

Studies in Potential Filaricides. 6. Synthesis of 3,8-Disubstituted 1,3,8-Triazabicyclo[4.4.0]decan-2-ones and -thiones[†]

Satyavan Sharma, R. Bindra, R. N. Iyer, and Nitya Anand*

Central Drug Research Institute, Lucknow, India. Received October 2, 1974

1,3,8-Triazabicyclo[4.4.0]decan-2-ones and -thiones having varying substituents at positions 3 and 8 have been synthesized. When tested in cotton rats for their antifilarial activity against *Litomosoides carinii*, some of the compounds showed moderate microfilaricidal activity.

3-Ethyl-8-methyl-1,3,8-triazabicyclo[4.4.0]decan-2-one (28), synthesized earlier in this laboratory as an analog of diethylcarbamazine (DEC) having greatly reduced conformational mobility, was found to possess marked microfilaricidal activity.¹ In a structure-activity relationship study, analogs of 28 with variations at positions 2, 3, and 8 have been prepared and screened for their biological activity and the results are reported in this communication.

In the earlier synthesis² of 28, the substituents which were to appear at positions 3 and 8 were present in the synthetic units (synthons) right from the initial step of the synthesis and, thus, the method was not suitable for carrying out variations at these positions. Therefore, alternative routes for the synthesis of compounds of the type 28 were investigated and the method described below provides the needed flexibility for carrying out these variations.

Chemistry. Condensation of *N*-alkylethylenediamines 1-4³ with *N*-alkylmaleamic acids 5-7⁴ gave *N*-alkyl-2-(1-alkyl-2-oxo-3-piperazinyl)acetamides 8-13 (Table I). It is noteworthy that in these condensations, of the two possible position isomers, mainly 8-13 are formed, obviously due to lesser steric hindrance offered by the primary amines over the secondary amines in the Michael addition. The structures of the amides 8-13 were confirmed by an alternative synthesis of 13. Thus, treatment of *N*-benzylethylenediamine (4) with diethyl maleate or fumarate (62) gave 4-benzyl-2-carbethoxymethylpiperazin-3-one (63) which on reaction with ethylamine yielded 13.

The amides 8-13 were reduced with LiAlH₄ to 1-alkyl-3-(2-alkylamino)ethylpiperazines 14-19 (Table II). The amines 14-19, on treatment with ethyl chloroformate yielded the corresponding ethoxycarbonyl derivatives 20-24⁴ (Table II) which were conveniently cyclized with NaOEt to 3,8-disubstituted 1,3,8-triazabicyclo[4.4.0]decan-2-ones 25-30.² Reaction of 17-19 with COCl₂ gave 28-30 in one step but in lower yields.

Catalytic hydrogenation of 30 using Pd/C as catalyst yielded 3-ethyl-1,3,8-triazabicyclo[4.4.0]decan-2-one (36) which proved to be a very useful intermediate for preparing 28 as also its other 8-substituted analogs 37-61 (Table III). Methylation of 36 with HCOOH-HCHO gave 28 while treatment with various alkyl or aralkyl halides, epoxides, alkyl and aryl isocyanates, and isothiocyanates gave other 8-substituted derivatives (see Scheme I).

Reaction of 16, 17, and 19 with thiophosgene yielded 3,8-disubstituted 1,3,8-triazabicyclo[4.4.0]decane-2-thiones 33-35. 1-Benzyl-3-(2-benzylamino)ethylpiperazine (16) failed to react with ethyl chloroformate which is very likely due to steric hindrance offered by the bulky benzyl group at 3'-N position which hinders the approach of ethyl chloroformate to the secondary nitrogen of the piperazine ring. However, 16 on reaction with COCl₂ furnished the required 3,8-dibenzyl-1,3,8-triazabicyclo[4.4.0]decan-2-one (27).

Catalytic hydrogenation of 27 using Pd/C or treatment with ethyl chloroformate followed by the hydrolysis of the resulting ethoxycarbonyl derivative 32 with HBr⁵ removed only the 8-benzyl protection giving 31. This 8-debenzylation was confirmed by the NMR studies of 27, 31, and 32. In the NMR spectrum of 27, the signal at δ 3.41 has been assigned to NCH₂Ph and the signal at δ 4.43 for the more deshielded CONCH₂Ph protons. Debenzylation of 27, either with Pd/C or the ClCOOEt-HBr method, gives 31 and 32, respectively, both of which do not show absorption corresponding to NCH₂Ph at δ 3.41, while the signal for CONCH₂Ph around δ 4.43 is retained in both the compounds, thus indicating the preferential removal of 8-benzyl group.

Biological Activity. All the compounds synthesized were evaluated for their in vivo antifilarial activity in cotton rats infected with *Litomosoides carinii* by the technique of Hawking and Sewell⁶ in the division of chemotherapy of this Institute. The compounds were administered intraperitoneally daily for 6 days at dosages of 30 and 10 mg/kg using three animals per experimental group. In this test only 25, 26, 36, and 48 showed 80-90% reduction of blood microfilarial count at a dose of 30 mg/kg in comparison with the same order of activity exhibited by 28 and DEC at 1 and 6 mg/kg, respectively.¹ Other compounds were inactive at 30 mg/kg. None of the compounds possessed any action on adult worms.

The results thus show that (1) substitution of the 3-ethyl group by methyl as in 25 results in reduction of activity; (2) removal of the 8-methyl group as in 36 or increasing the size of the alkyl group also brings about either lowering or loss of activity; (3) substitution of C=O by C=S (34) causes complete disappearance of activity in 28.

The above data suggest that in addition to the electron density on the three-component nitrogen atoms and the geometry of the molecule, the bulk of the groups around those atoms and the associated steric factors are also important in determining the antifilarial activity in this class of compounds.

Some of the compounds were also subjected to pharmacological screening, but the only noteworthy activity shown was the antiinflammatory activity. When tested in mice according to the method of Winter et al.⁷ at a dose of 40 mg/kg po, 48 caused 32% inhibition of carrageenin-induced edema as compared to 31% inhibition by phenylbutazone at 25 mg/kg.

Experimental Section

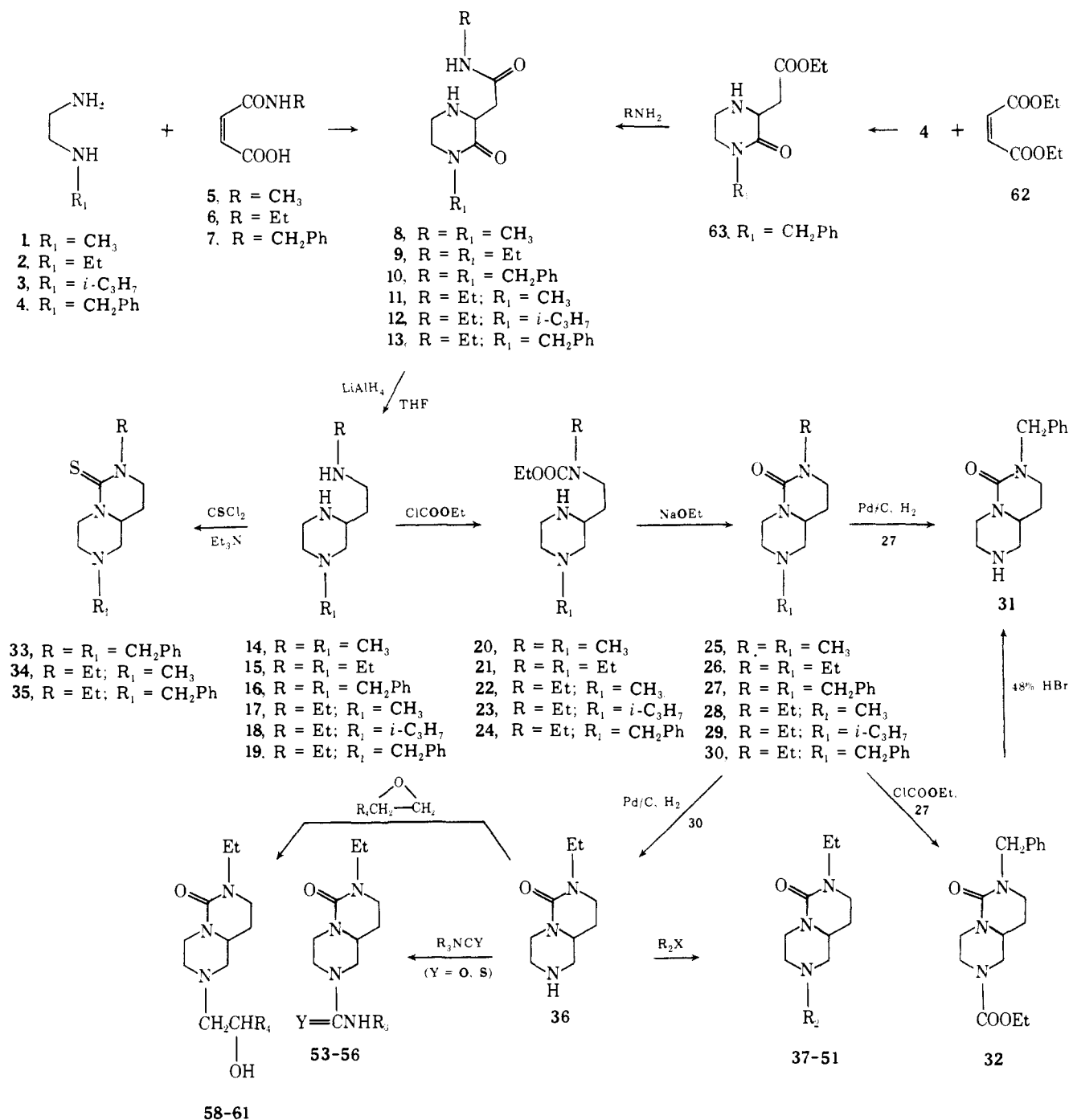
Melting and boiling points are uncorrected; melting points were taken in a sulfuric acid bath. IR spectra were recorded on 137 and 337 Perkin-Elmer infracord spectrophotometers, while NMR spectra were taken on a Varian A-60-D spectrometer. The mass spectra were determined with a Hitachi RMU-6E single focusing spectrometer. Where analyses are indicated by symbols of the elements, the analytical results were within $\pm 0.4\%$ of the theoretical values.

Synthesis of *N*-Substituted 2-(1-Substituted-2-oxo-3-piperazinyl)acetamides. Method A. *N*-Ethyl-2-(1-methyl-2-oxo-3-

*Communication No. 2001 from Central Drug Research Institute, Lucknow, India.

[†]The exact location of the -COOEt group has not been assigned. Its position has been given arbitrarily.

Scheme I



piperazinyl)acetamide (11). A solution of 1 (10 g, 0.135 mol) in dioxane (50 ml) was added dropwise to a stirred solution of 6 (19.3 g, 0.135 mol) in dioxane (100 ml). The reaction mixture was refluxed for 12 hr and cooled to room temperature, the clear solution was decanted off from a small amount of oil, and the solvent was removed to give the product as a thick oil. Compounds 8–10 and 12 were also made by the same method.

Method B. N-Ethyl-2-(1-benzyl-2-oxo-3-piperazinyl)acetamide (13). A solution of 4 (15.0 g, 0.1 mol) in dry ether (75 ml) was added dropwise to a stirred solution of diethyl fumarate 62 (17.2 g, 0.1 mol) in dry ether (75 ml) kept at room temperature and the reaction mixture stirred at the same temperature for 4 hr. The solvent was removed to get 4-benzyl-2-carbethoxymethylpiperazin-3-one (63) as a thick oil: yield 26.0 g (94%). Anal. ($\text{C}_{15}\text{H}_{20}\text{N}_2\text{O}_3$) C, H, N.

A mixture of 63 (20 g, 0.07 mol), EtNH_2 (10 ml), and absolute EtOH (25 ml) was heated at 140° in a sealed tube for 72 hr. The solvent was removed in vacuo and the residue crystallized from benzene to give 13 as colorless flakes.

1-Benzyl-3-(2-ethylamino)ethylpiperazine (19). 13 (50 g, 0.18 mol) was added in portions to a slurry of LiAlH_4 (35 g, 0.9 mol) in dry THF (400 ml) kept in an ice bath and the reaction mixture refluxed for 18 hr. Solvent was removed; the residual mixture was cooled in ice and then suspended in ether. Excess of LiAlH_4 was decomposed by 10% NaOH solution, the thick colorless mass filtered off, solvent removed from filtrate, and the residue distilled under reduced pressure. Compounds 14–18 were also made by a similar procedure.

N-Ethoxycarbonyl-1-benzyl-3-(2-ethylamino)ethylpiperazine (24). To a stirred solution of 19 (72 g, 0.29 mol) in H_2O (175 ml), cooled to 0° , HCl was added to bring the pH of the solution to 3–3.5 and to this solution ethyl chloroformate (35 ml, 0.36 mol) was added dropwise maintaining the pH between 3 and 3.5. This range of pH was kept constant by the addition of an aqueous solution of CH_3COONa from time to time. Stirring was continued for an additional 1 hr and then the reaction mixture was kept in refrigerator overnight. The solution was washed with ether (100 ml), the aqueous layer was saturated with K_2CO_3 , the oil which sepa-

Table I. N-Substituted 2-(1-Substituted-2-oxo-3-piperazinyl)acetamides

No.	R	R ₁	Mp or bp (mm), °C	Yield, %	Formula	Analyses
8	CH ₃	CH ₃	180–185 (2 × 10 ⁻³)	41	C ₈ H ₁₅ N ₃ O ₂	C, H, N
9	C ₂ H ₅	C ₂ H ₅	Oil ^a	45	C ₁₀ H ₁₉ N ₃ O ₂	C, H
10	C ₆ H ₅ CH ₂	C ₆ H ₅ CH ₂	95	52	C ₂₀ H ₂₃ N ₃ O ₂	C, H, N
11	C ₂ H ₅	CH ₃	180 (5 × 10 ⁻³)	41	C ₉ H ₁₇ N ₃ O ₂	C, H
12	C ₂ H ₅	<i>i</i> -C ₃ H ₇	155–157 (2 × 10 ⁻²)	21	C ₁₁ H ₂₁ N ₃ O ₂	C, H, N
13	C ₂ H ₅	C ₆ H ₅ CH ₂	112	80	C ₁₅ H ₂₁ N ₃ O ₂	C, H, N

^aPurified by chromatography over a basic alumina column using C₆H₆ as eluent.

Table II. 1,3-Disubstituted Piperazines

No.	R	R ₁	R ₂	Bp (mm), °C	Yield, %	Formula	Analyses
14	CH ₃	CH ₃	H	130–135 (6)	72	C ₈ H ₁₃ N ₃	C, H, N
15	C ₂ H ₅	C ₂ H ₅	H	130 (17)	76	C ₁₀ H ₂₃ N ₃	C, H
16	C ₆ H ₅ CH ₂	C ₆ H ₅ CH ₂	H	168–170 (1 × 10 ⁻²)	96	C ₂₀ H ₂₇ N ₃	C, H
17	C ₂ H ₅	CH ₃	H	110–112 (8)	80	C ₉ H ₂₁ N ₃	C, H
18	C ₂ H ₅	<i>i</i> -C ₃ H ₇	H	118–122 (20)	57	C ₁₁ H ₂₅ N ₃	C, H, N
19	C ₂ H ₅	C ₆ H ₅ CH ₂	H	175–180 (8)	90	C ₁₅ H ₂₅ N ₃	C, H, N
20	CH ₃	CH ₃	CO ₂ C ₂ H ₅	100–103 (2 × 10 ⁻³)	70	C ₁₁ H ₂₃ N ₃ O ₂	C, H, N
21	C ₂ H ₅	C ₂ H ₅	CO ₂ C ₂ H ₅	110–115 (2 × 10 ⁻³)	90	C ₁₃ H ₂₇ N ₃ O ₂	C, H, N
22	C ₂ H ₅	CH ₃	CO ₂ C ₂ H ₅	Oil ^a	82	C ₁₂ H ₂₅ N ₃ O ₂	C, H, N
23	C ₂ H ₅	<i>i</i> -C ₃ H ₇	CO ₂ C ₂ H ₅	110–111 (2 × 10 ⁻³)	76	C ₁₄ H ₂₉ N ₃ O ₂	C, H
24	C ₂ H ₅	C ₆ H ₅ CH ₂	CO ₂ C ₂ H ₅	140–145 (1 × 10 ⁻²)	86	C ₁₆ H ₂₉ N ₃ O ₂	C, H, N

^aPurified by chromatography over a basic alumina column using CHCl₃ as eluent.

rated was extracted with ether (3 × 200 ml), combined extracts were dried (Na₂SO₄), and solvent was removed to get the product as an oil. Compounds 20–23 were prepared using the same method.

3-Ethyl-8-benzyl-1,3,8-triazabicyclo[4.4.0]decan-2-one (30). A solution of 24 (80 g, 0.25 mol) and NaOEt (Na, 7.0 g, 0.3 mol, and absolute EtOH, 250 ml) in absolute EtOH (100 ml) was refluxed for 36 hr. Solvent was removed thoroughly from the reaction mixture, the residue treated with 50% NaOH solution, and the aqueous phase extracted with CH₂Cl₂ (3 × 200 ml). The combined extracts were dried (Na₂SO₄) and solvent was removed to get the product as an oil. Compounds 25, 26, 28, and 29 were also obtained by the similar manner.

3-Ethyl-1,3,8-triazabicyclo[4.4.0]decan-2-one (36). A solution of 30 (78 g, 0.28 mol) in glacial AcOH (100 ml) was added to a suspension of 5% Pd/C (31.2 g) in glacial AcOH (200 ml) and hydrogenated in a Parr hydrogenator at 3.50 kg/cm² for 24 hr. The catalyst was removed by filtration and solvent removed under reduced pressure. The free base was obtained from the residue either by passing dry NH₃ in the CHCl₃ solution of the residue and filtering the separated CH₃COONH₄ or by treating the ice-cooled C₆H₆ solution of the residue with 50% NaOH and extracting the aqueous phase with C₆H₆ (3 × 150 ml). The solvent was removed and the product obtained as an oil. 27 was also debenzylated in a similar manner to give 31.

3-Ethyl-8-methyl-1,3,8-triazabicyclo[4.4.0]decan-2-one (28). HCHO (25 ml, 40%) was added dropwise to a solution of 36 (5.0 g, 0.027 mol) in 85% HCOOH (7.5 ml) kept at 80° and the mixture heated at the same temperature for 8 hr. Excess of HCOOH and HCHO were removed under reduced pressure and the residue

was treated with 50% NaOH solution (25 ml). The separated oil was extracted with CH₂Cl₂ (4 × 30 ml), the combined extracts were dried (Na₂SO₄), and the solvent was removed to get the product as an oil.

3-Ethyl-8-substituted 1,3,8-Triazabicyclo[4.4.0]decan-2-ones (37–61). Starting with 36, various 8-substituted compounds were prepared by one of the following methods.

Method C. A mixture of 36 (2 g, 0.011 mol), *n*-butyl iodide (1.3 ml, 0.011 mol), anhydrous K₂CO₃ (3 g), and dry acetone (50 ml) was refluxed for 24 hr, the inorganic salts were filtered off, and the solvent was removed from the filtrate to give 3-ethyl-8-*n*-butyl-1,3,8-triazabicyclo[4.4.0]decan-2-one (38) as an oil. By a similar method compounds 39–51 were also prepared.

Method D. PhNCO (0.81 ml, 0.008 mol) in dry C₆H₆ (10 ml) was added dropwise to an ice-cold solution of 36 (1.5 g, 0.008 mol) in dry C₆H₆ (25 ml); the mixture was kept for 12 hr at room temperature and finally refluxed for 1 hr. The residue, obtained on removal of the solvent, was crystallized from C₆H₆–petroleum ether to get 3-ethyl-8-phenylcarbonyl-1,3,8-triazabicyclo[4.4.0]decan-2-one (54). Compounds 53, 55, and 56 were made in a similar manner.

Method E. A mixture of 36 (1 g, 0.0054 mol) and 1,2-epoxy-3-phenoxypropane (0.81 g, 0.0054 mol) in EtOH (30 ml) was refluxed for 6 hr. Removal of solvent from the reaction mixture gave 3-ethyl-8-(3-phenoxy-2-hydroxypropyl)-1,3,8-triazabicyclo[4.4.0]decan-2-one (60) as an oil which was purified by chromatography over a basic alumina column using C₆H₆ as eluent. Compounds 58, 59, and 61 were also prepared by this method.

3-Ethyl-8-guanyl-1,3,8-triazabicyclo[4.4.0]decan-2-one (52).

Table III. 3,8-Disubstituted 1,3,8-Triazabicyclo[4.4.0]decan-2-ones and-thiones

No.	R	R ₁	Y	Method	Mp or bp (mm), °C	Yield, %	Formula	Analyses	MED ^c × 6 days ip, mg/kg
DEC									6
25	CH ₃	CH ₃	O	A ^e	95-98 (2 × 10 ⁻³)	70	C ₉ H ₁₇ N ₃ O	C, H, N	30, 10 (inactive)
26	C ₂ H ₅	C ₂ H ₅	O	A	140-145 (2 × 10 ⁻²)	81	C ₁₁ H ₂₁ N ₃ O	C, H	30, 10 (inactive)
27	C ₆ H ₅ CH ₂	C ₆ H ₅ CH ₂	O	A	180-185 (2 × 10 ⁻²)	27	C ₂₁ H ₂₅ N ₃ O	C, H, N	i ^d
28	C ₂ H ₅	CH ₃	O	B ^f	130-132 (5 × 10 ⁻²) ^a	90	C ₁₀ H ₁₉ N ₃ O	C, H	1
29	C ₂ H ₅	<i>i</i> -C ₃ H ₇	O	A	115-120 (2 × 10 ⁻²)	50	C ₁₂ H ₂₃ N ₃ O	C, H, N	i
30	C ₂ H ₅	C ₆ H ₅ CH ₂	O	B	165 (5 × 10 ⁻³)	90	C ₁₆ H ₂₃ N ₃ O	C, H	i
31	C ₆ H ₅ CH ₂	H	O	A	Oil	64	C ₁₄ H ₁₉ N ₃ O	C, H, N	i
32	C ₆ H ₅ CH ₂	CO ₂ C ₂ H ₅	O	A	Oil	61	C ₁₇ H ₂₃ N ₃ O ₃	C, H	i
33	C ₆ H ₅ CH ₂	C ₆ H ₅ CH ₂	S	A	Oil	54	C ₂₁ H ₂₅ N ₃ S	C, H, N	i
34	C ₂ H ₅	CH ₃	S	A	130-133 (5 × 10 ⁻³)	75	C ₁₀ H ₁₉ N ₃ S	C, H, N	i
35	C ₂ H ₅	C ₆ H ₅ CH ₂	S	B	210-215 (5 × 10 ⁻³)	43	C ₁₆ H ₂₃ N ₃ S	C, H, N	i
36	C ₂ H ₅	H	O	B	125-128 (2 × 10 ⁻²)	80	C ₉ H ₁₇ N ₃ O	C, H	30, 10 (inactive)
37	C ₂ H ₅	CH ₂ CH ₂ CH ₃	O	C	130 (5 × 10 ⁻³)	83	C ₁₂ H ₂₃ N ₃ O	C, H	i
38	C ₂ H ₅	CH ₂ (CH ₂) ₂ CH ₃	O	C	145 (5 × 10 ⁻³)	76	C ₁₃ H ₂₅ N ₃ O	C, H, N	i
39	C ₂ H ₅	CH ₂ (CH ₂) ₃ CH ₃	O	C	Oil	76	C ₁₄ H ₂₇ N ₃ O	C, H, N	i
40	C ₂ H ₅	CH ₂ CH ₂ CH(CH ₃) ₂	O	C	116 (2 × 10 ⁻²)	38	C ₁₄ H ₂₇ N ₃ O	C, H, N	i
41	C ₂ H ₅	CH ₂ (CH ₂) ₄ CH ₃	O	C	Oil	78	C ₁₅ H ₂₉ N ₃ O	C, H, N	i
42	C ₂ H ₅	CH ₂ (CH ₂) ₅ CH ₃	O	C	142 (4 × 10 ⁻²)	64	C ₁₆ H ₃₁ N ₃ O	C, H, N	i
43	C ₂ H ₅	CH ₂ (CH ₂) ₆ CH ₃	O	C	165-170 (2 × 10 ⁻²)	94	C ₁₇ H ₃₃ N ₃ O	C, H, N	i
44	C ₂ H ₅	CH ₂ (CH ₂) ₈ CH ₃	O	C	Oil	58	C ₁₉ H ₃₇ N ₃ O	C, H	i
45	C ₂ H ₅	COOC ₂ H ₅	O	C	Oil	76	C ₁₂ H ₂₁ N ₃ O ₃	C, H	i
46	C ₂ H ₅	CH ₂ COOC ₂ H ₅	O	C	150 (2 × 10 ⁻²)	32	C ₁₃ H ₂₃ N ₃ O ₃	C, H, N	i
47	C ₂ H ₅	CON(C ₂ H ₅) ₂	O	C	162 (5 × 10 ⁻²)	93	C ₁₄ H ₂₆ N ₄ O ₂	C, H, N	i
48	C ₂ H ₅	CH ₂ CH ₂ C ₆ H ₅	O	C	180 (4 × 10 ⁻²)	80	C ₁₇ H ₂₅ N ₃ O	C, H, N	30, 10 (inactive)
49	C ₂ H ₅	CH ₂ CH ₂ N(C ₂ H ₅) ₂	O	C	138-140 (5 × 10 ⁻³)	53	C ₁₅ H ₃₀ N ₄ O	C, H, N	i
50	C ₂ H ₅	CH ₂ CH ₂ N(CH ₃) ₂	O	C	Oil	70	C ₁₃ H ₂₆ N ₄ O	C, H	i
51	C ₂ H ₅	CH ₂ (CH ₂) ₂ N(CH ₃) ₂	O	C	148-150 (5 × 10 ⁻²)	70	C ₁₄ H ₂₈ N ₄ O	C, H, N	i
52	C ₂ H ₅	HN=CNH ₂	O	F ^g	Oil	83	C ₁₀ H ₁₉ N ₅ O	C, H	i
53	C ₂ H ₅	CONHC ₂ H ₅	O	D	151-153	92	C ₁₂ H ₂₂ N ₄ O ₂	C, H	i
54	C ₂ H ₅	CONHC ₆ H ₅	O	D	90-92	50	C ₁₆ H ₂₂ N ₄ O ₂	C, H, N	i
55	C ₂ H ₅	CSNHC ₂ H ₅	O	D	140-142	81	C ₁₂ H ₂₂ N ₄ OS	C, H	i
56	C ₂ H ₅	CSNHC ₆ H ₅	O	D	195-196	38	C ₁₆ H ₂₂ N ₄ OS	C, H	i
57	C ₂ H ₅	CH ₃ ^b	O	F	143-145	47	C ₁₀ H ₁₉ N ₃ O ₂	C, H, N	i
58	C ₂ H ₅	CH ₂ CH(OH)C ₆ H ₅	O	E	Oil ^h	84	C ₁₇ H ₂₅ N ₃ O ₂	C, H	i
59	C ₂ H ₅	CH ₂ CH(OH)CH ₂ p-C ₆ H ₄ CH ₂ O	O	E	Oil	75	C ₁₈ H ₂₆ C ₁ N ₃ O ₃	C, H, N	i
60	C ₂ H ₅	CH ₂ CH(OH)CH ₂ O p-C ₆ H ₄ CH ₂ O	O	E	Oil	83	C ₁₈ H ₂₇ N ₃ O ₃ ·H ₂ O	C, H	i
61	C ₂ H ₅	CH ₂ CH(OH)CH ₂ O (CH ₃) ₂ CH	O	E	Oil	62	C ₁₅ H ₂₉ N ₃ O ₃	C, H	i

^aLit. mp 130-132° (5 × 10⁻²). ^bN-Oxide. ^cMinimum effective dose to clear 90% of the pretreatment circulating microfilariae. ^dInactive at 30 mg/kg. ^eRefers to *N*-alkylethylenediamine and *N*-alkylmaleamic acid route. ^fRefers to *N*-benzylethylenediamine and diethyl fumarate route. ^gMethod described in the Experimental Section. ^hAll the compounds, obtained as oils, were purified by chromatography over a basic alumina column using benzene as eluent.

A mixture of **36** (1 g, 0.0054 mol) and S-methylthiourea sulfate (1.01 g, 0.0054 mol) in 65% EtOH (30 ml) was refluxed for 24 hr until no more methyl mercaptan was evolved. The solvent was removed from the reaction mixture and the residue treated with 50% NaOH solution (25 ml). The separated oil was extracted with C₆H₆ (3 × 50 ml), the combined extracts were dried (Na₂SO₄), and solvent was removed to get the product as a thick oil.

3-Ethyl-8-methyl-1,3,8-triazabicyclo[4.4.0]decan-2-one 8-Oxide (57). A solution of **28** (1.5 g, 0.007 mol) in H₂O (2 ml) was treated with 30% H₂O₂ (2 ml) and the reaction mixture stirred for 1 hr at 60°. Excess of H₂O₂ was decomposed by adding KMnO₄ solution to the reaction mixture and concentrated under reduced pressure, and the residue was extracted with hot C₆H₆ (3 × 50 ml). The combined extracts were dried (Na₂SO₄), the solvent was removed, and the residue was crystallized from C₆H₆-petroleum ether.

3,8-Dibenzyl-1,3,8-triazabicyclo[4.4.0]decan-2-one (27). A mixture of **16** (20 g, 0.06 mol) and Et₃N (18 ml, 0.128 mol) in dry CHCl₃ (200 ml) was added dropwise with stirring to COCl₂ (15 ml) kept below -20° when a vigorous exothermic reaction took place and a thick mass of Et₃N·HCl separated out. After the addition was over, the reaction mixture was stirred for an additional 12 hr at room temperature and solvent removed in vacuo. The residue was taken up in dry C₆H₆ (200 ml), the insoluble Et₃N·HCl filtered off, and the solvent removed from the filtrate to get the product as an oil.

3-Ethyl-8-methyl-1,3,8-triazabicyclo[4.4.0]decane-2-thione (34). A solution of CSCl₂ (3.1 ml, 0.035 mol) in dry CHCl₃ (25 ml) was added dropwise under stirring to an ice-cold mixture of **17** (6 g, 0.035 mol) and Et₃N (9.8 ml, 0.07 mol) in dry CHCl₃ (100 ml) and the reaction mixture stirred for 1 hr at room temperature and then refluxed for 1 hr. Solvent was removed in vacuo and the residue taken up in dry C₆H₆ (100 ml). The insoluble Et₃N·HCl was filtered off, the solvent removed from filtrate, and the residue distilled under reduced pressure. Using this procedure compounds **33** and **35** were also made.

3-Benzyl-8-carbethoxy-1,3,8-triazabicyclo[4.4.0]decan-2-one (32). A solution of ethyl chloroformate (2 ml, 0.02 mol) in C₆H₆ (10 ml) was added dropwise to a cold solution of **27** (1.4 g,

0.004 mol) in C₆H₆ (40 ml) and the reaction mixture refluxed for 24 hr. The solvent was removed in vacuo, the residue cooled in ice and treated with 50% NaOH solution (10 ml), and the separated oil extracted with C₆H₆ (3 × 25 ml). Removal of the solvent from combined extracts yielded the product as an oil.

3-Benzyl-1,3,8-triazabicyclo[4.4.0]decan-2-one (31). A mixture of **32** (1 g, 0.003 mol) and 48% HBr solution (20 ml) in glacial AcOH (20 ml) was refluxed for 24 hr. The solvent was removed from the reaction mixture, the residue treated with 50% NaOH solution (25 ml), and the separated oil extracted with C₆H₆ (3 × 20 ml). The combined extracts were dried (Na₂SO₄) and solvent was removed to get the product as an oil.

Acknowledgments. The authors thank Drs. A. B. Sen and R. K. Chatterjee of the division of chemotherapy of this Institute for providing the antifilarial screening results, Dr. B. N. Dhawan and his associates for antiinflammatory data, and the staff of our Instrumentation Services for various spectroscopic and analytical data. One of us (S.S.) thanks CSIR, New Delhi, for the award of a junior research fellowship.

References and Notes

- (1) R. Saxena, R. N. Iyer, N. Anand, R. K. Chatterjee, and A. B. Sen, *J. Pharm. Pharmacol.*, **22**, 307 (1970).
- (2) R. Saxena, S. Sharma, R. N. Iyer, and N. Anand, *J. Med. Chem.*, **14**, 929 (1971).
- (3) D. K. Yung, L. G. Chatten, and D. P. MacLeod, *J. Pharm. Sci.*, **57**, 2073 (1968).
- (4) N. B. Mehta, A. P. Phillips, F. Fu Lui, and R. E. Brooks, *J. Org. Chem.*, **25**, 1012 (1960).
- (5) W. B. Wright, Jr., and H. J. Brabander, *J. Org. Chem.*, **26**, 4057 (1961).
- (6) F. Hawking and P. Sewell, *Br. J. Pharmacol. Chemother.*, **3**, 285 (1948).
- (7) C. A. Winter, E. A. Risley, and G. W. Nuss, *Proc. Soc. Exp. Biol. Med.*, **111**, 544 (1962).

Potential Bioreductive Alkylating Agents. 5. Antineoplastic Activity of Quinoline-5,8-diones, Naphthazarins, and Naphthoquinones

Ai Jeng Lin,* Brian J. Lillis, and Alan C. Sartorelli

Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06510. Received February 12, 1975

A number of 2-chloromethyl and 2-bromomethyl derivatives of naphthoquinones, quinolinediones, and naphthazarins were designed and synthesized as potential bioreductive alkylating agents, and the antitumor activity of these compounds was assessed in mice bearing Sarcoma 180 ascites cells. The results indicated that, with the exception of 3-benzamido-2-chloromethyl-1,4-naphthoquinone, which was inactive, all newly synthesized naphthoquinones possessed strong antitumor activity against this neoplasm. 6,7-Bis(bromomethyl)quinoline-5,8-dione had moderate inhibitory activity against Sarcoma 180 at its optimal daily dosage level of 15 mg/kg. 3-Bromo-2-bromomethyl- and 3-bromo-2-chloromethylnaphthazarin produced a moderate extension of the life span of tumor-bearing mice; whereas, in contrast, 6,7-dimethyl analogs of these agents were inactive when employed in daily doses up to 40 mg/kg of body weight.

The synthesis of a series of benzo- and naphthoquinone derivatives with common structural features (1), which were designed to generate a reactive species in cells, have been reported previously.¹⁻⁵ These agents were found to be potent inhibitors of (a) the growth of rodent tumors,¹⁻³ (b) the synthesis of DNA and RNA in these neoplastic cells in vitro,^{1,2} and (c) the coenzyme Q-mediated beef heart mitochondrial enzymes, NADH-oxidase and succinoxidase.^{2,4} The mechanism by which these compounds exert their cytotoxicity has been postulated⁶ to involve the enzymatic reduction of the quinone ring in vivo, presumably by an

NADPH-dependent quinone reducing system analogous to that acting on mitomycin C,⁷⁻⁹ to form a dihydroquinone, which spontaneously generates the reactive species, an o-quinone methide (2); this reactive intermediate may then act to alkylate cellular components. Chemical evidence¹⁰ has been obtained to substantiate the existence of an o-quinone methide from 2,3-dimethyl-5,6-bis(acetoxy-methyl)-1,4-benzoquinone (3).

As part of a study to develop new antineoplastic agents of this class with (a) greater therapeutic potency and (b) better water solubility (in salt form), the synthesis of a