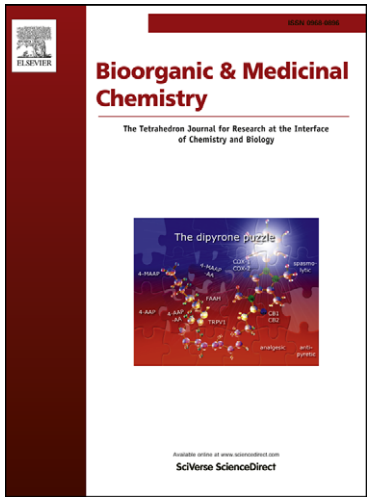

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Synthesis of new heterocyclic hybrids based on pyrazole and thiazolidinone scaffolds as potent inhibitors of tyrosinase

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Dedicated to my colleague late Prof. Yogesh Mundhe

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A B S T R A C T

As a part of ongoing studies in developing new Tyrosinase inhibitors, a class of structurally novel 2-(2,4- Dimethoxy phenylamino)-5 methylene-4-thiazolinone derivatives were synthesized by incorporating 2-(2,4-Dimethoxy-phenylamino)-thiazol-4-one with various 1-(1-methyl-buta-1,3-dienyl)-3-phenyl-1H-pyrazole-4-carbaldehyde. The results showed that some of the synthesized compounds exhibited significant inhibitory activities. Especially, 5-[3-(2-Chloro-phenyl)-1-phenyl-1H-pyrazol-4-ylmethylene]-2-(2,4-dimethoxy-phenylamino)-thiazol-4-one (5h) and 5-[3-(3-Chloro-phenyl)-1-phenyl-1H-pyrazol-4-ylmethylene]-2-(2,4-dimethoxy-phenylamino)-thiazol-4-one (5g) possessing 2-chloro-phenyl and 3-chloro-phenyl group exhibited the most potent Tyrosinase inhibitory activity with an IC_{50} value of 34.12 and 52.62 µM, respectively. The inhibition mechanism analysis of 5h and 5g thiazolidinone derivatives demonstrated that the inhibitory effects of the compounds on Tyrosinase were reversible and competitive. Preliminary structure activity relationships (SAR) analysis suggested that further development of such compounds might be of interest, as it manifests simple reversible slow binding inhibition against monophenolase and diphenolase.

Keywords: Thiazolidinone scaffolds, Tyrosinase inhibitors, Competitive inhibition, Heterocyclic hybrids

1. Introduction

Tyrosinase (monophenol or *o*-diphenol, oxygen oxidoreductase E.C. 1.14.18.1, syn polyphenol oxidase), also known as polyphenol oxidase (PPO) is a multifunctional copper containing enzyme from oxidase family, widely distributed in microorganisms, animals and plants.[1] Tyrosinase catalyzes melanin biosynthesis in two distinct reactions: in the *o*-hydroxylation of monophenols to *o*-diphenols (monophenolase) and in the oxidation of o-dipheols to *o*-quinones (Diphenolase).[2] Such Quinines could polymerize spontaneously due to high reactivity and can polymerize spontaneously to form high molecular weight melanin pigment by a series of reactions with amino acids and proteins.[3] Melanin is one of the most important natural biopolymer responsible for pigmentation, colour patterns of mammalian skin and protect skin from solar radiations.[4] However, this beneficial trait is most widely associated with some severe human maladies and threatening diseases due to the overproduction of melanin such as *senile lentigo*, freckles, melasma, chloasama and pigmented acne scars are of particular concern in women.[5] The treatment usually involves the use of medicine or medicinal cosmetics containing depigmenting agents or skin whitening agents.[6] Likewise Tyrosinase inhibitors were also used to treat the dermatological disorders related to melanin hyperaccumulation viz age spots, actinic damages, freckles and essential in cosmetics for depigmentation.[7,8] Tyrosinase has been also linked to neurodegeneration in patients suffering from Parkinson's disease.[9,10] In insects Tyrosinase is uniquely associated with three different biochemical processes like sclerotisation of cuticle, defensive encapsulation and melanisation of foreign organism and wound healing.[11] Thus, involvement of Tyrosinase in such key processes provide potential targets for developing safer and effective Tyrosinase inhibitors as insecticides and for their control.

Previous reports confirmed that Tyrosinase was one of the main causes of most fruits and vegetables quality loss during post harvest handling and processing, leading to faster

degradation and shorter shelf life.[12,13] Therefore, inhibition of melanin formation by Tyrosinase is also applicable to fruit and vegetable preservation by the alleviation of browning. Thus, unregulated action of Tyrosinase and excessive production of melanin will cause number of human disease aetiologies.[14] Hence, therapies to such diseases can be explored by Tyrosinase inhibition. Tyrosinase inhibitors had gain an insight as a therapeutic agent, as insecticide and antibrowning agent from last couple of decades. Tyrosinase inhibitors normally either render the copper within the active site inactive by chelation, obviating the substrate enzyme interactions or inhibit oxidation via an electrochemical process.[15]

Among the nitrogen containing heterocyclic skeletons, thiazolidin-4-ones, rigid molecules, are considered as a biologically privileged scaffold well-tolerated in human subjets[16, 17] and could be perspective models for the designing of new hybrids.[18] Keeping in view the above facts and continuing our program on the development of efficient methods to generate drug-like nitrogen-containing molecules.[19-22] We were interested in a simple synthesis of thiazolidinone analogues containing the pyrazole moiety and their evaluation as potent inhibitors of tyrosinase.

2. Results and Discussions

2.1 Synthesis

In the present investigation 2-(2,4-Dimethoxy-phenylamino)-5-methylene-thiazol-4-one (5a-5j) have been prepared by the condensation of 2-(2,4-Dimethoxy-phenylamino)-thiazol-4-one (3) and substituted 1,3-diphenyl-1H-pyrazole-4-carbaldehydes (4a-4j) by the known literature method[23] (Scheme I). Substituted 1,3-diphenyl-1H-pyrazole-4-carbaldehydes (4a-4j) were prepared by Vilsmeir–Haack reaction on acetophenonephenylhydrazones.[24] The resulting crud product was purified by recrystallization from acetic acid: DMF (2:1) to afford

title compound (5a-5j). The compounds structure was confirmed by spectral data (IR, ^{1}H NMR and MS).

2.2 Biological evaluation

The effect of thiazolidinone derivatives on Tyrosinase inhibition

For evaluating the tyrosinase inhibition, all the synthesised thiazolidinone scaffolds were subjected to tyrosinase inhibition assay with L-DOPA as substrate, according to the method reported by our groups with some slight modifications. The tyrosinase inhibition by kojic acid was ever reported, therefore, it was selected as reference compound. The IC_{50} values of thiazolidinone scaffolds against monophenolase and diphenolase were summarised in Table 1, and IC_{50} values of all these compounds were determined from logarithmic concentration–inhibition curves and given as means of three different experiments. From results, it was observed that compounds 5h, 5g, 5d and 5i exhibited potent inhibition of mushroom tyrosinase with an IC_{50} values ranged from 34.0 to 82.0 µM. Especially, compounds 5h and 5g possessing 2-chloro phenyl and 3-chloro phenyl substituents showed more potent inhibitory activities than the other compounds and kojic acid. Most of the thiazolidinone scaffolds viz 5a and 5j showed lower or negligible activities against tyrosinase.

Taken as an ensemble, the following general features of SAR can be deduced from compounds (5a–5j). When the activities of the scaffolds possessing different substituents viz methoxy, methyl and fluro, bromo and chloro compared, then it was observed that substituted thiazolidinones with halogen atom (-Cl, -F, -Br) exhibited more potent inhibitory activities than the thiazolidinones substituted with other groups. In addition, the activities of thiazolidinone derivatives substituted with chloro group at different positions on phenylamino-thiazol-4-one have different activities. The highest inhibitory activity obtained with derivative 5h, when derivative is substituted with 2-cholro phenyl group could form a more tight interaction with an enzyme.

From the Table 1, it was observed that with the increase of lipophilicity of the compound, the inhibitory activities increased gradually. These results showed that the inhibitory activities of 5-[3-(2-Chloro-phenyl)-1-phenyl-1H-pyrazol-4-ylmethylene]-2-(2,4-dimethoxy-phenylamino)-thiazol-4-one (5h), 5-[3-(3-Chloro-phenyl)-1-phenyl-1H-pyrazol-4-ylmethylene]-2-(2,4-dimethoxy-phenylamino)-thiazol-4-one (5g) analogues related to the lipophilicity showed the most potent inhibitory activity in all their homologues, and with the increase of the radius of halogen atom, the inhibitory activities decreased gradually. The

inhibitory activities of the halo substituted homologues were more potent than the inhibitory activities of other substituted thiazolidinones. This result might be related to different inhibitory mechanism. Since the chloro substituted thiazolidinones mainly depended on interaction with B site of Tyrosinase and may be due to the increase of the radius of halogen atom might cause stereohindrance for the inhibitors approaching the active site of the enzyme. However, substituted thiazolidinones with electron withdrawing groups are similar to benzaldehyde-type inhibitors, the Tyrosinase inhibitory mechanism of this type of inhibitors, come from the ability to form a Schiff base with a primary amino group in the enzyme.[26] The increase of lipophilicity might be benefited to the formation of Schiff base. As shown in Table 1, all of the thiazolidinone derivatives exhibited certain inhibitory activities against tyrosinase at the concentration of 200μM. The inhibitory activities of thiazolidinone scaffolds also be related to the structure of tyrosinase, which contained a type-B site which is other than a coupled binuclear copper active site in the catalytic core. Tyrosinase inhibition of compounds 5h, 5g and 5d depended on the competency of the chlorine atom that binds with B site, and tyrosinase would lose its catalysing ability after forming complex.[25]

Progress curve, a new simple graphical method described for the determination of inhibition type and inhibition constants of an enzyme reaction without any replot. It may be also helpful to detect the inhibition detection limit (IDL), which describes the limit of detection of competitive inhibition for end point assays as a function of the proportion of substrate converted into product, has been developed.[27] The progress curve obtained for oxidation of L-DOPA catalysed by mushroom Tyrosinase in presence of thiazolidinone scaffolds. The progress curve depicted, as the concentration of inhibitors raised, the residual enzyme activity drastically decreased showing inhibition of Tyrosinase (Fig.2). The enzyme activity inhibition was manifested by all inhibitors shown similar relationship between the enzyme activity and enzyme concentration. From the progress curve obtained, the compounds showing solid lines below the line of enzyme activity has indicative of enzyme inhibition and vice versa.

Inhibitory mechanism

The kinetic behaviors of the oxidation of L-tyrosine and L-DOPA, catalyzed by mushroom tyrosinase at different concentrations of compounds (5h, 5g, 5d and 5i) were studied. In this experiment, the initial velocity of the enzyme was monitored via dopachrome formation at 475 nm. As shown in Figure 1, the Lineweaver–Burk plots of 1/V versus 1/[S] result in a family of straight lines with the same y-axis intercept, as illustrated, respectively, for the four tyrosinase inhibitors. In the figure, the abscissa 1/[L-DOPA] is the reciprocal of the

concentrations of L-tyrosine, whereas the ordinate 1/V is the reciprocal of the change of the velocity, which reflects the reciprocal of tyrosinase activity. The arrangement of the family of lines in each graph indicated that these are competitive inhibitors [(1, Ki = 11.2 lM), (3, 0.77 lM), (4, 0.29 lM), and (5, 15.6 lM)], with their inhibitory activity toward tyrosinase decreasing with increasing concentration of the substrate. Most competitive inhibitors of tyrosinase have molecular structures which closely resemble that of the product of each respective step.

These results demonstrated that the inhibitory activity of compound 5h and 5g on the tyrosinase were reversible and competitive, suggesting that chlorophenyl substituted scaffolds more potent than inhibited the enzyme by binding to its binuclear active site reversibly. The dissociation constant value of L-DOPA binding with Tyrosinase was higher in presence of scaffold 5h and 5g, decreases in presence of other halide containing derivatives (5g, 5i, 5e and 5f). The present investigation reported that thiazolidinone scaffolds had potent inhibitory effects on the diphenolase activity of mushroom tyrosinase. Interestingly, compound 5h was found to be the most potent inhibitor with IC_{50} value of 34.12 µM. Preliminary structure activity relationships (SARs) analysis indicated that chlorophenyl moieties of 5h and 5g might play an important role in determining their inhibitory activities, (1) because of the chlorine atom might be binding with the B site other than the A active site, and Tyrosinase would lose its catalysing ability after such binding[25]; (2) carboxyl group might be effective group to the interaction of compound with the active site of tyrosinase.[22] The inhibition mechanism analysis of compounds 5h and 5g demonstrated that the inhibitory effects of the two compounds on the tyrosinase were reversible and competitive.

3. Conclusion

In the present study, a series of thiazolidinone scaffolds were synthesized and their inhibitory effects on mushroom Tyrosinase were evaluated. The results demonstrated that the analogues possessing halide substituents with phenylamino linker exhibited more potent inhibitory activities than the reference compound kojic acid. Analysis of the inhibition kinetics revealed that thiazolidinone scaffolds were reversible competitive inhibitors.

4. Experimental

4.1. General

Melting points were recorded in open capillaries with electrical melting point apparatus and were uncorrected. IR spectra (KBr disks) were recorded using a Perkin–Elmer 237 spectrophotometer. ^{1}H NMR (400 MHz) spectra were recorded on a Bruker Avance spectrometer using $CDCl_3$ as solvent. Mass spectra were recorded on a Shimadzu LCMSQP 1000 EX. All the reagents and solvents used were of analytical grade and were used as supplied unless otherwise stated. TLC was performed on silica gel coated plates for monitoring the reactions.

4.1.1. 2-chloro-N-(2,4-dimethoxy-phenyl)-acetamide (2)

Yield: 97 %.
MF/FWt: $C_{10}H_{12}ClNO_3$ /229
MP: 142 ^oC
IR cm $^{-1}$: 3408, 3105, 2964, 1668, 1543, 1496, 1282.
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.82 (d, 1H, *J*= 7.6 Hz), 6.61 (s, 1H), 6.52-6.50 (m, 1H), 4.21 (s, 2H), 3.87 (s, 1H), 3.86 (s, 3H), 3.79 (s, 3H).
MS: m/e 230 (M+1).

4.1.2. 2-(2,4-Dimethoxy-phenylamino)-thiazol-4-one (3)

Yield: 84 %.
MF/FWt: $C_{11}H_{12}N_2O_3S$ /252
MP: 168 ^oC
IR cm $^{-1}$: 3390, 3143, 3034, 1705, 1643.
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.15 (d, 1H, *J*= 8.8 Hz), 6.50-6.44 (m, 2H), 3.97 (s, 1H), 3.86 (s, 2H), 3.83 (s, 3H), 3.80 (s, 3H).
MS: m/e 253 (M+1).

4.1.3. 1-(1-methyl-buta-1,3-dienyl)-3-phenyl-1H-pyrazole-4-carbaldehyde[24] (4a-j)

4.1.4.1 2-(2,4-Dimethoxy-phenylamino)-5-methylene-thiazol-4-one (5a)

Yield: 72 %.

MF/FWt: $C_{27}H_{22}N_4O_3S$ /482

MP: 175 ^oC

IR cm $^{-1}$: 3120, 3053, 1710, 1666, 1340.

^1H NMR (400 MHz, $CDCl_3$): δ 8.06 (s, 1H), 7.90 (d, 2H, *J*= 8.3 Hz), 7.54 (d, 1H, *J*= 7.6 Hz), 7.66 (d, 2H, *J*=7.2 Hz), 7.51-7.46 (m, 2H), 7.43 (d, 2H, *J*= 6.8), 7.35 (t, 1H, *J*= 7.2 Hz), 7.26 (s, 1H), 7.09 (d, 1H, *J*= 8.3 Hz), 6.55 (t, 2H), 3.91 (s, 1H), 3.86 (s, 3H), 3.80 (s, 3H).

MS: m/e 483 (M+1).

4.1.4.2 2-(2,4-Dimethoxy-phenylamino)-5-(1-phenyl-3-p-tolyl-1H-pyrazol-4-ylmethyle- ne)-thiazol-4-one (5b)

Yield: 68 %.

MF/FWt: $C_{28}H_{24}N_4O_3S$ /496

MP: 175 ^oC

IR cm $^{-1}$: 3369, 3182, 3024, 1693, 1614.

^1H NMR (400 MHz, $CDCl_3$): δ 8.03 (s, 1H), 7.75 (d, 2H, *J*= 6.8 Hz), 7.73 (s, 1H), 7.54 (d, 2H, *J*= 7.6 Hz), 7.49-7.62 (m, 2H), 7.36 (s, 1H), 7.34-7.26 (m, 2H), 7.09 (d, 1H, *J*= 8.4 Hz), 6.99-6.52 (m, 2H), 3.91 (s, 1H), 3.86 (s, 3H), 3.83 (s, 3H), 2.42 (s, 3H).

MS: m/e 497 (M+1).

4.1.4.3 2-(2,4-Dimethoxy-phenylamino)-5-[3-(4-methoxy-phenyl)-1-phenyl-1H-pyrazol-4-ylmethylene]-thiazol-4-one (5c)

Yield: 65 %.

MF/FWt: $C_{28}H_{24}N_4O_4S$ /512

MP: 163 ^oC

IR cm $^{-1}$: 3369, 3076, 1666, 1610.

^1H NMR (400 MHz, $CDCl_3$): δ 8.02 (s, 1H), 7.87 (s, 1H), 7.73 (d, 1H, *J*= 8 Hz), 7.58 (d, 1H, *J*= 8 Hz), 7.49-7.46 (m, 2H), 7.31 (d, 2H, *J*= 6.8 Hz), 7.08 (d, 2H, *J*= 8.4 Hz), 7.01 (d, 1H, *J*= 8.4 Hz), 6.57-6.52 (m, 2H), 3.91 (s, 1H), 3.87 (s, 3H), 3.86 (s, 3H), 3.83 (s, 3H).

MS: m/e 513 (M+1).

4.1.4.4 5-[3-(4-Chloro-phenyl)-1-phenyl-1H-pyrazol-4-ylmethylene]-2-(2,4-dimethoxy-phenylamino)-thiazol-4-one (5d)

Yield: 68 %.

MF/FWt: $C_{27}H_{21}ClN_4O_3S$ /517

MP: 149 ^{o}C

IR cm $^{-1}$: 3383, 3120, 1708, 1668, 1346.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.09 (s, 1H), 7.92 (d, 1H, *J*= 7.2 Hz), 7.84 (d, 1H, *J*=7.2 Hz), 7.56-7.50 (m, 3H), 7.47 (s, 1H), 7.45-7.32 (m, 2H), 7.06 (d, 2H, *J*= 8 Hz), 6.87 (d, 1H, *J*= 6.8 Hz), 6.62 (s, 1H), 6.51-6.47 (m, 1H), 3.91 (s, 1H), 3.86 (s, 3H), 3.83 (s, 3H).

MS: m/e 518 (M+1).

4.1.4.5 5-[3-(4-bromo-phenyl)-1-phenyl-1H-pyrazol-4-ylmethylene]-2-(2,4-dimethoxy-phenylamino)-thiazol-4-one (5e)

Yield: 60 %.

MF/FWt: $C_{27}H_{21}BrN_4O_3S$ /560

MP: 167 ^{o}C

IR cm $^{-1}$: 3381, 3124, 1708, 1668, 134.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.39 (s, 1H), 7.92 (d, 1H, *J*= 7.2 Hz), 7.86 (d, 1H, *J*= 8.4 Hz), 7.58-7.51 (m, 3H), 7.48 (s, 1H), 7.40-7.31 (m, 2H), 7.09 (d, 2H, *J*= 7.6 Hz), 6.88 (d, 1H, *J*= 7.2 Hz), 6.60 (s, 1H), 6.50-6.48 (m, 1H), 3.91 (s, 1H), 3.88 (s, 3H), 3.83 (s, 3H).

MS: m/e 561 (M+1).

4.1.4.6 2-(2,4-Dimethoxy-phenylamino)-5-[3-(4-fluoro-phenyl)-1-phenyl-1H-pyrazol-4-ylmethylene]-thiazol-4-one (5f)

Yield: 53 %.

MF/FWt: $C_{27}H_{21}FN_4O_3S$ /500

MP: 167 ^{o}C

IR cm $^{-1}$: 3387, 3121, 1703, 1662.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.37 (s, 1H), 7.93 (d, 1H, *J*= 7.2 Hz), 7.87 (d, 1H, *J*= 7.2 Hz), 7.55-7.50 (m, 3H), 7.45 (s, 1H), 7.42-7.34 (m, 2H), 7.10 (d, 2H, *J*= 7.6 Hz), 6.87 (d, 1H, *J*= 6.8 Hz), 6.62 (s, 1H), 6.52-6.49 (m, 1H), 3.91 (s, 1H), 3.87 (s, 3H), 3.83 (s, 3H).

MS: m/e 501 (M+1).

4.1.4.7 5-[3-(3-Chloro-phenyl)-1-phenyl-1H-pyrazol-4-ylmethylene]-2-(2,4-dimethoxy-phenylamino)-thiazol-4-one (5g)

Yield: 50 %.

MF/FWt: $C_{27}H_{21}ClN_4O_3S$ /517

MP: 120 ^{o}C

IR cm $^{-1}$: 3380, 3051, 1710, 1655, 1317.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.04 (s, 1H), 7.86 (d, 1H, *J*= 7.2 Hz), 7.84 (d, 1H, *J*= 7.2 Hz), 7.55-7.53 (m, 3H), 7.45 (s, 1H), 7.37-7.33 (m, 2H), 7.11 (d, 2H, *J*= 7.6 Hz), 6.86 (d, 1H, *J*= 6.8 Hz), 6.63 (s, 1H), 6.50-6.45 (m, 1H), 3.91 (s, 1H), 3.86 (s, 3H), 3.82 (s, 3H).

MS: m/e 518 (M+1).

4.1.4.8 5-[3-(2-Chloro-phenyl)-1-phenyl-1H-pyrazol-4-ylmethylene]-2-(2,4-dimethoxy-phenylamino)-thiazol-4-one (5h)

Yield: 54 %.

MF/FWt: $C_{27}H_{21}ClN_4O_3S$ /517

MP: 155 ^{o}C

IR cm $^{-1}$: 3383, 3055, 1716, 1651, 1315.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.09 (s, 1H), 7.87 (d, 1H, *J*= 7.6 Hz), 7.84 (d, 1H, *J*= 7.8 Hz), 7.54-7.45 (m, 4H), 7.38-7.33 (m, 2H), 7.13 (d, 2H, *J*= 7.2 Hz), 6.85 (d, 1H, *J*= 7.6 Hz), 6.63 (s, 1H), 6.50-6.47 (m, 1H), 3.91 (s, 1H), 3.86 (s, 3H), 3.84 (s, 3H).

MS: m/e 518 (M+1).

4.1.4.9 5-[3-(2,4-Chloro-phenyl)-1-phenyl-1H-pyrazol-4-ylmethylene]-2-(2,4-dimethoxy-phenylamino)-thiazol-4-one (5i)

Yield: 54 %.

MF/FWt: $C_{27}H_{20}Cl_2N_4O_3S$ /551

MP: 169 ^{o}C

IR cm $^{-1}$: 3389, 3124, 1718, 1665.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.35 (s, 1H), 7.91 (d, 1H, *J*= 7.2 Hz), 7.89 (d, 1H, *J*= 7.6 Hz), 7.58-7.55 (m, 2H), 7.45 (s, 1H), 7.45-7.33 (m, 2H), 7.06 (m, 2H), 6.88 (m, 1H), 6.62 (s, 1H), 6.52-6.46 (m, 1H), 3.88 (s, 1H), 3.79 (s, 3H), 3.76 (s, 3H).

MS: m/e 552 (M+1).

4.1.4.10 2-(2,4-Dimethoxy-phenylamino)-5-[3-(2,4-dimethoxy-phenyl)-1-phenyl-1H-pyrazol-4-ylmethylene]-thiazol-4-one (5j)

Yield: 61 %.

MF/FWt: $C_{29}H_{26}N_4O_5S$ /542

MP: 177 ^{o}C

IR cm $^{-1}$: 3372, 3070, 1668, 1615.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.04 (s, 1H), 7.89 (d, 1H, *J*= 7.6 Hz), 7.85 (d, 1H, *J*= 6.8 Hz), 7.54-7.51 (m, 2H), 7.47 (s, 1H), 7.39-7.32 (m, 2H), 7.10 (d, 2H, *J*= 7.2 Hz), 6.86 (d, 1H, *J*= 7.6 Hz), 6.61 (s, 1H), 6.50-6.47 (m, 1H), 3.86 (s, 1H), 3.82 (s, 3H), 3.81 (s, 3H), 3.70 (s, 3H), 3.79 (s, 3H).

MS: m/e 543 (M+1).

4.2 Synthesis

4.2.1. General procedure for synthesis of 2-chloro-N-(2,4-dimethoxy-phenyl)-acetamide (2)

To a stirred solution of 2, 4-Dimethoxy-phenylamine (1.53 g, 10 mmol) **1** in DMF (15 mL), chloro-acetyl chloride (1.12 g, 10 mmol) was drop wise added under nitrogen and reaction mixture was stirred for 3h. at room temperature. After reaction was completed the contents were poured into ice-cold water. The solid mass was separated, filtered, washed with water and then purified by recrystalization from ethanol to get 2-chloro-N-(2,4-dimethoxy-phenyl)-acetamide **2**.

4.2.2. General procedure for synthesis of 2-(2,4-Dimethoxy-phenylamino)-thiazol-4-one (3)

To a stirred solution of 2-chloro-N-(2,4-dimethoxy-phenyl)-acetamide (2.29 g, 10 mmol) **2** in ethanol (15 mL), ammonium thiocyanate (0.76 g, 10 mmol) was added and reaction mixture was reflux for 1.5 h. After reaction was completed the contents were poured into ice-cold water. The solid mass was separated, filtered, washed with water and then purified by recrystallization from ethanol to get 2-(2,4-Dimethoxy-phenylamino)-thiazol-4-one **3.**

4.2.3. General procedure for synthesis of 1-(1-methyl-buta-1,3-dienyl)-3-phenyl-1H-pyrazole-4-carbaldehyde[24] (4a-j)

4.2.4. General procedure for synthesis of 2-(2,4-Dimethoxy-phenylamino)-5-methylene-thiazol-4-one (5a-j)

A well stirred solution of 2-(2,4-dimethoxy-phenylamino)-thiazol-4-one (0.504 g, 2 mmol) **3** in 17.5 mL of acetic acid was buffered with sodium acetate (4 mmol) and added with the 1-(1-methyl-buta-1,3-dienyl)-3-phenyl-1H-pyrazole-4-carbaldehyde (0.744 g, 3 mmol) **4a**. The solution was refluxed for 4 h and then poured into ice-cold water. The yellow

precipitate was filtered and washes with water, the resulting crud product was purified by recrystallization from acetic acid: DMF (2:1) to afford title compound **5a**.

4.3 Assay of tyrosinase activity

Mushroom tyrosinase (EC 1.14.18.1) (Sigma Chemical Co.) was used as described previously with some modifications, using either, L-DOPA (diphenolase) or L-tyrosine (monophenolase) as substrate[28]. Briefly, all the synthesised compounds were screened for the diphenolase inhibitory activity of tyrosinase using L-DOPA as substrate. All the compounds were dissolved in DMSO. The final concentration of DMSO in the test solution was 2.0%. Phosphate buffer, pH 6.8, was used to dilute the DMSO stock solution of test compounds. Thirty units of mushroom tyrosinase (0.5 mg/ml) were first pre-incubated with the compounds, in 50 mM phosphate buffer (pH 6.8), for 10 min at 25°C. Then the L-DOPA (0.5 mM) was added to the reaction mixture and the enzyme reaction was monitored by measuring the change in absorbance at 475 nm of formation of the DOPA chrome for 1 min.

The measurement was performed in triplicate for each concentration and averaged before further calculation. IC_{50} value, a concentration giving 50% inhibition of tyrosinase activity, was determined by interpolation of the dose–response curves. The percent of inhibition of tyrosinase reaction was calculated as following:

$$\text{Inhibition rate (\%)} = (B\text{-}S)/B \times 100$$

Here, the B and S are the absorbances for the blank and samples. Kojic acid was used as reference standard inhibitors for comparison.

4.4 Progress curves determination

All reactions were carried out using L-tyrosine as a substrate in 0.25 M phosphate buffer (pH 6.8) at 30°C. Enzyme activities were measured continuously for 15 min using a UV spectrophotometer. The kinetic parameters associated with time-dependent inhibition of tyrosinase, progress curves with 20 data points (30 s intervals) were obtained at different inhibitor concentrations using fixed substrate concentrations.

Acknowledgment

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References:

1. Song, K. K.; Huang, H.; Han, P.; Zhang, C. L.; Shi, Y.; Chen, Q. X. Biochem Biophys *Res Commun.* **2006,** *342*, 1147-1151
2. Chen, Q. X.; Liu, X. D.; Huang, H. *Biochemistry (Mosc.).* **2003**, *68*, 644-649
3. Fenoll, L. G.; Penalver, M. J.; Rodriguez-Lopez, J. N.; Varon, R.; Garcia-Canovas, F. J. *Int. J. Biochem. Cell Biol.* **2004**, *36*, 235
4. Lerch, K. *Life Chem. Rep.* **1987**, *5*, 221-234
5. Ito, S. *Pigment Cell Res.* **2003**, *16*, 230- 236
6. Martinez, M. V.; Whitaker, J. R. *Trends Food Sci. Tech.* **1995**, *6*, 195-200
7. Tripathi, R. K.; Hearing, V. J.; Urabe, K.; Aroca, P.; Spritz, R. A. *J. Biol. Chem.* **1992**, *267*, 23707-23712
8. Thanigaimalai, P.; Hoang, T. A. L.; Lee, S. C.; Sharma, V. K.; Yun, C. Y.; Roh, E. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2991-2993
9. Schallreuter, K. U.; Hasse, S.; Rokos, H.; Chavan, B.; Shalbaf, M.; Spencer, J. D. *Exp Dermatol.* **2009**, *18*, 680-688
10. Asanuma, A.; Miyazaki, I.; Ogawa, N. *Neurofox. Res.* **2003**, *5*, 165-176
11. Ashida, M.; Brey, P. T. *Proc Natl Acad Sci U S A.* **1995,** *92*, 10698-10702
12. Zhu, Y. J.; Zhou, H. T.; Hu, Y. H.; Tang, J. Y.; Su, M. X.; Guo, Y. J. *Food Chem.* **2011**, *124*, 298-302
13. Yi, W.; Cao, R.; Peng, W.; Wen, H.; Yan, Q.; Zhou, B. *Eur J Med Chem.* **2010**, *45*, 639-646
14. Kubo, I.; Nihei, K. I.; Shimizu, K. *Bioorg. Med. Chem. Lett.* **2004**, *12*, 5343
15. Matsuura, R.; Ukeda, H.; Sawamura, M. J. *Agric. Food Chem.* **2006**, *54*, 2309
16. Lesyk, R. B.; Zimenkovsky, B. *Curr. Org. Chem.* **2004**, *8*, 1547-1577
17. Verma, A.; Saraf, S. K. *Eur J Med Chem.* **2008**, *43*, 897-905

18. Prabhakar, Y. S.; Solomon, V. R.; Gupta, M. K.; Katti, S. B. *In Topics in Heterocyclic Chemistry;* Gupta, S. P., Ed.; Springer: Berlin, **2006**, *4*, 161–249

19. Solomon, V. R.; Haq, W.; Srivastava, K.; Puri, S. K.; Katti, S. B. *J. Med. Chem.* **2007**, *50*, 394–398

20. Bandgar, B. P.; Gawande, S. S.; Bodade, R. G.; Gawande, N. M.; Khobragade, C. N. *Bioorg. Med. Chem.* **2009**, *17*, 8168-8173

21. Bandgar, B. P.; Totre, J. V.; Gawande, S. S.; Khobragade, C. N.; Warangkar, S. C.; Kadam, P. D. *Bioorg. Med. Chem.* **2010**, *18*, 6149-6155

22. Bandgar, B. P.; Gawande, S. S.; Warangkar, S. C.; Totre, J. V. *Bioorg. Med. Chem.* **2010**, *18*, 3618-3624

23. Geronikaki, A.A.; Lagunin, A.A.; Hadjipavlou-Litina, D.I.; Eleftheriou, P.T.; Filimonov, D.A.; Poroikov, V.V.; Alam, I.; Saxena, A.K. *J. Med. Chem.* **2008**, *51*, 1601–1609

24. Kira, M. A.; Abdel-Raeman, M. O.; Gadalla, K. Z. *Tetrahedron Lett.* **1969**, *2*, 109.

25. Lim, G.G.; Imura, Y.; Yoshimura, E. *Protein J.* **2012**, *31*, 609-614.

26. Kubo, I.; Kinst-Hori, I.; Nihei, K.; Soria, F.; Takasaki, M.; Calderón, J. S. *J Agric Food Chem.* **1999**, *47*, 4574-4578

27. Yoshino, M.; Murakami, K. *J Enzyme Inhib Med Chem.* **2009,** *24*, 288-290.

28. Masamoto, Y.; Iida, S.; Kubo, M. *Planta Med.* **1980**, *40*, 361.

List of captions:

Scheme I. Synthesis of 2-(2,4-Dimethoxy-phenylamino)-5-methylene-thiazol-4-one (5a-j)

Figure 1. Lineweaver–Burk plots for the inhibition of the diphenolase activity of tyrosinase by compounds (5h and 5g). (A) Concentrations of compound 5h for curves were 0, 10, 20, and 30 μM; (B) compound 5g for curves were 0, 20, 30 and 40 μM; respectively. The final enzyme concentration was 2.0 μg/ml. The data shown in the fig is mean of three different experiments.

Figure 2. Progress curve for oxidation of L-DOPA catalyzed by mushroom tyrosinase in the presence of thiazolidinone scaffolds (5h > 5g> 5d > 5e > 5f> 5c> 5i > 5b). Data shown in the figure is mean of three different experiments.

Table 1. Tyrosinase inhibitory activities of different thiazolidinone scaffolds

Table 1. Tyrosinase inhibitory activities of different thiazolidinone scaffolds

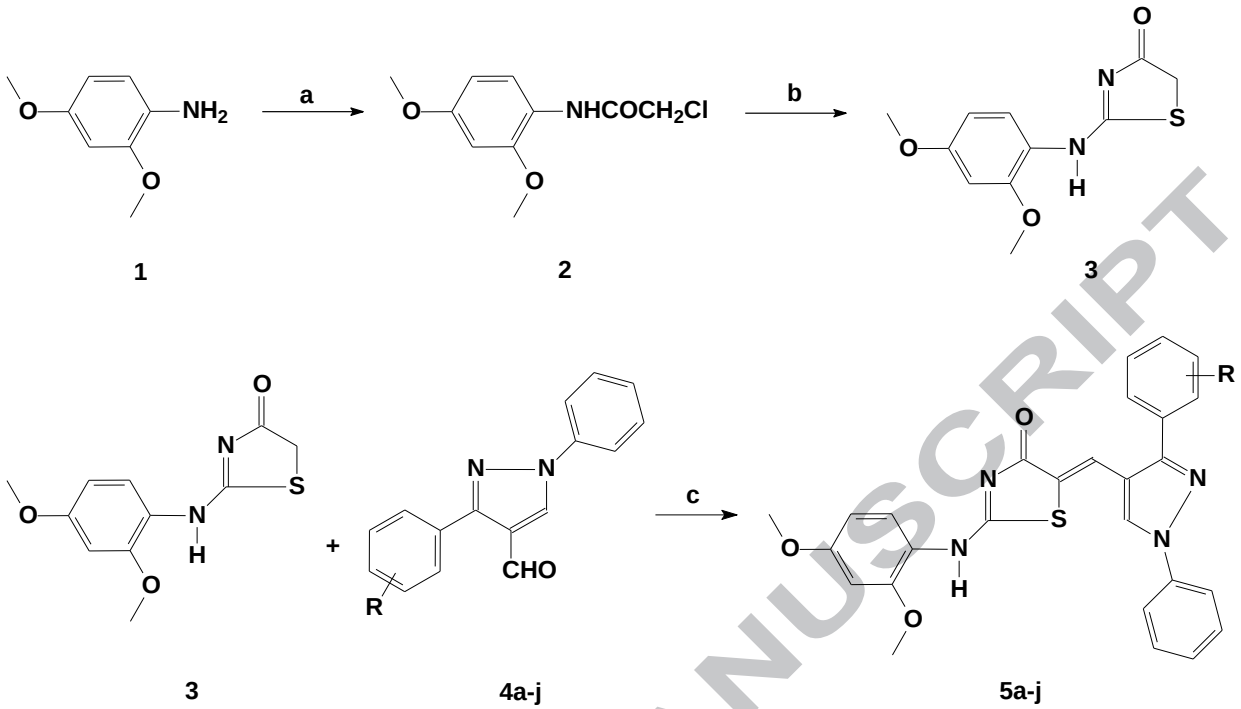
Compounds	CLog P[c]	IC_{50} (µM) [a]	Percent of inhibition[b]	Type of Inhibition Ki (µM)
5a	6.307	---	8.03	---
5b	6.806	194.02	13.5	---
5c	6.28147	114.05	46.8	---
5d	7.03856	74.02	37.97	Competitive 5.70
5e	7.18856	131.08	87.92	competitive 9.46
5f	6.46856	104.32	54.23	competitive 7.18
5g	7.03856	52.62	109.8	competitive 21.52
5h	6.78856	34.12	126.04	competitive 29.08
5i	7.50384	81.68	52.03	competitive 16.92
5j	5.77757	---	--	
Kojic acid		22.5	--	competitive 14.86

a Values were determined from logarithmic concentration–inhibition curves and are given as means of three experiments.

b Percent of inhibition of tyrosinase reaction at the 200 µM.

c Value of CLog P was obtained by Chem BioDraw Ultra 12.0.

Data shown in the table is mean of three different experiments



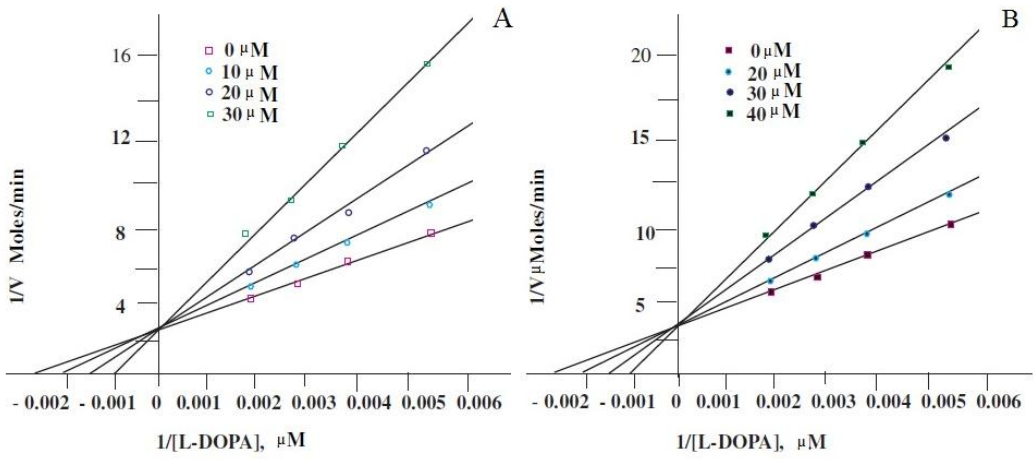
R= H, 4-CH_3, 4-OCH_3, 4-Cl, 4-Br, 4-F, 3-Cl, 2-Cl, 2,4-Cl, 2,4-OCH_3

Scheme-I

Reagent and conditions: a. $ClCOCH_2Cl$, N,N-DMF, r.t., 2 h.

b. NH_4SCN, ethanol, reflux, 2h.

c. CH_3COONa, CH_3COOH, reflux, 2-4 h.



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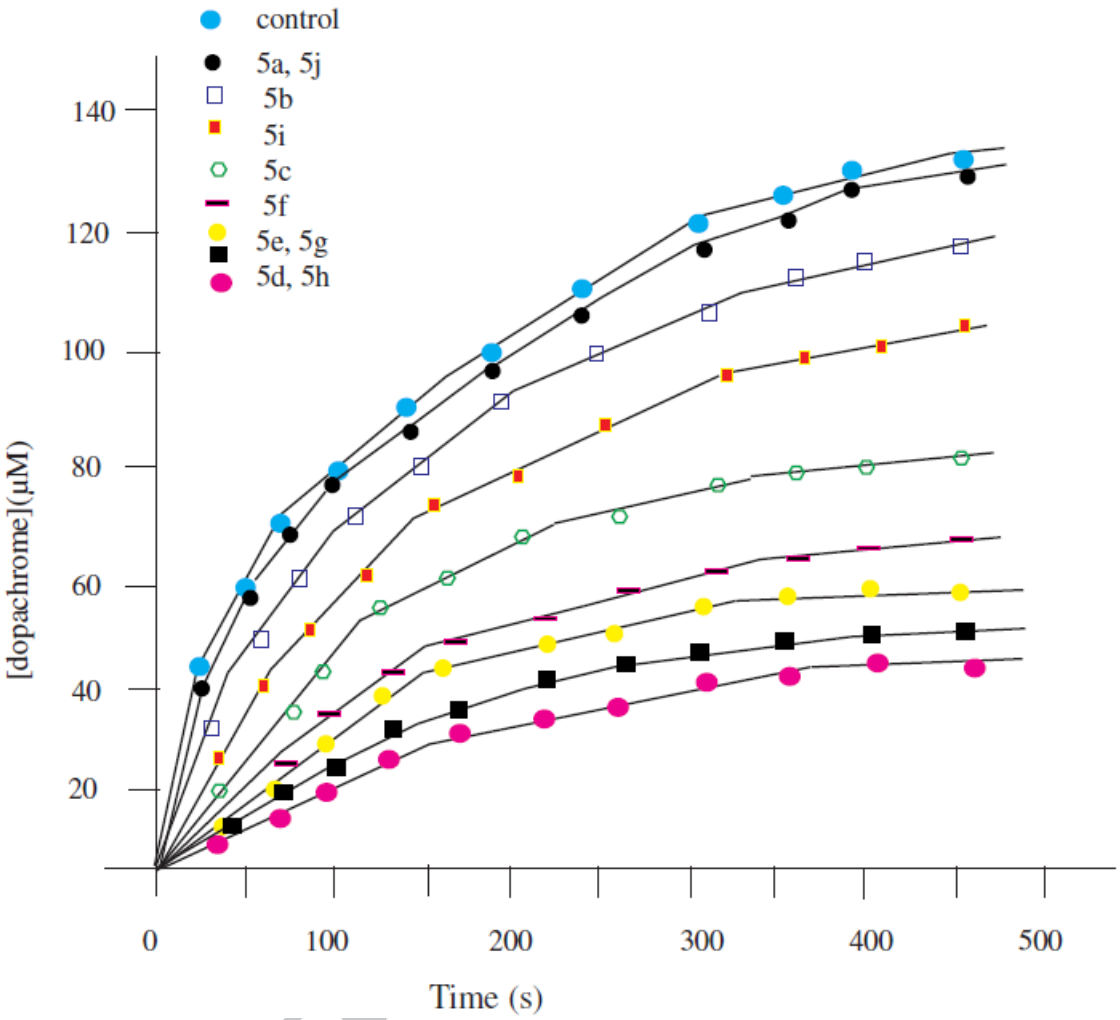

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Synthesis of new heterocyclic hybrids based on pyrazole and thiazolidinone scaffolds as potent inhibitors of tyrosinase

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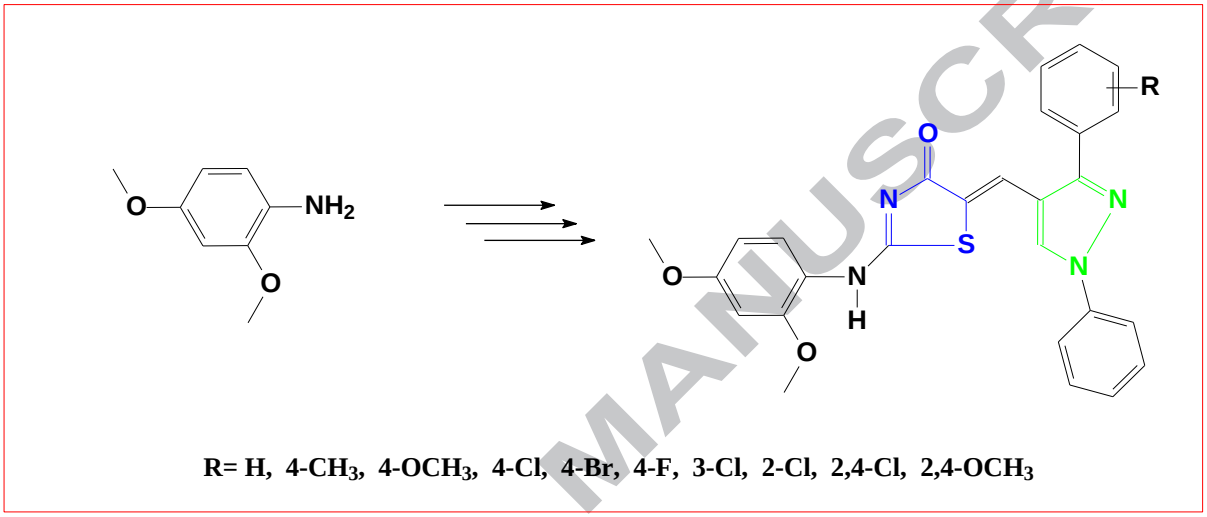

R= H, 4-CH_3, 4-OCH_3, 4-Cl, 4-Br, 4-F, 3-Cl, 2-Cl, 2,4-Cl, 2,4-OCH_3