Iodination of Isoeugenol Compounds and Its Potential for Therapy of HER-2 Breast Cancer by Molecular Docking

Dadan Suryasaputra, Athina Mardatillah(✉), and Putri Nurfadilah

Faculty of Pharmacy, Universitas Jenderal Achmad Yani, Cimahi, Indonesia
athinamardatillah@gmail.com

Abstract. This research used the synthesis of isoeugenol with iodide to improve anticancer activity on the isoeugenol compounds as the first step in the manufacture of radiopharmaceuticals. One of the anticancer mechanisms of isoeugenol is in silico, potentially inhibiting the target molecules on the growth of neoplasms, namely cyclooxygenase 2 (COX-2) and lipoxygenase 5 (LOX-5). Synthesis was conducted by several conditions to get optimum results, such as the oxidizer chloramine T variations of 40, 50, and 60 mg and pH variations of 5.8, 7, and 8. Separation was carried out using the Liquid-Liquid Extraction method, followed by monitoring using TLC with chloroform eluent, then the compound purification by TLC and characterized using a UV-Visible spectrophotometer and an infrared spectrophotometer. To visualize the interaction of the reaction product with the HER-2 protein, molecular docking was carried out using the Autodock tool. The UV spectrum of the synthetic compound had three peaks, namely 274 nm, 268 nm, and 263 nm, while the pure isoeugenol had one peak at 260 nm. The infrared spectrum of isoeugenol compounds with the synthetic compound generally had the same band, and there were differences in the synthesized compounds, namely the presence of C-I bonds, C = C groups of trans alkenes, and O-H groups with intramolecular hydrogen bonds. Based on UV-visible and infrared spectra, synthetic compounds were different from isoeugenol. Statistically, there was no significant difference; iodo-isoeugenol compounds could be formed in all pH and chloramine T variations used. Based on the molecular docking study results, the compound formed from the synthesis (iodo-isoeugenol1) had the greatest potential to interact with HER2 receptors with codes 3POZ and 6TG0 compared to other isomers.

Keywords: HER2 · isoeugenol · optimization · molecular docking · *iodo*-isoeugenol

A. Sri Wahyuni et al. (Eds.): ICB-Pharma 2022, AHCPS 3, pp. 227–239, 2023.
https://doi.org/10.2991/978-94-6463-050-3_19

1 Introduction

Isoeugenol is a compound of group phenylpropanoid, an isomer of eugenol. The synthesis of isoeugenol can be done through the isomerase eugenol using strong bases and high temperatures. Some research results have indicated that isoeugenol has a variety of anticancer activities, one of the mechanisms is in silico, potentially inhibiting the target molecules on the growth of neoplasms, namely cyclooxygenase 2 (COX-2) and lipoxygenase 5 (LOX-5) [1] (Fig. 1).

Iodine and its compounds have wide applications in the health sector. The iodide ion is an iodine atom with a charge of −1. Water-soluble iodine (I-) is a rate-limiting substrate for the synthesis of thyroid hormones used to treat thyroid disorders and the mechanisms underlying iodide transport, currently applied to treat advanced forms of thyroid cancer and non-thyroid cancer with iodide as a radioisotope [2].

Iodides can be widely used to mark medicinal compounds through iodination reactions. However, it is challenging to make iodo-isoeugenol from benzene iodination because benzene is a cyclic compound with each sp^2 hybridized carbon atom. It has six unhybridized p orbitals of delocalized double bonds, providing a negative charge around the carbon atoms that protects the benzene from attacking the nucleophile group. Thus, if iodine is used as a nucleophile in the benzene iodination process, iodobenzene will not be produced. Another method that can be employed to synthesize iodobenzene is iodination through an electrophilic substitution reaction using chloramine T oxidizer [3]. Chloramine T is a strong oxidizer, where the oxidizer can oxidize and facilitate one radioactive iodine atom with protein molecules [4], and chloramine T has a fast reaction rate [5]. The use of radioisotope iodide to treat cancer can be done in the form of radiopharmaceuticals [6].

Therefore, the background of this study is an effort to increase the anticancer activity of iodide and isoeugenol by synthesizing isoeugenol by iodide with a potent oxidizing agent, namely chloramine T.

This study aims to produce compounds suspected to be iodo-isoeugenol and determine the optimum conditions for synthesizing iodo-isoeugenol with the chloramine T oxidizer variations of 40, 50, and 60 mg and pH variations of pH 5.8, 7, and 8. This research is expected to be used as a reference in developing radiopharmaceuticals for cancer diagnostic and therapy.

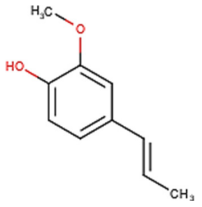


Fig. 1. The Structure of Isoeugenol

2 Research Method

2.1 Material

Isoeugenol, Sodium Iodide, chloramine T, sodium hydroxide (NaOH), Sodium metabisulfite, Silica GF254, chloroform, distilled water, potassium dihydrogen phosphate, ethyl acetate, silica GF254, filter paper and n-hexane.

2.2 Tool

The tools utilized among others were analytical scales equipment-laboratory glassware (Pyrex), separating funnel (Pyrex) 250 mL, pH meter (melter tolledo), magnetic stirrer, pressure vessel ATOS. Instrument: spectrophotometer UV-Vis (Type, Shimadzu), FTIR (Shimadzu ® Affinity R-1). Molecular docking used a laptop set with Core™ i5 processor, 8Gb of RAM, windows™ 10 operating system. The software used was Yasara® ver.21.8.26, Marvin sketch® ver. 20.20.0, Autodock tool® ver.1.5.6, and Discovery studio visualizer® client 2020.

2.3 Research Procedures

2.3.1 Preparation of the Solution

i) Isoeugenol Solution

To prepare a buffer solution with a pH of 5.8; 7; and 8, 50 mL of 0.2 M potassium dihydrogen phosphate was put into a measuring flask, and 200 mL was measured. Then, sodium hydroxide was added, respectively, 3.6 mL, 29.1 mL, and 46.1 mL. Then, water was added up to the mark (Table 1).

ii) Chloramine T Solution

As much as 60, 50, and 40 mg chloramine T were dissolved in 12.5 mL of phosphate buffer pH 5.8, 7, and 8 to obtain a 4.8, 4, and 3.2 mg/mL concentration.

iii) Sodium Metabisulfite Solution

A total of 120 mg of sodium metabisulfite was dissolved to 50 mL of phosphate buffer pH of 5.8, 7, and 8. Then, its concentration was 2.4 mg/mL.

Table 1. The Volume of NaOH into Buffer Solution [9]

pH	5.8	7	8
NaOH (mL)	3.6	29.1	46.1

Table 2. Composition of the Synthesis System. *This composition is a modification [7, 8]

No	Reactants	Number of reactants
	Isoeugenol	0.015 mmol/12.5 mL
	Chloramine T	0.22 mmol/12.5 mL
	Sodium metabisulfite	0.6 mmol/50 mL
	NaI	0.03 mmol/0.5 mL

iv) NaI Solution

As many as 4.75 mg of NaI was dissolved in 0.5 mL of phosphate buffer pH of 5.8, 7, and 8. Thus, the concentration was 4.75 mg/mL (Table 2).

2.3.2 Determination of pH Optimum

The pH variations in the phosphate buffer used were 5.8, 7, and 8. In Erlenmeyer, 12.5 mL of isoeugenol was added with various pH, 0.5 mL NaI, and 12.5 mL chloramine T. Solution was then stirred using a magnetic stirrer for 30 s. Afterward, 50 mL of sodium metabisulfite was added and stirred again, utilizing a magnetic stirrer for 10 s. Furthermore, LLE was carried out using chloroform and water as a solvent with a ratio of 1:1. The nonpolar layer was accommodated in the vial, and the TLC test was carried out.

2.3.3 Optimization Amount of Chloramine T

Variations in the amount of oxidizer used were chloramine T 60 mg/ 2.5 mL, 50 mg/12.5 mL, and 40 mg/12.5 mL for phosphate pH of 5.8, 7, and 8, respectively. A 5 mL of isoeugenol in various pH, 0.5 mL of NaI, and a variation of chloramine T were then stirred using a magnetic stirrer for 30 s, and 50 mL of sodium metabisulfite was added then stirred again using a magnetic stirrer for 10 s. Furthermore, LLE was carried out using chloroform and water as a solvent with a ratio of 1:1. The nonpolar layer was accommodated in the vial, and the thin-layer chromatography (TLC) test was carried out.

2.3.4 Monitoring and Purification of Synthesis Products

The nonpolar solution obtained from the LLE results was spotted using a capillary tube on the GF 254 silica plate measuring 1 × 7 cm, 3 × 7 cm and 8 × 7 cm. Then, elution was conducted using several eluents: ethyl acetate, chloroform, and n-hexane. The chromatogram was viewed under a UV lamp with 254 nm and 365 nm wavelengths. Then, the Rf value was calculated as a parameter of TLC. After obtaining a suitable mobile phase, preparative TLC was continued to obtain pure synthetic compound.

$$\mathrm{Rf} = \frac{distance\ moved\ by\ solute}{distance\ moved\ by\ solvent}$$

2.3.5 Characterization of Synthesis Products

i) Characterization by UV-Vis Spectrophotometer

The filtrate preparative TLC was measured using a UV-Vis spectrophotometer at a wavelength of 200–400 nm, and then the spectrum formed was compared with isoeugenol pure.

ii) Characterization by Infrared Spectrophotometer (IR)

The filtrate preparative TLC that had been dried was mixed with a bit of KBr, crushed to a homogeneous. A mixture of both was stored in the discs and pressed until solid. The spectrum formed from the compound result of the synthesis was then compared with the spectrum of isoeugenol pure.

2.3.6 Molecular Docking

This study began with the validation by redocking the original ligand on the receptor downloaded from the Protein Data Bank (PDB). Downloaded PDB codes must meet less than two resolution criteria and come from the human organism. After the receptor and native ligand were separated and prepared, validation was carried out by redocking. The redocking native ligand conformation was then superimposed with the initial native ligand conformation using the YASARA application. The RMSD (Root Mean Square Deviation) value would appear from the overlapping process. The next stage of this study was the molecular docking of isoeugenol compounds and their iodinated derivatives. The four derivative compounds resulting from the iodination of isoeugenol are based on the possibility of the iodine atom being substituted into the isoeugenol structure.

3 Results and Discussion

The iodination reaction through electrophilic substitution, namely the positively charged iodine (I^+) process, attacks a system with high electron density, such as an aromatic ring or double bonds, so that the result of this reaction will form a carbon-iodide bond and release positively charged species [6]. The possibility of entering the iodide atom is at the alpha carbon, the first carbon attached to the functional group, in addition to having a high electron density, the greater the electronegativity value and bond strength [10], there is also an OH group, which is ortho and para driving substituent [11]. The reaction is predicted as follows in Fig. 2.

The reaction results were monitored using Thin Layer Chromatography (TLC). In TLC, the Retention factor (Rf) parameter was used to see the migration of compounds; the best Rf value is between 0.2–0.8. The optimization result of the mobile phase chosen was chloroform because it gave an Rf value of 0.58 for all synthesized compounds, while for pure isoeugenol, the Rf value was 0.65. The chloroform fraction has not yet got a single spot from TLC monitoring from the ECC results. The preparative TLC method isolates the synthesized compound separated from the impurities and then characterizes it using a UV-Vis spectrophotometer and infrared (IR) spectrophotometer (Fig. 3).

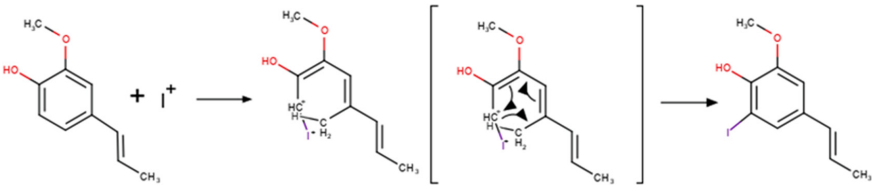


Fig. 2. The Reaction of Iodination of Isoeugenol

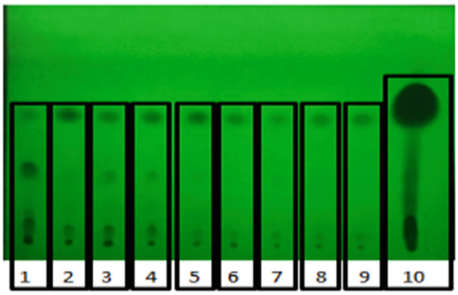


Fig. 3. Monitoring the Fraction of Chloroform with TLC

Description:
1 = The results of the synthesis pH 5.8 with chloramine T 40 mg.
2 = The results of the synthesis pH 5.8 with chloramine T 50 mg.
3 = The results of the synthesis pH 5.8 with chloramine T 60 mg.
4 = The results of the synthesis pH 7 with chloramine T 40 mg.
5 = The results of the synthesis pH 7 with chloramine T 50 mg.
6 = The results of the synthesis pH 7 with chloramine T 60 mg.
7 = The results of the synthesis pH 8 with chloramine T 40 mg.
8 = The results of the synthesis pH 8 with chloramine T 50 mg.
9 = The results of the synthesis pH 8 with chloramine T 60 mg.
10 = Isoeugenol pure.

The UV-Vis spectrum of the synthesized compound showed the formation of several new peaks at the maximum wavelength of 274 nm, 268 nm, and 263 nm with an absorbance value of 0.03–0.1 in the synthesis results of all pH and chloramine T variations. Pure isoeugenol in chloroform showed only one peak with a maximum wavelength at 260.8 nm. Hence, the spectrum of the synthesis results is a different compound from isoeugenol. Iodide is auxochrome that results in a bathochromic shift when attached to a chromophore. The use of chloroform solvent has a cut-off value at a wavelength of 233 nm, so that characterization with chloroform solvent is disturbed, and spectra can be read above λ 250–400 nm (Fig. 4).

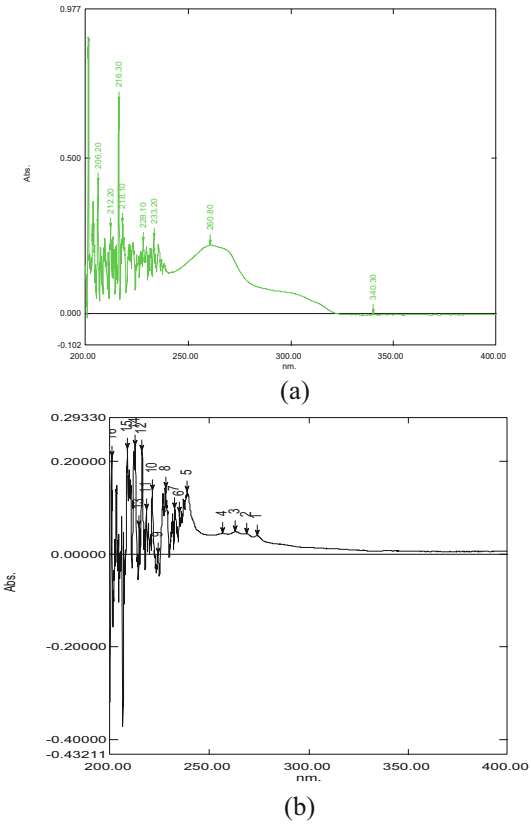


Fig. 4. Spectrum UV of (a) Isoeugenol Pure in the Chloroform and (b) the Results of the Synthesis

The IR spectrum of the synthesized compound is different from the isoeugenol. By comparing the IR spectrum of the synthesized compound with pure isoeugenol, it can be seen that isoeugenol had an absorption band at 3390.9 cm^{-1} (literature 3400–3300 cm^{-1}), showing the presence of an OH phenol group, while the synthesized compound had an absorption band at 3360 cm^{-1} (Fig. 5).

Moreover, the molecule has close donor and acceptor hydrogen bonds, resulting in the preferred intramolecular hydrogen bonding and shifting the band at a weaker frequency. There is an aromatic group C = C in pure isoeugenol, giving it an absorption band of 1512.2 cm^{-1} (1600–1475 cm^{-1}). However, the manufactured compound had a faint absorption, suggesting a change from cis to trans substituted. On the other hand, the generated molecule produced an absorption band at 536 cm^{-1} (600–485 cm^{-1}), indicating the presence of a C-I bond [12]. The IR spectra of the two substances are similar in general (Fig. 6).

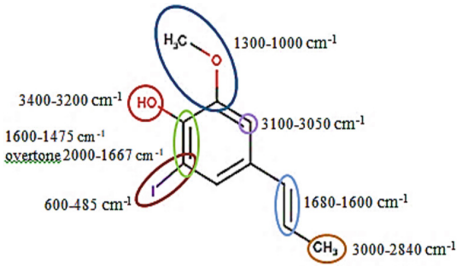


Fig. 5. The Interpretation of the IR Compound Results of the Synthesis

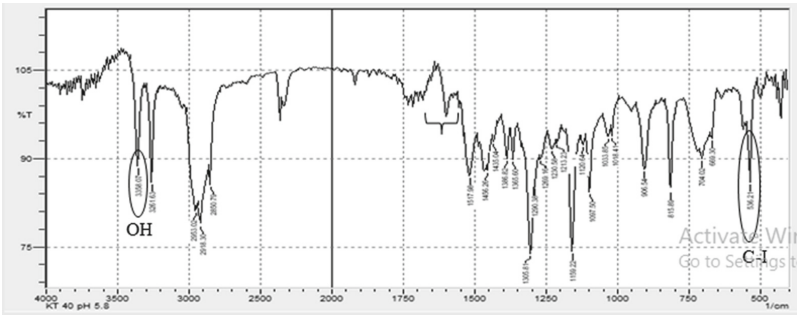
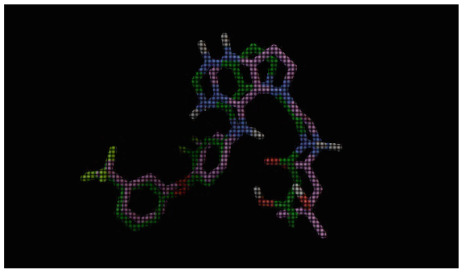


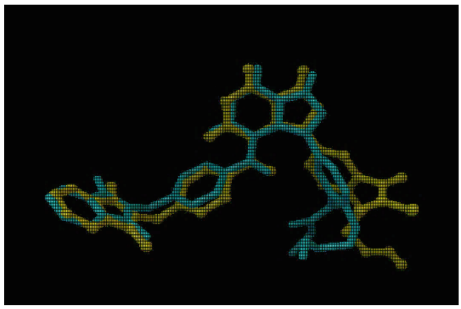
Fig. 6. IR Spectrum of Compound Results from the Synthesis

Table 3. Yield (%) of Synthetic Compounds at All Variations

pH	Chloramine T (mg)	Yield (%)
5.8	40	45.01
	50	17.78
	60	37.96
7	40	23.97
	50	35.16
	60	17.95
8	40	22.37
	50	20.18
	60	28.82



(a)



(b)

Fig. 7. Superimpose Initial Native Ligand (Green Colored) with After Redocking (Red or Yellow Colored) (a) 3POZ (b) 6TG0

Table 4. RMSD Value of Redocking

PDB code	Average value of RMSD (Å)
3POZ	1.156 ± 0.169
6TG0	1.653 ± 0.196

Table 5. Binding Energy (ΔG) and The Inhibition Constant (Ki)

HER2 PDB code	3POZ		6TG0	
Compound	ΔG (Kkal/mol)	Ki (μM)	ΔG (Kkal/mol)	Ki (μM)
Isoeu genol	−6.29	24.33	−7.18	5.49
iodo-isoeugenol1	−7.07	6.59	−9.11	0.21
iodo-isoeugenol2	−6.24	26.49	−7.9	1.62
iodo-isoeugenol3	−7.02	7.21	−8.22	0.94
iodo-isoeugenol4	−6.89	8.89	−8.75	0.38

An ANOVA test was conducted to evaluate the relationship between the variations with yield (Table 3). A significance value of 0.455 indicated no statistically significant difference in variations. It could be concluded that iodo isoeugenol could be formed in the above conditions.

From the TLC results, there was a difference between Rf synthetic compounds and isoeugenol. Then, the UV-Vis and IR spectra showed a similar pattern with isoeugenol, but there was a new peak, namely the C-I bond. Based on that, it can be assumed that synthesized compounds were different from isoeugenol. Then, the calculation using the statistical multiple linear regression ANOVA test revealed no significant difference in the variation in pH and chloramine T used.

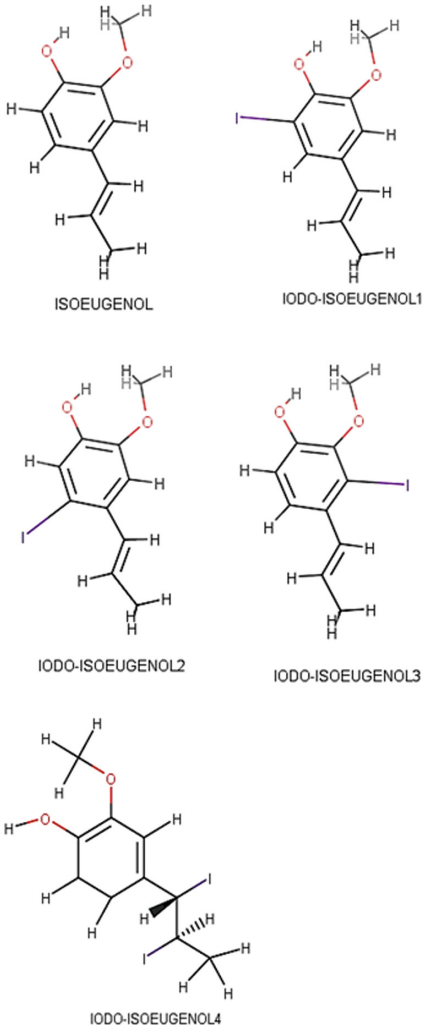
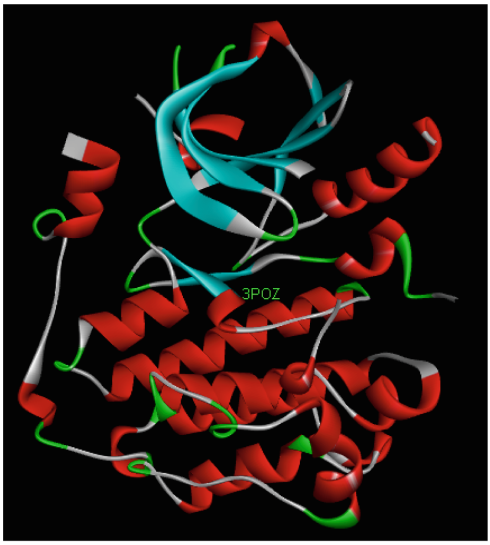


Fig. 8. Structure of the Test Ligand

3.1 Molecular Docking of Synthesized Compounds with HER2 Receptors

The downloaded PDB codes that met the resolution criteria of less than two and came from the human organism were 2 PDB codes, namely 3POZ and 6TG0 codes. After the receptor and native ligand were separated and prepared, the validation was carried out by redocking. The redocking native ligand conformation was then superimposed with the initial native ligand conformation using the YASARA application (Fig. 7). The RMSD (Root Mean Square Deviation) value was obtained from the overlapping process. The



(3POZ)

(6TG0)

Fig. 9. Structure of Target Receptors

RMSD was determined by comparing the position of the native ligand with the redocking ligands (Table 4).

The binding free energy (ΔG) and the inhibition constant (Ki) are shown in Table 5.

Based on the test results, in general, all test ligands could interact with HER2 receptors, both 3POZ and 6TG0, with the lowest binding free energy (ΔG) value = −7.07 kcal/mol for 3POZ receptors and the lowest ΔG value = −9.11 kcal/ mole for the 6TG0 receptor. The value of ΔG or free energy indicates the binding affinity of the ligand to the receptor. The smaller the free energy, the more stable the protein-ligand complex.

The molecular docking test also obtained the value of the inhibition constant (Ki). The inhibition constant (Ki) is the estimated value of the inhibitory ligand concentration on the target receptor. The smaller the value of Ki, the lower the concentration required to block the receptor. Table 5 displays that the lowest Ki value of the test compound was 0.21 μM for the 6TG0 receptor and 6.59 μM for the Ki receptor for the 3POZ receptor (Figs. 8 and 9).

4 Conclusion

1. Based on the Thin Layer Chromatography results, the spectrum of UV-Vis and IR spectrum of the compound synthesized was a different compound from isoeugenol.
2. The ANOVA test results on the variations of pH and chloramine T used showed no significant differences. Thus, the iodo-isoeugenol could be formed at all pH and chloramine T variations.
3. Based on the molecular docking study results, the compound formed from the synthesis (iodo-isoeugenol1) had the most significant potential to interact with HER2 receptors with codes 3POZ and 6TG0 compared to other isomers with the lowest binding energy and inhibition constant.

Recommendation Further characterization is required to ensure the formation of iodo-isoeugenol.

References

1. Zarlaha, A., Kourkoumelis, N., Stanojkovic, T. P., & Kovala-Demertzi, D. (2014). Cytotoxic activity of essential oil and extracts of Ocimum Basilicum against human carcinoma cells. Molecular docking study of isoeugenol as a potent cox and lox inhibitor. *Digest Journal of Nanomaterials and Biostructures*, *9*(3), 907–917.
2. Pesce, L., & Kopp, P. (2014). Iodide transport: implications for health and disease. *International Journal of Pediatric Endocrinology*, 1–12.
3. Oekar, N. K., Ws, A. H., Widyasari, E. M., Daruwati, I., Pradana, A. T., & Luthpi, M. (2013). Aplikasi Iptek Nuklir dalam Pengembangan Obat Bahan Alam, 174–183.
4. Tuasikal, B. ., Handayani, T., Priyatmojo, D., Trinugraha, A. ., Lelananingtyas, N., & Dinasdi. (2017). Teknik Nuklir Untuk Peningkatan Kesehatan Reproduksi Ternak

5. Hostettmann, K., Marston, A., & Hostettmann, M. (1997). *Preparative Chromatography Techniques Application in Natural Product Isolation.* (C. Messerschmidt & Rheinau, Eds.) (Second, Co). Springer.
6. Levita, J. dan M. (2019). *Radioiodinasi Pada Pembuatan Radiofarmaka* (1st ed.). yogyakarta: Deepublish.
7. Hermanson, G. T. (2013). Isotopic Labeling Techniques. *Bioconjugate Techniques*, 507–534.
8. Jesper V, C., Anders, F., & Lars, C. (1991). Iodination of phenols, *388*, 379–388.
9. Kementrian Kesehatan RI. (2014). *Farmakope Indonesia* (V). Jakarta: Kementrian Kesehatan RI.
10. Harborne, J. . (1996). *Metode Fitokimia Penuntun Cara Modern Menganalisis Tumbuhan* (terbitan k). Bandung: Institut Teknologi Bandung.
11. Gibbs, G. V., Hill, F. C., Boisen, M. B., & Downs, R. T. (1998). Power law relationships between bond length, bond strength and electron density distributions. *Physics and Chemistry of Minerals*, *25*(8), 585–590.
12. Ralph, Fessenden J & Joan, Fessenden S. (2010). *Dasar Dasar Kimia Organik*. Alih Bahasa : Dra. Sukmariah Maun, Dra. Kamiantri Anas, Dra. Tilda S. Sally. Binarupa Aksara Publisher, Jakarta. Hal. 569–576
13. Pavia, D. L., Lampman, G. M., Kriz, G. S., & Vyvyan, J. R. (2009). *INTRODUCTION TO SPECTROSCOPY*. (B. Kirksey, Ed.) (4th ed.). Bellingham, Washington: BROOKS/COLE CENGAGE Learning.
14. Elmar Krieger, Gert Vriend, YASARA View—molecular graphics for all devices—from smartphones to workstations, *Bioinformatics*, Volume 30, Issue 20, 15 October 2014, Pages 2981–2982, https://doi.org/10.1093/bioinformatics/btu426
15. Michel F. Sanner. Python: A Programming Language for Software Integration and Development. J. Mol. Graphics Mod., 1999, Vol 17, February. pp57–61