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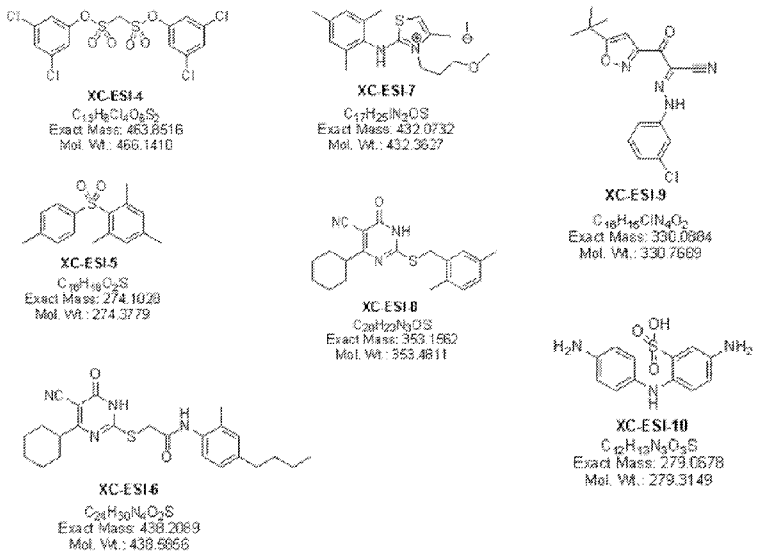
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(54) **Title**: ANTIMICROBIAL METHODS USING INHIBITORS OF EXCHANGE PROTEINS DIRECTLY ACTIVATED BY CAMP (EPAC)



(57) **Abstract**: Embodiments of the invention are directed to compositions and method for treating a viral infection. Embodiments of the invention are directed to medicine and health care. Certain embodiments are directed to methods of treating infectious disease. Additional embodiments are directed to the use of inhibitors of exchange proteins directly activated by cAMP (Epac) to treat viral infections.

FIG. 1

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ANTIMICROBIAL METHODS USING INHIBITORS OF EXCHANGE PROTEINS DIRECTLY ACTIVATED BY cAMP (Epac)

DESCRIPTION

PRIORITY CLAIM

[001] This application claims priority to and is a continuation-in-part of international application serial number PCT/US2013/025319 filed February 8, 2013.

FEDERAL FUNDING

[002] This invention was made with government support under grants R01GM066170 and R01GM106218 awarded by the National Institutes of Health. The government has certain rights in the invention.

TECHNICAL FIELD

[003] Embodiments of the invention are directed to medicine and health care. Certain embodiments are directed to methods of treating infectious disease. Additional embodiments are directed to the use of inhibitors of exchange proteins directly activated by cAMP (Epac) to treat viral infections.

BACKGROUND

[004] The outbreak of Middle East respiratory syndrome coronavirus (MERS-CoV) infections poses a threat to public health worldwide. MERS-CoV causes a severe acute respiratory syndrome (SARS)-like human respiratory disease, the infections emerged in Saudi Arabia in 2012 and subsequently has spread to eight other countries in the Middle East and to Europe (WHO-Middle East respiratory syndrome coronavirus (MERS-CoV; Breban et al. (2013) *Lancet.* 382:694-699). As of October 6, 2013, it has caused 136 confirmed human infections, including 58 deaths, a case-fatality of 43% (available on the world wide web at cdc.gov/coronavirus/mers/). Although the predicted pandemic potential of MERS is low (Assiri et al. (2013) *Lancet Infect. Dis.* 13:752-761), an increase with further evolution of MERS-CoV in nature is of concern. There remains a need for development of effective therapeutic approaches.

SUMMARY

[005] Certain embodiments are directed to compositions comprising an Epac specific inhibitor and methods for using such compositions to treat a subject or patient having or at risk of developing a microbial infection. In certain aspects the microbial infection is a viral infection.

[006] Certain embodiments are directed to methods for attenuating a viral infection or inhibiting viral replication in a subject having a viral infection comprising administering an Epac specific inhibitor to the subject. In certain aspects the viral infection is a Coronovirus or Flavivirus infection. The anti-microbial affects of Epac inhibitors is not limited to MERS-CoV or SARS-CoV. In certain aspects Epac inhibitors can be used on a broad spectrum of viruses, including but not limited to MERS-CoV, SARS-CoV, influenza, Rift Valley fever virus, Nipah virus, Marburg virus, avian H5N1 influenza virus, hepatitis C virus, vaccinia virus, HIV-1, or dengue virus infection. In certain aspects the viral infection can result in a severe acute respiratory syndrome (SARS). In a further aspect the SARS is the result of a SARS-CoV or MERS-CoV infection.

[007] An Epac specific inhibitor can be selected from α-[2-(3-chlorophenyl)hydrazinylidene]-5-(1,1-dimethylethyl)-b-oxo-3-isoxazolepropanenitrile (ESI-09); *N*-(5-*tert*-Butyl-isoxazol-3-yl)-2-[(3-chlorophenyl)-hydrazono]-2-cyanoacetamide (HJC0683); 2-[(3-Chlorophenyl)-hydrazono]-2-cyano-*N*-(5-methyl-isoxazol-3-yl)acetamide (HJC0692); 3-(5-*tert*-Butyl-isoxazol-3-yl)-2-[(3-chlorophenyl)-hydrazono]-3-oxo-propionitrile (HJC0680, ESI-09); 3-(5-*tert*-Butyl-isoxazol-3-yl)-2-[(2-chlorophenyl)-hydrazono]-3-oxo-propionitrile (HJC0693); 3-(5-*tert*-Butyl-isoxazol-3-yl)-2-[(4-chlorophenyl)-hydrazono]-3-oxo-propionitrile (HJC0694); 3-(5-*tert*-Butyl-isoxazol-3-yl)-3-oxo-2-(phenyl-hydrazono)-propionitrile (HJC0695); 3-(5-*tert*-Butyl-isoxazol-3-yl)-2-[(2,5-dichlorophenyl)-hydrazono]-3-oxo-propionitrile (HJC0696); 3-(5-*tert*-Butyl-isoxazol-3-yl)-3-oxo-2-(m-tolyl-hydrazono)propionitrile (HJC0712); 3-(5-*tert*-Butyl-isoxazol-3-yl)-3-oxo-2-[(3-trifluoromethyl-phenyl)-hydrazono]propionitrile (HJC0720); 3-(5-*tert*-Butyl-isoxazol-3-yl)-2-[(3-nitrophenyl)-hydrazono]-3-oxo-propionitrile (HJC0721); 3-(5-*tert*-Butyl-isoxazol-3-yl)-3-oxo-2-(p-tolyl-hydrazono)propionitrile (HJC0724); 3-(5-*tert*-Butyl-isoxazol-3-yl)-2-[(3,5-dichlorophenyl)-hydrazono]-3-oxo-propionitrile (HJC0726); 2-[(4-Bromophenyl)-hydrazono]-3-(5-*tert*-butyl-isoxazol-3-yl)-3-oxo-propionitrile (HJC0742); 2-[(3-Bromophenyl)-hydrazono]-3-(5-*tert*-butyl-isoxazol-3-yl)-3-oxo-propionitrile (HJC0743); 3-

(5-*tert*-Butyl-isoxazol-3-yl)-2-[(2,5-dimethylphenyl)-hydrazono]-3-oxo-propionitrile (HJC0744); 3-(5-*tert*-Butyl-isoxazol-3-yl)-3-oxo-2-(quinolin-6-yl-hydrazono)propionitrile (HJC0745); 3-(5-*tert*-Butyl-isoxazol-3-yl)-2-[(2,3-dichlorophenyl)-hydrazono]-3-oxo-propionitrile (HJC0750); 3-(5-*tert*-Butyl-isoxazol-3-yl)-2-[(3-ethynyl-phenyl)-hydrazono]-3-oxo-propionitrile (HJC0751); 3-{*N*'-[2-(5-*tert*-Butyl-isoxazol-3-yl)-1-cyano-2-oxo-ethylidene]-hydrazino}benzoic acid ethyl ester (HJC0752); 3-{*N*'-[2-(5-*tert*-Butyl-isoxazol-3-yl)-1-cyano-2-oxo-ethylidene]-hydrazino}benzonitrile (HJC0753); 2-[(3-Acetyl-phenyl)-hydrazono]-3-(5-*tert*-butyl-isoxazol-3-yl)-3-oxo-propionitrile (HJC0754); 3-(5-*tert*-Butyl-isoxazol-3-yl)-2-[(2,3-dimethylphenyl)-hydrazono]-3-oxo-propionitrile (HJC0755); 3-(5-*tert*-Butyl-isoxazol-3-yl)-2-[(3-hydroxymethylphenyl)-hydrazono]-3-oxo-propionitrile (HJC0756); 3-(5-*tert*-Butyl-isoxazol-3-yl)-2-(indan-5-yl-hydrazono)-3-oxo-propionitrile (HJC0757); 2-[(3,5-Bis-trifluoromethyl-phenyl)-hydrazono]-3-(5-*tert*-butyl-isoxazol-3-yl)-3-oxo-propionitrile (HJC0758); 2-{*N*'-[2-(5-*tert*-Butyl-isoxazol-3-yl)-1-cyano-2-oxo-ethylidene]-hydrazino}-6-chloro-benzoic acid (HJC0759); 3-(5-*tert*-Butyl-isoxazol-3-yl)-2-[(3-chloro-4-hydroxy-phenyl)-hydrazono]-3-oxo-propionitrile (HJC0760); 2-[(3-Chloro-phenyl)-hydrazono]-3-(5-methyl-isoxazol-3-yl)-3-oxo-propionitrile (HJC0768); or 2-[(3,5-Dichlorophenyl)-hydrazono]-3-(5-methyl-isoxazol-3-yl)-3-oxo-propionitrile (HJC0770).

[008] Other embodiments of the invention are discussed throughout this application. Any embodiment discussed with respect to one aspect of the invention applies to other aspects of the invention as well and vice versa. Each embodiment described herein is understood to be embodiments of the invention that are applicable to all aspects of the invention.

[009] As used herein, the term "IC_{50}" refers to an inhibitory dose that results in 50% of the maximum response obtained.

[010] The term half maximal effective concentration (EC_{50}) refers to the concentration of a drug that presents a response halfway between the baseline and maximum after some specified exposure time.

[011] As used herein, an "inhibitor" as described herein, for example, can inhibit directly or indirectly the activity of a protein. The term "Epac specific inhibitor" refers to a compound that decreases the activity of Epac in a cell without significantly binding and reducing the activity of non-Epac proteins in the cell. EPAC inhibitors include EPAC1

inhibitors and/or EPAC2 inhibitors. In some embodiments, the anti-viral agent inhibits EPAC1 (and may also inhibit EPAC2). In other embodiments, the anti-viral agent specifically inhibits EPAC1 (and does not significantly inhibit EPAC2).

[012] As used herein, an "inhibitor" as described herein, for example, can inhibit directly or indirectly the activity of a protein. The term "Epac specific inhibitor" refers to a compound that decreases the activity of Epac in a cell without significantly binding and reducing the activity of non-Epac proteins in the cell. EPAC inhibitors include EPAC1 inhibitors and/or EPAC2 inhibitors.

[013] An "effective amount" of an agent in reference to treating a disease or condition means an amount capable of decreasing, to some extent, a pathological condition or symptom resulting from a pathological condition. The term includes an amount capable of invoking a growth inhibitory, cytostatic and/or cytotoxic effect and/or apoptosis of the cancer or tumor cells.

[014] As used herein, the term "patient" or "subject" refers to a living mammalian organism, such as a human, monkey, cow, sheep, goat, dogs, cat, mouse, rat, guinea pig, or species thereof. In certain embodiments, the patient or subject is a primate. Non-limiting examples of human subjects are adults, juveniles, infants and fetuses.

[015] The terms "comprise," "have," and "include" are open-ended linking verbs. Any forms or tenses of one or more of these verbs, such as "comprises," "comprising," "has," "having," "includes," and "including," are also open-ended. For example, any method that "comprises," "has," or "includes" one or more steps is not limited to possessing only those one or more steps and also covers other unlisted steps.

[016] The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or."

[017] The use of the word "a" or "an" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one."

[018] Throughout this application, the term "about" is used to indicate that a value includes the inherent variation of error for the device, the method being employed to determine the value, or the variation that exists among the study subjects.

DESCRIPTION OF THE DRAWINGS

[019] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of the specification embodiments presented herein.

[020] FIG. 1. Chemical Structures of Hits and General Strategy to Create New Epac2 Probes.

[021] FIG. 2. Examples of compounds having a general formula of Formula III.

[022] FIG. 3. Examples of compounds having a general formula of Formula IV.

[023] FIG. 4. Examples of compounds having a general formula of Formula V.

[024] FIG. 5. Examples of compounds having a general formula of Formula VI.

[025] FIG. 6. Examples of compounds having a general formula of Formula VII.

[026] FIGs. 7A-7B. Relative potency of EPAC specific antagonists. (A) Dose-dependent competition of ESIs (open circles) and cAMP (closed squares) with 8-NBD-cAMP in binding to EPAC2. (B) Dose-dependent inhibition of EPAC1 (closed circles) or EPAC2 (open circles) GEF activity by ESI-05, ESI-07 and ESI-09 in the presence of 25 μM cAMP.

[027] FIG. 8. Effect of ESI-09 on type I and II PKA activity. Relative Type I (filled bars) and II (open bars) PKA holoenzyme activities in the presence of 100 μM cAMP plus vehicle control, 25 μM H-89, 25 μM ESI-05, 25 μM ESI-07, or 25 μM ESI-09. Data are presented in the format of means and standard deviations (n=3).

[028] FIGs. 9A-9B. Effects of EPAC2-specific antagonists on 007-AM–mediated cellular activation of Rap1. Serum-starved HEK293/EPAC2 cells or HEK293/EPAC1 cells with or without pretreatment of ESI-05 or ESI-07 for 5 min were stimulated with 10 μM 007-AM for 10 min. GTP-bound Rap1 (Rap1GTP) obtained by a Ral-GDSRBD-GST pull-down

assay and total cellular Rap1 were detected by immunoblotting with Rap1-specific antibody. (A) HEK293/EPAC2 cells treated with ESI-05. (B) HEK293/EPAC2 cells treated with ESI-07. (C) HEK293/EPAC1 cells treated with ESI-05 or ESI-07. Similar results were obtained with three independent experiments for each panel. A t test was used to determine statistical significance (*P < 0.05).

[029] FIG. 10. Effect of ESI-09 on EPAC-mediated PKB phosphorylation in HEK293/EPAC1, HEK293/EPAC2, and human vascular smooth muscle (hVSMC) cells. Serum-starved HEK293/EPAC1, HEK293/EPAC2, and hVSMC cells with or without pretreatment of 10 μM ESI-09 were stimulated with 10 μM 007-AM. Cell lysates were subjected to Western blot analysis as described under "Experimental Procedures" using anti-phospho-Ser473-specific (*PKB-P473*) and anti-phospho-Thr308-specific (*PKB-P308*) PKB antibodies. Similar results were obtained from three independent experiments.

[030] FIG. 11. Prior treatment with ESI-09, but not H89, protects permissive cells against MERS-CoV infection in a cell type-independent manner. Confluent Calu-3 cells were treated with DMSO (as control), H89, or ESI-09, all at 1 and 10 μM, for 2 hrs before MERS-CoV challenge at an MOI of 0.1. The effect of the different treatments on viral yield (A) was evaluated at 24 hrs pi. Similar experiments were also performed using Vero E6 cells (B). The effective concentrations of ESI-09 were determined by treating Calu-3 cells as described in (A) with serial two-fold dilutions of ESI-09 and compared yields of infectious virus at 24 hrs (MOI of 0.1) (C). The lactate dehydrogenase (LDH)-based cytotoxicity assay (Promega) was used to evaluate the drug's cytotoxic potential (D). Briefly, confluent Calu-3 and Vero E6 cells grown in 6-well plates were incubated with the indicated concentrations of ESI-09 for 24 hrs before assessing LDH released into the culture medium. Cells incubated with 50 μM DMSO were included as controls. *** $p < 0.001$, 1-way or 2-way ANOVA analysis. A representative of at least two independently conducted experiments of each type is presented.

[031] FIG. 12. Prior ESI-09 treatment is as effective in protecting Calu-3 cells against both MERS-CoV and SARS-CoV. Calu-3 cells grown in chamber slides were pretreated with 10 μM of DMSO, H89, or ESI-09 for 2 hrs, followed by infection with MERS-CoV (MOI of 0.1) for 24 hrs before assessing the expressions of CD26 and virus-specific antigen in infected versus mock-infected cultures by indirect immunofluorescent (IIF) staining. Briefly, paraformaldehyde (4%)-fixed infected Calu-3 cells were stained with goat anti-human CD26 (5 mg/ml, R&D) and rabbit-anti-MERS-CoV (1:200 dilution) antibodies (a generous gift

from Dr. Heinz Feldmann, NIH/Rocky Mountain Laboratories, Hamilton, Montana), followed by staining with either Alex488-conjugated, donkey anti-goat IgG or Alex568-conjugated donkey anti-rabbit IgG. DAPI was used to stain the nucleus of cells. Stained cultures were analyzed by using an inverted phase contrast fluorescence microscope (Olympus 1X51) (A). Cell-free supernatants harvested at 24 hrs pi were used to determine the yields of MERS-CoV (B). Confluent Vero E6 cells grown in 12-well plates were similarly subjected to treatment with 10 μM of DMSO, H89, or ESI-09 prior to infection with SARS-CoV (MOI of 0.1), followed by assessing the yield of virus in culture medium at 24 hrs pi. *** $p < 0.001$, 1-way ANOVA analysis. A representative of at least three independently conducted experiments is presented.

[032] FIG. 13. ESI-09 treatment is effective in inhibiting viral RNA replication and protein expression of MERS-CoV without affecting total CD26 expression and virus binding to Calu-3 cells. The amount of CD26 glycoprotein in the lysates of Calu-3 cells treated for 2 hrs with either 10 μM DMSO or ESI-09 were determined by Western blot analysis. Constitutively expressed β-actin was included as an internal control. The resulting protein bands were analyzed using ImageJ and the ratios between the densities of CD26 and β-actin within each cell type were compared for the effect of different treatments on CD26 expression (A). The expression of CD26 in Calu-3 cells treated with 10 μM DMSO or ESI-09 for 24 hrs was also monitored by IIF staining with goat-anti-human CD26/DPP4 antibodies and Alexa488-conjugated donkey-anti-goat immunoglobulin, as indicated in the text. DAPI staining of cellular nuclei was included (blue). The cultures were analyzed by using an inverted phase contrast fluorescence microscope (Olympus 1X51) (B). The binding efficiencies of MERS-CoV on the membranes of untreated and treated Calu-3 cells were evaluated as described in the text. Briefly, the differentially treated cells were incubated with MERS-CoV (MOI of 20) in an ice-bath for two hrs, washed thoroughly with ice-cold PBS, and subjected to 1-cycle of freeze-thaw before determining the titers of membrane-bound viral particles in Vero E6-based infection assays. Virus binding to untreated Calu-3 cells was defined as 100% (C). A representative of at least two independently conducted experiments to each subset of the study is presented. The effect of ESI-09 treatment on viral RNA replication and protein expression over time were also evaluated. Briefly, Calu-3 cells challenged with live or γ-inactivated MERS-CoV (MOI=5) were treated with DMSO or ESI-09 (10 μM) for indicated time points p.i. before subjecting to total RNA extraction and cell lysate preparation. Quantitative (q) RT-PCR analyses targeting virus-specific upstream E

gene and cellular GAPDH gene (as endogenous control) were used to monitor the kinetics of RNA replication. The intensity of mRNA of upstream E gene of each sample relative to that of GAPDH was calculated according to the standard $\Delta\Delta Ct$ method (37), and the average of mRNA signaling in duplicate samples is depicted (D). For determining the effect of ESI-09 treatment on the viral protein synthesis, Western blot analyses with a pair of rabbit anti-MERS-CoV antibodies (1:2,000) and horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (1:15,000, Cell Signaling Technology) were employed. (E).

[033] FIG. 14. Epac-1 gene knockdown (KD) results in a significantly reduced susceptibility of Calu-3 cells in response to both MERS-CoV and SARS-CoV infection. The phenotypes of stable Epac-1 KD-versus-control KD Calu-3 cells, established by shRNA lentiviral transduction, were determined by Western blot analyses. Epac-1 contents were compared, using the ratios of relative densities between protein bands of Epac-1 and β-actin (as control) as measured by ImageJ. The ratio between Epac-1 and β-actin in control KD cells was defined as 1 (A). The impact of Epac-1 KD on MERS-CoV and SARS-CoV replication was assessed after infection with each of the viruses at an MOI of 0.1 for 24 hrs. The resulting virus yields were assessed by Vero E6-based infection assays (B). ** $p < 0.01$, * $p < 0.05$, 2-way ANOVA analysis. A representative of three independently conducted experiments is presented.

[034] FIG. 15. Neither ESI-09 treatment nor MERS-CoV infection affects the expression and localization of Epac protein in Calu-3 cells. Calu-3 cells grown either in 12-well plates or in chamber-slides were infected with MERS-CoV or rMERS-CoV-RFP (MOI=5) for 1 hr, followed by DMSO or ESI-09 treatment (10 μM) for 6, 8, 12, 18, and/or 22 hrs before assessing the expression and localization of Epac protein. Specifically, Western blot analyses of the expression levels of Epac protein in response to DMSO-versus-ESI-09-versus-MERS-CoV infection over time were compared, using the ratios of relative densities between protein bands of Epac and GAPDH (as control) as measured by ImageJ. The ratio between Epac and GAPDH in mock-infected controls in each time point was defined as 1. For localizing the expression of Epac protein and MERS-CoV-RFP replication, indirect IF staining was used. Briefly, the Epac protein in differentially treated cells was revealed by using a pair of anti-Epac and its isotype-matching Alexa488-conjugated secondary antibodies, whereas direct IF was used to directly assess the replication of MERS-CoV-RFP, a general gift of Drs. Amy Sims and Ralph Baric (University of North Carolina, Chapel Hill),

under an inverted phase contrast fluorescence microscope (Olympus 1X51). DAPI was used to stain the nucleus of cells (blue). Epac expression (green, arrow) in uninfected, DMSO-treated (a) or ESI-09-treated (b) cells, MERS-CoV-RFP expression (red, arrowhead) in DMSO-treated (c-e) or ESI-09-treated (i-k) cells, Merged Epac and MERS-CoV-RFP expression in DMSO-treated (f-h) or ESI-09-treated (l-n) cells. A representative of two independently performed experiments is presented. Magnification: 400X.

[035] FIG. 16. ESI-09 is not virucidal, possesses an unusual wide and effective therapeutic window, and requires its continual presence in the infected cultures to be effective against both MERS-CoV and SARS-CoV infection in Calu-3 cells. Equal aliquots of MERS-CoV or SARS-CoV stocks were incubated at 37 ^{o}C for 2 hrs with an equal volume of MEM/2% FBS (M-2) medium, or 20 μM of either DMSO or ESI-09 for a final concentration of 10 μM each. The infectious virus yield was subsequently determined by Vero E6-based infection assays (A). To evaluate the duration of ESI-09 treatment needed to protect against MERS-CoV infection in Calu-3 cells, two sets of duplicate cell cultures were treated with 10 μM DMSO or ESI-09 for 2 hrs. After challenge with MERS-CoV (MOI of 0.1), one set was replenished with DMSO and ESI-09, respectively, whereas the other set was replenished with M-2 medium. The resulting supernatants were tested for virus yield at 24 hrs pi (B). To examine the therapeutic potential of ESI-09, confluent Calu- 3 cells grown in 12-well plates were treated with ESI-09 (10 μM) or DMSO at indicated time points, where 0 hr is defined as the time of MERS-CoV infection (MOIs of 0.1 and 5). The yield of progeny virus was assessed at 38 hrs (MOI of 0.1) (C) or 24 hrs (MOI of 5) (D) pi, as described elsewhere, and was used to evaluate the therapeutic potential. *** $p < 0.001$, 2-way ANOVA analysis. A representative of two independently conducted experiments is presented.

[036] FIG. 17. Illustrates the breadth of viruses on which Epac inhibitors have affect.

DESCRIPTION

[037] cAMP-mediated signaling regulates a myriad of important biological processes under both physiological and pathological conditions. In multi-cellular eukaryotic organisms, the effects of cAMP are transduced by the protein kinase A/cAMP-dependent protein kinase (PKA/cAPK) and the exchange protein directly activated by cAMP/cAMP-regulated guanine nucleotide exchange factor (EPAC/cAMP-GEF) (de Rooij et al. (1998) *Nature* 396: 474-477; Kawasaki et al. (1998) *Science* 282: 2275-2279). Since both PKA and EPAC are ubiquitously expressed in all tissues, an increase in intracellular cAMP levels will lead to the activation of both PKA and EPAC. Net physiological effects of cAMP entail the integration of EPAC- and PKA-dependent pathways in a spatial and temporal manner. Depending upon their relative abundance, distribution and localization, as well as the precise cellular environment, the two intracellular cAMP receptors may act independently, converge synergistically, or oppose each other in regulating a specific cellular function (Cheng et al. (2008) *Acta Biochim Biophys Sin* (Shanghai) 40: 651-662). Therefore, careful dissections of the individual role and relative contribution of EPAC and PKA within the overall cAMP signaling in various model systems are critical for further elucidating the mechanism of cAMP signaling, as well as essential for developing novel mechanism-based therapeutic strategies targeting specific cAMP-signaling components.

[038] There are two forms of EPAC, EPAC1 and EPAC2, which are encoded by separate genes, *EPAC1* and *EPAC2*, respectively. *EPAC1* is expressed ubiquitously with predominant expression in the thyroid, kidney, ovary, skeletal muscle, and specific brain regions. *EPAC2* is predominantly expressed in the brain and adrenal gland (de Rooij et al. (1998) *Nature* 396:474–477; Kawasaki et al. (1998) *Science* 282:2275–2279).

I. USE OF EPAC INHIBITORS AS ANTI-MICROBIALS

[039] Cyclic AMP is a universal second messenger that is evolutionally conserved in diverse form of lives, including human and pathogens such as bacterial, fungi and protozoa. It has been well recognized that cAMP play major roles in microbial virulence, ranging from a potent toxin to a master regulator of virulence gene expression. (MaDonough & Rodriguez (2012) *Nature Rev Microbiol* 10:27-38). As a major intracellular cAMP receptor, it is likely that EPAC proteins are important cellular targets for microbe infection. Intracellular levels of cAMP are tightly regulated by many cell type-specific isoforms of AC and phosphodiesterase (PDE), a family of enzymes that inhibit cAMP signaling by degrading

intracellular cAMP (Hanoune and Defer (2001) *Annu Rev Pharmacol Toxicol* 41:145–174; Willoughby and Cooper (2008) *Nat Methods*. 5:29-36). While the impact of cAMP on diverse cellular functions is complex, an elevated expression of intracellular cAMP generally suppresses host antimicrobial defense (Beavo and Brunton (2002) *Nat Rev Mol Cell Biol.* 3(9):710-718). A critical role for cAMP signaling in regulating host defense mechanisms is underscored by the fact that many pathogens, including viruses, establish infection in permissive hosts by having evolved strategies targeting the adenosine-cAMP axis to modulate the levels of intracellular cAMP (McDonough and Rodriguez (2011) *Nat Rev Microbiol.* 10(1):27-38).

[040] In certain aspects Epac specific inhibitors can be used for attenuating or preventing uptake of a microbe by a vascular endothelial cell. Endothelial and epithelial cell-cell junctions and barriers play a critical role in the dissemination of microbe infection. EPAC and its down-stream effector Rap1 have been shown to play an important role in cellular functions related to endothelial cell junctions and barrier (Kooistra et al. (2005) *FEBS Lett* 579:4966-4972; Baumer et al. (2009) *J Cell Physiol.* 220:716-726; Noda et al. (2010) *Mol Biol Cell* 21:584-596; Rampersad et al. *J. Biol Chem.* 285:33614-33622; Spindler et al (2011) *Am J Pathol* 178:2424-2436).

[041] Certain embodiments are directed to methods of suppressing microbe infection comprising administering an Epac specific inhibitor to a subject having or under the risk of microbe infection. In certain aspects the microbe is a bacteria, virus, or fungi. In other aspects the Epac specific inhibitor is selected from the Epac inhibitors described herein.

A. Coronavirus

[042] Coronaviruses are a species in the genera of virus belonging to one of two subfamilies *Coronavirinae* and *Torovirinae* in the family *Coronaviridae*. Coronaviruses are enveloped viruses with a positive-sense RNA genome and with a nucleocapsid of helical symmetry. The genomic size of coronaviruses ranges from approximately 26 to 32 kilobases, extraordinarily large for an RNA virus. Coronaviruses produce a 3' co-terminal nested set of subgenomic mRNA's during infection.

[043] Proteins that contribute to the overall structure of all coronaviruses are the spike (S), envelope (E), membrane (M) and nucleocapsid (N). In the specific case of the SARS coronavirus, a defined receptor-binding domain on S mediates the attachment of the virus to

its cellular receptor, angiotensin-converting enzyme 2 (ACE2). Some coronaviruses (specifically the members of Betacoronavirus subgroup A) also have a shorter spike-like protein called hemagglutinin esterase (HE).

[044] Coronaviruses primarily infect the upper respiratory and gastrointestinal tract of mammals and birds. Four to five different currently known strains of coronaviruses infect humans. Human coronavirus includes SARS-CoV, which is the virus that causes SARS. SARS-CoV has a unique pathogenesis because it causes both upper and lower respiratory tract infections and can also cause gastroenteritis. Coronaviruses are believed to cause a significant percentage of all common colds in human adults. The significance and economic impact of coronaviruses as causative agents of the common cold are hard to assess because, unlike rhinoviruses (another common cold virus), human coronaviruses are difficult to grow in the laboratory. Coronaviruses can even cause pneumonia, either direct viral pneumonia or a secondary bacterial pneumonia. The SARS-CoV was identified as the etiologic agent of an epidemic that resulted in over 8,000 infections, about 10% of which resulted in death (Li et al., *Science* 309(5742): 1864-68, 2005).

[045] Human coronaviruses include, but are not limited to Human coronavirus 229E, Human coronavirus OC43, SARS-CoV, Human Coronavirus NL63 (HCoV-NL63, New Haven coronavirus), Human coronavirus HKU1, and Middle East respiratory syndrome coronavirus (MERS-CoV), previously known as Novel coronavirus 2012 and HCoV-EMC.

B. Influenza

[046] Certain embodiments are directed to inhibiting replication of influenza virus in a subject. Influenza is an acute, highly infectious disease caused by the influenza virus. Infection occurs via the respiratory tract, and with seasonal strains recovery is usually quite rapid. However, particularly in elderly or debilitated patients, severe complications may result from secondary infection. Epidemic or pandemic strains, to which there is little or any natural immunity, may cause fulminate infection even in young and healthy individuals. The only therapeutic agents available are the neuraminidase inhibitors zanamivir (Relenza®; SmithKline Glaxo) and oseltamivir (Tamiflu®; Roche), andamantadine, which is less effective. Consequently control of the disease relies on immunization.

[047] Influenza virus is an orthomyxovirus, and there are three known types A, B, and C. Influenza A causes seasonal, epidemic or pandemic influenza in humans, and may also

cause epizootics in birds, pigs and horses. Influenza B and C are associated with sporadic outbreaks, usually among children and young adults. Influenza viruses are divided into strains or subtypes on the basis of antigenic differences in the HA and NA antigens. Each virus is designated by its type (A, B or C), the animal from which the strain was first isolated (designated only if non-human), the place of initial isolation, the strain number, the year of isolation, and the particular HA and NA antigens (designated by H and N respectively, with an identifying numeral).

[048] "Avian influenza" (AI) is caused by influenza A viruses which occur naturally among wild birds, such as ducks, geese and swans. Until an epizootic in Pennsylvania in 1983-84, AI was not regarded as a virulent disease.

[049] "Low pathogenic avian influenza" (LPAI) is common in birds and causes few problems. Wild birds, primarily waterfowl and shorebirds, are the natural reservoir of the low pathogenic strains of the virus (LPAI). Although reservoir birds typically do not develop any clinical signs due to LPAI virus, the virus may cause disease outbreaks in domestic chickens, turkeys and ducks.

[050] "Non-pathogenic avian influenza" is caused by avian influenza virus strains that are able to infect susceptible birds, but does not cause disease symptoms or disease outbreaks.

[051] Highly pathogenic avian influenza" (HPAI) is characterized by sudden onset, severe illness and rapid death of affected birds, and has a mortality rate approaching 100%. HPAI is a virulent and highly contagious viral disease that occurs in poultry and other birds. On rare occasions, highly pathogenic avian influenza can spread to humans and other animals, usually following direct contact with infected birds. LPAI and HPAI strains of avian influenza can readily be distinguished by their relative reproduction ratio, infectivity and mortality; HPAI has a significantly higher reproduction ratio, invariably infects susceptible birds such as chickens, and causes death of infected susceptible birds within approximately 6 days after infection. Only viruses which are of either H5 or H7 subtype are known to be highly pathogenic avian influenza viruses. It is thought that HPAI viruses arise from LPAI H5 or H7 viruses infecting chickens and turkeys after spread from free-living birds. At present it is assumed that all H5 and H7 viruses have this potential, and that mutation to virulence is a random event. For example, influenza virus strain H5N1 is highly

pathogenic, deadly to domestic fowl, and can be transmitted from birds to humans. There is no human immunity against HPAI, and no vaccine is available.

[052] Pandemic influenza is virulent human influenza that causes a global outbreak, or pandemic, of serious illness. Influenza A viruses may undergo genetic changes which result in major changes in antigenicity of both the hemagglutinin and the neuraminidase (i.e., antigenic shift). Antigenic shift is thought to result from the fact that influenza A can infect animals as well as humans. A mixed infection, in which strains from different species infect a single host, can lead to reassortment which results in a new influenza virus to which the human population is completely susceptible; an influenza pandemic may result. Because there is little natural immunity, the disease can spread easily from person to person. The most serious influenza pandemics occurred in 1918 ("Spanish flu"), 1957 ("Asian flu") and 1968 ("Hong Kong flu"). The 1918 influenza pandemic killed approximately 50 to 100 million people worldwide; the 1957 pandemic was responsible for 2 million deaths; and the 1968 outbreak caused about 1 million deaths.

[053] Seasonal or common influenza (interpandemic influenza) is a respiratory illness that can be readily transmitted from person to person. Most people have some immunity, and vaccines are available. These may be live, attenuated vaccines, killed virus (inactivated vaccines), or sub-unit ("split virus") vaccines. Other types of vaccine are in clinical trial. Small changes in antigenicity of the hemagglutinin or neuraminidase, known as antigenic drift, occur frequently. The population is no longer completely immune to the virus, and seasonal outbreaks of influenza occur. These antigenic changes also require the annual reformulation of influenza vaccines.

[054] A highly pathogenic influenza virus may be of any hemagglutinin type, including H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15 or H16. The highly pathogenic influenza virus may be one of any sub-type, including but not limited to H5N1, H5N2, H5N8, H5N9, H7N3, H7N7, and H9N2.

[055] Given the biology of influenza a non-specific treatment for the virus would be of benefit to the healthcare practitioner and eliminate the need to type the virus or reformulate vaccines on a periodic basis.

II. EPAC INHIBITORS

[056] Certain embodiments include methods that use compounds that inhibit Epac as medicaments for treating diseases or conditions involving Epac. Methods for synthesizing compounds that modulate Epac are described in a related application, PCT/US2013/025319 having an international filing date of February 8, 2013, which is incorporated herein by reference in its entirety.

[057] EPAC inhibitors can be identified and characterized using a high throughput assays. One such assay is a fluorescence-based high throughput assay for screening EPAC specific antagonists (Tsalkova et al. (2012) *PLoS. ONE.* 7: e30441). The assay is highly reproducible and simple to perform using the "mix and measure" format. A pilot screening led to the identification of small chemical compounds capable of specifically inhibiting cAMP-induced Epac activation while not affecting PKA activity, i.e., Epac specific inhibitors (ESI).

[058] *Primary screen assay* – Fluorescence intensity of 8-NBD-cAMP in complex with EPAC2 is used as the readout in the primary screen assay. Primary screen is performed in black 96-well or 384-well microplates. As an example, 50 nM EPAC2 solution is prepared in 20 mM Tris buffer, pH 7.5, containing 150 mM NaCl, 1 mM EDTA, and 1 mM DDT. 8-NBD-cAMP is added to EPAC2 solution up to 60 nM from a stock solution in water. Sample is dispensed into plate and test compounds added from 96-well mother plates. Samples with cAMP addition and no additions are used as a positive and a negative control. Fluorescence intensity signal from 8-NBD was recorded at room temperature (rt) before and after tested compounds are added using SpectaMaxM2 microplate reader (Molecular Devices, Silicon Valley, CA, USA) with excitation/emission wavelengths set at 470/540 nm.

[059] *Secondary confirmation assay* – Measurement of in vitro guanine nucleotide exchange factor (GEF) activity of EPAC was adapted from a well known fluorescence-based assay using a fluorescent guanine nucleotide analog (van den Berghe et al. (1997) *Oncogene* 15: 845-850), and used as a functional confirmation assay for the compounds identified from primary screen. Briefly, 0.2 μM of Rap1B(1-167) loaded with the fluorescent GDP analog (Mant-GDP), was incubated with EPAC in 50 mM Tris buffer pH 7.5, containing 50 mM NaCl, 5 mM $MgCl_2$, 1 mM DTT, and a 100-fold molar excess of unlabeled GDP (20 μM) in the presence of various concentrations of test compound and 25 μM cAMP. Exchange of Mant-GDP by GDP was measured as a decrease in fluorescence intensity over time using a

FluoroMax-3 spectrofluorometer with excitation/emission wavelengths set at 366/450 nm. Typically, decay in the fluorescence intensity was recorded over a time course of 6000 s with data points taken every 60 s.

[060] *Counter screening assay* - Kinase activity of the type I and II PKA holoenzyme are measured spectrophotometrically in a 96-well plate with a coupled enzyme assay as described previously (Cook et al. (1982) *Biochemistry* 21: 5794-5799). In this assay, the formation of ADP is coupled to the oxidation of NADH by the pyruvate kinase/lactate dehydrogenase reactions so the reaction rate can be determined by following the oxidation of NADH, reflected by a decrease in absorbance at 340 nm. The kinase reaction mixture (100 µl) contains 50 mM Mops (pH 7.0), 10 mM $MgCl_2$, 1 mM ATP, 1 mM PEP, 0.1 mM NADH, 8 U of pyruvate kinase, 15 U of lactate dehydrogenase, fixed amount of type I or type II PKA holoenzyme, and 0.1 mM cAMP, with or without 25 µM of test compound. Reactions are pre-equilibrated at room temperature and initiated by adding the Kemptide substrate (final concentration 0.26 mM). PKA activity measured in the presence of 25 µM H89, a selective PKA inhibitor, are used as a positive control of PKA inhibition.

[061] Once a compound is identified as having an Epac modulating activity, a number of analogs and variations are designed to produce an Epac inhibitor with appropriate pharmacologic characteristics.

[062] Using Epac assays, several Epac inhibitors have been identified that are capable of blocking biochemical and cellular cAMP-induced EPAC activation (Tsalkova et al. (2012) *Proc. Acad. Natl. Sci. USA.* 109:18613-18618). A number of chemical analogs of Epac specific inhibitors (ESI) have been synthesized and characterized (Chen et al. (2012) *Bioorganic & Medicinal Chemistry Letters.* 22:4038-4043; Chen et al. (2013) *J. Med. Chem.* 56(3):952-62; Chen et al. (2013) *Tetrahedron Lett.* 54(12):1546-1549). Some of these chemical analogs displayed more potent Epac inhibition activity and better pharmacological properties than parental compounds.

[063] In certain embodiments the Epac inhibitor is α-[2-(3-Chlorophenyl)hydrazinylidene]-5-(1,1-dimethylethyl)-b-oxo-3-isoxazolepropanenitrile (ESI-09).

Table 1: Apparent IC_{50} values of ESIs for competing with 8-NBD-cAMP in binding Epac2.

Compound	Apparent IC_{50} (μM)	Relative Potency (RA)*
cAMP	39 ± 2.0	1.0
ESI-04	6.7 ± 0.7	5.8
ESI-05	0.48 ± 0.03	81
ESI-06	1.0 ± 0.2	39
ESI-07	0.67 ± 0.03	57
ESI-08	8.7 ± 1.1	4.5
ESI-09	10 ± 1.2	3.9
ESI-10	18 ± 2.0	2.2

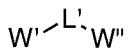
*RA = $IC_{50,\ cAMP}$/ $IC_{50,\ compound}$

Table 2: Apparent IC_{50} values of ESIs for suppressing Epac1 and Epac2 GEF activities.

Compound	EPAC1 IC_{50} (μM)	EPAC2 IC_{50} (μM)
ESI-05	NMA*	0.43 ± 0.06
ESI-07	NMA*	0.72 ± 0.08
ESI-09	3.2 ± 0.4	1.4 ± 0.1

*NMA: no measurable activity

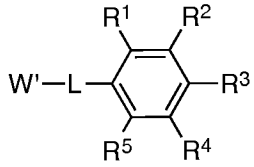
[064] Certain embodiments are directed to an isolated Exchange Protein Activated by cAMP (EPAC) modulating compound having a general formula of Formula I:



Formula I

where L' is -SO_2-, -NH-, or -C(O)-C(CN)=N-NH-; and W′ and W′′ are independently substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl.

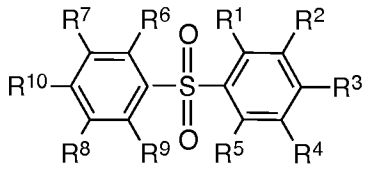
[065] Further embodiments are directed to an isolated Exchange Protein Activated by cAMP (EPAC) modulating compound having a general formula of Formula II:



Formula II

where R^1, R^2, R^3, R^4, and R^5 are independently hydrogen, hydroxyl, halogen, C_1-C_4 alkoxy; substituted or unsubstituted C_1-C_{10} alkyl, substituted or unsubstituted C_1-C_{10} heteroalkyl, substituted or unsubstituted C_5-C_7 cycloakyl, substituted or unsubstituted C_5-C_7 heterocyclyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, or C_1-C_5 alkylamine; L is $-SO_2-$ or -NH-; and W′ is as described above for Formula I. In a further aspect, L is $-SO_2-$. In certain aspects W′ is substituted phenyl or N-containing heteroaryl. In yet another aspect, a nitrogen in the N-containing heteroaryl is attached to L.

[066] An isolated Exchange Protein Activated by cAMP (EPAC) modulating compound having a general formula of Formula III:



Formula III

where R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9 , and R^{10} are independently hydrogen, hydroxyl, halogen, C_1-C_4 alkoxy, substituted or unsubstituted C_1-C_{10} alkyl, substituted or unsubstituted C_1-C_{10} heteroalkyl, substituted or unsubstituted C_5-C_7 cycloakyl, substituted or unsubstituted C_5-C_7 heterocyclyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, or C_1-C_5 alkylamine. In certain aspects R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, and R^{10} are independently hydrogen or C_1-C_{10} alkyl. In a further aspect, R^1, R^3, and R^5 are C_1-C_{10} alkyl; and R^2 and R^4 are hydrogen. In still further aspects, one or more of R^7, R^9, and R^{10} are C_1-C_{10} alkyl. In yet further aspects R^7, R^9, and R^{10} are C_1-C_{10} alkyl. In certain aspects R^{10} is substituted or unsubstituted C_1-C_4 alkyl or C_1-C_4 alkoxy. In yet other aspects, R^{10} is halide or halo-substituted heteroaryl.

[067] Certain embodiments are directed to a compound of Formula III where R^1, R^3, and R^5 are methyl; R^2 and R^4 are hydrogen; and (a) R^7, R^9, and R^{10} are C_1-C_{10} alkyl, and R^6 and

R^8 are hydrogen; (b) R^{10} is C_1-C_{10} alkyl, and R^6, R^7, R^8, R^9 are hydrogen; (c) R^{10} is C_1-C_4 alkoxy, and R^6, R^7, R^8, R^9 are hydrogen; (d) R^{10} is halogen, and R^6, R^7, R^8, R^9 are hydrogen; (e) R^{10} is hydroxyl, and R^6, R^7, R^8, R^9 are hydrogen; or (f) R^{10} is a halogen or C_{1-4} alkyl substituted pyridine, or a 2-, 4-, 5-, or 6-halo-pyridine, and R^6, R^7, R^8, R^9 are hydrogen.

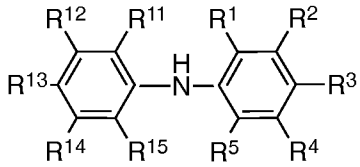
[068] Certain embodiments are directed to a compound of Formula III where R^1, R^3, and R^5 are methyl; R^2 and R^4 are hydrogen; and (a) R^7, R^9, and R^{10} are methyl, and R^6 and R^8 are hydrogen; (b) R^{10} is methyl, and R^6, R^7, R^8, R^9 are hydrogen; (c) R^{10} is methoxy, and R^6, R^7, R^8, R^9 are hydrogen; (d) R^{10} is iodo, and R^6, R^7, R^8, R^9 are hydrogen; (e) R^{10} is hydroxyl, and R^6, R^7, R^8, R^9 are hydrogen; or (f) R^{10} is 5-fluoro-pyridine and R^6, R^7, R^8, R^9 are hydrogen.

[069] Certain embodiments are directed to a compound of Formula III where R^3 is methyl; R^1, R^2, R^4, and R^5, are hydrogen; and (a) R^7, R^9, and R^{10} are C_1-C_{10} alkyl, and R^6 and R^8 are hydrogen; (b) R^{10} is C_1-C_{10} alkyl, and R^6, R^7, R^8, R^9 are hydrogen; (c) R^{10} is C_1-C_4 alkoxy, and R^6, R^7, R^8, R^9 are hydrogen; (d) R^{10} is halogen, and R^6, R^7, R^8, R^9 are hydrogen; (e) R^{10} is hydroxyl, and R^6, R^7, R^8, R^9 are hydrogen; or (f) R^{10} is a halogen, C_{1-4} alkyl substituted pyridine, or a 2-, 4-, 5-, or 6-halo-pyridine, and R^6, R^7, R^8, R^9 are hydrogen.

[070] Certain embodiments are directed to a compound of Formula III where R^3 is methyl; R^1, R^2, R^4, and R^5, are hydrogen; and (a) R^7, R^9, and R^{10} are methyl, and R^6 and R^8 are hydrogen; (b) R^{10} is methyl, and R^6, R^7, R^8, R^9 are hydrogen; (c) R^{10} is methoxy, and R^6, R^7, R^8, R^9 are hydrogen; (d) R^{10} is iodo, and R^6, R^7, R^8, R^9 are hydrogen; (e) R^{10} is hydroxyl, and R^6, R^7, R^8, R^9 are hydrogen; or (f) R^{10} is 5-fluoro-pyridine, and R^6, R^7, R^8, R^9 are hydrogen.

[071] In certain embodiments the compound of formula III is 1,3,5-trimethyl-2-(2,4,5-trimethyl-bensenesulfonyl)-benzene (HJC-2-71); 2-(4-methoxy-benzenesulfonyl)-1,3,5-trimethyl-benzene (HJC-2-82); 1,3,5-Trimethyl-2-(toluene-4-sulfonyl)-benzene (HJC-2-85); 4-(2,4,6-Trimethyl-benzenesulfonyl)-phenol (HJC-2-87); 2-(4-Iodo-benzenesulfonyl)-1,3,5-trimethyl-benzene (HJC-2-93); 2-Fluoro-5-[4-(2,4,6-trimethyl-benzenesulfonyl)-phenyl]-pyridine (HJC-2-97); or 1,2,4-Trimethyl-5-(toluene-4-sulfonyl)-benzene (HJC-2-98).

[072] Still a further embodiment is directed to an isolated Exchange Protein Activated by cAMP (EPAC) modulating compound having a general formula of Formula IV:



Formula IV

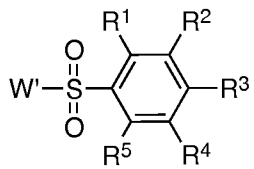
where R^1, R^2, R^3, R^4, and R^5 are as described for Formula III above; and R^{11}, R^{12}, R^{13}, R^{14}, and R^{15} are independently hydrogen, halogen, C_1-C_{10} alkyl, or C_1-C_{10} heteroalkyl. In certain aspects, R^1, R^3, and R^5 are C_1-C_{10} alkyl; and R^2 and R^4 are hydrogen. In a further aspect, R^{11}, R^{12}, R^{13}, R^{14}, and R^{15} are independently hydrogen, halogen, or C_1-C_{10} alkyl.

[073] Certain embodiments are directed to compounds of Formula IV where R^1, R^3, and R^5 are C_1-C_{10} alkyl; R^2 and R^4 are hydrogen; and (a) R^{11} and R^{14} are halogen, and R^{12}, R^{13}, and R^{15} are hydrogen; (b) R^{12} and R^{14} are halogen, and R^{11}, R^{13}, and R^{15} are hydrogen; or (c) R^{13} is C_1-C_{10} alkyl, and R^{11}, R^{12}, R^{14}, and R^{15} are hydrogen.

[074] Certain embodiments are directed to compounds of Formula IV where R^1, R^3, and R^5 are methyl; R^2 and R^4 are hydrogen; and (a) R^{11} and R^{14} are chloro, and R^{12}, R^{13}, and R^{15} are hydrogen; (b) R^{12} and R^{14} are chloro, and R^{11}, R^{13}, and R^{15} are hydrogen; or (c) R^{13} is methyl, and R^{11}, R^{12}, R^{14}, and R^{15} are hydrogen.

[075] In certain aspect the compound of formula IV is (3,5-Dichloro-phenyl)-(2,4,6-trimethyl-phenyl)-amine (HJC-2-83); *p*-Tolyl-(2,4,6-trimethyl-phenyl)-amine (HJC-2-89); or (2,5-Dichloro-phenyl)-(2,4,6-trimethyl-phenyl)-amine (HJC-3-38).

[076] Certain embodiments are directed to an isolated Exchange Protein Activated by cAMP (EPAC) modulating compound having a general formula of Formula V:



Formula V

where R^1, R^2, R^3, R^4, and R^5 are as described in Formula III above; and W′ is as described in Formula I above. In certain aspects, R^1, R^2, R^3, R^4, and R^5 are independently hydrogen, halogen, C_1-C_{10} alkyl, or C_1-C_{10} heteroalkyl. In certain aspects, W′ is substituted or

unsubstituted indole, substituted or unsubstituted azaindole, or substituted or unsubstituted pyrrole. In certain aspects, W′ is unsubstituted indole or unsubstituted azaindole. In a further aspect, W′ is pyrrole substituted with one or more C_1-C_{10} alkyl groups. In certain aspects, W′ is 1-ethylpyrrole or 2,4-dimethylpyrrole.

[077] Certain embodiments are directed to compounds of Formula V where R^1, R^3, and R^5 are C_1-C_{10} alkyl; R^2 and R^4 are hydrogen; and W′ is substituted or unsubstituted indole, substituted or unsubstituted azaindole, or substituted or unsubstituted pyrrole. In certain aspects, W′ is unsubstituted indole or unsubstituted azaindole. In a further aspect, W′ is pyrrole substituted with one or more C_1-C_{10} alkyl groups. In certain aspects, W′ is 1-ethylpyrrole or 2,4-dimethylpyrrole.

[078] Certain embodiments are directed to compounds of Formula V where R^1, R^3, and R^5 are methyl; R^2 and R^4 are hydrogen; and W′ is substituted or unsubstituted indole, substituted or unsubstituted azaindole, or substituted or unsubstituted pyrrole. In certain aspects, W′ is unsubstituted indole or unsubstituted 4-, 5-, 6-, or 7-azaindole. In a further aspect, W′ is pyrrole substituted with one or more methyl or ethyl. In certain aspects, W′ is 1-ethylpyrrole or 2,4-dimethylpyrrole.

[079] Certain embodiments are directed to compounds of Formula V where R^1 and R^3 are C_1-C_{10} alkyl; R^2, R^4, and R^5 are hydrogen; and W′ is substituted or unsubstituted indole, substituted or unsubstituted azaindole, or substituted or unsubstituted pyrrole. In certain aspects, W′ is unsubstituted indole or unsubstituted azaindole. In a further aspect, W′ is pyrrole substituted with one or more C_1-C_{10} alkyl groups. In certain aspects, W′ is 1-ethylpyrrole or 2,4-dimethylpyrrole.

[080] Certain embodiments are directed to compounds of Formula V where R^1 and R^3 are methyl; R^2, R^4, and R^5 are hydrogen; and W′ is substituted or unsubstituted indole, substituted or unsubstituted azaindole, or substituted or unsubstituted pyrrole. In certain aspects, W′ is unsubstituted indole or unsubstituted 4-, 5-, 6-, or 7-azaindole. In a further aspect, W′ is pyrrole substituted with one or more methyl or ethyl. In certain aspects, W′ is 1-ethylpyrrole or 2,4-dimethylpyrrole.

[081] Certain embodiments are directed to compounds of Formula V where R^2 and R^4 are C_1-C_{10} alkyl; R^1, R^3, and R^5 are hydrogen; and W′ is substituted or unsubstituted indole,

substituted or unsubstituted azaindole, or substituted or unsubstituted pyrrole. In certain aspects, W′ is unsubstituted indole or unsubstituted azaindole. In a further aspect, W′ is pyrrole substituted with one or more C_1-C_4 alkyl groups. In certain aspects, W′ is 1-ethylpyrrole or 2,4-dimethylpyrrole.

[082] Certain embodiments are directed to compounds of Formula V where R^2 and R^4 are methyl; R^1, R^3, and R^5 are hydrogen; and W′ is substituted or unsubstituted indole, substituted or unsubstituted azaindole, or substituted or unsubstituted pyrrole. In certain aspects, W′ is unsubstituted indole or unsubstituted 4-, 5-, 6-, or 7-azaindole. In a further aspect, W′ is pyrrole substituted with one or more methyl or ethyl. In certain aspects, W′ is 1-ethylpyrrole or 2,4-dimethylpyrrole.

[083] Certain embodiments are directed to compounds of Formula V where R^3 is C_1-C_{10} alkyl; R^1, R^2, R^4, and R^5 are hydrogen; and W′ is substituted or unsubstituted indole, substituted or unsubstituted azaindole, or substituted or unsubstituted pyrrole. In certain aspects, W′ is unsubstituted indole or unsubstituted azaindole. In a further aspect, W′ is pyrrole substituted with one or more C_1-C_{10} alkyl groups. In certain aspects, W′ is 1-ethylpyrrole or 2,4-dimethylpyrrole.

[084] Certain embodiments are directed to compounds of Formula V where R^3 is methyl; R^1, R^2, R^4, and R^5 are hydrogen; and W′ is substituted or unsubstituted indole, substituted or unsubstituted azaindole, or substituted or unsubstituted pyrrole. In certain aspects, W′ is unsubstituted indole or unsubstituted 4-, 5-, 6-, or 7-azaindole. In a further aspect, W′ is pyrrole substituted with one or more methyl or ethyl. In certain aspects, W′ is 1-ethylpyrrole or 2,4-dimethylpyrrole.

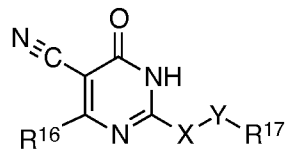
[085] Certain embodiments are directed to compounds of Formula V where R^1 is C_1-C_{10} alkyl; R^2, R^3, R^4, and R^5 are hydrogen; and W′ is substituted or unsubstituted indole, substituted or unsubstituted azaindole, or substituted or unsubstituted pyrrole. In certain aspects, W′ is unsubstituted indole or unsubstituted azaindole. In a further aspect, W′ is pyrrole substituted with one or more C_1-C_{10} alkyl groups. In certain aspects, W′ is 1-ethylpyrrole or 2,4-dimethylpyrrole.

[086] Certain embodiments are directed to compounds of Formula V where R^1 is methyl; R^2, R^3, R^4, and R^5 are hydrogen; and W′ is substituted or unsubstituted indole,

substituted or unsubstituted azaindole, or substituted or unsubstituted pyrrole. In certain aspects, W′ is unsubstituted indole or unsubstituted 4-, 5-, 6-, or 7-azaindole. In a further aspect, W′ is pyrrole substituted with one or more methyl or ethyl. In certain aspects, W′ is 1-ethylpyrrole or 2,4-dimethylpyrrole.

[087] In certain embodiments the compound of Formula V is 1-(2,4,6-Trimethyl-benzenesulfonyl)-1*H*-indole (HJC-2-77); 2-Ethyl-1-(2,4,6-trimethyl-benzenesulfonyl)-1*H*-pyrrole (HJC-2-79); 1-(2,4,6-Trimethyl-benzenesulfonyl)-1*H*-pyrrolo[2,3-b]pyridine (HJC-2-81); 1-(2,4,6-Trimethyl-benzenesulfonyl)-1*H*-pyrrolo[2,3-c]pyridine (HJC-3-21); 1-(2,4,6-Trimethyl-benzenesulfonyl)-1*H*-pyrrolo[3,2-c]pyridine (HJC-3-22); 1-(2,4,6-Trimethyl-benzenesulfonyl)-1*H*-pyrrolo[3,2-b]pyridine (HJC-3-23); 2-Ethyl-1-(toluene-4-sulfonyl)-1*H*-pyrrole (HJC-3-26); 2,4-Dimethyl-1-(2,4,6-trimethyl-benzenesulfonyl)-1H-pyrrole (HJC-3-50); 2-Ethyl-1-(toluene-2-sulfonyl)-1*H*-pyrrole (HJC-3-53); 1-(3,5-Dimethyl-benzenesulfonyl)-2-ethyl-1*H*-pyrrole (HJC-3-54); 1-(2,4-Dimethyl-benzenesulfonyl)-2-ethyl-1*H*-pyrrole (HJC-3-55); or 1-(2,4,6-Trimethyl-benzenesulfonyl)-1*H*-indole-5-carboxylic acid (HJC-3-62).

[088] Certain embodiments are directed to an isolated Exchange Protein Activated by cAMP (EPAC) modulating compound having a formula of:



Formula VI

where R^{16} is substituted or unsubstituted C_1-C_{10} alkyl, substituted or unsubstituted C_3-C_6 heteroalkyl, substituted or unsubstituted C_3-C_6 cycloalkyl, substituted or unsubstituted C_3-C_6 heterocyclyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl; R^{17} is hydrogen, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl; X is sulfur or nitrogen; and Y is a direct bond, -CH_2-, -$CH_2C(O)O$-, or –$CH_2C(O)N$-. Formula VI represents an alternative embodiment of Formula I, where W' is a substituted pyrimidine, and L is a particular linker designated by –X-Y-.

[089] Certain embodiments are directed to compounds of Formula VI where X is sulfur; Y is –CH_2-; R^{16} is as described above for Formula VI; and R^{17} is as described above for

Formula VI. In certain aspects R^{17} is as described above for Formula VI; and R^{16} is (a) C_3-C_6 cycloakyl, (b) C_6 cycloakyl, (c) C_5 cycloalkyl, (d) C_4 cycloalkyl, (e) C_3 cycloalkyl, (f) branched or linear C_1-C_{10} alkyl, or (g) branched C_3 alkyl. In certain aspects, R^{17} is substituted phenyl. In certain aspects, R^{17} is a C_1-C_{10} alkyl substituted phenyl. In further aspects, the substituted phenyl has 1, 2, or 3 C_1-C_{10} alkyl substituents. In certain aspects the C_1-C_{10} alkyl substituents are at positions 1, 3, and 5; 2 and 5; 2 and 4; 1 and 3; or 3 of the phenyl group. In a further aspect, R^{17} is 3,6-dimethylphenyl; 3,5-dimethylphenyl; or 2,4-dimethylphenyl. In yet a further aspect, R^{17} is 2,4,6-trimethylphenyl.

[090] Certain embodiments are directed to compounds of Formula VI where X is sulfur; Y is $-CH_2C(O)N-$; R^{16} is as described above for Formula VI; and R^{17} is as described above for Formula VI. In certain aspects R^{17} is as described above for Formula VI; and R^{16} is (a) C_3-C_6 cycloakyl, (b) C_6 cycloakyl, (c) C_5 cycloalkyl, (d) C_4 cycloalkyl, (e) C_3 cycloalkyl, (f) branched or linear C_1-C_{10} alkyl, or (g) branched C_3 alkyl. In certain aspects, R^{17} is substituted phenyl. In certain aspects, R^{17} is a C_1-C_{10} alkyl substituted phenyl. In further aspects, the substituted phenyl has 1, 2, or 3 C_1-C_{10} alkyl substituents. In certain aspects the C_1-C_{10} alkyl substituents are at positions 1, 3, and 5; 2 and 5; 2 and 4; 1 and 3; or 3 of the phenyl group. In a further aspect, R^{17} is 3,6-dimethylphenyl; 3,5-dimethylphenyl; or 2,4-dimethylphenyl. In yet a further aspect, R^{17} is 2,4,6-trimethylphenyl.

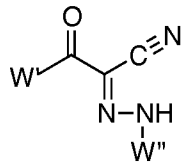
[091] Certain embodiments are directed to compounds of Formula VI where X is nitrogen; Y is $-CH_2-$; R^{16} is as described above for Formula VI; and R^{17} is as described above for Formula VI. In certain aspects R^{17} is as described above for Formula VI; and R^{16} is (a) C_3-C_6 cycloakyl, (b) C_6 cycloakyl, (c) C_5 cycloalkyl, (d) C_4 cycloalkyl, (e) C_3 cycloalkyl, (f) branched or linear C_1-C_{10} alkyl, or (g) branched C_3 alkyl. In certain aspects, R^{17} is substituted phenyl. In certain aspects, R^{17} is a C_1-C_{10} alkyl substituted phenyl. In further aspects, the substituted phenyl has 1, 2, or 3 C_1-C_{10} alkyl substituents. In certain aspects the C_1-C_{10} alkyl substituents are at positions 1, 3, and 5; 2 and 5; 2 and 4; 1 and 3; or 3 of the phenyl group. In a further aspect, R^{17} is 3,6-dimethylphenyl; 3,5-dimethylphenyl; or 2,4-dimethylphenyl. In yet a further aspect, R^{17} is 2,4,6-trimethylphenyl.

[092] Certain embodiments are directed to compounds of Formula VI where X is nitrogen; Y is a direct bond; R^{16} is as described above for Formula VI; and R^{17} is as described above for Formula VI. In certain aspects R^{17} is as described above for Formula VI; and R^{16} is (a) C_3-C_6 cycloakyl, (b) C_6 cycloakyl, (c) C_5 cycloalkyl, (d) C_4 cycloalkyl, (e) C_3 cycloalkyl,

(f) branched or linear C_1-C_{10} alkyl, or (g) branched C_3 alkyl. In certain aspects, R^{17} is substituted phenyl. In certain aspects, R^{17} is a C_1-C_{10} alkyl substituted phenyl. In further aspects, the substituted phenyl has 1, 2, or 3 C_1-C_{10} alkyl substituents. In certain aspects the C_1-C_{10} alkyl substituents are at positions 1, 3, and 5; 2 and 5; 2 and 4; 1 and 3; or 3 of the phenyl group. In a further aspect, R^{17} is 3,6-dimethylphenyl; 3,5-dimethylphenyl; or 2,4-dimethylphenyl. In yet a further aspect, R^{17} is 2,4,6-trimethylphenyl.

[093] In certain embodiments a compound of Formula VI is 4-Cyclohexyl-2-(2,5-dimethyl-benzylsulfanyl)-6-oxo-1,6-dihydro-pyrimidine-5-carbonitrile (HJC-1-65); 4-Cyclohexyl-2-(4-methyl-benzylsulfanyl)-6-oxo-1,6-dihydro-pyrimidine-5-carbonitrile (HJC-1-67); 4-Cyclohexyl-2-(3,5-dimethyl-benzylsulfanyl)-6-oxo-1,6-dihydro-pyrimidine-5-carbonitrile (HJC-1-72); 4-Cyclohexyl-2-(2,4-dimethyl-benzylsulfanyl)-6-oxo-1,6-dihydro-pyrimidine-5-carbonitrile (HJC-1-74); 2-Benzylsulfanyl-4-cyclohexyl-6-oxo-1,6-dihydro-pyrimidine-5-carbonitrile (HJC-1-76); 4-Cyclohexyl-6-oxo-2-(2,4,6-trimethyl-benzylsulfanyl)-1,6-dihydro-pyrimidine-5-carbonitrile (HJC-1-87); 2-(2,5-Dimethyl-benzylsulfanyl)-4-isopropyl-6-oxo-1,6-dihydro-pyrimidine-5-carbonitrile (HJC-1-95); 4-Cyclopentyl-2-(2,5-dimethyl-benzylsulfanyl)-6-oxo-1,6-dihydro-pyrimidine-5-carbonitrile (HJC-1-97); 4-Cyclopropyl-2-(2,5-dimethylbenzylsulfanyl)-6-oxo-1,6-dihydro-pyrimidine-5-carbonitrile (HJC-1-98); 4-Cyclohexyl-6-oxo-2-phenylamino-1,6-dihydro-pyrimidine-5-carbonitrile (HJC-1-99); 4-[5-Cyano-2-(2,5-dimethylbenzylsulfanyl)-6-oxo-1,6-dihydro-pyrimidin-4-yl]-piperidine-1-carboxylic acid tert-butyl ester (HJC-1-93); (5-Cyano-4-cyclohexyl-6-oxo-1,6-dihydro-pyrimidin-2-ylsulfanyl)-acetic acid (HJC-2-4); 2-(5-Cyano-4-cyclohexyl-6-oxo-1,6-dihydro-pyrimidin-2-ylsulfanyl)-*N*-(2,4,6-trimethyl-phenyl)-acetamide (HJC-3-33); or 2-(5-Cyano-4-cyclohexyl-6-oxo-1,6-dihydro-pyrimidin-2-ylsulfanyl)-*N*-*p*-tolyl-acetamide (HJC-3-35).

[094] Certain embodiments are directed to an isolated Exchange Protein Activated by cAMP (EPAC) modulating compound having a formula of:



Formula VII

in certain aspects W′ and W″ are as described for Formula I above.

[095] In certain embodiments W' is an unsubstituted or substituted isoxazole. In certain aspects the isoxazole is attached via the 3 position. In certain aspects the substituted isoxazole is a 4-substituted isoxazole, a 5-substituted isoxazole, or a 4,5-substituted isoxazole. In a particular aspect the substituted isoxazole is a 5-substituted isoxazole. In certain aspects the substituent is independently a branched or unbranched C_1 to C_{10} alkyl. In certain aspect the alkyl is a methyl, ethyl, n-propyl, iso-propyl, n-butyl, *sec*-butyl, *iso*-butyl, *tert*-butyl, *neo*-pentyl, n-pentyl, or isopenyl. In certain embodiments the isoxazole is a 5-methyl or 5 *tert*-butyl isoxazole. In a further aspect W' can be a substituted to unsubstituted phenyl.

[096] In certain embodiments W'' is a monocyclic or polycyclic, substituted or unsubstituted aryl or heteroaryl. In certain aspects W'' is a substituted phenyl or N-containing heteroaryl. In a further aspect the substituted phenyl is a 2; 3; 4; 5; 6; 2,3; 2,4; 2,5; 2,6; 3,4; 3,5; 3,6; 4,5; 4,6; or 5,6 substituted phenyl. In still further aspects the phenyl comprises one or more substituent selected from bromo, fluoro, chloro, iodo, C_1-C_4 alkyl, hydroxy, nitro, fluoromethyl, difluoromethyl, trifluoromethyl, nitrile, C_1-C_4 alkynyl, acetyl, C_1-C_4 hydroxyalkyl, C_1-C_4 alkoxy, or carboxyl group. In certain aspects W'' is a substituted or unsubstituted benzopyridine or a substituted or unsubstituted indane. In certain aspects W'' is a 3-chlorophenyl; 2-chlorophenyl; 4-chlorophenyl; phenyl; 3,6-dichlorophenyl; 3-methylphenyl, 3-trifluoromethylphenyl; 3-nitrophenyl; 4-methylphenyl, 3,5-dichlorophenyl; 4-bromophenyl; 3-bromophenyl; 3,6-dimethylphenyl; benzopyridine; 2,3-dichlorophenyl; 3-ethynyl; benzoic acid ethyl ester; 3-benzonitrile; 3-acetylphenyl; 2,3-methylphenyl; 3-ethoxyphenyl; indane; 3,5-di-trifluoromethylphenyl; 6-chloro-benzoic acid; or 3-chloro, 4-hydroxyphenyl.

[097] In certain aspects a compound of Formula VII is selected from *N*-(5-*tert*-Butyl-isoxazol-3-yl)-2-[(3-chlorophenyl)-hydrazono]-2-cyanoacetamide (HJC0683); 2-[(3-Chlorophenyl)-hydrazono]-2-cyano-*N*-(5-methyl-isoxazol-3-yl)acetamide (HJC0692); 3-(5-*tert*-Butyl-isoxazol-3-yl)-2-[(3-chlorophenyl)-hydrazono]-3-oxo-propionitrile (HJC0680, ESI-09); 3-(5-*tert*-Butyl-isoxazol-3-yl)-2-[(2-chlorophenyl)-hydrazono]-3-oxo-propionitrile (HJC0693); 3-(5-*tert*-Butyl-isoxazol-3-yl)-2-[(4-chlorophenyl)-hydrazono]-3-oxo-propionitrile (HJC0694); 3-(5-*tert*-Butyl-isoxazol-3-yl)-3-oxo-2-(phenyl-hydrazono)-propionitrile (HJC0695); 3-(5-*tert*-Butyl-isoxazol-3-yl)-2-[(2,5-dichlorophenyl)-hydrazono]-3-oxo-propionitrile (HJC0696); 3-(5-*tert*-Butyl-isoxazol-3-yl)-3-oxo-2-(m-tolyl-

hydrazono)propionitrile (HJC0712); 3-(5-*tert*-Butyl-isoxazol-3-yl)-3-oxo-2-[(3-trifluoromethyl-phenyl)-hydrazono]propionitrile (HJC0720); 3-(5-*tert*-Butyl-isoxazol-3-yl)-2-[(3-nitrophenyl)-hydrazono]-3-oxo-propionitrile (HJC0721); 3-(5-*tert*-Butyl-isoxazol-3-yl)-3-oxo-2-(p-tolyl-hydrazono)propionitrile (HJC0724); 3-(5-*tert*-Butyl-isoxazol-3-yl)-2-[(3,5-dichlorophenyl)-hydrazono]-3-oxo-propionitrile (HJC0726); 2-[(4-Bromophenyl)-hydrazono]-3-(5-*tert*-butyl-isoxazol-3-yl)-3-oxo-propionitrile (HJC0742); 2-[(3-Bromophenyl)-hydrazono]-3-(5-*tert*-butyl-isoxazol-3-yl)-3-oxo-propionitrile (HJC0743); 3-(5-*tert*-Butyl-isoxazol-3-yl)-2-[(2,5-dimethylphenyl)-hydrazono]-3-oxo-propionitrile (HJC0744); 3-(5-*tert*-Butyl-isoxazol-3-yl)-3-oxo-2-(quinolin-6-yl-hydrazono)propionitrile (HJC0745); 3-(5-*tert*-Butyl-isoxazol-3-yl)-2-[(2,3-dichlorophenyl)-hydrazono]-3-oxo-propionitrile (HJC0750); 3-(5-*tert*-Butyl-isoxazol-3-yl)-2-[(3-ethynyl-phenyl)-hydrazono]-3-oxo-propionitrile (HJC0751); 3-{*N*'-[2-(5-*tert*-Butyl-isoxazol-3-yl)-1-cyano-2-oxo-ethylidene]-hydrazino}benzoic acid ethyl ester (HJC0752); 3-{*N*'-[2-(5-*tert*-Butyl-isoxazol-3-yl)-1-cyano-2-oxo-ethylidene]-hydrazino}benzonitrile (HJC0753); 2-[(3-Acetyl-phenyl)-hydrazono]-3-(5-*tert*-butyl-isoxazol-3-yl)-3-oxo-propionitrile (HJC0754); 3-(5-*tert*-Butyl-isoxazol-3-yl)-2-[(2,3-dimethylphenyl)-hydrazono]-3-oxo-propionitrile (HJC0755); 3-(5-*tert*-Butyl-isoxazol-3-yl)-2-[(3-hydroxymethylphenyl)-hydrazono]-3-oxo-propionitrile (HJC0756); 3-(5-*tert*-Butyl-isoxazol-3-yl)-2-(indan-5-yl-hydrazono)-3-oxo-propionitrile (HJC0757); 2-[(3,5-Bis-trifluoromethyl-phenyl)-hydrazono]-3-(5-*tert*-butyl-isoxazol-3-yl)-3-oxo-propionitrile (HJC0758); 2-{*N*'-[2-(5-*tert*-Butyl-isoxazol-3-yl)-1-cyano-2-oxo-ethylidene]-hydrazino}-6-chloro-benzoic acid (HJC0759); 3-(5-*tert*-Butyl-isoxazol-3-yl)-2-[(3-chloro-4-hydroxy-phenyl)-hydrazono]-3-oxo-propionitrile (HJC0760); 2-[(3-Chloro-phenyl)-hydrazono]-3-(5-methyl-isoxazol-3-yl)-3-oxo-propionitrile (HJC0768); or 2-[(3,5-Dichlorophenyl)-hydrazono]-3-(5-methyl-isoxazol-3-yl)-3-oxo-propionitrile (HJC0770).

III. CHEMICAL DEFINITIONS

[098] Various chemical definitions related to EPAC modulating compounds are provided as follows.

[099] As used herein, "predominantly one enantiomer" means that the compound contains at least 85% of one enantiomer, or more preferably at least 90% of one enantiomer, or even more preferably at least 95% of one enantiomer, or most preferably at least 99% of one enantiomer. Similarly, the phrase "substantially free from other optical isomers" means that the composition contains at most 5% of another enantiomer or diastereomer, more

preferably 2% of another enantiomer or diastereomer, and most preferably 1% of another enantiomer or diastereomer. In certain aspects, one, both, or the predominant enantiomer forms or isomers are all covered.

[0100] As used herein, the term "nitro" means $-NO_2$; the term "halo" or "halogen" designates -F, -Cl, -Br or -I; the term "mercapto" means -SH; the term "cyano" means -CN; the term "azido" means $-N_3$; the term "silyl" means $-SiH_3$, and the term "hydroxy" means -OH.

[0101] The term "alkyl," by itself or as part of another substituent, means, unless otherwise stated, a linear (i.e. unbranched) or branched carbon chain of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 carbons, which may be fully saturated, monounsaturated, or polyunsaturated. An unsaturated alkyl group includes those having one or more carbon-carbon double bonds (alkenyl) and those having one or more carbon-carbon triple bonds (alkynyl). The groups, $-CH_3$ (Me, methyl), $-CH_2CH_3$ (Et, ethyl), $-CH_2CH_2CH_3$ (*n*-Pr, n-propyl), $-CH(CH_3)_2$ (*iso*-Pr, iso-propyl), $-CH_2CH_2CH_2CH_3$ (*n*-Bu, n-butyl), $-CH(CH_3)CH_2CH_3$ (*sec*-butyl), $-CH_2CH(CH_3)_2$ (*iso*-butyl), $-C(CH_3)_3$ (*tert*-butyl), $-CH_2C(CH_3)_3$ (*neo*-pentyl), are all non-limiting examples of alkyl groups.

[0102] The term "heteroalkyl," by itself or in combination with another term, means, unless otherwise stated, a linear or branched chain having at least one carbon atom and at least one heteroatom selected from the group consisting of O, N, S, P, and Si. In certain embodiments, the heteroatoms are selected from the group consisting of O, S, and N. The heteroatom(s) may be placed at any interior position of the heteroalkyl group or at the position at which the alkyl group is attached to the remainder of the molecule. Up to two heteroatoms may be consecutive. The following groups are all non-limiting examples of heteroalkyl groups: trifluoromethyl, $-CH_2F$, $-CH_2Cl$, $-CH_2Br$, $-CH_2OH$, $-CH_2OCH_3$, $-CH_2OCH_2CF_3$, $-CH_2OC(O)CH_3$, $-CH_2NH_2$, $-CH_2NHCH_3$, $-CH_2N(CH_3)_2$, $-CH_2CH_2Cl$, $-CH_2CH_2OH$, $CH_2CH_2OC(O)CH_3$, $-CH_2CH_2NHCO_2C(CH_3)_3$, and $-CH_2Si(CH_3)_3$.

[0103] The terms "cycloalkyl" and "heterocyclyl," by themselves or in combination with other terms, means cyclic versions of "alkyl" and "heteroalkyl", respectively. Additionally, for heterocyclyl, a heteroatom can occupy the position at which the heterocycle is attached to the remainder of the molecule. Examples of cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and cycloheptyl groups. Examples of heterocyclic

groups include indole, azetidinyl, pyrrolidinyl, pyrrolyl, pyrazolyl, oxetanyl, pyrazolinyl, imidazolyl, imidazolinyl, imidazolidinyl, oxazolyl, oxazolidinyl, isoxazolinyl, isoxazolyl, thiazolyl, thiadiazolyl, thiazolidinyl, isothiazolyl, isothiazolidinyl, furyl, tetrahydrofuryl, thienyl, oxadiazolyl, piperidinyl, piperazinyl, 2-oxopiperazinyl, 2-oxopiperidinyl, 2-oxopyrrolodinyl, 2-oxoazepinyl, azepinyl, hexahydrodiazepinyl, 4-piperidonyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, triazinyl, triazolyl, tetrazolyl, tetrahydropyranyl, morpholinyl, thiamorpholinyl, thiamorpholinyl sulfoxide, thiamorpholinyl sulfone, 1,3-dioxolane, tetrahydro-1,1-dioxothienyl, and the like.

[0104] The term "aryl" means a polyunsaturated, aromatic, hydrocarbon substituent. Aryl groups can be monocyclic or polycyclic (e.g., 2 to 3 rings that are fused together or linked covalently). The term "heteroaryl" refers to an aryl group that contains one to four heteroatoms selected from N, O, and S. A heteroaryl group can be attached to the remainder of the molecule through a carbon or heteroatom. Non-limiting examples of aryl and heteroaryl groups include phenyl, 4-azaindole, 5-azaindole, 6-azaindole, 7-azaindole, 1-naphthyl, 2-naphthyl, 4-biphenyl, 1-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl, 3-pyrazolyl, 2-imidazolyl, 4-imidazolyl, pyrazinyl, 2-oxazolyl, 4-oxazolyl, 2-phenyl-4-oxazolyl, 5-oxazolyl, 3-isoxazolyl, 4-isoxazolyl, 5-isoxazolyl, 2-thiazolyl, 4-thiazolyl, 5-thiazolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidyl, 4-pyrimidyl, 5-benzothiazolyl, purinyl, 2-benzimidazolyl, 5-indolyl, 1-isoquinolyl, 5-isoquinolyl, 2-quinoxalinyl, 5-quinoxalinyl, 3-quinolyl, and 6-quinolyl. Substituents for each of the above noted aryl and heteroaryl ring systems are selected from the group of acceptable substituents described below.

[0105] Various groups are described herein as substituted or unsubstituted (i.e., optionally substituted). Optionally substituted groups may include one or more substituents independently selected from: halogen, nitro, cyano, hydroxy, amino, mercapto, formyl, carboxy, oxo, carbamoyl, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, alkoxy, alkylthio, alkylamino, $(alkyl)_2$amino, alkylsulfinyl, alkylsulfonyl, arylsulfonyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl. In certain aspects the optional substituents may be further substituted with one or more substituents independently selected from: halogen, nitro, cyano, hydroxy, amino, mercapto, formyl, carboxy, carbamoyl, unsubstituted alkyl, unsubstituted heteroalkyl, alkoxy, alkylthio,

alkylamino, $(alkyl)_2$amino, alkylsulfinyl, alkylsulfonyl, arylsulfonyl, unsubstituted cycloalkyl, unsubstituted heterocyclyl, unsubstituted aryl, or unsubstituted heteroaryl. Examples of optional substituents include, but are not limited to: -OH, oxo (=O), -Cl, -F, -Br, C_{1-4}alkyl, phenyl, benzyl, $-NH_2$, $-NH(C_{1-4}alkyl)$, $-N(C_{1-4}alkyl)_2$, $-NO_2$, $-S(C_{1-4}alkyl)$, $-SO_2(C_{1-4}alkyl)$, $-CO_2(C_{1-4}alkyl)$, and $-O(C_{1-4}alkyl)$.

[0106] The term "alkoxy" means a group having the structure –OR', where R' is an optionally substituted alkyl or cycloalkyl group. The term "heteroalkoxy" similarly means a group having the structure -OR, where R is a heteroalkyl or heterocyclyl.

[0107] The term "amino" means a group having the structure –NR'R", where R' and R" are independently hydrogen or an optionally substituted alkyl, heteroalkyl, cycloalkyl, or heterocyclyl group. The term "amino" includes primary, secondary, and tertiary amines.

[0108] The term "oxo" as used herein means oxygen that is double bonded to a carbon atom.

[0109] The term "pharmaceutically acceptable salts," as used herein, refers to salts of compounds of this invention that are substantially non-toxic to living organisms. Typical pharmaceutically acceptable salts include those salts prepared by reaction of a compound of this invention with an inorganic or organic acid, or an organic base, depending on the substituents present on the compounds of the invention.

[0110] Non-limiting examples of inorganic acids which may be used to prepare pharmaceutically acceptable salts include: hydrochloric acid, phosphoric acid, sulfuric acid, hydrobromic acid, hydroiodic acid, phosphorous acid and the like. Examples of organic acids which may be used to prepare pharmaceutically acceptable salts include: aliphatic mono- and dicarboxylic acids, such as oxalic acid, carbonic acid, citric acid, succinic acid, phenyl- heteroatom-substituted alkanoic acids, aliphatic and aromatic sulfuric acids and the like. Pharmaceutically acceptable salts prepared from inorganic or organic acids thus include hydrochloride, hydrobromide, nitrate, sulfate, pyrosulfate, bisulfate, sulfite, bisulfate, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, hydroiodide, hydro fluoride, acetate, propionate, formate, oxalate, citrate, lactate, p-toluenesulfonate, methanesulfonate, maleate, and the like.

[0111] Suitable pharmaceutically acceptable salts may also be formed by reacting the agents of the invention with an organic base, such as methylamine, ethylamine, ethanolamine, lysine, ornithine and the like. Pharmaceutically acceptable salts include the salts formed between carboxylate or sulfonate groups found on some of the compounds of this invention and inorganic cations, such as sodium, potassium, ammonium, or calcium, or such organic cations as isopropylammonium, trimethylammonium, tetramethylammonium, and imidazolium.

[0112] It should be recognized that the particular anion or cation forming a part of any salt of this invention is not critical, so long as the salt, as a whole, is pharmacologically acceptable.

[0113] Additional examples of pharmaceutically acceptable salts and their methods of preparation and use are presented in Handbook of Pharmaceutical Salts: Properties, Selection and Use (2002), which is incorporated herein by reference.

[0114] An "isomer" of a first compound is a separate compound in which each molecule contains the same constituent atoms as the first compound, but where the three dimensional configuration of those atoms differs. Unless otherwise specified, the compounds described herein are meant to encompass their isomers as well. A "stereoisomer" is an isomer in which the same atoms are bonded to the same other atoms, but where the configuration of those atoms in three dimensions differs. "Enantiomers" are stereoisomers that are mirror images of each other, like left and right hands. "Diastereomers" are stereoisomers that are not enantiomers.

[0115] It is contemplated that any embodiment discussed in this specification can be implemented with respect to any method or composition of the invention, and vice versa. Furthermore, compositions of the invention can be used to achieve methods of the invention.

IV. PHARMACEUTICAL FORMULATIONS AND ADMINISTRATION

[0116] In certain aspects, the invention provides compositions comprising one or more Epac inhibitor with one or more of: a pharmaceutically acceptable diluent; a carrier; a solubilizer; an emulsifier; and/or a preservative. Such compositions may contain an effective amount of at least one Epac inhibitor. Thus, the use of one or more Epac inhibitor for the

preparation of a medicament is also included. Such compositions can be used in the treatment of a variety of Epac associated diseases or conditions, such as microbial infections.

[0117] An Epac inhibitor may be formulated into therapeutic compositions in a variety of dosage forms such as, but not limited to, liquid solutions or suspensions, tablets, pills, powders, suppositories, polymeric microcapsules or microvesicles, liposomes, and injectable or infusible solutions. The preferred form depends upon the mode of administration and the particular disease targeted. The compositions also preferably include pharmaceutically acceptable vehicles, or carriers well known in the art.

[0118] Acceptable formulation components for pharmaceutical preparations are nontoxic to recipients at the dosages and concentrations employed. In addition to the EPAC inhibitor(s), compositions may contain components for modifying, maintaining, or preserving, for example, the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption, or penetration of the composition. Suitable materials for formulating pharmaceutical compositions include, but are not limited to, amino acids (such as glycine, glutamine, asparagine, arginine or lysine); antimicrobials; antioxidants (such as ascorbic acid, sodium sulfite or sodium hydrogen-sulfite); buffers (such as acetate, borate, bicarbonate, Tris-HCl, citrates, phosphates or other organic acids); bulking agents (such as mannitol or glycine); chelating agents (such as ethylenediamine tetraacetic acid (EDTA)); complexing agents (such as caffeine, polyvinylpyrrolidone, beta-cyclodextrin or hydroxypropyl-beta-cyclodextrin); fillers; monosaccharides; disaccharides; and other carbohydrates (such as glucose, mannose or dextrins); proteins (such as serum albumin, gelatin or immunoglobulins); coloring, flavoring and diluting agents; emulsifying agents; hydrophilic polymers (such as polyvinylpyrrolidone); low molecular weight polypeptides; salt-forming counter ions (such as sodium); preservatives (such as benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid or hydrogen peroxide); solvents (such as glycerin, propylene glycol or polyethylene glycol); sugar alcohols (such as mannitol or sorbitol); suspending agents; surfactants or wetting agents (such as pluronics, PEG, sorbitan esters, polysorbates such as polysorbate 20, polysorbate 80, triton, tromethamine, lecithin, cholesterol, tyloxapal); stability enhancing agents (such as sucrose or sorbitol); tonicity enhancing agents (such as alkali metal halides, preferably sodium or potassium chloride, mannitol sorbitol); delivery vehicles; diluents; excipients and/or pharmaceutical adjuvants. (see *Remington's*

Pharmaceutical Sciences, 18 th Ed., (A. R. Gennaro, ed.), 1990, Mack Publishing Company), hereby incorporated by reference.

[0119] Formulation components are present in concentrations that are acceptable to the site of administration. Buffers are advantageously used to maintain the composition at physiological pH or at a slightly lower pH, typically within a pH range of from about 4.0 to about 8.5, or alternatively, between about 5.0 to 8.0. Pharmaceutical compositions can comprise TRIS buffer of about pH 6.5-8.5, or acetate buffer of about pH 4.0-5.5, which may further include sorbitol or a suitable substitute therefor.

[0120] The pharmaceutical composition to be used for *in vivo* administration is typically sterile. Sterilization may be accomplished by filtration through sterile filtration membranes. If the composition is lyophilized, sterilization may be conducted either prior to or following lyophilization and reconstitution. The composition for parenteral administration may be stored in lyophilized form or in a solution. In certain embodiments, parenteral compositions are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle, or a sterile pre-filled syringe ready to use for injection.

[0121] The above compositions can be administered using conventional modes of delivery including, but not limited to, intravenous, intraperitoneal, oral, and by perfusion. When administering the compositions by injection, the administration may be by continuous infusion or by single or multiple boluses. For parenteral administration, the EPAC modulating agents may be administered in a pyrogen-free, parenterally acceptable aqueous solution comprising the desired Epac inhibitor in a pharmaceutically acceptable vehicle. A particularly suitable vehicle for parenteral injection is sterile distilled water in which one or more Epac inhibitors are formulated as a sterile, isotonic solution, properly preserved.

[0122] Once the pharmaceutical composition of the invention has been formulated, it may be stored in sterile vials as a solution, suspension, gel, emulsion, solid, or as a dehydrated or lyophilized powder. Such formulations may be stored either in a ready-to-use form or in a form (e.g., lyophilized) that is reconstituted prior to administration.

[0123] If desired, stabilizers that are conventionally employed in pharmaceutical compositions, such as sucrose, trehalose, or glycine, may be used. Typically, such stabilizers will be added in minor amounts ranging from, for example, about 0.1% to about 0.5% (w/v).

Surfactant stabilizers, such as TWEEN®-20 or TWEEN®-80 (ICI Americas, Inc., Bridgewater, N.J., USA), may also be added in conventional amounts.

[0124] To determine the bioavailability of Epac inhibitors, an IP injection formulation was developed in which the compounds were dissolved in ethanol and then diluted 1:10 with a 10% Tween 80 in normal saline solution. This formulation was determined suitable by passing the simulated in vivo blood dilution assay. In vivo pharmacokinetic studies were performed in four week old female C57BL6/N mice. After one single intraperitoneal (IP) injection of the ESI-09 compound (10 mg/kg) in mice (n=5 for each time point), blood levels of ESI-09 were determined to be rapidly elevated reaching maximal values of 42,520 ng/ml (128 μM) at 0.5 hr with a half-life of 3.5 hrs. These results suggest that ESI-09 has an excellent bioactivity in vivo.

[0125] For the compounds of the present invention, alone or as part of a pharmaceutical composition, such doses are between about 0.001 mg/kg and 1 mg/kg body weight, preferably between about 1 and 100 μg/kg body weight, most preferably between 1 and 10 μg/kg body weight.

[0126] Therapeutically effective doses will be easily determined by one of skill in the art and will depend on the severity and course of the disease, the patient's health and response to treatment, the patient's age, weight, height, sex, previous medical history and the judgment of the treating physician.

[0127] In some methods of the invention, an Epac inhibitor is administered to a patient infected or at risk of infection by a microbe. In certain aspects the microbe is a virus. In such cases, embodiments may further involve treating the patient with the current standard of care for symptoms related to such an infection, e.g., fluids, mechanical ventilation, etc. Epac inhibitor compositions may be administered to the patient before, after, or at the same time as other therapies. Therapeutic compositions may be administered 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more times, and they may be administered every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 hours, or 1, 2, 3, 4, 5, 6, 7 days, or 1, 2, 3, 4, 5 weeks, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months.

V. EXAMPLES

[0128] The following examples as well as the figures are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples or figures represent techniques discovered by the inventors to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

EXAMPLE 1

INHIBITION OF EPAC INHIBITS VIRAL REPLICATION

[0129] Although intracellular cAMP plays a role in regulating host anti-microbial responses, its effect on MERS-CoV infection in permissive cells has not been previously investigated. The inventors have shown that human bronchial epithelial Calu-3 cells are highly permissive to MERS-CoV, resulting in acute and profound apoptosis (Tao et al. (2013) *J Virol.* 87(17):9953-9958). Since PKA and Epac serve as key mediators of cAMP signaling, to investigate if cAMP signaling participates in regulating the infection of virus, Calu-3 cells were pretreated with either H89 (LC Laboratories), a PKA-specific inhibitor (Marunaka and Niisato (2003) *Biochem Pharmacol.* 66(6):1083-1089), or an Epac-specific inhibitor (ESI)-09 (Almahariq et al. (2013) *Mol Pharmacol.* 83(1):122-128; Tsalkova et al. (2012) *Proc Natl Acad Sci U S A* 109(45):18613-18618), or DMSO (as carrier control) for 2 hrs before challenging the cells with MERS-CoV at a multiplicity of infection (MOI) of 0.1. Subsequent effects on infected cells were assessed by monitoring the formation of cytopathic effects (CPE) and yields of infectious progeny virus at 24 hrs post infection (pi).

[0130] The inventors found that prior treatment with ESI-09, but not H89, attenuated CPE formation (data not shown), and significantly reduced viral yields ($p < 0.001$) (FIG. 11A). To determine if ESI-09-mediated inhibition of MERS-CoV replication is limited to Calu-3 cells, the same experiment was performed using Vero E6 cells. FIG. 11B indicates that the ability of ESI-09 treatment to restrict MERS-CoV infection was cell type-independent, as results were similar with Vero E6 cells. It was also noted that significant reduction in virus yield occurred when cells were treated with ESI-09 at the concentrations between 5 to 40 μM in Calu-3 cells (FIG. 11C). As shown in FIG. 11D, the concentration of

ESI-09 required for causing 50% inhibition of cell survival (CC_{50}) was greater than 50 μM for both Calu-3 and Vero E6 cells, based on the LDH-based cytotoxicity assay (Promega), suggesting that the anti-MERS-CoV growth inhibition imposed by ESI-09 treatment at the concentration of 10 μM, was not because of drug cytotoxicity. To further investigate the effect of ESI-09 on MERS-CoV replication, Calu-3 cells grown in 8-well chamber slides (Nunc Lab-Tek) were treated with 10 μM of H89, ESI-09, or DMSO for 2 hrs prior to challenge with virus at an MOI of 0.1. The effect of ESI-09 was assessed by determining the yields of infectious virus and the expressions of CD26, the receptor of MERS-CoV (Raj et al. (2013) *Nature.* 495(7440):251-25421), and virus-specific antigens at 24 hrs p.i. by the standard indirect immunofluorescence (IIF) staining.

[0131] Stained specimens were analyzed with an inverted UV microscopy (Olympus 1X51). As shown in FIG. 12A, DMSO control and H89 treatment did not protect against MERS-CoV infection, as shown by the extensive CPE (i.e., detachment of monolayer) and readily detectable viral antigen. In contrast, Calu-3 cells treated with ESI-09 were almost fully protected, as indicated by unnoticeable CPE, and minimal expression of viral antigen. This capacity of ESI-09 to protect cells against MERS-CoV infection was consistent with the amount of infectious progeny viruses detected (FIG. 12B). To evaluate whether the anti-MERS-CoV activity of ESI-09 could be extended to include SARS-CoV, experiments were performed using the same treatment and infection strategy as described for MERS-CoV. Prior ESI-09, but not H89, treatment was also as effective in protecting cell cultures against SARS-CoV, resulting in nearly a 4-$\log_{10}$ reduction in viral titers (FIG. 12C).

[0132] As the extracellular domain of CD26 can be released into the circulation as soluble CD26 (Matsuno et al. (2007) *Biomark Insights.* 1:201-204; Ikushima et al. (2002) *Cell Immunol.* 215(1):106-110), studies were performed to determine whether ESI-09 treatment might reduce surface expression of CD26, thereby reducing MERS-CoV binding and subsequent virus replication. For this, the effect of DMSO versus ESI-09 treatment were compared, at 10 μM for 2 hrs, on CD26 expression in Calu-3 cells by both Western blotting and IIF. Whereas the total amount of CD26 was not affected by ESI-09 treatment (FIG. 13A), the pattern of CD26 expression on the membrane of Calu-3 cells was changed with ESI-09 treatment (FIG. 13B). In contrast to the relatively diffuse expression pattern in DMSO-treated cells, the expression of CD26 was rearranged, becoming more concentrated at

the cell membrane in response to ESI-09 treatment. It was also investigated whether such an altered pattern of CD26 expression would affect viral binding to Calu-3 cells.

[0133] For this study, Calu-3 cells untreated, DMSO-, H89-, or ESI-09-treated were incubated with an equal amount of infectious MERS-CoV (MOI of 20) in an ice bath for 2 hrs, washed thoroughly with ice-cold PBS to remove unbound viruses and submitted to one cycle of "freeze (-80°C) - thaw" in 100 μl of MEM/2% FCS medium to maximally retrieve membrane-bound viral particles for titrations. As shown in FIG. 13C, neither H89 nor ESI-09 treatment adversely influenced MERS-CoV binding to Calu-3 cells, when compared to untreated or DMSO-treated cells. To identify which stage(s) of virus's lifecycle downstream of the binding/adsorption might be affected by ESI-09 treatment, Calu-3 cells grown in 12-well plates were infected with live or gamma (γ)-inactivated (cobalt-60, 5 megarads) MERS-CoV (MOI=5) for 1 hrs at 4°C, followed by ESI-09 or DMSO treatment (10 μM) before harvesting total RNA and cell lysates at indicated time points p.i. for determining the kinetics of virus RNA replication by real-time (RT) reverse transcription Touch Thermal Cycler, Bio-Rad) and Western blot analyses. For quantifying viral RNA replication by RT-PCR, a region upstream of the envelope (E) gene (upE) was targeted, as described (Corman et al. (2012) *Euro Surveill.* 17(39). pii: 2028524) and the GAPDH gene as the internal control. As shown in FIG. 13D, ESI-09 treatment significantly inhibited genomic replication of virus, starting at 6 hrs, reaching the maximum at 8 hrs, and remained inhibitory at 12 hrs p.i. Viral RNA replication was not detected in cells challenged with γ-inactivated virus (data not shown). This ESI-09-mediated inhibitory kinetics of viral RNA replication was consistent with the expression of spike-surface glycoproteins (S) and the nucleocapsid (N) protein as revealed by Western blot analyses (FIG. 13E), thereby suggesting that inhibiting viral RNA replication and protein synthesis are likely antiviral mechanisms of ESI-09. Taken together, these results suggested that the cAMP-Epac, but not cAMP-PKA, signaling axis plays a role in the regulation of MERS-CoV replication in permissive cells.

[0134] To more definitely demonstrate that Epac proteins are important for sustaining viral replication, Epac1 gene knockdown (KD) Calu-3 cells were established by using the shRNA Lentiviral Transduction System (Sigma-Aldrich) (Abbas-Terki et al. (2002) *Hum Gene Ther.* 13(18):2197-2201). These KD cells enabled examination of the effect of Epac1 might have in regulating the replication of both MERS-CoV and SARS-CoV, and to validate the results attributed to the pharmacological inhibitor. As shown in FIG. 14A, Epac1

expression was reduced by ~ 50% in KD Calu-3 cells when compared to that in the control KD cells. To evaluate whether such a moderate reduction in Epac-1 expression could affect similar to the ESI-09 effect, both control and Epac-1 KD cells were infected with either MERS-CoV or SARS-CoV (MOI of 0.1) for 24 hrs before assessing virus yields. As shown in FIG. 14B, reducing Epac-1 expression by ~50 % was sufficient to significantly reduce the replication of MERS-CoV and SARS-CoV.

[0135] While the activity state of Epac, a multidomain mediator of cAMP signaling, is determined by its allosteric interaction with cAMP (Schmidt et al. (2013) *Pharmacol Rev.* 65(2):670-709), an increased transcriptional expression of Epac gene has been demonstrated in mice suffered from either myocardial hypertrophy or neointima formation induced by vacular injury (Yokoyama et al. (2008) *Am J Physiol Heart Circ Physiol.* 295(4):H1547-1555; Ulucan et al. (2007) *Am J Physiol Heart Circ Physiol.* 293(3):H1662-1672). Since Epac appears to play a previously unidentified role in supporting viral replication, it was determined whether its expression could be modified in response to acute MERS-CoV infection. Briefly, MERS-CoV-infected Calu-3 cells (MOI=5) grown in 12-well plates were treated with DMSO or ESI-09 (10 μM) for indicated time periods before harvesting supernatants and extracting cellular lysates for assessing virus titers and Epac protein expression. Early ESI-09 treatment resulted in profound reduction of virus titers especially at both 12 and 22 hrs p.i. (data not shown). Western blot analyses using mouse-anti-Epac (Santa Cruz,) or rabbit-anti-GAPDH antibody (Cell Signaling Technology) in combination of anti-mouse IgG-HRP (Biolab) or anti-rabbit IgG-HRP (Cell Signaling Technology) revealed that neither ESI-09 treatment nor MERS-CoV infection over time could significantly modulate the level of Epac protein expression (FIG. 15A). It was also determined if the expression of Epac can be co-localized with intracellular virus, in which Calu-3 cells grown in chamber-slides were infected with recombinant (r) MERS-CoV-expressing red fluorescence protein (RFP) at 4°C for 1 hr (28), followed by treatment with either DMSO or ESI-09 for indicated time periods before assessing the expression of Epac and MERS-CoV-RFP by IF. Consistent with Western blot results, the expression pattern and intensity of Epac (arrow) in Calu-3 cells was not affected by either MERS-CoV infection or ESI-09 treatment.

[0136] Additionally, its expression was not strictly co-localized with intracellular viruses either (arrowhead). While it is clear that prior ESI-09 treatment was effective in restricting MERS-CoV and SARS-CoV replication without compromising viral binding, it was further

evaluated whether the antiviral effect provided by ESI-09 could be attributed to a virucidal effect. For this test, an equal volume of SARS-CoV or MERS-CoV were incubated with MEM/2% FSC (M-2), DMSO (10μM), or ESI-09 (10 μM) at 37°C for 2 hrs before determining their effect on viral yields in Vero E6 cells. It was found that neither DMSO nor ESI-09 treatment had any noticeable direct effect on the resulting viral yields (FIG. 16A). To investigate if the antiviral effect of ESI-09 required its continuing presence in the culture system, duplicate sets of Calu-3 cell cultures were treated with DMSO vehicle or 10 μM ESI-09 for 2 hrs. One set was replenished with DMSO and ESI-09, respectively, after MERS-CoV challenge (MOI of 0.1), whereas the other set received M-2 medium without the additives. As shown in FIG. 16B, the ability of ESI-09 to inhibit viral replication appears to be reversible, as cells first treated with ESI-09 and replenished with M-2 medium without ESI-09 showed no evidence of virus inhibition. Finally, to determine if treatment of cells prior to challenge is a prerequisite for ESI-09's antiviral effect, the effect of adding ESI-09 at various times after initiating virus infection was examined. Briefly, Calu-3 cells were treated with ESI-09 at indicated time points (FIGs. 16C-16D), where 0 hr is defined as the time of viral challenge. Cell culture supernatants were harvested for assessing protective efficacy at either 38 hrs (MOI of 0.1) or 24 hrs (MOI of 5) post-challenge. Not only was the pre-challenge treatment unnecessary for protection, but treating infected cells (MOI of 0.1) with ESI-09 as late as 16 or 20 hrs (FIG. 16C) or treating 12 hrs post-challenge for those infected with an MOI of 5 (FIG. 16D) were effective in reducing viral replication, thereby suggesting the treatment late in infection could be beneficial. The effectiveness of such a delayed ESI-09 treatment in plunging the yields of virus in Calu-3 cells suggests that this antiviral drug might affect a late event(s) of the virus replication strategy, such as assembly and/or release, in addition to inhibiting synthesis of viral proteins and RNA replication (FIG. 13D-13E).

[0137] The anti-microbial affects of Epac inhibitors is not limited to MERS-CoV or SARS-CoV. To investigate whether the ability of ESI-09 to inhibit the replication of both MERS-CoV and SARS-CoV *in vitro* can be extended to other emerged pathogenic RNA viruses, confluent Calu-3 cell cultures were infected with Rift Valley fever virus (ZH501 strain), Nipah virus (Malaysia strain), Marburg virus (Angola strain), and the H5N1 subtype of avian influenza A virus (Vietnam/1203/04 strain) at a multiplicity of infection (MOI) of 1. The effect of treatment with either ESI-09 or DMSO vehicle (10 μM) on infected cells was assessed by monitoring either the yields of infectious progeny virus at appropriate time points post infection. It was found that ESI-09 treatment is effective in reducing the yields of

infectious RVFV, Nipah virus, Marburg virus, and avian H5N1 influenza virus as it was for both MERS-CoV and SARS-CoV (FIG. 17).

[0138] In summary, in these studies of the linkage of the cAMP signaling pathway and MERS-CoV infection, a previously unknown function of Epac1 protein in regulating the replication of both MERS-CoV and SARS-CoV in a cell-type independent manner was identified. These conclusions are based on the usage of both an Epac-specific inhibitor (ESI-09) and Epac-1 KD cells, and Calu-3 and Vero E6 tissue cultures. It was found that ESI-09 exerts an antiviral effect when used at a non-toxic concentration. In addition, it does so, not only without the need for treatment prior to infection, but also with an extended therapeutic window. Incidentally, adenosine and its analogs have been successfully investigated as potent inhibitors of the replication of hepatitis C virus, vaccinia virus, HIV-1, dengue virus, and other flaviviruses (Yin et al. (2009) *Proc Natl Acad Sci U S A.* 106(48):20435-20439; Manvar et al. (2013) Biochemistry. 52(2):432-444; Wu et al. (2010) *J Med Chem.* 53(22):7958-7966; Oh et al. (2010) *Nucleic Acids.* 29(10):721-733). The dual role of CD26 as the MERS-CoV receptor and an adenosine deaminase (ADA)-anchoring protein (Kameoka et al. (1993) *Science.* 261(5120):466-469; Gracia et al. (2013) *FASEB J.* 27:1048-1061; Dong et al. (1997) *J.Immunol.* 159:6070-6076; Raj et al. (2013) *J Virol.* doi: 10.1128/JVI.02935-13) provides a linkage between MERS-CoV infection and cAMP signaling. However, the potential role of the cAMP axis in the host response to MERS-CoV has yet to be studied. Nevertheless, these findings indicate that ESI-09 and its analogs are a new class of antiviral agents representing a strategy for combating MERS-CoV and other emerging and re-emerging virus infections.

CLAIMS

1. A method for inhibiting viral replication in a subject having a viral infection comprising administering an Epac inhibitor to the subject.

2. The method of claim 1, wherein the viral infection is a MERS-CoV, SARS-CoV, Rift Valley fever virus, Nipah virus, Marburg virus, H5N1 virus, hepatitis C virus, vaccinia virus, HIV-1, or dengue virus infection.

3. The method of claim 1, wherein the viral infection is an Influenza infection.

4. The method of claim 1, wherein the viral infection is a Coronovirus infection.

5. The method of claim 4, wherein the Coronovirus is a MERS-CoV virus.

6. The method of claim 1, wherein the Epac inhibitor is selected from 3-(5-*tert*-Butyl-isoxazol-3-yl)-2-[(3-chlorophenyl)-hydrazono]-3-oxo-propionitrile (HJC0680, ESI-09);*N*-(5-*tert*-Butyl-isoxazol-3-yl)-2-[(3-chlorophenyl)-hydrazono]-2-cyanoacetamide (HJC0683); 2-[(3-Chlorophenyl)-hydrazono]-2-cyano-*N*-(5-methyl-isoxazol-3-yl)acetamide (HJC0692); 3-(5-*tert*-Butyl-isoxazol-3-yl)-2-[(2-chlorophenyl)-hydrazono]-3-oxo-propionitrile (HJC0693); 3-(5-*tert*-Butyl-isoxazol-3-yl)-2-[(4-chlorophenyl)-hydrazono]-3-oxo-propionitrile (HJC0694); 3-(5-*tert*-Butyl-isoxazol-3-yl)-3-oxo-2-(phenyl-hydrazono)-propionitrile (HJC0695); 3-(5-*tert*-Butyl-isoxazol-3-yl)-2-[(2,5-dichlorophenyl)-hydrazono]-3-oxo-propionitrile (HJC0696); 3-(5-*tert*-Butyl-isoxazol-3-yl)-3-oxo-2-(m-tolyl-hydrazono)propionitrile (HJC0712); 3-(5-*tert*-Butyl-isoxazol-3-yl)-3-oxo-2-[(3-trifluoromethyl-phenyl)-hydrazono]propionitrile (HJC0720); 3-(5-*tert*-Butyl-isoxazol-3-yl)-2-[(3-nitrophenyl)-hydrazono]-3-oxo-propionitrile (HJC0721); 3-(5-*tert*-Butyl-isoxazol-3-yl)-3-oxo-2-(p-tolyl-hydrazono)propionitrile (HJC0724); 3-(5-*tert*-Butyl-isoxazol-3-yl)-2-[(3,5-dichlorophenyl)-hydrazono]-3-oxo-propionitrile (HJC0726); 2-[(4-Bromophenyl)-hydrazono]-3-(5-*tert*-butyl-isoxazol-3-yl)-3-oxo-propionitrile (HJC0742); 2-[(3-Bromophenyl)-hydrazono]-3-(5-*tert*-butyl-isoxazol-3-yl)-3-oxo-propionitrile (HJC0743); 3-(5-*tert*-Butyl-isoxazol-3-yl)-2-[(2,5-dimethylphenyl)-hydrazono]-3-oxo-propionitrile (HJC0744); 3-(5-*tert*-Butyl-isoxazol-3-yl)-3-oxo-2-(quinolin-6-yl-hydrazono)propionitrile (HJC0745); 3-(5-*tert*-Butyl-isoxazol-3-yl)-2-[(2,3-dichlorophenyl)-hydrazono]-3-oxo-

propionitrile (HJC0750); 3-(5-*tert*-Butyl-isoxazol-3-yl)-2-[(3-ethynyl-phenyl)-hydrazono]-3-oxo-propionitrile (HJC0751); 3-{*N*'-[2-(5-*tert*-Butyl-isoxazol-3-yl)-1-cyano-2-oxo-ethylidene]-hydrazino}benzoic acid ethyl ester (HJC0752); 3-{*N*'-[2-(5-*tert*-Butyl-isoxazol-3-yl)-1-cyano-2-oxo-ethylidene]-hydrazino}benzonitrile (HJC0753); 2-[(3-Acetyl-phenyl)-hydrazono]-3-(5-*tert*-butyl-isoxazol-3-yl)-3-oxo-propionitrile (HJC0754); 3-(5-*tert*-Butyl-isoxazol-3-yl)-2-[(2,3-dimethylphenyl)-hydrazono]-3-oxo-propionitrile (HJC0755); 3-(5-*tert*-Butyl-isoxazol-3-yl)-2-[(3-hydroxymethylphenyl)-hydrazono]-3-oxo-propionitrile (HJC0756); 3-(5-*tert*-Butyl-isoxazol-3-yl)-2-(indan-5-yl-hydrazono)-3-oxo-propionitrile (HJC0757); 2-[(3,5-Bis-trifluoromethyl-phenyl)-hydrazono]-3-(5-*tert*-butyl-isoxazol-3-yl)-3-oxo-propionitrile (HJC0758); 2-{*N*'-[2-(5-*tert*-Butyl-isoxazol-3-yl)-1-cyano-2-oxo-ethylidene]-hydrazino}-6-chloro-benzoic acid (HJC0759); 3-(5-*tert*-Butyl-isoxazol-3-yl)-2-[(3-chloro-4-hydroxy-phenyl)-hydrazono]-3-oxo-propionitrile (HJC0760); 2-[(3-Chloro-phenyl)-hydrazono]-3-(5-methyl-isoxazol-3-yl)-3-oxo-propionitrile (HJC0768); or 2-[(3,5-Dichlorophenyl)-hydrazono]-3-(5-methyl-isoxazol-3-yl)-3-oxo-propionitrile (HJC0770).

7. The method of claim 6, wherein the EPAC inhibitor is ESI-09.

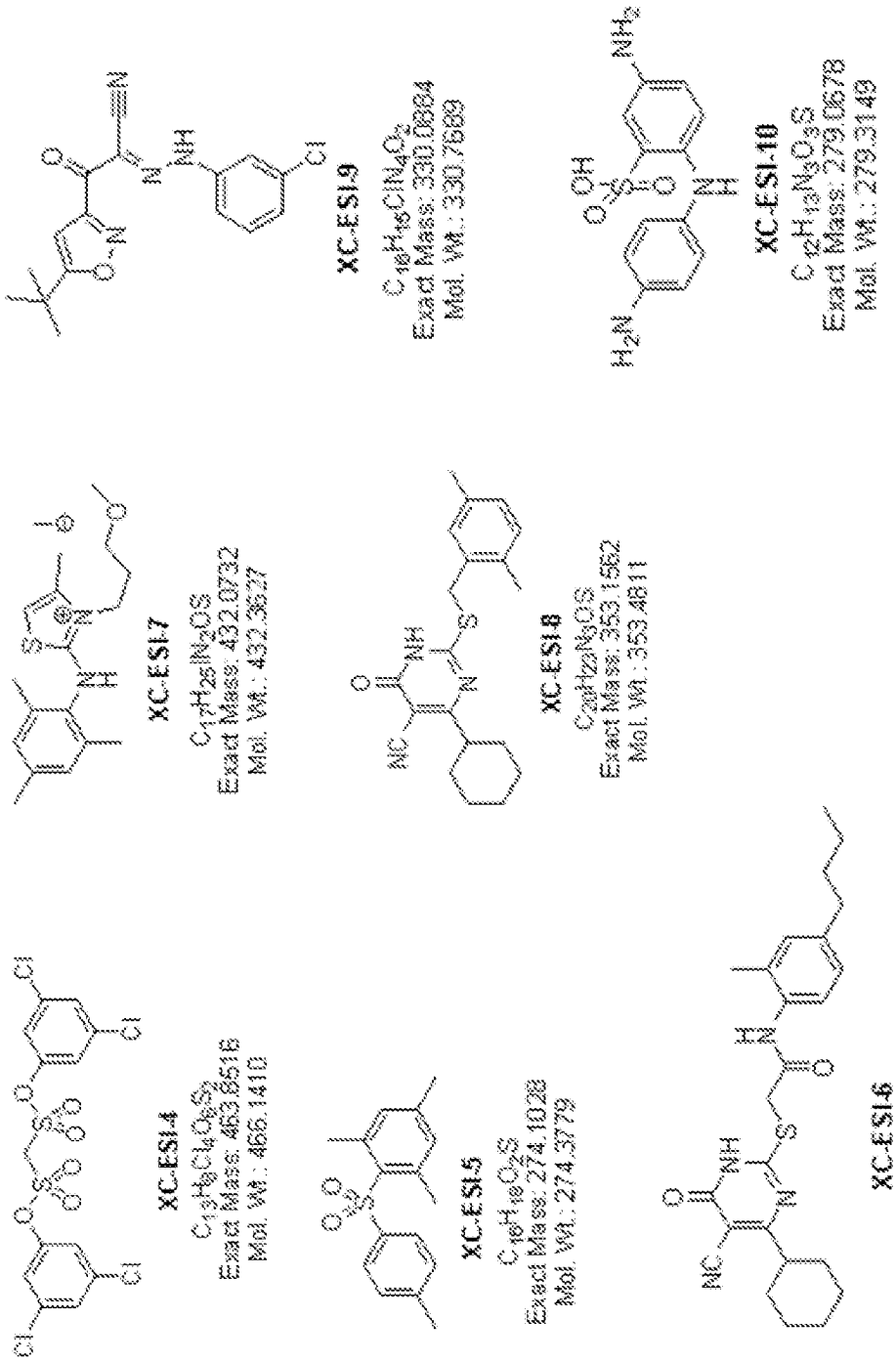

XC-ESI-4
$C_{19}H_8Cl_4O_4S_2$
Exact Mass: 463.8516
Mol. Wt.: 466.1410
XC-ESI-5
$C_{16}H_{18}O_2S$
Exact Mass: 274.1028
Mol. Wt.: 274.3779
XC-ESI-6
$C_{24}H_{30}N_4O_2S$
Exact Mass: 438.2089
Mol. Wt.: 438.5656
XC-ESI-7
$C_{17}H_{28}IN_2OS$
Exact Mass: 432.0732
Mol. Wt.: 432.3627
XC-ESI-8
$C_{20}H_{23}N_3OS$
Exact Mass: 353.1562
Mol. Wt.: 353.4811
XC-ESI-9
$C_{16}H_{15}ClN_4O_2$
Exact Mass: 330.0884
Mol. Wt.: 330.7689
XC-ESI-10
$C_{12}H_{13}N_3O_3S$
Exact Mass: 279.0678
Mol. Wt.: 279.3149

FIG. 1

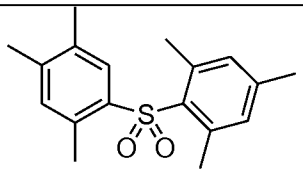
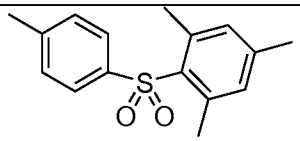
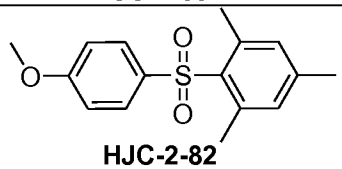
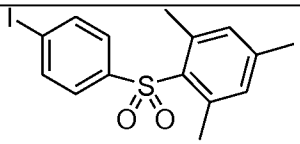
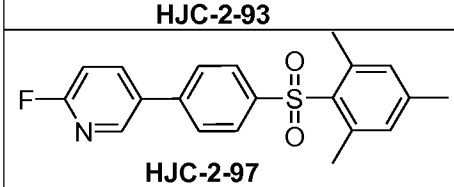
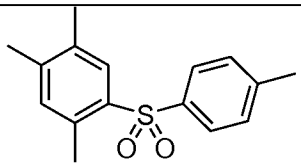
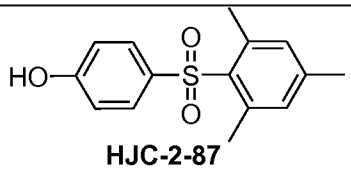
Chemical structure	IC_{50} (μM)
 HJC-2-71	0.7
 HJC-2-85	1.0
 HJC-2-82	1.9
 HJC-2-93	4-10*
 HJC-2-97	3-10*
 HJC-2-98	9-30*
 HJC-2-87	14

FIG. 2

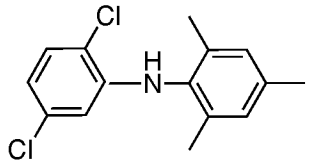
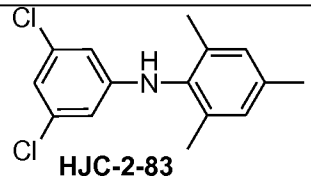
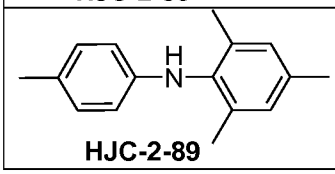
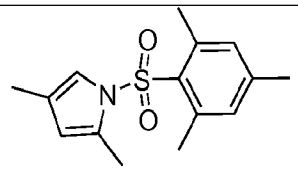
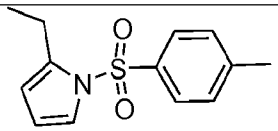
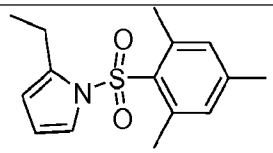
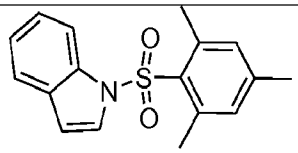
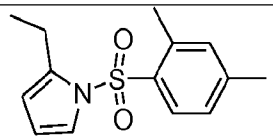
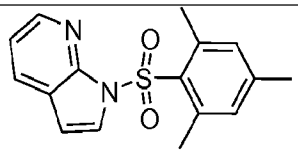
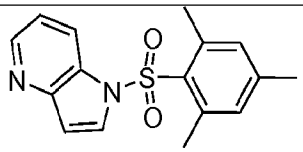
Chemical structure	IC_{50} (μM)
 **HJC-3-38**	0.4
 **HJC-2-83**	0.9
 **HJC-2-89**	3.8-10*

FIG. 3

Chemical structure	IC_{50} (μM)
 HJC-3-50	0.4*
 HJC-3-26	0.4*
 HJC-2-79	0.5
 HJC-2-77	1.2
 HJC-3-55	1.3*
 HJC-2-81	2.4
 HJC-3-23	3.8*

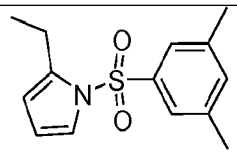
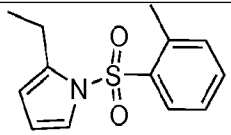
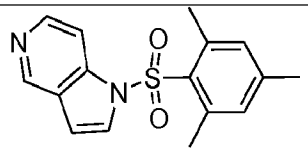
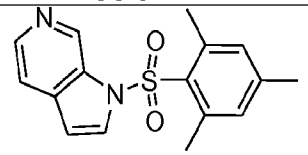
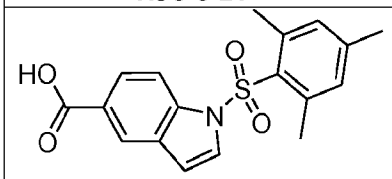
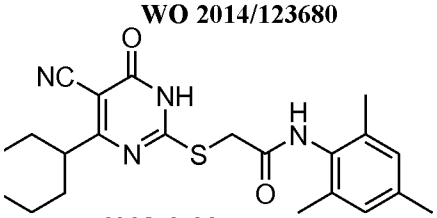
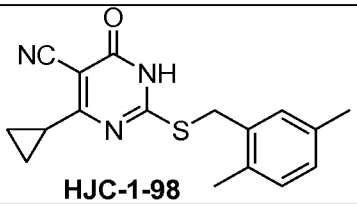
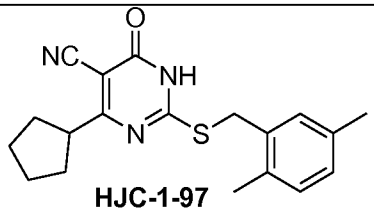
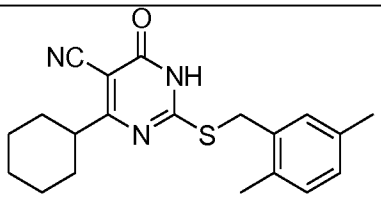
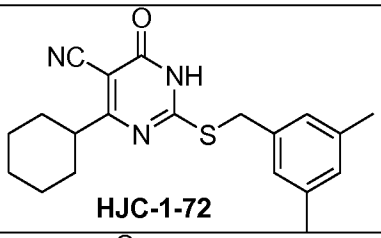
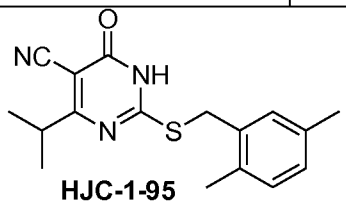
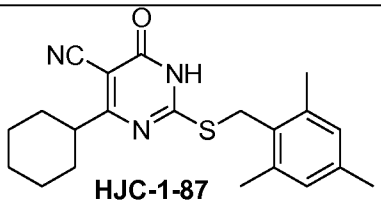
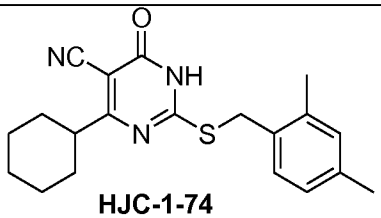
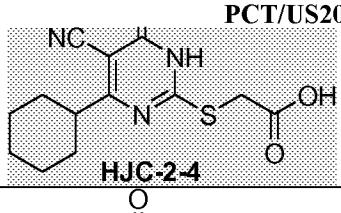
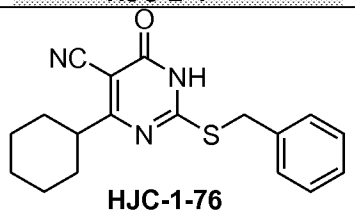
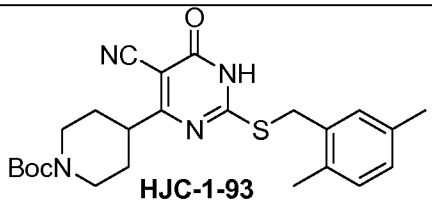
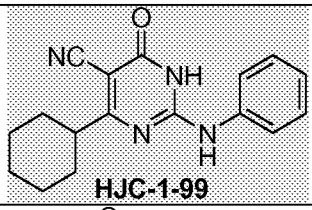
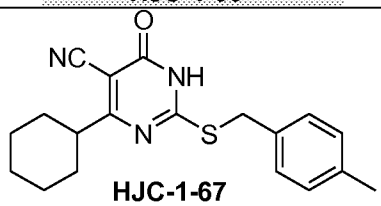
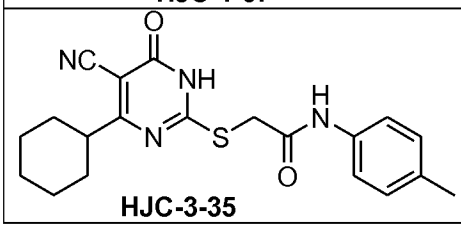
 HJC-3-54	4.7*
 HJC-3-53	5.3*
 HJC-3-22	8.9*
 HJC-3-21	13*
 HJC-3-62	11*

FIG. 4

 HJC-3-33	4
 HJC-1-98	4.0*
 HJC-1-97	5.9
 HJC-1-65	8.3
 HJC-1-72	7.0
 HJC-1-95	13
 HJC-1-87	12
 HJC-1-74	16

 HJC-2-4	High, CND*
 HJC-1-76	31
 HJC-1-93	29
 HJC-1-99	30-100
 HJC-1-67	38
 HJC-3-35	30-100

* Estimated IC_{50} due to high background at the en of titrations.
** IC_{50} high and cannot be determined

FIG. 5

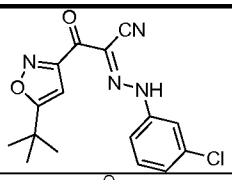
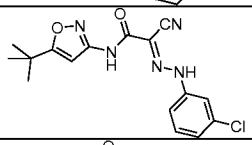
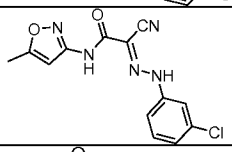
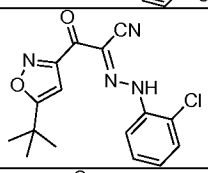
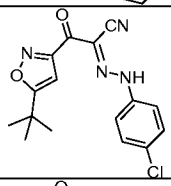
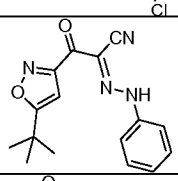
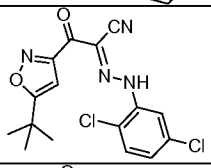
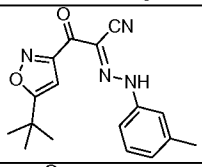
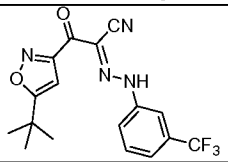
NO	Structure	HPLC Retention Time	HRMS	IC_{50} (μM)
ESI-09		21.72 min 99.6%	331.0969	4.4
HJC0683		20.97 min 96.7%	346.1074	>300
HJC0692		18.55 min 98.5%	304.0606	>300
HJC0693		22.77 min 96.6%	331.0969	34
HJC0694		21.74 min 98.1%	331.0963	20
HJC0695		20.50 min 99.4%	297.1355	73
HJC0696		23.69 min 97.1%	365.0576	7.7
HJC0712		21.29 min 99.0%	311.1514	22.7
HJC0720		21.80 min 96.0%	365.1230	15.6

FIG. 6

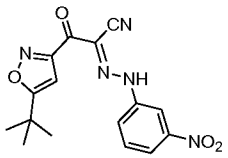
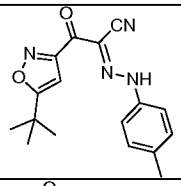
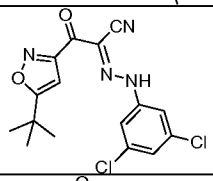
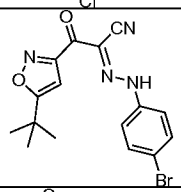
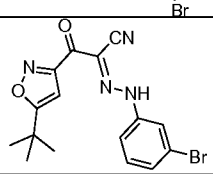
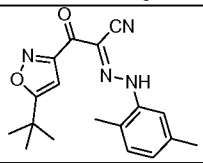
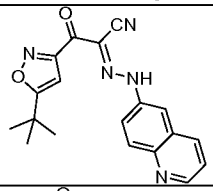
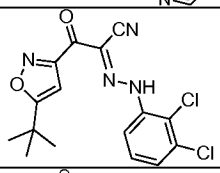
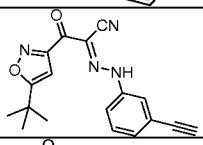
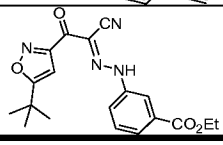
HJC0721		20.33 min 96.4%	342.1207	30
HJC0724		21.36 min 98.6%	311.1515	57
HJC0726		23.20 min 99.0%	365.0563	1.0
HJC0742		22.01 min 98.9%	375.0455	16
HJC0743		21.93 min 98.3%	375.0456	11
HJC0744		23.01 min 98.6%	325.1664	>300
HJC0745		16.09 min 97.8%	348.1458	77
HJC0750		23.74 min 97.5%	365.0568	25
HJC0751		20.83 min 96.2%	321.1350	72
HJC0752		21.53 min 98.8%	369.1558	85

FIG. 6 cont.

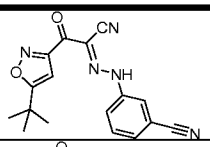
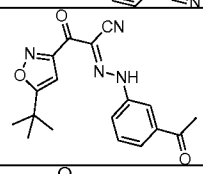
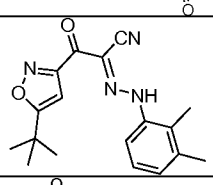
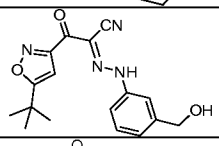
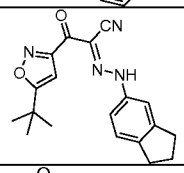
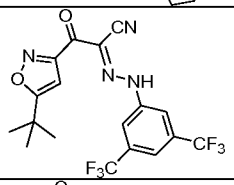
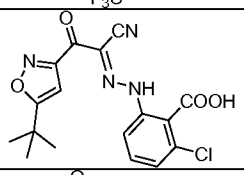
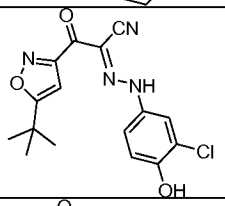
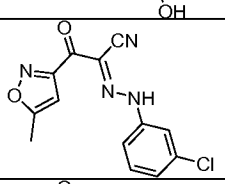
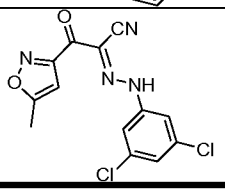
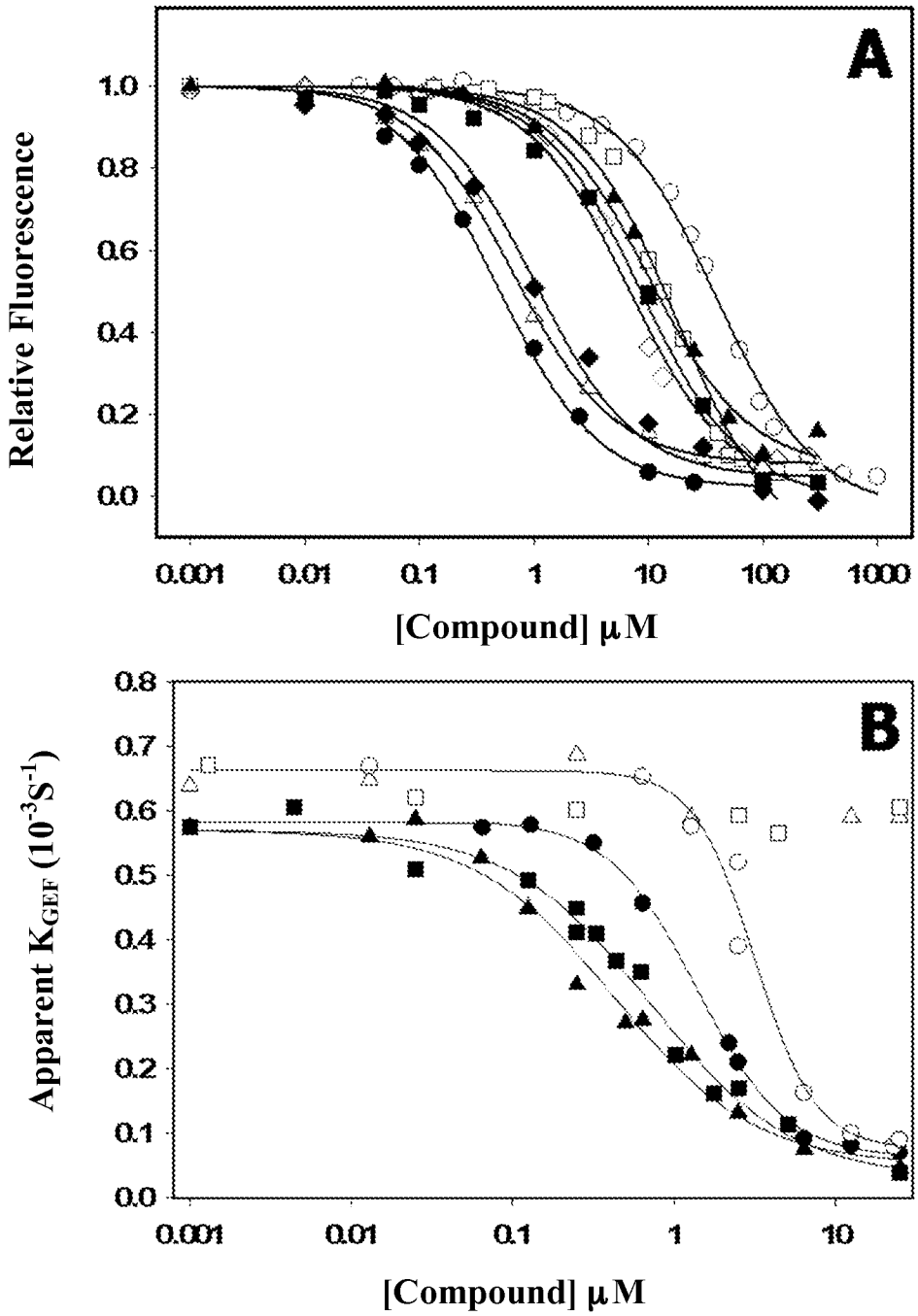
HJC0753		19.87 min 99.3%	322.1303	72
HJC0754		19.80 min 98.2%	339.1459	>300
HJC0755		22.69 min 95.7%	325.1666	270
HJC0756		17.86 min 99.6%	327.1457	>300
HJC0757		22.47 min 99.6%	337.1664	27
HJC0758		22.96 min 96.4%	433.1098	6.8
HJC0759		20.02 min 96.5%	375.0858	>300
HJC0760		18.89 min 99.0%	347.0909	>300
HJC0768		19.18 min 98.4%	289.0492	106
HJC0770		20.79 min 98.4%	323.0103	18

FIG. 6 cont.


A
Relative Fluorescence
1.0
0.8
0.6
0.4
0.2
0.0
0.001
0.01
0.1
1
10
100
1000
[Compound] μM
B
Apparent K_{GEF} ($10^{-3}S^{-1}$)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.001
0.01
0.1
1
10
[Compound] μM

FIGs. 7A-7B

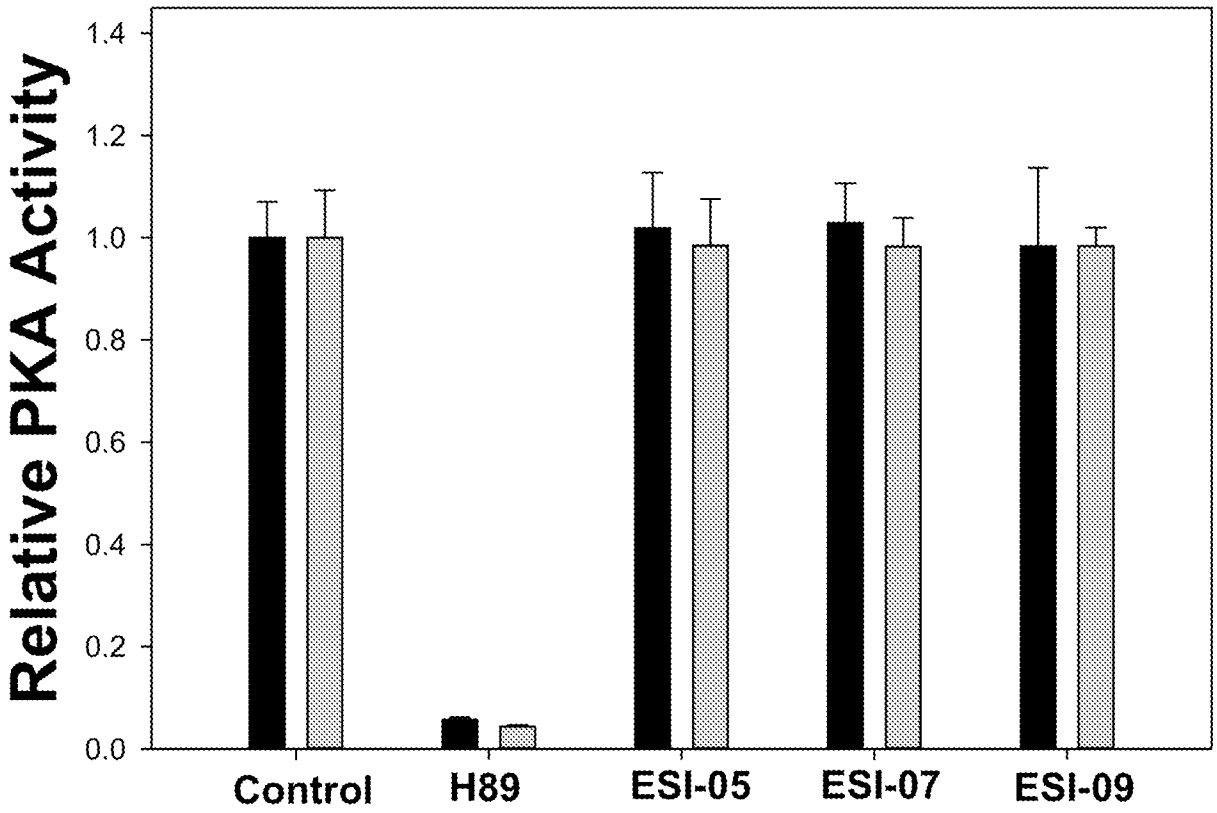
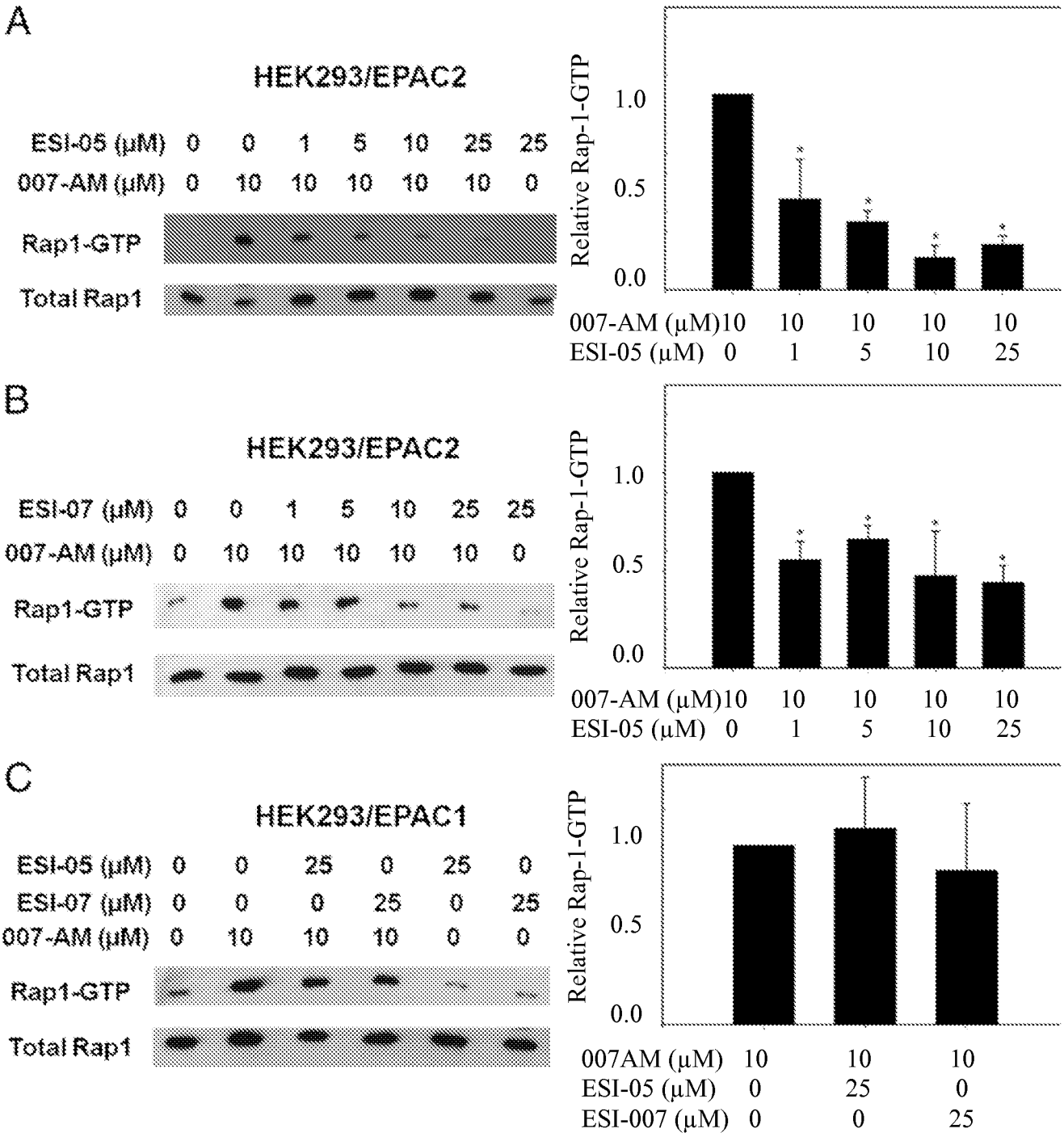

Relative PKA Activity
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Control
H89
ESI-05
ESI-07
ESI-09

FIG. 8


A
HEK293/EPAC2
ESI-05 (μM) 0 0 1 5 10 25 25
007-AM (μM) 0 10 10 10 10 10 0
Rap1-GTP
Total Rap1
Relative Rap-1-GTP
1.0
0.5
0.0
007-AM (μM) 10 10 10 10 10
ESI-05 (μM) 0 1 5 10 25
B
HEK293/EPAC2
ESI-07 (μM) 0 0 1 5 10 25 25
007-AM (μM) 0 10 10 10 10 10 0
Rap1-GTP
Total Rap1
Relative Rap-1-GTP
1.0
0.5
0.0
007-AM (μM) 10 10 10 10 10
ESI-05 (μM) 0 1 5 10 25
C
HEK293/EPAC1
ESI-05 (μM) 0 0 25 0 25 0
ESI-07 (μM) 0 0 0 25 0 25
007-AM (μM) 0 10 10 10 0 0
Rap1-GTP
Total Rap1
Relative Rap-1-GTP
1.0
0.5
0.0
007AM (μM) 10 10 10
ESI-05 (μM) 0 25 0
ESI-007 (μM) 0 0 25

FIGs. 9A-9C

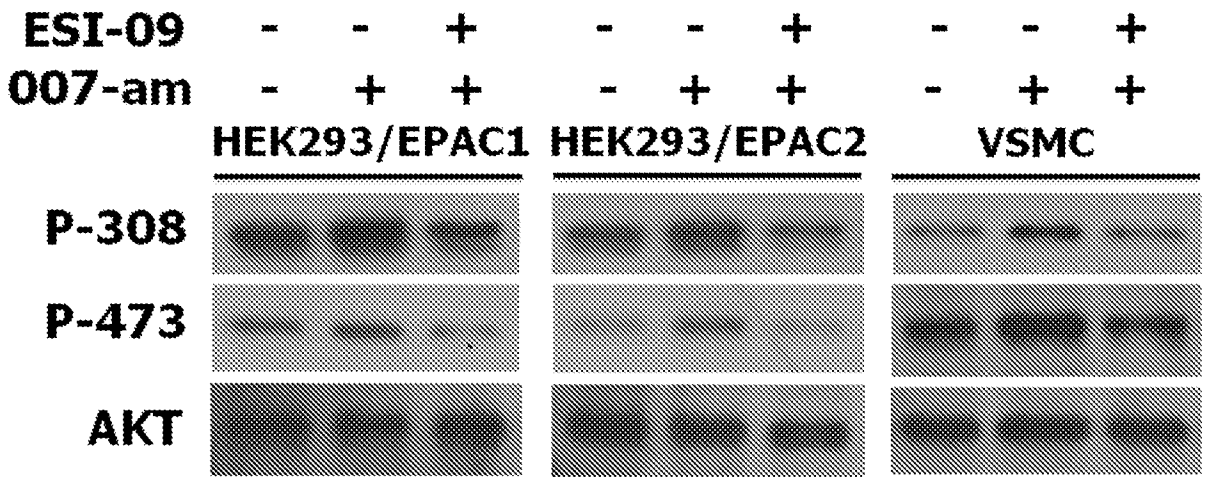


FIG. 10

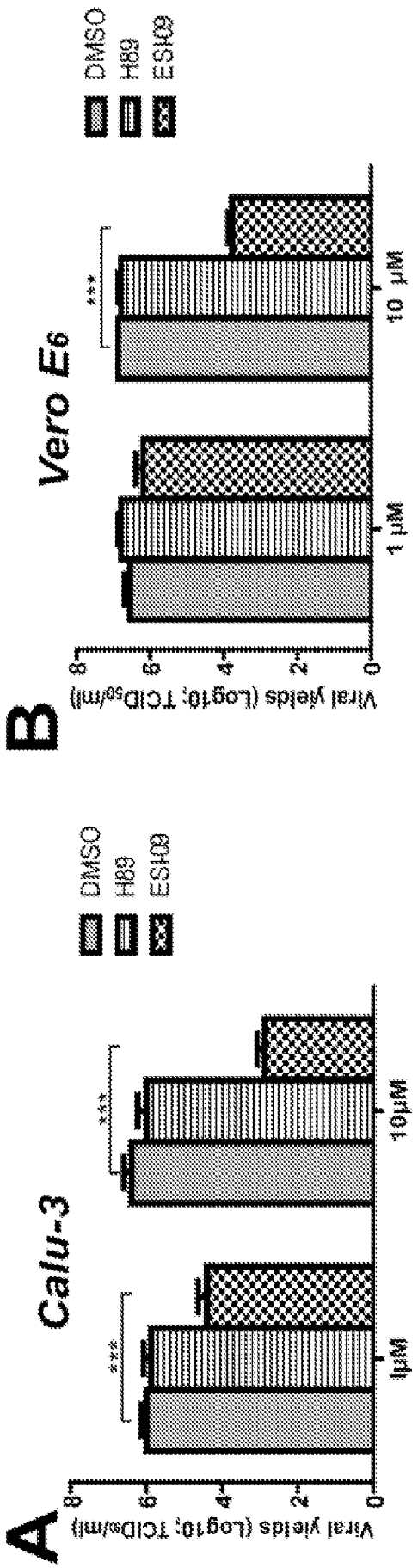

A
Calu-3
Viral yields (Log10; TCID50/ml)
0
2
4
6
8
***
***
1μM
10μM
DMSO
H89
ESI09
B
Vero E6
Viral yields (Log10; TCID50/ml)
0
2
4
6
8
***
1 μM
10 μM
DMSO
H89
ESI09

FIG. 11A-11B

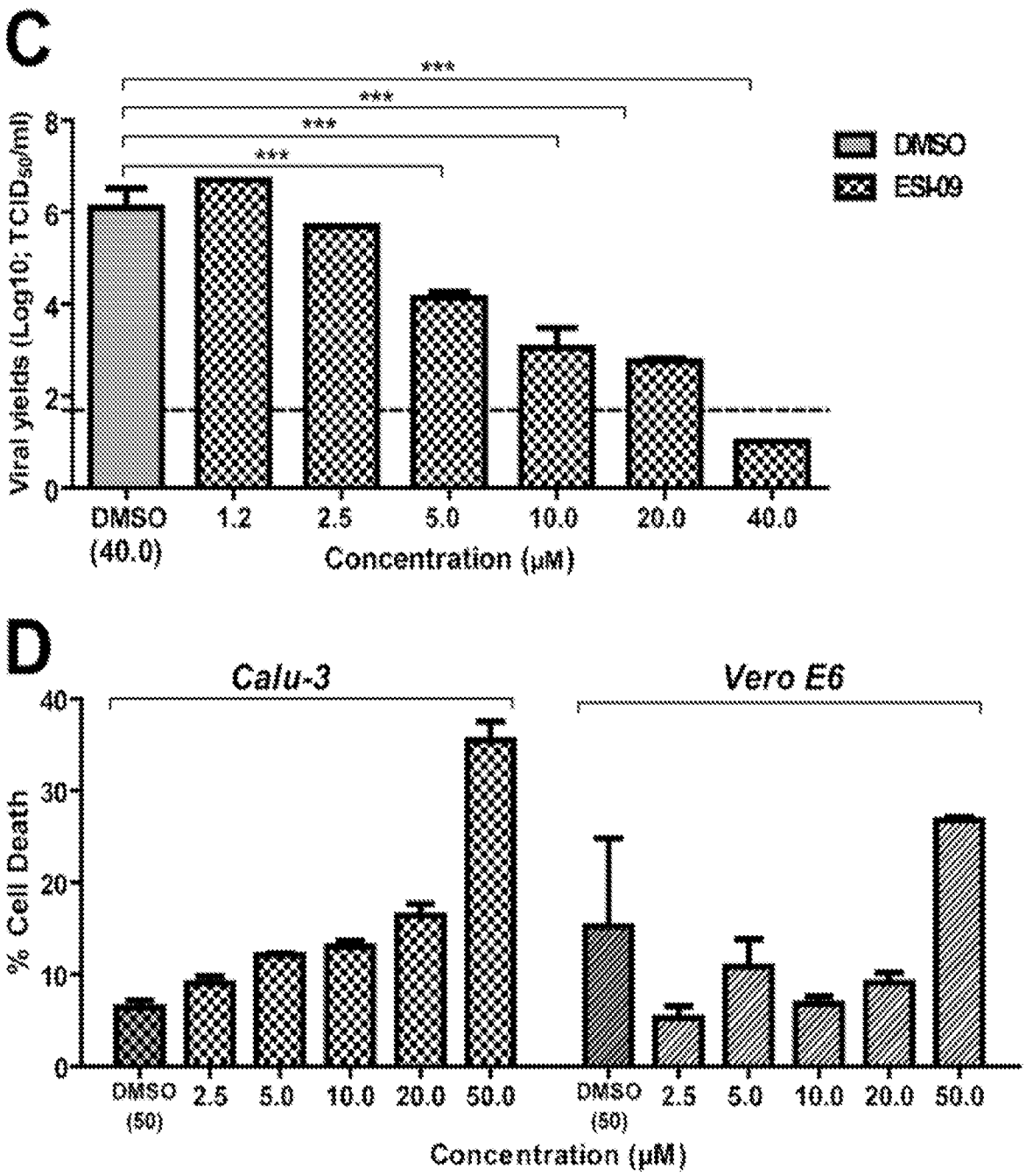

C
***
***
***
***
DMSO
ESI-09
Viral yields (Log10; TCID50/ml)
0
2
4
6
8
DMSO (40.0)
1.2
2.5
5.0
10.0
20.0
40.0
Concentration (μM)
D
Calu-3
Vero E6
% Cell Death
0
10
20
30
40
DMSO (50)
2.5
5.0
10.0
20.0
50.0
DMSO (50)
2.5
5.0
10.0
20.0
50.0
Concentration (μM)

FIG. 11C-11D

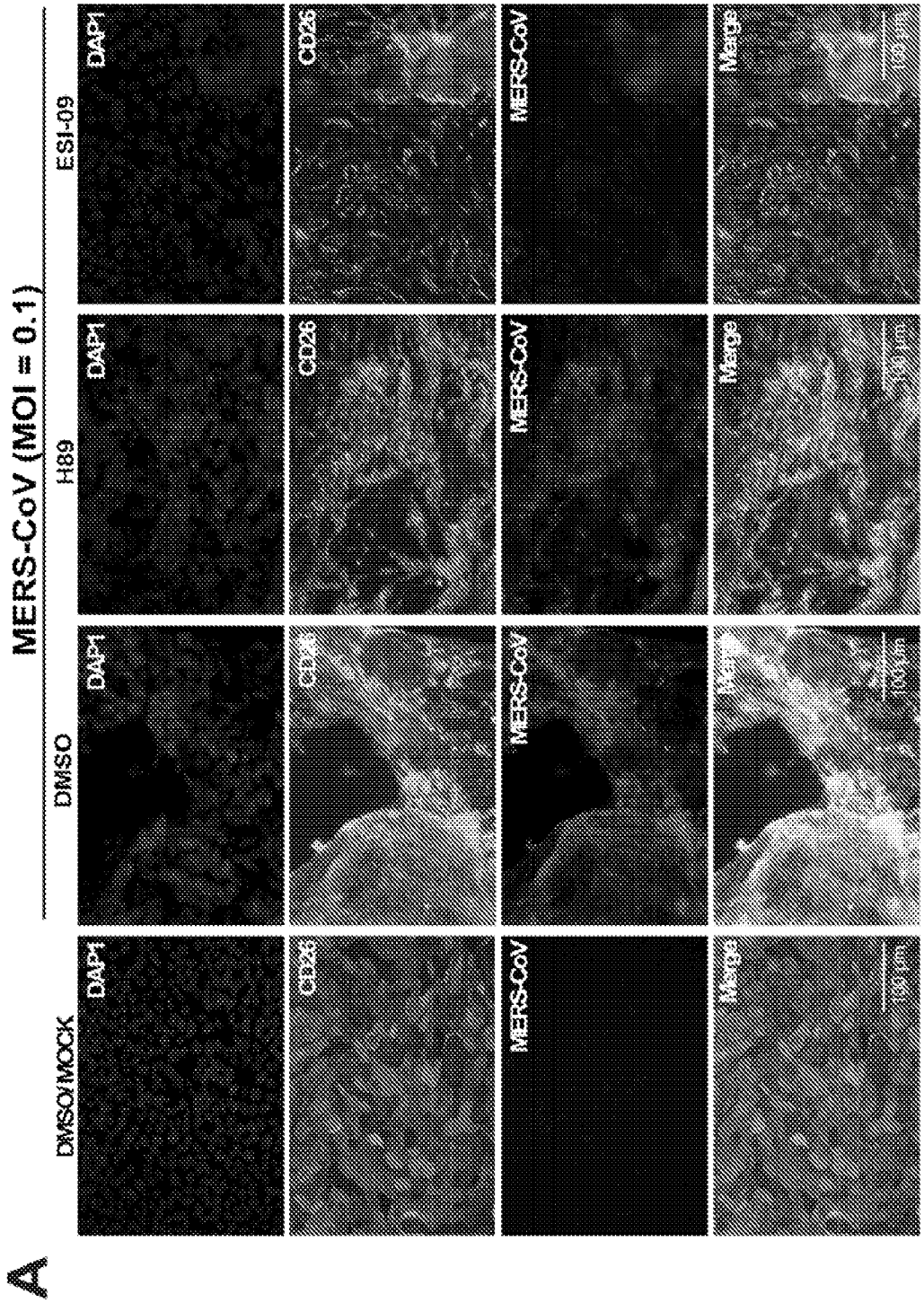

A
MERS-CoV (MOI = 0.1)
DMSO/MOCK
DMSO
H89
ES1-09
DAPI
CD26
MERS-CoV
Merge
100 μm

FIG. 12A

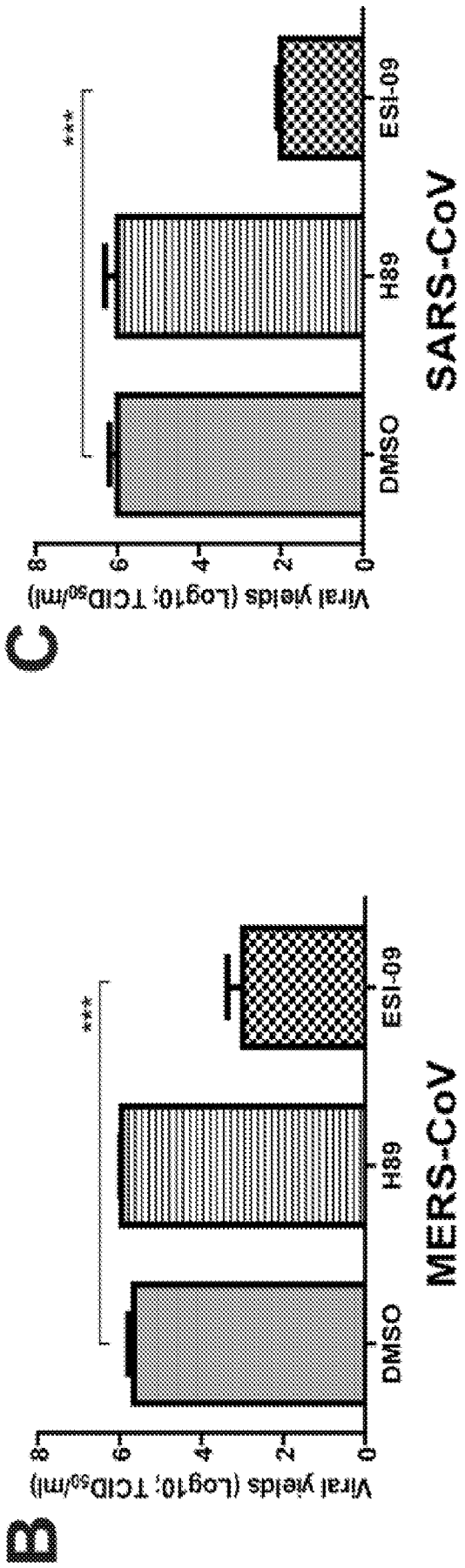
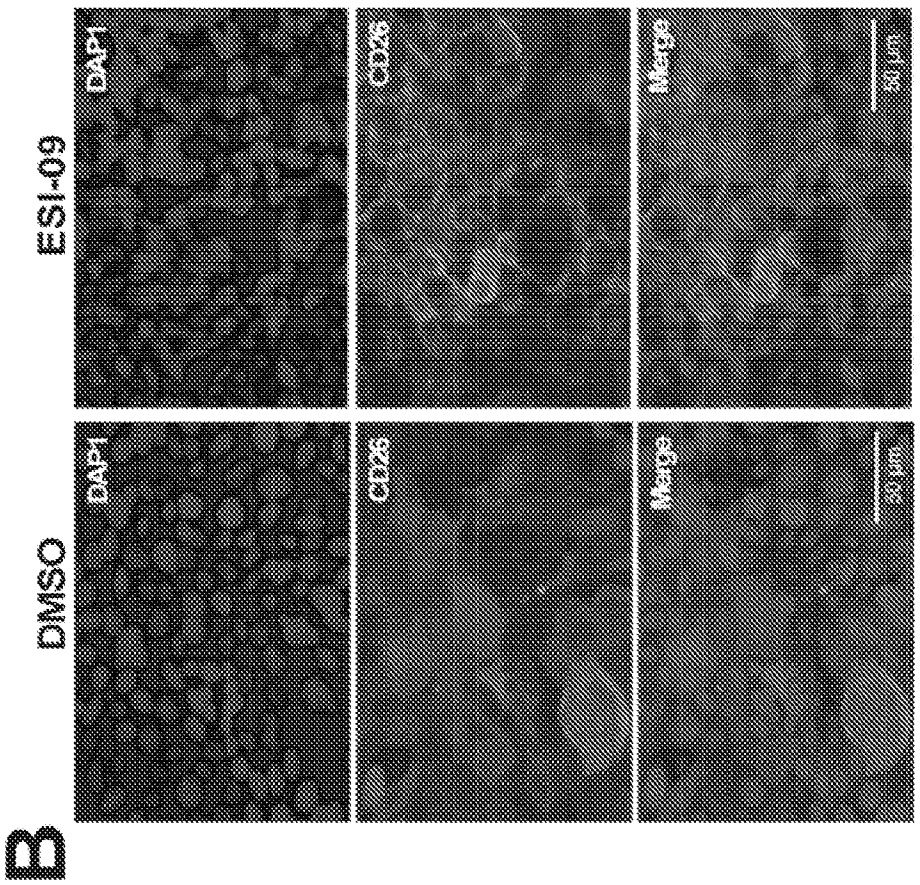

B
Viral yields (Log10; TCID50/ml)
0
2
4
6
8
***
DMSO
H89
ESI-09
MERS-CoV
C
Viral yields (Log10; TCID50/ml)
0
2
4
6
8
***
DMSO
H89
ESI-09
SARS-CoV

FIG. 12B-12C


B
DMSO
ESI-09
DAPI
CD26
Merge
50 μm

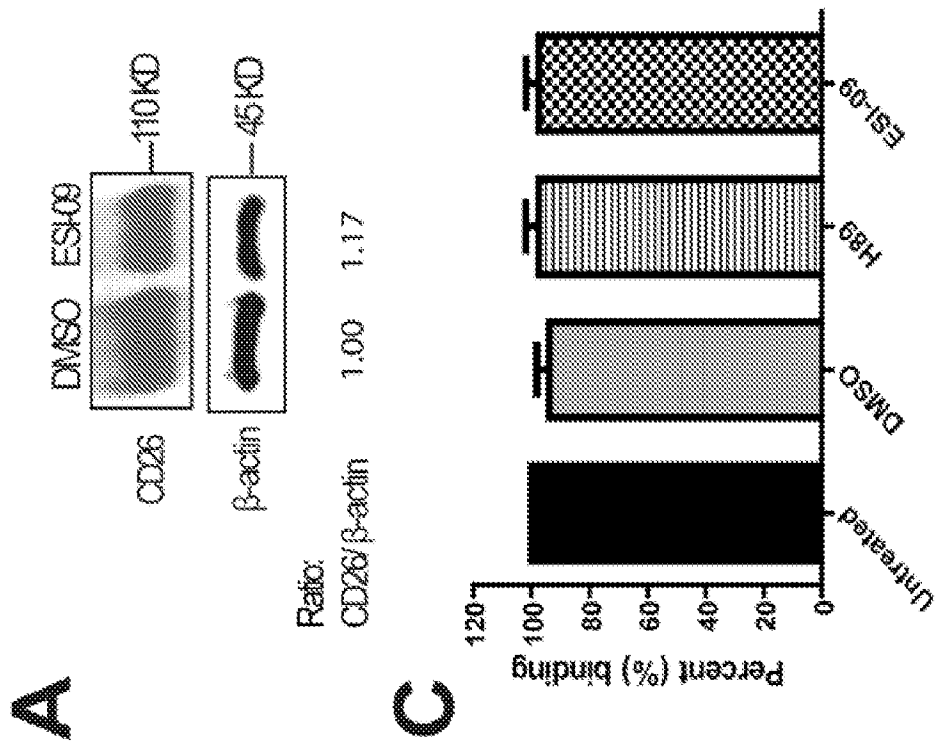

A
DMSO
ESI-09
110 KD
CD26
45 KD
β-actin
Ratio:
CD26/β-actin
1.00
1.17
C
Percent (%) binding
120
100
80
60
40
20
0
Untreated
DMSO
H89
ESI-09

FIG. 13A-13C

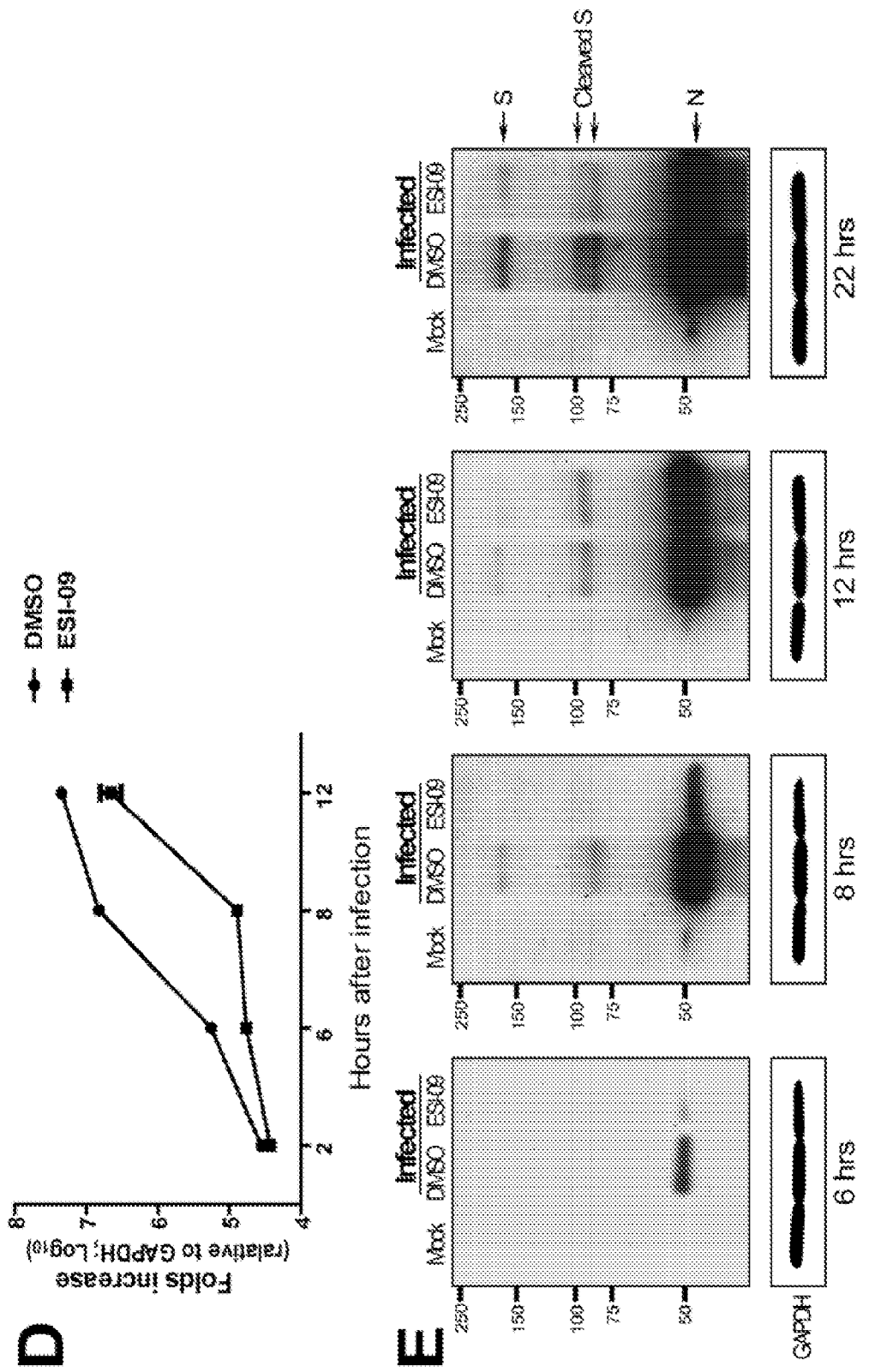

D
DMSO
ESI-09
Folds increase
(relative to GAPDH; Log10)
Hours after infection
E
Mock
DMSO
ESI-09
Infected
6 hrs
8 hrs
12 hrs
22 hrs
GAPDH
S
Cleaved S
N
250
150
100
75
50

FIG. 13D-13E

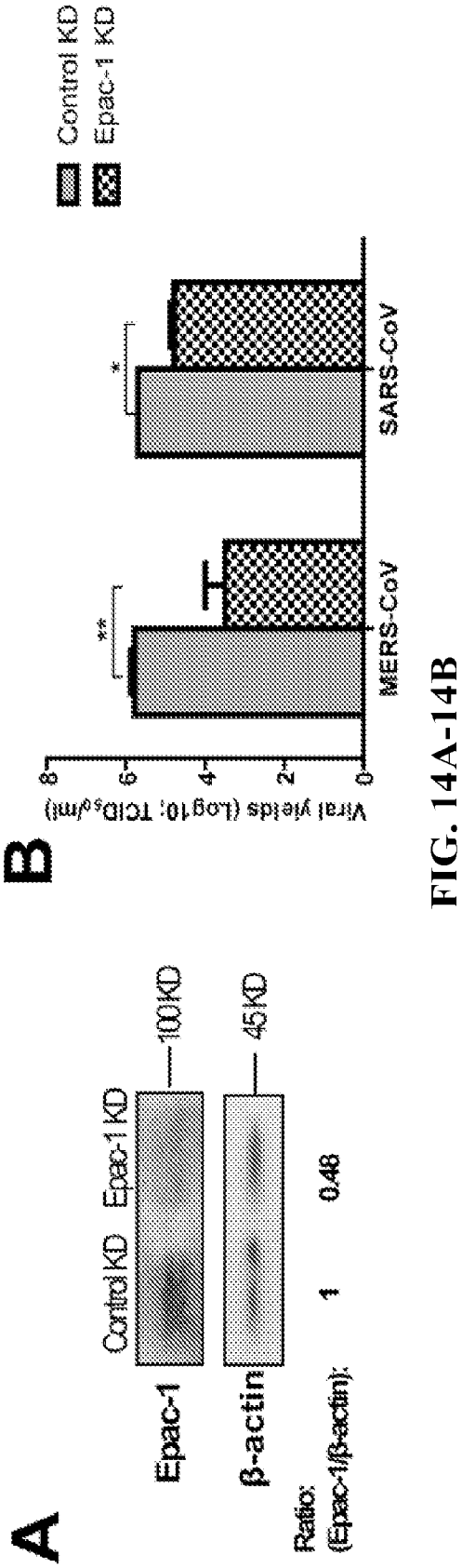

A
Epac-1
β-actin
Control KD
Epac-1 KD
100KD
45KD
Ratio:
(Epac-1/β-actin):
1
0.48
B
Viral yields (Log10; TCID50/ml)
0
2
4
6
8
**
*
MERS-CoV
SARS-CoV
Control KD
Epac-1 KD

FIG. 14A-14B

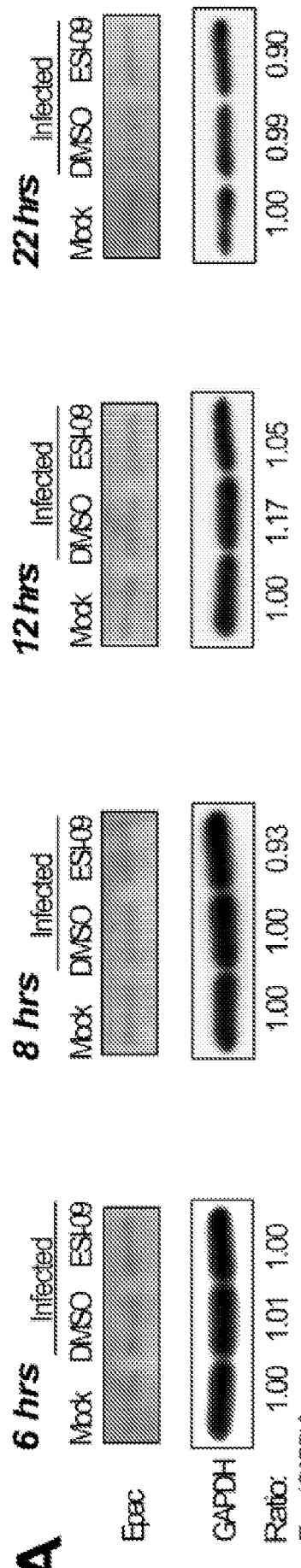

A
6 hrs
8 hrs
12 hrs
22 hrs
Infected
Mock
DMSO
ESI-09
Epac
GAPDH
Ratio:
(Epac/GAPDH)
1.00 1.01 1.00
1.00 1.00 0.93
1.00 1.17 1.05
1.00 0.99 0.90

FIG. 15A

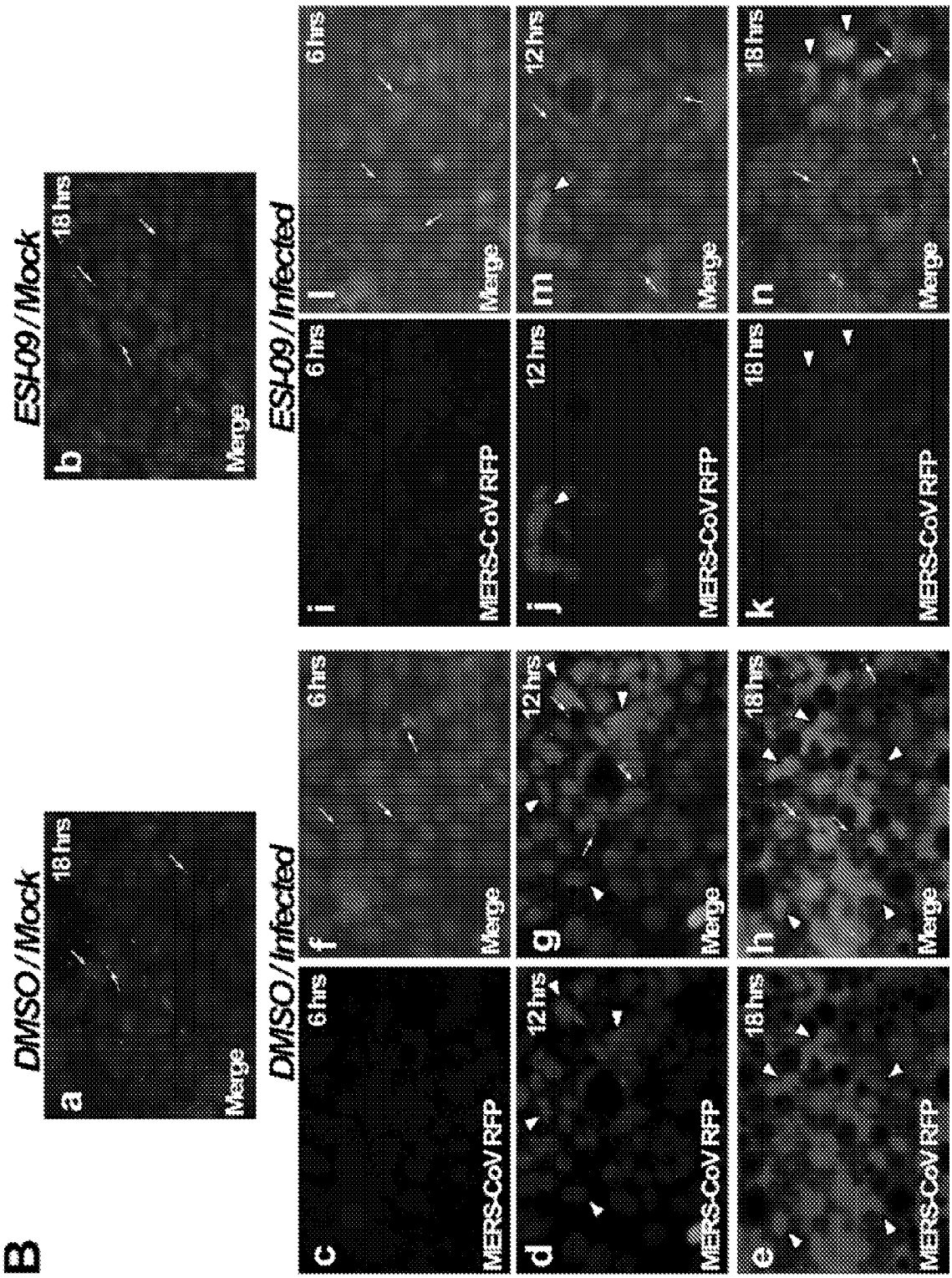

B
DMSO/Mock
ES-09/Mock
DMSO/Infected
ES-09/Infected
a
18 hrs
Merge
b
18 hrs
Merge
c
6 hrs
MERS-CoV RFP
d
12 hrs
MERS-CoV RFP
e
18 hrs
MERS-CoV RFP
f
6 hrs
Merge
g
12 hrs
Merge
h
18 hrs
Merge
i
6 hrs
MERS-CoV RFP
j
12 hrs
MERS-CoV RFP
k
18 hrs
MERS-CoV RFP
l
6 hrs
Merge
m
12 hrs
Merge
n
18 hrs
Merge

FIG. 15B

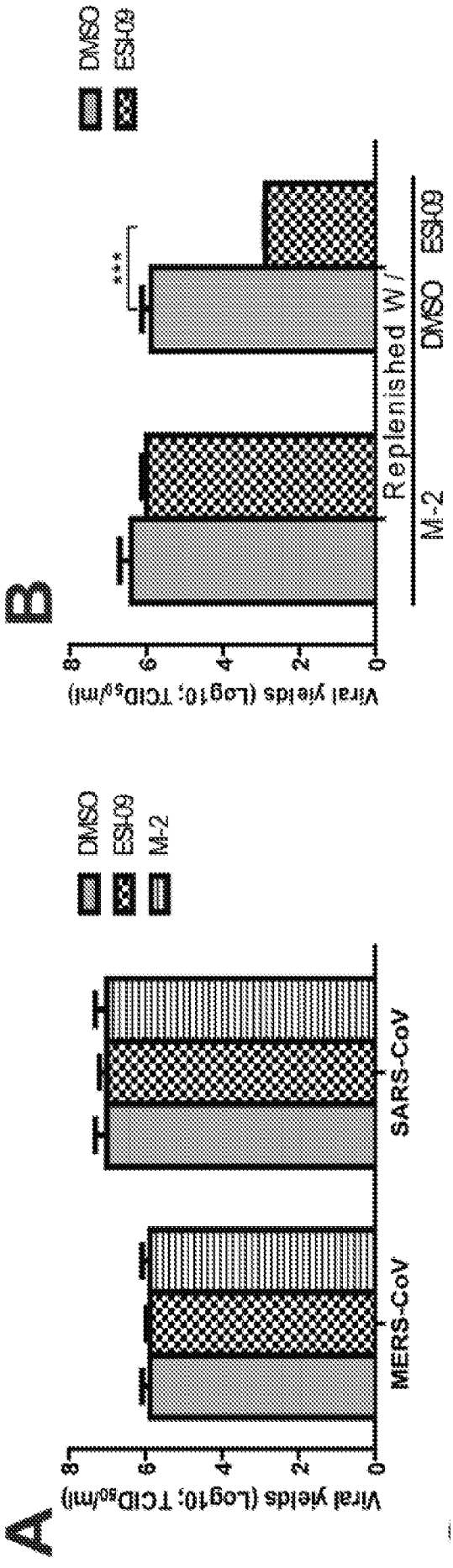

A
Viral yields (Log10; TCID50/ml)
8
6
4
2
0
MERS-CoV
SARS-CoV
DMSO
ESI-09
M-2
B
Viral yields (Log10; TCID50/ml)
8
6
4
2
0
***
Replenished W/
DMSO
ESI-09
M-2
DMSO
ESI-09

FIG. 16A-16B

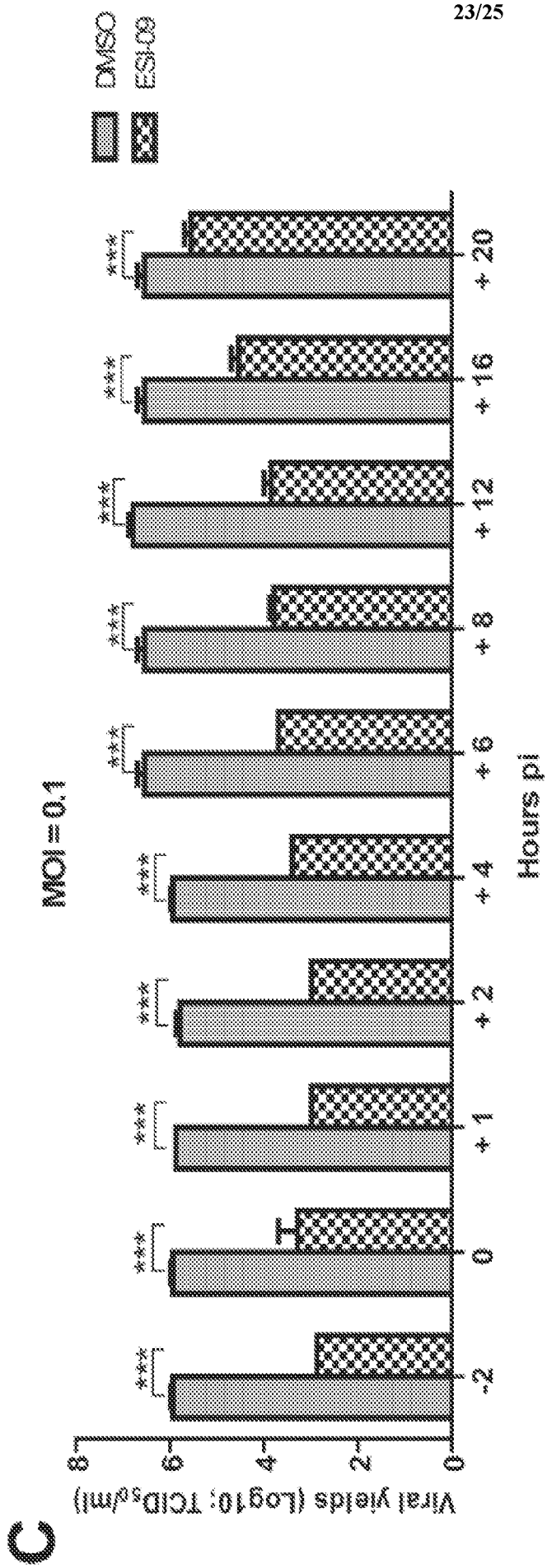

C
Viral yields (Log10; TCID50/ml)
0
2
4
6
8
MOI = 0.1
Hours pi
-2
0
+ 1
+ 2
+ 4
+ 6
+ 8
+ 12
+ 16
+ 20
***
DMSO
ES108

FIG. 16C

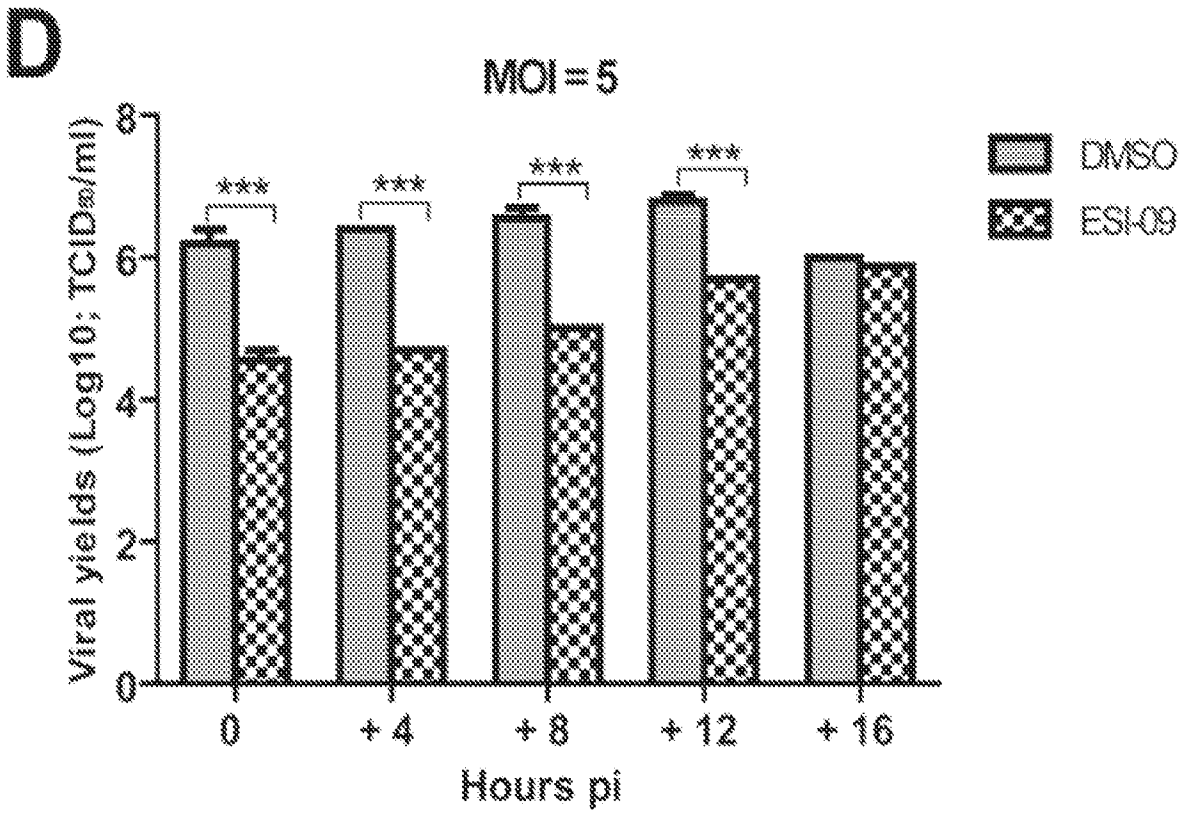

D
MOI = 5
Viral yields (Log10; TCID50/ml)
0
2
4
6
8
***
***
***
***
0
+ 4
+ 8
+ 12
+ 16
Hours pi
DMSO
ESI-09

FIG. 16D

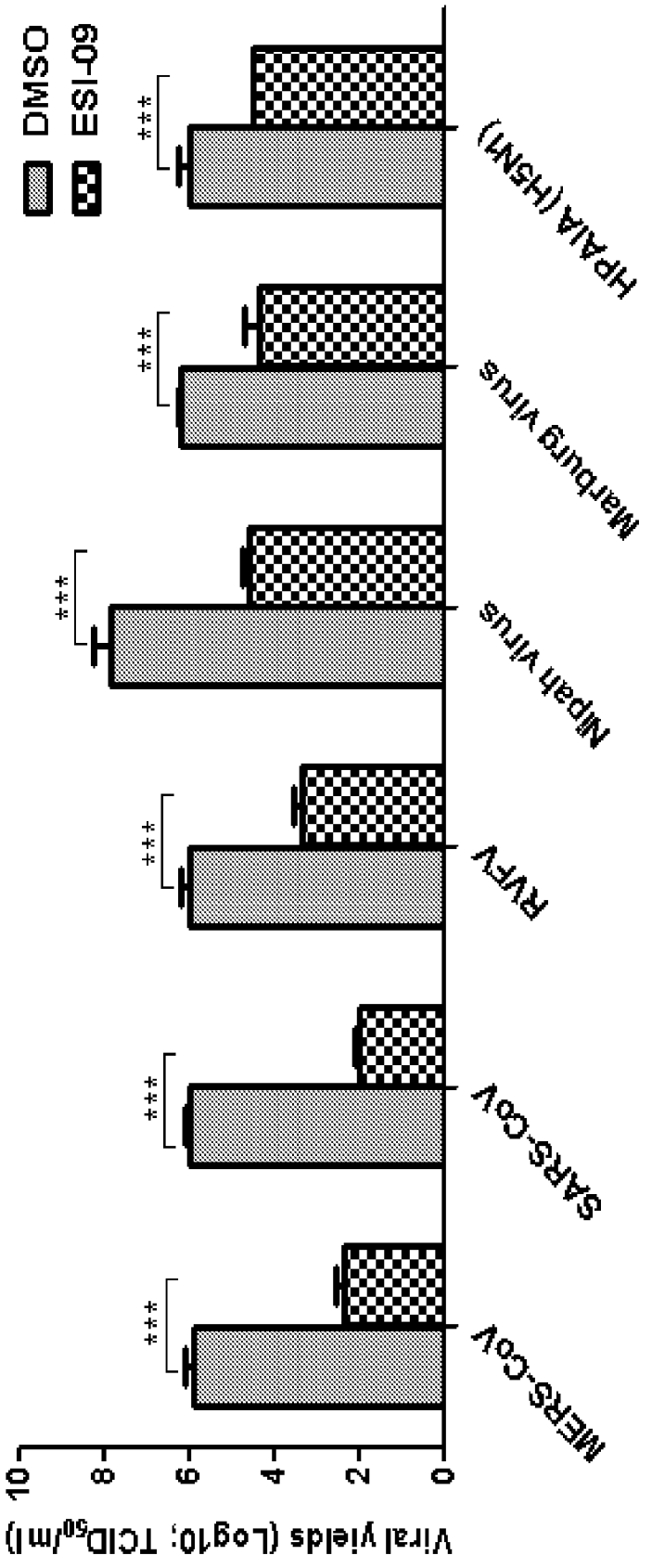

DMSO
ESI-09
Viral yields (Log10; TCID50/ml)
0
2
4
6
8
10
***
MERS-CoV
SARS-CoV
RVFV
Nipah Virus
Marburg Virus
HPAIA (H5N1)

FIG. 17

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/011975

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 31/136 (2014.01)
USPC - 435/375
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61K 31/136, 39/35, 39/395 (2014.01)
USPC - 424/191.1; 435/6, 375

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - A61K 31/10, 31/136, 31/277 (2014.02)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Orbit, Google Patents, PubMed, Google Scholar

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ALMAHARIQ et al. 'A Novel EPAC-Specific Inhibitor Suppresses Pancreatic Cancer Cell Migration and Invasion.' Molecular Pharmacology, 83: Pgs. 122-128, January 2013. entire document	1-7
A	US 2011/0060029 A1 (IWATSUBO et al) 10 March 2011 (10.03.2011) entire document	1-7
A	US 2007/0060548 A1 (LEVINE et al) 15 March 2007 (15.03.2007) entire document	1-7
P, X	WO 2013/119931 A1 (CHENG et al) 15 August 2013 (15.08.2013) entire document	1-7

☐ Further documents are listed in the continuation of Box C. ☐

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

Date of the actual completion of the international search	Date of mailing of the international search report
16 April 2014	07 MAY 2014

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)