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New Thiourea-, and Azomethine Derivatives of 4-Amino-5-[Hydroxy (Diphenyl)Methyl]-2,4-Dihydro-3*h*-1,2,4-Triazol-3-Thion with Potential Antimicrobial Activity

4-Amino-5-[Hidroksi(Difenil)Metil]-2,4-Dihidro-3*h*-1,2,4-Triazol-3-Tiyon Yapısından Elde Edilen Bazı Yeni Tiyoüre ve Azometin Türevleri ve Antimikrobiyel Aktivitelerinin İncelenmesi

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Abstract

New 5-[hydroxy(diphenyl)methyl]-4-{[(substituted phenyl)methylidene]amino}-2,4-dihydro-3*H*-1,2,4-triazol-3-thione, N-(substituted phenyl)-N'-{3-[hydroxy(diphenyl)methyl]-5-thioxo -1,5-dihydro-4H-1,2,4-triazol-4-yl}thiourea and [1,2,4]triazolo[3,4-b][1,3,4] thiadiazole derivatives were synthesized from 4-amino-5-[hydroxy(diphenyl)methyl] -2,4-dihydro-3*H*-1,2,4-triazol-5-thione. The structures of the new compounds were determined on the basis of analytical (C,H,N) and spectroscopic data (IR, ^{1}H-NMR , MS). All the compounds were evaluated for antimicrobial activity against *Escherichia coli* ATCC 8739, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 1539, *Candida albicans* ATCC 10231, *Staphylococcus aureus* ATCC 6538 and *Staphylococcus epidermis* ATCC 12228. Some of the compounds were found active against *S.aureus* and *S.epidermidis* (MİC 31.2-7.8mcg /ml).

Key words: 1,2,4-Triazole-3-thione, thiourea, [1,2,4]triazolo[3,4-b][1,3,4]thiadiazole, Antimicrobial activity.

Introduction

The prevalance of resistance and cross resistance against existing chemoterapeutic agents makes antimicrobial research increasingly attractive and stimulates the search for new compounds.

The 1,2,4-triazole nucleus and fused heterocyclic systems derived from it have been reported to demonstrate a wide spectrum of activities (Reid *et al.*,1976 ; Karow,1981 ;Hassan *et al.*, 1983,Eweiss *et al.*, 1986; Chaturvedi *et al.*, 1988; Habib *et al.*, 1997; Gülerman *et al.*, 1997; Zitouni *et al.*, 1999). Besides 1,2,4-triazoles, thiourea derivatives have been reported to exhibit antimicrobial activity (Rollas *et al.*, 1991 ; Küçükgüzel *et al.*, 2001)

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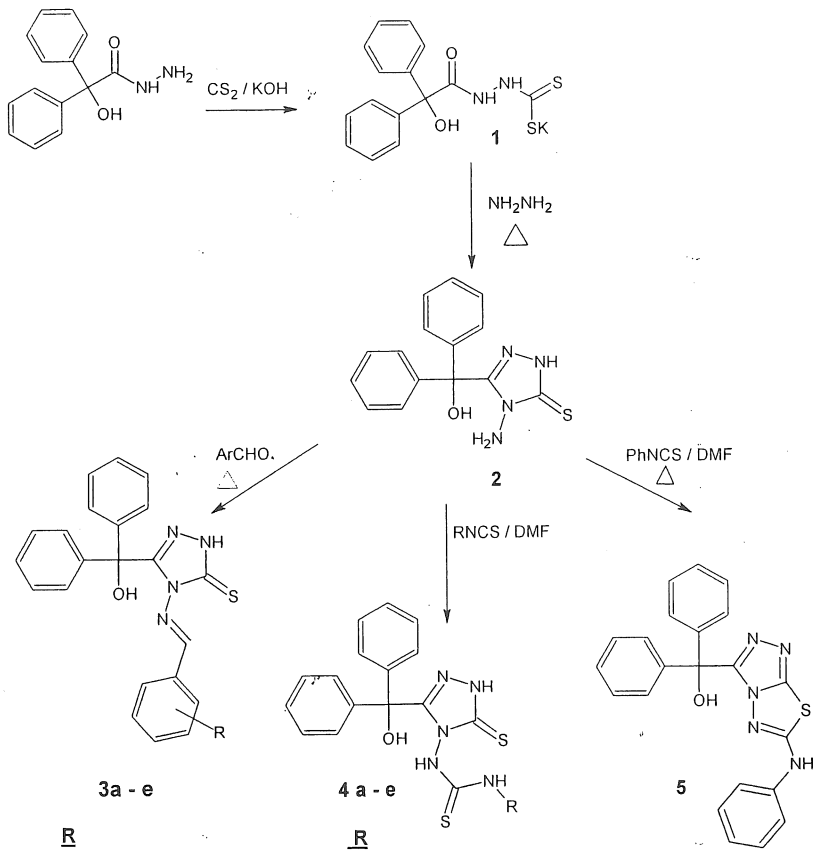
Prompted by these reports and in continuation of our previous work on the synthesis of heterocycles of pharmaceutical interest , we selected 4-amino-5-[hydroxy(diphenyl)methyl]-2,4-dihydro-3H-1,2,4-triazol-3-thione **2** , a versatile substrate for mono and difunctional electrophiles by virtue of its vicinal amino and thione groups as the key intermediate and prepared new derivatives which may exhibit antimicrobial activity.

Thus treatment of benzilic acid hydrazide in ethanolic potassium hydroxide with carbon disulfide afforded the potassium salt of 2-[hydroxy(diphenyl)acethyl]hydrazincarbodithioic acid **1** almost quantitatively (Ergenç *et al.*, 1996). **1** was reacted with hydrazine hydrate according to *Reid and Heindel* to obtain **2** , which was in turn condensed with aromatic aldehydes (Misra *et al.*, 1988) to give the azomethine derivatives **3a – e** .

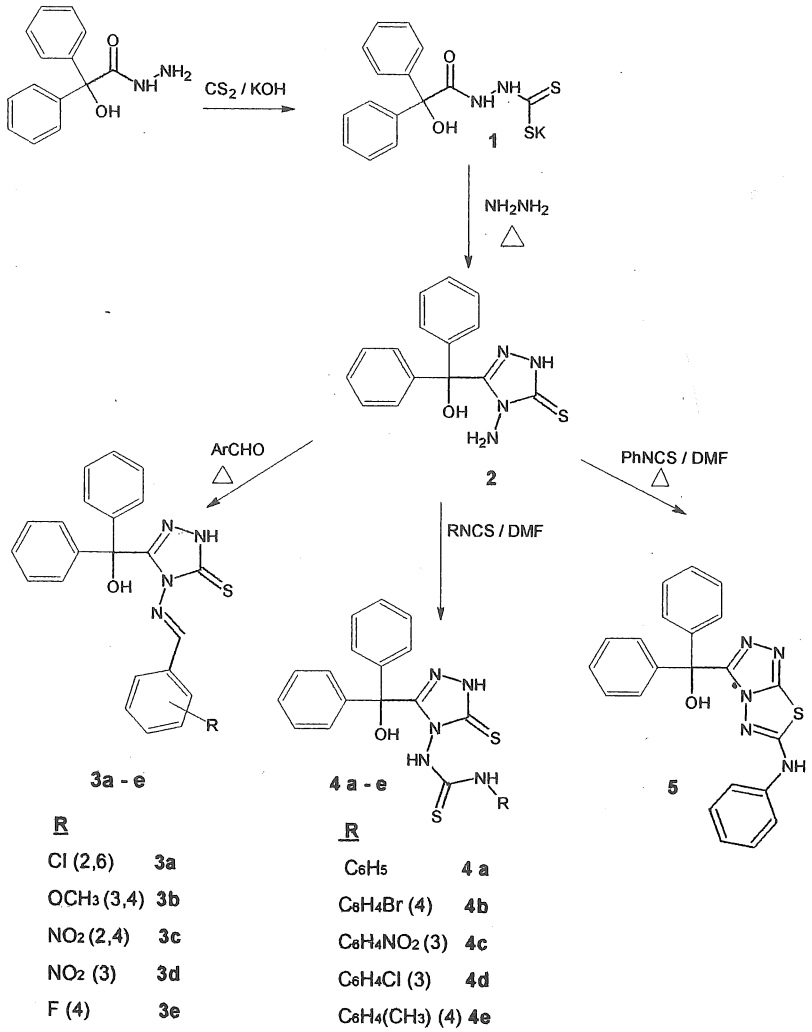
Reaction of **2** in dry dimethylformamide with appropriate isothiocyanates at room temperature (Chaturvedi *et al.*, 1988) yielded new thiourea derivatives **4a – e**. The same reaction was also carried out at an elevated temperature to achieve **5** , a new triazolo[3,4-b][1,3,4]thiadiazole derivative, according to a literature method (Misra *et al.*, 1988) .

The synthetic routes to **1-5** are outlined in in scheme 1. The structures of the synthesized compounds were confirmed by elemental analyses (C H N) and spectral data.(IR, ^{1}H-NMR , EIMS / CIMS). The molecular formulas , melting points , yields and analytical data of **1-5** are presented in the *table*.



R		R	
Cl (2,6)	**3a**	C_6H_5	**4 a**
OCH_3 (3,4)	**3b**	C_6H_4Br (4)	**4b**
NO_2 (2,4)	**3c**	$C_6H_4NO_2$ (3)	**4c**
NO_2 (3)	**3d**	C_6H_4Cl (3)	**4d**
F (4)	**3e**	$C_6H_4(CH_3)$ (4)	**4e**

Scheme 1



Scheme 1

Table

Compd.	Formula (M.W.)	Mp. (^{0}C)	Yield (%)	Analysis (cald. / found) C	H	N
1	$C_{15}H_{13}KN_2O_2S_2$ (356.50)	245 (d)	98.8	50.54 / 50.60	3.68 / 3.77	7.86 / 7.78
2	$C_{15}H_{14}N_4OS$ (298.37)	220	76.5	60.38 / 59.90	4.73 / 4.70	18.78 / 18.60
3a	$C_{22}H_{16}Cl_2N_4OS$ (455.36)	230	80	58.03 / 57.80	3.54 / 3.32	12.30 / 12.73
3b	$C_{24}H_{22}N_4O_3S$ (446.53)	235	71	64.56 / 64.01	4.97 / 5.20	12.55 /12.51
3c	$C_{22}H_{16}N_6O_5S$ (476.46)	240	64	55.46 / 56.17	3.38 / 3.63	17.64 / 17,56
3d	$C_{22}H_{17}N_5O_3S$ (431.47)	207	68	61.24 / 61.73	3.97 / 4.30	16.23 / 16.78

3e	$C_{22}H_{17}FN_4OS$ (404.47)	217	70	65.33 / 65.40	4.24 / 4.23	13.85 / 13.62
4a	$C_{22}H_{19}N_5OS_2$ (433.55)	187	47	60.95 / 60.56	4.42 / 4.44	16.15 / 16.78
4b	$C_{22}H_{18}BrN_5OS_2$ (512.45)	185	42	51.56 / 51.47	3.54 / 3.72	13.67 / 13.70
4c	$C_{22}H_{18}N_6O_3S_2$ (478.54)	205	48	55.22 / 55.02	3.79 / 3.89	17.56 / 17.45
4d	$C_{22}H_{18}ClN_5OS_2$ (467.97)	205	37	56.46 / 56.99	3.88 / 4.10	14.96 / 15.08
4e	$C_{23}H_{21}N_5OS_2$ (447.57)	220	35	61.72 / 60.81	4.72 / 4.59	15.64 / 15.49
5	$C_{22}H_{17}N_5OS$ (401.85)	192	12	65.81/ 65.92	4.77 / 4.78	17.44 / 17.68

Materials and Methods

Melting points were determined with a Buchi 530 melting point apparatus and are uncorrected.The spectra were recorded on PerkinElmer 1600 FT (IR) , Bruker AC 200 (200 MHz ^{1}H-NMR) and VG Zab Spec (70 eV /EIMS) instruments ,respectively.CIMS (CH_4) were provided by Sittingbourne Research Centre (UK). Elemental analyses were performed on a Carlo Erba 1106 elemental analyzer.

[Hydroxy(diphenyl)acetyl]hydrazinecarbodithioic acid potassium salt **1**

0.025 mol. Benzilic acid hydrazide was dissolved in 40 ml anhydrous ethanol containing 2.25 g KOH with constant stirring.To this solution 5 ml CS_2 were added.The yellow colored reaction mixture thus obtained was further agitated for 1h to effect complete solidification . After suction filtration the crude product was washed with dry ether and dried to afford **1** as a water soluble fine yellow powder.

IR (KBr) υ cm^{-1}:2920 (Ar C-H), 1660 (C=O), 1480,1420 (C=C), 1028 (C=S). *EIMS (m/z) (70 eV)*: 357(MH$^+$) (2), 224 (19) , 195 (5), 193 (3) ,165 (10), 183 (55) , 131 (3) 105 (100), 77 (67), 51 (28),44 (15).

4-Amino-5-[hydroxy(diphenyl)methyl]-2,4-dihydro-3H-1,2,4-triazol-3-thione **2**

To 7.12 g of **1** were added 4 ml water and 2 ml hydrazine hydrate and the reaction mixture thus obtained was refluxed until a white precipitate was obtained . The crude product was diluted with water,filtered and recrystallized from ethanol to yield **2** as fine colorless needles.

IR (KBr) υ cm^{-1} : 3440 (OH), 3300,3150 (NH), 3025 (Ar.C-H) , 1615 ,1500, 1440 (C=N, C=C) *^{1}H NMR (DMSO – d_6) δ ppm* : 5.14 (NH_2, 2H,s) 6.63 (OH,1H,s) 7.22-7.55 (Ph, 10H,m), 13.62 (NH,1H,s). *CIMS (CH_4) (m/z)* : 299 (MH$^+$) (35) , 281 (60), 211 (5),183 (100), 157 (2), 145 (4).

General procedure for the synthesis of **3a - e**

To equimolar amounts of **2** and an appropriate aldehyde in EtOH (10 ml) , few drops of concentrated H_2SO_4 were added and the reaction mixture was heated under reflux on a water both for 1 h . The crude product was precipitated by the addition of water and purified by washing with hot ethanol.

5-[hydroxy(diphenyl)methyl]-4-{[(2,6-dichlorophenyl)methylidene]amino}-2,4-dihydro-3H-1,2,4-triazol-3-thione **3 a**

IR (KBr) υ cm^{-1} : 3420 (OH), 3200 (NH), 3070, 3020 (Ar. C-H), 1600, 1570, 1475 (C=N, C=C),1035 (C=S). *^{1}H NMR (DMSO – d_6) δ ppm* : 6.57 (OH, 1H,s), 7.24-7.54 (Ph,13H,m), 10.36 (CH,1H,s), 14.18 (NH,1H,s). *EIMS (m/z) (70 eV)*: 455 (M$^+$) (0.4), 283 (25.3), 206 (80.72), 205 (18.57), 183 (7.24), 182 (31.8), 173 (14.92),105 (100), 101 (35.68), 77 (56.94).

5-[hydroxy(diphenyl)methyl]-4{[(3,4-dimethoxyhenyl)methylidene]amino}-2,4-dihydro-3H-1,2,4-triazol-3-thione **3 b**

IR (KBr) υ cm^{-1} : 3400 (OH), 3260 (NH),3060, 3020 (Ar. C-H), 1600, 1580, 1475 (C=N, C=C), 1050 (C=S). *^{1}H NMR (DMSO – d_6) δ ppm* : 6.74 (OH, 1H,s) 6.92-7.33 (Ph,13H,m), 10.43 (CH,1H,s), 13.95 (NH,1H,s). *EIMS (m/z) (70 eV)*: 283 (3.7), 206 (16.63),183 (6.82), 182 (41.04), 163 (78.15), 105 (100).

5-[hydroxy(diphenyl)methyl]-4-{[(2,4-dinitrophenyl)methylidene]amino}-2,4-dihydro -3H-1,2,4-triazol-3-thione **3c**

IR(KBr) υ cm^{-1} : 3460 (OH), 3260 (NH),3080, 3020 (Ar. C-H), 1610, 1560, 1480 (C=N,C=C), 1050 (C=S). *^{1}H NMR (DMSO – d_6) δ ppm :* 6.93 (OH, 1H,s),7.22-7.45 (Ph,13H,m) , 10.84 (CH,1H,s),14.19 (NH,1H,s). *EIMS (m/z) (70 eV):* 475 (3.64), 283 (5.85), 206 (16.63),193 (9.25), 182 (32.21), 165 (10.55) ,105 (100),101(21.36), 77 (58.29).

5-[hydroxy(diphenyl)methyl]-4-{[(3-nitrophenyl)methylidene]amino}-2,4-dihydro-3H-1,2,4-triazol-3-thione **3 d**

IR (KBr) υ cm^{-1} : 3430 (OH), 3320 (NH),3080, 3020 (Ar.C-H), 1610, 1560, 1520, 1470 (C=N,C=C), 1048 (C=S). *^{1}H NMR (DMSO – d_6) δ ppm* : 6.93 (OH, 1H,s),7.23-7.73 (Ph,14H,m) , 9.75 (CH,1H,s), 14.11 (NH,1H,s). *EIMS (m/z) (70 eV)*: 281 (14.32), 207(100), 182 (15.2), 105 (7.9) , 77(12.02).

5-[hydroxy(diphenyl)methyl]-4-{[(4-fluorophenyl)methylidene]amino}-2,4-dihydro-3H-1,2,4-triazol-3-thione **3 e**

IR (KBr) υ cm^{-1} : 3420 (OH), 3320 (NH),3080, 3020 (Ar.C-H), 1600, 1580, 1490 (C=N, C=C),1040 (C=S). ^{1}H NMR (DMSO – d_6) δ ppm : 6.80 (OH, 1H, s), 7.22-7.57 (Ph,14H,m) 9.32 (CH,1H,s), 14.02 (NH,1H,s). CIMS (CH$_4$) (m/z) : 405 (MH$^+$) (6.6), 387 (10), 266 (50), 183 (20), 122 (100), 105 (6.5) .

General procedure for the synthesis of **4a - e**

Equimolar amounts of **2** and an appropriate isothiocyanate in dry dimethylformamide were stirred for 1 h at room temperature. The resulting reaction mixture was poured into ice- water , filtered and recrystallized from ethanol / water.

N-Phenyl-N'-{3-[hydroxy(diphenyl)methyl]-5-thioxo-1,5-dihydro-4H-1,2,4-triazol-4-yl}-thiourea **4 a**

IR (KBr) υ cm^{-1} : 3425 (OH), 3290 (NH), 3155 (Ar.CH),1630,1593,1500 (C=N,C=C),1265 (C=S). *^{1}H NMR (DMSO – d_6) δ ppm* : 6.75 (OH,1H,s),7.1-7.3 (Ph,15H,m) , 9.84 (NH,2H,s), 13.65 (triazole NH,1H,s). *EIMS (m/z) (70 eV)*: 433 (M$^+$) (4), 385 (23),357 (18),341 (16), 311 (24),283 (15), 298 (76), 279 (87),183 (22),165 (37),135 (10),128 (14),152 (40), 118 (57), *105* (95) ,93 (50), 77 (100), 65 (22) , 61 (17), 60 (21), 59 (12).

N-(4-Bromophenyl)-N'-{3-[hydroxy(diphenyl)methyl]-5-thioxo-1,5-dihydro-4H-1,2,4-triazol-4-yl}-thiourea **4 b**

IR (KBr) υ cm^{-1}: 3430 (OH), 3300 (NH), 3120 (Ar.CH), 1610,1590,1500 (C=N,C=C), 1260 (C=S). *^{1}H NMR (DMSO – d_6) δ ppm*: 6.75 (OH,1H,s), 7.25-7.40 (Ph,14 H,m), 9.85 (NH,2H,s), 13.40 (triazole NH,1H,s).

N-(4-Nitrophenyl)-N'-{3-[hydroxy(diphenyl)methyl]-5-thioxo-1,5-dihydro-4H-1,2,4-triazol-4-yl}-thiourea **4 c**

IR (KBr) υ cm^{-1} : 3430 (OH), 3315 (NH), 3140 (Ar.CH), 1625,1580,1520 (C=N,C=C) ,1240 (C=S). *^{1}H NMR (DMSO – d_6) δ ppm*: 6.76 (OH,1H,s), 7.22-7.38 (Ph,14H,m) ,10.42 (NH,2H,s), 13.70 (triazole NH,1H,s).

N-(3-Chlorophenyl)-N'-{3-[hydroxy(diphenyl)methyl]-5-thioxo-1,5-dihydro-4H-1,2,4-triazol-4-yl}-thiourea **4 d**

IR (KBr) υ cm^{-1} : 3430 (OH), 3290 (NH), 3120 (Ar.CH) ,1615,1580,1520 (C=N,C=C), 1240 (C=S). *^{1}H NMR (DMSO – d_6) δ ppm* : 6.80 (OH,1H,s) , 7.20-7.38 (Ph,14H,m,) 10.02 (NH,2H,s), 13.30 (triazole NH,1H,s).

N-(4-Methylphenyl)-N'-{3-[hydroxy(diphenyl)methyl]-5-thioxo-1,5-dihydro-4H-1,2,4-triazol-4-yl}-thiourea **4 e**

IR (KBr) υ cm^{-1} : 3450 (OH), 3300 (NH), 3145 (Ar.CH), 1620,1590,1540 (C=N,C=C), 1240 (C=S). ^1H *NMR (DMSO – d_6)* δ *ppm* : 2.6 (CH_3,3H,s), 6.90 (OH,1H,s), 7.25-7.36 (Ph,14H,m), 10.03 (NH,2H,s), 13.08 (triazole NH,1H,s).

Synthesis of (6-Anilino-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)(diphenyl)methanol **5**

Equimolar amounts of **2** and an phenylisothiocyanate in dry dimethylformamide (20 ml) were refluxed for 24 h. The colorless crystalline product obtained after cooling was filtered and washed with ethanol to afford **5.**

IR (KBr)υ cm^{-1} : 3450 (OH), 3300 (NH) , 3100 , 1615,1585,1570 (C=N,C=C).

^1H *NMR(DMSO – d_6)* δ *ppm* : 6.63 (OH,1H,s), 7.41-7.54 (Ph,15H,m) , 9.94 (NH, 1H,s).

Results and Discussion

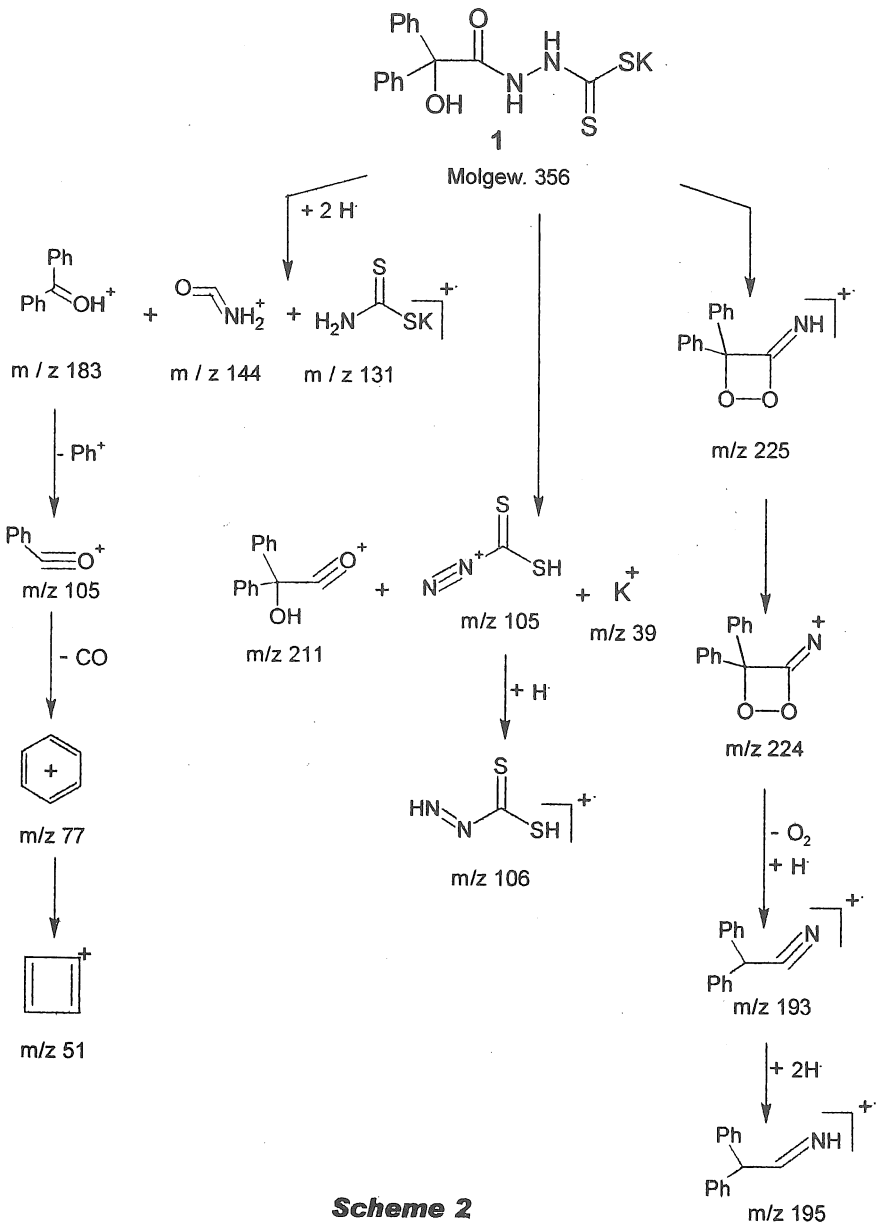
Absence of the C=O band of **1** at 1660 cm^{-1} and presence of an absorption at 1615 cm^{-1} attributed to the C=N function of the triazolinethione system in the IR spectrum and observation of a singlet at δ 5.14 ppm (NH_2, 2H) in the NMR spectrum of **2** provided evidence for the expected transformation. The IR Spectra af **3 a - e** showed characteristic bands at 3400 – 3460 cm^{-1}(OH) ,3320-3200 cm^{-1}(NH), 1610-1470 cm^1 (C=N; endocyclic / exocyclic and C=C), and 1035–1050cm^{-1} (C=S) (Misra at al.,1988 and Eweiss *et al.*, 1986). In the literature it has been stated that the sulphur at 3-pıosition of the 1,2,4-triazole ring is said to be incorporated as a thiole (Habib *et al.*, 1988) or thione (Eweiss *et al.*, 1986 ; İlhan *et al.*, 1996) function.The observation of a low field NH signal (δ = 13.95 – 14.19 ppm) and a low field N=CH signal (δ = 9.32 –10.84 ppm) showed that **3 a– e** existed only in the thione form.The paramagnetic shift observed for the N=CH proton, which generally absorbs at about δ 8.50 ppm (Hesse *et al.*, 1979), was attributed to the anisotropy of the thiocarbonyl group (İlhan et.al., 1996).

Absence of SH signals and presence of low field singlets at about δ 13.08-13.70 ppm assigned to the NH of the triazoline thione ring supported the thione form also in **4 a - e** .

The IR spectrum of **5** showed OH- and NH-bands at 3451 and 3300 cm^{-1}, respectively. The structure was further confirmed by the OH singlet at δ 6.63 ppm , the multiplet at δ 7.41- 7.54 ppm assigned to the aromatic protons and the characteristic broad NH peak at δ 9.94 ppm observed in the NMR spectrum.

The mass fragmentation routes of all the compounds were in accordance with the literature. (El Dawy *et al.*, 1983 ; Ergenç *et al.*, 1996).

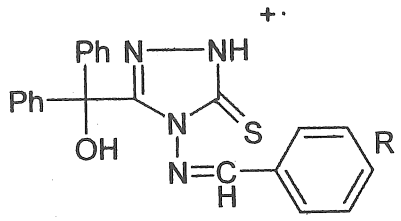
Compound **1** did not display a molecular ion under EI whereas the quasimolecular ion (MH^+ ,m/z 299,1.3 %) was observed in the CIMS (*scheme 2*)



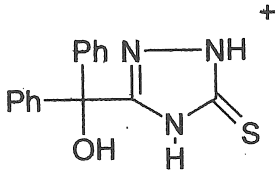
Scheme 2

3 a-e did not provide stable M^+ peaks under electron impact and showed only low intensity quasi molecular ions (MH^+) in the chemical ionization mass spectra. The major fragmentation route observed was the breaking of the N-N bond at 4-position of the 1,2,4-triazole ring (Ergenç *et al.*, 1996). The proposed mass fragmentation patterns of **3** and **4a** selected as examples, are presented in *schemes 3* and *4* respectively.

The in vitro antimicrobial activity of **3 a - e** and **4 a - e** was investigated against *Escherichia coli* ATCC 8739, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 1539, *Candida albicans* ATCC 10231, *Staphylococcus aureus* ATCC 6538 und *Staphylococcus epidermis* ATCC 12228 using the Müller-Hinton medium The following compounds inhibited the growth of *S.aureus* ATCC 6538 and / or *S.epidermis* ATCC 12228 at the cited concentrations (mcg / ml) . **3c** : 31.2 ; **3d** : 31.2 ; **4a** : 78.0 ; **4b** : 31.2 (*S.aureus* ATCC 6538) and **3c**:7.8 ; **3d** : 15.6 (*S.epidermis* ATCC 12228).

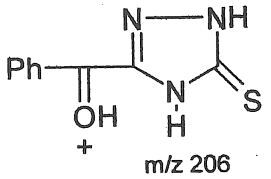


3 a-e



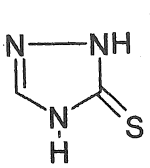
m/z 283

- Ph+ m/z 77



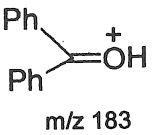
m/z 206

+H·



m/z 101

+



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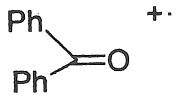
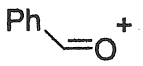


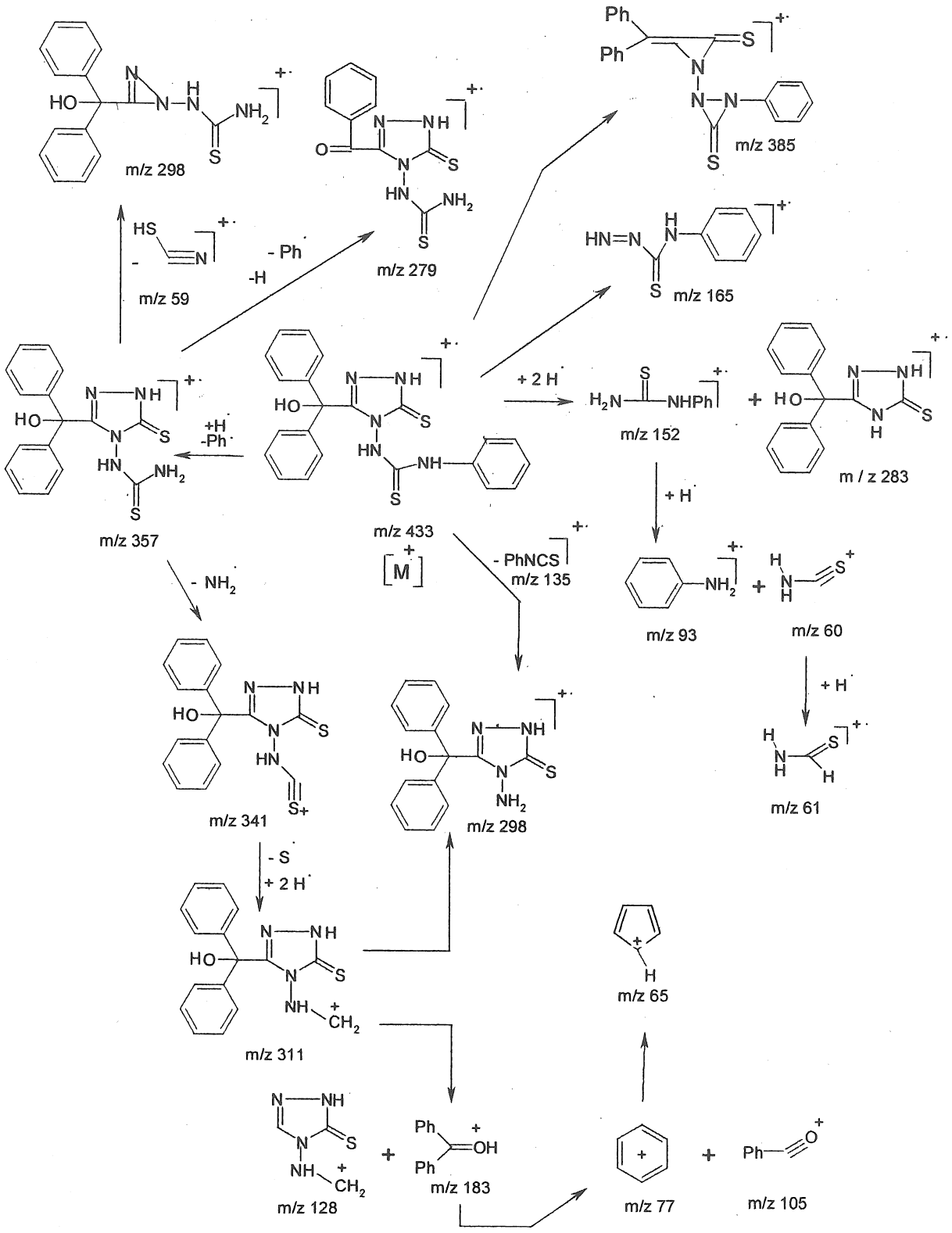
m/z 182

- Ph+ m/z 77



m/z 105

Scheme 3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m/z 165
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m / z 283
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m/z 61
m/z 135
m/z 341
m/z 298
m/z 311
m/z 65
m/z 128
m/z 183
m/z 77
m/z 105

Scheme 4

Özet

4-Amino-5-[hidroksi(difenil)metil]-2,4-dihidro-3H-1,2,4-triazol-5-tiyon yapısından hareketle 5-[hidroksi (difenil)metil]-4-{[(substtitüe fenil)metiliden]amino}-2,4-dihidro-3H-1,2,4-triazol -3-tiyon , N-(substitüe fenil)-N'-{3-[hidroksi(difenil)metil]-5-tiyokso-1,5-dihidro-4H-1,2,4-triazol-4-il}-tiyoüre ve [1,2,4]triazolo[3,4-b] [1,3,4]tiyadiazol yapısında maddeler sentezlenerek yapıları elementel analiz (C, H, N) ve spektral verilerin (IR, ^{1}H-NMR , MS) yardımıyla aydınlatılmış ve *Escherichia coli ATCC 8739, Klebsiella pneumoniae ATCC 4352,Pseudomonas aeruginosa ATCC 1539, Candida albicans ATCC 10231,Staphylococcus aureus ATCC 6538* ve *Staphylococcus epidermis ATCC 12228* suşlarına karşı antimikrobiyal etkileri incelenmiş ve bazı bileşiklerde *S. aureus* ve *S. epidermis'e* karşı aktivite saptanmıştır (MIK: 31.2 –78 mcg. / ml) .

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