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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}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 veryFew 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.

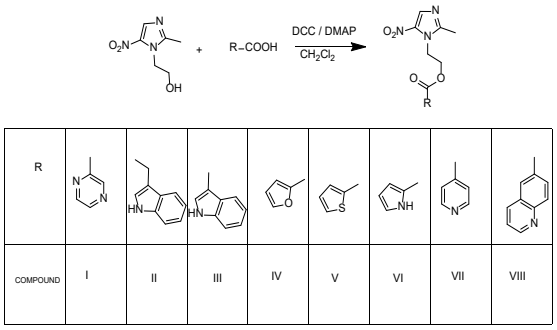
Material and Methods

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. ^{1}H &^{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-VII

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), ^{1}H-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). ^{1}H-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 CH_2), 1469.07(aliphatic CH,bend), 745.86 (Ar CH), 1348.25(-N-O Sym. str), 3435.24(-N-H,str). ^{1}H-NMR ($CDCl_3$,400 MHz); δ 8.06 (s,1H, MTZ-H), 2.49 (s,3H, MTZ-CH_3), 4.69-4.70 (t,2H,MTZ-CH_2), 4.72-4.73 (t,2H,MTZ-CH_2), 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). ^{13}C-NMR ($CDCl_3$) 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),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 CH_2), 3121.39(ArCH,str) 1468.77 (aliphatic CH,bend), 744.12 (Ar CH), 1364.48(-N-O Sym. str). ^{1}H-NMR ($CDCl_3$,400 MHz); δH 7.562 (s,1H, MTZ-H), 2.524 (s,3H, MTZ-CH_3), 4.610-4.621(t,2H,MTZ-CH_2), 4.681-4.692 (t,2H,MTZ-CH_2),7.121-7.130 (d,1H,Furan), 6.498-6.502 (d,1H,Furan), 7.943 (s,1H, Furan).^{13}C-NMR δ ppm; 139.7 (MTZ-C1), 133.370 (MTZ-C2), 151.223 (MTZ-C3), 14.431 (MTZ-CH_3 –C4), 45.311 (MTZ-CH_2 –C5), 63.018 (MTZ-CH_2 –C6), 157.946 (C=O –C7), 143.732 (FuranC8),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 CH_2), 3135.13(ArCH,str) 1468.07(aliphatic CH,bend), 677.16 (Ar CH), 1362.62(-N-O Sym. str), 737.25(-C-S,,str). ^{1}H-NMR ($CDCl_3$,400 MHz); δ 8.05 (s,1H, MTZ-H), 2.49 (s,3H, MTZ-CH_3), 4.60-4.63(t,2H,MTZ-CH_2), 4.71-4.73(t,2H,MTZ-CH_2),7.96-7.98(d,1H,thiophene,), 7.21-7.23 (t,1H, thiophene), 7.73-7.74(d,1H, thiophene).^{13}C-NMR δ ppm; 139.04 (MTZ-C1), 133.61 (MTZ-C2), 151.95 (MTZ-C3), 14.46(MTZ-CH_3 –C4), 45.16 (MTZ-CH_2 –C5), 63.49 (MTZ-CH_2 –C6), 161.36 (C=O –C7), 134.92 (FuranC8),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 (ν, cm^{-1}): 1701.67 (C=O -ester), 3220 (C=N str), 1533.80 (C-N str), 2893.03, 2869.13(aliphatic CH_2), 3090.88(ArCH,str) 1483.05(aliphatic CH,bend), 737.71 (Ar CH), 1364.36(-N-O Sym. str), 3382.95(-N-H,str). ^{1}H-NMR ($CDCl_3$,400 MHz); δ 8.04 (s,1H, MTZ-H), 2.45 (s,3H, MTZ-CH_3), 4.55-4.57(t,2H,MTZ-CH_2),4.66-4.68(t,2H,MTZ-CH_2),7.03(s,1H,pyrrole),6.16-6.18(d,1H,pyrrole),6.68(s, 1H,pyrrole) 11.91(s,1H,NH-pyrrole).^{13}C NMR ($CDCl_3$) δ ppm; 139.04 (MTZ-C1), 133.55 (MTZ-C2), 151.95 (MTZ-C3), 14.36 (MTZ-CH_3 –C4), 45.51 (MTZ-CH_2 –C5), 62.03 (MTZ-CH_2 –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) ethylisonicotinate (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 CH_2), 3055.89(ArCH,str) 1454.58(aliphatic CH,bend), 764..50 (Ar CH), 1366.51(-N-O Sym. str). ^{1}H-NMR ($CDCl_3$,400 MHz); δ 7.943 (s,1H, MTZ-H), 2.472 (s,3H, MTZ-CH_3), 4.676-4.696(t,2H,MTZ-CH_2,2.4Hz),4.703-4.724(t,2H,MTZ-CH_2,4.8Hz),7.678-7.693(d,1H, isonicotinate), 8.750-8.764 (d,1H, isonicotinate).^{13}C-NMR δ ppm; 138.5 (MTZ-C1), 133.283 (MTZ-C2), 150.854 (MTZ-C3), 14.305 (MTZ-CH_3 –C4), 44.903(MTZ-CH_2 –C5), 63.515 (MTZ-CH_2 –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 CH_2), 3025.33(ArCH,str) 1471.05(aliphatic CH,bend), 744.84(Ar CH), 1365.85(-N-O Sym. str).^{1}H-NMR ($CDCl_3$,400 MHz); δ 7.981 (s,1H, MTZ-H), 2.487 (s,3H, MTZ-CH_3), 4.721-4.740 (t,2H,MTZ-CH_2,2.8Hz), 4.755-4.782 (t,2H,MTZ-CH_2,2.8Hz),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).

^{13}C-NMR δ ppm; 139.081 (MTZ-C1), 133.274 (MTZ-C2), 150.669 (MTZ-C3), 14.315 (MTZ-CH_3 –C4), 45.156 (MTZ-CH_2 –C5), 63.116 (MTZ-CH_2 –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 wasfurnished 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}H-NMR, ^{13}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 acetateand 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 (1710–1738 cm^{-1}) along with precursor footprints of C-N and C=N stretching in metronidazole moiety by 3220 and 1530cm^{-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 δ ppm for one aromatic proton in all the compounds confirmed the incorporation of MTZ moiety in the compounds. Similarly, in carbon NMR the resonation 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-dihydroxyphenylalanine). It was conducted by mixing 800 µL of 0.05M phosphate buffer at pH 6.8, 100 µL of mushroom tyrosinase enzyme in PBS (1000U/mL), and the synthesized metronidazole derivatives (I-VIII) with varying concentrations viz., 50, 100, 150, 200 and 250 µM. The mixtures were properly sealed in a glass vial. The mixture thus obtained was preincubated at 30°C for 10 minutes, then, 500 µM of 0.05M 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 intervel. The following formula determined the percentage inhibition, $[(OD^0 - OD^1)/OD^0] \times 100\%$, 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-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 moderate inhibition against mushroom tyrosinase exhibiting the IC_{50} values in the range of 91.9 and 217.6 µM especially, compounds VII and VIII exhibited higher inhibitory properties against mushroom tyrosinase by 92.5 and 91.9 µM, respectively.

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

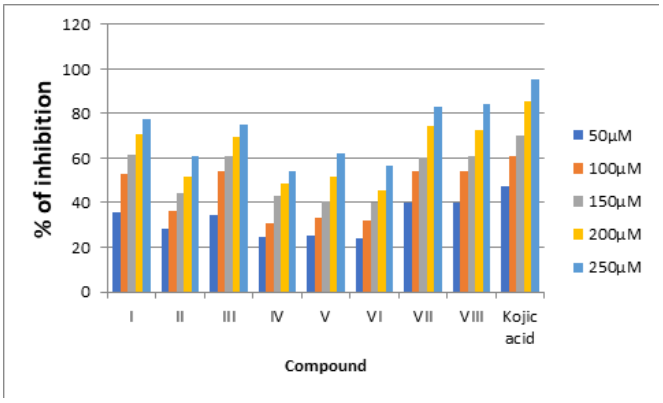


Fig. 1: % of inhibition of compounds and kojic acid

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 µM and there is no meaning to increase further loading of substrate the maximum was fixed to 250 µM. 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

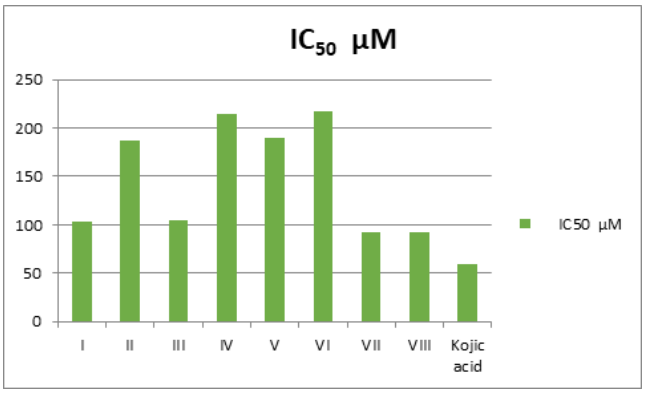


Fig. 2: IC_{50} values are compared with compounds and Kojic acid

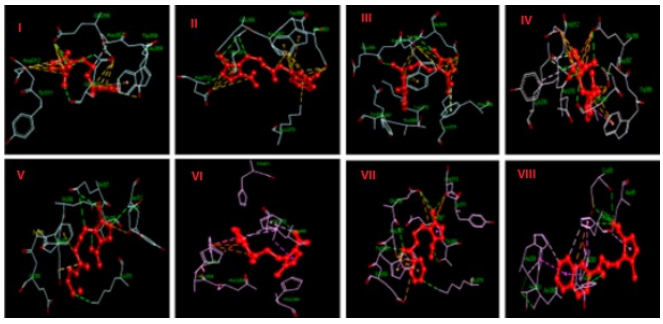


Fig. 3: Molecules docking studies of three-dimensional view interactions of the synthesized compounds I, II, III, IV, V, VI, VII and VIII with 2y9w protein.

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 µM, respectively. Whereas compoundIII 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 showsthatVII 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 natureof 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 µM, 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 VIIII, confirming the mushroom tyrosinase activity.

References

1. Chang TS. An updated review of tyrosinase inhibitors. Int. J. Mol. Sci. 2009 Jun; 10:2440-75. Available from: https://doi.org/10.3390/ijms10062440
2. Lee SG, Karadeniz F, SeoY, Kong CS. Anti-melanogenic effects of flavonoid glycosides from Limonium tetragonum (Thunb.) bullock via inhibition of tyrosinase and tyrosinase related proteins. Molecules.2017 Sep 5;22(9):1480. Available from: doi: 10.3390/molecules22091480
3. Matsuo K, Kobayashi M, Takuno Y, Kuwajima H, Ito H, Yoshida T. Anti-tyrosinase activity constituents of Arctostaphylos uva-ursi. YakugakuZasshi. 1997 Dec; 117 (12): 1028-32. Available from: https://doi.org/10.1248/yakushi1947.117.12_1028
4. Alam N,Yoon KN, Lee JS, Cho HJ, Lee TS. Consequence of the antioxidant activities and tyrosinase inhibitory effects of various extracts from the fruiting bodies of Pleurotus ferulae. Saudi.J.Biol. Sci. 2012 Jan; 19: 111-118. Available from:https:doi:10.1016/j.sjbs.2011.11.004
5. Kim NY, Kwon HS, Lee HY. Effect of inhibition on tyrosinase and melanogenesis of Agastache rugosa Kuntze by lactic acid bacteria fermentation. J. Cosmet. Dermatol. 2017 Sep; 16 (3):407-15. Available from: doi: 10.1111/jocd.12264
6. McEvily AJ. Iyengar R, Otwell WS. Inhibition of enzymatic browning in foods and beverages. Crit. Rev Food Sci. Nutr. 1992 Sep 29; 32(3): 253-73. Available from: https://doi.org/10.1080/10408399209527599
7. Frankos VH, Schmitt DF, Haws LC, McEvily AJ, Iyengar R, Miller SA,Clydesdale FM,Forbes AL,Sauer RM. Generally recognized as safe (GRAS) evaluation of 4-hexylresorcinol for use as a processing aid for prevention of melanosis in shrimp.Regul. Toxicol.Pharmacol.1991Oct;14(2):202-12.Available from:http://doi: 10.1016/0273-2300(91)90007-i
8. Orhan IE, Hassan Khan MT. Flavonoid derivatives as potent tyrosinase inhibitors – a survey of recent findings between 2008–2013.Curr. Top. Med. Chem. 2014; 14 (12):1486. Available from: doi: 10.2174/1568026614666140523120741
9. Jegal J, Park SA,Chung K,Chung,Lee J,Jeong EJ, Him KH,Yang MH. Tyrosinase inhibitory flavonoid from Juniperus communisfruits.

Biosci.Biotechnol.Biochem.2016 Dec; 80(12): 2311-2317.Available from: doi:10.1080/09168451.2016.1217146
10. Muhammad D, Hubert J, Lalun N,Renault JH, Bobichon H, Nour M, Nazabadioko LV.Isolation of flavonoids and triterpenoids from the fruits of Alphitonianeocaledonica and evaluation of their anti-oxidant, antityrosinase and cytotoxic activities.Phytochem. Anal. 2015 Mar; 26(2):137-44. Available from: doi: 10.1002/pca.2545
11. Erdogan Orhan I, Senol FS, Aslan Erdem S, Irem Tatli I, Kartal M, Alp S. Tyrosinase and cholinesterase inhibitory potential and flavonoid characterization of Viola odorata L. (Sweet Violet).Phytother. Re. 2015 Sep; 29(9):1304-10. Available from: doi: 10.1002/ptr.5378
12. Jones K, Hughes J, Hong M, Jia Q, Orndorff S. Modulation of melanogenesis by aloesin: a competitive inhibitor of tyrosinase. Pigment Cell Res. 2002 Sep 5; 15(5): 335-40. Available from: doi:10.1034/j.1600-0749.2002.02014.x.
13. Chen J, Xiaojing Yu, Huang Y. Inhibitory mechanisms of glabridin on tyrosinase. Spectrochim.Acta.A Mol.Biomol.Spectrosc. 2016 Nov 5;168:111-17. Available from: doi: 10.1016/j.saa.2016.06.008
14. Xia L, Idhayadhulla A, Lee Y, Kim SH, Wee YJ. Antioxidant and antibacterial evaluation of synthetic furomollugin and its diverse analogs.Medicinal Chemistry Research.2014Feb 12;23:3528-38. Available from: doi: https://doi.org/10.1007/s00044-014-0929-9
15. Gupta R, Sharma S, Singh R, Vishwakarma RA,Mignani S, Singh PP. Functionalized Nitroimidazole Scaffold Construction and Their Pharmaceutical Applications: A 1950–2021 Comprehensive Overview. Pharmaceuticals. 2022 Apr 30; 15(5):561. Available from: https://doi.org/10.3390/ph15050561
16. Hu YG, Gao ZP, Zheng YY, Hu CM, Lin J, Wu XZ, Zhang X, Zhou YS, Xiong Z, Zhu DY. Synthesis and Biological Activity Evaluation of 2-Cyanopyrrole Derivatives as Potential Tyrosinase Inhibitors. Front. Chem. 2022 Jun 17; 10: 914944. Available from: doi:10.3389/fchem.2022.914944
17. Maddila S, Pagadala R, Jonnalagadda SB. 1,2,4-Triazoles: a review of synthetic approaches and the biological activity. Lett. Org. Chem. 2013 July 19;10 (10): 693-714.Available form: doi:10.2174/1570178610101311261154 48
18. Crozet MD, Terme T,Vanelle P. Designing new 5-nitroimidazoles: Towards safer anti-infectious agents. Lett. Drug Des. Discov. 2014; 11: 531-59. Available from: doi: 10.2174/15701808106661311220106 15
19. Rajão MA, Furtado C, Alves CL, Passos-Silva DG, de Moura MB, Schamber-Reis BL,Kunrath-Lima M, Zuma AA, Vieira-da-Rocha JP, Garcia JBF. Unveiling Benznidazole's mechanism of action through over expression of DNA repair proteins in Trypanosoma cruzi. Environ. Mol. Mutagenesis. 2013 Dec 13; 55 (4):309-21. doi: Available form: 10.1002/em.21839
20. Rami M, Dubois L, Parvathaneni NK, Alterio V, van Kuijk SJA, Monti SM, Lambin P, De Simone G,Supuran CT, Winum JY. Hypoxia-Targeting Carbonic Anhydrase IX Inhibitors by a New Series of Nitroimidazole Sulfonamides/Sulfamides/Sulfamates. J. Med. Chem. 2013 Nov 14;56(21): 8512-20.Available form:doi: 10.1021/jm4009532
21. Kubo I,Kinst-Hori I. Tyrosinase inhibitors from cumin. J. Agric. Food Chem. 1998 Dec 3;46 (12): 5338-5341.Available form: https://doi.org/10.1021/jf980226
22. Duan YT, Wang ZC,SangYL,Tao XX, Teraiya SB, Wang PF,Wen Q, Zhou XJ, Ding L, Yang YH, Zhu HL. Design and synthesis of 2-styryl of 5-Nitroimidazole derivatives and antimicrobial activities as FabH inhibitors, Eur. J. Med. Chem. 2014 Apr 9;76: 387-96. Available from: doi:10.1016/j.ejmech.2014.02.004
23. Jorgensen MA, Manos J, Mendz GL Hazell SL. The mode of action of metronidazole in Helicobacter pylori: futile cycling or reduction.J.Antimicrob.Chemother. 1998Jan;41(1):67-75.Available from: doi: 10.1093/jac/41.1.67
24. Jarrad AM, Karoli T, Debnath A, Tay CY, Huang JX, Kaeslin G,Elliott AG,Miyamoto Y, Ramu S,Kavanagh AMJ, Zuegg J, Eckmann L, Mark A T Blaskovich, Cooper MA. Metronidazole-triazole conjugates: Activity against Clostridium difficile and parasites. Eur. J. Med. Chem. 2015 Aug 28;101: 96-102.Available form:doi:10.1016/j.ejmech.2015.06.019.1
25. Du QR, Dong Li D, Zhou Pi Y, Ran Li j, Jain Sun, FangxF, Zhongx WQ, Bin Gong H, Liang Zhu H. Novel 1,3,4-oxadiazole thioether derivatives targeting thymidylate synthase as dual anticancer/antimicrobial agents.Bioorg. Med. Chem. 2013 Apr 15;21(8): 2286-97. Available from: doi: 10.1016/j.bmc.2013.02.008
26. Feng Wang S, Yin Y,Qiao F, Wu X, Sha S, Zhang L, Liang Zhu H. Synthesis, molecular docking and biological evaluation of metronidazole derivatives containing piperazine skeleton as potential antibacterial agents.Bioorg. Med. Chem. 2014 Apr 15;22(8): 2409-15. Available from: doi: 10.1016/j.bmc.2014.03.004
27. Maddili SK, Zhen Li Z, Kannekanti VK, Bheemanaboina RRY. Tuniki B,Tangadanchu VKR, He Zhou C. Azoalkyl ether imidazo[2,1-b] benzothiazoles as potentially antimicrobial agents with novel structural skeleton. Bioorg. Med. Chem. Lett. 2018 Aug 1;28(14): 2426-31. Available from: doi: 10.1016/j.bmcl.2018.06.016
28. Ullah A, Iftikhar F,Arfan M, Batool Kazmi ST, Anjum MN,I San-Ul-Haq,Ayaz M, Farooq S, Rashid U. Amino acid conjugated antimicrobial drugs: Synthesis, lipophilicity- activity relationship, antibacterial and urease inhibition activity. Eur. J. Med. Chem. 2018 Feb 10; 145: 140-143.Available form:doi:10.1016/j. ejmech.2017.12.089
29. Qiu Li H, Chen Xu, Sen Li H,PingXiao Z, Shi L, Liank Zhu H. Metronidazole–Flavonoid Derivatives as Anti- Helicobacter pylori Agents with Potent Inhibitory Activity against HPE-Induced Interleukin-8 Production by AGS Cells.Chem.Med.Chem.2007 Sep;2(9): 1361-9. Available from: doi: 10.1002/cmdc.200700097
30. Feng Cui S, Ping Peng L, Zhen Zhang H, Rasheed S, Kumar KV, He Zhou C. Novel hybrids of metronidazole and quinolones: synthesis, bioactive evaluation, cytotoxicity, preliminary, antimicrobial mechanism and effect of metal ions on their transportation by human serum albumin. Eur. J. Med. Chem. 2014 Oct 30;86: 318-34. Available form: doi:10.1016/j.ejmech.2014.08.063
31. Zhang X, Sangani CB, Xin Jia L, Xian Gong P, Wang F, Jun-Fang Wang, Liang Zhu H. Synthesis and antibacterial evaluation of novel Schiff's base derivatives of nitroimidazole nuclei as potent E. coli FabH inhibitors. RSC Adv. 2014 Sep;4: 54217. Available from: doi: 10.1039/C4RA08567A
32. Qian Y, Jia Zhang H, Zhang H, Xu C, Zhao J, Liang Zhu H. Synthesis, molecular modeling, and biological evaluation of cinnamic acid metronidazole ester derivatives as novel anticancer agents, Bioorg. Med. Chem.2010 Jul 15;18(14): 4991-6. Available from:doi: 10.1016/j.bmc.2010.06.003
33. Sun J.Cheng Lv P, Jiao Guo F, Yi Wang X, Zhang Y, Hua Sheng G,Song Qian S, Liang Zhu H. Aromatic diacylhydrazine derivatives as a new class of polo-like kinase 1 (PLK1) inhibitors. Eur. J. Med. Chem. 2014 Jun 23;81: 420-6. Available from: doi: 10.1016/j.ejmech.2014.05.026
34. Wang Chen L, Fei Wang P, Jie Tang D, Xiang Tao X, Jun Man R, Yue Qiu H, Chang Wang Z, Chen Xu, Liang Zhu H. Metronidazole containing pyrazole derivatives potently inhibit tyrosyl-tRNA synthetase: design, synthesis, and biological evaluation. Chem. Biol. Drug Des. 2016 Oct;88(4):592-8.
35. Bkhaitan MM, Alarjah M, Mirza AZ,Abdalla AN, El-Said HM, Faidah HS. Preparation and biological evaluation of metronidazole derivatives with monoterpenes and eugenol. Chem. Biol. Drug Des.2018 Dec;92(6):1954-1962. Available from: https://doi.org/10.1111/cbdd.13366
36. Islam NM, Iskander MN, Microtubulin binding sites as target for developing anticancer agents. Mini-Rev. Med. Chem. 2004 Dec;4(10):1077-104. Available from: doi: 10.2174/1389557043402946
37. Samaneh Zolghadri, Asieh Bahrami, Mahmud Tareq Hassan Khan, J. MunozMunoz, F. Garcia-Molina, F. Garcia-Canovas & Ali Akbar Saboury (2019) A comprehensive review on tyrosinase inhibitors, Journal of Enzyme Inhibition and Medicinal Chemistry. 2019 Jan; 34(1), 279-309. Available from: https://doi.org/10.1080/14756366.2018.1545767
38. Chen MJ, Hung CC, Chen YR, Lai ST, Chan CF. Novel synthetic kojic acid-methimazole derivatives inhibit mushroom tyrosinase and melanogenesis. J Biosci Bioeng. 2016 Dec;122(6):666-672. Available from: https://doi: 10.1016/j.jbiosc.2016.06.002.

39. Elavarasu SR, Senthil S. Synthesis and Characterization of Metronidazole Derivatives for Tyrosinase Inhibitory Activity. Indian J. Heterocycl. Chem., 2023 Oct-Dec; 33(4): 445–451. Available from: https://doi.org/10.59467/IJHC.2023.33.445

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