

SYNTHESIS AND INVESTIGATION OF CERTAIN 3(5)-SUBSTITUTED-1,2,4-TRIAZOLE-5(3)-CARBOXYLIC ACID DERIVATIVES

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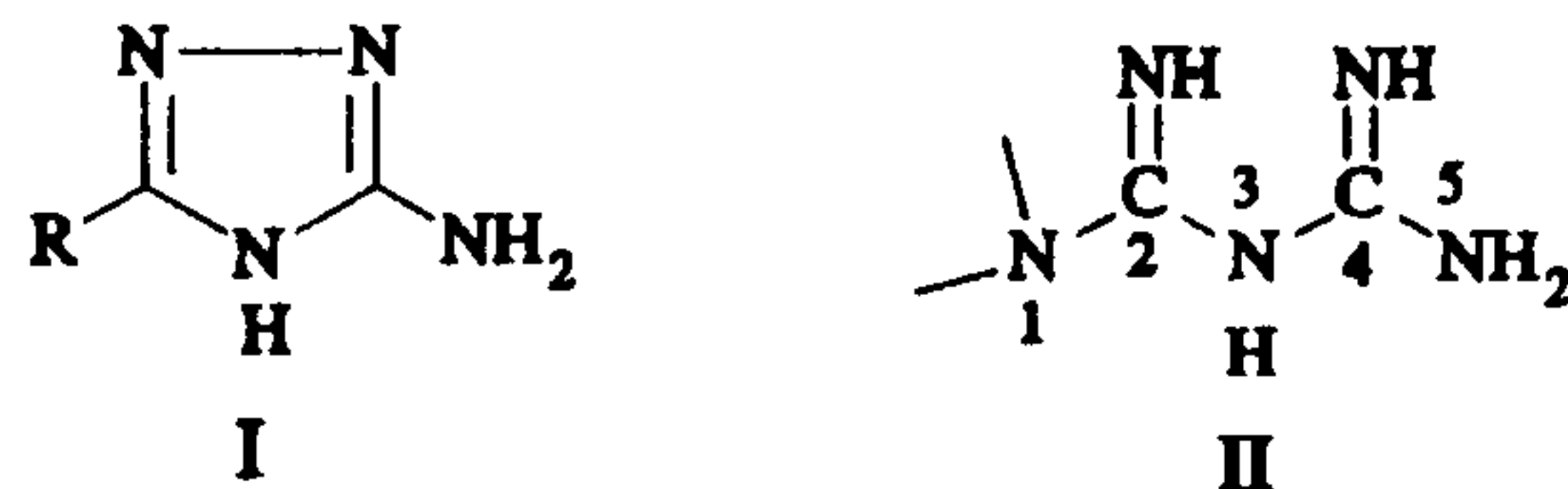
تم تحضير أميدات - هيدروكساميدات - هيدرازيدات - امينو سيميكربازيدات - سيميكاربازيدات وثيوسيميكاربازيدات ٣(٥)-محتلات او ٤(٥)-ترايازول-٥(٣)-حامض كربوكسيليك. ولقد تم اختبار معظم المركبات المحضرة كمضادات للبكتريا بنوعيتها، ولم تظهر لها أى فاعلية حتى تركيز ٢,٥ مجم/مل. ولقد تم اختيار مجموعات الامينوسيميكربازيدات (8h, 9h, 10h, 11h) والسيميكربازيدات (8i, 9i, 10i, 11i) والثيوسيميكربازيدات (8j, 9j, 10j) لدراسة تأثيرها على اختبار احتمال الجلوكوز عن طريق الفم كأختبار أولى لقياس هذه الفاعلية فى الفئران. وقد أظهرت هذه المركبات نتائج متباينة فى الفاعلية.

A series of 3(5)-substituted 1,2,4-triazole-5(3)-carboxylic acid amides, hydroxamic, hydrazide, iminosemicarbazide, semicarbazide and thiosemicarbazide have been synthesized. Most of the prepared compounds were tested for their growth inhibitory activity against Staph. aureus and E. coli and none of them revealed antibacterial activity at concentration up to 2.5 mg/mL. The 5(3)-iminosemicarbazides (8h, 9h, 10h, 11h), 5(3)-semicarbazides (8i, 9i, 10i, 11i) and 5(3)-thiosemicarbazides (8j, 9j, 10j) were chosen for evaluation of their potentialities to lower blood glucose level in rats. The oral glucose tolerance test was used as preliminary test for evaluation of hypoglycemic activity of these compounds. The tested compounds showed different onset and duration of action.

INTRODUCTION

3-Amino-5-substituted-amino-1,2,4-triazoles I can be regarded as constrained analogues of the biguanide II class of oral hypoglycemics.

In view of this analogy different substituents at 3 and 5 positions were synthesized aiming to disclose the hypoglycemic activity of the 1,2,4-triazole nucleus. In a British patent, the hypoglycemic activity of a series of 5-amino-3-dialkylamino-1,2,4-triazoles was reported.¹ In another series the monoalkylamino analogues were found inactive.² Trials to replace 5-amino group in I by benzene sulfamoyl³ or by sulfonylurea moiety⁴ yielded inactive hypoglycemic agents, meanwhile, derivatives with 3-aryl-5-mercaptosubstituents were able to show significant reduction in blood sugar level.⁴



On the other hand triazole ring had received a considerable attention as a polar component of different nitrofurans derivatives^{5,6} with enhanced antibacterial activity. Promising results were obtained on testing 3,4-diphenyl-1,2,4-triazole-5-mercaptoacetic acid derivatives for their activity on a number of pathogenic microorganisms,⁷ while 3-phenacylthio-5-substituted-4H-1,2,4-triazoles showed insignificant activity.⁸

In view of these findings we now report on *in vitro* antibacterial activity and effect on blood

glucose level in rats and the structure activity relationship of certain 3(5)-substituted-1,2,4-triazole-5(3)-carboxylic acid derivatives.

EXPERIMENTAL

Melting points were determined on electrothermal melting point apparatus and are uncorrected. Microanalysis and MS (carried out on LKB-GC MS 9000 s instrument) at 70 eV were performed at the microanalytical center, Faculty of Science, Cairo University. ¹H-NMR spectra were determined on EM-360 (60 MHz) in DMSO-d₆ (otherwise specified) using TMS as the internal standard. IR spectra were recorded on 470-Shimadzu infrared spectrophotometer as KBr discs. Product purity was checked by TLC kieselgel 60 F254. Yields given are those of the crude products.

Synthesis of methyl 3(5)-acetamido-1,2,4-triazole-5(3)-carboxylate **4** and the acid **6**

Compound **2**⁹ (2.84 g, 0.02 mole) and acetic anhydride (20 ml) were heated under reflux for 15 minutes. The cold mixture was then poured into water (25 ml) and refluxed for 15 minutes then concentrated under vacuum. The precipitate collected after cooling was then filtered and crystallized from water to give the ester **4** (2.8 g, 76%), m.p. 234-235°C; IR ν 3200, 3085, 1729, 1699 cm⁻¹; ¹H-NMR (δ ppm): 2.1 (s, 3H, CH₃CO), 3.9 (s, 3H, OCH₃), 11.7 (br, 1H, NHCOCH₃) exchangeable; 13.9 (br, 1H, N₁-H) exchangeable; MS: m/z 184 (M⁺, 7.6%), 185 (M+1, 7.9%), 153 (5.3%), 142 (57%), 111 (15%), 84 (11.5%), 82 (7.4%). Anal. Calcd for C₆H₈N₄O₃: C, 39.13; H, 4.38; N, 30.42, Found: C, 39.2; H, 4.6; N, 30.0.

The ester **4** (1.84 g, 0.01 mole) was dissolved in a solution of NaOH (0.6 g, 0.015 mole) in water (30 ml), and left at room temperature for 5 hours, then acidified with dilute HCl to pH 4. The formed precipitate was filtered off, and purified by dissolving in 1N NaOH and reprecipitated by dilute HCl to give the acid **6** (1.2 g, 70%); m.p. 294-295°C; IR ν 3200, 3150, 3050-2530, 1707, 1683 cm⁻¹; ¹H-NMR (δ ppm) 2.1 (s, 3H, CH₃CO), 7.85 (s,

1H, COOH) exchangeable; 11.4 (br, 1H, NHCOCH₃) exchangeable and 13.5 (br, 1H, N₁-H) exchangeable.

Anal. Calcd for C₅H₆N₄O₃: C, 35.30; H, 3.55; N, 32.93, Found: C, 35.6; H, 3.9; N, 33.0.

3(5)-Substituted-1,2,4-triazole-5(3)-carboxamides: (**8a,b**; **9a,b**; **10a,b**; and **11a,b**) General procedure

A solution of the esters **2-5** (7 mmole) in excess of amine (50 ml) was left overnight at room temperature then the reaction mixture was evaporated under reduced pressure and the residue of **8a,b**; **9a,b**; **10a,b**; or **11a,b** was crystallized from the appropriate solvent as shown in Tables (1-4).

Compound 8a: ¹H-NMR (δ ppm) 6 (br, 2H, -NH₂) exchangeable; 7.4 (br, 2H, -CONH₂) exchangeable and 12.5 (br, 1H, N₁-H) exchangeable.

Compound 8b: ¹H-NMR (δ ppm) 1 (t, 3H, CH₃); 3.2 (q, 2H, CH₂); 6.0 (br, 2H, -NH₂) exchangeable; 8.0 (t, 1H, CONH) exchangeable and 12.2 (br, 1H, N₁-H) exchangeable.

Compound 9a: ¹H-NMR (δ ppm) (methanol-d₄) 4.9 (br, 2H, CONH₂) exchangeable.

Compound 9b: ¹H-NMR (δ ppm) 1.0 (t, 3H, CH₃); 3.25 (p, 2H, CH₂); 8.9 (br, 1H, CONH) exchangeable and 15.3 (br, 1H, N₁-H) exchangeable.

Compound 10a: ¹H-NMR (δ ppm) 2.1 (s, 3H, CH₃CO); 7.5 (h, 2H, CONH₂) exchangeable; 11.4 (br, 1H, NHCOCH₃) exchangeable and 13.7 (br, 1H, N₁-H) exchangeable.

Compound 10b: ¹H-NMR (δ ppm) 1.13 (t, 3H, CH₃); 2.16 (s, 3H, CH₃CO); 3.3 (m, 2H, CH₂); 8.3 (t, 1H, CONH) exchangeable; 11.3 (br, 1H, NHCOCH₃) exchangeable and 13.6 (br, 1H, N₁-H) exchangeable.

Compound 11a: ¹H-NMR (δ ppm) 7.2-8 (br) exchangeable.

Compound 11b: $^1\text{H-NMR}$ (δ ppm) 1.2 (t, 3H, CH_3); 3 (q, 2H, CH_2) and 7.2 (br, 1H, CONH) exchangeable.

3(5)-Substituted-1,2,4-triazole-5(3)-carboxamides: (8c-e; 9c-e; 10c,d; and 11c,d)

To the ester **2** (1 g, 7 mmole) powdered ammonium chloride (0.3 g) and excess of the appropriate amine (50 mmole) was added then the mixture refluxed for 4 hours. The excess amine was distilled off under reduced pressure and the residue crystallized to yield **8c-e**; Table (1).

Compound 8c: $^1\text{H-NMR}$ (δ ppm) (pyridine- d_5) 1-2 (m, 10H, cyclohexyl); 4.1 (s, 1H, $-\text{CH}$ of cyclohexyl); 7.3 (br, 2H, $-\text{NH}_2$); 8.2 (br, 1H, CONH) exchangeable and 9.9 (br, 1H, $\text{N}_1\text{-H}$) exchangeable.

Compound 8d: $^1\text{H-NMR}$ (δ ppm) 4.4 (d, 2H, CH_2); 6.1 (br, 2H, $-\text{NH}_2$) exchangeable; 7.3 (s, 5H, C_6H_5); 8.5 (br, 1H, CONH) exchangeable and 12.7 (br, 1H, $\text{N}_1\text{-H}$) exchangeable.

Compound 8e: $^1\text{H-NMR}$ (δ ppm) 6.1 (br, 2H, NH_2) exchangeable; 6.9-7.9 (m, 5H, C_6H_5); 9.8 (br, 1H, CONH) exchangeable and 12.6 (br, 1H, $\text{N}_1\text{-H}$) exchangeable.

To a solution of **3**^{10,11} (1.13 g, 7 mmole) in ethanol (20 ml) the appropriate amine (9 mmole) was added and the mixture was stirred for 3 hours at 70-80°C. The reaction mixture was then concentrated under reduced pressure and the separated products **9c-e** were filtered and crystallized from the suitable solvent, Table (2).

Compound 9c: $^1\text{H-NMR}$ (δ ppm) 1-2 (m, 10H, cyclohexyl); 3.5 (s, 1H, CH of cyclohexyl) and 8.6 (d, 1H, CONH) exchangeable.

Compound 9d: $^1\text{H-NMR}$ (δ ppm) 4.3 (d, 2H, CH_2); 7.2 (s, 5H, C_6H_5) and 9.3 (t, 1H, CONH) exchangeable.

Compound 9e: $^1\text{H-NMR}$ (δ ppm) 7.2-7.9 (m, 5H, C_6H_5); 10.8 (br, 1H, CONH) exchangeable and 15.5 (br, 1H, $\text{N}_1\text{-H}$) exchangeable.

To a solution of **4** (1.84 g, 10 mmole) in methanol (30 ml) was added the appropriate amine (20 mmole) and the mixture refluxed for 30 minutes. After cooling the precipitated amides **10c,d** were filtered and crystallized from the suitable solvent, Table (3).

Compound 10c: $^1\text{H-NMR}$ (δ ppm) 0.8-1.8 (m, 11H, cyclohexyl); 2.0 (s, 3H, CH_3CO); 7.63 (d, 1H, CONH) exchangeable; 11.16 (br, 1H, NHCOCH_3) exchangeable and 13.5 (br, 1H, $\text{N}_1\text{-H}$) exchangeable.

Compound 10d: $^1\text{H-NMR}$ (δ ppm) 2.13 (s, 3H, CH_3CO); 4.4 (d, 2H, CH_2); 7.3 (s, 5H, C_6H_5); 8.73 (t, 1H, CONH) exchangeable; 11.4 (br, 1H, NHCOCH_3) exchangeable and 13.73 (br, 1H, $\text{N}_1\text{-H}$) exchangeable.

To a solution of **5**^{12,13} (1.72 g, 10 mmole) in methanol (15 ml) was added dropwise to a solution of the appropriate amine (11 mmole) in methanol (10 ml). After complete addition the reaction mixture was left overnight at room temperature and the residue **11c,d** crystallized from the appropriate solvent, Table (4).

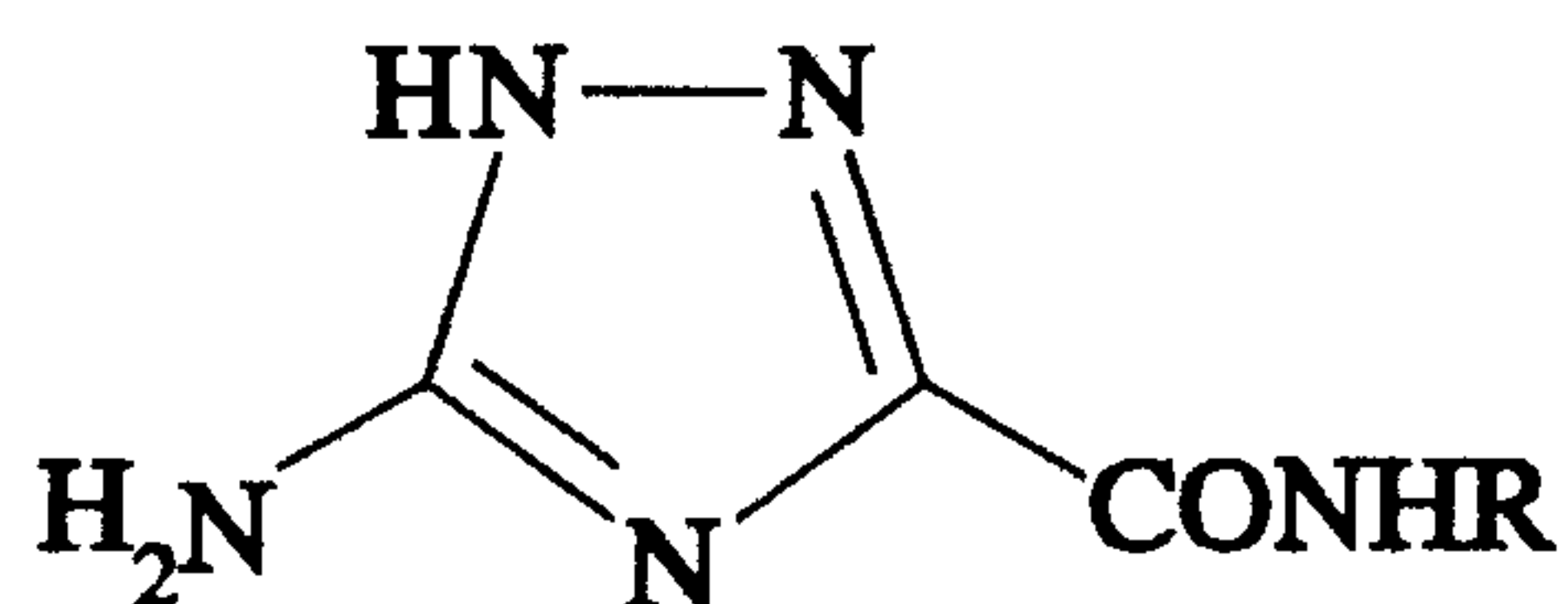
Compound 11c: $^1\text{H-NMR}$ (δ ppm) 0.9-2 (m, 11H, cyclohexyl) and 7.6-8.4 (br, 1H, CONH) exchangeable.

Compound 11d: $^1\text{H-NMR}$ (δ ppm) 4.4 (d, 2H, CH_2); 7.4 (s, 5H, C_6H_5) and 8.4 (t, 1H, CONH) exchangeable.

3(5)-Acetamido-1,2,4-triazolecarboxanilide (10e)

A mixture of **8e** (1.02 g, 5 mmole) and acetic anhydride (10 ml) was heated under reflux for 15 minutes, then poured while hot into water (20 ml) and refluxed for 15 minutes. The reaction mixture was concentrated under reduced pressure and **10e** was filtered and crystallized, Table (3).

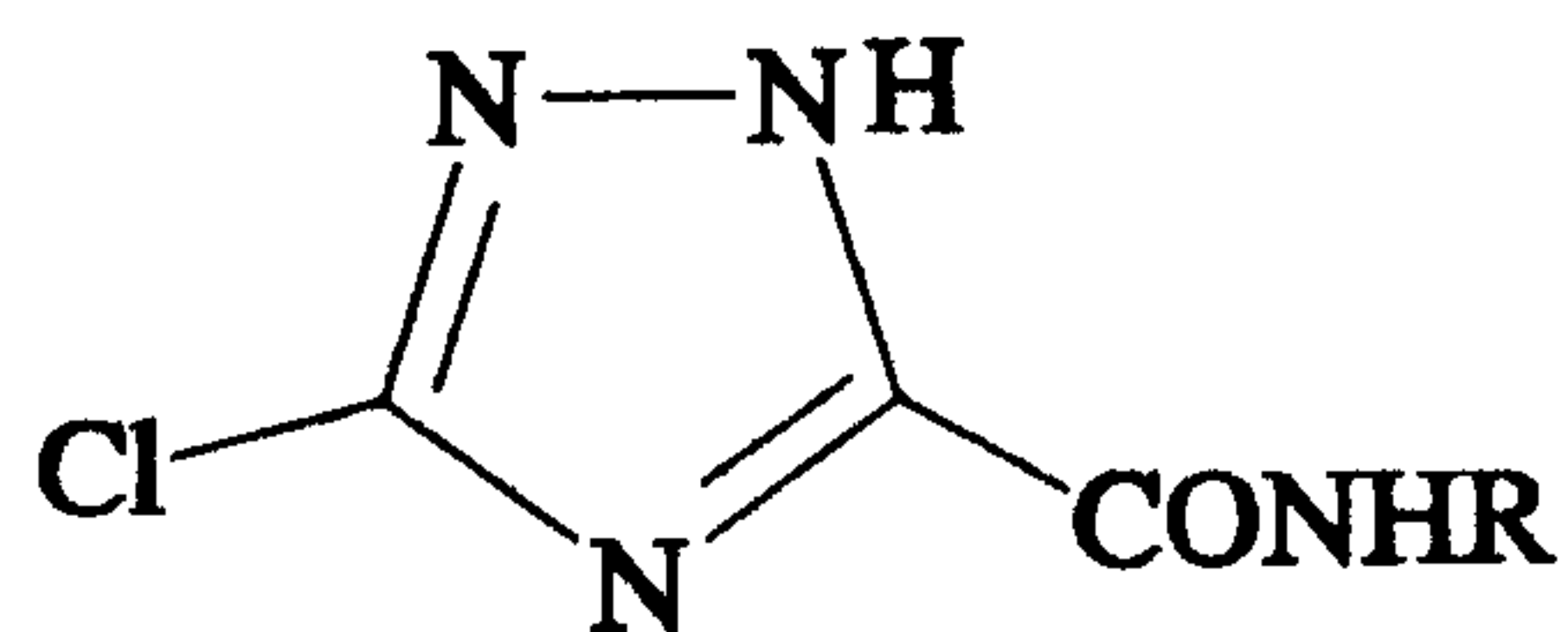
Compound 10e: $^1\text{H-NMR}$ (δ ppm) 2.1 (s, 3H, CH_3CO); 7-8 (m, 5H, C_6H_5); 10.16 (br, 1H, CONH) exchangeable; 11.6 (br, 1H, NHCOCH_3) exchangeable and 14 (br, 1H, $\text{N}_1\text{-H}$) exchangeable.

Table 1: Physical constants of 5-amino-1,2,4-triazole-3-carboxylic acid derivatives 8(a-j).

Comp. No.	R	m.p. (°C) (Cryst. solvent)	IR ν (cm ⁻¹)	Yield (%)	Mol. Formula (M. wt.)	Microanalysis, Calcd./Found		
						C%	H%	N%
8a	H	>300 aq. methanol	3335, 3145, 1693, 1579	85	C ₃ H ₅ N ₃ O ^a (127.10)	—	—	—
8b	C ₂ H ₅	231-232 water	3405, 3205, 3080, 1671	91	C ₉ H ₉ N ₃ O (155.16)	38.71 38.60	5.85 5.40	45.14 45.10
8c	C ₆ H ₁₁ (c)	252-253 water	3425, 3315, 3055, 1654	88	C ₉ H ₁₅ N ₃ O (209.06)	51.64 51.70	7.23 6.70	33.48 33.50
8d	CH ₂ C ₆ H ₅	245-246 aq. ethanol	3400, 3300, 3050, 1656	78	C ₉ H ₁₁ N ₃ O (217.23)	55.29 55.70	5.10 4.80	32.24 32.50
8e	C ₆ H ₅	255-256 water	3460, 3200, 3055, 1677	77	C ₉ H ₉ N ₃ O (203.20)	53.20 52.80	4.46 4.60	34.46 34.30
8f	OH	207-209 water	3375, 3220, 3150, 1664	78	C ₃ H ₅ N ₃ O ₂ (143.10)	25.18 25.50	3.52 3.80	48.94 48.60
8g	NH ₂	>300 glacial acetic acid	3420, 3120, 1690, 1557	66.5	C ₃ H ₆ N ₆ O ^a (142.12)	—	—	—
8h	NHC(NH)NH ₂	>300 aq. ethanol	3315, 3130, 1658, 1548	61.5	C ₄ H ₈ N ₈ O (184.16)	26.09 25.90	4.38 4.88	60.84 60.60
8i	NHCONH ₂	>300 aq. ethanol	3415, 3220, 1712-1652	69	C ₄ H ₇ N ₇ O ₂ (185.14)	25.95 25.80	3.81 4.00	52.96 52.50
8j	NHCSNH ₂	>300 aq. ethanol	3400, 3245, 1689, 1539	71	C ₄ H ₇ N ₇ OS ^b (201.21)	23.88 23.40	3.51 3.20	48.73 48.80

^aReported in reference [9]^bSulphur Calcd./found (15.9/16).

Table 2: Physical constants of 3(5)-chloro-1,2,4-triazole-5(3)-carboxylic acid derivatives 9(a-j).



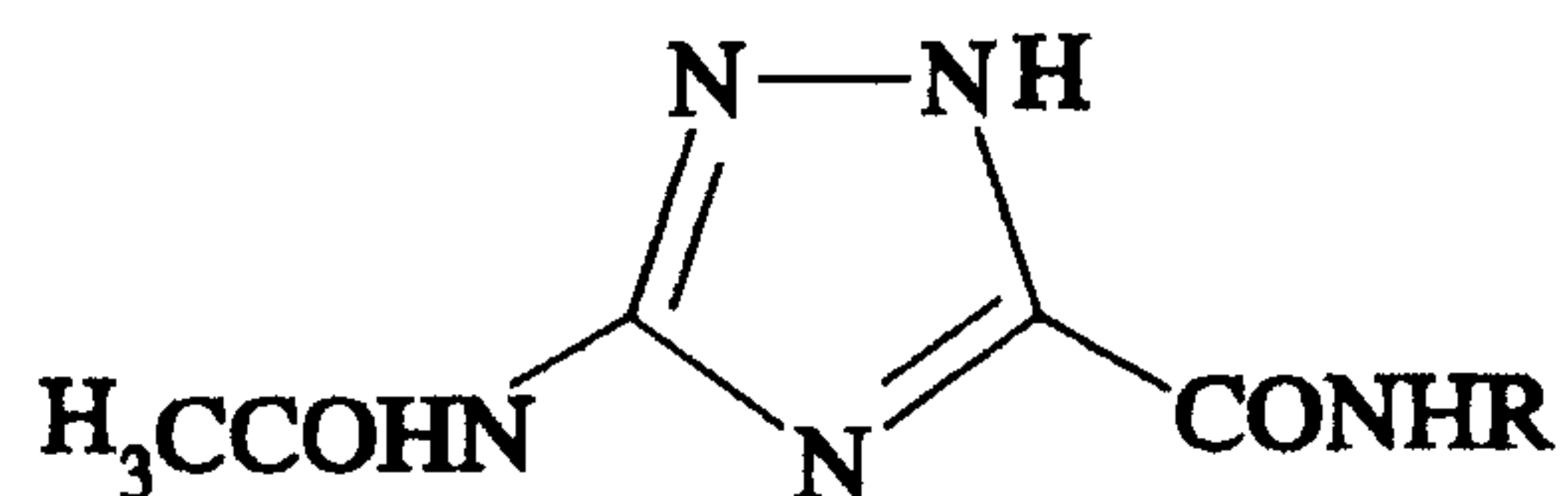
Comp. No.	R	m.p. (°C) (solvent)	IR ν (cm ⁻¹)	Yield (%)	Mol. Formula (M. wt.)	Microanalysis, Calcd./Found			
						C%	H%	N%	Cl%
9a	H	231-233 aq. ethanol	3365-3240, 3120, 1682	77	C ₃ H ₃ ClN ₄ O ^a (146.54)	—	—	—	—
9b	C ₂ H ₅	162-163 water	3335, 3140, 1659, 1572	71.5	C ₅ H ₇ ClN ₄ O (174.59)	34.40 34.60	4.04 3.80	32.09 31.70	20.31 20.00
9c	C ₆ H ₁₁ (c)	227-228 aq. methanol	3310, 3165 1662, 1562	91	C ₉ H ₁₃ ClN ₄ O (228.68)	47.27 47.40	5.7543 5.50	24.50 24.10	15.50 15.10
9d	CH ₂ C ₆ H ₅	173-174 aq. methanol	3335, 3145, 1656, 1566	73	C ₁₀ H ₉ ClN ₄ O (236.65)	50.75 51.40	3.83 3.90	23.68 23.90	14.98 15.00
9e	C ₆ H ₅	196-198 water	3275, 3110, 1665, 1557	63.5	C ₉ H ₇ ClN ₄ O (222.63)	48.55 48.70	3.17 3.00	25.16 25.20	15.92 15.70
9f	OH	156-167 water	3390, 2010, 1630, 1529	70	C ₃ H ₃ ClN ₄ O ₂ ^b (194.25)	19.01 19.00	3.19 3.20	29.56 29.50	18.70 18.70
9g	NH ₂	175-176 aq. ethanol	3350-3225, 1660, 1530	95	C ₃ H ₃ ClN ₅ O ^a (161.55)	22.30 22.00	2.50 2.60	43.35 43.50	21.94 21.90
9h	NHC(NH)NH ₂	169-170 (CHCl ₃ - CH ₃ OH)2:1	3440-3280, 1667, 1575	71	C ₄ H ₆ ClN ₇ O (203.59)	23.60 23.60	2.97 2.80	48.16 48.00	17.41 14.40
9i	NHCONH ₂	137-139 ethanol	3400-3200, 1687-1664	50	C ₄ H ₅ ClN ₆ O ₂ (204.58)	23.48 23.80	2.46 2.80	41.08 41.10	
9j	NHCSNH ₂	204-205 aq. methanol	3350, 3160, 1684, 1607	81	C ₄ H ₃ ClN ₆ OS (220.64)	21.77 21.80	2.28 2.30	38.09 38.50	16.06 16.00

^aReported in reference [11] ^bcontain 1.79 molecule of water.

3(5)-Nitro-1,2,4-triazolecarboxanilide (11e)

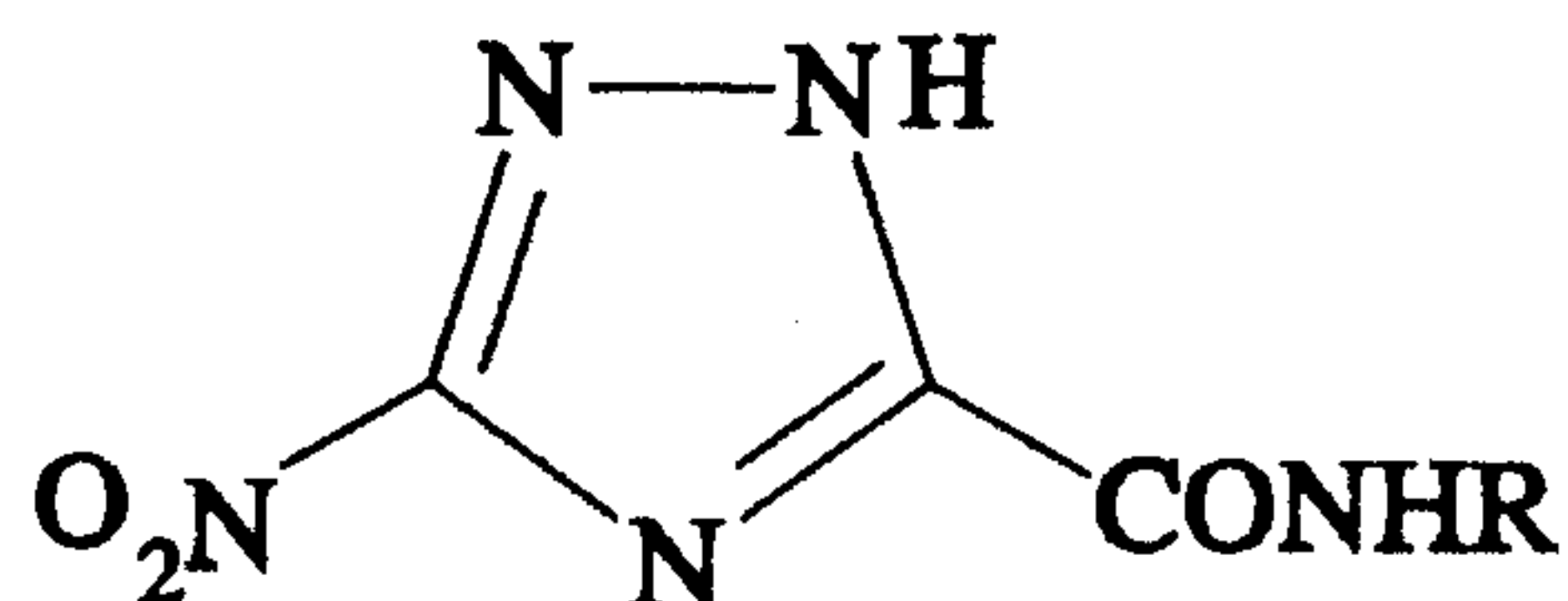
A solution of 8e (1.02 g, 5 mmole) in glacial acetic acid (10 ml) was added to an ice-cooled stirred solution of NaNO₂ (0.4 g) in H₂SO₄ (2 ml) and stirring was continued for 5 minutes. To the reaction mixture water (10 ml) was added, the solution warmed to 60°C and

solution of 10% NaNO₂ (50 ml) was then added and stirring continued for 1 hour keeping the temperature at 60°C. On cooling the formed precipitate was filtered off, and the residue 11e was crystallized, Table (4).

Table 3: Physical constants of 3(5)-acetamido-1,2,4-triazole-5(3)-carboxylic acid derivatives 10(a-j).

Comp. No.	R	m.p. (°C) (Cryst. solvent)	IR ν (cm ⁻¹)	Yield (%)	Mol. Formula (M. wt.)	Microanalysis, Calcd./Found		
						C%	H%	N%
10a	H	313-315 aq. ethanol	3375,3215,3105, 1689,1672	77	C ₅ H ₇ N ₅ O ₂ (169.14)	35.51 35.80	4.17 4.60	41.41 41.30
10b	C ₂ H ₅	293-294 aq. ethanol	3380,3285,3100, 1689,1668	81	C ₇ H ₁₁ N ₅ O ₂ (197.20)	42.64 42.70	5.62 6.00	35.51 35.90
10c	C ₆ H ₁₁ (c)	298-300 ethanol	3395,3285,3205, 1689,1666	60	C ₁₁ H ₁₇ N ₅ O ₂ (251.29)	52.58 52.30	6.82 6.80	27.87 27.40
10d	CH ₂ C ₆ H ₅	292-293 aq. ethanol	3395,3285,3100, 1680,1673	71	C ₁₂ H ₁₃ N ₅ O ₂ (259.27)	55.59 55.50	5.05 5.30	27.01 26.70
10e	C ₆ H ₅	>300 ethanol	3395,3275,3105, 1689,1667	73	C ₁₁ H ₁₁ N ₅ O ₂ (245.24)	53.87 53.50	4.52 4.80	28.56 28.60
10f	OH	265-267 methanol	3300,3260, 1685,1676	50	C ₅ H ₇ N ₅ O ₃ (185.14)	32.44 32.30	3.81 4.00	37.83 38.10
10g	NH ₂	275-277 water	3300,3260,3075, 1685,1661	87	C ₅ H ₈ N ₆ O ₂ (184.16)	32.61 32.40	4.38 4.70	45.63 45.60
10h	NHC(NH)NH ₂	169-170 water	3455,3330,3280, 1688,1655	82	C ₆ H ₁₀ N ₈ O ₂ (226.20)	31.86 31.60	4.46 4.80	— —
10i	NHCONH ₂	281-283 water	3425,3275,1686, 1668,1650	42	C ₆ H ₉ N ₇ O ₃ (227.18)	31.72 31.90	3.99 4.10	43.16 42.80
10j	NHCSNH ₂	250-251 water	3400,3360,3260, 1688,1661	49	C ₆ H ₉ N ₇ O ₂ S* (243.25)	29.63 29.90	3.73 4.00	40.31 40.00

*Analysis S; calcd. 13.18 found 13.1.

Table 4: Physical constants of 3(5)-nitro-1,2,4-triazole-5(3)-carboxylic acid derivatives 11(a-i).

Comp. No.	R	m.p. (°C) (Cryst. solvent)	IR ν (cm ⁻¹)	Yield (%)	Mol. Formula (M. wt.)	Microanalysis, Calcd./Found		
						C%	H%	N%
11a	H	213-214 methanol	3395,3235,3140, 1682,1510,1384	82	C ₃ H ₃ N ₃ O ₃ (157.09)	22.94 22.70	1.92 2.00	44.56 44.20
11b	C ₂ H ₅	195-196 water	3120,1640, 1532,1382	70	C ₅ H ₇ N ₃ O ₃ (185.14)	32.44 32.80	3.81 4.00	37.83 37.40
11c	C ₆ H ₁₁ (c)	169-170 acetone	3385,3105,1663, 1542,1381	50	C ₉ H ₁₃ N ₃ O ₃ (239.23)	45.19 45.40	5.48 5.90	29.27 28.90
11d	CH ₂ C ₆ H ₅	170-171 acetone benzene	3390,3175,1652, 1554,1381	60	C ₁₀ H ₉ N ₃ O ₃ (247.21)	48.56 48.47	3.67 4.25	28.33 28.40
11e	C ₆ H ₅	230-233 DMF/water	3350,3185,1685, 1551,1382	43	C ₉ H ₇ N ₃ O ₃ (233.19)	46.63 --	3.03 --	30.03 29.80
11f	OH	150-152 methanol	3365,3120,1660, 1550,1387	46	C ₃ H ₃ N ₃ O ₄ (173.09)	20.82 20.50	1.75 1.40	-- --
11g	NH ₂	177-179* water	3330,3215,3160, 1663,1542,1382	81	C ₃ H ₄ N ₆ O ₃ (172.10)	--	--	--
11h	NHC(NH)NH ₂	212-215 water	3400,3365,3240, 1665,1526,1389	68	C ₄ H ₆ N ₈ O ₃ (214.14)	22.44 22.00	2.82 3.20	52.33 52.40
11i	NHCONH ₂	208-210 aq. methanol	3440,3390,1671, 1648,1525,1381	37	C ₄ H ₅ N ₇ O ₄ (215.13)	22.33 22.30	2.34 2.50	45.58 45.20

*As reported ref. (14).

Compound 11e: ¹H-NMR (δ ppm) (trifluoroacetic acid) 7.2-8.3 (m, 5H, C₆H₅) and 9.4-9.6 (br, 1H, CONH) exchangeable

3(5)-Substituted-1,2,4-triazole hydroxamic acids (8f, 9f, 10f and 11f)

"A freshly prepared solution of hydroxylamine prepared by mixing solution of

H₂NOH, HCl (13.70 g, 200 mmole) in ethanol (50 ml) and KOH (14 g, 250 mmole) in ethanol (50 ml) was used in the following"

To a solution of 2 (1.0 g, 7 mmole) in ethanol (30 ml) was added the solution of hydroxylamine (25 ml) while stirring. The reaction mixture was then refluxed for 2 hours and the precipitate was filtered, washed with

methanol and then dissolved in 20% acetic acid (20 ml) and allowed to stand for 30 minutes. The precipitate **8f** was filtered and crystallized Table (1).

Compound 8f: $^1\text{H-NMR}$ (δ ppm) 3.35 (br, 1H, OH) exchangeable; 6 (br, 2H, NH_2) exchangeable; 8.9 (br, 1H, CONH) exchangeable and 10.7 (br, 1H, $\text{N}_1\text{-H}$) exchangeable.

To a solution of **3** (1.13 g, 7 mmole) in ethanol (20 ml) was added a solution of hydroxylamine (4 ml) with stirring. The reaction mixture was left for 1 hours at room temperature and the product **9f** worked up as given under **8f**, Table (2).

Compound 9f: $^1\text{H-NMR}$ (δ ppm) 8-10 (hump) exchangeable.

A solution of **4** (1.0 g, 5 mmole) in ethanol (20 ml) was added portionwise with stirring to a solution of hydroxylamine (50 ml) and the mixture was left at room temperature overnight and the product **10f** worked up as given under **8f**, Table (3).

Compound 10f: $^1\text{H-NMR}$ (δ ppm) 2.13 (s, 3H, CH_3CO) and 7.5-12.5 (br, 4H, NHOH, NHCOCH_3 , $\text{N}_1\text{-H}$) exchangeable.

A solution of **5** (0.86 g, 5 mmole) in methanol (5 ml) was added portionwise with stirring to a solution of hydroxylamine (10 ml) and left overnight at room temperature, and the product **11f** worked up as given under **8f**, Table (4).

Compound 11f: $^1\text{H-NMR}$ (δ ppm) 7-7.2 (hump) exchangeable.

3(5)-Substituted-1,2,4-triazole-5(3)-carboxylic acid hydrazides (**8g**, **9g**, **10g** and **11g**)

A mixture of **2** (1.0 g, 7 mmole) and hydrazine hydrate (5 ml) was heated under reflux for 5 hours. The precipitated **8g**⁹ was filtered, washed with water and crystallized, Table (1).

Compound 8g: $^1\text{H-NMR}$ (δ ppm) 3.3 (br, 2H,

CONHNH_2) exchangeable; 6 (br, 2H, NH_2) exchangeable; 8.2 (br, 1H, CONHNH_2) exchangeable and 10.9 (br, 1H, $\text{N}_1\text{-H}$) exchangeable.

A solution of **3** (1.13 g, 7 mmole) in methanol (20 ml) was added with stirring to hydrazine hydrate (0.5 ml) at room temperature. The solution was stirred at room temperature for 3 hours. Then the solution was concentrated at reduced pressure and the residue crystallized to give **9g**, Table (2).

Compound 9g: $^1\text{H-NMR}$ (δ ppm) 5.5 (br, 3H, CONHNH_2) exchangeable.

A suspension of **4** (1.84 g, 10 mmole) in methanol (40 ml) was added carefully to a stirred solution of hydrazine hydrate (2.5 ml) in methanol (20 ml). Stirring was continued at room temperature for 30 minutes and then left overnight. The formed precipitate of **10g** was filtered and crystallized, Table (3).

Compound 10g: $^1\text{H-NMR}$ (δ ppm) 2.1 (s, 3H, CH_3CO); 4-5 (br, 2H, CONHNH_2) exchangeable; 9.4 (br, 1H, CONHNH_2) exchangeable; 11.6 (br, 1H, NHCOCH_3) exchangeable and 13-14 (br, 1H, $\text{N}_1\text{-H}$) exchangeable.

A cooled solution of **5** (1.72 g, 10 mmole) in methanol (10 ml) was added dropwise to a stirred solution of hydrazine hydrate (0.6 ml) in methanol (10 ml), keeping the temperature below 10°C , then left overnight. The mixture worked up as **10g** to yield **11g**,¹⁴ Table (4).

Compound 11g: $^1\text{H-NMR}$ (δ ppm) 7.6-8 (br) exchangeable.

3(5)-Substituted-1,2,4-triazole-5(3)-iminosemicarbazides (**8h**, **9h**, **10h** and **11h**)

To a suspension of **8g** (1.0 g, 7 mmole) in water (30 ml) was added guanidine HCl (1.4 g, 16 mmole) and the reaction mixture heated under reflux for 6 hours. On cooling the precipitate **8h** was filtered and crystallized, Table (1).

Compound 8h: $^1\text{H-NMR}$ (δ ppm) 4.4 (br, 2H,

C(NH)NH₂) exchangeable; 6 (br, 3H, NH₂, CONHNH-) exchangeable; 9.2 (br, 2H, -C(NH)NH₂, CONHNH) exchangeable and 12.2 (br, 1H, N₁-H) exchangeable.

To a solution of 3 (1.13 g, 7 mmole) in ethanol (30 ml) aminoguanidine bicarbonate (1g, 7 mmole) was added and the mixture refluxed for 2 hours and the solvent was removed in vacuo. The product 9h was crystallized, Table (2).

Compound 9h: ¹H-NMR (δ ppm) 7.3 (br, 4H, NHC(NH)NH₂) exchangeable and 8.9 (br, 1H, CONH) exchangeable.

To a solution of 6 or 7 (12 mmole) in water (20 ml) warmed to 50-60°C, aminoguanidine bicarbonate (2 g, 15 mmole) was added gradually and the reaction mixture was heated on boiling water bath for 4 hours. On cooling the precipitated 10h or 11h was filtered and crystallized, Tables (3 & 4).

Compound 10h: ¹H-NMR (δ ppm) 2.1 (s, 3H, CH₃CO); 3.5-5.3 (br, 3H) exchangeable; 7.7 (br, 3H) exchangeable and 9.23-10.2 (br, 1H) exchangeable.

Compound 11h: ¹H-NMR (δ ppm) 7.4 (br, 4H, NHC(NH)NH₂) exchangeable and 8.5-9.5 (br, 1H, CONH) exchangeable.

3(5)-Substituted-1,2,4-triazole-5(3)-semicarbazides (8i, 9i, 10i and 11i)

Urea (1.5 g, 25 mmole) was added to a suspension of 8g (1 g, 7 mmole) in water and the stirred mixture was heated under reflux for 4 hours. On cooling the precipitated 8i was filtered, washed with water and crystallized, Table (1).

Compound 8i: ¹H-NMR (δ ppm) 5.8 (br, 2H, CONH₂) exchangeable; 6 (br, 2H, NH₂) exchangeable; 7.8 (br, 1H, CONHNH) exchangeable; 9.9 (br, 1H, CONH) exchangeable and 10.3 (br, 1H, N₁-H) exchangeable.

To the ester 3 (1.13 g, 7 mmole) in ethanol (30 ml) was added semicarbazide HCl (1 g, 7 mmole) in water. The reaction mixture was refluxed for 2 hours after which the solvent was

distilled under reduced pressure and 9i was crystallized, Table (2).

Compound 9i: ¹H-NMR (δ ppm) 5.9 (br, 2H, CONH₂) exchangeable; 7 (br, 1H, CONHNH) exchangeable and 7.7 (br, 1H, CONHNH) exchangeable.

A mixture of hydrazide 10g (1.84 g, 10 mmole), urea (1.2 g, 20 mmole) and glacial acetic acid (30 ml) was stirred at 100°C for 2 hours. On cooling 10i was filtered and crystallized, Table (3).

Compound 10i: ¹H-NMR (δ ppm) 2.16 (s, 3H, CH₃CO); 5.9 (s, 2H, CONH₂) exchangeable; 8 (s, 1H, CONHNH) exchangeable; 9.8 (s, 1H, CONHNH) exchangeable; 11.6 (br, 1H, NHCOCH₃) exchangeable and 13.83 (br, 1H, N₁-H) exchangeable.

A solution of the ester 5 (1.72 g, 10 mmole) in methanol (10 ml) was added gradually at room temperature with stirring to a freshly prepared solution of semicarbazide HCl (1.11 g, 10 mmole) already neutralized by sodium bicarbonate in water (10 ml) the reaction mixture was stirred overnight and the precipitated 11i filtered off and crystallized, Table (4).

Compound 11i: ¹H-NMR (δ ppm) 7.3-8.3 (br) exchangeable.

3(5)-Substituted-1,2,4-triazole-5(3)-thiosemicarbazides (8j, 9j and 10j)

A solution of the hydrazide 8g or 9g (7 mmole) and ammonium thiocyanate (1.5 g, 20 mmole) in 20% HCl (20 ml) was heated under reflux for 3 hours. The reaction mixture was concentrated under reduced pressure and the precipitated 8j or 9j was filtered and crystallized, Tables (1 & 2).

Compound 8j: ¹H-NMR (δ ppm) 6.1 (br, 2H, NH₂) exchangeable; 7.5 (br, 2H, CSNH₂) exchangeable; 9.3 (br, 1H, CONHNH) exchangeable; 9.95 (br, 1H, CONHNH) exchangeable and 10.3 (br, 1H, N₁-H) exchangeable.

Compound 9j: ¹H-NMR (δ ppm) 7-8 (br, 3H,

NHCSNH₂) exchangeable and 9.4 (br, 1H, CONH) exchangeable.

A mixture of the hydrazide 10g (1.84 g, 10 mmole) and ammoniumthiocyanate (1.5 g, 20 mmole) in glacial acetic acid (30 ml) was refluxed for 2 hours. After cooling the formed precipitate 10j was filtered, and crystallized, Table (3).

Compound 10j: ¹H-NMR (δ ppm) 2.1 (s, 3H, CH₃CO); 7.63 (br, 2H, CSNH₂) exchangeable; 9.33 (br, 1H, NHCS) exchangeable; 10.13 (br, 1H, CONHNH) exchangeable; 11.56 (br, 1H, NHCOCH₃) exchangeable and 13.83 (br, 1H, N₁-H) exchangeable.

Test of the antibacterial activity

The agar cup diffusion method was applied¹⁵ for testing the antibacterial activity of the compounds 4, 8a-f, 10a-g,i and 11g. One ml portion of DMSO was transferred into seven sterile Wasserman tubes. Then one ml of the solution of the tested compound in DMSO (5 mg/ml) was transferred to the first tube, mixed well and serially diluted with the same solvent contained in each of the other tubes so as to produce the following dilution; 2500, 1250, 625, 312, 156 and 78 μg/ml. An aliquot of 0.1 ml of each of the prepared dilutions were pipetted into the appropriate cup beginning with the lowest concentration (78 μg/ml), the last cup was used as control test for DMSO. The plates were left for one hour at room temperature then incubated at 37°C for 48 hours. The observed zone of inhibition was measured for each concentration of the tested compounds.

Glucose-tolerance test

The glucose level in plasma of the drown blood was determined by the glucose oxidase method.¹⁶ Glucose determination kit (Biocon) was reconstituted to afford freshly prepared working solution. The supplied vial of standard glucose solution 100 mg/ml was used. Groups of adult albino rats (150-250 g) of either sex were obtained from the animal house of Assiut University. Rats were divided into groups of four animals which were deprived of food for 12

hour prior to testing. Rats were orally glucose loaded by administration of 2 g/kg of a 50% solution of glucose using oesophageal canula. Test drugs were then administered orally in dose of 20 mg/kg (in 0.5% gum acacia). Glucose levels (mg/ml) were determined at 0, 30, 60, 120 minutes time intervals as in Table (5). Animals were anaesthetized with ether just before collection of blood samples. Blood samples were collected from the retro-orbital plexus of rats using a capillary tube previously moistened with heparin solution (50 unit/ml normal saline). In each collection, about 0.5 ml of blood was taken into heparinized tube (100 μl heparin solution) and centrifuged at 4500 r.p.m. for 15 minutes then plasma was withdrawn by micropipette and transferred into screw capped tubes. Aliquots of plasma or standard glucose (10 μl) were transferred into stoppered screw capped tube and to each tube one ml of working solution was added and incubated at 37°C for 15 minutes. The absorbance of the developed color was measured at 505 nm against a blank.

Concentration of glucose =

Absorbance of sample x Concentration of standard (mg/100 ml)

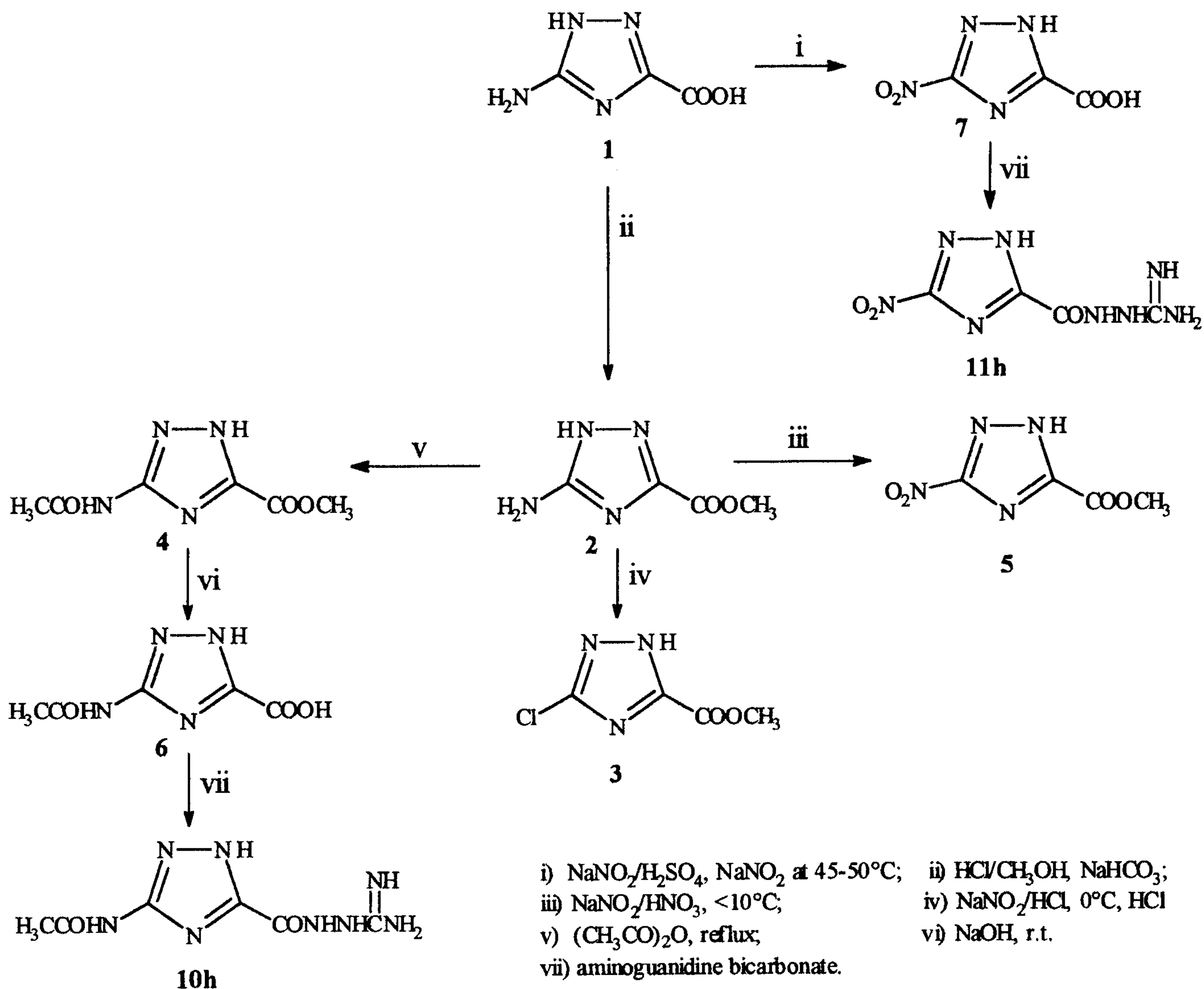
Absorbance of standard

The degree of variability in results was expressed as the mean ± standard error. The significance of differences between samples was determined using the students t-test. Difference was regarded as significant at $p < 0.05$ and highly significant at $p < 0.01$.

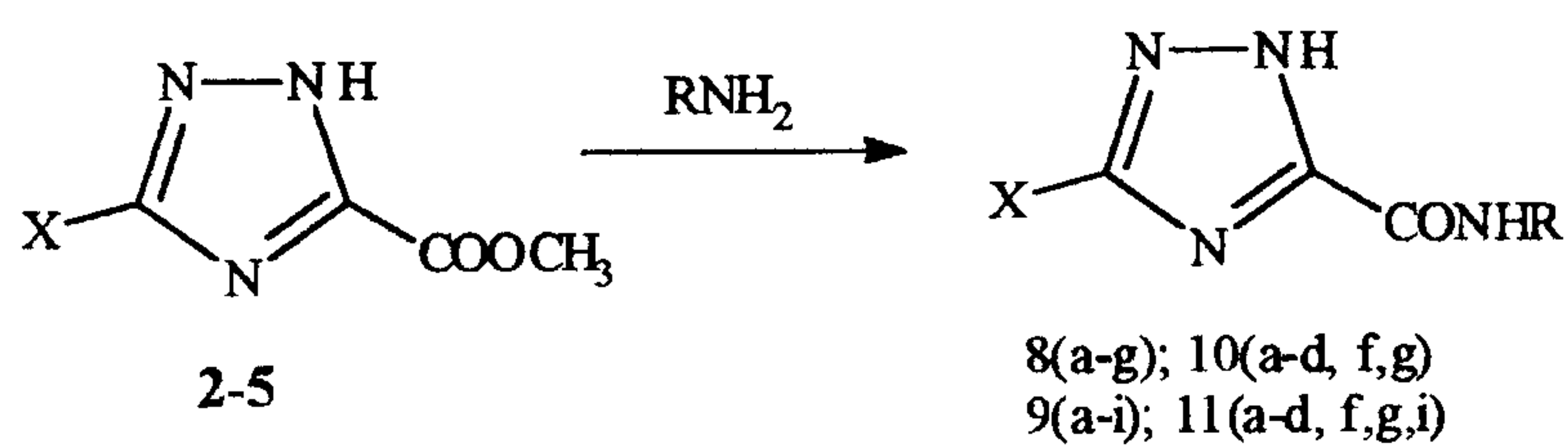
RESULTS AND DISCUSSION

Chemistry

For the synthesis of the 1,2,4-triazole 5(3)-carboxylic acid derivatives, the conventional method was applied where the 3(5)-substituted triazole esters were reacted with the amines under suitable reaction conditions as shown by Scheme 2. The starting esters were prepared by the derivatization of the amino group of the ester 2⁹ to yield the 3(5)-chloro 3^{10,11} and 3(5)-nitroester 5^{12,13}. 3(5)-Acetamido ester 4 was prepared by refluxing 2 with acetic anhydride.

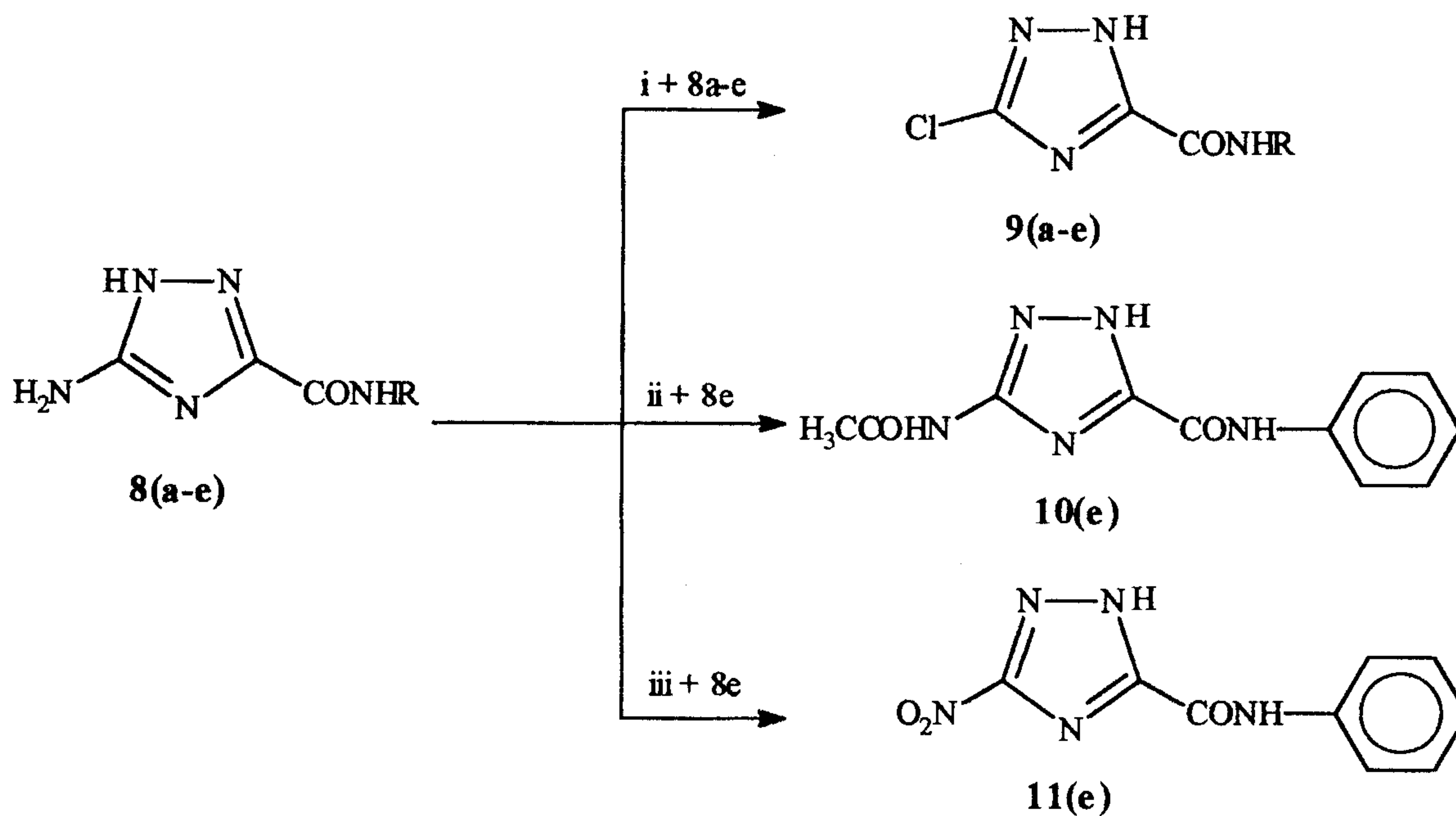


Scheme 1



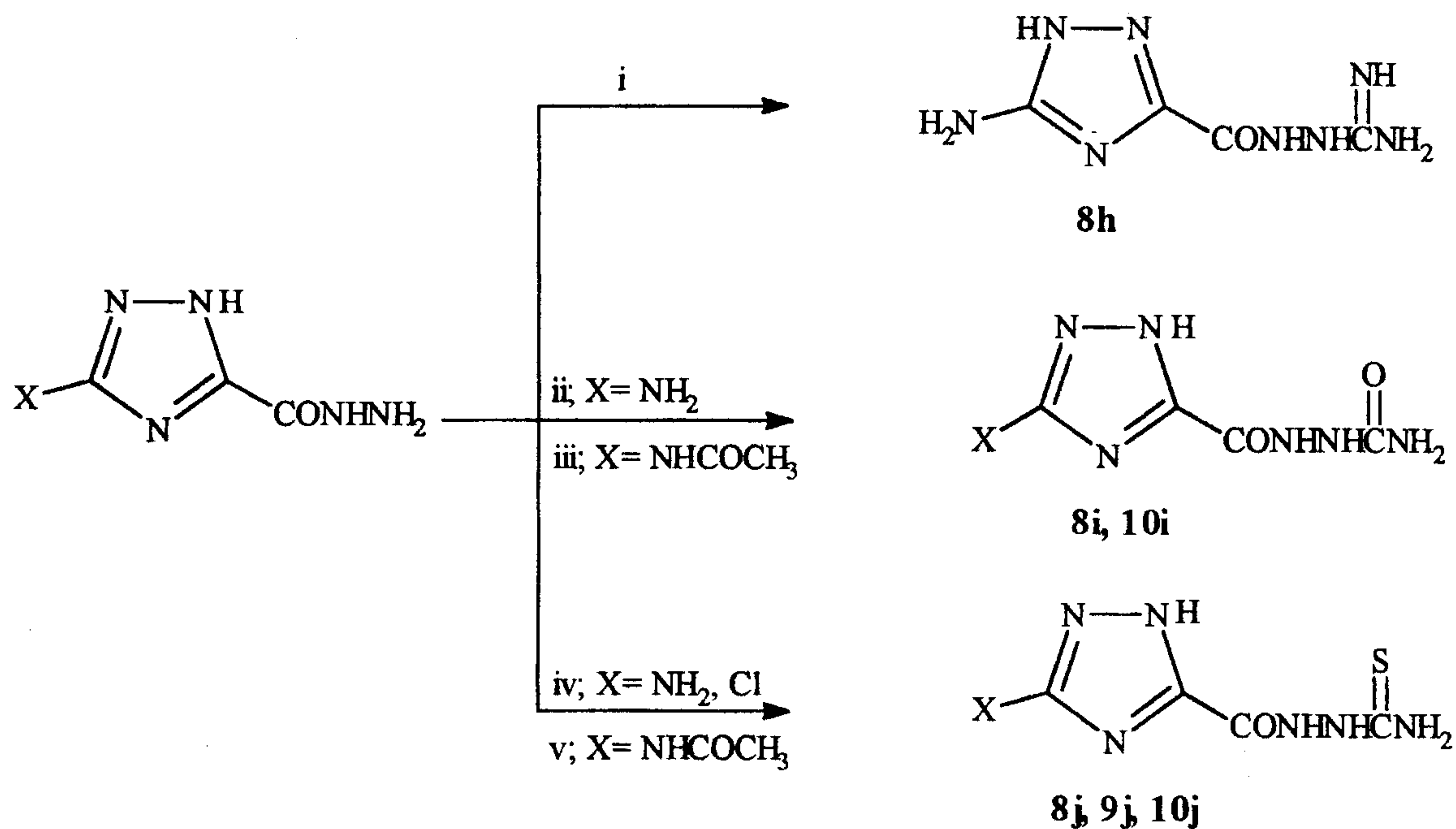
No.	X	R
2, 8	NH_2	a = H; b = Et; c = cyclohexyl
3, 9	Cl	d = $\text{CH}_2\text{C}_6\text{H}_5$; e = C_6H_5 ; f = OH
4, 10	NHCOCH_3	g = NH_2 ; h = $\text{NHC}(=\text{NH})\text{NH}_2$
5, 11	NO_2	i = NHCONH_2

Scheme 2



i) NaNO_2/HCl ; ii) $(\text{CH}_3\text{CO})_2\text{O}$, reflux; iii) $\text{NaNO}_2/\text{H}_2\text{SO}_4$, NaNO_2 60°C

Scheme 3



i) guanidine HCl; ii) urea/ H_2O ; iii) urea, AcOH; iv) $\text{NH}_4\text{SCN}/20\% \text{HCl}$; v) $\text{NH}_4\text{SCN}/\text{AcOH}$

Scheme 4

Further hydrolysis yielded the 3(5)-acetamido-5(3)-carboxylic acid **6**, Scheme 1. The acetamidocarboxylic acid **6** was not attainable by acetylation of **1** with acetylchloride or acetic anhydride at room temperature. Decarboxylation took place on heating **1** with acetic anhydride in glacial acetic acid to yield 3-acetamido-1,2,4-triazole. The latter was identified by matching the determined melting point with reported one (286-288°C) and by the disappearance of the band at 1702 cm⁻¹ corresponding to the C=O of the carboxyl group and the appearance of a new absorption band at 1689 of the C=O of acetamido group.¹⁷ Although most of the target compounds were prepared according to Scheme 2, there were some difficulties that hindered the generalization of the procedure to prepare 3(5)-chlorocaboxamides (**9a-e**), 3(5)-acetamidocarboxanilide (**10e**) and 3(5)-nitrocarboxanilide (**11e**).

In our hands it was more easy to substitute the 3(5)-amino group in the series (**8a-e**) by the required functionality as illustrated by Scheme 3. According to the nature of 3(5)-substituent on 1,2,4-triazole different synthones were used for the preparation of the imino-, semi- and thiosemicarbazides as shown by Scheme 4.

5(3)-Iminosemicarbazides were prepared by the reaction of the ester **3** or the acids **6**, and **7** with aminoguanidine bicarbonate to yield 3(5)-chloro (**9h**)-, 3(5)-acetamido (**10h**) and 3(5)-nitro (**11h**) derivatives. On the other hand 3(5)-amino 5(3)-iminosemicarbazide (**8h**) was prepared from the corresponding hydrazide **8g** and guanidine hydrochloride.

Reaction of the hydrazides **8g** and **10g** with urea in aqueous medium and in acetic acid respectively yielded the corresponding semicarbazides **8i** and **10i**. The semicarbazides **9i** and **11i** have been obtained from the reaction of the esters **3** and **5** with semicarbazide. The thiosemicarbazides were prepared from the hydrazides **8g**, **9g** and **10g** by reaction with ammonium thiocyanate in 20% HCl or in glacial acetic acid to yield **8j**, **9j** and **10j**, Scheme 4.

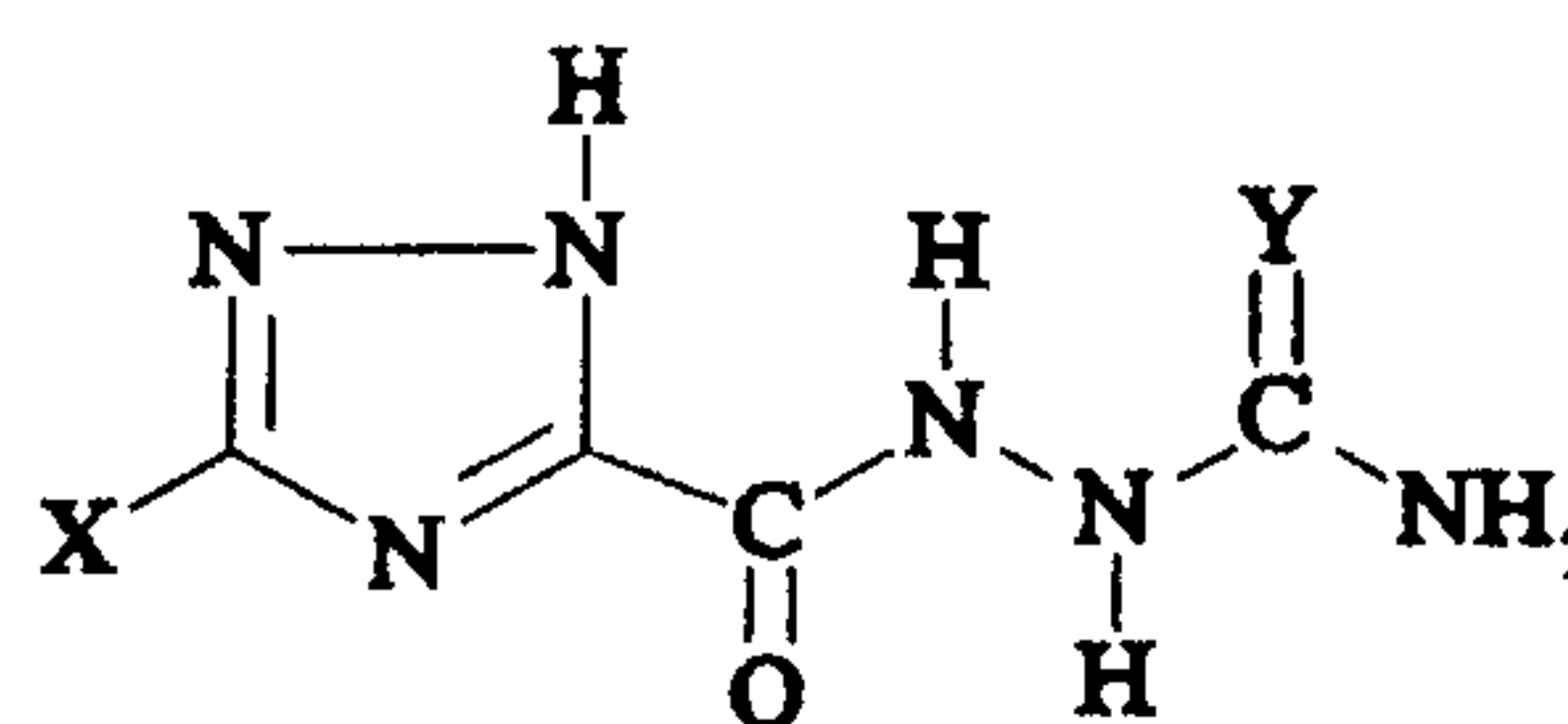
It is noteworthy that the preparation of 3(5)-nitro-1,2,4-triazole-5(3)-thiosemicarbazide was not attainable inspite of the different reaction conditioned attempted.

Activated 3(5)-nitrotriazole carboxylic acid

7 was carried out under mild condition using oxalylchloride, ethyl chloroformate or N,N'-carbonyldiimidazole reagents in THF or DMF and reacted with thiosemicarbazide at room temperature. Unsuccessful results were also obtained from the reaction of the methyl ester with thiosemicarbazide and from the reaction of the hydrazide (**11g**) with ammonium thiocyanate.

Biological results

The compounds of the 3(5)-amino-5(3)-carboxylic acid derivatives (**8a-j**), 3(5)-acetamido analogues **4**, (**10a-g,i**), and the 3(5)-nitro-5(3)-carboxylic acid hydrazide (**11g**) were tested for their growth inhibitory activity against *Staph. aureus* and *E. coli*. None of the tested compounds revealed antibacterial activity against the challenged strains at concentration up to 2.5 mg/ml.



X = NH₂, Cl, NHCOCH₃, NO₂; Y = NH, O, S

Fig. 1: Compounds subjected to glucose-tolerance test.

Reported 1,2,4-Triazoles, prepared to mimic constrained biguanide chain have revealed hypoglycemic potential.^{1,4}

The triazole derivatives depicted in Fig. 1 contains besides the 1,2,4-triazole ring a side chain that represents an isosteric substitute of noncyclized biguanide moiety. Emerged from the above observation the compounds **8h-j**, **9h-j**, **10h-j** and **11h,i** were chosen for evaluation of their potentialities to lower blood glucose level (BGL) in rats. Hyperglycemia was induced by an oral dose of 2 g/kg body weight of glucose solution (50% w/v). In the test groups of animals, each compound was given orally at a dose of 20 mg/kg body weight immediately after the glucose load. Results in rats are listed in Table 5. Thirty minutes after the administration of the tested compounds, the BGL relative to the control value was in the range of 84%, 81% and

Table 5: Effect of the investigated triazole derivatives (20 mg/kg) on blood glucose level^(a) in rats.

Compound No.	B.G.L. (mg/100 ml) at different time intervals (min)		
	30	60	120
Control (b)	122±5.8	109± 6.5	86.5±3.3
8h	103±2.3 ^(c)	109±11.5	103.5±13.6
8i	90±5.0 ^(d)	78.5±3.9 ^(d)	71±5.8 ^(c)
8j	146±8.8	93±3.9	85±5.8
9h	100±5.6 ^(c)	100.5±16.8	105±11.5
9i	94±9.9 ^(c)	96±3.9	74±8.5 ^(c)
9j	181±18	87±5.3 ^(c)	87±4.3
10h	96±8 ^(c)	99±2.6	106±6.7
10i	101±11.7	139±5.9	140±15
10j	148±10.1	155±17.6	129.5±10.7
11h	165±5	143±12.5	105±11
11i	154±10.3	99.5±12.9	72±5 ^(c)

(a) Each value represents the mean ± S.E. from 4 or 5 animals.

(b) Fasting blood glucose level 72±4, n= 24.

(c) Significantly different from the control, P < 0.05

(d) Significantly different from the control, P < 0.01.

78% for the derivatives 8h, 9h and 10h respectively, and 73%, 77% for the derivatives 8i and 9i. After one hour only 8i and 9j are 71% and 80% of the control value. After two hours 8i, 9i and 11i were 82%, 86% and 87% of the control value. It means that, the iminosemicarbazide series 8h, 9h and 10h have short duration while the semicarbazide 8i and 9i have an effect more prolonged. On the other hand the chlorothiosemicarbazide 9j and the nitrosemicarbazide 11i have shown delayed effect.

It seems that the observed variations of onset, and reduction in BGL relative to the control have resulted from mutual interaction between the 3(5)-substituted and the side chain attached to the triazole nucleus. This can be emphasized by the examination of the derivatives 8j, 10i,j and 11h which significantly did not affect the BGL or enhanced the level relative to the control value at the given time.

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