RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Prospects for multitarget lipid-raft-coated silica beads: A remarkable online biomaterial for discovering multitarget antitumor lead compounds.

Author's name, academic degrees and contacts

Caleb Kesse Firempong (PhD Medical Sciences, calebuse@yahoo.com) [1], Xia Cao (PhD Clinical Diagnostics, 841661898@qq.com), Shan-Shan Tong (PhD Clinical Diagnostics, 877274194@qq.com) [1], Jiangnan Yu (PhD Pharmaceutical Analysis, yjn@ujs.edu.cn) [1, 2]and Xuming Xu[1] (PhD Pharmaceutics/Drug Delivery; Post-Doc Gene Transfer and Tissue Engineering)

Authors' Affiliations

[1]Department of Pharmaceutics, School of Pharmacy, Centre for Nano Drug/Gene Deliveryand Tissue Engineering, Jiangsu University, Zhenjiang, P.R.China; [2]School of Pharmacy, China Pharmaceutical University, Nanjing, P.R.China.

Corresponding Author:

Ximing Xu[1], 301 Xuefu Rd, Zhenjiang, Jiangsu 212013, P.R. China. Phone: 86-511-8503845; Fax: 86-511-8503845; E-mail: xmxu@ujs.edu.cn

No potential conflicts of interest

Contents

1. Introduction
2. Prominent transmembrane activities of lipid rafts
3. Screening of antitumor lead compounds
4. Lipid rafts with highly expressed cancer-related receptors
5. Receptors as tools for rapid isolation of antitumor agents
6. Lipid rafts silica beads as an online antitumor screening biomaterial
7. Prospect for multiple receptor-based lipid raft biomaterials in cancer therapy
8. Challenges and future considerations in lipid raft screening applications.
9. Conclusion

Acknowledgement

References

ABSTRACT

A recently discovered receptor-rich lipid raft, which is linked to numerous transmembrane signal transduction pathways, has emerged as a medically significant biomaterial for screening antitumor agents. Compounds that interact with the biomaterial might potentially inhibit cancer cell growth, and thus be valuable as antitumor agents. Two standard anticancer drugs, lestaurtinib and gefitinib, interacted with the tropomyosin –related tyrosine kinase A (TrkA) receptor-rich lipid raft. The fact that they are both well known inhibitors of TrkA receptor strengthened the observed linkage. There is now a considerable interest in developing other related biomaterials to serve a similar purpose, and more importantly, support the concept of multitarget drug discovery. It is expected that new anticancer drugs strategically act on multiple pathways to achieve optimal therapeutic efficacies and decreased stimulation of acquired resistance. However, the current conventional approaches such as chemical screening systems and in-silico methods cannot fully satisfy the increasing demand for the therapeutic agents. It has therefore become imperative to explore other alternatives to increase the number of clinically important antitumor agents. Here, we report the prospect of establishing lipid raft biomaterial with well endowed multiple cancer-related receptors for screening antitumor leads that affect multiple pathways. This review also examines receptor-ligand interactions in the hunt for novel antitumor agents and the associated key receptors.

Keywords: antitumor, biomaterial, screening, lipid raft, multitarget, lead compounds, receptors, ligands, cancer treatment.

1. Introduction

Growing evidence that several effective drugs produce their actions via interactions with multiple targets is boosting the development of research areas that can challenge the reductionist approach.[1] Consistent with this, the concepts of drug repurposing,[2] polypharmacology,[3] chemogenomics,[4] phenotypic screening and high-throughput *in vivo* testing of mixture-based libraries[5] in an integrated manner have provided meaningful alternatives to the current paradigm of drug discovery. Thus, a shift from a single target to the more preferred multiple target approach. Although these platforms have provided clinically important multitarget lead compounds, their significant outputs are woefully inadequate in meeting the ever increasing demand for therapeutic drugs. As a result, there is an urgent need to investigate other promising approaches that could accelerate progress in this domain. To this end, establishing a biological model to effectively screen multitarget agents is a high priority for a successful anticancer drug discovery strategy.

Previous report on monotarget lipid raft biomaterial strongly signalled the possibility of developing a multitarget model for identifying antitumor components which can act on multiple pathways. The biological interactions between the receptor-rich biomaterial and its ligands , the associated thermodynamics and effective applications of the model was extensively investigated.[6] The presence of overexpressed tropomyosin –related tyrosine kinase A (TrkA) receptors on lipid raft silica beads (LRSB) was confirmed via immunofluorescence bioassays. The receptor specificity was also validated by TrkA target (lestaurtinib and gefitinib) and non-TrkA target (gemcitabine) standard anticancer drugs (Figure 1). The chromatograms of target drugs exhibited longer retention time as compared to non-target drugs. Likewise, bioactive LRSB isolate (Ethyl fraction) exhibited similar trend as the target drugs, and significantly inhibited cancer cell growth. However, these active components failed to exert cytotoxic activities in the presence of TrkA inhibitors (K252a and primary antibody). These findings support the interactions between the effective components and sufficiently expressed TrkA receptor in the lipid raft.

In spite of comprehensive studies on well-known cancer target receptors such as epidermal growth factor receptor (EGFR), vascular epidermal growth factor receptor (VEGFR) and Fas receptor (FasR), there is still very limited reporting regarding their bioscreening activities for potential antitumor agents.[6-7] So far the best known studies have been limited to cell membrane chromatography (CMC) with highly expressed receptors like EGFR and VEGFR. The medical benefits of employing these receptors for affinity screening cannot be overemphasized in our quest for effective chemotherapeutic drugs. Fortunately, the first TrkA receptor-rich lipid raft from U251 glioma cancer cells set the stage for obtaining other lipid rafts with multiple receptors from related cancer cells. Several bioscreening systems including immobilized DNA chromatography,[8] immobilized plasma protein chromatography,[9] immobilized liposome chromatography [10] and immobilized biomembrane chromatography[11] have been developed to detect interactions between a chemical and its target protein.[12] The yet to be established multitarget lipid raft belongs to this class of screening systems with the advantage of having enriched multiple receptors. The multitarget biomaterial can provide different sites for bioactive compounds, and more importantly, isolate compounds capable of acting on multiple pathways.

The desired multitarget lipid raft can overcome most of the problems associated with multitarget drug discovery, particularly longer processing time, high cost implications, low "hit" rates, and most significantly, the lack of correlation between *in vitro* and *in vivo* data. Technically, the feasibility of the proposed lipid raft biomaterial has been verified via its monotarget analogue, and it can be seen as an extension of the existing model.[6] Why does a multitarget lipid raft present such unique opportunities? Perhaps, it can offer a more realistic biological system with enriched diverse cancer-related receptors for bioactive compounds to locate and dock with. More importantly, it can isolate lead compounds from complex mixtures, especially natural products that could affect multiple pathways. The system might also provide experimentally verified data and enhance the search for multitarget agents. With the development of multitarget LRSB, the effective on-line pharmacological studies and rapid screens for multitarget agents could be easily accessible. However, it must be noted that present reports describe the exploratory stages, and there is a need for more comprehensive studies to demonstrate the clinical value of this novel technology.

2. Prominent transmembrane activities of lipid rafts

Certain cues for cell growth, survival and other physiological processes are transmitted through specialized sub-domains in the plasma membrane known as lipid rafts. [13] These rafts contain a variety of proteins, e.g., caveolins, flotillins, GPI-linked proteins, G proteins, src family kinases, EGF receptors, PDGF receptors, endothelin receptors, phosphotyrosine phosphatase, MAPK, protein kinase C and PI 3-kinase.[14] Such rafts regulate signal transduction by activating or suppressing phosphorylation cascades.[15] For instance, they can function as negative regulators of EGFR tyrosine phosphorylation,[16] thus, upon ligand binding and receptor activation, the EGFR migrates out of the rafts.[17] The membrane rafts might serve as a novel target in cancer therapy due to the ability to organize receptors and their downstream molecules. It has been reported that several growth factor signalling pathways depend on lipid raft. This is evident in the role of lipid rafts in the regulation of differentiation, apoptosis and cell migration, which is linked to invasiveness and metastasis.[18] The study also revealed certain synthetic and naturally occurring substances that are known to affect the lateral membrane organization in tumour cell growth which could provide potential or actual therapeutics. Even though most drugs bind to proteins and regulate their activities, some drugs act via a new therapeutic approach called membrane-lipid therapy (thus binding to lipids);.[19] Mollinedo and Gajate identified Fas/CD95 death receptor and lipid rafts as new targets for apoptosis-directed cancer therapy[20]. Similarly, the cannabinoid CB1 receptor antagonist rimonabant (SR141716) inhibited human breast cancer cell proliferation via a lipid raft-mediated mechanism[21].

Many "homing devices", including folate [22], transferrin[23] and peptides ,[24] have been installed on liposomes or nanoparticles as a means of achieving effective receptor-target tumour therapy. [25] Similarly, lipid rafts have been conjugated to certain drugs to impact lipophilicity. Simultaneously, the rafts are also linked to a target moiety that can be recognized by a specific transporter/receptor in cell membrane. The findings indicated that both the lipid and target moiety acted synergistically toward cellular uptake.[26] Nanogel surfaces decorated with alginate or mannosylated alginate were used to target dendritic cell receptors. The uptake of nanogel was dependent on endosomal-based processes. However, inhibition of lipid raft activities impaired the uptake, which was reduced, indicating the involvement of more than one entry routes. [27] In a related study, a drug delivery mechanism for target nanoparticles that utilizes direct cytoplasmic delivery into a target cell while avoiding endosomal incorporation

was observed in lipophilic delivery. This phenomenon entails the direct delivery of lipophilic substances to cell membrane via lipid mixing, and subsequent intracellular trafficking via lipid raft dependent processes. [28]

Effective delivery of therapeutic genes to designated target cells and their action at the intracellular site are crucial requirements for successful gene therapy. [29] Studies have suggested that lipid rafts of microvascular dermal endothelial cells are essential to Kaposi'sarcoma-associated herpes virus (KSHV) infection and gene expression. This could be due to their potential roles in the modulation of KSHV-induced PI3-K, RhoA-GTPase and Dia-2 signal molecules in post-binding and entry stages of infection.[30] The data suggest a possible role for lipid rafts in gene delivery. DNA nanoparticles (DNPs) are non-viral gene vectors with excellent *in vivo* potential. Disruption of lipid rafts by depletion of membrane cholesterol significantly inhibits DNP transfection, while the inhibition of other endocytic pathways has little effect. These events reveal the active involvement of lipid rafts in transfection. [31] Other reports have also shown that dendritic cell utilizes multiple endocytic routes for Synthetic Virus-Like Particle uptake which is dominated by lipid raft-mediated macropinocytosis.[32] Currently, there is limited information on the application of receptor-rich lipid raft biomaterial for screening antitumor agents. However, recent developments have ignited a special interest in this approach, as evidenced in reports of lipid raft bioscreening assays for antitumor components in complex natural mixtures[6].

3. Screening of antitumor lead compounds

New antitumor agents of pharmaceutical importance, described as "hits", could be identified from different sources such as natural/synthetic products, computational chemistry, chemical libraries and pharmaceutical biotechnology (Figure 2). The "hits" are pharmacologically, pharmacodynamically and pharmacokinetically enhanced via chemical or functional modification to obtain a lead compound. The lead usually has a known structure with well documented mechanism of action. The leads must also qualify as drug candidates that are safe for human clinical trials after optimization and developmental studies (Figure 2). [33] Generally, the initial routine bioscreening of any "hits" irrespective of their origin is based on cell/target assays using established cell lines to measure the cytotoxic activities (Figure 2). Some well-established methods commonly employed in these assays include colony formation methods, crystal violet methods, tritium-labelled thymidine uptake methods, and

MTT[3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] and WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium] bioassays. Toxicity studies of active agents on normal cells are investigated using canine kidney (MDCK), mouse fibroblasts (L929), Rhesus monkey kidney from Macaca mullata cells (LLC-MK2) and human peripheral blood mononuclear cells. [34] The agents or compounds that are found to be active and non-toxic in the *in vitro* models are further tested for *in vivo* antitumor efficacy. *In vivo* models, in which tumours are induced, using NOD-SCID, Balb C57BL/6 and athymic mice, are widely employed for this kind of investigations.[35] In fact, the entire process of screening for antitumor agents is characterized by low output, undue delays and high financial costs. For these reasons, several attempts are being made to solve some of these problems.

4. Lipid rafts with highly expressed cancer-related receptors

Several studies have reported overexpressed receptors on some cancer cells which are clearly absent in normal cells[36]. This situation can be clinically explored to discover more antitumor agents with high therapeutic efficacies. As microenvironments in the exoplasmic leaflet of cell membrane, lipid rafts might contain cancer-related receptors. Despite their reduced receptor numbers as compared to cell membrane, the rafts still demonstrate high specificity and increased receptor densities, which could lead to increased output of bioactive compounds.[6] Previous monotarget lipid raft, with abundant TrkA receptors, and the desired multitarget LRSB models, with prominent therapeutic receptors like EGFR, VEGFR and FasR can support the molecular basis of this clinically useful receptor-ligand screening bioassay.[37] In figure 3, the bioactive isolate from lipid raft (containing multiple receptors-FasR, TNFR, VEGFR, EGFR and HER2) can interact with any of the associated receptors in its antitumor applications. A follow up study could also easily ascertain the specific receptors involved in the receptor-ligand interactions. Most of these receptors are sufficiently expressed in predominant cancer cell lines (prostrate, breast and colon), and therefore, present a unique opportunity that can be therapeutically explored to provide more treatment options for patients.

TrkA receptors which can be activated to engage molecules for signal transduction are highly embedded in lipid rafts.[38] Therefore, TrkA-rich lipid raft could act as a linchpin from which a potent death signal could be launched to become a new promising anticancer target.[20, 39] The

identification of EGFR as an oncogene has led to the development of anticancer therapeutics which is directed against EGFR (called 'EGFR inhibitors'). Similarly, the overactivation of HER2 oncogene has been linked to the progression of certain aggressive cancers. Recently, this protein has become an important biomarker and therapeutic target for approximately 30% breast cancer patients.[40] The dysregulation of TNFR pathways results in a wide range of pathological conditions, including cancer, autoimmnune disease, inflammation and viral infection.[41] This explains the importance of these proteins as potential drug targets.[41c, 42] The membrane-anchored FasR ligand on adjacent cell causes the oligomerization of FasR receptor molecules in death-inducing signalling complex (DISC)[43]. This event could be mimicked by the binding of agonistic lead compounds which might be unravelled by a Fas-based LRSB screening system. Additionally, the vascular endothelial growth factor (VEGF), one of the important elements in regulating angiogenesis, is now a key focus in antiangiogenic therapy.[44] Thus, the VEGFR and its respective ligands, particularly VEGF, could play prominent roles in various cancer cells where they promote the growth of tumour blood vessels nurturing malignant cells.[45]

5. Receptors as tools for rapid isolation of antitumor agents

Antitumor drug discovery has become one of the most challenging and researched fields in cancer therapy due to the complex mechanism of tumour initiation and growth, as well as the difficulty in treating them .[46] Numerous screening techniques, based on target receptors or enzymes, and virtual screening system, using computer-aided drug design, have been employed for investigating lead compounds or drugs. [47] A target enzyme (DNA topoisomerase II) was employed to isolate antitumor agents from a mixture of compounds, and concurrently, the agents were analysed by high performance liquid chromatography/electrospray ionization-mass spectrometry (HPLC/ESI-MS).[48] Two combined receptors (nicotinic acetylcholine and oestrogen receptors) were used in liquid chromatography for online pharmacological studies, particularly the identification of lead candidates from complex biological or chemical mixtures.[49] Similarly, other efficient screening methods like immobilized β_2-Adrenoceptor (β_2-AR), immobilized human vitamin D3 receptor (hVDR) and TrkA receptor-rich lipid raft have been used for such purposes.[6, 50] Some target-based screening models for antitumor agents are presented in Table 1. Generally, these models are used to separate active compounds from complex natural products.

Currently, most studies focus on receptor-rich membranes to provide a platform for direct screening of active compounds. [51] These membranes usually have receptors, like EGFR and VEGR, which work in a form of bionic affinity chromatography. It is normally coupled to HPLC/MS to study the ligand–receptor interactions. EGFR is closely associated with tumour cell proliferation, angiogenesis, tumour invasion, metastasis and inhibition of apoptosis.[52] It is therefore a strategic target for screening tumour inhibitors. As a key receptor in the progression of tumour, VEGFR-2 has been an important target for antitumor therapeutic research[53]. Some small molecules, such as sunitinib and sorafinib, acting against neovascularisation by blocking the signal transduction pathway of VEGFR-2 have shown potent clinical effects. [54] In order to improve the ligand–receptor interaction studies and existing drug screening strategies aimed at specific receptors, some biological affinity systems with better specificity have to be designed. Currently, artificial cell cultures overexpressing VEGFR-2 on cell surface, named HEK293/VEGFR cell lines, have been constructed in a HEK293 engineered cell line[55]. The established HEK293/VEGFR-based CMC was considered as a model that could underline the performance of specific interactions between VEGFR-2 and its ligands.

6. Receptor-rich lipid rafts as an online biomaterial for screening antitumor agents

The online challenges in screening antitumor agents have always been: 1) designing limited effective bioscreening systems, 2) difficulty in reducing costs, and 3) lack of reconciling *in vitro* and *in vivo* data [63]. Over the years, CMC models have been able to address some of the problems. This technique has resulted in successful isolation of numerous bioactive components from complex samples that interact with membrane receptors. The dynamic simulation of the action of drug *in vivo* by the CMC presents a direct screening technique for active compounds.[37a, 64 59, 65] But the CMC is plagued with several issues regarding its selectivity, specificity, stability and service life span. These drawbacks are due to the use of homogenized cell membrane which contains receptors at relatively low densities and existing as different varieties.[37a, 64] The situation has impeded further development of CMC, and therefore, other alternatives are required.

For the first time, a novel TrkA receptor-rich lipid raft for identifying antitumor agents has been established (Figure 1). As a promising technology with inherent high selectivity and specificity, the monotarget lipid raft offers an efficient approach for online isolation and

analysis of active compounds. Previous reports have revealed that the biomaterial was stable and able to withstand extreme conditions[6b]. Fluorescent images of the biomaterial showed no obvious changes in intensity before and after packing the column. Most importantly, no fluorescence attenuation was observed in the lipid raft even after 30 days of continuous use. Further treatment with different mobile phases showed no effect on fluorescent intensity. In short, the long-lasting stability was a great improvement on the cell membrane-immobilized stationary phase (only 3 days or just 1 week under continuous usage).[66] Significantly, the lipid raft biomaterial provided unique retention behaviours for easy identification of active agents (Figure 1).

Currently, there is only one type of receptor-rich lipid raft biomaterial, and therefore, this concept can be extended to other related cells to develop more antitumor screening tools. It is possible to discover other lipid rafts with abundant multiple receptors to provide different targets for screening multitarget agents. Studies on the monotarget model have now paved the way for scientists to explore the possibility of designing a multitarget analogue of this biomaterial. Technically, both the mono- and multi- target models are likely to operate on the same principle with the latter having the benefit of providing multiple interaction sites.

7. Prospect of multiple receptor-based lipid raft biomaterial in cancer therapy

Multitarget agents have been increasingly explored [67] for enhanced therapeutic efficacies, improved safety profiles, and reduced resistance, [68] while limiting unwanted cross-reactivities via optimization of target selectivity. [69] Some clinically successful multitarget anticancer drugs include the anticancer kinase inhibitors sunitinib against PDGFR and VEGFR, dasatinib against Abl and Src, and lapatinib against EGFR and HER2. [70] However, these drugs are administered to few patients and the need to develop additional drugs could be a life-saving. For the past years, studies aiming at multitarget antitumor drug discovery have not been very successful, and the situation continues to greatly limit the number of validated leads. The desired multitarget biomaterial could significantly increase the true hit rate which will give rise to a larger number of therapeutic multitarget agents. Table 2 reveals some reported antitumor agents that act on multiple receptors with related target cancer diseases. The number of such agents could be remarkably increased if the current drug discovery protocols are improved and additional ones provided. The multitarget biomaterial will not only provide bioscreening, but also serve as a tool for understanding molecular-level

actions associated with cancers. . Comparatively, the specificity, stability and life span of the lipid raft will stand out among numerous antitumor bioscreening systems. [6b, 66] Hence, employing the biomaterial as routine mass screening system for potential antitumor agents will increase the number of leads for further development.

Establishing a multitarget biomaterial at this crucial moment could be very timely. Figure 1 shows the successful development of monotarget lipid raft for screening antitumor agents. The as yet undeveloped multitarget analogue can be seen as an improved version of the previous system. The compatibility of the proposed biomaterial to existing structures will be unquestionable as witnessed in the monotarget model. The biomaterial can also be easily constructed from a range of prominent receptor-rich cancer cell lines. Structure-activity relationship (SAR) studies can be facilitated since the system can support large scale bioscreening investigations. Preliminary information on lead compounds for different target receptors and even possible mechanistic actions prior to employing confirmatory assays could be ascertained. Most significantly, the multitarget LRSB can provide multiple sites for lead antitumor compounds which might affect multiple pathways. Now comprehensive studies in the application of lipid raft as screening biomaterial are in the exploratory stages, and additional work needs to be done to help generate such systems that will eventually enhance the number of multitarget agents employed in combating cancers.

8. Challenges and future considerations of lipid raft screening applications

Generally, the lipid raft screening system can suffer from problems relating to the search for multitarget antitumor agents. But the retrieval rate of the lead compounds could be highly enhanced with shortened processes, and even very strong correlation between *in vitro* and *in vivo* data. The capacity to effectively develop and identify sufficiently expressed receptors in cancer cells can be problematic. The use of appropriate technology to rapidly culture target cells within a reasonable time could also be a daunting task with some cost implications. Optimization of lipid raft extraction and technical modification of the biomaterial to improve its life span cannot be ignored. To further build therapeutically useful models, we need to have an in-depth molecular knowledge about the target receptors and also consider the role of co-receptors in modulating these activities. Establishing a new lipid raft model capable of screening multitarget antitumor agents will be characterized by high failures, but it is worth the effort. .

More studies should be directed at developing receptor-rich multitarget chemical screening systems. In that regard, understanding the mechanistic actions of these cancer-related receptors will not only become academically prudent, but also could lead to the development of highly specific drug inhibitors for clinical applications. It may also be necessary to modify existing chemical screening approaches from single to multiple target-oriented standard operating systems. As a necessity, knowledge on the whole receptor-based screening system including the ligands, stationary and mobile phases, and other accessory components requires a scientific upgrade. The efficient activities of the proposed multitarget model could also be realised by incorporating existing multitarget drug information on the current and future discovery. Now the scientific community has at its disposal a monotarget analogue of lipid raft biomaterial which is still in its infancy. In the opinion of these authors, there is a high probability of developing a multitarget analogue of the biomaterial for effective isolation of multitarget antitumor agents. The monotarget model was constructed from U251 cells; however, other cancer cells with sufficiently expressed receptors can also be employed. The final outcome could be clinically very important.

9. Conclusion

TrkA receptor-rich lipid raft biomaterial has been successfully employed to screen antitumor compounds which dramatically inhibited the growth of cancer cells. Comprehensive studies on this monotarget biomaterial in relation to its specificity, sensitivity, stability, life span and thermodynamic properties have been promising. The biomaterial has been easily adaptable to existing HPLC protocols for sample analysis. Most importantly, this monotarget model has paved way for the establishment of multitarget analogue which will operate on the same principle. This report therefore presents a promising would-be multitarget biomaterial for fast screening of antitumor lead components with the potential to interact with multiple targets.

Acknowledgments

We appreciate the support by National Natural Science Foundation of China (30973677, 81373371), Doctoral Fund of Ministry of Education of China (20113227110012) and Special Funds (JHB2012-37, GY2012049, GY2013055, GY2011028) in Zhenjiang, Jiangsu Province for all the projects related to multitarget potential of antitumor Chinese herbal medicine.

Figure legends

Figure 1: The development and on-line application of lipid raft biomaterial, with confirmatory MTT bioassay data. The figure illustrates the culture of U251 glioma cancer cells with overexpressed TrkA receptor. The extracted lipid raft was immobilized on activated silica beads to form lipid raft-coated silica beads as validated by fluorescence analysis. This biomaterial was then packed into a column to serve as a stationary phase for the on-line analysis of potential antitumor components (See chromatograms). The bioactive components, including standard anticancer drugs (gefitinib and lestaurtinib), and ether fraction revealed long retention times. The ether extract exhibited significant cytotoxicity against cancer cells as observed in the MTT analysis. This cytotoxicity was comparable to the standard anticancer drug, 5-Fluorouracil.

Figure 2: Schematic representation of anticancer drug discovery and development.

Figure 3: The possible multiple targets that can be affected by bioactive isolates from lipid raft-coated silica beads containing cancer-related receptors like Epidermal growth factor receptor (EGFR), Tumour necrosis factor receptor (TNFR), Human epidermal growth factor receptor 2 (HER2), Vascular epidermal growth factor receptor (VEGFR) and Fas receptor (Fas). Most anticancer drugs act via apoptotic (programmed cell death) induction and inhibition of angiogenesis in their antitumor activities.

Tables

Table 1: Target-based screening for antitumor agents in various mixtures

Molecular target	Screening model	Active compounds	Mixtures	Reference
β_2-Adrenoceptor	Purified receptor	Amygdalin	*Semen Armeniacae* Amarum	[50a]
DNA topoisomerase	Purified enzyme	Doxorubicin daunorubicin	Doxorubicin, Daunorubicin,pravastain	[48]
EGFR	HEK293/CMC	Asarinin	*Radix et Rhizoma Asari*	[56]
EGFR	HEK293/CMC	Vauquline Strychnine	*Semen Strychni*	[7]
EGFR	HEK293/CMC	Resveratrol	Rhizoma Polygoni Cuspidati	[57]
EGFR	A431/CMC	Taspine Caulophine	*Radix Caulophylli*	[58]
EGFR	A431/CMC	Oxymatrine Matrine	*Radix sophorae flavescentis*	[59]
Hsp90	Progesterone receptor chaperone complex	Capsaicin	Chemical library	[60]
Melan A	MU89	OBAA, Flunarizine, 17-AAG, Aphidicolin, Damnacanthal, PMA, Dantrolene, Glyburide	Chemical library	[61]
TrkA receptor	Lipid raft	NA	*Albizziae Cortex*	[6a, 62]
VEGFR-2	HEK293/CMC	Mesaconitine Aconitine Hypaconitine	*Galla chinensis* *Aconitum carmichaeli* Debx	[54]

Abbreviations: **A431**, epidermoid carcinoma cell line; **17-AAG**, 17-allylaminogeldanamycin; **CMC**, cell membrane chromatography; **DNA**, deoxyribonucleic acid; **EGFR**, epidermal growth factor receptor; **HEK293**, human embryonic kidney 293 cells; **Hsp90**, heat shock protein 90; **Melan A**, melanoma antigen. **MU89**, melanoma cell line; **OBAA**, 3-(4-octadecyl)benzoylacrylic acid; **PMA**, phorbol-12-myristate-13 acetate; **TrkA**, tropomyosin –related tyrosine kinase A; **VEGFR**, vascular endothelial growth factor receptor;

Table 2: Reported antitumor agents that interact with multiple receptors

Agent	Targeted disease	Multitarget action	Reference
ABT-869	Solid malignancies, acute myeloid leukemia	VEGFR-2, CSF1R	37c, 71
AMG-706	Thyroid cancers, non-small-cell lung cancer	VEGFR-2 , KIT	37c, 72
Berberine	Breast cancer, Prostate cancer, Cervical cancer	HER-2, AR, FasR	73
Celasterol	Prostate cancer; non-small-cell lung cancer; Breast cancer, human glioma	AR, FasR, DR-4/5, VEGFR-2	74
Curcumin	colon cancer, pancreatic cancer	ER-α, FasR, IR, EPCR, H2R, HER-2, EGFR, DR-5, IL-8R, CXCR4, LDLR, AHR, ITR,AR	75
Indirubin/ indirubin-3'-monoxime	Prostate cancer, Myeloid leukaemia	VEGFR-2, FGFR1	76
Lapatinib	Breast cancer	EGFR ERBB-2	37c, 77
Norcantharidin	Colorectal carcinoma, Colon cancer, Hepatoma cells	FasR, VEGFR-2, DR-5	78
Quercetin	Pancreatic cancer, Breast cancer	EGFR, ER-α	79
Resveratrol	Prostate cancer , breast cancer	AR, ER-α	80
Sunitinib	Gastrointestinal stromal tumor, renal cell carcinoma	VEGFR-2 KIT	37c, 81
Wogonin	Breast cancer, HUVEC	EGFR, ER-α, VEGFR-1	82
ZD-6474	Non-small-cell lung cancer, small-cell lung cancer, myeloma	EGFR VEGFR-2 RET	37c, 83

Abbreviations: **AR**, androgen receptor; **AHR**, aryl hydrocarbon receptor; **CSF1R**, colony stimulating factor 1 receptor; **CXCR-4**, C-X-C chemokine receptor type 4; **DR-4/5**, death receptor-4/5; **EGFR**, epidermal growth factor receptor; **EPCR**, endothelial protein C receptor; **ER-α**, oestrogen receptor-alpha; **FasR**, Fas receptor ; **FGFR-1**, fibroblast growth factor receptor 1; **HER-2**, human epidermal growth factor receptor 2; **H2R**, histamine (2) receptor; **IL-8-R**, interleukin 8-receptor; **IR**, integrin receptor; **KIT**, KIT receptor; **LDL-R**, low density lipoprotein–receptor; **RET**, RET receptor; **VEGFR-1/2**, vascular endothelial growth factor receptor-1/2;

References

1. Medina-Franco, J. L.; Giulianotti, M. A.; Welmaker, G. S.; Houghten, R. A., Shifting from the single to the multitarget paradigm in drug discovery. *Drug discovery today* **2013,** *18* (9), 495-501.
2. Aubé, J., Drug repurposing and the medicinal chemist. *ACS medicinal chemistry letters* **2012,** *3* (6), 442-444.
3. Paolini, G. V.; Shapland, R. H.; van Hoorn, W. P.; Mason, J. S.; Hopkins, A. L., Global mapping of pharmacological space. *Nature biotechnology* **2006,** *24* (7), 805-815.
4. Jacoby, E., Computational chemogenomics. *Wiley Interdisciplinary Reviews: Computational Molecular Science* **2011,** *1* (1), 57-67.
5. (a) Houghten, R. A.; Dooley, C. T.; Appel, J. R., In vitro and direct in vivo testing of mixture-based combinatorial libraries for the identification of highly active and specific opiate ligands. *The AAPS journal* **2006,** *8* (2), E371-E382; (b) Carroll, F. I.; Houghten, R. A., From rapid in vitro screening to rapid in vivo screening in the drug discovery process. *Neuropsychopharmacology* **2009,** *34* (1), 251-252.
6. (a) Tong, S.; Fu, M.; Cao, X.; Firempong, C. K.; Yi, C.; Zhen, Q.; Zhong, H.; Yu, J.; Xu, X., Lipid Raft Stationary Phase Chromatography for Screening Anti-tumor Components from Galla chinensis. *Chromatographia* **2014,** *77* (5-6), 419-429; (b) Tong, S.; Sun, C.; Cao, X.; Zheng, Q.; Zhang, H.; Firempong, C. K.; Feng, Y.; Yang, Y.; Yu, J.; Xu, X., Development and thermodynamic evaluation of novel lipid raft stationary phase chromatography for screening potential antitumor agents. *Biomedical Chromatography* **2014**.
7. Sun, M.; Guo, Y.; Dai, B.; Wang, C.; He, L., High‐expression EGFR/cell membrane chromatography‐online‐high‐performance liquid chromatography/mass spectrometry: rapid screening of EGFR antagonists from Semen Strychni. *Rapid Communications in Mass Spectrometry* **2012,** *26* (17), 2027-2032.
8. Su, X.; Hu, L.; Kong, L.; Lei, X.; Zou, H., Affinity chromatography with immobilized DNA stationary phase for biological fingerprinting analysis of traditional Chinese medicines. *Journal of chromatography A* **2007,** *1154* (1), 132-137.
9. (a) Noctor, T. A.; Wainer, I. W.; Hage, D. S., Allosteric and competitive displacement of drugs from human serum albumin by octanoic acid, as revealed by high-performance liquid affinity chromatography, on a human serum albumin-based stationary phase. *Journal of Chromatography B: Biomedical Sciences and Applications* **1992,** *577* (2), 305-315; (b) Vuignier, K.; Guillarme, D.; Veuthey, J.-L.; Carrupt, P.-A.; Schappler, J., High performance affinity chromatography (HPAC) as a high-throughput screening tool in drug discovery to study drug–plasma protein interactions. *Journal of pharmaceutical and biomedical analysis* **2013,** *74*, 205-212.
10. Zhang, C.; Li, J.; Xu, L.; Shi, Z.-G., Fast immobilized liposome chromatography based on penetrable silica microspheres for screening and analysis of permeable compounds. *Journal of Chromatography A* **2012,** *1233*, 78-84.
11. (a) Beigi, F.; Lundahl, P., Immobilized biomembrane chromatography of highly lipophilic drugs. *Journal of Chromatography A* **1999,** *852* (1), 313-317; (b) Moaddel, R.; Musyimi, H. K.; Sanghvi, M.; Bashore, C.; Frazier, C. R.; Khadeer, M.; Bhatia, P.; Wainer, I. W., Synthesis and characterization of a cellular membrane affinity chromatography column containing histamine 1 and $P2Y_1$ receptors: A multiple G-protein coupled receptor column. *Journal of pharmaceutical and biomedical analysis* **2010,** *52* (3), 416-419; (c) Habicht, K.; Frazier, C.; Singh, N.; Shimmo, R.; Wainer, I.; Moaddel, R., The synthesis and characterization of a nuclear membrane affinity chromatography column for the study of human breast cancer resistant protein (BCRP) using nuclear membranes obtained from the LN-229 cells. *Journal of pharmaceutical and biomedical analysis* **2013,** *72*, 159.

12. (a) Silverman, L.; Campbell, R.; Broach, J. R., New assay technologies for high-throughput screening. *Current opinion in chemical biology* **1998,** *2* (3), 397-403; (b) Stockwell, B. R., Exploring biology with small organic molecules. *Nature* **2004,** *432* (7019), 846-854; (c) Lipinski, C.; Hopkins, A., Navigating chemical space for biology and medicine. *Nature* **2004,** *432* (7019), 855-861.
13. (a) Pike, L. J., Lipid rafts bringing order to chaos. *Journal of lipid research* **2003,** *44* (4), 655-667; (b) van Deurs, B.; Roepstorff, K.; Hommelgaard, A. M.; Sandvig, K., Caveolae: anchored, multifunctional platforms in the lipid ocean. *Trends in cell biology* **2003,** *13* (2), 92-100.
14. (a) Brown, D. A.; Rose, J. K., Sorting of GPI-anchored proteins to glycolipid-enriched membrane subdomains during transport to the apical cell surface. *Cell* **1992,** *68* (3), 533-544; (b) Liu, P.; Ying, Y.; Ko, Y.-G.; Anderson, R. G., Localization of platelet-derived growth factor-stimulated phosphorylation cascade to caveolae. *Journal of Biological Chemistry* **1996,** *271* (17), 10299-10303; (c) Mineo, C.; James, G. L.; Smart, E. J.; Anderson, R. G., Localization of epidermal growth factor-stimulated Ras/Raf-1 interaction to caveolae membrane. *Journal of Biological Chemistry* **1996,** *271* (20), 11930-11935; (d) Sargiacomo, M.; Sudol, M.; Tang, Z.; Lisanti, M. P., Signal transducing molecules and glycosyl-phosphatidylinositol-linked proteins form a caveolin-rich insoluble complex in MDCK cells. *The Journal of cell biology* **1993,** *122* (4), 789-807; (e) Chun, M.; Liyanage, U. K.; Lisanti, M. P.; Lodish, H. F., Signal transduction of a G protein-coupled receptor in caveolae: colocalization of endothelin and its receptor with caveolin. *Proceedings of the National Academy of Sciences* **1994,** *91* (24), 11728-11732; (f) Gorodinsky, A.; Harris, D. A., Glycolipid-anchored proteins in neuroblastoma cells form detergent-resistant complexes without caveolin. *The Journal of cell biology* **1995,** *129* (3), 619-627; (g) Bickel, P. E.; Scherer, P. E.; Schnitzer, J. E.; Oh, P.; Lisanti, M. P.; Lodish, H. F., Flotillin and epidermal surface antigen define a new family of caveolae-associated integral membrane proteins. *Journal of Biological Chemistry* **1997,** *272* (21), 13793-13802.
15. Edidin, M., Membrane cholesterol, protein phosphorylation, and lipid rafts. *Science Signaling* **2001,** *2001* (67), pe1.
16. (a) Roepstorff, K.; Thomsen, P.; Sandvig, K.; van Deurs, B., Sequestration of epidermal growth factor receptors in non-caveolar lipid rafts inhibits ligand binding. *Journal of Biological Chemistry* **2002,** *277* (21), 18954-18960; (b) Takebayashi, M.; Hayashi, T.; Su, T. P., σ - 1 Receptors potentiate epidermal growth factor signaling towards neuritogenesis in PC12 cells: Potential relation to lipid raft reconstitution. *Synapse* **2004,** *53* (2), 90-103.
17. (a) Abulrob, A.; Giuseppin, S.; Andrade, M. F.; McDermid, A.; Moreno, M.; Stanimirovic, D., Interactions of EGFR and caveolin-1 in human glioblastoma cells: evidence that tyrosine phosphorylation regulates EGFR association with caveolae. *Oncogene* **2004,** *23* (41), 6967-6979; (b) Mineo, C.; Gill, G. N.; Anderson, R. G., Regulated migration of epidermal growth factor receptor from caveolae. *Journal of Biological Chemistry* **1999,** *274* (43), 30636-30643.
18. Hryniewicz-Jankowska, A.; Augoff, K.; Biernatowska, A.; Podkalicka, J.; Sikorski, A. F., Membrane rafts as a novel target in cancer therapy. *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer* **2014,** *1845* (2), 155-165.
19. Escribá, P. V., Membrane-lipid therapy: a new approach in molecular medicine. *Trends in molecular medicine* **2006,** *12* (1), 34-43.
20. Mollinedo, F.; Gajate, C., Fas/CD95 death receptor and lipid rafts: new targets for apoptosis-directed cancer therapy. *Drug Resistance Updates* **2006,** *9* (1), 51-73.
21. Sarnataro, D.; Pisanti, S.; Santoro, A.; Gazzerro, P.; Malfitano, A. M.; Laezza, C.; Bifulco, M., The cannabinoid CB1 receptor antagonist rimonabant (SR141716) inhibits human breast cancer cell proliferation through a lipid raft-mediated mechanism. *Molecular pharmacology* **2006,** *70* (4), 1298-1306.
22. Gabizon, A.; Tzemach, D.; Gorin, J.; Mak, L.; Amitay, Y.; Shmeeda, H.; Zalipsky, S., Improved therapeutic activity of folate-targeted liposomal doxorubicin in folate receptor-expressing tumor models. *Cancer chemotherapy and pharmacology* **2010,** *66* (1), 43-52.

23. Chiu, S.-J.; Liu, S.; Perrotti, D.; Marcucci, G.; Lee, R. J., Efficient delivery of a Bcl-2-specific antisense oligodeoxyribonucleotide (G3139) via transferrin receptor-targeted liposomes. *Journal of controlled release* **2006,** *112* (2), 199-207.
24. Han, H. D.; Mangala, L. S.; Lee, J. W.; Shahzad, M. M.; Kim, H. S.; Shen, D.; Nam, E. J.; Mora, E. M.; Stone, R. L.; Lu, C., Targeted gene silencing using RGD-labeled chitosan nanoparticles. *Clinical Cancer Research* **2010,** *16* (15), 3910-3922.
25. Yu, B.; Tai, H. C.; Xue, W.; Lee, L. J.; Lee, R. J., Receptor-targeted nanocarriers for therapeutic delivery to cancer. *Molecular membrane biology* **2010,** *27* (7), 286-298.
26. Vadlapudi, A. D.; Vadlapatla, R. K.; Kwatra, D.; Earla, R.; Samanta, S. K.; Pal, D.; Mitra, A. K., Targeted lipid based drug conjugates: a novel strategy for drug delivery. *International journal of pharmaceutics* **2012,** *434* (1), 315-324.
27. Thomann-Harwood, L.; Kaeuper, P.; Rossi, N.; Milona, P.; Herrmann, B.; McCullough, K., Nanogel vaccines targeting dendritic cells: contributions of the surface decoration and vaccine cargo on cell targeting and activation. *Journal of Controlled Release* **2013,** *166* (2), 95-105.
28. Partlow, K. C.; Lanza, G. M.; Wickline, S. A., Exploiting lipid raft transport with membrane targeted nanoparticles: a strategy for cytosolic drug delivery. *Biomaterials* **2008,** *29* (23), 3367-3375.
29. Khalil, I. A.; Kogure, K.; Akita, H.; Harashima, H., Uptake pathways and subsequent intracellular trafficking in nonviral gene delivery. *Pharmacological reviews* **2006,** *58* (1), 32-45.
30. Raghu, H.; Sharma-Walia, N.; Veettil, M. V.; Sadagopan, S.; Caballero, A.; Sivakumar, R.; Varga, L.; Bottero, V.; Chandran, B., Lipid rafts of primary endothelial cells are essential for Kaposi's sarcoma-associated herpesvirus/human herpesvirus 8-induced phosphatidylinositol 3-kinase and RhoA-GTPases critical for microtubule dynamics and nuclear delivery of viral DNA but dispensable for binding and entry. *Journal of virology* **2007,** *81* (15), 7941-7959.
31. Chen, X.; Shank, S.; Davis, P. B.; Ziady, A. G., Nucleolin-mediated cellular trafficking of DNA nanoparticle is lipid raft and microtubule dependent and can be modulated by glucocorticoid. *Molecular Therapy* **2011,** *19* (1), 93-102.
32. Sharma, R.; Ghasparian, A.; Robinson, J. A.; McCullough, K. C., Synthetic virus-like particles target dendritic cell lipid rafts for rapid endocytosis primarily but not exclusively by macropinocytosis. *PloS one* **2012,** *7* (8), e43248.
33. Pang, M.-H.; Kim, Y.; Jung, K. W.; Cho, S.; Lee, D. H., A series of case studies: practical methodology for identifying antinociceptive multi-target drugs. *Drug discovery today* **2012,** *17* (9), 425-434.
34. (a) Sanchez, I.; Calderon, J.; Ruiz, B.; Tellez, J.; Calzada, L.; Taboada, J., In vitro cytotoxicity of flavonoids against MK2 and C6 tumour cells. *Phytotherapy Research* **2001,** *15* (4), 290-293; (b) Franco‐Molina, M.; Gomez‐Flores, R.; Tamez‐Guerra, P.; Tamez‐Guerra, R.; Castillo‐Leon, L.; Rodríguez‐Padilla, C., In vitro immunopotentiating properties and tumour cell toxicity induced by Lophophora williamsii (peyote) cactus methanolic extract. *Phytotherapy Research* **2003,** *17* (9), 1076-1081; (c) Mena-Rejon, G.; Caamal-Fuentes, E.; Cantillo-Ciau, Z.; Cedillo-Rivera, R.; Flores-Guido, J.; Moo-Puc, R., In vitro cytotoxic activity of nine plants used in Mayan traditional medicine. *Journal of ethnopharmacology* **2009,** *121* (3), 462-465.
35. Alonso-Castro, A. J.; Villarreal, M. L.; Salazar-Olivo, L. A.; Gomez-Sanchez, M.; Dominguez, F.; Garcia-Carranca, A., Mexican medicinal plants used for cancer treatment: pharmacological, phytochemical and ethnobotanical studies. *Journal of ethnopharmacology* **2011,** *133* (3), 945-972.
36. Brodeur, G. M.; Minturn, J. E.; Ho, R.; Simpson, A. M.; Iyer, R.; Varela, C. R.; Light, J. E.; Kolla, V.; Evans, A. E., Trk receptor expression and inhibition in neuroblastomas. *Clinical Cancer Research* **2009,** *15* (10), 3244-3250.
37. (a) HOU, J., Evaluation of drug‐muscarinic receptor affinities using cell membrane chromatography and radioligand binding assay in guinea pig jejunum membrane. *Acta Pharmacologica Sinica* **2005,** *26* (1), 113-116; (b) Sadagopan, S.; Sharma-Walia, N.; Veettil, M. V.; Raghu, H.; Sivakumar, R.; Bottero, V.; Chandran, B., Kaposi's sarcoma-associated herpesvirus induces

sustained NF-κB activation during de novo infection of primary human dermal microvascular endothelial cells that is essential for viral gene expression. *Journal of virology* **2007,** *81* (8), 3949-3968; (c) Sathornsumetee, S.; Reardon, D. A., Targeting multiple kinases in glioblastoma multiforme. **2009**.

38. (a) Pike, L. J., Lipid rafts. *J. Lipid Res.* **2003,** *44* (4), 655-667; (b) Zhuang, L.; Kim, J.; Adam, R. M.; Solomon, K. R.; Freeman, M. R., Cholesterol targeting alters lipid raft composition and cell survival in prostate cancer cells and xenografts. *J. Clin. Invest.* **2005,** *115* (4), 959-968.

39. Patra, S. K., Dissecting lipid raft facilitated cell signaling pathways in cancer. *Biochim. Biophys. Acta* **2008,** *1785* (2), 182-206.

40. Mitri, Z.; Constantine, T.; O'Regan, R., The HER2 receptor in breast cancer: pathophysiology, clinical use, and new advances in therapy. *Chemotherapy research and practice* **2012,** *2012*.

41. (a) Hehlgans, T.; Pfeffer, K., The intriguing biology of the tumour necrosis factor/tumour necrosis factor receptor superfamily: players, rules and the games. *Immunology* **2005,** *115* (1), 1-20; (b) Clark, I. A., How TNF was recognized as a key mechanism of disease. *Cytokine & growth factor reviews* **2007,** *18* (3), 335-343; (c) Tansey, M. G.; Szymkowski, D. E., The TNF superfamily in 2009: new pathways, new indications, and new drugs. *Drug discovery today* **2009,** *14* (23), 1082-1088.

42. Grewal, I. S., Overview of TNF superfamily: a chest full of potential therapeutic targets. In *Therapeutic Targets of the TNF Superfamily*, Springer: 2009; pp 1-7.

43. Wang, L.; Yang, J. K.; Kabaleeswaran, V.; Rice, A. J.; Cruz, A. C.; Park, A. Y.; Yin, Q.; Damko, E.; Jang, S. B.; Raunser, S., The Fas-FADD death domain complex structure reveals the basis of DISC assembly and disease mutations. *Nature structural & molecular biology* **2010,** *17* (11), 1324-1329.

44. Yi, Z. F.; Cho, S. G.; Zhao, H.; Wu, Y. y.; Luo, J.; Li, D.; Yi, T.; Xu, X.; Wu, Z.; Liu, M., A novel peptide from human apolipoprotein (a) inhibits angiogenesis and tumor growth by targeting c‐Src phosphorylation in VEGF‐induced human umbilical endothelial cells. *International Journal of Cancer* **2009,** *124* (4), 843-852.

45. Ferrara, N., Vascular endothelial growth factor: basic science and clinical progress. *Endocrine reviews* **2004,** *25* (4), 581-611.

46. Sherr, C. J., Principles of tumor suppression. *Cell* **2004,** *116* (2), 235-246.

47. (a) Kingsmore, S. F.; Lindquist, I. E.; Mudge, J.; Gessler, D. D.; Beavis, W. D., Genome-wide association studies: progress and potential for drug discovery and development. *Nature Reviews Drug Discovery* **2008,** *7* (3), 221-230; (b) Araujo, R. P.; Liotta, L. A.; Petricoin, E. F., Proteins, drug targets and the mechanisms they control: the simple truth about complex networks. *Nature reviews Drug discovery* **2007,** *6* (11), 871-880.

48. Pan, Y.; Zhang, H.; Chen, Y., New approach for screening anti-tumor compounds. *Chinese Science Bulletin* **2003,** *48* (7), 630-633.

49. Moaddel, R.; Lu, L.; Baynham, M.; Wainer, I. W., Immobilized receptor-and transporter-based liquid chromatographic phases for on-line pharmacological and biochemical studies: a mini-review. *Journal of Chromatography B* **2002,** *768* (1), 41-53.

50. (a) Zheng, X.; Zhao, X.; Yang, R.; Wang, S.; Wei, Y.; Zheng, J., β2-Adrenoceptor affinity chromatography and its application in the screening of the active compounds from Semen Armeniacae Amarum. *Chinese Science Bulletin* **2008,** *53* (6), 842-847; (b) Arai, M. A.; Kobatake, E.; Koyano, T.; Kowithayakorn, T.; Kato, S.; Ishibashi, M., A method for the rapid discovery of naturally occurring products by proteins immobilized on magnetic beads and reverse affinity chromatography. *Chemistry–An Asian Journal* **2009,** *4* (12), 1802-1808.

51. (a) Liang, M.-J.; He, L.-C.; Yang, G.-D., Screening, analysis and in vitro vasodilatation of effective components from Ligusticum Chuanxiong. *Life sciences* **2005,** *78* (2), 128-133; (b) Hou, X.; Wang, S.; Hou, J.; He, L., Establishment of A431 cell membrane chromatography‐RPLC method for screening target components from Radix Caulophylli. *Journal of separation science* **2011,** *34* (5), 508-513.

52. Richard, J.; Sainsbury, C.; Needham, G.; Farndon, J.; Malcolm, A.; Harris, A., Epidermal-growth-factor receptor status as predictor of early recurrence of and death from breast cancer. *The Lancet* **1987,** *329* (8547), 1398-1402.
53. (a) Kerbel, R. S., Tumor angiogenesis. *New England Journal of Medicine* **2008,** *358* (19), 2039-2049; (b) Krause, D. S.; Van Etten, R. A., Tyrosine kinases as targets for cancer therapy. *New England Journal of Medicine* **2005,** *353* (2), 172-187; (c) Zhang, J.; Yang, P. L.; Gray, N. S., Targeting cancer with small molecule kinase inhibitors. *Nature Reviews Cancer* **2009,** *9* (1), 28-39.
54. Li, M.; Wang, S.; Zhang, Y.; He, L., An online coupled cell membrane chromatography with LC/MS method for screening compounds from Aconitum carmichaeli Debx. acting on VEGFR-2. *Journal of pharmaceutical and biomedical analysis* **2010,** *53* (4), 1063-1069.
55. Wang, L.; Ren, J.; Sun, M.; Wang, S., A combined cell membrane chromatography and online HPLC/MS method for screening compounds from Radix Caulophylli acting on the human α 1A-adrenoceptor. *Journal of pharmaceutical and biomedical analysis* **2010,** *51* (5), 1032-1036.
56. Han, S.; Huang, J.; Hou, J.; Wang, S., Screening epidermal growth factor receptor antagonists from Radix et Rhizoma Asari by two‐dimensional liquid chromatography. *Journal of separation science* **2014,** *37* (13), 1525-1532.
57. Sun, M.; Zhang, Y.-m.; Zhang, J.; Wang, S.-c.; He, L.-c., A high expression EGFR/cell membrane chromatography and online high performance liquid chromatography/mass spectrometry method for screening EGFR antagonists from Rhizoma Polygoni Cuspidati. *Acta Pharmaceutica Sinica B* **2011,** *1* (2), 115-120.
58. Sun, M.; Ren, J.; Du, H.; Zhang, Y.; Zhang, J.; Wang, S.; He, L., A combined A431 cell membrane chromatography and online high performance liquid chromatography/mass spectrometry method for screening compounds from total alkaloid of Radix Caulophylli acting on the human EGFR. *Journal of Chromatography B* **2010,** *878* (28), 2712-2718.
59. Wang, S.; Sun, M.; Zhang, Y.; Du, H.; He, L., A new A 431/cell membrane chromatography and online high performance liquid chromatography/mass spectrometry method for screening epidermal growth factor receptor antagonists from Radix sophorae flavescentis. *Journal of Chromatography A* **2010,** *1217* (32), 5246-5252.
60. Patwardhan, C. A.; Alfa, E.; Lu, S.; Chadli, A., Progesterone Receptor Chaperone Complex–Based High-Throughput Screening Assay Identification of Capsaicin as an Inhibitor of the Hsp90 Machine. *Journal of biomolecular screening* **2014**, 1087057114549147.
61. Haggerty, T. J.; Dunn, I. S.; Rose, L. B.; Newton, E. E.; Kurnick, J. T., A screening assay to identify agents that enhance T-cell recognition of human melanomas. *Assay and drug development technologies* **2012,** *10* (2), 187-201.
62. Tong, S.; Sun, C.; Cao, X.; Zheng, Q.; Zhang, H.; Firempong, C. K.; Feng, Y.; Yang, Y.; Yu, J.; Xu, X., Development and thermodynamic evaluation of novel lipid raft stationary phase chromatography for screening potential antitumor agents. *Biomedical Chromatography* **2014,** *28* (12), 1615-1623.
63. (a) Horrobin, D. F., Modern biomedical research: an internally self-consistent universe with little contact with medical reality? *Nature Reviews Drug Discovery* **2003,** *2* (2), 151-154; (b) Kubinyi, H., Drug research: myths, hype and reality. *Nature Reviews Drug Discovery* **2003,** *2* (8), 665-668.
64. Bing-Xiang, Y.; Jin, H.; Guang-De, Y.; Li-mei, Z.; Lang-Chong, H., Comparison of determination of drug–muscarinic receptor affinity by cell-membrane chromatography and by radioligand-binding assay with the cerebrum membrane of the rat. *Chromatographia* **2005,** *61* (7-8), 381-384.
65. He, L.; Yang, G.; Geng, X., Enzymatic activity and chromatographic characteristics of the cell membrane immobilized on silica surface. *Chinese Science Bulletin* **1999,** *44* (9), 826-831.
66. Hou, X.; Zhou, M.; Jiang, Q.; Wang, S.; He, L., A vascular smooth muscle/cell membrane chromatography–offline-gas chromatography/mass spectrometry method for recognition, separation and identification of active components from traditional Chinese medicines. *Journal of Chromatography A* **2009,** *1216* (42), 7081-7087.

67. (a) Smalley, K. S.; Haass, N. K.; Brafford, P. A.; Lioni, M.; Flaherty, K. T.; Herlyn, M., Multiple signaling pathways must be targeted to overcome drug resistance in cell lines derived from melanoma metastases. *Molecular cancer therapeutics* **2006,** *5* (5), 1136-1144; (b) Sergina, N. V.; Rausch, M.; Wang, D.; Blair, J.; Hann, B.; Shokat, K. M.; Moasser, M. M., Escape from HER-family tyrosine kinase inhibitor therapy by the kinase-inactive HER3. *Nature* **2007,** *445* (7126), 437-441.
68. Larder, B. A.; Kemp, S. D.; Harrigan, P. R., Potential mechanism for sustained antiretroviral efficacy of AZT-3TC combination therapy. *Science* **1995,** *269* (5224), 696-699.
69. Zhang, X.; Crespo, A.; Fernández, A., Turning promiscuous kinase inhibitors into safer drugs. *Trends in biotechnology* **2008,** *26* (6), 295-301.
70. (a) Krug, M.; Hilgeroth, A., Recent advances in the development of multi-kinase inhibitors. *Mini reviews in medicinal chemistry* **2008,** *8* (13), 1312-1327; (b) Adrian, G. L.; Marcel, V.; Robert, B. G.; Richard, T., A comparison of physicochemical property profiles of marketed oral drugs and orally bioavailable anti-cancer protein kinase inhibitors in clinical development. *Current topics in medicinal chemistry* **2007,** *7* (14), 1408-1422.
71. (a) Wong, C.-I.; Koh, T.-S.; Soo, R.; Hartono, S.; Thng, C.-H.; McKeegan, E.; Yong, W.-P.; Chen, C.-S.; Lee, S.-C.; Wong, J., Phase I and biomarker study of ABT-869, a multiple receptor tyrosine kinase inhibitor, in patients with refractory solid malignancies. *Journal of Clinical Oncology* **2009,** *27* (28), 4718-4726; (b) Shankar, D. B.; Li, J.; Tapang, P.; McCall, J. O.; Pease, L. J.; Dai, Y.; Wei, R.-Q.; Albert, D. H.; Bouska, J. J.; Osterling, D. J., ABT-869, a multitargeted receptor tyrosine kinase inhibitor: inhibition of FLT3 phosphorylation and signaling in acute myeloid leukemia. *Blood* **2007,** *109* (8), 3400-3408.
72. (a) Sherman, S. I., Early clinical studies of novel therapies for thyroid cancers. *Endocrinology and metabolism clinics of North America* **2008,** *37* (2), 511-524; (b) Lee, C. B.; Socinski, M. A., Vascular endothelial growth factor receptor tyrosine kinase inhibitors in non-small cell lung cancer: a review of recent clinical trials. *Reviews on recent clinical trials* **2007,** *2* (2), 117-120.
73. (a) Kuo, H.-P.; Chuang, T.-C.; Yeh, M.-H.; Hsu, S.-C.; Way, T.-D.; Chen, P.-Y.; Wang, S.-S.; Chang, Y.-H.; Kao, M.-C.; Liu, J.-Y., Growth suppression of HER2-overexpressing breast cancer cells by berberine via modulation of the HER2/PI3K/Akt signaling pathway. *Journal of agricultural and food chemistry* **2011,** *59* (15), 8216-8224; (b) Li, J.; Cao, B.; Liu, X.; Fu, X.; Xiong, Z.; Chen, L.; Sartor, O.; Dong, Y.; Zhang, H., Berberine suppresses androgen receptor signaling in prostate cancer. *Molecular cancer therapeutics* **2011,** *10* (8), 1346-1356; (c) Lu, B.; Hu, M.; Peng, J., Cytotoxicity of berberine on human cervical carcinoma HeLa cells through mitochondria, death receptor and MAPK pathways, and in-silico drug-target prediction. *Toxicology in vitro* **2010,** *24* (6), 1482-1490.
74. (a) Yang, H.; Chen, D.; Cui, Q. C.; Yuan, X.; Dou, Q. P., Celastrol, a triterpene extracted from the Chinese "Thunder of God Vine," is a potent proteasome inhibitor and suppresses human prostate cancer growth in nude mice. *Cancer research* **2006,** *66* (9), 4758-4765; (b) Mou, H.; Zheng, Y.; Zhao, P.; Bao, H.; Fang, W.; Xu, N., Celastrol induces apoptosis in non-small-cell lung cancer A549 cells through activation of mitochondria-and Fas/FasL-mediated pathways. *Toxicology in Vitro* **2011,** *25* (5), 1027-1032; (c) Sung, B.; Park, B.; Yadav, V. R.; Aggarwal, B. B., Celastrol, a triterpene, enhances TRAIL-induced apoptosis through the down-regulation of cell survival proteins and up-regulation of death receptors. *Journal of Biological Chemistry* **2010,** *285* (15), 11498-11507; (d) Huang, Y.; Zhou, Y.; Fan, Y.; Zhou, D., Celastrol inhibits the growth of human glioma xenografts in nude mice through suppressing VEGFR expression. *Cancer letters* **2008,** *264* (1), 101-106.
75. (a) Goel, A.; Kunnumakkara, A. B.; Aggarwal, B. B., Curcumin as "Curecumin": from kitchen to clinic. *Biochemical pharmacology* **2008,** *75* (4), 787-809; (b) Anand, P.; Sundaram, C.; Jhurani, S.; Kunnumakkara, A. B.; Aggarwal, B. B., Curcumin and cancer: an "old-age" disease with an "age-old" solution. *Cancer letters* **2008,** *267* (1), 133-164.
76. (a) Zhang, X.; Song, Y.; Wu, Y.; Dong, Y.; Lai, L.; Zhang, J.; Lu, B.; Dai, F.; He, L.; Liu, M., Indirubin inhibits tumor growth by antitumor angiogenesis via blocking VEGFR2‐mediated JAK/STAT3 signaling in endothelial cell. *International Journal of Cancer* **2011,** *129* (10), 2502-2511; (b)

Zhen, Y.; Sørensen, V.; Jin, Y.; Suo, Z.; Więdłocha, A., Indirubin-3′-monoxime inhibits autophosphorylation of FGFR1 and stimulates ERK1/2 activity via p38 MAPK. *Oncogene* **2007,** *26* (44), 6372-6385.

77. Schwartz, J., Current combination chemotherapy regimens for metastatic breast cancer. *American Journal of Health-System Pharmacy* **2009,** *66* (23 Supplement 6), S3-S8.

78. (a) Peng, F.; Wei, Y.-Q.; Tian, L.; Yang, L.; Zhao, X.; Lu, Y.; Mao, Y.-Q.; Kan, B.; Lei, S.; Wang, G.-S., Induction of apoptosis by norcantharidin in human colorectal carcinoma cell lines: involvement of the CD95 receptor/ligand. *Journal of cancer research and clinical oncology* **2002,** *128* (4), 223-230; (b) Zhang, L.; Ji, Q.; Liu, X.; Chen, X.; Chen, Z.; Qiu, Y.; Sun, J.; Cai, J.; Zhu, H.; Li, Q., Norcantharidin inhibits tumor angiogenesis via blocking VEGFR2/MEK/ERK signaling pathways. *Cancer science* **2013,** *104* (5), 604-610; (c) Yeh, C.-h.; Yang, Y.-y.; Huang, Y.-f.; Chow, K.-c.; Chen, M.-f., Induction of apoptosis in human Hep3B hepatoma cells by norcantharidin through a p53 independent pathway via TRAIL/DR5 signal transduction. *Chinese journal of integrative medicine* **2012,** *18*, 676-682.

79. (a) Lee, L.-T.; Huang, Y.-T.; Hwang, J.-J.; Lee, P.; Ke, F.-C.; Nair, M. P.; Kanadaswam, C.; Lee, M.-T., Blockade of the epidermal growth factor receptor tyrosine kinase activity by quercetin and luteolin leads to growth inhibition and apoptosis of pancreatic tumor cells. *Anticancer research* **2001,** *22* (3), 1615-1627; (b) Miodini, P.; Fioravanti, L.; Di Fronzo, G.; Cappelletti, V., The two phyto-oestrogens genistein and quercetin exert different effects on oestrogen receptor function. *British journal of cancer* **1999,** *80* (8), 1150.

80. (a) Mitchell, S. H.; Zhu, W.; Young, C. Y., Resveratrol inhibits the expression and function of the androgen receptor in LNCaP prostate cancer cells. *Cancer Research* **1999,** *59* (23), 5892-5895; (b) Pozo‐Guisado, E.; Lorenzo‐Benayas, M. J.; Fernández‐Salguero, P. M., Resveratrol modulates the phosphoinositide 3‐kinase pathway through an estrogen receptor α‐dependent mechanism: Relevance in cell proliferation. *International journal of cancer* **2004,** *109* (2), 167-173.

81. (a) Demetri, G. D.; van Oosterom, A. T.; Garrett, C. R.; Blackstein, M. E.; Shah, M. H.; Verweij, J.; McArthur, G.; Judson, I. R.; Heinrich, M. C.; Morgan, J. A., Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial. *The Lancet* **2006,** *368* (9544), 1329-1338; (b) Motzer, R. J.; Hutson, T. E.; Tomczak, P.; Michaelson, M. D.; Bukowski, R. M.; Rixe, O.; Oudard, S.; Negrier, S.; Szczylik, C.; Kim, S. T., Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. *New England Journal of Medicine* **2007,** *356* (2), 115-124.

82. (a) Chung, H.; Jung, Y. m.; Shin, D. H.; Lee, J. Y.; Oh, M. Y.; Kim, H. J.; Jang, K. S.; Jeon, S. J.; Son, K. H.; Kong, G., Anticancer effects of wogonin in both estrogen receptor‐positive and‐negative human breast cancer cell lines in vitro and in nude mice xenografts. *International Journal of Cancer* **2008,** *122* (4), 816-822; (b) Lin, C.-M.; Chang, H.; Chen, Y.-H.; Wu, I.-H.; Chiu, J.-H., Wogonin inhibits IL-6-induced angiogenesis via down-regulation of VEGF and VEGFR-1, not VEGFR-2. *Planta medica* **2006,** *72* (14), 1305-1310.

83. Bates, D., ZD-6474. AstraZeneca. *Current opinion in investigational drugs (London, England: 2000)* **2003,** *4* (12), 1468-1472.