Dipartimento di Scienze Farmaceutiche[1], Facoltà di Farmacia, Istituto di Farmacologia[2], Facoltà di Medicina, Città Universitaria, Università di Catania, Italy

Synthesis and pharmacological screening of 1,3,4-thiadiazino[2,3-*b*]quinazoline derivatives

A. Santagati[1], M. Modica[1], M. Santagati[1], V. M. C. Cutuli[2], N. G. Mangano[2] and A. Caruso[2]

Derivatives of 1,3,4-thiadiazino[2,3-*b*]quinazoline **7**, **9**, **9a**, **12**, **12a**, and **13** were prepared from the 3-amino-6-bromo-2,3-dihydro-2-thioxo-4-(1*H*)-quinazolinone (**2**) and its analogue without bromine. A series of the title derivatives with or without bromine was tested and the results of pharmacological screening are discussed.

1. Introduction

In the past few years we have synthesized and tested a series of derivatives of 1,3,4-thiadiazolo[2,3-*b*]quinazolin-5-one [1, 2], some of which displayed anti-inflammatory activity comparable to that of mefenamic acid. The above derivatives were envisaged [3] as potential N-substituted anthranilic acids, like mefenamic acid and its analogues, the anti-inflammatory and analgesic activities of which are well known; the anthranilic structure was included through the quinazolinone system, condensed with substituted 1,3,4-thiadiazole whose many derivatives display interesting biological activities [4, 5].

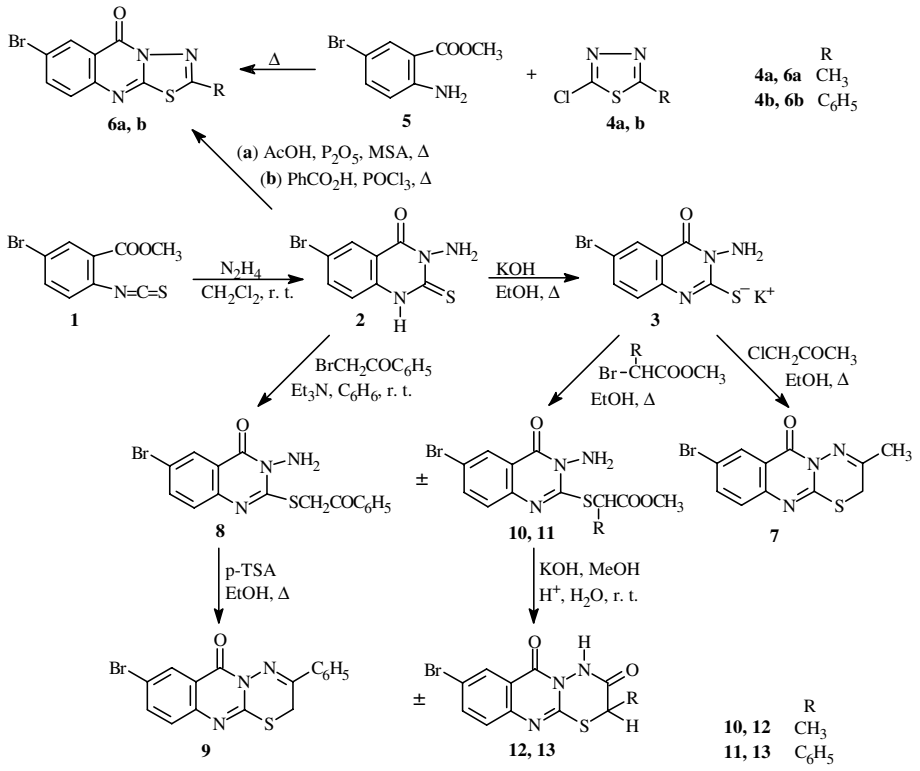
Recently, we have established [6] new methods for the synthesis of a large number of derivatives of the above system with high yields and of particularly for the preparation of quinazolinone derivatives fused with the substituted 1,3,4-thiadiazine ring. In this report, we describe the synthesis of some derivatives of the above heterocyclic systems using, as in the cited paper [6], the versatile intermediates 3-amino-6-bromo-2,3-dihydro-2-thioxo-4(1*H*)-quinazolinone (**2**) and its analogue without bromine. The derivatives with the 1,3,4-thiadiazine ring, synthesized with two analoguous derivatives without bromine [6], constitute two homologous series of derivatives and were tested for their analgesic and anti-inflammatory activities.

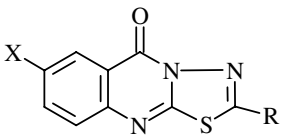
2. Investigations and results

2.1. Chemistry

The new derivatives of fused heterocyclo-bromoquinazolinones **7**, **9**, **12** and **13** were obtained starting from the versatile 3-amino-6-bromo-2,3-dihydro-2-thioxo-4(1*H*)-quinazolinone (**2**) prepared by adding the bromo-isothiocyanate **1** [7] to a stirred solution of hydrazine hydrate in dichloromethane at room temperature (Scheme). The amino-thioxo structure **2** was confirmed by analytical and spectral data, and independent syntheses; reaction of the bromo-amino-thioxo derivative **2** with a suitable acid under appropriate conditions gave the 7-bromo-2-methyl (**6a**) and 7-bromo-2-phenyl (**6b**) -5*H*-[1,3,4]thiadiazolo[2,3-*b*]quinazolin-5-ones, which were shown to be identical with those obtained by Russo et al. [2] from the condensa-

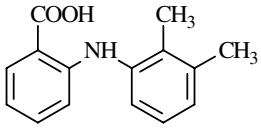
Scheme





X = H, Br, Cl
R = CH_3, SCH_3, C_6H_5

1,3,4-Thiadiazolo[2,3-*b*]quinazolin-5-one derivatives



Mefenamic acid

tion of the 3-bromo methyl ester **5** with 5-methyl- or 5-phenyl-2-chloro-1,3,4-thiadiazole **4a** and **4b**, respectively.

The 8-bromo-3-methyl (**7**) and 8-bromo-3-phenyl (**9**) -2*H*,6*H*-[1,3,4]thiadiazino[2,3-*b*]quinazolin-6-ones were obtained by boiling the potassium salt of the amino-thioxo derivative **3** and chloroacetone under reflux in ethanol, and by cyclizing in refluxing ethanol with a catalytic amount of p-toluensulfonic acid (p-TSA) the thio derivative **8**, obtained in benzene from the amino-thioxo derivative **2** and 2-bromoacetophenone in the presence of triethylamine.

Moreover, the (±)-8-bromo-2-methyl (**12**) and (±)-8-bromo-2-phenyl dione derivatives (**13**) were prepared by alkaline hydrolysis in methanol of the methyl esters **10** and **11** obtained from the potassium salt **3** and the methyl esters of (±)-2-bromopropionic acid and (±)-α-bromobenzeneacetic acid, respectively.

Derivatives without bromine **9a** and **12a** were prepared starting from the 3-amino-2,3-dihydro-2-thioxo-4(1*H*)-quinazolinone [6] and using the same methods as for the preparation of the bromo-derivative analogues **9** and **12**, by cyclization of the derivatives **8a** and **10a**, respectively.

The analytical and spectral data of all compounds agree with the proposed structures. In particular, in the IR and ^{1}H NMR spectra of tricycles **7**, **9** and **9a**, the absence of signals due to the NH_2 or NH group and the presence of signals in the region of δ 3.80–4.40 attributable to the protons adjacent to a sulfur atom, corroborated the formation of the double bond at the 3,4 position; the diones **12**, **12a** and **13** confirmed the amidic hydrogen in the IR spectra in the region of 3180–3195 cm^{-1} and in the region of δ 11.80–12.35 in the ^{1}H NMR spectra, while exhibited the C-2 proton as a quartet (**12** and **12a**) at δ 4.30 and as a singlet (**13**) at δ 5.61.

2.2. Pharmacology

We evaluated the analgesic and anti-inflammatory activities, behavioral effects and acute toxicity of the test compounds, the 8-bromo substituted tricycles **7**, **9**, **12**, and **13** and their homologues without bromine on the benzene **7a** [6], **9a**, **12a**, and **13a** [6], as well as the ulcerogenic potential of the most active compounds in terms of anti-inflammatory activity **9a** and **12a** compared to phenylbutazone (PBZ). Compounds **12a**, **12**, **13a**, and **13** were tested as racemates. The pharmacological data are summarized in the Table and the Fig.

The test compounds did not show any significant gross behavioral effects at doses of to up 1000 mg/kg po and 800 mg/kg ip in mice. At higher doses, the most typical signs of acute intoxication were motor uncoordination, bradypnoea and hypotonia. At these levels, death generally occurred 12–48 h after drug administration in 40–

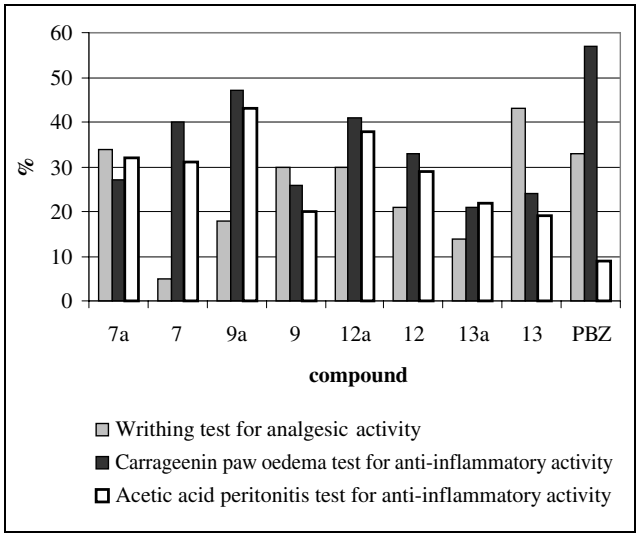
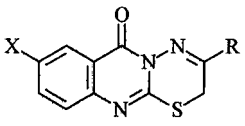
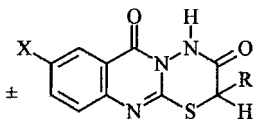


Fig.: Activity of test compounds versus controls

Table: Pharmacological data of compounds



7a, 7, 9a, 9



12a, 12, 13a, 13

Compd.	X	R	Acute toxicity Approximate LD_{50} (mg/kg) po	ip	Analgesic activity Phenylquinone Writhing-test (% protection ± SE)[a] 10 mg/kg	Anti-inflammatory activity: Carrageenin Paw oedema (% inhibition ± SE)[a] 100 mg/kg	Anti-inflammatory activity: Acetic acid peritonitis (% reduction ± SE)[a] 10 mg/kg	Ulcerogenic index 300 mg/kg × 2
7a	H	Me	>1000	>800	34 ± 12*	27 ± 6*	32 ± 8*	–
7	Br	Me	>1000	>800	5 ± 3	40 ± 5*	31 ± 6*	–
9a	H	Ph	~800	~400	18 ± 7*	47 ± 4*	43 ± 8*	80
9	Br	Ph	>1000	>800	30 ± 4*	26 ± 5*	20 ± 7*	–
12a	H	Me	~800	~400	30 ± 4*	41 ± 4*	38 ± 5*	80
12	Br	Me	>1000	>800	21 ± 8*	33 ± 4*	29 ± 7*	–
13a	H	Ph	>1000	>800	14 ± 6	21 ± 6*	22 ± 8*	–
13	Br	Ph	>1000	>800	43 ± 3*	24 ± 5*	19 ± 10*	–
PBZ			~700	~300	33 ± 3*	57 ± 3*	9 ± 6	275[b]

Oral administration for all tests.
[a] Values are percent of controls. $P < 0.05$ Student-Newman-Keuls test versus controls. [b] PBZ 100 mg/kg × 2

60% of the animals, whereas the surviving mice appeared to be normal throughout the 7-days observation period. Only compound **13** showed interesting activity in the phenylquinone-induced writhing test on mice at a dose of 10 mg/kg po, whereas the remaining compounds exhibited activity comparable to or lower than that of PBZ.
In the rat paw oedema test, only compounds **7**, **9a**, and **12a** showed a good activity at a dose of 100 mg/kg po.
In the acetic acid peritonitis assay, compounds **9a** and **12a** at a dose of 10 mg/kg showed an activity markedly greater than that of PBZ.
In screening for ulcerogenic potential, only compounds **9a** and **12a**, the most active in the anti-inflammatory activity, were evaluated; they were much less active in causing gastric lesions at an oral dose of 300 mg/kg × 2 compared to PBZ at 100 mg/kg × 2, under the same experimental conditions.

3. Discussion

The activities of brominated and non brominated analogues were compared, and the influence of bromine on the activities was examined.
No trend was observed in the analgesic activity; in fact, the activities of the bromo-3-methyl derivative **7** and the 3-methyl derivative **7a** were greatly different (5% versus 30%), as were those of the bromo-2-phenyl-dione derivative **13** and the 2-phenyl-dione derivative **13a** (43% versus 14%).
No trend was observed in the carrageenin test either, but the activities were slightly different.
In the acetic acid peritonitis test, all brominated derivatives showed activities lower than those of the corresponding non brominated derivatives.
Given the nature of the bromine group and the bulk of the methyl and phenyl group the above data are not sufficient, to plot a trend in structure-activity relationships; derivatives with other types of substituents are needed, as well as knowledge of the polarity.

4. Experimental

4.1. Chemistry

Melting points are uncorrected and were determined in open capillary tubes on a Gallenkamp apparatus. IR spectra were recorded on a Perkin Elmer 1600 Series FT-IR in KBr disks. Elemental analyses for C, H, N, and S were obtained on a Fisons-Carlo Erba EA1108 elemental analyzer and were within 0.4% of the theoretical values. The ^{1}H NMR spectra were recorded at 200 MHz on a Varian Inova-Unity 200 spectrometer in DMSO-d_6 solution, chemical shifts (δ) are reported in ppm from TMS as internal standard; coupling constants (J) are in Hertz. The purity of compounds was checked by TLC on Merck silica gel 60 F-254 plates. All commercial chemicals were purchased from Aldrich, Fluka, Merck and Carlo Erba and were used without further purification.

4.1.1. Methyl ester of 5-bromo-2-isothiocyanato-benzoic acid (**1**)

The isothiocyanate **1** was prepared according to the method described by Grafe I. et al. [7], starting from 5-bromo methyl anthranilate [8]; in the paper the properties of this isothiocyanate **1** are not indicated; the crude product was recrystallized from petroleum ether (35/60) to give **1** as a white powder (59%); m.p. 58–60 °C; IR (cm^{-1}): 2145 and 2085 (N=C=S), 1725 (C=O).
$C_9H_6BrNO_2S$

4.1.2. 3-Amino-6-bromo-2,3-dihydro-2-thioxo-4(1H)-quinazolinone (**2**) *and its potassium salt* (**3**)

A solution of **1** (6.0 mmol) in CH_2Cl_2 (20 ml) was added dropwise at room temperature to a stirred solution of hydrazine hydrate (0.3 ml, 98%, d = 1.03) in CH_2Cl_2 (20 ml). The mixture was stirred at room temperature for 3 h; the resulting solid was collected, washed with dichloromethane, dried and recrystallized from dimethylformamide to give **2** as a white powder (82%); m.p. 258–260 °C; IR (cm^{-1}): 3295 and 3180 broad (NH or NH_2), 1655 broad (C=O); ^{1}H NMR (DMSO-d_6): δ 6.38 (s, 2H, NH_2), 7.34–8.10 (m, 3H, ArH), 13.25 (br s, 1H, NH).
$C_8H_6BrN_3OS$
A suspension of the bromo-amino-thioxo derivative **2** (5.3 mmol) in a solution of KOH (0.3 g) in ethanol (50 ml) was heated under reflux for 1 h. The resulting solid was collected, washed with warm dioxane and dried to give **3** as a white powder (83%); m.p. 261–263 °C dec.; IR (cm^{-1}): 3210 and 3125 (NH or NH_2), 1640 (C=O).
$C_8H_5BrKN_3OS$

4.1.3. 7-Bromo-2-methyl-5H-1,3,4-thiadiazolo[2,3-b]quinazolin-5-one (**6a**)

A mixture of the bromo-amino-thioxo derivative **2** (3.95 mmol), phosphorous pentoxide (0.6 g), methanesulfonic acid (MSA) (1.3 ml, 19.8 mmol) and acetic acid (0.15 ml) was heated at 80 °C for 9 h. After cooling, the mixture was treated with 10% NaOH solution and the resulting solid was collected, washed with H_2O, dried and recrystallized from dioxane/ethanol/water to give **6a** as colourless needles (80%); m.p. 238–240 °C; IR (cm^{-1}): 1690 (C=O).
$C_{10}H_6BrN_3OS$

4.1.4. 7-Bromo-2-phenyl-5H-1,3,4-thiadiazolo[2,3-b]quinazolin-5-one (**6b**)

A mixture of the bromo-amino-thioxo derivative **2** (5.2 mmol), benzoic acid (5.2 mmol) and phosphorus oxychloride (4 ml) was refluxed with stirring for 40 min. After cooling, the mixture was poured into ice and 10% NaOH solution; the resulting solid was collected, washed with water, dried and recrystallized from ethanol/dioxane to give **6b** as light yellow needles (89%); m.p. 248–251 °C; IR (cm^{-1}): 1695 (C=O).
$C_{15}H_8BrN_3OS$
Compounds **6a** and **6b** were identical with respect to m.p., IR and TLC with the samples obtained by Russo et al [2], by condensation of the methyl ester of 5-bromo-anthranilic acid **5** [8] and 5-methyl **4a** [9] and 5-phenyl-2-chloro-1,3,4-thiadiazolo **4b** [10], respectively.

4.1.5. 8-Bromo-3-methyl-2H,6H-[1,3,4]thiadiazino[2,3-b]quinazolin-6-one (**7**)

A mixture of the potassium salt **3** (2.0 mmol) and chloroacetone (0.15 ml) in ethanol (20 ml) was refluxed for 2 h. The resulting solid was collected, washed with ethanol and then with H_2O, dried and recrystallized from ethanol to give **7** as white needles (38%); m.p. 239–240 °C; IR (cm^{-1}): 1695 (C=O); ^{1}H NMR (DMSO-d_6): δ 2.35 (s, 3H, CH_3), 3.81 (s, 2H, CH_2), 7.46–8.20 (m, 3H, ArH).
$C_{11}H_8BrN_3OS$

4.1.6. 3-Amino-6-bromo-2-[[2-phenyl-2-oxoethyl]thio]-3(3H)-quinazolinone (**8**)

A mixture of the bromo-amino-thioxo derivative **2** (2.2 mmol), 2-bromoacetophenone (2.2 mmol) and triethylamine (0.3 ml) in benzene (25 ml) was stirred at room temperature for 6h; the mixture was then poured into H_2O (200 ml) and the resulting solid was collected, washed with H_2O, dried and recrystallized from ethanol/H_2O to give **8** as an amorphous white powder (91%); m.p. 180–182 °C dec.; IR (cm^{-1}): 3320 and 3200 (NH_2), 1650 (C=O).
$C_{16}H_{12}BrN_3O_2S$

4.1.7. 3-Amino-2-[[2-phenyl-2-oxoethyl]thio]-3-(3H)-quinazolinone (**8a**)

Prepared according to the same procedure as **8**, using 3-amino-2,3-dihydro-2-thioxo-4(1*H*)-quinazolinone [6] instead of the bromo-amino-thioxo derivative **2**; the resulting solid was recrystallized from ethanol to give **8a** as an amorphous white powder (88%); m.p. 186–189 °C dec.; IR (cm^{-1}): 3320 and 3220 (NH_2), 1700 and 1670 (C=O).
$C_{16}H_{13}N_3O_2S$

4.1.8. 8-Bromo-3-phenyl-2H,6H-[1,3,4]thiadiazino[2,3-b]quinazolin-6-one (**9**)

p-Toluensulfonic acid (p-TSA) (30 mg) was added to a suspension of the bromo-thio derivative **8** (1.1 mmol) in refluxing ethanol (20 ml). The mixture was refluxed for 4 h; the resulting solid was collected, washed with warm ethanol, dried and recrystallized from dioxane/ethanol to give **9** as a yellow powder (72%); m.p. 250–253 °C; IR (cm^{-1}): 1695 (C=O); ^{1}H NMR (DMSO-d_6): δ 4.38 (s, 2H, CH_2), 7.50–8.22 (m, 8H, ArH).
$C_{16}H_{10}BrN_3OS$

4.1.9. 3-Phenyl-2H,6H-[1,3,4]thiadiazino[2,3-b]quinazolin-6-one (**9a**)

p-Toluensulfonic acid (p-TSA) (30 mg) was added to a suspension of the thio derivative **8a** (1.1 mmol) in refluxing ethanol (20 ml). The mixture was refluxed for 4 h, filtered while hot and the resulting solution cooled to room temperature: the solid separated was collected and recrystallized from ethanol to give **9a** as colourless needles (51%); m.p. 173–175 °C; IR (cm^{-1}): 1690 (C=O); ^{1}H NMR (DMSO-d_6): δ 4.40 (s, 2H, CH_2), 7.48–8.20 (m, 8H, ArH).
$C_{16}H_{11}N_3OS$

4.1.10. Methyl ester of (±)-2-[(3-amino-6-bromo-3,4-dihydro-4-oxo-2-quinazolinyl)thio]-propionic acid (**10**)

A mixture of the potassium salt **3** (1.8 mmol) and methyl (±)-2-bromopropionate (0.3 ml) in ethanol (30 ml) was refluxed for 3 h. The mixture was filtered and the filtrate poured into H_2O (200 ml): the resulting solid was collected, washed with H_2O, dried and recrystallized from ethanol/dioxane to give **10** as a white powder (40%); m.p. 168–171 °C; IR (cm^{-1}): 3305 and 3205 (NH_2), 1725 and 1670 (C=O).
$C_{12}H_{12}BrN_3O_3S$

4.1.11. Methyl ester of (±)-2-[(3-amino-3,4-dihydro-4-oxo-2-quinazolinyl)-thio]-propionic acid (**10a**)

Prepared according to the same procedure as **10**; using instead of the bromo derivative **3**, the potassium salt of 3-amino-2,3-dihydro-2-thioxo-4(1*H*)-quinazolinone [6]; compound **10a** (recrystallized from ethanol) was obtained as a white powder (75%); m.p. 140–142 °C; IR (cm^{-1}): 3320 and 3210 (NH_2), 1730 and 1675 (C=O).
$C_{12}H_{13}N_3O_3S$

4.1.12. Methyl ester of (±)-α-[(3-amino-6-bromo-3,4-dihydro-4-oxo-2-quinazolinyl)thio]-benzeneacetic acid (**11**)

A mixture of the potassium salt **3** (2.6 mmol) and methyl(±)-α-bromophenylacetate (2.8 mmol) in methanol (30 ml) was heated under reflux for 3 h. The mixture was filtered and the filtrate poured into H_2O (200 ml): the resulting solid was collected, washed with H_2O, dried and recrystallized from ethanol to give **11** as a white powder (77%); m.p. 238–240 °C; IR (cm^{-1}): 3315 and 3270 (NH_2), 1735 and 1685 (C=O).
$C_{17}H_{14}BrN_3O_3S$

4.1.13. (±)-8-Bromo-2-methyl-2H,6H-[1,3,4]thiadiazino[2,3-b]quinazoline-3,6(4H)-dione (**12**)

A suspension of the ester **10** (1.15 mmol) in a solution of NaOH (50 mg) in methanol (10 ml) and H_2O (4 ml) was stirred at room temperature for 14 h. The resulting solution was poured into H_2O (100 ml) and on acidification with HCl to pH 4–5 a white solid separated; the solid was collected, washed with H_2O, dried and recrystallized from dioxane/ethanol to give **12** as a white powder (40%); m.p. 210–212 °C; IR (cm^{-1}): 3185 broad (NH), 1695 and 1675 (C=O); ^{1}H NMR (DMSO-d_6): δ 1.42 (d, J = 7.2 Hz, 3 H, CH_3), 4.30 (q, J = 7.2, 1 H, CH), 7.34–8.22 (m, 3 H, ArH), 12.10 (br s, 1 H, NH).
$C_{11}H_8BrN_3O_2S$

4.1.14. (±)-2-Methyl-2H,6H-[1,3,4]thiadiazino[2,3-b]quinazoline-3,6-(4H)-dione (**12a**)

The same procedure as used for compound **12** starting from **10a**; compound **12a** recrystallized from ethanol was obtained as a white powder (52%); m.p. 234–236 °C; IR (cm^{-1}): 3195 broad (NH), 1695 and 1670 (C=O); ^{1}H NMR (DMSO-d_6): δ 1.42 (d, J = 7.2, 3 H, CH_3), 4.30 (q, J = 7.2, 1 H, CH), 7.50–8.16 (m, 4 H, ArH), 11.84 (s, 1 H, NH).
$C_{11}H_9N_3O_2S$

4.1.15. (±)-8-Bromo-2-phenyl-2H,6H-[1,3,4]thiadiazino-[2,3-b]quinazoline-3,6(4H)-dione (**13**)

A suspension of the ester **11** (1.5 mmol) in a solution of NaOH (80 mg) in methanol (13 ml) and H_2O (2 ml) was stirred at room temperature for 20 h. The mixture was filtered and the solution poured into H_2O (100 ml): on acidification with HCl to pH 4–5 a white solid separated; the solid was collected, washed with water and recrystallized from ethanol/dioxane to give **13** as a white solid (60%); m.p. 254–256 °C dec.; IR (cm^{-1}): 3180 (NH), 1695 and 1675 (C=O); ^{1}H NMR (DMSO-d_6): δ 5.61 (s, 1 H, CH), 7.31–8.23 (m, 3 H, ArH), 12.32 (s, 1 H, NH).
$C_{16}H_{10}BrN_3O_2S$

4.2. Pharmacology

4.2.1. Materials and methods

Swiss male mice (25–28 g) and Sprague-Dawley rats (120–159 g) were used. The animals were starved for about 12 h before administration and maintained at a temperature of 22 ± 2 °C. The test compounds and phenylbutazone (PBZ) were suspended in 0.5% aqueous methylcellulose solution and administered orally or intraperitoneally. Control animals received the same amounts of the vehicle.

4.2.2. Behavioral effects and acute toxicity in mice [11]

Irwin's multidimensional screening-evaluative procedure was used on groups of 5 animals. The compounds were administered at three doses orally (500–800-1000) mg/kg) or intraperitoneally (200–500-800) mg/kg). The animals were kept under blinded observation for 6 h and the symptomatology was checked again 24 h later. The approximate LD_{50} was obtained from mortality in the following 7-day period.

4.2.3. Analgesic activity: phenylbenzoquinone writhing test [12]

Analgesia was assessed by means of phenylbenzoquinone (PBQ)-induced writhing in groups of 4 male mice. Each mouse was given i.p. 0.25 ml of 0.02% PBQ in 6% ethanol and the number of writhes was counted for 5 min, beginning 5 min after the injection. The tested compounds and PBZ were administered orally (10 mg/kg) 60 min before PBQ. The analgesic effect was expressed as percentage protection in comparison with controls.

4.2.4. Anti-inflammatory activity

4.2.4.1. Carrageenin-induced oedema [13]

Groups of 4 rats were used. The tested compounds and PBZ were given orally at 100 mg/kg. 60 min later, 0.1 ml of 1% carrageenin solution was injected into the sub-plantar tissue of the right hind paw. The volume was measured by a mercury plethysmometer prior to the injection of carrageenin and 3 h later: the increase in volume of the paw 3 h after the injection of carrageenin was adopted as a measure of oedema. Swelling in treated animals was calculated as percentage inhibition in comparison with controls.

4.2.4.2. Acetic acid peritonitis [14]

Groups of 4 rats were tested. Peritonitis was produced by an i.p. injection of acetic acid (10 ml/kg of 0.5% aqueous solution). 30 min later rats were killed by ether and peritoneal exudate was collected and measured. The tested compounds and PBZ were given orally at a dose of 10 mg/kg, 60 min before injection of the acetic acid. The antiexudative response was expressed as the percentage reduction in volume of exudate compared with controls.

4.2.5. Ulcerogenic activity [15]

Groups of 4 rats were used. Compounds **9a** and **12a** were given orally (300 mg/kg) to animals fasted for 24 h and after 2 h the treatment was repeated again. 6 h after the first dose, each rat was sacrificed by ether inhalation, the stomach removed, opened along the greater curvature and examined with a dissecting microscope for the presence of gastric ulcers. The severity of mucosal damage (ulcerogenic index) was graded by means of scores from 0 (no lesion) to 4 (exceptionally severe lesions). In order to take into account the percentage of rats having ulcers, an index of ulceration was calculated using the following formula:

$$\frac{\text{mean degree of ulceration} \times \text{number animals with ulcers}}{\text{number of animals}} \times 100 = \text{Ulceration index}$$

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Prof. Andrea Santagati
Dipartimento di Scienze Farmaceutiche
Facoltà di Farmacia
Università di Catania
Viale A. Doria 6
95125 Catania
Italy