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Azo benzimidazole - A biologically active scaffold

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Abstract : Azo compounds are a very unique class of chemical compounds, drawing considerations in scientific research. Azo compounds are studied as class of organic colorants which have at least a conjugated chromophore azo ($-N=N-$) group in fusion with one or more aromatic or heterocyclic ring system. Benzimidazole derivatives are privileged intermediates for the development of molecules of pharmaceutical or biological interest. Benzimidazole derivatives have gathered wide applications in diverse therapeutic areas such as antiulcer, anticancer agents, and anthelmintic species to name just a few. Although many azo derivatives of benzimidazole nucleus has been reported in literature but only few of them have been evaluated for their biological potencies. This review focuses primarily on those derivatives which are evaluated as anticancer, antibacterial, antifungal, antitubercular, and other medicinal agents. This review may be helpful for the investigators on the basis of substitution pattern on the nucleus with an objective to assist medicinal chemists for developing an SAR on azo benzimidazoles or similar compounds.

Keywords : Azo benzimidazole, A biologically active scaffold.

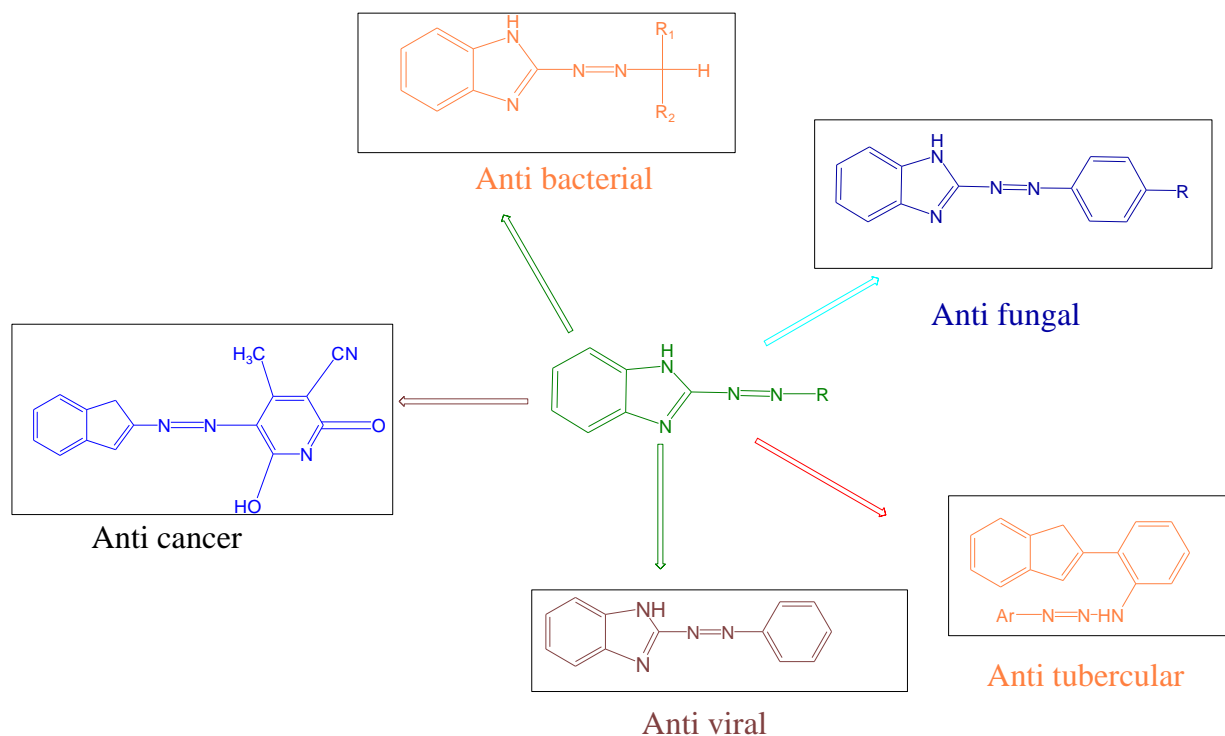
Introduction:

Azo compounds are compounds bearing the functional group diazenyl $R-N=N-R'$, in which R and R' can be either aryl or alkyl group. As per IUPAC, azo compounds are derivatives of diazene (diimide), $HN=NH$, wherein both hydrogens are substituted by hydro carbyl groups, e.g. $PhN=NPh$ azobenzene or diphenyldiazene. The more stable derivatives generally contain two aryl groups. The $N=N$ group is called an *azo group*. Aromatic azo compounds can be synthesized by azo coupling, which involves an electrophilic substitution reaction where an aryl diazonium cation is attacked by another aryl ring, substituted with electron-donating groups.

As noted above, aryl azo compounds are luminously colored and are useful as pigments and dyes. In chemical words, their colours are because of delocalization of π (π) electrons.

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Graphical abstract:

Azo compounds, such as methyl orange and methyl red, are used as acid-base indicators because their acid and salt forms have various colours. Azo pigments are colourless particles (typically earths or clays) that have been coloured using azo compounds. They have excellent colouring properties, again mainly in the yellow to red range, as well as light fastness (that is, colourfast when exposed to light). The light fastness depends not only on the properties of the organic azo compound, but also on the way they have been adsorbed on the pigment carrier. Azo pigments are advantageous because they are non-toxic.

Biological Activities:**Anti bacterial studies:**

Due to the current problem of bacterial infections caused by the increasing resistance of microorganisms to commonly used antibiotics and a limited number of drugs effective in combating with them, it is necessary to constantly search for new chemotherapeutic agents that will be more effective in the fight with microorganisms, less toxic and better tolerated by the patients. Many of the currently tested molecules have in their structure a substituted-1H-azo benzimidazole system and microbiological tests confirm great potential of this class of compounds as antimicrobial agents against Gram-positive and Gram-negative bacterial strains.

Mohammed A. Abdelgawad *et al.*, (2018) reported the reaction of *o*-phenylene diamine / *o*-aminothiophenol with *p*-amino benzoic acid to produce respective substituted benzimidazoles, which was then coupled with various aniline to produce different azo derivatives in good yield (3NQ and 4NQ) ¹.

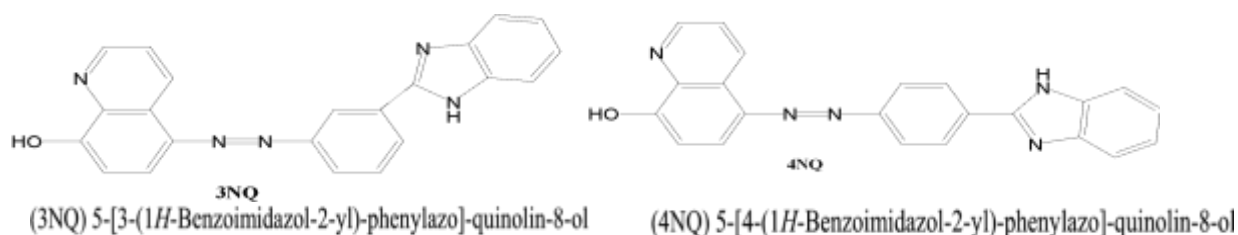


Fig.1 Novel azobenzimidazole derivatives (3NQ and 4NQ) with anti microbial and antifungal activity

3NQ and 4NQ showed a variation antimicrobial effect against each test pathogenic microorganisms. Compounds 3NQ and 4NQ showed a strong inhibitory effect and recorded 23 to 30 mm, but showed a moderate antifungal inhibition effect of 20 mm^{2,3}. The In vitro antimicrobial activity of the synthesized compounds was screened against gram positive (*Bacillus subtilis*) and gram negative (*Escherichia coli*) bacteria, yeast (*Candida albicans*) and filamentous fungi (*Aspergillus niger*). The MIC of the compound 3NQ and 4NQ were 50 µg/ml against *Bacillus subtilis* and *E. Coli* but it was 100 µg/ml and 250µg/mL against *C. albicans*⁴ and the MIC and ZOI values are shown in Table 1.

Table 1 MIC of 3NQ and 4NQ compounds against different pathogenic microorganisms

| Compound No | Inhibition zone diameter (mm) | MIC (mg/ml) |
|--------------------------|-------------------------------|-------------|
| <i>Bacillus subtilis</i> | | |
| 3NQ | 13 | 50 |
| 4NQ | 14 | 50 |
| Streptomycin | 14.00 | |
| <i>E. coli</i> | | |
| 3NQ | 14 | 50 |
| 4NQ | 13 | 50 |
| Streptomycin | 00.00 | |
| <i>C. albicans</i> | | |
| 3NQ | 11 | 100 |
| 4NQ | 13 | 250 |
| Streptomycin | 12.00 | |

According to Y.Lakshmi Narasimha Murthy *et al.*, (2013) 2-Amino benzimidazole was diazotized with ethyl cyanoacetate and malanonitrile in the presence of HCl and sodium nitrite in cold condition for the preparation of compound III a, and IIIb (in separate reactions) and the compounds are shown in Fig.2. Ni (II), Cu (II), and Ag (I) complexes were studied for their antibacterial and antifungal activities by nutrient agar and potato dextrose agar well diffusion method, the ZOI values are shown in Table 2⁵.

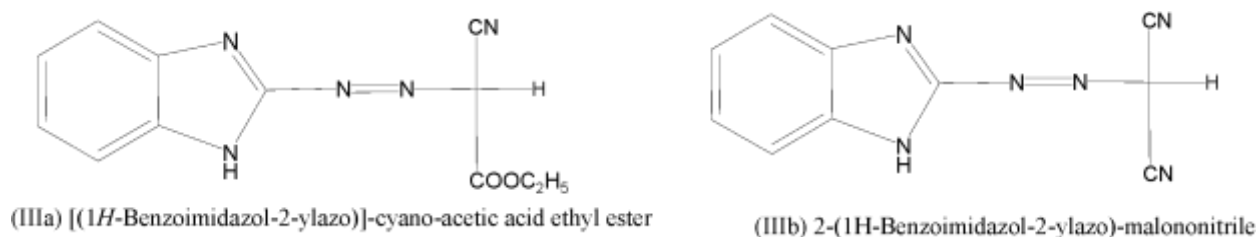


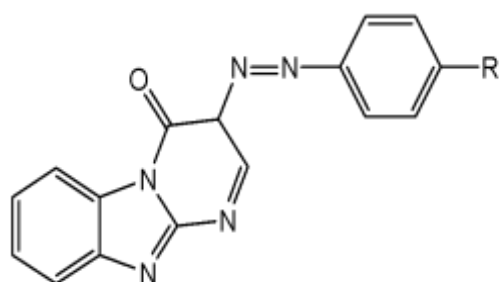
Fig. 2 Novel azo benzimidazole ligands (IIIa & IIIb) with anti microbial activity

According to Calinescu *et al.*, (2008) the synthesized compounds were evaluated against gram positive bacterial strains (*Bacillus subtilis* and *Staphylococcus aureus*) and gram negative bacterial strains (*Escherichia coli* and *Klebsiella pneumonia*). These were also tested against two fungal strains *Aspergillus niger* and *Candida albicans*. Compound III a showed excellent activities against all the species with zone of inhibition in the range of 7.5 mm-9.0mm, but compound III b was only active against *Escherichia coli* with zone of inhibition of 10.75 mm shown in Table 2 ⁶.

Table 2 Antimicrobial data of the investigated compounds (zone of inhibition in mm)

| Compounds | EC | KP | BS | SA | AN | CA |
|------------|-------|-------|-------|-------|-------|-------|
| IIIa | 08.50 | 09.00 | 08.00 | 07.50 | 08.50 | 09.00 |
| IIIb | 10.75 | NA | NA | NA | NA | NA |
| Ampicillin | 31.25 | NA | 29.50 | 30.50 | --- | --- |

P. Sharma *et al.*, (2011) reported that 1*H*-benzimidazol- 2-amine and ethyl 3-oxo-2-[(*E*)-phenyldiazenyl] butanoate was reacted with sodium ethoxide and the products (3a-r) was formed was dehydrated by using anhydrous sodium sulphate, the yield reported was 70-86 % and the compounds were shown in Fig.3.



R= -H,4-OC₂H₅,6-C₂H₅,6-NO₂,4-OCH₃,
4-Cl,6-OH,4-COOH,4-CH₃,4-OH,
6-CH₃,6-OCH₃,6-NO₂,4-C₂H₅,4-Br,
5-CH₃,5-Cl,5-OCH₃.

(3a-r) 3-Phenylozo-3*H*-benzo[4,5]imidazo[1,2-*a*]pyrimidin-4-one

Fig. 3 A substituted azo benzimidazole derivative with antibacterial activity

The newly synthesized compounds were screened for their antibacterial activity against *Pseudomonas diminuta*, *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* using broth micro-dilution method. All the synthesized compounds have shown excellent antimicrobial activities with favourable MIC values are shown in Table 3.⁷

Table 3 Invitro antimicrobial and cytotoxicity of synthesised compounds (3a-r)

| Sl. No. | Entry | R | <i>P. diminuta</i> | <i>B. subtilis</i> | <i>E. coli</i> | <i>S. aureus</i> |
|---------|-------|----------------------------------|--------------------|--------------------|----------------|------------------|
| 1 | 3a | -H | -3.0934 | -3.2574 | -3.1746 | -3.3222 |
| 2 | 3b | 4-OC ₂ H ₅ | -1.1761 | -1.3617 | -1.2553 | -1.4472 |
| 3 | 3c | 6-C ₂ H ₅ | -1.5563 | -1.7243 | -1.6532 | -1.7634 |
| 4 | 3d | 6-NO ₂ | -2.1303 | -2.2900 | -2.2041 | -2.3617 |
| 5 | 3e | 4-OCH ₃ | -2.2304 | -2.3802 | -2.3222 | -2.4698 |
| 6 | 3f | 4-Cl | -2.4232 | -2.5843 | -2.5119 | -2.6532 |
| 7 | 3g | 6-OH | -3.1761 | -3.3426 | -3.2565 | -3.4108 |
| 8 | 3h | 4-COOH | -2.4624 | -2.6021 | -2.5441 | -2.6902 |
| 9 | 3i | 4-CH ₃ | -2.5315 | -2.6928 | -2.6021 | -2.7559 |

| | | | | | | |
|----|------------|---------------------------------|---------|---------|---------|---------|
| 10 | 3j | 4-OH | -3.1303 | -3.3118 | -3.2041 | -3.3598 |
| 11 | 3k | 6-CH ₃ | -2.5740 | -2.7380 | -2.6628 | -2.8162 |
| 12 | 3l | 6-OCH ₃ | -2.3324 | -2.4843 | -2.4393 | -2.5563 |
| 13 | 3m | 4-NO ₂ | -1.9542 | -2.1139 | -2.0607 | -2.1903 |
| 14 | 3n | 4-C ₂ H ₅ | -1.3424 | -1.5441 | -1.4472 | -1.6232 |
| 15 | 3o | 4-Br | -1.7634 | -1.9294 | -1.8751 | -2.0253 |
| 16 | 3p | 5-CH ₃ | -2.6021 | -2.7076 | -2.6284 | -2.8062 |
| 17 | 3q | 5-Cl | -2.5575 | -2.6857 | -2.5911 | -2.7324 |
| 18 | 3r | 5-OCH ₃ | -2.3617 | -2.4698 | -2.3802 | -2.5441 |
| 19 | Ampicillin | -- | -3.2175 | -3.3802 | -3.2742 | -3.4150 |

According to Sujit Kumar Mohanty *et al.*, (2018) a series of some novel azo derivatives of benzimidazole were prepared by coupling diazonium derivative of benzimidazole with various appropriate aromatic compounds. This led to the synthesis of 10 azo benzimidazole derivatives (6a1-5 and 6b1-5)⁸ and the compounds are shown in Fig.4.

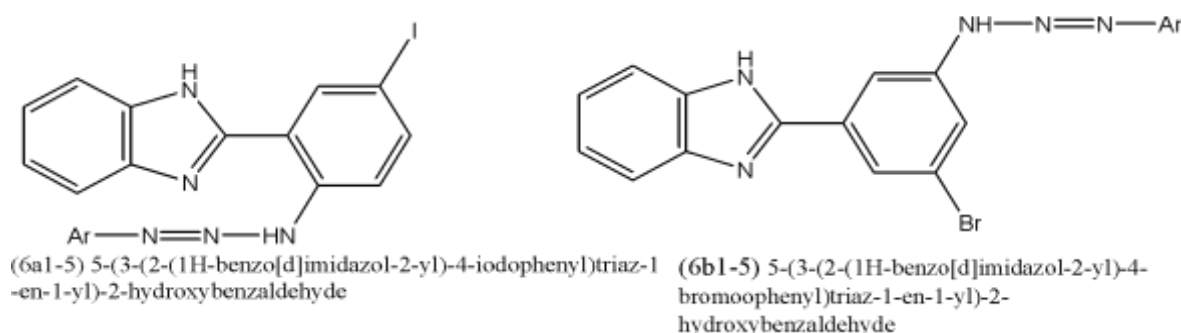


Fig. 4 Some novel substituted azo benzimidazole derivatives with Antibacterial, anti tubercular and Invitro cytotoxicity (6a & 6b 1-5)

The in-vitro antibacterial activities of all the synthesized compounds were tested against Gram positive bacteria (*Bacillus subtilis*) and Gram-negative bacteria (*Escherichia Coli*) by a standard serial dilution method⁹ using a stock solution of 100 mg/ml concentrations. Most of the synthesized compounds exhibited good to moderate inhibitory activities against *B. subtilis* and *E. coli*. All the azole derivatives of benzimidazole were potent antimicrobial agent, with an MIC value ranging from micro molar to sub micro molar. Especially, compounds 6a2, 6a3, 6b2 and 6b3 showed the best antimicrobial activity and the MIC values are shown in Table 4.

Table 4 Invitro antimicrobial and cytotoxicity of synthesised compounds (6a1-5 & 6b1-5)

| Sl No | Compounds | <i>B.subtilis</i> MIC (µM/ml) | <i>E.coli</i> MIC (µM/ml) | <i>M.tuberculosis</i> MIC (µM/ml) | Cytotoxicity MIC (µM/ml) |
|-------|-----------|-------------------------------|---------------------------|-----------------------------------|--------------------------|
| 1 | 6a1 | 0.0264 | 0.1043 | 0.795 | 70 |
| 2 | 6a2 | 0.0135 | 0.0144 | 0.134 | 17 |
| 3 | 6a3 | 0.0176 | 0.0132 | 0.128 | 13 |
| 4 | 6a4 | 0.1892 | 0.3452 | 0.657 | 55 |
| 5 | 6a5 | 0.0823 | 0.0568 | 0.368 | 120 |
| 6 | 6b1 | 0.1761 | 0.1334 | 0.760 | 70 |
| 7 | 6b2 | 0.0121 | 0.0145 | 0.127 | 11 |
| 8 | 6b3 | 0.0112 | 0.0133 | 0.119 | 15 |

| | | | | | |
|----|-------------|--------|--------|-------|-----|
| 9 | 6b4 | 0.1345 | 0.0671 | 0.543 | 112 |
| 10 | 6b5 | 0.2345 | 0.2812 | 0.346 | 40 |
| 11 | Norfloxacin | 0.0127 | 0.0130 | – | – |
| 12 | Isoniazid | – | – | 0.731 | – |
| 13 | Tamoxifen | – | – | – | 12 |

Khalid J. AL-adilee *et al.*, (2015)^{10, 11} reported the synthesis of azo dye compound 2-[2-(benzimidazolyl)azo]-5-amino phenol (BIAAP) by the diazotization coupling reaction with some modifications. The novel azo-schiff base ligand (BIADMebP), was synthesized by condensation of 4,4-dimethyl amino benzaldehyde with azo dye compound 2-[2-(benzimidazolyl)azo]-5-amino phenol (BIAAP) in 70 ml ethanol in the presences 4 drops of glacial acetic acid as a catalyst. Then the above compound was complexed with seven different metal complexes (Co, Ni, Cu, Zn, Pd, and Pt) the % yield was in a range of 64-87 % and the compound was shown in Fig. 5.

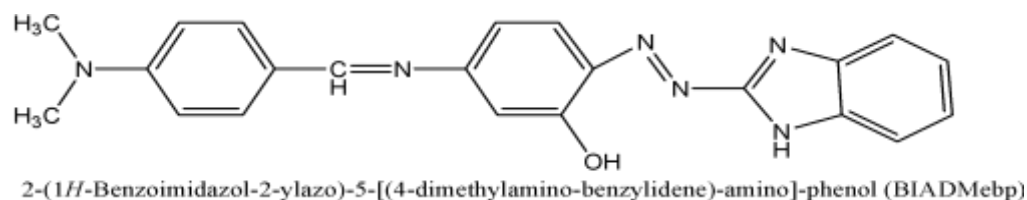


Fig. 5 Some azo-schiff based ligands with antibacterial and antifungal activity

The in vitro antibacterial activity of the azo Schiff base ligand (BIADMebP) and its metal complexes were tested against the bacteria *E.coli*, *staphylococcus*, *Candida albicans* and *Aspergillus niger*. Stock solutions were prepared by dissolving the ligand and its metal complexes in DMF. The diameter of the inhibition Zones was measured in millimetres. All metal complexes showed a remarkable antibacterial activity against bacterial species. The chelate complex revealed that all bacteria were sensitive except the Ni (II)-complex avers activity to *Escherichia coli* while Cu (II) and Zn (II) complexes was insensitive towards *candida albicans*¹². The ZOI values are shown in Table 5.

Table 5 Antibacterial activates data (zone of inhibition in mm) of azo schiff base ligand (BIADMebP) and its chelate complexes.

| Compounds | Anti bacterial activity | | Anti fungal activity | |
|--|-------------------------|-----------------------|-------------------------|-------------------------|
| | <i>E.coli</i> | <i>Staphylococcus</i> | <i>Candida albicans</i> | <i>Aspergillusniger</i> |
| LH=ligand | – | – | – | + |
| [Co(L) ₂].H ₂ O | ++ | ++ | + | ++ |
| [Ni(L) ₂].H ₂ O | – | + | +++ | +++ |
| [Cu(L) ₂].H ₂ O | +++ | ++ | – | + |
| [Zn(L) ₂].H ₂ O | ++ | + | – | + |
| [Pd(L)Cl].H ₂ O | + | ++ | + | ++ |
| [Pt(L) ₂].Cl ₂ | + | + | ++ | ++ |

Antifungal activity:

The number of fungal infections increased more than 200% during last two decades and constitute third most frequent of blood infections¹³. The *Candida* spp. is responsible for 75% of all hospital infections caused by fungi. The contemporaneous used medicines include polien (amphotericin B), azoles (fluconazole and itraconazole) and echinocandins. These drugs cause serious adverse effects so that the result of searching for new chemotherapeutic agents to treat mycobacterial infections is very important¹⁴.

Mohammed A. Abdelgawad *et al.* (2018) reported the reaction of *o*-phenylene diamine / *o*-aminothiophenol with *p*-amino benzoic acid to produce respective substituted benzimidazoles, which was then coupled with various aniline to produce different azo derivatives in good yield (3NQ and 4NQ) and the compounds are present in Fig.1. 3NQ and 4NQ showed a variation antimicrobial effect against each test pathogenic microorganisms. Compounds 3NQ and 4NQ showed a strong inhibitory effect and recorded 23 to 30 mm, but showed a moderate antifungal inhibition effect of 20 mm. The In vitro antimicrobial activity of the synthesized compounds was screened against gram positive (*Bacillus subtilis*) and gram negative (*Escherichia coli*) bacteria, yeast (*Candida albicans*) and filamentous fungi (*Aspergillus niger*). The MIC of the compound 3NQ and 4NQ were 50 µg/ml against *Bacillus subtilis* and *E. coli* but it was 100µg/ml and 250µg/mL against *C. Albicans*. The MIC values are present in Table 1.

Novinson *et al.* (1976)¹⁵ reported the diazotiation of 2-Amino benzimidazole with ethyl cyanoacetate and malanonitrile in the presence of HCl and sodium nitrite in cold condition for the preparation of compound III a, and III b (in separate reactions) and the compounds are present in Fig.2. Ni (II), Cu (II), and Ag (I) complexes were studied for their antibacterial and antifungal activities by nutrient agar and potato dextrose agar well diffusion method and the ZOI values are shown in Table 2.

In vitro antimicrobial activities of the synthesized dinucleating ligands and their Ni(II), Cu(II), and Ag(I) complexes were studied for their antifungal activities by nutrient agar and potato dextrose agar well diffusion method. The synthesized compounds were tested against two fungal strains *Aspergillus niger* and *Candida albicans*. The zone of inhibition of IIIa and IIIb for 50 µl of compound from 1 mg/ml and the ZOI values are shown in Table 2.

According to Khalid J. AL-adilee *et al.*, (2015) the anti fungal activity of the azo Schiff base ligand (BIADMebP) and its metal complexes were test against the bacteria *Escherichia Coli*, *staphylococcus*, *Candida albicans* and *Aspergillus niger*. Stock solutions were prepared by dissolving the ligand and its metal complexes in DMF. The diameter of the inhibition Zones measured in millimetres and all the tested metal complexes show a remarkable antibacterial activity against bacterial species. The chelate complex revealed that all bacteria was sensitive except the Ni (II)-complex avers activity to *Escherichia coli* while Cu (II) and Zn (II) complexes was insensitive to words *candida albicans* and the ZOI values are shown in Table 5.

Yazhen Ke *et al.* (2014)¹⁶ reported the reaction mixture of 5-amino-1H-benzimidazole (III-1, III-2 or III-3) water and concentrated HCl at 0°C, a solution of sodium nitrite in water was added drop wise while maintaining the temperature below 5°C, after stirring for 20 min, a solution of diazonium chloride was prepared. Subsequently, a solution of diazonium chloride was added gradually to a mixture of phenols (IV-1-IV-10), sodium hydroxide, ethanol and water at 0-5°C. After the addition of the above diazonium solution, the mixture was stirred for 3-6 h until a lot of precipitate was produced to give the target products V-1toV-28. The synthesized compounds were shown in Fig.6.

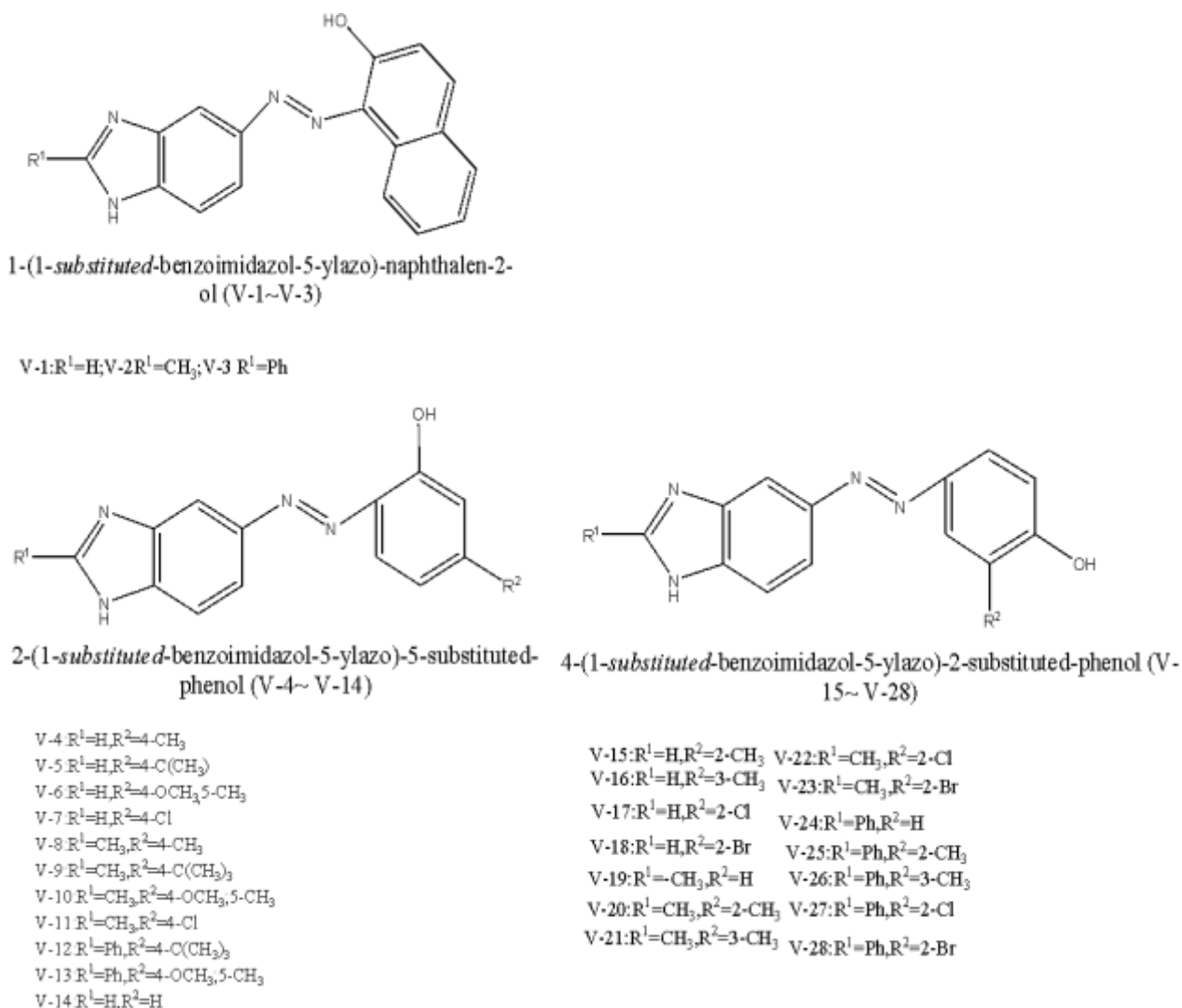


Fig. 6 Some substituted azo phenol benzimidazole derivatives with antifungal activity

Above series of benzimidazole-azophenol derivative were screened in vitro for their antifungal activities at 100 µg/mL against five phytopathogenic fungi such as *Fusarium graminearum*, *Alternaria solani*, *Valsa mali*, *Botrytis cinerea*, and *Curvularia lunata*. Hymexazol, a commercial agricultural fungicide, was used as a positive control. Compounds V-4-V-6, V-10, V-11, V-18, V-22, and V-24-V-26 showed good antifungal activity against *A. solani*; compounds V-4, V-5, V-8-V-11, V-19-V-22, and V-24-V-26 showed good antifungal activity against *V. mali*; compounds V-5, V-6, V-10, V-11, V-19, and V-22-V-26 showed good antifungal activity against *C. lunata*; compounds V-4 and V-5 showed good antifungal activity against *B. cinerea*; compounds V-4, V-5, V-8, and V-9 showed good antifungal activity against *F. graminearum*. Among them, compound V-5 exhibited a good and broad-spectrum of antifungal activities against five phytopathogenic fungi at the concentration of 100µg/mL. The benzimidazole-azonaphthol derivatives (V-1-V-4) generally exhibited less potent antifungal activities than benzimidazole-azo-phenol ones (V-4-V-28). To V-4-V-14, R¹ as the hydrogen atom is an important factor for their antifungal activities. For example, the inhibition rates of V-9 and V-12 at 100 µg/mL against *A. solani*, *V. mali*, *C. lunata*, *B. cinerea*, and *F. graminearum* were 40.2%/21.5%, 58.5%/29.4%, 39.2%/21.9%, 47.4%/21.5%, and 57.4%/3.9%, respectively and the ZOI values are shown in Table 6.

Table 6 % inhibition of Antifungal activity of compound V-1 to V-28

| Compounds | Antifungal Activities (Inhibition %) | | | | |
|------------|--------------------------------------|----------------|------------------|-------------------|-----------------------|
| | <i>A. solani</i> | <i>V. mali</i> | <i>C. lunata</i> | <i>B. cinerea</i> | <i>F. graminearum</i> |
| V-1 | 13.6 (±0.8) | 70.6 (±0.5) | 8.1 (±0.7) | 10.0 (±0.5) | 5.4 (±0.7) |
| V-2 | 16.8 (±1.6) | 35.0 (±1.1) | 14.6 (±0.7) | 13.8 (±0.5) | 12.8 (±0.5) |
| V-3 | 25.2 (±0.8) | 14.8 (±0.6) | 1.2 (±0.7) | 0.0 (±0.5) | 1.4 (±0.4) |
| V-4 | 50.5 (±1.6) | 70.6 (±0.5) | 22.6 (±1.6) | 73.1 (±0.6) | 51.4 (±1.3) |
| V-5 | 66.8 (±0.8) | 76.2 (±0.9) | 68.1 (±1.2) | 86.0 (±1.1) | 84.6 (±0.6) |
| V-6 | 58.4 (±2.1) | 47.7 (±0.5) | 53.1 (±1.8) | 15.6 (±1.1) | 36.6 (±0.9) |
| V-7 | 33.2 (±0.8) | 46.8 (±0.5) | 44.1 (±1.2) | 35.1 (±1.1) | 41.0 (±0.4) |
| V-8 | 35.1 (±0.8) | 57.9 (±0.5) | 22.9 (±2.1) | 42.5 (±1.0) | 51.9 (±1.2) |
| V-9 | 40.2 (±1.6) | 58.5 (±0.9) | 39.2 (±1.6) | 47.4 (±1.7) | 57.4 (±0.6) |
| V-10 | 51.4 (±1.6) | 52.9 (±0.5) | 67.0 (±1.6) | 21.4 (±2.0) | 45.3 (±0.7) |
| V-11 | 54.7 (±1.6) | 65.3 (±1.4) | 74.0 (±1.0) | 44.8 (±1.1) | 31.7 (±0.7) |
| V-12 | 21.5 (±1.4) | 29.4 (±1.8) | 21.9 (±1.0) | 21.5 (±1.2) | 3.9 (±0.9) |
| V-13 | 25.2 (±1.6) | 33.0 (±1.8) | 20.8 (±1.0) | 29.9 (±0.8) | 11.5 (±0.7) |
| V-14 | 42.5 (±1.4) | 49.7 (±1.8) | 35.1 (±1.6) | 24.7 (±0.5) | 34.2 (±0.9) |
| V-15 | 41.6 (±0.8) | 19.7 (±1.5) | 42.0 (±1.6) | 19.2 (±1.6) | 17.1 (±0.9) |
| V-16 | 43.0 (±1.6) | 26.5 (±1.0) | 25.4 (±0.6) | 18.1 (±0.8) | 5.8 (±0.9) |
| V-17 | 19.2 (±1.6) | 20.6 (±1.5) | 31.2 (±1.4) | 12.9 (±1.1) | 6.8 (±0.8) |
| V-18 | 57.5 (±1.6) | 26.7 (±1.0) | 47.0 (±1.9) | 24.8 (±1.6) | 11.1 (±0.6) |
| V-19 | 38.3 (±2.4) | 55.6 (±1.0) | 75.7 (±1.2) | 6.3 (±1.4) | 22.8 (±0.6) |
| V-20 | 45.3 (±1.4) | 67.5 (±1.5) | 26.7 (±0.7) | 30.1 (±0.5) | 36.6 (±1.3) |
| V-21 | 35.5 (±1.4) | 66.6 (±0.6) | 34.0 (±1.9) | 30.4 (±1.9) | 36.4 (±0.6) |
| V-22 | 54.2 (±0.8) | 57.6 (±1.9) | 79.8 (±0.7) | 33.6 (±0.5) | 44.9 (±0.7) |
| V-23 | 41.1 (±1.4) | 45.3 (±1.5) | 72.9 (±0.7) | 10.7 (±0.9) | 18.9 (±0.7) |
| V-24 | 57.7 (±4.1) | 60.8 (±0.6) | 74.9 (±1.4) | 32.0 (±1.4) | 42.0 (±1.2) |
| V-25 | 70.1 (±0.8) | 60.1 (±0.6) | 81.8 (±0.0) | 42.0 (±0.5) | 38.5 (±0.4) |
| V-26 | 58.4 (±0.8) | 49.8 (±0.0) | 62.8 (±0.7) | 22.6 (±0.5) | 37.5 (±0.7) |
| V-27 | 29.5 (±0.8) | 36.7 (±0.6) | 44.9 (±0.7) | 7.2 (±1.1) | 2.1 (±0.7) |
| V-28 | 29.5 (±0.8) | 41.5 (±0.6) | 15.8 (±0.7) | 5.7 (±0.5) | 8.4 (±0.4) |
| Hymexazole | 79.7 (±0.8) | 43.5 (±0.9) | 70.5 (±0.6) | 79.9 (±0.6) | 69.0 (±0.7) |

Antiviral activity:

Mohammad Ashfaq *et al.*, reported the reaction of aqueous NaNO₂ with aqueous aniline. The reaction mixture was stirred for 20 minutes at 0°C, then few drops of 2M HCl was added in the reaction mixture at 0°C with constant stirring for 3hr. A yellow colored benzene diazonium salt was obtained which was neutralized with 1M Na₂CO₃. Then 25 mM active methylene reagent was added drop wise in benzene diazonium chloride solution with constant stirring for 3-4 h until the final product of yellow colored benzene diazonium azo compound was obtained¹⁷. The compound was shown in Fig. 7.

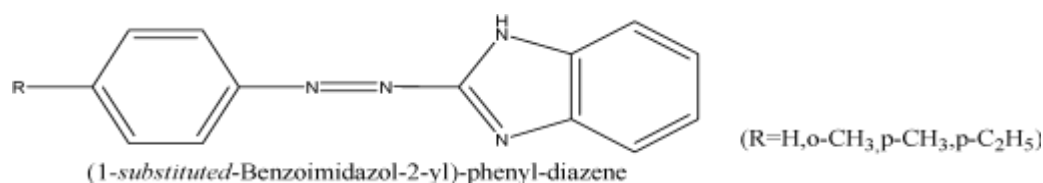


Fig. 7 Some substituted azo phenol compounds with antiviral activity

In vivo antiviral testing of the compound was carried out in developing chick embryos. Nine day-old embryonated chicken eggs were labelled according to the compound used. The Hemagglutination test in case of anti-NDV potential of compound for 100% at 0.1 mg/100 μ L and the compound inhibit 50% of against NDV and AIV (H9N2) viral growth shown in Table 7.

Table 7 Antiviral potential of azo compounds

| Compound | Concentration (Mg/100 μ L) | NDV | | AIV (H9N2) | |
|----------|-----------------------------------|----------|--------------|------------|--------------|
| | | HA titer | % inhibition | HA titer | % inhibition |
| 3 | 0.1 | 128 | 50 | 512 | 40 |

Antitubercular activity:

Tuberculosis is a pernicious infectious disease caused mainly by *Mycobacterium tuberculosis* (MTB) as well as *Mycobacterium bovis* and *Mycobacterium africanum*¹⁸. In agreement with World Health Organization (WHO) report from 2018 tuberculosis is on the list of ten most frequent causes of death throughout the world.

The pandemic of AIDS has notable impact on world complication of tuberculosis. One-third of the increase of incidences of tuberculosis in the last 5 years can be appertaining to HIV co-infection. Additional factor that is concerning the circumstances for the increase of morbidity on tuberculosis and for the increase of mortality is the emergence of new strains of *M. tuberculosis* that are resistant to some or all of current medicines against tuberculosis, which are named as multidrug resistant tuberculosis (MDR-TB)¹⁹. So that the result of increased resistance, newly synthesised compounds with new mechanism of actions are needed to combat with infections.

Sujit Kumar Mohanty, *et al.* (2018) reported that a series of some novel azo derivatives of benzimidazoles the synthesized compounds were shown in Fig.4. The synthesized compounds were subjected to in-vitro anti mycobacterial activity against *M.tuberculosis*²⁰. The 96 wells plates received 100 μ L of the middle brook 7H9 broth and serial dilution of compounds was made directly on the plate. The final drug concentrations were tested at convenient concentrations. A blue colour in the well was indicating as no bacterial growth, and pink colour was scored as growth. These Compounds are 6a2, 6a3, 6b2 and 6b3 are shown good MIC results in Table 4.

Anticancer activity:

Cancer is one of the deadliest diseases nowadays and is a great topic for research as the challenging task is to develop new entities with selectivity towards cancerous cells. It is an essential pharmacophore and an entitled structure in medicinal chemistry. According to the World Health Organisation (2015 survey), one in six deaths is due to cancer around the globe, accounting for 8.8 million deaths of which 70% of the cases were from low- and middle-income countries. In the efforts to develop suitable anticancer drugs, medicinal chemists have focussed on benzimidazole derivatives. This review article covers the current development of benzimidazole-based anticancer agents along with their SAR.

According to Dostanic, J, *et al.*, (2011) ²¹ a mixture of ethyl acetoacetate, cyanoacetamide, and potassium hydroxide in methanol was mixed and heated under reflux for 1 h. Then the solution was acidified with hydrochloric acid. Then the respective compounds of 2a were treated with potassium hydroxide at 0-5°C for 1 h and the compound 3a was obtained.

Moreover a mixture of ethyl benzoylacetate, cyanoacetamide, and potassium hydroxide in ethanol was mixed and heated under reflux for 20 h. Then the solution was acidified with hydrochloric acid. Then the respective compounds of 2b are treated with potassium hydroxide at 0-5°C for 1 h and the compound 3b was obtained. Presented in Fig. 8.

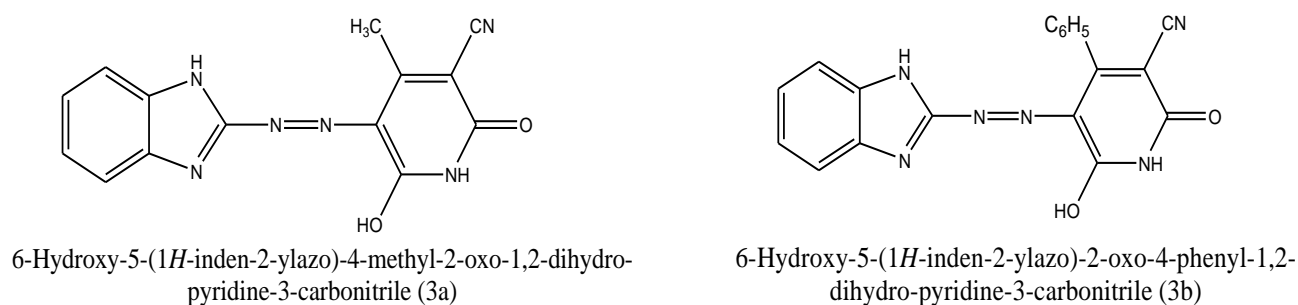


Fig. 8 Some novel substituted azodyes with anti proliferative activity

The antiproliferative activity of the investigated dyes against cancer cell lines HCT-116 and MDA-MB-231 has been tested ²². The assessment of the *in vitro* anti proliferative activity of the dyes against the human breast cancer MDA-MB-231 cell line was performed. It was noticed that both dyes expressed anti proliferative activities against MD-MB-231 cells potential for inhibition of MDA-MB-231 cell proliferation at lower concentrations showed in (Table 8). Dye 3b exhibited greater potential for inhibition of MDA-MB-231 cell proliferation compared to dye 3a, which expressed anti proliferative activity only at higher concentrations (10 and 1 μ M). The anti proliferative activities of dyes against those cells were not as satisfactory as the detected activities against the MD-MB-231 cells. Both dyes exhibited slight potential for inhibition of HCT-116 cell proliferation at lower concentrations showed in Table 9.

Table 8 % of inhibition (μ M) of proliferation of MDA-MB-231 cells

| SI No | Concentration | % of inhibition (μ M) of proliferation of MDA-MB-231 cells | |
|-------|---------------|---|----|
| | | 3a | 3b |
| 1 | 0.01 | 78 | 78 |
| 2 | 0.1 | 80 | 80 |
| 3 | 1 | 78 | 75 |
| 4 | 10 | 75 | 60 |

Table 9 % of inhibition (μ M) of proliferation of HCT-116 cells

| SI No | Concentration | % of inhibition (μ M) of proliferation of HCT-116 cells | |
|-------|---------------|--|----|
| | | 3a | 3b |
| 1 | 0.01 | 78 | 78 |
| 2 | 0.1 | 80 | 80 |
| 3 | 1 | 78 | 75 |
| 4 | 10 | 75 | 60 |

According to Sujit Kumar Mohanty, *et al.* (2018) series of some novel azo derivatives of benzimidazole were prepared by coupling diazonium derivative of benzimidazole with various convenient aromatic compounds. It was reported that the synthesized compounds were subjected to in-vitro cytotoxicity assay against Vero cells. The assay was engaged by the sulforhodamine B (SRB) method. All most of all the compounds exhibited weak cytotoxicity. Compounds 6a2, 6a3, 6b2, 6b3 compounds exhibited significant cytotoxic activity with lesser CTC50 value which shown in Table 4.

Summary:

In summary, this article presents a literature review of azo benzimidazole derivatives with antibacterial, antitubercular, antifungal and anti cancer, anti viral properties. In general, the antimicrobial activity seemed to be more dependent on the nature of the substituents rather the basic skeleton of azo benzimidazole. Presence of para substituted phenyl ring with electron withdrawing group produces better antifungal action when compared to electron donating group. Substitution of azo benzimidazole ring at 2-position with electron donating group produces better antifungal activity. Presence of meta substituted phenyl ring with electron withdrawing group produces better antifungal action when compared to electron donation group. Presence of electron withdrawing group in para position of azo benzimidazole ring increases the anti viral activity. Presence of heterocyclic substitution with electron withdrawing group at p-position increase anti cancer activity. Presence of electron donating group directly attached to the benzimidazoles increase the antibacterial activity against different micro organisms and at the same time, more effective and less toxic.

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