


Molecular Docking Studies of 3-diazo-1-methyl-1,3-dihydro-indol-2-one from *Anogeissus leiocarpus* leaves against some Inflammatory Mediators

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Abstract: Targeting key proteins in inflammatory pathways can be an effective strategy for developing new anti-inflammatory therapies. This study investigated the molecular docking of 3-diazo-1-methyl-1, 3-dihydro-indol-2-one against major proinflammatory targets such as cyclooxygenase 2 (COX-2), prostaglandin E₂ (PGE₂) synthase, tumor necrosis factor (TNF- α), interleukin 1 beta (IL-1 β), and N-methyl-D-Aspartate (NMDA) receptor. Three-dimensional structures of the protein targets were obtained from the protein databank, and the compound structure was retrieved from PubChem in an SDF file. Molecular docking was performed using AutoDock Vina Software, while 2D and 3D (surface) views of interaction visualizations were generated using Discovery Studio and PyMOL, respectively. The Physicochemical, lipophilicity, solubility, pharmacokinetics, and Lipinski drug-likeness were assessed using SwissADME Server. The docking results revealed that the compound showed the strongest binding affinity to COX-2 (-7.2 kcal/mol). ADME-T predictions suggest favorable oral bioavailability, metabolic stability, and low toxicity. These finding suggests that 3-diazo-1-methyl-1, 3-dihydro-indol-2-one may serve as a potential anti-inflammatory lead compound, warranting further *in vitro* and *in vivo* validation.

Keywords: 3-diazo-1-methyl-1, 3-dihydro-indol-2-one; COX-2; NMDA receptor; PGE synthase; IL-1 β ; TNF- α .

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1. Introduction

Inflammation is a protective response of the body in response to harmful physical, chemical, or biological stimuli [1]. The cardinal symptoms of inflammation are swelling, increased temperature at the site of injury, pain, and redness [2]. At the molecular level, inflammation is characterized by increased production of reactive oxygen species (ROS) by immune cells, which play a dual role defending against harmful stimuli but also contributing to tissue damage when excessively produced [3]. Prolonged ROS generation can lead to oxidative stress and chronic inflammation, contributing to the pathogenesis of a number of conditions such as arthritis, diabetes, cancer, cardiovascular, and neurological diseases [4]. Key mediators of inflammation include enzymes such as cyclooxygenase 2 (COX-2) and prostaglandin E₂ (PGE₂) synthase; cytokines like tumor necrosis factor (TNF- α) and interleukin 1 beta (IL-1 β); and receptors such as N-methyl-D-Aspartate (NMDA) receptors. These molecules play important roles in the initiation and sustaining of inflammatory responses [5].

COX-2 catalyzes the conversion of arachidonic acid into proinflammatory PGs such as PGE₂, which mediates fever, pain, and vasodilation at inflammation sites [6]. Certain cytokines are also key mediators in the inflammatory process. Interleukin-1 (IL-1) and tumor necrosis factor (TNF) are two important proinflammatory cytokines [5], which kick-start the cascade of reactions associated with inflammation [7]. They do so by triggering the dilation of blood vessels, mobilizing and enhancing the movement of immune cells to the site of infection. Moreover, the NMDA receptor, which is naturally responsible for synaptic plasticity and pain transmission, also participates in sensitizing the central nervous system to pain during inflammation [8]. Together, these molecules drive the complex cascade of acute and chronic inflammatory events.

To treat inflammatory disorders, a class of drugs known as non-steroidal anti-inflammatory drugs (NSAIDs) is commonly prescribed. The NSAIDs include ibuprofen, naproxen, diclofenac, celecoxib, mefenamic acid, indomethacin, and aspirin [9]. The prolonged use of these drugs results in mild to severe adverse reactions and side effects ranging from gastrointestinal to renal toxicity [10]. Consequent upon this, alternative medications with different mechanisms of action and hence, fewer or no side effects are required. Current efforts are channeled towards the exploration of phytochemicals that could serve as novel lead compounds for the discovery of new drugs [11]. In the past, this approach had led to the successful discovery of so many important drugs such as pilocarpine, taxol, quinine, atropine, artemisinin, aspirin, colchicine, quinidine, ephedrine, physostigmine, reserpine, vincristine, and vinblastine [11].

In our earlier study, we reported the anti-inflammatory, anti-nociceptive, and anti-pyretic activities of the leaves of *Anogeissus leiocarpus* and suggested that the compound 3-diazo-1-methyl-1, 3-dihydro-indol-2-one, a derivative of indolone, could be responsible for the observed activities of the plant [12]. Other indole derivatives have also been reported to possess anti-inflammatory, analgesic, and anti-pyretic activities [13, 14]. In this present study, we focus on *in silico* molecular docking of 3-diazo-1-methyl-1, 3-dihydro-indol-2-one against some inflammatory mediators such as COX-2, PGE synthase, TNF- α , IL-1 β , and NMDA receptor. Targeting these key proteins in the inflammatory pathways can be an effective strategy for developing new anti-inflammatory therapies. The molecular modeling with docking simulation strategy provides insights into the potential mechanisms by which the compound may inhibit

these inflammatory targets. In addition, we also employed computational tools to verify the ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties of the compound.

2. Materials and Methods

2.1. Protein target retrieval.

The crystal structure of targets: Human Cyclooxygenase-2 (PDB ID: 5IKR), NMDA receptor (PDB ID: 5EWJ), PGE-Synthase (PDB ID: 4AL1), TNF-alpha (PDB ID: 5MU8), and IL-1 beta (PDB ID: 4G6J). These protein structures were refined using Discovery Studio software.

2.2. Test ligand.

From our former study that evaluated the fractions of the aqueous extract of *Anogeissus leiocarpus* leaves against experimental models of inflammation, pain, and pyrexia [12], one of the GC-MS-identified active principles, 3-diazo-1-methyl-1,3-dihydro-indol-2-one, was selected for this study. The GC-MS details of the compound (#) are presented in Table 1 below as reported by Idakwoji et al. [12].

Table 1. GC-MS details of 3-diazo-1-methyl-1, 3-dihydro-indol-2-one.

Peak No.	Retention time (min)	Area (%)	Name of compound	Ref	CAS	Quality
1	33.6281	1.6272	Hexadecanoic acid, 15-methyl-, methyl ester	144336	006929-04-0	74
2	37.1472	1.5158	9-Oxabicyclo [6.1.0] nonane, cis-	11674	004925-71-7	37
3	45.5413	64.4608	Cholesterol	231246	000057-88-5	98
4#	46.5426	1.3785	3-Diazo-1-methyl-1,3-dihydro-indol-2-one	42696	003265-14-3	35
5	49.8614	31.0177	Cholest-4-en-3-one	229991	000601-57-0	99

2.3. Ligand retrieval.

The 3D format of 3-diazo-1-methyl-1, 3-dihydro-indol-2-one and anti-inflammatory standard drugs were downloaded from the Pubchem database in SDF format. These SDF files were converted to PDB using PyMOL prior to molecular docking studies [15].

2.4. Molecular docking protocol.

The docking studies were conducted to evaluate the binding affinity between 3-diazo-1-methyl-1, 3-dihydro-indol-2-one and selected inflammatory targets: COX-2, NMDA receptor, PGE-Synthase, TNF- α , and IL-1 β . All protein structures were prepared by removing water molecules, adding polar hydrogens, and assigning Gasteiger charges using AutoDock Tools. The active site investigation was performed using the Site Finder of Molecular Operating Environment [16].

Molecular docking was performed using AutoDock Vina Software [17]. Docking was performed using a grid box defined around the known active site of each protein to ensure accurate targeting of ligand binding. Each grid box was sized at 40 x 40 x 40 Å with a spacing of 1.0 Å, centered on the ligand-binding pocket of the target protein. AutoDock Vina's scoring function, based on empirical free energy estimation, was used to rank binding affinities in kcal/mol. For validation of docking accuracy, re-docking of co-crystallized ligands into their respective protein binding sites was conducted, and the root mean square deviation (RMSD)

between the docked pose and crystal conformation was evaluated. An RMSD value of ≤ 2.0 Å was acceptable for reliable reproduction of the binding mode.

Visualization of the protein-ligand interactions was done using Discovery Studio software and Pymol, producing both 2D interaction diagrams and 3D surface renderings of binding poses [15].

2.5. Drug-likeness/ ADMET analysis using the SwissADME tool.

The Physicochemical, lipophilicity, solubility, pharmacokinetics, and Lipinski drug-likeness of compounds and standard drugs were determined using SwissADME Server [18].

3. Results and Discussion

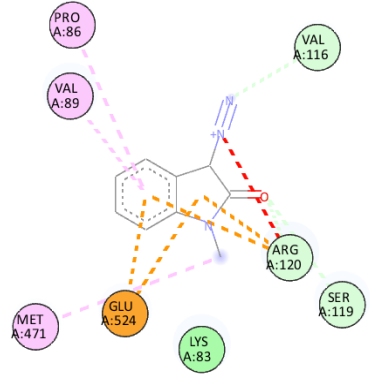
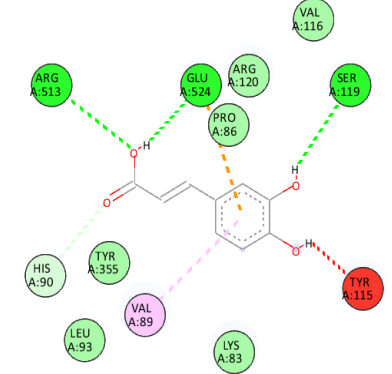
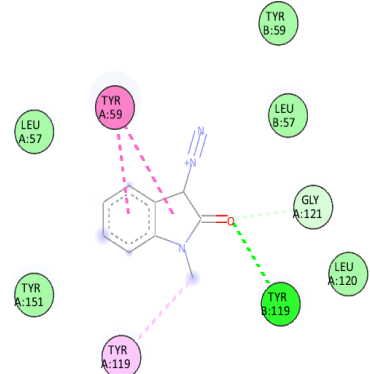
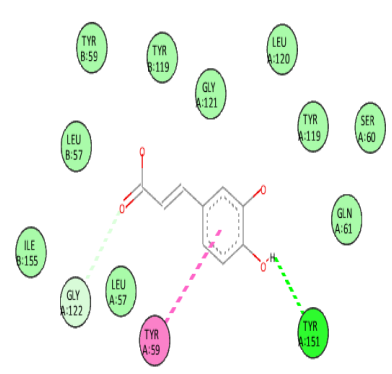
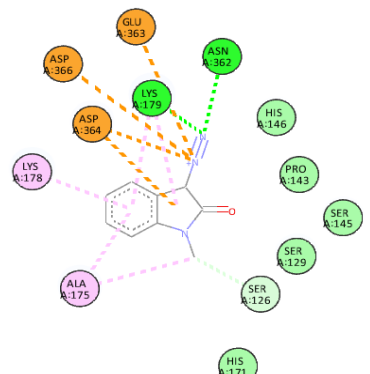
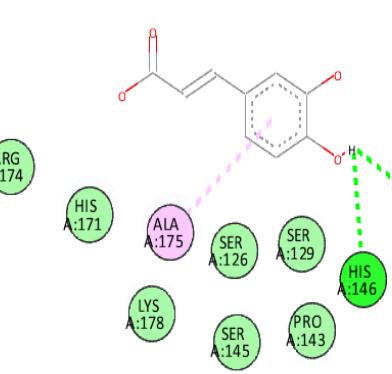
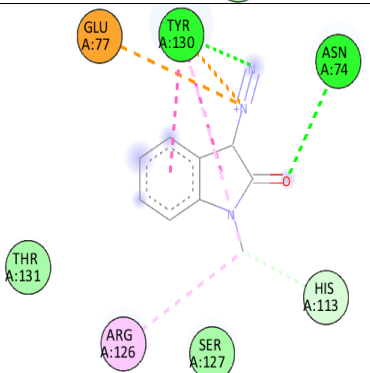
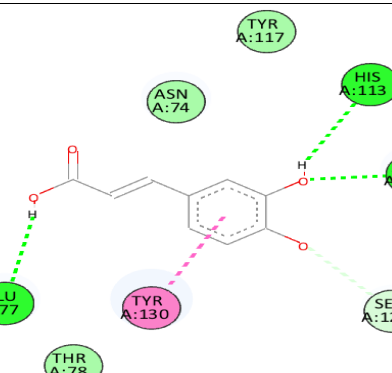
Table 2 shows the binding energies of 3-diazo-1-methyl-1, 3-dihydro-indol-2-one and the standard drug (Ibuprofen) against COX-2, PGE synthase, TNF- α , IL-1 β , and NMDA receptor. The ligand had binding affinities ranging from -4.9 to -7.5 kcal/mol. It had the highest binding affinity (-7.5 kcal/mol) with COX-2 and the least with PGE synthase (-4.9 kcal/mol). The results of the interactions of the ligand with the inflammatory mediators are presented in Table 3. With COX, the ligand showed a unique set of interactions, viz. Pi-alkyl and carbon-hydrogen interaction. The pi-alkyl interactions were with PRO86, VAL89, and MET471, while the carbon-hydrogen interactions were with VAL116, ARG120, and SER119. With TNF- α , the ligand showed carbon-hydrogen interaction with LEU57, TYR151, TYR59, GLY121, and LEU120. With the NMDA receptor, the ligand showed a unique set of interactions, viz. Pi-Carbon Hydrogen and pi-pi stacked interactions. The carbon-hydrogen bonds were with HIS146, PRO143, SER145, SER129, and HIS171, while the pi-pi stacked interactions were with LYS178 and ALA175. With PGE synthase, 3-diazo-1-methyl-1, 3-dihydro-indol-2-one showed conventional hydrogen bonding with TYR130 and ASN74. It also showed carbon-hydrogen bonding with THR131, SER127, and HIS113. With the NMDA receptor, the ligand showed conventional hydrogen interaction with SER43, TYR68, and carbon-hydrogen interaction with PRO87, LYS63, LEU62, LEU67, GLY61, and LYS65. Table 4 shows the 3D structures of 3-diazo-1-methyl-1, 3-dihydro-indol-2-one and the standard drug (Ibuprofen) against the mediators. Table 5 shows pharmacokinetic and drug likeness properties (ADMET) of 3-diazo-1-methyl-1, 3-dihydro-indol-2-one and ibuprofen. The compound possesses favorable ADMET profiles, indicating good oral bioavailability, metabolic stability, low toxicity, and also obeys Lipinski's rule.

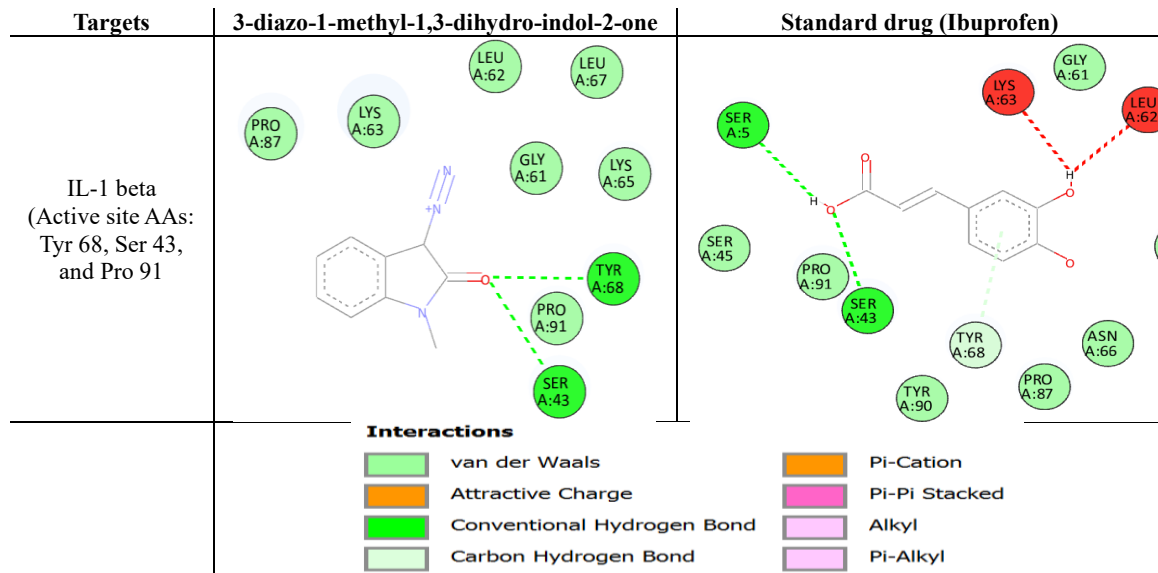
Table 2. Binding energies of 3-diazo-1-methyl-1, 3-dihydro-indol-2-one and standard drug (Ibuprofen) against some inflammatory mediators.

Targets	3-diazo-1-methyl-1,3-dihydro-indol-2-one	Standard drug (Ibuprofen)	RMSD value
COX-2	-7.5	-6.8	1.5
TNF- α	-5.9	-5.7	1.3
NMDA receptor	-5.8	-5.6	1.3
PGE-Synthase	-4.9	-5.2	1.8
IL-1 β	-5.3	-6.1	1.4

Docking results of the ligand with protein targets showing binding affinities (kcal/mol) and RMSD values (Å)

Table 3. 2D structures of 3-diazo-1-methyl-1, 3-dihydro-indol-2-one and standard drug (Ibuprofen) against some inflammatory mediators.

Targets	3-diazo-1-methyl-1,3-dihydro-indol-2-one	Standard drug (Ibuprofen)
<p>COX-2 (Active site AAs: Arg 120, Glu 524, Lys 83, Ser 119)</p>		
<p>TNF-alpha (Active site AAs: Tyr 59, Tyr 119, Tyr 151, Leu 57)</p>		
<p>NMDA receptor (Active site AAs: Asp 364, Lys 179, Asn 362, Ala 175, and Lys 178)</p>		
<p>PGE-Synthase (Active site AAs: Glu 77, and Tyr 130)</p>		



2D structures of the docking interactions of the ligand with protein targets, showing key interacting residues and types of interactions

Table 4. 3D structures of 3-diazo-1-methyl-1, 3-dihydro-indol-2-one and standard drug (Ibuprofen) against some inflammatory mediators.

Targets	3-diazo-1-methyl-1,3-dihydro-indol-2-one	Standard Drug (Ibuprofen)
COX-2		
TNF-alpha		
NMDA receptor		

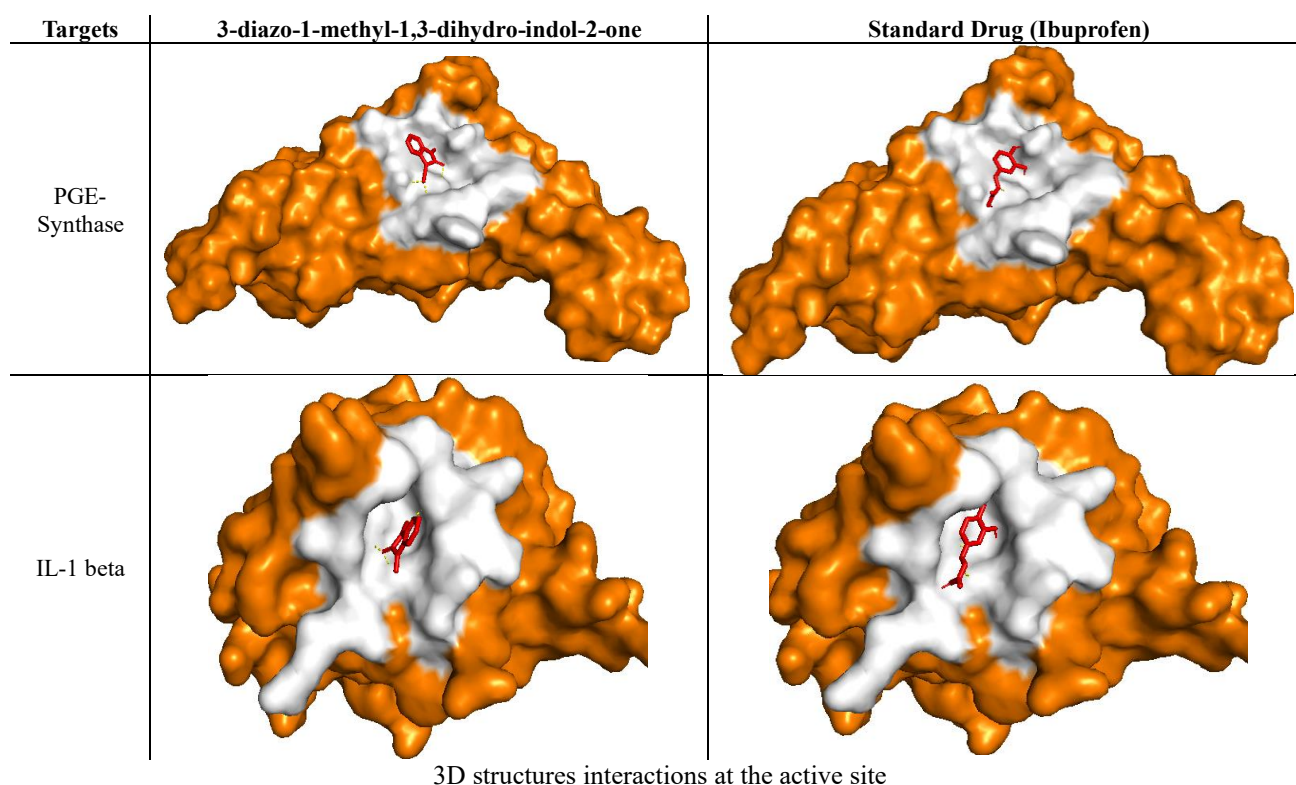


Table 5. Pharmacokinetic and drug likeness properties (ADMET) of 3-diazo-1-methyl-1, 3-dihydro-indol-2-one and standard drug (Ibuprofen).

Parameters	3-diazo-1-methyl-1,3-dihydro-indol-2-one	Ibuprofen
Phytochemical Properties		
Molecular formula	C ₉ H ₇ N ₃ O	C ₁₃ H ₁₈ O ₂
Molecular weight(g/mol)	173.17	206.28
Num. H-Bond acceptor	3	2
Num. H-Bond donor	0	1
Molar refractivity	48.08	62.18
Lipophilicity <i>CLogP_{o/w}</i> value	1.35	3.00
Water solubility	Soluble	Soluble
Druglikeness		
Obeys Lipinski rule?	Yes, 0-violation	Yes, 0-violation
Verber violations	Yes	Yes
Bioavailability score	0.85	0.85
Pharmacokinetics		
GI absorption	High	High
BBB permeant	yes	Yes
P – gp substrate	No	No
CYP1A2 inhibitor	Yes	No
CYP2C19 inhibitor	No	No
CYP2A9 inhibitor	No	No
CYP2A6 inhibitor	No	No
CYP3A4 inhibitor	No	No
Log K _p (cm/s)(skin permeation)	-5.09	-5.07

This table summarizes the predicted ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties of the ligand.

COX-2 contributes to the initiation of inflammation by converting arachidonic acid into prostaglandin H₂ (PGH₂), which serves as a precursor for other PGs, especially PGE₂ [19]. Non-steroidal anti-inflammatory drugs (NSAIDs) are usually the drugs of choice when it comes to inflammatory conditions. NSAIDs are inhibitors of both COX-1 and COX-2 enzymes, and they are classified as either non-complete or complete inhibitors [20]. NSAIDs include ibuprofen, naproxen, diclofenac, celecoxib, meloxicam, mefenamic acid, nimesulide, etodolac, and aspirin. The inhibition of COX by Aspirin is in a non-reversible manner, while

that of ibuprofen is a function of time. Some NSAIDs, such as meloxicam, etodolac, and nimesulide, have a greater affinity for COX-2 [21]. In this study, 3-diazo-1-methyl-1, 3-dihydro-indol-2-one showed special interactions such as Pi-alkyl and carbon-hydrogen interactions. The pi-alkyl interactions were with PRO86, VAL89, and MET471, while the carbon-hydrogen interactions were with VAL116, ARG120, and SER119. Pi-Alkyl interactions are non-covalent in nature, and they occur between the π -electron cloud of an aromatic ring, such as benzene, and the electron density of an alkyl group, such as methyl or ethyl groups. Pi-alkyl interactions are very important in stabilizing protein-ligand binding [22]. Carbon-hydrogen interactions are also weak non-covalent interactions between a carbon-hydrogen bond and an electron-rich region or group, such as a lone pair or electronegative atom. Carbon-hydrogen interactions, though usually not pronounced, play an important role in the stabilization of protein–ligand interactions [22]. These interactions might have contributed to the strong binding affinity (-7.5 kcal/ mol) of 3-diazo-1-methyl-1, 3-dihydro-indol-2-one with COX-2. This was higher than that of the reference ibuprofen. This observation suggests that the ligand may have a stronger affinity for COX-2 than ibuprofen, indicating its potential as a COX-2 inhibitor. However, this inference is based solely on *in silico* data and warrants further *in vitro* and *in vivo* studies to confirm its pharmacological relevance.

Prostaglandin E2 (PGE2) is responsible for a wide range of physiological and pathophysiological activities in the body [23]. PGE2 is implicated in the signature symptoms of inflammation: redness, swelling, and pain [24]. In a normal physiological state, PGE2 plays a key role in the initiation of immune response, regulation of blood pressure, and intestinal function [25]. In the central nervous system, it promotes pain transmission but reduces the synthesis of cytokine and prostaglandin via EP2 activation [26,27]. PGE2 is synthesized from PGH2 by the action of PGE synthase [28]. PGE synthase is found alongside COX-1 in the endoplasmic reticulum compartment [29]. The binding of PGE2 to its receptors can trigger the actions of immune cells such as macrophages, T/B lymphocytes, and dendritic cells in the location of the inflammation [30]. In this study, with PGE synthase, 3-diazo-1-methyl-1, 3-dihydro-indol-2-one showed a conventional hydrogen bond interaction with TYR130 and ASN74. It also showed carbon-hydrogen bonding with THR131, SER127, and HIS113. Hydrogen bonds are one of the primary forces that stabilize the binding of a ligand to the active site of an enzyme. 3-diazo-1-methyl-1, 3-dihydro-indol-2-one had a low binding affinity of -4.9 kcal/ mol with PGE synthase. The gastrointestinal and renal side effects of NSAIDs arise from their ability also to inhibit the COX-1 enzyme [31]. As such, inhibition of PGE synthase instead of COX could be a way to avoid some of these side effects. 3-diazo-1-methyl-1, 3-dihydro-indol-2-one in its present state is not a strong inhibitor of PGE synthase, as revealed by the binding affinity. However, chemical modifications to its structure with further *in vitro* and *in vivo* confirmatory tests might make it a good candidate for development into a potent PGE synthase inhibitor.

Cytokines play a key role in inflammatory processes and various chronic inflammatory diseases such as rheumatoid arthritis [32]. The major groups of cytokines responsible for inflammation and pain are necrosis factors (e.g., TNF- α) and the interleukin family (e.g., Interleukin-1 β) [5]. The action of TNF- α in inflammatory disease is not well understood yet; however, it is believed that it plays a role in the initiation and progression of the disease when there is overproduction by fibroblasts [33]. In this study, 3-diazo-1-methyl-1, 3-dihydro-indol-2-one showed carbon-hydrogen interaction with LEU57, TYR151, TYR59, GLY121 and LEU120 of TNF- α . The ligand had a moderate binding affinity of -5.9 kcal/ mol with TNF- α .

Currently, five TNF- α inhibitors are available for the treatment of several inflammatory diseases. These include the monoclonal antibodies- infliximab, adalimumab, golimumab, and certolizumab pegol, and the fusion protein- etanercept [33]. These inhibitors have affinity for all the forms of human TNF- α receptor, but with golimumab having the highest affinity and biological activity. Despite the effectiveness of these drugs, monoclonal antibody therapies are expensive, leading to high costs for patients and healthcare systems. With proper chemical modifications, 3-diazo-1-methyl-1, 3-dihydro-indol-2-one can come in as a substitute for these drugs. Studies have reported that targeting IL-1 β and components of the receptor for IL-1 β in various rodent models of arthritis effectively reduced inflammation. 3-diazo-1-methyl-1, 3-dihydro-indol-2-one showed conventional hydrogen interaction with SER43, TYR68 and carbon-hydrogen interaction with PRO87, LYS63, LEU62, LEU67, GLY61, LYS65. With a binding affinity of -5.3 kcal/mol, 3-diazo-1-methyl-1, 3-dihydro-indol-2-one could be a potential IL-1 β inhibitor. However, further *in vitro* and *in vivo* tests are required to validate this claim.

NMDA receptors are primarily known for their role in synaptic transmission, plasticity, and neurodevelopment in the central nervous system [34]. However, recent research has highlighted their involvement in inflammation, particularly in the context of neuroinflammation and peripheral inflammatory processes [35]. With the NMDA receptor, 3-diazo-1-methyl-1, 3-dihydro-indol-2-one showed a unique set of interactions, viz. Pi- Carbon Hydrogen and pi-pi stacked interactions. The carbon-hydrogen bonds were with HIS146, PRO143, SER145, SER129, and HIS171, while the pi-pi stacked interactions were with LYS178 and ALA175. Pi-pi stacking interactions occur between aromatic rings, and they play an important role in the accurate prediction of ligand binding and affinity. 3-diazo-1-methyl-1, 3-dihydro-indol-2-one had a moderate binding affinity of -5.8 kcal/mol with the NMDA receptor. The implication of 3-diazo-1-methyl-1, 3-dihydro-indol-2-one being a mild antagonist of NMDA receptors is that it may be free of the side effects that limit the use of current NMDA receptor antagonists such as ketamine and memantine [36]. The side effects usually result from completely blocking the receptors with strong antagonists. Therefore, a mild antagonist such as 3-diazo-1-methyl-1, 3-dihydro-indol-2-one could avert these side effects if these observed effects are validated in *in vitro* and *in vivo* screenings.

The pharmacokinetic properties and drug-likeness of 3-diazo-1-methyl-1, 3-dihydro-indol-2-one were also investigated in this study. The compound possesses excellent ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties. 3-diazo-1-methyl-1, 3-dihydro-indol-2-one also passed the drug-likeness test as it obeyed the five rules of Lipinski, which are- molecular weight (MW) of not more than 500 g/mol, hydrogen bond acceptors not more than 10, hydrogen bond donors not more than 5, LogP value less than 5, and number of rotatable bonds not less than 10 [37]. A violation of two or more of these rules implies that a molecule is not qualified to be an oral drug. 3-diazo-1-methyl-1, 3-dihydro-indol-2-one had zero violation and also did not inhibit the majority of the CYP enzymes.

4. Conclusions

Conclusively, the ligand had good binding affinities with the inflammatory mediators, especially with the COX-2 enzyme. Also, the compound possesses favorable ADMET profiles indicating good oral bioavailability, metabolic stability, and low toxicity. With further *in vitro* and *in vivo* confirmatory studies, 3-diazo-1-methyl-1, 3-dihydro-indol-2-one could be a

potential candidate for further development into an anti-inflammatory drug with multiple mechanisms of action.

Author Contributions

Conceptualization, P.I.; methodology, P.I., F.U., and C.O.; software, S.O.; validation, A.I., E.I., and P.N.; formal analysis, A.W.; resources, M.A. and H.M.; data curation, P.I.; writing—original draft preparation, P.I.; writing—review and editing, F.U., C.O., and P.N.; visualization, S.O.; supervision; project administration, A. I and M.H. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement

Not applicable.

Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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None.

Conflicts of Interest

The authors declare no conflict of interest.

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