 2H), 7.58 – 7.51 (m, 2H), 7.54 – 7.46 (m, 2H), 7.46 – 7.38 (m, 1H), 6.64 – 6.58 (m, 2H), 5.30 (s, 2H); **¹³C NMR** (100 MHz, DMSO d₆) δ 165.20, 157.68, (d, J = 11.9 Hz), 157.58, 150.43, 146.01, 137.45, 135.94, 129.49, 129.18, 128.99, 128.63, 125.89, 125.45, 124.60, 114.44, 114.07; **FT-IR (KBr, ν max (cm⁻¹)):** 3198 (Ar-CH), 1310 (C-N), 1657 (C = N), 1469 (weak, N = N), 1337 & 1480 (N = O), 1515 & 3080 (N-H); **ESI-MS:** 396.4 [M + H] + .

2.3.2. 4-{(E)-[4-(4-nitrophenyl)-6-phenylpyrimidin-2-yl]diazanyl}benzene-1,2diamine 5b

Molecular formula: C₂₂H₁₇N₇O₂; **Composition:** C (64.23%), H (4.16%), N (23.83%), O (7.78%); **Yield:** 84.07%; **M.P:** 260°C ± 1; **¹H NMR** (400 MHz, DMSO d₆) δ 8.70 (s, 1H), 8.26 – 8.20 (m, 2H), 8.12 – 8.06 (m, 2H), 7.92 – 7.86 (m, 2H), 7.58 – 7.50 (m, 2H), 7.46 – 7.38 (m, 1H), 6.95 (dd, J = 7.7, 2.2 Hz, 1H), 6.62 (d, J = 7.7 Hz, 1H), 6.52 (d, J = 2.2 Hz, 1H); **¹³C NMR** (100 MHz, DMSO d₆) δ 165.20, 157.63 (d, J = 39.7 Hz), 146.01, 137.72, 137.45, 136.73, 135.94, 129.49, 129.18, 128.99, 128.63, 127.64, 124.60, 120.18, 119.48, 114.44, 111.83; **FT-IR (KBr, ν max (cm⁻¹)):** 3195 (Ar-CH), 1317 (C-N), 1656 (C = N), 1495 (weak, N = N), 1335 & 1446 (N = O), 1513 & 3085 (N-H); **ESI-MS:** 411.4 [M + H] + .

Table 1 Physical data of synthesised of Azo Dyes.

Compound	R ₁	R ₂	R ₃	Ar	Physical state	
					Colour	State
5a	H	NO ₂	H		orange	Solid
5b	H	NO ₂	H		Brown	Solid
5c	H	Cl	H		orange	Solid
5d	H	Cl	H		Brown	Solid
5e	H	Cl	H		yellow	Solid
5f	H	Cl	H		Yellow	Solid
5g	H	Cl	H		Brown	Solid
5h	OCH ₃	OCH ₃	OCH ₃		Brown	Solid
5i	OCH ₃	OCH ₃	OCH ₃		Brown	Solid
5j	OCH ₃	OCH ₃	OCH ₃		yellow	Solid
5k	OCH ₃	OCH ₃	OCH ₃		Yellow	Solid
5l	OCH ₃	OCH ₃	OCH ₃		Brown	Solid

2.3.3. 4-[(Z)-[4-(4-chlorophenyl)-6-phenylpyrimidin-2-yl]diazanyl]aniline 5c

Molecular formula: C₂₂H₁₆ClN₅; **Composition:** C (64.48%), H (4.18%), N (18.15%), Cl (9.19%); **Yield:** 77.28%; **M.P.:** 218°C ± 1; **¹H NMR** (400 MHz, DMSO *d*₆) δ 8.70 (s, 1H), 7.94 – 7.86 (m, 4H), 7.55 – 6.58 (m, 2H), 5.30 (s, 2H); **¹³C NMR** (100 MHz, DMSO *d*₆) δ 165.20, 157.63 (d, *J* = 39.7 Hz), 150.43, 145.81, 137.45, 135.13, 132.88, 130.09, 129.49, 129.18, 128.76, 128.63, 125.45, 114.44, 114.07; **FT-IR (KBr, ν max (cm⁻¹)):** 3192 (Ar-CH), 1309 (C-N), 1654 (C = N), 1500 (weak, N = N), 1512 & 3079 (N-H), 1029 (C-Cl); **ESI-MS:** 385.8 [M + H]⁺.

2.3.4. 4-[(Z)-[4-(4-chlorophenyl)-6-phenylpyrimidin-2-yl]diazanyl]benzene-1,2-diamine 5d

Molecular formula: C₂₂H₁₇ClN₆; **Composition:** C (65.92%), H (4.27%), N (2.96%), Cl (8.84%); **Yield:** 77.82%; **M.P.:** 256°C ± 1; **¹H NMR** (400 MHz, DMSO *d*₆) δ 8.70 (s, 1H), 7.94 – 7.86 (m, 4H), 7.58 – 7.50 (m, 2H), 7.46 – 7.38 (m, 1H), 7.33 – 7.27 (m, 2H), 6.95 (dd, *J* = 7.7, 2.0 Hz, 1H), 6.62 (d, *J* = 7.7 Hz, 1H), 6.52 (d, *J* = 2.0 Hz, 1H); **¹³C NMR** (100 MHz, DMSO *d*₆) δ 165.20, 157.63 (d, *J* = 11.9 Hz), 137.72, 137.45, 136.73, 135.13, 132.88, 130.09, 129.49, 129.18, 128.69 (d, *J* = 16.5 Hz), 127.64, 120.18, 119.48, 114.44,

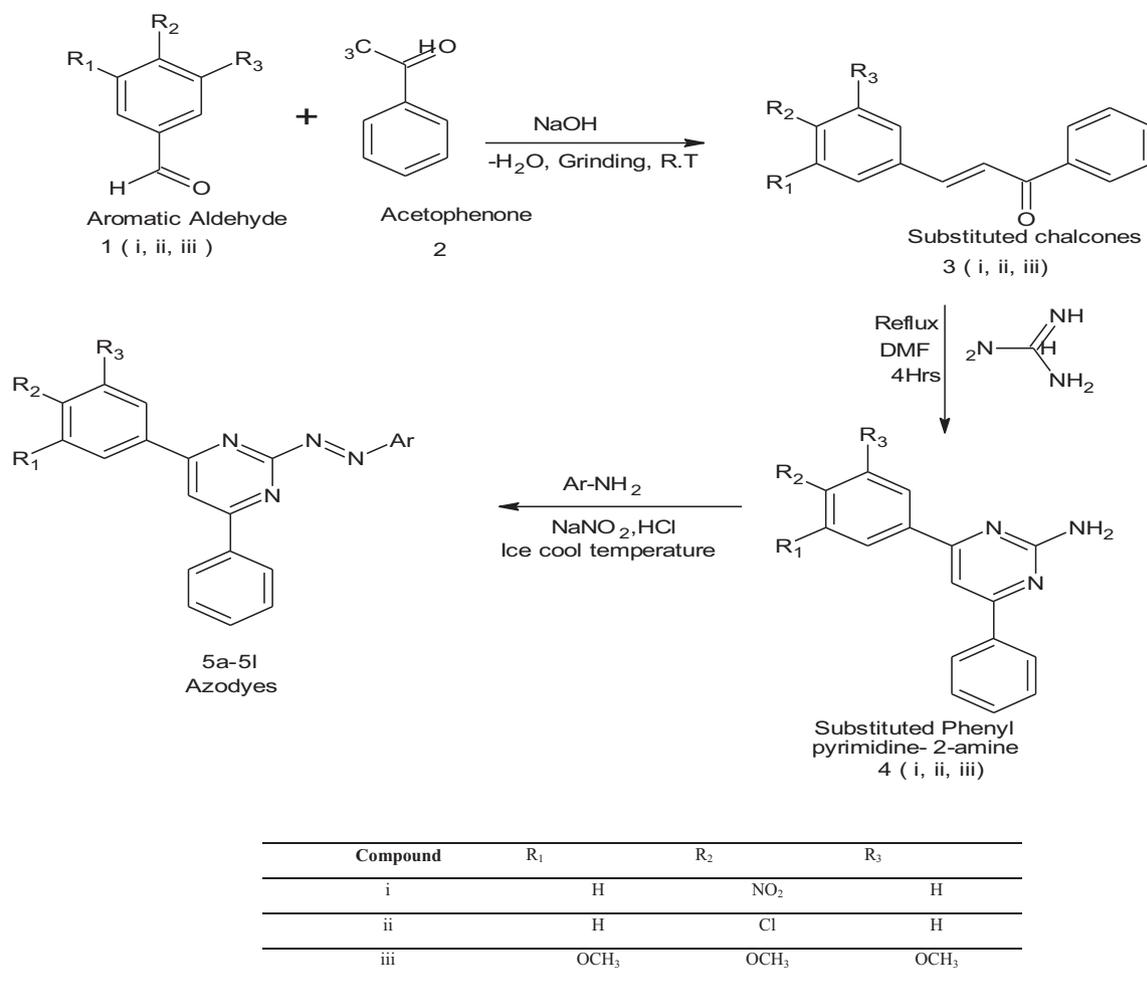
111.83; **FT-IR (KBr, ν max (cm⁻¹)):** 3194 (Ar-CH), 1309 (C-N), 1656 (C = N), 1431 (weak, N = N), 1501 & 3075 (N-H), 1032 (C-Cl); **ESI-MS:** 400.86 [M + H]⁺.

2.3.5. 1-(4-[(Z)-[4-(4-chlorophenyl)-6-phenylpyrimidin-2-yl]diazanyl]phenyl)methanamine 5e

Molecular formula: C₂₃H₁₈ClN₅; **Composition:** C (69.08%), H (4.54%), N (17.51%), Cl (8.87%); **Yield:** 80.63%; **M.P.:** 148°C ± 1; **¹H NMR** (400 MHz, DMSO *d*₆) δ 8.74 (t, *J* = 6.4 Hz, 2H), 8.70 (s, 1H), 7.94 – 7.84 (m, 6H), 7.58 – 7.50 (m, 2H), 7.46 – 7.38 (m, 1H), 7.36 – 7.27 (m, 4H), 4.13 – 4.07 (m, 2H); **¹³C NMR** (100 MHz, DMSO *d*₆) δ 165.20, 157.68, 157.58 (d, *J* = 11.9 Hz), 149.04, 140.20, 138.54 – 122.36 (m), 114.44 (d, *J* = 10.1 Hz), 45.59; **FT-IR (KBr, ν max (cm⁻¹)):** 3199 (Ar-CH), 2893 (Alif-CH), 1309 (C-N), 1656 (C = N), 1445 (weak, N = N), 1518 & 3070 (N-H), 1032 (C-Cl); **ESI-MS:** 399.87 [M + H]⁺.

2.3.6. 5-[(Z)-[4-(4-chlorophenyl)-6-phenylpyrimidin-2-yl]diazanyl]pyrimidin-2-amine 5f

Molecular formula: C₂₀H₁₄ClN₇; **Composition:** C (61.94%), H (3.64%), N (25.28%), Cl (9.14%); **Yield:** 78.03%; **M.P.:** 242°C ± 1; **¹H NMR** (400 MHz, DMSO *d*₆) δ 8.50 (s, 1H), 8.28 (s, 2H), 7.94 – 7.86 (m, 4H), 7.58 – 7.50 (m, 2H), 7.46 – 7.38



Scheme 1 Synthesis of Azodyes.

(m, 1H), 7.33 – 7.27 (m, 2H), 6.61 (s, 2H); ¹³C NMR (100 MHz, DMSO *d*₆) δ 169.92, 163.97, 160.65 (d, *J* = 2.5 Hz), 151.53, 139.89, 137.01 (d, *J* = 17.9 Hz), 132.30, 130.20 (d, *J* = 1.4 Hz), 128.95, 128.65 (d, *J* = 13.5 Hz), 115.68; FT-IR (KBr, ν max (cm⁻¹)): 3191 (Ar-CH), 1309 (C-N), 1656 (C = N), 1480 (weak, N = N), 1519 & 3072(N-H), 1032 (C-Cl); ESI-MS: 387.8 [M + H] + .

2.3.7. 4-[(*E*)-[4-(4-chlorophenyl)-6-phenylpyrimidin-2-yl]diazenyl]naphthalen-1-amine 5g

Molecular formula: C₂₆H₁₈ClN₅; **Composition:** C (71.64%), H (4.16%), N (16.07%), Cl (8.13%); **Yield:** 83.67%; **M.P.:** 216°C ± 1; ¹H NMR (400 MHz, DMSO *d*₆) δ 8.70 (s, 1H), 8.39 – 8.33 (m, 1H), 8.01 (dd, *J* = 7.7, 1.4 Hz, 1H), 7.94 – 7.86 (m, 5H), 7.63 (td, *J* = 7.5, 1.3 Hz, 1H), 7.58 – 7.50 (m, 2H), 7.46 – 7.38 (m, 2H), 7.33 – 7.27 (m, 2H), 6.93 (d, *J* = 7.7 Hz, 1H), 5.45 (s, 2H); ¹³C NMR (100 MHz, DMSO *d*₆) δ 165.20, 157.63 (d, *J* = 11.9 Hz), 146.22, 144.30, 137.45, 135.13, 132.88, 132.19, 130.09, 129.49, 129.18, 128.69 (d, *J* = 16.5 Hz), 128.13, 123.90 (d, *J* = 1.9 Hz), 122.51, 120.51, 114.40 (d, *J* = 10.1 Hz), 109.01; FT-IR (KBr, ν max (cm⁻¹)): 3192 (Ar-CH), 1309 (C-N), 1655 (C = N), 1501 (weak, N = N), 1510 & 3072(N-H), 1032 (C-Cl); ESI-MS: 435.9 [M + H] + .

2.3.8. 4-[(1*E*)-2-[4-phenyl-6-(3,4,5-trimethoxyphenyl)pyrimidin-2-yl]diazen-1-yl]aniline 5 h

Molecular formula: C₂₅H₂₃N₅O₃; **Composition:** C (68.01%), H (5.25%), N (15.86%), O (10.87%); **Yield:** 83.96%; **M.P.:** 198°C ± 1; ¹H NMR (400 MHz, DMSO *d*₆) δ 8.70 (s, 1H), 7.92 – 7.86 (m, 2H), 7.58 – 7.51 (m, 2H), 7.51 – 7.46 (m, 2H), 7.46 – 7.38 (m, 1H), 6.92 (s, 2H), 6.64 – 6.58 (m, 2H), 5.30 (s, 2H), 3.75 (s, 3H); ¹³C NMR (100 MHz, DMSO *d*₆) δ 164.69, 157.94, 157.71, 153.44, 150.43, 141.39, 137.45, 130.30, 129.49, 129.18, 128.63, 125.89, 125.45, 114.99, 114.07, 111.16, 60.68, 56.26; FT-IR (KBr, ν max (cm⁻¹)): 3191(Ar-CH), 2894 (Alif.-CH), 1276 (C-N), 1655 (C = N), 1010 & 1276(C-O), 1489 (weak, N = N), 1503&3070(N-H); ESI-MS: 441.4 [M + H] + .

2.3.9. 4-[(1*E*)-2-[4-phenyl-6-(3,4,5-trimethoxyphenyl)pyrimidin-2-yl]diazen-1-yl]benzene-1,2-diamine 5i

Molecular formula: C₂₅H₂₄N₆O₃; **Composition:** C (65.78%), H (5.30%), N (18.41%), O (10.51%); **Yield:** 79.32%; **M.P.:** 236°C ± 1; ¹H NMR (400 MHz, DMSO *d*₆) δ 8.70 (s, 1H), 7.92 – 7.86 (m, 2H), 7.58 – 7.50 (m, 2H), 7.46 – 7.38 (m, 1H), 6.95 (dd, *J* = 7.7, 2.2 Hz, 1H), 6.92 (s, 2H), 6.62 (d, *J* = 7.7 Hz, 1H), 6.52 (d, *J* = 2.2 Hz, 1H), 3.87 (s, 6H), 3.75 (s, 3H); ¹³C NMR (100 MHz, DMSO *d*₆) δ 164.69,

157.94, 157.71, 153.44, 141.39(d, $J = 575.2$ Hz), 138.86 – 135.66 (m), 133.97 – 124.13 (m), 119.83 (d, $J = 339.7$ Hz), 114.99, 111.83, 111.16, 60.68, 56.26(d, $J = 1767.9$ Hz); **FT-IR (KBr, ν max (cm^{-1}))**: 3191(Ar-CH), 2894 (Alif.-CH), 1318 (C-N), 1659 (C = N), 1017 & 1248(C-O), 1499 (weak, N = N), 1503&3072(N-H);**ESI-MS**: 456.4 [M + H] + .

2.3.10. 1-{4-[(1E)-2-[4-phenyl-6-(3,4,5-trimethoxyphenyl)pyrimidin-2-yl]diazene-1-yl]phenyl}methanamine 5j

Molecular formula: $\text{C}_{26}\text{H}_{25}\text{N}_5\text{O}_3$; **Composition**:C (68.56%), H (5.53%), N (15.37%), O (10.54%); **Yield**: 76.25%; **M.P.**: 142°C ± 1 ; **^1H NMR** (400 MHz, DMSO d_6) δ 8.77 – 8.68 (m, 3H), 7.88 (d, $J = 9.2, 8.0, 1.5$ Hz, 4H), 7.58 – 7.50 (m, 2H), 7.46 – 7.38 (m, 1H), 7.33 (dt, $J = 7.7, 1.0$ Hz, 2H), 6.92 (s, 2H), 4.10 (tt, $J = 6.2, 1.0$ Hz, 2H), 3.75 (s, 3H); **^{13}C NMR** (100 MHz, DMSO d_6) δ 164.69, 157.94, 157.71, 153.44, 140.80 (d, $J = 573.2$ Hz), 137.45, 130.30, 129.49, 129.18, 129.12, 128.89, 128.63, 124.11, 114.99, 111.16, 60.68, 56.26, 45.59; **FT-IR (KBr, ν max (cm^{-1}))**: 3194(Ar-CH), 2892 (Alif.-CH), 1318 (C-N), 1658 (C = N), 1017 & 1247 (C-O), 1499 (weak, N = N), 1502 & 3074(N-H);**ESI-MS**: 455.5 [M + H] + .

2.3.11. 4-[(1E)-2-[4-phenyl-6-(3,4,5-trimethoxyphenyl)pyrimidin-2-yl]diazene-1-yl]naphthalen-1-amine 5k

Molecular formula: $\text{C}_{29}\text{H}_{25}\text{N}_5\text{O}_3$; **Composition**:C (70.86%), H (5.13%), N (14.25%), O (9.76%); **Yield**: 82.36%; **M.P.**: 238°C ± 1 ; **^1H NMR** (400 MHz, DMSO d_6) δ 8.70 (s, 1H), 8.39 – 8.33 (m, 1H), 8.01 (dd, $J = 7.7, 1.2$ Hz, 1H), 7.94 – 7.86 (m, 3H), 7.63 (td, $J = 7.5, 1.3$ Hz, 1H), 7.58 – 7.50 (m, 2H), 7.46 – 7.38 (m, 2H), 6.93 (d, $J = 8.4$ Hz, 3H), 5.45 (s, 2H), 3.75 (s, 3H); **^{13}C NMR** (100 MHz, DMSO d_6) δ 170.42, 160.80, 158.40, 153.02, 146.25, 142.01, 139.74, 137.10, 130.16 (d, $J = 6.0$ Hz), 129.27, 128.95, 128.53 (d, $J = 10.2$ Hz), 127.93, 124.48 (d, $J = 6.8$ Hz), 122.76, 121.12, 116.55, 111.06, 108.15, 60.78, 56.26; **FT-IR (KBr, ν max (cm^{-1}))**: 3191(Ar-CH), 2894 (Alif.-CH), 1318 (C-N), 1658 (C = N), 1017 & 1247 (C-O), 1478 (weak, N = N), 1502 & 3073(N-H);**ESI-MS**: 491.5 [M + H] + .

2.3.12. 5-[(1E)-2-[4-phenyl-6-(3,4,5-trimethoxyphenyl)pyrimidin-2-yl]diazene-1-yl]pyrimidin-2-amine 5l

Molecular formula: $\text{C}_{23}\text{H}_{21}\text{N}_7\text{O}_3$; **Composition**:C (62.29%), H (4.77%), N (22.11%), O (10.82%); **Yield**: 75.13%; **M.P.**: 244°C ± 1 ; **^1H NMR** (400 MHz, DMSO d_6) δ 8.28 (s, 2H), 7.92 – 7.86 (m, 2H), 7.58 – 7.50 (m, 2H), 7.46 – 7.38 (m, 1H), 6.92 (s, 2H), 6.61 (s, 2H), 3.75 (s, 3H); **^{13}C NMR** (100 MHz, DMSO d_6) δ 164.02 (d, $J = 537.9$ Hz), 157.94, 157.71, 153.44, 148.53, 141.39, 137.45, 134.63 – 123.78 (m), 129.49, 129.18, 128.63, 114.99, 111.16, 60.68, 56.26(d, $J = 2121.4$ Hz); **FT-IR (KBr, ν max (cm^{-1}))**: 3192(Ar-CH), 2893 (Alif.-CH), 1318 (C-N), 1658 (C = N), 1017 & 1247 (C-O), 1468 (weak, N = N), 1502&3071(N-H);**ESI-MS**: 443.4 [M + H] + .

2.4. Computational study

The activity of compounds against CYP450 subtypes (CYP2D6, CYP3A4, CYP2C9, CYP1A2, and CYP2C19) was predicted using OCHEM. Bioactivity scores were projected by online Molinspiration cheminformatics. The drug-

likeness and toxicity properties were determined using the OSIRIS property explorer (Data warrior). The human brain permeability and intestinal absorption were predicted by the construction of a Brain Or IntestinaL EstimateD permeation (BOILED), -Egg model, using the Swiss-ADMET web tool.

2.5. Molecular docking:

Docking involved three steps i) protein preparation ii) ligand preparation iii) molecular docking. 2.5.1 Protein preparation:

Protein preparation was performed on the crystal structure of a complex formed between phospholipase A2 and aspirin (Pdb id: 3NT1) using protein preparation wizard (Schrodinger suite LLC) in which water molecules were removed, bond orders were assigned, hydrogens were added, creating zero bond orders to metals, creating disulfide bonds and deleting 5A distance water from the protein.

2.5.1. Ligand preparation

Ligand preparation was done using a pool of tools intended to produce high-quality 3D structures for a variety of drug-like structures beginning with 2D or 3D structures in SD or maestro format. The use of LIGPREP software produces single, low energy, 3D structure with correct chiralities for each of the planned structure. The structures of ligands were downloaded from the PubChem search beta (<https://pubchem.ncbi.nlm.nih.gov/>). Ligand structures were geometrically minimized using the OPLS_2005 force field by LIG PREP molecule of Maestro 11.1(Schrodinger LLC). The prepared ligands were used for docking

2.5.2. Molecular docking

docking was performed using Glide molecule version 11.1.011 in the Schrodinger suite. Induced fit docking was carried out using Schrodinger's Glide.

2.6. Biological evaluation of the synthesized compounds 5a-5l

2.6.1. Anti-inflammatory activity

2.6.1.1. Preparation of RBCs suspension (Juvekar et al., 2009; Sadique et al., 1989). Blood from the healthy human volunteer who has not taken any NSAIDs for two weeks before the experiment was conducted and was transferred into the centrifuge tubes which were then centrifuged at 3000 rpm for 10 min. The RBC's suspension was washed thrice with an equal volume of Normal saline (NS). The volume of blood was measured and reconstituted to 10% v/v suspension with NS.

2.6.1.2. Heat-induced hemolysis (Shinde et al., 1999). The mixture consisting of a 1 ml test sample in a concentration range of 50 – 250 $\mu\text{g}/\text{ml}$ and 1 ml of 10% RBCs suspension were taken into the centrifuge tubes. The tubes were incubated for 30 min on a water bath maintained at 56 °C which was then cooled under running tap water, the mixture was centrifuged at 2500 rpm for 5 min and the OD values of the supernatant were measured at 660 nm. Instead of a test sample, only saline was taken as the control. Aspirin was used as a reference drug.

The % inhibition of Haemolysis was calculated from the formula:

$$\% \text{Inhibition} = \frac{\text{O.D of control} - \text{O.D of test}}{\text{O.D of control}}$$

2.6.2. Anti-oxidant activity

DPPH Free Radical Scavenging Assay (Shridhar et al., 2016)

Sample stock solutions (1.0 mg/mL) were diluted to final concentrations of 2, 4, 6, 8, 10 g/mL, using D. water. One mL of a 0.1 mM DPPH ethanolic solution was added to 2.5 ml of sample solutions of different concentrations and allowed to react under room temperature. The OD values were recorded at 518 nm after 30 min and converted into the % antioxidant activity using the formula:

$$\% \text{Inhibition} = \frac{\text{O.D of control} - \text{O.D of test}}{\text{O.D of control}}$$

From the % Inhibition, the IC-50 values were determined.

3. Results and discussion

The titled compounds i.e. azodyes were synthesized in three steps via the formation of chalcones. Step-1 for chalcone preparation involved solvent-free condition, by frictional force applied through grinding various aldehydes with acetophenone in a mortar and a pestle. (Bathelemy et al., 2016). The observations showed that there was no difference in yield and purity. This method of preparation of chalcones reduced the cost of solvent, time, and usage of organic solvents such as alcohol. This method was more reliable in normal laboratory conditions.

step-2 involved the formation of substituted Phenyl pyrimidine-2-amine derivatives by cyclization of chalcones with guanidine hydrochloride at a temperature of 60 °C in presence of dimethylformamide. DMF is a polar aprotic hydrophilic solvent served as a good catalyst and a reagent. (Heravi et al., 2018) The free primary amino group was diazotized and coupled with various aromatic amines by conventional diazotization of the free amino group in step-3. The presence of azo group was characterized by IR spectroscopy at a frequency between 1500 and 1400 and by the absence of free NH-stretching.

The progress of the reaction and purity of the compounds was assessed by thin-layer chromatography and their melting points. Characterization was done by FTIR, proton and C-13 NMR spectroscopy done on Bruker at 400 and 100 MHz

The present work also focused on computational tools used to predict the properties, toxicities, and activity of the compounds on various targets.

3.1. OCHEM

Cytochrome P450 and its subtypes are an important set of enzymes involved in the metabolism of various drugs. The present work involves the *in-silico* study of the synthesized drug candidates by these enzymes. OCHEM (online chemical modeling), a web-based tool (Sushko et al., 2011), was chosen to evaluate the activity of the synthesized azo dyes against the cytochromes P450 (CYPs). OCHEM predicts the ability of a chemical entity as an inhibitor or non-inhibitor of CYP450 subtypes (CYP3A4, CYP2C19, CYP2D6, CYP1A2, CYP2C9).

The synthesized compounds were established to be inhibitors of the subtypes of cytochrome P450. The compound **5j** (inhibitors of CYP2D6), **5c**, **5d**, **5e**, **5h**, **5i**, **5j** (inhibitors of CYP2C19), **5a-5e**, **5g-5l** (inhibitors of CYP2C9), **5c**, **5d**, **5e**, **5f**, **5g** (inhibitors of CYP1A2), **5a-5e**, **5g-5l** (inhibitors of CYP3A4) & remaining were non-inhibitors of subtypes which was shown in the Table 2.

3.2. Molinspiration cheminformatics

Molinspiration cheminformatics (Nadeem et al., 2016) bioactivity score prediction for drug targets include GPCR ligands, kinase inhibitors, ion channel modulators, enzymes, and nuclear receptors. The larger is the bioactivity score, the higher is the probability of the compound to be active. Therefore, a molecule having a bioactivity score more than 0.00 is likely to possess biological activity, value -0.50 to 0.00 are moderately active and if the score is less than -0.50 it is presumed to be inactive

Compounds **5c-5l** were active, **5a** & **5b** were moderately active on GPCR ligand, **5c-5g**, **5i-5l** were active, remaining were moderately active on enzyme inhibitor, all the compounds were active on kinase inhibitor, **5e** was active, and remaining were moderately active on ion channel modulator, all of them were moderately active on nuclear receptor ligand & protease inhibitors, which was tabulated in Table 3 and have prospective for further structural alteration using QSAR studies.

3.3. Open source program OSIRIS property explorer

Druglikeness, a qualitative concept used in drug design for how “drug-like” a substance is concerning bioavailability usually given a score based on the Lipinski rule of five concepts. The *In-silico* toxicity properties and drug-likeness were obtained from OSIRIS (Sander et al., 2015), property explorer. Compounds **5a**, **5b**, **5c**, **5d**, **5e**, **5f**, **5h**, **5i**, **5j**, **5l** were non mutagenic, **5b**, **5d**, **5e**, **5g**, **5i**, **5j**, **5k** were non tumorigenic (Fig. 1) and **5a**, **5c**, **5d**, **5f**, **5k** were non irritant (Fig. 2)

3.4. Human intestinal absorption (HIA) and brain permeability prediction

Brain permeability and HIA are two important pharmacokinetic parameters that contribute to the bioavailability and cell permeability to a considerable degree. To predict the HIA and brain permeability, we constructed a BOILED-Egg model on the online Swiss ADMET tool by the tendering SMILE notations as input (Daina et al., 2017), which resulted in the creation of the BOILED-Egg model with WLOGP Vs TPSA on x&y-axis respectively (Fig. 3). Molecules that fall in the yellow region depict the BBB permeation whereas the white eclipse region represents gastrointestinal absorption. Among all the analyzed molecules, Compounds **5c**, **5e**, **5f** were well-absorbed but have no access to the brain. Compound **5j** showed passive intestinal absorption. Compounds **5e**, **5j** were substrates for P-gp while the others are nonsubstrates of P-gp efflux. None of the produced compounds could cross the BBB. The conclusion drawn from these studies would be useful to advance further for the enhancement of their pharmacokinetic properties.

Table 2 Activity of Azo compounds against their CYP450 sub types.

Compound	AMES	CYP1A2	CYP2C9	CYP2C19	CYP2D6	CYP3A4
5a	active	–	+	–	–	+
5b	active	–	+	–	–	+
5c	active	+	+	+	–	+
5d	active	+	+	+	–	+
5e	inactive	+	+	+	–	+
5f	inactive	+	–	–	–	–
5g	inactive	+	+	–	–	+
5h	active	–	+	+	–	+
5i	active	–	+	+	–	+
5j	inactive	–	+	+	+	+
5k	inactive	–	+	–	–	+
5l	inactive	–	+	–	–	+

Table 3 Bioactive score of azo compounds against targets.

S. No	compounds	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
1	5a	–0.01	–0.07	0.13	–0.42	–0.24	–0.05
2	5b	–0.05	–0.11	0.15	–0.44	–0.27	–0.03
3	5c	0.12	–0.03	0.26	–0.39	–0.18	0.00
4	5d	0.08	–0.08	0.27	–0.41	–0.21	0.03
5	5e	0.17	0.00	0.28	–0.47	–0.04	0.01
6	5f	0.19	–0.04	0.36	–0.30	–0.20	0.07
7	5g	0.13	–0.01	0.21	–0.33	–0.25	0.03
8	5h	0.05	–0.10	0.24	–0.40	–0.21	–0.01
9	5i	0.01	–0.13	0.25	–0.42	–0.24	0.01
10	5j	0.09	–0.07	0.25	–0.47	–0.10	0.00
11	5k	0.06	–0.13	0.20	–0.35	–0.27	0.01
12	5l	0.11	–0.10	0.32	–0.32	–0.23	0.05

3.5. Molecular docking

Inflammation common symptoms for various ailments and diseases occur due to various eicosanoids released by the action of COX enzymes on arachidonic acid due to cell or tissue damage. Though many classes of drugs were found to be the inhibitors for COX yet a perfect inhibitor was unknown. Several researchers are still working on COX inhibitors to bring out effective and safe drugs used in all types of inflammations. In this research, we used molecular docking to understand the interaction of azodyes with COX-2. GLIDE was used for docking studies. (Kumar et al., 2019). Amongst the synthesized compounds, it was observed that three compounds could dock into the COX-2 active site successfully. The G-Scores were of –6.23, –6.14, and –5.809 kcal/mol were obtained for 5c, 5d, and 5f respectively. The lower score was observed for 5c rationalizes the tighter binding of azodye analog into the COX-2 active site than that of the other two compounds. All the three compounds involved in the hydrogen bonding with a residue Tyr 355, which can be due to the bonding with azo nitrogen and pi interaction of phenyl ring with Tyr 355. The azo dye group was surrounded by residues Ser353, Leu 352, Val 349, Ala527, and Glu 524 of COX-2. Therefore using phenyl azo fragment of the molecule and

Tyr 355 contributed to stabilizing the ligand–enzyme complex. Molecular docking studies further supported the strong inhibitory activity of 5c compared to 5d and 5f (Fig. 4).

3.6. Anti-inflammatory activity

The heat-induced hemolysis method was used to measure the anti-inflammatory activity of the synthesized compounds. The synthesized compounds **5b**, **5c**, **5e**, **5h**, and **5j** exhibited membrane stabilization effect by inhibiting the lysis of the erythrocyte membrane. The percentage of membrane stabilization for synthesized compounds and standard were done at 50, 100, 150, 200, 250 µg/ml, and values were reported in the Table 4. Compound **5c**, **5j** showed maximum inhibition of **71.08%**, **71.91%** respectively which were closer to that of the standard aspirin (72.91%). The anti-inflammatory action of the synthesized compounds is completely concentration-dependent as seen from Fig. 5.

3.7. Antioxidant activity

DPPH assay is widely used to assess the compounds for their antioxidant activity because of its reliability and simple procedure. The results of the DPPH assay of the synthesized com-

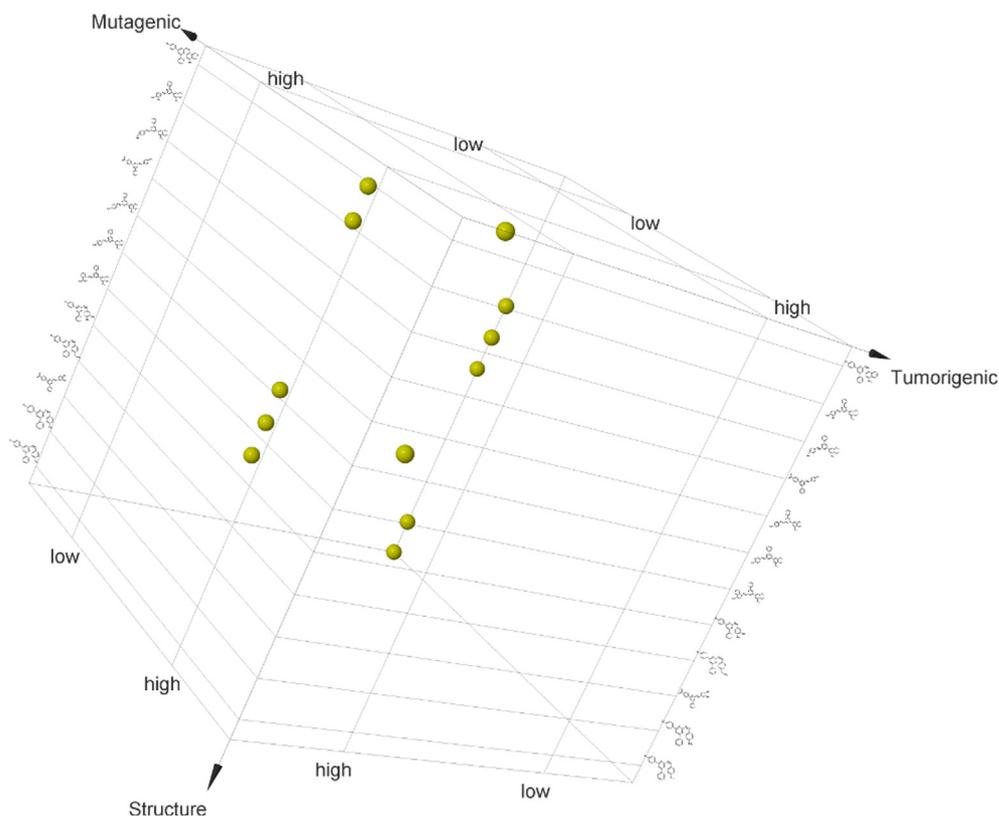


Fig. 1 3D Graph- Structure vs Tumorigenic vs Mutagenic.

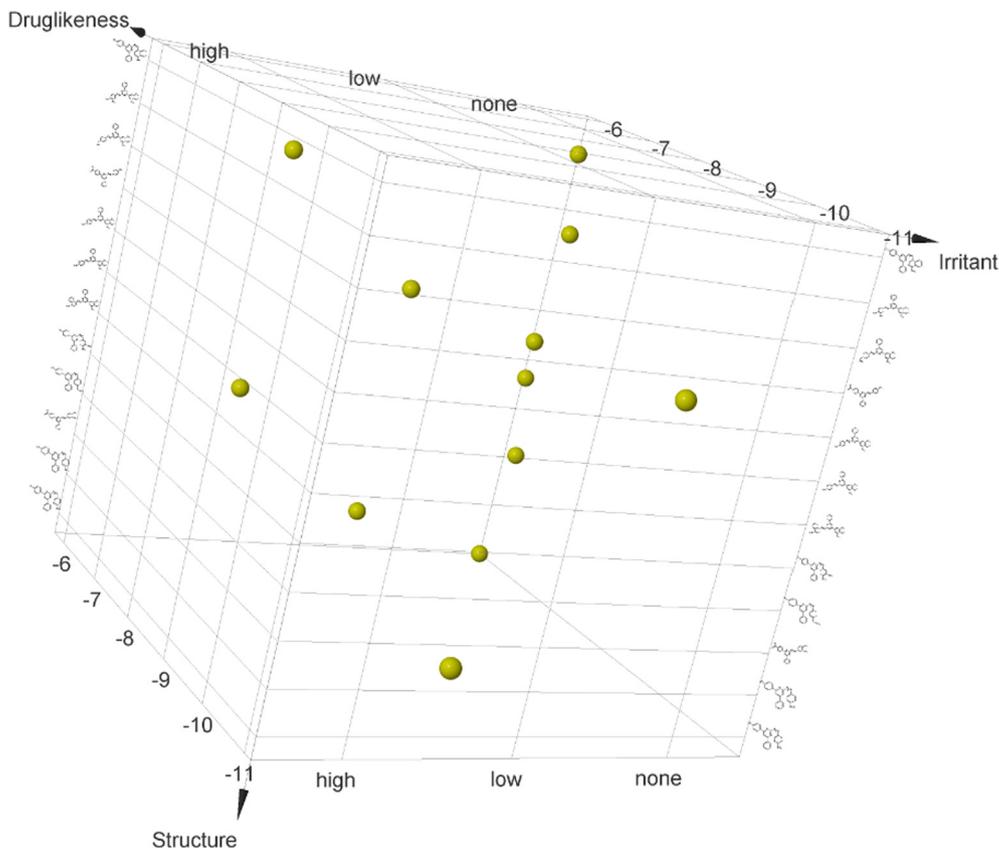


Fig. 2 3D Graph- Structure vs Tumorigenic vs Mutagenic.

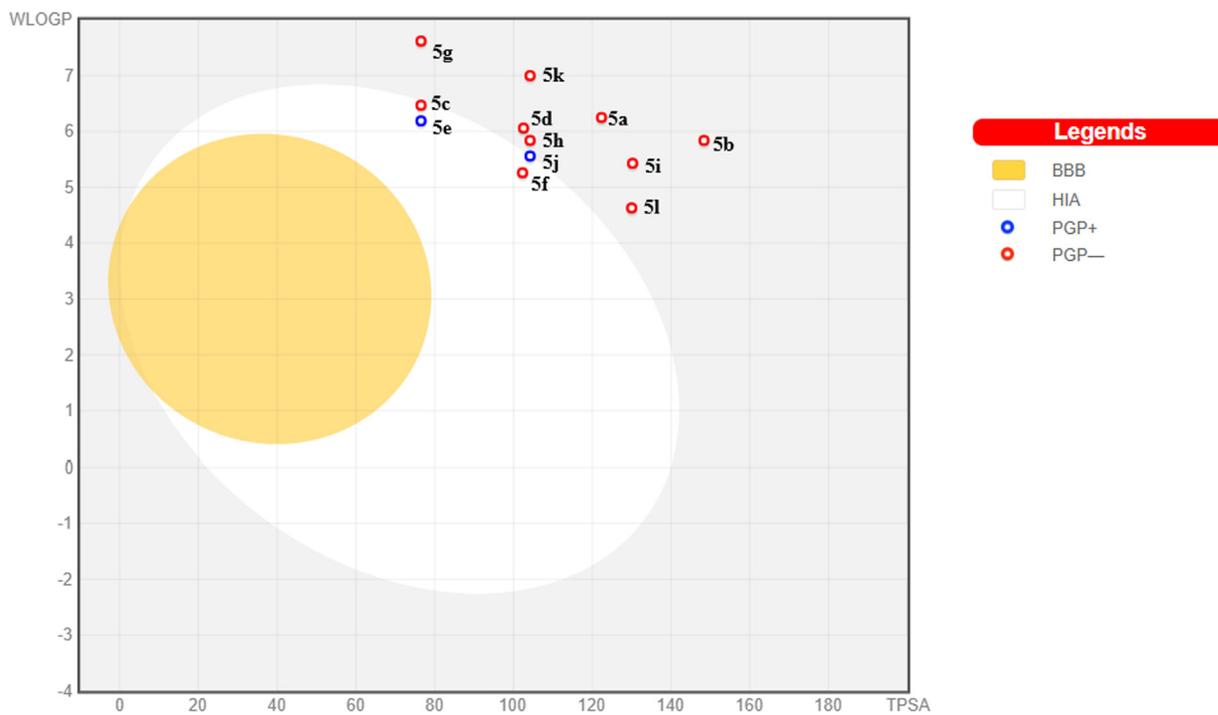


Fig. 3 Graphical representation of Brain Or Intestinal Estimated permeation. **HIA**: Human Intestinal Absorption **BBB**: Blood-Brain Barrier **PGP**: Permeability Glyco Protein.

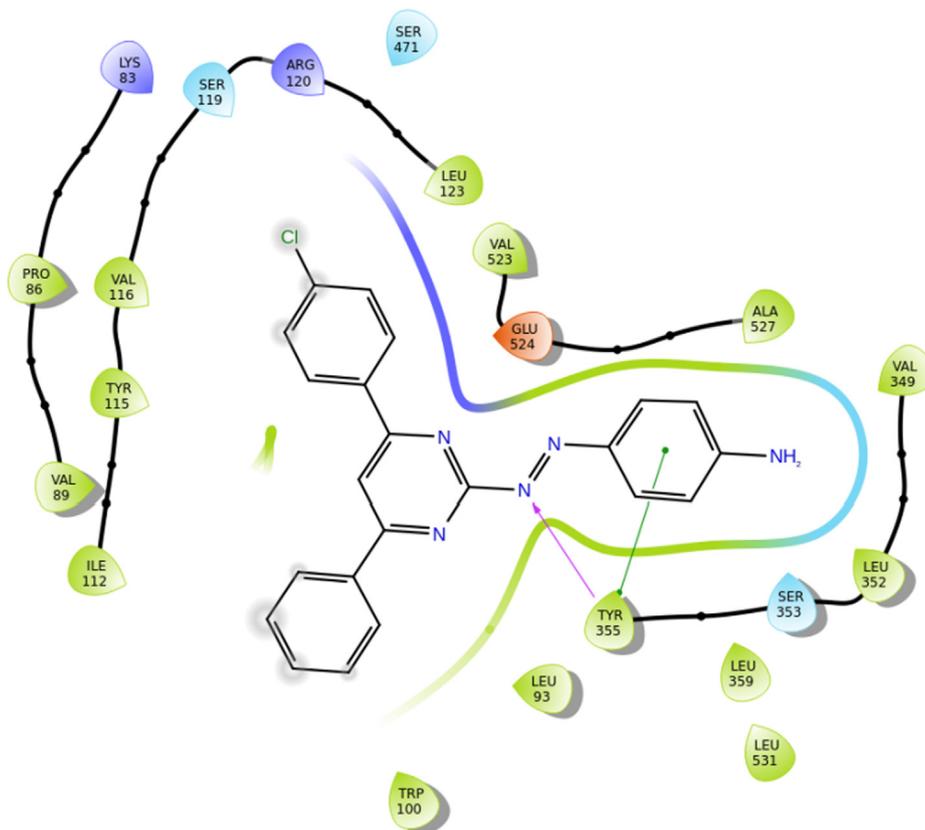
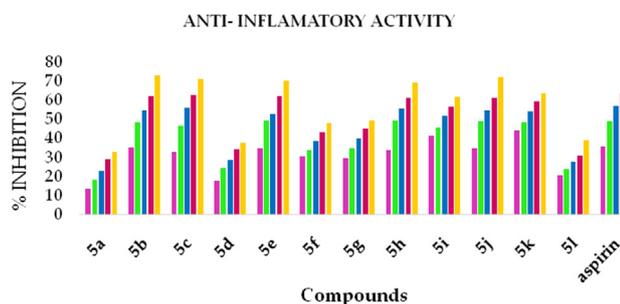


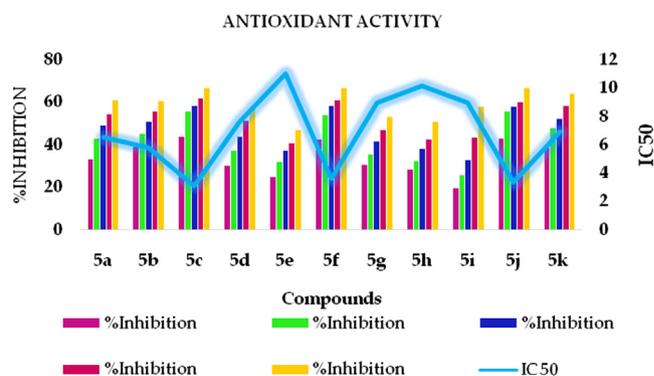
Fig. 4 Molecular docking; The interaction of compound 5c with 3NT1.

Table 4 Anti-inflammatory activity.

S. No	Compound	% inhibition				
		50 µg/ml	100 µg/ml	150 µg/ml	200 µg/ml	250 µg/ml
1	5a	39.32	44.94	52.19	63.31	70.53
2	5b	15.73	21.12	29.21	43.82	50.05
3	5c	24.49	26.74	33.11	40.22	49.21
4	5d	34.05	42.07	49.38	58.34	64.87
5	5e	19.24	23.48	29.59	35.59	42.80
6	5f	25.61	27.83	30.31	34.82	40.44
7	5g	33.23	40.28	46.42	57.23	66.58
8	5h	36.56	47.69	53.58	60.21	69.74
9	5i	26.54	32.85	42.81	49.95	52.91
10	5j	24.58	28.34	33.86	37.29	42.18
11	5k	27.24	32.89	40.47	49.57	55.23
12	5l	34.59	48.20	53.43	61.68	67.92
13	Aspirin	35.29	48.83	56.75	63.27	72.91

**Fig. 5** Concentration-dependent increase in the anti-inflammatory activity of Azo Dyes.

pounds are shown in Table 5. Lower the IC-50 value better than the DPPH activity. In the assay carried out the compounds 5c, 5f, 5j, 5l showed better activities. It was important to note that compounds 5c(3.13) has the IC-50 value closer to

**Fig. 6** Graphical representation of compounds vs % inhibition for anti-inflammatory activity.

that of ascorbic acid (3.03). A concentration-dependent increase in the antioxidant activity of the tested compounds was observed as shown in Fig. 6.

Table 5 Antioxidant activity.

S.No	Compound	IC50	% inhibition				
			2 µg/ml	4 µg/ml	6 µg/ml	8 µg/ml	10 µg/ml
1	5a	6.53	33.21	39.96	47.87	53.41	59.25
2	5b	5.82	39.25	36.21	41.14	48.62	53.54
3	5c	3.13	43.99	30.21	36.81	41.81	48.22
4	5d	7.66	30.41	34.26	40.31	44.59	51.19
5	5e	11.05	24.98	25.80	32.96	43.57	58.23
6	5f	3.61	42.63	48.21	53.57	58.92	57.14
7	5g	8.99	30.85	32.54	39.96	47.26	55.72
8	5h	10.19	28.64	37.44	43.80	51.62	57.92
9	5i	8.94	19.82	45.34	49.85	57.33	61.59
10	5j	3.35	43.01	52.31	55.88	59.67	63.29
11	5k	6.92	38.59	47.83	52.51	58.26	64.11
12	5l	3.91	40.96	42.56	47.16	50.81	57.33
13	Ascorbic acid	3.03	43.91	56.13	59.55	62.72	67.53

4. Conclusion

The results of the present study indicated that all the synthesized pyrimidine azo dyes exhibited antioxidant and anti-inflammatory activities with less toxicity. Among all the 12 compounds **5c** and **5j** were found to greatly influence the activities which may be due to the type of substituents present on the ring. The study carried out considered that the three-dimensional nature of chemical structures plays an important part in ligand-receptor binding and assists in providing an approach for further optimization and development of new leads.

Acknowledgement

This research has been funded by Scientific Research Deanship at the University of Ha'il – Saudi Arabia through project number RG-191339.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.arabjc.2020.09.050>.

References

- Abadi, A.H., Eissa, A.A.H., Hassan, G.S., 2003. Synthesis of novel 1,3,4-trisubstituted pyrazole derivatives and their evaluation as antitumor and antiangiogenic agents. *Chem. Pharm. Bull. (Tokyo)* 51, 838–844. <https://doi.org/10.1248/cpb.51.838>.
- Bathelemy, N., Charles, F., Pantaleon, A., Azeh, N., Estella, T.-F., Hortense, G., Aghem, F., Ronel, T., Olivier, A., Ngadjui, B., 2016. Synthesis and evaluation of antimicrobial properties of some chalcones. *Br. J. Pharm. Res.* 14, 1–11. <https://doi.org/10.9734/BJPR/2016/28243>.
- Bradshaw, T.D., Stevens, M.F.G., Westwell, A.D., 2001. The discovery of the potent and selective antitumour agent 2-(4-amino-3-methylphenyl) benzothiazole (DF 203) and related compounds. *Curr. Med. Chem.* 8, 203–210. <https://doi.org/https://doi.org/10.2174/0929867013373714>.
- Child, R.G., Wilkinson, R.G., Tomcu-Fucik, A., 1977. Effect of substrate orientation of the adhesion of polymer joints, in: *Chem. Abstr.* p. 6031.
- Daina, A., Michielin, O., Zoete, V., 2017. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci. Rep.* 7, 42717. <https://doi.org/10.1038/srep42717>.
- Derkowska-Zielinska, B., Gondek, E., Pokladko-Kowar, M., Kaczmarek-Kedziera, A., Kysil, A., Lakshminarayana, G., Krupka, O., 2020a. Photovoltaic cells with various azo dyes as components of the active layer. *Sol. Energy* 203, 19–24. <https://doi.org/10.1016/j.solener.2020.04.022>.
- Derkowska-Zielinska, B., Matczyszyn, K., Dudek, M., Samoc, M., Czapliski, R., Kaczmarek-Kedziera, A., Smokal, V., Biitseva, A., Krupka, O., 2019. All-optical poling and two-photon absorption in heterocyclic azo dyes with different side groups. *J. Phys. Chem. C* 123, 725–734. <https://doi.org/10.1021/acs.jpcc.8b10621>.
- Derkowska-Zielinska, B., Szmigiel, D., Kysil, A., Krupka, O., Kozanecka-Szmigiel, A., 2020b. Photoresponsive behavior of heterocyclic azo polymers with various functional groups. *J. Phys. Chem. C* 124, 939–944. <https://doi.org/10.1021/acs.jpcc.9b10495>.
- Garg, H.G., Prakash, C., 1972. Potential antidiabetics. 11. Preparation of 4-arylaazo-3,5-disubstituted-(2H)-1,2,6-thiadiazine 1,1-dioxides. *J. Med. Chem.* 15, 435–436. <https://doi.org/10.1021/jm00274a035>.
- Hamid, S.A., 2007. Characterization and thermal decomposition of indolydene aniline azo-dyes derivatives. *Jordan J. Chem.* 2, 133–144.
- Heravi, M.M., Ghavidel, M., Mohammadkhani, L., 2018. Beyond a solvent: triple roles of dimethylformamide in organic chemistry. *RSC Adv.* 8, 27832–27862. <https://doi.org/10.1039/C8RA04985H>.
- Juvekar, A., Sakat, S., Wankhede, S., Juvekar, M., Gambhire, M., 2009. Evaluation of antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata*. *Planta Med.* 75, PJ178. <https://doi.org/10.1055/s-0029-1234983>.
- Kini, S., Swain, S., Gandhi, A., 2007. Synthesis and evaluation of novel benzothiazole derivatives against human cervical cancer cell lines. *Indian J. Pharm. Sci.* 69, 46. <https://doi.org/10.4103/0250-474X.32107>.
- Koshti, S.M., Sonar, J.P., Sonawane, A.E., Pawar, Y.A., Nagle, P.S., Mahulikar, P.P., More, D.H., 2008. Synthesis of azo compounds containing thymol moiety.
- Kumar, D.T., Iyer, S., Christy, J.P., Siva, R., Tayubi, I.A., Doss, C.G.P., Zayed, H., 2019. A comparative computational approach toward pharmacological chaperones (NN-DNJ and ambroxol) on N370S and L444P mutations causing Gaucher's disease, in: *Advances in Protein Chemistry and Structural Biology*. Elsevier, pp. 315–339. <https://doi.org/https://doi.org/10.1016/bs.apcsb.2018.10.002>.
- Kumar, N., Drabu, S., Shalini, K., 2017. Synthesis and pharmacological screening of 4, 6-substituted di-(phenyl) pyrimidin-2-amines. *Arab. J. Chem.* 10, S877–S880. <https://doi.org/10.1016/j.arabjc.2012.12.023>.
- Nadeem, S., Sirajuddin, M., Ahmad, S., Tirmizi, S.A., Ali, M.I., Hameed, A., 2016. Synthesis, spectral characterization and in vitro antibacterial evaluation and Petra/Osiris/Molinspiration analyses of new Palladium(II) iodide complexes with thioamides. *Alexandria J. Med.* 52, 279–288. <https://doi.org/10.1016/j.ajme.2015.10.003>.
- Palleros, D.R., 2004. Solvent-free synthesis of chalcones. *J. Chem. Educ.* 81, 1345. <https://doi.org/doi:10.1021/ed081p1345>.
- Pathan, R.U., Borul, S.B., 2008. Synthesis and antimicrobial activity of Azo compounds containing drug moiety. *Orient. J. Chem.* 24, 1147–1148.
- Racane, L., Stojkovic, R., Tralic-Kulenovic, V., Karminski-Zamola, G., 2006. Synthesis and antitumor evaluation of novel derivatives of 6-amino-2-phenylbenzothiazoles. *Molecules* 11, 325–333. <https://doi.org/https://doi.org/10.3390/11050325>.
- Rathod, K.M., 2011. Synthesis and antimicrobial activity of azo compounds containing resorcinol moiety. *Asian J. Res. Chem.* 4, 734–736.
- Rathod, K.M., 2010. Synthesis and antimicrobial activity of azo compounds containing paracetamol moiety. *Orient. J. Chem.* 26, 1163.
- Rathod, K.M., Thakre, N.S., 2013. Synthesis and antimicrobial activity of azo compounds containing m-cresol moiety. *Chem. Sci. Trans.* 2, 25–28. <https://doi.org/10.7598/cst2013.254>.
- Sadique, J., Al-Rqobahs, W.A., Bughaith, E.I., Gindi, A.R., 1989. The bioactivity of certain medicinal plants on the stabilization of RBC membrane system. *Fitoterapia* 60, 525–532.
- Sah, P.P.T., 2010. The antitubercular activity of azo dyes. I. Bisazo dyes derived from tetrazotized bis(4-aminophenyl)-sulfone and various coupling components. *Recl. des Trav. Chim. Pays-Bas* 69, 1407–1434. <https://doi.org/10.1002/recl.19500691110>.
- Sah, P.P.T., Oneto, J.F., 2010. The antitubercular activity of azo dyes. II. Monazo dyes derived from diazotized sulfa drugs and bis (4-aminophenyl)-sulfone and bisazo dyes from tetrazotized bis (4-aminophenyl)-sulfone and sulfa drugs. *Recl. des Trav. Chim. des Pays-Bas* 69, 1435–1447. <https://doi.org/10.1002/recl.19500691111>.
- Sander, T., Freyss, J., von Korff, M., Rufener, C., 2015. DataWarrior: an open-source program for chemistry aware data visualization and analysis. *J. Chem. Inf. Model.* 55, 460–473. <https://doi.org/10.1021/ci500588j>.

- Shinde, U., Phadke, A., Nair, A., Mungantiwar, A., Dikshit, V., Saraf, M., 1999. Preliminary studies on the immunomodulatory activity of Cedrus deodara wood oil. *Fitoterapia* 70, 333–339. [https://doi.org/10.1016/S0367-326X\(99\)00031-3](https://doi.org/10.1016/S0367-326X(99)00031-3).
- Shridhar, A.H., Keshavayya, J., Hoskeri, J.H., 2012. Synthesis of 1, 3, 4-oxadiazole incorporated azo dye derivatives as a potent Biological activity molecules. *Int. J. Pharm. Pharm. Sci.* 4, 386–390.
- Shridhar, A.H., Keshavayya, J., Peethambar, S.K., Joy Hoskeri, H., 2016. Synthesis and biological activities of Bis alkyl 1,3,4-oxadiazole incorporated azo dye derivatives. *Arab. J. Chem.* 9, S1643–S1648. <https://doi.org/10.1016/j.arabjc.2012.04.018>.
- Sushko, I., Pandey, A., Novotarskyi, S., Körner, R., Rupp, M., Teetz, W., Brandmaier, S., Abdelaziz, A., Prokopenko, V., Tanchuk, V., Todeschini, R., Varnek, A., Marcou, G., Ertl, P., Potemkin, V., Grishina, M., Gasteiger, J., Baskin, I., Palyulin, V., Radchenko, E., Welsh, W., Kholodovych, V., Chekmarev, D., Cherkasov, A., Aires-de-Sousa, J., Zhang, Q.-Y., Bender, A., Nigsch, F., Patiny, L., Williams, A., Tkachenko, V., Tetko, I., 2011. Online chemical modeling environment (OCHEM): web platform for data storage, model development and publishing of chemical information. *J. Cheminform.* 3, P20. <https://doi.org/10.1186/1758-2946-3-S1-P20>.
- Venkatesh, P., N. Pandeya, S., 2009. Synthesis and Anti-inflammatory Activity of Some Novel 2,4-Diaryl-3,5-bis(arylimino)-1,2,4-thiadiazolidine Derivatives. *E-Journal Chem.* 6, 495–503. <https://doi.org/10.1155/2009/645131>.
- Yazdanbakhsh, M.R., Yousefi, H., Mamaghani, M., Moradi, E.O., Rassa, M., Pouramir, H., Bagheri, M., 2012. Synthesis, spectral characterization and antimicrobial activity of some new azo dyes derived from 4,6-dihydroxypyrimidine. *J. Mol. Liq.* 169, 21–26. <https://doi.org/10.1016/j.molliq.2012.03.003>.
- Zollinger, H., 2003. *Color chemistry: syntheses, properties, and applications of organic dyes and pigments.* John Wiley & Sons.