Original Article

IN SILICO SCREENING OF ANTITUBERCULAR AND ANTI-INFLAMMATORY ACTIVITY OF INDAZOLE BASED NATURAL ALKALOIDS AND A NOVEL INDAZOLE DERIVATIVE

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Abstract

The naturally occurring indazole scaffold containing alkaloids Nigeglanine, Nigellicine, and Nigellidine, which are derived from Nigella Sativa and Nigella Glandulifera, shows antibacterial, anti-inflammatory, anti-cancer, and other pharmacological actions. Recent studies have revealed the potential value of these molecules in a variety of biological conditions. Resistance and severe side effects, however, drive research into new and more effective anti-inflammatory and anti-tuberculosis medications. In this research molecular docking analysis along with ADMET and drug likeness prediction were carried out to evaluate the newly designed indazole scaffold as potent Enoyl-ACP (CoA) reductase enzyme and cox-2 inhibitor. Comparing with reference compound docking scores isoniazid (-5.21 kcal/mol) and indomethacin (-6.42 kcal/mol) compound BPM showed highest binding affinities (-7.66 and -7.46 kcal/mol) with respective enzymes. Depending on the specific ADMET risk factors and drug similarity for oral bioavailability, all indazole scaffolds shows significant activity. Present study demonstrated that newly designed indazole scaffold and natural alkaloids containing indazole nucleus could be the potential drug of choice against inflammation and tuberculosis.

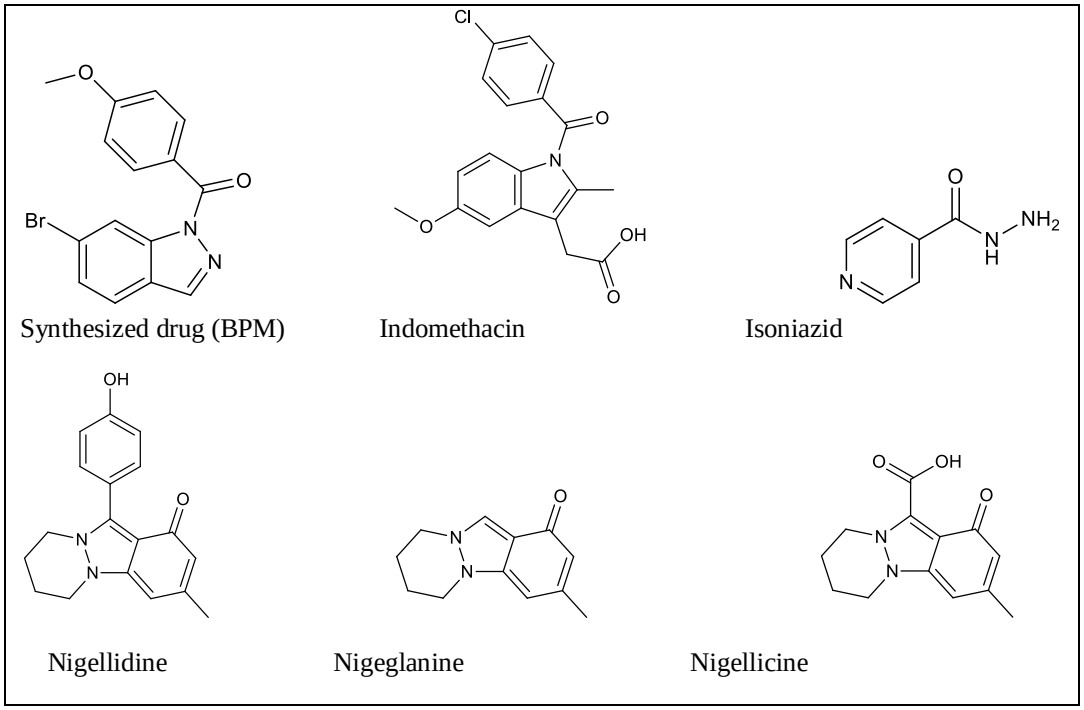
Keywords: ADMET; Indazole; Indomathacin; Molecular Docking; Nigella Glandulifera; Nigella Sativa.

1. INTRODUCTION

The naturally occurring alkaloids Nigeglanine, Nigellicine, and Nigellidine, which are derived from Nigella Sativa and Nigella Glandulifera, exhibit antibacterial, antifungal, analgesic, anti-inflammatory, anti-cancer, and numerous other actions [1,2]. Indazole is becoming more widely used as a medicine for treating various illnesses. Investigations carried out in recent decades have identified the potential usefulness of these compounds in several biological conditions such as apoptosis inhibition [3], anti-arthritis [4], anti-proliferative activity [5], antihypertensive agent [6], anti-psychotic [7], hypotensive [8], obesity [9], antineoplastic [10], anti-hyperlipidemic activity [11], agent as trichomonacidal activity [12], Analgesic and antipyretic [13] and anti-inflammatory [14].

A significant global public health issue is tuberculosis (TB). The real threat to global TB control is posed by drug resistance and the severe side effects of current TB treatment. Novel antitubercular drugs are therefore urgently required, especially those made from natural components or having minimum side effects [15]. Hence, in this context we have carried out synthesis of 1H-indazole derivative and a molecular docking to evaluate the effect of natural alkaloids containing indazole nucleus and a designed indazole compound on Enoyl-ACP (CoA) reductase enzyme and cyclooxygenase-2 enzyme along with its *in silico* ADMET prediction.

Figure I. Chemical structures of Indazole based compounds and reference drugs.



2. METHODS AND MATERIALS

2.1 Chemistry:

All commercially available chemicals were used without further purification. DMF used in reactions was distilled prior to use and other solvents used in reactions and solvents used for purification were distilled prior to use. Thin-layer chromatography (TLC) was performed on precoated aluminum plates and visualized by UV light (254 nm). Melting point was determined on a Buchi melting point apparatus and was uncorrected. IR spectra were measured on a Bruker FT-IR spectrometer. ^{1}H NMR spectra were recorded at 500 MHz on a Bruker spectrometer using $CDCl_3$ as the solvent.

2.2 Molecular Docking Studies:

This computational work was accomplished by using software Autodock 4.2.6.

2.2.1 Preparation of Ligand:

Using the ACD/ChemSketch software, compounds with an indazole scaffold and isoniazid and indomathacin was created. For conversion of 3D structures into .pdb file format Open Babel GUI 3.1.1 were used [16] and by applying 100 steps of the steepest descent and 10 steps of conjugate gradient in the UCSF-Chimera software, the energy of all the structures was minimized [17]. With the aid of Autodock tools, these structures were converted into PDBQT file format. [18-19].

2.2.2 Preparation of Receptor:

The three-dimensional coordinates of Enoyl-ACP (CoA) reductase enzyme (InhA) (PDB ID: 2AQK) and Cyclooxygenase-2 (PDB ID: 3NT1) was down-loaded from the PDB-RCSB databank [20-21]. The target proteins were pre-processed before molecular docking by adding missing H-atoms, defining bond orders, deleting any heterogeneous ligand and water molecules. The grid parameter (.gpf) files were prepared by adjusting the size dimension of grid box as 98 × 98 × 98 (0.5 Å spacing) for 2AQK and 113 × 99× 99 (0.5 Å spacing) 3NT1 proteins respectively. Finally the grid log files (.glg) and docking log files (.dlg) were prepared by launching Autogrid and autodock tools respectively. The docking was carried out with 10 docking runs for each ligand. The generations and the evaluations were both set to 27000 and 2500000, respectively. Finally analysis of the complexes was carried out using ADT. The types of docked interactions were carried out by discovery studio visualizer.

2.2.3 Prediction of ADMET and Drug-likeness properties:

SwissADME was used to predict the drug like, physicochemical properties and Absorption, Distribution, Metabolism, Excretion, and Toxicity of compounds [22-26].

3. RESULT AND DISCUSSION:

3.1 Procedure for the synthesis of compound BPM:

The starting compound 6-bromo-1H-indazole was obtained by using various reported methods. A stirred solution of 6-bromo-1H-indazole (1.0 equiv.), (4-methoxyphenyl) benzoic acid (1.5 equiv.), Na_2CO_3 (2 equiv.) and a coupling agent N-ethylcarbodiimide hydrochloride acid (1.5 equiv.) in DMF was stirred at R.T. for overnight. TLC (solvent ratio of 8:2; ethyl acetate: n-hexane). The solvent was removed under reduced pressure. The remaining solid was subjected to filtration. The crude product was purified by recrystallization using EtOH to afford the corresponding (6-bromo-1H-indazol-1-yl) (4-methoxyphenyl) methanone. Purified product was subjected for spectral analysis but the spectral data did not show the signals for desired product. The same results obtained by changing molar ratios of reagents. Hence we conclude that the desired product under this reaction conditions could not be formed.

3.2 Molecular docking studies of Compounds against 2AQK:

The natural alkaloid **nigellidine** showed the highest binding energy -7.89 kcal/mol and formed two conventional H-bonds with Thr39 and Leu63 amino acid residue with bond distance 2.03 and 2.09 Å respectively. It formed Carbon-H bonds with Ile95 and Gly14 amino acids. It formed alkyl and π-alkyl bond with Phe41 and π-σ bond with Ile16 amino acid residues of the receptor. Followed by compound **BPM** showed binding energy -7.66kcal/mol and no conventional H- bond interactions were observed. It formed Carbon-H bond with Ile95 amino acid. It formed π-π-staked bonds with Phe41 and Phe97 amino acid residues. It also formed π-alkyl bonds with Ile122 and Phe41 and alkyl bonds with Val65 and Ile122 amino acid residues. The compound **nigeglanine** showed the binding energy -6.61kcal/mol, formed one hydrogen bond with Val65 amino acid and bond distance was 2.10 Å. It formed alkyl and π-alkyl bond with amino acid Leu63. It formed π-π-staked bonds with Phe41 and π-σ bond with Ile95 amino acid residues. The binding interactions between 2AQK and indazole derivatives were shown in Table I and Figure II.

Figure II: A & B) 2D and 3D Docked complex of 2AQK and nigellidine, C & D) 2D and 3D Docked complex of 2AQK and BPM respectively.

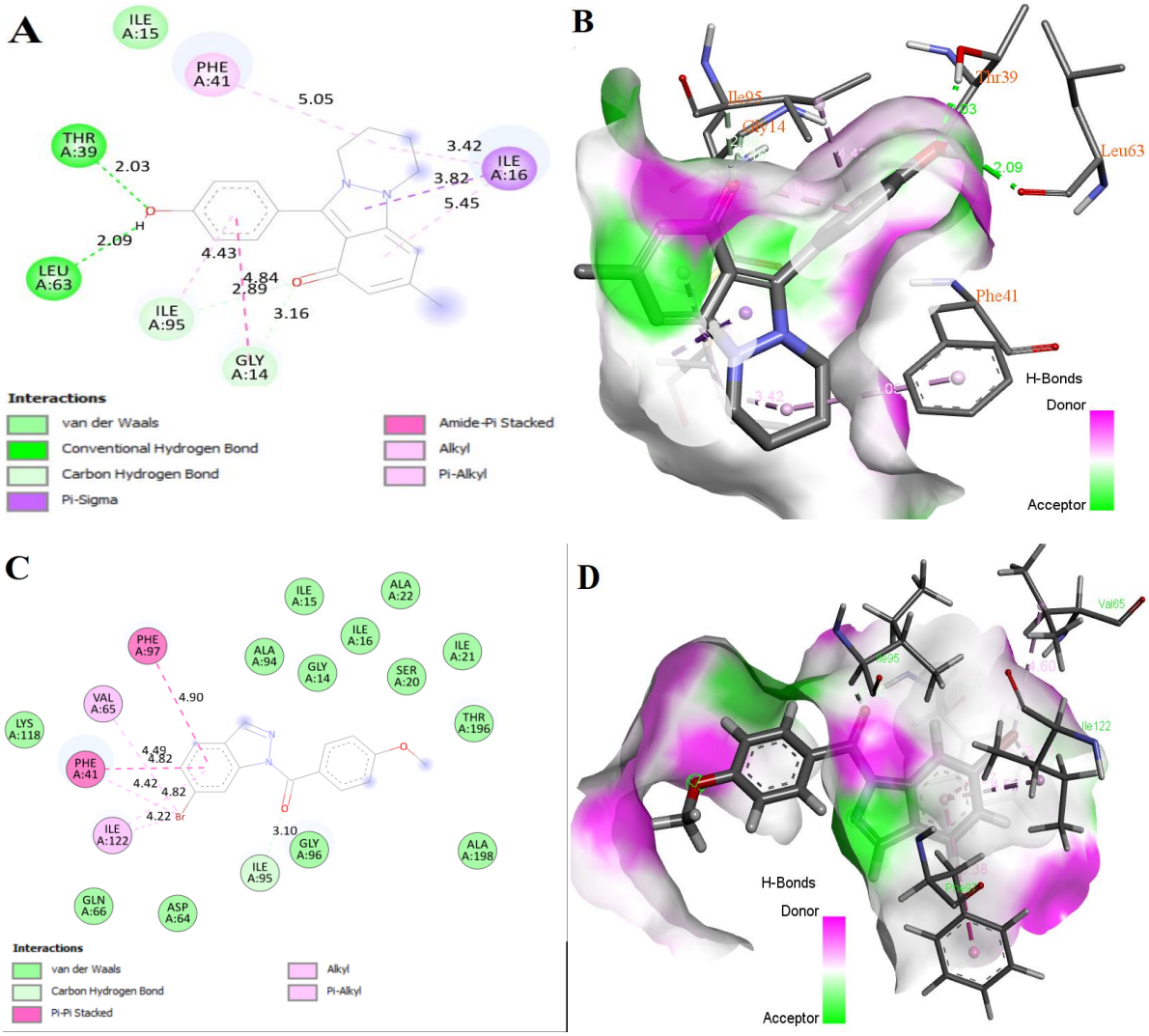


Table I. Interactions of newly designed and natural alkaloid containing indazole nucleus with 2AQK

Compd.	**Binding energies (kcal/mol)**	**Hydrogen bond**	**Bond Angle in (Å)**	**Other interactions (Hydrophobic etc.)**
BPM	-7.66	-	-	Ile95, Phe41,Phe97, Val65, Ile122, Lys118, Ala94, Ile15, Ile16,Ser20, Ile21, Thr196.
Nigellidine	-7.89	Thr39, Leu63	2.03, 2.09	Ile95, Gly14 , Phe41, Ile16
Nigeglanine	-6.61	Val65	2.10	Leu63, Phe41, Ile95
Nigellicine	-6.21	-	-	Asp148, Phe149, Ala191, Tyr158, Met103, Met161.

Isoniazid	-5.26	Gly14, Leu63	Asp64,	3.48, 3.24, 4.62	Ile15, Phe41, Thr39, Ser13, Val65, Gly40,Gln66, Ile122, Ile95

3.3 Molecular docking studies of Compounds against 3NT1:

Natural alkaloid **nigellidine** showed the highest binding energy -9.02 kcal/mol and formed two hydrogen bonds with Asn382 and Trp387 amino acid residue with bond distance 3.67 and 4.40 Å respectively. It creats alkyl bonds with Ala199, Leu390, Leu391 and π-alkyl bond with Leu391 amino acid residues. It also formed π-π-T shaped bond with His386 amino acid residue of the receptor. Followed by compound **nigellicine** showed binding energy of -8.02 kcal/mol and it formed 3 hydrogen bond interactions with Ser126, Gln372, Lys332 amino acid residues with bond distance 2.95, 3.09 and 3.82 Å respectively. It formed C-H bond with Ser121 and π -alkyl bond with Tyr122 amino acid. The compound **BPM** showed the binding energy value of -7.46 kcal/mol and no any hydrogen bond interactions were observed. It formed Cabon-H bond with Ala527 amino acid. It formed alkyl and π-alkyl bond with amino acid Leu63. It formed amide-π-staked bonds with Gly526, Met522 and π-alkyl bonds with Tyr385, Phe381, Trp387, Leu352, Val523, Val116, Tyr355, Leu531 and Ala527 as well as alkyl bonds with Val116 and Leu93 amino acids.The binding interactions between 2AQK and indazole derivatives were shown in Table II and Figure III.

Figure III: A & B) 2D and 3D Docked complex of 3NT1 and nigellidine, C & D) 2D and 3D Docked complex of 3NT1 and BPM respectively.

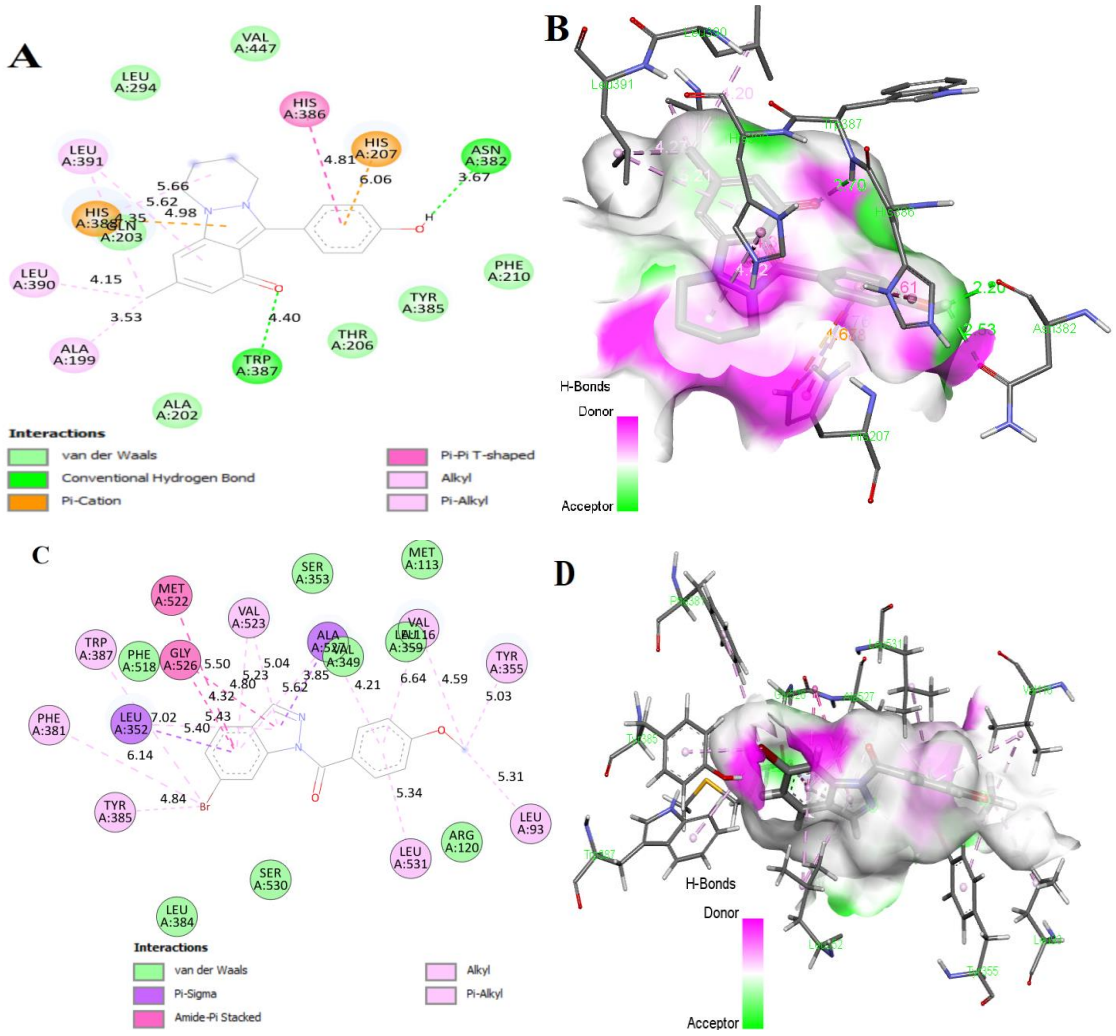


Table II. Types of interactions of newly designed and natural alkaloid containing indazole nucleus with 3NT1:

Entry	Binding affinities (kcal/mol)	Conventional Hydrogen bond	Bond Angle (Å)	Hydrophobic and other interactions
BPM	-7.46	-	-	Ala527, Gly526, Met522, Val116, Leu93, Tyr385, Phe381, Trp387, Leu352, Val349, Tyr355, Leu531, Ala527, Leu384, Ser530, Arg120, Lue539, Met113, Ser353, Phe518
Nigellidine	-9.02	Asn382, Trp387	3.67, 4.40	His386, Ala199, Leu390, Leu391, Ala202, Thr206, Tyr383, Phr210, Val447, Leu294, Gln203
Nigeglanine	-7.33	Tyr206	1.60	His386, His388, Leu391, Leu390, Ala199, His207
Nigellicine	-8.02	Ser126,Gln372, Lys332	2.95, 3.09, 3.82	Ser121, Tyr122, Asp125, Ile124, Phe371, Gln370, Thr118
Indomethacin	-6.42	-	-	Ser353, Tyr355, Tyr385, Gly526

3.4 Prediction of ADMET and Drug-likeness properties:

The drug-likeness properties of the natural alkaloids, reference drugs (isoniazid and indomethacin) and designed indazole compound (BPM) were predicted following Rule of Five. The entire tested compound passes rule with 0 violations. The number of HBD and HBA's for all tested compounds was less than 5 and 10 respectively. All the molecules having tPSA value < 140Å2. The low BBB penetration of all tested compounds (tPSA> 90.00 Å2), indicates that the adverse effects of CNS are either compact or inattentive. All the compounds were observed to have WLOGp values (low toxicity level or not) less than 5. These molecules have synthetic accessibility score < 5 (easily synthesizable in lab.). Bioavailability score of compounds was found to be 0.55 for BPM, nigellidine, nigeglanine and isoniazid while 0.85 for nigellicine and indomethacin. As a result, the newly designed compound is drug-like, orally bioavailable and active. All of the molecules under investigation showed higher GI absorption values, proving their high capacity for absorption by the human intestine. Except nigellicine all the compounds were permeant of Blood Brain Barrier. Except nigellidine remaining compounds served as non Pgp-substrate. BPM, nigellidine, nigeglanine and indomethacin were found to inhibit the CYP1A2 and nigellicine and isoniazid were observed to non-inhibitors of CYP1A2. The compound BPM and indomethacin were obserced to inhibit the CYP2C19 and CYP2C9 while nigellidine, nigellicine, nigeglanine and isoniazid were found to be non-inhibitors of CYP2C19 and CYP2C9. The entire tested compounds were found to be non-inhibitors of CYP2D6 except nigellidine. The ADME properties of tested compounds shown in figure IV and Table III.

Figure IV. Bioavailability (Radar plot); LIPO: Lipophilicity; FLEX: Flexibility; SIZE: Size; INSAT: Insaturation; INSOLU: Insolubility; POLAR: Polarity.

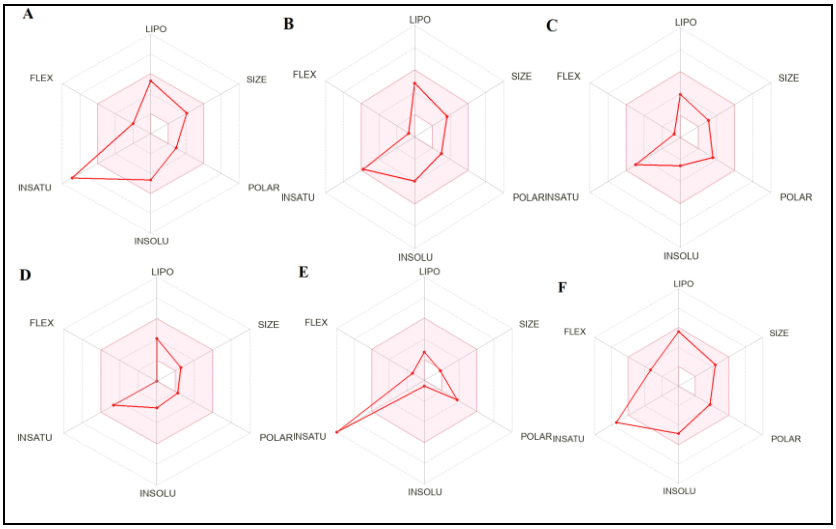


Table III. Drug likeness and ADME properties of compounds:

ADMET properties	**BPM**	**Nigellidine**	**Nigellicine**	**Nigeglanine**	**Isoniazid**	**Indomethacin**
MW	331.16	294.35	246.26	202.25	137.14	357.79
tPSA	44.12	47.16	64.23	26.93	68.01	68.53
WLOGP	3.5	3.28	1.6	1.91	-0.31	3.93
HBD	0	1	1	1	2	1
HBA	3	2	3	1	3	4
RO5 violation	0	0	0	0	0	0
RB	3	1	1	0	2	5
SA	2.14	2.86	2.51	2.32	1.24	2.51
GI-absorption	High	High	High	High	High	High
BBB-permeant	Yes	Yes	No	Yes	Yes	Yes
Pgp-substrate	No	Yes	No	No	No	No
Bioavailability-score	0.55	0.55	0.85	0.55	0.55	0.85

CYP1A-inhibitor	Yes	Yes	No	Yes	No	Yes
CYP2C19-inhibitor	Yes	No	No	No	No	Yes
CYP2C9-inhibitor	Yes	No	No	No	No	Yes
CYP2 D-inhibitor	No	Yes	No	No	No	No

MW=Molecular Weight; tPSA= Total Polar Surface Area; HBD= Hydrogen Bond Donars; HBA= Hydrogen Bond Acceptors; RB=Rotatable bonds; SA= Synthetic Asseccibility.

4. CONCLUSION:

This work addresses, a negative result of synthesis of a novel derivative of 1H-indazole and the molecular docking, ADMET properties prediction carried out on indazole based compound (BPM), natural alkaloids and standard reference isoniazide and indomethacin as InhA and COX-2 inhibitors. In this study, the natural alkaloid nigellidine (-7.89 kcal/mol) and BPM (-7.66 kcal/mol) has significant binding energies when docked with 2AQK and nigellidine (-9.02 kcal/mol), nigellicine (-8.02 kcal/mol) and BPM (-7.46 kcal/mol) showed promising binding affinity when docked with 3NT1. Comparing with reference compound docking scores isoniazid (-5.21 kcal/mol) and indomethacin (-6.42 kcal/mol) compound BPM showed highest binding affinities with respective enzymes.

Drug-likeness and ADME prediction shows that the newly synthesizable indazole derivative and natural alkaloids containing indazole nucleus, isoniazid and indomethacin are orally bioavailable with good GI absorption, toxicity level is low, and good permeability and follow Lipinski's Rule. Furthermore, all these indazole scaffolds were discovered to have good synthetic accessibility (<5) indicating that they are easy to synthesize in the lab. By considering all these negative and positive results, our research group is trying to develop 1H- substituted indazole derivatives which gives promising results against inflammation and tuberculosis.

All these results demonstrated that, after being improved further or under different reaction conditions BPM can be synthesizable and could be the preferred medication for the treatment of inflammation and mycobacterium TB.

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Conflict of Interest:

The authors declare no conflicts of interest.

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