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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF SCHIFF AND AZO-SCHIFF BASE LIGANDS

Alhagie JADAMA[a], Seda YUKSEKDANACI[b,*], Demet ASTLEY[a], Ihsan YASA[c]

ABSTRACT. Schiff and azo-Schiff base derivatives were synthesized, identified and evaluated for their antimicrobial and antifungal activities against gram-positive bacteria, gram-negative bacteria and fungi such as *Escherichia coli*, *Pseudomonas aureginosa*, *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhimurium*, *Saccharomyces cerevisiae and Candida albicans*. Chiral amides (**2a**-**e**) were synthesised by using D-Phenylglycine, L-Phenylalanine, L-valine, L-isoleucine and L-methionine. Experimental studies include the use of chiral amide derivatives (**2a**-**e**) of the five amino acids to synthesize the Schiff and azo-Schiff base derivatives of amides (**3a**-**e**, **4a**-**c** and **4e**). The structures of the compounds were determined by spectroscopic analyses (FTIR, ^{1}H-NMR, ^{13}C-NMR) and elemental analysis. Among the tested compounds, compound **3b** and **3d** were found to show the most potent inhibitory action against Gram positive and Gram negative bacteria.

Keywords: *Schiff base, azo-Schiff base, amino acid, amide, biological activity*

INTRODUCTION

Schiff base, named after Hugo Schiff, is a compound with the nitrogen atom connected to an aryl or alkyl group and functional group that contains a carbon-nitrogen double bond. And, azo compounds bear the functional group diazenyl R-N=N-R with R being an aryl or alkyl substituent.

[a] *Department of Chemistry, Faculty of Science, Ege University, Izmir, Turkey*
[b] *Department of Occupational Health and Safety, Faculty of Health Sciences, Uşak University, Uşak, Turkey*
[c] *Department of Biology, Faculty of Science, Ege University, Izmir, Turkey*
* *Corresponding author: seda.yuksekdanaci@usak.edu.tr*

Azo-Schiff base derivatives show various biological activities that include antibacterial [1-4], antifungal [5] and anticancer [6-8]. For example diazenyl chalcones derivatives showed good activity against *E. coli*, *S. enterica*, *B. Subtilis* and *A. fumigatus* [9]. Azo-linked salicylidenic Schiff bases have been tested for theirs antimicrobial activity and 4-((*E*)-(4-((*E*)-phenyldiazenyl)phenylimino) methyl) benzene-1,2,3-triol showed antimicrobial activity against *K. pneu-moniae*, *E. cloacae*, *E. coli*, *B. megaterium*, *S. aureus*, *M. luteus*, *M. smegmatis*, *P. aeruginosa* [10]. In another study, azo-Schiff base derivatives were sythesized via condensation of salicylaldehyde derivatives with thiosemicarbazide. These azo-Schiff base compounds exhibited antimicrobial activity against *B. Subtilis*, *S. aureus*, *E. coli* and *P. aereuginosa* [11]. In another study by the same group, azo-Schiff base compounds were synthesized by reacting 5-phenyl azo salicyladehyde derivatives with o-amino phenol. The synthesized compounds also exhibited very high antimicrobial activity against *P. aeruginosa* [12]. *E*-5-((4-nitro phenyldiazanyl) quinoline-8-ol has been tested for its antimicrobial activity and was found to have high antimicrobial effect against multi-drug resistant bacteria [13]. Slassi et al. synthesized imidazole based azo-Schiff base derivatives and evaluated their antifungal activity against selected fungi species. The synthesized derivarives showed good antifungal activity against *S. spiospermum, A. fumigatus,* and *C. albicans* [14].

In this study, D-Phenylglycine, L-Phenylalanine, L-valine, L-isoleucine and L-methionine were used for the synthesis of chiral amide derivatives (**2a**-**e**). The synthesized chiral amides derivatives were reacted with 2-hydroxy-1-naphthaldehyde and (*E*)-2-hydroxy-5-(phenyl diazenyl) benzaldehyde for the synthesis of Schiff base (**3a**-**e**) and azo-Schiff base compounds (**4a**-**c**, **4e**).

RESULTS AND DISCUSSION

Chemistry

In this study, five Schiff base and four azo-Schiff base amino acid derivatives were synthesised, characterized and investigated for their biological activities. D-Phenylglycine, L-Phenylalanine, L-valine, L-isoleucine and L-methionine were used as starting materials for the synthesis of amide structures (**2a**-**e**) (Scheme 1). For the synthesis of Schiff base derivatives (**3a**-**e**) 2-hydroxy-1-naphthaldehyde was used in methanol under reflux for 4 hours (Scheme 2). The yields of the products vary between 84 to 94 %.

(*E*)-2-hydroxy-5-(phenyldiazenyl) benzaldehyde was used for the synthesis of azo-Schiff base derivatives (**4a-c**, **4e**) (Scheme 3) and the yields for these products varied between 61 to 85 %. The structure of the final products was determined by using spectroscopic analyses (FTIR, ^{1}H-NMR, ^{13}C-NMR) and elemental analysis methods.

The FTIR spectra of the Schiff base derivatives (**3a**-**e**) showed characteristic bands for OH, C=N and C=O vibrations. Schiff bases show a absorption band due to O-H vibrations between 3557-3400 cm^{-1}. In the FTIR spectra, Schiff bases exhibit bands at approximately 1690 and approximately 1670 cm^{-1} that are assignable to vibrations of C=N and C=O stretchings (Table 1).

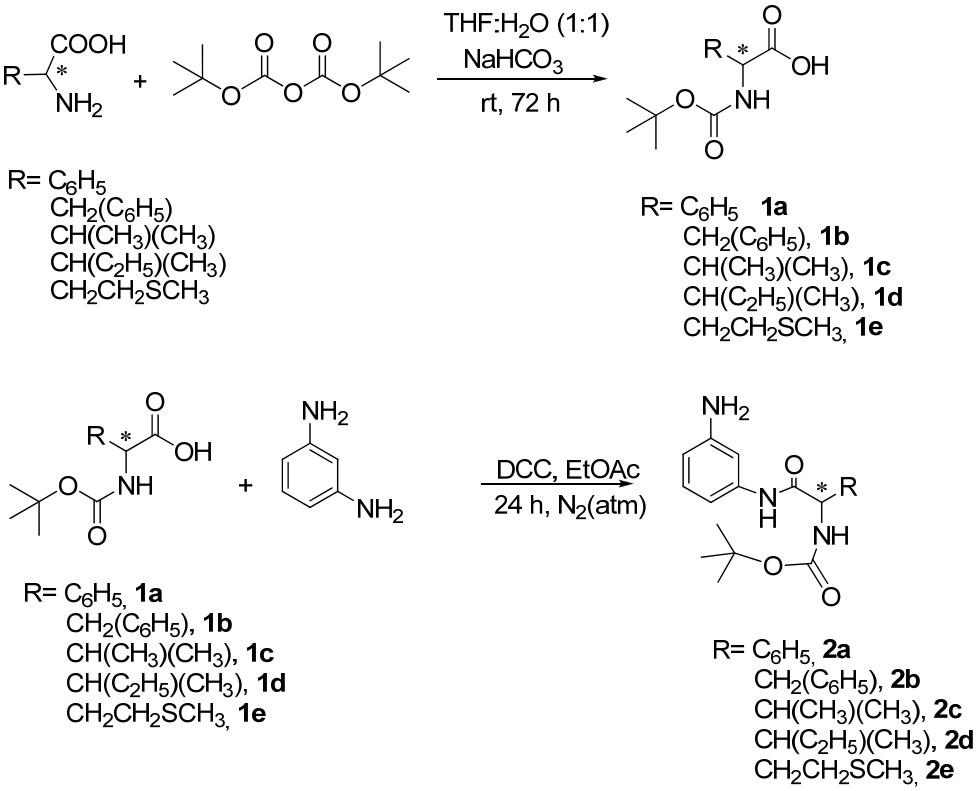
When the ^{1}H-NMRs of Schiff base derivatives (**3a**-**e**) are analyzed, we see that -OH protons are observed at 15.33, 15.68, 15.32, 15.31 and 15.29 ppm, respectively. The imine protons of the same compounds also gave signals at 9.21, 9.59, 9.30, 9.36 and 9.21 ppm, respectively.

When the ^{13}C-NMR spectra of the Schiff base derivatives (**3a**-**e**) are examined, the signals observed in the range of 172-169 ppm belong to the carbonyl groups, and the peak observed around 155 ppm belongs to the imine carbon. The chiral amino acid carbons of the same compounds also gave signals at 59.71, 57.11, 61.25, 60.22 and 54.62 ppm, respectively.

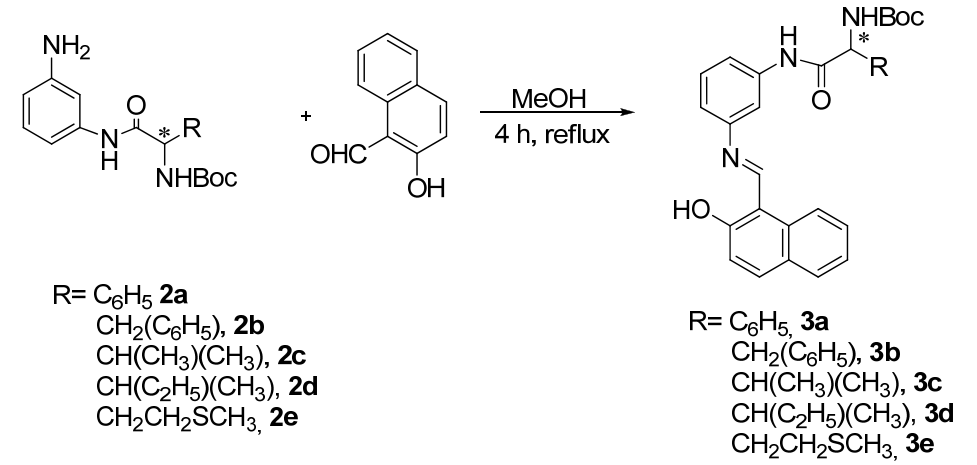
The FTIR spectra of the azo-Schiff base derivatives (**4a**-**c**,**4**-**e**) showed characteristic bands for OH and NH vibrations. Azo-Schiff bases (**4a**-**c**, **4e**) showed a absorption band due to O-H vibrations 3312, 3312, 3474 and 3474 cm^{-1}, respectively. And also Azo-Schiff bases showed a absorption band due to N-H vibrations 3228, 3327, 3263 and 3272 cm^{-1}, respectively (Table 1).

When the ^{1}H-NMRs of azo-Schiff base derivatives (**4a-c, 4e**) are analyzed, we see that –OH protons are observed at 13.78, 13.69, 13.72 and 13.69 ppm, respectively. The -CH protons in the amino acid structure are observed at 5.88, 5.62, 5.59 and 5.68 ppm, respectively. The imine protons of the same compounds also gave signals at 9.20, 8.95, 9.24 and 9.21 ppm, respectively.

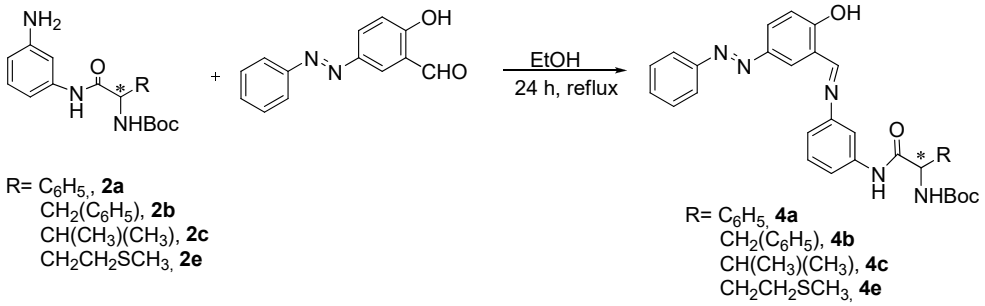
When the ^{13}C-NMR spectra of the Schiff base derivatives (**4a-c, 4e**) are examined, the signals observed in the range of 171.26-156.30 ppm belong to the carbonyl groups, and the peak observed around 162 ppm belongs to the imine carbon. The chiral amino acid carbons of the same compounds also gave signals at 59.52, 56.86, 61.20 and 54.52 ppm, respectively.



Scheme 1. Synthesis of chiral amide derivatives



Scheme 2. Synthesis of Schiff base derivatives



R= C_6H_5, **2a**
$CH_2(C_6H_5)$, **2b**
$CH(CH_3)(CH_3)$, **2c**
$CH_2CH_2SCH_3$, **2e**

R= C_6H_5, **4a**
$CH_2(C_6H_5)$, **4b**
$CH(CH_3)(CH_3)$, **4c**
$CH_2CH_2SCH_3$, **4e**

Scheme 3. Synthesis of azo-Schiff base derivatives

Table 1. Selected stretching frequencies of the synthesized final products (cm^{-1})

Compound	O-H	N-H	C=N	C=O	Ar. C-H	Ar. C=C	Alp. C-H
3a	3436	3315	1690	1663	2970	1525	2853
3b	3557	3328	1688	1660	3031	1527	2876
3c	3400	3316	1690	1668	3055	1505	2872
3d	3485	3330	1688	1662	2960	1526	2830
3e	3480	3301	1682	1672	3055	1546	2919
4a	3212	3328	1690	1669	3055	1598	2852
4b	3212	3327	1690	1673	3056	1599	2853
4c	3474	3263	1695	1628	3059	1544	3025
4e	3474	3272	1694	1673	3059	1544	2936

Biological Activity

The evaluated biological activities of all synthesized compounds (**3a-e**, **4a-c**, **4e**) were tested for their minimum inhibitory concentrations (MIC) (µg/ml) against the gram negative bacteria *E.coli, P. aureginosa* and *S. typhimurium,* gram positive bacteria *B. cereus,* and *S. aureus*, and fungi *S. cerevisiae* and *C. Albicans* and compared with the standard drugs Gentamicin and Tetracycline.

Compounds **3a**, **3b**, **3d**, **3e**, **4a** and **4e** showed an inhibitory effect on the Gram negative bacterium *P. aureginosa* (0.19 µg/ml). The effect of the six compounds listed in the smallest MIC value indicates the sensitivity of *P. aureginosa* to these compounds, and therefore its potential to be used for antimicrobial purposes in cases where *P. aureginosa* is targeted with additional studies. It was observed that *E. coli* was generally resistant to synthesized compounds tested in the study (**3a-e**, **4a-c**, **4e**). Except compound **3e** (1.56 µg/ml), MIC values were determined as 6.25 µg/ml and above for the rest. In the *in vitro*

antimicrobial activity determination of all compounds, *S. aureus* exhibit resistance to all derivatives, except for compound **3d** (0.39 µg/ml) and compound **3b** (0.19 µg/ml). A strong antimicrobial effect against *B. cereus* was not detected in the compounds synthesized in the study. *S. typhimurium* was sensitive to the compund **3d** (0.39 µg/ml). And *S. cerevisiae* was sensetive to the compund **3b** (1.56 µg/ml). Compounds **3a**, **3b**, and **3c** showed an inhibitory effect on the pathogenic yeast *C. albicans*. Among the all synthesized compounds used in the MIC test, compounds **3b** and **3d** were observed to have broad spectrum (Gram positive and Gram negative) antimicrobial activity. According to our findings, compounds **3b** and **3d** have the potential to be candidates for new antimicrobials. A detailed comparison of antimocrobial activities of all synthesized derivatives and the standard drugs used for activity comparison is summarized in the MIC table (Table 2).

Table 2. MIC values of Schiff and azo-Schiff base derivatives against bacteria and C. fungi (µg/ml)

Compound	*E. coli*	*P. aureginosa*	*S. aureus*	*B. cereus*	*S. thyphimirium*	*S. cerevisiae*	*C. albicans*
3a	12,5	0,19	12,5	25	12,5	6,25	6,25
3b	12,5	0,19	0,19	12,5	6,25	1,56	6,25
3c	12,5	12,5	50	50	50	25	6,25
3d	6,25	0,19	0,39	50	0,39	25	25
3e	1,56	0,19	25	50	25	25	25
4a	12,5	0,19	50	50	50	25	12,5
4b	12,5	25	50	50	50	12,5	12,5
4c	25	50	50	50	25	12,5	12,5
4e	25	0,19	25	50	25	12,5	12,5
Gentamicin	1,56	1,56	0,39	0,39	0,39	25	25
Tetracycline	3,125	25	0,78	1,56	1,56	25	12,5

CONCLUSIONS

Schiff base and azo-Schiff base amino acid derivatives (**3a-e**, **4a-c, 4e**) were synthesised and evaluated for their antimicrobial and antifungal activities against *Escherichia coli*, *Pseudomonas aureginosa*, *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhimurium*, *Saccharomyces cerevisiae and Candida albicans*. The synthesised Schiff and azo-Schiff base derivatives show different activity against the test organisms. Compounds **3b** and **3d** seem to show better results with different test organisms compared with the other synthesised compounds.

EXPERIMENTAL SECTION

Materials

The Perkin Elmer Spectrum 100 FTIR Spectrometer was used for infrared spectra studies. All reagents were obtained from Merck, Sigma-Aldrich and Alfa Aesar. Melting points were recorded with an electro thermal digital melting points apparatus. TLC analyses were carried out on precoated aluminium plates and coloumn chromatography was performed on Silica gel 60 F254 (Merck). UV active components were observed under the UV lamp for TLC. ^{1}H-NMR and ^{13}C-NMR spectra were recorded using 400 MHz Varian NMR spectrometer.

General procedure for the synthesis of *N*-(tert-butoxycarbonyl)-amino acid (1a-e)

The protection reaction of amine groups was carried out with the same method as in our previous publication [15]. A mixture of di-*tert*-butyl dicarbonate (1.1 equiv) in 40 ml tetrahydrofuran was added to a mixture of an amino acid (1 equiv) and sodium bicarbonate (4 equiv) dissolved in 40 ml of water. The reaction mixture was continued for 72 hours until completion at room temperature. Completion of the reaction was confirmed by TLC (Hexane/EtOAc 1:1) and the remaining THF was evaporated. 2M HCl was added to pH 2 and water layer extracted with EtOAc (4×20ml), dried with sodium sulfate and concentrated in vacuo to give the desired product. D-Phenlyglycine, L-phenylalanine, L-isoleucine, L- Valine and L-methionine were obtained in 86%, 87%, 93%, 89 % and 85% yields respectively.

General procedure for the synthesis of *N*-(*tert*-butoxycarbonyl)-amino acid-benzene-1,2-diamine (2a-e)

N-(*tert*-butoxycarbonyl)-amino acids (1 equiv) and *m*-phenylenediamine (1 equiv) were dissolved in 12 ml EtOAc with stirring at room temperature. A solution of *N,N'*-dicyclohexylcarbodiimide (1 equiv) dissolved in 8 ml EtOAc was added in drops for 20 mins to the preceeding mixture and the reaction was allowed to complete at room temperature for 6 hours. Upon completion, it was filtered and filtrate was washed twice with citric/tartaric acid solution 5% w/w and evaporated to dryness. The residue was purified by SiO_2 gel column chromatography (DCM/EtOAc 2:1) to give the desired compounds.

tert-Butyl 2-(3-aminophenylamino)-2-oxo-1-phenylethylcarbamate (2a)

White solid, yield: 82%, mp: 101-103 ^{o}C, IR (KBr, cm^{-1}): 3321, 3010, 2924, 2855, 1668, 1494, 1164. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.65 (s, 1H, NH), 7.47 (d, *J*=5.6 Hz, 2H, Ar-H), 7.29 (d, *J*= 6.2 Hz, 3H, Ar-H), 6.95 (d,

J=7.9 Hz, 1H, Ar-H), 6.91 (d, *J*=7.6 Hz, 1H, Ar-H), 6.70 (d, *J*= 7.0 Hz, 1H, Ar-H), 6.32 (d, *J*=7.6 Hz, 1H, Ar-H), 6.11 (s, 1H, NH), 5.54 (s, 1H, CH), 3.58 (bs, 2H, NH_2), 1.41 (s, 9H, 3xCH_3). ^{13}C-NMR (100MHz, $CDCl_3$) δ (ppm): 169.08, 155.69, 147.19, 138.52, 137.56, 132.43, 130.91, 129.49, 128.93, 128.35, 127.35, 111.29, 110.14, 106.93, 80.44, 59.12, 28.35. Anal. calc. for $C_{19}H_{23}N_3O_3$: C, 66.84; H, 6.79; N, 12.31. Found: C, 66.80; H, 6.77; N, 12.28.

tert-Butyl 1-(3-aminophenylamino)-1-oxo-3-phenylpropan-2-ylcarbamate (2b)

White solid, yield: 75%, mp: 145-147 °C, IR (KBr, cm^{-1}): 3433, 3322, 3292, 3026, 2964, 2925, 2852, 1691, 1661, 1523, 1162. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.31-7.25 (m, 3H, Ar-H), 7.19 (t, *J*=7.7 Hz, 1H, Ar-H), 6.97 (d, *J*=8.2 Hz, 1H, Ar-H), 6.92-6.88 (m, 2H, Ar-H), 6.68 (d, 1H, *J*=8.4 Hz, Ar-H), 6.26 (dd, 1H, *J*=7.9 Hz, *J*=1.3 Hz, Ar-H), 5.03 (s, 1H, CH), 4.33-4.28 (m, 1H, NH), 3.33 (s, 2H, NH_2), 3.23 (s, 1H, NH), 2.98 (dd, *J*=13.6 Hz, *J*=4.4 Hz, 1H, CH_{2b}); 2.91 (t, *J*=10.0 Hz, 1H, CH_{2a}); 1.30 (s, 9H, 3xCH_3). ^{13}C-NMR (100MHz, $CDCl_3$) δ (ppm): 170.80, 170.29, 155.78, 149.43, 139.94, 138.44, 129.68, 129.32, 128.43, 126.67, 109.88, 107.74, 105.48, 78.48, 58.74, 38.00, 28.60. Anal. calc. for $C_{20}H_{25}N_3O_3$: C, 67.58; H, 7.09; N, 11.82. Found: C, 67.52; H, 7.06; N, 11.80.

tert-Butyl 1-(3-aminophenylamino)-3-methyl-1-oxobutan-2-ylcarbamate (2c)

White solid, yield: 86%, mp: 119-122 °C, IR (KBr, cm^{-1}): 3312, 3047, 2969, 2933, 2874, 1667, 1614, 1164. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.84 (s, 1H, NH), 6.95 (t, *J*=7.9 Hz, 2H, Ar-H), 6.79 (d, *J*=7.1 Hz, 1H, Ar-H), 6.34 (d, *J*=7.5 Hz, 1H, Ar-H), 5.70 (d, *J*=8.7 Hz, 1H, CH), 4.16 (s, 1H, NH), 3.63 (s, 2H, NH_2), 2.13 (d, *J*=6.7 Hz, 1H, $CH(CH_3)_2$), 1.40 (s, 9H, 3xCH_3), 0.99 (t, J=6.8 Hz, 6H, 2xCH_3). ^{13}C-NMR (100MHz, $CDCl_3$) δ (ppm): 170.94, 156.41, 147.13, 138.75, 129.46, 111.05, 110.22, 106.90, 79.90, 60.96, 31.15, 28.34, 19.32. Anal. calc. for $C_{16}H_{25}N_3O_3$: C, 62.52; H, 8.20; N, 13.67. Found: C, 62.50; H, 8.22; N, 13.62.

tert-Butyl 1-(3-aminophenylamino)-3-methyl-1-oxopentan-2-ylcarbamate (2d)

White solid, yield: 85%, mp: 130-131 °C, IR (KBr, cm^{-1}): 3310, 2966, 2933, 2877, 1664, 1528, 1164. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.77 (s, 1H, NH), 6.96 (t, *J*=7.6 Hz, 2H, Ar-H), 6.79 (d, *J*=7.0 Hz, 1H, Ar-H), 6.35 (d, *J*=7.5 Hz, 1H, Ar-H), 5.62 (d, *J*=8.7 Hz, 1H, CH), 4.18 (s, 1H, NH), 3.63 (s,

2H, NH_2), 1.91 (s, 1H, CH_{2a}-CH_3), 1.61 (s, 1H, CH_{2b}-CH_3), 1.40 (s, 9H, 3xCH_3), 1.21-1.12 (m, 1H, CH-CH_3), 0.98 (d, *J*=6.8 Hz, 3H, CH_3), 0.89 (t, *J*=7.6 Hz, 3H, CH_3). ^{13}C-NMR (*100MHz*, $CDCl_3$) δ (ppm): 170.94, 156.35, 147.10, 138.73, 129.47, 111.03, 110.19, 106.85, 79.99, 60.01, 37.23, 28.33, 24.94, 15.50, 11.05. Anal. calc. for $C_{17}H_{27}N_3O_3$: C, 63.53; H, 8.47; N, 13.07. Found: C, 63.49; H, 8.49; N, 13.07.

tert-Butyl 1-(3-aminophenylamino)-4-(methylthio)-1-oxobutan-2-ylcarbamate (2e)

White solid, yield: 64%, mp: 77-80 °C, IR (KBr, cm^{-1}): 3311, 3047, 2977, 2928, 2869, 1667, 1615, 1165. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.86 (s, 1H, NH), 6.98 (t, *J*=8.0 Hz, 2H, Ar-H), 6.76 (d, *J*=7.5 Hz, 1H, Ar-H), 6.36 (d, *J*=7.7 Hz, 1H, Ar-H), 5.80 (d, *J*=7.5 Hz, 1H, CH), 4.49 (d, *J*=6.0 Hz, 1H, NH), 3.65 (bs, 2H, NH_2), 2.59 (t, *J*=7.3 Hz, 2H, S-CH_2), 2.14-2.09 (m, 1H, CH-CH_{2a}), 2.06 (s, 3H, S-CH_3), 2.02-1.95 (m, 1H, CH-CH_{2a}), 1.40 (s, 9H, 3xCH_3). ^{13}C-NMR (100MHz, $CDCl_3$) δ (ppm): 170.53, 156.19, 147.20, 138.64, 129.60, 111.24, 110.09, 106.78, 80.32, 54.31, 31.94, 30.26, 28.33, 15.31. Anal. calc. for $C_{16}H_{25}N_3O_3S$: C, 56.61; H, 7.15; N, 12.42. Found: C, 56.59; H, 7.18; N, 12.40.

General procedure for the synthesis *N*-(*tert*-butoxycarbonyl)-amino acid-2- hydroxy-1-naphthaldehyde (3a-e)

N-(*tert*-butoxycarbonyl)-amino acid-benzene-1,3-diamine (1 equiv) was dissolved in 15 ml methanol. An equimolar amount of 2-hydroxy-1-naphthaldehyde was added to the above mixture under reflux for at least 4 hours with the addition of a trace amount of acetic acid. The reaction was monitored with with TLC (DCM/EtOAc 2:1). The reaction mixture was cooled down upon completion, filtered and crystallized from ethanol to obtained desired product.

(*R*,*E*)-*tert*-Butyl-2-(3-((2-hydroxynaphthalen-1-yl)methyleneamino) phenylamino)-2-oxo-1-phenyl ethylcarbamate (3a)

Orange solid, yield: 84%, mp: 201-203 °C, IR (KBr, cm^{-1}): 3436, 3315, 2970, 2925, 2853, 1690, 1663, 1525, 1171. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 15.33 (s, 1H, OH), 9.21 (s, 1H, N=CH), 9.07 (bs, 1H,Ar-H), 8.17 (d, *J*=8.2 Hz, 1H, Ar-H), 7.69 (d, *J*=9.2 Hz, 1H, Ar-H), 7.62 (d, *J*=7.8 Hz, 1H, Ar-H), 7.58 (d, *J*=8.2 Hz, 2H, Ar-H), 7.49 (s, 1H, Ar-H), 7-41-7.34 (m, *J*=7.4 Hz, 3H, Ar-H), 7.29 (d, *J*=7.4 Hz, 1H, Ar-H), 7.25 (d, *J*=8.6 Hz, 1H, Ar-H), 7.15 (d, *J*=8.9 Hz, 1H, Ar-H), 6.95 (d, 1H, *J*=9.0 Hz, Ar-H), 6.86 (d, *J*=6.3 Hz, 1H,

Ar-H), 5.80 (d, *J*=6.8 Hz, 1H, CH), 5.58 (bs, 1H, NH), 1.44 (s, 9H, 3xCH_3). ^{13}C-NMR (100MHz, $CDCl_3$) δ (ppm): 172.17, 169.17, 164.94, 156.20, 153.74, 144.42, 139.20, 137.02, 136.54, 133.43, 129.72, 129.19, 128.84, 128.04, 127.54, 127.06, 124.48, 123.38, 122.76, 119.23, 118.59, 117.23, 116.58, 109.98, 108.67, 81.01, 59.71, 28.34. Anal. calc. for $C_{30}H_{29}N_3O_4$: C, 72.71; H, 5.90; N, 8.48. Found: C, 72.68; H, 5.93; N, 8.46.

(*S*,*E*)-*tert*-Butyl-1-(3-((2-hydroxynaphthalen-1-yl)methyleneamino) phenylamino)-1-oxo-3-phenyl propan-2-ylcarbamate (3b)

Orange solid, yield: 86%, mp: 206-208 °C, IR (KBr, cm^{-1}): 3557, 3303, 3328, 3031, 2978, 2876, 1688, 1660, 1527, 1160. ^{1}H-NMR (400 MHz, DMSO-D_6) δ (ppm): 15.68 (s, 1H, OH), 10.17 (s, 1H, NH), 9.59 (s, 1H, N=CH), 8.43 (d, *J*=8.4 Hz, 1H, Ar-H), 7.92 (d, *J*=9.2 Hz, 1H, Ar-H), 7.78 (d, *J*=7.8 Hz, 1H, Ar-H), 7.72 (s, 1H, Ar-H), 7.55 (t, *J*=7.8 Hz, 1H, Ar-H), 7.49 (d, *J*= 4.7 Hz, 1H, Ar-H), 7.42 (d, *J*=4.8 Hz, 2H, Ar-H), 7.35 (t, *J*=7.7 Hz, 3H, Ar-H), 7.29 (t, *J*= 7.2 Hz, 2H, Ar-H), 7.20 (d, *J*=7.2 Hz, 1H, Ar-H), 7.13 (d, *J*=8.0 Hz, 1H, Ar-H), 6.98 (d, *J*=9.1 Hz, 1H, CH), 4.34 (bs, 1H, NH), 3.03 (d, *J*=10.0 Hz, 1H, Ar-CH_{2b}), 2.86 (t, *J*=10.8 Hz, 1H, Ar-CH_{2a}), 1.31 (s, 9H, 3xCH_3). ^{13}C-NMR (100MHz, DMSO-D_6) δ (ppm): 171.54, 171.18, 155.89, 144.68, 140.51, 138.33, 137.46, 133.55, 130.45, 129.68, 129.49, 128.60, 128.50, 127.11, 126.76, 123.96, 122.63, 120.56, 117.76, 114.94, 112.35, 108.86, 78.60, 57.11, 37.90, 28.61. Anal. calc. for $C_{31}H_{31}N_3O_4$: C, 73.06; H, 6.13; N, 8.25. Found: C, 73.10; H, 6.14; N, 8.23.

(*S*,*E*)-*tert*-Butyl-1-(3-((2-hydroxynaphthalen-1-yl)methyleneamino) phenylamino)-3-methyl-1-oxo butan-2-ylcarbamate (3c)

Orange solid, yield: 91%, mp: 175-177 °C, IR (KBr, cm^{-1}): 3400, 3316, 3055, 2965, 2929, 2872, 2400, 1690, 1668, 1640, 1505, 1162. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 15.32 (s, 1H, OH), 9.30 (s, 1H, N=CH), 9.09 (s, 1H, Ar-H), 8.04 (d, *J*=7.2 Hz, 1H, Ar-H), 7.65 (d, *J*=9.1 Hz, 1H, Ar-H), 7.57 (d, *J*=5.8 Hz, 2H, Ar-H), 7.42 (t, *J*=7.2 Hz, 1H, Ar-H), 7.24 (t, *J*=6.3 Hz, 2H, Ar-H), 7.12 (s, 1H, Ar-H), 6.94 (d, 1H, *J*=8.9 Hz, Ar-H), 6.85 (s, 1H, NH), 5.62 (d, *J*=8.3 Hz, 1H, CH), 4.26 (s, 1H, NH), 2.21 (d, *J*=5.4 Hz, 1H, CH-(CH_3)$_2$), 1.45 (s, 9H, 3xCH_3), 1.10 (d, *J*=6.2 Hz, 6H, 2xCH_3). ^{13}C-NMR (100MHz, $CDCl_3$) δ (ppm): 171.98, 171.23, 156.89, 153.57, 144.45, 139.24, 136.95, 133.33, 129.71, 129.13, 128.02, 127.01, 123.36, 122.67, 119.11, 117.29, 116.41, 110.09, 108.60, 80.48, 61.25, 30.98, 28.38, 19.38, 18.63. Anal. calc. for $C_{27}H_{31}N_3O_4$: C, 70.26; H, 6.77; N, 9.10. Found: C, 70.27; H, 6.74; N, 9.07.

tert-Butyl (2*S*,3*S*)-1-(3-((*E*)-(2-hydroxynaphthalen-1-yl) methyleneamino)phenylamino)-3-methyl-1-oxopentan-2-ylcarbamate (3d)

Orange solid, yield: 94%, mp: 197-200 °C, IR (KBr, cm^{-1}): 3485, 3330, 2960, 2926, 2830, 1688, 1662, 1526, 1174. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 15.31 (s, 1H, OH), 9.36 (s, 1H, N=CH), 9.08 (s, 1H, Ar-H), 8.04 (d, *J*=8.4 Hz, 1H, Ar-H), 7.64 (d, *J*=9.0 Hz, 1H, Ar-H), 7.57 (d, *J*=8.6 Hz, 2H, Ar-H), 7.43 (t, *J*=7.2 Hz, 1H, Ar-H), 7.27-7.22 (m, 2H, Ar-H), 7.10 (t, *J*=7.2 Hz, 1H, Ar-H), 6.94 (d, *J*=9.1 Hz, 1H, Ar-H), 6.84 (d ,*J*=8.5 Hz, 1H, NH), 5.62 (d, *J*=8.5 Hz, 1H, CH), 4.30 (s, 1H, NH), 1.97 (d, *J*=5.6 Hz, 1H, CH-CH_3), 1.44 (s, 9H, 3xCH_3), 1.34-1.24 (m, 2H, CH_2), 1.08 (d, *J*=6.7 Hz, 3H, CH-CH_3), 0.95 (t, *J*=7.4 Hz, 3H, CH_2-CH_3). ^{13}C-NMR (100MHz, $CDCl_3$) δ (ppm): 171.92, 171.40, 156.87, 153.53, 144.44, 139.26, 136.91, 133.33, 129.70, 129.11, 128.02, 127.01, 123.35, 122.65, 119.12, 117.23, 116.46, 109.96, 108.59, 80.47, 60.22, 37.13, 28.38, 25.16, 15.57, 10.92. Anal. calc. for $C_{28}H_{33}N_3O_4$: C, 70.71; H, 6.99; N, 8.84. Found: C, 70.67; H, 6.95; N, 8.80.

(*S*,*E*)-*tert*-Butyl-1-(3-((2-hydroxynaphthalen-1-yl) methyleneamino)phenylamino)-4-(methylthio)-1-oxobutan-2-ylcarbamate (3e)

Orange solid, yield: 92%, mp: 156-158 °C, IR (KBr, cm^{-1}): 3480, 3301, 3055, 2978, 2919, 1682, 1672, 1546, 1165. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 15.29 (s, 1H, OH), 9.21 (s, 1H, N=CH), 9.13 (s, 1H, Ar-H), 8.02 (d, *J*=7.3 Hz, 1H, Ar-H), 7.67 (d, *J*=7.2 Hz, 1H, Ar-H), 7.60 (d, *J*=7.5 Hz, 2H, Ar-H), 7.44 (app. t, *J*=6.2 Hz, 1H, Ar-H), 7.27 (d, *J*=8.4 Hz, 2H, Ar-H), 7.20 (s, 1H, Ar-H), 6.94 (s, 2H, Ar-H+NH), 5.71 (s, 1H, CH), 4.57 (s, 1H, NH), 2.66 (t, *J*=6.3 Hz, 2H, S-CH_2), 2.21 (s, 1H, CH-CH_{2b}), 2.11 (s, 4H, S-CH_3+CH-CH_{2a}), 1.45 (s, 9H, 3xCH_3). ^{13}C-NMR (100MHz, $CDCl_3$) δ (ppm): 170.80, 156.55, 153.88, 144.87, 139.13, 136.99, 133.28, 129.89, 129.19, 128.08, 127.10, 123.47, 122.53, 119.03, 118.60, 117.48, 116.54, 110.58, 108.68, 80.80, 54.62, 31.63, 30.41, 28.38, 15.43. Anal. calc. for $C_{27}H_{31}N_3O_4S$: C, 65.70; H, 6.33; N, 8.51. Found: C, 65.67; H, 6.31; N, 8.47.

General procedure for the synthesis *N*-(*tert*-butoxycarbonyl)-amino acid-phenyldiazenyl salicylaldehyde (4a-c, 4e)

N-(*tert*-butoxycarbonyl)-amino acid-benzene-1,3-diamine (1 equiv) were dissolved in 35 ml ethanol. An equimolar amount of phenyldiazenyl salicylaldehyde (1 equiv) was added to the above mixture under reflux for at least 24 hours with the addition of a trace amount of acetic acid. The reaction

was monitored with TLC (DCM/EtOAc 2:1). The reaction mixture was cooled down upon completion, filtered and crystallized from ethanol to obtained final product.

tert-Butyl-(*R*)-2-(3-((*E*)-2-hydroxy-5-((*E*)phenyldiazenyl)benzylideneamino)phenylamino)-2-oxo-1-phenylethylcarbamate (4a)

Orange solid, yield: 70%, mp: 183-185 °C, IR (KBr, cm^{-1}): 3328, 3212, 3055, 2924, 2852, 1690, 1669, 1640, 1598, 1167. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 13.78 (s, 1H, OH), 9.20 (s, 1H, N=CH), 8.59 (s, 1H, Ar-H), 7.95 (app d, *J*=5.6 Hz, 2H, Ar-H), 7.88 (d, *J*= 8.0 Hz, 2H, Ar-H), 7.59 (d, *J*=9.1 Hz, 2H, Ar-H), 7.53 (t, *J*=8.0 Hz, 3H, Ar-H), 7.46-7.42 (m, 1H, Ar-H), 7.39-7.33 (m, 3H, Ar-H), 7.23 (d, *J*=7.7 Hz, 1H, Ar-H), 7.16 (t, *J*=7.6 Hz, 1H, Ar-H), 7.06 (d, *J*=9.4 Hz, 1H, Ar-H), 6.86 (d, *J*=6.9 Hz, 1H, NH), 5.88 (d, *J*=7.2 Hz, 1H, CH), 5.64 (d, *J*=5.5 Hz, 1H, NH), 1.48 (s, 9H, 3xCH_3). ^{13}C-NMR (100MHz, $CDCl_3$) δ (ppm): 169.33, 164.19, 162.01, 162.00, 156.30, 152.56, 147.52, 145.39, 139.01, 139.00, 136.45, 130.45, 129.50, 129.15, 129.04, 128.81, 128.27, 127.56, 127.31, 122.65, 118.68, 118.31, 118.11, 117.71, 111.56, 81.01, 59.52, 28.42. Anal. calc. for $C_{32}H_{31}N_5O_4$: C, 69.93; H, 5.69; N, 12.74. Found: C, 69.95; H, 5.67; N, 12.75.

tert-Butyl-(*S*)-1-(3-((*E*)-2-hydroxy-5-((*E*)-phenyldiazenyl)benzylideneamino)phenylamino)-1-oxo-3-phenylpropan-2-ylcarbamate (4b)

Orange solid, yield: 85%, mp: 191-193 °C, IR (KBr, cm^{-1}): 3327, 3212, 3056, 2984, 2926, 2853, 1690, 1673, 1640, 1599, 1519, 1164. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 13.69 (s, 1H, OH), 8.95 (s, 1H, N=CH), 8.52 (s, 1H, Ar-H), 7.98 (d, *J*=8.7 Hz, 1H, Ar-H), 7.89 (d, *J*=7.0 Hz, 3H, Ar-H), 7.54 (s, 1H, Ar-H), 7.52 (t, *J*=7.2 Hz, 2H, Ar-H), 7.45 (d, *J*=7.3 Hz, 1H, Ar-H) 7.27 (app d, *J*=3.7 Hz, 4H, Ar-H), 7.18 (s, 2H, Ar-H), 7.08 (d, *J*=8.7 Hz, 1H, Ar-H), 6.91 (s, 1H, Ar-H), 5.62 (d, *J*=6.7 Hz, 1H, CH), 4.74 (bs, 1H, NH), 3.23 (dd, *J*=13.3 Hz, *J*=6.1 Hz, 1H, CH_{2a}), 3.13 (dd, *J*=13.4 Hz, *J*=8.1 Hz, 1H, CH_{2b}), 1.42 (s, 9H, 3xCH_3). ^{13}C-NMR (100MHz, $CDCl_3$) δ (ppm): 170.61, 164.03, 163.12, 162.18, 156.39, 152.53, 151.62, 147.92, 145.40, 138.75, 136.51, 130.50, 129.68, 129.24, 129.05, 128.74, 128.07, 127.52, 127.03, 122.64, 121.72, 118.66, 118.45, 118.05, 117.84, 111.91, 80.73, 56.86, 38.66, 28.35. Anal. calc. for $C_{33}H_{33}N_5O_4$: C, 70.32; H, 5.90; N, 12.43. Found: C, 70.34; H, 5.91; N, 12.41.

tert-Butyl-(*S*)-1-(3-((*E*)-2-hydroxy-5-((*E*)-phenyldiazenyl)benzylideneamino)phenyl amino)-3-methyl-1-oxobutan-2-ylcarbamate (4c)

Orange solid, yield: 61%, mp: 172-174 °C, IR (KBr, cm^{-1}): 3474, 3263, 3059, 3025, 2984, 1695, 1628, 1544, 1164. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 13.72 (s, 1H, OH), 9.24 (s, 1H, N=CH), 8.54 (s, 1H, Ar-H), 7.92 (t, *J*=7.9 Hz, 2H, Ar-H), 7.87 (d, *J*=7.5 Hz, 2H, Ar-H), 7.59 (s, 1H, Ar-H), 7.49 (t, *J*=7.0 Hz, 2H, Ar-H), 7.44 (d, *J*=7.0 Hz, 1H, Ar-H), 7.28 (d, *J*=7.6 Hz, 1H, Ar-H), 7.16 (t, *J*=6.8 Hz, 1H, Ar-H), 7.05 (d, *J*=8.6 Hz, 1H, Ar-H), 6.87 (d, *J*=6.8 Hz, 1H, NH), 5.59 (d, *J*=8.5 Hz, 1H, CH), 4.26 (app t, *J*=7.6 Hz, 1H, NH), 2.19 (d, *J*=6.8 Hz, 1H, $CH(CH_3)_2$), 1.47 (s, 9H, 3xCH_3), 1.10 (d, *J*=6.2 Hz, 6H, 2xCH_3). ^{13}C-NMR (100MHz, $CDCl_3$) δ (ppm): 171.26, 164.09, 162.00, 156.85, 152.53, 147.69, 145.35, 138.96, 130.44, 129.58, 129.02, 128.15, 127.36, 122.63, 118.66, 118.33, 118.05, 117.66, 111.63, 80.48, 61.20, 30.95, 28.41, 19.41, 18.70. Anal. calc. for $C_{29}H_{33}N_5O_4$: C, 67.55; H, 6.45; N, 13.58. Found: C, 67.54; H, 6.43; N, 13.52.

tert-Butyl-(*S*)-1-(3-((*E*)-2-hydroxy-5-((*E*)-phenyldiazenyl)benzylideneamino)phenyl amino)-4-(methylthio)-1-oxobutan-2-ylcarbamate (4e)

Orange solid, yield: 64%, mp: 182-184 °C, IR (KBr, cm^{-1}): 3474, 3272, 3059, 3024, 2936, 1694, 1673, 1544, 1184. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 13.69 (s, 1H, OH), 9.21 (s, 1H, N=CH), 8.58 (s, 1H, Ar-H), 7.96 (t, *J*=9.0 Hz, 2H, Ar-H), 7.87 (d, *J*=7.4 Hz, 2H, Ar-H), 7.60 (s, 1H, Ar-H), 7.49 (t, *J*=7.4 Hz, 2H, Ar-H), 7.44 (t, *J*=7.2 Hz, 1H, Ar-H), 7.32 (d, *J*=7.7 Hz, 1H, Ar-H), 7.23 (t, *J*=7.6 Hz, 1H, Ar-H), 7.06 (d, *J*=8.8 Hz, 1H, Ar-H), 6.93 (d, *J*=7.2 Hz, 1H, NH), 5.68 (d, *J*=7.7 Hz, 1H, CH), 4.59 (d, *J*=7.0 Hz, 1H, NH), 2.68 (t, *J*=7.2 Hz, 2H, S-CH_2), 2.25-2.17 (m, 1H, CH-CH_{2b}), 2.12 (s, 3H, S-CH_3), 2.09-2.02 (m, 1H, CH-CH_{2a}), 1.46 (s, 9H, 3xCH_3). ^{13}C-NMR (100MHz, $CDCl_3$) δ (ppm): 170.81, 164.03, 162.24, 156.56, 152.51, 147.98, 145.40, 138.89, 130.49, 129.74, 129.04, 128.12, 127.45, 122.62, 118.67, 118.41, 118.07, 117.71, 111.99, 80.80, 54.52, 31.57, 30.38, 28.39, 15.41. Anal. calc. for $C_{29}H_{33}N_5O_4S$: C, 63.60; H, 6.07; N, 12.79. Found: C, 63.62; H, 6.04; N, 12.77.

Biological Activity

The biological activities of the target compounds were carried out to ascertain the minimum inhibitory concentrations (MIC μg/ml) of derivatives against the gram negative bacteria E.coli (ATCC 12228), P. aureginosa (ATCC 27853) and S. typhimurium (CCM 5445), gram positive bacteria B.

Cereus (ATCC 6633), and S. aureus (ATCC 6538-P); fungi S. cerevisiae (ATCC 9763) and C. Albicans (ATCC 10239). All strains were stored as frozen stocks containing 30% glycerol at -2 °C. Stock strains were cultured on TSA (Tryptic Soy Agar) plates prior to experiment. For the determination of MIC values, double strength MHB (Mueller Hington Broth) medium was inoculated and left to incubate at 37 °C overnight. The experiment was carried out in 96 microplate wells according to the method reported as standard (Clinical and Laboratory Standards Institute, 2011). All products (**3a**-**e**, **4a**-**c**, **4e**) whose antimicromial activity was determined on the strains in the MIC test were prepared in 10% DMSO solution.

The stock concentration of all antimicrobial compounds was set at 100 µg/ml. Fresh cultures of standard strains were prepared to have 0.5 McFarland turbidity. 50 µl of bacterial suspensions were transferred to each well. Then, each antimicrobial compound solution to be tested was added to the wells in a volume of 50 µl. Positive and negative control groups were formed into microplate wells 11 and 12, respectively. Only the relevant test microorganism was transferred to the positive controls and only the relevant antimicrobial compound to the negative controls. The prepared microplates were left to incubation at 37 °C for 24 hours. After the incubation, the activities of the antimicrobial compounds in the wells were evaluated according to the lack of turbidity, which indicates the inhibition of test bacteria, and the formation of red color when 1% TTC solution was dropped. The concentration of the compound in the end wells with no turbidity and red color formation observed was accepted as the MIC values for the microorganism concerned. Antimicrobial activities of the all compounds tested were determined by the MIC (Minimum inhibitory concentration test) and 50 µg/ml was realized as 0.19 µg/ml.

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REFERENCES

1. W. A. Zoubi; A. A. S. Al-Hamdani; , S. D. Ahmed; Y. G. Ko; *Appl. Organometal. Chem.*, **2018**, *32*, 3895-3910
2. S. Slassi; A. El-Ghayoury; M. Aarjane; K. Yamni; A. Amine; *Appl. Organometal. Chem.*, **2020**, *34*, 5503-5512
3. M. S. Kasare; P. P. Dhavan; B. L. Jadhav; S. D. Pawar; *Chemistry Select,* **2019**, *4*, 10792–10797

4. M. Gulcan; S. Özdemir; A. Dündar; E. İspir; M. Z. Kurtoğlu; *Anorg. Allg. Chem.*. **2014**, *640*, 1754-1762
5. A. Kakanejadifarda; V. Khojasteha; A. Zabardastia; F. Azarbani; *Org. Chem. Res.*, **2018**, *4*, 210-226
6. H. M. Hessoon; F. F. Karam; *Egypt. J. Chem.*, **2022**, *65*, 327-334
7. K. J. Al-Adilee; S. R. Hasan; *IOP Conf. Ser.: Earth Environ. Sci.*, **2021**, *790*, 12031-12051
8. P. A. Channar; A. Saeed; D. Shahzad; F. A. Larik; M. Hassan; H. Raza; Q. Abbas; S. Seo; *Chem. Biol. Drug Des.*, **2018**, *92*, 1692-1698
9. H. Kaur; J. Singh; B. Narasimhan; *BMC Chemistry*, **2019**, *13*, 1-19
10. E. İspir; *Dyes and Pigment*, **2009**, *82*, 13-19
11. M. S. Refat; I. M. El-Deen; Z. M. Anwer; S. El-Ghol; *J. Coord. Chem.*, **2009**, *10*, 1709-1718
12. M. S. Refat; I. M. El-Deen; R. R. Amin; S. El-Ghol; *Toxicol. Environ. Chem.*, **2010**, *92*, 1093-1110
13. I. N. Witwit; Z. Y. Motaweq; H. M. Mubark; *J. Pharm. Sci. & Res.*, **2018**, *10*, 3074-3083
14. S. Slassi; A. Fix-Tailler; G. Larcher; A. Amine; A. El-Ghayoury; *Heteroat. Chem.*, **2019**, *2019*, 1-8
15. E. Isik; D. Astley; S. Yuksekdanaci; I. Yasa; *Lett. Drug Des. Discov.*, **2020**, *17*, 1372-1379.