# The Chemistry of Formazans and Tetrazolium Salts

## Formazanlar ve Tetrazolyum Tuzlarının Kimyası

Review Article

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**ABSTRACT**

In this rewiev, the structure of formazans and of tetrazolium salts has been described. Furthermore, importance and usage of formazan-tetrazolyum systems have been explained. Tautomerization properties and geometric isomers based on conjugate π-system of formazans have been examined. In addition, common synthesis of these compounds and UV-visible, IR, $^1$H NMR spectrums used in explanation of structures have been discussed.

**Key Words**
Formazan, Tetrazolium salts, Tautomerization, Chelate form

**ÖZET**

Bu çalışmada tetrazolyum tuzları ve formazanların yapıları tanımlanmıştır. Ayrıca formazan-tetrazolyum sistemlerinin önemi ve kullanım alanları açıklanmıştır. Formazanların sahip oldukları conjugate π-system kaynaklanan tautomerleşme özellikleri ve geometrik izomerleri incelenmiştir. Bu bileşiklerin en yaygın kullanılan sentezleri ve yapılarının açıklanmasında önemli olan UV-visible, IR ve $^1$H NMR spektrumları tartışılmıştır.

**Anahtar Kelimeler**
Formazan, tetrazolyum tuzları, tautomeri, şelat yapı

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## INTRODUCTION

This review aims at providing a general survey of the chemistry of formazans and of tetrazolium salts. Tetrazolium salts and formazans have been known in chemistry for a hundered years. In 1894, von Pechmann and Runge described the synthesis of certain tetrazolium salts using the oxidation of formazan compounds [1,2]. This oxidative process was important for its reversibility by a variety of reducing agents [3]. The tetrazolium –formazan couple is a special redox system acting as a proton acceptor or as an oxidant [4]. In 1941, Kuhn and Jerchel initiated their biological application [5,6], and Reid contributed a documented survey of their significance in chemistry and biology [7]. Formazan/tetrazolium system is described as the marker of vitality [8]. When given to a living organism, tetrazolium salts are reduced back to formazan depending upon the viability of the organism. These compounds are used in Brucella-ring test in milk [9]. Formazan/tetrazolium system is quite useful in the determination of the effect and the selection of anti-cancer drugs [10]. In 1976, Altman reviewed the literature on tetrazolium salts and their reduction properties [11]. The tetrazolium to formazan reactions are now widely exploited as indicators of reducing systems with applications in chemistry and biology [3]. Formazans are also well known to have antiviral, antiinflammatory-analgesic, antifertility, antitubercular activity, antimicrobial activities [12,13]. This feature is the cause of an increasing interest in the chemistry of formazans.

These enable the viability of the organism be tested by monitoring formazan formation with spectroscopy. That is why the spectroscopic investigation of formazans is of importance.

## STRUCTURE OF TETRAZOLIUM SALTS AND FORMAZANS

Tetrazolium salts (Figure 1) are organic compounds, which can be implied as quaternized tetrazoles. Almost all the known tetrazolium salts are derived from (2H) tetrazole, although the series derived from (1H) tetrazole is theoretically possible [14]. Their five membered unsaturated ring contains two double bonds, one carbon and four nitrogen atoms, one of which is quarternary positively charged [4]. As a result, the compounds have salt-like properties.

The double bonds in the tetrazolium ring are not strictly localized, they can be distributed in two different patterns, so as to form two isomers (Scheme 1).

Tetrazolium salts are colorless or faintly yellow compounds and they are reduced to deeply coloured compounds known as formazans. Tetrazolium salts in general are stable and crystalline and are formed with weak acids. Many of these salts, in particular the chlorides, are soluble in water and give solutions

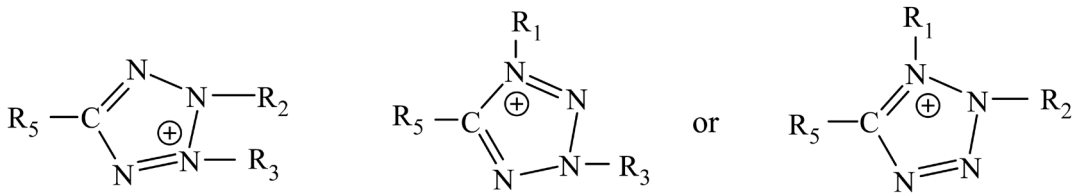
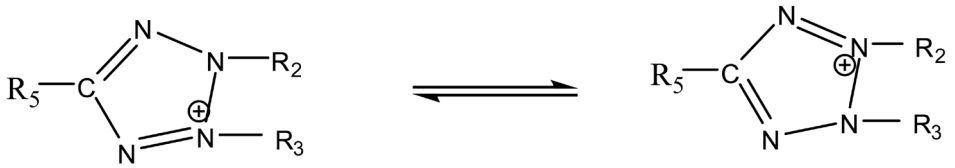



**Figure 1.** Tetrazolium salts.



**Scheme 1.** Two isomers of 2-H-tetrazolium salt.

of neutral pH. All these chlorides are readily soluble in methanol and ethanol. The bromides and iodides are progressively less soluble in these solvents [15].

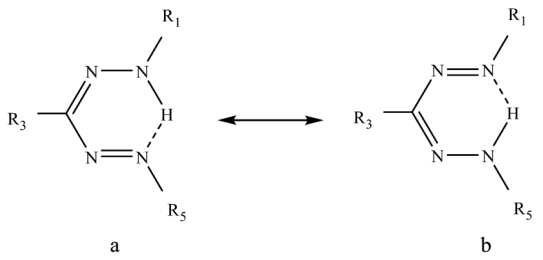
Formazans are characterized by intense colors, ranging from cherry red to a deep purplish black and contain the characteristic chain of atoms –N=N–C=N–NH– [16]. Formazans are generally solids of relatively low melting point in spite of large the size of the molecules. Triarylformazans are often particularly soluble in chloroform and acetone; in water the solubility appears to be negligible, the solvent being colored.

Their structure was first revealed by Bamberger and by von Pechmann [17] who agreed to call them formazyl compounds. In 1933, German usage is exemplified by Beilstein, in which the compound is termed formazan. The compound is substituted with three phenyl groups at R, R′, R″ which is called 1,3,5-triphenylformazan.

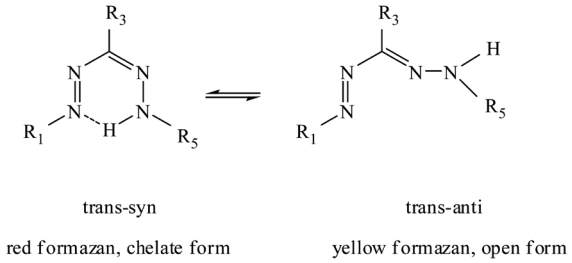
The structure of formazans was rather complex. The tautomerism of formazans, first described by Penchmann [15], but his results were inconclusive. In 1941 Hunter and Roberts [18] conclusively established for several pairs of formazans that the individuals in each pair were identical, although previously were described as tautomeric. They suggested that formazans were resonance hybrids with a chelated hydrogen-bridge structure. These workers therefore proposed an internally coordinated hydrogen-bond structure, which can exist in two mesomeric forms, a and b. The formazan molecule thus appears to be a resonance hybrid of these forms (Scheme 2).

Hausser et al [19], showed that some formazans could be changed from red to yellow forms upon exposure to visible light. A formazan molecule allows the existence of four possible structures due to geometrical isomerism about the two double bonds (C = N, syn – anti and N = N, cis – trans), the possibilities of tautomerism being ignored for the present. The stereoisomers can be indicated as shown in Figure 2.

As seen from Figure 3, cis-anti and trans-anti forms do not show chelate structure due to position of N-H, on the other hand cis-syn and trans-syn are observed to contain chelate structure involving hydrogen bonding. As the steric reason occures in cis-syn, it is hardly possible to show chelate structure. Trans-syn form is the most favorable for the chelate structure. Formazan molecules involving hydrogen bridge are red while those which do not show chelate form are yellow (Scheme 3).




**Scheme 2.** Mesomeric forms of formazans.




**Scheme 3.** Colors of formazans.

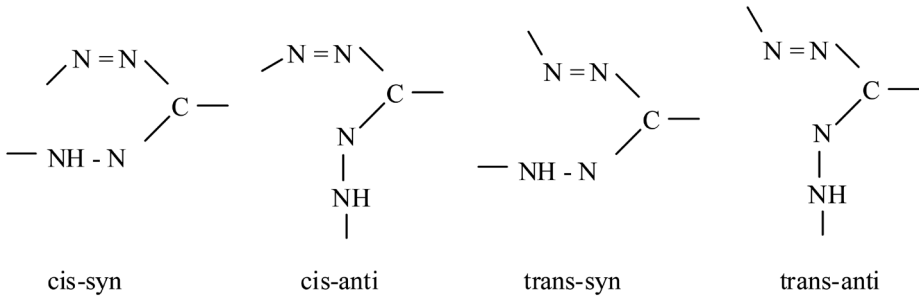
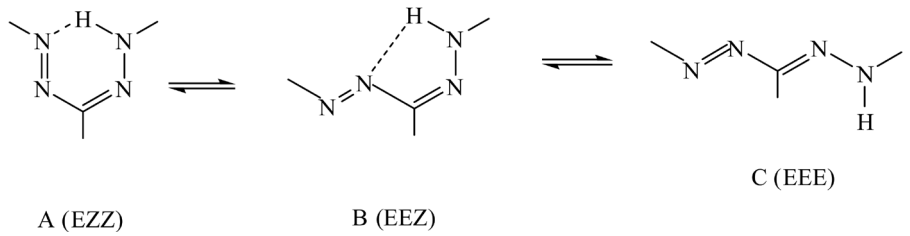
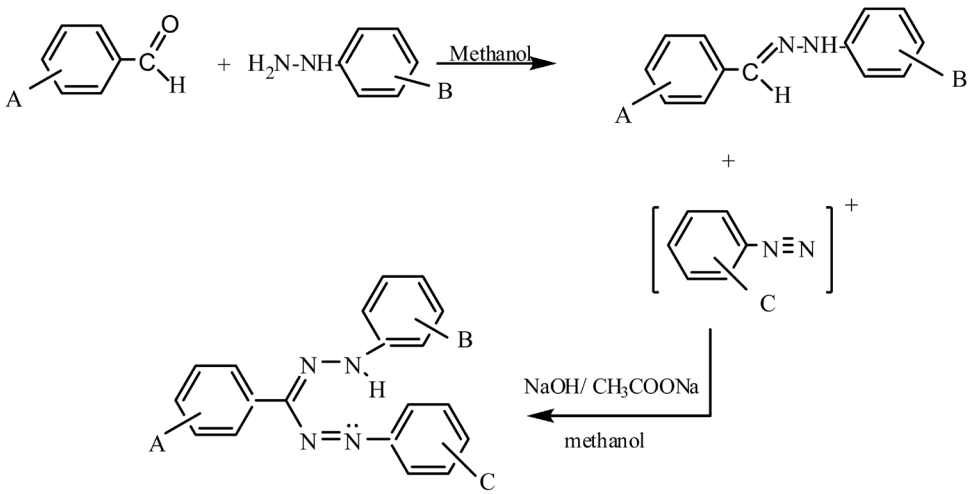



**Figure 2.** Stereoisomers of formazan.




**Scheme** 4. Three configurational combinations of formazans.




**Scheme 5.** Synthesis of formazans by the reaction of diazonium salts on arylhydrazones.

The presence of two multiple bonds in the formazan chain and the N5 atom with a lone pair electron brings about the formation of an overall conjugated system.

Recently, Lipunova has designated configuration of formazan structures in terms of E and Z instead of cis, trans, or syn, anti [20].

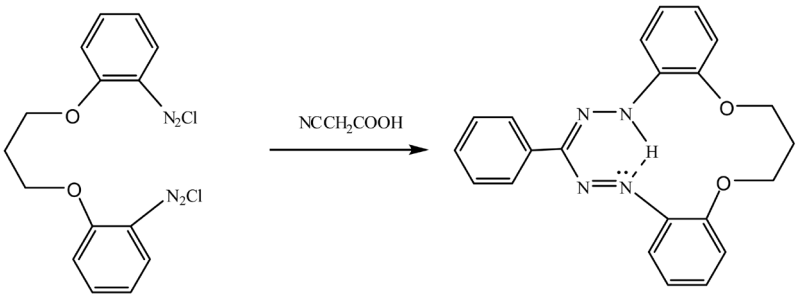
X-Ray diffraction studies of a large series of formazans revealed three configurational combinations in the azohydrazone chains of the crystals, EZZ, EEZ and EEE. The configurations refer to the bond sequences N1–N2, N2–C3 and C3–N4 (Scheme 4).

The configuration of formazans is largely determined by the steric effect of the substituent at the carbon atom. Thus EZZ isomers (structure A) are characteristic of formazans with a bulky substituent $R_3$ (Ph, But, $NO_2$, etc.). In addition EZZ configuration is stabilized by a bridging N-H···N intramolecular hydrogen bond in the six-membered chelate ring. The results of ab initio quantum-chemical calculations of all possible conformations of these compounds suggest that the EZZ configuration is the most stable [21]. The EEZ isomer is stabilized by the $N_2$-$HN_5$ bond in the five-membered ring (structure B), while the EEE form is stabilized by intermolecular H-bonds. In N-azaheterocyclic formazans, which crystallise in the imino tautomeric form, the intramolecular $N_4$–HN (heterocycle) H-bond provides additional stabilization.

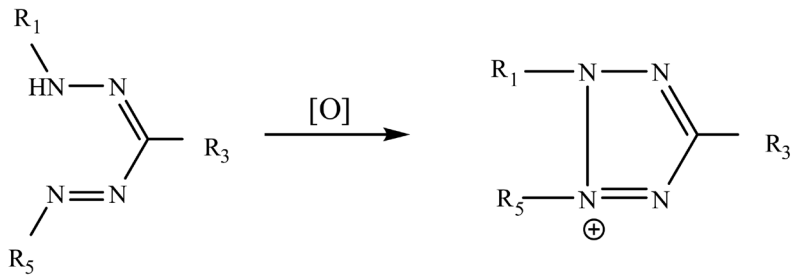
## SYNTHESIS OF FORMAZANS

### By the reaction of diazonium salts on arylhydrazones

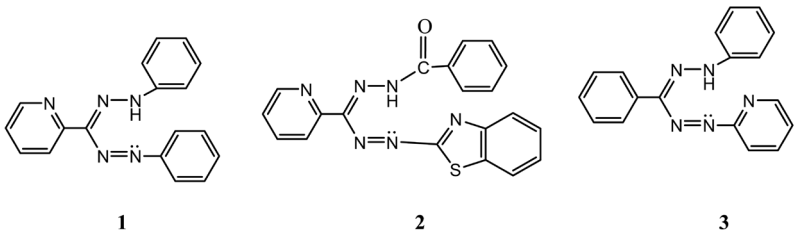
There are various methods proposed for the synthesis of formazans in the literature. The first one is the condensation of aromatic and aliphatic aldehydes with phenylhydrazine and the coupling reaction of the resulting hydrazones with diazonium salts (Scheme 5)[22]. The majority of known formazans are generated by this method, which is the standard one for the triaryl formazans.




**Scheme 6.** Synthesis of the macrocyclic crown-formazan.




**Scheme 7.** Synthesis of tetrazolium salts.



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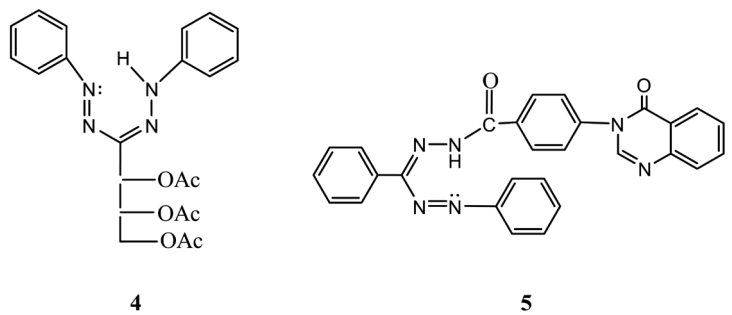


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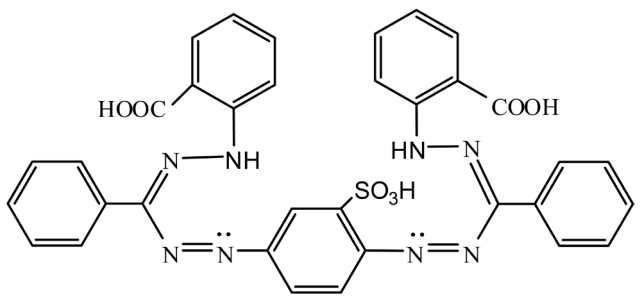


Many formazans were synthesized with different substituted aldehyde, hydrazine and amines, by coupling reaction method, Not only substituted starting material, but also heterocyclic derivatives of aldehyde, hydrazine and amines were used to synthesize formazans. For example, Frolova et al synthesized 1,5-Diphenyl-3-(pyridyl)formazans (1) with pyridinecarbaldehyde phenylhydrazone [23]. Desai generated heterocyclic formazans (2) with the diazonium salt of 2-amino-benzothiazole and thiophene-2-aldehyde benzoylhydrazone [24] Mariappan et al prepared formazans with diazonium salt of heteroarylamine (3) [25]. The monophenylhydrazones of aldehydo sugars couple normally with benzenediazonium chloride. In this way, the glucose, galactose, and mannose molecules have been linked to the formazan system. Mady et al synthesized pent-2-enose (4) formazans which were used with monosaccharides instead of aldehyde [26]. Babu and Nadendla used antranilic acid as a starting material and synthesized 3-(4-hydrazinobenzoyl)-2-methyl-3-quanizolin-4-one (5) [6].

When compound of two amino groups as phenylenediamine was used in formation reaction of formazans, bis-formazans were formed as a result of two-side diazotization. For example, 1,4-bis-[3,3′-phenyl-5,5′-(o-carboxyphenyl)-formaz-1-yl]-benzene-o-sulphonic acid was prepared by 2,5-diaminobenzene sulphonic acid (6) [27] .

### By the reaction of diazonium salts on compounds containing active methylene groups

Diazonium salts are coupled with a variety of compounds containing active methylene groups, such as aldehydes, ketones, nitroalkanes, malonic acid esters, cyanacetic acid ester, betadiketones, etc. The substitution of one diazonium ion in the methylene group is achieved to give the azo compound, which rearranges to a phenylhydrazone. When two molecules of diazonium salt are used, a second diazonium ion is coupled to the phenylhydrazone molecule previously formed [4,15].

The macrocyclic crown-formazans were prepared by this method with pyruvic acid and arylpyruvic acids in 1994 (Scheme 6)[28].

### By the reduction of tetrazolium salts

Formazans can be prepared by the reduction of tetrazolium salts. Since tetrazolium salts are prepared only by the oxidation of formazan, this method has no synthetic importance.

### By the modifications of substituents present in formazans

Formazans may be converted to other formazans by effecting changes in functional groups substituted in the molecule. The hydrolysis of ester and nitrile substituents to the carboxylic acids and of N-acyl groups to free amino groups have been reported in particular cases. Nitro groups have been reduced to amines, carboxylic acids have been esterified through the silver salts and the decarboxylation of C-carboxyl compounds has been described [15].

## SYNTHESIS OF TETRAZOLIUM SALTS

The tetrazolium salts have been prepared by the oxidation of formazans; some of which have been obtained by the modification of structures present in an already existent tetrazolium nucleus.

Various oxidants were used in the past for the oxidation of formazans to the tetrazolium salts (Scheme 7). These include mercuric oxide, nitric acid, isoamylnitrite, N-bromo succinimide, potassium permanganate, lead tetraacetate and t-butyl hypochlorite. Different oxidants have been used under different conditions. Although oxidation occurs in different reagent and condition, all mechanisms occur in similar manner and at the same time by dehydrogenation and cyclization. The disapperance of formazan color shows that oxidation reaction is complete and tetrazolium salts are formed. Ishıyama and co-workers synthesized novel tetrazolium salts, 2-benzothiazolyl-3-(4-carboxy-2-methoxyphenyl)-5-[4-(2-sulfoethylcarbamoyl) phenyl]-2Htetrazolium and 2,2′-dibenzothiazolyl-5, 5′-bis[4-di(2-sulfoethyl) carbamoylphenyl]-3,3′-(3, 3′-dimethoxy-4,4′-biphenylene)ditetrazolium, disodium salt by the oxidation of formazans [29,30].

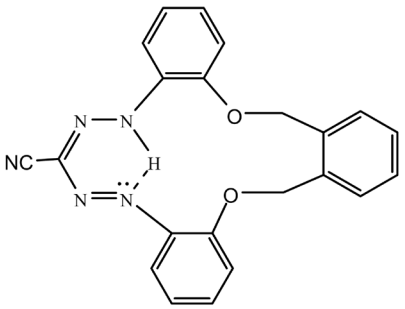
## SPECTRAL PROPERTIES

Because synthesis of formazans and tetrazolium salts were based on previous years, UV-vis and IR were used frequently during the first studies of these compounds. Besides, being a colored

compound and changing wavelength depending on substituents increased the importance of UV spectra. Today, although some methods as NMR and X-ray diffraction are used to eluciate these structure, UV studies remain important and new studies also employ UV spectrometry.

The spectra of triaryl formazans exhibit four distinctive absorption bants (A,B,C, and D), one in the visible and the others in the UV range [31]. The first band (A) observed in the wavelength range of 216-239 nm is assigned to that of the phenyl moiety. The second band (B, 240-285 nm) is attributed to the low energy p-p* transition of the phenyl moiety. The third band (C) within the 300- 350 nm range and the sharp peak is due to the p-p* transition within the hydrogen chelate ring formed by the azo and hydrazone group and the tautomerization occuring within this ring.

The fourth broad band is characteristic of the formazan structure due to p-p* transitions within the N = N group influenced by charge transfer within the whole molecule. The band is generally observed at 410-500 nm and shifted to 600 nm depending upon the structure. The visible absorption is very intensive, with the extinction coefficients registering values between 13.000-23.000 for mono-formazans and 35.000- 50.000 for di-formazans [6].

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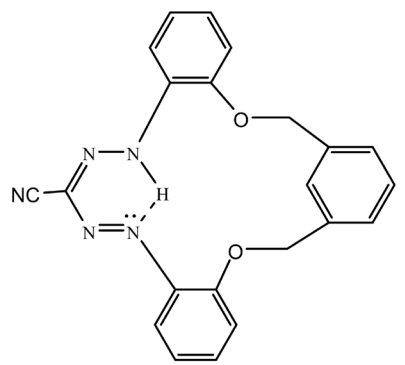
Tetrazolium salts in solution are so colorless compunds that the visible spectra of them are nearly free of absorption, while they display broad maxima in the UV range between 240-300 nm by their chemical sctructure with conjugated double bonds. This single absorption range is shaped by substituent attached salt. In recent years, although studies on tetrazolium salts have attracted great interest of scientists, their $^1$H NMR and IR spectra have not been examined thoroughly.

There are notable absorption bands in the IR spectra of formazans. These are C=N, N-H and N=N absorption bands. Shifting toward lower or higher frequency of these bands determine chelate or non-chelate structure. The C=N stretching band at 1500-1510 cm$^{-1}$ shows chelate structures. On the contrary, the C=N stretching band at 1551-1561 cm$^{-1}$ shows non-chelate structures [27, 32]. The shifts of these band to higher frequencies are explained in terms of the rupture of hydrogen bond and the loss of the resonance stabilization of the six-membered chelate ring. Similarly, the lower values of the N=N stretching band are due to the intramolecular hydrogen bond and to some chelate ring resonance. The N=N stretching band of the chelate form of TPF is located at 1357 cm$^{-1}$ while non-chelate form is located at 1418 cm$^{-1}$ [20]. In addition, the lower values of absorption bands N-H 3011-3090 cm$^{-1}$, showed chelate structure . The majority of formazans with this form are generally characterized by the lack of N-H absorption band. Chelate structures have a six-membered conjugated system that p-electrons are delocalized. Because of this, double bond characterize decreases. And the stretching bands of C=N, N=N and N-H were observed at lower frequencies.

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The N-H signal of formazan in the NMR spectrum is indicative in evaluating the structure. N-H signal in the downfield region at $\delta$ 16 exhibits intramoleculer hydrogen bonding, while upfield shifts of this signal at $\delta$ 10 indicate a weakening of the intramoleculer hydrogen bonding. Abbas examined N-H signals of macrocyclic bis formazans

in the $^1$H NMR spectrum and compared the positions of the NH signals in the $^1$H NMR spectra of the proposed structures 7 and 8. Compound 7 exhibited sharp NH signal in the downfield region at $\delta$ 15.70, indicative of an intra-molecular hydrogen bond. On the other hand, the NH signal of the macrocyclic formazan 8 was found at $\delta$ 11.94. These upfield shifts of the NH signal indicate a weakening of the intra-molecular hydrogen bond, which can be attributed to the increased size of the macrocycles 8 compared with that of 7 [33].

## CONCLUDING REMARKS

Formazans and tetrazolium salts were first synthesized over a century ago, but still attract attention of chemists, biologists, technologists and other specialists. In recent years, antiviral, antiinflammatory-analgesic, antifertility, antitubercular activity, antimicrobial activities, anticancer properties of formazans and tetrazolium salts have been published. This review aimes to describe the structure, synthesis and spectral properties of formazans and tetrazolium salts for highlighting the future applications in several bioactive phenomena.

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