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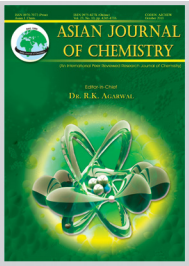

Synthesis and Antimicrobial Effects of 1,3,5-Substituted Phenyl Formazans

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In this study, novel formazans with various substituents on 1,3,5-phenyl rings were synthesized and their structures were elucidated with the use of elemental analysis, mass, ^{1}H NMR, ^{13}C NMR, IR, UV-VIS spectra. Also, antimicrobial effects of formazans were tested against selected microorganism, *Staphylococus aureus, S. epidermidis, S. saprophyticus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae*. Moreover, the antiyeast effects of the formazans are seen on the *Candida kefir, C. glabrata, C. tropicalis, Cryptococcus neofarmans, Saccharomyces cerevisiae*. In the present study, it was generally observed that the formazans were very active against *Candida kefir, C. tropicalis, Cryptococcus neofarmans* and *Saccharomyces cerevisiae*.

Key Words: Formazan, Antimicrobial effect, Synthesis.

INTRODUCTION

Formazans are coloured compounds due to in their structure π-π* transitions of π-electrons. Since Pechman synthesized[1] the first formazans afterward there have been numerous formazans synthesized and their structural features, tautomeric and photochromic isomers were investigated[2-5]. There were several studies on the synthesis of metal complexes[6,7]. Their complexes are also coloured compounds. The redox behaviours of these complexes were also evaluated. Formazans form tetrazolium salt when they are oxidized[8]. Tetrazolium salts are reduced back to formazans by the enzymes in the cell and stain the tissue. Tetrazolium- Formazan system is classified as a marker of vitality[9], there were used at the screening of anti cancer drugs and the determination of activity on tumor cell [10, 11], determined sperm viability[12]. Formazans have other important medical applications but they are toxic in nature, which prevented its routine in health sector. In this study, novel formazans with various substituents on 1- and 3-phenyl rings have been synthesized. Their structures were elucidated and their spectral behaviours are investigated by elemental analysis, mass, ^{1}H NMR, ^{13}C NMR, IR, UV-VIS spectra. The effect of substituents was also determined λ_{max} shift values by the use of UV-VIS spectra. The goal of this study was to synthesize the less toxic compounds for the use of medical purposes and develop new dyes for the dyeing industry. Also the factors effecting the colour of the compounds were elucidated by the use of λ_{max} values. Furthermore, we evaluated antimicrobial effects of formazans against some microorganism. The newly synthesized formazans were evaluated for their antimicrobial activity against *Staphylococus aureus, S. epidermidis, S. saprophyticus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae*. Moreover, the antiyeast effects of the formazans are seen on the *Candida kefir, C. glabrata, C. tropicalis, Cryptococcus neofarmans, Saccharomyces cerevisiae*. In our work, we observed the formazans in general are more active against yeast.

EXPERIMENTAL

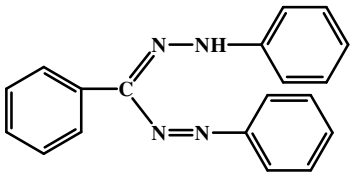
The UV-VIS spectra of all the formazans synthesized in this study were taken with UNICAM UV2-100 UV/Visible spectrophotometer using 1 cm quartz cells in 10^{-4} mol L^{-1} CH_3OH using 325 nm UV lambda in range of 200-600 nm. The IR spectra were obtained by MATTSON 100-FT-IR spectrophotometer between 4000-400 cm^{-1} using KBr pellets. ^{1}H NMR spectral studies were performed with BRUKER 400 MHz spectrophotometer using $CDCl_3$, 10^{-4} mol L^{-1}. All the elemental analysis studies were carried out by the use of LECO-CHNS-932 elemental analyzer.

Synthesis of 1.3.5-triphenylformazan: Benzaldehyde phenylhydrazone was synthesized by the reaction of benzaldehyde (2.12 g, 0.02 mol) with phenylhydrazine (2.16 g, 0.02 mol) in a methanolic medium at the pH value of 5-6. It was dissolved in appropriate amount of methanol and mixed with the basic buffer solution prepared with sodium hydroxide (2.50 g), sodium acetate (3.50 g) and methanol (200 mL). In

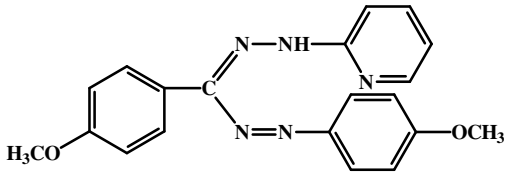
another flask diazonium chloride solution was prepared by the common way with aniline (1.86 g, 0.02 mol) concentrated HCl (5 mL) and sodium nitrite (1.50 g) at 0-5 ºC. Ice cubes were added into the medium in order to keep the temperature at 0-5 ºC. Benzenediazonium chloride solution was added to basic buffer benzaldehyde phenylhydrazone solution it in dropwise manner with constant stirring. The mixture was stirred for 2 h forming bright red coloured formazan. It was kept in the fridge for 2 days and recrystallized from methanol. All the other formazans were synthesized by the same way.

Structural formulae of the formazans of synthesized were shown in following:

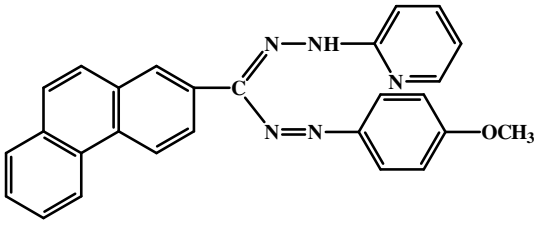
1,3,5-triphenylformazan (TPF): m.w. 300, m.p. 172-173 ºC; cheery-red colur; yield 78 %; m.p. lit: 170-173 ºC. Anal. calcd. (%) for $C_{19}H_{16}N_4$: C, 76.00; H, 5.33; N, 18.66. Found: C, 76.06; H, 5.32; N, 18.62.



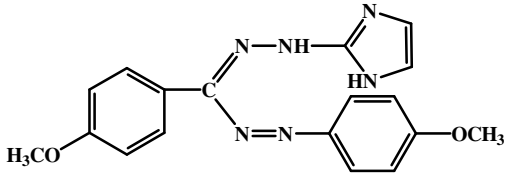
1-(4-Methoxyphenyl)-3-(4-methoxyphenyl)-5-(pyridino-2-il)-formazan (MMPyF): m.f. $C_{20}H_{19}N_5O_2$; m.w. calcd.: 361, found: 362.1; m.p.: 126-128 ºC.



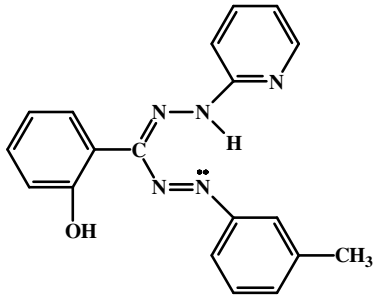
1-(4-Methoxyphenyl)-3-(9-phenantrenalde-hyde)-5-(pyridino-2-il)-formazan (MPPyF): m.f. $C_{27}H_{21}N_5O$; m.w. calcd.: 431, found: 431.1, m.p.: 108-112 ºC.



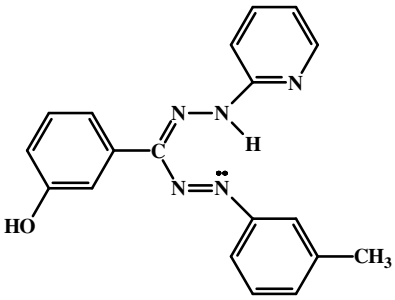
1-(4-Methoxyphenyl)-3-(4-methoxyphenyl)-5-(imidazol-2-il)-formazan (MMIF): m.f. $C_{18}H_{18}N_6O_2$; m.w. calcd.: 350, found: 350.1; m.p.: 198-202 ºC.



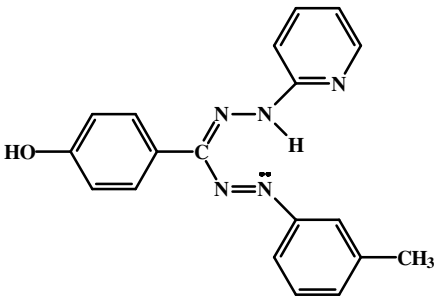
1-(4-Methylphenyl)-3-(2-hydroxyphenyl)-5-(pyridino-2-il)-formazan (*o*-MHPyF): m.w. calcd.: 331, found: 304.



1-(4-Methylphenyl)-3-(3-hydroxyphenyl)-5-(pyridino-2-il)-formazan (*m*-MHPyF); m.w. calcd.: 331, found: 336.



1-(4-Methylphenyl)-3-(4-hydroxyphenyl)-5-(pyridino-2-il)-formazan (*p*-MHPyF): m.w. calcd.: 331, found: 341.



Antimicrobial studies: In this work, *Staphylococus aureus, S. epidermidis, S. saprophyticus* (Gram positive), *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae* (Gram negative), *Candida kefir, C. glabrata, C. tropicalis, Cryptococcus neofarmans* and *Saccharomyces cerevisiae* (Yeast) were used to investigate the antibacterial and antifungal activities of formazans. The bacterial subcultures for *Staphylococus aureus, S. epidermidis, S. saprophyticus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Candida kefir, C. glabrata, C. tropicalis, Cryptococcus neofarmans* and *Saccharomyces cerevisiae* were obtained from Gazi University, Faculty of Science, Department of Biology. The compounds were tested for their antimicrobial activity by the well-diffusion method[13]. *Staphylococus aureus, S. epidermidis, S. saprophyticus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae* liquid cultures were prepared in brain heart infusion broth (BHI) for their antimicrobial tests. *Candida kefir, C. glabrata, C. tropicalis, Cryptococcus neofarmans* and *Saccharomyces cerevisiae* liquid cultures were prepared in

TABLE-1
IN VITRO ANTIMICROBIAL ACTIVITY OF FORMAZANS AGAINST SOME MICROORGANISMS

Bacteria strains	Diameter of zone (mm)						
	TPF	*o*-MHPyF	*m*-MHPyf	*p*-MHPyF	MMIF	MPPyF	MMPyf
S. aureus	12	2	-	6	-	2	10
S. epidermidis	-	-	-	8	-	-	8
S. saprophyticus	-	-	-	-	-	4	10
P. aeruginosa	-	2	-	-	-	-	-
K. pneumoniae	2	2	4	-	4	-	-
E. coli	-	3	2	-	-	-	-
C. kefir	-	4	4	2	-	4	20
C. glabrata	-	2	2	2	-	-	-
C. tropicalis	20	2	6	4	2	4	6
Cryptococcus neoformans	8	-	2	2	4	6	12
S. cerevisiae	10	2	2	2	4	4	12

Symbol (-) reveals that the compounds have any activity against to the microorganisms

Saburoud dextrose broth (SDB) for their antifungal tests. Each compound was dissolved in dimethyl sulfoxide and sterilated using a Milipore membrane filter (0.45 mm). DMSO was found to have no antimicrobial activity against any of the test organisms. About 1 cm^3 of a 24 h broth culture containing 10^6 cfu cm^{-3} was placed in sterile Petri dishes. Moltent nutrient Agar (15 cm^3) kept at 45 ºC was then poured into the Petri dishes and allowed to solidity. Then 6 mm diameter holes were punched carefully using a sterile cork borer and were completely filled with the test solutions. The plates were incubated for 24 h at 37 ºC for bacteria and 24-48 h at 30 ºC for yeast. After 24 h, the inhibition zone, which appeared around the holes in each plate was measured.

RESULTS AND DISCUSSION

The bacteriological and fungicidal activities of the newly synthesized compounds formazans were determined against eleven bacteria and yeast. The data for the antimicrobial tests are summarized in Table-1.

Formazans used in this study had different antibacterial and antifungal activity *in vitro* against the tested bacterial isolates and yeasts. We were generally observed that the newly synthesized compounds formazans are very active against *Candida kefir*, *C. tropicalis*, *Cryptococcus neofarmans* and *Saccharomyces cerevisiae*. The highest inhibition of growth occurred in MMPyF against *C. kefir*. Among the synthesized compounds, MMPyf showed well activity, against *S. aureus, S. epidermidis, S. saprophyticus, C. kefir, C. tropicalis, Cryptococcus neoformans, S. cerevisiae*. The other synthesized compounds showed moderate activity against used some bacteria and yeast. In the world, researchers synthesized different formazan derivatives and screened for their antimicrobial activities against bacteria and yeast. Marjadi *et al.*[14] synthesized ten substituted formazan derivatives (**3a-j**) by coupling Schiff base with appropriate aryl diazonium chlorides in pyridine. The synthesized compounds were screened for their antimicrobial activities against *B. subtilis, S. typhi and E. coli* and antifungal activity against *C. albicans* at a concentration of 40 µg/mL in DMF by cup-plate method. The synthesized compounds showed different activity against microorganisms.

A series of novel uracil formazans (**5a-r**) were synthesized by Samel and Pai[15] and the structures of the compounds have been confirmed by elemental analysis and spectral analysis. Furthermore; the synthesized compounds were screened for their antibacterial activity against *E. coli* and *S. aureus* and antifungal activity against *S. cerevisiae* and *C. albicans* at a concentration of 60 µg/mL in DMF by cup-plate method. Desai and Desai[16] synthesized ten formazan compounds. These compounds had been screened for their antifungal activity against *Candida albicans* (ATCC-64550), *Candida krusei* (ATCC-14243) and *Candida parapsilosis* (ATCC-22019) and antibacterial activity against *Escherchia coli* (ATCC-6538), *Staphylococcus aureus* (ATCC-6538), *Pseudomonas aeruginosa* (ATCC-1539) and *Bacillus substilis* (ATCC-6633).

From the present study, it is concluded that present results are different from the studies previously conducted by several authors. These differences may be due to use of different microorganisms and formazan derivatives.

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