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(54) Titre : COMPOSITIONS ET PROCEDES POUR LA DETECTION DE CELLULES CANCEREUSES EXPRIMANT PDE3A OU SLFN12

(54) Title: COMPOSITIONS AND METHODS FOR CANCER EXPRESSING PDE3A OR SLFN12

(57) Abrégé/Abstract:
The present invention features improved methods of identifying patients having cancer (e.g., melanoma, adenocarcinoma, lung, cervical, liver or breast cancer) using biomarkers (e.g., PDE3A, SLFN12) that correlate with drug sensitivity and consequently treating a stratified patient population with an agent of the invention (e.g., DNMDP, zardaverine, and anagrelide).

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[Continued on next page]

(54) Title: COMPOSITIONS AND METHODS FOR CANCER EXPRESSING PDE3A OR SLFN12

FIG. 1A

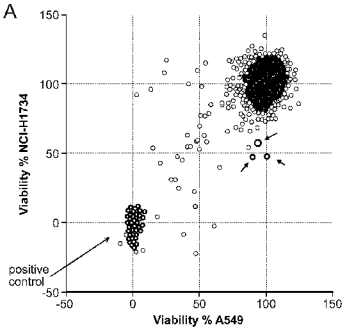


FIG. 1B

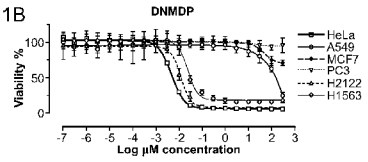


FIG. 1C

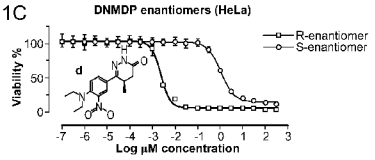
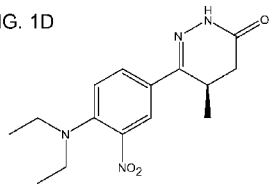


FIG. 1D



(57) Abstract: The present invention features improved methods of identifying patients having cancer (e.g., melanoma, adenocarcinoma, lung, cervical, liver or breast cancer) using biomarkers (e.g., PDE3A, SLFN12) that correlate with drug sensitivity and consequently treating a stratified patient population with an agent of the invention (e.g., DNMDP, zardaverine, and anagrelide).

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COMPOSITIONS AND METHODS FOR CANCER EXPRESSING PDE3A OR SLFN12

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 62/204,875 filed August 13, 2015.

STATEMENT OF RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH

This invention was made with support from the United States (US) Government under Grant No. 3U54HG005032 awarded by the National Institutes of Health. The US Government has certain rights in the invention.

BACKGROUND OF THE INVENTION

Cancer kills over 550,000 people in the United States and over 8 million people world-wide each year. New agents, including small molecules, molecules that impact tissue-specific growth requirements, and immunomodulatory agents, have been shown to benefit a subset of patients whose cancers have unique genomic mutations or other characteristics. Unfortunately, many cancer patients are still left without effective therapeutic options.

One approach to identify new anti-cancer agents is phenotypic screening to discover novel small molecules displaying strong selectivity between cancer cell lines, followed by chemogenomics to identify the cell features associated with drug response. In the 1990s, Weinstein and colleagues demonstrated that the cytotoxic profile of a compound can be used to identify cellular characteristics, such as gene-expression profiles and DNA copy number that correlate with drug sensitivity. The ability to identify the features of cancer cell lines that mediate their response to small molecules has strongly increased in recent years with automated high-throughput chemosensitivity testing of large panels of cell lines coupled with comprehensive genomic and phenotypic characterization of the cell lines. Phenotypic observations of small-molecule sensitivity can be linked to expression patterns or somatic alterations, as in the case of *SLFN11* expression in cancer cell lines sensitive to irinotecan treatment, and an *EWS-FLI1* rearrangement in cancer cell lines sensitive to PARP inhibitors, respectively.

Methods of characterizing malignancies at a molecular level are useful for stratifying patients, thereby quickly directing them to effective therapies. Improved methods for characterizing the responsiveness of subjects having cancer are urgently required.

SUMMARY OF THE INVENTION

Date Reçue/Date Received 2023-07-25

As described below, the present invention features methods of identifying patients having a cancer (e.g., melanoma, adenocarcinoma, lung, cervical, liver, endometrium, lung, hematopoetic / lymphoid, ovarian, cervical, soft-tissue sarcoma, leiomyosarcoma, urinary tract, pancreas, thyroid, kidney, glioblastoma, or breast cancer) that is sensitive to treatment with a phosphodiesterase 3A (PDE3A) modulator (e.g., 6-(4-(diethylamino)-3-nitrophenyl)-5-methyl-4,5-dihydropyridazin-3(2H)-one, zardaverine, and anagrelide) by detecting co-expression of *PDE3A* and *Schlafen 12* (*SLFN12*) polynucleotides or polypeptides in a cancer cell derived from such patients.

In one embodiment, the present invention provides a method of killing or reducing the survival of a cancer cell selected as responsive to a phosphodiesterase 3A (PDE3A) modulator. The method includes the step of contacting the cell with a PDE3A modulator, where the cell was selected as having an increase in *PDE3A* and/or *Schlafen 12* (*SLFN12*) polypeptide or polynucleotide relative to a reference, thereby reducing the survival of the cancer cell. In another embodiment, the present invention provides a method of reducing cancer cell proliferation in a subject pre-selected as having a cancer that is responsive to a PDE3A modulator. The method comprises administering to the subject a PDE3A modulator, wherein the subject is pre-selected by detecting an increase in PDE3A and/or SLFN12 polypeptide or polynucleotide levels relative to a reference, thereby reducing cancer cell proliferation in the subject. In one embodiment, the subject is pre-selected by detecting an increase in PDE3A and/or SLFN12 polypeptide or polynucleotide levels. In some embodiments, the PDE3A modulator is selected from the group consisting of 6-(4-(diethylamino)-3-nitrophenyl)-5-methyl-4,5-dihydropyridazin-3(2H)-one (DNMDP), zardaverine, and anagrelide.

In another embodiment, the present invention provides a method of identifying a subject having a cancer responsive to PDE3A modulation. The method includes the step of detecting an increase in the level of a PDE3A and/or SLFN12 polypeptide or polynucleotide in a biological sample of the subject relative to a reference, thereby identifying the subject as responsive to PDE3A modulation. In one embodiment, an increase in the level of PDE3A and SFLN1 polypeptide or polynucleotide is detected.

In some embodiments, the increase in the level of PDE3A and/or SLFN12 polypeptide is detected by a method selected from the group consisting of immunoblotting, mass spectrometry, and immunoprecipitation. In some other embodiments, the increase in the level of *PDE3A*, and/or *SLFN12* polynucleotide is detected by a method selected from the group consisting of quantitative PCR, Northern Blot, microarray, mass spectrometry, and in situ hybridization. In some embodiments, the activity of PDE3A is reduced. The PDE3A modulator may be administered orally. The PDE3A modulator may be administered by intravenous injection.

In some embodiments, the cancer cell is a melanoma, endometrium, lung, hematopoetic / lymphoid, ovarian, cervical, soft-tissue sarcoma, leiomyosarcoma, urinary tract, pancreas, thyroid, kidney, glioblastoma, or breast cancer. In some other embodiments, the cancer cell is not a B-cell

proliferative type cancer. In some embodiments, the cancer cell is not multiple myeloma. In some embodiments, the biological sample is a tissue sample.

In another aspect, the present invention provides a kit for identifying a subject having cancer as responsive to PD3A modulation, the kit comprising a capture reagent that binds PDE3A and/or a capture reagent that binds SLFN12. In one embodiment, the kit comprises a capture reagent that binds PDE3A and a capture reagent that binds SLFN12.

In yet another aspect, the present invention provides a kit for decreasing cancer cell proliferation in a subject pre-selected as responsive to a PDE3A modulator, the kit comprising DNMDP, zardaverine, and/or anagrelide.

The invention provides methods for treating subjects having cancer identified as responsive to treatment with a PDE3A modulator by detecting co-expression of PDE3A and/or Schlafen 12 (SLFN12) polynucleotides or polypeptides in the cancer. Compositions and articles defined by the invention were isolated or otherwise manufactured in connection with the examples provided below. Other features and advantages of the invention will be apparent from the detailed description, and from the claims.

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The present invention as claimed relates to:

- use of a phosphodiesterase 3A (PDE3A) modulator for killing or reducing the survival of a cancer cell selected as responsive to the PDE3A modulator, wherein the cell was selected as having an increase in the level of a PDE3A and/or a Schlafen 12 (SLFN12) polypeptide or polynucleotide relative to a non-responsive cancer cell or healthy control cell, and the PDE3A modulator is selected from the group consisting of 6-(4-(diethylamino)-3-nitropheny1)-5-methy1-4,5-dihydropyridazin-3(2H)-one (DNMDP), zardaverine, anagrelide, and pharmaceutically acceptable salts thereof;

- use of a PDE3A modulator for reducing cancer cell proliferation in a subject pre-selected as having a cancer that is responsive to the PDE3A modulator, wherein the subject is pre-selected by detecting an increase in the level of PDE3A and/or SLFN12 polypeptide or polynucleotide relative to a non-responsive cancer cell or healthy control cell, and the PDE3A modulator is selected from the group consisting of DNMDP, zardaverine, anagrelide, and pharmaceutically acceptable salts thereof;

- a method of identifying a subject having a cancer cell responsive to a PDE3A modulator selected from the group consisting of DNMDP, zardaverine, anagrelide, and pharmaceutically acceptable salts thereof, the method comprising detecting an increase in a PDE3A and/or SLFN12 polypeptide or polynucleotide level in a biological sample of the subject relative to a non-responsive cancer cell or healthy control cell, thereby identifying said subject as having a cancer cell responsive to PDE3A modulation;

- a method of identifying a subject having a cancer cell that is resistant to a PDE3A modulator selected from the group consisting of DNMDP, zardaverine, anagrelide, and pharmaceutically acceptable salts thereof, the method comprising detecting a decrease in the level of a SLFN12 polypeptide or polynucleotide level in a biological sample of the subject relative to a responsive cancer cell or healthy control cell, thereby identifying said subject as having a cancer cell resistant to PDE3A modulation;

- a kit for identifying a subject having cancer responsive to a PDE3A modulator selected from the group consisting of DNMDP, zardaverine, anagrelide, and pharmaceutically acceptable salts thereof, the kit comprising a first capture reagent that binds a PDE3A polypeptide or polynucleotide and a second capture reagent that binds SLFN12 polypeptide or polynucleotide;

- a kit for decreasing cancer cell proliferation in a subject responsive to a PDE3A modulator selected from the group consisting of DNMDP, zardaverine, anagrelide,

Date Reçue/Date Received 2023-07-25

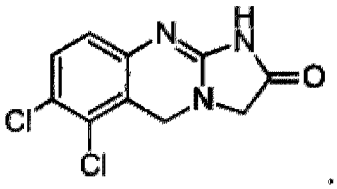
and pharmaceutically acceptable salts thereof, the kit comprising (a) a first capture reagent that binds a PDE3A polypeptide or polynucleotide; (b) a second capture reagent that binds SLFN12 polypeptide or polynucleotide; and (c) an effective amount of DNMDP, zardaverine, and/or anagrelide, or a pharmaceutically acceptable salt thereof; and

- use of a PDE3A modulator in the manufacture of a medicament for the treatment of cancer pre-selected as responsive to the PDE3A modulator, wherein the cancer is pre-selected by detecting an increase in the level of PDE3A and/or SLFN12 polypeptide or polynucleotide relative to a non-responsive cancer cell or healthy control cell, and the PDE3A modulator is selected from the group consisting of DNMDP, zardaverine, anagrelide, and pharmaceutically acceptable salts thereof.

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs. The following references provide one of skill with a general definition of many of the terms used in this invention: Singleton et al., Dictionary of Microbiology and Molecular Biology (2nd ed. 1994); The Cambridge Dictionary of Science and Technology (Walker ed., 1988); The Glossary of Genetics, 5th Ed., R. Rieger et al. (eds.), Springer Verlag (1991); and Hale & Marham, The Harper Collins Dictionary of Biology (1991). As used herein, the following terms have the meanings ascribed to them below, unless specified otherwise.

By "Anagrelide" (IUPAC Name 6,7-dichloro-1,5-dihydroimidazo (2,1-b)quinazolin-2(3*H*)-one) is meant a small molecule phosphodiesterase inhibitor having the following structure:



By "Cilostamide" (IUPAC Name *N*-cyclohexyl-*N*-methyl-4-[(2-oxo-1*H*-quinolin-6-yl)oxy]butanamide) is meant a small molecule inhibitor having the following structure:

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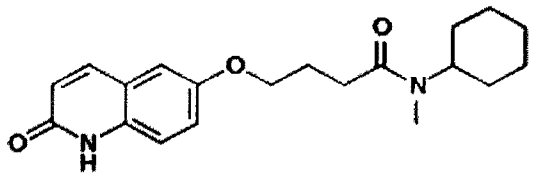
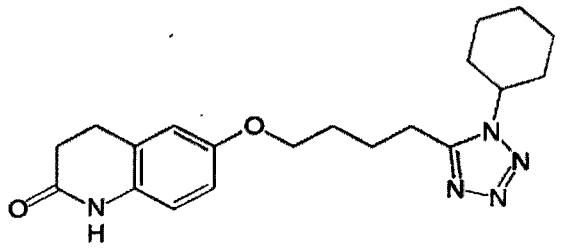
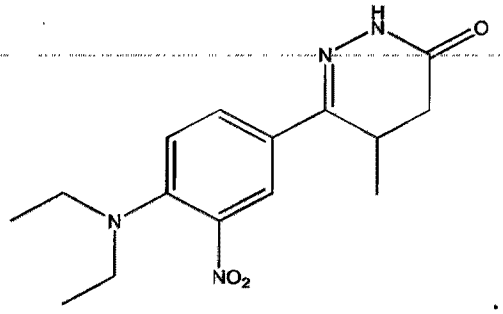


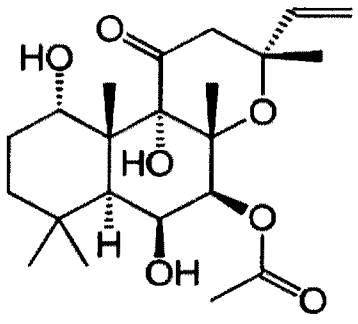
By "Cilostazol" (IUPAC Name 6-[4-(1-cyclohexyl-1*H*-tetrazol-5-yl)butoxy]-3,4-dihydro-2(1*H*)-quinolinone) is meant a small molecule inhibitor having the following structure:



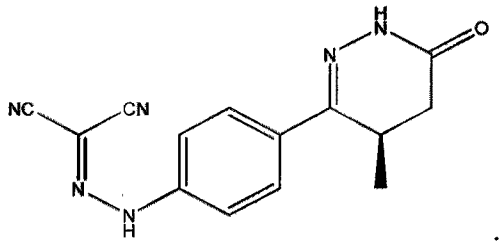
By "DNDMP" (IUPAC Name 6-(4-(diethylamino)-3-nitrophenyl)-5-methyl-4,5-dihydropyridazin-3(2H)-one) is meant a small molecule inhibitor having the following structure:



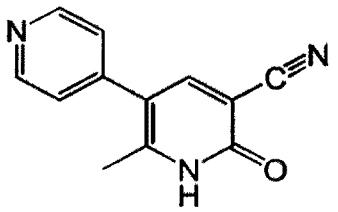
By "Forskolin" (IUPAC Name (3*R*,4a*R*,5*S*,6*S*,6a*S*,10*S*,10a*R*,10b*S*)-6,10,10b-Trihydroxy-3,4a,7,7,10a-pentamethyl-1-oxo-3-vinyldodecahydro-1*H*-benzo[*f*]chromen-5-ylacetate) is meant a small molecule inhibitor having the following structure:



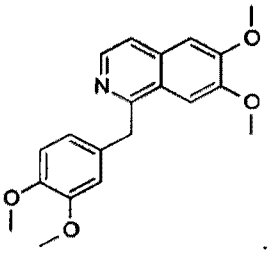
By "Levosimendan" (IUPAC Name (E)-2-cyano-1-methyl-3-(4-(4-methyl-6-oxo-1,4,5,6-tetrahydropyridazin-3-yl)phenyl)guanidine) is meant a small molecule inhibitor having the following structure:



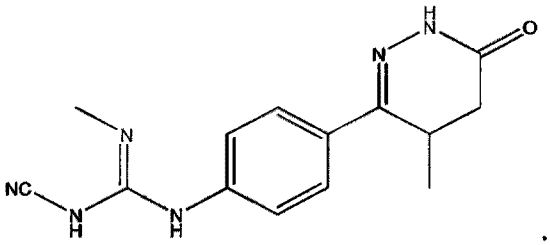
By "Milrinone" (IUPAC Name 2-methyl-6-oxo-1,6-dihydro-3,4'-bipyridine-5-carbonitrile) is meant a small molecule inhibitor having the following structure:



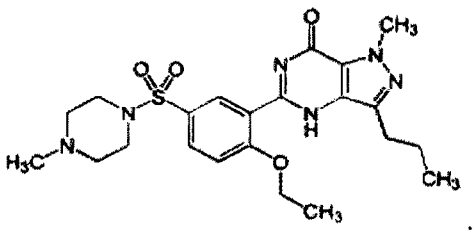
By "Papaverine" (IUPAC Name 1-(3,4-dimethoxybenzyl)-6,7-dimethoxyisoquinoline) is meant a small molecule inhibitor having the following structure:



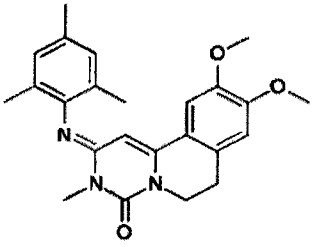
By "Siguazodan" (IUPAC Name (E)-2-cyano-1-methyl-3-(4-(4-methyl-6-oxo-1,4,5,6-tetrahydropyridazin-3-yl)phenyl)guanidine) is meant a small molecule inhibitor having the following structure:



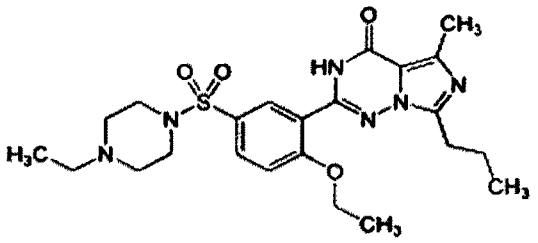
By "Sildenafil" (IUPAC Name 1-[4-ethoxy-3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenylsulfonyl]-4-methylpiperazine) is meant a small molecule inhibitor having the following structure:



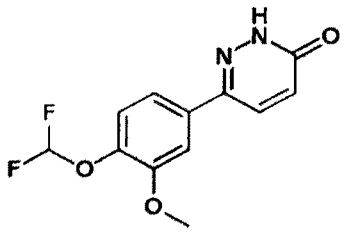
By "Trequinsin" (IUPAC Name 9,10-dimethoxy-3-methyl-2-(2,4,6-trimethylphenyl)imino-6,7-dihydropyrimido[6,1-*a*]isoquinolin-4-one) is meant a small molecule inhibitor having the following structure:



By "Vardenifil" (IUPAC Name 4-[2-ethoxy-5-(4-ethylpiperazin-1-yl)sulfonyl-phenyl]-9-methyl-7-propyl-3,5,6,8-tetrazabicyclo[4.3.0]nona-3,7,9-trien-2-one) is meant a small molecule inhibitor having the following structure:



By "Zardaverine (IUPAC Name 3-[4-(Difluoromethoxy)-3-methoxyphenyl]-1*H*-pyridazin-6-one)" is meant a small molecule inhibitor having the following structure:



In some other embodiments, any one of the compounds Cilostamide, Cilostazol, DNDMP, Levosimendan, Milrinone, Papaverine, Siguazodan, Sildenafil, Trequinsin, Vardenifil, and

Zardaverine is a small molecule phosphodiesterase inhibitor. In another embodiment, forskolin may be used in a method of the invention.

By "PDE3A polypeptide" is meant a protein or fragment thereof having at least 85% amino acid sequence identity to the sequence provided at NCBI Ref No. NP_000912.3 that catalyzes the hydrolysis of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). An exemplary human full-length PDE3A amino acid sequence is provided below:

MAVPGDAARVRDKPVHSGVSQAPTAGRDCHHRADPASPRDSGCRGCWGDLVLQPLRSSRKLSSALCAGSLSFLLA
LLVRLVRGEVGCDLEQCKEAAAAEEEEAAPGAEGGVFPGPRGGAPGGGARLSPWLQPSALLFSLLCAFFWMGLYL
LRAGVRLPLAVALLAACCGGEALVQIGLGVGEDHLLSLPAAGVVLSCLAAATWLVLRLRLGVLMIALTSAVRTVS
LISLERFKVAWRPYLAYLAGVLGILLARYVEQILPQSAEAAPREHLGSQLIAGTKEDIPVFKRRRRSSSVVSAEM
SGCSSKSHRRTSLPCIPREQLMGHSEWDHKRGPRGSQSSGTSITVDIAVMGEAHGLITDLLADPSLPPNVCTSLR
AVSNLLSTQLTFQAIHKPRVNPVTSLSENYTCSDSEESSEKDKLAIPKRLRRSLPPGLLRRVSSTWTTTTSATGL
PTLEPAPVRRDRSTSIKLQEAPSSSPDSWNNPVMMTLTKSRSFTSSYAISAANHVKAKKQSRPGALAKISPLSSP
CSSPLQGTPASSLVSKISAVQFPESADTTAKQSLGSHRALTYTQSAPDLSPQILTPPVICSSCGRPYSQGNPADE
PLERSGVATRTPSRTDDTAQVTSDYETNNNSDSSDIVQNEDETECLREPLRKASACSTYAPETMMFLDKPILAPE
PLVMDNLDSIMEQLNTWNFPIFDLVENIGRKCGRILSQVSYRLFEDMGLFEAFKIPIREFMNYFHALEIGYRDIP
YHNRIHATDVLHAVWYLTTQPIPGLSTVINDHGSTSDSDSDSGFTHGHMGYVFSKTYNVTDDKYGCLSGNIPALE
LMALYVAAAMHDYDHPGRTNAFLVATSAPQAVLYNDRSVLENHHAAAAWNLFMSRPEYNFLINLDHVEFKHFRFL
VIEAILATDLKKHFDFVAKFNGKVNDDVGIDWTNENDRLLVCQMCIKLADINGPAKCKELHLQWTDGIVNEFYEQ
GDEEASLGLPISPFMDRSAPQLANLQESFISHIVGPLCNSYDSAGLMPGKWVEDSDESGDTDDPEEEEEEAPAPN
EEETCENNESPKKKTFKRRKIYCQITQHLLQNHKMWKKVIEEEQRLAGIENQSLDQTPQSHSSEQIQAIKEEEEE
KGKPRGEEIPTQKPDQ (SEQ ID NO.: 3)

Three PDE3A isoforms are known: PDE3A1, PDE3A2, and PDE3A3. PDE3A1 comprises amino acids 146-1141, PDE3A2 isoform 2 comprises amino acids 299-1141, and PDE3A3 comprises amino acids 483-1141 of the full-length PDE3A amino acid sequence.

By "*PDE3A* polynucleotide" is meant any nucleic acid molecule, including DNA and RNA, encoding a *PDE3A* polypeptide or fragment thereof. An exemplary *PDE3A* nucleic acid sequence is provided at NCBI Ref: NM_000921.4:

```
        1   gggggccact gggaattcag tgaagagggc accctatacc atggcagtgc
ccggcgacgc
       61  tgcacgagtc agggacaagc ccgtccacag tggggtgagt caagccccca
cggcgggccg
      121 ggactgccac catcgtgcgg accccgcatc gccgcgggac tcgggctgcc gtggctgctg
      181 gggagacctg gtgctgcagc cgctccggag ctctcggaaa ctttcctccg cgctgtgcgc
      241 gggctccctg tcctttctgc tggcgctgct ggtgaggctg gtccgcgggg aggtcggctg
      301 tgacctggag cagtgtaagg aggcggcggc ggcggaggag gaggaagcag ccccgggagc
      361 agaaggggcc gtcttcccgg ggcctcgggg aggtgctccc gggggcggtg cgcggctcag
      421 cccctggctg cagccctcgg cgctgctctt cagtctcctg tgtgccttct tctggatggg
      481 cttgtacctc ctgcgcgccg gggtgcgcct gcctctggct gtcgcgctgc tggccgcctg
      541 ctgcgggggg gaagcgctcg tccagattgg gctgggcgtc ggggaggatc acttactctc
      601 actccccgcc gcgggggtgg tgctcagctg cttggccgcc gcgacatggc tggtgctgag
      661 gctgaggctg ggcgtcctca tgatcgcctt gactagcgcg gtcaggaccg tgtccctcat
      721 ttccttagag aggttcaagg tcgcctggag accttacctg gcgtacctgg ccggcgtgct
      781 ggggatcctc ttggccaggt acgtggaaca aatcttgccg cagtccgcgg aggcggctcc
      841 aagggagcat ttggggtccc agctgattgc tgggaccaag gaagatatcc cggtgtttaa
      901 gaggaggagg cggtccagct ccgtcgtgtc cgccgagatg tccggctgca gcagcaagtc
      961 ccatcggagg acctccctgc cctgtatacc gagggaacag ctcatggggc attcagaatg
     1021 ggaccacaaa cgagggccaa gaggatcaca gtcttcagga accagtatta ctgtggacat
     1081 cgccgtcatg ggcgaggccc acggcctcat taccgacctc ctggcagacc cttctcttcc
```

1141 accaaacgtg tgcacatcct tgagagccgt gagcaacttg ctcagcacac agctcacctt
1201 ccaggccatt cacaagccca gagtgaatcc cgtcacttcg ctcagtgaaa actatacctg
1261 ttctgactct gaagagagct ctgaaaaaga caagcttgct attccaaagc gcctgagaag
1321 gagtttgcct cctggcttgt tgagacgagt ttcttccact tggaccacca ccacctcggc
1381 cacaggtcta cccaccttgg agcctgcacc agtacggaga gaccgcagca ccagcatcaa
1441 actgcaggaa gcaccttcat ccagtcctga ttcttggaat aatccagtga tgatgaccct
1501 caccaaaagc agatccttta cttcatccta tgctatttct gcagctaacc atgtaaaggc
1561 taaaaagcaa agtcgaccag gtgccctcgc taaaatttca cctctttcat cgccctgctc
1621 ctcacctctc caagggactc ctgccagcag cctggtcagc aaaatttctg cagtgcagtt
1681 tccagaatct gctgacacaa ctgccaaaca aagcctaggt tctcacaggg ccttaactta
1741 cactcagagt gccccagacc tatcccctca aatcctgact ccacctgtta tatgtagcag
1801 ctgtggcaga ccatattccc aagggaatcc tgctgatgag cccctggaga gaagtggggt
1861 agccactcgg acaccaagta gaacagatga cactgctcaa gttacctctg attatgaaac
1921 caataacaac agtgacagca gtgacattgt acagaatgaa gatgaaacag agtgcctgag
1981 agagcctctg aggaaagcat cggcttgcag cacctatgct cctgagacca tgatgtttct
2041 ggacaaacca attcttgctc ccgaacctct tgtcatggat aacctggact caattatgga
2101 gcagctaaat acttggaatt ttccaatttt tgatttagtg gaaaatatag gaagaaaatg
2161 tggccgtatt cttagtcagg tatcttacag acttttgaa gacatgggcc tctttgaagc
2221 ttttaaaatt ccaattaggg aatttatgaa ttattttcat gctttggaga ttggatatag
2281 ggatattcct tatcataaca gaatccatgc cactgatgtt ttacatgctg tttggtatct
2341 tactacacag cctattccag gcctctcaac tgtgattaat gatcatggtt caaccagtga
2401 ttcagattct gacagtggat ttacacatgg acatatggga tatgtattct caaaaacgta
2461 taatgtgaca gatgataaat acggatgtct gtctgggaat atccctgcct tggagttgat
2521 ggcgctgtat gtggctgcag ccatgcacga ttatgatcat ccaggaagga ctaatgcttt
2581 cctggttgca actagtgctc ctcaggcggt gctatataac gatcgttcag ttttggagaa
2641 tcatcacgca gctgctgcat ggaatctttt catgtcccgg ccagagtata acttcttaat
2701 taaccttgac catgtggaat ttaagcattt ccgtttcctt gtcattgaag caattttggc
2761 cactgacctg aagaaacact ttgacttcgt agccaaattt aatggcaagg taaatgatga
2821 tgttggaata gattggacca atgaaaatga tcgtctactg gtttgtcaaa tgtgtataaa
2881 gttggctgat atcaatggtc cagctaaatg taaagaactc catcttcagt ggacagatgg
2941 tattgtcaat gaattttatg aacagggtga tgaagaggcc agccttggat tacccataag
3001 ccccttcatg gatcgttctg ctcctcagct ggccaacctt caggaatcct tcatctctca
3061 cattgtgggg cctctgtgca actcctatga ttcagcagga ctaatgcctg gaaaatgggt
3121 ggaagacagc gatgagtcag gagatactga tgacccagaa gaagaggagg aagaagcacc
3181 agcaccaaat gaagaggaaa cctgtgaaaa taatgaatct ccaaaaaaga agactttcaa
3241 aaggagaaaa atctactgcc aaataactca gcacctctta cagaaccaca agatgtggaa
3301 gaaagtcatt gaagaggagc aacggttggc aggcatagaa aatcaatccc tggaccagac
3361 ccctcagtcg cactcttcag aacagatcca ggctatcaag gaagaagaag aagagaaagg
3421 gaaaccaaga ggcgaggaga taccaaccca aaagccagac cagtgacaat ggatagaatg
3481 ggctgtgttt ccaaacagat tgacttgtca aagactctct tcaagccagc acaacattta
3541 gacacaacac tgtagaaatt tgagatgggc aaatggctat tgcattttgg gattcttcgc
3601 attttgtgtg tatattttta cagtgaggta cattgttaaa aacttttgc tcaaagaagc
3661 tttcacattg caacaccagc ttctaaggat tttttaagga gggaatatat atgtgtgtgt
3721 gtatataagc tcccacatag atacatgtaa aacatattca cacccatgca cgcacacaca
3781 tacacactga aggccacgat tgctggctcc acaatttagt aacatttata ttaagatata
3841 tatatagtgg tcactgtgat ataataaatc ataaaggaaa ccaaatcaca aaggagatgg
3901 tgtggcttag caaggaaaca gtgcaggaaa tgtaggttac caactaagca gcttttgctc
3961 ttagtactga gggatgaaag ttccagagca ttatttgaat tctgatacat cctgccaaca
4021 ctgtgtgtgt gtgtgtgtgt gtgtgtgtgt gtgtgtgtgt gtgtgaaaga gagacagaag
4081 ggaatggttt gagagggtgc ttgtgtgcat gtgtgtgcat atgtaaagag atttttgtgg
4141 tttaagtaac tcagaatagc tgtagcaaat gactgaatac atgtgaacaa acagaaggaa
4201 gttcactctg gagtgtcttt gggaggcagc cattccaaat gccctcctcc atttagcttc
4261 aataaagggc cttttgctga tggagggcac tcaagggctg ggtgagaggg ccacgtgttt
4321 ggtattacat tactgctatg caccacttga aggagctcta tcaccagcct caaacccgaa
4381 agactgaggc attttccagt ctacttgcct aatgaatgta taggaactgt ctatgagtat
4441 ggatgtcact caactaagat caaatcacca tttaagggga tggcattctt tatacctaaa
4501 cacctaagag ctgaagtcag gtcttttaat caggttagaa ttctaaatga tgccagagaa
4561 ggcttgggaa attgtacttc agcgtgatag cctgtgtctt cttaatttgc tgcaaaatat
4621 gtggtagaga aagaaaagga aacagaaaaa tcactctggg ttatatagca agagatgaag
4681 gagaatattt caacacaggg tttttgtgtt gacataggaa aagcctgatt cttggcaact

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4741 gttgtagttt gtctttcagg ggtgaaggtc ccactgacaa cccctgttgt ggtgttccac
4801 acgctgtttg ttggggtagc ttccatcggc agtctggccc attgtcagtc atgcttcttc
4861 tggccgggga gattatagag agattgtttg aagattgggt tattattgaa agtcttttt
4921 tttgtttgtt ttgttttggt ttgtttgttt atctacactt gtttatgctg tgagccaaac
4981 ctctatttaa aaagttgata ctcactttca atattttatt tcatattatt atatatgtca
5041 tgatagttat cttgatgtaa atatgaagat ttttttgttt ctgtagatag taaactcttt
5101 ttttaaaaaa ggaaaaggga aacattttta taaagttata ttttaatcac catttttata
5161 cattgtagtt ctctccaagc ccagtaagag aatgatgatt catttgcatg gaggtcgatg
5221 gacaaccaat catctacctt ttctaattta aatgataatc tgatatagtt ttattgccag
5281 ttaaatgagg atgctgcaaa gcatgttttt tcactagtaa cttttgctaa ctgaatgaat
5341 tctgggtcca tatctcccag atgaaaaact gttaaccaat accatatttt atagttggtg
5401 tccatttctt tccaacactg tttgttatga ttcttccttg agtacttata tacagacctg
5461 ctcattatct aaacaatctt accttctaag taaaccttga ttgtgatttc cagtttttat
5521 tttctctgac gtagtagaaa ggaatgttta cattaaaaat acttttgttt ctcataaatg
5581 gatattgtac tccccccttt caaagcatta ttttacaata attcatggca ttttaaaaaa
5641 taaggcaaag ataatacgac aaaaaatata catggtttca aggcaaattc tccaataagt
5701 tggaaaatgt aaaaaggatc aagtggatgc agcctctacc taaataatta aaatatattt
5761 cagtatattt ctgaattaac accaggtctt cattatttag aacttactaa attgttttca
5821 ttttcttagt tttacctgtg tatctccatg tttgcaaaaa ttactataag tcaaattttg
5881 ccagtgaatt taactatttt tctttccttg caattaaggg gaaaaaagca tttatcttat
5941 cttctcatac cccttgcatc taagtactta gcaaagtcaa tattttccca ttttccaaat
6001 gcgtccatct ctaacataaa tattaattga acatagagct atgtttggag tgagtggact
6061 ggcaggacag ttggaagtcc atcacagtct attgacagtt tcatcaaagc tgtatagtcc
6121 aactagtggg gcagcttggc tactatggtg gaagtctcag caaactgcct ggttttgttt
6181 gtttgttttg ttttaaggta caggaaataa gaggaataat agtggccaaa gcaattagaa
6241 catcttcatt ccagaactgt gttcagcaat ccaggcagat tgatacattt ttctttaaaa
6301 ataaattgct attacagcta gacgtcaatt gggataaata aagggatgaa gatccactaa
6361 gtttgtgact ttcatacaca cccagtacat ctcaaaggat gctaagggac attttctgcc
6421 agtagagttc tccccctttt tggtgacagc aatattatta tgttcacatc taactccaga
6481 gcttacttcc tgtggtgcca atgtatttgt tgcaatttac tacatttta tatgagccta
6541 tttataggtg ccattaaact caggtctttc aaatgaaaga gtttctagcc cacttaggga
6601 aaaagataat tgtttagaaa accataaaat caatggtagg aaaagttgga actggttacc
6661 tggatgccat ggttctctgt taaataaagt aagagaccag gtgtattctg agtgtcatca
6721 gtgttatttt cagcatgcta ataaatgtct ttccggttat atatctatct aaattaacct
6781 ttaaaatatt ggtttccttg ataaaagcac cacttttgct tttgttagct gtaatatttt
6841 ttgtcattta gataagacct ggtttggctc tcaataaaag atgaagacag tagctctgta
6901 cagggatata tctatattag tcttcatctg atgaatgaag aaatttttctc atattatgtt
6961 caagaaagta tttacttcct aaaaatagaa ttcccgattc tgtctatttt ggttgaatac
7021 cagaacaaat ctttccgttg caatcccagt aaaacgaaag aaaaggaata tcttacagac
7081 tgttcatatt agatgtatgt agactgttaa tttgcaattt ccccatattt cctgcctatc
7141 ttacccagat aactttcttt gaaggtaaaa gctgtgcaaa aggcatgaga ctcaggccta
7201 ctctttgttt aaatgatgga aaaatataaa ttattttcta agtaataaaa gtataaaaat
7261 tatcattata aataaagtct aaagtttgaa attattaatt taaaaaaaaa aaaaaaaaa
```

(SEQ ID NO.: 4)

By "Schlafen 12 (SLFN12) polypeptide" is meant a protein or fragment thereof having at least 85% amino acid sequence identity to the sequence provided at NCBI Ref No. NP_060512.3 that interacts with PDE3A when bound to anagrelide, zardaverine or DNMDP and related compounds. An exemplary human SLFN12 amino acid sequence is provided below:

```
MNISVDLETNYAELVLDVGRVTLGENSRKKMKDCKLRKKQNESVSRAMCALLNSGGGVIKAEIENEDYSYTKDGI
GLDLENSFSNILLFVPEYLDFMQNGNYFLIFVKSWSLNTSGLRITTLSSNLYKRDITSAKVMNATAALEFLKDMK
KTRGRLYLRPELLAKRPCVDIQEENNMKALAGVFFDRTELDRKEKLTFTESTHVEIKNFSTEKLLQRIKEILPQY
VSAFANTDGGYLFIGLNEDKEIIGFKAEMSDLDDLEREIEKSIRKMPVHHFCMEKKKINYSCKFLGVYDKGSLCG
YVCALRVERFCCAVFAKEPDSWHVKDNRVMQLTRKEWIQFMVEAEPKFSSSYEEVISQINTSLPAPHSWPLLEWQ
```

RQRHHCPGLSGRITYTPENLCRKLFLQHEGLKQLICEEMDSVRKGSLIFSRSWSVDLGLQENHKVLCDALLISQD
SPPVLYTFHMVQDEEFKGYSTQTALTLKQKLAKIGGYTKKVCVMTKIFYLSPEGMTSCQYDLRSQVIYPESYYFT
RRKYLLKALFKALKRLKSLRDQFSFAENLYQIIGIDCFQKNDKKMFKSCRRLT (SEQ ID NO.: 5)

By "*Schlafen 12* (*SLFN12*) polynucleotide" is meant any nucleic acid molecule, including DNA and RNA, encoding a SLFN12 polypeptide or fragment thereof. An exemplary *SLFN12* nucleic acid sequence is provided at NCBI Ref: NM_018042.4:

```
   1 tttgtaactt cacttcagcc tcccattgat cgctttctgc aaccattcag actgatctcg
  61 ggctcctatt tcatttacat tgtgtgcaca ccaagtaacc agtgggaaaa ctttagaggg
 121 tacttaaacc ccagaaaatt ctgaaaccgg gctcttgagc cgctatcctc gggcctgctc
 181 ccaccctgtg gagtgcactt tcgttttcaa taaatctctg cttttgttgc ttcattcttt
 241 ccttgctttg tttgtgtgtt tgtccagttc tttgttcaac acgccaagaa cctggacact
 301 cttcactggt aacatatttt ggcaagccaa ccaggagaaa agaatttctg cttggacact
 361 gcatagctgc tgggaaaatg aacatcagtg ttgatttgga aacgaattat gccgagttgg
 421 ttctagatgt gggaagagtc actcttggag agaacagtag gaaaaaaatg aaggattgta
 481 aactgagaaa aaagcagaat gaaagtgtct cacgagctat gtgtgctctg ctcaattctg
 541 gaggggagt gatcaaggct gaaattgaga atgaagacta tagttataca aaagatggaa
 601 taggactaga tttggaaaat tcttttagta acattctgtt atttgttcct gagtacttag
 661 acttcatgca gaatggtaac tactttctga tttttgtgaa gtcatggagc ttgaacacct
 721 ctggtctgcg gattaccacc ttgagctcca atttgtacaa aagagatata acatctgcaa
 781 aagtcatgaa tgccactgct gcactggagt tcctcaaaga catgaaaaag actagaggga
 841 gattgtattt aagaccagaa ttgctggcaa agaggccctg tgttgatata caagaagaaa
 901 ataacatgaa ggccttggcc ggggtttttt ttgatagaac agaacttgat cggaaagaaa
 961 aattgacctt tactgaatcc acacatgttg aaattaaaaa cttctcgaca gaaaagttgt
1021 tacaacgaat taaagagatt ctccctcaat atgtttctgc atttgcaaat actgatggag
1081 gatatttgtt cattggttta aatgaagata aagaaataat tggctttaaa gcagagatga
1141 gtgacctcga tgacttagaa agagaaatcg aaaagtccat taggaagatg cctgtgcatc
1201 acttctgtat ggagaagaag aagataaatt attcatgcaa attccttgga gtatatgata
1261 aaggaagtct ttgtggatat gtctgtgcac tcagagtgga gcgcttctgc tgtgcagtgt
1321 ttgctaaaga gcctgattcc tggcatgtga aagataaccg tgtgatgcag ttgaccagga
1381 aggaatggat ccagttcatg gtggaggctg aaccaaaatt ttccagttca tatgaagagg
1441 tgatctctca aataaatacg tcattacctg ctccccacag ttggcctctt ttggaatggc
1501 aacggcagag acatcactgt ccagggctat caggaaggat aacgtatact ccagaaaacc
1561 tttgcagaaa actgttctta caacatgaag gacttaagca attaatatgt gaagaaatgg
1621 actctgtcag aaagggctca ctgatcttct ctaggagctg gtctgtggat ctgggcttgc
1681 aagagaacca caaagtcctc tgtgatgctc ttctgatttc ccaggacagt cctccagtcc
1741 tatacacctt ccacatggta caggatgagg agtttaaagg ctattctaca caaactgccc
1801 taaccttaaa gcagaagctg gcaaaaattg gtggttacac taaaaaagtg tgtgtcatga
1861 caaagatctt ctacttgagc cctgaaggca tgacaagctg ccagtatgat ttaaggtcgc
1921 aagtaattta ccctgaatcc tactatttta caagaaggaa atacttgctg aaagcccttt
1981 ttaaagcctt aaagagactc aagtctctga gagaccagtt ttcctttgca gaaaatctat
2041 accagataat cggtatagat tgctttcaga agaatgataa aaagatgttt aaatcttgtc
2101 gaaggctcac ctgatggaaa atggactggg ctactgagat atttttcatt atatatttga
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2161 taacattctc taattctgtg aaaatatttc tttgaaaact ttgcaagtta agcaacttaa
2221 tgtgatgttg gataattggg ttttgtctat tttcacttct ccctaaataa tcttcacaga
2281 tattgtttga gggatattag gaaaattaat ttgttaactc gtctgtgcac agtattattt
2341 actctgtctg tagttcctga ataaattttc ttccatgctt gaactgggaa aattgcaaca
2401 cttttattct taatgacaac agtgaaaatc tcccagcata tacctagaaa acaattataa
2461 cttacaaaag attatccttg atgaaactca gaatttccac agtgggaatg aataagaagg
2521 caaaactcat (SEQ ID NO.: 6)

In some aspects, the compound is an isomer. "Isomers" are different compounds that have the same molecular formula. "Stereoisomers" are isomers that differ only in the way the atoms are arranged in space. As used herein, the term "isomer" includes any and all geometric isomers and stereoisomers. For example, "isomers" include geometric double bond cis- and trans-isomers, also termed E- and Z-isomers; R- and S-enantiomers; diastereomers, (d)-isomers and (l)-isomers, racemic mixtures thereof; and other mixtures thereof, as falling within the scope of this invention.

Geometric isomers can be represented by the symbol ----- which denotes a bond that can be a single, double or triple bond as described herein. Provided herein are various geometric isomers and mixtures thereof resulting from the arrangement of substituents around a carbon-carbon double bond or arrangement of substituents around a carbocyclic ring. Substituents around a carbon-carbon double bond are designated as being in the "Z" or "E" configuration wherein the terms "Z" and "E" are used in accordance with IUPAC standards. Unless otherwise specified, structures depicting double bonds encompass both the "E" and "Z" isomers.

Substituents around a carbon-carbon double bond alternatively can be referred to as "cis" or "trans," where "cis" represents substituents on the same side of the double bond and "trans" represents substituents on opposite sides of the double bond. The arrangement of substituents around a carbocyclic ring can also be designated as "cis" or "trans." The term "cis" represents substituents on the same side of the plane of the ring, and the term "trans" represents substituents on opposite sides of the plane of the ring. Mixtures of compounds wherein the substituents are disposed on both the same and opposite sides of plane of the ring are designated "cis/trans."

The term "enantiomers" refers to a pair of stereoisomers that are non-superimposable mirror images of each other. An atom having an asymmetric set of substituents can give rise to an enantiomer. A mixture of a pair of enantiomers in any proportion can be known as a "racemic" mixture. The term "(±)" is used to designate a racemic mixture where appropriate. "Diastereoisomers" are stereoisomers that have at least two asymmetric atoms, but which are not mirror-images of each other. The absolute stereochemistry is specified according to the Cahn-Ingold-Prelog R-S system. When a compound is an enantiomer, the stereochemistry at each chiral carbon can be specified by either R or S. Resolved compounds whose absolute configuration is unknown can be designated (+) or (-) depending on the direction (dextro- or levorotatory) which they rotate plane polarized light at the

wavelength of the sodium D line. Certain of the compounds described herein contain one or more asymmetric centers and can thus give rise to enantiomers, diastereomers, and other stereoisomeric forms that can be defined, in terms of absolute stereochemistry at each asymmetric atom, as (R)- or (S)-. The present chemical entities, pharmaceutical compositions and methods are meant to include all such possible isomers, including racemic mixtures, optically substantially pure forms and intermediate mixtures.

Optically active (R)- and (S)-isomers can be prepared, for example, using chiral synthons or chiral reagents, or resolved using conventional techniques. Enantiomers can be isolated from racemic mixtures by any method known to those skilled in the art, including chiral high pressure liquid chromatography (HPLC), the formation and crystallization of chiral salts, or prepared by asymmetric syntheses.

Optical isomers can be obtained by resolution of the racemic mixtures according to conventional processes, e.g., by formation of diastereoisomeric salts, by treatment with an optically active acid or base. Examples of appropriate acids are tartaric, diacetyltartaric, dibenzoyltartaric, ditoluoyltartaric, and camphorsulfonic acid. The separation of the mixture of diastereoisomers by crystallization followed by liberation of the optically active bases from these salts affords separation of the isomers. Another method involves synthesis of covalent diastereoisomeric molecules by reacting disclosed compounds with an optically pure acid in an activated form or an optically pure isocyanate. The synthesized diastereoisomers can be separated by conventional means such as chromatography, distillation, crystallization or sublimation, and then hydrolyzed to deliver the enantiomerically enriched compound. Optically active compounds can also be obtained by using active starting materials. In some embodiments, these isomers can be in the form of a free acid, a free base, an ester or a salt.

In certain embodiments, the compound of the invention can be a tautomer. As used herein, the term "tautomer" is a type of isomer that includes two or more interconvertible compounds resulting from at least one formal migration of a hydrogen atom and at least one change in valency (e.g., a single bond to a double bond, a triple bond to a single bond, or vice versa). "Tautomerization" includes prototropic or proton-shift tautomerization, which is considered a subset of acid-base chemistry. "Prototropic tautomerization" or "proton-shift tautomerization" involves the migration of a proton accompanied by changes in bond order. The exact ratio of the tautomers depends on several factors, including temperature, solvent, and pH. Where tautomerization is possible (e.g., in solution), a chemical equilibrium of tautomers can be reached. Tautomerizations (i.e., the reaction providing a tautomeric pair) can be catalyzed by acid or base, or can occur without the action or presence of an external agent. Exemplary tautomerizations include, but are not limited to, keto-to-enol; amide-to-imide; lactam-to-lactim; enamine-to-imine; and enamine-to-(a different) enamine tautomerizations. A specific example of keto-enol tautomerization is the interconversion of pentane-2,4-dione and 4-

hydroxypent-3-en-2-one tautomers. Another example of tautomerization is phenol-keto tautomerization. A specific example of phenol-keto tautomerization is the interconversion of pyridin-4-ol and pyridin-4(1H)-one tautomers.

All chiral, diastereomeric, racemic, and geometric isomeric forms of a structure are intended, unless specific stereochemistry or isomeric form is specifically indicated. All processes used to prepare compounds of the present invention and intermediates made therein are considered to be part of the present invention. All tautomers of shown or described compounds are also considered to be part of the present invention.

By "agent" is meant any small molecule chemical compound, antibody, nucleic acid molecule, or polypeptide, or fragments thereof.

By "ameliorate" is meant decrease, suppress, attenuate, diminish, arrest, or stabilize the development or progression of a disease.

By "alteration" is meant a change (increase or decrease) in the expression levels or activity of a gene or polypeptide as detected by standard art known methods such as those described herein. As used herein, an alteration includes an about 10% change in expression levels, preferably an about 25% change, more preferably an about40% change, and most preferably an about 50% or greater change in expression levels. "

By "analog" is meant a molecule that is not identical, but has analogous functional or structural features. For example, a polypeptide analog retains the biological activity of a corresponding naturally-occurring polypeptide, while having certain biochemical modifications that enhance the analog's function relative to a naturally occurring polypeptide. Such biochemical modifications could increase the analog's protease resistance, membrane permeability, or half-life, without altering, for example, ligand binding. An analog may include an unnatural amino acid.

In this disclosure, "comprises," "comprising," "containing" and "having" and the like can have the meaning ascribed to them in U.S. Patent law and can mean " includes," "including," and the like; "consisting essentially of" or "consists essentially" likewise has the meaning ascribed in U.S. Patent law and the term is open-ended, allowing for the presence of more than that which is recited so long as basic or novel characteristics of that which is recited is not changed by the presence of more than that which is recited, but excludes prior art embodiments.

"Detect" refers to identifying the presence, absence or amount of the analyte to be detected. In particular embodiments, the analyte is a PDE3A or SLFN12 polypeptide.

By "disease" is meant any condition or disorder that damages or interferes with the normal function of a cell, tissue, or organ. Examples of diseases include melanoma, adenocarcinoma, lung cancer, cervical cancer, liver cancer and breast cancer.

By "effective amount" is meant the amount of a compound described herein required to ameliorate the symptoms of a disease relative to an untreated patient. The effective amount of active

compound(s) used to practice the present invention for therapeutic treatment of a disease varies depending upon the manner of administration, the age, body weight, and general health of the subject. Ultimately, the attending physician or veterinarian will decide the appropriate amount and dosage regimen. Such amount is referred to as an "effective" amount. In one embodiment, the compound is DNMDP, zardaverine, or anagrelide.

The invention provides a number of targets that are useful for the development of highly specific drugs to treat or a disorder characterized by the methods delineated herein. In addition, the methods of the invention provide a facile means to identify therapies that are safe for use in subjects. In addition, the methods of the invention provide a route for analyzing virtually any number of compounds for effects on a disease described herein with high-volume throughput, high sensitivity, and low complexity.

By "fragment" is meant a portion of a polypeptide or nucleic acid molecule. This portion contains, preferably, at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, or about 90% of the entire length of the reference nucleic acid molecule or polypeptide. A fragment may contain about 10, about 20, about 30, about 40, about 50, about 60, about 70, about 80, about 90, about 100, about 200, about 300, about 400, about 500, about 600, about 700, about 800, about 900, or about 1000 nucleotides or amino acids.

"Hybridization" means hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleobases. For example, adenine and thymine are complementary nucleobases that pair through the formation of hydrogen bonds.

By "marker" or "biomarker" is meant any protein or polynucleotide having an alteration in expression level or activity (e.g., at the protein or mRNA level) that is associated with a disease or disorder. In particular embodiments, a marker of the invention is PDE3A or SLFN12.

By "modulator" is meant any agent that binds to a polypeptide and alters a biological function or activity of the polypeptide. A modulator includes, without limitation, agents that reduce or eliminate a biological function or activity of a polypeptide (e.g., an "inhibitor"). For example, a modulator may inhibit a catalytic activity of a polypeptide. A modulator includes, without limitation, agents that increase or decrease binding of a polypeptide to another agent. For example, a modulator may promote binding of a polypeptide to another polypeptide. In some embodiments, a modulator of PDE3A polypeptide is DNMDP. In some other embodiments, the modulator of PDE3A polypeptide is anagrelide or zardaverine.

By "reference" is meant a standard or control condition.

Nucleic acid molecules useful in the methods of the invention include any nucleic acid molecule that encodes a polypeptide of the invention or a fragment thereof. Such nucleic acid molecules need not be 100% identical with an endogenous nucleic acid sequence, but will typically exhibit substantial identity. Polynucleotides having "substantial identity" to an endogenous sequence

are typically capable of hybridizing with at least one strand of a double-stranded nucleic acid molecule. Nucleic acid molecules useful in the methods of the invention include any nucleic acid molecule that encodes a polypeptide of the invention or a fragment thereof. Such nucleic acid molecules need not be 100% identical with an endogenous nucleic acid sequence, but will typically exhibit substantial identity. Polynucleotides having "substantial identity" to an endogenous sequence are typically capable of hybridizing with at least one strand of a double-stranded nucleic acid molecule. By "hybridize" is meant pair to form a double-stranded molecule between complementary polynucleotide sequences (e.g., a gene described herein), or portions thereof, under various conditions of stringency. (See, e.g., Wahl, G. M. and S. L. Berger (1987) Methods Enzymol. 152:399; Kimmel, A. R. (1987) Methods Enzymol. 152:507).

For example, stringent salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, preferably less than about 500 mM NaCl and 50 mM trisodium citrate, and more preferably less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent, e.g., formamide, while high stringency hybridization can be obtained in the presence of at least about 35% formamide, and more preferably at least about 50% formamide. Stringent temperature conditions will ordinarily include temperatures of at least about 30° C, more preferably of at least about 37° C, and most preferably of at least about 42° C. Varying additional parameters, such as hybridization time, the concentration of detergent, e.g., sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those skilled in the art. Various levels of stringency are accomplished by combining these various conditions as needed. In a preferred: embodiment, hybridization will occur at 30° C in 750 mM NaCl, 75 mM trisodium citrate, and 1% SDS. In a more preferred embodiment, hybridization will occur at 37° C in 500 mM NaCl, 50 mM trisodium citrate, 1% SDS, 35% formamide, and 100 μg/ml denatured salmon sperm DNA (ssDNA). In a most preferred embodiment, hybridization will occur at 42° C in 250 mM NaCl, 25 mM trisodium citrate, 1% SDS, 50% formamide, and 200 μg/ml ssDNA. Useful variations on these conditions will be readily apparent to those skilled in the art.

For most applications, washing steps that follow hybridization will also vary in stringency. Wash stringency conditions can be defined by salt concentration and by temperature. As above, wash stringency can be increased by decreasing salt concentration or by increasing temperature. For example, stringent salt concentration for the wash steps will preferably be less than about 30 mM NaCl and 3 mM trisodium citrate, and most preferably less than about 15 mM NaCl and 1.5 mM trisodium citrate. Stringent temperature conditions for the wash steps will ordinarily include a temperature of at least about 25° C, more preferably of at least about 42° C, and even more preferably of at least about 68° C. In a preferred embodiment, wash steps will occur at 25° C in 30 mM NaCl, 3 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at 42 C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash

steps will occur at 68° C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. Additional variations on these conditions will be readily apparent to those skilled in the art. Hybridization techniques are well known to those skilled in the art and are described, for example, in Benton and Davis (Science 196:180, 1977); Grunstein and Hogness (Proc. Natl. Acad. Sci., USA 72:3961, 1975); Ausubel et al. (Current Protocols in Molecular Biology, Wiley Interscience, New York, 2001); Berger and Kimmel (Guide to Molecular Cloning Techniques, 1987, Academic Press, New York); and Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York.

By "substantially identical" is meant a polypeptide or nucleic acid molecule exhibiting at least 50% identity to a reference amino acid sequence (for example, any one of the amino acid sequences described herein) or nucleic acid sequence (for example, any one of the nucleic acid sequences described herein). Preferably, such a sequence is at least 60%, more preferably 80% or 85%, and more preferably 90%, 95% or even 99% identical at the amino acid level or nucleic acid to the sequence used for comparison.

Sequence identity is typically measured using sequence analysis software (for example, Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wis. 53705, BLAST, BESTFIT, GAP, or PILEUP/PRETTYBOX programs). Such software matches identical or similar sequences by assigning degrees of homology to various substitutions, deletions, and/or other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. In an exemplary approach to determining the degree of identity, a BLAST program may be used, with a probability score between e^{-3} and e^{-100} indicating a closely related sequence.

By "subject" is meant a mammal, including, but not limited to, a human or non-human mammal, such as a bovine, equine, canine, ovine, or feline.

Ranges provided herein are understood to be shorthand for all of the values within the range. For example, a range of 1 to 50 is understood to include any number, combination of numbers, or sub-range from the group consisting 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50.

As used herein, the terms "treat," treating," "treatment," and the like refer to reducing or ameliorating a disorder and/or symptoms associated therewith. It will be appreciated that, although not precluded, treating a disorder or condition does not require that the disorder, condition or symptoms associated therewith be completely eliminated.

Unless specifically stated or obvious from context, as used herein, the term "or" is understood to be inclusive. Unless specifically stated or obvious from context, as used herein, the terms "a", "an", and "the" are understood to be singular or plural.

Unless specifically stated or obvious from context, as used herein, the term "about" is understood as within a range of normal tolerance in the art, for example within 2 standard deviations of the mean. About can be understood as within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. Unless otherwise clear from context, all numerical values provided herein are modified by the term about.

The recitation of a listing of chemical groups in any definition of a variable herein includes definitions of that variable as any single group or combination of listed groups. The recitation of an embodiment for a variable or aspect herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

Any compositions or methods provided herein can be combined with one or more of any of the other compositions and methods provided herein.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1D show identification and characterization of 6-(4-(diethylamino)-3-nitrophenyl)-5-methyl-4,5-dihydropyridazin-3(2H)-one (DNMDP), a potent and selective cancer cell cytotoxic agent. Figure 1A is a scatterplot of 1924 compounds showing mean survival of TP53 mutant NCI-H1734 cells, which is a non-small cell lung cancer cell line, and TP53 wild-type A549 cells, another lung cancer cell line, after 48 hours of treatment at concentrations of 10 μM. DNMDP is indicated with a large arrowhead. Other compounds that selectively killed NCI-H1734 cells are indicated with a small arrow. Positive control staurosporine is indicated with a long arrow. Figure 1B is a linear graph showing a panel of cell lines that was treated with the indicated concentrations of DNMDP for 48 hours. Figure 1C is a linear graph showing the HeLa cell line that was treated with indicated concentrations of the separated enantiomers of DNMDP for 48 hours. The (R)-enantiomer had a 500-fold lower EC_{50} compared to the (S)-enantiomer. Figure 1D is a structure of (R)-DNMDP.

Figure 2 shows that DNMDP selectively killed NCI-H1734 and did not affect cell viability in A549. NCI-H1734 and A549 cell lines were treated with indicated compounds and concentrations for 48 hours.

Figure 3 shows the synthesis scheme of (R)-6-(4-(diethylamino)-3-nitrophenyl)-5-methyl-4,5-dihydropyridazin-3(2H)-one (R)-DNMDP) and analogues. Reaction conditions are as follows: (**a**) Ac_2O, (91%); (**b**) 90% HNO_3, H_2SO_4, (19%); (**c**) NaOH, MeOH/H_2O, (100%), then CH_3CHO, $NaBH(OAc)_3$, (7%); (**d**) $(BrCH_2CH_2)_2O$, K_2CO_3, DMF, (46%); (**e**) CH_3CHO, $NaBH_3CN$, MeOH, (82%).

Figures 4A-4C show super-critical fluid (SCF) chromatographs of 6-(4-(diethylamino)-3-nitrophenyl)-5-methyl-4,5-dihydropyridazin-3(2H)-one (DNMDP) (top to bottom: ES+, diode array, ES- traces). Figure 4A are three chromatographs showing Peak 1 (CRO separation); Figure 4B are three chromatographs showing Peak 2 (CRO separation); Figure 4C are three chromatographs showing synthesized (R)-DNMDP (5:95 ratio peaks 1:2 by uv).

Figures 5A-5C show that *Phosphodiesterase 3A* (*PDE3A*) expression correlated with sensitivity to 6-(4-(diethylamino)-3-nitrophenyl)-5-methyl-4,5-dihydropyridazin-3(2H)-one (DNMDP), but inhibition of PDE3A mediated cAMP hydrolysis did not correlate with cytotoxicity. Figure 5A is a scatterplot showing correlation between DNMDP sensitivity and expression of 18,988 genes in 766 genomically characterized cell lines. Cell lines were treated for 72 hours with concentrations ranging from 66.4 μM – 2 nM in 2-fold step dilutions. The Z-score for Pearson correlation between PDE3A expression and sensitivity to DNMDP is 8.5. Figure 5B is a scatterplot showing results from cell lines from panel A that were treated with 480 compounds. DNMDP showed the best correlation between *PDE3A* expression and sensitivity. Figure 5C is a scatterplot showing published PDE3 inhibitor IC_{50} values and EC_{50} values of HeLa cells treated with indicated compounds up to 10 μM for 48 hours. DNMDP IC_{50} concentration for PDE3A inhibition was determined in Figure 7B.

Figures 6A-6C show chemical structures of 6-(4-(diethylamino)-3-nitrophenyl)-5-methyl-4,5-dihydropyridazin-3(2H)-one (DNMDP), siguazodan and levosimendan, respectively.

Figures 7A and 7B are graphs showing determination of Phosphodiesterase 3A (PDE3A) *in vitro* IC_{50} of 6-(4-(diethylamino)-3-nitrophenyl)-5-methyl-4,5-dihydropyridazin-3(2H)-one (DNMDP). Figure 7A shows PDE3A *in vitro* inhibition with indicated concentrations of positive control trequinsin (IC_{50} curve was performed by Caliper). Figure 7B shows PDE3A *in vitro* inhibition with indicated concentrations of DNMDP (IC_{50} curve was performed by Caliper).

Figures 8A and 8B are graphs showing that induction of cAMP signaling did not phenocopy cytotoxicity induced by 6-(4-(diethylamino)-3-nitrophenyl)-5-methyl-4,5-dihydropyridazin-3(2H)-one (DNMDP). Forskolin: FSK. Figure 8A shows cAMP concentrations that were measured 1 hour after treatment with indicated compounds and concentration in HeLa cells. Figure 8B shows viability of HeLa cells that were treated with indicated compounds and concentrations for 48 hours.

Figures 9A-9C show that non-lethal Phosphodiesterase 3 (PDE3) inhibitors rescued cell death induced by 6-(4-(diethylamino)-3-nitrophenyl)-5-methyl-4,5-dihydropyridazin-3(2H)-one (DNMDP) by competing for the binding of PDE3A. Figure 9A is a scatterplot showing viability of HeLa cells that were treated with 1600 bioactive compounds at a concentration of 20 μM in combination with 30 nM (EC70) of DNMDP for 48 hours. The viability was calculated as a percentage of the untreated DMSO control. Figure 9B is a linear graph showing viability of HeLa cells that were treated with DNMDP in combination with indicated concentrations of non-lethal PDE3 and pan-PDE inhibitors

for 48 hours. Figure 9C shows a SDS-PAGE gel depicting the result of affinity purification performed on 200 μg of HeLa cell lysate using a DNMDP linker-analogue tethered to a solid phase with the same rescue characteristic as non-lethal PDE3 inhibitors. Indicated compounds were co-incubated with the linker-analogue. The affinity purified fraction was run on an SDS-PAGE gel and probed for PDE3A.

Figures 10A and 10B show the structure and rescue phenotype of linker-compound tert-butyl (R)-(2-(2-(2-(ethyl(4-(4-methyl-6-oxo-1,4,5,6-tetrahydropyridazin-3-yl)phenyl)amino)ethoxy) ethoxy)ethyl)carbamate (DNMDP)-2L. Figure 10A shows the structure of DNMDP-2L. Figure 10B is a graph showing the viability of HeLa cells that were treated with indicated compounds and concentrations for 48 hours.

Figures 11A-11C show that Phosphodiesterase 3A (PDE3A) was not essential in sensitive cell lines, but was required for relaying the cytotoxic signal. Figure 11A is a Western blot. HeLa cells were infected with Cas9 and indicated guide RNAs (sgRNA) against *PDE3A*. Western blots were probed for PDE3A at indicated time points. Figure 11B is a bar graph showing percent rescue of HeLa cells that were infected with indicated sgRNAs for two weeks and treated with 1 μM of 6-(4-(diethylamino)-3-nitrophenyl)-5-methyl-4,5-dihydropyridazin-3(2H)-one (DNMDP) for 48 hours. Percent rescue was normalized to the Cas9-only control. Figure 11C is a plot showing viability of cells infected with indicated sgRNAs and treated with various concentrations of 6-(4-(diethylamino)-3-nitrophenyl)-5-methyl-4,5-dihydropyridazin-3(2H)-one (DNMDP).

Figures 12A and 12B are a Western blot and a graph showing that reduction of Phosphodiesterase 3A (PDE3A) protein level caused resistance to 6-(4-(diethylamino)-3-nitrophenyl)-5-methyl-4,5-dihydropyridazin-3(2H)-one (DNMDP). In Figure 12A HeLa cells were treated with scrambled control siRNA or a combination of four different siRNAs targeting PDE3A. Cells were lysed at indicated time-points and immunoblotted for PDE3A and Actin. Figure 12B is a linear graph showing viability of HeLa cells that were treated with indicated concentrations of DNMDP analogue 3 for 48 hours.

Figures 13A-13C show that Phosphodiesterase 3A (PDE3A) immunoprecipitation in the presence of 6-(4-(diethylamino)-3-nitrophenyl)-5-methyl-4,5-dihydropyridazin-3(2H)-one (DNMDP) revealed novel SIRT7 and SLFN12 interaction. Figure 13A shows a schematic overview of the affinity enrichment followed by quantitative proteomics of PDE3A performed in HeLa cells. All cells were treated for four hours prior to lysis with 10 μM of indicated compounds. The presence of all compounds was maintained throughout the experiment including washing steps. Figure 13B is a scatterplot showing $\log_2$ ratios for proteins that were enriched in anti-PDE3A immunoprecipitates in the DMSO treated HeLa cells compared to anti-PDE3A immunoprecipitates in the presence of blocking peptide specific to the PDE3A antibody; each dot represents a protein. Figure 13C is a scatterplot showing Log_2 ratios of changes of proteins bound to PDE3A in the presence of DNMDP

versus trequinsin. Each dot represents the average of two replicates per condition for an individual protein. In all cases, the data plotted passed the Bland-Altman test with 95% confidence interval for reproducibility.

Figures 14A-14C show results of replicate PDE3A-protein interaction studies using PDE3A as bait under different conditions. Each scatterplot showed $\log_2$ ratios of two replicates for proteins that were enriched by PDE3A under different conditions over enrichment by PDE3A in the presence of blocking peptide. Each dot represents the $\log_2$ ratio for that particular protein, medium gray dots correspond to a Benjamini-Hochberg adjusted p value <0.01, light gray dots represent proteins that fall outside of the Blandt-Altman test for reproducibility within a 95% confidence interval. In Figure 14A protein enrichment was accomplished by immunoprecipitation using anti-PDE3A. In Figure 14B protein enrichment was accomplished by immunoprecipitation using anti-PDE3A in the presence of DNMDP. In Figure 14C protein enrichment was accomplished by immunoprecipitation using anti-PDE3A in the presence of trequinsin.

Figures 15A-15E show that cell lines with dual expression of *SLFN12* and *PDE3A* were significantly enriched for DNMDP-sensitive cell lines. Figure 15A is a scatterplot showing mRNA robust multichip average (RMA) expression values for *PDE3A* and *SLFN12* from the Cancer Cell Line Encyclopedia (CCLE) database (a detailed genetic characterization of a large panel of human cancer cell lines;) with sensitive cell lines indicated (Barretina et al., Nature 483, 603–607, 2012). 21 sensitive cell lines were binned in three groups of 7 based on area under the curve (AUC) rank. Figure 15B is a bar graph showing results of a Fisher's exact test on DNMDP sensitivity of cell lines with high expression of both *SLFN12* and *PDE3A* (RMA Log2 > 5) compared to other cell lines. The top half of the bar on the right indicates melanoma cell lines. Figure 15C is a scatterplot showing mRNA RPKM+1 expression values for *PDE3A* and *SLFN12* from RNA sequencing data. Figure 15D is a bar graph showing qPCR expression changes of *SLFN12* in HeLa cells transduced with shSLFN12 normalized to GAPDH. Figure 15E is a plot showing viability of HeLa cells transduced with indicated shRNA reagents and treated with indicated concentrations of DNMDP for 72 hours.

Figures 16A and 16B are scatter plots showing that *SLFN12* expression was amongst the top genes correlating with DNMDP sensitivity. Figure 16A shows the correlation between DNMDP sensitivity and expression of 18,988 genes in 766 genomically characterized cell lines. Cell lines were treated for 72 hours with concentrations ranging from 66.4 μM – 2 nM in 2-fold step dilutions. Figure 16B is a scatterplot showing a correlation between DNMDP sensitivity and expression of 18,988 genes in 766 genomically characterized cell lines. Expression levels were corrected for PDE3A expression as described earlier (Kim et al., Genetica 131, 151–156, 2007). Cell lines were treated for 72 hours with concentrations ranging from 66.4 μM – 2 nM in 2-fold step dilutions.

Figures 17A-7B show that DNMDP induces apoptosis in HeLa cells. Figure 17A is a plot showing viability of HeLa cells treated for 48 hours with indicated concentrations of DNMDP.

Caspase-Glo represents Caspase 3/7 activity indicating induction of apoptosis. CellTiter-Glo reflects viability. Figure 17B is an immunoblot. HeLa cells were treated for 36 hours with indicated compounds and concentrations. HeLa cells were harvested and immunoblotted for PARP-cleavage products, indicative of apoptosis.

Figure 18 is a scatterplot of *PDE3A* mRNA expression and sensitivity to DNMDP of 766 cancer cell lines.

Figure 19 is an immunoblot showing that DNMDP induces interaction between PDE3A and SIRT7 and SLFN12 in HeLa cells. HeLa cells were transfected with indicated plasmids and treated with indicated compounds with a final concentration of 10 μM for four hours. Endogenous PDE3A was immunoprecipitated and immunoblotted for V5 to identify novel interaction with SIRT7 and SLFN12 (upper two panels). Immunoprecipitate input was immunoblotted for PDE3A and V5 (lower two panels). V5-SLFN12 was undetectable in whole cell lysate.

Figure 20 is an immunoblot showing confirmation of mass spectrometric results herein using affinity reagents. Figure 20 shows that DNMDP and (weakly) anagrelide, but not trequinsin, induced PDE3A and SFLN12 complex formation.

Figure 21 is a set of tables showing that SLFN12 is lost in cells that have acquired resistance to DNMDP.

Figure 22 is a plot showing sensitization of a DNMDP-resistant cell line by expression of SLFN12 or expression of SFLN12 and PDE3A.

Figure 23 is a scatter plot showing sensitivity of Leiomyosarcomas (LMS) to PDE3A modulation based on SLFN12 expression level.

Table 1 shows sensitivity data of 766 cancer cell lines treated with DNMDP. Cell lines were treated for 72 hours with concentrations ranging from 66.4 μM – 2 nM in 2-fold step dilutions.

Table 2 shows results from panel of 19 phosphodiesterase inhibition reactions performed by Caliper. DNMDP concentration was 100 nM.

Table 3 shows RPKM values of SLFN12 and PDE3A expression in multiple healthy tissue types.

Table 4 showing Leiomyosarcoma sensitivity to DNMDP

Table 5 shows binding of DNMDP to PDE3A(677-1141).

Compositions and articles defined by the invention were isolated or otherwise manufactured in connection with the examples provided below. Other features and advantages of the invention will be apparent from the detailed description, and from the claims.

DETAILED DESCRIPTION

As described below, the present invention features improved methods of identifying patients having cancer (e.g., melanoma, endometrium, lung, hematopoetic / lymphoid, ovarian, cervical, soft-

tissue sarcoma, leiomyosarcoma, urinary tract, pancreas, thyroid, kidney, glioblastoma, or breast cancer)) that is sensitive to treatment with a phosphodiesterase 3A (PDE3A) modulator by detecting co-expression of PDE3A and Schlafen 12 (SLFN12) polypeptides or polynucleotides in a cancer cell derived from such patients. The invention is based at least in part on the discovery that sensitivity to phosphodiesterase 3A modulators, such as 6-(4-(diethylamino)-3-nitrophenyl)-5-methyl-4,5-dihydropyridazin-3(2H)-one, or DNMDP, in 766 cancer cell lines correlated with expression of the phosphodiesterase 3A gene, *PDE3A*. Like DNMDP, a subset of PDE3A inhibitors kill selected cancer cells while others do not; these cell-sparing PDE3A inhibitors instead block DNMDP induced cytotoxicity. Furthermore, PDE3A depletion leads to DNMDP resistance. DNMDP binding to PDE3A promotes an interaction between PDE3A and Sirtuin 7 (SIRT7) and Schlafen 12 (SLFN12), suggesting a neomorphic activity, and *SLFN12* and *PDE3A* co-expression correlated with DNMDP sensitivity. These results indicate that PDE3A modulators are promising cancer therapeutic agents and demonstrate the power of chemogenomics in small-molecule discovery and target-identification.

Accordingly, the invention provides methods of selecting a subject as having a cancer that responds to a PDE3A modulator, where the selection method involves detecting co-expression of *PDE3A* and *Schlafen 12* (*SLFN12*) polypeptides or polynucleotides, in a cancer cell derived from such subjects.

PDE3A Modulator

The identification of PDE3A modulators was made in connection with a phenotypic screen designed to identify cytotoxic small molecules in a mutant *tp53* background. A chemogenomics approach complements target-driven drug development programs, which consists of extensive *in vitro* and *in vivo* target validation, and can also be referred to as reverse chemogenomics (Zheng et al., Curr Issues Mol Biol 4, 33–43, 2002). Many U.S. Food and Drug Administration (FDA)-approved targeted therapies have been developed this way, among them small-molecule kinase inhibitors that target oncogenic somatic driver mutations (Moffat et al., Nat Rev Drug Discov 13, 588–602, 2014). However, the discovery and development of targeted therapies is often hampered by limitations in knowledge of the biological function of the target, its mechanism of action, and the available chemical matter to selectively inhibit the target.

Phenotypic screening can discover novel targets for cancer therapy whose specific molecular mechanism is often elucidated by future studies (Swinney et al., Nat Rev Drug Discov 10, 507–519, 2011). In recent years, two classes of anti-cancer drugs found by unbiased phenotypic screening efforts have been approved by the FDA. Lenalidomide and pomalidomide were found to be modulators of an E3-ligase that alter the affinity of its target, leading to degradation of lineage specific transcription factors (Krönke et al., Science 343, 301–305, 2014; Lu et al., Science 343, 305–309, 2014), whereas romidepsin and vorinostat were later identified as histone deacetylase (HDAC)

inhibitors (Moffat et al., Nat Rev Drug Discov 13, 588–602, 2014; Nakajima et al., Exp. Cell Res. 241, 126–133, 1998, Marks et al., Nat Biotechnol 25, 84–90, 2007).

Tumor suppressor alterations are suitable targets for phenotypic screening as they are not directly targetable with small molecules, although synthetic lethal approaches such as olaparib treatment of BRCA1/BRCA2 mutant cancers have proven to be effective. According to current knowledge, the *tp53* tumor suppressor gene is the most frequently mutated across human cancer, with somatic mutations detected in 36% of 4742 cancers subjected to whole exome sequencing. Despite many attempts, no compounds that selectively kill *tp53* mutant cells have been identified.

A phenotypic screen developed to identify small molecules causing synthetic lethality in *tp53* mutant cancer cells enabled the serendipitous discovery of a class of cancer-selective cytotoxic agents which act as modulators of phosphodiesterase 3A (PDE3A), as described herein below. Cyclic nucleotide phosphodiesterases catalyze the hydrolysis of second messenger molecules cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), and are important in many physiological processes. Several phosphodiesterase inhibitors have been approved for clinical treatment, including PDE3 inhibitors milrinone, cilostazol, and levosimendan for cardiovascular indications and inhibition of platelet coagulation, as well as the PDE3 inhibitor anagrelide for thrombocythemia. PDE5 inhibitors, e.g. vardenafil, are used for smooth muscle disorders including erectile dysfunction and pulmonary arterial hypertension, and the PDE4 inhibitor roflumilast reduces exacerbations from chronic obstructive pulmonary disease (COPD).

Phosphodiesterase inhibitors act by direct inhibition of their targets or by allosteric modulation; for example, structural analysis of PDE4 has led to the design of PDE4D and PDE4B allosteric modulators (Burgin et al., Nat Biotechnol 28, 63–70, 2010; Gurney et al., Neurotherapeutics 12, 49–56, 2015). The data provided herein below indicates that the cancer cytotoxic phosphodiesterase modulator DNMDP likely acts through a similar allosteric mechanism.

Accordingly, the invention provides methods for identifying subjects that have a malignancy that is likely to respond to PDE3A modulator treatment based on the level of *PDE3A* and *SLFN12* expression in a subject biological sample comprising a cancer cell. In some embodiments, the PDE3A modulator is DNMDP. In some other embodiments, the PDE3A modulator is anagrelide or zardaverine.

Compound Forms and Salts

The compounds of the present invention include the compounds themselves, as well as their salts and their prodrugs, if applicable. A salt, for example, can be formed between an anion and a positively charged substituent (e.g., amino) on a compound described herein. Suitable anions include chloride, bromide, iodide, sulfate, nitrate, phosphate, citrate, methanesulfonate, trifluoroacetate, and acetate. Likewise, a salt can also be formed between a cation and a negatively charged substituent

(e.g., carboxylate) on a compound described herein. Suitable cations include sodium ion, potassium ion, magnesium ion, calcium ion, and an ammonium cation such as tetramethylammonium ion. Examples of prodrugs include C_{1-6} alkyl esters of carboxylic acid groups, which, upon administration to a subject, are capable of providing active compounds.

Pharmaceutically acceptable salts of the compounds of the present disclosure include those derived from pharmaceutically acceptable inorganic and organic acids and bases. As used herein, the term "pharmaceutically acceptable salt" refers to a salt formed by the addition of a pharmaceutically acceptable acid or base to a compound disclosed herein. As used herein, the phrase "pharmaceutically acceptable" refers to a substance that is acceptable for use in pharmaceutical applications from a toxicological perspective and does not adversely interact with the active ingredient.

Examples of suitable acid salts include acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptanoate, glycolate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, palmoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, salicylate, succinate, sulfate, tartrate, thiocyanate, tosylate and undecanoate. Other acids, such as oxalic, while not in themselves pharmaceutically acceptable, may be employed in the preparation of salts useful as intermediates in obtaining the compounds of the present invention and their pharmaceutically acceptable acid addition salts. Salts derived from appropriate bases include alkali metal (e.g., sodium), alkaline earth metal (e.g., magnesium), ammonium and N-(alkyl)$_4^+$ salts. The present invention also envisions the quaternization of any basic nitrogen-containing groups of the compounds disclosed herein. Water or oil-soluble or dispersible products may be obtained by such quaternization. Salt forms of the compounds of any of the formulae herein can be amino acid salts of carboxyl groups (e.g., L-arginine, -lysine, -histidine salts).

Lists of suitable salts are found in Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Company, Easton, Pa., 1985, p. 1418; Journal of Pharmaceutical Science, 66, 2 (1977); and "Pharmaceutical Salts: Properties, Selection, and Use A Handbook; Wermuth, C. G. and Stahl, P. H. (eds.) Verlag Helvetica Chimica Acta, Zurich, 2002 [ISBN 3-906390-26-8].

The neutral forms of the compounds may be regenerated by contacting the salt with a base or acid and isolating the parent compound in the conventional manner. The parent form of the compound differs from the various salt forms in certain physical properties, such as solubility in polar solvents, but otherwise the salts are equivalent to the parent form of the compound for the purposes of the present invention.

In addition to salt forms, the present invention provides compounds which are in a prodrug

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form. Prodrugs of the compounds described herein are those compounds that undergo chemical changes under physiological conditions to provide the compounds of the present invention. Additionally, prodrugs can be converted to the compounds of the present invention by chemical or biochemical methods in an ex vivo environment. For example, prodrugs can be slowly converted to the compounds of the present invention when placed in a transdermal patch reservoir with a suitable enzyme or chemical reagent. Prodrugs are often useful because, in some situations, they may be easier to administer than the parent drug. They may, for instance, be more bioavailable by oral administration than the parent drug. The prodrug may also have improved solubility in pharmacological compositions over the parent drug. A wide variety of prodrug derivatives are known in the art, such as those that rely on hydrolytic cleavage or oxidative activation of the prodrug. An example, without limitation, of a prodrug would be a compound of the present invention which is administered as an ester (the "prodrug"), but then is metabolically hydrolyzed to the carboxylic acid, the active entity. Additional examples include peptidyl derivatives of a compound of the present invention.

The present invention also includes various hydrate and solvate forms of the compounds.

The compounds of the present invention may also contain unnatural proportions of atomic isotopes at one or more of the atoms that constitute such compounds. For example, the compounds may be radiolabeled with radioactive isotopes, such as for example tritium (^{3}H), iodine-125 (^{125}I) or carbon-14 (^{14}C). All isotopic variations of the compounds of the present invention, whether radioactive or not, are intended to be encompassed within the scope of the present invention.

Diagnostics

The present invention features diagnostic assays for the characterization of cancer. In one embodiment, levels of *PDE3A* and/or *Schlafen 12* (*SLFN12*) polynucleotides or polypeptides are measured in a subject sample and used as an indicator of cancer that is responsive to treatment with a PDE3A modulator. Levels of *PDE3A* and/or *Schlafen 12* polynucleotides may be measured by standard methods, such as quantitative PCR, Northern Blot, microarray, mass spectrometry, and in situ hybridization. Standard methods may be used to measure levels of PDE3A and/or Schlafen 12, polypeptides in a biological sample derived from a tumor. Such methods include immunoassay, ELISA, western blotting using an antibody that binds PDE3A and/or Schlafen 12 and radioimmunoassay. Elevated levels of *PDE3A* and *Schlafen 12* polynucleotides or polypeptides relative to a reference are considered a positive indicator of cancer that is responsive to treatment with a PDE3A modulator.

Types of biological samples

In characterizing the responsiveness of a malignancy in a subject to PDE3A modulator treatment, the level of PDE3A and/or SLFN12 expression is measured in different types of biologic samples. In one embodiment, the biologic sample is a tumor sample.

PDE3A and/or SLFN12 expression is higher in a sample obtained from a subject that is responsive to PDE3A modulator treatment than the level of expression in a non-responsive subject. In another embodiment, PDE3A and/or SLFN12 is at least about 5, 10, 20, or 30-fold higher in a subject with a malignancy than in a healthy control. Fold change values are determined using any method known in the art. In one embodiment, change is determined by calculating the difference in expression of PDE3A and/or SLFN12 in a cancer cell vs the level present in a non-responsive cancer cell or the level present in a corresponding healthy control cell.

Selection of a treatment method

As reported herein below, subjects suffering from a malignancy may be tested for PDE3A and/or SLFN12 expression in the course of selecting a treatment method. Patients characterized as having increased PDE3A and/or SLFN12 relative to a reference level are identified as responsive to PDE3A modulator treatment.

Kits

The invention provides kits for characterizing the responsiveness or resistance of a subject to PDE3A modulator treatment.

Also provided herein are kits that can include a therapeutic composition containing an effective amount of a PDE3A modulator in, e.g., unit dosage form.

In one embodiment, a diagnostic kit of the invention provides a reagent for measuring relative expression of PDE3A and SLFN12. Such reagents include capture molecules (e.g., antibodies that recognize PDE3A and SLFN12 polypeptides or nucleic acid probes that hybridize with PDE3A and SLFN12 polynucleotides).

In some embodiments, the kit comprises a sterile container which includes a therapeutic or diagnostic composition; such containers can be boxes, ampoules, bottles, vials, tubes, bags, pouches, blister-packs, or other suitable container forms known in the art. Such containers can be made of plastic, glass, laminated paper, metal foil, or other materials suitable for holding medicaments.

In one embodiment, a kit of the invention comprises reagents for measuring PDE3A and/or SLFN12 levels. If desired, the kit further comprises instructions for measuring PDE3A and/or SLFN12 and/or instructions for administering the PDE3A modulator to a subject having a malignancy, e.g., a malignancy selected as responsive to PDE3A modulator treatment. In particular embodiments, the instructions include at least one of the following: description of the therapeutic agent; dosage schedule and administration for treatment or prevention of malignancy or symptoms

thereof; precautions; warnings; indications; counter-indications; over dosage information; adverse reactions; animal pharmacology; clinical studies; and/or references. The instructions may be printed directly on the container (when present), or as a label applied to the container, or as a separate sheet, pamphlet, card, or folder supplied in or with the container.

The practice of the present invention employs, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are well within the purview of the skilled artisan. Such techniques are explained fully in the literature, such as, "Molecular Cloning: A Laboratory Manual", second edition (Sambrook, 1989); "Oligonucleotide Synthesis" (Gait, 1984); "Animal Cell Culture" (Freshney, 1987); "Methods in Enzymology" "Handbook of Experimental Immunology" (Weir, 1996); "Gene Transfer Vectors for Mammalian Cells" (Miller and Calos, 1987); "Current Protocols in Molecular Biology" (Ausubel, 1987); "PCR: The Polymerase Chain Reaction", (Mullis, 1994); "Current Protocols in Immunology" (Coligan, 1991). These techniques are applicable to the production of the polynucleotides and polypeptides of the invention, and, as such, may be considered in making and practicing the invention. Particularly useful techniques for particular embodiments will be discussed in the sections that follow.

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the assay, screening, and therapeutic methods of the invention, and are not intended to limit the scope of the invention.

EXAMPLES

Example 1. Identification of a cell-selective cytotoxic small molecule

To identify anti-cancer compounds with cell-selective cytotoxic activity, an unbiased chemical screen was performed in two lung adenocarcinoma cell lines, A549 and NCI-H1734, both of which harbor oncogenic *KRAS* mutations and truncating *STK11* mutations, and which were *TP53* wild type and mutant (R273L), respectively. 1,924 compounds were screened from the Molecular Libraries Small-Molecule Repository validation set in the A549 and NCI-H1734 cell lines at a single concentration of 10 μM in 384-well format in duplicate. As a proxy for cellular viability, ATP content was measured after 48 hours of compound treatment.

Three compounds showed a selective reduction in cell viability for the NCI-H1734 cell line compared to the A549 cell line, with an approximately 50% reduction in the NCI-H1734 cell line, which is > 4 median absolute deviations from the median in the negative direction, compared to a minimal change of < 1 median absolute deviations from the median in the A549 cell line (Figure 1A). Retesting the three compounds in a dose–response analysis validated that one compound, 6-(4-

(diethylamino)-3-nitrophenyl)-5-methyl-4,5-dihydropyridazin-3(*2H*)-one, or DNMDP, was specifically toxic to the NCI-H1734 cell line (Figure 2).

Testing of additional cell lines with DNMDP showed clear cell-selective cytotoxicity, with an EC_{50} between 10 and 100 nM for two additional lung adenocarcinoma cell lines, NCI-H1563 and NCI-H2122, and for HeLa cervical carcinoma cells, but an EC_{50} greater than 1 μM for A549, MCF7, and PC3 cells (Figure 1B; Figure 1C). Caspase activity was detected by a caspase-sensitive luciferase assay and by poly ADP ribose polymerase (PARP) cleavage in HeLa cells upon DNMDP treatment, indicating that sensitive cells undergo apoptosis after DNMDP exposure (Figures 17A-17B). To characterize cellular sensitivity to DNMDP further, 766 genomically characterized cancer cell lines were screened for DMNDP sensitivity at concentrations ranging from 66.4 μM to 2 nM in 2-fold dilution steps for 72 hours. From these cell lines, 22 cell lines were categorized as sensitive with a robust Z-score lower than -4, which represented multiple lineages including multiple melanoma cell lines, amongst others (Table 1).

Next, the DNMDP enantiomers were separated by chiral super-critical fluid (SCF) chromatography. One enantiomer was 500-fold more potent in HeLa cells than the other (Figures 1C and D). The (R)-enantiomer was synthesized from commercially available starting materials (Figure 3). This synthesized enantiomer had similar activity to the more potent separated material and was identical by chiral SCF chromatography, confirming stereochemistry of the active enantiomer (Figures 4A-4C). Two (R)-des-nitro analogues of DNMDP were synthesized, both of which tested similarly to (R)-DNMDP (Figure 3). Figures 4A-4C show super-critical fluid (SCF) chromatographs of 6-(4-(diethylamino)-3-nitrophenyl)-5-methyl-4,5-dihydropyridazin-3(2H)-one (DNMDP) (top to bottom: ES+, diode array, ES- traces). Figure 4A shows Peak 1 (CRO separation); Figure 4B shows Peak 2 (CRO separation); and Figure 4C shows synthesized (R)-DNMDP (5:95 ratio peaks 1:2 by uv).

Table 1: Sensitivity data of 766 cancer cell lines treated with DNMDP

Cell line	Lineage	DNMDP AUC	Robust Z-score
COV318	OVARY	0.095838	-6.863450362
IGR37	SKIN	0.41146	-6.532158389
JHUEM1	ENDOMETRIUM	0.53468	-6.402820773
HEL	HAEMATOPOIETIC AND LYMPHOID TISSUE	0.57955	-6.355723071
CORL51	LUNG	0.59436	-6.340177786
HEL9217	HAEMATOPOIETIC AND LYMPHOID TISSUE	0.75005	-6.176758102
NCIH1563	LUNG	1.0887	-5.821294837
SKMEL3	SKIN	1.2215	-5.681901594
NCIH2122	LUNG	1.3105	-5.58848293

RVH421	SKIN	1.4556	-5.436179018
HUT78	HAEMATOPOIETIC AND LYMPHOID TISSUE	1.5307	-5.35735046
DKMG	CENTRAL NERVOUS SYSTEM	1.7217	-5.156867709
GB1	CENTRAL NERVOUS SYSTEM	1.8269	-5.046444748
G292CLONEA141B1	BONE	1.9664	-4.900018865
HMCB	SKIN	1.9762	-4.889732315
A2058	SKIN	2.0833	-4.777315024
NCIH1734	LUNG	2.2179	-4.636032415
NCIH196	LUNG	2.5263	-4.312320999
LI7	LIVER	2.5414	-4.296471315
JHOM1	OVARY	2.7006	-4.129367368
COLO741	SKIN	2.7231	-4.10575029
HS578T	BREAST	2.8012	-4.023772788
K029AX	SKIN	2.9362	-3.88207032
MONOMAC1	HAEMATOPOIETIC AND LYMPHOID TISSUE	2.9692	-3.847431939
HT1197	URINARY TRACT	3.0929	-3.717590492
NCIH520	LUNG	3.1351	-3.67329535
CAL78	BONE	3.1711	-3.635508025
NCIH647	LUNG	3.2187	-3.585544785
CGTHW1	THYROID	3.4296	-3.36417404
NCIH1666	LUNG	3.6097	-3.175132451
L33	PANCREAS	3.625	-3.159072838
UACC62	SKIN	3.9116	-2.858243747
CAS1	CENTRAL NERVOUS SYSTEM	3.9993	-2.766189625
CAL51	BREAST	4.0017	-2.76367047
OSRC2	KIDNEY	4.326	-2.423269652
X8505C	THYROID	4.3418	-2.406685215
SH4	SKIN	4.3672	-2.380024158
NCIH1395	LUNG	4.4473	-2.29594736
SNU503	LARGE INTESTINE	4.5692	-2.16799528
HS729	SOFT TISSUE	4.6518	-2.081294362
SW579	THYROID	4.697	-2.033850277
YH13	CENTRAL NERVOUS SYSTEM	4.7007	-2.029966579
DBTRG05MG	CENTRAL NERVOUS SYSTEM	4.7415	-1.987140944
SEM	HAEMATOPOIETIC AND LYMPHOID TISSUE	4.7433	-1.985251578
HS852T	SKIN	4.7511	-1.977064324
SNU449	LIVER	4.752	-1.976119641
NCIH2286	LUNG	4.7782	-1.948618866
JHOS2	OVARY	4.8254	-1.899075485
BICR31	UPPER AERODIGESTIVE TRACT	4.8356	-1.888369076
IGR1	SKIN	4.8613	-1.861393125

JHUEM3	ENDOMETRIUM	4.93	-1.789282313
SNU387	LIVER	4.9639	-1.753699249
UMUC1	URINARY TRACT	4.9933	-1.7228396
X8305C	THYROID	5.0004	-1.7153871
NCIH1915	LUNG	5.0031	-1.712553051
P31FUJ	HAEMATOPOIETIC AND LYMPHOID TISSUE	5.0106	-1.704680691
COLO678	LARGE INTESTINE	5.0245	-1.690090585
EOL1	HAEMATOPOIETIC AND LYMPHOID TISSUE	5.0478	-1.665633789
KNS42	CENTRAL NERVOUS SYSTEM	5.0791	-1.632779809
SW1783	CENTRAL NERVOUS SYSTEM	5.1161	-1.593942837
HS940T	SKIN	5.1573	-1.550697343
SNU685	ENDOMETRIUM	5.206	-1.499579489
BCPAP	THYROID	5.2336	-1.470609207
COLO829	SKIN	5.2432	-1.460532587
DM3	PLEURA	5.2635	-1.439224734
OCUM1	STOMACH	5.2843	-1.417392058
M059K	CENTRAL NERVOUS SYSTEM	5.3059	-1.394719663
MG63	BONE	5.3943	-1.301930788
NCIH2172	LUNG	5.4245	-1.270231421
CAOV3	OVARY	5.4646	-1.228140539
PEER	HAEMATOPOIETIC AND LYMPHOID TISSUE	5.4754	-1.216804342
HS839T	SKIN	5.5232	-1.166631172
CORL105	LUNG	5.5442	-1.144588566
SNU5	STOMACH	5.5498	-1.138710537
MFE296	ENDOMETRIUM	5.5618	-1.126114762
NCIH854	LUNG	5.576	-1.111209762
NCIH146	LUNG	5.5773	-1.10984522
NCIH2081	LUNG	5.5811	-1.105856558
COV644	OVARY	5.5849	-1.101867896
VCAP	PROSTATE	5.5863	-1.100398388
BICR18	UPPER AERODIGESTIVE TRACT	5.6	-1.086018212
RH18	SOFT TISSUE	5.6283	-1.056313176
KPNYN	AUTONOMIC GANGLIA	5.6717	-1.010758457
KPNSI9S	AUTONOMIC GANGLIA	5.6827	-0.99921233
SKCO1	LARGE INTESTINE	5.688	-0.993649196
MV411	HAEMATOPOIETIC AND LYMPHOID TISSUE	5.6905	-0.991025076
COV362	OVARY	5.6913	-0.990185358
NCO2	HAEMATOPOIETIC AND LYMPHOID TISSUE	5.7088	-0.971816519
JHH4	LIVER	5.71	-0.970556942
NCIH2141	LUNG	5.7218	-0.958171096

LXF289	LUNG	5.734	-0.945365392
MEWO	SKIN	5.738	-0.9411668
TE125T	SOFT TISSUE	5.744	-0.934868913
SNU869	BILIARY TRACT	5.7543	-0.924057539
LNCAPCLONEFGC	PROSTATE	5.7557	-0.922588032
NCIH2009	LUNG	5.7594	-0.918704335
SKNBE2	AUTONOMIC GANGLIA	5.7717	-0.905793666
IALM	LUNG	5.775	-0.902329827
DU145	PROSTATE	5.7825	-0.894457468
HCC1419	BREAST	5.7835	-0.89340782
NALM6	HAEMATOPOIETIC AND LYMPHOID TISSUE	5.7872	-0.889524123
PECAPJ15	UPPER AERODIGESTIVE TRACT	5.789	-0.887634757
LU99	LUNG	5.8016	-0.874409193
LAMA84	HAEMATOPOIETIC AND LYMPHOID TISSUE	5.8201	-0.854990707
ONCODG1	OVARY	5.8296	-0.845019051
HS888T	BONE	5.8353	-0.839036058
SKNSH	AUTONOMIC GANGLIA	5.8424	-0.831583558
TUHR14TKB	KIDNEY	5.8451	-0.828749509
PF382	HAEMATOPOIETIC AND LYMPHOID TISSUE	5.8519	-0.821611903
ALLSIL	HAEMATOPOIETIC AND LYMPHOID TISSUE	5.8724	-0.800094121
KMS34	HAEMATOPOIETIC AND LYMPHOID TISSUE	5.8799	-0.792221762
BICR6	UPPER AERODIGESTIVE TRACT	5.8837	-0.788233099
GRANTA519	HAEMATOPOIETIC AND LYMPHOID TISSUE	5.8937	-0.77773662
OCIAML2	HAEMATOPOIETIC AND LYMPHOID TISSUE	5.8945	-0.776896902
SUIT2	PANCREAS	5.8956	-0.775742289
BT549	BREAST	5.9226	-0.747401796
KMS28BM	HAEMATOPOIETIC AND LYMPHOID TISSUE	5.9369	-0.732391831
HCC1428	BREAST	5.9402	-0.728927992
HCC1500	BREAST	5.9451	-0.723784718
A549	LUNG	5.9509	-0.71769676
KCL22	HAEMATOPOIETIC AND LYMPHOID TISSUE	5.9598	-0.708354893
COLO679	SKIN	5.9634	-0.704576161
SKMEL5	SKIN	5.9639	-0.704051337
HCC1395	BREAST	5.9716	-0.695969048
NCIH1435	LUNG	5.9756	-0.691770456
LOUNH91	LUNG	5.9793	-0.687886759
RPMI8402	HAEMATOPOIETIC AND LYMPHOID TISSUE	5.9827	-0.684317956
COLO668	LUNG	5.9969	-0.669412956

SKLU1	LUNG	6.0109	-0.654717885
KMS12BM	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.0135	-0.6519888
SNU1272	KIDNEY	6.0226	-0.642437004
MOLM6	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.0447	-0.619239786
EPLC272H	LUNG	6.0469	-0.61693056
SCC4	UPPER AERODIGESTIVE TRACT	6.0502	-0.613466722
LMSU	STOMACH	6.0528	-0.610737638
KMS20	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.0542	-0.60926813
G402	SOFT TISSUE	6.0606	-0.602550384
KYSE410	OESOPHAGUS	6.0741	-0.588380137
L540	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.0807	-0.581452461
MOLT13	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.084	-0.577988623
L1236	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.0853	-0.57662408
LP1	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.1029	-0.558150277
SNU620	STOMACH	6.1039	-0.557100629
MALME3M	SKIN	6.112	-0.548598481
GSU	STOMACH	6.1172	-0.543140312
MCF7	BREAST	6.1256	-0.53432327
COLO800	SKIN	6.1272	-0.532643833
MKN7	STOMACH	6.1453	-0.513645206
SNU119	OVARY	6.1473	-0.51154591
U118MG	CENTRAL NERVOUS SYSTEM	6.1481	-0.510706192
OCILY19	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.1512	-0.507452283
RKN	SOFT TISSUE	6.1579	-0.500419642
DV90	LUNG	6.1676	-0.490238057
NCIH1355	LUNG	6.171	-0.486669254
KMM1	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.1723	-0.485304712
NCIH1184	LUNG	6.1776	-0.479741578
U937	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.1777	-0.479636613
EJM	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.1782	-0.479111789
C32	SKIN	6.1786	-0.47869193
NCIH23	LUNG	6.1854	-0.471554324
RERFLCAD1	LUNG	6.1862	-0.470714606
T3M10	LUNG	6.1867	-0.470189782
U266B1	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.1906	-0.466096155
CAL54	KIDNEY	6.1949	-0.461582669
DND41	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.1979	-0.458433726

PC14	LUNG	6.2003	-0.455914571
KMS11	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.2008	-0.455389747
DMS53	LUNG	6.2061	-0.449826613
SNU1214	UPPER AERODIGESTIVE TRACT	6.2071	-0.448776965
GOS3	CENTRAL NERVOUS SYSTEM	6.2076	-0.448252141
TE8	OESOPHAGUS	6.2119	-0.443738655
ECGI10	OESOPHAGUS	6.2151	-0.440379781
KO52	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.2174	-0.437965591
NCIH1793	LUNG	6.2189	-0.436391119
NB4	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.219	-0.436286155
NCIH1105	LUNG	6.2191	-0.43618119
OCILY10	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.222	-0.433137211
NCIH69	LUNG	6.2243	-0.430723021
A673	BONE	6.2304	-0.424320168
HCC4006	LUNG	6.2335	-0.42106626
SCC9	UPPER AERODIGESTIVE TRACT	6.2351	-0.419386823
OAW28	OVARY	6.2381	-0.416237879
BXPC3	PANCREAS	6.2387	-0.415608091
ISTMES1	PLEURA	6.2389	-0.415398161
SKMM2	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.2396	-0.414663408
NCIN87	STOMACH	6.24	-0.414243548
T98G	CENTRAL NERVOUS SYSTEM	6.2412	-0.412983971
GP2D	LARGE INTESTINE	6.2536	-0.399968337
FTC238	THYROID	6.2564	-0.397029323
KMS27	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.2607	-0.392515837
SNU201	CENTRAL NERVOUS SYSTEM	6.2618	-0.391361224
BC3C	URINARY TRACT	6.266	-0.386952703
RS411	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.2689	-0.383908724
TALL1	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.2742	-0.37834559
RT4	URINARY TRACT	6.2742	-0.37834559
SKOV3	OVARY	6.2773	-0.375091681
RERFLCAD2	LUNG	6.2783	-0.374042033
KHM1B	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.2859	-0.366064709
KASUMI2	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.2904	-0.361341294
MOLT16	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.2966	-0.354833477
NUDUL1	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.2966	-0.354833477
KMS18	HAEMATOPOIETIC AND	6.2973	-0.354098723

	LYMPHOID TISSUE		
MDAMB175VII	BREAST	6.2981	-0.353259005
RMGI	OVARY	6.3019	-0.349270343
KIJK	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.305	-0.346016434
OCIAML5	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.3062	-0.344756857
KMRC20	KIDNEY	6.3063	-0.344651892
LU65	LUNG	6.3082	-0.342657561
JIMT1	BREAST	6.3087	-0.342132737
SNU8	OVARY	6.3089	-0.341922807
KALS1	CENTRAL NERVOUS SYSTEM	6.3098	-0.340978124
SCABER	URINARY TRACT	6.322	-0.32817242
OVMANA	OVARY	6.3268	-0.32313411
TUHR10TKB	KIDNEY	6.3302	-0.319565307
SUPM2	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.3314	-0.318305729
JMSU1	URINARY TRACT	6.3317	-0.317990835
NCIH446	LUNG	6.3331	-0.316521328
COV434	OVARY	6.3341	-0.31547168
HCC38	BREAST	6.3361	-0.313372384
KMRC2	KIDNEY	6.3393	-0.310013511
SNU478	BILIARY TRACT	6.3432	-0.305919884
SUDHL1	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.3444	-0.304660306
CMLT1	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.3494	-0.299412067
UACC257	SKIN	6.3508	-0.29794256
NCIH1339	LUNG	6.3509	-0.297837595
M07E	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.3511	-0.297627665
KMRC3	KIDNEY	6.3514	-0.297312771
NCIH1693	LUNG	6.3603	-0.287970905
MM1S	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.3604	-0.28786594
HCC1143	BREAST	6.3611	-0.287131186
KATOIII	STOMACH	6.3642	-0.283877278
MDAMB453	BREAST	6.3691	-0.278734003
J82	URINARY TRACT	6.3718	-0.275899954
CAL27	UPPER AERODIGESTIVE TRACT	6.3725	-0.2751652
HS766T	PANCREAS	6.3727	-0.274955271
HCT8	LARGE INTESTINE	6.3733	-0.274325482
NCIH1581	LUNG	6.3747	-0.272855975
REH	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.3759	-0.271596397
MPP89	PLEURA	6.3817	-0.265508439
SNU761	LIVER	6.3819	-0.26529851

RH30	SOFT TISSUE	6.3841	-0.262989284
KURAMOCHI	OVARY	6.3842	-0.26288432
HS936T	SKIN	6.385	-0.262044601
HCC15	LUNG	6.3861	-0.260889989
F36P	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.388	-0.258895657
PANC0504	PANCREAS	6.3894	-0.25742615
NOMO1	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.3925	-0.254172242
SKUT1	SOFT TISSUE	6.3987	-0.247664425
CCK81	LARGE INTESTINE	6.4043	-0.241786397
NCIH211	LUNG	6.4058	-0.240211925
NH6	AUTONOMIC GANGLIA	6.4066	-0.239372206
BECKER	CENTRAL NERVOUS SYSTEM	6.4161	-0.229400551
NCIH1869	LUNG	6.4177	-0.227721114
ASPC1	PANCREAS	6.4186	-0.226776431
VMCUB1	URINARY TRACT	6.4199	-0.225411889
SNU398	LIVER	6.4206	-0.224677136
THP1	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.4214	-0.223837417
HS611T	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.4224	-0.222787769
ONS76	CENTRAL NERVOUS SYSTEM	6.4253	-0.21974379
LOVO	LARGE INTESTINE	6.4266	-0.218379248
GMS10	CENTRAL NERVOUS SYSTEM	6.4313	-0.213445903
RKO	LARGE INTESTINE	6.4316	-0.213131009
ZR7530	BREAST	6.4339	-0.210716818
FU97	STOMACH	6.4421	-0.202109705
OCILY3	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.4442	-0.199905445
BV173	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.4448	-0.199275656
NCIH1568	LUNG	6.4489	-0.1949721
NCIH1155	LUNG	6.4497	-0.194132381
JURKAT	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.4524	-0.191298332
CW2	LARGE INTESTINE	6.4567	-0.186784846
RD	SOFT TISSUE	6.4567	-0.186784846
RERFLCAI	LUNG	6.4571	-0.186364987
YD10B	UPPER AERODIGESTIVE TRACT	6.4579	-0.185525268
SF295	CENTRAL NERVOUS SYSTEM	6.4581	-0.185315339
JJN3	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.4585	-0.18489548
EB1	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.4633	-0.17985717
KNS60	CENTRAL NERVOUS SYSTEM	6.4642	-0.178912487
X697	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.4674	-0.175553613

TOV21G	OVARY	6.4695	-0.173349353
JHH5	LIVER	6.4703	-0.172509634
OVTOKO	OVARY	6.4718	-0.170935162
WM1799	SKIN	6.4744	-0.168206078
PL21	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.4754	-0.16715643
CA46	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.4772	-0.165267064
PATU8988S	PANCREAS	6.479	-0.163377697
HCC44	LUNG	6.4794	-0.162957838
KARPAS299	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.4827	-0.159494
PANC0327	PANCREAS	6.4856	-0.156450021
YD8	UPPER AERODIGESTIVE TRACT	6.4856	-0.156450021
GDM1	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.4875	-0.15445569
IM95	STOMACH	6.4877	-0.154245761
HCT15	LARGE INTESTINE	6.4918	-0.149942204
WM793	SKIN	6.4939	-0.147737944
SHP77	LUNG	6.5008	-0.140495373
X8MGBA	CENTRAL NERVOUS SYSTEM	6.5012	-0.140075514
OUMS23	LARGE INTESTINE	6.5015	-0.139760619
SW1116	LARGE INTESTINE	6.5032	-0.137976218
NCIH1703	LUNG	6.5035	-0.137661324
HLF	LIVER	6.5042	-0.13692657
REC1	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.5051	-0.135981887
ML1	THYROID	6.5066	-0.134407415
HOS	BONE	6.5069	-0.134092521
SW837	LARGE INTESTINE	6.5072	-0.133777626
EHEB	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.5124	-0.128319457
HUH28	BILIARY TRACT	6.5145	-0.126115197
MDAMB157	BREAST	6.5173	-0.123176182
CHP212	AUTONOMIC GANGLIA	6.5178	-0.122651359
RMUGS	OVARY	6.52	-0.120342133
NCIH2106	LUNG	6.5249	-0.115198858
SKLMS1	SOFT TISSUE	6.5254	-0.114674034
X647V	URINARY TRACT	6.5257	-0.11435914
HS294T	SKIN	6.5258	-0.114254175
CHAGOK1	LUNG	6.5292	-0.110685372
NCIH2228	LUNG	6.5304	-0.109425795
MHHCALL3	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.5324	-0.107326499
TE6	OESOPHAGUS	6.5328	-0.10690664
MHHES1	BONE	6.5353	-0.10428252

X42MGBA	CENTRAL NERVOUS SYSTEM	6.5397	-0.099664069
SH10TC	STOMACH	6.5448	-0.094310865
HCC202	BREAST	6.5484	-0.090532132
ACHN	KIDNEY	6.5518	-0.08696333
SCC25	UPPER AERODIGESTIVE TRACT	6.5527	-0.086018646
PANC0403	PANCREAS	6.5578	-0.080665442
A2780	OVARY	6.5613	-0.076991674
EBC1	LUNG	6.5617	-0.076571815
SW620	LARGE INTESTINE	6.5658	-0.072268259
SKMEL31	SKIN	6.5659	-0.072163294
PK45H	PANCREAS	6.5666	-0.07142854
NCIH2030	LUNG	6.5688	-0.069119315
SKMES1	LUNG	6.5724	-0.065340583
NAMALWA	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.5738	-0.063871075
CAL12T	LUNG	6.5741	-0.063556181
HPBALL	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.5743	-0.063346251
HT1080	SOFT TISSUE	6.5745	-0.063136322
OE33	OESOPHAGUS	6.5749	-0.062716463
SR786	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.5751	-0.062506533
NCIH929	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.5755	-0.062086674
OVCAR4	OVARY	6.5755	-0.062086674
T47D	BREAST	6.5764	-0.061141991
HCC1937	BREAST	6.5773	-0.060197308
SKHEP1	LIVER	6.5773	-0.060197308
KMS26	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.5778	-0.059672484
SNU1066	UPPER AERODIGESTIVE TRACT	6.5779	-0.059567519
SUPHD1	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.5802	-0.057153329
L428	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.5828	-0.054424244
PLCPRF5	LIVER	6.584	-0.053164667
MSTO211H	PLEURA	6.5871	-0.049910758
GA10	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.59	-0.046866779
HSC2	UPPER AERODIGESTIVE TRACT	6.59	-0.046866779
MKN74	STOMACH	6.5911	-0.045712167
TOLEDO	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.5926	-0.044137695
KARPAS620	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.5931	-0.043612871
CALU6	LUNG	6.5932	-0.043507906
SNU1196	BILIARY TRACT	6.5947	-0.041933434

HGC27	STOMACH	6.595	-0.04161854
NCIH716	LARGE INTESTINE	6.5964	-0.040149033
HDMYZ	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.5974	-0.039099385
A3KAW	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.6031	-0.033116392
SNGM	ENDOMETRIUM	6.6038	-0.032381638
CAL851	BREAST	6.6074	-0.028602906
JHUEM2	ENDOMETRIUM	6.608	-0.027973117
LN18	CENTRAL NERVOUS SYSTEM	6.6106	-0.025244032
VMRCRCZ	KIDNEY	6.6107	-0.025139067
TE10	OESOPHAGUS	6.6127	-0.023039772
CAKI2	KIDNEY	6.614	-0.021675229
PK1	PANCREAS	6.6156	-0.019995793
TE1	OESOPHAGUS	6.6158	-0.019785863
IGR39	SKIN	6.6163	-0.019261039
NCIH1781	LUNG	6.6169	-0.01863125
A253	SALIVARY GLAND	6.6238	-0.01138868
NCIH727	LUNG	6.6253	-0.009814208
G361	SKIN	6.6284	-0.006560299
TYKNU	OVARY	6.6296	-0.005300722
SNU1041	UPPER AERODIGESTIVE TRACT	6.6307	-0.004146109
JL1	PLEURA	6.6309	-0.00393618
SNU283	LARGE INTESTINE	6.6315	-0.003306391
HCT116	LARGE INTESTINE	6.632	-0.002781567
LS1034	LARGE INTESTINE	6.6323	-0.002466673
EFO21	OVARY	6.633	-0.001731919
DMS114	LUNG	6.6335	-0.001207095
SNU1077	ENDOMETRIUM	6.6342	-0.000472342
DAOY	CENTRAL NERVOUS SYSTEM	6.6343	-0.000367377
NCIH2342	LUNG	6.6346	-5.24824E-05
MOLP8	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.6347	5.24824E-05
BHT101	THYROID	6.6351	0.000472342
TE5	OESOPHAGUS	6.6355	0.000892201
PSN1	PANCREAS	6.6403	0.005930511
NCIH2170	LUNG	6.6424	0.008134771
RCHACV	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.6426	0.008344701
HUH6	LIVER	6.6437	0.009499314
NCIH838	LUNG	6.6448	0.010653926
YAPC	PANCREAS	6.6485	0.014537624
KYSE450	OESOPHAGUS	6.6505	0.016636919
RERFLCMS	LUNG	6.6512	0.017371673
OVISE	OVARY	6.6514	0.017581603

HT55	LARGE INTESTINE	6.6554	0.021780194
SNU899	UPPER AERODIGESTIVE TRACT	6.662	0.02870787
NCIH226	LUNG	6.6624	0.02912773
X639V	URINARY TRACT	6.6635	0.030282342
TE14	OESOPHAGUS	6.6652	0.032066744
MKN45	STOMACH	6.6662	0.033116392
UMUC3	URINARY TRACT	6.6662	0.033116392
HEC6	ENDOMETRIUM	6.6667	0.033641216
X253JBV	URINARY TRACT	6.6694	0.036475265
SKMEL24	SKIN	6.6712	0.038364631
VMRCLCD	LUNG	6.6718	0.03899442
DLD1	LARGE INTESTINE	6.6751	0.042458258
ECC12	STOMACH	6.6785	0.046027061
WSUDLCL2	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.6801	0.047706498
PFEIFFER	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.6804	0.048021392
NCIH2087	LUNG	6.6806	0.048231322
NCIH2029	LUNG	6.6826	0.050330617
SJSA1	BONE	6.6844	0.052219984
A172	CENTRAL NERVOUS SYSTEM	6.6858	0.053689491
SNU1033	LARGE INTESTINE	6.6873	0.055263963
TM31	CENTRAL NERVOUS SYSTEM	6.6885	0.05652354
X2313287	STOMACH	6.6886	0.056628505
SQ1	LUNG	6.6945	0.062821428
SUPT11	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.695	0.063346251
NCIH2023	LUNG	6.6954	0.063766111
HCC1569	BREAST	6.6976	0.066075336
TT2609C02	THYROID	6.7014	0.070063998
SW1990	PANCREAS	6.7019	0.070588822
OVSAHO	OVARY	6.7028	0.071533505
NCIH841	LUNG	6.7036	0.072373224
ME1	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.7039	0.072688118
COLO205	LARGE INTESTINE	6.7052	0.07405266
TCCSUP	URINARY TRACT	6.7056	0.074472519
TE11	OESOPHAGUS	6.7063	0.075207273
TE4	OESOPHAGUS	6.707	0.075942026
NCIH1694	LUNG	6.7095	0.078566146
KP4	PANCREAS	6.7102	0.0793009
CL11	LARGE INTESTINE	6.711	0.080140618
NCIH596	LUNG	6.7123	0.08150516
OCIAML3	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.7152	0.084549139

KMH2	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.7155	0.084864034
PK59	PANCREAS	6.7163	0.085703752
HDLM2	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.7172	0.086648435
ES2	OVARY	6.7183	0.087803048
SKNDZ	AUTONOMIC GANGLIA	6.7192	0.088747731
NCIH650	LUNG	6.7194	0.088957661
CAL62	THYROID	6.721	0.090637097
MDAMB231	BREAST	6.7222	0.091896675
HARA	LUNG	6.7238	0.093576111
MFE319	ENDOMETRIUM	6.7242	0.093995971
LCLC103H	LUNG	6.7269	0.09683002
OE19	OESOPHAGUS	6.7273	0.097249879
HT144	SKIN	6.7297	0.099769034
HEC251	ENDOMETRIUM	6.7301	0.100188893
A4FUK	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.7317	0.10186833
K562	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.7319	0.102078259
HEC59	ENDOMETRIUM	6.7321	0.102288189
NCIH1341	LUNG	6.7337	0.103967626
A204	SOFT TISSUE	6.7338	0.10407259
OV7	OVARY	6.7346	0.104912309
OV90	OVARY	6.7381	0.108586076
HCC827	LUNG	6.7384	0.108900971
DU4475	BREAST	6.742	0.112679703
SKMEL1	SKIN	6.742	0.112679703
KYSE70	OESOPHAGUS	6.7428	0.113519422
CHP126	AUTONOMIC GANGLIA	6.7459	0.11677333
DETROIT562	UPPER AERODIGESTIVE TRACT	6.7465	0.117403119
CMK	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.7483	0.119292485
X769P	KIDNEY	6.7486	0.11960738
DEL	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.7494	0.120447098
PANC0813	PANCREAS	6.751	0.122126535
COLO201	LARGE INTESTINE	6.752	0.123176182
SKNMC	BONE	6.7533	0.124540725
CALU3	LUNG	6.7536	0.124855619
SNU1076	UPPER AERODIGESTIVE TRACT	6.7574	0.128844281
HCC78	LUNG	6.7625	0.134197486
ESS1	ENDOMETRIUM	6.7626	0.13430245
NCIH1755	LUNG	6.771	0.143119493
HPAFII	PANCREAS	6.7751	0.147423049

CAKI1	KIDNEY	6.7755	0.147842908
COLO783	SKIN	6.778	0.150467028
NCIH2405	LUNG	6.7785	0.150991852
KNS81	CENTRAL NERVOUS SYSTEM	6.7793	0.15183157
HCC95	LUNG	6.7794	0.151936535
HL60	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.7796	0.152146465
FADU	UPPER AERODIGESTIVE TRACT	6.7809	0.153511007
TE617T	SOFT TISSUE	6.782	0.15466562
KMBC2	URINARY TRACT	6.7837	0.156450021
HCC1171	LUNG	6.7838	0.156554986
CAPAN1	PANCREAS	6.786	0.158864211
CORL88	LUNG	6.7915	0.164637275
PECAPJ49	UPPER AERODIGESTIVE TRACT	6.7927	0.165896852
SF126	CENTRAL NERVOUS SYSTEM	6.7933	0.166526641
GSS	STOMACH	6.794	0.167261395
U87MG	CENTRAL NERVOUS SYSTEM	6.7949	0.168206078
HEYA8	OVARY	6.7972	0.170620268
HT1376	URINARY TRACT	6.7994	0.172929493
COLO792	SKIN	6.7997	0.173244388
SKMEL2	SKIN	6.8019	0.175553613
NCIH460	LUNG	6.8048	0.178597592
KU1919	URINARY TRACT	6.8061	0.179962134
SNU407	LARGE INTESTINE	6.8062	0.180067099
KU812	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.8063	0.180172064
NCIH747	LARGE INTESTINE	6.8075	0.181431642
A101D	SKIN	6.8089	0.182901149
PATU8988T	PANCREAS	6.8099	0.183950797
HS895T	SKIN	6.8118	0.185945128
HMC18	BREAST	6.8147	0.188989107
X253J	URINARY TRACT	6.8153	0.189618895
TE9	OESOPHAGUS	6.8154	0.18972386
LS123	LARGE INTESTINE	6.8175	0.191928121
MCAS	OVARY	6.8199	0.194447276
SW403	LARGE INTESTINE	6.8208	0.195391959
MDST8	LARGE INTESTINE	6.8209	0.195496924
RCM1	LARGE INTESTINE	6.8231	0.197806149
NCIH1650	LUNG	6.825	0.19980048
RPMI8226	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.8256	0.200430269
SUDHL8	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.8258	0.200640198
HEPG2	LIVER	6.8274	0.202319635

HT115	LARGE INTESTINE	6.8303	0.205363614
KYSE520	OESOPHAGUS	6.8305	0.205573544
ISHIKAWAHERAKLIO02ER	ENDOMETRIUM	6.8313	0.206413262
RT112	URINARY TRACT	6.8313	0.206413262
SNU308	BILIARY TRACT	6.8314	0.206518227
HCC1806	BREAST	6.8314	0.206518227
NCIH2085	LUNG	6.8317	0.206833121
EFO27	OVARY	6.832	0.207148015
NCIH2052	PLEURA	6.8321	0.20725298
HSC4	UPPER AERODIGESTIVE TRACT	6.8327	0.207882769
KYSE140	OESOPHAGUS	6.836	0.211346607
LC1SQSF	LUNG	6.8361	0.211451572
KMRC1	KIDNEY	6.8362	0.211556537
HUPT3	PANCREAS	6.837	0.212396255
NCIH1838	LUNG	6.8375	0.212921079
T24	URINARY TRACT	6.8383	0.213760797
WM115	SKIN	6.8396	0.21512534
KASUMI1	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.8439	0.219638826
GAMG	CENTRAL NERVOUS SYSTEM	6.8471	0.222997699
SBC5	LUNG	6.8494	0.225411889
WM2664	SKIN	6.8521	0.228245938
D283MED	CENTRAL NERVOUS SYSTEM	6.857	0.233389213
MIAPACA2	PANCREAS	6.8607	0.23727291
BL70	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.8619	0.238532488
NCIH1623	LUNG	6.862	0.238637453
BHY	UPPER AERODIGESTIVE TRACT	6.8627	0.239372206
OVCAR8	OVARY	6.8637	0.240421854
SNU840	OVARY	6.8651	0.241891361
CFPAC1	PANCREAS	6.8671	0.243990657
HS944T	SKIN	6.8697	0.246719742
LK2	LUNG	6.8724	0.249553791
JHH1	LIVER	6.8737	0.250918333
OVKATE	OVARY	6.8742	0.251443157
T84	LARGE INTESTINE	6.8791	0.256586432
SW1573	LUNG	6.8813	0.258895657
KYSE30	OESOPHAGUS	6.8825	0.260155235
DANG	PANCREAS	6.8825	0.260155235
SU8686	PANCREAS	6.8851	0.26288432
YD15	SALIVARY GLAND	6.8858	0.263619073
COLO680N	OESOPHAGUS	6.8864	0.264248862
SUDHL6	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.887	0.264878651

SNU626	CENTRAL NERVOUS SYSTEM	6.8886	0.266558087
SNU1105	CENTRAL NERVOUS SYSTEM	6.8918	0.269916961
BT20	BREAST	6.8931	0.271281503
FTC133	THYROID	6.8949	0.273170869
P12ICHIKAWA	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.8951	0.273380799
NCIH292	LUNG	6.8954	0.273695693
JHH2	LIVER	6.9004	0.278943933
RCC10RGB	KIDNEY	6.9009	0.279468757
JHOC5	OVARY	6.9036	0.282302806
X786O	KIDNEY	6.9057	0.284507067
AN3CA	ENDOMETRIUM	6.9081	0.287026222
KP3	PANCREAS	6.909	0.287970905
HEC151	ENDOMETRIUM	6.9099	0.288915588
KE39	STOMACH	6.9103	0.289335447
HS822T	BONE	6.9115	0.290595024
A375	SKIN	6.9117	0.290804954
MORCPR	LUNG	6.9126	0.291749637
C2BBE1	LARGE INTESTINE	6.9144	0.293639003
NCIH2452	PLEURA	6.9169	0.296263123
TCCPAN2	PANCREAS	6.9184	0.297837595
VMRCRCW	KIDNEY	6.9222	0.301826257
NCIH810	LUNG	6.9222	0.301826257
PC3	PROSTATE	6.9226	0.302246116
MDAMB435S	SKIN	6.9227	0.302351081
NCIH322	LUNG	6.9254	0.30518513
MOLP2	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.928	0.307914215
HCC366	LUNG	6.9295	0.309488687
KELLY	AUTONOMIC GANGLIA	6.9352	0.31547168
AGS	STOMACH	6.9378	0.318200764
MDAMB468	BREAST	6.9388	0.319250412
SNUC5	LARGE INTESTINE	6.939	0.319460342
HCC1195	LUNG	6.941	0.321559638
NB1	AUTONOMIC GANGLIA	6.9466	0.327437666
NCIH2126	LUNG	6.9473	0.32817242
HT	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.9476	0.328487314
SW48	LARGE INTESTINE	6.9505	0.331531293
QGP1	PANCREAS	6.9517	0.33279087
NUGC3	STOMACH	6.9527	0.333840518
SNU719	STOMACH	6.9544	0.33562492
SKES1	BONE	6.9576	0.338983793
OVK18	OVARY	6.9579	0.339298688
HEC1B	ENDOMETRIUM	6.9583	0.339718547

KLE	ENDOMETRIUM	6.9584	0.339823511
HEC50B	ENDOMETRIUM	6.9622	0.343812174
TF1	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.9682	0.350110061
AM38	CENTRAL NERVOUS SYSTEM	6.9715	0.353573899
HCC1954	BREAST	6.9728	0.354938441
MELHO	SKIN	6.9769	0.359241998
EN	ENDOMETRIUM	6.9773	0.359661857
HCC2108	LUNG	6.9789	0.361341294
X22RV1	PROSTATE	6.9813	0.363860449
PATU8902	PANCREAS	6.9874	0.370263301
LN229	CENTRAL NERVOUS SYSTEM	6.9883	0.371207984
GI1	CENTRAL NERVOUS SYSTEM	6.9897	0.372677491
SNU213	PANCREAS	6.9923	0.375406576
COLO684	ENDOMETRIUM	6.993	0.376141329
SNU738	CENTRAL NERVOUS SYSTEM	6.9945	0.377715801
JK1	HAEMATOPOIETIC AND LYMPHOID TISSUE	6.9966	0.379920062
KYSE510	OESOPHAGUS	6.9987	0.382124322
NCIH1299	LUNG	6.9991	0.382544181
IGROV1	OVARY	7.0026	0.386217949
ACCMESO1	PLEURA	7.0033	0.386952703
BICR16	UPPER AERODIGESTIVE TRACT	7.0071	0.390941365
HCC2279	LUNG	7.0072	0.39104633
PANC1	PANCREAS	7.0096	0.393565485
CCFSTTG1	CENTRAL NERVOUS SYSTEM	7.0119	0.395979675
SNU668	STOMACH	7.0126	0.396714428
SW1271	LUNG	7.0143	0.39849883
SUDHL4	HAEMATOPOIETIC AND LYMPHOID TISSUE	7.0162	0.400493161
GCT	SOFT TISSUE	7.0174	0.401752738
TT	THYROID	7.0183	0.402697421
DMS454	LUNG	7.019	0.403432175
LS180	LARGE INTESTINE	7.0225	0.407105943
SNU182	LIVER	7.0252	0.409939992
KNS62	LUNG	7.0253	0.410044957
OC314	OVARY	7.0273	0.412144253
RH41	SOFT TISSUE	7.0285	0.41340383
NCIH1373	LUNG	7.0318	0.416867668
BEN	LUNG	7.0341	0.419281858
MESSA	SOFT TISSUE	7.0401	0.425579746
HEC1A	ENDOMETRIUM	7.0465	0.432297493
L363	HAEMATOPOIETIC AND LYMPHOID TISSUE	7.0473	0.433137211
CAL29	URINARY TRACT	7.0497	0.435656366

RAJI	HAEMATOPOIETIC AND LYMPHOID TISSUE	7.0524	0.438490415
ZR751	BREAST	7.054	0.440169852
KYSE180	OESOPHAGUS	7.0541	0.440274817
LOXIMVI	SKIN	7.058	0.444368444
YD38	UPPER AERODIGESTIVE TRACT	7.06	0.446467739
SNU410	PANCREAS	7.0646	0.45129612
NCIH2291	LUNG	7.0654	0.452135838
PANC0203	PANCREAS	7.0662	0.452975556
NCIH1792	LUNG	7.0701	0.457069183
SW1088	CENTRAL NERVOUS SYSTEM	7.0786	0.46599119
SKMEL30	SKIN	7.079	0.46641105
KM12	LARGE INTESTINE	7.0792	0.466620979
HEC108	ENDOMETRIUM	7.0804	0.467880557
NCIH526	LUNG	7.0825	0.470084817
NCIH661	LUNG	7.0832	0.470819571
KYSE150	OESOPHAGUS	7.0859	0.47365362
TUHR4TKB	KIDNEY	7.0861	0.47386355
U251MG	CENTRAL NERVOUS SYSTEM	7.091	0.479006825
MKN1	STOMACH	7.0915	0.479531649
DMS273	LUNG	7.0958	0.484045135
HS683	CENTRAL NERVOUS SYSTEM	7.0975	0.485829536
HS746T	STOMACH	7.1012	0.489713233
OAW42	OVARY	7.1038	0.492442318
KYO1	HAEMATOPOIETIC AND LYMPHOID TISSUE	7.1048	0.493491966
HS688AT	SKIN	7.1049	0.493596931
SIGM5	HAEMATOPOIETIC AND LYMPHOID TISSUE	7.1077	0.496535945
HUCCT1	BILIARY TRACT	7.1094	0.498320346
HS819T	BONE	7.1097	0.498635241
HCC1588	LUNG	7.1149	0.50409341
KPL1	BREAST	7.1178	0.507137389
KE97	HAEMATOPOIETIC AND LYMPHOID TISSUE	7.1187	0.508082072
HCC2218	BREAST	7.1208	0.510286332
OCIM1	HAEMATOPOIETIC AND LYMPHOID TISSUE	7.1253	0.515009748
NCIH441	LUNG	7.1284	0.518263657
NCIH1092	LUNG	7.139	0.529389924
SKMEL28	SKIN	7.1392	0.529599854
HPAC	PANCREAS	7.1394	0.529809784
SAOS2	BONE	7.1406	0.531069361
RL952	ENDOMETRIUM	7.1432	0.533798446
SKNAS	AUTONOMIC GANGLIA	7.145	0.535687812

CAL148	BREAST	7.1477	0.538521861
DMS79	LUNG	7.1572	0.548493516
EFE184	ENDOMETRIUM	7.1614	0.552902038
SUPT1	HAEMATOPOIETIC AND LYMPHOID TISSUE	7.167	0.558780066
NMCG1	CENTRAL NERVOUS SYSTEM	7.1746	0.56675739
NCIH358	LUNG	7.1753	0.567492144
TE441T	SOFT TISSUE	7.1772	0.569486475
MELJUSO	SKIN	7.1877	0.580507778
IPC298	SKIN	7.1984	0.59173901
SW1353	BONE	7.1985	0.591843975
CAL33	UPPER AERODIGESTIVE TRACT	7.2038	0.597407109
SNU489	CENTRAL NERVOUS SYSTEM	7.2056	0.599296475
LCLC97TM1	LUNG	7.2086	0.602445419
BICR56	UPPER AERODIGESTIVE TRACT	7.2108	0.604754644
NCIH508	LARGE INTESTINE	7.2176	0.61189225
HSC3	UPPER AERODIGESTIVE TRACT	7.2237	0.618295103
SNU878	LIVER	7.2238	0.618400067
CAMA1	BREAST	7.2254	0.620079504
LS411N	LARGE INTESTINE	7.2279	0.622703624
YKG1	CENTRAL NERVOUS SYSTEM	7.2376	0.632885208
JHH6	LIVER	7.2377	0.632990173
KG1C	CENTRAL NERVOUS SYSTEM	7.238	0.633305068
BT474	BREAST	7.2422	0.637713589
SNU1079	BILIARY TRACT	7.2463	0.642017145
KARPAS422	HAEMATOPOIETIC AND LYMPHOID TISSUE	7.2487	0.6445363
HEC265	ENDOMETRIUM	7.2509	0.646845526
NCIH2444	LUNG	7.2606	0.65702711
NUDHL1	HAEMATOPOIETIC AND LYMPHOID TISSUE	7.2677	0.664479611
AMO1	HAEMATOPOIETIC AND LYMPHOID TISSUE	7.2764	0.673611547
HCC1833	LUNG	7.2887	0.686522217
SNUC4	LARGE INTESTINE	7.2927	0.690720808
HDQP1	BREAST	7.2935	0.691560527
OV56	OVARY	7.2957	0.693869752
P3HR1	HAEMATOPOIETIC AND LYMPHOID TISSUE	7.2973	0.695549189
NUGC4	STOMACH	7.2991	0.697438555
U2OS	BONE	7.3013	0.69974778
SNU886	LIVER	7.3032	0.701742112
NCIH28	PLEURA	7.3081	0.706885386
SNU601	STOMACH	7.3091	0.707935034

ECC10	STOMACH	7.3182	0.71748683
LS513	LARGE INTESTINE	7.3199	0.719271232
CAL120	BREAST	7.32	0.719376196
SNU1040	LARGE INTESTINE	7.3288	0.728613098
NCIH2171	LUNG	7.3416	0.742048591
SUDHL5	HAEMATOPOIETIC AND LYMPHOID TISSUE	7.3508	0.751705352
BFTC905	URINARY TRACT	7.3514	0.752335141
HT29	LARGE INTESTINE	7.364	0.765560705
RPMI7951	SKIN	7.375	0.777106832
AML193	HAEMATOPOIETIC AND LYMPHOID TISSUE	7.3753	0.777421726
MEC1	HAEMATOPOIETIC AND LYMPHOID TISSUE	7.376	0.778156479
HEP3B217	LIVER	7.4062	0.809855846
SNU475	LIVER	7.4091	0.812899825
HUH1	LIVER	7.4298	0.834627537
HUPT4	PANCREAS	7.4555	0.861603488
IMR32	AUTONOMIC GANGLIA	7.4593	0.865592151
NCIH889	LUNG	7.4952	0.903274511
HCC2935	LUNG	7.5084	0.917129863
MC116	HAEMATOPOIETIC AND LYMPHOID TISSUE	7.5146	0.92363768
X5637	URINARY TRACT	7.5183	0.927521377
SKM1	HAEMATOPOIETIC AND LYMPHOID TISSUE	7.5234	0.932874582
SKBR3	BREAST	7.5494	0.960165427
EM2	HAEMATOPOIETIC AND LYMPHOID TISSUE	7.5755	0.987561238
RI1	HAEMATOPOIETIC AND LYMPHOID TISSUE	7.5915	1.004355605
SIMA	AUTONOMIC GANGLIA	7.6032	1.016636485
FUOV1	OVARY	7.6122	1.026083316
SNUC2A	LARGE INTESTINE	7.6165	1.030596802
SNU61	LARGE INTESTINE	7.6228	1.037209584
CAPAN2	PANCREAS	7.6273	1.041933
SNU216	STOMACH	7.6319	1.04676138
MOLM13	HAEMATOPOIETIC AND LYMPHOID TISSUE	7.646	1.061561416
HUNS1	HAEMATOPOIETIC AND LYMPHOID TISSUE	7.6648	1.081294796
HCC1438	LUNG	7.7264	1.145953108
NCIH2196	LUNG	7.7386	1.158758812
SNU466	CENTRAL NERVOUS SYSTEM	7.7589	1.180066665
SUDHL10	HAEMATOPOIETIC AND LYMPHOID TISSUE	7.7977	1.220793004
SNU46	UPPER AERODIGESTIVE TRACT	7.8035	1.226880962
CALU1	LUNG	7.8185	1.242625681

BFTC909	KIDNEY	7.9189	1.348010331
JVM3	HAEMATOPOIETIC AND LYMPHOID TISSUE	7.961	1.392200508
MHHCALL4	HAEMATOPOIETIC AND LYMPHOID TISSUE	8.031	1.465675862
JURLMK1	HAEMATOPOIETIC AND LYMPHOID TISSUE	8.1126	1.551327131
KE37	HAEMATOPOIETIC AND LYMPHOID TISSUE	8.1163	1.555210829
S117	SOFT TISSUE	8.2668	1.713182839
KMS21BM	HAEMATOPOIETIC AND LYMPHOID TISSUE	8.3309	1.780465271
KYM1	SOFT TISSUE	8.4417	1.896766259
CORL95	LUNG	8.5762	2.037943903
MHHNB11	AUTONOMIC GANGLIA	8.8255	2.299621128
MDAMB361	BREAST	9.2909	2.788127266

Example 2. Identification of PDE3A as a putative target of DNMDP

Given the potent cell-selective growth inhibition by 6-(4-(diethylamino)-3-nitrophenyl)-5-methyl-4,5-dihydropyridazin-3(2H)-one (DNMDP), its mechanism of action was examined in more detail. To determine the molecular target of DNMDP, chemogenomic analysis was performed of the 766 tested cell lines, previously characterized for mutations, copy number, and gene expression features as part of the Cancer Cell Line Encyclopedia (CCLE, Barretina et al., 2012), to look for correlation between these genomic features and DNMDP sensitivity. Analysis of Pearson correlations between DNMDP sensitivity and expression of individual genes across the cell line set showed a strong correlation with expression of the *PDE3A* gene, encoding phosphodiesterase 3A (Figure 5A). The correlation between DNMDP sensitivity and *PDE3A* expression is not perfect (Figure 18), and it is possible that some errors are introduced due to the high-throughput nature of the cell line sensitivity characterization, as manual validation for all 766 cell lines was not logistically feasible. Mutation and copy number features, in contrast, did not correlate with DNMDP sensitivity. Conversely, of 480 compounds tested, DNMDP sensitivity was the closest correlate of *PDE3A* expression (Figure 5B), indicating that cancer cell lines with high *PDE3A* expression were more distinctly sensitive to DNMDP than to any other tested compound. In contrast to the motivation of the initial screen, there was no correlation between *TP53* mutation, or other measures of p53 function, and DNMDP sensitivity.

Given these results and the clear structural similarity of DNMDP to known PDE3 inhibitors, e.g., levosimendan and siguazodan (Figures 6A-6C), biochemical analysis of DNMDP against 19 phosphodiesterases representing 11 PDE super families was performed. At a concentration of 100 nM, DNMDP specifically inhibited both PDE3A and PDE3B, weakly inhibited PDE10, and had little or no detectable effect on other phosphodiesterases (Table 2).

Because of the cellular correlation between *PDE3A* expression and DNMDP sensitivity, the *in vitro* inhibition of PDE3A and PDE3B by DNMDP, and the structural similarity of DNMDP to known PDE3 inhibitors, it was analyzed whether all PDE3 inhibitors would exhibit a similar cytotoxic profile to DNMDP. Surprisingly, there was almost no correlation between IC_{50} for *in vitro* enzymatic PDE3A inhibition and HeLa cell cytotoxicity across a series of tested compounds (Figure 5C and Figure 7A and 7B). Indeed, the potent PDE3 inhibitor trequinsin (PDE3 IC_{50} = 0.25 nM, Ruppert et al., Life Sci. 31, 2037–2043, 1982) did not affect HeLa cell viability in any detectable way. Despite their differential effects on HeLa cell viability, the non-cytotoxic PDE3 inhibitor trequinsin and the potent cytotoxic compound DNMDP had similar effects on intracellular cAMP levels in forskolin-treated HeLa cells (Figures 8A and 8B). This result indicates that inhibition of the cAMP and cGMP hydrolysis functions of PDE3A was not sufficient for the cytotoxic activity of DNMDP.

Table 2: Results of phosphodiesterase inhibition reactions

PDE	% inh. #1	% inh. #2	% inhibition
PDE1A1	3	7	5
PDE1B	-5	0	-2
PDE1C	2	9	5
PDE2A	6	10	8
PDE3A	95	95	95
PDE3B	98	97	97
PDE4A1A	14	18	16
PDE4B1	21	20	21
PDE4C1	10	14	12
PDE4D3	14	16	15
PDE4D7	19	20	20
PDE5A1	16	16	16
PDE7A	24	20	22
PDE7B	5	11	8
PDE8A1	10	12	11
PDE9A2	0	5	2
PDE10A1	61	65	63
PDE10A2	67	70	68
PDE11A	14	18	16

Example 3. Target validation of PDE3A

The complex relationship between phosphodiesterase 3A (PDE3A) inhibition and cell killing, in which 6-(4-(diethylamino)-3-nitrophenyl)-5-methyl-4,5-dihydropyridazin-3(2H)-one (DNMDP) and some PDE3 inhibitors kill HeLa and other DNMDP-sensitive cells, whereas others PDE3 inhibitors do not affect cell viability, indicated several possible interpretations including: 1) the cytotoxic activity might be PDE3-independent and due to action on a different protein though screening 234 kinases found no kinase inhibition by 10 μM DNMDP; 2) cytotoxic and non-cytotoxic PDE3 inhibitors might bind to different sites within the protein and exert distinct activities; or 3) the cytotoxic and non-cytotoxic PDE3 inhibitors might bind to the PDE3 active sites but have different effects on the conformation and activity of the protein. This third possibility might be unexpected, but allosteric modulators of PDE4 have been shown to bind the PDE4 active site and interact with

upstream (UCR2), and downstream (CR3) regulatory domains and thereby stabilize specific inactive conformations (Burgin et al., Nat Biotechnol 28, 63–70, 2010). Most importantly, PDE4 competitive inhibitors and PDE4 allosteric modulators with similar IC_{50}s for cAMP hydrolysis in vitro had different cellular activities and safety profiles in animal studies (Burgin et al., Nat Biotechnol 28, 63–70, 2010). To evaluate whether PDE inhibitors or other small molecules compete with DNMDP, the PHARMAKON 1600 collection of 1600 bioactive compounds (PHARMAKON 1600 is a unique collection of 1600 known drugs from US and International Pharmacopeia) was screened to identify compounds that were able to rescue cell death induced by DNMDP. HeLa cells were co-treated with 30 nM DNMDP (the EC_{70} concentration) and 20 μM of each bioactive compound. Cell viability after 48-hour treatment was assessed by ATP consumption as described earlier. The five most potent compounds that rescued cell death induced by DNMDP were all PDE inhibitors, and the three most potent compounds, levosimendan, milrinone, and cilostazol, were all selective PDE3 inhibitors (Figure 9A).

In follow-up experiments, it was confirmed that cilostamide, levosimendan, milrinone, and several other non-cytotoxic selective PDE3 inhibitors were able to rescue DNMDP cytotoxicity in a dose-dependent manner (Figure 9B). The most potent DNMDP competitor was trequinsin, with an "RC_{50}" (the concentration at which it achieved 50% rescue) of < 1 nM; in contrast, PDE5 inhibitors such as sildenafil and vardenafil, as well as the pan-PDE inhibitors idubulast and dipyridamole, were not effective competitors up to 10 μM concentrations in this assay (Figure 9B). This indicated that non-cytotoxic PDE3 inhibitors and DNMDP compete for binding to the same molecular target that is mediating the cytotoxic phenotype.

To identify the molecular target of DNMDP, an affinity purification was performed using an (*R*)-des-nitro-DNMDP solid-phase tethered linker analogue (Figure 10A) incubated with HeLa cell lysate. This linker analogue had the same DNMDP cytotoxicity rescue phenotype as non-cytotoxic PDE3 inhibitors described above (Figure 10B), indicating that it too bound to the same molecular target. It was competed for the molecular target by adding either an excess of trequinsin or separate enantiomers of DNMDP, where only the (*R*)-enantiomer was cytotoxic. Immunoblotting for PDE3A of the affinity purified material showed that PDE3A indeed binds to the linker analogue. Binding of PDE3A to the linker analogue was blocked by both trequinsin and (*R*)-DNMDP, but not by the non-cytotoxic enantiomer (*S*)-DNMDP (Figure 9C). Thus both trequinsin and (*R*)-DNMDP prevented the binding of PDE3A to the tethered DNMDP analogue, and it was concluded that both molecules bind PDE3A directly.

Based on the observations that DNMDP-sensitive cells expressed high levels of *PDE3A*, and that DNMDP competed with non-cytotoxic inhibitors for PDE3A binding, it was hypothesized that DNMDP mediated its cytotoxic phenotype through the interaction with PDE3A and that PDE3A abundance was a direct cellular determinant of DNMDP sensitivity. To validate this hypothesis, the

effect of reducing levels of PDE3A on the response to DNMDP was tested. A clustered regularly interspaced short palindromic (CRISPR)-associated CAS9 enzyme that was targeted with three guide RNAs (sgRNA) targeting three different sites in the *PDE3A* locus led to complete loss of PDE3A expression (Cong et al., Science 339, 819–823, 2013) sgRNA2 and sgRNA3 almost completely reduced PDE3A protein levels, whereas sgRNA1 had a moderate effect on PDE3A expression (Figure 11A). Importantly, both sgRNA2 and sgRNA3 led to significant rescue of toxicity by an active cytotoxic DNMDP analog, **3** (Figures 11A and 11B and Figures 5A-5C). Both sgRNA2 and sgRNA3 led to significant rescue of toxicity by DNMDP (Figure 11C). Changes in proliferation rate or morphology in HeLa cells with reduced PDE3A expression were not observed, indicating that PDE3A was not required for cell survival. In an independent approach using an siRNA smart-pool containing four different siRNAs targeting *PDE3A*, PDE3A expression was reduced in HeLa cell line with a maximum efficiency of 70% between 24 and 72 hours after transfection. HeLa cells treated with siPDE3A had a higher EC_{50} to a DNMDP analog compared to the control siRNA condition (Figures 12A and 12B). Without being bound by theory it was concluded that DNMDP cytotoxicity requires PDE3A, and that DNMDP likely modulates the function of PDE3A.

Example 4. Determining the mechanism of action of DNMDP

The dependence of 6-(4-(diethylamino)-3-nitrophenyl)-5-methyl-4,5-dihydropyridazin-3(2H)-one (DNMDP) cytotoxicity on phosphodiesterase 3A (PDE3A) protein abundance indicated a possible mechanism similar to that recently observed for lenalidomide, which acts by a neomorphic or hypermorphic mechanism by stabilizing an interaction between cereblon and IKAROS Family Zinc Finger 1 (IKZF1) and IKZF3 (Krönke et al., Science 343, 301–305, 2014; Lu et al., Science 343, 305–309, 2014). In addition, PDE4 allosteric modulators, but not competitive inhibitors, have been shown to bind and stabilize a "closed" protein conformation that has independently been shown to uniquely bind the PDE4-partner protein DISC1 (Millar et al., Science 310, 1187–1191, 2005). The protein complexes in which PDE3A resides were characterized under normal conditions, and it was examined how these complexes change when PDE3A is bound to DNMDP or the non-cytotoxic PDE3 inhibitor trequinsin. PDE3A and interacting proteins from Hela cells were immunoprecipitated in the presence of DNMDP and trequinsin followed by labeling with isobaric stable isotope tags for relative abundance and quantitation by mass spectrometry (iTRAQ/MS, Figure 13A). PDE3A immunoprecipitates from HeLa cells were enriched for multiple protein phosphatase subunits including protein phosphatase 2 subunits (PPP2CA, PPP2R1A, PPP2R1B, PPP2R2A, PPP2R2D), calcineurin (PPP3R1, PPP3CA, Beca et al., Circ. Res. 112, 289–297, 2013), 14-3-3 (YWHAB, YWHAQ, YWHAG, YWHAZ, Pozuelo Rubio et al., Biochem. J. 392, 163–172, 2005), and tubulin (TUBA1C, TUBA1B) family members (Figure 13B and Figure 14A). In addition, it was found that

PDE3A and PDE3B reside in the same protein complex, which has been previously reported (Malovannaya et al., Cell 145, 787–799, 2011).

Binding of DNMDP altered the composition of interacting proteins that were co-immunoprecipitated with PDE3A. Proteins that were specifically enriched in PDE3A immunoprecipitates after treatment with DNMDP included Sirtuin 7 (SIRT7) and Schlafen 12 (SLFN12) (Figure 13C and Figure 14B). These proteins specifically interacted with PDE3A in the presence of DNMDP, and were not observed in the trequinsin treated control, whereas a known PDE3B interactor, abhydrolase domain-containing protein 15 (ABHD15, Chavez et al., Biochem. Biophys. Res. Commun. 342, 1218–1222, 2006), was enriched in the immunoprecipitate from trequinsin-treated cells (Figure 13C and Figure 14C). The interaction promoted by DNMDP between PDE3A and both SIRT7 and SLFN12 was validated with affinity reagents. Immunoprecipitation of endogenous PDE3A in HeLa cells treated with DNMDP, but not DMSO or trequinsin, enhanced complex formation of ectopically expressed V5-tagged SIRT7 and SLFN12 with PDE3A, as evidenced by coimmunoprecipitation (Figure 19). Figure 20 further shows that DNMDP and (weakly) anagrelide, but not trequinsin, induced PDE3A and SFLN12 complex formation.

Similar to PDE3A, overexpression of SLFN12 appears to have a cytotoxic effect in DNMDP sensitive cell lines, contributing to the difficulty of detecting SLFN12 in whole cell lysates.

The enhanced interaction of PDE3A with SIRT7 and SLFN12 indicated the possibility that one or more of these interacting proteins might contribute to DNMDP sensitivity. *SIRT7* mRNA expression was relatively constant among all cells tested, but the co-expression of *SLFN12* and *PDE3A* mRNA showed a strong correlation with DNMDP sensitivity; almost all DNMDP-sensitive cell lines expressed high levels of *SLFN12* (Figure 15A-15C). Importantly, almost half of sensitive cell lines expressing high levels of *SLFN12* and *PDE3A* were found to be melanoma cell lines (Figure 15B). *SLFN12* expression alone was also one of the top genes correlating with sensitivity to DNMDP, corroborating the hypothesis that SLFN12 could be functionally involved in DNMDP-induced cytotoxicity (Figure 16A). Moreover, when correcting for *PDE3A* expression, *SLFN12* expression was the top correlating gene with DNMDP sensitivity (Figure 16B). To assess whether SLFN12 is required for the cytotoxic phenotype of DMNDP, we reduced *SLFN12* mRNA expression by 60% by knockdown with two shRNAs in HeLa cells (Figure 15D). Similar to reduction in PDE3A expression, reduction of SLFN12 expression did not result in cytotoxicity, and in fact decreased sensitivity to DNMDP (Figure 15E). These results show that SLFN12, like PDE3A, is required for the cytotoxic phenotype of DMNDP. Characterization of normal expression of *SLFN12* and *PDE3A* by the GTEX consortium (Pierson, E. et al. PLoS Comput. Biol. 11, e1004220 (2015)) shows low expression of *SLFN12* in normal tissues, while high co-expression of both *PDE3A* and *SLFN12* is rarely observed (Table 3). This could suggest that on-target toxicity of DNMDP and related compounds may be potentially limited.

Table 3: RPKM values of *SLFN12* and *PDE3A* expression in multiple healthy tissue types

	SLFN12 (RPKM)		*PDE3A* (RPKM)		
	Mean	**SD**	**Mean**	**SD**	n
Adipose - Subcutaneous	2.14	0.70	4.76	2.03	128
Adipose - Visceral (Omentum)	2.43	1.03	4.24	1.94	31
Adrenal Gland	3.01	0.83	0.34	0.21	52
Artery - Aorta	2.10	0.71	16.15	5.12	82
Artery - Coronary	1.80	0.80	17.72	6.52	44
Artery - Tibial	1.09	0.49	12.98	6.35	137
Bladder	1.38	0.57	1.33	0.40	11
Brain - Amygdala	0.37	0.23	0.96	0.34	26
Brain - Anterior cingulate cortex (BA24)	0.28	0.16	0.77	0.45	22
Brain - Caudate (basal ganglia)	0.40	0.23	1.27	0.37	36
Brain - Cerebellar Hemisphere	0.11	0.07	2.73	1.49	29
Brain - Cerebellum	0.19	0.10	2.40	0.98	31
Brain - Cortex	0.25	0.12	0.56	0.59	25
Brain - Frontal Cortex (BA9)	0.26	0.15	0.54	0.33	28
Brain - Hippocampus	0.39	0.31	0.82	0.38	28
Brain - Hypothalamus	0.46	0.29	0.93	0.48	30
Brain - Nucleus accumbens (basal ganglia)	0.28	0.16	1.11	0.41	32
Brain - Putamen (basal ganglia)	0.29	0.18	0.91	0.33	24
Brain - Spinal cord (cervical c-1)	0.50	0.32	0.65	0.55	19
Brain - Substantia nigra	0.62	0.50	0.82	0.47	27
Breast - Mammary Tissue	2.48	0.74	8.19	2.85	66
Cells - EBV-transformed lymphocytes	4.70	1.57	0.02	0.01	54
Cells - Transformed fibroblasts	5.34	2.27	0.58	0.60	155
Colon - Sigmoid	1.58	0.50	10.24	3.48	13
Colon - Transverse	0.99	0.47	11.24	4.32	45
Esophagus - Gastroesophageal Junction	1.14	0.31	16.82	5.63	22

Esophagus - Mucosa	1.01	0.45	0.82	1.32	106
Esophagus - Muscularis	1.29	0.35	[redacted]	[redacted]	99
Fallopian Tube	2.32	0.86	[redacted]	[redacted]	6
Heart - Atrial Appendage	1.05	0.38	[redacted]	[redacted]	38
Heart - Left Ventricle	0.81	0.38	[redacted]	[redacted]	95
Kidney - Cortex	1.21	1.07	1.40	0.84	8
Liver	0.29	0.16	0.49	0.28	34
Lung	2.83	1.12	2.78	1.48	133
Minor Salivary Gland	1.75	0.61	0.62	0.44	5
Muscle - Skeletal	0.25	0.18	0.84	0.42	157
Nerve - Tibial	2.82	0.87	[redacted]	[redacted]	114
Ovary	1.92	0.57	2.17	1.13	35
Pancreas	0.52	0.27	2.65	0.86	65
Pituitary	0.47	0.23	1.04	0.47	22
Prostate	1.41	0.57	[redacted]	[redacted]	42
Skin - Not Sun Exposed (Suprapubic)	0.76	0.37	0.66	0.34	41
Skin - Sun Exposed (Lower leg)	0.63	0.31	1.00	0.69	126
Small Intestine - Terminal Ileum	1.61	0.72	[redacted]	[redacted]	17
Spleen	3.46	0.92	1.18	0.46	34
Stomach	1.10	0.40	[redacted]	[redacted]	81
Testis	0.49	0.19	0.43	0.20	60
Thyroid	3.19	0.96	2.59	1.34	120
Uterus	1.99	0.56	[redacted]	[redacted]	32
Vagina	1.39	1.39	2.49	2.49	34
Whole Blood	1.40	1.10	0.06	0.05	191

Figure 21 shows that SLFN12 is lost in cells that have acquired resistance to DNMDP. Cell lines initially sensitive to DNMDP were made resistant by persistent exposure to DNMDP and subsequently analyzed by RNA-seq. One gene was downregulated in both HeLa and H2122: SLFN 12. Accordingly, a reduction in levels of SLFN 12 indicates that cells have become resistant to DNMDP and other PDE3A modulators.

Figure 22 shows sensitization of a DNMDP-resistant cell line by expression of SLFN12 or expression of SFLN12 and PDE3A. Expression of SLFN12 was sufficient to confer DNMDP sensitivity to A549 cells. Adding PDE3A expression led to further sensitization.

Leiomyosarcomas are malignant smooth muscle tumors. Patient tumor samples from leiomyosarcomas were analyzed for PDE3A and SLFN12 expression to characterize sensitivity of leiomyosarcomas (LMS) to DNMDP. Leiomyosarcomas are thought to be sensitive to DNMDP due to prevalence among high purity TCGA samples expressing elevated levels of PDE3A and SLFN12 (Figure 23, Table 4). P value for association of biomarker expression with leiomyosarcoma lineage: 0.0001.

Table 4. Leiomyosarcomas Characterization

	Marker Expression Indicates DNMDP sensitive	**Marker Expression Indicates DNMDP not sensitive**
LMS	17	31
Not LMS	38	1516

Differential scanning fluorimetry (DSF) was used to demonstrate binding of DNMDP to purified PDE3A catalytic domain, PDE3A(677-1141). In this experiment, 5 μM hsPDE3A(640-1141) was incubated in the absence or presence of 100 μM compounds, as indicated in Table 5. Binding buffer: 20 mM Hepes pH 7.4, 100 μM TCEP, 1 mM $MgCl_2$, 150 mM NaCl.

Table 5. Binding of DNMDP to PDE3A(677-1141)

	T_m (°C)	ΔT_m (°C)
$PDE3A_{677-1141}$	52.4 ± 0.0	
$PDE3A_{677-1141}$ + DNMDP	58.4 ± 0.0	6.0
$PDE3A_{677-1141}$ + Anagrelide	56.6 ± 0.0	4.2
$PDE3A_{677-1141}$ + Trequinsin	66.2 ± 0.0	14.2

Using chemogenomics, a class of compounds was discovered, exemplified by DNMDP, that targeted a novel cancer dependency by small-molecule modulation of PDE3A. These compounds bound PDE3A in a mutually exclusive manner with non-cytotoxic PDE3 inhibitors and exerted a

neomorphic or hypermorphic effect on the function of PDE3A, leading to a change in its protein-protein interactions. One unique protein-interaction partner, SLFN12, was highly expressed in DNMDP-sensitive cell lines, indicating a functional role in the pathway through which the cytotoxic signal was relayed. As a result, DNMDP was both selective and potent across a large panel of cancer cell lines.

Here, a novel cytotoxic compound was identified with great selectivity and low-nM potency against cancer cell lines across multiple lineages. Using gene-expression correlates for chemogenomics, PDE3A was identified as the putative target of this small molecule, DNMDP. Interestingly, loss of PDE3A expression resulted in resistance to DNMDP. Moreover, PDE3A immunoprecipitation followed by isobaric stable isotope tags for relative abundance and quantitation by mass spectrometry (iTRAQ/MS) identified SLFN12 and SIRT7 as novel protein-protein interaction partners of PDE3A upon DNMDP binding, possibly due to allosteric modulation of the function of PDE3A. Importantly, *SLFN12* expression was the top correlating gene with DNMDP sensitivity when corrected for *PDE3A* expression. Single gene or multi-gene expression correlations have shown to help elucidate the mechanism of action and relevant signaling pathways of small molecules. A novel biochemical target for cancer treatment was identified that is unlikely to have been found by target identification approaches such as loss-of-function screens or genomic analysis.

PDE3A belongs to the superfamily of phosphodiesterases and together with PDE3B forms the PDE3 family. The PDE3 family has dual substrate affinity and hydrolyses both cAMP and cGMP. Expression of *PDE3A* is highest in the cardiovascular system, platelets, kidney, and oocytes (Ahmad et al., Horm Metab Res 44, 776–785, 2012). The clinical PDE3 inhibitor cilostazol has been developed to treat intermittent claudication, as PDE3A inhibition in platelets impairs activation and platelet coagulation (Bedenis et al., Cochrane Database Syst Rev 10, CD003748, 2014). Other PDE3 inhibitors, such as milrinone, amrinone, and levosimendan, are indicated to treat congestive heart failure, where the combination of vasodilation and elevated cardiac cAMP levels increases cardiac contractility (Movsesian et al., Curr Opin Pharmacol 11, 707–713, 2011). None of these clinical inhibitors were able to replicate the cytotoxic phenotype of DNMDP, indicating that cyclic nucleotide hydrolysis was not sufficient to induce cell death in DNMDP-sensitive cell lines.

Interestingly however, other PDE3 inhibitors such as zardaverine, anagrelide, and quazinone have been reported previously to have cell cytotoxic characteristics in a select number of cancer cell lines (Sun et al., PLoS ONE 9, e90627, 2014; Fryknäs et al., J Biomol Screen 11, 457–468, 2006). In concordance with the present findings, other PDE3 and PDE4 inhibitors were found not to replicate the cytotoxic phenotype of zardaverine where retinoblastoma protein retinoblastoma 1 (RB1) expression was reported to separate zardaverine sensitive cell lines from non-sensitive cell lines (Sun et al., PLoS ONE 9, e90627, 2014). This finding was in contrast to the present data where a correlation between cytotoxic activities of DNMDP and copy-number or mRNA expression of *RB1*

was not identified. Another PDE3 inhibitor, anagrelide, uniquely inhibited megakaryocyte differentiation, resulting in apoptosis. Other PDE3 inhibitors tested did not have this activity (Wang et al., Br. J. Pharmacol. 146, 324–332, 2005; Espasandin, Y. et al., J. Thromb. Haemost. n/a–n/a, 2015, doi:10.1111/jth.12850). It was hypothesized that the reported effects of zardaverine on cell viability and anagrelide on megakaryocyte differentiation are mediated through the same PDE3A modulation as described in this study.

Multiple PDE3 inhibitors were competitive inhibitors and have been shown to occupy the catalytic binding site of cAMP and cGMP (Card et al., Structure 12, 2233–2247, 2004; Zhan et al., Mol. Pharmacol. 62, 514–520, 2002). In addition, zardaverine has been co-crystalized in a complex with PDE4D, where it occupies the cAMP-binding site, and has been modeled to bind PDE3B in a similar manner (Lee et al., FEBS Lett. 530, 53–58, 2002). Given the structural similarity of DNMDP to zardaverine and that DNMDP inhibited both PDE3A and PDE3B, it was hypothesized that the binding mode of DNMDP is very similar to that of zardaverine. This indicated that in addition to acting as a cAMP/cGMP-competitive inhibitor, DNMDP allosterically induces a conformation that is responsible for its cytotoxic phenotype. Allosteric modulation of phosphodiesterases has been described previously for PDE4, where small molecules bound in the active site and simultaneously interacted with regulatory domains that came across the PDE4 active site. As a result, allosteric modulators stabilized a protein conformation that has been shown to differentially bind different PDE4 partner proteins (Burgin et al., Nat Biotechnol 28, 63–70, 2010).

The study of proteins associated with PDE3A might illuminate both its normal function and the way in which PDE3A modulators such as DNMDP kill cancer cells. PDE3A interacted with protein phosphatase 2 subunits, which are implicated in oncogenic viral transformation and are mutated in human cancers (Nagao et al., Int. Symp. Princess Takamatsu Cancer Res. Fund 20, 177–184, 1989; Imielinski et al., Cell 150, 1107–1120, 2012; Lawrence et al., Nature 499, 214–218, 2013), indicating a role for PDE3A in cancer cell signaling. Even though these interactions were not induced by DNMDP binding, the importance of the protein phosphatases in cancer biology would warrant further research.

The enhanced interaction between PDE3A and SLFN12, facilitated by DNMDP binding to PDE3A, and the correlation between sensitivity to DNMDP with *SLFN12* expression strongly indicated that it is necessary to understand the functional impact of the PDE3A-SLFN12 interaction. However, little is known at this time about the functional role of SLFN12 in human physiology and cancer biology. *SLFN12* is part of the *schlafen* gene family that diverges largely between humans and rodents. The large difference is due to rapid gene evolution and positive selection (Bustos et al., Gene 447, 1–11, 2009). Therefore, *SLFN12* has no murine orthologue, preventing the study of SLFN12 in a well-understood model organism. The single publication on SLFN12 showed modulation of prostate cancer cell lines after ectopic expression of SLFN12 (Kovalenko et al., J. Surg. Res. 190, 177–184,

2014). Additional studies into the function of SLFN12 and its interaction with PDE3A could elucidate the mechanism of DNMDP cytotoxicity. Two observations indicated that DNMDP acted as a neomorph or hypermorph on PDE3A function: 1) DNMDP-sensitive cancer cell lines did not depend on PDE3A expression for survival, but rather PDE3A knock-down led to DNMDP resistance; and 2) DNMDP induced or enhanced protein-protein interactions upon binding to PDE3A. Lenalidomide was an example of a small molecule that acted as a neomorph or hypermorph rather than as an enzymatic inhibitor. Lenalidomide modulated a specific protein-protein interaction between the cereblon ubiquitin ligase and Ikaros transcription factors, which were then subsequently targeted for degradation (Krönke et al., Science 343, 301–305, 2014; Lu et al., Science 343, 305–309, 2014). By analogy, DNMDP might directly stabilize a PDE3A-SLFN12 interaction, or DNMDP could allosterically stabilize a PDE3 conformation that binds SLFN12. Either of these mechanisms could result in a neo- or hypermorphic phenotype. Further characterization of the neomorphic phenotype induced by DNMDP might facilitate synthesis of small molecules that will not inhibit cyclic nucleotide hydrolysis by PDE3A. Toxicity profiles of such small molecules should differ from PDE3 inhibitors prescribed for cardiovascular indications.

This study has uncovered a previously unknown role for PDE3A in cancer maintenance, in which its function can be modified by a subset of PDE3 inhibitors, resulting in toxicity to a subset of cancer cell lines. These data indicated that DNMDP and its analogs had a hyper- or neomorphic effect on PDE3A, leading to cellular toxicity, which was corroborated by cells becoming less sensitive to DNMDP with decreasing levels of cellular PDE3A. These observations are comparable with other reports of allosteric modulation of phosphodiesterases (Burgin et al., Nat Biotechnol 28, 63–70, 2010), indicating that DNMDP and analogues may have similar effects on PDE3A. The exact mechanism of cell-selective cytotoxicity remains unknown for now; however, further studies into the novel interactions with SLFN12, and perhaps SIRT7, might be informative.

In summary, the study herein used differential cytotoxicity screening to discover a cancer cell cytotoxic small molecule, DNMDP. Profiling of DNMDP in 766 genomically-characterized cancer cell lines revealed stereospecific nanomolar efficacy in about 3% of cell lines tested. A search for genomic features that indicated sensitivity revealed that elevated PDE3A expression strongly correlated with DNMDP response. DNMDP inhibited PDE3A and PDE3B, with little or no activity towards other PDEs. However, unexpectedly, most other PDE3A inhibitors tested did not phenocopy DNMDP, including the potent and selective PDE3A inhibitor, trequinsin. Co-treatment of DNMDP-sensitive cells with trequinsin competed away the cancer cell cytotoxic activity of DNMDP, and knockout of PDE3A rescued the otherwise sensitive cells from DNMDP-induced cytotoxicity, leading us to hypothesize that PDE3A is required for cancer cell killing by DNMDP, which induces a neomorphic alteration of PDE3A. Mass spectrometric analysis of PDE3A immunoprecipitates alone or in the presence of DNMDP or trequinsin revealed differential binding of SLFN12 and SIRT7 only

in the presence of DNMDP. Similar to PDE3A, SLFN12 expression levels were elevated in DNMDP-sensitive cell lines, and knock down of SLFN12 with shRNA decreased sensitivity of cells to DNMDP, indicating that DNMDP-induced complex formation of PDE3A with SLFN12 is critical to the cancer cell cytotoxic phenotype. Results herein therefore implicate PDE3A modulators as candidate cancer therapeutic agents and demonstrate the power of chemogenomics in small molecule discovery.

The experiments above were performed with the following methods and materials.

Compound library screening in NCI-H1734 and A549 cell lines

1500 NCI-H1734 or 1000 A549 cells were plated in a 384-well plate in 40 µl of RPMI supplemented with 10% Fetal Bovine Serum and 1% Pen/Strep. 24 hours after plating, a compound-library of 1924 small molecules was added at a concentration of 10 µM. Staurosporine was used a positive control for cytotoxicity at a concentration of 10 µM, and DMSO was used a negative control at a concentration of 1%. All compounds were incubated for 48 hours with indicated small molecules. After 48 hours, 384-well plates were removed from the incubator and allowed to cool to room temperature for 20 minutes. Cell viability was assessed by adding 40 µl of a 25% CELLTITERGLO® (Promega) in PBS with a THERMO COMBI™ or multichannel-pipette and incubated for 10 minutes. The luminescence signal was read using a Perkin-Elmer EnVision. Viability percentage was calculated by normalizing to DMSO controls.

Compound sensitivity testing in cell lines

1000 HeLa (DMEM), 1000 A549 (RPMI), 500 MCF-7 (DMEM), 4000 PC3 (F12-K), 1000 NCI-H2122 (RPMI) or 1500 NCI-H1563 (RPMI) cells were plated in a 384-well plate in 40 µl of corresponding growth media supplemented with 10% Fetal Bovine Serum. 24 hours after plating, indicated compounds were added at indicated concentrations and incubated for 48 hours. Cell viability was assessed as described in *Compound library screening in NCI-H1734 and A549 cell lines.*

Caspase activity in HeLa cells

1000 HeLa cells were plated in 384-well plate in 40 µl of corresponding growth media supplemented with 10% Fetal Bovine Serum. 24 hours after plating, indicated compounds were added at indicated concentrations and incubated for 48 hours. Caspase-Glo from Promega was added according to the manufacturers recommendations and luminescence was determined as described in *Compound library screening in NCI-H1734 and A549 cell lines.*

Large-scale cell-line viability measurements

The sensitivity of 777 cancer cell lines (CCLs) was measured drawn from 23 different lineages to DNMDP. Each cell line was plated in its preferred media in white opaque 1536-plates at a density of 500 cells/well. After incubating overnight, DNMDP was added by acoustic transfer at 16 concentrations ranging from 66.4 μM – 2 nM in 2-fold steps in duplicate (Labcyte Echo 555, Labcyte Inc., Sunnyvale, CA). After 72 hours treatment, cellular ATP levels were measured as a surrogate for viability (CELLTITERGLO®, Promega Corporation, Madison, WI) according to manufacturer's protocols using a ViewLux Microplate Imager (PerkinElmer, Waltham, MA) and normalized to background (media-only) and vehicle (DMSO)-treated control wells.

Concentration response curves were fit using nonlinear fits to 2- or 3-parameter sigmoid functions through all 16 concentrations with the low-concentration asymptote set to the DMSO-normalized value, and an optimal 8-point dose curve spanning the range of compound-sensitivity was identified. The area under the 8-point dose curve (AUC) was computed by numeric integration as a metric for sensitivity for further analysis. Similar sensitivity measurements have been obtained for a collection of 480 other compounds, enabling analyses that identify cell lines responding uniquely to DNMDP (see Broad Institute Cancer Therapeutics Response Portal, a dataset to identify comprehensively relationships between genetic and lineage features of human cancer cell lines and small-molecule sensitivities for complete list of compounds).

Correlation of sensitivity measurements with basal gene expression

Gene-centric robust multichip average (RMA)-normalized basal mRNA gene expression data measured on the Affymetrix GeneChip Human Genome U133 Plus 2.0 Array were downloaded from the Cancer Cell Line Encyclopedia (CCLE, a detailed genetic characterization of a large panel of human cancer cell lines; Barretina et al., Nature 483, 603–607, 2012). Pearson correlation coefficients were calculated between gene expression (18,988 transcripts) and areas under the curve (AUCs) across 760 overlapping CCLs. For comparisons across small molecules exposed to differing numbers of CCLs, correlation coefficients were transformed using Fisher's transformation.

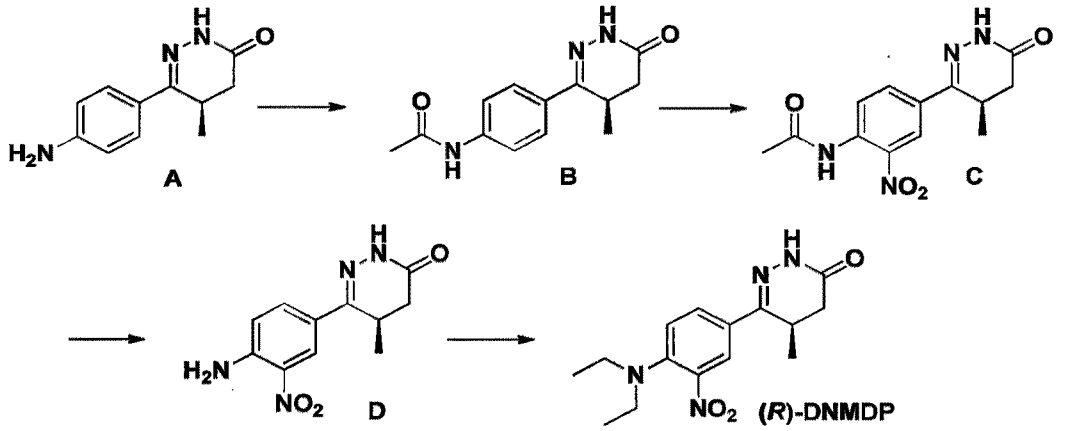
Chemistry Experimental Methods

General details

All reactions were carried out under nitrogen (N2) atmosphere. All reagents and solvents were purchased from commercial vendors and used as received. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker (300 or 400 MHz ^{1}H, 75 or 101 MHz ^{13}C) spectrometer. Proton and carbon chemical shifts are reported in ppm (δ) referenced to the NMR solvent. Data are reported as follows: chemical shifts, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet; coupling constant(s) in Hz). Flash chromatography was performed using 40-60 μm Silica

Gel (60 Å mesh) on a Teledyne Isco Combiflash Rf. Tandem Liquid Chromatography/Mass Spectrometry (LC/MS) was performed on a Waters 2795 separations module and 3100 mass detector with a Waters Symmetry C18 column (3.5 µm, 4.6 X 100 mm) with a gradient of 0-100% CH3CN in water over 2.5 min with constant 0.1% formic acid. Analytical thin layer chromatography (TLC) was performed on EM Reagent 0.25 mm silica gel 60-F plates. Elemental analysis was performed by Robertson Microlit Laboratories, Ledgewood NJ.

Synthesis of (R)-DNMDP



In 5 mL of acetic anhydride, 2.00 g (9.84 mmol) of (R)-6-(4-aminophenyl)-5-methyl-4,5-dihydropyridazin-3(*2H*)-one (**A**, Toronto Research Chemicals) was stirred 1 hour before addition of 30 mL water, filtration, rinsing the solids with water and drying to yield 2.20 g of product **B** (91%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.92 (s, 1H), 10.13 (s, 1H), 7.74 (d, J = 8.9, 2H), 7.65 (d, J = 8.8, 2H), 3.41 – 3.33 (m, 1H), 2.68 (dd, J = 6.8, 16.8, 1H), 2.23 (d, J = 16.7, 1H), 2.08 (s, 3H), 1.07 (d, J = 7.3, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.50, 166.27, 152.25, 140.27, 129.24, 126.24, 118.70, 33.47, 26.91, 24.02, 15.87. HPLC: R_t 0.72 min, purity > 95%. MS: 246 (M + 1).

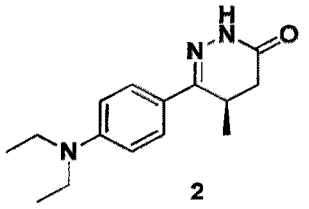
To 3.09 g of **B** (15.3 mmol) dissolved in 30 mL of sulfuric acid and cooled in an ice bath was added 0.72 mL of 90% nitric acid (15 mmol) in 8 mL sulfuric acid via an addition funnel over 10 minutes. After stirring 1 hour the mixture was poured onto ice. The yellow solid was filtered off and the water was rinsed several times with EtOAc before drying and combining with the yellow solid. Chromatography with 40-60% EtOAc in hexane yielded 1.12 g (25%) of product as a yellow solid which was recrystallized from EtOAc. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.13 (s, 1H), 10.41 (s, 1H), 8.25 (d, J = 1.8, 1H), 8.07 (dd, J = 1.8, 8.6, 1H), 7.71 (d, J = 8.6, 1H), 3.55 – 3.40 (m, 1H), 2.74 (dd, J = 6.9, 16.8, 1H), 2.27 (d, J = 16.8, 1H), 2.09 (s, 3H), 1.08 (d, J = 7.2, 3H). ^{13}C NMR (75 MHz, DMSO-d6) δ 168.57, 166.31, 150.37, 142.19, 131.69, 131.32, 130.60, 125.07, 121.70, 33.30, 26.81,

23.44, 15.64. TLC: Rf 0.25 (1:1 EtOAc:hexane). HPLC: R_t 0.87 min, purity > 95%. MS: 291 (M + 1). HRMS Exact Mass (M + 1): 291.1088. Found: 291.1091

To 58 mg of C (0.20 mmol) dissolved in 10 mL of MeOH was added a solution of 48 mg NaOH (1.2 mmol) in 0.5 mL water. After 1 hour the reaction was concentrated, water was added and rinsed with EtOAc, the EtOAc was dried and concentrated to give 48 mg (93%) of product **D**. ^{1}H NMR (300 MHz, DMSO-d6) δ 10.92 (s, 1H), 8.28 (d, J = 2.0, 1H), 7.87 (dd, J = 2.1, 9.0, 1H), 7.76 (s, 2H), 7.06 (d, J = 9.0, 1H), 3.33 (s, 1H), 2.67 (dd, J = 6.8, 16.8, 1H), 2.22 (d, J = 16.6, 1H), 1.06 (d, J = 7.3, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.25, 151.12, 146.69, 132.72, 129.80, 122.57, 122.19, 119.80, 33.43, 26.70, 15.77. MS: 249 (M + 1).

To 35 mg of amine **D** (0.14 mmol) dissolved in 0.5 mL Dimethylformamide (DMF) was added 70 mg of acetaldehyde (1.6 mmol) and 170 mg of $NaBH(OAc)_3$ (0.80 mmol) and 10 µL, (0.2 mmol) of HOAc. After stirring 3 hours, water and EtOAc were added, the EtOAc separated, dried, concentrated and chromatographed with 30-50% EtOAc in hexane to isolate 3 mg of the (R)-DNMDP (7%). The synthesized material was identical to purchased racemic material by TLC, HPLC and ^{1}H NMR. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.58 (s, 1H), 8.04 (d, J = 2.3, 1H), 7.84 (dd, J = 2.3, 9.0, 1H), 7.11 (d, J = 9.0, 1H), 3.30-3.36 (m, 1H), 3.26 (q, J = 7.1, 4H), 2.71 (dd, J = 6.8, 16.9, 1H), 2.48 (d, J = 17.0, 1H), 1.25 (d, J = 7.4, 3H), 1.16 (t, J = 7.1, 6H). TLC: Rf 0.25 (1:1 EtOAc:hexane). HPLC: R_t 1.27 min, purity > 95%. MS: 305 (M + 1). Exact Mass (M + 1): 305.1608 Found: 305.1616. ^{13}C NMR (75 MHz, $CDCl_3$, purchased material) δ 166.28, 152.02, 145.24, 141.21, 129.77, 124.94, 123.94, 121.00, 46.10, 33.80, 27.81, 16.24, 12.56.

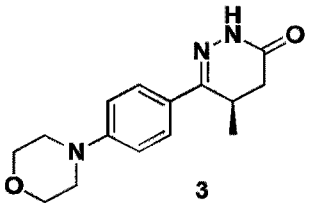
The optical purity of (R)-DNMDP was determined using chiral SCF chromatography and comparison to commercially available racemic material: Column: ChiralPak AS-H, 250 x 4.6 mm, 5µm, Mobile Phase Modifier: 100% Methanol, Gradient: 5 to 50% Methanol over 10 minutes, Flow Rate: 4 mL/min, Back Pressure: 100 bar, Column Temperature: 40 C. UV detection was from 200-400 nm. Retention times of separated isomers: 5.36, 6.64 minutes; retention time of (R)-DNMDP, 6.60 minutes, 1:19 ratio of enantiomers detected.



2

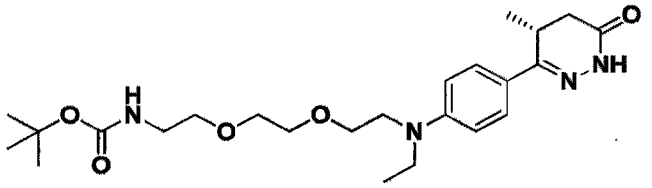
2. To 200 mg (0.98 mmol) of **A** dissolved in 5 mL of MeOH was added 87 mg of acetaldehyde (2.0 mmol), 113 uL of HOAc (2.0 mmol) and 124 mg (2.0 mmol) of $NaBH_3CN$ and the reaction was stirred overnight at room temperature. The next day the same quantity of reagents were added and the reaction stirred another 24 hours. The mixture was concentrated and partitioned

between CH_2Cl_2 and water, the CH_2Cl_2 was separated, dried, and concentrated before chromatography with 20-40% EtOAc in hexane isolated 210 mg of product as a white solid (82%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.95 (s, 1H), 7.64 (d, J = 8.7, 2H), 6.66 (d, J = 8.7, 2H), 3.37 (dd, J = 9.6, 16.4, 5H), 2.67 (dd, J = 6.5, 16.8, 1H), 2.43 (d, J = 16.8, 1H), 1.41 – 1.02 (m, 10H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.82, 154.55, 148.79, 127.32, 120.81, 111.08, 44.32, 33.92, 27.74, 16.37, 12.50. TLC: Rf 0.25 (1:1 EtOAc:hexane). HPLC: R_t 1.05 min, purity > 95%. MS: 260 (M + 1). HRMS Exact Mass (M + 1): 260.1757. Found: 260.1764



3

3. To 200 mg (0.984 mmol) of **A** dissolved in 1 mL of Dimethylformamide (DMF) was added 250 μL (2.00 mmol) of bis (2-bromoethyl) ether and 400 mg of K2CO3 and the mixture was stirred overnight at 60 °C. The next day another 250 μL of bis (2-bromoethyl) ether and 170 mg of K2CO3 were added. After 3 hours, EtOAc and water were added, the water was rinsed with EtOAc, the combined EtOAc washes were dried and concentrated. Chromatography with 0-4% MeOH in CH2Cl2 yielded 125 mg of product (46%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.61 (s, 1H), 7.68 (d, J = 8.8, 2H), 6.92 (d, J = 8.8, 2H), 3.99 – 3.76 (m, 4H), 3.44 – 3.31 (m, 1H), 3.29 – 3.22 (m, 4H), 2.70 (dd, J = 6.7, 16.8, 1H), 2.46 (d, J = 16.7, 1H), 1.24 (d, J = 7.3, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.64, 154.05, 152.18, 127.10, 125.33, 114.73, 66.69, 48.33, 33.93, 27.94, 16.36. TLC: R_f 0.1 (1:50 MeOH:CH_2Cl_2). HPLC: R_t 1.05 min, purity > 95%. MS: 274 (M + 1). HRMS: calcd. 274.1556 (M + 1); found 274.1552. Anal. Calcd. for $C_{15}H_{19}N_3O_2$: C, 65.91; H, 7.01; N, 15.37; Found. 65.81, H, 6.66, N, 15.26.



DNMDP-2L. To 130 mg of **A** (0.64 mmol) dissolved in 0.4 mL of Dimethylformamide (DMF) was added 100 mg of tert-butyl 2-(2-(2-bromoethoxy)ethoxy)-ethylcarbamate (Toronto Research Chemical, 0.32 mmol) and 90 mg of K_2CO^3 (64 mmol) and the mixture was stirred at 60 °C overnight. After cooling, water was added and rinsed several times with EtOAc. The combined

EtOAc layers were dried, concentrated, and chromatographed with 50-70% EtOAc to yield 81 mg of product (58%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.06 (s, 1H), 7.59 (d, J = 8.8 Hz, 2H), 6.62 (d, J = 8.8 Hz, 2H), 5.15 (s, 1H), 4.53 (s, 1H), 3.72 (t, J = 5.2 Hz, 2H), 3.65 (s, 4H), 3.55 (t, J = 5.2 Hz, 2H), 3.32 (m, 5H), 2.67 (dd, J = 16.8, 6.7 Hz, 1H), 2.42 (d, J = 16.4 Hz, 1H), 1.44 (s, 9H), 1.22 (d, J = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.83, 155.99, 154.45, 149.64, 127.33, 123.24, 112.58, 79.28, 70.30, 70.26, 70.22, 69.45, 43.14, 40.39, 33.96, 28.43, 27.89, 16.40; HPLC: R_t 2.50 min (7.5 min run), purity > 95%. MS: 435 (M + 1). This product (0.19 mmol) was dissolved in 1 mL MeOH and to the solution was added acetaldehyde (50 uL, 0.89 mmol), 10 uL HOAc (0.2 mmol) and 12 mg $NaBH_3CN$ (0.19 mmol). After 1 hour, $NaHCO_3$(aq) and CH_2Cl_2 were added, the CH_2Cl_2 was separated and the water washed twice with CH_2Cl_2. The combined CH_2Cl_2 was dried, concentrated, and chromatography with 60-70% EtOAc in hexane yielded 71 mg of product as a clear oil (82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.91 (s, 1H), 7.63 (d, J = 8.9 Hz, 2H), 6.69 (d, J = 8.9 Hz, 2H), 5.07 (s, 1H), 3.65 (t, J = 6.0 Hz, 2H), 3.61 (s, 4H), 3.55 (dt, J = 9.9, 5.5 Hz, 4H), 3.46 (q, J = 7.0 Hz, 2H), 3.38 - 3.22 (m, 3H), 2.67 (dd, J = 16.8, 6.7 Hz, 1H), 2.43 (d, J = 16.7 Hz, 1H), 1.45 (s, 10H), 1.23 (d, J = 7.3 Hz, 3H), 1.18 (t, J = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.84, 155.96, 154.46, 148.89, 127.35, 121.38, 111.28, 79.22, 70.68, 70.27, 70.24, 68.74, 49.95, 45.49, 40.32, 33.97, 28.43, 27.80, 16.43, 12.14. R_t 2.99 min (7.5 min run), purity > 95%. MS: 463 (M + 1).

Attachment to resin

To a solution of 18 mg of DNMDP-2L (0.04 mmol) in 0.8 mL of CH_2Cl_2 was added 0.2 mL of trifluoroacetic acid (TFA) and the solution was stirred 2 h before concentration and dissolution in 0.5 mL DMSO. To this was added 10 uL of Et_3N (0.07 mmol) and 12 mg of *N,N'*-disuccinimidyl carbonate (DSC) (0.05 mmol) and the solution was stirred overnight. LC analysis indicated the reaction was not complete, another 25 mg of N,N'-disuccinimidyl carbonate (0.1 mmol) was added. LC analysis after 2 hours showed ca. 5:1 ratio of DSC product:amine. A 1 mL sample of Affi-Gel 102 resin was rinsed five times with DMSO with a centrifuge, then suspended in 0.5 mL DMSO. To the resin was added 30 uL of the DSC product solution and 25 uL Et3N and the mixture was swirled. After 2 days, LC analysis of the DMSO solution showed complete disappearance of the DCS adduct; the underivatized amine was still present. The DMSO was removed by centrifuge and decanted and the resin was rinsed several times with DMSO and stored in PBS buffer.

Bioactives screen to rescue DNMDP induced cytotoxicity

1000 HeLa cells were plated in a 384-well plate in 40 µl of DMEM supplemented with 10% Fetal Bovine Serum and 1% Pen/Strep. 24 hours after plating, a compound-library of 1600 bioactive molecules (Pharmacon) was added at a concentration of 20 µM. In parallel to bioactive compound

incubation, DNMDP was added to a final concentration of 30 nM and incubated for 48 hours. Cell viability was assessed as described in Compound library screening in NCI-H1734 and A549 cell lines.

Linker-affinity purification of molecular target of DNMDP and immunoblotting

HeLa cells were washed with ice-cold PBS before lysed with NP-40 lysis buffer (150 mM NaCl, 10% glycerol, 50 mM Tris-Cl pH 8.0, 50 mM $MgCl_2$, 1% NP-40) supplemented with EDTA-free protease inhibitors (Roche) and phosphatase inhibitor mixtures I and II (Calbiochem). Cell lysates were incubated on ice for at least 2 minutes and subsequently centrifuged for 10 minutes at 4° C at 15,700 x g after which the supernatant was quantified using BCA protein assay kit (Pierce). 200 µg total HeLa cell lysate was incubated with 3 µl Affi-Gel 102 resin (BioRad) coupled to affinity linker DNMDP-2L in a total volume of 400 µl for four hours. Prior to incubation, indicated compounds were added to affinity purifications at a final concentration of 10 µM. Samples were washed three times with lysis buffer containing corresponding compound concentrations of 10 µM. Proteins bound to Affi-Gel 102 resin were reduced, denatured, and separated using Tris-Glycine gels (Novex) and transferred to nitrocellulose membranes using the iBlot transfer system (Novex). Membranes were incubated overnight at 4° C with primary antibodies against PDE3A (1:1000, Bethyl). Incubation with secondary antibodies (1:20,000, LI-COR Biosciences) for two hours at room temperature and subsequent detection (Odyssey Imaging System, LI-COR Biosciences) were performed according to manufacturer's recommendations.

PARP-cleavage immunoblotting

HeLa cells were treated with indicated concentration of DNMDP and staurosporine for 36 hours. HeLa cells were lysed and processed as described in *Linker-affinity purification of molecular target of DNMDP and immunoblotting*. Membranes were incubated with an antibody against PARP (1:1000, Cell Signaling #9532) and actin and subsequently imaged as described in *Linker-affinity purification of molecular target of DNMDP and immunoblotting*.

Targeting PDE3A locus using CRISPR

CRISPR target sites were identified using the MIT CRISPR Design Tool (online MIT CRISPR design portal). For cloning of sgRNAs, forward and reverse oligos were annealed, phosphorylated and ligated into BsmBI-digested pXPR_BRD001. Oligo sequences are as follows:

sgRNA	Forward oligo	Reverse oligo
PDE3A_sg1	CACCGTTTTCACTGAGCGAAGTGA (SEQ ID NO.: 7)	AAACTCACTTCGCTCAGTGAAAAC (SEQ ID NO.: 8)
PDE3A_sg2	CACCGAGACAAGCTTGCTATTCCAA (SEQ ID NO.: 9)	AAACTTGGAATAGCAAGCTTGTCTC (SEQ ID NO.: 10)
PDE3A_sg3	CACCGGCACTCTGAGTGTAAGTTA (SEQ ID NO.: 11)	AAACTAACTTACACTCAGAGTGCC (SEQ ID NO.: 12)

To produce lentivirus, 293T cells were co-transfected with pXPR_BRD001, psPAX2 and pMD2.G using calcium phosphate. Infected HeLa cells were selected with 2ug/ml of puromycin.

Reduction of PDE3A expression using siRNA

HeLa cells were plated in 96-well plates and transfected after 24 hours with PDE3A and Non-Targeting siRNA smartpools (On Target Plus, Thermo Scientific) according to the manufacturers recommendations. HeLa cell lysate was obtained 24 hours and 72 hours after transfection and immunoblotted for PDE3A and Actin (1:20,000, Cell Signaling) as described in *Linker-affinity purification of molecular target of DNMDP and immunoblotting.* HeLa cells were treated for 48 hours with indicated concentrations of Compound 3. Cell viability was assessed as described in Compound library screening in NCI-H1734 and A549 cell lines.

Measuring cellular cAMP concentrations in HeLa cells

5000 HeLa cells were plated in 96-well plates. 24 hours after plating, HeLa cells were incubated for one hour with indicated compounds at indicated concentrations. cAMP levels were determined with the CAMP-GLO™ assay (Promega) according to the manufacturers recommendations. Cellular concentrations of cAMP were determined by normalizing to a standard curve generated according to the manufacturers recommendations.

Extended Proteomics Methods for PDE3A-protein interaction studies

Immunoprecipitation of PDE3A in HeLa cells

HeLa cells were treated for four hours prior to lysis with 10 μM of indicated compounds: DMSO, DNMDP and trequinsin. HeLa cells were lysed with ModRipa lysis buffer (1%NP-40: 50 mM Tris-HCl, pH 7.8, 150 mM NaCl, 0.1% sodium deoxycholate, 1 mM EDTA) supplemented with protease and phosphatase inhibitors as described in *Linker-affinity purification of molecular target of DNMDP and immunoblotting*, and indicated compounds as described above to a final concentration of 10 μM. 13 mg of HeLa total cell lysate was incubated with 0.5% PDE3A antibody (Bethyl) and incubated overnight. Blocking peptide (Bethyl) against the PDE3A antibody was added simultaneously with the PDE3A antibody in the corresponding condition. Total cell lysate and antibody mixture was then incubated with 10 μl Protein A Plus Agarose (Fisher Scientific) for 30 minutes at 4° C. Protein A Plus Agarose was then washed two times with lysis buffer containing indicated compounds at a concentration of 10 μM. Finally, Protein A Plus Agarose was washed once with lysis buffer containing no NP-40 and indicated compounds at a concentration of 10 μM.

On-bead digest

The beads from immunopurification were washed once with IP lysis buffer, then three times with PBS, the three different lysates of each replicate were resuspended in 90 uL digestion buffer (2M Urea, 50 mM Tris HCl), 2 ug of sequencing grade trypsin added, 1 hour shaking at 700 rpm. The supernatant was removed and placed in a fresh tube. The beads were then washed twice with 50 uL digestion buffer and combined with the supernatant. The combined supernatants were reduced (2 uL 500 mM DTT, 30 minutes, room temperature), alkylated (4 uL 500 mM IAA, 45 minutes, dark) and a longer overnight digestion performed: 2 ug (4 uL) trypsin, shake overnight. The samples were then quenched with 20 uL 10% folic acid (FA) and desalted on 10 mg SEP-PAK® columns.

iTRAQ labeling of peptides and strong cation exchange (scx) fractionation

Desalted peptides were labeled with isobaric tags for relative and absolute quantification (iTRAQ)- reagents according to the manufacturer's instructions (AB Sciex, Foster City, CA). Peptides were dissolved in 30 µl of 0.5 M TEAB pH 8.5 solution and labeling reagent was added in 70 ul of ethanol. After 1 hour incubation the reaction was stopped with 50 mM Tris/HCl pH 7.5. Differentially labeled peptides were mixed and subsequently desalted on 10 mg SEP-PAK® columns.

	iTRAQ labeling			
	114	115	116	117
Rep1	Blocking peptide	No addition	DNMDP	trequinsin
Rep2	Blocking peptide	No addition	DNMDP	trequinsin

SCX fractionation of the differentially labelled and combined peptides was done as described in Rappsilber et al. (Rappsilber et al., Nat Protoc 2, 1896–1906, 2007), with 6 pH steps (buffers- all contain 25% acetonitrile) as below:

1: ammonium acetate 50 mM pH 4.5,
2: ammonium acetate 50 mM pH 5.5,
3: ammonium acetate 50 mM pH 6.5,
4: ammonium bicarbonate 50 mM pH 8,
5: ammonium hydroxide 0.1% pH 9,
6: ammonium hydroxide 0.1% pH 11.

Empore SCX disk used to make stop-and-go-extraction-tips (StageTips) as described in the paper.

MS analysis

Reconstituted peptides were separated on an online nanoflow EASY-NLC™ 1000 UHPLC system (Thermo Fisher Scientific) and analyzed on a benchtop Orbitrap Q EXACTIVE™ mass spectrometer (Thermo Fisher Scientific). The peptide samples were injected onto a capillary column (PICOFRIT® with 10 μm tip opening/ 75 μm diameter, New Objective, PF360-75-10-N-5) packed in-house with 20 cm C18 silica material (1.9 μm REPROSIL-PUR® C18-AQ medium, Dr. Maisch GmbH, r119.aq). The UHPLC setup was connected with a custom-fit microadapting tee (360 μm, IDEX Health & Science, UH-753), and capillary columns were heated to 50 °C in column heater sleeves (Phoenix-ST) to reduce backpressure during UHPLC separation. Injected peptides were separated at a flow rate of 200 nL/min with a linear 80 min gradient from 100% solvent A (3% acetonitrile, 0.1% formic acid) to 30% solvent B (90% acetonitrile, 0.1% formic acid), followed by a linear 6 min gradient from 30% solvent B to 90% solvent B. Each sample was run for 120 minutes, including sample loading and column equilibration times. The Q EXACTIVE™ instrument was operated in the data-dependent mode acquiring high-energy collisional dissociation (HCD) MS/MS scans (R=17,500) after each MS1 scan (R=70,000) on the 12 top most abundant ions using an MS1 ion target of 3x 106 ions and an MS2 target of 5x104 ions. The maximum ion time utilized for the MS/MS scans was 120 ms; the HCD-normalized collision energy was set to 27; the dynamic exclusion time was set to 20s, and the peptide match and isotope exclusion functions were enabled.

Quantification and identification of peptides and proteins

All mass spectra were processed using the Spectrum Mill software package v4.1 beta (Agilent Technologies) which includes modules developed by Applicants for isobaric tags for relative and absolute quantification (iTRAQ)-based quantification. Precursor ion quantification was done using extracted ion chromatograms (XIC's) for each precursor ion. The peak area for the XIC of each precursor ion subjected to MS/MS was calculated automatically by the Spectrum Mill software in the intervening high-resolution MS1 scans of the liquid chromatography (LC)-MS/MS runs using narrow windows around each individual member of the isotope cluster. Peak widths in both the time and m/z domains were dynamically determined based on MS scan resolution, precursor charge and m/z, subject to quality metrics on the relative distribution of the peaks in the isotope cluster vs theoretical. Similar MS/MS spectra acquired on the same precursor m/z in the same dissociation mode within +/- 60 seconds were merged. MS/MS spectra with precursor charge >7 and poor quality MS/MS spectra, which failed the quality filter by not having a sequence tag length > 1 (i.e., minimum of 3 masses separated by the in-chain mass of an amino acid) were excluded from searching.

For peptide identification MS/MS spectra were searched against human Universal Protein Resource (Uniprot) database to which a set of common laboratory contaminant proteins was appended. Search parameters included: ESI-Q EXACTIVE™-HCD scoring parameters, trypsin

enzyme specificity with a maximum of two missed cleavages, 40% minimum matched peak intensity, +/- 20 ppm precursor mass tolerance, +/- 20 ppm product mass tolerance, and carbamidomethylation of cysteines and iTRAQ labeling of lysines and peptide n-termini as fixed modifications. Allowed variable modifications were oxidation of methionine, N-terminal acetylation, Pyroglutamic acid (N-termQ),Deamidated (N),Pyro Carbamidomethyl Cys (N-termC),with a precursor MH+ shift range of -18 to 64 Da. Identities interpreted for individual spectra were automatically designated as valid by optimizing score and delta rank1-rank2 score thresholds separately for each precursor charge state in each liquid chromatography (LC)-MS/MS while allowing a maximum target-decoy-based false-discovery rate (FDR) of 1.0% at the spectrum level.

In calculating scores at the protein level and reporting the identified proteins, redundancy is addressed in the following manner: the protein score is the sum of the scores of distinct peptides. A distinct peptide is the single highest scoring instance of a peptide detected through an MS/MS spectrum. MS/MS spectra for a particular peptide may have been recorded multiple times, (i.e. as different precursor charge states, isolated from adjacent SCX fractions, modified by oxidation of Met) but are still counted as a single distinct peptide. When a peptide sequence >8 residues long is contained in multiple protein entries in the sequence database, the proteins are grouped together and the highest scoring one and its accession number are reported. In some cases when the protein sequences are grouped in this manner there are distinct peptides which uniquely represent a lower scoring member of the group (isoforms or family members). Each of these instances spawns a subgroup and multiple subgroups are reported and counted towards the total number of proteins. iTRAQ ratios were obtained from the protein-comparisons export table in Spectrum Mill. To obtain iTRAQ protein ratios the median was calculated over all distinct peptides assigned to a protein subgroup in each replicate. To assign interacting proteins the Limma package in the R environment was used to calculate moderated t-test p, as described previously and added Blandt-Altman testing to filter out proteins for which the CI for reproducibility was below 95% (Udeshi et al., Mol Cell Proteomics 11, 148–159, 2012).

Validation of DNMDP-induced PDE3A protein interactions using immunoprecipitation and immunoblotting

HeLa cells were transfected with ORF overexpression constructs expressing V5-tagged SIRT7, V5-tagged SLFN12, or V5-tagged GFP. ORF expression constructs were obtained from the TRC (clone IDs: TRCN0000468231, TRCN0000476272, ccsbBroad304_99997). At 72 hours post transfection, cells were treated with 10 μM DNMDP or trequinsin for 4 hours followed by lysis using the ModRipa lysis buffer and immunoprecipitation of PDE3A. For each condition, 2 mg total protein lysate was incubated with 1 μg of anti-PDE3A antibody at 4° C overnight, after which 7.5 μl each of Protein A- and Protein G- Dynabeads (Life Technologies 10001D and 10003D) were added and

incubated for another 1 hour. Beads were washed and bound proteins were eluted with 30 µl of LDS PAGE gel loading buffer. Input (~60 µg total protein lysate) and IP products were resolved on 4-12% Tris-Glycine PAGE gels and immunoblotted with an anti-V5 antibody (Life Technologies R96205, 1:5000), the Bethyl anti-PDE3A antibody (1:1000), and secondary antibodies from LiCOR Biosciences (Cat.# 926-32210 and 926068021, each at 1:10,000). Blots were washed and imaged using a LiCOR Odyssey infrared imager.

Knockdown of SLFN12 expression using shRNA and testing for drug sensitivity

Constructs expressing shRNAs targeting *SLFN12*, or the control vector, were packaged into lentiviruses and delivered into HeLa cells by viral transduction. Three *SLFN12*-targeting shRNAs were used, all of which were obtained from the TRC (CloneIDs: TRCN0000152141 and TRCN0000153520). Infected cells were selected using 1 µg/ml puromycin for 3 days and then grown in non-selective media for 3 more days. Cells were then plated into 384-well assay plates and tested for drug sensitivity as described above. Knockdown of *SLFN12* was validated by qPCR. Total RNA was extracted using kit reagents (RNeasy Mini Kit (Qiagen #74104) and QIAschredder (Qiagen #79656)). cDNA was generated using kit reagents (SuperScript III First-Strand Synthesis System (Life Technologies #18080-051)). qPCR was performed for *GAPDH* and *SLFN12* (Life Technologies Hs00430118_m1) according to the manufacturer's recommendations. *SLFN12* expression was normalized to corresponding samples *GAPDH* ct-values.

Other Embodiments

From the foregoing description, it will be apparent that variations and modifications may be made to the invention described herein to adopt it to various usages and conditions. Such embodiments are also within the scope of the following claims.

The recitation of a listing of elements in any definition of a variable herein includes definitions of that variable as any single element or combination (or subcombination) of listed elements. The recitation of an embodiment herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

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CLAIMS:

1. Use of a phosphodiesterase 3A (PDE3A) modulator for killing or reducing the survival of a cancer cell selected as responsive to the PDE3A modulator, wherein the cell was selected as having an increase in the level of a PDE3A and/or a Schlafen 12 (SLFN12) polypeptide or polynucleotide relative to a non-responsive cancer cell or healthy control cell, and the PDE3A modulator is selected from the group consisting of 6-(4-(diethylamino)-3-nitropheny1)-5-methy1-4,5-dihydropyridazin-3(2H)-one (DNMDP), zardaverine, anagrelide, and pharmaceutically acceptable salts thereof.

2. Use of a PDE3A modulator for reducing cancer cell proliferation in a subject pre-selected as having a cancer that is responsive to the PDE3A modulator, wherein the subject is pre-selected by detecting an increase in the level of PDE3A and/or SLFN12 polypeptide or polynucleotide relative to a non-responsive cancer cell or healthy control cell, and the PDE3A modulator is selected from the group consisting of DNMDP, zardaverine, anagrelide, and pharmaceutically acceptable salts thereof.

3. A method of identifying a subject having a cancer cell responsive to a PDE3A modulator selected from the group consisting of DNMDP, zardaverine, anagrelide, and pharmaceutically acceptable salts thereof, the method comprising detecting an increase in a PDE3A and/or SLFN12 polypeptide or polynucleotide level in a biological sample of the subject relative to a non-responsive cancer cell or healthy control cell, thereby identifying said subject as having a cancer cell responsive to PDE3A modulation.

4. A method of identifying a subject having a cancer cell that is resistant to a PDE3A modulator selected from the group consisting of DNMDP, zardaverine, anagrelide, and pharmaceutically acceptable salts thereof, the method comprising detecting a decrease in the level of a SLFN12 polypeptide or polynucleotide level in a biological sample of the subject relative to a responsive cancer cell or healthy control cell, thereby identifying said subject as having a cancer cell resistant to PDE3A modulation.

5. The use or method of any one of claims 1-4, wherein the level of PDE3A or SLFN12 is detected by a method selected from the group consisting of immunoblotting, mass spectrometry, and immunoprecipitation.

6. The use or method of any one of claims 1-4, wherein the level of PDE3A or SLFN12 polynucleotide is detected by a method selected from the group consisting of quantitative PCR, Northern Blot, microarray, mass spectrometry, and in situ hybridization.

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7. The use or method of any one of claims 1-6, wherein the cancer cell is a melanoma, endometrium, lung, hematopoietic / lymphoid, ovarian, cervical, soft-tissue sarcoma, leiomyosarcoma, urinary tract, pancreas, thyroid, kidney, glioblastoma, or breast cancer cell.

8. The use or method of any one of claims 1-6, wherein the cancer cell is not a B-cell proliferative type cancer.

9. The use or method of any one of claims 1-6, wherein the cancer cell is not multiple myeloma.

10. The use of claim 2, wherein the PDE3A modulator is for administration orally.

11. The use of claim 2, wherein the PDE3A modulator is for administration by intravenous injection.

12. The method of claim 3 or 4, wherein the biological sample is a tissue sample comprising a cancer cell.

13. A kit for identifying a subject having cancer responsive to a PDE3A modulator selected from the group consisting of DNMDP, zardaverine, anagrelide, and pharmaceutically acceptable salts thereof, the kit comprising a first capture reagent that binds a PDE3A polypeptide or polynucleotide and a second capture reagent that binds SLFN12 polypeptide or polynucleotide.

14. A kit for decreasing cancer cell proliferation in a subject responsive to a PDE3A modulator selected from the group consisting of DNMDP, zardaverine, anagrelide, and pharmaceutically acceptable salts thereof, the kit comprising (a) a first capture reagent that binds a PDE3A polypeptide or polynucleotide; (b) a second capture reagent that binds SLFN12 polypeptide or polynucleotide; and (c) an effective amount of DNMDP, zardaverine, and/or anagrelide, or a pharmaceutically acceptable salt thereof.

15. Use of a PDE3A modulator in the manufacture of a medicament for the treatment of cancer pre-selected as responsive to the PDE3A modulator, wherein the cancer is pre-selected by detecting an increase in the level of PDE3A and/or SLFN12 polypeptide or polynucleotide relative to a non-responsive cancer cell or healthy control cell, and the PDE3A modulator is selected from the group consisting of DNMDP, zardaverine, anagrelide, and pharmaceutically acceptable salts thereof.

16. The use of claim 15, wherein the cancer is a melanoma, endometrium, lung, hematopoietic / lymphoid, ovarian, cervical, soft-tissue sarcoma, leiomyosarcoma, urinary tract, pancreas, thyroid, kidney, glioblastoma, or breast cancer.

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FIG. 1A

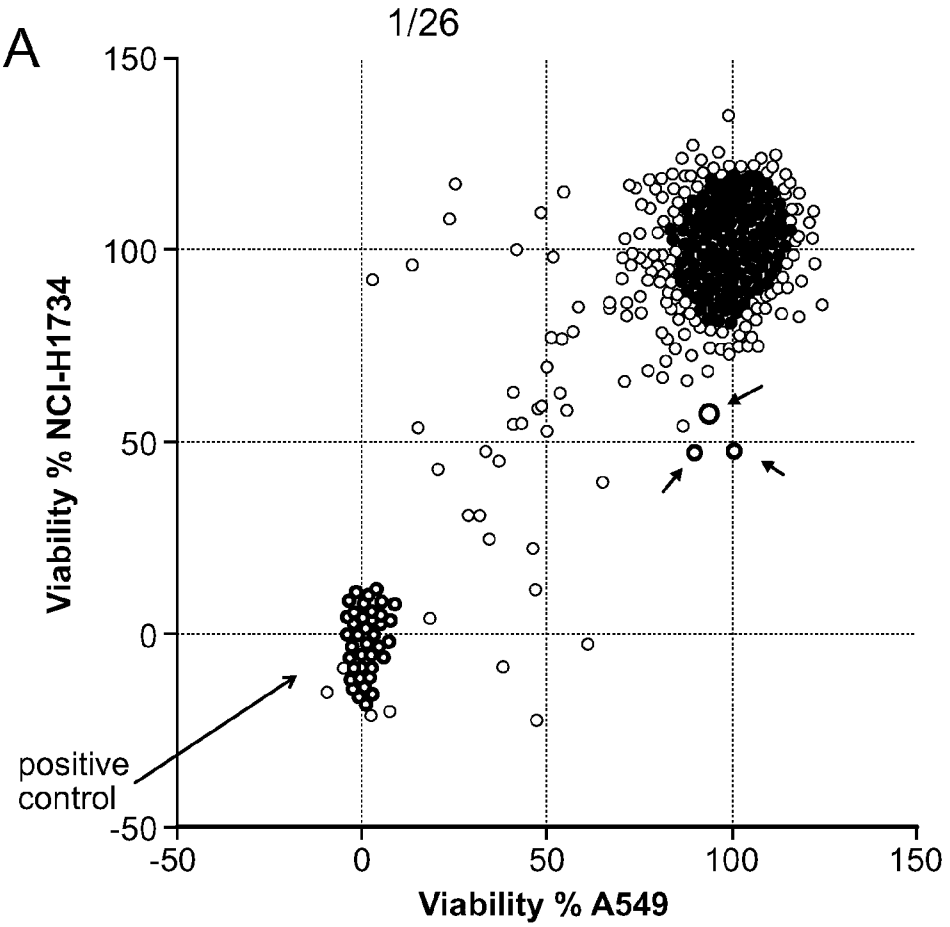


FIG. 1B

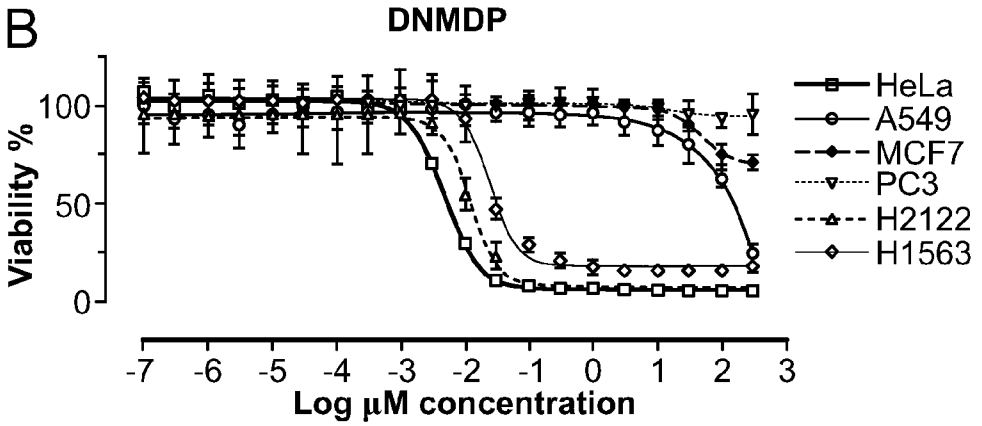


FIG. 1C

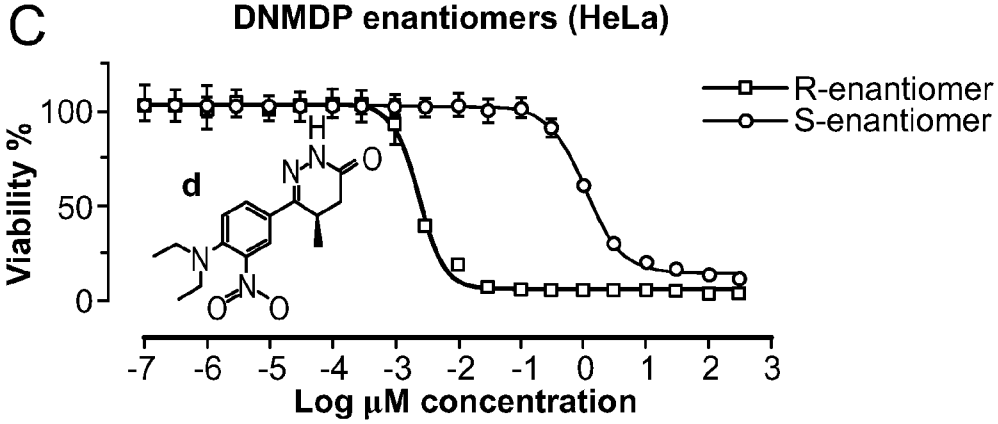


FIG. 1D

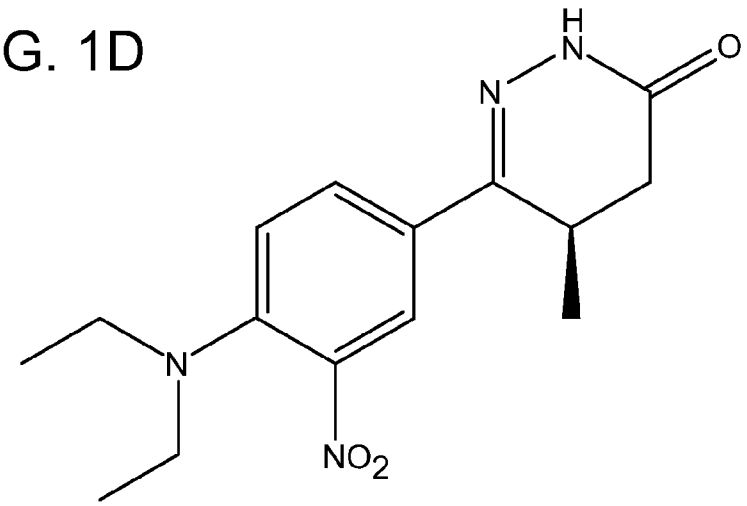

H
N
N
O
N
NO2

FIG. 2

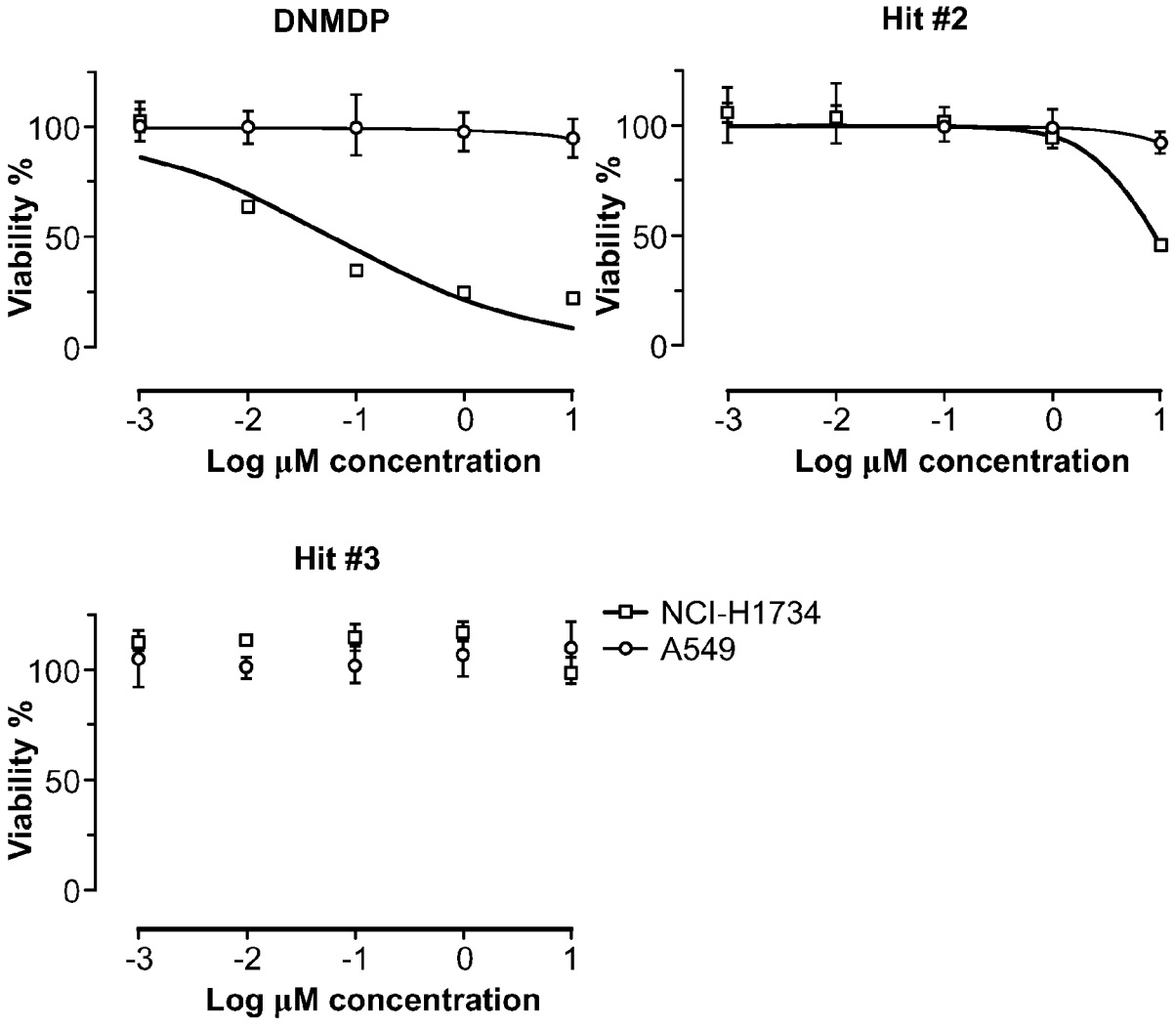

DNMDP
Hit #2
Hit #3
Viability %
100
50
0
-3
-2
-1
0
1
Log µM concentration
NCI-H1734
A549

FIG. 3

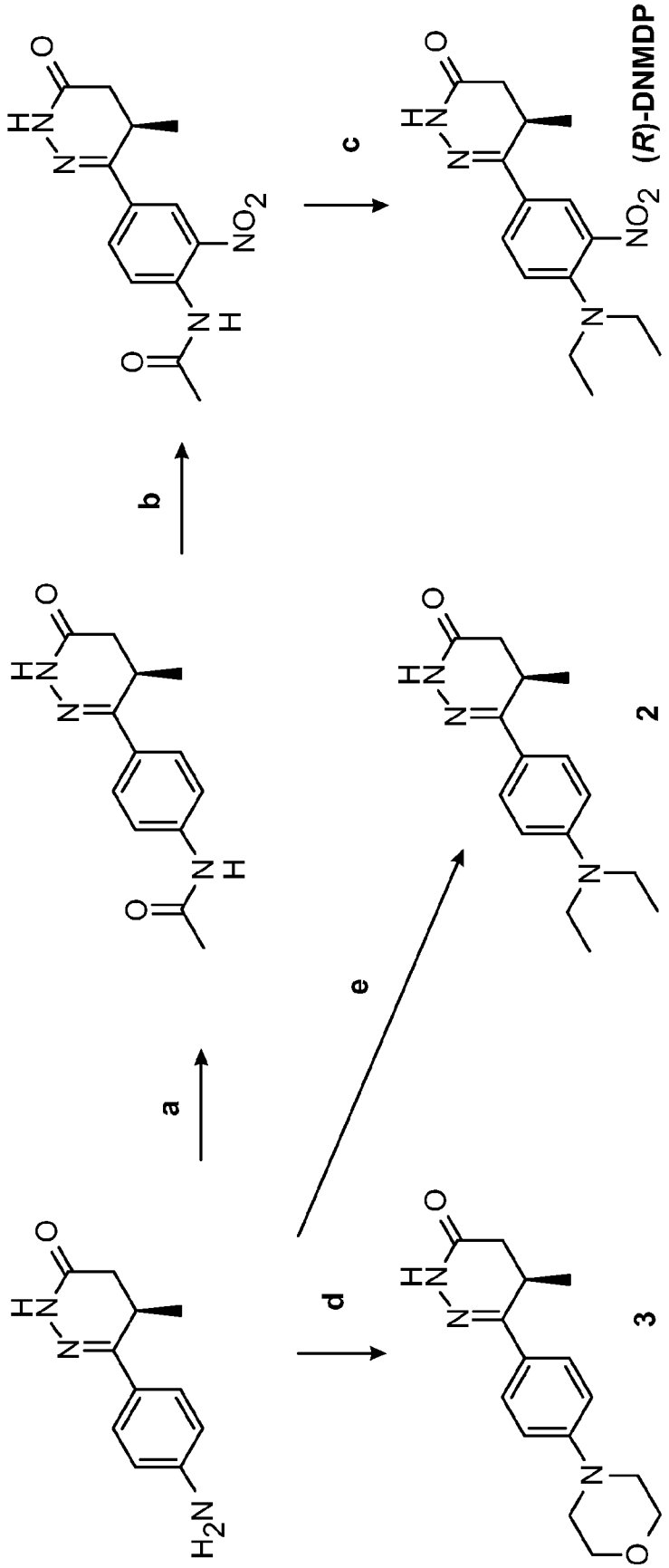


FIG. 4A

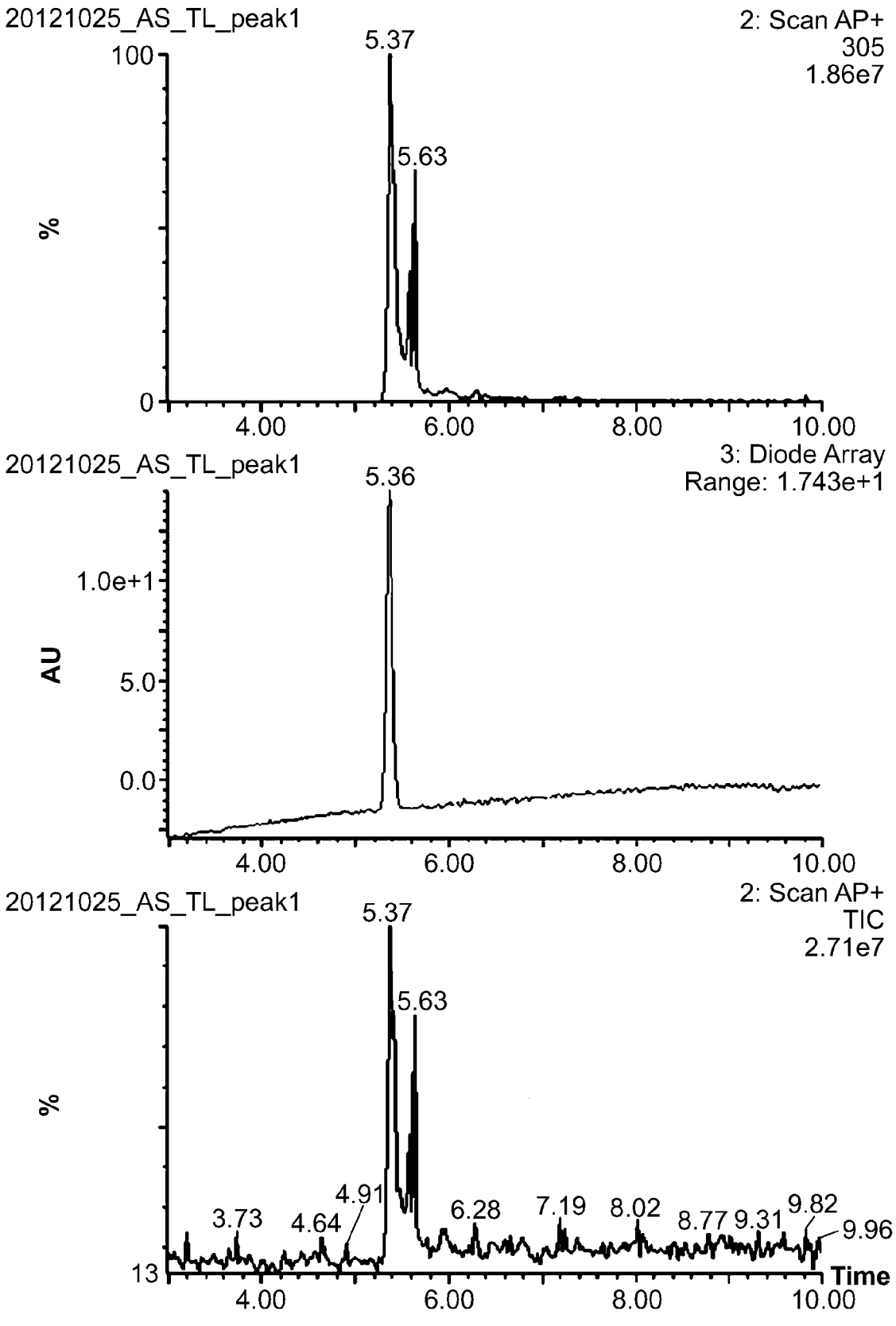

20121025_AS_TL_peak1
2: Scan AP+
305
1.86e7
5.37
5.63
100
%
0
4.00
6.00
8.00
10.00
20121025_AS_TL_peak1
3: Diode Array
Range: 1.743e+1
5.36
1.0e+1
AU
5.0
0.0
4.00
6.00
8.00
10.00
20121025_AS_TL_peak1
2: Scan AP+
TIC
2.71e7
5.37
5.63
4.91
3.73
4.64
6.28
7.19
8.02
8.77
9.31
9.82
9.96
%
13
Time
4.00
6.00
8.00
10.00

FIG. 4B

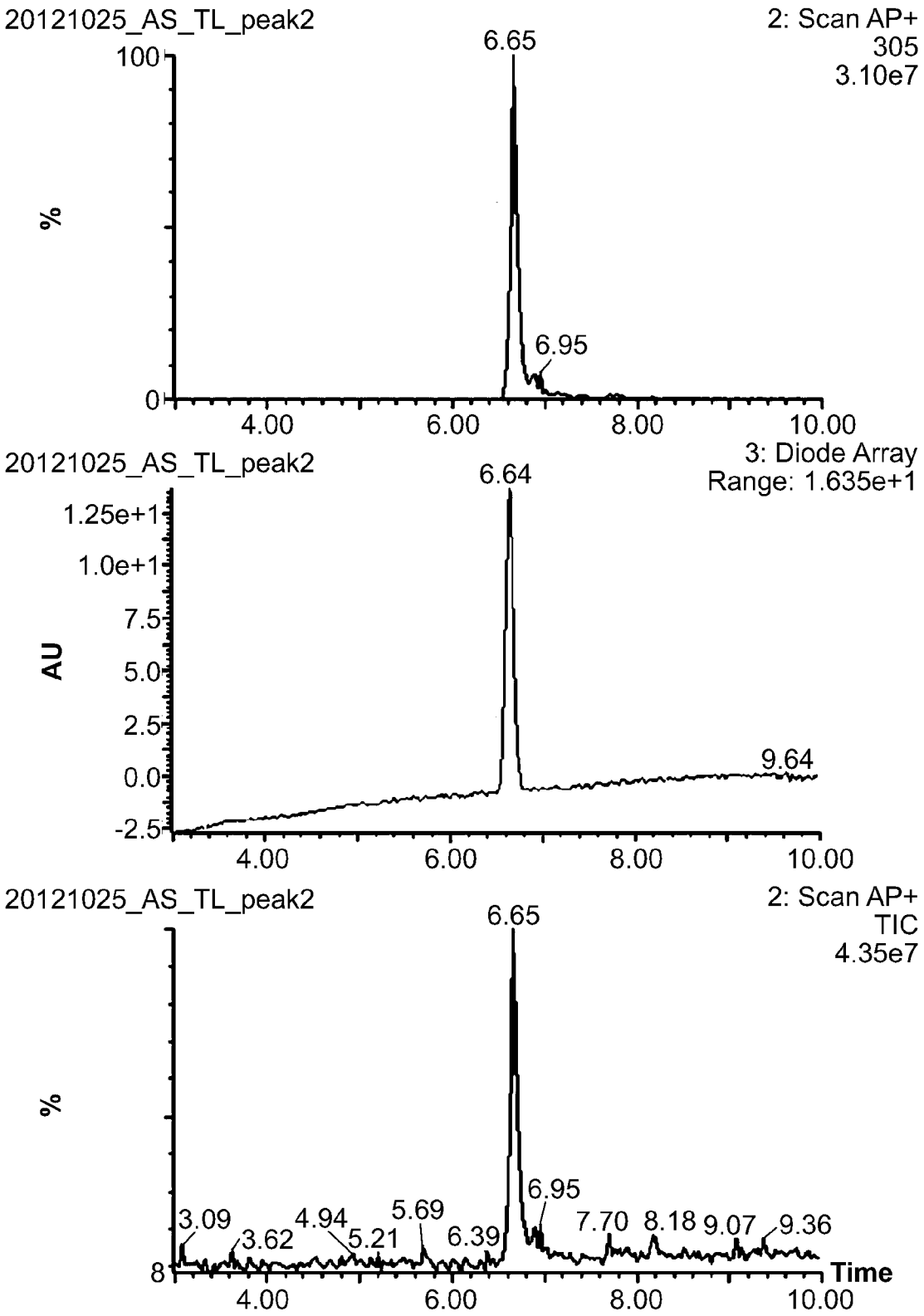

20121025_AS_TL_peak2
2: Scan AP+
305
3.10e7
6.65
6.95
100
%
0
4.00
6.00
8.00
10.00
20121025_AS_TL_peak2
3: Diode Array
Range: 1.635e+1
6.64
9.64
1.25e+1
1.0e+1
7.5
5.0
2.5
0.0
-2.5
AU
4.00
6.00
8.00
10.00
20121025_AS_TL_peak2
2: Scan AP+
TIC
4.35e7
6.65
6.95
3.09
3.62
4.94
5.21
5.69
6.39
7.70
8.18
9.07
9.36
%
8
Time
4.00
6.00
8.00
10.00

FIG. 4C

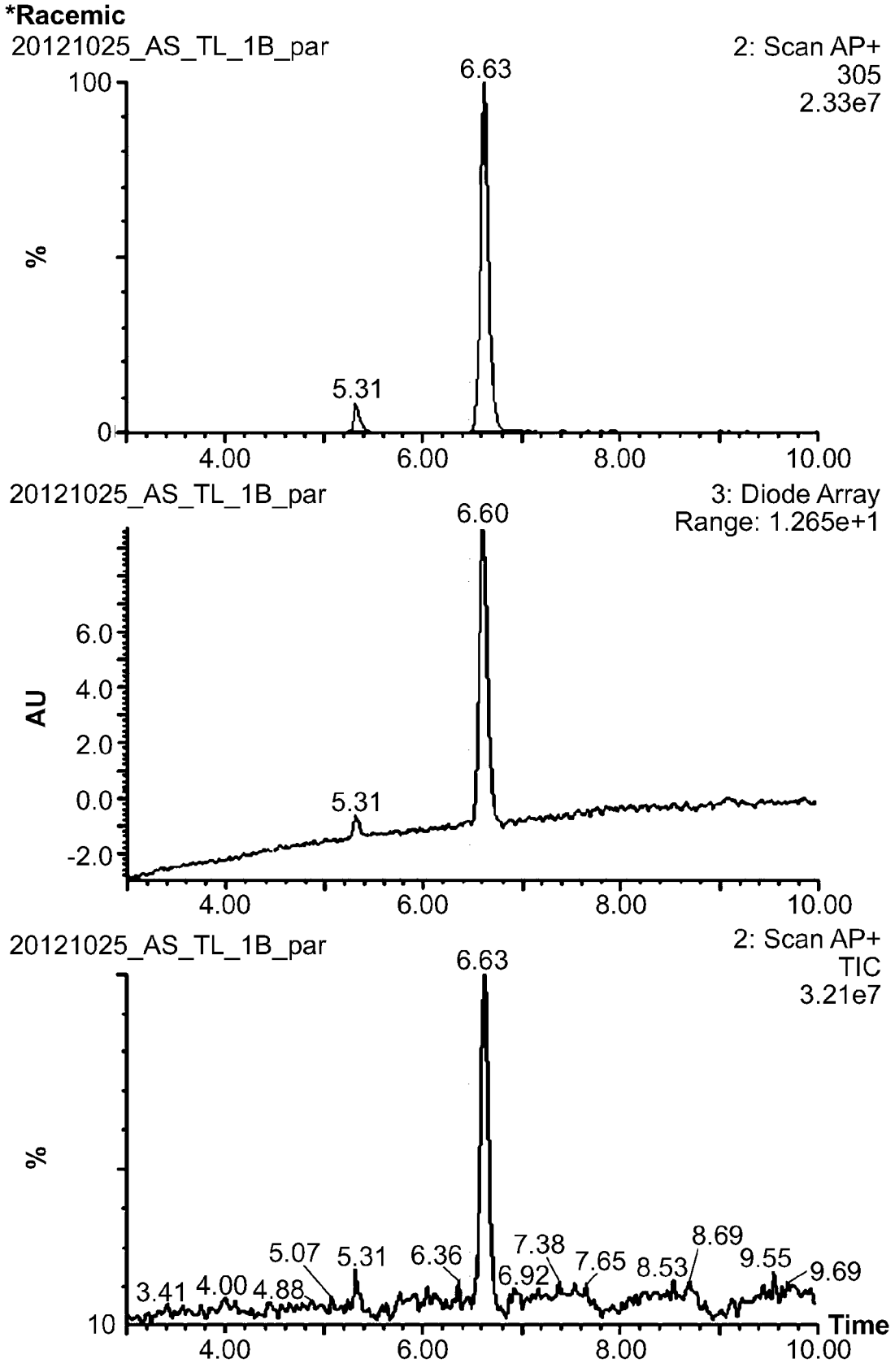

*Racemic
20121025_AS_TL_1B_par
2: Scan AP+
305
2.33e7
6.63
100
%
5.31
0
4.00
6.00
8.00
10.00
20121025_AS_TL_1B_par
3: Diode Array
Range: 1.265e+1
6.60
AU
6.0
4.0
2.0
0.0
-2.0
5.31
4.00
6.00
8.00
10.00
20121025_AS_TL_1B_par
2: Scan AP+
TIC
3.21e7
6.63
%
5.07
5.31
6.36
7.38
6.92
7.65
8.53
8.69
9.55
9.69
3.41
4.00
4.88
10
Time
4.00
6.00
8.00
10.00

FIG. 5A

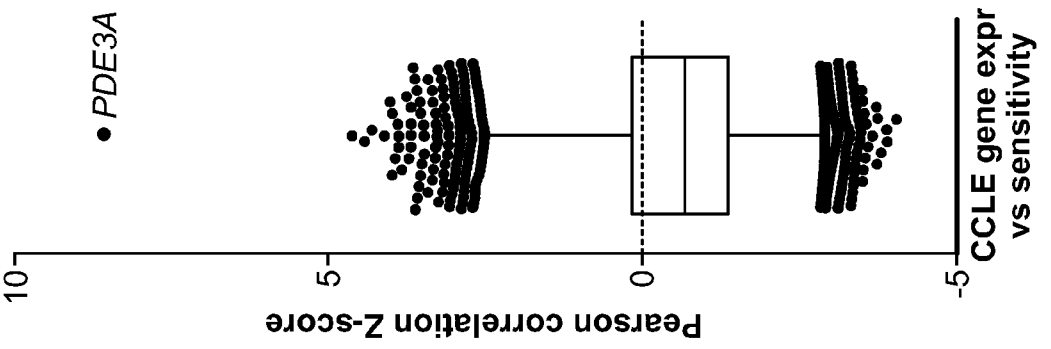


FIG. 5B

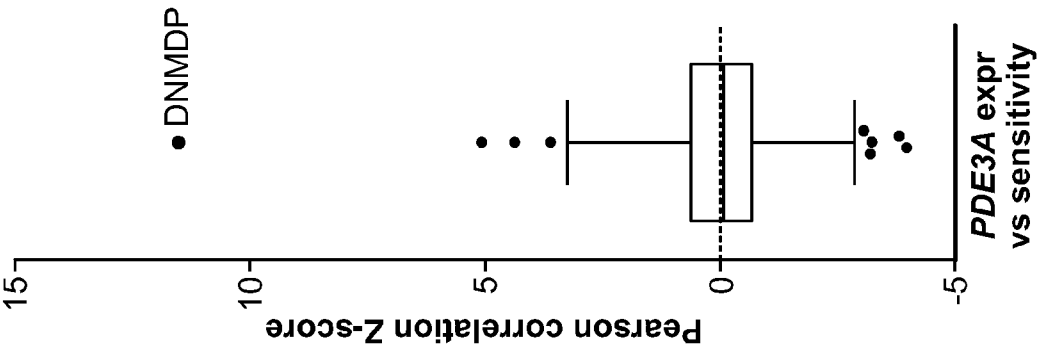


FIG. 5C

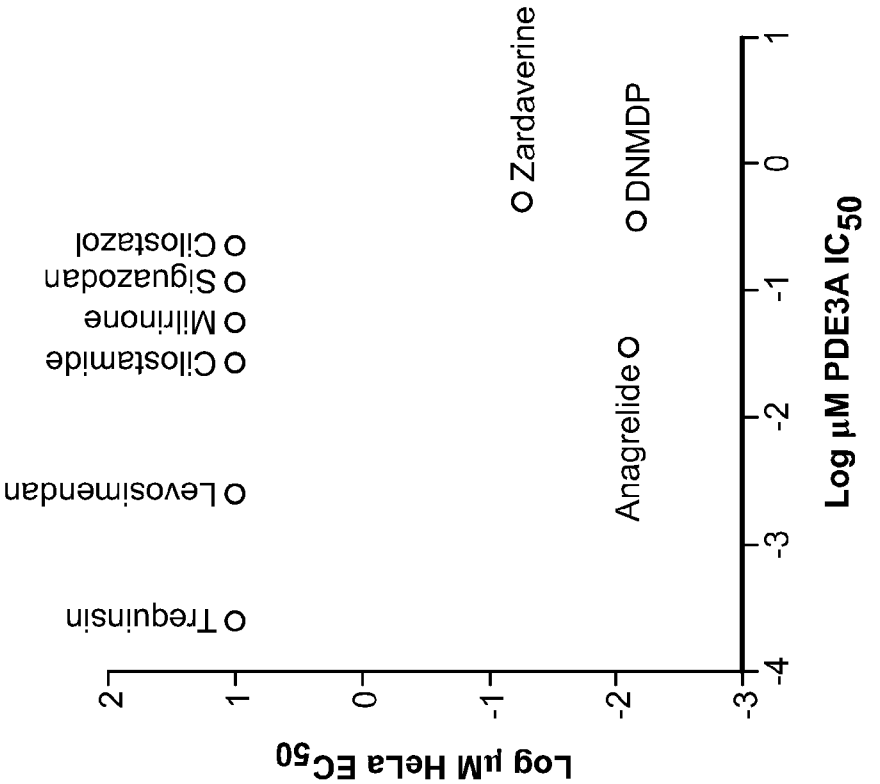


FIG. 6A

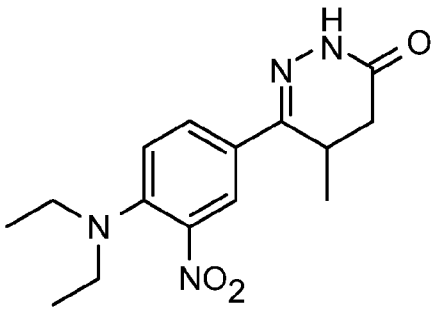


FIG. 6B

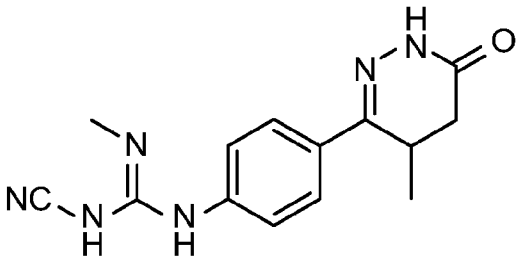


FIG. 6C

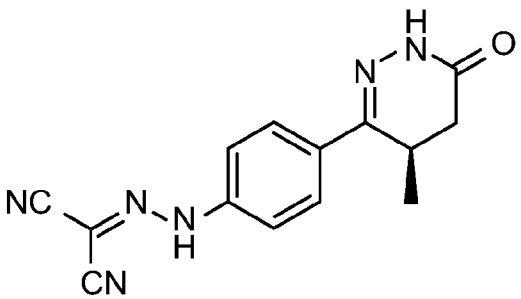


FIG. 7A

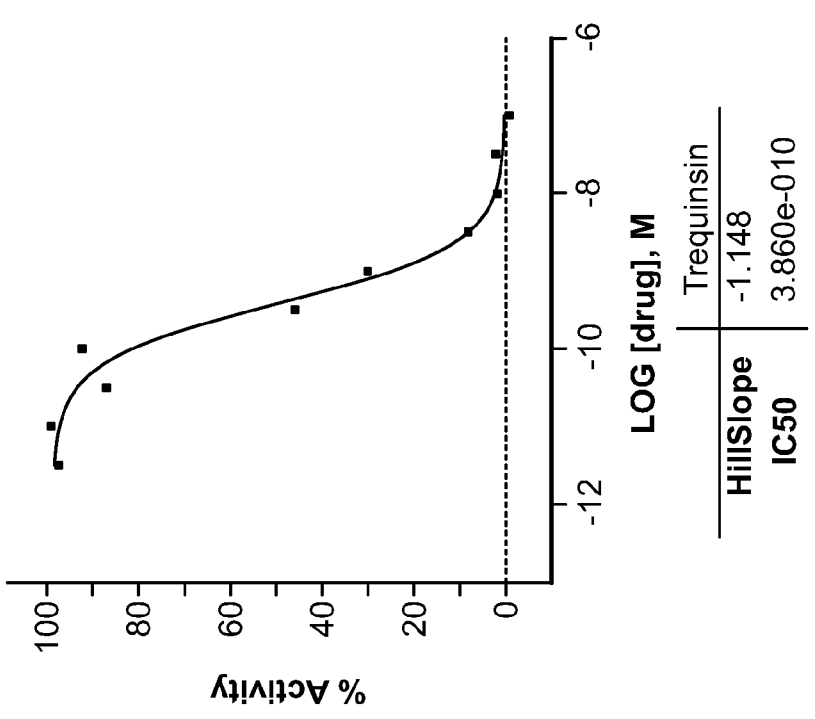

% Activity
100
80
60
40
20
0
-12
-10
-8
-6
LOG [drug], M
Trequinsin
HillSlope -1.148
IC50 3.860e-010

FIG. 7B

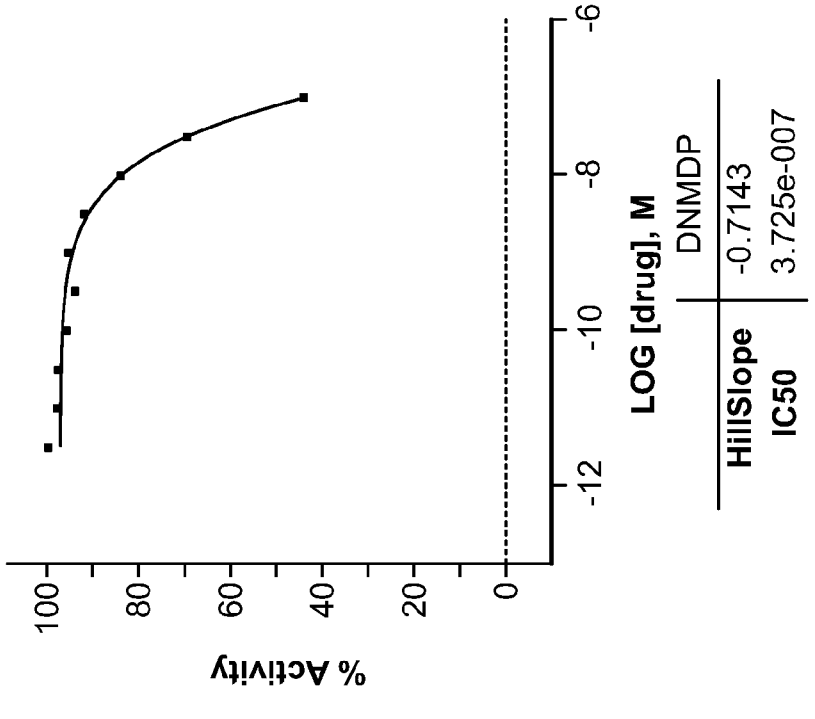

% Activity
100
80
60
40
20
0
-12
-10
-8
-6
LOG [drug], M
DNMDP
HillSlope -0.7143
IC50 3.725e-007

FIG. 8A

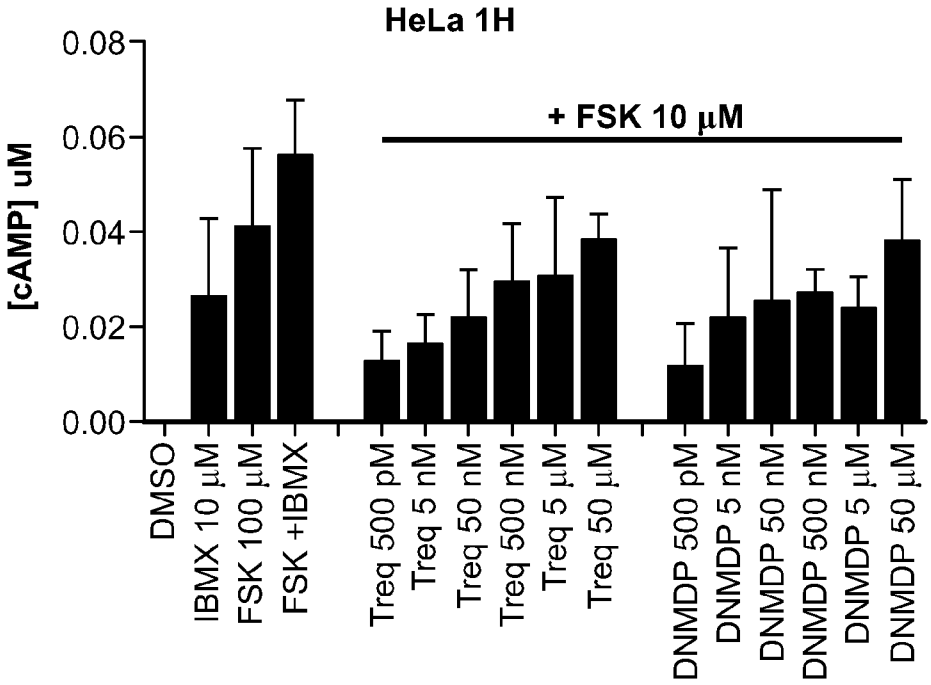

HeLa 1H
+ FSK 10 µM
[cAMP] uM
0.08
0.06
0.04
0.02
0.00
DMSO
IBMX 10 µM
FSK 100 µM
FSK +IBMX
Treq 500 pM
Treq 5 nM
Treq 50 nM
Treq 500 nM
Treq 5 µM
Treq 50 µM
DNMDP 500 pM
DNMDP 5 nM
DNMDP 50 nM
DNMDP 500 nM
DNMDP 5 µM
DNMDP 50 µM

FIG. 8B

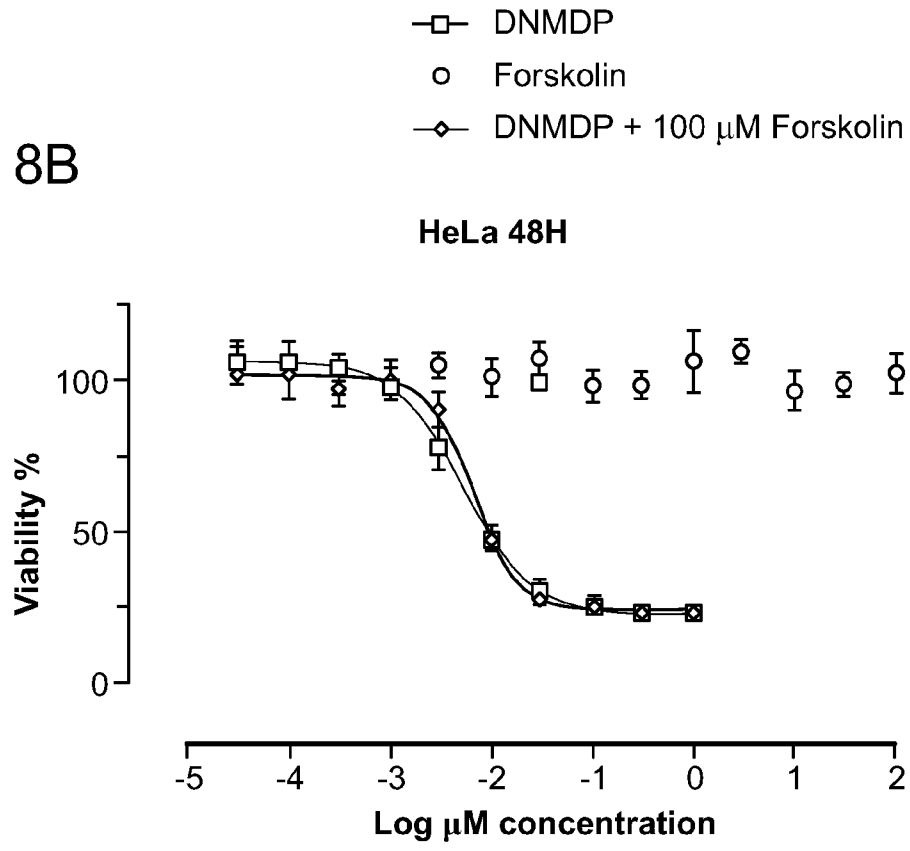

DNMDP
Forskolin
DNMDP + 100 µM Forskolin
HeLa 48H
Viability %
100
50
0
-5
-4
-3
-2
-1
0
1
2
Log µM concentration

FIG. 9A

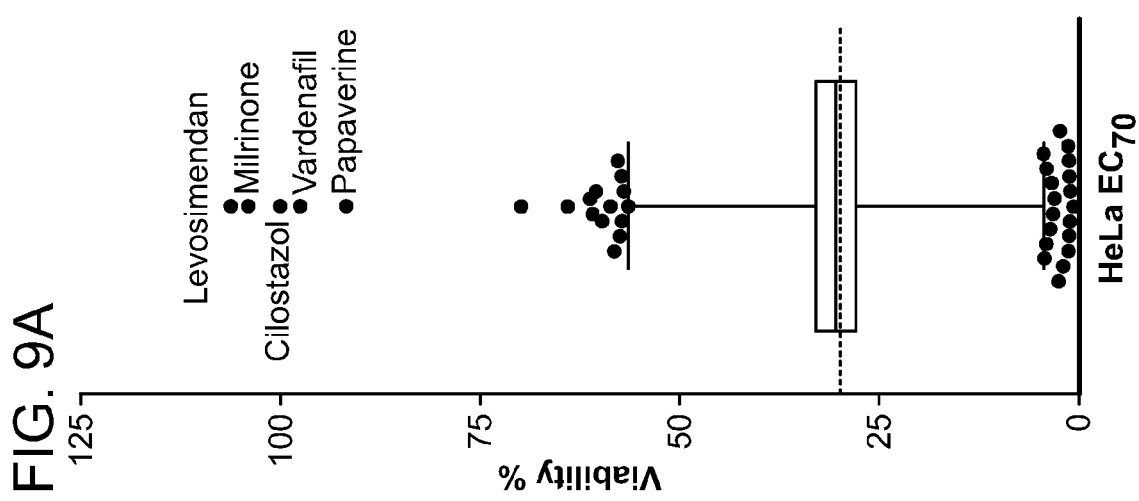


FIG. 9B

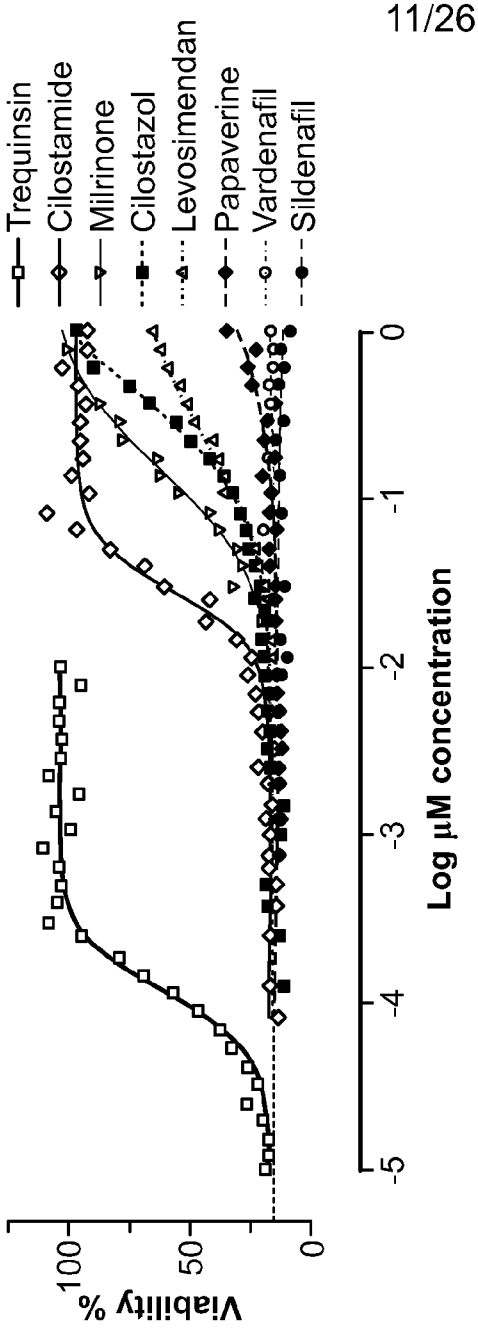


FIG. 9C

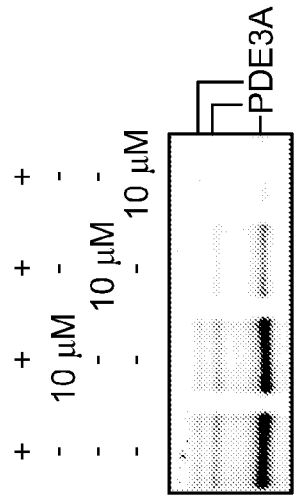


FIG. 10A

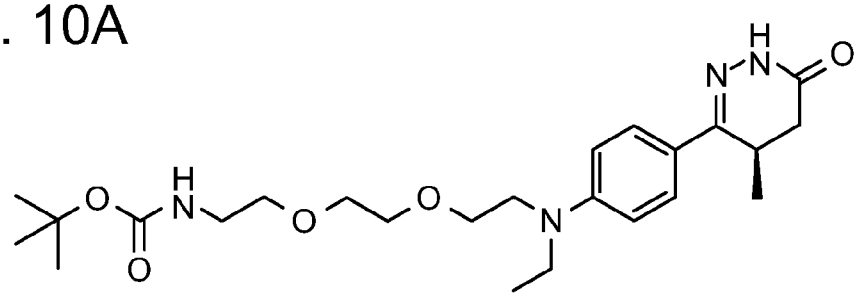


FIG. 10B

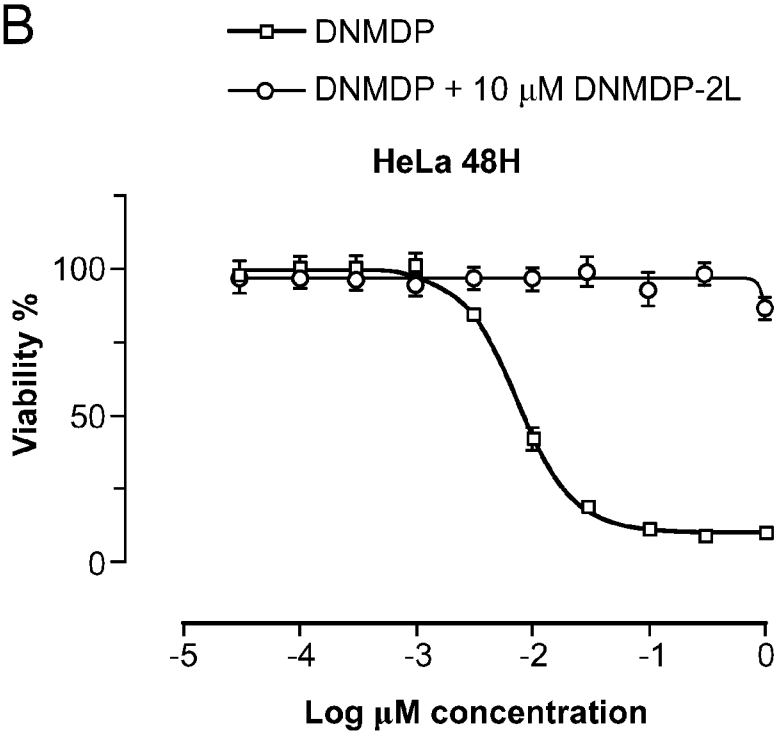


FIG. 11A

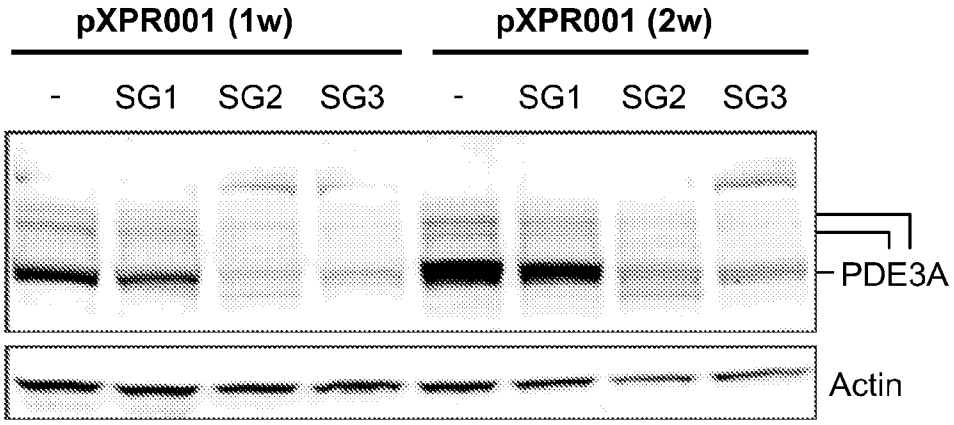


FIG. 11B

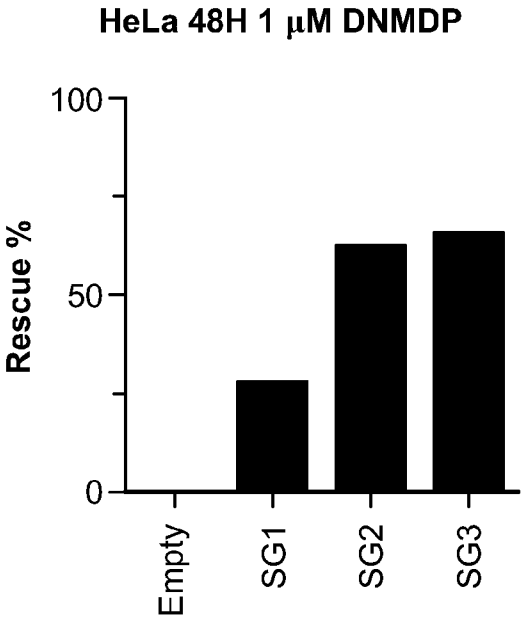


FIG. 11C

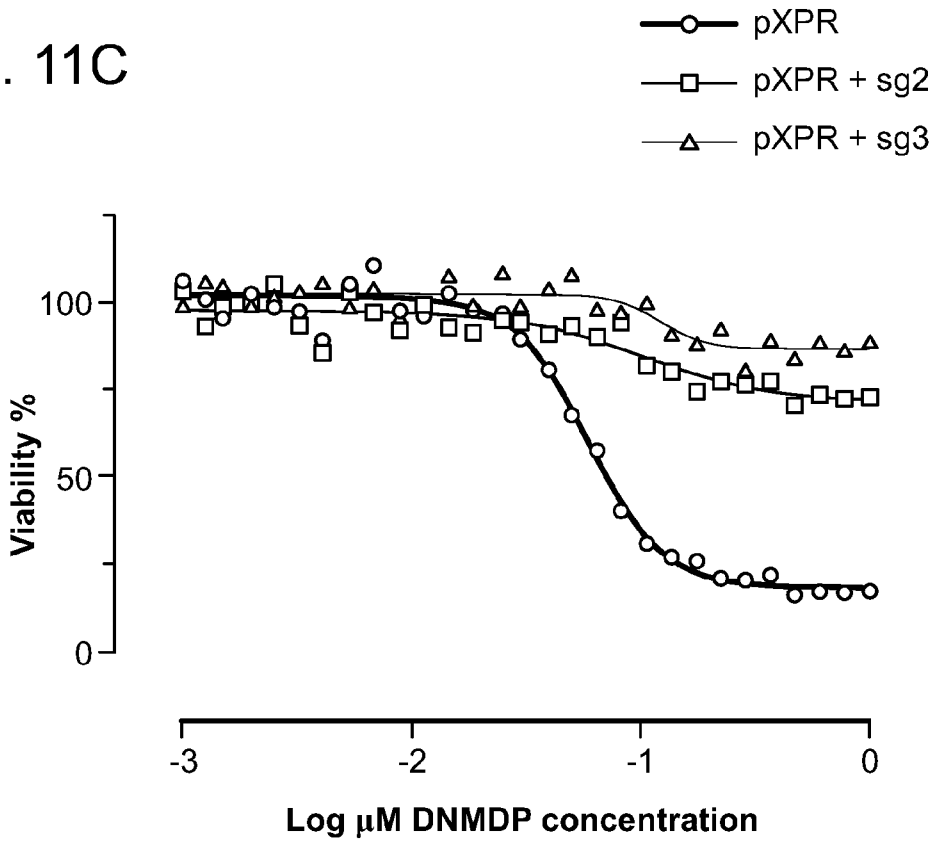


FIG. 12A

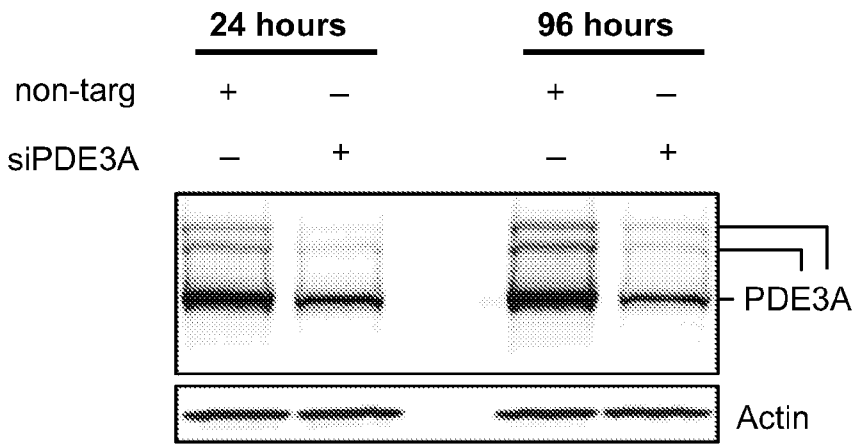


FIG. 12B

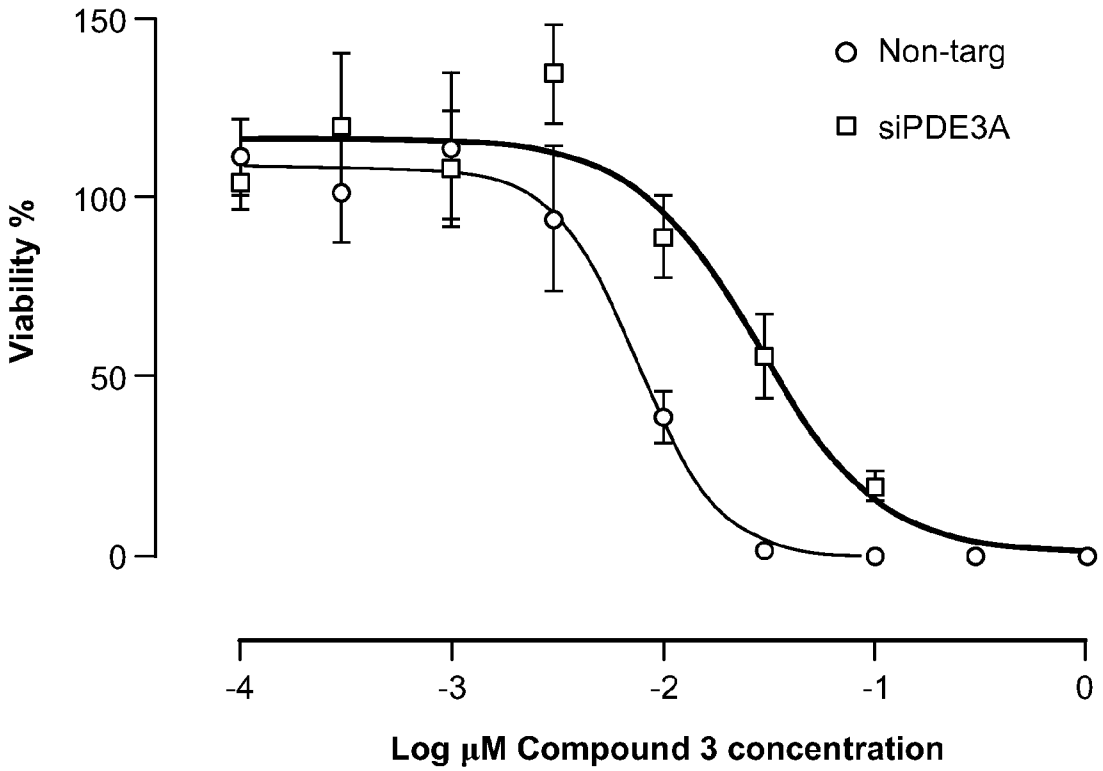


FIG. 13A

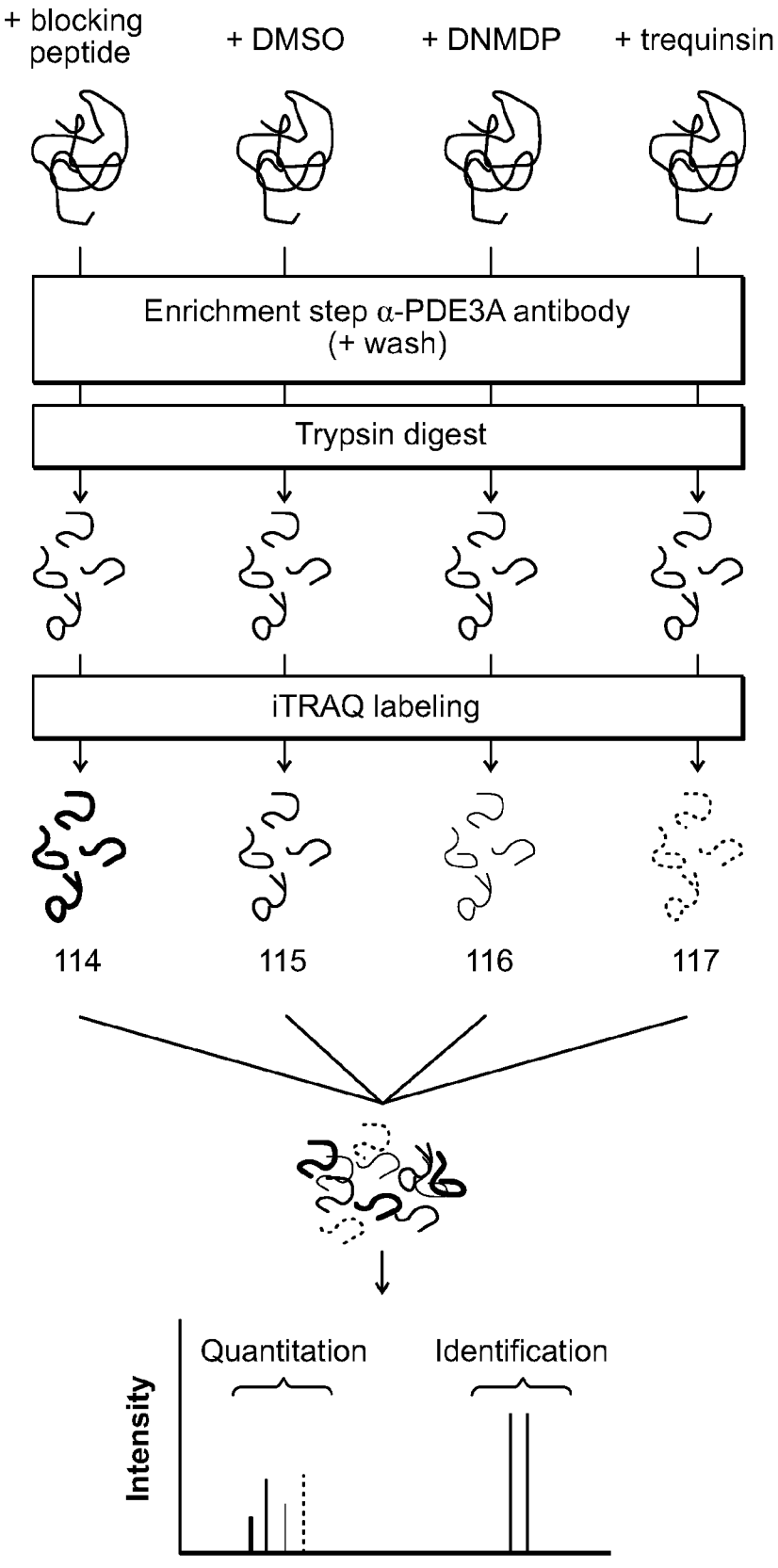

+ blocking peptide
+ DMSO
+ DNMDP
+ trequinsin
Enrichment step α-PDE3A antibody (+ wash)
Trypsin digest
iTRAQ labeling
114
115
116
117
Quantitation
Identification
Intensity

FIG. 13B

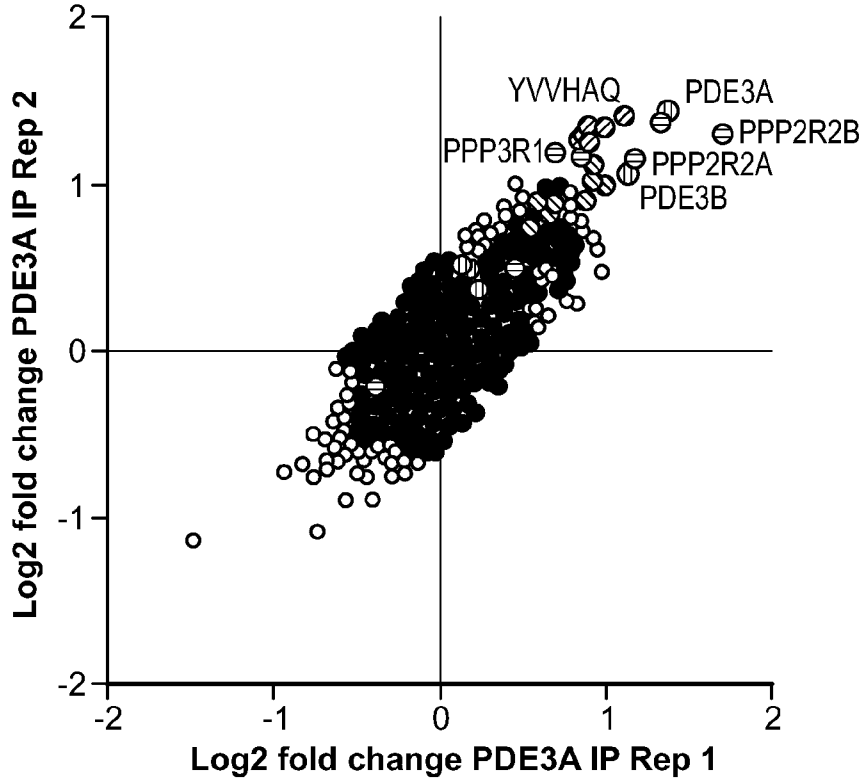


FIG. 13C

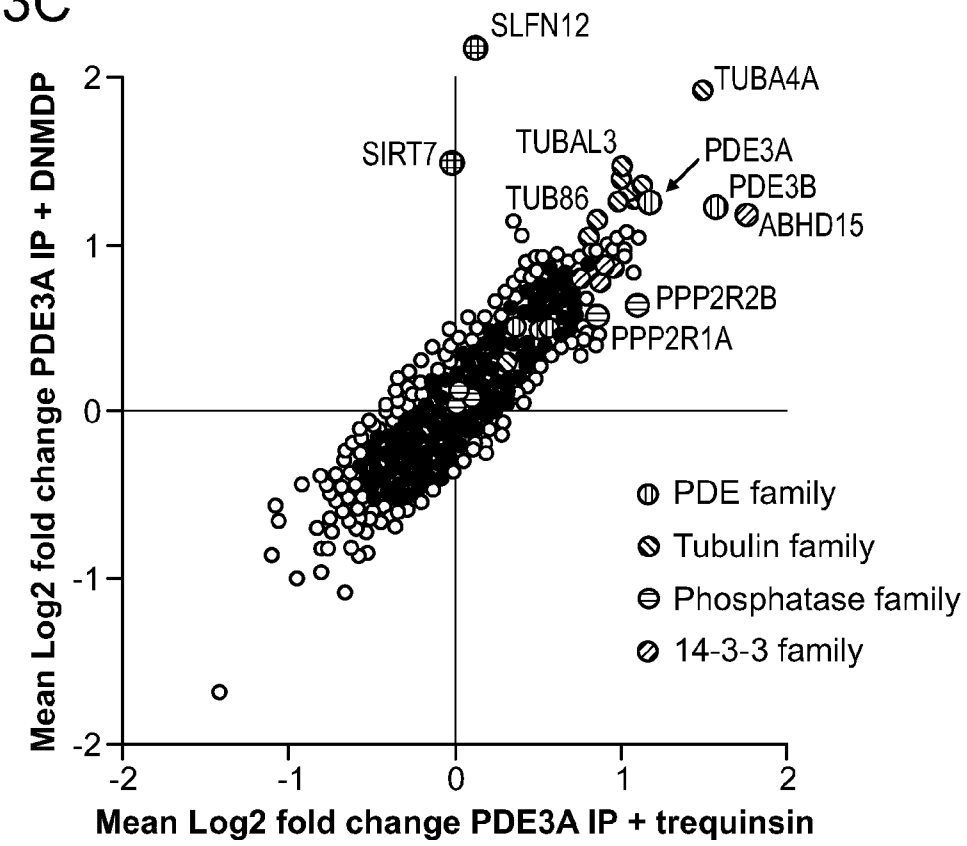


FIG. 14A

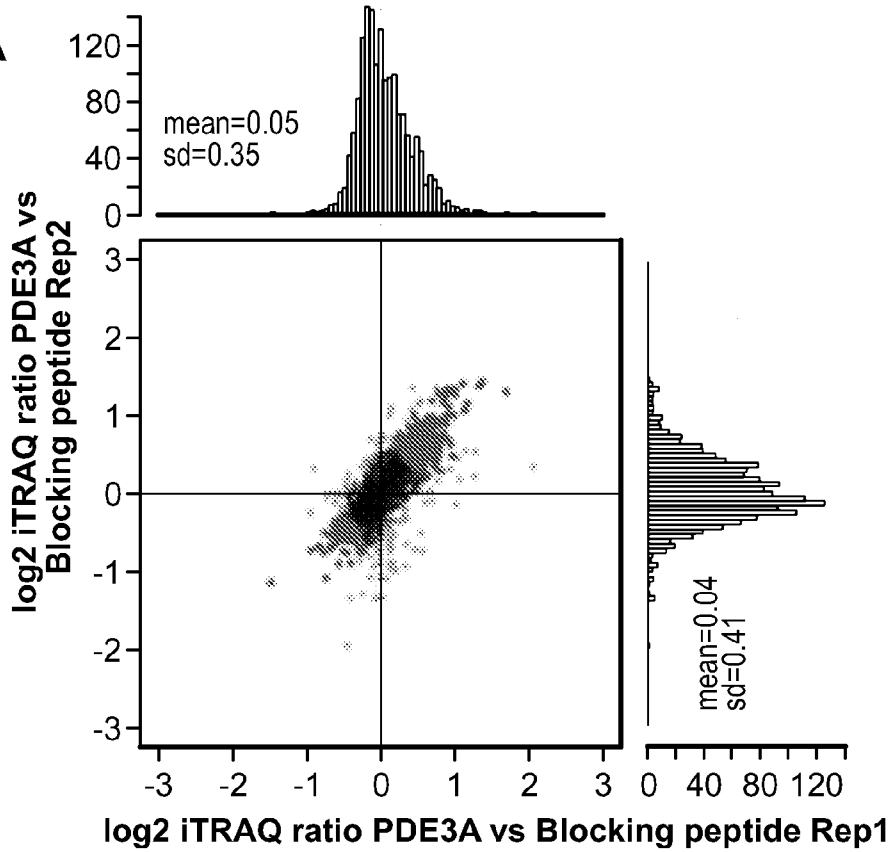


FIG. 14B

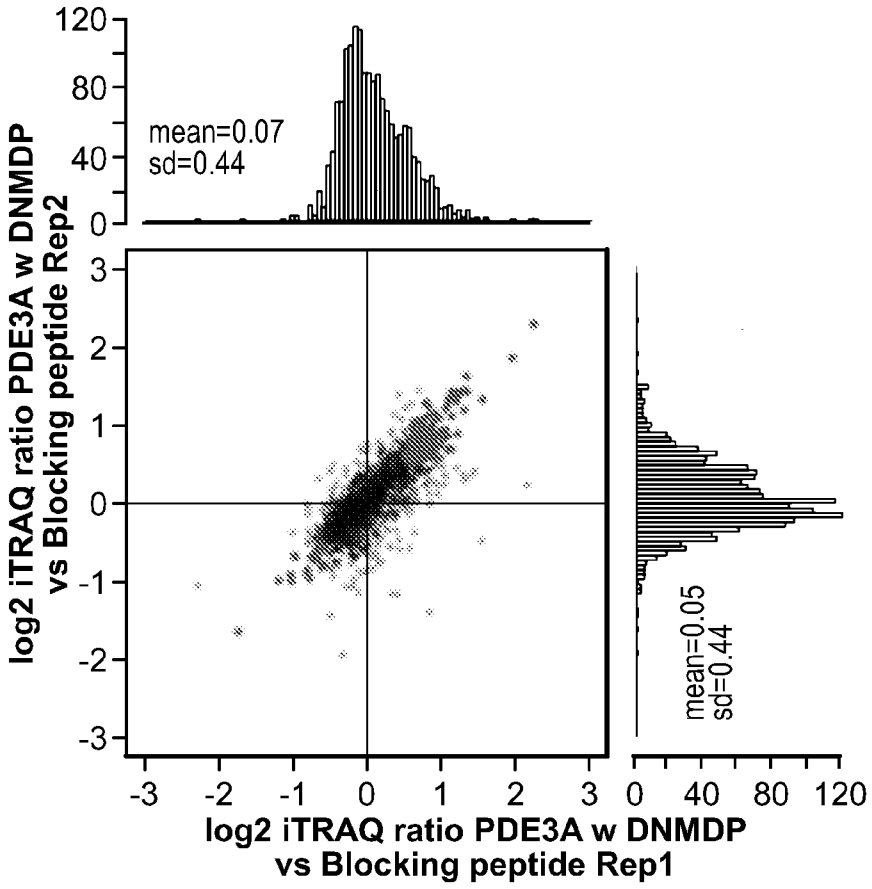


FIG. 14C

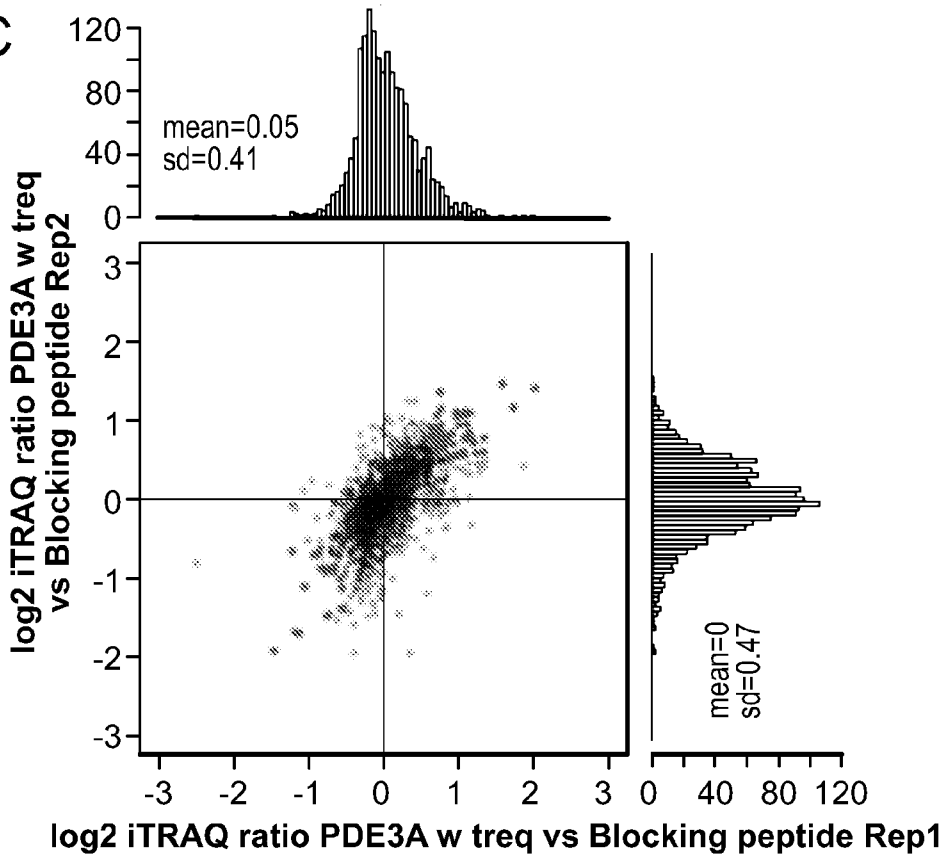


FIG. 15A

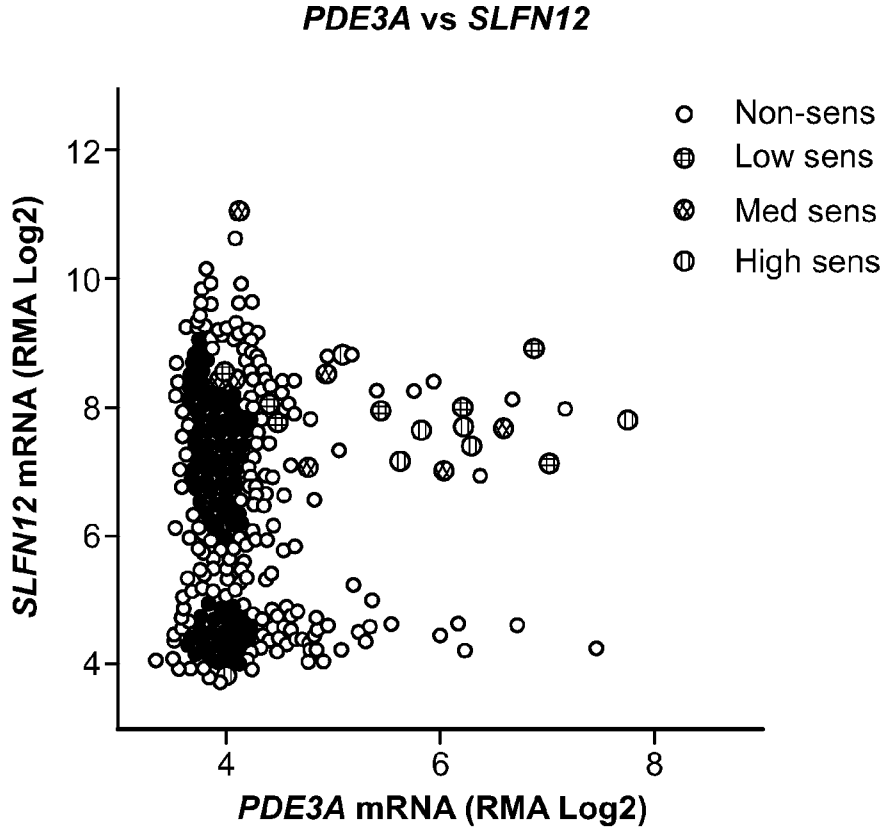


FIG. 15B

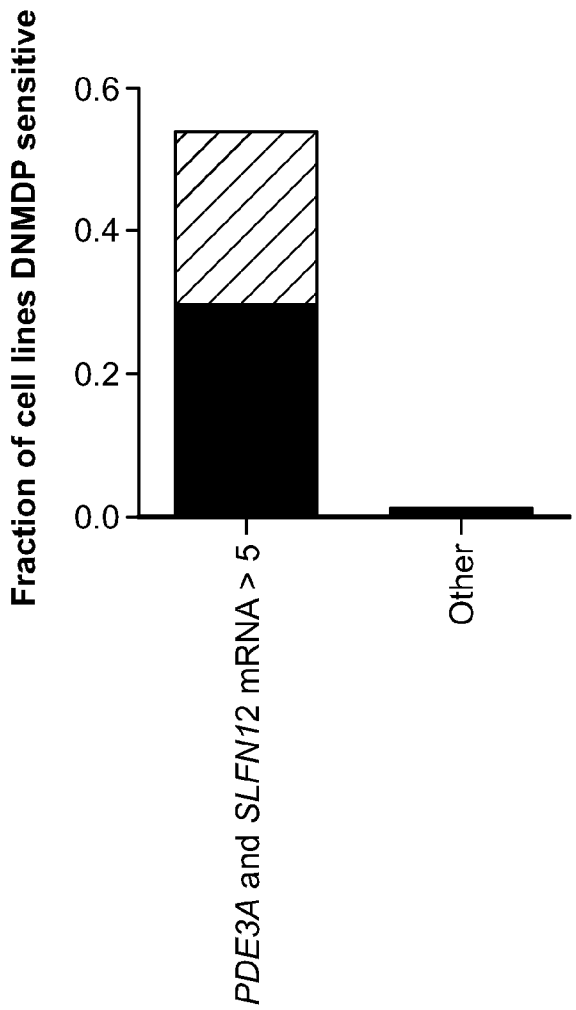


FIG. 15C

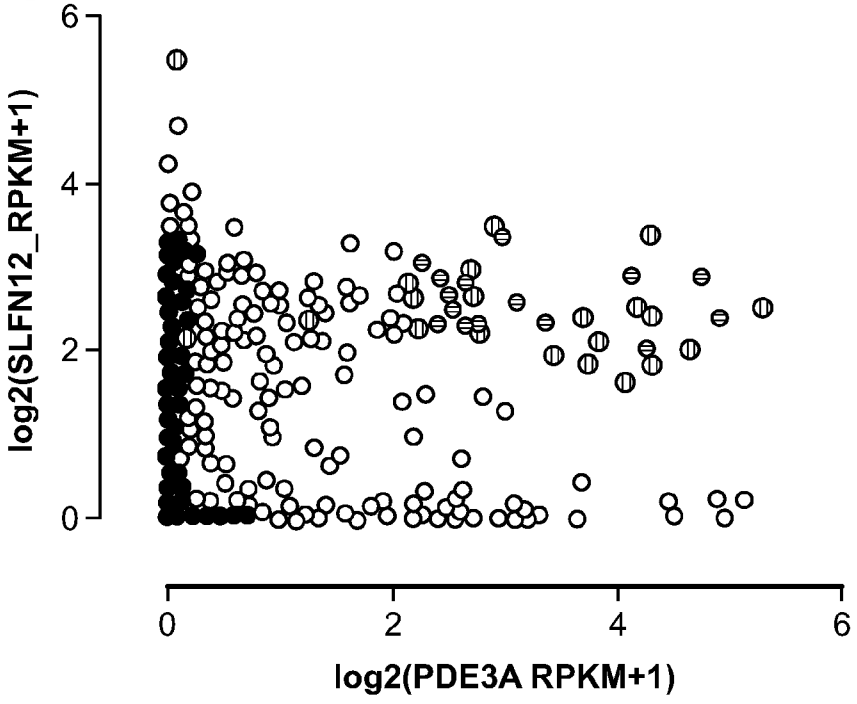


FIG. 15D

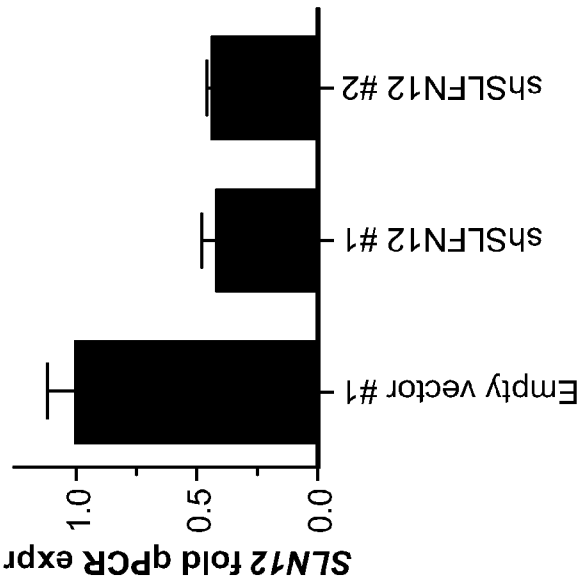


FIG. 15E

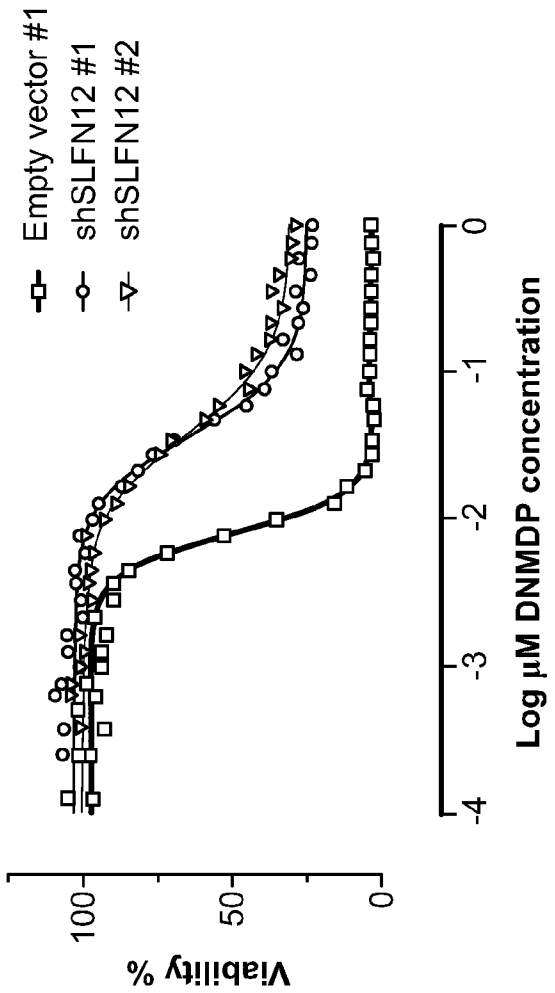


FIG. 16A

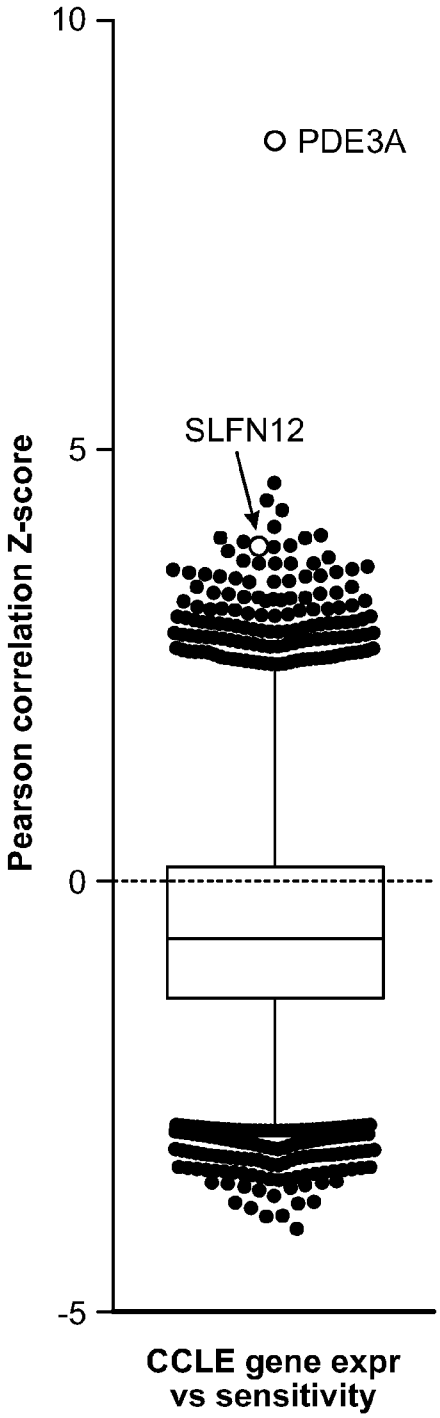


FIG. 16B

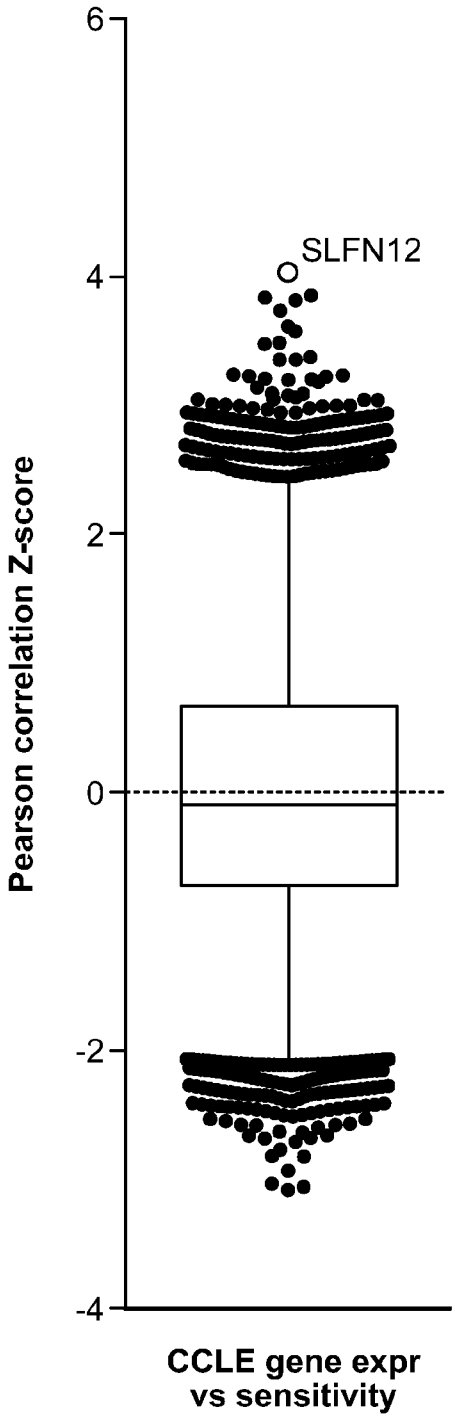


FIG. 17A

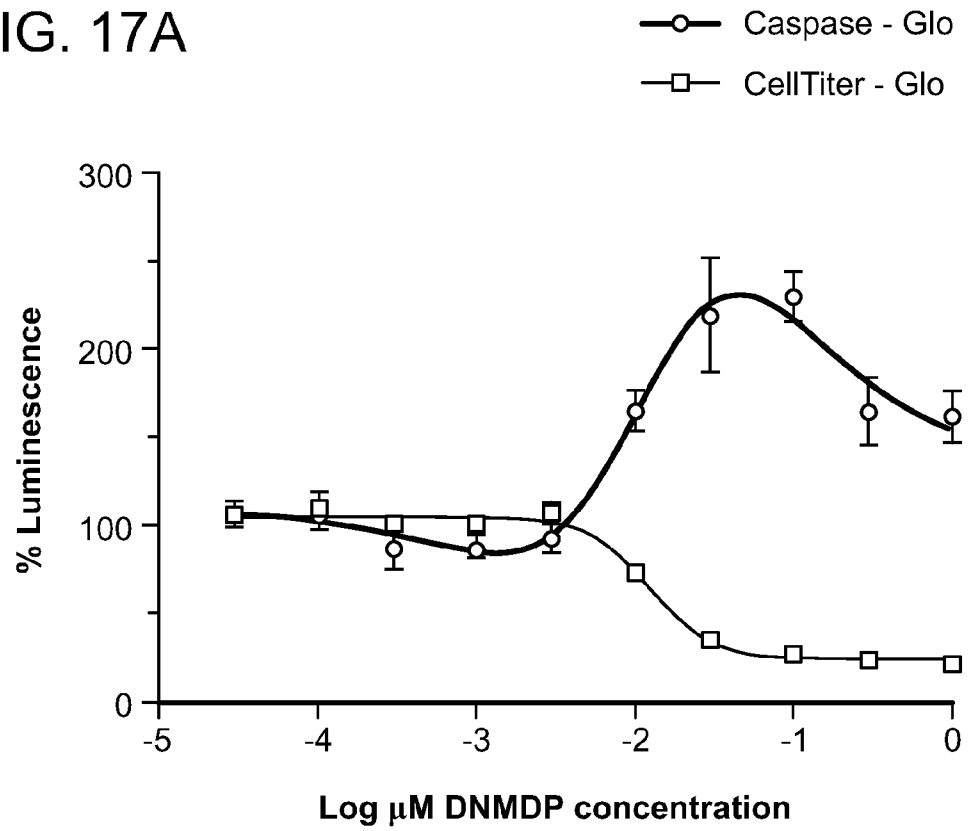
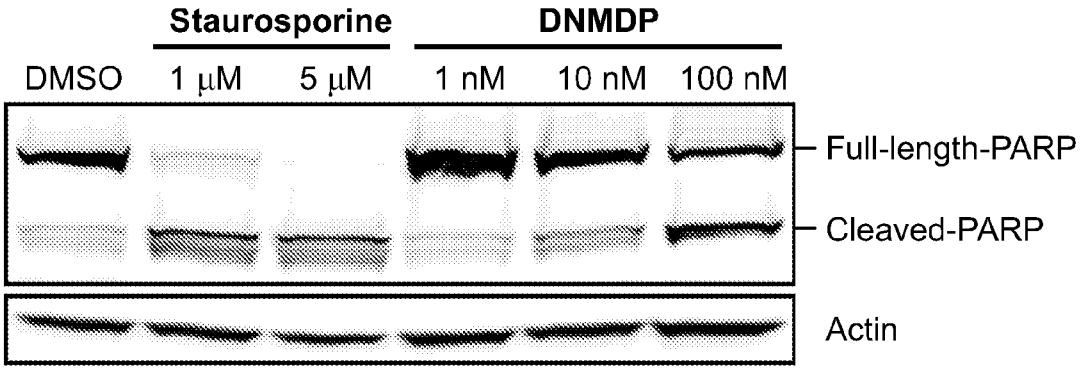


FIG. 17B



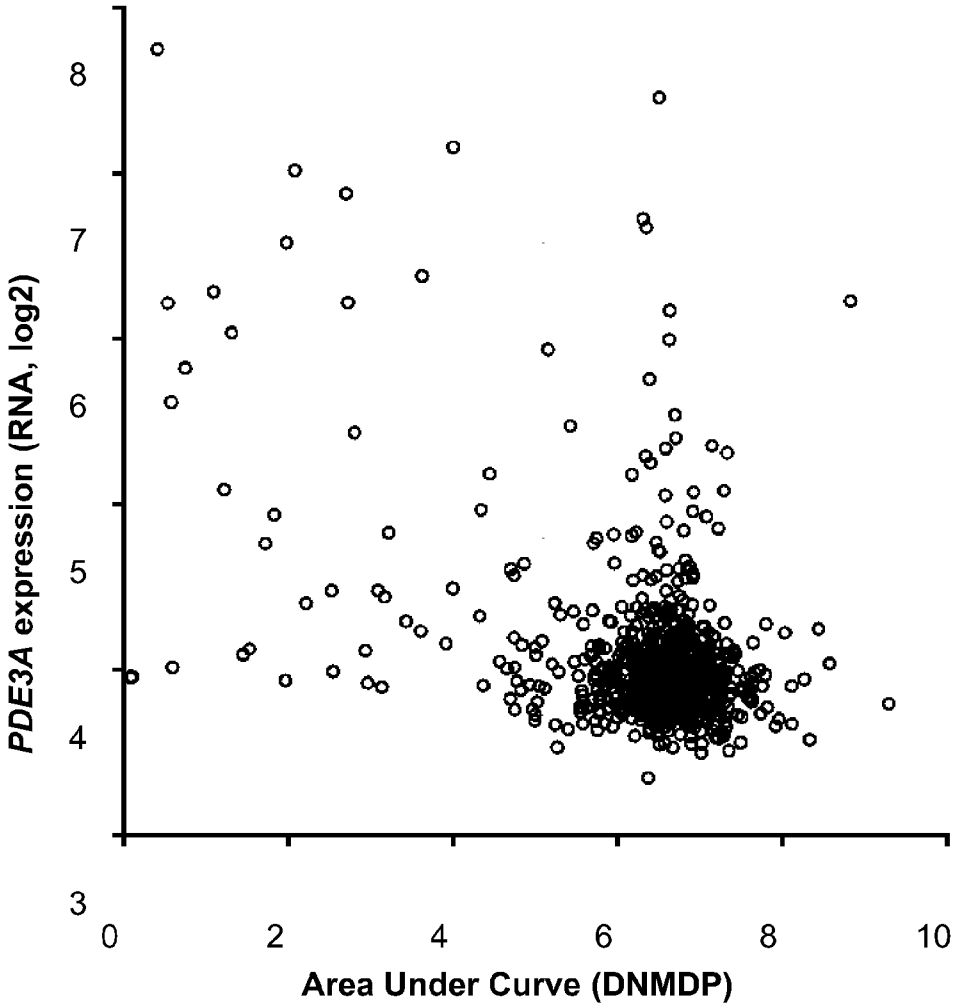

PDE3A expression (RNA, log2)
8
7
6
5
4
3
0
2
4
6
8
10
Area Under Curve (DNMDP)

FIG. 18

FIG. 19

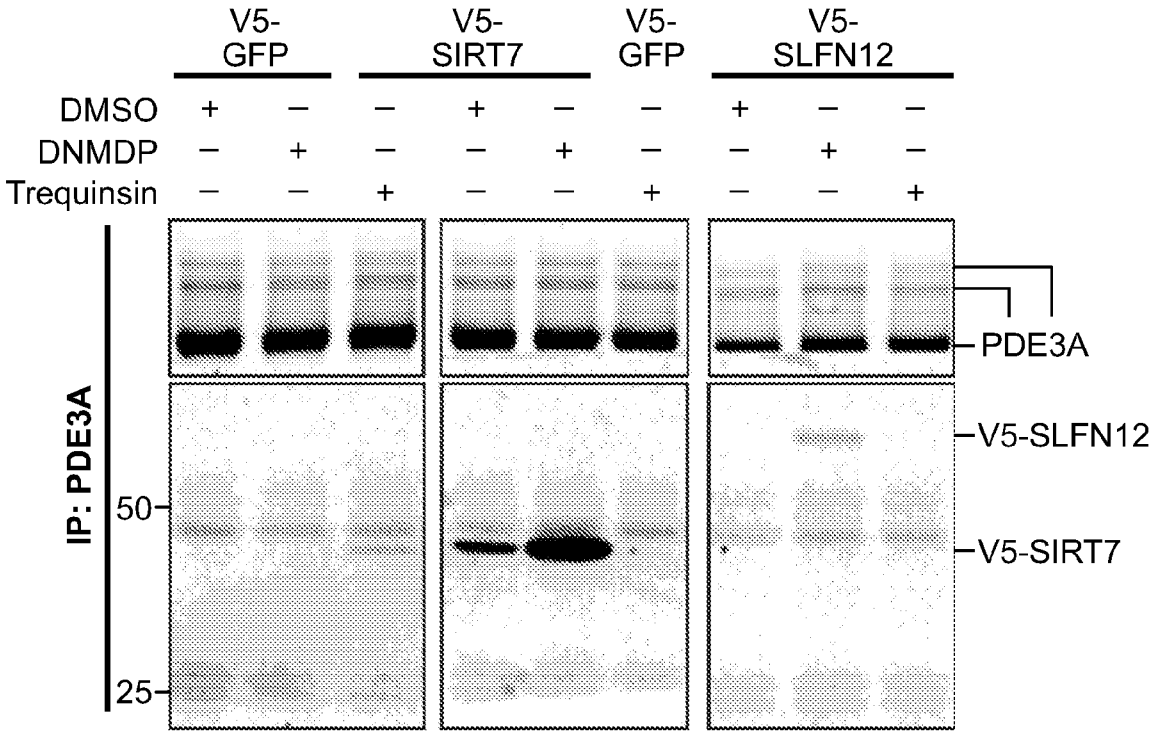

V5-GFP
V5-SIRT7
V5-GFP
V5-SLFN12
DMSO
DNMDP
Trequinsin
IP: PDE3A
50
25
PDE3A
V5-SLFN12
V5-SIRT7

FIG. 20

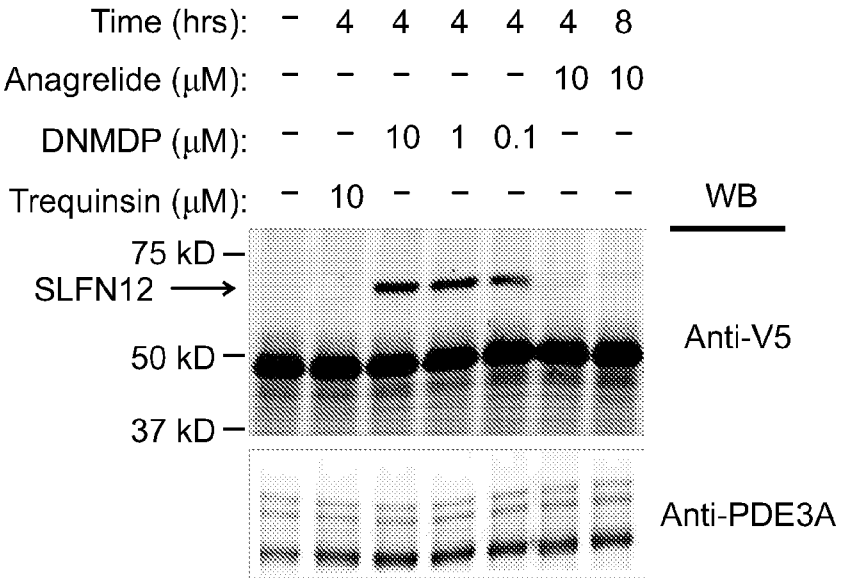

Time (hrs): – 4 4 4 4 4 8
Anagrelide (μM): – – – – – 10 10
DNMDP (μM): – – 10 1 0.1 – –
Trequinsin (μM): – 10 – – – – –
WB
75 kD
SLFN12
50 kD
37 kD
Anti-V5
Anti-PDE3A

FIG. 21

	HeLa parental line		HeLa resistant line	
Gene_Name	Hela_FPKM	Hela-P2_FPKM	Hela-Res1_FPKM	Hela-Res2_FPKM
SLFN12	12.51	12.63	0.15	0
PDE3A	56.24	62.15	53.46	54.91

	H2122 parental line		H2122 resistant line	
Gene_Name	H2122-P1_FPKM	H2122-P2_FPKM	H2122-Res1_FPKM	H2122-Res2_FPKM
SLFN12	4.69	5.15	0.33	0.46
PDE3A	25.52	25.24	15.82	15.13

FIG. 22

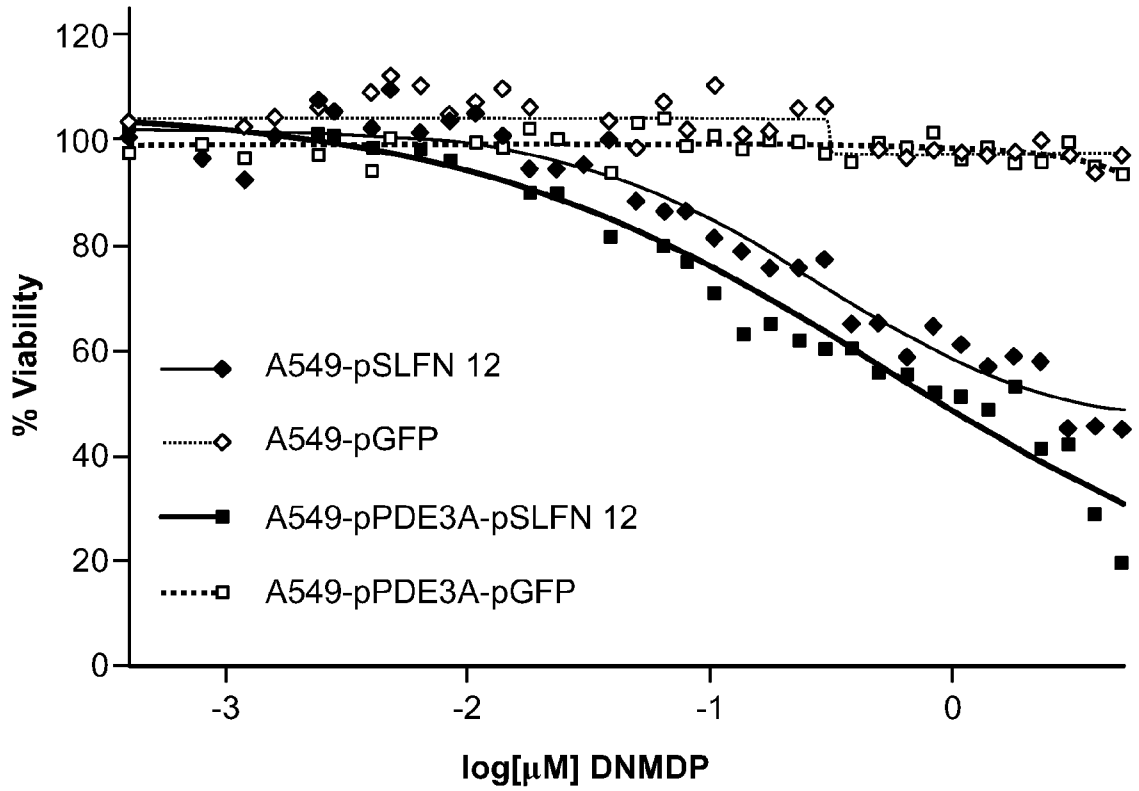
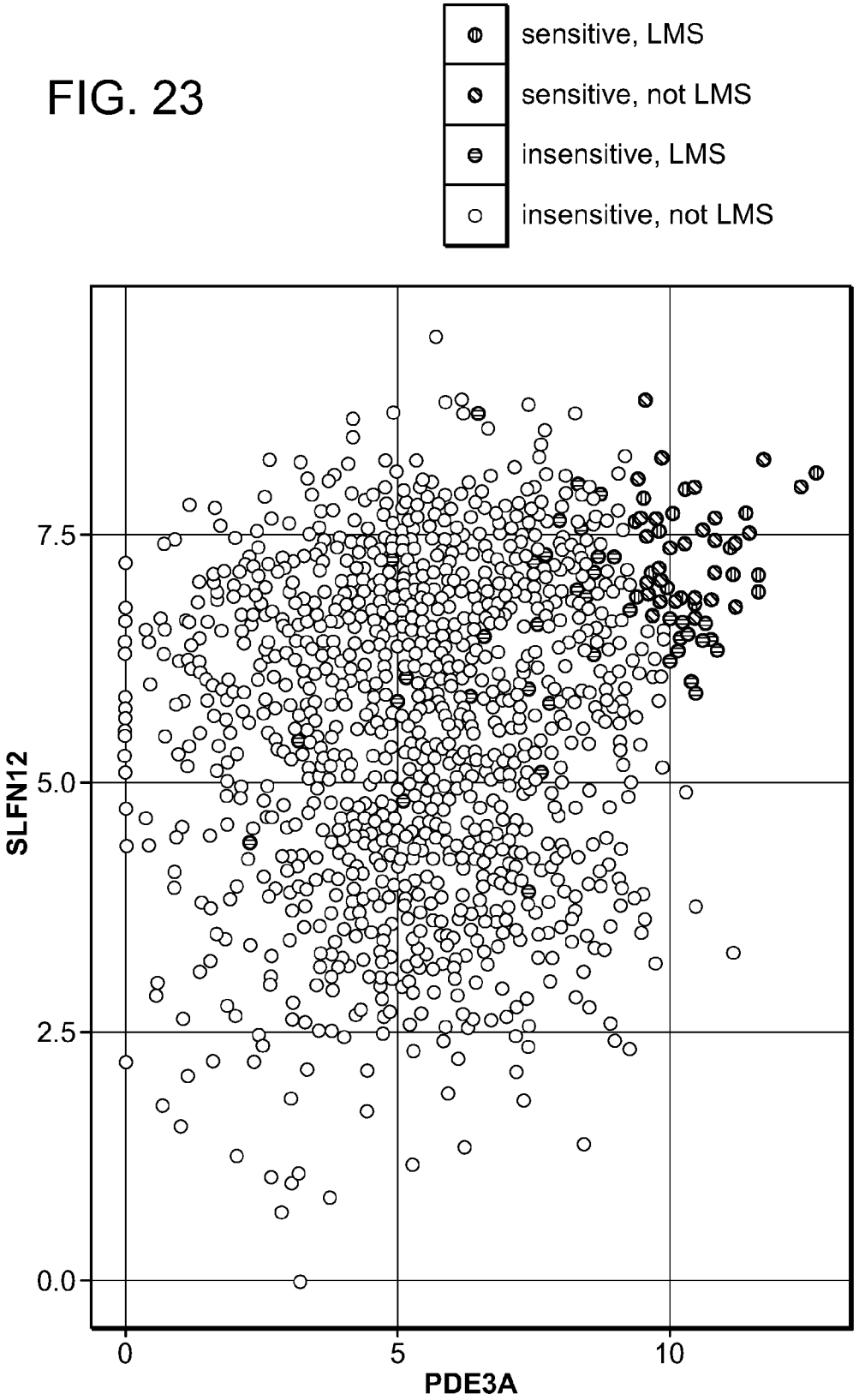


FIG. 23


sensitive, LMS
sensitive, not LMS
insensitive, LMS
insensitive, not LMS
SLFN12
7.5
5.0
2.5
0.0
0
5
10
PDE3A