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Synthesis, Characterization, and Docking Study of a Novel Indole Derivative Containing a Tosyl Moiety as Anti-Oxidant Agent

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Abstract: Indole derivatives are key components of natural products and possess a wide range of biological and pharmaceutical applications. Here, we present the synthesis of a new indole derivative, namely 2-(1-ethyl-5-nitro-1*H*-indole-7-carbonyl)butyl 4-methylbenzenesulfonate. The structural elucidation of this compound was accomplished through comprehensive spectroscopic analysis, including Fourier-transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR), and high-resolution mass spectrometry (HRMS). Our molecular docking study revealed that this compound exhibits strong affinity towards tyrosinase, making it a promising candidate as an antioxidant agent.

Keywords: indole derivative; tosyl group; molecular docking; antioxidant agent

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1. Introduction

Tyrosinase is a copper-containing glycoprotein recongized for the oxidation of phenol to *o*-quinone [1] and tyrosine to dopaquinone [2], which is the rate-limiting step in melanin production. This enzyme is also involved in the enzymatic browning of fruits, fungi, and vegetables [3,4]. Therefore, tyrosinase inhibitors are essential in food preservation, cosmetics, and biomedical research applications.

On the other hand, heterocyclic compounds are ubiquitous in pharmaceuticals, appearing in the majority of marketed drugs. Among these, indole derivatives are commonly used in a range array of pharmaceutical applications. This includes their use as anti-cancer [5], antioxidant [6], anti-microbial [7], and anti-viral agents [8]. Remarkably, the introduction of the tosyl group in the structure of indole has yielded numerous bioactive compounds with different activities such as larvicidal effects [9], antioxidant activity [10], and anti-viral activity [11] (Figure 1).

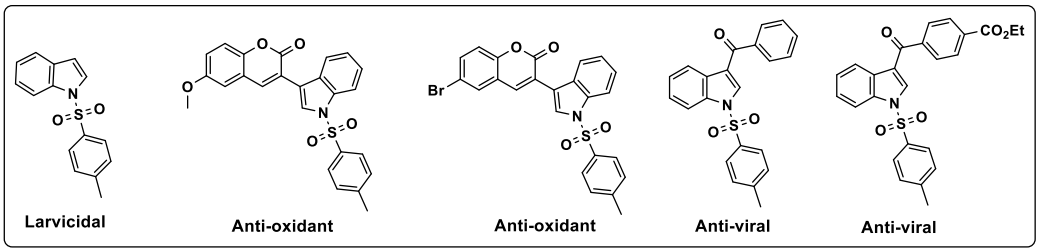


Figure 1. Biological activities of some tosylindole derivatives.

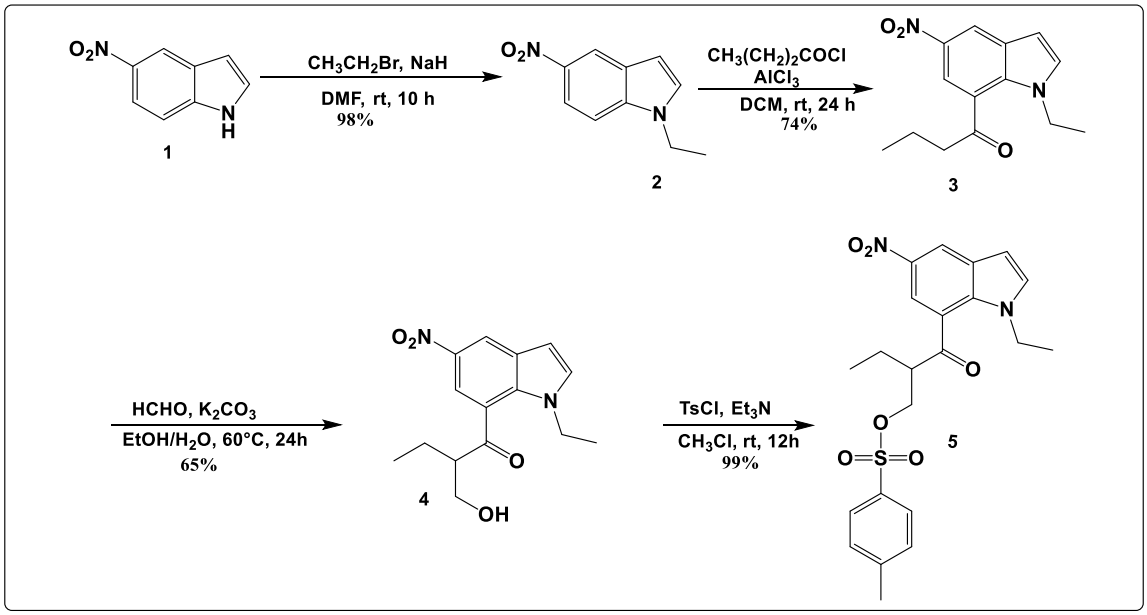
To the best of our knowledge, all bioactive indole derivatives containing a tosyl group are currently reported to be N-tosylated compounds. Interestingly, there are no documented cases in the literature of O-tosyl indoles serving as antioxidant agents. Therefore, we

decided to synthesize a novel indole derivative with an O-tosyl moiety and to study its potential antioxidant activity through molecular docking. Our focus was on evaluating the properties of this O-tosylated analog, given the predominance of N-tosylated derivatives in the existing literature.

2. Results and Discussion

2.1. Synthesis and Characterization

Our targeted compound was prepared according to the synthesis pathway in Scheme 1. The process of synthesis started with the *N*-alkylation of 5-nitroindazole (**1**) using the procedure reported by Bortolozzi, R. et al. [12] to give intermediate **2** in a very good yield (98%). This alkylation step was crucial for protecting the indole nitrogen from reacting with butyryl chloride in the subsequent step and for enhancing the hydrophobic interactions of the final compound with the target enzyme, thereby improving its binding affinity and overall activity. Next, intermediate **2** was acylated with butyryl chloride in the presence of aluminum chloride ($AlCl_3$), affording intermediate **3** with a 74% yield. Thereafter, a condensation reaction using formaldehyde in the presence of potassium carbonate (K_2CO_3) was performed to yield 65% of compound **4**. Lastly, the hydroxy group of **4** was easily tosylated under mild conditions using *p*-toluenesulfonyl chloride in the presence of triethylamine (Et_3N) as a base in chloroform, leading to compound **5** in a very good yield (99%), as illustrated in Scheme 1.



Scheme 1. Synthesis pathway of compound **5**.

The structure of compound **5** was then structurally elucidated through spectroscopic techniques. By HRMS (Figure 2), its molecular formula was determined to be $C_{22}H_{24}N_2O_6S$, with a molecular ion peak at *m*/*z* 445.0154 [M+1]+. FTIR spectroscopy effectively monitored the conversion of the hydroxy group in compound **4** to the tosyl group in compound **5**. In the IR spectrum of compound **4** (Figure 3A), the hydroxy group is indicated by a band at 3451.88 cm^{-1}. This band is absent in the IR spectrum of compound **5** (Figure 3B), confirming the transformation. In the ^1H NMR spectrum (Figure 4), the presence of eight aromatic protons appearing between 7.27 ppm and 9.29 ppm supports the structure of **5**. Furthermore, the presence of a singlet peak in the aliphatic region with an integration of three protons corresponding to Ar-CH_3 further demonstrates the presence of the tosyl group. As expected, the ^{13}C NMR spectrum (Figure 5) indicates the presence of 20 carbons, which is in accordance with the structure of **5**, since there are equivalent carbons in the tosyl group.

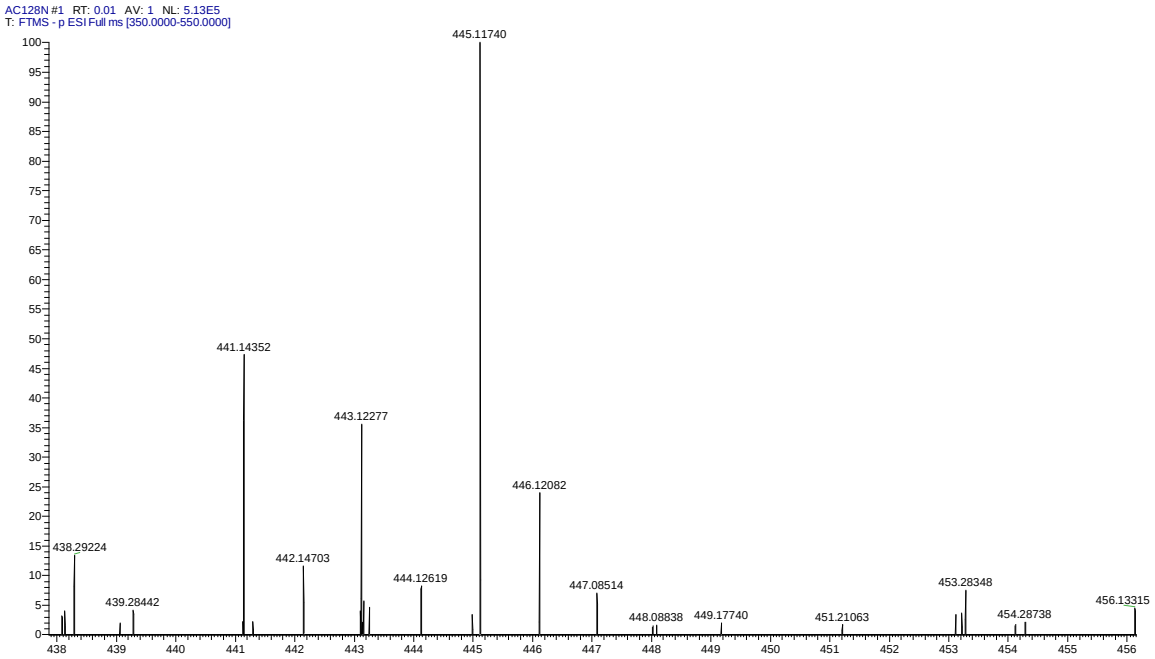


Figure 2. HRMS spectrum of **5**.

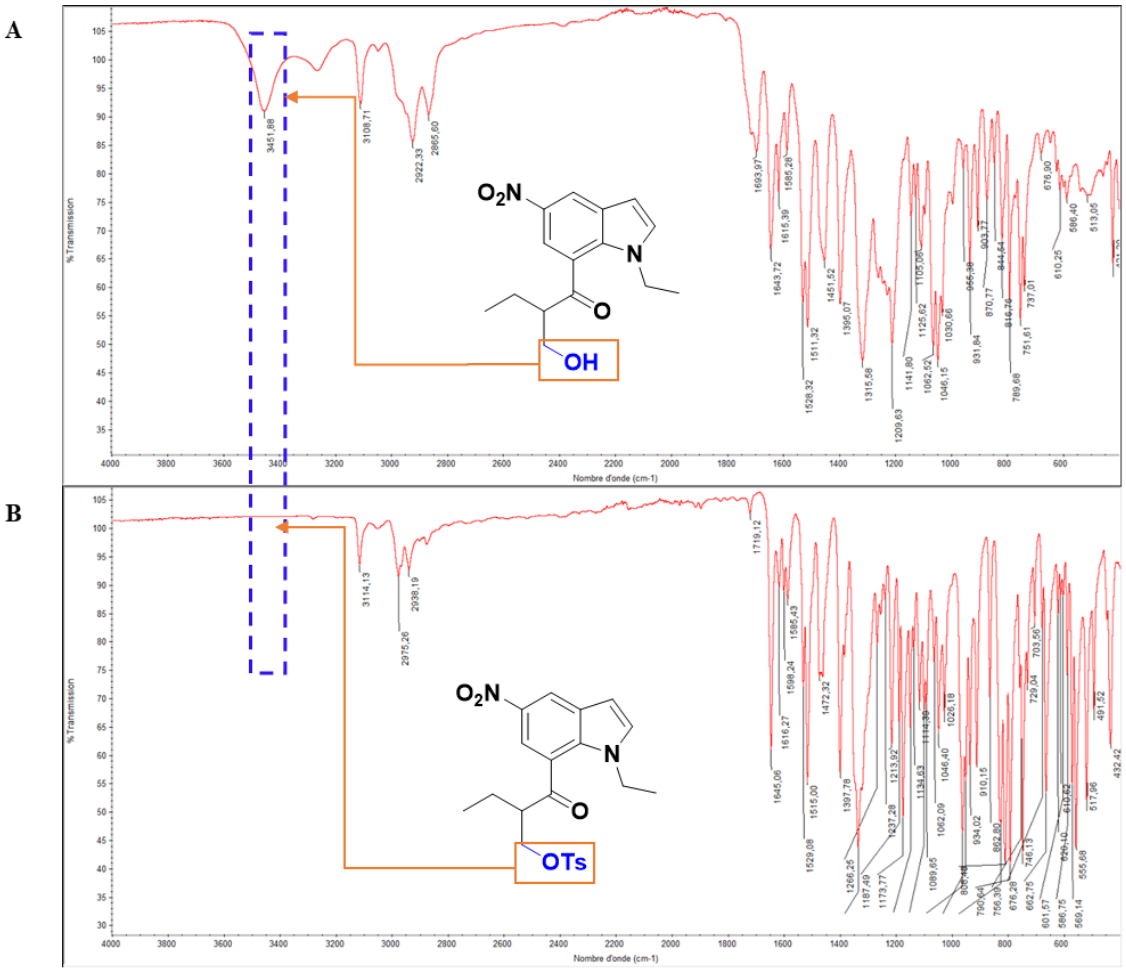


Figure 3. (**A**) the IR spectrum of compound **4;** (**B**) the IR spectrum of compound **5**.

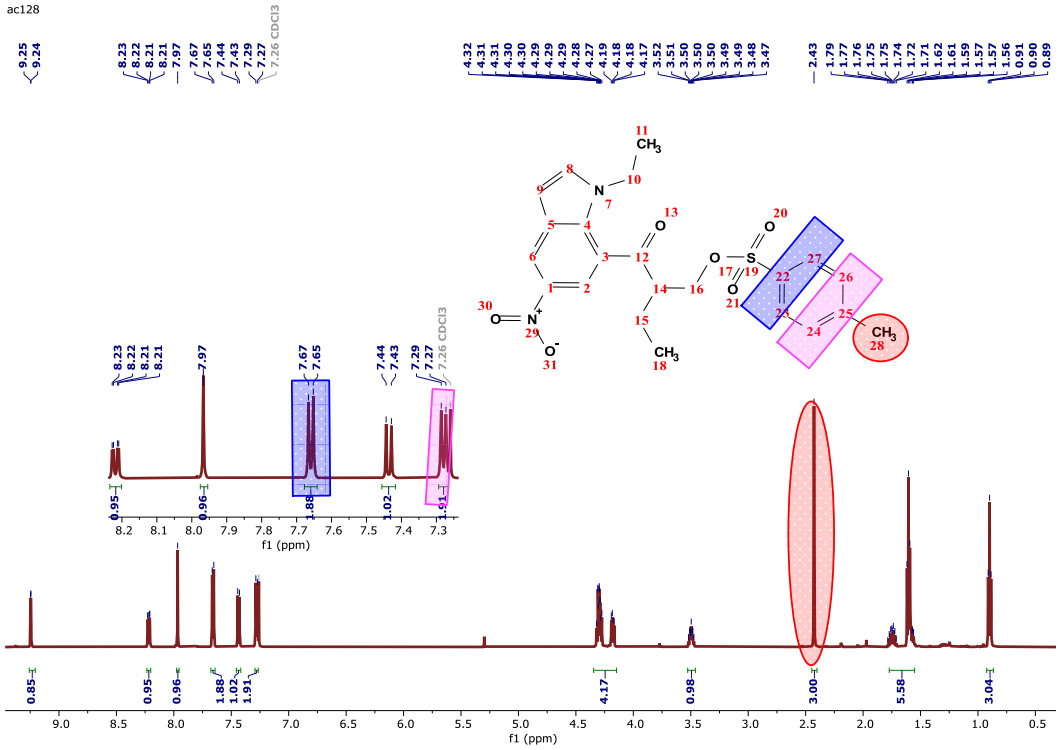


Figure 4. The ^{1}H NMR spectrum of **5** in $CDCl_3$.

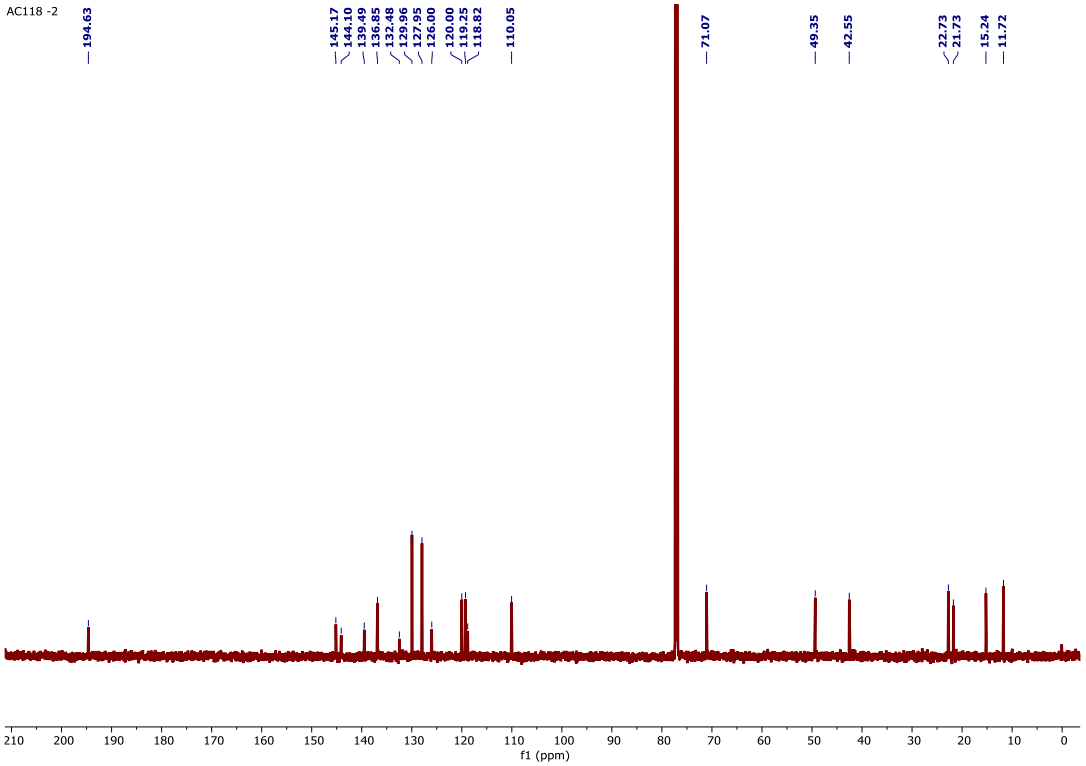


Figure 5. The ^{13}C spectrum of **5** in $CDCl_3$.

2.2. Docking Studies

In order to investigate the possible antioxidant activity of compound **5**, we used the crystal structure of tyrosinase from *Bacillus megaterium* (PDB ID: 3NM8), retrieved from the RSCB protein data bank (https://www.rcsb.org/). As illustrated in Table 1, the

compound presented a docking score in the order of −10.86 Kca/mol which suggested that it could bind to tyrosinase spontaneously, indicating good protein ligand affinity. Enzymatic inhibition can theoretically occur through the ligand occupancy of the enzyme active site by non-covalent interactions [13]. Accordingly, compound **5** potentially possesses potent inhibition capacity as it effectively fits into the active site of tyrosinase and forms favorable interactions with its amino acid residues. This includes hydrogen bonds with Lys47, Π-alkyl, and alkyl–alkyl with Ala 44, Ala 40, Lys 47, Thr 267, Ile 139, and Phe 48 as well as an amide- Π stacking interaction with Ile 39, as shown in Figure 6B,C.

Table 1. Docking results of compound **5**.

Compound	Docking Score (Kcal/mol)	Interactions	Interacting Residues
4	−8.53	H-bond	Ala 44
		Π-alkyl	Lys 47
		Alkyl–alkyl	Ala40, Phe 48
5	−10.86	H-bond	Lys 47
		Π–alkyl	Ala 44, Ala40, Lys 47, Thr267
		Alkyl–alkyl	Ile 139, Phe 48
		Amide–Π stacking	Ile 39

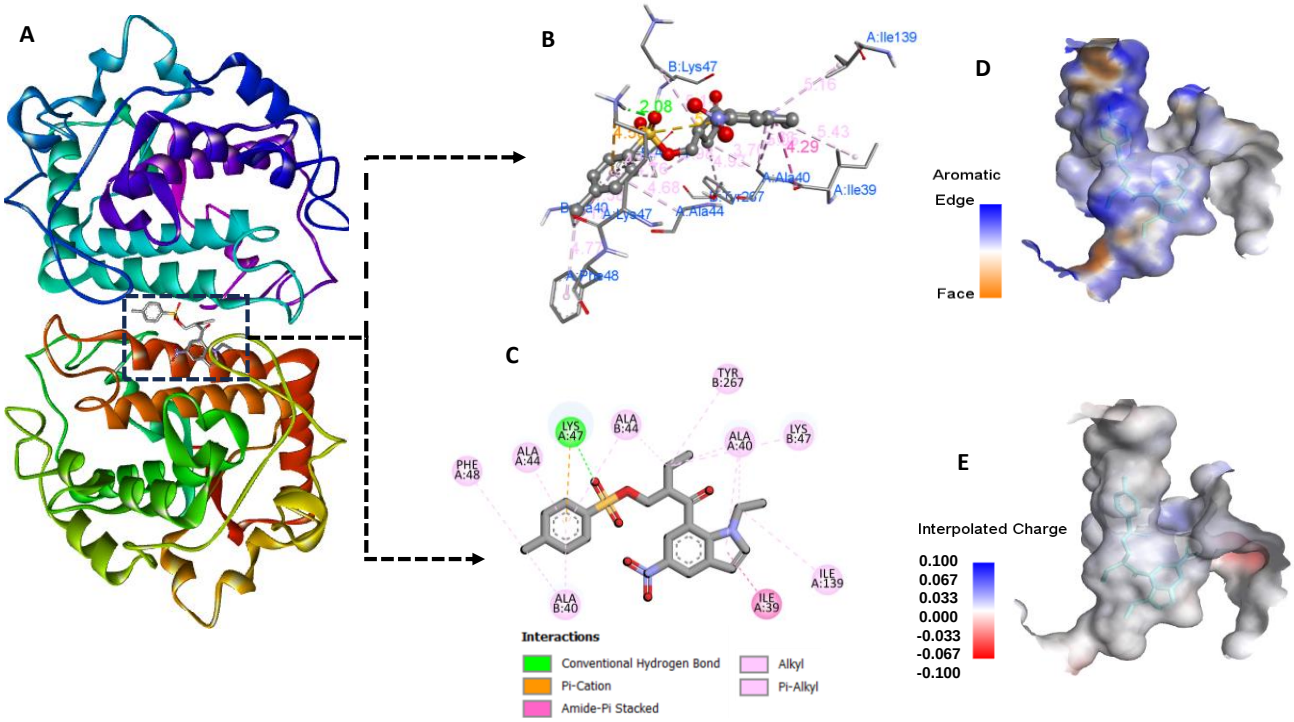


Figure 6. (**A**) Compound **5** fits in the active site of tyrosinase; (**B**,**C**) 3D and 2D representations of interacting residues; (**D**,**E**) surface representations of **5** within tyrosinase active site.

Furthermore, a significant observation was made when comparing compound **5** with its hydroxy analog compound **4**. The introduction of the tosyl group in compound **5** increased its affinity for the target enzyme tyrosinase. This is evidenced by a difference of −2.15 kcal/mol in the docking scores, indicating that compound **5** requires less energy to form a complex with tyrosinase compared to compound **4**. Additionally, while the hydroxy group of compound **4** engages in a single interaction with Ala 44 (Figure 7), the tosyl group engages in several interactions with amino acid residues, making the ligand–protein complex more stable. These interactions include the benzene ring of the tosyl group forming π-π and π–alkyl interactions with Ala 44 and Phe 48, as well as the sulfonyl

group forming hydrogen bonds with Lys 47. While several N-tosyl indole derivatives are currently reported as antioxidant agents [6], these findings indicate that O-tosyl indole derivatives also hold promise as effective antioxidant agents.

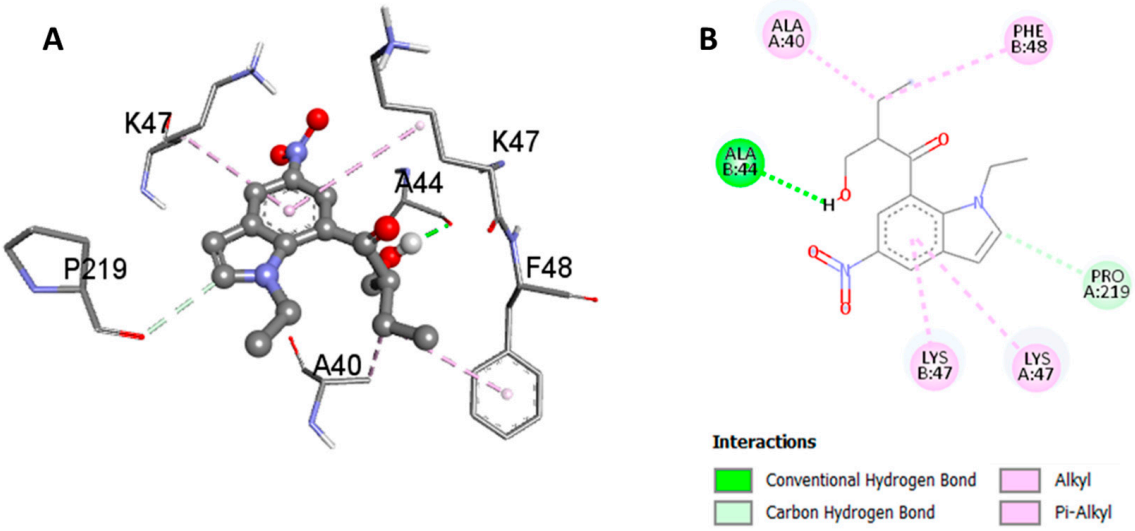


Figure 7. Three-dimensional (**A**) and two-dimensional (**B**) representations of interacting residues with compound **4**.

3. Materials and Methods

3.1. General Procedures

All chemicals utilized in this research were procured from commercial suppliers (Alfa Aesar, Ward Hill, MA, USA and Sigma Aldrich, Burlington, MA, USA) and were employed without further purification. The progression of reactions was tracked through thin-layer chromatography (TLC). The determination of melting points was conducted using the Stuart-SMP30 apparatus (Stuart, Cincinnati, OH, USA). FTIR spectra were recorded in the range of 400–4000 cm^{-1}, using a Thermo Scientific Nicolet IS50 FTIR (Thermo Scientific, Waltham, MA, USA). The ^{1}H and ^{13}C NMR spectra were recorded in an appropriate deuterated solvent solution on a Jeol 600 MHz spectrometer (Jeol, Tokyo, Japan) at room temperature with chemical shifts reported in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal reference. Mass spectra were acquired using an Exactive Plus Orbitrap mass spectrometer coupled with an LC-MS-MS system (Thermo Scientific, Waltham, MA, USA).

3.2. Synthesis

Intermediate **2** was prepared as described in [12]. (See Figure S1 for the ^{1}H NMR spectrum.)

1-(1-Ethyl-5-nitro-1*H*-indol-7-yl)butan-1-one (**3**). (See Figures S2 and S3 for NMR spectra.)

$AlCl_3$ (2 mmol) was added dropwise to a solution of **2** (1.5 mmol) in dichloromethane (DCM) at 0 °C, and the mixture was stirred for 30 min. After allowing the reaction mixture to rise to ambient temperature, butyryl chloride (1.5 mmol) was added, and the resulting mixture was stirred for 12 h. After the total consumption of **2** as indicated by thin-layer chromatography (TLC), the resultant mixture was quenched with water (3 mL) and extracted with ethyl acetate (EtOAc) (2 × 20 mL). The combined organic layers were washed with brine and dried over magnesium sulfate ($MgSO_4$), and the solvent was removed under vacuum. The crude product was then separated by column chromatography elution with hexane/DCM (3/2 (*v*/*v*)) to afford a yellow solid. Yield: 74%. m.p:113–114 °C. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 9.31 (d, *J* = 2.3 Hz, 1H), 8.19 (dd, *J* = 9.1, 2.3 Hz, 1H), 7.90 (s, 1H), 7.40 (d, *J* = 9.0 Hz, 1H), 4.28 (q, *J* = 7.3 Hz, 2H), 2.85 (t, *J* = 7.4 Hz, 2H), 1.82 (h, *J* = 7.4 Hz,

2H), 1.58 (t, *J* = 7.4 Hz, 3H), 1.03 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR ($CDCl_3$, 151MHz) δ 195.32, 143.88, 139.30, 135.69, 125.99, 119.95, 118.86, 118.53, 109.83, 42.32, 41.98, 18.32, 15.29, 14.10. IR (neat cm^{-1}): ν = 1392–1529 (NO_2), 1650 (C=O). HRMS (+ESI) *m*/*z*: $[M+H]^+$ calculated for $C_{14}H_{17}N_2O_3$: 261,1161, found, 261,0845.

1-(1-Ethyl-5-nitro-1*H*-indol-7-yl)-2-(hydroxymethyl)butan-1-one (**4**). (See Figures S4 and S5 for NMR spectra.)

In a vigorously stirred solution of **3** (0.39 mmol) and formaldehyde (1.17 mmol) in ethanol (EtOH) (5mL), a solution of K_2CO_3 (0.47 mmol) in 1 mL of water and 1 mL of EtOH was added. The resulting mixture was stirred for 24 h at 60 °C. After the total consumption of the starting material, confirmed by TLC, the solvent was removed, and the crude product was extracted three times with EtOAc. The resulting organic phases were combined, dried over $MgSO_4$, and then evaporated to dryness. The crude product was subsequently separated by column chromatography, using an eluent composed of DCM/EtOAc (5/2), to obtain a yellow solid. Yield: 65%. m.p: 120–121 °C. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 9.25 (d, *J* = 2.3 Hz, 1H), 8.15 (dd, *J* = 9.1, 1.9 Hz, 1H), 7.96 (s, 1H), 7.39 (d, *J* = 9.0 Hz, 1H), 4.29 (q, *J* = 7.4 Hz, 2H), 3.93 (ddt, *J* = 86.1, 10.5, 4.6 Hz, 2H), 3.26 (dd, *J* = 7.2, 3.9 Hz, 1H), 2.61 (s, 1H OH), 1.76 (ddt, *J* = 73.1, 14.1, 7.0 Hz, 2H), 1.59 (t, *J* = 7.4 Hz, 3H), 0.98 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 199.00, 144.07, 139.46, 136.52, 126.04, 119.99, 119.12, 118.84, 109.94, 63.50, 52.03, 42.46, 22.89, 15.21, 12.21. IR (neat cm^{-1}): ν = 1395–1528 (NO_2), 1693 (C=O), 3451 (OH). HRMS (+ESI) *m*/*z*: $[M+H]^+$ calculated for $C_{15}H_{19}N_2O_4$: 291.1267, found, 291,0738.

2-(1-Ethyl-5-nitro-1*H*-indole-7-carbonyl)butyl 4-methylbenzenesulfonate (**5**).

To a stirred solution of **4** (0.36 mmol) and Et_3N (0.72 mmol) in chloroform ($CHCl_3$) at 0 °C, *p*-toluenesulfonyl chloride (0.54 mmol) was gradually added. The reaction was allowed to progress to room temperature and stirred for 12 h. Upon completion of the reaction, the solvent was evaporated. The crude product underwent extraction three times with DCM (6 mL) and the organic layers were combined, washed with brine, and dried over $MgSO_4$. Subsequently, the solvent was evaporated. Column chromatography elution with (hexane/DCM (1/2)) yielded the desired compound **5** as a white solid. Yield: 99%. m.p: 151–152 °C.^{1}H NMR ($CDCl_3$, 600 MHz) δ 9.25 (d, *J* = 2.3 Hz, 1H), 8.22 (dd, *J* = 9.0, 2.3 Hz, 1H), 7.97 (s, 1H), 7.66 (d, *J* = 8.3 Hz, 2H), 7.44 (d, *J* = 9.0 Hz, 1H), 7.28 (d, *J* = 8.0 Hz, 2H), 4.35 − 4.12 (m, 4H), 3.55 − 3.40 (m, 1H), 2.43 (s, 3H), 1.82 − 1.54 (m, 5H), 0.90 (t, *J* = 7.5 Hz, 3H).^{13}C NMR ($CDCl_3$, 151 MHz) δ 194.63, 145.17, 144.10, 139.49, 136.85, 132.48, 129.96, 127.95, 126.00, 120.00, 119.25, 118.82, 110.05, 71.07, 49.35, 42.55, 22.74, 21.73, 15.24, 11.72. IR (neat cm^{-1}): ν = 1397–1529 (NO_2), 1645 (C=O). HRMS (+ESI) *m*/*z*: $[M+H]^+$ calculated for $C_{22}H_{25}N_2O_6S$: 445.1355, found, 445.0154.

3.3. Molecular Docking

A 3D structure of tyrosinase (pdb id; 3NM8) was retrieved in PDB format from the RSCB protein data website (https://www.rcsb.org/). This pdb structure and its preparation was accomplished by BIOVIA Discovery Studio Visualizer by removing water molecules and unnecessary chains. Thereafter, AutoDockTools (1.5.7) allowed us to add Kollman chargers and to compute Gasteiger charges. We then created the PDBQT format of the protein. As for the ligands, we used Chemdraw (16.0) to sketch the 2D structure followed by energy optimization using the MM2 algorithm within Chem 3D (16.0) software.

3.3.1. Docking Study

Molecular docking is a computational technique that predicts the preferred orientation of a ligand to a target protein when bound to each other to form a stable complex. In this study, molecular docking was performed using the Lamarckian Genetic Algorithm (LGA) provided by autodock4.0 on the MGL tools 1.5.7 graphical platform. A grid box was defined around the active site of the receptor to specify the docking search space. The grid parameters, including the center coordinates and dimensions, were set based on methodology outlined by Tamanna. N et al. [1]. The docking calculations were executed, generating

multiple conformations of the ligand bound to the receptor. The best poses were chosen based on their corresponding binding energies and were then used for further visualization.

3.3.2. Visualization of Docking Results

After saving the output PDBQT file of the best pose, BIOVIA Discovery Studio Visualizer was used to study the multiple interactions established between ligands and protein.

4. Conclusions

This communication presents the synthesis of a novel indole derivative bearing a tosyl group, namely 2-(1-ethyl-5-nitro-1*H*-indole-7-carbonyl)butyl 4-methylbenzenesulfonate (**5**). This compound was prepared in four steps starting from the commercially available 6-nitroindole **1** using mild conditions. The structural characterization of **5** was achieved using numerous spectroscopic methods including HRMS, FTIR, ^{1}H, and ^{13}C NMR. Using molecular docking, we showed that compound **5** exhibited good affinity to tyrosinase (3NM8), suggesting it has potential antioxidant activity.

Supplementary Materials: Figure S1: ^{1}H NMR spectrum of **2**, Figures S2 and S3: ^{1}H NMR and ^{13}C NMR spectra of **3**, Figures S4 and S5: ^{1}H NMR and ^{13}C NMR spectra of **4**, Figure S6: HRMS spectrum of **3**, Figure S7: HRMS spectrum of **4**.

Author Contributions: Conceptualization, S.E.K. and N.E.B.; methodology, S.E.K.; software, A.C.; validation, S.E.K. and N.E.B.; formal analysis, S.E.K.; investigation, A.C.; resources, S.E.K.; data curation, A.C.; writing—original draft preparation A.C.; writing—review and editing, S.E.K. and N.E.B.; visualization, S.E.K. and N.E.B.; supervision, S.E.K. and N.E.B.; project administration, S.E.K.; funding acquisition, S.E.K. All authors have read and agreed to the published version of the manuscript.

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

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