Pharmaceutical Chemistry Journal, Vol. 28, No. 9, 1994

SYNTHESIS AND ANTI-INFLAMMATORY ACTIVITY OF 2,2'-DICYCLOHEXYLSULFIDE DERIVATIVES

V. P. Krivonogov, G. A. Tolstikov, B. I. Galimov, Yu. I. Murinov, D. N. Lazareva, V. A. Davydova, and I. I. Krivonogova

UDC 615.442.938.07

Despite the existence of a quite large number of steroidal and nonsteroidal anti-inflammatory agents, the search for new antiphlogistics receives ever more study both from synthetic chemists and pharmacologists and clinicians, because the use of most of the existing anti-inflammatories is limited by poor tolerance and unwanted side effects [1, 2].

We report here the synthesis of 2,2'-dicyclohexylsulfide derivatives and the screening of members of this series for compounds with high anti-inflammatory activity and low toxicity. The compounds and their properties have not previously been described.

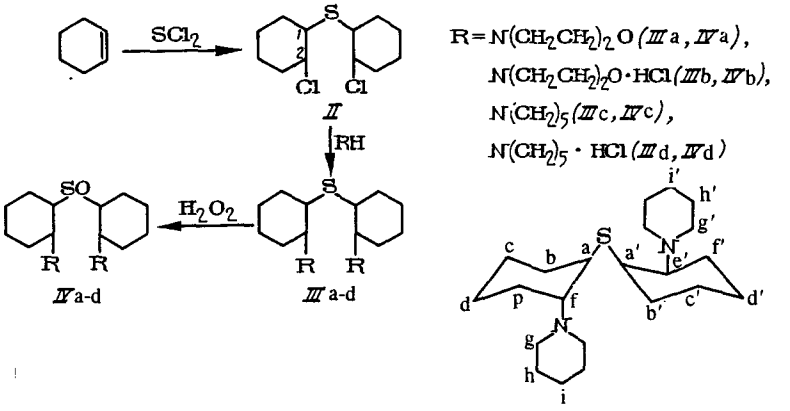
2,2'-Dicyclohexylsulfide derivatives were prepared by treatment of cyclohexene (compound I) with SCl_2 [3] followed by reaction of the resulting dichlorodicyclohexylsulfide with secondary amines at 150-180°C [4]. Yields of desired products were 86-90% in terms of the dichloride (compound II).

This scheme was used to prepare 2,2'-dimorpholinyldicyclohexylsulfide (IIIa) and 2,2'-dipiperidinyldicyclohexylsulfide (IIIc), which are easily oxidized with hydrogen peroxide at room temperature in the presence of 1 N HCl. After evaporation of water, this was used to prepare the dihydrochlorides of 2,2'-dimorpholinyldicyclohexylsulfoxide (IVb) and 2,2'-dipiperidinyldicyclohexylsulfoxide (IVd), treatment of which with 25% ammonia solution and extraction with benzene gives 2,2'-dimorpholinyldicyclohexylsulfoxide (IVa) and 2,2'-dipiperidinyldicyclohexylsulfoxide (IVc).

The dihydrochlorides of 2,2'-dimorpholinyldicyclohexylsulfide (IIIb) and 2,2'-dipiperidinyldicyclohexylsulfide (IIId) were prepared by passage of gaseous HCl through benzene solutions of these compounds to constant weight, followed by evaporation of solvent.

The purity and structure of compounds were demonstrated by IR [5, 6] and PMR spectra, and by elemental analysis.

Scheme 1



PMR spectral signals were assigned without difficulty, as a large number of compounds was studied. Structural fragments of these compounds are in atlases of PMR spectra [7, 8].

Institute of Organic Chemistry, Urals Scientific Center, Russian Academy of Sciences, Ufa. Translated from Khimiko-farmatsevticheskii Zhurnal, No. 9, pp. 29-33, September, 1994. Original article submitted November 29, 1993.

0091-150X/94/2809-0641$12.50

PMR spectra of reaction products were recorded on a Tesla BS-487B (Czechoslovakia) apparatus with a working frequency of 80 MHz. Proton chemical shifts with respect to hexamethyldisilane were expressed in terms of δ (ppm). IR spectra were taken on a Carl Zeiss, Jena IR-20 spectrophotometer (Germany) using NaCl and LiF prisms as liquid films or as suspensions in vaseline. Melting temperatures were determined using a Boetius apparatus (Germany). Elemental analyses corresponded to expected results.

2,2'-Dichlorodicyclohexylsulfide (II). Freshly prepared compound I (201 g, 2.45 mole) in 300 ml of CH_2Cl_2, dried over $CaCl_2$, was cooled to −20 to −25°C; this temperature was maintained as 123.6 g (1.2 mole) of freshly prepared SCl_2 in 100 ml of CH_2Cl_2 was added dropwise over 2 h; the reaction mixture was then brought to room temperature and kept for 2 h (the solution was transparent). The heat exchanger was replaced with a distillation tube and the CH_2Cl_2 was evaporated at a water bath temperature of 40°C using a tap-operated vacuum pump; the yield was 303 g (94%) of a viscous transparent liquid, which crystallized on standing for 2 days. Crystals were collected by filtration using a Schott funnel, and the yield was 258 g (80%) of 2,2'-dichlorodicyclohexylsulfide (II) with a trans-diequatorial orientation of substituents; the melting temperature was 74°C, the formula was $C_{12}H_{20}Cl_2S$. The IR spectrum (ν_{max}, cm^{-1}) was 625-670 (C−S), 710-770 (CCl). A strong band at 727 cm^{-1} is characteristic of the CCl equatorial bond. Other absorption bands around 693 cm^{-1} demonstrate the trans-diequatorial orientation of substituents [5].

2,2'-Dimorpholinyldicyclohexylsulfide (IIIa). 2,2'-Dichlorodicyclohexylsulfide (106.8 g, 0.4 mole) was mixed with 278 g (3.2 mole) of morpholine, and the reaction mixture was heated to the boiling point of morpholine (120-150°C); the reaction was mixed at this temperature for 6 h. After cooling, a crystalline mass was obtained, which was dissolved in 400 ml of water. The insoluble crystals were collected by filtration using a Schott filter and washed with water to yield 126 g (86%) of crystals which were highly soluble in benzene and chloroform and less soluble in ether and acetone. Recrystallization from alcohol yielded 110 g of pink crystals with a melting temperature of 122-124°C (alcohol). The formula was $C_{20}H_{36}N_2O_2S$.

The IR spectrum (ν_{max}, cm^{-1}): 1090-1140 (antisymmetrical vibrations of C−O−C), 1120 (symmetrical vibrations of C−O−C), 800-1200 (valent vibrations of C−N and stretching vibrations of N−H), 1457 (deformational vibrations of CH), 3000-2850 (valent vibrations of CH) [6]. The PMR spectrum ($CDCl_3$, δ, ppm) was: 0.97-1.47 (m, $8H^{c, c', d, d'}$), 1.6-2.17 (m, $8H^{b, b', e, e'}$), 2.17-2.93 (m, $12H^{g, g', f, f', a, a'}$), 3.53 (t, j = 5.0; $8H^{h, h'}$).

2,2'-Dimorpholinyldicyclohexylsulfide Dihydrochloride (IIIb). 2,2'-Dimorpholinyldicyclohexylsulfide (IIIa) (11.0 g, 0.03 mole) was dissolved in 150 ml of benzene; this solution was cooled with water and gaseous HCl was passed through it to complete saturation (weight increase of 2 g); benzene was evaporated *in vacuo* using a tap pump, and the residue was dried *in vacuo* to yield 13 g (100%) of 2,2'-dimorpholinyldicyclohexylsulfide dihydrochloride, with a melting temperature of 220-221°C; this was highly soluble in water (0.1 g dissolved in 2 ml of water) and methanol and was insoluble in benzene and ether. The formula was $C_{20}H_{36}N_2O_2S \cdot 2HCl$.

The IR spectrum (ν_{max}, cm^{-1}) was: 600-660 (C−S), 1010-1250 (N≡), 1120 (C−O−C), 2460, 2580, (N≡·HCl). The PMR spectrum (D_2O, δ, ppm) was: 0.83-2.6 (m, $16H^{2b, 2d, 2e, 2c}$), 2.6-3.27 (m, $12H^{2a, 2f, 4h}$), 3.45 (m, $8H^{4g}$).

2,2'-Dimorpholinyldicyclohexylsulfoxide (IVa). 2,2'-Dimorpholinyldicyclohexylsulfide (IIIa) (36.8 g, 0.1 mole) was dissolved in 200 ml of benzene, and 200 ml of 1 N HCl was added; 11.3 g (0.1 mole) of 30% hydrogen peroxide was then added over 30 min with cooling using cold water. The reaction mixture was mixed at room temperature for 8 h and then kept overnight. The next day, the resulting complex was diluted with 25 ml of 25% aqueous ammonia and extracted with benzene (3 × 20 ml). The combined benzene extracts were dried over $MgSO_4$, and the benzene was evaporated *in vacuo* using a tap pump. The residue weighed 34.5 g (90%), and was a viscous, ductile glassy mass; it crystallized upon standing. After recrystallization from benzene, a yield of 30 g (80%) of 2,2'-dimorpholinyldicyclohexylsulfoxide was obtained, with a melting temperature of 112-114°C (benzene). The formula was $C_{20}H_{36}N_2O_3S$.

The IR spectrum (ν_{max}, cm^{-1}) was: 1020, 1040 (valent vibrations of S=O), 560-570 (deformational vibrations of SO), 1120 (symmetrical vibrations of C−O−C), 1070 (antisymmetrical vibrations of C−O−C), 1210, 1245 (characteristic for tertiary amines). The PMR spectrum (C_6D_6, δ, ppm) was: 0.73-1.25 (m, $8H^{c, d, c', d'}$), 1.35-1.71 (m, $H8^{b, d}$), 1.88-2.75 (m, 12 $H^{g, g'f, f', a, a'}$), 3.38-2.63 (m, $8H^{h, h'}$).

2,2'-Dipiperidinyldicyclohexylsulfide (IIIc). 2,2'-Dichlorodicyclohexylsulfide (II) (98.8 g, 0.366 mole) was mixed with 272 g (3.2 mole) of piperidine. The reaction mixture was heated to 110°C (water bath temperature 130°C), and was mixed at this temperature for 6 h and left overnight. On the next day, the reaction mixture was diluted with water to dissolve the quaternary salt of piperidine with HCl, and this was extracted with ether. After evaporation of the ether, 134 g of liquid was

TABLE 1. Comparison of the Acute Toxicities of Compounds with Known Anti-inflammatory Substances

Compound	I.p. LD_{50}, mg/kg
IVb	4800 (3423±5785)
IIIb	2370 (1762±3177)
IVa	3100 (2818±3410)
IIIa	3900 (3507±4337)
IIIc	1080 (1140±1114)
IIId	4225 (3799±4704)
IVc	1120 (889±2008)
IVd	860 (651,5±1135.2)
Voltaren	380
Butadione	250
Brufen	370
Aspirin	3000
Indomethacin	30

TABLE 2. Effects of 2,2'-Dicyclohexylsulfide Deriva-tives on Carragheenan Inflammation in Rats

Compound	Dose of 1/50 LD_{50}	Anti-inflammatory effect, %
IVb	96	27
IIIb	47	42
IIId	84	0
IIIc	22	43
IVa	62	24
IIIa	78	14
IVd	17	0
IVc	22	15
Voltaren	8	50

obtained, from which crystallization with acetone yielded 101 g (76%) of 2,2'-dipiperidinyldicyclohexylsulfide (IIIc), with a melting temperature of 68-69°C (alcohol). The formula was $C_{22}H_{40}N_2S$.

The IR spectrum (ν_{max}, cm^{-1}) was: 760, 780, 820, 850, 865, 880, 900, 975, 1020, 1040, 1050, 1110, 1125, 1140, 1160, 1170, 1200, 1210, 1225, 1270, 1289, 1310, 1330, 1350, 1380, 1465, 2740, 2770, 2800, 2860, 2940. The PMR spectrum ($CDCl_3$, δ, ppm) was: 0.83-2.08 (m, $28H^{b, c, d, e, b', c', d', e', h, h', i, i'}$), 2.80-3.17 (m, $12H^{g, g', f, f', a, a'}$).

2,2'-Dipiperidinyldicyclohexylsulfide Dihydrochloride (IIId). This was prepared by 2,2'-dipiperidinyldicylcohexylsulfide (IIIc) as described for compound IIIb. The yield was 99%, the melting temperature was 252-254°C, the compound was highly soluble in water (0.1 g of IIId dissolved in 1 ml of water) and methanol, and insoluble in ether and benzene. The formula was $C_{22}H_{40}N_2OS \cdot 2HCl$.

The IR spectrum (ν_{max}, cm^{-1}) was: 560, 660 (C−S), 1110-1270 (N≡), 2440, 2540 (=N−HCl). The PMR spectrum (D_2O, δ, ppm) was: 1.83 (m, $28H^{2b, 2c, 2d, 2e, 4h, 2i}$), 2.6-3.7 (m, $12H^{4g, 2a, 2f}$).

2,2'-Dipiperidinyldicyclohexylsulfoxide (IVb). This was prepared from 2,2'-dipiperidinyldicyclohexylsulfide (IIIc) as described for IVa. The yield was 90%, the melting temperature was 39-42°C (benzene). The formula was $C_{22}H_{40}N_2OS$.

The IR spectrum (ν_{max}, cm^{-1}) was: 1040 (valent vibrations of SO), 560, 680 (deformational vibrations of SO), 1105, 1120, 1140, 1160, 1210, 1220, 1260 (typical of tertiary amines. The PMR spectrum (CCl_4, δ, ppm) was: 0.94-1.88 (m, $28H^{b, c, d, e, b', c', d', e', 2h, 2h', 2i', 2i}$), 1.88-2.80 (m, $12H^{2g, 2g', f, f', a, a'}$).

2,2'-Dipiperidinyldicyclohexylsulfoxide Dihydrochloride (IVd). 2,2'-Dipiperidinyldicyclohexylsulfide (IIIb) (5.47 g, 0.015 mole) was mixed with 50 ml of benzene and 30 ml of 1 N HCl, and 1.9 ml of 30% hydrogen peroxide was added dropwise while cooling with water; the reaction mixture was mixed at room temperature for 8 h, after which the water and benzene were evaporated to yield 6.63 g (100%) of 2,2'-dipiperidinyldicyclohexylsulfoxide dihydrochloride (IVd), which was a thick, yellow, oil-like liquid which, on drying *in vacuo*, became a viscous glassy mass. The compound was highly soluble in water, methanol, and acetone, was insoluble in ether, hexane, and benzene.

The IR spectrum (ν_{max}, cm^{-1}) was: 625 (deformational vibrations of SO groups), 1040 (valent vibrations of SO groups), 1130, 1170 (typical of tertiary amines), 2560, 2649, 2700 (the quaternary amine salt), 3400 (H_2O). The PMR spectrum (D_2O, δ, ppm) was: 1.83 (m, $28H^{b, c, d, e, b', c', d', e', h, h', i, i'}$), 2.7-4.3 (m, $12H^{g, g', f, f', a, a'}$).

TABLE 3. The Effects of 2,2'-Dicyclohexylsulfide Derivatives on Inflammation Induced by Carragheenan in Mice

Compound	Dose, mg/kg	Number of mice	% increase in footpad edema at 4 h compared to initial	p	Anti-inflammatory effect, %
IIIa	50	8	54,2±3,9	<0,01	33
IIIc	50	8	54,6±4,6	<0,01	33
IIId	50	8	35,5±6,3	<0,02	55
IVa	50	8	58,63±2,66	<0,01	27
IIIb	50	8	49,1±2,8	<0,002	39
IVb	50	8	58,51±5,65	<0,02	27
IVc	50	8	45,7±5,7	<0,002	44
IVd	50	8	69,08±3,84	<0,01	14
Voltaren	8	7	39,1±4,3	<0,001	50
Control	—	7	81,0±6,0	—	

TABLE 4. The Effects of 2,2'-Dicyclohexylsulfide Derivatives on Inflammation Induced by Carragheenan in Rats

Compound	Dose, mg/kg	Number of rats	Mean increase in footpad volume, % of initial		p for rats		Anti-inflammatory effect, %	
			after 4 h	after 24 h	after 4 h	after 24 h	after 4 h	after 24 h
IIIa	50	6	45,22±5,1	25,99±5,13	<0,01	<0,01	26	25
IIIb	50	6	31,09±2,89	22,48±4,64	<0,01	<0,01	49	30
IIIc	50	6	34,87±3,59	20,59±4,1	<0,02	<0,01	42	37
IIId	50	6	38,33±6,20	19,99±7,39	<0,005	<0,01	49	37
IVa	50	8	48,67±6,9	25,45±6,93	<0,01	<0,01	21	35
IVb	50	8	34,26±2,1	15,84±2,17	<0,01	<0,02	44	50
IVc	50	8	24,26±2,36	13,5±3,38	<0,002	<0,01	60	59
IVd	50	8	32,06±2,93	13,31±1,68	<0,01	<0,01	47	59
Voltaren	8	8	29,45±4,59	14,93±2,53	<0,01	<0,02	52	53
Control	—	8	60,68±6,15	32,47±5,29	—	—	—	—

2,2'-Dimorpholinyldicyclohexylsulfoxide Dihydrochloride (IVb). This was prepared with a 100% yield by the same method used for IVb, using 5.5 g (0.015 mole) of 2,2'-dimorpholinyldicyclohexylsulfide (IVa), 30 ml of 1 N HCl, and 1.9 ml of 30% hydrogen peroxide. 2,2'-Dimorpholinyldicyclohexylsulfoxide dihydrochloride was a light brown, viscous, glassy mass, which was highly soluble in water and methanol and insoluble in hexane and ether.

The IR spectrum (ν_{max}, cm^{-1}) was: 625 (δ, SO), 1050 (ν, SO), 1120 (C−O−C), 1130-1160 (N≡), 2560, 2650, 2700 [$(R^3NH)^+A^-$). The PMR spectrum (D_2O, δ, ppm) was: 1.17-3.00 (m, $16H^{b, c, d, e, b', c', d', e'}$), 3.03-3.83 (m, $12H^{2g, 2g', f, f', a, a'}$), 3.83-4.61 (m, $8H^{2i,2i'}$).

PHARMACOLOGICAL METHODS

The acute toxicities of the compounds was studied in 320 white mongrel mice of both sexes, which received single i.p. doses. LD_{50} values were calculated as described by Litchfield and Wilcoxon on the tenth day of observation [9]. The results are presented in Table 1.

The compounds synthesized were less toxic (see Table 1) by a factor of 3-18 as compared with butadione, by 3-12 as compared with Voltaren, and by 29-160 as compared to indomethacin.

These data indicate that the compounds synthesized here are members of toxicity class III, i.e., moderately toxic substances.

TABLE 5. Anti-inflammatory Effects of Compounds IVb and IIIb in Comparison with that of Voltaren in Carragheenan Edema

Compound	ED_{50}, mg/kg	Mean increase in footpad volume, %	Anti-inflammatory effect, %
IVb	170	72.8±9.4	47
IIIb	62	75.2±13.7	46
Voltaren	8	87.2±11.2	37
Control	—	13.97±24.5	—

TABLE 6. The Effects of 2,2'-Dicyclohexylsulfide Derivatives on the Ulcerogenic Actions of Indomethacin (20 mg/kg) and Acetylsalicylic Acid (150 mg/kg b.i.d.)

Compound	Number of rats	Dose, mg/kg	Indomethacin		Number of rats	Acetylsalicylic acid	
			mean number of ulcers at 24 h	ulcers present		mean number of ulcers at 24 h	ulcers present
Control	4	—	4	In all 4 rats: 8, 2, 3, 3	5	9	In 3 of 5
Voltaren	5	8	2	In 1 of 5 rats; stomach contents brown	4	3	In 2 of 4
IVb	4	170	None	Brown mucus in plicae	4	3	In 2 of 4
IIIb	4	62	None	Light-colored stomach contents	—	—	—

TABLE 7. Therapeutic Indexes (TI)

Compound	LD_{50}, mg/kg	ED_{50}, mg/kg	TI
Voltaren	380	8	47,5
IVb	4800	170	28
IIIb	2470	62	40

Anti-inflammatory activities were studied using an inflammation model based on dosage with 0.1 ml of 1% aqueous carragheenan in rats or with doses of 0.05 ml in mice, given under the footpad aponeurosis. Anti-inflammatory activity was assessed in terms of the reduction in inflammatory edema in comparison with Voltaren. Compounds were given at a dose of 50 mg/kg and at 1/50 of the LD_{50}, 1 h before induction of inflammation. Some compounds were compared with Voltaren by dosage at the ED_{50} (the mean effective dose calculated by the Litchfield–Wilcoxon method). Measurements were made at the time of maximum inflammation (4 h after carragheenan).

Comparative studies of the anti-inflammatory effects of substances at a dose of 1/50 the ED_{50} showed that most 2,2'-dicyclohexylsulfide derivatives had anti-inflammatory activity, reducing the intensity of carragheenan edema. The results are presented in Table 2.

The effects of compounds IIIb and IIIc at this dose were similar to those of Voltaren.

Comparison of the anti-inflammatory effects of 2,2'-dicyclohexylsulfide derivatives with that of Voltaren at the LD_{50}, using the carragheenan edema model in mice and rats, using doses of 50 mg/kg (Tables 3 and 4) showed that the compounds had antiphlogistic activity. Derivatives IIId, IIIb, and IVc had activities in mice similar to that of Voltaren. In rats, IIId and IVc, and IIIc and IVd were more effective.

ED_{50} values were determined for some active and highly soluble compounds, and their effects were compared with that of Voltaren (Table 5).

The effects of compounds IVb and IIIb at doses equal to the ED_{50} were similar to that of Voltaren.

Compound IIIb differed from many anti-inflammatory therapeutic agents in lacking any ulcerogenic effect, as demonstrated in terms of induction of ulcers with acetylsalicylic acid and indomethacin in rats (Table 6).

Thus, a number of 2,2'-dicyclohexylsulfide derivatives, i.e., IIId, IIIb, IVc, and IVb, have anti-inflammatory effects which, in the cases of these four compounds, are comparable with the actions of Voltaren.

The compounds were less toxic than butadione, Voltaren, and indomethacin.

The therapeutic ratio of compound IIIb was similar to that of Voltaren (Table 7).

Since a number of these novel 2,2'-dicyclohexylsulfide derivatives were found to have high anti-inflammatory activities, further studies on their pharmacological properties should be carried out.

REFERENCES

1. F. P. Trinus, N. A. Mokhort, and V. M. Klebanov, Non-Steroidal Anti-Inflammatory Agents [in Russian], Kiev (1975).
2. A. S. Saratikov, G. P. Pritsep, and V. E. Yavorskaya, Anti-Inflammatory Agents of the Pyrazole Group [in Russian], Tomsk (1975).
3. G. A. Tolstikov, N. N. Novitskaya, R. G. Kantyukova, et al., Tetrahedron, **34**, No. 17, 2655-2661 (1978).
4. V. P. Krivonogov, R. M. Shayakhmetova, V. I. Dronov, et al., Zhurn. Priklad. Khim., No. 11, 2505-2510 (1981).
5. N. N. Novitskaya, R. G. Kantyukova, V. A. Ponomareva, et al., Monomer Chemistry and Physical Chemistry [in Russian], Ufa (1975), pp. 104-120.
6. M. D. Mashkovskii, Therapeutic Agents [in Russian], Moscow (1978), Vol. 1, pp. 177, 186, 190, 192, 198.
7. NMR Spectra Catalog, compiled by N. S. Bhacca, D. P. Hollis, L. F. Johnson, E. A. Pier, and J. N. Shoolerg, Instrument Division, Varian Associates (1962, 1963). Lithographed in the USA by the National Press.
8. H. A. Szymanskii and R. E. Jelin, NMR Band Handbook, New York–Washington (1968).
9. Litchfield and Wilcoxon, cited in: M. L. Belen'kii, Elements of the Quantitative Analysis of Pharmacological Effects [in Russian], Riga (1959), pp. 171-174.