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Synthesis, characterization, molecular docking, ADMET properties and *in vitro* anti-inflammatory screening of some isoxazoline derivatives

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This paper describes the synthesis of isoxazoline derivatives using Bi_2O_3-TiO_2 nanocatalyst. The (E)-3-(2-amino-3,5-dibromophenyl)-1-(substituedphenyl)prop-2-en-1-one have been treated with hydroxylamine hydrochloride in the presence of nanocatalyst Bi_2O_3-TiO_2 and ethanol as solvent to yield 2,4-dibromo-6-(3-(substitutedphenyl)-4,5-dihydroisoxazol-5-yl)aniline **8-14**. The final product has been separated and purified by ethanol recrystallization. The obtained final product **8-14** has been characterized by elemental analysis, FT-IR, ^{1}H and ^{13}C NMR spectral studies. Synthetic transformation details, small catalytic amounts, excellent product yields and suitable solvent for the formation of these isoxazoline scaffolds are elucidated. Synthesized derivatives have been screened for their *in vitro* anti-inflammatory activity using HRBC method. The obtained *in vitro* results have been compared with the molecular docking with protein, DNA Gyrase (PDB ID: 3U2D), Cyclooxygenase-1 (PDB ID: 3N8V) and Cyclooxygenase-2 (PDB ID: 3LN1) enzyme using Schrodinger suit Maestro 11.2 version. *In silico* ADMET screening has also been performed by qikprop module of Schrodinger suit.

Keywords: Bi_2O_3-TiO_2 nano catalyst, Docking, ADMET

Heterocyclic compounds containing isoxazoline ring serve as valuable synthetic templates for synthesizing novel compounds with enhancing therapeutic properties[1]. These structures form the basis for numerous pharmaceutical, agrochemical and biologically active compounds[2]. The slight modification in the chemical structure of isooxazoline ring throws new vision on the research expansion of structure – activity relationship into molecular interactions at the receptor level[3]. The isooxazoline derivatives have differential activities and mechanical action towards immunological disorders such as infection, inflammation, impaired immune responsiveness and autoimmune disorders[4]. The synthesis of novel isoxazoline derivatives remain a main focus of medicinal research hence more attention has been given to the synthesis of isoxazoline derivatives as a source of new antibacterial agents[5]. Isoxazoline derivatives have been reported to possess antifungal, antibacterial, anticonvulsant, anti-inflammatory, anti-viral, analgesic, antitumor, chemotherapy activity. Penicillin derivatives containing isoxazoline ring were found to be antibacterial agent[6-12]. Recently, various nano catalysts have gained much attention due to their greater surface area per unit mass[13]. In recent past, among the other nano catalysts, metal oxides such as ZnO, CuO, SiO_2, CeO_2, Fe_3O_4, CaO, ZrO_2, *etc*. in nano form[14-20], hence a new series of isoxazoline derivatives have been synthesized with Bi_2O_3-TiO_2 nanocatalyst for an enhanced photocatalytic rate of hydrogen evolution.

Non-steroidal anti-inflammatory drugs (NSAIDs) are the backbone for the management of pain which arises due to inflammatory diseases. These drugs suppress natural processes that are responsible for inflammation[21]. A number of non-selective non steroidal anti- inflammatory drugs (NSNS-AIDs) such as indomethacin, ibuprofen, phenylbutazone, oxyphenylbutazone, diclofenac, fenoprofen, caprofen, benoxaprofen, sulindac and aspirin, *etc*. are available in the market[22]. Celecoxib, rofecoxib and meloxicam are known as COX-2 selective non- steroidal anti-inflammatory drugs. These drugs have improved gastrointestinal safety but may cause acute renal failure. Since serious side effects are associated with the use of various NSNSAIDs and COX-2 selective NSAIDs, they cannot be taken for long time

continuously[23]. Before describing different approaches about the development of safer anti-inflammatory drugs it will be worthwhile to review what is known as the mode of action of non-steroidal anti- inflammatory drugs[24]. From the above description it is clear that non selective as well as COX-2 selective anti-inflammatory drugs have some advantages and some disadvantages associated with them and hence there is a need to develop safer anti-inflammatory drugs. Hence, our synthesized isooxazoline derivatives will be a promising drug candidate to overcome such serious side effects as an anti – inflammatory moiety.

The computational tools in drug discovery limiting the use of animal models and for helping the rational designing of safe drug candidates[25]. Thus, the results of docking can be extremely beneficial in finding drugs that are effective against particular diseases. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using scoring functions. Structure-based design has emerged as a new tool in medicinal chemistry[26]. Recently Sundaramurthi *et al*.[27] demonstrated the usefulness of docking in short-listing potential candidates and subsequently confirmed its efficiency by *in vitro* testing on M.tuberculosis. We employed the same principle of molecular docking and followed it up with the use of stringent scoring functions to enhance the accuracy of our results. The set of molecules identified by us in this study are very likely to serve as potential leads in the search for new drugs with anti-inflammatory and antioxidant activities.

The critical concepts are whether such compound exhibit pharmacokinetic drug-like properties and that will cause safety concerns in man are cleared by most important considerations of ADME studies[28]. ADME studies computes for every molecule: the chemical structure, physicochemical properties (such as molecular weight, topological polar surface area considering phosphorous and sulphur as polar atoms, molar refractivity, *etc*), lipophilicity, water solubility, bioavailability radar, pharmacokinetics, the skin permeability coefficient, drug-likeness and medicinal chemistry[29]. The success of our synthesized isooxazoline derivatives depends on its ADME characters in human body and early prediction of DME toxicity for drug likeness of a new ligand helps in reducing the probability of its failure at the drug development stage[30].

Many isoxazoline derivatives are proved to have good anti-inflammatory activity and also reported various substitutes at 3rd position of the isoxazolines aromatic ring, which made for analysis as analgesic, anti-inflammatory agents[31]. Inflammation is a protective response that involves immune cells, blood vessels, and molecular mediators. To eliminate the initial cause of cell injury, clear out necrotic cells and tissues damaged from the original insult and the inflammatory process is the purpose of inflammation and also to initiate tissue repair[32]. In this light hereby we prepared many Isoxazoline derivatives which screened for anti-inflammatory activity by *in vitro* techniques.

Experimental Section

Catalytic synthesis of isoxazoline

In order to get effective results, the reaction conditions were optimized. For this purpose, (E)-3-(3-(2-amino-3,5-dibromophenyl)acryloyl)benzonitrile, hydroxylamine hydrochloride were used as the model substrate for the synthesis of isoxazoline derivatives **8-14**. The reaction was monitored by TLC technique using ethyl acetate–hexane (3:7 v/v) as the solvent system. The reaction conditions were optimized in terms of the following reaction variables.

Initially, a blank reaction was carried out using (E)-3-(3-(2-amino-3,5-dibromophenyl)acryloyl) benzonitrile, hydroxylamine hydrochloride for the synthesis of isoxazoline derivatives **8-14** at 100°C which resulted in no formation of isoxazoline product even after 15 h. The same reaction was carried out using a catalytic amount of Bi_2O_3 - TiO_2 which afforded the desired substituted isoxazoline with 88% yield in 7 h.

To check the effectiveness of nanocrystalline Bi_2O_3 - TiO_2 with different catalysts we tried Bi_2O_3, TiO_2, Bi_2O_3 - TiO_2 for the condensation reaction of isoxazoline. Bi_2O_3 gave poor yield while TiO_2 gave good yield but required more time as compared to Bi_2O_3 - TiO_2. We observed that Bi_2O_3 - TiO_2 gave good yield with less time compared to other nanocatalyst and the results are shown in Table 1 Thus, it is confirmed from our studies that Bi_2O_3 - TiO_2 was superior for the condensation reaction with good yield in short time.

To optimize the amount of catalyst required for the condensation we tried various mol equivalents of the

Table 1 — Effect of catalyst on reaction time and yield

Entry	Catalyst	Time (h)	Yield (%)
1	Nano-Bi_2O_3	12	70
2	Nano-TiO_2	12	72
3	Nano-Bi_2O_3-TiO_2	7	88

catalyst compared to the quantity of the isoxazoline (Table 2). It was found that when the reaction was carried out with 0.5 mol equivalents reaction was 88%. The condensation reaction was carried out in different solvents such as DMF, MeOH, EtOH, and CH_3CN and the results clearly demonstrated that ethanol was found to be the good choice which is shown in Table 3.The yields of the reaction under ethanol solvent conditions were greater and the reaction times were generally shorter than the conventional method.

In order to study the possibility of reusability, the catalyst was filtered, washed with methanol and calcined at 200°C in an oven for 2 h. The reusability of the catalyst was checked for several successive runs under identical reaction conditions. The catalyst was found to be stable and reusable even after 5 cycles without appreciable loss in activity.

(E)-3-(2-Amino-3,5-dibromophenyl)-1-(substituted-phenyl)prop-2-en-1-one **1-7** were treated with hydroxylamine hydrochloride in the presence of nanocatalyst Bi_2O_3-TiO_2 and ethanol as solvent to yield 2,4-dibromo-6-(3-(substitutedphenyl)-4,5-dihydroisoxazol-5-yl)aniline **8-14**. The final product separated and purified by ethanol recrystallization. The obtained final product **8-14** was characterized by elemental analysis, FT-IR, ^1H and ^{13}C NMR spectral studies. Synthetic route is given in the Scheme 1.

Spectral Properties of Synthesized Compounds

3-(5-(2-Amino-3,5-dibromophenyl)-4,5-dihydroisoxazol-3-yl) benzonitrile, 8

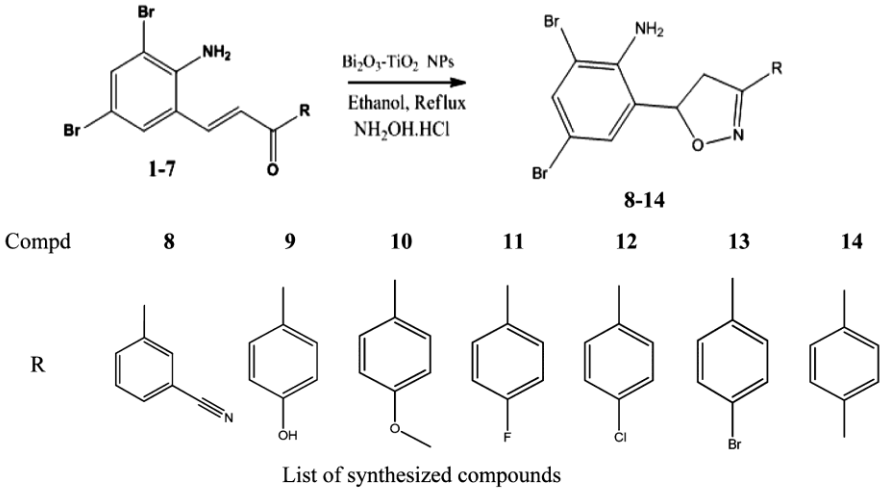
Yield 88%. m.p.192°C. Mol. Formula: $C_{16}H_{11}Br_2N_3O$. Appearance – Pale yellow solid. FTIR (KBr): 3059 (aromatic C-H), 2941 (Ali-CH), 3471 (-NH_2), 2185 (CN), 1614 cm^{-1} (C=N); ^1H NMR (DMSO-d_6, 400 MHz): δ 7.44 – 7.97 (m, aromatic protons, 9H), 3.56 (dd,CHa, 1H), 3.72 (dd, CHb, 1H), 5.81 (dd, CHx, 1H), 4.60 (s, NH_2, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 109.16, 112.31, 112.48, 117.87, 124.71, 128.88, 130.04, 134.08, 135.25, 140.76 (aromatic carbons), 156.7 (C=N), 40.4 (CH_2). 77.8 (CH). Anal. Calcd: C, 45.64; H, 2.63. Found: C, 46.76; H, 2.21%.

Table 2 — Effect of mole percentage of catalyst

Entry	(mol) of Bi_2O_3-TiO_2	Time (h)	Yield (%)
1	0.1	12	70
2	0.4	10	72
3	0.5	7	88
4	1	7	88

Table 3 — Effect of solvent on reaction

Entry	Solvent	Temperature (°C)	Time (h)	Yield (%)
1	MeOH	Reflux	12	75
2	EtOH	Reflux	7	88
3	DMF	Reflux	15	Trace
4	CH_3CN	Reflux	15	60
5	CH_2Cl_2	Reflux	15	Trace



List of synthesized compounds

- 3-(5-(2-amino-3,5-dibromophenyl)-4,5-dihydroisoxazol-3-yl)benzonitrile **(8)**
- 4-(5-(2-amino-3,5-dibromophenyl)-4,5-dihydroisoxazol-3-yl)phenol **(9)**
- 2,4-dibromo-6-(3-(4-methoxyphenyl)-4,5-dihydroisoxazol-5-yl)aniline **(10)**
- 2,4-dibromo-6-(3-(4-fluorophenyl)-4,5-dihydroisoxazol-5-yl)aniline **(11)**
- 2,4-dibromo-6-(3-(4-chlorophenyl)-4,5-dihydroisoxazol-5-yl)aniline **(12)**
- 2,4-dibromo-6-(3-(4-bromophenyl)-4,5-dihydroisoxazol-5-yl)aniline **(13)**
- 2,4-dibromo-6-(3-(p-tolyl)-4,5-dihydroisoxazol-5-yl)aniline **(14)**

Scheme 1

4-(5-(2-Amino-3,5-dibromophenyl)-4,5-dihydroisoxazol-3-yl)phenol, 9

Yield 76%. m.p.204°C. Mol. Formula: $C_{15}H_{12}Br_2N_2O_2$. Appearance - Pale yellow solid. FTIR (KBr): 3062 (aromatic C-H), 2941 (Ali-CH), 3475 (-NH_2), 2193 (CN), 1595 cm^{-1} (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 6.43 – 7.20 (m, aromatic protons, 2H), 3.19 (dd,CHa, 1H), 3.32 (dd, CHb, 1H), 5.42 (dd, CHx, 1H), 4.18 (s, NH_2, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 113.3, 113.38, 116.43, 117.43, 121.58, 28.46, 129.29, 129.34, 131.09, 121.11, 135.70, 139.39, (aromatic carbons), 155.3 (C=N), 40.5 (CH_2). 78.5 (CH). Anal. Calcd: C, 43.72; H, 2.94. Found: C, 44.55; H, 2.52%.

2,4-Dibromo-6-(3-(4-methoxyphenyl)-4,5-dihydroisoxazol-5-yl)aniline, 10

Yield 71%. m.p.227°C. Mol. Formula: $C_{16}H_{14}Br_2N_2O_2$. Appearance - Pale yellow solid. FTIR (KBr): 3059 (aromatic C-H), 2947 (Ali-CH), 3475 (-NH_2), 2194 (CN), 1595 cm^{-1} (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 6.57 – 7.27 (m, aromatic protons, 2H), 3.23 (dd,CHa, 1H), 3.41 (dd, CHb, 1H), 5.50 (dd, CHx, 1H), 4.26 (s, NH_2, 2H), 3.36 (s, OCH_3, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 109.16, 112.48, 114.30, 123.14, 124.70, 128.77, 130.68, 135.2, 140.76 (aromatic carbons), 155.3 (C=N), 40.4 (CH_2). 77.8 (CH), 55.3 (OCH_3). Anal. Calcd: C, 45.10; H, 3.31. Found: C, 46.80; H, 3.09%.

2,4-Dibromo-6-(3-(4-fluorophenyl)-4,5-dihydroisoxazol-5-yl)aniline, 11

Yield 73%. m.p.255°C. Mol. Formula: $C_{15}H_{11}Br_2FN_2O$. Appearance - Pale yellow solid. FTIR (KBr): 3053 (aromatic C-H), 2924 (Ali-CH), 3388 (-NH_2), 2237 (CN), 1587 cm^{-1} (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 6.75 – 7.36 (m, aromatic protons, 2H), 3.11 (dd,CHa, 1H), 3.26 (dd, CHb, 1H), 5.37 (dd, CHx, 1H), 4.14 (s, NH_2, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 114.01,115.38, 115.56, 117.45,117.65, 28.71, 128.74, 129.86, 129.94, 131.42, 132.30, 135.93, 141.09 (aromatic carbons), 155.5 (C=N), 40.1 (CH_2). 78.4 (CH). Anal. Calcd: C, 43.51; H, 2.68. Found: C, 44.65; H, 2.13%.

2,4-Dibromo-6-(3-(4-chlorophenyl)-4,5-dihydroisoxazol-5-yl)aniline, 12

Yield 75%. m.p.243°C. Mol. Formula: $C_{15}H_{11}Br_2ClN_2O$. Appearance - Pale yellow solid. FTIR (KBr): 3055 (aromatic C-H), 2922 (Ali-CH), 3390 (-NH_2), 2264 (CN), 1600 cm^{-1} (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.33 – 7.57 (m, aromatic protons, 2H), 3.53 (dd,CHa, 1H), 3.67 (dd, CHb, 1H), 5.79 (dd, CHx, 1H), 4.56 (s, NH_2, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 109.16, 112.48, 124.69, 127.70, 128.51, 129.35, 130.68, 135.25, 136.07, 140.76 (aromatic carbons), 154.6 (C=N), 40.4 (CH_2). 77.8 (CH). Anal. Calcd: C, 41.85; H, 2.58. Found: C, 40.78; H, 2.55%.

2,4-Dibromo-6-(3-(4-bromophenyl)-4,5-dihydroisoxazol-5-yl)aniline, 13

Yield 76%. m.p.278°C. Mol. Formula: $C_{15}H_{11}Br_3N_2O$. Appearance - Pale yellow solid. FTIR (KBr): 3059 (aromatic C-H), 2945 (Ali-CH), 3479 (-NH_2), 2196 (CN), 1587 cm^{-1} (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.28 – 7.60 (m, aromatic protons, 2H), 3.39 (dd,CHa, 1H), 3.54 (dd, CHb, 1H), 5.66 (dd, CHx, 1H), 4.43 (s, NH_2, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 110.15, 113.36, 115.79, 116.99, 118.81, 124.15, 125.52, 129.88, 131.67, 136.24, 141.65 144.5 (aromatic carbons), 160.4 (C=N), 40.3 (CH_2). 78.8 (CH). Anal. Calcd: C, 37.93; H, 2.33. Found: C, 39.35; H, 2.29%.

2,4-Dibromo-6-(3-(*p*-tolyl)-4,5-dihydroisoxazol-5-yl)aniline, 14

Yield 79%. m.p.237°C. Mol. Formula: $C_{16}H_{14}Br_2N_2O$. Appearance - Pale yellow solid. FTIR (KBr): 3055 (aromatic C-H), 2947 (Ali-CH), 3427 (-NH_2), 2200 (CN), 1614 cm^{-1} (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.16 – 7.56 (m, aromatic protons, 2H), 3.53 (dd,CHa, 1H), 3.68 (dd, CHb, 1H), 5.79 (dd, CHx, 1H), 4.57 (s, NH_2, 2H), 2.28 (s, CH_3, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 109.16, 112.48, 124.69, 26.51, 127.37, 129.79, 130.68, 132.25, 140.76, 141.24 (aromatic carbons), 155.7 (C=N), 40.4 (CH2), 77.8 (CH), 21.3 (-CH_3). Anal. Calcd: C, 46.86; H, 3.44. Found: C, 46.17; H, 3.86%.

Results and Discussion

Docking studies of isoxazoline derivative with protein receptors of 3U2D, 3LN1 and 3N8V

Molecular docking was used to understand the binding modes and to support the antibacterial activity of the synthesized compounds obtained experimentally, also to elucidate new information for further structural optimization. Considering DNA Gyrase (PDB ID: 3U2D), Cyclooxygenase-1 (PDB ID: 3N8V) and Cyclooxygenase-2 (PDB ID: 3LN1) as the target receptor, comparative and automated docking studies with newly synthesized isoxazoline compounds was performed to determine the best *in silico* conformation.

Table 4 shows the Binding Energy and Inhibition Constant of seven compounds including the standard against all the three receptor proteins. The results showed that the binding energies of **8-14** were lower than that of diclofenac in its interaction with three protein receptor 3LN1, 3N8V and 3U2D (Table 4). Among the sequence of compounds **9**, **14** and **10** delivered outstanding binding energy against 3LN1, 3N8V and 3U2D proteins with binding energies –7.89, –5.78 and –3.91 k.cal/mol respectively. Similarly, 14 showed on greater dock score (–7.62 k.cal/mol) against 3LN1 protein; **9** showed on greater dock score (–5.78 k.cal/mol) against 3N8V protein and 3U2D showed better dock score (–3.916 kcal/mol). The docking results were compared with human trial drugs such as diclofenac. The outcome gives information to show an excellent result on proteins.

The analysis of the active site of DNA Gyrase (PDB ID: 3U2D) enzyme, reveals that all the synthesized compounds **8-14** are making various close contacts with the residues lining the active site of DNA Gyrase (PDB ID: 3U2D) enzyme, the interacting amino acids of compound **9**, **10** and **11** are shown in Fig. 1. The analysis of best scoring pose of

Table 4 — Docking scores of synthesized isoxazoline derivatives **8-14** with various protein receptors

Compd	3LN1	3N8V	3U2D
8	–5.453	–4.947	–3.573
9	–7.89	–5.783	–3.664
10	–3.341	–5.11	–3.916
11	–6.611	–5.528	–3.712
12	–6.548	–5.373	–3.281
13	–7.036	–5.431	–3.265
14	–7.62	–5.785	–2.82
Diclofenac	–8.567	–7.126	–7.896

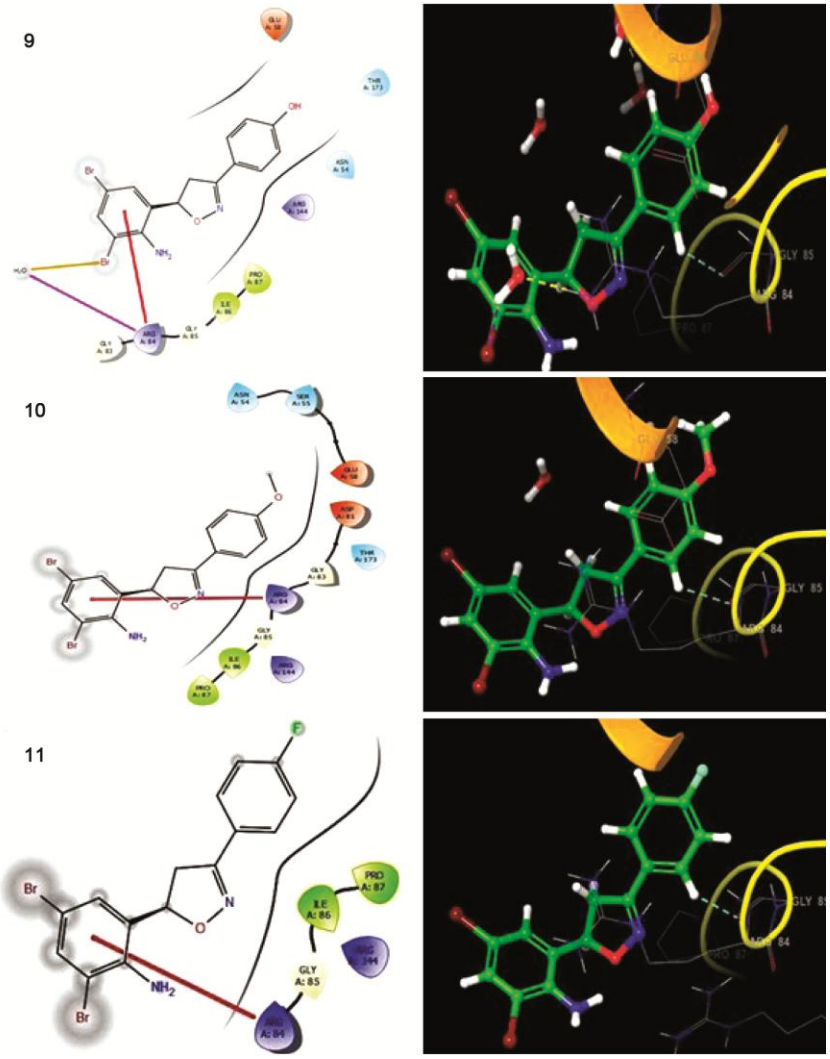


Fig. 1 — 2D and 3D pictorial representation of docking interactions of isoxazoline derivative **9**, **10** and **11** with DNA Gyrase (PDB ID: 3U2D)

compounds (stable conformation) in the DNA Gyrase (PDB ID: 3U2D) enzyme pocket showed significant hydrogen bonding as well as hydrophobic interactions between them (Fig. 1). The isoxazoline phenyl group of compound **9**, **10** and **11** exhibits π-anion interactions with the residues Arg84. The compound **9** exhibits hydropobic interactions with the residues Gly85, Ile86, Pro87, Arg144, Asn84, Thr173, Glu58 of DNA Gyrase (PDB ID: 3U2D) . The isoxazoline phenyl group of compounds **9**, **10** and **11** exhibit π-anion interactions with the residues Arg84. The compound **9**, **10** and **11** exhibits hydropobic interactions with the residues Gly85, Ile86, Pro87, Arg144, Asn84, Thr173, Glu58 of DNA Gyrase (PDB ID: 3U2D). The analysis of best scoring pose of compounds in the Cyclooxygenase-1 (PDB ID: 3N8V) enzyme pocket showed significant hydrogen bonding as well as hydrophobic interactions between them (Fig. 2). The isoxazoline hydroxyl group (-OH) of compound **9** exhibits hydrogen bond interactions with the residues Glu524 and Arg83. The isoxazoline bromide group (-Br) of compound **11** exhibits hydrogen bond interactions with the residues Arg83.The compounds **9**, **11** and **14** exhibit hydropobic interactions with the residues Ile89, Leu92, Leu93, Val119, Val116, Lbj115, Leu99, Trp100, Val103, Arg123, Leu123 of Cyclooxygenase-1 (PDB ID: 3N8V).

The analysis of best scoring pose of compounds in the Cyclooxygenase-2 (PDB ID: 3LN1) enzyme pocket showed significant hydrogen bonding as well as hydrophobic interactions between them (Fig. 3). The isoxazoline hydroxyl group (-OH) of compound **9** exhibits hydrogen bond interactions with the residues

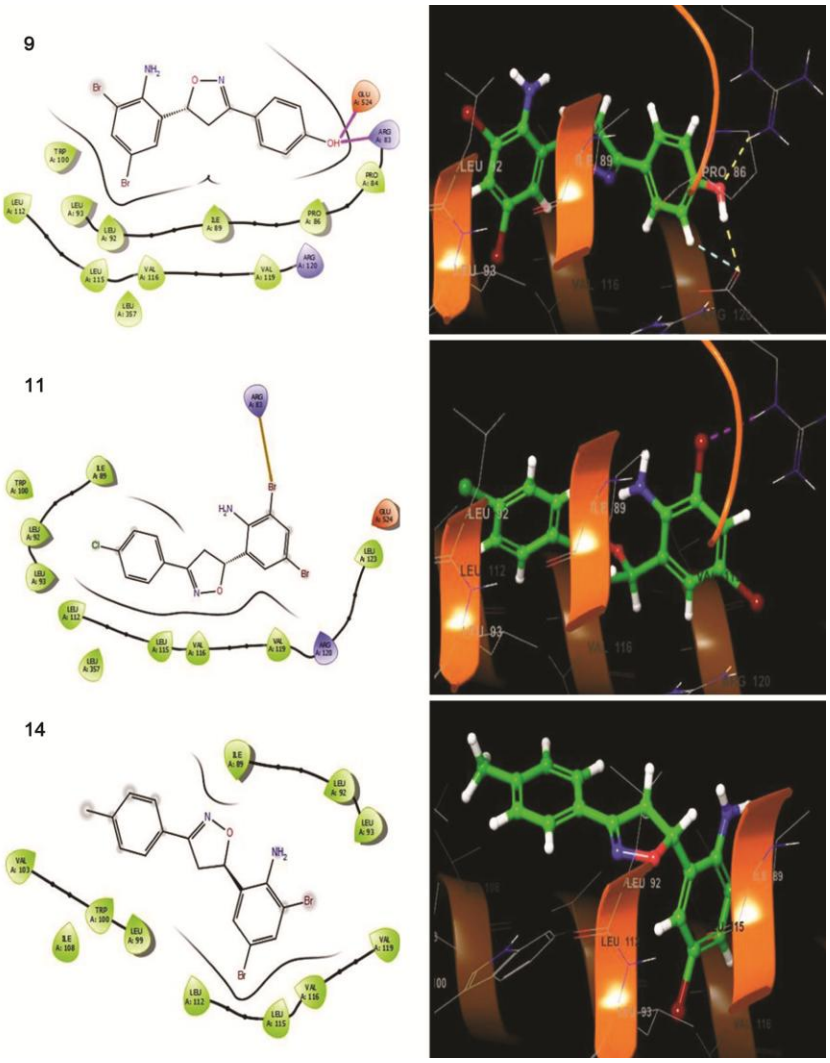


Fig. 2 — 2D and 3D pictorial representation of docking interactions of isoxazoline derivative **9**, **11** and **14** with Cyclooxygenase-1 (PDB ID: 3N8V)

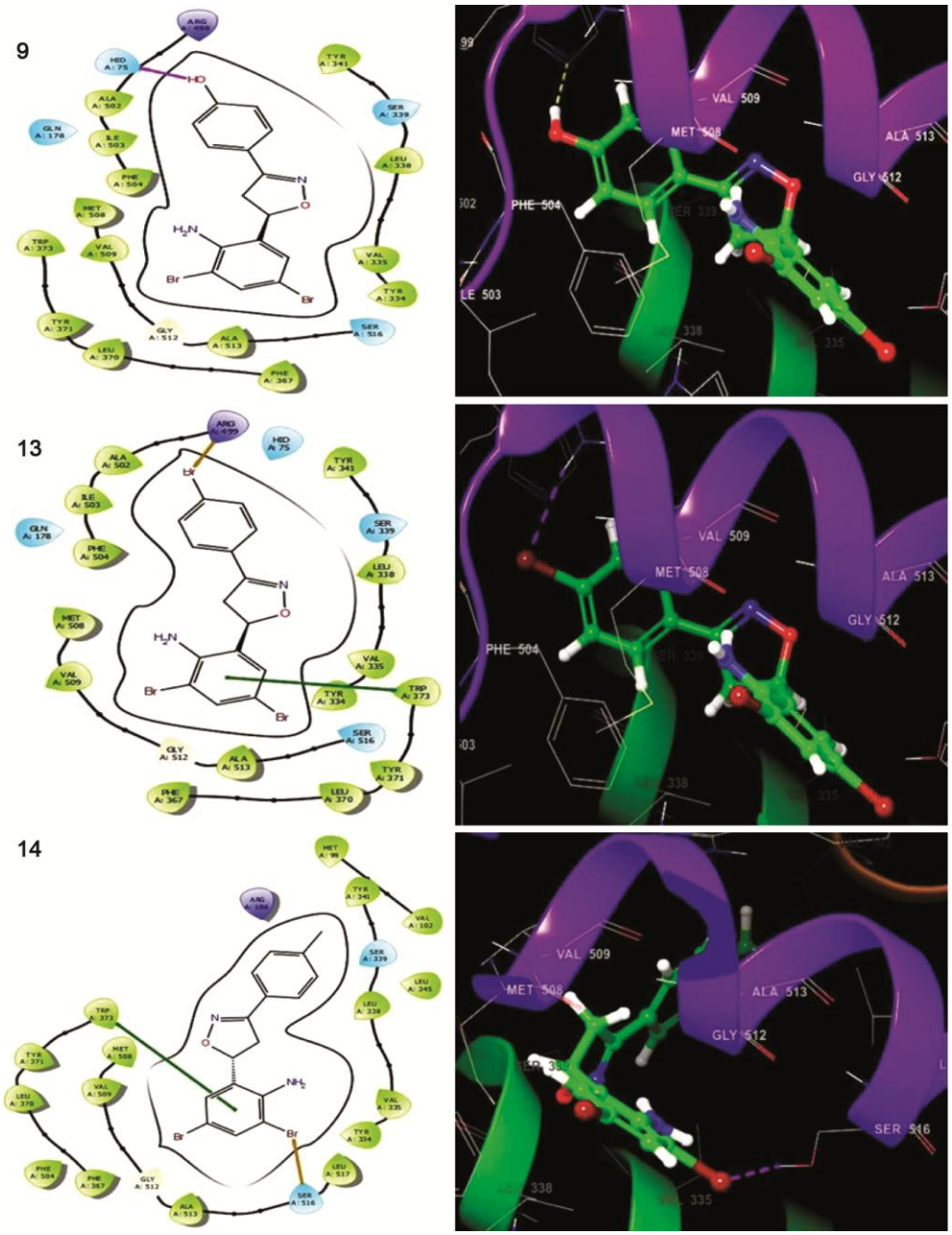


Fig. 3 — 2D and 3D pictorial representation of docking interactions of isoxazoline derivative **9**, **13** and **14** with Cyclooxygenase-2 (PDB ID: 3LN1

Hid75. The isoxazoline bromide group (-Br) of compound **13** and **14** exhibits hydrogen bond interactions with the residues Arg499 and Ser516. The isoxazoline phenyl group of compound **13** and **14** exhibits π-anion bond interactions with the residues Trp373. The compound **9**, **13** and **14** exhibits hydropobic interactions with the residues Met508, Val509, Glu512, Ala513, Leu517, Tyr234, Val335, Leu378, Ser379, Tyr341, Phe504 of Cyclooxygenase-2 (PDB ID: 3LN1).

Prediction of Pharmacokinetic Properties

The two-dimensional (2D) structures of the ligand **8-14** were imported to thecomputational program module *Qikprop* Schrödinger software for the *in silico* determination pharmacokinetic properties. The statistical parameters based on the ADME properties prediction of compounds **8-14** are shown in Table 5 and Table 6.

The synthesized molecules were further evaluated for their drug-like behavior through ADME

(Absorption, Distribution, Metabolism and Excretion) properties. Also, all the seven identified molecules are in the acceptable range and fulfilling the Lipinski's rule of five criteria's like molecular weight <500 daltons <5 hydrogen bond donors <10 hydrogen bond acceptors, volume (from 500.0 to 2000.0) and logP < 5. Estimation of absorption and distribution of drugs within the body, the Predicted octanol/water partition coefficient (QPlogPo/w, –2.0 – 6.5), water solubility (QplogS, –6.5 – 0.5), cell permeability (QPPCaco, <25 poor, >500 great) and Predicted apparent MDCK cell permeability in nm/sec (QPPMDCK , <25 poor, >500 great) ranged between –3.0 to –4.30; –5.01 to –6.34; 367 to 1775 and 1103 to 10000, respectively (Table 5 and Table 6). All these pharmacokinetic parameters of synthesized molecules are within the acceptable range and thereby renowned as drug-like molecules.

The pharmacokinetic properties predictions of compounds **8-14** imply that all compounds possess the drug like properties. The results conceded that no violation is found in agreement with Lipinski's rule of five. Additionally, the molecular mass of compounds was ranges from 410 to 474 a.m.u. The number of hydrogen bond donor ranges from 1.5 to 2.5 whereas the hydrogen bond acceptor values vary from 3.7 to 5.2. In addition, the partition coefficient values of all compounds are less than five. Van der Waals surface area of polar nitrogen and oxygen atoms (PSA, 7.0 – 200.0) ranged from 47 to 73.

All the tested compounds (**8-14**) have maximum percentage of human oral absorption. The aqueous solubility (QPlogS) parameter and IC50 values of HERG K+ channel blockage (QPlogHERG, below –5) of all the tested compounds possess admissible parameters (–4.2 to –4.5).The prediction of blood brain barrier permeability (QplogBB, –3.0 – 1.2) for the tested compounds was appraised and all the compounds were predicted to have adequatable values ranges from –0.154 to –0.250.

Table 5 — Qikprop studies with physical descriptors of isoxazoline derivatives

Compd	mol_MW	Volume	PSA	donorHB	accptHB
8	421.09	986.293	73.238	1.5	5.2
9	412.08	943.235	69.991	2.5	4.45
10	426.107	997.393	55.632	1.5	4.45
11	414.071	936.762	47.431	1.5	3.7
12	430.526	964.813	47.437	1.5	3.7
13	474.977	973.164	47.43	1.5	3.7
14	410.107	980.591	47.456	1.5	3.7

Anti-inflammatory activity of isoxazoline derivatives (8-14)

The body's innate and adaptive immune systems cause inflammation as a non-specific protective reaction to tissue injury. Infections and tissue damage cause an inflammatory response of greater intensity, whereas tissue dysfunction can result in an inflammatory response of lesser intensity[33]. Inflammatory cascade have four major steps: start, progression, termination, and resolution (in that order). There is a reversal of the inflammatory response to the homeostatic state involved in the resolution of inflammation. A lack of resolution results in a prolonged inlammatory response[34]. Numerous diseases, such as rheumatoid arthritis and cancer as well as cognitive and nervous system disorders (such as dementia, stroke and brain injury), have been linked to chronic inflammation[35]. Chronic inflammation also contributes to the development of inflammatory bowel disease and obesity. It is also linked to asthma and atherosclerosis. Chronic inflammation also contributes to the development of many neurodegenerative diseases[36,37]. The anti-inflammatory, analgesic, and anti-pyretic effects of non-steroidal anti-inflammatory medicines (NSAIDs) make them extremely popular around the world. Long-term use of NSAIDs, on the other hand, can have a number of negative consequences, some of which can be life-threatening if ignored. Asthenia, heartburn, and dyspepsia are the most prevalent side effects of this medication. Worsening side effects include platelet and prostaglandin inhibition, raised blood pressure, drug-induced asthma, renal failure and hepatic toxicity[38-42].

Table 6 — Summary of average pharmacokinetic property distributions of isoxazoline derivatives

Compd	QPlogPo/w	QPlogS	QPlogHERG	QPPCaco	QPPMDCK	% HOA
8	–3.036	–6.345	–4.526	367.966	1103.431	90.643
9	–3.007	–5.011	–4.268	539.422	1668.48	93.451
10	–3.877	–5.586	–4.336	1774.212	6042.06	100
11	–3.994	–5.671	–4.281	1775.758	10000	100
12	–4.25	–6.042	–4.33	1772.68	10000	100
13	–4.323	–6.143	–4.35	1775.075	10000	100
14	–4.065	–5.869	–4.33	1771.84	6033.484	100

Many diseases associated with inflammation are caused by aberrant activation of enzymes such as inducible nitric oxide synthase (iNOS)10, cyclooxygenase 1 (COX1), and cyclooxygenase 2 (COX2). Inhibition of their catalytic function may have anti-inflammatory effects[43,44]. Drug interactions, especially in patients with liver or kidney disease, might have catastrophic consequences when treating inflammatory disorders that may be linked to certain microbial infections[45,46]. To add to that, from the perspective of pharmacoeconomics, and in an effort to improve patient compliance, the quest for a strong analgesic with fewer side effects, as well as an anti-inflammatory and anti-microbial drug, has taken centre stage in recent years[47] Bacterial infection can also lead to inflammation and pain, which are common side effects. It is common practice to prescribe two different classes of drugs at the same time (chemotherapeutics and analgesics, as well as anti-inflammatory medications)[48]. A compound with all three of these properties is rare. That's why our goal is to discover a chemical that possesses all three of these properties at the same time. We discovered that the isoxazole ring is one of the moieties studied while looking for such a molecule. It is therefore crucial to continue working on anti-inflammatory chemical research. Isoxazoles, have been identified as anti- inflammatory agents when it comes to the design of various novel structures. Because of its vast range of biological activity and therapeutic possibilities, isoxazoline, a five-membered heterocycle with one oxygen atom and one nitrogen atom at adjacent poistions, is of tremendous importance. It is attention-grabbing to point out that all of the compounds in this series had some anti-inflammatory properties. The most active compound, **14** showed even stronger anti-inflammatory effects (71.03%) than standard drug diclofenac (67.59%). The compound **14** exhibited most potent anti-inflammatory activity amongst the newly synthesized molecules **8-14**. Methyl derivative compound **14** exhibited better activity than chalcones. Compounds **11** (66.21%), **12** (64.83%) and **13** (62.07%) exhibit high activity and they inhibit the growth to a remarkable extent, which may be due to the presence of fluro, chloro, and bromo substituents on the benzene ring, besides the presence of isoxazole skeletons. When compared to other compounds, the isoxazoline derivatives with halogen substituents demonstrated greater activity, according to the results. It has been observed that the compound **10** (58.62%) having methoxy at *para*-position and the compound **9** (61.38%) having hydroxy group as substituent on phenyl ring was the modest activity. The compound having methyl group and halogen group at *para*-position as substituent at phenyl group elicited potent anti-inflammatory activity and when compound substituted with methoxy and cyano group at *para*-position on phenyl ring, and then the compounds showed lower anti-inflammatory activity. It has been observed that among the newly synthesized series the compound **8** having substituted with cyano group on phenyl ring at *para*-position exhibited modest inhibition activity (57.24%). The compound **9** has shown better antiinflammatory activity (61.38%) than the compound **8** (57.24%). The compound **8** and **10** were found to possess modest anti-inflammatory activity (Table 7).

Structural activity relationship showed that compounds in which all compounds sequentially for this series. First, comparing derivatives **8-14** with different electron donating and withdrawing substituent on the benzene ring, the order of activity was 4-CH_3> 4-F> 4-Cl> 4-Br> 4-OH > 3-CN > 4-OCH_3. Electron-withdrawing groups seemed to be a

Table 7 — Anti-inflammatory activity of isoxazoline derivatives **8-14**

Compd	Conc. g /mL	Absorbance (nm) (Mean ± SEM)	Protection (%)
8	200	0.62	57.24
	100	0.71	51.03
	50	0.85	41.38
9	200	0.56	61.38
	100	0.79	45.52
	50	0.91	37.24
10	200	0.6	58.62
	100	0.73	49.66
	50	0.88	39.31
11	200	0.49	66.21
	100	0.65	55.17
	50	0.78	46.21
12	200	0.51	64.83
	100	0.68	53.10
	50	0.84	42.07
13	200	0.55	62.07
	100	0.67	53.79
	50	0.85	41.38
14	200	0.42	71.03
	100	0.65	55.17
	50	0.78	46.21
Diclofenac sodium (Standard)	200	0.47	67.59
	100	0.59	59.31
	50	0.75	48.28
Control	Unknown	1.45	0

Table 8 — Anti-inflammatory activity of synthesized chalcone derivatives **1-7** using HRBC assay

Compd	Conc. g /mL	Absorbance (nm) (Mean ± SEM)	% Protection
1	200	0.54	62.76
	100	0.72	50.34
	50	0.89	38.62
2	200	0.62	57.24
	100	0.84	42.07
	50	0.91	37.24
3	200	0.55	62.07
	100	0.76	47.59
	50	0.92	36.55
4	200	0.69	52.41
	100	0.81	44.14
	50	0.95	34.48
5	200	0.59	59.31
	100	0.7	51.72
	50	0.88	39.31
6	200	0.65	55.17
	100	0.77	46.90
	50	0.86	40.69
7	200	0.53	63.45
	100	0.74	48.97
	50	0.85	41.38
Diclofenac sodium (Standard)	200	0.47	67.59
	100	0.59	59.31
	50	0.75	48.28
Control	Unknown	1.45	0

more beneficial structural feature than electron-withdrawing groups for anti-inflammatory activity. Second, for compounds **8** (with cyano group), substitutions at the third position appeared to modest influence the activity. Hydroxy substitution seemed to be a more beneficial structural feature than electron-donating groups for anti-inflammatory activity. Substituted methyl and electron withdrawing group of isoxazoline were showed better inflammatory activity. It may be concluded from the results that most of the isoxazolines (**8-14**) possessed more potent anti-inflammatory activity than their corresponding chalcones **1-7** (Table 8). As a result, it's critical to base the development of novel synthetic techniques and the design of new isoxazole derivatives on the most up-to-date knowledge promising from recent research.

Conculsion

This paper describes the synthesis of isoxazoline derivatives using Bi_2O_3-TiO_2 nanocatalyst. The obtained final product **8-14** was characterized by elemental analysis, FT-IR, ^1H and ^{13}C NMR spectral studies. Synthesized derivatives screened for their *in vitro* anti-inflammatory activity using HRBC method. The obtained *in vitro* results were compared with the molecular docking with protein. *In silico* ADMET screening also performed.

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