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SYNTHESIS AND ANTIMICROBIAL EVALUATION OF SOME QUINAZOLINONE DERIVATIVES

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تم فى هذا البحث تشييد العديد من مركبات الكينازولينون المحمل عليها فى موضع ٢ بعض مشتقات الثيادیازول خلال رابطة الثيوفينيل وقد تم اثبات التركيب البنائى لهذه المركبات بواسطة التحليل الدقى ودراسة أطيافها فى الأشعة تحت الحمراء والرنين النووى المغناطيسى.
كما تمت دراسة تأثير هذه المركبات على بعض البكتريا الموجبة الجرام والسالبة الجرام وكذلك أحد أنواع الفطر.

Several quinazolinone compounds were prepared having thiadiazole moiety joined to the 4(3H)-quinazolinone ring at 2-position through thiomethyl bridge. The antimicrobial activities of the prepared compounds have been investigated.

INTRODUCTION

Quinazolinone derivatives are known to possess diverse biological actions including anti-inflammatory[1-3], anti-microbial[4-6], anti-convulsant[7-9], histamine antagonists[10,11] and many other biological actions. This has promoted the synthesis of some thiadiazole derivatives of 4 (3H)-quinazolinone with the hope that such engagement might enhance the anti-microbial activity.

EXPERIMENTAL

All melting points were recorded in open glass capillaries and are uncorrected. The IR spectra were performed in Nujol mulls or KBr tablet on Beckmann 4210 spectrophotometer. The ^{1}H-NMR spectra were scanned on Varian EM-360L spectrometer, using TMS as an internal standard. Microanalysis were carried out at the Microanalytical Unit, Faculty of Science, Cairo University.

3-Aryl-2-(2-amino-1,3,4-thiadiazol-5-yl) thiomethyl-4(3H)-quinazolinones (IIIa-c)

To a well-stirred mixture of 2-amino-5-mercapto-1,3,4-thiadizole (II) (0.01 mol, 1.33 g)[12] and equimolar amount of anhydrous K_2CO_3 (1.38 g) in dry acetone (20 ml) was added gradually a solution of the appropriate 3-aryl-2-bromomethyl-4(3H)-quinazolinone (Ia-c)[13,14] (0.01 mol) in dry acetone (20 ml). The reaction mixture was stirred at room temperature for 3 hrs. The separated crystals were filtered, washed with water, dried and recrystallized from aqueous DMF (Table 1).

IR (KBr) for compound (IIIa) cm^{-1}: 3400-3250 (NH_2), 1685 (C=O), 1630 (C=N), 1320 (δ CH_2-S). ^{1}H-NMR for compound (IIIa) (DMSO-d_6), δ ppm: 4.9 (s, 2H, $\underline{CH_2}$-S), 7.10-7.83 (m, 10H, Ar-H, quin-$C_{6,7,8}$-H & NH_2), 8.36 (dd, J_1= 8, J_2= 1.5 Hz, 1H, qui-C_5-H).

3-Aryl-2-(6-aryl-imidazo[2,1-b]-1,3,4-thiadiaozl-2-yl) thiomethyl-4(3H)-quinazolinones (IVa-f)

To a solution of the selected (IIIa-c) (0.1 mol) in absolute ethanol/dry chloroform mixture (1:1, 20 ml) the appropriate phenacyl bromide (0.1 mole)[13] was added. The reaction mixture was heated under reflux for 4 hrs. The solvents were evaporated under vacuum, the residue was dissolved in ethanol (20 ml) and fused sodium acetate was then added. The mixture was heated for 10 min., then allowed to cool. The separated

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Table 1: 3-Aryl-2-(2-amino-1,3,4-thiadiazole-5-yl)thiomethyl-4(3H)-quinazolinones (IIIa-c).

Comp. No.	R	Yield %	M.P. °C	M. formula (M. wt.)	Analysis % (Calcd./Found)			
					C	H	N	S
IIIa	H	75	231-2	$C_{17}H_{13}N_5OS_2$ (367.45)	55.56 55.20	3.56 3.60	19.05 18.80	17.45 17.20
b	Br	92	237-8	$C_{17}H_{12}BrN_5OS_2$ (446.35)	45.74 45.90	2.70 2.40	15.69 15.80	14.36 14.10
c	Cl	84	213-4	$C_{17}H_{12}ClN_5OS_2$ (401.90)	59.80 60.00	3.00 2.80	17.42 17.10	15.95 16.20

Table 2: 3-Aryl-2-(6-aryl imidazo[2,1-b]-1,3,4-thiadiazol-2-yl)-thiomethyl-4(3H)-quinazolinones (IVa-f).

Comp. No.	R^1	R^2	Yield %	M.P. °C	M. formula (M. wt.)	Analysis % (Calcd./Found)				
						C	H	N	S	halogen
IVa	H	H	62	220-1	$C_{25}H_{17}N_5OS_2$ (467.58)	64.22 64.10	3.66 3.80	14.97 15.10	13.71 14.00	- -
b	Br	H	74	224-6	$C_{25}H_{16}BrN_5OS_2$ (546.48)	54.94 55.10	2.95 2.70	12.81 12.50	11.73 11.90	14.62 14.30
c	Cl	H	82	201-2	$C_{25}H_{16}ClN_5OS_2$ (502.03)	59.81 59.60	3.21 3.30	13.95 13.80	12.77 13.00	7.06 7.20
d	H	Br	81	228-9	$C_{25}H_{16}BrN_5OS_2$ (546.48)	54.94 54.80	2.95 3.20	12.81 12.90	11.73 12.10	14.62 14.80
e	Br	Br	85	232-3	$C_{25}H_{15}Br_2N_5OS_2$ (625.38)	48.01 47.80	2.41 2.20	11.19 11.40	10.25 9.90	25.25 25.80
f	Cl	Br	76	203-4	$C_{25}H_{15}BrClN_5OS_2$ (580.93)	51.69 51.30	2.60 2.90	12.06 11.90	11.03 10.80	13.58 13.50 Br

solid was filtered, washed with water, dried and recrystallized from aqueous DMF. (Table 2). IR (KBr) for compound (IVa) cm^{-1}: 1685 (C=O), 1625 (C=N), 1335 (δ CH_2-S). ^{1}H-NMR for compound (IVa) ($CDCl_3$), δ ppm: 4.8 (s, 2H, CH_2-S), 7.1-7.9 (m, 13H, Ar-H, imidazothiazole-C_5-H & quin-$C_{6,7,8}$-H), 8.3 (dd, J_1= 8, J_2= 1.5 Hz, 1H quin-C_5-H).

3-Aryl-2-[2-(substitutedcarbonylamino)-1,3,4-thiadiazol-5-yl] thiometyl-4(3H)-quinazolinones (Va-f)

To a cold solution of the selected (IIIa-c) (0.01 mol) in chloroform (15 ml), the appropriate acid chloride (0.01 mol) and triethylamine (0.011 mol, 1.5 ml) were added. The reaction mixture was stirred for 1 hr. The

solvent was evaporated on a water bath nearly to dryness. The residue was digested with water, filtered, dried and recrystallized from aqueous ethanol (Table 3). IR (KBr) for compound (Va) cm^{-1}: 3190-3160 (br. band ν NH), 1690 (acyclic amide C=O), 1680 (quin-C=O), 1640 (C=N), 1330 (CH_2-S).

^{1}H-NMR for compound (Va) (DMSO-d_6), δ ppm: 2.4 (s, 3H, CO$\underline{CH_3}$), 5.0 (s, 2H, $\underline{CH_2}$-S), 7.0-8.1 (m, 9H, Ar-H, NH & quin-$C_{6,7,8}$-H), 8.3 (dd, J_1= 8, J_2= 1.5 Hz, 1H, quin-C_5-H).

3-Aryl-2-[2-(phenylcarbamoylamino)-1,3,4-thiadiazol-5-yl]-thiomethyl-4(3H)-quinazolinones (VIa-c)

To a solution of the selected (IIIa-c) (0.01 mol) in dry benzene (20 ml) phenyl isocyanate[15] (0.011 mol, 1.49 g) was added. The reaction mixture was heated under reflux for 4 hrs, concentrated and cooled. The obtained precipitate after addition of pet. ether (20 ml) was filtered and recrystallized from benzene (Table 4). IR (Nujol) for compound (VIa) cm^{-1}: 3400 (NH), 1710 (NH-C=O), 1690 (quin-C=O), 1640 (C=N). ^{1}H-NMR for compound (VIa) ($CDCl_3$), δ ppm: 4.7 (s, 2H, $\underline{CH_2}$-S). 7.1-7.9 (m, 15H, Ar-H, NH, quin-$C_{6,7,8}$-H), 8.2 (dd, J_1= 8, J_2= 1.5 Hz, 1H, quin-C_5-H).

3-Aryl-2-[2-(ethoxycarbonylacetamido)-1,3,4-thiodiazol-5-yl] thiomethyl-4(3H)-quinazolinones (VIIa-c)

A mixture of the selected (IIIa-c) (0.01 mol) and diethyl malonate (0.01 mol, 1.6 g) in chlorobenzene (20 ml) was heated udner reflux for 5 hrs[16], then allowed to attain room temperature. The deposited crystals were separated, dried and recrystallized from aqueous DMF (Table 5). IR (Nujol) for compound (VIIa) cm^{-1}: 3120 (NH, 1720 (ester C=O), 1685 (amide-C=O), 1630 (C=O), 1280, 1050 (C-O-C). ^{1}H-NMR for compound (VIIa) (DMSO-d_6), δ ppm: 1.2 (t, 3H, CH_2-CH_3), 3.6 (s, 2H, $\underline{CH_2}$-CO), 4.2 (q, 2H, $\underline{CH_2}$-CH_3), 4.7 (s, 2H, $\underline{CH_2}$-S), 6.9-7.8 (m, 9H, Ar-H, NH, quin-$C_{6,7,8}$-H), 8.3 (dd, J_1= 8, J_2= 1.5 Hz, 1H, quin-C_5-H).

Table3: 3-Aryl-2-[2-(Substitutedcarbonlyamino)-1,3,4-thiadiazol-5-yl]-thiomethyl-4(3H)-quinazolinones (Va-f).

Comp. No.	R^1	R^2	Yield %	M.P. °C	M. formula (M. wt.)	Analysis % (Calcd./Found)				
						C	H	N	S	halogen
Va	H	CH_3	82	201-2	$C_{19}H_{15}N_5O_2S_2$ (409.49)	55.73 55.60	3.69 3.80	17.10 16.90	15.65 15.40	- -
b	Br	CH_3	87	228-9	$C_{19}H_{14}BrN_5O_2S_2$ (488.39)	46.72 46.50	2.89 3.10	14.34 14.10	13.13 12.80	16.36 16.50
c	Cl	CH_3	89	208-9	$C_{19}H_{14}ClN_5O_2S_2$ (443.94)	51.40 51.60	3.18 3.00	15.79 15.60	14.44 14.20	7.98 8.01
d	H	C_6H_5	64	236-7	$C_{24}H_{17}N_5O_2S_2$ (471.56)	61.13 61.40	3.63 3.40	14.85 15.10	13.59 13.80	- -
e	Br	C_6H_5	71	245-6	$C_{24}H_{16}BrN_5O_2S_2$ (550.46)	52.36 52.10	2.93 3.10	12.72 12.50	11.64 11.80	14.51 14.80
f	Cl	C_6H_5	90	216-8	$C_{24}H_{16}ClN_5O_2S_2$ (506.01)	56.96 57.20	3.18 3.40	13.84 14.00	12.67 12.40	7.00 6.80

Table 4: 3-Aryl-2-[2-(phenyl carbonlyamino)-1,3,4-thiadiazol-5-yl]-thiomethyl-4(3H)-quinazolinones (VIa-f).

Comp. No.	R^1	Yield %	M.P. °C	M. formula (M. wt.)	Analysis % (Calcd./Found)				
					C	H	N	S	halogen
VIa	H	81	219.10	$C_{24}H_{18}N_6O_2S_2$ (486.58)	59.24 59.50	3.72 3.60	17.27 17.10	13.17 13.40	- -
b	Br	76	229-30	$C_{24}H_{17}BrN_6O_2S_2$ (565.48)	50.97 51.10	3.03 2.90	14.86 14.50	11.34 11.50	14.13 14.30
c	Cl	75	209-11	$C_{24}H_{17}ClN_6O_2S_2$ (521.03)	55.32 55.20	3.29 3.50	16.13 16.30	12.30 12.10	6.80 7.10

Table 5: 3-Aryl-2-[2-(ethoxycarbonylacetamido)-1,3,4-thiadiazol-5-yl]-thiomethyl-4(3H)-quinazolinones (VIIa-c).

Comp. No.	R^1	Yield %	M.P. °C	M. formula (M. wt.)	Analysis % (Calcd./Found)				
					C	H	N	S	halogen
VIIa	H	72	224-5	$C_{22}H_{19}N_5O_4S_2$ (481.56)	54.87 55.10	3.98 4.10	14.54 14.60	13.31 13.00	- -
b	Br	86	243-4	$C_{22}H_{18}BrN_5O_4S_2$ (560.46)	47.14 46.80	3.23 3.40	12.49 12.20	11.44 11.10	14.26 14.50
c	Cl	79	231-2	$C_{22}H_{18}ClN_5O_4S_2$ (516.01)	51.21 51.50	3.52 3.40	13.37 13.30	12.42 12.70	- -

Table 6: 3-Aryl-2-(1,3,4-thiadiazol[3,2-a]pyrimidine-5,7-dion-2-yl)thiomethyl-4(3H)-quinazolinones (VIIIa-c).

Comp. No.	R^1	Yield %	M.P. °C	M. formula (M. wt.)	Analysis % (Calcd./Found)				
					C	H	N	S	halogen
VIIIa	H	70	211-2	$C_{20}H_{13}N_5O_3S_2$ (435.49)	55.16 55.30	3.00 3.20	16.08 15.80	14.73 14.50	- -
b	Br	87	232-4	$C_{20}H_{12}BrN_5O_3S_2$ (514.39)	46.70 47.00	2.35 2.60	13.61 13.50	12.47 12.60	15.53 15.20
c	Cl	82	227-8	$C_{20}H_{12}ClN_5O_3S_2$ (469.94)	51.12 51.40	2.57 2.40	14.90 15.10	13.64 13.30	7.54 7.70

3-Aryl-2-(1,3,4-thiadiazolo[3,2-a]-pyrimidine-5,7-dion-2-yl) thiomethyl-4)3H)-quinazolinones (VIIIa-c)

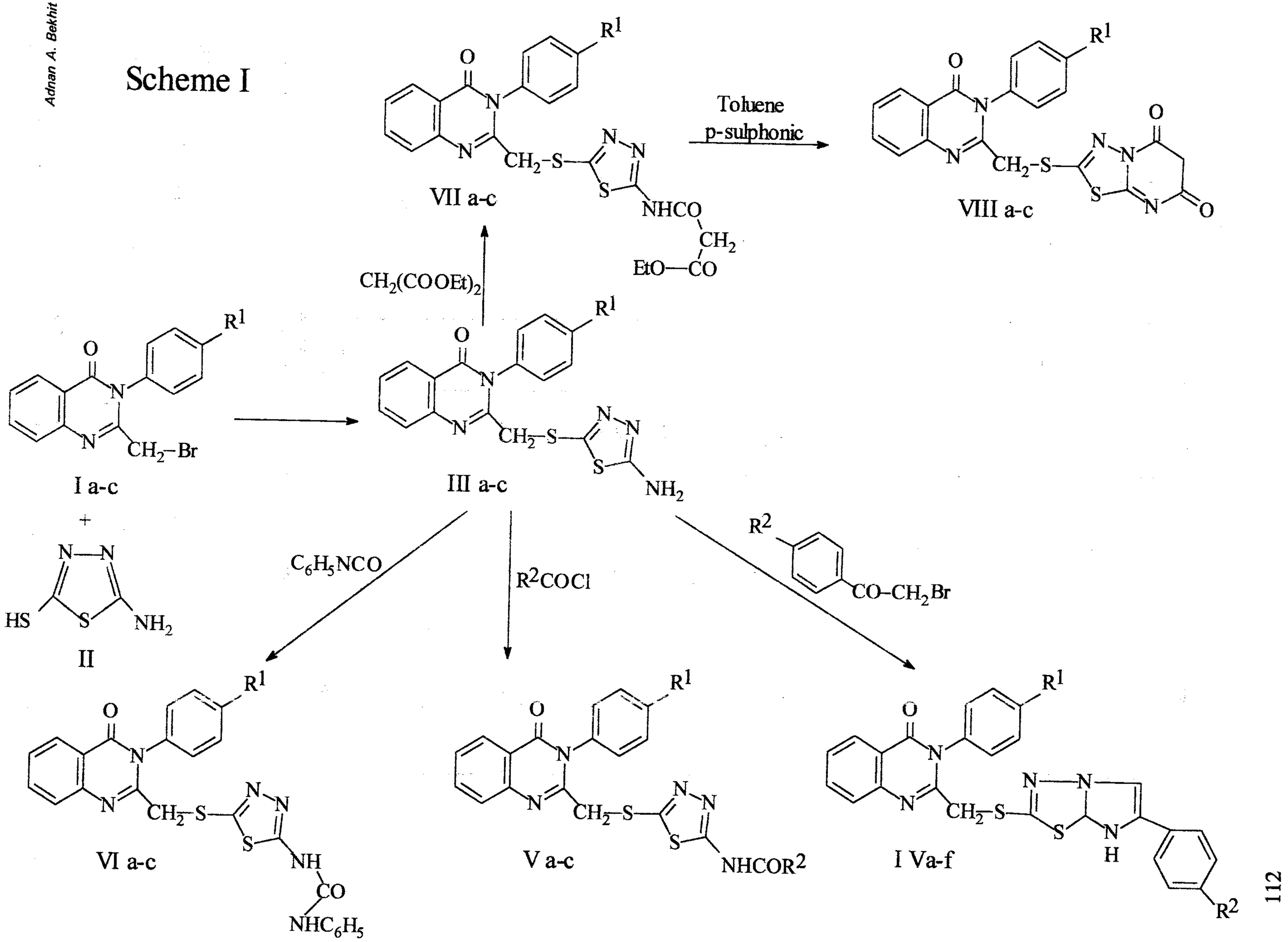
A suspension of the appropriate (VIIIa-c) (0.001 mol) and 100 mg toluene-p-sulphonic acid monohydrate in toluene (20 ml)[16] was heated under reflux for 20 hrs. The reaction mixture was cooled and the separated solid product was filtered, dried and recrystallized from aqueous ethanol (Table 6). IR (Nujol) for compound (VIIIa) cm^{-1}: 1685 (quin-C=O), 1660 (C=O), 1635 (C=N). ^{1}H-NMR for compound (VIIIa) ($CDCl_3$), δ ppm: 4.4 (s, 2H, pyrimidine-CH2), 4.8 (s, 2H, $\underline{CH_2}$-S), 7-7.9 (m, 8H, Ar-H & quin-$C_{6,7,8}$-H), 8.2 (dd, J_1= 8, J_2= 1.5 Hz, 1H, quin-C_5-H).

Table 7: The inhibition zones (I.Z.) in mm and minimal inhibitory concentration (MIC) in μg/ml.

Compound No.	*E. coli*		*S. aureus*		*C. albicans*
	I.Z.	MIC	I.Z.	MIC	I.Z.
III a	16	200	12	100	18
b	-	-	16	100	22
c	12	200	34	12.5	16
IV a	14	100	32	25	24
b	16	200	22	50	28
c	24	100	18	100	22
d	20	100	24	50	22
e	14	200	28	50	14
f	26	50	20	100	18
V a	14	200	20	100	25
b	-	-	30	50	28
c	-	-	32	12.5	28
d	-	-	28	50	27
e	28	50	20	100	32
f	12	200	22	100	30
VI a	16	200	24	50	34
b	-	-	14	100	18
c	18	200	20	100	26
VII a	-	-	-	-	31
b	22	100	16	200	
c	26	50	32	50	
VIII a	18	50	22	100	
b	32	25	24	100	
c	18	100	28	50	
Penicillin G	-	-	42	4	
Streptomycin	28	6			
Clotrimazole*					47

* Cansten®, Bayer.

Scheme I


R1
O
N
N
CH2–S
N
N
S
NHCO
CH2
EtO—CO
VII a-c
Toluene
p-sulphonic
VIII a-c
CH2(COOEt)2
CH2–Br
I a-c
III a-c
NH2
+
HS
S
NH2
II
C6H5NCO
R2COCl
R2
CO–CH2Br
VI a-c
NH
CO
NHC6H5
V a-c
NHCOR2
I Va-f
H

Preliminary antimicrobial testing

The antimicrobial activity for all the synthesized compounds was preliminary evaluated for *in vitro* activity against *Staphylococcus aureus* NCTC 4163 as Gram-positive bacteria, *Escherichia coli* 5933 as Gram-negative bacteria and *Candida albicans* 3501 for fungus using the cup diffusion technique[17]. Further evaluation was then carried out on compounds that exhibited reasonable inhibition zones ($\geq$ 20 mm), to determine their minimal inhibitory concentration (MIC) values using two fold serial dilution method.

For inhibition zone measurement, the compounds were dissolved in propylene glycol in a concentration of 1 mg/ml sterile nutrient agar (oxoid) which was incubated with the tested organisms. Solution of Penicillin G, Streptomycin or Clotrimazole (0.1%) in propylene glycol was used as a standard. The inhibition zones were measured in mm.

For the MIC measurement, the serial dilution method was adopted. The results are recorded in Table 7.

RESULTS AND DISCUSSION

The designed compounds were prepared as outlined in scheme I. The key compounds, 3-aryl-2(2-amino-1,3,4-thiadiazol-5-yl)thiomethyl-4(3H)-quinazolinones (IIIa-c), were considered as very fruitful intermediates for the preparation of diverse fused heterocyclic systems. These intermediates in their turn were successfully obtained by heating (Ia-c) with 2-amino-5-mercapto-1,3,4-thiadiazole (II). Treating (IIIa-c) with the appropriate p-substituted phenacyl bromide revealed the corresponding imidazo[2,1-b]thiazole derivatives (IVa-f), whereas heating (IIIa-c) under reflux with acetyl or benzoyl chloride resulted in the corresponding substituted carbamoylamino-1,3,4-thiadiazole derivatives (Va-f). On the other hand, condensation of (IIIa-c) with phenyl isocyanate achieved the corresponding phenylcarbamoylamino-1,3,4-thiadiazole derivatives (VIa-c). Moreover, when compounds (IIIa-c) were heated separately with diethyl malonate in chlorobenzene[16] the corresponding(ethoxycarbonylacetamido)-1,3,4-thiodiazole derivatives (VIIa-c) were obtained. The latter compounds underwent cyclization into the corresponding thiazolo[3,2-a]pyrimidine-5,7-dione derivatives (VIIIa-c) by heating in toluene-p-sulphonc acid[16].

It became obvious from the aforementioned antimicrobial testing (Table 7), that compounds having thiadiazole moiety with free amino group posses slight activity against *Staphylococcus aureus* and *Candida albicans*. Compounds with imidazo[2,1-b]1,3,4-thiadiazol moiety (IVc,d&f) proved to be active against both *Escherichia coli* and *Candida albicans*, whereas compound (IVe) showed activity against *Staphylococcus aureus*. Moreover, when the free amino group was acylated, the activity was enhanced against *Staphylococcus* and *Candida albicans*, as clearly shown in compounds (Va,c,&d). On the other hand, when the amino group was converted into the corresponding (phenyl-carbamoylamino) function (VIa-c), the activity was greatly diminished. Furthermore, conversion of the free amino group into the (ethoxycarbonylacetamido) moiety (VIIb,c) resulted in activity comparable to that of streptomycin. Compound (VIIIb) having thiadiazolo[3,2-a]pyrimidine-5,7-dione moiety exhibited activity superior than streptomycin against *Escherichia coli*.

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