In silico Study of New Five-Membered Heterocyclic Derivatives Bearing (1,3,4-oxadiazole and 1,3,4-thiadiazole) As Promising Cyclooxygenase Inhibitors

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Abstract :

A novel series of pyrazole, oxadiazole and thiadiazole bearing Nabumetone moiety were designed, synthesized, and evaluated for their anti-inflammatory activity against cyclooxygenase enzyme 2, after Insilico assay (by molecular docking study) a best set has been synthesized and characterized.

The activity of the compounds was predicted by a molecular docking research utilizing the GOLD software tool from the Cambridge Crystallographic Data Base. We tested them in real in vivo as anti-inflammatory agents using egg white procedure. Due to their hydrogen bonding interaction with crucial amino acids in COX-2 isozymes Arg120, Tyr355, and Ser530, all tested compounds in molecular docking demonstrated significant activities compared with diclofenac, naproxen, and 6MNA as reference drugs. The data obtained from docking studies were highly correlated with that obtained from the in vivo assay in which compounds 3c, 6c, and 7c showed the best docking PLP fitness which were 91.35, 89.66, and 92.09 respectively with COX-2. Other compounds 2c, 4c, 5c, 6a, 6b, showed a PLP fitness above 80. Many of the non-steroidal anti-inflammatory drugs (NSAIDs) currently marketed produce severe gastro-toxic side effects and have low selectivity toward COX-2 enzyme. The benefits of producing NSAIDs without these side effects and with higher selectivity are obvious, particularly for patients requiring long-term therapy. The aim of this investigation was to produce novel NSAIDs, based on Nabumetone, that exhibit little or no gastro-toxicity and higher selectivity. This research offered helpful direction for the identification of novel pyrazole and thiadiazole anti-inflammatory compounds.

Key Words: Cambridge Crystallographic Data Center, Lipinski Rule, Molecular Docking, Nabumetone.

استخدام الحاسوب (للالتحام الجزيئي) لدراسة مشتقات حلقية غير متجانسة جديدة مكونة من حلقة خماسية (1،3،4-أوكساديازول و1،3،4-ثياديازول) كمثبطات واعدة لانزيمات السيكلواوكسجينيز.

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الخلاصة:
تم تصميم سلسلة جديدة من البيرازول والأوكساديازول والثياديازول الحاملة لجزيئة دواء النابوميتون ، وتصنيعها وتقييمها لنشاطها المضاد للالتهابات ضد إنزيم سيكلوأوكسيجيناز 2 ، بعد اختبار برمجيات الحاسوب (من خلال دراسة الالتحام الجزيئي) تم تصنيع أفضل مجموعة وتمييزها. بعد التنبؤ بنشاطهم من خلال دراسة الالتحام الجزيئي باستخدام أداة برنامج Cambridge Crystallographic Data Base (GOLD) ، قمنا باختبارهم في الجسم الحي كعوامل مضادة للالتهابات باستخدام إجراء بياض البيض. نظرًا لتفاعل الرابطة الهيدروجينية مع الأحماض الأمينية الحاسمة في إنزيمات COX-2 Arg120 و Tyr355 و Ser530 ، أظهرت جميع المركبات تحت الاختبار في الالتحام الجزيئي أنشطة مهمة مقارنةً بالديكلوفيناك والنابروكسين و MNA 6 كأدوية مرجعية. كانت البيانات التي تم الحصول عليها من دراسات الالتحام مرتبطة ارتباطًا وثيقًا بتلك التي تم الحصول عليها من الفحص في الجسم الحي حيث أظهرت المركبات c3 وc6 وc7 أفضل التحام جزيئي PLP والتي كانت 91.35 و89.66 و92.09 على التوالي مع COX-2. أظهرت المركبات الأخرى 2c, 4c, 5c, 6a, 6b، التحام PLP أعلى من 80. العديد من العقاقير غير الستيرويدية المضادة للالتهابات (NSAIDs) التي يتم تسويقها حاليًا تنتج آثارًا جانبية شديدة السمية المعوية ولها انتقائية منخفضة تجاه إنزيم COX-2. إن فوائد إنتاج مضادات الالتهاب غير الستيروئيدية بدون هذه الآثار الجانبية والانتقائية العالية واضحة، خاصة للمرضى الذين يحتاجون إلى علاج طويل الأمد. كان الهدف من هذا البحث هو إنتاج مضادات الالتهاب غير الستيروئيدية الجديدة، استنادًا إلى نابوميتون، والتي تظهر سمية معدية قليلة أو معدومة وانتقائية أعلى. قدم هذا البحث توجيهًا مفيدًا لتحديد مركبات البيرازول والثياديازول الجديدة المضادة للالتهابات.

الكلمات المفتاحية: مركز بيانات التصوير البلوري في كامردج، قاعدة لبنسكي، الالتحام الجزيئي، نابيوميتون

Introduction

In recent years, the design and development of novel pharmaceutical agents have been significantly driven by computational methods and tools that facilitate in silico studies (1). One such area of research focuses on the synthesis of heterocyclic compounds, particularly those bearing the 1,3,4-oxadiazole and 1,3,4-thiadiazole moieties, which have shown immense promise as potential cyclooxygenase (COX) inhibitors (2). The inhibition of COX enzymes, specifically COX-1 and COX-2, has been a key therapeutic strategy for various inflammatory conditions, pain relief, osteoarthritis, low back pain, rheumatoid arthritis and as chemo-preventive agents in cancer (3).

1,3,4-oxadiazole and 1,3,4-thiadiazole are five-membered heterocyclic ring systems that have garnered considerable attention due to their diverse pharmacological activities and structural versatility. These heterocycles are characterized by the presence of nitrogen, oxygen, and sulfur atoms, allowing them to exhibit a wide range of biological effects through interactions with specific molecular targets (4).

The 1,3,4-oxadiazole ring is composed of two nitrogen atoms and one oxygen atom, while the 1,3,4-thiadiazole ring contains two nitrogen atoms and one sulfur atom. This subtle difference in composition imparts distinct physicochemical properties to each heterocycle, making them attractive candidates for drug design and development. Researchers have found that subtle modifications to the heterocyclic structures can significantly influence the biological activity and selectivity of the compounds (5).

The inhibition of cyclooxygenase enzymes, particularly COX-2, has been a major focus in drug discovery, as COX-2 is associated with inflammation and pain, while COX-1 plays a vital role in maintaining physiological functions, including gastric mucosal integrity and platelet aggregation. Therefore, selective COX-2 inhibitors have been sought after to minimize adverse effects associated with non-selective COX inhibitors, such as

gastrointestinal complications and bleeding disorders (6).

The structure-activity relationship (SAR) is a critical aspect of drug design that focuses on understanding how changes in the molecular structure of a compound affect its biological activity. In the context of COX-2 inhibitors, SAR studies have revealed key structural features necessary for potent and selective inhibition include: the presence of a carboxylic acid group: The carboxylic acid moiety in COX-2 inhibitors forms hydrogen bonds with key amino acid residues in the active site, contributing to strong interactions and improved binding affinity, hydrophobic substituents: Hydrophobic groups attached to the core scaffold enhance the lipophilicity of the molecule, enabling it to fit snugly within the hydrophobic channel of the COX-2 active site, and size and flexibility: Optimal COX-2 inhibitors often possess a specific size and flexibility that allows them to access and interact with critical amino acid residues within the active site (7,8).

Strategies for Targeting COX-2 Enzyme includes: Selective COX-2 Inhibition: One of the primary strategies in targeting the COX-2 enzyme is to develop selective COX-2 inhibitors that spare COX-1 activity. This selectivity reduces the risk of adverse effects associated with non-selective COX inhibitors, such as gastrointestinal complications, covalent and Non-Covalent Inhibitors: COX-2 inhibitors can be designed as covalent or non-covalent inhibitors. Covalent inhibitors form a covalent bond with the enzyme, while non-covalent inhibitors rely on reversible interactions, such as hydrogen bonding and van der Waals forces, virtual Screening and Molecular Docking: In silico methods like virtual screening and molecular docking play a crucial role in identifying potential COX-2 inhibitors. Virtual screening involves screening large compound libraries to identify promising candidates, while molecular docking predicts the binding interactions between ligands and the COX-2 active site, natural Products and Combinatorial Chemistry: Natural products have been a valuable source of lead compounds for COX-2 inhibition. Combinatorial chemistry techniques enable the synthesis of diverse compound libraries to explore new chemical space for potential COX-2 inhibitors, and finally; dual COX and LOX Inhibition: Some researchers have explored the dual inhibition of COX and lipoxygenase (LOX) enzymes as a strategy to achieve enhanced anti-inflammatory effects and reduce side effects (9,10).

To achieve the goal of developing potent and selective COX inhibitors, computational methods have proven to be invaluable. Molecular docking, a widely employed computational technique, allows researchers to predict the binding interactions between ligands and target proteins. [11] In this study, the GOLD suite was employed for molecular docking analysis to gain insights into the binding modes and affinities of the newly designed 1,3,4-oxadiazole and 1,3,4-thiadiazole derivatives with the active sites of COX enzymes (12).

Furthermore, in silico ADME (Absorption, Distribution, Metabolism, and Excretion) evaluation is crucial in the early stages of drug discovery to assess the pharmacokinetic properties of potential drug candidates. The Swiss ADME website is a reliable and user-friendly platform that aids researchers in predicting key ADME parameters, helping to identify compounds with favorable pharmacokinetic profiles and increased chances of successful development (13).

Methodology

The computational approach used in this investigation is further elucidated in figure 1. The investigation of molecular docking

for the compounds (1-7c) **table 1**. was conducted using the CCDC GOLD Suite (version 2022.2.0). The visualization of protein structures, ligands, hydrogen bonding interactions, short contacts, and bond length estimates was performed using the CCDC Hermes visualizer program (version 2022.2.0). ChemDraw version 20.1 was used to generate two-dimensional representations of the ligands, whereas ChemBio 3D version 20.1 was employed to construct three-dimensional models and optimize the ligands' energy. The Swiss ADME server was used to provide predictions on the pharmacokinetic characteristics, namely absorption, distribution, metabolism, and excretion (ADME), of the synthesized pharmaceutical compounds (14).

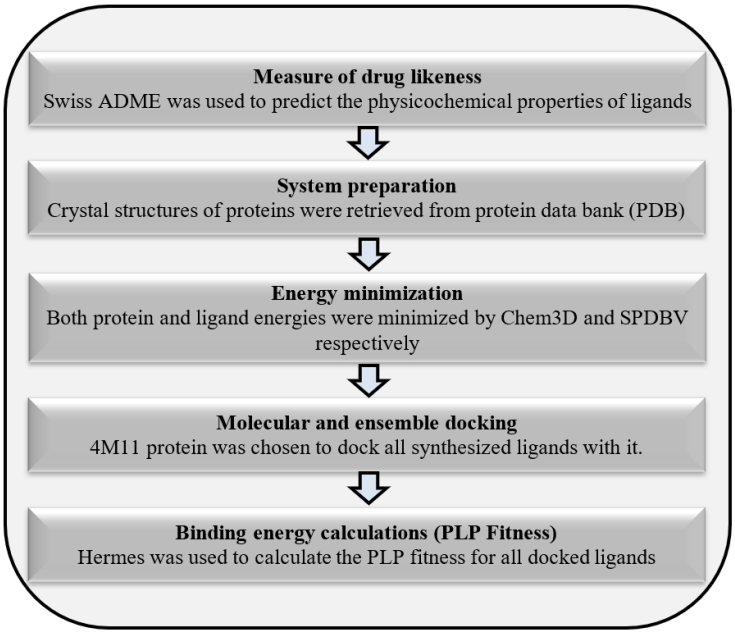
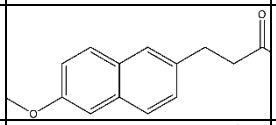
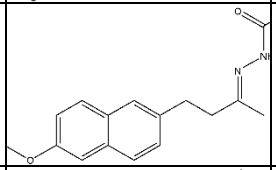
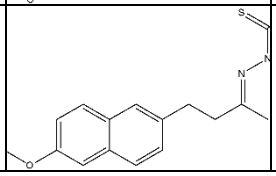
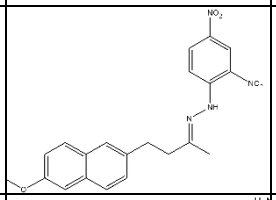
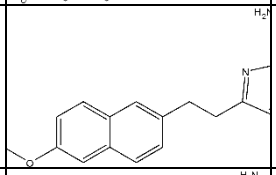
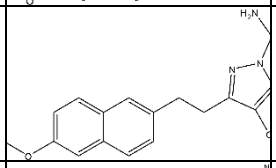
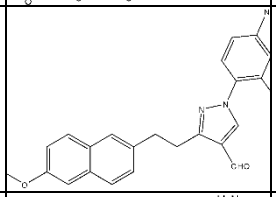
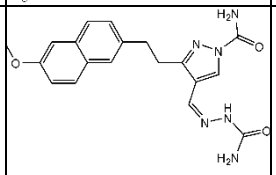
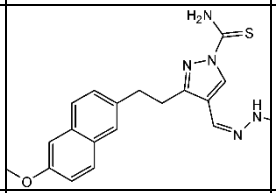
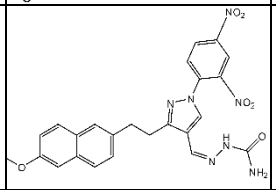
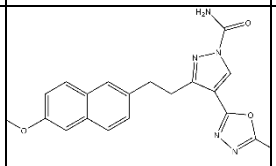
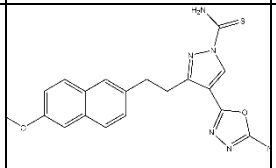
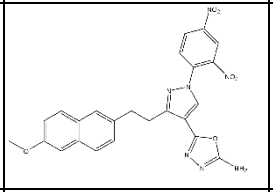
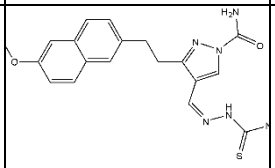
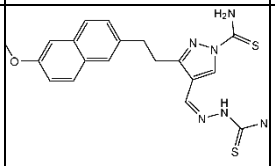
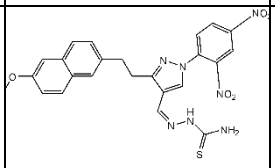
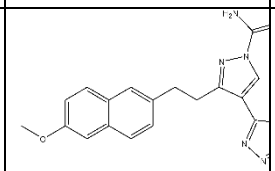
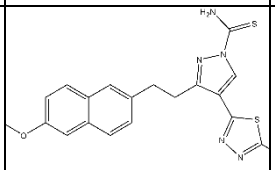
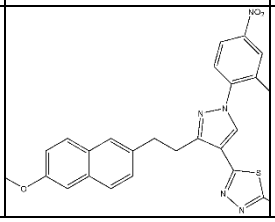


Figure 1: Outline of computational protocol.

Table 1: Structures and names of synthesized compounds.

Comp.	Structure	IUPAC/ Smile Name
1		4-(6-methoxynaphthalen-2-yl)butan-2-one COC1=CC2=CC=C(CCC(C)=O)C=C2C=C1
2a		(E)-2-(4-(6-methoxynaphthalen-2-yl)butan-2-ylidene)hydrazine-1-carboxamide COC1=CC2=CC=C(CC\C(C)=N\NC(N)=O)C=C2C=C1
2b		(E)-2-(4-(6-methoxynaphthalen-2-yl)butan-2-ylidene)hydrazine-1-carbothioamide COC1=CC2=CC=C(CC\C(C)=N\NC(N)=S)C=C2C=C1

Comp.	Structure	IUPAC/ Smile Name
2c		(E)-1-(2,4-dinitrophenyl)-2-(4-(6-methoxynaphthalen-2-yl)butan-2-ylidene)hydrazine COC1=CC2=CC=C(CC\C(C)=N\NC3=C(C=C(C=C3)[N+]([O-])=O)[N+]([O-])=O)C=C2C=C1
3a		4-formyl-3-(2-(6-methoxynaphthalen-2-yl)ethyl)-1H-pyrazole-1-carboxamide COC1=CC2=CC=C(CCC3=NN(C=C3C=O)C(N)=O)C=C2C=C1
3b		4-formyl-3-(2-(6-methoxynaphthalen-2-yl)ethyl)-1H-pyrazole-1-carbothioamide COC1=CC2=CC=C(CCC3=NN(C=C3C=O)C(N)=S)C=C2C=C1
3c		1-(2,4-dinitrophenyl)-3-(2-(6-methoxynaphthalen-2-yl)ethyl)-1H-pyrazole-4-carbaldehyde COC1=CC2=CC=C(CCC3=NN(C=C3C=O)C3=C(C=C(C=C3)[N+]([O-])=O)[N+]([O-])=O)C=C2C=C1
4a		(Z)-4-(((hydrazinecarbonyl)imino)methyl)-3-(2-(6-methoxynaphthalen-2-yl)ethyl)-1H-pyrazole-1-carboxamide COC1=CC2=CC=C(CCC3=NN(C=C3\C=N/NC(N)=O)C(N)=O)C=C2C=C1
4b		(Z)-N-((1-carbamothioyl-3-(2-(6-methoxynaphthalen-2-yl)ethyl)-1H-pyrazol-4-yl)methylene)hydrazinecarboxamide COC1=CC2=CC=C(CCC3=NN(C=C3\C=N/NC(N)=O)C(N)=S)C=C2C=C1
4c		(Z)-N-((1-(2,4-dinitrophenyl)-3-(2-(6-methoxynaphthalen-2-yl)ethyl)-1H-pyrazol-4-yl)methylene)hydrazinecarboxamide COC1=CC2=CC=C(CCC3=NN(C=C3\C=N/NC(N)=O)C3=C(C=C(C=C3)[N+]([O-])=O)[N+]([O-])=O)C=C2C=C1
5a		4-(5-amino-1,3,4-thiadiazol-2-yl)-3-(2-(6-methoxynaphthalen-2-yl)ethyl)-1H-pyrazole-1-carboxamide COC1=CC2=CC=C(CCC3=NN(C=C3C3=NN=C(N)O3)C(N)=O)C=C2C=C1
5b		4-(5-amino-1,3,4-oxadiazol-2-yl)-3-(2-(6-methoxynaphthalen-2-yl)ethyl)-1H-pyrazole-1-carbothioamide COC1=CC2=CC=C(CCC3=NN(C=C3C3=NN=C(N)O3)C(N)=S)C=C2C=C1

Comp.	Structure	IUPAC/ Smile Name
5c		5-(1-(2,4-dinitrophenyl)-3-(2-(6-methoxynaphthalen-2-yl)ethyl)-1H-pyrazol-4-yl)-1,3,4-oxadiazol-2-amine COC1=CC2=CC=C(CCC3=NN(C=C3C3=NN=C(N)O3)C3=C(C=C(C=C3)[N+]([O-])=O)[N+]([O-])=O)C=C2C=C1
6a		(Z)-4-(((hydrazinecarbonothioyl)imino)methyl)-3-(2-(6-methoxynaphthalen-2-yl)ethyl)-1H-pyrazole-1-carboxamide COC1=CC2=CC=C(CCC3=NN(C=C3\C=N/NC(N)=S)C(N)=O)C=C2C=C1
6b		(Z)-4-(((hydrazinecarbonothioyl)imino)methyl)-3-(2-(6-methoxynaphthalen-2-yl)ethyl)-1H-pyrazole-1-carbothioamide COC1=CC2=CC=C(CCC3=NN(C=C3\C=N/NC(N)=S)C(N)=S)C=C2C=C1
6c		(Z)-N-((1-(2,4-dinitrophenyl)-3-(2-(6-methoxynaphthalen-2-yl)ethyl)-1H-pyrazol-4-yl)methylene)hydrazinecarbothioamide COC1=CC2=CC=C(CCC3=NN(C=C3\C=N/NC(N)=S)C3=C(C=C(C=C3)[N+]([O-])=O)[N+]([O-])=O)C=C2C=C1
7a		4-(5-amino-1,3,4-thiadiazol-2-yl)-3-(2-(6-methoxynaphthalen-2-yl)ethyl)-1H-pyrazole-1-carboxamide COC1=CC2=CC=C(CCC3=NN(C=C3C3=NN=C(N)S3)C(N)=O)C=C2C=C1
7b		4-(5-amino-1,3,4-thiadiazol-2-yl)-3-(2-(6-methoxynaphthalen-2-yl)ethyl)-1H-pyrazole-1-carbothioamide COC1=CC2=CC=C(CCC3=NN(C=C3C3=NN=C(N)S3)C(N)=S)C=C2C=C1
7c		5-(1-(2,4-dinitrophenyl)-3-(2-(6-methoxynaphthalen-2-yl)ethyl)-1H-pyrazol-4-yl)-1,3,4-thiadiazol-2-amine COC1=CC2=CC=C(CCC3=NN(C=C3C3=NN=C(N)S3)C3=C(C=C(C=C3)[N+]([O-])=O)[N+]([O-])=O)C=C2C=C1

ADME Methods

Using the Swiss ADME program, which predicts physicochemical characteristics and pharmacokinetic features, we drew all of the ligands (1–7c) in Chem Sketch (v. 12). BOILED-EGG was utilized to determine the small molecule's lipophilicity and polarity (15,16).

Preparing protein receptor and ligands:

Swiss PDB Viewer (v. 3.7) was used to fill in missing atoms in the crystal structures of cyclooxygenase COX-1 [PDB ID: 3N8Z] and COX-2 [PDB ID: 4M11] from the Protein Data Bank (PDB). In order to assure the appropriate ionization and tautomeric states of amino acid residues, we rectified the crystal structures of the proteins obtained by downloading by

introducing hydrogen atoms. Our synthesized ligands' energies were minimized using the MM2 force field in CheBio3D (v.20.0).

Docking approaches:

Molecular docking was performed using the commercially available version of Genetic Optimization for Ligand Docking (GOLD) (v. 2022.2.0) (16,17). In addition, the receptors for the docking procedure were prepared using the Hermes visualizer program included in the GOLD Suite. All of the protein residues in the downloaded protein structure complexes within ten Ao of the reference ligands constitute the binding location used for GOLD docking.

For the purpose of ensemble docking (18), five COX-2 proteins (1pxx, 4m11, 3LN1, 3KK6, and 5kIR) were downloaded from the PDB database. Therefore, 4m11 was selected for the docking study method. CCDC Superstar was used to locate the cavity and active site. The protein's reference ligand was utilized to calculate the active site radius (10 A^o).

The experimental design used the chemscore kinase as the foundation. The assessment criteria were computed with the ChemPLP algorithm. The docking parameters were maintained at their normal values, and the solutions were assessed using the Piecewise Linear Potential (PLP) fitness function. The determination of protein-ligand steric complementarity is achieved by the use of ChemPLP, a computational algorithm that incorporates distance and angle-dependent hydrogen interactions. The evaluation of the interaction between the amino acid residues of the COX-1 and COX-2 proteins and the synthesized ligands was conducted by the analysis of docking data, including the binding mode, docked posture, and binding free energy. The present study aimed to assess the interaction between the amino acid residues of proteins COX-1 and COX-2 and our manufactured ligands by investigating the docking data, including the binding mode, docked posture, and binding free energy.

Through the integration of molecular docking and ADME evaluation, this study aimed to identify new and potent COX inhibitors among the 1,3,4-oxadiazole and 1,3,4-thiadiazole derivatives, potentially paving the way for the discovery of safer and more effective anti-inflammatory agents.

Results

ADME Results

Adsorption, Distribution, Metabolism, and Excretion (ADME) analysis was performed on all produced chemical compounds **table2.** Orally given drugs, as a general rule, should have a molecular weight (M.wt) of less than 500, less than 5 hydrogen bond donor sites, and fewer than 10 hydrogen bond acceptor sites, according to the Lipinski rule.

Since topological polar surface area (TPSA) is an additional essential feature associated with drugs bioavailability, we computed it as well. Therefore, molecules having a TPSA >140 A^o are assumed to have poor oral bioavailability since they are absorbed passively (19). Our results showed that compounds 2a-c, 3a-c, 4a, 4c, & 5a) have TPSA below 140, and the bioavailability for all ligands was 0.55 which mean that all ligands reach the systemic circulation, while compounds 4b, 5b, 5c, 6a-c, & 7-c have TPSA more than 140, with bioavailability score 0.55 except 4c, 5c, 6c, 7c showed bioavailability score 0.17.

Table2. ADME properties of synthesized compounds

#	MW	tatable bo	ond accep	-bond don	MR	TPSA	iLOGP	ESOL Log S	ESOL Solubility (mg/ml)	ESOL Solubility (mol/l)
1	228.29	4	2	0	70.03	26.3	2.74	-3.37	9.80E-02	4.29E-04
2a	285.34	6	3	2	84.42	76.71	1.88	-3.01	2.76E-01	9.67E-04
2b	301.41	6	2	2	91.62	91.73	3.09	-3.49	9.71E-02	3.22E-04
2c	408.41	8	6	1	119.98	125.26	3.08	-5.65	9.07E-04	2.22E-06
3a	323.35	6	4	1	90.43	87.21	2.5	-3.47	1.09E-01	3.36E-04
3b	339.41	6	3	1	97.63	102.23	2.53	-3.95	3.80E-02	1.12E-04
3c	446.41	8	7	0	124.85	135.76	2.59	-5.38	1.86E-03	4.17E-06
4a	380.4	8	5	3	104.6	137.62	1.87	-3.13	2.81E-01	7.39E-04
4b	396.47	8	4	3	111.8	152.64	1.67	-3.61	9.75E-02	2.46E-04
4c	503.47	10	8	2	139.02	186.17	1.7	-5.05	4.46E-03	8.86E-06
5a	378.38	6	6	2	102.74	135.08	2.63	-3.68	7.92E-02	2.09E-04
5b	394.45	6	5	2	109.94	150.1	2.62	-4.16	2.75E-02	6.97E-05
5c	501.45	8	9	1	137.16	183.63	2.81	-5.57	1.36E-03	2.70E-06
6a	396.47	8	4	3	111.8	152.64	2.71	-3.61	9.75E-02	2.46E-04
6b	412.53	8	3	3	119	167.66	2.8	-4.09	3.38E-02	8.19E-05
6c	519.53	10	7	2	146.22	201.19	2.76	-5.53	1.53E-03	2.95E-06
7a	394.45	6	5	2	108.35	150.18	2.82	-4.16	2.71E-02	6.87E-05
7b	410.52	6	4	2	115.55	165.2	2.63	-4.64	9.39E-03	2.29E-05
7c	517.52	8	8	1	142.77	198.73	2.57	-6.05	4.59E-04	8.87E-07

#	ESOL Class	Ali Log S	Ali Class	cos-IT Log	GI absorption	BBB permeant	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor
1	Soluble	-3.3	Soluble	-5.35	High	Yes	No	Yes	Yes
2a	Soluble	-3.55	Soluble	-5.13	High	Yes	No	Yes	No
2b	Soluble	-4.49	erately sol	-5.32	High	No	No	Yes	Yes
2c	Moderately soluble	-7.81	oorly solub	-6.73	Low	No	Yes	No	Yes
3a	Soluble	-3.96	Soluble	-5.06	High	No	No	Yes	Yes
3b	Soluble	-4.89	erately sol	-5.25	High	No	No	Yes	Yes
3c	Moderately soluble	-7.06	oorly solub	-6.64	Low	No	No	No	Yes
4a	Soluble	-4.19	erately sol	-4.8	High	No	Yes	No	No
4b	Soluble	-5.13	erately sol	-4.99	Low	No	No	No	No
4c	Moderately soluble	-7.3	oorly solub	-6.37	Low	No	No	No	No
5a	Soluble	-4.63	erately sol	-5.7	High	No	Yes	Yes	No
5b	Moderately soluble	-5.57	erately sol	-5.89	Low	No	No	No	No
5c	Moderately soluble	-7.73	oorly solub	-7.26	Low	No	No	No	Yes
6a	Soluble	-5.13	erately sol	-4.99	Low	No	No	No	No
6b	Moderately soluble	-6.07	oorly solub	-5.18	Low	No	No	No	Yes
6c	Moderately soluble	-8.24	oorly solub	-6.55	Low	No	No	No	No
7a	Moderately soluble	-5.58	erately sol	-5.74	Low	No	No	No	No
7b	Moderately soluble	-6.52	oorly solub	-5.93	Low	No	No	No	Yes
7c	Poorly soluble	-8.68	oorly solub	-7.3	Low	No	No	No	Yes

#	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	log Kp (cm/s)	Lipinski #violations	Bioavailability Score
1	No	Yes	No	-5.51	0	0.55
2a	No	No	No	-6.41	0	0.55
2b	Yes	No	Yes	-6.08	0	0.55
2c	Yes	No	Yes	-4.94	0	0.55
3a	Yes	No	No	-6.51	0	0.55
3b	Yes	No	Yes	-6.18	0	0.55
3c	Yes	No	Yes	-5.84	0	0.55
4a	No	No	No	-7.42	0	0.55
4b	No	No	No	-7.09	0	0.55
4c	Yes	No	Yes	-6.74	2	0.17
5a	No	No	No	-7.07	0	0.55
5b	Yes	No	Yes	-6.75	0	0.55
5c	Yes	No	Yes	-6.4	2	0.17
6a	Yes	No	Yes	-7.09	0	0.55
6b	Yes	No	Yes	-6.76	0	0.55
6c	Yes	No	Yes	-6.42	2	0.17
7a	Yes	No	Yes	-6.74	0	0.55
7b	Yes	No	Yes	-6.41	0	0.55
7c	No	No	Yes	-6.06	2	0.17

Docking Results

The molecular interactions between the active binding sites of the protein target and the synthesized compounds 1-7c were investigated using docking studies conducted using the GOLD Suite program. These experiments aimed to estimate the selectivity and binding energies of the created compounds for COX-1 and COX-2.

The PLP fitness of compounds 1–7c, 6MNA, diclofenac, and naproxen was assessed in relation to their ability to form complexes in the active sites of COXs. The inhibitory action of these compounds was then compared. **Table 3** presents the range of PLP fitness values for the docked compounds on COX 1 and COX 2, which vary from 49.32 to 72.71 and 62.35 to 92.09, respectively. **Table 4** displays the 3D configurations of many compounds that were produced. These structures exhibit hydrogen bonding and establish close interactions with significant amino acids. The consistency between our docking findings and experimental data obtained from an in vivo examination is quite close.

In order minimize the potential for inadvertent selection of an unsuitable protein model, enhance pose prediction and virtual screening enrichments, and ensure the accuracy of the docking process, we conducted ensemble docking as the initial step, employing a set of five distinct COX-2 proteins.

Hydrogen bonds and short contacts were identified using docking analysis to be present between the final ligand library and the following residues: Arg120, Tyr355, Ser530, Val116, Tyr385, Gly526, Val523, Trp387, Ala527, Leu531, Leu534, Leu345, Leu539, Val89, and Val349.

The determination of short contacts and hydrogen bonding distances between a specific protein atom and our synthesized ligands (20) relies on the measurement of bond lengths below 3Å and the inclusion of GOLD.

The brief contacts are characterized by several interaction forces, including as van der Waals, electrostatic, steric, pi-pi stacking, and dipole-dipole interactions.

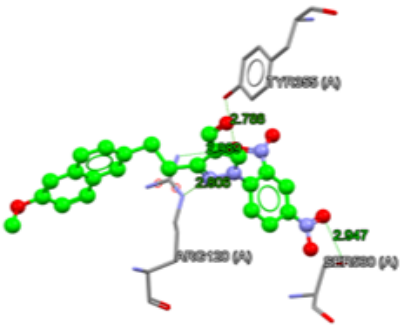
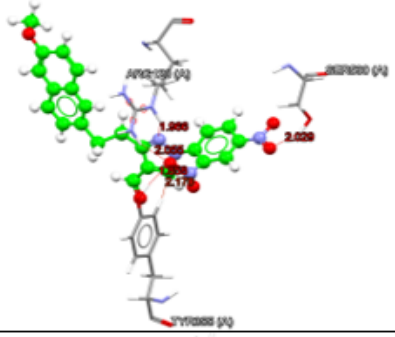
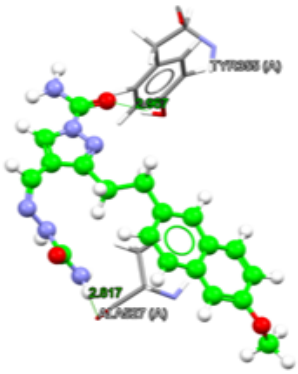
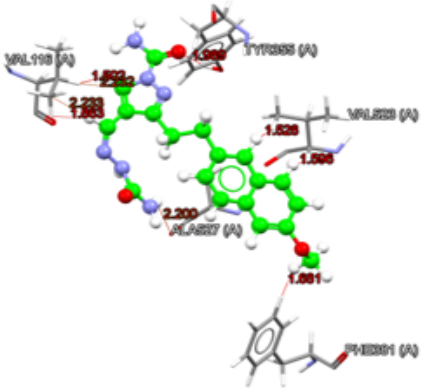
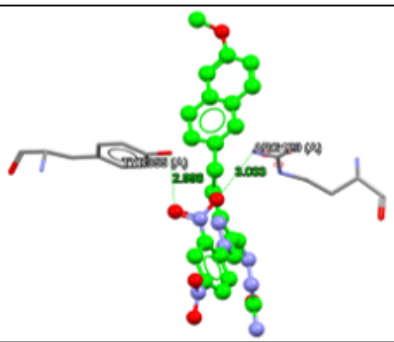
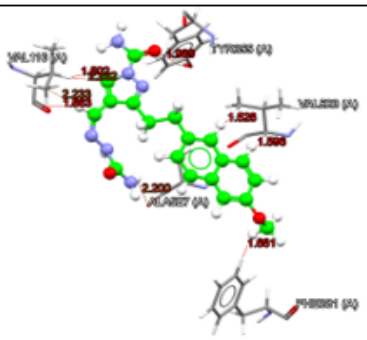
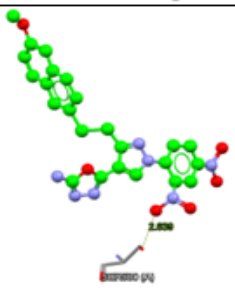
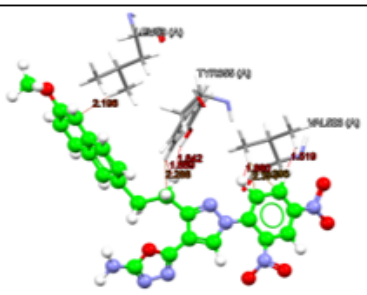
The binding of five authorized NSAIDs (as shown in **Table 5**) involves hydrogen bond interactions between Arg120 and Tyr355. These interactions are seen in compounds 2a, 2c, 3a, 3c, 4c, 5a, and 7a. Compounds 3c, 5c, and 6c have hydrogen bonding interactions with Ser530, which serves as the binding site for diclofenac, lumiracoxib, and tolfenamic acid. Compound 7c has a single hydrogen bond with Tyr355, similar to the hydrogen bonding seen in aspirin.

Table 3: The present study investigates the binding energies of Nabumetone derivatives and reference nonsteroidal anti-inflammatory drugs (NSAIDs) when docked with cyclooxygenase-2 (COX-2) ** and cyclooxygenase-1 (COX-1).*.

Compounds	COX-2 (PLP Fitness) Kcal/Mol	Amino Acids Included in H-bonding	Amino Acids Included in Hydrophobic Interactions	COX-1 (PLP Fitness) Kcal/Mol
1	60.22	Tyr355, Arg120	Gly526, Val523, Trp387, Arg120 and Tyr355	67.36
2a	66.85	Tyr355, Arg120	Trp387, Gly526	65.12
2b	62.35	Arg120	Trp387, Tyr355, Arg120, Leu384	62.02
2c	80.97	Tyr355, Arg120	Arg120, Ser530, Tyr115	62.51
3a	74.75	Tyr355, Arg120	Trp387, Tyr355, Arg120, Ser530	68.03
3b	72.32	Tyr385, Arg120	Leu531, Arg120, Val523, Trp 387,	66.98

Compounds	COX-2 (PLP Fitness) Kcal/Mol	Amino Acids Included in H-bonding	Amino Acids Included in Hydrophobic Interactions	COX-1 (PLP Fitness) Kcal/Mol
			Leu 384	
3c	**91.35**	Arg120, Tyr355, Ser530	Tyr355, Arg120, Ser530,	49.36
4a	80.47	Ala527, Tyr355	Ala527, Phe381, Val116, Val523, Tyr355	61.52
4b	70.08	Tyr355, Tyr115, Ala 527	Tyr115, Arg120, Tyr355, Ala527	63.25
4c	83.69	Tyr355, Arg120	Tyr355, Leu93, Val89, Arg120	51.89
5a	72.80	Tyr355, Arg120	Arg120, Tyr355, Val523, Gly526, Tyr385, Trp387, Leu384	63.17
5b	68.98	Ala527, Arg120	Leu531, Ala527, Arg120, Leu359, Tyr355, Ser353	72.71
5c	80.40	Ser530	Leu531, Leu93, Tyr355, Val 523	58.19
6a	85.06	Arg120	Val 523, Arg120, Leu359, tyr355	60.08
6b	84.56	Arg120, Ala 527	Leu359, Val349, Val116, Ala527, Arg120, Tyr355, Trp387, Leu384, Gly526, Val523	59.87
6c	**89.66**	Ser530, Tyr115	Leu531, Ser530, Tyr115,	51.79
7a	74.32	Tyr355, Arg120	Val116, Ala527, Arg120, Tyr355, Gly526, Leu384, Trp387,	60.38
7b	72.47	Arg120	Ile345, Leu531, Val349, Ala527, Val523, Trp387	61.89
7c	**92.09**	Tyr355	Val116, Ala527, Tyr355, Arg120	49.32
Diclofenac	71.7	Ser530, Tyr385	Ala527, Val349, Gly526 and Trp387	68.60
Naproxen	74.23	Arg120, Tyr355	Ser530, Ala527, Gly526, Val349, Leu352 and Val523	63.12

Table 4: 3D structure of some synthesized compounds* binding to active amino acids.

	H-Bond	Short Contact
3c		
4a		
4c		
5c		

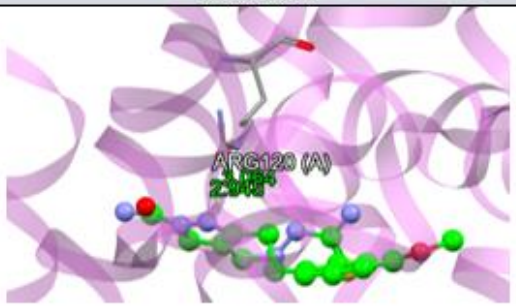
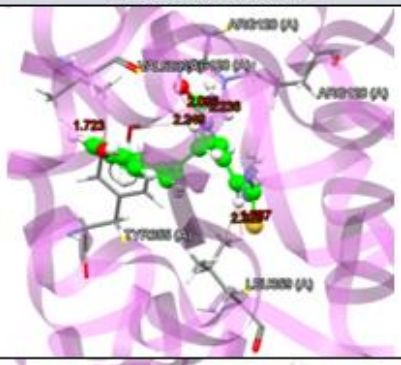
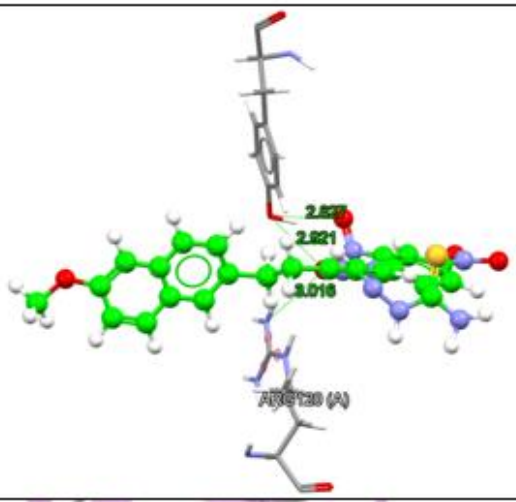
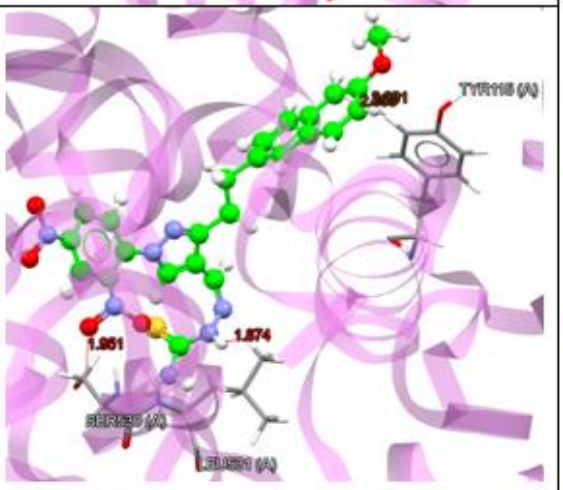
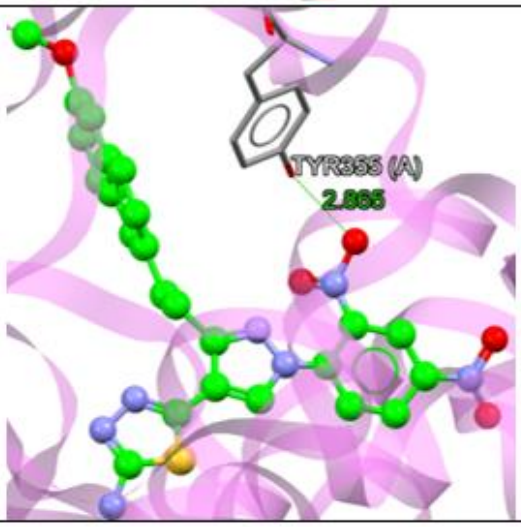
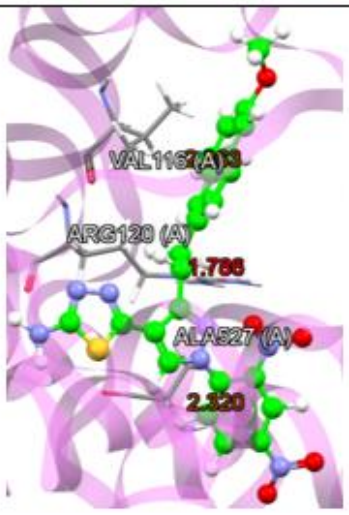
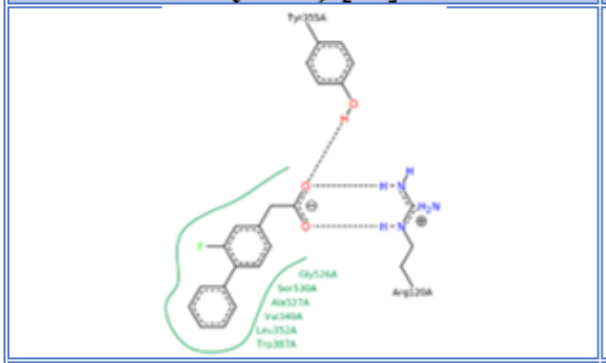
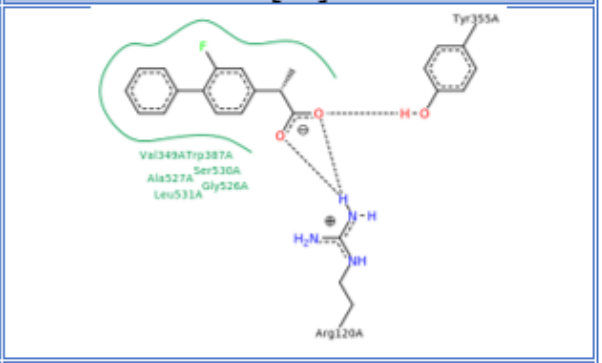
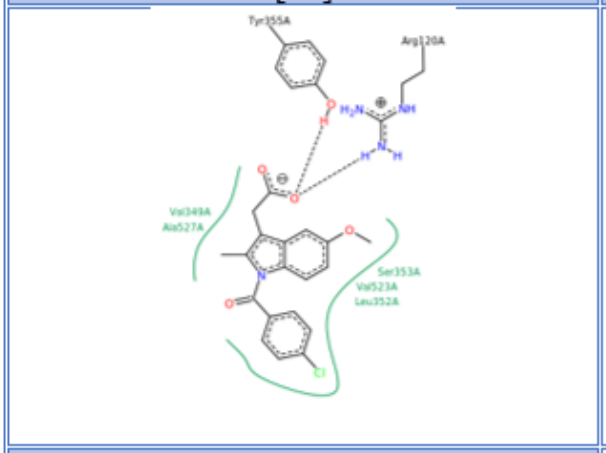
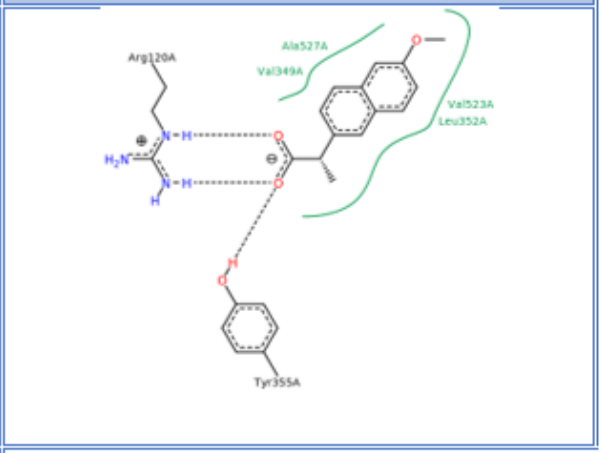
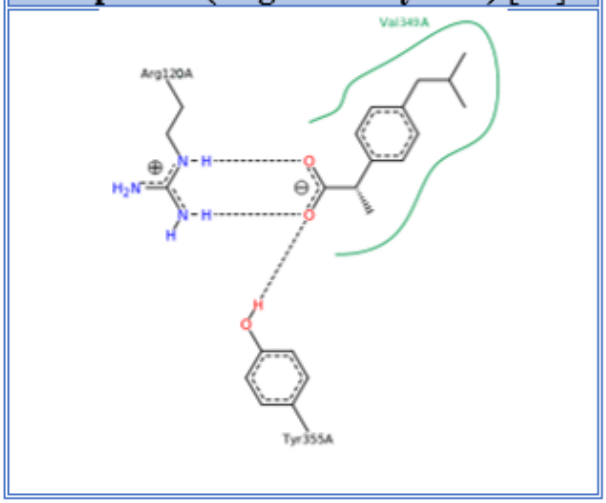
	H-Bond	Short Contact
6a		
6c		
7c		

Table 5: The binding site interaction of the five approved NSAIDs showing H-bonding with Arg120 and Tyr355.

Des-methylflurbiprofen (Arg120 & Tyr355) [21]	**Flurbiprofen (Arg120 & Tyr355)** [22]
	
Indomethacin (Arg120 & Tyr355) [23]	**Naproxen (Arg120 & Tyr355)** [22]
	
Ibuprofen (Arg120 & Tyr355) [24]	
	

Discussion

All compounds in the study satisfied Lipinski's criterion, with the exception of compounds 4c, 5c, 6c, and 7c.

Additionally, it satisfied the criteria of topological descriptors and molecular drug-likeness structural keys such as LogP and Log S. The measure of the amount of absorption of a molecule from the gut after oral delivery is known as the GI absorption score. The absorption would exhibit a high level of excellence if the outcome were to be significantly elevated. In the current investigation, it was seen that the gastrointestinal (GI) absorption of the majority of compounds was found to be high, indicating their potential for efficient absorption from the intestinal tract. However, it should be noted that compounds 2c, 3c, 4b, 4c, 5b, 5c, 6a-c, and 7a-c exhibited lower GI absorption, suggesting a reduced likelihood of effective absorption from the intestine for these specific compounds.

Due to the disparity in size between the COX-2 active site and the COX-1 active site, the insertion of synthesized compounds with substantial structures into the COX-1 enzyme pocket poses a challenge. However, certain synthesized compounds exhibit favorable docking outcomes with COXs and are capable of fitting within the COX-2 active site, as evidenced by the data presented in **table 5**. The compounds 3c, 6c, and 7c exhibited the greatest docking PLP fitness values while interacting with COX-2, with respective values of 91.35, 89.66, and 92.09. Similarly, these compounds also shown high docking PLP fitness values when interacting with COX-1, with respective values of 49.36, 51.79, and 49.32. The PLP fitness values for the other compounds, as shown in **table 3**, exceeded 80. These compounds included 2c, 4c, 5c, 6a, and 6b.

Conclusion

In conclusion, the investigation of 1,3,4-oxadiazole and 1,3,4-thiadiazole derivatives as COX inhibitors through in silico methods represents a promising approach in drug discovery and development. The computational tools utilized, such as the GOLD suite for docking and the Swiss ADME website for ADME prediction, significantly contribute to streamlining the identification and optimization of potential drug candidates. By exploiting the structural versatility and pharmacological properties of these heterocyclic rings, researchers strive to contribute to the advancement of pharmaceutical science and provide novel therapeutic options for various diseases and conditions.

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