SYNTHESIS AND ANTITUMOR ACTIVITY OF EPOXY HETEROCYCLIC COMPOUNDS.

1. GLYCIDYL TRIAZOLONE AND IMIDAZOLONE DERIVATIVES

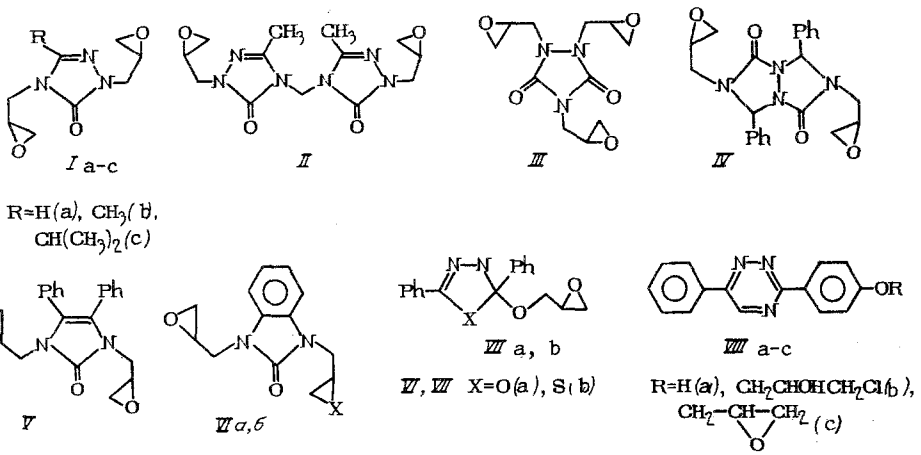
N. I. Korotkikh, G. A. Losev,
V. F. Lipnitskii, S. G. Kalistratov,
A. S. Sokolova, and O. P. Shvaika

UDC 615.015.11:547.78+547.79+547.87

Research on the antitumor properties of epoxide compounds has been comparatively limited over the years. Antitumor activity has been established for the prospidin epoxide analogue [9] and more recently the German firm Henckel has shown an interest in the glycidyl derivatives of cyclic ureas, which were tested on the P-388 leukemia strain [6]. Prolongation of lifespan for the test animals reached at best 150-200% compared to the control. Unfortunately the authors presented no data on the antitumor activity of these substances with respect to solid tumor strains.

We have already investigated the activity of the series of epoxy heterocyclic compounds having amide and pyridine nitrogen atoms in combinations that are encountered in many physiologically active substances and that are capable of accelerating oxirane addition reactions [4], leading us to expect that the compounds examined would be highly effective. In the current report we have made a comparative study of epoxide derivatives of the triazolone series, namely the 1,2,4-triazolones (Ia-c), bistriazolone II, triazolidinedione III and dioxotriazolidinotriazolidine, and the imidazole series: imidazolone V, benzimidazolone VIa, its thiirane analogue VIb, and monoglycidyloxy derivatives of oxa- and thiadiazolines (VIIa and VIIb) and triazine (VIIIc).

Compounds Ia-c were synthesized using semicarbazide as the starting reagent, as outlined in [2]; compound II was formed by reacting 1,2,4-triazolone-5 with formaldehyde, then condensing the resultant bistriazonolylmethanes with epichlorhydrin and dehydrochlorinating the condensation products with alkalis [5]. Compounds III-VIa were obtained in a similar way from the corresponding azolones [1, 7]. Compounds IV and VIb-VIIIa are described for the first time. Thiiranoepoxide compound VIa was synthesized using diglycidylbenzimidazolone VIa and thiourea as the starting reagents. Oxa- and thiadiazolines VIIa and VIIb were obtained by reacting the appropriate oxa- and thiadiazole salts with the trialkylammonium glycidylates formed in situ by mixing triethylamine with glycidol as described in [11]. The ac-triazine derivative VIIIa was synthesized using the method outlined in [3] from phenacyl bromide and p-hydroxybenzhydrazide; the intermediate chlorhydrin VIIIb afforded its glycidyl ester VIIIc.



Institute of Physico-organic Chemistry and Carbon Chemistry, Ukrainian Academy of Sciences, Donetsk. Center for the Chemistry of Medicines, All-Union Pharmaceutical Chemistry Research Institute. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 27, No. 1, pp. 51-54, January, 1993. Original article submitted April 4, 1991.

0091-150X/93/2701-0067$12.50 © 1993 Plenum Publishing Corporation

TABLE 1. Yields, Principal Constants and Elemental Analysis Data for New Compounds II, IV, VIIa, VIIb and VIIIa-c

Compound	Yield, %	R_f	Melting point, °C (crystallization solvent)	Empirical formula
II	77	0,39	134—137 (ethanol)	$C_{13}H_{18}N_6O_4$
IV	95	0,82	158—162 (dimethylformamide)	$C_{22}H_{12}N_4O_4$
VIIa	78	0,70	99—101 (methanol)	$C_{23}H_{20}N_2O_3$
VIIb	45	—	140—143 (hexane)	$C_{23}H_{20}N_2O_2S$
VIIIa	24	0,62	241—242 (acetic acid)	$C_{15}H_{11}N_3O$
VIIIb	57	0,65	153—156 (ethanol)	$C_{18}H_{16}ClNO_2$
VIIIc	94	0,88	155—157(ethanol)	$C_{18}H_{15}N_3O_2$

Note. Chlorine content in compound VIIIb was 10.2% (calculated 10.4%). R_f of compounds VIIIa and VIIIb were determined in a 2:1 acetone—benzene system.

The composition and structure of compounds IV, VIIa, VIIb and VIIIa-c were confirmed by elemental analysis, epoxide and thiirane group content measurements and the molecular weights of the substances (Table 1), and were corroborated by spectral data (Table 2). For example, the PMR spectra of epoxide derivatives I.V, VIIa, VIIb and VIIIc exhibited the characteristic oxirane ring signals (δ of 2.33-2.68 ppm for CH_2—O and 2.9-3.3 ppm for CH—O) and CH_2—N group signals of the glycidyl fragment (3.2-4.2 ppm). In the IR spectra the most typical absorption bands were the epoxide ring CH_2 (ν of 3060-3070 cm^{-1}) and the C=O (ν of 1705 cm^{-1}, compound IV) and C=N bands (ν of 1560-1590 cm^{-1}, compounds VIIa, VIIb and VIIIa-c).

Compounds I, II and IV-VI comprised mixtures of chromatographically indistinguishable recemates (R, R/S, S) and meso-forms (R, S); compound III was a mixture of two racemates (R, R, R/S, S, S and R, R, S/S, S, R). No separation or evaluation of the contents of individual forms was undertaken.

EXPERIMENTAL (CHEMICAL)

Epoxide compounds Ia-c, II, III and IV were purified by means of column chromatography using 100/160 μm silica gel adsorbent and 10:1 and 5:1 chloroform—methanol elutriator. The other compounds and epoxides III and IV were purified by recrystallization from appropriate solvents. PMR spectra were recorded on a Tesla BS-467C (60 MHz), internal standard TMS or HMDS. IR spectra were taken on a UR-20 instrument with slit program 4 and registration speed of 160 cm^{-1}/min.

Thin-layer chromatography was performed using Silufol silica gel (Czechoslovakia), with a 10:1 chloroform—methanol mixture as elutriator and iodine vapor as developer. Molecular weights were determined by the cryoscopic method in water (compound II) and in benzene (compounds IV, VIIIb and VIIIc). Epoxide group content (EGC) was found the means of argentometry in conformity with state standard GOST 12497—78 by reacting the compound with hydrogen chloride in acetone (back titration).

Diglycidyl-1,2,4-triazolones-5 (Ia-c, II, IV). A sample of 0.34 g (2 mmoles) of tetraethylammonium chloride was added to a suspension of 0.1 mole of the corresponding triazolone in 157 ml (2 moles) of epichlorhydrin. The reaction mixture was heated to 80-90°C (compounds Ia-c, II) or 100°C (compounds III, IV) and stirred at this temperature for 2-3 h (compounds Ia-c), 6-11 h (compound II) or 1 h (compound III). During this period the epichlorhydrin was observed to dissolve and undergo complete addition at the two functional NH groups. After this solution had been cooled to 30-50°C 8.8 g (0.22 moles) of caustic soda were added with vigorous stirring over 1 h, the stirring being continued for a further 3-9 h. When the resultant precipitate had been filtered off, the filtrate was evaporated in vacuum. Colorless or yellowish viscous liquids were obtained in the residue. From these liquids only epoxide Ib was successfully isolated in the crystalline form (mp 47-50°C) by extracting with hexane or by means of column chromatography (see also [2]).

5-Glycidyloxy-2,4,5-triphenyl-Δ^2-1,3,4-oxa- or thiadiazolines (VIIa and VIIb). To 5 ml of freshly-distilled glycidol were added 1.5 ml (10 mmoles) of anhydrous triethylamine (the

TABLE 2. Spectral Data for New Compounds II, IV, VIIa, VIIb and VIIIa-c

Compound	IR spectra, ν, cm^{-1}	PMR spectra, δ, ppm				
		epoxide group proton signals		CH_2–N(O)	CH arom.	other characteristic signals
		CH_2O	CH–O			
II	307w (CH_2O), 1728s (C=O), 1596s (C=N)	2.33m, 2.62m	3.02m	3.22-40.2m 5.45s	–	2.20s (CH_3)
IV	3090s (CHN), 3065w (CH_2O), 1735s, 1723s (C=O), 1600ar. (C=$C_{arom.}$)	2.40-2.75m	275-3.12m	4.02d 4.09d, 3, 12m	7.48m	6.30s 6.40s (CHN)
VIIa	3065w (CH_2O), 1630w, 1600s, 1505s (C=N C=$C_{arom.}$)	2.40m	2.97m	3.43m	7.23m	–
VIIb	3070w (CH_2O), 1600s, 1560m, 1500s (C=N/C=$C_{arom.}$)	2.73m	3.27m	3.83m	7.43m	–
VIIIa	3330m, broad (O–H), 3085w (CHN), 1605w, 1590s (C=N/C=$C_{arom.}$)	–	–	–	6.77-8.59m	9.41s (CHN) 10.22s (O–H)
VIIIb	3270m, broad (O–H), 3065w (CHN), 1620s, 1562m (C=N/C=$C_{arom.}$)	–	–	4.04m	7.12-8.49m	9.37s (CHN)
VIIIc	3085w (CHN), 3060w (CH_2O), 1584w, 1563m (C=N/C=$C_{arom.}$)	2.68m	3.24m	4.07d	6.87-8.63m	8.84s (CHN)

Note. IR spectra taken in Nujol. PMR spectra recorded in deuterochloroform (compounds II and IV), and carbon tetrachloride (compounds VIIa and VIIb).

mixture was heated slightly). Then 4 g (10 mmoles) of 2,3,5-triphenyl-1,3,4-oxa- or thiadiazolium perchlorate were added in small amounts with stirring. After it had been heated to 60°C (~10 min), then cooled, 50 ml or water were added to the mixture, which was stirred. The viscous oily product that precipitated from the solution was crystallized from methanol (see Tables 1 and 2).

3-[4-(2-Hydroxy-3-chloropropyl)oxyphenyl]-6-phenyl-1,2,4-triazine (VIIIb). To a suspension of 1.25 g (5.02 mmoles) of triazine VIIIa in 7.8 ml (0.1 mole) of epichlorhydrin were added 0.06 g of 25% aqueous caustic soda solution and 0.017 g (0.1 mmole) of tetraethylammonium chloride. After the mixture had been stirred at 80-85°C for 1-5 h, the resultant solution was evaporated to dryness in vacuum at 60°C and triturated with 1 ml of alcohol. The precipitate was filtered off and dried (see Tables 1 and 2).

3-(4-Glycidyloxyphenyl)-6-phenyl-1,2,4-triazone (VIIIc). A sample of 0.017 g (0.1 mmole) of tetraethylammonium chloride and 0.24 g (6 mmoles) of caustic soda were added to a solution of 2.04 g (6 mmoles) of chlorhydrin VIIIb in 9.5 ml of epichlorhydrin. The reaction mixture was stirred for 15 min at 50°C and for 1 h at 80°C. After the suspension had been cooled, the precipitate was filtered off and the filtrate evaporated in vacuum at 50°C. Then 3-4 ml of hexane were added to the semi-crystalline precipitate, which was filtered off and dried (see Tables 1 and 2).

EXPERIMENTAL (BIOLOGICAL)

Experiments on the toxicity and antitumor activity of the new glydicyl triazolone and imidazolone derivatives I-VIIIc were carried out with outbred white mice and rats of both sexes weighing 18-20 g and 110-130 g respectively, and on BDF_1 and $C_{57}BL_6$ line mice using the standard methods [8, 10]. The water-insoluble compounds (IV, V, VIa, VIb and VIIIc) were studied as suspensions prepared ex temporae using a 10% polyvinylpyrrolidone solution;

TABLE 3. Toxicity and Antitumor Activity of Epoxy Heterocyclic Compounds Ia-c, II-V, VIa, VIb, VIIa, VIIb and VIIIc

Compound	Toxicity, mg/kg		Antitumor activity						
	LD_{50}	MTD	Jensen sarcoma	M-1 sarcoma	180 sarcoma	Walker carcino-	B-16 melanoma	P-388 leukemia*	L_{1210} leukemia*
Ia	72	47	26/92	16/43	—	24/43	40/58	45/160	—
Ib	85	55	13/94	21/67	50/83	—	50/84	—	40/226
Ic	110	70	40/18	—	—	—	—	—	—
II	380	170	42/90	50/51	—	75/98	60/50	160/220	172/170
III	40	25	15/63	10/20	—	15/59	20/48	20/160	20/130
IV	290	190	85/—50	—	185/16	—	—	—	—
V	—	200	85/—60	—	150/28	—	—	—	—
VIa	120	76	55/90	50/75	—	50/79	75/50	60/139	—
VIb	—	48	30/3	30/10	—	—	—	30/138	40/109
VIIa	—	110	60/10	—	—	—	—	—	—
VIIb	—	90	60/11	—	—	—	—	—	—
VIIIc	—	123	60/39	—	—	—	—	—	—

Note. Dose (mg/kg) is shown in the numerator, tumor growth inhibition (I, %) in the denominator. I with a minus sign indicates tumor growth stimulation.
*Increase in mean lifepan of the animals, %.

the water-soluble compounds (Ia-c, II and III) were investigated in isotonic sodium chloride solution.

The toxicity of the substances was tested on mice by means of a single intraperitoneal injection. Median lethal dose (LD_{50}) causing death in 50% of the expressed animals was found for compounds Ia-c, II, III, IV and VIa, while maximum tolerable doses (MTD) were established for all the substances. For compounds Ic, V, VIb, VIIb and VIIIc the tolerability was determined using a single intraperitoneal injection.

Antitumor activity was tested on rats and mice using transplanted Jensen, M-1 and 180 sarcomas, Walker carcinosarcoma (WCS), B-16 melanoma, and P-388 and L_{1210} leukoses. The compounds were administered intraperitoneally (Ia-c, II, III, VIa, VIb) or perorally (IV, V, VIIa, VIIb and VIIIc) over 6-7 days (rats) or 5-7 days (mice) in 1/3-1/8 LD_{50} dosages. Therapeutic effect was assessed in terms of the percentage inhibition of tumor growth (I, %), and in the case of the leukoses from the increase in mean lifespan (IML) of the animals. The data obtained was statistically processed using the Student-Fisher method.

The tests the substances exhibited moderate toxicity: LD_{50} was 40-380 mg/kg for compounds Ia-c, II, III, IV and VIa, and MTD was 25-200 mg/kg for all the compounds (Table 3). The data presented in Table 3 demonstrates that the compounds with pyridine nitrogen atoms (Ia-c, II and VIIIc) displayed high antitumor activity towards most of the solid tumor strains (e.g., I reached 95-96% with respect to the Jensen sarcoma). The bifunctional water-soluble structures Ia-c and II exhibited a greater antitumor effect than the monoepoxides VIIa, VIIb and VIIIc. The greatest antiblastic effect on the P-388 and L_{1210} leukoses came from diglycicyltriazolones Ia-c, particularly compound Ib (IML of up to 226%), and from their bisheterocyclic analogues (IML of up to 220%). Compound II also showed a high antitumor effect towards the solid tumor strains (I of up to 98% with respect to the Walker carcinosarcoma) and appreciably lower toxicity (below half) compared to the mononuclear analogues Ia and Ib. It is interesting that the antitumor effect of compounds Ia-c and II was greater than that of the triepoxide analogue III, while their toxicity was lower. This suggests that the pyridine nitrogen atoms have a significant influence on these properties. Surprisingly, triazolidine IV and imidazolone V revealed no antitumor activity whatsoever, some stimulation of tumor development even being observed (by 50-60% in the case of the Jensen sarcoma). At the same time diglycidylbenzimidazolone VIa displayed a high antiblastic effect (I of up to 90%), although this compound is a structural analogue of epoxide V, When the oxirane ring in compound VIa was replaced by a thiirane ring (compound VIb), antileukosis activity was virtually unaffected (138% and 139% for compounds VIb and VIa respectively), but antitumor activity towards solid tumor strains droped sharply (with respect to the Jensen sarcoma I was 3% for compound VIb and 90% for compound VIa). In addition substance VIb was seen to have a pronouncedly greater toxicity than diepoxide VIa (see Table 3). The lower antitumor activity of thiiranoepoxide VIb compared to the diepoxide analogue VIa was probably due to the lower alkylating activity of the thiirane ring relative to the oxirane ring. Finally, it is worth mentioning the noticeably greater antitumor effect of monoepoxide VIIIc (I of 39%) as against that of azolines VIIa and VIIb (I of 10-11%). This shows that it is worthwhile investigating diaryl-ac-triazines having higher functionality.

In summary, our investigations have enabled new classes of epoxy heterocyclic compounds to be uncovered with high antitumor activity (triazolones II and II). The research also showed that the effect of the heterocyclic ring structure on the antitumor activity and toxicity of this class of compound conforms to a number of empirical rules.

LITERATURE CITED

1. USSR Patent No. 271005. Otkrytiya, No. 17, 76 (1970).
2. USSR Patent No. 1002290. Otkrytiya, No. 9, 76 (1983).
3. N. I. Korotkikh, A. Yu. Chervinskii, S. N. Baranov, et al., Zh. Org. Khim., 15, No. 5, 962-971 (1979).
4. N. I. Korotkikh, V. N. Artemov, and O. P. Shvaika, Dokl. Akad. Nauk, 290, No. 3, 624-627 (1986).
5. N. I. Korotkikh and O. P. Shvaika, New Epoxy Heterocyclic Azole and Azine Series Monomers [in Russian], Dep. at VINITI, P. 146-V 88 (1988), pp. 1-25.
6. European Patent No. 0053503. Izobret. SSSR i za Rubezh., 57, No. 15, 72 (1982).
7. FRG Patent No. 3102373. Izobret. SSSR i za Rubezh., 57, No. 1, 61 (1983).
8. V. A. Chernov, Experimental Chemotherapy Methods [in Russian], Moscow (1971), pp. 357-403.
9. V. A. Chernov, Trudy VNII Khim.-farm. Inst., No. 3, 9-11 (1973).
10. Experimental Assessment of Antitumor Preparations in USSR and USA [in Russian], Moscow (1980), p. 295.

ANTIVIRAL ACTIVITY OF 1-METHYLASCORBIGEN IN EXPERIMENTAL ARBOVIRUS INFECTIONS

L. K. Berezina, T. A. Posevaya,
I. F. Barinskii, A. M. Korolev,
V. E. Zakharov, and M. N. Preobrazhenskaya

UDC 615.281.8.076.9

The search for preparations capable of raising the immune reactivity of the organism toward viral and bacterial infections is very urgent. The present work is dedicated to the study of the possible protection of mice from death induced by some arbovirus infections using the new immunomodulator 1-methylascorbigen. Ascorbigen, an indole-containing derivative of L-ascarbic acid, was isolated from cabbage juice [1, 2]; the natural compound and its analogs, including 1-methylascorbigen, were obtained synthetically [3].

The study of 1-methylascorbigen showed that it is an active immunomodulator, which increases the activity of natural killers and stimulates the generation of cytotoxic T-lymphocytes in the allogenic mixed culture of lymphocytes. When it is injected ip in mice, it induces a significant increase in the number of cells of the peritoneal exudate on account of monocytes, macrophages, activated lymphocytes, and neutrophils [4]. These data impelled us to study the possible utilization of 1-methylascorbigen to protect animals from viral infections. With in vitro experiments, 1-methylascorbigen did not exhibit any antibacterial or antiviral activity.

EXPERIMENTAL

The following bunyaviruses were utilized for infection: Issyk-Kul strain LEIV-315K, white hare strain LEIV 11552 Taimyr, Rift Valley fever (RVF) strain 8-87L, and Crimean hemorrhagic fever (CHF) strain LEIV 10145 Uzbekistan. The virus of Western equine encephalomyelitis (WEE) (California strain) pertaining to the alpha viruses was also utilized. In the case of the bunyaviruses, the virus-containing suspension of the brain of white mice with the infective titer of 7.0-8.5 log LD_{50}/0.03 ml, which was introduced sc (0.1 ml) or intracerebrally (CHF) (0.01 ml), was utilized. The virus of WEE was introduced im using the volume of 0.1 ml at the dose of 10 LD_{50}. Hybrid white mice of the mass 6-8 g and 10-12 g were utilized. The in vivo activiral activity was determined in mice using the indicator of protection with percentages

D. I. Ivanovskii Institute of Virology, Russian Academy of Medical Sciences. Scientific-Research Institute of New Antibiotics, Russian Academy of Medical Sciences Moscow.

Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 27, No. 1, pp. 55-56, January, 1993. Original article submitted February 25, 1992.