Syntheses of Novel 3-Amino-2(1*H*)-thioxo-4(3*H*)-quinazolinones and Evaluation of Their Immunotropic Activity. Part III [1)]

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Summary

The synthesis of two series of derivatives containing the quinazolinone-4 moiety is described. 3-Amino-2(1*H*)-thioxo-4(3*H*)-quinazolinone (**1**) was subjected to reactions with halogenoketones and halogenoaldehydes, leading to the production of the corresponding ketones, aldehydes, Schiff bases, and 6-oxo-1,4,5-thiadiazin[2,3-*b*]quinazoline derivatives. Subsequently, **1** was condensed with selected α,β-unsaturated carbonyl compounds, aldehydes, ketones, acid chlorides, and esters. The compounds were tested for their potential activity in a model of humoral and cellular immune response. The tests showed that the compounds exhibited differential immunotropic activities. Of particular interest is compound **19**, exhibiting a strong stimulatory activity with regard to cellular immune response and compound **16** exerting a strong inhibitory action in both types of the immune response.

Introduction

Quinazolinone-4 derivatives have been found to be biologically versatile compounds, having antimalarial, hypnotic, anticonvulsant, antipyretic, analgesic, antiinflammatory, diuretic, antihypertensive, antitubercular (febrifugine), bronchodilator, and other diverse activities [1,2]. Over 30 drugs, derivatives of quinazolinone-4, have so far been registered worldwide, most of them being diuretics and drugs for the Central Nervous System [3,4]. Only a few of them, e.g. Methaqualone, Metolazone, Ketanserin, play an important role in a modern pharmacotherapy.

There are some compounds among the quinazolinone-4 derivatives synthesized recently which have not been yet described pharmacologically, e.g. radiosensitizing [5], antiulcer [6], anticancer [7] agents.

Tricyclic quinazolinone-4 derivatives are also biologically active, exhibiting CNS depressive [8], analgesic [9], and antibacterial (*Mycobacterium tuberculosis* and *M. leprae*)[10] activity. In our recent review[11] we presented the most interesting examples of anticonvulsant and antiinflammatory active quinazolinones-4 synthesized after 1990 and also some other derivatives belonging to a new class of pharmacologically active compounds, i.e. angiotensin II and cholecystokinin receptor antagonists.

[1)] Part II. W. Nawrocka, J. J. Staśko, *Polish J. Chem.* **1997**, *71*, 792–796.

Results and Discussion

Chemistry

The reactions of 3-amino-2(1*H*)-thioxo-4(3*H*)-quinazolinone (**1**) with acetic acid derivatives[12] and cyanogen bromide[13] have been described previously. The aim of this work was to examine a possibility of the synthesis of novel derivatives of 6-oxo-1,4,5-thiadiazin[2,3-*b*]quinazoline and the products of the reaction of 3-amino-2(1*H*)-thioxo-4(3*H*)-quinazolinone (**1**) with selected α,β-unsaturated carbonyl compounds. Another goal of this study was to investigate effects of the synthesized compounds upon the development of humoral and cellular immune response.

Quinazolinone **1** and some halogenoketones and halogenoaldehydes were the substrates for the synthesis of tricyclic derivatives. The reaction with phenacyl bromide in glacial acetic acid at room temperature yields 3-amino-2-phenacylthio-4(3*H*)-quinazolinone (**2**) as the product of *S*-alkylation. Under the above conditions, reactions with other ketones were not observed. While heating compound **1** with phenacyl bromide undergoes cyclization to product **3**, with 4-methoxyphenacyl bromide forms a mixture of ketone **4** and tricyclic **5** and finally with chloroacetone, 3-amino-2-acetonylothio-4(3*H*)-quinazolinone (**6**) is obtained. Increasing the heating time allowed a single, tricyclic product **5** to be obtained but not in the case of the cyclization reaction with chloroacetone (Scheme 1).

Heating of sodium salt of quinazolinone **1a** [12] with chloroacetone in boiling diethylene glycol dimethyl ether (diglyme) resulted in formation of 2*H*-3-methyl-6-oxo-1,4,5-thiadiazin[2,3-*b*]quinazoline (**7**).

The structures of the products obtained were confirmed by the results of elemental analysis and IR, ^{1}H NMR, and MS spectra. The bands 3400 and 3320 cm^{-1} characteristic for the -NH_2 groups and 1700 cm^{-1} characteristic for the C=O groups are present in the IR spectra of the compounds **2**, **4**, **6**. In addition, the ^{1}H NMR spectra displayed two singlet peaks at δ 6.63 ppm and 5.17 ppm, which were assigned to the NH_2 and -SCH_2CO- protons, respectively. The shift in position of methylene protons can be explained by the interaction of the CH_2 group and the shielding effect due to the vicinity of the protons. In the MS spectra of ketones **2**, **4**, and **6**, splittings at the α carbon were recorded. Assignment of cyclic structures **3**, **5**, and **7** was based on the observation that NH_2 groups were absent in their IR and ^{1}H NMR spectra and the -SCH_2C= protons were shifted to higher fields, δ 4.94–4.49 ppm.

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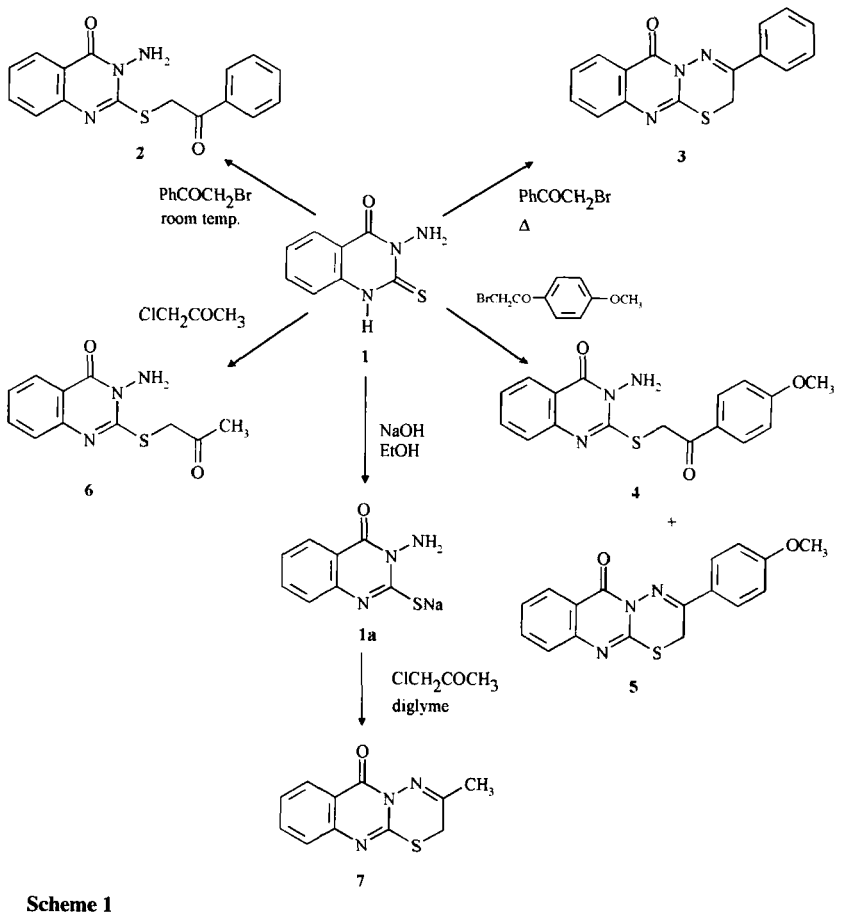
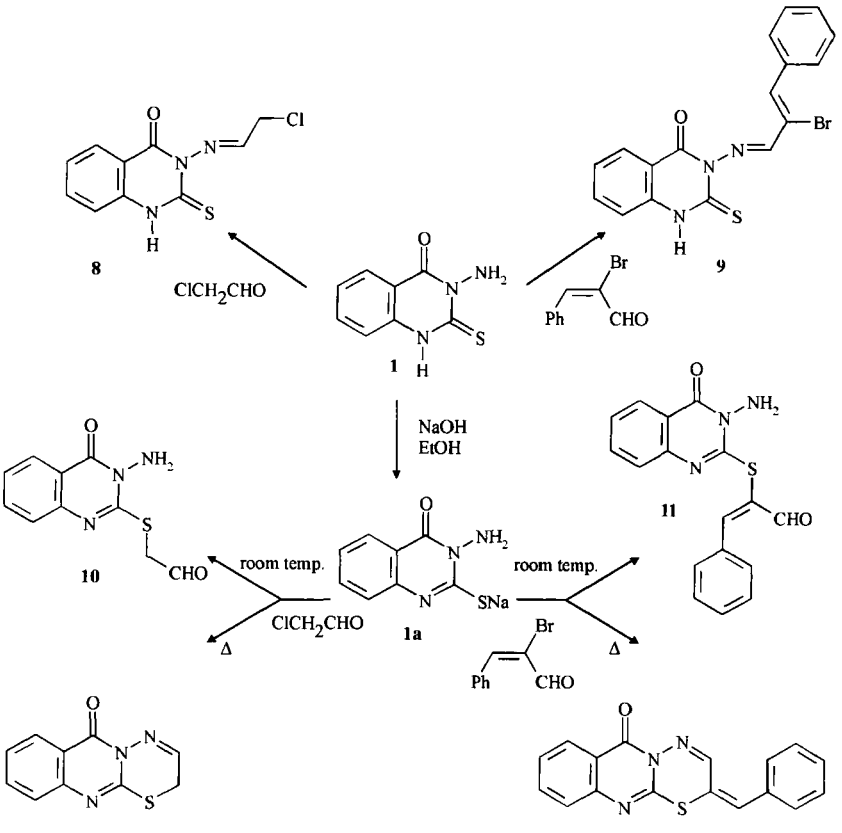
Quinazolinone **1** was then subjected to reaction with chloroacetic and α-bromocinnamic aldehydes in glacial acetic acid (Scheme 2). After such treatment cyclic products **12** and **13**, aldehydes **10** and **11**, Shiff bases **8** and **9** or mixtures of them could be expected. However, in both reactions a similar array of products containing Schiff bases **8** and **9** was found. Assignment of their structures results from the following observations. The NH_2 and CHO groups are not present in the IR and ^1H NMR spectra of the compounds **8** and **9**. Their MS spectra display base peaks *m/z* 36 and *m/z* 80, indicating the elimination of HCl and HBr from products **8** and **9**, respectively.

In the reaction of quinazolinone **1a** with halogenoaldehydes in diglyme *S*-alkylation was observed at room temperature, resulting in the formation of aldehydes **10** and **11**. Their structures were confirmed by ^1H NMR spectra, each of which contained signals at δ 6.67, 6.51 ppm and δ 9.73, 9.57 ppm corresponding to $-NH_2$ and -CHO groups, respectively. When the reaction mixture was heated to boiling temperature two tricyclic products **12** and **13** were obtained. Both signals related to $-NH_2$ and CHO groups disappeared when the cyclization was accomplished and methine proton signals were present in the spectra.

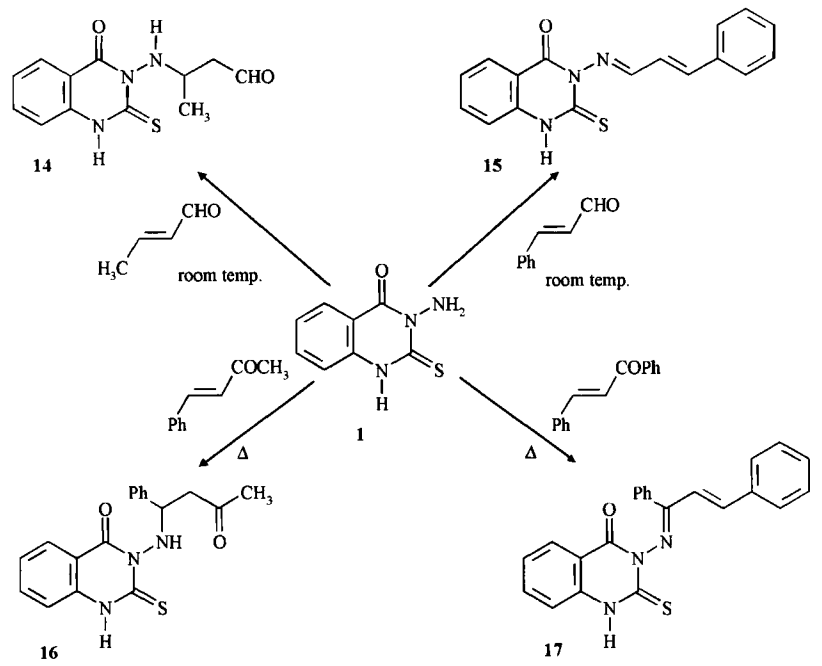
The new series of compounds was formed in the reactions of quinazolinone **1** with selected α,β-unsaturated carbonyl compounds. Quinazolinone **1** reacted in glacial acetic acid with α,β-unsaturated crotonoyl and cinnamoyl aldehydes yielding corresponding aldehyde **14** and Schiff base **15** (Scheme 3) at room temperature. Thus both quinazolinone **1** and 2-amino-5-oxo-1,3,4-thiadiazol[2,3-*b*]quinazoline, described earlier[13], undergo similar rearrangement. Subsequent application of α,β-unsaturated ketones, benzylideneacetone, or benzylideneacetophenone in boiling ethanol led to the formation of ketone **16**, the product of addition to double bond or imine **17.**



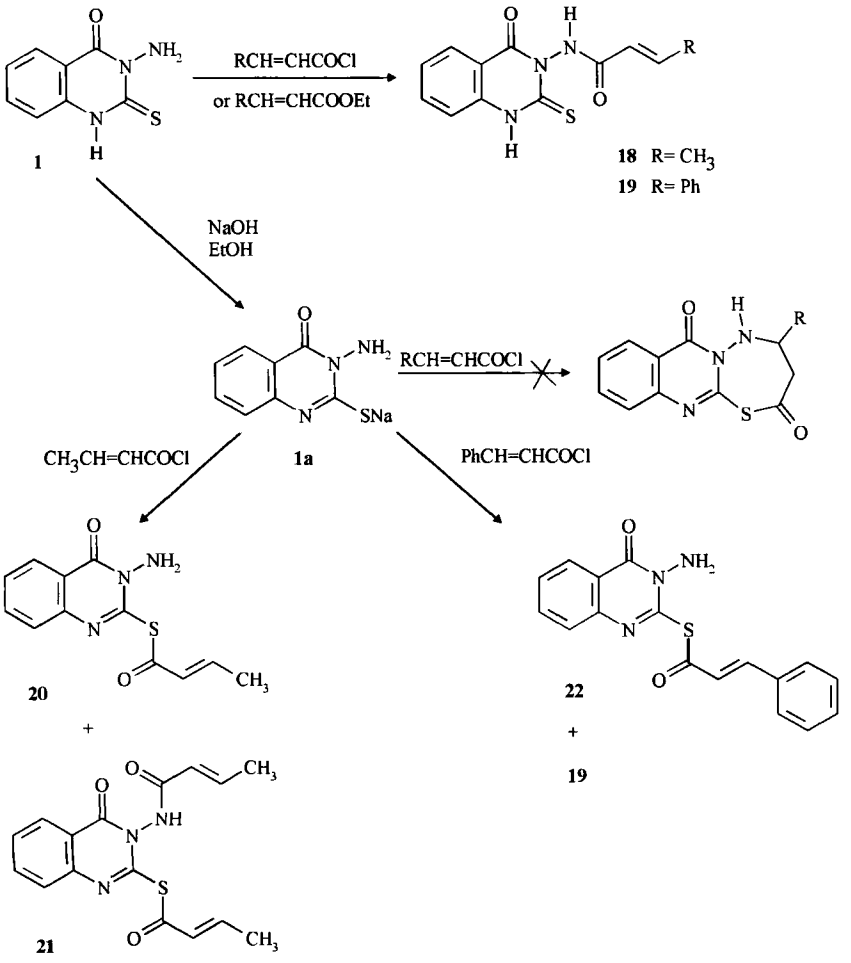
Scheme 1



Scheme 2



Scheme 3



Scheme 4

Similarly to the MS spectra of ketones **2**, **4**, **6**, the spectrum of ketone **16** displays the peaks related to the fragmentation at the α carbon position. In the spectrum of imine derivative **17**, the signals due to the fragmentation of α,β C-C bond adjacent to imine nitrogen were recorded. The products **14–17** of two last reactions depend on the substituent at the double bond, carbonylic groups and experimental conditions. The subsequent reaction of 3-amino-2(1*H*)-thioxo-4(3*H*)-quinazolinone (**1**) with chlorides of α,β-unsaturated acids: crotonoyl or cinnamoyl in THF at room temperature (Scheme 4) yielded in both cases amides: 3-crotonoylamino-2(1*H*)-thioxo- quinazolinone (**18**) and 3-cinnamoyl- amino-2(1*H*)-thioxo-quinazolinone (**19**). Amides **18** and **19** were also obtained when quinazolinone **1** was heated to its boiling temperature together with ethyl crotonate or ethyl cinnamate in diglyme. After such treatment of quinazolinone **1a** formation of tricyclic derivatives was expected. In both reactions there were obtained mixtures as evidenced by TLC. Analytical data confirmed that in the reaction of crotonoyl chloride, 3-amino-2-crotonoylthio-4(3*H*)-quinazolinone (**20**) and small amounts of 3-crotonoylamino-2-crotonoylthio- 4(3*H*)-quinazolinone (**21**) were formed. In the case of the cinnamoyl chloride reaction, 3-amino-2-cinnamoylthio-4(3*H*)-quinazolinone (**22**) and trace amounts of amide **19** were produced. The structures of the compounds **20** and **22** were confirmed by ^{1}H NMR spectra in which amino and vinylene protons signals are present. The obtained compounds **2–22** contain in their structure pharmacofore groups. Among them there are tricyclic derivatives containing 1,3,4-thiadiazines[14,15] condensed with quinazolinone-4, Schiff bases[15,16] including cinnamylideneamino groups[17], ketones[18], amides of cinnamic acid[19]. Such chemical structures suggest their biological activities.

Immunology

Effect of the compounds on the humoral and cellular immune responses to sheep erythrocytes in mice

The compounds were given to mice intraperitoneally, at doses of 10 and 100 μg/mouse, 3 h after immunization. The reference compounds – cyclosporin A and levamisole – were administered in a similar way. On day 4 following immunization the number of antibody secreting cells (PFC) was determined according to Jerne in a modification of Mishell and Dutton's method [20]. The results presented in Table 1 show that the preparations exerted different actions on generation of the humoral immune response *in vivo*. Preparation **19** did not exhibit statistically significant actions in both studied doses.

Table 1. Effects of the compound on the humoral immune response in CBA mice.

Compound	μg/mouse	X Mean	±SE	*P* Student test
Control		1164	128	
CSA	10	893	30	NS
	100	524	29	<0.001
Levamisole	10	1253	104	NS
	100	1857	138	<0.01
19	10	950	41	NS
	100	1554	198	NS
22	10	1332	143	NS
	100	1794	58	<0.01
16	10	1066	118	NS
	100	515	18	<0.001

Preparation **22** raised the PFC number at a dose of 100 μg/mouse; this effect was comparable to that of levamisole. In contrast, preparation **16** exhibited, at the higher dose, a strong inhibitory action, similar to that observed for cyclosporin A.

Table 2 demonstrates the effects of the compounds on the development of delayed hypersensitivity to SRBC in 129Ao/Boy mice. The preparations were given 3 h after sensitization of mice with SRBC. After 4 days the delayed hypersensitivity reaction was elicited by administration of an intradermal dose of SRBC (immunological tests).

Table 2. Effect of the compounds on delayed type hypersensitivity to SRBC in 129 mice.

Compound	μg/mouse	X Mean	±SE	*P* Student test
Control		13.37	0.84	
CSA	10	10.50	0.75	<0.05
	100	6.84	1.11	<0.01
Levamisole	10	13.27	1.61	NS
	100	15.50	1.11	NS
19	10	12.00	0.84	NS
	100	20.17	1.93	<0.01
22	10	12.00	0.42	NS
	100	18.00	1.43	<0.01
16	10	9.00	0.62	<0.01
	100	6.17	0.42	<0.001

Preparation **19** (Table 2) at a dose of 100 μg showed a strong stimulatory action in this type of the immune response. Similar, although a weaker action, was exerted by preparation **22**. On the other hand, preparation **16** demonstrated a very strong suppressive property, even at the low dose. Its action was stronger than that of cyclosporin A.

Structure-Activity Relationship

The studied compounds exhibited different and statistically significant immunotropic activities. Amide **19** appeared to be a strong stimulator of the cellular response, thioester **22** was a general stimulator of both types of the immune response, and ketone **16** was a universal, strong inhibitor of the immune response.

Derivatives **16** and **22** in which amine group at position 3 of quinazolinone **1** is substituted by carbonyl substituents exhibit a strong immunotropic activity. On the other hand, substitution at position 2, compound **22**, leads to a weaker action stimulating the cellular immune response.

It seems that compounds **19** and **16** are most interesting and could be the subject of further studies aimed at their potential applications in therapy. Compounds showing similar activity to **19** are thus able to stimulate the cellular immune response in a selective way, and may be useful for combating infections caused by intracellular pathogens, where development of the cellular immune response is desirable.

Evaluating preparation **16**, which appeared to strongly suppress both types of the immune response with an activity comparable to that of CSA, we may envisage its application for diminution of the immune reactivity of patients subjected to transplantations. The potential therapeutic application of the described compounds would depend, however, on their low toxicity, accessibility and effectiveness of oral treatment.

Experimental

Chemistry

Melting points (uncorrected) were measured with a Boethius melting point apparatus. Analyses were performed on a Perkin Elmer 2400 analyser and satisfactory results within ± 0.04% of calculated values were obtained for the new compounds. IR spectra (in KBr) were recorded with an IR 75 spectrophotometer, ^{1}H NMR spectra on a Tesla BS 587 (80 MHz) and a Bruker ADVANCE DRX 300 using TMS as an internal standard. Mass spectra were determined on a GCMS -LK 82091 spectrometer at an ionisation energy of 15 or 70 eV. The course of reaction and the purity of products were checked by TLC (Kiesselgel G. Merck) in diethyl ether : ethanol = 5:1 for eluation.

The reaction of 3-amino-2(1H)-thioxo-4(3H)-quinazolinone (1) with halogenoketones: phenacyl bromide, 4-methoxyphenacyl bromide, and chloroacetone, 2–6

A mixture of quinazoline **1** (1.93g, 0.01 mol), 0.01 mol of appropriate halogenoketones (phenacyl bromide, 4-methoxyphenacyl bromide or chloroacetone) and glacial acetic acid (30 ml) was stirred at room temperature for 72 h for compound **2** or stirred and refluxed for 5–10 h for compounds **3–6**. The precipitate was collected by filtration, neutralized with 5% aq. $NaHCO_3$ solution, washed with water, dried, and crystallized.

The mixture of derivatives **4** and **5** was extracted with boiling *n*-butanol (4 × 50 ml). The extract was concentrated and compound **4** was obtained. Insoluble residue was crystallized from DMF to obtain compound **5**.

3-Amino-2-phenacylthio-4(3H)-quinazolinone (2)

Yield 2.70 g (87%), white solid from *n*-BuOH or acetonitrile, mp 150–151 °C.– IR (KBr): ν cm^{-1} = 3400, 3280 (NH_2), 1700, 1680 (CO), 1490 (C=N), 1450 (-CH_2-), 1210, 705 (C-S), 770 (CH aromatic).– ^{1}H-NMR (DMSO): δ = 5.17 (s, 2H), 6.52–6.73 (m, 2H), 6.63 (s, 2H), 6.86–7.37 (m, 3H), 7.51–7.73 (m, 2H), 8.03–8.15 (m, 2H).– Anal.: $C_{16}H_{13}N_3O_2S$ (311.36).– MS (70 eV): *m/z* (%) = 313 (10), 311 (99), 206 (5), 160 (7), 121 (5), 120 (89), 118 (27), 106 (13), 105 (100), 28 (5).

3-Phenyl-2H-1,4,5-thiadiazin[2,3,-b]iunazolin-6-one (3)

Yield 1.90 g (65%), white powder from DMF, mp 205–207 °C.

IR (KBr): ν cm^{-1} = 2900 (CH), 1670 (CO), 1490 (C=N), 1490 (-CH_2-), 1200, 760 (CH), 705 (C-S).– ^{1}H-NMR (DMSO): δ = 4.92 (s, 2H), 6.61–6.71 (m, 2H), 6.81–7.26 (m, 2H), 7.36–7.71 (m, 3H), 7.97–8.08 (m, 2H).– Anal.: $C_{16}H_{11}N_3OS$ (293.34).

3-Amino-2(p-methoxyphenacylthio)-4(3H)-quinazolinone (4)

Yield 1.26 g (37%), crystalized from *n*-BuOH, mp 276–278 °C.– IR (KBr): ν cm^{-1} = 3420, 3300 (NH_2), 2900 (CH or $ArOCH_3$), 1700, 1680 (CO), 1485 (C=N), 1450 (-CH_2-), 1200, 760 (CH, aromatic), 705 (S-CH).– ^{1}H-NMR (DMSO): δ = 3.87 (s, 3H), 5.16 (s, 2H), 6.43 (s, 2H), 7.10 (d, *J* = 8 Hz, 2H), 7.71 (m, 4H), 8.17 (m, 2H).– Anal.: $C_{17}H_{15}N_3O_3S$ (341.38).– MS (70 eV): *m/z* (%) = 343 (5), 342 (11), 341 (64), 162 (9), 144 (8), 137 (4), 136 (54), 135 (100), 121 (20), 107 (27), 102 (12), 92 (26), 77 (55).

3-(p-Methoxyphenyl)-2H-1,4,5-thiadiazin[2,3-b]quinazolin-6-one (5)

Yield 1.40 g (43%), white solid from DMF, mp 176–178 °C.– IR (KBr): ν cm^{-1} = 2900 (CH), 2840 ($ArOCH_3$), 1700 (CO), 1660 (C=N), 1320 (-N=), 1200, 770 (CH aromatic), 705 (C-S).– ^{1}H-NMR (DMSO): δ = 3.88 (s, 3H), 4.50 (s, 2H), 7.10 (d, *J* = 8 Hz, 2H), 7.54 (m, 2H), 7.87 (m, 4H).– Anal.: $C_{17}H_{13}N_3O_2S$ (323.37).

2-Aceontylthio-3-amino-4(3H)-quinazolinone (6)

Yield 2.12 g (85%), white crystals from *n*-BuOH, mp 150–152 °C.– IR (KBr): ν cm^{-1} = 3400, 3320 (NH_2), 2900 (CH_3), 1710, 1690 (CO), 1480 (C=N, -CH_2-), 770 (CH aromatic), 705 (C-S).– ^{1}H-NMR (DMSO): δ = 2.45 (s, 3H), 5.16 (s, 2H), 6.74 (br, 2H), 6.93–7.26 (m, 1H), 7.37–8.13 (m, 3H).– Anal.: $C_{11}H_{11}N_3O_2S$ (249.29).– MS (15 eV): *m/z* (%) = 251 (5), 250 (12), 249 (100), 207 (12), 160 (11), 120 (78), 108 (38), 105(10), 91 (8), 43 (3).

Reaction of quinazolinone ***1a*** *with chloroacetone* ***7****, chloroacetyl aldehyde,* ***10, 12*** *and α-bromocinnamic aldehyde,* ***11,13****.*

A mixture of quinazolinone **1a** [12] (2.16g, 0.01 mol) and 0.01 mol of chloroacetone or chloroacetyl aldehyde or α-bromocinnamic aldehyde in diglyme (30 ml) was stirred at room temperature for compounds **10**, **11**, or stirred and refluxed for 5 h for compounds **7**, **12**, **13**. On completion of the reaction the mixture was cooled. The precipitate was collected by filtration, washed with diethyl ether and water, dried, and crystallized.

3-Methyl-2H-1,4,5-thiadiazin[2,3-b]quinazolin-6-one (7)

Yield 2.00 g (87%), white precipitate from *n*-BuOH, mp 198.5–201 °C.– IR (KBr): ν cm^{-1} = 2900 (CH), 1690 (CO), 1540 (C=N), 1460 (-CH_2-), 1000, 770 (CH aromatic), 705 (C-S).– ^{1}H-NMR (DMSO): δ = 2.35 (s, 3H), 4.49 (s, 2H), 7.56 (t, 1H), 7.66 (d, J = 8.2 Hz, 1H), 7.87 (m, 1H), 8.25 (m, 1H).– Anal.: $C_{11}H_9N_3OS$ (231.27).

3-Amino-2-formylmethylthio-4(3H)-quinazolinone (10)

Yield 1.74 g (74%), yellow powder from ethanol, mp 210–212 °C.– IR (KBr): ν cm^{-1} = 3400, 3320 (NH_2), 2900, 2750 (CH), 1720 (-CH_2COH), 1690 (CO), 1560 (C=N), 1350 (NH_2), 760 (CH aromatic).– ^{1}H-NMR (DMSO): δ = 4.43 (d, 2H), 6.67 (s, 2H), 7.04 (m, 1H, ArH_8), 7.43 (m, 1H, ArH_6), 7.55 (m, 1H, ArH_7), 7.85 (m, 1H, ArH_5), 9.73 (t, 1H).– Anal.: $C_{10}H_9N_3O_2S$ (235.26).– MS (15 eV): *m/z* (%) = 236 (5), 235 (38), 234 (3), 209 (16), 208 (100), 207 (78), 179 (17), 161 (22), 131 (64),105 (70), 103 (54), 77(60), 43 (1).

3-Amino-2-(α-formylstyrylthio)-4(3H)-quinazolinone (11)

Yield 2.15 g (67%), yellow powder from *n*-BuOH, mp 162–165 °C.– IR (KBr): ν cm^{-1} = 3400, 3340 (NH_2), 3000, 2800 (CH), 1710 (C=CCHO), 1680 (CO), 1630 (C=N), 1405, 760 (CH), 705 (C-S).– ^{1}H-NMR (DMSO): δ = 6.51 (s, 2H), 7.10–7.83 (m, 6H), 7.85–7.93 (m, 3H), 8.30 (s, 1H), 9.57 (s, 1H).– Anal.: $C_{17}H_{13}N_3O_2S$ (323.37).– MS (15 eV): *m/z* (%) = 325 (6), 324 (15), 323 (75), 235 (11), 193 (59), 160 (30), 131 (44), 120 (99), 119 (17), 118 (100), 92 (11), 77 (10), 59 (11).

2H-1,4,5-Thiadiazin[2,3-b]quinazolin-6-one (12)

Yield 1.40 g (65%), yellow powder from ethanol, mp 262–264 °C.– IR (KBr): ν cm^{-1} = 3300 (N=CH), 2920 (-CH_2-), 1690 (CO), 1650 (C=N), 1480 (-CH_2-), 760 (CH, aromatic), 705 (C-S).– ^{1}H-NMR (DMSO): δ = 3.30 (d, *J* = 5 Hz, 2H), 6.89 (d, *J* = 8.1 Hz, 1H, ArH_8), 7.27 (m, 1H, ArH_6), 7.46 (m, 1H, ArH_7), 7.57 (m, 1H, ArH_5), 7.72 (t, 1H).– Anal.: $C_{10}H_7N_3OS$ (217.25).– MS (15 eV): *m/z* (%) = 219 (4), 218 (8), 217 (12), 202 (7), 193 (100), 178 (27), 177 (61), 175 (31), 162 (47), 120 (97), 119 (31), 104 (20), 92 (23), 77 (7), 76 (45).

2-Benzylidene-1,4,5-thiadiazin[2,3-b]quinazolin-6-one (13)

Yield 1.60 g (52%), dark-yellow powder from DMF, mp 274–277 °C.– IR (KBr): ν cm^{-1} = 3300 (C=N), 2900 (CH), 1690 (CO), 1630 (C=CH-Ph), 1480 (C=N), 1150, 760 (CH, aromatic), 705 (C-S).– ^{1}H-NMR (DMSO): δ = 6.87 (m, 3H), 7.75 (m, 2H), 7.85 (m, 2H), 7.92 (m, 2H), 8.43 (s, 1H), 8.48 (s, 1H).– Anal.: $C_{17}H_{11}N_3OS$ (305.35).– MS (15 eV): *m/z* (%) = 305 (4), 246 (9), 245 (44), 212 (90), 204 (43), 193 (56), 178 (78), 162 (76), 120 (94), 119 (100), 92 (14).

Reaction of quinazolinone 1 with chloroacetyl, α-bromocinnamoyl, crotonoyl, and cinnamoyl aldehydes, ***8, 9, 14, 15***

The compounds **8**, **9**, **14**, and **15** were prepared similarly as described previously[13].

3-(2-Chloroethylideneamino)-2(1H)-thio-4(3H)-quinazolinone (8)

Yield 1.60 g (65%), yellow precipitate from ethanol, mp 163–165 °C.– IR (KBr): ν cm^{-1} = 3300 (N=CH), 2900 (CH), 1690 (CO), 1670 (C=N), 1250 (NH-C=S, -CH_2Cl), 760 (CH aromatic).– ^{1}H-NMR (DMSO): δ = 4.12 (m, 2H), 5.89 (m, 1H), 6.97–7.58 (m, 2H), 7.72–7.97 (m, 2H), 14.84 (br, 1H).– Anal.: $C_{10}H_8N_3OSCl$ (253.71).– MS (15 eV): *m/z* (%) = 255 (7), 254 (3), 253 (20), 204 (40), 193 (15), 163 (12), 146 (27), 119 (43), 92 (16), 59 (5), 38 (31), 36 (100).

3-(-Bromocinnamylidene)amino-2(1)-thioxo-4(3H)-quinazolinone (9)

Yield 2.40 g (62%), yellow powder from ethanol, mp 209–210 °C.– IR (KBr): ν cm^{-1} = 3400 (N=CH), 3010 (C=C), 1690 (CO), 1670 (C=N), 1630 (C=CPh), 1200 (C=S), 760 (CH, aromatic).– ^{1}H-NMR (DMSO): δ = 6.68–7.42 (m, 4H), 7.47–7.64 (m, 6H), 8.22 (t, 1H), 13.86 (s, 1H).– Anal.: $C_{17}H_{12}N_3OSBr$ (386.27).– MS (15 eV): *m/z* (%) = 307 (8), 306 (8), 305 (94), 253 (35), 235 (22), 193 (60), 162 (25), 145 (21), 120 (55), 82 (97), 81 (10), 80 (100), 79 (11), 77 (17).

3-Methyl-3[2(1H)-thioxo-4(3H)-oxo-quinazolin-3-ylamino]-propanal-1 (14)

Yield 1.45 g (55%), yellow powder from ethanol, mp 205–206 °C.– IR (KBr): ν cm^{-1} = 3320, 1625 (NH), 2940, 2730, 1390 (CH), 1730 (-CH_2-COH), 1695 (CO), 1500 (NHC=S), 760 (CH, aromatic).– ^{1}H-NMR (DMSO): δ = 1.25 (d, *J* = 6.3 Hz, 3H), 2.40 (m, 2H), 3.82 (m, 1H), 5.24 (s, 1H), 6.81 (m, 1H), 7.30 (m, 3H), 9.47 (t, 1H).– Anal.: $C_{12}H_{13}N_3O_2S$ (263.31).

3-Cinnamylideneamino-2(1H)-thioxo-4(3H)-quinazolinone (15)

Yield 1.85 g (60%), yellow precipitate from ethanol, mp 182–185 °C.– IR (KBr): ν cm^{-1} = 3300 (NH), 2920 (CH), 1680 (CO), 1665 (CH=CH trans), 1630 (C=CHPh, C=N), 1530 (NH-CS), 1170, 760 (CH, aromatic).– ^{1}H-NMR (DMSO): δ = 6.81 (m, 5H), 7.05–7.76 (m, 6H), 8.15 (t, 1H), 14.60 (br, 1H).– Anal.: $C_{17}H_{13}N_3OS$ (307.37).

*Reactions of 3-amino-2(1H)-thioxo-4(3H)-quinazolinone (**1**) with α,β-unsaturated ketones: benzylideneacetone or benzylideneacetophenone, **16, 17***

A mixture of quinazoline **1** (1.93g, 0.01 mol) and benzylideneacetone (1.46g, 0.01 mol) or benzylideneacetophenone (2.08g, 0.01 mol) in ethanol (50 ml) was refluxed for 10 h. The white precipitate was collected by filtration, washed with diethyl ether, and crystallized.

*4-Phenyl-4[2(1H)-thioxo-4(3H)-oxo-quinazolin-3-ylamino]-butan-2-one (**16**)*

Yield 3.01 g (88%), white compound from CH_3CN, mp 155–157 °C.– IR (KBr): ν cm^{-1} = 3350 (NH), 2900 (CH), 1720, 1690 (CO), 1630 (NH), 1430 (-CH_2CO), 1360 (CH_3CO), 1170 (ArCOAl, CH), 760 (CH, aromatic), 700 (C-S).– ^{1}H-NMR (DMSO): δ = 2.18 (s, 3H), 3.68 (m, 2H), 5.72 (m, 1H), 6.14–7.60 (m, 10 H), 14.35 (br, 1H).– Anal.: $C_{18}H_{17}N_3O_2S$ (339.41).– MS (15 eV): *m/z* (%) = 341 (3), 340 (11), 339 (66), 193 (98), 162 (11), 120 (100), 118 (31), 104 (4), 43 (95).

*3-(α-Phenylcinnamylideneamino)-2(1H)-thioxo-4(3H)-quinazolinone (**17**)*

Yield 2.20 g (58%), white powder from CH_3CN, mp 192–194 °C.– IR (KBr): ν cm^{-1} = 3050 (CH=CH), 1680 (CO), 1660 (CH=CH trans), 1620 (-CH=CH-Ph), 1420 (N-CS-N), 760 (CH, aromatic).– ^{1}H-NMR (DMSO): δ = 6.70–8.05 (m, 16 H), 14.50 (br, 1H).– Anal.: $C_{23}H_{17}N_3OS$ (383.47).– MS (15 eV): *m/z* (%) = 383 (50), 281 (23), 277 (53), 252 (15), 235 (34), 219 (53), 162 (21), 145 (20), 119 (16), 105 (100), 59 (18).

*Reaction of 3-amino-2(1H)-thioxo-4(3H)-quinazolinone (**1**) with α,β-unsaturated acid chlorides: crotonoyl or cinnamoyl chlorides, **18, 19**.*

Method A. Compound **1** (1.93g, 0.01 mol) was suspended n anhydrous THF (50 ml), then 0.01 mol of the appropriate acid chlorides (crotonoyl or cinnamoyl) in 20 ml of THF was added dropwise with mechanical stirring at room temperature. Stirring was continued for 10 h. The mixture was evaporated *in vacuo*, water was added (ca 50 ml), and the mixture was neutralized with 5% aq. $NaHCO_3$ solution. The crude product was filtered off, washed with water, dried, and crystallized from *n*-butanol.

Method B. A mixture of quinazoline **1** (1.93g, 0.01 mol) and an appropriate ester: ethyl crotonate or ethyl cinnamate (0.01 mol) in diglyme (30 ml) was refluxed for 10 h. After cooling the solid obtained was filtered off, washed with diethyl ether and crystallized from *n*-butanol.

*3-Crotonoylamino-2(1H)-thioxo-4(3H)-quinazolinone (**18**)*

Yield 2.10 g (80%), white solid from *n*-BuOH, mp 204–206 °C.– IR (KBr): ν cm^{-1} = 3260 (NHCO), 3050 (CH=CH), 2900 (CH), 2820 (CH_3), 1720 (NHCO), 1670(NCO, RCH=CHR′ trans), 1630, 1290 (RCH=CHR′ trans), 1170 (CH, aromatic).– ^{1}H-NMR (DMSO): δ = 1.89 (d, *J* = 6.4 Hz, 3H), 6.11 (d, *J* = 16.9 Hz, 1H), 6.80 (m, 1H), 7.31 (m, 1H, ArH_8), 7.63 (m, 1H, ArH_6), 7.81 (m, 1H, ArH_7), 7.99 (d, *J* = 8.2 Hz, 1H, ArH_5), 9.88 (s, 1H, D_2O exchangeable), 14.50 (br, 1H, exchangeable D_2O).– Anal.: $C_{12}H_{11}N_3O_2N$ (261.30).

*3-Cinnamoylamino-2(1H)-thioxo-4(3H)-quinazolinone (**19**)*

Yield 2.52 g (78%), cream-coloured precipitate from *n*-BuOH, mp 224–225.5 °C.– IR (KBr): ν cm^{-1} = 3280 (NHCO), 3040 (CH=CH trans), 2900 (CH), 1680 (NCO), 1660 (COCH=CH), 1630 (CO), 1290 (CH=CH trans), 1170 (-Ph), 1140 (C=S), 760 (CH, aromatic).– ^{1}H-NMR (DMSO): δ = 6.82 (d, *J* = 15.80 Hz, 1H), 7.40 (m, 4H), 7.60 (d, *J* = 15.8 Hz, 1H), 7.66 (m, 3H), 7.80 (m, 1H), 8.01 (m, 1H), 10.15 (s, 1H, exchangeable D_2O), 14.75 (br, 1H, exchangeable D_2O).– Anal.: $C_{17}H_{13}N_3O_2S$ (323.37).

*Reaction of quinazoline **1a** with crotonoyl or cinnamoyl chlorides, **20–22**.*

The derivatives **20–22** were prepared similarly as described [12]. The mixtures of compounds **20, 21, and 19, 22** were crystallized from *n*-butanol. Products **20**, **22** were found in the precipitate while **19**, **21** in filtrate. Analytical and spectral data were identical with those of derivatives **18**, **19** obtained by method A.

*3-Amino-2-crotonoylthio-4(3H)-quinazolinone (**20**)*

Yield 1.88 g (72%), white precipitate from *n*-BuOH, mp 183–185 °C.– IR (KBr): ν cm^{-1} = 3420, 3370 (NH_2), 2940 (CH), 1710,1700 (CO), 1620 (NH, COC=C), 1430 (N-CS-N), 1310 (CHR=CHR′ trans), 780 (CH), 700 (C-S).– ^{1}H-NMR (DMSO): δ = 1.85 (d, *J* = 6.4 Hz, 3H), 6.34 (m, 1H), 6.56 (s, 2H), 6.86 (m, 1H), 7.28 (m, 1H, ArH_8), 7.64 (m, 1h, ArH_6), 7.90 (m, 1H, ArH_7), 8.10 (m, 1H, ArH_5).– Anal.: $C_{12}H_{11}N_3O_2S$ (261.30).

*3-Crotonoylamino-2-crotonoylthio-4-(3H)-quinazolinone (**21**)*

Yield 0.26 g (8%), white powder from *n*-BuOH, mp 260–263 °C.– IR (KBr): ν cm^{-1} = 3250 (NHCO), 3050 (CH=CH), 2830 (CH), 1740, 1690 (CO), 1630 (NHCO, C=N), 1550 (NH, C-N), 760 (CH).– Anal.: $C_{16}H_{15}N_3O_3S$ (329.37).– MS (70 eV): *m/z* (%) = 330 (1), 329 (16), 261 (4), 243 (5), 193 (5), 188 (44), 120 (8), 69 (100), 41 (79), 39 (16).

*3-Amino-2-cinnamoylthio-4(3H)-quinazolinone (**22**)*

Yield 1.92 g (60%), white product from *n*-BuOH, mp 178–181 °C.– IR (KBr): ν cm^{-1} = 3400, 3300 (NH_2), 3040 (CH=CH trans), 1710 (CO), 1630 (C=CPh), 1570, 1330 (NH), 1270 (-N=), 1110 (CH), 890 (NH), 705 (C-S).– ^{1}H-NMR (DMSO): δ = 6.52 (s, 2H), 6.67 (m, 1H), 6.93 (m, 1H), 7.35 (m, 1H), 7.50 (m, 3H), 7.76 (m, 1H), 7.88 (t, 3H), 7.98 (d, *J* = 15.9 Hz, 1H).– Anal.:$C_{17}H_{13}N_3O_2S$ (323.37).– MS (70 eV): *m/z* (%) = 324 (3), 323 (14), 263 (2), 193 (6), 132 (15), 131 (100), 120 (16), 103 (45), 77 (16).

Immunological tests and statistical evaluation

Animals: Mice of CBA and 129Ao/Boy strains (10–12 week old) derived from the Animal Breeding Facility of the Institute of Immunology, Wrocław, Poland.

Preparation of the compounds

The compounds were initially dissolved in a mixture of cremophor (Sigma) and ethanol, then diluted with 0.5% NaCl. The compounds were given into mice in a volume of 0.2 ml at doses of 10 μg × 100 μg/mouse 3 hours after immunization with SRBC.

Determination of the humoral immune response to SRBC was performed according to Jerne in a modification of Mishell and Dutton [20]. The results were expressed as a mean value of plaque-forming cells (PFC) per 10^6 of spleen cells ± standard error. The number of mice per group = 5.

Test for delayed hypersensitivity to SRBC was performed according to Lagrange [21]. The results are shown as mean values of the foot pad swelling and expressed in DTH units (1 unit = 0.1 mm). The number of mice per group = 6.

The results are shown as mean values from 5 or 6 mice ± standard error. The statistical significance (*p*) was calculated using t-Student test.

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