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Research Article

Synthesis and Characterization of Metronidazole and Heterocycle Ester Dyads for Tyrosinase Inhibitory Activity

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ABSTRACT

Tyrosinase is an important copper-based enzyme mainly involved in skin pigmentation. The inhibition of the tyrosinase enzyme attracts importance in cosmetic and medicinal chemistry industry for its applications in skin whitening and anti-browning agents for humans as well as in food, and agriculture industries. Imidazole-based metronidazole and its derivatives are widely accepted drug for wide range of diseases. Therefore, the present report involves the synthesis of heterocyclic derivatives of metronidazole and the investigation of its efficacy towards tyrosinase inhibitory activity. A series of metronidazole esters were synthesized and their chemical structures were confirmed using spectral techniques like, infrared spectroscopy (IR), proton nuclear magnetic resonance ($^1\text{H-NMR}$), and liquid chromatography-mass spectrometry (LC-MS). All the compounds were evaluated for its tyrosinase inhibitory activity by oxidation of 3,4-dihydroxyphenylalanine in the presence of the synthesized esters (I-VIII) with kojic acid as standard. Among the synthesized compounds, VII (isonicotinic ester) and VIII (quinoline ester) demonstrated significant activity IC_{50} values 92.5 and 91.8 μM , respectively. Further molecular docking experiments were carried out for the synthesized compounds with 2y9w protein exhibited a greater number of physical interactions for compounds VII and VIII than the other compounds in the series, confirming the mushroom tyrosinase activity.

INTRODUCTION

Tyrosinase inhibitors in the past few decades have gained attraction for its melanogenic applications in mammalian, fruits, and fungi. Melanogenesis is a process for the formation of macromolecular pigments usually catalyzed by tyrosinase enzyme. Several such tyrosinase enzyme inhibitors have been identified and studied from natural as well as synthetic sources.^[1-5] Polyphenols^[6,7] and flavonoids^[8-11] are most investigated from natural resources, while polyhydroxy coumarins,^[12] chalcones^[13] and some heterocycles^[14] from synthetic sources produced effective results with very low poison effects. Imidazole derivatives are important among heterocycles found as active tyrosinase inhibitors.^[15,16]

On the other hand, metronidazole (MTZ), an important bactericidal compound, was found useful against various

diseases caused by gram-negative anaerobic bacteria and various protozoans.^[17,18] MTZ was functionalized/derivatized through its functions like methyl group,^[19] nitro group^[20] and the pendant ethanolic group^[21] and reported for several biological activities. Particularly, the hydroxyl function of the ethanolic group is the most favorite for the researchers for its synthetic ease and it easily be converted or linked to several other chemical fragments.^[22] Many chemical modifications and its consequences on biological properties were studied except for the nitro group in MTZ due to its valuable contribution to various biological activity. Several research groups have attempted to derivatize MTZ, particularly altering two groups viz., (i) *N*-linked alkyl group (ii) methyl group in imidazole ring for its anticancer, antibacterial and antifungal properties.^[23] Very few modifications have been reported in the case of 2-methyl group modification in MTZ. The methyl group

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is usually converted to the styryl group with varying substitutions.^[24] Various conjugates attached through ethoxy linkage were reported with 1,2,3-triazole,^[25] oxadiazole,^[26] piperazine,^[27] benzothiazole,^[28] triazine,^[29] flavonoids,^[30] other heterocycles.^[31] Few conjugates linked through ester linkages with groups like, phenyl,^[32] cinnamic,^[33] salicylic,^[34] pyrazole,^[35] terpenoids^[36] have also been synthesized but very few researchers were attempted to functionalize in both arms.^[37]

Methimazole derivatives exhibit excellent tyrosinase inhibitor activity in the range of 4.8 ± 1.4 nM IC_{50} values and interestingly, their structural traces are more like MTZ.^[38] The proven antibacterial activity of metronidazole and its relationship with tyrosinase inhibitory properties encourage us to investigate the derivatives of metronidazole for antityrosinase activity. We have investigated and reported some of the MTZ ester derivatives with various aromatic hydrocarbons that showed good inhibition against tyrosinase enzymes.^[39] Modeling studies show that hydrocarbon ester fails to produce interaction with protein sites by cyclic hydrocarbons, whereas the imidazole fragment produced the major bonding interactions. We believe that polarity of the heterocycles could promote more interactions and generally heterocycles and proven candidates for a wide range of biological activities. Hence, we attempted to make some heterocyclic ester derivatives of metronidazole and investigated its tyrosinase inhibitor activity and molecular docking studies.

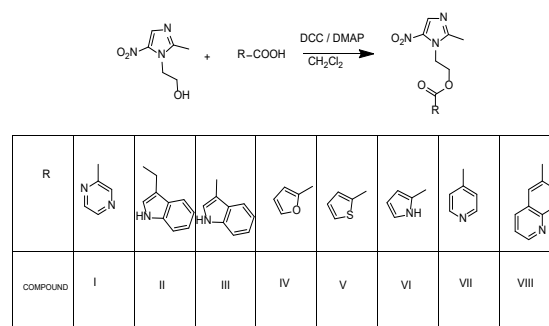
MATERIAL AND METHODS

Synthesis

The solvents and starting materials used for this study were purchased from SRL, India, and used without purification. Precoated TLC plates purchased from Merck (60 F254) were used to follow up on the reaction and visualized by the iodine vapors adsorption method and illumination under UV light. Melting points of the samples were determined by the instrument made by REMI, India. FTIR spectra were recorded on Agilent Fourier transform infrared spectroscopy (Cary 630 FTIR) using the KBr pellet method. 1H & ^{13}C -NMR were recorded on JEOL – 600 MHz. Chemical shift values (δ) measured in ppm scale using tetra methyl silane (TMS) as the internal standard. Molecular mass data was recorded on TOF-MS ES+. The purity of the synthesized compounds was determined using Waters, USA using BEHC18 column with acetonitrile as mobile phase.

General Procedure for Synthesis of Compounds I-VIII

The scheme of synthesis for the metronidazole derivatives was depicted in Scheme 1. To the mixture of 0.38 mmol of metronidazole (1 eq.), 0.38 mmol of corresponding heterocyclic carboxylic acids (1equiv), and 0.41 mmol of DCC (1.2 eq.), 0.05 mmol of DMAP was added in dry



Scheme 1: Scheme of the synthesis of compounds I-VIII

dichloromethane (10 mL) at ambient temperature and stirred for overnight. The reaction was monitored through thin-layer chromatography (TLC). A pale white precipitate formed as DCC-Urea was filtered and it was washed several times with 5% aqueous ammonium acetate solution followed by water. After the complete removal of unreacted DCC through washings, the crude product was obtained by removing the solvent using rotary vacuum evaporator. The crude product was subjected to column chromatography using silica gel as adsorbent and hexane: ethyl acetate gradient mixture as mobile phase to get pure white colored solid as product. The purified substance was recrystallized using ethanol.

2-(2-methyl-5-nitroimidazolyl)ethyl pyrazine-2-carboxylate (I)

Yield: 84%; m.pt. 120°C; FTIR (cm^{-1}): 1723.18 (ester C=O), 3220 (C=N str), 1532.90 (C-N str), 2914.08, 3054 (aliphatic CH_2 str), 3015.12 (ArCH, str), 1438.18 (Aliphatic CH, bend), 741 (Ar CH), 1367.37 (-N-O Sym. str), 1H -NMR ($CDCl_3$, 400 MHz); δH 7.973 (s, 1H, MTZ-H), 2.604 (s, 3H, MTZ- CH_3), 4.764–4.784 (t, 2H, MTZ- CH_2 , 8Hz), 4.787–4.807 (t, 2H, MTZ- CH_2 , 8Hz), 8.706 (s, 1H, Pyrazine), 8.798–8.806 (d, 1H, Pyrazine), 9.257 (s, 1H, Pyrazine). ^{13}C -NMR δ ppm; 142.642 (MTZ-C1), 133.409 (MTZ-C2), 151.369 (MTZ-C3), 14.518 (MTZ- CH_3 -C4), 45.07 (MTZ- CH_2 -C5), 64.264 (MTZ- CH_2 -C6), 163.74 (C=O -C7), 139 (Pyrazine C8), 144.559 (Pyrazine C9), 144.559 (Pyrazine C10), 146.456 (Pyrazine C11). EIMS m/z : 277.9767 (M^+).

2-(2-methyl-5-nitroimidazolyl)ethyl 2-(3-indolyl)acetate (II)

Yield: 82%; m.pt. 122°C; FTIR (cm^{-1}): 1649.28 (C=O -ester), 3220 (C=N str), 1594.94 (C-N str), 2920.05, 2859.27 (aliphatic CH_2), 3043.12 (ArCH, str), 1483.05 (aliphatic CH, bend), 766.84 (Ar CH), 1343.38 (-N-O Sym. str), 3433.42 (-N-H, str). 1H -NMR ($CDCl_3$, 400 MHz); δH 8.014 (s, 1H, MTZ-H), 2.311 (s, 3H, MTZ- CH_3), 4.359–4.384 (t, 2H, MTZ- CH_2 , 8Hz), 4.522–4.547 (t, 2H, MTZ- CH_2 , 8Hz), 3.699 (s, 2H, Indole- CH_2), 7.157–7.161 (d, 1H, Indol), 7.036–7.074 (d, 1H, Indol), 6.924–6.921 (t, 1H, Indol), 7.319–7.361 (t, 1H, Indol), 10.932 (s, NH-indole).

^{13}C -NMR δ ppm; 139.081 (MTZ-C1), 133.62 (MTZ-C2), 152.216 (MTZ-C3), 14.324 (MTZ- CH_3 -C4), 45.351 (MTZ- CH_2 -C5), 63.058 (MTZ- CH_2 -C6), 171.781 (C=O -C7),



31.049 (Indol C8), 107.033 (Indol C9), 124.721 (Indol C10), 136.669 (Indol C11), 127.581 (Indol C12), 118.854 (Indol C13), 119.146 (Indol C14), 121.686 (Indol C15), 112.005 (Indol C16). EIMS m/z : 329.0625 (M^+).

2-(2-methyl-5-nitroimidazolyl)ethyl indole-2-carboxylate (III)

Yield : 82%; m.pt. 122°C; 1687.57 (C=O -ester), 3220 (C=N str), 1529.27 (C-N str), 2974.25, 2855.44 (aliphatic CH₂), 1469.07 (aliphatic CH, bend), 745.86 (Ar CH), 1348.25 (N-O Sym. str), 3435.24 (N-H, str). ¹H-NMR (CDCl₃, 400 MHz); δ 8.06 (s, 1H, MTZ-H), 2.49 (s, 3H, MTZ-CH₃), 4.69-4.70 (t, 2H, MTZ-CH₂), 4.72-4.73 (t, 2H, MTZ-CH₂), 7.25-7.29 (d, 1H, Indol), 7.66-7.68 (d, 1H, Indol), 7.43-7.45 (t, 1H, Indol), 7.09-7.11 (t, 1H, Indol-H), 7.05-7.07 (t, 1H, Indol), 11.92 (s, NH-Indol). ¹³C-NMR (CDCl₃) δ ppm; 139.081 (MTZ-C1), 133.62 (MTZ-C2), 152.216 (MTZ-C3), 14.324 (MTZ-CH₃-C4), 45.351 (MTZ-CH₂-C5), 63.058 (MTZ-CH₂-C6), 171.781 (C=O -C7), 31.049 (Indol C8), 107.033 (Indol C9), 124.721 (Indol C10), 136.669 (Indol C11), 127.581 (Indol C12), 119.146 (Indol C13), 121.686 (Indol C14), 112.005 (Indol C15). EIMS m/z : 315.1101 (M^+).

2-(2-methyl-5-nitroimidazolyl)ethyl furan-2-carboxylate (IV)

Yield : 85%; m.pt. 100°C; FTIR (cm⁻¹): 1714.43 (C=O -ester), 3220 (C=N str), 1531.72 (C-N str), 2968.88, 2925.24 (aliphatic CH₂), 3121.39 (ArCH, str), 1468.77 (aliphatic CH, bend), 744.12 (Ar CH), 1364.48 (N-O Sym. str). ¹H-NMR (CDCl₃, 400 MHz); δ 7.562 (s, 1H, MTZ-H), 2.524 (s, 3H, MTZ-CH₃), 4.610-4.621 (t, 2H, MTZ-CH₂), 4.681-4.692 (t, 2H, MTZ-CH₂), 7.121-7.130 (d, 1H, Furan), 6.498-6.502 (d, 1H, Furan), 7.943 (s, 1H, Furan). ¹³C-NMR δ ppm; 139.7 (MTZ-C1), 133.370 (MTZ-C2), 151.223 (MTZ-C3), 14.431 (MTZ-CH₃-C4), 45.311 (MTZ-CH₂-C5), 63.018 (MTZ-CH₂-C6), 157.946 (C=O -C7), 143.732 (Furan C8), 119.117 (Furan C9), 112.287 (Furan C10), 147.089 (Furan C11). EIMS m/z : 265.961 (M^+).

2-(2-methyl-5-nitroimidazolyl)ethyl thiophene-2-carboxylate (V)

Yield : 85%; m.pt. 81°C; FTIR (cm⁻¹): 1699.71 (C=O -ester), 3220 (C=N str), 1526.07 (C-N str), 2968.81, 2925.73 (aliphatic CH₂), 3135.13 (ArCH, str), 1468.07 (aliphatic CH, bend), 677.16 (Ar CH), 1362.62 (N-O Sym. str), 737.25 (C-S, str). ¹H-NMR (CDCl₃, 400 MHz); δ 8.05 (s, 1H, MTZ-H), 2.49 (s, 3H, MTZ-CH₃), 4.60-4.63 (t, 2H, MTZ-CH₂), 4.71-4.73 (t, 2H, MTZ-CH₂), 7.96-7.98 (d, 1H, thiophene), 7.21-7.23 (t, 1H, thiophene), 7.73-7.74 (d, 1H, thiophene). ¹³C-NMR δ ppm; 139.04 (MTZ-C1), 133.61 (MTZ-C2), 151.95 (MTZ-C3), 14.46 (MTZ-CH₃-C4), 45.16 (MTZ-CH₂-C5), 63.49 (MTZ-CH₂-C6), 161.36 (C=O -C7), 134.92 (Furan C8), 134.50 (thiophene C9), 128.96 (thiophene C10), 132.49 (thiophene C11). EIMS m/z : 282.0496 (M^+).

2-(2-methyl-5-nitroimidazolyl)ethyl 1H-pyrrole-2-carboxylate (VI)

Yield : 87%; m.pt. 85°C; FTIR (v, cm⁻¹): 1701.67 (C=O -ester), 3220 (C=N str), 1533.80 (C-N str), 2893.03, 2869.13 (aliphatic CH₂), 3090.88 (ArCH, str), 1483.05 (aliphatic CH, bend),

737.71 (Ar CH), 1364.36 (N-O Sym. str), 3382.95 (N-H, str). ¹H-NMR (CDCl₃, 400 MHz); δ 8.04 (s, 1H, MTZ-H), 2.45 (s, 3H, MTZ-CH₃), 4.55-4.57 (t, 2H, MTZ-CH₂), 4.66-4.68 (t, 2H, MTZ-CH₂), 7.03 (s, 1H, pyrrole), 6.16-6.18 (d, 1H, pyrrole), 6.68 (s, 1H, pyrrole), 11.91 (s, 1H, NH-pyrrole). ¹³C-NMR (CDCl₃) δ ppm; 139.04 (MTZ-C1), 133.55 (MTZ-C2), 151.95 (MTZ-C3), 14.36 (MTZ-CH₃-C4), 45.51 (MTZ-CH₂-C5), 62.03 (MTZ-CH₂-C6), 160.28 (C=O -C7), 121.50 (pyrrole C8), 115.68 (pyrrole C9), 110.19 (pyrrole C10), 125.22 (pyrrole C11). EIMS m/z : 266.1522 (M^+).

2-(2-methyl-5-nitroimidazolyl)ethyl isonicotinate (VII)

Yield : 87%; m.pt. 85°C; FTIR (cm⁻¹): 1729.04 (C=O -ester), 3220 (C=N str), 1526.61 (C-N str), 2920.05, 2859.27 (aliphatic CH₂), 3055.89 (ArCH, str), 1454.58 (aliphatic CH, bend), 764.50 (ArCH), 1366.51 (N-O Sym. str). ¹H-NMR (CDCl₃, 400 MHz); δ 7.943 (s, 1H, MTZ-H), 2.472 (s, 3H, MTZ-CH₃), 4.676-4.696 (t, 2H, MTZ-CH₂, 2.4 Hz), 4.703-4.724 (t, 2H, MTZ-CH₂, 4.8 Hz), 7.678-7.693 (d, 1H, isonicotinate), 8.750-8.764 (d, 1H, isonicotinate). ¹³C-NMR δ ppm; 138.5 (MTZ-C1), 133.283 (MTZ-C2), 150.854 (MTZ-C3), 14.305 (MTZ-CH₃-C4), 44.903 (MTZ-CH₂-C5), 63.515 (MTZ-CH₂-C6), 164.631 (C=O -C7), 136.154 (isonicotinate C8), 122.542 (isonicotinate C9), 150.572 (isonicotinate C10). EIMS m/z : 277.9767 (M^+).

2-(2-methyl-5-nitroimidazolyl)ethyl quinoline-6-carboxylate (VIII)

Yield : 82%; m.pt. 122°C; FTIR (cm⁻¹): 1718.45 (C=O -ester), 3220 (C=N str), 1529.45 (C-N str), 2962.72, 2923.72 (aliphatic CH₂), 3025.33 (ArCH, str), 1471.05 (aliphatic CH, bend), 744.84 (Ar CH), 1365.85 (N-O Sym. str). ¹H-NMR (CDCl₃, 400 MHz); δ 7.981 (s, 1H, MTZ-H), 2.487 (s, 3H, MTZ-CH₃), 4.721-4.740 (t, 2H, MTZ-CH₂, 2.8 Hz), 4.755-4.782 (t, 2H, MTZ-CH₂, 2.8 Hz), 8.141-8.152 (d, 1H, quinoline), 8.170-8.174 (d, 1H, quinoline), 9.004-9.019 (d, 1H, quinoline), 7.466-7.498 (d, 1H, quinoline), 8.234-8.258 (d, 1H, quinoline), 8.457 (d, 1H, quinoline). ¹³C-NMR δ ppm; 139.081 (MTZ-C1), 133.274 (MTZ-C2), 150.669 (MTZ-C3), 14.315 (MTZ-CH₃-C4), 45.156 (MTZ-CH₂-C5), 63.116 (MTZ-CH₂-C6), 165.555 (C=O -C7), 126.920 (quinoline C8), 128.438 (quinoline C9), 127.397 (quinoline C10), 150.232 (quinoline C11), 152.907 (quinoline C12), 122.075 (quinoline C13), 137.360 (quinoline C14), 130.219 (quinoline C15), 131.308 (quinoline C16). EIMS m/z : 327.9789 (M^+).

RESULTS AND DISCUSSION

Chemistry

The synthetic method of metronidazole esters was furnished in Scheme 1. The targeted compounds (I-VIII) were synthesized by the esterification procedure in the presence of DCC/DMAP catalyst in a polar solvent medium with good yields. The chemical structure of the MTZ esters viz., I-VIII were confirmed by the

spectroscopic methods viz., $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, and IR. Mass spectroscopic details are in accordance with the expected molecular weight values. Their solubility was also surveyed; these compounds are soluble in DMF, DMSO, CHCl_3 , ethyl acetate and CH_3OH , but they are insoluble in non-polar solvents and water. IR spectral investigation of the compounds confirmed the presence of the ester group ($1710\text{--}1738\text{ cm}^{-1}$) along with precursor footprints of C-N and C=N stretching in metronidazole moiety by 3220 and 1530 cm^{-1} respectively. Both proton and carbon NMR spectral investigation of all the compounds showed the characteristics of resonating signals of all protons and carbons in the respective chemical structures of I-VIII. A singlet in the range of $7.9\text{ }\delta$ ppm for one aromatic proton in all the compounds confirmed the incorporation of MTZ moiety in the compounds. Similarly, in carbon NMR the resonance peaks around 139 , 133 and 150δ ppm ascribed for three carbon atoms of imidazole ring of MTZ, respectively. LC-MS studies of the compounds showed 99.2 to 99.9% purity and the molecular mass were exactly matched with theoretical calculations of the respective chemical structure of the compounds.

Tyrosinase Inhibitory Assay

The assay of inhibitory activity of the synthesized MTZ esters I-VIII against mushroom tyrosinase enzyme were performed as per the procedure reported elsewhere.^[38] The commercial mushroom tyrosinase was purchased from Sigma. The inhibitory activity measurement was performed by determining the oxidation rate of the substrate L-DOPA ($3,4\text{-dihydroxyphenylalanine}$). It was conducted by mixing $800\text{ }\mu\text{L}$ of 0.05 M phosphate buffer at pH 6.8 , $100\text{ }\mu\text{L}$ of mushroom tyrosinase enzyme in PBS (1000 U/mL), and the synthesized metronidazole derivatives (I-VIII) with varying concentrations viz., 50 , 100 , 150 , 200 and $250\text{ }\mu\text{M}$. The mixtures were properly sealed in a glass vial. The mixture thus obtained was preincubated at 30°C for 10 minutes, then, $500\text{ }\mu\text{M}$ of 0.05 M L-DOPA dissolved in PBS was added. Immediately after the addition, the change in absorbance value in UV-vis spectrometer was measured at 475 nm at regular time interval. The following formula determined the percentage inhibition, $[(\text{OD}^0 - \text{OD}^1)/\text{OD}^0] \times 100\%$, where OD^0 is the absorbance without the inhibitor, and OD^1 is the absorbance with the inhibitor. From the dose response curve of inhibition efficiency, IC_{50} values were calculated. For comparison purpose kojic acid was used control and standard. The experiment was conducted in duplicate for confirmation.

The IC_{50} values of metronidazole derivatives (I-VIII) showing tyrosinase inhibitory values are tabulated in Table 1. Screening experiments were performed by individually oxidizing $3,4\text{-dihydroxyphenylalanine}$ along with synthesized metronidazole derivatives. From the screening results, a bar chart (Fig. 1) and Table 1, it was observed that the synthesized compounds exhibited

Table 1: IC_{50} values of the synthesized compounds I-VIII

Compound	% of inhibition					IC_{50} μM
	50	100	150	200	250	
I	35.87	53.00	61.22	70.54	77.31	102.7
II	28.41	36.08	44.22	51.66	60.57	187.3
III	34.53	54.10	60.87	69.34	75.19	104.3
IV	24.87	30.74	43.07	48.69	54.33	213.9
V	25.41	33.05	40.87	51.84	62.05	190.1
VI	23.98	32.15	39.85	45.28	56.63	217.6
VII	39.85	54.05	60.31	74.1	82.96	92.5
VIII	40.25	53.97	61.07	72.85	84.22	91.9
Kojic acid	47.58	61.02	69.79	85.41	95.35	59.2

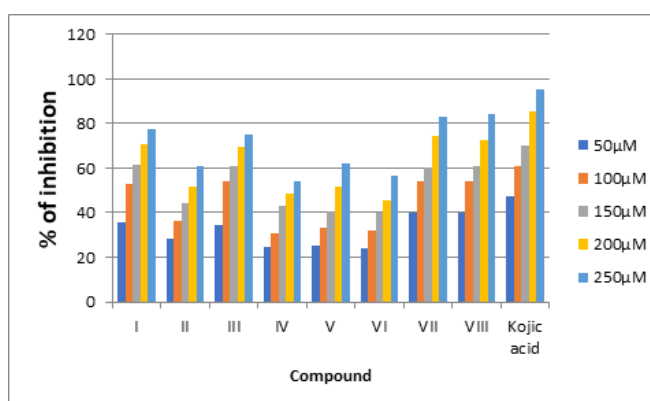


Fig. 1: % of inhibition of compounds and kojic acid

moderate inhibition against mushroom tyrosinase exhibiting the IC_{50} values in the range of 91.9 and $217.6\text{ }\mu\text{M}$ especially, compounds VII and VIII exhibited higher inhibitory properties against mushroom tyrosinase by 92.5 and $91.9\text{ }\mu\text{M}$, respectively.

The comparison of inhibitory activity was compiled and exhibited through bar chart are given in Fig. 1, the observed value is presented in Table 1. It reveals that, the percentage inhibition was increased by regular increments while increasing the substrate concentration as given in Table 1 in all the cases. It also indicated that, the activity has not attained its saturation even after $250\text{ }\mu\text{M}$ and there is no meaning to increase further loading of substrate the maximum was fixed to $250\text{ }\mu\text{M}$. The inhibitory effect on the tyrosinase activity of compounds I-VIII were compared with kojic acid. The compounds VII and VIII are exhibited very close activity with the standard.

The IC_{50} values of all the compounds and standard were plotted in Fig. 2. It reveals that compounds I, VII and VIII showed closer values to kojic acid and the remaining compounds did not show promising activity. These compounds possess either oxygen/sulfur heterocycle or nitrogen with acidic proton. Interestingly, the three compounds exhibited good activity among the synthesized



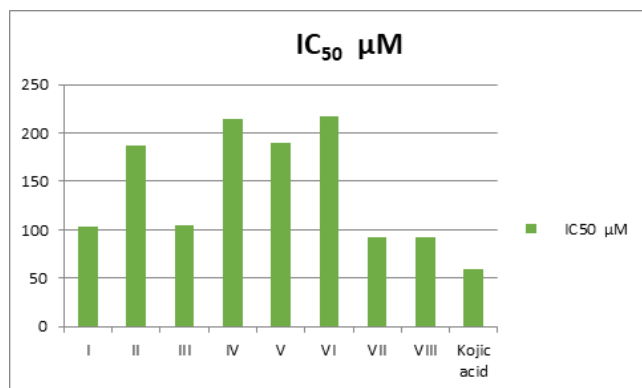


Fig. 2: IC₅₀ values are compared with compounds and Kojic acid

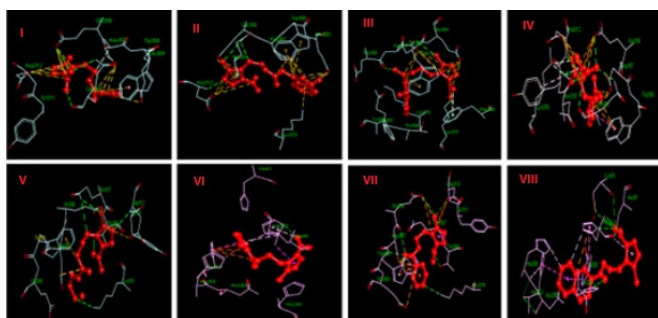


Fig. 3: Molecules docking studies of three-dimensional view interactions of the synthesized compounds I, II, III, IV, V, VI, VII and VIII with 2y9w protein.

compounds and possess pyridine traces, which is generally basic in nature. electronic environment raised due to the non-bonding electron pair on the nitrogen of pyridine might assist to produce more interactions with protein sites.

Molecular Docking studies

The synthesis and molecular docking investigations of synthesized hetero-aromatic substituted imidazole derivatives with 2y9w protein molecules are performed. Fig. 3 depicts the interaction of the synthesized compound with the protein 2y9w and Table 2 lists the docking results. The specific characteristics of the substitutions contained in the molecules have an impact on the docking findings. VIII had the most protein linkages out of all the synthesized compounds owing to three types of hydrogen bond interactions with 10 hydrogen bonding interactions, including pi-donor hydrogen bonding, normal hydrogen bonding and carbon-hydrogen bonding interactions. Asparagine, histidine, and cysteine, which are present in the protein at different locations and with variable bond lengths, were involved in these interactions. One of them, residue HIS85, was found to have strong, conventional hydrogen bonding relationship and a bond length of 2.84 Å. In addition, compound VII contains nine interactions, including two types of hydrogen bonding interactions, including the pi-donor hydrogen bond interaction and the conventional hydrogen bond interaction. These nine interactions, which are LYS379, THR308, ASP129, GLU356, TRP358, ASP357 and TRP358, all share seven amino acid

Table 2: Binding energy, inhibition constant and types of interactions details of the compounds I-VIII

Compound	Binding energy (kcal/mol)	Inhibition constant	No of hydrogen bonding	Hydrogen bonding and amino acid residue
I	-2.91	7.37 mM	4	GLN307(2.71Å), GLU356(3.37Å), GLN307(2.98Å) Normal hydrogen bonding interactions ASP357(3.44Å) carbon-hydrogen bonding interaction
II	-3.32	3.7 mM	3	ASP312(2.61Å), GLN307(3.24Å), GLN307(2.99Å) are Normal hydrogen bonding interactions
III	-1.6	67.71 mM	5	ASP357(3.37Å) ASP357(3.10Å) GLU356(2.38Å)LYS379(3.03Å) are Normal hydrogen bonding interactionsPHE368(3.70Å) pi-donor hydrogen boning interaction
IV	-2.53	13.97 mM	7	GLN307(2.70Å)ASN310(3.13Å) ASP312(2.92Å)GLU356(2.96Å) GLN307(2.64Å) conventional hydrogen bonding interaction LYS376(3.36Å) carbon hydrogen bonding interaction LYS376(3.28Å) carbon- hydrogen bonding interaction
V	-4.25	761.84 μM	5	LYS379 (3.28 Å), THR308(3.32Å) ASP312(3.14Å) GLU356(2.95Å), GLN307(3.15Å)are normal hydrogen bonding interactions
VI	-2.98	6.52 mM	3	HIS85(3.16Å) Normal hydrogen bonding interaction HIS85(3.69Å) Pi-donor hydrogen bond interaction HIS85(2.99Å) carbon-hydrogen bonding interaction
VII	-3.62	2.21 mM	9	LYS379(2.95Å)THR308(2.58Å) ASP3129(3.15Å)GLU356(3.26Å), GLU356(3.27Å) TRP358(2.75Å) ASP357(2.64Å) are hydrogen bond interactionsTRP358(2.98Å) Pi- donor hydrogen bond interaction TRP358(3.51Å) Pi-donor hydrogen bonding interaction
VIII	-4.92	249.44 μM	10	HIS244(3.19Å), HIS85(3.23Å),HIS85(2.84Å) CYS83(3.49Å) CYS83(2.98Å) HIS263(3.10Å) are hydrogen bond interactionsASN81(4.10Å) Pi-donor hydrogen bond interaction HIS259(3.34Å) , HIS259(3.14Å) and HIS85(3.62Å) arecarbon- hydrogen bond interactions

residues. With a bond length of 2.58, this conventional hydrogen bonding contact also has significant interaction with residue THR308. Compound IV has the third-highest interactions with protein molecules and has seven interactions with five different amino acid residues. Of all of these interactions, GLN307 has the shortest bond length (2.70 Å), indicating the intensity of the association. Compounds V and III have five interactions with five different amino acid residues through conventional hydrogen bonding and pi-donor hydrogen bonding, respectively. Compound I was the second-to-last chemical in this line-up, involving three regular hydrogen bonds and one carbon-hydrogen bond through the amino acid residues of GLN307, GLU356, GLN307, and ASP357. With the smallest hydrogen bond length of 2.71, GLN307 has shown the strongest involvement of these residues. The two compounds II and VI interact with protein molecules with lower binding energies, producing only three interactions. Compound II having only two amino acid residues was involved in the conventional hydrogen bonding interactions that are ASP312 and GLN307. The compound VI also shows interactions with only single amino acid residues that are HIS85 with three interactions in different modes: Normal hydrogen bonding interaction, pi-donor type hydrogen bond and carbon-hydrogen bond interaction. The detailed bond length of each compound amino acid residue interaction has been listed in Table 2. Apart from these interactions another parameter also decides the interaction ability of the compounds viz., the binding energy, inhibition constant value of the proteins. Of course, both values are directly proposed to each other, but there is no rule that they should be correlated with several interactions. An impressive result regarding the constant value of binding and inhibition was obtained. The compound VIII which is having more number interactions with various amino acid residues, however, shows very less binding energy and inhibition constant thus being obtained to be -4.92 kcal/mol and 249.44 μ M, respectively. Whereas compound III has lowest binding energy and inhibition constant of -1.6 kcal/mol and 67.71 mM respectively, it had less interaction than compound B8. The two chemicals with the fewest interactions, II and VI, exhibit moderate binding energy and inhibition constant.

This result clearly shows that VII and VIII have a greater number of ligand-protein interactions, mainly dominated by hydrogen bond type and pi donor attractions. Compounds that possess oxygen (IV) and sulphur (V) produced the least number of attractions and showed very little activity. Comparing the results from IC_{50} values and docking study, nitrogen heterocycles containing ester derivatives of MTZ produced effective inhibition and a greater number of bonding interactions. This result tempts to suggest, the electron donating nature of pyridine-based derivatives produced effective inhibition

due to more interactions generated with acidic acceptor sites of protein. Binding energy data of the compounds also supports the decision.

CONCLUSION

Metronidazole ester derivatives using various heterocyclic fragments were synthesized, and their chemical structures were confirmed using various spectral techniques. The oxidation of the 3,4-dihydroxyphenylalanine method determined the mushroom tyrosinase activity of the synthesized compounds. Tyrosinase inhibitory activity was compared with kojic acid as a reference and found that all those molecules exhibited moderate activity, particularly compounds isonicotinic ester (VII), quinoline ester (VIII), derivatives showed decent activity with IC_{50} values 92.5 and 91.8 μ M, respectively. Molecular docking studies and IC_{50} values suggested that ligand with electron donor fragments will have much interactions with more number of protein sites there it produces more tyrosinase activity. Docking studies with 2y9w protein also support this by exhibiting more physical interactions for compounds VII and VIII, confirming the mushroom tyrosinase activity.

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