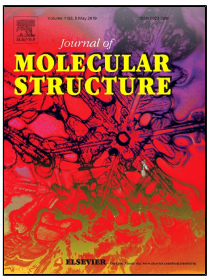
Accepted Manuscript



Synthesis, structural investigations and *in vitro* biological evaluation of N, N-dimethyl aniline derivatives based azo dyes as potential pharmacological agents

Vinodkumar, J. Keshavayya

PII:	S0022-2860(19)30312-6
DOI:	10.1016/j.molstruc.2019.03.042
Reference:	MOLSTR 26311
To appear in:	*Journal of Molecular Structure*
Received Date:	13 November 2018
Accepted Date:	13 March 2019

Please cite this article as: Vinodkumar, J. Keshavayya, Synthesis, structural investigations and *in vitro* biological evaluation of N, N-dimethyl aniline derivatives based azo dyes as potential pharmacological agents, *Journal of Molecular Structure* (2019), doi: 10.1016/j.molstruc.2019.03.042

Synthesis, structural investigations and *in vitro* biological evaluation of N, N-dimethyl aniline derivatives based azo dyes as potential pharmacological agents

Vinodkumar and J Keshavayya*

Department of Chemistry, School of Chemical Sciences,
Jnanasahyadri, Kuvempu University,
Shankaraghatta - 577 451, Shimoga, Karnataka, India
Email: jathikeshavayya1959@gmail.com

Abstract

Three novel bioactive azo dyes were synthesized by a classical diazo-coupling method using sulfamethazine with three different N, N-dimethyl aniline derivatives as coupling components under appropriate experimental conditions. The structural features of the newly synthesized compounds were explored by using various analytical and spectroscopic techniques. Further, these compounds were assessed for the different pharmacological activities such as antimicrobial, anti-tuberculosis, antioxidant and anti-inflammatory properties and results indicated that, these azo compounds exhibited effective pharmacological activities.

Keywords: Sulfamethazine; Azo dye; Biocidal activity; Anti-tuberculosis; Anti-inflammatory; DPPH assay.

1. Introduction

Azo dyes are the dynamic class of organic compounds which have received great attention and have been widely used in the various fields of science and technology such as in

pharmaceuticals, polymers, electronics, electrochemical, biological and analytical investigations [**1-5**]. These dyes are highly coloured and due to their tremendous ability to impart entire spectrum of colours, they have been used for the past decades as significant dyes and pigments and they represent nearly 60-70% of the commercial dyes worldwide [**6-7**]. Further, these compounds are being utilized as separating agents in a mixture of metal ions [**8**]. The azo dye containing heterocyclic ring has received much attention in pharmaceuticals industry compared to those with the simple carbocyclic aromatic system due to their extraordinary colouring properties, tinctorial strength, brightness, fastness properties and distinct bathochromic effect [**9-10**]. Among the various heterocyclic azo dyes, those containing nitrogen atom are actively involved in the different pharmacological activities such as antitumor [**11-13**], anti-inflammatory, antimycotic [**14**], antimicrobial as well as antioxidant [**15-17**].

Although, various drugs are available in the market for treating most of the dreadful diseases, many of such drugs are having still innumerable undesirable properties such as toxicity, drug resistance *etc*. Hence, there is a need to discover potent drugs to mitigate the impending clinical challenges. Additionally, sulfamethazine drugs are well-known antibiotics for the treatment of bacterial infections and they have shown potent pharmacological activities because of their non-toxicity and specific action against target diseases [**18**].

In the recent years, a good lot of work has been carried out in the field of azo dyes and their metal complexes in our laboratory. Further these compounds have been characterized by using various physicochemical methods and tested for various biological activities [**19-22**]. In continuation of the above work, the present study has been carried out in aiming at the synthesis of novel bioactive compounds derived from sulfamethazine moiety and their structural investigations by various analytical techniques. Further, these compounds have also

assessed for various pharmacological applications such as *in vitro* biocidal, anti-tuberculosis, anti-inflammatory as well as antioxidant activities.

2. Experimental

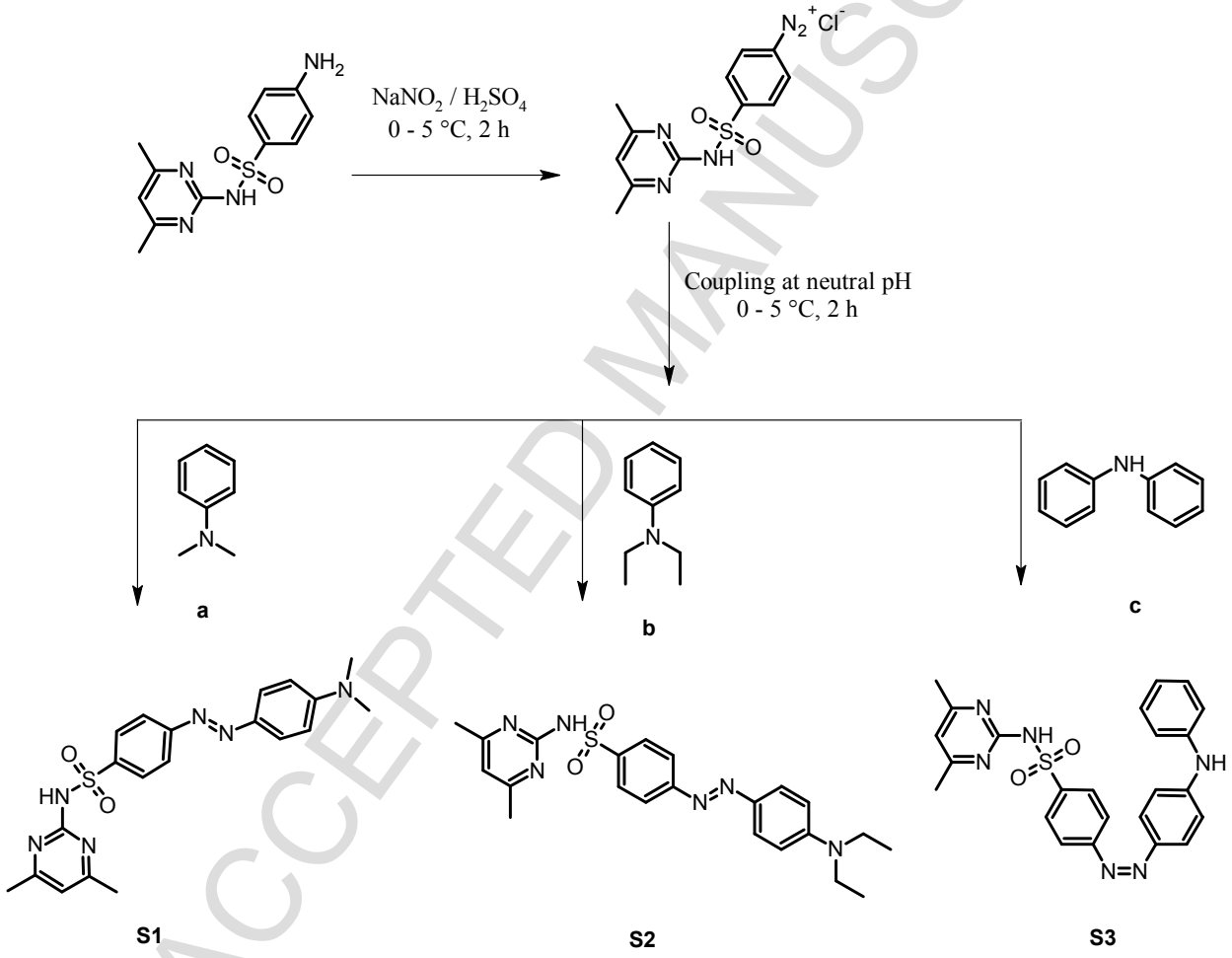
2.1. Materials and Methods

All the chemicals, solvents and reagents were of analytical grade and were used as received without further purification. Progress of the reactions was monitored by thin layer chromatography performed on silica gel coated aluminium sheets. Melting points were taken in an open capillary tube using electro thermal instrument without any correction. The UV-Visible spectra of the newly synthesized dyes were recorded on Shimadzu UV Probe spectrophotometer in the range of 200-800 nm with two solvents DMSO (dimethylsulphoxide) and DMF (dimethylformamide) at a concentration of 10^{-6} mol L^{-1}. Infrared spectra of the azo compounds were obtained by using Bruker FT-IR spectrophotometer instrument with KBr disc method and absorption bands were recorded in the range of 400- 4000 cm^{-1}. The ^{1}H NMR spectra were recorded in DMSO-d_6 solvent using a Bruker Ascend 400 MHz instrument and their chemical shifts were expressed as δ values. Mass spectra of the dye molecules were recorded at 70 eV of electron energy using Waters Model-SynaptG2 USA instrument.

2.2. Synthesis of Sulfamethazine azo dyes ***1(S1-S2)***:

Heterocyclic amine (2 mmol) was dissolved in dilute hydrochloric acid (2 mL HCl in 3 mL of H_2O) and cooled to 0-5 ℃ in an ice bath. To this reaction mixture, previously prepared a solution of sodium nitrite (2 mmol) in 2 mL sulphuric acid was added dropwise and the mixture was stirred for about 2 h. The resulting diazonium salt solution was utilized for coupling reaction by adding to the ethanolic solution of N, N-dimethyl aniline (2 mmol)

derivatives at the same temperature and stirring was continued for another 2 h. A neutral condition of the reaction mixture was maintained by the simultaneous addition of required volume of saturated sodium bicarbonate solution and progress of the reaction was monitored by TLC. The crude product was filtered off, washed several times with water, dried and recrystallized from ethanol and corresponding azo derivatives were obtained in order to good yields. The overall reaction path for the synthesis of azo dyes was depicted in the **Scheme-1**.



Scheme-1: Synthetic route for the preparation of azo dyes, **S1-S3**

*2.2.1. Synthesis of N-(4, 6-dimethylpyrimidin-2-yl)-4-{(E)-[4, 4- (dimethylamino) phenyl] diazenyl} benzenesulfonamide (**S1**)*

The dye was orange colour solid with 65 % yield, m.p. 192-195 ^{0}C. ^{1}H NMR (DMSO-d_6): δ 2.26 (s, 6H, CH_3 attached to pyridine ring), 3.08 (s, 6H, CH_3 attached to phenyl ring), 6.76 (s, 1H, Ar-H), 6.84 (d, 2H, Ar-H, *J*=8.8 Hz), 7.80-7.87 (m, 4H, Ar-H), 8.08 (d, 2H, Ar-H, *J*=8.4 Hz), 11.69 (s, 1H, NH). FT-IR (KBr): N=N (1597cm^{-1}), Ar-C-H (2967 cm^{-1}), -N-H (3229 cm^{-1}). LC-MS (m/z) = 410 $[M+H]^+$. Anal.cal (%) for $C_{20}H_{22}N_6O_2S$: C (58.52), H (5.40), N (20.47), O (7.80), S (7.81). Found: C (58.47), H (5.32), N (20.35), S (7.76).

*2.2.2. Synthesis of N-(4, 6-dimethylpyrimidin-2-yl)-4-{(E)-[4, 4- (diethylamino) phenyl] diazenyl} benzenesulfonamide (**S2**)*

The dye was reddish-orange colour solid with 62 % yield, m.p. 194-198 ^{0}C. ^{1}H NMR (DMSO-d_6): δ 1.15 (t, 6H, CH_3, *J*=6.8 Hz), 2.26 (s, 6H, CH_3 attached to pyridine ring), 3.46 (q, 4H, CH_2, *J*=6.8 Hz), 6.76 (s, 1H, Ar-H), 6.76 (s, Ar-H), 6.81 (d, 2H, Ar-H, *J*=9.2 Hz), 7.78-7.84 (m, 4H, Ar-H), 8.08 (d, 2H, Ar-H, *J*=8.4 Hz), 11.59 (s, 1H, NH). FT-IR (KBr): N=N (1598 cm^{-1}), Ar-C-H (2971 cm^{-1}), NH (3230 cm^{-1}). LC-MS (m/z) = 438 $[M+H]^+$. Anal.cal (%) for $C_{22}H_{26}N_6O_2S$: C (60.25), H (5.98), N (19.16), O (7.30), S (7.31). Found: C (60.12), H (5.79), N (18.98), S (7.28).

*2.2.3. Synthesis of 4-{(Z)-[4-(phenylamino) phenyl] diazenyl}-N-(4, 6-dimethylpyrimidin-2-yl) benzenesulfonamide (**S3**)*

The dye was dark orange colour solid with 60 % yield, m.p. 203-208 ^{0}C. ^{1}H NMR (DMSO-d_6): δ 2.26 (s, 6H, CH_3 attached to pyridine ring), 6.79 (s, 1H, Ar-H), 6.81-9.03 (m, 13H, Ar-H), 11.64 (s, 2H, NH). FT-IR (KBr): N=N (1586 cm^{-1}), Ar-C-H (3032 cm^{-1}), NH (3361 cm^{-1}).

LC-MS (m/z) = 459 $[M+H]^+$. Anal.cal (%) for $C_{24}H_{22}N_6O_2S$: C (62.86), H (4.84), N (18.33), O (6.98), S (6.99). Found: C (62.74), H (4.76), N (18.12), S (6.87).

2.3. Antimicrobial activity

In the present study, the novel heterocyclic azo dyes (S1-S3) were tested for growth inhibitory effect against pathogenic microorganisms by tube dilution technique [**23-24**]. The organisms used for the antimicrobial investigation are *Escherichia faecalis* (Gram positive bacteria), *Pseudomonas aeruginosa* and *Escherichia coli* (Gram negative bacteria) and fungal strains are *Candida albicans, Aspergillus flavus* and *Aspergillus niger*. Briefly, 20 µL of the azo compound was added into the 380 µL of BHI (Brain heart infusion) broth and from the above mixture, 200 µL was taken and added into the tube containing 200 µL of fresh BHI broth. From the above, 200 µL of the test solution was taken and serially diluted to next 9 tubes separately of each samples in order to get the final concentration of the test compounds in the range of 100-0.4 mg mL^{-1}. From the previously cultured respective microorganisms, 5 µL was taken and added into 200 µL BHI broth and this culture suspension was transferred into the serially diluted tubes and incubated for 24 h at 37 ^{0}C. At the end of the incubation, the tubes were checked for the appearance of turbidity. For antibacterial and antifungal activities, Ciprofloxacin and Fluconazole were used as positive controls respectively and DMSO as a negative control. Further, the results of the above activities were recorded in terms of minimum inhibitory concentration (MIC).

2.4. Anti-tuberculosis activity

The anti-tuberculosis activities of the synthesized azo compounds were investigated against *Mycobacterium Tuberculosis* (H37 RV strain, **ATCC-27294**) by employing the microplate Alamar Blue assay (MABA) technique. This method is non-hazardous, thermally stable and

shows good correlation with proportional and BACTEC radiometric method. Briefly, 200 µL of sterile deionised water was added to the sterile 96 wells plate in order to minimize the evaporation of medium in the test wells during incubation. To this added 100 µL of the Middlebrook 7H9 broth and serial dilution of the compounds were made directly on the plate. Finally, different azo dyes were studied in the concentrations range of 100-0.2 $\mu g\ mL^{-1}$. Plates were enclosed and preserved with parafilm and incubated for five days at 37 ºC. After completion of this period, 25 µL of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 h. The development of blue color in the wells indicates the bactericidal nature of the synthesized compounds while pink color exhibits inertness nature of the dye compound towards the bacterial growth. MIC method was defined as lowest drug concentration which prevents the color change from blue to pink [**25**].

2.5. Anti-inflammatory activity

Anti-inflammatory activity of the target compounds was investigated by Gelatin Zymography electrophoresis method [**26-27**]. The non-continuous electrophoresis gels were prepared and 50 µL of matrix metalloproteinase (MMP) sample was mixed with 50 µL of the azo compounds and incubated for 1 h. Further, the non-reducing buffer was mixed with equal quantity of negative control (MMP) as well as positive control (MMP + tetracycline hydrochloride). The electrophoresis was run at 50 V for 15 min and later increased to 100 V. After completion of the experiment, gels were washed with SDS (sodium dodecyl sulfate) surfactant followed by zymogram renaturing buffer for 1 h to remove the remaining surfactant and allow the gels for renaturing of the proteins. The gels were further incubated in zymogram incubation buffer for overnight at 37 ^{0}C. Finally, the gels were stained with Coomassie blue R-250 and the development of white bands indicated the presence of

gelatinases with the lower bands representing gelatinases-A (MMP-2) which are about 72 kDa while the upper bands are gelatinases-B (MMP-9) which are about 95 kDa.

2.6. DPPH radical scavenging activity

The antioxidant activities of azo compounds were studied by using DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay [**28**]. An aliquot of 25 µL of different concentrations (40 µg, 80 µg and 120 µg) of azo compounds in DMSO was added to a microplate separately and mixed with 175 µL of 0.086 mM ethanolic solution of DPPH. The mixture was incubated at ambient temperature for 30 minutes and scavenging effect of the azo dyes was analyzed at 517 nm while taking ascorbic acid as a standard. In this test, a solution of radical was decolourized after reduction with an antioxidant (AH) or a radical (R•) in accordance with the proposed reaction [**29**].

DPPH• + AH → DPPH-H + A•, **DPPH• + R• → DPPH-R**

The DPPH test is based on the capability of the stable free DPPH radical to react with hydrogen donors. The percentage of inhibition activity was calculated by using the formula given below and IC_{50} (Inhibitory concentration) was also calculated.

$$\% \text{ inhibition of DPPH radical} = [(A_{br}-A_{ar})/ A_{br} \times 100]$$

Where A_{br} is the absorbance before reaction and A_{ar} is the absorbance after reaction has taken place.

3. Results and Discussion

We have reported the three novel azo dyes having sulfamethazine moiety and their synthetic route is depicted in **Scheme 1**. The target compounds **S1-S3** were synthesized from facile, efficient and economical reactants by classical diazotization technique. Physical and analytical data of the prepared azo compounds were summarized in **Table-1**. Structural confirmations of the azo dyes were carried out by various physicochemical methods such as FT-IR, ^{1}H NMR, LC-MS, elemental analysis and electronic spectroscopic methods. Spectral data were found to be in good agreement with the proposed structure of the prepared azo compounds.

<Table-1>

3.1. IR spectral data

Infrared spectra of the azo compounds **S1-S3** were given in **Figs. 1-3** and all the compounds have shown a strong absorption peak in the region of 2971-3032 cm^{-1} are because of aromatic CH stretching bonds respectively. The peaks at 1586-1598 cm^{-1} are due to C=C stretching and 1231-1268 cm^{-1} are due to aliphatic CH stretching. The absorption peaks at 1597-1721 cm^{-1} are assigned to C=N stretching. Strong absorption peaks present at 3229-3361 cm^{-1}, 1497-1521 cm^{-1} are due to the NH and azo groups respectively.

< Fig-1>

< Fig-2>

< Fig-3>

3.2. *^{1}H NMR spectral data*

Proton NMR spectra of the synthesized compounds were recorded in DMSO-d6 using Bruker spectrophotometer and spectra are given in **Figs. 4-6**. It is an additional support for the structural confirmation of azo compounds. The number of protons present in the azo compounds was achieved from chemical shift (δ in ppm). The spectra resonated a triplet at δ 1.13-1.17 ppm for methyl proton, a singlet at δ 2.26 ppm corresponding to methyl group attached to pyrimidine ring, a singlet resonated at δ 3.08 ppm with respect to dimethylaniline proton, a quartet at δ 3.45-3.50 ppm for methylene proton, a singlet at δ 6.76 ppm related to aromatic proton, a doublet at δ 6.81-6.83 ppm corresponding to aromatic protons, a multiplet at δ 7.78-7.87 ppm corresponding to aromatic protons, a doublet at δ 8.08-8.10 ppm corresponding to aromatic protons, a singlet at δ 11.59-11.64 ppm related to NH proton, but compound S3 shows multiplet at δ 6.81-9.03 ppm which is related to aromatic protons.

< Fig-4>

< Fig-5>

< Fig-6>

3.3. Mass spectral data

Mass spectra of the prepared azo dyes shows that the molecular ion peaks are in good agreement with their suggested empirical formula given in **Table-1** and spectra are given in **Figs. 7-9** The synthesized azo compounds **S1**, **S2** and **S3** showed $[M+H]^{+}$ peaks at m/z 410, 438 and 459 respectively which were related to the molecular weight of the dyes.

< Fig-7>

< Fig-8>

< Fig-9>

3.4. Electronic absorption spectra

<Table-2>

The electronic absorption spectra of the synthesized azo dyes **S1-S3** were recorded in two solvents (DMSO, DMF) in the range of 200-800 nm and the results are summarized in **Table-2**. All the dyes exhibit main absorption band in the range of 444 - 468 nm which can be assigned as **n→π*** electronic transition of an azo chromophore. This is due to the interaction between a solvent molecule and nitrogen atom of the prepared azo compounds having non-bonding electrons (**Figs.10-11**) [**30**]. From the findings given in **Table-2**, it is revealed that substituents effect also plays a very important role in the absorption spectra of dyes **S1-S3** and also introducing the electron donating substituents like CH_3, C_2H_5, and C_6H_5 on the diazo component, the bathochromic shift is facilitated as an account of the interaction between the hydrogen atom of dyes with solvent molecules and increasing polarity of the dye system in the excited state [**31**]. Furthermore, all these azo dyes exhibited the molar absorpitivity it the range of 3.979 - 4.270 which account for the intense colour of the dyes in solution.

< Fig-10>

< Fig-11>

3.5. Antimicrobial activity

A multidrug resistance of bacterial strains continues to be a great challenge due to their biochemical and morphological modifications and need for the novel drug has motivated much attention. Therefore, the azo group containing heterocyclic moiety have received significant interest due to their wide spectrum of biological and pharmacological applications [**32-33**]. In the present study, the *in vitro* antibacterial activities of synthesized dyes were studied by MIC method against three pathogenic bacterial strains while taking ciprofloxacin drug as a positive control. The results are summarised in the **Table-3**, and it is evident that all the compounds **S1-S3** shown efficient antibacterial activity against tested bacterial strains, except for *P.aeruginosa* which exhibited resistant in all the azo dyes, whereas *E.coli* showed mild sensitiveness towards dyes, **S3** displayed excellent antibacterial activity in *E. fecalis*.

Similarly, compounds **S1-S3** exhibited potential antifungal properties against *C.albicans, A.flavus* and *A.niger* strains and results are as shown in **Table-3**. The antifungal activities of azo compounds are in the order, **S1** > **S3** > **S2,** with **S1** exhibiting high activity compared to the other compounds which showed moderate effects. From the results, it is clear that tested azo compounds have efficient bactericidal and fungicidal properties.

<Table-3>

3.6. Anti-tuberculosis activity

Tuberculosis is one of the challenging infectious diseases for therapeutic field usually infected by the *Mycobacterium tuberculosis* which affects lungs and other parts of the body and even sometimes it may lead to death. To overcome these problems new drugs were

developed in modern years but, the utility is limited due to their adverse effects [**34**]. In the present study, the synthesized azo dyes were screened for their anti-tuberculosis activity against *M. tuberculosis* and the results are shown in **Table-4**. The results reveal that all the compounds exhibited significant resistant against *M.tuberculosis*. Among these dyes, **S3** displayed good anti-tuberculosis activity than the other two compounds which are shown moderate activity with anti-Tb drugs such as pyrazinamide, ciprofloxacin and compatible with streptomycin as shown in **Fig-12**.

<Table-4>

< Fig-12>

3.7. Anti-inflammatory activity

Inflammatory is a complex biological reaction of body tissues to harmful stimuli, such as a pathogen, damaged cells or irritant and protective nature of the body. It indicates to remove the initial cause of cell injury and initiate the tissue repair. Nowadays, large numbers of medicines are developed in related to anti-inflammatory agents some of them are aspirin, ibuprofen, and diclofenac [**35**]. The above investigation expected to through delight in understanding the impact of azo compounds on anti-inflammatory activity against two enzymes MMP-2, MMP-9 and results are represented in **Fig-13** and **Fig-14**. From the obtained data all the compounds have shown significant inhibition effect against MMP-2 and MMP-9 enzymes. Out of these studied dyes, **S2** shows highly potent anti-inflammatory activity against MMP-2 enzyme and others exhibits reasonable activity. In case of MMP-9 enzyme **S3**, **S1** and **S2** compounds shows good to moderate anti-inflammatory activity.

< Fig-13>

< Fig-14>

3.8. Antioxidant activity

Antioxidants are the molecules that consume the free radicals produced by the oxidation process thereby prevents the living tissues from injury and abnormal metabolic system and some of the antioxidants are ascorbic acid, thiols *etc*. In recent years, synthetic antioxidants are found to be more advantageous than natural ones. But, usage of the synthetic antioxidants is constrained because of their toxicity and resistance. Hence in this point of view, there is a need to develop a new class of organic compounds which are less toxic, stable, cheap and do not show any side effect [**36**]. Therefore, in the present investigation, azo compounds are assessed for their scavenging activity with the ease of DPPH assay. The radical activity of target compounds were tested at different concentrations 40 µg, 80 µg and 120 µg is shown in **Fig-15**. From the results, it is obvious that activity is completely concentration dependent and among these compounds **S2** displayed good activity, while **S1** and **S3** exhibit moderate activity compared with standard ascorbic acid.

< Fig-15>

4. Conclusions

In summary, we have successfully synthesized three novel bioactive azo compounds by the well known diazo-coupling method and their structural features were accomplished on the basis of spectral data. Electronic absorption spectral study specifies that, the prepared azo compounds containing excellent colouring properties due to the presence of chromophores in their structures. Further, these azo dyes proved to have significant antibiotic property against tested pathogens and among the tested dyes **S3** exhibits significant bactericidal activity than **S1** and **S2**, while in case of fungicidal activity **S1** shows efficient inhibition effect towards antifungal nature. All the synthesized compounds displayed promising anti-tuberculosis activity against *M. Tuberculosis*, among them **S3** shown higher activity than the remaining compounds and compatible with the standard drug Streptomycin. Compound **S2** displayed excellent anti-inflammatory effect against MMP-2 enzyme and **S3** also exhibited noticeable anti-inflammatory action against MMP-9 enzyme. Antioxidant property of the synthesized compounds was examined by using DPPH scavenging assay, among the tested compounds **S2** shows good antioxidant activity. From the present study, we can develop an advanced strategy by the combination of two or more pharmacological properties in a single compound which may be useful in the treatment of multiple diseases. Hence, these synthesized azo compounds were found to be interesting lead molecules for curing multiple diseases enabling to build a platform for the designing of novel drugs with multiple therapeutic properties.

Therefore, the results herein reported may be used in the pharmaceutical area for developing potential drugs in the future.

Acknowledgement

One of the author, Mr. Vinodkumar very thankful to the Department of Post Graduate Studies and Research in Chemistry, Kuvempu University, Shimoga for furnishing laboratory amenities. Authors are also grateful to Manipal University, Manipal for allocating spectral information and Maratha Mandal's central research laboratory, Belgaum for providing biological studies.

References

[01] Marmion D.M, "Handbook of US Colourant", WILEY, New York, (1999) 23-26.

[02] M.S. Refat, M.Y. EI-Sayed, A.M.A. Adam, *J. Mol. Struct*. 1038 (2013) 62-72.

[03] Jyotirmaya Sahoo, Suman Kumar Makep and Paidesetty Sudhir Kumar. *J. Taibah university*, 9 (2015) 187-195.

[04] Demirbas, D. Sahin, N. Demirbas, and S. A. Karaoglu, *Eur. J. Med. Chem*. 44 (7) (2009) 2896-2903.

[05] Gung, B. and Taylor, R. *J. Chem. Ed*, (2004) 1630-1632.

[06] Marc Loudon, organic chemistry, 5th ed., (23.10 A-B) (2010) 1139-1144.

[07] T. Pooja, C. Malay, *Indian J. Eng. Mater. Sci*. 11 (2004) 499-504.

[08] A.F. Shoair, A.A. El-Bindary, A.Z. El-Sonbati, N.M. Beshry. *J. Mol liq.* 215 (2016) 740-748.

[09] Divyesh R. Patel, Naitik B. Patel, Bhavesh M. Patel and Keshav C. Patel. *J. Saudi Chem. Soc*, 18 (2014) 902-913.

[10] Franker Amen Imadegbor, Kasali Ademola Bello, Abdul Raheem Giwa. IOSR *J. Poly. Text. Engg*, 1 (2) (Jan 2014) 46-52.

[11] Hari Raghav Maradiya, *J. Saudi Chem. Soc*, 16 (2012) 69-74.

[12] A.R. Katritzky, A.F. Pozharskii, A. Soldatenkov, 2nd ed., John Wiley & Sons, Hoboken, (2011) 2549-2552.

[13] K. S. Bhat, B. Poojary, D. J. Prasad, P. Naik, and B. S. Holla, *Eur. J. Med. Chem*. 44 (12) (2009) 5066-5070.

[14] G. B. Bagihalli, P. G. Avaji, S. A. Patil, and P. S. Badami, *Eur. J. Med. Chem*. 43 (12) (2008) 2639-2649.

[15] E. R. Fernandez, J. L. Manzano, J. J. Benito, R. Hermosa, E. Monte, and J. J. Criado, *J. Inorg Biochem*, 99 (8) (2005) 1558-1572.

[16] M.A. Ali, A.H. Mirza, R.J. Butcher, M.T.H. Tarafder, T.B. Keat, A.M. Ali, *J. Inorg. Biochem.* 92 (2002) 141-148.

[17] A.B. Beshir, S.K. Guchhait, J.A. Gascon, G. Fenteany, *Bioorg. Med. Chem. Lett.* 18 (2008) 498-504.

[18] Papich, M.G, 3rd ed., St. Louis, MO: Elsevier Saunders, (2011) 723-724.

[19] C.T. Keerthi Kumar, J.Keshavayya, T. Rajesh, S.K. Peethamber, R.A. Shoukat Ali, *Chem Sci Trans*, 2(4) (2013) 1346-1351.

[20] S. Harisha, J. Keshavayya, B.E. Kumaraswamy, C.C.Vishwanath, *Dyes pigm*, 136 (2017) 742-753.

[21] N.M. Mallikarjuna, J. Keshavayya, M.R. Maliyappa, R.A. Shoukat Ali, Venkatesha Talavar, *J. Mol.str*, 1165 (2018) 28-36.

[22] Vinodkumar, J. Keshavayya, M. Pandurangappa, B.N. Ravi, *CDC*, 17-18 (2018) 13-29.

[23] Antimicrobial susceptibility testing protocols. Schwalbe, Moore and Goodwin, Crc Press (2007) 139-209.

[24] P. Klahn, M. Bronstrup, *Microbiol. Immunol.* 398 (2016) 365-417.

[25] Zongli Ren, Juanjuan Chen, and Raouf A. Khalil, *Methods Mol Biol*, 1626 (2017) 79-102.

[26] Vijayalakshmi D, Dhandapani R, Jayaveni S, Jithendra P , Rose C, Mandal A B, *J. Ethnopharmacology*, 141 (2012) 542-546.

[27] Blois M S. *Nature*, 181 (1958) 1199-1200.

[28] Oyaizu. M, *Japanese journal of nutrition,* 44 (1986) 307-315.

[29] Parejo L, Codina C, Petrakis C, Kefalas P, *J. Pharm. Toxicol. Meth, 44 (*2000) 507-512.

[30] Motaleb Ghasemian, Ali Kakanejadifard, Farideh Azarbani, Abedin Zabardasti, Sahar Kakanejadifard. *J. Mol. Struct.*195 (2014) 35-39.

[31] G.G. Mohamed, *Spectrochim, Acta,* 64A (2006) 188–194.

[32] N. Raman, A. Sakthivel, N. Parvin, *Spectrochim Acta part A Mol Biomol Spectrosc,* 125 (2014) 404-413.

[33] Lelia Pacheco Correa Barros, Karina Pena Del Rio, Tatiane dos Santos Conceicao Carvalho, Maria do Carmo Freire Ribeiro Pinto, Kelly Cristina Gallan de Moura, Priscila Cristina Bartolomeu Halicki, Daniela Fernandes Ramos, Pedro Eduardo Almeida da Silva, *Tuberculosis*, 111(2018) 198-201.

[34] Maria, C. S, Lourenco, Marcus, V. N deSouza., Alessandra, C. Pinheiro, Marcelle de L., Ferreira, Rasnisb, B. Goncalves, Thais Cristina, M. Nogneira, Monica, A. Peralta. ARKIVOC (xv) (2007)181-191.

[35] Ferrero M L, Nielsen O H, Andersen P S, Girardin S E, Nielsen, Andersen, Girardin. *Clin. Exp. Immunol.* 147 (2) (2007) 227-235.

[36] Christen W G, Glynn R J, Chew E Y, Buring J E, *Ophthalmology,* 115(5) (2008) 822-829.

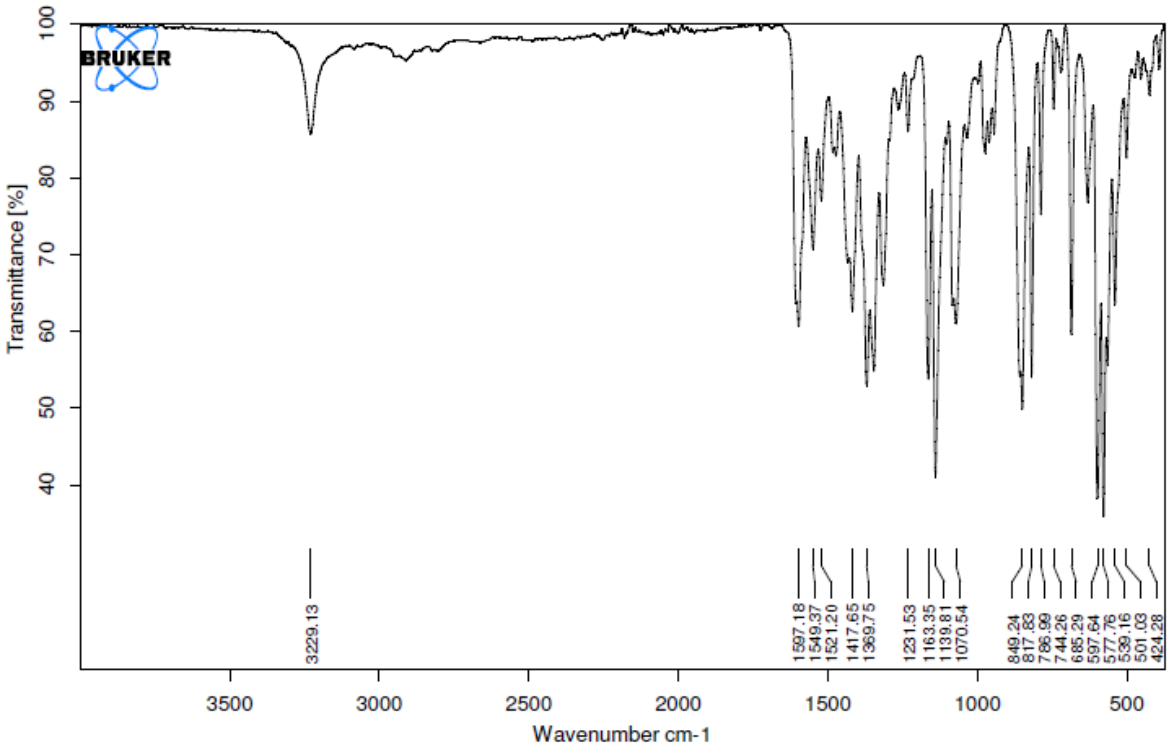


Fig-1 IR spectrum of compound **S1**

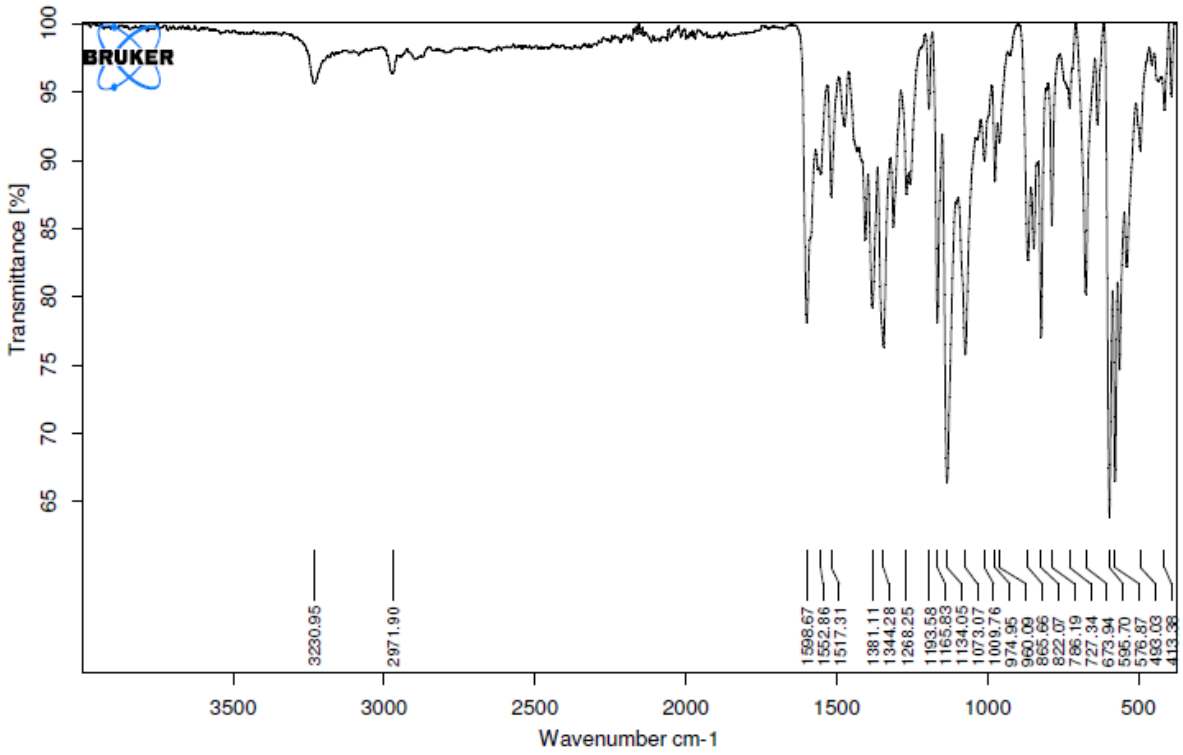


Fig-2 IR spectrum of compound **S2**

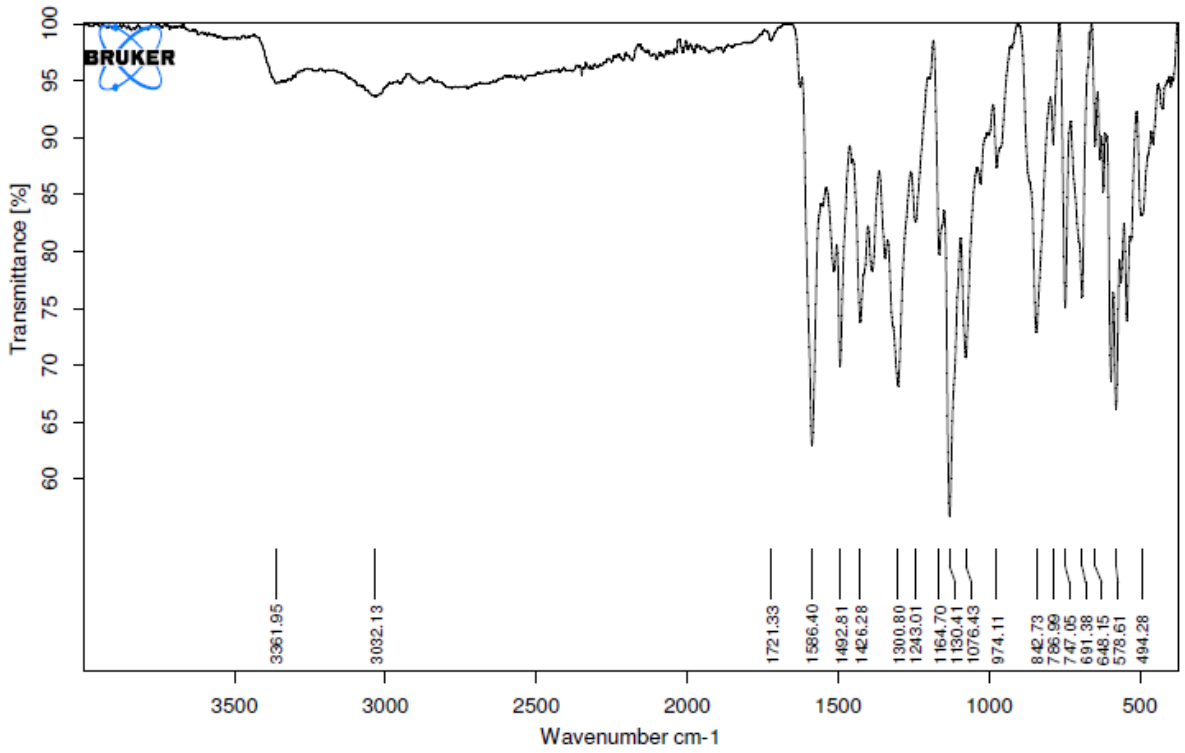


Fig-3 IR spectrum of compound **S3**

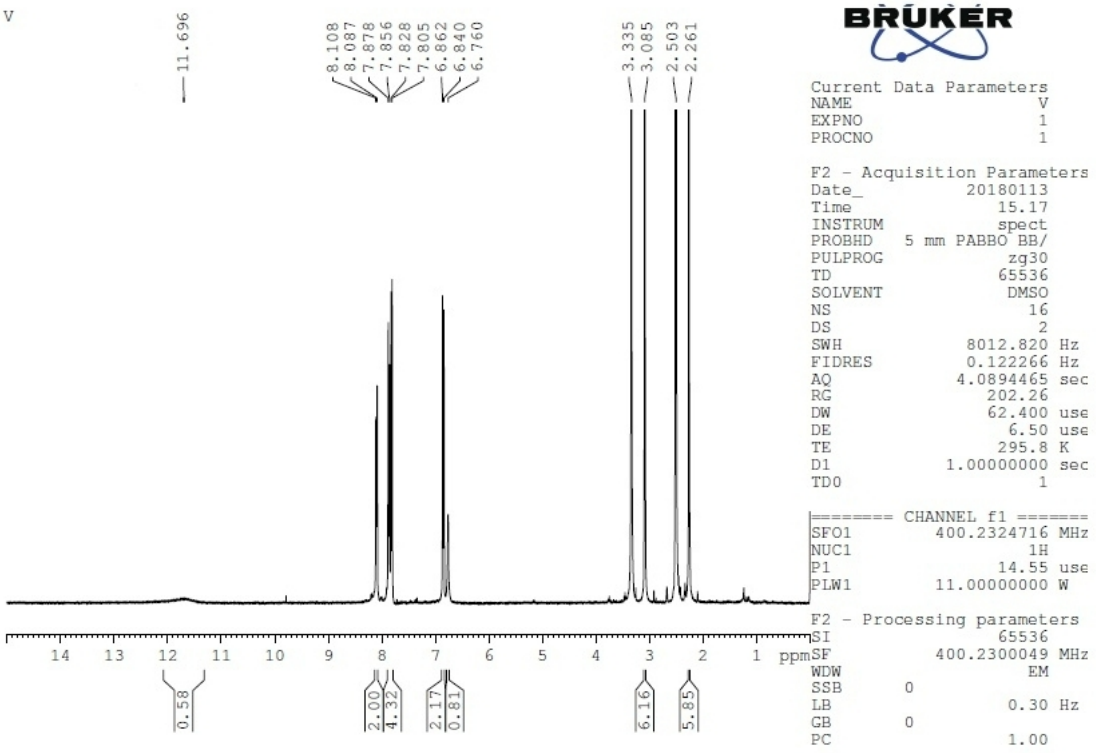


Fig-4 ^{1}H NMR spectrum of compound of **S1**

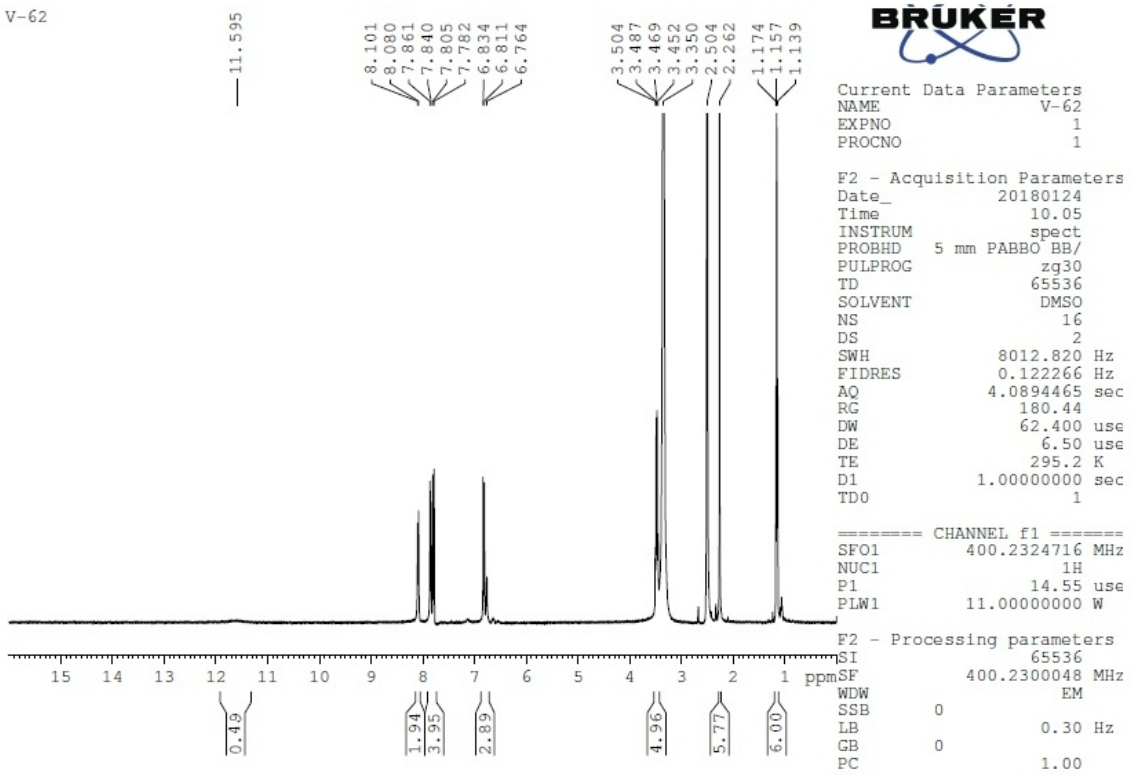


Fig-5 ^{1}H NMR spectrum of compound of **S2**

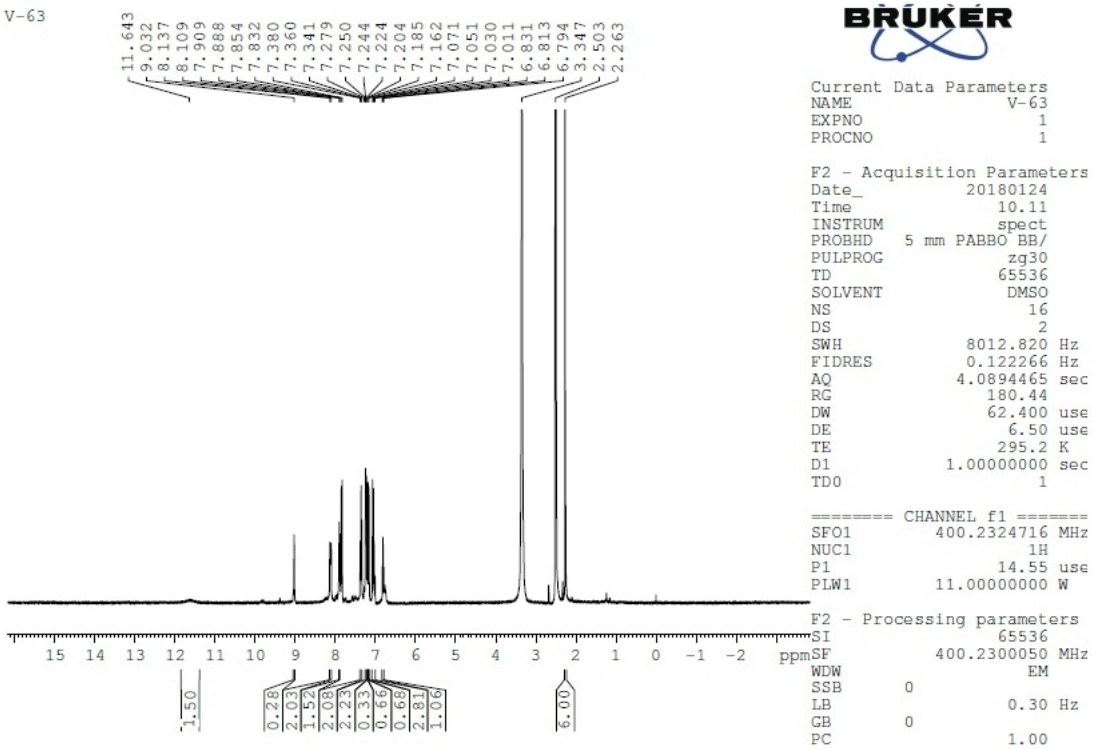


Fig-6 ^{1}H NMR spectrum of compound of **S3**

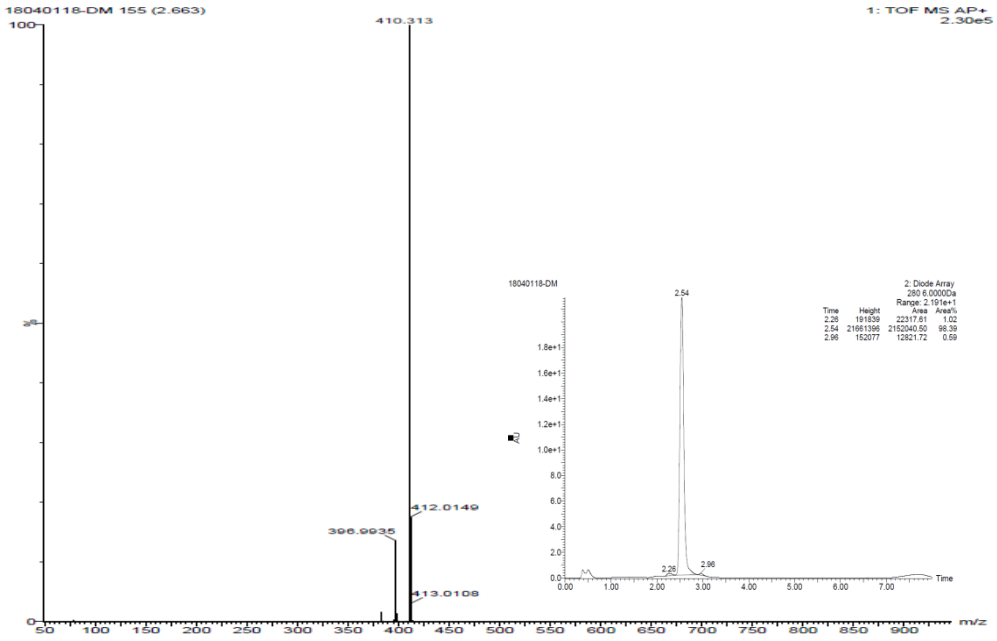


Fig-7 LC-MS spectrum of compound **S1**

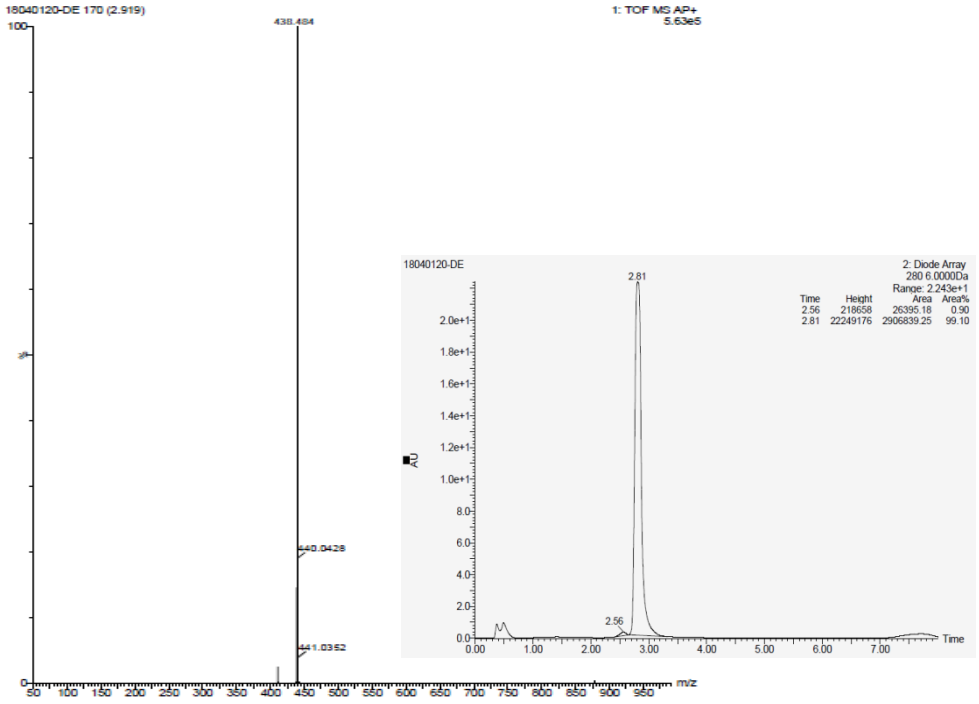


Fig-8 LC-MS spectrum of compound **S2**

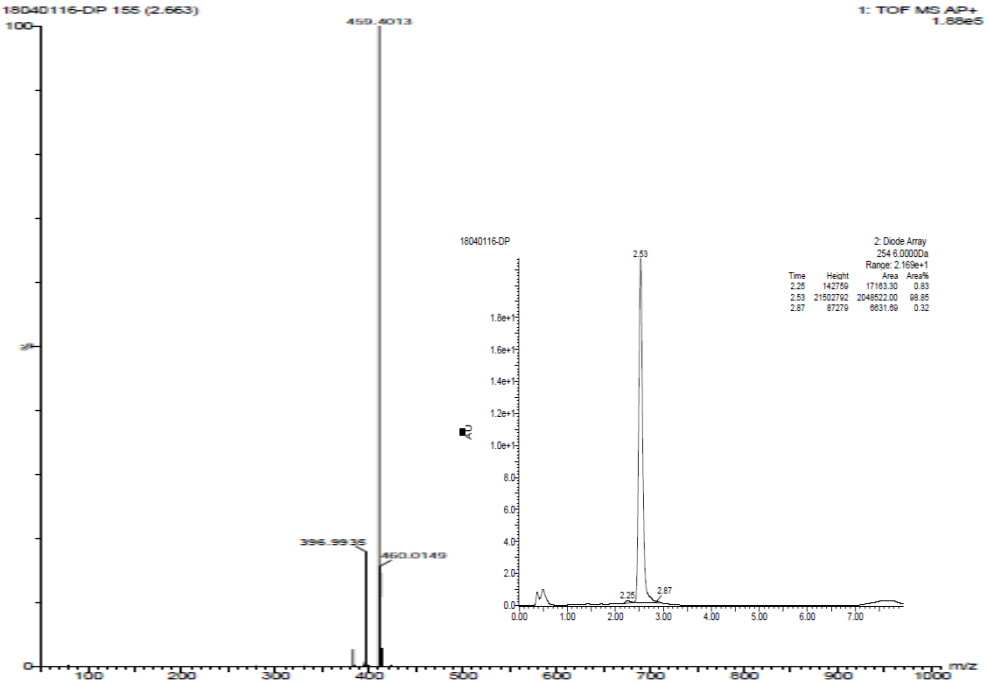


Fig-9 LC-MS spectrum of compound **S3**

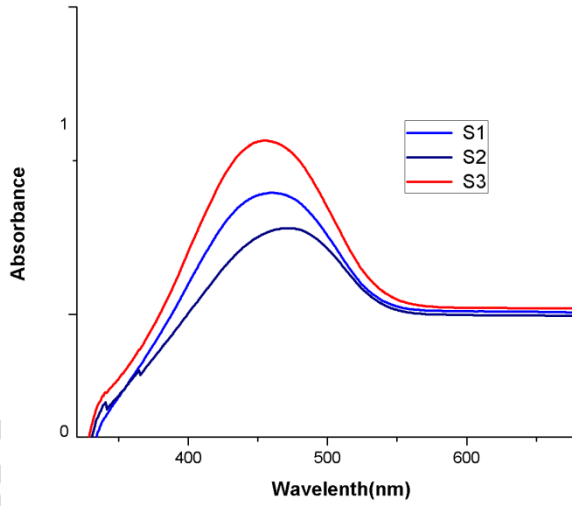


Fig-10**.** UV-Visible spectra of the dyes **S1-S3** in DMSO

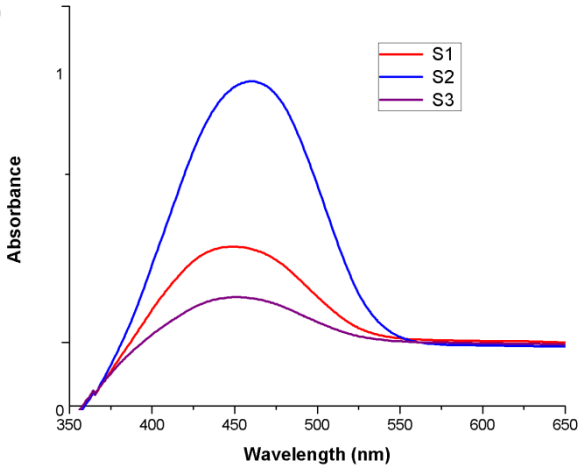


Fig-11. UV-Visible spectra of the dyes **S1-S3** in DMF

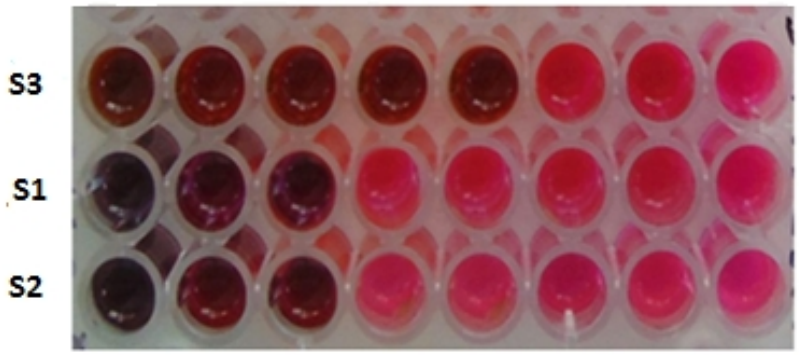


Fig-12. Anti-tuberculosis activity of the dyes, **S1-S3**

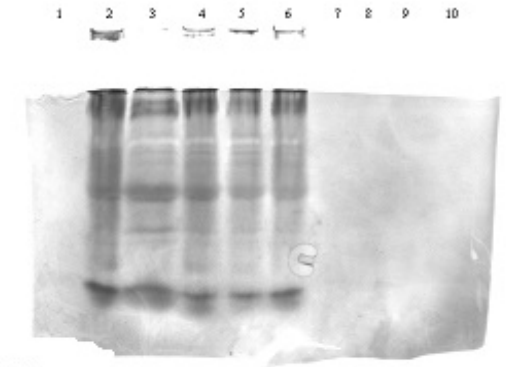


Fig-13. Electrophoresis bands of the dyes **S1-S3** showing anti-inflammatory activity.

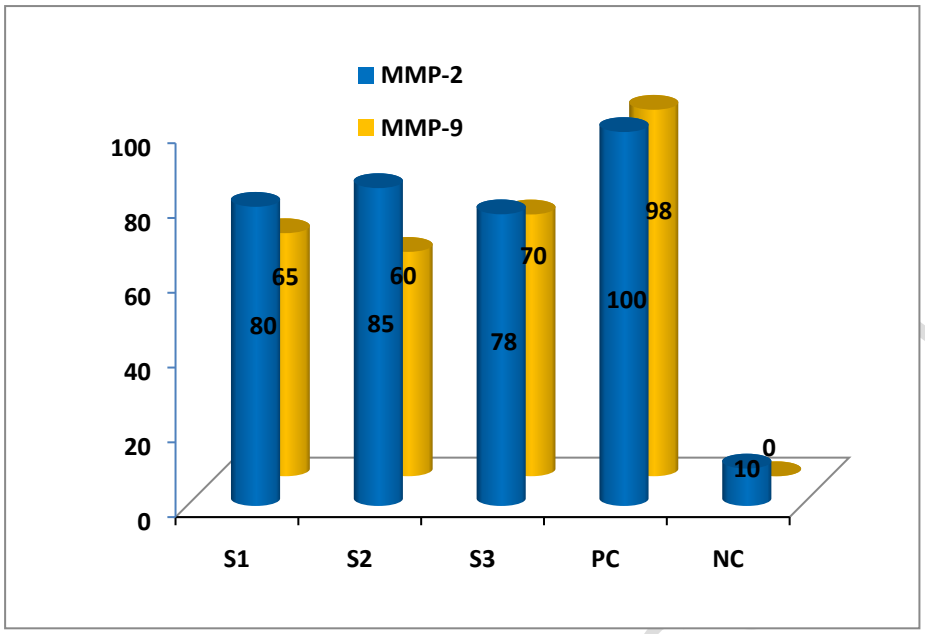


Fig-14. Anti-inflammatory activity of the dyes, **S1-S3**

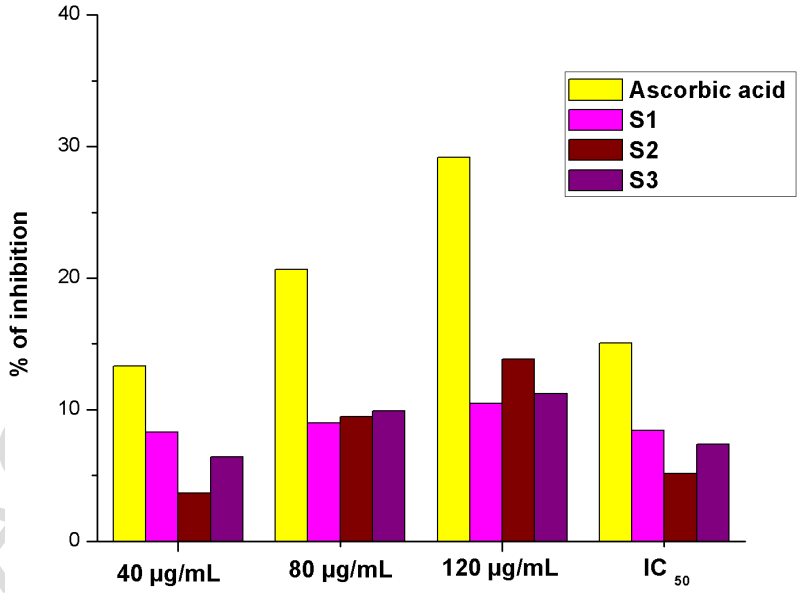


Fig-15. DPPH activity of the dyes, **S1-S3**

Research Highlights

- Synthesis of azo compounds by a conventional diazo-coupling method which is facile and effective.
- Sulfamethazine compounds were known for their antimicrobial properties.
- Sulfamethazine incorporated azo dyes are also exhibited effective pharmacological properties.
- In future, these kinds of dyes can be used in the development of multiple therapeutic drugs by inserting multiple properties in a single compound.

Table 1: Physical and analytical data of the dyes **S1-S3**

Compound	Mol. Formula.	Mol. Mass.	M.P (°C)	Colour	Yield (%)
S1	$C_{13}H_{11}N_5O_4S$	409	192-195	Orange	65
S2	$C_{22}H_{26}N_6O_2S$	438	194-198	Reddish-orange	62
S3	$C_{24}H_{22}N_6O_2S$	458	203-208	Dark orange	60

Table 2: UV-Visible spectral data of the dyes **S1-S3**

Compounds	λ_{max}(nm) **DMSO**	**log ε**	Compounds	λ_{max}(nm) **DMF**	**log ε**
S1	453	4.270	**S1**	444	3.979
S2	457	4.120	**S2**	459	4.413
S3	468	3.979	**S3**	451	3.659

Table 3: Antibacterial and antifungal activity of the dyes **S1-S3**

Samples	**100 mg/mL**	**50 mg/mL**	**25 mg/mL**	**12.5 mg/mL**	**6.25 mg/mL**	**3.12 mg/mL**	**1.6 mg/mL**	**0.8 mg/mL**	**0.4 mg/mL**
E.fecalis									
S1	S	S	S	S	S	S	R	R	R
S2	S	S	S	S	S	S	S	S	R
S3	S	S	S	S	S	S	S	S	S
E.coli									
S1	S	S	R	R	R	R	R	R	R
S2	S	S	R	R	R	R	R	R	R
S3	S	S	S	R	R	R	R	R	R
P.aeruginosa									
S1	S	S	S	S	R	R	R	R	R
S2	S	S	S	R	R	R	R	R	R
S3	S	S	S	R	R	R	R	R	R

C.albicans									
S1	S	S	S	S	S	S	R	R	R
S2	S	S	R	R	R	R	R	R	R
S3	S	S	S	R	R	R	R	R	S
A.flavus									
S1	S	S	S	S	S	S	R	R	R
S2	S	S	S	S	S	S	S	R	R
S3	S	S	S	S	S	S	S	S	R
A.niger									
S1	S	S	S	S	S	S	S	S	S
S2	S	S	S	S	S	S	S	S	R
S3	S	S	S	S	S	S	S	R	R

S- Sensitive

R- Resistant

Table 4: Anti-tuberculosis activity of the dyes, **S1-S3**

Compounds	**100 µg/mL**	**50 µg/mL**	**25 µg/mL**	**12.5 µg/mL**	**6.25 µg/mL**	**3.12 µg/mL**	**1.6 µg/mL**	**0.8 µg/mL**
S1	S	S	R	R	R	R	R	R
S2	S	S	R	R	R	R	R	R
S3	S	S	S	S	S	R	R	R

S-Sensitive

R- Resistant