

## CONJUGATED AROMATIC COMPOUNDS FOR DIAGNOSIS AND THERAPY

### STATEMENT OF GOVERNMENT SUPPORT

The U.S. Government may have certain rights in the invention. Some work related to the  
5 development of this invention was carried out under National Cancer Institute Grant Number R43  
CA89772-01.

### CROSS REFERENCE TO RELATED APPLICATION

This application claims the benefit of the earlier filing date of U.S. Provisional Application  
10 No. 60/678,089, filed May 4, 2005, which is incorporated herein by reference in its entirety.

### FIELD

This invention concerns methods of monitoring and altering immune function, for example  
in the diagnosis and/or treatment of infectious and neoplastic disease. It also discloses methods of  
15 treating lesions, such as hyperproliferative lesions associated with infectious pathogens and/or  
inflammation.

### BACKGROUND

Immunotherapy involves the modulation of a subject's immune response to improve an  
20 innate ability to retain health. It has been recognized that there is a relationship between  
electromagnetic fields and biological functions, such as immunity. For example, U.S. Patent No.  
4,670,386 discloses that the expression of strongly antigenic tumor specific antigens is induced by  
exposure of tumor cells to high frequency electromagnetic radiation that produces cyclic, rapid,  
alternating changes of polarity in dipolar molecular components of cancer cells. However, one of the  
25 drawbacks of very high frequency radiation is that it causes unwanted and potentially damaging  
heating of biological tissue.

The relationship between electromagnetic radiation and immunity is also illustrated by U.S.  
Patent No. 6,038,478, which discloses that lymphocytes can be attracted to a desired location in the  
body by applying electrodes to the desired location and stimulating the tissue with sufficient electric  
30 current to attract lymphocytes. Low energy alternating current magnetic fields were used to induce  
an immune response in U.S. Patent Publication 2002/0072646. Similarly, WO 02/062418 discloses  
enhancing immune function by exposing a subject to a magnetic field, or to very high frequency  
electromagnetic fields that are angularly modulated.

As noted in chapter 50 of *The Biomedical Engineering Handbook* (CRC Press 1995),  
35 biologic systems frequently have electrical activity associated with them. This activity can be a  
constant DC electric field, a constant flux of charge-carrying particles or current, or a time-varying  
electric field or current associated with some time-dependent or biochemical phenomenon.

Bioelectric phenomena are associated with the distribution of ions or charged molecules in a biologic structure and the changes in this distribution resulting from specific processes.

Electromagnetic bioimpedance measurements have been used for diagnostic purposes. For example, WO 01/076475 discloses use of an alternating magnetic field to induce electrical eddy  
5 currents in biological tissue. An oscillator circuit is used to generate current in a coil adjacent targeted tissue. Since the amplitude of the resultant voltage is proportional to the conductivity of the tissue, changes in bioimpedance are used to detect changes in the tissue that are associated with tumors, such as prostate tumors. Another tissue impedance measuring device for differentiating tissue types is disclosed in WO 01/67098. The disclosure of both of these PCT publications (WO  
10 01/076475 and WO 01/67098) is incorporated herein by reference.

The effect of electromagnetic fields on biological organisms and their cellular components has previously been appreciated. However, it has been difficult to take advantage of this inter-relationship, and there has been a need to more effectively couple the immune system to external sources of modulating electromagnetic radiation.

15 Protein phosphatases are composed of at least two separate and distinct families: the protein serine/threonine phosphatases and the protein tyrosine phosphatases (PTPs). Human protein tyrosine phosphatases (human PTPs) are a large and diverse family of proteins present in all eukaryotes. Each PTP is composed of at least one conserved domain characterized by an 11-residue sequence motif containing cysteine and arginine residues that are known to be essential for catalytic activities. The  
20 sequences of PTP share no similarity to serine or threonine, acid or alkaline phosphatases. The diversity in structure within the PTP family results primarily from the variety of non-catalytic sequences attached to the NH<sub>2</sub>- or COOH- termini of the catalytic domain. There are numerous PTPs involved in intracellular phosphate metabolism and domains. The diversity of the extracellular segments presumably reflects the variety of ligands to which the PTPs are exposed and catalyze  
25 phosphate transfer.

PTPs generally are classified into two subgroups. The first subgroup is made up of the low molecular weight, intracellular enzymes that contain a single conserved catalytic phosphatase domain. All known intracellular type PTPs contain a single conserved catalytic phosphatase domain. Examples of the first group of PTPs include placental PTP 1B, T-cell PTP, rat brain PTP, neuronal  
30 phosphatase (STEP), and cytoplasmic phosphatases that contain a region of homology to cytoskeletal proteins.

The second subgroup of PTPs comprises high molecular weight, receptor-linked PTPs, termed R-PTPs. The R-PTPs generally include an intracellular catalytic region, a single transmembrane segment, and a putative ligand-binding extracellular domain. The structures and sizes  
35 of the putative ligand-binding extracellular "receptor" domains of R-PTPs are quite divergent, in contrast to the intracellular catalytic regions of R-PTPs which are highly homologous. All R-PTPs have two tandemly duplicated catalytic phosphatase homology domains, with the prominent

exception of an R-PTP termed HPTP $\beta$ , which has only one catalytic phosphatase domain. (Tsai et al., J. Biol. Chem. 266(16):10534-10543 (1991)).

One example of R-PTPs is the leukocyte common antigen (LCA) (Ralph, S. J., EMBO J. 6:1251-1257 (1987)). LCA is a family of high molecular weight glycoproteins expressed on the surface of all leukocytes and their hemopoietic progenitors. A remarkable degree of similarity is detected with the sequence of LCA from several species (Charbonneau et al., Proc. Natl. Acad. Sci. USA 85:7182-7186 (1988)). LCA is referred to in the literature by different names, including T200, B220 for the B cell form, the mouse allotypic marker Ly-5, and more recently CD45 (Cobbold et al., Leucocyte Typing III, ed. A. J. McMichael et al., pp. 788-803 (1987)). CD45 is believed to play a critical role in T cell activation. These studies are reviewed in Weiss A., Ann. Rev. Genet. 25:487-510 (1991).

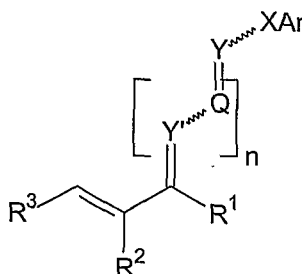
Another example of R-PTPs is the leukocyte common antigen related molecule (LAR) (Streuli et al., J. Exp. Med. 168:1523-1530 (1988)). In addition, published application W092/01050 discloses human R-PTP- $\alpha$ ,  $\beta$  and  $\gamma$  and reports on the nature of the structural homologies found among the conserved domains of these three R-PTP and other members of this protein family.

The extracellular PTPs are related to surface recognition and adhesion molecules of leukocyte cell surface recognition. The PTPs are not only associated with human cells, but also present in prokaryotes and viruses, and bacteria. In the pathogenic bacterium *Yersinia*, the causative agent of bubonic plague, the Yop2b tyrosine-specific PTP is an essential virulence determinant.

Numerous studies have demonstrated the importance of PTPs in physiological processes. Phenotypic defects and hyperproliferative behavior of T- and B-lymphocytes, granulocytes and macrophages are considered to be key issues in the development of cancer and autoimmune diseases.

### SUMMARY OF THE DISCLOSURE

Disclosed herein are compounds, compositions and methods for using such compounds and compositions for the modulation, particularly stimulation, of an immune response, which is useful, for example, in the treatment of cancer. In one embodiment, the compounds have the formula



wherein Ar comprises an aryl group substituted with at least one electron withdrawing group;

X is NH, CH<sub>2</sub>, O, —, SO<sub>2</sub>, or B;

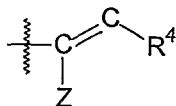
Q is —(CH=CH)<sub>m</sub>—CH—;

Y and Y' independently are selected from N, CH and B;

n is 0 or 1;

m is from 0 to 6;

R<sup>1</sup> is H, lower alkyl, optionally substituted phenyl or



5 wherein R<sup>4</sup> is an optionally substituted phenyl group;

R<sup>2</sup> is optionally substituted lower alkyl, aralkyl, thioalkyl, amidoalkyl, amino, amino alkyl, nitro, azido; together with Z forms an optionally substituted 4–7 membered carbocyclic or heterocyclic ring; or together with R<sup>4</sup> forms an optionally substituted phenyl group;

10 R<sup>3</sup> with R<sup>2</sup> forms an optionally substituted phenyl group or is an optionally substituted phenyl group; and

Z is H, lower alkyl, substituted alkyl, thioalkyl, nitrile, amino, or together with R<sup>2</sup> forms an optionally substituted 4–7 membered carbocyclic or heterocyclic ring.

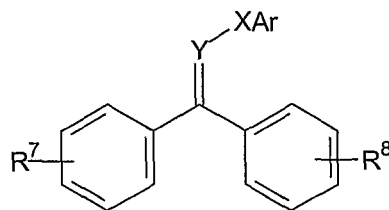
15 In one embodiment, the compounds are derivatized with a biomolecule, such as a carbohydrate, amino acid, peptide, fatty acid, nucleoside or nucleic acids. In one aspect, such derivatization is used to enhance a compound's solubility and/or target a compound to a particular tissue, cell or receptor. In one embodiment, an optionally substituted phenyl group, as recited with respect to the general formula above, comprises such a biomolecule.

In one embodiment, the disclosed compounds are resonance modulators effective to stimulate a therapeutic response. Accordingly, disclosed herein is a method for stimulating a  
20 therapeutic response (such as an immune response) by administering to a subject in need of therapy (such as immunostimulation) a therapeutically effective amount of a resonance modulating compound that possesses resonating intramolecular dipole moments (or electron densities) that allow it to interact with biological environments. Administration of the compound can take many forms, including, without limitation, topical application to a target area, insertion of pellets into the skin,  
25 placement in diseased organs, and inhalation. The resonance modulating compounds are capable of stimulating an immune response characterized in part by infiltration of immune cells, such as lymphocytes, into a target region in the vicinity of the resonance modulating compound. The electromagnetic properties of the target region also change as the immune cells enter the target area, and these electromagnetic changes can be detected (for example by electromagnetic signals provided  
30 by the resonance modulator) to measure the adequacy of a subject's immune response. Deficiencies of the immune response can be quickly detected in this manner, for example by the absence of an expected aggregation of immune cells, and appropriate therapeutic or preventative interventions taken. One such intervention is to expose the resonance modulating compound to an external electromagnetic stimulus that enhances the immune response both locally in the target area, and  
35 remotely throughout the body. Examples of such electromagnetic stimuli are electrical current

flowing across an electrical potential through the compound, an induced magnetic field, or radiant energy (such as laser energy) applied to the compound.

- Particular examples of resonance modulator compounds include aryl compounds, particularly those having an aryl group substituted with electron withdrawing groups. In one  
 5 embodiment resonance modulator compounds have the general formula set forth above.

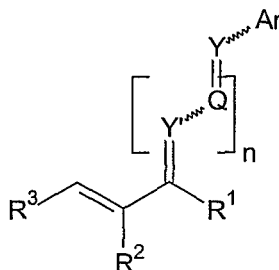
In certain embodiments, disclosed compounds include, for example, compounds having the formula above; wherein n is 0, such as compounds of the formula



- wherein  $R^7$  and  $R^8$  independently are  $-OR^5$ ,  $-SR^5$ ,  $-SO_2N(R^5)_2$ ,  $-SO_2CN$ ,  $-SO_2R^6$ ,  $-SO_2N_3$ ,  
 10  $N_3$ ,  $-CN$ ,  $-N(R^5)C(O)R^6$ ,  $-R^6$ ;

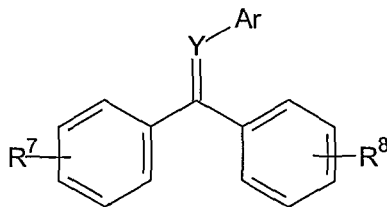
each  $R^5$  independently is H, lower alkyl, carbohydrate, fatty acid, amino acid, peptide, nucleoside or nucleic acid;  $R^6$  is lower alkyl or C-glycoside; and Ar, X and Y are as described above.

In another embodiment, X represents a bond. Exemplary structures wherein X represents a bond include



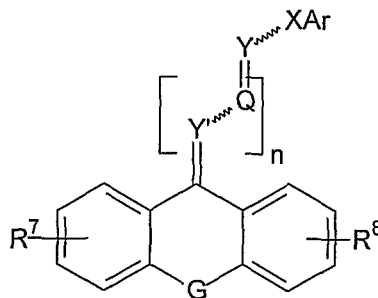
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Exemplary embodiments of such compounds, when  $n = 0$ , are represented by the formula



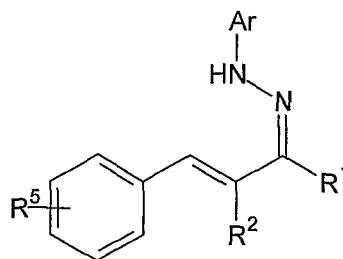
wherein Ar, Y,  $R^7$  and  $R^8$  are as defined above.

In one embodiment, the compounds have the formula



wherein G represents  $-\text{CH}_2-$ ,  $-\text{C}(\text{O})-$ ,  $-\text{N}-$ ,  $-$ ,  $-\text{O}-$ , or  $-\text{S}-$ ; n is 0 or 1; and Y', Q, Y, X, Ar,  $\text{R}^7$  and  $\text{R}^8$  are as set forth above.

Other compounds disclosed herein include those having the formula

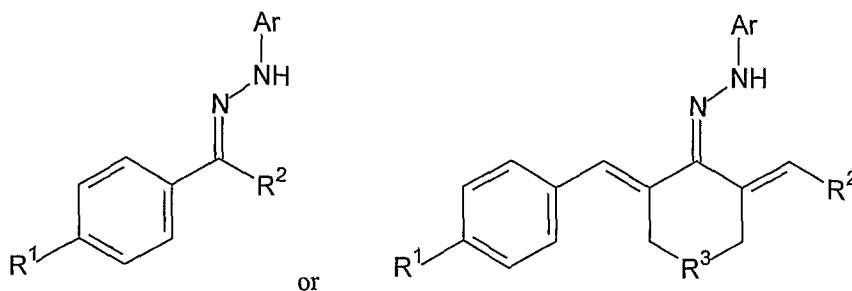


wherein  $\text{R}^5$  is H,  $-\text{OR}^6$ ,  $-\text{SR}^6$ ,  $-\text{SO}_2\text{N}(\text{R}^6)_2$ ,  $-\text{SO}_2\text{CN}$ ,  $-\text{SO}_2\text{R}^7$ ,  $-\text{SO}_2\text{N}_3$ ,  $\text{N}_3$ ,  $-\text{CN}$ ,  $-\text{N}(\text{R}^6)\text{C}(\text{O})\text{R}^7$ , or  $-\text{R}^7$ ;

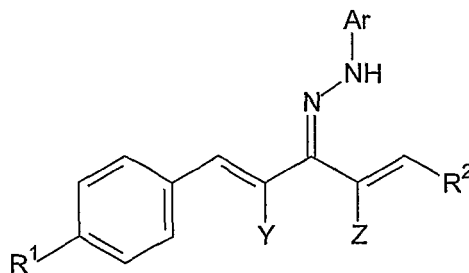
$\text{R}^1$  and  $\text{R}^6$  independently are H or lower alkyl;

$\text{R}^2$  and  $\text{R}^7$  independently are lower alkyl; and Ar is an aryl group as set forth above.

Particular embodiments of compounds disclosed herein include arylhydrazones. Other examples include aryl nitrohydrazones, such as phenylhydrazones, such as polyaryl mononitro- or dinitrophenylhydrazones, for example



or



wherein R<sup>1</sup> is hydrogen, hydroxy, hydroxyphenyl (such as 2- or 4-hydroxyphenyl), acetate, phosphate, azido, nitrile, amino, dimethylamino, sulfate, methylsulfonate, phosphate, succinate;

R<sup>2</sup> is an unsubstituted (C<sub>6</sub>H<sub>5</sub>) or substituted phenyl group such C<sub>6</sub>H<sub>4</sub>OH, C<sub>6</sub>H<sub>4</sub>N<sub>3</sub>, C<sub>6</sub>H<sub>4</sub>CN, 4-HO-C<sub>6</sub>H<sub>4</sub>-C<sub>6</sub>H<sub>4</sub>, C<sub>6</sub>H<sub>4</sub>OPO<sub>2</sub>OH, C<sub>6</sub>H<sub>4</sub>OSO<sub>2</sub>H, C<sub>6</sub>H<sub>4</sub>NH<sub>2</sub>, C<sub>6</sub>H<sub>4</sub>NHMe<sub>2</sub>, C<sub>6</sub>H<sub>4</sub>OSO<sub>2</sub>Me, C<sub>6</sub>H<sub>4</sub>OCO(CH<sub>2</sub>)<sub>x</sub>CO<sub>2</sub>H, or C<sub>6</sub>H<sub>5</sub>Cl;

Ar is nitrophenyl, such as C<sub>6</sub>H<sub>3</sub>-2,4(NO<sub>2</sub>)<sub>2</sub>, C<sub>6</sub>H<sub>4</sub>-4(NO<sub>2</sub>), C<sub>6</sub>H<sub>4</sub>-3(NO<sub>2</sub>), or C<sub>6</sub>H<sub>3</sub>-2,4(NO<sub>2</sub>)<sub>2</sub>;

R<sup>3</sup> = -O-, -S-, -CH<sub>2</sub>-, -N-, —, -CHA- and -CHOA-; where A=aryl, ester, amide, lipid, carbohydrate, or peptide;

Y = H, (CH)<sub>x</sub>CH<sub>3</sub> (x = 0–12), -S-CH<sub>3</sub>, nitrile, amino, nitro, azido, succinate, or amide; and Z = H, (CH)<sub>x</sub>CH<sub>3</sub> (x = 0–12), -S-CH<sub>3</sub>, nitrile, amino, nitro, azido, succinate, or amide.

In particular embodiments, these compounds possess resonating intramolecular dipole movements that are believed to be capable of electrostatic interaction with biological environments, and in particular examples interact with the extracellular catalytic receptors of R-PTP, such as those found on the R-PTP CD45+ PTP subtype, or with lymphocytes in general. This interaction attracts lymphocytes (such as T-lymphocytes) to target regions to which the compound has been applied. Although the resonance modulating compounds are capable of non-covalent affiliations, they typically lack substantial chemical reactivity and produce no local chemical reactions. They nonetheless appear to activate immune function, for example by mobilizing lymphocytes (for example T-lymphocytes such as CD45+ T-lymphocytes, for example CD45RO+ or CD45RA+ lymphocytes) from lymphatic networks, and concentrate immunological activity in the targeted regions of the body that have been exposed to the compound. The resonance modulator compounds are also capable of stimulating the immune system at locations remote from the targeted region, for example in the spleen, by its interactions with the distributed networks of immune cells, such as those found in the lymphatic system. This mobilization of immune response can be used to treat infections or tumors.

The ability of the disclosed compounds to activate immune system activity remote from the site of application of the compound to the body allows the compound to act as an immune stimulator in the substantial absence of cytotoxicity or other pharmacological action (such as an estrogenic or anti-estrogenic activity) on the target cell itself. Since the compound is activating an immune response that affects the target cell, the body's natural defenses are elicited to indirectly achieve a desired therapeutic effect. Hence the drug can remain on the surface of the body (for example on the skin), and have its remote immunological effect without penetrating the skin to contact a target cell

(such as a dysplastic, neoplastic, or virally infected cell). The drug can also be dispensed in new dosage forms, such as tablets or crystals, which provide prolonged contact with an environment in which immunological stimulation is desired without necessarily bringing the drug into contact with cells that are the target of the therapeutic intervention. Other such dosage forms include containers  
5 (such as sealed bandages or impermeable pouches) that may be affixed to the skin to exert their remote immunoactivating property without even directly contacting the body of the subject or penetrating into or through the skin, or by introducing the dosage form into the skin where it exerts its effect in solid (including crystalline) form. Dosage forms can also include tablets or suppositories, which can be introduced into body cavities other than blood vessels (such as the vagina, anus or  
10 peritoneum) to dissolve in body fluids that contact a diseased tissue (such as the vaginal cervix, anal epithelium, or peritoneal metastases).

Certain embodiments of the disclosed compounds are cytotoxic. Exemplary cytotoxic compounds nucleate or self-assemble at a particular tissue, cell or receptor, for example a target cell. In one aspect, such compounds are selectively cytotoxic for a particular cell-type. Targeted self-  
15 aggregation at a tumor cell in certain examples is able to achieve localized increases in activity of the drug.

In particular embodiments, the compound is administered by applying it to the skin of the subject, for example by applying it topically in a gel to the surface of the skin, or otherwise introducing it into the skin, for example by intradermal placement of crystals or pellets of the  
20 compound. However the compound can also be administered by introducing the compound into the body, for example into a diseased organ or a tumor (such as a malignant tumor or metastatic lesion), to stimulate a local immune response, mobilize lymphocytes from the lymphatic system, and direct an immune response at the target organ or tumor. The compound can also be administered in an aerosol preparation for tracheobronchial or oropharyngeal administration. In one example, the compound is  
25 applied topically to or adjacent a metastatic or epithelial lesion, such as a chest wall breast cancer lesion or a cervical epithelial carcinoma. The compound is applied to the epithelial or epidermal surface of the subject on an area that is in therapeutically sufficient proximity to the lesion to stimulate the immune response in the target region. In the example of a superficial lesion (such as a chest wall recurrence of breast tumor or a cervical or anal epithelial cancer), the compound is applied  
30 directly to the lesion.

In other embodiments, the compound is not applied to a cutaneous lesion (such as a primary tumor or cutaneous metastasis) but is instead placed on the body surface over a subjacent tumor within the body that the compound does not contact, such that the tumor and compound are separated by an integumentary structure through which the compound does not penetrate. Hence the compound  
35 can be applied on or over the skin of the subject to have an anti-tumor effect on neoplastic cells deeper within the body, such as metastatic cells within the lymphatic system, or tumors within organs or structures such as the uterus, breast, anus or prostate.

The disclosed compounds can be used to treat an internal or external body surface lesion. Such lesions can include an anogenital lesion, such as a genital or anal lesion, for example a wart or malignancy, such as a primary malignancy. In particular disclosed examples the lesion is an internal lesion, such as a genital wart of the female reproductive tract, such as a cervical wart. In one  
5 embodiment the lesion is a precancerous lesion. Indeed, in some examples the lesion is a papillomavirus associated lesion, such as an HPV-induced lesion. In yet other examples the lesion is a non-venereal wart, such as a plantar wart, filiform wart, flat wart or seborrheic wart. In certain embodiments, the disclosed compounds can be used to exert a prophylactic effect, such as by treating a precancerous lesion and preventing or delaying neoplasia of the tissue. The compounds are  
10 particularly suitable for treating genital lesions caused by a variety of pathologies, including cancers of the vagina and cancers of the external genitalia, such as primary cancers of the penis or vulva.

In particular examples, the compound is applied topically to the body surface lesion or introduced into the skin, for example by injection or placement of a pellet into the lesion. Alternatively the agent is applied in a pad to the lesion, and the pad is left in place over the lesion for  
15 an extended period of time, such as one or more days or a week or more.

In other examples, the lesion is a skin lesion induced by infection or inflammation. The lesion may be a virally induced lesion, such as a wart, for example a papillomavirus induced wart. Alternatively, the lesion being treated is a bacterial (for example staphylococcus) or viral (for example herpes virus) infection of the skin, or a skin neoplasm (for example a skin cancer such as a  
20 squamous or basal cell carcinoma or a primary melanoma).

The immune stimulating activity or PTP activating activity or immunomodulating activity of the compound can be enhanced by exposing the compound to an electromagnetic field (such as a time varying electric field, a time varying magnetic field, and/or a radiating electromagnetic field), for example a field that induces increased resonance modulation of the compound. For example, such a  
25 field can be induced by placing a magnetic probe in the vicinity of the compound that has been administered to the subject, or by providing a current that flows through the compound between two electrodes, or by a laser that irradiates the compound with laser energy. In particular embodiments, activity of the immune system can be conveniently up-regulated by applying the compound to a target region of the skin using a convenient patch or pellet, and inducing the electromagnetic field in  
30 a manner that increases the resonance modulation of the compound. In this manner, the resonance modulator on the skin surface acts as a convenient coupler between the externally applied electromagnetic field and the immune system, which can increase immune function particularly locally at the site of the resonance modulator. However, it is also believed that immune function can be enhanced systemically, remote from the resonance modulating compound.

35 The ability of the compound to interact with PTPs (such as components of the immune system) also allows the compound to be used to monitor immune function. It has been observed that application of the compound to a target region (for example in a patch applied to the skin) mobilizes PTP expressing cells, such as cellular components of the immune system. The attraction of the

immune cells apparently occurs by electrostatically interacting with and attracting dendritic cells and other early phase lymphoid cells. These cells aggregate in the vicinity of the resonance modulator, and change electromagnetic characteristics (such as bioimpedance) in the target region in which they aggregate. The changed electromagnetic characteristics are detectable, for example, as a change in amplitude of the voltage difference detectable across the target area, even in the absence of an applied voltage potential across the electrodes. In particular embodiments, electrodes are placed in contact with the compound, and the inherent varying voltages are produced by resonance modulation of the compound over time. The amplitude of these varying voltage potentials is used to monitor immune function. For example, a reduction in voltage amplitude between the electrodes as compared to a normal control is an indication of impaired immune function.

The amplitude of the waveform (or other electromagnetic characteristics) may be monitored to determine a response of the subject's immune system to the compound. An increase in the amplitude of the voltage over time indicates that an immune response has occurred (and that immune effector cells such as lymphocytes have migrated to the target region). If the increase in amplitude falls below a predetermined threshold (such as an expected increase of at least 10%, 25% or 50%), then further diagnostic or therapeutic interventions may be undertaken to assess or correct the reasons for the impaired immunity. For example, a rigorous search can be undertaken for infectious, toxic or neoplastic causes of the impaired immune response. Alternatively, the immune response can be upregulated by exposing the resonance modulating compound to an electromagnetic field that induces increased resonance modulation of the compound. Another approach would be to initiate treatment of the subject with appropriate anti-infective or anti-neoplastic chemotherapeutic agents.

The disclosed agents are particularly effective for treating a tumor, by administering the agent to the subject (for example by topical application to a target region over or adjacent a cutaneous metastasis). An external electromagnetic field may then optionally be applied to the agent to increase its resonance modulation and thereby increase its immunostimulant effect. The external electromagnetic field may be produced, for example, by a magnetic probe that induces a localized magnetic field, an induced external current applied across electrodes, or a laser that stimulates resonance modulation of the resonance modulating compound. Resulting increased mobilization of antigen presenting cells (such as dendritic cells) and immune effector cells (such as T-cells) to the target region helps direct immunosurveillance and immune effector activity to the tumor target. The agents can be used, for example, to treat a cervical, anal or vaginal carcinoma, such as a carcinoma associated with an HPV infection in which HPV has induced a malignant transformation of the cells.

In particular embodiments, the disclosed agents can stimulate an immune response either through or to a RPTP expressing cell, such as a CD45<sup>+</sup> cell, such as a CD45<sup>+</sup> T-lymphocyte or a RPTP or CD45<sup>+</sup> expressing infected cell. An example of such a cell would be a lymphocyte produced in response to a virally infected cell, such as a cell infected with papillomavirus, for example human papillomavirus (HPV), or an immunodeficiency virus (such as HIV).

In particular methods of monitoring an immune response, compound is placed in contact with the subject (for example on or in the skin of the subject). In one aspect of the method the compound is a resonance modulator so that the inherent resonance modulation of the compound yields characteristic waveforms of the compound. The waveforms (such as alternation of voltage  
5 potentials or other electromagnetic waves) detected from the compound are monitored to detect waveforms produced by the compound that are associated with altered immune function. In particular embodiments, the waveforms are monitored by detecting voltage changes in the compound over time, for example by detecting waveforms that have a decreased amplitude from an expected increase that would be seen over time. In some embodiments of the method, once the waveforms  
10 have been detected that are associated with altered immune function, a diagnostic or therapeutic intervention may be undertaken in response to the detection of the waveforms.

For example, the intervention can be a diagnostic intervention designed to detect a specific feature of the altered immunity (such as decreased number or function or particular cells involved in the immune response, such as T-lymphocytes) or a cause of the altered immunity (such as infection  
15 with an immunodeficiency virus, or the presence of a neoplastic condition, such as a tumor, that alters immune function). Alternatively, detection of altered immunity results in a therapeutic intervention, such as administration of an anti-neoplastic or anti-infectious therapy (such as an antibiotic or antiviral drug), and/or modulating resonance of the compound to enhance immune function. Modulating resonance of the compound can include applying an induced electromagnetic field to the  
20 compound that enhances resonance modulation of the compound, which in turn improves immune function. In particular embodiments, the improved immune function constitutes improved mobilization of T-lymphocytes (such as CD45+ T-lymphocytes) to the site of application of the resonance modulating compound.

The disclosed methods also include a method of treating a tumor by exposing the tumor to a  
25 therapeutically effective dose of a resonance modulating compound, and applying an external electromagnetic field to the compound to increase its resonance modulation of the compound and thereby increase an anti-tumor effect of the compound to treat the tumor. Alternatively, the method can be used to treat an infection (such as a bacterial infection like *Yersinia pestis* infection or a viral infection, such as an HPV or HIV infection, or other infections in which a PTP is expressed by the  
30 pathogen) by administering a therapeutically effective amount of the compound. In particular examples, the therapeutic amount is sufficient to interact with a PTP extracellular receptor of a cell to activate the receptor. In particular examples of either treatment, the compound is applied to the skin of the subject, or to an area of infection or neoplasia. The compound may, for example, be applied as a topical gel to the skin of the subject, or to urogenital or anogenital epithelium (such as anal, vaginal  
35 or cervical epithelium) that is infected with a papillomavirus (such as HPV). In some examples, the epithelium is dysplastic or metaplastic epithelium, such as cervical intraepithelial or high grade or squamous intraepithelial neoplasms (CIN/HSIL), anal intraepithelial neoplasms (AIN/HSIL) or a squamous carcinoma. In particular examples, the compound is applied as a 0.25% gel that is applied

topically to the subject for a period sufficient to have a therapeutic effect, such as at least five days. In particular methods of treatment, gel is applied daily, and/or the effective amount is at least 2 grams of a topical gel containing at least 0.25% of the compound.

The unusual properties of the disclosed compounds also permit them to be used in methods of concentrating dendritic cells and/or lymphocytes, either *in vitro* or *in vivo*. For example, RPTP+ cells, such as lymphocytes (for example CD45+ cells, such as T-lymphocytes) can be concentrated from biological tissue (or in culture) by exposing the biological tissue (or cells) to an effective amount of the agent. In one example, that agent is applied to the skin of a subject to concentrate RPTP+ cells (such as lymphocytes) at and around the site of application of the agent. However, RPTP+ cells, such as lymphocytes and/or dendritic cells, can be attracted to any target region of the body in which the agent is introduced, such as a breast, colon or prostate tumor. Alternatively, the agent can be introduced into a tissue culture that contains RPTP+ cells such as lymphocytes and/or dendritic cells to attract them to the agent. Selective concentrations of sub-populations of cells can be achieved in this manner. The agent can also be used to increase the concentration of CD45RO+ and CD45RB+ cells, for example by inducing expression of these cell surface markers. In particular embodiments, selective expression of CD45RO+ occurs, which is involved in cytotoxic activity.

Also disclosed herein is a method for stimulating platelet levels, for example by stimulating platelet production, for example in the treatment of thrombocytopenia. One embodiment of this method includes treating a subject having reduced platelet count with a compound disclosed herein. In certain examples the thrombocytopenic subject has reduced platelet levels as a result of a neoplastic, autoimmune or infectious disorder. In particular examples, the subject has an autoimmune thrombocytopenia, or toxic thrombocytopenia, for example induced by cytotoxic antineoplastic drugs.

The foregoing and other objects, features, and advantages of the disclosed compounds, compositions and methods for their use will become more apparent from the following detailed description of several embodiments which proceeds with reference to the accompanying figures.

### BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 is a schematic illustration of the interaction between a conjugated aryl hydrazone and the CD45+ T-lymphocyte surface receptor.

FIG. 2 illustrates the structural formula of A-007.

FIG. 3 is a schematic illustration of the resonance structures of a conjugated aryl hydrazone.

### DETAILED DESCRIPTION

#### I. Abbreviations

**A-007:** 4,4'-dihydroxybenzophenone-2,4-dinitrophenylhydrazone

**AMTR:** A-007 Magnetic Transistor Resonator

**An:** Antigen

**APC:** Antigen Presenting Cell

**BDP-DNP:** 2,6-Dibenzylidenecyclohexanone-2,4-dinitrophenylhydrazone

**DC:** Dendritic Cell

**DNP:** Dinitrophenylhydrazone

5 **PTP:** Protein Tyrosine Phosphatase

## II. Terms

Unless otherwise noted, technical terms are used according to conventional usage.

Definitions of common terms in molecular biology may be found in Benjamin Lewin, *Genes V*, published by Oxford University Press, 1994 (ISBN 0-19-854287-9); Kendrew *et al.* (eds.), *The Encyclopedia of Molecular Biology*, published by Blackwell Science Ltd., 1994 (ISBN 0-632-02182-9); and Robert A. Meyers (ed.), *Molecular Biology and Biotechnology: a Comprehensive Desk Reference*, published by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8). Variables such as used throughout the disclosure are the same variables as previously defined unless stated to the contrary.

15 If any of these terms conflict with a document that has been incorporated by reference, the meanings of terms set forth in this document will control.

In order to facilitate review of the various embodiments of this disclosure, the following explanations of specific terms are provided:

**Alkyl group:** An alkyl group is a branched or unbranched saturated hydrocarbon group of 1 to 24 carbon atoms, such as methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, isobutyl, *t*-butyl, pentyl, 20 hexyl, heptyl, octyl, decyl, tetradecyl, hexadecyl, eicosyl, tetracosyl and the like. A "lower alkyl" group is a saturated branched or unbranched hydrocarbon having from 1 to 10 carbon atoms.

**Aryl group:** Aryl group denotes any carbon-based aromatic group including, but not limited to, phenyl, naphthyl, fluorenyl, etc. The term "aromatic" also includes "heteroaryl groups." The term heteroaryl refers to an aromatic group that has at least one heteroatom incorporated within 25 the ring of the aromatic group. Examples of heteroatoms include, without limitation, nitrogen, oxygen, sulfur, and phosphorous. The aryl group can be substituted with one or more groups including, but not limited to, alkyl, alkynyl, alkenyl, aryl, halide, nitro, amino, ester, ketone, aldehyde, hydroxy, carboxylic acid, or alkoxy, or the aryl group can be unsubstituted.

30 Similarly, the term "aralkyl" refers to an aryl group having an alkyl group, as defined above, attached to the aryl group. An example of an aralkyl group is a benzyl group.

**Animal:** Living multi-cellular vertebrate organisms, a category that includes, for example, mammals and birds. The term mammal includes both human and non-human mammals. Similarly, the term "subject" includes both human and veterinary subjects.

35 **Antigen:** A compound, composition, or substance that can stimulate the production of antibodies or a T-cell response in an animal, including compositions that are injected or absorbed into an animal. An antigen reacts with the products of specific humoral or cellular immunity, including

those induced by heterologous immunogens. The term "antigen" includes all related antigenic epitopes.

**Cancerous:** Referring to a malignant tumor or lesion. Examples include primary cancer of the penis, vulva, vagina, uterine cervix, or integument. An example of a cancer of the penis is an invasive squamous cell carcinoma. One example of cancer of the vagina is squamous cell carcinoma. Any of these tumors can be treated with the methods described herein.

**CD Markers:** Cluster of differentiation (CD) markers that can serve as cell surface markers for different classes of lymphocytes. A systematic nomenclature has been developed in which CD has been determined by the binding of certain monoclonal antibodies to certain human leukocyte antigens (HLA). Further information about CD markers is disclosed, for example in Roitt et al., Immunology (6<sup>th</sup> Edition), 2001. A certain subset of CD marker is the CD45 marker, which is a leukocyte common antigen (LCA). CD45 RA, CD45RB and CD45RO are restricted LCAs that are a subset of CD45<sup>+</sup> cells, and are further described in Roitt, et al., Appendix 2.

Certain characteristics of these LCAs are shown in the following table:

| Name   | Expression                                                    | M. W.   | Functions                                                                                                                                          | Other names                                |
|--------|---------------------------------------------------------------|---------|----------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------|
| CD45   | leukocytes                                                    | 180-240 | tyrosine phosphatase, augments signaling through antigen receptor of B and T cells, multiple isoforms result from alternative splicing (see below) | Leukocyte common antigen (LCA), T200, B220 |
| CD45RO | T cell subsets, B cell subsets, monocytes, macrophages        | 180     | isoform of CD45 containing none of the A, B and C exons                                                                                            |                                            |
| CD45RA | B cells, T cell subsets (naive T cells) monocytes             | 205-220 | isoforms of CD45 containing the A exon                                                                                                             |                                            |
| CD45RB | T cell subsets, B cells, monocytes, macrophages, granulocytes | 190-220 | isoforms of CD45 containing the B exon                                                                                                             | T200                                       |

**Chemotaxis:** Movement of an organism or a single cell, such as a leukocyte, in response to a chemical compound. As used herein, chemotaxis can occur in response to any physical property of the compound, including electrostatic properties.

**Chemotherapy; chemotherapeutic agents:** As used herein, any chemical agent with therapeutic usefulness in the treatment of diseases, for example diseases characterized by abnormal cell growth, such as neoplasms. In one embodiment, a chemotherapeutic agent is an agent of use in treating neoplasms such as solid tumors. One of skill in the art can readily identify a chemotherapeutic agent of use (e.g. see Slapak and Kufe, *Principles of Cancer Therapy*, Chapter 86 in Harrison's Principles of Internal Medicine, 14th edition; Perry et al., *Chemotherapy*, Ch. 17 in Abeloff, *Clinical Oncology* 2<sup>nd</sup> ed., © 2000 Churchill Livingstone, Inc; Baltzer L, Berkery R (eds): *Oncology Pocket Guide to Chemotherapy*, 2nd ed. St. Louis, Mosby-Year Book, 1995; Fischer DS, Knobf MF, Durivage HJ (eds): *The Cancer Chemotherapy Handbook*, 4th ed. St. Louis, Mosby-Year Book, 1993).

**Derivative:** Derivative is used to refer to a compound or portion of a compound that is derived from or is theoretically derivable from a parent compound.

**Dendritic cell (DC):** Dendritic cells are the principle antigen presenting cells (APCs) involved in primary immune responses. Dendritic cells include plasmacytoid dendritic cells and myeloid dendritic cells. Their major function is to identify and process antigen in tissues, migrate to lymphoid organs and present antigenic information in order to activate the T-cell cascade. Immature dendritic cells originate in the bone marrow and reside in the periphery as immature cells.

DCs are capable of evolving from immature, antigen-capturing cells to mature, antigen-presenting, T cell-priming cells; converting antigens into immunogens and expressing molecules such as cytokines, chemokines, costimulatory molecules and proteases to initiate an immune response.

**Hydrazone:** A compound with the structure  $R_2C=NNR_2$ , differing from a ketone or aldehyde by the replacement of the double bonded oxygen with the  $=NNR_2$ . A hydrazone is generally formed by the condensation of a hydrazine with a carbonyl group. An aryl hydrazone is a hydrazone in which at least one of the R groups is an aryl group, for example a phenyl group (a phenylhydrazone). A nitrophenylhydrazone is a phenylhydrazone having one or more  $NO_2$  substitutions on the phenyl ring.

**Immune response:** A response of an organism to a foreign (non-self) agent. An immune response to a stimulus is implemented by cells of the immune system, such as a B-lymphocyte, or a T-lymphocyte. In one embodiment, the response is specific for a particular antigen (an "antigen-specific response").

**Infectious agent:** An agent that can infect a subject, including, but not limited to, viruses, bacteria, and fungi.

**Inherent electromagnetic waveforms:** Waveforms that are produced as a characteristic of a compound, independent of actively induced electromagnetic phenomena, such as intentional application of electrical currents, electrical potentials, or magnetic fields. An example of a waveform

is the waveform produced by alternating voltages over time, for example waveforms that alternate between a positive and negative potential, often in a predictable manner (for example as defined by a sine wave). Waveforms can be monitored by a variety of electromagnetic monitoring devices, such as a volt meters that measures an electrical potential across two electrodes in contact with the compound.

**Intervention:** An intervention is an action taken to detect or affect a physiologic or medical state of a subject. A diagnostic intervention detects the state of the subject, for example by performing a laboratory test, such as a blood test, biopsy, imaging study or physical examination. A therapeutic intervention affects the state of the subject, for example by performing surgery, administering a drug or other treatment, or performing any other therapeutic procedure.

**Isolated:** An "isolated" biological component (such as a dendritic cell or lymphocyte, or a population of those cells) has been substantially separated or purified away from other biological components in the cell of the organism in which the component naturally occurs.

**Laboratory evidence of impaired immunity:** Objective laboratory data that is generally medically accepted as evidence of reduced immunity, such as a white blood cell count that is below accepted norms in a particular laboratory, reduced lymphocyte (such as T-lymphocyte) concentration, or evidence of generalized impaired activity of any cell of the immune system. In particular examples, the data demonstrate impaired cellular immunity or humoral immunity, or both.

**Lacking chemical reactivity:** Certain resonance modulating compounds are electrostatically active but substantially non-reactive under physiological conditions (in the body) because of their resonance stabilization. Such lack of chemical reactivity results in the compound being excreted substantially completely unchanged after systemic (such as oral or intravenous) administration. An example of this lack of reactivity is seen, for example, by an absence of N-methylation of A-007 in the body

**Leukocyte:** Cells in the blood, also termed "white cells," that are involved in defending the body against infective organisms and foreign substances. Leukocytes are produced in the bone marrow. There are 5 main types of white blood cell, subdivided between 2 main groups: polymorphonuclear leukocytes (neutrophils, eosinophils, basophils) and mononuclear leukocytes (monocytes and lymphocytes). When an infection is present, the production of leukocytes increases.

**Neoplasm:** An abnormal cellular proliferation, which includes benign and malignant tumors, as well as other proliferative disorders.

**Optional or optionally:** These terms indicate that the subsequently described event or circumstance can but need not occur, and that the description includes instances where said event or circumstance occurs and instances where it does not.

**Papillomavirus:** Papillomaviruses are small, nonenveloped viruses with an icosahedral symmetry, capsomere, and a double-strand circular DNA genome of about 8,000 bp. All papillomaviruses have a similar genetic organization. The viral genome is divided into an early region which encodes the genes required for viral DNA replication and cellular transformation, a late

region that codes for the capsid proteins, and a regulatory region that contains the origin of replication and many of the control elements for transcription and replication.

Papillomaviruses have a high degree of species specificity. There are no known examples of natural transmission of human papillomavirus (HPV) to other species. Papillomaviruses also display a marked degree of cellular tropism, infecting only surface squamous epithelia of the skin or mucosa and producing for the most part benign epithelial tumors. Specific viral types appear to have a preference for either cutaneous or mucosal types. For example, HPV-11 does not readily infect cutaneous epithelium from other body sites but can infect mucosal epithelium of either the genital or the respiratory tract. However the papillomaviruses induce cellular proliferation and transformation that can lead to the development of invasive cancers. HPV infections have been associated with the development of cervical and anal cancers.

Genital warts are usually caused by HPV. Papilloma viruses cause small growths (warts) on the skin and mucous membranes. Infection of the genital and anal regions with HPV can cause warts (anogenital condyloma) on the penis, vulva, urethra, vagina, cervix, and around the anus (perianal). More than fifty different types of HPV have been classified. Several types, including 6 and 11, are associated with raised, rough, easily visible genital warts (especially in women). Other types are associated with flat warts. More importantly, several types are associated with pre-malignant and malignant changes in the cervix (abnormal Pap smears). These include types 16, 18, 31, 39, 45, 51, and 52. Research also shows that the presence of both HPV and herpes virus together is a good predictor of the development of cervical cancer.

Lesions on the external genitalia are easily recognized. On the penis, genital warts tend to be drier and more limited than on the female genitalia or around the anus of either sex. They grow best in the moist genital area, and are raised, rough, flesh-colored "warty" appearing tumors that may occur singly or in clusters. Left untreated, warts around the anus and vulva may rapidly enlarge, taking on a "cauliflower-like" appearance. In women, HPV can invade the vagina and cervix. These warts are flat and not easily visible without special procedures. Because HPV can lead to pre-malignant changes in the cervix (cervical dysplasia), it is important that this condition be diagnosed and treated. Regular Pap smears are important for detecting HPV.

**Papillomavirus associated lesion:** A lesion in which there is evidence of a papillomavirus infection, such as identification of an HPV associated with the lesion.

**Pharmaceutical agent or drug:** A chemical compound or composition capable of inducing a desired therapeutic or prophylactic effect when properly administered to a subject. Pharmaceutical agents include, but are not limited to, chemotherapeutic agents and anti-infective agents.

**Pharmaceutically acceptable carriers:** The pharmaceutically acceptable carriers useful in this invention are conventional. *Remington's Pharmaceutical Sciences*, by E. W. Martin, Mack Publishing Co., Easton, PA, 19th Edition (1995), describes compositions and formulations suitable for pharmaceutical delivery of the compounds herein disclosed.

In general, the nature of the carrier will depend on the particular mode of administration being employed. For instance, parenteral formulations usually comprise injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol or the like as a vehicle. For solid compositions (*e.g.*, powder, pill, tablet, or capsule forms), conventional non-toxic solid carriers can include, for example, pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. In addition to biologically-neutral carriers, pharmaceutical compositions to be administered can contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate.

**Purified:** The term "purified" does not require absolute purity; rather, it is intended as a relative term. Thus, for example, a purified preparation of lymphocytes is one in which the lymphocytes are present in a greater concentration than in its natural environment within the body.

**Resonance Modulator:** A compound that possesses resonating intramolecular dipole movements (or electrical densities) that allow it to electrostatically interact with biological environments. A resonance modulator is also characterized by the emission of oscillating frequency waves generated by the compound's intramolecular resonance. This resonance is believed to convey the electrical dispositions for interactions with cells of the immune system (such as dendritic cells) to up-regulate and/or enhance immunity. Resonance modulators are capable of attracting immune cells, concentrating them in a target region of the peripheral immune system adjacent the resonance modulator, and in some instances have a distant effect on circulating immune cells (such as immune cells in the peripheral blood and lymphoid tissue such as a lymph node or the spleen). Many resonance modulators have a crystalline structure. Examples of assays for selecting resonance modulator candidates are disclosed in Example 14.

**Sigma waves:** Sigma waves that are recorded from crystals and tissue culture are waves over and above the base line of electrical energy that is generated from the heart and other biorhythms within the body. The sigma waves therefore measure voltage changes with time and amplitude increases with time after exposure to A-007.

**Subject in need of immunostimulation:** A subject having a condition that would benefit from general or specific stimulation of the immune system. Examples include subjects with immune deficiencies (such as persons infected with HIV or who have recently received chemotherapy or other immunosuppressive drugs), and persons with conditions that could be improved by the stimulation of an immune response (such as subject infected with a pathogen or tumor that alters immune function, such as a lymphoma). In certain examples, the subject is in need of immunostimulation for a condition other than a tumor, for example because of infection or immunocompromise (for example infectious or pharmaceutically induced immunodeficiency). In other examples, the subject in need of immunostimulation is a subject having laboratory evidence of impaired immune function.

**Therapeutically effective dose:** A dose sufficient to inhibit or prevent advancement, or to cause regression of the disease, or which is capable of relieving symptoms caused by the disease, such as pain or swelling.

**Thrombocytopenia:** is a term for a reduced platelet (thrombocyte) count. Normal  
5 thrombocyte levels are known to those of skill in the art, and those levels are easily determined by automated means. Normal levels differ among laboratories depending on the detection means used. However, thrombocytopenia leads to reduced clotting and pathological bleeding (for example from the gums, nose, or internal organs).

**Waveforms associated with altered immune function:** Waveforms that are noted to be  
10 present in subject having an enhanced or decreased immune function. For example, the amplitude of voltage potential waveforms increases over time after a resonance modulating compound is applied to a subject. The increase in amplitude is a consequence of normal immune function, and is indicative of the mobilization of immune cells (such as lymphocytes) and their migration to the site of application of the compound. However, an increase in amplitude that is below that seen in the same  
15 subject at a baseline measurement taken during health, or an increase below a range that is statistically normal in a population of subjects, can be taken as associated with altered (impaired) immune function. Conversely, an increase in amplitude that is greater than normal (as determined for a particular individual or a population) is an indication of supra-normal immune function.

Unless otherwise explained, all technical and scientific terms used herein have the same  
20 meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. The singular terms "a," "an," and "the" include plural referents unless context clearly indicates otherwise. Similarly, the word "or" is intended to include "and" unless the context clearly indicates otherwise. The term "comprises" means "includes." In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

25

### **III. Descriptions of Several Embodiments**

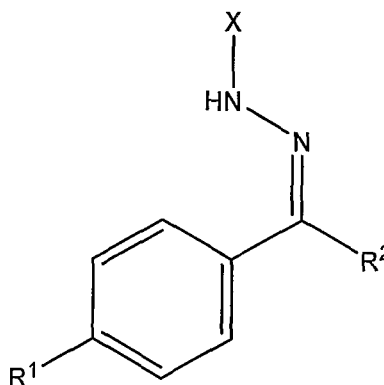
Particular methods disclosed herein concern the use of hydrazone compounds to treat  
infectious, neoplastic and/or inflammatory conditions. Exemplary methods include convenient and  
effective treatment for body surface lesions, such as infectious, neoplastic or inflammatory lesions,  
30 for example warts.

Without being bound to theory, certain compounds act as resonance modulators and act as  
activators of PTPs. In certain examples, the compound acts as an immunostimulant and/or as a  
coupling agent between the immune system and external monitors or modulators. The compounds  
are believed to interact with PTPs, for example interacting with cellular components of the immune  
35 system (such as CD45<sup>+</sup> receptor lymphocytes or dendritic cells) to promote maturation of immune cells, and recruitment of other cellular components of immunity in an immune response. The ability of compounds to attract immune cells to the vicinity of the modulator also allows an immune response to be directed to a target region within the body (such as a tumor) where an immune

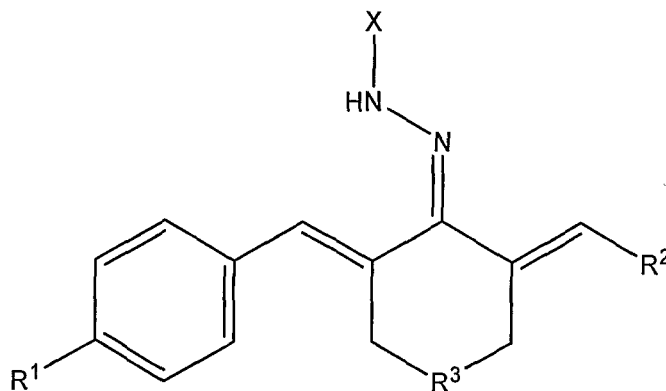
response is needed for treatment of a localized condition. However, in certain embodiments, the compounds also are capable of eliciting an immune response at distant sites, such as remote lymphatic tissue or metastatic lesions. The compounds therefore provide a novel interface with the immune system that allows information about immune status to be collected and interventions (including manipulation of immune function) to be performed.

Resonance structures illustrate composite electronic structures of compounds in which electron density is delocalized. Multiple alternative formal structures are said to be resonance structures, and a molecule can be described as a resonance hybrid of these structures. The conjugated aromatic compounds disclosed herein generally comprise multiple alternative formal structures having delocalized electron density.

An particular example of a resonance modulator disclosed herein is a 2,4-dinitrophenylhydrazone (referred to as A-007), wherein the compound is



and  $R^1$  is OH,  $R^2$  is  $C_6H_4OH$  and X is  $C_6H_3-2,4(NO_2)_2$ . Many other examples of resonance modulating compounds are also disclosed herein, some of which are shown in the attached FIGS. 12, 16 and 17. These include formyl and acetylbarbituric phenylhydrazone analogs. Another example is 2,6-dibenzylidenecyclohexanone-2,4-dinitrophenylhydrazone



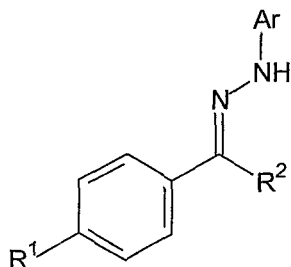
wherein X is a nitrophenyl ( $C_6H_3-2,4(NO_2)_2$ ),  $R^1$  is H,  $R^2$  is unsubstituted phenyl, and  $R^3$  is  $CH_2$ .

The resonance modulator compounds disclosed herein, including 4,4'-dihydroxybenzophenone-2,4-dinitrophenylhydrazone (A-007), are particularly suitable to act as a sensor and immune modulator, because they generally have an affinity for cell membrane receptors. One example of this affinity for RPTP+ cell membrane receptors is the interaction between A-007 and the CD45+ T-lymphocyte surface receptor, which is illustrated in FIG. 1. These interactions are believed to induce maturation of the cells with which they interact, to promote the immune response.

### Example 1

#### Resonance Modulation with Phenylhydrazones

This example discloses particularly suitable conjugated aromatic compounds having both modulating and sensor properties. Certain such compounds include polyaryl mononitro- or dinitrophenylhydrazones, such as



wherein  $R^1$  is hydrogen, hydroxy, 2- or 4-hydroxyphenyl, acetate, phosphate, azido, nitrile, amino, dimethylamino, sulfate, methylsulfonate, phosphate, nitroso, succinate or another water soluble electrophilic group capable of hydrogen bonding;  $R^2$  is  $C_6H_5$ ,  $C_6H_4OH$ ,  $C_6H_4N_3$ ,  $C_6H_4CN$ , 4-HO- $C_6H_4-C_6H_4$ ,  $C_6H_4OPO_2OH$ ,  $C_6H_4OSO_2H$ ,  $C_6H_4NH_2$ ,  $C_6H_4NHMe_2$ ,  $C_6H_4OSO_2Me$ ,  $C_6H_4OCO(CH_2)_xCO_2H$ , or  $C_6H_5Cl$ ; and Ar is  $C_6H_3-2,4(NO_2)_2$ ,  $C_6H_4-4(NO_2)$ ,  $C_6H_4-3(NO_2)$ , or  $C_6H_3-2,4(NO_2)_2$ .

As illustrated in FIG. 3, these chemical structures are able to resonate between multiple dipolar configurations of conjugated electron rich/poor areas that allows/permits migrating intermolecular hydrogen bonding, as well as nucleophilic/electrophilic attractions with complementary sites to exist for moments in time. As illustrated in FIG. 1, these resonating mini-dipoles are capable of attracting for example -SH, -COOH,  $-NH_2-CH-NH_3^+$  that are present in amino acids (such as cysteine, threonine, arginine), carbohydrates (mucopolysaccharides) and glycoproteins, all of which are essential components of cellular regulators in the immune system. For example, simple interactions with 3 or 4 amino acids on the surface of the CD45+RA receptor on a lymphocyte is believed to induce the expression of a modified surface protein (RO, RB) capable of initiating the T-cell cascade with influxes of CD4+/CD8+ into tissues, organs and the circulation. In the "moment in time" during which the hydrogen bonding and attractions are modulated via electrical stimulation, the transient electrostatic interactions that intrinsically exist with these structures are increased and can be quantified with NMR spectroscopy.

The presence of multiple electronegative moieties capable of multiple resonance structures, attached through conjugated and aryl bonds, generates oscillating frequency waves. Certain resonance modulators described herein (such as A-007) have a sufficient "electron count" in that they have an excess of electrons for electrostatic interactions. The excess allows attraction of electrophilic/nucleophilic centers that are present in proteins and other biomolecules, and they do not need to react (via a transfer of electrons and covalent bond formations) for structure stabilization. Instead, simple "flirtations" with the environment are believed to induce changes in other polymolecular configurations, such as the CD45+ receptor, without changing the structure of the resonance modulator.

In a particularly disclosed embodiment of a resonance modulator has the formula above, wherein  $R^1$  is OH,  $R^2$  is  $C_6H_4OH$  and X is  $C_6H_3-2,4(NO_2)_2$  (A-007). This compound is highly electronegative. Moreover, A-007 can be represented in several resonance forms, as illustrated in FIG. 3, as can additional compounds disclosed herein. Representative oscillating frequency waves generated by the hydrazones electrostatically interact with dendritic cells and other early phase lymphoid cellular elements to promote immunity. The interactions can be incorporated into one or more Dirac equation(s) to formulate quantum molecular changes in the skin associated with influxes of potentially selective and diagnostic lymphocyte components.

Stabilization of certain resonance forms of the disclosed compounds is believed to occur upon binding, which results in alteration of frequency and electrical effluxes from the test areas as the resonance modulator interacts with lymphocytes. The interactions typically are non-covalent interactions, such as electrostatic interactions. The cycle of resonance will be disturbed by this interaction, and such interference has been monitored (for example by detecting changes in waveforms of voltage over time) and used as an indicator of activity. Variations in the waveform patterns indicate the presence of an evolving cellular immune response (such as the infiltration of dendritic cells and lymphocytes). An absence of expected waveforms or other detected patterns of interference are also useful for detecting an abnormal immune response that requires further investigation and/or treatment. Hence the resonance modulator acts as a coupling agent between immune activity and an external monitor.

The resonance modulator is capable not only of detecting cellular immune activity, but it also couples the immune system to external physical modulators that can be used to alter this activity. For example, a magnetic resonator probe may be used to apply a magnetic field to the surface of the skin, for example by applying the probe to or adjacent the skin surface. The magnetic probe activates the resonance modulator, stimulates resonant energy efflux and promotes tissue T-cell contact. Thus, if an individual is not able to induce an immune modulation via unstimulated T-cell-resonance modulator interactions, the further polarization provided by an external programmed magnetic field can provide added stimulus to the immune response. Use of such an external magnetic field with A-007 as the resonance modulator is referred to as an AMTR (A-007 Magnetic Transistor Resonator).

Some of the disclosed methods therefore concern the use of various resonance modulators, such as hydrazones, that are able to function as quantum chemical modulators/sensors/transmitters of immune profiles. These agents are therefore useful in documenting and treating changes in epithelial surfaces (such as the skin) that are connected *via* biological networks with lymphatic circuits.

- 5 Peripheral modulation of lymphocytes and precursor cells in the skin is an early warning network for systemic lymphocytes and a natural mechanism by which living systems detect foreign changes and objects (viruses, bacteria, chemicals, cancer cells, etc). Invasion by cancer and foreign chemical/biological objects can produce electrical and emergent behavioral changes that require monitoring/alterations to insure health. Hence the ability to monitor the status of this peripheral  
10 modulation provides an important new medical diagnostic and therapeutic tool.

### Example 2

#### Anti-neoplastic and Immune Modulating Characteristics of 4,4'-Dihydroxybenzophenone-2,4-dinitrophenylhydrazone (A-007)

- 15 This example demonstrates that the disclosed compounds, including 4,4'-Dihydroxybenzophenone-2,4-dinitrophenylhydrazone, have significant anticancer activity in anticancer trials (IND 47,470). Specifically, among fifty-three (53) people treated with topical A-007 (as a 0.25% gel), 37% objective remissions have been observed with ten complete responses.

20 **Table 1. Responses of Cutaneous Metastases to A-007 0.25% Gel Applied Topically to Cancer Lesions Twice Daily**

| Cancer         | #  | Applied (g) | M/F # | Response                | Duration (wks) | Toxicities    |
|----------------|----|-------------|-------|-------------------------|----------------|---------------|
| Breast         | 27 | 0.01 - 2.43 | 30 F  | 5 CR, 7 PR,<br>6 stable | 6 wks-14.5 mos | 7 skin (mild) |
| Melanoma       | 5  | 0.02 - 0.50 | 3F/2M | 1 PR, 3 NR              | 3-15           | 2 Skin (mild) |
| KS             | 4  | 0.02 - 0.22 | 1F/3M | 2 PR, 2 NR              | 11             | 0             |
| H/N (Sq. Cell) | 1  | 0.091       | 1M    | NR                      | 0              | 0             |
| NHL            | 4  | 0.02 - 0.16 | 1F/3M | 1 PR                    | 3.5 yrs        | 0             |
| Angio-sarcoma  | 2  | 0.02 - 0.06 | 1F/1M | 2 NR                    | 0              | 0             |
| Anogenital     | 10 | 0.05        | 9F/1M | 5 CR, 2 PR              | 1.8+ yrs       | 0             |

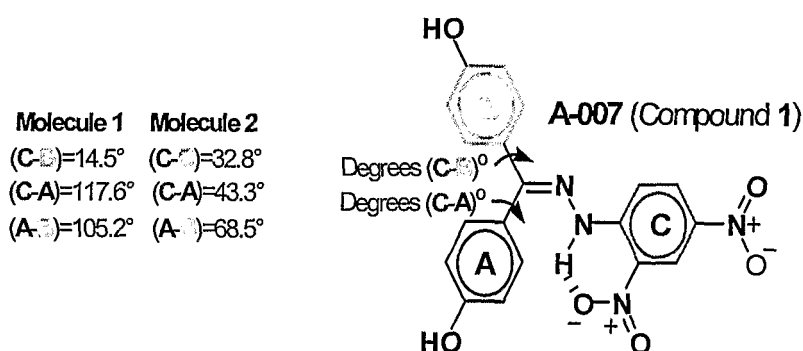
*Note: KS = Kaposi's sarcoma; H/N=head/neck; NHL=non-Hodgkins' lymphoma; CR=complete response; PR=partial response >50%; NR=no response). All patients treated had received at least 2-regimens of systemic therapy (local surgery for the anogenital cancers) prior to initiating A-007.*

25

Certain disclosed compounds do not act via a cytotoxic mechanism. This is evidenced, for example, by a substantial absence of A-007 induced local or systemic toxicity. Histochemical assays,

from biopsies of human skin topically treated with A-007, confirmed that increased infiltrates of a variety of T-lymphocytes (CD4+, CD3+, CD8+, and CD45+) had occurred during treatment. Increased skin infiltrates of CD11c+ dendritic cells were also observed in treated areas. Immunohistochemical (IHC) studies to date have found that immune modulation had occurred *in vitro* and *in vivo* following exposure to A-007. Hence the compounds disclosed herein can have a therapeutic effect without diffuse cytotoxicity that often causes collateral clinical harm.

X-ray crystallography data revealed that A-007 (as monoclinic crystals) exists essentially as two unique molecules, which differ only in the orientation within the bis-diphenylmethane group, where the rings are approximately perpendicular to each other (and rotated approximately 90° from the orientation of the rings in each rotamer) as shown in the accompanying figure. Both rotamers showed strong intramolecular hydrogen bonds between the -NH of the -HN=N=C- moiety and an oxygen of the *o*-nitro group. Thus, there are at least three unique moieties present in A-007 that may contribute to its overall biological activity - a dihydroxy-diphenylmethane, a hydrazone and a dinitrophenyl moiety. However, despite A-007's high electrophilicity, it substantially lacks chemical reactivity under physiological conditions in the human body. For example, the NH on the hydrazone group might ordinarily be predicted to undergo *in vivo* methylation or acylation, but such modifications are substantially absent when A-007 is administered to a human subject. Indeed, the drug is excreted substantially unchanged. Furthermore, A-007 is very insoluble in body fluids and is not extensively absorbed into the blood. A-007 has not been detected in the blood from any of the subjects who have been treated with topical A-007. Plasma assays of A-007 verify its stability.



X-ray crystallography characteristics of A-007

25

### Example 3

#### Effect on the Immune System

The lymphatic system includes lymphatic vessels that communicate with other structures and organs that contain lymphatic tissue in a specialized form of reticular connective tissue. Lymphatic vessels include lymph capillaries, which combine into larger lymph vessels (lymphatics) that resemble veins in structure, but have thinner walls and more valves. Lymph nodes are distributed throughout the body, with the most intense concentration in the face and neck, axillae, thoracic cavity, intestines, groin, elbows and knees. Shallow lymphatic channels of the skin generally

follow veins, while deeper lymphatics generally follow arteries. These lymphatics function to network lymph fluid throughout the body, but they are also an important distributed aspect of the immune system that functions in surveillance and defense against foreign cells, such as microbes and cancer cells.

5           The lymphatic system contains numerous types of lymphocytes. Some of these lymphocytes are T-cells that destroy foreign cells directly or indirectly by releasing cytotoxic substances. Other lymphocytes are B-cells that differentiate into plasma cells that secrete antibodies against antigens to help eliminate them. The lymph nodes filter foreign material carried by the lymph fluid, so that segregated foreign material can then be destroyed by phagocytosis. The spleen, thymus and tonsils  
10       are other lymphatic organs that produce B-cells, T-cells, and other lymphocytes.

          When tumor cells are present in the dermis and epidermis, dendritic cells from the lymph system interact with the tumor cells, and recruit other immune effector cells (such as T-lymphocytes) from lymphatics.

          CD45<sup>+</sup> surface receptors are present on lymphoendothelial cells, and in particular on  
15       dendritic cells. Dendritic cells (DC) are antigen-presenting cells (APCs) involved in the initiation of the immune response. Serving as immune system sentinels, DCs are responsible for antigen (An) acquisition and subsequent transport to T-lymphocyte rich areas. The DCs are present in lymphatic tissues, such as peripheral cutaneous tissue, as well as in lymphoid organs. Once immature DCs interact with an antigen and become activated, the mature APCs are capable of specific immune  
20       responses. Secondary lymphoid organs, such as the skin, recruit both naive T-lymphocytes and An-stimulated DCs (APC) into T-cell rich lymphoid zones/networks (nodes, etc). Co-localizing these early immune responses constitutes cognitive T-cell activation.

          Both cancer and administered chemicals can produce emergent behavior in healthy skin with collective distributed intelligence of lymphocyte populations. Effective recognition responses require  
25       both DC (APCs) and lymphocyte cytokine effectors. Because chemicals and tumor cells often have limited expression of microhistochemical (MHC) antigens and lack co-stimulatory molecules, they are not effective modulators of APCs. One mechanism for the development and progression of cancer and allied diseases is lack of MHC antigenic properties that would otherwise produce emergent behavior in lymphatic networks. Similarly, life-threatening chemical and/or biological  
30       contacts can also induce emergent lymphatic behavioral patterns. The agents disclosed herein, including A-007, are organic molecules, that are sufficiently electrically endowed to act as a hapten/An, and/or through electromagnetic field effects (EFE), to modulate or up-regulate emergent lymphocyte networks. The disclosed compounds include a new class of renaissance molecules referred to herein as *resonance modulators*. It is believed that up-regulation of the CD45<sup>+</sup> receptor is  
35       one initiation site for the A-007-induced immune modulations that are observed in patients with cancer.

          CD45<sup>+</sup> is expressed on dendritic cells, lymphocytes, monocytes, and leukocytes, as well as some neoplastic cells, as a protein tyrosine phosphatase (PTP), which together with other members of

the PTPs, are responsible for phosphorylating tyrosine residues. Blockade of the CD45+ receptor sites with anti-CD45 antibodies inhibits T-cell activation and prevents mitogen (lectin) activation of naive T-cells. CD45+ receptor surfaces contain arginine, serine/threonine and cysteine moieties, which can bind to and/or transfer natural ligands to the surface of APCs, as well as hydrolyze tyrosyl phosphates. Certain of the disclosed compounds, including, for example, A-007 do not inhibit or block CD45+, but up-regulate CD45+ lymphocytes and dendritic cells (to APCs) via electrostatic/non-covalent binding with Arg, Cys, Ser/ Threo, etc as illustrated in FIG. 1. Different resonance states of A-007 are capable of interacting with different immune cells.

A-007-activated DCs are capable of initiating mitotic events with naïve human blood peripheral mononuclear cells (PBMC) and up-regulating both CD45+ and CD11c+ receptors in human peripheral dendritic cells. Dendritic cells in cancer tissue are up-regulated from CD45RA+ to CD45RO+/CD45RB+ following exposure to topical A-007 during topical treatment of skin lesions. Thus, A-007 is not an inhibitor of CD45+, but an up-regulator or modulator of the molecular sites. These properties allow a crystal or implanted skin pellet of A-007 to increase both local and more remote immune cell populations (such as splenic dendritic cell populations), and increase concentrations of immune effector cells, such as CD8+ cytotoxic lymphocytes (CTL). The influence that functional group substitutions may have on A-007's intra-/intermolecular hydrogen bonding and electrostatic interactions is presented below.

This evidence demonstrates that A-007 has the ability to interact peripherally in the skin and other epithelial surfaces with dendritic cells to induce maturation of the antigen presenting cell (APC). In addition, at least one cell surface receptor (CD45+) appears to be up-regulated by A-007. It is believed that this initial interaction is via dendritic and other lymphocyte precursors, such as antigen presenting cells. Through this chemical interaction, APCs and other early recognition cells are capable of being sensed and modulated with maturation of the lymphocyte recognition cascade - CD4+, CD8+, etc. and associated cytokines.

Some of the unusual properties of A-007 that make it suitable as an immune modulator are illustrated by the ability of its crystals to align in an unusual pattern when evaporated on a glass slide between two electrical wires through which a current was introduced. This arrangement of the crystals was obtained by evaporating an alcoholic (5%) solution of A-007 in air on a glass slide between two wires respectively connected to the positive and negative leads of a 9 volt battery. The A-007 condenses in a circumferential pattern at the positive pole, rather than as a diffuse "spot" over the entire field. When evaporation of the alcoholic solution occurs in a magnetic field, a single line of tightly agglutinated crystals forms down the middle of the slide. Hence A-007 has electromagnetic properties that interact with induced electromagnetic fields. This characteristic can also be used as a screening test for other resonance modulators.

These unusual electromagnetic properties of A-007 are further illustrated by the conductance of this compound. This was measured from A-007 crystals, to which two microelectrodes were attached using the magnification of a dissecting microscope. The electrodes were separated by about

1.5 - 2 mm on the crystal surface and the energy was measured from the contact. The Fluke ScopeMeter was used to record the frequency (Hz) generated. No external applied current was involved; hence the measured frequencies represent a natural physical property of the crystals. These excursions from the baseline frequency are believed to be a translational type of energy that is  
5 accumulated from the environment and intermittently released. The baseline frequency is likely from ambient electromagnetic radiation. Excursions from the baseline frequency of at least 30-40 Hz is generated by the resonance of the crystals by the intermittent excursions (indicated by the peaks in frequency) from the baseline frequency of about 60 Hz. This ability to emit natural frequencies of energy (for example at a frequency of at least 20 Hz from baseline, for example to between 30-40  
10 Hz), and/or at least once every five minutes (for example at least once every three minutes) is another example of a characteristic of a resonance modulator that allows resonance modulators to be screened and selected for further testing.

When a crystal or a pressure pressed pellet of the chemical is submersed into a tissue culture media with naive human lymphocytes (obtained from peripheral blood), lymphocytes and dendritic  
15 cells aggregate around the crystal or pellet. Crystals (0.5 mg) of A-007 were placed into RPMI media containing 5% bovine serum albumin, antibiotic preservatives penicillin/streptomycin, and naive lymphocytes obtained from the buffy coat of blood from a healthy person. After contact with the crystal of A-007, there is agglutination and increased mitotic activity, as well as colonization of activated dendritic cells into the area of the A-007 resonance modulator agent.

20 The ability of an agent to induce aggregation of naive lymphocytes in culture around a test agent is another factor to be considered in determining whether the agent is a resonance modulator suitable for use in the methods disclosed herein. The ability to induce aggregation within one hour is a particularly strong indication that the compound is suitable for further investigation. "Aggregation" refers to a substantial increase in the number of lymphocytes, such as an increase visible by  
25 microscopy.

Another unusual electromagnetic characteristic of the resonance modulator is that an increase in amplitude of an electromagnetic wave (such as current) can be measured as lymphocytes mature and agglutinate around the crystal.

To obtain these waveforms, A-007 was condensed on to sterile glass microscope slides, as  
30 shown in Fig 4. The dried slide was immersed into a petri dish with RPMI (5% bovine albumin), and a pair of sterile microelectrodes were inserted into the media (about 5 cm apart) and placed in contact with the opposite ends of the line of A-007 and the voltage recorded. The A-007 is not soluble in the media and remains on the slide with continuity. No induction currents were present; hence the measured currents were an inherent characteristic of the resonance modulating compound.

35 After 1-hour of exposure to A-007, there was an increase in amplitude of the measured voltage over time as the lymphocytes migrate to and attach to the A-007 crystals. Since the crystals do not dissolve, they remain available for continuous interactions. *In vitro*, the lymphocytes eventually die after about 24 hours from lack of cytokines needed for cellular perpetuation.

Hence another characteristic that can be used to select resonance modulators is an ability to increase the amplitude of alternating voltages in an inherent current produced by the resonance modulator when the compound is placed in culture with naive lymphocytes.

5

#### Example 4

##### Monitoring Immune Function

As a consequence of their extraordinary properties, the compounds described herein (including resonance modulators) can be used to monitor electrical activity associated with the immune system. Exemplary compounds can be used as an analytical device to measure and control  
10 immune characteristics, in which there is a linear relationship between T-lymphocyte and frequency responses. The microbalance that exists is due to the fact that mass sensitivity of a 50 Hz A-007 crystal is approximately  $0.057 \text{ Hzcm}^2\text{ng}^{-1}$ , which is approximately 50 times higher than that of an electronic fine-balance with a sensitivity of  $0.1 \mu\text{g}$ . The crystals can be used to measure electromagnetic properties of cellular elements. In particular embodiments, crystals are chosen the  
15 have a mass sensitivity of at least about 0.01, 0.03 or  $0.05 \text{ Hzcm}^2\text{ng}^{-1}$ . The crystals can be combined with quartz micro-crystals to amplify the electrical interactions (piezoelectric resonator effects) and improve transmission. In particular examples, the quartz microcrystals would be provided in an amount of 10-50% of the total weight of the composition.

The crystals are therefore capable of readily transforming interactions between RPTP+ cell  
20 (such as lymphocyte) populations and equivalent electrical circuits of the cutaneous tissues which permit a complete description of the oscillations in the presence of the hydrazones and other resonance modulators. Basically, the resonance modulators serve as a resonator of dipolar movements and electrostatic interactions with cellular elements, such as cells of the tissue and peripheral immune system. The resonance modulator allows these interactions to be monitored for  
25 diagnostic purposes, and altered for therapeutic interventions.

The ability to use resonance modulating compounds to monitor immune function permits early diagnosis of a subject's altered ability to recognize a biological insult, such as a toxin or foreign antigen. For example, a subject who has been exposed to a foreign chemical or biological agent may not recognize the exposure and may therefore not respond appropriately. However if the resonance  
30 modulator detects a change in function of the immune system, this altered immune status serves as a sentinel event that alerts the subject to a possible unknown toxic exposure. Similarly, subjects exposed to environmental stresses (such as virus/bacteria and traumatic events) may have dendritic cells that do not recognize foreign viruses, chemicals or cancer. Functional impairment of the dendritic cells may allow significant penetration of immune defenses and a threat to life via emergent  
35 behavior mechanisms.

The resonance modulators may be used in methods to detect and quantitate changes in cellular profiles of the skin that are associated with normal body immunity and natural surveillance activity. Animal studies and human studies have shown that a resonance modulator such as A-007 is

not absorbed from the skin but will attract and peripherally activate populations of dendritic cells, CD8<sup>+</sup> cytotoxic lymphocytes and other cellular populations that are needed for the natural modulation toward foreign exposure or irritation. In the event that the body does not respond to the presence of the proposed sensor/stimulant, then aggressive health monitoring and therapies may be pursued.

The monitoring device can take the form of a patch that maintains the resonance modulator in contact with the skin of a subject. The patch can be periodically attached to a voltage meter, for example a voltage meter that provides output in the form of a waveform tracing of the type obtained from an oscilloscope. The amplitude of voltage changes over time is then evaluated to determine how the amplitude changes compare to changes that are observed in healthy subjects. In one example, the amplitude of the voltage waveforms would be expected to increase by a predetermined value (for example at least 10% or 25%) in the presence of the resonance modulator. An amplitude change that is less than the predetermined value is taken as an indicator of a pathologic insult, such as exposure to a toxin or pathogen. This result can indicate the need for more specific testing, such as detection of environmental toxins or pathogens, or imaging tests to detect a tumors or progression of tumors. Alternatively, the abnormal test can prompt the initiation of therapy (or more aggressive therapy), such as the administration of chemotherapeutic agents.

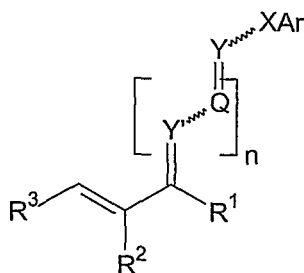
The inherent amplitude and amplitude changes that would be seen would differ for each resonance modulating compound. Hence a specific amplitude change cannot be expressed for all resonance modulating compounds. Nonetheless, it is the recognition of this characteristic of resonance modulators, and their ability to act as an interface for immune function, that serves as the basis of the disclosed methods. Now that this characteristic has been identified, the specific inherent amplitudes generated by each compound and the changes in amplitude that would be seen in specific disease situations or in generalized immunocompromise, can be determined.

In some embodiments, a piezoelectric crystal may be introduced into the resonance modulator compound to provide maximum A-007 transmission through a transducer effect.

### Example 5

#### Additional Examples of Compounds

A variety of compounds having immunomodulatory and/or antineoplastic activity are disclosed herein, many of these compounds also have resonance modulating properties. These compounds are capable of treating body surface lesions such as warts and cancers, such as anogenital, genital, reproductive tract, or skin cancers. In one embodiment, the compounds have the general formula



wherein Ar comprises an aryl group substituted with at least one electron withdrawing group;

X is NH, CH<sub>2</sub>, O, —, SO<sub>2</sub>, or B;

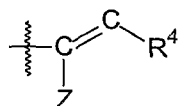
Q is  $-(CH=CH)_m-CH-$ ;

5 Y and Y' independently are selected from N, CH and B;

n is 0 or 1;

m is from 0 to 6;

R<sup>1</sup> is H, lower alkyl, optionally substituted phenyl or



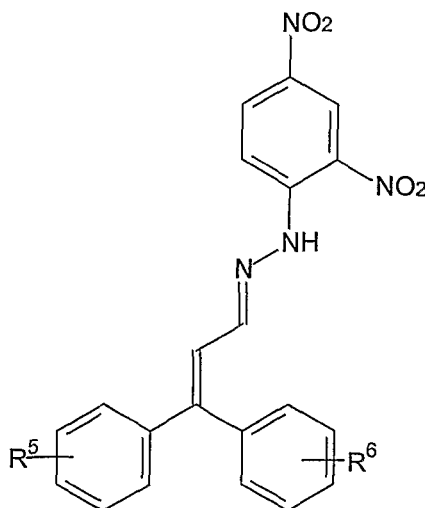
10 wherein R<sup>4</sup> is an optionally substituted phenyl group;

R<sup>2</sup> is optionally substituted lower alkyl, aralkyl, thioalkyl, amidoalkyl, amino, amino alkyl, nitro, azido; together with Z forms an optionally substituted 4–7 membered carbocyclic or heterocyclic ring; or together with R<sup>4</sup> forms an optionally substituted phenyl group;

15 R<sup>3</sup> with R<sup>2</sup> forms an optionally substituted phenyl group or is an optionally substituted phenyl group; and

Z is H, lower alkyl, substituted alkyl, thioalkyl, nitrile, amino, or together with R<sup>2</sup> forms an optionally substituted 4–7 membered carbocyclic or heterocyclic ring.

Certain embodiments include compounds of the formula above, wherein n is 1 or more. In one embodiment, such compounds are represented by the formula

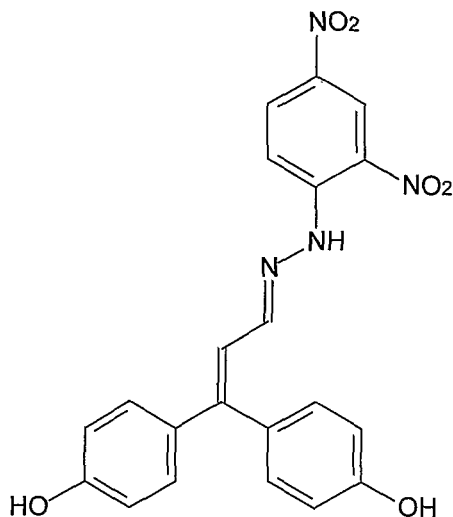


wherein  $R^5$  and  $R^6$  independently are  $-OR^7$ ,  $-SR^7$ ,  $-SO_2N(R^7)_2$ ,  $-SO_2CN$ ,  $-SO_2R^8$ ,  $-SO_2N_3$ ,  $N_3$ ,  $-CN$ ,  $-N(R^7)C(O)R^8$ ,  $-R^7$ ;

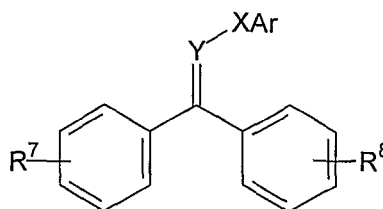
$R^7$  is H, lower alkyl, carbohydrate, fatty acid, amino acid, peptide, nucleoside or nucleic acid; and

5  $R^8$  is lower alkyl or C-glycoside.

In another embodiment, a compound has the formula



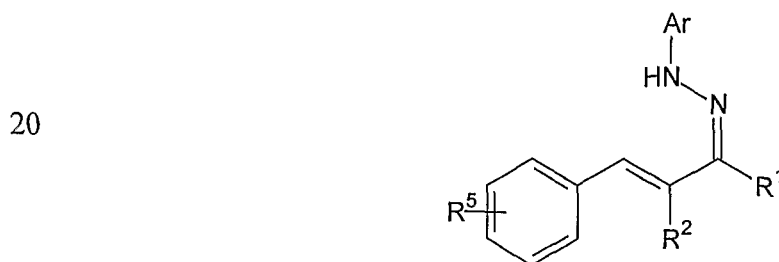
10 In certain embodiments, the compounds having the general formula set forth above have n equal 0. Certain examples of such compounds have the formula



wherein  $R^7$  and  $R^8$  independently are  $-OR^5$ ,  $-SR^5$ ,  $-SO_2N(R^5)_2$ ,  $-SO_2CN$ ,  $-SO_2R^6$ ,  $-SO_2N_3$ ,  $N_3$ ,  $-CN$ ,  $-N(R^5)C(O)R^6$ ,  $-R^6$ ;

15 each  $R^5$  independently is H or lower alkyl;  $R^6$  is lower alkyl; and Ar, X and Y are as described above.

Other compounds disclosed herein include those having the formula

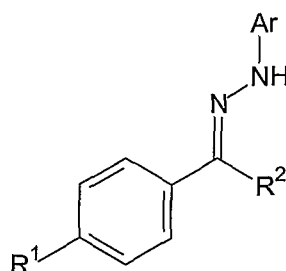


wherein  $R^5$  is H,  $-OR^6$ ,  $-SR^6$ ,  $-SO_2N(R^6)_2$ ,  $-SO_2CN$ ,  $-SO_2R^7$ ,  $-SO_2N_3$ ,  $N_3$ ,  $-CN$ ,  $-N(R^6)C(O)R^7$ , or  $-R^7$ ;

$R^1$  and  $R^6$  independently are H or lower alkyl;

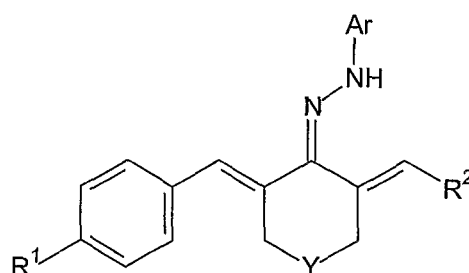
$R^2$  and  $R^7$  independently are lower alkyl; and Ar is an aryl group as set forth above.

- 5 In one particular example, the compound is a mono or polyaryl mononitro- or dinitrophenylhydrazone such as



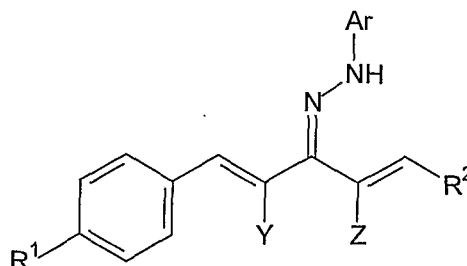
- 10 wherein  $R^1$  is hydrogen, hydroxy, 2- or 4-hydroxyphenyl, acetate, nitroso, phosphate, azido, nitrile, amino, dimethylamino, sulfate, methylsulfonate, phosphate, succinate or another water soluble electrophilic group capable of hydrogen bonding;  $R^2$  is  $C_6H_4OH$ ,  $C_6H_4N_3$ ,  $C_6H_4CN$ , 4-HO- $C_6H_4$ - $C_6H_4$ ,  $C_6H_4OPO_2OH$ ,  $C_6H_4OSO_2H$ ,  $C_6H_4NH_2$ ,  $C_6H_4NHMe_2$ ,  $C_6H_4OSO_2Me$ ,  $C_6H_4OCO(CH_2)_xCO_2H$ , or  $C_6H_5Cl$ ; and Ar represents an aryl group substituted with one or more nitro moiety. In certain
- 15 embodiments, Ar comprises  $C_6H_3$ -2,4( $NO_2$ )<sub>2</sub>,  $C_6H_4$ -4( $NO_2$ ),  $C_6H_4$ -3( $NO_2$ ), or  $C_6H_3$ -2,4( $NO_2$ )<sub>2</sub>. In a particular example,  $R^1$  is OH,  $R^2$  is  $C_6H_4OH$  and X is  $C_6H_3$ -2,4( $NO_2$ )<sub>2</sub>.

In one embodiment, useful compounds are represented by the structure:



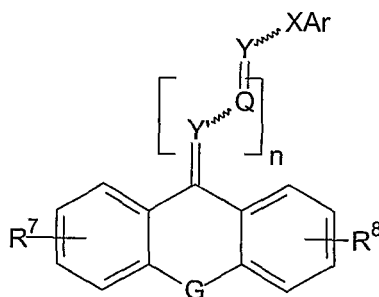
- 20 wherein  $R_1$  is hydrogen, hydroxyl, 2- or 4-hydroxyphenyl, acetate, phosphate, azido, nitrile, amino, dimethylamino, sulfate, methylsulfonate, phosphate, succinate or another water soluble electrophilic group capable of hydrogen bonding;  $R_2$  is  $C_6H_5$ ,  $C_6H_4OH$ ,  $C_6H_5$ ,  $C_6H_4N_3$ ,  $C_6H_4CN$ , 4-HO- $C_6H_4$ - $C_6H_4$ ,  $C_6H_4OPO_2OH$ ,  $C_6H_4OSO_2H$ ,  $C_6H_4NH_2$ ,  $C_6H_4NHMe_2$ ,  $C_6H_4OSO_2Me$ ,  $C_6H_4OCO(CH_2)_xCO_2H$ , or  $C_6H_5Cl$ ; X is  $C_6H_3$ -2,4( $NO_2$ )<sub>2</sub>,  $C_6H_4$ -4( $NO_2$ ),  $C_6H_4$ -3( $NO_2$ ), or  $C_6H_3$ -2,4( $NO_2$ )<sub>2</sub>; and Y is -O-, -S-, -
- 25  $CH_2$ -, -N-, —, -CHA- or -CHOA-, wherein A is aryl, ester, amide, lipid, carbohydrate, or peptide residues. In a particular example,  $R^1$  is OH,  $R^2$  is  $C_6H_4OH$ , and Ar is  $C_6H_3$ -2, 4( $NO_2$ )<sub>2</sub>.

In another embodiment, the compounds have the structure:



wherein  $R_1$  is hydrogen, hydroxyl, 2- or 4-hydroxyphenyl, acetate, phosphate, azido, nitrile, amino, dimethylamino, sulfate, methylsulfonate, phosphate, succinate or another water soluble electrophilic group capable of hydrogen bonding;  $R_2$  is  $C_6H_4OH$ ,  $C_6H_4N_3$ ,  $C_6H_4CN$ ,  $CH_3$ , 4-HO- $C_6H_4$ - $C_6H_4$ ,  $C_6H_4OPO_2OH$ ,  $C_6H_4OSO_2H$ ,  $C_6H_4NH_2$ ,  $C_6H_4NHMe_2$ ,  $C_6H_4OSO_2Me$ ,  $C_6H_4OCO(CH_2)_xCO_2H$ , or  $C_6H_5Cl$ ;  $X$  is  $C_6H_3$ -2, 4( $NO_2$ )<sub>2</sub>,  $C_6H_4$ -4( $NO_2$ ),  $C_6H_4$ -3( $NO_2$ ), or  $C_6H_3$ -2, 4( $NO_2$ )<sub>2</sub>;  $Y$  is H,  $(CH)_xCH_3$  ( $x=0-12$ ), -S- $CH_3$ , nitrile, amino, nitro, azido, succinate, or amide; and  $Z$  is H,  $(CH)_xCH_3$  ( $x=0-12$ ), -S- $CH_3$ , nitrile, amino, nitro, azido, succinate, or amide. In a particular example,  $R_1$  is H or OH,  $R_2$  is  $C_6H_4OH$  or  $C_6H_5$  and Ar is  $C_6H_3$ -2, 4( $NO_2$ )<sub>2</sub>.

Additional useful compounds are disclosed in WO 94/05276 (the '276 publication), which is incorporated herein by reference. Compounds disclosed in the '276 publication have now been found to have immunomodulatory and/or resonance modulating activity. Embodiments of such compounds have the formula:



wherein G represents - $CH_2$ -, -C(O)-, -N-, -, -O-, or -S-;  $n$  is 0 or 1;  $R^7$  and  $R^8$  independently are - $OR^5$ , - $SR^5$ , - $SO_2N(R^5)_2$ , - $SO_2CN$ , - $SO_2R^6$ , - $SO_2N_3$ ,  $N_3$ , -CN, - $N(R^5)C(O)R^6$ , - $R^6$ ; Ar comprises an aryl group substituted with at least one electron withdrawing group;

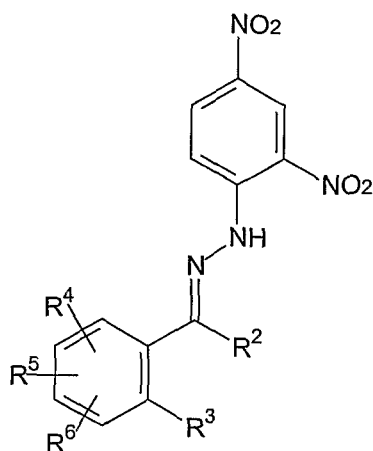
$X$  is NH,  $CH_2$ , O,  $SO_2$ , or B;

$Q$  is  $-(CH=CH)_m-CH-$ ;

$Y$  and  $Y'$  independently are selected from N, CH and B;

each  $R^5$  independently is H or lower alkyl; and  $R^6$  is lower alkyl.

Certain embodiments of compounds disclosed herein are illustrated by the following formula:



wherein R<sup>2</sup> is H, lower alkyl or optionally substituted phenyl; R<sup>3</sup> is H, or together with R<sup>2</sup> forms an optionally substituted heterocyclic or carbocyclic ring, which optionally is fused to an aryl group; R<sup>4</sup>, R<sup>5</sup> and R<sup>6</sup> independently are selected from H,  
 5 -OH and -OR<sup>7</sup>.

The structures and synthetic methods for many of the described compounds have been previously described in U.S. Patent No. 4,732,904, which is incorporated herein by reference. Some compounds that were not described in that patent include 10-[(2,4-dinitrophenyl)hydrazono]-1,2-dihydroxy-10H-anthracen-9-one (DNPA). The synthesis of this compound was performed by  
 10 dissolving 0.1 mole of 1,2-dihydroxy- 9,10-anthraquinone in 50 ml of ethanol and 5 ml sulfuric acid. Gently the solution was heated to 60°C with stirring and 0.1 mole of 2,4-dinitrophenylhydrazine in 50 ml of ethanol was added slowly with continued stirring. The solution was heated for 2-hrs and cooled in a refrigerator. Deep red crystals of DNPA appeared; m.p. 240-242°C; yield 92%. The  
 15 product analyzed for C<sub>20</sub>H<sub>12</sub>N<sub>4</sub>O<sub>7</sub> - found: C, 63.98; H, 3.46; N, 11.13; NMR and mass spectra agreement. Additional compounds suitable for use in the presently disclosed compositions and methods are disclosed in WO 2004/078174, published September 16, 2004. The compounds disclosed in this publication are incorporated herein by reference.

Additional examples of resonance modulators are provided in Example 13.

20

### Example 6

#### Formulations for Administration to Subjects

The agents can be provided in many forms, such as a crystal, pellet or gel/cream. The agent can be implanted, for example, subcutaneously or applied to an epithelial surface of the patient whose immune system maybe impaired, or who may have come in contact with a lethal or toxic material  
 25 capable of down-regulating their immune system. The agent can be incorporated into any form (such as a patch, or a two-dimensional or three-dimensional matrix) that brings it into electromagnetic contact with a target site. "Electromagnetic contact" refers to a sufficient proximity to exert its immune modulating effect as described herein. In some examples, the agent is applied to or over a lesion (such as a tumor or dysplastic epithelium) that is being treated. The agents can also be

chemically modified if desired, for example to form polymers. The agent is also useful to detect changes in immune function that are associated with cancer, in which there is a loss of auto-modulation of the immune system.

5 In one example, a pellet of A-007 was prepared by pressing 50 mg of the pure compound and sizing it to 16-gauge. Bulk A-007 was prepared using GLP/GMP procedures, with no additives. Depending upon the dissolution properties of the 100% A-007 pellet, additives (stearic acid, povidone, etc) or other pharmaceutically acceptable carriers may be added to it. Pellets may be manufactured, for example, in 25 mg, 50 mg and 75 mg doses. Adjustments in pellet concentrations may be made according to the observed physical properties (such as dissolution rates) and animal  
10 toxicity. Therapeutically effective doses can be determined by known means, and doses to be administered can be varied depending on the condition being treated, or the severity of a disease.

The agents can also be provided in the form of a gel, such as a preparation of propylene glycol/methyl cellulose, for application to a target area (such as a portion of the body) in which it is desired to focus an immune response. The gels may be provided in a tube, such as 50 mg of the  
15 active agent suspended in the gel. These gels are particularly convenient for application to the skin or other epithelial surface (for example skin of the chest wall, back, skin of the foot, face or anogenital area, or cervical mucosa). In some embodiments, the gel is dispensed from an elongated applicator tube (such as a rectal or vaginal applicator), for example to apply it rectally or intravaginally.

Alternatively, the pellet can be inserted into a target region, such as the chest wall. In other  
20 examples, the agent is suspended in tetrahydrofuran (THF) or another non-toxic aerosol, and introduced into the tracheobronchial or oropharyngeal area to treat cancer of the oral cavity or upper respiratory tract.

It has been found that the pellet stimulates the deposition of uric acid crystals, which can result in painful nodules. Application to the surface of the skin is therefore more preferred than  
25 insertion into the skin, but either approach will produce the immune modulation described herein.

### Example 7

#### External Monitors of Immune Activity

As previously noted, the described agents can both monitor and/or up-regulate immune  
30 profiles in the presence of emergent behavioral alterations that are associated with pathological conditions.

In its simplest form, the monitor is a device for detecting changes in electromagnetic properties of the agent, such as a resonance modulator, as it interacts with a biological system. The interaction can be observed either *in vitro* or *in vivo*. Hence the monitor can be applied to a subject,  
35 or to biological samples taken from the subject (such as a tissue culture), for further analysis.

The resonance modulating agent (A-007) is present on the skin of a patient with lymphoma, as a 0.25% propylene glycol/methyl cellulose gel (0.5 g). Although shown as a gel, it could also be applied as a cream or simple crystals (50 mg) or a pellet (50 - 100 mg) that can be applied on to or

inserted into the epithelial surface or the skin (or other surface) with a 16-gauge trochar needle. In one embodiment, the agent is applied to the skin as an orange gel, and covered with a 2 x 3 Tegaderm® patch with two spaced apart attached skin surface electrode tabs of the type used as electrocardiogram leads. The electrodes are positioned on either side of the gel, in a position that  
5 allows them to measure a voltage potential across the gel. The two probes of a voltage meter are then contacted, one probe to each electrode tab. The voltage meter preferably is associated with an oscilloscope that measures changes in voltage over time, so that the amplitude of the detected voltage over time can be seen. In particular embodiments, the voltage meter is a Fluke® Scopemeter 192/196/199 having software that permits the voltage waveforms to be filtered to remove background  
10 voltages, and to be viewed, recorded, measured and analyzed on a computer. Changes in the amplitude of voltage over time are then observed to monitor immune response to the resonance modulator.

After establishing a baseline amplitude and/or frequency at the time the resonance modulator is applied to the skin, the electrodes are then subsequently attached to the oscilloscope to monitor  
15 amplitude and frequency of electrical transmission from the test site. For a resonance modulator such as A-007, the amplitude of the voltage changes over time will increase.

After the patch was in place seven days on a normal subject, the tips of the voltmeter probes were again attached to the electrode leads, and the changes in voltage over time were recorded. A measurement was made of the electrical activity associated with agglutination and increased mitotic  
20 activity brought about by migration of activated dendritic cells into the target area of the patch. The amplitude of the waveform (as measured from zero) increased by about 25%, from 3 volts to 4 volts.

Although this example has illustrated measurement of changes in electrical activity with a voltmeter, electrodes could also be sensed with an external laser beam sensor that detects changes in local chemical - cellular interactions. Since the chemical is capable of conducting electrical energy  
25 via resonance, the chemical will provide signals of stages of cellular interaction associations. For example, a molecular scanner such as a laser could be used to excite the chemical or assay at its absorption wavelength, which is 404 nm for A-007. One such molecular scanner is an Edmund Industrial Optics solid state tunable laser (wave length 425 nm) that is programmed to record or generate specific excitations associated with A-007 resonance and cellular interactions. These high-  
30 performance lasers may be programmed to recognize A-007 spectral characteristics on a nano scale much like a bar code scanner.

### Example 8

#### Enhancement of Immune Function Modulation

35 The disclosed compounds also provide a relatively simple method to enhance immune function, for example in subjects suffering from impaired immunity. Such subjects may, for example, have an immunodeficiency disease (such as HIV/AIDS) or a toxin induced immune defect (such as leucopenia induced by an antineoplastic chemotherapy drug or an environmental toxin).

Other subjects may have an impaired immunity of the kind often seen in subjects with malignant tumors. Alternatively, the subject may have objective laboratory evidence of impaired immunity, regardless of whether the ultimate cause of the impaired immunity is known. The compound can act as a coupling agent or interface with the immune system that allows the immune system to be not only monitored, but also therapeutically manipulated.

As already shown, a targeted immune response can be directed to a target region (such as the vicinity of a tumor) by introducing the agent into or adjacent the target area. Exposing the resonance modulator to an electromagnetic field that stimulates the electromagnetic properties of the agent then stimulates the resonance modulator. For example, the agent can be exposed to an external magnetic or electrical field. One example of such an electromagnetic field is one that signals or tunes the frequency that would provide the highest concentration of a resonant form of the resonance modulator (such as one of the forms of A-007 shown in FIG. 3) to provide maximum interface with cellular receptor surfaces. In this example, a laser would be chosen to apply an electromagnetic field in the form of laser energy that possesses similar wave lengths (400-450) to those demonstrated by A-007. Although A-007 does not have lasing properties, a programmed laser could recognize its spectra characteristics. A simple magnetic field generated by 2 AA or 3 V batteries could non-specifically activate a surface gel of A-007 or 9 V for a subcutaneous pellet. A more specific tunable laser would be able to scan the area and be more specific about the optimal electromagnetic field to apply. Selecting a desired frequency will increase the amplitude of the voltage changes over time seen in the resonance modulator, and in turn enhance or activate an immune response.

To treat a tumor that is present in both the dermis and epidermis a conjugated aromatic compound is applied to the surface of the skin over a target area that contains the tumor. Although the compound will attract dendritic cells and lymphocytes into the target area, tumors can often evade normal immune surveillance, and it is helpful to further stimulate the immune response by heightening activation of the resonance modulator. A magnetic probe positioned over the area where the compound has been applied to the skin can provide such heightened activation. The magnetic probe is connected to a device that includes an oscillator circuit, a frequency counter, a voltage supply, and an oscilloscope. The device is activated to apply a magnetic pulse to the compound to enhance its resonance modulating properties.

An example of a suitable magnetic pulse generator is The Magnetic Pulser, which is a High Intensity Momentary Time-Variant Pulsed DC Magnetic Field Therapy Generator MODEL #: MPG5 available from Health Canada. The Magnetic Pulser (MPG5) is designed to generate an intense (~43,133 Gauss), momentary (~2.5mS) pulsed DC magnetic field that can be used to stimulate the resonance modulator compound. The magnetic pulse can be applied for a sustained period of time, sufficient to enhance the activity of the compound. For example, the pulsed DC magnetic field could be applied for 5-60 minutes or longer.

A simple magnetic field generated by 2 AA or 3 V batteries would activate a surface gel of A-007, or a 9 V battery would activate a subcutaneous pellet. A more specific tunable laser would be

able to scan the area (such as an area to which the compound has been topically applied) to provide a more specific indication of the electromagnetic field that is to be applied. Selecting a desired frequency of the field applied would maximize the amplitude of the energy produced by the resonance modulator. The duration of time for which the electromagnetic field is applied will depend  
5 on the subject's immune status; longer periods of stimulation would be helpful for subject's having a poorer immune status. Although a general magnetic field may be used, a preferred approach is to use a wavelength or energy specific tunable transmitter for wide regions of treatment. The effect of the treatment can be assessed by monitoring immune status with easily available modalities, such as peripheral T-cell flow cytometry analysis. These readily available and convenient tests provide  
10 additional guidance in the selection of characteristics of an applied electromagnetic field for a particular resonance modulator.

In one example, the applied electromagnetic stimulus is an electric field of the type applied in U.S. Patent No. 6,190,893, which is incorporated by reference. The device for electrical stimulation includes a gold wire cathode extending along one edge of the target area, and a silver wire  
15 anode positioned along an opposing edge of the target area. An EG&G Princeton Applied Research Potentiostat/Galvostat Model 263A (Oakridge, TN) is used as the source of constant potential. The electrical stimulus can be applied for a period of at least one hour, at a steady potential of 100 mV.

Many other devices are available for applying the electromagnetic field to the resonance modulator in a human body or a tissue culture. For example, PCT Publication WO 02/102457, which  
20 is incorporated herein by reference, discloses such an apparatus that includes a transducer and a generator to apply an AC signal to the transducer, such that an electromagnetic field is generated with a basic frequency between 0.1 Hz and 4000 Hz. A similar device is also shown in U.S. Patent No. 5,968,527, which is incorporated by reference.

In some situations it is desirable to use a bioimpedance device to both provide a stimulus to  
25 the resonance modulator, and monitor the electrical properties of the target area to which the resonance modulator has been applied. Direct bioimpedance measuring systems use a current generator to generate a continuous, constant amplitude and frequency current through a human or animal body segment, for the purpose of measuring tissue conductance. Frequencies in the range of 30KHz-30 MHz have typically been used. Impedance to the continuous current flow in the body  
30 segment generates a voltage difference across the body segment, and a bioimpedance meter measures the impedance in the body segment. Examples of bioimpedance devices are shown in WO 01/76475; U.S. Patent No. 4,805,621; and U.S. Patent No. 5,529,072. The bioimpedance probe is placed over the target area to which the resonance modulator has been applied, and the voltage difference is introduced across the target area. Changes in bioimpedance are also used to monitor the status of the  
35 subject's immune system. It is believed that an increased bioimpedance would be seen as immune status improves.

### Example 9

#### Use of the Disclosed Compounds in Treatment of Lymphoma

An A-007 0.25% gel (0.5 g) was applied to the skin of a 42 year old patient with lymphoma, as described in Example 7, and there was improvement in the patient's peripheral lymphocyte profile (as measured by a total lymphocyte count) as well as an increase in amplitude of the sigma waves, indicating that there was increased activity in the skin associated with A-007. The sensing was conducted with a Fluke® ScopeMeter oscilloscope and a PC program (192/196/199) capable of recording and sorting environmental background noise. The 25% increase in voltage amplitude was considered a positive modulation of immunity in this particular example, which was consistent with the improvement in the lymphocyte profile.

### Example 10

#### Use of the Compounds in Treatment of Cervical Cancer

A 23 year female with early cervical cancer-in-situ who applied the A-007 0.25% gel (2 g) daily for 5-days experienced a dramatic cure that has lasted for over one year. The cancer cells in the epidermis disappeared, resulting in a normal appearing epithelium with influxes of CD45+ T-lymphocytes. The responses are associated with increased organization of T-lymphocyte patterns and disappearance of cancer cells.

### Example 11

#### Protein Tyrosine Phosphatases

Human protein tyrosine phosphatases (PTPs) are a large and diverse family of proteins present in all eukaryotes. Each PTP is composed of at least one conserved domain characterized by an 11-residue sequence motif containing cysteine and arginine residues, the latter are known to be essential for catalytic activities. The sequences of PTP share no similarity to serine or threonine, acid or alkaline phosphatases. The diversity in structure within the PTP family results primarily from the variety of non-catalytic sequences attached to the NH<sub>2</sub>- or COOH- termini of the catalytic domain. There are numerous PTPs involved in intracellular phosphate metabolism and domains. The diversity of the extra cellular segments presumably reflects the variety of ligands to which the PTPs are exposed and catalyze phosphate transfer.

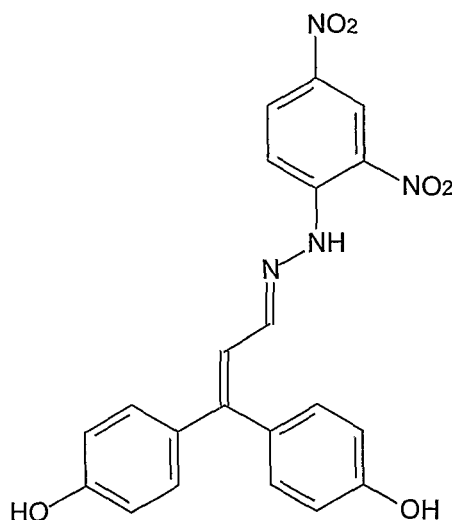
The extracellular-PTPs are one class of PTP receptors that are related to surface recognition and adhesion molecules of leukocyte cell surface recognition. The PTPs are not only associated with human cells, but also present in prokaryotes and viruses, and bacteria. In the pathogenic bacterium *Yersinia*, the causative agent of bubonic plague, the Yop2b tyrosine-specific PTP is an essential virulence determinant.

Numerous studies have demonstrated the importance of PTPs in physiological processes. Phenotypic defects and hyperproliferative behavior of T-and B-lymphocytes, granulocytes and macrophages are considered to be key issues in the development of cancer and autoimmune diseases.

The catalytic domain for the PTPs has been described in crystallographic studies, as reviewed in Z. Jia, et al., Structural Basis for Phosphotyrosine Peptide Recognition by Protein Tyrosine Phosphatase 1B, *Science* **268**: 1754-1758, 1995. This reference and Z. Xu et al., Negative regulation of CD45 by differential homodimerization of the alternatively spliced isoforms, *Nature Immunology* **3**:764-771, 2002, disclose the various types of cells, bacteria and viruses that express PTPs. The present example reviews the interactions and results for aryl hydrazones as PTP modulators.

A review of 4,4'-dihydroxybenzophenone-2,4-dinitrophenylhydrazone (A-007) and its structure property relationship (FIGS. 1 and 3) reveals that it does not satisfy Lipinski's "Rule of 5". The "Rule of 5" predicts that for absorption or permeation, a drug is more likely to have less than five hydrogen-bond donors, less than ten hydrogen bond receptors, the molecular weight is less than 500 and/or the calculated log P is less than 5 (CACH Group, Fujitsu, Beaverton, OR). Obviously A-007 and the other hydrazones have greater than 5 hydrogen bond donors as seen by crystallography (Klein, C.L., Gray, D., and Stevens, E.D., Crystal and molecular structures of benzophenone phenylhydrazone derivatives with anticancer activity, *Structural Chemistry* **4**: 377-383, 1993). Thus the external binding to lymphocytes and cancer cells that are described in this patent is in agreement with structural-behavioral observations of the A-007 type aryl hydrazones, in that the drug is substantially not absorbed through the skin to which it is applied. Its effects are therefore achieved over a distance, as would be expected from an electromagnetic effect.

As illustrated by FIG. 1, A-007 fits well into extracellular catalytic receptors of CD45+ PTP subtype. Moreover, other compounds disclosed herein, including



also fit well with these receptors.

Cervical cancer cells (that had been transformed through HPV-induced mutations) undergo cell death in the presence of crystals of A-007; supporting A-007's ability, through its resonance to catalyze HS – SH dimerization of cysteine residues up-grading the receptor and death. This has been verified in patients with cervical cancer (Table 2). A topical gel with 0.25% of A-007 was administered intravaginally in a dose of 2 grams daily for 5 days. A-007 induced cell death was not observed with benign fibroblasts or healthy epithelial cells that did not contain or had not been

transformed by HPV. Topical application of the compounds, including resonance modulators such as A-007 to cervical/vaginal epithelial membranes in subjects infected with HPV results in elimination of cancer cells, as well as the elimination of virus particles, and an up-regulation of CD45RO+CD4+ and CD8+ T-lymphocytes. The ultimate therapeutic effect of the topical application of A-007 was induction of cell death in HIV infected cells (Table 2 and FIGS 10 and 13). As can be seen in Table 2, A-007 increased the presence of T-lymphocytes, and particularly CD45+, CD123+, CD4+ and CD8+ cells. In particular examples, an increase in CD45+, CD4+ and CD8+ cells was particularly noted. In particular, increases of CD45RO+, CD45RA+ and CD45RB+ were noted.

10 **Table 2. Effects of A-007 (0.25% Gel) Applied Intravaginally to Humans with Cancer\***

| Patient/<br>Histology      | HPV**<br>(Response)                                       | Response***<br>(Cancer) | T-Cell Profile*<br>(Tissue/blood)                              | Toxicity | Amount<br>A-007<br>Applied<br>(mg) |
|----------------------------|-----------------------------------------------------------|-------------------------|----------------------------------------------------------------|----------|------------------------------------|
| Vaginal<br>Cancer<br>(V-1) | No virus                                                  | >75%<br>4 mos           | CD45+(↑100%)<br>CD8+ (↑25%)                                    | None     | 2.5                                |
| Vaginal<br>Cancer<br>(V-2) | + (CR)                                                    | CR<br>3.6 yrs           | CD45+ NA<br>CD4+ (↑ 20%)<br>CD8+ (↑ 25%)                       | None     | 2.5                                |
| CIN II<br>(V-3)            | + (CR)<br>3.6 yrs                                         | CR<br>3.6 yrs           | CD45 CD4+ (↑ 38%)<br>CD8+ (↑ 12%)                              | None     | 2.5                                |
| CA In<br>Situ<br>(V-7)     | + (CR)<br>3.1 yrs                                         | CR<br>3.1 yrs           | CD45RO+ (↑ 50%)<br>CD4+ (↑ 40%)                                | None     | 2.5                                |
| CIN I<br>(V-8)             | + (CR)<br>3 yrs                                           | CR<br>3 yrs             | CD45RO+ (↑ 50%)<br>CD8+ (↑ 50%)                                | None     | 5                                  |
| CIN I<br>(V-9)             | +<br>Relocated<br>(verbal<br>report -<br>NED)<br>2.7+ yrs | + (CR)<br>2.7 yrs       | CD45RO+ (↑ 80%)<br>CD4+ (↑ 15%)<br>CD8+ (↑ 20%)                | None     | 2.5                                |
| CIN II<br>(V-11)           | +<br>HR → LR<br>1.6 yr                                    | CR<br>2.5 yrs           | CD45+ (↑20%)<br>CD45RB+ (↑53%)<br>CD123+ (↑21%)<br>CD8+ (↑28%) | None     | 2.5                                |
| CA/CIN<br>II<br>(V-12)     | +<br>HR → LR<br>2 yrs                                     | CR<br>1.6 yrs           | CD45RO+ (↑ 67%)<br>CD4+ (↑ 55%)<br>CD8+ (↑ 36%)                | None     | 5                                  |

| Patient/<br>Histology        | HPV**<br>(Response)     | Response***<br>(Cancer) | T-Cell Profile*<br>(Tissue/blood)                 | Toxicity | Amount<br>A-007<br>Applied<br>(mg) |
|------------------------------|-------------------------|-------------------------|---------------------------------------------------|----------|------------------------------------|
| Cervical<br>Cancer<br>(V-13) | +<br>HR → LR<br>2.2 yrs | CR<br>2.2 yrs           | CD45RB+ (↑ 72%)<br>CD45RA+ (↑ 14%)<br>CD4+ (↑ 9%) | None     | 2.5                                |
| CIN<br>II/warts<br>(V-14)    | + (CR)<br>1.6 yrs       | CR<br>1.6 yrs           | CD45+ (↑ 45%)<br>CD45RA+ (↑ 50%)<br>CD8+ (↑ 60%)  | None     | 5                                  |
| CIN<br>I/VAIN I<br>(V-15)    | + (CR)<br>1.6 yrs       | CR<br>1.6 yrs           | CD45+ (↑ 30%)<br>CD4+ (↑ 10%)<br>CD8+ (↑ 10%)     | None     | 5                                  |
| CIN<br>II/warts<br>(V-16)    | + (CR)<br>1.5 yrs       | CR<br>1.5 yrs           | CD45+ (↑ 15%)<br>CD45RO+ (↑ 31%)<br>CD8/4+ NC     | None     | 2.5                                |
| CIN I<br>(V-17)              | + (CR)<br>1.2 yrs       | CR<br>1.2 yrs           | CD45+ (↑ 16%)<br>CD8+ (↑ 30%)                     | None     | 2.5                                |

\*The gel (0.25%) was administered intravaginal (2 g daily for five days; could be repeated every 3 - 6 weeks).

\*\*Presence & changes in HPV titers. Note: patients V-4 to V-6 and V-10 were evaluated but not treated. CR – complete response. \*\*\*Response and duration from first response date. NA – not available. NC – no change.

5 +Changes that occurred with local treatment. Two pathologists reviewed the IHC results, independently. No change in CD11c noted. NED – no evidence of disease.

10 Although shown as a gel in this example, the disclosed compounds could also be applied as a cream or simple crystals (50 mg) or a pellet (50 - 100 mg) that can be applied on to or inserted into the epithelial surface or the skin (or other surface) with a 16-gauge trochar needle for short-term treatments.

15 HeLa cancer cells (HPV+) undergo necrobiosis and death when incubated with crystals of A-007 for 24 hours. Clear healthy cancer cells migrate to the crystal. Then through cell membrane chemical interphase and modulation of the PTP receptors the cells down regulate, darken with pyknotic changes associated with DNA agglutination resulting in death (lower clumped dark staining cells). The attraction that the crystals have for the cells is associated with intramolecular resonance and associated magnetic patterns.

Naive T-lymphocytes are also activated in the presence of A-007 with agglutination of activated lymphocytes+.

20 Thus, A-007 and other disclosed compounds, such as the described hydrazones, can play a unique role in not only sensitizing HPV positive epithelial cells to attack by activated T-cells (Table 2) but also has direct effects on HPV positive cells and A-007 resulting in cell and virus death (Table 2). In the latter Table patients were no longer infected with virus after treatments, which illustrates

that the agent has anti-viral properties as well. Thus, A-007 has both up-regulation of CD45+ activities in T-lymphocytes, as well as extracellular membrane deregulation and elimination of cells infected with HIV.

These findings suggest that both HPV infected cervical epithelial cells and T-lymphocytes contain extracellular membrane bound PTPs with which the disclosed agents, including A-007 can interact. The HPV virus may contain an active extracellular PTP receptor, which when exposed to the compounds disclosed herein can undergo a deregulation and death. Regardless of the actual mechanism, the disclosed compounds have been found to have effective anti-viral activity against HPV. Moreover, it is believed that T-lymphocytes are activated by a resonance modulator, such as A-007, to promote the presence of surface membrane CD45+RO PTPs, which is believed to occur through HS – SH formation within the catalytic receptor site. CD45+RA T-lymphocytes represent resting or naive cells that are not capable of attacking foreign cells, hence the conversion to CD45+RO markers represents an activation of the cytotoxic arm of the cellular immune system. As Table 2 illustrates, the resonance modulator had substantial anti-viral (anti-HPV) and anti-neoplastic activity. The proportion of CD45+RO cells increases substantially after treatment with the agent.

Cells that express PTPs, such as *Yersinia pestis* pathogens, or PTP+ cells that are infected with pathogens (such as HPV infected epithelial cells) can also agglutinate with A-007. This agglutination is believed to occur by oxidation of cysteine and disulfide bond formation. This bond formation disrupts cell integrity and cell death - releasing virus. In the presence of A-007, viral PTP cell membrane auto-oxidation occurs with viral inactivation. Most of the patients in Table 1 also possessed HPV+ hyperplastic cervical cells containing HPV induced intranuclear changes (CIN) that cleared with A-007 therapy. This further emphasizes that the disclosed compounds, including resonance modulators such as A-007, have the ability to destroy benign and hyperplastic cells infected with HPV, as well as malignant cells.

Anticancer and antiviral activities for hydrazone analogs, such as 2,6-dibenzylidenecyclohexaone-2, 4-dinitrophenyl hydrazone, which has improved binding affinities and up-regulation for the PTP CD45 receptor is also potentiated through exposure to ultraviolet light. Ultraviolet light induces Diels-Alder reactions between A-007 and arginine's diimine moiety and adduct formation with cysteine's HS- moiety resulting in up-regulation of the CD45 receptor (FIG. 1). Hence the immune modulating, anti-viral and anti-tumor activities of such resonance modulators can be enhanced by exposing the compound to ultraviolet light.

#### Example 12

##### Anti-neoplastic and Immune Modulating Characteristics of 4,4'-Dihydroxybenzophenone-2,4-dinitrophenylhydrazone (A-007)

4,4'-Dihydroxybenzophenone-2,4-dinitrophenylhydrazone (A-007, compound 1 in Figure 12), has produced significant anticancer activities in patients with anal squamous cell cancer (Table

3). A topical gel containing 0.25% of A-007 (2 grams per day of gel) was applied intra-anally daily for five days, and the observed results are shown in Table 3.

**Table 3. Effects of A-007 (0.25% Gel) Applied Anal to Cancer\***

| Patient/<br>Histology**        | HPV***<br>(Response) | Response<br>(Cancer) | T-Cell Profile<br>(Tissue)                                                    | Toxicity | Amount of<br>A-007 Applied<br>(mg) |
|--------------------------------|----------------------|----------------------|-------------------------------------------------------------------------------|----------|------------------------------------|
| Anal Cancer (A-1) <sup>+</sup> | +<br>CR – 1.9 yr     | 100%<br>2.6 yrs+     | CD45+ (↑10%)<br>CD45RO+ (↑25%)<br>CD8+ (↑10%)                                 | None     | 2.5                                |
| Anal Cancer (A-2) <sup>+</sup> | None<br>Detected     | 100%<br>2.0 yrs+     | D45RA+ (↑63%)<br>CD45RB+ (↑23%)<br>CD4+ (↑13%)<br>CD8+ (↑2%)<br>CD11C+ (↑15%) | None     | 2.5                                |
| Anal Cancer (A-3) <sup>+</sup> | None<br>Detected     | 100%<br>1.6 yr +     | CD45+ (NC)<br>All others NC                                                   | None     | 2.5                                |
| Anal Cancer (A-4)              | None<br>Detected     | NC/stable<br>1.5 yr+ | CD45ROCD8+ (↑<br>30%)<br>CD45RA+ (↑ 11%)                                      | None     | 5.0                                |
| Anal Cancer (A-5)              | + HIV/HPV<br>(NR)    | Stable x 6<br>wk     | CD45RA+ (↓10%)<br>CD45RB+ (↑20%)<br>CD4/8 NC                                  | None     | 5.0                                |

5 \*The gel (0.25%) was administered topical to anal area (daily for five days; could be repeated every 3-6 weeks); \*\*Histology – squamous cells or adenocarcinoma; \*\*\*Response and duration from first response date. \*\*\*Changes in HPV titers; NC – no change; <sup>+</sup>Changes that occurred with treatment. <sup>+</sup>Pts underwent surgery post 5-day therapy.

10

### Example 13

#### Further Examples of Disclosed Compounds

2,6-Dibenzylidenecyclohexanone-2, 4-dinitrophenylhydrazone (BDP-DNP) has 100-fold greater immunomodulating anticancer activities relative to A-007 as demonstrated by the impact of BDP-DNP on cell growth for malignant squamous cell cancer (SCCA-HM) growing in culture (pre-  
15 post Rx). After 24 hours incubation with BDP-DNP (0.4 mcg/mL) in tissue culture media (L. R. Morgan, US Patent. 5,270,172) the lymphocytes underwent an impressive stimulation (large white clumps of activated T-lymphocytes) and destruction of the cancer cells. No cancer cells are detected.

#### Example 14

##### Characteristics of Some Active Compounds

As disclosed in the preceding examples, compounds disclosed herein provide both a diagnostic and therapeutic interface with the immune system, and which independently have anti-tumor and anti-viral activities (particularly against HPV and HPV infected cells, such as vaginal and cervical cancers associated with HPV infection.

Compounds have been disclosed that have one or more of the following characteristics, for example at least three or five or all of the following characteristics:

Specific attraction of CD45+ lymphocytes (for example CD45RO+ and CD45RA+ lymphocytes) to the resonance modulator compound, either in culture or when applied to a surface (such as the skin) of a living subject.

Upregulation of CD45RA+ to CD45RO+ lymphocytes, for example when applied to an epithelium of a subject.

Possession of inherent electromagnetic properties, for example the ability to form a single line of agglutinated crystals between positively and negatively charged points when the crystals are evaporated, or the emission of electromagnetic energy.

Possession of properties that affect lymphocyte migration, for example inducing aggregation of naive lymphocytes in culture.

Certain examples of the compounds are also characterized by an ability to increase the amplitude of alternating voltages waves measured from the compound after it is applied to the skin of an individual. In certain examples, the increased amplitude is reduced in subjects having impaired immunity, such as impaired lymphocyte function.

In one embodiment, substantially no absorption through the skin is observed when the compound is applied to the skin. In one embodiment, such compounds do not satisfy Lipinski's "Rule of 5." When applied to the skin such compounds may substantially completely remain on the surface of the skin, without transdermal flux.

Potential resonance modulators can also be selected based on chemical structures that suggest resonance modulation, namely the structural characteristics described earlier in this specification. For example, polyaryl compounds with electronegativity can be selected, such as Casodex®, Napreelan®, Eulexin®, and Bextra®.

Once candidates have been selected as potential resonance modulators, they can be easily assayed to determine whether they induce lymphocyte blastogenesis and activate lymphocytes. Such an assay is found in Morgan et al., *Anticancer Research* 13:1763-1768 (1993), which is incorporated by reference. See also Janossy et al., *Clin. Exp. Immunol.* 14:581-596 (1973). Briefly, uptake of [<sup>3</sup>H]thymidine was measured for lymphocytes in culture. Lymphocytes were cultured in RPMI-1640 tissue culture medium supplemented with 10% fetal bovine serum (FBS). Following stimulation with the resonance modulator, tritiated thymidine (2-20 µCi/ml, specific activity 2 Ci/mmol) was added to the cells and incubated with them. The uptake of [<sup>3</sup>H]thymidine was stopped by the addition of cold

10% trichloroacetic acid after a selected period of time, and the radioactivity of the samples measured in a scintillation counter. The % lymphoblast was determined by counting the number of blasts per high powered field (hpf) while % activation was determined by uptake of the tritiated thymidine. The increase voltage was measured with an oscilloscope as in Example 7.

5

**Table 4**  
**Prediction of Resonance Modulating Properties**

| <i>Drug/Compound</i> | <i>% Lymphoblast*</i> | <i>% Activation*</i> | <i>Increased voltage**</i> |
|----------------------|-----------------------|----------------------|----------------------------|
| A-007                | 75%                   | 100%                 | 54%                        |
| Compound 38          | 100%                  | 100%                 | 69%                        |
| DNPA (in example 5)  | 50%                   | 75%                  | 22%                        |
| Fludamide ( Eulexin) | 0%                    | 0%                   | 5%                         |

10 \*The procedure described in Fan, Morgan, et al, (Adoptive Immunotherapy of Advanced Renal Cell Cancer using PHA-stimulated Autologous Lymphocytes, Anticancer Research 16, 230-239, 1993) was used to quantitate the lymphoblasts and lymphocyte activation. This paper is incorporated herein by reference.

\*\*Measured the increased amplitude with an oscilloscope.

15 This procedure illustrates a correlation between increased amplitude of voltage signals with enhanced activation of lymphocytes. In particular, greater increases in amplitude were observed with more vigorous enhancement of lymphocyte activation. This assay can be used to quickly screen resonance modulating agent candidates for immune activating activity. For example, using this assay, an increased voltage amplitude of at least 20% (for example an increased amplitude of at least 50%) can be used as a measure of resonance modulation activity on the immune system. Resonance modulation candidates satisfying such criteria (for example an increase in amplitude of at least 20%) are then selected for further use and study.

### Example 15

#### Topical Preparations

25 The compounds disclosed herein can be prepared as topical preparations for application to the skin as immunomodulators, anti-viral agents, or anti-neoplastic preparations.

In one example, the compound comprises 2,6-Dibenzylidenecyclohexanone-2, 4-dinitrophenylhydrazone (BDP-DNP). It is placed in a topical preparation for application to an epithelial surface, for example by application to malignant epithelium, such as a urogenital neoplasm, such as an anal, vaginal or cervical neoplasm, such as cervical CIN.

30

The compound can be used in methods of treating a tumor (neoplasm) by administering to an affected subject a therapeutically effective amount of the agent to induce regression or elimination of the tumor cells. In particular examples, administering the BDP-DMP comprises applying the BDP-DNP topically to the tumor, although it can also include other forms of administration, such as oral, inhalational, injected or subcutaneous administration.

#### Example 16

##### Inhalational Preparations

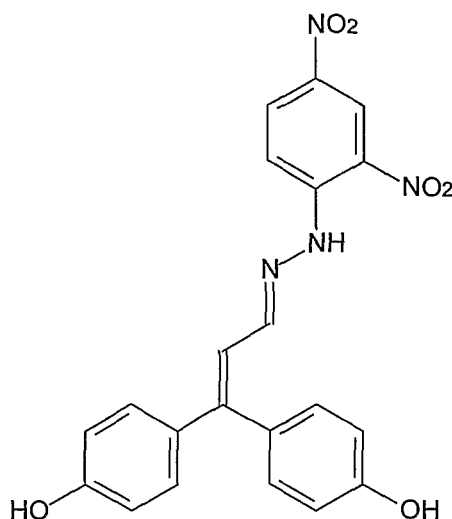
The compound (in this example A-007) in tetrahydrofuran (THF) in 5% solution was aerosoled into the respiratory system of mice under 15 psi of pressure for 30 sec. By releasing the aerosol into a closed chamber in which the mice were present. The treatment resulted in no deaths among the treated mice. Five days later the mice were sacrificed and the pulmonary system was examined by resecting the trachea and lungs en block. Histological examination of the specimens revealed that the bronchial epithelium had increased lymphocyte infiltration (CD8+ and CD4+). Identical patterns of T-cell modulation were seen as with topical applications to the cervix and skin. Controls were treatment with the THF alone with no increases in lymphocyte infiltration observed.

#### Example 17

##### Active Agent-Receptor Interactions

This example describes modeling studies used to evaluate the binding of compounds disclosed herein with receptors, such as CD45. These studies were conducted with Sybyl 6.9.2 (Tripos Inc, St Louis, MO). The models of the compounds were made from Sybyl fragments and then optimized using the MMFF94 force field (with MMFF94 charges). These models were then docked into the 1BZC crystal structure active site using FlexX 1.13 and the Drug Score scoring function. FlexX does an incremental fragment based docking where various base fragments of the ligand are docked and then the rest of the ligand is built up from this base fragment placement. Thus, all possible orientations and conformations of the ligand are sampled.

Figure 19 illustrates the docking of the compound



with CD45 according to this method. In these studies, the illustrated compound has a better binding score than A-007 largely because it is held tighter by ARG 47 at the entrance to the pocket with close contacts to the dihydroxybiphenyl moiety of the compound illustrated above.

- 5           The illustrated compound has also been found to aggregate where it binds to CD45 and exert a direct cytotoxic activity.

### Example 18

#### General procedure for preparation of hydrazones

- 10           Method 1: Substituted phenylhydrazines (0.148 mol) were suspended in MeOH (300 mL) at 50°C and concentrated sulfuric acid (20 mL) added with stirring at 50°C. After the hydrazine dissolved, the substituted benzophenone or aryl ketone (0.1 mol) in MeOH (300 mL) was added with stirring at 50°C over 10 minutes and then stirred for another 30 min. The solvent was evaporated (75%), water (500 mL) added, and the precipitate washed with 3% aqueous NaHCO<sub>3</sub>. Recrystallizations were from EtOH,  
15           MeOH, or acetic acid. Complete elemental and spectral analysis were conducted on all products.

- Method 2: A second general method proceeds as follows: Substituted aryl hydrazines (2.7 mmol) were dissolved in 15 mL MeOH and 2.1 mmol of the ketone added with stirring. Dowex 50W-50X2-100 cation exchange resin (0.5 g) [Dow Corp., Baton Rouge, LA] was added. The suspension was refluxed for 1 h at 75°C on a water bath. The mixture was filtered hot under water vacuum  
20           pressure and the resin washed with 3 x 0.5 mL of MeOH. The contents of the collection flask were allowed to cool to room temperature and 20 mL of distilled water added. The colored precipitates were filtered, dried and recrystallized from ETOH or MeOH. Representative yields were in the 80% range.

- N*-Benzhydrylidene-*N'*-(2,4-dinitrophenyl)hydrazine was prepared using method 1, yielding yellow  
25           crystals in 82% recovery from EtOH or MeOH; mp 232-234°C; IR: 3295(N-H), 2928(Ar-H), 1612(C=N), 1590, 1504, 1416, 1334, 1127 and 1091, 834, 777, 698 and 608 cm<sup>-1</sup>; <sup>1</sup>H-NMR(DMSO, 400 MHz): δ 11.25(1 H, s, N-H), 9.07(1 H, s), 8.37 (1 H, d, *J* = 8 Hz), 8.22(1 H, d, *J* = 8 Hz), 7.67 (4 H, m, *J* = 8 Hz) 7.41 (6 H, m, *J* = 8 Hz); Anal. Calcd for C<sub>19</sub>H<sub>14</sub>N<sub>4</sub>O<sub>4</sub>: C, 62.98; H, 3.86; N, 15.46. Found: C, 63.17; H, 4.06; N, 15.49.

4-Hydroxybenzophenone-2,4-dinitrophenylhydrazone was prepared using method 2, and was obtained as red needles that recrystallized from ETOH - yield 80%; mp 224-225°C; IR: 3541(N-H), 3271(N-H), 3098(O-H), 2905(Ar-H), 1621(C=N), 1591, 1511, 1418, 1329, 1276, 1130, 1083, 829 and 698 cm<sup>-1</sup>; <sup>1</sup>H-NMR(DMSO, 400 MHz): δ 11.23(1/2 H, s, N-H), 10.99(1/2 H, s, N-H), 10.1(1 H, s, "broad" OH), 8.8(1 H, s), 8.41 (1 H, d, *J* = 8 Hz), 8.19(1 H, d, *J* = 8 Hz), 7.67 (2 H, m, *J* = 8 Hz), 7.48 (3 H, m, *J* = 8 Hz), 7.28(1 H, dd, *J* = 8 Hz), 7.04(1 H, dd, *J* = 8 Hz), 6.84(1 H, dd, *J* = 8 Hz); Anal. Calc'd. for C<sub>19</sub>H<sub>14</sub>N<sub>4</sub>O<sub>5</sub>: C, 59.07; H, 3.62. Found: C, 59.71, H, 3.84.

2,2',4,4'-Tetrahydroxybenzophenon-2,4-dinitrophenylhydrazone was synthesized as deep red crystals that recrystallized from EtOH - yield 75%; mp 290-291°C; IR: 3631(N-H), 3491(N-H), 3272(O-H), 2905(Ar-H), 1614(C=N), 1518, 1415, 1322, 1206, 1143, 1107, 974, 864 and 827 cm<sup>-1</sup>; <sup>1</sup>H-NMR(DMSO, 400 MHz): δ 11.59 (1 H, s, O-H), 11.15(1 H, s, N-H), 10.12(2 H, s, O-H), 9.91(1 H, s, O-H), 8.85 (1 H, s), 8.48 (1 H, d, *J* = 8 Hz), 8.19(1 H, d, *J* = 8 Hz), 7.61 (1 H, d, *J* = 8 Hz), 6.95 (1 H, dd, *J* = 8 Hz), 6.8(1 H, dd, *J* = 8 Hz), 6.57(1 H, s), 6.46(1 H, dd, *J* = 8 Hz), 6.38(1 H, s), 6.31(1 H, dd, *J* = 8 Hz); Anal. Calc'd. for C<sub>19</sub>H<sub>14</sub>N<sub>4</sub>O<sub>8</sub>: C, 53.52; H, 3.28; N, 13.14. Found: C, 51.79; H, 3.19; N, 13.36.

1,2-Dihydroxy-10-anthraquinone-2,4-dinitrophenylhydrazone was synthesized from the base quinone and 2,4-DNP using General Method 1. Yield was 92%. The reaction mixture was refluxed for 48 h. The compound precipitated from the reaction upon cooling and recrystallized from dioxane/hexane as red microcrystals; mp 240-242°C; IR: 3502(N-H), 3287(N-H), 3113(O-H), 2925(Ar-H), 2559(Ar-H), 1618(C=N), 1595, 1501, 1441, 1335, 1298, 1135, 1091 and 781 cm<sup>-1</sup>; <sup>1</sup>H-NMR(DMSO, 300 MHz): δ 11.97(1 H, s, N-H), 8.86(1 H, s), 8.47(1 H, d, *J* = 6), 8.38(1 H, s), 8.24 (1 H, d, *J* = 12 Hz), 8.17(2 H, m), 8.12 (2 H, m), 7.89 (4 H, m), 7.75 (3 H, m).

### Example 19

#### Stimulation of Platelet Levels

Another unexpected property of the disclosed compounds, such as resonance modulators, for example A-007, is their ability to increase platelet production in subjects who are administered the drugs. For example, subjects receiving A-007 have been found to have enlarged spleens and increased platelet production. This increase in platelet production is particularly advantageous for subjects suffering from thrombocytopenia, for example induced by immune or neoplastic disease or cytotoxic drugs used in the treatment of such diseases. For example A-007 or resonance modulators or other compounds disclosed herein that are capable of stimulating platelet production are administered to a subject in need of such stimulation. The drugs are administered in any manner described herein.

Mice that have received A-007 in accordance with the disclosed methods have been found to have enlarged spleens (splenomegaly) and elevated platelet production. The compounds disclosed herein are therefore capable of providing extramedullary megakaryocyte and platelet production (outside of the bone marrow).

**Example 20****Treatment of Body Surface Lesions**

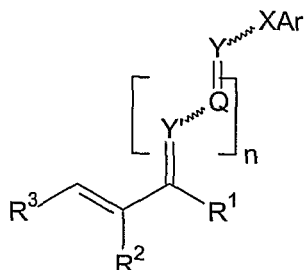
5 The agents disclosed herein can be used in the treatment of a broad variety of body surface lesions, such as warts, HSV blisters, psoriasis and skin cancers. These lesions are treated by applying the agent topically to the lesion for a sufficient period of time to induce disappearance or regression of the lesions. For example, the agent is applied to the surface of the lesion in a 0.25% gel, in a dose of 2 g per day, for 3-10 days. The treatment can be repeated as needed until resolution of the lesion has been achieved.

10 Other treatment regimens can be used. For example, a higher or lower dosage of the active ingredient can be used as clinically appropriate. Therapeutically effective amounts of the drug can be determined by clinicians depending on the clinical circumstances. For example, a total dose of 1-10 g, 2-5 g, or 2-3 g can be administered topically during a treatment regimen. Dosage regimens can be once a day (for example nightly), twice a day, or more often. The total duration of treatment can vary  
15 depending on the clinical circumstances (for example daily for 3-10 days, for example daily for 5 days), and the treatment regimens can be repeated at intervals as necessary. For example, treatment can be administered daily for 5 days followed by 25 days of no treatment followed by another course of daily treatment for 5 days.

20 Having illustrated and described the principles of the invention in several examples, it should be apparent to those skilled in the art that the invention can be modified in specific details without departing from such principles. I claim all modifications coming within the spirit and scope of the following claims.

I claim:

1. A compound other than A-007 of the formula



- 5 wherein Ar comprises an aryl group substituted with at least one electron withdrawing group;

X is NH, CH<sub>2</sub>, O, —, SO<sub>2</sub>, or B;

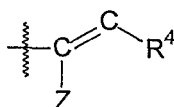
Q is —(CH=CH)<sub>m</sub>—CH—;

Y and Y' independently are selected from N, CH and B;

n is 0 or 1;

- 10 m is from 0 to 6;

R<sup>1</sup> is H, lower alkyl, optionally substituted phenyl or



wherein R<sup>4</sup> is an optionally substituted phenyl group;

- 15 R<sup>2</sup> is optionally substituted lower alkyl, aralkyl, thioalkyl, amidoalkyl, amino, amino alkyl, nitro, azido; together with Z forms an optionally substituted 4–7 membered carbocyclic or heterocyclic ring; or together with R<sup>3</sup> forms an optionally substituted phenyl group;

R<sup>3</sup> with R<sup>2</sup> forms an optionally substituted phenyl group or is an optionally substituted phenyl group; and

- 20 Z is H, lower alkyl, substituted alkyl, thioalkyl, nitrile, amino, or together with R<sup>2</sup> forms an optionally substituted 4–7 membered carbocyclic or heterocyclic ring.

2. The compound of claim 1 wherein when X is NH and Y is N, n is 1, R<sup>1</sup> is R<sup>5</sup> or both; and

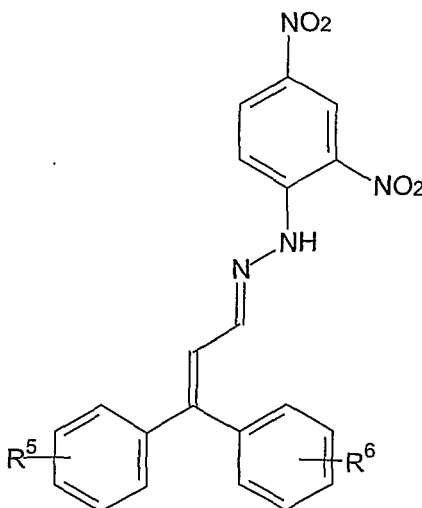
R<sup>5</sup> is H or lower alkyl.

25

3. The compound of claim 1, wherein Ar is aryl substituted with one or more halogen, haloalkyl, acyl, carboxy, carboxamido, carboalkoxy, sulfonic acid, cyano or nitro group.

- 30 4. The compound of claim 1, wherein Ar comprises at least two electron withdrawing groups.

5. The compound of claim 4, wherein Ar is a dinitrophenyl group.
6. The compound of claim 1, wherein R<sup>1</sup> is phenyl substituted with one or more group selected from -OR<sup>5</sup>, -SR<sup>5</sup>, -SO<sub>2</sub>N(R<sup>5</sup>)<sub>2</sub>, -SO<sub>2</sub>CN, -SO<sub>2</sub>R<sup>5</sup>, -SO<sub>2</sub>N<sub>3</sub>, N<sub>3</sub>, -CN, -N(R<sup>5</sup>)C(O)R<sup>6</sup>, -R<sup>6</sup>, or combinations thereof;
- each R<sup>5</sup> independently is H, lower alkyl, carbohydrate, fatty acid, amino acid, peptide, nucleoside or nucleic acid; and
- R<sup>6</sup> is lower alkyl or C-glycoside.
7. The compound of claim 1, wherein R<sup>2</sup> together with R<sup>3</sup> forms an optionally substituted phenyl group
8. The compound of claim 1, wherein R<sup>3</sup> is phenyl substituted with one or more group selected from -OR<sup>5</sup>, -SR<sup>5</sup>, -SO<sub>2</sub>N(R<sup>5</sup>)<sub>2</sub>, -SO<sub>2</sub>CN, -SO<sub>2</sub>R<sup>5</sup>, -SO<sub>2</sub>N<sub>3</sub>, N<sub>3</sub>, -CN, -N(R<sup>5</sup>)C(O)R<sup>6</sup>, -R<sup>6</sup>, or combinations thereof.
9. The compound of claim 1, wherein n is 0.
10. The compound of claim 1, wherein m is 1, 2 or 3.
11. The compound of claim 1, wherein m is 0 or 1.
12. The compound of claim 1, wherein both n and m are 1.
13. The compound of claim 1, wherein n is 1 and m is 0.
14. The compound of claim 13, having the formula



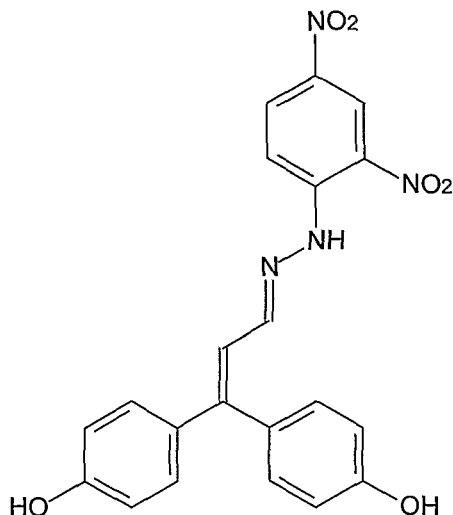
wherein  $R^5$  and  $R^6$  independently are  $-OR^7$ ,  $-SR^7$ ,  $-SO_2N(R^7)_2$ ,  $-SO_2CN$ ,  $-SO_2R^8$ ,  $-SO_2N_3$ ,  $N_3$ ,  $-CN$ ,  $-N(R^7)C(O)R^8$ ,  $-R^7$ ;

$R^7$  is H or lower alkyl; and

$R^8$  is lower alkyl.

5

15. The compound of claim 14, having the formula



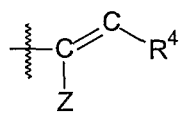
10

16. The compound of claim 1, wherein

X is  $CH_2$ , O,  $SO_2$ , B;

n is 0;

$R^1$  is an optionally substituted phenyl group or



$R^4$  is an optionally substituted phenyl group;

15

$R^2$  is optionally substituted lower alkyl or together with Z forms an optionally substituted 4-7 membered carbocyclic or heterocyclic ring; or together with  $R^4$  forms an optionally substituted phenyl group; and

$R^3$  is an optionally substituted phenyl group.

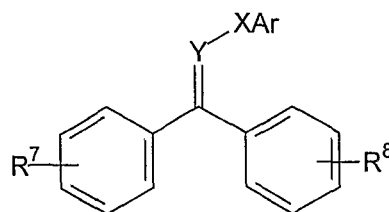
20

17. The compound of claim 16, wherein  $R^1$  is phenyl substituted with one or more group selected from  $-OR^5$ ,  $-SR^5$ ,  $-SO_2NH_2$ ,  $-SO_2CN$ ,  $-SO_2R^5$ ,  $-SO_2N_3$ ,  $N_3$ ,  $-CN$ ,  $-N(R^5)C(O)R^6$ ,  $-R^6$ , or combinations thereof.

25

18. The compound of claim 16, wherein  $R^2$  and  $R^3$  form a phenyl group.

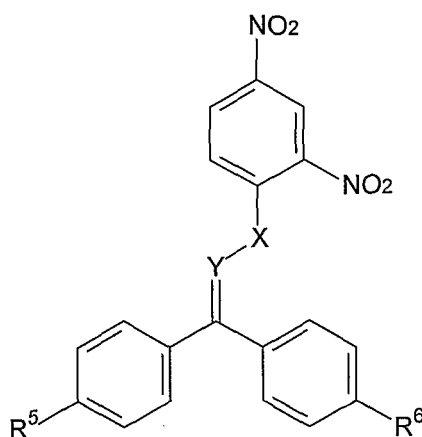
19. The compound of claim 18, having the formula



wherein  $R^7$  and  $R^8$  independently are  $-OR^5$ ,  $-SR^5$ ,  $-SO_2N(R^5)_2$ ,  $-SO_2CN$ ,  $-SO_2R^6$ ,  $-SO_2N_3$ ,  $N_3$ ,  $-CN$ ,  $-N(R^5)C(O)R^6$ ,  $-R^6$ ;

- 5 each  $R^5$  independently is H or lower alkyl; and  
 $R^6$  is lower alkyl.

20. The compound of claim 19 having the formula



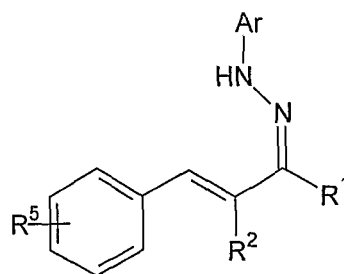
10

21. The compound of claim 20, wherein  $R^5$  and  $R^6$  are  $-OH$ .

22. The compound of claim 21, wherein X is  $CH_2$  and Y is N or CH.

15

23. The compound of claim 22, having the formula



20

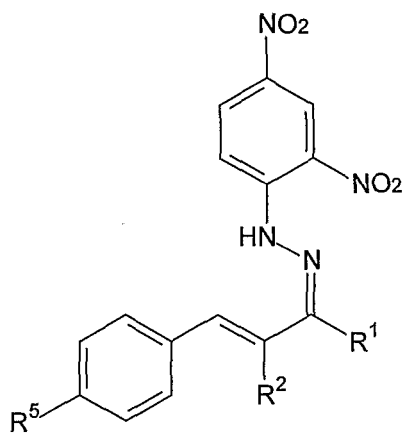
- 25 wherein  $R^5$  is H,  $-OR^6$ ,  $-SR^6$ ,  $-SO_2N(R^6)_2$ ,  $-SO_2CN$ ,  $-SO_2R^7$ ,  $-SO_2N_3$ ,  $N_3$ ,  $-CN$ ,  $-N(R^6)C(O)R^7$ , or  $-R^7$ ;

$R^1$  and  $R^6$  independently are H or lower alkyl; and  
 $R^2$  and  $R^7$  independently are lower alkyl.

24. The compound of claim 23, wherein Ar comprises a nitrophenyl group.

25. The compound of claim 24, wherein Ar comprises a dinitrophenyl group.

26. The compound of claim 25, having the formula

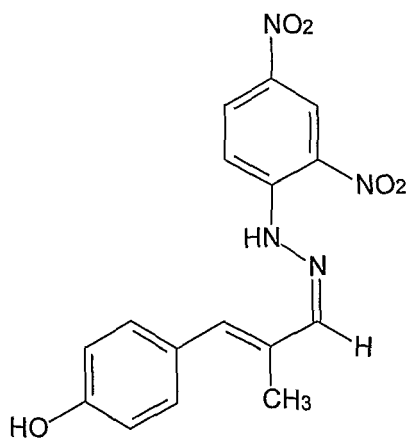


27. The compound of claim 26, wherein  $R^1$  is H.

28. The compound of claim 27, wherein  $R^2$  is methyl or ethyl.

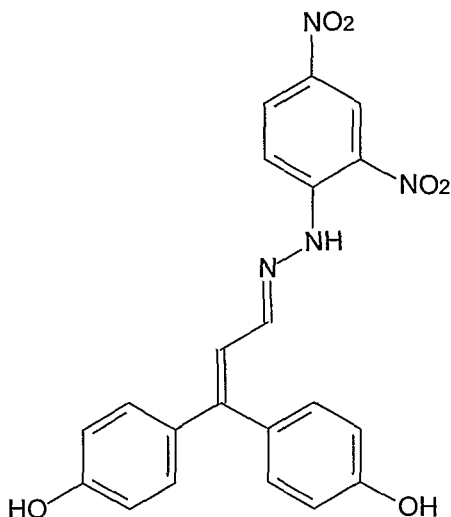
29. The compound of claim 28, wherein  $R^5$  represents  $-OR^6$ .

30. The compound of claim 29, having the formula



31. A pharmaceutical composition, comprising a compound of claim 1 and a pharmaceutically acceptable carrier.

32. The composition of claim 31, wherein the compound has the formula



5

33. The composition of claim 31, wherein the composition comprises a topical gel or solution.

10 34. The composition of claim 33, wherein the topical gel or solution comprises at least about 0.25% of the compound.

35. The composition of claim 31, wherein the composition comprises a sustained release dosage form.

15

36. The composition of claim 35, wherein the sustained release dosage form comprises a tablet or crystal.

20 37. The composition of claim 31, wherein the composition is suitable for delivery via inhalation.

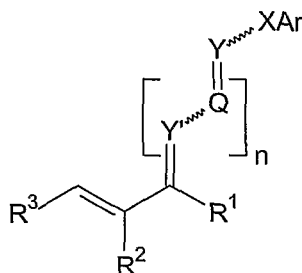
38. The composition of claim 37, wherein the composition comprises an aerosol.

25 39. A pharmaceutical composition comprising a compound of claim 6 and a pharmaceutically acceptable carrier.

40. A pharmaceutical composition comprising a compound of claim 16 and a pharmaceutically acceptable carrier.

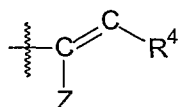
41. A method of stimulating an immune response of a subject in need of immunostimulation, comprising:

- 5 selecting a subject in need of immunostimulation;  
administering to the subject in need of immunostimulation a therapeutically effective amount of a resonance modulating compound other than A-007 having the formula



wherein Ar comprises an aryl group substituted with at least one electron withdrawing group;

- 10 X is NH, CH<sub>2</sub>, O, —, SO<sub>2</sub>, or B;  
Q is —(CH=CH)<sub>m</sub>—CH—;  
Y and Y' independently are selected from N, CH and B;  
n is from 0 or 1;  
m is from 0 to 6;  
15 R<sup>1</sup> is H, lower alkyl, optionally substituted phenyl or



wherein R<sup>4</sup> is an optionally substituted phenyl group;

- R<sup>2</sup> is optionally substituted lower alkyl, aralkyl, thioalkyl, amidoalkyl, amino, amino alkyl, nitro, azido; together with Z forms an optionally substituted 4–7 membered carbocyclic or heterocyclic ring; or together with R<sup>3</sup> forms an optionally substituted phenyl group;  
20 R<sup>3</sup> with R<sup>2</sup> forms an optionally substituted phenyl group or is an optionally substituted phenyl group; and  
Z is H, lower alkyl, substituted alkyl, thioalkyl, nitrile, amino, or together with R<sup>3</sup> forms an optionally substituted 4–7 membered carbocyclic or heterocyclic ring.

25

42. The method of claim 41, wherein selecting the subject in need of immunostimulation comprises selecting a subject who has laboratory evidence of impaired immunity.

43. The method of claim 41, wherein selecting the subject comprises selecting a subject  
30 who is infected with an infectious pathogen.

44. The method of claim 43, wherein the infectious pathogen is a virus.
45. The method of claim 44, wherein the pathogen is a human papillomavirus.
- 5 46. The method of claim 41, wherein selecting the subject comprises selecting a subject who has dysplasia of the anus or uterine cervix.
47. The method of claim 46, wherein the subject is a subject who has dysplasia induced by infection with a papillomavirus.
- 10 48. The method of claim 41, wherein administering the compound comprises administering the resonance modulating compound to the subject intra-vaginally or intra-anally.
49. The method of claim 48, wherein administering the compound comprises
- 15 introducing a sustained release dosage form intra-vaginally or intra-anally.
50. The method of claim 49, wherein the sustained release dosage form comprises a tablet or crystal.
- 20 51. The method of claim 41, wherein administering the compound comprises administering the compound topically at a site other than a cutaneous lesion.
52. The method of claim 41, wherein administering the compound comprises administering the compound topically on a skin surface site, wherein the skin surface is substantially
- 25 impermeant to the compound, and the immune response is stimulated remote from the skin surface site.
53. The method of claim 51, wherein administering the compound comprises administering the compound inhalationally.
- 30 54. The method of claim 53, wherein the compound is administered inhalationally to a subject having a respiratory epithelium infection or tumor.
55. The method of claim 41, wherein the immune response is stimulated in a lymphatic
- 35 network.
56. The method of claim 41, wherein the compound is applied to a hyperproliferative body surface lesion.

57. The method of claim 56, wherein the hyperproliferative body surface lesion is an anal, cervical, oral, penile, vaginal or vulval lesion.
- 5 58. The method of claim 56, wherein the hyperproliferative body surface lesion is a precancerous lesion.
59. The method of claim 58, wherein the precancerous lesion comprises actinic keratosis, Bowenoid actinic keratosis, arsenical keratosis, Bowen's disease, viral keratosis,  
10 leukoplakia, erythroplakia of queyrat, a nevus or a wart.
60. The method of claim 56, wherein the lesion is a primary neoplastic lesion selected from anal cancer, cervical cancer, basal or squamous cell carcinoma, melanoma, penile cancer, vulval cancer, vaginal cancer or cancer of the oral mucosa.
- 15 61. The method of claim 56, wherein the body surface lesion is a psoriasis lesion.
62. The method of claim 41, wherein Ar is aryl substituted with one or more halogen, haloalkyl, acyl, carboxy, carboxamido, carboalkoxy, sulfonic acid, cyano or nitro group.
- 20 63. The method of claim 62, wherein the resonance modulating compound is a nitrophenylhydrazone.
64. The method of claim 62, wherein the resonance modulating compound is a  
25 dinitrophenylhydrazone.
65. The method of claim 64, wherein the resonance modulating compound is a 2,4-dinitrophenylhydrazone.
- 30 66. The method of claim 41, wherein Ar comprises a polycyclic group.
67. The method of claim 41, wherein Ar comprises a substituted phenyl group.
68. The method of claim 41, wherein Ar comprises at least two electron withdrawing  
35 substituents.
69. The method of claim 68, wherein Ar is a dinitrophenyl group.

70. The method of claim 41, wherein n is 0.

71. The method of claim 41, wherein m is 1, 2 or 3.

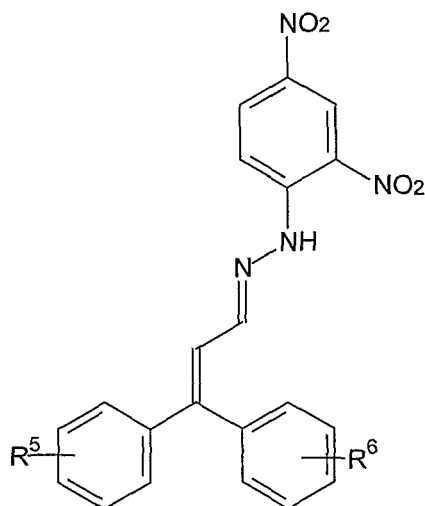
5 72. The method of claim 41, wherein m is 0 or 1.

73. The method of claim 41, wherein both n and m are 1.

74. The method of claim 41, wherein n is 1 and m is 0.

10

75. The method of claim 41, wherein the resonance modulating compound has the formula

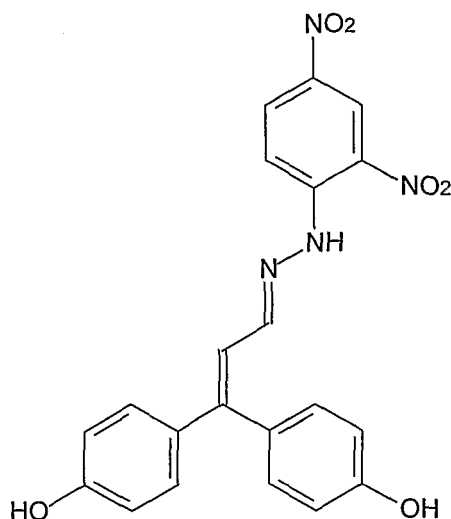


15 wherein  $R^5$  and  $R^6$  independently are  $-OR^7$ ,  $-SR^7$ ,  $-SO_2N(R^7)_2$ ,  $-SO_2CN$ ,  $-SO_2R^8$ ,  $-SO_2N_3$ ,  $N_3$ ,  $-CN$ ,  $-N(R^7)C(O)R^8$ ,  $-R^7$ ;

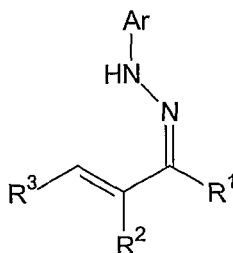
$R^7$  is H or lower alkyl; and

$R^8$  is lower alkyl.

20 76. The method of claim 41, wherein the resonance modulating compound has the formula



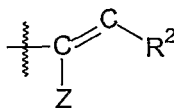
77. The method of claim 41, wherein the resonance modulating compound comprises:



5

X is a dinitrophenyl group;

R<sup>1</sup> is H, lower alkyl, optionally substituted phenyl or



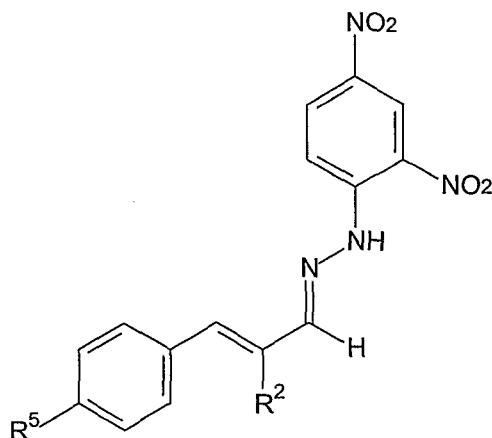
10 wherein R<sup>2</sup> is lower alkyl or an optionally substituted phenyl group;

R<sup>3</sup> is optionally substituted lower alkyl, thioalkyl, amidoalkyl, amino, amino alkyl, nitro, azido; together with Z forms an optionally substituted 4-7 membered carbocyclic or heterocyclic ring; or together with R<sup>4</sup> forms an optionally substituted phenyl group;

15 R<sup>4</sup> with R<sup>3</sup> forms an optionally substituted phenyl group or is an optionally substituted phenyl group.

78. The method according to claim 77, wherein R<sup>1</sup> is H.

20 79. The method according to claim 78, wherein the resonance modulating compound has the formula



wherein R<sup>2</sup> is lower alkyl;

R<sup>5</sup> is H, OH, -OR<sup>6</sup>, -SO<sub>2</sub>NH<sub>2</sub>, N<sub>3</sub>, -CN, -SO<sub>2</sub>CN, -SO<sub>2</sub>N<sub>3</sub> or lower alkyl; and

R<sup>6</sup> is lower alkyl.

5

80. The method of claim 41, wherein administering the compound comprises applying the compound to an epithelial surface of the subject.

81. The method of claim 80, wherein applying the compound to the epithelial surface of the subject comprises applying the compound to the surface of skin.

10

82. The method of claim 80, wherein applying the compound to an epithelial surface of the subject comprises introducing the compound into the skin.

15

83. The method of claim 41, wherein the method of stimulating an immune response comprises a method of treating a tumor by stimulating the immune response of the subject.

84. The method of claim 81, wherein applying the compound to the skin of the subject comprises applying the compound to an area of skin that is over or adjacent a tumor to stimulate the immune response of the subject.

20

85. The method of claim 84, wherein the tumor is a malignant tumor.

86. The method of claim 85, wherein the tumor is a metastasis.

25

87. The method of claim 41, further comprising enhancing immune stimulation by exposing the compound to an electromagnetic field that induces increased resonance modulation of the compound.

88. The method of claim 41, wherein the electromagnetic field is produced by a magnetic probe.

89. The method of claim 88, wherein the electromagnetic field is produced by a current flowing between two electrodes.

90. The method of claim 41, further comprising monitoring an amplitude of a waveform generated by resonance modulation of the compound to determine a response of the subject's immune system to the compound.

91. The method of claim 90, further comprising exposing the compound to an electromagnetic field to induce increased resonance modulation of the compound if the amplitude is below a therapeutically desired amplitude.

92. The method of claim 41, wherein the method of stimulating an immune response comprises a method of stimulating an immune response by or against a cell that expresses an extracellular membrane-bound protein tyrosine phosphatase.

93. The method of claim 92, wherein the cell is a T lymphocyte, and the method of stimulating the immune response comprises stimulating the immune response by the T-lymphocyte against an antigen.

94. The method of claim 93, wherein the cell is a cell that is infected with a CD45+ virus, and the method of stimulating the immune response comprises stimulating the immune response against the cell that is infected with the virus.

95. The method of claim 94, wherein the virus is a papillomavirus or a retrovirus.

96. The method of claim 95, wherein the virus is a papillomavirus.

97. The method of claim 96, wherein the virus is a human papillomavirus.

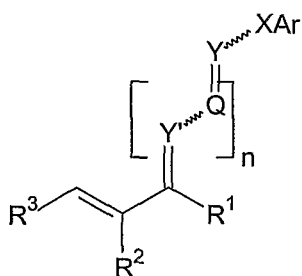
98. The method of claim 94, wherein the virus is a retrovirus.

99. The method of claim 98, wherein the virus is a human immunodeficiency virus.

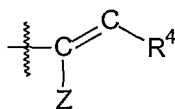
100. The method of claim 92, wherein the cell is a cell that is infected with a virus comprising an active extracellular membrane-bound protein tyrosine phosphatase, and the method of

stimulating the immune response comprises stimulating the immune response against the cell that is infected with the virus.

101. A method of monitoring an immune response of a subject, comprising:  
 5 placing a resonance modulating compound in contact with the subject to produce inherent electromagnetic waveforms characteristic of the resonance modulating compound compound other than A-007 having the formula



- 10 wherein Ar comprises an aryl group substituted with at least one electron withdrawing group;  
 X is NH, CH₂, O, —, SO₂, or B;  
 Q is  $-(CH=CH)_m-CH-$ ;  
 Y and Y' independently are selected from N, CH and B;  
 n is 0 or 1;  
 15 m is from 0 to 6;  
 R¹ is H, lower alkyl, optionally substituted phenyl or

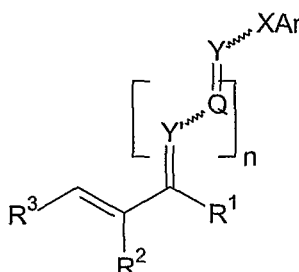


- wherein R⁴ is an optionally substituted phenyl group;  
 R² is optionally substituted lower alkyl, aralkyl, thioalkyl, amidoalkyl, amino, amino alkyl,  
 20 nitro, azido; together with Z forms an optionally substituted 4–7 membered carbocyclic or heterocyclic ring; or together with R³ forms an optionally substituted phenyl group;  
 R³ with R² forms an optionally substituted phenyl group or is an optionally substituted phenyl group;  
 Z is H, lower alkyl, substituted alkyl, thioalkyl, nitrile, amino, or together with R² forms an  
 25 optionally substituted 4–7 membered carbocyclic or heterocyclic ring; when X is NH and Y is N, n is 1, R¹ is R⁵ or both; and  
 R⁵ is H or lower alkyl; wherein the electromagnetic waveforms indicate an immune state of the subject; and  
 monitoring waveforms produced by the compound for waveforms associated with altered  
 30 immune function.

102. The method of claim 101, wherein monitoring the waveforms comprises detecting voltage changes across the compound over time, without introducing an external current or voltage potential.

5 103. The method of claim 101, wherein monitoring the waveforms associated with altered immune function comprise detecting waveforms having a decreased amplitude.

104. A method of treating a cancerous or precancerous lesion, comprising applying to the lesion an effective amount of a compound comprising a compound other than A-007 having the  
10 formula



wherein Ar comprises an aryl group substituted with at least one electron withdrawing group;

X is NH, CH<sub>2</sub>, O, —, SO<sub>2</sub>, or B;

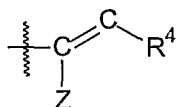
15 Q is —(CH=CH)<sub>m</sub>—CH—;

Y and Y' independently are selected from N, CH and B;

n is 0 or 1;

m is from 0 to 6;

R<sup>1</sup> is H, lower alkyl, optionally substituted phenyl or



20

wherein R<sup>4</sup> is an optionally substituted phenyl group;

R<sup>2</sup> is optionally substituted lower alkyl, aralkyl, thioalkyl, amidoalkyl, amino, amino alkyl, nitro, azido; together with Z forms an optionally substituted 4–7 membered carbocyclic or heterocyclic ring; or together with R<sup>3</sup> forms an optionally substituted phenyl group;

25 R<sup>3</sup> with R<sup>2</sup> forms an optionally substituted phenyl group or is an optionally substituted phenyl group;

Z is H, lower alkyl, substituted alkyl, thioalkyl, nitrile, amino, or together with R<sup>2</sup> forms an optionally substituted 4–7 membered carbocyclic or heterocyclic ring; when X is NH and Y is N, n is 1, R<sup>1</sup> is R<sup>5</sup> or both; and

30 R<sup>5</sup> is H or lower alkyl.

105. The method of claim 104, wherein the lesion is a hyperproliferative body surface lesion.

5 106. The method of claim 104, wherein the hyperproliferative body surface lesion is a precancerous lesion.

107. The method of claim 105, wherein the lesion is an anal, cervical, oral, penile, vaginal or vulval lesion.

10 108. The method of claim 106, wherein the precancerous lesion comprises actinic keratosis, Bowenoid actinic keratosis, arsenical keratosis, Bowen's disease, viral keratosis, leukoplakia, erythroplakia of queyrat, a nevus or a wart.

15 109. The method of claim 106, wherein the precancerous lesion is papilloma virus associated lesion.

110. The method of claim 104, wherein the lesion is a primary neoplastic lesion.

20 111. The method of claim 110, wherein the neoplastic lesion is anal cancer, cervical cancer, basal or squamous cell carcinoma, melanoma, penile cancer, vulval cancer, vaginal cancer or cancer of the oral mucosa.

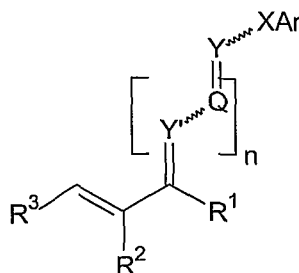
25 112. The method of claim 110, wherein the primary neoplastic lesion comprises a metastatic neoplastic lesion.

113. The method of claim 110, wherein the lesion is a penile lesion.

114. The method of claim 110, wherein the lesion is a vulval or vaginal lesion.

30 115. The method of claim 105, wherein the hyperproliferative body surface lesion is an internal body surface lesion.

35 116. A method of concentrating lymphocytes from biological tissue, comprising exposing the biological tissue to an effective amount of a compound other than A-007 having the formula



wherein Ar comprises an aryl group substituted with at least one electron withdrawing group;

X is NH, CH<sub>2</sub>, O, —, SO<sub>2</sub>, or B;

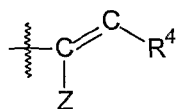
Q is —(CH=CH)<sub>m</sub>—CH—;

5 Y and Y' independently are selected from N, CH and B;

n is 0 or 1;

m is from 0 to 6;

R<sup>1</sup> is H, lower alkyl, optionally substituted phenyl or



10 wherein R<sup>4</sup> is an optionally substituted phenyl group;

R<sup>2</sup> is optionally substituted lower alkyl, aralkyl, thioalkyl, amidoalkyl, amino, amino alkyl, nitro, azido; together with Z forms an optionally substituted 4–7 membered carbocyclic or heterocyclic ring; or together with R<sup>3</sup> forms an optionally substituted phenyl group;

15 R<sup>3</sup> with R<sup>2</sup> forms an optionally substituted phenyl group or is an optionally substituted phenyl group;

Z is H, lower alkyl, substituted alkyl, thioalkyl, nitrile, amino, or together with R<sup>2</sup> forms an optionally substituted 4–7 membered carbocyclic or heterocyclic ring; when X is NH and Y is N, n is 1, R<sup>1</sup> is R<sup>5</sup> or both; and

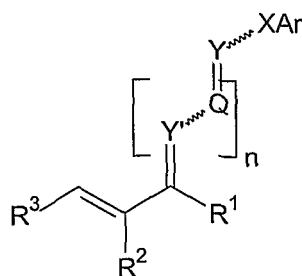
R<sup>5</sup> is H or lower alkyl.

20

117. The method of claim 116, wherein exposing the living organism to the effective amount of the compound comprises applying the compound to skin of the living organism.

118. The method of claim 117, wherein applying the compound to the skin comprises  
25 applying a topical preparation of the compound to the surface of the skin.

119. A method of treating an infection in a subject, comprising:  
administering to the subject a therapeutically effective amount of a compound other than A-007  
having the formula



wherein Ar comprises an aryl group substituted with at least one electron withdrawing group;

X is NH, CH<sub>2</sub>, O, —, SO<sub>2</sub>, or B;

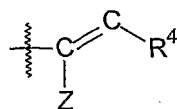
Q is  $-(\text{CH}=\text{CH})_m-\text{CH}-$ ;

5 Y and Y' independently are selected from N, CH and B;

n is 0 or 1;

m is from 0 to 6;

R<sup>1</sup> is H, lower alkyl, optionally substituted phenyl or



10 wherein R<sup>4</sup> is an optionally substituted phenyl group;

R<sup>2</sup> is optionally substituted lower alkyl, aralkyl, thioalkyl, amidoalkyl, amino, amino alkyl, nitro, azido; together with Z forms an optionally substituted 4–7 membered carbocyclic or heterocyclic ring; or together with R<sup>3</sup> forms an optionally substituted phenyl group;

15 R<sup>3</sup> with R<sup>2</sup> forms an optionally substituted phenyl group or is an optionally substituted phenyl group;

Z is H, lower alkyl, substituted alkyl, thioalkyl, nitrile, amino, or together with R<sup>2</sup> forms an optionally substituted 4–7 membered carbocyclic or heterocyclic ring; when X is NH and Y is N, n is 1, R<sup>1</sup> is R<sup>5</sup> or both; and

R<sup>5</sup> is H or lower alkyl.

20 that interacts with a PTP extracellular receptor of a cell to activate the receptor.

120. The method of claim 119, further comprising applying an external electromagnetic field to the compound to increase a resonance modulation of the compound, and thereby increase an immunostimulant effect of the compound to treat the infection.

25

121. The method of claim 119, wherein the infection is a virus infection.

122. The method of claim 121, wherein the virus is a papillomavirus or a retrovirus.

30

123. The method of claim 122, wherein the virus is a papillomavirus.

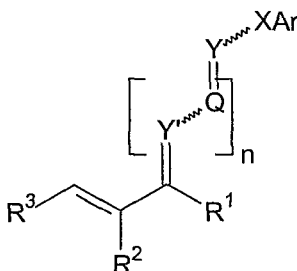
124. The method of claim 123, wherein the virus is a human papillomavirus.
125. The method of claim 122, wherein the virus is a retrovirus.
- 5 126. The method of claim 125, wherein the virus is a human immunodeficiency virus.
127. The method of claim 119, wherein the infection is a bacterial infection.
128. The method of claim 119, wherein the cell is a T-lymphocyte.
- 10 129. The method of claim 128, wherein the cell is a CD45+ lymphocyte.
130. The method of claim 129, wherein the cell is a CD45+ pathogen.
- 15 131. The method of claim 130, wherein the pathogen is human papillomavirus.
132. The method of claim 119, further comprising enhancing interaction between the compound and the receptor by exposing the compound and cell to ultraviolet radiation.
- 20 133. The method of claim 119, wherein administering the compound to the subject comprises applying the compound to dysplastic or metaplastic epithelium.
134. The method of claim 133, wherein the epithelium is urogenital epithelium.
- 25 135. The method of claim 134, wherein the urogenital epithelium is vaginal, cervical or anal epithelium.
136. The method of claim 133, wherein administering the compound to the subject comprises applying the compound to a squamous cell cancer.
- 30 137. The method of claim 136, wherein the tumor is an anal squamous cell cancer.
138. The method of claim 119, wherein administering comprises applying a topical gel.
- 35 139. The method of claim 138, wherein the topical gel comprises about 0.25% of the compound.

140. The method of claim 138, wherein applying the compound to the subject comprises applying the compound at least daily for a sufficient period to produce a therapeutic effect.

141. The method of claim 141, wherein applying the compound comprises applying the compound at least daily for at least five days.

142. The method of claim 119, wherein the therapeutically effective amount of the compound is at least 2 grams of a topical gel per day, wherein the gel comprises at least about 0.25% of the compound.

143. A method of activating a cellular protein tyrosine phosphatase present in a cell membrane, comprising exposing the cell to an amount of a resonance modulator compound a compound other than A-007 of the formula



wherein Ar comprises an aryl group substituted with at least one electron withdrawing group;

X is NH, CH<sub>2</sub>, O, —, SO<sub>2</sub>, or B;

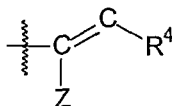
Q is  $-(CH=CH)_m-CH-$ ;

Y and Y' independently are selected from N, CH and B;

n is 0 or 1;

m is from 0 to 6;

R<sup>1</sup> is H, lower alkyl, optionally substituted phenyl or



wherein R<sup>4</sup> is an optionally substituted phenyl group;

R<sup>2</sup> is optionally substituted lower alkyl, aralkyl, thioalkyl, amidoalkyl, amino, amino alkyl, nitro, azido; together with Z forms an optionally substituted 4–7 membered carbocyclic or heterocyclic ring; or together with R<sup>3</sup> forms an optionally substituted phenyl group;

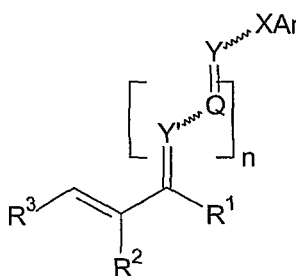
R<sup>3</sup> with R<sup>2</sup> forms an optionally substituted phenyl group or is an optionally substituted phenyl group; and

Z is H, lower alkyl, substituted alkyl, thioalkyl, nitrile, amino, or together with R<sup>2</sup> forms an optionally substituted 4–7 membered carbocyclic or heterocyclic ring.

sufficient to activate the protein tyrosine phosphatase.

144. The method of claim 143, wherein exposing the cell to the amount of the resonance modulator comprises applying the resonance modulator topically to or adjacent the cell.

- 5 145. A method of stimulating an immune response of a subject in need of immunostimulation, comprising:  
 selecting a subject in need of immunostimulation;  
 administering to the subject a therapeutically effective amount of a compound other than A-007 of the formula



10

wherein Ar comprises an aryl group substituted with at least one electron withdrawing group;

X is NH, CH<sub>2</sub>, O, —, SO<sub>2</sub>, or B;

Q is —(CH=CH)<sub>m</sub>—CH—;

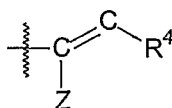
Y and Y' independently are selected from N, CH and B;

15

n is 0 or 1;

m is from 0 to 6;

R<sup>1</sup> is H, lower alkyl, optionally substituted phenyl or



wherein R<sup>4</sup> is an optionally substituted phenyl group;

20

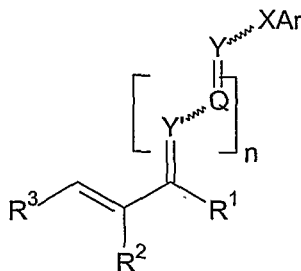
R<sup>2</sup> is optionally substituted lower alkyl, aralkyl, thioalkyl, amidoalkyl, amino, amino alkyl, nitro, azido; together with Z forms an optionally substituted 4–7 membered carbocyclic or heterocyclic ring; or together with R<sup>3</sup> forms an optionally substituted phenyl group;

R<sup>3</sup> with R<sup>2</sup> forms an optionally substituted phenyl group or is an optionally substituted phenyl group; and

25

Z is H, lower alkyl, substituted alkyl, thioalkyl, nitrile, amino, or together with R<sup>2</sup> forms an optionally substituted 4–7 membered carbocyclic or heterocyclic ring.

146. A method for treating a subject having a hyperproliferative disorder, comprising:  
 selecting a subject in need of immunostimulation;  
 administering to the subject a therapeutically effective amount of a cytotoxic compound of the formula
- 30



wherein Ar comprises an aryl group substituted with at least one electron withdrawing group;

X is NH, CH<sub>2</sub>, O, —, SO<sub>2</sub>, or B;

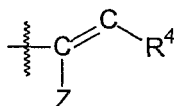
Q is —(CH=CH)<sub>m</sub>—CH—;

5 Y and Y' independently are selected from N, CH and B;

n is 0 or 1;

m is from 0 to 6;

R<sup>1</sup> is H, lower alkyl, optionally substituted phenyl or



10 wherein R<sup>4</sup> is an optionally substituted phenyl group;

R<sup>2</sup> is optionally substituted lower alkyl, aralkyl, thioalkyl, amidoalkyl, amino, amino alkyl, nitro, azido; together with Z forms an optionally substituted 4–7 membered carbocyclic or heterocyclic ring; or together with R<sup>3</sup> forms an optionally substituted phenyl group;

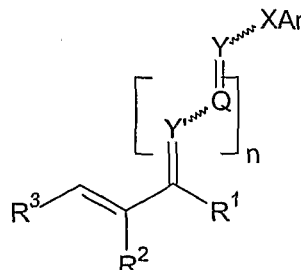
15 R<sup>3</sup> with R<sup>2</sup> forms an optionally substituted phenyl group or is an optionally substituted phenyl group; and

Z is H, lower alkyl, substituted alkyl, thioalkyl, nitrile, amino, or together with R<sup>2</sup> forms an optionally substituted 4–7 membered carbocyclic or heterocyclic ring.

20 147. A method for treating thrombocytopenia, comprising:

selecting a subject having thrombocytopenia;

administering to the subject a therapeutically effective amount of a cytotoxic compound other than A-007 having of the formula



wherein Ar comprises an aryl group substituted with at least one electron withdrawing group;

25 X is NH, CH<sub>2</sub>, O, —, SO<sub>2</sub>, or B;

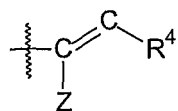
Q is  $-(CH=CH)_m-CH-$ ;

Y and Y' independently are selected from N, CH and B;

n is 0 or 1;

m is from 0 to 6;

5 R<sup>1</sup> is H, lower alkyl, optionally substituted phenyl or



wherein R<sup>4</sup> is an optionally substituted phenyl group;

R<sup>2</sup> is optionally substituted lower alkyl, aralkyl, thioalkyl, amidoalkyl, amino, amino alkyl, nitro, azido; together with Z forms an optionally substituted 4–7 membered carbocyclic or

10 heterocyclic ring; or together with R<sup>3</sup> forms an optionally substituted phenyl group;

R<sup>3</sup> with R<sup>2</sup> forms an optionally substituted phenyl group or is an optionally substituted phenyl group; and

Z is H, lower alkyl, substituted alkyl, thioalkyl, nitrile, amino, or together with R<sup>2</sup> forms an optionally substituted 4–7 membered carbocyclic or heterocyclic ring.

15

148. The method of claim 147, wherein the compound is

