Synthesis, Antimicrobial, Antioxidant and Structural Studies of Some New Sulfua Drug Containing an Azo-azomethine Group

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Abstract

A new series of azo-azomethine compounds derived from sulfadiazine were prepared. The compounds were prepared, starting from the coupling of the sulfadiazine diazonium salt with 2-hydroxy-3-methoxy benzaldehyde, followed by condensation with 4-bromoaniline, 2-chloroaniline, 2-methyl-3-chloroaniline, sulfadiazine, 2,4-dimethylaniline, and 2-hydroxyaniline, respectively. All compounds were characterized by CHN analyses, FT-IR, and NMR spectroscopic data. The antimicrobial activity of all synthesized compounds was studied against gram-positive and gram-negative bacteria and fungi. They were screened for their antibacterial activities towards the gram-positive Staphylococcus aureus and the gram-negative Escherichia coli, as well as their antifungal activities against Aspergillus niger, Candida albicans, and Candida glabrata, to evaluate their antimicrobial potential. Furthermore, their antioxidant activities were investigated by using the β-Carotene bleaching method. QSAR Properties and Molecular properties of all compounds were obtained by using Hyperchem software.

Keywords: Antimicrobial activity; Azo-azomethine; QSAR properties; Sulfadiazine.

1. Introduction

It is well known that azo compounds with one or more azo group (–N=N–) group, separated by two phenyl rings were used in printing systems, biological staining, textile industry, and various photochemical productions [1,2,3,4,5]. Azo compounds were also used in foodstuffs [6] because of their low toxicity, less allergic reactions, and have no hyperactivity effect. In addition, the azo-Schiff bases were reported to display various antimicrobial, anticancer, and antioxidant activities, and several other pharmacological properties [7,8,9,10]. On the other hand, sulfadiazine is an antibacterial prescription medicine for the prevention and treatment of certain types of bacterial infections, including the treatment of canccroids, Toxoplasma gondii encephalitis, urinary tract infections, and other infections [11,12].

It is worth noting that the pyrimidine moiety is one of the most widespread heterocycles in biologically occurring compounds, such as uracil, thymine cytosine, and vitamin B1[13]. Pyrimidine derivatives have a wide spectrum of therapeutic actions such as antimicrobial, anti-HIV, anticancer and other biological activity [14]. Due to the prebiotic nature of pyrimidine to living cells in biodiversity, it is a highly featured idea for the development of molecules of biological and pharmaceutical interest. Several synthetic methods for the pyrimidine heterocyclic synthesis were developed in literature [14-18].

Quantitative structure-activity relationship (QSAR) analysis is an efficient method for building mathematical models, which attempts to find a statistically significant correlation between the chemical structure and the physiochemical properties of drugs, such as toxicity, metabolism, drug and drug interactions, and carcinogenicity [19].

Thus, the present work describes the synthesis of a new series of azo-azomethine compounds based on sulfadiazine to develop new antimicrobial agents and their biological activities will be evaluated. Moreover, the antioxidant activities will be also investigated and the QSAR analysis will be discussed.
2. Experimental

Materials and Reagents

2-chloroaniline, 4-bromoaniline, 2-methyl-3-chloroaniline, 2-hydroxyaniline and 2,4-dimethoxyaniline were purchased from Fluka and used without further purification. Sodium carbonate, conc. Hydrochloric acid, sodium nitrite, 2-hydroxy-3-methoxy benzaldehyde (ortho- Vanillin), butylated hydroxyl toluene (BHT), and sulfadiazine were obtained from Sigma-Aldrich. All solvents were supplied from the Fluka company.

Physical measurements

The FT-IR spectra as KBr discs were recorded in the range 400–4000 cm⁻¹ using Shimadzu FT-IR model 8400 instrument. The spectra of ¹H NMR was measured on a Bruker at 400 MHz, with TMS as an internal reference and using DMSO-d₆ as a solvent. Carbon, hydrogen, and nitrogen elemental analysis data are obtained by the Euro vector EA 3000A Elemental analyzer. Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected.

Synthesis

4-[(3-Formyl-4-hydroxy-5-methoxyphenyl)diazenyl]-N-(pyrimidin-2-yl)benzenesulfonamide (1)

Compound 1 was prepared according to a literature method [12] by the following method:

A solution of sulfadiazine (1.25g; 0.5 mmol) in 10mL of 2N of hydrochloric acid was added at 0°C to 5°C. Then, 30 mL of a solution of NaNO₂ (0.55 g, 7.5 mmol) was added dropwise to the above solution. The reaction mixture was stirred for 2 h at -5°C. The solid product was washed several times with cold water and crystallized from absolute ethanol. The purity of each compound was checked by TLC (EtOH / CHCl₃; 1:9). Yield: 53%, m.p. 109-110 °C. IR (KBr) cm⁻¹: 3446(OH), 3380(NH), 1570(C=N sulfa), 1570(C=N), 1366(SO₂), 1257(C-O), 963(S-N). \( \delta / ppm: 12.89 (s, 1H, OH) ; 8.95 (s, 1H, CH=N); 11.01(s, 1H, NH) ; 3.82 (s, 3H, OCH₃) ; 6.93(t, 1H, Ar-H) ; 7.05(dd, 2H, Ar-H) ; 7.27(s, 1H, Ar-H), 7.77(dd, 2H, Ar-H), 8.01(dd, 2H, Ar-H), 8.43(dd, 2H, Ar-H), 8.72(s, 1H, Ar-H), 8.48(dd, 2H, Ar-H). \)

Calcd. for C₂₃H₁₉BrN₂O₅S: C, 50.60; H, 3.72; N, 14.59.

4-[(3-(2-Chlorophenyl)iminomethyl)-4-hydroxy-5-methoxyphenyl)diazenyl]-N-(pyrimidin-2-yl)benzenesulfonamide (3)

Yellow solid, Rf:0.9, yield: 57%, m.p. 115-117 °C. IR (KBr) cm⁻¹: 3450(OH), 3440(N-H), 3070(CH-arom), 2962(CH-ariph), 1616(CH=N), 1570(C=N sulfa), 1465(N=O), 1366(SO₂), 1203(SO₂), 1253(C-O), 912(S-N). \( \delta / ppm: 12.87 (s, 1H, OH) ; 8.92 (s, 1H, CH=N), 11.20 (s, 1H, NH); 3.80 (s, 3H, OCH₃) ; 6.92(t, 1H, Ar-H), 7.25(t, 1H, Ar-H), 7.27(s, 1H, Ar-H), 7.32(t, 1H, Ar-H), 7.57(d, 1H, Ar-H), 8.03(dd, 2H, Ar-H), 8.47(dd, 2H, Ar-H), 8.51(d, 2H, Ar-H), 8.56(s, 1H, Ar-H). \)


4-[(3-(3-Chloro-2-methylphenyl)iminomethyl)-4-hydroxy-5-methoxyphenyl)diazenyl]-N-(pyrimidin-2-yl)benzenesulfonamide (4)

Orange crystals, Rf:0.8, yield: 67%, m.p. 110-112 °C. IR (KBr) cm⁻¹: 3448(OH), 3441(N-H), 3063(CH-arom), 2966(CH-ariph), 1612(CH=N), 1562(C=N).
SYNTHESIS, ANTIMICROBIAL, ANTIOXIDANT AND STRUCTURAL STUDIES

sulfadiazine, 1,465 (N=S), 1365 (SO₂), 1180 (SO₂), 1263 (C=O), 968 (S-N).

1H NMR (400 MHz, DMSO-d₆): δ / ppm: 12.87 (s, 1H, OH); 8.83 (s, 1H, CH=N); 11.19 (s, 1H, NH); 3.81 (s, 3H, OCH₃); 2.36 (s, 3H, CH₃); 6.93 (d, 1H, Ar-H); 7.02 (t, 1H, Ar-H); 7.27 (s, 1H, Ar-H); 7.39 (t, 1H, Ar-H); 7.55 (d, 1H, Ar-H); 8.03 (dd, 2H, Ar-H); 8.46 (dd, 2H, Ar-H); 8.53 (d, 2H, Ar-H); 8.56 (s, 1H, Ar-H).

Anal. Calculated for C₁₅₀₅(N=Nsulfa), 1454 (N=N), 1365 (SO₂ arom), 2938 (CH₃ aliph) cm⁻¹: C, 52.09; H, 4.00; N, 19.42.

Antimicrobial activity

The antimicrobial activity of all synthesized compounds (i.e., compounds 1 - 7) was tested against Gram-positive Staphylococcus aureus (ATCC 25923) and Gram-negative Escherichia coli (ATTC 25922). The antifungal activity for all compounds was tested against candida albicans, Aspergillus niger, and candida glabrata. by the shake flask method.

Antioxidant Activity

The β-carotene bleaching method is based on the loss of the yellow color of β-carotene because of its reaction with radicals formed by linoleic acid oxidation in an emulsion and according to previous methods[21-23]. β-Carotene (0.2 mL/mg) was dissolved in chloroform and linoleic acid (0.02 mL) and Tween 20 (0.2 mL) were added to 1 mL of this solution. Chloroform was evaporated under vacuum at 50 °C and distilled water was added; then the emulsion was vigorously shaken for two minutes. The emulsion (3.8 mL) was added to a tube containing 0.2 mL of solutions of 0.1 mg/mL of reference compound butylated hydroxytoluene (BHT), and a synthesized compound. The reaction was followed by spectrophotometry at λ = 470 nm and the test emulsion was incubated in a water bath at 45 °C for 2 h when the absorbance was measured again. The absorbance was measured at 15 min. interval to observe the rate of bleaching of β-Carotene [24]. The results obtained were compared with the reference antioxidant BHT and expressed as a percentage of antioxidant activity (%A) using the following formula:

%A = 1 - [(Ao - A₁) / (*Ao - *A₀)] x 100

where A₀ is the initial absorbance and A₁ is the final absorbance measured for the test sample and *A₀ is the initial absorbance and *A₁ is the final absorbance measured for the control.

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Results and discussion

4-((3-Formyl-4-hydroxy-5-methoxyphenyl)diazenyl)-N-(pyrimidin-2-yl)benzenesulfonamide (1) was prepared from sulfadiazine, which was diazotized with NaNO₂ and HCl to form the corresponding diazonium salt, and then treated with 2-hydroxy-3-methoxy benzaldehyde at 0°C, Scheme 1. Compound 1 was reacted with 4-bromoaniline, 2-chloroaniline, 2-methyl-3-chloroaniline, sulfadiazine, 4-dimethylamline, and 2-hydroxyanline, to afford compounds 2, 3, 4, 5, 6, and 7, respectively in good yield, Scheme 1. All compounds (1-7) gave satisfactory elemental analyses (see, Experimental Section). They are stable solids and non-hygroscopic. All compounds were confirmed by FT-IR spectroscopy. The infrared spectra of studied compounds displayed strong bands between 3435 – 3479 cm⁻¹, which can be attributed to the stretching vibration of OH. Furthermore, the IR spectra of all compounds showed absorption bands due to the stretching vibrations of C-O between 1257 - 1226 cm⁻¹, and C=N sulfia between 1581-1500 cm⁻¹, SO₂ at the range 1368 – 1327 cm⁻¹ and 1203 – 1153 cm⁻¹ for asymmetric and symmetric vibration. The formation of azo-azomethine compounds was confirmed by the presence of two characteristic bands namely N=N together with C=N groups at the range 1430 - 1470 cm⁻¹ and 1612-1651 cm⁻¹ respectively [25].

Antioxidant Activity

The antioxidant can be defined as a substance that reduces damage due to free radicals. Phenol compounds which owned OH act as reducing agent by donating a hydrogen atom to free radical, so that it leads to the elimination of oxygen species (ROS) which create during biochemical operation in the body system [26,27]. The antioxidant activity of compounds 1–7 have been compared with BHT. These compounds (i.e. 1-7) containing hydroxyl groups that are very useful for scavenging free radicals and prevent damaging cells [28]. The ability of the compounds to inhibit β-carotene was evaluated using BHT as the reference, as seen in Figures 1 and 2. As shown in Table 1, the
significant antioxidant properties were observed for
the synthetic compounds 3 and 2, while compounds
such as 5 and 6 possess less effectiveness. The
antioxidant activity of these compounds changed with
the change of substructures [22]. Therefore, the
antioxidant activity of these new compounds is as
follows:

\[3 > 2 > 1 > 7 = 4 > 6 > 5\]

As it is well known that highly antioxidant materials
should be associated with high antibacterial activities.
No clear relations between the antioxidant and
antibacterial behaviours of these investigated
compounds were observed, as illustrated, for example,
compound 1 showed the highest antibacterial activity,
but moderate activity as an antioxidant, Tables 1 and
2.

**Figure 1.** Antioxidant activity of compounds 1, 2, and 5 (c, control)

**Figure 2.** Antioxidant activity of compounds 4, 3, and 7 (c, control)

**Antimicrobial activity**

Compounds 1–7 were tested for their antimicrobial
activity in vitro against human pathogens. These tests were carried against
are gram-positive *Staphylococcus aureus* (*S. aureus*)
and gram-negative *Escherichia coli* (*E. coli*). Furthermore, compounds 1–7 were screened for
antifungal activity against *Candida albicans*,
*Candida glabrata*, and *Aspergillus niger* by agar
well diffusion method. The results of the antimicrobial
testing data are presented in Table 2 and Figures 3
and 4. The investigation of antimicrobial testing data
shown that the studied compounds displayed a good to
moderate activity against the selected microorganism
at a concentration of 30 mg/mL. Thus, in comparison
with amoxicillin, compound 1 showed a good activity,
while compounds 2, 3, 4, 5, 6 and 7 show moderate
activity against *E. Coli* and *S. aureus*, Table 2.

In comparison with standard amoxicillin, compounds
(1, 3, 4, and 5) were showed moderate activity against
*S. aureus* while the same compounds showed low
effectiveness towards *E. Coli* expect compound 1
which showed a moderate activity toward *E. Coli*.
Compound 1–7 display an excellent to moderate
antifungal activity against *C. albicans* and *Aspergillus
niger* except compound 4 which showed a moderate
activity towards them. On the other hand, all the
synthesized compounds were displayed moderate
activity toward *C. glabrata* except compound 2,
which shows no effectiveness, Table 1. The inhibition zone
of all compounds against bacteria and fungi at a
concentration of 30 mg/mL is represented in Figs. 3
and 4.

One of the main components of our synthesized
compounds was sulfadiazine, it is an antibiotic that is
a member of sulfa drugs. Many types of research have
given clear, detailed information on the biological
activity of Schiff bases which have proven efficacy
and effectiveness as strong bactericidal factors [29].

**Figure 3.** Inhibition Zone of all compounds against
bacteria at a concentration of 30 mg/ml.

**Figure 4.** Inhibition Zone of all compounds against
fungi at a concentration of 30 mg/mL.

**Computational study**

Scheme 2 shows a graphical representation of the
Sulfadiazine Azo-azomethine compounds
described in this work the optimized geometrical
structures of the systems calculated performing
semi-empirical methods. Semi-empirical self-consistent
field molecular orbital (SCF-MO) the method at the AM1 level of theory.
These optimized structures are essential to study the
geometrical parameters of compounds and give
information about their point groups.

The optimization of structures is an essential step to proceed with other meaningful calculations.

Table 3 shows some of the selected QSAR properties of the investigated compounds. The trends of surface area and volume values are quite clear since it is obvious that the compounds which have bulky chemical group have larger values of surface and volume. In the present calculations, the total volume of the studied series varied from 1087.56 to 1607.46 Å³. The calculated log p values of the studied compounds vary from 3.89 to 6.97, this indicates that the studied molecules are lipophilic.

The calculated refractivity and polarizability of the studied compounds have indicated different values for each one of them. This means that the structural change of molecules has an observable effect on the theoretical refractivity and polarizability values for the two series, (Table 3).

**Scheme 2.** The optimized structures of Sulfadiazine Azo-azomethine compounds

### 4. Conclusion

A new series of azo-azomethine compounds derived from sulfadiazine was prepared. Antimicrobial and antioxidant activity was evaluated for these compounds. The compounds show moderate antimicrobials activities against *Staphylocuses aureas* and Escherichia coli, and good to moderate activities against three types of pathogenic fungi. The most elegant result as antimicrobial activity was obtained for compounds 3, 5, and 7 while the synthesized compounds 2, 3 showed high activities as an antioxidant agent.

**Conflict of interest**
The author declares that he has no conflict of interest.

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**Table 1:** Effectiveness of synthesized compounds (azo – azomethine) as an antioxidant

<table>
<thead>
<tr>
<th>Comp. symbol</th>
<th>A₀</th>
<th>Aᵣ</th>
<th>A₀*</th>
<th>Aᵣ*</th>
<th>A %</th>
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<td>BHT</td>
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<td>2.364</td>
<td>2.057</td>
<td>1.803</td>
<td>73</td>
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<tr>
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<td>2.126</td>
<td>2.057</td>
<td>1.803</td>
<td>45</td>
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<tr>
<td>2</td>
<td>2.312</td>
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<td>2.057</td>
<td>1.803</td>
<td>50</td>
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<tr>
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<td>2.003</td>
<td>2.057</td>
<td>1.803</td>
<td>61</td>
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<td>4</td>
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<td>2.128</td>
<td>2.057</td>
<td>1.803</td>
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<tr>
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<td>2.133</td>
<td>2.057</td>
<td>1.803</td>
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<td>6</td>
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<tr>
<td>7</td>
<td>2.333</td>
<td>2.181</td>
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<td>1.803</td>
<td>40</td>
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</table>

**Table 2:** The inhibition zones of the synthesized compounds against selected microbes.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Bacteria</th>
<th>Fungal</th>
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<td>Escherichia coli</td>
<td>Staphylocuses aureas</td>
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<td>49</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>22</td>
</tr>
<tr>
<td>3</td>
<td>21</td>
<td>25</td>
</tr>
<tr>
<td>4</td>
<td>22</td>
<td>30</td>
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</table>
Table 3. Selected QSAR properties of compounds 1-7 for the AM1 optimized structures.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Surface area (approx) ( (\text{Å}^2) )</th>
<th>Surface area (grid) ( (\text{Å}^2) )</th>
<th>Volume ( (\text{Å}^3) )</th>
<th>Hydration energy (kcal/mol)</th>
<th>Log P</th>
<th>Refractivity ( (\text{Å}^3) )</th>
<th>Polarizability ( (\text{Å}^3) )</th>
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<tbody>
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References


