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Research Article

ANTIOXIDANT ACTIVITY OF SOME NEWLY PREPARED SYMMETRICALLY AZO DYES DERIVED FROM SULFA DRUGS

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ABSTRACT

Objective: Sulfa drugs (sulfonamides) were the first type of drugs largely medically used for preventive and chemotherapeutic agents against various types of diseases. To the date, much research has been directed toward the synthesis sulfa drug derivatives such as azo-sulfa drug compounds. The aim of the present study is to synthesize of azo-sulfa compounds as antioxidant agents.

Methods: First, three of sulfa drugs react with 4-methoxy-1,2-Naphthoquinone in aqueous medium by stirring at room temperature to give symmetrically azo-sulfa compounds. The colored compounds which formed were examined their structures by infrared and proton nuclear magnetic resonance spectral techniques.

Results: Three symmetrically azo-sulfa compounds were tested as antioxidant agents compared with ascorbic acid (AA) using 2,2-diphenyl-1-picrylhydrazyl method. The results indicated that these compounds had good activities (57.79–73.69%) at 30 µg/ml, which had less activity than AA (81.34%) at the same concentration. These results referred the IC_{50} which had values (15.23–21.35 µg/ml), whereas AA had 7.59 µg/ml.

Conclusion: Attachment of heterocyclic rings containing nitrogen and oxygen (isoxazole) on the azo-sulfa compounds can enhance the antioxidant activity as compared with the heterocyclic rings containing nitrogen only (pyrimidine) and without heterocyclic rings, which enhanced the lipophilicity which may increase the bioavailability and efficacy of the drug.

Keywords: Antioxidant, Sulfonamide, Azo compounds.

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INTRODUCTION

Sulfa compounds have been used widely for a long time in the treatment of diverse diseases [1]. In the medicinal uses, there are more than 30 drugs which are commercial used in markets, including antihypertensive, antibacterial, antiprotozoal, antifungal, and anti-inflammatory [2].

One of the important clinically used sulfa drugs was called prontosil which is azo-sulfa drug that gave protective action against streptococci in animals. Prontosil has two effective features, and it was active *in vivo*, while it is ineffective *in vitro*. These features led to the fact that this drug was not the active [3,4]. Azo dyes have wide applications, these compounds have been used as cosmetics because of more stable than natural colours and used as pharmaceutical products [5]. According to stability, azo-dyes are stable in different ranges of pH, they do not vanish when exposed to oxygen or light, and some of them are soluble in water. However, azo dyes have not the ability to dissolve in fat or oily phases [6].

Antioxidants are organic or inorganic compounds have ability to reduce or limit oxidative damage effects in the structures of biological tissues by passivizing free radicals. These classes of compounds may be added to the foods to increases their shelf life by process called lipid peroxidation retardation. Accordingly, these types of compounds have been used in wide area as food additives to avert the degradation of food, and they play a significant role to prevent many diseases and aging which lifestyle-related, being closely related to the reactive oxygen species (ROS) and lipid peroxidation formation in the body [7-9]. Antioxidant compounds are species widely utilized against the free radicals which reduce oxidative stress in the cell. Natural and chemical sources may be containing these antioxidant compounds [10].

2,2-diphenyl-1-picrylhydrazyl (DPPH) tests have been widely applied to antioxidant activity screening because this class of compounds can accommodate many tested samples in a short time and sensitive for detecting active ingredients at low concentrations [11]. When DPPH radicals face the materials with proton donating such as an antioxidant, it would be scavenged and the absorbance is reduced. Thus, the DPPH radicals were used by many researchers to assay the scavenging activity of some synthesized and natural compounds [12].

The present study includes the synthesis and characterization of three types of symmetrically azo-dyes which derived from sulfa drugs and tested them as synthesized antioxidant compounds using DPPH method. Moreover, the effect of different concentrations of the compounds on the antioxidant activity compared with standard antioxidant drug (ascorbic acid [AA]).

MATERIALS AND METHODS

Materials

1. The chemicals used were procured from the following companies, sulfa drugs and 2,2'-diphenylpicryl-1-hydrazyl (DPPH) from Sigma-Aldrich Chemical Co. AA from Merck Ltd. Ethanol was analytical grade from SD fine chemicals Ltd., Mumbai, India.
2. Melting points were determined on the uncorrected open capillary tube on Campbell Melting Point Apparatus.
3. Infrared (IR) spectra were taken using Fourier-transform IR Shimadzu model 8400 spectrophotometer and KBr disk at Pharmaceutical Chemistry Department, College of Pharmacy, Basrah University. Only principal peaks are reported and expressed in cm^{-1}.
4. Proton nuclear magnetic resonance spectra (^{1}H-NMR) were recorded on Bruker model ultra-shield 400 MHz

spectrophotometers (Switzerland) at Tehran University, Iran. Chemical shifts are expressed as δ value (ppm), and dimethyl sulfoxide (DMSO)-d6 was used as a solvent and tetramethylsilane as an internal standard.

5. While citing ^{1}H-NMR data, the following abbreviations have been used S (singlet), d (doublet), t (triplet), and m (multiplet).
6. The CHNS analysis measurements for the synthesized compounds were performed at the Analytical Laboratory of Tehran University, Iran, using EuroVector model EA3000A (Italy).
7. Spectral and absorbance measurements were made with a Jena Model 1100, UV-Visible spectrophotometer (Germany) in Pharmaceutical Chemistry Department, College of Pharmacy, Basrah University. Wavelengths are expressed as nm.

Synthesis of compounds

An aqueous solution of 4-methoxy-1,2-naphthoquinone (0.001 mole) in small amount of water was stirred on magnetic stirrer achieved to complete dissolved solution. Sulfa drugs (sulfisoxazole, sulfamerazine, or sulfaguanidine) (0.002 mole) was added gradually by small amounts for a period of 15 min to the above aqueous solution at room temperature with vigorously stirring. After complete addition, the mixture was stirred for further time (about 15–30 min) at room temperature and the resulting product was formed. The bright colored solid precipitate of desired product was filtered off and washed with cooled mixture of water and methanol. The product was recrystallized from H_2O: DMF (2:8) and dried under vacuum [13]. The physical properties are listed in Table 1.

Antioxidant Activity

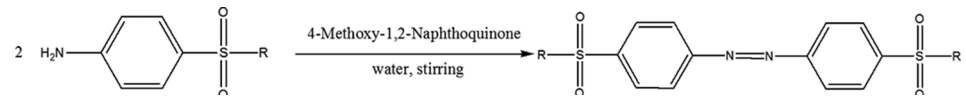
Briefly, solution of DPPH with concentration 0.1 mM was prepared using ethanol as solvent. From the solution above, 0.5 ml was withdrawn and added to 1.5 ml of azo-sulfa compound solution in ethanol at different concentrations (5–30 g/ml). The resulting mixture was shaken vigorously by mechanical shaker and left to stand for 30 min incubation period at room temperature. The absorbance of the clear resulting solution was measured at 517 nm using ethanol as blank. The lowering in absorbance of the resulting mixture indicates a higher activity of DPPH free radical scavenging. Azo-sulfa compound concentration providing 50% inhibition (IC_{50}) was calculated using the graph by plotting inhibition percentage against azo-sulfa compound concentration. Ascorbic acid (AA) was used as positive controls, and all tests were carried on triplicates [14].

The capability to scavenge the DPPH radical was calculated using the following equation [8]:

$$\text{DPPH scavenging effect (\%)} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

Statistical analysis

Data are expressed as a mean ± SD. Total variations present in a set of data were estimated by one-way analysis of variance and comparisons were made between the treated groups. All data were analyzed using Duncan's multiple range test. $P < 0.05$ was considered as the level statistical significance.



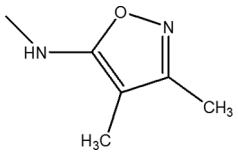
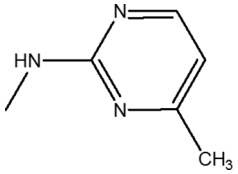
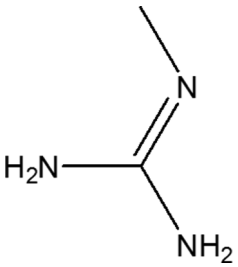
Compound	R	Name
SF		N-(3,4-dimethylisoxazol-5-yl)-4-((4-(N-(3,4-dimethylisoxazol-5-yl) sulfamoyl) phenyl) diazenyl) benzenesulfonamide
SM		4,4'-(diazene-1,2-diyl) bis (N-(4-methylpyrimidin-2-yl) benzenesulfonamide)
SQ		N-(diaminomethylene)-4-((4-(N-(diaminomethylene) sulfamoyl) phenyl) diazenyl) benzenesulfonamide

Table 1: The characterization of the prepared azo compounds

Compound	Molecular formula	Molecular weight (g/mol)	Crystal Color	Molecular pressure (°C)	Yield (%)	Elemental analysis found (Calcd.)			
						C%	H%	N%	S%
SF	$C_{22}H_{22}N_6O_6S_2$	530.58	Red	170	87	49.80 (48.25)	4.18 (4.02)	15.84 (16.24)	12.09 (12.21)
SM	$C_{22}H_{20}N_8O_4S_2$	524.58	Orange	230 dec.	82	50.37 (49.54)	3.84 (3.68)	21.36 (21.61)	12.23 (13.02)
SQ	$C_{14}H_{16}N_8O_4S_2$	424.46	Deep red	191	79	39.62 (40.56)	3.80 (3.74)	26.40 (25.88)	15.11 (15.43)

RESULTS AND DISCUSSION

^{1}H-NMR spectra

^{1}H-NMR spectra of the prepared azo dyes were performed in deuterated DMSO solutions with tetramethylsilane as an internal standard. All these spectra showed a peak at 2.5 ppm which was due to DMSO solvent, and some spectra showed a sharp peak at 3.33 ppm due to dissolved water in DMSO [15,16]. Figs. 1-3 represent the ^{P1P}H-NMR spectra of the azo compounds and Table 2 represents the data of Figs. 1-3.

Figs. 1-3 represent the ^{1}H-NMR spectra of the three azo compounds. Two of these compounds exhibited characteristic aliphatic systems as follows; compound SF gave two singlet signals at 2.268 and 2.284 ppm which related to twelve protons of four methyl groups in isoxazole rings. The second compound SM gave two singlet signals at 2.293 and 2.328 ppm related to six protons of the -CH_3 groups in pyrimidine rings. The compound SQ is lacked to these types of aliphatic protons which not give signals in the high field.

For the compounds SF and SM, there are signals at low field which were represented by singlet signals due to proton of –NH- group of sulfa fragment at 10.945 and 11.372 ppm, respectively. The third compound SQ showed broad signal at 5.717 ppm related to protons of -NH_2 groups in the aliphatic moiety.

Multiplet signals are in the range 6.352–8.299 ppm which attributed to twelve protons of the aromatic systems of phenyl and heterocyclic rings for the compound SF, as shown in Table 2, whereas, for the compounds SM and SQ, there are multiplet signals in the range of 6.352–8.293 ppm and 6.233–8.219 ppm which attributed to the protons of phenyl rings for compounds SM and SQ, respectively, as shown in Table 2.

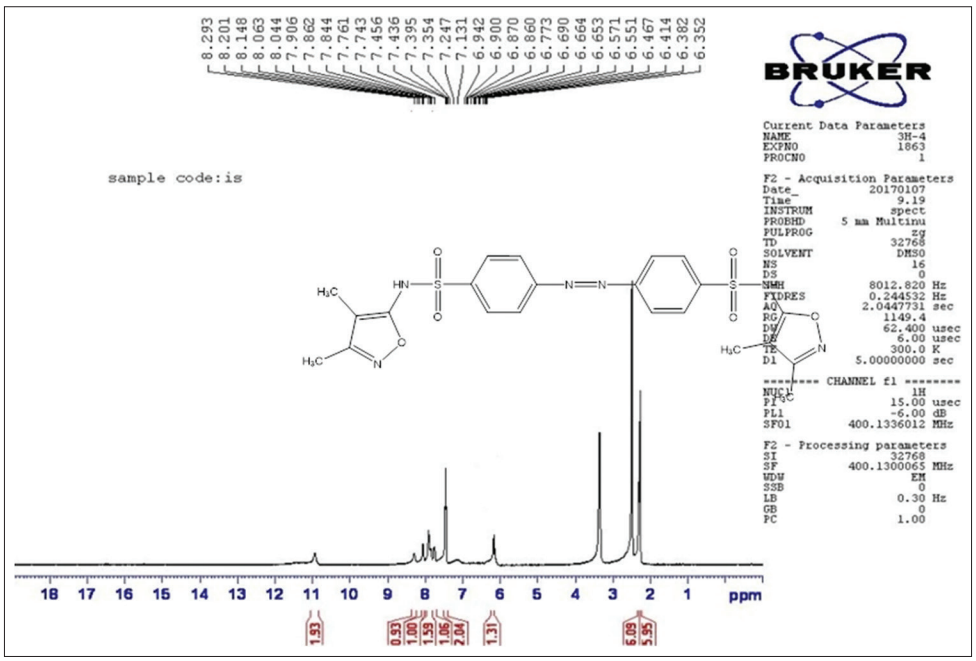


Fig. 1:^{1}H-nuclear magnetic resonance spectrum of compound SF

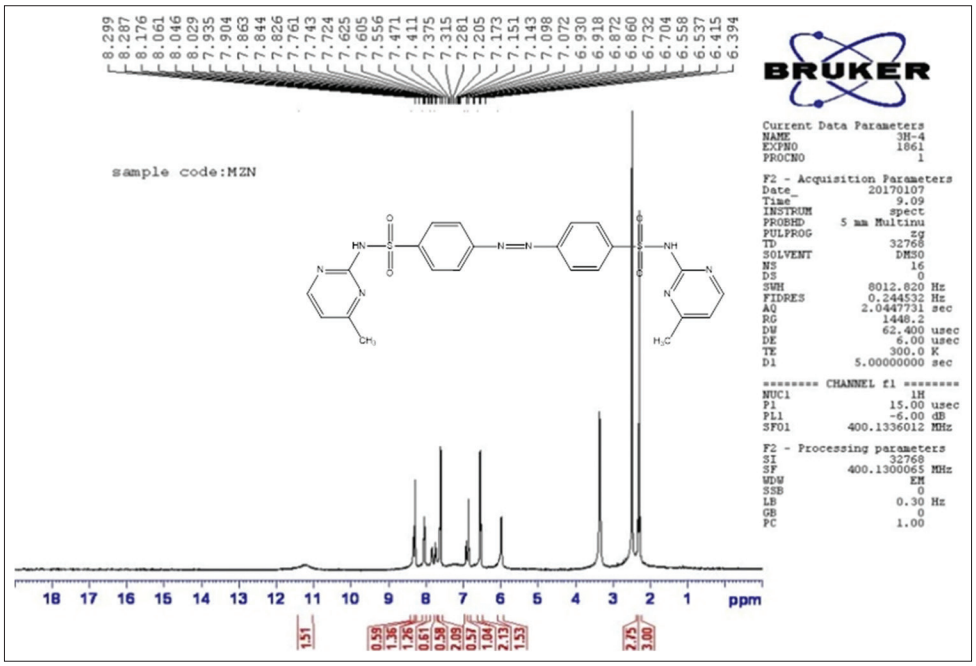


Fig. 2:^{1}H-nuclear magnetic resonance spectrum of compound SM

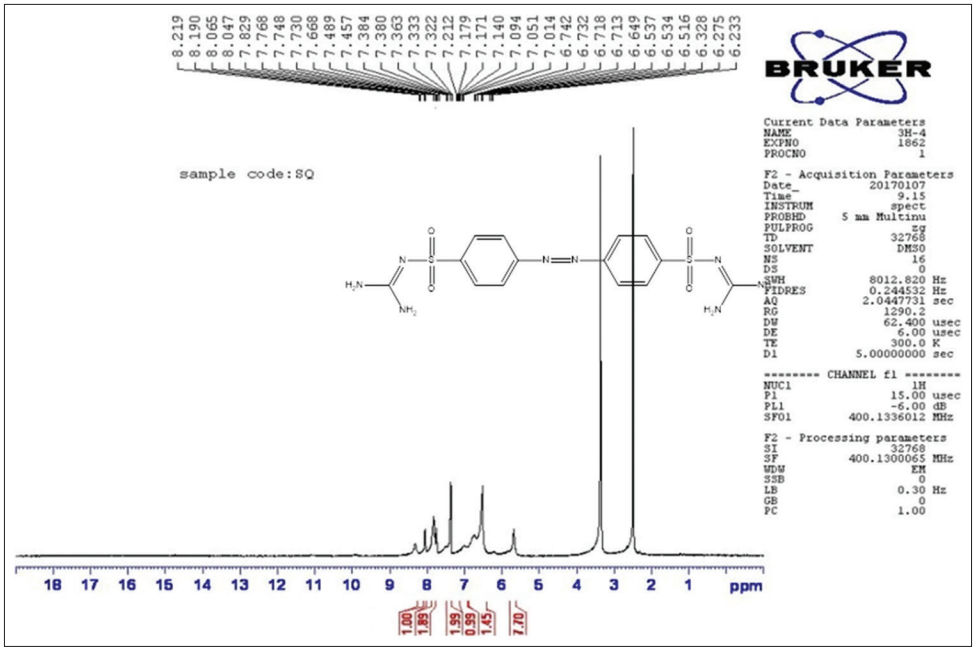


Fig. 3: ^{1}H-nuclear magnetic resonance spectrum of compound SQ

Table 2: ^{1}H-nuclear magnetic resonance spectrum data of synthesized compounds

Compound	d ppm			
	-CH_3	-NH_2	Aromatic system	-NH-
SF	2.268 (s) (6H) 2.284 (s) (6H)	-	6.352–8.293 (m) (8H)	10.945 (s) (2H)
SM	2.293 (s) (3H) 2.328 (s) (3H)	-	6.394–8.299 (m) (12H)	11.372 (s) (2H)
SQ	-	5.717 (b) (8H)	6.233–8.219 (m) (8H)	-

Table 3: FT-IR data of the synthesized compounds in cm^{-1}

Compound	n (N-H) str.	n (C-H) str. Arom.	n (C-H) str. Aliph.	n (C=N) str.	n (C=C) str.	n (N=N) str.	n (C-N) str.	n (C-H) bend. Arom.
SF	3370 m	3143 w	2912 w	1620 m	1597 m	1473 s	1303 s	829 s
SM	3356 m	3183 w	2954 m	1627 m	1693 s	1492 s	1330 m	891 s
SQ	3425 m 3332 m	3132 w	-	1616 s	1597 s	1496 s	1284 m	813 s

FT-IR spectra

FT-IR spectra of the synthesized compounds were carried out using KBr disc method. The characterized bands are given in Table 3.

The IR spectra of the azo-sulfa compounds were performed by the KBr disc method. Table 3 represents the data of the important bands of the IR spectra of SF, SM, and SQ compounds. The IR data of all compounds showed medium bands at 3425–3332 cm^{-1} which is characteristic of the N-H stretching [17] of these compounds. The IR spectra of the compounds showed strong-medium bands at 1627–1616 cm^{-1} and 1496–1473 cm^{-1} which are characteristic of the C = N and N = N stretching, respectively [17]. The compounds showed strong-medium bands at 1330–1284 cm^{-1} which referred to C-N stretching. Weak absorption bands at 3183–3132 cm^{-1} attributed to stretching vibration of C-H aromatic systems.

Antioxidant reactivity

The researchers used different methods for antioxidant activity assays in the literature references. Using DPPH radical scavenging activity is common method to investigate the antioxidant activities for different classes of natural and synthetic materials because it is quick and simple method.

As with the most antioxidant assays, the spectrophotometer instrument was used in the DPPH measurements at the wavelength of 517 nm. The results indicate that, by mixing the antioxidant samples with DPPH reagent solution, the color is clearly turned from purple to yellow by time. The changing in color is determined by measuring absorbance intensity with a spectrophotometer at 517 nm. Antioxidant results of the synthesized compounds (SF, SM, and SQ) with respect to AA are reported in Table 4.

In our study and according to chemical structural features, there were three different types of synthesized compounds. It is obvious that structural change gave clean refer vision about the bioactivity which trends to structural modification of molecules. When structure altered, the biological activity will change in a regular trend. Without exception, this has been reflected in the case of the sulfonamide group which maybe showed a tendency of antioxidant activity. We indicated that all three compounds had good antioxidant activity but less effect than AA. Table 5 refers the antioxidant activity % of the prepared compounds which gave the values between 57.79, 59.82, and 73.69 for SQ, SM, and SF, respectively, compared with 81.34 for AA, as shown in Fig. 4.

Furthermore, differences in the aromatic groups (pyrimidine, isoxazole, and primary amines) may be enhanced the antioxidant potency. We found that compound SF has higher IC_{50} (15.23 µg/ml) as compared with another two compounds SM and SQ (20.71 and 21.35 µg/ml), respectively, as shown in Table 5.

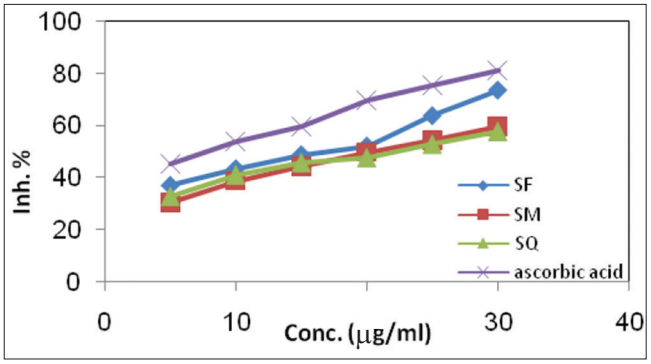


Fig. 4: Free radical scavenging effects % of the azo-sulfa compounds and ascorbic acid at different concentrations

Table 4: Percentage inhibition of different concentrations of azo-sulfa compounds and ascorbic acid

Concentration (µg/ml)	Compound			
	SF	SM	SQ	AA
5	37.11±0.02	30.47±0.11	33.08±0.05	45.54±0.24
10	43.49±0.08	38.89±0.35	41.29±0.14	54.09±0.09
15	48.87±0.12	44.39±0.57	45.91±0.22	59.82±0.47
20	52.17±0.14	49.88±0.43	47.79±0.09	69.70±0.37
25	63.89±0.11	54.60±1.24	52.93±0.41	75.65±0.62
30	73.69±1.01	59.82±0.51	57.79±0.58	81.34±0.27

Table 5: DPPH radical scavenging IC50 values of azo-sulfa compounds and ascorbic acid

Compound	IC50 value (µg/ml)
SF	15.23±0.11
SM	20.71±0.23
SQ	21.35±0.41
AA	7.59±0.08

The present investigation emphasized mainly on two important things. One of these is to the synthesis of molecules having N and O heterocyclic moieties is determine their antioxidant efficacy. Nitrogen and oxygen-containing heterocyclic organic compounds show interesting chemical property as well as biological activity [18]. Furthermore, each of the compounds contains a high percentage of nitrogen or oxygen gave enhancement to the reactivity. Therefore, it might have enhanced the power to absorb free radicals, especially ROS and reactive nitrogen species (RNS).

CONCLUSION

Three novel symmetrically azo-sulfonamides compounds such as SF, SM, and SQ were synthesized, characterized, and evaluated for their antioxidant test. Most of the synthesized compounds showed promising antioxidant activity compared with AA, suggesting a possible clinical significance of novel compounds. Compound SF showed remarkable results as compared with the standard compound AA.

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