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Bioactive PI3-kinase/Akt/mTOR Inhibitors in Targeted Lung Cancer Therapy

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Supplementary file 1

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Table S1. Summary of potential alkaloids targeting Akt/mTOR pathway in lung cancer cells.

Compd.	Mechanism of action	Assays	Ref.
Cytisine (**1**)	– Growth inhibition in cell lines including A549, NCIH23 and NCI-H460, – Cell cycle arrest in G2/M phase in A549, – Induction of mitochondrial apoptosis in A549 and NCI-H460 cancer cells, – Increasing expression levels of p-38, p-JNK, BAD, cytochrome c, cleaved caspase and	– Cell counting kit-8 assay, – Flow cytometry using Annexine V-FITC/propidium iodide staining, – Western blotting, Measurement of ROS level with	[1]

	PARP, decreasing expression levels of Bcl-2, p-ERK, p-STAT3 and NF-κB (p65).	DCFH-DA, – Measurement of tumor volume using formula 1/2(L×W2).	
Matrine (**2**)	– Reduction in cell viability of A549 and 95D cancer cell, – Induction of early apoptotic pathway in dose and time-dependent manner, – Inactivation of Akt in a dose-dependent manner.	– MTT assay, – Flow cytometry, – Western blotting.	[2]
Wasoxymatrine (**3**)	– Suppression of cell proliferation in A549, H1974 and HCC287 cancer cells, – Decrease in EGRF phosphorylation and inactivation of ERK1/2 and Akt, – Cell cycle arrest in G0/G1 phase, – Tumor growth inhibition in HCC827 xenograft model	– MTS assay, – Western blotting, – Flow cytometry by using Annexine V-FITC/propidium iodide staining, Calculation of tumor volume using formula 1/2(L×W2)), – Immunohistochemical analysis.	[3]
Evodiamine (**4**)	– Induction of arrest in G2/S phase, Inhibition of cell proliferation and Induction of apoptosis and ROS production in A549 cancer cells, – Downregulation of mRNA expression levels of survivin, Bcl-2, cyclin B1, p-Src, SHH and GLI1, – Reduction in telomerase activity, mRNA expression level of GLI1 and SHH and intracellular concentration of GSH, – Decrease in NF-κB and Akt phosphorylation levels.	– MTS assay, – Flow cytometry, – Immunoblotting analysis, – SYBR Green real-time PCR, Commercial GSH determination kit, – Immunoblotting and Western blotting.	[4]
Hirsutine (**5**)	– Selective inhibition of cell proliferation and Induction caspase-dependent apoptosis in A549 cells, – Decreasing mitochondrial membrane potential (ΔΨ) and depletion of ATP, Induction of release cytochrome c and ROS generation, – Increasing association between cyclophilin-D (CypD) and adenine nucleotide translocator 1 (ANT1), – Decrease in Rho-associated coiled-coil kinase (ROCK1) level and increase of p-PTEN levels, – Inactivation of P13K/Akt pathway.	– Cell counting kit-8 assay, – Flow cytometry by using Annexine V-FITC/propidium iodide staining, Confocal microscopy with JC-1 staining, – ATP determination kit, – Western blot analysis, – DCF-DA staining, Immunoprecipitation assay.	[5]
Chaetoglobosin K (**6**)	– Inhibition of cell growth in H2009 and WB-ras cells – Decrease in JNK and Akt phosphorylation – Decrease in phosphorylation of active sites of c-JUN, ATF-2, MDM2 and Rac1	– Cell growth assay with counting cells per dish with hemocytometer, Western blotting.	[6]
Neferine (**7**)	– Reduction in cell viability of A549 cells, – Acidic vesicular organelles (AVO) formation as the characteristic of autophagy, – Down regulation of Akt/m-TOR pathway, – Hyper-generation of ROS, depletion of GSH levels and lipid peroxidation (LPO) content, – Increased p53, Bax, caspase-3 and caspase-9 expression and decrease in Bcl-2 gene expression,	– MTT assay, – Acridine orange staining, – Western blotting, – Fluorometric assay, – Light microscopy Real time-quantitative PCR analysis (RT-qPCR).	[7] [8]

	– Decrease in gene expressions of PI3K/Akt/mTOR, – Decrease in mRNA expressions of COX-2, NF-κB, VEGF and CYP2E1.		
Noscapine (**8**)	– Inhibition of cell proliferation in H460 and A549 cells, – Reduction in tumor volume in nude mice bearing H460 xenograft lung tumor, – Combination of noscapine and cisplatin induced apoptosis in H460, A549 cells and nude mice bearing H460 xenograft lung tumor, – Increase in expression of p53, p21, caspase-3, cleaved caspase-3, cleaved PARP and Bax and decrease in expression of Akt, p-Akt, cyclin D1, survivin, Bcl-2 in H460 and A549 cells treated with combination of noscapine and cisplatin.	– Crystal violet dye assay, – TUNEL assay, – Western blotting.	[9]
Krukovine (**9**)	– Growth inhibition, Inhibition of cell colony formation, Induction of cell cycle arrest at G1 phase and apoptosis in H460 and A549 cancer cells, – Inhibition of RAF/ERK pathway and Akt inactivation.	– MTT assay, – Colony formation assay by staining with crystal violet, – Flow cytometry and Western blotting.	[10]
Tetrandrine (**10**)	– Decreasing proliferation of A549 cancer cells and Induction of apoptosis, – Decreasing Bcl-x, increasing Bid and Bax levels and Suppression of Akt and ERK phosphorylation.	– MTT assay, – Flow cytometry by using Annexine V-FITC/propidium iodide staining, – Western blotting.	[11]
Berberine (**11**)	– Inhibition of cell proliferation and migration and colony formation in A549 and H1299 cells, – Increasing apoptotic cells in A549 and H1299 cells, – Increasing expression levels of cleaved caspase3/7/9, cleaved PARP, Bax protein and decreasing Bcl-2 protein, – Decrease in phosphorylated Akt and ERK1/2 levels, COX-2 expression, – Inhibition of expression of AP-2α, AP-2β, hTERT, HIF-1α, VEGF, at mRNA and protein levels, – Translocation of p50 and p65 NF-κB from cell nuclei to cytoplasm and Inhibition in binding of p50/p65 NF-κB to COX-2.	– Wound-healing assay, – MTT assay, – Anchorage independent colony formation assay, – Annexin-V staining-based fluorescence-activated cell sorter, – Western blotting, RT-PCR, – Immunofluorescence assay, – Streptavidin-agarose pulldown assay.	[12]
Reniermycin M (**12**)	– Growth inhibition of H460 cancer cells, – Inhibition of aggregate formation in a dose-dependent manner, – Decreasing p-Akt, p-ERK, BCL2 and MCL1 levels in a dose-dependent manner.	– Water-soluble tetrazolium salt assay, – Light microscopy, – Western blotting	[13]
Solasodine (**13**)	– Cytotoxic activity on A549 cancer cells – Suppressing cell invasion in dose-dependent manner – Suppression of mRNA expression of MMP-2, MMP-9 and extracellular inducer of matrix metalloproteinase (EMMPRIN) – Elevation of expression of tissue inhibitor of metalloproteinase-1 (TIMP-1), TIMP-2 and reversion-inducing cysteine-rich protein with kazal motifs (RECK) – Down-regulation of microRNA-21 expression and PI3K/Akt phosphorylation in dose-dependent manner.	– MTT assay, – Boyden Chamber Invasion assay, – RT-qPCR, – SDS-PAGE and western blotting.	[14]
Mahanine (**14**)	– Significant growth inhibition in A549 and H1299 cells,	– MTT assay and cells counting with hemocytometer	[15]

	– Deactivation of Akt and reduction in m-TOR, PDK1 and Rictor level, – Induction of apoptosis through suppression of Rictor.	after staining with trypan blue, – Immunoblot and qPCR analysis, – Annexine V-Cyc3 detection assay.	
Piperlongumine (**15**)	– Inhibition of growth, proliferation and colony formation in H549 and A549/DTX cancer cells, – Induction of apoptosis and autophagy in H460 and A549/DTX cancer cells, – Cleavage of PARP, reduction of Bcl-2 level, – Induction of cell cycle arrest in S phase and ROS generation, – Down regulation of cyclin D1, CDK4, CDK6 and Rb, – Decreasing Akt phosphorylation and increasing ERK1/2 phosphorylation, – NF-κB inactivation through Akt dephosphorylation.	– MTT assay, – Colony formation assay by staining with hamatoxlin, – Flow cytometry, – Western blotting, – TUNEL assay, – Immunohistochemical assay.	[16] [17]
Ethoxysanguinarine (**16**)	– Reduction in gene expression of CIP2A in A549 cells, – Down regulation of CIP2A in H1975 and A549 cells through a time-dependent manner, – Down regulation of p-Akt and p-Myc in H1975 and A549 cells – Up regulation of PP2A, – Cleavage of PARP, decrease in procaspase-8 and procaspase-9 in H1975 and A549 cell, – Inhibition of cell proliferation and induction of apoptosis in H1975 and A549 cells.	– RT-qPCR, – Western blotting, MTT assay, – Flow cytometry by using Annexine V-FITC/propidium iodide staining.	[18]
Dicentrine (**17**)	– Reduction in cell viability, invasion and migration in A549 cancer cells, – Increasing apoptotic cells – Increasing cleavage of caspase-3, caspase-8, caspase-9, PARP and death-inducing signaling complex (DISC) formation – Overexpression of c-IAP2, c-FLIP and Bcl-xl – Inhibition of TNF-α induced expression of MT1-MMP, MMP-9, plasminogen activator receptor (Upar), intercellular adhesion molecule 1 (ICAM-1) and Cox-2, – Inhibition of TNF-α induced NF-Kb, AP-1 activation and TNF-α-induced Akt phosphorylation.	– MTT assay, – PI staining assay and flow cytometry analysis, – Modified Boyden chamber, – Western blotting.	[19]
Leonurine hydrochloride (**18**)	– Inhibition of cell proliferation and cell cycle arrest at G0/G1 phase in H292 cells, – Induction apoptosis in H292 cells – Increasing MAPK phosphorylation and decrease Akt phosphorylation, – Increasing ROS accumulation and mRNA ratio of Bax/Bcl-2 and mRNA levels of caspase-3 and caspase-9.	– MTT assay, – Flow cytometry, – Western blotting, – RT-q PCR	[20]

Table S2. Summary of potential flavonoids targeting Akt/mTOR pathway in lung cancer cells

Compd.	Mechanism of action	Assays	Ref.
Naringenin (**19**)	– Reduction of A549 cells migration, – Inhibition of MMP-2 and MMP-9 activities,	– Wound healing, – Boyden chamber assays,	[21]

	– Reduction in phosphorylation of the AKT/total AKT ratio.	– Gelatin zymography analysis, – RT-PCR and Western blotting.	
Eriodictyol (**20**)	– Cytotoxic activity in A549 cells, – Increased apoptotic cell death, DNA damage and cell cycle arrest in G2/M phase, – Increasing Bax/Bcl-2 ratio, – Downregulation of m-TOR, pm-TOR and PI3K/Akt protein expression levels.	– MTT assay, – Annexin V/PI staining and flow cytometry, – Comet assay, – Muse Cell Analyzer and Muse Cell Cycle Kit, – DOC6 and flow cytometry, – Western blotting.	[22]
Chrysin (**21**)	– Growth inhibition and apoptosis by inhibition of Akt/mTOR activation, – Promotion of A549 cell death and growth inhibition by increasing doxorubicin-induced AMPK activation.	– Western blotting, – MTT assay and PI staining.	[23]
Apigenin (**22**)	– Decreasing cell viability, proliferation and migration in A549 cell, – Decrease in Akt, p-Akt levels, MMP-2, MMP-9, GSK-3β, and HEF1 expression – Downregulation of N-cadherin, and the EMT-promoting transcription factors, – Suppression of Snail family mediated to AKT inactivation – Suppression of clonogenic growth in A549 and H1299 cells, – Cleavaging procaspase-8, procaspase-9, procaspase-3, and PARP in cells treated with combination of apigenin and TRAIL, – Increase in levels of Bax and Bad and reduction in Bcl-2 and Bcl-xl levels in cells treated with combination of apigenin and TRAIL, – Inhibition of NF-kB p65 nuclear translocation – Decrease in c-FLIP expression in cells treated with combination of apigenin and TRAIL, – Decrease in phosphorylation of AKT and PI3K after co-treatment of apigenin and TRAIL, – Increasing phospho-JNK in cells co-treated with apigenin and TRAIL, – Upregulating DR4 and DR5 levels, elevation of p53 level, activation of MAPK p38, and suppression of the MAPK ERK in cells co-treated with apigenin and TRAIL.	– MTT assay, – Colony formation assay, – Wound-healing assay, – Transwell assay, – Western blotting, – Transwell migration and invasion assays, – Colony-forming assays, – Annexin V-FITC kit.	[24] [25, 26]
Baicalein (**23**)	– Enhancement of diamminedichloroplatinum (CDDP) cytotoxicity and early apoptosis in CDDP-resistant A549 cells, – Reduction in tumor growth of A549/CDDP xenograft model, – Inhibition invasion and migration induced by CDDP, – Blocking PI3K/Akt and NF-κB activity in A549/CDDP – Decrease in anti-apoptotic genes including c-IAP1, c-IAP2, survivin and Bcl-Xl.	– MTT assay, – Annexin V/PI staining, – Weighting tumor size, – Western blotting.	[27]
Luteolin (**24**)	– Blocking TGF-β1-induced EMT and EMT-related gene expression in A549 cells, – Blocking TGF-β1-induced E-cadherin downregulation via Snail, – Reverse of TGF-β1-induced decrease in IκBα, – Inhibition of TGF-β1-induced phosphorylation of PI3K/Akt–mTOR pathway, – Block of NF-κB binding to the Snail promoter region induced by TGF-β1.	– Trypan blue exclusion method, – Annexin-V and PI staining, – Western blot and RT-PCR analysis, – Chromatin immunoprecipitation assay.	[28]
Fisetin (**25**)	– Decreased cell viability in A549 and H1792 cells, – Interaction with mTOR complex in two sites and inhibition of downstream targets of mTOR including 4E-BP1, EIF4E and p70S6K, – Activation of PTEN, as a tumor suppressor gene, – Significant increase in phosphorylation of AMPKα, – Inhibition of PI3K and Akt phosphorylation, – Increase in TSC2 protein level at dose-dependent manner.	– MTT assay, – Docking study, – Immunoblot analysis, – Western blotting and Phospho-Act ELISA kit, – Immunoblot analysis and chemiluminescence detection.	[29]
Kaempferol (**26**)	– Reduction in cell viability in A549 cells and Induction of apoptosis, – Decreasing Bcl-2 and Bcl-xL levels in a dose-dependent manner and p85 subunit of PI3K and total Akt, – Increasing expression of Bad and Bax, – Dose-dependent phosphorylation of MAPK, MEK1/2, c-Jun and cleaved PARP, – Down-regulation of mRNA and protein levels of cyclinD1,	– MTT assay, – TUNEL assay, – Western blotting, – Cell Counting Kit-8 (CCK-8) assay, – RT-qPCR, – Fluorescein isothiocynate (FITC)-conjugated annexin V and propidium iodide	[31] [32]

	– Down-regulation of Bcl-2 and upregulation of Bax, cleaved caspase-3 and cleaved caspase-9, – Decreasing phosphorylated levels of PI3K and AKT, – Upregulation of miR-340 expression, mRNA and protein expression levels of PTEN.	[30].	
Quercetin (**27**)	– Suppression of migratory and invasive abilities in A549 and HCC827 cells, – Induction of E-cadherin expression and suppression of N-cadherin and vimentin, – Upregulation of p-AKT, – Sensitization of TRAIL-induced cytotoxicity in H460, A549, H2009 and H1299 cells, – Induction of apoptosis in H460 cells treated with TRAIL plus quercetin, – Cleavaging PARP and activation of caspase-8 and -3 in cells treated with TRAIL plus quercetin, – Inhibition of Akt phosphorylation and survivin in cells treated with TRAIL plus quercetin, – Decrease in total Akt protein, phosphorylation of MEK1/2, ERK, JNK and c-Jun.	– MTT assay, – Western blotting, – Wound-closure, – Transwell migration and Matrigel invasion assays, – Immunofluorescence microscopy, LDH release assay, Staining with acridine orange-ethidium bromide and observation with microscope.	[33] [34] [35]
Tangeretin (**28**)	– Suppression of IL-1β induced COX-2 protein and m-RNA expression in A549 cells, – Suppression of phosphorylation of ERK, p38 MAPK, JNK, and Akt induced by IL-1β in A549 cells, – Inhibition of translocation of p65 NF-kB and degradation of IkBa induced by IL-1β, – Inhibition of endogenous COX-2 expression in H1299 cells.	– Western blotting and RT-PCR.	[36]
Nobiletin (**29**)	– Decrease in cell viability in A549/ADR cells, – Decrease in expression levels of MRP1 and neuroblastoma-derived MYC (MYCN), – Decrease in GSK3β phosphorylation, – Induction of apoptosis A549/ADR Cells and reduction in tumor volume, – Cleavage of caspase-3 and c-PARP and the decrease in MRP1, survival and Bcl-xL.	– MTT assay, – Western blotting, – Confocal microscopy, – Flow cytometry using PI.	[37]
Artocarpin (**30**)	– Proliferation inhibition of A549, H226 and H1299 cells, – Induction of apoptosis and ROS generation, – Induction of MAPK, ERK1/2 and Akt phosphorylation, – Time-dependent promotion of phosphorylation of p53 and expression of PUMA, cytochrome c, Apaf-1 and cleaved caspase 3, – NF-κB activation.	– MTT assay, – ELISA assay, – Western blotting, – Annexin-V-FITC/PI, – Immunocytochemistry & Receptor gene assay.	[38]
Kushenol z (**31**)	– Inhibition of NSCLS cells proliferation, – Induction apoptotic body formation and apoptosis, – Upregulating the ratio of Bax/Bcl-2 and cleavaging of caspase 3 and 9, – Upregulated ERS marker, C/EBP Homologous Protein (CHOP), – Increasing cleavage of caspase 7 and caspase12, – Increasing the activity of PKA, inhibiting cAMP-PDE and Akt/mTOR pathway.	– Cell counting kit-8 (CCK-8) assay, – Hoechst 33258 nuclear staining, – Annexin V/PI staining, – Western blotting.	[39]
Glabridin (**32**)	– Decrease in A549 cell migration and invasion, – Increase in E-cadherin level and decrease in vimentin, – Decrease in angiogenesis of A549 cells, expression of αv and b3 integrin, phosphorylation of FAK, phosphorylation forms of AKT.	– Transwell migration and wound-healing assay, – Immunoblot analysis, – BD Bio-Coat Angiogenesis System, Immunoblot analysis, – Immunoblot assay and Rho activation assay kit.	[40]
Sotetsuflavone (**33**)	– Dose-dependent inhibition of A549 cell migration and invasion, – EMT reversing by upregulation of E-cadherin and downregulation of N-cadherin, vimentin and snail, – Decreasing HIF-1α, NF-κB and TNF-α expression, – Downregulation of MMP-9 and MMP-13 expression, – Inhibition of PI3K/Akt pathway in time and dose-dependent manners.	– Scratch test and Transwell invasion assay, – Western blotting, Immunoflufluorescence assay, – PCR (RT-PCR)	[41]
Vitexin (**34**)	– Dose-dependent reduction in cell viability in A549 cells, – Induction of apoptosis, – Increasing loss of MMP,	– MTT assay, – Flow cytometry analysis via Annexin-V/PI double staining, – Weighting tumor volume,	[42]

	– Downregulation of Bcl2/Bax ratio and upregulation of cleavd-caspase-3, – Reduction in p-Akt, p-P13K and p-mTOR levels.	– JC-1 staining, – Western blotting.	
Luteoloside (**35**)	– Cytotoxic activity in A549 and H292 cells, – Cell cycle arrest in G0/G1 phase and induction of autophagy, – Upregulation of LC3-II and Beclin-1 expression and decrease of p62 and p-mTOR, – Decrease in CyclinD1, CDK4 and Cyclin E, – Downregulation of Erk1/2, inhibition of p-AKT, p-P70S6K and p-mTOR.	– MTT assay, – Flow cytometry, – MDC as florescent probe, – Western blotting, – H2DCFDA prob and flow cytometry.	[43]
Vicenin II (**36**)	– Reduction in cell viability of H23 cells both alone and in combination with radiation, – Increasing ROS generation in combination with radiation, – Increasing PI3KCA protein and mRNA levels in combination with radiation, – Lowering levels of pAkt, Akt, and Akt1 mRNA in combination with radiation, – Lowering levels of Bcl-2 protein and Bcl-2, Bcl-xL mRNA levels in combination with radiation.	– MTT assay and colony forming assay, – Protein carbonyl assay by using 2,4 dinitrophenylhydrazine (DNPH), – DCFH-DA, – Western blotting, – Quantitative real-time PCR, – Ultra-structural study.	[44]
Astragaline (**37**)	– Selective cytotoxicity in A549 and H1299 cells, – Induction of apoptosis in A549 and H1299 cells, – Inhibition of the clonogenic growth of H1299 and A549 cells, – Cleavaging of caspase-8, -9, -3, and PARP, – Decreasing the expression of Bcl-xl and Bcl-2 and increasing in the expression of Bad and Bax, – Decrease in p38 and ERK phosphorylation in a dose-dependent manner, – Inhibition of the nucleus translocation of NF-κB.	– MTT assay, – Propidium iodide staining [30], – Colony-forming assay, – Western blotting.	[45]

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