from (at least) duplicate incubations at a given drug concentration.

Thiol-Neutralization Assay.—Solutions of drugs in DMF (0.2 ml) were mixed with 0.5 ml of 0.4 mM solutions of 2-mercaptoethanol, cysteine hydrochloride, or glutathione in 0.1 *M* sodium phosphate, pH 7.4. After standing for 2 min at 25°, residual thiol was measured by the coloration produced (and read immediately at 412 mμ) on adding excess Ellman's reagent [5,5′-dithio(2-nitrobenzoic acid)] in 0.1 *M* sodium phosphate, pH 7.4. Appropriate blanks were established with drugs and thiol and drugs and Ellman's reagent. Relative thiol-blocking activity was determined as the molar ratio (drug:thiol) to neutralize 50% of the thiol, using N-ethylmaleimide as reference.

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[3-(2-Mercaptoethylamino)propyl]oxamide and Related Compounds as Potential Antiradiation Agents[1]

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Thiols and the corresponding hydrogen thiosulfate esters were prepared as potential radioprotective agents from [ω-(1-aziridinyl)alkyl]oxamides by ring-opening reactions. Of 18 such compounds prepared, only [3-(2-mercaptoethylamino)propyl]oxamide (**6a**) showed considerable radioprotective activity in mice.

In the course of a continuing search for superior antiradiation agents through modifications of 2-aminoethanethiol, appropriate ring openings of the known[2] N,N′-bis[3-(1-aziridinyl)propyl]oxamide (**2b**) were effected as an entry into the area of 2-(ω-acylaminoalkylamino)ethanethiols and related compounds. The terminal substituent in this case is an oxamoyl group, and the resulting products were N,N′-bis[3-(2-mercaptoethylamino)propyl]oxamide (**3b**) dihydrochloride and the corresponding bis(hydrogen thiosulfate) (**3c**). As shown in Scheme I and described in the Experimental Section, variations of the general reaction sequence led to other oxamide derivatives (**3a**, **b**, **e** and **6a–p**). Such compounds are, in effect, oxamoylated analogs of the recently described S-2-(ω-aminoalkylamino)ethyl dihydrogen phosphorothioates, which showed an exceptionally high level of radioprotective activity.[3] Ring-opened products were limited, however, to thiols and the corresponding hydrogen thiosulfate esters, since, as an example, the treatment of [3-(1-aziridinyl)propyl]oxamide (**5a**) with Na_3SPO_3 in H_2O in the presence of 2 molar equiv of AcOH resulted in the isolation of an impure dihydrogen phosphorothioate ester.

The preparation of N-[3-(1-aziridinyl)propyl]-N′-methyloxamide (**5c**) from ethyl [3-(1-aziridinyl)propyl]-oxamate (**4**) is an exception to the general route and was followed after difficulties had been encountered in the separation of the required intermediate, ethyl methyloxamate, from N,N′-dimethyloxamide following the reaction of diethyl oxalate with $MeNH_2$. Analytically pure N-[3-(1-aziridinyl)propyl]-N′-cyclohexyloxamide (**5d**) was obtained by the alternative route, *i.e.*, the reaction of **4** with cyclohexylamine, although the general route was also effective. Hydrogen thiosulfate esters were prepared by aziridine-ring openings with either $Na_2S_2O_3$ and AcOH[4] or $(NH_4)_2S_2O_3$.[3,5] The thiol **6p** hydrochloride was not obtained pure but was converted into pure [3-(2-phenyl-3-thiazolidinyl)propyl]-oxamic acid 2-phenylhydrazide (**7**) with benzaldehyde.

[3-(2-Mercaptoethylamino)propyl]oxamide (**6a**) hydrochloride was the only end product among those described here that showed appreciable radioprotective activity in mice in tests carried out at the Walter Reed Army Institute of Research, Washington, D. C.[6] The approximate LD_{50} dose of **6a** was 700 mg/kg; a dose of 400 mg/kg of **6a** administered intraperitoneally 30 min prior to irradiation (1000 R, γ rays) gave 53% survival as compared to 0% among untreated control mice, and a dose of 200 mg/kg gave 40% survival. All the other thiols and thiosulfates tested were nonprotective with the exception that the thiosulfate **6b** and the thiol **6c** gave slight protection at a high dose level relative to the respective LD_{50} dose.

Experimental Section[7]

1-(2-Aminoethyl)aziridine (**1a**), bp 126°, was prepared from 2-(2-aminoethylamino)ethanol (1.0 mole) in 17% yield by a published procedure[8] (lit.[8] bp 126–127.5°). On a larger scale (4.8 moles of the alcohol) rearrangement of **1a** to piperazine was predominant, and the yield of **1a** was only 1%.

N,N′-Bis[ω-(1-aziridinyl)alkyl]oxamides (**2**) were prepared by the method reported by Bestian[2] for the preparation of **2b**. A solution of diethyl oxalate (7.30 g, 50.0 mmoles) in EtOAc (10 ml) was added slowly to a stirred solution of 100 mmoles of the appropriate aziridine (**1a**, **1b**,[2,3] or **1c**[3]) in EtOAc (50 ml). The mixture was allowed to stand at 25° for 3 hr and was then refrigerated. The crystalline product was collected and washed with EtOAc: **2a** (mp 159–160°) was obtained in 84% yield; **2b** (mp 142°, lit.[2]

(1) This investigation was supported by the U. S. Army Medical Research and Development Command under Contract No. DA-49-193-MD-2028.

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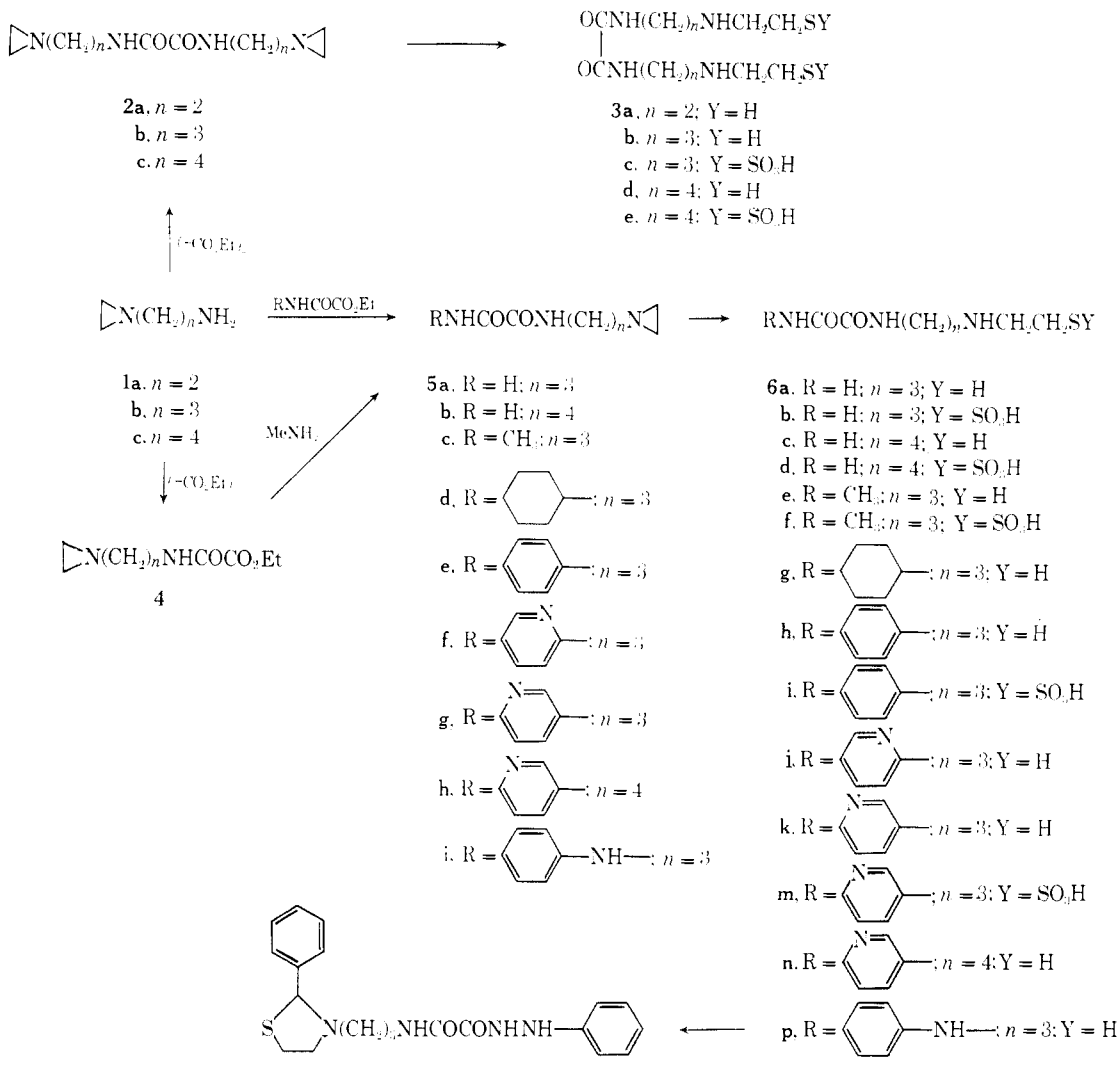
(6) For details of the testing procedure see L. Field, A. Ferretti, R. R. Crenshaw, and T. C. Owen, *J. Med. Chem.*, **7**, 39 (1964).

(7) All analytical samples were dried *in vacuo* over P_2O_5 at room temperature unless another temperature is specified. Melting points were determined with a Kofler Heizbank unless noted otherwise. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within 0.4% of the theoretical values.

(8) G. P. Jones, A. Langsjoen, M. M. C. Neumann, and J. Zomlefer, *J. Org. Chem.*, **9**, 125 (1944).

SCHEME I



mp 139°), in 85% yield; and **2c** (mp 115°), in 81% yield. *Anal.* ($C_{10}H_{18}N_4O_2$, **2a**) C, H, N; ($C_{14}H_{26}N_4O_2$, **2c**) C, H, N.

N,N′-Bis[ω-(2-mercaptoethylamino)alkyl]oxamide (3a, 3b, and 3d) Dihydrochlorides.—The appropriate aziridine (**2a**, **b**, or **c**, 8.00 mmoles) was added to a solution of H_2S (2.00 g, 58.7 mmoles) in MeOH (50 ml) at −20°. The resulting solution was warmed slowly to 0° and maintained at this temperautre in a tightly stoppered flask for 16 hr. The solution was concentrated to ~25 ml (~10 ml in the case of **2c**) *in vacuo*, filtered under N_2, and treated with 5.8 *N* dry HCl in *i*-PrOH (2.90 ml, 16.8 mmoles). Addition of Et_2O (50 ml) to the mixture and refrigeration gave a white crystalline product, which was collected and washed with Et_2O; **3a**·2HCl (mp ~263° dec) was obtained in 87% yield; **3b**·2HCl (mp 257° dec[9]), in 85% yield; and **3d**·2HCl (mp 262–263°), in 90% yield. *Anal.* ($C_{10}H_{22}N_4O_2S_2$·2HCl, **3a**·2HCl) C, H, N; SH: calcd, 18.01; found, 16.3; ($C_{12}H_{26}N_4O_2S_2$·2HCl, **3b**·2HCl) C, H, N, SH; ($C_{14}H_{30}N_4O_2S_2$·2HCl, **3d**·2HCl) C, H, N, S; SH: calcd, 15.62; found, 13.6.

N,N′-Bis[3-(2-mercaptoethylamino)propyl]oxamide-S,S′-disulfonic Acid (3c).—The bisaziridine **2b** (2.00 g, 7.86 mmoles) was added in small portions to a solution of $Na_2S_2O_3$·$5H_2O$ (3.90 g, 15.7 mmoles) in H_2O (6 ml) at 0°. The mixture was stirred at 0° for 1 hr and then treated dropwise with glacial AcOH (945 mg, 15.7 mmoles). After 30 min at 0°, additional AcOH (945 mg) was added and stirring was continued at 0° for 1 hr and at 25° for 1 hr. The resulting mixture was refrigerated for 16 hr, and the white crystalline **3c** was collected and washed (H_2O, 5 ml); yield 3.13 g (83%), mp ~197° dec. *Anal.* ($C_{12}H_{26}N_4O_8S_4$) C, H, N.

N,N′-Bis[4-(2-mercaptoethylamino)butyl]oxamide-S,S′-disulfonic Acid (3e).—A solution of $(NH_4)_2S_2O_3$ (1.78 g, 12.0 mmoles) and **2c** (1.69 g, 6.00 mmoles) in H_2O (50 ml) was placed under water aspirator vacuum on a rotary evaporator at 25° for 3 hr. The evaporation was continued at 40° until a pasty residue remained. The residue was redissolved in H_2O (25 ml) and again evaporated at 40° to give a solid, which was stirred with warm MeOH (25 ml), refrigerated, collected, and dried at 60° to give **3e** as a hygroscopic solid, yield 2.96 g (97%), mp 142–144° with softening from 119°.[9] *Anal.* ($C_{14}H_{30}N_4O_8S_2$) C, H, N, S.

Ethyl [3-(1-Aziridinyl)propyl]oxamate (4).—A stirred solution of freshly distilled diethyl oxalate (58.4 g, 0.400 mole) in EtOAc (100 ml) at 0° was treated dropwise over a period of 4 hr with a solution of **1b** (20.0 g, 0.200 mole) in EtOAc (200 ml). The resulting solution was stirred at 25° for 45 min and then heated at 70° (0.025 mm) on a rotary evaporator to remove EtOAc and excess diethyl oxalate. The residual oil was refrigerated for 3 days and filtered under N_2; yield 35.7 g (89%), $n^{21}D$ 1.4968. *Anal.* ($C_9H_{16}N_2O_3$) C, H, N.

[3-(1-Aziridinyl)propyl]oxamide (5a).—A solution of **1b** (6.95 g, 69.4 mmoles) in anhydrous EtOAc (10 ml) was added dropwise to a stirred mixture of ethyl oxamate[10] (8.13 g, 69.4 mmoles) in EtOAc (50 ml). The resulting mixture was stirred at 34° for 2 hr and refrigerated. The white crystalline **5a** was collected, washed (EtOAc), and dried at 60°; yield 10.8 g (91%), mp 161°. *Anal.* ($C_7H_{13}N_3O_2$) C, H, N.

[4-(1-Aziridinyl)butyl]oxamide (5b).—**1c** (3.43 g, 30.0 mmoles) was added to a filtered solution of ethyl oxamate[10] (3.52 g, 30.0 mmoles) in EtOAc (60 ml) at 60°. The resulting solution was held at 25° for 2 hr and then refrigerated for 64 hr. The crystalline **5b** was collected and washed with cold EtOAc; yield 5.37 g (97%), mp 153°. *Anal.* ($C_8H_{15}N_3O_2$) C, H, N.

(9) Determined with a Mel-Temp apparatus.

(10) A. Weddige, *J. Prakt. Chem.*, **10**, 196 (1874).

N-[3-(1-Aziridinyl)propyl]-N′-methyloxamide (5c, Table I).—A solution of $MeNH_2$ (4.55 g, 0.150 mole) in anhydrous EtOAc (50 ml) was added rapidly to a stirred solution of **4** (20.0 g, 0.100 mole) in EtOAc (50 ml) at 0°. The resulting solution was stirred at 25° for 1 hr and refrigerated. The white crystalline product was collected and washed with EtOAc; yield 17.5 g.

TABLE I

N′-SUBSTITUTED N-[ω-(1-AZIRIDINYL)ALKYL]OXAMIDES

No.	Yield, %	Mp, °C	Formula	Analyses
5c	95	134–135	$C_8H_{15}N_3O_2$	C, H, N
5d	88	149	$C_{13}H_{23}N_3O_2$	C, H, N
5e	92	114	$C_{13}H_{17}N_3O_2$	C, H, N
5f	97	75	$C_{12}H_{16}N_4O_2$	C, H, N
5g	80	118	$C_{12}H_{16}N_4O_2$	C, H, N
5h	93	123	$C_{13}H_{18}N_4O_2$	C, H, N
5i	93	151	$C_{13}H_{18}N_4O_2$	C, H, N

Ethyl (3-pyridyl)oxamate was prepared by a modification of a literature procedure for the preparation of ethyl (2-pyridyl)-oxamate.[11] Ethyl oxalyl chloride (13.7 g, 0.100 mole) was added dropwise to a stirred solution of 3-aminopyridine (9.41 g, 0.100 mole) in pyridine (10 ml) at 0°. The resulting solution was stirred at 25° for 1 hr, diluted with H_2O (40 ml), and refrigerated. The crystalline product was collected and washed with cold H_2O; yield 9.02 g (46%), mp 100°. *Anal.* ($C_9H_{10}N_2O_3$) C, H, N.

N′-Substituted N-[ω-(1-Aziridinyl)alkyl]oxamides (5d–i, Table I).—**1b** or **1c** (50.0 mmoles) was added to a filtered solution of the appropriate oxamate ester (ethyl cyclohexyloxamate,[12] ethyl oxanilate,[13] ethyl (2-pyridyl)oxamate,[11] ethyl (3-pyridyl)-oxamate, or ethyl hydrogen oxalate 2-phenylhydrazide[14]) (50.0 mmoles) in EtOAc (50 ml). After 3 hr at 25° the reaction mixture was refrigerated, and the crystalline oxamide was collected and washed with cold EtOAc. (The oxamide **5d**, mp 149°, was also prepared by addition of cyclohexylamine to a solution of **4** in EtOAc.)

[ω-(2-Mercaptoethylamino)alkyl]oxamides (6a, c, e, g, h, j, k, n) Hydrochlorides (Table II).—MeOH (50–75 ml) was saturated

TABLE II

[ω-(2-MERCAPTOETHYLAMINO)ALKYL]OXAMIDE HYDROCHLORIDES

No.	Yield, %	Mp, °C	Formula	Analyses
6a	86	241	$C_7H_{15}N_3O_2S \cdot HCl$	C, H, N, SH
6c	91	252	$C_8H_{17}N_3O_2S \cdot HCl$	C, H, N, S; SH[a]
6e	84	235	$C_8H_{17}N_3O_2S \cdot HCl$	C, H, N; S,[b] SH[c]
6g[d]	45	Indefinite	$C_{13}H_{25}N_3O_2S$	C, H, N, S; SH[e]
6h	93	257–258	$C_{13}H_{19}N_3O_2S \cdot HCl$	C, H, N, S, SH
6j	99	169–172[f]	$C_{12}H_{18}N_4O_2S \cdot 2HCl$	C, H, N, S, SH
6k	100	192–194[f]	$C_{12}H_{18}N_4O_2S \cdot 2HCl$	C, H, N, S, SH
6n	99	182–184[f]	$C_{13}H_{20}N_4O_2S \cdot 2HCl$	C, H, N, S, SH

[a] SH: calcd, 12.93; found, 12.5. [b] S: calcd, 12.54; found, 12.0. [c] SH: calcd, 12.93; found, 12.4. [d] Isolated as free base. [e] SH: calcd, 11.51; found 11.1. [f] Determined with a Mel-Temp apparatus.

with H_2S at 0°. A slow stream of H_2S was bubbled through the stirred solution while the appropriate aziridine **5** (10.0 mmoles) was added in small portions. The resulting mixture was stirred at 0° for 15 min, refrigerated for 16 hr in a tightly stoppered flask, evaporated to half-volume on a rotary evaporator, and filtered under N_2. The thiol **6g** was isolated as the free base by evaporation of the filtrate to dryness *in vacuo* and recrystallization of the residue from EtOH (30 ml). The other thiols were prepared by addition of ~4 *N* dry HCl in 1-propanol (10.5 mmoles) (25.0 mmoles for **6j**, **k**, **m**) to the filtrate. Et_2O (50-100 ml) was added to the resulting mixture, and the precipitated hydrochlorides were collected and washed with Et_2O (**6h**·HCl was collected without the addition of Et_2O).

TABLE III

S-2-(ω-OXAMIDOALKYLAMINO)ETHYL HYDROGEN THIOSULFATES

No.	Yield, %	Mp, °C	Formula	Analyses
6b	35	Indefinite	$C_7H_{15}N_3O_5S_2$	C, H, N, S
6d	81	188	$C_8H_{17}N_3O_5S_2$	C, H, N, S
6f	93	Indefinite	$C_8H_{17}N_3O_5S_2$	C, H, N, S
6i	74	248	$C_{13}H_{19}N_3O_5S_2$	C, H, N, S
6m	73	Indefinite	$C_{12}H_{18}N_4O_5S_2$	C, H, N, S

S-2-(ω-Oxamidoalkylamino)ethyl Hydrogen Thiosulfates (6b, d, f, i, m, Table III). A. 6b.—The aziridine **5a** (3.00 g, 17.5 mmoles) was added in small portions to a stirred solution of $Na_2S_2O_3 \cdot 5H_2O$ (4.35 g, 17.5 mmoles) in H_2O (9 ml) at 0°. The suspension was stirred at 0° for 1 hr, treated dropwise with AcOH (1.05 g, 17.5 mmoles), and stirred an additional hour at 0°. The solid was broken up with a glass rod, and the mixture was stirred 30 min, treated dropwise with additional AcOH (1.05 g), stirred 30 min longer, and filtered. The filtrate was held at 0° for 16 hr and evaporated to dryness *in vacuo*. The hygroscopic residue was triturated in EtOH (five 30-ml portions), dried *in vacuo* over P_2O_5, and dissolved in hot MeOH (50 ml). Refrigeration of the solution gave **6b** as an amorphous solid, which was collected, washed (MeOH), and dried at 60°.

B. 6d.—A mixture of **5b** (3.71 g, 20.0 mmoles) and $(NH_4)_2S_2O_3$ (3.56 g, 2.40 mmoles) in 2:1 H_2O–EtOH (30 ml) was stirred until complete solution occurred (10 min). The reaction mixture was then placed under aspirator vacuum on a rotary evaporator at 25° for 1 hr, and the evaporation was continued at 35° until a pasty residue remained. The residue was redissolved in H_2O–EtOH (30 ml) and again evaporated at 35° to give crude **6d**, which was recrystallized twice from H_2O–EtOH.

C. 6f.—A mixture of $(NH_4)_2S_2O_3$ (2.43 g, 16.4 mmoles) and **5c** (3.04 g, 16.4 mmoles) in H_2O (50 ml) was stirred at 25–35° for 1 hr. The resulting solution was placed under aspirator vacuum on a rotary evaporator at 25° for 1 hr. The evaporation was continued at 40° to give a pasty residue, which was redissolved in H_2O (50 ml). Evaporation of the solution *in vacuo* at 40° gave pure **6f** as a hygroscopic solid.

D. 6i.—The aziridine **5e** (2.47 g, 10.0 mmoles) was added in small portions to a stirred solution of $Na_2S_2O_3 \cdot 5H_2O$ (2.48 g, 10.0 mmoles) in H_2O (35 ml) at 0°. The resulting mixture was treated dropwise with AcOH (0.600 g, 10.0 mmoles), stirred for 1 hr at 0°, treated with more AcOH (0.600 g), and stirred for an additional hour at 0° and then at 25° for 1 hr. The crude **6i** was collected and recrystallized from H_2O (130 ml).

E. 6m.—A mixture of $(NH_4)_2S_2O_3$ (1.54 g, 10.4 mmoles) and **5g** (2.58 g, 10.4 mmoles) in 3:1 H_2O–EtOH (20 ml) was stirred at 30° until complete solution occurred (15 min). The resulting solution was placed under aspirator vacuum on a rotary evaporator at 25° for 1 hr and evaporated to dryness at 40°. The residual **6m** was recrystallized from H_2O (10 ml) and dried at 78°.

[3-(2-Phenyl-3-thiazolidinyl)propyl]oxamic Acid 2-Phenylhydrazide (7).—A solution of crude **6p**·HCl (333 mg, ~1.00 mmole), which was prepared from **5i** in ~85% yield by the general procedure described above, and $NaOAc \cdot 3H_2O$ (136 mg, 1.00 mmoles) in $AcNMe_2$ (1 ml) was stirred for 10 min, filtered, and treated with PhCHO (106 mg, 1.00 mmoles). The solution was refiltered after 10 min, heated at 70° for 5 min, cooled to 25°, and treated dropwise with H_2O (2 ml). The gummy precipitate crystallized and was collected and washed with H_2O; yield 203 mg (53%), mp 120–122°.[9] *Anal.* $C_{20}H_{24}N_4O_2S$) C, H, N, S.

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