Mini-Reviews in Medicinal Chemistry, 2018, *18*, 1548-1558

REVIEW ARTICLE

Biomedical Applications of Aromatic Azo Compounds

Yousaf Ali[1,2], Shafida Abd Hamid[1*] and Umer Rashid[3]

[1]*Kulliyyah of Science, International Islamic University Malaysia, Bandar Indera Mahkota, 25200 Kuantan, Malaysia;* [2]*Department of Chemistry, Sarhad University of Science and Information Technology, Peshawar, Pakistan;* [3]*Department of Chemistry, COMSATS University Islamabad, Abbottabad Campus 22060, Pakistan*

ARTICLE HISTORY

Received: July 04, 2017
Revised: February 19, 2018
Accepted: May 14, 2018

DOI:
10.2174/1389557518666180524113111

Abstract: Azo dyes are widely used in textile, fiber, cosmetic, leather, paint and printing industries. Besides their characteristic coloring function, azo compounds are reported as antibacterial, antiviral, antifungal and cytotoxic agents. They have the ability to be used as drug carriers, either by acting as a 'cargo' that entrap therapeutic agents or by prodrug approach. The drug is released by internal or external stimuli in the region of interest, as observed in colon-targeted drug delivery. Besides drug-like and drug carrier properties, a number of azo dyes are used in cellular staining to visualize cellular components and metabolic processes. However, the biological significance of azo compounds, especially in cancer chemotherapy, is still in its infancy. This may be linked to early findings that declared azo compounds as one of the possible causes of cancer and mutagenesis. Currently, researchers are screening the aromatic azo compounds for their potential biomedical use, including cancer diagnosis and therapy. In this review, we highlight the medical applications of azo compounds, particularly related to cancer research. The biomedical significance of *cis-trans* interchange and negative implications of azo compounds are also discussed in brief.

Keywords: Azo dyes, cancer, drug delivery, antimicrobial, Azo compounds, antiviral.

1. INTRODUCTION

Compounds containing at least one R^1-N=N-R^2 functional group are called azo compounds. The R^1 and R^2 may be alkyl or aryl group, giving two distinct classes of azo compounds. The name azo comes from '*azote*', a French word used for nitrogen [1]. Aliphatic azo compounds are mostly colorless and relatively less stable than the aryl azo compounds. The C-N bond of an azoalkane cleaves at high temperature or upon irradiation, giving nitrogen gas and radicals. This property enables some alkyl azo compounds to act as radical initiators. For example, azobisisobutyronitrile (AIBN) is used as radical initiator in the polymerization of unsaturated monomers to make plastics [2, 3]. On the other hand, aromatic azo compounds are more common and highly stable. The azo moiety (–N=N-) in this class is conjugated with two, identical or different, mono- or polycyclic aromatic rings. The presence of aryl groups on both sides of -N=N- group extend the delocalized system and make this class more stable. The conjugated/delocalized system absorbs light in the visible range (400-700 nm) and gives characteristic colors to azo compounds, thus called "azo dyes" [4]. They possess deep bright colors, in particular red, orange and yellow. Blue and brown colors are rare. The nature of substituents attached to the aromatic ring and their positions determine the color of the azo compound. In general, the more extensive the conjugated π system of a compound, the longer the wavelength (λ) of visible light absorbed and vice versa [5]. That is why CH_3-N=N-CH_3 is colorless while Ph-N=N-Ph is orange (Fig. **1**).

Colorless Orange

Fig. (1). Examples of aliphatic (colorless) and aromatic (orange) azo compounds. (*The color version of the figure is available in the electronic copy of the article*).

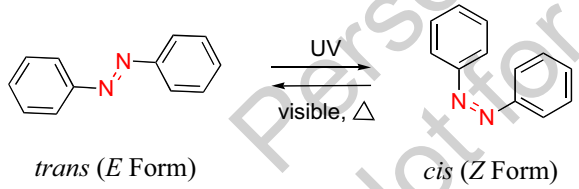
Aromatic azo compounds do not occur in nature and belong to the synthetic class of dyes. The history of azo dyes started in the 1860s when Bismarck brown and aniline yellow were synthesized in the laboratory [6]. Currently, around 10,000 of aromatic azo compounds have been reported. Hamon *et al.* [7] summarized various pathways for the synthesis of azo compounds, which include oxidation of aromatic amines, reduction of aromatic compounds having

*Address correspondence to this author at the Kulliyyah of Science, International Islamic University Malaysia, Bandar Indera Mahkota, 25200 Kuantan, Malaysia; Tel: +60 9570 5003; Fax: +60 9571 571 6783;
E-mail: shafida@iium.edu.my

nitroso group, coupling reaction of arylamines with nitroso compounds, oxidation of hydrazines and reduction of azoxybenzene derivatives. Merino *et al.* [8] reviewed the mechanistic aspects of various methods used for azo synthesis. The usual pathway follows two steps; the formation of diazonium salt and its subsequent reaction with a coupling component (aryl ring). As diazonium salts are usually unstable at room temperature, these reactions are conducted at 0-5°C.

2. ISOMERISM IN AZO COMPOUNDS

Azo compounds exist in two configurations, the *trans* or "*E*" form and the *cis* or "*Z*" form. In general, the *E* isomer is more stable than *Z* isomer. For a simple molecule like azobenzene, the energy difference between the ground state of these two isomers is about 50 kJ/mol. The *cis-trans* interchange happens with external stimuli, such as light or heat. Upon exposure to light of a certain wavelength (350 nm), the stable *trans* form generally photo-isomerizes to *cis* form. In the case of azobenzene the conversion results in a change of the dipole moment from 0.5 D to 3.1 D while end to end distance decreases by about 3.5 Å [9-11]. Thermal or/and photochemical (450 nm) treatment switch back the *cis* configuration into *trans* configuration (Fig. **2**). Thus the conformation of compounds changes without bond breaking. Interestingly, the two isomeric forms with distinct properties usually show different behavior in cellular system. The nature of substituents on the phenyl rings greatly influences the geometry of azo compounds and subsequently their properties and applications. The geometry of the aromatic rings is indicated by ^{1}H NMR spectroscopy. The signals of the *cis* isomer appear relatively at higher field than the corresponding *trans* isomer [12].



trans (*E* Form) *cis* (*Z* Form)

Fig. (2). Light/heat induced isomerization of azobenzene.

3. APPLICATIONS OF AZO COMPOUNDS

Azo compounds are widely used in cosmetics, food, textile and pharmaceutical industries as colorants and additives. Besides their coloring function, they also show biological activities such as antibiotic, antifungal, cytotoxic and antiproliferative properties. One of the important applications of azo compounds is their use in drug delivery, especially in colon-specific drug delivery system. This review is mainly about the medical applications of azo compounds, particularly in cancer research. The target is not to give a full account of all the related reports but rather to highlight the key data that elaborate the current and potential biomedical aspect of azo compounds. The biomedical significance of *cis-trans* interchange and negative implications of azo compounds are highlighted in brief.

3.1. Azo Compounds as Drug Carriers

Azo compounds have the ability to act as drug carriers, either by acting as a 'cargo' for active species or by prodrug approach. The prodrug (also called predrug or proagent) was termed by Albert in 1958 for pharmacologically inactive entity which is converted to its parent active form by chemical or/and enzymatic method [13, 14]. The active drug and the nontoxic promoiety are released by internal or external stimuli in a targeted site within the body. A series of publication about prodrug concept is already presented by Karaman's group [15-18]. The cleavable character of azo bonds and their applications is recently reviewed by Mulu *et al.* [19].

Azo prodrugs are designed for specific release of therapeutic amines in colon [20]. Therapeutic agents are conjugated *via* azo linkage and are subsequently released by the action of azoreductase enzyme. The azoreductase sensitive system is useful in colon-targeted drug delivery for the treatment of related diseases, such as colorectal cancer, inflammatory bowel disease and amoebiases [21]. The enzyme acts as a trigger for releasing drug agent from the drug carrier. The main advantage of this approach is that the administrated drug bypasses the acidic environment of the stomach, which is the prime requirement in colon specific drug delivery. For example, the conjugation of 5-aminosalicylic acid, an anti-inflammatory drug, with polyamidoamine dendrimer *via* azo linkage produces a stable product that safely reached into the colon and releases the active drug upon azo bond reduction [22]. Kennedy *et al.* [23] synthesized the mutual azo prodrugs from antimicrobial peptides and non-steroidal anti-inflammatory (NSAIDS) agents. Azoreductase splits the prodrug into two distinct therapeutic agents, antimicrobial peptide and NSAID (Fig. **3A**) that target the infection and inflammation caused by *Clostridium difficile*.

Gemcitabine, methotrexate and oxaliplatin are commonly used anticancer drugs. In 2013, Sharma *et al.* [24] prepared azo-linked prodrugs of these compounds (Fig. **3B-D**) and their analogs and studied their subsequent release in colon site. They performed azoreductase assay in the presence of rat fecal and found that 70-80% of drug contents were released from the synthesized prodrugs. As these azo prodrugs were found stable both in acidic and basic buffers, they are supposed to be safe in upper gastro intestinal tract environment. Cytotoxic assay of the compounds showed good activity against colorectal cancer cell lines. In a separate study [25], the same group reported the *ex vivo* release (85-90%) of anticancer drugs conjugated from polyphosphazene-based prodrugs.

However, this approach is not applicable in wide-range, as it is limited to drug molecules that have aromatic amine for azo linkage. Therefore, such modification is only useful for those colorectal drugs which have amine functional group that can be diazotized to make azo linkage with suitable carrier.

The external stimuli that cause *cis-trans* interchange of azo compounds have therapeutic applications in terms of drug delivery. Recently, Wang *et al.* [26] reviewed stimuli-responsive dendrimers for the treatment of various diseases,

emphasizing on cancer. A comprehensive review describing light switchable active molecules (including azo compounds) is published by Mayer and Heckel [27].

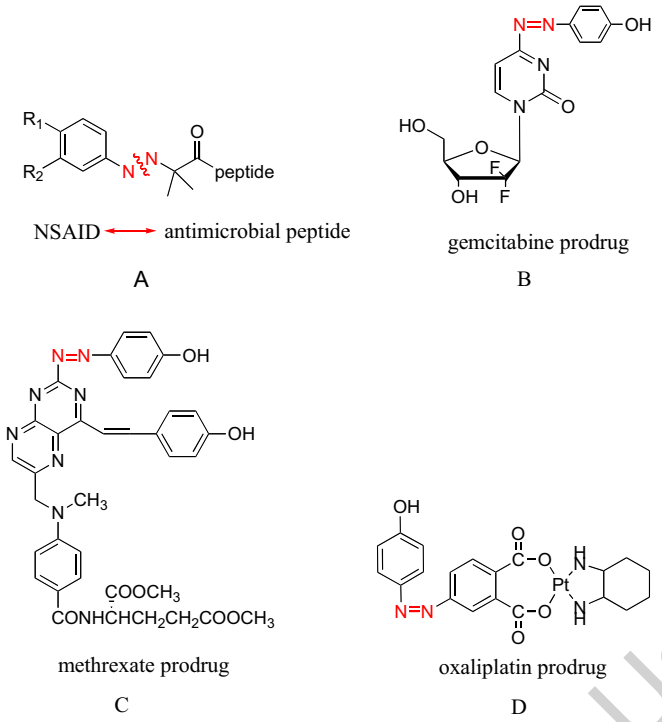


Fig. (3 A-D). Examples of azo prodrugs.

The encapsulated contents (drug) can be released from liposome if photoisomerizable azo moieties are incorporated into its bilayer membrane. Yagai *et al.* [28] incorporated azobenzene-based phospholipids into the bilayer membrane of liposome. They found that the conformational change of azobenzene from *trans* to *cis* upon UV illumination disrupted the liposome bilayer packing, and thus allowed the release of entrapped compounds. Similarly, Tong *et al.* [29], synthesized amphiphilic diblock copolymer containing both hydrophobic and hydrophilic segments, made of azobenzene polymethacrylate (PAzoMA) and poly(acrylic acid)(PAA), respectively. A reversible *trans-cis* photoisomerization was observed in the azobenzene (hydrophobic segment) by changing the UV/Vis light irradiation, which caused change in the morphology of polymer. A similar morphological change was also observed in Mesoporous Silica Nanoparticles (MSNs) bearing azobenzene moiety. The *cis-trans* interchange of azo part altered the geometry of nanoparticles that subsequently released the entrapped therapeutic agents from the pores of MSN [30]. Mas *et al.* [31] reported that the entrapped chemotherapeutic drug loaded in the silica mesoporous material can be capped by azopyridine derivative, which acts as a "gate keeper". The gate was opened by the action of azoreductase enzyme. A similar approach was applied by Li *et al.* [32] , where the opening/release of entrapped contents occurred as a result of azo bond cleavage by the action of azoreductase. Such approach could potentially be applied in colon-targeted drug delivery system. Looking forward, Wang *et al.* [33] did post-modification of MSN, using azobenzene/β-cyclodextrin as supramolecular valves. The loaded anticancer drug (doxorubicin) was released from the nanopores of MSNs by red light that caused *trans-cis* interchange of azo part.

Despite these facts, light irradiation of wavelength below 700 nm has certain limitations. It can only penetrate up to 1 cm deep into living tissues. Therefore UV or blue light can act as a triggering agent in medical conditions associated with skin or exterior layers of some internal organs. On the other hand, red light effectively penetrates tissue in most organisms, and thus extends studies on whole living animals [34]. The use of Near-Infrared (NIR) light is more promising as hemoglobin/water and lipids have their lowest absorption coefficient in this region. Therefore, such light is more useful for triggering a drug release in interior areas of the body [35]. Besides functioning as triggering agent, NIR fluorescent core–shell silica-based nanoparticles, known as Cornell dots (or C dots), have also been recently permitted by FDA for human stage I molecular imaging of cancer [36].

Some MSNs have been modified to degrade thermally and release the entrapped drug. In 2012, Saint-Cricq *et al.* [37] designed such thermally degradable drug delivery nanoparticles in which the drug could be released by the action of magnetic field. The core-shell Fe_3O_4@SiO_2 mesoporous silica nanoparticles (MSN) were impregnated with rhodamine 6G, a model therapeutic that has similar size to many anticancer agents and possess good thermal and optical stability. The nanoparticles were coated with thermo responsive azo-functionalised polymer (poly(ethylene glycol))(PEG). The results of their experiments showed that the rhodamine 6G was well-trapped inside the mesoporous silica nanoparticles by the polymeric caps and was successfully released from MSN-Azo-PEG particles through heating caused by high frequency oscillating magnetic field.

3.2. Azo Compounds as Anticancer Agent

To date, there is no azo compound marketed as anticancer drug. However, based on *in vitro* and *in vivo* studies, the anticancer potential of various azo compounds has been suggested by a few researchers. Sulfasalazine (Azulfidine®) (Fig. **4A**), a well-known anti-inflammatory drug that also showed antifibrogenesis effect [38], was previously recommended for brain cancer fits [39]. A clinical trial study of sulfasalazine was completed, in which its effect on glutamate levels was determined in patients with glioma by magnetic resonance spectroscopy [40]. Some recent reports that described the ability of other azo compounds as an anticancer agent are highlighted below.

Ran *et al.* [41] reported some azo and azoxy-based Schiff base derivatives as antiproliferative and cytotoxic agents against HeLa cell lines. The structures of active compounds are given in (Fig. **4B-D**). The bioactivities of azo compounds were found to be better than the compounds having azoxy groups. Thiophene ring-based azo compounds were evaluated for their cytotoxic potential by Farghaly *et al.* [42]. Some of these azo compounds exhibited better antitumor activity against *Ehrlich Ascites Carcinoma* tumor cells compared to doxorubicin. In another study [43], the same group synthesized fused azolotriazino-benzosuberones and evalu-

ated their antitumor activity. The *in vivo* efficacy was studied against murine Colon 38 cancer and compared to standard anticancer drug, etoposide. The GI_{50} values of the tested compounds were found in the range of 0.6-0.7 for different derivatives. Further, relaxation assay performed to examine the effects of the same series and etoposide on the inhibition of topoisomerase II activity showed that all the examined compounds were more active than etoposide.

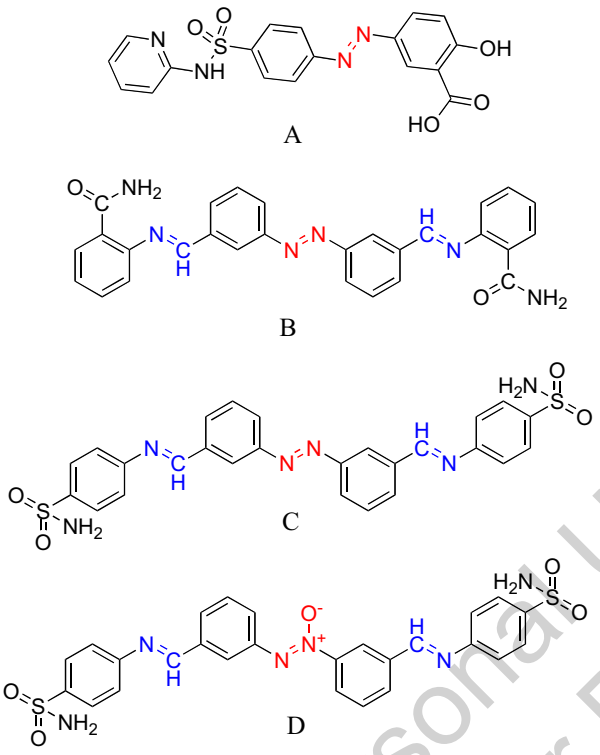


Fig. (4). Structures of active azo compounds; Sulfasalazine **(A)**, azo **(B, C)** and azoxy-based Schiff base derivatives **(D)**.

Overexpression of protein tyrosine phosphatase receptor-type Z (PTPRZ) was reported in glioblastoma and other tumors such as neuroblastoma gastric cancers and small-cell lung carcinoma. The intracellular delivery of acid red 27 (food dye) and its analogues showed inhibition of PTPRZ activity in C6 glioblastoma cells and suppressed their migration and proliferation *in vitro*. However, low membrane permeability and substantial instability of these compounds in living cells were declared as two major upsets that need further investigation [44]. Some azo dyes have been studied for therapy and diagnosis of diseases associated with central nervous system. Various examples of such dyes are listed and discussed by Kaur *et al.* [45].

4. ACTIVITY-CONFORMATION RELATIONSHIP OF AZO COMPOUNDS

Structural configuration specifies the medical stand of chemical compounds. The *cis-trans* interchange of azo compounds has momentous role in their therapeutic stand. For example, *cis* platin, a well-known anticancer drug, recommended in almost all types of solid cancer and mutagenesis, displays different pharmacological activity than *trans* platin [46]. These two isomeric forms usually show different activity in cellular system. For example, Engdahl *et al.* [47] investigated azo-combretastatin A4 (azo-CA4) (Fig. **5A**) as light-activated tubulin polymerization inhibitor. The photoisomerization of azo-CA4 was done by a simple consumer grade LED flashlight. *In vitro* inhibition of tubulin polymerization by azo-CA4 is significantly increased in the presence of isomerizing light. The cytotoxicity of the azo-combretastin A4 compound was conducted using human cervical cancer (HeLa) cells. During MTT assay they found that azo-CA4 showed little to no toxicity in the absence of light even at high concentration (100 μM). However, in the presence of light, azo-CA4 resulted in complete cell death at concentration as low as 500 nM (200-fold more in bioactivity). These findings support the work of Borowiak *et al.* [48] who explained the light dependent mechanism of action of these compounds. They found that *cis* isomers (under blue light) are up to 250 times more cytotoxic than *trans* isomers (kept in the dark). On the same note, Sheldon *et al.* [49] reported the automatic turn-off activity of Azo-CA4 and demonstrated the distinct effects of *cis* and *trans* isomeric forms. The potency of these compounds against human umbilical vein endothelial cells was enhanced 13-35 fold upon illumination. In the presence of light, the compound adopted *cis* conformation and has EC_{50} values in nM range. Additionally, over the time, the compound automatically reverted back to its less toxic *trans* form and has the potential to turn off its activity automatically with time. Due to this switchable potency, similar compounds might have the ability for automatic turn-off mechanism that might be helpful in site specific drugs controlling and antitumor activity. Later on, Rastogi *et al.* [50] synthesized analogue of Azo-CA4 (Fig. **5B**) replacing the methoxy group of ring A with ethoxy group and observed improved activity against HeLa and H157 cancer cell lines.

Enzyme properties and activity can be changed with light by introducing azo-bridges into enzyme inhibitors and activators. Ferreira *et al.* [51] presented a report on azobenzene-derived photoswitchable RET kinase inhibitor (Fig. **5C**). Both cell-free and live-cell experiments were performed and photoisomerization from the *E* to *Z* form was achieved *in situ* with a concomitant decline in the inhibitory result. In cell free assay, the IC_{50} values for the two photo-isomers were found to be 150 nM (*E* form) and 580 nM *(Z* form*)*. In cell-based functional assay, using beta galactosidase-based enzyme fragment complementation technology to give enzyme activity correlated luminescence readout, the IC_{50} values for the two photo-isomers (*E* and Z forms) were observed to be 3.8 μM and 12 μM, respectively.

5. COORDINATION CHEMISTRY OF AZO COMPOUNDS AND THEIR APPLICATIONS

Metal coordination compounds show biological applications and are used as therapeutic agents [52, 53]. Transition metals are predominantly favored compared to other metals as they can adopt a wide range of coordination numbers and oxidation states. Azo compounds can form metal complexes with the nitrogen atoms of azo functional group or/and through other donors like OH, NH_2, C=O or SH that are pre-

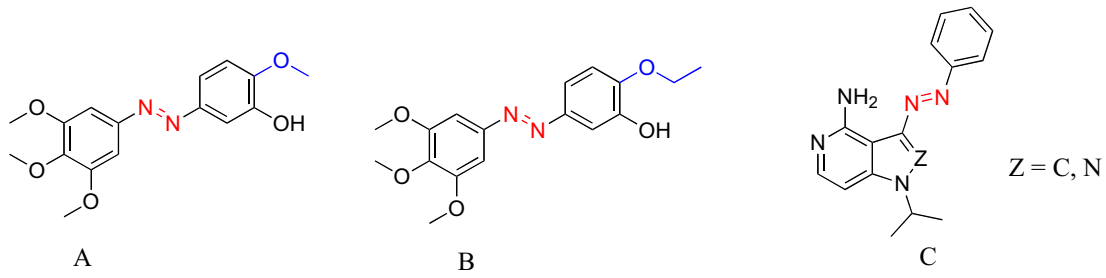


Fig. (5). Structures of azo-CA4 **(A)** analogue of azo-CA4 **(B)** and azobenzene-derived RET kinase inhibitor **(C)**.

sent in the azo compound. However, as the azo groups have weak donor properties, the presence of these donating groups in congenial position(s), most likely in conjugation with azo group, is imperative for the formation of stable complexes [54]. However, there are some cases whereby these complexes sometimes showed better applications than the parent azo compound. For example, when the antibacterial potential of sulfamethoxazolyl-azo-*p*-cresol and its copper (II) complexes were investigated, the complexes showed better activity than sulfamethoxazolyl-azo-*p*-cresol alone [55]. In a study conducted by Mahmoud *et al.* [56] a series of new azo compounds and their complexes with various metals *i.e.* Cu(II), Zn(II) Cd(II)), Mn(II), Cr(III), Fe(III), Co(II), Ni(II) were studied for their antifungal, antibacterial and anticancer activities. All these metal complexes except Mn(II) showed significant antibacterial activity compared to parent azo ligand. However, only Co(II), Ni(II) and Cd(II) complexes exhibited antifungal activities due to lipophilic nature. In anticancer evaluation against breast carcinoma (MCF7 cell line), Zn(II) complex presented the maximum anticancer potential with IC_{50} value of 12.0 $1mL^{-1}$. Sarigul *et al.* [57] investigated the antiproliferative activities of azo-azomethine compounds and their copper (II) complexes against HeLa cell line. The compounds were found to exhibit higher anticancer activity than 5-fluorouracil at various concentrations (25, 50 and 100 µg mL^{-1}). Similarly, the metal complexes of 2-amino-1,2,4-triazole azo dyes have shown antibacterial as well as antifungal activities [58].

The antidiabetic activities of triorganitin (IV) complexes with azo-carboxylates were reported by Roy *et al.* [59]. Investigation on α-glucosidase enzyme showed better results than acarbose, the standard drug. In a separate study, the same group synthesized diorganotin (IV) complexes with (*E*)-5-((2-carboxyphenyl)diazenyl)-2-hydroxy benzoic acid and examined their α-glucosidase enzyme inhibition potential for their antidiabetic properties evaluation. They also showed better antidiabetic activity than acarbose. Some azo-containing schiff base ruthenium(II) complexes have been recommended to be used in drug, food and cosmetic industries Due to strong antioxidant activity [60].

However, there are cases where some complexes showed less activity than the parent azo compounds. For example, Gaber *et al.* [61] evaluated the antitumor activity of triazole and thiadiazole-based azo compounds and their complexes with copper nanoparticles. All the azo complexes exhibited less cytotoxicity than the respective free azo ligands. Similarly, Sarigul *et al.* [62] evaluated some copper (II) complexes with azo dyes that were inactive against different bacterial strains.

Solid tumors contain regions with very low concentration of intracellular oxygen, known as hypoxia. This condition has a role in the tumor growth and resistance to chemotherapy. Sun, *et al.* [63] reported the use of azo-based iridium (III) complexes as phosphorescent probes for the detection of hypoxia. The azo group was reduced by azoreductase under hypoxic condition that gave highly phosphorescent amine. Similarly, azo complexes of rhodium and ruthenium showed good activity *against Escherichia coli* and *Mycobacterium tuberculosis* bacterial species [64].

Other than complexes, Li *et al.* [65] have reported an azo-based probe for the determination of H_2S concentrations in living cells. The non-fluorescent azo compounds were reacted with sulfide and reduced to fluorescent products. The designed probe was applied to enumerate endogenous sulfide in mouse blood serum/tissues. Some azo-based probe when applied for cellular imaging, also showed magnificent response to the changes of mitochondrial GSH [66]. It is worth to mention here that various classes of azo compounds are being reported as probes [67, 68]. For example, a comprehensive review describing the applications of fluorescent probes in hypoxia including various examples of azo-based probes and their applications has been published by Elmes *et al.* [69]. Other than hypoxia, some azo-based probes are also reported for the imaging of neurofibrillary tangles in patients with Alzheimer's disease [70].

6. ANTIMICROBIAL AND ANTIVIRAL PROPERTIES

The first azo dye that got medical attention was prontosil, (sulphonamido-chrysoidin) (Fig. **6A**). This antibacterial drug was discovered in 1932 by Bayer's laboratory [71, 72] . Prontosil was also proposed to treat systemic disease such as septicemia [73]. The sulphonamide group was recognized as the basic therapeutic part of prontosil, which led to the development of sulphonamide class of drugs [74, 75]. A notable number of research to explore the antimicrobial activities of azo compounds containing sulpha group has been done. More recently, Moanta *et al.* [76] synthesized (4-(phenyldiazenyl)phenyl benzene sulfonate (Fig. **6B**) that showed excellent results against *S. aureus* and *C. albicans* bacterial strains. Similarly, sulfamoylphenylazo-thiophene and/or thiazole derivatives showed moderate antibacterial activity [77].

Besides compounds with sulpha group(s), the antimicrobial activity of other compounds containing azo group has been investigated through *in vitro*, *in vivo*, and *in silico* studies [78, 79]. According to some reports, the introduction of azo group has improved more than 60% of the antibacterial activities than the parent molecule [80]. In particular, azo-metal complexes, Schiff bases [81, 82], azo compounds of pyrimidine [83, 84] and other therapeutically recognized classes of organic compounds, such as enamines, pyrazole, thiazole and triazole have shown excellent antimicrobial activities [85, 86]. For example, benzotriazole-azo-phenol/aniline derivatives showed better antifungal activities (3.5–10.8 folds) than carbendazim against *Curvularia lunata and Alternaria alternate* [87].

Azo compounds also showed antiviral (including anti-HIV) activities for example, azodicarbonamide (ADA) (Fig. **6C**), an aliphatic azo dye, was previously reported as anti-HIV agent [88]. Rice group [89], demonstrated that ADA exerted its effect by targeting the zinc binding site of the HIV-1 nucleocapsid p7 (NCp7) protein and resulted in the ejection of zinc-finger, the negative part. A bisazo compound with anti-HIV activity was reported by Ono *et al.* [90] and further explored by Poli *et al.* [91] through clinical trial. In 2014, Gomha *et al.* [92] synthesized pyrazolo[4,3-d]isoxazole backbone scaffold that bears a phenyldiazenyltriazinyl or phenyldiazenylthiazolyl side chain. The compounds were evaluated against two viral strains of HIV-1 (RF and IIIB) which showed excellent antiviral ability with EC_{50} values in the sub-nanomolar range. The most active derivative was *p*-chlorophenyldiazenylthiazolyl (Fig. **6D**). To explore the molecular basis of their actions, the compounds were all tested against the HIV-1 viral enzyme reverse transcriptase (HIV-1 RT) and the most active RT inhibitor showed IC_{50} 0.016 nM. In a study conducted by Marich *et al.* [93], pyrimidine analogues (Fig. **6E**) with azo functional group showed promising anti-HIV results, while Thomas's group reported diphenylpyrazolodiazene (Fig. **6F**) as HIV-1 Nef function inhibitor [94, 95]. However, in a recent study, when the azo linker in B9 was replaced with one- or two-carbon bond, similar antiretroviral activities with enhanced oral bioavailability was observed [96].

7. AZO DRUGS IN MEDICAL USE

Currently, there are only a few azo compounds in medical use. Besides Prontosil and Sulfasalazine, briefly discussed above, the other two azo compounds used as drugs are Phenazopyridine and Balsalazide (Fig. **7A** and **7B**, respectively). Phenazopyridine (Pyridium®) is often prescribed to relieve the pain and irritation caused during urinary tract infections (UTI) due to surgery, endoscopic procedures, catheter or injury [97]. Balsalazide (Colazal®, Colazide®) belongs to the same family as sulfasalazine and is clinically used for the treatment of ulcerative colitis (UC) [98]. Olsalazine (Dipentum®) is another example of this family which has the same mechanism of action [99]. The medical efficacy of these drugs is attributed to 5-aminosalicylic acids (5-ASA). 5-ASA is not used as such because it is absorbed rapidly in the upper intestine region before it reaches the target colonic site. Therefore, its azo prodrugs are synthesized for safe and targeted delivery in the treatment of inflammatory bowel diseases [100].

8. AZO DYES IN STAINING/HISTOLOGY

Dyes are used in cellular staining to improve the visualization of the cells/cellular components and metabolic process. Sometimes they are applied to differentiate between cells of different sources and status, living or dead [101, 102]. The first synthetic aniline dye (mauve) was accidentally synthesized by William Perkins in 1856 while trying to

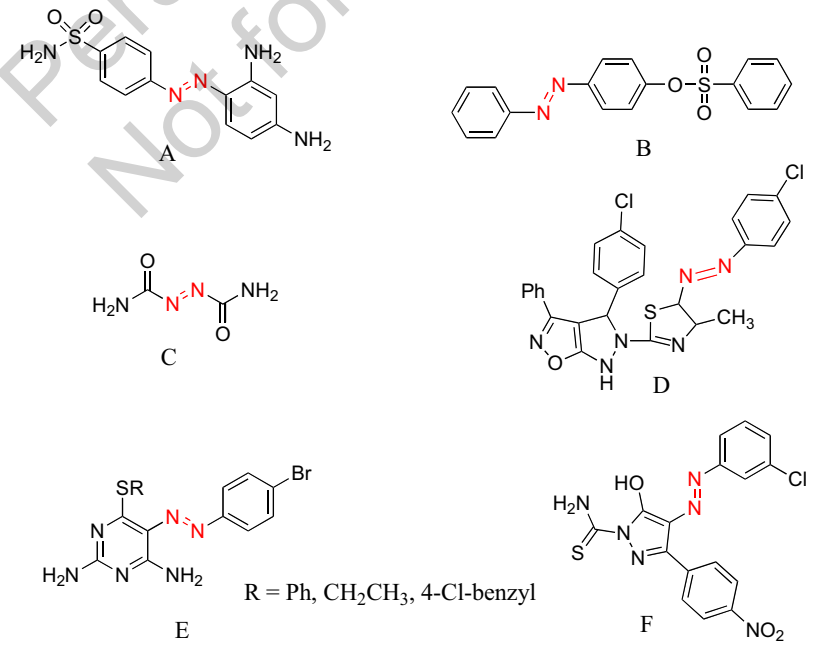
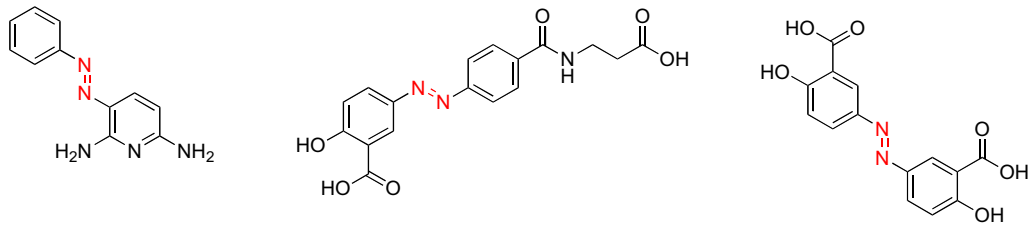


Fig. (6). Azo compounds showing antimicrobial **(A-B)** and antiviral **(C-F)** properties.



A B C

Fig. (7) Structures of Phenazopyridine **(A)**, Balsalazide **(B)** and Olsalazine **(C)**.

synthesize quinine, an antimalaria drug [103]. It was later discovered that the dye stains microbial and animal cells differently, which sets a basis for microbial screening. Acid-fast stain was used to detect tuberculosis bacteria, *Mycobacterium tuberculosis* [104], while Trypan blue, a diazo dye selectively imparted blue color to dead tissues/cells. It was also used in ophthalmic cataract surgery to visualize the capsulorhexis [105, 106]. Sudan stain test was used to express the level of fecal fat during the diagnosis of steatorrhoea (a condition of surplus fat in feces) [107] and the Oil Red O staining techniques were used for fingermark enhancement [108]. Sava *et al.* [109] synthesized diazo compounds and studied their staining properties on human central nervous system sections (cerebral cortex, cerebellum cortex and spinal cord). Each structure was clearly revealed by staining with new diazo dye.

Chromoendoscopy is a diagnostic technique used to detect cancer in the gastrointestinal tract. In this medical procedure, stains are used during endoscopy to visualize the differences in mucosa and detect dysplastic and malignant changes in the gastro intestinal tract. It is often recommended in surveillance of the esophagus (for Barrett's esophagus), examining polyps in the colon and in surveillance of dysplasia in inflammatory bowel disease [110]. Congo red is one of the stains used in chromo-endoscopy [111], which changes color from red to dark blue (or black) when exposed to acidic medium and thus is used to highlight sites of excessive acid production. In combination with methylene blue dye, Congo red is also used to stain gastric intestinal metaplasia, for early gastric cancer screening and in the evaluation of post-vagotomy patients [112].

9. CARCINOGENIC AND NON-CARCINOGENIC AZO COMPOUNDS

In 1859, Ludwig WC Rehn, a German Surgeon, reported for the first time the carcinogenic potential of dyes. He noticed an unusual incidence of bladder tumors in people working in dye industry and termed that “aniline cancer” [113]. In the following years, animal studies confirmed the ability of coal-tar dyes to cause liver, lung and bladder cancers [114, 115]. However, aromatic amines cannot be generalized and declared as carcinogenic/mutagenic as there are various aromatic amine-based drugs and dyes that are approved by FDA.

Azo compounds are usually resistance to aerobic conditions but are readily reduced by the action of intestinal flora [116]. In mammalian systems, azoreduction is generally catalyzed by bacterial azoreductase enzymes in the intestinal tract and by hepatic azoreductases in the liver. The hepatic azoreductases are less active and less common than bacterial azoreductases [117]. The reduction of azo dyes gives compounds that might be more or less toxic as compared to parent molecules. For example, the reduction of Direct Black 38 (also Acid Red 85) releases highly carcinogenic aromatic amine, benzidine [118]. Similarly, aromatic amines used in hair dyes react with atmospheric pollutants and form other carcinogenic derivatives. The *o*-toluidine (a genotoxic carcinogen) has been detected in blood samples of hairdressers even with usage of protective glove. Medical conditions associated with azo compounds and their metabolites include frequent headaches in adults, neurotoxicity, genotoxicity and carcinogenicity [119]. The carcinogenicity of these compounds is due to their metabolic conversion to electrophilic species, that interact with electron-rich sites of DNA and cause DNA adducts, mutations and subsequent adverse effects. Therefore, the enzyme-induced formation of genotoxic metabolites should be considered prior to the synthesis of azo dyes for practical use. The human and ecological risks associated with various azo dyes and their metabolites has been discussed by Chequer *et al.* [120].

Appropriate structural modification can reduce or eliminate their negative part. A report on benzidine analogues by Chung *et al.* [121] revealed that the addition of a sulfonic acid moiety and in some cases, complexation of benzidine with a metal ion decreased the mutagenicity of benzidine. The nature of substituent(s) and their position on the basic skeleton, also determine the mutagenicity/carcinogenicity of azo compounds. For example, 2-methoxy-4 aminoazobenzene and 3-methoxy-4-aminoazobenzene have the same skeleton but with different position of methoxy group. However, under similar conditions, the first compound is a weak mutagen whereas the later was found to exhibit strong hepatocarcinogen in rats and highly mutagenic in *Escherichia coli* and *Salmonella typhimurium* [122]. Similarly, some reports revealed that dyes containing amino group at *para* position were carcinogenic while the *ortho* isomers of the compounds were not [123, 124]. Sulphonation of azo compounds decreases the toxicity by influencing the metabolism and consequently their urinary excretion. Therefore, most of the sulphonated dyes are allowed to be used in cosmetics, foods and in medication [125].

CONCLUSION AND FUTURE PERSPECTIVE

Azo compounds are used in food, pharmaceutical, cosmetic and textile industries as additives and colorants. They show many types of biological activities, targeting viruses,

bacteria, fungi *etc*. Their potential use as drugs, drug carriers and in medical diagnosis, describe their multidirectional therapeutic stand. The *cis-trans* interchange is an additional feature which further enhances their applications. Currently, only a few azo compounds are prescribed as medicines. The issue of adverse effects, like carcinogenicity/mutagenicity associated with azo compounds and their metabolites need to be addressed by more systematic approach. The use of computational method will be a good addition to determine the structure-activity relationship of azo compounds and their metabolites, and to predict their mechanism of action. Molecular docking of various azo compounds showed good interaction with different receptors [126]. The development of QSAR/QPARs that correlate the relative carcinogenicity/ mutagenicity of azo compounds and their derivatives can help to identify the factors that change their relative mutagenicity. Attempts in this connection are already made by a few researchers. [122, 127]. Among the acceptable daily intake (ADI) of different food colorants, the less dangerous color is blue followed by yellow and lastly green [128]. This is an additional aspect which may be considered in designing safe azo drugs. The interlinked multi-disciplinary and systemic studies may nullify the adverse effects of azo compounds and lead towards the production of medicinal colorants.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge MOSTI (06-01-08-SF0147) and MOHE (ERGS12-017-0017) for supporting the research team.

REFERENCES

[1] Haubrich, W.S. Medical meanings: A glossary of word origins. ACP Press: **2003**.
[2] Woo, Y.-T.; Lai, D.Y.; Arcos, J.C. Aliphatic and polyhalogenated carcinogens: structural bases and biological mechanisms. Academic Press: **2013**.
[3] Mooter, G.; Maris, B.; Samyn, C.; Augustijns, P.; Kinget, R. Use of azo polymers for colon-specific drug delivery. *J. Pharm. Sci*., **1997**, *86*(12), 1321-1327.
[4] Christie, R. Colour chemistry. Royal Society of Chemistry: **2014**.
[5] Zollinger, H., Color chemistry: Syntheses, properties, and applications of organic dyes and pigments. John Wiley & Sons: **2003**.
[6] Waring, D.R.; Hallas, G. The chemistry and application of dyes. Springer Science & Business Media: **2013**.
[7] Hamon, F.; Djedaini-Pilard, F.; Barbot, F.; Len, C. Azobenzenes—Synthesis and carbohydrate applications. *Tetrahedron*, **2009**, *65*(49), 10105-10123.
[8] Merino, E. Synthesis of azobenzenes: The coloured pieces of molecular materials. *Chem. Soc. Rev*., **2011**, *40*(7), 3835-3853.
[9] Bullock, D.; Cumper, C.; Vogel, A. 989. Physical properties and chemical constitution. Part XLIII. The electric dipole moments of azobenzene, azopyridines, and azoquinolines. *J. Chem. Soc. (Resumed)*, **1965**, 5316-5323.
[10] Bandara, H.D.; Burdette, S.C., Photoisomerization in different classes of azobenzene. *Chem. Soc. Rev*., **2012**, *41*(5), 1809-1825.
[11] Brieke, C.; Rohrbach, F.; Gottschalk, A.; Mayer, G.; Heckel, A. Light‐controlled tools. *Angew. Chem. Intl. Edit*., **2012**, *51*(34), 8446-8476.
[12] Merino, E.; Ribagorda, M., Control over molecular motion using the cis–trans photoisomerization of the azo group. *Beilstein J. Org. Chem*., **2012**, *8*(1), 1071-1090.
[13] Albert, A. Chemical aspects of selective toxicity. *Nature*, **1958**, *182*(4633), 421.
[14] Huttunen, K.M.; Raunio, H.; Rautio, J. Prodrugs from serendipity to rational design. *Pharmacol. Rev*., **2011**, *63*(3), 750-771.
[15] Karaman, R., Prodrugs Design based on inter-and intramolecular chemical processes. *Chem. Biol. Drug Des*., **2013**, *82*(6), 643-668.
[16] Karaman, R.; Fattash, B.; Qtait, A., The future of prodrugs–design by quantum mechanics methods. *Expert Opin. Drug Discov*., **2013**, *10*(5), 713-729.
[17] Karaman, R., Using predrugs to optimize drug candidates. *Expert Opin. Drug Discov.,* **2014**, *9*(12), 1405-1419.
[18] Horani, W.; Thawabteh, A.; Scrano, L.; Bufo, S. A.; Mecca, G.; Karaman, R., Anti-cancer Prodrugs-Three Decades of Design. *World J. Pharm. Pharmaceut. Sci.*, **2015**, *4*(7), 1751-1779.
[19] Mutlu, H.; Geiselhart, C.M.; Barner-Kowollik, C. Untapped potential for debonding on demand: The wonderful world of azo-compounds. *Mater. Horizons*, **2018**, *5*, 162-183.
[20] Roldo, M.; Barbu, E.; Brown, J.F.; Laight, D.W.; Smart, J.D.; Tsibouklis, J. Azo compounds in colon-specific drug delivery. *Expert Opin. Drug Discov*., **2007**, *4*(5), 547-560.
[21] Chourasia, M.; Jain, S., Pharmaceutical approaches to colon targeted drug delivery systems. *J. Pharm. Pharm. Sci.,* **2003**, *6*(1), 33-66.
[22] Wiwattanapatapee, R.; Lomlim, L.; Saramunee, K., Dendrimers conjugates for colonic delivery of 5-aminosalicylic acid. *J. Control Release,* **2003**, *88*(1), 1-9.
[23] Kennedy, D.A.; Vembu, N.; Fronczek, F.R.; Devocelle, M. Synthesis of mutual azo prodrugs of anti-inflammatory agents and peptides facilitated by α-aminoisobutyric acid. *J. Org. Chem*., **2011**, *76*(23), 9641-9647.
[24] Sharma, R.; Rawal, R.K.; Gaba, T.; Singla, N.; Malhotra, M.; Matharoo, S.; Bhardwaj, T. Design, synthesis and ex vivo evaluation of colon-specific azo based prodrugs of anticancer agents. *Bioorg. Med. Chem. Lett*., **2013**, *23*(19), 5332-5338.
[25] Sharma, R.; Rawal, R. K.; Malhotra, M.; Sharma, A.; Bhardwaj, T. Design, synthesis and *ex-vivo* release studies of colon-specific polyphosphazene anticancer drug conjugates. *Bioorg. Med. Chem*., **2014**, *22*(3), 1104-1114.
[26] Wang, H.; Huang, Q.; Chang, H.; Xiao, J.; Cheng, Y. Stimuli-responsive dendrimers in drug delivery. *Biomater. Sci*., **2016**, *4*(3), 375-390.
[27] Mayer, G.; Heckel, A. Biologically active molecules with a "light switch". *Angew. Chem. Int. Edit*., **2006**, *45*(30), 4900-4921.
[28] Yagai, S.; Karatsu, T.; Kitamura, A. Photocontrollable self-assembly. *Chem. Eur. J*., **2005**, *11*(14), 4054-4063.
[29] Tong, X.; Wang, G.; Soldera, A.; Zhao, Y. How can azobenzene block copolymer vesicles be dissociated and reformed by light? *J. Phys. Chem. B.,* **2005**, *109*(43), 20281-20287.
[30] Barhoumi, A.; Liu, Q.; Kohane, D.S. Ultraviolet light-mediated drug delivery: Principles, applications, and challenges. *J. Control Release,* **2015**, *219*, 31-42.
[31] Mas, N.; Agostini, A.; Mondragón, L.; Bernardos, A.; Sancenón, F.; Marcos, M.D.; Martínez-Máñez, R.; Costero, A.M.; Gil, S.; Merino‐Sanjuán, M. Enzyme-Responsive silica mesoporous supports capped with azopyridinium salts for controlled delivery applications. *Chem. Eur. J*., **2013**, *19*(4), 1346-1356.
[32] Li, X.; Tang, T.; Zhou, Y.; Zhang, Y.; Sun, Y. Applicability of enzyme-responsive mesoporous silica supports capped with bridged silsesquioxane for colon-specific drug delivery. *Micropor. Mesopor. Mat*., **2014**, *184*, 83-89.
[33] Wang, D.; Wu, S. Red-light-responsive supramolecular valves for photo-controlled drug release from mesoporous nanoparticles. Langmuir, **2016**, *32*(2), 632-636.
[34] Samanta, S.; Beharry, A. A.; Sadovski, O.; McCormick, T. M.; Babalhavaeji, A.; Tropepe, V.; Woolley, G. A. Photoswitching azo compounds *in vivo* with red light. *J. Am. Chem. Soc*., **2013**, *135*(26), 9777-9784.

[35] Alvarez-Lorenzo, C.; Bromberg, L.; Concheiro, A. Light-sensitive intelligent drug delivery systems. *Photochem. Photobiol*., **2009**, 85(4), 848-860.

[36] Alvarez-Lorenzo, C.; Concheiro, A. Smart drug delivery systems: From fundamentals to the clinic. *Chem. Commun.,* **2014**, *50*(58), 7743-7765.

[37] Saint-Cricq, P.; Deshayes, S.; Zink, J.; Kasko, A. Magnetic field activated drug delivery using thermodegradable azo-functionalised PEG-coated core-shell mesoporous silica nanoparticles. *Nanoscale*, **2015**, *7*(31), 13168-13172.

[38] Wang, Y.-R.; Tian, F.-L.; Yan, M.-X.; Fan, J.-H.; Wang, L.-Y.; Kuang, R.-G.; Li, Y.-Q. Sulfasalazine inhibits inflammation and fibrogenesis in pancreas *via* NF-κB signaling pathway in rats with oxidative stress-induced pancreatic injury. *Drug Des. Dev. Ther*., **2016**, *10*, 1743.

[39] Sontheimer, H.; Bridges, R.J. Sulfasalazine for brain cancer fits. *Expert Opin. Investig. Drugs*, **2012**, *21*(5), 575-578.

[40] Robert, S.M.; Buckingham, S.C.; Campbell, S.L.; Robel, S.; Holt, K.T.; Ogunrinu-Babarinde, T.; Warren, P.P.; White, D.M.; Reid, M.A.; Eschbacher, J.M. SLC7A11 expression is associated with seizures and predicts poor survival in patients with malignant glioma. *Sci. Transl. Med*., **2015**, *7*(289), 289ra86-289ra86.

[41] Su, R.; Lü, L.; Zheng, S.; Jin, Y.; An, S. Synthesis and characterization of novel azo-containing or azoxy-containing Schiff bases and their antiproliferative and cytotoxic activities. *Chem. Res. Chin. Univ*., **2015**, *31*(1), 60-64.

[42] Farghaly, T.A.; Abdallah, Z.A. Synthesis, azo-hydrazone tautomerism and antitumor screening of N-(3-ethoxycarbonyl-4, 5, 6, 7-tetrahydro-benzo [b] thien-2-yl)-2-arylhydrazono-3-oxobutanamide derivatives. *Arkivoc*, **2008**, *17*, 295-305.

[43] Farghaly, T.A.; Abdalla, M.M. Synthesis, tautomerism, and antimicrobial, anti-HCV, anti-SSPE, antioxidant, and antitumor activities of arylazobenzosuberones. *Bioorg. Med. Chem*., **2009**, *17*(23), 8012-8019.

[44] Fujikawa, A.; Nagahira, A.; Sugawara, H.; Ishii, K.; Imajo, S.; Matsumoto, M.; Kuboyama, K.; Suzuki, R.; Tanga, N.; Noda, M. Small-molecule inhibition of PTPRZ reduces tumor growth in a rat model of glioblastoma. *Sci. Rep*., **2016**, *6*, 20473.

[45] Kaur, H.; Yadav, S.; Narasimhan, B. Diazenyl Derivatives as Therapeutic and Diagnostic Agents Acting on Central Nervous System. *Cent. Nerv. Syst. Agents Med. Chem. (Formerly Curr. Med. Chem.-Central Nervous System Agents),* **2015**, *15*(1), 42-51.

[46] Coluccia, M.; Natile, G. Trans-platinum complexes in cancer therapy. *Anticancer Agents Med. Chem. (Formerly Curr. Med. Chem.-Anti-Cancer Agents),* **2007**, *7*(1), 111-123.

[47] Engdahl, A. J.; Torres, E. A.; Lock, S. E.; Engdahl, T.B.; Mertz, P.S.; Streu, C.N. Synthesis, characterization, and bioactivity of the photoisomerizable tubulin polymerization inhibitor azo-combretastatin A4. *Org. Lett.*, **2015**, *17*(18), 4546-4549.

[48] Borowiak, M.; Nahaboo, W.; Reynders, M.; Nekolla, K.; Jalinot, P.; Hasserodt, J.; Rehberg, M.; Delattre, M.; Zahler, S.; Vollmar, A. Photoswitchable inhibitors of microtubule dynamics optically control mitosis and cell death. *Cell*, **2015**, *162*(2), 403-411.

[49] Sheldon, J.E.; Dcona, M.M.; Lyons, C.E.; Hackett, J.C.; Hartman, M.C. Photoswitchable anticancer activity *via* trans–cis isomerization of a combretastatin A-4 analog. *Org. Biomol. Chem*., **2016**, *14*(1), 40-49.

[50] Rastogi, S.K.; Zhao, Z.; Barrett, S.L.; Shelton, S.D.; Zafferani, M.; Anderson, H.E.; Blumenthal, M.O.; Jones, L.R.; Wang, L.; Li, X. Photoresponsive azo-combretastatin A-4 analogues. *Eur. J. Med. Chem*., **2018**, *143*, 1-7.

[51] Ferreira, R.; Nilsson, J. R.; Solano, C.; Andréasson, J.; Grøtli, M., Design, Synthesis and Inhibitory Activity of Photoswitchable RET Kinase Inhibitors. *Sci. Rep*., **2015**, 5.

[52] Thompson, K. H.; Orvig, C. Metal complexes in medicinal chemistry: New vistas and challenges in drug design. *Dalton Trans.*, **2006**, *1*(6), 761-764.

[53] Fricker, S.P. Metal compounds in cancer therapy. Springer Science & Business Media: 2012.

[54] Al-Jibouri, M.N. Synthesis AND Characterization of transition Metal Complexes With Azo Ligand Derived from 4-Hydroxy-6-Methyl-2-Pyranone. *Europ. Chem. Bull*., **2014**, *3*(5), 447-451.

[55] Sahu, N.; Das, D.; Mondal, S.; Roy, S.; Dutta, P.; Sepay, N.; Gupta, S.; López-Torres, E.; Sinha, C. The structural characterization and biological activity of sulfamethoxazolyl-azo-p-cresol, its copper (ii) complex and their theoretical studies. *J. Chem*., **2016**, *40*(6), 5019-5031.

[56] Mahmoud, W.H.; Omar, M.; Sayed, F.N. Synthesis, spectral characterization, thermal, anticancer and antimicrobial studies of bidentate azo dye metal complexes. *J. Therm. Anal. Calorim*., **2016**, *124*(2), 1-19.

[57] Sarigul, M.; Sari, A.; Kose, M.; McKee, V.; Elmastas, M.; Demirtas, I.; Kurtoglu, M. New bio-active azo-azomethine based Cu (II) complexes. *Inorg. Chim. Acta*, **2016**, *444*, 166-175.

[58] Khedr, A.M.; Gaber, M.; El-Zaher, A.; Eman, H. Synthesis, Structural Characterization, and Antimicrobial Activities of Mn (II), Co (II), Ni (II), Cu (II) and Zn (II) Complexes of Triazole-based Azodyes. *Chin. J. Chem*., **2011**, *29*(6), 1124-1132.

[59] Roy, M.; Roy, S.; Singh, K.S.; Kalita, J.; Singh, S.S. Synthesis, characterisation and anti-diabetic activities of triorganotin (IV) azo-carboxylates derived from amino benzoic acids and resorcinol: Crystal structure and topological study of a 48 membered macrocyclic-tetrameric trimethyltin (IV) complex. *Inorg. Chim. Acta,* **2016**, *439*, 164-172.

[60] Inan, A.; Ikiz, M.; Taylan, S.; Bilgin, S.; Genç, N.; Sayin, K.; Ceyhan, G.; Muhammet, K.; Kazancı, A.; Ispir, E. Antiproliferative, antioxidant, computational and electrochemical studies of new azo-containing Schiff Base Ruthenium (II) complexes. *New J. Chem*., **2018**, *42*, 2952-2963.

[61] Gaber, M.; El-Sayed, Y.; El-Baradie, K.; Fahmy, R. Complex formation, thermal behavior and stability competition between Cu (II) ion and Cu 0 nanoparticles with some new azo dyes. Antioxidant and *in vitro* cytotoxic activity. *Spectrochim. Acta Mol. Biomol. Spectrosc*., **2013**, *107*, 359-370.

[62] Sarigul, M.; Deveci, P.; Kose, M.; Arslan, U.; Dagi, H.T.; Kurtoglu, M. New tridentate azo–azomethines and their copper (II) complexes: Synthesis, solvent effect on tautomerism, electrochemical and biological studies. *J. Mol. Struct*., **2015**, *1096*, 64-73.

[63] Sun, L.; Li, G.; Chen, X.; Chen, Y.; Jin, C.; Ji, L.; Chao, H. Azo-Based Iridium (III) complexes as multicolor phosphorescent probes to detect hypoxia in 3D multicellular tumor spheroids. *Sci. Rep*., **2015**, *5***.**

[64] Mehrotra, R.; Shukla, S.N.; Gaur, P. Synthesis and spectroscopic characterization of antibacterial rhodium and ruthenium organometallics molded by C–H activation at the ortho position of a phenyl ring. *J. Coord. Chem*., **2015**, *68*(4), 650-661.

[65] Li, X.; Cheng, J.; Gong, Y.; Yang, B.; Hu, Y., Mapping hydrogen sulfide in rats with a novel azo-based fluorescent probe. *Biosens. Bioelectron*., **2015**, *65*, 302-306.

[66] Lim, S.-Y.; Hong, K.-H.; Kim, D.I.; Kwon, H.; Kim, H.-J. Tunable heptamethine–azo dye conjugate as an NIR fluorescent probe for the selective detection of mitochondrial glutathione over cysteine and homocysteine. *J. Am. Chem. Soc*., **2014**, *136*(19), 7018-7025.

[67] Gómez-Vallejo, V.; Borrell, J.I.; Llop, J. A convenient synthesis of 13 N-labelled azo compounds: A new route for the preparation of amyloid imaging PET probes. *Eur. J. Med. Chem*., **2010**, *45*(11), 5318-5323.

[68] Uddin, M.I.; Evans, S.M.; Craft, J.R.; Marnett, L.J.; Uddin, M.J.; Jayagopal, A. Applications of azo-based probes for imaging retinal hypoxia. *Med. Chem. Lett*., **2015**, *6*(4), 445-449.

[69] Elmes, R.B., Bioreductive fluorescent imaging agents: Applications to tumour hypoxia. *Chem. Commun.,* **2016**, *52*(58), 8935-8956.

[70] Matsumura, K.; Ono, M.; Kimura, H.; Ueda, M.; Nakamoto, Y.; Togashi, K.; Okamoto, Y.; Ihara, M.; Takahashi, R.; Saji, H. 18F-labeled phenyldiazenyl benzothiazole for *in vivo* imaging of neurofibrillary tangles in Alzheimer's disease brains. *Med. Chem. Lett.,* **2011**, *3*(1), 58-62.

[71] Bentley, R. Different roads to discovery; Prontosil (hence sulfa drugs) and penicillin (hence β-lactams). *J. Ind. Microbiol. Biotechnol*., **2009**, *36*(6), 775-786.

[72] Lesch, J.E. The first miracle drugs: How the sulfa drugs transformed medicine. Oxford University Press, USA: **2007**.

[73] Browning, C. Chemotherapy by sulphanilamide drugs. *Brit. Med. J.,* **1939**, *2*(4100), 265.

[74] Otten, H. Domagk and the development of the sulphonamides. *J. Antimicrob. Chemoth.,* **1986**, *17*(6), 689-690.

[75] Lyddiard, D.; Jones, G.L.; Greatrex, B.W. Keeping it simple: lessons from the golden era of antibiotic discovery. *FEMS Microbiol. Lett.,* **2016**, *363*(8), fnw084.

[76] Moanta, A.; Ionescu, C.; Dragoi, M.; Tutunaru, B.; Rotaru, P. A new azo-ester: 4-(phenyldiazenyl) phenyl benzene sulfonate—spectral, thermal, and electrochemical behavior and its antimicrobial activity. *J. Therm. Anal. Calorim*., **2015**, *120*(2), 1151-1161.

[77] Fadda, A.A.; El-badrawy, A.M.; Refat, H.M.; Abdel-Latif, E. Synthesis of some new 2-Substituted-4-Sulfamoylphenylazo-Thiophene and/or thiazole derivatives as antibacterial agents. *Phosphorus Sulfur Silicon Relat. Elem*., **2016**, *191*(5), 778-785.

[78] Piotto, S.; Concilio, S.; Sessa, L.; Porta, A.; Calabrese, E.C.; Zanfardino, A.; Varcamonti, M.; Iannelli, P. Small azobenzene derivatives active against bacteria and fungi. *Eur. J. Med. Chem*., **2013**, *68*, 178-184.

[79] Al-Masoudi, W.A.; Al-Asadi, R.H.; Othman, R.M.; Al‐Masoudi, N.A. Synthesis, antimicrobial activity, computational and modeling studies of some new organotellurium compounds containing azo moities. *Eur. J. Chem.,* **2015**, *6*(4), 374-380.

[80] Mkpenie, V.; Ebong, G.; Obot, I.; Abasiekong, B. Evaluation of the effect of azo group on the biological activity of 1-(4-methylphenylazo)-2-naphthol. *J. Chem*., **2008**, *5*(3), 431-434.

[81] Jarrahpour, A.; Fathi, J.; Mimouni, M.; Hadda, T.B.; Sheikh, J.; Chohan, Z.; Parvez, A. Petra, Osiris and Molinspiration (POM) together as a successful support in drug design: Antibacterial activity and biopharmaceutical characterization of some azo Schiff bases. *Med. Chem. Res*., **2012**, *21*(8), 1984-1990.

[82] Alaghaz, A.-N.M.; Ammar, Y.A.; Bayoumi, H.A.; Aldhlmani, S.A. Synthesis, spectral characterization, thermal analysis, molecular modeling and antimicrobial activity of new potentially N_2O_2 azo-dye Schiff base complexes. *J. Mol. Struct*., **2014**, *1074*, 359-375.

[83] Shaikh, A.; Meshram, J.S. Design, synthesis and pharmacological assay of novel azo derivatives of dihydropyrimidinones. *Cogent Chem.*, **2015**, *1*(1), 1019809.

[84] Gulcan, M.; Özdemir, S.; Dündar, A.; İspir, E.; Kurtoğlu, M. Mononuclear complexes based on pyrimidine ring azo schiff-base ligand: Synthesis, characterization, antioxidant, antibacterial, and thermal investigations. *Z. Anorg. Allg. Chem*., **2014**, *640*(8-9), 1754-1762.

[85] Sahoo, J.; Mekap, S.K.; Kumar, P.S. Synthesis, spectral characterization of some new 3-Heteroaryl azo 4-Hydroxy coumarin derivatives and their antimicrobial evaluation. *J. Taibah Univ. Sci.,* **2015**, *9*(2), 187-195.

[86] Rizk, H.; Ibrahim, S.; El-Borai, M. Synthesis, fastness properties, color assessment and antimicrobial activity of some azo reactive dyes having pyrazole moiety. *Dyes Pigm*., **2015**, *112*, 86-92.

[87] Lv, M.; Ma, J.; Li, Q.; Xu, H. Discovery of benzotriazole-azo-phenol/aniline derivatives as antifungal agents. *Bioorg. Med. Chem. Lett*., **2018**, *28*(2), 181-187.

[88] Vandevelde, M.; Witvrouw, M.; Schmit, J.; Sprecher, S.; De Clercq, E.; Tassignon, J. ADA, a potential anti-HIV drug. *AIDS Res. Hum. Retrov*., **1996**, *12*(7), 567-568.

[89] Rice, W.G.; Turpin, J.A.; Huang, M.; Clanton, D.; Buckheit Jr, R.W.; Covell, D.G.; Wallqvist, A.; McDonnell, N.B.; DeGuzman, R.N.; Summers, M.F. Azodicarbonamide inhibits HIV-1 replication by targeting the nucleocapsid protein. *Nat. Med*., **1997**, *3*(3), 341-345.

[90] Ono, M.; Wada, Y.; Wu, Y.; Nemori, R.; Jinbo, Y.; Wang, H.; Lo, K.-M.; Yamaguchi, N.; Brunkhorst, B.; Otomo, H. FP-21399 blocks HIV envelope protein-mediated membrane fusion and concentrates in lymph nodes. *Nat. Biotechnol*., **1997**, *15*(4), 343-348.

[91] Poli, G.; Vicenzi, E. FP-21399 (Lexigen Pharmaceuticals). *IDrugs* **2001**, *4*(11), 1293-1295.

[92] Gomha, S.M.; Badrey, M.G.; Abdalla, M.M.; Arafa, R.K. Novel anti-HIV-1 NNRTIs based on a pyrazolo [4, 3-d] isoxazole backbone scaffold: design, synthesis and insights into the molecular basis of action. *Med. Chem. Comm.,* **2014**, *5*(11), 1685-1692.

[93] Marich, Y.A.; Al-Salihi, N.J.; Al-Masoudi, N.A. Synthesis, anti-HIV activity and molecular modeling study of some new pyrimidine analogues. *Eur. J. Chem*., **2014**, *5*(4), 588-594.

[94] Iyer, P.C.; Zhao, J.; Emert-Sedlak, L.A.; Moore, K.K.; Smithgall, T.E.; Day, B.W. Synthesis and structure–activity analysis of diphenylpyrazolodiazene inhibitors of the HIV-1 Nef virulence factor. *Bioorg. Med. Chem. Lett*., **2014**, *24*(7), 1702-1706.

[95] Emert-Sedlak, L.A.; Narute, P.; Shu, S.T.; Poe, J.A.; Shi, H.; Yanamala, N.; Alvarado, J.J.; Lazo, J.S.; Yeh, J.I.; Johnston, P.A. Effector kinase coupling enables high-throughput screens for direct HIV-1 Nef antagonists with antiretroviral activity. *Chem. Biol.,* **2013**, *20*(1), 82-91.

[96] Emert-Sedlak, L.A.; Loughran, H.M.; Shi, H.; Kulp, J.L.; Shu, S.T.; Zhao, J.; Day, B.W.; Wrobel, J.E.; Reitz, A.B.; Smithgall, T.E. Synthesis and evaluation of orally active small molecule HIV-1 Nef antagonists. *Bioorg. Med. Chem. Lett*., **2016**, *26*(5), 1480-1484.

[97] Gaines, K.K. Phenazopyridine hydrochloride: The use and abuse of an old standby for UTI. *Urol. Nurs*., **2004**, *24*(3), 207.

[98] Green, J.R.; Lobo, A.J.; Holdsworth, C.D.; Leicester, R.J.; Gibson, J.A.; Kerr, G.D.; Hodgson, H.J.; Parkins, K.J.; Taylor, M.D.; Group, A.I. Balsalazide is more effective and better tolerated than mesalamine in the treatment of acute ulcerative colitis. *Gastroenterology*, **1998**, *114*(1), 15-22.

[99] Ibrahim, M.A.; Elagawany, M.; Ibrahim, T.S. Green and catalyst-free synthesis of olsalazine analogs. *Green Chem. Lett. Rev*., **2016**, *9*(2), 91-95.

[100] Dhaneshwar, S.S.; Kandpal, M.; Gairola, N.; Kadam, S. Dextran: A promising macromolecular drug carrier. *Indian J. Pharm. Sci*., **2006**, *68*(6), 705.

[101] Veuthey, T.; Herrera, G.; Dodero, V.I. Dyes and stains: From molecular structure to histological application. *Front. Biosci. (Landmark Ed*), **2014**, *19*, 91-112.

[102] Sabnis, R.W. Handbook of biological dyes and stains: synthesis and industrial applications. John Wiley & Sons: 2010.

[103] Gurr, E. Synthetic dyes in biology, medicine and chemistry. Elsevier: **2012**.

[104] Ryan, G. J.; Shapiro, H. M.; Lenaerts, A. J., Improving acid-fast fluorescent staining for the detection of mycobacteria using a new nucleic acid staining approach. *Tuberculosis,* **2014**, *94*(5), 511-518.

[105] Melles, G.R.; de Waard, P.W.; Pameyer, J.H.; Beekhuis, W.H. Trypan blue capsule staining to visualizethe capsulorhexis in cataract surgery. *J. Cataract Refr. Surg*., **1999**, *25*(1), 7-9.

[106] Wainwright, M. The use of dyes in modern biomedicine. *Biotech. Histochem*., **2003**, *78*(3-4), 147-155.

[107] Khouri, M.; Huang, G.; Shiau, Y. Sudan stain of fecal fat: new insight into an old test. *Gastroenterology*, **1989**, *96*(2), 421-27.

[108] Braasch, K.; de la Hunty, M.; Deppe, J.; Spindler, X.; Cantu, A. A.; Maynard, P.; Lennard, C.; Roux, C. Nile red: Alternative to physical developer for the detection of latent fingermarks on wet porous surfaces? *Forensic Sci. Intl.,* **2013**, *230*(1), 74-80.

[109] Sava, C.; Mehedinti, R.; Hincu, M. M., A series of new dyes for central nervous system sections staining. Note 2. *Farmacia,* **2009**, *57*(3), 355-365.

[110] Wong, K. S. L.; Adler, D.; Chand, B.; Conway, J.; Croffie, J.; Disario, J.; Mishkin, D.; Shah, R.; Somogyi, L.; Tierney, W. Chromoendoscopy. *Gastrointest. Endosc*., **2007**, *66*(4), 639-649.

[111] Hamilton, J.W.; Thune, R.G.; Morrissey, J.F. Symptomatic ectopic gastric epithelium of the cervical esophagus. *Digest. Dis. Sci*., **1986**, *31*(4), 337-342.

[112] Trivedi, P.; Braden, B. Indications, stains and techniques in chromoendoscopy. *QJM*, **2013**, *106*(2), 117-131.

[113] Rehn, L. Blasengeschwulste bei Fuchsin-Arbeitern. *Arch. Klin. Chir*., **1895**, 50, 588-600.

[114] Luch, A. Nature and nurture–lessons from chemical carcinogenesis. *Nat. Rev. Cancer*, **2005**, *5*(3), 113-125.

[115] Yap, K.K.; Neuhaus, S.J. Making cancer visible–Dyes in surgical oncology. *Surg. Oncol*., **2016**, *25*(1), 30-36.

[116] Chengalroyen, M.; Dabbs, E. The microbial degradation of azo dyes: minireview. *World J. Microbiol. Biotech*., **2013**, *29*(3), 389-399.

[117] Puvaneswari, N.; Muthukrishnan, J.; Gunasekaran, P. Toxicity assessment and microbial degradation of azo dyes. *Indian J. Exp. Biol.,* **2006**, *44*(8), 618.

[118] Rafii, F.; Hall, J.; Cerniglia, C. Mutagenicity of azo dyes used in foods, drugs and cosmetics before and after reduction by Clostridium species from the human intestinal tract. *Food Chem. Toxicol.,* **1997**, *35*(9), 897-901.

[119] Yamjala, K.; Nainar, M. S.; Ramisetti, N. R., Methods for the analysis of azo dyes employed in food industry–a review. *Food Chem.,* **2016**, *192*, 813-824.

[120] Chequer, F.M.D.; Dorta, D.J.; de Oliveira, D.P. Azo dyes and their metabolites: does the discharge of the azo dye into water bodies represent human and ecological risks? INTECH Open Access Publisher: **2011**.

[121] Chung, K.-T.; Chen, S.-C.; Claxton, L.D. Review of the Salmonella typhimurium mutagenicity of benzidine, benzidine analogues, and benzidine-based dyes. Mutat. *Res.-Rev. Mutat*., **2006**, *612*(1), 58-76.

[122] Garg, A.; Bhat, K.L.; Bock, C.W. Mutagenicity of aminoazobenzene dyes and related structures: A QSAR/QPAR investigation. *Dyes Pigm*., **2002**, *55*(1), 35-52.

[123] Longstaff, E. An assessment and categorisation of the animal carcinogenicity data on selected dyestuffs and an extrapolation of those data to an evaluation of the relative carcinogenic risk to man. *Dyes Pigm*., **1983**, *4*(4), 243-304.

[124] Gregory, P. Azo dyes: Structure-carcinogenicity relationships. *Dyes Pigm*., **1986**, *7*(1), 45-56.

[125] Bafana, A.; Devi, S.S.; Chakrabarti, T. Azo dyes: past, present and the future. *Environ. Rev*., **2011**, *19,* 350-371.

[126] El-Bindary, A.; Ghoneim, M.; Diab, M.; El-Sonbati, A.; Shoair, A.; El-Kader, M.A. Molecular docking, potentiometric and thermodynamic studies of some azo compounds. *J. Solution Chem*., 1-19, DOI: 10.1007/s10953-016-0486-6.

[127] Benigni, R.; Passerini, L. Carcinogenicity of the aromatic amines: from structure–activity relationships to mechanisms of action and risk assessment. *Mutat. Res.-Rev. Mutat*., **2002**, *511*(3), 191-206.

[128] Martins, N.; Roriz, C.L.; Morales, P.; Barros, L.; Ferreira, I.C. Food colorants: Challenges, opportunities and current desires of agro-industries to ensure consumer expectations and regulatory practices. *Trends Food Sci. Tech*., **2016**, *52*, 1-15.