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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISION DE NUEVAS CREACIONES
E. S. D.

Re: Expediente No. **07.050.827**
Solicitud de patente a nombre de **JANSSEN PHARMACEUTICA N. V.**

Yo, DILIA MARIA RODRIGUEZ D'ALJEMAN, actuando como apoderada sustituta de acuerdo con la sustitución adjunta, identificada como aparece al pie de mi firma, domiciliada en la carrera 11 A No. 86-53, piso 6, obrando como apoderada de la sociedad **JANSSEN PHARMACEUTICA N. V.**, domiciliada en Beerse, Bélgica, solicitante de la patente de la referencia, en respuesta al oficio No. 04006 notificado por fijación en lista el 4 de marzo de 2010, relacionado con el concepto técnico de fondo No. 259, me permito informar lo siguiente:

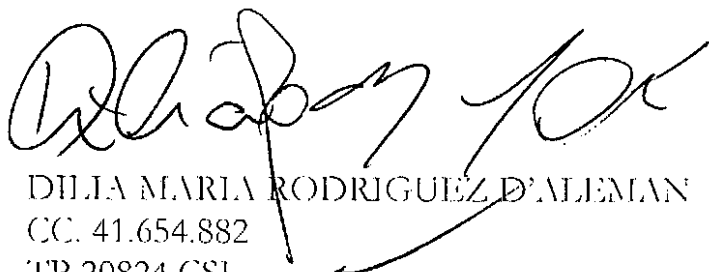
Acompañamos copia informal de los siguientes documentos:

MOFFETT, Robert B., Rober Andre., SKALLETZKY, Louis, "Antiulcer agents. P-Aminobenzamido aromatic compounds". JOL. MED. CHEM., 1971, vol 14, no. 10, pp. 963-968: Publication Date (Web): October, 1971. DOI 10.1021/jm00292a019; y

NILSSON, Jonas W. y Col. "Solid-Phase Síntesis of libraries Generated from a 4-phenyl-2-carboxy-piperazine scaffold". J. Comb. Chem., 2001, 3(6), pp. 546-553. DOI: 10.1021/cc010013o. Publication Date (Web): September 6, 2001.

Con la presentación ante ese despacho de estos dos artículos se da cabal respuesta al requerimiento exigido por el señor Examinador con base en lo manifestado en el artículo 46 de la Decisión 486.

Señor Superintendente,



DILIA MARIA RODRIGUEZ D'ALJEMAN
CC. 41.654.882
TP 20824 CSJ
NRA

Clarke, Modet & C^o

COLOMBIA

SEÑORES

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

DIVISIÓN DE NUEVAS CREACIONES

E. S. D.

REF:

EXPEDIENTE NR. : 07.050.827
SOLICITUD DE PATENTE A NOMBRE DE JANSSEN
PHARMACEUTICA N.V.

Yo, **JIMENA ESCOBAR URIBE**, identificada como aparece al pie de mi firma, domiciliada en la Carrera 11 No. 86-53 Piso 6, atentamente digo a usted que sustituyo el poder con que obro a la doctora **DILIA RODRIGUEZ D'ALEMAN**, domiciliada en Bogotá, D.C., portadora de la tarjeta profesional No. 20.824, con las mismas facultades con que me fueron conferidas para este caso.



JIMENA ESCOBAR URIBE

C.C. 39.779.452 de Usaquén

T.P. 68.228

Acepto sustitución

DILIA RODRIGUEZ D'ALEMAN

C.C. 41.654.882 de Bogotá

T.P. 20.824

PRESENTACION PERSONAL

El anterior escrito fue presentado ante el
NOTARIO ONCE DEL CIRCULO DE BOGOTA, D.C.

personalmente por Jimeno Escobar

quien exhibió la c.c. 3999432 Uquén

y Tarjeta Profesional No. 683.728 S.S.

29 SEP 2010

Fecha

Jimeno Escobar

Guillermo Chávez Cristancho
NOTARIO

[Handwritten signature and stamp]
Guillermo Chávez Cristancho
NOTARIO II DEL CIRCULO DE BOGOTA, D.C.

Antiulcer Agents. p-Aminobenzamido Aromatic Compounds¹

ROBERT BRUCE MOFFETT,* ANDRÉ ROBERT, AND LOUIS L. SKALETZKY

Research Laboratories of The Upjohn Company, Kalamazoo, Michigan 49001

Received April 26, 1971

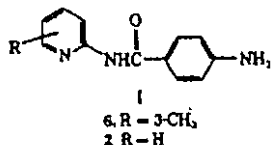
P.J. 10-1971

Pg 3-568-(6)

A series of 2-(p-aminobenzamido)pyridines (1) were found to have a powerful inhibiting effect on experimental gastric ulcers in rats. Attachment of the p-aminobenzamido group at other positions on the pyridine ring, or substitution of other nitrogen heterocycles for pyridine, greatly decreased activity. p-Aminobenzanilide was found to be highly active, but quite toxic. The compds were prepd from p-nitrobenzoyl chloride and the requisite aromatic amine, followed by reduction of the NO₂ group. A number of intermediates and analogs are reported.

Gastrointestinal ulcers are commonly treated by diet, antacids, anticholinergics, or surgery. Anticholinergics reduce the amount of gastric juice and its acidity and also the motility of the stomach and intestine. Although widely used, they are by no means the final answer. Gastric ulcers can be produced in rats by restraint, forced exertion, pylorus ligation, or by administration of certain adrenal cortical hormones. Anticholinergics are active in preventing all these types of ulcers; however, it has been our goal to find an antiulcer agent that does not work by anticholinergic mechanism.

For screening, compds were selected that were thought not to be anticholinergic either on the basis of structure or because they were inactive in the Magnus or other anticholinergic test. Any compd found to prevent exertion ulcers² in rats was retested as an anticholinergic. If inactive at doses much higher than the antiulcer dose, it was studied for prevention of all the above kinds of ulcers. Thus, a series of compds (1) was discovered, many of which showed antiulcer activity.¹



The most active was 6. This is related to the weak antibacterial "carbopyridin"³ (2) which in turn is related to sulfapyridine. Compd 2 was found to be a much weaker antiulcer agent than 6. Likewise, changing the positions of the various groups on the rings, adding other groups, or changing their nature, reduced or eliminated activity (Table I). The replacement of the pyridine ring by various other heterocyclic rings (Table II) also greatly reduced or eliminated activity. Surprisingly, substitution of a benzene ring in place of the pyridine ring gave a very active anilide (70). However, this was found to be quite toxic.

It is interesting that many of these compds were found to exhibit mild CNS-depressant activity in intact mice. However, there was no close correspondence between antiulcer and CNS-depressant activities, and

the better antiulcer agents were active at doses much lower than the depressive doses. Although certain known CNS depressants, for example chlorpromazine, chlorthalidoxepoxide, phenobarbital, meprobamate, etc., were shown to prevent exertion ulcers in rats, the doses required produced overt depression. Tables I and II include a rough indication of CNS-depressant activity in mice of our compd. Several well-known drugs are included for comparison.

The aminobenzamido compounds were made by reduction of the corresponding nitrobenzamides (method B). These were prepared from the appropriate amino heterocycle or aniline and nitrobenzoyl chloride (method A). Representative procedures are given in the Experimental Section. In several cases the reaction of substituted benzoyl chlorides with 2-aminopyridines yielded *N,N*-dibenzoyl compds (Table III). These were inactive as antiulcer agents, but could be easily hydrolyzed to the desired benzamidopyridines (method D). This dibenzoylation could be avoided by the use of the appropriate benzoic anhydride (when available) in place of the acid chloride.

In the benzoylation reaction of certain of the amino-heterocyclic compounds, the possibility is recognized that the benzoyl moiety might go on a ring N instead of the NH₂ group. However, the structures (52, 54, 57, 59, 62, 64) are formulated in Table II on the basis of analogy with the literature.¹⁻⁴

In one case (tetramethylbenzoylation of adenine), two isomers were isolated. Although definitive structure proof is not available, 64 is formulated, on the basis of nmr, as the NH₂-substituted derivative, and 65 as a ring-substituted compd, possibly a mixture of the 7 and 9 derivatives.

Experimental Section¹⁰

2-(p-Nitrobenzamido)-3-picoline (5) (Method A).—To a cold soln of 15 g (0.0906 mole) of p-nitrobenzoyl chloride in 20 ml of pyridine and 25 ml of CHCl₃ was added 4.7 g (0.0906 mole) of

(5) R. G. Fargher and F. L. Pyman, *J. Chem. Soc.*, 118, 317 (1919).

(6) P. N. Criss and J. R. Hoover, U. S. Patent 3,336,191 (1967); *Chem. Abstr.*, 64, 56585 (1968).

(7) G. Cipani and V. Grignani, *Lehr. PSR Zinol. Abstr. Vene. Kim. Ser.*, 263 (1963); *Chem. Abstr.*, 69, 17190f (1965).

(8) V. P. Bchilpashov, B. L. Porokova, V. A. Krasova, Yu. N. Shilovsk, and I. Ya. Postovskii, *Zh. Org. Khim.*, 1, 2320 (1963); *Chem. Abstr.*, 64, 11030d (1966).

(9) M. W. Bullock, J. J. Hand, and E. L. R. Stokstad, *J. Org. Chem.*, 29, 566 (1957).

(10) Melting points were taken in capillary tubes with a partial immersion thermometer. Calibration of the apparatus against standard compounds showed no need for correction. Infrared spectra were obtained on all pure compounds and nmr (Varian A-60) on representative examples. These were in accordance with the proposed structures. Where analyses are indicated only by symbols of the elements or functions, anal. results obtained for these elements or functions were within $\pm 0.4\%$ of theory.

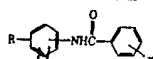
(1) Presented in part at the Great Lakes Regional American Chemical Society Meeting, DeKalb, Ill., June 8, 1969.

(2) A. Robert, J. J. Northam, J. E. Nezamis, and J. P. Phillips, *Amer. J. Dig. Dis.*, 16, 467 (1970).

(3) A. Robert and L. L. Skletzky, U. S. Patent 3,418,320 (1968); Netherlands Application 661284; *Chem. Abstr.*, 64, 56445 (1968).

(4) R. Kuhn, E. F. Moller, G. Wende, and H. Reibert, *Chem. Ber.*, 76, 711 (1943).

TABLE I
BENZAMIDOPYRIDINIZES



No.	Position of NHCO on pyridine ring	R	X	Method of prep.	Crysol solvent	Yield, %	Mp, °C	Empirical formula	Analysis	LD ₅₀ , mg/kg	CNS depression, mg/kg	Average acute LD ₅₀ , mg/kg
1	2	H	4-NO ₂	A	n-BuOH	80	239-241	C ₁₂ H ₁₀ N ₂ O ₂		>1000	300	
2	2	H	4-NH ₂	B	EtOH	80	153-156	C ₁₂ H ₁₀ N ₂ O	C, H, N	850	1000	50
3	2	H	4-NH ₂ ·2HCl	C	MeOH	93	>310	C ₁₂ H ₁₀ Cl ₂ N ₂ O				
4	2	H	4-NHCOCH ₃	f	DMF-H ₂ O	81	203-204	C ₁₃ H ₁₀ N ₂ O ₂	C, H, N	>1000	100	
5	2	3-CH ₃	4-NO ₂	A	AcOH-H ₂ O	75	190-197	C ₁₃ H ₁₀ N ₂ O ₂	C, H, N	>1000		
6	2	3-CH ₃	4-NH ₂	B		48	177-179	C ₁₃ H ₁₀ N ₂ O	C, H, N	562	300	35
7	2	3-CH ₃	4-NH ₂ ·2HCl	C	MeOH	94	>340 dec	C ₁₃ H ₁₀ Cl ₂ N ₂ O	C, H, Cl, N	562	100	35
8	2	3-CH ₃	4-NH ₂ ·maleate	f	EtOH	75	134-135.5	C ₁₃ H ₁₀ N ₂ O ₂	C, H, N	562	100	55
9	2	3-CH ₃	4-NHCOCH ₃ ·2H ₂ O	f	EtOH-BuOH-H ₂ O	63	206	C ₁₃ H ₁₀ N ₂ O ₂ ·2H ₂ O	C, H, N	>1000		
10	2	3-CH ₃	4-N(CH ₃) ₂	f	i-PrOH	77	182-185	C ₁₃ H ₁₀ N ₂ O	C, H, N	>1000	300	75
11	2	3-CH ₃ (CH ₃ COO ⁺)	4-NO ₂	A	EtOH	66	125.5-127	C ₁₃ H ₁₀ N ₂ O ₂	C, H, N	>1000	100	
12	2	3-CH ₃ (CH ₃ COO ⁺)	4-NH ₂	B	EtOAc	48	170-171.5	C ₁₃ H ₁₀ N ₂ O	C, H, N	562	100	
13	2	3-CH ₃	3-NO ₂	A	CHCl ₃ -hexane	7.4	166-168	C ₁₃ H ₁₀ N ₂ O ₂	C, H, N	>1000		
14	2	3-CH ₃	3-NH ₂	B	i-PrOH	53	177-179	C ₁₃ H ₁₀ N ₂ O	C, H, N			60
15	2	3-CH ₃	2-NH ₂	f	CHCl ₃ -hexane	32	147-149	C ₁₃ H ₁₀ N ₂ O	C, H, N	562	100	90
16	2	3-CH ₃	H	f	PhH-hexane	56	128.5-129.5	C ₁₃ H ₁₀ N ₂ O	C, H, N	562	30	50
17	2	3-CH ₃	4-Cl	D	PhH-hexane	71	145.5-146	C ₁₃ H ₉ N ₂ O	C, H, N	562	300	60
18	2	3-CH ₃	4-Cl	D	EtOH-H ₂ O	76	181-182	C ₁₃ H ₉ ClN ₂ O	C, H, Cl, N	750	300	
19	2	3-CH ₃	3,4,5-(OCH ₃) ₃	D	i-PrOH	92	157.5-159	C ₁₃ H ₁₀ N ₂ O ₂	C, H, N	562	1000	
20	2	4-CH ₃	4-NO ₂	A	AcOH	75	191-192	C ₁₃ H ₁₀ N ₂ O ₂	C, H, N			
21	2	4-CH ₃	4-NH ₂	B	95% EtOH	55	144-145	C ₁₃ H ₁₀ N ₂ O	C, H, N	300	300	40 (toxic)
22	2	5-CH ₃	4-NO ₂	A	EtOH	80	170-180	C ₁₃ H ₁₀ N ₂ O ₂	C, H, N	>1000		
23	2	5-CH ₃	4-NH ₂	B	CHCl ₃ -hexane	79	167-168	C ₁₃ H ₁₀ N ₂ O	C, H, N	562	100	35
24	2	6-CH ₃	4-NO ₂	A	EtOH	54	133-138	C ₁₃ H ₁₀ N ₂ O ₂	C, H, N	472		
25	2	6-CH ₃	4-NH ₂	B	DMF-H ₂ O	80	173-174	C ₁₃ H ₁₀ N ₂ O	C, H, N	178	30	75
26	2	4,5-(CH ₃) ₂	4-NO ₂	A	EtOAc	62	169.5-161	C ₁₃ H ₁₀ N ₂ O ₂	C, H, N	>1000		
27	2	4,5-(CH ₃) ₂	4-NH ₂	B	95% EtOH	82	184-185.5	C ₁₃ H ₁₀ N ₂ O	C, H, N	711	300	50
28	2	5,6-(CH ₃) ₂	4-NO ₂	A	95% EtOH	28	161.5-162.5	C ₁₃ H ₁₀ N ₂ O	C, H, N			
29	2	5,6-(CH ₃) ₂	4-NH ₂	B	EtOH	87	149.5-151	C ₁₃ H ₁₀ N ₂ O	C, H, N	750		50
30	2	5-Cl	4-NO ₂	A	EtOAc	70	233-234	C ₁₃ H ₉ ClN ₂ O	C, H, Cl, N	>1000		
31	2	5-Cl	4-NH ₂	f	EtOH	28	191.5-193	C ₁₃ H ₉ ClN ₂ O	C, H, Cl, N	>1000	1000	50
32	2	3-COOH	4-NO ₂	A	AcOH-H ₂ O	31	275-285	C ₁₃ H ₉ N ₂ O ₂	C, H, N	562		
33	2	3-COOH	4-NH ₂	B	H ₂ O	42	260-281 dec	C ₁₃ H ₉ N ₂ O ₂	C, H, N	56	100	
34	2	6-NHCOCH ₃ ·p-NH ₂	4-NO ₂	A	AcOH	81	203-204	C ₁₃ H ₁₀ N ₂ O ₂	C, H, N			
35	2	6-NHCOCH ₃ ·p-NH ₂	4-NH ₂	B	Me ₂ CO-H ₂ O	50	250-252	C ₁₃ H ₁₀ N ₂ O ₂	C, H, N			
36	2	OC ₂ H ₄ CH ₃	4-NO ₂	D	i-PrOH	44	139.5-140.5	C ₁₃ H ₁₀ N ₂ O ₂	C, H, N	>1000	300	

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37	3	H	4-NO ₂	A	EtOH-MeOH	98	190.5-192	C ₁₄ H ₁₁ N ₃ O ₂	C, H, N	422	100
38	3	H	4-NH ₂	B	MeOH	86	209.5-211	C ₁₄ H ₁₁ N ₃ O	C, H, N	602	100
39	3	H	4-NH ₂ , HCl	J	H ₂ O-i-PrOH	78	244.5-245.5	C ₁₄ H ₁₁ ClN ₃ O	C, H, Cl, N	902	80
40	3	2,6-(CH ₃) ₂	4-NO ₂	A	MeOH	75	205-206.5	C ₁₄ H ₁₁ N ₃ O ₂	C, H, N	>1000	80
41	3	2,6-(CH ₃) ₂	4-NO ₂	B	MeOH	98	172.5-173.5	C ₁₄ H ₁₁ N ₃ O ₂	C, H, N	502	80
42	4	H	4-NO ₂	A	MeOH	44	244-245	C ₁₄ H ₁₁ N ₃ O ₂	C, H, N	49	80
43	4	H	4-NH ₂	B	MeOH	85	258-257	C ₁₄ H ₁₁ N ₃ O	C, H, N	208	80
44	4	H	4-NH ₂ , 2HCl	C	MeOH	97	>350	C ₁₄ H ₁₁ ClN ₃ O	C, H, Cl, N	12	80
45	4	H	H ^a	A	H ₂ O	78	202-205	C ₁₄ H ₁₁ N ₃ O	C, H, N	18	80
46	4	H	4-CH ₃	A	EtOAc	36	178.5-181	C ₁₄ H ₁₁ N ₃ O	C, H, N	75	80
47	4	H	2,3,5,6-(CH ₃) ₄	J	MeOH-H ₂ O	84	285-312 dec	C ₁₄ H ₁₁ CH ₃ O	C, H, Cl, N	30	80
48	4	3-Me	4-NO ₂	A	Me ₂ CO	79	168-168	C ₁₄ H ₁₁ N ₃ O ₂	C, H, N	10	80
49	4	3-Me	4-NH ₂	B	EtOH	85	205-208.5	C ₁₄ H ₁₁ N ₃ O	C, H, N	10	80

^a Yields are based on material melting not less than 2° below highest mp obtained. ^b See ref 10. ^c Lethality and CNS activity were determined in mice. For method see R. B. Moffett, A. B. Hanze, and P. H. Seay, *J. Med. Chem.*, **7**, 174 (1964). Table I, footnotes a and b. The lowest dose showing definite CNS depression is given. A (—) indicates no depression was observed. In some cases mild stimulation was observed. A blank indicates this compound was not tested. ^d The procedure for producing ulceration ulcers in rats has been reported. ^e ED₅₀ is the oral dose necessary to reduce ulcer formation by 50% taking into account both the number and severity of the ulcers. A (—) indicates the compound was substantially inactive. A blank indicates the compound was not tested. ^f See ref 4. ^g Specific prep given in Experimental Section. ^h Prepared in these laboratories by Mr. James E. Stafford. ⁱ Contains a Me group on the amide N. ^j The p-nitrobenzoyl chloride soln was added to the 2-(methylamino)-3-picoline in CHCl₃ and pyridine below 50° and stirred at room temp for 2 hr. The crude product was chromatographed on SiO₂ and eluted with 5% MeOH in CH₂Cl₂. ^k Hydrogenation in n-BuOH. ^l The product of hydrogenation was used in the reaction mixt. It was cooled in vacuo, dissolved in 3 l. of H₂O, filtered hot from catalyst, and cooled. ^m Prep from 2,6-diaminopyridine and an excess of p-nitrobenzoyl chloride. ⁿ Hydrogenation in glacial AcOH with PtO, in place of Pd/C. The crystal and dried product was found by Karl Fischer anal. to contain 0.84% H₂O (equiv to 0.18 H₂O/mol). ^o E. Koenigs, G. Kinne, and W. Weiss, *Chem. Ber.*, **57**, 1172 (1924). ^p Too toxic to test.

2-amino-3-picolines, and the soln was stirred on a steam bath overnight. The mixt was dried with 50 ml of EtOH yielding 15.4 g (75%) of yellow cryst, mp 186-188°. A sample was recrystd from AcOH-H₂O, mp 186-187°.

2-(p-Aminobenzamido)-3-picoline (6) (Method B).—A mixt of 13.58 g (0.027 mole) of 6 and 150 ml of 70% aq AcOH was hydrogenated with 1 g of 30% Pd/C at 3.5 kg/cm² and 25°. After filt and evapn in vacuo at 50°, the product was dissolved in CHCl₃, washed (5% NaHCO₃, water), and again evapd. The amorphous solid crystd slowly from EtOH giving 11 g of light tan cryst contg EtOH, mp 170-174.5° (after sintering at 90°). This was sublimed at 0.002 mm in a bath up to 172° yielding 5.8 g (48.5%) of nearly white solid, mp 177-179°, contg no solvent. Principal spectra bands were: ir (Nujol mull) 3450, 3400, 3320, 3210 (NH); 1640 cm⁻¹ (amide CO) and numerous arom bands; uv (EtOH) 296 and 218 mμ; and nmr (DMP-d₄) δ 2.27 (s, 3, CH₃), 9.95 (broad s, 1, CONH), 5.7 (broad s, 2, NH₂), and between 6.0 and 8.4 (m's 7, arom H's).

Dihydrochloride (7) (Method C).—A soln of 20.0 g (0.088 mole) of 6 in 200 ml of MeOH was strongly acidified with methanolic HCl. The resulting cryst were collected, washed (i-PrOH), and dried, yielding 24.4 g (94%) of white solid, mp some dec at 240-250°, but not all melted at 340°.

Maleate (Salt) (8).—A soln of 20.0 g (0.088 mole) of 6 in 200 ml of MeOH was treated with a soln of 11.6 g (0.1 mole) of maleic acid in 50 ml of MeOH. On diln with 100 ml of i-PrOH, 22.6 g (75%) of yellow cryst was obtained, mp 132-134°. Recrystn from 125 ml of EtOH gave 17 g of yellow cryst, mp 134-135.5°.

4'-(2-Pyridylcarbamoyl)acetanilide (4).—A soln of 3.0 g (0.014 mole) of 2 in 10 ml of Ac₂O and 10 ml of AcOH was heated on a steam bath for 15 min, coned in vacuo, and dild with aq Na₂CO₃. The product was collected, washed (H₂O), and recrystd from DMF-H₂O, giving 2.9 g (81%) of solid, mp 203-204°.

4'-[(3-Methyl-2-pyridyl)carbamoyl]acetanilide·2H₂O (9).—A soln of 3 g (0.013 mole) of 6 in 75 ml of AcOH was refluxed for 6 hr, coned in vacuo, and dild with water. The product was collected, dried, and recrystd from a mixt of 10 ml of 95% EtOH and 5 ml of BuOH yielding 2.6 g (65%) of solid, mp 206°. This was found by Karl Fischer anal. to be the dihydrate. Calcd (C₁₄H₁₇N₃O₄·2H₂O): H₂O, 11.80; found H₂O, 11.74.

2-[p-(Dimethylamino)benzamido]-3-picoline (10).—A soln of 27.2 g (0.1 mole) of 6 in 200 ml of MeOH, 20 ml of AcOH, and 30 ml (0.37 mole) of 37% aq CH₃O was hydrogenated with 2 g of 30% Pd/C at 3.5 kg/cm² and 25°. The theor amt of H₂ was absorbed in 5 hr. After filt, the soln was evapd in vacuo, mixed with ice water, and adjusted to pH 8. The solid was collected, washed (aq NaHCO₃, H₂O), and dried, giving 23.4 g of nearly white solid, mp 178-182.5°. This was recrystd from 300 ml of i-PrOH yielding 19.6 g (77%) of light tan cryst, mp 179-184°. A second recrystn from i-PrOH gave cryst, mp 182-185°.

2-(o-Aminobenzamido)-3-picoline (15).—A soln of 33 g (0.3 mole) of 2-amino-3-picoline and 16.5 g (0.1 mole) of isoaltic anhydride in 100 ml of DMF was heated under reflux for 1 hr, poured into ice water contg 10 g of Na₂CO₃, and extd with Et₂O. The Et₂O soln was extd with dil HCl, and the ext was basified with Na₂CO₃. The resulting oil was extd with Et₂O, washed (satd NaCl), and dried (MgSO₄). Evapn left an oil which was chromatd on SiO₂ and eluted with 5% MeOH in CH₂Cl₂. The product solidified on trituration with Et₂O-hexane and was recrystd from CHCl₃-hexane, yielding 7.3 g (32%) of cryst, mp 147-149°.

2-Benzamido-3-picoline (16).—A soln of 21.6 g (0.2 mole) of 2-amino-3-picoline and 22.6 g (0.1 mole) of Bz₂O in 500 ml of ether was allowed to stand at room temp for 24 hr and evapd to dryness in vacuo. The residue was dissolved in CH₂Cl₂, washed (10% Na₂CO₃, H₂O, satd NaCl), and dried (MgSO₄). Evapn left a gum which crystd on trituration with Et₂O-hexane, and was recrystd first from aq EtOH then from PhH-hexane yielding 11.85 g (56%) of white solid, mp 128.5-130°.

3,3',4,4',5,5'-Hexamethoxy-N-(3-methyl-2-pyridyl)dibenzamide (77).—To a soln of 46.2 g (0.2 mole) of 3,4,5-trimethoxybenzoyl chloride in 100 ml of dry pyridine was added 10.8 g (0.1 mole) of 2-amino-3-picoline. After stirring on a steam bath for 2 hr, most of the pyridine was distd in vacuo and the residue was shaken with ice water. The product was collected, washed (H₂O, aq Na₂CO₃, H₂O), and dried, giving 43.1 g of solid, mp 151-156°. Recrystn from 520 ml of i-PrOH yielded 38.4 g (77%) of white cryst, mp 158-160.5°. The dibenzoyl structure was confirmed by nmr and mass spec: ir (Nujol mull) 1700, 1685 cm⁻¹ (C=O) and no NH/OH absorption; nmr (CDCl₃) δ 2.33

TABLE II

No.	Ar	X	Method of prep	Cryst solvent	Yield, %	Mp, °C	Empirical formula	Analysis, C, H, N	LD ₅₀ , mg/kg	CNS depression, mg/kg	Acute toxicity ED ₅₀ , mg/kg
50		4-NO ₂	A	EtOH	55	183-184	C ₁₄ H ₁₁ N ₂ O ₂	C, H, N	>1000	1000	
51		4-NH ₂	B	EtOH-H ₂ O	61	229-229	C ₁₄ H ₁₁ N ₂ O	C, H, N	>1000	300	
52		4-NO ₂	A	DMF	44	>380	C ₁₄ H ₁₁ N ₂ O ₂	C, H, N	562		
53		4-NH ₂	B	MeOEtOH	74	273.5 dec	C ₁₄ H ₁₁ N ₂ O	C, H, N	422		60
54		4-NO ₂	A	MeOEtOH	44	309-311	C ₁₄ H ₁₁ N ₂ O ₂	C, H, N	>1000		
55		4-NH ₂	B'	MeOEtOH-MeOH	10'	275-277	C ₁₄ H ₁₁ N ₂ O	C, H, N			
56		4-NH ₂ ·2HCl	f	MeOH	60'	>360	C ₁₄ H ₁₁ Cl ₂ N ₂ O	C, H, Cl, N	178	300	75
57		4-NO ₂	A	DMF	82	338-339	C ₁₄ H ₁₁ N ₂ O ₂	C, H, N	>1000		
58		4-NH ₂	B	DMF-MeOH	63	303-306 dec	C ₁₄ H ₁₁ N ₂ O	C, H, N	750	300	
59		4-NO ₂	A	DMF	85	273-273.5	C ₁₄ H ₁₁ N ₂ O ₂	C, H, N	562		
60		4-NH ₂	B	DMF-MeOH	82	298.5-299	C ₁₄ H ₁₁ N ₂ O	C, H, N	178		
61		4-NO ₂	A	MeOEtOH	60	255 dec	C ₁₄ H ₁₁ ClN ₂ O ₂	C, H, Cl, N	>1000	300	
62		4-NO ₂	A	DMF	85	>360	C ₁₄ H ₁₁ N ₂ O ₂	C, H, N	750	300	

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63		4-NH ₂	B ¹	EtOH	89	243-244.5	C ₁₀ H ₁₀ N ₄ O	C, H, N	>1000	300	50
64		2,3,4,5-(CH ₃) ₄	f	Xylene	52	187-189	C ₁₀ H ₁₀ N ₄ O	C, H, N	>1000	1000	
65		2,3,4,5-(CH ₃) ₄		MeOEtOH ⁺	6.3	280.5-283.5	C ₁₀ H ₁₀ N ₄ O	C, H, N	>1000	100	
66		4-NO ₂	A	DMF	67	346-348 dec	C ₁₀ H ₈ N ₄ O ₂	C, H, N, S	582		
67		4-NH ₂	B ¹	i-PrOH	48	235-237	C ₁₀ H ₁₀ N ₄ O ₂	C, H, N, S	750		
68		4-NO ₂	A	DMF-EtOOMe	82	294.5-296	C ₁₀ H ₈ N ₄ O ₂	C, H, N, S	>1000		
69		4-NH ₂	B ¹	EtOOMe	68	199.5-201.5	C ₁₀ H ₁₀ N ₄ O ₂	C, H, N, S	318		
70		4-NH ₂	f			234-241 ⁺	C ₁₀ H ₁₀ N ₄ O		422	100	40 ⁺
71		4-NO ₂	A ⁺	EtOH		161-162 ⁺	C ₁₀ H ₈ N ₄ O ₂	N	>1000	300	
72		4-NO ₂	B ¹	CHCl ₃ -benzene		155-156 ⁺	C ₁₀ H ₈ N ₄ O ₂	N	104	30	
73		4-NH ₂	f			122-124 ⁺	C ₁₀ H ₁₀ N ₄ O		750	100	
74		4-NH ₂	B ¹	Ph	91	116-118 ⁺	C ₁₀ H ₁₀ N ₄ O	C, H, N	1000	300	
81	Atropine sulfate						C ₁₇ H ₂₇ N ₃ O ₆		318	100	1.0
82	Sodium phenobarbital						C ₁₂ H ₁₁ N ₃ NaO ₃		200	100	50 ⁺
83	Chlorpromazine-HCl						C ₁₆ H ₁₄ ClN ₂ O		150	20	1.5 ⁺
84	Chlordiazepoxide-HCl						C ₁₅ H ₁₀ ClN ₂ O ₂		200	50	20 ⁺
85	Meprobamate						C ₁₂ H ₁₅ N ₃ O ₂		600	200	100

⁺ See footnote a, Table I. ⁺ See ref 10. ⁺ See footnote c, Table I. ⁺ See footnote d, Table I. ⁺ 2-Methoxyethanol. ⁺ See Experimental Section. ⁺ Cipena, et al., report mp 337°. ⁺ Shchigolov, et al., report mp 273-274° dec. ⁺ Various fractions from DMF-MeOH had dec points from 294 to 299° all had identical IR spectra. ⁺ Hydrogenated in 2-methoxyethanol; 3.5 days were required to complete reaction. ⁺ Part of the product was insol in the aq. AcOH react mixt and after filtn was extd from the catalyst with warm DMF. The rest was obtd by neutralisation of the filtrate. ⁺ Obtained from K and K Labs. Inc., Plainview, N. Y. ⁺ G. Lochemann and T. Lobenstein, *Chem. Ber.*, 75, 1911 (1942), report mp 137-138°. ⁺ This compd showed good after preventing activity but produced splenomegaly as a side effect. ⁺ H. Adams and L. M. Werbel, *J. Amer. Chem. Soc.*, 60, 3799 (1938), report mp 199-191.5°. ⁺ G. Lochemann and H. Kübler, *Chem. Ber.*, 80, 479 (1947), report mp 132-133°. ⁺ Too toxic to test. ⁺ A. Piotti, *Chem. Ber.*, 14, 1319 (1931). ⁺ Prep'd by Dr. R. S. Hai in these laboratories by method B in EtOH with PtO₂ catalyst. The lit. shows a wide discrepancy in reports of mp. See Shab, *J. Indian Inst. Sci.*, 7, 207 (1920); *Brit. J. Med.*, 14, 210. ⁺ Administered sc in this test.

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TABLE III

No.	R	X	Crystall solvent	Yield, %	Mp, °C	Empirical formula	Analysis ^a
75	CH ₃	4-CH ₃	n-BuOH	24	225-227	C ₁₂ H ₁₂ N ₂ O ₂	C, H, N
76	CH ₃	4-Cl	n-BuOH	27	218-220	C ₁₀ H ₁₀ Cl ₂ N ₂ O ₂	C, H, Cl, N
77	CH ₃	3,4,5-(OCH ₃) ₃	i-PrOH	77	158-160.5	C ₁₆ H ₁₈ N ₂ O ₆	C, H, N
78	CH ₃	4-N(CH ₃) ₂	TMU ^b	17	213-235	C ₁₆ H ₁₈ N ₄ O ₂	C, H, N
79	OCH ₂ CH ₃	4-NO ₂	i-PrOH	48	172-173.5	C ₁₂ H ₁₀ N ₂ O ₄	C, H, N
80	NO ₂	4-NO ₂	MeOEtOH ^c	18	213.5-214.5	C ₁₁ H ₁₀ N ₂ O ₄	C, H, N

^a See footnote a, Table I. All compds in Table III were prepd essentially by method A except as noted. ^b See ref 10. ^c The crude product was dissolved in CHCl₃, washed (aq Na₂CO₃, H₂O), and dried (MgSO₄). After filtration and evapn the product was crystd from the solvent indicated. ^d Prep specifically described in the Experimental Section. ^e p-Dimethylaminobenzoyl chloride·HCl was washed (NaOH, H₂O), dried (Na₂SO₄), and evapd. Crystn from 2-methoxyethanol or DMF gave product contg solvent, difficult to remove. Recrystn from tetramethylurea (TMU) gave solvent-free material. ^f The crude product from a 0.36-mole run was refluxed for 2.5 hr with 8 l of MeOH, 670 ml of H₂O, and 300 ml of concd HCl. A solid insol in the mixt was recrystd from 2-methoxyethanol giving white solid which was surprisingly found to be the di-p-nitrobenzoyl compd: *ir* (Nujol mull) 1720, 1705 (C=O), 1600, 1525, 1335, 1235, 835 cm⁻¹ etc., no NH/OH peaks; nmr (DMSO-d₆) only aromatic H's between δ 4.3 and 5.6 integrating for 3 on pyridine and 8 on benzene.

(a, CH₃ on pyridine ring) 7.78, 7.87 (two s's, 18, OCH₃), and between 7.05 and 8.50 (m's 7, aromatic H); mass spec, mol wt, calcd 496.5, found 498.

2-(3,4,5-Trimethoxybenzamido)-3-picoline (19) (Method D).—A soln of 20 g (0.04 mole) of 77 in 500 ml of EtOH, 450 ml of water, and 50 ml of concd HCl was refluxed for 1 hr and evapd in *vacuo* at 50° to a small vol. The residue was dild with ice water, strongly acidified with HCl, and extd twice with Et₂O. The aq soln was adjusted to pH 8 yielding 11.2 g (92%) of white solid, mp 157-158°. This was recrystd from i-PrOH giving 10 g of white cryst, mp 157.5-159°.

2-(p-Aminobenzamido)-5-chloropyridine (31).—A mixt of 50 g (0.18 mole) of 30 and 300 ml of MeOH was hydrogenated with 5 g of 5% Pt/C and sulfid¹¹ at 41 kg/cm² and 145°. The product was dissolved in CH₂Cl₂, filt'd from catalyst, and evapd in *vacuo*. The residue was cryst from EtOH yielding 12.3 g (28%) of tan cryst, mp 191.5-193°.

Attempt to carry out this reduction with Pd/C (method B) yielded only the Cl-free product 2.

2-(p-Aminobenzamido)pyridine·HCl (39).—A soln of 24.0 g (0.113 mole) of 37 in 225 ml of 4% aq HCl was dild with 1.8 l of i-PrOH yielding 25.6 g (78%) of fluffy white cryst of monohydrochloride, mp 244.5-245.5°.

2,3,5,6-Tetramethyl-N-4-pyridylbenzamide·HCl (47).—A soln of 14.3 g (0.04 mole) of 2,3,5,6-tetramethylbenzoic acid and 65 ml of SOCl₂ in 100 ml of PhH was refluxed for 3.5 hr and evapd to dryness in *vacuo*. This crude acid chloride in 50 ml of PhH was added to a soln (9.4 g, 0.1 mole) of 4-aminopyridine and 13.8 ml (0.1 mole) of Et₃N in 100 ml of THF. After refluxing 1.5 hr and standing overnight, water, PhH, and a little AcOH were added, and the mixt was concd in *vacuo*. The solid was collected, dried, dissolved in 150 ml of MeOH and 75 ml of water at the boiling point, acidified with a few drops of aq HCl, filt'd, and concd to 125 ml. On cooling 14.9 g (64%) of white solid was obtained, mp 285-312° dec.

2-(p-Aminobenzamido)benzimidazole (55).—This was prepd from 14.2 g (0.05 mole) of 53 by method B, but much of the product sep'd as a solid in the reaction mixt. This was filt'd and the soln neutralized. The resulting free base was recrystd from 50% methoxyethanol in MeOH giving 1.6 g (10%) of cryst, mp 275-277°.

(11) From Engelhard Industries, Inc., Newark, N. J.

Dihydrochloride (56).—The solid (55) was extd from the catalyst with DMF and the soln was evapd in *vacuo*. The resulting solid free base in MeOH was converted to the hydrochloride with an excess of ethanolic HCl yielding 9.75 g (60%) of tan solid which did not melt up to 360°. The total yield (base and hydrochloride) was 70%.

N-(2,3,5,6-Tetramethylbenzoyl)adenine (64) and 7- and/or 9-(2,3,5,6-Tetramethylbenzoyl)adenine (65).—A soln of 14.3 g (0.09 mole) of 2,3,5,6-tetramethylbenzoic acid in 50 ml of SOCl₂ and 50 ml of PhH was refluxed for 2 hr and evapd in *vacuo*. The crude acid chloride was dissolved in 50 ml of PhH and added to a suspension of 10.8 g of adenine in 80 ml of dry pyridine. After stirring on a steam bath for 3 hr, the soln was evapd in *vacuo*. The residual solid was pulverized and stirred on a steam bath for 0.5 hr with 200 ml of 5% NaHCO₃. The crude solid was collected, washed (H₂O), dried, and crystd from 600 ml of xylene giving 16.9 g of tan solid. This was boiled with 1 l of MeOH and filt'd from 2.1 g of solid (see below). The MeOH soln was evapd to dryness, and the residue was recrystd from 600 ml of xylene yielding 12.2 g (52%) of 64: mp 187-189°; *tlc* (on SiO₂, developed with 5% MeOH in CHCl₃) showed only one spot; *ir* (Nujol mull) 3360, 3310, 3240, 3150 (NH), 1710, 1685 (C=O, C=N), and 1290 cm⁻¹ (CN or C=C); nmr (CDCl₃) δ 2.10 (s, 6, CH₃), 2.27 (s, 6, CH₃), 6.33 (exchangeable with D₂O, broad s, 2, NH's), 7.12, 7.91, 8.50 (s's, 1 each, arom H's).

The above solid, insol in MeOH, was recrystd from 40 ml of 2-methoxyethanol giving 1.5 g of 65: mp 260.5-263.5°; *tlc* (on SiO₂, developed with 5% MeOH in CHCl₃) showed only one spot; *ir* 3480, 3430, 3300, 3280, 3230, 3140, 3130 (NH), 1750, 1720 (C=O), 1640 cm⁻¹ (C=O, C=N or C=C); nmr (DMF-d₇) δ 2.11 (d, 6, *J* = 5 Hz, CH₃), 2.28 (d, 6, *J* = 2 Hz, CH₃), 7.22, 8.10, and 8.41 (d's, 3 arom H's), 7.47 and 7.70 (broad s's, 2, exchangeable with D₂O, NH). Mass spec mol wt, calcd 295.35; found 295.

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Solid-Phase Synthesis of Libraries Generated from a 4-Phenyl-2-carboxy-piperazine Scaffold†

Jonas W. Nilsson,¹ Fredrik Thorstensson,¹ Ingemar Kvarnström,¹ Tudor Oprca,^{5,6} Bertil Samuelsson,^{3,4,†} and Ingemar Nilsson^{*,6}

Department of Chemistry, Linköping University, S-581 83 Linköping, Sweden, and Department of Medicinal Chemistry, AstraZeneca R&D Mölndal, S-43183 Mölndal, Sweden

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Strategies for finding novel structures of therapeutical interest are discussed. The rationale for the selection of the two scaffolds *N*4-(*m*-aminophenyl)-piperazine-2-carboxylic acid **E** and *N*4-(*o*-aminophenyl)-piperazine-2-carboxylic acid **F** is described. The synthesis of the appropriate precursors to scaffold **E** and **F** and their use in solid-phase chemistry are described. A 160-member library was produced combining these novel piperazine scaffolds with eight sulfonyl chlorides/acid chlorides and 10 amines. The compound library prepared was analyzed using LC-MS, showing the expected base peak in all wells at an average purity of 82%.

Introduction

The pharmaceutical industry is under continuous pressure to prepare and evaluate novel structural motifs to meet an ever-increasing number of new protein targets being discovered and evaluated for therapeutic use. The chemistry goal is to find structural motifs with broad applications for drug targets in different therapeutic areas. 1,4-Benzodiazepines belong to a well-known class of bioactive compounds that are anxiolytics and antihypnotics,¹ cholecystokinin receptor A and B antagonists,² and opioid receptor ligands,³ that show several other activities,⁴ and which have been successfully developed into marketed drugs. Not surprisingly, the synthesis of 1,4-benzodiazepines libraries was explored very early using combinatorial chemistry.⁵ More recently, biphenyl structural motifs have been incorporated into molecules showing broad biological activity including antihypertensive (antagonists of the angiotensin II receptor),⁶ antihyperglycemic,⁷ endothelin-A (ET_A) receptor antagonists,⁸ and cyclooxygenase-2 (COX-2) inhibitor⁹ activity.

The advent of combinatorial chemistry has revolutionized drug discovery making large, diverse and drug-like compound libraries available for screening against new protein targets in a rapid and cost efficient manner. Numerous recent publications have highlighted this development, describing the synthesis of compound libraries based on novel scaffolds.¹⁰ However, the initial efforts in combinatorial chemistry mainly focused on large and diverse libraries primarily

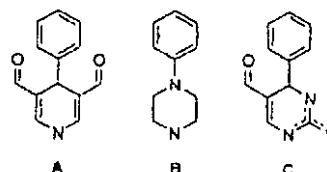


Figure 1. Structural motifs frequently observed in biologically active compounds.

driven by chemistry has so far had limited success. Notably, the current trend is directed toward the design and synthesis of smaller and target based iterative libraries.

Notwithstanding the current focus based on the latter approach, there is a need for exploring ideas for general lead generation libraries, thus discovering hitherto unexplored structural classes with useful biological activities. As part of an effort to develop novel drug-like motifs amenable for combinatorial diversification, we have searched for biologically relevant templates in the MDDR¹¹ (MACCS-II Drug Data Report) database, by defining small sets of structural motifs attributed to broad biological activities. Three such motifs frequently observed in biologically active compounds were annotated (Figure 1). Template A was found in 673 dihydropyridines, template B in 2271 phenyl-piperazines, and template C in 140 other compounds. Drugs or drug candidates incorporating these three templates show a very wide range of pharmacological activities. These are presented in Table 1, and summarized as follows: 15 dihydropyridines are marketed drugs, while 11 are in phase II or III, covering a total of five therapeutic areas; 16 phenyl-piperazines are marketed drugs, while 23 phenyl-piperazines are currently in phase II or III, covering 18 therapeutic indications. In total, there are 65 structures in phase II (or higher), covering 23 therapeutic areas. Ten structures containing the template C are in the preclinical evaluation stage, covering three therapeutic areas. The absence of phase I (or higher) status for compounds having the template C is indicative of the

† This is the first publication in a series of scaffolds generated around this theme.

* To whom correspondence should be addressed. Tel: 46-31-7761161. Fax: 46-31-7763839. E-mail: Ingemar.Nilsson@astrazeneca.com.

¹ Linköping University.

² AstraZeneca R&D Mölndal.

³ Present address: EST Lead Informatics, AstraZeneca R&D Mölndal, S-43183 Mölndal, Sweden.

⁴ Present address: Medivir AB, Lunastigen 7, S-141 44 Huddinge, Sweden.

⁵ Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm, Sweden.

Table 1. Structure-Activity Profile for the Three Templates Discussed in Figure 1^a

class	example	main therapeutic activity	nr. str. ^b	status
template A (dihydropyridines)	Felodipine	Ca-channel blocker (antihypertensive)	14	launched
	Nimodipine	neuronal injury inhibitor	1	launched
	Furildipine	Ca-channel blocker (antihypertensive)	3	phase III
	Cronidipine	Ca-channel blocker (antihypertensive)	4	phase II
	Modipafant	PAF antagonist	2	phase II
	YM-15430-1	β -1 adrenergic blocker	1	phase II
template B (phenyl piperazines)	Niguldipine	anticoagulant enhancer	1	phase II
	Norflaxacin	antibacterial-quinolone	6	launched
	Naflopidil	α -1 adrenergic blocker (prostate)	2	launched
	Trazodone	antidepressant	2	launched
	Nefazodone	5 HT _{2A} antagonist (antidepressant)	1	launched
	Vesnarinone	phosphodiesterase III inhibitor (cardiotonic)	1	launched
	Levodropropizine	antitussive	1	launched
	Itraconazole	antifungal	1	launched
	Dapiprazole	α -2 adrenergic agonist (glaucoma)	1	launched
	Oxyptertine	anxiolytic	1	launched
	Arpiprazole	antipsychotic	1	phase III
	Rifalazil	antimycobacterial	1	phase III
	Fananserine	antipsychotic	4	phase II
	Upidosin	α -1 adrenergic blocker (prostate)	3	phase II
	Flivanserin	antidepressant	3	phase II
	Ensacutin	cognition disorders	2	phase II
	Saperconazole	antifungal	2	phase II
	Ro-23-9424	antibacterial-quinolone	1	phase II
	R-68151	lipooxygenase inhibitor (antipsoriatic)	1	phase II
	R-61837	antiviral	1	phase II
	UP-5222-04	analgesic	1	phase II
	AZ01279880	gp11b/IIIa antagonist (antiaggregant)	1	phase II
	TAK-044	endothelin antagonist (renal failure)	1	phase II
	CGP-43371	hypolipidemic	1	phase II
template C	SQ-32321	Ca-channel blocker (antihypertensive)	5	preclinical
	SNAP-6145-(+)	α -1 adrenergic blocker (prostate)	4	preclinical
	MDDR_266261	antiinflammatory	1	preclinical

^a Based on the 3084 compounds that contain these scaffolds retrieved from the February 2000 version of the MDDR database. Compounds in phase II (or higher) are shown for templates A and B, but only compounds in preclinical testing are disclosed for template C. ^b "nr. str." is the number of structures in each category. A representative for each class is given in the "example" column. See text for details.

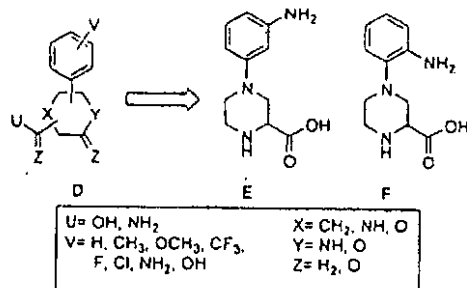


Figure 2. Generic structure and the two 4-phenyl-2-carboxy-piperazine scaffolds used.

novelty of this particular class of structures for pharmaceutical applications. All three templates have been shown to block Ca-channels.

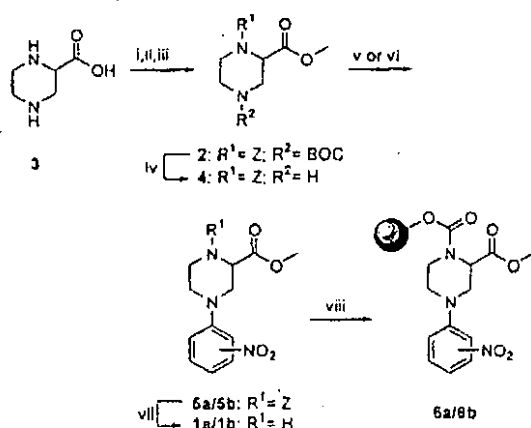
On the basis of these findings, we have therefore reasoned that derivatives of the generic structure D (Figure 2) would be of general interest due to their structural similarity to the bioactive templates shown in Figure 1. The goal is to explore spatially defined structures having ring heteroatoms and appropriately located functional groups that allow for diversification in well-defined directions. This will immediately translate into a good understanding of the pharmacophore, and the interactions with the biological target once activity is obtained. Consequently, we have undertaken

the synthesis of several scaffolds derived from the general template D, and subsequently developed a synthesis strategy for production of libraries around these scaffolds. In this paper, we report on the synthesis of two of the selected scaffolds (E and F) and their exploration using solid-phase chemistry. The selection of scaffolds E and F was made on the basis of synthetic feasibility and provides three points of diversification and furthermore provides appealing prospects for libraries with different directional vectors. Gratifyingly, this structural motif appears in compounds possessing antibacterial¹² and oxytocin receptor antagonist¹³ activities but most importantly remains an unexplored framework for therapeutic exploration.

Results and Discussion

These novel scaffolds (E and F) provide a fairly rigid framework, with well-defined directional vectors of the functional groups where diversity can be introduced, thus providing straightforward information for structure-activity relationships. The strategy adopted for the test library was to prepare the regioisomers 1a/1b (Scheme 1) in solution and subsequently attach and derivatize these scaffolds on solid support. The immobilized scaffolds were subsequently reacted with 8 different electrophiles (acid chlorides and sulfonyl chlorides) and 10 different amines (primary and secondary) to produce the 160 members in this library.

Scheme 1. Synthesis of Scaffolds.



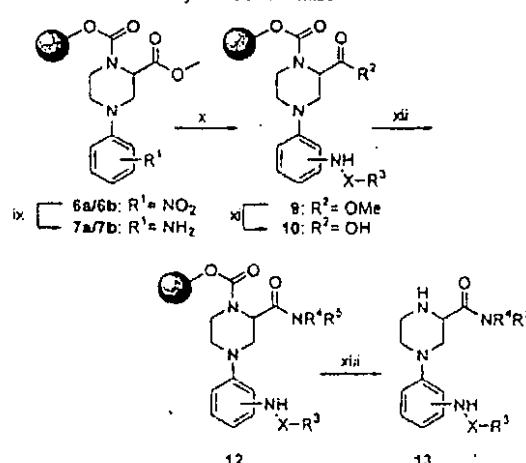
* Reagents and conditions: (i) BOC-ON, pH 11, dioxane/H₂O 1:1; (ii) Z-Cl, pH 9.5; (iii) CH₃I, NaHCO₃ (aq), adogen 464, DCM; (iv) HCl, dioxane; (v) 2-fluoronitrobenzene, TEA, 60 °C to give 5a (*o*-NO₂); (vi) Pd(OAc)₂, BINAP, CsCO₃, 3-bromonitrobenzene, toluene, 100 °C to give 5b (*m*-NO₂); (vii) TFMSA, anisole, DCM; (viii) *p*-nitrophenyl carbonate Wang resin,¹⁶ DIPEA, DMF, 80 °C.

Synthesis and Immobilizing of the Scaffolds. For the synthesis of the scaffold 2, piperazine-2-carboxylic acid 3 (Scheme 1) was selectively monoprotected with 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetone (BOC-ON) in dioxane/water at pH 11 adjusted with aqueous sodium hydroxide. The pH was then lowered with hydrochloric acid to 9.5, and benzyl chloroformate (Z-Cl) was added. The diprotected piperazine-2-carboxylic acid was then esterified using iodomethane and aqueous sodium bicarbonate under phase transfer conditions in DCM (dichloromethane) to yield the orthogonally protected piperazine-2-carboxylic acid 2 in 78% yield.¹⁴ The *tert*-butoxycarbonyl group was cleaved to yield 4 by treating 2 with hydrochloric acid in dioxane. Reacting Z-protected piperazine 4 with *o*-fluoro-nitrobenzene for 5 days gave 5a in 95% yield. However, direct aromatic substitution using the corresponding *m*-fluoro-nitrobenzene failed as expected due to the less activating effect of *m*-nitro substituent. Instead 5b was synthesized in 78% yield using palladium acetate, BINAP ((*R*)-(+)-2,2'-bis(diphenylphosphino)-1,1'-binaphthyl), *m*-bromo-nitrobenzene, and cesium carbonate in toluene at 100 °C.¹⁵ Deprotection of 5a/5b using trifluoromethanesulfonic acid (TFMSA) in DCM gave the free amines 1a and 1b in 92% and 85% yields correspondingly.

The scaffolds 1a/1b were immobilized onto the *p*-nitrophenyl carbonate activated Wang resin¹⁶ using DIPEA (diisopropylethylamine) in DMF (dimethylformamide). The loading (81% for 6a and 61% for 6b) was surprising considering the poor nucleophilicity of the N1 of piperazine-2-carboxylates.¹⁷ Longer reaction times did not improve loadings.

Solid-Phase Reactions. There are several methods reported for reduction of nitro compounds on solid support,^{18–20} and CrCl₂ (chromium(II)chloride)¹⁹ in DMF and SnCl₄·H₂O (tin(IV)chloride monohydrate)¹⁸ in DMF were examined. Very slow reaction was observed using the former.²¹ Premature cleavage of the Wang-linker has been reported using SnCl₄·

Scheme 2. Chemistry on Solid Phase.



* Reagents and conditions: (ix) SnCl₄·H₂O, DMF; (x) R¹XCl, X = SO₂ or CO (8), pyridine, DCM; (xi) TMSOK, THF; (xii) NHR²R³ (11), PyBOP, NMM, DMF; (xiii) TFA, 30% in DCM.

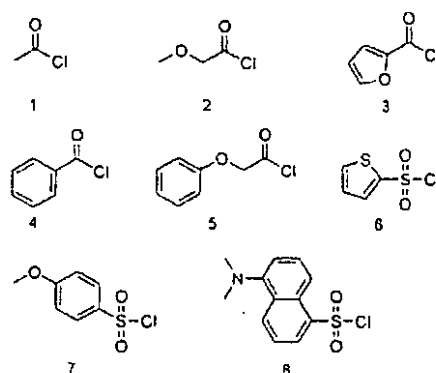


Figure 3. Diversity reagents 8 (1–8).

H₂O due to formation of hydrogen chloride and/or Sn(IV) Lewis acid capabilities.^{22,18} However, reduction of 6a/6b using SnCl₄·H₂O in DMF for 18 h at room temperature produced a clean reduction to the anilines 7a/7b (Scheme 2) with no resin cleavage observed.²² Subsequently 7a/7b were coupled in DCM/pyridine with the acid chlorides and sulfonyl chlorides in chemset 8 (Figure 3). The esters 9 were hydrolyzed to the corresponding carboxylic acids using potassium trimethylsilanoate in THF (tetrahydrofuran). The free acids 10 were then coupled with the amines in chemset 11 (Figure 4) using pyBOP (benzotriazol-1-yl-oxytripyrrolidino-phosphonium hexafluorophosphate) and *N*-methylmorpholine (NMM) in DMF to yield the amides 12. The resin bound products were cleaved from the resin with 40% trifluoroacetic acid (TFA) in DCM to provide the TFA salts 13 of the 160-member library.

Quality Assessment and Purification. The library was analyzed for every compound using HPLC/UV/ESI-MS. All wells showed a base peak consistent with the expected product (MS). The initial analysis showed a purity of above 80% for 126 of the 160 members based on UV light absorption.²⁴ To further raise the quality of the library, 39

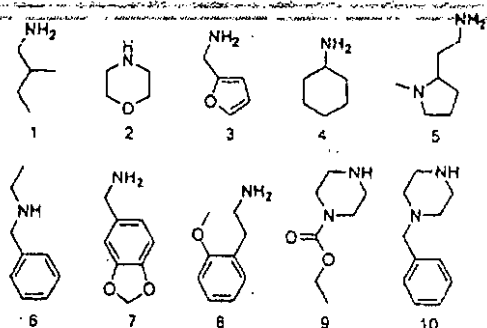


Figure 4. Diversity reagents 11(1-10).

Table 2. Amount of *p*-Hydroxy Benzylated Product in Different Sets/Subsets

set/substrate	13-(Bn-OH) _n ^a
13a	7.9%
13a(1-8,3)	25.0%
13a(1-8,7)	13.1%
13b	19.6%
13b(1-8,8)	43.6%

^a The average percentage of sets/subsets of 13 converted to the impurity in which electrophilic substitution with *p*-hydroxy benzyl cation has taken place.

members were subjected to preparative HPLC purification using mass-triggered fraction collection. After purification, a second more thorough analysis²⁵ of the entire library showed that only 96 of 160 members had purity above 80%. However, 134 of 160 of the compounds showed purity above 70%, and in no case the purity was less than 40%. The library showed an average purity of 82%. Notable is that almost all of the impurities could be identified by their mass.

The most prominent impurities (10% out of 18%) could be assigned to molecules with a mass 106 or 212 more than the expected mass. A plausible explanation for this is that during the cleavage of 12, some of the Wang-linker is liberated as *p*-hydroxy benzyl cation, which could add to the members of 13, by electrophilic substitution. In accordance with that 13b, which is more prone to electrophilic aromatic substitution than 13a, also shows a higher amount of this impurity. Some impurity trends depending on the reacted amines (chemset 11) are also evident. The products resulting from the more activated aromatic amines 11{3}, 11{7}, and 11{8} (Figure 4) show impurities above average (Table 2).

The other identified impurities (4% out of 18%) consist of *p*-hydroxy aniline, acylated by chemset 8 (Figure 3). These correspond to the reduced version of *p*-nitrophenyl carbonate Wang-resin that has been acylated during the conversion of 7a/7b to 9, and then released from the resin at the cleavage step (12 to 13). The remaining impurities (4% out of 18%) were not characterized.

Treating the resins 6a/6b with a good nucleophile after the immobilization and before the reduction step (6a/6b to 7a/7b) could eliminate the *p*-hydroxy aniline impurity by minimizing the unreacted *p*-nitrophenyl carbonate Wang-resin. Furthermore, the cleavage step (12 to 13) could include some cation scavenger to minimize adduct to the target molecules.

Table 3. Purity and Yields for a Selection of the Library

compd	purity (%)	yield (%) ^a	pur. ^b	compd	purity (%)	yield (%) ^a	pur. ^b
13a(1,2)	87	58		13a(7,10)	85	74	
13a(1,5)	92	63		13a(8,10)	79	63	
13a(2,3)	71	18		13b(1,2)	64	45	
13a(3,1)	91	67		13b(2,2)	77	49	
13a(3,4)	91	70		13b(3,2)	69	41	
13a(3,6)	100	38	yes	13b(4,5)	84	62	
13a(3,10)	87	75		13b(5,9)	75	58	
13a(4,5)	97	63		13b(6,1)	72	46	
13a(4,10)	93	73		13b(6,8)	85	11	yes
13a(5,3)	70	30		13b(7,9)	58	64	
13a(5,7)	84	51		13b(8,5)	78	60	
13a(6,2)	94	80		13b(8,8)	88	19	yes
13a(7,5)	94	84		13b(8,10)	76	33	

^a Yield based on NMR integrals with hexamethyldisiloxane as standard. ^b Indicates whether the compound was purified on preparative HPLC or not.

A selection of the library was subjected to yield determination using NMR. This selection of 20 members was made on basis of evenly distributed compounds from a PCA analysis including 60 chemical descriptors.²⁶ The results from the NMR analysis are summarized in Table 3.

Library Profiling. Virtual and/or existing compound collections (10²–10⁶ compounds) are screened for their content in druglike^{27,28} and permeable²⁹ compounds. Two druglike scoring schemes, one based on Daylight fingerprints³⁰ and one based on estimated physicochemical properties,³¹ have been implemented in-house.³² Briefly, a druglike molecule is assigned a value closer to 1 (i.e., it is classified as belonging to the MDDR¹¹ database), whereas a nondruglike compound is assigned a value closer to 0 (i.e., it is classified as belonging to the ACD³³ database). A value of 0.5 in the druglike scores denotes an equal probability to be classified as "drug" and "nondrug".

Lipinski and co-workers²⁹ suggested, after studying a subset of 2245 drugs from the World Drug Index, that poor passive permeability is more likely when a chemical structure exhibits more than 5 hydrogen bond donors (HDO, expressed as the sum of O-Hs and N-Hs), when the molecular weight (MW) is over 500, when the calculated logarithm of the octanol/water partition coefficient³⁴ (CLOGP) is over 5, and when there are more than 10 H-bond acceptors (HAC, expressed as the sum of Ns and Os). Any pairwise combination of the following conditions: MW > 500, CLOGP > 5, HDO > 5, and HAC > 10, may result in compounds with poor permeability. We recently suggested³⁵ that leadlike libraries should include small, polar compounds with MW below 350 and CLOGP below 3.0.

Within this framework, we have analyzed the contents of the 4-phenyl-2-carboxy-piperazine library to evaluate its contents in terms of druglike and leadlike compounds, as follows: This library was deemed to contain only druglike compounds, since all of them have DFPS ≥ 0.7 and PPFS ≥ 0.7, where DFPS and PPFS are the Daylight-FingerPrint-Score and the Property-and-Pharmacophore-Feature-Score, that evaluate the druglike quality of a compound.³² All the compounds in the 4-phenyl-2-carboxy-piperazine library have a probability higher than 0.7 to be assigned to the MDDR database, as opposed to the ACD database. Furthermore, 152

out of 160 compounds (95%) are within the "rule-of-5" cutoff, i.e., they do not violate more than one of the "rule of 5" criteria. Also, 118 compounds (73.75%) do not violate any of the "rule of 5" criteria, whereas 34 (21.25%) violate one of the rules. In conclusion, the 4-phenyl-2-carboxy-piperazine library contains at least 95% druglike structures that do not violate the "rule of 5", therefore are expected to yield a good percentage of good-permeability compounds.

We note that only 36 compounds (22.5%) satisfied the leadlike criteria (CLOGP ≤ 4.0 , and MW ≤ 400), in addition to being druglike. This is due to the inherent nature of this library, expected to be druglike, but not leadlike according to the previous definition,³⁵ for the following reason: The scaffolds depicted in Figure 2 have low MW (205 Da) and CLOGP (-0.04), allowing for diverse alterations in the substitution pattern at the disconnection moieties, i.e., at the amine and at the keto groups in Figure 2. Therefore, large variations in MW and CLOGP are permitted in the final compounds. These alterations can furthermore be chosen to meet the "rule-of-5" and druglike criteria, implying that this library is both "druglike" and "leadlike" in the same time.

Conclusions

This paper demonstrates one approach to enrich a compound collection to be used in biological screening in the early lead discovery phase. The focus of this test library is not directed toward a given drug target but rather toward the discovery of novel fairly rigid frameworks with diversity functions that cover a pre-defined structural space. We have also emphasized the concept of leadlike and druglike molecules (e.g., within the constraints imposed by the "rule of 5",²⁹ but less stringent with respect to "leadlike"³⁵), to provide good starting points for lead exploration once a compound is confirmed as a hit in biological screens. A screening library composed of 160 spatially separated compounds³⁶ has been generated using two regioisomers of the scaffold diversified at two positions with eight electrophiles and 10 amines. The methodology is well adapted for creating larger libraries as well as for using other sets of electrophiles and nucleophiles. The further exploration of the scaffolds E and F and other modifications of the general scaffold D are ongoing.

Experimental Section

General. Toluene was distilled over calcium hydride prior to use. TLC analysis was performed on Merck precoated 60 F₂₅₄ plates. Column chromatography was performed using silica gel 60 (0.040–0.063 mm, Merck). Organic phases were dried over magnesium sulfate monohydrate. Concentrations were performed by rotary evaporation. NMR spectra were recorded on a Bruker AC-F 250 instrument using chloroform-*d*₃ as solvent and tetramethylsilane as an internal standard. HPLC/MS analyses of all samples were performed on a VG Fisons ESI Platform 2 (MassLynx) equipped with positive (ES+) and negative (ES-) electrospray detection and with a photodiode array detector. Samples (~1 mM, 5 μ L) were injected on a reversed-phase column (Waters Xterra MS C8 2.5 μ m, 4.6 $\times$ 50 mm), with a linear gradient of 0.1 M NH₄OAc/CH₃CN going from 95/5 to 0/100 for 4 min with a flow

rate of 1.5 mL/min. Mass spectra were collected from 100 to 800 au for both ES+ and ES-, and UV spectra were recorded between 220 and 350 nm. Quantifications were based on the total absorption in the range 220–250 nm. Purification was performed on a semipreparative HPLC system with mass trace triggered fraction collection (Micro-mass, model LCZ 2000, Fraction Lynx). Samples were separated on a Waters Xterra C8 5 μ m, 20 $\times$ 100 mm column with 0.1 M NH₄OAc/CH₃CN through a linear generic gradients going from 95/5 to 0/100 for 8 min with a flow rate of 25 mL/min. High-resolution mass spectrometry (HRMS) was run on a Finnigan MAT900 equipped with an electrospray ion source. Samples were infused at 1 μ L/min.

Synthesis of Scaffolds: Racemic N1-(Benzyloxycarbonyl)-N4-(*tert*-butoxycarbonyl)-piperazine-2-carboxylic Acid, Methyl Ester¹⁴ (2). Piperazine-2-carboxylic acid dihydrochloride (20.5 g, 101 mmol) was dissolved in a 1:1 mixture of water and 1,4-dioxane (660 mL). Using 50% aqueous sodium hydroxide, the solution was adjusted to pH 11. BOC-ON (27.4 g, 111 mmol) dissolved in 1,4-dioxane (160 mL) was slowly added during a period of 1 h. The pH was kept constant at 11 during the addition. The solution was then stirred for three additional hours, during which the pH was again kept constant at 11. The resulting solution was then cooled with an ice bath, and the pH was lowered to pH 9.5 using aqueous hydrochloric acid (6 M). The solution was kept at pH 9.5 during a dropwise addition of benzyl chloroformate (21.4 mL, 152 mmol) over a period of 45 min. After approximately 4 h, the temperature was raised to ambient. The solution was left stirring overnight (16 h). The solution was washed with diethyl ether (4 $\times$ 200 mL), acidified to pH 1–2, and extracted with ethyl acetate (4 $\times$ 200 mL). The combined ethyl acetate extracts were dried, filtered, and concentrated. The resulting yellow oil was dissolved in saturated aqueous sodium bicarbonate (200 mL) and treated with a solution of 40 g of Adogen 464 and 15 mL of iodomethane in 200 mL of DCM. The mixture was stirred for 48 h and was then extracted with DCM (2 $\times$ 200 mL). The combined organic extracts were washed with water (200 mL), dried, and evaporated. The resulting oil was dissolved again in DCM (100 mL) and left to crystallize. Several recrystallizations from DCM gave 2 as pure crystals (29.2 g, 78%). Melting point and NMR was in accordance with literature.¹⁴ A more extensive NMR analysis is given. ¹H NMR (250.13 MHz) δ 7.42–7.21 (m, 5H), 5.24–5.06 (m, 2H), 4.81–4.33 (m, 2H), 4.15–3.81 (m, 2H), [3.74 and 3.69]³⁷ (s, 3H), 3.38–3.21 (m, 1H), 3.18–3.02 (dd, *J* = 3.6, 11.4 Hz, 1H), 2.97–2.79 (m, 1H), 1.55–1.38 (m, 9H). ¹³C NMR (62.90 MHz) δ 170.1, 155.8, 153.8, 136.2, 128.5, 128.2, 127.9, 80.3, [67.7 and 67.6]³⁷ [54.6 and 54.1]³⁷ 52.4, 44.6, 42.4, [41.2 and 40.9].³⁷

Racemic N1-(Benzyloxycarbonyl)-piperazine-2-carboxylic Acid, Methyl Ester (4). Compound 2 (20 g, 53 mmol) was dissolved in 1,4-dioxane (100 mL). To this solution was added dropwise 60 mL of concentrated hydrochloric acid (12 M). After 1 h the solvents were evaporated, and the residue was taken up in water and washed with toluene. The aqueous phase was extracted once with 500 mL of DCM with 5% triethylamine and then thrice with DCM. The

combined organic extracts were dried, filtered, and concentrated to afford **4** (14.7 g, 100%) as an oil. ^1H NMR (250.13 MHz) δ 7.38–7.22 (m, 5H), 5.20–5.05 (m, 2H), 4.80–4.60 (m, 1H), 3.90–3.82 (m, 1H), [3.77 and 3.72] ^{37} (s, 3H), 3.59–3.42 (m, 1H), 3.25–3.02 (m, 1H), 3.00–2.81 (m, 2H), 2.79–2.62 (m, 1H), 1.82 (bs, 1H). ^{13}C NMR (62.90 MHz) δ 171.2, [156.3 and 155.8], ^{37} 136.5, 128.9, 128.3, 128.1, 127.9, 127.6, [67.1 and 66.9], ^{37} [55.2 and 54.7], ^{37} 52.0, [47.1 and 47.0], ^{37} 45.1, [42.3 and 42.0]. ^{37}

Racemic N1-(Benzyloxycarbonyl)-N4-(*o*-nitrophenyl)-piperazine-2-carboxylic Acid, Methyl Ester (5a). Amine **4** (14.7 g, 53 mmol) was dissolved in triethylamine (14.7 mL, 106 mmol), *o*-nitro-fluorobenzene (11.3 mL, 106 mmol) was added, and the solution was heated at 60 °C for 5 days. Excess reagent was removed under reduced pressure, and the resulting yellow-red oil was purified with flash chromatography (toluene/ethyl acetate 9:1) to yield **5a** (20.1 g, 95%) as a yellow oil. ^1H NMR (250.13 MHz) δ 7.68–7.65 (m, 1H), 7.49–7.46 (m, 1H), 7.37–7.33 (m, 5H), 7.20–7.11 (m, 2H), 5.19–5.17 (m, 2H), 4.89–4.76 (m, 1H), 4.08–3.96 (m, 1H), [3.78 and 3.72] ^{37} (s, 3H), 3.75–3.66 (m, 1H), 3.61–3.42 (m, 1H), 3.19–3.09 (m, 2H), 2.95–2.81 (m, 1H). ^{13}C NMR (62.90 MHz) δ [170.3 and 170.1], ^{37} [156.4 and 155.7], ^{37} 145.3, 144.9, 136.3, 133.1, 128.6, 128.2, 127.9, 125.1, 123.9, 122.5, 67.7, [55.2 and 54.8], ^{37} 53.2, 52.6, 51.8, [41.9 and 41.6]. ^{37} HRMS m/z 422.1339 [(M + Na) $^+$ calcd for $\text{C}_{20}\text{H}_{21}\text{N}_3\text{O}_6\text{Na}^+$ 422.1328].

Racemic N1-(Benzyloxycarbonyl)-N4-(*m*-nitrophenyl)-piperazine-2-carboxylic Acid, Methyl Ester (5b). An oven-dried (130 °C, 6 h) Schlenktube was charged with anhydrous toluene (5 mL), amine **4** (104 mg, 374 μmol), 1-bromo-3-nitrobenzene (50.0 mg, 249 μmol), palladium(II)-acetate (0.56 mg, 2.5 μmol), and (*R*)-(+)-2,2'-Bis(diphenylphosphino)-1,1'-binaphthyl (BINAP) (2.21 mg, 3.74 μmol) under argon. After 30 min of complex formation, oven-dried (130 °C, 6 h) cesium carbonate (122 mg, 374 μmol) was added, and the tube was heated at 100 °C for 20 h. The solution was allowed to cool to room temperature, diluted with diethyl ether (20 mL), and washed twice with water. The organic phase was dried and concentrated. The reaction mixture was purified with flash column chromatography (toluene/ethyl acetate 9:1) to give **5b** as a yellow oil (71.2 mg, 178 μmol , 72% yield). ^1H NMR (250.13 MHz) δ 7.78–7.61 (m, 2H), 7.46–7.28 (m, 5H), 7.25–7.19 (m, 2H), 5.35–5.10 (m, 2H), 5.03–4.90 (m, 1H), 4.30–4.08 (m, 2H), [3.77 and 3.72] ^{37} (s, 3H), 3.62–3.31 (m, 2H), 3.15 (dd, J = 3.93, 12.55 Hz, 1H), 2.96–2.82 (m, 1H). ^{13}C NMR (62.90 MHz) 170.3, [155.7 and 155.2], ^{37} 151.4, 149.2, 136.2, 129.9, 128.6, 128.2, 128.0, 125.3, 122.6, 115.0, 110.7, [67.8 and 67.7], ^{37} 55.2, 54.8, 52.7, [50.9 and 50.6], ^{37} 47.8, [41.3 and 41.1]. ^{37} HRMS m/z 422.1340 [(M + Na) $^+$ calcd for $\text{C}_{20}\text{H}_{21}\text{N}_3\text{O}_6\text{Na}^+$ 422.1328].

Racemic N4-(*o*-Nitrophenyl)-piperazine-2-carboxylic Acid, Methyl Ester (1a). Anisole (22 mL, 201 mmol) was added to a solution of **5a** (20.1 g, 50 mmol) in 500 mL of DCM. A solution of TFMSA (44.4 mL, 502 mmol), in 200 mL of DCM, was added dropwise to the reaction mixture. After 10 min the reaction mixture was gently poured into a vigorously stirred aqueous solution of sodium dihydrogen

phosphate monohydrate (137 g, 1.01 mol). The resulting mixture was extracted thrice with diethyl ether/triethylamine (2:1, 3 $\times$ 100 mL), and the combined organic phases were dried and concentrated. Purification by flash column chromatography (ethanol/ethyl acetate 1:1) gave **1a** as a yellow oil (12.2 g, 46 mmol, 92% yield). ^1H NMR (250.13 MHz) δ 7.73 (dd, J = 1.5, 8.1 Hz, 1H), 7.54–7.47 (m, 1H), 7.22 (dd, J = 0.9, 7.7 Hz, 1H), 7.14–7.07 (m, 1H), 4.12 (bs, 1H), 3.73 (dd, J = 3.2, 7.7 Hz, 1H), 3.76 (s, 3H), 3.45 (dd, J = 3.1, 11.7 Hz, 1H), 3.23–2.87 (m, 5H). ^{13}C NMR (62.90 MHz) δ 171.8, 145.8, 144.3, 133.4, 125.6, 122.7, 121.9, 57.0, 54.0, 52.5, 52.2, 44.4. The pure free base **1a** was dissolved in methanol, treated with 1.5 equiv of concentrated hydrochloric acid, concentrated, and recrystallized from methanol to give **1a**·HCl as a yellow solid, suitable for storing: mp 198 °C (dec, darkens at 80 °C). ^1H NMR (250.13 MHz, D₂O, shifts relative to H₂O = 4.75 ppm) δ 7.80 (dd, J = 1.5, 8.4 Hz, 1H), 7.62–7.56 (m, 1H), 7.33 (d, J = 8.1 Hz, 1H), 7.26–7.20 (m, 1H), 4.44 (dd, J = 3.7, 7.7 Hz, 1H), 3.81 (s, 3H), 3.67–3.51 (m, 2H), 3.44–3.12 (m, 4H). ^{13}C NMR (62.90 MHz, D₂O, shifts relative to acetone = 30.9 ppm) δ 168.5, 144.8, 144.6, 135.4, 126.3, 125.9, 123.7, 56.1, 54.5, 51.5, 49.3, 43.2. HRMS m/z 266.1137 [(M + H) $^+$ calcd for $\text{C}_{12}\text{H}_{16}\text{N}_3\text{O}_4$ + 266.1141].

Racemic N4-(*m*-Nitrophenyl)-piperazine-2-carboxylic Acid, Methyl Ester (1b). Synthesis was performed using the same method as for *o*-nitro compound **1a**. Purification by flash column chromatography (ethanol/ethyl acetate 1:1) gave **1b** as a yellow oil (85% yield). ^1H NMR 7.62 (s, 1H), 7.55 (d, J = 1.46 Hz, 1H), 7.38–7.25 (m, 1H), 7.15 (dd, J = 1.8, 8.4 Hz, 1H), 3.78 (s, 3H), 3.67–3.52 (m, 2H), 3.40–3.15 (m, 3H), 3.13–2.87 (m, 2H), 2.62 (bs, 1H). ^{13}C NMR (62.90 MHz) 171.1, 151.1, 148.4, 129.0, 120.9, 113.1, 109.1, 56.1, 51.5, 50.0, 47.8, 43.1. HRMS m/z 266.1139 [(M + H) $^+$ calcd for $\text{C}_{12}\text{H}_{16}\text{N}_3\text{O}_4$ + 266.1141].

General Remarks for Solid-Phase Reactions. All solid-phase reactions were checked for completion by taking small samples of the resin, which was subsequently rinsed and cleaved with 30% TFA in DCM. The cleavage solution was evaporated and subjected to reversed-phase HPLC analysis. In those cases where the amount of solvent is not explicitly stated, the minimal amount to achieve good swelling was used. Glassware was silylated by treatment with a 10% solution of dichlorodimethylsilane in DCM followed by rinsing with DCM.

Racemic N1-(PS-Wang)-N4-(*o*/*m*-Nitrophenyl)-piperazine-2-carboxylic Acid, Methyl Esters (6a/6b). PS-Wang resin (2.5 g, 0.89 mmol/g, 2.23 mmol) was suspended in DCM (18 mL). The suspension was cooled on an ice bath, and *p*-nitrophenyl chloroformate (1.35 g, 6.68 mmol) dissolved in DCM (5 mL) was added. After mixing, the suspension was treated with NMM (0.73 mL, 6.68 mmol). The mixture was shaken in an ice bath for 2 h, and then overnight at room temperature. The suspension was transferred to a fritted reservoir. The supernatant was removed with applied pressure of nitrogen gas, and the resin was rinsed three times with DCM. The treatment with *p*-nitrophenyl chloroformate and NMM was repeated once. The resin was then rinsed alternating with DCM and diethyl ether

(repeated three times) and then with DMF (five times). DMF was then added until the resin was swelled, and the suspension was transferred to a silylated round-bottomed flask. This was then treated with either a solution of racemic *N*4-(*o*-nitrophenyl)-piperazine-2-carboxylic acid, methyl ester hydrochloride salt 1a·HCl (2.02 g, 6.68 mmol) and DIPEA (1.16 mL, 6.68 mmol) in DMF (5 mL) or a solution of racemic *N*4-(*m*-nitrophenyl)-piperazine-2-carboxylic acid, methyl ester (1b) (1.77 g, 6.68 mmol) in DMF (5 mL). To the scaffold treated resin was then added additional DIPEA (1.16 mL, 6.68 mmol). The flask was heated at 80 °C and shaken for 20 h. The resin was then transferred to a fritted reservoir and the supernatant was removed by applied nitrogen gas. The resin was rinsed once with DMF. The supernatant and first rinse were collected, evaporated, and purified on column chromatography to isolate excess scaffolds 1a/1b. Then the resin was rinsed with DMF four times, then alternating with DCM and diethyl ether (five times), and finally with diethyl ether three times. The resin was then dried under vacuum for several days. The loading was 0.57 mmol/g (81%) for *o*-substituted (6a) and 0.43 mmol/g (61%) for *m*-substituted (6b).¹¹

Racemic *N*1-(PS-Wang)-*N*4-(*o/m*-Aminophenyl)-piperazine-2-carboxylic Acid, Methyl Esters (7a/7b). Racemic *N*1-(PS-Wang)-*N*4-(*o/m*-nitrophenyl)-piperazine-2-carboxylic acid, methyl esters (6a/6b) (10 g, loading 0.43–0.57 mmol/g) were each suspended in DMF in a fritted reservoir and treated with a solution of SnCl₂·H₂O (26.7 g, 118 mmol, 21–27 equiv) in DMF (15 mL). After shaking for 18 h, the supernatants were removed with applied nitrogen pressure, and the resin was rinsed with DMF (five times), methanol (two times), DCM (two times), and diethyl ether (two times). The resin was then dried under vacuum for several days and then stored under argon.

Library Synthesis. Racemic *N*1-(PS-Wang)-*N*4-(*o*-aminophenyl)-piperazine-2-carboxylic acid, methyl ester (7a) and racemic *N*1-(PS-Wang)-*N*4-(*m*-aminophenyl)-piperazine-2-carboxylic acid, methyl ester (7b) were each divided onto a plate with 96 fused fritted 1 mL reservoirs. A total of 80 reservoirs were used on each plate with an average of 56 mg resin per reservoir for 7a and 65 mg for 7b. This resulted in a typical loading of about 30 μmol scaffold per reservoir. The resin was swelled in DCM and sucked dry, then rinsed with DCM:pyridine (2:1) (2 × 400 μL) and sucked dry. Eight different electrophiles (diversity reagents 8) in DCM (300 μL, 1.27 M, 380 μmol, 12 equiv) were added in 10 reservoirs each on both plates. More DCM:pyridine (2:1) was added until good swelling (approximately 200 μL). Mixing was achieved by rotating the closed plates for 15 h. The supernatants were removed by suction, and the resin was rinsed with DCM (5 × 400 μL), methanol (2 × 400 μL), DCM (5 × 400 μL), and tetrahydrofuran (THF) (4 × 400 μL). This furnished the amides and sulfonyl amides 9. The resin was sucked dry, and potassium trimethylsilanoate in THF (600 μL, 0.625 M, 380 μmol, 12 equiv) was added. After mixing for 4 h, the resin was sucked dry and rinsed with THF (5 × 800 μL), THF:acetic acid (3:1) (4 × 800 μL), and DMF (5 × 400 μL). This yielded the methylester deprotected 10. The resin was again sucked dry. PyBOP in

DMF (562 μL, 0.333 M, 187 μmol, 6 equiv) was added. Ten different amines (diversity reagents 11) (200 μL, 0.95 M, 190 μmol, 6 equiv) were added to eight reservoirs each on both plates orthogonal to the electrophile addition. Finally NMM in DMF (90 μL, 4.22 M, 380 μmol, 12 equiv) was added, and the plates were rotated for 10 h to give 12. After sucking dry and washing with DMF (5 × 800 μL), methanol (3 × 800 μL), and DCM (5 × 800 μL), cleavage was performed by adding 400 μL of DCM and 300 μL of TFA and mixing for 10 min. The cleavage solutions were collected in two 96-well plates. Residual products were rinsed of the resin with DCM (3 × 200 μL) and also collected. Solvents were evaporated in a vacuum centrifuge at 45 °C and 5 mbar overnight, and 13 were collected.

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