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## Ligand fishing as a tool to screen natural products with anticancer potential

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## 1 **Ligand fishing as a tool to screen natural products with anticancer potential**

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6  
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### 17 18 **ABSTRACT**

19 Cancer is the second leading cause of death in the world and its incidence is expected to  
20 increase with the aging of the world's population and globalization of risk factors. Natural  
21 products and their derivatives have provided a significant number of approved anticancer  
22 drugs and the development of robust and selective screening assays for the identification of  
23 lead anticancer natural products is essential in the challenge of developing personalized  
24 targeted therapies tailored to the genetic and molecular characteristics of tumors. To this end,  
25 the ligand fishing assay is a remarkable tool to rapidly and rigorously screen complex  
26 matrices, such as plant extracts, for the isolation and identification of specific ligands that  
27 bind to relevant pharmacological targets. In this paper, we review the application of ligand  
28 fishing with cancer-related targets to screen natural product extracts for the isolation and  
29 identification of selective ligands. We provide critical analysis of the system configurations,  
30 targets, and key phytochemical classes related to the field of anticancer research. Based on  
31 the data collected, ligand fishing emerges as a robust and powerful screening system for the  
32 rapid discovery of new anticancer drugs from natural resources. It is currently an  
33 underexplored strategy according to its considerable potential.

34 **Keywords:** Affinity chromatography, cancer, drug discovery, ligand fishing, natural  
35 products

36  
37 **List of abbreviations:** 2-AG, 2-arachidonylglycerol; AA, arachidonic acid; ACS, American  
38 Chemical Society BDNF, brain-derived neurotrophic factor; CM, cell membrane; COXs,  
39 cyclooxygenases; cPLA<sub>2</sub>, cytosolic phospholipase 2; CTAC, hexadecyltrimethylammonium

chloride; CYP450, cytochrome P450; DAG, diacylglycerol; ECM, extracellular matrix; EGFR, epidermal growth factor receptor; FDA, Food and Drug Administration; FGF, fibroblast growth factor; FGFR4, fibroblast growth factor receptor 4; GO, graphene oxide; GSK-3 $\beta$ , glycogen synthase kinase 3 $\beta$ ; GST, glutathione-S-transferase; HF, hollow fiber; HSPs, heat shock proteins; IOMN, iron oxide magnetic nanoparticle; IP<sub>3</sub>, inositol triphosphate; iPLA<sub>2</sub>, calcium-independent phospholipase A<sub>2</sub>; JAK2, Janus kinase 2; LDH, lactate dehydrogenase; LOXs, lipoxygenases; MAGL, monoacylglycerol lipase; MAPK, mitogen-activated protein kinase; MAPKAPK2, MAPK-activated protein kinase 2; MKK3, mitogen-activated protein kinase kinase 3; MMPs, matrix metalloproteinases; MPs, magnetic particles; NAD, nicotinamide adenine dinucleotide; NGF, nerve growth factor; OGO, oriented layered graphene oxide pad; PAF, platelet-activating factor; PAI-1, plasminogen activator inhibitor; PI3K, phosphatidylinositol 3 kinase; PGs, prostaglandins; PGE<sub>2</sub>, prostaglandin E<sub>2</sub>; PLA<sub>2</sub>s, phospholipases A<sub>2</sub>; PKC, protein kinase C; PLC, phospholipase C; QD, quantum dots; QR2, quinone reductase 2; SIRT, sirtuin; sPLA<sub>2</sub>, secreted phospholipase A<sub>2</sub>; STAT3, signal transducer and activator of transcription 3; TCMs, traditional Chinese medicines; TrkB receptor, tropomyosin-related kinase B receptor; UF, ultrafiltration; uPA, urokinase plasminogen activator; uPAR, urokinase plasminogen activator receptor; UHPLC-LTQ, ultra-high-performance liquid chromatography-linear trap quadrupole; UPLC-Q-TOF/MS, ultra-high performance liquid chromatography-quadrupole-time-of-flight mass spectrometry

## 1. Introduction

With 10 million deaths by 2020, corresponding to nearly one in six, cancer is the second leading cause of death in the world [1]. The most common cancers are breast, lung, colon, and prostate. The incidence of cancer is rapidly increasing worldwide because of the aging population, spread of viral and bacterial infections, pollution, and globalization of behavioral and dietary risk factors, including obesity, lack of exercise, tobacco and alcohol use, as well as low fruit and vegetable consumption [2].

Although many cancers can be cured if detected early and treated effectively, many patients still die for a variety of reasons, including lack of accessible treatments in poor countries, inability to complete treatment in patients at risk of heart or kidney failure, late diagnosis of metastatic tumors, natural and acquired resistance to treatments, anticancer drugs toxicity, and insensitivity of cancer stem cells to treatment with surgery, chemotherapy, radiation or immunotherapy [2–7]. Cancer cells develop multiple adaptative mechanisms to survive, become persistent against metabolic, hypoxic, nutritional, and therapeutic pressure, and escape detection and destruction by the immune system. Understanding these characteristics has led to major advances in the development of targeted drugs acting on the deregulation of cell metabolism, proliferative signaling, epigenetic reprogramming, cell cycle deregulation, tumor angiogenesis, and resistance to apoptosis and antitumor immunity [8]. Thus, cancer treatments are currently moving from standardized and validated protocols for each type of cancer to personalized protocols based on the genetic and molecular characteristics of tumors. From this perspective, the discovery of molecules with new modes

of action and the identification of key cellular pharmacological targets for the survival of cancer cells have opened the way to important therapeutic innovations [9].

Natural products have provided considerable value to the development of anticancer drugs because of their unique chemical scaffolds and original mechanisms of action. A variety of naturally derived agents are used in cancer chemotherapy, whether as active, preventive, or chemosensitizing drugs and the identification and evaluation of natural anticancer molecules is one of the most active areas of research in medicinal chemistry and biotechnology [10]. However, the screening and purification of natural products are often laborious, time-consuming, and expensive. In this sense, rapid and efficient methods need to be developed, especially for purifying bioactive compounds from complex matrices. In exploring these gaps, ligand fishing has emerged as an innovative, efficient, and specific high-throughput screening method designed to save research groups time and resources in the search for new chemical hits. This strategy consists in purifying a molecule with a selective affinity for a biological molecular target of therapeutic interest from a complex mixture of natural products obtained by extraction from biomass. To date, a large number of ligand fishing techniques have been developed with various protocols and efficiently optimized to identify ligands from plant, animal, or bacterial extracts. Combined with highly sensitive high-resolution analytical methods (e.g., LC-HRMS and NMR), ligand fishing allows for the rapid identification and purification of fished ligands, accelerating the pharmacological evaluation and characterization of bioactive natural products [11–13].

In this paper, we aimed to propose an exhaustive updated review and critical analysis of the ligand fishing technique as an effective screening strategy to identify natural products with anticancer potential. We reviewed the contribution of this technique to the identification of anticancer natural products in various biological extracts, discussed the different technical approaches developed and explored its gaps and possible improvements.

## 2. Materials and Methods

### 2.1 Search strategy

The present systematic review was performed based on original reports published in the scientific literature from all years until the 1<sup>st</sup> of October 2022. The scientific databases chosen for this work were Science Direct, PubMed, Google Scholar, Scopus, and Web of Science, using the following descriptors: “ligand fishing”, “natural products” and “cancer”. The obtained results were filtered to remain with research articles only. Moreover, we did not contact authors to retrieve unpublished data or unavailable full texts.

### 2.2 Inclusion/exclusion criteria

Papers were included in this study if the authors used ligand fishing to screen natural products, if the authors developed a proof of concept with known ligands to rationalize the method, and if the target was directly related to cancer pathophysiology. Only articles published in English and that met the inclusion criteria described above were selected for further evaluation. Manuscripts that did not satisfy the inclusion criteria, reviews, meta-

1  
2  
3 122 analysis, abstracts, conferences, editorials, letters, case reports, thesis, conferences, and  
4  
5 123 manuscripts with full text not available were excluded from the present review.

6 124 Two independent investigators (L.A.M.S. Duarte-Filho and P.C.O. Oliveira) were in  
7  
8 125 charge to select the articles evaluating the title, abstract and full text. All retrieved articles  
9  
10 126 were thoroughly examined in an attempt to identify the ones that did not fit into the inclusion  
11  
12 127 criteria properly. In case of divergence in the selection of manuscripts, two additional expert  
13  
14 128 reviewers were consulted (L. Picot and M.C. Moraes). Manually added papers were also  
15  
16 129 retrieved from the references of the articles selected through our systematic search.

### 17 130 *2.3 Data Collection*

18  
19 131 All data were submitted to standardized forms to comprehensively collect information  
20  
21 132 about the country and year of publication, diversity of ligand fishing techniques and  
22  
23 133 experimental conditions, cancer targets and their respective cancer types, natural products,  
24  
25 134 identified ligands, and their chemical classes, as well as pre-clinical assessments (when  
26  
27 135 applicable). Furthermore, the selected data here presented was verified based on the expertise  
28  
29 136 of the authors and summarized into figures, tables, and diagrams for facilitated  
30  
31 137 comprehension of the numerical results.

## 32 138 **3. Results and Discussion**

### 33 139 **3.1. Bibliometric data**

34  
35 140 The initial systematic search produced 569 results, of which 175 were from Science Direct,  
36  
37 141 19 from PubMed, 242 from Google Scholar, 118 from Scopus, and 15 from Web of Science.  
38  
39 142 One hundred and eleven articles were excluded because of duplicity. The remaining 458  
40  
41 143 articles were screened and 403 were excluded after reading of the title and abstract.  
42  
43 144 Afterward, we remained with 55 articles for a full examination, of which 3 could not be  
44  
45 145 retrieved and 17 were excluded due to divergencies with the inclusion criteria previously set.  
46  
47 146 In addition, 32 studies from reference lists of selected articles were manually added, of which  
48  
49 147 1 could not be retrieved and 8 were excluded after full-text analysis. At last, the total number  
50  
51 148 of articles selected for the present review was 58. The works included in the present review  
52  
53 149 were sorted according to the country where the research was developed, year of publication,  
54  
55 150 ligand fishing technique, anticancer targets, and respective cancer types. Additionally, details  
56  
57 151 concerning the natural product (family, species, parts used, ligands fished, and chemical  
58  
59 152 class) as well as the type of extraction, solvents, and buffers employed were also gathered  
60  
153 **(Tables S1 and S2 respectively).**

154 According to our data, approximately 83% of the selected works (n=48) were from  
155  
156 155 China, 5.1% from the USA (n=3), 3.4% from a cooperation China-USA (n=2), 3.4% from  
157  
158 156 China-South Korea (n=2), 1.7% from USA-Turkey (n=1), 1.7% from USA-Taiwan (n=1)  
159  
160 157 and 1.7% from Brazil (n=1), as shown in **Figure 1**. Considering the year of publication, the  
161  
158 first seven years (2011-2017) account for 36.2% (n=21) of the works published. On the other  
159  
160 159 hand, publications from the last four years (2018-2022) account for 63.8% of the included  
160  
161 160 articles (n=37) (**Figure 2**).

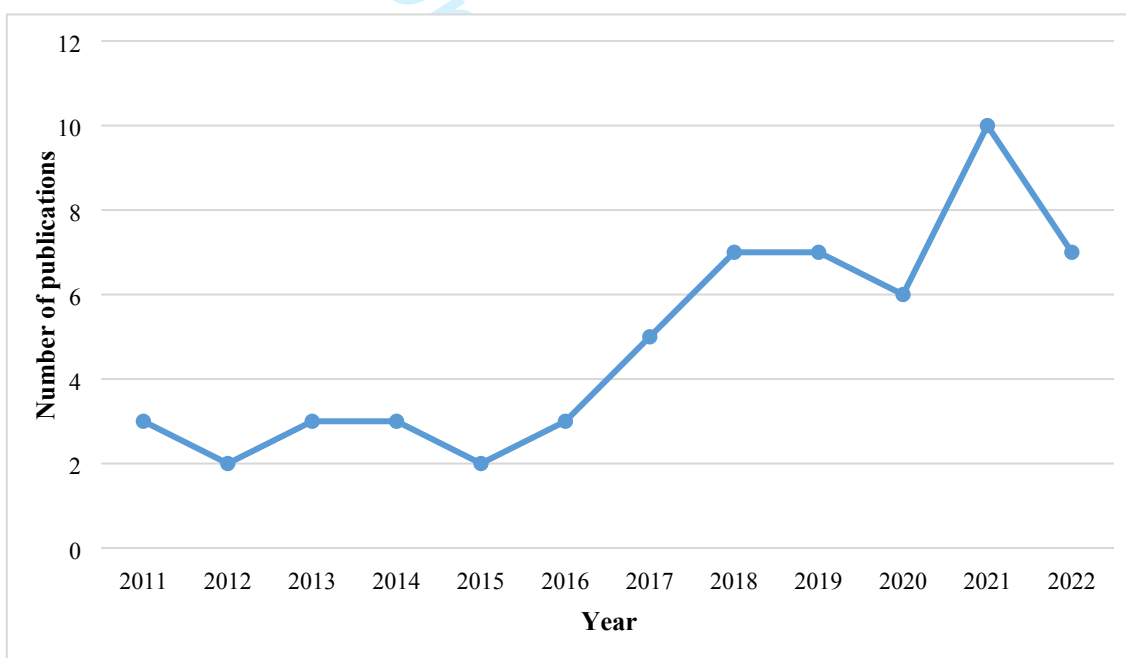


162 Figure 1 – Geographical distribution of selected studies



163

164 Additional assessments of the fished ligands were also part of the investigations. In  
 165 this regard, more than two-thirds (n=40 – 69%) of the selected articles performed *in vitro*  
 166 assays in order to investigate the enzymatic inhibition and/or *in vitro* anticancer potential of  
 167 the fished-out ligands.



168

169 Figure 2 – Evolution of the annual number of world publications dealing with the  
 170 identification of anticancer natural products using a ligand fishing strategy.

### 171 3.2. Diversity of ligand fishing techniques developed to isolate anticancer natural 172 products

173 According to the numbers shown in Figure 2, ligand fishing is a technique that has  
 174 raised increasing interest in the last years and is gaining more attention from the scientific  
 175 community as a versatile and powerful purification assay. The possibility of using a complex  
 176 matrix with minimal sample preparation represents one of its major advantages, as it results  
 177 in an economy of time and resources. Various techniques to bind ligands and separate bound  
 178 ligands interacting with the molecular target from unbound ligands have been developed. The  
 179 macromolecular target may be free in solution or immobilized on solid supports, and bound  
 180 ligands may be finally dissociated from the complex matrix using a variety of techniques

181 such as ultrafiltration, centrifugation, or magnetic separation. Although immobilized targets  
 182 usually demand more preparation time and reagents, they also provide better chemical and  
 183 thermal stability and, especially, reusability for the system [14,15]. The use of solid support  
 184 opens many possibilities and the diversity of immobilization chemistry and methodology  
 185 results in a wide range of protocols and system configurations [16]. However, despite the  
 186 benefits, immobilization is not mandatory for the ligand fishing assay.

187

### 188 3.2.1. Free biological target

189 Ligand fishing methodologies that use the free biological target in solution require a  
 190 step to recover the ligand-target complex from the solution. The isolation of the complex is  
 191 based on the difference in size or mass of the complex with the remaining elements of the  
 192 complex matrix. In the literature, techniques such as ultrafiltration and centrifugation are  
 193 often used to separate the components by differences in size and mass.

194

#### 195 *3.2.1.1. Ultrafiltration*

196 Ultrafiltration (UF) membranes can selectively retain macromolecules and  
 197 consequently any ligand-target complex with a molecular weight higher than the pore size,  
 198 but not small substances (although non-specific interactions between the UF membrane and  
 199 small compounds may occur through electrostatic or low energy non-covalent forces) as  
 200 shown by Figure 3. In this sense, it is possible to use the target in its free form, eliminating  
 201 all the steps required for biomolecules immobilization, but more importantly, the UF  
 202 membranes usually present weak interaction with the components of a mixture, so the ligands  
 203 with no affinity for the target will readily cross this physical barrier [13,17] The necessity to  
 204 perform several washes to completely remove the unbound components is considered a  
 205 drawback of this technique, as well as the need for high pressure to eliminate non-targeted  
 206 molecules. Therefore, the washing steps are important to avoid retention by non-specific  
 207 interactions.

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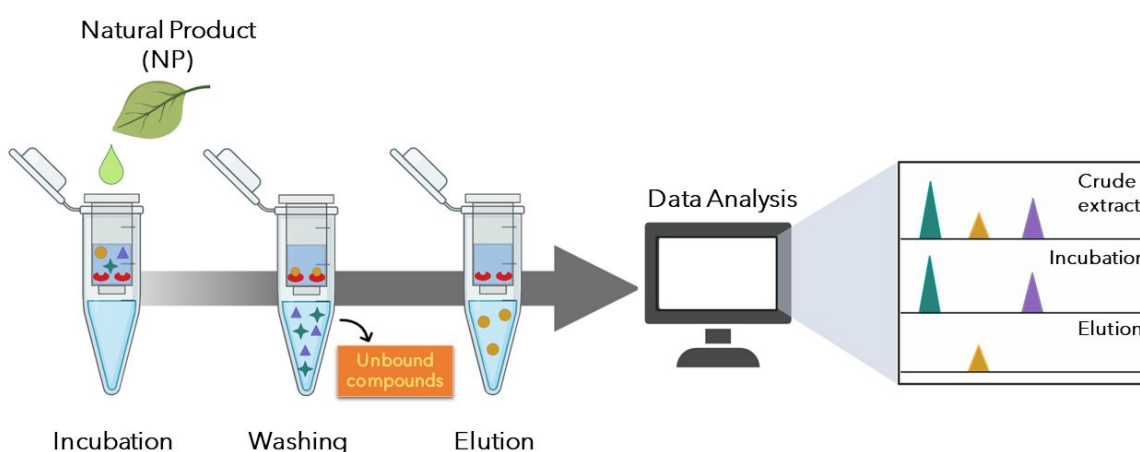
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208 Figure 3 – Schematic illustration of ligand fishing assay using ultrafiltration membrane. In  
 209 this configuration, it is used a physical barrier (membrane) to retain the target. As a result,  
 210 the ligand-target complex can be isolated, and the washing of weakly bound ligands and  
 211 elution of ligands with strong affinity can be promptly executed.



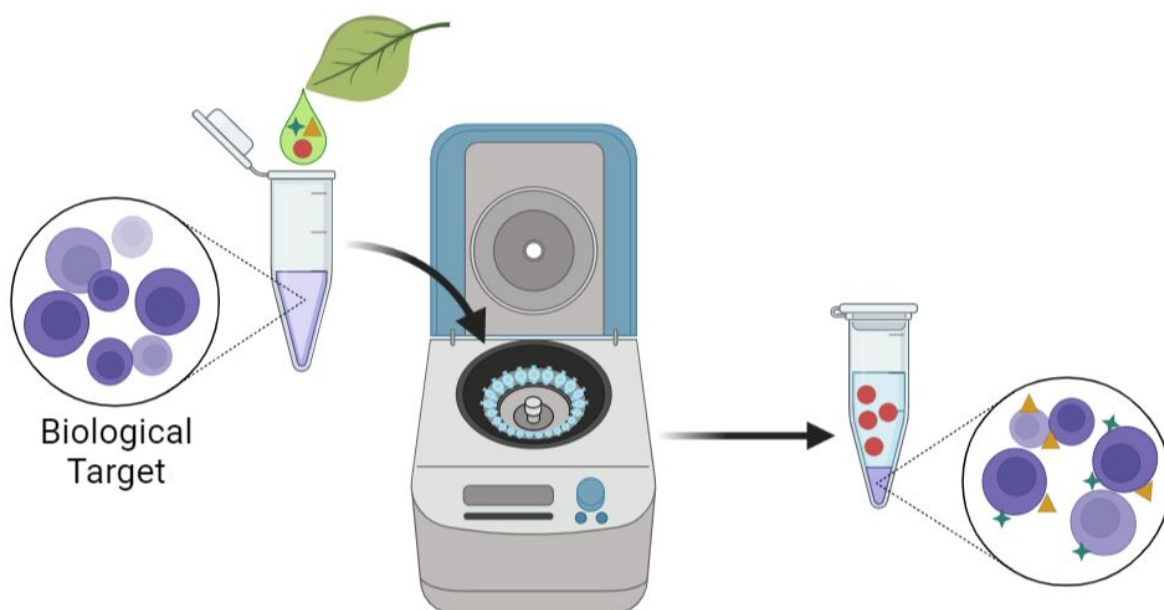
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2  
3 212 In two different works, *Glycyrrhiza uralensis* Fisch. root and mango (*Mangifera*  
4 213 *indica* L.) leaves extracts were screened for tyrosinase inhibitors using the ligand fishing  
5 214 assay associated with the UF technique [18,19]. Liu et al. [18] compared the performance of  
6 215 UF and magnetic support to identify ligands in the *G. uralensis* Fisch. extract. Even though  
7 216 both techniques were able to recognize the same ligands, it was observed different responses  
8 217 for each assay. Comparing methodologies, the two presented easy operation, however, the  
9 218 UF process demanded more time (more than 1 hour) while the usage of magnetic particles  
10 219 resulted in a 20-min assay. UF is usually presented as a fast technique, however, this time  
11 220 can be shortened when considering other techniques. The authors argued that this method has  
12 221 easy operation and fast response with few parameters to adjust [18]. Ma and collaborators  
13 222 [12] applied UF to identify DNA-binding substances from several herbal extracts. After  
14 223 evaluating different membranes and conditions, they chose a modified poly (ether sulfone)  
15 224 membrane that was able to retain the target and operated under a variety of eluting solvents.  
16 225 Once established, this UF affinity assay was evaluated and it was able to fish out 10 ligands  
17 226 (berberine, coptisine, palmitine, daurisolone, dauricine, fangchinoline, tetrandrine,  
18 227 actinomycin D, actinomycin V, and actinomycin X0 $\beta$ ) proving to be a reliable method for  
19 228 the recognition of potential ligands in a complex matrix.

### 229 3.2.1.2. Centrifugation

230 Centrifugation is particularly useful for suspensions that contain components with  
231 large differences in density and sedimentation time, such as macromolecules (cells, DNA,  
232 etc.) versus low molecular weight molecules. This property is thus particularly tailored to the  
233 separation of ligands bound on target macromolecules from unbound molecules (Figure 4).

234 To explore *Bufo melanostictus* Schneider venom as a source of anti-cancer  
235 components, Ren et al. [11] used a simple procedure to perform the ligand fishing assay. In  
236 this work, the biological targets were HepG2 and MCF-7 cells, and the affinity assay was  
237 conducted in the cell culture flask, where the growth medium was replaced by a toad venom  
238 solution. After incubation, the cells were washed and centrifugated and the venom molecules  
239 bound to the cells were eluted using methanol as organic solvent. Comparison of the raw toad  
240 venom composition with the eluted compounds, using UPLC-MS analysis, allowed them to  
241 identify the toxins that interacted with the cellular targets [11]. Similarly, Wei and  
242 collaborators [20] performed this cell affinity-based assay to screen bioactive components in  
243 a *Curcuma longa* L. extract. Using centrifugation, PC3 cells incubated with the extract were  
244 decanted and separated from unbound molecules and washed to elute the ligands. After  
245 concentration and analysis by UHPLC-LTQ Orbitrap MS, three curcuminoids (curcumin,  
246 demethoxycurcumin, and bisdemethoxycurcumin) with significant anticancer activity were  
247 identified [20].

248



249

250 Figure 4 – Ligand fishing assay using centrifugation as a method to isolate the  
 251 macromolecule and unbound ligands (low-mass fraction) through the action of centripetal  
 252 force. Therefore, the supernatant can be easily retrieved, and the ligand-target complex can  
 253 proceed to the next steps (washing and elution).

### 254 3.2.2. Grafting of the biological target on a solid support

255 Solid supports are usually used for enzyme immobilization, and a large panel of  
 256 materials and strategies can be used. Amino acids present in the macromolecule can be used  
 257 for the formation of covalent bonds between the support and the target. Electrostatic forces  
 258 and weak interactions, such as Van der Waals, can also promote immobilization. The solid  
 259 supports can be classified as inorganic, such as silica, metals, metallic oxides, and carbon, or  
 260 organic like natural and synthetic polymers [15,21].

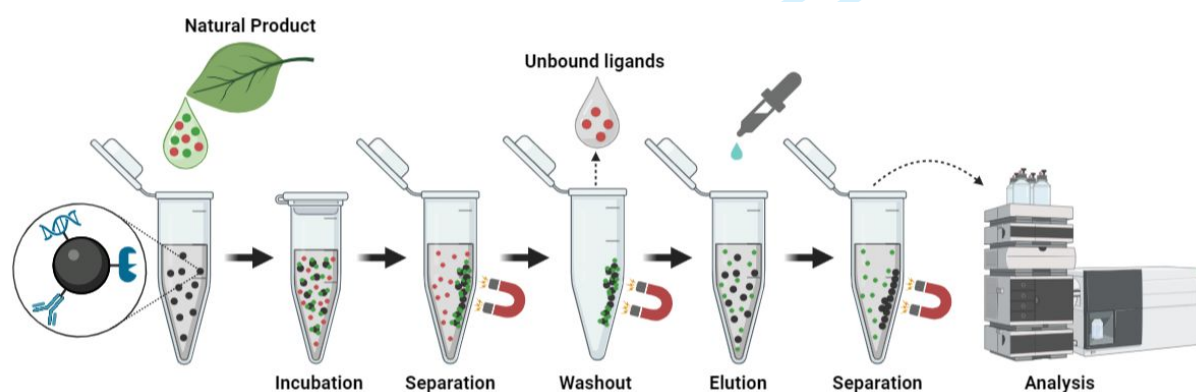
#### 261 *3.2.2.1. Magnetic particles*

262 The convenience of this technique resides in the superparamagnetic properties of the  
 263 particles, which allow the practical and fast isolation of interesting ligands using an external  
 264 electromagnetic field applied by a simple magnet. These characteristic conserves the  
 265 colloidal stability of nano-sized magnetic particles since they do not present remaining  
 266 magnetization after the removal of the external field, theoretically avoiding agglomeration  
 267 [22]. Beyond superparamagnetic properties, the surface functionalization of magnetic  
 268 particles confers versatile chemical features to this material that are responsible for its wide  
 269 range of applications [23–26].

270 Iron oxide magnetic nanoparticles (IOMN) have multiple applications in biomedical  
 271 sciences due to their unique physicochemical properties. However, their chemical reactivity  
 272 and high surface energy also represent two important issues able to reduce the  
 273 superparamagnetic properties of this material. In this sense, IOMN silica-coating is an  
 274 interesting procedure to enhance the chemical stability and reduce agglomeration and  
 275 eventual cytotoxicity of this material [27]. In a sophisticated experiment, Yi et al. [28]  
 276 fabricated a polydopamine-functionalized silica-coated iron oxide mesoporous magnetic  
 277 particle meant to immobilize glutathione-S-transferase (GST) on its surface. The authors  
 278 used a hexadecyltrimethylammonium chloride (CTAC) template to create mesopores in the

279 silica-coated IOMN, aiming to screen different plant extracts (*P. frutescens* (L.) Britt leaves,  
 280 *A. orientale* (Sam.) Juz. roots, and *C. sinensis* (L.) Kuntze extracts). As a result, they  
 281 successfully fished and identified six compounds, of which two ((-)-Epigallocatechin-3-O-  
 282 gallate and (-)-Epicatechin-3-gallate) presented IC<sub>50</sub> values for (GST) lower than 100μM  
 283 [28]. It is important to note that mesoporous silica offers a larger surface area in comparison  
 284 to ordinary silica coating, allowing the attachment of more functional groups to the  
 285 nanocomposite, hence increasing fishing yields. [29].

286 Although several techniques are available for protein immobilization on the surface  
 287 of magnetic particles, most of these approaches are not adequate to immobilize  
 288 transmembrane receptors, which in turn are the targets of more than one-third of all approved  
 289 drugs [30,31]. Indeed, to bind relevant bioactive ligands, these receptors must keep their  
 290 physiological tridimensional structure to expose functional binding domains, and their  
 291 purification and grafting on magnetic particles may lead to denaturation. Exploring this  
 292 limitation, three works developed cell membrane-coated magnetic nanoparticles  
 293 overexpressing certain types of receptors involved in cancer pathophysiology. Arituluk and  
 294 colleagues [32] engineered SH-SY5Y neuroblastoma cells overexpressing tropomyosin-  
 295 related kinase B (TrkB) receptors to encapsulate IOMN and screen Gotu kola (*Centella*  
 296 *asiatica* (L.) Urban) plant extract seeking ligands. Since the TrkB receptor is a  
 297 transmembrane integral protein and its functionality depends greatly on the integrity of the  
 298 phospholipids in its vicinity, using TrkB-enriched membrane fragments instead of purified  
 299 TrkB receptors represents a very interesting strategy to simulate more realistic conditions.  
 300 However, some validated tyrosine kinase receptor inhibitors, such as sunitinib and  
 301 nintedanib, act through the receptor's intracellular domains, which in turn are unlikely to be  
 302 accessible in this fishing system since most of the intracellular space is occupied by the  
 303 magnetic particles [33,34].

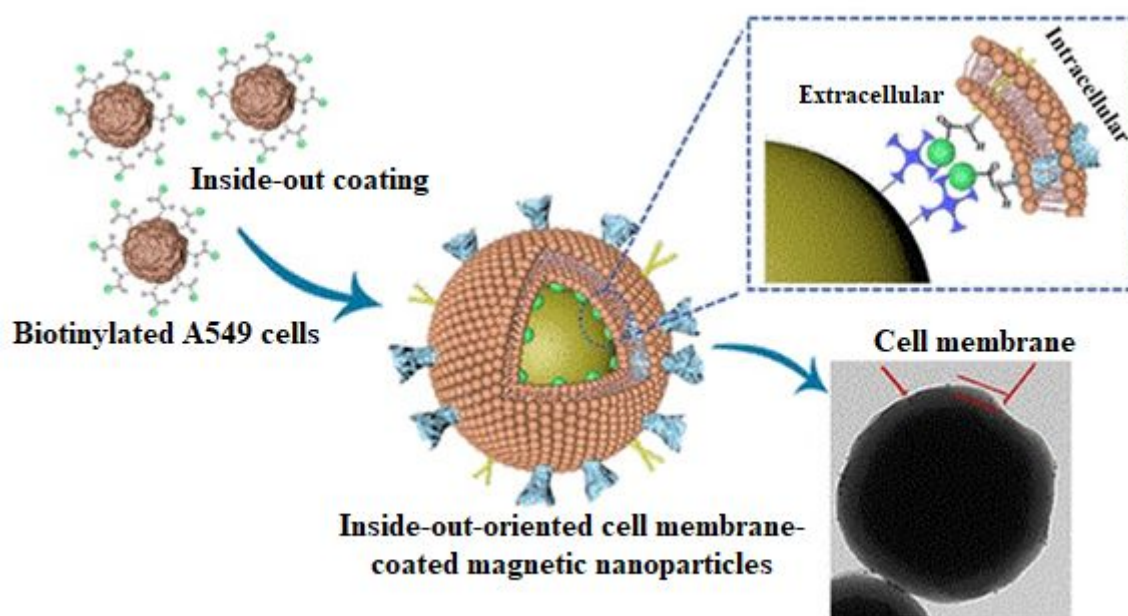


304 Figure 5 – Ligand fishing using magnetic particles (MPs). The MPs can be used to  
 305 immobilize a variety of targets such as DNA, antibodies, and other proteins. After successful  
 306 immobilization, MPs are incubated with the natural product. By affinity, the green compound  
 307 is adsorbed by MPs whereas the red compound remains unbound. With the aid of an external  
 308 magnetic field, the separation step occurs. In it, the complex MPs-green compound is retained  
 309 inside the tube whereas the unbound red compound is washed out. The washout step is  
 310 repeated multiple times to mitigate unspecific binding. Then, the complex MPs-green

311 compound is eluted using an organic solvent. Finally, a magnetic field is applied once again  
 312 to recover MPs whereas the green compound-containing supernatant is brought to analysis.

313 In another study, Bu and collaborators [35] addressed this limitation. They  
 314 functionalized carboxy-terminated iron oxide nanoparticles with streptavidin and  
 315 biotinylated human lung adenocarcinoma epithelial (A549) cell fragments overexpressing  
 316 the epidermal growth factor receptor (EGFR) to obtain inside-out-oriented cell membrane  
 317 magnetic nanoparticles. With this system, the authors intended to fish out ligands from  
 318 *Strychnos nux-vomica* L. extract that act in the intracellular domains of this receptor. As one  
 319 of the negative controls, they used outside-out-oriented magnetic nanoparticles and observed  
 320 that erlotinib and gefitinib, two known EGFR inhibitors, were more efficiently fished out by  
 321 the inside-out-oriented magnetic nanoparticles in comparison to the outside-side-out one,  
 322 highlighting the importance of transmembrane receptors binding site accessibility. Figure 6  
 323 shows the orientation of the cell membrane concerning the encapsulated magnetic  
 324 nanoparticle.

325 The fabrication and functionalization of magnetic particles can be diversified and  
 326 adapted to confer desired physicochemical properties to this material. However, controlling  
 327 parameters such as particle size, shape, stability, and dispersibility in specific solvents is  
 328 often a challenging task [36] Furthermore, the characterization of nanomaterials involves  
 329 expensive analytical techniques and equipment, which in turn represent a bottleneck to the  
 330 study of such materials [37]. In this regard, commercially available functionalized magnetic  
 331 beads represent a feasible alternative, as explored by Yasuda et al. and Singh et al. [38,39]



332  
 333 Figure 6 – Representation of the inside-out-oriented cell membrane-coated magnetic  
 334 nanoparticle. Reprinted (adapted) with permission from [35]. Copyright 2021 American  
 335 Chemical Society (ACS).

336 Based on the above-mentioned, magnetic particles represent versatile and very  
 337 efficient supports for ligand fishing assays, able to be modified, adapted, and associated with  
 338 different approaches in order to meet realistic physiological conditions depending on the  
 339 target and its location inside the body.



340

341 *3.2.2.2. Hollow Fiber*

342 The hollow fibers (HF) are organic polymeric membranes with pore diameter and  
343 inner cavities that operate as physical barriers capable to entrap the biological target, in a  
344 similar way to ultrafiltration membranes. Hollow fibers have been used as a carrier for  
345 proteins, cells, and liposomes [40,41]. The main characteristics of HF include its  
346 biocompatibility, large surface area, high preconcentration capacity, and good recovery.  
347 Additionally, this approach has an easy operation and fast response. The ligand fishing assay  
348 that uses HF (Figure 7) requires usual laboratory instrumentation, however, as in other  
349 techniques, false positives are problematic. For this reason, the washing solvent is a critical  
350 part of the assay.

351 Zhao et al. [42] developed an efficient approach based on adsorbed hollow fiber to  
352 screen and identify potential tyrosinase inhibitors in *Pueraria lobata* (Willd.) Ohwi extract.  
353 Seven tyrosinase ligands were fished out in this study, and three compounds had their  
354 inhibitory effect confirmed by *in vitro* assays. In 2019, Li and collaborators [43] used hollow  
355 fiber to screen a series of coumarins and *Angelicae dahurica* Fisch. extract against human  
356 hepatoma and renal tubular cells. The same system was applied to different sample matrices.  
357 The first application was to screen the bioactive components directly from the plant extract,  
358 as an *in vitro* approach. In the second study, the same herbal extract was orally administered  
359 to rats. After five hours, abdominal aortal blood and rats' kidney and liver tissues were  
360 collected and submitted to the HF fishing assay. In this *in vivo* assay, the authors aimed to  
361 track which substances were able to reach these target organs. For all applications the hollow  
362 fiber affinity-based assay was successful in the recognition and isolation of binders,  
363 indicating the versatility of this method [43].

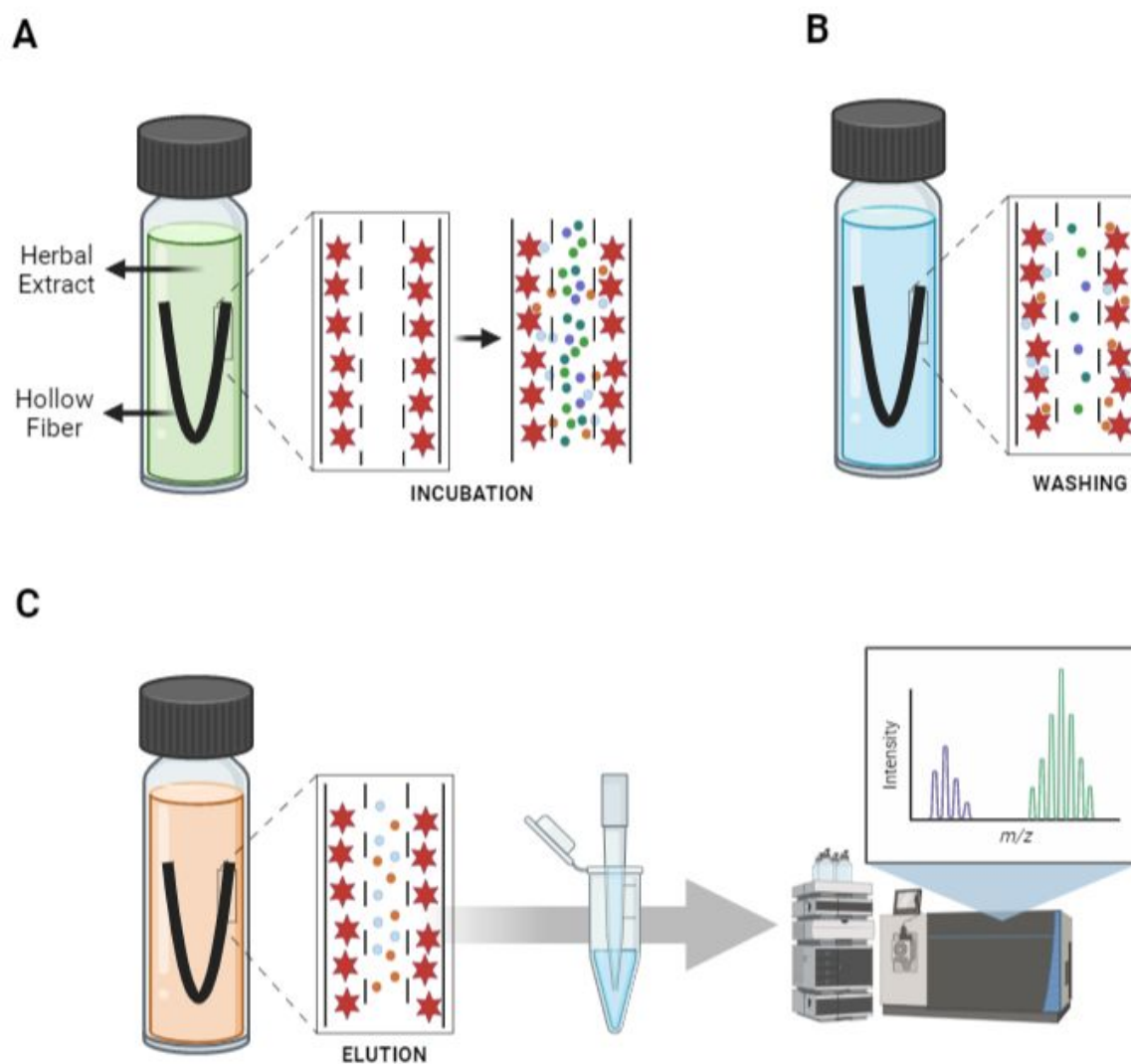


Figure 7 – Ligand fishing using hollow fiber (A: Incubation step - the biotarget is trapped inside the fiber allowing diffusion and interaction of ligands; B: Washing step: the hollow fiber was removed from extract solution and cleaned to remove unbound substances; C: Elution step: the ligand-target complex is disrupted, the bound ligands are eluted and proceeded to analysis)

### 3.2.2.3 Other immobilization techniques

This topic presents distinct systems for screening bioactive components from natural products with anticancer potential, which from now on will be referred to as miscellaneous. The application of different approaches allows the study of new characteristics in the development of ligand fishing assays. In this regard, the majority of ligand fishing studies here discussed used innovative configurations, supports, or immobilization approaches. In three insightful studies, the Heat shock protein 90 (Hsp 90) was grafted in mesoporous silica beads associated with InP/ZnS [44,45] and CdTe [46] quantum dots (QD). The support used combines the feature of *in-situ* imaging through the fluorescence response of QD with the ligand fishing technique. The QD was used as fluorescent probes in an imaging assay, this way the ligand-target complex can be directly tested in a cell-based assay. Using this support the authors propose not only to screen and identify potential binders by ligand fishing assay but also to assess the biological activity of fished ligands on HeLa and MCF-7 cells. The application of these silica nanocomposites was successful in the screening of potential inhibitors of Hsp 90 on *Alisma plantago-aquatica* L., *Tripterygium Wilfordii* Hook. f., and



1  
2  
3 386 *Curcuma longa* L., in which, for the first time, four ligands (zingiberenol, (6R,7R)-  
4 387 bisabolone, (*E*)-Atlantone and 6-(1-Hydro-xymethylvinyl)-4,8a-dimethyl-3,5,6,7,8,8a-  
5 388 hexahydro-1H-naphthalen-2-one) were associated to this extract.

8 389 Another example includes the use of biotinylated triplex DNA on agarose beads. In  
9 this approach, Xu and collaborators [47] used affinity immobilization of the target. They  
10 390 proposed a screening of a known collection of small molecules to validate the methodology  
11 391 and applied it to search for ligands in the *Phellodendron chinense* Schneid cortexes. The  
12 392 developed screening assay was able to fish two components (berberine and palmatine) from  
13 393 the complex mixture. Even though carbon-based materials have numerous applications, Zhou  
14 394 et al. [48] state that this is the first time that graphene oxide (GO)/oriented layered graphene  
15 395 oxide pad (OGO) is used as solid support for the immobilization of cell membranes (CM) in  
16 396 ligand fishing assay. In the search for EGFR inhibitors, CM from H1819 and A549 cells were  
17 397 selected to coat GO/OGO. This system was used to screen potential ligands from the Comfrey  
18 398 rhizome extract (*Lithospermum erythrorhizon* Siebold & Zucc.). During the evaluation, OGO  
19 399 presented a better performance than GO with a higher target load and better stability. The  
20 400 H1819-CM@OGO proved to be a better target for fishing EGFR inhibitors. The developed  
21 401 platform successfully fished three components from the extract (salvianolic acid B,  
22 402 deoxyshikonin, and shikonin) that showed a strong biological response against H1819 cells  
23 403 [48].  
24 404

25 405 In order to better understand the well-known anticancer activity of a Traditional  
26 406 Chinese Medicine (*Tripterygium wilfordii* Hook. f.), Tang and colleagues [51] used a  
27 407 singular approach to uncover more information regarding bioactive components and  
28 408 mechanism of action. In this work, the authors synthesized nanoparticles of CaCO<sub>3</sub> that were  
29 409 covered by MCF-7 cell membranes. The exposed receptors in the cell membrane were  
30 410 available for interaction with potential ligands in the plant extract. The differential of this  
31 411 solid support resides in its capacity to hydrolase in an acidic medium. This feature permits  
32 412 the support's removal preserving the target and the release of the bound ligands. This affinity  
33 413 assay fished out seven ligands from the *T. wilfordii* extract. The most potent fished ligand  
34 414 (celastrol) was used in the second application of the CaCO<sub>3</sub> nanoparticles - a target protein  
35 415 fishing assay. For this assay, the celastrol was fixed into the support surface and it was used  
36 416 to screen the protein collection from the MCF-7 cells digestion. The novelty in this  
37 417 bioanalytical method is the use of the same support for both ligand and protein fishing, which  
38 418 the authors named as a none-loss target release. Therefore, besides the screening of bioactive  
39 419 substances in complex mixtures this strategy could be used to fish potential protein targets  
40 420 [51]

As a summary of the works here discussed, we observed that most of them (n=20 – 34.5%) used miscellaneous approaches to screen natural products. Additionally, magnetic particles (n=17 – 29.3%), ultrafiltration (n=12 – 20.7%), hollow fiber (n=8 – 13.8%), and dialysis (n=1 – 1.7%) were also performed in an attempt to find anticancer leads from natural products (**figure 8**).

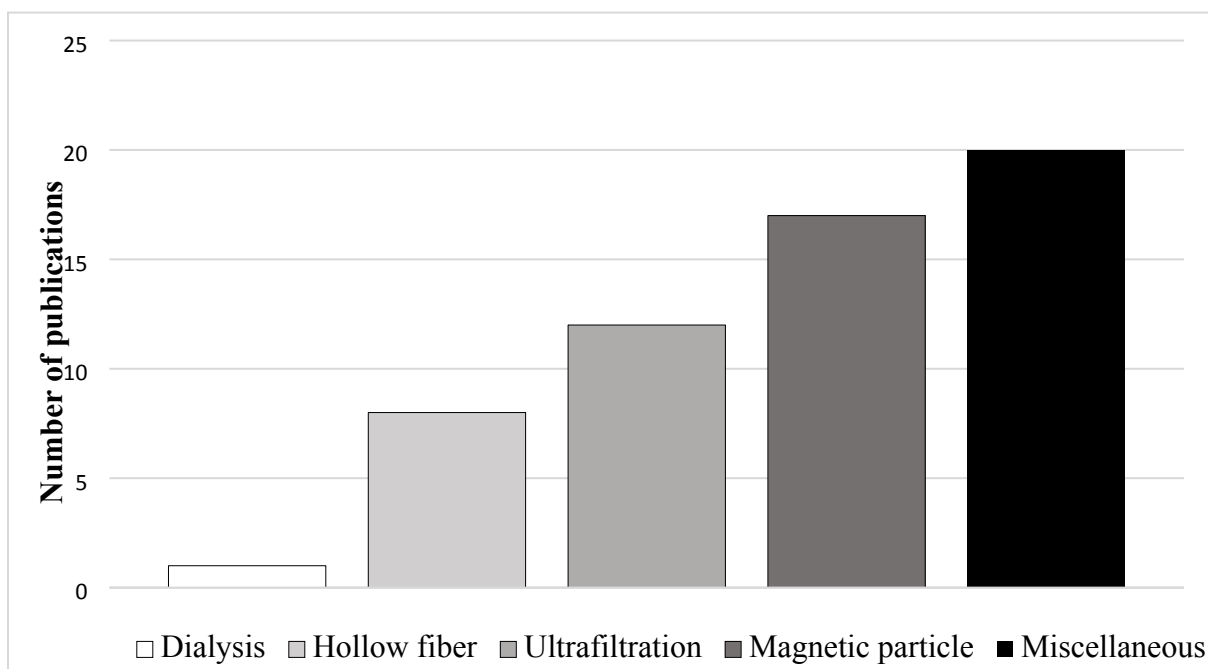


Figure 8 – Number of articles sorted by ligand fishing technique. Each bar represents the number of works included in each category.

As discussed in this section, the ligand fishing assays can be presented in different system configurations. Most of the works analyzed in this review fit the offline definition. This means that the isolation and identification of potential ligands are performed in two independent and physically disconnected steps [52]. Even though this configuration has proven to be useful, it is important to mention that there are different approaches to perform this affinity assay. Online assays are the ones that automatically insert the samples into the system, where the analyst has minimal interference. In more detail, this technique uses a bypass to transfer aliquots from one line to another [52,53]. Besides avoiding errors from preparation due to the automated transferring of samples and aliquots, this continuous flow can represent an economy of time and resources. These advantages are especially relevant in the screening of small biologically active compounds. To the best of our knowledge, no articles were found applying online ligand fishing using cancer-related targets to screen bioactive substances. Certainly, each assay has benefits and limitations but the fact that there is a gap in the literature for this type of system configuration indicates that there is still a lot to explore.

### 3.3. Anticancer natural products identified by ligand fishing

**Table S1** presents the list of natural products identified from various natural sources as binders of specific molecular or cellular targets dysregulated in different cancers, using the ligand fishing strategy. For each molecule identified, the chemical class is indicated. Analysis of the selected works showed that flavonoids (n=23), alkaloids (n=17), and phenolic

1  
2  
3 449 acids (n=13) were the most fished secondary metabolites, followed by terpenoids (n=11),  
4 450 coumarins (n=5), anthraquinones (n=5), and steroids (n=4). Ligands from other chemical  
5 451 classes (i.e., saponins, tannins, lignans, etc.) were fished in 16 works (**figure 9A**).  
6  
7 452 Unsurprisingly, these classes have been extensively studied for their anticancer potential,  
8 453 which allowed the discovery of remarkable anticancer compounds such as vinca alkaloids,  
9 454 camptothecin, and paclitaxel [54–61]

10 455 Flavonoids are one of the largest classes of secondary metabolites, corresponding to  
11 456 about 60% of all natural polyphenols, with approximately 8000 molecules [62,63]. Their  
12 457 biological activity is usually attributed to a high antioxidant power [64], however, it is also  
13 458 well known that flavonoids can act on several pathways, including some involved with cancer  
14 459 pathophysiology. For example, *in vitro* studies have demonstrated that flavonoids can  
15 460 modulate several protein kinases, epidermal growth factor receptors, vascular endothelial  
16 461 growth factor receptors, and cytoskeleton dynamics, besides inhibiting some metabolizing  
17 462 enzymes that activate a large number of pro-carcinogens [61,65-67]. In total, 96 flavonoids  
18 463 from 23 works were identified as binders of molecular targets related to cancer,  
19 464 demonstrating the potency of this technique to rapidly screen and identify flavonoids with  
20 465 anticancer potential. Among them, polymethoxylated flavones are interesting molecules that  
21 466 have been intensively studied for their anticancer potential. These natural products are  
22 467 versatile pharmacological agents that can modulate different pathways involved with cell  
23 468 proliferation, apoptosis, and cytoskeleton dynamics [68-70]. Moreover, Cheng and  
24 469 collaborators [71] for instance have identified for the first time eight polymethoxylated  
25 470 flavones in the fruit peels of *Citrus reticulata* Blanco that act as inhibitors of the matrix  
26 471 metalloproteinase 2. It is important to note that although *C. reticulata* is a well-studied plant  
27 472 species, these ligands were only detected after the implementation of a ligand fishing  
28 473 approach, which unveils the power of the technique in identifying undiscovered compounds.

29 474 The fact that flavonoids are abundant in nature and act by several mechanisms may  
30 475 explain why this class has been the most fished one among the selected articles. Quercetin  
31 476 and kaempferol, for example, are known to exhibit a pleiotropic enzymatic inhibition activity.  
32 477 It should also be noted that flavonoids can aggregate and form colloidal suspensions in *in*  
33 478 *vitro* assays, with a high potential to unspecifically bind and inhibit enzymes, which could  
34 479 produce false-positive results, misleading drug discovery research [72-74]. In this sense,  
35 480 none of the studies dealing with ligand fishing and identifying flavonoids have addressed the  
36 481 possible formation of colloidal aggregates, although this process in physiological conditions  
37 482 is unconfirmed [75,76].

38 483 The most successful class of natural products being used to treat cancer is probably  
39 484 alkaloids. They represent a highly diverse group of compounds containing cyclic structures  
40 485 with at least one basic nitrogen atom incorporated within [77,78]. There are approximately  
41 486 12,000 different alkaloids identified and classified according to their chemical structures,  
42 487 biosynthetic pathways, and biological activities. Since the 1960s, alkaloids have been  
43 488 recognized as an important class in the treatment of cancer, with the vinca alkaloids being  
44 489 the first plant-derived antimitotic agents to be introduced into the drug market [60]. Alkaloids  
45 490 are considered important sources of anticancer drugs, with several preclinical and clinical

1  
2  
3 491 studies being conducted [79]. They act through complex mechanisms, affecting multiple  
4 492 targets, and therefore can be useful to treat various forms of cancers [78]. Camptothecin, for  
5 493 instance, is a monoterpene pentacyclic quinoline alkaloid first extracted from the leaves of  
6 494 *Camptotheca acuminata* that originated irinotecan and topotecan, two FDA-approved  
7 495 topoisomerase-1 inhibitors able to treat metastatic ovarian, colorectal, and primary colon  
8 496 cancers. Another successful example is taxol, a  $\beta$ -tubulin-binding alkaloid extracted from the  
9 497 barks of *Taxus brevifolia* Nutt. that was approved in the 1980s for the treatment of Kaposi  
10 498 sarcoma, ovarian, breast, colon lung, bladder, prostate, melanoma, esophageal, and neck  
11 499 cancers [80].

12 500 Given the importance of alkaloids to the discovery of new anticancer agents,  
13 501 seventeen studies have successfully fished and characterized 37 alkaloids. However, some  
14 502 minor constituents like the ones that were reported for the first time in the complex venom  
15 503 of *Bufo melanostictus* Schneider, such as compound 18 ( $C_{14}H_{17}O_4N_2$ ), were referred to as  
16 504 unknown due to their novelty [11]. This highlights the importance of sensitive chemical  
17 505 screening methods, such as ligand fishing, in the discovery of new alkaloids with unexplored  
18 506 pharmacological potential.

19 507

### 20 508 **3.4. Natural sources screened for anticancer compounds using the Ligand Fishing** 21 509 **strategy**

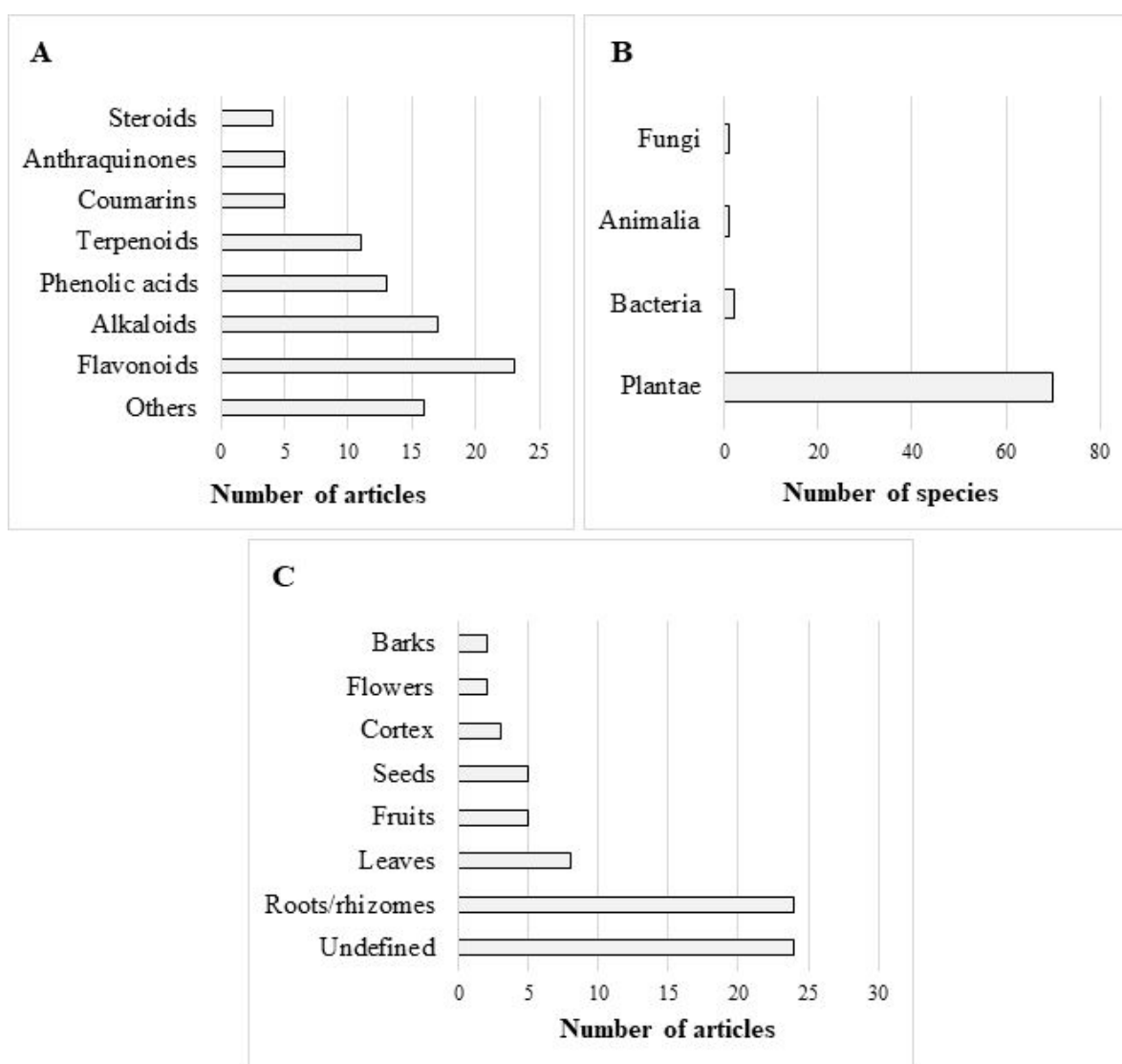
22 510 Among the taxonomic kingdoms of the species searched (n=74), the Plantae kingdom  
23 511 stands out as the most studied one, with 94,6% of all species in the selected articles (n=70)  
24 512 (**figure 9B and Table S1**). Different parts of plants were investigated in these studies (**table**  
25 513 **S1**), the roots/rhizomes being the most studied ones (n=24), followed by the leaves (n=8),  
26 514 fruits (n=5), seeds (n=5), cortex (n=3), flowers (n=2) and barks (n=2). Interestingly, there  
27 515 was a considerable number of studies (n=24) that did not specify the part used, being referred  
28 516 to as undefined (**figure 9C and table S1**).

29 517 It is also worth mentioning that most ligand fishing experiments performed to identify  
30 518 anticancer natural products were made with terrestrial plant extracts (**Figure 9B**). However,  
31 519 there is a huge and undiscovered potential in marine organisms [87][89], which were barely  
32 520 screened by ligand fishing studies for anticancer leads. The group of Choi and collaborators  
33 521 [82] was the only one that screened a marine-derived natural product. They fished out  
34 522 tetrangulol methyl ether, a potent quinone reductase-2 inhibitor ( $IC_{50} = 0.16 \mu M$ ), from an  
35 523 extract of *Actinomyces* sp. derived from marine sediment. Indeed, marine-derived natural  
36 524 products from seaweeds, microalgae, and plants have shown great potential, not only for  
37 525 cancerology but also for human health [83–85]. In this sense, they represent an immense  
38 526 reservoir of interesting natural molecules, and as such, should be better explored using the  
39 527 ligand fishing strategy.

40 528

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531

532 **Figure 9** – Graphical summary of ligands fished (A), the taxonomic kingdom of the screened  
 533 natural product (B), and the parts used to make the extraction (C).

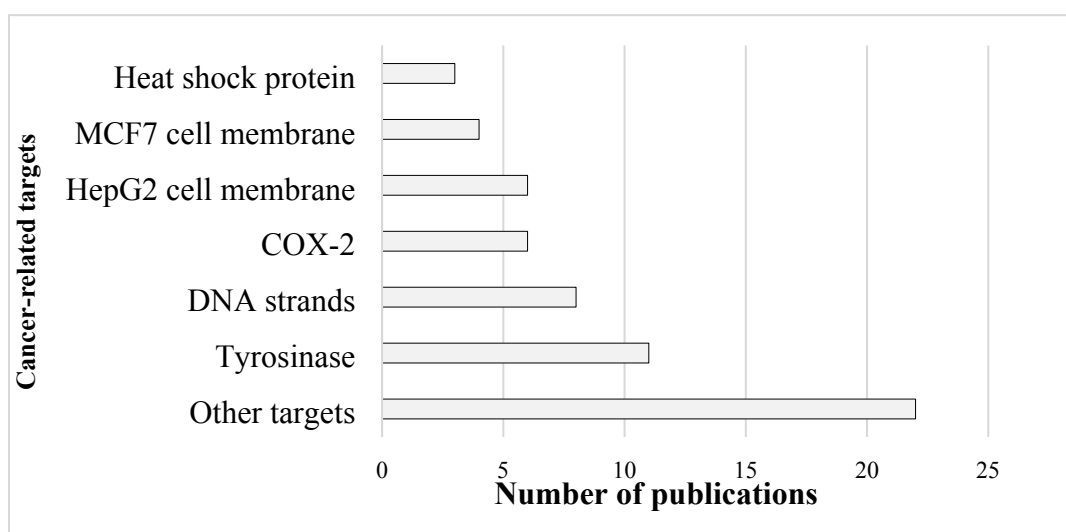
534

### 535 3.5. Cancer-related targets

536 Most newly approved anticancer drugs explore specific oncoproteins encoded by  
 537 mutated genes. However, the number of identified genes is extremely small compared to the  
 538 diversity of cancer targets described in the literature, which makes the oncogene  
 539 determination and search for new target inhibitors two complementary approaches in  
 540 anticancer drug discovery [86]. Hereupon, in this section, we focus on the latter aspect of  
 541 anticancer drug discovery, discussing closely related cancer targets, general pathways, and  
 542 other technologies involved in the ligand fishing works selected for this review. Among  
 543 cancer molecular targets, tyrosinase stands out as the most studied one (n=11 – 18.3%),  
 544 followed by DNA (n=8 – 13.3%) the cell membrane of HepG2 cells (n=6 – 10.4%),  
 545 cyclooxygenase-2 (n=6 – 10.4%), cell membrane of MCF7 cells (n=4 – 6.6%) and heat shock  
 546 protein 90 (n=3 – 6.3%). Other targets were also employed, such as lactate dehydrogenase,  
 547 glutathione-S-transferase, fibroblast growth factor receptor 4 (FGFR4), and tropomyosin-  
 548 related kinase B (TrkB) receptor among others (**figure 10** and **table S2**).

549





550 **Figure 10** – Main anticancer targets exploited in natural products ligand fishing studies.

551 Each bar represents the number of studies included

552

### 553 3.5.1. Proteins

554 Proteins are the most explored targets for disease treatment and their modulating  
 555 agents represent the vast majority of all anticancer drugs approved by the FDA [87]. Not  
 556 surprisingly, proteins were the most used targets to construct ligand fishing systems able to  
 557 capture anticancer compounds from natural products.

558

#### 559 *3.5.1.1 Tyrosinase*

560 Tyrosinase is an enzyme involved in the production of melanin in different organisms  
 561 such as animals and plants. In humans, tyrosinase is closely related to melanogenesis and its  
 562 inhibitors may represent an important clinical strategy to treat melanoma [88]. In this sense,  
 563 tyrosinase inhibitors were sought by different ligand fishing approaches such as hollow fiber  
 564 [42], ultrafiltration [19,88–94], and magnetic particles [95,96], in an attempt to efficiently  
 565 capture ligands with affinity for this target.

566 Yang and colleagues [89], for example, successfully fished out two derivatives from  
 567 quercetin and kaempferol (quercetin-3-O-(6-O-malonyl)- $\beta$ -D-glucopyranoside and  
 568 kaempferol-3-O-(6-O-malonyl)- $\beta$ -D-glucopyranoside, respectively) from the *Morus alba* L.  
 569 leaf extract as promising tyrosinase inhibitors. However, it is important to note that the  
 570 oxidation of phenolic compounds by the active tyrosinase contributes to the formation of  
 571 reactive species, stimulating apoptosis via the activation of p53. Therefore, the indirect  
 572 cytotoxic effect of tyrosinase depends on its activations, instead of inhibition [96]. Indeed,  
 573 the prodrug-activating feature of tyrosinase has been exploited to improve melanoma  
 574 treatment since melanoma cells distinguish from normal cells, among other characteristics,  
 575 by the overexpression of tyrosinase, which in turn favors cytotoxicity towards cancer over  
 576 normal cells [97].

577 On the other hand, the exaggerated production of melanin in melanoma cells confers  
 578 radioprotection and resistance to photodynamic therapy. Additionally, melanin can chelate  
 579 chemotherapeutic agents like cyclophosphamide whereas cell whitening promotes



1  
2  
3 580 sensitization, indicating that tyrosinase inhibitors may represent a feasible alternative for  
4  
5 581 melanoma chemosensitization [98].

6 582

7  
8 583 *3.5.1.2. Cyclooxygenase-2 (COX-2)*

9  
10 584 Cyclooxygenase-2 (COX-2) is one of the most studied targets involved with  
11  
12 585 inflammation. COX-2 inhibitors are widely used to contain inflammation since they present  
13  
14 586 fewer side effects than COX-1 inhibitors. These enzymes catalyze the oxidation of fatty acids  
15  
16 587 to produce eicosanoids such as prostaglandins (PG), which in turn are closely related to the  
17  
18 588 transformation of normal hyperplastic into neoplastic cells. In this regard, several levels of  
19  
20 589 evidence indicate that the inhibition of cyclooxygenases, particularly COX-2, is intimately  
21  
22 590 linked to a significant risk reduction for cancer development. Furthermore, COX-2 is  
23  
24 591 overexpressed in various cancer types such as breast [99], colon [100], cervical [101], and  
25  
26 592 non-small cell lung cancer [102]. Additionally, COX-2 overexpression is associated with  
27  
28 593 poor prognosis in the brain, ovarian, uterine, hepatocarcinoma, and melanoma, among other  
29  
30 594 cancers, which makes this enzyme an important target for anticancer therapy research [103].

31 595 The prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) is one of the most important eicosanoids produced by  
32  
33 596 COXs, having the ability to stimulate several intracellular pathways involved in cancer  
34  
35 597 pathophysiology. For instance, PGE<sub>2</sub> can promote apoptosis resistance via upregulation of  
36  
37 598 Bcl-2, stimulate angiogenesis via upregulation of vascular endothelial growth factor (VEGF),  
38  
39 599 metastasis through increase in metalloproteinases types 2 and 9, tumor growth via  
40  
41 600 upregulation of epidermal growth factor receptor (EGFR) and invasion via PI3K-AKT  
42  
43 601 signaling pathway [104].

44 602 Based on the importance of COX-2 for cancerology, six works employed this target  
45  
46 603 in the development of ligand fishing assays to screen natural products, aiming to find  
47  
48 604 innovative inhibitors. Four of the articles focused on the role of COX-2 inhibitors in cancer  
49  
50 605 therapy [105-108], whereas two works focused on the anti-inflammatory features of the  
51  
52 606 fished inhibitors [109, 110]. In any case, COX-2 inhibitors can be extremely valuable in both  
53  
54 607 scenarios. Celecoxib, for instance, is a widely used selective COX-2 inhibitor that promotes  
55  
56 608 local anti-inflammatory response as well as acts as a chemopreventive agent, reducing  
57  
58 609 recurrences of adenoma and breast cancer by more than 40% [111].

59 610

60 611 *3.5.1.3 Phospholipases A<sub>2</sub> (PLA<sub>2</sub>s)*

612 Phospholipases A<sub>2</sub> (PLA<sub>2</sub>s) are enzymes belonging to the esterase superfamily that  
613  
614 613 catalyze the hydrolyzation of the acyl bond of glycerophospholipids to release  
615  
616 614 lysophospholipids and free fatty acids, being subdivided in cytosolic (cPLA<sub>2</sub>), calcium-  
617  
618 615 independent (iPLA<sub>2</sub>), secreted (sPLA<sub>2</sub>), lysosomal, adipose-specific PLA<sub>2</sub>s and platelet-  
619  
620 616 activating factor (PAF) [112]. Among its different isoforms, cPLA<sub>2</sub>α, iPLA<sub>2</sub>β, sPLA<sub>2</sub>-IIA,  
617  
618 617 and sPLA<sub>2</sub>-III have demonstrated involvement with human carcinogenesis, therefore being  
619  
620 618 able to be considered for anticancer therapies. Arachidonic acid (AA) is, undoubtedly, one  
619  
620 619 of the most important products of PLA<sub>2</sub>s' action. It is a glycerophospholipid derivative that  
620  
620 620 is transformed into eicosanoids closely related to inflammation and tumorigenesis by the

1  
2  
3 621 action of cyclooxygenases (COXs), lipoxygenases (LOXs), and cytochrome p450 (CYP450)  
4  
5 622 enzymes [113].

6  
7 623 Based on this, numerous studies have demonstrated the dual role of PLA<sub>2</sub>s isoforms  
8  
9 624 in tumorigenesis. The sPLA<sub>2</sub>, for instance, acts as a tumor suppressor in gastric and intestinal  
10  
11 625 cancers and as a tumor promoter in breast, lung, prostate, ovarian, and esophageal cancers  
12  
13 626 [113], whereas cPLA<sub>2</sub> is mostly tumorigenic, except for colon cancer where it acts as a tumor  
14  
15 627 suppressor [114]. This highlights the importance of targeting the appropriate isoforms of  
16  
17 628 PLA<sub>2</sub>s depending on the type of cancer.

18  
19 629 Given the above-mentioned, PLA<sub>2</sub> inhibitors may be promising pharmacological  
20  
21 630 tools to treat cancer. In this regard, Wei and collaborators [115] elaborated a magnetic  
22  
23 631 nanoparticle-based ligand fishing system with immobilized porcine PLA<sub>2</sub> in an attempt to  
24  
25 632 find inhibitors from *Pseudopithomyces* sp. extract. In it, they fished six inhibitors and tested  
26  
27 633 them against human lung carcinoma (A549), bone marrow neuroblastoma (SH-SY5Y), and  
28  
29 634 cervical cancer (HeLa) cell lines. Interestingly, the authors found good potency of fusaristatin  
30  
31 635 C (IC<sub>50</sub> = 10.10 μM) against A549 cells, but not against SH-SY5Y and HeLa cells, which  
32  
33 636 may correlate with the alleged role of PLA<sub>2</sub>s in specific types of cancers.

#### 32 33 637 34 638 *3.5.1.4 Lactate dehydrogenase (LDH)*

35  
36 639 Lactate dehydrogenase (LDH) is a tetrameric enzyme formed by two subunits (A and  
37  
38 640 B) with different body distributions. While subunit A is more present in the liver and  
39  
40 641 muscles, subunit B predominates in the heart, being both upregulated in different types of  
41  
42 642 cancer [116]. Differently from normal cells, cancer cells obtain most of their energy from  
43  
44 643 glycolysis and subsequent conversion of pyruvate into lactate by LDH, even under normal  
45  
46 644 aerobic conditions [117]. This distinguished activity pattern turns LDH into an interesting  
47  
48 645 target in the search for inhibitors with anticancer potential since diminished levels of LDH  
49  
50 646 activity reduce tumorigenicity causing minor side effects [116,118]. Exploring this  
51  
52 647 potentiality, Cheng and collaborators [119] fabricated a bovine LDH-grafted magnetic  
53  
54 648 particle capable of rapidly screening natural inhibitors from two complex matrices. In it, the  
55  
56 649 authors successfully fished out twelve different ligands of which at least six (emodin, rhein,  
57  
58 650 aloe-emodin, chrysophanic acid, lunatin, and (-)-epicatechin-3-O-gallate) have  
59  
60 651 demonstrated promising activity against cancer cells by other research groups [120-125].  
62  
63 652 However, in this work, the authors did not perform enzymatic assays to check inhibition in  
64  
65 653 the presence of the fished-out ligands, not being possible to confirm their alleged LDH  
66  
67 654 inhibiting potential.

#### 68 69 655 70 656 *3.5.1.5 Glutathione S-transferase (GST)*

71  
72 657 Glutathione S-transferase (GST) is a family of enzymes that catalyze the conjugation  
73  
74 658 of glutathione into different types of electrophilic substances, being responsible for phase II  
75  
76 659 detoxification. Additionally, GST (μ and π classes) participates in other cellular processes  
77  
78 660 including apoptosis, carcinogenesis, tumor formation, and multidrug resistance, in which it  
79  
80 661 undermines the chemotherapy drug efficacy by extrusion and inhibits the MAPK pathways

1  
2  
3 662 to avoid tumor cell apoptosis [126]. Indeed, the GST isozyme GSTP1 ( $\pi 1$ ) is overexpressed  
4  
5 663 in different types of cancer (glioma, gastric, prostate, and breast cancer) and its  
6  
7 664 inhibition/knockdown increases the response to anticancer-approved drugs [127]. For these  
8  
9 665 reasons, GST inhibitors have been considered for anticancer drug discovery and treatment,  
10  
11 666 being used as an immobilized target in the ligand fishing system developed by Yi and  
12  
13 667 colleagues [28]. In it, the authors engineered a mesoporous silica magnetic particle with GST  
14  
15 668 immobilized on its surface intending to screen natural products with inhibitory potential  
16  
17 669 against GST. With it, they could efficiently fish seven ligands, of which three presented high  
18  
19 670 binding affinity for GST, being them rosmarinic acid, (-)-epigallocatechin-3-O-gallate, and  
20  
21 671 (-)-epicatechin-3-gallate [28].

22  
23 672 GST inhibitors are classified according to their activity in specific binding sites,  
24  
25 673 namely G- and H-sites. G-site inhibitors are glutathione-based molecules since this specific  
26  
27 674 site only accepts glutathione as a substrate. On the other hand, H-site inhibitors are more  
28  
29 675 structurally diversified, making the development of inhibitors quite challenging [127]. One  
30  
31 676 way or the other, natural products are interesting sources of GST inhibitors and when  
32  
33 677 associated with ligand fishing approaches, this enables fast screening and discovery of new  
34  
35 678 chemosensitizers to aid current cancer chemotherapy.

36  
37 679

#### 38 680 3.5.1.6 Quinone reductase-2 (QR2)

39  
40 681 Like GST, quinone reductase-2 (QR2) is a phase II enzyme that protects the body  
41  
42 682 against toxic and reactive chemical species, although its role in cancer appears to be less  
43  
44 683 evident. Yet, QR2 inhibitors have been hypothesized as cancer chemopreventive agents,  
45  
46 684 resveratrol being one of the most studied natural products for this purpose [128,129]. Indeed,  
47  
48 685 resveratrol has been associated with all three steps of carcinogenesis and as a QR2 inhibitor,  
49  
50 686 it seems to regulate the degradation of cyclin D1 by the proteasome via the AKT-GSK-3 $\beta$   
51  
52 687 pathway, limiting cell growth. Moreover, QR2 knockdown results in slower proliferation and  
53  
54 688 cell cycle arrest at the G1 phase, indicating that this enzyme plays a key role in cell division  
55  
56 689 [130].

57  
58 690 From this perspective, Choi and collaborators [82] screened different natural products  
59  
60 691 seeking QR2 inhibitors by a ligand fishing assay. They incubated human recombinant QR2  
61  
62 692 with marine sediment bacteria (*Actinomyces* sp.) and hop (*Humulus lupulus* L.) extracts,  
63  
64 693 being able to fish out and identify three different inhibitors, one from bacteria (tetrangulol  
65  
66 694 methyl ether) and two from hops (xanthohumol and xanthohumol D). As compared to  
67  
68 695 resveratrol ( $IC_{50} = 5.1 \mu M$ ), xanthohumol and xanthohumol D showed lower activity ( $IC_{50} >$   
69  
70 696  $100 \mu M$ ) whereas tetrangulol methyl ether was quite potent ( $IC_{50} = 0.16 \mu M$ ) [131]. In fact,  
71  
72 697 the latter compound has shown interesting cytotoxicity against different types of cancer cells  
73  
74 698 (KB, MCF-7, and NCI-H187) [132], which may also correlate with its potent inhibitory  
75  
76 699 activity against QR2.

77  
78 700

#### 79 701 3.5.1.7 Heat-shock proteins (HSPs)

1  
2  
3 702 The heat-shock proteins (HSP) are mammalian chaperones that regulate protein  
4  
5 703 folding and refolding in the face of stressful conditions such as abrupt temperature changes  
6  
7 704 and exposure to high concentrations of reactive oxygen species (ROS). In this sense, HSPs  
8  
9 705 represent the primary line of defense against stress-related cellular events, therefore being  
10  
11 706 related to various stress-originated diseases. For these reasons, HSP has been widely  
12  
13 707 considered in the search for new drugs, especially in cancerology. In this regard, HSPs play  
14  
15 708 a key role in carcinogenesis surveillance since cancer cells require distinct and often mutated  
16  
17 709 biochemical machinery to favor proliferation, invasion, metastasis, evasion from apoptosis,  
18  
19 710 and drug resistance [133].

20  
21 711 Among the families of HSPs (HSP27, HSP40, HSP60, HSP70, and HSP90), HSP90  
22  
23 712 is the most studied one. This protein is overexpressed in several types of cancer and is related  
24  
25 713 to a bad prognosis in cholangiocarcinoma, glioblastoma, gastric, lung, and breast cancers.  
26  
27 714 The body of evidence correlating HSP90 to cancer is vast. The increased expression of  
28  
29 715 HSP90, for example, is responsible for the activation and stabilization of oncogenic protein  
30  
31 716 kinases, such as STAT3, JAK2, AKT, PI3K, and MAPK, which ultimately stimulate cancer  
32  
33 717 cell growth and tumor progression [134].

34  
35 718 In the field of ligand fishing, HSP90 has been explored as a target in the search for  
36  
37 719 new anticancer agents. Three works from the same research group used quantum dots as a  
38  
39 720 support to immobilize HSP90 and screen natural products for drug leads [44-46]. The group  
40  
41 721 found multiple HSP90 inhibitors, some with characterized cytotoxicity *in vitro* and antitumor  
42  
43 722 effect *in vivo* [135-137]. However, developing HSP90 inhibitors is quite challenging as they  
44  
45 723 frequently fail in clinical trials either due to high toxicity or lack of beneficial effects  
46  
47 724 [138,139].

48  
49 725

#### 50 51 726 3.5.1.8 Sirtuin 6 (SIRT6)

52  
53 727 Sirtuins are nicotinamide adenine dinucleotide-dependent (NAD<sup>+</sup>) proteins belonging  
54  
55 728 to the class III histone deacetylase family, being involved in DNA damage repair, stress  
56  
57 729 response, ribosome biogenesis, cell proliferation, and cell death [140]. There are seven  
58  
59 730 isoforms of sirtuins (SIRT1-7), classified according to their function and location. Sirtuin 6  
60  
731 (SIRT6), in particular, is a nuclear deacetylase involved in the modulation of various  
732  
733 732 biological processes such as inflammation, metabolism, aging, tumor suppression, and  
734  
735 733 carcinogenesis. The reduction of SIRT6 levels is associated with the human premature aging  
736  
737 734 disorder and restoration of its activity reduces cell senescence. Additionally, SIRT6  
738  
739 735 possesses both tumor suppressor and oncogenic properties that will emerge depending on the  
740  
741 736 type of cell. In colorectal, ovarian, and pancreatic cancers, for example, functional SIRT6  
742  
743 737 inhibits progression whereas its upregulation is related to hepatocarcinoma, skin, lung,  
744  
745 738 breast, and prostate cancers [141].

746  
747 739 In this context, two similar works used immobilized SIRT6 onto the surface of  
748  
749 740 magnetic particles to screen *Trigonella foenum-graecum* seed extract in an attempt to find  
750  
751 741 interesting ligands [38,39] In the first, the authors develop the fishing system using catalytic  
752  
753 742 mutant SIRT6 and heat-inactivated SIRT6 as negative controls, being able to identify

1  
2  
3 743 quercetin and vitexin as inhibitors. In the second work, the authors used the same fishing  
4 744 technique but with a different identification approach, being able to identify orientin and  
5 745 fourteen other SIRT6 modulators for the first time.  
6  
7  
8 746

#### 9 747 3.5.1.9 $\alpha_v\beta_3$ Integrin

10 748 Cancer invasion and metastasis strongly depend on the partial degradation of the  
11  
12 749 extracellular matrix and the formation of new blood vessels [142]. Integrins are a protein  
13  
14 750 family responsible for cell-cell and cell-extracellular matrix adhesion as well as angiogenesis,  
15  
16 751 being the  $\alpha_v\beta_3$  integrin (also known as vitronectin receptor) the most important one [143]. In  
17  
18 752 fact, this protein is highly expressed in newborn vessels whereas is lacking in resting  
19  
20 753 endothelial cells [144]. In this sense,  $\alpha_v\beta_3$  integrin has been explored as an anticancer target  
21  
22 754 since the inhibition of its activity has been associated with endothelial apoptosis and  
23  
24 755 inhibition of angiogenesis, which is a crucial step in tumor formation and growth [145,146].  
25  
26 756 Cilengitide, for example, is one of the most studied  $\alpha_v\beta_3$  antagonists, being able to potentiate  
27  
28 757 the radiotherapy outcomes in patients with glioma [147].

29  
30 758 Having this challenge in mind, Uliana and colleagues [148] developed a ligand  
31  
32 759 fishing system with immobilized  $\alpha_v\beta_3$  integrin able to efficiently fish out [10]-gingerol from  
33  
34 760 the ginger extract. The [10]-gingerol has been tested as an adjuvant to the treatment with  
35  
36 761 doxorubicin using *in vitro* and *in vivo* models of triple-negative breast cancer where it was  
37  
38 762 able to reduce metastasis, chemotherapy-related weight loss, and hepatotoxicity [149].  
39

#### 40 763 3.5.1.10 Tropomyosin-related kinase B (TrkB) receptor

41  
42 764 The tropomyosin-related kinase is a family of tyrosine kinase receptors involved in  
43  
44 765 neural development and cell functioning, survival, and proliferation. As tyrosine kinase  
45  
46 766 receptors, after neurotrophin stimulus, they undergo a dynamic homodimerization with  
47  
48 767 subsequent transactivation of the tyrosine kinase domains which in turn allows the  
49  
50 768 recruitment of cytoplasmic proteins and activation of intracellular pathways [150].  
51  
52 769 Alterations in these receptors, either via mutations or rearrangements, have been related to  
53  
54 770 oncogenesis, therefore making these proteins potential anticancer targets [151]. They are  
55  
56 771 divided into three types depending on their activating neurotrophin: the TrkA, stimulated by  
57  
58 772 the nerve growth factor (NGF), the TrkB, which is essentially activated by the brain-derived  
59  
60 773 neurotrophic factor (BDNF) and the TrkC which in turn is stimulated in the presence of the  
774 neurotrophin-3 [152]. Preclinical studies have demonstrated that TrkA and TrkB inhibitors  
775 are capable of attenuating the proliferation and aggressiveness of pancreatic, neuroblastoma,  
776 and neuroendocrine tumors via apoptosis induction [153]. Additionally, the pan-Trk inhibitor  
777 (TrkA, B, and C inhibitor) entrectinib (Rozlytrek®), recently approved in Japan, the USA,  
778 and the European Union has demonstrated great results in the treatment of solid tumors in  
779 patients with specific genetic conditions [154].  
780

781 Aware of the potential of Trk inhibitors, Arituluk and colleagues [32] developed a  
782 ligand fishing assay using magnetic nanoparticles coated with TrkB-overexpressing SH-  
783 SY5Y cell membranes to screen a Chinese natural product in the search for new inhibitors.



1  
2  
3 784 Despite some limitations, this model proved to be a fast-track in the screen of TrkB inhibitors  
4  
5 785 from a very complex natural matrix, with a great degree of specificity as it was able to  
6  
7 786 discriminate between TrkB binders and non-binders mixed in equimolar concentration [32].  
8  
9 787

#### 10 788 *3.5.1.11 Fibroblast growth factor receptor 4 (FGFR4)*

11  
12 789 Fibroblast growth factor receptor 4 (FGFR4) is a highly conserved tyrosine kinase  
13  
14 790 receptor involved in various physiological processes such as tissue growth and repair,  
15  
16 791 production of bile acids, metabolism, and muscle differentiation. Upon activation by the  
17  
18 792 fibroblast growth factor (FGF) in the extracellular domains, the FGFR4 undergoes  
19  
20 793 dimerization and transphosphorylation (intracellular domains), which in turn allows the  
21  
22 794 direct activation of the PLC $\gamma$ -IP $_3$ -DAG-PKC pathway and indirect (via FGFR substrate 2 –  
23  
24 795 FRS2) activation of RAS-RAF-MAPK and PI3K-AKT pathways [155]. Moreover, FGFR4  
25  
26 796 overexpression and mutation as well as upregulation of its endogenous agonists are closely  
27  
28 797 related to the development of cancer, especially breast cancer [156]. Despite that, the  
29  
30 798 oncogenic functions of FGFR4 seem to vary greatly depending on the type of cancer as it is  
31  
32 799 related to poor prognosis in colorectal, esophageal, liver, rhabdomyosarcoma, and lung  
33  
34 800 cancers and with better survival in cervical, cholangiocarcinoma, pancreatic and head and  
35  
36 801 neck cancers [157].

37 802 Based on the close relationship between FGFR4 receptors and cancer, Bu and  
38  
39 803 colleagues [158] used a previously engineered HEK293 cell transfected with the Y367C  
40  
41 804 mutation [159] to produce a mutant FGFR4-expressing cell membrane-coated silica particle  
42  
43 805 to screen a TCM product. It is important to note that the FGFR4 Y367C mutation is a  
44  
45 806 dominant oncogene in MDA-MB453 breast cancer cells [160] and as such, it is a good model  
46  
47 807 for the screening of anticancer compounds through cell membrane ligand fishing assay. As a  
48  
49 808 result, the authors fished out four alkaloids of which one (napellonine) had inhibitory action  
50  
51 809 on FGFR4, with IC $_{50}$  of 133 $\mu$ g/mL. Yet according to the authors, in comparison to the  
52  
53 810 standard FGFR4 inhibitor PD173074 (IC $_{50}$  = 71.3 $\mu$ g/mL), napellonine would need structural  
54  
55 811 modifications to increase its potency towards FGFR4 [158].  
56  
57 812

#### 58 813 *3.5.1.12 Urokinase plasminogen activator (uPA)*

59  
60 814 The urokinase plasminogen activator (uPA) is an essential protease involved in the  
815  
816 815 mechanism of conversion of plasminogen into plasmin. The extracellular matrix (ECM) is  
817  
818 816 particularly affected by the activation of the uPA receptor (uPAR) by uPA as it is degraded  
819  
820 817 by plasmin-activated metalloproteinases. The activation of this system is regulated by the  
821  
822 818 plasminogen activator inhibitor 1 and 2 (PAI-1/2), which in turn signals for internalization  
823  
824 819 of the complex uPA-uPAR. This phenomenon is crucial for various biological processes like  
820  
821 820 embryogenesis and wound healing as well as in pathological states such as cancer invasion  
822  
823 821 and metastasis [161]. Furthermore, the uPA system can facilitate cancer metastasis by  
824  
825 822 different mechanisms other than ECM degradation. The binding of uPA to the uPAR can  
826  
827 823 activate MAPK pathways (Ras-Raf-MEK-ERK and Rac1-MKK3-p38-MAPKAPK2) which  
828  
829 824 are involved with cell growth, differentiation, survival, migration, and apoptosis [162]. The



1  
2  
3 825 invasion of breast cancer cells, for example, is thought to be mediated by uPA via the p38-  
4 826 MAPK pathway with the aid of transmembrane integrins since the uPAR lacks  
5 827 transmembrane and cytosolic domains. Additionally, PAI-1 has a dual role in cancer since it  
6 828 stimulates the expression and internalization of the uPA-uPAR via low-density lipoproteins.  
7 829 Interestingly, this endogenous inhibitor has been associated with a poor cancer prognosis.  
8 830 Indeed, PAI-1's ability to limit excessive degradation of the ECM seems to help cancer cells  
9 831 to invade other tissues and spread through the bloodstream. Furthermore, PAI-1 also  
10 832 stimulates angiogenesis, apparently by exerting the same control over the ECM [163].  
11 833 Previous reports showed that the uPA antagonist amiloride could modulate uPA gene  
12 834 expression at transcription and post-transcriptional levels, proving to be effective against  
13 835 cancer in preclinical models [164].

14 836 From this panorama, Li and collaborators [165] developed a fishing system designed  
15 837 to screen natural uPA ligands from traditional Chinese medicines (TCMs). With it, they  
16 838 screened forty-two TCMs to find seven uPA ligands, of which pentagalloylglucose and 28-  
17 839 *O*- $\beta$ -D-glucopyranosyl pomolic acid presented good inhibitory potential against uPA ( $IC_{50}$  =  
18 840 1.639  $\mu$ M and 37.82  $\mu$ M respectively). The high throughput nature of this approach allowed  
19 841 the authors to screen dozens of complex matrices, with thousands of chemical entities each,  
20 842 in a very short time as compared to classical natural product screenings, which makes this  
21 843 technique suitable for accelerated natural product screen and drug discovery.

22 844

### 23 845 3.5.1.13 Matrix Metalloproteinase-2 (MMP-2)

24 846 Matrix metalloproteinases (MMPs) are a large family of zinc-dependent proteases  
25 847 that act by cleaving and rebuilding the extracellular matrix in connective tissues. These  
26 848 enzymes participate in physiological processes such as apoptosis, ovulation, inflammation,  
27 849 angiogenesis, neural growth, bone remodeling, embryonic development, and organ  
28 850 morphogenesis. On the other hand, pathological processes such as cancer invasion and  
29 851 metastasis occur with the aid of MMPs, highlighting the importance of these enzymes to  
30 852 cancerology [166]. There are more than 20 isoforms of MMPs, each having specific  
31 853 substrates, tissue distribution, molecular mass, and gene coding. Among them, gelatinases  
32 854 (MMP-2 and 9) are particularly involved in cancer invasion and metastasis as their increased  
33 855 expression has been related to the invasiveness of gliomas [167], malignant transformations  
34 856 after human papilloma virus infections [168], reduced survival in patients with primary breast  
35 857 carcinoma [169] and esophageal cancer [170].

36 858 In this context, Tao and colleagues [171] constructed a very interesting ligand fishing  
37 859 system that combines immobilization on magnetic particles with a microfluidic chip system.  
38 860 In it, the authors prepared and characterized a quantum dot magnetic particle with  
39 861 immobilized MMP-2 before submitting it to a microfluidic chip. Through two different  
40 862 channels (b and c), they injected the MMP-2-functionalized magnetic particles and the fruit  
41 863 extract of *Rosa roxburghii* Tratt. After incubation, a small ring magnet trapped the magnetic  
42 864 particles, and a wash solvent (phosphate buffer solution) was pumped through two other  
43 865 channels (d and f) to remove unspecifically bound ligands that were collected through two

1  
2  
3 866 additional channels (e and g). In the end, the dissociating solvent (30% acetonitrile in water)  
4  
5 867 was pumped through another channel (h) to separate the ligands from the MMP-2-  
6  
7 868 functionalized magnetic particles. Finally, the eluted ligands were pumped into a UPLC-Q-  
8  
9 869 TOF/MS system to be analyzed. Among the twelve ligands fished and identified, ellagic acid  
10  
11 870 stands out as it is a well-known MMP-2 inhibitor [171,172]. This polyphenolic compound  
12  
13 871 possesses antiproliferative activity in various types of cancer cells, such as glioblastoma (U87  
14  
15 872 and U118), human osteogenic sarcoma (CRL1343), human colorectal adenocarcinoma  
16  
17 873 (Caco-2), human prostate cancer (DU 145) and breast cancer (Hs 578T and MCF-7)  
18  
19 874 [173-175]. Furthermore, the other eleven ligands fished (niga-ichigoside F<sub>1</sub>, rosamultin,  
20  
21 875 kajiichigoside F<sub>1</sub>, zebirioside O, peduncloside, potentillanoside A, quadranoside VIII,  
22  
23 876 corilagin, oreganol A, medicagenic acid, and valerenic acid) were described as MMP-2  
24  
25 877 inhibitors for the first time, evidencing the high throughput potential of this approach to  
26  
27 878 isolate new molecules that could be useful for cancer treatment [171].  
28  
29 879

#### 30 880 3.5.1.14 Monoacylglycerol lipase (MAGL)

31 881 The monoacylglycerol lipase (MAGL) is a soluble enzyme that catalyzes the  
32  
33 882 hydrolyzation of monoacylglycerols to glycerol and fatty acids. Moreover, this enzyme is  
34  
35 883 responsible for the hydrolysis of the endocannabinoid 2-arachidonoylglycerol (2-AG),  
36  
37 884 releasing arachidonic acid, which in turn is a major precursor of pro-inflammatory agents  
38  
39 885 that are involved in cancer progression, such as prostaglandins. In aggressive types of cancer,  
40  
41 886 MAGL provides the free fatty acid supply that triggers downstream pro-tumorigenic  
42  
43 887 pathways [176]. Nevertheless, MAGL is highly active in melanoma, ovarian, breast,  
44  
45 888 prostate, and liver cancer cells [177].

46 889 In this context, Mei and collaborators [178] engineered yeast cells (KM71H *Pichia*  
47  
48 890 *pastoris*) to expose MAGL on their surface. In a preliminary study, the authors screened  
49  
50 891 twelve TCMs (Corydalis Rhizoma, Salvia Miltiorrhiza Radix et Rhizoma, Chuanxiong  
51  
52 892 Rhizoma, Carthami Flos, Andrographis Herba, Eucommiae Cortex, Asari Radix et Rhizoma,  
53  
54 893 Ginkgo Folium, Cinnamomum Ramulus, Rhei Radix et Rhizoma, Acorus Tatarinowii  
55  
56 894 Rhizoma, and Paridis Rhizoma) to verify their inhibitory potential against MAGL. From it,  
57  
58 895 three TCMs had inhibitory activity greater than 50%, Corydalis Rhizoma (*Corydalis*  
59  
60 896 *yanhusuo* W. T. Wang) being the most active one (66.6% of inhibition). After the  
897 characterization of the ligand fishing system with fluorescent antibodies, the authors  
898 incubated the modified yeast with the extract of *Corydalis yanhusuo* to search for MAGL  
899 inhibitors. Among the seven ligands fished and characterized, the dehydrocorydaline  
900 (presented the highest relative binding rate in comparison with the control (JZL184) [178].  
901 In another study, dehydrocorydaline demonstrated anticancer activity *in vitro* against MCF-  
902 7 cells, promoting apoptosis via the Bax-Bcl-2 signaling pathway [179]. On the other hand,  
903 it was not demonstrated yet if the anticancer effect of DHC is also mediated through the  
904 inhibition of MAGL, which can be an important research question for subsequent studies.

#### 905 906 3.5.2 DNA

1  
2  
3 907 DNA structural characterization has advanced tremendously in the last decades.  
4  
5 908 Various possible arrangements differ significantly from the B-form of DNA proposed by  
6  
7 909 Watson and Crick in the 1950s. While the DNA B-form is relatively conserved in most cells,  
8  
9 910 other forms such as slipped, cruciform, triplex, and quadruplex are much more susceptible to  
10  
11 911 mutations, arousing great interest in the field of cancer-related drug discovery [180,181].  
12  
13 912 DNA G-quadruplexes, for instance, are guanine-rich DNA strands that can inhibit telomerase  
14  
15 913 under physiological conditions, being this enzyme overexpressed in various types of cancer.  
16  
17 914 Telomerase overexpression is associated with apoptosis suppression and the formation of  
18  
19 915 spontaneous tumors in animal models [189][191]. In this sense, small molecules that act as  
20  
21 916 G-quadruplex stabilizers represent an interesting alternative to limit telomerase activity and  
22  
23 917 therefore its oncogenic effects. Telomestatin, for example, is a potent telomerase inhibitor  
24  
25 918 derived from *Streptomyces anulatus* that has shown promising anticancer activity both *in*  
26  
27 919 *vivo* and *in vitro* [183,184].

28  
29 920 Based on our survey, eight works used DNA strands (double, triple, and quadruple)  
30  
31 921 as smart baits to fish out ligands from natural products with anticancer potential. Different  
32  
33 922 approaches were used to apply DNA strands into ligand fishing settings, including dialysis  
34  
35 923 [185], ultrafiltration [12], magnetic particles [186], and microfluid chips [187,188] as well  
36  
37 924 as other less trivial supports such as streptavidin-coated 96-well plates [49,50] and agarose  
38  
39 925 beads [47]. The DNA-bound ligands were tested in preclinical settings in two specific reports.  
40  
41 926 Ma and colleagues [12] found seven DNA binders from 62 plant samples, of which two  
42  
43 927 (daurisolone and tetrandrine) were active against various triple-negative breast cancer and  
44  
45 928 pediatric cancer cell lines ( $GI_{50} < 20\mu\text{M}$ ). In another report, Gao and colleagues [194][196]  
46  
47 929 found four DNA-bound alkaloids (sanguinarine, chelerythrine, protopine, and  
48  
49 930 allocryptopine). They found no cytotoxic activity for protopine and allocryptopine whereas  
50  
51 931 chelerythrine and sanguinarine presented cytotoxicity against both normal (LO2 cell line)  
52  
53 932 and liver cancer cells (HepG2 cell line) [187]. In fact, DNA binders are known for their  
54  
55 933 undistinguishable toxicity against cancer and normal cells, which dramatically limits their  
56  
57 934 utilization in clinical settings [189].

### 58 935 3.5.3 Cancer cell membranes

59  
60 936 In the late 1990s, He and colleagues [190] developed a technique that used cell  
937 membranes for the fabrication of stationary phases applied in chromatographic studies. This  
938 innovative approach reproduces *in vivo* drug-receptor interactions and adsorbs ligands on the  
939 cell membrane. Coupled with it, high-performance liquid chromatography (HPLC) systems  
940 separated the retained ligands for further analysis, giving rise to cell membrane  
941 chromatography (CMC). From then on, various adaptations of the technique were performed  
942 to adequate to different research topics, including ligand fishing with a focus on anticancer  
943 drug discovery.

944 In this review, several papers used a great variety of cancer cell lines to perform ligand  
945 fishing assays such as PC3 (human androgen-refractory prostate cancer) [20], MCF-7 (breast  
946 cancer) [11,51,191], HepG2 (hepatoma) [11,192], HCT116 (colorectal cancer) [193], A549  
947 (lung carcinoma) [35], SKOV-3 (human ovarian cancer) [190,194], ACHN (renal

1  
2  
3 948 adenocarcinoma) [190,195,196], HEK293 cells (immortalized human embryonic kidney  
4 cells) [197] among others. The rationale of the techniques resides in the slight differences  
5 949 between normal and cancer cells as well as the preservation of membrane receptors' vicinity.  
6 950  
7 In this sense, cancer cells are used as smart baits to fish out ligands according to their affinity  
8 951 for cell membrane surface structures, mainly receptors. Some cell membrane ligand fishing  
9 952 studies have induced the overexpression of membrane receptors to potentialize the adsorption  
10 953 of ligands. Hu et al. [197], for example, used HEK293 cells overexpressing the epidermal  
11 954 growth factor receptors (EGFR) to encapsulate graphene nanoparticles, which were capable  
12 955 of fishing out luteolin and caffeic acid from the extract of *Taraxacum mongolicum* Hand.-  
13 956 *Mazz.* Ligand fishing systems with membrane-anchored receptors are more realistic than free  
14 957 or immobilized receptors since the cell membrane play an important role in the conformation  
15 958 of these targets that may affect the affinity of ligands [197].  
16 959  
17 960

#### 961 **4. Concluding Remarks**

962 Natural products are an important source of bioactive compounds. Therefore, the  
963 potential for discovering new anticancer drug leads is undeniable. Despite that, classical  
964 screenings are poorly efficient and quite unspecific. To address these issues, ligand fishing  
965 approaches have emerged in the last two decades as a better option to accelerate the hit-to-  
966 lead discovery process. In this review, we gathered detailed information from the scientific  
967 literature aiming to provide a reasonable summary of findings, highlighting successful cases  
968 as well as some limitations of the techniques. This approach's versatility permits various  
969 suitable configurations depending on the target and its particularities. In this sense, a great  
970 number of different anticancer targets, such as proteins, DNA, and cancer cells themselves,  
971 were employed to fabricate robust ligand fishing systems capable of identifying new  
972 bioactive compounds. Nevertheless, we observed clear tendencies among the articles, in  
973 terms of natural products and the location where the research was developed. Another  
974 important piece of information is that a significant number of studies do not perform  
975 anticancer preclinical evaluations. Therefore, there is a need for follow-up studies designed  
976 to shed light on the ligands' alleged biological activities.

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#### 981 **Conflict of interest statement**

982 The authors have no conflict of interest to declare

#### 983 **Data availability statement**

984 All data are already available in the current scientific literature. Therefore, data sharing does  
985 not apply as the authors have not performed new experimental assays.

986

1  
2  
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4

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Species	Part	Extraction	Technique & Target	Ligands Fished	Chemical Class	Ref
<i>Aconitum szechenyianum</i> Gay (Ranunculaceae)	Roots	Reflux – EtOH 60%	Miscellaneous (FGFR4)	<b>1)</b> Napellonine, <b>2)</b> 12-Epinapelline, <b>3)</b> Benzoylaconitine and <b>4)</b> 3-Deoxyaconitine	Alkaloids	[158]
<i>Acorus tatarinowii</i> Schott (Acoraceae)	Undefined	Ultrasonication – EtOH 70%	Miscellaneous (MAGL)	-	-	[178]
<i>Actinomyces sp.</i> (Actinomycetaceae)	Whole	Liquid-liquid extraction – acetone-EtOAc-H <sub>2</sub> O	Ultrafiltration (QR-2)	<b>1)</b> Tetrangulol methyl ether	Anthraquinone	[82]
<i>Alisma orientale</i> (Sam.) Juz. (Alismataceae)	Roots	Ultrasonication – MeOH:H <sub>2</sub> O (7:3)	Magnetic particle (GST)	-	-	[28]
<i>Alisma plantago-aquatica</i> L. (Alismataceae)	Undefined	Reflux – EtOH 85%	Miscellaneous (Hsp 90α)	<b>1)</b> Alisol F, <b>2)</b> 16-oxo-11-deoxy-Alisol A, <b>3)</b> Alisol F 24-acetate, <b>4)</b> Alisol L 23-acetate, <b>5)</b> 16-oxo-11-anhydro-Alisol A, <b>6)</b> Alisol H, <b>7)</b> Alisol B-23-acetate, <b>8)</b> Alisol C, <b>9)</b> 3-Methyl-5,6- dimethoxy-2,3-dihydro-1H-indene-1-one, <b>10)</b> (1S,3α,7αβ)-1- Acetyloctahydro- 4α-hydroxy-7β- isopropyl 4-methyl-1H-indene, <b>11)</b> Corymbolone, <b>12)</b> (-)-Spathulenol, <b>13)</b> n-Hexadecanoic acid, <b>14)</b> Hexadecanoic acid ethyl ester, <b>15)</b> Linoleic acid ethyl ester, <b>16)</b> 13,17-Seco-5α-pregn-13(18)-en-20-one, <b>17)</b> Lupeol, <b>18)</b> Lupeol acetate, <b>19)</b> Taraxasterol and <b>20)</b> Betulinaldehyde	Fatty acids, steroids and terpenoids	[45]
<i>Andrographis paniculata</i> (Burm. f.) Nees (Acanthaceae)	Undefined	Ultrasonication – EtOH 70%	Miscellaneous (MAGL)	-	-	[178]

<i>Anemarrhena asphodeloides</i> Bunge (Asparagaceae)	Rhizomes	Percolation – EtOH 95%	Ultrafiltration (COX2)	<b>1) Neomangiferin, 2) Phellodendrine, 3) Mangiferin, 4) Magnoflorine, 5) 3-O-feruloylquinnic acid, 6) (–)-Oblongine, 7) Menisperine, 8) Demethyleneberberine, 9) Oxoberberine, 10) Berberrubine, 11) Jatrorrhizine, 12) Timosaponin B, 13) Timosaponin B-II, 14) Timosaponin A-I, 15) Palmatine, 16) Berberine, 17) Sanleng acid, 18) Timosaponin A-III, 19) Anemarrhenasaponin I, 20) cis-Hinokiresinol</b>	Alkaloids, xanthenes and saponins	[105]
<i>Angelicae dahurica</i> Fisch. (Apiaceae)	Roots	Ultrasonication – MeOH	Hollow fiber (HepG-2 cells)	<b>1) Xanthotoxol, 2) Xanthotoxin, 3) Psoralen, 4) Bergapten, 5) Oxypeucedanin, 6) Imperatorin, 7) Isoimperatorin.</b>	Coumarins	[43]
<i>Artemisia capillaris</i> Thunb (Asteraceae)	Undefined	Decoction – H <sub>2</sub> O	Hollow fiber (HepG-2 and ACHN cells)	<b>1) Chlorogenic acid, 2) p-Hydroxyacetophenone</b>	Phenolic acid and terpenoid	[196]
<i>Asarum heterotropoides</i> F. Schmidt. (Aristolochiaceae)	Undefined	Ultrasonication – EtOH 70%	Miscellaneous (MAGL)	-	-	[178]
<i>Astragalus membranaceus</i> (Fisch.) Bunge (Fabaceae)	Undefined	Ultrasonication – MeOH	Magnetic particle (EGFR/HEK293 cells)	-	-	[197]
<i>Atractylodes macrocephala</i> Koidz (Asteraceae)	Roots	Infusion – H <sub>2</sub> O	Magnetic particle (Tyrosinase)	<b>1) Gallic acid, 2) Albiflorin, 3) Paeoniflorin, 4) Liquiritin apioside, 5) Liquiritin, 6) Galloylpaeoniflorin, 7) Ononin, 8) Isoliquiritigenin, 9) Glycyrrhizic acid, 10) Oxypaeoniflora, 11) Benzoylpaeoniflorin, 12) Benzoyloxypaeoniflorin, 13) Mudanpioside C, 14) Paeonolide and 15) Apiopaeonoside</b>	Phenolic acid, terpenoids saponins, and glycosides	[95]
<i>Bufo melanostictus</i> Schneider (Bufonidae)	Secretion (venom)	Reflux – EtOH and H <sub>2</sub> O	Miscellaneous (HepG2 and MCF-7 cells)	<b>1) Serotonin, 2) N-methyl serotonin, 3) Bufotenidine, 4) Suberoyl arginine, 5) Arenobufagin, 6) Telocinobufatoxin, 7) Bufotalin, 8) 3-(N-pimeloyl argininyl) bufalin, 9) Cinobufotalin, 10) Bufalitoxin, 11) 3-(N-pimeloyl argininyl) resibufogenin, 12) Bufalin, 13) Resibufotoxin, 14) Cinobufotoxin, 15) 3-(N-azelayl argininyl) resibufogenin, 16) Cinobufagin and 17) Resibufogenin</b>	Alkaloids, amino acid, bufogenins and bufotoxins	[11]
<i>Camellia sinensis</i> (L.) Kuntze (Theaceae)	Leaves	Ultrasonication – MeOH:H <sub>2</sub> O (7:3)	Magnetic particle (GST)	<b>1) Caffeic acid, 2) Rosmarinic acid, 3) Gallic acid, 4) (–)-Epigallocatechin-3-O-gallate, 5) (–)-Epicatechin-3-gallate and 6) Quercetin-3-O-glucoside</b>	Phenolic acids and flavonoids	[28]

1 2 3 4 5 6	<i>Camellia sinensis</i> (L.) Kuntze (Theaceae)	Leaves	-	Magnetic particle (COX2)	<b>1)</b> (-)-gallocatechin, <b>2)</b> (-)-epigallocatechin, <b>3)</b> (-)-epicatechin, <b>4)</b> (-)-epigallocatechin-3-gallate, <b>5)</b> (-)-gallocatechin-3-gallate, <b>6)</b> (-)-epigallocatechin-3-(3''-O-methyl)-gallate, <b>7)</b> (-)-epicatechin-3-gallate, <b>8)</b> (-)-epicatechin-3-(3''-O-methyl)-gallate	Flavonoids	[106]
7 8 9 10 11 12	<i>Camellia sinensis</i> (L.) Kuntze (Theaceae)	Leaves	Ultrasonication – MeOH 70%	Magnetic particle (COX2)	<b>1)</b> 3-Galloylquinic acid, <b>2)</b> 5-Galloylquinic acid, <b>3)</b> 3-p-Coumaroylquinic acid, <b>4)</b> 5-p-Coumaroylquinic acid, <b>5)</b> 5-caffeoylquinic acid, <b>6)</b> (-)-Epicatechin, <b>7)</b> (-)-Epicatechin 3-O-gallate, <b>8)</b> Quercetin 3-O-rhamnosylgalactoside, <b>9)</b> Quercetin 3-O-glucoside, <b>10)</b> Kaempferol 3-O-glucosylrutinoside, <b>11)</b> Kaempferol-3-O-rutinoside, <b>12)</b> Kaempferol 3-O-glucoside	Phenolic acids and flavonoids	[107]
13 14 15 16 17 18	<i>Carthamus tinctorius</i> L. (Asteraceae)	Undefined	Ultrasonication – EtOH 70%	Miscellaneous (MAGL)	-	-	[178]
19 20 21 22 23 24 25 26 27	<i>Cassia obtusifolia</i> L. (Fabaceae)	Seeds	Decoction – EtOH 75%	Miscellaneous (HepG2 cells)	<b>1)</b> Aurantio-obtusin, <b>2)</b> 1-desmethyllaurantio-obtusin, <b>3)</b> Chryso-obtusin, <b>4)</b> Obtusin, and <b>5)</b> Questin	Anthraquinones	[193]
28 29 30 31 32	<i>Cecropia obtusifolia</i> Bertol. (Urticaceae)	Undefined	Reflux – MeOH; Ultrasound – HCl 8%; Liquid- liquid – HCl- CHCl <sub>3</sub>	Hollow fiber (HCT116 cells)	<b>1)</b> Aloe-emodin and <b>2)</b> Physcion	Anthraquinones	[194]
33 34 35 36	<i>Centella asiatica</i> (L.) Urban (Apiaceae)	Undefined	-	Magnetic particle (TrkB)	<b>1)</b> Dicafeoylquinic acid, <b>2)</b> O-cafeoylquinic acid, <b>3)</b> Madecassic acid, <b>4)</b> Castillicetin, <b>5)</b> Quercetin, <b>6)</b> Stigmasterol, <b>7)</b> Pomolic acid, <b>8)</b> Naringin	Phenolic acids, terpenoids, steroid and flavonoids	[32]
37 38 39 40	<i>Cibotium barometz</i> (L.) J.Sm. (Cibotiaceae)	Rhizomes	Ultrasonication – MeOH	Magnetic particle (EGFR/HEK293 cells)	-	-	[197]
41 42 43	<i>Cinnamomum cassia</i> (L.) J.Presl (Lauraceae)	Undefined	Ultrasonication – EtOH 70%	Miscellaneous (MAGL)	-	-	[178]

<i>Cinnamomum cassia</i> (L.) J.Presl (Lauraceae)	Cortex	Percolation – EtOH 95%	Ultrafiltration (COX2)	<b>1) Neomangiferin, 2) Phellodendrine, 3) Mangiferin, 4) Magnoflorine, 5) 3-O-feruloylquinnic acid, 6) (–)-Oblongine, 7) Menisperine, 8) Demethyleneberberine, 9) Oxoberberine, 10) Berberrubine, 11) Jatrorrhizine, 12) Timosaponin B, 13) Timosaponin B-II, 14) Timosaponin A-I, 15) Palmatine, 16) Berberine, 17) Sanleng acid, 18) Timosaponin A-III, 19) Anemarrhenasaponin I, 20) cis-Hinokiresinol</b>	Alkaloids, xanthenes and saponins	[105]
<i>Citrus reticulata</i> Blanco (Rutaceae)	Fruits (peels)	Percolation – EtOH 75%	Miscellaneous (MCF-7 cells)	<b>1) 5,7,8,30,40-Pentamethoxyflavanone, 2) 3,5,6,7,8,40-Hexamethoxyflavanone, 3) 5,7,8,30,40,50-Hexamethoxyflavone, 4) 7-Hydroxy-5,6,8,30,40-pentamethoxyflavone, 5) Isosinensetin, 6) 30-Hydroxy-5,6,7,8,40-pentamethoxyflavone or 40-Hydroxy-5,6,7,8,30-pentamethoxyflavone, 7) Sinensetin, 8) 5,6,7,8,40-Pentamethoxyflavanone, 9) 3-Hydroxy-5,6,7,8,40-pentamethoxyflavone, 10) Tetramethyl-O-isoscutellarein, 11) Tetramethyl-O-scutellarein, 12) Nobiletin, 13) 3,5,6,7,8,30,40-Heptamethoxyflavone, 14) 5,6,7,8,30,40-Hexamethoxyflavanone, 15) 5-Hydroxy-6,7,8,30,40-pentamethoxyflavone, 16) Tangeretin</b>	Flavonoids	[71]
<i>Cnidium monnieri</i> (L.) Cuss. (Apiaceae)	Fruits	Reflux – MeOH	Hollow fiber (SGC7901, RAW264.7 and SHSY5Y cells)	<b>1) Psoralen, 2) Bergapten, 3) Imperatorin and 4) Osthole</b>	Coumarins	[41]
<i>Coptis chinensis</i> Franch. (Ranunculaceae)	Roots	Solid-liquid extraction – MeOH; Liquid- liquid partition – EtOAc-H <sub>2</sub> O	Ultrafiltration (DNA)	<b>1) Berberine, 2) Coptisine and 3) Palmitine</b>	Alkaloids	[12]
<i>Coptis chinensis</i> Franch. (Ranunculaceae)	Rhizomes	Ultrasonication – EtOH 75%	Miscellaneous (DNA)	<b>1) Epiberberine, 2) Coptisine, 3) Jatrorrhizine, 4) Berberrubine and 5) Columbamine</b>	Alkaloids	[49]
<i>Coptis chinensis</i> Franch. (Ranunculaceae)	Rhizomes	-	Magnetic particle (DNA)	<b>1) Columbamine, 2) Epiberberine, 3) Jatrorrhizine, 4) Palmatine and 5) Berberine</b>	Alkaloids	[186]
<i>Coptis chinensis</i> Franch. (Ranunculaceae)	Undefined	Decoction – MeOH 20%	Hollow fiber (MCF7, MADB106, and SGC7901 cells)	<b>1) Jatrorrhizine, 2) Epiberine, 3) Coptisine, 4) Palmatine, 5) Berberine</b>	Alkaloids	[40]

1 2 3	<i>Corydalis yanhusuo</i> W. T. Wang (Papaveraceae)	Rhizomes	Ultrasonication – EtOH 70%	Miscellaneous (MAGL)	<b>1)</b> Tetrahydropalmatin, <b>2)</b> Tetrahydrocoptisine, <b>3)</b> Coptisine, <b>4)</b> Fumaricine, <b>5)</b> Palmatine, <b>6)</b> Berberine and <b>7)</b> Dehydrocorydaline	Alkaloids	[178]
4 5 6	<i>Curcuma longa</i> L. (Zingiberaceae)	Rhizomes	Maceration and ultrasonication – MeOH	Miscellaneous (PC3-cells)	<b>1)</b> Curcumin, <b>2)</b> Demethoxycurcumin and <b>3)</b> Bisdemethoxycurcumin	Phenolic pigments	[20]
7 8 9 10	<i>Curcuma longa</i> L. (Zingiberaceae)	Rhizomes	Percolation – EtOH 95%	Miscellaneous (Hsp 90 $\alpha$ )	<b>1)</b> Zingiberenol, <b>2)</b> Ar-turmerone, <b>3)</b> Curlone, <b>4)</b> (6R,7R)-bisabolone, <b>5)</b> (E)-Atlantone, <b>6)</b> 6-(1-Hydroxymethylvinyl), <b>7)</b> 4,8a-dimethyl-3,5,6,7,8,8a-hexahydro-1H-naphthalen-2-one	Terpenoids	[44]
11 12 13 14	<i>Curcuma longa</i> L. (Zingiberaceae)	Rhizomes	Ultrasonication - MeOH	Hollow fiber (HepG-2, SKOV-3, and ACHN cells)	<b>1)</b> Bisdemethoxycurcumin, <b>2)</b> Demethoxycurcumin and <b>3)</b> Curcumin	Phenolic pigments	[195]
15 16 17 18 19	<i>Cynanchum otophyllum</i> C.K.Schneid. (Apocynaceae)	Undefined	Ultrasonication – MeOH	Magnetic particle (EGFR/HEK293 cells)	-	-	[197]
20 21 22	<i>Eucommia ulmoides</i> Oliv. (Eucommiaceae)	Undefined	Ultrasonication – EtOH 70%	Miscellaneous (MAGL)	-	-	[178]
23 24 25 26	<i>Gardenia jasminoides</i> J. Ellis (Rubiaceae)	Undefined	Decoction – H <sub>2</sub> O	Hollow fiber (HepG-2 and ACHN cells)	<b>1)</b> Chlorogenic acid, <b>2)</b> Geniposide, <b>3)</b> Crocin	Phenolic acid, glycoside and carotenoid	[196]
27 28 29 30 31 32 33 34 35 36 37	<i>Gastrodia elata</i> Blume. (Orchidiaceae)	Undefined	-	Ultrafiltration (Tyrosinase)	<b>1)</b> 12-15 Heneicosadienoic acid, Octadecanoic acid, 2- hydroxylpropyl esters, <b>2)</b> Unknown, <b>3)</b> 3-(4-tert-butylphenyl)-2-methylpropanal, <b>4)</b> 4,4'-Dihydroxybenzylsulfid, <b>5)</b> Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester <b>6)</b> 9-Octadecenamamide, (trans-), <b>7)</b> Unknown, <b>8)</b> Unknown, <b>9)</b> 9-Octadecenamamide, (cis-), <b>10)</b> 4,4'-Dihydroxydiphenyl ether, <b>11)</b> 4-(1-(4-hydroxyphenyl)phenyl)phenol, <b>12)</b> Octadecanoic acid, 2,3-dihydroxypropyl ester, <b>13)</b> 4,4'-Dimethoxydiphenylmethane, <b>14)</b> 4,4'-Methylenediphenol, <b>15)</b> 2,4-Bis(4-hydroxybenzyl) phenol, <b>16)</b> 4-Hydroxybenzyl methyl ether	Fatty acids, amides, polyphenols and purines	[90]
38 39 40 41 42	<i>Ginkgo biloba</i> L. (Ginkgoaceae)	Undefined	Ultrasonication – EtOH 70%	Miscellaneous (MAGL)	-	-	[178]



1 2 3 4 5 6	<i>Glycyrrhiza uralensis</i> Fisch. (Fabaceae)	Roots	Percolation – EtOH 95%; Liquid-liquid partition – H <sub>2</sub> O- Petroleum ether- EtOAc-n-BuOH	Magnetic particle (Tyrosinase)	<b>1)</b> Liquiritin apioside, <b>2)</b> Neolicucuroside, <b>3)</b> Liquiritigenin, <b>4)</b> Licorice saponin, <b>5)</b> Chrysoeriol, <b>6)</b> Dihydrodaidzein, <b>7)</b> Formononetin, <b>8)</b> Glycyrrhisoflavanone, <b>9)</b> Glycyrrhizic acid, <b>10)</b> Glycycoumarin, <b>11)</b> Pratensein	Flavonoids, saponin and coumarin	[18]
7 8 9 10 11 12	<i>Glycyrrhiza uralensis</i> Fisch. (Fabaceae)	Roots	Infusion – H <sub>2</sub> O:Honey (85%)	Magnetic particle (Tyrosinase)	<b>1)</b> Gallic acid, <b>2)</b> Albiflorin, <b>3)</b> Paeoniflorin, <b>4)</b> Liquiritin apioside, <b>5)</b> Liquiritin, <b>6)</b> Galloylpaeoniflorin, <b>7)</b> Ononin, <b>8)</b> Isoliquiritigenin, <b>9)</b> Glycyrrhizic acid, <b>10)</b> Oxypaeoniflora, <b>11)</b> Benzoylpaeoniflorin, <b>12)</b> Benzoyloxypaeoniflorin, <b>13)</b> Mudanpioside C, <b>14)</b> Paeonolide and <b>15)</b> Apiopaeonoside	Phenolic acid, terpenoid saponins, and glycosides	[95]
13 14 15 16	<i>Humulus lupulus</i> L. (Cannabaceae)	Flowers	Liquid-liquid extraction - MeOH-CHCl <sub>3</sub>	Ultrafiltration (QR-2)	<b>1)</b> Xanthohumol and <b>2)</b> Xanthohumol D	Flavonoids	[82]
17 18 19 20	<i>Ligusticum chuanxiong</i> Hort. (Apiaceae)	-	Ultrasonication – EtOH 70%	Miscellaneous (MAGL)	-	-	[178]
21 22 23 24 25	<i>Ligustrum lucidum</i> W.T.Aiton (Oleaceae)	Fruits	Ultrasonication – MeOH	Magnetic particle (EGFR/HEK293 cells)	-	-	[197]
26 27 28 29 30 31	<i>Lithospermum erythrorhizon</i> Siebold & Zucc. (Boraginaceae)	Rhizomes	Reflux- Deionized H <sub>2</sub> O	Miscellaneous (A549 and H1819 cells)	<b>1)</b> Salvianolic acid B, <b>2)</b> Deoxyshikonin and <b>3)</b> Shikonin	Phenolic acid and quinones	[48]
32 33 34 35	<i>Macleaya cordata</i> (Willd.) R. Br. (Papaveraceae)	Seeds	Ultrasonication – EtOH (85%, pH 2)	Miscellaneous (DNA)	<b>1)</b> Sanguinarine, <b>2)</b> Chelerythrine, <b>3)</b> Protopine and <b>4)</b> Allocryptopine	Alkaloids	[187]
36 37 38 39 40 41	<i>Macleaya cordata</i> (Willd.) R.Br. (Papaveraceae)	Seeds	Ultrasonication – MeOH (85%, pH 2)	Ultrafiltration (DNA)	<b>1)</b> Chelerythrine, <b>2)</b> Sanguinarine, <b>3)</b> Protopine and <b>4)</b> Allocryptopine	Alkaloids	[188]

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25	<i>Mangifera indica</i> L. (Anacardiaceae)	Leaves	Ultrasonication – EtOH (75%)- EtOAc- <i>n</i> -BuOH	Ultrafiltration (Tyrosinase)	<b>1)</b> Gallic acid, <b>2)</b> Iriflophenone 3-C-glucoside, <b>3)</b> Mangiferin, <b>4)</b> Protocatechuic acid, <b>5)</b> Iriflophenone 3-C-(2'-O-galloyl)-glucoside, <b>6)</b> 6'-O-Galloyl-mangiferin, <b>7)</b> Maclurin 3-C-(2'-O-p-hydroxybenzoyl)-glucoside, <b>8)</b> Iriflophenone 3-C-(2',6'-di-O-galloyl)-glucoside, <b>9)</b> Hyperoside, <b>10)</b> Isoquercitrin, <b>11)</b> Ethyl gallate, <b>12)</b> Iriflophenone 3-C-(2'-O-p-hydroxybenzoyl)-glucoside, <b>13)</b> Quercetin-3-O-xyloside, <b>14)</b> 3-O-Galloyl shikimic acid, <b>15)</b> 3-O-Galloyl quinic acid, <b>16)</b> Maclurin 3-C-(2'-O-galloyl)-glucoside, <b>17)</b> 3,5-Di-O-galloyl quinic acid, <b>18)</b> 5-O-Digalloyl quinic acid, <b>19)</b> Digallic acid, <b>20)</b> Isomangiferin, <b>21)</b> 1,4,6-Tri-O-galloyl glucoside, <b>22)</b> Maclurin 3-C-(2',3'-di-O-galloyl)-glucoside, <b>23)</b> 1,3-Digalleoyl acetone, <b>24)</b> 1,3,4,6-Tetra-O-galloyl glucoside, <b>25)</b> 6'-O-(p-Hydroxybenzoyl) mangiferin, <b>26)</b> Epicatechin gallate, <b>27)</b> 1,2,3,4,6-Penta-O-galloyl glucoside, <b>28)</b> Iriflophenone 3-C-(2',3',6'-tri-O-galloyl)-glucoside, <b>29)</b> Luteolin-7-O-glucoside, <b>30)</b> Quercetin-3-O-arapyranoside, <b>31)</b> Quercetin-3-O-arafuranoside, <b>32)</b> Kaempferol-3-O-glucoside, <b>33)</b> Quercetin-3-O-rhamnoside, <b>34)</b> Ethyl 2,4-dihydroxy-3-galloyl oxybenzoate, <b>35)</b> Ethyl digallate, <b>36)</b> 7-O-Methyl quercetin-3-O-rhamnoside	Phenolic acids, xanthenes, benzophenones, flavonoids, quinic and shikimic acid derivatives	[19]
26 27 28 29 30	<i>Menispermum dauricum</i> DC. (Menispermaceae)	Undefined	Solid-liquid extraction – MeOH; Liquid-liquid partition – EtOAc-H <sub>2</sub> O	Ultrafiltration (DNA)	<b>1)</b> Daurisoline and <b>2)</b> Dauricine	Alkaloids	[12]
31 32 33 34 35 36 37 38	<i>Morus alba</i> L. (Moraceae)	Leaves	Solid-liquid extraction – EtOH 50%; Liquid-liquid partition – H <sub>2</sub> O-petroleum ether-EtOAc	Ultrafiltration (Tyrosinase)	<b>1)</b> Neochlorogenic acid, <b>2)</b> Chlorogenic acid, <b>3)</b> Cryptochlorogenic acid, <b>4)</b> Rutin, <b>5)</b> Isoquercitrin, <b>6)</b> Kaempferol-3-O- $\alpha$ -L-rhamnopyranosyl-(1–6)- $\beta$ -D-glucopyranoside, <b>7)</b> Quercetin-3-O-(6-O-malonyl)- $\beta$ -D-glucopyranoside, <b>8)</b> 3,4-Dicaffeoylquinic acid, <b>9)</b> Astragalinal, <b>10)</b> 3,5-Dicaffeoylquinic acid, <b>11)</b> 4,5-Dicaffeoylquinic acid, <b>12)</b> Kaempferol-3-O-(6-O-malonyl)- $\beta$ -D-glucopyranoside	Phenolic acids and flavonoids	[89]
39 40 41 42	<i>Morus alba</i> L. (Moraceae)	Fruits	Ultrasonication – MeOH	Magnetic particle (EGFR/HEK293 cells)	-	-	[197]

<i>Oroxylum indicum</i> L. (Bignoniaceae)	Seeds	Ultrasonication – MeOH 50%	Miscellaneous (Tyrosinase)	<b>1) Oroxin B, 2) Kaempferid-3-O-beta-D-gentiobioside, 3) Chrysin-7-O-beta-D-gentiobioside, 4) Oroxin A, 5) Baicalein and 6) Chrysin</b>	Flavonoids	[94]
<i>Otholobium pubescens</i> (Poir.) J.W. Grimes (Fabaceae)	Leaves and flowers	Percolation – CH <sub>2</sub> Cl <sub>2</sub> -MeOH- MeOH: H <sub>2</sub> O (1:1)-H <sub>2</sub> O	Miscellaneous (Tyrosinase)	<b>1) Daidzin, 2) Isoorientin, 3) Isovitexin, 4) Daidzein, 5) Genistein, 6) 3-(5-hydroxybenzofuran-6-yl) propanoic acid</b>	Flavonoids	[17]
<i>Paeonia lactiflora</i> Pall (Paeoniaceae)	Roots	Infusion – H <sub>2</sub> O	Magnetic particle (Tyrosinase)	<b>1) Gallic acid, 2) Albiflorin, 3) Paeoniflorin, 4) Liquiritin apioside, 5) Liquiritin, 6) Galloylpaeoniflorin, 7) Ononin, 8) Isoliquiritigenin, 9) Glycyrrhizic acid, 10) Oxypaeoniflora, 11) Benzoylpaeoniflorin, 12) Benzoyloxypaeoniflorin, 13) Mudanpioside C, 14) Paeonolide and 15) Apiopaeonoside</b>	Phenolic acid, terpenoids saponins, and glycosides	[95]
<i>Paris polyphylla</i> Sm. (Melanthiaceae)	Undefined	Ultrasonication – EtOH 70%	Miscellaneous (MAGL)	-	-	[178]
<i>Perilla frutescens</i> (L.) Britt (Lamiaceae)	Leaves	Ultrasonication – MeOH:H <sub>2</sub> O (7:3)	Magnetic particle (GST)	<b>1) Caffeic acid, 2) Rosmarinic acid, 3) Gallic acid, 4) (-)-Epigallocatechin-3-O-gallate, 5) (-)-Epicatechin-3-gallate and 6) Quercetin-3-O-glucoside</b>	Phenolic acids and flavonoids	[28]
<i>Phellodendron chinense</i> C.K.Schneid. (Ranunculaceae)	Cortex	Ultrasonication – EtOH 75%	Miscellaneous (DNA)	<b>1) Epiberberine, 2) Coptisine, 3) Jatrorrhizine, 4) Berberrubine and 5) Columbamine</b>	Alkaloids	[49]
<i>Phellodendron chinense</i> C.K.Schneid. (Rutaceae)	Barks	Ultrasonication – EtOH 75%	Miscellaneous (DNA)	<b>1) Berberine and 2) Palmatine</b>	Alkaloids	[47]
<i>Phellodendron chinense</i> C.K.Schneid. (Rutaceae)	Cortex	Percolation – EtOH 95%	Ultrafiltration (COX2)	<b>1) Neomangiferin, 2) Phellodendrine, 3) Mangiferin, 4) Magnoflorine, 5) 3-O-feruloylquinnic acid, 6) (-)-Oblongine, 7) Menisperine, 8) Demethyleneberberine, 9) Oxoberberine, 10) Berberrubine, 11) Jatrorrhizine, 12) Timosaponin B, 13) Timosaponin B-II, 14) Timosaponin A-I, 15) Palmatine, 16) Berberine, 17) Sanleng acid, 18) Timosaponin A-III, 19) Anemarrhenasaponin I, 20) cis-Hinokiresinol</b>	Alkaloids, xanthenes and saponins	[105]

1	<i>Phellodendron chinense</i> Schneid. (Rutaceae)	Barks	Infusion – EtOH:H <sub>2</sub> O (1:3)	Dialysis (DNA)	1) Berberine	Alkaloid	[185]
2							
3							
4	<i>Polygonum cuspidatum</i> Siebold & Zucc. (Polygonaceae)	Undefined	Ultrasonication – EtOH 70%	Magnetic particle (LDH)	1) (-)-Epicatechin-3-O-gallate, 2) Trihydroxystilbene-3-O-(6''-galloyl)-glucoside, 3) Emodin-1-O-glucoside, 4) Emodin-8-O-(6-malonyl)-glucoside, 5) Torachryson sulfate, 6) Emodin	Flavonoid, stilbenoid, naphthol and anthraquinones	[119]
5							
6							
7							
8							
9	<i>Polygonum cuspidatum</i> Siebold & Zucc. (Polygonaceae)	Rhizomes	Reflux – CHCl <sub>3</sub> :H <sub>2</sub> SO <sub>4</sub> (5:4)	Hollow fiber (HCT116 cells)	1) Aloe-emodin, 2) Emodin and 3) Physcion	Anthraquinones	[194]
10							
11							
12							
13							
14	<i>Polygonum multiflorum</i> (Thunb.) Moldenke (Polygonaceae)	Rhizomes	Reflux – MeOH; Ultrasound – HCl 8%; Liquid-liquid – HCl-CHCl <sub>3</sub>	Hollow fiber (HCT116 cells)	1) Aloe-emodin, 2) Emodin, 3) Physcion, 4) Rhein and 5) Chrysophanol	Anthraquinones	[194]
15							
16							
17							
18							
19	<i>Pseudopithomyces sp.</i> (Didymosphaeriaceae)	Whole	Solid-liquid extraction – MeOH-EtOAc	Magnetic particle (PLA2)	1) Cyclo-(4-hydroxyl-Pro-Leu), 2) Cyclo-(Pro-Val), 3) Fusaristatin C, 4) Ergosterol peroxide, 5) Ergosterol and 6) Cerevisterol	Cyclic peptides and Steroids	[115]
20							
21							
22							
23	<i>Psoralea corylifolia</i> L. (Fabaceae)	Fruits	Reflux – MeOH	Hollow fiber (SGC7901, RAW264.7 and SHSY5Y cells)	1) Psoralen and 2) Isopsoralen	Coumarins	[41]
24							
25							
26							
27	<i>Pterocephalus hookeri</i> (C.B. Clarke) Diels (Caprifoliaceae)	Undefined	Percolation – EtOH 95%	Miscellaneous (COX2)	1) Swertisin, 2) Scopoletin	Flavonoid and coumarin	[110]
28							
29							
30							
31	<i>Pterocephalus hookeri</i> (C.B. Clarke) Diels (Caprifoliaceae)	Whole	Percolation – EtOH 95%	Miscellaneous (COX2)	1) Sylvestroside I	Terpenoid	[108]
32							
33							
34							
35	<i>Pueraria lobata</i> (Willd.) Ohwi (Fabaceae)	Undefined	Reflux – EtOH 75%	Hollow fiber (Tyrosinase)	1) Daidzein, 2) 3'-Methoxy puerarin, 3) 3'-Hydroxy puerarin 4) Puerarin, 5) Puerarin 6''-O-xyloside 6) Puerarin-4'-O-glucoside, 7) Puerarin aposide	Flavonoids	[42]
36							
37							
38							
39	<i>Pueraria lobata</i> (Willd.) Ohwi (Fabaceae)	Roots	Ultrasonication – MeOH 75%	Ultrafiltration (Tyrosinase)	1) Puerarin, 2) Mirificin, 3) Daidzin and 4) Genistin	Flavonoids	[91]
40							
41							
42							
43							
44							
45							
46							

<i>Pueraria lobata</i> (Willd.) Ohwi (Fabaceae)	Roots	Reflux – EtOH:H <sub>2</sub> O (75%); Liquid- liquid extraction – petroleum ether-EtOAc	Ultrafiltration (Tyrosinase)	<b>1) 3'-Hydroxypuerarin, 2) Puerarin, 3) Puerarin-6"-O-xyloside, 4) Daidzin, 5) Genistin, 6) 6"-O-acetyldaidzin and 7) Daidzein</b>	Flavonoids	[93]
<i>Radix et Rhizoma Rhei</i> (Polygonaceae)	Roots	Decoction – H <sub>2</sub> O	Hollow fiber (HepG-2 and ACHN cells)	<b>1) Rhein</b>	Anthraquinone	[196]
<i>Rheum palmatum</i> L. (Polygonaceae)	Undefined	Ultrasonication – EtOH 70%	Miscellaneous (MAGL)	-	-	[178]
<i>Rheum rhabarbarum</i> L. (Polygonaceae)	Undefined	Ultrasonication – EtOH 70%	Magnetic particle (LDH)	<b>1) (-)-Epicatechin-3-O-gallate, 2) ω-Hydroxyemodin, 3) Emodin-1-O-glucoside, 4) Lunatin, 5) Chrysophanic acid, 6) Rhein-O-(acetyl)-glucoside, 7) Aloe emodin, 8) Rhein and 9) Emodin</b>	Flavonoids and anthraquinones	[119]
<i>Rhus chinensis</i> Mill. (Anacardiaceae)	Undefined	Ultrasonication – MeOH 75%	Miscellaneous (uPA)	<b>1) Gallic acid, 2) Methyl gallate, 3) Pentagalloylglucose, 4) Ellagic acid, 5) Ziyu-glycoside I, 6) 28-O-β-D-glucopyranosyl pomolic acid, and 7) 1β-Hydroxyeuscapic acid</b>	Phenolic acid, tannins, saponins and terpenoid	[165]
<i>Rosa roxburghii</i> Tratt. (Rosaceae)	Fruits	Ultrasonication – EtOH 70%	Miscellaneous (MMP-2)	<b>1) Oreganol A, 2) Corilagin, 3) Ellagic acid, 4) Zebirioside O, 5) Niga-ichigoside F1, 6) Peduncloside, 7) Rosamultin, 8) Kajiichigoside F1, 9) Potentillanoside A, 10) Quadranoside VIII, 11) Valerenic acid and 12) Medicagenic acid</b>	Phenolic compound, tannin, phenolic acid, saponin and terpenoids	[171]
<i>Salvia miltiorrhiza</i> Bge. (Lamiaceae)	Undefined	Ultrasonication – EtOH 70%	Miscellaneous (MAGL)	-	-	[178]
<i>Sanguisorba officinalis</i> L. (Rosaceae)	Undefined	Ultrasonication – MeOH 75%	Miscellaneous (uPA)	<b>1) Gallic acid, 2) Methyl gallate, 3) Pentagalloylglucose, 4) Ellagic acid, 5) Ziyu-glycoside I, 6) 28-O-β-D-glucopyranosyl pomolic acid, and 7) 1β-Hydroxyeuscapic acid</b>	Phenolic acid, tannins, saponins and terpenoid	[165]
<i>Saussurea laniceps</i> Hand.-Mazz. (Asteraceae)	Aerial parts	Reflux – H <sub>2</sub> O	Magnetic particle (COX2)	<b>1) Skimmin, 2) Scopolin, 3) Umbelliferone, 4) Scopoletin</b>	Coumarins	[109]
<i>Schisandra chinensis</i> (Turcz)	Undefined	Ultrasonication - MeOH	Hollow fiber	<b>1) Schisandrol A, 2) Schizandrin A, 3) Schisantherin A and 4) Schizandrin C</b>	Lignans and tannin	[41]



(Schisandraceae)			(SGC7901, RAW264.7, SHSY5Y cells)			
<i>Scutellaria baicalensis</i> Georgi (Lamiaceae)	Undefined	Ultrasonication – EtOH 70%	Magnetic particle (Tyrosinase)	<b>1) Baicalin, 2) Baicalein, 3) Wogonin and 4) Oroxylin A</b>	Flavonoids	[26]
<i>Scutellaria barbata</i> D. Don (Lamiaceae)	Undefined	Ultrasonication – MeOH	Magnetic particle (EGFR/HEK293 cells)	-	-	[197]
<i>Selaginella doederleinii</i> Hieron. (Selaginellaceae)	Undefined	Reflux – EtOH 70% and EtAc	Magnetic particle (LDH)	<b>1) Amentoflavone and 2) Robustafavone</b>	Flavonoids	[25]
<i>Smilax china</i> L. (Smilacaceae)	Rhizomes	Infusion – H <sub>2</sub> O	Magnetic particle (Tyrosinase)	<b>1) Gallic acid, 2) Albiflorin, 3) Paeoniflorin, 4) Liquiritin apioside, 5) Liquiritin, 6) Galloylpaeoniflorin, 7) Ononin, 8) Isoliquiritigenin, 9) Glycyrrhizic acid, 10) Oxypaeoniflora, 11) Benzoylpaeoniflorin, 12) Benzoyloxypaeoniflorin, 13) Mudanpioside C, 14) Paeonolide and 15) Apiopaeonoside</b>	Phenolic acid, terpenoids saponins, and glycosides	[95]
<i>Sophora flavescens</i> Aiton (Fabaceae)	Roots	Infusion – H <sub>2</sub> O	Hollow fiber (MCF-7, ACHN, SKOV-3, and HepG-2 cells)	<b>1) Oxymatrine, 2) Matrine, 3) Sophoridine and 4) Oxysophocarpine</b>	Alkaloids	[191]
<i>Sophora flavescens</i> Aiton (Fabaceae)	Undefined	Ultrasonication – EtOH 70%	Magnetic particle (Tyrosinase)	<b>1) Kurarinone and 2) Sophoraflavanone G</b>	Flavonoids	[26]
<i>Stephania tetrandra</i> S. Moore (Menispermaceae)	Undefined	Solid-liquid extraction – MeOH; Liquid-liquid partition – EtOAc-H <sub>2</sub> O	Ultrafiltration (DNA)	<b>1) Fangchinoline and 2) Tetrandrine</b>	Alkaloids	[12]
<i>Stephania tetrandra</i> S. Moore (Menispermaceae)	Undefined	Ultrasonication – EtOH 75%	Miscellaneous (DNA)	<b>1) Fangchinoline and 2) Tetrandrine</b>	Alkaloids	[50]
<i>Streptomyces antibioticus</i> (Streptomycetaceae)	Whole	Liquid-liquid extraction - Medium-EtOAc	Ultrafiltration (DNA)	<b>1) Actinomycin D, 2) Actinomycin V and 3) Actinomycin X0<math>\beta</math></b>	Actinomycins	[12]
<i>Strychnos nux-vomica</i> L. (Loganiaceae)	Undefined	Reflux – EtOH (60%, v/v)	Magnetic particle (EGFR/A549 cells)	<b>1) 10,11-dimethoxystrychnine and 2) Loganetin</b>	Alkaloid and terpenoid	[35]

1 2 3 4	<i>Taraxacum mongolicum</i> Hand.-Mazz (Asteraceae)	Undefined	Ultrasonication – MeOH	Magnetic particle (EGFR/HEK293 cells)	1) Luteolin and 2) Caffeic acid	Flavonoid and phenolic acid	[197]
5 6 7	<i>Trigonella foenum-graecum</i> L. (Fabaceae)	Seeds	Maceration – EtOH 72%	Magnetic particle (SIRT6)	1) Orientin	Flavonoid	[39]
8 9 10	<i>Trigonella foenum-graecum</i> L. (Fabaceae)	Seeds	-	Magnetic particle (SIRT6)	1) Quercetin and 2) Vitexin	Flavonoids	[38]
11 12 13 14	<i>Tripterygium wilfordii</i> Hook. f. (Celastraceae)	Undefined	Reflux – EtOH	Miscellaneous (MCF-7 cells)	1) Genistein, 2) Tripteryl E-3, 3) Wilforlide A, 4) Cangononine, 5) Wilforine, 6) Regeol C, 7) Celastrol	Flavonoid, terpenoids and alkaloid	[51]
15 16 17 18 19 20 21	<i>Tripterygium wilfordii</i> Hook. f. (Celastraceae)	Rhizomes	Percolation and ultrasonication – EtOH 95%	Miscellaneous (Hsp 90)	1) Demecolcine, 2) Wilforine, 3) Triptotriterpenic acid, 4) Celastrol, 5) R1-Barrigenol, 6) 24R,25-dihydroxycholecalciferol, 7) Ursodeoxycholic acid, 8) 8,14-Seco-3,19-epoxyandrostane-8,14-dione, 17-acetoxy-3 $\beta$ -methoxy-4,4-dimethyl, 9) Spirost-8-en-11-one, 10) Propanoic acid, 11) Demecolcine, 12) Bufa-20,22-dienolide, 13) Ergosta-5,22-dien-3-ol, 14) $\beta$ -Sitosterol and 15) Betulinaldehyde	Alkaloids, terpenoids and steroids	[46]
22 23 24	<i>Vaccinium corymbosum</i> L. (Ericaceae)	Leaves	Reflux – Petroleum ether-EtOH 70%	Miscellaneous (HepG2 cells)	1) Chlorogenic acid, 2) Rutin, 3) Isoquercitrin, 4) Cyanidin-3-O-glucose, 5) Iridin and 6) Quercetin	Phenolic acid and flavonoids	[192]
25 26 27 28	<i>Xanthium strumarium</i> L. (Asteraceae)	Fruits	Reflux – CH <sub>2</sub> Cl <sub>2</sub> -MeOH	Ultrafiltration (Tyrosinase)	1) Protocatechuic acid, 2) 3,5- Di-O-caffeoylquinic acid, 3) 1,5-Di-O-caffeoylquinic acid and 4) Chlorogenic acid	Phenolic acids	[92]
29 30 31 32	<i>Zingiber officinale</i> Roscoe. (Zingiberaceae)	Rhizomes	Vortexing - EtOH	Magnetic particle ( $\alpha$ V $\beta$ 3 integrin)	1) [10]-gingerol	Phenolic compound	[148]

**Table S1** – Natural products screened through ligand fishing approaches sorted by species in alphabetical order. Complementary information regarding the family, the parts used, the type of extraction, the extraction solvent, the ligand fishing technique with the respective cancer-related target, the ligands fished with their chemical classification as well as the bibliographical reference is also provided.

Ligand Fishing Technique	Ligand Fishing Medium	Target	Type of Cancer	Ligands Fished	Ref
<b>Dialysis</b>	Ultrapure water	DNA (G-quadruplex)	Various	<b>1) Berberine</b>	[185]
<b>Hollow Fiber</b>	Phosphate buffer	HCT116 cells	Colorectal cancer	<b>1) Aloe-emodin, 2) Emodin, 3) Physcion, 4) Rhein and 5) Chrysophanol</b>	[194]
	CMC-Na solution 5%	HepG-2 cells	Hepatoma	<b>1) Xanthotoxol, 2) Xanthotoxin, 3) Psoralen, 4) Bergapten, 5) Oxypeucedanin, 6) Imperatorin, 7) Isoimperatorin.</b>	[43]
	MeOH 5%	HepG-2, SKOV-3, and ACHN cells	Hepatoma, human ovarian cancer, and human renal carcinoma	<b>1) Bisdemethoxycurcumin, 2) Demethoxycurcumin and 3) Curcumin</b>	[195]
	Double-distilled water	MCF-7, ACHN, SKOV-3, and HepG-2 cells	Breast cancer, human renal carcinoma, human ovarian cancer, and hepatoma	<b>1) Oxymatrine, 2) Matrine, 3) Sophoridine and 4) Oxysophocarpine</b>	[191]
	Phosphate buffer	SGC7901, RAW264.7, SHSY5Y cells	Gastric cancer, neuroblastoma and leukemia	<b>1) Psoralen, 2) Bergapten, 3) Imperatorin 4) Osthole 5) Isopsoralen 6) Schisandrol A, 7) Schizandrin A, 8) Schisantherin A and 9) Schizandrin C</b>	[41]
	Phosphate buffer	Tyrosinase	Melanoma	<b>1) Daidzein, 2) 3'-Methoxy puerarin, 3) 3'-Hydroxy puerarin 4) Puerarin, 5) Puerarin 6''-O-xyloside 6) Puerarin-4'-O-glucoside, 7) Puerarin aposide</b>	[42]
	Phosphate buffer	HepG-2 and ACHN cells	Hepatoma and human renal carcinoma	<b>1) Chlorogenic acid, 2) p-Hydroxyacetophenone 3) Geniposide, 4) Crocin 5) Rhein</b>	[196]
Double-distilled water	MCF7, MADB106, and SGC7901 cells	Breast cancer and gastric cancer	<b>1) Jatrorrhizine, 2) Epiberine, 3) Coptisine, 4) Palmatine, 5) Berberine</b>	[40]	

<b>Magnetic particles</b>	Phosphate buffer	EGFR/A549 cells	Lung carcinoma	<b>1)</b> 10,11-dimethoxystrychnine and <b>2)</b> Loganetin	[35]
	Phosphate buffer	Cyclooxygenase-2 (COX2)	Various	<b>1)</b> (-)-gallocatechin, <b>2)</b> (-)-epigallocatechin, <b>3)</b> (-)-epicatechin, <b>4)</b> (-)-epigallocatechin-3-gallate, <b>5)</b> (-)-gallocatechin-3-gallate, <b>6)</b> (-)-epigallocatechin-3-(3''-O-methyl)-gallate, <b>7)</b> (-)-epicatechin-3-gallate, <b>8)</b> (-)-epicatechin-3-(3''-O-methyl)-gallate	[106]
	Tris-HCl buffer	Cyclooxygenase-2 (COX2)	Various	<b>1)</b> Skimmin, <b>2)</b> Scopolin, <b>3)</b> Umbelliferone, <b>4)</b> Scopoletin	[109]
	Tris-HCl buffer	Cyclooxygenase-2 (COX2)	Various	<b>1)</b> 3-Galloylquinic acid, <b>2)</b> 5-Galloylquinic acid, <b>3)</b> 3-p-Coumaroylquinic acid, <b>4)</b> 5-p-Coumaroylquinic acid, <b>5)</b> 5-caffeoylquinic acid, <b>6)</b> (-)-Epicatechin, <b>7)</b> (-)-Epicatechin 3-O-gallate, <b>8)</b> Quercetin 3-O-rhamnosylgalactoside, <b>9)</b> Quercetin 3-O-glucoside, <b>10)</b> Kaempferol 3-O-glucosylrutinoside, <b>11)</b> Kaempferol-3-O-rutinoside, <b>12)</b> Kaempferol 3-O-glucoside	[107]
	Phosphate buffer	DNA (ds)	Various	<b>1)</b> Columbamine, <b>2)</b> Epiberberine, <b>3)</b> Jatrorrhizine, <b>4)</b> Palmatine and <b>5)</b> Berberine	[186]
	Distilled water	EGFR/HEK293 cells	Glioma	<b>1)</b> Luteolin and <b>2)</b> Caffeic acid	[197]
	Phosphate buffer	Glutathione S-transferase (GST)	Various (multidrug resistance)	<b>1)</b> Caffeic acid, <b>2)</b> Rosmarinic acid, <b>3)</b> Gallic acid, <b>4)</b> (-)-Epigallocatechin-3-O-gallate, <b>5)</b> (-)-Epicatechin-3-gallate and <b>6)</b> Quercetin-3-O-glucoside	[28]
	Acetate buffer	Lactate dehydrogenase (LDH)	Various	<b>1)</b> (-)-Epicatechin-3-O-gallate, <b>2)</b> Trihydroxystilbene-3-O-(6''-galloyl)-glucoside, <b>3)</b> Emodin-1-O-glucoside, <b>4)</b> Emodin-8-O-(6-malonyl)-glucoside, <b>5)</b> Torachryson sulfate, <b>6)</b> Emodin, <b>7)</b> ω-Hydroxyemodin, <b>8)</b> Lunatin, <b>9)</b> Chrysophanic acid, <b>10)</b> Rhein-O-(acetyl)-glucoside, <b>11)</b> Aloe emodin, <b>12)</b> Rhein	[119]

1	Tris-HCl buffer	Phospholipase A2 (PLA2)	Various	<b>1) Cyclo-(4-hydroxyl-Pro-Leu), 2) Cyclo-(Pro-Val), 3) Fusaristatin C, 4) Ergosterol peroxide, 5) Ergosterol and 6) Cerevisterol</b>	[115]
2	Ammonium acetate buffer	Sirtuin 6 (SIRT6)	Various	<b>1) Orientin</b>	[39]
3	Phosphate buffer	Sirtuin 6 (SIRT6)	Various	<b>1) Quercetin and 2) Vitexin</b>	[38]
4	Tris-HCl buffer	Tropomyosin receptor kinase B (TrkB)	Various	<b>1) Dicafeoylquinic acid, 2) O-cafeoylquinic acid, 3) Madecassic acid, 4) Castillicetin, 5) Quercetin, 6) Stigmasterol, 7) Pomolic acid and 8) Naringin</b>	[32]
5	Phosphate buffer	Tyrosinase	Melanoma	<b>1) Liquiritin apioside, 2) Neolicuroside, 3) Liquiritigenin, 4) Licorice saponin, 5) Chrysoeriol, 6) Dihydrodiadzein, 7) Formononetin, 8) Glycyrrhisoflavanone, 9) Glycyrrhizic acid, 10) Glycycomarin and 11) Pratensein</b>	[18]
6	Phosphate buffer	Tyrosinase	Melanoma	<b>1) Gallic acid, 2) Albiflorin, 3) Paeoniflorin, 4) Liquiritin apioside, 5) Liquiritin, 6) Galloylpaeoniflorin, 7) Ononin, 8) Isoliquiritigenin, 9) Glycyrrhizic acid, 10) Oxypaeoniflora, 11) Benzoylpaeoniflorin, 12) Benzoyloxypaeoniflorin, 13) Mudanpioside C, 14) Paeonolide and 15) Apiopaeonoside</b>	[95]
7	Phosphate buffer	$\alpha$ V $\beta$ 3 integrin	Various	<b>1) [10]-gingerol</b>	[148]



	Acetate buffer	Lactate dehydrogenase (LDH)	Various	<b>1) Amentoflavone and 2) Robustaflavone</b>	[25]
	Tris-HCl buffer	Tyrosinase	Melanoma	<b>1) Baicalin, 2) Baicalein, 3) Wogonin, 4) Oroxylin A, 5) Kurarinone, 6) Sophoraflavanone G</b>	[26]
<b>Ultrafiltration</b>	Phosphate buffer	Cyclooxygenase-2 (COX2)	Various	<b>1) Neomangiferin, 2) Phellodendrine, 3) Mangiferin, 4) Magnoflorine, 5) 3-O-feruloylquinnic acid, 6) (-)-Oblongine, 7) Menisperine, 8) Demethyleneberberine, 9) Oxoberberine, 10) Berberrubine, 11) Jatrorrhizine, 12) Timosaponin B, 13) Timosaponin B-II, 14) Timosaponin A-I, 15) Palmatine, 16) Berberine, 17) Sanleng acid, 18) Timosaponin A-III, 19) Anemarrhenasaponin I, 20) cis-Hinokiresinol</b>	[105]
	Distilled water, MeOH or MeOH-distilled water	DNA	Various	<b>1) Berberine, 2) Coptisine, 3) Palmitine 4) Daurisoline, 5) Dauricine, 6) Fangchinoline, 7) Tetrandrine, 8) Actinomycin D, 9) Actinomycin V and 10) Actinomycin X0<math>\beta</math></b>	[12]
	Buffer (50 mM KCl, 10 mM KH <sub>2</sub> PO <sub>4</sub> , and 1 mM K <sub>2</sub> EDTA, pH 7.4)	DNA (G-quadruplex)	Various	<b>1) Chelerythrine, 2) Sanguinarine, 3) Protopine and 4) Allocryptopine</b>	[188]
	Tris-HCl buffer	Quinone reductase-2 (QR-2)	Various (chemoprevention)	<b>1) Tetrangulol methyl ether, 2) Xanthohumol and 3) Xanthohumol D</b>	[82]
	Phosphate buffer	Tyrosinase	Melanoma	<b>1) Neochlorogenic acid, 2) Chlorogenic acid, 3) Cryptochlorogenic acid, 4) Rutin, 5) Isoquercitrin, 6) Kaempferol-3-O-<math>\alpha</math>-L-rhamnopyranosyl-(1-6)-<math>\beta</math>-d-glucopyranoside, 7) Quercetin-3-O-(6-O-malonyl)-<math>\beta</math>-D-glucopyranoside, 8) 3,4-Dicaffeoylquinnic acid, 9) Astragalinalin, 10) 3,5-Dicaffeoylquinnic acid, 11) 4,5-Dicaffeoylquinnic acid, 12) Kaempferol-3-O-(6-O-malonyl)-<math>\beta</math>-D-glucopyranoside</b>	[89]

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5	Potassium phosphate	Tyrosinase	Melanoma	<p><b>1)</b> 12-15 Heneicosadienoic acid, Octadecanoic acid, 2- hydroxylpropyl esters, <b>2)</b> Unknown, <b>3)</b> 3-(4-tert-butylphenyl)-2-methylpropanal, <b>4)</b> 4,4'-Dihydroxybenzylsulfid, <b>5)</b> Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester <b>6)</b> 9-Octadecenamide, (trans-), <b>7)</b> Unknown, <b>8)</b> Unknown, <b>9)</b> 9-Octadecenamide, (cis-), <b>10)</b> 4,4'-Dihydroxydiphenyl ether, <b>11)</b> 4-(1-(4-hydroxyphenyl)phenyl)phenol, <b>12)</b> Octadecanoic acid, 2,3-dihydroxypropyl ester, <b>13)</b> 4,4'-Dimethoxydiphenylmethane, <b>14)</b> 4,4'-Methylenediphenol, <b>15)</b> 2,4-Bis(4-hydroxybenzyl) phenol, <b>16)</b> 4-Hydroxybenzyl methyl ether</p>	[90]
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13	Potassium phosphate	Tyrosinase	Melanoma	<p><b>1)</b> Gallic acid, <b>2)</b> Iriflophenone 3-C-glucoside, <b>3)</b> Mangiferin, <b>4)</b> Protocatechuic acid, <b>5)</b> Iriflophenone 3-C-(2'-O-galloyl)-glucoside, <b>6)</b> 6'-O-Galloyl-mangiferin, <b>7)</b> Maclurin 3-C-(2'-O-p-hydroxybenzoyl)-glucoside, <b>8)</b> Iriflophenone 3-C-(2',6'-di-O-galloyl)-glucoside, <b>9)</b> Hyperoside, <b>10)</b> Isoquercitrin, <b>11)</b> Ethyl gallate, <b>12)</b> Iriflophenone 3-C-(2'-O-p-hydroxybenzoyl)-glucoside, <b>13)</b> Quercetin-3-O-xyloside, <b>14)</b> 3-O-Galloyl shikimic acid, <b>15)</b> 3-O-Galloyl quinic acid, <b>16)</b> Maclurin 3-C-(2'-O-galloyl)-glucoside, <b>17)</b> 3,5-Di-O-galloyl quinic acid, <b>18)</b> 5-O-Digalloyl quinic acid, <b>19)</b> Digallic acid, <b>20)</b> Isomangiferin, <b>21)</b> 1,4,6-Tri-O-galloyl glucoside, <b>22)</b> Maclurin 3-C-(2',3'-di-O-galloyl)-glucoside, <b>23)</b> 1,3-Digalleoyl acetone, <b>24)</b> 1,3,4,6-Tetra-O-galloyl glucoside, <b>25)</b> 6'-O-(p-Hydroxybenzoyl) mangiferin, <b>26)</b> Epicatechin gallate, <b>27)</b> 1,2,3,4,6-Penta-O-galloyl glucoside, <b>28)</b> Iriflophenone 3-C-(2',3',6'-tri-O-galloyl)-glucoside, <b>29)</b> Luteolin-7-O-glucoside, <b>30)</b> Quercetin-3-O-arapyanoside, <b>31)</b> Quercetin-3-O-arafuranoside, <b>32)</b> Kaempferol-3-O-glucoside, <b>33)</b> Quercetin-3-O-rhamnoside, <b>34)</b> Ethyl 2,4-dihydroxy-3-galloyl oxybenzoate, <b>35)</b> Ethyl digallate, <b>36)</b> 7-O-Methyl quercetin-3-O-rhamnoside</p>	[19]
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23	Potassium phosphate	Tyrosinase	Melanoma		
24	buffer + Ethyl acetate				
25	(fraction)				
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36	Phosphate buffer	Tyrosinase	Melanoma	<p><b>1)</b> Daidzin, <b>2)</b> Isoorientin, <b>3)</b> Isovitexin, <b>4)</b> Daidzein, <b>5)</b> Genistein, <b>6)</b> 3-(5-hydroxybenzofuran-6-yl) propanoic acid</p>	[17]
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1		MeOH + Potassium phosphate buffer	Tyrosinase	Melanoma	<b>1)</b> Protocatechuic acid, <b>2)</b> 3,5- Di-O-caffeoylquinic acid, <b>3)</b> 1,5-Di-O-caffeoylquinic acid and <b>4)</b> Chlorogenic acid	[92]
2						
3						
4		Phosphate buffer	Tyrosinase	Melanoma	<b>1)</b> 3'-Hydroxypuerarin, <b>2)</b> Puerarin, <b>3)</b> Puerarin-6''-O-xyloside, <b>4)</b> Daidzin, <b>5)</b> Genistin, <b>6)</b> 6''-O-acetyldaidzin and <b>7)</b> Daidzein	[93]
5						
6						
7		Phosphate buffer	Tyrosinase	Melanoma	<b>1)</b> Oroxin B, <b>2)</b> Kaempferid-3-O-beta-D-gentiobioside, <b>3)</b> Chrysin-7-O-beta-D-gentiobioside, <b>4)</b> Oroxin A, <b>5)</b> Baicalein and <b>6)</b> Chrysin	[94]
8						
9						
10		Phosphate buffer	Tyrosinase	Melanoma	<b>1)</b> Puerarin, <b>2)</b> Mirificin, <b>3)</b> Daidzin and <b>4)</b> Genistin	[91]
11						
12		Phosphate buffer	Urokinase-type plasminogen activator	Prognostic marker (breast, prostate, and bladder cancer)	<b>1)</b> Gallic acid, <b>2)</b> Methyl gallate, <b>3)</b> Pentagalloylglucose, <b>4)</b> Ellagic acid, <b>5)</b> Ziyu-glycoside I, <b>6)</b> 28-O-β-D-glucopyranosyl pomolic acid, and <b>7)</b> 1β-Hydroxyeuscapic acid	[165]
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18	<b>Miscellaneous</b>	Gradient EtOH-Deionized water	Cyclooxygenase-2 (COX2)	Various	<b>1)</b> Sylvestroside I	[108]
19						
20		Gradient MeOH-Deionized water	Cyclooxygenase-2 (COX2)	Various	<b>1)</b> Swertisin, <b>2)</b> Scopoletin	[110]
21						
22		Isopropyl alcohol-Ammonium acetate buffer	Fibroblast growth factor receptor 4 (FGFR4)	Various	<b>1)</b> Napellonine, <b>2)</b> 12-Epinapelline, <b>3)</b> Benzoylaconitine and <b>4)</b> 3-Deoxyaconitine	[158]
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29		DMSO-Tris-HCl buffer	DNA - G-quadruplex (HT24 and HT22); Duplex sequence (DNA26); DNA (Calf thymus DNA)	Various	<b>1)</b> Sanguinarine, <b>2)</b> Chelerythrine, <b>3)</b> Protopine and <b>4)</b> Allocryptopine	[187]
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1	Buffer (50 mM KCl, 10 mM KH <sub>2</sub> PO <sub>4</sub> , and 1 mM K <sub>2</sub> EDTA, pH 7.4)	DNA (G-quadruplex)	Various	<b>1) Chelerythrine, 2) Sanguinarine, 3) Protopine and 4) Allocryptopine</b>	[188]
2					
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4					
5	Buffer II (20 mM NH <sub>4</sub> OAC, 5 mM Mg(AC) <sub>2</sub> , pH 5.5)	DNA (triplex)	Various	<b>1) Fangchinoline and 2) Tetrandrine</b>	[50]
6					
7					
8					
9					
10	Buffer II (20 mM NH <sub>4</sub> OAC, 5 mM MgCl <sub>2</sub> , pH 5.5)	DNA (triplex)	Various	<b>1) Berberine and 2) Palmatine</b>	[47]
11					
12					
13					
14	Buffer II (20 mM NH <sub>4</sub> OAC, 5 mM Mg(AC) <sub>2</sub> , pH 5.5)	DNA (triplex)	Various	<b>1) Epiberberine, 2) Coptisine, 3) Jatrorrhizine, 4) Berberrubine and 5) Columbamine</b>	[49]
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20	Serum-free DMEM	HepG2 and MCF-7 cells	Hepatoma and breast cancer	<b>1) Serotonin, 2) N-methyl serotonin, 3) Bufotenidine, 4) Suberoyl arginine, 5) Arenobufagin, 6) Telocinobufatoxin, 7) Bufotalin, 8) 3-(N-pimeloyl argininy) bufalin, 9) Cinobufotalin, 10) Bufalitoxin, 11) 3-(N-pimeloyl argininy) resibufogenin, 12) Bufalin, 13) Resibufotoxin, 14) Cinobufotoxin, 15) 3-(N-azelayl argininy) resibufogenin, 16) Cinobufagin and 17) Resibufogenin</b>	[11]
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27					
28	Phosphate buffer	HepG2 cells	Hepatoma	<b>1) Chlorogenic acid, 2) Rutin, 3) Isoquercitrin, 4) Cyanidin-3-O-glucose, 5) Iridin and 6) Quercetin</b>	[192]
29					
30					
31					
32	Undefined	Hsp 90	Various	<b>1) Demecolcine, 2) Wilforine, 3) Triptotriterpenic acid, 4) Celastrol, 5) R1-Barrigenol, 6) 24R,25-dihydroxycholecalciferol, 7) Ursodeoxycholic acid, 8) 8,14-Seco-3,19-epoxyandrostane-8,14-dione, 17-acetoxy-3<math>\beta</math>-methoxy-4,4-dimethyl, 9) Spirost-8-en-11-one, 10) Propanoic acid, 11) Demecolcine, 12) Bufa-20,22-dienolide, 13) Ergosta-5,22-dien-3-ol, 14) <math>\beta</math>-Sitosterol and 15) Betulinaldehyde</b>	[46]
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	Undefined	Hsp 90 $\alpha$	Various	1) Alisol F, 2) 16-oxo-11-deoxy-Alisol A, 3) Alisol F 24-acetate, 4) Alisol L 23-acetate, 5) 16-oxo-11-anhydro-Alisol A, 6) Alisol H, 7) Alisol B-23-acetate, 8) Alisol C, 9) 3-Methyl-5,6- dimethoxy-2,3-dihydro-1H-indene-1-one, 10) (1S,3 $\alpha$ ,7 $\beta$ )-1- Acetyloctahydro- 4 $\alpha$ -hydroxy-7 $\beta$ - isopropyl 4-methyl-1H-indene, 11) Corymbolone, 12) (-)-Spathulenol, 13) n-Hexadecanoic acid, 14) Hexadecanoic acid ethyl ester, 15) Linoleic acid ethyl ester, 16) 13,17-Seco-5 $\alpha$ -pregn-13(18)-en-20-one, 17) Lupeol, 18) Lupeol acetate, 19) Taraxasterol and 20) Betulinaldehyde	[45]																																								
	Undefined	Hsp 90 $\alpha$	Various	1) Zingiberenol, 2) Ar-turmerone, 3) Curlone, 4) (6R,7R)-bisabolone, 5) (E)-Atlantone, 6) 6-(1-Hydroxymethylvinyl), 7) 4,8a-dimethyl-3,5,6,7,8,8a-hexahydro-1H-naphthalen-2-one	[44]																																								
	EtOH 50%	MCF-7 cells	Breast cancer	1) Genistein, 2) Tripteryl E-3, 3) Wilforlide A, 4) Cangononine, 5) Wilforine, 6) Regeol C, 7) Celastrol	[51]																																								
	DMSO-RPMI-1640 medium	PC3-cells	Human androgen-refractory prostate cancer	1) Curcumin, 2) Demethoxycurcumin and 3) Bisdemethoxycurcumin	[20]																																								
	Phosphate buffer	Matrix Metalloproteinase-2 (MMP-2)	Various	1) Oreganol A, 2) Corilagin, 3) Ellagic acid, 4) Zebirioside O, 5) Nigaichigoside F1, 6) Peduncloside, 7) Rosamultin, 8) Kajiichigoside F1, 9) Potentillanoside A, 10) Quadranoside VIII, 11) Valerenic acid and 12) Medicagenic acid	[171]																																								
	Tris-HCl buffer	Monoacylglycerol lipase (MAGL)	Various	1) Tetrahydropalmatin, 2) Tetrahydrocoptisine, 3) Coptisine, 4) Fumaricine, 5) Palmatine, 6) Berberine and 7) Dehydrocorydaline	[178]																																								
	Serum-free Dulbecco's modified eagle medium (DMEM)	HepG2 cells	Hepatoma	1) Aurantio-obtusin, 2) 1-desmethyaurantio-obtusin, 3) Chryso-obtusin, 4) Obtusin, and 5) Questin	[193]																																								
	Phosphate buffer	A549 and H1819 cells	Lung carcinoma	1) Salvianolic acid B, 2) Deoxyshikonin and 3) Shikonin	[48]																																								



	Serum-free Dulbecco's modified eagle medium (DMEM)	MCF-7 cells	Breast cancer	<b>1)</b> 5,7,8,30,40-Pentamethoxyflavanone, <b>2)</b> 3,5,6,7,8,40-Hexamethoxyflavanone, <b>3)</b> 5,7,8,30,40,50-Hexamethoxyflavone, <b>4)</b> 7-Hydroxy-5,6,8,30,40-pentamethoxyflavone, <b>5)</b> Isosinensetin, <b>6)</b> 30-Hydroxy-5,6,7,8,40-pentamethoxyflavone or 40-Hydroxy-5,6,7,8,30-pentamethoxyflavone, <b>7)</b> Sinensetin, <b>8)</b> 5,6,7,8,40-Pentamethoxyflavanone, <b>9)</b> 3-Hydroxy-5,6,7,8,40-pentamethoxyflavone, <b>10)</b> Tetramethyl-O-isoscutellarein, <b>11)</b> Tetramethyl-O-scutellarein, <b>12)</b> Nobiletin, <b>13)</b> 3,5,6,7,8,30,40-Heptamethoxyflavone, <b>14)</b> 5,6,7,8,30,40-Hexamethoxyflavanone, <b>15)</b> 5-Hydroxy-6,7,8,30,40-pentamethoxyflavone, <b>16)</b> Tangeretin	[71]
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**Table S2** – Ligand fishing studies grouped by technique. Information concerning the ligand fishing medium, the cancer-related target with type of cancer involved, the ligands fished, and the respective bibliographical reference is also provided.