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Synthesis, biological evaluation and molecular docking analysis of novel benzopyrimidinone derivatives as potential anti-tyrosinase agents

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ABSTRACT

2-substitued-benzopyrimidinones **2** were synthesized in high to excellent yields in a single step *via* condensation of 2-aminobenzamide **1** with some aryl-aldehydes in the presence of iodine. Cyclocondensation reaction of hydrazides **3** which were obtained in two steps from benzopyrimidinones **2**, with some electrophilic species such as 2,4-pentandione, 2,5-hexandione, 1-phenylbutan-1,3-dione and cyclic anyhdrides provided the new compounds **4a–c**, **5a–c**, **6a–c**, **7a–c**, **8a–c** and **9a–c**. The synthesized compounds were characterized by spectroscopic means. They were also evaluated for their anti-tyrosinase potential. The structure-activity relationship (SAR) was discussed on the basis of the molecular docking analysis.

1. Introduction

Tyrosinase is a copper containing oxidase that widely exists in microorganisms, animals and plants and is responsible for biosynthesis of melanin pigment in skin, hair and eyes by catalyzing the oxidation of phenol to o-quinone [1–3].

In addition, enzymatic browning in plant-derived food product is an undesirable reaction that is responsible for discoloration of fresh fruits, vegetables, mushrooms and loss in nutritional quality [4].

On another side, human skin color is mainly determined by the production of the dark color pigment melanin, which has an important photoprotective function against ultraviolet (UV) inducing skin damage [5–7], present in the basal layer of the epidermis [8].

Note that, the production of an abnormal amount of melanin pigmentation in different specific parts of the skin may cause aesthetic problem in humans and hyperpigmentary disorders, such as, postinflammatory, hyperpigmentation, maturational dyschromia, periorbital hyperpigmentation, melasma age spots, freckles and melanoma [9,10]. Recently, unregulated tyrosine action has also been reported to be linked with Parkinson's and other neurodegenerative diseases [11–13].

In this context, the development of tyrosinase inhibitors has promising potential in the agricultural sector for insecticides and anti-browning of vegetables and fruits [14–16], medicinal for treatment of hyper-pigmentation disorders and cosmetic for whitening agents products [17–18].

So, the search for tyrosinase inhibitors is a current research topic in the context of preventing hyper-pigmentation and browning effect. Many natural and synthetic inhibitor compounds have already been reported in the literature. Thus, kojic acid, kojic acid octanoates, salicylhydroxamic acid, catechins, hydroquinone, resveratrol and oxyresveratrol representing building blocks of natural products, were also described for their tyrosinase inhibition properties [19–21]. Some other known pyrimidine-fused tyrosinase inhibitors such as 6-methyl-3-phenethyl-3,4-dihydro-1*H*-benzopyrimidin-2-thione (Fig. 1A) [22], 2-(4-Fluorophenyl)-benzopyrimidinone (Fig. 1-B) [23] and pyranotriazolopyrimidines (Fig. 1C) [24] have been found to be promising depigmenting agents.

In the other hand, pyrazole and their derivatives received great attention due to their biological activities as anticancer and anti-5-lipoxygenase [25], antiviral agents [26], antimalarial agents [27], potent carbonic anhydrase and acetylcholinesterase inhibitors [28].

In addition, some of them have shown good anti-tyrosinase activity (Fig. 2A) [29]. Furthermore, some previous studies haves reported that pyrrole pharmacophore possess various biological activities such as antitumor [30], cytotoxic [31], anticancer [32] and anti-tyrosinase activities (Fig. 2B) [33]. Also pyrrolidinedione derivatives are core

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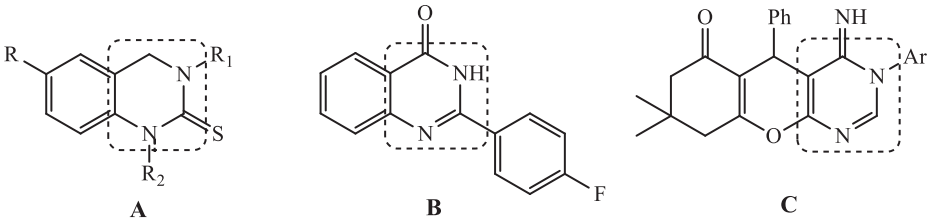


Fig. 1. Previously reported Pyrimidine derivatives as anti-tyrosinase agents.

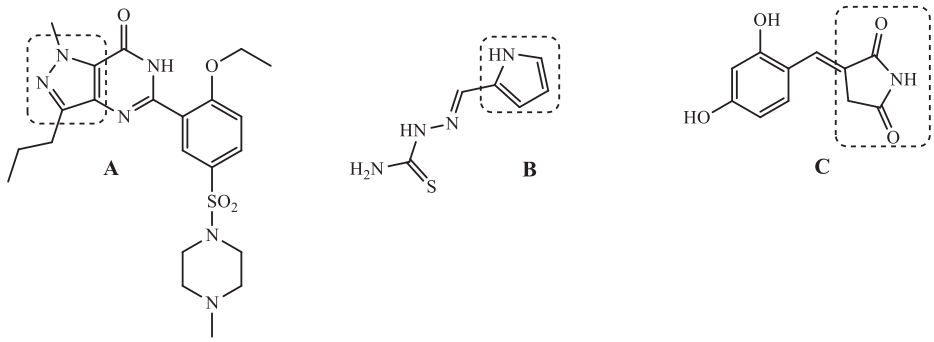


Fig. 2. Examples of pyrrole, pyrazole and pyrrolidinedione-fused anti-tyrosinase agents.

structural units found in natural products and have been described as antibiotics with an unknown mode of action [34–35]. Thus some of them have shown antimicrobial [36] and anti-tyrosinase properties (Fig. 2C) [37].

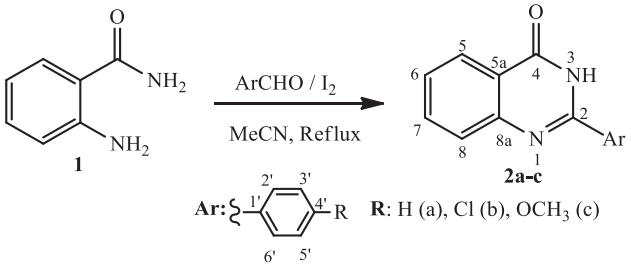
Encouraged by these findings, in the present work, we have designed and synthesized some new compounds through combination of benzopyrimidinone moiety with different pharmacophores such as pyrrole, pyrazole and pyrrolidinedione. Hereby we are reporting for the first time the anti-tyrosinase activities of the newly synthesized compounds. Furthermore, the structure-activity relationship (SAR) was supported by the molecular docking analysis.

2. Results and discussion

2.1. Chemistry

According to the procedure adopted by Rahmouni et al. [25], we reported the preparation of key intermediate benzopyrimidinone **2** through a condensation of stoichiometric mixture of 2-aminobenzamide **1** and aryl-aldehyde in acetonitrile under reflux for 24 h in the presence of equimolar amount of iodine (Scheme 1). The structures of these compounds were confirmed according to their spectral data. In fact the ^{1}H NMR spectra of compounds **2** showed, in addition to the signals corresponding to the aromatic protons introduced by the 2-aminobenzamide moiety and the aryl-aldehyde, the presence of the singlet related to the NH proton at δ_H 12.26–12.53. Moreover, the ^{13}C NMR spectral data of these compounds confirm the proposed structures.

Our approach to the target molecules **4–9** firstly started by the construction of the hydrazide skeleton type **3**, *via* condensation reaction



Scheme 1. Synthetic pathway to benzopyrimidinones **2a–c.**

of benzopyrimidinones **2** with ethyl chloroacetate to give the corresponding esters, which were treated by hydrazine hydrate in ethanol at room temperature. Benzopyrimidine hydrazides **3a-c** were prepared in good

yields (58–72%) (Scheme 2).

The ^{1}H NMR spectra of compounds **3** showed essentially the presence of a singlet relative to the methylene group ($H_{1''}$) at δ_H 5.11–5.14 and the appearance of new signals, attributable to the NH and NH_2 protons at δ_H 9.48–9.50 and 3.65–4.32, respectively.

The ^{13}C NMR spectra of these compounds showed essentially the appearance of a new signal at δ_C 64.1–64.3 and 166.1–166 correlates to the methylene carbons $C_{1''}$ and the carbonyl function $C_{2''}$ respectively.

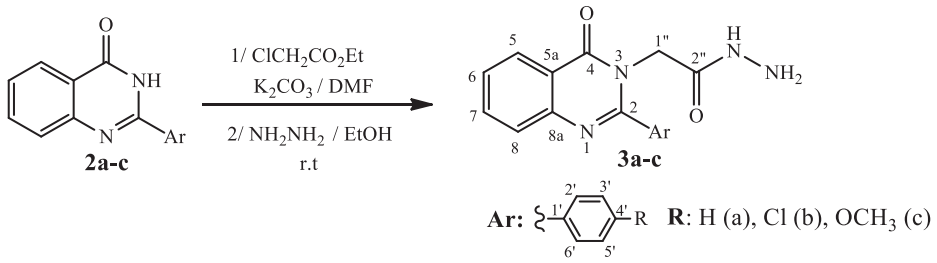
Hydrazides of type **3** represent an essential intermediate for the formation of several heterocyclic compounds [38–40] known for their high reactivity and their utility as building blocks. This has aroused our interest for their use to synthesize the desired compounds **4–9**.

Firstly, the reaction of hydrazides **3a–c** with hexane-2,5-dione in refluxing dioxane in the presence of a catalytic amount of acetic acid afforded the corresponding pyrrole **4a–c** derivatives (Scheme 3). The formed new compounds **4** were characterized by their ^{1}H and ^{13}C. The ^{1}H NMR spectra of compounds **4a–c** showed, in addition to the signals relative to protons introduced by the benzopyrimidinone moiety, the presence of a singlet at δ_H 8.47–11.07 due to the NH group and two signals at δ_H 1.89–2.00 and at δ_H 5.40–5.75 relative to methyl groups and ethylene protons respectively, for which chemical shifts and multiplicities were in good agreement with the proposed structures.

Similarly, condensation of precursors **3** with pentane-2,4-dione or 1-phenyl-1,3-butanedione in refluxing dioxane for 8 h in the presence a catalytic amount of acetic acid gave the new hydroxylated pyrazole derivatives **5–6** (Scheme 4).

The structures of these compounds have been identified by their spectroscopic data. For example, the ^{1}H NMR spectra of compounds **5** showed essentially the appearance of a new signal corresponding to the OH proton at δ_H 4.34–4.45 and two doublets (J = 18.6 Hz) at δ_H 2.79–2.86 and 2.86–3.11 relative to the CH_2 group ($H_{4'''}$) and the disappearance of the signals relative to the NH and NH_2 protons in compounds **3** at δ_H 9.48–950 and 3.65–4.32, respectively. The ^{13}C NMR spectra of the same compounds showed essentially the appearance of new signals relative to carbons $C_{4'''}$ and $C_{5'''}$ at δ_C 63.3–63.9 and 91.4–91.9 respectively.

The hydroxylated pyrazole formation could be explained by the



Scheme 2. Synthetic pathway to benzopyrimidine hydrazides **3a–c.**

stability induced by the formation of a six-center pseudocycle *via* the hydrogen bond (Scheme 4).

Finally, the hydrazides **3** were further reacted with a series of cyclic anhydrides (phthalic, naphthalic or tricyclic anhydrides) under refluxing dioxane in the presence of a catalytic amount of acetic acid for 8 h to give compounds **7–9** in good yields (Scheme 5).

The structures of all the new compounds **7**, **8** and **9** have been assigned from their spectral data ^{1}H NMR and ^{13}C NMR.

On the other hand, the high resolution mass spectra (ESI-HRMS) of all the synthesized compounds showed the correct protonated molecular ion peaks $[M+H]^{+}$, which were compatible with the desired structures.

2.2. Biological activity

2.2.1. Anti-tyrosinase activity

All the synthesized compounds have been evaluated for their *in vitro* anti-tyrosinase activity.

According to the IC_{50} values given in Table 1, most synthesized compounds showed interesting anti-tyrosinase potential (IC_{50} = 46.54 ± 1.46–15.5 ± 0.82 μM) compared to kojic acid used as a positive control (13.75 ± 0.68 μM). The benzopyrimidinone **2a** with unsubstituted phenyl did not exhibit any activity against tyrosinase. In contrary, compounds **2b and 2c** with a substituted phenyl (4-ClPh, 4-OCH_3Ph, respectively) showed interesting inhibitory effect of tyrosinase. For the hydrazide compounds **3a-c**, only compound **3b** with a 4-Cl-Ph was active with an IC_{50} value of 20.10 ± 1.00 μM. It is clear that the chlorine atom and its electronic effect was in favor of the high activity of compound **3b**.

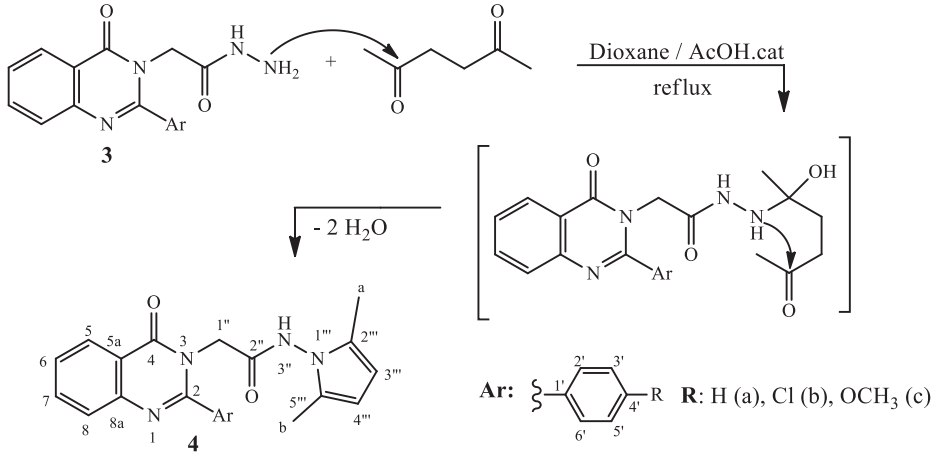
On the other hand, only compound **4a** displayed a modest activity with an IC_{50} value of 46.54 ± 1.46 μM. This finding shows that the copresence of the pyrrole and the phenyl groups in the molecule (**4a**) is in favor of the anti-tyrosinase activity. The inactivity of compound **4b** compared to its bioactive hydrazide **3b** clearly shows the importance of the nature of the substituent attached to the 4-position of the phenyl groups (4-Cl-C_6H_4) and the conversion of hydrazide into pyrrole ring. On the other hand, the prepared pyrazole derivatives **5a,c** exhibited anti-tyrosinase capacity with IC_{50} values of 15.5 ± 0.82 μM and 23.90 ± 1.08 μM, respectively, except **5b** (4-Cl-Ph) which is found to be inactive. As shown in Table 1, compound **5a** exhibits an anti-tyrosinase potential comparable to that of kojic acid (IC_{50} = 15.50 ± 0.82 μM). In this series, the unsubstituted aromatic ring (case of **5a**) was in favor of this activity.

The compounds **6a–c** were found to be inactive towards tyrosinase enzyme compared to their analogues **5a–c**. This results show clearly the importance of the nature of the group attached at C-5″ (CH_3 in **5** and Ph in **6**) to have this activity. However the presence of the methyl group in **5a–c** is in favor of their anti-tyrosinase activity. The steric hindrance of the phenyl group in compounds **6** could possibly explain the loss of this activity compared to the methyl group in their analogues **5**. However, the activity of compounds **7c** (IC_{50} = 23.18 ± 0.77 μM), **8c** (IC_{50} = 31.77 ± 1.88 μM) and **9c** (IC_{50} = 27.90 ± 1.14 μM) could be explained by the co-existence of the 4-OCH_3-Ph group and the polycyclic moiety introduced by the cyclic anhydrides used. The nature of the substituent in the aromatic ring introduced by the hydrazide (**3**) seems to have an influence on the activity of this new series of compounds. However, the presence of the 4-OCH_3 as a substituent in the aromatic ring of these derivatives (**7c**, **8c** and **9c**) characterized by its donor mesomeric effect (+M) may explain this difference of activity between all these analogues.

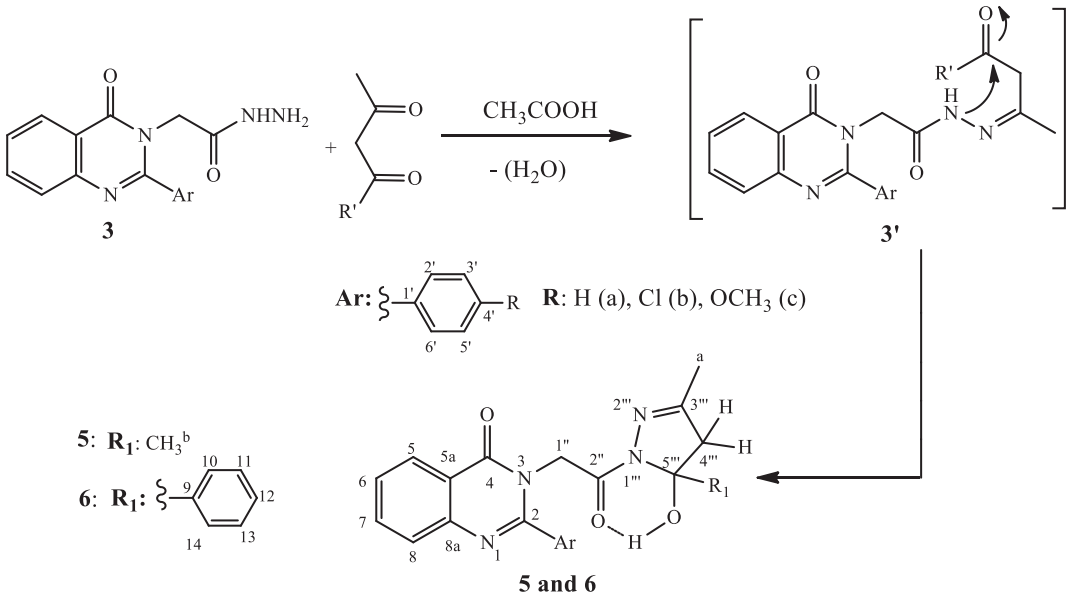
2.2.2. Molecular docking studies

Tyrosinase crystal structure is composed of tetramer subunits chain-A, chain-B, chain- C and chain-D respectively with the sequence length of 391 amino acids. It is a binuclear a the copper-containing enzyme that catalyzes the conversion of a monophenol (tyrosine) and /or o-diphenol (L-DOPA) in its corresponding o-quinone derivative [41].

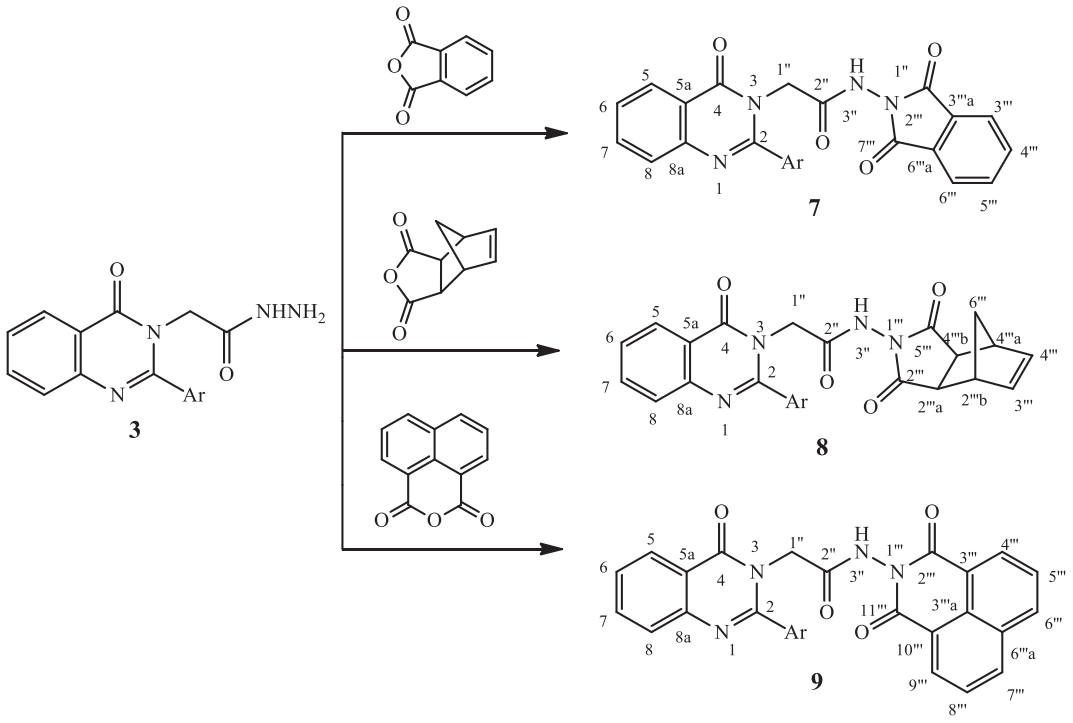
In-depth docking analysis has been performed to elucidate the interactions of the active antityrosinase agents **2c**, **3b**, **4a**, **5a** and **7c** at the tropolone binding domain of binuclear copper-containing an



Scheme 3. Synthetic route of pyrrole derivatives **4a–c.**



Scheme 4. Synthetic pathway to pyrazole derivatives **5a-c** and **6a–c**.



Scheme 5. Synthetic pathway to compounds **7a–c**, **8a–c** and **9a–c.**

enzyme (PDB: 2Y9X) by using Autodock 4.2 (Table 2). The majority of anti-tyrosinase agents reported thus far, resembles the binding interaction of tropolone within the hydrophobic pocket (Fig. 3).

2.2.2.1. Docking interactions for the most active anti-tyrosinase agents. **Docking interactions for 2c:** Quinazolin-4(3*H*)-one pharmacophore is involved in the Pi-lone pair (green color) and Pi-alkyl interactions (pink color) with the carbonyl group of HIS-A-94 and VAL-A-299 respectively. The 2-(4-methoxyphenyl) group is responsible for the unfavorable bumps (red color) with GLU-A-98 and HIS-A-296 in that phenyl ring and 4-methoxy group is involved in the Pi-alkyl, Pi-sigma and carbon-carbon binding interactions with PRO-A-91, LEU-A-303, VAL-A-299 respectively (Fig. 4).

Docking interactions for 7c: 4-oxoquinazolin-3(4*H*)-yl pharmacophore is involved in Pi-alkyl interactions (pink color) with TRP-A-101 and TRP-A-293. In addition to this some metal-acceptor (grey color) and carbon-carbon, interactions (light green color) have been observed in between the carbonyl functional group of the ring and TYR-A-97, GLU-A-98. The 2-(4-methoxyphenyl) ring is involved in Pi-cation interactions (golden color) with TRP-A-293, PHE-A-292, HIS-A-295, and HIS-A-296. Some unfavorable bumps (red color) have been observed with 4-methoxy group and HIS-A-295, HIS-A-263 and MET-A-280. In addition to this, the amide functional group is involved in the conventional hydrogen binding interactions with HIS-A-94 (green color). The *N*-(1,3-dioxoisoindolin-2-yl) ring is involved in Pi-cation (HIS-A-94, ARG-A-95), Pi-sigma (ASP-A-300, VAL-A-299, ARG-A-95), and Pi-

Table 1
Anti-tyrosinase activity of compounds **2a–c**, **3a–c**, **4a–c**, **5a–c**, **6a–c**, **7a–c**, **8a–c** and **9a–e**.

Product	IC_{50} (μM)[a]
2a	na[b]
2b	26.84 ± 1.05
2c	25.00 ± 1.20
3a	na
3b	20.10 ± 1.00
3c	na
4a	46.54 ± 1.46
4b	na
4c	na
5a	15.5 ± 0.82
5b	na
5c	23.90 ± 1.08
6a	na
6b	na
6c	na
7a	na
7b	na
7c	23.18 ± 0.77
8a	na
8b	na
8c	31.77 ± 1.88
9a	na
9b	na
9c	27.90 ± 1.14
Kojic acid[c]	13.75 ± 0.68

[a] The concentration at which 50% of tyrosinase is inhibited (mean ± SD, n = 3).
[b] non active.
[c] positive control.

Table 2
Binding energies of promising anti-tyrosinase agents.

Binding energy (kcal/mol)						
Tropolone	−5.33	−5.22	−5.18	−5.18	−4.95	−5.01
4a	−7.20	−7.12	−7.09	−7.09	−7.04	−6.93
5a	−7.45	− 7.28	−7.21	−7.19	−7.09	−7.00
3b	−7.12	−7.20	−7.00	−6.50	−6.20	− 6.12
7c	−6.37	−6.16	−6.04	−5.91	−5.41	−5.37
2c	−6.18	−6.05	−5.91	−5.63	−5.30	−5.26

Alkyl interactions (LEU-A303 and VAL-A-299) respectively (Fig. 4).

Docking interactions for 3b: 4-oxoquinazolin-3(4*H*)-yl pharmacophore is involved in the Pi-Alkyl interactions with PRO-A-91, LEU-A-255, LEU-A-303 and some Pi-sigma interactions with VAL-A-299 and PRO-A-91 respectively. The 2-(2-(4-chlorophenyl) ring is involved in the Pi-Alkyl interactions with TRP-A-293. In addition to this acetohydrazide functional group is involved in the conventional hydrogen binding interactions with TYR-A-365, ARG-A-20, CYS-A-297, HIS-A-296, GLU-A-98, TRP-A-293, and HIS-A-296, respectively (Fig. 4).

Docking interactions for 5a: 2-phenylquinazolin-4(3*H*)-one ring is involved in the Pi-Alkyl interactions with LEU-A-255 and some conventional hydrogen bonding interactions were observed with HIS-A-296. The Phenyl ring is involved in Pi-Alkyl, Pi-lone pair interactions with ASP-A-300 and in Pi-anion interactions with ARG-A-95. In addition to this 3-(2-(5-hydroxy-3,5-dimethyl-4,5-dihydro-1*H*-pyrazol-1-yl group is involved in conventional hydrogen binding, Pi-Alkyl, and Pi-sigma interactions with HIS-A-94, with TYR-A-97, TRP-A-101, and HIS-A-296, respectively (Fig. 4).

3. Conclusion

In this research work, we did designed and synthesized some new molecules with the combination of benzopyrimidinones with pyrrole, pyrazole and pyrrolidinedione **4–9** *via* the cyclocondensation reaction of hydrazides **3**, previously prepared from 2-aminobenzamide **1** in three steps, with some electrophilic entities like diketones and three cyclic anyhydrides. The anti-tyrosinase activity of the newly prepared compounds **4–9** was evaluated. Most of the tested compounds showed interesting anti-tyrosinase activity and the structure-activity relationship was explained by the molecular docking analysis. Compounds **2b**, **2c**, **3b**, **4a**, **5a**, **5c**, **7c**, **8c** and **9c** were found to be the most anti-tyrosinase agents. It has been found that the compound **5a** displayed the highest activity with an IC_{50} value of 13.75 ± 0.68 μM, which was comparable to kojic acid used as positive control. The results of Molecular docking indicates that compound **5a** showed a good fit within the pocket site of the protein molecular surface and had a minimum binding energy of −7.45 kcal/mol in comparison with the tropolone. Hereby in conclusion the anti-tyrosinase activity of most tested compounds depends of the nature and the electronic effect of the substituents attached to the aromatic ring and the pyrrole or pyrazole moiety of compounds **4** and **5**.

4. Experimental section

4.1. General information

All reactions were monitored by TLC using aluminium sheets of Merck silica gel 60 F254, 0.2 mm. Melting temperatures were determined on an electrothermal 9002 apparatus and were reported uncorrected. NMR spectra were recorded on a Bruker AC-300 spectrometer at 300 MHz (^{1}H) and 75 MHz (^{13}C). All chemical shifts were reported as δ values (ppm) relative to residual non deuterated solvent. The anti-tyrosinase activity was measured using a UV–Visible ELISA spectro-photometric reader (Type of microplate: 96 and 384 wells, Wavelength range: 200–1000 nm, Selection of the wavelength: monochromator, Spectrum scanning speed < 10 s from 200 to 1000 nm, Incubation: from room temperature (25 °C) + 2 °C to 45 °C.

4.1.1. General procedure for the synthesis of 2-aryl-benzopyrimidinone *2*

To a mixture of 2-aminobenzamide **1** (5 mmol) and various arylaldehydes (5 mmol) in dry acetonitrile (50 mL), molecular iodine (5 mmol) was added. After the reaction was completed, the mixture was cooled to room temperature. A solution of sodium thiosulphate (5%) was added and the resulted solid was filtered off and washed with water. The crude product was recrystallized from ethanol.

*4.1.1.1. 2-phenylbenzopyrimidin-4(3H)-one **2a**.* White solid, yield: 55%. mp: 250–252 °C. ES-HRMS $[M+H]^+$ calcd. for $(C_{14}H_{11}N_2O)^+$: 223.0871, found: 223.0880. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 7.56 (m, 4H, H_{arom}), 7.74 (d, 1H, H_8, J = 7.5 Hz), 7.82 (td, 1H, H_7, J = 8.1 Hz, J = 1.5 Hz), 8.18 (m, 3H, H_{arom}), 12.47 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 121.9, 125.8, 126.4, 127.3, 127.7, 128.5, 131.2, 132.7, 134.4, 148.6, 152.3, 162.2.

*4.1.1.2. 2-(4-chlorophenyl)benzopyrimidin-4(3H)-one **2b**.* White solid, Yield: 68%. mp > 300 °C. ES-HRMS $[M+H]^+$ calcd. for $(C_{14}H_{10}ClN_2O)^+$: 257.0482, found: 257.0491. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 7.73 (m, 5H, H_{arom}), 8.19 (d, 3H, H_{arom}, J = 6.6 Hz), 12.53 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 121.0, 125.8, 126.6, 127.3, 128.6, 129.5, 131.6, 134.5, 148.5, 151.4, 162.2.

*4.1.1.3. 2-(4-methoxyphenyl) benzopyrimidin-4(3H)-one **2c**.* White solid, Yield: 60%. mp: 240–242 °C. ES-HRMS $[M+H]^+$ calcd. for $(C_{15}H_{13}N_2O_2)^+$: 253.0977, found: 253.0981. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 3.77 (s, 3H, OCH_3), 6.99 (d, 2H, $H_{3',5'}$, J = 8.7 Hz), 7.39 (t, 1H, H_6, J = 7.8 Hz), 7.6 (d, 1H, H_8, J = 8.1 Hz), 7.72 (td, 1H,H_7, J = 8.1 Hz, J = 1.2 Hz), 8.04 (dd, 1H, H_5, J = 8.1 Hz, J = 0.9 Hz), 8.10 (d, 2H, $H_{2',6'}$, J = 8.7 Hz), 12.26 (s, 1H, NH). ^{13}C NMR

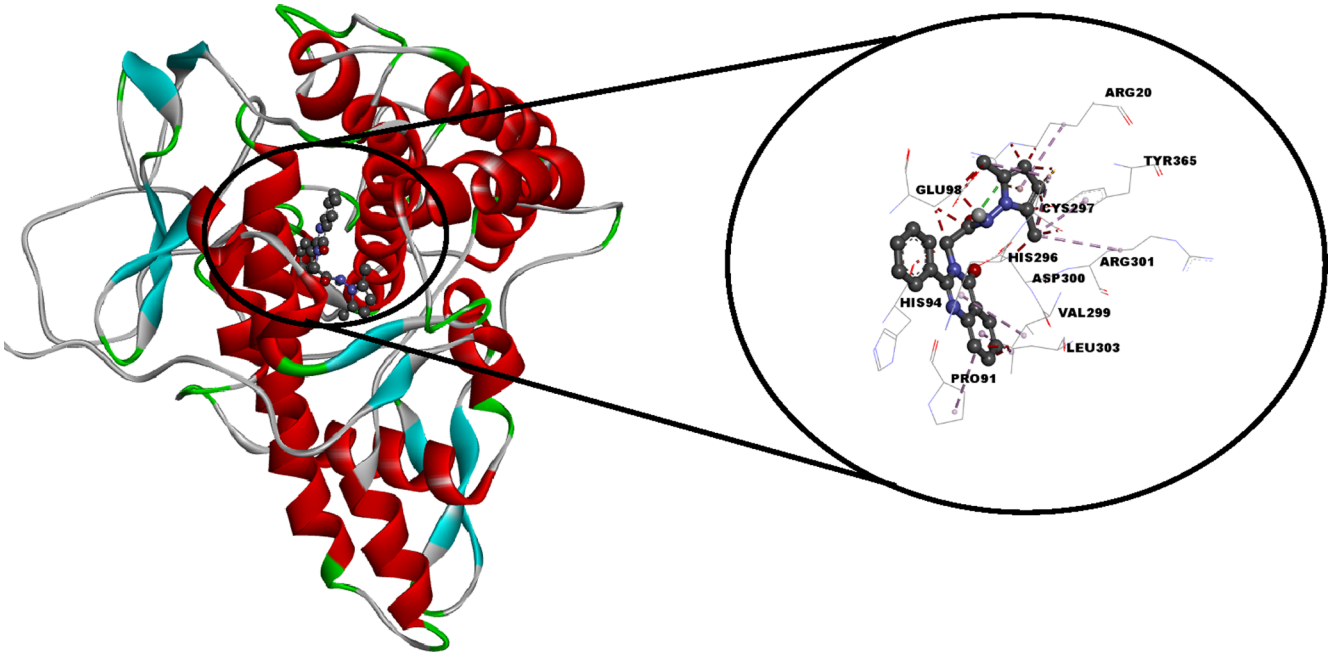


Fig. 3. Binding site of Conjugate **4a**with in tropolone hydrophobic cavity of PDB:2Y9X, 2D-interactions with HIS-A-296 and ARG-A-20 (conventional hydrogen bond interactions in green color), hydrophobic cavity composed of surrounding aminoacids sequence LEU-A-303, PRO-A-91, VAL-A-299, CYS-A-297, ARG-A-301 (alkyl interactions) and Pi-Alkyl bonds with TYR-A-365 and ARG-A-301, respectively.

(75 MHz, DMSO-d_6): δ (ppm) = 55.4, 113.9, 120.6, 124.8, 125.7, 126.0, 127.1, 129.4, 134.4, 148.8, 151.9, 161.8, 162.3.

*4.1.2. General procedure for preparation of hydrazides **3***

Equimolar solution 1 mmol of 2-arylbenzopyrimidinones **2**, anhydrous potassium carbonate and ethyl chloroacetate was refluxed in dry DMF (40 mL) with continuous stirring for 6 h. The reaction mixture was allowed to cool and then it was poured in water and extracted using EtOAC. The organic layer was dried over anhydrous $MgSO_4$. After evaporation of the solvent, the residual was treated with an excess of hydrazine hydrate in ethanol at room temperature for 1 h. The precipitated solid formed was filtered, washed with ethanol, and dried to obtain compounds **3a–c**.

*4.1.2.1. 2-(4-oxo-2-phenylbenzopyrimidin-3(4H)-yl)acetohydrazide **3a***. Yellow solid, Yield: 58%. mp: 240–242 °C. ES-HRMS $[M+H]^+$ calcd. for $(C_{16}H_{15}N_4O_2)^+$: 295.1195, found: 259.1204. ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm) = 3.47 (s, 2H, NH_2), 5.14 (s, 2H,$H_{1''}$), 7,53 (m, 3H, H_{arom}), 7.65 (m, 1H, H_6), 7.94 (m, 2H, $H_{7,8}$), 8.28 (d, 1H, H_5, J = 8.1 Hz), 8.47 (m, 2H, H_{arom}), 9.5 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ (ppm) = 64.2, 114.4, 123.7, 127.0, 127.4, 128.0, 128.5, 130.7, 134.3, 137.1, 151.3, 158.6, 165.7, 166.2.

*4.1.2.2. 2-(4-oxo-2-(4-chlorophenyl)benzopyrimidin-3(4H)-yl) acetohydrazide **3b***. Yellow solid, Yield: 64%. mp: 240–242 °C. ES-HRMS $[M+H]^+$ calcd. for $(C_{16}H_{14}ClN_4O_2)^+$: 329.0805, found: 329.0814. ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm) = 4.32 (s, 2H, NH_2), 5.12 (s, 2H, $H_{1''}$), 7.55 (d, 2H, $H_{2',6'}$, J = 8.7 Hz), 7.64 (m, 1H, H_6), 7.95 (m, 2H, $H_{7,8}$), 8.29 (d, 1H, H_5, J = 8.1 Hz), 8.46 (d, 2H, $H_{3',5'}$, J = 8.7 Hz), 9.48 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ (ppm) = 64.3, 114.4, 123.8, 127.2, 127.4, 128.6, 129.8, 134.4, 135.7, 136.0, 151.2, 157.6, 165.8, 166.1.

*4.1.2.3. 2-(2-(4-methoxyphenyl)-4-oxo benzopyrimidin −3(4H)-yl) acetohydrazide **3c***. Yellow solid, Yield: 72%. mp: 192–194 °C. ES-HRMS $[M+H]^+$ calcd. for $(C_{17}H_{17}N_4O_3)^+$: 325.1301, found: 325.1310. ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm) = 3.83 (s, 3H, OCH_3), 4.34 (s, 2H, NH_2), 5.52 (s, 2H, $H_{1''}$), 7.04 (d, 2H, $H_{3',5'}$, J = 8.1 Hz), 7.59 (m, 1H, H_6), 7.90 (m, 2H, $H_{7,8}$), 8.24 (d, 1H, H_5, J = 7.8 Hz), 8.41 (d, 2H, $H_{2',6'}$, J = 8.1 Hz), 9.48 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ (ppm) = 55.2, 64.1, 113.8, 114.0, 123.7, 126.5, 127.2, 129.6, 129.8, 134.3, 151.4, 158.5, 161.5, 165.4, 166.3.

*4.1.3. General procedure for preparation of compounds **4***

A mixture of appropriate hydrazide **3** (1 mmol) and 2,5-hexanedione (1 mmol) was stirred in reflux of dry dioxane in the presence of catalytic amount of acetic acid (0.001 mmol, 0.06 mL). After 8 h, the reaction mixture was cooled to room temperature and the dioxane was removed in vacuo and the residue was purified by silica gel chromatography (petroleum ether/ ethyl acetate, 70:30) to give the compounds **4**.

*4.1.3.1. N-(2,5-dimethyl-1H-pyrrol-1-yl)-2-(4-oxo-2-phenylbenzopyrimidin-3(4H)-yl)acetamide **4a***. Light brown solid, Yield: 51%. m.p: 252–254 °C. ES-HRMS $[M+H]^+$ calcd. for $(C_{22}H_{20}N_4O_2)^+$: 373.1670, found: 373.1679. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 1.89 (s, 6H, $2CH_3$), 5.42 (s, 2H, $H_{1''}$), 5.59 (s, 2H, $H_{3''',4'''}$), 7.54 (s, 3H, H_{arom}), 7.70 (m, 1H, H_6), 8.00 (s, 2H, $H_{7,8}$), 8.34 (d, 1H, H_5, J = 8.1 Hz), 8.56 (s, 2H, H_{arom}), 11.07 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 10.8, 64.1, 103.0, 114.4, 123.8, 126.9, 127.1, 127.5, 128.1, 128.4, 130.9, 134.4, 136.9, 151.4, 158.5, 165.7, 166.8.

*4.1.3.2. 2-(2-(4-chlorophenyl)-4-benzopyrimidin-3(4H)-yl)-N-(2,5-dimethyl-1H-pyrrol-1-yl)acetamide **4b***. Light brown, Yield: 58%. mp: 264–266 °C. ES-HRMS $[M+H]^+$ calcd. for $(C_{22}H_{19}ClN_4O_2)^+$: 407.1275, found: 407.1284. ^{1}H NMR (300 MHz,$CDCl_3$): δ (ppm) = 1.98 (s, 6H, $2CH_3$), 5.40 (s, 2H, $H_{1''}$), 5.74 (s, 2H, $H_{3''',4'''}$), 7.44 (d, 2H, $H_{2',6'}$, J = 8.1 Hz), 7.60 (t, 1H, H_6, J = 7.5 Hz); 7.90 (t, 1H, H_7, J = 7.8 Hz), 8.03 (d, 1H, H_8, J = 8.4 Hz), 8.17 (d, 1H, H_5, J = 7.8 Hz), 8.41 (s, 1H, NH), 8.48 (d, 2H, $H_{3',5'}$, J = 8.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 10.5, 64.5, 103.9, 113.8, 122.3, 126.8, 127.2, 127.9, 128.2, 129.3, 133.9, 135.0, 136.8, 151.8, 158.1, 164.7, 166.1.

*4.1.3.3. N-(2,5-dimethyl-1H-pyrrol-1-yl)-2-(2-(4-methoxyphenyl)-4-oxobenzopyrimidin-3(4H)-yl)acetamide **4c***. Light brown, Yield: 68%.

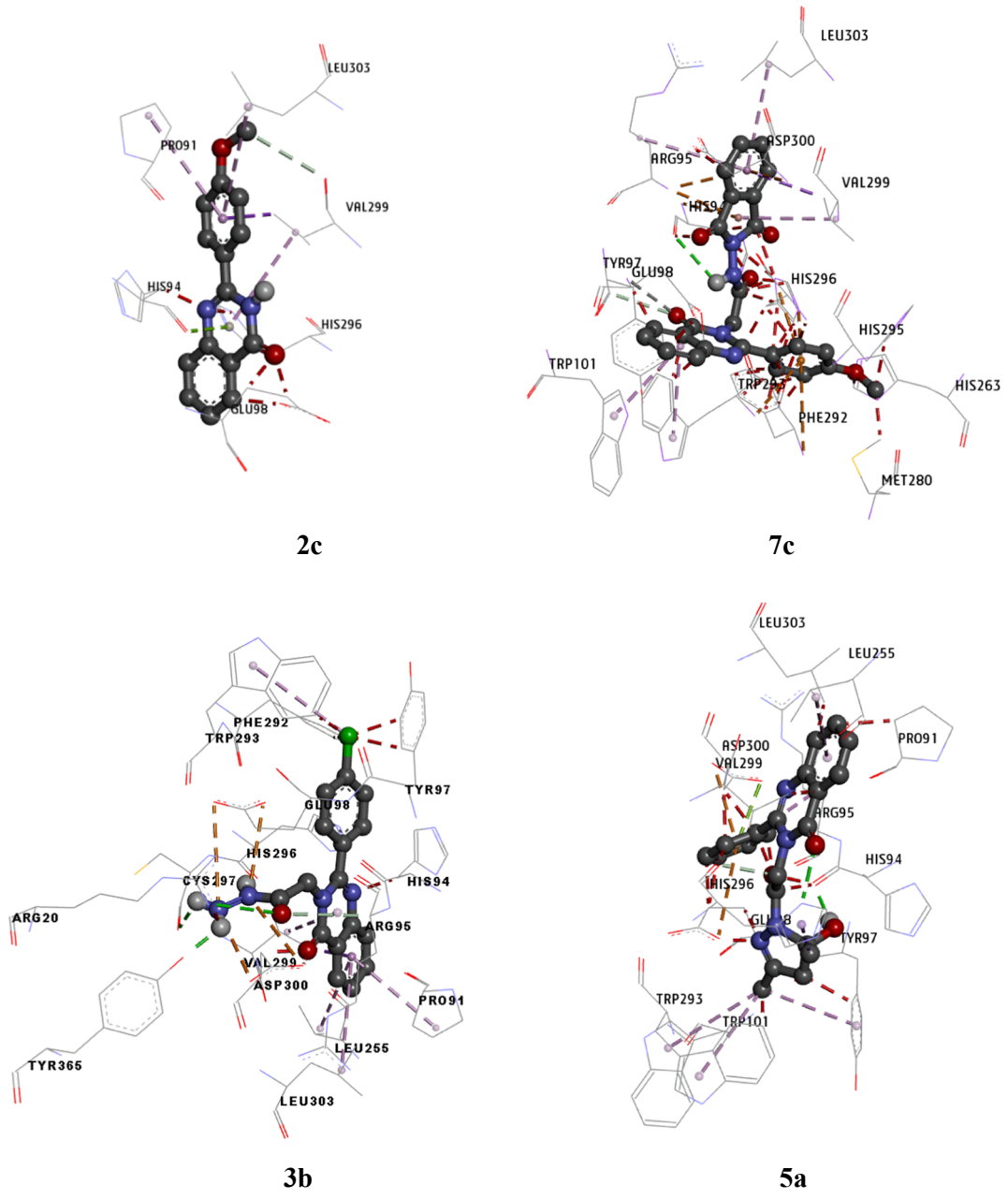


Fig. 4. Binding site of conjugate **2c**, **7c**, **3b** and **5a** (most effective anti-tyrosinase agent) in the tropolone hydrophobic cavity of PDB: 2Y9X.

mp: 220–222 °C. ES-HRMS $[M+H]^+$ calcd. for $(C_{23}H_{22}N_4O_3)^+$: 403.1792, found: 403.1801. ^1H NMR (300 MHz,$CDCl_3$): δ (ppm) = 2.00 (s, 6H, $2CH_3$), 3.89 (s, 3H, OCH_3), 5.41 (s, 2H, $H_{1''}$), 5.75 (s, 2H, $H_{3'',4''}$), 6.99 (d, 2H, $H_{3',5'}$, J = 8.7 Hz), 7.55 (t, 1H, H_6, J = 7.5 Hz.), 7.87 (t, 1H, H_7, J = 7.5 Hz), 8.00 (d, 1H, H_8, J = 8.4 Hz), 8.15 (d, 1H, H_5, J = 8.1 Hz), 8.47 (s, 1H, NH), 8.53 (d, 2H, $H_{2',6'}$, J = 8.7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 10.5, 54.8, 64.5, 103.9, 113.4, 113.5, 122.2, 126.1, 127.2, 127.7, 129.2, 129.7, 133.7, 152.0, 158.9, 161.7, 164.5, 166.3.

4.1.4. General procedure for the synthesis of compounds 5 and 6

A mixture of appropriate hydrazide **3** (1 mmol) and 2,4-pentanedione (1 mmol) or 1-phenyl-1,3-butanedione (1 mmol) was stirred in reflux of dry dioxane in the presence of catalytic amount of acetic acid (0.001 mmol, 0.06 mL). After 8 h, the reaction mixture was cooled to room temperature and the dioxane was removed *in vacuo* and the residue was purified by silica gel chromatography (petroleum ether / ethyl acetate, 60:40) to yield compounds **5** or compounds **6**.

4.1.4.1. 3-(2-(5-hydroxy-3,5-dimethyl-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl)-2-phenylbenzopyrimidin-4(3H)-one ***5a***. White solid, Yield: 76%. mp: 178–180 °C. ES-HRMS $[M + H]^+$ calcd. for $(C_{21}H_{20}N_4O_3)^+$: 377.1641, found: 377.1653. ^1H NMR (300 MHz,$CDCl_3$): δ (ppm) = 1.84 (s, 3H, $CH_3^{(a)}$), 2.13 (s, 3H, $CH_3^{(b)}$), 2.80 (d, 1H, $H_{4''}$, J = 18.3 Hz), 3.05 (d, 1H, $H_{4''}$, J = 18.3 Hz), 4.34 (s, 1H, OH), 5.47 (d, 1H, $H_{1''}$, J = 15.6 Hz), 5.61 (d, 1H, $H_{1''}$, J = 15.6 Hz), 7.44 (m, 3H, H_{arom}), 7.54 (td, 1H, H_6, J = 8.1 Hz, J = 1.2 Hz), 7.83 (td, 1H, H_7, J = 8.4 Hz, J = 1.5 Hz), 7.99 (d, 1H, H_8, J = 8.4 Hz), 8.30 (d, 1H, H_5, J = 8.1 Hz), 8.50 (m, 2H, H_{arom}). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 16.1, 26.9, 51.2, 63.8, 91.9, 115.0, 123.6, 126.5, 127.9, 128.2, 128.5, 130.3, 133.6, 138.0, 152.2, 155.6, 159.6, 166.1, 166.6.

4.1.4.2. 3-(2-(5-hydroxy-3,5-dimethyl-4,5-dihydro-1H-pyrazol-1-yl)-2oxoethyl)-2(4-chlorophenyl)benzopyrimidin-4(3H)-one ***5b***. White solid,

Yield: 58%. mp: 96–98 °C. ES-HRMS $[M+H]^+$ calcd. for $(C_{21}H_{19}ClN_4O_3)^+$: 411.1224, found: 411.1236. ^1H NMR (300 MHz,$CDCl_3$): δ (ppm) = 1.82 (s, 3H, $CH_3^{(a)}$), 2.11 (s, 3H, $CH_3^{(b)}$), 2.79 (d, 1H, $H_{4''}$, J = 18.6 Hz), 2.85 (d, 2H, $H_{4''}$, J = 18.6 Hz), 5.46 (d, 1H, $H_{1''}$, J = 15.6 Hz), 5.55 (d, 1H, $H_{1''}$, J = 15.6 Hz), 7.40 (d, 2H, $H_{2',6'}$, J = 9.0 Hz), 7.54 (td, 1H, H_6, J = 8.1 Hz, J = 1.2 Hz), 7.83 (td, 1H, H_7, J = 8.4 Hz, J = 1.2 Hz), 7.98 (d, 1H, H_8, J = 8.4 Hz), 8.27 (dd, 1H, H_5, J = 8.4 Hz, J = 1.2 Hz), 8.42 (d, 2H, $H_{3',5'}$, J = 9.0 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 16.1, 26.9, 51.2, 63.9, 91.8, 114.9, 123.7, 126.7, 127.9, 128.4, 129.8, 130.1, 133.7, 136.5, 152.1, 155.8, 158.5, 166.1, 166.5.

4.1.4.3. 3-(2-(5-hydroxy-3,5-dimethyl-4,5-dihydro-1H-pyrazol-1-yl)-2oxoethyl)-2(4-methoxyphenyl)benzopyrimidin-4(3H)-one ***5c***. White solid, Yield: 66%. mp: 110–112 °C. ES-HRMS $[M+H]^+$ calcd. for $(C_{22}H_{22}N_4O_4)^+$: 407.1730, found: 407.1742. ^1H NMR (300 MHz,$CDCl_3$): δ (ppm) = 1.84 (s, 3H, $CH_3^{(a)}$), 2.11 (s, 3H, $CH_3^{(b)}$), 2.85 (d, 1H, $H_{4''}$, J = 18.6 Hz), 3.03 (d, 1H, $H_{4''}$, J = 18.6 Hz), 3.87 (s, 3H, OCH_3), 4.45 (s, 1H, OH), 5.46 (d, 1H, $H_{1''}$, J = 15.6 Hz), 5.58 (d, 1H, $H_{1''}$, J = 15.6 Hz), 6.95 (d, 2H, $H_{3',5'}$, J = 6.9 Hz), 7.49 (t, 1H, H_6, J = 7.2 Hz), 7.80 (td, 1H, H_7, J = 8.4 Hz, J = 1.2 Hz), 7.97 (d, 1H, H_8, J = 8.4 Hz), 8.29 (d, 1H, H_5, J = 7.5 Hz), 8.48 (d, 2H, $H_{2',6'}$, J = 6.9 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 15.6, 26.4, 50.7, 54.8, 63.3, 91.3, 113.1, 114.2, 123.1, 125.5, 127.1, 129.6, 130.0, 133.0, 151.8, 155.1, 158.9, 161.2, 165.4, 166.1.

4.1.4.4. 3-(2-(5-hydroxy-3-methyl-5-phenyl-4, 5-dihydro-1H-pyrazol-1-yl)-2oxoethyl)-2 -phenylbenzopyrimidin-4(3H)-one ***6a***. Yellow solid, Yield: 65%. mp: 191–193 °C. ES-HRMS $[M+H]^+$ calcd. for $(C_{26}H_{22}N_4O_3)^+$: 439.1770, found: 439.1782. ^1H NMR (300 MHz,$CDCl_3$): δ (ppm) = 2,15 (s, 3H, $CH_3^{(a)}$), 2.95 (d, 1H, $H_{4''}$, J = 18.6 Hz), 3.29 (d, 1H, $H_{4''}$, J = 18.6 Hz), 5.48 (d, 1H, $H_{1''}$, J = 15.6 Hz), 5.88 (d, 1H, $H_{1''}$, J = 15.6 Hz), 7.04 (m, 3H, H_{arom}), 7.34 (d, 2H, H_{arom}, J = 7.0 Hz), 7.51 (m, 4H, H_{arom}), 7.79 (t, 1H, H_7, J = 8.4 Hz), 7.96 (d, 1H, H_8, J = 8.4 Hz), 8.23 (d, 1H, H_5, J = 8.1 Hz), 8.56 (m, 2H, H_{arom}). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 15.5, 53.5, 63.3, 93.4, 114.5, 123.0, 123.5, 126.0, 127.3, 127.5, 127.8, 128.1, 128.1, 129.8, 133.1, 137.6, 142.2, 151.6, 155.1, 159.1, 165.6, 165.8.

4.1.4.5. 3-(2-(5-hydroxy-3-methyl-5-phenyl-4,5-dihydro-1H-pyrazol-1-yl)-2oxoethyl)-2-(4-chlorophenyl)benzopyrimidin-4(3H)-one ***6b***. Yellow solid, Yield: 52%. mp: 186–188 °C. ES-HRMS $[M+H]^+$ calcd. for $(C_{26}H_{21}ClN_4O_3)^+$: 473.1380, found: 473.1392. ^1H NMR (300 MHz,$CDCl_3$): δ (ppm) = 2.10 (s, 3H, $CH_3^{(a)}$), 2.89 (d, 1H, $H_{4''}$, J = 18.9 Hz), 3.23 (d, 1H, $H_{4''}$, J = 18.9 Hz), 5.43 (d, 1H, $H_{1''}$, J = 15.6 Hz), 5.74 (d, 1H, $H_{1''}$, J = 15.6 Hz), 7.05 (m, 3H, H_{arom}), 7.28 (d, 2H, H_{arom}, J = 6.9 Hz), 7.43 (m, 3H, H_{arom}), 7.74 (td, 1H, H_7, J = 8.4 Hz, J = 1.5 Hz), 7.90 (d, 1H, H_8, J = 8.4 Hz), 8.19 (dd, 1H, H_5, J = 8.1 Hz, J = 0.9 Hz), 8.46 (d, 2H, H_{arom}, J = 8.7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 16.0, 53.9, 63.8, 93.9, 114.9, 123.6, 123.9, 126.7, 127.8, 128.1, 128.5, 128.6, 129.9, 133.7, 136.5, 136.6, 142.7, 152.0, 155.6, 158.5, 166.1, 166.3.

4.1.4.6. 3-(2-(5-hydroxy-3-methyl-5-phenyl-4, 5-dihydro-1H-pyrazol-1-yl)-2oxoethyl)-2-(4-methoxyphenyl)benzopyrimidin-4(3H)-one ***6c***. Yellow solid, Yield: 58%. mp: 160–162 °C. ES-HRMS $[M+H]^+$ calcd. for $(C_{27}H_{24}N_4O_4)^+$: 469.1876, found: 469.1888. ^1H NMR (300 MHz,$CDCl_3$): δ (ppm) = 2.16 (s, 3H, $CH_3^{(a)}$), 2.95 (d, 1H, $H_{4''}$, J = 18.9 Hz), 3.29 (d, 1H, $H_{4''}$, J = 18.9 Hz), 3.90 (s, 3H, OCH_3), 5.48 (d, 1H, $H_{1''}$, J = 15.6 Hz), 5.84 (d, 1H, $H_{1''}$, J = 15.6 Hz), 7.01 (d, 2H, $H_{3',5'}$, J = 9.0 Hz), 7.10 (m, 3H, H_{arom}), 7.37 (d, 2H, H_{arom}, J = 8.1 Hz), 7.46 (t, 1H, H_6, J = 7.8 Hz), 7.77 (td, 1H, H_7, J = 8.1 Hz, J = 1.2 Hz), 7.92 (d, 1H, H_8, J = 8.4 Hz), 8.22 (d, 1H, H_5, J = 8.1 Hz), 8.52 (d, 2H, $H_{2',6'}$, J = 9.0 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 15.5, 53.4, 54.8, 63.2, 93.4, 113.1, 114.2, 123.1, 123.5, 125.5, 127.1, 127.6, 128.1, 129.7, 130.3, 133.0, 142.2, 151.7, 155.0, 158.9, 161.2, 165.4, 165.9.

4.1.5. General procedure for preparation of compounds ***7–9(a-c)***

A mixture of appropriate hydrazide **3** (1 mmol) and cyclic anhydride (1 mmol) was stirred in reflux of dry dioxane in the presence of catalytic amount of acetic acid (0.001 mmol, 0.06 mL). After 8 h, the reaction mixture was cooled to room temperature and the dioxane was removed in vacuo and the residue was purified by silica gel chromatography (petroleum ether / ethyl acetate, 60:40) to yield compounds **7–9**.

4.1.5.1. N-(1,3-dioxoisoindolin-2-yl)-2-(4-oxo-2-phenylbenzopyrimidin-3(4H)-yl)acetamide ***7a***. White solid, Yield: 57%. mp: 232–243 °C. ES-HRMS $[M+H]^+$ calcd. for $(C_{24}H_{16}N_4O_4)^+$: 425.1249, found: 425.1261. ^1H NMR (300 MHz, DMSO-d_6): δ (ppm) = 5.49 (s, 2H, $H_{1''}$), 7.54 (m, 3H,H_{arom}), 7.70 ((t, 1H, H_6, J = 8.1 Hz), 7.97 (m, 6H, H_{arom}), 8.32 (d, 1H, H_5, J = 8.1 Hz), 8.60 (m, 2H, H_{arom}), 11.20 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 63.6, 114.2, 123.5, 123.7, 127.1, 127.5, 128.4, 128.5, 129.7, 130.4, 134.4, 135.2, 137.0, 151.4, 158.8, 164.8, 166.7, 168.3.

4.1.5.2. 2-(2-(4-chlorophenyl)-4-oxobenzopyrimidin-3(4H)-yl)-N-(1,3-dioxoisoindolin-2-yl)acetamide ***7b***. White solid, Yield: 55%. mp: > 300 °C. ES-HRMS $[M+H]^+$ calcd. for $(C_{24}H_{15}ClN_4O_4)^+$: 459.0860, found: 459.0872. ^1H NMR (300 MHz, DMSO-d_6): δ (ppm) = 5.39 (s, 2H, $H_{1''}$), 7.56 (d, 2H, $H_{2',6'}$, J = 8,4 Hz), 7.63 (s, 1H, H_8), 7.88 (m, 6H, H_{arom}), 8.26 (d, 1H, H_5, J = 8.1 Hz), 8.55 (d, 1H, $H_{3',5'}$, J = 8.4 Hz), 11.10 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 68.5, 119.0, 128.3, 128.6, 132.2, 132.3, 133.3, 134.1, 134.9, 139.4, 140.5, 141.6, 133.4, 156.0, 162.5, 169.6, 170.3, 171.5.

4.1.5.3. N-(1,3-dioxoisoindolin-2-yl)-2-(2-(4-methoxyphenyl)-4-oxobenzopyrimidin-3(4H)-yl)acetamide ***7c***. White solid, Yield: 73%. mp: 232–234 °C. ES-HRMS $[M+H]^+$ calcd. for $(C_{25}H_{18}N_4O_5)^+$: 455.1355, found: 455.1367. ^1H NMR (300 MHz, DMSO-d_6): δ (ppm) = 3.88 (s, 3H, OCH_3), 5.46 (s, 2H, $H_{1''}$), 7.10 (d, 2H, $H_{3',5'}$, J = 7.8 Hz), 7.65 (s, 1H, H_8), 7.96 (m, 6H, H_{arom}), 8.28 (d, 1H, H_5, J = 7.5 Hz), 8.54 (d, 1H, $H_{2',6'}$, J = 7.8 Hz), 11.15 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 55.3, 63.5, 113.8, 113.9, 123.5, 123.7, 125.3, 126.6, 127.3, 129.3, 130.1, 132.5, 134.4, 135.3, 1151.5, 158.6, 164.8, 165.2, 166.8.

4.1.5.4. N-(1,3-dioxo-3a,4,7,7a-tetrahydro-1H-4,7-methanoisoindol-2(3H)-yl)-2-(4-oxo-2-phenylbenzopyrimidin-3(4H)-yl)acetamide ***8a***. White solid, Yield: 70%. mp: 242–244 °C. ES-HRMS $[M+H]^+$ calcd. for $(C_{25}H_{20}N_4O_4)^+$: 441.1563, found: 441.1572. ^1H NMR (300 MHz,$CDCl_3$): δ (ppm) = 1.55 (d, 1H, $H_{6'''}$, J = 9.0 Hz), 1.76 (d, 1H, $H_{6'''}$, J = 9.0 Hz), 3.38 (s, 2H, $H_{4'''b,2'''a}$), 3.46 (s, 2H, $H_{4'''a,2'''b}$), 5.37 (s, 2H, $H_{1''}$), 6.19 (s, 2H, $H_{3''',4'''}$), 7.53 (m, 4H, H_{arom}), 7.88 (t, 1H, H_7, J = 8.1 Hz), 8.04 (d, 1H, H_8, J = 7.8 Hz), 8.16 (d, 1H, H_5, J = 8.1 Hz), 8.28 (s, 1H, NH), 8.54 (m, 2H, H_{arom}). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 44.3, 45.0, 51.7, 64.7, 114.4, 123.0, 126.9, 128.3, 128.5, 128.6, 130.8, 134.1, 134.7, 137.2, 152.3, 159.6, 165.1, 165.6, 173.3.

4.1.5.5. 2-(2-(4-chlorophenyl)-4-oxobenzopyrimidin-3(4H)-yl)-N-(1,3-dioxo-3a,4,7,7a-tetrahydro-1H-4,7-methanoisoindol-2(3H)-yl)acetamide ***8b***. White solid, Yield: 58%. mp: 216–218 °C. ES-HRMS $[M+H]^+$ calcd. for $(C_{25}H_{19}ClN_4O_4)^+$: 475.1173, found: 475.1183. ^1H NMR (300 MHz, DMSO-d_6): δ (ppm) = 1.45 (d, 1H, $H_{6'''}$, J = 8.4 Hz), 1.49 (d, 1H, $H_{6'''}$, J = 8.4 Hz), 3.23 (s, 2H, $H_{4'''b,2'''a}$), 3.38 (s, 2H, $H_{4'''a,2'''b}$), 5.22 (s, 2H, $H_{1''}$), 5.99 (s, 2H, $H_{3''',4'''}$), 7.49 (d, 2H, $H_{2',6'}$, J = 8,7 Hz), 7.62 (m, 1H, H_6), 7.91 (m, 2H, H_{arom}), 8.21 (d, 1H, H_5, J = 8.1 Hz), 8.84 (d, 2H, $H_{3',5'}$, J = 8.7 Hz), 10.77 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 43.5, 44.3, 51.3, 63.6, 114.2, 123.5, 127.4, 127.5, 128.5, 130.1, 134.4, 134.6, 135.7, 135.8, 151.2, 157.7, 164.7, 165.5, 173.9.

4.1.5.6. 2-(2-(4-methoxyphenyl)-4-oxobenzopyrimidin-3(4H)-yl)-N-(1,3-dioxo-3a,4,7,7a-tetrahydro-1H-4,7-methanoisoindol-2(3H)-yl)acetamide ***8c***. White solid, Yield: 69%. mp: 232–234 °C. ES-HRMS $[M+H]^+$ calcd. for $(C_{26}H_{22}N_4O_5)^+$: 471.1682, found: 471.1691. ^{1}H NMR (300 MHz,$CDCl_3$): δ (ppm) = 1.52 (d, 1H, $H_{6'''}$, J = 8.4 Hz), 1.74 (d, 1H, $H_{6'''}$, J = 8.7 Hz), 3.35 (s, 2H, $H_{4'''b,2'''a}$), 3.43 (s, 2H, $H_{4'''a,2'''b}$), 3.87 (s, 3H, OCH_3), 5.28 (s, 2H, $H_{1''}$), 6.16 (s, 2H, $H_{3''',4'''}$), 6.98 (d, 2H, $H_{3',5'}$, J = 7.8 Hz), 7.44 (t, 1H, H_6, J = 7.8 Hz), 7.78 (t, 1H, H_7, J = 7.8 Hz), 7.93 (d, 1H, H_8, J = 8.4 Hz), 8.04 (d, 1H, H_5, J = 8.1 Hz), 8.42 (d, 2H, $H_{2',6'}$, J = 7.8 Hz), 8.54 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 43.8, 44.5, 51.2, 54.8, 64.0, 113.3, 113.5, 122.5, 125.8, 127.3, 129.4, 129.7, 133.4, 134.2, 151.8, 158.9, 161.5, 164.4, 165.3, 172.9.

4.1.5.7. N-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)-2-(4-oxo-2-phenylbenzopyrimidin-3(4H)-yl)acetamide ***9a***. White solid, Yield: 52%. mp: 256–258 °C, ES-HRMS $[M+H]^+$ calcd. for $(C_{28}H_{18}N_4O_4)^+$: 475.1406, found: 475.1419. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 5.52 (s, 2H, $H_{1''}$), 7.60 (m, 3H, H_{arom}), 7.68 (td, 1H, H_6, J = 8.1 Hz, J = 1.8 Hz), 7.87 (t, 2H, H_{arom}, J = 7,5 Hz), 7.97 (m, 2H, H_{arom}), 8.32 (d, 1H, H_5, J = 8.1 Hz), 8.50 (m, 4H, H_{arom}), 8.65 (m, 2H, H_{arom}), 11.27 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 63.6, 114.2, 121.6, 123.6, 127.1, 127.3, 127.4, 127.5, 128.4, 128.5, 129.6, 130.8, 131.3, 131.5, 134.4, 135.1, 151.4, 158.8, 161.4, 165.5, 166.1.

4.1.5.8. 2-(2-(4-chlorophenyl)-4-oxobenzopyrimidin-3(4H)-yl)-N-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)acetamide ***9b***. Yield: 45%. mp: > 300 °C. ES-HRMS $[M+H]^+$ calcd. for $(C_{28}H_{17}ClN_4O_4)^+$: 509.1024, found: 509.1037. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 5.51 (s, 2H, $H_{1''}$), 7.62 (d, 2H, $H_{2',6'}$, J = 8.1 Hz), 7.70 (s, 1H, H_8), 7.89 (m, 4H, H_{arom}), 8.32 (d, 1H, H_5, J = 7.8 Hz), 8.51 (m, 4H, H_{arom}), 8.63 (d, 2H, $H_{3',5'}$, J = 7.8 Hz), 11.21 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 63.8, 114.3, 121.6, 123.6, 127.1, 127.3, 127.4, 127.5, 128.5, 129.6, 130.2, 131.4, 134.5, 135.1, 135.7, 136.0, 151.3, 157.8, 161.4, 165.6, 166.1.

4.1.5.9. N-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)-2-(2-(4-methoxyphenyl)-4-oxobenzopyrimidin-3(4H)-yl)acetamide ***9c***. White solid, Yield: 64%. mp: 247–249 °C. ES-HRMS $[M+H]^+$ calcd. for $(C_{29}H_{20}N_4O_5)^+$: 505.1512, found: 505.1525. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 3.82 (s, 3H, OCH_3), 5.44 (s, 2H, $H_{1''}$), 7.08 (d, 2H, $H_{3',5'}$, J = 8.1 Hz), 7.57 (s, 1H, H_8), 7.80–7.88 (m, 4H, H_{arom}), 8.27 (d, 1H, H_5, J = 7.8 Hz), 8.46 (t, 3H, H_{arom}, J = 9.0 Hz), 8.54 (d, 2H, $H_{2',6'}$, J = 8.4 Hz), 11.18 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 55.3, 63.6, 113.9, 114.0, 121.6, 122.9, 123.6, 126.6, 127.2, 127.3, 127.4, 129.7, 130.2, 131.5, 134.3, 135.1, 151.6, 158.8, 161.4, 161.7, 165.3, 166.2.

4.2. Biological evaluation

4.2.1. Antityrosinase activity

Anti-tyrosinase activity of all the prepared compounds was conducted as described by Gardelly et al. with some modifications [42]. The reaction mixture containing 880 µL of 2 mM substrate (LDopa dissolved in 50 mM phosphate buffer, pH 6.8) was added to 100 µL of the tested compound diluted in 10% DMSO solution at different concentrations. The reaction was initiated by addition of enzyme (tyrosinase: 20 µL, 1000 U/mL in phosphate buffer) to the substrate solution, and the inhibitor (hydroquinone (1 mM)). The assay mixture was then incubated at 25 °C for 10 min after which the absorbance was measured at 475 nm using a spectrophotometer. The effect of inhibition was determined by the maximum decrease in the amount of dopachrome formed and the absorbance was measured by spectrophotometry at 475 nm, a wavelength at which all the compounds tested do not absorb. A blank assay (without tyrosinase) was conducted. The inhibitory activity was determined by the diminution of the maximum dopachrome formed quantity. Tyrosinase inhibition was calculated according to the following equation: (%) = (A − B)/A × 100 where A represents the optical density of the tyrosinase enzyme and B represents the optical density of the test product. The assay was carried out in triplicate. Sample concentration providing 50% inhibition (IC_{50}) was obtained plotting the inhibition percentage against sample concentrations. Kojic acid was used as positive control. All assays were performed in triplicate.

4.2.2. Molecular docking procedure

The optimization of all the geometries of scaffolds was performed with Gaussian 09 semi-empirical PM3 force-field method [43]. The crystal structures of PDB (PDB: 2Y9X) was obtained from the RSCB protein data bank. [41] Docking studies were performed using Autodock 4.2 software [44]. The visualisation and analysis of interactions were performed using Pymol, version 0.99 [45].

Declaration of Competing Interest

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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