4. A. G. Makhsumov, A. K. Khadzhiev, I. K. Gul'mirzaeva, et al., Physiologically Active Compounds [in Russian], Kiev (1983), No. 15, pp. 62-66.
5. M. I. Prokhorova and Z. N. Tupikova, A Comprehensive Laboratory Handbook on Carbohydrate and Lipid Metabolism [in Russian], Leningrad (1965), pp. 157-158.

HETEROCYCLIC SEMICARBAZONES AND THIOSEMICARBAZONES. XLIX. ANTIINFLAMMATORY ACTIVITY OF ISATIN THIOSEMICARBAZONES AND THEIR CYCLIZATION PRODUCTS

A. B. Tomchin, I. L. Zhmykhova, M. M. Ponomareva, L. G. Pastushenkov, and É. G. Gromova

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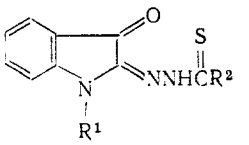
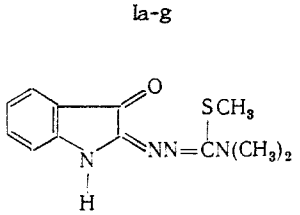
The aim of this investigation was to find active compounds in the isatin series, and to establish relationships between structure and antiinflammatory activity. The isatin thioacyl hydrazones (I-IV) have been obtained, having different radicals attached to the indole nitrogen, and also the number, structures, and positions of the hydrazone groupings at $C_{(2)}$ or $C_{(3)}$. For comparison with these compounds. some of their reaction products have been obtaine in which triazine and thiadiazine rings are formed (V-IX), together with isatin derivatives which do not contain the thiosemicarbazone side chain (X). All these compounds were insoluble in water, with the exception of (IXa, b) and (Xc, d) as their hydrochlorides.

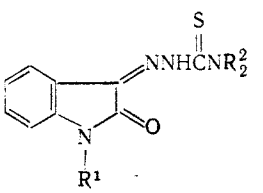


Ia-g

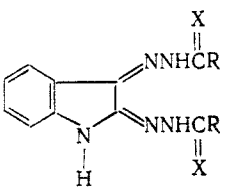
Ia-g: $R^1 = H$ (a-c); CH_3 (d-g); $R^2 = NH_2$ (a), $N(CH_3)_2$ (b,d), SCH_3 (c), NHC_6H_5 (e), $NHC_6H_4OCH_3$-p (f), $NHC_6H_4COOC_2H_5$-p (g)



II

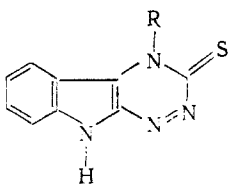


IIIa-c

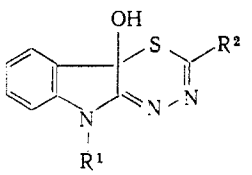


IVa,b

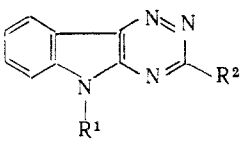
IIIa-c $R^1 = H$ (a), $CH_2COOC_2H_5$ (b), $COCH_3$ (c); $R^2 = H$ (a, b), CH_3 (c) IVa: X = S; $R = NH_2$; IVb X = O; $R = C_2H_5$



Va, b



VIa-h



VIIa-d

Va, b. R = H (a), NH_2 (b); VIa-h, VIIa-d: $R^1 = H$ (VIa,b,f,h; VIIa-d), CH_3 (VIc-e); VIa-h: $R^2 = NH_2$ (a), $N(CH_3)_2$ (b, c), NHC_6H_5 (d), $NHC_6H_4OCH_3$-p (e), C_6H_5 (f), $C_6H_4OCH_3$-p (g), C_6H_4Cl-m (h); VII(a-d) $R^2 = SCH_2CH{=}CH_2$ (a), SH (b), $SCHC_6H_5COC_6H_5$ (c), $NHNH_2$ (d)

Antiinflammatory activity was examined in three models of aseptic pathological inflammation, namely thermal burns, pulmonary adrenalin edema, and cotton wool granulemia. In the thermal burn model, activity was shown by (I), (III-VII), and (X). In the pulmonary adrenalin edema model, activity was observed in a smaller number of compounds, but the structure-activity relationships were substantially the same. In the cotton wool granulemia model, activity was shown by (Ia, b), (IIIc), (Va), (VIIa), (VIII), (IXa, b), and (Xd).

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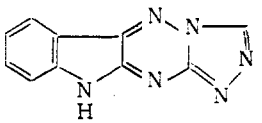
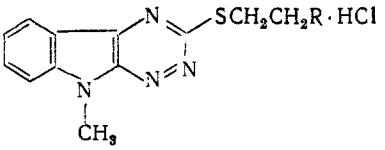
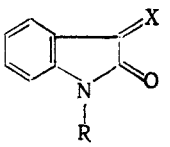


VIII



IXa, b



Xa-f

IX a R = $N(CH_3)_2$; IXb: R = N⟨⟩; Xa-f X = O (a-d), NC_6H_5 (e),

$NC_6H_4SO_2NH_2$-p (f); R = H (a,e,f), CH_2N⟨⟩ (b), $CH_2CH_2N(CH_3)_2$ (c),

$CH_2CH_2N(C_2H_5)_2$ (d)

Compounds previously reported Ia-c [13]; Id, e, g, VId, h [11]; If [10]; II [7]; IIIa [2]; IIIb [17]; IVa, b [8]; Va [4]; Vb [6]; VIa-c, f, g [9]; VIIa [19]; VIIb [3]; VIIc [12]; VIId [5]; Xb [20]; Xe [18].

The most active compounds in all the models were the isatin 2-thiosemicarbazones (Ia, b, d) and the 3-mercapto-1,2,4-triazino[6,5-b]-indoles (Va, b). High activity was also shown by some derivatives of the isomeric heterocyclic system 1,2,4-triazino[5,6-b]indole [(VIIa) and (VIII)].

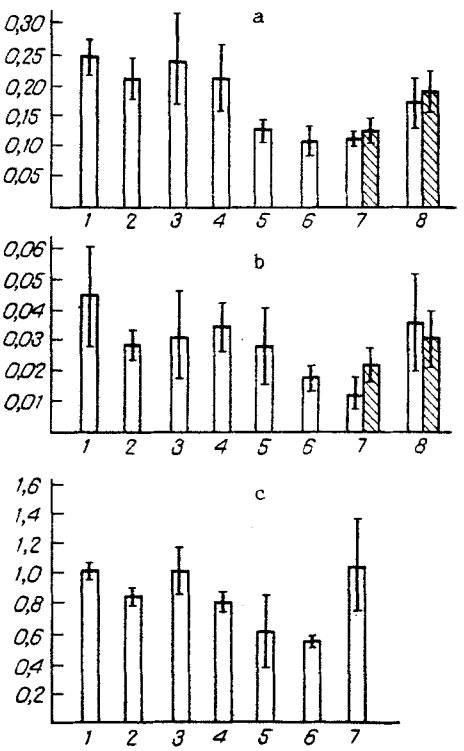


Fig. 1. Effects of isatin 2-thiosemicarbazones (Ia, b, d) and 1,2,4-triazino[6,5-b]indoles (Va, b) on exudative (a), proliferative (b) (administered intraperitoneally in DMSO), and adrenalin edema of the lungs (c) (administered intraperitoneally in oil). The light columns represent therapeutic treatment (three days following operation), and shaded columns therapeutic-prophylactic treatment (three days before and three days after operation). a), b): 1) DMSO; 2) indomethacin, 1 mg/kg; 3) 2.5% sodium carbonate solution; 4), 5), 6), 7), 8) compounds (Id), (Ia), (Ib), (Va), and (Vb) in a dose of 15 mg/kg; c): 1) control (oil); 2) indomethacin 20 mg/kg; 3), 4), 5), 6), 7), compounds (Id), (Ia), (Ib), Va), and (Vb) in doses of 50 mg/kg.

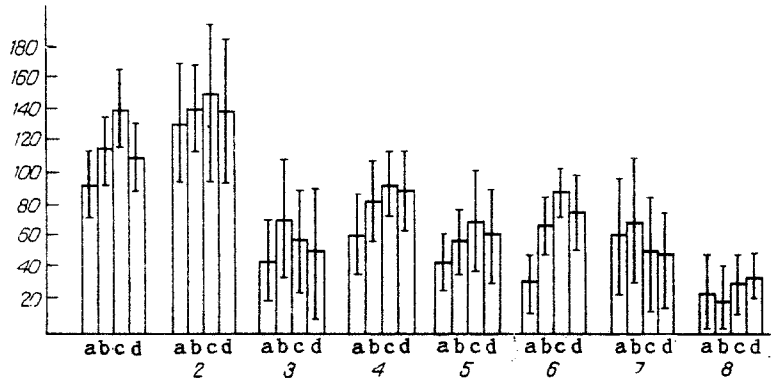


Fig. 2. Effects of isatin 2-thiosemicarbazones (Ia, b, d) and 1,2,4-triazino[6,5-b]indoles (Va, b) on increases in volume of rat extremities in model thermal burns. 1) Control (oil), 2) control (DMSO), 3) indomethacin, 20 mg/kg, 4) 5), 6) compounds (Id, a, b) administered intraperitoneally in DMSO in a dose of 15 mg/kg, 7), 8) compounds (Va, b), administered subcutaneously in oil, 50 mg/kg. (a) After 1.5 h, (b) after 3 h, (c) after 5 h, (d), after 24 h.

The test results for the most active of these water-insoluble compounds are given in Figs. 1 and 2. High activity was also shown by the water-soluble 1,2,4-triazino[5,6-b]indole (IXa). This compound, when administered in therapeutic and therapeutic-prophylactic modes in a dose of 10 mg/kg, reduced fluid formation in moist granulemia by factors of 3.1 and 2.2, respectively, over the controls, and increased granulation tissue by 2.6 and 2.4 times. In the thermal burn model, inflammation developed very slowly over the 24 h observation period. On average, the increase in volume of the extremity was decreased by a factor of 2.3. Hence, the most active of the isatin derivatives are more active antiinflammatory agents than indomethancin [14].

Isatin 2-thiosemicarbazones are more active than the 3-isomers [15]. This relationship is also characteristic of their cyclization products. The 2-hydrazones are also more active than the 2,3-bishydrazones. In the 2- and 3-thiacylhydrazones, the most active compounds do not carry substituents on the indole nitrogen. Introduction of an alkylthio group reduces activity, and the introduction of a phenyl group into the terminal amino-group in 2-thiosemicarbazones results in loss of activity, or even promotes inflammation. In the thiadiazines (VI), the introduction of electron-donor substituents into the benzene ring has a favorable effect. The presence in the triazine or thiadiazine rings in (V) or (VI) of amino- or dialkylamino-groups favors the development of activity.

In addition to antiinflammatory activity, we also studied the acute toxicity of the compounds. Only (IIIc), (IXb), and (IXc) were significantly toxic. The remaining compounds were active in doses not exceeding 1/10 of the LD_{50}, and therefore had sufficient breadth of therapeutic effect. In addition, the intraperitoneal administration of (Ia, b, d), (Va, b), (VIIa), (Viii), and (IXa) in doses of 10-15 mg/kg, i.e., approximately 1/10 of the LD_{50}, over six days did not result in any apparent toxic reactions in the gastrointestinal tract or adversely affect the general condition of the animals, in contrast to indomethacin. These isatin derivatives are therefore less toxic than indomethacin.

EXPERIMENTAL (CHEMICAL PART)

The homogeneity of all the compounds was checked by TLC, in addition to other methods. UV spectra were obtained on an SF-20 instrument for $2 \cdot 10^{-5}$ M solutions. The yields and constants of the compounds are given in Table 1.

1-Acetylisatin 3-(4,4-Dimethyl)thiosemicarbazone (IIIc). Finely-ground 1-acetylisatin (2 g, 10.5 mmole) and 1.38 g (11.6 mmole) of 4,4-dimethylsemicarbazide were mixed in 18 ml of glacial acetic acid, and the mixture was stirred for 10 h and kept overnight. It was then filtered, and the solid washed with acetic acid (2 × 3 ml) and ether (2 × 3 ml), and dried at 80°C to give (IIIc) as fine lemon-yellow crystals.

TABLE 1. Constants, Yields, and Elemental Analyses of Compounds Prepared

Compound	Yield, %	mp, °C (crystallization solvent)	Found, %				Empirical formula	Calculated, %				Absorption maximum for solution in alcohol (water)	
			C	H	N	S		C	H	N	S	λ_{max}, nm	lg ε
III c	99	165 (propyl alcohol)	53,74 53,66	5,16 4,92	19,01 19,12	10,77 10,89	$C_{13}H_{14}N_4O_2S$	53,78	4,86	19,30	11,04	273 pl 350	3,67 3,72
VI e	91	219 (DMF—propyl alcohol, 1:1)	60,23 60,34	4,91 5,05	16,78 16,71	9,81 9,71	$C_{17}H_{16}N_4O_2S$	59,98	4,74	16,46	9,42	288 366 452	4,01 3,98 3,70
IX a	52,1[a]	249 (alcohol)	51,54 51,62	5,68 5,80	21,25 21,17	10,01 10,10	$C_{14}H_{17}N_5S \cdot HC$[b]	51,92	5,60	21,62	9,90	270 336 428	4,55 3,93 3,16
IX b	83[a]	234 (butyl alcohol)	55,94 55,81	6,15 6,11	18,96 19,13	9,13 9,04	$C_{14}H_{21}N_5S \cdot HC$[c]	56,11	6,10	19,24	8,81	216,5 270 302 pl 336 428[e]	4,46 4,58 3,67 3,93 3,19
X c	99,2[a]	217 (butyl alcohol)	56,21 56,34	6,47 6,36	10,99 10,89	—	$C_{12}H_{14}N_2O_2 \cdot HCl$[d]	56,58	5,94	11,00	—	244,5 250 304	4,30 4,21 3,13
X d	95,4[a]	184 (isopropyl alcohol)	59,24 59,42	6,94 7,04	9,60 9,78	—	$C_{14}H_{18}N_2O_2 \cdot HCl$	59,46	6,77	9,91	—	244 249,5 303	4,28 4,20 3,08
X f	60	266 (acetic acid)	55,59 55,76	3,76 3,71	13,77 13,66	10,92 10,85	$C_{14}H_{11}N_3O_3S$	55,80	3,68	13,95	10,64	249,5 300 411 e	4,40 3,49 3,41

[a]Yield of base given.
[b]Found, %: Cl 10.73; 10.92. Calculated, %: 10.95.
[c]Found, %: Cl 9.83; 9.71. Calculated, %: Cl 9.74.
[d]Found, %: Cl 14.08; 14.14. Calculated, %: 13.81.
[e]The position and intensity of this band were measured for a $2 \cdot 10^{-4}$ M solution.

2-p-Methoxyphenylamino-9b-hydroxy-5-methyl-1,3,4-thiadiazino[5,6-b]-indole (VIe)* was obtained by treating the thiosemicarbazone (If) with hydrochloric acid, as for the compound not containing a methoxy group [11]. The product was isolated as fine cherry-red crystals.

3-(2-Piperidinoethylthio)-9-methyl-1,2,4-triazino[6,5-b]indole Hydrochloride (IXb). To a solution of 2.7 g (12.5 mmole) of (Va) in 350 ml of 1 N aqueous sodium hydroxide was added a solution of 2.41 g (13.1 mmole) of 2-piperidinoethyl chloride hydrochloride in 10 ml of water, and the mixture stirred and kept for two days. The product separated as a dark raspberry-red oil. The aqueous layer was separated by decantation, and extracted with ether (2 × 150 ml). The oil was dissolved in the ether extract, and the solution dried over anhydrous sodium sulfate and saturated with gaseous hydrogen chloride. The hydrochloride separated as an oil, which crystallized on trituration. The solid was filtered off, washed with ether, and dried in vacuo over phosphoric anhydride to give 3.4 g of (IXb). Recrystallization and drying at 105°C gave small bright yellow crystals.

3-(2-Diemthylaminoethylthio)-9-methyl-1,2,4-triazino[6,5-b]indole Hydrochloride (IXa) was obtained as for (IXb), by reacting (Va) with 2-dimethylaminoethyl chloride hydrochloride. The base was almost quantitatively converted into the hydrochloride, isolated as lustrous yellow crystals.

1-(2-Dimethylaminoethyl)isatin Hydrochloride (Xc). The free base of this compound and (Xd) were obtained previously, and used directly in solution for the synthesis of the thiosemicarbazones without isolation and identification [16]. To a mixture of 28.74 g (0.169 mole) of isatin sodium salt and 70 ml of anhydrous DMF was added 23.4 ml (0.2 mole) of freshly distilled 2-dimethylaminoethyl chloride, and the mixture heated with stirring for 1 h on a boiling water bath. The mixture was kept overnight, filtered, and the filtrate evaporated under reduced pressure to give 36.7 g (99.2%) of the crude base of (Xc) as a reddish-brown oil. This was dissolved in 120 ml of anhydrous methanol, and the solution saturated with hydrogen chloride. The hydrochloride, which separated as an oil, was filtered off, dissolved at the boil in 365 ml of n-butanol, and seeded to accelerate crystallization. After keeping overnight, the solid was filtered off, washed with butanol (2 × 90 ml) and ether (4 × 10 ml), and dried in vacuo over phosphoric anhydride to give 7.77 g of (Xc) as yellow crystals.

1-(2-Diethylaminoethyl)isatin hydrochloride (Xd) was obtained as for (Xc), as fine orange crystals.

3-(4-Sulfamido)phenylimino-2-oxoindoline (Xf). A mixture of 8 g (0.0544 mole) of isatin 9.5 g (0.0551 mole) of p-aminosulfanilamide, and 50 ml of glacial acetic acid was boiled under reflux for 1.5 h. The mixture was then filtered hot, and the solid washed three times with boiling acetic acid and three times with ether to give (Xf) as yellow crystals.

EXPERIMENTAL (BIOLOGICAL PART)

For the model pulmonary adrenalin edema, mongrel white mice of both sexes weighing 18-20 g were used, and for the other models, white rats weighing 180-200 g, at least six animals per test. In the model thermal burns, the inflammatory process was measured by the increase in volume of the rear extremity following immersion in water heater to 51°C. The volume of the extremity was determined oncometrically after 1.5, 3.5, and 24 h. The normal volume of the extremity was taken as 100%. Pulmonary edema was induced by subcutaneous administration of an 0.05% solution of adrenalin hydrochloride in a dose of 5 mg/kg. Development of the inflammatory process was assessed by the "pulmonary coefficient," defined as the ratio of the weight of the lungs in grams per 100 g of body weight. In series I tests, the drugs were administered subcutaneously, in one dose for prophylaxis one hour before application of the irritant used to induce inflammation, as a 2% suspension in peach oil in the thermal burn model in a dose of 50 mg/kg, and pulmonary adrenalin edema model in doses of 20 and 50 mg/kg. In the model pulmonary edema, compounds (VII-X) were administered intraperitoneally in doses of 10 or 50 mg/kg, water-soluble compounds being given as 1% aqueous solutions, and insoluble compounds as 1% solutions in DMS [1]. The effects of the drugs on the exudative and proliferative components were measured by the method of Aleshinskaya and Mokhort in the cotton wool granulemia model. The test compounds were administered intraperitoneally for three days after operation, in doses of 10-15 mg/kg as solutions in water or dimethyl sulfoxide. The ef-

*We thank V. S. Dmitrukha for his assistance in carrying out this part of the work.

fects on the exudative component were assessed by the difference in mass between the freshly separated granulemias and after drying to constant weight, and the effects on the proliferative component by the differences in the weights of the dried capsules and the globules themselves.

The control group of animals received the appropriate solvents. In addition, for purposes of comparison another group of animals was treated intraperitoneally with indomethacin as a 1% solution in 2.5% sodium carbonate, in a dose of 20 mg/kg (in the cotton wool granulemia model, 1 mg/kg).

The results were evaluated statistically, the arithmetic means and their confidence limits being calculated. The scatter of values in the thermal burn model did not exceed 12%, and in the other models 8%.

The acute toxicities of the compounds when given as suspensions in oil were lower than when given as solutions, but they could not be measured accurately as a result of inadequate uptake. Acute toxicities were therefore determined only for solutions, by the intravenous route.

LITERATURE CITED

1. V. N. Barshchikov, A. M. Tentsova, and A. S. Azhgikhin, Farmatsiya, No. 4, 70-74 (1967).
2. G. I. Zhungietu and M. A. Rekhter, Isatin and Its Derivatives [in Russian], Shtiintsa, Kishinev, p. 109.
3. I. S. Ioffe, A. B. Tomchin, and E. N. Zhukova, Zh. Obshch. Khim., 39, No. 1, 78-83 (1969
4. I. S. Ioffe, A. B. Tomchin, and G. A. Shirokii, Zh. Org. Khim., 7, No. 1, 179-183 (1971).
5. A. B. Tomchin, M. A. Ignat'eva, and G. F. Masyuta, Khim.-farm. Zh., No. 3, 23-24 (1972).
6. A. B. Tomchin, I.S. Ioffe, and G. A. Shirokii, Zh. Org. Khim., 8, No. 2, 400-404 (1972).
7. A. B. Tomchin, G. A. Shirokii, and V. S. Dmitrukha, Khim. Geterotsikl. Soedin., No. 1, 83-88 (1976).
8. A. B. Tomchin, V. S. Dmitrukha, and P. S. Pel'kis, Zh. Org. Khim., 13, No. 4, 878-884 (1977).
9. A. B. Tomchin and G. A. Shirokii, ibid., No. 2, 404-411.
10. A. B. Tomchin, K. N. Zelenin, and G. A. Shirokii, Khim. Geterotsikl. Soedin., No. 3, 355 (1979).
11. A. B. Tomchin and G. A. Shirokii, Zh. Org. Khim., 15, No. 4, 855-865 (1979).
12. A. B. Tomchin, ibid., 18, No. 6, 1272-1280 (1982).
13. A. B. Tomchin, V.S. Dmitrukha, V. I. Il'enko, and V. G. Platonov, Khim.-farm. Zh., No. 6, 691-697 (1983).
14. F. P. Trinus and B. M. Klebanov, Fiziol. Akt. Veshch. No. 12, 3-12 (1980).
15. K. C. Joshi, Pharmazie, 37, 1-12 (1982).
16. F. Knotz, Sci. Pharm. (Wien), 38, 98-106 (1970).
17. F. Knotz, ibid., 163-172.
18. R. Pummerer and M. Goettler, Ber. Chem. Ges., 43, 1376-1386 (1910).
19. V. J. Ram, Arch. Pharm. (Weinheim), 313, 108-113 (1980).
20. R. S. Varma and W. L. Nobles, J. Heterocyc. Chem., 3, 462-466 (1966).