[54] **METHODS FOR TREATING BONE DEFICIT CONDITIONS WITH BENZOTHIAZOLE**

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Related U.S. Application Data

[63] Continuation of application No. 08/735,881, Oct. 23, 1996, abandoned.

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[52] **U.S. Cl.** **514/447**; 514/430; 514/439; 514/443

[58] **Field of Search** 514/447, 430, 514/439, 443

[56] **References Cited**

U.S. PATENT DOCUMENTS

5,280,040	1/1994	Labroo et al.	514/422
5,441,964	8/1995	Bryant et al.	514/324
5,523,309	6/1996	Bryant et al.	514/320
5,622,974	4/1997	Muehl	514/320

FOREIGN PATENT DOCUMENTS

WO93/10113 A1	5/1993	WIPO .	
WO95/10513 A1	4/1995	WIPO .	

OTHER PUBLICATIONS

Chem. abstr. vol. 127, abstract No. 127:17703, Petrie et al. Preparation of (hetero aromatic compounds for treating bone deficit conditions, WO–97/15308 (Eng.) (1997).

Chem. abstr. vol. 107, abst. No. 107:109578, Watts et al., Studies on the ligand specificity and potential identify of microsomal antiestrogen–binding sites, Mol. Pharmocol. 1987, 31(5), 541–51.

Chem abstr. vol. 108, abstract No. 108:69162, Jordan et al., Effects of antiestrogens on bone in castrated and intact female rats, Breast Cancer Res. Treat. 1987, 10(1), 31–5.

Chem. abstr. vol. 115, abstract No. 115:8533, Schwarz et al. 1,2–diphenyl–1–pyridybut–1–enes–potential antigestrogens. part 1. synthesis Arch. Pharm. 1991, 324(4), 223–9.

Neelam et al. Structure–activity relationship of antiestrogens: A study using triarylbutenone, benzofuran and triayrlfuran analogues as models for triarylethylenes and triarylpropenones. J. Med. chem. 1989, vol. 32, pp. 1700–1707, see entire article.

Von Angerer et al. Studies on heterocycle–based pure estrogen antagonists. Ann. N.Y. Academy Sciences. 1995, vol. 761, pp. 176–191, see especially pp. 178–180.

Mundy, G.R., "Cytokines and Growth Factors in the Regulation of Bone Remodeling," *Journal of Bone and Mineral Research* (1993) 8(2):S505–S510.

Mundy, G.R., "Regulation of Bone Formation by Bone Morphogenetic Proteins and Other Growth Factors," *Clinical Orthopaedics and Related Research* (1996) 324:24–28.

Harris, S.E., et al., "Effects of Transforming Growth Factor β on Bone Nodule Formation and Expression of Bone Morphogenetic Protein 2, Osteocalcin, Osteopontin, Alkaline Phosphatase, and Type I Collagen mRNA in Long–Term Cultures of Fetal Rat Calvarial Osteoblasts," *Journal of Bone and Mineral Research* (1994) 9(6):855–863.

Stein, G.S., et al., "Bone cell differentiation: a functionally coupled relationship between expression of cell–growth–and tissue–specific genes," *Current Opinion in Cell Biology* (1990) 2:1018–1027.

Urist, M.R., "Bone: Formation by Autoinduction," *Science* (1965) 150:893–899.

Wozney, J.M., et al., "Novel Regulators of Bone Formation: Molecular Clones and Activities," *Science* (1988) 242:1528–1534.

Wozney, J.M., "The Bone Morphogenetic Protein Family and Osteogenesis," *Molecular Reproduction and Development* (1992) 32:160–167.

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Attorney, Agent, or Firm—Morrison & Foerster LLP

[57] **ABSTRACT**

Compounds containing two aromatic systems covalently linked through a linker containing one or more atoms, or "linker" defined as including a covalent bond per se so as to space the aromatic systems at a distance 1.5–15 Å, are effective in treating conditions associated with bone deficits. The compounds can be administered to vertebrate subjects alone or in combination with additional agents that promote bone growth or that inhibit bone resorption. They can be screened for activity prior to administration by assessing their ability to effect the transcription of a reporter gene coupled to a promoter associated with a bone morphogenetic protein and/or their ability to stimulate calvarial growth in model animal systems.

7 Claims, 91 Drawing Sheets

At least 1N Ar^1 –linker – Ar^2 1.5–15A		(I)
Ar^1	Ar^2	
contains 5-membered heterocycle	substituted or unsubsituted benzene	II-A
contains 5-membered heterocycle	substituted or unsubstituted naphthalene	II-B
contains 5-membered heterocycle	contains 6-membered heterocycle	II-C
contains 5-membered heterocycle	contains 5-membered heterocycle	II-D
contains 6-membered heterocycle	substituted or unsubstituted benzene	II-E
contains 6-membered heterocycle	substituted or unsubstituted naphthalene	II-F
contains 6-membered heterocycle	contains 6-membered heterocycle	II-G
substituted or unsubstituted naphthalene	substituted or unsubstituted benzene	II-H
substituted or unsubstituted naphthalene	substituted or unsubstituted naphthalene	II-I
substituted or unsubstituted benzene	substituted or unsubstituted benzene	II-J

FIG. 1

2T3L−C9 CELLS			10/1/96				
5x 10 3 CELLS/WELL							
	uM	READ 1	READ 2	AVERAGE	INDUCTION	AVE−BASAL	%MAX
OS−8	100.000	0.21	0.22	0.22	0.18	−0.99	−17.90
	31.250	3.96	4.44	4.20	3.49	3.00	54.26
	9.766	6.99	6.46	6.72	5.59	5.52	100.00
	3.052	4.62	4.88	4.75	3.95	3.55	64.22
	0.954	3.13	3.16	3.14	2.61	1.94	35.12
	0.298	2.75	2.59	2.67	2.22	1.47	26.58
	0.093	2.10	2.04	2.07	1.72	0.87	15.77
	0.029	1.56	1.71	1.63	1.36	0.43	7.60
	0.0091	1.45	1.42	1.44	1.19	0.23	4.21
	0.0028	1.28	1.37	1.33	1.10	0.12	2.25
	0.0000	1.32	1.30	1.31			
	0.0000	1.20	1.00	1.10			
		AVERAGE BASAL		1.20			

FIG. 2

NNC#	MOL.WEIGHT	CONCENTRATION		%RESPONSE	
50-0194	430.33				
50-0194		100.00	uM	−19.190	
		31.25	uM	32.450	
		9.77	uM	−14.240	
		3.05	uM	−11.330	
		953.67	nM	−12.790	
		298.02	nM	−13.450	
		93.13	nM	−12.290	
		29.10	nM	−9.440	
		9.09	nM	−6.450	
		2.84	nM	−8.130	
		888.18	pM	−3.320	
50-0195	275.36				
50-0195		100.00	uM	−4.630	
		31.25	uM	16.790	
		9.77	uM	62.830	
		3.05	uM	102.720	
		953.67	nM	60.860	
		298.02	nM	32.450	
		93.13	nM	19.340	
		29.10	nM	17.220	
		9.09	nM	5.640	
		2.84	nM	4.840	
		888.18	pM	5.640	
50-0196	276.30				
50-0196		100.00	uM	−16.210	
		31.25	uM	−8.560	
		9.77	uM	11.620	
		3.05	uM	27.790	
		953.67	nM	18.390	
		298.02	nM	6.230	
		93.13	nM	12.420	
		29.10	nM	12.630	
		9.09	nM	6.590	
		2.84	nM	7.970	
		888.18	pM	5.060	
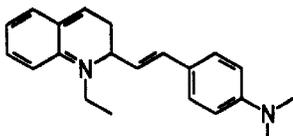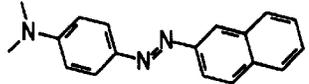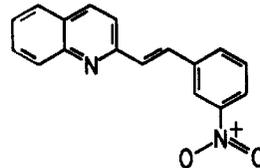
FIG. 3A
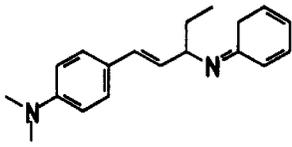
50-0197	274.37				
50-0197		100.00	uM	-18.250	
		31.25	uM	-14.980	
		9.77	uM	4.040	
		3.05	uM	93.790	
		953.67	nM	205.530	
		298.02	nM	242.920	
		93.13	nM	195.890	
		29.10	nM	115.320	
		9.09	nM	85.630	
		2.84	nM	54.380	
		888.18	pM	33.180	
59-0008	254.32				
59-0019	59-0019				
59-0019		100.00	uM	-22.240	
		31.25	uM	-22.670	
		9.77	uM	-17.470	
		3.05	uM	74.490	
		953.67	nM	198.080	
		298.02	nM	258.340	
		93.13	nM	225.350	
		29.10	nM	75.220	
		9.09	nM	24.030	
		2.84	nM	34.480	
		888.18	pM	-3.740	
59-0020	266.73				
59-0020		100.00	uM	-16.510	
		31.25	uM	-16.040	
		9.77	uM	-0.270	
		3.05	uM	96.490	
		953.67	nM	153.320	
		298.02	nM	110.240	
		93.13	nM	60.030	
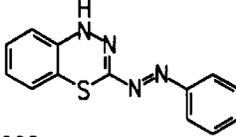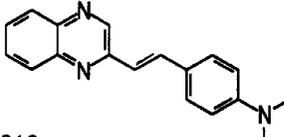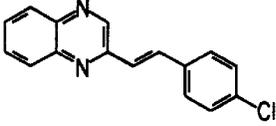
FIG. 3B

		29.10	nM	37.870	
		9.09	nM	24.820	
		2.84	nM	20.500	
		888.18	pM	13.310	

FIG. 3C
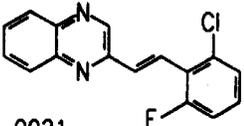
59-0021	284.72				
59-0021		100.00	uM	-16.310	
		31.25	uM	-12.850	
		9.77	uM	84.130	
		3.05	uM	89.940	
		953.67	nM	65.750	
		298.02	nM	33.940	
		93.13	nM	22.560	
		29.10	nM	25.020	
		9.09	nM	13.910	
		2.84	nM	33.270	
		888.18	pM	15.500	
59-0022	266.37				
59-0022		100.00	uM	7.250	
		31.25	uM	-2.070	
		9.77	uM	-0.270	
		3.05	uM	4.390	
		953.67	nM	3.060	
		298.02	nM	-1.800	
		93.13	nM	-0.200	
		29.10	nM	-3.270	
		9.09	nM	1.130	
		2.84	nM	2.590	
		888.18	pM	2.460	
59-0023	239.28				
59-0023		100.00	uM	-12.720	
		31.25	uM	33.140	
		9.77	uM	56.500	
		3.05	uM	29.550	
		953.67	nM	25.360	
		298.02	nM	15.700	
		93.13	nM	7.380	
		29.10	nM	9.710	
		9.09	nM	1.000	
		2.84	nM	4.520	
		888.18	pM	-0.010	
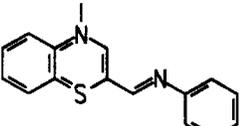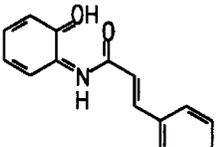
FIG. 3D
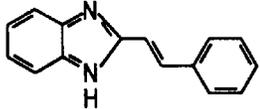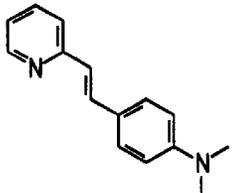
59-0024	220.28				
59-0025	224.31				
59-0025		100.00	uM	−25.590	
		31.25	uM	14.150	
		9.77	uM	50.690	
		3.05	uM	57.880	
		953.67	nM	38.900	
		298.02	nM	28.530	
		93.13	nM	19.660	
		29.10	nM	17.490	
		9.09	nM	−0.600	
		2.84	nM	−4.190	
		888.18	pM	4.670	
59-0026	248.29				
59-0026		100.00	uM	−29.830	
		31.25	uM	−9.440	
		9.77	uM	−10.470	
		3.05	uM	46.220	
		953.67	nM	107.760	
		298.02	nM	86.720	
		93.13	nM	36.850	
		29.10	nM	26.720	
		9.09	nM	8.520	
		2.84	nM	−1.240	
		888.18	pM	4.020	
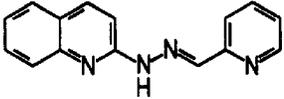
FIG. 3E
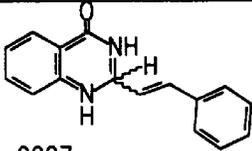
59-0027	250.30				
59-0027		100.00	uM	89.810	
		31.25	uM	54.670	
		9.77	uM	44.940	
		3.05	uM	23.780	
		953.67	nM	8.380	
		298.02	nM	6.330	
		93.13	nM	7.360	
		29.10	nM	3.380	
		9.09	nM	−1.620	
		2.84	nM	−3.670	
		888.18	pM	−0.720	
59-0028	226.28				
59-0028		100.00	uM	−26.750	
		31.25	uM	−16.740	
		9.77	uM	29.550	
		3.05	uM	100.580	
		953.67	nM	54.940	
		298.02	nM	31.340	
		93.13	nM	7.500	
		29.10	nM	7.500	
		9.09	nM	7.880	
		2.84	nM	3.140	
		888.18	pM	4.670	
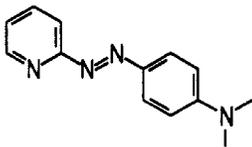
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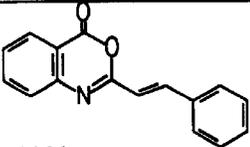
59-0029	249.27				
59-0029		100.00	uM	−15.150	
		31.25	uM	41.940	
		9.77	uM	36.630	
		3.05	uM	7.120	
		953.67	nM	21.880	
		298.02	nM	15.540	
		93.13	nM	1.810	
		29.10	nM	1.370	
		9.09	nM	12.140	
		2.84	nM	−4.230	
		888.18	pM	9.040	
59-0030A	233.28				
59-0030A		100.00	uM	−27.970	
		31.25	uM	−22.830	
		9.77	uM	−5.420	
		3.05	uM	57.280	
		953.67	nM	72.620	
		298.02	nM	53.000	
		93.13	nM	29.990	
		29.10	nM	14.630	
		9.09	nM	3.870	
		2.84	nM	6.970	
		888.18	pM	1.810	
59-0031	231.30				
59-0031		100.00	uM	−25.790	
		31.25	uM	−17.810	
		9.77	uM	20.840	
		3.05	uM	87.380	
		953.67	nM	49.320	
		298.02	nM	43.110	
		93.13	nM	29.530	
		29.10	nM	1.810	
		9.09	nM	1.220	
		2.84	nM	−0.550	
		888.18	pM	4.160	
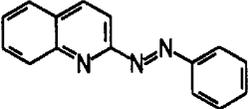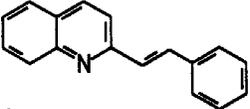
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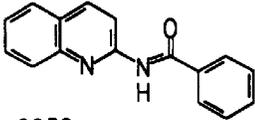
59-0032	248.29				
59-0032		100.00	uM	−7.780	
		31.25	uM	40.750	
		9.77	uM	42.820	
		3.05	uM	25.700	
		953.67	nM	31.170	
		298.02	nM	34.410	
		93.13	nM	3.570	
		29.10	nM	4.320	
		9.09	nM	−10.000	
		2.84	nM	5.650	
		888.18	pM	11.990	
59-0033	248.29				
59-0033		100.00	uM	−28.180	
		31.25	uM	−11.590	
		9.77	uM	55.300	
		3.05	uM	49.710	
		953.67	nM	47.410	
		298.02	nM	0.250	
		93.13	nM	7.980	
		29.10	nM	−8.940	
		9.09	nM	−7.630	
		2.84	nM	−0.400	
		888.18	pM	−5.980	
59-0034	268.34				
59-0034		100.00	uM	−28.51	
		31.25	uM	24	
		9.77	uM	73.58	
		3.05	uM	37.91	
		953.67	nM	20.09	
		298.02	nM	16.87	
		93.13	nM	15.23	
		29.10	nM	28.83	
		9.09	nM	9.08	
		2.84	nM	23.02	
		888.18	pM	−0.32	
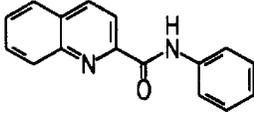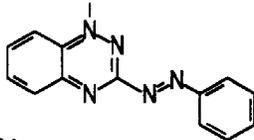
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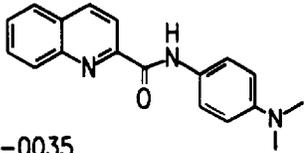
59-0035	291.36				
59-0035		100.00	uM	−14.92	
		31.25	uM	29.17	
		9.77	uM	15.87	
		3.05	uM	18.8	
		953.67	nM	3.88	
		298.02	nM	6.15	
		93.13	nM	3.22	
		29.10	nM	−10.03	
		9.09	nM	15.58	
		2.84	nM	−3.56	
		888.18	pM	−7.13	
59-0036	262.31				
59-0036		100.00	uM	−0.98	
		31.25	uM	−3.25	
		9.77	uM	−4.54	
		3.05	uM	−1.95	
		953.67	nM	0.32	
		298.02	nM	−6.49	
		93.13	nM	−17.19	
		29.10	nM	−0.66	
		9.09	nM	−5.52	
		2.84	nM	−9.4	
		888.18	pM	−16.53	
59-0037	308.00				
59-0037		100.00	uM	−10.69	
		31.25	uM	−11.99	
		9.77	uM	−10.03	
		3.05	uM	−19.11	
		953.67	nM	−9.4	
		298.02	nM	2.27	
		93.13	nM	−2.9	
		29.10	nM	−10.69	
		9.09	nM	2.59	
		2.84	nM	0.66	
		888.18	pM	−2.59	
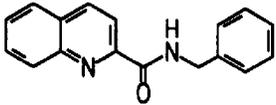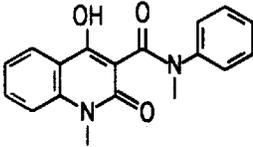
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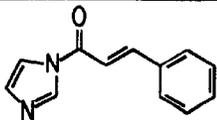
59-0038	291.36			
59-0038		100.00	uM	−23.430
		31.25	uM	−8.390
		9.77	uM	−0.100
		3.05	uM	−2.860
		953.67	nM	−2.240
		298.02	nM	3.900
		93.13	nM	6.350
		29.10	nM	1.150
		9.09	nM	6.960
		2.84	nM	−4.390
		888.18	pM	0.380
59-0039	312.35			
59-0039		100.00	uM	14.170
		31.25	uM	7.620
		9.77	uM	1.940
		3.05	uM	−3.140
		953.67	nM	−7.770
		298.02	nM	−5.980
		93.13	nM	−8.820
		29.10	nM	−2.390
		9.09	nM	−16.580
		2.84	nM	−4.480
		888.18	pM	−0.450
59-0040	290.37			
59-0040		100.00	uM	−20.400
		31.25	uM	−17.310
		9.77	uM	−8.110
		3.05	uM	32.180
		953.67	nM	36.180
		298.02	nM	17.440
		93.13	nM	2.040
		29.10	nM	10.350
		9.09	nM	6.070
		2.84	nM	6.960
		888.18	pM	13.440
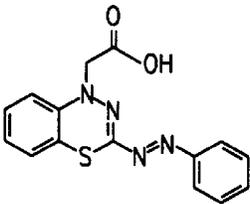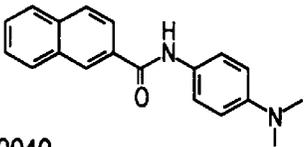
FIG. 3J
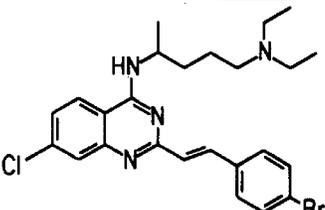
59-0041	501.90				
59-0041		100.00	uM	-18.37	
		31.25	uM	-17.33	
		9.77	uM	-5.11	
		3.05	uM	3.31	
		953.67	nM	-0.77	
		298.02	nM	-1.56	
		93.13	nM	3.55	
		29.10	nM	-11.24	
		9.09	nM	0.25	
		2.84	nM	-0.27	
		888.18	pM	2.02	
59-0042	281.36				
59-0042		100.00	uM	163.51	
		31.25	uM	-7.67	
		9.77	uM	9.41	
		3.05	uM	0.75	
		953.67	nM	6.11	
		298.02	nM	3.82	
		93.13	nM	2.54	
		29.10	nM	4.07	
		9.09	nM	-9.73	
		2.84	nM	-0.02	
		888.18	pM	18.37	
59-0043	280.29				
59-0043		100.00	uM	20.66	
		31.25	uM	7.4	
		9.77	uM	-1.29	
		3.05	uM	-2.31	
		953.67	nM	1.54	
		298.02	nM	-0.79	
		93.13	nM	1.52	
		29.10	nM	2.79	
		9.09	nM	-0.27	
		2.84	nM	8.92	
		888.18	pM	-4.34	
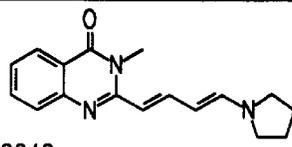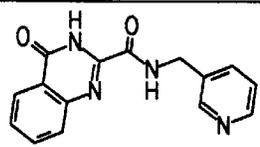
FIG. 3K
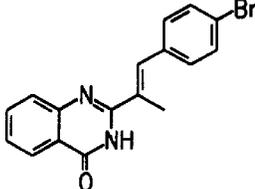
59-0044	341.21				
59-0044		100.00	uM	7.38	
		31.25	uM	11.72	
		9.77	uM	12.49	
		3.05	uM	-0.52	
		953.67	nM	0.5	
		298.02	nM	6.11	
		93.13	nM	-1.54	
		29.10	nM	19.14	
		9.09	nM	7.13	
		2.84	nM	-2.06	
		888.18	pM	5.84	
59-0045	283.33				
59-0045		100.00	uM	52.37	64.460
		31.25	uM	148.43	192.960
		9.77	uM	204.47	422.540
		3.05	uM	280.3	437.020
		953.67	nM	254.82	410.890
		298.02	nM	218.21	266.090
		93.13	nM	196.98	183.730
		29.10	nM	96.06	80.440
		9.09	nM	67.35	55.530
		2.84	nM	52.99	44.160
59-0046	389.37				
59-0046		100.00	uM	79.33	
		31.25	uM	2.24	
		9.77	uM	-1.67	
		3.05	uM	-6.18	
		953.67	nM	0.001	
		298.02	nM	-3.63	
		93.13	nM	-0.84	
		29.10	nM	-8.42	
		9.09	nM	3.92	
		2.84	nM	0.3	
		888.18	pM	5.61	
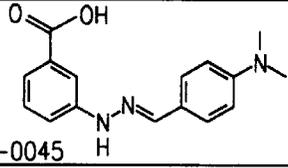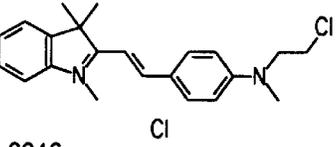
FIG. 3L
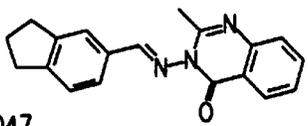
59-0047	303.37				
59-0047		100.00	uM	-6.73	
		31.25	uM	10.38	
		9.77	uM	-6.16	
		3.05	uM	-1.39	
		953.67	nM	-10.11	
		298.02	nM	-4.49	
		93.13	nM	-7.28	
		29.10	nM	-12.34	
		9.09	nM	-3.08	
		2.84	nM	-2.26	
		888.18	pM	-5.34	
59-0048	384.50				
59-0048		100.00	uM	-6.73	
		31.25	uM	0.27	
		9.77	uM	-5.61	
		3.05	uM	-2.26	
		953.67	nM	-12.89	
		298.02	nM	-1.69	
		93.13	nM	-4.77	
		29.10	nM	-8.14	
		9.09	nM	-3.92	
		2.84	nM	-11.2	
		888.18	pM	-4.77	
59-0049	251.29				
59-0049		100.00	uM	4.49	
		31.25	uM	0	
		9.77	uM	-4.77	
		3.05	uM	1.96	
		953.67	nM	8.69	
		298.02	nM	-5.04	
		93.13	nM	-2.24	
		29.10	nM	1.69	
		9.09	nM	-4.49	
		2.84	nM	2.24	
		888.18	pM	-0.3	
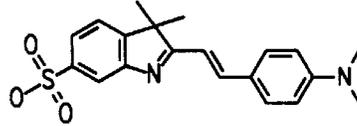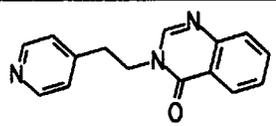
FIG. 3M
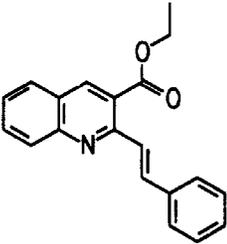
59-0050	303.36				
59-0050		100.00	uM	45.79	
		31.25	uM	10.02	
		9.77	uM	11.29	
		3.05	uM	−4.68	
		953.67	nM	−6.92	
		298.02	nM	−5.65	
		93.13	nM	1.69	
		29.10	nM	−7.57	
		9.09	nM	−12.05	
		2.84	nM	−13.63	
		888.18	pM	5.2	
59-0051	251.35				
59-0051		100.00	uM	32.36	
		31.25	uM	−18.42	
		9.77	uM	−0.55	
		3.05	uM	−13.94	
		953.67	nM	−12.02	
		298.02	nM	−14.59	
		93.13	nM	−7.55	
		29.10	nM	−11.4	
		9.09	nM	−14.91	
		2.84	nM	−10.74	
		888.18	pM	−20.03	
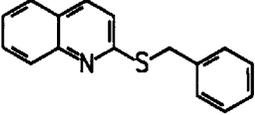
FIG. 3N
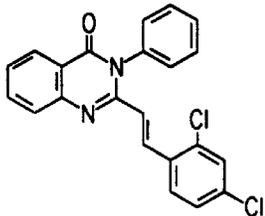
59-0052	393.28				
59-0052		100.00	uM	−21.62	
		31.25	uM	−13.32	
		9.77	uM	−21.31	
		3.05	uM	−11.08	
		953.67	nM	−20.66	
		298.02	nM	−17.14	
		93.13	nM	−16.49	
		29.10	nM	−11.4	
		9.09	nM	−10.74	
		2.84	nM	−11.08	
		888.18	pM	−14.59	
59-0053	354.41				
59-0053		100.00	uM	−17.14	
		31.25	uM	−21.31	
		9.77	uM	−9.47	
		3.05	uM	−11.08	
		953.67	nM	−0.83	
		298.02	nM	−11.4	
		93.13	nM	−9.47	
		29.10	nM	−19.72	
		9.09	nM	−18.45	
		2.84	nM	−10.09	
		888.18	pM	−2.76	
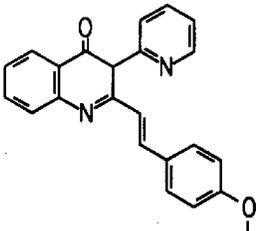
FIG. 30
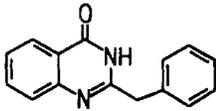
59-0054	236.28				
59-0054		100.00	uM	-20.04	
		31.25	uM	-6.95	
		9.77	uM	8.3	
		3.05	uM	-3.37	
		953.67	nM	-2.4	
		298.02	nM	-0.99	
		93.13	nM	-0.99	
		29.10	nM	-1.94	
		9.09	nM	5.92	
		2.84	nM	-2.17	
		888.18	pM	-9.31	
59-0055	425.51				
59-0055		100.00	uM	-13.76	
		31.25	uM	-9.51	
		9.77	uM	-2.02	
		3.05	uM	3.24	
		953.67	nM	-6.27	
		298.02	nM	-4.05	
		93.13	nM	-1.62	
		29.10	nM	-7.49	
		9.09	nM	-7.09	
		2.84	nM	-3.04	
59-0056	512.34				
59-0056		100.00	uM	-1.42	
		31.25	uM	-4.87	
		9.77	uM	0.18	
		3.05	uM	3.84	
		953.67	nM	-5.07	
		298.02	nM	-7.29	
		93.13	nM	0.001	
		29.10	nM	-4.25	
		9.09	nM	-1.02	
		2.84	nM	-3.85	
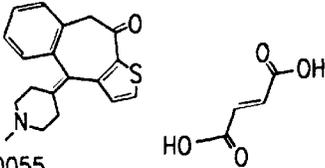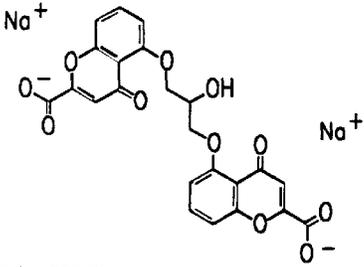
FIG. 3P
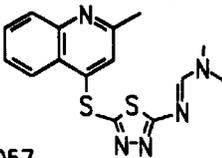
59-0057					
59-0057		100.00	uM	-24.150	
		31.25	uM	-24.300	
		9.77	uM	-5.980	
		3.05	uM	-11.500	
		953.67	nM	-13.000	
		298.02	nM	-6.280	
		93.13	nM	-12.550	
		29.10	nM	-6.870	
		9.09	nM	-8.520	
		2.84	nM	-16.290	
59-0058					
59-0058		100.00	uM	4.170	
		31.25	uM	7.620	
		9.77	uM	-1.790	
		3.05	uM	-7.320	
		953.67	nM	-1.940	
		298.02	nM	-6.870	
		93.13	nM	-1.490	
		29.10	nM	-8.370	
		9.09	nM	-5.080	
		2.84	nM	-12.400	
59-0059					
59-0059		100.00	uM	-18.700	
		31.25	uM	-16.140	
		9.77	uM	-3.090	
		3.05	uM	0.150	
		953.67	nM	6.010	
		298.02	nM	-1.910	
		93.13	nM	-1.760	
		29.10	nM	-9.100	
		9.09	nM	-8.220	
		2.84	nM	-5.720	
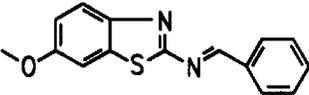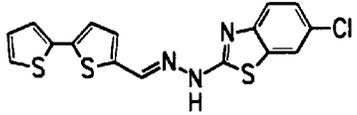
FIG. 3Q
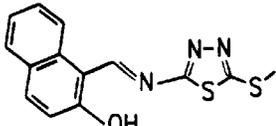
59-0060				
59-0060		100.00	uM	-4.250	
		31.25	uM	-14.520	
		9.77	uM	1.030	
		3.05	uM	-1.180	
		953.67	nM	-13.200	
		298.02	nM	-0.740	
		93.13	nM	-3.670	
		29.10	nM	-7.340	
		9.09	nM	-1.310	
		2.84	nM	0.290	
59-0061					
59-0061		100.00	uM	-17.890	
		31.25	uM	-18.770	
		9.77	uM	-17.170	
		3.05	uM	-14.080	
		953.67	nM	-17.020	
		298.02	nM	-7.190	
		93.13	nM	-1.910	
		29.10	nM	-0.440	
		9.09	nM	-6.010	
		2.84	nM	-4.560	
59-0062					
59-0062		100.00	uM	-13.940	
		31.25	uM	-12.910	
		9.77	uM	-4.560	
		3.05	uM	-4.540	
		953.67	nM	-6.900	
		298.02	nM	-4.100	
		93.13	nM	-1.620	
		29.10	nM	3.230	
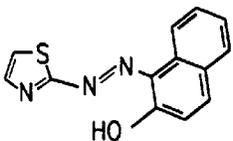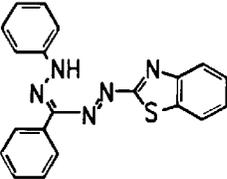
FIG. 3R

		9.09	nM	8.070	
		2.84	nM	0.440	
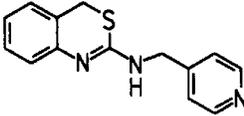
59-0063

59-0063		100.00	uM	-2.510	
		31.25	uM	-6.130	
		9.77	uM	-8.950	
		3.05	uM	-8.020	
		953.67	nM	-8.010	
		298.02	nM	-2.520	
		93.13	nM	-5.810	
		29.10	nM	-3.450	
		9.09	nM	-4.390	
		2.84	nM	-6.280	
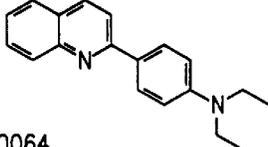
59-0064

59-0064		100.00	uM	-23.090	
		31.25	uM	-21.040	
		9.77	uM	78.400	
		3.05	uM	155.220	
		953.67	nM	113.120	
		298.02	nM	30.640	
		93.13	nM	15.240	
		29.10	nM	22.150	
		9.09	nM	-0.770	
		2.84	nM	4.410	
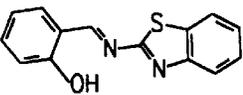
59-0065

59-0065		100.00	uM	-2.030	
		31.05	uM	-2.980	
		9.77	uM	-15.240	
		3.05	uM	-15.400	
		953.67	nM	-15.240	
		298.02	nM	-10.520	
		93.13	nM	-13.830	
		29.10	nM	-5.810	
		9.09	nM	-3.620	
		2.84	nM	-7.070	

FIG. 3S
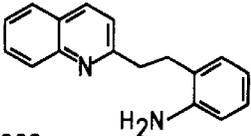
59-0066

		59-0066				
		100.00	uM	10.060	
		31.25	uM	2.680	
		9.77	uM	10.850	
		3.05	uM	14.610	
		953.67	nM	0.950	
		298.02	nM	3.780	
		93.13	nM	1.730	
		29.10	nM	-2.820	
		9.09	nM	-2.820	
		2.84	nM	-3.920	
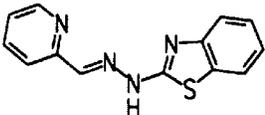
59-0067

		59-0067				
		100.00	uM	-24.040	
		31.25	uM	-24.890	
		9.77	uM	-1.450	
		3.05	uM	60.900	
		953.67	nM	133.860	
		298.02	nM	75.330	
		93.13	nM	28.760	
		29.10	nM	20.070	
		9.09	nM	4.980	
		2.84	nM	4.450	
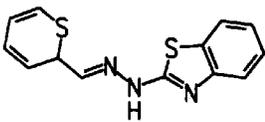
59-0068

		59-0068				
		100.00	uM	-22.130	
		31.25	uM	-7.880	
		9.77	uM	93.900	
		3.05	uM	81.060	
		953.67	nM	22.330	
		298.02	nM	17.300	
		93.13	nM	8.460	
		29.10	nM	-3.530	
		9.09	nM	-4.230	
		2.84	nM	-6.140	

FIG. 3T
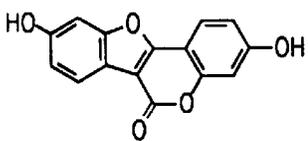
59-0069				
59-0069		100.00	uM	5.490
		31.25	uM	9.670
		9.77	uM	16.090
		3.05	uM	-7.180
		953.67	nM	-2.840
		298.02	nM	-3.710
		93.13	nM	-11.180
		29.10	nM	-5.790
		9.09	nM	-7.180
		2.84	nM	-4.750
59-0070				
59-0070		100.00	uM	-25.930
		31.25	uM	-23.000
		9.77	uM	36.060
		3.05	uM	214.280
		953.67	nM	158.530
		298.02	nM	72.890
		93.13	nM	20.940
		29.10	nM	7.760
		9.09	nM	7.590
		2.84	nM	-8.400
59-0071				
59-0071		100.00	uM	-18.650
		31.25	uM	-15.540
		9.77	uM	17.060
		3.05	uM	176.090
		953.67	nM	76.070
		298.02	nM	31.260
		93.13	nM	16.410
		29.10	nM	4.870
		9.09	nM	-7.330
		2.84	nM	-4.660
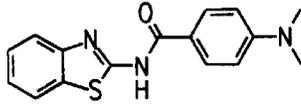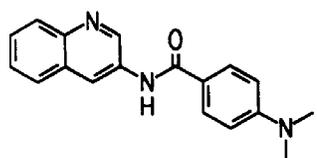
FIG. 3U
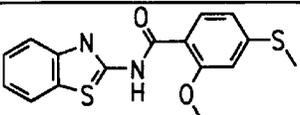
59-0072				
59-0072	100.00	uM	−19.750	
	31.25	uM	−18.650	
	9.77	uM	−18.430	
	3.05	uM	−15.770	
	953.67	nM	9.970	
	298.02	nM	74.740	
	93.13	nM	175.430	
	29.10	nM	213.580	
	9.09	nM	164.320	
	2.84	nM	119.100	
	888.18	pM	60.770	
59-0073				
59-0073	100.00	uM	−3.010	
	31.25	uM	−4.830	
	9.77	uM	−9.660	
	3.05	uM	−4.680	
	953.67	nM	−6.500	
	298.02	nM	−2.510	
	93.13	nM	7.140	
	29.10	nM	0.97	
	9.09	nM	−5.5	
	2.84	nM	5.3	
59-0074				
59-0074	100.00	uM	−2.85	
	31.25	uM	2.14	
	9.77	uM	−4.85	
	3.05	uM	−3.5	
	953.67	nM	−4.85	
	298.02	nM	9.95	
	93.13	nM	4.47	
	29.10	nM	−8	
	9.09	nM	−4.17	
	2.84	nM	6.97	
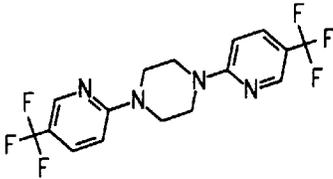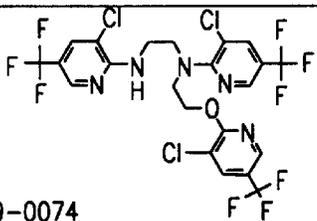
FIG. 3V
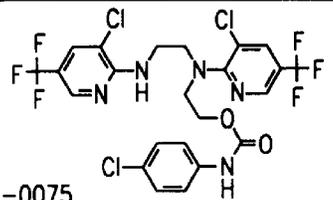
59-0075					
59-0075		100.00	uM	-0.68	
		31.25	uM	-10.16	
		9.77	uM	-5.35	
		3.05	uM	-6.5	
		953.67	nM	-0.85	
		298.02	nM	5.97	
		93.13	nM	0.97	
		29.10	nM	-2.35	
		9.09	nM	0.32	
		2.84	nM	10.47	
59-0076					
59-0076		100.00	uM	-19.12	
		31.25	uM	9.29	
		9.77	uM	10.63	
		3.05	uM	22.43	
		953.67	nM	19.93	
		298.02	nM	3.47	
		93.13	nM	19.93	
		29.10	nM	10.63	
		9.09	nM	14.28	
		2.84	nM	11.3	
59-0077					
59-0077		100.00	uM	-20.96	
		31.25	uM	-16.23	
		9.77	uM	-10.58	
		3.05	uM	-11.96	
		953.67	nM	-19.44	
		298.02	nM	-17.3	
		93.13	nM	-13.79	
		29.10	nM	-15.62	
		9.09	nM	-14.09	
		2.84	nM	-14.4	
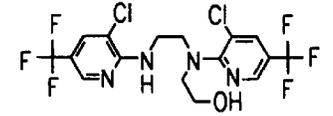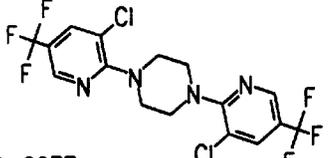
FIG. 3W
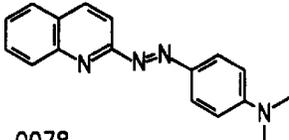
59-0078				
59-0078		100.00	uM	-26.540	
		31.25	uM	-22.560	
		9.77	uM	71.530	
		3.05	uM	207.960	
		953.67	nM	379.230	
		298.02	nM	241.460	
		93.13	nM	136.100	
		29.10	nM	84.020	
		9.09	nM	50.350	
		2.84	nM	56.600	
		888.18	pM	92.520	
59-0079				
59-0079		100.00	uM	-34.980	
		31.25	uM	-21.390	
		9.77	uM	37.200	
		3.05	uM	122.580	
		953.67	nM	69.010	
		298.02	nM	64.000	
		93.13	nM	46.490	
		29.10	nM	30.310	
		9.09	nM	33.490	
		2.84	nM	29.760	
59-0080				
59-0080		100.00	uM	5.390	
		31.25	uM	5.560	
		9.77	uM	6.440	
		3.05	uM	2.440	
		953.67	nM	-5.030	
		298.02	nM	7.660	
		93.13	nM	-3.630	
		29.10	nM	3.650	
		9.09	nM	1.050	
		2.84	nM	6.940	
59-0081				
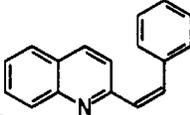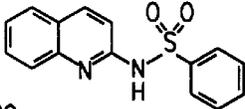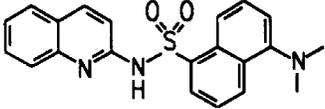
FIG. 3X

59-0081		100.00	uM	62.840	
		31.25	uM	11.300	
		9.77	uM	−8.670	
		3.05	uM	2.440	
		953.67	nM	−5.200	
		298.02	nM	−2.080	
		93.13	nM	1.220	
		29.10	nM	−2.250	
		9.09	nM	1.050	
		2.84	nM	−3.300	
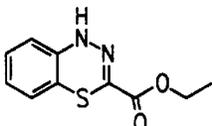
59-0082

59-0082		100.00	uM	111.79	
		31.25	uM	62.68	
		9.77	uM	32.36	
		3.05	uM	9.11	
		953.67	nM	−10.62	
		298.02	nM	−1.86	
		93.13	nM	−6.89	
		29.10	nM	−3.91	
		9.09	nM	2.22	
		2.84	nM	16.36	

59-0083

59-0083		100.00	uM	48.93	
		31.25	uM	40.91	
		9.77	uM	25.85	
		3.05	uM	17.85	
		953.67	nM	8.55	
		298.02	nM	3.9	
		93.13	nM	2.05	
		29.10	nM	7.99	
		9.09	nM	−3.91	
		2.84	nM	3.35	
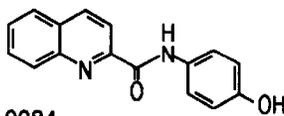
59-0084

59-0084		100.00	uM	37.670	
		31.25	uM	26.050	
		9.77	uM	9.210	
		3.05	uM	10.070	

FIG. 3Y

		953.67	nM	21.700
		298.02	nM	5.900
		93.13	nM	4.870
		29.10	nM	−10.920
		9.09	nM	10.080
		2.84	nM	−2.080
59-0085				
59-0085		100.00	uM	17.070
		31.25	uM	41.890
		9.77	uM	18.500
		3.05	uM	20.340
		953.67	nM	22.490
		298.02	nM	8.090
		93.13	nM	11.790
		29.10	nM	1.240
		9.09	nM	−0.760
		2.84	nM	5.940
59-0086				
59-0086		100.00	uM	30.750
		31.25	uM	31.190
		9.77	uM	14.790
		3.05	uM	13.500
		953.67	nM	14.080
		298.02	nM	3.940
		93.13	nM	9.370
		29.10	nM	−2.610
		9.09	nM	−5.040
		2.84	nM	1.530
59-0087				
59-0087		100.00	uM	10.660
		31.25	uM	11.080
		9.77	uM	3.100
		3.05	uM	−1.320
		953.67	nM	17.070
		298.02	nM	7.950
		93.13	nM	−4.460
		29.10	nM	4.510
		9.09	nM	−0.470
		2.84	nM	9.660
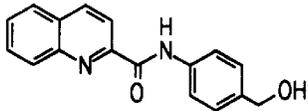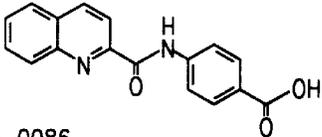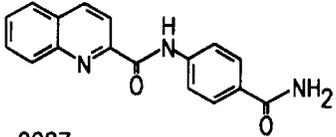
FIG. 3Z
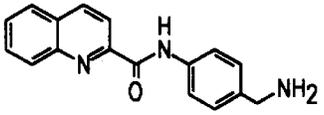
59-0088					
59-0088		100.00	uM		
		31.25	uM		
		9.77	uM		
		3.05	uM		
		953.67	nM		
		298.02	nM		
		93.13	nM		
		29.10	nM		
		9.09	nM		
		2.84	nM		
59-0089					
59-0089		100.00	uM	60.09	
		31.25	uM	116.25	
		9.77	uM	65.85	
		3.05	uM	36.1	
		953.67	nM	37.96	
		298.02	nM	18.42	
		93.13	nM	6.33	
		29.10	nM	13.58	
		9.09	nM	0.75	
		2.84	nM	-5.77	
59-0090					
59-0090		100.00	uM	32.77	
		31.25	uM	24.63	
		9.77	uM	19.5	
		3.05	uM	41.31	
		953.67	nM	9.8	
		298.02	nM	-1.76	
		93.13	nM	3.53	
		29.10	nM	2.95	
		9.09	nM	2.95	
		2.84	nM	7.8	
59-0091					
59-0091		100.00	uM	0.26	
		31.25	uM	13.54	
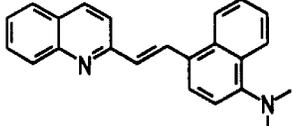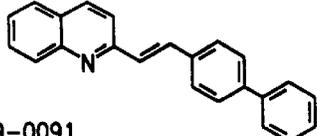
FIG. 3AA

		9.77	uM	95.94	
		3.05	uM	87.71	
		953.67	nM	44.17	
		298.02	nM	38.26	
		93.13	nM	23.87	
		29.10	nM	21.65	
		9.09	nM	10.95	
		2.84	nM	20.92	
59-0092					
59-0092		100.00	uM	−11.56	
		31.25	uM	17.84	
		9.77	uM	50.19	
		3.05	uM	25.84	
		953.67	nM	14.4	
		298.02	nM	6.77	
		93.13	nM	8.62	
		29.10	nM	2.22	
		9.09	nM	8.38	
		2.84	nM	1	
59-0093					
59-0093		100.00	uM	−11.67	
		31.25	uM	15.02	
		9.77	uM	35.44	
		3.05	uM	29.89	
		953.67	nM	22.88	
		298.02	nM	19.56	
		93.13	nM	5.18	
		29.10	nM	7.39	
		9.09	nM	4.56	
		2.84	nM	5.9	
59-0094					
59-0094		100.00	uM	−17.69	
		31.25	uM	45.15	
		9.77	uM	24.97	
		3.05	uM	19.81	
		953.67	nM	9.35	
		298.02	nM	1.36	
		93.13	nM	9.24	
		29.10	nM	−0.48	
		9.09	nM	6.16	
		2.84	nM	1.61	
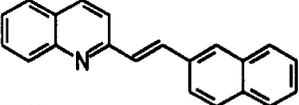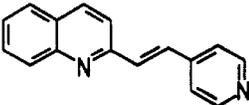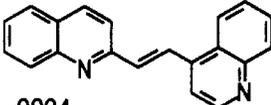
FIG. 3BB
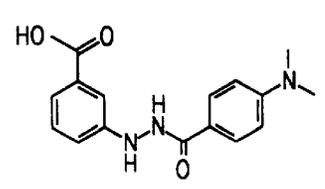
59-0095

	100.00	uM		44.7
	31.25	uM		47.61
	9.77	uM		12.78
	3.05	uM		21.49
	953.67	nM		15.01
	298.02	nM		10.22
	93.13	nM		13.98
	29.10	nM		20.31
	9.09	nM		10.9
	2.84	nM		9.21
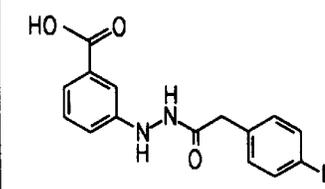
59-0096

	100.00	uM		413.05
	31.25	uM		287.23
	9.77	uM		137.38
	3.05	uM		78.5
	953.67	nM		49.13
	298.02	nM		50.68
	93.13	nM		47.95
	29.10	nM		26.28
	9.09	nM		18.75
	2.84	nM		22.17
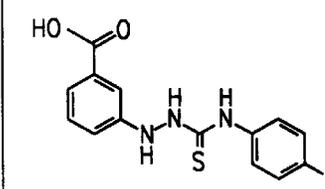
59-0097

	100.00	uM		77.47
	31.25	uM		201.9
	9.77	uM		160.93
	3.05	uM		61.44
	953.67	nM		47.78
	298.02	nM		51.54
	93.13	nM		34.64
	29.10	nM		43.18
	9.09	nM		39.91
	2.84	nM		27.13

FIG. 3CC
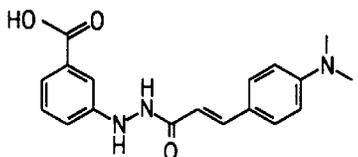
59-0098

59-0098		100.00	uM		−1.38
		31.25	uM		186.89
		9.77	uM		221.7
		3.05	uM		164.69
		953.67	nM		96.94
		298.02	nM		68.25
		93.13	nM		57
		29.10	nM		51.88
		9.09	nM		41.29
		2.84	nM		33.43
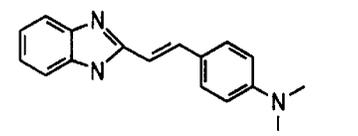
59-0099

59-0099		100.00	uM	13.040	
		31.25	uM	56.880	
		9.77	uM	119.340	
		3.05	uM	237.420	
		953.67	nM	285.440	
		298.02	nM	164.610	
		93.13	nM	123.300	
		29.10	nM	69.240	
		9.09	nM	44.500	
		2.84	nM	47.390	
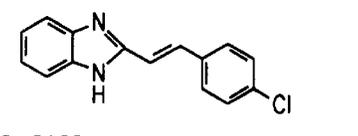
59-0100

59-0100		100.00	uM	−10.020	
		31.25	uM	−10.730	
		9.77	uM	30.340	
		3.05	uM	114.410	
		953.67	nM	77.540	
		298.02	nM	40.290	
		93.13	nM	35.730	
		29.10	nM	28.290	
		9.09	nM	17.480	
		2.84	nM	11.470	
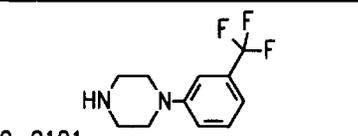
59-0101

59-0101		100.00	uM	26.370	

FIG. 3DD

		31.25	uM	12.440	
		9.77	uM	−0.780	
		3.05	uM	10.280	
		953.67	nM	2.110	
		298.02	nM	7.860	
		93.13	nM	1.140	
		29.10	nM	2.820	
		9.09	nM	4.150	
		2.84	nM	5.590	
59-0102	284.34				
59-0102		100.00	uM	−24.350	
		31.25	uM	−11.140	
		9.77	uM	63.540	
		3.05	uM	121.320	
		953.67	nM	79.530	
		298.02	nM	72.460	
		93.13	nM	66.290	
		29.10	nM	45.690	
		9.09	nM	27.260	
		2.84	nM	42.330	
		888.18	nM	33.430	
59-0103	313.38				
		100.00	uM	−29.69	
		31.25	uM	−29.53	
		9.77	uM	−28.22	
		3.05	uM	−27.72	
		953.67	nM	−5.58	
		298.02	nM	54.15	
		93.13	nM	170.95	
		29.10	nM	222.87	
		9.09	nM	210.39	
		2.84	nM	203.4	
		0.80	nM	114.55	
59-0104	297.31				
		100.00	uM	−29.84	
		31.25	uM	−26.72	
		9.77	uM	−29.2	
		3.05	uM	−27.05	
		953.67	nM	24.37	
		298.02	nM	196.42	
		93.13	nM	213.89	

FIG. 3EE

Structure / ID	MW	Conc.	Unit	Value	
		29.10	nM	220.04	
		9.09	nM	245.42	
		2.84	nM	182.45	
		0.80	nM	119.55	
59-0105	267.29				
		100.00	uM	-25.72	
		31.25	uM	-15.89	
		9.77	uM	31.7	
		3.05	uM	54.17	
		953.67	nM	53.67	
		298.02	nM	41.35	
		93.13	nM	44.5	
		29.10	nM	39.02	
		9.09	nM	25.38	
		2.84	nM	31.7	
		0.80	nM	18.05	
59-0106	297.31				
		100.00	uM	-14.05	
		31.25	uM	223.52	
		9.77	uM	202.58	
		3.05	uM	107.73	
		953.67	nM	71.3	
		298.02	nM	44.84	
		93.13	nM	26.54	
		29.10	nM	23.05	
		9.09	nM	27.87	
		2.84	nM	12.23	
		0.80	nM	11.4	
59-0107	332.38				
		100.00	uM	48.55	
		31.25	uM	22.87	
		9.77	uM	7.19	
		3.05	uM	0.65	
		953.67	nM	11.12	
		298.02	nM	-3.92	
		93.13	nM	1.09	
		29.10	nM	-15.69	
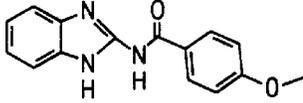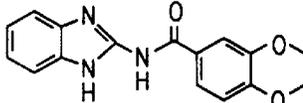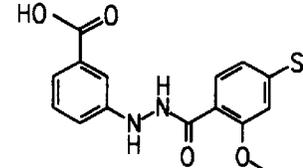
FIG. 3FF

		9.09	nM	−11.32	
		2.84	nM	−2.62	
		0.80	nM	−16.11	
59−0108	316.31				
		100.00	uM	227.73	
		31.25	uM	96.02	
		9.77	uM	58.57	
		3.05	uM	37.23	
		953.67	nM	18.94	
		298.02	nM	25.68	
		93.13	nM	−4.8	
		29.10	nM	2.62	
		9.09	nM	−4.8	
		2.84	nM	3.92	
		0.80	nM	4.14	
59−0109	316.31				
		100.00	uM	43.12	
		31.25	uM	27.64	
		9.77	uM	5.89	
		3.05	uM	6.32	
		953.67	nM	13.51	
		298.02	nM	7.85	
		93.13	nM	3.71	
		29.10	nM	−3.27	
		9.09	nM	5.01	
		2.84	nM	−4.58	
		0.80	nM	6.98	
59−0110	286.29				
		100.00	uM	65.11	
		31.25	uM	67.05	
		9.77	uM	35.27	
		3.05	uM	25.26	
		953.67	nM	27.01	
		298.02	nM	15.24	
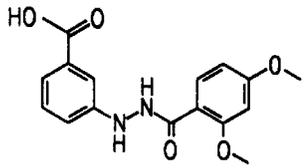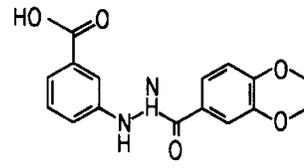
FIG. 3GG

		93.13	nM	10.68	
		29.10	nM	5.89	
		9.09	nM	5.45	
		2.84	nM	10.24	
		0.80	nM	4.14	
59-0111	152.15				
		100.00	uM	23.360	
		31.25	uM	22.330	
		9.77	uM	12.260	
		3.05	uM	5.390	
		953.67	nM	2.190	
		298.02	nM	1.230	
		93.13	nM	2.430	
		29.10	nM	6.350	
		9.09	nM	4.350	
		2.84	nM	4.350	
		0.80	nM	3.230	
59-0112	149.19				
		100.00	uM	2.670	
		31.25	uM	4.670	
		9.77	uM	2.750	
		3.05	uM	3.790	
		953.67	nM	4.270	
		298.02	nM	1.150	
		93.13	nM	9.630	
		29.10	nM	0.920	
		9.09	nM	0.510	
		2.84	nM	12.900	
		0.80	nM	2.990	
59-0113	274.37				
		100.00	uM	22.010	
		31.25	uM	25.940	
		9.77	uM	7.500	
		3.05	uM	3.070	
		953.67	nM	-0.760	
		298.02	nM	-4.690	
		93.13	nM	-4.790	
		29.10	nM	5.090	
		9.09	nM	0.150	
		2.84	nM	-0.250	
		0.80	nM	0.150	
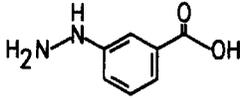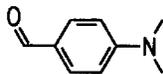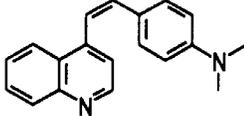
FIG. 3HH

59-0114	475.54				
		100.00	uM	52.030	
		31.25	uM	36.120	
		9.77	uM	25.840	
		3.05	uM	16.670	
		953.67	nM	12.540	
		298.02	nM	9.420	
		93.13	nM	−1.060	
		29.10	nM	2.160	
		9.09	nM	−6.000	
		2.84	nM	2.470	
		0.80	nM	−1.460	
59-0115	318.87				
		100.00	uM	73.700	
		31.25	uM	2.770	
		9.77	uM	−10.430	
		3.05	uM	−12.340	
		953.67	nM	−13.750	
		298.02	nM	−13.960	
		93.13	nM	−11.940	
		29.10	nM	−9.830	
		9.09	nM	−8.820	
		2.84	nM	−0.950	
		0.80	nM	−0.050	
59-0116	269.30				
		100.00	uM	31.380	
		31.25	uM	109.060	
		9.77	uM	231.070	
		3.05	uM	240.670	
		953.67	nM	132.020	
		298.02	nM	75.820	
		93.13	nM	53.250	
		29.10	nM	47.500	
		9.09	nM	39.440	
		2.84	nM	42.170	
		0.80	nM	31.180	
59-0117	268.38				
		100.00	uM	−68.520	

FIG. 3II

		31.25	uM	−7.450	
		9.77	uM	111.630	
		3.05	uM	64.340	
		953.67	nM	4.740	
		298.02	nM	−19.270	
		93.13	nM	−26.660	
		29.10	nM	−28.880	
		9.09	nM	−42.180	
		2.84	nM	−41.300	
		0.80	nM	−39.220	
59-0118	313.36				
		100.00	uM	−67.170	
		31.25	uM	−56.580	
		9.77	uM	−58.060	
		3.05	uM	−55.720	
		953.67	nM	−48.200	
		298.02	nM	−50.300	
		93.13	nM	−33.310	
		29.10	nM	−47.340	
		9.09	nM	−49.310	
		2.84	nM	−56.200	
		0.80	nM	−57.310	
59-0119	314.34				
		100.00	uM	167.500	
		31.25	uM	−29.240	
		9.77	uM	−57.800	
		3.05	uM	−52.030	
		953.67	nM	−54.240	
		298.02	nM	−53.870	
		93.13	nM	−38.110	
		29.10	nM	−55.100	
		9.09	nM	−52.270	
		2.84	nM	−53.500	
		0.80	nM	−43.650	
59-0120	504.49				
		100.00	uM	−82.790	
		31.25	uM	−80.470	
		9.77	uM	−66.800	
		3.05	uM	−50.790	
		953.67	nM	−54.240	
		298.02	nM	−45.250	
		93.13	nM	−50.660	

FIG. 3JJ

		29.10	nM	−50.300
		9.09	nM	−50.300
		2.84	nM	−50.300
		0.80	nM	−43.280
59-0121	245.29			
		100.00	uM	−79.690
		31.25	uM	−75.590
		9.77	uM	25.650
		3.05	uM	94.850
		953.67	nM	43.910
		298.02	nM	−1.800
		93.13	nM	−4.150
		29.10	nM	−22.050
		9.09	nM	−31.110
		2.84	nM	−26.760
		0.80	nM	−28.270
59-0122	333.39			
		100.00	uM	−19.050
		31.25	uM	−12.080
		9.77	uM	−7.610
		3.05	uM	25.210
		953.67	nM	83.580
		298.02	nM	87.220
		93.13	nM	63.890
		29.10	nM	42.680
		9.09	nM	45.320
		2.84	nM	37.780
		0.80	nM	27.030
59-0123	347.42			
		100.00	uM	34.430
		31.25	uM	34.710
		9.77	uM	38.620
		3.05	uM	55.100
		953.67	nM	51.900
		298.02	nM	41.410
		93.13	nM	29.970
		29.10	uM	13.760
		9.09	nM	17.120
		2.84	nM	13.480
		0.80	nM	1.190
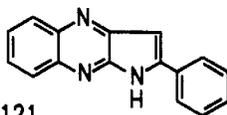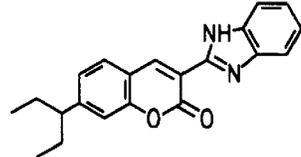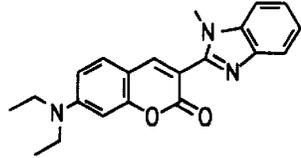
FIG. 3KK
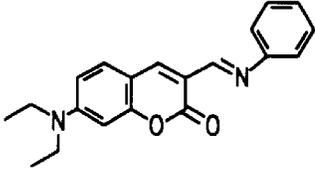
59-0124	350.44				
		100.00	uM	56.640	
		31.25	uM	81.500	
		9.77	uM	145.880	
		3.05	uM	135.830	
		953.67	nM	268.990	
		298.02	nM	224.290	
		93.13	nM	134.850	
		29.10	nM	91.690	
		9.09	nM	80.390	
		2.84	nM	63.060	
		0.80	nM	51.460	
59-0125	372.45				
		100.00	uM	-6.780	
		31.25	uM	67.530	
		9.77	uM	54.120	
		3.05	uM	28.700	
		953.67	nM	21.580	
		298.02	nM	22.280	
		93.13	nM	22.700	
		29.10	nM	1.630	
		9.09	nM	15.700	
		2.84	nM	9.840	
		0.80	nM	8.460	
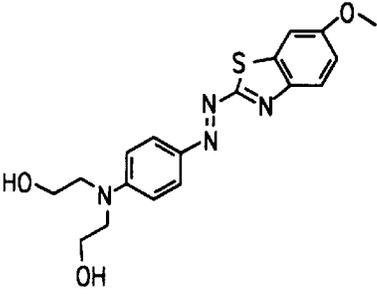
FIG. 3LL
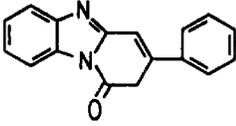
59-0126	260.30				
		100.00	uM	−17.390	
		31.25	uM	−13.100	
		9.77	uM	9.270	
		3.05	uM	40.530	
		953.67	nM	21.390	
		298.02	nM	25.660	
		93.13	nM	9.430	
		29.10	nM	6.360	
		9.09	nM	6.510	
		2.84	nM	0.080	
		0.80	nM	3.750	
59-0127	329.41				
		100.00	uM	−20.610	
		31.25	uM	−21.820	
		9.77	uM	−6.060	
		3.05	uM	−3.900	
		953.67	nM	−8.820	
		298.02	nM	−6.200	
		93.13	nM	11.880	
		29.10	nM	1.610	
		9.09	nM	3.600	
		2.84	nM	−2.070	
		0.80	nM	4.220	
59-0128	436.34				
		100.00	uM		
		31.25	uM		
		9.77	uM		
		3.05	uM		
		953.67	nM		
		298.02	nM		
		93.13	nM		
		29.10	nM		
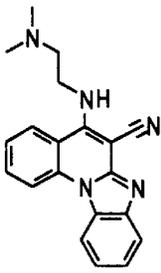
FIG. 3MM

		9.09	nM		
		2.84	nM		
		0.80	nM		
59-0129	277.71				
		100.00	uM	−20.46	
		31.25	uM	−21.21	
		9.77	uM	44.36	
		3.05	uM	4.38	
		953.67	nM	5.9	
		298.02	nM	3.6	
		93.13	nM	2.07	
		29.10	nM	4.22	
		9.09	nM	−0.68	
		2.84	nM	12.48	
		0.80	nM	−0.53	
59-0130	287.34				
		100.00	uM	4.38	
		31.25	uM	8.35	
		9.77	uM	5.91	
		3.05	uM	4.98	
		953.67	nM	0.39	
		298.02	nM	8.66	
		93.13	nM	2.85	
		29.10	nM	3.6	
		9.09	nM	4.36	
		2.84	nM	8.96	
		0.80	nM	24.75	
59-0131	331.22				
		100.00	uM	8.75	
		31.25	uM	0.12	
		9.77	uM	−10.38	
		3.05	uM	−6.39	
		953.67	nM	−2.81	
		298.02	nM	1.61	
		93.13	nM	−1.98	
		29.10	nM	−2.59	
		9.09	nM	0.14	
		2.84	nM	−5.77	

FIG. 3NN

		0.80	nM	−0.5	

59-0132	313.32				
		100.00	uM	−17.1	
		31.25	uM	−14.81	
		9.77	uM	−14.37	
		3.05	uM	−12.92	
		953.67	nM	−13.54	
		298.02	nM	−10.38	
		93.13	nM	−3.65	
		29.10	nM	−7.66	
		9.09	nM	−6.18	
		2.84	nM	−9.97	
		0.80	nM	−2.81	

59-0133	327.34				
		100.00	uM	−16.04	
		31.25	uM	−16.91	
		9.77	uM	−17.31	
		3.05	uM	−16.7	
		953.67	nM	−9.34	
		298.02	nM	−12.69	
		93.13	nM	−11.23	
		29.10	nM	−17.74	
		9.09	nM	6.02	
		2.84	nM	−4.71	
		0.80	nM	0.55	

FIG. 300
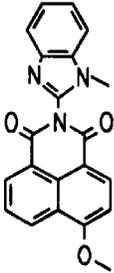
59-0134	357.37				
		100.00	uM		
		31.25	uM		
		9.77	uM		
		3.05	uM		
		953.67	nM		
		298.02	nM		
		93.13	nM		
		29.10	nM		
		9.09	nM		
		2.84	nM		
		0.80	nM		
59-0135	356.39				
		100.00	uM	−21.3	
		31.25	uM	−14.16	
		9.77	uM	−1.98	
		3.05	uM	0.97	
		953.67	nM	11.68	
		298.02	nM	−1.13	
		93.13	nM	−1.55	
		29.10	nM	−2.81	
		9.09	nM	12.11	
		2.84	nM	−5.75	
		0.80	nM	4.54	
59-0136	411.87				
		100.00	uM		
		31.25	uM		
		9.77	uM		
		3.05	uM		
		953.67	nM		
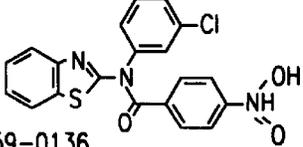
FIG. 3PP

		298.02	nM	
		93.13	nM	
		29.10	nM	
		9.09	nM	
		2.84	nM	
		0.80	nM	
59-0137	296.71			
		100.00	uM	
		31.25	uM	
		9.77	uM	
		3.05	uM	
		953.67	nM	
		298.02	nM	
		93.13	nM	
		29.10	nM	
		9.09	nM	
		2.84	nM	
		0.80	nM	
59-0138	340.81			
		100.00	uM	-6.91
		31.25	uM	-12.68
		9.77	uM	4.59
		3.05	uM	32.61
		953.67	nM	19.07
		298.02	nM	8.18
		93.13	nM	2.26
		29.10	nM	12.22
		9.09	nM	56.42
		2.84	nM	7.24
		0.80	nM	1.63
59-0139	340.43			
		100.00	uM	45.53
		31.25	uM	44.59
		9.77	uM	53.62
		3.05	uM	30.42
		953.67	nM	28.25
		298.02	uM	20.31
		93.13	nM	18.6
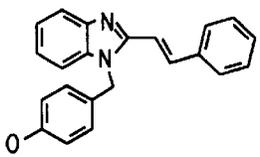
FIG. 3QQ

		29.10	nM	14.4
		9.09	nM	13.93
		2.84	nM	18.61
		0.80	nM	10.05
59-0140	289.17			
		100.00	uM	
		31.25	uM	
		9.77	uM	
		3.05	uM	
		953.67	nM	
		298.02	nM	
		93.13	nM	
		29.10	nM	
		9.09	nM	
		2.84	nM	
		0.80	nM	
59-0141	437.33			
		100.00	uM	-6.76
		31.25	uM	5.69
		9.77	uM	19.85
		3.05	uM	43.96
		953.67	nM	44.73
		298.02	nM	37.12
		93.13	nM	24.36
		29.10	nM	18.6
		9.09	nM	26.7
		2.84	nM	15.96
		0.80	nM	7.87
59-0142	379.29			
		100.00	uM	9.43
		31.25	uM	33.72
		9.77	uM	47.33
		3.05	uM	40.19
		953.67	nM	36.53
		298.02	uM	29.94
		93.13	nM	22.11
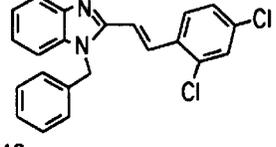
FIG. 3RR

		29.10	nM	20.9
		9.09	nM	19.14
		2.84	nM	10.38
		0.80	nM	17.12
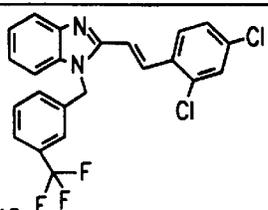
59-0143	447.29			
		100.00	uM	0.4
		31.25	uM	34.39
		9.77	uM	42.21
		3.05	uM	50.57
		953.67	nM	36.94
		298.02	nM	27.23
		93.13	nM	16.99
		29.10	nM	19.27
		9.09	nM	14.42
		2.84	nM	11.33
		0.80	nM	23.72
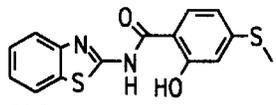
59-0144	316.40			
		100.00	uM	-14.59
		31.25	uM	-4.44
		9.77	uM	47.1
		3.05	uM	53.89
		953.67	nM	43.11
		298.02	nM	29.2
		93.13	nM	18.5
		29.10	nM	12.9
		9.09	nM	5.54
		2.84	nM	3.71
		0.80	nM	5.87
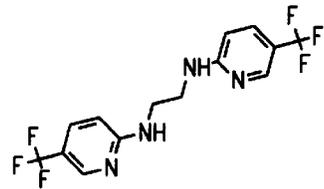
59-0145	350.27			
		100.00	uM	435.91
		31.25	uM	422.15
		9.77	uM	446.93
		3.05	uM	434.17
		953.67	nM	238.34
		298.02	uM	45.99
		93.13	nM	9.22
		29.10	uM	7.71
		9.09	nM	0.11

FIG. 3SS
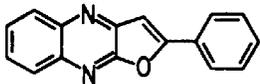
		2.84	nM	6.27
		0.80	nM	3.55
59-0146	246.27			
		100.00	uM	−63.05
		31.25	uM	4.42
		9.77	uM	−13.73
		3.05	uM	−16.45
		953.67	nM	−35.47
		298.02	nM	−51.25
		93.13	nM	−50.13
		29.10	nM	−42.92
		9.09	nM	−45.64
		2.84	nM	−56.58
		0.80	nM	−39.68
59-0147	314.36			
		100.00	uM	−85
		31.25	uM	−85
		9.77	uM	−80.29
		3.05	uM	−41.67
		953.67	nM	78.69
		298.02	nM	269.13
		93.13	nM	323.59
		29.10	nM	339.88
		9.09	nM	270.48
		2.84	nM	245.58
		0.80	nM	180.33
59-0148	291.35			
		100.00	uM	−68.38
		31.25	uM	−36.33
		9.77	uM	−2.3
		3.05	uM	12.12
		953.67	nM	−2.42
		298.02	nM	−16.21
		93.13	nM	−30.87
		29.10	nM	−35.58
		9.09	nM	−39.07
		2.84	nM	−41.18
		0.80	nM	−45.53
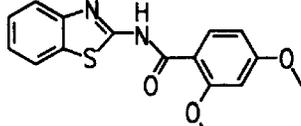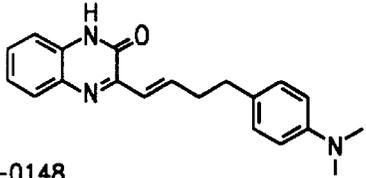
FIG. 3TT
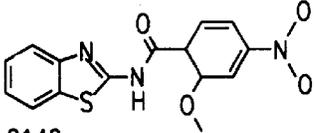
59-0149	329.33				
		100.00	uM	−16.9	
		31.25	uM	−1.8	
		9.77	uM	−0.53	
		3.05	uM	15.29	
		953.67	nM	78.78	
		298.02	nM	163.5	
		93.13	nM	223.57	
		29.10	nM	173.93	
		9.09	nM	122.3	
		2.84	nM	98.02	
		0.80	nM	69.06	
59-0150	304.39				
		100.00	uM	63.32	
		31.25	uM	193.32	
		9.77	uM	419.26	
		3.05	uM	497.21	
		953.67	nM	295.19	
		298.02	nM	193.35	
		93.13	nM	99.46	
		29.10	nM	69.96	
		9.09	nM	59	
		2.84	nM	52.16	
		0.80	nM	48.75	
59-0151	278.311				
59-0151		100.00	uM	−6.660	
		31.25	uM	16.240	
		9.77	uM	18.300	
		3.05	uM	11.690	
		953.67	nM	8.500	
		298.02	nM	9.070	
		93.13	nM	6.110	
		29.10	nM	5.880	
		9.09	nM	7.700	
		2.84	nM	2.000	
		0.80	nM	1.210	
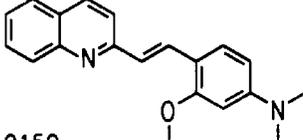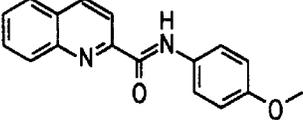
FIG. 3UU
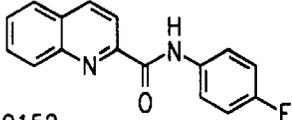
59-0152	266.275				
59-0152		100.00	uM	−6.890	
		31.25	uM	12.490	
		9.77	uM	21.950	
		3.05	uM	12.820	
		953.67	nM	7.350	
		298.02	nM	4.290	
		93.13	nM	9.750	
		29.10	nM	4.860	
		9.09	nM	1.320	
		2.84	nM	4.280	
		0.80	nM	4.160	
59-0153	282.73				
59-0153		100.00	uM	−4.150	
		31.25	uM	−0.390	
		9.77	uM	11.120	
		3.05	uM	14.540	
		953.67	nM	9.520	
		298.02	nM	11.570	
		93.13	nM	−0.160	
		29.10	nM	1.550	
		9.09	nM	−0.960	
		2.84	nM	4.730	
		0.80	nM	5.650	
59-0154	262.312				
59-0154		100.00	uM	0.290	
		31.25	uM	24.670	
		9.77	uM	15.680	
		3.05	uM	14.540	
		953.67	nM	13.170	
		298.02	nM	5.540	
		93.13	nM	2.690	
		29.10	nM	−1.190	
		9.09	nM	2.460	
		2.84	nM	4.170	
		0.80	nM	1.890	
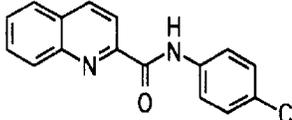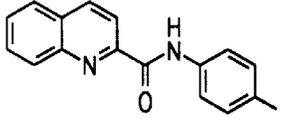
FIG. 3VV
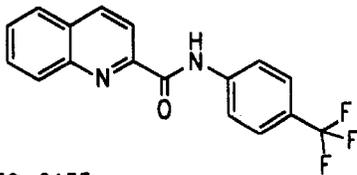
59-0155	316.282					
59-0155		100.00	uM	−2.950		
		31.25	uM	1.900		
		9.77	uM	−9.450		
		3.05	uM	−0.220		
		953.67	nM	0.690		
		298.02	nM	5.090		
		93.13	nM	−3.250		
		29.10	nM	0.530		
		9.09	nM	−1.900		
		2.84	nM	9.480		
		0.80	nM	−1.130		
59-0156	333.391					
59-0156		100.00	uM	5.840		
		31.25	uM	2.050		
		9.77	uM	7.960		
		3.05	uM	6.890		
		953.67	nM	−0.370		
		298.02	nM	−1.880		
		93.13	nM	−3.550		
		29.10	nM	−7.340		
		9.09	nM	−1.590		
		2.84	nM	2.650		
		0.80	nM	2.500		
59-0157	290.366					
59-0157		100.00	uM	−6.440		
		31.25	uM	14.920		
		9.77	uM	19.930		
		3.05	uM	11.440		
		953.67	nM	8.570		
		298.02	nM	−7.190		
		93.13	nM	0.080		
		29.10	nM	−0.230		
		9.09	nM	−4.460		
		2.84	nM	2.200		
		0.80	nM	9.920		

FIG. 3WW
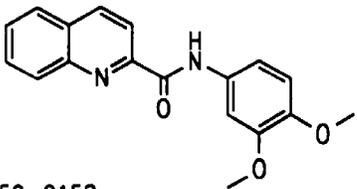
59-0158	308.337					
59-0158		100.00	uM	5.980		
		31.25	uM	3.720		
		9.77	uM	16.140		
		3.05	uM	27.060		
		953.67	nM	9.930		
		298.02	nM	11.900		
		93.13	nM	2.810		
		29.10	nM	3.110		
		9.09	nM	0.690		
		2.84	nM	1.900		
		0.80	nM	7.970		
59-0159	308.337					
59-0159		100.00	uM	2.790		
		31.25	uM	13.530		
		9.77	uM	4.700		
		3.05	uM	10.910		
		953.67	nM	2.800		
		298.02	nM	9.710		
		93.13	nM	4.830		
		29.10	nM	0.650		
		9.09	nM	5.900		
		2.84	nM	6.610		
		0.80	nM	6.250		
59-0160	319.408					
59-0160		100.00	uM	-5.060		
		31.25	uM	-3.390		
		9.77	uM	5.300		
		3.05	uM	15.910		
		953.67	nM	6.610		
		298.02	nM	11.380		
		93.13	nM	4.460		
		29.10	nM	3.520		
		9.09	nM	4.700		
		2.84	nM	-0.650		
		0.80	nM	7.560		
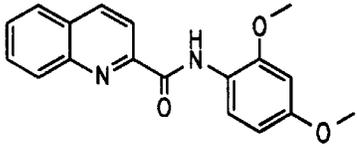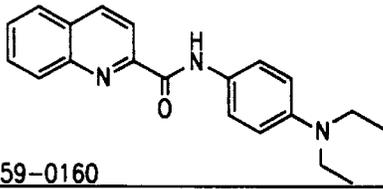
FIG. 3XX

59-0196	323.201		
59-0196		100.00	uM
		31.25	uM
		9.77	uM
		3.05	uM
		953.67	nM
		298.02	nM
		93.13	nM
		29.10	nM
		9.09	nM
		2.84	nM
		0.80	nM
59-0197	323.201		
59-0197		100.00	uM
		31.25	uM
		9.77	uM
		3.05	uM
		953.67	nM
		298.02	nM
		93.13	nM
		29.10	nM
		9.09	nM
		2.84	nM
		0.80	nM
59-0198	261.324		
59-0198		100.00	uM
		31.25	uM
		9.77	uM
		3.05	uM
		953.67	nM
		298.02	nM
		93.13	nM
		29.10	nM
		9.09	nM
		2.84	nM
		0.80	nM
59-0199	291.35		
59-0199		100.00	uM
		31.25	uM

FIG. 3YY

		Value	Unit			
		9.77	uM			
		3.05	uM			
		953.67	nM			
		298.02	nM			
		93.13	nM			
		29.10	nM			
		9.09	nM			
		2.84	nM			
		0.80	nM			
59-0200	342.351					
59-0200		100.00	uM			
		31.25	uM			
		9.77	uM			
		3.05	uM			
		953.67	nM			
		298.02	nM			
		93.13	nM			
		29.10	nM			
		9.09	nM			
		2.84	nM			
		0.80	nM			
59-0201	331.328					
59-0201		100.00	uM			
		31.25	uM			
		9.77	uM			
		3.05	uM			
		953.67	nM			
		298.02	nM			
		93.13	nM			
		29.10	nM			
		9.09	nM			
		2.84	nM			
		0.80	nM			
59-0202	300.336					
59-0202		100.00	uM			
		31.25	uM			
		9.77	uM			
		3.05	uM			
		953.67	nM			
		298.02	nM			
		93.13	nM			
		29.10	nM			

FIG. 3ZZ

		9.09 nM			
		2.84 nM			
		0.80 nM			
59-0203	292.338				
59-0203		100.00 uM			
		31.25 uM			
		9.77 uM			
		3.05 uM			
		953.67 nM			
		298.02 nM			
		93.13 nM			
		29.10 nM			
		9.09 nM			
		2.84 nM			
		0.80 nM			
59-0204	344.389				
59-0204		100.00 uM			
		31.25 uM			
		9.77 uM			
		3.05 uM			
		953.67 nM			
		298.02 nM			
		93.13 nM			
		29.10 nM			
		9.09 nM			
		2.84 nM			
		0.80 nM			
59-0205	318.782				
59-0205		100.00 uM			
		31.25 uM			
		9.77 uM			
		3.05 uM			
		953.67 nM			
		298.02 nM			
		93.13 nM			
		29.10 nM			
		9.09 nM			
		2.84 nM			
		0.80 nM			

FIG. 3AAA

59-0206							
59-0206	348.808						
		100.00	uM				
		31.25	uM				
		9.77	uM				
		3.05	uM				
		953.67	nM				
		298.02	nM				
		93.13	nM				
		29.10	nM				
		9.09	nM				
		2.84	nM				
		0.80	nM				
59-0207	348.808						
59-0207		100.00	uM				
		31.25	uM				
		9.77	uM				
		3.05	uM				
		953.67	nM				
		298.02	nM				
		93.13	nM				
		29.10	nM				
		9.09	nM				
		2.84	nM				
		0.80	nM				
59-0208	338.307						
59-0208		100.00	uM				
		31.25	uM				
		9.77	uM				
		3.05	uM				
		953.67	nM				
		298.02	nM				
		93.13	nM				
		29.10	nM				
		9.09	nM				
		2.84	nM				
		0.80	nM				

FIG. 3BBB

59-0209	247.297						
59-0209		100.00	uM				
		31.25	uM				
		9.77	uM				
		3.05	uM				
		953.67	nM				
		298.02	nM				
		93.13	nM				
		29.10	nM				
		9.09	nM				
		2.84	nM				
		0.80	nM				
59-0210	297.376						
59-0210		100.00	uM				
		31.25	uM				
		9.77	uM				
		3.05	uM				
		953.67	nM				
		298.02	nM				
		93.13	nM				
		29.10	nM				
		9.09	nM				
		2.84	nM				
		0.80	nM				
59-8000	298.342						
59-8000		100.00	uM				
		31.25	uM				
		9.77	uM				
		3.05	uM				
		953.67	nM				
		298.02	nM				
		93.13	nM				
		29.10	nM				
		9.09	nM				
		2.84	nM				
		0.80	nM				

FIG. 3CCC

59-8001	255.273						
59-8001		100.00	uM				
		31.25	uM				
		9.77	uM				
		3.05	uM				
		953.67	nM				
		298.02	nM				
		93.13	nM				
		29.10	nM				
		9.09	nM				
		2.84	nM				
		0.80	nM				
59-8002	302.286						
59-8002		100.00	uM				
		31.25	uM				
		9.77	uM				
		3.05	uM				
		953.67	nM				
		298.02	nM				
		93.13	nM				
		29.10	nM				
		9.09	nM				
		2.84	nM				
		0.80	nM				
59-8003	270.288						
59-8003		100.00	uM				
		31.25	uM				
		9.77	uM				
		3.05	uM				
		953.67	nM				
		298.02	nM				
		93.13	nM				
		29.10	nM				
		9.09	nM				
		2.84	nM				
		0.80	nM				

FIG. 3DDD

59-8004	331.371						
59-8004		100.00	uM				
		31.25	uM				
		9.77	uM				
		3.05	uM				
		953.67	nM				
		298.02	nM				
		93.13	nM				
		29.10	nM				
		9.09	nM				
		2.84	nM				
		0.80	nM				
59-8005	299.326						
59-8005		100.00	uM				
		31.25	uM				
		9.77	uM				
		3.05	uM				
		953.67	nM				
		298.02	nM				
		93.13	nM				
		29.10	nM				
		9.09	nM				
		2.84	nM				
		0.80	nM				
59-8006	327.38						
59-8006		100.00	uM				
		31.25	uM				
		9.77	uM				
		3.05	uM				
		953.67	nM				
		298.02	nM				
		93.13	nM				
		29.10	nM				
		9.09	nM				
		2.84	nM				
		0.80	nM				

FIG. 3EEE

59-8007	297.354						
59-8007		100.00	uM				
		31.25	uM				
		9.77	uM				
		3.05	uM				
		953.67	nM				
		298.02	nM				
		93.13	nM				
		29.10	nM				
		9.09	nM				
		2.84	nM				
		0.80	nM				
59-8008	261.299						
59-8008		100.00	uM				
		31.25	uM				
		9.77	uM				
		3.05	uM				
		953.67	nM				
		298.02	nM				
		93.13	nM				
		29.10	nM				
		9.09	nM				
		2.84	nM				
		0.80	nM				
59-8009	289.313						
59-8009		100.00	uM				
		31.25	uM				
		9.77	uM				
		3.05	uM				
		953.67	nM				
		298.02	nM				
		93.13	nM				
		29.10	nM				
		9.09	nM				

FIG. 3FFF

		2.84	nM			
		0.80	nM			
59-8010	261.299					
59-8010		100.00	uM			
		31.25	uM			
		9.77	uM			
		3.05	uM			
		953.67	nM			
		298.02	nM			
		93.13	nM			
		29.10	nM			
		9.09	nM			
		2.84	nM			
		0.80	nM			
59-8011	285.299					
59-8011		100.00	uM			
		31.25	uM			
		9.77	uM			
		3.05	uM			
		953.67	nM			
		298.02	nM			
		93.13	nM			
		29.10	nM			
		9.09	nM			
		2.84	nM			
		0.80	nM			
59-8012	294.285					
59-8012		100.00	uM			
		31.25	uM			
		9.77	uM			
		3.05	uM			
		953.67	nM			
		298.02	nM			

FIG. 3GGG

		93.13	nM			
		29.10	nM			
		9.09	nM			
		2.84	nM			
		0.80	nM			
59-8013	301.364					
59-8013		100.00	uM			
		31.25	uM			
		9.77	uM			
		3.05	uM			
		953.67	nM			
		298.02	nM			
		93.13	nM			
		29.10	nM			
		9.09	nM			
		2.84	nM			
		0.80	nM			
59-8014	377.396					
59-8014		100.00	uM			
		31.25	uM			
		9.77	uM			
		3.05	uM			
		953.67	nM			
		298.02	nM			
		93.13	nM			
		29.10	nM			
		9.09	nM			
		2.84	nM			
		0.80	nM			
59-8015	285.299					
59-8015		100.00	uM			
		31.25	uM			
		9.77	uM			
		3.05	uM			

FIG. 3HHH

		953.67	nM			
		298.02	nM			
		93.13	nM			
		29.10	nM			
		9.09	nM			
		2.84	nM			
		0.80	nM			
59-8016	285.299					
59-8016		100.00	uM			
		31.25	uM			
		9.77	uM			
		3.05	uM			
		953.67	nM			
		298.02	nM			
		93.13	nM			
		29.10	nM			
		9.09	nM			
		2.84	nM			
		0.80	nM			

FIG. 3III

CHEMISTRY		CONCENTRATION		ABA-S
51-2229				
51-2229		100.00	uM	125.320
		10.00		28.260
	210.236	2.00		20.140
		0.40		-9.740
		0.08		-9.710
92-3052				
92-3052		131.056	uM	-9.28
		13.106		113.80
	381.516	2.621		12.61
		0.524		20.25
		0.105		24.45
92-3390				
92-3390		145.012	uM	-8.05
		14.501		31.57
	344.798	2.900		139.68
		0.580		49.82
		0.116		21.01
92-3552				
92-3552		214.326	uM	108.15
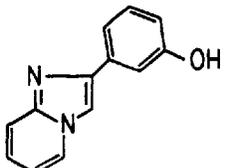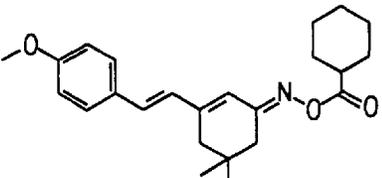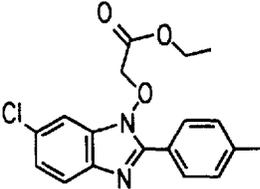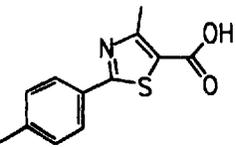
FIG. 4A

	21.433		69.74
233.289	4.287		31.59
	0.857		39.70
	0.171		18.29
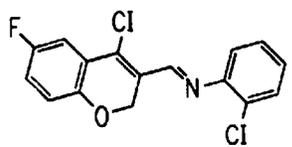
92-6353

92-6353	155.199	uM	
	31.040		204.14
322.166	15.520		154.94
	3.104		28.09
	1.552		
	0.310		3.53
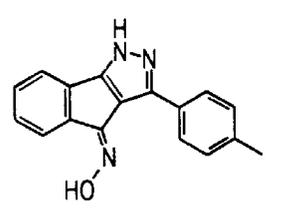
92-8007

92-8007	181.613	uM	−16.65
	36.323		58.65
275.311	18.161		142.33
	3.632		45.65
	1.816		
	0.363		4.47
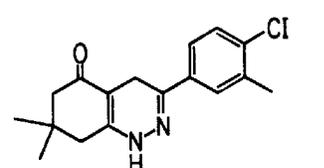
92-8215

92-8215	165.123	uM	32.90
	33.025		151.06
302.805	16.512		132.29
	3.302		59.90
	1.651		
	0.330		23.34

FIG. 4B
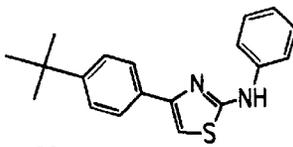
92-8258				
92-8258		162.102	uM	−16.65
		32.420		157.44
	308.447	16.210		101.04
		3.242		39.02
		1.621		
		0.324		12.78
92-8362				
92-8362		154.647	uM	136.79
		30.929		137.00
	323.318	15.465		65.02
		3.093		17.34
		1.546		
		0.309		0.41
92-8372				
92-8372		150.045	uM	63.76
		30.009		134.71
	333.234	15.004		92.06
		3.001		31.35
		1.500		
		0.300		13.20
92-9183				
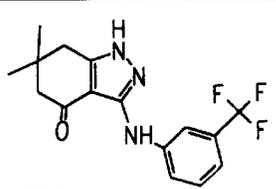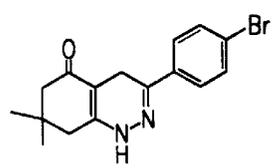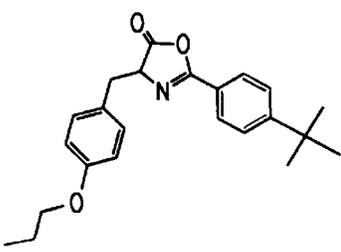
FIG. 4C

92-9183		137.568	uM	-22.80
		13.757		16.61
	363.457	2.751		101.96
		1.376		
		0.550		58.17
		0.110		38.47
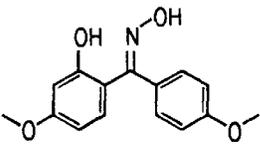
(structure) 93-0215

93-0215		182.957	uM	115.230
		18.296		88.110
	273.288	3.659		20.870
		0.732		-28.680
		0.146		5.250
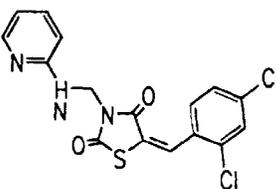
(structure) 93-0399

93-0399		131.491	uM	128.130
		13.149		38.560
	380.253	2.630		41.240
		0.526		-4.910
		0.105		3.910
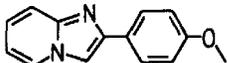
(structure) 93-0587

93-0587		222.953	uM	178.130
		22.295		60.410
	224.263	4.459		-0.180
		0.892		-3.470
		0.178		-8.460
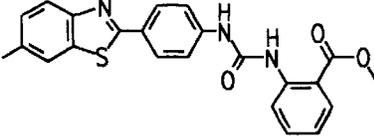
(structure) 93-1327

93-1327		119.764	uM	-42.000
		11.976		119.130
	417.487	2.395		67.930
		0.479		8.520

FIG. 4D
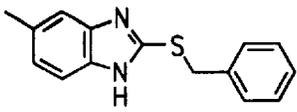
		0.096		14.870
93-1340				
93-1340		196.576	uM	-31.290
		19.658		127.340
	254.355	3.932		35.710
		0.786		37.630
		0.157		7.280
93-1474				
93-1474		145.940	uM	-45.110
		14.594		110.290
	342.607	2.919		35.080
		0.584		109.040
		0.117		40.130
93-1766				
93-1766		144.348	uM	
		14.435		
	346.366	2.887		
		0.577		
		0.115		
93-1866				
93-1866		148.214	uM	75.940
		14.821		173.150
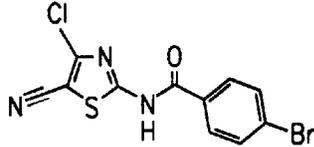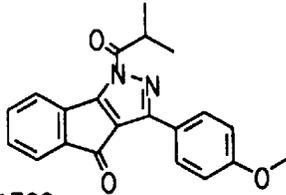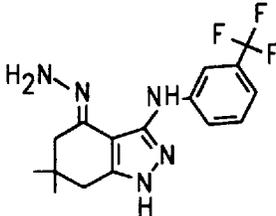
FIG. 4E
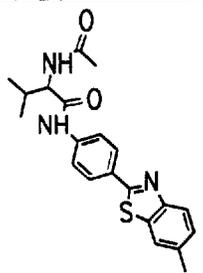
850-7377			
850-7377		131.062	uM
		13.106	
	381.498	2.621	
		0.524	
		0.105	

-50.32
68.27
116.61
61.26
25.86
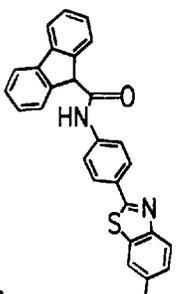
850-7413			
850-7413		111.964	uM
		11.196	
	446.572	2.239	
		0.448	
		0.090	

-40.44
-2.55
157.01
78.73
23.91
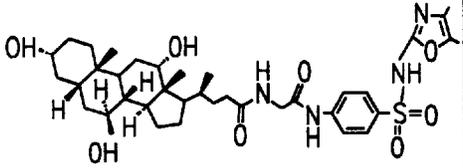
850-7449			
850-7449		69.938	uM
		6.994	
	714.923	1.399	
		0.280	
		0.056	

-42.42
73.79
112.16
75.24
26.36

FIG. 4F

93-7485				
93-7485		143.099	uM	-42.91
		14.310		28.36
	349.409	2.862		153.04
		0.572		74.27
		0.114		50.28
93-7991				
93-7991		127.367	uM	-16.87
		12.737		8.95
	392.585	2.547		105.51
		0.509		47.53
		0.102		54.26
850-8170				
850-8170		101.513	uM	-33.79
		10.151		158.65
	492.55	2.030		126.27
		0.406		43.05
		0.061		50.00
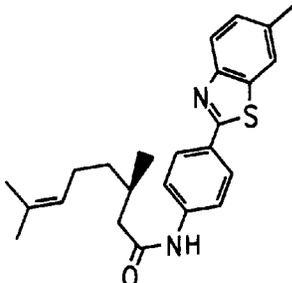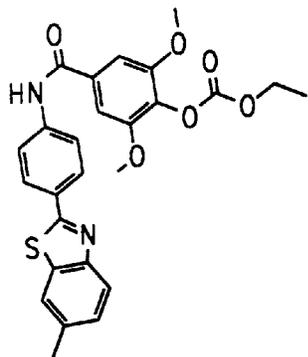
FIG. 4G
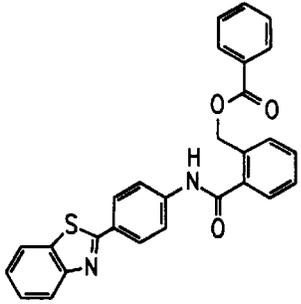
850-8205		
850-8205	104.478 uM	-39.52
	10.448	51.18
478.57	2.090	163.82
	0.418	106.06
	0.084	73.68
850-8241		
850-8241	82.279 uM	-2.07
	8.226	181.77
607.685	1.646	118.23
	0.329	66.73
	0.066	36.14
850-8278		
850-8278	139.101 uM	-40.09
	13.910	39.00
359.451	2.782	182.38
	0.556	122.84
	0.111	78.90
850-8387		
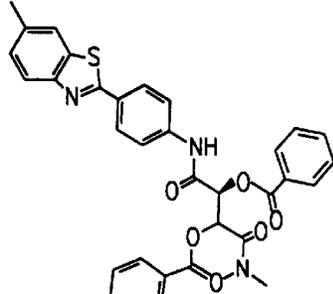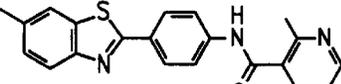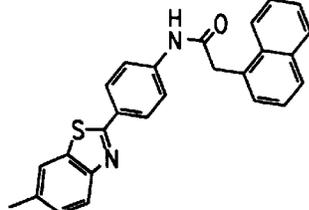
FIG. 4H

850-8387		122.392	uM	-17.06
		12.239		130.31
	408.523	2.448		129.75
		0.490		62.69
		0.098		40.74
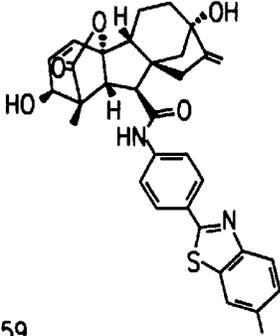
850-8459

850-8459		87.921	uM	-21.13
		8.792		11.30
	568.692	1.758		131.92
		0.352		71.13
		0.070		58.55
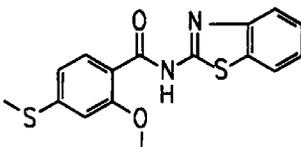
850-8613

850-8613		151.319	uM	-26.05
		15.132		85.55
	330.428	3.026		381.37
		0.605		255.32
		0.121		122.93
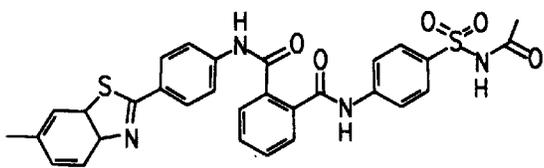
850-8637

850-8637		85.518	uM	-25.17
		8.552		33.35
	584.673	1.710		122.49
		0.342		57.19
		0.068		37.42

FIG. 41
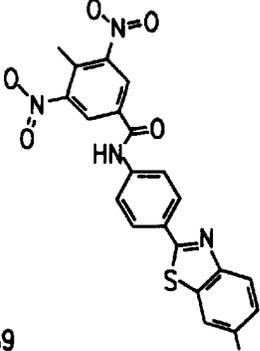
850-8889				
850-8889		111.493	uM	-17.470
		11.149		142.970
	448.457	2.230		74.150
		0.446		21.010
		0.089		8.530
850-8964				
850-8964		95.156	uM	-30.92
		9.516		44.99
	525.454	1.903		126.29
		0.381		49.84
		0.076		44.99
850-9071				
850-9071		109.998	uM	-24.620
		11.000		84.120
	454.552	2.200		149.030
		0.440		54.540
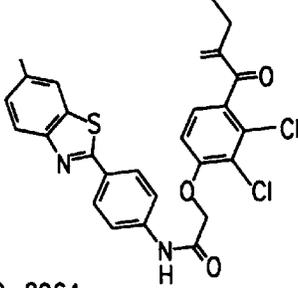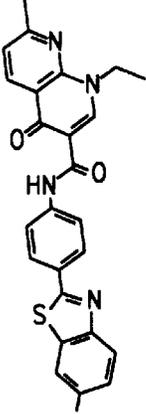
FIG. 4J
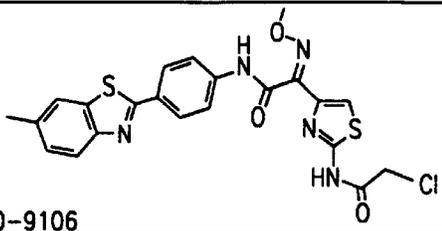
		0.088		23.540
850-9106				
850-9106		100.000	uM	-15.710
		10.000		99.820
	499.999	2.000		111.960
		0.400		74.500
		0.080		23.150
850-9142				
850-9142		85.596	uM	-14.980
		8.560		165.770
	584.138	1.712		66.650
		0.342		27.780
		0.068		0.670
850-9179				
850-9179		105.357	uM	-24.630
		10.536		105.200
	474.579	2.107		89.280
		0.421		46.110
		0.064		19.160
850-9212				
850-9212		92.139	uM	-26.580
		9.214		40.900
	542.657	1.843		111.690
		0.369		76.950
		0.074		30.840
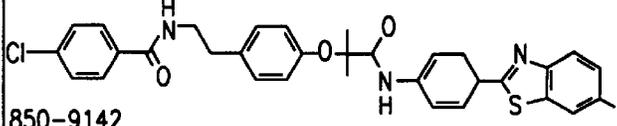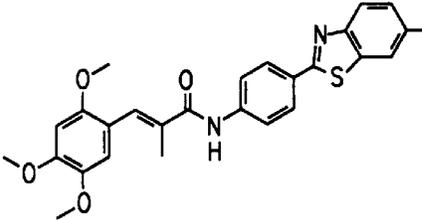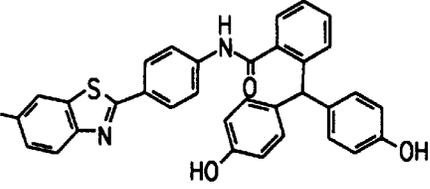
FIG. 4K
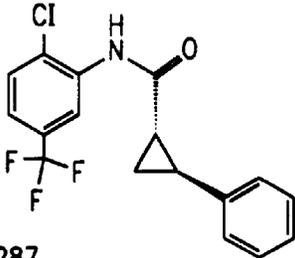
850-9287				
850-9287		147.170	uM	−15.82
		14.717		15.82
	339.744	2.943		130.71
		0.589		91.11
		0.118		69.05
850-9356				
850-9356		99.506	uM	−24.650
		9.951		83.140
	502.482	1.990		168.810
		0.396		45.470
		0.080		9.740
850-9467				
850-9467		120.646	uM	−19.800
		12.065		112.990
	414.436	2.413		122.730
		0.483		43.520
		0.097		33.140
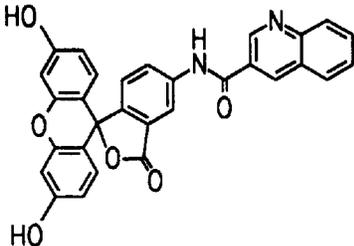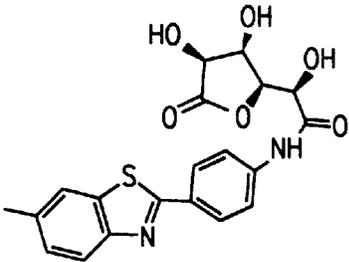
FIG. 4L

850-9576				
850-9576		111.724	uM	−27.430
		11.172		90.560
	447.532	2.234		101.610
		0.447		44.900
		0.089		19.930
895-0262				
895-0262		166.019	uM	−19.18
		33.204		−12.60
	301.169	16.602		148.28
		3.320		−2.23
		0.332		−3.07
895-0268				
895-0268		128.383	uM	−18.87
		25.677		40.25
	369.458	12.836		169.96
		2.568		195.29
		0.257		14.02
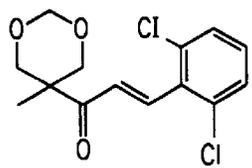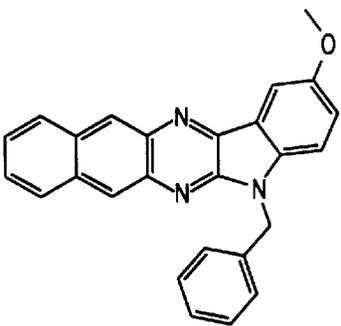
FIG. 4M
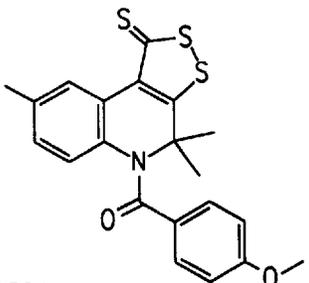
895-0594				-21.63
895-0594		120.896	uM	-21.63
		12.090		25.89
	413.58	2.418		122.10
		0.484		75.32
		0.097		39.42
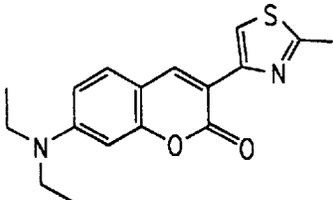
895-0857				
895-0857		159.026	uM	-30.46
		15.903		146.74
	314.407	3.181		74.54
		0.636		25.82
		0.127		3.66
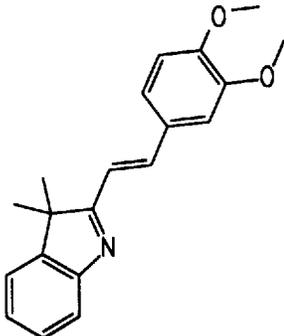
895-0964				
895-0964		162.655	uM	-31.06
		16.265		325.06
	307.393	3.253		87.51
		0.651		40.39
		0.130		16.03

FIG. 4N
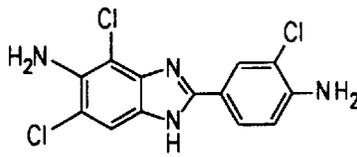
895−1161		
895−1161	152.625	uM
	15.263	
327.602	3.053	
	0.611	
	0.122	
895−1420		
895−1420	220.965	uM
	22.097	
226.279	4.419	
	0.884	
	0.177	
895−1679		
895−1679	180.910	uM
	18.091	
276.383	3.618	
	0.724	
	0.145	
895−1691		
895−1691	182.992	uM
	18.292	
273.34	3.658	
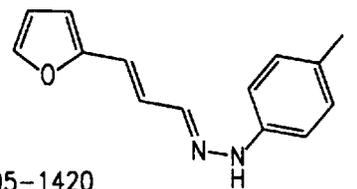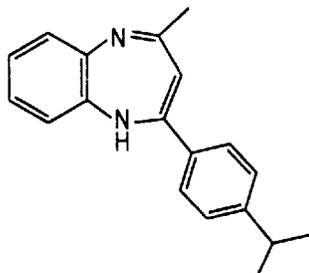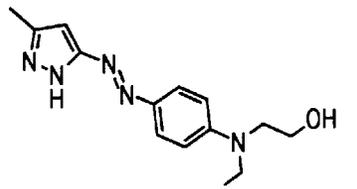
− 5.51
109.31
56.06
29.49
24.71
−19.47
110.90
49.94
33.65
20.06
−30.36
111.72
102.83
18.01
0.44
−16.29
50.84
105.70

FIG. 40
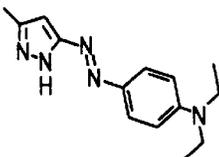
		0.732		60.23
		0.146		23.42
895-1754				
895-1754		194.295	uM	-31.44
		19.430		132.78
	257.341	3.886		75.39
		0.777		39.30
		0.155		16.19
895-1888				
895-1888		212.504	uM	-33.65
		21.250		29.75
	235.286	4.250		148.84
		0.850		73.77
		0.170		28.14
895-2474				
895-2474		184.952	uM	-20.74
		18.495		128.69
	270.335	3.699		66.37
		0.740		43.27
		0.148		19.44
895-2475				
895-2475		162.159	uM	265.41
		16.216		287.86
	308.337	3.243		227.34
		0.649		65.40
		0.130		28.96
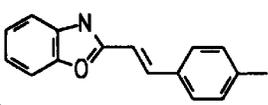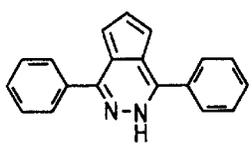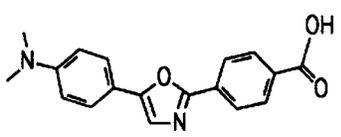
FIG. 4P
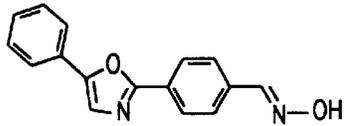
895-2544				
895-2544		189.186	uM	17.53
		18.919		136.50
	264.284	3.784		59.15
		0.757		24.75
		0.151		11.86
895-3113				
895-3113		160.067	uM	−22.22
		16.007		224.52
	312.372	3.201		68.46
		0.640		43.36
		0.128		30.56
895-3306				
895-3306		172.170	uM	−23.24
		17.217		38.63
	290.41	3.443		333.10
		0.689		164.63
		0.136		64.33
895-3810				
895-3810		196.973	uM	89.79
		19.897		106.75
	251.289	3.979		73.78
		0.796		33.45
		0.159		16.86
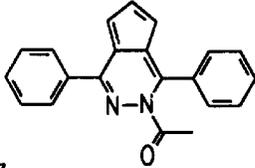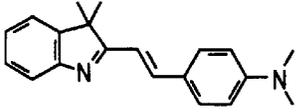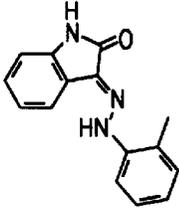
FIG. 4Q
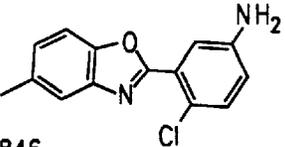
895-3846		
895-3846	193.267	uM
	19.327	
258.708	3.865	
	0.773	
	0.155	

-21.41
13.40
114.46
52.12
38.29
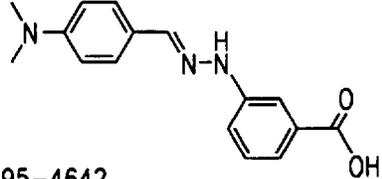
895-4642		
895-4642	176.473	uM
	17.647	
283.331	3.529	
	0.706	
	0.141	

6.97
383.99
447.51
304.86
100.45
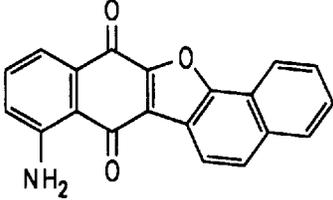
895-4843		
895-4843	159.581	uM
	15.958	
313.312	3.192	
	0.638	
	0.128	

-17.18
24.54
100.12
60.37
27.85
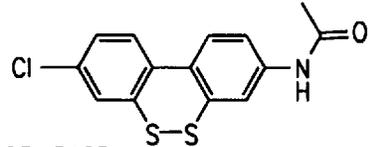
895-5185		
895-5185	162.433	uM
	16.243	
307.821	3.249	
	0.650	
	0.130	

- 6.47
213.42
107.83
46.75
18.27

FIG. 4R

895–5960				
895–5960		103.348	uM	−10.03
		10.335		156.04
	483.796	2.067		62.07
		0.413		34.47
		0.083		7.24
895–6353				
895–6353		167.555	uM	−10.45
		16.755		21.59
	298.408	3.351		101.77
		0.670		54.91
		0.134		24.15
895–6643				
895–6643		145.862	uM	100.09
		14.586		74.25
	342.786	2.917		16.86
		0.583		−0.89
		0.117		−7.94
895–7828				
895–7828		184.973	uM	−32.44
		18.497		−29.24
	270.31	3.699		85.15
		0.740		125.64
		0.148		−30.80
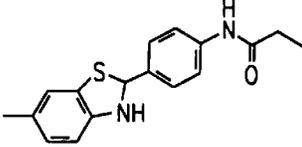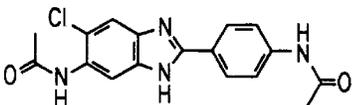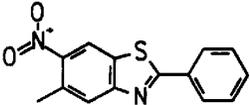
FIG. 4S
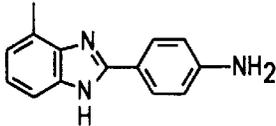
895-7985				
895-7985		223.935	uM	122.070
		22.394		3.900
	223.279	4.479		-7.790
		0.896		5.520
		0.179		-2.270
895-7997				
895-7997		176.461	uM	
		17.646		
	283.349	3.529		
		0.706		
		0.141		
895-8053				
895-8053		134.398	uM	
		13.440		
	372.03	2.666		
		0.538		
		0.108		
895-8137				
895-8137		169.326	uM	
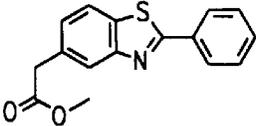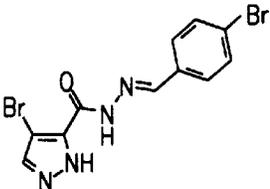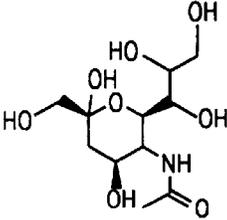
FIG. 4T

	16.933			
295.288	3.387			
	0.677			
	0.135			
895-8185				
895-8185	219.057	uM		
	21.906			
228.251	4.361			
	0.876			
	0.175			
895-8286				
895-8286	142.765	uM	142.210	
	14.277		40.390	
350.225	2.855		17.850	
	0.571		−10.890	
	0.114		6.580	
895-8383				
895-8383	191.774	uM	−44.020	
	19.177		76.480	
260.724	3.835		135.940	
	0.767		77.030	
	0.153		37.630	
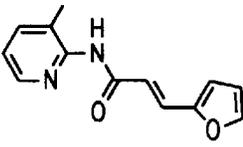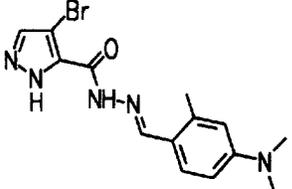
FIG. 4U

895-8862			
895-8862		165.876	uM
		16.588	
	301.43	3.318	
		0.664	
		0.133	

54.72
159.21
113.97
41.96
38.28
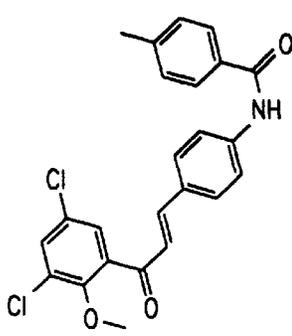
895-9683			
895-9683		113.552	uM
		11.355	
	440.326	2.271	
		0.454	
		0.091	

-20.67
201.56
12.55
0.62
-0.69
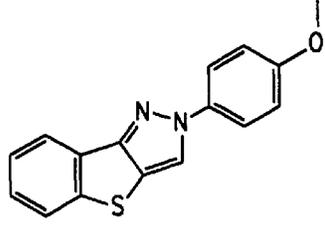
895-9896			
895-9896		178.349	uM
		17.835	
	280.349	3.567	
		0.713	
		0.143	

-29.16
0.62
182.84
118.55
42.75

FIG. 4V

896-0122				
896-0122		190.610	uM	−14.15
		19.061		151.42
	262.316	3.812		56.90
		0.762		19.20
		0.152		11.42
896-0246				
896-0246		154.888	uM	−17.57
		15.489		34.35
	322.814	3.096		102.03
		0.620		46.52
		0.124		20.52
896-0255				
896-0255		123.000	uM	−17.14
		12.300		67.75
	406.504	2.480		168.78
		0.492		61.27
		0.098		49.97
896-0345				
896-0345		107.532	uM	−18.86
		10.753		77.80
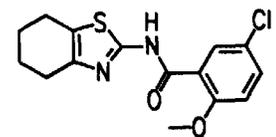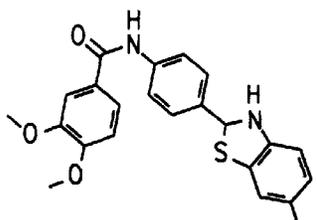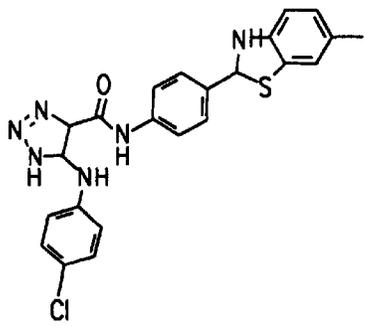
FIG. 4W

464.979	2.151			188.94
	0.430			106.12
	0.086			37.18
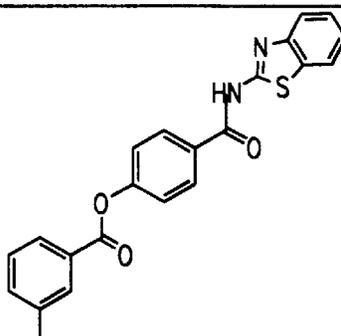
896-0390

896-0390	128.718	uM		-16.90
	12.872			87.23
388.445	2.574			210.25
	0.515			73.35
	0.103			28.25
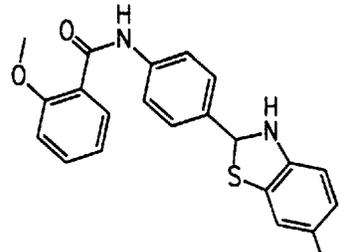
896-0535

896-0535	132.810	uM		-10.41
	13.281			73.84
376.478	2.656			199.80
	0.531			102.12
	0.106			35.72
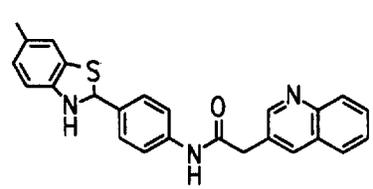
896-0554

896-0554	121.499	uM		-16.32
	12.150			105.48
411.527	2.430			115.43
	0.486			53.88
	0.097			27.03

FIG. 4X
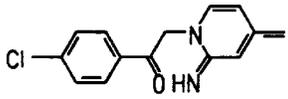
896-0686				
896-0686		191.774	uM	-19.80
		19.177		176.04
	260.724	3.835		115.02
		0.767		97.67
		0.153		25.27
896-0692				
896-0692		131.269	uM	22.78
		13.127		149.23
	380.897	2.625		78.33
		0.525		51.06
		0.105		46.12
896-0719				
896-0719		91.950	uM	-6.49
		9.195		187.43
	543.774	1.839		127.43
		0.366		50.04
		0.074		36.16
896-0773				
896-0773		147.228	uM	-13.94
		14.723		175.33
	339.609	2.945		221.91
		0.589		52.48
		0.118		32.99
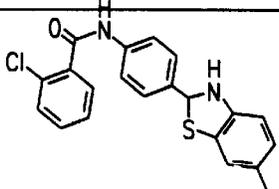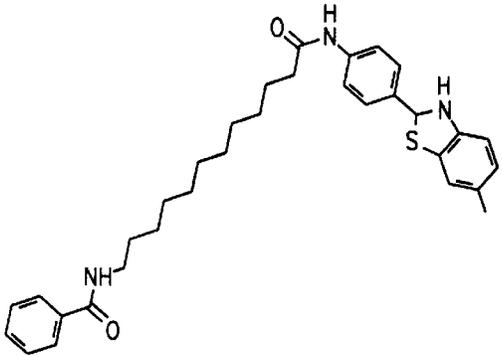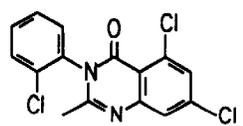
FIG. 4Y

896-0819

896-0819		124.219	uM	-16.20
		12.422		70.03
	402.516	2.484		165.79
		0.497		82.61
		0.099		49.06

896-0853

896-0853		157.546	uM	-27.06
		15.755		75.38
	317.367	3.151		208.69
		0.630		33.08
		0.126		32.63

896-0921

896-0921		174.583	uM	-19.59
		17.458		44.07
	266.397	3.492		103.23
		0.698		54.02
		0.140		23.86

FIG. 4Z
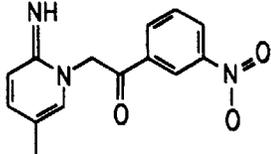
896-0936				
896-0936		184.314	uM	−16.20
		18.431		153.61
	271.276	3.686		184.53
		0.737		79.16
		0.147		32.61
896-0959				
896-0959		103.796	uM	−1.73
		10.380		102.48
	461.703	2.076		61.61
		0.415		63.56
		0.083		48.27
896-1201				
896-1201		106.343	uM	−45.70
		10.834		92.57
	461.496	2.167		191.83
		0.433		47.22
		0.087		58.25
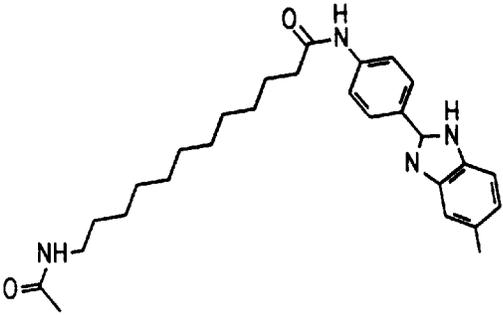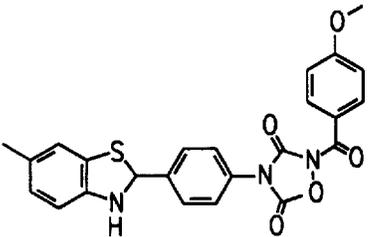
FIG. 4AA
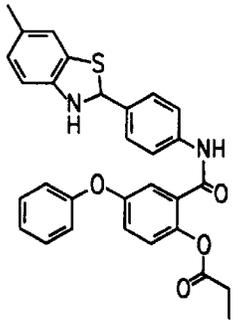
896-1301				
896-1301		97.922	uM	-24.32
		9.792		102.49
	510.612	1.958		139.28
		0.392		97.89
		0.078		23.45
896-1349				
896-1349		115.883	uM	-39.92
		11.588		55.08
	431.47	2.318		122.68
		0.464		67.25
		0.093		3.39
896-1362				
896-1362		142.749	uM	1,073.91
		14.275		1,082.17
	360.266	2.855		884.71
		0.571		-9.82
		0.114		-20.37
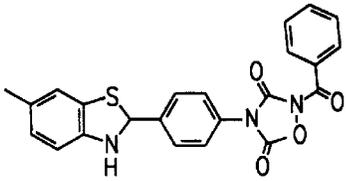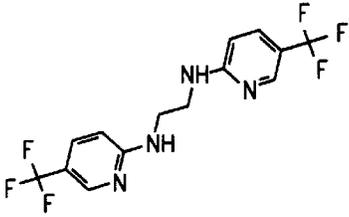
FIG. 4BB

METHODS FOR TREATING BONE DEFICIT CONDITIONS WITH BENZOTHIAZOLE

This is a continuation of U.S. application Ser. No. 08/735,881 filed Oct. 23, 1986, now abandoned.

TECHNICAL FIELD

The invention relates to compositions and methods for use in limiting undesired bone loss in a vertebrate at risk of such bone loss, in treating conditions that are characterized by undesired bone loss or by the need for bone growth, in treating fractures, and in treating cartilage disorders. More specifically, the invention concerns the use of specific classes of compounds identified or characterized by a high throughput screening assay.

BACKGROUND ART

Bone is not a static tissue. It is subject to constant breakdown and resynthesis in a complex process mediated by osteoblasts, which produce new bone, and osteoclasts, which destroy bone. The activities of these cells are regulated by a large number of cytokines and growth factors, many of which have now been identified and cloned. Mundy has described the current knowledge related to these factors (Mundy, G. R. *Clin Orthop* 324:24–28, 1996; Mundy, G. R. *J Bone Miner Res* 8:S505–10, 1993).

Although there is a great deal of information available on the factors which influence the breakdown and resorption of bone, information on growth factors which stimulate the formation of new bone is more limited. Investigators have searched for sources of such activities, and have found that bone tissue itself is a storehouse for factors which have the capacity for stimulating bone cells. Thus, extracts of bovine bone tissue obtained from slaughterhouses contain not only structural proteins which are responsible for maintaining the structural integrity of bone, but also biologically active bone growth factors which can stimulate bone cells to proliferate. Among these latter factors are transforming growth factor β, the heparin-binding growth factors (acidic and basic fibroblast growth factor), the insulin-like growth factors (insulin-like growth factor I and insulin-like growth factor II), and a recently described family of proteins called bone morphogenetic proteins (BMPs). All of these growth factors have effects on other types of cells, as well as on bone cells.

The BMPs are novel factors in the extended transforming growth factor β superfamily. They were first identified by Wozney J. et al. *Science* (1988) 242:1528–34, using gene cloning techniques, following earlier descriptions characterizing the biological activity in extracts of demineralized bone (Urist M. *Science* (1965) 150:893–99). Recombinant BMP2 and BMP4 can induce new bone formation when they are injected locally into the subcutaneous tissues of rats (Wozney J. *Molec Reprod Dev* (1992) 32:160–67). These factors are expressed by normal osteoblasts as they differentiate, and have been shown to stimulate osteoblast differentiation and bone nodule formation in vitro as well as bone formation in vivo (Harris S. et al. *J. Bone Miner Res* (1994) 9:855–63). This latter property suggests potential usefulness as therapeutic agents in diseases which result in bone loss.

The cells which are responsible for forming bone are osteoblasts. As osteoblasts differentiate from precursors to mature bone-forming cells, they express and secrete a number of enzymes and structural proteins of the bone matrix, including Type-I collagen, osteocalcin, osteopontin and alkaline phosphatase (Stein G. et al. *Curr Opin Cell Biol*

(1990) 2:1018–27; Harris S. et al. (1994), supra). They also synthesize a number of growth regulatory peptides which are stored in the bone matrix, and are presumably responsible for normal bone formation. These growth regulatory peptides include the BMPs (Harris S. et al. (1994), supra). In studies of primary cultures of fetal rat calvarial osteoblasts, BMPs 1, 2, 3, 4, and 6 are expressed by cultured cells prior to the formation of mineralized bone nodules (Harris S. et al. (1994), supra). Like alkaline phosphatase, osteocalcin and osteopontin, the BMPs are expressed by cultured osteoblasts as they proliferate and differentiate.

Although the BMPs are potent stimulators of bone formation in vitro and in vivo, there are disadvantages to their use as therapeutic agents to enhance bone healing. Receptors for the bone morphogenetic proteins have been identified in many tissues, and the BMPs themselves are expressed in a large variety of tissues in specific temporal and spatial patterns. This suggests that BMPs may have effects on many tissues other than bone, potentially limiting their usefulness as therapeutic agents when administered systemically. Moreover, since they are peptides, they would have to be administered by injection. These disadvantages impose severe limitations to the development of BMPs as therapeutic agents.

There is a plethora of conditions which are characterized by the need to enhance bone formation. Perhaps the most obvious is the case of bone fractures, where it would be desirable to stimulate bone growth and to hasten and complete bone repair. Agents that enhance bone formation would also be useful in facial reconstruction procedures. Other bone deficit conditions include bone segmental defects, periodontal disease, metastatic bone disease, osteolytic bone disease and conditions where connective tissue repair would be beneficial, such as healing or regeneration of cartilage defects or injury. Also of great significance is the chronic condition of osteoporosis, including age-related osteoporosis and osteoporosis associated with post-menopausal hormone status. Other conditions characterized by the need for bone growth include primary and secondary hyperparathyroidism, disuse osteoporosis, diabetes-related osteoporosis, and glucocorticoid-related osteoporosis. In addition, or alternatively, the compounds of the present invention may modulate metabolism, proliferation and/or differentiation of normal or aberrant cells or tissues.

There are currently no satisfactory pharmaceutical approaches to managing any of these conditions. Bone fractures are still treated exclusively using casts, braces, anchoring devices and other strictly mechanical means. Further bone deterioration associated with post-menopausal osteoporosis has been decreased or prevented with estrogens or bisphosphonates.

U.S. Pat. No. 5,280,040 discloses a class of compounds which are 3,4-diaryl chromans. These compounds can be considered derivatives of 2,3,4 triphenyl butanol, where the hydroxy at the 1-position forms an ether with the ortho position of the phenyl group substituted at the 4-position of the butanol. The parent 3,4-diaryl chromans do not contain nitrogen atoms in the aromatic moieties or their linkers. A preferred compound, centchroman, contains a nitrogen substituent only in one of the substituents on a phenyl moiety. These compounds are disclosed in the '040 patent as useful in the treatment of osteoporosis.

The present invention discloses compounds useful for limiting or treating bone deficit conditions, and for other uses that should be apparent to those skilled in the art from the teachings herein.

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DISCLOSURE OF THE INVENTION

The invention provides compounds that can be administered as ordinary pharmaceuticals and have the metabolic effect of enhancing bone growth. The compounds of the invention can be identified using an assay for their ability to activate control elements associated with these factors. Thus, the invention is directed to methods and compositions for stimulating the growth of skeletal (bone) tissue, which methods and compositions use, as active ingredients, compounds wherein two aromatic systems are coupled so as to be spaced apart from each other by about 1.5 to about 15 Angstroms. The thus-linked systems (including the linker coupling them) generally include at least one nitrogen atom.

Therefore, the compounds useful in the invention can be described as having the formula Ar^1-linker-Ar^2, wherein each of Ar^1 and Ar^2 is independently an aromatic system and the linker portion of the formula spaces Ar^1 and Ar^2 apart by a distance of approximately 1.5–15 Angstroms. Ar^1, Ar^2 and the linker may optionally be substituted with non interfering substituents. In the useful compounds, there is generally at least one nitrogen atom in either Ar^1, Ar^2 and/or the linker, independent of any substituents thereon. Preferably, the compounds of the invention contain at least one additional heteroatom selected from the group consisting of N, S and O, independent of any substituent.

Thus, in one aspect, the invention is directed to a method to treat a condition in a vertebrate animal characterized by a deficiency in, or need for, bone growth replacement and/or an undesirable level of bone resorption, which method comprises administering to a vertebrate subject in need of such treatment an effective amount of a compound of the formula:

(1)

wherein

each R^1 and R^2 is independently a non-interfering substituent;

m is an integer of 0–4;

the dotted line represents an optional π bond; and

L is a flexible non-conjugating linker.

In other aspects, the invention relates to pharmaceutical compositions for use in the method.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 gives a schematic representation of the compounds used as active ingredients in the methods of the invention.

FIG. 2 shows the dose response curve for a positive control compound, designated 59-0008.

FIGS. 3 and 4 show illustrative compounds of the invention and the results obtained with them in an in vitro test.

MODES OF CARRYING OUT THE INVENTION

A rapid throughput screening test for compounds capable of stimulating expression of a reporter gene linked to a BMP promoter (a surrogate for the production of bone morphogenetic factors that are endogenously produced) is described in U.S. application Ser. No. 08/458,434, filed Jun. 2, 1995, the contents of which are incorporated herein by reference.

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This assay is also described as a portion of a study of immortalized murine osteoblasts (derived from a mouse expressing a transgene composed of a BMP2 promoter driving expression of T-antigen) in Ghosh-Choudhery, N. et al. *Endocrinology* (1996) 137:331–39. In this study, the immortalized cells were stably transfected with a plasmid containing a luciferase reporter gene driven by a mouse BMP2 promoter (–2736/114 bp), and responded in a dose-dependent manner to recombinant human BMP2.

Briefly, the assay utilizes cells transformed permanently or transiently with constructs in which the promoter of a bone morphogenetic protein, specifically BMP2 or BMP4, is coupled to a reporter gene, typically luciferase. These transformed cells are then evaluated for the production of the reporter gene product; compounds that activate the BMP promoter will drive production of the reporter protein, which can be readily assayed. Over 40,000 compounds have been subjected to this rapid screening technique, and only a very small percentage are able to elicit a level of production of luciferase 5-fold greater than that produced by vehicle. Compounds that activate the BMP promoter share certain structural characteristics not present in inactive compounds. The active compounds ("BMP promoter-active compounds" or "active compounds") are useful in promoting bone or cartilage growth, and thus in the treatment of vertebrates in need of bone or cartilage growth.

BMP promoter-active compounds can be examined in a variety of other assays that test specificity and toxicity. For instance, non-BMP promoters or response elements can be linked to a reporter gene and inserted into an appropriate host cell. Cytotoxicity can be determined by visual or microscopic examination of BMP promoter- and/or non-BMP promoter-reporter gene-containing cells, for instance. Alternatively, nucleic acid and/or protein synthesis by the cells can be monitored. For in vivo assays, tissues may be removed and examined visually or microscopically, and optionally examined in conjunction with dyes or stains that facilitate histologic examination. In assessing in vivo assay results, it may also be useful to examine biodistribution of the test compound, using conventional medicinal chemistry/animal model techniques.

As used herein, "limit" or "limiting" and "treat" or "treatment" are interchangeable terms. The terms include a postponement of development of bone deficit symptoms and/or a reduction in the severity of such symptoms that will or are expected to develop. The terms further include ameliorating existing bone or cartilage deficit symptoms, preventing additional symptoms, ameliorating or preventing the underlying metabolic causes of symptoms, preventing or reversing bone resorption and/or encouraging bone growth. Thus, the terms denote that a beneficial result has been conferred on a vertebrate subject with a cartilage, bone or skeletal deficit, or with the potential to develop such deficit.

By "bone deficit" is meant an imbalance in the ratio of bone formation to bone resorption, such that, if unmodified, the subject will exhibit less bone than desirable, or the subject's bones will be less intact and coherent than desired. Bone deficit may also result from fracture, from surgical intervention or from dental or periodontal disease. By "cartilage defect" is meant damaged cartilage, less cartilage than desired, or cartilage that is less intact and coherent than desired.

Representative uses of the compounds of the present invention include: repair of bone defects and deficiencies, such as those occuring in closed, open and non-union fractures; prophylactic use in closed and open fracture

reduction; promotion of bone healing in plastic surgery; stimulation of bone ingrowth into non-cemented prosthetic joints and dental implants; elevation of peak bone mass in pre-menopausal women; treatment of growth deficiencies; treatment of peridontal disease and defects, and other tooth repair processes; increase in bone formation during distraction osteogenesis; and treatment of other skeletal disorders, such as age-related osteoporosis, post-menopausal osteoporosis, glucocorticoid-induced osteoporosis or disuse osteoporosis and arthritis. The compounds of the present invention can also be useful in repair of congenital, trauma-induced or surgical resection of bone (for instance, for cancer treatment), and in cosmetic surgery. Further, the compounds of the present invention can be used for limiting or treating cartilage defects or disorders, and may be useful in wound healing or tissue repair.

Bone or cartilage deficit or defect can be treated in vertebrate subjects by administering compounds of the invention which have been identified through suitable screening assays and which exhibit certain structural characteristics. The compositions of the invention may be administered systemically or locally. For systemic use, the compounds herein are formulated for parenteral (e.g., intravenous, subcutaneous, intramuscular, intraperitoneal, intranasal or transdermal) or enteral (e.g., oral or rectal) delivery according to conventional methods. Intravenous administration will be by a series of injections or by continuous infusion over an extended period. Administration by injection or other routes of discretely spaced administration will generally be performed at intervals ranging from weekly to once to three times daily. Alternatively, the compounds disclosed herein may be administered in a cyclical manner (administration of disclosed compound; followed by no administration; followed by administration of disclosed compound, and the like). Treatment will continue until the desired outcome is achieved. In general, pharmaceutical formulations will include a compound of the present invention in combination with a pharmaceutically acceptable vehicle, such as saline, buffered saline, 5% dextrose in water, borate-buffered saline containing trace metals or the like. Formulations may further include one or more excipients, preservatives, solubilizers, buffering agents, albumin to prevent protein loss on vial surfaces, lubricants, fillers, stabilizers, etc. Methods of formulation are well known in the art and are disclosed, for example, in *Remington's Pharmaceutical Sciences*, Gennaro, ed., Mack Publishing Co., Easton Pa., 1990, which is incorporated herein by reference. Pharmaceutical compositions for use within the present invention can be in the form of sterile, non-pyrogenic liquid solutions or suspensions, coated capsules, suppositories, lyophilized powders, transdermal patches or other forms known in the art. Local administration may be by injection at the site of injury or defect, or by insertion or attachment of a solid carrier at the site, or by direct, topical application of a viscous liquid. For local administration, the delivery vehicle preferably provides a matrix for the growing bone or cartilage, and more preferably is a vehicle that can be absorbed by the subject without adverse effects.

Delivery of compounds herein to wound sites may be enhanced by the use of controlled-release compositions, such as those described in pending U.S. patent application Ser. No. 07/871,246 (corresponding to WIPO publication WO 93/20859, which is incorporated herein by reference in its entirety). Films of this type are particularly useful as coatings for prosthetic devices and surgical implants. The films may, for example, be wrapped around the outer sur-

faces of surgical screws, rods, pins, plates and the like. Implantable devices of this type are routinely used in orthopedic surgery. The films can also be used to coat bone filling materials, such as hydroxyapatite blocks, demineralized bone matrix plugs, collagen matrices and the like. In general, a film or device as described herein is applied to the bone at the fracture site. Application is generally by implantation into the bone or attachment to the surface using standard surgical procedures.

In addition to the copolymers and carriers noted above, the biodegradable films and matrices may include other active or inert components. Of particular interest are those agents that promote tissue growth or infiltration, such as growth factors. Exemplary growth factors for this purpose include epidermal growth factor (EGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), transforming growth factors (TGFs), parathyroid hormone (PTH), leukemia inhibitory factor (LIF), and insulin-like growth factors (IGFs). Agents that promote bone growth, such as bone morphogenetic proteins (U.S. Pat. No. 4,761, 471; PCT Publication WO 90/11366), osteogenin (Sampath et al. *Proc. Natl. Acad. Sci. USA* (1987) 84:7109–13) and NaF (Tencer et al. *J. Biomed. Mat. Res.* (1989) 23:571–89) are also preferred. Biodegradable films or matrices include calcium sulfate, tricalcium phosphate, hydroxyapatite, polylactic acid, polyanhydrides, bone or dermal collagen, pure proteins, extracellular matrix components and combinations thereof. Such biodegradable materials may be used in combination with non-biodegradable materials, to provide desired mechanical, cosmetic or tissue or matrix interface properties.

Alternative methods for delivery of compounds of the present invention include use of ALZET osmotic minipumps (Alza Corp., Palo Alto, Calif.); sustained release matrix materials such as those disclosed in Wang et al. (PCT Publication WO 90/11366); electrically charged dextran beads, as disclosed in Bao et al. (PCT Publication WO 92/03125); collagen-based delivery systems, for example, as disclosed in Ksander et al. *Ann. Surg.* (1990) 211(3):288–94; methylcellulose gel systems, as disclosed in Beck et al. *J. Bone Min. Res.* (1991) 6(11):1257–65; and alginate-based systems, as disclosed in Edelman et al. *Biomaterials* (1991) 12:619–26. Other methods well known in the art for sustained local delivery in bone include porous coated metal protheses that can be impregnated and solid plastic rods with therapeutic compositions incorporated within them.

The compounds of the present invention may also be used in conjunction with agents that inhibit bone resorption. Antiresorptive agents, such as estrogen, bisphosphonates and calcitonin, are preferred for this purpose. More specifically, the compounds disclosed herein may be administered for a period of time (for instance, months to years) sufficient to obtain correction of a bone deficit condition. Once the bone deficit condition has been corrected, the vertebrate can be administered an anti-resorptive compound to maintain the corrected bone condition. Alternatively, the compounds disclosed herein may be administered with an anti-resorptive compound in a cyclical manner (administration of disclosed compound, followed by anti-resorptive, followed by disclosed compound, and the like).

In additional formulations, conventional preparations such as those described below may be used.

Aqueous suspensions may contain the active ingredient in admixture with pharmacologically acceptable excipients, comprising suspending agents, such as methyl cellulose; and wetting agents, such as lecithin, lysolethicin or long-chain

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fatty alcohols. The said aqueous suspensions may also contain preservatives, coloring agents, flavoring agents and sweetening agents in accordance with industry standards.

Preparations for topical and local application comprise aerosol sprays, lotions, gels and ointments in pharmaceutically appropriate vehicles which may comprise lower aliphatic alcohols, polyglycols such as glycerol, polyethylene glycol, esters of fatty acids, oils and fats, and silicones. The preparations may further comprise antioxidants, such as ascorbic acid or tocopherol, and preservatives, such as p-hydroxybenzoic acid esters.

Parenteral preparations comprise particularly sterile or sterilized products. Injectable compositions may be provided containing the active compound and any of the well known injectable carriers. These may contain salts for regulating the osmotic pressure.

If desired, the osteogenic agents can be incorporated into liposomes by any of the reported methods of preparing liposomes for use in treating various pathogenic conditions. The present compositions may utilize the compounds noted above incorporated in liposomes in order to direct these compounds to macrophages, monocytes, other cells and tissues and organs which take up the liposomal composition. The liposome-incorporated compounds of the invention can be utilized by parenteral administration, to allow for the efficacious use of lower doses of the compounds. Ligands may also be incorporated to further focus the specificity of the liposomes.

Suitable conventional methods of liposome preparation include, but are not limited to, those disclosed by Bangham, A. D. et al. *J Mol Biol* (1965) 23:238–252, Olson, F. et al. *Biochim Biophys Acta* (1979) 557:9–23, Szoka, F. et al. *Proc Natl Acad Sci USA* (1978) 75:4194–4198, Mayhew, E. et al. Biochim Biophys (1984) 775:169175, Kim, S. et al. *Biochim Biophys Acta* (1983) 728:339:348, and Mayer, et al. *Biochim Biophys Acta* (1986) 858:161–168.

The liposomes may be made from the present compounds in combination with any of the conventional synthetic or natural phospholipid liposome materials including phospholipids from natural sources such as egg, plant or animal sources such as phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, sphingomyelin, phosphatidylserine, or phosphatidylinositol. Synthetic phospholipids that may also be used, include, but are not limited to: dimyristoylphosphatidylcholine, dioleoylphosphatidylcholine, dipalmitoylphosphatidylcholine and distearoylphosphatidycholine, and the corresponding synthetic phosphatidylethanolamines and phosphatidylglycerols. Cholesterol or other sterols, cholesterol hemisuccinate, glycolipids, cerebrosides, fatty acids, gangliosides, sphingolipids, 1,2-bis(oleoyloxy)-3-(trimethyl ammonio) propane (DOTAP), N-[1-(2,3-dioleoyl) propyl-N,N,N-trimethylammonium chloride (DOTMA), and other cationic lipids may be incorporated into the liposomes, as is known to those skilled in the art. The relative amounts of phospholipid and additives used in the liposomes may be varied if desired. The preferred ranges are from about 60 to 90 mole percent of the phospholipid; cholesterol, cholesterol hemisuccinate, fatty acids or cationic lipids may be used in amounts ranging from 0 to 50 mole percent. The amounts of the present compounds incorporated into the lipid layer of liposomes can be varied with the concentration of their lipids ranging from about 0.01 to about 50 mole percent.

Using conventional methods, approximately 20 to 30% of the compound present in solution can be entrapped in liposomes; thus, approximately 70 to 80% of the active compound is wasted. In contrast, where the compound is incorporated into liposomes, virtually all of the compound is incorporated into the liposome, and essentially none of the active compound is wasted.

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The liposomes with the above formulations may be made still more specific for their intended targets with the incorporation of monoclonal antibodies or other ligands specific for a target. For example, monoclonal antibodies to the BMP receptor may be incorporated into the liposome by linkage to phosphatidylethanolamine (PE) incorporated into the liposome by the method of Leserman, L. et al. *Nature* (1980) 288:602–604.

Veterinary uses of the disclosed compounds are also contemplated. Such uses would include limitation or treatment of bone or cartilage deficits or defects in domestic animals, livestock and thoroughbred horses. The compounds described herein can also modify a target tissue or organ environment, so as to attract bone-forming cells to an environment in need of such cells.

The compounds of the present invention may also be used to stimulate growth of bone-forming cells or their precursors, or to induce differentiation of bone-forming cell precursors, either in vitro or ex vivo. As used herein, the term "precursor cell" refers to a cell that is committed to a differentiation pathway, but that generally does not express markers or function as a mature, fully differentiated cell. As used herein, the term "mesenchymal cells" or "mesenchymal stem cells" refers to pluripotent progenitor cells that are capable of dividing many times, and whose progeny will give rise to skeletal tissues, including cartilage, bone, tendon, ligament, marrow stroma and connective tissue (see A. Caplan *J. Orthop. Res.* (1991) 9:641–50). As used herein, the term "osteogenic cells" includes osteoblasts and osteoblast precursor cells. More particularly, the disclosed compounds are useful for stimulating a cell population containing marrow mesenchymal cells, thereby increasing the number of osteogenic cells in that cell population. In a preferred method, hematopoietic cells are removed from the cell population, either before or after stimulation with the disclosed compounds. Through practice of such methods, osteogenic cells may be expanded. The expanded osteogenic cells can be infused (or reinfused) into a vertebrate subject in need thereof. For instance, a subject's own mesenchymal stem cells can be exposed to compounds of the present invention ex vivo, and the resultant osteogenic cells could be infused or directed to a desired site within the subject, where further proliferation and/or differentiation of the osteogenic cells can occur without immunorejection. Alternatively, the cell population exposed to the disclosed compounds may be immortalized human fetal osteoblastic or osteogenic cells. If such cells are infused or implanted in a vertebrate subject, it may be advantageous to "immunoprotect" these non-self cells, or to immunosuppress (preferably locally) the recipient to enhance transplantation and bone or cartilage repair.

Within the present invention, an "effective amount" of a composition is that amount which produces a statistically significant effect. For example, an "effective amount" for therapeutic uses is the amount of the composition comprising an active compound herein required to provide a clinically significant increase in healing rates in fracture repair; reversal of bone loss in osteoporosis; reversal of cartilage defects or disorders; prevention or delay of onset of osteoporosis; stimulation and/or augmentation of bone formation in fracture non-unions and distraction osteogenesis; increase and/or acceleration of bone growth into prosthetic devices; and repair of dental defects. Such effective amounts will be determined using routine optimization techniques and are dependent on the particular condition to be treated, the condition of the patient, the route of administration, the formulation, and the judgment of the practitioner and other factors evident to those skilled in the art. The dosage required for the compounds of the invention (for example, in osteoporosis where an increase in bone formation is desired) is manifested as a statistically significant difference in bone

mass between treatment and control groups. This difference in bone mass may be seen, for example, as a 5–20% or more increase in bone mass in the treatment group. Other measurements of clinically significant increases in healing may include, for example, tests for breaking strength and tension, breaking strength and torsion, 4-point bending, increased connectivity in bone biopsies and other biomechanical tests well known to those skilled in the art. General guidance for treatment regimens is obtained from experiments carried out in animal models of the disease of interest.

The dosage of the compounds of the invention will vary according to the extent and severity of the need for treatment, the activity of the administered compound, the general health of the subject, and other considerations well known to the skilled artisan. Generally, they can be administered to a typical human on a daily basis on an oral dose of about 0.1 mg/kg–1000 mg/kg, and more preferably from about 1 mg/kg to about 200 mg/kg. The parenteral dose will appropriately be 20–100% of the oral dose.

Screening Assays

The osteogenic activity of the compounds used in the methods of the invention can be verified using in vitro screening techniques, such as the assessment of transcription of a reporter gene coupled to a bone morphogenetic protein-associated promoter, as described above, or in alternative assays such as the following:

Technique for Neonatal Mouse Calvaria Assay (In vitro)

This assay is similar to that described by Gowen M. & Mundy G. J Immunol (1986) 136:2478–82. Briefly, four days after birth, the front and parietal bones of ICR Swiss white mouse pups are removed by microdissection and split along the sagittal suture. The bones are incubated in BGJb medium (Irvine Scientific, Santa Ana, Calif.) plus 0.02% (or lower concentration) β-methylcyclodextrin, wherein the medium also contains test or control substances, at 37° C. in a humidified atmosphere of 5% CO_2 and 95% air for 96 hours.

Following this, the bones are removed from the incubation media and fixed in 10% buffered formalin for 24–48 hours, decalcified in 14% EDTA for 1 week, processed through graded alcohols; and embedded in paraffin wax. Three μm sections of the calvaria are prepared. Representative sections are selected for histomorphometric assessment of bone formation and bone resorption. Bone changes are measured on sections cut 200 μm apart. Osteoblasts and osteoclasts are identified by their distinctive morphology.

Other auxiliary assays can be used as controls to determine non-BMP promoter-mediated effects of test compounds. For example, mitogenic activity can be measured using screening assays featuring a serum-response element (SRE) as a promoter and a luciferase reporter gene. More specifically, these screening assays can detect signalling through SRE-mediated pathways, such as the protein kinase C pathway. For instance, an osteoblast activator SRE-luciferase screen and an insulin mimetic SRE-luciferase screen are useful for this purpose. Similarly, test compound stimulation of cAMP response element (CRE)-mediated pathways can also be assayed. For instance, cells transfected with receptors for PTH and calcitonin (two bone-active agents) can be used in CRE-luciferase screens to detect elevated cAMP levels. Thus, the BMP promoter specificity of a test compound can be examined through use of these types of auxiliary assays.

In Vivo Assay of Effects of Compounds on Murine Calvarial Bone Growth

Male ICR Swiss white mice, aged 4–6 weeks and weighing 13–26 gm, are employed, using 4–5 mice per group. The calvarial bone growth assay is performed as described in PCT application WO 95/24211. Briefly, the test compound or appropriate control vehicle is injected into the subcutaneous tissue over the right calvaria of normal mice.

Typically, the control vehicle is the vehicle in which the compound was solubilized, and is PBS containing 5% DMSO or is PBS containing Tween (2 μl/10 ml). The animals are sacrificed on day 14 and bone growth measured by histomorphometry. Bone samples for quantitation are cleaned from adjacent tissues and fixed in 10% buffered formalin for 24–48 hours, decalcified in 14% EDTA for 1–3 weeks, processed through graded alcohols; and embedded in paraffin wax. Three to five μm sections of the calvaria are prepared, and representative sections are selected for histomorphometric assessment of the effects on bone formation and bone resorption. Sections are measured by using a camera lucida attachment to trace directly the microscopic image onto a digitizing plate. Bone changes are measured on sections cut 200 μm apart, over 4 adjacent 1×1 mm fields on both the injected and noninjected sides of the calvaria. New bone is identified by its characteristic woven structure, and osteoclasts and osteoblasts are identified by their distinctive morphology. Histomorphometry software (OsteoMeasure, Osteometrix, Inc., Atlanta) is used to process digitizer input to determine cell counts and measure areas or perimeters.

Additional In Vivo Assays

Lead compounds can be further tested in intact animals using an in vivo, dosing assay. Prototypical dosing may be accomplished by subcutaneous, intraperitoneal or oral administration, and may be performed by injection, sustained release or other delivery techniques. The time period for administration of test compound may vary (for instance, 28 days as well as 35 days may be appropriate). An exemplary, in vivo subcutaneous dosing assay may be conducted as follows:

In a typical study, 70 three-month-old female Sprague-Dawley rats are weight-matched and divided into seven groups, with ten animals in each group. This includes a baseline control group of animals sacrificed at the initiation of the study; a control group administered vehicle only; a PBS-treated control group; and a positive control group administered a compound (non-protein or protein) known to promote bone growth. Three dosage levels of the compound to be tested are administered to the remaining three groups.

Briefly, test compound, positive control compound, PBS, or vehicle alone is administered subcutaneously once per day for 35 days. All animals are injected with calcein nine days and two days before sacrifice (two injections of calcein administered each designated day). Weekly body weights are determined. At the end of the 35-day cycle, the animals are weighed and bled by orbital or cardiac puncture. Serum calcium, phosphate, osteocalcin, and CBCs are determined. Both leg bones (femur and tibia) and lumbar vertebrae are removed, cleaned of adhering soft tissue, and stored in 70% ethanol for evaluation, as performed by peripheral quantitative computed tomography (pQCT; Ferretti, J. Bone (1995) 17:353S–64S), dual energy X-ray absorptiometry (DEXA; Laval-Jeantet A. et al. Calcif Tissue Intl (1995) 56:14–18; J. Casez et al. Bone and Mineral (1994) 26:61–68) and/or histomorphometry. The effect of test compounds on bone remodeling can thus be evaluated.

Lead compounds can also be tested in acute ovariectomized animals (prevention model) using an in vivo dosing assay. Such assays may also include an estrogen-treated group as a control. An exemplary subcutaneous dosing assay is performed as follows:

In a typical study, 80 three-month-old female Sprague-Dawley rats are weight-matched and divided into eight groups, with ten animals in each group. This includes a baseline control group of animals sacrificed at the initiation of the study; three control groups (sham ovariectomized (sham OVX)+vehicle only; ovariectomized (OVX)+vehicle only; PBS-treated OVX); and a control OVX group that is administered a compound known to promote bone growth. Three dosage levels of the compound to be tested are administered to the remaining three groups of OVX animals.

Since ovariectomy (OVX) induces hyperphagia, all OVX animals are pair-fed with sham OVX animals throughout the 35 day study. Briefly, test compound, positive control compound, PBS, or vehicle alone is administered subcutaneously once per day for 35 days. Alternatively, test compound can be formulated in implantable pellets that are implanted for 35 days, or may be administered orally, such as by gastric gavage. All animals, including sham OVX/vehicle and OVX/vehicle groups, are injected intraperitoneally with calcein nine days and two days before sacrifice (two injections of calcein administered each designated day, to ensure proper labeling of newly formed bone). Weekly body weights are determined. At the end of the 35-day cycle, the animals' blood and tissues are processed as described above.

Lead compounds may also be tested in chronic OVX animals (treatment model). An exemplary protocol for treatment of established bone loss in ovariectomized animals that can be used to assess efficacy of anabolic agents may be performed as follows. Briefly, 80 to 100 six month old female, Sprague-Dawley rats are subjected to sham surgery (sham OVX) or ovariectomy (OVX) at time 0, and 10 rats are sacrificed to serve as baseline controls. Body weights are recorded weekly during the experiment. After approximately 6 weeks of bone depletion (42 days), 10 sham OVX and 10 OVX rats are randomly selected for sacrifice as depletion period controls. Of the remaining animals, 10 sham OVX and 10 OVX rats are used as placebo-treated controls. The remaining OVX animals are treated with 3 to 5 doses of test drug for a period of 5 weeks (35 days). As a postitive control, a group of OVX rats can be treated with an agent such as PTH, a known anabolic agent in this model (Kimmel et al. *Endocrinology* (1993) 132:1577–84). To determine effects on bone formation, the following procedure can be followed. The femurs, tibiae and lumbar vertebrae 1 to 4 are excised and collected. The proximal left and right tibiae are used for pQCT measurements, cancellous bone mineral density (BMD) (gravimetric determination), and histology, while the midshaft of each tibiae is subjected to cortical BMD or histology. The femurs are prepared for pQCT scanning of the midshaft prior to biomechanical testing. With respect to lumbar vertebrae (LV), LV2 are processed for BMD (pQCT may also be performed); LV3 are prepared for undecalcified bone histology; and LV4 are processed for mechanical testing.

Nature of the Compounds Useful in the Invention

All of the compounds of the invention contain two aromatic systems, Ar^1 and Ar^2, spaced apart by a linker at a distance of 1.5–15 Å, and all generally contain at least one nitrogen atom. A summary of the structural features of the compounds included within the invention is shown in FIG. 1.

As shown, Ar^1 and Ar^2 may include various preferred embodiments. These are selected from the group consisting of a substituted or unsubstituted aromatic ring system containing a five-membered heterocycle; a substituted or unsubstituted aromatic ring system containing a six-membered heterocycle; a substituted or unsubstituted naphthalene moiety; and a substituted or unsubstituted benzene moiety. There are 16 possible combinations of these embodiments if Ar^1 and Ar^2, are considered distinguishable. As will be clear, however, the designation of one aromatic system as Ar^1 and the other as Ar^2 is arbitrary; thus there are only ten possible combinations. However, for simplicity, Ar^1 and Ar^2 are designated separately with the realization that the choice is arbitrarily made. All linkers described herein if not palindromic, are considered link Ar^1 to Ar^2 or vice-versa whether or not the complementary orientation is explicitly shown. Thus, if Ar^1 and Ar^2 are different and a linker is specified as —CONR, it is understood that also included is the linker —NRCO—.

The non-interfering substituents on the aromatic system represented by Ar^1 and the non-interfering substituents on the aromatic system represented by Ar^2 are represented in the formulae herein by R^1 and R^2, respectively. Generally, these substituents can be of wide variety. Among substituents that do not interfere with the beneficial effect of the compounds of the invention on bone in treated subjects are included alkyl (1–6C, preferably lower alkyl 1–4C), including straight or branched-chain forms thereof, alkenyl (1–6C, preferably 1–4C), alkynyl (1–6C, preferably 1–4C), all of which can be straight or branched chains and may contain further substituents; halogens, including F, Cl, Br and I; siloxy, OR, SR, NR_2, OOCR, COOR, NCOR, NCOOR, and benzoyl, CF_3, —OCF_3, SCF_3, —$N(CF_3)_2$, CN, SO, SO_2R and SO_3R wherein R is alkyl (1–6C) or is H. Where R^1 or R^2 substituents are in adjacent positions in the aromatic system, they may form a ring. Further, rings may be included in substituents which contain sufficient carbon and heteroatoms to provide this possibility.

Preferred non-interfering substituents include hydrocarbyl groups of 1–6C, including saturated and unsaturated, linear or branched hydrocarbyl as well as hydrocarbyl groups containing ring systems; halo groups, alkoxy, hydroxy, amino, monoalkyl- and dialkylamino where the alkyl groups are 1–6C, CN_3, CF, and COOR.

Although the number of R^1 and R^2 may typically be 0–4 or 0–5 depending on the available positions in the aromatic system, preferred embodiments include those wherein the number of R^1 is 0, 1 or 2 and of R^2 is 0, 1 or 2.

The linker group, L, may be a covalent bond or any group having a valence of at least two and covering a linear distance of from about 1.5 to about 15 Angstroms, including those that contain cyclic moieties, that meet this spatial requirement. Useful linkers are divided, by definition herein, into three general categories: (1) flexible non-conjugating linkers, (2) flexible conjugating linkers, and (3) constrained linkers. The preferred choice of linker will depend on the choices for Ar^1 and Ar^2.

As defined herein, flexible non-conjugating linkers are those that link only one position of Ar^1 to one position of Ar^2, and provide only a single covalent bond or a single chain between Ar^1 and Ar^2. The chain may contain branches, but may not contain π-bonds (except in the branches) or cyclic portions in the chain. The linker atoms in the chain itself rotate freely around single covalent bonds, and thus the linker has more than two degrees of freedom. Particularly useful flexible non-conjugating linkers, besides a covalent bond, are those of the formulae: —NR—, —CR_2—, —S—, or —O—, wherein R is H or alkyl (1–6C), more preferably H or lower alkyl (1–4C) and more preferably H. Also preferred are those of the formulae: —NRCO—, —CONR—, —CR_2S—, —SCR_2—, —OCR_2—, —CR_2O—, —NRNR—, —CR_2CR_2—, —$NRSO_2$—, —SO_2NR—, —CR_2CO—, —$COCR_2$—, and —NR— NR—CO—CR_2— and its complement —CR_2—CO— NR—NR—, including the isosteres thereof. Also preferred are those of the formulae: —$NR(CR_2)_2NR$—, —$O(CR_2)_2O$—, and —$S(CR_2)_2S$—, including the isosteres thereof. The optimum choice of linker is dependent on the nature of Ar^1 and Ar^2.

Flexible conjugating linkers are those that link only one position of Ar^1 to one position of Ar^2, but incorporate at least one double or triple bond or one or more cyclic systems and thus have only two degrees of freedom. A flexible conjugating linker may form a completely conjugated π-bond linking system between Ar^1 and Ar^2, thus providing for co-planarity of Ar^1 and Ar^2. Examples of useful flexible conjugating linkers include: —RC=CR—; —N=N—; —C=C—; —RC=N—; —N=CR—; —NR—N=CR—; —NR—NR—CO—CR=CR—; and the like, where R is H or alkyl (1–6C); preferably H or lower alkyl (1–4C); and more preferably H.

Constrained linkers are those that have more than one point of attachment to either or both Ar^1 and Ar^2 and, thus, generally allow for only one degree of freedom. Constrained linkers most frequently form fused 5- or 6-membered cyclic moieties with Ar^1 and/or Ar^2 where either Ar^1 or Ar^2 has at least one substituent appropriately positioned to form a second covalent bond with the linker, e.g., where Ar^2 is a phenyl group with a reactive, ortho-positioned substituent, or is derivatized to the linker directly at the ortho position. (Although the aromatic moieties should properly be refined to as phenylene or naphthylene in such cases, generally the term "phenyl" or "naphthyl" is used herein to include both monovalent and bivalent forms of these moieties). Examples of particularly useful constrained linkers include
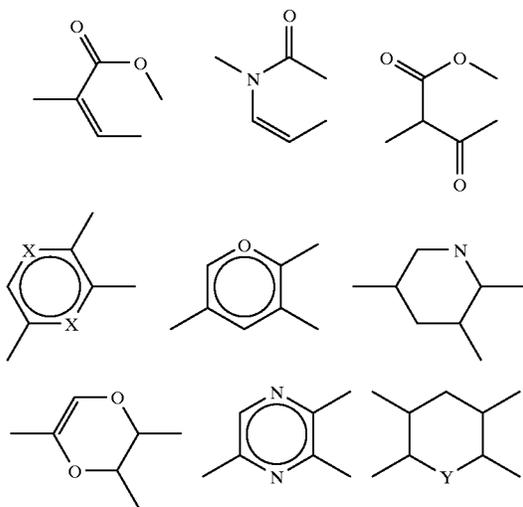
and the like, where X is O, N, S or CR, and Y is CR$_2$ or C=O.

Ar^1 is an aromatic system containing a five-membered heterocycle, of the formula:
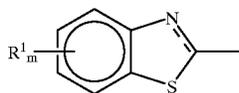
wherein the dotted line represents an optional π-bond;

each R^1 is independently a non-interfering substituent; and

m is an integer of 0–4.

The nature of the R^1 and R^2 is outlined above. Preferably, L is selected from the group consisting of —NRCO— and —CONR—, wherein R is H or alkyl (1–6C) and R^2 is —NR$_2$ or —OR where each R is independently H or alkyl (1–6C). The preferred compound is selected from the group consisting of Compound Nos. 59-0102 and 59-0070.

Synthesis of the Compounds Useful in the Invention

Many of the compounds useful in the invention are commercially available and can be synthesized by art-known methods. Those compounds useful in the invention which are new compounds, can similarly be obtained by methods generally known in the art.

The following examples are intended to illustrate, but not to limit, the invention.

Preparation A

Compound 59-0008 was synthesized according to the procedure of McDonald, W. S., et al. Chem Comm (1969)

392–393; Irving, H. N. N. H. et al. Anal Chim Acta (1970) 49:261–266. Briefly, 10.0 g of dithizone was taken up in 100 ml EtOH and 50 ml AcOH and heated at reflux for 18 h. After cooling, this was diluted first with 100 ml water and then with 50 ml 1N NaOH. This was then further neutralized by the addition of 6N NaOH to bring the pH to 5.0. This deep purple mixture was then concentrated on a rotavapor to remove organics. Once the liquid had lost all of its purple color, this was filtered to collect the dark precipitate. Purification by flash chromatography (4.5×25.7 cm; EtAc/Hep. (1:4); R$_f$ 0.22) followed by recrystalization from EtOH gave 2.15 g (25% yield) of dark purple crystals, mp=184–185° C. ^{1}H NMR (CDCl$_3$) 7.90 (d of d, J$_1$=7.7, J$_2$=2.2, 2H), 7.64 (hump, 1H), 7.49 (m, 3H), 7.02 (m, 1H), 6.91 (m, 2H), 6.55 (d, J=8.1, 1H). MS (EI) 254 (47, M+), 105 (26), 77 [100], 51 (27). HRMS (EI, M+) 254.0626 (calcd 254.0626182). Anal. Calcd for C$_{13}$H$_{10}$N$_4$S: C, 61.40; H, 3.96; N, 22.03. Found: C, 61.40; H, 4.20; N, 22.06.

EXAMPLE 1

High Throughput Screening

Several thousand compounds were tested in the assay system set forth in U.S. Ser. No. 08/458,434, filed Jun. 2, 1995, and incorporated herein by reference. The standard positive control was a compound of the invention, 59-0008 (also denoted "OS8"), which is of the formula:

"OS8"
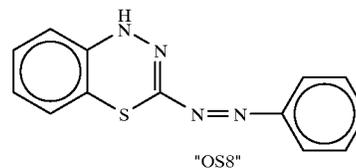
In more detail, the 2T3-BMP-2-LUC cells, a stably transformed osteoblast cell line described in Ghosh-Choudhury et al. Endocrinology (1996) 137:331–39, referenced above, was employed. The cells were cultured using α-MEM, 10% FCS with 1% penicillin/streptomycin and 1% glutamine ("plating medium"), and were split 1:5 once per week. For the assay, the cells were resuspended in a plating medium containing 4% FCS, plated in microtiter plates at a concentration of 5×10^3 cells (in 50 μl)/well, and incubated for 24 hours at 37° C. in 5% CO$_2$. To initiate the assay, 50 μl of the test compound or the control in DMSO was added at 2× concentration to each well, so that the final volume was 100 μl. The final serum concentration was 2% FCS, and the final DMSO concentration was 1%. Compound 59-0008 (10 μM) was used as a positive control.

The treated cells were incubated for 24 hours at 37° C. and 5% CO$_2$. The medium was then removed, and the cells were rinsed three times with PBS. After removal of excess PBS, 25 μl of 1× cell culture lysing reagent (Promega #E153A) was added to each well and incubated for at least ten minutes. Optionally, the plates/samples could be frozen at this point. To each well was added 50 μl of luciferase substrate (Promega #E152A; 10 ml Promega luciferase assay buffer per 7 mg Promega luciferase assay substrate). Luminescence was measured on an automated 96-well luminometer, and was expressed as either picograms of luciferase activity per well or as picograms of luciferase activity per microgram of protein.

In this assay, compound 59-0008 (3-phenylazo-1H-4,1,2-benzothiadiazine) exhibited a pattern of reactivity, as shown in FIG. 2. The activity for compound 59-0008 was maximal at a concentration of approximately 3–10 μM and, more particularly, at about 3 μM, and thus provided a response of

approximately 175 light emission units. Accordingly, other tested compounds were evaluated at various concentrations, and these results were compared to the results obtained for 59-0008 at 10 μM (which value was normalized to 100). For instance, any tested compound in FIG. 3 and FIG. 4 that showed greater activity than 10 μM of 59-0008 would result in a value over 100.

As shown in FIG. 3 (46 sheets) and FIG. 4 (28 sheets), several compounds were found to be particularly effective.

EXAMPLE 2

In Vivo Calvarial Bone Growth Data

Compound 59-0008 was assayed in vivo according to the procedure described previously (see "In vivo Assay of Effects of Compounds on Murine Calvarial Bone Growth", supra). As compared to a vehicle control, compound 59-0008 induced a 4-fold increase in width of new calvarial bone.

EXAMPLE 3

Chondrogenic Activity

Compounds 59-008, 59-0102 and 50-0197 were assayed for effects on the differentiation of cartilage cells, as compared to the action of recombinant human BMP-2. Briefly, a mouse clonal chondrogenic cell line, TMC-23, was isolated and cloned from costal cartilage of transgenic mice containing the BMP-2 gene control region driving SV-40 large T-antigen, generated as described in Ghosh-Choudhury et al *Endocrinology* 137:331–39, 1996. These cells were cultured in DMEM/10% FCS, and were shown to express T-antigen, and also to produce aggrecan (toluidine blue staining at pH 1.0) and Type-II collagen (immunostaining) by 7 days after confluence.

For measurement of alkaline phosphatase (ALP) activity, the technique of LF Bonewald et al. *J Biol Chem* (1992) 267:8943–49, was employed. Briefly, TMC-23 cells were plated in 96 well microtiter plates in DMEM containing 10% FCS at 4×10³ cells/well. Two days after plating, the cells were confluent and the medium was replaced with fresh medium containing 10% FCS and different concentrations of compounds or recombinant BMP-2. After an additional 2 or 5 days incubation, the plates were washed twice with PBS, and then lysing solution (0.05% Triton X-100) was added (100 μl/well). The cells were lysed by three freeze-thaw cycles of –70° C. (30 min), followed by 37° C. (30 min with shaking). Twenty microliters of cell lysates were assayed with 80 μl of 5 mM p-nitrophenol phosphate in 1.5 M 2-amino-2-methyl-propanol buffer, pH 10.3 (Sigma ALP kit, Sigma Chemical Co., St. Louis, Mo.) for 10 min at 37° C. The reaction was stopped by the addition of 100 μl of 0.5 M NaOH. The spectrophotometric absorbance at 405 nm was compared to that of p-nitrophenol standards to estimate ALP activity in the samples. The protein content of the cell lysates was determined by the Bio-Rad protein assay kit (Bio-Rad, Hercules, Calif.). Specific activity was calculated using these two parameters.

At day 2, compounds 59-0008 (10^{-9} M), 59-0102 (10^{-7} M) and 59-0197 (10^{-9} M) increased ALP levels approxi-

mately 3-, 2- and 2.5-fold, respectively, as compared to the vehicle control. Recombinant BMP2 at 100, 50 or 10 ng/ml induced ALP levels approximately 10-, 4- or 1.5-fold, respectively, as compared to the vehicle control.

EXAMPLE 4

Synthesis of Exemplary Compounds

Compounds of the invention can be synthesized by the procedures described in Dryanska, V. and Ivanov, K. *Synthesis* (1976) 1:37–8, using the described embodiment of Ar^2 and the appropriate analog of the benzothiazole embodied in Ar^1.

Alternates to the olefin linker described can also be prepared using standard methods.

We claim:

1. A method to treat a condition in a vertebrate animal characterized by a deficiency in, or need for, bone growth replacement and/or an undesirable level of bone resorption, which method comprises administering to a vertebrate subject in need of such treatment an effective amount of a compound of the formula:

(1)
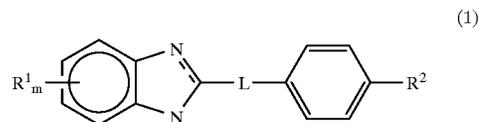
wherein

each R^1 and R^2 is independently a non-interfering substituent;

m is an integer of 0–4;

the dotted line represents an optional π bond; and

L is a flexible non-conjugating linker.

2. The method of claim 1 wherein L is selected from the group consisting of —NRCO— and —CONR—, wherein R is H or alkyl (1–6C).

3. The method of claim 1 wherein R^2 is —NR₂ or —OR where each R is independently H or alkyl (1–6C).

4. The method of claim 1 wherein said compound is selected from the group consisting of Compound Nos. 59-0102 and 59-0070.

5. The method of claim 1 wherein said condition is osteoporosis, bone fracture or deficiency, primary or secondary hyperparathyroidism, periodontal disease or defect, metastatic bone disease, osteolytic bone disease, post-plastic surgery, post-prosthetic joint surgery, or post-dental implantation.

6. The method of claim 1 which further comprises administering to said subject one or more agents that promote bone growth or that inhibit bone resorption.

7. The method of claim 6 wherein said agents are selected from the group consisting of bone morphogenetic factors, anti-resorptive agents, osteogenic factors, cartilage-derived morphogenic proteins, growth hormones, and differentiating factors.

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