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Original Article

DEVELOPMENT OF 1H-INDAZOLE DERIVATIVES AS ANTI-INFLAMMATORY AGENTS USING COMPUTATIONAL METHODS

RAJESH B. NANAWARE, ANURUDDHA R. CHABUKSWAR*, PRAJAKTA V. ADSULE, SWATI C. JAGDALE, KUNAL G. RAUT

School of Health Sciences and Technology, Department of Pharmaceutical Sciences, Dr. Vishwanath Karad MIT World Peace University, Pune-411038, Maharashtra, India

***Corresponding author: Anuruddha R. Chabukswar; *Email: anuruddha.chabukswar@mitwpu.edu.in**

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ABSTRACT

Objective: Due to the rising prevalence of disorders linked to inflammation, there is a greater emphasis on the discovery and development of anti-inflammatory drugs, with a focus on producing new structural compounds.

Methods: In this research, molecular docking and Molecular Dynamics (MD) simulation study were carried out to evaluate the 1H-indazole analogs as potent anti-inflammatory agents.

Results: The compounds containing difluorophenyl, para-toulene and 4-methoxyphenyl group shows significant binding results (9.11, 8.80 and 8.46 kcal/mol respectively) when docked with Cyclooxygenase-2 (COX-2) enzyme 3NT1. The results of the MD simulation indicated that test compound BDF was relatively stable in the COX-2 enzymes active sites. The compound BDF-3NT1 demonstrated substantial affinities for binding with all of its aimed targets following a dynamic Molecular Mechanics with Generalized Born Surface Area (MM-GBSA) analysis.

Conclusion: In accordance to this study, newly developed 1H-indazole compounds have the potential for treating inflammation.

Keywords: 1H-Indazole, Anti-inflammatory agents, Cyclooxygenase-2, Molecular docking, MD Simulations, MMGBSA

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INTRODUCTION

Several biologically active compounds are made up of heterocycles with thiazole, sulphur, and nitrogen moieties as their fundamental components. Part of the body's immunological reaction to an external stimulus is inflammation. It is helpful initially because it starts the healing process. It is concerning, though, because inflammation has the ability to self-replicate, causing new inflammation in reaction to pre-existing inflammation. Worldwide, inflammatory diseases are extremely frequent and can go uncontrolled. In severe cases, such as severe allergies, autoimmune disorders, and organ rejection, inflammation can be fatal [1].

Numerous illnesses have been related to chronic inflammation, such as pulmonary disease, diabetes, cancer, arthritis, Alzheimer's disease, and Cardio Vascular Diseases (CVD) [1-6]. Most Non-Steroidal Anti-inflammatory Drugs (NSAIDs) prevent prostaglandin and thromboxane production by blocking the actions of COX-1 and COX-2 [7]. The desired anti-inflammatory, analgesic, and antipyretic effects result from inhibiting COX-2, while the undesired side effects, including as gastrointestinal bleeding [8, 9], renal issues [10], and impacts on the Central Nervous System (CNS), result from inhibiting COX-1 [11]. While corticosteroids and NSAIDs are widely used to treat chronic inflammation, there is a need for safer and more effective treatments. Innovative techniques to drug development, such as immune system regulation and targeted molecular interventions, may lead to advancements in anti-inflammatory drug therapy [1-5].

Indazoles are heterocyclic compounds that include nitrogen and have been discovered to have a wide range of pharmacological applications [12]. Numerous biological activities, including antimicrobial agents [13], cancer treatment [14], anti-inflammatory [15] antiviral in nature [16], anti-platelet therapy [17], antispermatogenic [18], and 5-HT6 antagonists [19] are just a few of the many biological activities demonstrated by these heterocyclic compounds.

Therefore, in this context, the current work focuses on the computational study of newly designed COX-2 inhibitors through the use of in silico molecular docking, MD simulations, and MM/GBSA investigations. In our previous study, these newly synthesized 1H-indazole derivatives showed significant analgesic and anti-inflammatory (*in vivo)* activity. Here, we carried out *in silico* investigation of the anti-inflammatory activity of these compounds to find out the binding affinities and stability of 1H-indazole derivatives with the cyclooxygenase enzyme.

MATERIALS AND METHODS

Intel Core CPU i5-4570, running at 3.2GHz and RAM 4GB was used to perform this computational task.

Molecular docking

Construction of ligand

Twelve newly synthesized compounds were gotten from our previous work [20] and used in this research. Using the Open Babel GUI, 3D structures have been converted to PDB file format [21]. All of the structures energy [22] was minimized using UCSF-Chimera software, and Autodock tools 4.2.6 were then used to convert the results into PDBQT [23, 24]. Table 1 showed the 2D structures of 1H-Indazole analogs.

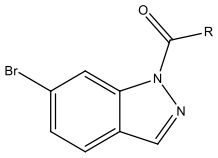
Receptor preparation

The 3D crystal structure of the COX-2 enzyme was downloaded from the Protein Data Bank (https://www.rcsb.org); PDB ID: 3NT1 [25]. Water molecules, ions, and other ligands were removed from the proteins, and then polar-H and Kollman charges were added. Using the Auto Dock Tools (ADT) graphical user interface, PDBQT files for the receptor protein were generated following the assignment of AD4 charges.

Generation of grid

To create the grid parameter (.gpf) files, the grid box size for the 3NT1 protein was modified to 102×100×112 (0.5Å spacing). In the docking experiment, ten docking runs were carried out for every ligand using the genetic search Lamarkain algorithm in an attempt to find optimum conformational space for the ligand. The generations and the number of assessments were set at 2500000 and 27000, respectively. The ligand shape with the highest binding energy was chosen to examine close intramolecular bonds to the receptor.

Table 1: Structures of 1H-indazole analogs



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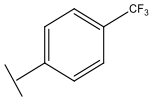
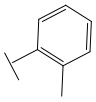
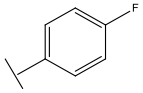
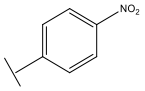
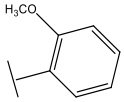
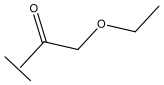
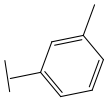
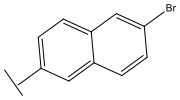
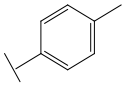
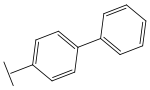
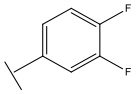
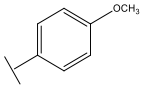
BCF		BOT	
BPF		BPN	
BOM		BETX	
BMT		BBN	
BPT		BDP	
BDF		BPM	

MD simulation study

MD simulation studies were conducted on the docked compounds 3NT1+BDF and 3NT1+NPS, 3NT1+APO (no ligand), [26] utilizing the Desmond 2020.1 of Schrodinger, LLC. The OPLS-2005 explicit solvent model with SPC water molecules and force field [27-30] were employed in this system. Na+ions were added to the system together with 0.15M sodium chloride solution to balance the charge in order to stimulate the physiological environment. First, the system was retrained over the protein complex using 100ps of NVT ensemble to achieve equilibrium. A 12-ps NPT ensemble short-run equilibrium and reduction followed next. The NPT ensemble was configured using the Nose-Hoover chain coupling scheme [31], and it was kept at these parameters for the duration of the simulations: 27 °C, 1.0ps relaxation period, and 1 bar pressure. The method of Martyna-Tuckerman-Klein chain coupling with a 2ps relaxation time, the barostat method [32] was used to control pressure. The Ewald method [33] with a particle mesh was utilized to calculate the electrostatic interactions across long distances, and the radius for the coulomb interactions has been set at 9Å. At a time step of 2fs, bonded forces were computed for every trajectory employing the RESPA integrator. In order to monitor the stability of the MD simulations, the following metrics were computed: Solvent Accessible Surface Area (SAS Area), Radius of Gyration (Rg), (Root mean Square Fluctuation) RMSF, and (Root mean Square Deviation) RMSD.

Binding free energy calculations

We calculated the binding free energies of each complex using MM-GBSA computations and molecular mechanics. Using a 100-step sample size, the MM-GBSA binding free energy in the simulation trajectory and the final 50 frames of the OPLS 2005 force field were estimated using the Python script thermal_mmgbsa. py. The binding free energy of prime MM-GBSA (kcal/mol) was determined by applying the additivity principle, which entails adding up each unique energy module, such as a covalent, H-bond, van der Waals, lipophilic, solvation and stacking of ligand and protein.

ΔG_{bind} equation: $\Delta G_{bind} = \Delta G_{MM} + \Delta G_{Solv} - \Delta G_{SA}$

RESULTS AND DISCUSSION

Molecular docking

Compounds showing highest binding energies: with Arg120 and Tyr355 amino acid residues, 6-bromo-1H-indazol-1-yl-(3, 4-difluorophenyl) methanone; BDF formed 2 conventional hydrogen bonds with bond distances of 5.03 and 6.00 Å, exhibiting the maximum binding energy value of-9.11kcal/mol. It formed a C-H bond with Ala527. It created π-stacked amide bonds with Gly526 and π-alkyl bonds with the amino acid residues Tyr385, Phe149, Trp387, Phe381, Leu352, Val523, Val116, Leu531 and Ala527**.** After that, compound (6-bromo-1H-indazol-1-yl) (p-tolyl) methanone; BPT showed a binding affinity value of-8.80 kcal/mol and formed one C-H bond interaction involving the residues Ala527 and bond distance of 3.74 Å between them. It created an amide-π-staked connection with the Gly526 amino acid residue in addition to a hydrogen bond. Additionally, it created π-alkyl bonds with the amino acid residues Leu359 and Tyr385, Phe381, Trp387, Phe381, Leu352, Val523, Val116, Leu531, Leu359, and Ala527. With the amino acid Ala527, the compound 6-bromo-1H-indazol-1-yl-(4-fluorophenyl) methanone; BPF formed a single carbon-hydrogen bond with a bond distance of 3.77 Å, exhibiting a binding energy value of-8.49kcal/mol. It bonded with the amino acid residue Gly526 to form an amide-π-staked bond. Additionally, it created π-alkyl bonds with the receptors amino acid residues, Tyr385, Trp387, Phe381, Leu352, Val523, Val116, Leu531, Leu359, and Ala527. If we compare the results of indazole derivatives with the reference drug indomethacin, it was found that the 3NT1-indomethacin complex exhibiting the binding energy-6.42 kcal/mol and does not showed any hydrogen bond, but the other interacting residues were common. Protein structure and stability may be influenced by amide stacking interactions, which are stronger than many other interactions and may compete with hydrogen bonds [34, 35]. Indomethacin and all the novel indazoles showed an amide-π-staked connection with the Gly526 amino acid and hence formed a stable complex. To a large extent, amide stacking remains unclear. The interaction between 3NT1 and indazole analog were shown in table 2 and fig. 1.

Table 2: Interaction of indazole derivatives with 3NT1 binding site residues

Molecule	Binding affinities (kcal/mol)	H-bonds	Angles (Å)	Hydrophobic and other interacting residues
BCF	-7.63	Arg120, Tyr385	3.82, 5.73	Ala527, Gly526, Leu352, Leu359, Leu531, Leu93, Met522, Phe381, Phe518, Ser353, Ser530, Trp387, Tyr348, Tyr355, Val16, Val349, Val523.
BPF	-8.44	Tyr385	4.02	Ala527, Arg120, Gly526, Ile345, Leu352, Leu359, Leu384, Leu531, Leu93, Met113, Met522, Phe381, Phe518, Ser353, Ser530, Trp387, Tyr348, Tyr355, Tyr385, Val16, Val349, Val523.
BOM	-7.3	----	----	Ala527, Arg120, Gly526, Leu352, Leu359, Leu384, Leu531, Met113, Met522, Phe381, Phe518, Ser353, Ser530, Trp387, Tyr348, Tyr355, Tyr385, Val16, Val349, Val523.
BPT	-8.80	----	----	Ala527, Arg120, Gly526, Leu359, Leu384, Leu352, Leu93, Met113, Met522, Phe357, Phe381, Phe518, Ser530, Ser353, Trp387, Tyr355, Tyr385, Val116, Val349, Val523.
BDF	-9.11	Arg120, Tyr355	5.03, 6.0	Ala527, Gly526, Leu359, Leu384, Leu352, Leu531, Met113, Met522, Phe357, Phe381, Phe581, Ser530, Ser 353, Trp387, Tyr385, Val116, Val349.
BOT	-8.07	Arg120	3.82	Ala527, Gly526, Ile345, Leu359, Leu384, Leu352, Leu531, Met113, Met522, Phe381, Phe518, Ser530, Ser 353, Trp387, Tyr385, Val116, Val349, Val525.
BPN	-7.88	Arg120	3.85	Ala527, Gly526, Ile345, Leu359, Leu384, Leu352, Leu531, Leu93, Met113, Met522, Phe518, Phe357, Phe381,, Ser530, Ser 353, Trp387, Tyr385, Val116, Val349, Val525.
BETX	-7.49	Tyr385	3.54	Ala527, Arg120, Gly526, Leu359, Leu384, Leu352, Leu531, Met113, Met522, Phe518, Phe381, Ser530, Ser353, Trp387, Tyr355, Val116, Val349, Val525.
BBN	8.20	Arg120	3.50	Ala527, Gly526, Leu359, Leu384, Leu352, Leu531, Met113, Met522, Phe518, Ser530, Ser353, Trp387, Tyr385, Tyr355, Val116, Val349, Val523.
BDP	-7.19	Arg120	4.12	Ala527, Gly526, Ile112, Leu359, Leu384, Leu352, Leu531, Phe381, Phe518, Ser530, Ser353, Trp387, Tyr115, Tyr385, Tyr355, Val116, Val523, Val527.
BPM	-8.46	Tyr355	3.93	Ala527, Arg120, Gly526, Leu359, Leu384, Leu352, Leu531, Leu93, Phe381, Phe518, Ser530, Ser353, Trp387, Tyr385, Val116, Val523.
Indomethacin	-6.42	---	--	Ala527, Arg120, Gly526, Ile517, Leu359, Leu384, Leu352, Leu531, Leu93, Met522, Phe381, Phe518, Ser530, Ser353, Trp387, Tyr385, Val116, Val349, Val523.

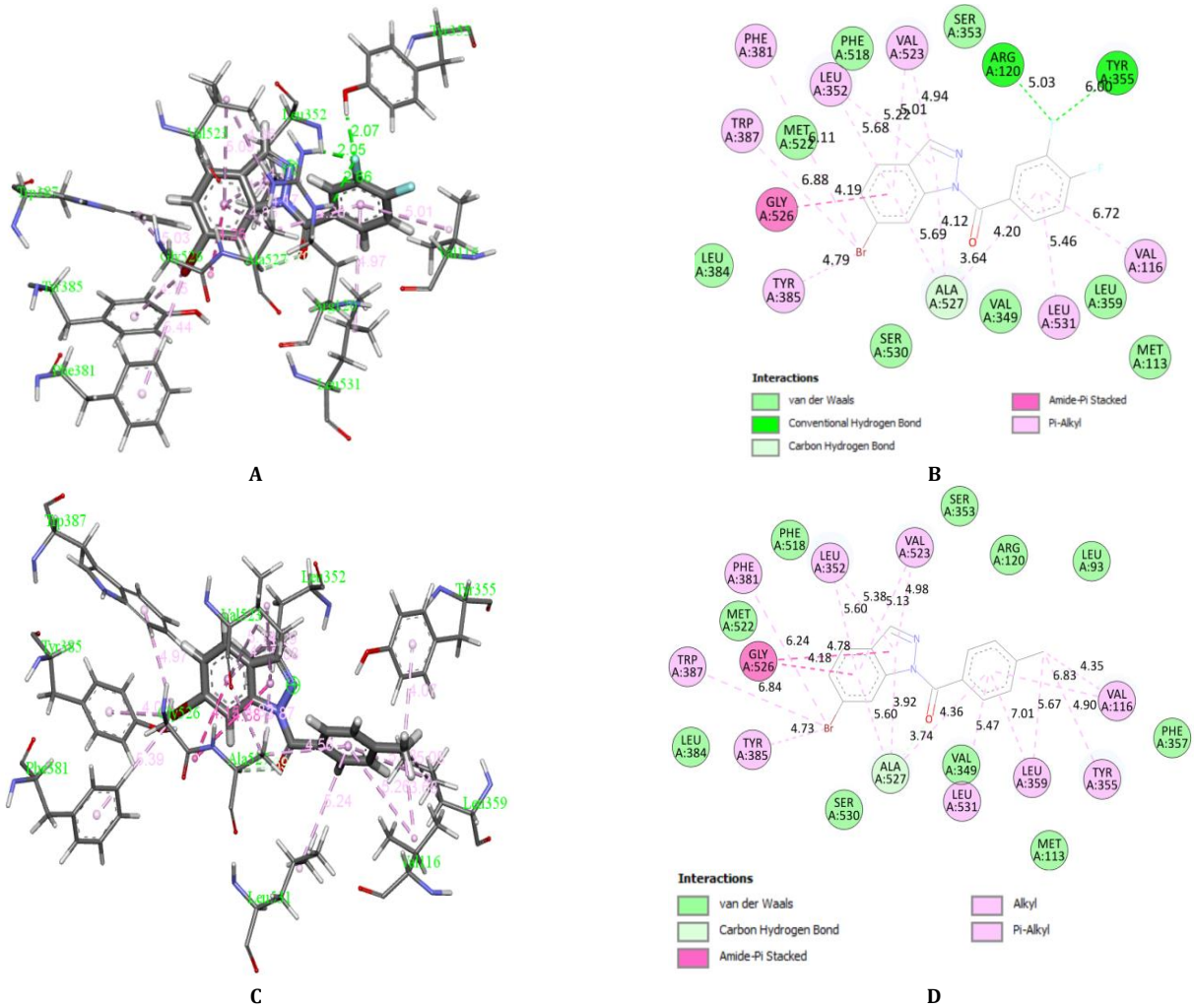


Fig. 1: **A and B: 3D and 2D complex of BDF with 3NT1. C and D: BPT with 3NT1**

Molecular dynamic simulation study

The stability and convergence of the 3NT1-apo (only protein), 3NT1+BDF (with ligand), and 3NT1+NPS (with native ligand) complexes were studied using simulations based on molecular dynamics and experiments. A stable conformation was produced by the 100 ns simulation, according to the RMSD data. When 3NT1 combined with BDF, the RMSD found to be 2.5 Å, compared to a very high 4.2 Å (3NT1-Apo) Cα-backbone when it was detected alone (fig. 2A). Whereas the other ligand NPS bound 3NT1 Cα-backbone, the total RMSD was shown to be 4.1 Å (fig. 2A). Only the 3NT1+BDF complex showed good convergence and stable conformation. As a result, it's feasible to make assumptions that the complex formed by BDF bound to 3NT1 is quite stable because of the enhanced affinities of the ligand.

The 3NT1-Apo proteins RMSF plot showed little spikes of fluctuation, with the possible exception of residues 50–60, which have greater flexibility. During the 100ns simulation, there was less fluctuation in the residuals (fig. 2B). While 3NT1 was bound by BDF and NPS, it showed 30-50, 60-70, 90-95, and 110-120, respectively, showing that the residues conformations of amino acid were flexible (fig. 2B). Consequently, RMSF graphs can be used to infer it, while proteins are in ligand-bound conformations during the simulation process, their structures do not change.

One metric that can be used to assess the protein's compactness is the Rg. The Rg of the 3NT1-Apo Cα-backbone in this case was found to be consistently between 24.2 and 24.4Å (fig. 2C). While the protein attached to NPS showed an increase in peak up to 40 ns before falling down, the protein bound to BDF showed a decrease of peak from 24.2 to 24 Å (fig. 2C). A protein in its ligand-bound state has an unprecedentedly high degree of stability in its gyration. Peak lowering signifies a highly compact structure. A stable, strongly interacting complex is suggested by the presence of significant no. of H-bonds between the protein and ligand.

The number of H-bonds between 3NT1 and BDF remained statistically significant over the course of a 100-millisecond simulation (fig. 2D). It is shown that the number of hydrogen bonds between 3NT1 and BDF is one, which is the average (fig. 2D). While between NPS and 3NT1, the average numbers of hydrogen bonds up to 40ns is two while later settled in number 1 (fig. 2D). Molecular docking examinations were conducted; however, they yielded no results, which substantiated the MD simulation discovered a hydrogen bond between the protein and the ligand.

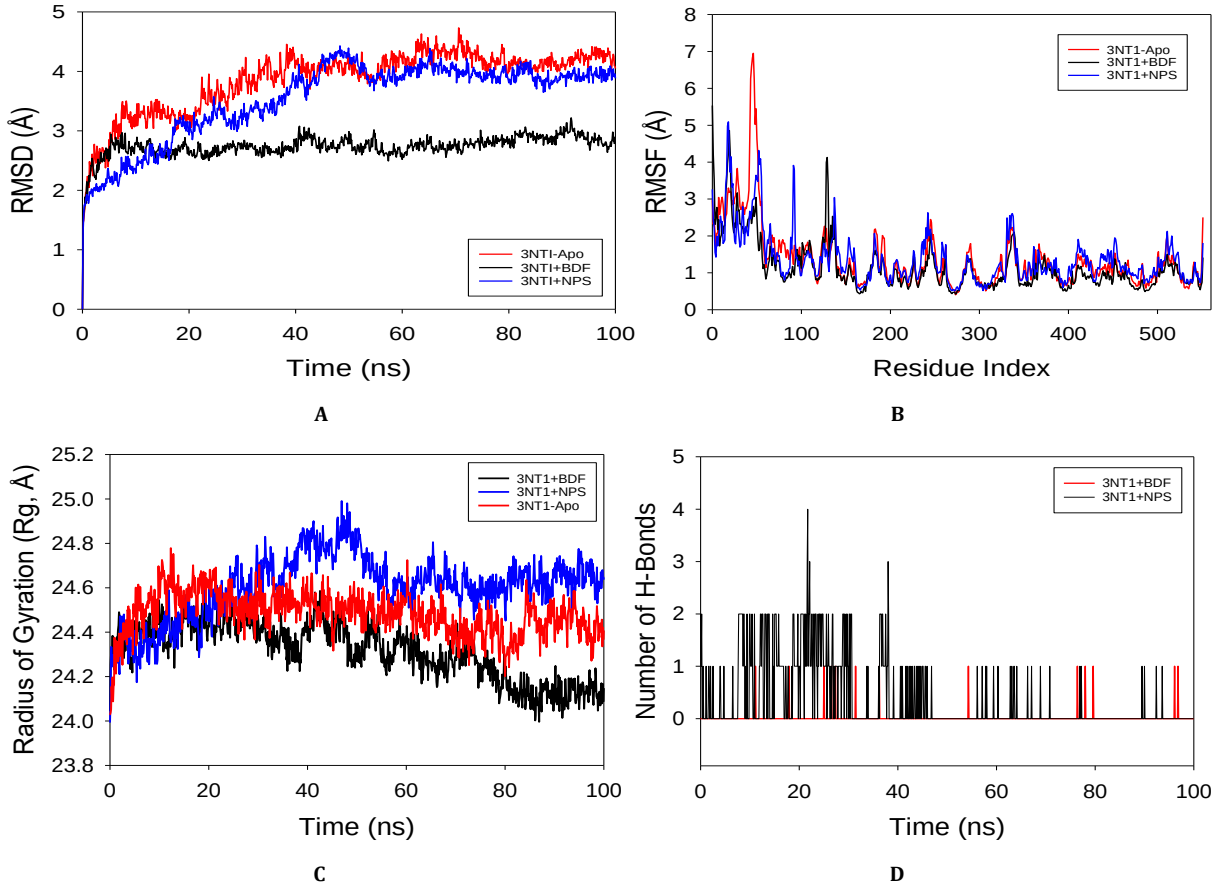


Fig. 2: MD simulation analysis (A) Cα backbone of 3NT1-Apo (red), 3NT1+BDF (Black) and 3NT1+NPS (Blue) (B) RMSF: 3NT1-Apo (red), 3NT1+BDF (Black) and 3NT1+NPS (Blue). (C) Rg: 3NT1-Apo (red), 3NT1+BDF (Black) and 3NT1+NPS (Blue). (D) H-bonds in 3NT1+BDF (Red) and 3NT1+NPS (Black)

Post dynamic MMGBSA binding free energy analysis

Employing the MD simulation, the binding free energy and other contributing energy for 3NT1+BDF and 3NT1+NPS were determined in the form of MM-GBSA. The data suggested that GbindLipo, Gbindvd W, and Gbind Coulomb had the greatest effects on the simulated complexes stability. However, GbindSolvGB and Gbind Covalent were in charge of the opposite outcome. We discovered that the binding free energy in the 3NT1+BDF complex was greater than that of 3NT1+NPS. These results validated the promise of BDF, demonstrated its efficacy in binding the target protein, and ability to create stable protein-ligand complexes. Both the systems (3NT1 with BDF and NPS) showed higher negative binding free energy values i. e., (ΔGbind) =-56.83 kcal/mol and-54.81 kcal/mol, respectively and suggest greater ligand binding in the receptor's active region. Table 3 represents components of binding free energy for the 3NT1+BDF and 3NT1+NPS.

Table 3: Components of binding free energy for the 3NT1+BDF and 3NT1+NPS

Energies (kcal/mol)	3NT1+BDF	3NT1+NPS
ΔG_{bind}	-56.83±2.1	-54.81±3.2
ΔG_{bind}Lipo	-20.09±2.3	-18.20±0.86
ΔG_{bind}vdW	-47.48±2.17	-41.26±1.46
ΔG_{bind}Coulomb	-3.46±1.01	-12.41±2.1
$\Delta G_{bind}H_{bond}$	-0.007±0.0	-0.75±0.34
ΔG_{bind}SolvGB	15.28±2.27	18.39±1.4
ΔG_{bind}Covalent	1.10±0.5	0.744±0.22

CONCLUSION

In order to identify possible COX-2 inhibitors, we carried out docking of 1H-indazole derivatives atomically. According to a docking study, these analogues significantly bind to the 3NT1 enzyme. In this work, compound 6-bromo-1H-indazol-1-yl-(3, 4-fluorophenyl) methanone i. e. BDF containing difluoro group 9.11kcal/mol and (6-bromo-1H-indazol-1-yl) (p-tolyl) methanone i. e. BPT, containing para-toluene group 8.80kcal/mol has highest binding energy. Test substance BDF was found to be considerably stable in the active site of the COX-2 enzyme, according to the findings from the MD simulation. After conducting a dynamic MM-GBSA investigation, the compound BDF-3NT1 showed significant binding affinities with all of its selected targets. Previous synthesis and *in vivo* studies conducted by our research team and current *in silico* study confirms the effectiveness of 1H-indazole analogues as analgesic and anti-inflammatory agent and it would be a promising compound for the treatment of inflammation as a selective COX-2 inhibitor. To determine whether indazole analogues are beneficial in human clinical trials, more investigation is needed.

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Nil

AUTHORS CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author RBN designed the study, wrote the protocol, done the analyses of the study and reviewed manuscript. PVA and SCJ performed the statistical analysis. Author ARC wrote the first draft of the manuscript and reviewed manuscript. Author KGR done literature review. All authors read and approved the final manuscript.

CONFLICT OF INTERESTS

The authors declare no conflicts of interest

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