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(54) Title: MOLECULAR INHIBITORS OF THE Wnt/ β -CATENIN PATHWAY

(57) Abstract: The present invention is directed toward a method of treating a subject for a condition mediated by aberrant Wnt/ β -catenin signaling by selecting a subject with a condition mediated by aberrant Wnt/ β -catenin signaling and administering to the selected subject a compound selected from the group consisting of those set forth in Table 1, Table 2, and a pharmaceutically acceptable salt thereof. A method of similarly modulating the Wnt/ β -catenin pathway in a subject is also discussed.



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MOLECULAR ACTIVATORS OF THE Wnt/ β -CATENIN PATHWAY

[0001] This application claims benefit of U.S. Provisional Patent Application
5 Serial No. 61/140,655, filed December 24, 2008, which is hereby incorporated by
reference in its entirety.

FIELD OF THE INVENTION

[0002] This invention relates to molecular activators of the Wnt/ β -catenin
10 pathway.

BACKGROUND OF THE INVENTION

[0003] Wnt/ β -catenin signaling regulates cell fate and proliferation during
development, homeostasis, and disease. The canonical Wnt pathway describes a
15 series of events that occur when Wnt proteins bind to cell-surface receptors of the
Frizzled family, causing the receptors to activate Dishevelled family proteins and
ultimately resulting in a change in the amount of β -catenin that reaches the nucleus.
Dishevelled (DSH) is a key component of a membrane-associated Wnt receptor
complex which, when activated by Wnt binding Frizzled, inhibits a second complex
20 of proteins that includes axin, GSK-3, and the protein APC. The axin/GSK-3/APC
complex normally promotes the proteolytic degradation of the β -catenin intracellular
signaling molecule. After this " β -catenin destruction complex" is inhibited, a pool of
cytoplasmic β -catenin stabilizes, and some β -catenin is able to enter the nucleus and
interact with TCF/LEF family transcription factors to promote specific gene
25 expression.

[0004] Numerous diseases have been linked to aberrant Wnt/ β -catenin
signaling and several conditions (Moon RT, "WNT and Beta-catenin Signaling:
Diseases and Therapies," *Nat Rev Gen* 5(9):691-701 (2004)). It is also clear that
activation of Wnt/ β -catenin signaling may be therapeutic for a variety of other

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indications including those involving a deficit in stem/progenitor cells. Lithium chloride is currently the only FDA approved small molecule modulator of Wnt/ β -catenin signaling. The narrow therapeutic range of lithium combined with the vast number of diseases linked to Wnt/ β -catenin signaling begs the discovery of additional small molecule modulators.

[0005] The present invention is directed at identifying small molecule modulators of Wnt/ β -catenin signaling.

SUMMARY OF THE INVENTION

10 [0006] One aspect of the present invention is directed toward a method of treating a subject for a condition mediated by aberrant Wnt/ β -catenin signaling by selecting a subject with a condition mediated by aberrant Wnt/ β -catenin signaling and administering to the selected subject a compound selected from the group consisting of those set forth in Table 1, Table 2, and a pharmaceutically acceptable salt thereof.

15 [0007] Another aspect of the present invention is directed toward a method of activating the Wnt/ β -catenin pathway in a subject including selecting a subject in need of Wnt/ β -catenin pathway activating and administering to the selected subject a compound selected from the group consisting of those set forth in Table 1, Table 2, and a pharmaceutically acceptable salt thereof.

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BRIEF DESCRIPTION OF THE DRAWINGS

[0008] Figures 1A-G illustrate that nuclear β -catenin predicts improved survival in melanoma patients and correlates with decreased tumor proliferation. Figure 1A is a graph showing that patients with the highest levels of nuclear β -catenin (upper tertile) exhibit an increased survival probability by Kaplan-Meier analysis compared to patients in the middle and lower tertile. This trend was statistically significant by log-rank test. Figure 1B is a graph showing metastases separated into those with the highest nuclear β -catenin levels (upper 20%, n=46) and those with lower nuclear β -catenin levels (remaining 80%, n=179). Kaplan-Meier analysis showed a significantly increased survival probability in patients with the highest amount of nuclear β -catenin (Gehan-Breslow-Wilcoxon test). Figure 1C is a graph

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showing the subset of patients with available data on tumor depth (Breslow thickness) analyzed by Kaplan-Meier survival curves. Tumors were grouped based on the AJCC tumor staging guidelines for tumor depth into T1 (0-1.00 mm, n=35), T2 (1.01-2.00 mm, n=26), T3 (2.01-4.00 mm, n=32) or T4 (>4.00 mm, n=20). The survival curves exhibited an extremely significant trend by log-rank test. Figure 1D and Figure 1E are graphs showing tumors grouped by tumor staging depth evaluated for proliferation (Figure 1D) and for expression of nuclear β -catenin (Figure 1E). Bars show the mean and standard deviation for each group, while gray dots represent individual tumors. The horizontal dotted lines represent the mean Ki-67 and nuclear β -catenin seen for all tumors in the array. As expected, increasing tumor depth is associated with increased proliferation. By contrast, levels of nuclear β -catenin decrease with increasing tumor depth, suggesting that activation of Wnt/ β -catenin signaling is lost with melanoma progression. The trend for both %Ki-67 and nuclear β -catenin was extremely significant by ANOVA (* $p < 0.002$). Figure 1F is a histogram showing primary tumors stratified into tertiles based on levels of nuclear β -catenin (see Figure 5), and the distribution of proliferation as measured by %Ki-67 was assessed in each tertile. Patients with the highest levels of nuclear β -catenin (upper tertile, n=39) showed a lower mean %Ki-67 than patients in the middle tertile (n=39) or the lower tertile (n=40). This trend was extremely significant by ANOVA (* $p < 0.0001$). The histogram illustrates that tumors with the lowest levels of nuclear β -catenin (lower tertile) show a clear shift towards higher proliferation compared to patients with the highest levels of nuclear β -catenin (upper tertile). Figure 1G is a graph showing normalized levels of nuclear β -catenin in primary tumors plotted against proliferation as measured by %Ki-67, and a Deming regression analysis (diagonal line) reveals an extremely significant inverse correlation between levels of nuclear β -catenin and proliferation as measured by Ki-67 (slope = -1.089 ± 0.24).

[0009] Figures 2A-G illustrate activation of Wnt/ β -catenin signaling changes melanoma cell fate. Figure 2A is a photograph showing B16 cells expressing GFP, WNT3A or WNT5A isolated at equivalent confluency, spun down and photographed in a 96-well plate, demonstrating the marked difference in pigmentation seen in melanoma cells expressing WNT3A. Figure 2B shows expression of WNT5A was

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confirmed by immunoblotting of cell lysates. Figure 2C shows immunofluorescent staining demonstrating increased nuclear β -catenin in B16 cells expressing WNT3A, consistent with activation of the Wnt/ β -catenin pathway. Figure 2D is a histogram showing conditioned media from B16:GFP, B16:WNT3A and B16:WNT5A cells incubated with a human melanoma cell line stably transduced to express firefly luciferase under the control of a TCF-based Wnt/ β -catenin-responsive promoter. Media from B16:WNT3A cells activate the reporter, indicating that these cells secrete active WNT3A. Figure 2E is a histogram showing expression of the Wnt/ β -catenin target gene *Axin2* measured by quantitative real-time PCR and normalized to *Gapdh*. Upregulation of *Axin2* is seen in WNT3A cells, indicating activation of the Wnt/ β -catenin pathway. Figure 2F is a histogram showing proliferation of cells expressing GFP, WNT3A or WNT5A was measured by hemacytometer after six days of culture (shaded bars, left y-axis) or by MTT assay after three days of culture (unshaded bars, right y-axis). Bars represent the average and standard deviation of three to six biological replicates. The inhibition of proliferation seen with WNT3A cells is extremely significant by ANOVA with both proliferation assays (* $p < 0.001$). Figure 2G is a histogram showing cell cycle analysis where cells expressing WNT3A demonstrated a decreased population in S phase and an increased population in G1 compared to cells expressing GFP or WNT5A. Bars indicate the average and standard deviation of three biologic replicates, and the data shown are representative of five individual experiments, each with at least three biologic replicates per condition. The changes observed in %G1 and %S with the WNT3A cells is extremely significant by ANOVA (* $p < 0.001$).

[0010] Figures 3A-E illustrate that elevation of melanocyte differentiation markers by WNT3A corresponds with decreased tumor growth and metastasis *in vivo*. Figure 3A is a heatmap of whole genome expression profiles of WNT3A or WNT5A cell lines compared to gene expression in GFP cells, which served as the reference sample. Three biologic replicates were analyzed for each cell line. The heatmap illustrates the differences between the most significant regulated genes in WNT3A cells compared to WNT5A cells by unpaired t-test. Genes that were among the most significantly regulated in WNT3A cells are listed with normalized fold-change ($\log_2$) compared to GFP cells shown in parentheses. The most significantly regulated genes

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include known Wnt/ β -catenin targets, genes involved in melanocyte and neural crest differentiation, and genes implicated in melanoma prognosis or therapeutics. Figure 3B is a histogram showing several genes selected for validation using real-time quantitative PCR (qPCR), including genes implicated in melanocyte differentiation (*Met*, *Kit*, *Sox9*, *Mitf*, *Si/Gp100*), melanoma biology (*Trpm1*, *Kit*, *Mme*, *Mlze*), and genes that are known Wnt target genes (*Axin2*, *Met*, *Sox9*). Genes that were upregulated in WNT3A cells by transcriptional profiling are all upregulated by qPCR, while genes that are downregulated in WNT3A cells on the array (*Mlze*, *Mme*) are also downregulated by qPCR. Genes upregulated in WNT3A cells are universally downregulated in the WNT5A cells, providing evidence that WNT5A can antagonize transcription of Wnt/ β -catenin gene targets in melanoma cells, even in the absence of WNT3A. Data are expressed as log2-transformed fold-change compared to B16:GFP cells, and are representative of three or more experiments with similar results. Figure 3C is a histogram showing gene changes induced by WNT3A inhibited upon treatment with β -catenin siRNA (20nM) compared to control siRNA (20nM). Data are expressed as log2-transformed fold-change in cells treated with β -catenin siRNA compared to control siRNA. Figure 3D is a graph showing tumor explants demonstrating that B16 cells expressing *WNT3A* form smaller tumors than cells expressing GFP or WNT5A. Data are expressed as the mean and standard deviation from four mice for each tested cell line. The experiment shown is representative of four independent experiments with the same result, all involving at least four mice for each cell line tested. The decrease in tumor size with WNT3A was highly significant by ANOVA at 14 days post-implantation (* $p=0.004$). Figure 3E is a plot showing metastases to the popliteal sentinel lymph node bed evaluated by Firefly luciferase assay, demonstrating significantly decreased metastases in tumors expressing WNT3A.

[0011] Figures 4 A-D illustrate figures related to tumor microarray analysis. Figure 4A is a histogram depicting the distribution of nuclear β -catenin staining in the cohort of primary tumors. The bar below shows the cut-offs for the three tertiles used for analysis of survival in Figure 1. Figure 4B is a histogram depicting survival analysis in metastases. The upper 20% was selected based on both the population distribution and the absolute levels of nuclear β -catenin, which correspond roughly

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with the upper tertile of the population. Figure 4C is a plot showing levels of nuclear β -catenin compared in primary tumors and metastases/recurrences, showing a decrease in nuclear β -catenin in metastases/recurrences that approximated statistical significance using an unpaired two-tailed t-test. This data supports the hypothesis that Wnt/ β -catenin signaling is lost with melanoma progression. Figure 4D is a plot comparing %Ki-67 with another marker of proliferation, %PCNA. Deming regression analysis gave an extremely significant correlation, with a slope of 1.04 suggesting that proliferation was robustly measured by %Ki-67.

[0012] Figures 5A-D illustrate Wnt expression in the context of human melanoma. Figure 5A is a table showing data from the NCBI Gene Expression Omnibus used to evaluate the expression of Wnt isoforms in benign nevi and melanoma tumors (see also Barrett et al., *Nucleic Acids Res.* D760-5 (2007), which is hereby incorporated by reference in its entirety). The datasets used include GDS1375 (Talantov et al., *Clin. Cancer Res.* 11(20):7234-42 (2005), which is hereby incorporated by reference in its entirety) and GDS1989 (Smith et al., *Cancer Biol. Ther.* 4(9):1018-29 (2005), which is hereby incorporated by reference in its entirety). The primary expression data is shown, and the above table summarizes the data from these two datasets. The data summarization is based on the reported 'detection call' of the Affymetrix data used for all three datasets, and the scale indicates the percentage of samples with 'present' calls on the expression of the different Wnt isoforms. In the primary data presented above, 'absent' calls are faded out. Scoring was as follows: 0 calls were 'absent' in all samples; + represents up to 25% of specimens have expression; ++ represents 25- 50% of specimens have expression; +++ represents 50-75% of specimens have expression; ++++ represents 75-100% of specimens have expression. Few Wnt isoforms are expressed by melanoma tumors based on this transcriptional profiling, and only *wnt3*, *wnt4*, *wnt5a* and *wnt6* were detected in melanomas from both gene datasets. Figures 5B and Figure 5C are histograms showing the human melanoma cell lines Mel375 (Figure 5B) and UACC 1273 (Figure 5C) were transduced with lentiviral constructs for encoding either GFP or WNT3A. Cells were counted after 3-7 days by hemacytometer and the panels above are representative of multiple experiments with similar results. The bars represent the average and standard deviation from three biologic replicates. P-values

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for two-tailed t-tests were statistically significant (* $p < 0.05$). Expression of WNT3A also led to a consistent and reproducible decrease in proliferation by MTT assay. No consistent effect on proliferation was seen with expression of WNT5A, again similar to the B 16 cell lines. Figure 5D is a histogram showing human melanoma cell lines cultured for 3-7 days in the presence of either 10mM sodium chloride or 10mM lithium chloride. Proliferation was measured by hemacytometer or MTT assay, and normalized to growth observed in the samples cultured in 10mM sodium chloride. Lithium chloride inhibited proliferation in all human melanoma cell lines tested.

[0013] Figures 6A-F illustrate inhibitors of GSK3 activate Wnt/ β -catenin signaling and inhibit proliferation of B16 melanoma cells. Figure 6A and Figure 6B are photographs showing immunofluorescent staining of β -catenin demonstrates increased nuclear β -catenin in B16 cells treated with 10mM lithium chloride or 1 μ M BIO compared to control cells treated with 10mM sodium chloride or DMSO, respectively, consistent with activation of the Wnt/ β -catenin pathway by lithium and BIO. Figure 6C and Figure 6D are histograms showing quantitative PCR demonstrates increased *Axin2* levels in B 16 cells treated with 10mM lithium chloride or 1 μ M BIO compared to control cells, also consistent with activation of the Wnt/ β -catenin pathway by both drugs. Figure 6E and Figure 6F are histograms showing representative MTT proliferation assays and demonstrate the decreased proliferation seen in B16 cells treated with 10mM lithium chloride or 1 μ M BIO compared to control cells. Bars represent the mean and standard deviation of three to six biologic replicates. The difference is extremely significant by unpaired two-tailed t-test ($p < 0.001$).

[0014] Figures 7A-C illustrate microarray analysis of B16 cells expressing WNT3A and WNT5A. Figure 7A and Figure 7B are Venn diagrams which compare the genes upregulated and downregulated in B16 cells expressing WNT3A or WNT5A compared to control B16 cells expressing GFP, which served as the reference for Agilent whole mouse genome two-channel arrays. Very few genes were regulated by WNT5A compared to WNT3A, consistent with previous results in human melanoma cells. Figure 7C shows B16 melanoma cells transfected for 72 hours with either control siRNA or siRNA targeting murine β -catenin were analyzed by immunoblotting to assess knockdown of β -catenin protein. The siRNA sequences

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(SEQ ID NOs: 1-3) tested are on the right. It was found that siRNA #2 and #3 produced marked knockdown of β -catenin protein and for the validation of microarray target genes presented in Figure 3. Cells were transfected with a pool consisting of 10nM of siRNA #2 and #3 to minimize off-target effects of each individual siRNA.

5 **[0015]** Figure 8 illustrates a model for differentiation therapy using Wnt/ β -catenin activators in melanoma. This is a schematic diagram depicting a model of melanoma arising through transformation of differentiated melanocytes and nevus (mole) cells or from melanocytic progenitor cells, taking into account that clinical melanomas arise both from established melanocytic lesions and also *de novo* (Barnhill
10 et al., *Pathology of Melanocytic Nevi and Malignant Melanoma* (2004), which is hereby incorporated by reference in its entirety). Based readouts of differentiation such as gene expression profiles, previous studies have found that melanoma progression appears to correlate with the loss of expression of melanocytic markers. Additionally, this model also incorporates the concept of cancer stem cells (or tumor
15 initiating cells) in melanoma (Hendrix et al., *Nat. Rev. Cancer* 7:246 (2007), which is hereby incorporated by reference in its entirety), which give rise to highly proliferative bulk tumor cells, and are themselves highly resistant to conventional chemotherapy in the context of melanoma and other cancer stem cell models. Based on the finding that WNT3A is one of only three factors needed to generate functional
20 melanocytes from embryonic stem cells (Fang et al., *Stem Cells* 24:1668 (2006), which is hereby incorporated by reference in its entirety), as well as the well-described requirement for Wnt/ β -catenin signaling in melanocyte development from animal models (Dorsky et al., *Nature* 396:370 (1998), which is hereby incorporated by reference in its entirety), the leveraging of this pathway to force cell fate changes
25 in melanoma offers an attractive choice for therapeutic manipulation. The findings herein, as well as other supporting published results (Bachmann et al., *Clin. Cancer Res.* 11:8606 (2005); Kageshita et al., *Br. J. Dermatol.* 145:210 (2001), which are hereby incorporated by reference in their entirety) documenting the loss of β -catenin with melanoma progression and decreased survival are depicted below the model.
30 The present data suggests that using activators of Wnt/ β -catenin signaling in melanoma can force differentiation in both bulk tumor cells and cancer stem cells to promote cell fates associated with less aggressive tumors through the reactivation of

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melanocyte-associated transcriptional programs that are downregulated or lost during normal melanoma progression. The goal of differentiation therapy using Wnt/ β -catenin activators would be to elicit changes in tumor cell properties through reprogramming of cell, generating tumors that are less aggressive, less proliferative, or potentially more susceptible to currently available melanoma therapies. The availability of several previously FDA-approved activators of Wnt/ β -catenin signaling, including riluzole, can facilitate the rapid testing of this therapeutic approach in clinical trials.

10 **DETAILED DESCRIPTION OF THE INVENTION**

[0016] One aspect of the present invention is directed toward a method of treating a subject for a condition mediated by aberrant Wnt/ β -catenin signaling by selecting a subject with a condition mediated by aberrant Wnt/ β -catenin signaling and administering to the selected subject a compound selected from the group consisting of those set forth in Table 1, Table 2, and a pharmaceutically acceptable salt thereof.

15 [0017] In a preferred embodiment of this and other aspects described herein, the subject is human.

[0018] The condition which can be treated in accordance with this aspect of the present invention can be any one of the following: cancer (malignant melanoma, colorectal cancer, renal, liver, lung, breast, prostate, ovarian, parathyroid, leukemias, etc), bone mass diseases, fracture repair, FEVR, diabetes mellitus, cord blood transplants, psychiatric disease (e.g., bipolar depression), neurodegenerative disease (Alzheimer's, ALS), hair loss, diseases linked to loss of stem/progenitor cells, conditions improved by increasing stem/progenitor cell populations, HIV, and tooth

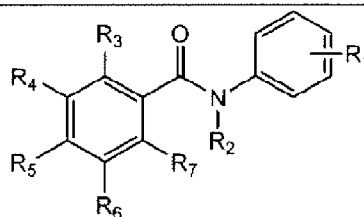
20 agensis.

[0019] Another aspect of the present invention is directed toward a method of activating the Wnt/ β -catenin pathway in a subject including selecting a subject in need of a Wnt/ β -catenin pathway activating and administering to the selected subject a compound selected from the group consisting of those set forth in Table 1, Table 2, and a pharmaceutically acceptable salt thereof.

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Table 1 - Activators/Enhancers

Family I	wherein: ----- represents an optional double bond; and R₁-R₆ are independently H, halogen, -OH, -OR₇, -C(O)R₇, -OC(O)R₇, -OC(O)NHR₇, -NH₂, -NHR₇, -NR₇R₈, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, monocyclic aryl, arylalkyl, or a mono or bicyclic heterocycle or heteroaryl containing 1 to 5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur, each of R₁-R₆ optionally substituted from 1 to 5 times with substituents selected from the group consisting of H, halogen, -OH, -OR₉, -NH₂, -NHR₉, -NR₉R₁₀, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, monocyclic aryl, and arylalkyl, wherein R₇-R₁₀ are defined as below; R₇-R₁₀ are independently H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, monocyclic aryl, arylalkyl, or a 3- to 6- membered heterocycle containing 1 to 5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur, each of R₇ and R₈ optionally substituted from 1 to 5 times with substituents selected from the group consisting of H, halogen, -OH, -NH₂, R₁₁OC(O)-, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, and C₃-C₆ cycloalkyl, each optionally substituted from 1 to 5 times with selected from the group consisting of H, halogen, -OH, and -NH₂, wherein R₁₁ is defined as below; R₁₁ is independently H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, or monocyclic aryl.
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Family II

wherein:

R_1 - R_7 are independently H, halogen, -OH, -OR₈, -NH₂, -NHR₈, -NR₈R₉, -CHO, -C(O)OH, -C(O)OR₈, -C(O)NHR₈, -C(O)NR₈R₉, -N(H)C(O)OR₈, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, monocyclic aryl, arylalkyl, or a mono or bicyclic heterocycle containing 1 to 5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur, each one of R_1 - R_7 optionally substituted from 1 to 5 times with substituents selected from the group consisting of H, halogen, -OH, -OR₁₀, -NH₂, -NHR₁₀, -NR₁₀R₁₁, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, monocyclic aryl, and arylalkyl, wherein R_8 - R_{10} are defined as below;

or R_4 and R_5 or R_5 and R_6 can combine to form a 3- to 6-membered carbocycle or heterocycle containing 1 to 5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur, wherein the carbocycle or heterocycle is optionally substituted 1 to 5 times with substituents selected from the group consisting of H, halogen, cyano, -OH, -NH₂, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, monocyclic aryl, and arylalkyl; and

R_8 - R_{10} are independently H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, monocyclic aryl, arylalkyl, or a mono or bicyclic heterocycle or heteroaryl containing 1 to 5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur, each one of R_8 , R_{10} optionally substituted from 1 to 5 times with substituents selected from the group consisting of H, halogen, cyano, -OH, -NH₂, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, monocyclic aryl, arylalkyl, and a mono or bicyclic heterocycle or heteroaryl containing 1 to 5 heteroatoms selected from the group consisting of

	oxygen, nitrogen, and sulfur.
Family III	wherein: carbon atoms designated * are in the R or S configuration; and R_1-R_9 are independently H, halogen, -OH, -OR₁₀, -NH₂, -NHR₁₀, -NR₁₀R₁₁, -CHO, -C(O)OH, -C(O)OR₁₀, -C(O)NHR₁₀, -C(O)NR₁₀R₁₁, -N(H)C(O)OR₁₀, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, monocyclic aryl, arylalkyl, or a mono or bicyclic heterocycle containing 1 to 5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur, each one of R_1-R_9 optionally substituted from 1 to 5 times with substituents selected from the group consisting of H, halogen, -OH, -C(O)OH, -CR₁₀R₁₁R₁₂, -OR₁₂, -NH₂, -NHR₁₂, -NR₁₂R₁₃, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, monocyclic aryl, adamantyl, and arylalkyl, wherein R_{10}-R_{13} are defined as below; R_{10}-R_{13} are independently H, halogen, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, monocyclic aryl, arylalkyl, or a mono or bicyclic heterocycle or heteroaryl containing 1 to 5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur, each one of R_{10}-R_{13} optionally substituted from 1 to 5 times with substituents selected from the group consisting of H, halogen, cyano, -OH, -NH₂, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₁-C₆ alkoxy, monocyclic aryl, arylalkyl, and a mono or bicyclic heterocycle or heteroaryl containing 1 to 5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur, wherein each one of R_{10}-R_{13} substituents is optionally substituted from 1 to 5 times with

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	substituents selected from the group H, -OH, -NH₂, -OCF₃, -NHR₁₄, -NR₁₄R₁₅, cyano, and nitro, wherein R₁₄ and R₁₅ are defined as below; R₁₄ and R₁₅ are independently H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, monocyclic aryl, arylalkyl, or a mono or bicyclic heterocycle or heteroaryl containing 1 to 5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur; or R₁₄ and R₁₅ can combine to form a 5- to 6-membered heterocycle or heteroaryl containing 1 to 5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur, each optionally substituted from 1 to 5 times with substituents selected from the group consisting of H, -OH, -NH₂, cyano, and nitro.
Family IV	wherein: carbon atoms designated * are in the R or S configuration; and R₁-R₅ are independently H, halogen, -OH, -NH₂, -CHO, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, monocyclic aryl, arylalkyl, or a mono or bicyclic heterocycle containing 1 to 5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur, each one of R₁-R₅ optionally substituted from 1 to 5 times with substituents selected from the group consisting of H, halogen, -OH, -OR₆, and -NH₂, wherein R₆ is defined as below; R₆ is H, -OH, -NH₂, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆

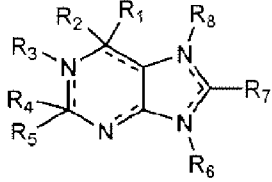
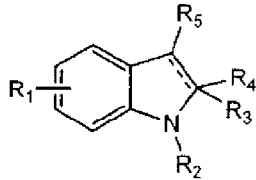
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	cycloalkyl, or monocyclic aryl.
Family V	wherein: R_1-R_7 are independently H, halogen, nitro, -OH, -OR₈, -OC(O)R₈, -OS(O)R₈, -OS(O)₂R₈, -NH₂, -NHR₈, -NR₈R₉, -CHO, -C(O)OH, -C(O)OR₈, -C(O)NHR₈, -C(O)NR₈R₉, -N(H)C(O)OR₈, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, monocyclic aryl, arylalkyl, or a mono or bicyclic heterocycle containing 1 to 5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur, each one of R_1-R_7 optionally substituted from 1 to 5 times with substituents selected from the group consisting of H, halogen, -OH, -OR₁₀, -NH₂, -NHR₁₀, -NR₁₀R₁₁, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, monocyclic aryl, and arylalkyl, wherein R_8-R_{11} are defined as below; or R_6 and R_7 can combine to form a 3- to 6-membered heterocycle or heteraryl containing 1 to 5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur, wherein each atoms of the heterocycle or heteroaryl is optionally substituted 1 to 5 times with substituents selected from the group consisting of H, halogen, -OH, cyano, -NH₂, and -N(H)C(O)OR₈, wherein R_8 is defined as below. R_8-R_{11} are independently H, C₁-C₁₅ alkyl, C₂-C₁₅ alkenyl, C₂-C₁₅ alkynyl, C₃-

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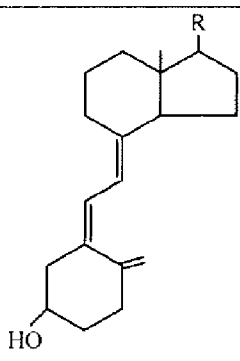
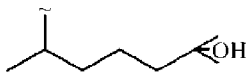
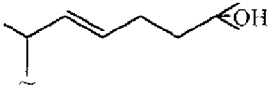
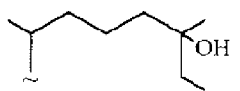
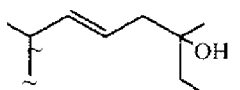
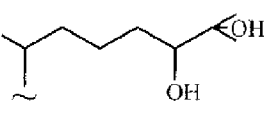
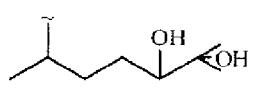
	C₆ cycloalkyl, monocyclic aryl, arylalkyl, or a mono or bicyclic heterocycle containing 1 to 5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur each optionally substituted with substituents selected from the group consisting of H, halogen, nitro, -OH, -OR₁₂, -OC(O)R₁₂, -NHC(O)R₁₂, wherein R₁₂ is defined below; and R₁₂ is independently H, C₁-C₁₅ alkyl, C₂-C₁₅ alkenyl, C₂-C₁₅ alkynyl, or C₃-C₆ cycloalkyl.
Family VI	wherein: R₁-R₃ are independently H, halogen, -OH, -NH₂, C₁-C₆ alkyl, -NO₂, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, monocyclic aryl, arylalkyl, or a mono or bicyclic heterocycle containing 1 to 5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur, each one of R₁-R₃ optionally substituted from 1 to 5 times with substituents selected from the group consisting of H, halogen, -OH, -OR₄, -NH₂, -NHR₄, -NR₄R₅, -NO₂, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, monocyclic aryl, and arylalkyl, wherein R₄ and R₅ are defined as below; R₄ and R₅ are independently H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, monocyclic aryl, arylalkyl, or a mono or bicyclic heterocycle or heteroaryl containing 1 to 5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur, each one of R₄ and R₅ optionally substituted from 1 to 5 times with substituents selected from the group consisting of H, halogen, cyano, -OH, -NH₂, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆

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	cycloalkyl, monocyclic aryl, arylalkyl, and a mono or bicyclic heterocycle or heteroaryl containing 1 to 5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur.
Family VII	 wherein: ----- represents an optional double bond; and R₁-R₈ are independently H, halogen, -OH, -NH₂, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, monocyclic aryl, arylalkyl, or a mono or bicyclic heterocycle containing 1 to 5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur, each one of R₁-R₈ optionally substituted from 1 to 5 times with substituents selected from the group consisting of H, halogen, -OH, -NH₂, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, monocyclic aryl, and arylalkyl, wherein each of R₁-R₈ substituents is optionally substituted from 1 to 5 times with substituents selected from the group consisting of H, -OH, -NH₂, halogen, cyano, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, monocyclic aryl, and arylalkyl; or R₁ and R₂ or R₄ and R₅ can combine to form (=O) or (=S);
Family VIII	

	wherein: ----- represents an optional double bond; and R₁-R₅ are independently H, halogen, -OH, -OR₆, -CHR₆, -NH₂, C₁-C₈ alkyl, methynyl, C₂-C₈ alkenyl, C₂-C₈ alkynyl, C₁-C₈ alkylcarboxyl, C₃-C₆ cycloalkyl, monocyclic aryl, arylalkyl, or a mono or bicyclic heterocycle or heteroaryl containing 1 to 5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur, each one of R₁-R₅ optionally substituted from 1 to 5 times with substituents selected from the group consisting of H, halogen, -OH, -NH₂, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, monocyclic aryl, and arylalkyl, wherein R₆ is defined as below; or R₃ and R₄ can combine to form (=O), or (=S). R₆ is H, C₁-C₈ alkyl, C₂-C₈ alkenyl, C₂-C₈ alkynyl, C₃-C₆ cycloalkyl, monocyclic aryl, arylalkyl, or a mono or bicyclic heterocycle containing 1 to 5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur.
Family IX	wherein: R¹-R³ are independently hydrogen, C₁-C₁₂ alkyl, or C₃-C₇ heterocycle, each optionally substituted from 1 to 3 times with substituents selected from the group consisting of pyrrolidine, C₁-C₁₂ monoalkylamino, C₁-C₁₂ dialkylamino, and C₃-C₇ heterocycle containing 1 to 5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur, each one of R¹-R³ substituents

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	optionally substituted from 1 to 4 times with substituents selected from hydrogen and C ₁ -C ₁₂ alkyl
Family X	 where R is a steroid homo side-chain defined by the structures I, II, III, IV, V and VI below:  I  II  III  IV  V  VI

Family XI	wherein: carbon atoms designated * are independently in the R or S configuration; and X is optionally oxygen or sulfur; R₁-R₃ and R₅ are independently H, halogen, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₄-C₇ cycloalkylalkyl, C₁-C₆ alkoxy, or C₁-C₆ amidoalkyl, each optionally substituted from 1 to 3 times with substituents selected from the group consisting of monocyclic aryl, monocyclic heteroaryl, arylalkyl, cyano, halogen, -NH₂, and hydroxy group, wherein the monocyclic aryl or heteroaryl containing 1-5 heteroatoms selected from the group consisting of nitrogen, sulfur, and oxygen, is optionally substituted from 1 to 3 times with substituents selected from the group consisting of C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₄-C₇ cycloalkylalkyl, C₁-C₆ alkoxy, arylalkoxy, and arylalkyl, each optionally substituted with substituents selected from the group consisting of halogen, hydroxy, -NH₂, and cyano; R₄ is a monocyclic aryl optionally substituted with substituents selected from the group consisting of halogen, hydroxy, -NH₂, cyano, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₄-C₇ cycloalkylalkyl, C₁-C₆ alkoxy, and C₁-C₆ alkoxyethyl; R₅-R₇ are independently halogen, H, -C(O)N(R₁₁)(R₁₂), C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₄-C₇ cycloalkylalkyl, monocyclic
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aryl, or arylalkyl, each optionally substituted from 1 to 3 times with substituents selected from the group consisting of phenyl, cyclocarbamoyl, cyano, halogen, $-NH_2$, hydroxy, $R_{12}C(O)N(R_{11})-$, and $R_{12}NHC(O)O-$ group, wherein the phenyl group is optionally substituted with substituents selected from the group consisting of halogen, hydroxy, $-NH_2$, and cyano, wherein R_{11} and R_{12} are defined below;

or R_6 and R_7 can combine to form a fused 5 or 6-membered monocyclic heterocycle or heteroaryl containing 1-5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur, optionally substituted with substituents selected from the group consisting of C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, C_3 - C_6 cycloalkyl, C_4 - C_7 cycloalkylalkyl, monocyclic aryl, and monocyclic heteroaryl, wherein the heteroaryl containing 1-5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur, is optionally substituted with substituents selected from the group consisting of halogen, hydroxy, and cyano;

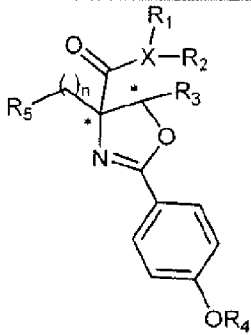
R_8 - R_{10} are independently halogen, hydroxy, $R_{12}NHC(O)O(R_5)-$, $R_{12}C(O)N(R_{11})-$, $R_{12}NHC(O)O-$, $-NH_2$, cyano, H, C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, C_3 - C_6 cycloalkyl, C_4 - C_7 cycloalkylalkyl, C_1 - C_6 alkoxy, C_1 - C_6 alkoxyethyl, C_1 - C_6 amidoalkyl, 4- to 6- membered lactam, or C_3 - C_6 cyclocarbamoyl, each optionally substituted from 1 to 3 times with substituents selected from the group consisting of halogen, hydroxy, $-NH_2$, cyano, $R_{13}NHC(O)O-$, wherein R_{12} - R_{13} are defined as below;

R_{11} - R_{13} are independently H, C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, C_3 - C_6 cycloalkyl, C_4 - C_7 cycloalkylalkyl, C_1 - C_6 alkoxy, 5- to 6- monocyclic aryl, or arylalkyl; each optionally substituted from 1 to 3 times with substituents selected from the group consisting of H, C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, C_3 - C_6 cycloalkyl, C_4 - C_7 cycloalkylalkyl, C_1 - C_6 alkoxy, halogen, $-C(O)NHOH$, hydroxy, $-NH_2$, and cyano;

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	or R₁₁ and R₁₂ can combine to form a mono or bicyclic heterocycle or heteroaryl containing 1 to 5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur.
Family XII	wherein: carbon atoms designated * are independently in the R or S configuration; and ----- represents an optional double bond; X is oxygen, sulfur, or NR₅, wherein R₅ is defined as below; R₁-R₄ are independently halogen, hydroxy, -NH₂, cyano, H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₄-C₇ cycloalkylalkyl, aryl alkyl, or mono or bicyclic heterocycle containing 1 to 5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur, -C(O)OCH₂CH₃, -(C₆H₅)O(C₆H₅), each optionally substituted from 1 to 3 times with substituents selected from the group consisting of C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkyl, C₃-C₆ cycloalkyl, monocyclic aryl, or mono or bicyclic heterocycle containing 1 to 5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur, cyano, halogen, -NH₂, R₅OC(O)-, .OR₅, and hydroxy group, wherein said phenyl group is optionally substituted with substituents selected from the group consisting of halogen, hydroxy, -NH₂, and cyano; or R₂ and R₃ can combine to form a fused 3 to 6-membered monocyclic heterocycle containing 1-5 heteroatoms selected from the group consisting of

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	oxygen, nitrogen, and sulfur, optionally substituted with substituents selected from the group consisting of H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₄-C₇ cycloalkylalkyl, monocyclic aryl, and monocyclic heteroaryl wherein the heteroaryl containing 1-5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur, is optionally substituted with substituents selected from the group consisting of halogen, hydroxy, and cyano; R₅ is independently H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, monocyclic aryl, R₆OC(O)NH-, OR₁OR₂ and -OR₆; R₆ is H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, monocyclic aryl, arylalkyl, or a mono or bicyclic heterocycle or heteroaryl containing 1 to 5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur, each optionally substituted with halogen, hydroxyl, cyano, -NH₂, C₁-C₂ alkyl, C₁-C₂ alkoxy, C₂-C₆ alkenyl, heteroarylalkoxy, arylalkoxy, and C₃-C₆ cycloalkyl, wherein the heteroaryl contains 1 to 5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur.
Family XIII	 wherein: the carbon atom designated * is in the R or S configuration; and X is oxygen, nitrogen, or -CH-;

	n is an integer from 0 to 6; R₁ and R₂ are independently O, H, hydroxyl, C(O), -NH₂, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₁-C₆ alkoxy, C₂-C₁₀ alkyletheryl, arylalkyl, or C₄-C₇ cycloalkylalkyl, each optionally substituted from 1 to 3 times with substituents selected from the group consisting of phenyl, cyano, halogen, N₃, -CH₂N₃, -NH₂, hydroxy, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, and mono or bicyclic heteroaryl or heterocycle containing 1 to 5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur, wherein the phenyl group is optionally substituted from 1 to 3 times with substituents selected from the group consisting of N₃, -CH₂N₃, -NH₂, and cyano; or R₁ and R₂ can combine to form a 5- or 6-membered monocyclic heterocycle containing 1-5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur, optionally substituted from 1 to 4 times with substituents selected from the group consisting of H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, and C₄-C₇ cycloalkylalkyl; R₃ and R₅ are independently R₆OC(O)-, R₆SO₂-, R₆SO-, R₆S-, or 5- or 6-membered monocyclic aryl or heteroaryl optionally substituted from 1 to 3 times with substituents selected from the group consisting of H, N₃, -CH₂N₃, -NH₂, hydroxy, cyano, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, and monocyclic aryl, wherein R₆ is defined as below; R₄ is independently H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, or C₄-C₇ cycloalkylalkyl, C₁-C₆ alkoxy, C₂-C₁₀ alkyletheryl, or arylalkyl, each optionally substituted from 1 to 3 times with substituents selected from the group consisting of phenyl, -OH, halogen, N₃, -CH₂N₃, -NH₂, cyano, and R₆NHC(O)O-, wherein R₆ is defined as below; R₆ is H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ alkynyl, C₃-C₆
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	cycloalkyl, mono cyclic aryl, arylalkyl or a mono or bicyclic heterocycle or heteroaryl containing 1 to 5 hereoatoms selected from the group consisting of oxygen, nitrogen, and sulfur, each optionally substituted with H, halogen, hydroxyl, cyano, -NH₂, and -C(O)NHOH; with the proviso that when X is oxygen, one of R₁ or R₂ is absent.
Family XIV	wherein: carbon atoms designated * are independently in the R or S configuration; and X is oxygen or nitrogen; R₁ and R₂ are independently H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, or C₄-C₇ cycloalkylalkyl, and arylalkyl, each optionally substituted from 1 to 3 times with substituents selected from the group consisting of phenyl, cyano, halogen, -NH₂, and hydroxy group, wherein the phenyl group is optionally substituted from 1 to 3 times with substituents selected from the group consisting of N₃, -CH₂N₃, -NH₂, and cyano; or R₁ and R₂ can combine to form a 3- to 10-membered monocyclic heterocycle containing 1-5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur, optionally substituted from 1 to 3 times with substituents selected from the group consisting of H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₄-C₇ cycloalkylalkyl, monocyclic aryl, and

	monocyclic heteroaryl wherein the monocyclic aryl or heteroaryl containing 1-5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur, and each of the monocyclic aryl or heteroaryl is optionally substituted with substituents selected from the group consisting of halogen, hydroxy, NH_2, and cyano; $\text{R}_3\text{-R}_5$ are independently 5- or 6-membered monocyclic aryl or heteroaryl optionally substituted with substituents selected from the group consisting of hydroxy, halogen, $-\text{NH}_2$, cyano, $\text{C}_1\text{-C}_6$ alkyl, $\text{C}_2\text{-C}_6$ alkenyl, $\text{C}_2\text{-C}_6$ alkynyl, $\text{C}_3\text{-C}_6$ cycloalkyl, $\text{C}_4\text{-C}_7$ cycloalkylalkyl, $\text{C}_1\text{-C}_6$ alkoxy carbonyl, $\text{C}_1\text{-C}_6$ alkoxy carboxyl, $\text{C}_1\text{-C}_6$ alkoxy carbamoyl, and $-\text{OR}_8$ each optionally substituted from 1 to 3 times with substituents selected from the group consisting of $\text{C}_1\text{-C}_6$ alkyl, $\text{C}_1\text{-C}_6$ alkenyl, $\text{C}_1\text{-C}_6$ alkynyl, phenyl, and hydroxyphenyl, wherein R_8 is defined as below; R_6 is independently H, $\text{C}_1\text{-C}_6$ alkyl, $\text{C}_2\text{-C}_6$ alkenyl, $\text{C}_2\text{-C}_6$ alkynyl, $\text{C}_3\text{-C}_6$ cycloalkyl, $\text{C}_4\text{-C}_7$ cycloalkylalkyl, $\text{R}_9\text{O}-(\text{CH}_2)_n\text{-OC(O)-}$, or $\text{R}_9\text{-OC(O)-}$, wherein n is an integer from 1 to 6 R_9 is defined as below; R_7 is independently H, $\text{C}_1\text{-C}_6$ alkyl, $\text{C}_2\text{-C}_6$ alkenyl, $\text{C}_2\text{-C}_6$ alkynyl, $\text{C}_3\text{-C}_6$ cycloalkyl, or $\text{C}_4\text{-C}_7$ cycloalkylalkyl, each optionally substituted from 1 to 3 times with substituents selected from the group consisting of phenyl, $\text{C}_3\text{-C}_{10}$ cycloalkyl, $\text{C}_4\text{-C}_{11}$ cycloalkylalkyl, $\text{C}_3\text{-C}_{10}$ cycloalkenyl, $\text{C}_4\text{-C}_{11}$ cycloalkenylalkyl, each optionally substituted from 1 to 3 times with substituents selected from the group consisting of $\text{C}_1\text{-C}_6$ alkyl, $\text{C}_2\text{-C}_6$ alkenyl, $\text{C}_2\text{-C}_6$ alkynyl, $\text{C}_3\text{-C}_{10}$ cycloalkyl, $\text{C}_4\text{-C}_{11}$ cycloalkylalkyl, $\text{C}_3\text{-C}_{10}$ cycloalkenyl, $\text{C}_3\text{-C}_{10}$ cycloalkenylalkyl, $\text{C}_1\text{-C}_6$ alkoxy, $\text{C}_1\text{-C}_6$ alkoxy etheryl, $\text{C}_1\text{-C}_6$ alkoxy carbonyl, $\text{C}_1\text{-C}_6$ alkoxy carboxyl, $-\text{OH}$, halogen, $-\text{NH}_2$, and cyano; R_8 is independently H, $\text{C}_1\text{-C}_6$ alkyl, $\text{C}_2\text{-C}_6$ alkenyl, $\text{C}_2\text{-C}_6$ alkynyl, $\text{C}_3\text{-C}_6$ cycloalkyl, $\text{C}_4\text{-C}_7$ cycloalkylalkyl, $\text{C}_1\text{-C}_6$ alkoxy, $\text{C}_1\text{-C}_6$ alkoxy carboxyl, $\text{C}_1\text{-C}_6$ alkoxy carbamoyl, each optionally substituted 1 to 5 times with substituents
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	selected from the group consisting of H, -OH, -NH₂, cyano, and C₁-C₆ alkyl; R₉ is independently H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₄-C₇ cycloalkylalkyl; with the proviso that when X is oxygen, one of R₁ or R₂ is absent.
Family XV	wherein: X and Y are independently C, O, N, or S; ----- represents an optional double bond; and R₁-R₆ are independently H, halogen, -OH, -NH₂, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, monocyclic aryl, arylalkyl, or a mono or bicyclic heterocycle containing 1 to 5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur, each of R₁-R₆ optionally substituted from 1 to 5 times with substituents selected from the group consisting of (=S), (=O), H, halogen, -OH, -NH₂, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₁-C₆ alkoxy, monocyclic aryl, and arylalkyl; or R₅ and R₆ can combine to form (=O) or (=S).
Family XVI	

wherein:

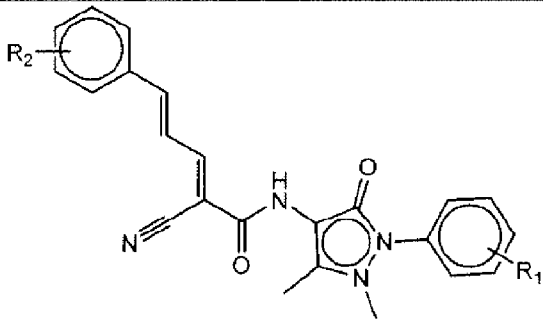
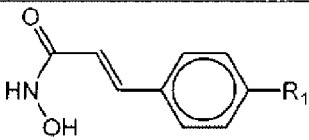
Carbon atoms designated * are in the R or S configuration; and

X is O or S;

R₁-R₃ are independently H, halogen, -OH, -OR₄, -NH₂, -NHR₄, -NR₄R₅, -CHO, -COOH, -COOR₄, -CONHR₄, -CONR₄R₅, -N(H)C(O)OR₄, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, mono or polycyclic aryl, arylalkyl, or a mono or bicyclic heterocycle containing 1 to 5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur, each one of R₁-R₃ optionally substituted from 1 to 5 times with substituents selected from the group consisting of H, halogen, -OH, -OR₄, -NH₂, -NHR₄, -NR₄R₅, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, mono or polycyclic aryl, and arylalkyl, each of R₁-R₃ substituents is optionally substituted from 1 to 5 times with substituents selected from H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, mono or polycyclic aryl, and arylalkyl, and or a mono or bicyclic heterocycle containing 1 to 5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur; wherein R₄ and R₅ are defined as below;

R₄ and R₅ are independently H, halogen, -OH, -NH₂, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, monocyclic aryl, arylalkyl, each optionally substituted from 1 to 3 times with substituents selected from the group consisting of H, halogen, -OH, and -NH₂;

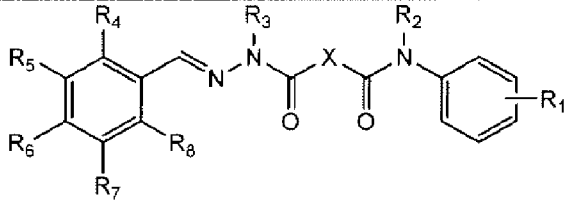
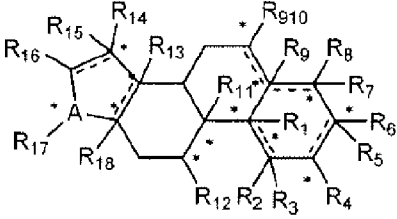
or R₄ and R₅ can combine to form 3- to 6-membered heterocycle or heteroaryl containing 1 to 5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur, each heterocycle or heteroaryl optionally substituted with substituents selected from the group consisting of H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, monocyclic aryl, arylalkyl, and or a mono or bicyclic heterocycle containing 1 to 5 heteroatoms selected from the

	group consisting of oxygen, nitrogen, and sulfur.
Family XVII	 wherein: R₁ and R₂ are independently H, halogen, -OH, -NH₂, -NO₂, -CHO, -COOH, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, monocyclic aryl, arylalkyl, or a mono or bicyclic heterocycle containing 1 to 5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur.
Family XVIII	 wherein: R₁ is independently H, -OH, -OR₂, -NH₂, -NHR₂, -NR₂R₃, -R₂NR₃, C₁-C₆ alkyl, C₁-C₆ alkoxy, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, monocyclic aryl, arylalkyl, or a mono or bicyclic heterocycle containing 1 to 5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur, each optionally substituted from 1 to 5 times with substituents selected from the group consisting of H, halogen, -OH, -NH₂, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, monocyclic aryl, arylalkyl, and a mono or bicyclic heterocycle containing 1 to 5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur, wherein R₂ and R₃ are defined as below;

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	R₂ and R₃ are independently H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, monocyclic aryl, arylalkyl, and a mono or bicyclic heterocycle containing 1 to 5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur.
Family XIX	wherein: n is an integer from 1 to 2; R₁-R₃ are independently H, -NH₂, -NO₂, -COOH, -C(O)NH₂, -C(O)NHOH, -OH, -OR₄, C₁-C₈ alkyl, C₂-C₈ alkenyl, C₂-C₈ alkynyl, C₃-C₈ cycloalkyl, C₄-C₉ cycloalkylalkyl, arylalkyl, mono or polycyclic aryl, a mono or bicyclic heterocycle, or heteroaryl containing 1 to 5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur, each of R₁-R₄ optionally substituted from 1 to 3 times with substituents selected from the group consisting of cyano, halogen, -NO₂, H, C₁-C₈ alkyl, C₁-C₈ alkoxy, aryloxy, C₂-C₈ alkenyl, C₂-C₈ alkynyl, C₃-C₈ cycloalkyl, C₄-C₉ cycloalkylalkyl, mono or polycyclic aryl, mono or bicyclic heterocycle or heteroaryl containing 1 to 5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur, N₃, -CH₂N₃, -NH₂, -COOH, -C(O)NH₂, -C(O)NHOH, R₄OC(O)N(R₅)-, and hydroxy group; R₄ and R₅ are H, C₁-C₈ alkyl, C₂-C₈ alkenyl, C₂-C₈ alkynyl, C₃-C₈ cycloalkyl, C₄-C₉ cycloalkylalkyl, arylalkyl, mono or polycyclic aryl, a mono or bicyclic heterocycle, or heteroaryl containing 1 to 5 heteroatoms selected from the

- 30 -

	group consisting of oxygen, nitrogen, and sulfur.
Family XX	 wherein: X is optionally C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₃-C₆ cycloalkenyl, 5- to 6- membered monocyclic aryl, or heteroaryl containing 1-5 heteroatoms selected from the group consisting of oxygen, sulfur, or nitrogen. R₁-R₈ are independently H, hydroxy, halogen, -NH₂, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₃-C₆ cycloalkenyl, 5- to 6- membered monocyclic aryl, or heteroaryl containing 1-5 heteroatoms selected from the group consisting of oxygen, sulfur; or R₅ and R₆ can combine to form a mono carbocycle or heterocycle, wherein the heterocycle contains 1-5 heteroatoms selected from the group consisting of oxygen, sulfur, or nitrogen.
Family XXI	 wherein: carbon atoms designated * are independently in the R or S configuration; and

----- represents an optional double bond;

A is a CR₁₉ or O;

R₁-R₁₈ are optionally and independently H, -OH, R₁₉C(O)-, R₁₉C(O)O-, -OR₁₉, halogen, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₄-C₇ cycloalkylalkyl, C₁-C₁₀ alkoxy-carboxyl, C₁-C₁₀ alkoxy-carbamoyl, C₁-C₁₀ alkoxy-carbonyl, aryloxy, arylalkoxy, or C₁-C₁₀ hydroxyketoalkyl, each optionally substituted from 1 to 3 times with substituents selected from the group consisting of hydroxy, -NH₂, cyano, halogen, and a mono or bicyclic heterocycle, or heteroaryl each containing 1 to 5 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur, each one of R₁-R₁₈ substituents optionally substituted from 1 to 3 times with substituents selected from the group consisting of H, -OH, halogen, (=O), (=S), C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₄-C₇ cycloalkylalkyl, C₁-C₁₀ alkoxy-carboxyl, C₁-C₁₀ alkoxy-carbamoyl, C₁-C₁₀ alkoxy-carbonyl, aryloxy, and arylalkoxy, wherein R₁₉ is defined as below;

or R₂ and R₃ can combine to form a carbonyl group;

R₆ and R₆ can combine to form the carbonyl group;

R₇ and R₈ can combine to form the carbonyl group;

R₁₄ and R₁₅ can combine to form the carbonyl group;

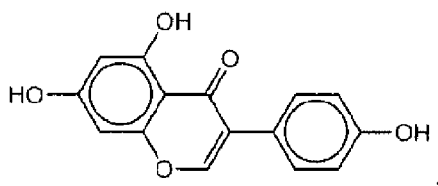
R₅ and R₆, R₇ and R₈, R₆ and R₉ and R₁₅ and R₁₆ can combine to form independently a 3- to 6-membered cycloalkyl, or heterocycle containing 1 to 5 heteroatoms selected from the group consisting of nitrogen, sulfur, and oxygen, wherein said heterocycle is optionally substituted with substituents selected from the group consisting of H, -OH, halogen, C₁-C₆ alkyl, C₂-C₆

alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₄-C₇ cycloalkylalkyl;

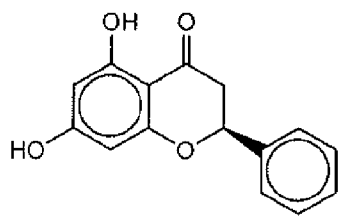
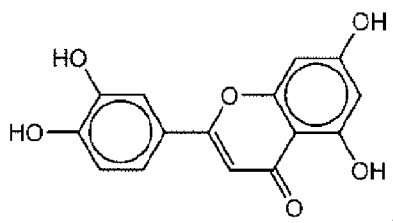
R₁₉ is independently H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₄-C₇ cycloalkylalkyl, C₁-C₁₀ alkoxy carbonyl, C₁-C₁₀ alkoxy carbamoyl, C₁-C₁₀ alkoxy carbonyl, aryloxy, arylalkoxy, or C₁-C₁₀ hydroxyketoalkyl, each optionally substituted from 1 to 3 times with substituents selected from the group consisting of hydroxy, -NH₂, cyano, and halogen.

with the proviso that when A is oxygen, R₁₆ is absent.

[0020] Suitable compounds of Family I have the following structures:

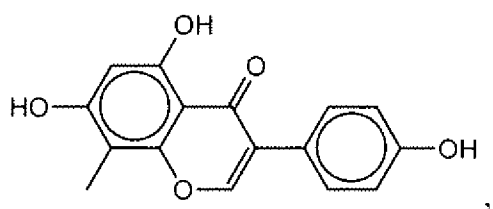
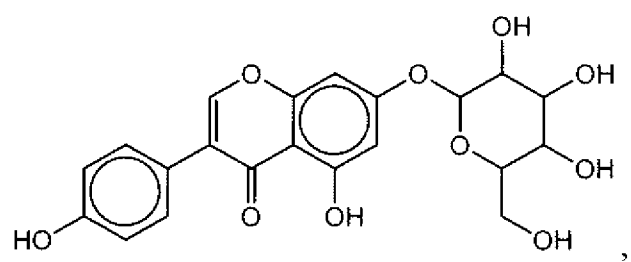


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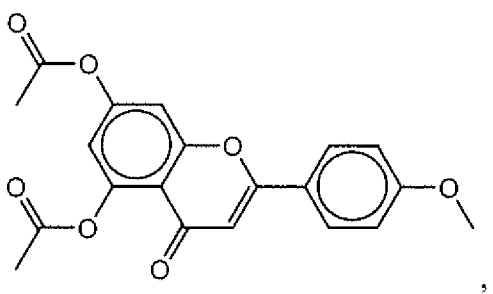
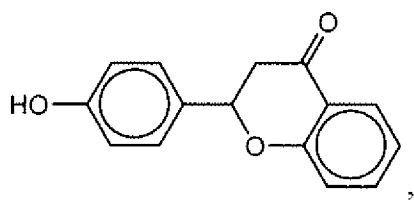


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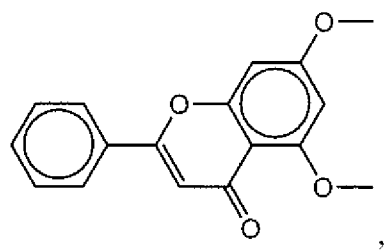
- 33 -



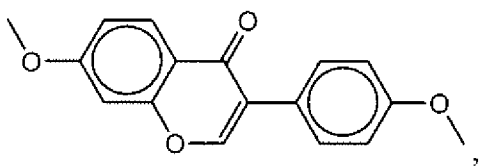
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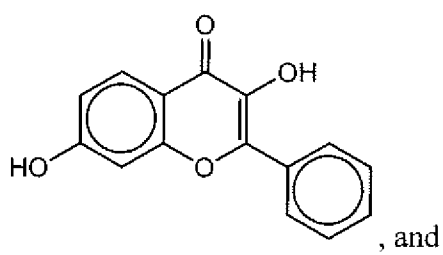
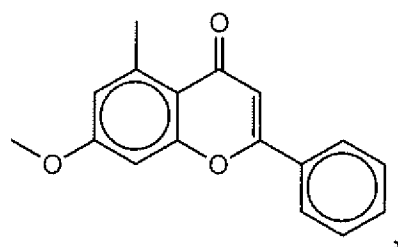
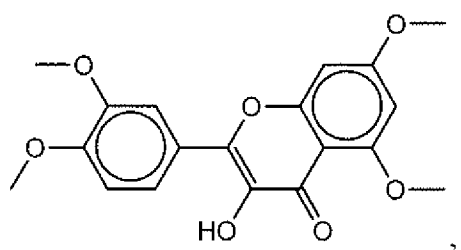
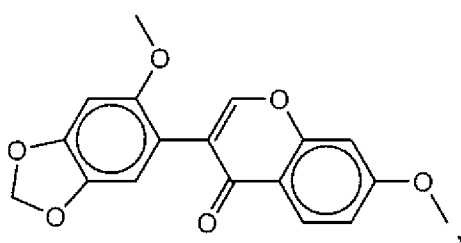
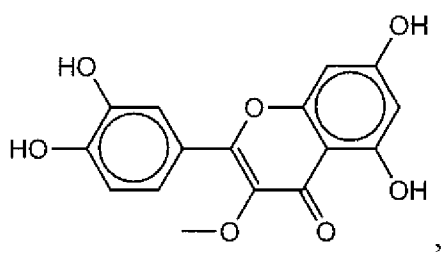
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- 34 -

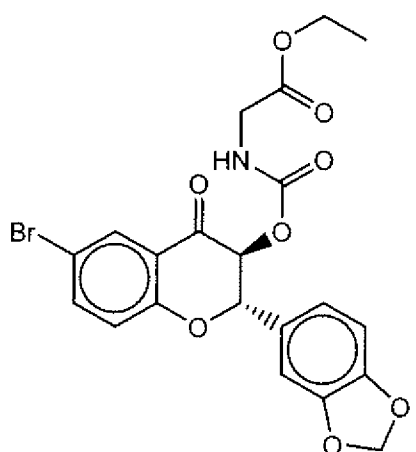


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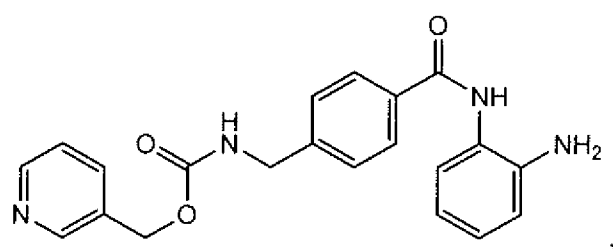
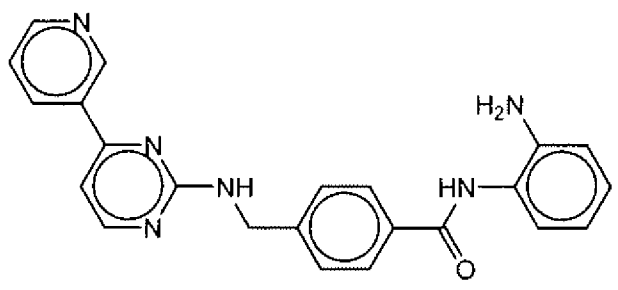
- 35 -



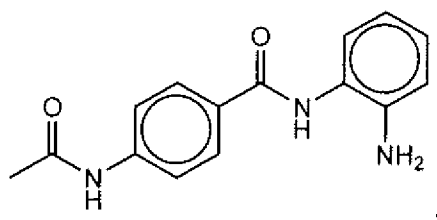
[0021]

Suitable compounds of Family II have the following structures:

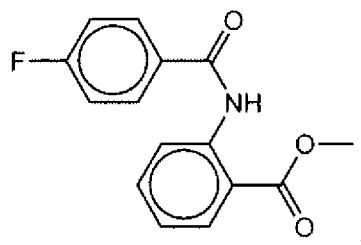
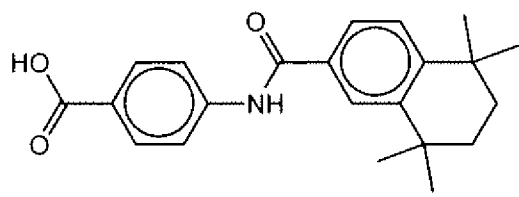
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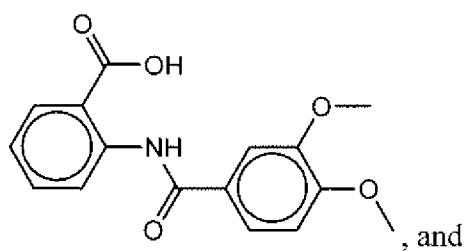
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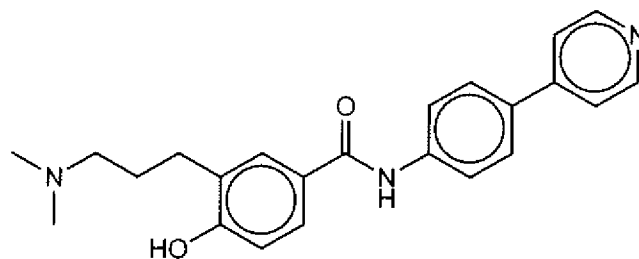
- 36 -



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, and

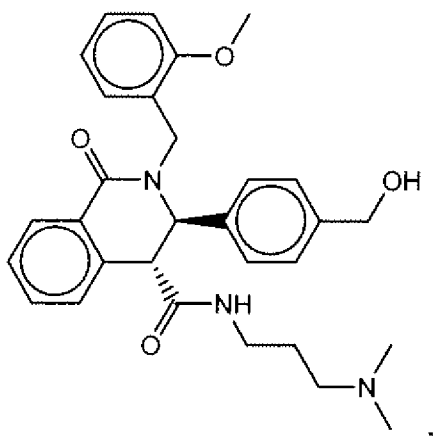
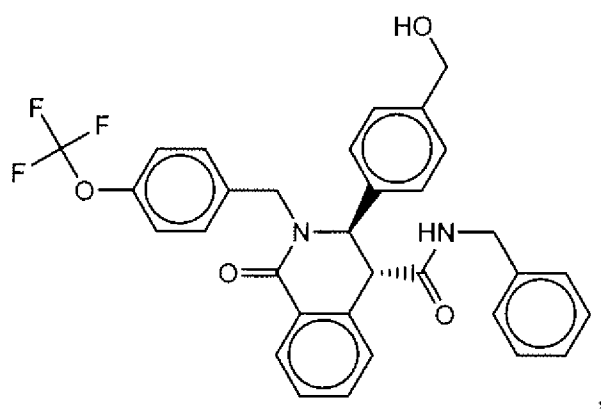
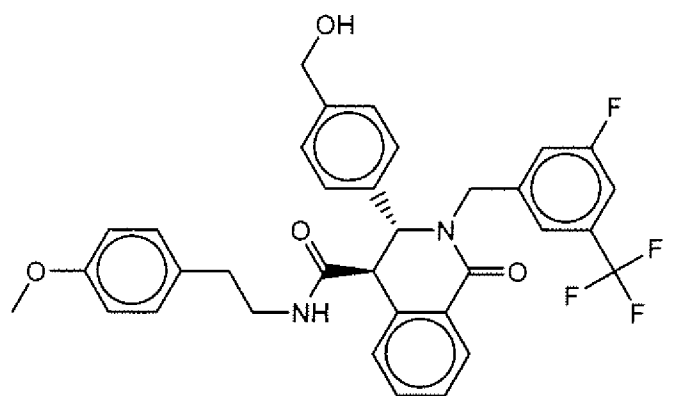


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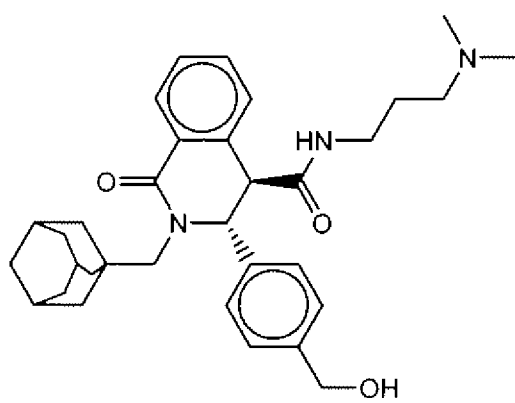
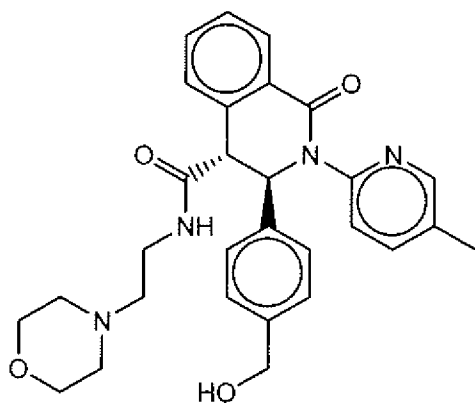
[0022]

Suitable compounds of Family III have the following structures:

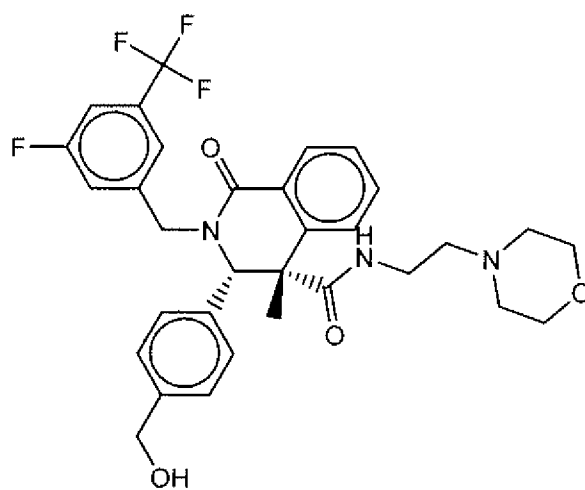
- 37 -



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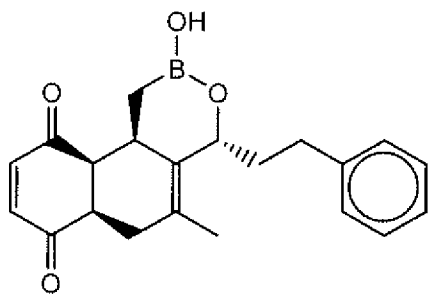
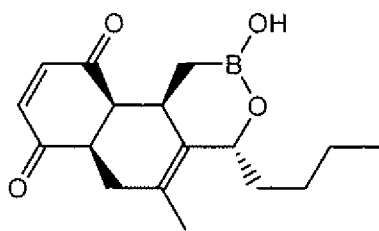
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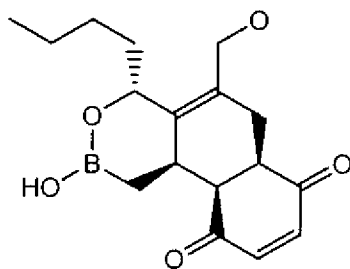
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[0023] Example of suitable compounds of Family IV have the following structures:

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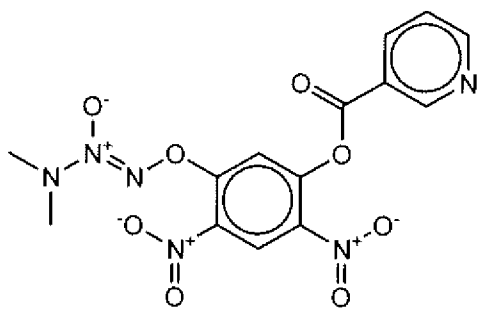
, and



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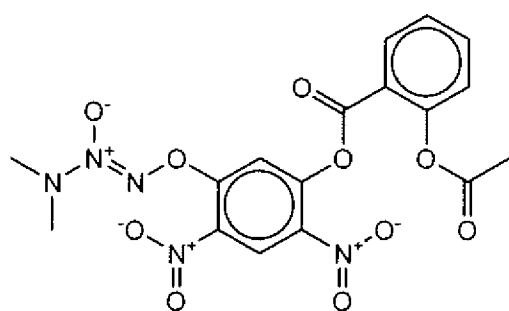
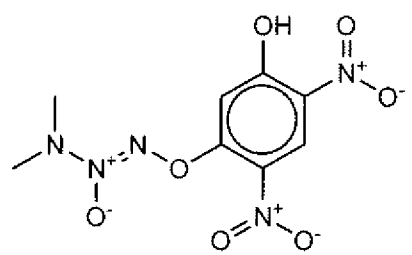
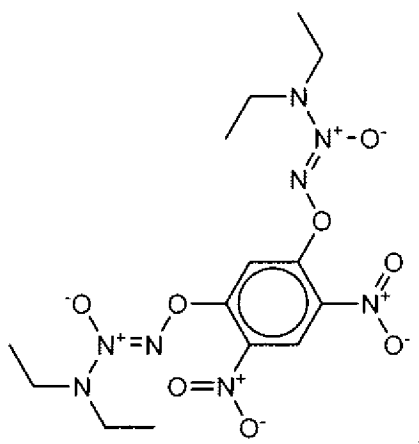
[0024]

Suitable compounds of Family V have the following structures:

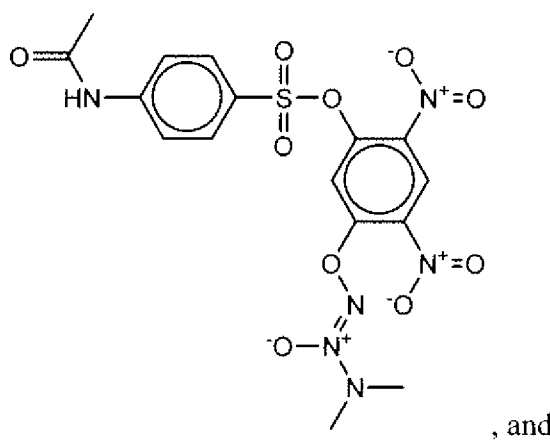


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- 40 -

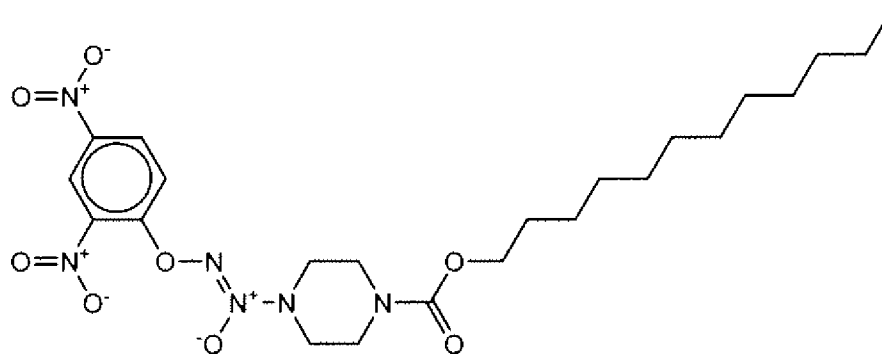


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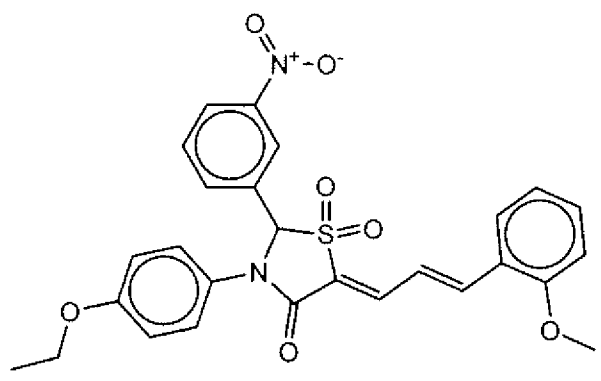


, and

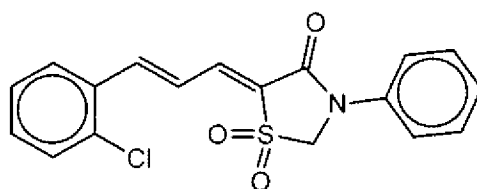
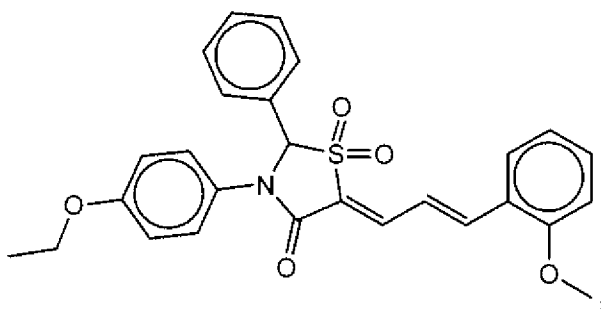
- 41 -



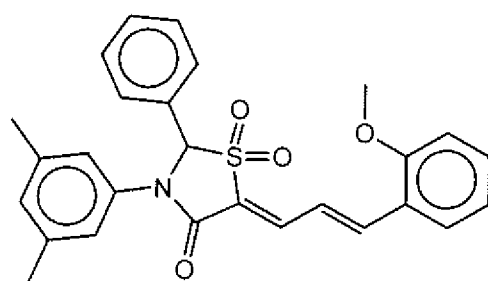
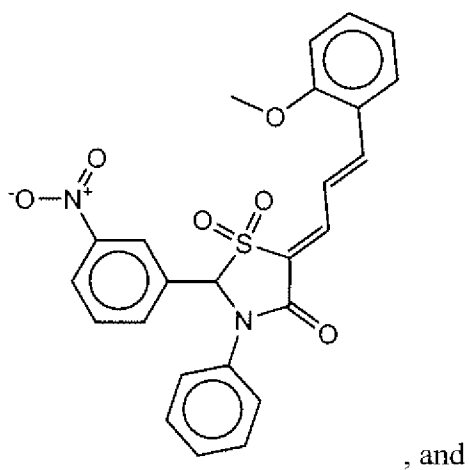
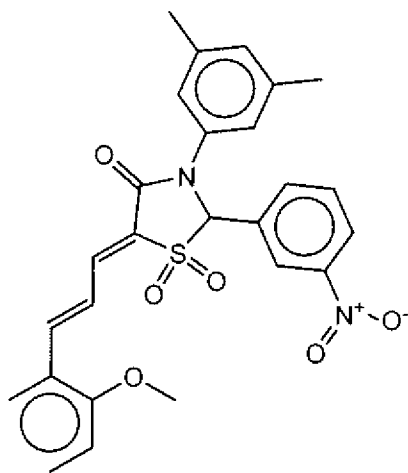
[0025] Suitable compounds of Family VI have the following structures:



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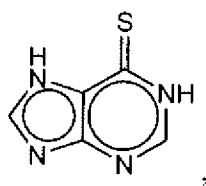
- 42 -



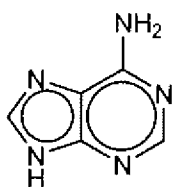
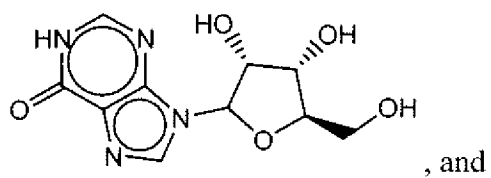
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[0026]

Suitable compounds of Family VII have the following structures:

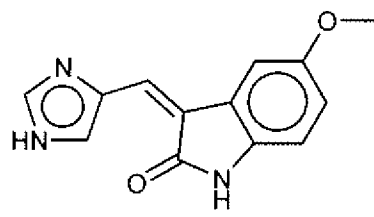
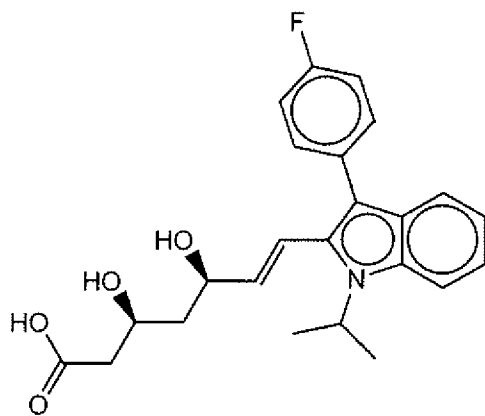
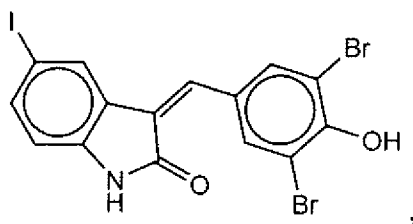


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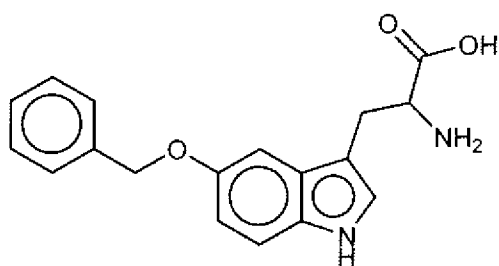
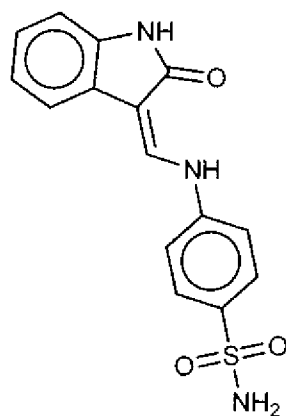


5 [0027]

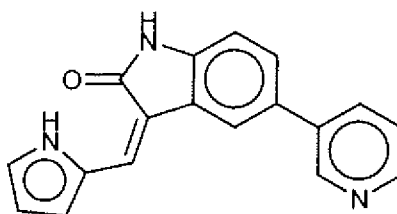
Suitable compounds of Family VIII have the following structures:



- 44 -



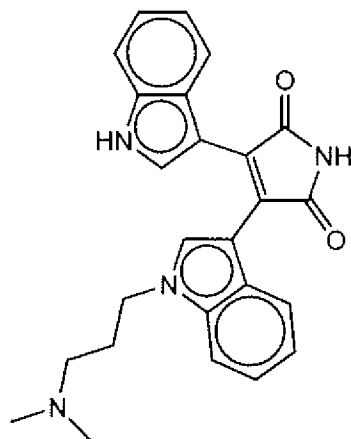
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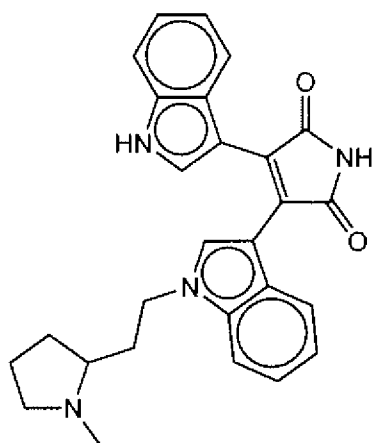
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[0028]

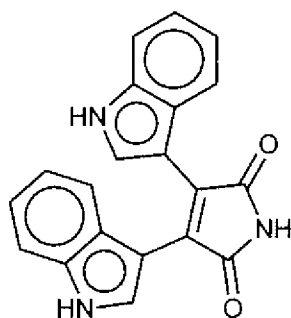
Suitable compounds of Family IX have the following structures:



- 45 -

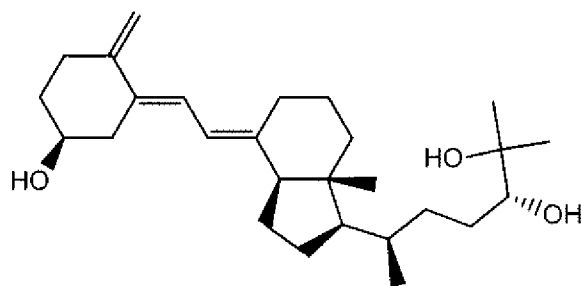


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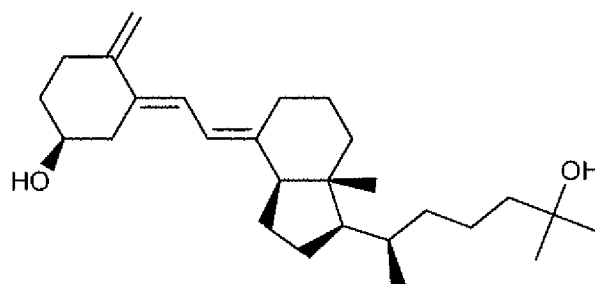


[0029]

Suitable compounds of Family X have the following structures:



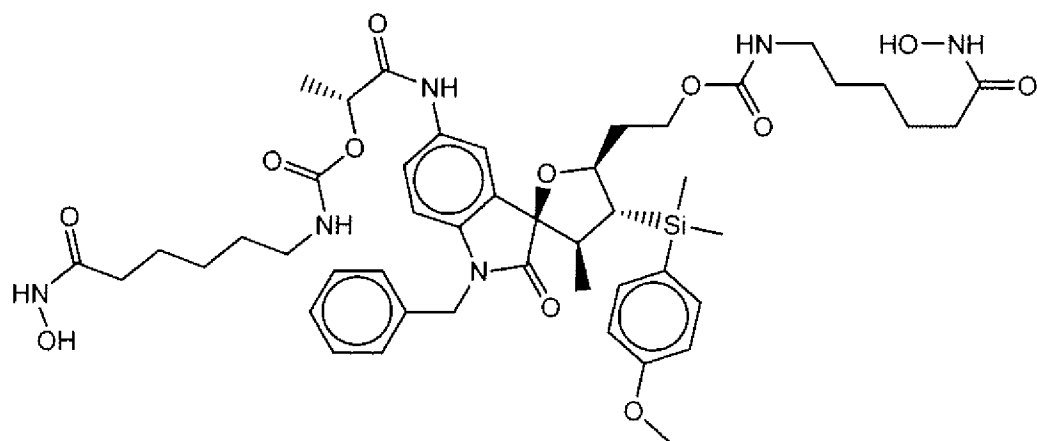
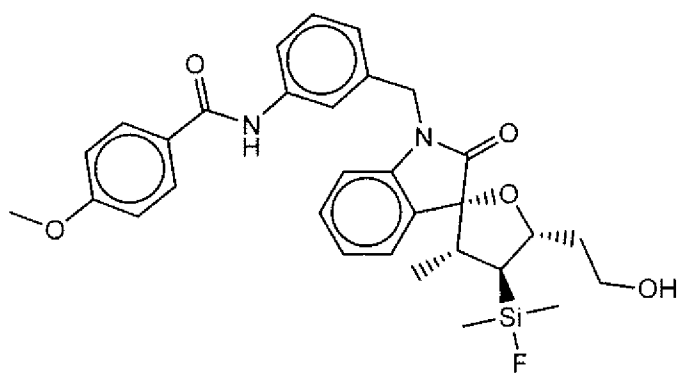
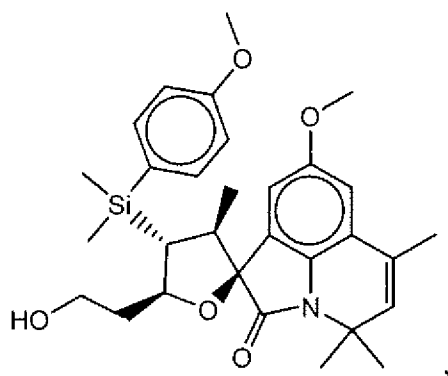
and



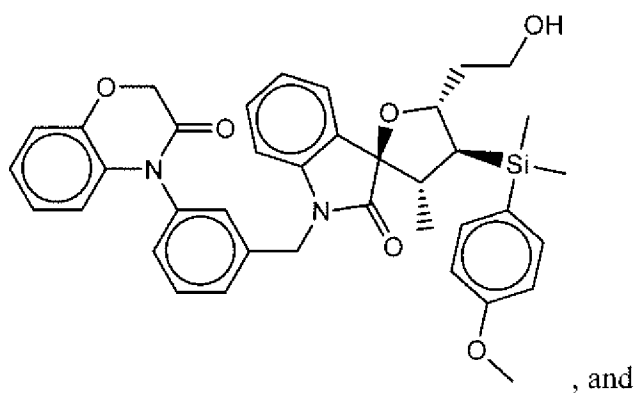
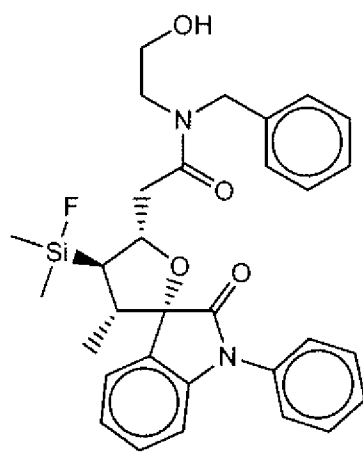
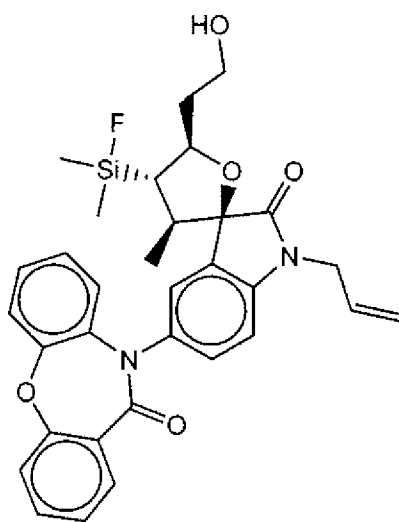
[0030]

Suitable compounds of Family XI have the following structures:

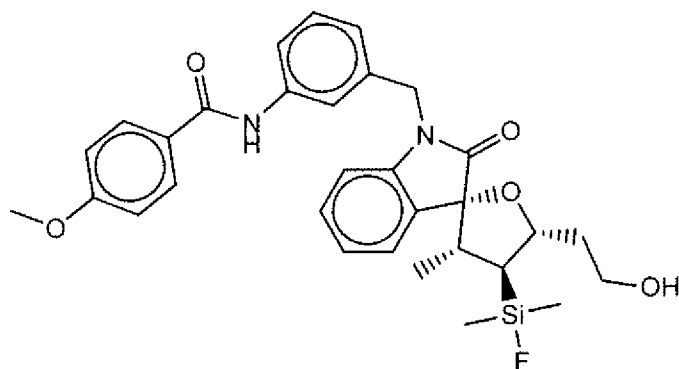
- 46 -



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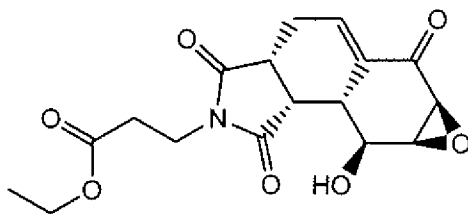


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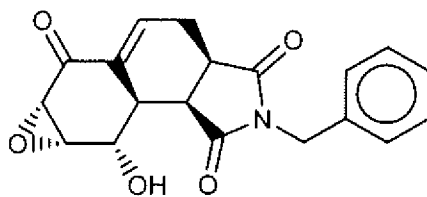
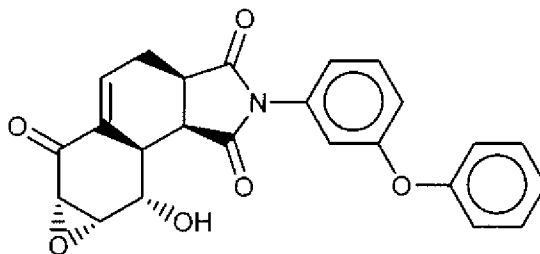
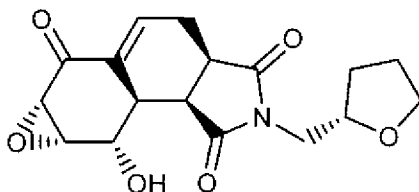


[0031]

Suitable compounds of Family XII have the following structures:

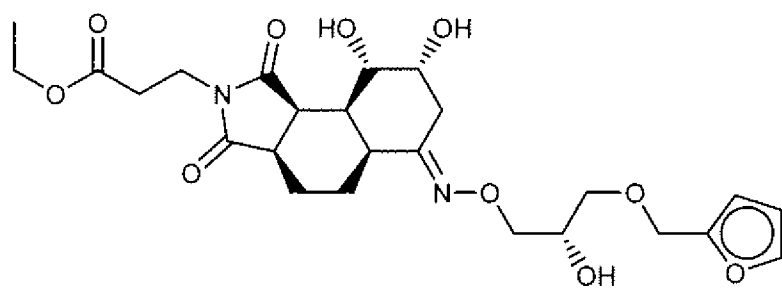
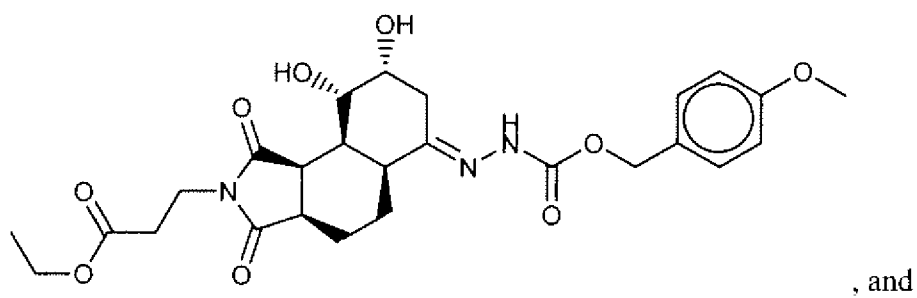


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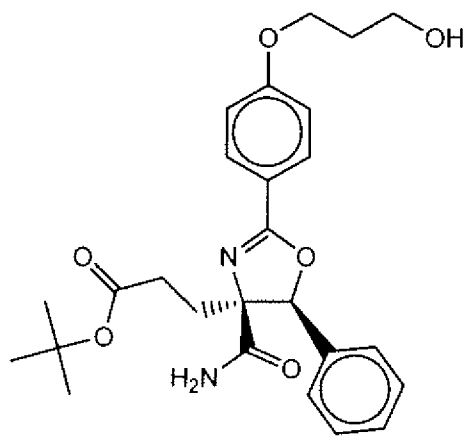
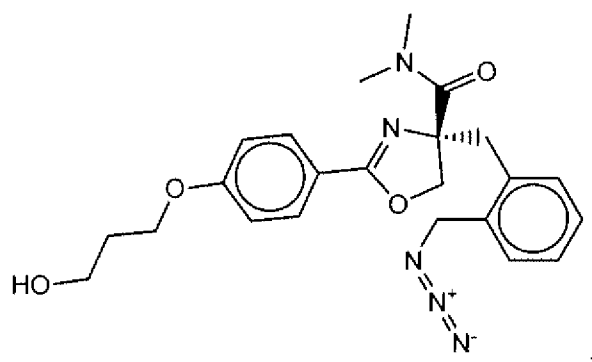
- 49 -



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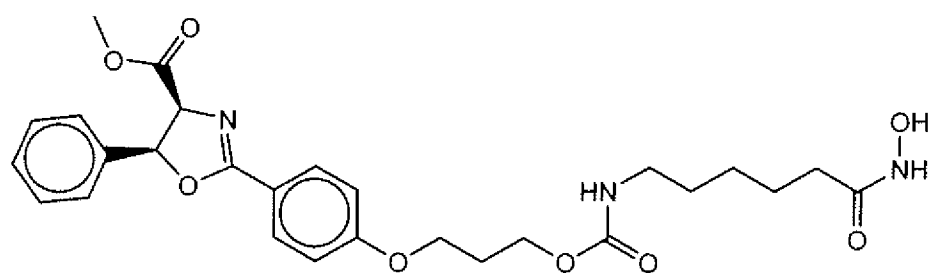
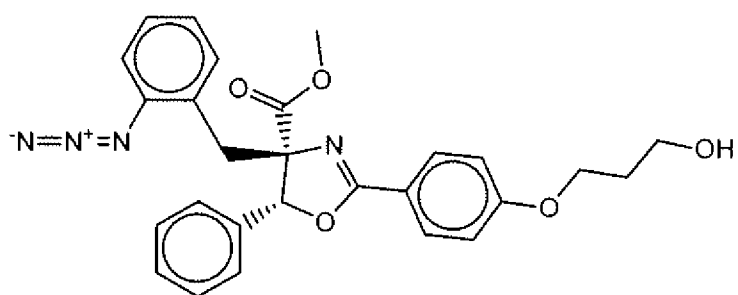
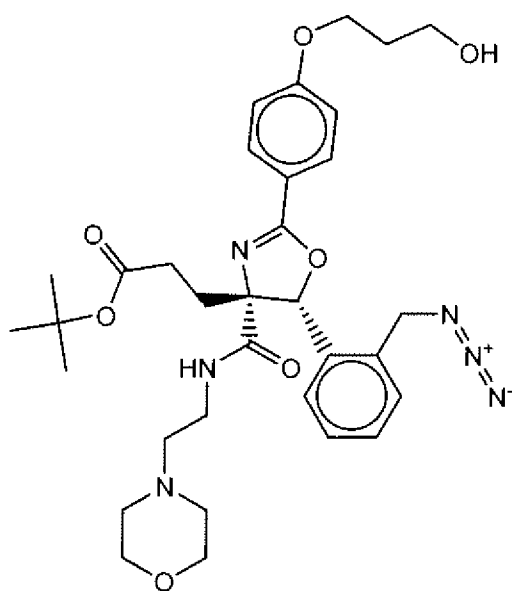
[0032]

Suitable compounds of Family XIII have the following structures:

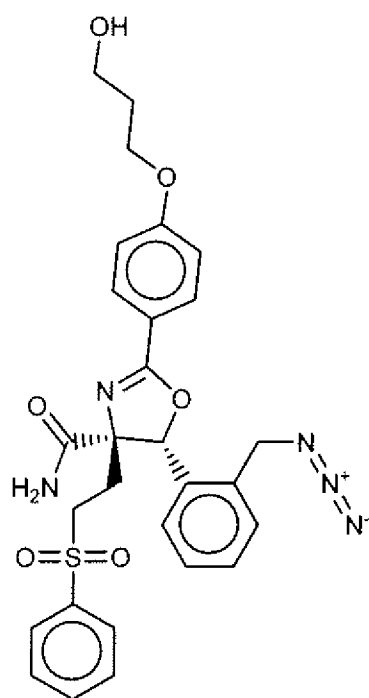
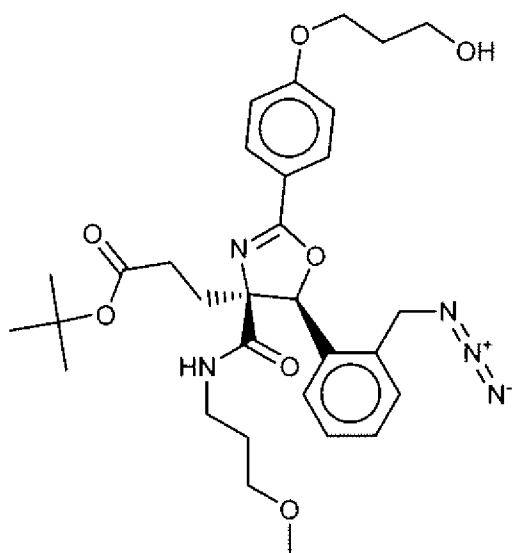


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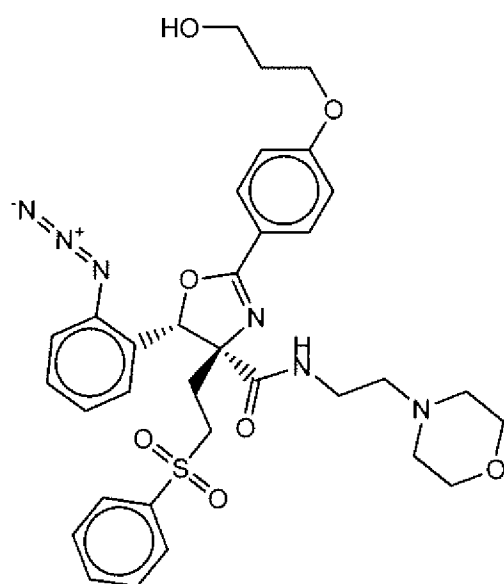
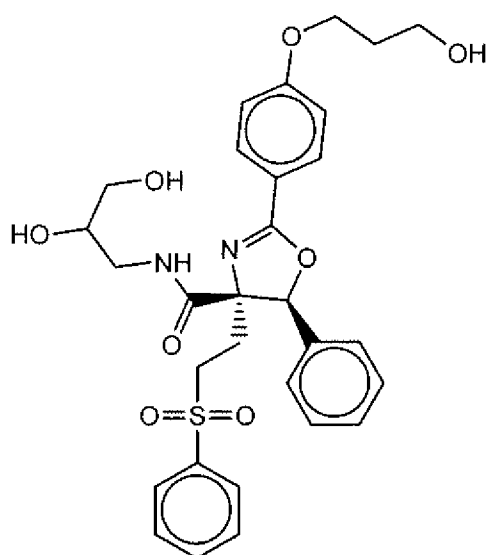
- 50 -



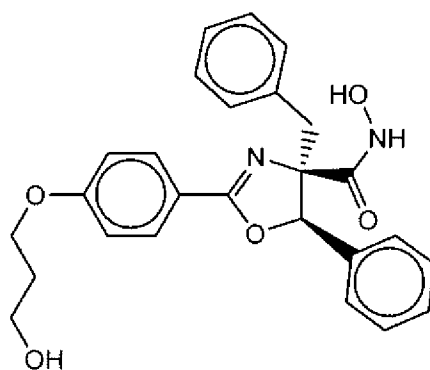
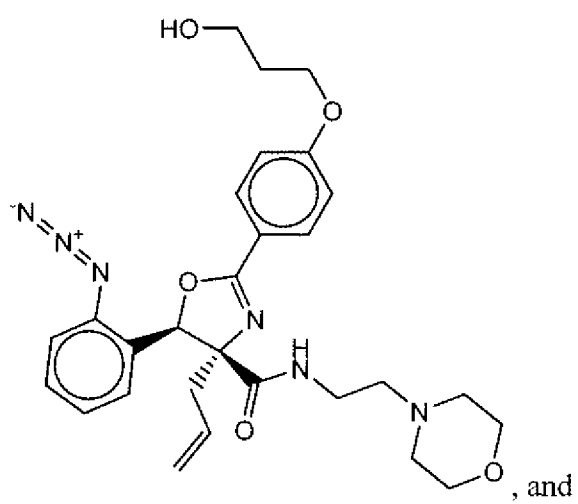
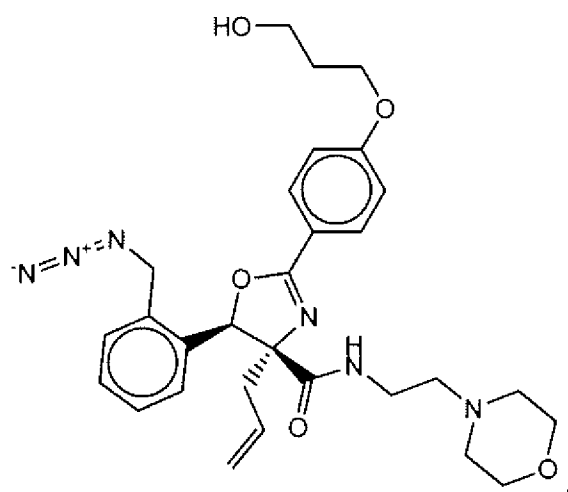
- 51 -



- 52 -

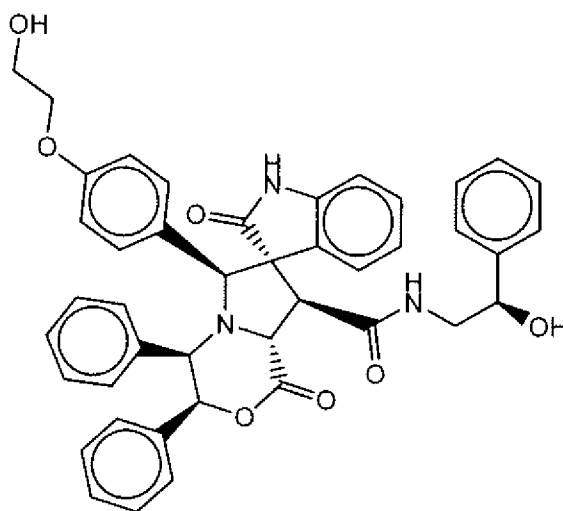
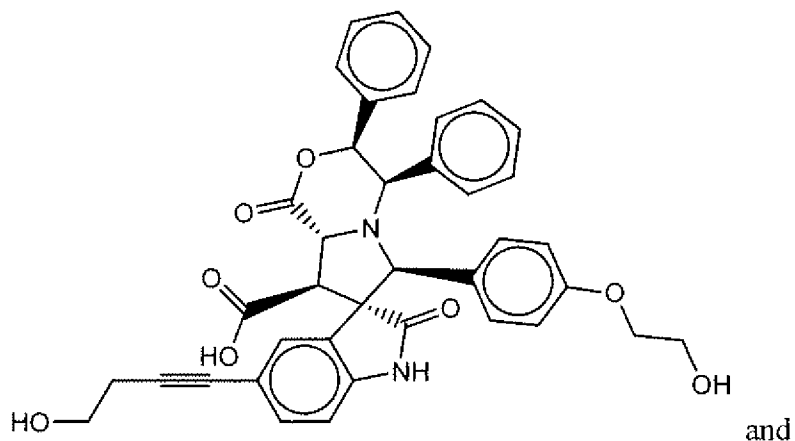


- 53 -



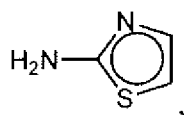
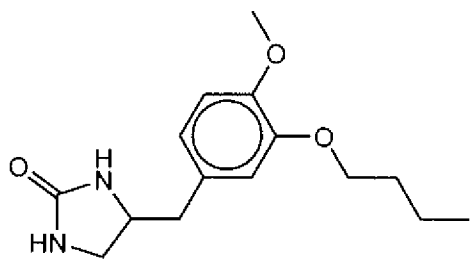
- 54 -

[0033] Suitable compounds of Family XIV have the following structures:



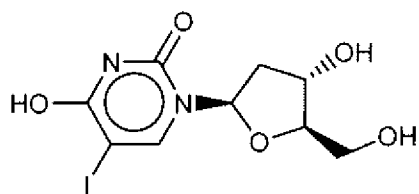
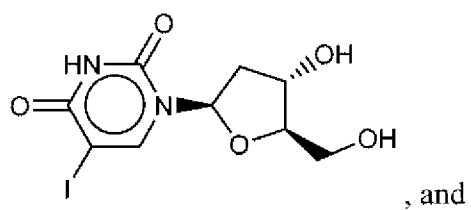
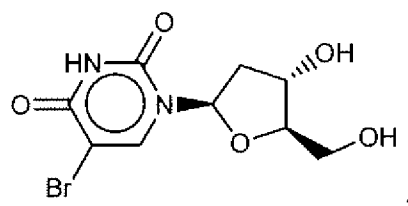
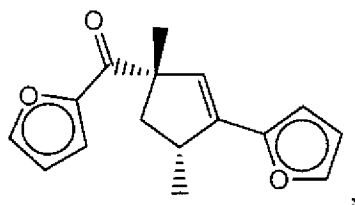
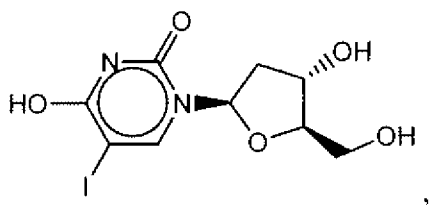
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[0034] Suitable compounds of Family XV have the following structures:



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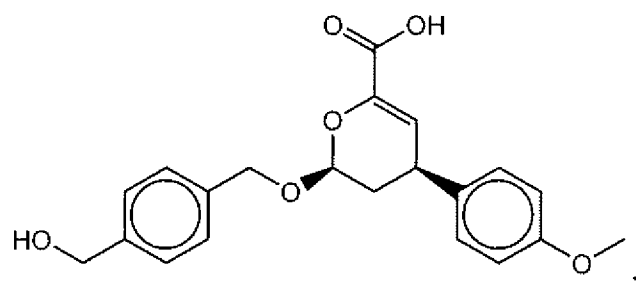
- 55 -



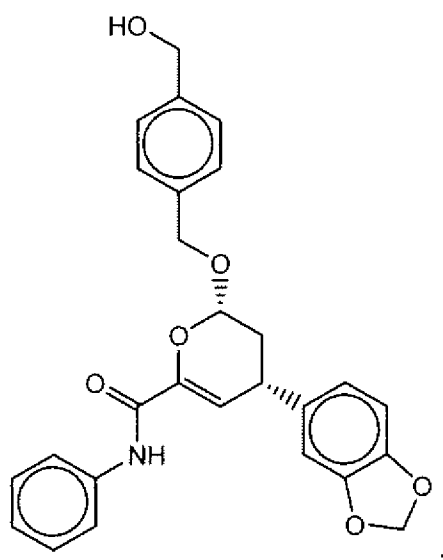
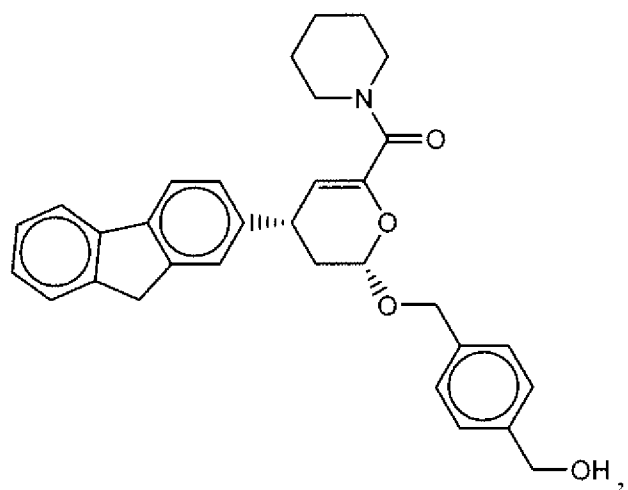
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[0035]

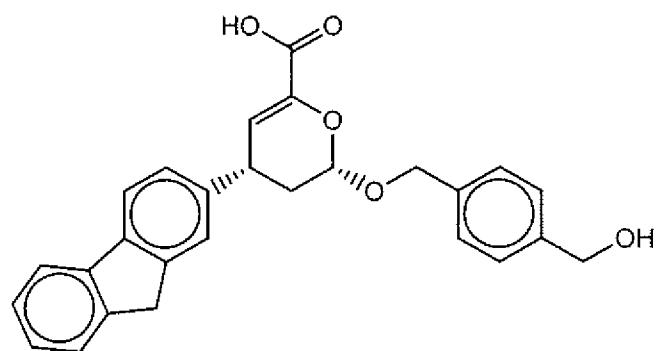
Suitable compounds of Family XVI have the following structures:



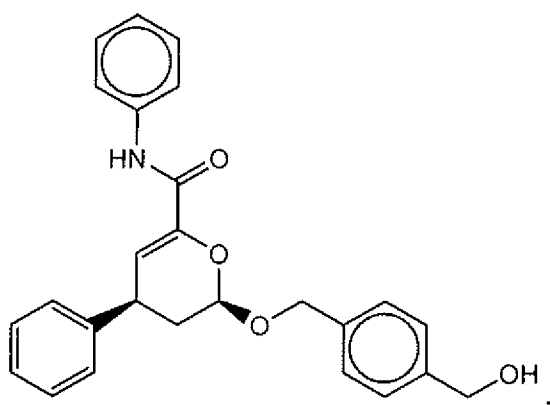
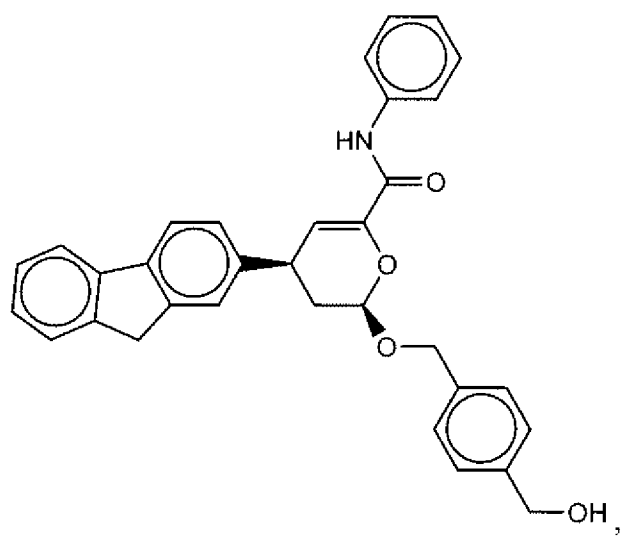
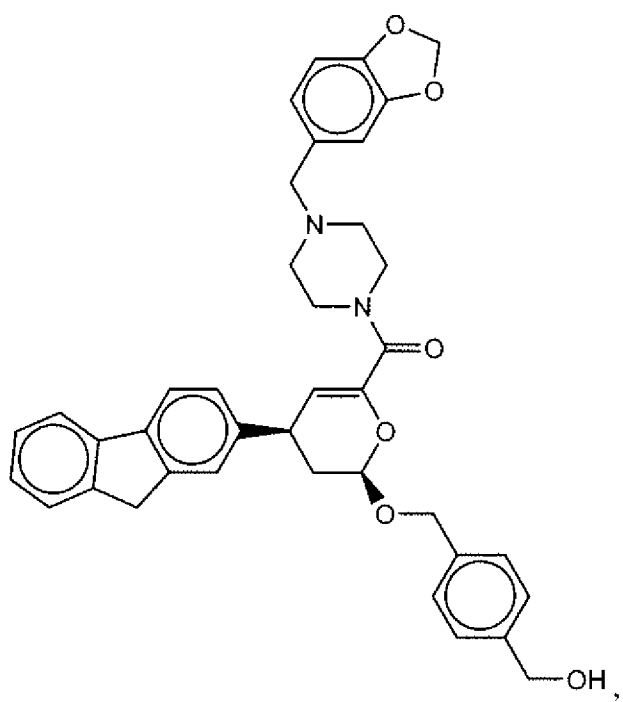
- 56 -



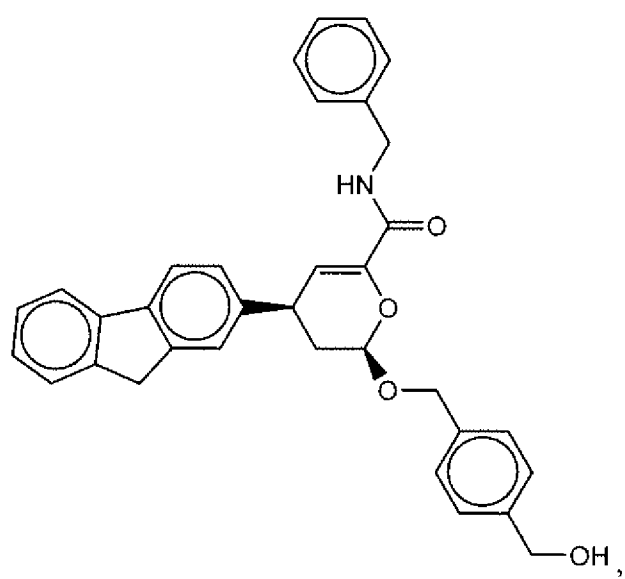
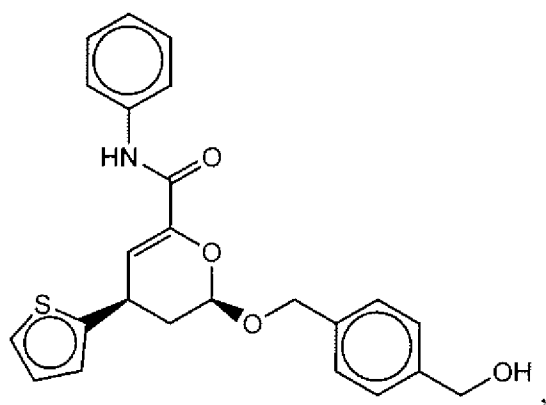
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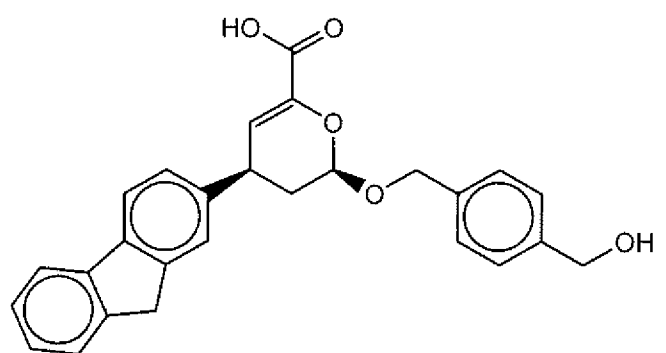
- 57 -



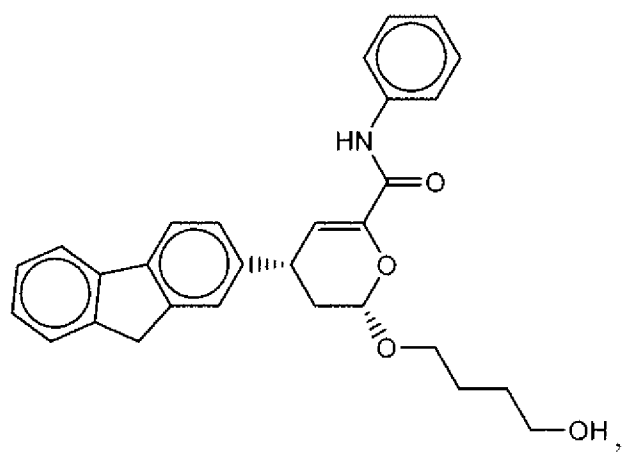
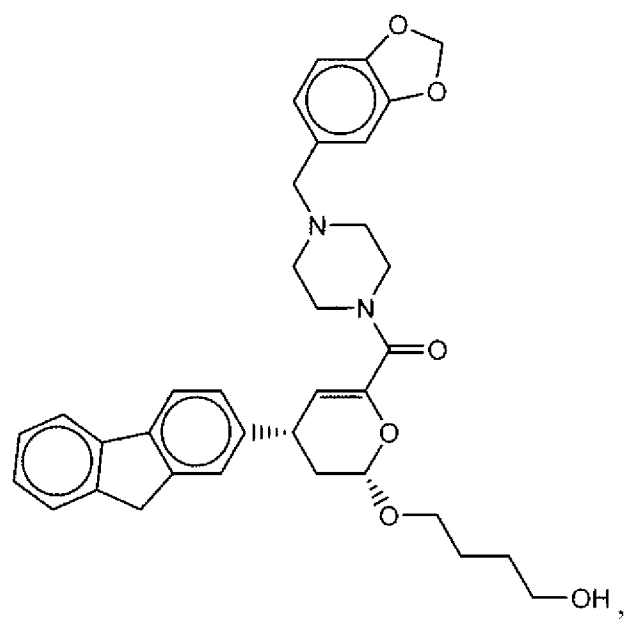
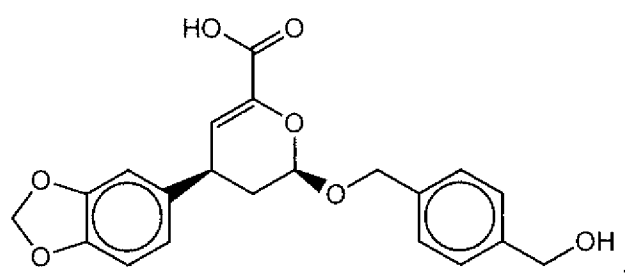
- 58 -



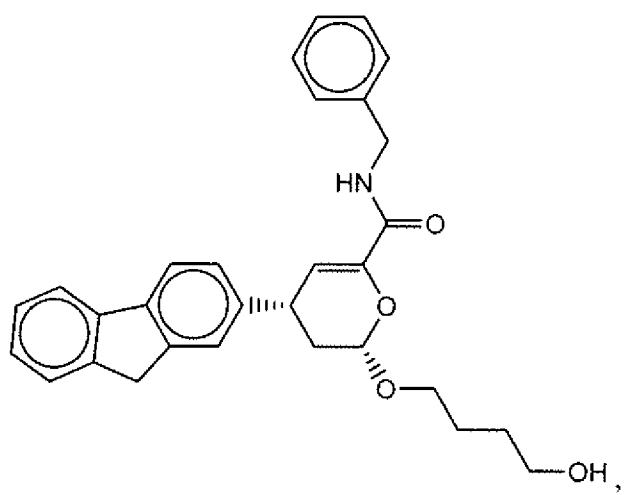
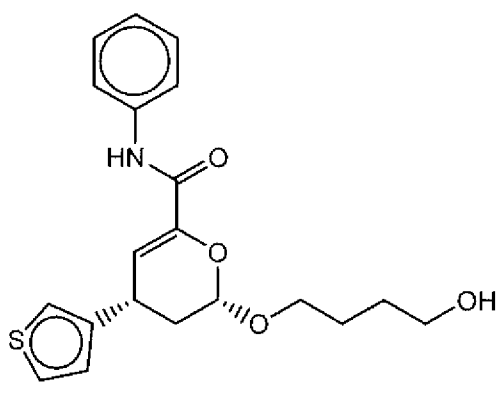
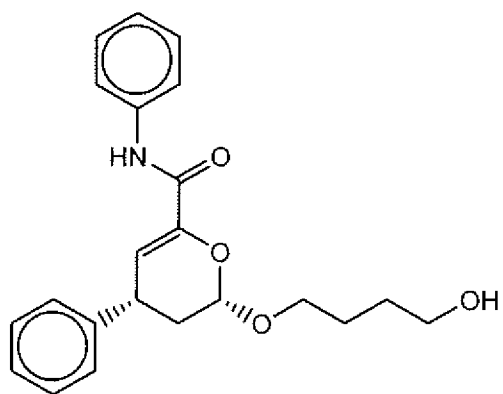
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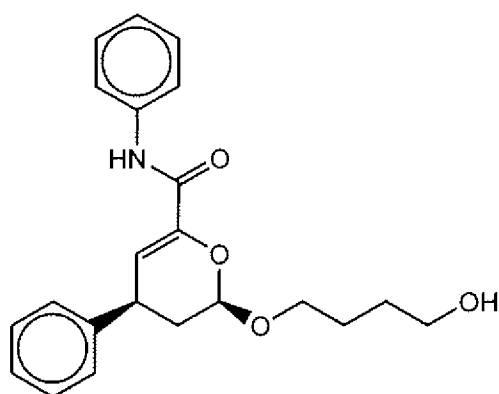
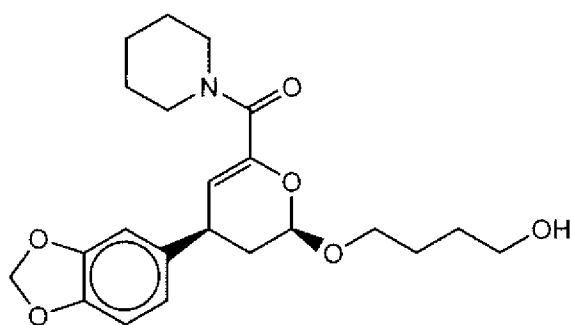
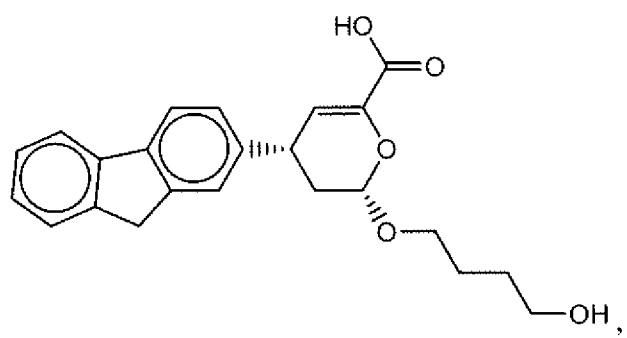
- 59 -



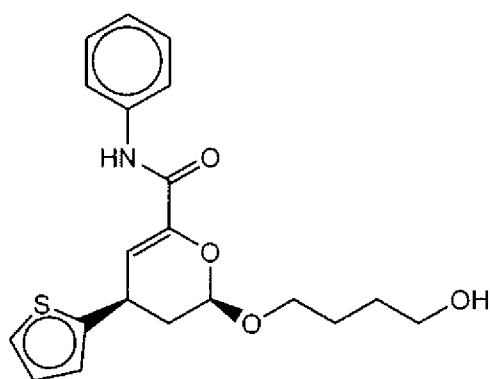
- 60 -



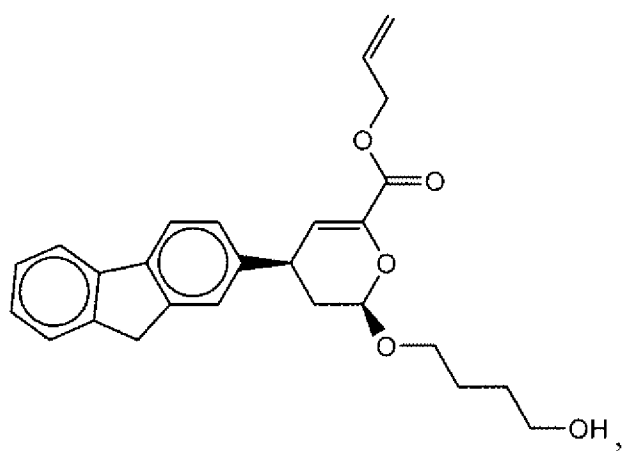
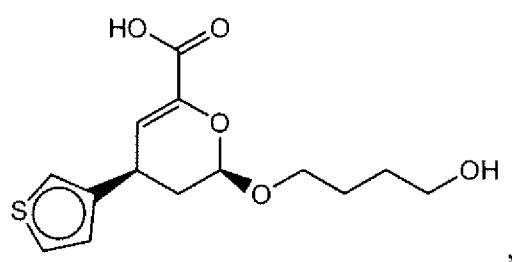
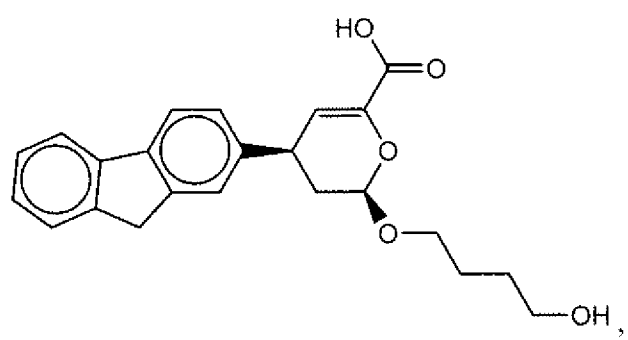
- 61 -



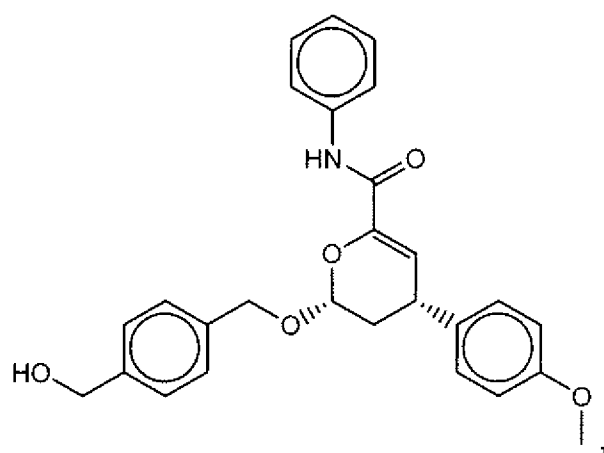
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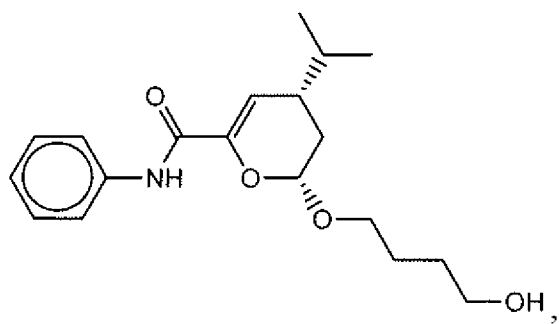
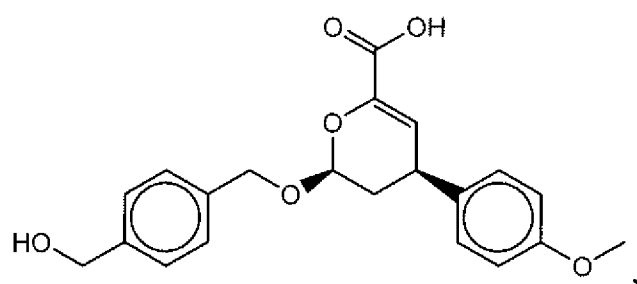
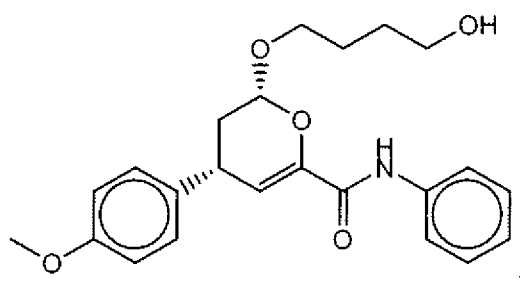
- 62 -



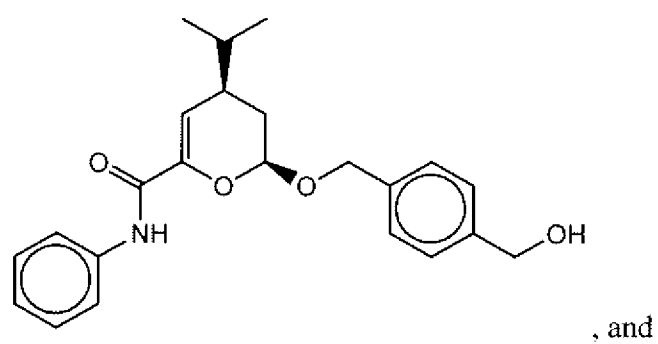
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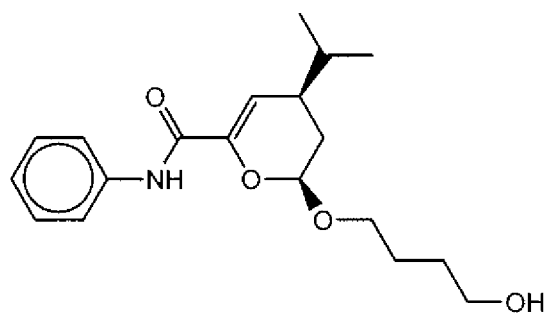
- 63 -



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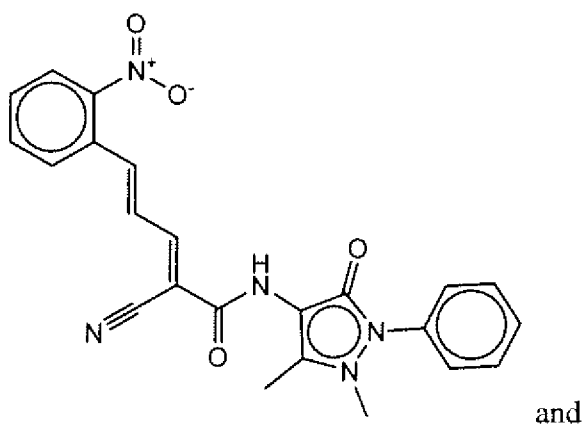


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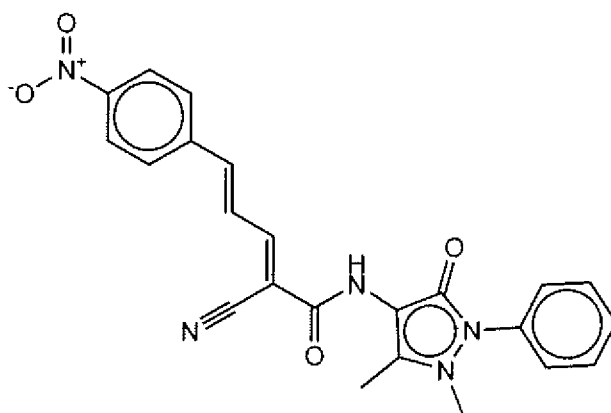


[0036]

Suitable compounds of Family XVII have the following structures:

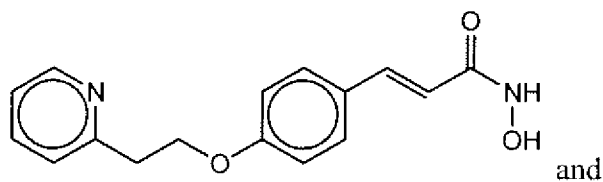


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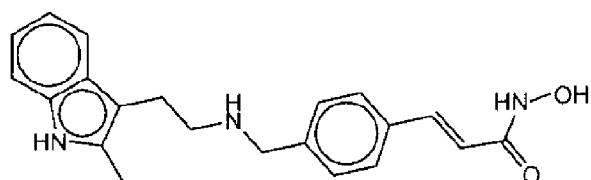
[0037]

Suitable compounds of Family XVIII have the following structures:

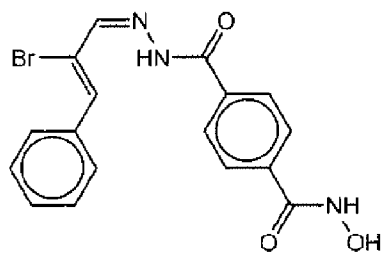


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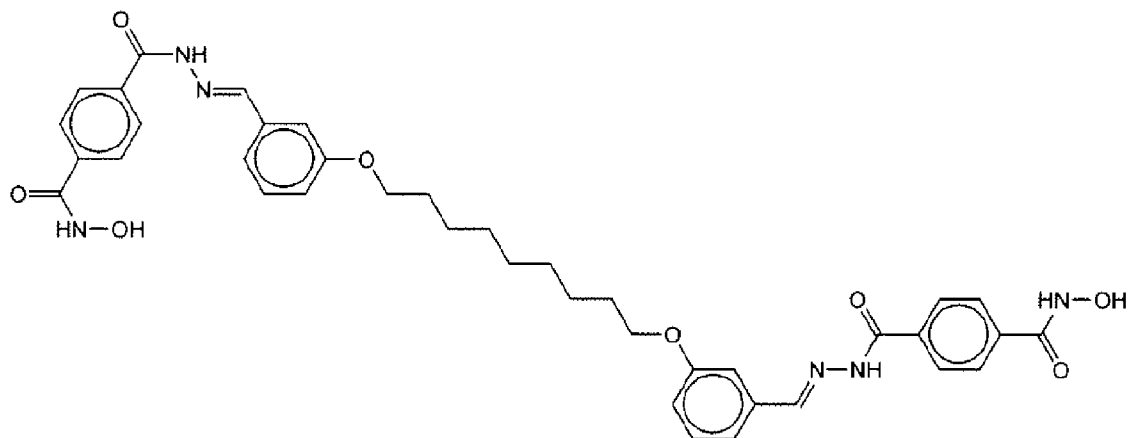
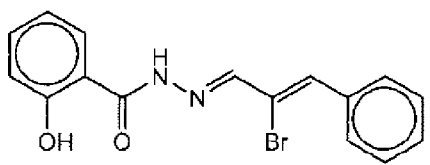
- 65 -



[0038] Suitable compounds of Family XIX have the following structures:

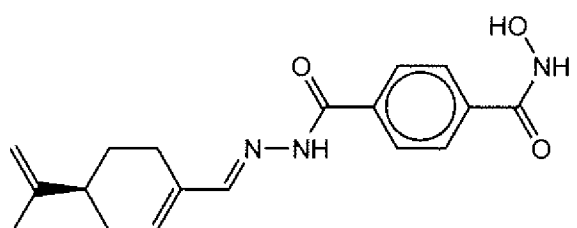
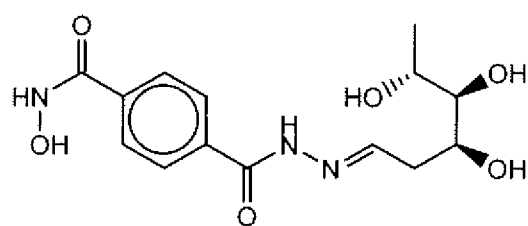
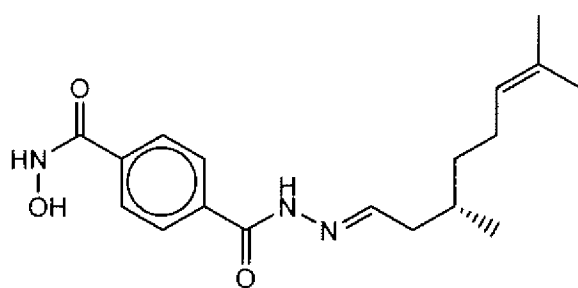
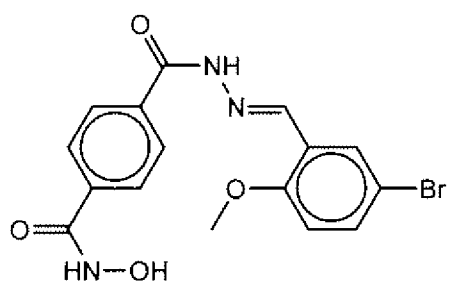
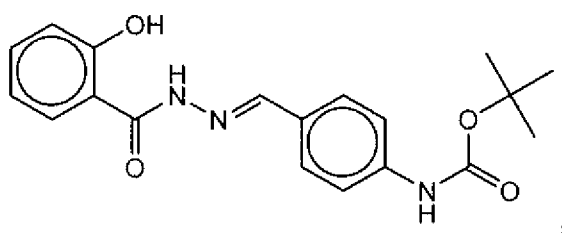


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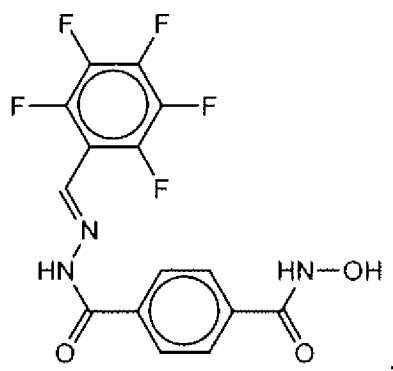
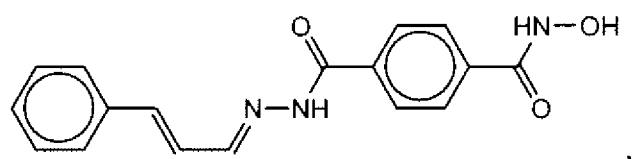
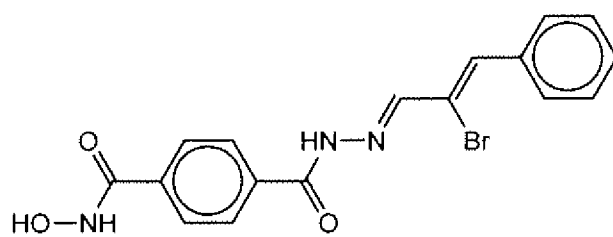
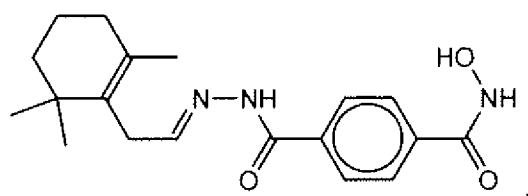
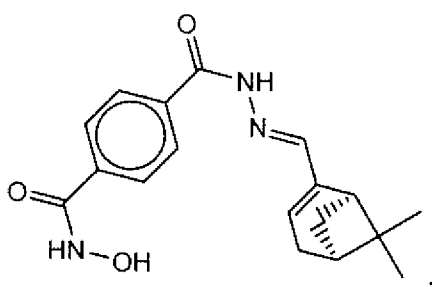


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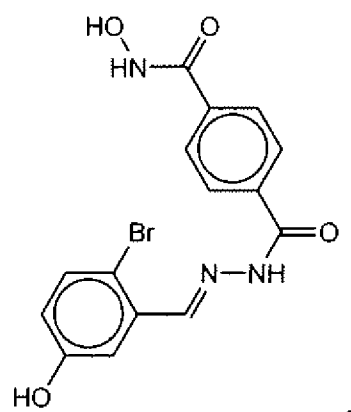
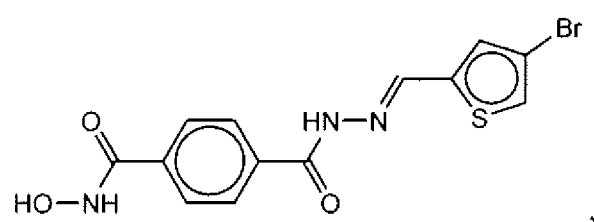
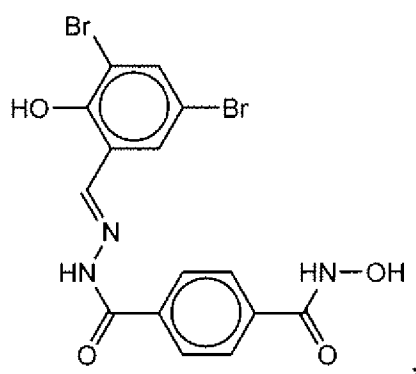
- 67 -



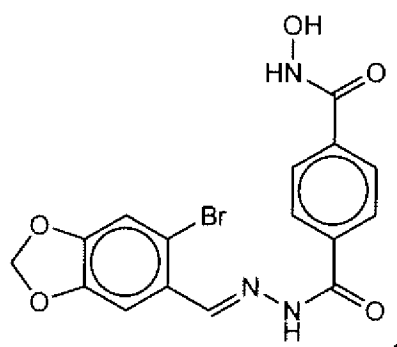
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10

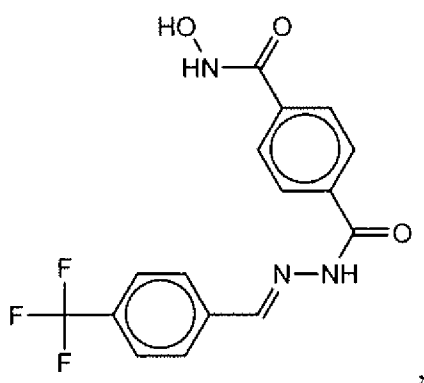
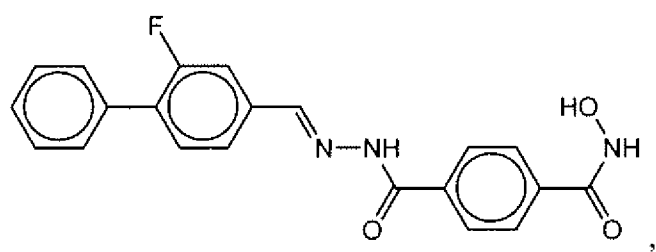
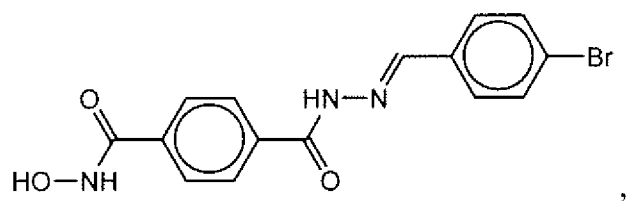
- 68 -



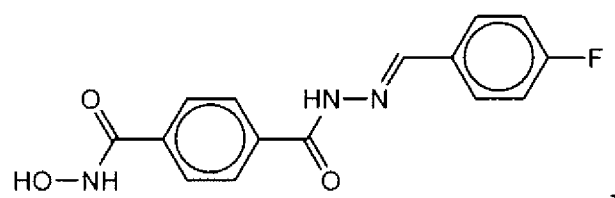
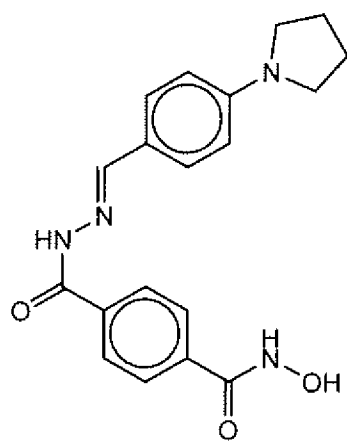
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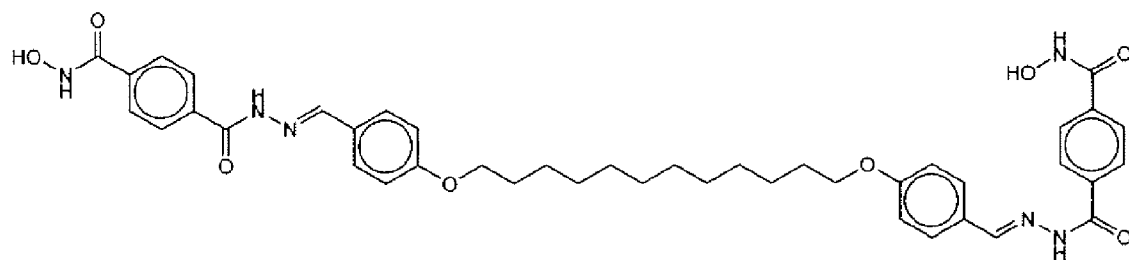
- 69 -



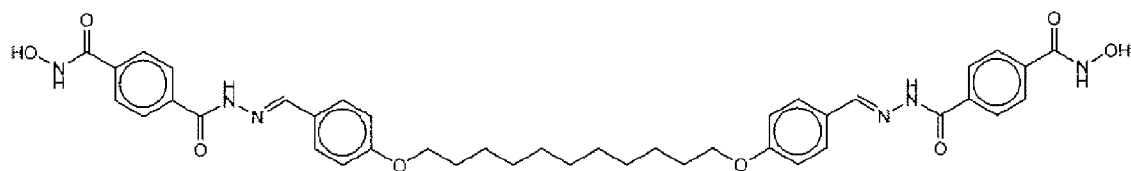
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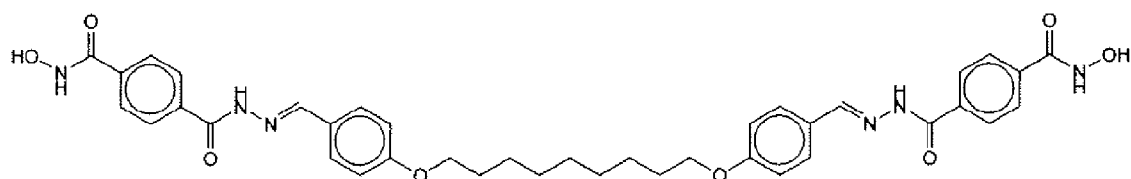
- 70 -



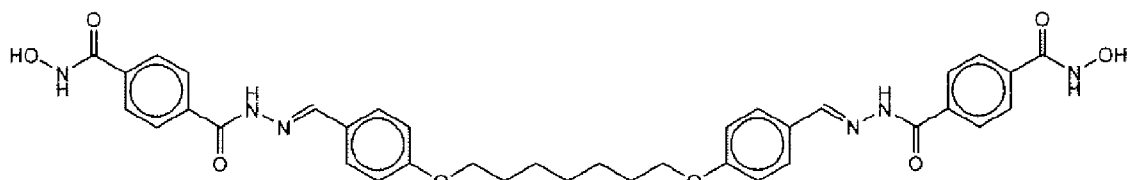
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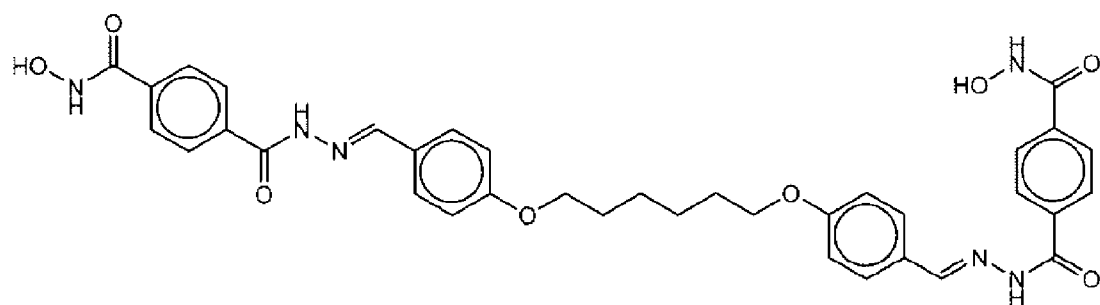
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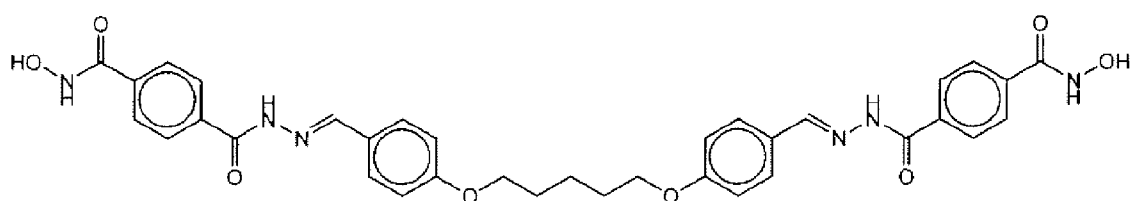
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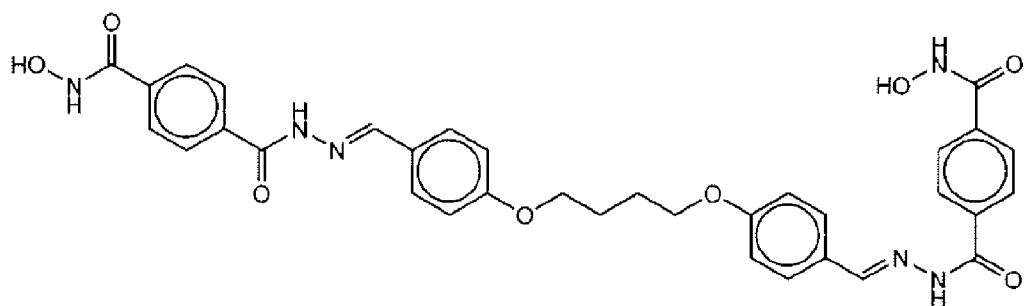


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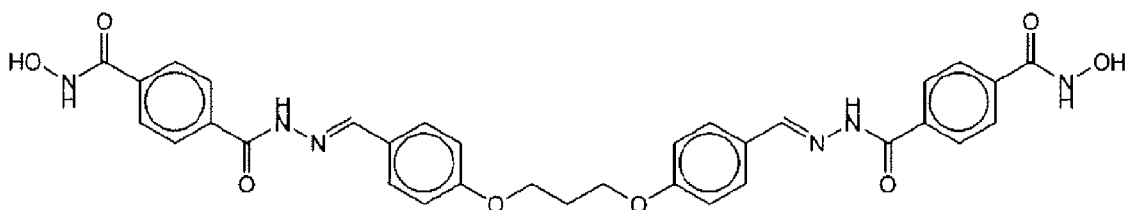
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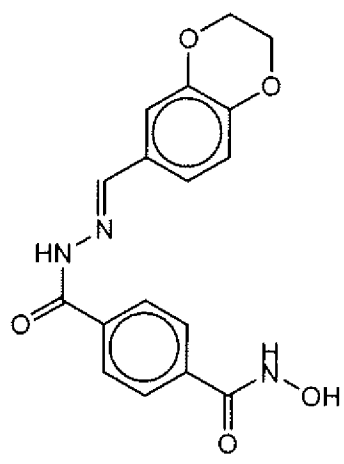
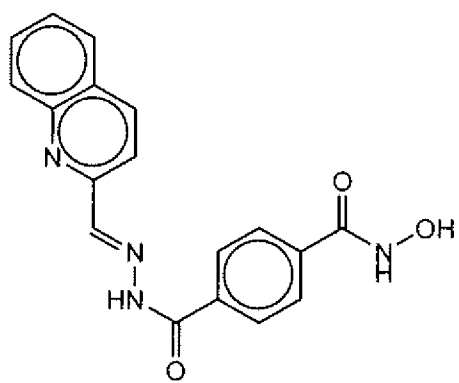
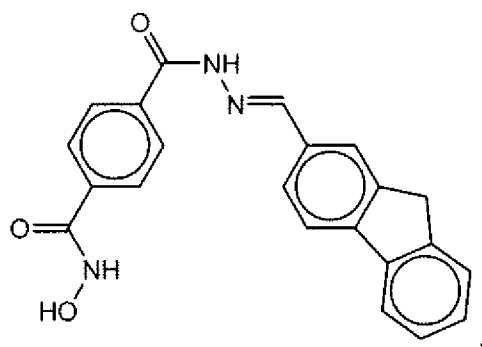
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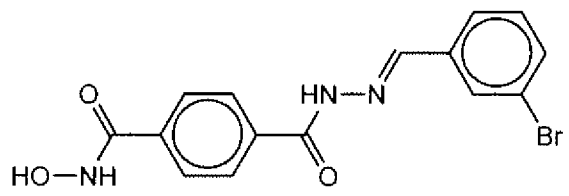


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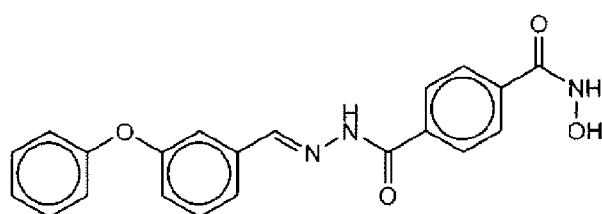
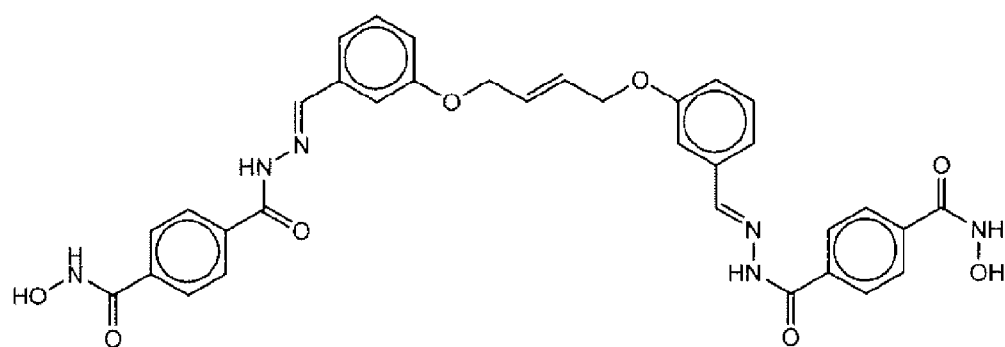
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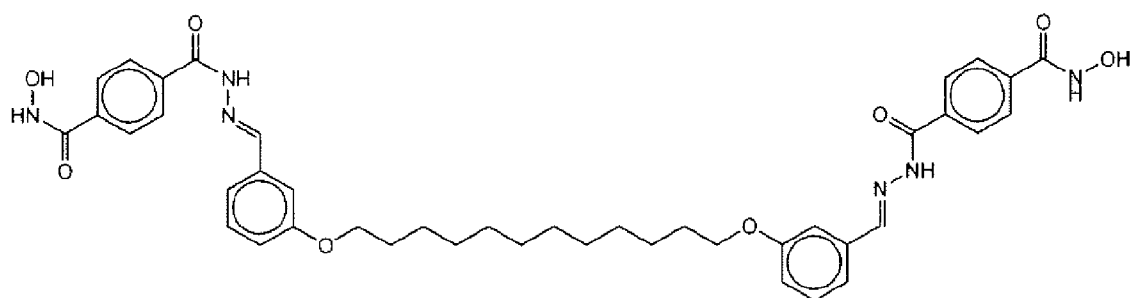
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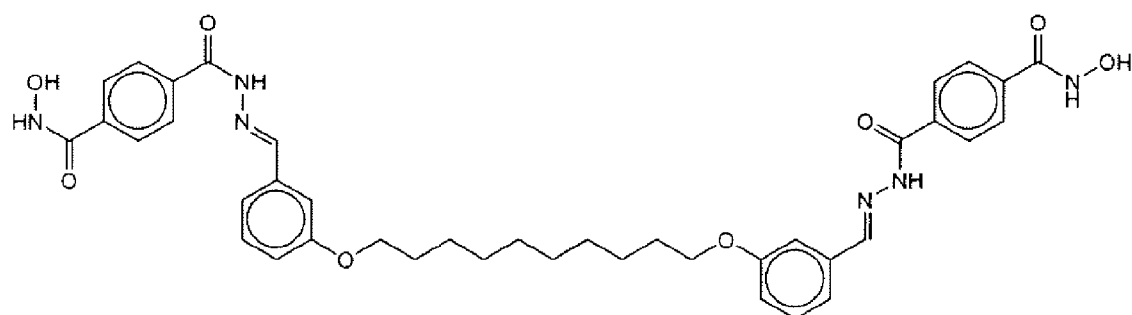
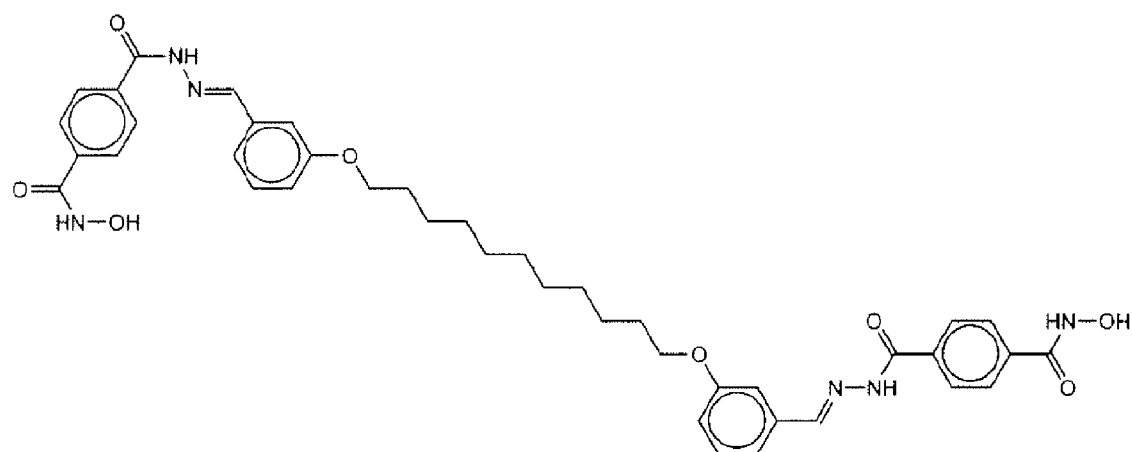
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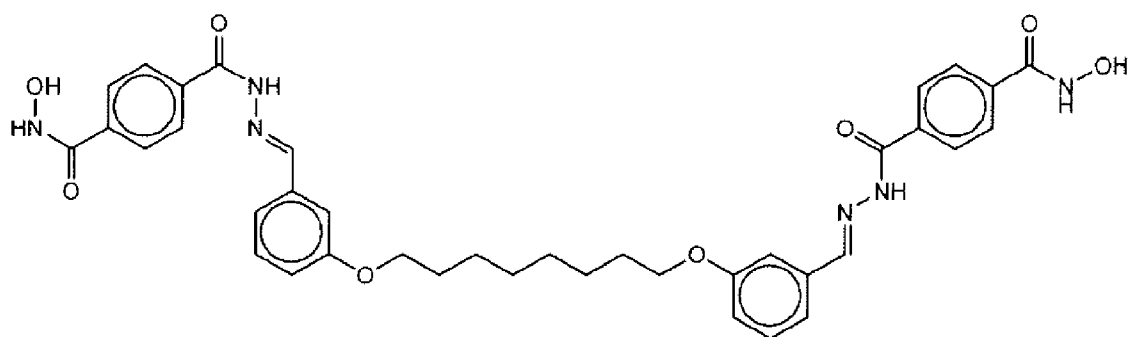
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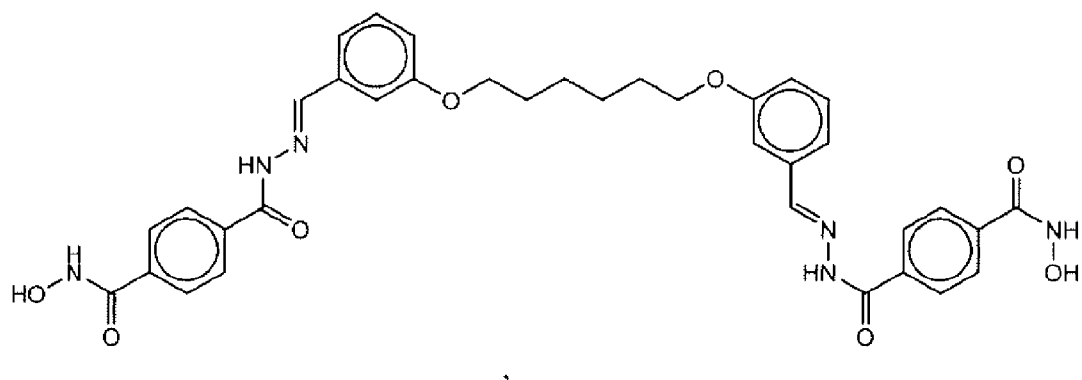
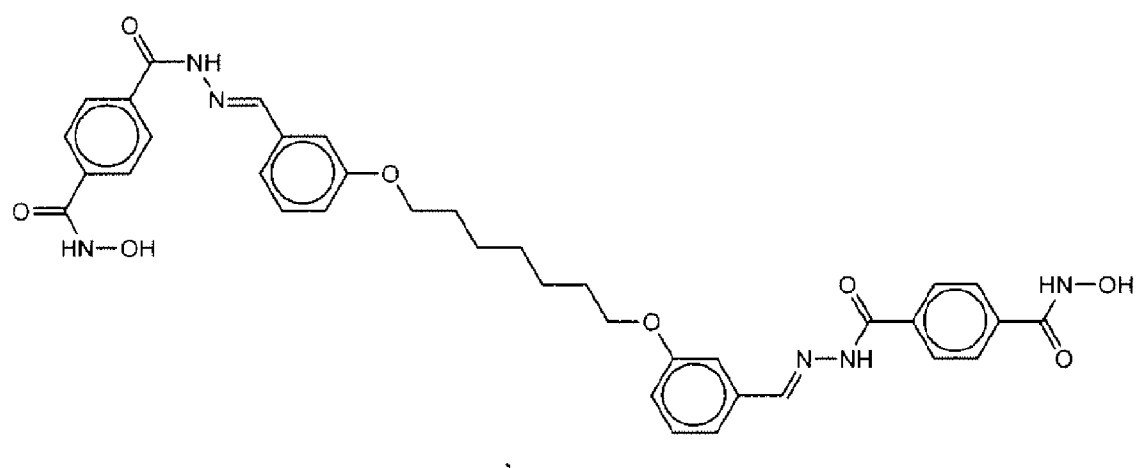
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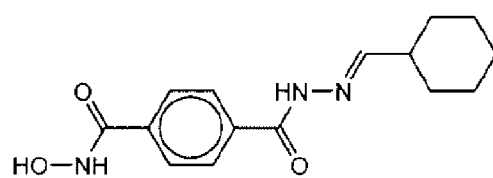
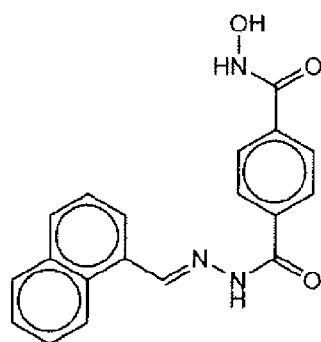
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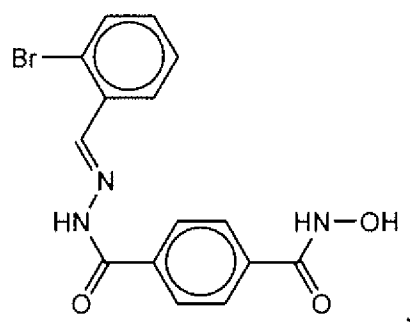
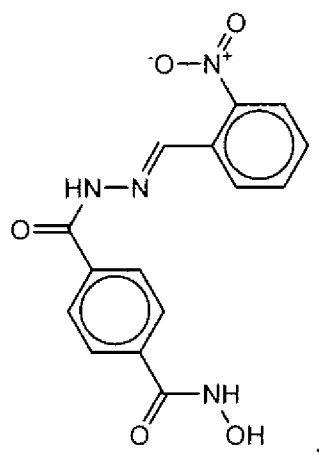
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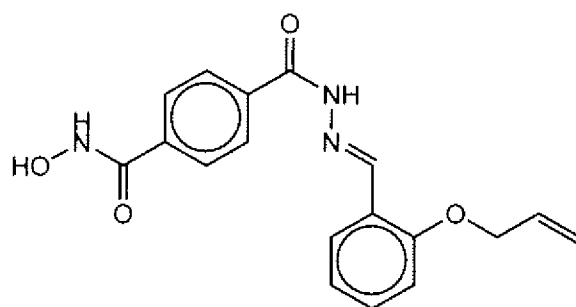
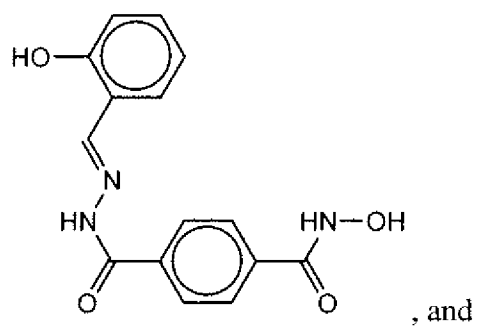
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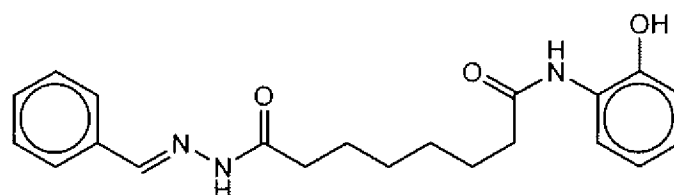
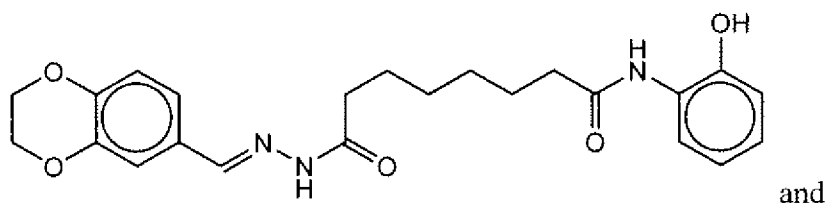


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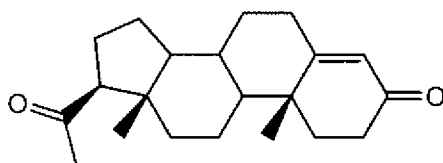
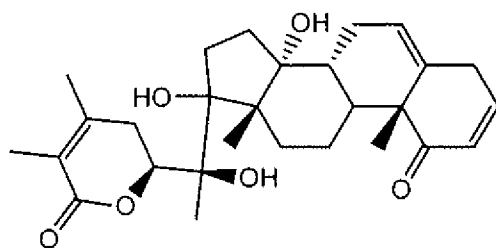
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[0039] Suitable compounds of Family XX have the following structures:



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[0040] Suitable compounds of Family XXI have the following structures:



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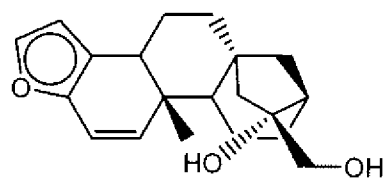
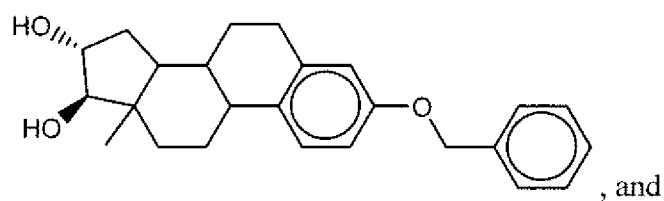
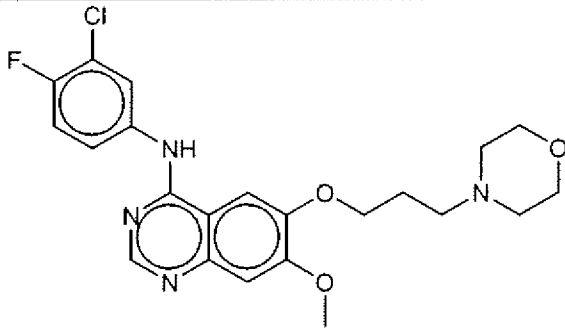
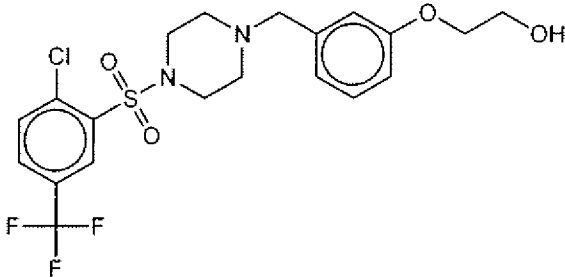
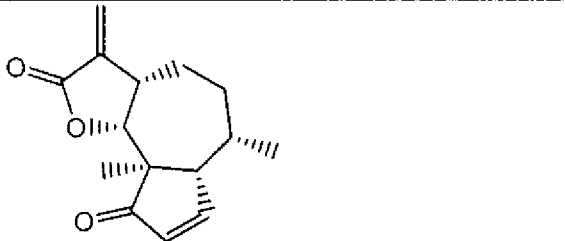
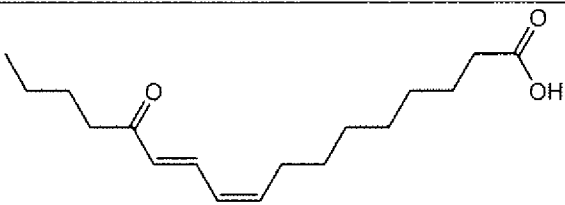
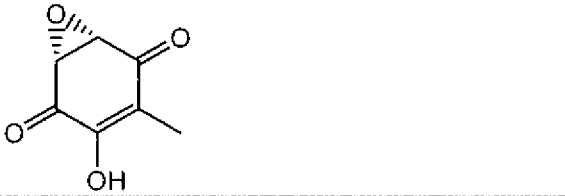
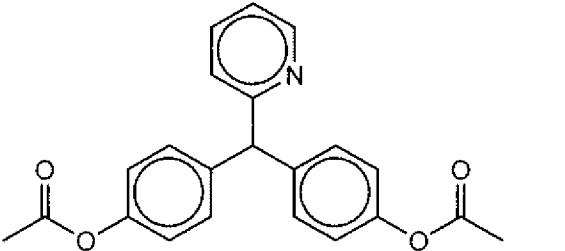
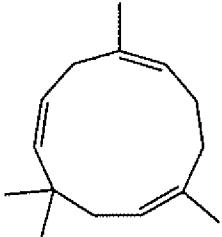
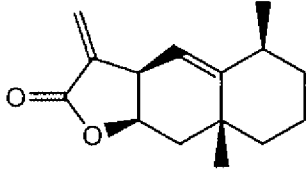
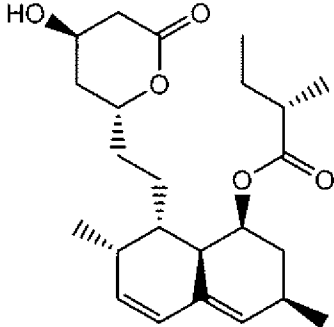
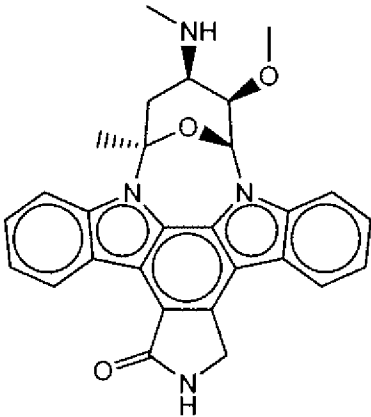
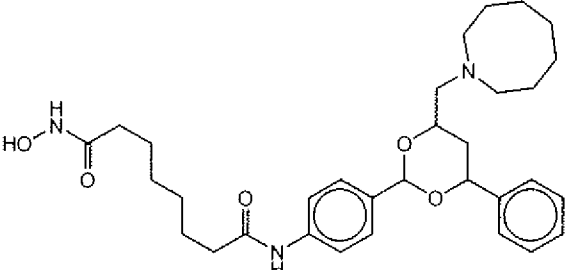
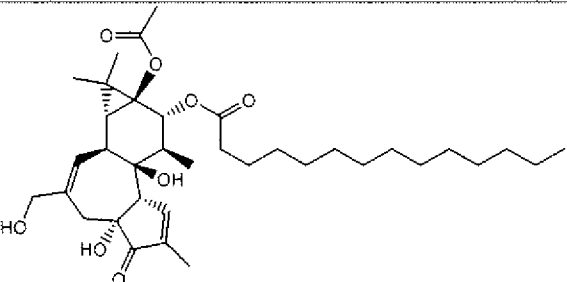


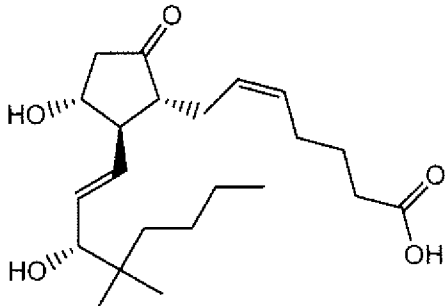
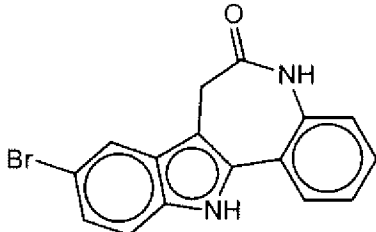
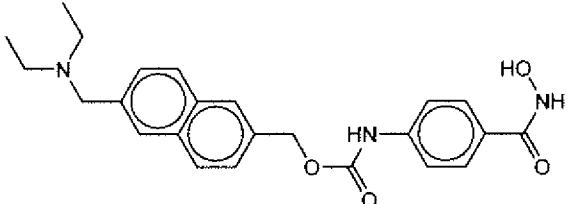
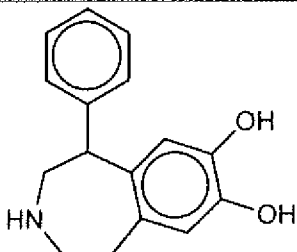
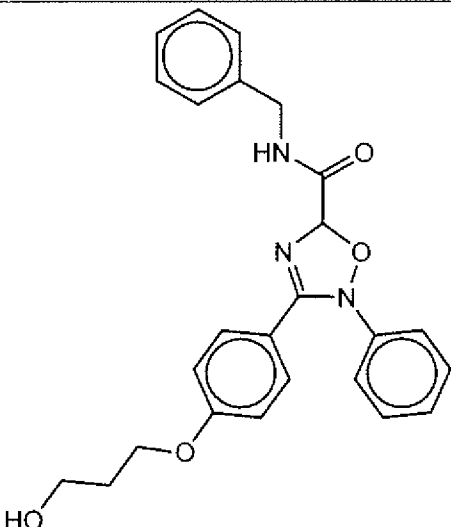
Table 2 - Other Suitable Compounds

Compound 1	
Compound 2	
Compound 3	
Compound 4	
Compound 5	
Compound 6	

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Compound 7	
Compound 8	
Compound 9	
Compound 10	
Compound 11	
Compound 12	

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Compound 13	
Compound 14	
Compound 15	
Compound 16	
Compound 17	

[0041] The compounds of the present invention can be administered orally, parenterally, for example, subcutaneously, intravenously, intramuscularly,

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intraperitoneally, by intranasal instillation, by inhalation, or by application to mucous membranes, such as, that of the nose, throat, and bronchial tubes. They may be administered alone or with suitable pharmaceutical carriers, and can be in solid or liquid form such as, tablets, capsules, powders, solutions, suspensions, or emulsions.

5 **[0042]** The active compounds of the present invention may be orally administered, for example, with an inert diluent, or with an assimilable edible carrier, or they may be enclosed in hard or soft shell capsules, or they may be compressed into tablets, or they may be incorporated directly with the food of the diet. For oral therapeutic administration, these active compounds may be incorporated with
10 excipients and used in the form of tablets, capsules, elixirs, suspensions, syrups, and the like. Such compositions and preparations should contain at least 0.1% of active compound. The percentage of the compound in these compositions may, of course, be varied and may conveniently be between about 2% to about 60% of the weight of the unit. The amount of active compound in such therapeutically useful compositions
15 is such that a suitable dosage will be obtained. Preferred compositions according to the present invention are prepared so that an oral dosage unit contains between about 1 and 250 mg of active compound.

[0043] The tablets, capsules, and the like may also contain a binder such as gum tragacanth, acacia, corn starch, or gelatin; excipients such as dicalcium
20 phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose, or saccharin. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier, such as a fatty oil.

[0044] Various other materials may be present as coatings or to modify the
25 physical form of the dosage unit. For instance, tablets may be coated with shellac, sugar, or both. A syrup may contain, in addition to active ingredient, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye, and flavoring such as cherry or orange flavor.

[0045] These active compounds may also be administered parenterally.
30 Solutions or suspensions of these active compounds can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be

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prepared in glycerol, liquid polyethylene glycols, and mixtures thereof in oils.

Illustrative oils are those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, or mineral oil. In general, water, saline, aqueous dextrose and related sugar solution, and glycols such as, propylene glycol or

5 polyethylene glycol, are preferred liquid carriers, particularly for injectable solutions. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

[0046] The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous
10 preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol
15 (e.g., glycerol, propylene glycol, and liquid polyethylene glycol), suitable mixtures thereof, and vegetable oils.

[0047] The compounds of the present invention may also be administered directly to the airways in the form of an aerosol. For use as aerosols, the compounds of the present invention in solution or suspension may be packaged in a pressurized
20 aerosol container together with suitable propellants, for example, hydrocarbon propellants like propane, butane, or isobutane with conventional adjuvants. The materials of the present invention also may be administered in a non-pressurized form such as in a nebulizer or atomizer.

[0048] The compounds of the present invention may also be administered
25 directly to the airways in the form of a dry powder. For use as a dry powder, the compounds of the present invention may be administered by use of an inhaler. Exemplary inhalers include metered dose inhalers and dry powdered inhalers. A metered dose inhaler or "MDI" is a pressure resistant canister or container filled with a product such as a pharmaceutical composition dissolved in a liquefied propellant or
30 micronized particles suspended in a liquefied propellant. The correct dosage of the composition is delivered to the patient. A dry powder inhaler is a system operable

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with a source of pressurized air to produce dry powder particles of a pharmaceutical composition that is compacted into a very small volume. For inhalation, the system has a plurality of chambers or blisters each containing a single dose of the pharmaceutical composition and a select element for releasing a single dose .

- 5 **[0049]** Suitable powder compositions include, by way of illustration, powdered preparations of the active ingredients thoroughly intermixed with lactose or other inert powders acceptable for intrabronchial administration. The powder compositions can be administered via an aerosol dispenser or encased in a breakable capsule which may be inserted by the patient into a device that punctures the capsule
10 and blows the powder out in a steady stream suitable for inhalation. The compositions can include propellants, surfactants and co-solvents and may be filled into conventional aerosol containers that are closed by a suitable metering valve.

EXAMPLES

15 **Example 1 -- Cell Lines**

- [0050]** B16 murine melanoma cells expressing firefly luciferase were used as the parental line for experiments described herein (Murakami et al., *Cancer Res.* 62:7328 (2002), which is hereby incorporated by reference in its entirety). Human melanoma UACC 1273 and M92047 cell lines are as described in Bittner et al.,
20 *Nature* 406:536 (2000), which is hereby incorporated by reference in its entirety). The human melanoma cell lines Mel375, A2058, Mel 29.6 and Mel501 were obtained from Fred Hutchinson Cancer Research Institute; Seattle, WA. The murine cell line HT22, a subclone of the HT4 hippocampal cell line, was obtained from The Salk Institute for Biological Studies. Sequences for human WNT3A and WNT5A were
25 amplified by polymerase chain reaction (PCR) and cloned into third generation lentiviral vectors derived from backbone vectors (Dull et al., *J. Virol.* 72:8463 (1998), which is hereby incorporated by reference in its entirety). These lentiviral vectors contained an EF 1-alpha promoter driving a bi-cistronic message encoding human Wnt isoforms plus GFP. Cells were sorted by fluorescence activated cell sorting
30 (FACS) for GFP expression, with the goal of obtaining cells with approximately equivalent levels of GFP expression.

Example 2 -- Cell culture

[0051] B16 murine melanoma cells were cultured in Dulbeccos modified Eagle's media (DMEM) supplemented with 2% Fetal Bovine Serum, and 1% antibiotic/antimycotic (Invitrogen; Grand Island, NY) (Murakami et al., *Cancer Res.* 62:7328 (2002), which is hereby incorporated by reference in its entirety). The human melanoma lines Mel375, M92047, A2058, Mel 29.6, Mel501 and Mel526 were cultured in DMEM supplemented with 2% FBS and 1% antibiotic/antimycotic. UACC1273 cells were cultured in RPMI (Invitrogen; Grand Island, NY) supplemented with 2% FBS and 1% antibiotic/antimycotic. All cell lines were cultured in the presence of 0.02% Plasmocin (InvivoGen; San Diego, CA). Synthetic siRNAs (Invitrogen; Grand Island, NY) were transfected into cultured cells at a final concentration of 20nM using Lipofectamine 2000 (Invitrogen; Grand Island, NY). HT22 cells were cultured in DMEM supplemented with 10% FBS and 1% antibiotic/antimycotic. Sequences for β -catenin siRNA are described in Figure 8.

Example 3 -- Conditioned Media and Measurement of Wnt Pathway Activation Using a Reporter Assay

[0052] Conditioned media was collected from sub-confluent melanoma cell lines, and this media was tested for its ability to activate Wnt/ β -catenin signaling in UACC1273 cells stably transduced with a previously described Wnt/ β -catenin-responsive firefly luciferase reporter and a constitutive *Renilla* luciferase gene used for normalization (Major et al., *Science* 316:1043 (2007), which is hereby incorporated by reference in its entirety). Conditioned media from B16 melanoma cells was spun down to clear cell debris and then incubated with reporter cells overnight. Activation of the Wnt/ β -catenin reporter was measured using a dual luciferase reporter (DLR) assay kit (Promega; Madison, WI).

Example 4 -- RNA Purification From B16 Melanoma Cells and PCR Analysis

[0053] Cells were cultured for approximately 72 hours until they reached 80-90% confluency. RNA was purified using the RNeasy kit using the manufacturer's protocol (Qiagen; Maryland, MD). cDNA was synthesized using Superscript Reverse

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Transcriptase (Invitrogen; Grand Island, NY). Light Cycler FastStart DNA Master SYBR GreenI (Roche; Mannheim, Germany) was used for real-time PCR as previously described (Major et al., *Science* 316:1043 (2007), which is hereby incorporated by reference in its entirety). Quantitative PCR results presented in the manuscript are representative of experiments performed on a minimum of three biologic replicates.

Example 5 -- Cell Proliferation Assays

[0054] For cell counts by hemacytometer, cells were seeded at a uniform density (usually between 10,000 to 25,000 cells per well) in a 12 or 24 well tissue culture plate in the appropriate media. At the end of 3-7 days, cells were trypsinized, resuspended in the appropriate media and counted. Dead cells were identified by 0.4% Trypan Blue stain and excluded from hemacytometer measurements. Cell proliferation experiments were performed with a minimum of six biologic replicates. Similar results were observed for all cell lines using the MTT assay (ATCC; Manassas, VA), performed according to manufacturer's protocol. For relative cell proliferation assays of B16: GFP cells incubated with lithium chloride or sodium chloride, cell proliferation was measured by luciferase assay. Cell cycle analysis was performed using DAPI-staining and flow cytometry. The Ki-67 rabbit monoclonal antibody was purchased from ThermoFisher (catalog no. RM-9106).

Example 6 -- Immunohistochemistry and Immunoblotting Studies

[0055] A polyclonal rabbit anti- β -catenin antibody was used for detection of β -catenin (1:1000 dilution for immunoblot, 1:200 dilution for immunohistochemistry). Cells were grown on 18mm glass coverslips, for 48-72 hours, fixed using 4% paraformaldehyde, permeabilized using 0.25% Triton X- 100, and then blocked with 10% goat serum. Goat anti-rabbit Alexa Fluor-568 antibody (Molecular Probes; Eugene, OR) was diluted 1:1000. Cells were counterstained for nucleic acid with DAPI (Molecular Probes; Eugene, OR). Paraffin-embedded nevus sections were stained using an antibody dilution of 1:200. Cellular lysates were obtained by lysing cells on plate with a 0.1% NP-40 based buffer and analyzed by NuPage 4-12%

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gradient gels (Invitrogen; Grand Island, NY). The WNT5A antibody was obtained from Cell Signaling Technologies (Danvers, MA).

Example 7 -- Tumor Microarrays

5 **[0056]** Tumor microarrays were assembled at the Yale Tissue Microarray Facility. Staining and scoring of tissue microarrays was performed using automated quantification (AQUA) as previously described (Camp et al., *Nat. Med.* 8:1323 (2002), which is hereby incorporated by reference in its entirety). Statistical analysis, including Kaplan-Meier survival probabilities, ANOVA, and t-tests, was performed
10 using the GraphPad Prism software package (GraphPad Software; La Jolla, CA).

Example 8 -- cDNA Microarrays

[0057] Agilent whole mouse genome array analysis was performed through the microarray core facility at the Huntsman Cancer Institute (Salt Lake City, UT).
15 Data analysis, including the t-test (Pan, *Bioinformatics* 18:546 (2002), which is hereby incorporated by reference in its entirety) was performed using the TM4 microarray software suite, which is freely available online (Saeed et al., *Biotechniques* 34:374 (2003), which is hereby incorporated by reference in its entirety). Two-channel hybridizations were performed with labeled cDNA isolated from three
20 biologic replicates each for cells expressing either WNT3A or WNT5A, using cDNA from GFP-expressing cells as the reference sample. These studies revealed gene sets regulated in both WNT3A and WNT5A cells (Figure 7), which were then filtered to obtain the top 10% of most variant genes in the WNT3A and WNT5A datasets. Subsequently, an unpaired two-tailed t-test analysis was used to identify genes that
25 were significantly different between the most variant genes in the WNT3A and WNT5A replicate samples, using an arbitrary p-value of $p < 0.04$ as a cut-off. The rationale for further comparing the regulated genes in WNT3A cells to those in WNT5A cells was based the finding that WNT5A did not have significant phenotypic effects (pigmentation, proliferation or cell cycle), and this subsequent comparison
30 allowed identification of potentially important genes regulated by WNT3A that might be missed by setting arbitrary cut-off values for significant genes (i.e. 2-fold upregulated or 50% downregulated).

Example 9 -- High Throughput Small Molecule Screen

[0058] Compounds were dissolved in dimethylsulphoxide (DMSO). For the primary screen, performed in duplicate, HT22 cells stably expressing the beta-catenin activated reporter (BAR) were cultured in growth medium (DMEM/10% FBS/1% antibiotic). 3000 cells per well were transferred to 384-well clear bottom plates (Nalgene Nunc; Rochester, NY) in 30uL of growth medium. The following day, 100nL of compound and 10uL of either growth media or WNT3A conditioned media (E.C.₅₀ dose) was transferred to the cells. The next day each well was imaged using transmitted light with the ImageXpress Micro (Molecular devices; Sunnyvale, CA) followed by the addition of 10uL of Steady-Glo (Promega; Madison, WI) as per the manufacture's instructions, and luminescence measurement on an EnVision Multilabel plate reader (PerkinElmer; Waltham, MA). Viability was scored by analyzing the ImageXpress images. As described in detail in Seiler et al. (Seiler et al., *Nucleic Acids Res.* 36:D351 (2008), which is hereby incorporated by reference in its entirety), each compound well received an algebraically signed Z-score corresponding to the number of standard deviations it fell above or below the mean of a well-defined mock-treatment distribution of DMSO controls. Z-score normalized data from the growth media stimulus group were sorted by average percent change. The fold-increase over the background of DMSO controls for each treatment was also calculated. The top 50 compounds with the greatest percent change of activity with the growth media were then resorted based on the percent change with the WNT3A stimulus.

Example 10 -- Nuclear β -catenin Correlates With Improved Patient Survival

[0059] Using the expression of nuclear β -catenin as a clinical surrogate marker for Wnt/ β -catenin activation (Bachmann et al., *Clin. Cancer Res.* 11:8606 (2005); T. Kageshita et al., *Br. J. Dermatol.* 145:210 (2001); Maeldandsmo et al., *Clin. Cancer Res.* 9:3383 (2003), which are hereby incorporated by reference in their entirety), a tumor microarray composed of 343 cores (118 primary tumors, 225 metastases) from patient tumors (Camp et al., *Nat. Med.* 8:1323 (2002), which is hereby incorporated by reference in its entirety) was scored. Survival probabilities for

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patients were estimated using Kaplan-Meier analysis after stratifying primary tumors into tertiles based on nuclear β -catenin expression (Figure 4). This analysis reveals that higher expression of nuclear β -catenin in both primary tumors (Figure 1A) and in metastases and recurrences (Figure 1B) predicts significantly increased patient survival. Also, levels of nuclear β -catenin are lower in metastases and recurrences compared to primary tumors (Figure 4). These findings confirm and extend previous reports of improved prognosis with elevated nuclear β -catenin in melanoma (Bachmann et al., *Clin. Cancer Res.* 11:8606 (2005); T. Kageshita et al., *Br. J. Dermatol.* 145:210 (2001); Maeldandsmo et al., *Clin. Cancer Res.* 9:3383 (2003), which are hereby incorporated by reference in their entirety).

Example 11 -- Nuclear β -catenin is Negatively Correlated with Proliferation

[0060] As tumor depth measurements (Breslow thickness) were obtained for 113 primary tumors in our array cohort, this sub-group of patients was analyzed based on the Breslow thickness stratification used as reported (Thompson, J. A., *Semin. Oncol.* 29:361 (2002), which is hereby incorporated by reference in its entirety). Increasing tumor depth is correlated with a lower probability of survival (Figure 1C) and with a higher degree of proliferation, which is measured by the percentage of cells expressing Ki-67 (Figure 1D). By contrast, nuclear β -catenin levels are highest for shallow tumors (T1) and decrease significantly with increased tumor depth (Figure 1E).

[0061] The percentage of tumors staining positive is then analyzed for the cellular proliferative marker Ki-67 (%Ki-67). Strikingly, distribution histograms of %Ki-67 staining in primary tumors stratified by expression of nuclear β -catenin show a statistically significant shift towards increased proliferation (elevated %Ki-67 staining) in the groups with lower nuclear β -catenin (Figure 1F). It is shown that there is no correlation between expression of α -catenin and %Ki-67 staining, and PCNA is used as an independent marker of proliferation (Figure 4). Taken together these data demonstrate that elevated nuclear β -catenin is negatively associated with proliferation as measured by either tumor size/depth, or by the markers Ki-67 and PCNA.

Example 12 -- Activation of Wnt/ β -catenin Signaling Changes Melanoma Cell Fate

[0062] Wnts, which can activate or antagonize β -catenin signaling, were
5 investigated in order to elicit changes in melanoma cells cultured *in vitro* that might
be consistent with the above clinical data. Since melanoma tumors appear to express
WNT3A (Figure 5), which has a pivotal role in the regulation of melanocyte biology
(Dorsky et al., *Genes Dev.* 14:158 (2000); Fang et al., *Stem Cells* 24:1668 (2006),
which are hereby incorporated by reference in their entirety), and they express
10 WNT5A, which is elevated in melanoma metastases (Bittner et al., *Nature* 406:536
(2000); Weeraratna et al., *Oncogene* 23:2264 (2004), which are hereby incorporated
by reference in their entirety), B16 mouse melanoma cells were transduced with
lentivirus constructs encoding WNT3A, WNT5A, or a GFP control.

[0063] B16:WNT3A cells exhibit strikingly increased pigmentation compared
15 to GFP or WNT5A cells (Figure 2A). Scoring cells for nuclear accumulation of β -
catenin revealed that only cells expressing WNT3A, and not WNT5A or GFP, exhibit
elevated β -catenin (Figure 2C). As a positive control it was shown that conditioned
media (CM) from B16 cells expressing WNT3A activates a β -catenin-responsive
reporter in UACC1273 melanoma cells (Figure 2D), confirming that these cells were
20 secreting active WNT3A. Also, it was shown that B16 cells expressing WNT3A
exhibit marked increases in expression of the β -catenin target gene *Axin2* (Jho et al.,
Mol. Cell Biol. 22:1172 (2002), which is hereby incorporated by reference in its
entirety) compared to B16:GFP cells (Figure 2E).

[0064] *In vitro* cell proliferation studies using the MTT cell proliferation assay
25 showed that B16 cells expressing WNT3A exhibit decreased proliferation compared
to cells expressing GFP or WNT5A (Figure 2F). This finding was paralleled in
human cell lines (Figure 5). Cell cycle profiles were then compared to the Wnt-
transduced melanoma cell lines, and found that cells expressing WNT3A exhibit an
increased population in G1, with a decreased population in S phase, compared to
30 control cells (Figure 2G). Together, these data suggest that WNT3A can induce
differentiation of the melanoma cells to a cell fate that is more pigmented and less
proliferative.

Example 13 -- Elevation of Melanocyte Differentiation Markers by *WNT3A*

[0065] Next, a genome-wide transcriptional profiling was performed to gain further insights into the consequences of expression of WNT3A and WNT5A, which revealed that levels of transcripts elevated by WNT3A were actually reduced by WNT5A (Figure 3B). Among the most highly significant genes elevated by WNT3A (Figure 3A) are *Axin2* (Jho et al., *Mol. Cell Biol.* 22:1172 (2002), which is hereby incorporated by reference in its entirety) and *Tcf7* (Roose et al., *Science* 285:1923 (1999), which is hereby incorporated by reference in its entirety), which are direct targets of Wnt/ β -catenin signaling; *Mme* and *Mlze*, downregulated genes previously linked to melanoma progression (Watabe et al., *Jpn. J. Cancer Res.* 92:140 (2001); Bilalovic et al., *Mod. Pathol.* 17:1251 (2004), which are hereby incorporated by reference in their entirety); *Mitf*, linked to pigment cell fate, and *Trpm1*, *Met*, *Sox9* and *Kit*, which are highly expressed during melanocyte and neural crest development (Loftus et al., *Proc. Natl. Acad. Sci. U S A* 96:9277 (1999, which is hereby incorporated by reference in its entirety)). To confirm the array data levels of selected transcripts were measured by quantitative PCR (Figure 3B). To establish that the effects of WNT3A on gene expression were specific, it was demonstrated that the changes in gene expression were antagonized by β -catenin siRNA (Figure 3C). The transcriptional profiling thus supports the conclusion, evident from visual examination of cells (Figure 2A), that WNT3A promotes melanoma cells adopting characteristics of melanocyte differentiation.

Example 14 -- *WNT3A* Reduces Melanoma Tumor Size and Metastasis in Mice

[0066] While expression of *Trpm1* was elevated by WNT3A (Figure 3B), its expression is usually reduced during melanoma progression. Taken with the observed changes in cell fate and proliferation seen in cells expressing WNT3A, this led to the prediction that cells expressing WNT3A would form less proliferative and less aggressive tumors in vivo. Indeed, implantation of WNT3A-transduced B16 cells into the footpads of C57BL/6 mice, significantly decreased tumor growth compared to B16 cells transduced with GFP or WNT5A (Figure 3D) and decreased metastases to popliteal lymph nodes (Figure 3E).

Example 15 -- A High-throughput Screen for Therapeutic Activators of Wnt/ β -catenin Signaling.

[0067] In support of the hypothesis that activation of Wnt/ β -catenin inhibits melanoma growth, treatment of B16 cells with the GSK3 inhibitors lithium chloride (LiCl) or 6-bromoindirubin-3'-oxime (BIO) also resulted in decreased proliferation of cultured cells (Figure 6). Precluding further consideration of either compound in murine therapeutic trials it was found that lithium and BIO exhibit several obstacles relating to both toxicity and, in the case of lithium, to difficulty maintaining adequate serum levels in mice. Consequently, identifying novel activators of Wnt/ β -catenin signaling that would have improved therapeutic efficacy and tolerance became a target.

[0068] A high-throughput screen of >60% of the FDA-approved panel of biologically active small molecules was performed using a Wnt-responsive luciferase reporter system to identify compounds that could either activate Wnt/ β -catenin signaling on their own, or synergize with WNT3A to enhance reporter activation.

[0069] Data in support of a novel "differentiation therapy" for treating melanoma with agents that activate β -catenin signaling are presented in Figure 8. Mechanistically, it is proposed that activating Wnt/ β -catenin signaling forces melanoma cells to adopt a more differentiated cell fate, resembling melanocytes, which are intrinsically less motile, less proliferative, and thus less deadly. The concept that different states of differentiation and pluripotency exist in melanoma is not new, and in fact recent research has focused on markers that may better identify so-called melanoma stem cells, or tumor initiating cells (Zabierowski et al., *Cancer Cell* 13:185 (2008); Schatton et al., *Nature* 451:345 (2008); Grichnik et al., *J. Invest. Dermatol.* 126:142 (2006), which are hereby incorporated by reference in their entirety). The forced differentiation of these tumor initiating populations into cell fates that are either more benign (i.e. slower growing, or less metastatic) or more treatable provides a new approach that could prove beneficial in concert with current therapies based on cytotoxicity or immunomodulation.

[0070] For the activation of β -catenin signaling to be considered as a therapy one would need reasonable assurance that enhancing Wnt/ β -catenin signaling in

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melanoma will not have the undesirable consequence of promoting proliferation. In support of the present data, a recent study found that expression of a stabilized β -catenin mutant (β -cat^{STA}) in mice did not increase proliferation of melanocytic cells, which is entirely consistent with our findings (Delmas et al., *Genes Dev.* 21:2923 (2007), which is hereby incorporated by reference in its entirety). This study also found that restricting the expression of β -cat^{STA} to melanocytes did not lead to any melanomas over a 2-year period (Delmas et al., *Genes Dev.* 21:2923 (2007), which is hereby incorporated by reference in its entirety). Additionally, other published reports (Ballin et al., *Br. J. Cancer* 48:83 (1983); Penso et al., *Mol. Genet. Metab.* 78:74 (2003); Kang et al., *Arch. Dermatol. Res.* 294:426 (2002), which are hereby incorporated by reference in their entirety) confirm our observed inhibition of melanoma cell proliferation upon treatment with lithium chloride, which is a pharmacologic activator of Wnt/ β -catenin signaling. Available data therefore strongly suggest that activation of Wnt/ β -catenin signaling is not by itself oncogenic in the context of melanoma.

[0071] Herein, it is suggested that future studies should consider a combination therapy in which activation of Wnt/ β -catenin signaling forces higher expression of *Kit*, which may increase their sensitivity to imatinib or sunitinib. Given that current therapeutic strategies have proven largely ineffective for metastatic melanoma, a “differentiation therapy” involving activators of β -catenin signaling, used as monotherapy or in combination therapy, may provide a new alternative for treating this disease.

[0072] Although preferred embodiments have been depicted and described in detail herein, it will be apparent to those skilled in the relevant art that various modifications, additions, substitutions, and the like can be made without departing from the spirit of the invention and these are therefore considered to be within the scope of the invention as defined in the claims which follow.

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WHAT IS CLAIMED:

1. A method of treating a subject for a condition mediated by aberrant Wnt/ β -catenin signaling, said method comprising:
selecting a subject with a condition mediated by aberrant Wnt/ β -catenin signaling and
administering to the selected subject a compound selected from the group consisting of those set forth in Table 1, Table 2, and a pharmaceutically acceptable salt thereof.
2. The method of claim 1, wherein the subject is human.
3. The method of claim 1, wherein a compound of Table 1 or a pharmaceutically acceptable salt thereof is administered.
4. The method of claim 1, wherein a compound of Table 2 or a pharmaceutically acceptable salt thereof is administered.
5. The method of claim 1, wherein the condition is selected from the group consisting of cancer, bone mass diseases, fracture repair, FEVR, diabetes mellitus, cord blood transplants, psychiatric disease, neurodegenerative disease, hair loss, diseases linked to loss of stem/progenitor cells, conditions improved by increasing stem/progenitor cell populations, HIV, and tooth agenesis.
6. A method of activating the Wnt/ β -catenin pathway in a subject comprising:
selecting a subject in need of Wnt/ β -catenin pathway activating and
administering to the selected subject a compound selected from the group consisting of those set forth in Table 1, Table 2, and a pharmaceutically acceptable salt thereof.

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7. The method of claim 6, wherein a compound of Table 1 or a pharmaceutically acceptable salt thereof is administered.

8. The method of claim 6, wherein a compound of Table 2 or a
5 pharmaceutically acceptable salt thereof is administered.

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Figure 1

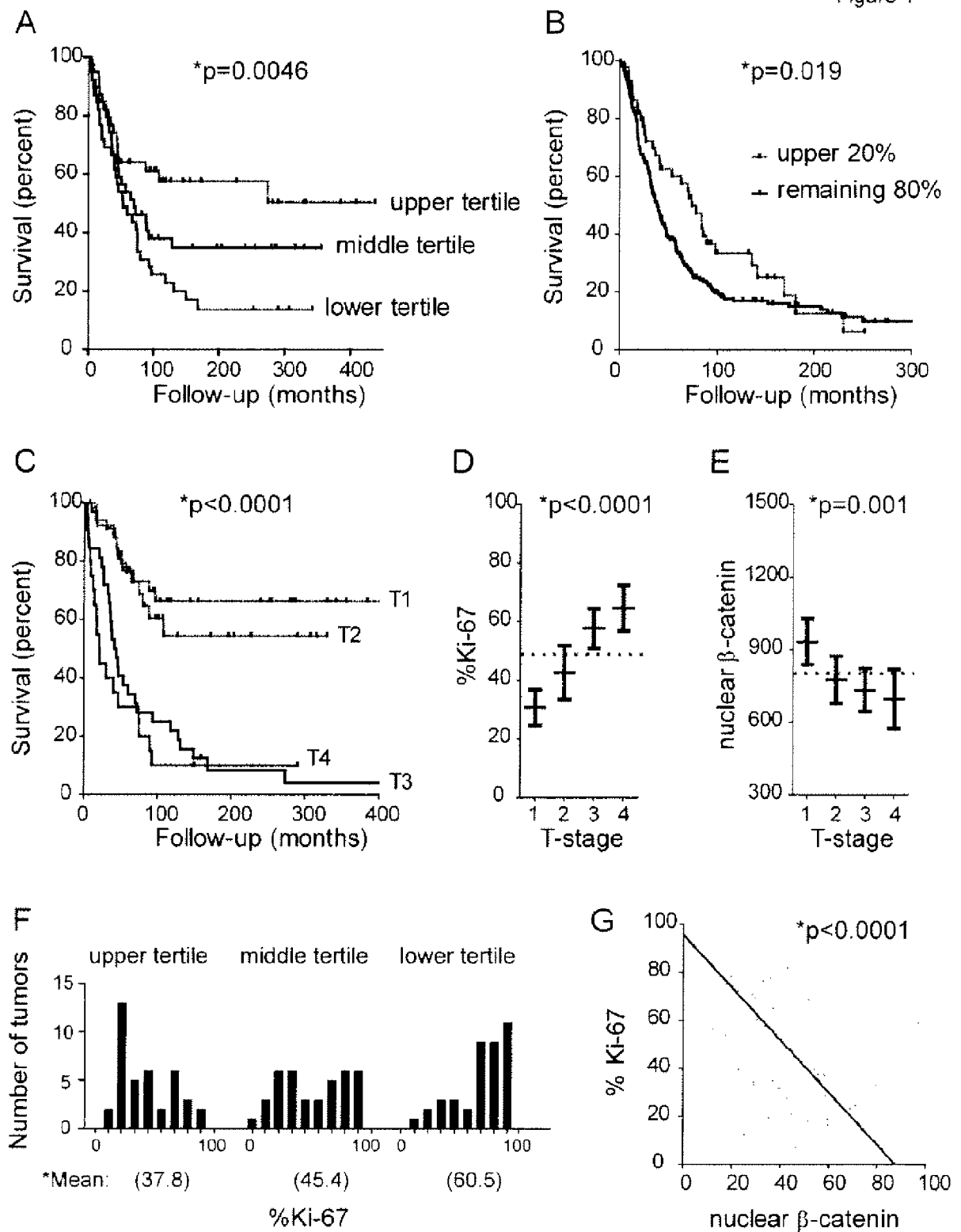


Figure 2

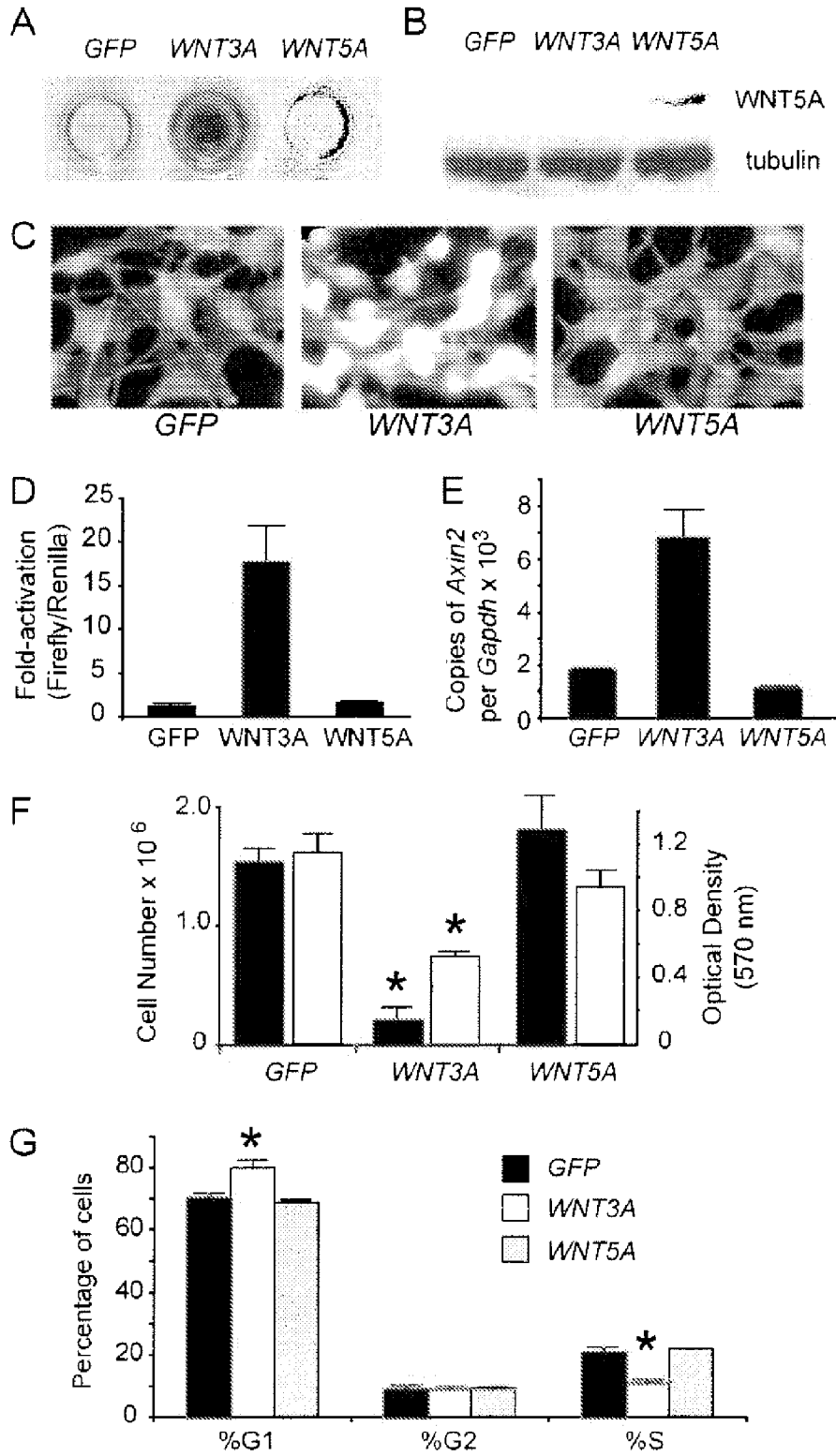
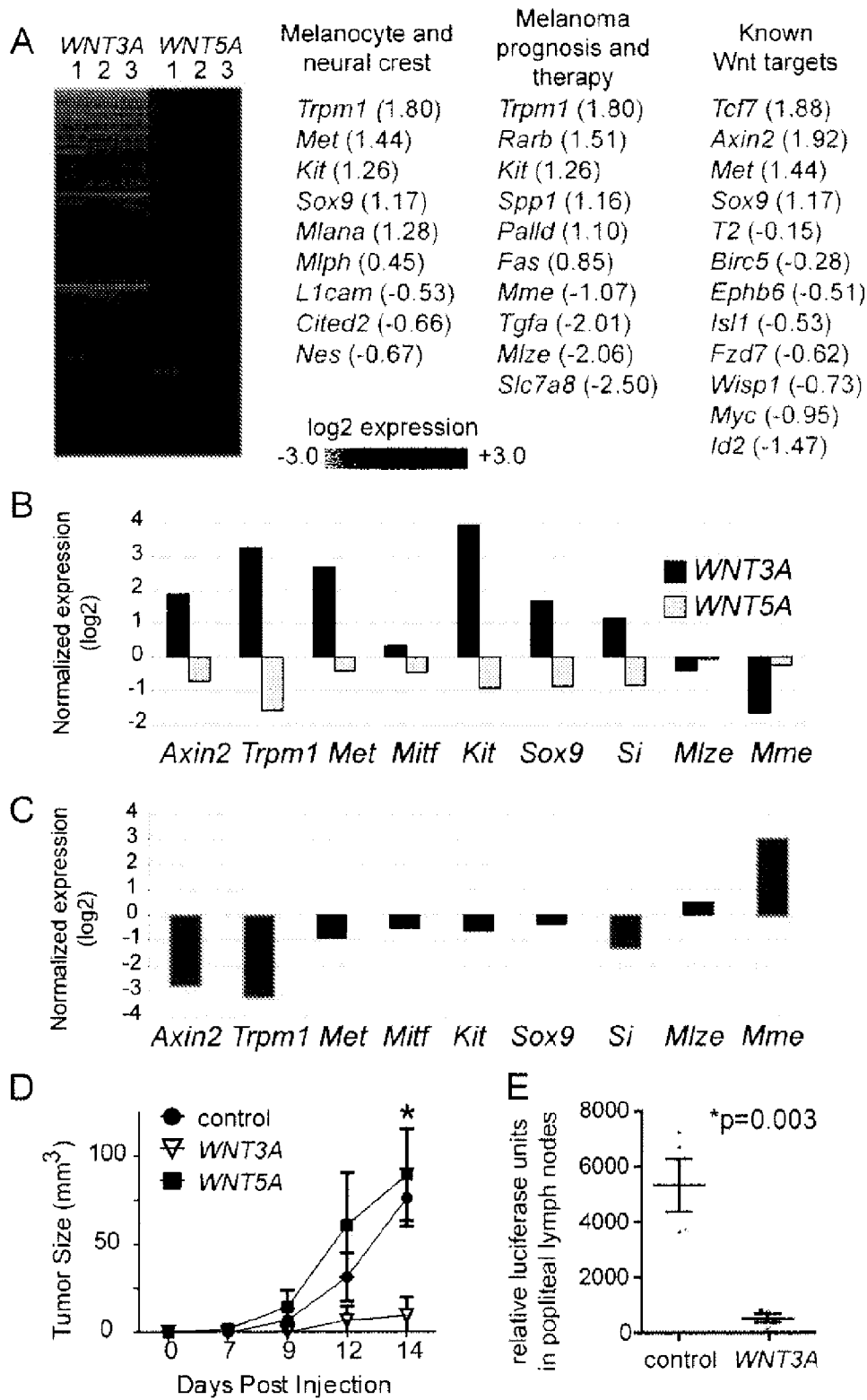
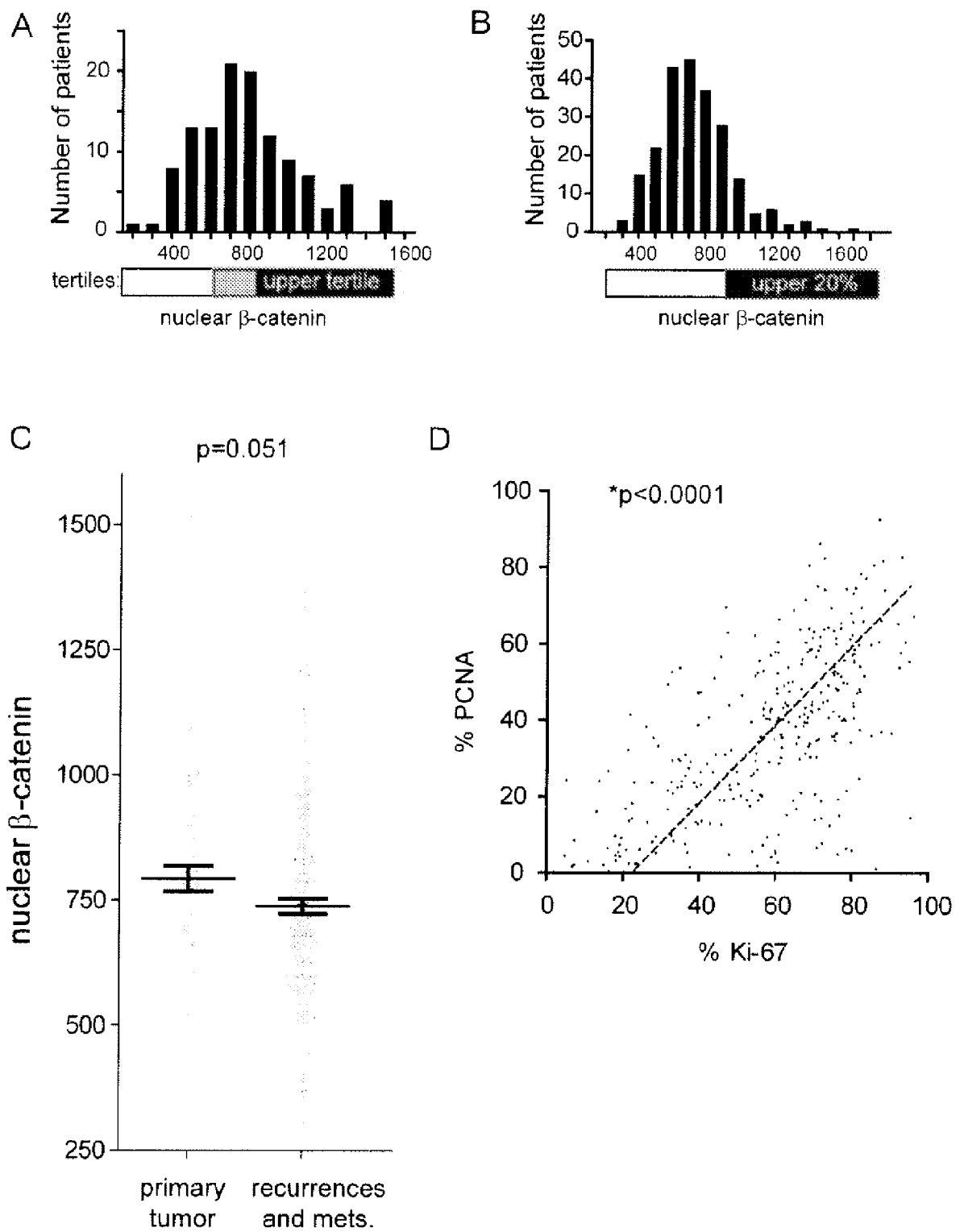


Figure 3



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Figure 4



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Figure 5

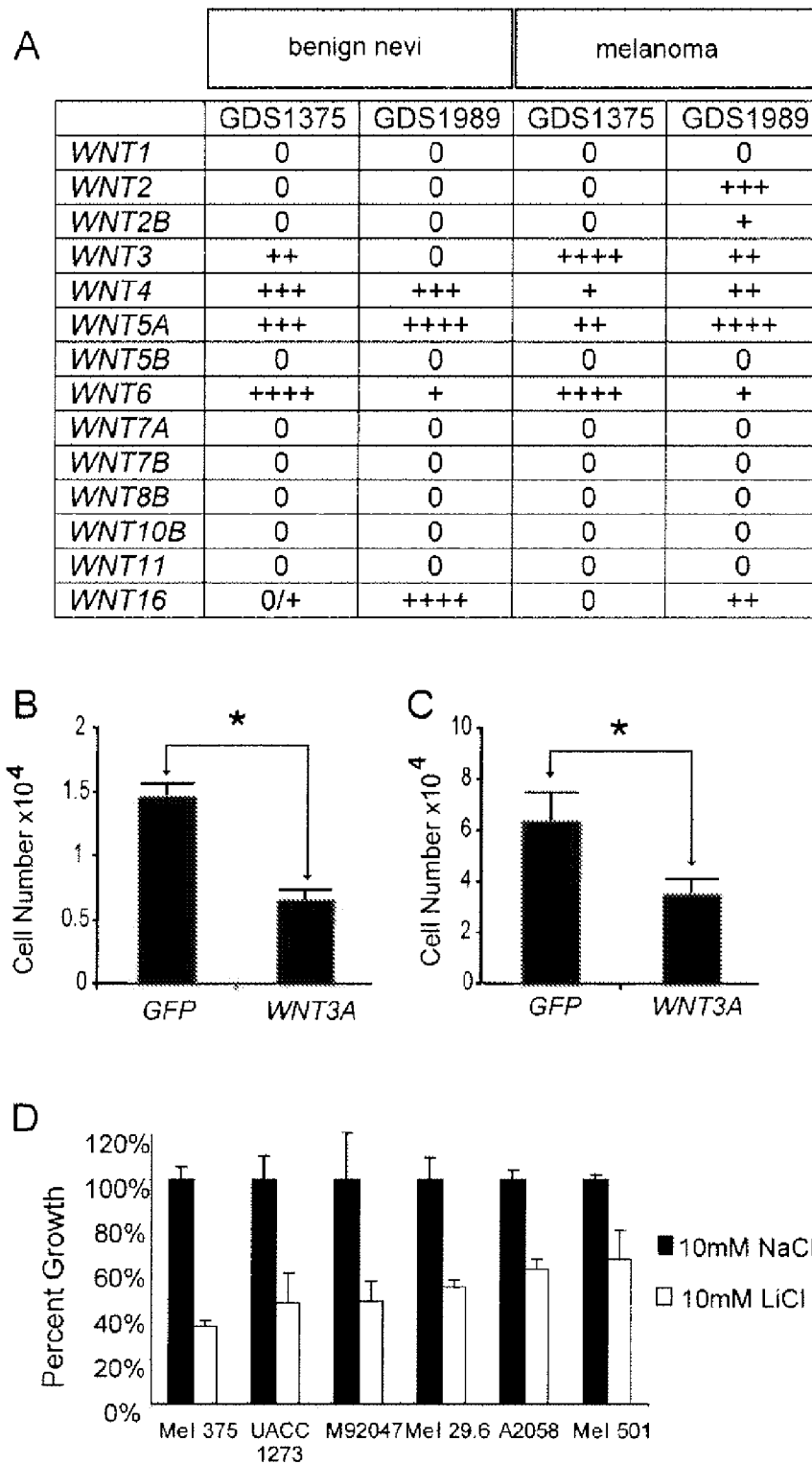


Figure 6

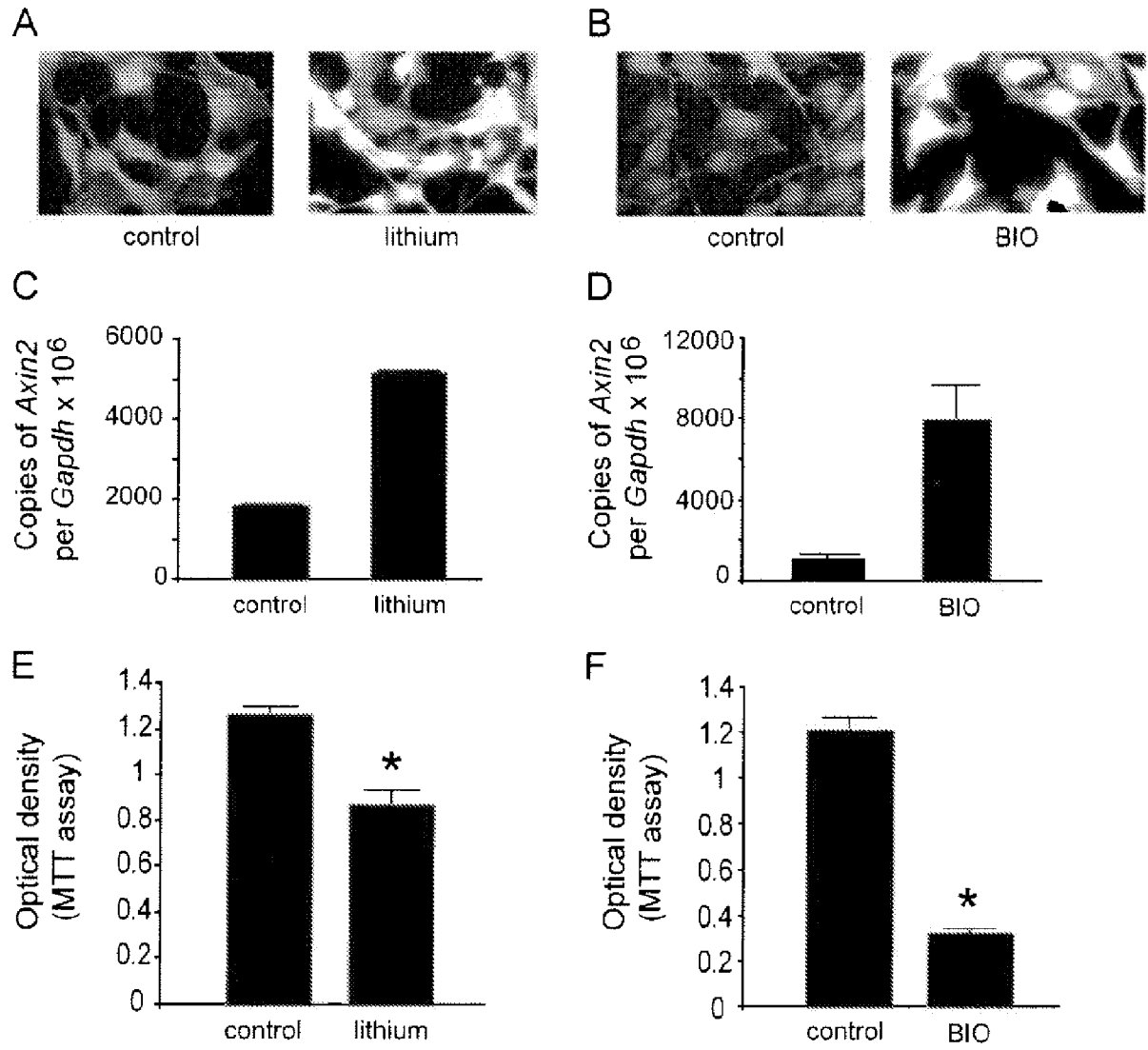


Figure 7

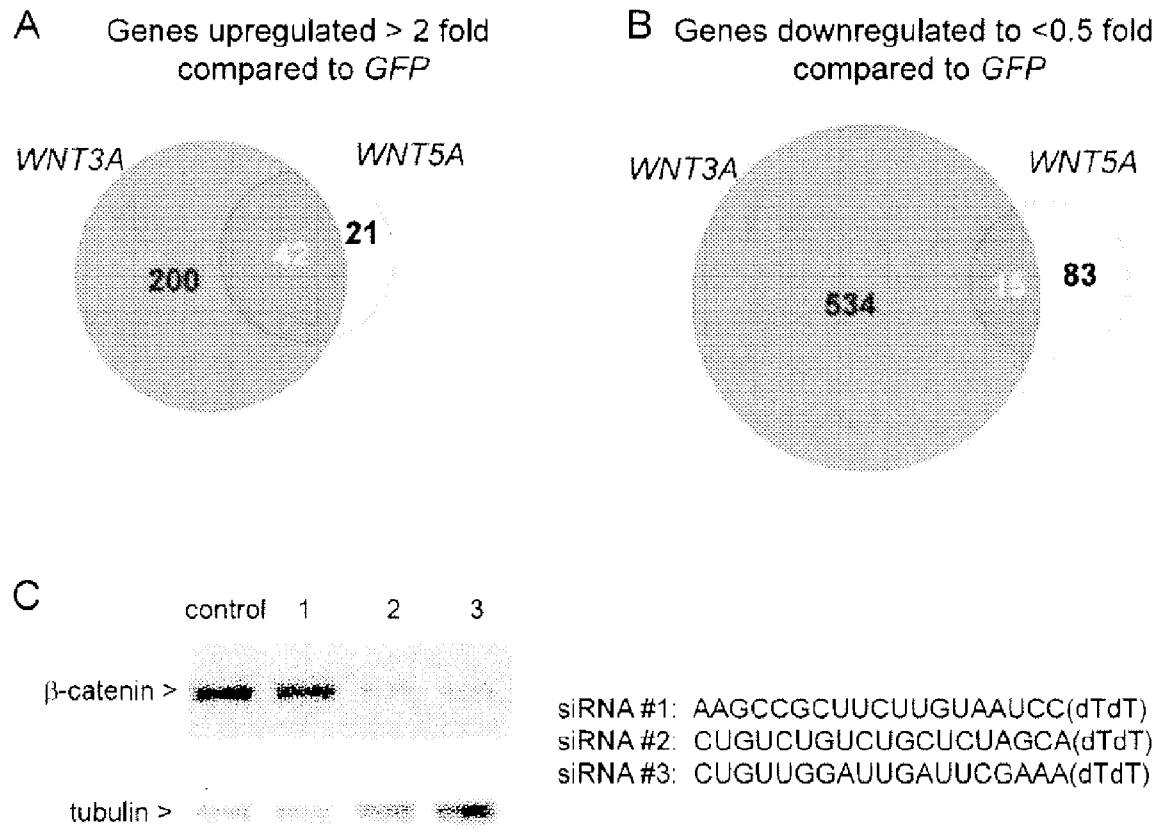
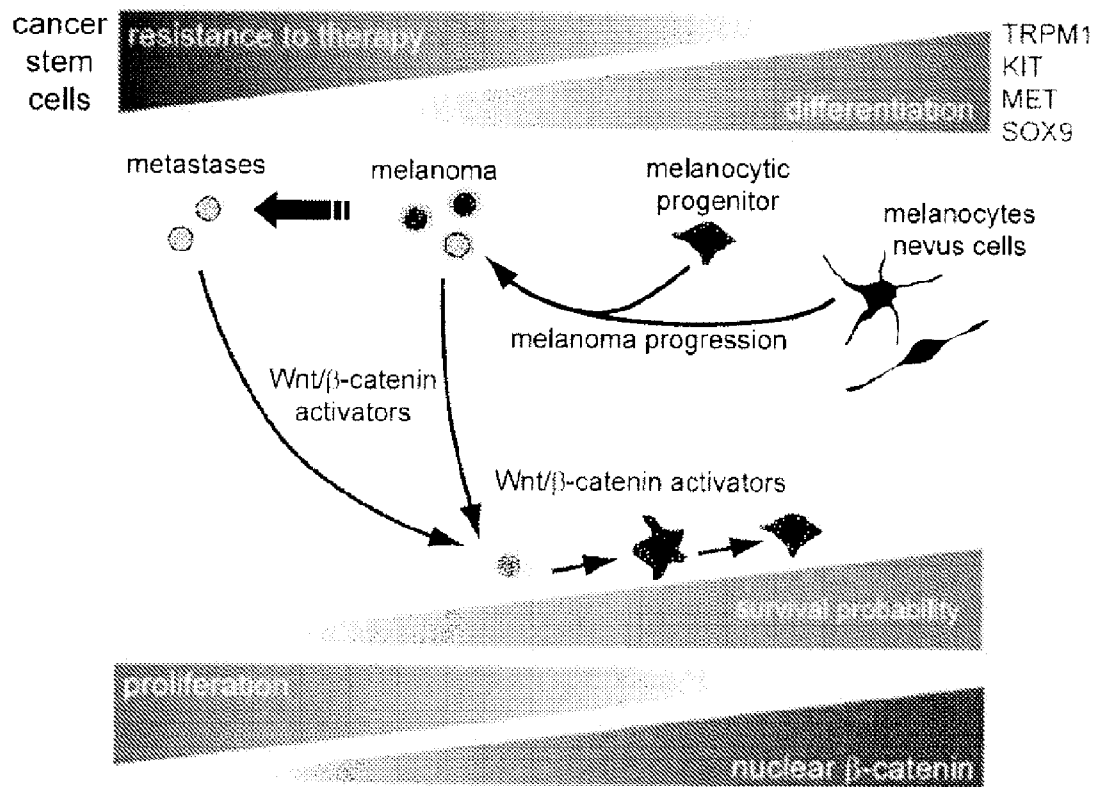


Figure 8



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 09/69003

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A01N 43/16; A61K 31/35 (2010.01)

USPC - 514/456

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8)-A01N 43/16; A61K 31/35 (2010.01)

USPC- 514/456

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

USPC- 514/183, 451, 449, 453, 455

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

PubWEST(PGPB,USPT,USOC,EPAB,JPAB); Google Patents; Google Scholar, wikipedia

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 6,924,141 B2 (MORGAN et al.) 02 August 2005 (02.08.2005) (col 1 ln 25-50; col 2 ln 5-50; col 7 ln 1-10; col 13 ln 60-65)	1-3, and 5-7
Y	(PALING et al.) Regulation of Embryonic Stem Cell Self-renewal by Phosphoinositide 3-Kinase-dependent Signaling* THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 279, No. 46, Issue of November 12, pp. 48063-48070, 2004 (pg 48064 col 2 para 4; pg 48066 Fig. 2; pg 48068 col 2 para 1).	1-3, and 5-7
A	Y294002. Cell Signaling Technology (online) 22 Aug 2007 [retrieved on 12 Apr 2010] Retrieved from the internet URL:<http://www.cellsignal.com/pdf/9901.pdf>. 1 pg	1-3, and 5-7
A	US 20040198750 A1 (GREEN et al.) 7 Oct 2004 (07.10.2004) para [0031]; [0034]; [0062]	1 and 5
A	US 20080312316 A1 (CULSHAW et al.) 18 Dec 2008 (18.12.2008) para [0002]; [0058]	1 and 5

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

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

08 April 2010 (08.04.2010)

Date of mailing of the international search report

22 APR 2010

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-3201

Authorized officer:

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PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 09/69003

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I-XXI: Claims 1-3 and 5-7 are drawn to a method of treating a subject for a condition mediated by aberrant Wnt/b-catenin signaling, and a method of activating the Wnt/b-catenin pathway in a subject, wherei each group is limited to one of the 21 compounds listed in Table 1, and Group I is limited to a compound of Family I, as listed in Table 1.

Groups XXII-XXXVIII: Claims 1, 2, 4-6, and 8 are drawn to a method of treating a subject for a condition mediated by aberrant Wnt/b-catenin signaling, and a method of activating the Wnt/b-catenin pathway in a subject, where each group is limited to one of the 17 compounds listed in Table 2.

*****continued on extra sheet*****

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-3 and 5-7, limited to a compound of Family I of Table I

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 09/69003

Continued from Box III - Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

The groups listed above do not relate to a single general inventive concept under PCT Rule 13.1 because under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons.

The groups are drawn to methods of using structurally distinct compounds for modulating Wnt/b-catenin signaling. Although several compounds in the listed groups are indole or indoline derivatives, this shared feature does not represent an improvement over the prior art of US 2006/0160876 A1 to Chao et al., which teaches an indole derivative (para [0020]) for treating a condition mediated by aberrant Wnt/b-catenin signaling (para [0191]; claim 56).

Accordingly, unity of invention is lacking.