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(54) Title: IL-8 RECEPTOR ANTAGONISTS

(57) Abstract: This invention relates to the novel use of sulfonamide squaramides in the treatment of disease states mediated by the chemokine, Interleukin-8 (IL-8).



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## IL-8 RECEPTOR ANTAGONISTS

### FIELD OF THE INVENTION

This invention relates to a novel group of sulfonamide squaramide  
5 compounds, processes for the preparation thereof, the use thereof in treating IL-8,  
GRO $\alpha$ , GRO $\beta$ , GRO $\gamma$ , NAP-2, and ENA-78 mediated diseases and pharmaceutical  
compositions for use in such therapy.

### BACKGROUND OF THE INVENTION

Many different names have been applied to Interleukin-8 (IL-8), such as  
10 neutrophil attractant/activation protein-1 (NAP-1), monocyte derived neutrophil  
chemotactic factor (MDNCF), neutrophil activating factor (NAF), and T-cell  
lymphocyte chemotactic factor. Interleukin-8 is a chemoattractant for neutrophils,  
basophils, and a subset of T-cells. It is produced by a majority of nucleated cells  
including macrophages, fibroblasts, endothelial and epithelial cells exposed to TNF,  
15 IL-1 $\alpha$ , IL-1 $\beta$  or LPS, and by neutrophils themselves when exposed to LPS or  
chemotactic factors such as FMLP. M. Baggiolini et al, J. Clin. Invest. 84, 1045  
(1989); J. Schroder et al, J. Immunol. 139, 3474 (1987) and J. Immunol. 144, 2223  
(1990) ; Strieter, et al, Science 243, 1467 (1989) and J. Biol. Chem. 264, 10621  
(1989); Cassatella et al, J. Immunol. 148, 3216 (1992).

20 GRO $\alpha$ , GRO $\beta$ , GRO $\gamma$  and NAP-2 also belong to the chemokine  $\alpha$  family.  
Like IL-8 these chemokines have also been referred to by different names. For  
instance GRO $\alpha$ ,  $\beta$ ,  $\gamma$  have been referred to as MGS $\alpha$ ,  $\beta$  and  $\gamma$  respectively  
(Melanoma Growth Stimulating Activity), see Richmond et al, J. Cell Physiology  
129, 375 (1986) and Chang et al, J. Immunol 148, 451 (1992). All of the chemokines  
25 of the  $\alpha$ -family which possess the ELR motif directly preceding the CXC motif bind  
to the IL-8 B receptor.

IL-8, GRO $\alpha$ , GRO $\beta$ , GRO $\gamma$ , NAP-2 and ENA-78 stimulate a number of  
functions in vitro. They have all been shown to have chemoattractant properties for  
neutrophils, while IL-8 and GRO $\alpha$  have demonstrated T-lymphocytes, and  
30 basophiles chemotactic activity. In addition IL-8 can induce histamine release from

basophils from both normal and atopic individuals. GRO- $\alpha$  and IL-8 can in addition, induce lysosomal enzyme release and respiratory burst from neutrophils. IL-8 has also been shown to increase the surface expression of Mac-1 (CD11b/CD18) on neutrophils without de novo protein synthesis. This may contribute to increased  
5 adhesion of the neutrophils to vascular endothelial cells. Many known diseases are characterized by massive neutrophil infiltration. As IL-8, Gro $\alpha$ , GRO $\beta$ , GRO $\gamma$  and NAP-2 promote the accumulation and activation of neutrophils, these chemokines have been implicated in a wide range of acute and chronic inflammatory disorders including psoriasis and rheumatoid arthritis, Baggiolini et al, FEBS Lett. 307, 97  
10 (1992); Miller et al, Crit. Rev. Immunol. 12, 17 (1992); Oppenheim et al, Annu. Rev. Immunol. 9, 617 (1991); Seitz et al., J. Clin. Invest. 87, 463 (1991); Miller et al., Am. Rev. Respir. Dis. 146, 427 (1992); Donnelly et al., Lancet 341, 643 (1993). In addition the ELR chemokines (those containing the amino acids ELR motif just prior to the CXC motif) have also been implicated in angiostasis, Strieter et al,  
15 Science 258, 1798 (1992).

In vitro, IL-8, Gro $\alpha$ , GRO $\beta$ , GRO $\gamma$ , and NAP-2 induce neutrophil shape change, chemotaxis, granule release, and respiratory burst, by binding to and activating receptors of the seven-transmembrane, G-protein-linked family, in particular by binding to IL-8 receptors, most notably the B-receptor, Thomas et al.,  
20 J. Biol. Chem. 266, 14839 (1991); and Holmes et al., Science 253, 1278 (1991). The development of non-peptide small molecule antagonists for members of this receptor family has precedent. For a review see R. Freidinger in: Progress in Drug Research, Vol. 40, pp. 33-98, Birkhauser Verlag, Basel 1993. Hence, the IL-8 receptor represents a promising target for the development of novel anti-inflammatory agents.

25 Two high affinity human IL-8 receptors (77% homology) have been characterized: IL-8R $\alpha$ , which binds only IL-8 with high affinity, and IL-8RB, which has high affinity for IL-8 as well as for GRO- $\alpha$ , GRO $\beta$ , GRO $\gamma$  and NAP-2. See Holmes et al., supra; Murphy et al., Science 253, 1280 (1991); Lee et al., J. Biol. Chem. 267, 16283 (1992); LaRosa et al., J. Biol. Chem. 267, 25402 (1992); and  
30 Gayle et al., J. Biol. Chem. 268, 7283 (1993).

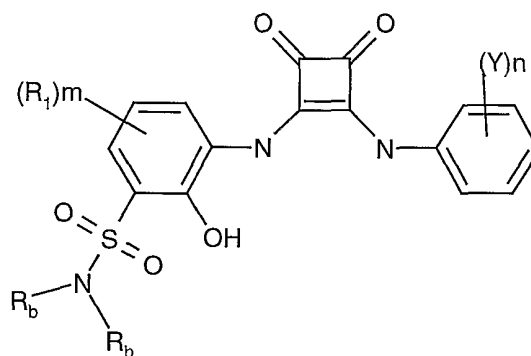
There remains a need for treatment, in this field, for compounds which are capable of binding to the IL-8  $\alpha$  or  $\beta$  receptor. Therefore, conditions associated with an increase in IL-8 production (which is responsible for chemotaxis of neutrophil and T-cells subsets into the inflammatory site) would benefit by compounds which are inhibitors of IL-8 receptor binding.

### SUMMARY OF THE INVENTION

This invention provides for a method of treating a chemokine mediated disease, wherein the chemokine is one which binds to an IL-8  $\alpha$  or  $\beta$  receptor and which method comprises administering an effective amount of a compound of Formula (I) or a pharmaceutically acceptable salt thereof. In particular the chemokine is IL-8.

This invention also relates to a method of inhibiting the binding of IL-8 to its receptors in a mammal in need thereof which comprises administering to said mammal an effective amount of a compound of Formula (I).

Compounds of Formula (I) useful in the present invention are represented by the structure:



(I)

wherein:

$R_1$  is independently selected from the group consisting of hydrogen, halogen, nitro, cyano, halosubstituted  $C_{1-10}$  alkyl,  $C_{1-10}$  alkyl,  $C_{2-10}$  alkenyl,  $C_{1-10}$  alkoxy, halosubstituted  $C_{1-10}$  alkoxy, azide,  $(CR_8R_8)_q S(O)_t R_4$ , hydroxy, hydroxy  $C_{1-4}$  alkyl, aryl, aryl  $C_{1-4}$  alkyl, aryloxy, aryl  $C_{1-4}$  alkyloxy, heteroaryl, heteroarylalkyl, heterocyclic, heterocyclic  $C_{1-4}$  alkyl, heteroaryl  $C_{1-4}$  alkyloxy,

aryl C<sub>2-10</sub> alkenyl, heteroaryl C<sub>2-10</sub> alkenyl, heterocyclic C<sub>2-10</sub> alkenyl,  
 (CR<sub>8</sub>R<sub>8</sub>)<sub>q</sub>NR<sub>4</sub>R<sub>5</sub>, C<sub>2-10</sub> alkenyl C(O)NR<sub>4</sub>R<sub>5</sub>, (CR<sub>8</sub>R<sub>8</sub>)<sub>q</sub> C(O)NR<sub>4</sub>R<sub>5</sub>,  
 (CR<sub>8</sub>R<sub>8</sub>)<sub>q</sub> C(O)NR<sub>4</sub>R<sub>10</sub>, S(O)<sub>3</sub>H, S(O)<sub>3</sub>R<sub>8</sub>, (CR<sub>8</sub>R<sub>8</sub>)<sub>q</sub> C(O)R<sub>11</sub>, C<sub>2-10</sub> alkenyl  
 C(O)R<sub>11</sub>, C<sub>2-10</sub> alkenyl C(O)OR<sub>11</sub>(CR<sub>8</sub>R<sub>8</sub>)<sub>q</sub> C(O)OR<sub>12</sub>, (CR<sub>8</sub>R<sub>8</sub>)<sub>q</sub> OC(O)  
 5 R<sub>11</sub>, (CR<sub>8</sub>R<sub>8</sub>)<sub>q</sub>NR<sub>4</sub>C(O)R<sub>11</sub>, (CR<sub>8</sub>R<sub>8</sub>)<sub>q</sub> NHS(O)<sub>2</sub>R<sub>17</sub>, (CR<sub>8</sub>R<sub>8</sub>)<sub>q</sub> and  
 S(O)<sub>2</sub>NR<sub>4</sub>R<sub>5</sub>; or two R<sub>1</sub> moieties together y form O-(CH<sub>2</sub>)<sub>5</sub>O- or a 5 to 6  
 membered unsaturated ring;  
 R<sub>b</sub> is independently selected from the group consisting of hydrogen, NR<sub>6</sub>R<sub>7</sub>, OH, OR<sub>a</sub>,  
 C<sub>1-5</sub>alkyl, aryl, arylC<sub>1-4</sub>alkyl, aryl C<sub>2-4</sub>alkenyl; cycloalkyl, cycloalkyl C<sub>1-5</sub> alkyl,  
 10 heteroaryl, heteroarylC<sub>1-4</sub>alkyl, heteroarylC<sub>2-4</sub> alkenyl, heterocyclic, heterocyclic  
 C<sub>1-4</sub>alkyl, and a heterocyclic C<sub>2-4</sub>alkenyl moiety; all of which moieties may be  
 optionally substituted one to three times independently by halogen, nitro,  
 halosubstituted C<sub>1-4</sub> alkyl, C<sub>1-4</sub> alkyl; amino, mono or di-C<sub>1-4</sub> alkyl substituted  
 amine, OR<sub>a</sub>, C(O)R<sub>a</sub>, NR<sub>a</sub>C(O)OR<sub>a</sub>, OC(O)NR<sub>6</sub>R<sub>7</sub>, hydroxy, NR<sub>9</sub>C(O)R<sub>a</sub>,  
 15 S(O)<sub>m</sub>R<sub>a</sub>, C(O)NR<sub>6</sub>R<sub>7</sub>, C(O)OH, C(O)OR<sub>a</sub>, S(O)<sub>2</sub>NR<sub>6</sub>R<sub>7</sub>, and NHS(O)<sub>2</sub>R<sub>a</sub>; or the  
 two R<sub>b</sub> substituents can join to form a 3-10 membered ring, optionally substituted and  
 containing, in addition to carbon, independently, 1 to 3 NR<sub>a</sub>, O, S, SO, or SO<sub>2</sub>  
 moieties which can be optionally unsaturated;  
 q is 0, or an integer having a value of 1 to 10;  
 20 t is 0, or an integer having a value of 1 or 2;  
 s is an integer having a value of 1 to 3;  
 R<sub>4</sub> and R<sub>5</sub> are independently selected from the group consisting of hydrogen,  
 optionally substituted C<sub>1-4</sub> alkyl, optionally substituted aryl, optionally  
 substituted aryl C<sub>1-4</sub>alkyl, optionally substituted heteroaryl, optionally  
 25 substituted heteroaryl C<sub>1-4</sub>alkyl, heterocyclic, and heterocyclicC<sub>1-4</sub> alkyl; or R<sub>4</sub>  
 and R<sub>5</sub> together with the nitrogen to which they are attached form a 5 to 7  
 member ring which optionally comprises an additional heteroatom selected from  
 oxygen, nitrogen or sulfur;

- Y is independently selected from the group consisting of hydrogen, halogen, nitro, cyano, halosubstituted C<sub>1-10</sub> alkyl, C<sub>1-10</sub> alkyl, C<sub>2-10</sub> alkenyl, C<sub>1-10</sub> alkoxy, halosubstituted C<sub>1-10</sub> alkoxy, azide, (CR<sub>8</sub>R<sub>8</sub>)<sub>q</sub> S(O)<sub>t</sub>R<sub>4</sub>, hydroxy, hydroxyC<sub>1-4</sub>alkyl, aryl, aryl C<sub>1-4</sub> alkyl, aryloxy, arylC<sub>1-4</sub> alkyloxy, heteroaryl, heteroarylalkyl, heteroaryl C<sub>1-4</sub> alkyloxy, heterocyclic, heterocyclic C<sub>1-4</sub>alkyl; aryl C<sub>2-10</sub> alkenyl, heteroaryl C<sub>2-10</sub> alkenyl, heterocyclic C<sub>2-10</sub> alkenyl, (CR<sub>8</sub>R<sub>8</sub>)<sub>q</sub> NR<sub>4</sub>R<sub>5</sub>, C<sub>2-10</sub> alkenyl C(O)NR<sub>4</sub>R<sub>5</sub>, (CR<sub>8</sub>R<sub>8</sub>)<sub>q</sub> C(O)NR<sub>4</sub>R<sub>5</sub>, (CR<sub>8</sub>R<sub>8</sub>)<sub>q</sub> C(O)NR<sub>4</sub>R<sub>10</sub>, S(O)<sub>3</sub>H, S(O)<sub>3</sub>R<sub>8</sub>, (CR<sub>8</sub>R<sub>8</sub>)<sub>q</sub> C(O)R<sub>11</sub>, C<sub>2-10</sub> alkenyl C(O)R<sub>11</sub>, C<sub>2-10</sub> alkenyl C(O)OR<sub>11</sub>, C(O)R<sub>11</sub>, (CR<sub>8</sub>R<sub>8</sub>)<sub>q</sub> C(O)OR<sub>12</sub>, (CR<sub>8</sub>R<sub>8</sub>)<sub>q</sub> OC(O) R<sub>11</sub>, (CR<sub>8</sub>R<sub>8</sub>)<sub>q</sub> NR<sub>4</sub>C(O)R<sub>11</sub>, (CR<sub>8</sub>R<sub>8</sub>)<sub>q</sub> NHS(O)<sub>2</sub>R<sub>d</sub>, and (CR<sub>8</sub>R<sub>8</sub>)<sub>q</sub> S(O)<sub>2</sub>NR<sub>4</sub>R<sub>5</sub>; or two Y moieties together form O-(CH<sub>2</sub>)<sub>s</sub>O- or a 5 to 6 membered unsaturated ring;
- n is an integer having a value of 1 to 5;
- m is an integer having a value of 1 to 4;
- R<sub>8</sub> is hydrogen or C<sub>1-4</sub> alkyl;
- R<sub>10</sub> is C<sub>1-10</sub> alkyl C(O)<sub>2</sub>R<sub>8</sub>;
- R<sub>11</sub> is selected from the group consisting of hydrogen, C<sub>1-4</sub> alkyl, optionally substituted aryl, optionally substituted aryl C<sub>1-4</sub>alkyl, optionally substituted heteroaryl, optionally substituted heteroarylC<sub>1-4</sub>alkyl, optionally substituted heterocyclic, and optionally substituted heterocyclicC<sub>1-4</sub>alkyl;
- R<sub>12</sub> is selected from the group consisting of hydrogen, C<sub>1-10</sub> alkyl, optionally substituted aryl and optionally substituted arylalkyl; and
- R<sub>17</sub> is selected from the group consisting of C<sub>1-4</sub>alkyl, aryl, arylalkyl, heteroaryl, heteroarylC<sub>1-4</sub>alkyl, heterocyclic, and heterocyclicC<sub>1-4</sub>alkyl, wherein the aryl, heteroaryl and heterocyclic rings are all optionally substituted.

## DETAILED DESCRIPTION OF THE INVENTION

The compounds of Formula (I) may also be used in association with the veterinary treatment of mammals, other than humans, in need of inhibition of IL-8 or other chemokines which bind to the IL-8RA and RB receptors. Chemokine mediated diseases for treatment, therapeutically or prophylactically, in animals include disease states such as those noted herein in the Methods of Treatment section.

The following terms, as used herein, refer to:

- "halo" - all halogens, that is chloro, fluoro, bromo and iodo.
- 10 • "C<sub>2-5</sub>alkyl" or "alkyl" - both straight and branched chain moieties of 2 to 5 carbon atoms, unless the chain length is otherwise limited, including, but not limited to, methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec-butyl, iso-butyl, tert-butyl, n-pentyl and the like.
- The term "alkenyl" is used herein at all occurrences to mean straight or  
15 branched chain moieties of 2-10 carbon atoms, unless the chain length is limited thereto, including, but not limited to ethenyl, 1-propenyl, 2-propenyl, 2-methyl-1-propenyl, 1-butenyl, 2-butenyl and the like.
- "aryl" - phenyl and naphthyl;
- "heteroaryl" (on its own or in any combination, such as "heteroaryloxy", or  
20 "heteroaryl alkyl") - a 5-10 membered aromatic ring system in which one or more rings contain one or more heteroatoms selected from the group consisting of N, O or S, such as, but not limited, to pyrrole, pyrazole, furan, thiophene, quinoline, isoquinoline, quinazolinyl, pyridine, pyrimidine, oxazole, thiazole, thiadiazole, triazole, imidazole, or benzimidazole.
- 25 • "heterocyclic" (on its own or in any combination, such as "heterocyclicalkyl") - a saturated or partially unsaturated 4-10 membered ring system in which one or more rings contain one or more heteroatoms selected from the group consisting of N, O, or S; such as, but not limited to, pyrrolidine, piperidine, piperazine, morpholine, tetrahydropyran, or imidazolidine.

• The term "arylalkyl" or "heteroarylalkyl" or "heterocyclicalkyl" is used herein to mean C<sub>1-10</sub> alkyl, as defined above, attached to an aryl, heteroaryl or heterocyclic moiety, as also defined herein, unless otherwise indicated.

Illustrative compounds of Formula (I) include:

- 5 3-[4-Chloro-2-hydroxy-3-(4-methyl-piperazine-1-sulfonyl)-phenylamino]-4-phenylamino-cyclobut-3-ene-1,2-dione;
- 3-[4-Chloro-2-hydroxy-3-(4-methyl-piperazine-1-sulfonyl)-phenylamino]-4-(2-chloro-phenylamino)-cyclobut-3-ene-1,2-dione;
- 3-[4-Chloro-2-hydroxy-3-(4-methyl-piperazine-1-sulfonyl)-phenylamino]-4-(2-bromo-phenylamino)-cyclobut-3-ene-1,2-dione;
- 10 3-[4-Chloro-2-hydroxy-3-(4-methyl-piperazine-1-sulfonyl)-phenylamino]-4-(2-fluoro-phenylamino)-cyclobut-3-ene-1,2-dione;
- 3-[4-Chloro-2-hydroxy-3-(4-methyl-piperazine-1-sulfonyl)-phenylamino]-4-(4-fluoro-phenylamino)-cyclobut-3-ene-1,2-dione;
- 15 3-[4-Chloro-2-hydroxy-3-(4-methyl-piperazine-1-sulfonyl)-phenylamino]-4-(2-methoxy-phenylamino)-cyclobut-3-ene-1,2-dione;
- 3-[4-Chloro-2-hydroxy-3-(4-methyl-piperazine-1-sulfonyl)-phenylamino]-4-(2,6-difluoro-phenylamino)-cyclobut-3-ene-1,2-dione;
- 3-[4-Chloro-2-hydroxy-3-(4-methyl-piperazine-1-sulfonyl)-phenylamino]-4-(2,4-difluoro-phenylamino)-cyclobut-3-ene-1,2-dione;
- 20 3-[3-((S)-3-Amino-pyrrolidine-1-sulfonyl)-4-chloro-2-hydroxy-phenylamino]-4-phenylamino-cyclobut-3-ene-1,2-dione;
- 6-Chloro-N-(2-diethylamino-ethyl)-3-(3,4-dioxo-2-phenylamino-cyclobut-1-enylamino)-2-hydroxy-benzenesulfonamide;
- 25 6-Chloro-3-[2-(2-chloro-phenylamino)-3,4-dioxo-cyclobut-1-enylamino]-N-(2-diethylamino-ethyl)-2-hydroxy-benzenesulfonamide;
- 6-Chloro-3-[2-(2-bromo-phenylamino)-3,4-dioxo-cyclobut-1-enylamino]-N-(2-diethylamino-ethyl)-2-hydroxy-benzenesulfonamide;
- (1-{6-Chloro-3-[2-phenylamino-3,4-dioxo-cyclobut-1-enylamino]-2-hydroxy-benzenesulfonyl}-piperidin-4-yl)-carbamic acid tert-butylester;
- 30



- 3-[3-([1,4<sup>7</sup>]Bipiperidiny1-1'-sulfonyl)-4-chloro-2-hydroxy-phenylamino]-4-phenylamino-cyclobut-3-ene-1,2-dione;
- 3-[4-Chloro-2-hydroxy-3-(piperazine-1-sulfonyl)-phenylamino]-4-phenylamino-cyclobut-3-ene-1,2-dione;
- 5 3-[4-Chloro-2-hydroxy-3-(4-methyl-[1,4]diazepane-1-sulfonyl)-phenylamino]-4-phenylamino-cyclobut-3-ene-1,2-dione;
- 3-[4-Chloro-2-hydroxy-3-(4-methyl-[1,4]diazepane-1-sulfonyl)-phenylamino]-4-(2-chloro-phenylamino)-cyclobut-3-ene-1,2-dione;
- 6-Chloro-3-(3,4-dioxo-2-phenylamino-cyclobut-1-enylamino)-2-hydroxy-
- 10 benzenesulfonamide;
- 3-[2-(2-Bromo-phenylamino)-3,4-dioxo-cyclobut-1-enylamino]-6-chloro-2-hydroxy-benzenesulfonamide;
- 6-Chloro-3-[2-(2-chloro-phenylamino)-3,4-dioxo-cyclobut-1-enylamino]-2-hydroxy-benzenesulfonamide;
- 15 6-Chloro-2-hydroxy-3-[2-(2-methoxy-phenylamino)-3,4-dioxo-cyclobut-1-enylamino]-benzenesulfonamide;
- 6-Chloro-3-[3,4-dioxo-2-(2-phenoxy-phenylamino)-cyclobut-1-enylamino]-2-hydroxy-benzenesulfonamide;
- 6-Chloro-3-[2-(4-fluoro-phenylamino)-3,4-dioxo-cyclobut-1-enylamino]-2-hydroxy-
- 20 benzenesulfonamide or a suitable salt thereof.

### Methods of Preparation

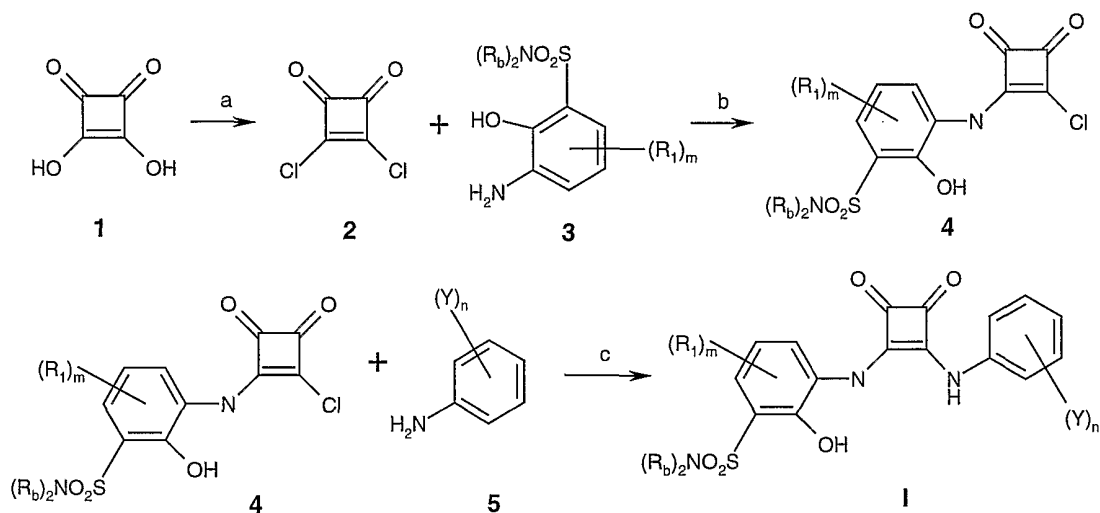
The compounds of Formula (I) may be obtained by applying synthetic procedures, some of which are illustrated in the Schemes below. The synthesis

25 provided for in these Schemes is applicable for producing compounds of Formula (I) having a variety of different R, R<sub>1</sub>, and aryl groups which are reacted, employing optional substituents which are suitably protected, to achieve compatibility with the reactions outlined herein. Subsequent deprotection, in those cases, then affords compounds of the nature generally disclosed. Once the guanidine nucleus has been

30 established, further compounds of these formulas may be prepared by applying standard techniques for functional group interconversion, well known in the art.

While the schemes are shown with compounds only of Formula (I) this is merely for illustration purposes only.

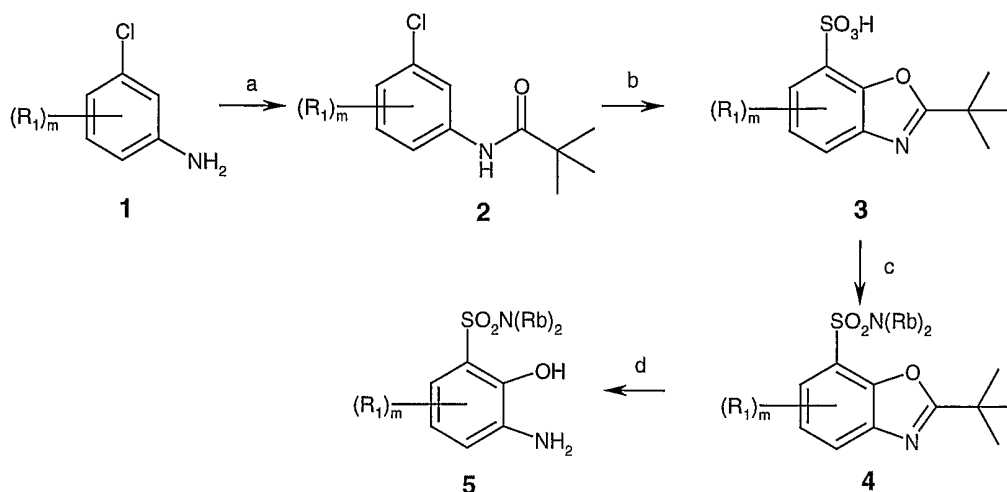
### Scheme 1



5

a.) (COCl)<sub>2</sub>, 45°C; b.) THF; c.) DMSO, rt or 45°C.

The desired compounds of formula (I) can be prepared as outlined in Scheme 1. Dichlorosquarate 2 can be prepared from squaric acid 1 using standard chlorination methods well known in the art such as oxalyl chloride and catalytic amounts of DMF in methylene chloride and heating at 45°C. Reacting 10 dichlorosquarate 2 with the desired phenol 3 in an organic solvent such as THF gives the mono-chlorosquarate 4. Reacting mono-chlorosquarate 4 with the desired aniline 5 in an organic solvent such as DMSO at room temperature or heating at 45°C gives the target compound of formula (I).

**Scheme 2**

a.) PivCl, TEA; b.) i. BuLi (2eq), THF, -40°C; ii. SO<sub>2</sub>; c.) i. SO<sub>2</sub>Cl<sub>2</sub>, ii. HN(R<sub>b</sub>)<sub>2</sub>, TEA;  
 d.) H<sub>2</sub>SO<sub>4</sub>, H<sub>2</sub>O.

If the desired phenolanylamine 3 in Scheme 1 is not commercially available, it can be prepared as outlined in Scheme 2. Commercially available substituted 3-chloroanilines 1 can be converted to the amide 2 using standard conditions well known in the art such as pivavolyl chloride and triethylamine in a suitable organic solvent such as methylene chloride. The amide 2 can be converted to the benzoxazole 3 using an excess amount of a strong base such as butyllithium in a suitable organic solvent such as THF under reduced reaction temperatures between -20 and -40°C followed by quenching the reaction with sulfur dioxide gas. The sulfonic acid 3 can be converted to the sulfonamide 4 using standard conditions well known in the art such as sulfurylchloride in a suitable organic solvent such as methylene chloride to give the intermediate sunfonyl chloride. The sulfonyl chloride intermediate can be transformed to the sulfonamide 4 using standard conditons well known in the art by reacting it with the amine HN(R<sub>b</sub>)<sub>2</sub> in the presence of a suitable amine base such as triethylamine in a suitable organic solvent such as methylene chloride. The desired phenolanylamine 5 can be obtained from the benzoxazole 4 using standard hydrolysis conditions well known in the art such as sulfuric acid in water and heating at 85°C.

## SYNTHETIC EXAMPLES

The invention will now be described by reference to the following examples which are merely illustrative and are not to be construed as a limitation of the scope of the present invention. All temperatures are given in degrees centigrade, all solvents are highest available purity and all reactions run under anhydrous conditions in an argon atmosphere unless otherwise indicated.

In the Examples, all temperatures are in degrees Centigrade (°C). Mass spectra were performed upon a VG Zab mass spectrometer using fast atom bombardment, unless otherwise indicated. <sup>1</sup>H-NMR (hereinafter "NMR") spectra were recorded at 250 MHz using a Bruker AM 250 or Am 400 spectrometer. Multiplicities indicated are: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet and br indicates a broad signal. Sat. indicates a saturated solution, eq indicates the proportion of a molar equivalent of reagent relative to the principal reactant.

### Example 1

#### **Preparation of 3-[4-Chloro-2-hydroxy-3-(4-methyl-piperazine-1-sulfonyl)-phenylamino]-4-phenylamino-cyclobut-3-ene-1,2-dione.**

*N*-(3,4-Dichloro-phenyl)-2,2-dimethyl-propionamide

3,4-dichloroaniline (150g) in TBME (1L) was cooled to 10-15 °C. 30% aq NaOH (141 g, 1.14 equiv) was added, and the solution stirred vigorously via overhead mechanical stirrer. Trimethylacetyl chloride ("PivCl", 126mL) was added at such a rate as to keep the internal temperature below 30 °C. During this addition, the solution mixture becomes thick with white solid product. When the addition was complete (10-15 min), the mixture was heated to 30-35 °C for 1 hr, and then allowed to cool. The reaction mixture was held at -5 °C (overnight), and then filtered, rinsing first with 90:10 water/MeOH (600mL) and then water (900mL). Drying under vacuum yielded 195g (86%) product, as off-white crystals. LCMS m/z 246(M-H)<sup>+</sup>.

2-*tert*-Butyl-6-chloro-benzooxazole-7-sulfonyl chloride

The solution of *N*-(3,4-dichloro-phenyl)-2,2-dimethyl-propionamide (10g, 41mmol) in dry THF (100mL) was cooled to -72 °C under argon. *n*-Butyl lithium (1.6M in hexane, 64mL, 102mmol) was added dropwise. The solution warmed to ca. -50 °C over 45 minutes, and then was kept in the -25 - -10 °C range for 2 hrs.

5 The solution was then re-cooled to -78 °C, and sulfur dioxide was bubbled through the solution for 30min. The solution was then allowed to warm to room temperature for 2h, and a Ar stream was bubbled through the solution, with a gas outlet provided so that any excess sulfur dioxide could escape during the warming. The THF solution was cooled in an ice bath, and sulfuryl chloride (3.58mL, 44.9mmol) was

10 added dropwise. After a few minutes, the solution was warmed to room temperature for overnight. The mixture was concentrated, diluted with ethyl acetate and washed with water. Decolorizing carbon was added and the mixture was filtered. The resulting solution was dried (sodium sulfate), filtered and concentrated to afford the title compound (12.4g, 98%). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.92 (d, 1H, J=8.5 Hz), 7.57 (d,

15 1H, J=8.4 Hz), 1.57 (s, 9H).

General procedure for the synthesis of sulfonylamides.

*2-tert*-Butyl-6-chloro-7-(4-methyl-piperazine-1-sulfonyl)-benzooxazole

To a solution of *2-tert*-butyl-6-chloro-benzooxazole-7-sulfonyl chloride (2.69g, 8.73mmol) and triethylamine (2.44mL, 17.5mmol) in THF (60mL) at 0 °C was

20 added 1-methylpiperazine (0.98mL, 8.83mmol). The reaction was warmed to room temperature and allowed to stir overnight. The solution was concentrated and then diluted with water and extracted with ethyl acetate (3 times). The combined organic layers were dried with MgSO<sub>4</sub>, filtered, and concentrated. Flash chromatography

25 (80% ethyl acetate/ 20% Ethanol) on silica gel gave the title compound (2.45g, 76%). EI-MS *m/z* 372(M+H)<sup>+</sup>.

General procedure for the hydrolysis of the benzooxazole to the desired aniline.

30 6-Amino-3-chloro-2-(4-methyl-piperazine-1-sulfonyl)-phenol

To a solution of 2-*tert*-Butyl-6-chloro-7-(4-methyl-piperazine-1-sulfonyl)-benzooxazole (2.44g, 6.56mmol) in 1,4-dioxane (20mL) was treated with water (4mL) and conc. H<sub>2</sub>SO<sub>4</sub> (4mL). The mixture was heated to 85 °C for 14h. The reaction was cooled to room temperature, and then basified to pH=14 with 25% aq NaOH. washed. The mixture was extracted with ethyl acetate (3 times), dried with MgSO<sub>4</sub>, filtered, and concentrated to afford the title compound (1.35g, 68%). EI-MS *m/z* 306(M+H)<sup>+</sup>.

#### 3,4-Dichloro-cyclobut-3-ene-1,2-dione

To a solution of 3,4-dihydroxy-cyclobut-3-ene-1,2-dione (10g, 88mmol) in dichloromethane (100mL) at 0 °C was added DMF (10 drops) followed by dropwise addition of oxalyl chloride (16.8mL, 193mmol) over 10 min. The reaction was warmed to room temperature and then heated to 45 °C for 4h. The reaction was cooled to room temperature and concentrated to afford the title compound (11g, 83%). <sup>13</sup>C NMR (100MHz, CDCl<sub>3</sub>) δ 190, 188.

General procedure for synthesis of phenyl monochloro squarate.

#### 3-Chloro-4-[4-chloro-2-hydroxy-3-(4-methyl-piperazine-1-sulfonyl)-phenylamino]-cyclobut-3-ene-1,2-dione

To a solution of 3,4-dichloro-cyclobut-3-ene-1,2-dione (509mg, 3.37mmol) in THF (43mL) at 0 °C was added a solution of 6-amino-3-chloro-2-(4-methyl-piperazine-1-sulfonyl)-phenol (1.03g, 3.37mmol) in THF (13mL) over 20 min. The reaction was warmed to room temperature and stirred for 14h. The resulting mixture was concentrated to afford the title compound (1.40g, 99%). LCMS *m/z* 421(M+H)<sup>+</sup>.

General procedure for the synthesis of the biphenyl squarate.

#### 3-[4-Chloro-2-hydroxy-3-(4-methyl-piperazine-1-sulfonyl)-phenylamino]-4-phenylamino-cyclobut-3-ene-1,2-dione

A solution of 3-chloro-4-[4-chloro-2-hydroxy-3-(4-methyl-piperazine-1-sulfonyl)-phenylamino]-cyclobut-3-ene-1,2-dione (1.10g, 2.62mmol) in DMF (10.0mL) was treated with aniline (0.270mL, 2.96mmol) for 14h at room temperature. Water (25mL) and 1N HCl (10mL) was added to the reaction and the  
5 resulting precipitate was centrifuged down and the water decanted off. Methanol was added to the solid and the resulting mixture was concentrated. Dichloromethane (50mL) was added to the solid, and then triethylamine (2mL) was added to dissolve most of the solid. The mixture was stirred for 1h and then filtered through celite and concentrated. The resulting solid was pumped on overnight, and then diluted with  
10 DCM (50mL). HCl (4N in 1,4- dioxane, 4.0mL) was added to the solution, and the resulting precipitate was filtered to afford the title compound (520mg, 42%). LCMS  $m/z$  477(M+H)<sup>+</sup>.

### Example 2

15 **Preparation of 3-(2-Bromo-phenylamino)-4-[4-chloro-2-hydroxy-3-(4-methyl-piperazine-1-sulfonyl)-phenylamino]-cyclobut-3-ene-1,2-dione.**

Following the general procedure for the synthesis of biphenyl squarate outlined in example 1, 3-chloro-4-[4-chloro-2-hydroxy-3-(4-methyl-piperazine-1-sulfonyl)-phenylamino]-cyclobut-3-ene-1,2-dione (150mg, 0.36mmol) and 2-  
20 bromoaniline (112mg, 0.651mmol) in DMF (1.7mL) were reacted at 39 °C to afford the title compound (98.4mg, 50%). Purification with triethylamine/DCM was not needed, only a DCM wash of the centrifuged solid was required. LCMS  $m/z$  555(M-H)<sup>+</sup>.

**Example 3****Preparation of 3-[4-Chloro-2-hydroxy-3-(4-methyl-piperazine-1-sulfonyl)-phenylamino]-4-(2-chloro-phenylamino)-cyclobut-3-ene-1,2-dione.**

5

Following the general procedure for the synthesis of biphenyl squarate outlined in example 1, 3-chloro-4-[4-chloro-2-hydroxy-3-(4-methyl-piperazine-1-sulfonyl)-phenylamino]-cyclobut-3-ene-1,2-dione (150mg, 0.36mmol) and 2-chloroaniline (0.069mL, 0.65mmol) in DMF (1.6mL) were reacted at 40 °C to afford the title compound (57.5mg, 32%). Purification with triethylamine/DCM was not needed, only a DCM wash of the centrifuged solid was required. LCMS m/z 511(M-H)<sup>+</sup>.

10

**Example 4****Preparation of 3-[4-Chloro-2-hydroxy-3-(4-methyl-piperazine-1-sulfonyl)-phenylamino]-4-(2-methoxy-phenylamino)-cyclobut-3-ene-1,2-dione.**

15

Following the general procedure for the synthesis of biphenyl squarate outlined in example 1, 3-chloro-4-[4-chloro-2-hydroxy-3-(4-methyl-piperazine-1-sulfonyl)-phenylamino]-cyclobut-3-ene-1,2-dione (152mg, 0.36mmol) and 2-methoxyaniline (0.060mL, 0.53mmol) in DMF (1.6mL) were reacted at room temperature for 48h to afford the title compound (119mg, 66%). Purification with triethylamine/DCM was not needed, only a DCM wash of the centrifuged solid was required. LCMS m/z 507(M-H)<sup>+</sup>.

20

25



**Example 5****Preparation of 3-[4-Chloro-2-hydroxy-3-(4-methyl-piperazine-1-sulfonyl)-phenylamino]-4-(2-fluoro-phenylamino)-cyclobut-3-ene-1,2-dione.**

5           Following the general procedure for the synthesis of biphenyl squarate outlined in example 1, 3-chloro-4-[4-chloro-2-hydroxy-3-(4-methyl-piperazine-1-sulfonyl)-phenylamino]-cyclobut-3-ene-1,2-dione (152mg, 0.36mmol) and 2-fluoroaniline (0.050mL, 0.52mmol) in DMF (1.6mL) were reacted at room temperature to afford the title compound (118mg, 69%). Purification with  
10 triethylamine/DCM was not needed, only a DCM wash of the centrifuged solid was required. LCMS m/z 495(M-H)<sup>+</sup>.

**Example 6****Preparation of 3-[4-Chloro-2-hydroxy-3-(4-methyl-piperazine-1-sulfonyl)-phenylamino]-4-(4-fluoro-phenylamino)-cyclobut-3-ene-1,2-dione.**  
15

          Following the general procedure for the synthesis of biphenyl squarate outlined in example 1, 3-chloro-4-[4-chloro-2-hydroxy-3-(4-methyl-piperazine-1-sulfonyl)-phenylamino]-cyclobut-3-ene-1,2-dione (150mg, 0.36mmol) and 4-  
20 fluoroaniline (0.048mL, 0.51mmol) in DMF (1.6mL) were reacted at room temperature to afford the title compound (123mg, 73%). Purification with triethylamine/DCM was not needed, only a DCM wash of the centrifuged solid was required. LCMS m/z 495(M-H)<sup>+</sup>.

**Example 7**  
25**Preparation of 3-[4-Chloro-2-hydroxy-3-(4-methyl-piperazine-1-sulfonyl)-phenylamino]-4-(2,6-difluoro-phenylamino)-cyclobut-3-ene-1,2-dione.**

          Following the general procedure for the synthesis of biphenyl squarate  
30 outlined in example 1, 3-chloro-4-[4-chloro-2-hydroxy-3-(4-methyl-piperazine-1-

sulfonyl)-phenylamino]-cyclobut-3-ene-1,2-dione (150mg, 0.36mmol) and 2,6-difluoroaniline (0.068mL, 0.63mmol) in DMF (1.6mL) were reacted at 40 °C to afford the title compound (124mg, 68%). Purification with triethylamine/DCM was not needed, only a DCM wash of the centrifuged solid was required. LCMS m/z

5 513(M-H)<sup>+</sup>.

### **Example 8**

#### **Preparation of 3-[4-Chloro-2-hydroxy-3-(4-methyl-piperazine-1-sulfonyl)-phenylamino]-4-(2,4-difluoro-phenylamino)-cyclobut-3-ene-1,2-dione.**

10

Following the general procedure for the synthesis of biphenyl squarate outlined in example 1, 3-chloro-4-[4-chloro-2-hydroxy-3-(4-methyl-piperazine-1-sulfonyl)-phenylamino]-cyclobut-3-ene-1,2-dione (150mg, 0.36mmol) and 2,4-difluoroaniline (0.055mL, 0.54mmol) in DMF (1.6mL) were reacted to afford the

15 title compound (135mg, 75%). Purification with triethylamine/DCM was not needed, only a DCM wash of the centrifuged solid was required. LCMS m/z 513(M-H)<sup>+</sup>.

### **Example 9**

#### **Preparation of 3-(2-Chloro-4-fluoro-phenylamino)-4-[4-chloro-2-hydroxy-3-(4-methyl-piperazine-1-sulfonyl)-phenylamino]-cyclobut-3-ene-1,2-dione.**

Following the general procedure for the synthesis of biphenyl squarate outlined in example 1, 3-chloro-4-[4-chloro-2-hydroxy-3-(4-methyl-piperazine-1-sulfonyl)-phenylamino]-cyclobut-3-ene-1,2-dione (150mg, 0.36mmol) and 2-chloro-4-fluoroaniline (0.090mL, 0.75mmol) in DMF (1.6mL) were reacted at 40 °C for

25 48h. Gilson preparatory HPLC was used (intead of triethylamine/DCM) to isolate the title compound (68.3mg, 19%). LCMS m/z 529(M-H)<sup>+</sup>.

30

### **Example 10**

**Preparation of 3-[4-Chloro-2-hydroxy-3-(piperazine-1-sulfonyl)-phenylamino]-4-phenylamino-cyclobut-3-ene-1,2-dione.**

4-(2-*tert*-Butyl-6-chloro-benzooxazole-7-sulfonyl)-piperazine-1-carboxylic acid *tert*-butyl ester

5           Following the general procedure for the synthesis of sulfonylamides outlined in example 1, 2-*tert*-butyl-6-chloro-benzooxazole-7-sulfonyl chloride (5.0g, 16.2mmol), triethylamine (2.4mL, 17.2mmol), and piperazine-carboxylic acid *tert*-butyl ester (3.62g, 19.4mmol) were reacted in THF (50mL) to afford the title compound (5.44g, 67%). LCMS m/z 402(M-H)<sup>+</sup> (desired -Boc).

10

6-Amino-3-chloro-2-(piperazine-1-sulfonyl)-phenol

          Following the general procedure for the hydrolysis of the benzooxazole to the desired aniline outlined in example 1, 4-(2-*tert*-butyl-6-chloro-benzooxazole-7-sulfonyl)-piperazine-1-carboxylic acid *tert*-butyl ester (2.0g, 4.3mmol), water  
15 (3.65mL), and H<sub>2</sub>SO<sub>4</sub> (3.65mL) in 1,4-dioxane (60mL) were reacted to afford the title compound (1.22g, 96%). LCMS m/z 292(M-H)<sup>+</sup>.

4-(3-Amino-6-chloro-2-hydroxy-benzenesulfonyl)-piperazine-1-carboxylic acid *tert*-butyl ester

20           To a solution of 6-amino-3-chloro-2-(piperazine-1-sulfonyl)-phenol (303mg, 1.04mmol) in DCM (12mL) was added di-*tert*-butyl dicarbonate (229mg, 1.05mmol) and the reaction mixture was allowed to stir at room temperature for 18h. The reaction was diluted with DCM and washed with 1N HCl (2 times). The organic layer was dried with MgSO<sub>4</sub>, filtered, and concentrated to afford the title compound  
25 (361mg, 89%). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 6.89 (d, 1H, J=8.48 Hz), 6.82 (d, 1H, J=8.48 Hz), 3.36 (m, 4H), 3.19 (m, 4H), 1.38 (s, 9H).

4-[6-Chloro-3-(2-chloro-3,4-dioxo-cyclobut-1-enylamino)-2-hydroxy-benzenesulfonyl]-piperazine-1-carboxylic acid *tert*-butyl ester

Following the general procedure for synthesis of phenyl monochloro squarate outlined in example 1, 4-(3-amino-6-chloro-2-hydroxy-benzenesulfonyl)-piperazine-1-carboxylic acid *tert*-butyl ester (359mg, 0.916mmol) and 3,4-dichloro-cyclobut-3-ene-1,2-dione (135mg, 0.893mmol) were reacted in THF (7.0mL) to  
5 form the title compound (410mg, 89%). LCMS m/z 406(M-H)<sup>+</sup> (desired-Boc).

3-[4-Chloro-2-hydroxy-3-(piperazine-1-sulfonyl)-phenylamino]-4-phenylamino-cyclobut-3-ene-1,2-dione

Following the general procedure for the synthesis of biphenyl squarate outlined in example 1, 4-[6-chloro-3-(2-chloro-3,4-dioxo-cyclobut-1-enylamino)-2-hydroxy-benzenesulfonyl]-piperazine-1-carboxylic acid *tert*-butyl ester (410mg, 0.81mmol) and aniline (0.146mL, 1.60mmol) were reacted in DMF (3.0mL) to afford the title compound (92.7mg, 25%). LCMS m/z 463(M-H)<sup>+</sup>.

15

### Example 11

**Preparation of 3-[4-Chloro-2-hydroxy-3-(4-methyl-[1,4]diazepane-1-sulfonyl)-phenylamino]-4-phenylamino-cyclobut-3-ene-1,2-dione.**

2-*tert*-Butyl-6-chloro-7-(4-methyl-[1,4]diazepane-1-sulfonyl)-benzoxazole

Following the general procedure for the synthesis of sulfonylamides outlined  
20 in example 1, 2-*tert*-butyl-6-chloro-benzoxazole-7-sulfonyl chloride (3.17g, 10.3mmol), triethylamine (2.90mL, 20.8mmol), and 1-methylhomopiperazine (1.29mL, 10.4mmol) were reacted in THF (80mL) to afford the title compound (2.21g, 56%). LCMS m/z 386(M-H)<sup>+</sup>.

25 6-Amino-3-chloro-2-(4-methyl-[1,4]diazepane-1-sulfonyl)-phenol

Following the general procedure for the hydrolysis of the benzoxazole to the desired aniline outlined in example 1, 2-*tert*-butyl-6-chloro-7-(4-methyl-[1,4]diazepane-1-sulfonyl)-benzoxazole (2.20g, 5.70mmol), water (3.0mL), and H<sub>2</sub>SO<sub>4</sub> (3.0mL) in 1,4-dioxane (25mL) were reacted to afford the title compound  
30 (1.39g, 77%). LCMS m/z 320(M-H)<sup>+</sup>.

3-Chloro-4-[4-chloro-2-hydroxy-3-(4-methyl-[1,4]diazepane-1-sulfonyl)-phenylamino]-cyclobut-3-ene-1,2-dione

Following the general procedure for synthesis of phenyl monochloro squarate outlined in example 1, 6-amino-3-chloro-2-(4-methyl-[1,4]diazepane-1-sulfonyl)-phenol (1.388g, 4.34mmol) and 3,4-dichloro-cyclobut-3-ene-1,2-dione (657mg, 4.34mmol) were reacted in THF (70mL) to form the title compound (1.303g, 70%). LCMS m/z 434(M-H)<sup>+</sup>.

3-[4-Chloro-2-hydroxy-3-(4-methyl-[1,4]diazepane-1-sulfonyl)-phenylamino]-4-phenylamino-cyclobut-3-ene-1,2-dione

Following the general procedure for the synthesis of biphenyl squarate outlined in example 1, 3-chloro-4-[4-chloro-2-hydroxy-3-(4-methyl-[1,4]diazepane-1-sulfonyl)-phenylamino]-cyclobut-3-ene-1,2-dione (150mg, 0.35mmol) and aniline (0.041mL, 0.45mmol) were reacted in DMF (1.6mL) to afford the title compound (26.3mg, 16%). LCMS m/z 491(M-H)<sup>+</sup>.

### **Example 12**

**Preparation of 3-[4-Chloro-2-hydroxy-3-(4-methyl-[1,4]diazepane-1-sulfonyl)-phenylamino]-4-(2-chloro-phenylamino)-cyclobut-3-ene-1,2-dione.**

Following the general procedure for the synthesis of biphenyl squarate outlined in example 1, 3-chloro-4-[4-chloro-2-hydroxy-3-(4-methyl-[1,4]diazepane-1-sulfonyl)-phenylamino]-cyclobut-3-ene-1,2-dione (147mg, 0.34mmol) and 2-chloroaniline (0.12mL, 1.14mmol) were reacted in DMF (1.6mL) at 38 °C for 48h to afford the title compound (53.6mg, 30%). Purification with triethylamine/DCM was not needed, only a DCM wash of the centrifuged solid was required. LCMS m/z 525(M-H)<sup>+</sup>.

### **Example 13**

**Preparation of 6-Chloro-*N*-(2-diethylamino-ethyl)-3-(3,4-dioxo-2-phenylamino-cyclobut-1-enylamino)-2-hydroxy-*N*-methyl-benzenesulfonamide.**

2-*tert*-Butyl-6-chloro-benzoxazole-7-sulfonic acid (2-diethylamino-ethyl)-methyl-amide

5           Following the general procedure for the synthesis of sulfonylamides outlined in example 1, 2-*tert*-butyl-6-chloro-benzoxazole-7-sulfonyl chloride (2.50g, 8.12mmol), triethylamine (2.26mL, 16.2mmol), and *N,N*-diethyl-*N'*-methylenediamine (1.34mL, 8.28mmol) were reacted in THF (50mL) to afford the title compound (1.73g, 53%). EI-MS *m/z* 402(M-H)<sup>+</sup>.

10

3-Amino-6-chloro-*N*-(2-diethylamino-ethyl)-2-hydroxy-*N*-methyl-benzenesulfonamide

          Following the general procedure for the hydrolysis of the benzoxazole to the desired aniline outlined in example 1, 2-*tert*-butyl-6-chloro-benzoxazole-7-sulfonic  
15   acid (2-diethylamino-ethyl)-methyl-amide (860mg, 2.14mmol), water (1.7mL), and H<sub>2</sub>SO<sub>4</sub> (1.7mL) in 1,4-dioxane (13mL) were reacted to afford the title compound (615mg, 87%). EI-MS *m/z* 336(M-H)<sup>+</sup>.

6-Chloro-3-(2-chloro-3,4-dioxo-cyclobut-1-enylamino)-*N*-(2-diethylamino-ethyl)-2-  
20   hydroxy-*N*-methyl-benzenesulfonamide

          Following the general procedure for synthesis of phenyl monochloro squarate outlined in example 1, 3-amino-6-chloro-*N*-(2-diethylamino-ethyl)-2-  
hydroxy-*N*-methyl-benzenesulfonamide (98mg, 0.292mmol) and 3,4-dichloro-cyclobut-3-ene-1,2-dione (76.4mg, 0.50mmol) were reacted in THF (2.8mL) to form  
25   the title compound (92.8mg, 71%). EI-MS *m/z* 450(M-H)<sup>+</sup>.

6-Chloro-*N*-(2-diethylamino-ethyl)-3-(3,4-dioxo-2-phenylamino-cyclobut-1-enylamino)-2-hydroxy-*N*-methyl-benzenesulfonamide

          Following the general procedure for the synthesis of biphenyl squarate  
30   outlined in example 1, 6-chloro-3-(2-chloro-3,4-dioxo-cyclobut-1-enylamino)-*N*-(2-

diethylamino-ethyl)-2-hydroxy-*N*-methyl-benzenesulfonamide (98mg, 0.22mmol) and aniline (0.0265mL, 0.29mmol) were reacted in DMF (1.2mL) to afford the title compound (35.0mg, 32%). LCMS  $m/z$  507(M-H)<sup>+</sup>.

5

#### **Example 14**

##### **Preparation of 6-Chloro-3-[2-(2-chloro-phenylamino)-3,4-dioxo-cyclobut-1-enylamino]-*N*-(2-diethylamino-ethyl)-2-hydroxy-*N*-methyl-benzenesulfonamide.**

Following the general procedure for the synthesis of biphenyl squarate outlined in example 1, 6-chloro-3-(2-chloro-3,4-dioxo-cyclobut-1-enylamino)-*N*-(2-diethylamino-ethyl)-2-hydroxy-*N*-methyl-benzenesulfonamide (99.5mg, 0.221mmol) and 2-chloroaniline (0.054mL, 0.51mmol) were reacted in DMF (1.2mL) to afford the title compound (18.3mg, 17%). Purification with triethylamine/DCM was not needed, only a DCM wash of the centrifuged solid was required. LCMS  $m/z$  541(M-H)<sup>+</sup>.

15

#### **Example 15**

##### **Preparation of 3-[2-(2-Bromo-phenylamino)-3,4-dioxo-cyclobut-1-enylamino]-6-chloro-*N*-(2-diethylamino-ethyl)-2-hydroxy-*N*-methyl-benzenesulfonamide.**

20

Following the general procedure for the synthesis of biphenyl squarate outlined in example 1, 6-chloro-3-(2-chloro-3,4-dioxo-cyclobut-1-enylamino)-*N*-(2-diethylamino-ethyl)-2-hydroxy-*N*-methyl-benzenesulfonamide (100mg, 0.22mmol) and 2-bromoaniline (0.0478g, 0.277mmol) were reacted in DMF (1.2mL) to afford the title compound (19.2mg, 15%). Purification with triethylamine/DCM was not needed, only a DCM wash of the centrifuged solid was required. LCMS  $m/z$  585(M-H)<sup>+</sup>.

25

#### **Example 16**

**Preparation of 3-[3-([1,4']Bipiperidinyl-1'-sulfonyl)-4-chloro-2-hydroxy-phenylamino]-4-phenylamino-cyclobut-3-ene-1,2-dione.**

1'-(2-*tert*-Butyl-6-chloro-benzoxazole-7-sulfonyl)-[1,4']bipiperidinyl

5        Following the general procedure for the synthesis of sulfonylamides outlined in example 1, 2-*tert*-butyl-6-chloro-benzoxazole-7-sulfonyl chloride (2.50g, 8.12mmol), triethylamine (2.26mL, 16.2mmol), and 4-piperidino-piperidine (1.368g, 8.13mmol) were reacted in THF (50mL) to afford the title compound (1.65g, 46%). EI-MS  $m/z$  440(M-H)<sup>+</sup>.

10

6-Amino-2-([1,4']bipiperidinyl-1'-sulfonyl)-3-chloro-phenol

      Following the general procedure for the hydrolysis of the benzoxazole to the desired aniline outlined in example 1, 1'-(2-*tert*-butyl-6-chloro-benzoxazole-7-sulfonyl)-[1,4']bipiperidinyl (827mg, 1.88mmol), water (1.7mL), and H<sub>2</sub>SO<sub>4</sub> (1.7mL) in 1,4-dioxane (13mL) were reacted to afford the title compound (623mg, 89%). EI-MS  $m/z$  374(M-H)<sup>+</sup>.

15

3-[3-([1,4']Bipiperidinyl-1'-sulfonyl)-4-chloro-2-hydroxy-phenylamino]-4-chloro-cyclobut-3-ene-1,2-dione

20

      Following the general procedure for synthesis of phenyl monochloro squarate outlined in example 1, 6-amino-2-([1,4']bipiperidinyl-1'-sulfonyl)-3-chloro-phenol (267mg, 0.714mmol) and 3,4-dichloro-cyclobut-3-ene-1,2-dione (107mg, 0.718mmol) were reacted in THF (8.0mL) to form the title compound (250mg, 72%). EI-MS  $m/z$  490(M-H)<sup>+</sup>.

25

3-[3-([1,4']Bipiperidinyl-1'-sulfonyl)-4-chloro-2-hydroxy-phenylamino]-4-phenylamino-cyclobut-3-ene-1,2-dione

      Following the general procedure for the synthesis of biphenyl squarate outlined in example 1, 3-[3-([1,4']bipiperidinyl-1'-sulfonyl)-4-chloro-2-hydroxy-phenylamino]-4-chloro-cyclobut-3-ene-1,2-dione (345mg, 0.706mmol) and aniline

30



(0.080mL, 0.877mmol) were reacted in DMF (5.0mL) to afford the title compound (196mg, 52%). LCMS  $m/z$  545(M-H)<sup>+</sup>,

### Example 17

#### 5 **Preparation of 3-[3-(Amino-piperidine-1-sulfonyl)-4-chloro-2-hydroxy-phenylamino]-4-phenylamino-cyclobut-3-ene-1,2-dione.**

[1-(2-*tert*-Butyl-6-chloro-benzoxazole-7-sulfonyl)-piperidin-4-yl]-carbamic acid *tert*-butyl ester

10 Following the general procedure for the synthesis of sulfonylamides outlined in example 1, 2-*tert*-butyl-6-chloro-benzoxazole-7-sulfonyl chloride (5.05g, 16.4mmol), triethylamine (4.57mL, 32.8mmol), and 4-N-Boc-aminopiperidine (3.288g, 16.4mmol) were reacted in THF (125mL) to afford the title compound (4.18g, 54%). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  7.98 (d, 1H, J=8.48 Hz), 7.63 (d, 1H, J=8.47  
15 Hz), 3.73 (d, 2H), 3.35 (bs, 2H), 2.92 (m, 2H), 1.75 (d, 2H), 1.35 (s, 10H).

6-Amino-2-(4-amino-piperidine-1-sulfonyl)-3-chloro-phenol

Following the general procedure for the hydrolysis of the benzoxazole to the desired aniline outlined in example 1, [1-(2-*tert*-butyl-6-chloro-benzoxazole-7-  
20 sulfonyl)-piperidin-4-yl]-carbamic acid *tert*-butyl ester (4.18g, 8.86mmol), water (5.5mL), and H<sub>2</sub>SO<sub>4</sub> (5.5mL) in 1,4-dioxane (55mL) were reacted to afford the title compound (2.03g, 75%). LCMS  $m/z$  306(M-H)<sup>+</sup>.

[1-(3-Amino-6-chloro-2-hydroxy-benzenesulfonyl)-piperiden-4-yl]-carbamic acid  
25 *tert*-butyl ester

To a solution of 6-amino-2-(4-amino-piperidine-1-sulfonyl)-3-chloro-phenol (290mg, 0.95mmol) in DCM (8.2mL) was added di-*tert*-butyl dicarbonate (207mg, 0.95mmol) and the reaction mixture was allowed to stir at room temperature for 18h. The reaction was diluted with DCM and washed with 1N HCl (2 times). The

organic layer was dried with  $\text{MgSO}_4$ , filtered, and concentrated to afford the title compound (242mg, 63%). EI-MS  $m/z$  405(M-H)<sup>-</sup>.

5 {1-[6-Chloro-3-(2-chloro-3,4-dioxo-cyclobut-1-enylamino)-2-hydroxy-benzenesulfonyl]-piperidin-4-yl}-carbamic acid *tert*-butyl ester

Following the general procedure for synthesis of phenyl monochloro squarate outlined in example 1, [1-(3-Amino-6-chloro-2-hydroxy-benzenesulfonyl)-piperidin-4-yl]-carbamic acid *tert*-butyl ester (803mg, 1.97mmol) and 3,4-dichloro-cyclobut-3-ene-1,2-dione (295mg, 1.96mmol) were reacted in THF (54mL) to form  
10 the title compound (741mg, 72%). LCMS  $m/z$  420(M-H)<sup>+</sup> (desired-Boc).

3-[3-(Amino-piperidine-1-sulfonyl)-4-chloro-2-hydroxy-phenylamino]-4-phenylamino-cyclobut-3-ene-1,2-dione

Following the general procedure for the synthesis of biphenyl squarate  
15 outlined in example 1, {1-[6-chloro-3-(2-chloro-3,4-dioxo-cyclobut-1-enylamino)-2-hydroxy-benzenesulfonyl]-piperidin-4-yl}-carbamic acid *tert*-butyl ester (249mg, 0.47mmol) and aniline (0.057mL, 0.62mmol) were reacted in DMF (2.2mL) to afford the title compound (119mg, 54%). LCMS  $m/z$  477(M-H)<sup>+</sup> (No Boc peak in NMR).

**Example 18**

**Preparation of {1-[6-Chloro-3-(3,4-dioxo-2-phenylamino-cyclobut-1-enylamino)-2-hydroxy-benzenesulfonyl]-piperidin-4-yl}-carbamic acid *tert*-butyl ester.**

Following the general procedure for the synthesis of biphenyl squarate outlined in example 1, {1-[6-chloro-3-(2-chloro-3,4-dioxo-cyclobut-1-enylamino)-2-hydroxy-benzenesulfonyl]-piperidin-4-yl}-carbamic acid *tert*-butyl ester (179mg, 0.344mmol) and aniline (0.037mL, 0.406mmol) were reacted in THF/DMF (3.0mL/0.5mL) to afford the title compound (36.3mg, 18%). Purification with triethylamine/DCM was not used. Desired compound slowly fell out of water wash. LCMS m/z 477(M-H)<sup>+</sup>.

**Example 19**

**Preparation of 3-[3-((R)-3-Amino-pyrrolidine-1-sulfonyl)-4-chloro-2-hydroxy-phenylamino]-4-phenylamino-cyclobut-3-ene-1,2-dione**

[(R)-1-(2-*tert*-Butyl-6-chloro-benzooxazole-7-sulfonyl)-pyrrolidin-3-yl]-carbamic acid *tert*-butyl ester

Following the general procedure for the synthesis of sulfonylamides outlined in example 1, 2-*tert*-butyl-6-chloro-benzooxazole-7-sulfonyl chloride (3.0g, 9.74mmol), triethylamine (1.63mL, 11.7mmol), and (R)-pyrrolidin-3-yl-carbamic acid *tert*-butyl ester (2.18g, 11.7mmol) were reacted in THF (30mL) to afford the title compound (3.0g, 67%). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.77 (d, 1H, J=8.5 Hz), 7.48 (d, 1H, J=8.5 Hz), 4.67 (bm, 1H), 4.22 (bm, 1H), 3.66 (bm, 2H), 3.52 (bm, 1H), 3.41 (bm, 1H), 2.19 (bm, 1H), 1.90 (bm, 1H), 1.48 (s, 9H).

6-Amino-2-((R)-3-amino-pyrrolidine-1-sulfonyl)-3-chloro-phenol

Following the general procedure for the hydrolysis of the benzoxazole to the desired aniline outlined in example 1, [(R)-1-(2-*tert*-butyl-6-chloro-benzoxazole-7-sulfonyl)-pyrrolidin-3-yl]-carbamic acid *tert*-butyl ester (2.0g, 4.31mmol), water (3.6mL), and H<sub>2</sub>SO<sub>4</sub> (3.6mL) in 1,4-dioxane (60mL) were reacted to afford the title compound (1.2g, 94%). LCMS m/z 292(M-H)<sup>+</sup>.

[(R)-1-(3-Amino-6-chloro-2-hydroxy-benzenesulfonyl)-pyrrolidin-3-yl]-carbamic acid *tert*-butyl ester

To a solution of 6-amino-2-((R)-3-amino-pyrrolidine-1-sulfonyl)-3-chloro-phenol (300mg, 1.02mmol) in DCM (8.5mL) was added di-*tert*-butyl dicarbonate (237mg, 1.08mmol) and the reaction mixture was allowed to stir at room temperature for 18h. The reaction was diluted with DCM and washed with 1N HCl (2 times). The organic layer was dried with MgSO<sub>4</sub>, filtered, and concentrated to afford the title compound (365mg, 91%). EI-MS m/z 391(M-H)<sup>-</sup>.

{(R)-1-[6-Chloro-3-(2-chloro-3,4-dioxo-cyclobut-1-enylamino)-2-hydroxy-benzenesulfonyl]-pyrrolidin-3-yl}-carbamic acid *tert*-butyl ester

Following the general procedure for synthesis of phenyl monochloro squarate outlined in example 1, [(R)-1-(3-amino-6-chloro-2-hydroxy-benzenesulfonyl)-pyrrolidin-3-yl]-carbamic acid *tert*-butyl ester (365mg, 0.93mmol) and 3,4-dichloro-cyclobut-3-ene-1,2-dione (140mg, 0.93mmol) were reacted in THF (7.0mL) to form the title compound (386mg, 82%). EI-MS m/z 506(M-H)<sup>-</sup>.

3-[3-((R)-3-Amino-pyrrolidine-1-sulfonyl)-4-chloro-2-hydroxy-phenylamino]-4-phenylamino-cyclobut-3-ene-1,2-dione

Following the general procedure for the synthesis of biphenyl squarate outlined in example 1, {(R)-1-[6-Chloro-3-(2-chloro-3,4-dioxo-cyclobut-1-enylamino)-2-hydroxy-benzenesulfonyl]-pyrrolidin-3-yl}-carbamic acid *tert*-butyl ester (470mg, 0.93mmol) and aniline (0.10mL, 1.09mmol) were reacted in DMF

(4.0mL) to afford the title compound (23.4mg, 6%). LCMS  $m/z$  463(M-H)<sup>+</sup> (No Boc peak in NMR).

### **Example 20**

5    **Synthesis of 6-Chloro-3-(3,4-dioxo-2-phenylamino-cyclobut-1-enylamino)-2-hydroxy-benzenesulfonamide.**

Synthesis of 6-Chloro-3-(2-chloro-3,4-dioxo-cyclobut-1-enylamino)-2-hydroxy-benzenesulfonamide:

Following the general procedure for the synthesis of phenyl monochloro  
10    squarates outlined in example 1; dichlorosquarate (4.5mmol) and 3-amino-6-chloro-2-hydroxy-benzenesulfonamide (4.5mmol) were stirred in THF (1mL) for 24 hrs to give 0.78g (50%) of 6-chloro-3-(2-chloro-3,4-dioxo-cyclobut-1-enylamino)-2-hydroxy-benzenesulfonamide which was used in the next step without further purification.

15    Synthesis of 6-Chloro-3-(3,4-dioxo-2-phenylamino-cyclobut-1-enylamino)-2-hydroxy-benzenesulfonamide.

Following the general procedure for the synthesis of diphenylsquarates outlined in example 1; 6-chloro-3-(2-chloro-3,4-dioxo-cyclobut-1-enylamino)-2-  
20    hydroxy-benzenesulfonamide (0.86mmol) and aniline (1.7mmol) were stirred in DMSO (1.0mL) for 24 hrs to give 6-Chloro-3-(3,4-dioxo-2-phenylamino-cyclobut-1-enylamino)-2-hydroxy-benzenesulfonamide. LCMS  $m/z$  396 (M-H)<sup>+</sup>.

### **Example 21**

25    **Synthesis of 3-[2-(2-bromo-phenylamino)-3,4-dioxo-cyclobut-1-enylamino]-6-chloro-2-hydroxy-benzenesulfonamide.**

Following the general procedure for the synthesis of diphenylsquarates outlined in example 1; 6-chloro-3-(2-chloro-3,4-dioxo-cyclobut-1-enylamino)-2-  
30    hydroxy-benzenesulfonamide (0.29mmol) and 2-bromoaniline (0.86mmol) were stirred in DMSO (3mL) for 24 hrs to give 60mg (44%) of 3-[2-(2-bromo-

phenylamino)-3,4-dioxo-cyclobut-1-enylamino]-6-chloro-2-hydroxy-benzenesulfonamide. LCMS m/z 474 (M-H)<sup>+</sup>.

#### **Example 22**

##### **5    Synthesis of 3-[2-(2-chloro-phenylamino)-3,4-dioxo-cyclobut-1-enylamino]-6-chloro-2-hydroxy-benzenesulfonamide.**

Following the general procedure for the synthesis of diphenylsquarates outlined in example 1; 6-chloro-3-(2-chloro-3,4-dioxo-cyclobut-1-enylamino)-2-hydroxy-benzenesulfonamide (0.86mmol) and 2-chloroaniline (1.7mmol) were  
10    stirred in DMSO (1.0mL) for 24 hrs to give 19mg (5%) of 3-[2-(2-chloro-phenylamino)-3,4-dioxo-cyclobut-1-enylamino]-6-chloro-2-hydroxy-benzenesulfonamide. LCMS m/z 430 (M-H)<sup>+</sup>.

#### **Example 23**

##### **15    Synthesis of 3-[2-(2-methoxy-phenylamino)-3,4-dioxo-cyclobut-1-enylamino]-6-chloro-2-hydroxy-benzenesulfonamide.**

Following the general procedure for the synthesis of diphenylsquarates  
20    outlined in example 1; 6-chloro-3-(2-chloro-3,4-dioxo-cyclobut-1-enylamino)-2-hydroxy-benzenesulfonamide (2.4mmol) and 2-methoxyaniline (7.3mmol) were stirred in DMSO (3mL) for 24 hrs to give 0.57g (55%) of 3-[2-(2-methoxy-phenylamino)-3,4-dioxo-cyclobut-1-enylamino]-6-chloro-2-hydroxy-benzenesulfonamide. LCMS m/z 425 (M-H)<sup>+</sup>.

**Example 24****Synthesis of 3-[2-(2-phenoxy-phenylamino)-3,4-dioxo-cyclobut-1-enylamino]-6-chloro-2-hydroxy-benzenesulfonamide.**

5

Following the general procedure for the synthesis of diphenylsquarates outlined in example 1; 6-chloro-3-(2-chloro-3,4-dioxo-cyclobut-1-enylamino)-2-hydroxy-benzenesulfonamide (0.43mmol) and 2-phenoxyaniline (1.3mmol) were stirred in DMSO (3mL) for 24 hrs to give 49mg (24%) of 3-[2-(2-phenoxy-phenylamino)-3,4-dioxo-cyclobut-1-enylamino]-6-chloro-2-hydroxy-benzenesulfonamide. LCMS m/z 489 (M-H)<sup>+</sup>.

10

**Example 25****Synthesis of 3-[2-(4-fluoro-phenylamino)-3,4-dioxo-cyclobut-1-enylamino]-6-chloro-2-hydroxy-benzenesulfonamide.**

15

Following the general procedure for the synthesis of diphenylsquarates outlined in example 1; 6-chloro-3-(2-chloro-3,4-dioxo-cyclobut-1-enylamino)-2-hydroxy-benzenesulfonamide (0.67mmol) and 4-fluoroaniline (1.3mmol) were stirred in DMSO (1.0mL) for 24 hrs to give 3-[2-(4-fluoro-phenylamino)-3,4-dioxo-cyclobut-1-enylamino]-6-chloro-2-hydroxy-benzenesulfonamide. LCMS m/z 412 (M-H)<sup>+</sup>.

20

**METHOD OF TREATMENT**

25

The compounds of Formula (I) or a pharmaceutically acceptable salt thereof can be used in the manufacture of a medicament for the prophylactic or therapeutic treatment of any disease state in a human, or other mammal, which is exacerbated or caused by excessive or unregulated IL-8 cytokine production by such mammal's cell, such as but not limited to monocytes and/or macrophages, or other chemokines which bind to the IL-8  $\alpha$  or  $\beta$  receptor, also referred to as the type I or type II receptor.

30

Accordingly, the present invention provides a method of treating a chemokine mediated disease, wherein the chemokine is one which binds to an IL-8  $\alpha$  or  $\beta$  receptor and which method comprises administering an effective amount of a compound of Formula (I) or a pharmaceutically acceptable salt thereof. In

5 particular, the chemokines are IL-8, GRO $\alpha$ , GRO $\beta$ , GRO $\gamma$ , NAP-2 or ENA-78.

For purposes herein, the compounds of Formula (I) and (II) all have the same dosages, and formulations as that of Formula (I) are used interchangeably.

The compounds of Formula (I) are administered in an amount sufficient to inhibit cytokine function, in particular IL-8, GRO $\alpha$ , GRO $\beta$ , GRO $\gamma$ , NAP-2 or ENA-78, such that they are biologically regulated down to normal levels of physiological function, or in some case to subnormal levels, so as to ameliorate the disease state. Abnormal levels of IL-8, GRO $\alpha$ , GRO $\beta$ , GRO $\gamma$ , NAP-2 or ENA-78 for instance in the context of the present invention, constitute: (i) levels of free IL-8 greater than or equal to 1 picogram per mL; (ii) any cell associated IL-8, GRO $\alpha$ , GRO $\beta$ , GRO $\gamma$ , NAP-2 or  
10 ENA-78 above normal physiological levels; or (iii) the presence IL-8, GRO $\alpha$ , GRO $\beta$ , GRO $\gamma$ , NAP-2 or ENA-78 above basal levels in cells or tissues in IL-8, GRO $\alpha$ , GRO $\beta$ , GRO $\gamma$ , NAP-2 or ENA-78 respectively, is produced.

There are many disease states in which excessive or unregulated IL-8 production is implicated in exacerbating and/or causing the disease. Chemokine mediated diseases include psoriasis, atopic dermatitis, osteo arthritis, rheumatoid  
20 arthritis, asthma, chronic obstructive pulmonary disease, adult respiratory distress syndrome, inflammatory bowel disease, Crohn's disease, ulcerative colitis, stroke, septic shock, multiple sclerosis, endotoxic shock, gram negative sepsis, toxic shock syndrome, cardiac and renal reperfusion injury, glomerulonephritis, thrombosis, graft vs. host reaction, Alzheimer's disease, allograft rejections, malaria, restenosis, angiogenesis, atherosclerosis, osteoporosis, gingivitis and undesired hematopoietic stem cells release and diseases caused by respiratory viruses, herpes viruses, and hepatitis viruses, meningitis, cystic fibrosis, pre-term labor, cough, pruritus, multi-organ dysfunction, trauma, strains, sprains, contusions, psoriatic arthritis, herpes,  
25 encephalitis, CNS vasculitis, traumatic brain injury, CNS tumors, subarachnoid hemorrhage, post surgical trauma, interstitial pneumonitis, hypersensitivity, crystal



induced arthritis, acute and chronic pancreatitis, acute alcoholic hepatitis, necrotizing enterocolitis, chronic sinusitis, uveitis, polymyositis, vasculitis, acne, gastric and duodenal ulcers, celiac disease, esophagitis, glossitis, airflow obstruction, airway hyperresponsiveness, bronchiolitis obliterans organizing pneumonia, bronchiectasis, bronchiolitis, bronchiolitis obliterans, chronic bronchitis, cor pulmonae, dyspnea, emphysema, hypercapnea, hyperinflation, hypoxemia, hyperoxia-induced inflammations, hypoxia, surgical lung volume reduction, pulmonary fibrosis, pulmonary hypertension, right ventricular hypertrophy, sarcoidosis, small airway disease, ventilation-perfusion mismatching, wheeze, colds and lupus.

These diseases are primarily characterized by massive neutrophil infiltration, T-cell infiltration, or neovascular growth, and are associated with increased IL-8, GRO $\alpha$ , GRO $\beta$ , GRO $\gamma$ , NAP-2 or ENA-78 production which is responsible for the chemotaxis of neutrophils into the inflammatory site or the directional growth of endothelial cells. In contrast to other inflammatory cytokines (IL-1, TNF, and IL-6), IL-8, GRO $\alpha$ , GRO $\beta$ , GRO $\gamma$ , NAP-2 or ENA-78 have the unique property of promoting neutrophil chemotaxis, enzyme release including but not limited to elastase release as well as superoxide production and activation. The  $\alpha$ -chemokines but particularly, GRO $\alpha$ , GRO $\beta$ , GRO $\gamma$ , NAP-2 or ENA-78, working through the IL-8 type I or II receptor can promote the neovascularization of tumors by promoting the directional growth of endothelial cells. Therefore, the inhibition of IL-8 induced chemotaxis or activation would lead to a direct reduction in the neutrophil infiltration.

Recent evidence also implicates the role of chemokines in the treatment of HIV infections, Littleman *et al.*, Nature 381, pp. 661 (1996) and Koup *et al.*, Nature 381, pp. 667 (1996).

Present evidence also indicates the use of IL-8 inhibitors in the treatment of atherosclerosis. The first reference, Boisvert *et al.*, J. Clin. Invest., 1998, 101:353-363 shows, through bone marrow transplantation, that the absence of IL-8 receptors on stem cells (and, therefore, on monocytes/macrophages) leads to a reduction in the development of atherosclerotic plaques in LDL receptor deficient mice. Additional

supporting references are: Apostolopoulos, et al., Arterioscler. Thromb. Vasc. Biol. 1996, 16:1007-1012; Liu, et al., Arterioscler. Thromb. Vasc. Biol. 1997, 17:317-323; Rus, et al., Atherosclerosis. 1996, 127:263-271.; Wang et al., J. Biol. Chem. 1996, 271:8837-8842; Yue, et al., Eur. J. Pharmacol. 1993, 240:81-84; Koch, et al.,  
5 Am. J. Pathol., 1993, 142:1423-1431.; Lee, et al., Immunol. Lett., 1996, 53, 109-113.; and Terkeltaub et al., Arterioscler. Thromb., 1994, 14:47-53.

The present invention also provides for a means of treating, in an acute setting, as well as preventing, in those individuals deemed susceptible to, CNS injuries by the chemokine receptor antagonist compounds of Formula (I).

10 CNS injuries as defined herein include both open or penetrating head trauma, such as by surgery, or a closed head trauma injury, such as by an injury to the head region. Also included within this definition is ischemic stroke, particularly to the brain area.

Ischemic stroke may be defined as a focal neurologic disorder that results  
15 from insufficient blood supply to a particular brain area, usually as a consequence of an embolus, thrombi, or local atheromatous closure of the blood vessel. The role of inflammatory cytokines in this area has been emerging and the present invention provides a mean for the potential treatment of these injuries. Relatively little treatment, for an acute injury such as these has been available.

20 , TNF- $\alpha$  is a cytokine with proinflammatory actions, including endothelial leukocyte adhesion molecule expression. Leukocytes infiltrate into ischemic brain lesions and hence compounds which inhibit or decrease levels of TNF would be useful for treatment of ischemic brain injury. See Liu *et al.*, Stroke, Vol. 25., No. 7, pp. 1481-88 (1994) whose disclosure is incorporated herein by reference.

25 Models of closed head injuries and treatment with mixed 5-LO/CO agents is discussed in Shohami *et al.*, J. of Vasc & Clinical Physiology and Pharmacology, Vol. 3, No. 2, pp. 99-107 (1992) whose disclosure is incorporated herein by reference. Treatment, which reduced edema formation, was found to improve functional outcome in those animals treated.

30 The compounds of Formula (I) are administered in an amount sufficient to inhibit IL-8, binding to the IL-8 alpha or beta receptors, from binding to these

receptors, such as evidenced by a reduction in neutrophil chemotaxis and activation. The discovery that the compounds of Formula (I) are inhibitors of IL-8 binding is based upon the effects of the compounds of Formulas (I) in the *in vitro* receptor binding assays which are described herein. The compounds of Formula (I) have  
5 been shown to be inhibitors of type II IL-8 receptors.

As used herein, the term "IL-8 mediated disease or disease state" refers to any and all disease states in which IL-8, GRO $\alpha$ , GRO $\beta$ , GRO $\gamma$ , NAP-2 or ENA-78 plays a role, either by production of IL-8, GRO $\alpha$ , GRO $\beta$ , GRO $\gamma$ , NAP-2 or ENA-78 themselves, or by IL-8, GRO $\alpha$ , GRO $\beta$ , GRO $\gamma$ , NAP-2 or ENA-78 causing another  
10 monokine to be released, such as but not limited to IL-1, IL-6 or TNF. A disease state in which, for instance, IL-1 is a major component, and whose production or action, is exacerbated or secreted in response to IL-8, would therefore be considered a disease state mediated by IL-8.

As used herein, the term "chemokine mediated disease or disease state" refers  
15 to any and all disease states in which a chemokine which binds to an IL-8  $\alpha$  or  $\beta$  receptor plays a role, such as but not limited to IL-8, GRO- $\alpha$ , GRO- $\beta$ , GRO $\gamma$ , NAP-2 or ENA-78. This would include a disease state in which, IL-8 plays a role, either by production of IL-8 itself, or by IL-8 causing another monokine to be released, such as but not limited to IL-1, IL-6 or TNF. A disease state in which, for instance,  
20 IL-1 is a major component, and whose production or action, is exacerbated or secreted in response to IL-8, would therefore be considered a disease stated mediated by IL-8.

As used herein, the term "cytokine" refers to any secreted polypeptide that affects the functions of cells and is a molecule, which modulates interactions  
25 between cells in the immune, inflammatory or hematopoietic response. A cytokine includes, but is not limited to, monokines and lymphokines, regardless of which cells produce them. For instance, a monokine is generally referred to as being produced and secreted by a mononuclear cell, such as a macrophage and/or monocyte. Many other cells however also produce monokines, such as natural killer  
30 cells, fibroblasts, basophils, neutrophils, endothelial cells, brain astrocytes, bone marrow stromal cells, epidermal keratinocytes and B-lymphocytes. Lymphokines are

generally referred to as being produced by lymphocyte cells. Examples of cytokines include, but are not limited to, Interleukin-1 (IL-1), Interleukin-6 (IL-6), Interleukin-8 (IL-8), Tumor Necrosis Factor-alpha (TNF- $\alpha$ ) and Tumor Necrosis Factor beta (TNF- $\beta$ ).

5           As used herein, the term "chemokine" refers to any secreted polypeptide that affects the functions of cells and is a molecule which modulates interactions between cells in the immune, inflammatory or hematopoietic response, similar to the term "cytokine" above. A chemokine is primarily secreted through cell transmembranes and causes chemotaxis and activation of specific white blood cells and leukocytes,  
10   neutrophils, monocytes, macrophages, T-cells, B-cells, endothelial cells and smooth muscle cells. Examples of chemokines include, but are not limited to IL-8, GRO- $\alpha$ , GRO- $\beta$ , GRO- $\gamma$ , NAP-2, ENA-78, IP-10, MIP-1 $\alpha$ , MIP- $\beta$ , PF4, and MCP 1, 2, and 3.

          In order to use a compound of Formula (I) or a pharmaceutically acceptable salt thereof in therapy, it will normally be formulated into a pharmaceutical  
15   composition in accordance with standard pharmaceutical practice. This invention, therefore, also relates to a pharmaceutical composition comprising an effective, non-toxic amount of a compound of Formula (I) and a pharmaceutically acceptable carrier or diluent.

          Compounds of Formula (I), pharmaceutically acceptable salts thereof and  
20   pharmaceutical compositions incorporating such may conveniently be administered by any of the routes conventionally used for drug administration, for instance, orally, topically, parenterally or by inhalation. The compounds of Formula (I) may be administered in conventional dosage forms prepared by combining a compound of Formula (I) with standard pharmaceutical carriers according to conventional  
25   procedures. The compounds of Formula (I) may also be administered in conventional dosages in combination with a known, second therapeutically active compound. These procedures may involve mixing, granulating and compressing or dissolving the ingredients as appropriate to the desired preparation. It will be appreciated that the form and character of the pharmaceutically acceptable character  
30   or diluent is dictated by the amount of active ingredient with which it is to be combined, the route of administration and other well-known variables. The

carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

The pharmaceutical carrier employed may be, for example, either a solid or liquid. Exemplary of solid carriers are lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, stearic acid and the like. Exemplary of liquid carriers are syrup, peanut oil, olive oil, water and the like. Similarly, the carrier or diluent may include time delay material well known to the art, such as glyceryl mono-stearate or glyceryl distearate alone or with a wax.

A wide variety of pharmaceutical forms can be employed. Thus, if a solid carrier is used, the preparation can be tableted, placed in a hard gelatin capsule in powder or pellet form or in the form of a troche or lozenge. The amount of solid carrier will vary widely but preferably will be from about 25mg. to about 1g. When a liquid carrier is used, the preparation will be in the form of a syrup, emulsion, soft gelatin capsule, sterile injectable liquid such as an ampule or nonaqueous liquid suspension.

Compounds of Formula (I) may be administered topically, that is by non-systemic administration. This includes the application of a compound of Formula (I) externally to the epidermis or the buccal cavity and the instillation of such a compound into the ear, eye and nose, such that the compound does not significantly enter the blood stream. In contrast, systemic administration refers to oral, intravenous, intraperitoneal and intramuscular administration.

Formulations suitable for topical administration include liquid or semi-liquid preparations suitable for penetration through the skin to the site of inflammation such as liniments, lotions, creams, ointments or pastes, and drops suitable for administration to the eye, ear or nose. The active ingredient may comprise, for topical administration, from 0.001% to 10% w/w, for instance from 1% to 2% by weight of the Formulation. It may however comprise as much as 10% w/w but preferably will comprise less than 5% w/w, more preferably from 0.1% to 1% w/w of the Formulation.

Lotions according to the present invention include those suitable for application to the skin or eye. An eye lotion may comprise a sterile aqueous solution

optionally containing a bactericide and may be prepared by methods similar to those for the preparation of drops. Lotions or liniments for application to the skin may also include an agent to hasten drying and to cool the skin, such as an alcohol or acetone, and/or a moisturizer such as glycerol or an oil such as castor oil or arachis oil.

Creams, ointments or pastes according to the present invention are semi-solid formulations of the active ingredient for external application. They may be made by mixing the active ingredient in finely-divided or powdered form, alone or in solution or suspension in an aqueous or non-aqueous fluid, with the aid of suitable machinery, with a greasy or non-greasy base. The base may comprise hydrocarbons such as hard, soft or liquid paraffin, glycerol, beeswax, a metallic soap; a mucilage; an oil of natural origin such as almond, corn, arachis, castor or olive oil; wool fat or its derivatives or a fatty acid such as steric or oleic acid together with an alcohol such as propylene glycol or a macrogel. The formulation may incorporate any suitable surface active agent such as an anionic, cationic or non-ionic surfactant such as a sorbitan ester or a polyoxyethylene derivative thereof. Suspending agents such as natural gums, cellulose derivatives or inorganic materials such as siliceous silicas, and other ingredients such as lanolin, may also be included.

Drops according to the present invention may comprise sterile aqueous or oily solutions or suspensions and may be prepared by dissolving the active ingredient in a suitable aqueous solution of a bactericidal and/or fungicidal agent and/or any other suitable preservative, and preferably including a surface active agent. The resulting solution may then be clarified by filtration, transferred to a suitable container which is then sealed and sterilized by autoclaving or maintaining at 98-100 °C. for half an hour. Alternatively, the solution may be sterilized by filtration and transferred to the container by an aseptic technique. Examples of bactericidal and fungicidal agents suitable for inclusion in the drops are phenylmercuric nitrate or acetate (0.002%), benzalkonium chloride (0.01%) and chlorhexidine acetate (0.01%). Suitable solvents for the preparation of an oily solution include glycerol, diluted alcohol and propylene glycol.

Compounds of formula (I) may be administered parenterally, that is by intravenous, intramuscular, subcutaneous intranasal, intrarectal, intravaginal or intraperitoneal administration. The subcutaneous and intramuscular forms of parenteral administration are generally preferred. Appropriate dosage forms for such administration may be prepared by conventional techniques. Compounds of Formula (I) may also be administered by inhalation, that is by intranasal and oral inhalation administration. Appropriate dosage forms for such administration, such as an aerosol formulation or a metered dose inhaler, may be prepared by conventional techniques.

For all methods of use disclosed herein for the compounds of Formula (I), the daily oral dosage regimen will preferably be from about 0.01 to about 80 mg/kg of total body weight. The daily parenteral dosage regimen about 0.001 to about 80 mg/kg of total body weight. The daily topical dosage regimen will preferably be from 0.1 mg to 150 mg, administered one to four, preferably two or three times daily. The daily inhalation dosage regimen will preferably be from about 0.01 mg/kg to about 1 mg/kg per day. It will also be recognized by one of skill in the art that the optimal quantity and spacing of individual dosages of a compound of Formula (I) or a pharmaceutically acceptable salt thereof will be determined by the nature and extent of the condition being treated, the form, route and site of administration, and the particular patient being treated, and that such optimums can be determined by conventional techniques. It will also be appreciated by one of skill in the art that the optimal course of treatment, i.e., the number of doses of a compound of Formula (I) or a pharmaceutically acceptable salt thereof given per day for a defined number of days, can be ascertained by those skilled in the art using conventional course of treatment determination tests.

The invention will now be described by reference to the following biological examples which are merely illustrative and are not to be construed as a limitation of the scope of the present invention.

### BIOLOGICAL EXAMPLES

The IL-8, and Gro- $\alpha$  chemokine inhibitory effects of compounds of the present invention were determined by the following in vitro assay:

**Receptor Binding Assays:**

[<sup>125</sup>I] IL-8 (human recombinant) was obtained from Amersham Corp.,  
Arlington Heights, IL, with specific activity 2000 Ci/mmol. Gro- $\alpha$  was obtained  
from NEN- New England Nuclear. All other chemicals were of analytical grade.

5 High levels of recombinant human IL-8 type  $\alpha$  and  $\beta$  receptors were individually  
expressed in Chinese hamster ovary cells as described previously (Holmes, et al.,  
Science, 1991, 253, 1278). The Chinese hamster ovary membranes were  
homogenized according to a previously described protocol (Haour, et al., J Biol  
Chem., 249 pp 2195-2205 (1974)). Except that the homogenization buffer was  
10 changed to 10mM Tris-HCL, 1mM MgSO<sub>4</sub>, 0.5mM EDTA (ethylene-diaminetetra-  
acetic acid), 1mMPMSF ( $\alpha$ -toluenesulphonyl fluoride), 0.5 mg/L Leupeptin, pH 7.5.  
Membrane protein concentration was determined using Pierce Co. micro-assay kit  
using bovine serum albumin as a standard. All assays were performed in a 96-well  
micro plate format. Each reaction mixture contained <sup>125</sup>I IL-8 (0.25 nM) or <sup>125</sup>I  
15 Gro- $\alpha$  and 0.5  $\mu$ g/mL of IL-8R $\alpha$  or 1.0  $\mu$ g/mL of IL-8R $\beta$  membranes in 20 mM Bis-  
Trispropane and 0.4 mM Tris HCl buffers, pH 8.0, containing 1.2 mM MgSO<sub>4</sub>, 0.1  
mM EDTA, 25 mM NaCl and 0.03% CHAPS. In addition, drug or compound of  
interest was added which had been pre-dissolved in DMSO so as to reach a final  
concentration of between 0.01nM and 100  $\mu$ M. The assay was initiated by addition  
20 of <sup>125</sup>I-IL-8. After 1 hour at room temperature the plate was harvested using a  
Tomtec 96-well harvester onto a glass fiber filtermat blocked with 1%  
polyethylenimine/0.5% BSA and washed 3 times with 25 mM NaCl, 10 mM  
TrisHCl, 1 mM MgSO<sub>4</sub>, 0.5 mM EDTA, 0.03 % CHAPS, pH 7.4. The filter was  
then dried and counted on the Betaplate liquid scintillation counter. The  
25 recombinant IL-8 R $\alpha$ , or Type I, receptor is also referred to herein as the non-  
permissive receptor and the recombinant IL-8 R $\beta$ , or Type II, receptor is referred to  
as the permissive receptor.

All of the exemplified compounds of Formulas (I) noted herein in the  
Synthetic Chemistry Section, Example 1 to 15, demonstrated an IC<sub>50</sub> from about 45  
30 to about <1  $\mu$ g/mL in the permissive models for IL-8 receptor inhibition. Of those



compounds tested, Examples 1 to 12 were also found to be inhibitors of Gro- $\alpha$  binding at about the same level.

**Chemotaxis Assay :**

The in vitro inhibitory properties of these compounds are determined in the neutrophil chemotaxis assay as described in Current Protocols in Immunology, vol I, Suppl 1, Unit 6.12.3., whose disclosure is incorporated herein by reference in its entirety. Neutrophils were isolated from human blood as described in Current Protocols in Immunology Vol I, Suppl 1 Unit 7.23.1, whose disclosure is incorporated herein by reference in its entirety. The chemoattractants IL-8, GRO- $\alpha$ , GRO- $\beta$ , GRO- $\gamma$  and NAP-2 are placed in the bottom chamber of a 48 multiwell chamber (Neuro Probe, Cabin John, MD) at a concentration between 0.1 and 100 nM. The two chambers are separated by a 5  $\mu$ m polycarbonate filter. When compounds of this invention are tested, they are mixed with the cells (0.001 - 1000 nM) just prior to the addition of the cells to the upper chamber. Incubation is allowed to proceed for between about 45 and 90 min at about 37°C in a humidified incubator with 5% CO<sub>2</sub>. At the end of the incubation period, the polycarbonate membrane is removed and the top side washed, the membrane then stained using the Diff Quick staining protocol (Baxter Products, McGaw Park, IL, USA). Cells which have chemotaxed to the chemokine are visually counted using a microscope. Generally, four fields are counted for each sample, these numbers are averaged to give the average number of cells which had migrated. Each sample is tested in triplicate and each compound repeated at least four times. To certain cells (positive control cells) no compound is added, these cells represent the maximum chemotactic response of the cells. In the case where a negative control (unstimulated) is desired, no chemokine is added to the bottom chamber. The difference between the positive control and the negative control represents the chemotactic activity of the cells.

**Elastase Release Assay:**

The compounds of this invention are tested for their ability to prevent Elastase release from human neutrophils. Neutrophils are isolated from human blood as described in Current Protocols in Immunology Vol I, Suppl 1 Unit 7.23.1. PMNs  $0.88 \times 10^6$  cells suspended in Ringer's Solution (NaCl 118, KCl 4.56,

NaHCO<sub>3</sub> 25, KH<sub>2</sub>PO<sub>4</sub> 1.03, Glucose 11.1, HEPES 5 mM, pH 7.4) are placed in each well of a 96 well plate in a volume of 50 ul. To this plate is added the test compound (0.001 - 1000 nM) in a volume of 50 ul, Cytochalasin B in a volume of 50 ul (20ug/ml) and Ringers buffer in a volume of 50 ul. These cells are allowed to warm (37 °C, 5% CO<sub>2</sub>, 95% RH) for 5 min before IL-8, GRO $\alpha$ , GRO $\beta$ , GRO $\gamma$  or NAP-2 at a final concentration of 0.01 - 1000 nM was added. The reaction is allowed to proceed for 45 min before the 96 well plate is centrifuged (800 xg 5 min) and 100 ul of the supernatant removed. This supernatant is added to a second 96 well plate followed by an artificial elastase substrate (MeOSuc-Ala-Ala-Pro-Val-AMC, Nova Biochem, La Jolla, CA) to a final concentration of 6 ug/ml dissolved in phosphate buffered saline. Immediately, the plate is placed in a fluorescent 96 well plate reader (Cytofluor 2350, Millipore, Bedford, MA) and data collected at 3 min intervals according to the method of Nakajima et al J. Biol Chem 254 4027 (1979). The amount of Elastase released from the PMNs is calculated by measuring the rate of MeOSuc-Ala-Ala-Pro-Val-AMC degradation.

#### **TNF- $\alpha$ in Traumatic Brain Injury Assay**

The present assay provides for examination of the expression of tumor necrosis factor mRNA in specific brain regions which follow experimentally induced lateral fluid-percussion traumatic brain injury (TBI) in rats. Adult Sprague-Dawley rats (n=42) were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and subjected to lateral fluid-percussion brain injury of moderate severity (2.4 atm.) centered over the left temporoparietal cortex (n=18), or "sham" treatment (anesthesia and surgery without injury, n=18). Animals are sacrificed by decapitation at 1, 6 and 24 hr. post injury, brains removed, and tissue samples of left (injured) parietal cortex (LC), corresponding area in the contralateral right cortex (RC), cortex adjacent to injured parietal cortex (LA), corresponding adjacent area in the right cortex (RA), left hippocampus (LH) and right hippocampus (RH) are prepared. Total RNA was isolated and Northern blot hybridization is performed and quantitated relative to an TNF- $\alpha$  positive control RNA (macrophage = 100%). A marked increase of TNF-  $\alpha$  mRNA expression is observed in LH (104 $\pm$ 17% of positive control, p < 0.05 compared with sham), LC (105 $\pm$ 21%, p < 0.05) and LA (69 $\pm$ 8%, p < 0.01) in the

traumatized hemisphere 1 hr. following injury. An increased TNF-  $\alpha$  mRNA expression is also observed in LH ( $46 \pm 8\%$ ,  $p < 0.05$ ), LC ( $30 \pm 3\%$ ,  $p < 0.01$ ) and LA ( $32 \pm 3\%$ ,  $p < 0.01$ ) at 6 hr. which resolves by 24 hr. following injury. In the contralateral hemisphere, expression of TNF-  $\alpha$  mRNA is increased in RH ( $46 \pm 2\%$ ,  $p < 0.01$ ), RC ( $4 \pm 3\%$ ) and RA ( $22 \pm 8\%$ ) at 1 hr. and in RH ( $28 \pm 11\%$ ), RC ( $7 \pm 5\%$ ) and RA ( $26 \pm 6\%$ ,  $p < 0.05$ ) at 6 hr. but not at 24 hr. following injury. In sham (surgery without injury) or naive animals, no consistent changes in expression of TNF-  $\alpha$  mRNA are observed in any of the 6 brain areas in either hemisphere at any times. These results indicate that following parasagittal fluid-percussion brain injury, the temporal expression of TNF- $\alpha$  mRNA is altered in specific brain regions, including those of the non-traumatized hemisphere. Since TNF- $\alpha$  is able to induce nerve growth factor (NGF) and stimulate the release of other cytokines from activated astrocytes, this post-traumatic alteration in gene expression of TNF- $\alpha$  plays an important role in both the acute and regenerative response to CNS trauma.

#### 15 **CNS Injury model for IL- $\beta$ mRNA**

This assay characterizes the regional expression of interleukin-1 $\beta$  (IL-1 $\beta$ ) mRNA in specific brain regions following experimental lateral fluid-percussion traumatic brain injury (TBI) in rats. Adult Sprague-Dawley rats ( $n=42$ ) are anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and subjected to lateral fluid-percussion brain injury of moderate severity (2.4 atm.) centered over the left temporoparietal cortex ( $n=18$ ), or "sham" treatment (anesthesia and surgery without injury). Animals are sacrificed at 1, 6 and 24 hr. post injury, brains removed, and tissue samples of left (injured) parietal cortex (LC), corresponding area in the contralateral right cortex (RC), cortex adjacent to injured parietal cortex (LA), corresponding adjacent area in the right cortex (RA), left hippocampus (LH) and right hippocampus (RH) are prepared. Total RNA is isolated and Northern blot hybridization was performed and the quantity of brain tissue IL-1 $\beta$  mRNA is presented as percent relative radioactivity of IL-1 $\beta$  positive macrophage RNA which was loaded on same gel. At 1 hr. following brain injury, a marked and significant increase in expression of IL-1 $\beta$  mRNA is observed in LC ( $20.0 \pm 0.7\%$  of positive control,  $n=6$ ,  $p < 0.05$  compared with sham animal), LH

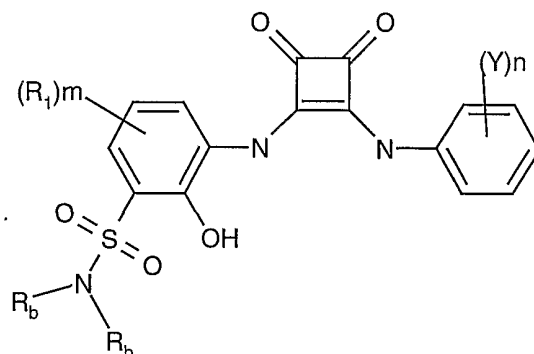
( $24.5 \pm 0.9\%$ ,  $p < 0.05$ ) and LA ( $21.5 \pm 3.1\%$ ,  $p < 0.05$ ) in the injured hemisphere, which remained elevated up to 6 hr. post injury in the LC ( $4.0 \pm 0.4\%$ ,  $n=6$ ,  $p < 0.05$ ) and LH ( $5.0 \pm 1.3\%$ ,  $p < 0.05$ ). In sham or naive animals, no expression of IL- $1\beta$  mRNA is observed in any of the respective brain areas. These results  
5 indicate that following TBI, the temporal expression of IL- $1\beta$  mRNA is regionally stimulated in specific brain regions. These regional changes in cytokines, such as IL- $1\beta$  play a role in the post-traumatic.

All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if  
10 each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

The above description fully discloses the invention including preferred embodiments thereof. Modifications and improvements of the embodiments specifically disclosed herein are within the scope of the following claims.  
15 Without further elaboration, it is believed that one skilled in the area can, using the preceding description, utilize the present invention to its fullest extent. Therefore the Examples herein are to be construed as merely illustrative and not a limitation of the scope of the present invention in any way. The embodiments of the invention in which an exclusive property or privilege is claimed are defined as  
20 follows.

What is claimed is:

1. A compound of the formula:



(I)

wherein:

R<sub>1</sub> is independently selected from the group consisting of hydrogen, halogen, nitro, cyano, halosubstituted C<sub>1-10</sub> alkyl, C<sub>1-10</sub> alkyl, C<sub>2-10</sub> alkenyl, C<sub>1-10</sub> alkoxy, halosubstituted C<sub>1-10</sub> alkoxy, azide, (CR<sub>8</sub>R<sub>8</sub>)<sub>q</sub> S(O)<sub>t</sub>R<sub>4</sub>, hydroxy, hydroxy C<sub>1-4</sub>alkyl, aryl, aryl C<sub>1-4</sub> alkyl, aryloxy, aryl C<sub>1-4</sub> alkyloxy, heteroaryl, heteroarylalkyl, heterocyclic, heterocyclic C<sub>1-4</sub>alkyl, heteroaryl C<sub>1-4</sub> alkyloxy, aryl C<sub>2-10</sub> alkenyl, heteroaryl C<sub>2-10</sub> alkenyl, heterocyclic C<sub>2-10</sub> alkenyl, (CR<sub>8</sub>R<sub>8</sub>)<sub>q</sub>NR<sub>4</sub>R<sub>5</sub>, C<sub>2-10</sub> alkenyl C(O)NR<sub>4</sub>R<sub>5</sub>, (CR<sub>8</sub>R<sub>8</sub>)<sub>q</sub> C(O)NR<sub>4</sub>R<sub>5</sub>, (CR<sub>8</sub>R<sub>8</sub>)<sub>q</sub> C(O)NR<sub>4</sub>R<sub>10</sub>, S(O)<sub>3</sub>H, S(O)<sub>3</sub>R<sub>8</sub>, (CR<sub>8</sub>R<sub>8</sub>)<sub>q</sub> C(O)R<sub>11</sub>, C<sub>2-10</sub> alkenyl C(O)R<sub>11</sub>, C<sub>2-10</sub> alkenyl C(O)OR<sub>11</sub>(CR<sub>8</sub>R<sub>8</sub>)<sub>q</sub> C(O)OR<sub>12</sub>, (CR<sub>8</sub>R<sub>8</sub>)<sub>q</sub> OC(O)R<sub>11</sub>, (CR<sub>8</sub>R<sub>8</sub>)<sub>q</sub>NR<sub>4</sub>C(O)R<sub>11</sub>, (CR<sub>8</sub>R<sub>8</sub>)<sub>q</sub> NHS(O)<sub>2</sub>R<sub>17</sub>, (CR<sub>8</sub>R<sub>8</sub>)<sub>q</sub> and S(O)<sub>2</sub>NR<sub>4</sub>R<sub>5</sub>; or two R<sub>1</sub> moieties together y form O-(CH<sub>2</sub>)<sub>5</sub>O- or a 5 to 6 membered unsaturated ring;

R<sub>b</sub> is independently selected from the group consisting of hydrogen, NR<sub>6</sub>R<sub>7</sub>, OH, OR<sub>a</sub>, C<sub>1-5</sub>alkyl, aryl, arylC<sub>1-4</sub>alkyl, aryl C<sub>2-4</sub>alkenyl; cycloalkyl, cycloalkyl C<sub>1-5</sub> alkyl, heteroaryl, heteroarylC<sub>1-4</sub>alkyl, heteroarylC<sub>2-4</sub> alkenyl, heterocyclic, heterocyclic C<sub>1-4</sub>alkyl, and a heterocyclic C<sub>2-4</sub>alkenyl moiety; all of which moieties may be optionally substituted one to three times independently by halogen, nitro, halosubstituted C<sub>1-4</sub> alkyl, C<sub>1-4</sub> alkyl; amino, mono or di-C<sub>1-4</sub> alkyl substituted

- amine,  $OR_a$ ,  $C(O)R_a$ ,  $NR_aC(O)OR_a$ ,  $OC(O)NR_6R_7$ , hydroxy,  $NR_9C(O)R_a$ ,  $S(O)_mR_a$ ,  $C(O)NR_6R_7$ ,  $C(O)OH$ ,  $C(O)OR_a$ ,  $S(O)_2NR_6R_7$ , and  $NHS(O)_2R_a$ ; or the two  $R_b$  substituents can join to form a 3-10 membered ring, optionally substituted and containing, in addition to carbon, independently, 1 to 3  $NR_a$ , O, S, SO, or  $SO_2$
- 5 moieties which can be optionally unsaturated;
- $q$  is 0, or an integer having a value of 1 to 10;
- $t$  is 0, or an integer having a value of 1 or 2;
- $s$  is an integer having a value of 1 to 3;
- $R_4$  and  $R_5$  are independently selected from the group consisting of hydrogen,
- 10 optionally substituted  $C_{1-4}$  alkyl, optionally substituted aryl, optionally substituted aryl  $C_{1-4}$ alkyl, optionally substituted heteroaryl, optionally substituted heteroaryl  $C_{1-4}$ alkyl, heterocyclic, and heterocyclic  $C_{1-4}$  alkyl; or  $R_4$  and  $R_5$  together with the nitrogen to which they are attached form a 5 to 7 member ring which optionally comprises an additional heteroatom selected from
- 15 oxygen, nitrogen or sulfur;
- $Y$  is independently selected from the group consisting of hydrogen, halogen, nitro, cyano, halosubstituted  $C_{1-10}$  alkyl,  $C_{1-10}$  alkyl,  $C_{2-10}$  alkenyl,  $C_{1-10}$  alkoxy, halosubstituted  $C_{1-10}$  alkoxy, azide,  $(CR_8R_8)_q S(O)_tR_4$ , hydroxy, hydroxy  $C_{1-4}$ alkyl, aryl, aryl  $C_{1-4}$  alkyl, aryloxy, aryl  $C_{1-4}$  alkyloxy, heteroaryl,
- 20 heteroarylalkyl, heteroaryl  $C_{1-4}$  alkyloxy, heterocyclic, heterocyclic  $C_{1-4}$ alkyl; aryl  $C_{2-10}$  alkenyl, heteroaryl  $C_{2-10}$  alkenyl, heterocyclic  $C_{2-10}$  alkenyl,  $(CR_8R_8)_q NR_4R_5$ ,  $C_{2-10}$  alkenyl  $C(O)NR_4R_5$ ,  $(CR_8R_8)_q C(O)NR_4R_5$ ,  $(CR_8R_8)_q C(O)NR_4R_{10}$ ,  $S(O)_3H$ ,  $S(O)_3R_8$ ,  $(CR_8R_8)_q C(O)R_{11}$ ,  $C_{2-10}$  alkenyl  $C(O)R_{11}$ ,  $C_{2-10}$  alkenyl  $C(O)OR_{11}$ ,  $C(O)R_{11}$ ,  $(CR_8R_8)_q C(O)OR_{12}$ ,
- 25  $(CR_8R_8)_q OC(O) R_{11}$ ,  $(CR_8R_8)_q NR_4C(O)R_{11}$ ,  $(CR_8R_8)_q NHS(O)_2R_d$ , and  $(CR_8R_8)_q S(O)_2NR_4R_5$ ; or two  $Y$  moieties together form  $O-(CH_2)_sO-$  or a 5 to 6 membered unsaturated ring;
- $n$  is an integer having a value of 1 to 5;
- $m$  is an integer having a value of 1 to 4;

R<sub>8</sub> is hydrogen or C<sub>1-4</sub> alkyl;

R<sub>10</sub> is C<sub>1-10</sub> alkyl C(O)<sub>2</sub>R<sub>8</sub>;

R<sub>11</sub> is selected from the group consisting of hydrogen, C<sub>1-4</sub> alkyl, optionally substituted aryl, optionally substituted aryl C<sub>1-4</sub>alkyl, optionally substituted heteroaryl, optionally substituted heteroarylC<sub>1-4</sub>alkyl, optionally substituted heterocyclic, and optionally substituted heterocyclicC<sub>1-4</sub>alkyl;

R<sub>12</sub> is selected from the group consisting of hydrogen, C<sub>1-10</sub> alkyl, optionally substituted aryl and optionally substituted arylalkyl;

R<sub>17</sub> is selected from the group consisting of C<sub>1-4</sub>alkyl, aryl, arylalkyl, heteroaryl, heteroarylC<sub>1-4</sub>alkyl, heterocyclic, and heterocyclicC<sub>1-4</sub>alkyl, wherein the aryl, heteroaryl and heterocyclic rings are all optionally substituted.

2. The compound according to Claim 1 which is:

- 3-[4-Chloro-2-hydroxy-3-(4-methyl-piperazine-1-sulfonyl)-phenylamino]-4-phenylamino-cyclobut-3-ene-1,2-dione;
- 3-[4-Chloro-2-hydroxy-3-(4-methyl-piperazine-1-sulfonyl)-phenylamino]-4-(2-chloro-phenylamino)-cyclobut-3-ene-1,2-dione;
- 3-[4-Chloro-2-hydroxy-3-(4-methyl-piperazine-1-sulfonyl)-phenylamino]-4-(2-bromo-phenylamino)-cyclobut-3-ene-1,2-dione;
- 3-[4-Chloro-2-hydroxy-3-(4-methyl-piperazine-1-sulfonyl)-phenylamino]-4-(2-fluoro-phenylamino)-cyclobut-3-ene-1,2-dione;
- 3-[4-Chloro-2-hydroxy-3-(4-methyl-piperazine-1-sulfonyl)-phenylamino]-4-(4-fluoro-phenylamino)-cyclobut-3-ene-1,2-dione;
- 3-[4-Chloro-2-hydroxy-3-(4-methyl-piperazine-1-sulfonyl)-phenylamino]-4-(2-methoxy-phenylamino)-cyclobut-3-ene-1,2-dione;
- 3-[4-Chloro-2-hydroxy-3-(4-methyl-piperazine-1-sulfonyl)-phenylamino]-4-(2,6-difluoro-phenylamino)-cyclobut-3-ene-1,2-dione;
- 3-[4-Chloro-2-hydroxy-3-(4-methyl-piperazine-1-sulfonyl)-phenylamino]-4-(2,4-difluoro-phenylamino)-cyclobut-3-ene-1,2-dione;

- 3-[3-((S)-3-Amino-pyrrolidine-1-sulfonyl)-4-chloro-2-hydroxy-phenylamino]-4-phenylamino-cyclobut-3-ene-1,2-dione;
- 6-Chloro-N-(2-diethylamino-ethyl)-3-(3,4-dioxo-2-phenylamino-cyclobut-1-enylamino)-2-hydroxy-benzenesulfonamide;
- 5 6-Chloro-3-[2-(2-chloro-phenylamino)-3,4-dioxo-cyclobut-1-enylamino]- N-(2-diethylamino-ethyl)-2-hydroxy-benzenesulfonamide;
- 6-Chloro-3-[2-(2-bromo-phenylamino)-3,4-dioxo-cyclobut-1-enylamino]- N-(2-diethylamino-ethyl)-2-hydroxy-benzenesulfonamide;
- (1-{6-Chloro-3-[2-phenylamino-3,4-dioxo-cyclobut-1-enylamino]-2-hydroxy-
- 10 benzenesulfonyl}-piperidin-4-yl)-carbamic acid tert-butylester;
- 3-[3-([1,4']Bipiperidinyl-1'-sulfonyl)-4-chloro-2-hydroxy-phenylamino]-4-phenylamino-cyclobut-3-ene-1,2-dione;
- 3-[4-Chloro-2-hydroxy-3-(piperazine-1-sulfonyl)-phenylamino]-4-phenylamino-cyclobut-3-ene-1,2-dione;
- 15 3-[4-Chloro-2-hydroxy-3-(4-methyl-[1,4]diazepane-1-sulfonyl)-phenylamino]-4-phenylamino-cyclobut-3-ene-1,2-dione;
- 3-[4-Chloro-2-hydroxy-3-(4-methyl-[1,4]diazepane-1-sulfonyl)-phenylamino]-4-(2-chloro-phenylamino)-cyclobut-3-ene-1,2-dione;
- 6-Chloro-3-(3,4-dioxo-2-phenylamino-cyclobut-1-enylamino)-2-hydroxy-
- 20 benzenesulfonamide;
- 3-[2-(2-Bromo-phenylamino)-3,4-dioxo-cyclobut-1-enylamino]-6-chloro-2-hydroxy-benzenesulfonamide;
- 6-Chloro-3-[2-(2-chloro-phenylamino)-3,4-dioxo-cyclobut-1-enylamino]-2-hydroxy-benzenesulfonamide;
- 25 6-Chloro-2-hydroxy-3-[2-(2-methoxy-phenylamino)-3,4-dioxo-cyclobut-1-enylamino]-benzenesulfonamide;
- 6-Chloro-3-[3,4-dioxo-2-(2-phenoxy-phenylamino)-cyclobut-1-enylamino]-2-hydroxy-benzenesulfonamide;
- 6-Chloro-3-[2-(4-fluoro-phenylamino)-3,4-dioxo-cyclobut-1-enylamino]-2-hydroxy-
- 30 benzenesulfonamide or a suitable salt thereof.



3. A pharmaceutical composition comprising an effective amount of a compound according to Claim 1, and a pharmaceutically acceptable carrier or diluent.
- 5 4. A method of treating a chemokine mediated disease state, wherein the chemokine binds to an IL-8  $\alpha$  or  $\beta$  receptor in a mammal, which comprises administering to said mammal an effective amount of a compound of the formula according to claim 1.
- 10 5. The method according to Claim 4 wherein the mammal is afflicted with a chemokine mediated disease selected from psoriasis, atopic dermatitis, osteo arthritis, rheumatoid arthritis, asthma, chronic obstructive pulmonary disease, adult respiratory distress syndrome, inflammatory bowel disease, Crohn's disease, ulcerative colitis, stroke, septic shock, multiple sclerosis, endotoxic shock, gram  
15 negative sepsis, toxic shock syndrome, cardiac and renal reperfusion injury, glomerulonephritis, thrombosis, graft vs. host reaction, Alzheimer's disease, allograft rejections, malaria, restenosis, angiogenesis, atherosclerosis, osteoporosis, gingivitis and undesired hematopoietic stem cells release and diseases caused by respiratory viruses, herpes viruses, and hepatitis viruses, meningitis, cystic fibrosis, pre-term  
20 labor, cough, pruritus, multi-organ dysfunction, trauma, strains, sprains, contusions, psoriatic arthritis, herpes, encephalitis, CNS vasculitis, traumatic brain injury, CNS tumors, subarachnoid hemorrhage, post surgical trauma, interstitial pneumonitis, hypersensitivity, crystal induced arthritis, acute and chronic pancreatitis, acute alcoholic hepatitis, necrotizing enterocolitis, chronic sinusitis, uveitis, polymyositis,  
25 vasculitis, acne, gastric and duodenal ulcers, celiac disease, esophagitis, glossitis, airflow obstruction, airway hyperresponsiveness, bronchiolitis obliterans organizing pneumonia, bronchiectasis, bronchiolitis, bronchiolitis obliterans, chronic bronchitis, cor pulmonae, dyspnea, emphysema, hypercapnea, hyperinflation, hypoxemia, hyperoxia-induced inflammations, hypoxia, surgical lung volume reduction,  
30 pulmonary fibrosis, pulmonary hypertension, right ventricular hypertrophy,

sarcoidosis, small airway disease, ventilation-perfusion mismatching, wheeze, colds  
and lupus.

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US02/01413

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 540/575; 544/383; 546/191,235; 548/542; 564/86; 514/218, 252.12, 316, 331, 424, 603.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
FILE USPATFULL

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE STRUCTURE SEARCH

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	WO 01/92202 A1 (PALOVICH ET AL) 06 DECEMBER 2001, see specially pages 3-7.	1-5

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

"A"	document defining the general state of the art which is not considered to be of particular relevance	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

01 MAY 2002

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

International application No.  
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A. CLASSIFICATION OF SUBJECT MATTER:

IPC (7):

C07D 207/48, 211/96, 295/26, 243/06; C07C 311/16; A61K 31/18, 31/40, 31/4468, 31/445,  
31/495, 31/551.

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

540/575; 544/383; 546/191,235; 548/542; 564/86; 514/218, 252.12, 316, 331, 424, 603.