matography (silica gel, 50% Et_2O–petroleum ether) gave 23.1 g (73.5%) of 4-(benzyloxy)-3-nitro-*N,N*-di-*n*-propylphenethylamine, which was used without further purification. To a solution of the 23.1 g (0.065 mol) of the above nitro compound in 100 mL of MeOH was added 0.35 g of PtO_2 and sufficient dry HCl gas in Et_2O to partially neutralize the amine. The mixture was hydrogenated at 60 psi with shaking. The catalyst was removed by filtration and the solvent by evaporation in vacuo. The residue was dissolved in *i*-PrOH and made acidic by the addition of ethereal HCl, Et_2O was added slowly, and the salt was filtered; 24.8 g (96%, 71% overall).

3-Formamido-4-hydroxy-*N,N*-*n*-dipropylphenethylamine Hydrochloride (46). A solution of 5.0 g (0.015 mol) of the free base of **45** in 150 mL of ethyl formate was refluxed overnight. The ethyl formate was removed in vacuo and the residual oil dissolved in EtOAc/Et_2O. A small amount of white solid was removed by filtration, and the solvents were again removed in vacuo. This crude product (5.2 g) was used without further purification. A solution of 1.75 g (0.0049 mol) of the above crude formyl derivative in 50 mL of MeOH containing a small amount of EtOAc was hydrogenated at 60 psi with shaking in the presence of 0.75 g of 10% Pd/C. After 1 h the catalyst was removed by filtration and the solvents were removed by evaporation in vacuo to give 1.3 g of an oily product. It was converted to the hydrochloride salt by the addition of ethereal HCl to a solution in MeOH, 0.925 g.

3-Acetamido-4-hydroxy-*N,N*-di-*n*-propylphenethylamine Hydrochloride (47). A solution of 2.1 g (0.0064 mol) of the free base of **45** in 75 mL of Ac_2O was stirred overnight at room temperature. The Ac_2O was removed in vacuo to give 2.4 g of a tan oil. This oil was dissolved in a mixture of 50 mL of MeOH and 10 mL of EtOAc and hydrogenated with shaking at 60 psi in the presence of 1.0 g of 10% Pd/C. The catalyst was filtered and the filtrate was concentrated to an oil in vacuo. This was converted to the hydrochloride in methanol with use of ethereal HCl, 1.75 g.

Assay for Inhibition of Adrenergic Neurotransmission in the Isolated Perfused Rabbit Ear Artery (REA). A 2–4-cm segment of central ear artery is mounted in a narrow cylindrical chamber where it is simultaneously perfused intraluminally and superfused extraluminally with oxygenated Krebs solution. Drugs can be administered by means of either the intraluminal or extraluminal flow. Changes in arterial diameter are reflected as changes in intraluminal perfusion pressure. At 4-min intervals, the vascular sympathetic nerves are excited by pulses from an electronic stimulator delivered through platinum electrodes present in the chamber. The test drug is administered in increasing concentration. Each concentration is allowed to remain in contact with the tissue for 4 min. The drug concentration is increased immediately following the response to nerve stimulation. If the effect of dopaminergic blockade is to be determined (*S*)-sulpiride superfusion is begun after obtaining the initial concentration–effect curve for the test compound. Following a 30-min equilibration period, the curve is repeated in the presence of (*S*)-sulpiride.

Acknowledgment. We thank Calab Jervay and Robert Zeid for assistance with the biological assays and Arnold Krog for help in preparing synthetic intermediates. The assistance of the Analytical, Physical and Structural Chemistry Department in the determination of elemental composition is also appreciated.

Registry No. 1, 81654-47-9; **2**, 81654-48-0; **3**, 81654-49-1; **4**, 85763-09-3; **5**, 101566-00-1; **6**·HCl, 101566-01-2; **7a**, 91374-22-0; **8a**, 91374-23-1; **8b**, 101566-02-3; **9a**, 97351-95-6; **10a**, 91374-25-3; **10b**, 101566-03-4; 11, 24954-62-9; **12**, 101566-04-5; **13**, 101566-05-6; **14**, 81654-50-4; **14**, 81654-50-4; **15**, 101566-06-7; **16**, 101566-07-8; **17**·HCl, 101566-08-9; **18**, 101566-09-0; **18**·HBr, 101566-10-3; **19**, 81654-51-5; **20**, 81654-52-6; **21**, 81654-54-8; **21**·HCl, 81654-53-7; **22**, 85763-08-2; **22**·HBr, 81654-59-3; **23**·HCl, 101566-11-4; **24**, 101566-12-5; **24**·HBr, 101566-13-6; **25**, 85763-10-6; **25**·HCl, 81654-56-0; **26**, 81654-62-8; **26**·HBr, 81654-57-1; **27**·HCl, 91374-19-5; **28**, 91374-21-9; **28**·HCl, 91374-20-8; **29**·HCl, 101566-14-7; **30**, 101566-15-8; **31**, 101566-16-9; **31**·HBr, 101566-17-0; **32**, 101566-18-1; **33**, 101566-19-2; **33**·HCl, 101566-20-5; **34**, 101566-21-6; **34**·HBr, 101566-22-7; **35**·HCl, 101566-23-8; **36**, 101566-24-9; **37**, 101566-25-0; **38**, 101566-26-1; **38**·HCl, 101566-27-2; **39**, 101566-28-3; **39**·HCl, 101566-29-4; **40**, 101566-30-7; **41**, 101566-31-8; **41**·HCl, 101566-32-9; **42**, 96886-45-2; **43**·HBr, 101566-33-0; **44**, 96886-47-4; **45**·2HCl, 96886-48-5; **46**, 101566-34-1; **46**·HCl, 101566-35-2; **47**, 101566-36-3; **47**·HCl, 101566-37-4; 4-methoxybenzeneethanamine, 55-81-2; propionaldehyde, 123-38-6; 1,2-ethanedithiol, 540-63-6; 4-methylphenylacetaldehyde, 5703-26-4; 5-chloro-1-phenyl-1*H*-tetrazole, 14210-25-4; 2-methyl-3-nitrophenylacetic acid, 23876-15-5; 4-methoxybenzeneethaneamine, 55-81-2; propionyl chloride, 79-03-8; *N*-[2-(4-methoxyphenyl)ethyl]propanamide, 67191-51-9; phenylpyruvic acid, 156-06-9; 6-(2-hydroxyethyl)-2(3*H*)-indolone, 101566-38-5; 4-methoxyphenylacetyl chloride, 4693-91-8; 4-(benzyloxy)-3-nitro-*N,N*-di-*m*-propylphenethylamine, 96886-51-0.

Synthesis, Saludiuretic, and Antihypertensive Activity of 6,7-Disubstituted 1(2*H*)- and 3,4-Dihydro-1(2*H*)-phthalazinones

S. Cherkez, J. Herzig, and H. Yellin*

Teva Pharmaceutical Industries Ltd., 61 013 Tel-Aviv, Israel. *Received May 16, 1984*

The synthesis of the isomeric series 6-chloro-7-sulfamoyl- and 7-chloro-6-sulfamoyl-1(2*H*)-phthalazinones (**1** and **2**) and 6-chloro-7-sulfamoyl- and 7-chloro-6-sulfamoyl-3,4-dihydo-1(2*H*)-phthalazinones (**3** and **4**), combining structural features characteristic to furosemide and hydralazine, is described, the mechanism of the formation of **1** and **2** is discussed, and their structure–activities relationships are studied. Preliminary screening in the rat shows that series **1** and **3** exhibit diuretic and saluretic activity similar to that of chlorothiazide with, however, Na^+/K^+ ratios more favorable than chlorothiazide and furosemide. The compounds of series **2** and **4** are practically inactive. All four series show initial antihypertensive activity lower than that of hydralazine. However, compounds **1a**, **1c**, and **4a** show a higher activity at 8 and/or 24 h after administration and thus may offer a unique combination of a "loop" diuresis with direct long-acting peripheral vasodilating effects.

Many diuretics and saluretics possess an aromatic nucleus with a halogen, pseudohalogen, or a phenoxy group in the position ortho to and an electronegative group in the position meta to a sulfonamide group.[1] Among those that have found wide use are furosemide (**5**), chlorothiazide (**6**), hydrochlorothiazide (**7**), and bumetanide (**15**) (Figure 1).

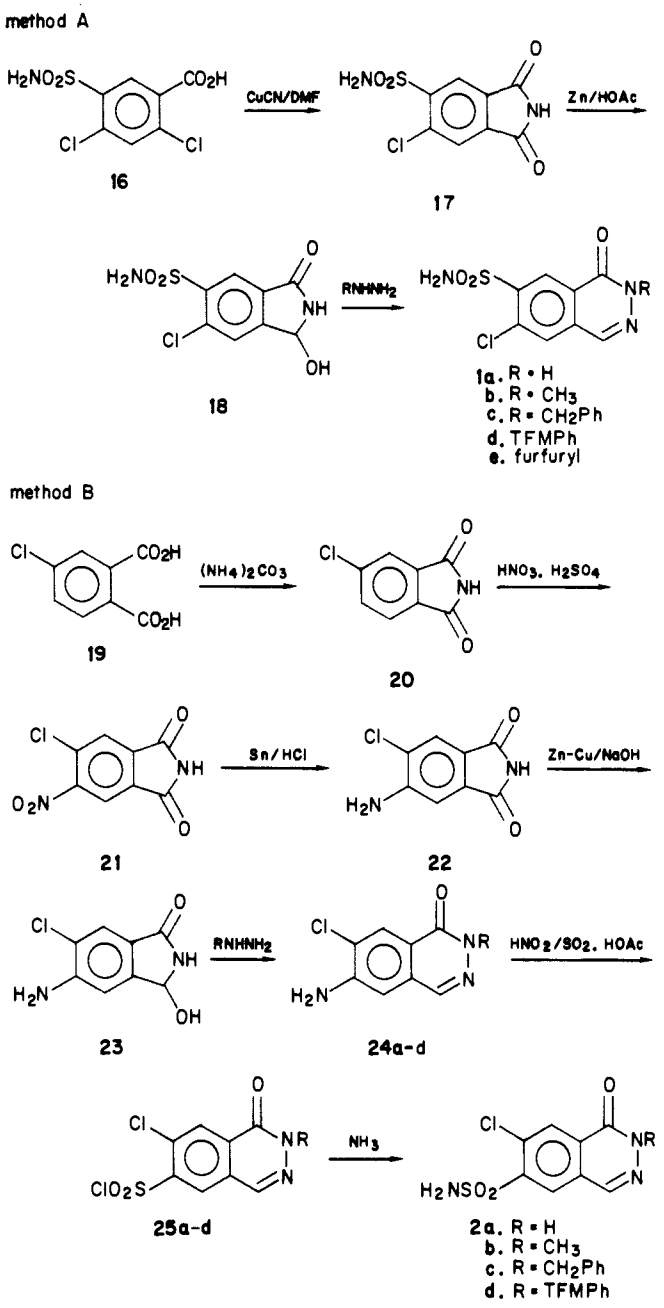
Compounds having cyclic or exocyclic –N–N– moieties (hydralazine, dihydralazine, compounds **11**–**14**[2-4]) are

(1) Feit, P. *J. Med. Chem.* 1971, *14*, 432.

(2) Kikuo, A.; Kiyoshi, S. Jpn. Patent 7455680; *Chem. Abstr.* 1974, *81*, 136167).

0022-2623/86/1829-0947$01.50/0 © 1986 American Chemical Society

Scheme I. Synthetic Sequences for Series 1 and 2



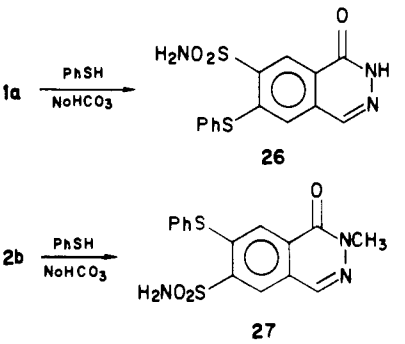
known to possess antihypertensive activity via a peripheral vasodilating effect.

We have synthesized a class of compounds combining structural features of both the furosemide and the hydralazine type. 1(2*H*)-Phthalazinone, which is one of the metabolites of hydralazine,[5] was selected as the basis for the synthesis of series **1** and **2**.

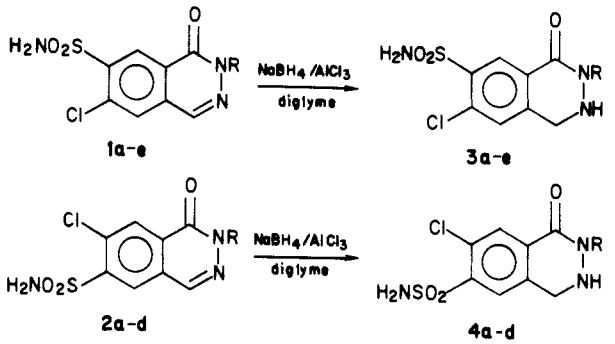
As it is known that reduction of similar diuretic agents often results in enhanced diuretic activity (e.g., hydrochlorothiazide vs. chlorothiazide), we also prepared the analogous series **3** and **4**.

As the activity of some diuretics is confined to specific structural isomers, we have examined the structure–activity relationship (SAR) of the two isomeric compounds in both the 1(2*H*)- and 1(2*H*,3*H*,4*H*)-phthalazinone series

Scheme II



Scheme III



with respect to their diuretic, saluretic, and antihypertensive activities.

Chemistry

Two distinct regiospecific pathways have been used for the synthesis of the two isomeric 1(2*H*)-phthalazinone series **1** and **2**, as shown in Scheme I. The structure of the two series was unequivocally assigned on the basis of their NMR data and a key intermediate of known structure **18**.

Synthesis. While the synthetic sequence in series **1** (method A) is short and straightforward, that of series **2** (method B) involves a seven-stage synthesis (Scheme I).

Method A. The key intermediate 4-chloro-5-sulfamoylphthalimide **17**,[6] obtained by the reaction of 2,4-dichlorosulfamoylbenzoic acid (**16**) with cuprous cyanide in DMF,[7] was reduced by Zn/acetic acid to the corresponding 3-hydroxyphthalimidine **18**. Condensation of the latter with hydrazines afforded the phthalazinones **1a–e**.

Method B. 4-Chlorophthalimide (**20**), obtained by fusion of **19** with $(NH_4)_2CO_3$, was converted to the phthalimide **22** by nitration and subsequent Sn/HCl reduction. Zn/Cu reduction in basic medium afforded the 3-hydroxyphthalimidine **23**, from which, by reaction with hydrazines, the corresponding 6-amino-7-chlorophthalazinones **24a–d** were obtained. The amino group was converted to sulfamoyl by applying the Schiemann reaction conditions, followed by treatment with liquid ammonia, thus affording the phthalazinones **2a–d**.

It is likely that the reduction step in both sequences determines the regiochemistry of the final products. The electron-withdrawing sulfamoyl group and the electron-donating amino group on the phenyl ring lead to opposite orienting effects and consequently to different courses of reduction.

(3) Delmar Chemicals Ltd, 1974, Brit. Pat. 1417946.
(4) De Ponti, C.; Bardi, U.; Merchetti, M. *Arzneim.-Forsch.* **1976**, *26*, 2089.
(5) (a) Zimmer, H.; Glaser, R.; Kokosa, J.; Garteiz, D.; Hess, E.; Litwin, A. *J. Med. Chem.* **1975**, *18*, 1031. (b) Zak, S.; Gilleran, T.; Karliner, J.; Lukas, G. *J. Med. Chem.* **1974**, *17*, 381.
(6) This compound has been reported to be prepared via a four-step procedure; see ref 7a.
(7) (a) Lee, G. E.; Wragg, W. R. May & Baker, Brit. Pat. 733968, 1961. (b) Cherkez, S.; Herzig, J.; Yellin, H. Teva Pharmaceutical Ind. Ltd Brit. Pat. 1571742, 1977.

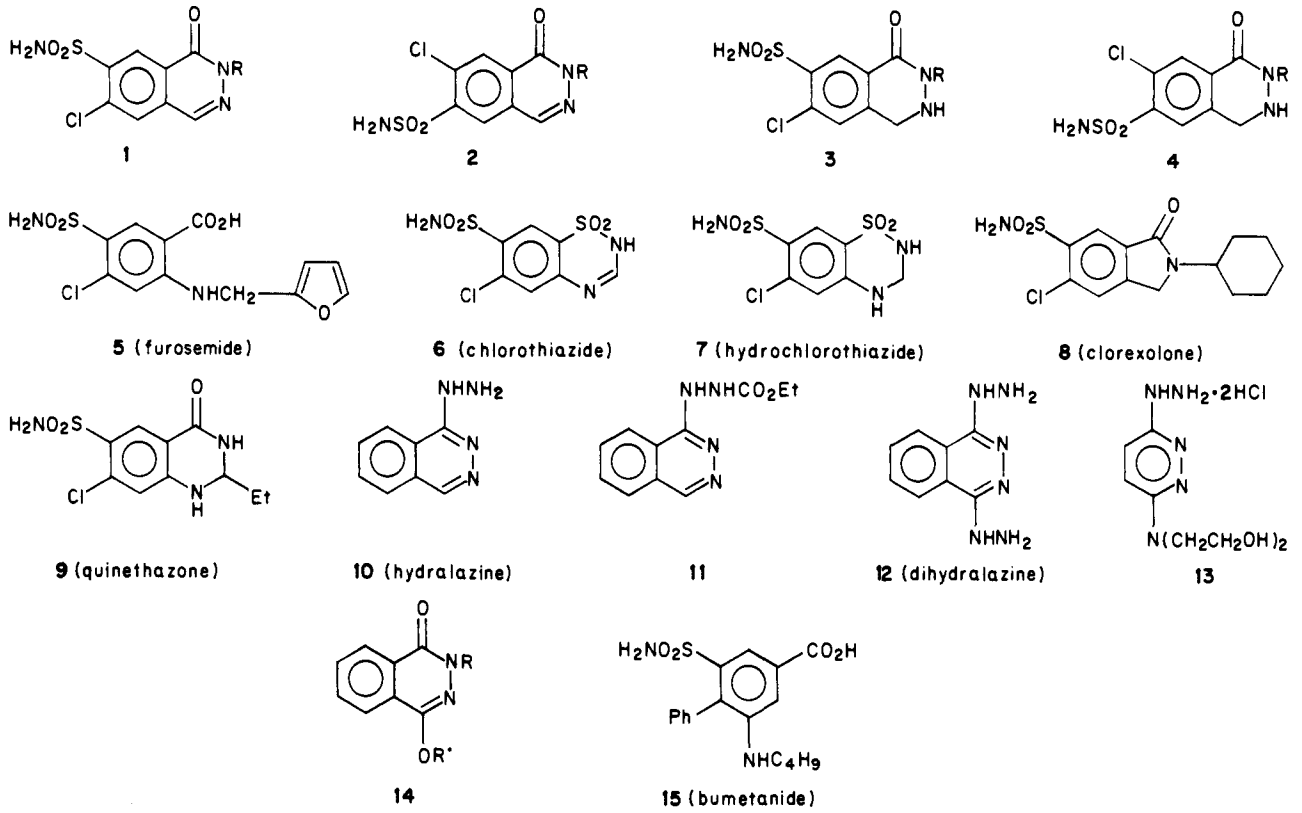


Figure 1.

Two compounds of the (phenylthio)phthalazinone series (**26**, **27**) were prepared by reacting the corresponding chlorophthalazinones with sodium thiophenoxide (see Scheme II). A similar reaction with sodium phenoxide failed, probably due to the lower nucleophilicity of the phenoxide ion.

The compounds of series **3** and **4** were synthesized by the reduction of the two corresponding 1(2*H*)-phthalazinones **1** and **2** with $NaBH_4/AlCl_3$ in diglyme[8,9] (Scheme III). This reagent was found to effectively reduce the conjugated C=N bond while leaving the carbonyl group intact. The physical, analytical, and spectral properties of phthalazinones **1–4** and of their intermediates are given in Tables I and II.

Spectral Data. NMR. A detailed discussion of the NMR pattern as a function of the relative position of the substituents is presented below.

IR. The C=O absorption bands of **1a** and **2a** (1655, 1648 cm^{-1}) are in good agreement with the reported data (1658 cm^{-1} for an unsubstituted 1(2*H*)-phthalazinone[10]). The other compounds have similar absorptions. The two SO_2 bands appear, as expected,[11] in the 1330–1360- and 1160–1180-cm^{-1} regions. The C=O absorption bands (1636–1668 cm^{-1}) of **3** and **4** are very similar to those of the corresponding 1(2*H*)-phthalazinones, the reduction of the C=N bond in the 3,4-position having almost no effect on the absorption of the carbonyl in the 1-position.

MS. The mass spectral fragmentation patterns of series **1** and **2** are very similar, both exhibiting a moderate to high intensity molecular ion peak and fragmentations involving the characteristic[12] cleavage of the N–N bond. The fragmentation pattern is largely determined by the substituent R. Thus, for R = H, Me, and furfuryl, M^+ is of high intensity and fragmentations of the pyridazine ring are of moderate intensity. For R = CH_2Ph and CF_3Ph, R^+ is the base peak and all other fragmentations are of minor importance.

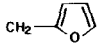
Compounds of series **3** and **4** also exhibit a molecular ion peak of moderate to high intensity, depending upon the nature of the substituent R. Both series fragment in similar patterns.

Reaction of 3-Hydroxyphthalimidines with Hydrazines. Mechanism and Intermediates. The reaction mechanism of 3-hydroxyphthalimidine (**28**) (also described as the open ring tautomer **35**)[18] with substituted hydrazines has been widely discussed in the early literature;[14-16] phthalazinone **29**, pseudophthalazinone[13] **30**, and phthalimidine **31** have all been suggested as possible products (Figure 2). Phthalazinones also have been reported[17] to be obtained from phthalaldehydic acid (or its tautomer 3-hydroxyphthalide **32**) via hydrazone or pseudohyrazone intermediates (**33** and **34**, respectively).

We have proceeded to study the mechanism of phthalazinone formation, giving special attention to (a) the influence of the substituent R on the course of the reaction and (b) possible intermediates. Two types of intermediates have been isolated upon reacting 3-hydroxyphthalimidine **18** with the various hydrazines, one for R = H (**36**) and one

(8) Cohen, E.; Klarberg, B.; Vanghan, J. R., Jr *J. Am. Chem. Soc.* **1960**, *82*, 2731.
(9) Brown, H. C. "Organic Syntheses Via Boranes"; Wiley: New York, 1975; Chapter 1, p 2.
(10) Mason, S. F. *J. Chem. Soc.* **1957**, 4874.
(11) Bellamy, L. J. "The IR Spectra of Complex Molecules"; Methuen: London, 1958; p 363.
(12) Zyakun, A. M.; Grablyauskas, K. Y.; Kost, A. N.; Zaretskii, V. I.; Vulfson, N. S. *Izv. Akad. Nauk SSSR, Ser. Khim.* **1970**, 2080.
(13) "Heterocyclic Compounds"; Elderfield, R. C., Ed.; Wiley: New York, 1957; Vol. 6, p 194.
(14) Dunet, A.; Wilemart, A. *Bull. Soc. Chim. Fr.* **1948**, 1081.
(15) Teppena, J. *Recl. Trav. Chim. Pays-Bas* **1923**, *42*, 30.
(16) Lechat, P. *C. R. Hebd. Seances Acad. Sci.* **1958**, *246*, 2771.
(17) Vaughan, W. R. *Chem. Rev.* **1948**, *43*, 447.
(18) (a) Lechat, P. *C. R. Hebd. Seances Acad. Sci.* **1957**, *46*, 326. (b) Flitsch, W. *Chem. Ber.* **1970**, *103*, 3205.

Table I. 1(2*H*)-Phthalazinones (**1a–e, 2a–d, 26, 27**) and 1(2*H*,3*H*,4*H*)-Phthalazinones (**3a–e, 4a–d**)[a]

no.	R	formula[b]	mp, °C	cryst/slurry solvent	yield,[c] %	IR, cm^{-1}	MS, *m/e*	^1H NMR (Me_2SO-d_6), δ				
								C_4-H	C_5-H	C_8-H	SO_2NH_2	other protons
1a	H	$C_8H_6N_3O_3SCl$	>310	H_2O	46[d]	3480, 3330, 3200, 1650 (C=O), 1610, 1560, 1470, 1345	259 (M^+, 100), 243 (7), 202 (5), 179 (39), 151 (11), 124 (48)	8.43 (s)	8.3 (s)	8.78	8.0 (br m)	
1b	CH_3	$C_9H_8N_3O_3SCl$	257–259	H_2O	33[d]	3260, 3080, 1645 (C=O), 1633, 1593, 1555, 1427, 1399	273 (M^+, 75), 245 (100), 202 (15), 193 (9), 165 (25), 122 (35)	8.45 (s)	8.3 (s)	8.8 (s)	8.04 (s)	3.72 (s, 3 H, CH_3)
1c	CH_2Ph	$C_{15}H_{12}N_3O_3SCl$	236–237	EtOH	30[d]	3370, 3240, 1638 (C=O), 1579, 1338, 1165	349 (M^+, 46), 321 (10), 245 (91), 91 (100)	8.5 (s)	8.29 (s)	8.8 (s)	8.0 (s)	5.32 (s, 2 H, CH_2), 7.3 (s, 5 H, Ph)
1d	TFMPh	$C_{15}H_9N_3O_3SClF_3$	212	H_2O	48 (88)[e]	3400, 3295, 3090, 1660 (C=O), 1585, 1455, 1387, 1332	403 (M^+, 33), 323 (4), 202 (6), 145 (100)	8.62 (s)	8.35 (s)	8.8 (s)	7.7–8.1 (m, 6 H, SO_2NH_2, TFMPh)	
1e		$C_{13}H_{10}N_3O_4SCl$	225	Et_2O	43[d]	3400, 3300, 3095, 1653 (C=O), 1583, 1386, 1320, 1175	339 (M^+, 100), 311 (18), 245 (95), 202 (8), 122 (10)	8.47 (s)	8.28 (s)	8.78 (s)	8.0 (s)	5.3 (s, 2 H, CH_2), 6.4 (s, 2 H, C_3'-H, C_4'-H), 7.58 (s, 1 H, C_5'-H)
26	H	$C_{14}H_{11}N_3O_3S_2$	310	Et_2O	45[f]	3480, 3260, 3150, 3000, 2900, 1666 (C=O), 1582, 1470, 1440, 1348, 1245	333 (M^+, 100), 253 (45), 210 (23), 183 (35)	8.22 (s)	7.38 (s)	8.74 (s)	7.93 (s)	7.62 (s, 5 H, Ph)
2a	H	$C_8H_6N_3O_3SCl$	>300	MeOH	55[g]	3320, 3240, 3090, 1648 (C=O), 1592, 1448, 1387, 1362, 1330	259 (M^+, 100), 243 (2), 202 (5), 179 (25), 151 (7), 124 (24)	8.61 (s)	8.65 (s)	8.36 (s)	8.0 (br s)	13.0 (s, 1 H, NH)
2b	CH_3	$C_9H_8N_3O_3SCl$	280	$CHCl_3$	80[g]	3290, 3200, 3090, 1637 (C=O), 1578, 1542, 1458, 1387, 1367, 1350	273 (M^+, 100), 245 (94), 202 (13), 193 (12), 165 (35), 122 (20)	8.64 (s)	8.66 (s)	8.4 (s)	8.0 (br s)	3.75 (s, 3 H, CH_3)
2c	CH_2Ph	$C_{15}H_{12}N_3O_3SCl$	193–195	$CHCl_3$/Et_2O (1:1)	66[g]	3360, 3230, 3100, 1645 (C=O),	349 (M^+, 15), 321 (5), 245 (37), 91	8.7 (s, 2 H)		8.4 (s)	8.02 (s)	5.32 (s, 2 H, CH_2), 7.35 (s, 5 H, Ph)

						1577, 1535, 1455, 1387, 1342	(100)					
2d	TFMPh	$C_{15}H_9N_3O_3SClF_3$	172–175	Et_2O	53[g]	3390, 3270, 3100, 1660 (C=O), 1586, 1547, 1495, 1456, 1388	403 (M^+, 50), 323 (4), 202 (6), 145 (100)	8.76 (s)	8.88 (s)	8.45 (s)	7.7–8.2 (m, 6 H, SO_2NH_2, TFMPh)	
27	CH_3	$C_{15}H_{13}N_3O_3S_2$	279–282	Et_2O	79[f]	3380, 3280, 1645 (C=O), 1585, 1540, 1364, 1340, 1170	347 (M^+, 100), 267 (22), 238 (11), 195 (35)	8.55 (s, 2 H)		*i*	7.92 (s)	3.1 (s, 3 H, CH_3), 7.62 (s, 5 H, Ph)
3a	H	$C_8H_8N_3O_3SCl$	149–151		46[h]	3200, 1632 (C=O), 1595, 1450, 1348, 1192, 1160	261 (M^+, 75), 260 (100), 231 (15), 203 (1), 180 (24), 123 (25)	4.02 (s, 2 H)	7.62 (s)	8.38 (s)	7.72 (s)	
3b	CH_3	$C_9H_{10}N_3O_3SCl$	255–257		47[h]	3260, 3090, 1648, 1632 (C=O), 1595, 1555, 1428, 1372	275 (M^+, 100), 274 (40), 273 (15), 247 (13), 245 (32), 231 (78), 203 (12), 123 (55)	4.08 (br s, 2 H)	7.6 (s)	8.4 (s)	7.73 (s)	3.07 (s, 3 H, CH_3)
3c	CH_2Ph	$C_{15}H_{14}N_3O_3SCl$	232–235	Et_2O	55[h]	3320, 3180, 3070, 1631 (C=O), 1595, 1445, 1375, 1330, 1157	351 (M^+, 20), 349 (2), 260 (2), 247 (8), 245 (7), 231 (5), 218 (3), 123 (5), 91 (100)	4.05 (d, J = 7.5, 2 H)	7.6 (s)	8.45	7.75 (s)	4.72 (s, 2 H, CH_2), 6.13 (br t, J = 7.5, 1 H, NH), 7.3 (s, 5 H, Ph)
3d	TFMPh	$C_{15}H_{11}N_3O_3SF_3Cl$	145–148	Et_2O	72[h]	3210, 1668 (C=O), 1595, 1455, 1336, 1170, 1130	405 (M^+, 90), 403 (90), 231 (35), 145 (100)	4.35 (s, 2 H)	*j*	8.55 (s)	7.7 (s)	7.5–7.85 (m, 5 H, TFMPh)
3e	CH_2Ph	$C_{13}H_{12}N_3O_4SCl$	148–151	Et_2O	69[h]	3250, 3060, 1635 (C=O), 1595, 1437, 1340, 1330, 1157	341 (M^+, 2), 339 (1), 247 (1), 245 (1), 123 (1), 81 (100)	4.05 (d, J = 7.5, 2 H)	7.6 (br s)	8.43 (s)	7.75 (s)	4.7 (s, 2 H, CH_2), 6.38 (br s, 2 H, C_3'-H, C_4'-H), 7.6 (br s, 1 H, C_5'-H)
4a	H	$C_8H_8N_3O_3SCl$	152		33[h]	3220, 1660 (C=O), 1450, 1340, 1195	261 (M^+, 51), 260 (77), 231 (8), 203 (16), 123 (100)	4.03 (m, 2 H)	7.9 (s, 2 H)		7.6–7.7 (m)	
4b	CH_3	$C_9H_{10}N_3O_3SCl$	255–258		27[h]	3220, 1642 (C=O), 1455, 1347, 1283, 1193,	275 (M^+, 50), 274 (32), 273 (11), 245 (10),	4.13 (d, J = 8.0, 2 H)	7.92 (s, 2 H)		7.8 (br s)	3.12 (s, 3 H, CH_3), 6.11 (t, J = 8.0, 1 H, NH)

Table I (Continued)

no.	R	formula[b]	mp, °C	cryst/slurry solvent	yield,[c] %	IR, cm^{-1}	MS, *m/e*	^{1}H NMR (Me_2SO-d_6), δ				
								C_4-H	C_5-H	C_8-H	SO_2NH_2	other protons
						1176	231 (31), 202 (25), 123 (100)					
4c	CH_2Ph	$C_{15}H_{14}N_3O_3SCl$	195–198	Et_2O	53[h]	3350, 3270, 3100, 1646 (C=O), 1560, 1445, 1347, 1165	351 (M^+, 12), 260 (2), 231 (4), 203 (3), 123 (6), 91 (100)	4.1 (d, J = 9.0, 2 H)	7.95 (s)	8.13 (s)	7.72	4.76 (s, 2 H, CH_2), 6.19 (br t, J = 9.0, 1 H, NH), 7.39 (s, 5 H, Ph)
4d	TFMPh	$C_{15}H_{11}N_3O_3SFCl_3$	140–143	$CHCl_3$	59[h]	3230, 1650 (C=O), 1450, 1400, 1340, 1195, 1170	405 (M^+, 100), 403 (25), 377 (10), 231 (28), 203 (8), 145 (26), 123 (7)	4.28 (m, 2 H)		8.08 (m, 2 H)		7.48–8.0 (m, 6 H, SO_2NH_2, TFMPh)

[a] For general experimental data, see Experimental Section. [b] The microanalyses were in satisfactory agreement with the calculated values (C, H, N, O, S, Cl for **1a–e**, **2a,b**; C, H, N, S, F, Cl for **1d**; C, H, N, O, S for **2c**; C, H, N for **2c,d**) within ±0.4%. [c] Yields were in no case optimized. [d] The yield refers to the reaction of **18** with the corresponding hydrazine. [e] The first number refers to the overall yield of **1d** based on **18**; the number in parentheses indicates the yield of the cyclization of **37**. [f] The yield refers to the reaction of **1a** (or **2b**) with sodium thiophenoxide. [g] The yield refers to the final step, namely, conversion of sulfonyl chlorides **25** to the corresponding sulfonamides **2**. [h] Yield of reduction step. [i] The signals of C_8-H and the aromatic protons are superimposed.

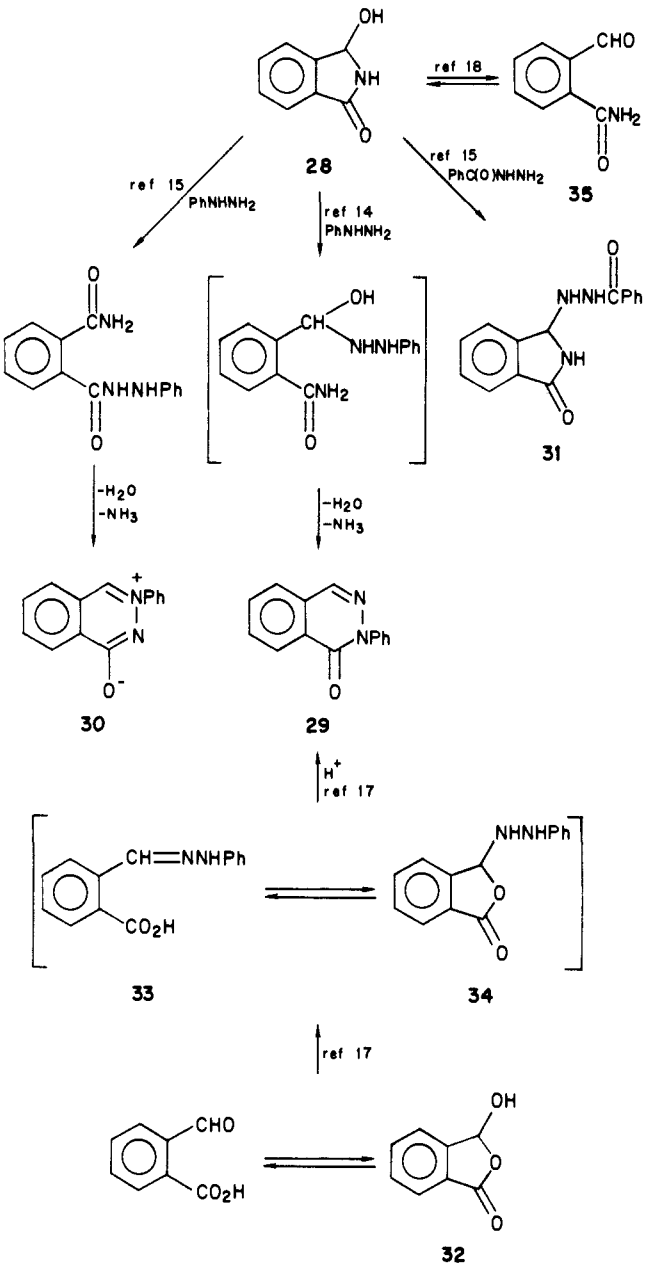
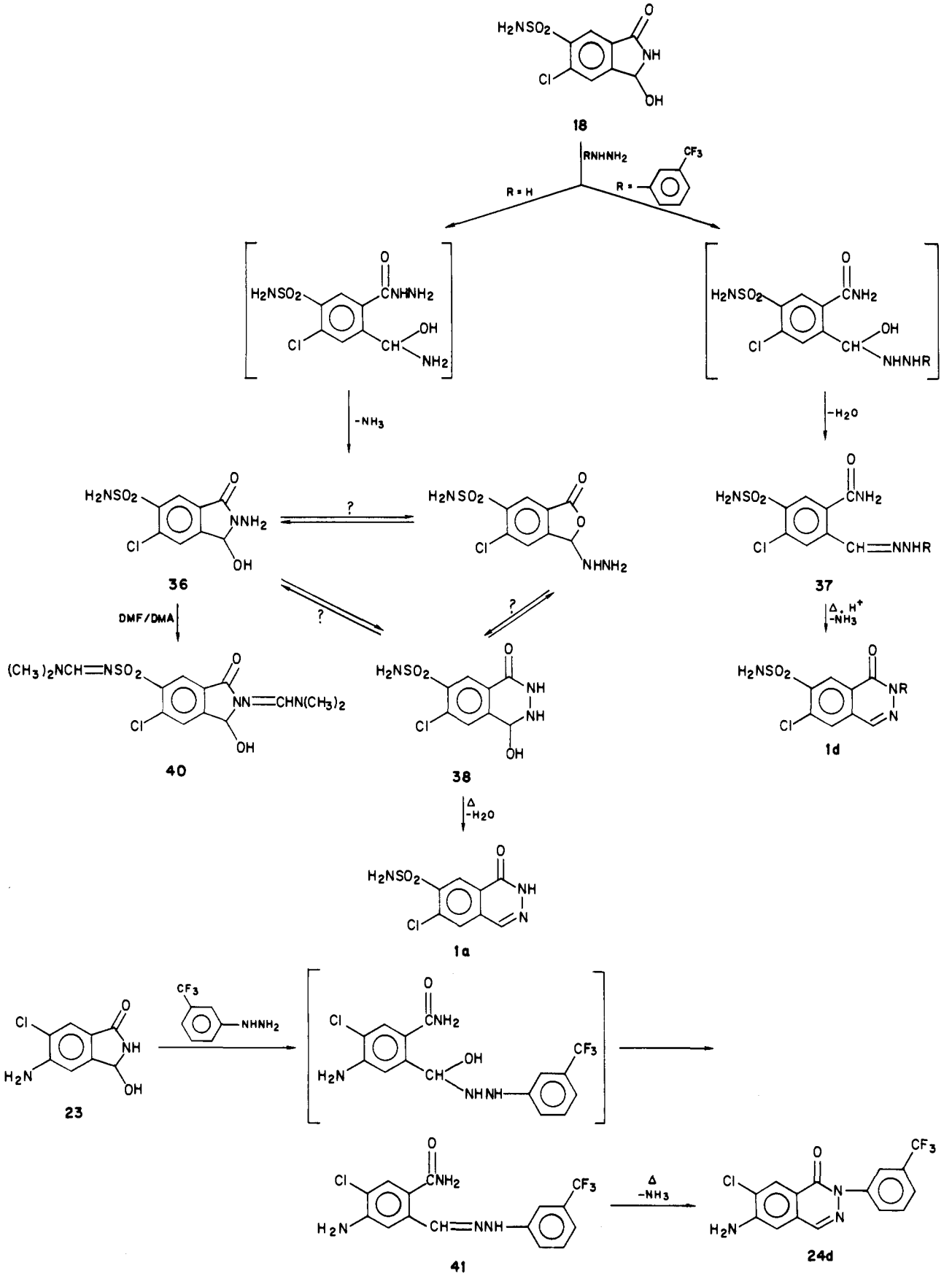


Figure 2.

for R = CF_3Ph (**37**). Compound **41**, a structural analogue of **37**, has been isolated from the reaction of **23** with [*m*-(trifluoromethyl)phenyl]-hydrazine. All three intermediates (**36**, **37**, **41**) were readily converted to the corresponding 1(2*H*)-phthalazinones by applying heat and/or acidic medium. The postulated reaction mechanisms are presented in Scheme IV.

For R = H, of the three possible intermediates **36**, **38**, **39** corresponding to $C_8H_8N_3O_4SCl$, **36** seems to be the correct structure, based on spectral data as follows. (a) The presence of an exocyclic amino group was established by reacting **36** with dimethylformamide dimethyl acetal. Both the sulfamoyl and the NNH_2 groups reacted, forming the bisformamidine **40**. (b) The NMR pattern of **36** is very similar to that of its precursor **18**. In addition to the common features, the signal at 4.75 ppm, exchangeable in D_2O, is assigned to the NNH_2 moiety. (c) The C=O absorption (1700 cm^{-1}) indicates clearly the presence of a five-membered phthalimidine ring. The carbonyl group in a phthalazinone such as **38** would absorb at about 1650 cm^{-1} and a lactone carbonyl as in the phthalide **39** at about 1750 cm^{-1}. Thus, two alternative mechanisms are possible

Scheme IV



(Scheme IV), depending on the substituent R.

Structure Assignment. The correct regiochemistry was assigned to series 1 and 2 on the basis of interpretation of NMR data and on conversion of 18 to a compound of known structure. The regiochemistry of series 3 and 4 is determined by that of the starting series 1 and 2.

By ^{1}H NMR. The corresponding deuterio analogues (deuterated at H-4) of **1a** and **2a** have been prepared. By comparison of the NMR spectra of the deuterated and nondeuterated compounds it was found (Figure 3) that the

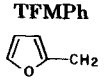
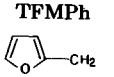
Table II. Starting Materials 17–23, 6-Amino- and 6-(Chlorosulfonyl)-7-chloro-1(2*H*)-phthalazinones (**24a–d** and **25a–d**), and Intermediates **36**, **37**, **40**, **41**, and **43**[a]

no.	mp, °C	cryst/slurry solvent	yield,[b] %	IR, cm^{-1}	MS, *m/e*	^1H NMR (Me_2SO-d_6), δ	remarks
17	290	dioxane	33	3320, 3090, 1793, 1720, 1537, 1390, 1350, 1180, 1150	260 (M^+, 100), 225 (10), 217 (18), 180 (38)	8.04 (s, 2 H, SO_2NH_2), 8.17 (s, 1 H, C_3-H), 8.35 (s, 1 H, C_6-H), 11.75 (s, 1 H, NH)	mp lit.[21] 292 °C
18	272	dil HCl	34	3400, 3310, 3270, 1700, (C=O), 1610, 1535, 1460, 1345	262 (M^+, 100), 245 (75) 234 (27), 227 (55), 182 (68)	5.96 (d, J = 9 Hz, 1 H, C_3-H), 6.6 (d, J = 9 Hz, 1 H, OH), 7.82 (m, 3 H, C_4-H, SO_2NH_2), 8.15 (s, 1 H, C_7-H), 9.2 (br s, 1 H, NH)	
20	196		91[c]	3240, 1760, 1725, 1700, 1350, 1300	181 (M^+, 100), 146 (41), 138 (92), 110 (84), 75 (84)	7.7 (s, 3 H, Ph), 11.3 (br s, 1 H, NH)	
		i-PrOH	77[d]				
21	192	H_2O	66			812 (s, 1 H, C_3-H), 8.35 (s, 1 H, C_6-H), 11.6 (br s, 1 H, NH)	mp lit.[21] 198 °C
22	312	H_2O	80			6.4 (s, 2 H, NH_2), 6.95 (s, 1 H, C_6-H), 7.4 (s, 1 H, C_3-H), 10.5 (br s, 1 H, NH)	mp lit.[21] 314 °C
23	>300	H_2O	61	3500–3080, 1690, 1622, 1485, 1450	198 (M^+, 100), 181 (80), 170 (20), 154 (45)	5.85 (br s, 1 H, C_3-H), 6.2 (br s, 2 H, NH_2), 7.05 (s, 1 H, C_4-H), 7.55 (s, 1 H, C_7-H), 8.6 (br s, 1 H, NH)	
24a	>310	H_2O	77	3450–3000, 1680, 1633, 1494, 1385, 1325, 1291	195 (M^+, 100), 167 (15), 138 (64)	6.55 (br s, 2 H, NH_2), 7.1 (s, 1 H, C_5-H), 8.05 (s, 1 H, C_8-H), 8.18 (s, 1 H, C_4-H), 12.3 (m, 1 H, NH)	
24b	>300	H_2O	60	3500–3200, 1630, 1560, 1490, 1435	209 (M^+, 60), 181 (100), 138 (59)	3.68 (s, 3 H, CH_3), 6.8 (s, 2 H, NH_2), 7.34 (s, 1 H, C_5-H), 8.3 (s, 1 H, C_8-H), 8.45 (s, 1 H, C_4-H)	
24c	190–193	Me_2SO/H_2O	37	3500–3200, 1633, 1571, 1492, 1430, 1380	285 (M^+, 14), 257 (15), 181 (20), 138 (10), 91 (100)	5.3 (s, 2 H, CH_2), 6.0–6.9 (m, 2 H, NH_2), 7.35 (s, 5 H, Ph), 7.1 (s, 1 H, C_5-H), 8.1 (s, 1 H, C_8-H), 8.27 (s, 1 H, C_4-H)	
24d	148	H_2O	45 (90)[e]	3550–3200, 1647, 1630, 1572, 1497, 1450, 1375, 1337	338 (65), 145 (81), 138 (100)	6.72 (br s, 2 H, NH_2), 7.12, 7.8, 8.0, 8.1, 8.4 (7 H, TFMPh, C_8-H, C_5-H, C_4-H)	
25a	225	H_2O	68				
25b	189	H_2O	52				
25c	153–156	H_2O	61				
25d	105	H_2O	55				
36	>310	MeOH	47	3310, 3200–3080, 1700 (C=O), 1620, 1424, 1386, 1334	277 (M^+, 15), 275 (2), 259 (100), 243 (6), 196 (38), 195 (25), 179 (27), 124 (21)	4.75 (s, 2 H, NNH_2), 5.67 (s, 1 H, C_3-H), 7.35 (br s, 3 H, SO_2NH_2, OH), 7.82 (s, 1 H, C_4-H), 8.12 (s, 1 H, C_7-H)	formula $C_8H_8N_3O_4SCl$[f]
37	253	Et_2O	54	3450, 3220, 3050, 1630 (C=O), 1615, 1535, 1410, 1360, 1330	420 (M^+, 11), 403 (49), 323 (7), 245 (53), 202 (3), 145 (100)	7.05–7.8 (m, 6 H, SO_2NH_2, TFMPh), 7.98 (s, 1 H), 8.15 (s, 1 H), 8.22 (s, 1 H), C_3-H, C_6-H, benzal), 11.2 (br s, 1 H, NNH)	formula $C_{15}H_{12}N_4O_3F_3ClS$[f]
40	220–222	MeOH	38	3260, 2930, 1710, 1692, 1642, 1622, 1425, 1341, 1285	387 (M^+, 100), 316 (65)	2.95 (s, 9 H, 3 CH_3), 3.21 (s, 3 H, CH_3), 5.87 (m, 1 H, C_3-H), 7.0 (m, 1 H, OH), 7.77 (s, 1 H, C_4-H), 8.0 (s, 1 H, Me_2NCH=N), 8.17 (s, 1 H, C_7-H), 8.36 (s, 1 H, Me_2NCH=NSO_2)	
41	219	Et_2O	50	3550–3200, 1626, 1612, 1551, 1480, 1465, 1396, 1337	356 (M^+, 30), 339 (77), 196 (35), 179 (100), 145 (81)	5.85 (br s, 2 H, NH_2), 7.35 (br s, 4 H, TFMPh), 3.5 (s, 2 H, C_6-H, benzal), 8.5 (s, 1 H, C_3-H), 10.9 (s, 1 H, NNH)	formula $C_{15}H_{12}N_4OF_3Cl$[f]
43	146–147	H_2O	55	1750 (C=O), 1618,	168 (M^+, 33), 139 (100),	5.38 (s, 2 H, CH_2), 7.8–8.8 (m, 3 H, Ph)	

1590, 1460, 1430, 1360, 1315, 1273, 1121, 997, 887, 830, 767, 675	111 (40)	

[a] For general experimental data, see Experimental Section. [b] Yields were in no case optimized. [c] Prepared from 4-chlorophthalic acid. [d] Prepared from 2,4-dichlorobenzoic acid. [e] The first number refers to the overall yield of **24d** based on **23**; the number in parentheses indicates the yield of the cyclization of 41. [f] The microanalyses were in satisfactory agreement with the calculated values (C, H, N, O, S, Cl for **36**, C, H, N, S, Cl, F for **37**, and C, H, N, F, Cl for 41) within ±0.4%.

Table III. Diuretic and Saluretic Activity[a] in the Rat of Phthalazinone Series 1–4

		diuretic activity, mL			saluretic activity,[b] mequiv/kg											
					0–5				5–24				0–24			
compd	R	0–5	5–24	0–24	Na^+	K^+	Cl^-	Na^+/K^+	Na^+	K^+	Cl^-	Na^+/K^+	Na^+	K^+	Cl^-	Na^+/K^+
1a	H	7.7 ± 0.6	3.6 ± 0.5	11.3 ± 0.8	8.16	2.55	9.87	3.18	4.21	2.51	4.10	1.68	12.37	5.06	13.97	2.44
1b	CH_3	6.1 ± 1.2	2.7 ± 0.3	8.8 ± 1.3	8.70	2.67	7.27	3.27	2.40	2.09	2.30	1.15	11.1	4.76	9.57	2.33
1c	CH_2Ph	5.9 ± 1.1	4.9 ± 1.2	10.8 ± 0.1	6.40	1.94	7.97	3.29	5.92	3.36	5.72	1.78	12.32	5.3	13.69	2.32
1d	TFMPh	2.2 ± 0.7	4.4 ± 1.4	6.6 ± 1.9	2.18	1.27		1.72	4.07	3.04		1.33	6.25	4.31		1.45
1e		1.8 ± 0.7	1.0 ± 0.6	2.8 ± 1.2	3.68	1.58	3.80	2.83	2.40	2.16	1.46	1.11	6.08	3.74	5.26	1.63
26	H	2.9 ± 2.8	4.7 ± 4.3	7.6 ± 2.9	2.90	1.45	3.97	2.0	4.97	4.25	4.47	1.17	7.87	5.70	8.44	1.38
2a	H	5.6 ± 0.6	3.2 ± 0.7	8.8 ± 0.3	6.14	2.51	4.10	2.44	4.30	3.19	5.02	1.35	10.44	5.70	9.12	1.83
2b	CH_3	2.2 ± 0.7	2.6 ± 1.1	4.8 ± 1.4	2.58	1.24	2.22	2.10	5.26	3.92	5.0	1.34	7.84	5.16	7.22	1.52
2c	CH_2Ph	2.6 ± 0.9	3.6 ± 1.8	6.2 ± 2.5	2.50	1.84	2.90	1.36	6.29	3.90	6.0	1.62	8.79	5.74	8.90	1.53
2d	TFMPh	0.9 ± 0.4	4.0 ± 1.1	4.9 ± 0.8	0.82	0.49		1.66	6.59	4.67		1.41	7.41	5.16		1.44
27	CH_3	2.1 ± 0.8	3.8 ± 1.0	5.9 ± 1.9	2.05	1.20	2.33	1.70	6.55	3.42	5.98	1.92	8.60	4.62	8.31	1.86
3a	H	6.5 ± 1.5	6.2 ± 1.0	12.7 ± 0.9	6.55	2.18	8.32	3.0	4.36	3.45	4.16	1.27	10.91	5.63	12.48	1.94
3b	CH_3	5.0 ± 0.7	6.8 ± 0.7	11.8 ± 1.3	4.45	2.55	6.20	1.74	5.98	5.27	3.15	1.13	10.43	7.82	9.35	1.33
3c	CH_2Ph	6.6 ± 1.0	6.6 ± 0.6	13.2 ± 1.3	6.90	2.61	8.69	2.65	5.70	4.18	5.0	1.36	12.60	6.79	13.69	1.86
3d	TFMPh	4.9 ± 1.6	6.7 ± 1.0	11.6 ± 1.7	6.17	1.99	7.91	3.10	4.90	3.42	4.69	1.43	11.07	5.41	12.60	2.05
3e		6.7 ± 1.8	3.8 ± 1.6	10.5 ± 3.4	6.74	2.38	8.45	2.83	3.09	3.37	2.98	0.92	9.83	5.75	11.43	1.71
4a	H	2.9 ± 0.5	3.7 ± 0.8	6.6 ± 0.3	3.23	1.58	3.49	2.04	5.84	3.94	6.28	1.48	9.07	5.52	9.77	1.64
4b	CH_3	2.7 ± 2.1	3.4 ± 1.2	6.1 ± 2.4	2.41	1.35	2.76	1.78	4.94	3.0	5.29	1.64	7.35	4.35	8.05	1.69
4c	CH_2Ph	4.0 ± 1.6	3.6 ± 0.9	7.6 ± 1.4	4.01	1.92	5.14	2.10	5.50	3.29	5.39	1.67	9.51	5.21	10.53	1.83
4d	TFMPh	1.6 ± 0.1	4.5 ± 0.9	6.1 ± 0.8	1.26	0.69	2.07	1.82	5.60	3.84	6.66	1.46	6.86	4.53	8.73	1.51
furosemide		19.2 ± 0.6	3.0 ± 0.2	22.0 ± 0.8	13.20	4.36	16.2	3.03	3.02	4.42	1.65	0.68	16.22	8.78	17.85	1.85
chlorothiazide		7.2 ± 0.3	4.7 ± 0.2	11.9 ± 0.6	7.74	3.75	8.80	2.06	5.03	4.14	4.75	1.21	12.77	7.89	13.55	1.62
saline		2.4 ± 0.2	3.3 ± 0.2	5.7 ± 0.3	2.10	1.38	2.60	1.52	4.70	3.48	4.03	1.35	6.80	4.86	6.63	1.40

[a] The experimental details of the diuretic and saluretic tests are described in the Experimental Section. [b] Standard deviations for the saluretic data have been calculated and are less than 10% of the mean values.

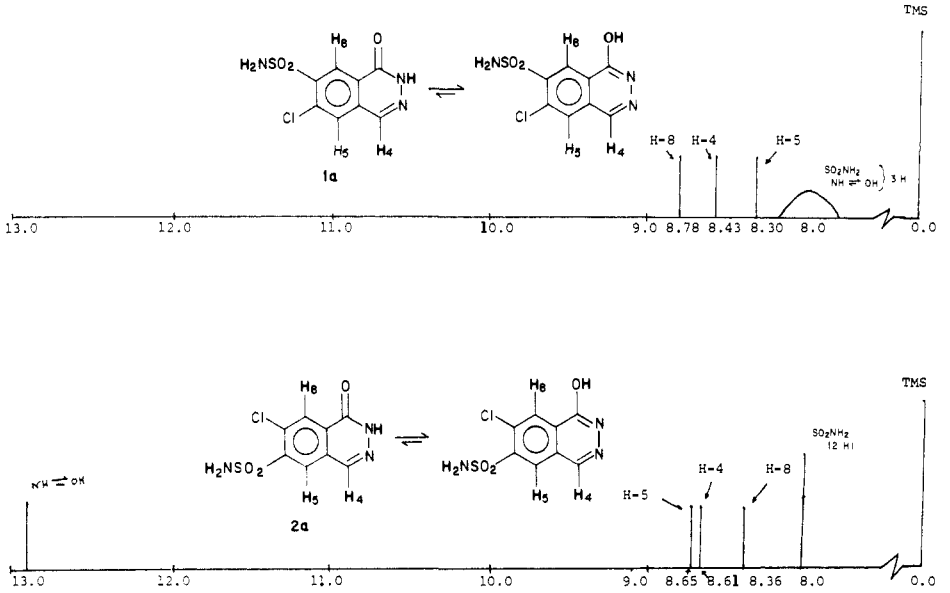


Figure 3. ^1H NMR spectra (8–13-ppm region) of **1a** and **2a**.

signals of the vinylic protons H-4 are situated between those of the aromatic protons H-5 and H-8. The most downfield signal in both series was assigned to the proton ortho to the powerful electron-withdrawing sulfamoyl group (H-8 in **1a** and H-5 in **2a**).

On the basis of considerations of the electronic effects of the sulfamoyl and carbonyl groups, it may be shown that the difference in chemical shift of the aromatic protons in **1a** should be larger than that of **2a**. Indeed, $\Delta\delta$(H5–H8) was found to be 0.48 and 0.29 ppm, respectively. The SO_2NH_2 and [CONH $\rightleftarrows$ C(OH)=N] signals appear as sharp singlets in the spectrum of **2a**, whereas for **1a** no NH peak can be observed in the 9–14-ppm region and the SO_2NH_2 appears as a very broad peak (see Figure 3). This may be explained by the proximity of the carbonyl and the sulfamoyl group in **1a** and subsequent intramolecular exchange.

By changing the nature of the substituent at the 6-position in **1a** from Cl to SPh (a strong electron donating group), an 0.62-ppm upfield shift of the H-5 signal is observed, while H-4 and H-8 remain practically unaffected. The NMR data of **3** and **4** further support the structure assignment presented above. Thus, in series **3**, the chemical shift difference $\Delta\delta$(H5–H8) is considerable (~0.8 ppm) due to their different magnetic environments, while in **4** both H-5 and H-8 have about the same δ value, and in all but one case (R = CH_2Ph), the two signals are superimposed. An attempt to assign the three relevant protons (H-4, H-5, H-8) in **1a** and **2a** by determination of the Overhauser effect of two adjacent protons (H-4 and H-5) in **2a** proved unsuccessful and no enhancement was observed. This is probably due to the small separation in chemical shifts (0.04 ppm) between H-4 and H-5.

By Chemical Synthesis. By converting **18**, a key intermediate in series **1**, to a product of known structure, the regiochemistry of this intermediate and consequently of the series as a whole was unequivocally established. Thus, **18** was treated with Zn–Cu in alkaline medium at elevated temperature, thereby losing the sulfamoyl group and converting the phthalimidine ring to phthalide **43**.

The same phthalide was also obtained from 4-chloro-2-methylbenzoic acid **42** (a commercial compound of known structure) by allylic bromination and spontaneous in situ dehydrobromination (see Scheme V). The identity of the lactones was proved by NMR, MS, IR, and mixture melting point. Thus, a 7-sulfamoyl structure could be unequivocally assigned to series **1** and subsequently a 6-sulfamoyl structure to series **2**.

Scheme V

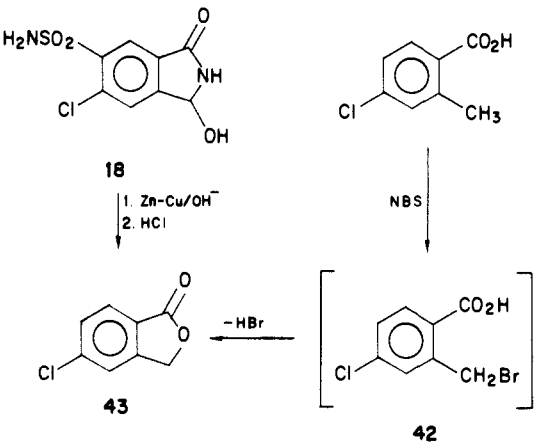


Table IV. Antihypertensive Activity[a] in the Rat of Selected Phthalazinones

compd	drop in systolic blood pressure, mmHg		
	1 h postdose	4 h postdose	24 h postdose
1a	13.7 ± 15.5	33.7 ± 11.0	38.0 ± 10.7
1b	31.7 ± 7.1	47.7 ± 8.7	26.3 ± 23.3
1c	23.0 ± 12.1	36.0 ± 11.1	47.0 ± 11.4
2b	29.7 ± 12.3	47.0 ± 17.8	19.3 ± 10.9
2c	35.3 ± 12.9	54.3 ± 17.3	4.7 ± 19.8
2d	35.6 ± 11.0	54.0 ± 16.5	27.7 ± 23.2
3a	24.7 ± 6.6	31.0 ± 15.6	27.0 ± 6.8
3b	31.3 ± 16.0	45.0 ± 12.1	22.7 ± 20.6
3e	25.3 ± 18.1	31.3 ± 20.3	23.0 ± 15.8
4a	34.7 ± 10.8	51.0 ± 24.9	47.7 ± 25.0
furosemide	35.8 ± 16.3	59.2 ± 16.5	5.3 ± 12.8
hydrochlorothiazide	28.6 ± 9.3	36.7 ± 15.5	18.0 ± 10.1
hydralazine	72.4 ± 33.8	75.1 ± 33.4	35.3 ± 25.0

[a] The experimental details of the tests are described in the Experimental Section.

Pharmacology

All compounds were subjected to a preliminary screening for diuretic, saluretic, and antihypertensive activity in the

Table V. Antihypertensive Screening of 1c (SHR)

	time following administration, h			
	control (0)	4	8	24
BP[a]	181.2 ± 37.4	163.2 ± 4.6	149.0 ± 1.5	170.2 ± 3.7
ΔBP[a]		23.4 ± 7.9[c]	40.2 ± 9.2[c]	14.6 ± 6.6[d]
heart rate[b]	not measured	399.6 ± 15.2	411.6 ± 8.7	402.0 ± 12.7

[a] Blood pressure, mmHg ± SEM. [b] Beats/minute + SEM. [c] $p < 0.05$. [d] $0.05 < p < 0.10$.

rat, and the results are summarized in Tables III–V.

Diuresis and Saluresis. Generally, compounds **1a–c** and **3a–e** exhibited moderate to good diuretic and saluretic activity. When compared to chlorothiazide, compounds **3a** and **3c** exhibited significantly better diuretic activity ($p < 0.05$), whereas compounds **1a**, **3b**, **3d**, and **3e** showed similar activity ($p > 0.05$) at 0–24 h level. The Na^+/K^+ ratios of **1a–c** and **3a,c–e** were similar or superior to those of furosemide.

The remaining compounds of series **1**, namely, **1d**, **1e**, and compound **26**, showed practically no diuretic or saluretic activity. In series **2**, only one compound (**2a**) exhibited a weak diuretic and a moderate saluretic activity; all the others (**2b–d**, **27**) were, as expected, practically inactive, and thus, this series as a whole may be considered devoid of significant diuretic activity.

All compounds of series **3** exhibited higher diuretic activity than those of series **1**, as is the case with the analogous hydrochlorothiazide vs. chlorothiazide.

The compounds of series **4** were moderately saluretic but showed practically no diuretic activity. The results for series **2** and **4** are in agreement with the general observation that a specific regiochemistry (the sulfamoyl and carboxyl groups oriented on the same side of the molecule) is essential for diuretic activity. Substitution of the Cl by the phenylthio group affords no enhancement of activity in series **2** (**27**) and has an adverse effect on activity in series **1** (**26**).

Antihypertensive Activity. In Table IV are listed those compounds that exhibited significant antihypertensive activity, i.e., comparable to or stronger than that of hydrochlorothiazide at the 1-, 4-, and 24-h time frames. When compared to hydralazine, compounds **1a–c**, **2b–d**, **3a,b,e**, and **4a** showed weaker activity at the 1- and 4-h time frames; however, the activity of **1a**, **1c**, and **4a** was stronger at 24-h postdose. Thus, these compounds may offer a unique combination of a "loop" diuresis with direct arterial vasodilating effects of long duration.

Compound **1c** was singled out for further screening. Its antihypertensive activity was determined in spontaneously hypertensive rats (SHR; T.N.O., Holland), ranging in weight from 250 to 350 g, and having a spontaneous BP base level of at least 170 mmHg (as measured by the indirect tail cuff plethysmographic method). The test compound was administered po at a single dose of 75 mg/kg to a group of five rats. Both indirect blood pressure and heart rate were recorded prior to and at 4, 8, and 24 h after administration. Their mean group values are presented in Table V, which shows that **1c** has significant antihypertensive activity in nonanesthetized SHR, reducing blood pressure by about 40 mmHg with the peak effect occurring about 8 h after administration.

The general profile of compound **1c** was determined in groups of three male mice (18–25 g of body weight, starved for 4–6 h) at doses of 50, 100, and 300 mg/kg, by observing motor activity reflexes, sedation, catalepsy, myosis, and mydriasis. No adverse effects have been detected. There was also no apparent difference between controls and experimental animals at all dose levels in the effect on body weight within 48 h after compound administration.

Experimental Section

The melting points were obtained on a Thomas-Hoover Unimelt capillary melting point apparatus and are uncorrected. Elemental analyses were performed by the Analytical Laboratories, Engleskirchen, West Germany. IR spectra were recorded as KBr pellets on a Perkin-Elmer 177 infrared spectrophotometer and are given in reciprocal centimeters. NMR spectra were taken on a JEOL 60 NMR spectrometer; all chemical shifts are given in δ units (ppm) downfield from Me_4Si, and the J values are given in hertz. Mass spectra were recorded with a Hitachi Perkin-Elmer RMU 6 mass spectrometer at 70 eV and are given as m/e units with their relative intensity. Monitoring of reactions and homogeneity of intermediates and final products were determined by TLC on Merck silica gel 60-F-254 plates developed with the solvent system $CHCl_3$/MeOH.

4-Chloro-5-sulfamoylphthalimide[7b] (**17**). A mixture of 2,4-dichloro-5-sulfamylbenzoic acid (**16**; 324 g, 1.2 mol); CuCN (123.5 g, 1.2 mol), and DMF (180 mL) was heated gently to 160 °C and kept at this temperature for 2 h. The hot suspension was then poured onto a solution containing $FeCl_3$ (240 g, 1.49 mol), water (760 mL), and concentrated HCl (100 mL), stirred for 20 min, allowed to cool to room temperature, and filtered. The solid was collected, washed thoroughly with water, and dried to give (after crystallization from dioxane) 102 g (33%) of **17**.

5-Chloro-3-hydroxy-6-sulfamoylphthalimidine (**18**). A suspension of **17** (48.0 g, 185 mmol) in MeOH (260 mL) and AcOH (260 mL) was added portionwise to a stirred and cooled mixture of Zn dust (51.0 g, 785 mmol) and water (80 mL), while the temperature was maintained at 10–15 °C. The mixture was stirred at 45 °C for 6 h and filtered. The collected solid was slurried in water (200 mL), filtered, dried, and extracted with MeOH (1.2 L). The methanolic solution was evaporated to dryness, and the residue was purified by slurrying in 10% HCl to remove any residual Zn salts, washed with water, and dried to give 16.5 g (34%) of **18**.

Isolation of Intermediate 5-Chloro-3-hydroxy-6-sulfamoyl-2-aminophthalimidine (**36**). A solution of **18** (2.0 g, 7.6 mmol) in hydrazine hydrate (2.0 g, 40 mmol) and water (2 mL) was stirred at room temperature for $\sim^1/_2$ hour. The mixture was poured onto ice, and the solid was collected and washed with water, treated with MeOH, filtered, and dried to give 1.0 g (47%) of **36**.

6-Chloro-7-sulfamoyl-1(2*H*)-phthalazinone[14] (**1a**). A solution of **18** (2.0 g, 7.6 mmol) in hydrazine hydrate (10 g, 200 mmol) and water (10 mL) was stirred at room temperature for 1 h. The solid was collected, slurried in cold water, filtered, and dried to give 0.90 g (46%) of **1a**.

6-Chloro-2-methyl-7-sulfamoyl-1(2*H*)-phthalazinone (**1b**). Methylhydrazine (3.45 g, 75 mmol) was added to a solution of **18** (5.0 g, 19 mmol) in water (10 mL) and 45% NaOH (6.5 mL) and stirred at 40 °C for 5 h under N_2. The reaction mixture was stirred overnight at room temperature and cooled to 0 °C, and the solid was collected and treated with 10% HCl (~2 mL). The product was washed thoroughly with water and dried to give 1.7 g (33%) of **1b**.

2-Benzyl-6-chloro-7-sulfamoyl-1(2*H*)-phthalazinone (**1c**). A mixture of **18** (1.0 g, 3.8 mmol), benzylhydrazine (2.2 g, 18 mmol), water (4 mL), and EtOH (3 mL) was stirred at 85 °C for 5 h under N_2. It was then evaporated to dryness and the oily residue was treated with 10% HCl (~30 mL). The resulting suspension was stirred at 10 °C for 1 h and filtered. The solid was washed with water, dried, treated with EtOH, filtered, and dried to give 0.40 g (30%) of **1c**.

6-Chloro-2-furfuryl-7-sulfamoyl-1(2*H*)-phthalazinone (**1e**). To a mixture of **18** (4.0 g, 15.3 mmol) and water (25 mL) was added a solution of furfurylhydrazine (8.8 g, 79 mmol) in EtOH (20 mL). The reaction mixture was stirred at 85 °C for 2 h under N_2. The mixture was acidified with 10% HCl, and the solid was collected, washed, dried, treated with Et_2O, filtered, and dried to give 2.2 g (43%) of **1e**.

Isolation of Intermediate 2-(Aminocarbonyl)-5-chloro-4-sulfamoylbenzaldehyde [*m*-(Trifluoromethyl)phenyl]-hydrazone (**37**). To a mixture of **18** (3.5 g, 13.4 mmol) and 14

mL of water was added a solution of [*m*-(trifluoromethyl)-phenyl]hydrazine (15.7 g, 89 mmol) in EtOH (21 mL). The mixture was stirred at 85 °C, under N_2, for 6 h. During this period two additional portions of $CF_3PhNHNH_2$ (3.0 g, 17 mmol each) were added. The reaction mixture was cooled to 0 °C, and the yellow solid was collected, washed with water, dried, treated with Et_2O, filtered, and dried to give 3.03 g (54%) of **37**.

6-Chloro-7-sulfamoyl-2-[*m*-(trifluoromethyl)phenyl]-1-(2*H*)-phthalazinone (1d). Concentrated HCl (2.5 mL) was added to a solution of **37** (2.5 g, 6 mmol) in DMF (12.5 mL) and the solution was stirred at 100 °C for 10 h. During this period, three additional portions of concentrated HCl (2.5 mL each) were added. The reaction mixture was poured onto ice, and the resulting solid was collected, washed thoroughly with water, and dried to give 2.15 g (88%) of **1d**.

6-(Phenylthio)-7-sulfamoyl-1(2*H*)-phthalazinone[19] (26). To a suspension of **1a** (0.50 g, 2 mmol) in 1 M $NaHCO_3$ (9 mL) was added thiophenol (0.4 mL). The mixture was stirred at 100 °C for 4 h, cooled to room temperature, and adjusted to pH 8 with dilute HCl. The solid was collected, washed thoroughly with water and ether, and dried to give 0.30 g (45%) of **26**.

4-Chlorophthalimide (20). (a) From 4-Chlorophthalic Acid[20] (19). Compound **19** prepared from its monosodium salt (201.5 g, 1.052 mol) was fused with ammonium carbonate (137.8 g, 1.435 mol), which has been previously ground in a mortar. The mixture was kept at ~300 °C for 2 h, with occasional shaking and reintroduction of the sublimed material. The dark hot melt was poured into an evaporating dish. The brownish solid was ground to a fine dust, 165 g (91%) of **20**.

(b) From 2,4-Dichlorobenzoic Acid. A mixture of 2,4-dichlorobenzoic acid (573 g, 3 mol), CuCN (322.5 g, 3.6 mol), and pyridine (300 mL) was heated to 130 °C. After a sudden rise of temperature (200 °C), the mixture was cooled to 100 °C and poured into 10% HCl (2 L). Water (~2 L) was added and the mixture was filtered. The solid was refluxed twice with acetone ($2 \times 1^1/_2$ L), and the filtrates were evaporated to dryness. The residue was refluxed with 2-propanol ($1^1/_2$ L), and the solid was collected and dried to give 416 g (77%) of **20**.

4-Chloro-5-nitrophthalimide[21] (21). A solution of **20** (15 g, 82 mmol) in 20% oleum (150 mL) and fuming nitric acid (18 mL) was heated at 80 °C for $^1/_2$ h. The mixture was cooled to room temperature and poured slowly onto ice (1.5 kg). The solid was collected, slurried twice in water, and dried to give 12 g (66%) of **21**.

4-Chloro-5-aminophthalimide[21] (22). Compound **21** (8.0 g, 35 mmol) was added to a solution of 28 g of $SnCl_2 \cdot 2H_2O$ in 150 mL of concentrated HCl, and the mixture was heated at 60 °C for $^1/_2$ h. It was then cooled to 0 °C, and the solid was collected, slurried in water, and dried to give 5.5 g (80%) of **22**.

5-Amino-6-chloro-3-hydroxyphthalimidine[22] (23). Compound **22** (5.8 g, 29.6 mmol) was added portionwise during ~$^1/_2$ hour to a stirred and cooled mixture of Zn dust (2.2 g, 33.8 mgat), $CuSO_4 \cdot 5H_2O$ (0.03 g), and 2 N NaOH (36 mL). The product was collected, slurried in water, and dried to give 3.6 g (61%) of **23**.

6-Amino-7-chloro-1(2*H*)-phthalazinone[14] (24a). A mixture of **23** (4.0 g, 20 mmol), 50% hydrazine hydrate (6 mL), and water (20 mL) was stirred at 95 °C for 2 h under N_2. The mixture was cooled to room temperature and the product was collected, washed with water, and dried to give 3.0 g (77%) of **24a**.

6-Amino-7-chloro-1(2*H*)-phthalazinone (24b). A mixture of **23** (4.0 g, 20 mmol), methylhydrazine (9 mL), and water (30 mL) was stirred at 90 °C for 3 h under N_2. The reaction mixture was cooled in an ice bath, and the product was collected, washed with water, and dried to give 2.5 g (60%) of **24b**.

6-Amino-2-benzyl-7-chloro-1(2*H*)-phthalazinone (24c). A mixture of **23** (8.0 g, 40 mmol), benzylhydrazine (32 g, 260 mmol), water (65 mL), and EtOH (45 mL) was stirred at 95 °C for 20 h under N_2. During this period an additional portion of benzylhydrazine (10 g, 80 mmol) in water (20 mL) and EtOH (15 mL) was added. The reaction mixture was filtered hot, the filtrate was cooled to 0 °C, and the solid was collected, slurried first in 10% HCl, then in water, and finally in benzene, and dried, giving 4.3 g of crude product. This was purified by heating in Me_2SO at 175 °C for 2 h, pouring the hot reaction mixture onto ice and collecting the solid (3.2 g). Another 1.0 g was obtained by evaporation of the filtrate and treatment of the residue with 10% HCl. Overall yield: 4.2 g (37%) of **24c**.

2-(Aminocarbonyl)-4-chloro-5-aminobenzaldehyde [*m*-(Trifluoromethyl)phenyl]hydrazone (41). A mixture of **23** (10 g, 51 mmol), $CF_3PhNHNH_2$ (37 g, 210 mmol), water (60 mL), and EtOH (60 mL) was stirred at 95 °C for 12 h under N_2. During this period an additional portion of $CF_3PhNHNH_2$ (5.0 g, 28 mmol) was added. The reaction mixture was cooled to 0 °C and filtered, and the solid was collected, slurried in ether, and dried to give 8.75 g (50%) of **41**.

6-Amino-7-chloro-2-[*m*-(trifluoromethyl)phenyl]-1-(2*H*)-phthalazinone (24d). A suspension of **41** (7.0 g, 20 mmol) in Me_2SO (30 mL) was heated at 180 °C for 3 h. The hot solution was cooled to 100 °C and poured onto ice. The solid was collected, slurried in water, and dried to give 6.0 g (90%) of **24d**.

2-Substituted 7-Chloro-6-(chlorosulfonyl)-1(2*H*)-phthalazinones[21] 25a–d. General procedure: $NaNO_2$ solution (20 mmol in 6 mL of water) was added dropwise to a mixture of **24** (10 mmol) in concentrated HCl (23 mL) while the temperature was maintained at 0–5 °C. The mixture was stirred at 5–10 °C for 20 min and poured into glacial acetic acid (15 mL) saturated with SO_2 and containing CuCl (135 mg). The reaction product was collected, slurried in water, and dried. Yields and melting points are listed in Table II.

2-Substituted 7-Chloro-6-sulfamoyl-1(2*H*)-phthalazinones[21] 2a–d. General procedure: A mixture of **25** (11 mmol) in liquid ammonia (60 mL) was stirred for 6 h at −60 °C. After allowing the ammonia to evaporate, the residue was triturated with water, collected, and heated with concentrated HCl at 90 °C for 1 h. The mixture was cooled, and the solid was collected, slurried in water, and dried. The crude product was further purified by heating it in an appropriate solvent. Yields and melting points are given in Table II.

2-Methyl-7-(phenylthio)-6-sulfamoyl-1(2*H*)-phthalazinone[19] (27). Compound **2b** (200 mg, 0.7 mmol) was suspended in 1 N $NaHCO_3$ (2 mL), and thiophenol (150 mg, 1.5 mmol) was added. The mixture was heated at 100 °C for 6 h, cooled to room temperature, and adjusted to pH 8 by dilute HCl. The solid was collected, washed well with water, and ether and dried to give 200 mg (79%) of **27**.

5-Chloro-6-[[(dimethylamino)methylene]sulfamoyl]-3-hydroxy-2-[[(dimethylamino)methylene]amino]phthalimidine (40). *N,N*-Dimethylformamide dimethyl acetal (145 mg, 1.2 mmol) was added to a suspension of 5-chloro-6-sulfamoyl-3-hydroxy-2-aminophthalimidine (**36**) (75 mg, 0.27 mmol) in absolute MeOH (3 mL). The reaction mixture was stirred for 1 h at room temperature and for 4 h at 50 °C. It was then concentrated under air and the precipitated solid collected and dried to give 40 mg (38%) of **40**.

General Procedure for the Preparation of 3,4-Dihydro-1(2*H*)-phthalazinones 3 and 4.[8] $AlCl_3$ (1.6 mmol) was added to dry diglyme (90 mL) with cooling and stirring under N_2. The mixtue was allowed to reach room temperature, and the phthalazinone (1.4 mmol) was added. A solution of $NaBH_4$ (8 mmol) in dry diglyme (30 mL) was then added dropwise. The mixture was heated for $^3/_4$ h at 40 °C (compounds **3b–e**, **4c,d**); in some cases (**3a**, **4a,b**), additional heating at 70 °C for 1 h was necessary for completion of reaction. The reaction mixture was then cooled to 10 °C, and water (10 mL) followed by 10% HCl (3 mL) was added carefully. The clear yellowish solution was evaporated to dryness. The residue was either extracted with $CHCl_3$ (in a Soxhlet) for 3 h (R = H, CH_3) or slurried in an appropriate solvent (R = Bz, CF_3NHNH_2, furfuryl). The resulting solid was then slurried in water and dried.

Preparation of 43. (1) A suspension of 4-chloro-2-methylbenzoic acid (ICN Pharmaceutical Inc.; 0.5 g, 2.9 mmol), *N*-bromosuccinimide (0.59 g, 2.9 mmol), and dibenzoyl peroxide (7 mg) in carbon tetrachloride (5 mL) was refluxed for 1 h. The reaction mixture was evaporated to dryness and the residue was suspended in a 10% sodium bicarbonate solution. The solid was

(19) Feit, P.; Tvaermose Nielsen, O. B. *J. Med. Chem.* **1972**, *15*, 79.
(20) "Organic Syntheses"; Wiley: New York, 1944; Collect. Vol. I, p 457.
(21) Cornish, E. J.; Lee, G. E.; Wragg, W. R. *J. Pharm. Pharmacol.* **1966**, *18*, 65.
(22) Reissert, A. *Ber. Dtsch. Chem. Ges.* **1913**, *46*, 1484.

collected, washed, and dried to give 0.2 g (60%) of **43**.

(2) By Reduction of 18. Compound **18** (500 mg, 2 mmol) was added portionwise to a stirred and cooled mixture of Zn dust (320 mg, 5 mmol), $CuSO_4 \cdot 5H_2O$ (2 mg), water (0.5 mL), and 20% NaOH (1 mL). The mixture was stirred in the cold for $^1/_2$ h, diluted with water (2 mL), and heated at 90 °C for 1 h. The Zn was filtered off, and the filtrate was concentrated to about half its volume and acidified (concentrated HCl) in the cold to pH 3. The crude 4-chloro-2-(hydroxymethyl)benzoic acid thus obtained was cyclized by heating in concentrated HCl (1 mL) at 90 °C for $^1/_2$ h. The solid was collected, washed well with water, and dried to give **43** (55%). The spectral data (NMR, IR, MS) of the 5-chlorophthalide prepared in this way are identical with those of the authentic phthalide obtained as described above. No depression was observed in a mixture melting point.

Pharmacology. For saludiuretic screening, all test compounds were prepared as a solution or as a suspension in saline and fed with a stomach tube to male "Sabra" rats (weight 170–200 g) of the Hebrew University, Jerusalem. The animals received 50 mg/kg in a loading volume of 25 mL of saline/kg of body weight. Eight rats were used for testing each compound. Four test compounds and two controls (furosemide or saline, eight rats each) were tested simultaneously on each experimental day. The rats were starved 18 h prior to the feeding and, following administration of the test compounds, were immediately placed in metabolic cages (two animals/cage) in a sound-proof 22 ± 1 °C, well-ventilated, 12:12 h illuminated room. No food or water was supplied during the experimental period. The urine was collected into plastic graduate measuring cylinders (each containing 2 drops of toluene), measured, and analyzed 5 and 24 h after administration of the compound. pH was measured immediately upon collection of the urine. The samples were centrifuged at 2000 rpm, and chlorides were titrated, after acidification with HNO_3, by $Hg(NO_3)_2$ in the presence of diphenylcarbazone as indicator. Na^+ and K^+ were determined by atomic absorption with a Perkin-Elmer Model 403 spectrophotometer.

For antihypertensive screening, groups of six Sprague–Dawley rats, of 250–350 g of body weight, with a systolic blood pressure (BP) of minimum 170 mmHg, were treated daily subcutaneously with 10 mg/kg doca and saline. When the BP reached 170 mmHg (usually after 3–4 weeks of doca/saline treatment), the test compounds were given orally in doses of 25 mg/kg in 5 mL/kg of 1% methylcellulose. BP was determined by the tail-cuff method prior to and at 1, 4, and 24 h following administration. Hydralazine (4 mg/kg), hydrochlorothiazide (25 mg/kg), and furosemide (25 mg/kg) served as positive controls.

Summary statistics (mean and standard deviation) for all compounds tested were calculated, and comparisons between compounds analyzed were made by the Student's *t* test for independent samples.

Acknowledgment. Diuresis and doca/saline experiments were conducted by Dr. J. Shani at the School of Pharmacy, The Hebrew University of Jerusalem, Israel. SHR experiments were peformed at the Israel Institute for Biological Research, Ness-Ziona, Israel. The assistance of S. Fenster in the statistical significance calculations is gratefully acknowledged.

Registry No. **1a**, 100448-25-7; **1b**, 100448-26-8; **1c**, 100448-27-9; **1d**, 100448-28-0; **1e**, 100448-29-1; **2a**, 100448-31-5; **2b**, 100448-32-6; **2c**, 100448-33-7; **2d**, 100448-34-8; **3a**, 100448-36-0; **3b**, 100448-37-1; **3c**, 100448-38-2; **3d**, 100448-39-3; **3e**, 100448-40-6; **4a**, 100448-41-7; **4b**, 100448-42-8; **4c**, 100448-43-9; **4d**, 100448-44-0; **16**, 2736-23-4; **17**, 3861-99-2; **18**, 100448-45-1; **19**, 89-20-3; **20**, 7147-90-2; **21**, 6015-57-2; **22**, 5566-48-3; **23**, 100448-46-2; **24a**, 100448-47-3; **24b**, 100448-48-4; **24c**, 100448-49-5; **24d**, 100448-50-8; **25a**, 100448-51-9; **25b**, 100448-52-0; **25c**, 100448-53-1; **25d**, 100448-54-2; **26**, 100448-30-4; **27**, 100448-35-9; **36**, 100448-55-3; **37**, 100448-56-4; **40**, 100448-57-5; **41**, 100448-58-6; **43**, 54109-03-4; furfurylhydrazine, 6885-12-7; 4-chloro-2-(hydroxymethyl)benzoic acid, 100448-59-7; hydrazine, 302-01-2; methylhydrazine, 60-34-4; benzylhydrazine, 555-96-4; [*m*-(trifluoromethyl)phenyl]hydrazine, 368-78-5; thiophenol, 108-98-5; 2,4-dichlorobenzoic acid, 50-84-0; *N*,*N*-dimethylformamide dimethyl acetal, 4637-24-5; 4-chloro-2-methylbenzoic acid, 7499-07-2; CuCN, 544-92-3.

Modified Di- and Tripeptides of the C-Terminal Portion of Oxytocin and Vasopressin as Possible Cognition Activation Agents

E. D. Nicolaides,*† F. J. Tinney,† J. S. Kaltenbronn,† J. T. Repine,† D. A. DeJohn,† E. A. Lunney,† W. H. Roark,† J. G. Marriott,‡ R. E. Davis,‡ and R. E. Voigtman‡

Departments of Chemistry and Pharmacology, Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, Michigan 48105. *Received June 24, 1985*

A number of peptides and modified peptides were synthesized and studied for their ability to reverse electroconvulsive shock-induced amnesia in rodents. A few of these peptides were selected for secondary evaluation in tests of short-term memory in rats and aged rhesus monkeys. A number of the peptides and modified peptides were active in the amnesia reversal test. In selected secondary tests, however, the chosen compounds failed to show significant activity in enhancing memory. New methods for preparing methyleneamino and methyleneoxy isosteres of peptides are reported. Other modified peptides also included methylenethio, methylenesulfonyl, and ethylene isosteres in place of the normal peptide amide bond.

The ability of peptides to influence cognitive functions has been the subject of several recent investigations.[1-5] de Wied has shown that lysine vasopressin delays the extinction of active avoidance behavior in the intact rat and Walter et al. have reported on the protective effects of neurohypophyseal hormones and analogues and C-terminal fragments of these hormones on the amnestic action of puromycin in mice.

An interest in the area of pharmacological treatments for cognitive dysfunctions or impairments has been actively pursued in these laboratories for some time. Previous reports covered the activity of 3-(aryloxy)pyridines[6] and

† Department of Chemistry.
‡ Department of Pharmacology.

(1) DeWied, D. *Nature (London)* **1971**, *232*, 58–60.
(2) Bohus, B.; Gispen, W. H.; DeWeid, D. *Neuroendocrinology* **1973**, *11*, 137.
(3) Walter, R.; Hoffman, P. L.; Flexner, J. B.; Flexner, L. B. *Proc. Natl. Acad. Sci.* **1975**, *72*, 4180.
(4) Flexner, J. B.; Flexner, L. B.; Walter, R.; Hoffman, P. L., *Pharmacol. Biochem. Behav.* **1978**, *8*, 93.
(5) Medveder, V. I.; Bakharev, V. D.; Kaurov, O. A. *Fiziol. Zh. SSSR I. M. Sechenova* **1982**, *68*, 1322.
(6) Butler, D. E.; Poschel, B. P. H.; Marriott, J. G. *J. Med. Chem.* **1981**, *24*, 346.

0022-2623/86/1829-0959$01.50/0 © 1986 American Chemical Society