New azo-azomethine derivative of sulfanilamide: Synthesis, Characterization, Spectroscopic, Antimicrobial and Antioxidant activity study

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Abstract. A series of azo-azomethine compounds (Sb1-Sb5) have been synthesized with magnificent yield by condensation reaction of 4-((3-formyl-4-hydroxy-5-methoxyphenyl)diazenyl)enzenesulfonamide and aniline derivatives. The new azo compound was prepared from sulfanilamide by converting it to diazonium salt followed by coupling reaction with 2-hydroxy-3-methoxybenzaldehyde in alkaline medium. The structures of synthesized azo and azo-azomethine compounds have been established based on their spectral data (FT-IR, 1HNMR, 13CNMR) and elemental analysis (C,H,N). The purity of compound and evaluation of Rf value were determined by TLC. The antimicrobial activity of azo-azomethine compounds have been tested in vitro against bacteria (Staphylococcus aureus, Escherichia coli and Klebsiella pneumonia) and fungi (Candida glabrata, Candida albicane and Aspergillus niger) by agar diffusion method, to assess their inhibiting potential. Also the antioxidant efficiency of azo-azomethine compounds have been calculated.

Keywords: azo-azomethine, azo dyes, sulfanilamide, aniline derivatives.

Introduction

Azo compounds are characterized by the presence of the azo moiety (–N=N–) in their structure, conjugated with two, distinct or identical, mono- or polycyclic aromatic or heteroaromatic systems. Because of their specific physico-chemical properties and biological activities, they have got a broad application in pharmaceutical, cosmetic, food, dyeing or textile industry and analytical chemistry. However, the most typical and popular field of utility remains as their coloring function. Medical importance of azo compounds is well known for their antibiotic, antifungal and anti-HIV properties. [1, 2]

The azo dye derived from the antibacterial drugs sulfonamides were the first effective chemotherapeutic agents that could be used systematically for the cure of bacterial infection in humans. A series of azo dyes containing the sulfonamide functional group were synthesized as potential antimicrobial agents. Today, there are a few sulfonamides and especially sulfonamide–trimethoprim combination that are used extensively for opportunistic infection in the patients with AIDS.
Schiff bases derived from sulfa drugs and aromatic aldehydes or ketones were more stable from other compounds because of magnificent resonance [5]. Many studies have proved that therapeutic properties of sulfonamides compounds became better when changed to Schiff bases(Schiff bases derived from drugs which have some hetero cyclic rings has been proved that it can be used as hard tonics and diuretic substances)[6]

Schiff bases are used as substrates in the preparation of a large amount of bioactive and industrial compounds. In addition, Schiff bases are well-known to have biological activities such as antibacterial, antifungal, antitumor, antiviral, anti-HIV-1, antiproliferative, herbicidal and anti-influenza A virus activities. It has been suggested that azomethine linkage (C=N) might be responsible for the biological activities of Schiff bases [7, 8]

This group of compounds is recognized as great biological activity and they play an important role in biological systems, for instance rhodopsin, halorhodopsin, bacterio rhodopsin the retinal molecule is connected to peptide through NH₂ group of lysine residue forming a Schiff base. furthermore Schiff bases are observed in various enzymes such as tryptophan synthase, transaminases, transketolases etc. [9]

Most chemists and scientific researchers have achieved many applications in a matter of biological properties of the Schiff base and their metal complexes to guarantee the best to usefulness. [10]

EXPERIMENTAL

Materials and Reagents

2-chloroaniline, p-Toluidine, 4- amino acetophenone, 2- hydroxyaniline and 2, 4-dimethylaniline was obtained from Fluka. HCl, NaNO₂, Sodium carbonate, 2-hydroxy 3- methoxy benzaldehyd and sulphanilamide from sigma-Aldrich product. All solvent used of analytical grade from Fluka and used without further purification... The measurements of melting point were done on Bauchi 510.

Instrumentation

The solid state FTIR spectra of the compounds were record on shimadzu FT –IR model 8400 S Spectrophotometer using KBr pellets in the range 4000 - 400 cm⁻¹. The spectra of ¹H NMR and ¹³CNMR were done in a Brucker spectrophotometer (400 MHZ) and using DMSO –d6 as solvent and TMS as internal standard. Elemental analysis (C, H, N) were recorded by Euro vector model 3000 A (Italy).

Synthesis of 4-((3-formyl-4-hydroxy-5-methoxyphenyl)diazenyl) benzenesulfonamide (A):

The compound was prepared as described by Erdem [11]. A solution of sulfanilamide 5 mmol (0.86 g) in 2N of HCl (10 mL) was stirred until a clear solution was obtained. The mixture was cooled to 0–5 °C, then sodium nitrate 7.5 mmol (0.5 g) in was added dropwise with stirring, maintaining the temperature below 5 °C. After the addition was completed, the solution was stirred for an additional 45 min. ortho vanillin 5 mmol (0.76 g) was dissolved in 30 mL of aqueous solution containing 20 mmol of Na₂CO₃. The mixture cooled to 0–5 °C in an ice bath, then gradually added to the solution of the cold diazonium salt of sulfanilamide. The resulting mixture was continually stirred at 0–5 °C for 2 h and keeping pH about 6–7. The originating precipitate filtered, washed several times with water and recrystallized from EtOH to give the desired azo compound. The purity azo compound was evaluated by thin layer chromatography by using ethanol / chloroform (1:9).
Synthesis of Sulfanilamide Azo-azomethine (Sb1-Sb5):

The Schiff-base was set by the usual condensation reaction, in which equimolar 1 mmol (0.3 g) of azo compound and the aniline derivatives 1mmol were liquefied in least possible quantity of methanol, also glacial acetic acid (2-3 drops) was added and refluxed for about 5-6 hrs, the response of the product was tested by TLC using acetone / chloroform (7:3), pure compounds were obtained by cooling the reaction mixture to freezing temperature. The precipitate was filtered off and washed with cold absolute methanol and dried in air.[11]

Table 1. The various synthetic preparation of azo – azomethine compounds are summarized in Scheme 1.

Antimicrobial activity

The compounds were screened in vitro for antimicrobial properties. The panel of pathogens involved Staphylococcus aureus as a Gram-positive bacterium, Escherichia coli as a Gram-negative bacterium, Klebsiella and fungal species Candida glabrata, Candida albicans and Aspergillus niger. This test was performed by agar diffusion method [12]. Dimethyl sulfoxide was used as a solvent and as control for disc sensitivity. 0.2 mL of fungal inocula and bacterial inocula were placed on the surface of Sabouraud dextrose agar (S.D.A) medium and Nutrient agar (N.A) medium respectively and then spread.
with L- shape glass rod .the plates were left for 10 minutes. The tested compounds and amoxicillin (as the reference drug ) were dissolved in DMSO with concentration include 0.03g /mL for every compound .The synthesis compounds were placed in central pore which include 0.1 m L .bacterial plates were incubated at (37 ± 2 °C) .while fungal plates were incubated at (25 ± 2 °C) for 24 hr ,the inhibition zones of each isolate were recorded in millimeter unit.

**Antioxidant assay using the β-carotene bleaching method**

The oxidative losses of -carotene/linoleic acid emulsion were used to assess the anti-oxidation ability of the synthetic organic compounds . 0.02 ml of linoleic acid and 0.2 ml of Tween 20 were placed in a round flask. And (1 ml ) -carotene ( 0.2 mg / ml in chloroform ) was added to the flask . The mixture was evaporated to dryness . 50 ml of distilled water was added to the mixture and shaking, 3.8 ml of the mixture was then dosed with 0.2 ml of corresponding concentration of tested sample or reference (BHT) compound .the control consist of 0.2 ml of DMSO instead of compound .as soon as the mixture was added to each tube , the zero time absorbance was read at 470 nm , the samples were then subjected to thermal autoxidation at 45 °C in a water bath for 2 h . absorbance was measured at 15 min intervals to monitor the rate of bleaching of -carotene [13].Antioxidant activity (AA) was calculated using the following equation:

\[
\%\text{AA} = 1 - \frac{(A_i - A_t)}{(A_i - A_t)} \times 100
\]

Where  
\( A_i \) : is the measured absorbance value of sample at zero time.
\( A_t \) : is the measured absorbance value of control at zero time.
\( A_t \) : is the measured absorbance value of sample after incubation (105)min at 45 °C.
\( A_t \) : is the measured absorbance value of control after incubation (105)min at 45 °C.

**Table 1: The symbol, Synthetic formula, IUPAC name, analytical and physical data of the prepared compounds**

| Co m. | Sym b. | Structural formula | IUPAC name | Mol.for mula | M.wt | m.p °C | Rf | Appearance | Yield % | Elemental analysis CHN partical (theoretical) | C% | H % | N% |
|-------|-------|-------------------|------------|-------------|------|-------|----|------------|---------|-----------------------------------------------|-----|-----|-----|
| A     |       | ![Structural formula](image) | (E)-4-((3-formyl-4-hydroxy-5 -methoxyphenyl) diazenyl) benzene sulfonamide | C_{14}H_{13}O_{5}N_{3}S | 335 | 200 | 0.6 | Maroon powder | 48 | 49.81 | 50.14 | 3.6 | 5.9 | 1.3 |
| Sb1   |       | ![Structural formula](image) | | C_{20}H_{17}O_{4}N_{4}S | 444.5 | 120 | 0.7 | Light orange crystals | 42 | 53.48 | 53.99 | 3.5 | 3.8 | 3.4 |
Results and Discussion

The azo-azomethine compounds (Sb1-Sb5) were prepared via reaction of aniline derivatives with azo compound (A). The prepared compounds are solid Compound, stable in air, the elemental analysis C, H, N are in agreement with suggested formula. The physical properties, percent yield and Rf values, are cited in Table -1.
Spectroscopic Analysis

Their spectroscopic analysis are in agreement with the empirical structures. The infrared spectra show the position and the intensities of the peaks which corresponds to various groups present in each compound. Moreover their elemental analyses are in conform with the theoretical expectations.

Infrared spectra

The infrared of prepared compounds (A, Sb1 - Sb5) shows characteristic bonds at (1465-1467) cm⁻¹ that be attributed to the stretching vibration of the azo group (-N=N-) . All the infrared spectra of the compounds were characterized by a broad band at 3375–3568 cm⁻¹ which correspond to the O-H stretching vibration. IR spectra of the Schiff bases showed the absence of bands at 1647 cm⁻¹ which attribute to carbonyl (C=O) stretching vibrations and, instead, the appearance of a strong new band at 1589–1633 cm⁻¹ that assigned to the azomethine (C=N) linkage, which indicates the reaction between the amino and aldehyde moieties of the starting reagents no more exist and have been converted into the respective Schiff base linkages. In addition, the strong band at 1226–1265 cm⁻¹ can corresponding to the phenolic C-O stretching vibration.

Furthermore the SO₂ moiety appears featured bands at the range 1330-1369 cm⁻¹ and in 1130-1199 cm⁻¹ which assigned to asymmetrical and symmetrical stretching vibration respectively of SO₂ [14, 15].

The spectral data of these synthesized compounds have been summarized in Table 2.

Table -2- FT-IR of synthetic amide (cm⁻¹,KBr disc ) (s : strong, m :medium ,w: weak ,br :broad)

| Com. | v(OH ) cm⁻¹ | v(NH₂) sulfa cm⁻¹ | v(C=O) cm⁻¹ | v(CH=N ) cm⁻¹ | v(N=N cm⁻¹ ) | v(SO₂ ) Asym. Sym. cm⁻¹ | v(C-O ) cm⁻¹ |
|------|-------------|-----------------|-------------|--------------|-------------|----------------|----------------|
| A    | 3568 m      | 3267-3363 s     | 1647 s      | –            | 1465 s      | 1330 s         | 1257 s         |
| Sb1  | 3448 br     | 3281 br         | 1614 s      | 1465 s       |             | 1334           | 1246 s         |
| Sb2  | 3421 m      | 3300 br         | 1616 s      | 1465 s       |             | 1369w          | 1253 s         |
| Sb3  | 3448 w      | 3300 br         | 1612 s      | 1467 s       |             | 1363 m         | 1255 s         |
| Sb4  | 3375 s      | 3246-3306 s     | 1633 s      | 1467 s       |             | 1365m          | 1226 s         |
| Sb5  | 3448m       | 3340br          | 1678 s      | 1589 s       | 1465 s      | 1357s          | 1265 s         |

NMR Spectra

The ¹H NMR and ¹³C NMR spectral data of synthesized compounds have been listed in Table3. The ¹HNMR spectral data analysis were carried out for these compounds given results indicating the compatibility of the proposed structures. Some spectra of compounds showed in Figures 1, 2 and 3 respectively. The ¹HNMR spectra of the azo compound (A) shows a single signal at δ (10.091) ppm which attributed to the proton of azo-aldehyde group. All the compounds are characterized by showing singlet signal at δ (9.79-14.00) ppm and which can be assigned to phenolic group (OH) . Also multiplet
signals that appear at δ 6.94 to 8.03 can be attributed to aromatic rings of these compounds, while we can have observed in azo-azomethine compounds (Sb1-Sb5) disappearance of the signal of azo-aldehyde group and instead of it a new single appears at δ (8.89-9.01) ppm which attributed to the proton of azomethine group (HC=N).

Furthermore, all azo-azomethine compounds have singlet signal at δ (6.83-6.99) ppm that due to the presence of two protons of (NH₂) group of sulfanilamide which innervate the desired results.

In addition, the methoxy substituent of the o-vanillin was observed as a strong singlet at δ 3.81–3.92 ppm. [16]

Similarly, the ¹³C NMR spectra shows signal at the range δ 163.51–160.88 ppm is due to the imine functional group (C=N) which confirms the formation of the Schiff base from new azo compound and aniline derivatives .[17] Additionally, the signals of aromatic carbon of these synthesized compounds represented at δ 112–162 ppm, while the signal of the methoxy carbon observed at the range δ 56.2–56.3 ppm. The ¹³C NMR spectral data of the azo-azo Schiff bases are in accord with suggested structures.

Table3: ¹HNMR spectral data of compounds (A, Sb1-Sb5)

| Symbol of com. | Structure | ¹H NMR | ¹³C NMR |
|---------------|-----------|--------|---------|
| A             | ![Image](image1) | 10.09 (s, 1H, C=O)  
10.67 (m, 1H, OH)  
3.92 (m, 3H, OCH₃)  
6.96-7.9 (m, 10H, Ar-H)  
6.93 (m, 2H, NH₂) | 56.3 (Ph-OCH₃)  
116-155 (Aromatic carbon)  
191 (CHO) |
| Sb1           | ![Image](image2) | 9.01 (s, 1H, CH=N)  
13.27 (m, 1H, OH)  
3.83 (m, 3H, OCH₃)  
7.33-7.61 (m, 10H, Ar-H)  
6.92 (m, 2H, NH₂) | 56.1 (Ph-OCH₃)  
112–155.9 (Aromatic carbon)  
160 (C=N) |
| Sb2           | ![Image](image3) | 8.891 (m, 1H, CH=N)  
13.7 (m, 1H, OH)  
3.82 (m, 3H, OCH₃)  
6.99-7.37 (m, 9H, Ar-H)  
6.834 (m, 2H, NH₂)  
2.305 (s, 6H, 2CH₃) | 56.2 (Ph-OCH₃)  
115.9 - 151 (Aromatic carbon)  
162.6 (C=N) |
| Sb3           | ![Image](image4) | 8.94 (m, 1H, CH=N)  
13.3 (m, 1H, OH)  
3.82 (m, 3H, OCH₃)  
6.94-7.32 (m, 9H, Ar-H)  
6.816 (m, 2H, NH₂)  
2.33 (s, 3H, CH₃) | 56.3 (Ph-OCH₃)  
115.9 – 151(Aromatic carbon)  
163 (C=N) |
8.96 (s, 1H, CH=N)  
14.00 (m, 1H, OHa) 
9.79 (m, 1H, OHb) 
3.81 (m, 3H, OCH₃) 
7.09-7.37 (m, 10H, Ar-H) 
6.995 (m, 2H, NH₂) 

56.1 (Ph-OCH₃),  
115 - 155 (Aromatic carbon),  
160 (C=N)

6.930 (m, 2H, NH₂) 
2.59 (s, 3H, CH₃)

Sb4

9.00 (m, 1H, CH=N)  
12.76 (m, 1H, OH) 
3.83 (m, 3H, OCH₃) 
7.15-8.03 (m, 10H, Ar-H) 
6.930 (m, 2H, NH₂) 
2.59 (s, 3H, CH₃)

56.1 (Ph-OCH₃),  
112 - 155 (Aromatic carbon),  
160 (C=N)

Sb5

Fig -1 - ¹HNMR spectrum of Sb2 compound
Fig. 2 - $^{13}$CNMR spectrum of Sb3 compound

Fig. 3 - $^{13}$CNMR NMR spectrum of Sb2 compound
Antioxidant Activity

Damage to cells caused by free radicals is believed to play a central role in the aging process and in disease progression. Antioxidants are our first line of defense against free radical damage, and are critical for maintaining optimum health and wellbeing. [18] The phenolic compounds, act as reducing agents by trapping free radicals, by acting as chelators, by donating hydrogen, and by quenching singlet oxygen. These highly reactive species are present in biological systems and may oxidize lipids, proteins, nucleic acids, which may initiate degenerative heart disease.[19]

The antioxidant activity of our synthesized compounds could be attributed to demesne these compounds hydroxyl group which have ability of scavenging free radical. It was estimated the activity of compounds (A ,Sb1- Sb5) as antioxidant compounds depending upon the relationship between absorbance and time as showing in Table4 and Figures( 4 , 5) and comparable those with BHT, and with application of previous mathematical ,the highest activity compound is Sb4 possess high activity comparable to the standard BHT and that because the existence of another phenolic hydroxyl group which can enhances the antioxidant properties , also the compounds A ,Sb1, Sb3 and Sb5 possess high activities to the standard BHT. while the compound Sb2 there was not any activity detected. Finally, based on antioxidant activity study, the actively order of these compounds take this way; Sb4 = Sb3 = Sb1 A Sb5 Sb2.

Table 4: Effectiveness results of prepared compounds as antioxidants compared with BHT (Solvent used DMSO)

| sample | Ai | At | Ai | At | AA% |
|--------|----|----|----|----|-----|
| BHT    | 2.436 | 2.364 | 2.078 | 1.813 | 73 |
| A      | 2.108 | 2.014 | 2.078 | 1.813 | 65 |
| Sb1    | 2.127 | 2.039 | 2.078 | 1.813 | 67 |
| Sb2    | 2.153 | 1.897 | 2.078 | 1.813 | 4 |
| Sb3    | 2.149 | 2.060 | 2.078 | 1.813 | 67 |
| Sb4    | 2.115 | 2.042 | 2.078 | 1.813 | 72 |
| Sb5    | 2.228 | 2.115 | 2.078 | 1.813 | 58 |

Fig. 4 - antioxidant activity of A Sb1  Sb2
In vitro studies of the antifungal and antibacterial activities of the investigated compounds against bacteria (S. aureus, E. coli and K. pneumonia) and fungi (C. glabrata, C. albicane and A. niger) were carried out using the modified Kirby–Bauer disc diffusion method. Almost all the compounds exhibited antibacterial activity against the studied microbes at concentration 30 mg/ml.

In comparison of the three kinds of studies strain we can observed that the prepared compounds have high to moderate antibacterial activity.

It is found that compound (A, Sb4, Sb5) are more effective against Eschericahia coli and, Staphylococcus aureus than Sb1, Sb2, Sb3 which have moderate activity. The compound Sb5 have the higher activity against K. pneumonia than other compound which show moderate activity.

The maximum antifungal activity was shown by Sb5 followed by Sb4 and A. Compound Sb2 showed moderate antifungal activity whereas compound Sb3 showed no activity against C. albicane and Aspergillus niger and moderate activity against C. glabrata.

The mechanism of action of sulfonamide is competitively inhibit the action of dihydropteroate synthase and blocking the net biosynthesis of folate coenzymes, therefore its bacteriostatic.

As we know the compounds containing Schiff base tend to powerfully acts as potent bactericidal agents to kill microorganisms Also, another reason for this increasing activity can be also accrediting to immerse solubility of these compounds which breakthrough the layers of lipid velum of the microorganism. Additionally, the o-vanillin-based compounds exhibited higher activity against organisms. This could be due to the presence of the methoxy group at the ortho position of the aldehyde moiety of the compound. [16,17].
Table -5- in vitro antimicrobial activity result of the synthesized compounds

|          | K. pneumonia | E- coli | S. aureas | C. glabrata | C. albicane | Aspergillus niger |
|----------|--------------|---------|-----------|-------------|-------------|------------------|
| A        | 20           | 45      | 49        | 22          | 40          | 40               |
| Sb1      | 20           | 21      | 20        | 20          | 20          | 0                |
| Sb2      | 19           | 18      | 18        | 21          | 20          | 40               |
| Sb3      | 21           | 20      | 17        | 25          | 0           | 0                |
| Sb4      | 20           | 49      | 45        | 40          | 40          | 40               |
| Sb5      | 25           | 32      | 35        | 40          | 50          | 40               |
| Amoxicillin | 40    | 38      | 40        |             |             |                  |
| sulfadiazine   | 0      |         |           |             |             |                  |
| Nystatin  |              |         |           |             | 20          | 40               | 0                |

*Staphylococcus aureus, Escherichia coli and Klepsilia pneumonia*

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