Design and synthesis of novel potent anticoagulant and anti-tyrosinase pyranopyrimidines and pyranotriazolopyrimidines: Insights from molecular docking and SAR analysis

Meriem Debbabia, Vijaykumar D. Nimbarte, Samia Chekir, Sarra Chortani, Anis Romdhane, Hichem Benjannet,⁎

Laboratory of Heterocyclic Chemistry, Natural Products and Reactivity (LR11ES39), Team: Medicinal Chemistry and Natural Products, Faculty of Science of Monastir, University of Monastir, Avenue of Environment, 5019 Monastir, Tunisia
Laboratory of Chemistry, URCOM, EA 3221, INC3M CNRS-F3038, UFR of Science and Technology, University of Le Havre, BP 1123, 25 rue Philippe Lebon, 76063 Le Havre Cedex, France

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ABSTRACT

Pyrimidine-fused compounds are of great interest for the discovery of potent bioactive agents. This study describes the synthesis of novel pyranopyrimidines 3a-f and pyranotriazolopyrimidines 4a-d derivatives via the cyclocondensation reaction of α-functionalized iminoether 2, which was obtained from 2-amino-3-cyanopyrane 1, with a series of primary aromatic amines and hydrazides, respectively. Structures of all synthesized compounds were established on the basis of spectroscopic methods including 1H NMR, 13C NMR and ES-HRMS. They were finally tested for their anticoagulant and anti-tyrosinase activities. Significant results have been obtained and the structure-activity relationship (SAR) was discussed with the help of molecular docking analysis.

1. Introduction

Designing of novel class of bioactive heterocycles and develop efficient methods for their synthesis with predefined functionalities is a challenging task in modern organic chemistry. Pyrimidines constitute an interesting group of heterocyclic compounds many of which possess wide-spread pharmacological properties and their chemistry and bioactivities are receiving considerable attention and has been documented in several reports. Furthermore, the multiple biological activities of these heterocyclic compounds are of increasing interest as antibacterial, antiviral and antitumor. In addition, some of them have shown anticoagulant and anti-tyrosinase activities.

On another hand, pyrane-fused compounds have, for a long time, attracted the interests of both synthetic and biological researchers alike because of their various chemical and biological properties. Many of them displayed a broad range of biological activities such as analgesic, antituberculosis, antiviral, anticoagulant and anti-tyrosinase.

Furthermore, triazoles are a class of compounds, which occupies a special role in nature. They have attracted intense interest in recent years because of their diverse pharmacological properties like antibacterial, analgesic, anti-inflammatory and particularly anticoagulant and anti-tyrosinase agents.

In view of the above observations and as a continuation of our previous work on the synthesis of a new fused-pyrimidine scaffold, we report here the synthesis of some new condensed pyrimidine derivatives 3 and 4 bearing in their structures fragments such as pyrane, pyrimidine and triazole, commonly found in anticoagulant and anti-tyrosinase agents.

All synthesized compounds 1, 2, 3a-f and 4a-d were evaluated for their anticoagulant by measuring the aPTT and for their anti-tyrosinase potency and the structure-activity relationship (SAR) was discussed with the help of molecular docking analysis.

2. Results and discussion

2.1. Chemistry

The 2-amino22123-cyanopyrane 1 has served as a key starting material. It was prepared via one-pot three-component reaction, of aryaldhyde, malononitrile and 5,5-dimethylcyclohexane-1,3-dione.
construction of a variety of poly-heterocycles [28,29]. For this purpose and as described in Schemes 2 and 3, our approach to the target systems 3 and 4 was firstly started by the construction of the α-functionalized iminoether 2, via condensation reaction of precursor 1 with triethylorthoformate under reflux of acetic anhydride (Scheme 1) [30].

The structures of compounds 3 have been assigned from their $^1$H NMR and $^{13}$C NMR spectra. Then, the reflux of iminoether 2 with some primary aromatic amines in ethanol for 5 h and in the presence of a catalytic amount of acetic acid led to new the pyranopyrimidines 3 (Table 1). Plausible pathway involves two successive nucleophilic additions of the $\text{NH}_2$ group on the imidic carbon and on the cyano function (Scheme 2).

The structures of compounds 3 have been assigned from their $^1$H NMR and mass spectrometry (ES-HRMS) analytical data. In fact, the $^1$H NMR spectra of compounds 3 showed, in addition to the new signals corresponding to the protons introduced by the intermediate 2, the appearance of new signals due to the primary aromatic amine and the appearance of the signals relative to the ethoxy group protons. Analysis of $^{13}$C NMR spectra of the same compounds showed the appearance of new signals relative to carbons introduced by the primary aromatic amine used, in addition to the disappearance of three signals at $\delta_C$ 13.2, 63.8 and 118.3 attributable to $\text{CH}_3\text{CH}_2\text{O}$, $\text{CH}_3\text{CH}_2\text{O}$ and $\text{CN}$, respectively.

In the second part of this work, a series of new pyranopyrimidine derivatives containing the 1,2,4-triazolo moiety 4a-d has been prepared, by cyclodepsilation reaction of the precursor 2, via its imidic carbon and nitrile functional group with appropriate acid hydrazides according to the procedure described by Abdel-Aziz et al. [31] (Scheme 3). This reaction was conducted in three solvents (dioxane, ethanol and toluene) and the best yields (60–85%) were obtained with dioxane (Table 2).

Plausible pathway involves two successive nucleophilic additions of $\text{NH}_2$ group on the imidic carbon then on the cyano function followed by dehydrocyclization to give the new pyranotriazolopyrimidines 4a-d (Scheme 3).

The formed compounds 4 were characterized by their $^1$H NMR, $^{13}$C NMR and mass spectrometry (ES-HRMS) spectral data.

Further, the $^1$H NMR spectra of new pyranotriazolopyrimidines 4a-d showed the disappearance of signals (triplet and quadruplet) relative to the ethoxy group of the α-functionalized iminoether 2 and the appearance of new signals, attributable to protons introduced by the appropriate hydrazide, of which chemical shifts and multiplicities are in agreement with the proposed structures.

Analysis of $^{13}$C NMR spectra of compounds 4a-d showed, in addition of the signals relative to the hydrazide moiety carbons, essentially the new signal at $\delta_C$ 152.3 relative to carbon C2, the disappearance of two signals at $\delta_C$ 13.2 and 63.8 corresponding to the ethoxy group carbons.

The ESI-HRMS showed the correct protonated molecular ion peaks [M + H]$^+$ for all examined compounds 3 and 4.

2.2. Biological activity

All the synthesized compounds 1, 2, 3a-f and 4a-d have been evaluated for their anticoagulant and anti-tyrosinase activities.

2.2.1. Anticoagulant activity

Anticoagulants have been introduced therapeutically for over half a century and are widely used in various fields such as surgery and cardiology. Since their introduction in therapeutics, important advances have been made, allowing a better understanding of their mechanism of action, the intimate mechanisms of thrombosis, the role of platelets and the endothelium. Thus, with the progress of methods of synthesis and the isolation from natural sources, new molecules were discovered.

Anticoagulants block the vitamin K cycle by acting on epoxide reductase and inhibiting the quinonereductase responsible for the transformation of vitamin K epoxide (KO) into vitamin K quinone, responsible for blood coagulation.

The in vitro anticoagulant activity of all the synthesized compounds 1, 2, 3a-f and 4a-d was evaluated by measuring the activated Partial Thromboplastin Time (aPTT) in normal human plasma. As shown in Table 3, all the synthesized compounds have displayed good anticoagulant activities expressed by the significant prolongation of aPTT in a concentration–dependent manner ranging from 53.7 to 72.8 s compared to that of the negative control (aPTT = 33.0 s).

From the results obtained, it has been found that the iminoether 2 exhibited the highest activity (aPTT = 72.8 ± 0.9 s) followed by the starting product 1 (aPTT = 68.3 ± 0.8 s) compared to the plasma as negative control (aPTT = 33.50 ± 0.05 s). The prepared pyranopyrimidines 3a-f exhibited good anticoagulant activity with aPTT values ranging from 53.7 ± 0.9 to 63.2 ± 1.1 s. In this series, compound 3f bearing a naphthyl group displayed the highest activity (aPTT = 63.2 ± 1.1 s) followed by 3c with a 4–C$_2$H$_5$P$_2$ (aPTT = 61.8 ± 1.0 s) whereas 3b (3–CH$_3$P$_2$) was found to be the less active (aPTT = 53.7 ± 0.9 s) but remains more effective than the negative control (plasma). This difference in activity could be explained by the nature and the position of the alky group attached to the aromatic ring. Compound 3e with a 4–OCH$_3$P$_2$–Ph showed a slightly higher activity than its analogue 3d (3–C$_2$H$_5$P$_2$) (aPTT = 57.0 ± 1.0 and 54.8 ± 0.8 s, respectively). This finding could be due to the nature of the substituent and its own electronic effect. Indeed, the methoxy group
exerted a mesomeric donor effect whereas chlorine atom exerted both a mesomeric donor and an inductive attractor effects. The derivative 3a (Ph) (aPTT = 57.1 ± 0.7 s) displayed an anticoagulant capacity slightly higher than 3b (3-CH₃Ph) and 3d (3-ClPh). This result shows the ineffectiveness of these two substituents in the aryl group at 3-position compared to the hydrogen atom.

On the other hand, the pyranotriazolopyrimidines 4a-d (aPTT = 55.1 ± 1.1 – 63.4 ± 0.7 s) were found to be anticoagulant compared to the negative control (plasma). The derivative 4c (R = CH₃CN) exhibited the highest activity with an aPTT value of 63.4 ± 0.7 s followed by 4b (R = CH₃) (aPTT = 60.1 ± 1.2 s). The comparison of the anticoagulant activity of 4b and 4c with that of the non substituted triazole derivative 4a (aPTT = 59.4 ± 0.8 s) does not show the impact of these two substituents introduced (CH₃ and CH₂CN) to improve this activity. Moreover, the coumarin moiety in compound 4d (aPTT = 55.1 ± 1 s) did not seem to bring any anticoagulant activity by comparison to 4a. This result could be explained by the extension of the size of the molecule and the decrease of its polarity which influence its solubility. All the tested compounds remain less active than heparin (aPTT = 125.2 ± 3.8 s, cc = 10 μg/mL) used as positive control.

2.2.2. Anti-tyrosinase activity

Tyrosinase is an enzyme present in the skin that activates the transformation of tyrosine (amino acid) into dark-colored pigment melanin. Its absence or mutation of its gene lead to a decrease or even a halting of the pigmentation. The in vitro anti-tyrosinase activity of compounds 1, 2, 3a-f and 4a-d were assessed by measuring the inhibition percentage (PI %). The results presented in Table 3 showed that most of tested compounds displayed significant anti-tyrosinase activity with percent of inhibition ranging from 73.77 ± 1.10 to 98.84 ± 1.81% compared to that of kojic acid used as a positive control.

### Table 1
Synthesis of pyranopyrimidines 3a-f.

<table>
<thead>
<tr>
<th></th>
<th>3a</th>
<th>3b</th>
<th>3c</th>
<th>3d</th>
<th>3e</th>
<th>3f</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ar</td>
<td>Ph</td>
<td>3-CH₃Ph</td>
<td>4-C₃H₇Ph</td>
<td>3-ClPh</td>
<td>4-OCH₃Ph</td>
<td>1-naphthyl</td>
</tr>
<tr>
<td>Yield (%)</td>
<td>67</td>
<td>74</td>
<td>80</td>
<td>76</td>
<td>86</td>
<td>84</td>
</tr>
</tbody>
</table>

![Scheme 1. Synthetic pathway to the precursors 1 and 2.](image)

![Fig. 3. Target compounds.](image)
exhibited the highest tyrosinase inhibitor with a PI value of 98.84 ± 1.81% whereas the starting compound 1 was the less active one against tyrosinase (PI = 73.77 ± 1.10%), hence the importance to modify the primary amine function into imine.

On the other hand, from the series of compounds 3a-f, only 3a, 3c and 3d displayed anti-tyrosinase capacity with PI values of 91.35 ± 1.00, 84.14 ± 2.00 and 94.23 ± 1.45%, respectively. It is clear that the chlorine atom and its 3-position of the aromatic ring were in favor of the high activity of compound 3d. These results show that the attachment of a methyl group at the 3-position on the aromatic ring (compound 3b) results in the loss of activity compared with compound 3a with an unsubstituted aromatic ring (PI = 91.35 ± 1.00%), whereas, the introduction of an ethyl group at the 4-position of the aromatic ring in compound 3c has generated an interesting anti-tyrosinase activity (PI = 84.14 ± 2.00%).

The inactivity of compound 3e (4-OCH₃Ph) compared to its bioactive analogue 3c (4-C₂H₅Ph) clearly shows the importance of the nature of the substituent attached to the 4-position of the aromatic ring in addition to the electronic effects (+I and/or +M) that can apply. The inactivity of the compound 3f (naphtyl) compared to its analogue 3a (Ph) (PI = 91.35 ± 1.00%) suggests that the extension of the conjugation might not be in favor of this activity. The difference in size and spatial arrangement between these two molecules could also be at the origin of the total loss of this activity by replacing a phenyl by a naphthyl group.

On the other hand, only the compound 4b (R = CH₃) was found to be inactive towards tyrosinase enzyme. Compound 4c (R = CH₂CN) exhibited the highest activity (PI = 93.94 ± 1.58%) followed by 4d with a coumarin system attached to its triazole ring.
Table 3
Anticoagulant and anti-tyrosinase activities of compounds 1, 2, 3a-f and 4a-d.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>aPTT (s)</th>
<th>PII (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>68.3 ± 0.8</td>
<td>73.77 ± 1.1</td>
</tr>
<tr>
<td>2</td>
<td>72.8 ± 0.9</td>
<td>98.84 ± 1.81</td>
</tr>
<tr>
<td>3a</td>
<td>57.1 ± 0.7</td>
<td>91.35 ± 1.00</td>
</tr>
<tr>
<td>3b</td>
<td>53.7 ± 0.9</td>
<td>na</td>
</tr>
<tr>
<td>3c</td>
<td>61.8 ± 1.0</td>
<td>84.14 ± 2.00</td>
</tr>
<tr>
<td>3d</td>
<td>54.8 ± 0.8</td>
<td>94.23 ± 1.45</td>
</tr>
<tr>
<td>3e</td>
<td>57.0 ± 1.0</td>
<td>na</td>
</tr>
<tr>
<td>3f</td>
<td>63.2 ± 1.1</td>
<td>na</td>
</tr>
<tr>
<td>4a</td>
<td>59.4 ± 0.8</td>
<td>78.09 ± 0.94</td>
</tr>
<tr>
<td>4b</td>
<td>60.1 ± 1.2</td>
<td>na</td>
</tr>
<tr>
<td>4c</td>
<td>63.4 ± 0.7</td>
<td>93.94 ± 1.58</td>
</tr>
<tr>
<td>4d</td>
<td>55.1 ± 1.1</td>
<td>90.12 ± 2.10</td>
</tr>
<tr>
<td>Plasma⁴</td>
<td>33.50 ± 0.05</td>
<td>na</td>
</tr>
<tr>
<td>Heparin&lt;sup&gt;+&lt;/sup&gt;</td>
<td>125.2 ± 3.8</td>
<td>na</td>
</tr>
<tr>
<td>Kojic acid&lt;sup&gt;+&lt;/sup&gt;</td>
<td>85.50 ± 1.00</td>
<td>na</td>
</tr>
</tbody>
</table>

* Expressed by measuring the activated Partial Thromboplastin Time (aPTT) (s) at the concentration of 1000 μg/mL and was the mean of three replicates (mean ± SD, n = 3).
* Capacities were represented as percent of inhibition (mean ± SD, n = 3).
* Non-active.
* Negative control.
* Positive control at the concentration of 10 μg/mL.
* Positive control.

(P1 = 90.12 ± 2.10%). The electron-withdrawing cyano group in compound 4c is certainly at the origin of the high activity compared to that of compound 4a where this group is replaced by a hydrogen atom (P1 = 78.09 ± 0.94%). The compounds 4c and 4d were found more effective than the positive control (P1 = 85.50 ± 1.00%). These findings reveal the importance of the nature of the substituent borne by the triazole.

2.2.3. Molecular docking studies
Tyrosinase is a binuclear copper-containing enzyme that catalyzes the conversion of a monophenol (tyrosine) and/or o-diphenol (L-DOPA) in its corresponding o-quinone derivative. The crystal structure is mainly composed tetramer subunit chain-A, chain-B, Chain-C and chain-D, respectively with the sequence length of 391 [32].

In depth docking analysis has been performed and investigated were carried out to elucidate the interaction of this class of scaffolds within the hydrophobic binding pocket of tropolone in PDB: 2Y9X and to investigate the binding mode and binding energies of 1, 2, 3a, 3b, 3c, 3d, 3e, 3f, 4a, 4b, 4c and 4d (Table 4) at the tropolone binding domain of binuclear copper containing enzyme (PDB: 2Y9X) by using auto dock 4.2. The majority of anti-tyrosinase agents that targets the tropolone binding site, reported thus far, resemble the binding interaction of tropolone within the hydrophobic binding site. Amongst all designed scaffolds most active set of compounds in terms of binding energies are within the conjugates 2, 3a, 3b, 3c and 3d found to be most active in terms of binding energy and interactions within the tropolone binding cavity of PDB: 2Y9X (Figs. 4 and 5).

In depth structure activity relationship of anti-tyrosinase agents (Fig. 5) suggest that “4-imino-8,8-dimethyl-3,5-diphenyl-5,7,8,9-tetrahydro-3H-chromeno[2,3-d]pyrimidin-6(4H)-one” core with ring A, B, C, D and E is most essential platform for the design of conjugates 3a, 3b, 3c, 3d, 3e and 3f. In conclusion, we observed that dimethyl group at position 8 of basic pharmacophore is essential for pi-alkyl type of interaction with surrounding amino acid sequence within the binding cavity of tropolone in addition to this 4-imino functional group gives it a unique character to get ionized and form hydrogen bonds with pocket amino acids (Fig. 6). Basic pharmacophore is a blend of saturated and unsaturated system which is essential for the pi-pi and pi-alkyl interactions which may be responsible for anti-tyrosinase property. Coming to diphenyl ring substitution at position 3 and 5 (most essential) respectively suggests that aliphatic substitutions in phenyl ring E may explore probability to have promising anti-tyrosinase activity and binding interactions, but in contrast bulkier ring or extension of this 3-rings pharmacophore to 4 ring system is a demerit for binding energies which we observed for conjugates 4a, 4b, 4c and 4d, respectively.

Binding site of conjugate 3d (most effective anti-tyrosinase agent from the series 3) in hydrophobic cavity of PDB: 2Y9X (Fig. 7), 2D-interactions in between 4 and imino group and some unfavorable bumps in red color code with GLU-A-98 (conventional hydrogen bond interactions in green color), Chlorine substitution in ring E is involved in conventional hydrogen bond interaction with TRP-A-293 (green color) and some unfavorable bumps (red color) with TRP-A-293, PHE-A-292 were observed due to Pi-Pi electron clouds in ring E, apart from this Pi-alkyl interactions were observed with TYR-A-9 in ring E, some more unfavorable bumps were observed with GLU-A-98, ARG-A-95, ASP-A-300 (Pi-amine in golden color), LEU-A-303 and VAL-A-299, respectively. Phenyl ring D is involved in Pi-Pi interactions with ARG-A-95 similarly some Pi-alkyl, Pi-Pi and conventional bonds were observed in Ring A with PRO-A-91, VAL-A-299, PHE-A-241 and LEU-A-255, respectively.

3. Conclusion
In this paper we have synthesized a series of new pyranopyrimidine- and pyranotriazolopyrimidine-based bioactive heterocyclic compounds 3 and 4 by reacting the α-functionalized-iminoether 2, previously prepared from α-aminocarbonitrile 1, with a series of primary aromatic amines and hydrazides, respectively. The newly prepared compounds 3a-f and 4a-d have been tested for their possible anticoagulant and anti-tyrosinase activities. The results showed that most compounds exhibited interesting activity and in depth docking analysis reveals the structure activity relationship which is relevant with the findings of biological evaluations. It has been found that the iminoether 2 exhibited the highest anticoagulant activity and tyrosinase inhibition amongst all compounds synthesized and tested. Most pyranopyrimidines 3 and pyranotriazolopyrimidines 4 also displayed interesting activity In depth docking analysis leads to a conclusion that 4-imino-8,8-dimethyl-3,5-diphenyl-5,7,8,9-tetrahydro-3H-chromeno[2,3-d]pyrimidin-6(4H)-one is absolutely important for buildup and improvement of antityrosinase activity of conjugates. Insilico SAR studies were supported with biological evaluation and in most of the cases the SAR showed the nature and the electron effect of the substituents on the aromatic ring or on the triazole moiety of pyranopyrimidines 3 and pyranotriazolopyrimidines 4, respectively to improve and to show significant binding interaction proven with Insilico docking studies.

4. Materials and methods
4.1. Chemistry
All reactions were monitored by TLC using aluminium sheets of Merck silica gel 60 F254, 0.2 mm. Melting points were determined on a Büchi S10 apparatus using capillary tubes.

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 and coupling constants (J) in Hertz. Mass spectra were obtained with ESI-TOF (LCT, Waters) using the reflectron mode in the positive ion mode.

4.1.1. General procedure for the synthesis of 2-amino-3-cyanopyranes

To a stirred stoichiometric mixture of arylaldehyde (10 mmol), malononitrile (10 mmol) and 5,5-dimethylcyclohexane-1,3-dione (10 mmol) in absolute ethanol (30 mL) was added a catalytic amount of piperidine and then was refluxed for 2 h. The solid product formed is filtered, dried and purified by recrystallization from ethanol to give compound 1 in good yield 97%.

4.1.1.1. 2-amino-9,10-dimethyl-5-oxo-4-phenyl-5,6,7,8-tetrahydro-4H-chromene-carbonitrile 1

Whitesolid, yield 97%, m.p: 240°C, 1H NMR (CDCl$_3$, 300 MHz) δ: H: 1.04 (3H, s, H$_9$), 1.11 (3H, s, H$_{10}$), 2.21 (2H, s, H$_8$), 2.45 (2H, s, H$_6$), 4.41 (1H, s, H$_4$), 4.51 (2H, s, NH$_2$), 7.26 (5H, s, Harom). 13C NMR (CDCl$_3$, 75 MHz) δ: 27.6, 28.7, 32.11, 35.5, 40.7, 50.7, 63.9, 114.2, 118.3, 157.5, 161.3, 195.5.

4.1.2. General procedure for the synthesis of the α-functionalized iminoether

The mixture of 1 (0.01 mmol) and triethylorthoformate (0.01 mmol) in acetic anhydride (30 mL) was refluxed for 1 h. The solvent was then removed under reduced pressure. The remaining solid was recrystallized from ethanol to give compound 2.

4.1.2.1. Ethyl N-(3-cyano-7,7-dimethyl-5-oxo-4-phenyl-5,6,7,8-tetrahydro-4H-chromen-2-yl) formimidate 2

Whitesolid, yield: 88%, m.p: 148°C, 1H NMR (CDCl$_3$, 300 MHz) δ: 1.03–1.05 (6H, 4s, H$_9$, H$_{10}$), 1.36 (3H, t, J = 6.9 Hz, H$_{13}$), 2.25 (2H, d, J = 16.5 Hz, H$_8$), 2.53 (2H, d, J = 12 Hz, H$_6$), 4.41 (2H, q, J = 6.9 Hz, H$_{12}$), 4.61 (1H, s, H$_4$), 7.16–7.92 (5H, m, Harom), 8.21 (1H, s, H$_{11}$). 13C NMR (CDCl$_3$, 75 MHz) δ: 13.2, 27.1, 28.2, 31.6, 35.1, 40.3, 50.2, 63.8, 83.4, 112.6, 126.5, 128.3, 155.4, 160.3, 161.5, 195.0, 126.8.

4.1.3. General procedure for the synthesis of the pyranopyrimidines 3a-f

The appropriate primary amine (0.001 mol) was added to the iminoether 2 (0.001 mol), and the mixture was stirred at reflux of ethanol (20 mL) and a few drops of acetic acid for 6 h. After cooling, the
precipitated solid was filtered, washed with cold ethanol, and dried to obtain compounds 3a-f.

4.1.3.1. 4-imino-8,8-dimethyl-3,5-diphenyl-5,7,8,9-tetrahydro-3H-chromeno[2,3-d]pyrimidin-6(4H)-one 3a. Yellow solid, yield: 67%, m.p: 254 °C. ESI-HRMS [M+H]⁺ calcld. for (C25H24N3O2)⁺: 398.1869, found: 398.1888. ¹H NMR (DMSO-d₆, 300 MHz) δ_H: 0.97–1.07 (6H, 2s, H₁₁+₁₂), 2.19 (1H, d, J = 16.2 Hz, H₉a), 2.35 (1H, d, J = 16.2 Hz, H₂b), 2.57 (1H, d, J = 17.7 Hz, H₇a), 2.65 (1H, d, J = 17.7 Hz, H₇b), 5.45 (1H, s, H₅), 6.99–7.54 (10H, m, H arom), 8.28 (1H, s, H₂), 8.60 (1H, s, H₁₃). ¹³C NMR (DMSO-d₆, 75 MHz) δ_C: 26.5, 28.5, 31.4, 31.8, 39.4, 50.15, 121.7, 128.2, 99.72, 114.27, 155.9, 158.9, 161.4, 163.7, 195.5.

4.1.3.2. 4-imino-8,8-dimethyl-3,5-diphenyl-5,7,8,9-tetrahydro-3H-chromeno[2,3-d]pyrimidin-6(4H)-one 3b. White solid, yield: 74%, m.p: 172 °C. ESI-HRMS [M+H]⁺ calcld. for (C₂₆H₂₆N₃O₂)⁺: 412.2045. ¹H NMR (CDCl₃, 300 MHz) δ_H: 1.01–1.12 (6H, 2s, H₁₁, H₁₂), 2.29 (2H, d, J = 5.4 Hz, H₉), 2.31 (3H, s, CH₃-Ph), 2.61 (2H, s, H₂), 2.89 (1H, s, H₅), 6.31 (1H, s, H₂), 6.98–7.47 (9H, m, H arom), 8.42 (1H, s, H₁₃). ¹³C NMR (CDCl₃, 75 MHz) δ_C: 20.8, 26.9, 28.3, 31.65, 33.9, 40.6, 50.2, 98.4, 113.8, 118.1–128.6, 155.9, 158.9, 160.7, 162.6, 195.5.

4.1.3.3. 3-(4-ethylphenyl)-4-imino-8,8-dimethyl-5-phenyl-5,7,8,9-tetrahydro-3H-chromeno[2,3-d]pyrimidin-6(4H)-one 3c. Yellow solid, yield: 80%, m.p: 202 °C. ESI-HRMS [M+H]⁺ calcld. for (C₂₇H₂₈N₃O₂)⁺: 426.2182 found 426.2203. ¹H NMR (CDCl₃, 300 MHz) δ_H: 1.01–1.19 (6H, 2s, H₁₁, H₁₂), 1.29 (2H, d, J = 6.6 Hz, H₂), 2.25 (2H, s, H₂), 2.67 (3H, s, CH₃-Ph), 4.92 (1H, s, H₅), 6.67 (1H, s, H₂), 7.15–7.49 (9H, m, H arom), 8.42 (1H, s, H₁₃). ¹³C NMR (CDCl₃, 75 MHz) δ_C: 15.4, 27.4, 28.8, 28.2, 32.1, 34.4, 41.2, 50.7, 98.7, 114.3, 121.7, 129.1, 156.6, 159.6, 161.2, 163.1, 196.0.

Fig. 5. Structure activity relationship of designed scaffolds for anti-tyrosinase agents.

4.1.3.4. (3-chlorophenyl)-4-imino-8,8-dimethyl-5-phenyl-7,8,9-tetrahydro-3H-chromeno[2,3-d]pyrimidin-6 (4H)–one 3d. White solid, yield: 76%, m.p: 164°C. ESI-HRMS [M+H]+ calcld. for (C25H23ClN3O2)+: 432.1479 found 432.1498. 1H NMR (CDCl3, 300MHz) δH: 1.01–1.12 (6H, 2s, H12, H11), 2.25 (2H, s, H9), 2.66 (2H, s, H7), 3.78 (1H, s, H13), 4.95 (1H, s, H5), 6.68–7.54 (9H, m, Harom), 8.48 (1H, s, H2). 13C NMR (CDCl3, 75MHz) δC: 26.8, 28.3, 31.6, 33.8, 40.6, 50.2, 98.9, 113.7, 118.4–129.7, 156.1, 158.6, 160.9, 162.6, 195.5.

4.1.3.5. 4-imino-3-(4-methoxyphenyl)-8,8-dimethyl-5-phenyl-5,7,8,9-tetrahydro-3H-chromeno[2,3-d]pyrimidin-6 (4H)–one 3e. Yellow solid, yield: 86%, m.p: 218°C. ESI-HRMS [M+H]+ calcld. for (C26H26N3O3)+: 428.1974 found 428.1994. 1H NMR (CDCl3, 300MHz) δH: 1.02–1.19 (6H, 2s, H11, H12), 2.19 (2H, s, H9), 2.65 (2H, s, H7), 3.78 (3H, s, OCH3), 4.95 (1H, s, H5), 6.50–7.45 (9H, m, Harom), 8.39 (1H, s, H2). 13C NMR (CDCl3, 75MHz) δC: 26.9–28.3, 31.6, 33.8, 40.7, 50.2, 54.9, 98.02, 113.7, 123.2, 130.4, 156.4, 159.3, 160.7, 162.7, 195.6.

4.1.3.6. 4-imino-8,8-dimethyl-3-(naphthalen-1-yl)-5-phenyl-5,7,8,9-tetrahydro-3H-chromeno[2,3-d]pyrimidin-6 (4H)–one 3f. White solid, yield: 84%, m.p: 234°C. ESI-HRMS [M+H]+ calcld. for (C29H26N3O2)+: 448.2025 found 448.2045. 1H NMR (CDCl3, 300MHz) δH: 1.00–1.19 (6H, 2s, H12, H11), 2.22 (2H, s, H9), 2.65 (2H, s, H7), 5.12 (1H, s, H5), 6.81–7.87 (12H, m, Harom), 8.35 (1H, s, H2), 7.43 (1H, s, H13). 13C NMR (CDCl3, 75MHz) δC: 27.0–28.2, 31.6, 34.0, 40.7, 50.2, 54.9, 98.02, 113.7, 123.2, 130.4, 156.4, 159.3, 160.7, 162.7, 195.6.

4.1.4. General procedure for the synthesis of the pyranotriazolopyrimidines 4a-d

Equimolar solution (15 mmol) of imino ether 2 and hydrazide was refluxed in dry dioxane (40 mL) with continuous stirring for 24 h under argon atmosphere. The crude product was purified by column chromatography (PE:EtOAc 4:6) to give compound 4a-d.

4.1.4.1. 9,9-dimethyl-12-phenyl-9,10-dihydro-8H-chromeno[3,2-e] [1,2,4]triazolo[1,5-c]pyrimidin-11(12H)-one 4a. White solid, yield: 85%, m.p: 223°C. ESI-HRMS [M+H]+ calcld. for (C20H19N4O2)+: 347.1508 found 347.1530. 1H NMR (CDCl3, 300MHz) δH: 1.08–1.10 (6H, 2s, H14, H15), 2.19 (2H, d, J = 16.2Hz, H8a), 2.29 (2H, d, J = 16.2Hz, H8b), 2.58 (2H, d, J = 3Hz, H10a), 2.69 (2H, d, J = 3Hz, H10b), 5.43 (1H, s, H12), 7.05–7.39 (5H, m, Harom), 8.23 (1H, s, H5), 9.04 (1H, s, H2). 13C NMR (CDCl3, 75MHz) δC: 27.5, 29.0, 32.3, 34.7, 41.1, 50.7, 104.0, 126.6, 127.8, 113.3, 138.5, 152.3, 156.9, 152.7, 163.6, 195.9.

4.1.4.2. 2,9,9-trimethyl-12-phenyl-9,10-dihydro-8H-chromeno[3,2-e][1,2,4]triazolo[1,5-c]pyrimidin-11(12H)–one 4b. White solid, yield: 60%, m.p: 226°C. ESI-HRMS [M+H]+ calcld. for (C21H21N4O2)+: 361.1665 found 361.1684. 1H NMR (CDCl3, 300MHz) δH: 1.10–1.16 (6H, 2s, H15, H14), 2.24 (2H, d, J = 16.2Hz, H8a), 2.36 (2H, d, J = 16.2Hz, H8b), 2.53 (3H, s, CH3), 2.61 (2H, d, J = 17.7Hz, H10a), 2.75 (2H, d, J = 17.7Hz, H10b), 5.49 (1H, s, H12), 7.13–7.45 (5H, m, Harom), 8.97 (1H, s, H2). 13C NMR (CDCl3, 75MHz) δC: 27.0, 28.5, 29.0, 32.3, 34.0, 40.7, 50.2, 54.9, 98.02, 113.5, 123.7, 128.5, 137.6, 152.3, 152.4, 163.0, 167.2, 195.0.

4.1.4.3. 2-(9,9-dimethyl-11-oxo-12-phenyl-9,10,11,12-tetrahydro-8H-chromeno[3,2-e][1,2,4]triazolo[1,5-c]pyrimidin-2-yl) acetonitrile 4c. White solid, yield: 73%, m.p: 228°C. ESI-HRMS [M+H]+ calcld. for (C22H20N5O2)+: 386.1617 found 386.1636. 1H NMR (CDCl3, 300MHz) δH: 1.14–1.25 (6H, 2s, H14, H15), 2.29 (2H, d, J = 16.5Hz, H8a), 2.39 (2H, d, J = 16.5Hz, H8b), 2.65 (2H, d, J = 17.7Hz, H10a), 2.78 (2H, d, J = 17.7Hz, H10b), 3.99 (2H, d, J = 17.7Hz, H10a), 7.13–7.84 (5H, m, Harom), 9.06 (1H, s, H2). 13C NMR (CDCl3, 75MHz) δC: 24.3, 27.5, 31.6, 34.0, 40.7, 50.2, 52.2, 104.0, 126.6, 127.8, 113.3, 137.6, 152.3, 152.4, 163.0, 167.2, 195.0.


4.2. Biological evaluation

4.2.1. Anticoagulant activity

The anticoagulant activity of the synthesized compounds were evaluated by measuring the aPTT (Thromboplastin partial activation time) using Platelet LS reagent (Trinity Biotech PLC, Cwicklow, Ireland). The coagulation test was carried out using normal human citrate plasma poor in platelets. In a coagulometer bowl, 45 μL of normal citrate plasma pool containing 5 μL of the tested product solution at concentrations of 1000 μg/mL are incubated for 1 min at 37°C, 50 μL of citrate plasm pool containing 5μL of the tested product solution at 63.5, 101.9, 104.9, 113.8, 127.9, 137.8, 152.5, 152.9, 160.6, 163.0, 63.5, 101.9, 104.9, 137.8, 127.9, 152.5, 152.9, 160.6, 163.0, 164.9, 195.0.

4.2.2. Anti-tyrosinase activity

The mushroom tyrosinase inhibition effect was determined using L-tyrosine (1 mM) as the substrate. Hydroquinone (1 mM) was used as a tyrosinase inhibitor. The substrate and inhibitor were prepared in 0.1 M phosphate buffer pH 6.5. Inhibition of tyrosinase was tested in a reaction mixture containing 1.960 mL of phosphate buffer, 2 mL of L-tyrosine (1 mM), 20 μL of fungus tyrosinase and 20 μL of hydroquinone (1 mM). The reaction was initiated by adding enzyme to the substrate solution and the inhibitor. The reaction and all solutions were thermostated at 25°C. 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 test was performed in triplicate. The percentage inhibition of tyrosinase activity at the concentration at which all the compounds tested do not absorb. A blank assay 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 test was performed in triplicate. The percentage inhibition of tyrosinase activity at the concentration at which all the compounds tested do not absorb. A blank assay 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 test was performed in triplicate. The percentage inhibition of tyrosinase activity at the concentration at which all the compounds tested do not absorb. A blank assay 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 test was performed in triplicate.

4.2.3. Molecular docking procedure

The optimization of all the geometries is carried out in Gaussian 09 using PM3 semi-empirical method [35]. The crystal structures of PDB (PDB: 2Y9X) were obtained from the RSCB protein data bank [32]. Docking studies were performed using AutoDock 4.2 software [36]. The Analysis of intermolecular interactions has been performed using Pymol, v. 0.99 [37].

Declaration of interest

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

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bijoorg.2018.10.004.

References

[12] G. Zhang, Y. Zhang, J. Yang, R. Zhang, W. Yang, R. Wang, X.H. Yang, 4,5-dihydronaphthalene-1(2H)-one as a positive control and the test was conducted in triplicate [33].


[37] The PyMOL Molecular Graphics System, Version 1.5.0.4 Schrödinger.