Synthesis and biological evaluation of some pyrazolone based Schiff base derivatives as enzymes inhibitors, antioxidant, and anticancer agents

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Research Article

Keywords: Pyrazolone, Antioxidant activity, Cervical cancer, Enzymes inhibitors

DOI: https://doi.org/10.21203/rs.3.rs-540190/v1

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Abstract

Drugs with sufficient efficacy for use in the therapy of Alzheimer and other diseases caused by oxidative stress have not yet been produced until today despite the great efforts. Therefore, many people all over the world are experiencing serious health problems due to these diseases nowadays. In the current study, a series of pyrazolone based Schiff base derivatives (2a-e) (except 2a) as target molecules were successfully synthesized for the first time, and then structurally illuminated by using FT-IR, $^1$H NMR and $^{13}$C NMR. Their inhibition activities on acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and tyrosinase enzymes were extensively tested, respectively. Furthermore, the synthesized molecules were also investigated for antioxidant and anticancer activities. The potential in vitro cytotoxic activities of the title molecules were examined on HeLa cancer and 3T3 mouse normal fibroblast cell lines using MTT assay. Our results showed that 1b ($IC_{50}$: 9.497 mM) and 2a ($IC_{50}$: 30.49 mM) significantly decreased the proliferation of HeLa cells. On the other hand, the apoptotic effect of 1b and 2a were investigated with acridine orange/propidium iodide double staining. The apoptotic cell ratios of the molecules treated with 1b and 2a were determined as 60 and 64%, respectively. While 2b was found to be a very active molecule in antioxidant activities assays in ABTS cation radical scavenging ($IC_{50}$:17.95±0.47 mM) and CUPRAC ($A_{0.5}$:48.73±0.52 mM) activities, 2c had a very active molecule in AChE, BChE and tyrosinase inhibitory activities with 82.79±1.03, 91.39±1.06 and 92.60±1.80 inhibition%, respectively. Also, the target molecules (2a-e) showed better antioxidant and enzyme inhibitory activities than those of ester derivatives (1a-e).

Introduction

Cancer is the deadliest health problem after cardiovascular system diseases. The most basic method employed in the therapy of cancer, which is a preventable and treatable disease, is chemotherapy. Therefore, the discovery and the development of new chemotherapeutics nowadays have become a necessity in order to overcome disadvantages of multidrug resistance [1, 2]. Cervical cancer is known worldwide as the fourth type most prevalent cancer influencing female. It was reported in 2018 that this disease caused almost 570,000 diagnosed cases and 311,000 deaths [3, 4]. In spite of the increasing risk of this type of cancer, no specific treatments are currently available for the patients [2–4]. Extensive tissue damage occurs at the end of most established treatments such as cryotherapy, chemotherapy and surgical excision [5, 6]. In addition, it has been known that the drugs approved for the treatment of cervical cancer by the Food and Drug Administration (FDA) have serious side effects and fall short due to their lack of specificity [7]. Today, the discovery studies of novel drugs that have the potential to be used in the treatment of this disease are ongoing uninterruptedly [8, 9].

Antioxidants, which are molecules to decrease the negative effects of free radicals in living metabolism, have a significant influence in delaying and prohibiting the progression of diverse chronic diseases [10, 11]. In recent years, the search for new drugs with antioxidant effects has increased in drug design
studies, because multifunctional drugs with antioxidant effects are thought to be effective in eliminating free radicals and drug side effects that take place as a result of metabolic events [12–14].

Alzheimer's Disease (AD) is defined as a progressive neurodegenerative disease that causes irreversible loss of cognitive skills and memory. It is the most common cause of dementia in advanced age [15–17]. AD was first defined in 1906 by German psychiatrist and neuropathologist, Alois Alzheimer [18]. This disease is the most prevalent disease among neurodegenerative diseases. Although the prevalence of the disease is 10% over the age of 65, this rate rises to 30% over the age of 85 [19]. In the population over the age of 60, the rate doubles every passing five years. Although it does not depend entirely on gender, it is stated that the risk of AD is higher in females over the age of 85 compared to male [20]. It is predicted that AD will be one of the most serious health problems in the future [21, 22]. Nowadays, inhibition of AChE and BChE enzymes that hydrolyze acetylcholine (ACh) and butyrylcholine (BCh) neurotransmitters have become a therapy option for this disease [23, 24]. For this reason, many researchers have tried to discover new inhibitors of both enzymes for the treatment of AD [25, 26].

Tyrosinase enzyme, which is found in animals, plants, fungi and microorganisms [26], belongs to the polyphenol oxidase (PPO) enzyme class [27]. This enzyme takes the first place among the PPO enzymes that have been best defined and characterized by its structure until now [28]. Tyrosinase enzyme, which is found in almost all living structures, performs different physiological tasks according to the organism type [28]. This enzyme is the main enzyme responsible for the production of melanin pigment in fungi and vertebrates [29]. When there is any injury and tissue damage in plants, it causes browning as a result of enzymatic darkening of phenolic compounds [27, 3, 30]. Tyrosinase not only provides color formation in the pigmenting process in mammals, but it also has a homeostatic effect in preventing UV damage [31–33]. However, in the event that the production pathway of tyrosinase is disrupted for any reason, especially, hyperpigmentation problems often occur. These molecules, used as hypopigmentation agents or tyrosinase inhibitors, can be obtained by chemical or biological means [34].

Schiff bases, namely imines, are usually the condensation products of primary amines with carbonyl compounds under certain conditions [35]. Schiff base derivatives, carrying an azomethine (-CH=N-) functional group, were first reported by Hugo Schiff [36–38]. These compounds have determined to possess a wide spectrum of biological effects such as anticancer [39], antimicrobial [40, 41], antioxidant [42], tyrosinase [43] and cholinesterase properties, etc. [44]. Apart from these, they are also used as used as catalysts, intermediates in organic synthesis, pigments and dyes and as polymer stabilizers [37]. In these days, a large number of researchers working in the field of medicinal chemistry are trying to obtain novel enzyme inhibitors, antioxidant and anticancer agents, which will have the desired efficacy, by using the molecules belonging to different classes of organic compounds [42–44]. Schiff base derivatives are also among the compound classes whose biological activities have been studied most when compared to other compound classes.

Pyrazolone, a five-membered heterocyclic compounds that contains two adjacent nitrogen atoms, can be considered as a derivative of pyrazole which has an additional carbonyl (C = O) group. Since pyrazolone
derivatives have various biological significances such as anti-inflammatory, antipyretic, analgesic, hypoglycemic, antimicrobial, and antioxidant, they attracted a significant attention of medicinal chemists [45, 46]. Schiff bases synthetically derived from 4-aminoantipyrine (4-AAP) are significant pyrazolone analogs, which have multifunctional traits including antifungal, antibacterial, antimalarial, antiviral, anti-inflammatory and antipyretic properties [47, 48]. Nowadays, 4-AAP derivatives are employed as significant biomodel compounds in medicinal and biological fields [49, 50].

The above observations encouraged us to carry out a study targeting the discovery of new enzymes inhibitors, antioxidant and anticancer agents. Thus, it was aimed to investigate anticancer, antioxidant, anticholinesterase and tyrosinase activities of some Schiff base analogs of 4-AAP (2a-e) in vitro conditions in this study. All molecules obtained within the scope of the study were characterized in detail by spectroscopic methods (FT-IR, $^1$H NMR and $^{13}$C NMR).

**Results And Discussion**

**Synthesis and characterization**

In this study, a series of 4-AAP Schiff base derivatives (2a-e) as target molecules were successfully prepared in high purity in two steps according to the pathway shown in Scheme 1. In the first step, the vanillin ester derivatives (1a-e) were easily synthesized with $78-86\%$ yield by the esterification reaction of vanillin with an appropriate benzoyl chloride derivative in a molar ratio of 1:1 in pyridine as solvent. In the second step, target molecules (2a-e) were quickly obtained with $79-87\%$ with yield by treatment of 4-AAP with the corresponding ester derivative in the molar ratio of 1:1 in ethanol as solvent. From these synthesized target molecules, 2b-e are novel but 2a are already known in the literature [51]. All reactions were performed by refluxing in 50 mL reaction flasks for 1 h under reflux with magnetic stirrer. The structural elucidation of all synthesized molecules was performed by $^1$H, $^{13}$C NMR, and FT-IR, respectively.

IR spectra of the Schiff base derivatives (2a-e) afforded the C-H stretching bands of the aromatic rings (3104-2959 cm$^{-1}$), C = O stretching bands of the pyrazolone ring (1646-1635 cm$^{-1}$) and the ester group (1755-1743 cm$^{-1}$). In $^1$H NMR spectra, C-H proton belonging to the imine group (CH = N), indicating that Schiff base was formed, resonated as a singlet peak at 9.62-9.61 ppm. The methyl groups in the 1$^{st}$ and 5$^{th}$ positions of the heterocyclic pyrazolone ring were observed as two singlets at 3.21-3.20 ppm and 2.49 ppm, respectively. In addition, the protons of the methoxy group were also found to resonate at 3.90-3.85 ppm as a singlet. The protons of the aromatic rings were recorded in the aromatic region between 9.12 and 7.12 ppm with splitting in the form of doublet, triplet, doublet of doublet or multiplet. When $^{13}$C NMR spectra of Schiff bases were examined, it was seen that the carbons of the methyl groups on the pyrazolone ring resonated at 35.81-35.78 and 10.31-10.29 ppm, respectively. The signal of the carbon of C = O group of the pyrazolone ring appeared at 160.03-159.99 ppm. The signal of the carbons of CH=N group was observed at 153.85-153.59 ppm. Other aromatic carbon signals were detected as 152.73-110.86 ppm.
Biological evaluation

Cytotoxic effects of the synthesized molecules

All synthesized molecules were examined for anti-tumor potential against HeLa (human cervical carcinoma), and 3T3 mouse fibroblast cell lines (control cell line) using MTT test. IC$_{50}$ values of all molecules are shown in Table 1. IC$_{50}$ values of these molecules showed different effects on each cell lines, and those with common effects were screened. Thus, the molecule with a concentration of less than 50 mM and low cell viability among all molecules was chosen as the drug candidate. In order to find potential candidates for the anticancer agents, the molecules cytotoxic to cancer cells and harmless to normal cells were selected. IC$_{50}$ values of 1b and 2a on HeLa cells showed the expected effect. 2e appeared to have the same anti-survival effect in two cell lines. However, it also showed toxic activity on normal 3T3 cells. Therefore, we selected 1b and 2a for concentration activities of IC50 in HeLa cells for ongoing our cellular studies.

![Table 1. IC$_{50}$ values of all synthesized molecules](image)

| Compounds | HeLa  | 3T3  |
|-----------|-------|------|
| 1a        | 321.78| 130.97|
| 1b        | 9.497 | 16.73|
| 1c        | ND    | ND   |
| 1d        | 338.85| 84.60|
| 1e        | 209.69| 472.55|
| 2a        | 30.49 | 66.7 |
| 2b        | 104.49| 1130.57|
| 2c        | 174.23| 40.54|
| 2d        | 165.32| 1416.06|
| 2e        | 22.71 | 14.33|

The morphological effects of 1b and 2a on HeLa cervical cancer cells were examined with images taken at 10x magnification (Fig. 1a). When compared with the control group cells, it was observed that cells incubated with 1b and 2a had shrinkage and divergence from each other. After 24 hours of incubation, the viability of HeLa cells incubated with 1b and 2a decreased to 48% and 41%, respectively (Fig. 1b, 1c).

In a similar study, Teran et al. synthesized Schiff base derivatives of 4-AAP and stated that the IC$_{50}$ doses of these molecules on mammalian macrophage cells were between 0.11-0.15 mg/mL [52]. When compared with this study, it is seen that (2a-e) synthesized in our study are less cytotoxic to normal cells.
Apoptotic effects of the synthesized molecules

Acridine orange and propidium iodide are dyes used to differentiate between living and non-living cells. Acridine orange dyes living cells, and gives them a green fluorescent, while propidium iodide stains dead cells and emits red fluorescence [53]. To investigate the apoptotic effects of 1b and 2a, HeLa cells were treated with 10 μM doses of these molecules. It was found that cells treated with 1b and 2a emitted more red fluorescence compared to the control group (Fig.2a). On the other hand, the apoptotic cell ratios of the molecules treated with 1b and 2a were 60% and 64%, respectively. In control cells, the rate of apoptotic cells was calculated as 22% (Fig. 2b). These results confirm that the apoptotic pathways of HeLa cells treated with 1b and 2a are activated.

Antioxidant activity results

Of synthesized Schiff base derivatives, 2a, 2b, 2c and 2e showed strong antioxidant activities in ABTS cation radical scavenging and CUPRAC assays, the most active one of which is 2b with IC$_{50}$:17.95±0.47 mM value which is very close to the BHA compound (IC$_{50}$:17.59±0.10 mM) used as a standard. The rest of Schiff bases (2a-e) had no activity in DPPH free radical scavenging and metal chelating assays with IC$_{50}$ values >1000 μM (Table 2). While (2a-e) showed good activity, (1a-e) demonstrated very weak activity. The most active synthesized compound is 2e. 2e (A$_{0.5}$:43.75±0.62 mM) and 2b (A$_{0.5}$:48.73±0.52 mM) have better activity than α-TOC (A$_{0.5}$:50.58±0.39 mM) used as a standard (Table 2). Synthesized molecules demonstrated no activities in DPPH free radical scavenging and metal chelating assays (Table 2).

Table 2 Antioxidant activity results*
| Samples | DPPH Free Radical | ABTS Cation Radical | Metal Chelate | CUPRAC | A<sub>0.5</sub> values (mM)<sup>b</sup> |
|---------|-------------------|---------------------|--------------|--------|---------------------------------|
| 1a      | >1000             | >100                | >1000        |        | 503.13±2.05                     |
| 1b      | >1000             | >1000               | >1000        | >1000  |                                |
| 1c      | >1000             | >1000               | >1000        |        | 644.17±1.84                     |
| 1d      | >1000             | >1000               | >1000        |        | 545.29±1.28                     |
| 1e      | >1000             | >1000               | >1000        |        | 280.86±1.16                     |
| 2a      | >1000             | 22.49±0.59          | >1000        |        | 55.45±0.76                      |
| 2b      | >1000             | 17.95±0.47          | >1000        |        | 48.73±0.52                      |
| 2c      | >1000             | 28.98±0.52          | >1000        |        | 89.16±0.48                      |
| 2d      | >1000             | >1000               | >1000        |        | 118.52±1.26                     |
| 2e      | >1000             | 87.16±0.75          | >1000        |        | 43.75±0.62                      |
| BHA     | 48.37±0.58        | 17.59±0.10          | -            |        | 18.44±0.15                      |
| α-TOC   | 16.30±0.79        | 9.74±0.42           | -            |        | 50.58±0.39                      |
| BHT     | 354.31±1.23       | 13.25±0.27          | -            |        | 27.68±0.24                      |
| EDTA    | -                 | -                   | 26.82±0.10   | -      |                                |

*Values are means of three parallel measurement ± Standard deviation, n=3

aValues were given as IC<sub>50</sub> for DPPH free radical, ABTS cation radical scavenging and metal chelating activities

bValues were given as A<sub>0.5</sub> for CUPRAC activity

**Enzyme inhibitory activity results**

For acetylcholinesterase inhibitory activity, galanthamine was used to be the standard with 81.78±0.62% inhibition value. 2c with 82.79±1.03% inhibition value showed the best anti AChE activity among all tested compounds. 2d also showed strong activity in AChE enzyme inhibitory activity (74.35±0.43% inhibition). We can say that (2a-e) showed better activity than (1a-e) looking at Table 3.

2a, 2c and 2e demonstrated very strong activity against BChE enzyme with 98.79±1.47, 91.39±1.06 and 84.88±1.08 inhibition %, respectively, better than galanthamine (76.58±1.08% inhibition) used as standard. Also, it can be seen in Table 3 that (2a-e) showed better activity than (1a-e).
Kojic acid was used as standard compound in tyrosinase enzyme inhibitory activity with 69.38±0.80 inhibition %. 2c and 2a showed very strong tyrosinase enzyme inhibitory activity, with 92.60±1.80 and 74.98±1.33 % inhibition, respectively, which are better than kojic acid. Again, it is possible to say that (2a-e) showed better activity than (1a-e) (Table 3).

**Table 3** Enzyme inhibition activities results*

| Comp. | AChE       | BChE       | Tyrosinase   |
|-------|------------|------------|--------------|
| 1a    | NA         | 14.77±0.39 | 12.68±0.08   |
| 1b    | 15.34±0.08 | 20.96±1.08 | 16.17±0.90   |
| 1c    | 7.32±0.00  | 50.00±0.85 | NA           |
| 1d    | NA         | NA         | NA           |
| 1e    | 58.30±1.04 | 53.06±0.71 | 21.74±0.57   |
| 2a    | 47.01±0.57 | 98.79±1.47 | 74.98±1.33   |
| 2b    | 49.44±0.93 | 41.56±0.60 | 25.11±0.80   |
| 2c    | 82.79±1.03 | 91.39±1.06 | 92.60±1.80   |
| 2d    | 74.35±0.43 | 63.29±0.37 | 6.98±0.09    |
| 2e    | 46.16±0.75 | 84.88±1.08 | 38.09±0.54   |
| Galantamine<sup>a</sup> | 81.78±0.62f | 76.58±1.08 | -            |
| Kojic acid<sup>b</sup> | - | - | 69.38±0.80 |

*All enzyme inhibition activity values were given as inhibition % at 200 mg/mL

<sup>a</sup> Standard compound for AChE and BChE

<sup>b</sup> Standard compound for Tyrosinase

NA: Not Active

**Conclusion**

In this study, a series of Schiff base derivatives of 4-AAP (2a-e) were successfully prepared, fully characterized by some spectral techniques; and their antioxidant activities were examined by using four different methods, respectively. The inhibitory effects of the synthesized molecules against AChE, BChE, and tyrosinase enzymes were extensively screened. According to the results obtained from all methods applied, 2b showed the best antioxidant activity. (2a-e) generally showed better antioxidant activity than (1a-e), except for DPPH free radical scavenging and metal chelating activity. 2c exhibited a very potent
activity in AChE and tyrosinase inhibitory activities. In addition, according to the results achieved from all procedures used, 2a showed the best enzyme inhibitory activities. Besides these, the cytotoxic effects of these molecules were also evaluated on HeLa human cervical cancer and 3T3 mouse fibroblast cell lines. Compared to other compounds, 1b and 2a showed the highest cytotoxic effect on HeLa cells.

**Materials And Methods**

**Chemicals and instrumentations**

Unless otherwise stated, all chemicals used in this study were obtained from the representatives of Aldrich and Sigma-Aldrich Companies, and employed without further purification. Melting points of the synthesized molecules were determined in open capillary tubes by using a melting point apparatus (Barnstead IA9100 Electrothermal Digital Melting Points Apparatus), and uncorrected. Characterization of all compounds was performed by using Fourier Transform Infrared Spectrometer (FTIR Agilent Cary 630 with ATR), and $^1$H and $^{13}$C NMR spectra (Bruker Avance 500 MHz spectrometer by using DMSO-$d_6$ as solvent and tetramethylsilane (TMS) as an internal standard at 500 MHz and 125 MHz, respectively.

**General procedures for biological studies**

**Anticancer Activity**

The synthesized molecules were investigated for their anticancer activities using the cervical cancer cell line (HeLa). Mouse normal fibroblasts cell line (3T3) was used as a control cell. The anticancer activity was evaluated using MTT assay [54]. HeLa cancer cells were maintained in DMEM medium (including 100 U/mL penicillin, 100 mg/mL streptomycin, 10% FBS and 2 mM L-glutamine). 3T3 mouse normal fibroblast cells were also maintained in DMEM medium. Cells were incubated at 37 °C in a humidified atmosphere with 5% CO$_2$. Firstly, cells were removed from the flask bottom with trypsin-EDTA solution (0.25%, Invitrogen) and seeded into 96-well plates (1x10$^4$ cells/well). The cells were incubated for 24 hours to adhere to the bottom of the wells. After the adherence, cell viability was determined for all compound effects. The different concentrations (1, 10, 100, 1000 mM) of each molecule were added in each well and incubated for 24 h, respectively. Then, the mediums of the cells were discharged, and cells were washed with PBS. 100 µL fresh medium added to each well. After this step, a 10 µL MTT solution (5mg/mL) was added to each well. Cells were also incubated for 4 h in growth condition for labeling the cells. At the end of the incubation, the medium in the wells was discharged and 100 µL of DMSO was added to each well to dissolve the formed formazan dye. The absorbance of color change from formazan precipitate solubility was measured at 570 nm using ELISA reader (Epoch, Biotek, USA). MTT assay was carried out triplicate. Besides, IC$_{50}$ values of the molecules on HeLa and 3T3 cells were calculated by using AATbio IC50 calculator.

**PI/AO Double Staining**
PI/AO double staining was performed to determine the apoptotic effect of the synthesized molecules on HeLa cells. A density of $1 \times 10^5$ HeLa cells were firstly seeded in a six well plate. After 24 h of incubation, cells were treated with an IC$_{50}$ concentration of 1b and 2a. Control cells were maintained with PBS buffer. After incubating for 24 h, cells were washed twice with DPBS; and 2 mL of fresh medium was added onto the cells. Subsequently, 10 μg/mL acridine orange and 10 μg/mL propidium iodide were added into cell medium after 24 h of incubation. Cells were incubated for 10 min for staining. Then, staining cells were washed with DPBS to remove the excess dye; and 2 mL of fresh medium was also added onto the cells. Images of cells were captured under a fluorescence microscope (Olympus BX51, Japan).

**Antioxidant methods**

In this study, antioxidant activity of all synthesized molecules were determined according to slightly modified modern versions of previously reported methods. For the determination of antioxidant activity of each title molecule, four different methods known in the literature and frequently used were preferred. These methods are 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) cation radical decolorisation assay, metal chelating activity and Cupric Reducing Antioxidant Capacity (CUPRAC) assay. IC$_{50}$ values in the current study were determined by a concentration-inhibition graph. A$_{0.5}$ values were computed by a concentration-absorbance graph.

**DPPH free radical scavenging assay**

The DPPH radical scavenging activity of Schiff base derivatives (2a-e) and vanillin ester derivatives (1a-e) was measured by a spectrophotometric method [55]. This procedure was based on the reduction in ethanol solution of DPPH. For this, 2, 5, 10 and 20 µL of 1 mM stock solutions of each molecule were firstly prepared, and then each of the prepared solutions was completed to 40 µL with DMSO, respectively. Afterwards, 160 µL of 0.1 mM of DPPH solutions was added into each well in the microplate, separately. Then, the resulting solution was allowed to react for about 30 min at room temperature in the dark. Finally, the absorbance was measured at 517 nm against a blank, respectively. In this study, in order to calculate the inhibition of DPPH in percent (I %), the following formula was utilized:

$$\text{Inhibition \%} = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100$$

where $A_{\text{control}}$ is the absorbance of the control reaction (containing all reagents except for the tested molecules), and $A_{\text{sample}}$ is the absorbance of the tested molecules. BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene) and α-TOC (α-Tocopherol) in this process were employed as positive control. All tests were repeated three times one after the other.

**ABTS cation radical scavenging assay**

The inhibition of decolorisation percent of ABTS$^+$ cation radical of Schiff base derivatives (2a-e) and vanillin ester derivatives 1a-e in this study was determined the inhibition percentage as a function of time
and concentration, and then the results obtained were assessed by comparison with BHT, BHA and α-TOC molecules employed as standards[56]. For this, 2, 5, 10 and 20 µL of 1 mM stock solutions of each molecule were firstly prepared, and then each of the prepared solutions was completed to 40 µL with DMSO, respectively. Afterwards, these solutions were added to each well respectively, and then, 160 mL of 7 mM ABTS solutions were added into each well in the microplate, separately. After that, the mixture was allowed to react for about 6 min at room temperature. Finally, the absorbance was measured at 734 nm. In this study, in order to calculate ABTS cation radical decolorisation activity as inhibition%, the following formula was utilized:

\[
\text{Inhibition}\% = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100
\]

where A is the absorbance. All tests were repeated three times one after the other.

**Metal chelating activity**

Metal chelating ability of Schiff base derivatives (2a-e) and vanillin ester derivatives (1a-e) was investigated according to the method of Dinis et al., [57]. For this, 2, 5, 10 and 20 µL of 1 mM stock solutions of each molecule were pipetted to each well, and then each of the samples were completed to 188 µL with DMSO and then 4 µL of 2 mM ferrous (II) chloride was added to the resulting solution, separately. After these processes, the reaction was initiated by the adding 8 µL of 5 mM ferrozine to the obtained solution. The mixture was allowed to react for about 10 min at room temperature. Finally, the absorbance was measured at 562 nm against a blank, respectively. The results obtained were stated as percentage of inhibition of the ferrozine-Fe2+ complex formation. In order to calculate the percentage inhibition of the ferrozine-Fe2+ complex formation, the following formula was utilized:

\[
\text{Metal chelating ability}\% = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100
\]

where A is the absorbance. EDTA in this process was employed as a positive control. All tests were repeated three times one after the other.

**CUPRAC assay**

This method consists of the reduction of Cu(II)-neocuproine into its colored form Cu(I)- neocuproine chelate in the presence of antioxidant molecules [58]. For this, 2.5, 6.25, 12.5, and 25 µL of 1 mM stock solutions of each molecule were pipetted to each well and then 61 µL of 10 mM CuCl2, 61 µL of 7.5 mM neocuproine, and 61 µL of 1 M of NH4OAc solutions were added into prepared solutions at different concentrations, respectively. After obtaining the complex during the experiment, the absorbance was carefully measured at 450 nm Finally, A_{0.5} values obtained from a concentration-absorbance graph were compared with standard molecules, BHA, BHT and α-tocopherol. All tests were repeated three times one after the other.

**Enzyme inhibition activity assays**
Anti-tyrosinase activity and anti-cholinesterase assay of all prepared molecules in this study were investigated according to modified modern versions of the earlier reported methods.

**Anti-cholinesterase assay**

The inhibitory effect of Schiff base derivatives (2a-e) and vanillin ester derivatives (1a-e) on AChE and BChE enzymes activities in the current study was determined according to slightly altered spectrophotometric method of Ellman et al., [59]. For this, all synthesized molecules were firstly dissolved in DMSO to obtain stock solutions at 4 mM concentration. Afterwards, aliquots of 150 µL of 100 mM sodium phosphate buffer (pH 8.0), 10 µL of sample solution and 20 µL BChE (or AChE) solution were mixed, and then, the resulting solution was incubated for about 15 min at 25 °C. After these processes, 10 µL of Ellman's reagent [5, 5′-dithiobis (2-nitrobenzoic acid), DTNB] was added to the solution. Finally, the reaction was initiated by the addition of 10 µL of butyrylthiocholine iodide (or acetylthiocholine iodide) as substrate to the obtained solution. After 30 min, the absorbances were measured at 412 nm. The final solution of tested molecules was 200 µL. In this study, in order to calculate the percentage of both enzyme inhibitions, the following formula was utilized:

\[
\text{Inhibition} \, (\%) = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100
\]

where A is the absorbance. Galantamine in this process was employed as a positive control. All tests were repeated three times one after the other.

**Anti tyrosinase activity**

Anti-tyrosinase activity of Schiff base derivatives (2a-e) and vanillin ester derivatives (1a-e) was determined according to the method designed by Hearing and Jimenez [60]. For this, all synthesized molecules were firstly dissolved in DMSO to obtain stock solutions at 4 mM concentration. Afterwards, aliquots of 150 µL of 100 mM sodium phosphate buffer (pH 6.8), 10 µL of sample solution and 20 µL tyrosinase solution were mixed, and then, the resulting solution was shaking for 3 minutes and incubated for 10 min at 37 °C. After these processes, 20 µL of DOPA solution which is used as substrate was added to the mixture. After 10 min at 37 °C, the absorbances were measured at 475 nm. In order to calculate the percentage of all enzyme inhibitions, the following formula was utilized:

\[
\text{Inhibition} \, (\%) = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100
\]

where A is absorbance. Kojic acid for the positive control was employed as an inhibitor. All tests were repeated three times one after the other.

**Statistical analysis**

The results of the antioxidant, anti-cholinesterase and tyrosinase activity assays in this work are stated as the mean ± SD of three parallel measurements. The statistical significance was forecasted by utilizing a Student's t-test, where p value < 0.05 was considered significant.
Experimental procedure for synthesis of title molecules (1a-e) and (2a-e)

General procedure for synthesis of ester derivatives of vanillin (1a-e)

A mixture of vanillin (5 mmol) and an appropriate benzoyl chloride derivative (5 mmol) in pyridine (20 mL) in 100 mL two-neck round-bottomed flask equipped with a magnetic stirrer, reflux condenser and thermometer were heated under reflux at 115 ºC for 1 h with continuous stirring [61]. After completion of the reaction, the mixture was cooled to room temperature and poured into 100 mL of icy water. Afterward it was held for 6 h at room temperature, the formed precipitate was filtered off, washed with 50 mL of distilled water, dried under air suction, and crystallized from ethanol to get pure ester derivative.

Spectral data for all compounds (1a-e)

4-Formyl-2-methoxyphenyl benzoate (1a)

White solid, yield: 81 %, m.p. 75-76 °C (lit. [62] m.p. 75-76 °C). FT-IR (ATR): $\nu_{\text{max}}$ (cm$^{-1}$), 3061, 2960 (C-H$_{\text{arom}}$), 2830, 2735 (C-H$_{\text{aldehyde}}$), 1732 (C=O$_{\text{ester}}$), 1697 (C=O$_{\text{aldehyde}}$), 1594 (C=C). $^1$H NMR (500 MHz; DMSO-$d_6$, ppm): $\delta$ 10.02 (s, 1H, CH$_{\text{aldehyde}}$), 8.17–8.12 (m, 2H, H$_{\text{arom}}$), 7.78 (t, $J$= 7.5 Hz, 1H, H$_{\text{arom}}$), 7.80–7.76 (m, 4H, H$_{\text{arom}}$), 7.54 (d, $J$= 8.0 Hz, 1H, H$_{\text{arom}}$), 3.87 (s, 3H, OCH$_3$).

$^{13}$C NMR (125 MHz; DMSO-$d_6$, ppm): $\delta$ 192.55 (C=O$_{\text{aldehyde}}$), 164.07 (C=O$_{\text{ester}}$), 152.13, 144.77, 135.75, 134.78, 130.40, 129.56, 128.69, 124.39, 124.12, 112.44 (C$_{\text{arom}}$), 56.59 (OCH$_3$).

4-Formyl-2-methoxyphenyl 2-nitrobenzoate (1b)

White solid, yield: 78 %, m.p. 121-122 °C (lit. [63] m.p. 111.0-112.5 °C). FT-IR (ATR): $\nu_{\text{max}}$ (cm$^{-1}$), 3075, 2960 (C=H$_{\text{arom}}$), 2826, 2736 (C-H$_{\text{aldehyde}}$), 1747 (C=O$_{\text{ester}}$), 1696 (C=O$_{\text{aldehyde}}$), 1599 (C=C$_{\text{arom}}$), 1526 (NO$_2_{\text{asym}}$), 1345 (NO$_2_{\text{sym}}$). $^1$H NMR (500 MHz; DMSO-$d_6$, ppm): $\delta$ 10.02 (s, 1H, CH$_{\text{aldehyde}}$), 8.20–8.10 (m, 2H, H$_{\text{arom}}$), 7.99–7.93 (m, 2H, H$_{\text{arom}}$), 7.71–7.66 (m, 2H, H$_{\text{arom}}$), 7.52 (d, $J$= 8.0 Hz, 1H, H$_{\text{arom}}$), 3.93 (s, 3H, OCH$_3$).

$^{13}$C NMR (125 MHz; DMSO-$d_6$, ppm): $\delta$ 192.55 (C=O$_{\text{aldehyde}}$), 164.07 (C=O$_{\text{ester}}$), 152.13, 144.77, 135.75, 134.78, 130.40, 129.56, 128.69, 124.39, 124.12, 112.44 (C$_{\text{arom}}$), 56.59 (OCH$_3$).

4-Formyl-2-methoxyphenyl 3-nitrobenzoate (1c)

White solid, yield: 83 %, m.p. 125-126 °C (lit. [64] m.p. 114-115 °C). FT-IR (ATR): $\nu_{\text{max}}$ (cm$^{-1}$), 3087, 2933 (C=H$_{\text{arom}}$), 2842, 2740 (C-H$_{\text{aldehyde}}$), 1740 (C=O$_{\text{ester}}$), 1686 (C=O$_{\text{aldehyde}}$), 1595 (C=C$_{\text{arom}}$), 1528 (NO$_2_{\text{asym}}$), 1344 (NO$_2_{\text{sym}}$). $^1$H NMR (500 MHz; DMSO-$d_6$, ppm): $\delta$ 10.03 (s, 1H, CH$_{\text{aldehyde}}$), 8.81–8.76 (m, 1H, H$_{\text{arom}}$), 8.62–8.60 (m, 1H, H$_{\text{arom}}$), 8.56–8.54 (m, 1H, H$_{\text{arom}}$), 7.94 (t, $J$= 8.0 Hz, 1H, H$_{\text{arom}}$), 7.72–7.64 (m, 2H, H$_{\text{arom}}$), 7.60 (d, $J$= 8.0 Hz, 1H, H$_{\text{arom}}$), 3.93 (s, 3H, OCH$_3$).

$^{13}$C NMR (125 MHz; DMSO-$d_6$, ppm): $\delta$ 192.57 (C=O$_{\text{aldehyde}}$), 162.43 (C=O$_{\text{ester}}$), 151.93, 148.58, 144.27, 136.37, 136.01, 131.60, 130.20, 129.20, 124.74, 124.29, 124.09, 112.60 (C$_{\text{arom}}$), 56.69 (OCH$_3$).
4-Formyl-2-methoxyphenyl 4-nitrobenzoate (1d)

White solid, yield: 86 %, m.p. 188-189 °C (lit. [64] m.p. 189-190 °C). FT-IR (ATR): $\nu_{\text{max}}$ (cm$^{-1}$), 3109, 2948 (C-H$_{\text{arom}}$), 2848, 2754 (C-H$_{\text{aldehyde}}$), 1744 (C=O$_{\text{ester}}$), 1697 (C=O$_{\text{aldehyde}}$), 1596 (C=C$_{\text{arom}}$), 1521 (NO$_2_{\text{asym}}$), 1347 (NO$_2_{\text{sym}}$). $^1$H NMR (500 MHz; DMSO-$d_6$ ppm): $\delta$ 10.03 (s, 1H, CH$_{\text{aldehyde}}$), 8.45–8.36 (m, 4H, H$_{\text{arom}}$), 7.70–7.65 (m, 2H, H$_{\text{arom}}$), 7.59 (d, $J$ = 8.0 Hz, 1H, H$_{\text{arom}}$), 3.88 (s, 3H, OCH$_3$)

4-Formyl-2-methoxyphenyl 3,5-dinitrobenzoate (1e)

White solid, yield: 80%, m.p. 162-163 °C (lit. [65] m.p. 163-164 °C). FT-IR (ATR): $\nu_{\text{max}}$ (cm$^{-1}$), 3109, 2948 (C-H$_{\text{arom}}$), 2848, 2754 (C-H$_{\text{aldehyde}}$), 1744 (C=O$_{\text{ester}}$), 1697 (C=O$_{\text{aldehyde}}$), 1596 (C=C$_{\text{arom}}$), 1521 (NO$_2_{\text{asym}}$), 1347 (sym., NO$_2$). $^1$H NMR (500 MHz; DMSO-$d_6$ ppm): $\delta$ 10.03 (s, 1H, CH$_{\text{aldehyde}}$), 9.13 (t, $J$ = 2.0 Hz, 1H, H$_{\text{arom}}$), 9.08 (d, $J$ = 2.0 Hz, 2H, H$_{\text{arom}}$), 7.72–7.63 (m, 43H, H$_{\text{arom}}$), 3.89 (s, 3H, OCH$_3$).

General procedure for synthesis of target molecules (2a-e)

A mixture of 4-amino-1,5-dimethyl-2-phenylpyrazol-3-one (1 mmol) and the corresponding ester derivative (1 mmol) was dissolved in anhydrous ethanol (10 mL) in 50 mL two-neck round-bottomed flask equipped with a magnetic stirrer, reflux condenser and thermometer. The reaction mixture was heated gently with continuous stirring at 80 °C under reflux for 2 h. After the completion of the reaction, the mixture was allowed to cool to room temperature, and then, the obtained crude product was removed by filtration, washed several times with petroleum ether. The residue was crystallized from ethanol to give the target molecule.

Spectral data for all compounds (2a-e)

4-[[4-(benzoyloxy)-3-methoxyphenylmethyleneamino]-1,2-dihydro-1,5-dimethyl-2-phenyl-3$H$pyrazol-3-one (2a)

Light yellow powder, yield: 81%, m.p. 206-207 °C (lit. [51] m.p. 195-197 °C). FT-IR (ATR): $\nu_{\text{max}}$ (cm$^{-1}$), 3065, 3037 (C-H$_{\text{arom}}$), 2936, 2839 (C-H$_{\text{aliph}}$), 1743 (C=O$_{\text{ester}}$), 1646 (C=O$_{\text{pyrazolone}}$), 1579, 1484 (C=C$_{\text{arom}}$ and C=N$_{\text{imine}}$). $^1$H NMR (500 MHz; DMSO-$d_6$ ppm): $\delta$ 9.61 (s, 1H, CH$_{\text{imine}}$), 8.17–8.11 (m, 2H, H$_{\text{arom}}$), 7.79–7.74 (m, 1H H$_{\text{arom}}$), 7.65–7.60 (m, 3H, H$_{\text{arom}}$), 7.56–7.52 (m, 2H, H$_{\text{arom}}$), 7.46 (dd, $J$ = 8.0, 1.5 Hz, 1H, H$_{\text{arom}}$), 7.41-7.37 (m, 3H, H$_{\text{arom}}$), 7.34 (d, $J$ = 8.0 Hz, 1H, H$_{\text{arom}}$), 3.85 (s, 3H, OCH$_3$), 3.20 (s, 3H, –N-CH$_3$), 2.49 (s, 3H, =C-CH$_3$). $^{13}$C NMR (125 MHz; DMSO-$d_6$ ppm): $\delta$ 164.39 (C=O$_{\text{ester}}$), 160.03 (C=O$_{\text{pyrazolone}}$), 157.99, 149.08, 143.92, 139.42, 131.53, 129.92, 124.21, 124.05, 123.88, 112.75 (C$_{\text{arom}}$), 56.74 (OCH$_3$).
153.85 (C=N_{imine}), 152.73, 151.74, 141.34, 137.24, 135.03, 134.62, 130.33, 129.65, 129.53, 129.01, 127.42, 125.13, 123.81, 120.71, 116.64, 110.86 (C_{arom}), 56.34 (OCH₃), 35.81 (−N-CH₃), 10.31 (−C-CH₃).

4-[[4-(2-nitrobenzoyloxy)-3-methoxyphenyl][methylene]amino]-1,2-dihydro-1,5-dimethyl-2-phenyl-3H-pyrazol-3-one (2b)

Light yellow powder, yield: 87%, m.p. 229-230 °C. FT-IR (ATR): υ_{max} (cm⁻¹), 3069, 2959 (C-H_{arom}), 2937, 2870 (C-H_{aliph}), 1750 (C=O_{ester}), 1635 (C=O_{pyrazolone}), 1571, 1494 (C=C_{arom} and C=N_{imine}), 1529 (NO₂_{asym}), 1348 (NO₂_{sym}). ¹H NMR (500 MHz; DMSO-d₆, ppm): δ 9.61 (s, 1H, CH_{imine}), 8.20-8.15 (m, 1H, H_{arom}), 8.12-8.08 (m, 1H H_{arom}), 7.97-7.92 (m, 2H, H_{arom}), 7.65 (d, J= 1.5 Hz, 1H, H_{arom}), 7.57-7.52 (m, 2H, H_{arom}), 7.48 (dd, J= 8.0, 1.5 Hz, 1H, H_{arom}), 7.40-7.37 (m, 3H, H_{arom}), 7.32 (d, J= 8.0 Hz, 1H, H_{arom}), 3.90 (s, 3H, OCH₃), 3.20 (s, 3H, −N-CH₃), 2.49 (s, 3H, =C-CH₃). ¹³C NMR (125 MHz; DMSO-d₆, ppm): δ 162.99 (C=O_{ester}), 159.99 (C=O_{pyrazolone}), 153.61 (C=N_{imine}), 152.75, 151.57, 148.63, 140.49, 137.71, 135.00, 134.29, 134.26, 130.89, 129.65, 127.46, 125.28, 125.18, 124.90, 123.14, 120.69, 116.54, 111.13 (C_{arom}), 56.51 (OCH₃), 35.77 (−N-CH₃), 10.30 (=C-CH₃).

4-[[4-(3-nitrobenzoyloxy)-3-methoxyphenyl][methylene]amino]-1,2-dihydro-1,5-dimethyl-2-phenyl-3H-pyrazol-3-one (2c)

Yellow powder, yield: 85%, m.p. 229-230 °C. FT-IR (ATR): υ_{max} (cm⁻¹), 3072, 2970 (C-H_{arom}), 2941, 2867 (C-H_{aliph}), 1747 (C=O_{ester}), 1645 (C=O_{pyrazolone}), 1575, 1462 (C=C_{arom} and C=N_{imine}), 1531 (NO₂_{asym}), 1344 (NO₂_{sym}). ¹H NMR (500 MHz; DMSO-d₆, ppm): δ 9.61 (s, 1H, CH_{imine}), 8.81-8.76 (m, 1H, H_{arom}), 8.62-8.54 (m, 2H H_{arom}), 7.93 (t, J= 8.0 Hz, 1H, H_{arom}), 7.66 (d, J= 1.5 Hz, 1H, H_{arom}), 7.57-7.53 (m, 2H, H_{arom}), 7.48 (dd, J= 8.0, 1.5 Hz, 1H, H_{arom}), 7.41-7.37 (m, 4H, H_{arom}), 3.86 (s, 3H, OCH₃), 3.20 (s, 3H, −N-CH₃), 2.49 (s, 3H, =C-CH₃). ¹³C NMR (125 MHz; DMSO-d₆, ppm): δ 162.76 (C=O_{ester}), 160.00 (C=O_{pyrazolone}), 153.71 (C=N_{imine}), 152.73, 151.53, 148.58, 140.91, 137.57, 136.33, 135.00, 131.58, 130.50, 129.66, 129.06, 127.47, 125.18, 124.69, 123.69, 120.70, 116.57, 110.96 (C_{arom}), 56.41 (OCH₃), 35.78 (−N-CH₃), 10.30 (=C-CH₃).

4-[[4-(4-nitrobenzoyloxy)-3-methoxyphenyl][methylene]amino]-1,2-dihydro-1,5-dimethyl-2-phenyl-3H-pyrazol-3-one (2d)

Yellow powder, yield: 79%, m.p. 235-237 °C. FT-IR (ATR): υ_{max} (cm⁻¹), 3104, 3011 (C-H_{arom}), 2940, 2867 (C-H_{aliph}), 1740 (C=O_{ester}), 1645 (C=O_{pyrazolone}), 1579, 1491 (C=C_{arom} and C=N_{imine}), 1525 (NO₂_{asym}), 1347 (NO₂_{sym}). ¹H NMR (500 MHz; DMSO-d₆, ppm): δ 9.62 (s, 1H, CH_{imine}), 8.45-8.41 (m, 2H, H_{arom}), 8.40-8.36 (m, 2H, H_{arom}), 7.66 (d, J= 1.5 Hz, 1H, H_{arom}), 7.57-7.53 (m, 2H, H_{arom}), 7.48 (dd, J= 8.0, 1.5 Hz, 1H, H_{arom}), 7.42-7.38 (m, 4H, H_{arom}), 3.87 (s, 3H, OCH₃), 3.21 (s, 3H, −N-CH₃), 2.49 (s, 3H, =C-CH₃). ¹³C NMR (125 MHz; DMSO-d₆, ppm): δ 163.00 (C=O_{ester}), 159.99 (C=O_{pyrazolone}), 153.71 (C=N_{imine}), 152.73, 151.50,
151.18, 140.95, 137.56, 135.00, 134.36, 131.84, 129.65, 127.45, 125.16, 124.63, 123.66, 120.70, 116.58, 110.95 (C_{arom}), 56.40 (OCH₃), 35.78 (–N-CH₃), 10.29 (=C-CH₃).

4-[[[4-(3,5-dinitrobenzoyloxy)-3-methoxyphenyl]methylene]amino]-1,2-dihydro-1,5-dimethyl-2-phenyl-3H-pyrazol-3-one (2e)

Yellow powder, yield: 80%, m.p. 227-228 °C. FT-IR (ATR): \( \nu_{\text{max}} \) (cm\(^{-1}\)), 3095, 3071 (C-H\(_{arom}\)), 2968, 2876 (C-H\(_{aliph}\)), 1755 (C=O\(_{ester}\)), 1573, 1488 (C=C\(_{arom}\) and C=N\(_{imine}\)), 1544 (NO\(_{2asym}\)), 1344 (NO\(_{2sym}\)).

\(^1\)H NMR (500 MHz; DMSO-d\(_6\), ppm): \( \delta \) 9.61 (s, 1H, CH\(_{imine}\)), 9.12 (d, \( J = 2.0 \) Hz, 1H, H\(_{arom}\)), 7.67 (s, 1H, H\(_{arom}\)), 7.54 (t, \( J = 7.5 \) Hz, 2H, H\(_{arom}\)), 7.50 (d, \( J = 8.0 \) Hz, 1H, H\(_{arom}\)), 7.44 (d, \( J = 8.0 \) Hz, 1H, H\(_{arom}\)), 7.41-7.37 (m, 3H, H\(_{arom}\)), 3.87 (s, 3H, OCH₃), 3.21 (s, 3H, –N-CH₃), 2.49 (s, 3H, =C-CH₃).

\(^{13}\)C NMR (125 MHz; DMSO-d\(_6\), ppm): \( \delta \) 161.30 (C=O\(_{ester}\)), 159.98 (C=O\(_{pyrazolone}\)), 153.59 (C=N\(_{imine}\)), 152.76, 151.39, 149.08, 140.60, 137.83, 135.00, 131.79, 129.87, 129.66, 127.47, 125.19, 123.76, 123.58, 120.67, 116.54, 111.07 (C\(_{arom}\)), 56.46 (OCH₃), 35.78 (–N-CH₃), 10.30 (=C-CH₃).

Declarations

Conflict of Interest: The authors declare that they have no conflict of interest.

References

1. Doll R, Peto R. The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. JNCI: Journal of the National Cancer Institute. 1981;66(6):1192-308.

2. Danaei G, Vander Hoorn S, Lopez AD, Murray CJ, Ezzati M, group CRAc. Causes of cancer in the world: comparative risk assessment of nine behavioural and environmental risk factors. The Lancet. 2005;366(9499):1784-93.

3. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2018;68(6):394-424.

4. Buskwoefie A, David-West G, Clare CA. A review of cervical cancer: incidence and disparities. Journal of the National Medical Association. 2020;112(2):229-32.

5. Nuzzo G, Giulianite F, Ardito F, Vellone M, Pozzo C, Cassano A et al. Liver resection for primarily unresectable colorectal metastases downsized by chemotherapy. Journal of Gastrointestinal Surgery. 2007;11(3):318-24.

6. Yan DB, Clingan P, Morris DL. Hepatic cryotherapy and regional chemotherapy with or without resection for liver metastases from colorectal carcinoma: how many are too many? Cancer. 2003;98(2):320-30.

7. Busch C-J, Becker B, Kriegs M, Gatzemeier F, Krüger K, Möckelmann N et al. Similar cisplatin sensitivity of HPV-positive and-negative HNSCC cell lines. Oncotarget. 2016;7(24):35832.
8. Waks AG, Winer EP. Breast cancer treatment: a review. Jama. 2019;321(3):288-300.
9. Miller A, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. cancer. 1981;47(1):207-14.
10. Willcox JK, Ash SL, Catignani GL. Antioxidants and prevention of chronic disease. Critical reviews in food science and nutrition. 2004;44(4):275-95.
11. Rao A, Agarwal S. Role of lycopene as antioxidant carotenoid in the prevention of chronic diseases: a review. Nutrition research. 1999;19(2):305-23.
12. Zhang H-Y, Yang D-P, Tang G-Y. Multipotent antioxidants: from screening to design. Drug Discovery Today. 2006;11(15-16):749-54.
13. Behl C, Moosmann B. Oxidative nerve cell death in Alzheimers disease and stroke: antioxidants as neuroprotective compounds. 2002.
14. Devasagayam T, Tilak J, Boloor K, Sane KS, Ghaskadbi SS, Lele R. Free radicals and antioxidants in human health: current status and future prospects. Japi. 2004;52(794804):4.
15. Jedynak BM, Lang A, Liu B, Katz E, Zhang Y, Wyman BT et al. A computational neurodegenerative disease progression score: method and results with the Alzheimer's disease neuroimaging initiative cohort. Neuroimage. 2012;63(3):1478-86.
16. Gong Y, Chang L, Viola KL, Lacor PN, Lambert MP, Finch CE et al. Alzheimer’s disease-affected brain: presence of oligomeric Aβ ligands (ADDLs) suggests a molecular basis for reversible memory loss. Proceedings of the National Academy of Sciences. 2003;100(18):10417-22.
17. Luo Q, Chen Y. Long noncoding RNAs and Alzheimer’s disease. Clinical interventions in aging. 2016;11:867.
18. Vishal S, Sourabh A, Harkirat S. Alois Alzheimer (1864–1915) and the Alzheimer syndrome. Journal of medical biography. 2011;19(1):32-3.
19. Ozansoy M, Başak AN. Tauopathies: A Distinct Class of Neurodegenerative Disorders. Turkish Journal of Neurology.13(Supp: 1):1-29.
20. Wang X-P, Ding H-L. Alzheimer’s disease: epidemiology, genetics, and beyond. Neuroscience bulletin. 2008;24(2):105.
21. Lyketsos CG, Steele C, Baker L, Galik E, Kopunek S, Steinberg M et al. Major and minor depression in Alzheimer’s disease: prevalence and impact. Journal of Neuropsychiatry and Clinical Neurosciences. 1997;9(4):556-61.
22. Norins LC. Licensed anti-microbial drugs logical for clinical trials against pathogens currently suspected in Alzheimer’s disease. Antibiotics. 2021;10(3):327.
23. Kazancioglu EA, Senturk M. Synthesis of N-phenylsulfonamide derivatives and investigation of some esterase enzymes inhibiting properties. Bioorganic Chemistry. 2020;104:104279.
24. Arslan T, Ceylan MB, Baş H, Biyiklioglu Z, Senturk M. Design, synthesis, characterization of peripherally tetra-pyridine-triazole-substituted phthalocyanines and their inhibitory effects on
cholinesterases (AChE/BChE) and carbonic anhydrases (hCA I, II and IX). Dalton Transactions. 2020;49(1):203-9.

25. Telpoukhovskaia MA, Patrick BO, Rodríguez-Rodríguez C, Orvig C. In silico to in vitro screening of hydroxypyridinones as acetylcholinesterase inhibitors. Bioorganic & medicinal chemistry letters. 2016;26(6):1624-8.

26. Bajda M, Więckowska A, Hebda M, Guzior N, Sotriffer CA, Malawska B. Structure-based search for new inhibitors of cholinesterases. International journal of molecular sciences. 2013;14(3):5608-32.

27. Athipornchai A, Niyomtham N, Pabuprapap W, Ajavakom V, Duca M, Azoulay S et al. Potent Tyrosinase Inhibitory Activity of Curcuminoid Analogues and Inhibition Kinetics Studies. Cosmetics. 2021;8(2):35.

28. Ramu P, Vimal S, Suresh P, Saravanakumar U, Sethuraman V, Anandhavelu S. Electrochemically Deposited Porous Graphene–Polypyrrole–Polyphenol Oxidase for Dopamine Biosensor. Electroanalysis. 2021;33(3):774-80.

29. Sepehri N, Iraji A, Yavari A, Asgari MS, Zamani S, Hosseini S et al. The natural-based optimization of kojic acid conjugated to different thio-quinazolinones as potential anti-melanogenesis agents with tyrosinase inhibitory activity. Bioorganic & Medicinal Chemistry. 2021;36:116044.

30. Zolghadri S, Bahrami A, Hassan Khan MT, Munoz-Munoz J, Garcia-Molina F, Garcia-Canovas F et al. A comprehensive review on tyrosinase inhibitors. Journal of Enzyme Inhibition and Medicinal Chemistry. 2019;34(1):279-309.

31. Kaya ED, Türkhan A, Gür F, Gür B. A novel method for explaining the product inhibition mechanisms via molecular docking: inhibition studies for tyrosinase from Agaricus bisporus. Journal of Biomolecular Structure and Dynamics. 2021:1-14.

32. Wu Y, Huo D, Chen G, Yan A. SAR and QSAR research on tyrosinase inhibitors using machine learning methods. SAR and QSAR in Environmental Research. 2021;32(2):85-110.

33. Jeong S-M-G, Yoon T-J. Development of Pigmentation-Regulating Agents by Drug Repositioning. International Journal of Molecular Sciences. 2021;22(8):3894.

34. Choi M-H, Yang S-H, Kim D-S, Kim ND, Shin H-J, Liu K. Novel Quercetin Derivative of 3, 7-Dioleylquercetin Shows Less Toxicity and Highly Potent Tyrosinase Inhibition Activity. International journal of molecular sciences. 2021;22(8):4264.

35. Kaya İ, Daban S, Şenol D. Synthesis and characterization of Schiff base, Co (II) and Cu (II) metal complexes and poly (phenoxy-imine) s containing pyridine unit. Inorganica Chimica Acta. 2021;515:120040.

36. Kolcu F, Erdener D, Kaya İ. Synthesis and characterization of a highly selective turn-on fluorescent chemosensor for Sn2+ derived from diimine Schiff base. Synthetic Metals. 2021;272:116668.

37. Iacopetta D, Ceramella J, Catalano A, Saturnino C, Bonomo MG, Franchini C et al. Schiff Bases: Interesting Scaffolds with Promising Antitumoral Properties. Applied Sciences. 2021;11(4):1877.

38. Gerengi H, Cakmak R, Dag B, Solomon MM, Tuysuz HAA, Kaya E. Synthesis and anticorrosion studies of 4-[(2-nitroacetophenonylidene)-amino]-antipyrine on SAE 1012 carbon steel in 15 wt.% HCl
solution. Journal of Adhesion Science and Technology. 2020;34(22):2448-66.

39. Bashiri M, Jarrahpour A, Nabavizadeh SM, Karimian S, Rastegari B, Haddadi E et al. Potent antiproliferative active agents: novel bis Schiff bases and bis spiro β-lactams bearing isatin tethered with butylene and phenylene as spacer and DNA/BSA binding behavior as well as studying molecular docking. Medicinal Chemistry Research. 2021;30(1):258-84.

40. Aslan HG, Akkoç S, Kökbudak Z, Aydin L. Synthesis, characterization, and antimicrobial and catalytic activity of a new Schiff base and its metal (II) complexes. Journal of the Iranian Chemical Society. 2017;14(11):2263-73.

41. Sönmez M, Sogukomerogullari HG, Öztçemel F, Berber İ. Synthesis and biological evaluation of a novel ONS tridentate Schiff base bearing pyrimidine ring and some metal complexes. Medicinal Chemistry Research. 2014;23(7):3451-7. doi:10.1007/s00044-014-0925-0.

42. Saleem MF, Khan MA, Ahmad I, Aslam N, Khurshid U. Synthesis and characterization of some new Schiff base derivatives of gabapentin, and assessment of their antibacterial, antioxidant and anticonvulsant activities. Tropical Journal of Pharmaceutical Research. 2021;20(1):145-53.

43. Tang J, Liu J, Wu F. Molecular docking studies and biological evaluation of 1, 3, 4-thiadiazole derivatives bearing Schiff base moieties as tyrosinase inhibitors. Bioorganic chemistry. 2016;69:29-36.

44. Sumrra SH, Zafar W, Asghar ML, Mushtaq F, Raza MA, Nazar MF et al. Computational investigation of molecular structures, spectroscopic properties, cholinesterase inhibition and antibacterial activities of triazole Schiff bases endowed metal chelates. Journal of Molecular Structure. 2021;1238:130382.

45. Tok F, Koçyiğit-Kaymakçıoğlu B, Sağlık BN, Levent S, Özky Y, Kaplancıklı ZA. Synthesis and biological evaluation of new pyrazolone Schiff bases as monoamine oxidase and cholinesterase inhibitors. Bioorganic chemistry. 2019;84:41-50.

46. Orabi EA, Orabi MA, Mahross MH, Abdel-Hakim M. Computational investigation of the structure and antioxidant activity of some pyrazole and pyrazolone derivatives. Journal of Saudi Chemical Society. 2018;22(6):705-14.

47. Raman N, Johnson Raja S, Sakthivel A. Transition metal complexes with Schiff-base ligands: 4-aminoantipyrine based derivatives—a review. Journal of Coordination Chemistry. 2009;62(5):691-709.

48. Alam MS, Lee D-U. Molecular structure, spectral (FT-IR, FT-Raman, Uv-Vis, and fluorescent) properties and quantum chemical analyses of azomethine derivative of 4-aminoantipyrine. Journal of Molecular Structure. 2021;1227:129512.

49. Arora R, Sharma R, Tageza A, Grewal AS, Saini B, Arora S et al. Design and synthesis of novel 4-aminophenazone Schiff bases by grinding technique as prospective anti-inflammatory agents. J Appl Pharm Sci. 2021;11:048-53.

50. Hu J, Qi J, Luo Y, Yin T, Wang J, Wang C et al. Synthesis, crystal structure and anticancer activities of an unusual inorganic–organic hybrid complex with a sandwicched ribbon structure. Arabian Journal
of Chemistry. 2021;14(5):103117.

51. Amal HE, N. 4-[2-(p-Acetamidobenzylidenehydrazino)-4-thiazolyl)] antipyrine. Istanbul University Science Faculty Magazine, Seri C: [Astronomy-Physics-Chemistry]. 1958; 23: 38-41.

52. Teran R, Guevara R, Mora J, Dobronski L, Barreiro-Costa O, Beske T et al. Characterization of antimicrobial, antioxidant, and leishmanicidal activities of Schiff base derivatives of 4-aminoantipyrine. Molecules. 2019;24(15):2696.

53. Bank HL. Rapid assessment of islet viability with acridine orange and propidium iodide. In vitro cellular & developmental biology. 1988;24(4):266-73.

54. Erdogan O, Abbak M, Demirbolat GM, Birtekocak F, Aksel M, Pasa S et al. Green synthesis of silver nanoparticles via Cynara scolymus leaf extracts: The characterization, anticancer potential with photodynamic therapy in MCF7 cells. PLoS One. 2019;14(6):e0216496.

55. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958;181(4617):1199-200.

56. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free radical biology and medicine. 1999;26(9-10):1231-7.

57. Dinis TC, Madeira VM, Almeida LM. Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Archives of biochemistry and biophysics. 1994;315(1):161-9.

58. Apak R, Güçlü K, Özyürek M, Karademir SE. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. Journal of agricultural and food chemistry. 2004;52(26):7970-81.

59. Ellman GL, Courtney KD, Andres Jr V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical pharmacology. 1961;7(2):88-95.

60. Hearing VJ, Jiménez M. Mammalian tyrosinase—the critical regulatory control point in melanocyte pigmentation. International Journal of Biochemistry. 1987;19(12):1141-7.

61. Topal G, Tombak A, Yigitalp E, Batibay D, Kilicoglu T, Oacak YS. Diester Molecules for Organic-Based Electrical and Photoelectrical Devices. Journal of Electronic Materials. 2017;46(7):3958-64.

62. Rosenmund K, Schapiro D. Synthetische Versuche in der Reihe der Antihelmintika. Archiv der Pharmazie. 1934;272(10-34):313-23.

63. Liu B, Cheng L, Hu P, Xu F, Li D, Gu W-J et al. Iron-catalyzed oxidative C–C (vinyl) σ-bond cleavage of allylarenes to aryl aldehydes at room temperature with ambient air. Chemical Communications. 2019;55(33):4817-20.

64. Dikusar E, Kozlov N. Esters Derived from Vanillin and Vanillal and Aromatic and Functionalized Aliphatic Carboxylic Acids. Russian journal of organic chemistry. 2005;41(7).

65. Dikusar E. New esters of vanillin and vanillal with some alkane-and arenecarboxylic acids. Russian journal of applied chemistry. 2006;79(6):1035-7.
Figures

Figure 1

a) The morphological images of HeLa cells treated with IC50 concentrations of 1b and 2a b) Cell viability measurement of HeLa cell lines after treated with 1b (1-1000 μM) for 24 h c) Cell viability measurement of HeLa cell lines after treated with 2a (1-1000 μM) for 24 h.
Figure 2

a) AO/PI double staining of 1b and 2a for 24 h treatment in HeLa cells
b) Percentage of AO/PI double staining of 1b and 2a for 24 h treatment in HeLa cells

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