Review

Future Prospective of Radiopharmaceuticals from Natural Compounds Using Iodine Radioisotopes as Theranostic Agents

Wiwit Nurhidayah 1,2, Luthfi Utami Setyawati 2,3, Isti Daruwati 3,4, Amirah Mohd Gazzali 5, Toto Subroto 1 and Muchtaridi Muchtaridi 2,3,*

1 Department of Chemistry, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Sumedang 45363, Indonesia
2 Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Padjadjaran University, Sumedang 45363, Indonesia
3 Research Collaboration Centre for Theranostic Radiopharmaceuticals, National Research and Innovation Agency (BRIN), Sumedang 45363, Indonesia
4 Research Center for Radioisotope, Radiopharmaceutical, and Biodosimetry Technology, Research Organization for Nuclear Energy, National Research and Innovation Agency (BRIN), Serpong 15310, Indonesia
5 School of Pharmaceutical Sciences, Universiti Sains Malaysia, USM, Penang 11800, Malaysia
* Correspondence: muchtaridi@unpad.ac.id; Tel.: +62-22-878428888

Abstract: Natural compounds provide precursors with various pharmacological activities and play an important role in discovering new chemical entities, including radiopharmaceuticals. In the development of new radiopharmaceuticals, iodine radioisotopes are widely used and interact with complex compounds including natural products. However, the development of radiopharmaceuticals from natural compounds with iodine radioisotopes has not been widely explored. This review summarizes the development of radiopharmaceuticals from natural compounds using iodine radioisotopes in the last 10 years, as well as discusses the challenges and strategies to improve future discovery of radiopharmaceuticals from natural resources. Literature research was conducted via PubMed, from which 32 research articles related to the development of natural compounds labeled with iodine radioisotopes were reported. From the literature, the challenges in developing radiopharmaceuticals from natural compounds were the purity and biodistribution. Despite the challenges, the development of radiopharmaceuticals from natural compounds is a golden opportunity for nuclear medicine advancement.

Keywords: iodine radioisotopes; natural compounds; radiopharmaceuticals

1. Introduction

Radiopharmaceuticals are drugs (pharmaceutical agents) that labeled with radioactive. They could be applied as theranostic agents. Radiopharmaceuticals are required to be target-specific, safe, and effective [1,2]. A radionuclide used for diagnostic purposes usually emits gamma rays, for example, technetium-99m, zirconium-89, indium-111, fluorine-18, xenon-133, iodine-123, and iodine-125. A radionuclide for therapeutic purposes emits alpha or beta rays, such as yttrium-90, iodine-131, samarium-153, lutetium-177, and astatine-211 [3,4]. In addition, the selection of radionuclides also considers half-life, energy, toxicity, and availability in nature. For example, 111In has the ideal SPECT imaging properties but has high cell DNA toxicity. Another example is zirconium-89, whose low pharmacokinetic properties are a perfect radionuclide for antibody labeling. However, the disadvantage is it can provide increased energy and penetrating photons during high abundance production [3].

A pharmaceutical agent can deliver a radiopharmaceutical to a target due to its specific and selective affinity for the target enzyme, protein, or receptor. The consideration in selecting a pharmaceutical agent is the ability to maintain its target specificity and selectivity
after radiolabeling [5], and some of the common examples include small molecules such as NaI, peptides, and proteins. However, they still have various stability, specificity, and selectivity problems. These problems prompt the research for new pharmaceutical agents and among the class of compounds with the potential to be developed as natural-based compounds. Natural compounds are known as precursors with various pharmacological effects such as antioxidants, antibacterial, and anticancer activities. The affinity of natural compounds for disease targeting is also favorable in their development as pharmaceutical agents. To achieve this aim, suitable synthesis reactions that will allow stable binding of radionuclides to the natural compounds chosen are needed [6].

One of the radionuclides that are predicted to bind well with natural compounds is iodine isotopes [7]. Iodine has several isotopes, including iodine-123, iodine-124, iodine-125, and iodine-131 (Table 1). The labeling of natural compounds with iodine isotopes in the discovery of radiopharmaceuticals for diagnostics and therapeutics of various diseases is very promising. Natural compounds that act as pharmaceutical agents will deliver iodine isotopes to the target. The radiation emitted by the iodine isotopes will then function as either a diagnostic or a therapeutic agent (Figure 1).

Table 1. Radioisotopes of iodine.

| Radioisotope | Half Life | Emission Type | Application | Refs. |
|--------------|-----------|---------------|-------------|-------|
| iodine-123   | 13.2 h    | Gamma, EC 
EC/aug | SPECT 
3 diagnostic | [8–10] |
| iodine-124   | 4.8 days  | Positron      | PET 
2 diagnostic | [11–13] |
| iodine-125   | 60 days   | Gamma, EC Aug | Preclinical study, Radiotherapy SPECT 
3 diagnostic | [14,15] |
| iodine-131   | 8.04 days | Gamma, beta   | Radiotherapy SPECT 
2 and PET 
2 diagnostic | [8–10] |

1 EC: Electron capture, 2 PET: Positron emission tomography, 3 SPECT: Single photon emission tomography.

Although very promising, the development of radiopharmaceuticals from natural compounds using iodine isotopes is limited. More information needs to be gathered in order to understand the limitations and challenges of this approach, and to drive effective strategies for the research and development process. This review collects data of radiopharmaceuticals from natural compounds using iodine radioisotopes that were reported in the last ten years. The stages and the challenges were reviewed, and potential strategies were discussed to escalate the development of the radiopharmaceuticals. The information gained in this review will help researchers to advance the research and development of radiopharmaceuticals and nuclear medicine practice in the future.

![Figure 1. Basic scheme of natural-based radiopharmaceuticals with iodine radioisotope.](image-url)
2. Differences between Radiopharmaceuticals from Natural Compounds with Other Radiopharmaceuticals

Radiopharmaceuticals from natural compounds are radiopharmaceuticals that use natural compounds as the ligand. The ligand will then selectively interact with target tissues; thus, it has the ability to selectively deliver radionuclides. This interaction can occur pharmacologically, immunologically, or metabolically, and they may be reversible. After the interaction and the binding of the ligand with its target, the bonded radiopharmaceutical can be internalized and stored in the target cells. It is hence very crucial for the ligand to effectively, at a low concentration, prevent any pharmacological activity or side effects on the target [5].

In comparison with radiopharmaceuticals from natural compounds, conventional radiopharmaceuticals usually utilize small molecules, peptides, and proteins as ligands. Small molecules such as amino acids, fatty acids, nucleotides, and small inorganic molecules enable the targeting of intracellular regions because small molecules can penetrate semipermeable membranes easily. The examples of radiopharmaceuticals that use small molecules are $[^{123}]$NaI, $[^{123}]$ioflupane, and $[^{123}]$iobenguane for neuroblastoma tumors [5]. $[^{123}]$NaI is a substrate for sodium iodide symporter for thyroid imaging. The presence of a parathyroid adenoma is characterized by areas of cellular tissue which do not exhibit trapping of $[^{123}]$NaI [16]. $[^{123}]$ioflupane provides sensitive results in the diagnosis of Parkinson’s disease even in its early stages, based on the pattern of $[^{123}]$ioflupane uptake on SPECT images, which can be interpreted as normal activity if it shows no dopaminergic deficit [17]. $[^{123}]$iobenguana was applied to neuroblastoma tumors by targeting the norepinephrine transporter (NET) [18].

Radiopharmaceuticals that use peptides or proteins usually target specific receptors of tumor or cancer cells. Peptide cells can diffuse rapidly into target tissues and show longer accumulation in tumor cells. However, the disadvantage of using peptides or proteins as ligands is the potential for radio nephrotoxicity due to the high accumulation of peptides in the kidney. One example of a protein as a ligand is the Designed ankyrin repeat proteins (DARPins) labeled with iodine-124, iodine-125 and iodine-131 which is aimed to evaluate human epidermal growth factor receptor 2 (HER2) expression levels in breast and gastroesophageal cancer [19].

The difference between radiopharmaceuticals from natural compounds and other radiopharmaceuticals is also found in the stages of development of these drugs as shown in Figure 2. In general, the stages of development of other radiopharmaceuticals consist of identifying the molecular targets and synthesizing pharmaceutical compounds to be used as ligands (small molecules or peptides). Suitable radiopharmaceutical synthesis reaction will then be selected, and the evaluation of the synthesized radiopharmaceutical will be carried out. Meanwhile, radiopharmaceuticals from natural compounds tend to have a longer development stage. The initial stage of the development of radiopharmaceuticals from natural compounds is the discovery of natural compounds themselves. Research usually starts from exploring the sources of natural products in nature. After that, the lead compound will be identified, and the natural compounds will be isolated and identified by their structure elucidation. Subsequently, the molecular targets and pharmacological activities will be identified [6,20]. The next step is the selection of an appropriate radiopharmaceutical synthesis reaction based on the structure and the targets of the natural compounds. Some natural compounds also require structure modifications to get the best radiopharmaceutical synthesis results. Then, just like other radiopharmaceuticals, the radiopharmaceuticals from natural compound will also be characterized and evaluated based on the following criteria; the stability, physicochemical characteristics, cellular uptake, preclinical studies, dosimetry prediction, and clinical studies [6].
Figure 2. Development of radiopharmaceuticals from natural compounds in comparison to the conventional radiopharmaceuticals.

3. Available Literature on from Natural Compounds with Iodine Radioisotopes in Last 10-Year Period

This review summarizes the reported studies on radiopharmaceuticals from natural compounds with iodine radioisotopes in the last ten years, between 2013 and 2022. The data obtained from various research are presented in Figure 3.

Figure 3. Research of radiopharmaceuticals from natural compounds in last 10 years.

From 2013–2022, 32 analyses on radiopharmaceuticals from natural compounds with iodine radioisotopes were conducted. The natural compounds are mostly isolated from plants, and their pharmacological effects are identified. These natural compounds are labeled with iodine radioisotopes to develop several therapeutic or diagnostic agents for various diseases, including nineteen for tumor or cancer, one for urinary tract dysfunction,
one for Alzheimer’s disease, seven for necrotic myocardium, one for neuroblastoma, one for ischemic stroke, one for determination of natural compound toxicity, and another one was just labelled as radiotracer unspecified. As described in Figure 2, the discovery and development of radiopharmaceuticals have a long process. After conducting radiolabeling reaction or radiopharmaceutical synthesis, several evaluations need to be passed, including stability, physicochemical, cellular uptake, preclinical, dosimetry, and clinical studies. From the recent research based on the collected data from the 32 studies, 1 was in the synthesis stage, 1 was in the physicochemical study stage, 7 were in the cellular uptake study stage, 21 were in the preclinical study stage, 2 were in the dosimetry prediction stage and none of them reached the clinical study stage. Detailed information is listed in Table 2.

Table 2. Recent report of radiopharmaceuticals from natural compound with iodine radioisotopes and their importance of development/application.

| Year | Natural Compounds | Sources | Pharmacological Activities | Radioisotope | Application | Recent Research Reported | Refs. |
|------|-------------------|---------|-----------------------------|--------------|-------------|--------------------------|-------|
| 2013 | Hydroxytyrosol     | olive leaves extract | Anticancer (breast, colon, prostate, and thyroid cancer) | iodine-131 | Cancer therapy | Preclinical study | [21–26] |
|      | Khellin            | Ammi visnaga fruits | Activity against kidney disease and vitiligo, anticancer | iodine-125 | Urinary tract imaging | Preclinical study | [27,28] |
|      | Hypericin          | Hypericum perforatum L. | Antiviral, necrosis avidity and anticancer | iodine-131 | Cancer therapy | Dosimetry prediction | [29,30] |
|      | Hypericin          | Hypericum perforatum L. | Antiviral, necrosis avidity and anticancer activity | iodine-123 | Cancer therapy | Dosimetry prediction | [30,31] |
|      | Lawsone            | Lawsonia inermis | Anticancer, antioxidant, and antibacterial | iodine-131 | Cancer theranostic | Preclinical study | [32–34] |
|      | Homoisoflavonoids  | Hyacinthaceae and Caesalpinioideae | Formation, extension, and destabilization of Aβ aggregates | iodine-125 | diagnostic of b-amyloid plaques in Alzheimer’s disease | Preclinical study | [35] |
|      | Gingko flavonoids  | Egb761 extract of Gingko Biloba extract | Anticancer | iodine-131 | Cancer diagnostic | Cellular uptake | [36] |
|      | Sinnidine A        | Cassia Senna L. | Structure similar to hypericin so it is predicted to have necrosis affinity like hypericin | iodine-131 | Myocardial infarction imaging | Preclinical study | [37] |
|      | Protohypericin     | Hypericum perforatum | Structure similar to hypericin so it is predicted to have necrosis affinity like hypericin | iodine-131 | Cancer theranostic | Preclinical study | [38] |
|      | Sennoside B        | Cassia senna L. | Structure similar to hypericin so it is predicted to have necrosis affinity like hypericin | iodine-131 | Necrosis-avid tracer | Preclinical study | [39] |
|      | Hesperetin         | citrus fruits | Anti-inflammatory, antioxidant, anticancer, antiviral, antiallergic, and neuroprotective | iodine-123 | Radiotracer for some disease | Preclinical study | [40–42] |
|      | Rutin              | citrus leaves | Antitumor, cytotoxic, anti-inflammatory, antiestrogenic, antimicrobial, antiallergic, and antioxidant | iodine-125 | Cancer diagnostic | Preclinical study | [43,44] |
|      | Rhein              | Cassia fistula L. | Necrotic myocardium | iodine-131 | Myocardium necrosis imaging | Preclinical study | [45–47] |
| Year | Natural Compounds | Sources | Pharmacological Activities | Radioisotope | Application | Recent Research Reported | Refs. |
|------|-------------------|---------|---------------------------|--------------|-------------|--------------------------|-------|
| 2017 | Eugenol | Syzygium aromaticum | Anticancer (prostate, breast, colon, and cervical cancer) | iodine-131 | Cancer therapy | Cellular uptake | [48–51] |
|      | Quercetin | vegetables, fruits, leaves, and grains | Anticancer | iodine-131 | Thyroid cancer therapy | Preclinical study | [52–54] |
|      | Arbutin | fresh fruit of the California buckeye | A tyrosinase inhibitor and antiinflammation | iodine-131 | Tumor diagnostic | Preclinical study | [55–57] |
|      | Vitexin | Passiflora caerulea L. | Necrosis-avid activity | iodine-131 | Myocardium necrosis imaging | Preclinical study | [58] |
|      | Naphthazarine | Juglans Mandshurica Maxim | Necrosis-avid activity | iodine-131 | Myocardium necrosis imaging | Preclinical study | [59,60] |
|      | Plumbagin | Plumbago zeylanica | Necrosis-avid activity | iodine-131 | Myocardium necrosis imaging | Preclinical study | [60,61] |
|      | Juglone | leaves and nuts of various plants from the Juglandaceae family | Necrosis-avid activity | iodine-131 | Myocardium necrosis imaging | Preclinical study | [60,62] |
| 2018 | Resveratrol | grapes, peanut, and Polygonum cuspidatum root | Anti-inflammatory, antikapoptotic, neuroprotective antiinflammation, and immunological regulatory | iodine-131 | Neuroblastoma cells imaging | Cellular uptake | [63–67] |
|      | Geristein | Soybeans | Anticancer (Breast cancer) | iodine-131 | Breast cancer diagnostic | Synthesis | [68–71] |
|      | 6-Gingerol | ginger-roots extract | Anticancer (breast cancer) | iodine-131 | Breast cancer diagnostic | Cellular uptake | [72–74] |
|      | 6-Shogaol | ginger-roots extract | Anticancer (breast cancer) | iodine-131 | Breast cancer diagnostic | Cellular uptake | [72–74] |
|      | Thymoquinone | Nigella sativa | Anticancer | iodine-131 | Cancer theranostic | Cellular uptake | [75] |
|      | FATQCSNPs (Folic acid-chitosan nanoparticles loaded with thymoquinone) | Nigella sativa | Anticancer | iodine-131 | Cancer theranostic | Cellular uptake | [75–77] |
| 2019 | Rutin | Several fruits and vegetables | Anticancer | iodine-131 | Cancer diagnostic | Physicochemical study | [78] |
|      | Ferulic acid | Several fruits and vegetables | Anticancer, antidiabetic, and activity against several neurodegenerative and cardiovascular diseases | iodine-131 | Cancer theranostic | Preclinical study | [79–82] |
|      | Khellin | Annona squamosa fruits | Anticancer | iodine-131 | Cancer theranostic | Preclinical study | [83] |
| 2020 | Zaeralenone | cereal crops | Ability to bind competitively with estrogen receptors | iodine-125 | Study the effect of Lactobacillus Plantarum on biodistribution pattern of Zaeralenone | Preclinical study | [84–86] |
|      | Riboflavin | meat, fish and fowl, eggs, dairy products, green vegetables, mushrooms, and almonds | Activity against nervous system diseases | iodine-131 | Ischemic stroke diagnostic | Preclinical study | [87–89] |
|      | Shikonin | Lithospermum erythrizon | Anticancer (lung cancer) | iodine-131 | Lung cancer diagnostic | Preclinical study | [90–92] |

4. Synthesis of Radiopharmaceuticals from Natural Compounds with Iodine Radioisotopes

In the development of radiopharmaceuticals, researchers commonly favor two kinds of synthesis reactions: (1) synthesis with nonradioactive iodine (iodine-127), and (2) radiosynthesis with iodine radioisotope. The purpose of nonradioactive synthesis is to predict
the structure of the objective compound by elucidating the structure using MS (Mass Spectroscopy) and NMR (Nuclear Magnetic Resonance). Radiosynthesis was to be tested for radiochemical purity. They are usually carried out through two reaction mechanisms, namely (1) electrophilic substitution and (2) nucleophilic substitution [7,93].

4.1. Electrophilic Substitutions

Electrophilic substitution reactions occur when iodine substitutes hydrogen on electron-rich aromatic rings such as phenol and group-substituted benzene rings. In general, iodine is available in the form of NaI solution therefore it needs to be converted into an electropositive form before reacting with pharmaceutical compounds using oxidizing agents, iodo-deprotonation, and iodo-demetallation. The oxidizing agent converts iodine to its electropositive form by oxidizing it thus its oxidation number increases. There are two types of oxidizing agent: (1) oxidizing agents containing halogens, and (2) oxidizing agents without halogens. Oxidizing agents with halogens include chloramine-T, iodine, and N-chlorosuccinimide, meanwhile oxidizing agents without halogens include tert-butyl hydroperoxide, peracetic acid and hydrogen peroxide [94]. Iododeprotonation usually occurs in aromatic compounds that have an electron-rich ring activated by OH, NH$_2$, or OMe [95]. Iododemetalization is reaction using organometallic precursors such as trialkylstannyl, trialkysilyl, or boronic acid derivatives [93].

4.2. Nucleophilic Substitution

Nucleophilic substitution reactions consist of several methods, including halogen exchange, isotope exchange, radioiodo-dediazonisation, and copper-assisted halogen exchange. The halogen exchange method occurs when radioactive iodine substitutes a halogen (bromine or chlorine) in the pharmaceutical compound [93]. This reaction requires extreme conditions. Zmuda et al. (2015) synthesized a tracer for Poly (ADP-ribose) Polymerase-1 (PARP-1) with solid state halogen exchange radioiodination method using bromination. The reaction took place under extreme conditions whereby the reaction temperature was 210 °C with an incubation time of 0.5 h. The authors reported the radiochemical yield obtained was 36.5 ± 7.2% [93,96].

The isotope exchange reaction was carried out by substituting the iodine present in the ligand with radioactive iodine. This reaction usually occurs under reflux with solvents. The solvents used are acetone, dichloromethane, acetonitrile, water, ethanol, or methyl ethyl ketone. Sadeghzadeh et al. synthesized 4-benzyl-1-(3-[125$^I$Iodobenzylsulfonyl)piperidine and 4-(3-[125$^I$Iodobenzyl)-1-(benzylsulfonyl)piperazine using this reaction. It used a wet method using different organic solvents, such as propylene glycol at elevated temperatures (100–200 °C) where the results showed that the purity obtained was 70% [93,97]. Radioiodo–diazenisation is a radioiodination method of compounds with a diazonium group. The reaction was conducted by substituting diazonium with radioiodine. It is usually carried out at low temperatures with the help of sodium nitrate. The reaction proceeds by the SN1 mechanism [93]. Copper-assisted exchange is a nucleophilic substitution reaction that uses copper as a catalyst. The reaction can occur via isotopic or halogen exchange. M. Hagimori et al. synthesized matrix metalloproteinase-12 (MMP-12) using a copper-assisted exchange. The reaction was conducted at 140 °C for 60 min, with high purity product [93,98].

4.3. Synthesis of Radiopharmaceuticals from Natural Compound with Iodine Radioisotopes in the Last 10 Years

The synthesis of radiopharmaceuticals from natural compounds is a challenging process. Natural compounds are expected to be labeled as stable and produce high radiochemical purity. The natural compounds to be labeled are mostly isolated from plants. Prior to labeling, they are usually characterized by LC/MS, HPLC, or NMR. Based on the literature study, several research articles reported the characterization method of natural compounds, but several articles have not reported it. Out of the 32 radiopharmaceuti-
cals of natural compounds using iodine radioisotope, 17 reported the natural compound characterization method, while 15 have not reported it. From the data collected in the last 10 years, all reported compounds were synthesized through electrophilic substitution reactions. This is because natural compounds would usually have an electron-rich aromatic ring. Of the 32 labeled compounds, 31 were synthesized in the presence of oxidizing agents, and 1 through iodo–destannylation. As oxidizing agents, 20 compounds used iodogen, 9 with chloramine-T, and 2 compounds with peracetic acids. Detailed information is listed in Table 3.

Table 3. Characterization of natural compounds and synthesis of radiopharmaceuticals candidates from natural compounds using iodine radioisotope that were reported in the last 10 years (2013–2022).

| Natural Compound | Characterization | Synthesis | Iodinated Natural Compound | Characterization | Refs. |
|------------------|------------------|-----------|---------------------------|------------------|-------|
| Hydroxytyrosol   | LC-MS (liquid chromatography–mass spectrometry) with positive mode [M+H] showed m/z 155. | iodogen   | $[^{[13]}I]$hydroxytyrosol | Structure was characterized by $^1H$ NMR and $^{13}C$ NMR Radiochemical Purity > 95% (by TLRC) | [26] |
| Khellin          | Not reported     | chloramine-T | $[^{[13]}I]$khellin       | Radiochemical Purity < 95% (by TLRC) | [26] |
| Hypericin        | HPLC-UV with retention time of 7.85 min | iodogen   | $[^{[13]}I]$hypericin   | HPLC with retention time of 11.57 min Radiochemical Purity: >95% (by HPLC) | [29,30] |
| Hypericin        | HPLC-UV with retention time of 7.85 min | iodogen   | $[^{[13]}I]$hypericin   | HPLC with retention time of 11.57 min Radiochemical Purity: >95% (by HPLC) | [30,31] |
| Lawson           | Structure was characterized by $^1H$ NMR and $^{13}C$ NMR | iodogen   | $[^{[13]}I]$lawson       | Structure was characterized by $^1H$ NMR and $^{13}C$ NMR Radiochemical Purity: <95% (by TLRC) | [33] |
| Homoisofoflavonoid | Structure was characterized by $^1H$ NMR and $^{13}C$ NMR | iododestannylation | $[^{[13]}I]$Homoisofoflavonoid | Structure was characterized by $^1H$ NMR and $^{13}C$ NMR Radiochemical Purity: >95% (by HPLC) | [34] |
| GFLAS            | Characterized by HPLC | iodogen   | $[^{[13]}I]$GFLAS      | Predicted structure have not reported Radiochemical Purity: <95% (by TLRC) | [36] |
| Natural Compound | Characterization | Synthesis | Iodinated Natural Compound | Characterization | Refs. |
|------------------|------------------|-----------|-----------------------------|------------------|-------|
| Sennoside A      | HPLC-UV with retention time of 9.98 min | iodogen | [131I]sennoside A | HPLC-UV with a retention time of 11.26 min Radiochemical Purity: >95% | [37] |
| Protohypericin   | HPLC-MS/MS [M+H]+ with m/z 505 Structure was characterized by 1H NMR and 13C NMR | iodogen | [131I]protohypericin | Radiochemical Purity: >95% (by HPLC) | [38] |
| Sennoside B      | HPLC with retention time of 7.09 min | iodogen | [131I]sennoside B | HPLC with retention time 9.55 min Radiochemical Purity: >95% (by HPLC) | [3] |
| Hesperetin       | LC/MS with [M+H]+ show m/z of 427 Structure was characterized by NMR | peracetic acid | [125I]hesperetin | Structure characterized by NMR and COSY analysis Radiochemical Purity: >95% (by HPLC) | [42] |
| Rutin            | Structure was characterized by NMR LC MS [M+H]+ with m/z of 737 | chloramine-T | [123I]rutin | Structure was characterized by NMR Radiochemical Purity: >95% (by HPLC) | [44] |
| Natural Compound | Characterization | Synthesis | Iodinated Natural Compound | Characterization | Refs. |
|------------------|------------------|-----------|-----------------------------|------------------|------|
| Rhein            | Not reported     | peracetic acid | ^{131}I|rhein               | Structure was characterized by NMR LC MS [M+H]^+ with m/z of 408.9 Radiochemical Purity: >95% (by HPLC) | [47] |
| Eugenol          | LC MS [M+H]^+ with m/z of 164.80 HPLC with retention time of 12.456 min | iodoogen | ^{131}I|eugenol         | Structure was characterized by NMR Radiochemical Purity: >95% (by TLRC) | [51] |
| Quercetin        | Not reported     | chloramine-T | ^{131}I|quercetin          | LC/MS characterization Radiochemical Purity: >95% (by HPLC) | [54] |
| Arbutin          | HPLC with retention time of 19.9 min | chloramine-T | ^{131}I|arbutin          | HPLC with retention time of 19.9 min Radiochemical Purity: >95% | [57] |
| Vitexin          | Not reported     | iodogen | ^{131}I|vitexin           | Structure was characterized by NMR Radiochemical Purity: >95% (by HPLC) | [58] |
| Napthazarine     | Not reported     | iodogen | ^{131}I|napthazarine      | HPLC with retention time of 8.53 min Radiochemical Purity: >95% (by HPLC) | [60] |
| Plumbagin        | Not reported     | iodogen | ^{131}I|plumbagin        | Radiochemical Purity: >95% (by TLRC) | [60] |
| Juglone          | Not reported     | iodogen | ^{131}I|juglone           | Radiochemical Purity: >95% (by TLRC) | [60] |
Table 3. Cont.

| Natural Compound | Characterization | Synthesis | Iodinated Natural Compound | Characterization | Refs. |
|------------------|------------------|-----------|-----------------------------|------------------|------|
| Resveratrol      | Structure was characterized by NMR LC/MS [M+H]+ with m/z of 229.08 | iodogen | [131I]resveratrol | Structure was characterized by NMR Radiochemical Purity: >95% (by TLRC) | [67] |
| Genistein        | Not reported     | chloramine-T | [131I]genistein | Predicted structure have not reported | Not reported Radiochemical Purity: >95% (by TLRC) | [71] |
| 6-Gingerol       | Not reported     | iodogen | [131I]6-gingerol | Predicted structure have not reported | Radiochemical Purity: >95% (by TLRC) | [74] |
| 6-Shogaol        | Not reported     | iodogen | [131I]6-shogaol | Predicted structure have not reported | Radiochemical Purity: >95% (by TLRC) | [74] |
| Thymoquinone     | Characterized by FTIR has C-H (2950–2800 cm⁻¹), C=O aromatic (1625–1440 cm⁻¹) and C=O ketones (1700–1665 cm⁻¹) | iodogen | [131I]thymoquinone | Predicted structure have not reported | Radiochemical Purity: <95% (by TLRC) | [75] |
| FATQCSNPs        | Characterized by FTIR has amine stretch in Chitosan (3550–3250 cm⁻¹), OH from Folic acid (3200–2500 cm⁻¹), C = O ketones from thymoquinone (1690 cm⁻¹), C = O carboxylic acid from Folic acid (1715 cm⁻¹), C-C (1360–1100 cm⁻¹) and C-O (1320–1210 cm⁻¹) | iodogen | [131I]FATQCSNPs | Predicted structure have not reported | Radiochemical Purity < 95% (by TLRC) | [75] |
| Rutin            | Not reported     | chloramine-T | [131I]rutin | Radiochemical Purity: <95% (by TLRC) | [78] |
| Ferulic acid     | Not reported     | chloramine-T | [131I]ferulic acid | LC/MS showed m/z 321.02 HPLC with retention time 17 min Radiochemical Purity: >95% | [82] |
| Khellin          | Not reported     | iodogen | [131I]khellin | Radiochemical Purity: >95% (by HPLC) | [83] |
| Zearalenone      | HPLC with retention time of 14.7 min | chloramine-T | [131I]zearalenone | HPLC with retention time of 15.8 min Purity: >95% (by HPLC) | [86] |
Table 3. Cont.

| Natural Compound | Characterization | Synthesis | Iodinated Natural Compound | Characterization | Refs. |
|------------------|------------------|-----------|----------------------------|------------------|-------|
| Riboflavin       | Not reported     | iodogen   | $[^{131}I]$-riboflavin     | Radiochemical Purity: >95% (by paper chromatography) | [89]  |
| Shikonin         | Not reported     | chloramine-T | $[^{131}I]$shikonin       | Structure was characterized by NMR; HPLC Rt of 8.14; Radiochemical Purity: <95% | [92]  |

The synthesis method and type of oxidizing agent used in the synthesis, and the purity of the 32 radiopharmaceutical candidates are described in Figure 4. The results of radiosynthesis showed that 24 compounds (77%) had radiochemical purity above 95% while 8 (23%) had a purity lower than 95%.

Figure 4. (a) Synthesis method and type of oxidizing agents used; (b) Purity of the 32 candidates of natural compound-based radiopharmaceuticals.

5. Evaluations of Radiopharmaceuticals from Natural Compounds with Iodine Radioisotopes

As illustrated in Figure 2, the evaluation of radiopharmaceuticals includes stability tests, physicochemical analysis, cellular uptake study, preclinical study, dosimetry prediction, and clinical study. All radiopharmaceuticals, including those derived from natural compounds, will need to go through these evaluations before approval can be granted for human use. Table 4 presents the evaluation of the 32 radioiodinated natural compound as collected and analyzed for this review. From the data, 22 of them reported stability tests, 8 of them reported physicochemical analysis, 10 of them reported cellular uptake study, 23 of them reported preclinical study, 2 of them reported dosimetry prediction and non-reported clinical study.
| Compound          | Stability | Log P  | Cell Uptake                                           | Preclinical Study                                                                                      | Dosimetry                                                                 | Refs.    |
|-------------------|-----------|--------|------------------------------------------------------|--------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------|----------|
| $[^{131}I]$hydroxytyrosol | <4 h      | $-0.41 \pm 0.12$ | Cellular uptake on Hutu80 (37.10%) > Caco2 (27.80%) > MCF7 (14.9%) > PC3 (14.50%) | Biodistribution: highest uptake in bladder, stomach, and intestine. | Not reported                                                               | [26]     |
| $[^{125}I]$khellin | >24 h     | Not reported | Not reported                                        | Biodistribution: The highest uptake in heart, lung, and spleen.                                        | Not reported                                                               | [28]     |
| $[^{131}I]$hypericin | Not reported | Not reported | Not reported                                        | Biodistribution: low uptake in necrosis cells but higher in lung, spleen, liver. High absorbed radiation dose in necrotic tissues. | [29,30] |
| $[^{131}I]$hypericin | Not reported | Not reported | Not reported                                        | Biodistribution: high uptake in necrosis cells but lower in lung, spleen, liver. High absorbed radiation dose in necrotic tissues. | [30,31] |
| $[^{131}I]$lawson | <4 h      | $-0.26 \pm 0.06$ | Keratinocyte (25.46%) > BJ (5.43%) > MCF7 (5.32%) > Caco2 (5.28%) | Biodistribution: highest uptake in uterus, breast and ovary (female mice); and prostate (male mice) | Not reported                                                               | [33]     |
| $[^{125}I]$homoisoflavonoids | Not reported | Not reported | Not reported                                        | Biodistribution: high uptake in necrosis cells, liver, spleen, and kidney. Not reported | [34]     |
| $[^{131}I]$GFLAS    | >24 h     | $-0.99 \pm 0.03$ | Cellular uptake on PC3 > MCF7                        | Pharmacokinetics: AUC of 634.65 MBq/Lxh, clearance 0.02 L/h/kg. The elimination half-life ($t_{1/2}$) of 11.75 hours SPECT/CT image shows high accumulation of radioactivity in necrotic tissue. Biodistribution: high uptake in necrotic tissues, liver, spleen and kidney | Not reported                                                               | [36]     |
| $[^{131}I]$sennidin A | In vivo stability > 48 h | $-1.11 \pm 0.02$ | Not reported                                        | Biodistribution: the highest ratio of target/non-target tissues was 11.7 Pharmacokinetics: concentration after injection in blood 99.451±4.442 MBq/L. $t_{1/2}$ was 14.9 h using noncompartmental analyses (show fast blood clearance) SPECT-CT, autoradiography, and histological staining showed high uptake in necrotic tissues | Not reported                                                               | [37]     |
| $[^{131}I]$protohypericin | Not reported | Not reported | Not reported                                        | Pharmacokinetics: the highest biodistribution: concentration after injection in blood 99.451±4.442 MBq/L. $t_{1/2}$ was 14.9 h using noncompartmental analyses (show fast blood clearance) SPECT-CT, autoradiography, and histological staining showed high uptake in necrotic tissues | Not reported                                                               | [38]     |
| $[^{131}I]$sennoside B | Not reported | Not reported | Not reported                                        | SPECT-CT showed selective accumulation of radioactivity in the necrotic tissues. Pharmacokinetics $t_{1/2}$ 8.6 h (fast clearance from blood) | Not reported                                                               | [3]      |
| $[^{123}I]$hesperetin | <4 h      | Not reported | Not reported                                        | The highest biodistribution: highest uptake in necrotic liver, necrotic muscle and kidney (fast clearance from blood) | Not reported                                                               | [42]     |
Table 4. Cont.

| Compound         | Stability     | Log P | Cell Uptake                        | Preclinical Study                                                                                     | Dosimetry | Refs. |
|------------------|---------------|-------|------------------------------------|------------------------------------------------------------------------------------------------------|-----------|-------|
| [{\textsuperscript{125}I}]rutin | Not reported  | Not reported | Not reported                       | Biodistribution and SPECT/CT studies in mice oral administration: high biodistribution uptake in stomach and small intestine, intravenous administration: highest biodistribution uptake in liver and small intestine | Not reported | [44]  |
| [{\textsuperscript{131}I}]rhein | >24 h         | Not reported | Not reported                       | Stability > 24 h                                                                                       | Pharmacokinetics: t_{1/2} 8.2 ± 0.49 h, Biodistribution: has optimum heart-to-blood, heart-to-liver and heart-to-lung ratios. | Not reported | [47]  |
| [{\textsuperscript{131}I}]eugenol | In vivo stability > 48 h | 1.50 ± 0.15 | In 4 h, cellular uptake on PC3 (54.35%) > MCF7 (45.68%) > Caco-2 (36.60%) | Not reported                                                                                         | Not reported | [51]  |
| [{\textsuperscript{131}I}]quercetin | Not reported  | Not reported | Cellular uptake in human thyroid: TT cell lines > FTC-133 cell lines > DRO cell lines, Cells viability study with CCK-8 assay showed the rate of proliferation inhibition of [{\textsuperscript{131}I}]qQuercetin ≥ [{\textsuperscript{131}I}]pQuercetin > qQuercetin > iodine-131[125]I | Biodistribution: the highest biodistribution uptake in tumors. In vivo therapeutic efficacy study in tumors showed that a single dose can suppress tumor growth with mild side effects. | Not reported | [54]  |
| [{\textsuperscript{131}I}]arbutin | Not reported  | Not reported | Not reported                       | The biodistribution study in CT26 tumor model mice were showed the highest uptake in bladder and kidney | Not reported | [57]  |
| [{\textsuperscript{131}I}]vitexin | 1.48 ± 0.06   | Not reported | Not reported                       | Pharmacokinetics: t_{1/2} 5.3 h, Biodistribution: necrotic-viable myocardium ratio of 5.0 ± 0.9, SPECT/CT: clear necrosis imaging on CA4P-treated W256 tumors, In vivo blocking study: could be blocked 51.95% and 64.29% by EB and cold vitexin | Not reported | [58]  |
| [{\textsuperscript{131}I}]napthazarin | Not reported  | Not reported | Not reported                       | Biodistribution: high necrotic-to-viable ratio and necrosis-to-blood ratio, Pharmacokinetic: t_{1/2} 4.73 h, SPECT/CT: necrotic myocardium could be clearly visualized in vitro DNA-binding; napthazarin could bind to DNA through intercalation in vivo blocking study: necrotic muscle could be significantly blocked by excessive ethidium bromide (a typical DNA intercalator) and cold napthazarin with 63.49 and 71.96% decline. | Not reported | [60]  |
| [{\textsuperscript{131}I}]plumbagin | >12 h         | Not reported | Not reported                       | Biodistribution: exhibited higher DNA-binding 5.60 × 10⁴ M⁻¹                                            | Not reported | [60]  |
| [{\textsuperscript{131}I}]juglone | >12 h         | Not reported | Not reported                       | Biodistribution: exhibited higher DNA-binding 7.53 × 10⁴ M⁻¹                                            | Not reported | [60]  |
| Compound          | Stability     | Log P  | Cellular Uptake                                                                 | Preclinical Study                                      | Dosimetry                                             | Refs. |
|-------------------|---------------|--------|--------------------------------------------------------------------------------|--------------------------------------------------------|-------------------------------------------------------|-------|
| [\(^{131}\)I]resveratrol | >24 h         | 0.48 ± 0.2 | Cellular uptake on human neuroblastoma cell lines SK-N-AS (24.24%) > SH-SY5Y (15.04%) | Not reported                                            | Not reported                                          | [67]  |
| [\(^{131}\)I]genistein  |               |        |                                                                                  | Evaluation have not reported                           |                                                      | [71]  |
| [\(^{131}\)I]6-gingerol  | Not reported  |        | Cellular uptake in breast cancer cell lines MDA-MB-231: [\(^{131}\)I]-6-shogaol > [\(^{131}\)I]-6-gGingerol | Not reported                                            | Not reported                                          | [74]  |
| [\(^{131}\)I]6-shogaol  | Not reported  |        | Cellular uptake in breast cancer cell lines MCF7: [\(^{131}\)I]-6-shogaol similar to [\(^{131}\)I]-6-g-Gingerol | Not reported                                            | Not reported                                          | [74]  |
| [\(^{131}\)I]thymoquinone | 4 h           |        | Cellular uptake: SKOV3 (7.3%) > Caco-2 (5.75%) (in dose 200–1000 ng/mL)          | Not reported                                            | Not reported                                          | [75]  |
| [\(^{131}\)I]FATQCSNPs | 4 h           |        | Cellular uptake: SKOV3 (12.38%) > Caco-2 (6.73%) (in dose 200–1000 ng/mL)        | Not reported                                            | Not reported                                          | [75]  |
| [\(^{131}\)I]rutin    |               | 0.44 ± 0.16 |                                                                                  | Not reported                                            | Not reported                                          | [78]  |
| [\(^{131}\)I]ferulic acid | >24 h         |        |                                                                                  | Not reported                                            | Not reported                                          | [82]  |
| [\(^{131}\)I]khellin  | >24 h         |        |                                                                                  | Biocirculation: %ID/gram in tumor s 4.35 ± 0.41 with tumor to muscle ratio 2.79 | Not reported                                          | [83]  |
| [\(^{125}\)I]zearealenone | >24 h         |        |                                                                                  | Biocirculation: the highest uptake in kidney, liver, intestine, tumor | Not reported                                          | [86]  |
| [\(^{131}\)I]riboflavin |               |        |                                                                                  | SPECT/CT image: uptake in the cerebral injury normal brain | Not reported                                          | [89]  |
| [\(^{131}\)I]shikonin  |               |        |                                                                                  | Autoradiography: infarcted to normal brain ratio 3.63   | Not reported                                          | [92]  |

Table 4. Cont.
The stability of radiopharmaceuticals is affected by several factors such as pH, light, and temperature. It needs to be stored under various storage conditions [98]. From 22 radioiodinated natural compounds that reported stability tests, the stability was classified into two groups: stability ≥ 24 h and <24 h, with a total of 14 and 8 compounds, respectively.

The physicochemical analysis consists of lipophilicity and protein binding characterizations. Lipophilicity, quantified as Log D or Log P, is a crucial parameter in estimating radiopharmaceuticals absorption, distribution, metabolism, and excretion (ADME) [99,100]. Besides lipophilicity, protein binding affects the biodistribution and clearance of radiopharmaceuticals. It has a positive correlation with lipophilicity [101].

Cellular uptake study aims to determine the specificity of a radiopharmaceutical towards its target by using cells or tissues that expresses the target [4,102]. The tested radiopharmaceutical will be incubated with cultured cells and cell uptake will be calculated as the percentage of radioactivity in cells compared to total radioactivity [103]. The selection of cell lines used depends on the specific target of the radiopharmaceutical on the receptor or on certain physiological conditions. Some of the cell lines that are often used include: Hutu80 (human gastrointestinal tumor cell lines), Caco-2 (human colon adenocarcinoma cells), MCF7 (human breast adenocarcinoma cells), PC3 (human prostate carcinoma cells), Keratinocyte (Human normal epidermal keratinocyte cells), B (Human normal foreskin fibroblast cells), TT, FTC-133, and DRO (human thyroid cell lines), SK-N-AS and SH-SY5Y (human neuroblastoma cell lines), MDA-MB-231 (the triple-negative breast cancer cell lines), and SKOV3 (human ovarian cancer cell lines). After cellular uptake, preclinical study will be conducted using experimental animals. In general, it consists of biodistribution, pharmacokinetics, and toxicity studies. A biodistribution study will allow the determination of radiopharmaceutical uptake in the animal organs, which will be calculated as %ID/g [104]. Based on the reported biodistribution study data, several compounds ([131]Ihydroxytyrosol, [123]Ihesperetin, [125]Irutin, [131]Ikhellin, and [125]Izearalenone) have a high accumulation pattern in certain organs, especially the thyroid, intestine and stomach. A pharmacokinetic study is needed to determine the pharmacokinetic parameters such as elimination rate constant (Kₑ), the volume of distribution (Vd), area under the curve [105], and clearance and time to maximum concentration (Tₘₐₓ) [106]. Radiopharmaceuticals are expected to have rapid blood clearance and short t₁/₂ elimination so that they can be excreted rapidly from the blood. Based on data collected, [131]Isennidin A, [131]Ipseudohypericin, [131]Icentereside B, [131]Irhein, [131]Ivitexin, [131]Inapatrazarin, and [131]Ishikonin reported pharmacokinetic study with t₁/₂ elimination value of 11.75, 14.9, 8.6, 8.2, 5.3, 4.73, and 0.675 h, respectively. Toxicity study aims to evaluate the safety of radiopharmaceuticals. Koziorowski et al. (2016) stated that in radiopharmaceuticals, acute toxicity tests were carried out to predict the effect of overdose whereas subacute, chronic, teratogenic, mutagenic and carcinogenic toxicity were not required for radiopharmaceuticals [107]. The schematic diagram of the preclinical study is depicted in Figure 5.

Dosimetry prediction is a procedure in the determination of the absorbed dose as the amount of energy absorbed per unit mass in all irradiated tissues or organs of interest. The aim was to determine the reference level of irradiation for every new radiopharmaceutical or estimate the absorbed dose for routinely used radiopharmaceuticals [108]. The final stage of radiopharmaceutical development is clinical study. This stage is carried out based on the regulations set in each region or country because, in general, each region or country would have different regulations regarding the rules of radiopharmaceutical-based clinical trials [5].
Dosimetry prediction is a procedure in the determination of the absorbed dose as the amount of energy absorbed per unit mass in all irradiated tissues or organs. The aim was to determine the reference levels of irradiation for every new radiopharmaceutical or estimate the absorbed dose for routinely used radiopharmaceuticals [108].

The final stage of radiopharmaceutical development is clinical study. This stage is carried out based on the regulations set in each region or country because, in general, each region or country would have different regulations regarding the rules of radiopharmaceutical-based clinical trials [5].

Table 5. Challenges in the development of natural product-based radiopharmaceuticals.

| No | Challenges                                      | Cases on Previous Studies                                                                 |
|----|-------------------------------------------------|------------------------------------------------------------------------------------------|
| 1. | Problem related to radiochemical purity         | Radiolabeled compounds have low radiochemical purity (RCP < 95%): [125I]khellin, [131I]lawson, [131I]GFLAS, [131I]sermridin A, [131I]lithmoquinone, [131I]FATQCSNPs, [131I]rutin, and [131I]shikonin |
| 2. | Problem related to biodistribution              | The biodistribution pattern was high in certain organs, especially the thyroid, intestine and stomach: [131I]hydroxytyrosol, [123I]hesperetin, [125I]rutin, [131I]khellin, and [125I]zearalenone |

6. Challenge and Strategies

The development stages of radiopharmaceuticals from natural compounds with iodine radioisotope are rather long and challenging. The challenges reported in previous studies were mostly related to radiochemical purity and biodistribution, as shown in Table 5. However, some radiolabeled compounds such as [131I]igenistein have not been tested in vitro and in vivo, so further development is needed.

6.1. Problem Related to Radiochemical Purity and the Strategies

Radiochemical purity is a crucial quality control factor in radiopharmaceutical development. The radiochemical impurities of iodine are free I⁻ and I₂ which affect the safety and accuracy of radiopharmaceuticals. The first strategy to obtain the maximum radiochemical purity is to select a suitable radioiodination method based on the steric characteristics of the natural compound as a substrate to be labeled. In addition, critical point optimization in the radioiodination method is important because it can minimize the formation of impurities. The first strategy is to optimize the critical point of radioiodination. [7,94]. Substrate characteristics and critical reaction points should be considered in selecting the radioiodination reaction method, as summarized in Table 6.
Table 6. Critical points and considerations of radioiodination reaction method as a strategy for increasing the radiochemical purity.

| Radioiodination Method                  | Critical Point that Needs to Be Optimized | Considerations                                                                 | Refs.          |
|----------------------------------------|-----------------------------------------|--------------------------------------------------------------------------------|----------------|
| Electrophilic substitution             | pH                                      | pH should be neutral, weak acid, or weak basic media.                           |                |
|                                        | CAT concentration                       | Excessive concentration causes oxidative side reactions such as polymerization, chlorination, and denaturation of the substrate. | [28,109–115]  |
|                                        | Temperature                             | Temperature to achieve the energy required for substitute H+ from the aromatic ring with radioactive iodonium ion. |                |
| Chloramine-T (CAT)                     | pH                                      | pH should be 7–8                                                                | [116–119]      |
|                                        | Iodogen concentration                   | Excessive concentration causes precipitates on the walls of the reaction vessel causing a low radiochemical purity. |                |
|                                        | Solvent                                 | Solvent: substrate in DMSO solvent showed with higher radiochemical purity RCP than substrate in aqueous solvent. |                |
| N-halosuccinimides (N-chlorosuccinimide and N-iodosuccinimide) | pH                                      | pH: N-iodosuccinimide with high activity in a strong acid medium                | [93,120,121]  |
|                                        | Mediators                               | Mediators such as NGA or mAB                                                     |                |
| Nucleophilic Substitutions (halogen and isotopic exchange) | Temperature                             | High temperature is required                                                     | [93]           |
|                                        | Reaction time                           | Reaction time: reactions take a long reaction time                             |                |

Selection of the suitable radioiodination method and optimization of the critical point in radioiodination could lead to high radiochemical purity. However, if the radiochemical purity is still lower than the required purity (>95%), another strategy that can be conducted is purification to separate impurities from the radiopharmaceuticals. The selection of the purification method depends on the molecular weight, lipophilicity, and molecular charge of the radiopharmaceuticals. Purification methods that can be applied include HPLC (High-Performance Liquid Chromatography), SPE (Solid Phase Extraction), SEC (Size-Exclusion Chromatography), and IEC (Ion-Exchange Chromatography).

One of the most widely used purification methods is the HPLC. The separation of compounds occurs due to differences in solute interactions and the column that lead to different elution rates for each component. As a result, it will provide high purity resolution. The parameters to consider in HPLC purification are polarity, flow rate, pH, the lipophilicity of the mobile phase, sample matrix, type of stationary phase, and temperature. SPE is widely chosen because it is simple, fast, and able to separate dissolved or suspended compounds from other compounds in the mixture based on their physical and chemical properties. Kim et al. (2019) conducted purification of $\text{[131]}\text{I}$metaiodobenzylguanidine using solid phase extraction and obtained a higher amount of product and lower exposure of operator to radiation [122]. SPE is commonly used in the separation of macromolecules that consist of substances with different molar masses. The SEC chromatography column uses porous polymeric beads. The pore size determines the dimensions of the compounds to be separated. Molecules with smaller size than the pores can enter the pores and retain, while the molecule with larger size than the pores will pass through the spaces between the packing material. In this way, the molecules with the highest molecular weight will be obtained in the first fraction. Lemps et al. performed purification of $\text{[125]}\text{I}$bevacizumab using the SEC method and obtained a radiochemical purity of 99.5% after purification [123]. IEC is a separation method for ions and polar molecules [124]. IEC consists of anion and cation exchange. Cation-exchange chromatography uses a negatively charged stationary phase that can separate cations from other ions. By contrast, anion-exchange chromatography uses a positively charged stationary phase that can separate anions from the other ions. Before conducting an IEC, the stationary phase should be achieved through electroneutrality. Visser et al. performed a purification of $\text{[131]}\text{I}$c-MOV18 which was a radiopharmaceutical candidate for therapy for ovarian cancer. The impurities were removed by purification using Dowex AG1-X8 (BioRad, Utrecht, The Netherlands) anion-exchange resin in PBS [125,126].
6.2. Problem Related to Biodistribution and the Strategies

Ideally, radiopharmaceuticals are required to have high specificity, rapid accumulation in target organs, and a high target-to-nontarget ratio [127]. Based on previous studies, some labeled compounds showed high biodistribution in other organs compared to the targeted organs. Altered biodistribution in vivo affected the accuracy of imaging and radiopharmaceutical therapy. This problem occurred due to the presence of other compounds, such as impurities (free I−) or residues that could be uptaken in organs and detected as radiopharmaceuticals. Spetz et al. conducted a study to determine the biodistribution of free 125I− and 131I− in rats and reported the highest biodistribution in the thyroid gland and stomach. In primary conditions, iodine was localized in the thyroid. In addition, the iodine uptake in the stomach occurred due to the expression of an iodine transport medium named Na+/I− sodium iodide symporter in the stomach [128].

The formation of impurity free iodide indicates low in vivo stability of C-I in the radiopharmaceuticals due to the deiodination reaction. The accumulation of free iodide as impurity in the thyroid, stomach, and intestine reduces the target-to-background ratio of the diagnostic agent so that the diagnostic results are biased. In therapeutic applications, it increases the accumulation of radioactivity in the non-target organs, which could lead to adverse reactions in these healthy organs. This in vivo deiodination reaction is caused by several enzymes including deiodinase enzymes, cytochromes P450 (CYP450) enzymes, and nonspecific nucleophilic enzymes. Deiodinase enzymes promote deiodination reactions in iodinated aromatic rings such as ortho–iodo–phenols. CYP450 enzymes promote deiodination via xenobiotics oxidation reactions, whereas nonspecific nucleophilic enzymes promote deiodination at electrophilic carbon atoms [129].

The first strategy to decrease free iodine accumulation in non-target organs is to design radiopharmaceuticals resistant to deiodination reactions with modification structural. In general, compounds with an arene group are stable to deiodination. In the iodination of the arene group, metaiodoarene is more resistant to deionization than orthoiodoarene and paraiodoarene. In addition, iodination at sp2 carbon atoms is usually more stable to deiodination reactions than iodination at sp and sp3 carbon atoms. Radioiodination of the vinyl group is also stable against in vivo deiodination reactions. However, the phenol and aniline groups have poor in vivo stability. Their stability can be improved by adding electron-donating substituents such as OCH3 to the aromatic ring. On the other hand, the addition of electron-withdrawing groups can decrease in vivo stability [129]. The resistance of some radioiodinated groups against in vivo deiodination is listed in Table 7.

Table 7. The resistance of some radioiodinated groups against in vivo deiodination [129].

| Resistant to Deiodination | Non-Resistant to Deiodination |
|--------------------------|------------------------------|
| Iodinated carbon sp2     | Iodinated carbon sp and sp3  |
| Iodoarenes              | Iodoaniline                  |
| Iodovinyl                | Iodophenols                  |
| Iodoallyl                | Radioiodinated nitrogen-containing (quinozalines, indoles, or imidazoles), and sulfur-containing (thiophenes) heterocycles |
| Radioiodinated oxygen-containing heterocycles | |

Compton et al. (1993) conducted the radioiodination of Δ9-Tetrahydrocannabinol (Δ9-THC) to produce 2-iodo-Δ9-THC and 5′-iodo-Δ9-THC. Δ9-THC is a natural compound isolated from Cannabis Sativa. The target of radioiodinated Δ9-THC is the imaging cannabinoid system. The structure of Δ9-THC, 2-iodo-Δ9-THC, and 5′-iodo-Δ9-THC are shown in Figure 6a. The in vivo study of 5′-iodo-Δ9-THC showed a poor in vivo profile due to the deiodination reaction (shown in Figure 6b). The position of iodine on a terminal sp3 carbon atom of linear pentylo moiety allows 5′-iodo-Δ9-THC to be susceptible to in vivo deiodination caused by CYP450. Cavina et al. (2016) provide solutions for structural
modifications that are expected to increase the stability against deiodination, including
the position of iodine on the iodo-ethoxy group, iodine on the cubane position, iodine on
carbon sp2 at the vinyl terminal, or iodine on C sp2 in the terminal allyl moiety [129]. The
structural modification is shown in Figure 6c. Based on this case, structural modification of
natural compounds provides a promising strategy to increase the stability of radioiodinated
natural compounds against in vivo deiodination.

![Figure 6](image)

**Figure 6.** (a) Structure of Δ²-THC, 2-ido-Δ⁸-THC and 5′-ido-Δ⁸-THC; (b) The mechanism of
deiodination reaction of 5′-ido-Δ⁸-THC; (c) Recommendation structure of iodinated THC which is
stable against deiodination reaction [130].

The second strategy to increase the in vivo stability of natural compound-based ra-
diopharmaceutical candidates is to label them with a linker. This linker will form a stable
chemical bond between the iodine radioisotope and the natural compounds [5], and it
must have a good in vivo stability. Kim et al. (2016) conducted radioiodination of cetux-
imab with the linker (N-(4-isothiocyanatobenzyl)-2-(3-(tributylstannyl)phenyl) acetamide
(IBPA). It was reported that [125I]IBPA-cetuximab had a more stable binding and higher
internalization in mice bearing LS174T tumor xenografts compared to [125I]cetuximab [130].

Another strategy that can be applied is to increase the target specificity by using
nanoparticles. Nanoparticles play a role in increasing the penetration of compounds
cross biological membranes so that they can effectively deliver therapeutic agents and
reduce the side effects of conventional delivery techniques. Nanoparticles can increase the
delivery specificity of radiopharmaceuticals towards its target by conjugating the targeting
molecule (ligand) on the nanoparticles’ surface [131]. This technique was carried out by
Ince et al. (2016) who produced the [131I]FATQCSNPs (folic acid-chitosan nanoparticles
loaded with thymoquinone) described earlier, with ovarian cancer cells as the target. $[^{131}]$FATQCSNPs incorporated thymoquinone isolated from *Nigella sativa* in a folic acid-chitosan nanoparticles [74]. Folic acid is a small molecule that is useful as a ligand that helps in the internalization of pharmaceuticals into cancer cells [75]. Folic acid was used due to ovarian cancer cells that were marked with overexpression of folic acid. The encapsulation of thymoquinone within folic acid-chitosan nanoparticles has improved the delivery of thymoquinone, especially with the presence of folic acid that helps to increase the specificity of delivery and increase cellular uptake. Both $[^{131}]$thymoquinone and $[^{131}]$FATQSCNPs were developed for diagnostic and therapy of cancer. The radioiodinated complex showed a higher uptake in SKOV3 cells as compared to $[^{131}]$thymoquinone [76]. A schematic diagram of $[^{131}]$FATQSCNPs is shown in Figure 7.

![Schematic diagram](image)

Figure 7. A schematic diagram of $[^{131}]$FATQSCNPs.

7. Methods

This review was conducted based on the results of the collection and analysis of articles obtained from the PubMed database with the following keywords: “radioiodination of natural compound”; “radiopharmaceutical natural compound”; “radioiodination of flavonoid”; “radioiodination of alkaloid”; “radioiodination reaction mechanism”; “design AND challenge new radiopharmaceutical”; “radiopharmaceutical AND natural product AND iodine”.

The inclusion criteria of the main article were articles that discuss radioiodination of natural compounds using the English language and published within the range years of 2013–2022. The inclusion criteria of the supporting articles were articles that discuss radiopharmaceuticals in general, iodine isotopes, and the pharmacological effects of natural compounds. Exclusion criteria were articles that were published more than 10 years ago for main articles, 20 years ago for supporting articles, and non-relevant articles to the topic discussed in this review.

Based on the search conducted using the aforementioned keywords, 942 journals were obtained and 512 articles were discarded as they were published more than 10 years ago (for main articles) and 20 years ago (for supporting articles), while 299 articles were non-relevant articles to the topic discussed in this review. This step has reduced the number of articles to 131 consisting of 102 supporting articles and 29 articles discussing the radioiodination of natural compounds with iodine radioisotope. The literature search flow is shown in Figure 8.
8. Future, Prospect, and Conclusions

The development of radiopharmaceuticals from natural compounds with iodine radioisotope is a long process with several challenges. Thirty-two radioiodinated natural compounds were collected from a literature study of the last 10 years. To determine the challenges that radiopharmaceutical researchers found in natural compounds, we reviewed 32 compounds from their synthesis to their evaluation results. These challenges are classified into two groups: (1) challenges related to chemical purity, and (2) challenges related to biodistribution. We discussed strategies that could be applied to resolve these challenges.

Based on the data, 8 of the 32 radioiodinated natural compounds collected had radiophysical purity problems. The first strategy offered is to optimize the critical point in the synthesis reaction to obtain the optimum synthesis conditions. The second strategy is the purification of synthetic products in several ways, including High-Performance Liquid Chromatography (HPLC), SPE (Solid Phase Extraction), (SPE), SEC (Size-Exclusion Chromatography), and IEC (Ion-Exchange Chromatography). The purification method can separate the product from impurities.

Based on the evaluation results, five radioiodinated natural compounds have problems with their biodistribution. Unspecified accumulation is characterized by high accumulation in the stomach, intestines, and thyroid. This unspecific accumulation shows poor in vivo stability due to the deiodination reaction. Several strategies to solve this problem include designing radiopharmaceuticals resistant to in vivo deiodination by structural modification, radiiodination with linkers, and application of nanoparticles.

Despite the challenges, the development of radiopharmaceuticals from natural compounds using iodine radioisotope offers a bright future in the development of radiopharmaceuticals. This review provides information that researchers undertaking further research can consider. The strategies offered in this review are expected to encourage improvement in research related to natural product-based radiopharmaceuticals with iodine radioisotopes as theranostic agents for various diseases.
Author Contributions: W.N. and M.M. contributed to design the concept and the content of manuscript, literature search, and manuscript preparation. I.D., L.U.S. and A.M.G. contributed to manuscript review. T.S. and M.M. contributed to design the concept and the content of manuscript, manuscript review, and M.M. responsible as guarantor. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by PMDSU Scholarship, grant number 2064/UN6.3.1/PT.00/2022.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors thank the Ministry of Education and Culture of Republic of Indonesia for financially support the review through PMDSU scholarship.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Sgouros, G.; Bodei, L.; McDevitt, M.R.; Nedrow, J.R. Radiopharmaceutical therapy in cancer: Clinical advances and challenges. Nat. Rev. Drug Discov. 2020, 19, 589–608. [CrossRef] [PubMed]
2. Lau, J.; Rousseau, E.; Kwon, D.; Lin, K.-S.; Chen, X. Insight into the Development of PET Radiopharmaceuticals for Oncology. Cancers 2020, 12, 1312. [CrossRef] [PubMed]
3. Holik, H.A.; Ibrahim, F.M.; Elaine, A.A.; Putra, B.D.; Achmad, A.; Kartamihardja, A.H.S. The Chemical Scaffold of Theranostic Radiopharmaceuticals: Radionuclide, Bifunctional Chelator, and Pharmacokinetics Modifying Linker. Molecules 2022, 27, 3062. [CrossRef] [PubMed]
4. Payolla, F.; Massabni, A.; Orvig, C. Radiopharmaceuticals for diagnosis in nuclear medicine: A short review. Eclét. Quim. J. 2019, 44, 11–19. [CrossRef]
5. Vermeulen, K.; Vandomme, M.; Bormans, G.; Cleeren, F. Design and Challenges of Radiopharmaceuticals. Semin. Nucl. Med. 2019, 49, 339–356. [CrossRef]
6. Wongso, H. Natural product-based Radiopharmaceuticals:Focus on curcumin and its analogs, flavonoids, and marine peptides. J. Pharm. Anal. 2021, 12, 380–393. [CrossRef]
7. Vaidyanathan, G.; Zalutsky, M.R. The Radiopharmaceutical Chemistry of the Radioisotopes of Iodine. In Radiopharmaceutical Chemistry; Lewis, J.S., Windhorst, A.D., Zeglis, B.M., Eds.; Springer International Publishing: Berlin/Heidelberg, Germany, 2019; pp. 391–408. [CrossRef]
8. Morphis, M.; van Staden, J.A.; du Raan, H.; Ljungberg, M. Validation of a SIMIND Monte Carlo modelled gamma camera for Iodine-123 and Iodine-131 imaging. Heliyon 2021, 7, e07196. [CrossRef]
9. Yordanova, A.; Eppard, E.; Karpig, S.; Bundschuh, R.A.; Schönberger, S.; Gonzalez-Carmona, M.; Feldmann, G.; Ahmadzadehfar, H.; Essler, M. Theranostics in nuclear medicine practice. OncoTargets Ther. 2017, 10, 4821–4828. [CrossRef]
10. Silverstein, E.B. Radioidine: The classic theranostic agent. Semin. Nucl. Med. 2012, 42, 164–170. [CrossRef]
11. Treglia, G.; Muoio, B.; Giovanella, L.; Salvatori, M. The role of positron emission tomosynthesis and positron emission tomosynthesis/computed tomography in thyroid tumours: An overview. Eur. Arch. Oto-Rhino-Laryngol. 2013, 270, 1783–1787. [CrossRef]
12. Braghiorioli, A.M.; Waissmann, W.; da Silva, J.B.; dos Santos, G.R. Production of iodine-124 and its applications in nuclear medicine. Appl. Radiat. Isot. 2014, 90, 138–148. [CrossRef] [PubMed]
13. Cascini, G.L.; Asabella, A.N.; Notaristefano, A.; Restuccia, A.; Ferrari, C.; Rubini, D.; Altini, C.; Rubini, G. 124 Iodine: A longer-life radiopharmaceutical. OncoTargets Ther. 2017, 10, 1209–1220. [CrossRef] [PubMed]
14. Schwartz, S.B.; Thon, N.; Nikolajek, K.; Niyazi, M.; Tonn, J.C.; Belke, C.; Beadle, B.M. A choice of radionuclide: Comparative outcomes and toxicity of ruthenium-106 and iodine-125 in the definitive treatment of uveal melanoma. Pr. Radiat. Oncol. 2015, 5, e169–e176. [CrossRef] [PubMed]
15. Pelletier-Galarneau, M.; Sogbein, O.O.; Dinh, L.; Mitran, B.; Garousi, J.; Rinne, S.; Löblom, J.; Orlova, A.; Deyev, S.; et al. Comparison of tumor-targeting properties of directly and indirectly radioiodinated designed ankyrin repeat protein (DARPin) G3 variants for molecular imaging of HER2. Int. J. Oncol. 2019, 54, 1209–1220. [CrossRef]
16. Hanson, J. A Hundred Years in the Elucidation of the Structures of Natural Products. Sci. Prog. 2017, 100, 63–79. [CrossRef]
21. Chimento, A.; Casaburi, I.; Rosano, C.; Avena, P.; De Luca, A.; Campana, C.; Martire, E.; Santolla, M.F.; Maggiolini, M.; Pezzi, V.; et al. Oleuropein and hydroxytyrosol activate P2Y12-R2-dependent pathways leading to apoptosis of ER-negative SKBR3 breast cancer cells. *Mol. Nutr. Food Res.* 2014, 58, 478–489. [CrossRef]

22. Siriani, R.; Chimento, A.; De Luca, A.; Casaburi, I.; Rizza, P.; Onofrio, A.; Iacobetta, D.; Puoci, F.; Andò, S.; Maggiolini, M.; et al. Oleuropein and hydroxytyrosol inhibit MCF-7 breast cancer cell proliferation interfering with ERK1/2 activation. *Mol. Nutr. Amp. Food Res.* 2010, 54, 833–840. [CrossRef] [PubMed]

23. Luo, C.; Li, Y.; Wang, H.; Cui, Y.; Feng, Z.; Li, H.; Li, Y.; Wang, Y.; Wurtz, K.; Weber, P.; et al. Hydroxytyrosol promotes superoxide production and defects in autophagy leading to anti-proliferation and apoptosis on human prostate cancer cells. *Curr. Cancer Drug Targets* 2013, 13, 625–639. [CrossRef]

24. Sun, L.; Luo, C.; Liu, J. Hydroxytyrosol induces apoptosis in human colon cancer cells through ROS generation. *Food Funct.* 2014, 5, 1909–1914. [CrossRef] [PubMed]

25. Toteda, G.; Lupinacci, S.; Vizza, D.; Bonofiglio, R.; Perri, E.; Bonofiglio, M.; Lofaro, D.; La Russa, A.; Leone, F.; Gigliotti, P.; et al. High doses of hydroxytyrosol induce apoptosis in papillary and follicular thyroid cancer cells. *J. Endocrinol. Invest.* 2017, 40, 153–162. [CrossRef] [PubMed]

26. Ozkan, M.; Muftuler, F.; Yurt, A.; Medine, I.; Unak, P. Isolation of Hydroxytyrosol from olive leaves extract, radioiodination and investigation of bioaffinity using in vivo/in vitro methods. *Radschim. Acta* 2013, 101, 585–593. [CrossRef]

27. Khalil, N.; Bishr, M.; Desouky, S.; Salama, O. *Ammi visnaga* L., a Potential Medicinal Plant: A Review. *Molecules* 2020, 25, 301. [CrossRef]

28. Khater, S.I.; Kandil, S.A.; Hussien, H. Preparation of radioiodinated khellin for the urinary tract imaging. *J. Radioanal. Nucl. Chem.* 2013, 295, 1939–1944. [CrossRef]

29. Mullaiharam, A.R.; Nirmala, H. St John’s wort (*Hypericum perforatum* L.): A Review of its Chemistry, Pharmacology and Clinical properties. *Int. J. Res. Phytochem. Pharmacol.* 2018, 1, 5–11. [CrossRef]

30. Cona, M.M.; Koole, M.; Feng, Y.; Liu; Verbruggen, A.; Oyen, R.; Ni, Y. Biodistribution and radiation dosimetry of radioiodinated hypericin as a cancer therapeutic. *Int. J. Oncol.* 2014, 44, 819–829. [CrossRef]

31. Aras, O.; Takan, G.; Kilcar, A.Y.; Muftuler, F.Z.B. Extraction and radioiodination of Gingko flavonoids and monitoring the cellular properties. *Int. J. Res. Phytochem. Pharmacol. Sci.* 2013, 125–131. [CrossRef] [PubMed]

32. Pour, A.P.; Farahbaksh, H. *Lawsonia inermis* L. leaves aqueous extract as a natural antioxidant and antibacterial product. *Nat. Prod. Res.* 2020, 34, 3399–3403. [CrossRef]

33. Tekin, V.; Muftuler, F.Z.B.; Yurt Kilcar, A.; Unak, P. Radioiodination and biodistribution of isolated lawson compound from *Lawsonia inermis* (henna) leaves extract. *J. Radioanal. Nucl. Chem.* 2014, 302, 225–232. [CrossRef]

34. Tekin, V.; Biber Muftuler, F.Z.; Guldu, O.K.; Kilcar, A.Y.; Medine, E.I.; Yavuz, M.; Unak, P.; Timur, S. Biological affinity evaluation of Lawsonia inermis origin Lawsone compound and its radioiodinated form via in vitro methods. *J. Radioanal. Nucl. Chem.* 2015, 303, 701–708. [CrossRef]

35. Gal, C.; Zhao, Z.; Nan, D.D.; Yin, B.; Hu, J. Homoisoflavonoids as potential imaging agents for β-amyloid plaques in Alzheimer’s disease. *Eur. J. Med. Chem.* 2014, 76, 125–131. [CrossRef] [PubMed]

36. Aras, O.; Takan, G.; Kilcar, A.Y.; Muftuler, F.Z.B. Extraction and radioiodination of Gingko flavonoids and monitoring the cellular incorporation. *J. Radioanal. Nucl. Chem.* 2011, 301, 271–278. [CrossRef]

37. Jiang, C.; Gao, M.; Li, Y.; Huang, D.; Yao, N.; Ji, Y.; Liu, X.; Zhang, D.; Wang, X.; Yin, Z.; et al. Exploring diagnostic potentials of radioiodinated sennarin A in rat model of reperfused myocardial infarction. *Int. J. Pharm.* 2015, 495, 31–40. [CrossRef]

38. Liu, X.; Feng, Y.; Jiang, C.; Lou, B.; Li, Y.; Liu, W.; Yao, N.; Gao, M.; Ji, Y.; Wang, Q.; et al. Radiopharmaceutical evaluation of (131I)-protophypericin as a necrosis avid compound. *J. Drug Target.* 2015, 23, 417–426. [CrossRef]

39. Zhang, D.; Huang, D.; Ji, Y.; Jiang, C.; Li, Y.; Gao, M.; Yao, N.; Liu, X.; Shao, H.; Jing, S.; et al. Experimental evaluation of radioiodinated sennoside B as a necrosis-avid tracer agent. *J. Drug Target.* 2015, 23, 180–190. [CrossRef]

40. Yang, H.L.; Chen, S.C.; Kumar, K.S.; Yu, K.N.; Chao, P.D.L.; Tsai, S.Y.; Hou, Y.C.; Hseu, Y.C. Antioxidant and anti-inflammatory potential of hesperetin metabolites obtained from hesperetin-administered rat serum: An ex vivo approach. *J. Agric. Food Chem.* 2012, 60, 522–532. [CrossRef]

41. Shin, K.C.; Nam, H.K.; Oh, D.K. Hydrolysis of flavanone glycosides by β-glucosidase from Pyrococcus furiosus and its application to the production of flavanone aglycones from citrus extracts. *J. Agric. Food Chem.* 2013, 61, 11532–11540. [CrossRef]

42. Jeon, J.; Ma, S.-Y.; Choi, D.; Kang, J.; Nam, Y.; Yoon, S.; Park, S. Radiosynthesis of 123I-labeled hesperetin for biodistribution study of orally administered hesperetin. *J. Radioanal. Nucl. Chem.* 2015, 306, 437–443. [CrossRef]

43. Chua, L.S. A review on plant-based rutin extraction methods and its pharmaceutical activities. *J. Ethnopharmacol.* 2013, 150, 805–817. [CrossRef] [PubMed]

44. Choi, M.H.; Rho, J.K.; Kang, J.A.; Shim, H.E.; Nam, Y.R.; Yoon, S.; Kim, H.R.; Choi, D.S.; Park, S.H.; Jang, B.-S.; et al. Efficient radiolabeling of rutin with 125I and biodistribution study of radiolabeled rutin. *J. Radioanal. Nucl. Chem.* 2016, 308, 477–483. [CrossRef]

45. Antonisamy, P.; Agastian, P.; Kang, C.W.; Kim, N.S.; Kim, J.H. Anti-inflammatory activity of rhein isolated from the flowers of *Cassia fistula* L. and possible underlying mechanisms. *Saudi J. Biol. Sci.* 2019, 26, 96–104. [CrossRef] [PubMed]
46. Zhang, D.; Jin, Q.; Ni, Y.; Zhang, J. Discovery of necrosis avidity of rhein and its applications in necrosis imaging. J. Drug Target. 2020, 28, 904–912. [CrossRef]

47. Wang, Q.; Yang, S.; Jiang, C.; Li, J.; Wang, C.; Chen, L.; Jin, Q.; Song, S.; Feng, Y.; Ni, Y.; et al. Discovery of Radioiodinated Monomeric Anthraquinones as a Novel Class of Necrosis Avid Agents for Early Imaging of Necrotic Myocardium. Sci. Rep. 2016, 6, 21341. [CrossRef]

48. Al-Sharif, I.; Remmal, A.; Aboussekhra, A. Eugenol triggers apoptosis in breast cancer cells through E2F1/survivin down-regulation. BMC Cancer 2013, 13, 600. [CrossRef]

49. Vidiyva, N.; Devaraj, S.N. Induction of apoptosis by eugenol in human breast cancer cells. Indian J. Exp Biol. 2011, 49, 871–878.

50. Ghosh, R.; Ganapathy, M.; Alworth, W.L.; Chan, D.C.; Kumar, A.P. Combination of 2-methoxyestradiol (2-ME2) and eugenol for apoptosis induction synergistically in androgen independent prostate cancer cells. J. Steroid Biochem. Mol. Biol. 2009, 113, 25–35.

51. Dervis, E.; Kilcar, A.Y.; Medine, E.I.; Tekin, V.; Uygur, E.; Muftuler, F.Z.B. In Vitro Incorporation of Radioiodinated Eugenol on Adenocarcinoma Cell Lines (Caco2, MCF7, and PC3). Cancer Biother. Radiopharm. 2017, 32, 75–81. [CrossRef]

52. Gibellini, L.; Pinti, M.; Nasi, M.; Montagna, J.P.; De Biasi, S.; Roat, E.; Bertoncelli, L.; Cooper, E.L.; Cossarizza, A. Quercetin and cancer chemoprevention. Evid Based Complement. Altern. Med. 2011, 2011, 591356. [CrossRef] [PubMed]

53. Jin, F.; Wu, Q.; Lu, Y.F.; Gong, Q.H.; Shi, J.S. Neuroprotective effect of resveratrol on 6-OHDA-induced Parkinson’s disease in rats. Front. Neurosci. 2012, 6, 532–541. [CrossRef]

54. Leis, K.; Baska, A.; Bereźnicka, W.; Marjańska, A.; Mazur, E.; Lewandowski, B.T.; Kałózny, K.; Gałazka, P. Resveratrol in the treatment of neuroblastoma: A review. Molecules 2018, 23, 797–809. [CrossRef] [PubMed]

55. Palensina, G.; Rosita, L.; Sagala, Z. Isolation of Arbutin from Leaves and Fruits of Buni (Antidesma Bunius L. Spreng) As Tyrosinase Enzyme Inhibitor. Bioinform. Biomed. Res. J. 2021, 4, 8–18. [CrossRef]

56. Ebadollahi, S.H.; Pouramir, M.; Zabihi, E.; Golpour, M.; Aghajanpour-Mir, M. The Effect of Arbutin on The Expression of Tumor Suppressor P53, BAX/BCL-2 Ratio and Oxidative Stress Induced by Tert-Butyl Hydroperoxide in Fibroblast and LNCap Cell Lines. Cell J. 2012, 22, 532–541. [CrossRef]

57. Huynh, P.T.; Ha, Y.S.; Lee, W.; Yoo, J. Radio-Iodinated arbutin for tumor imaging. J. Radiopharm. Mol. Probes 2015, 29, 174–179. [CrossRef]

58. Liang, J.; Sun, Z.; Zhang, D.; Yang, S.; Jiang, C.; Li, J.; Wang, C.; Chen, L.; Jin, Q.; Song, S.; Feng, Y.; Ni, Y.; et al. Discovery of Radioiodinated Flavonoids as Necrosis-Avid Agents and Application in Early Assessment of Tumor Necrosis. Mol. Pharm. 2018, 15, 207–215. [CrossRef]

59. Chen, G.; Pi, X.M.; Yu, C.Y. A new naphthalenone isolated from the green walnut husks of Juglans mandshurica Maxim. Nat. Prod. Res. 2015, 29, 174–179. [CrossRef]

60. Su, C.; Zhang, D.; Bao, N.; Ji, A.; Feng, Y.; Chen, L.; Ni, Y.; Zhang, J.; Yin, Z.-Q. Evaluation of Radioiodinated 1,4-Naphthoquinones as Necrosis Avid Agents for Rapid Myocardium Necrosis Imaging. Mol. Imaging Biol. 2017, 20, 74–84. [CrossRef]

61. Chen, C.A.; Chang, H.H.; Kao, C.Y.; Tsai, J.H.; Chen, Y.J. Plumbagin, isolated from Plumbago zeylanica, induces cell death through apoptosis in human pancreatic cancer cells. Pancreatology 2009, 9, 797–809. [CrossRef]

62. Aminin, D.; Polonik, S. 1,4-Naphthoquinones: Some Biological Properties and Application. Chem. Pharm. Bull. 2020, 68, 46–57. [CrossRef] [PubMed]

63. Peñalver, P.; Belmonte-Reche, E.; Adán, N.; Caro, M.; Mateos-Martín, M.L.; Delgado, M.; González-Rey, E.; Morales, J.C. Alkylated resveratrol prodrugs and metabolites as potential therapeutics for neurodegenerative diseases. Eur. J. Med. Chem. 2018, 146, 123–138. [CrossRef]

64. Jin, F.; Wu, Q.; Lu, Y.F.; Gong, Q.H.; Shi, J.S. Neuroprotective effect of resveratrol on 6-OHDA-induced Parkinson’s disease in rats. Eur. J. Pharmacol. 2008, 600, 78–82. [CrossRef]

65. Guimón, J.; Guimón, P. How ready-to-use therapeutic food shapes a new technological regime to treat child malnutrition. Technol. Forecast. Soc. Chang. 2012, 79, 1319–1327. [CrossRef]

66. Leis, K.; Baska, A.; Bereźnicka, W.; Marjańska, A.; Mazur, E.; Lewandowski, B.T.; Kahužny, K.; Gaļazka, P. Resveratrol in the treatment of neuroblastoma: A review. Rev. Neurosci. 2020, 31, 873–881. [CrossRef] [PubMed]

67. Caratay, K.B.; Kilcar, A.Y.; Guldú, O.K.; Medine, E.I.; Muftuler, F.Z.B. Isolation of resveratrol from peanut sprouts, radioiodination and investigation of its bioactivity on neuroblastoma cell lines. J. Radioanal. Nucl. Chem. 2020, 325, 75–84. [CrossRef]

68. Sakai, T.; Kogiso, M. Soy isoﬂavones and immunity. J. Med. Investig. 2008, 55, 167–173. [CrossRef]

69. Yuseran, H.; Hartoyo, E.; Nurseta, T.; Kalim, H. Molecular docking of genistein on estrogen receptors, promoter region of BCLX, caspase-3, Ki-67, cyclin D1, and telomere activity. J. Taibah Univ. Med. Sci. 2019, 14, 79–87. [CrossRef]

70. Rajah, T.T.; Du, N.; Drews, N.; Cohn, R. Genistein in the presence of 17beta-estradiol inhibits proliferation of ERbeta breast cancer cells. Pharmacology 2009, 84, 68–73. [CrossRef]

71. Ramdhani, D.; Widyasari, E.M.; Sriyani, M.E.; Armanda, Q.P.; Watabe, H. Iodine-131 labeled genistein as a potential radiotracer for breast cancer. Heliyon 2020, 6, e04780. [CrossRef]

72. Sp, N.; Kang, D.Y.; Lee, J.M.; Bae, S.W.; Kang, K.J. Potential Antitumor Effects of 6-Gingerol in p53-Dependent Mitochondrial Apoptosis and Inhibition of Tumor Sphere Formation in Breast Cancer Cells. Int. J. Mol. Sci. 2021, 22, 4660. [CrossRef] [PubMed]

73. Ray, A.; Vasudevan, S.; Sengupta, S. 6- Shogaol Inhibits Breast Cancer Cells and Stem Cell-Like Spheroids by Modulation of Notch Signaling Pathway and Induction of Autophagic Cell Death. PLoS ONE 2015, 10, e0137614. [CrossRef] [PubMed]
74. Karatay, K.B.; Kulçar, A.Y.; Derviş, E.; Müftüer, F.Z.B. Radioiodinated Ginger Compounds (6-gingerol and 6-shogaol) and Incorporation Assays on Breast Cancer Cells. Anticancer Agents Med. Chem. 2020, 20, 1129–1139. [CrossRef]

75. Ince, I.; Yıldırım, Y.; Güler, G.; Medine, E.; Ballica, G.; Kışdemir, B.C.; Göker, E. Synthesis and characterization of folic acid-chitosan nanoparticles loaded with thymoquinone to target ovarian cancer cells. J. Radioanal. Nucl. Chem. 2020, 324, 71–85. [CrossRef]

76. Woo, C.C.; Kumar, A.P.; Sethi, G.; Tan, K.H. Thymoquinone: Potential cure for inflammatory disorders and cancer. Biochem. Pharm. 2012, 83, 443–451. [CrossRef] [PubMed]

77. Sriyani, M.E.; Nuraeni, W.; Rosyidiah, E.; Widyasari, E.M.; Saraswati, A.; Shintia, M. Quality control and stability study of the [131]I-rutin produced in acidic condition. AIP Conf. Proc. 2021, 2381, 020802. [CrossRef]

78. Karatay, N.; Pruthi, V. Potential applications of ferulic acid from natural sources. Biotechnol. Rep. 2014, 4, 86–93. [CrossRef]

79. Bulas, S.; Bedoukian, E.C.; O'Neil, E.C.; Krantz, I.D.; Yum, S.W.; Liu, G.T.; Aleman, T.S. Ocular Biomarkers of Riboflavin Ischemic Stroke with Radioiodinated Riboflavin in Rat MCAO Models via Riboflavin Transporter Targeting. ACS Chem. Neurosci. 2012, 3, 2584–2593. [CrossRef]

80. Destito, G.; Yeh, R.; Rae, C.S.; Finn, M.G.; Manchester, M. Folic Acid-Mediated Targeting of Cowpea Mosaic Virus Particles to Tumor Cells. Chem. Biol. 2007, 14, 1152–1162. [CrossRef]

81. Zhang, X.D.; Wu, Q.; Yang, S.H. Ferulic acid promoting apoptosis in human osteosarcoma cell lines. Biochem. Pharm. 2012, 83, 443–451. [CrossRef] [PubMed]

82. Sedik, G.A.; Rizq, R.S.A.; Ibrahim, I.T.; Elzanfaly, E.S.; Motaleb, M.A. Miniaturized chromatographic systems for radiochemical purity evaluation of (131)I-Ferulic acid as a new candidate in nuclear medicine applications. Appl. Radiat. Isot. 2021, 167, 109370. [CrossRef] [PubMed]

83. Subramanian, V.S.; Sabui, S.; Teafatiller, T.; Bohl, J.A.; Said, H.M. Structure/functional aspects of the human riboflavin transporter-SLC52A3: Role of the predicted glycosylation and substrate-interacting sites. Am. J. Physiol.-Cell Physiol. 2017, 313, C228–C238. [CrossRef]

84. Selim, A.A.; Essa, B.M.; Abdelmonem, I.M.; Amin, M.A.; Sarhan, M.O. Extraction, purification and radioiodination of Khellin as cancer theranostic agent. Appl. Radiat. Isot. 2021, 178, 109970. [CrossRef] [PubMed]

85. Li, J.; Chen, Y.; Peng, C.; Hong, X.; Liu, H.; Fang, J.; Zhuang, R.; Pan, W.; Zhang, D.; Guo, Z.; et al. Micro-SPECT Imaging of Acute Metalloproteinase-12. J. Radioanal. Nucl. Chem. 2015, 302, 1163–1168. [CrossRef] [PubMed]

86. Taha, F.; Malviya, G.; Blair, A.; Boyd, M.; Chalmers, A.J.; Sutherland, A.; Pimlott, S.L. Synthesis and Evaluation of a Phosphorylase Inhibitor and Its Preliminary Evaluation as a Potential SPECT Tracer for Angiogenic Enzyme Expression. J. Med. Chem. 2014, 57, 109370. [CrossRef] [PubMed]

87. Yeh, Y.C.; Liu, T.J.; Lai, H.C. Shikonin Induces Apoptosis, Necrosis, and Premature Senescence of Human A549 Lung Cancer Tumor Cells through Upregulation of p53 Expression. Evid.-Based Complement. Altern. Med. 2015, 2015, 620383. [CrossRef]

88. Li, J.; Chen, Y.; Peng, C.; Hong, X.; Liu, H.; Fang, J.; Zhuang, R.; Pan, W.; Zhang, D.; Guo, Z.; et al. Micro-SPECT Imaging of Acute Ischemic Stroke with Radioiodinated Riboflavin in Rat MCAO Models via Riboflavin Transporter Targeting. ACS Chem. Neurosci. 2022, 13, 1966–1973. [CrossRef]

89. Takahashi, M.; Seki, K.-i.; Nishijima, K.-i.; Zhao, S.; Kuge, Y.; Tamaki, N.; Ohkura, K. Synthesis of a radioiodinated thymidine phosphorylase inhibitor and its preliminary evaluation as a potential SPECT tracer for angiogenic enzyme expression. J. Label. Compd. Radiopharm. 2008, 51, 384–387. [CrossRef]

90. Zhang, X.D.; Wu, Q.; Yang, S.H. Ferulic acid promoting apoptosis in human osteosarcoma cell lines. Pak. J. Med. Sci. 2017, 33, 127–131. [CrossRef]

91. Jeung, Y.J.; Kim, H.G.; Ahn, J.; Lee, H.J.; Lee, S.B.; Won, M.; Jung, C.R.; Im, J.Y.; Kim, B.K.; Park, S.K.; et al. Shikonin induces apoptosis of lung cancer cells via activation of FOXO3a/EGFR/SIRT1 signaling antagonized by p300. Biochim. Biophys. Acta 2016, 1863, 2584–2593. [CrossRef]

92. Selim, A.A.; Motaleb, M.A.; Fayez, H.A. Lung Cancer-Targeted [131I]-Iodoshikonin as Theranostic Agent: Radiolabeling, In Vivo Pharmacokinetics and Biodistribution. Pharm. Chem. J. 2020, 55, 1163–1168. [CrossRef]

93. Moustaq, S.; Jeon, J.; Shaheen, A.; Jang, B.S.; Park, S.H. Critical analysis of radioiodination techniques for micro and macro organic molecules. J. Radioanal. Nucl. Chem. 2016, 309, 859–899. [CrossRef]

94. Subramanian, V.S.; Sabui, S.; Teafatiller, T.; Bohl, J.A.; Said, H.M. Structure/functional aspects of the human riboflavin transporter-SLC52A3: Role of the predicted glycosylation and substrate-interacting sites. Am. J. Physiol.-Cell Physiol. 2017, 313, C228–C238. [CrossRef]

95. Nagaraju, Y.; Ravi, R.; Sivadas, A.; Chandrupatla, G.; Nagendra, H.; Yarlagadda, S.; Malviya, G.; Parthasarathy, R. Synthesis, T-2 toxin by lactic acid bacteria. Food Sci. Biotechnol. 2012, 21, 1677–1683. [CrossRef]

96. Sadeghzadeh, M.; Daha, F.J.; Sheibani, S.; Erfani, M. Radioiodination of 4-benzyl-1-(3-iodobenzylsulfonyl)piperidine, 4-(3-iodobenzyl)-1-(benzylsulfonyl)piperazine and their derivatives via isotopic and non-isotopic exchange reactions. J. Radioanal. Nucl. Chem. 2014, 302, 1119–1125. [CrossRef]

97. Lee, Y.-S. Radiopharmaceuticals for Molecular Imaging. Open Nucl. Med. J. 2010, 2, 178–185. [CrossRef]

98. Lee, Y.-S. Radiopharmaceuticals for Molecular Imaging. Open Nucl. Med. J. 2010, 2, 178–185. [CrossRef]

99. Waterhouse, R.N. Determination of lipophilicity and its use as a predictor of blood–brain barrier penetration of molecular imaging agents. Mol. Imaging Biol. 2003, 5, 376–389. [CrossRef]
100. Arnott, J.; Lobo, S. The influence of lipophilicity in drug discovery and design. Expert Opin. Drug Discov. 2012, 7, 863–875. [CrossRef]

101. De Kruijff, N.A.; Huber, W.; Müller, F.; Kansy, M.; Gerber, P.R. Predicting plasma protein binding of drugs: A new approach. Biochem. Pharm. 2002, 64, 1355–1374. [CrossRef]

102. Kratochwil, N.A.; Huber, W.; Walterbeek, H.T.; Denkova, A.G. A Critical Review of Alpha Radionuclide Therapy—How to Deal with Recoiling Daughters? Pharmaceuticals 2015, 8, 321–336. [CrossRef] [PubMed]

103. Ilem-Ozdemir, D.; Ekinci, M.; Gundogdu, E.; Asikoglu, M. Estimating Binding Capability of Radiopharmaceuticals by Cell Culture Studies. Int. J. Med. Nano Res. 2016, 3, 014. [CrossRef]

104. Motaleb, M.A.; Ibrahim, I.T.; Sayyed, M.E.; Awad, G.A.S. (131)I-trazodone: Preparation, quality control and in vivo biodistribution study by intrananal and intravenous routes as a hopeful brain imaging radiopharmaceutical. Rev. Esp Med. Nucl. Imagen Mol. 2017, 36, 371–376. [CrossRef] [PubMed]

105. Rebischung, C.; Hoffmann, D.; Stafeni, L.; Desruet, M.D.; Müftüler, F.; Ichedef, C.A.; Gumuser, F. In vivo biodistribution of 131 I labeled bleomycin for the study of peripheral benzodiazepine binding sites (PBBS). J. Label. Compd. Radiopharm. 2001, 48, 459–459. [CrossRef]

106. Shuryak, I.; Dadachova, E. New Approaches for Modeling Radiopharmaceutical Pharmacokinetics Using Continuous Distributions of Rates. J. Nucl. Med. 2015, 56, 1622–1628. [CrossRef]

107. Avcıbaşı, U.; Demiroğlu, H.; Ballinger, J.; Elsinga, P.; Ferrari, V.; Peitl, P.K.; Todde, S.; Mindt, T.L. Position paper on requirements for toxicological studies in the specific case of radiopharmaceuticals. EJNMNI Radiopharm. Chem. 2016, 1, 1. [CrossRef]

108. Koziorowski, J.; Behe, M.; Decristiforo, C.; Avcıbaşı, U.; Demiroğlu, H.; Unak, P.; Müftüler, F.; Ichedef, C.A.; Gumuser, F. Oxidative radiodination of aripiprazole by chloramine-T as a route to a potential brain imaging agent: A mechanistic approach. Radiopharmaceuticals 2015, 55, 116–122. [CrossRef]

109. El-Tawoosy, M.; Ibrahim, I. Radioiodination and biological evaluation of salbutamol as a β2-adrenoceptor agonist. Radiochemistry 2012, 54, 401–406. [CrossRef]

110. El-Azony, K.; El-Mehty, A.; Seddik, U.; Khater, S. Radioiodination and bioevaluation of nitrofurantoin for urinary tract imaging. J. Label. Compd. Radiopharm. 2012, 55, 315–319. [CrossRef]

111. Kiess, A.P.; Mirn, I.; Chen, Y.; Hobbs, R.; Sgouros, G.; Mease, R.; Pullambhatla, M.; Shen, C.J.; Foss, C.A.; Pomper, M.G. Auger therapeutic applications of commonly used radiopharmaceuticals in diagnostic nuclear medicine—A review. J. Nucl. Med. Mol. Imaging 2011, 38, 2269–2281. [CrossRef]

112. El-Tawoosy, M.; Ibrahim, I. Radioiodination and biological evaluation of salbutamol as a β2-adrenoceptor agonist. Radiochemistry 2012, 54, 401–406. [CrossRef]

113. Amin, A.; Soliman, S.; El-Aziz, H.; El-Enein, S. Radioiodination of Zaleplon and Its in-vivo Biologic Behavior in Mice: An Imaging Probe for Brain. Int. J. Chem. 2013, 6, 17. [CrossRef]

114. Amin, A.; Soliman, S.; El-Aziz, H.; El-Enein, S. Radioiodination of Zaleplon and Its in-vivo Biologic Behavior in Mice: An Imaging Probe for Brain. Int. J. Chem. 2013, 6, 17. [CrossRef]

115. Amin, A.; Soliman, S.; El-Aziz, H.; El-Enein, S. Radioiodination of Zaleplon and Its in-vivo Biologic Behavior in Mice: An Imaging Probe for Brain. Int. J. Chem. 2013, 6, 17. [CrossRef]

116. Amin, A.; Soliman, S.; El-Aziz, H.; El-Enein, S. Radioiodination of Zaleplon and Its in-vivo Biologic Behavior in Mice: An Imaging Probe for Brain. Int. J. Chem. 2013, 6, 17. [CrossRef]

117. Amin, A.; Soliman, S.; El-Aziz, H.; El-Enein, S. Radioiodination of Zaleplon and Its in-vivo Biologic Behavior in Mice: An Imaging Probe for Brain. Int. J. Chem. 2013, 6, 17. [CrossRef]

118. Amin, A.; Soliman, S.; El-Aziz, H.; El-Enein, S. Radioiodination of Zaleplon and Its in-vivo Biologic Behavior in Mice: An Imaging Probe for Brain. Int. J. Chem. 2013, 6, 17. [CrossRef]

119. Amin, A.; Soliman, S.; El-Aziz, H.; El-Enein, S. Radioiodination of Zaleplon and Its in-vivo Biologic Behavior in Mice: An Imaging Probe for Brain. Int. J. Chem. 2013, 6, 17. [CrossRef]

120. Amin, A.; Soliman, S.; El-Aziz, H.; El-Enein, S. Radioiodination of Zaleplon and Its in-vivo Biologic Behavior in Mice: An Imaging Probe for Brain. Int. J. Chem. 2013, 6, 17. [CrossRef]

121. Amin, A.; Soliman, S.; El-Aziz, H.; El-Enein, S. Radioiodination of Zaleplon and Its in-vivo Biologic Behavior in Mice: An Imaging Probe for Brain. Int. J. Chem. 2013, 6, 17. [CrossRef]

122. Amin, A.; Soliman, S.; El-Aziz, H.; El-Enein, S. Radioiodination of Zaleplon and Its in-vivo Biologic Behavior in Mice: An Imaging Probe for Brain. Int. J. Chem. 2013, 6, 17. [CrossRef]

123. Amin, A.; Soliman, S.; El-Aziz, H.; El-Enein, S. Radioiodination of Zaleplon and Its in-vivo Biologic Behavior in Mice: An Imaging Probe for Brain. Int. J. Chem. 2013, 6, 17. [CrossRef]

124. Amin, A.; Soliman, S.; El-Aziz, H.; El-Enein, S. Radioiodination of Zaleplon and Its in-vivo Biologic Behavior in Mice: An Imaging Probe for Brain. Int. J. Chem. 2013, 6, 17. [CrossRef]

125. Amin, A.; Soliman, S.; El-Aziz, H.; El-Enein, S. Radioiodination of Zaleplon and Its in-vivo Biologic Behavior in Mice: An Imaging Probe for Brain. Int. J. Chem. 2013, 6, 17. [CrossRef]
126. Molavipordanjani, S.; Tolmachev, V.; Hosseinimehr, S.J. Basic and practical concepts of radiopharmaceutical purification methods. *Drug Discov. Today* **2019**, *24*, 315–324. [CrossRef] [PubMed]

127. Amin, A.; Farrag, N.; AbdEl-Bary, A. Iodine-125-Chlorambucil as Possible Radio Anticancer for Diagnosis and Therapy of Cancer: Preparation and Tissue Distribution. *Br. J. Pharm. Res.* **2014**, *4*, 1873–1885. [CrossRef]

128. Spetz, J.; Rudqvist, N.; Forssell-Aronsson, E. Biodistribution and dosimetry of free 211At, 125I- and 131I- in rats. *Cancer Biother. Radiopharm.* **2013**, *28*, 657–664. [CrossRef]

129. Cavina, L.; van der Born, D.; Klaren, P.H.M.; Feiters, M.C.; Boerman, O.C.; Rutjes, F. Design of Radioiodinated Pharmaceuticals: Structural Features Affecting Metabolic Stability towards in Vivo Deiodination. *Eur. J. Org. Chem.* **2017**, *2017*, 3387–3414. [CrossRef]

130. Kim, E.J.; Kim, B.S.; Choi, D.B.; Chi, S.G.; Choi, T.H. Enhanced tumor retention of radioiodinated anti-epidermal growth factor receptor antibody using novel bifunctional iodination linker for radioimmunotherapy. *Oncol. Rep.* **2016**, *35*, 3159–3168. [CrossRef]

131. Tong, R.; Kohane, D.S. New Strategies in Cancer Nanomedicine. *Annu Rev. Pharm. Toxicol.* **2016**, *56*, 41–57. [CrossRef]