Molecular Diversity
https://doi.org/10.1007/s11030-020-10045-x

ORIGINAL ARTICLE

Novel quinolinone–pyrazoline hybrids: synthesis and evaluation of antioxidant and lipoxygenase inhibitory activity

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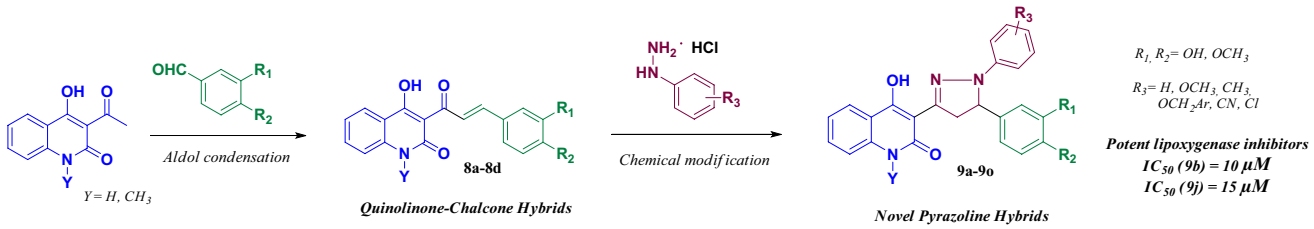
Received: 3 December 2019 / Accepted: 29 January 2020

Abstract

The present project deals with the investigation of structure–activity relationship of several quinolinone–chalcone and quinolinone–pyrazoline hybrids, in an effort to discover promising antioxidant and anti-inflammatory agents. In order to accomplish this goal, four bioactive hybrid quinolinone–chalcone compounds (**8a**–**8d**) were synthesized via an aldol condensation reaction, which were then chemically modified, forming fifteen new pyrazoline analogues (**9a**–**9o**). All the synthesized analogues were in vitro evaluated in terms of their antioxidant and soybean lipoxygenase (LOX) inhibitory activity. Among all the pyrazoline derivatives, compounds **9b** and **9m** were found to possess the best combined activity, whereas **9b** analogue exhibited the most potent LOX inhibitory activity, with IC_{50} value 10 μM. The in silico docking results revealed that the synthetic pyrazoline analogue **9b** showed high AutoDock Vina score (− 10.3 kcal/mol), while all the tested derivatives presented allosteric interactions with the enzyme.

Graphic Abstract



Keywords Quinolinones · Chalcones · Pyrazolines · Lipoxygenase · LOX inhibition · Antioxidant activity

Introduction

Inflammation is part of the body's defense mechanism, and it can be beneficial as a healing process, by which the immune system recognizes and removes harmful stimuli. Acute inflammation is induced by tissue damage due to trauma, microbial invasion or noxious compounds. Chronic inflammation is a persistent phenomenon that can last from months to years and plays a central role in some of the most challenging diseases of our time, including neurodegenerative and heart diseases, rheumatoid arthritis, diabetes, asthma, and even cancer.

Lipoxygenases (LOXs) are a heterogeneous family of structurally related non-heme iron-containing enzymes that

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11030-020-10045-x) contains supplementary material, which is available to authorized users.

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Published online: 17 February 2020

catalyze the insertion of molecular oxygen into polyunsaturated fatty acids, such as arachidonic acid or linoleic acid. They are classified as 5-, 8-, 12, and 15- LOXs according to their selectivity to oxygenate fatty acids in a specific position [1–4].

Over the years, several strategies have been developed in order to block the arachidonic acid pathway and different compounds have been identified as 5-LOX inhibitors [5–8]. Up to now, Zileuton is the only approved potent and selective 5-LOX inhibitor for the treatment of asthma [9, 10]. However, several 5-LOX inhibitors are now under clinical investigation for the treatment of cardiovascular diseases and vascular inflammation, atherosclerosis, asthma and for knee osteoarthritis [11–13]. Furthermore, recent studies examine the relationship between arachidonic acid cascade and carcinogenesis, revealing novel targets for the treatment of cancer [14–16]. It has been demonstrated that 5-LOX plays an important role in regulating cellular proliferation and there are 5-LOX inhibitor pharmacophores which can also cause cell death in prostate cancer cells [2].

Chronic inflammation and oxidative stress are two commonly associated conditions involved in the pathophysiology of cancers, diabetes, cardiovascular and pulmonary diseases, and others. Oxidative stress occurs when the balance between antioxidants and reactive oxygen species (ROS) is disrupted because of either depletion of antioxidants or accumulation of ROS.

The main feature of an antioxidant is its ability to bind free radicals, which are produced during the inflammation process by phagocytic leukocytes that invade the tissue. Moreover, ROS are involved in the biosynthesis of prostaglandins and in the cyclooxygenase (COX) and LOX mediated conversion of arachidonic acid into proinflammatory intermediates [17–19]. Therefore, the development of novel drugs that combine anti-inflammatory and antioxidant activity could be beneficial for the treatment of several diseases [20].

Hybrid molecules combine the structural frame of two or more different pharmacophores which have already been exploited in drug development, and they are designed in order to provide novel drugs with enhanced activity [21–23]. Quinolinones and chalcones are two scaffolds that have shown potential activity in many biological tests, and they are widely used in the development of new hybrid compounds.

Quinolinones are heterocyclic nitrogen compounds which are mainly found as alkaloids in a variety of natural products, as well as synthetic analogues (Fig. 1) [24, 25]. Furthermore, compounds containing the heterocyclic system of 4-substituted-2-quinolinone as a building block exhibit a wide variety of biological and pharmacological properties and are useful intermediates in the synthesis of a large number of bioactive molecules [26–28].

Chalcones are natural products which have been recognized as the precursors to the biosynthesis of flavonoids and isoflavonoids [29]. They are α,β-unsaturated carbonyl compounds, which are characterized by the presence of a three carbon bridge with a double bond (Fig. 1) [30]. Chalcones and their derivatives consist "privileged structures" and they show many interesting biological properties, such as anti-inflammatory [20, 31, 32], anti-parasitic [33], antimicrobial [34], antibacterial [32, 35], antioxidant [31, 36], cytotoxic [37], anticancer [31, 38] and so on.

Following the above trend, the aim of the present research is the synthesis of bioactive hybrid quinolinone–chalcone compounds, and the chemical modification of the above system, in order to form new pyrazoline analogues (Fig. 1).

Pyrazolines (Fig. 1) are well-known and important five-membered heterocyclic compounds, possessing two adjacent nitrogen atoms in the ring and only one endocyclic double bond. [39]. Depending on the position of the double bond, there are three possible partially reduced forms of pyrazoline structure, which are namely 1-pyrazoline, 2-pyrazoline or 3-pyrazoline which can exist in equilibrium with each other. Among them, 2-pyrazoline is more stable than the rest of the reduced forms, while it seems to be the most frequently studied form of pyrazoline [40].

Pyrazoline derivatives have been reported to exhibit a wide range of pharmacological activities such as antimicrobial [41–43], anticancer [44, 45], anti-inflammatory [46], antioxidant [47, 48], antidepressant [49] and so on. Out of all these biological activities of pyrazolines and their derivatives, the anti-inflammatory activity seems to concentrate the interest of researchers, while there is a

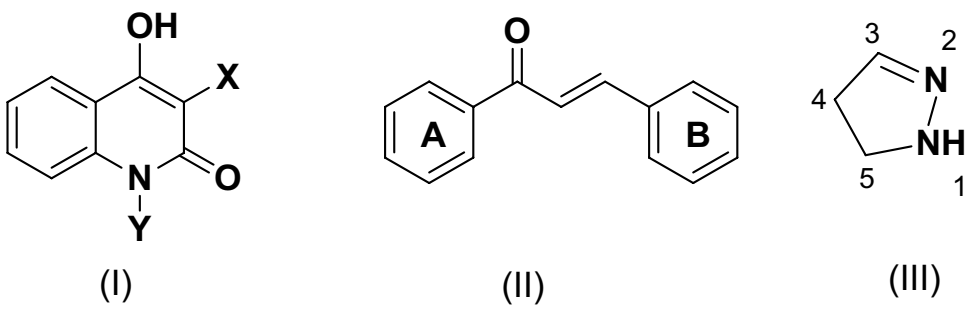
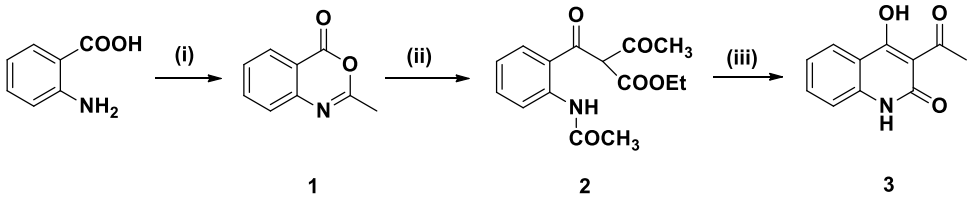
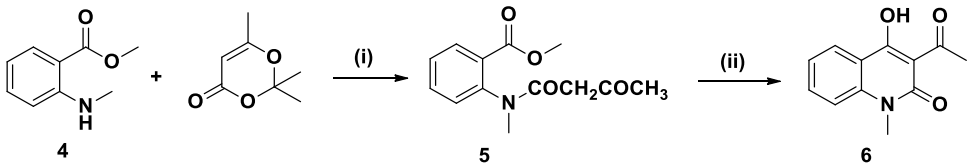


Fig. 1 General structure of the quinolinone (**I**), chalcone (**II**) and pyrazoline (**III**) molecular scaffolds



Scheme 1 Synthesis of 3-acetyl-4-hydroxy-2-quinolinone (**3**). Reagents and conditions: (i) $(CH_3CO)_2O$, 130 °C, 2 h (ii) CH_3COCH_2COOEt, t-BuOK, t-BuOH, rt (iii) aq Na_2CO_3/NaOH, rt



Scheme 2 Synthesis of 3-acetyl-4-hydroxy-1-methyl-2-quinolinone (**6**). Reagents and conditions: (i) toluene, 110 °C, 2 h (ii) $NaOCH_2CH_3$, 77 °C, 2 h

plethora of information available in the literature which is growing steadily over the years [50–56].

In 2019, Stefanes and coworkers synthesized a series of new pyrazoline derivatives, which were then evaluated for their anti-leukemic activity, revealing two of the compounds as promising candidates against acute leukemia [57]. Mumtaz and his group focused on the current need of new potential agents over the treatment of neurodegenerative disorders, such as Parkinson's disease or Alzheimer's disease, synthesizing several derivatives of thioureas and pyrazolines, which were then evaluated via two assays. Results showed one of the pyrazoline derivatives as the most potent acetylcholinesterase inhibitor, with $IC_{50} = 123 \pm 51$ nM [58].

Furthermore, the evaluation of the anti-inflammatory activity of pyrazolines still stands out in the literature, since several corresponding studies were published in 2019. Cai et al. [59] synthesized a number of novel steroidal derivatives bearing pyrazoline structure, among others, identifying one of them as the most potent anti-inflammatory agent, with an IC_{50} value of 0.86 μM on NO production in LPS induced RAW 264.7 cells. Earlier the same year, Sethiya and his research group synthesized some new pyrazoline derivatives, using ultrasonic irradiation, which demonstrated remarkable anti-inflammatory activity [60]. Chandel et al. [61] synthesized coumarin-based pyrazolines and evaluated them in terms of their in vitro and in vivo anti-inflammatory activity. Experimental results revealed one of the tested compounds as highly active anti-inflammatory agent and suggested it to be used as lead compound for the development of effective inhibitors.

With regard to the synthesis of pyrazolines, literature offers a wide variety of methods depending on the reactivity of molecules and the need of the chemist [62]. However, the most popular method is the one of Fischer and Knoevenagel, i.e., the reaction of α,β-unsaturated ketones with phenyl hydrazine in acetic acid under refluxing conditions [63, 64].

Table 1 New quinolinone–chalcone hybrids

Code	Y	R_1	R_2
8a	H	OCH_3	OCH_3
8b	H	OCH_3	OH
8c	CH_3	OCH_3	OCH_3
8d	CH_3	OCH_3	OH

Results and discussion

Chemistry

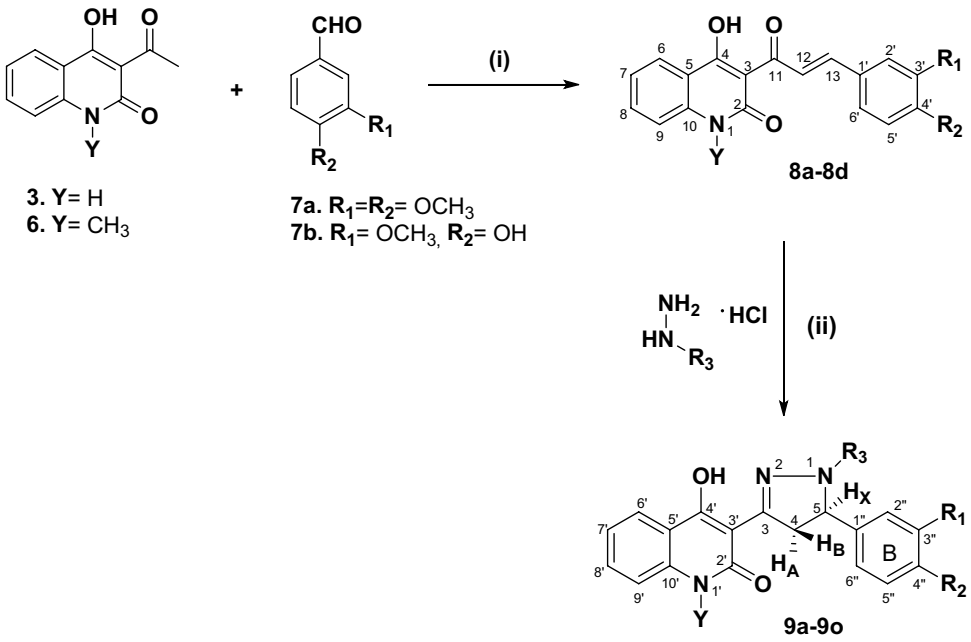
The desired starting quinolinones differ to the substituent Y attached to the nitrogen of the heterocyclic ring, and they were synthesized through different synthetic routes.

3-Acetyl-4-hydroxy-2-quinolinone (**3**) was synthesized using our previously developed methodology which includes C-acylation of ethyl acetoacetate by 2-methyl-3,1-benzoxazin-4-one (**1**) in a basic environment, followed by a cyclization reaction of the C-acylation product **2** in aqueous solution of Na_2CO_3 and NaOH (Scheme 1) [33, 65].

In order to investigate the role of the substituent Y, we synthesized quinolinone **6**, which bears a methyl group at this position. The synthesis was accomplished using our previous methodology, via an acylation reaction of secondary amine **4**, followed by a cyclization reaction of the acylation product **5**, in basic environment (Scheme 2) [33].

The desired four quinolinone–chalcone hybrids (Table 1) were finally synthesized according to Scheme 3, using compounds **3** and **6** as starting materials in an aldol condensation reaction with two different benzaldehydes. It is a methodology that has already been developed in a previous work of our laboratory, [33].

Proceeding with the synthesis and in order to further investigate the structure–activity relationship of the final compounds, we attempted a structural modification, which



Scheme 3 Synthesis of quinolinone-chalcone hybrid compounds and of new pyrazoline analogues. Reagents and conditions: (i) Piperidine, 78 °C, 5–24 h (ii) CH_3COOH/EtOH, reflux, 120 °C

was carried out to the α,β-unsaturated carbonyl system of compounds **8**, leading to pyrazoline derivatives **9** (Table 2).

Various conditions were investigated in order to synthesize the target pyrazoline derivatives. After optimization of the reaction conditions, we finally synthesized 15 new molecules by refluxing the corresponding quinolinone–chalcone compound (**8**) with a variety of hydrazine derivatives in glacial acetic acid (Scheme 3).

The structures of all the new pyrazolines were elucidated by ^{1}H-NMR, ^{13}C-NMR and HR-MS. In the ^{1}H-NMR of the compounds, all the three protons H_A, H_B and H_X attached to the C_4 and C_5 carbon atoms of the pyrazoline ring, respectively, were found to give an ABX spin system. The methylene protons of C_4 resonated as a pair of doublet of doublet peaks at regions 3.43–3.66 ppm (C_4-H_A) and 4.14–4.33 ppm (C_4-H_B). The methine proton of C_5 also appeared as a doublet of doublet peak at the region 4.96–5.48 ppm, due to its vicinal coupling with the two magnetically non-equivalent protons of the position C_4 of the pyrazoline ring. Among the three signals of H_A, H_B and H_X protons, the most deshielded one is attributed to the methine of C_5, due to its close proximity to the nitrogen of the pyrazoline ring and to ring B of the chalcone moiety.

Biology

The new derivatives were tested for their antioxidant activity in vitro based on their capacity to scavenge the stable free radical DPPH, as well as their ability to inhibit lipid peroxidation of linoleic acid induced by AAPH radical.

The first one is a fast, simple, cost-effective and widely used method, where the DPPH stable free radical reacts directly with the antioxidant and is decolorized. The scavenging effect of the synthesized compounds on the DPPH radical was evaluated according to the methods of Hadjipavlou et al. [66, 67].

AAPH-induced linoleic acid oxidation has been developed as a quick and reliable method for measuring the antioxidant activity. The thermal free radical producer (AAPH) generates free radicals in the solution which cause the oxidation of linoleic acid, and the method is a measure of how effective antioxidants protect against lipid peroxidation in vitro. Oxidation of exogenous linoleic acid by AAPH is followed by UV spectrophotometry in a highly diluted sample [68].

Furthermore, for the evaluation of their anti-inflammatory activity, all new molecules were tested as inhibitors of soybean LOX, which is a plant enzyme with satisfactory homology with the human 5-LOX, and the results can be considered as an indication of the anti-inflammatory activity of new analogues. The results of this study are presented in Table 3.

Evaluation of antioxidant and soybean LOX inhibitory activity

The results of the DPPH radical scavenging activity of the majority of the tested analogues showed good interaction with the DPPH radical at 100 μM concentration. In general, the interaction is altered in relation to the different substituents and more specifically in relation to the presence or not

Table 2 New pyrazoline analogues

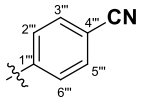
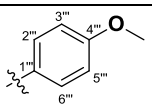
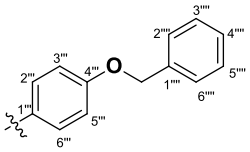
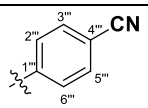
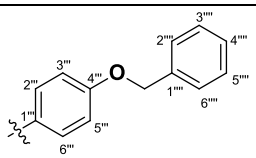
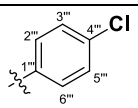
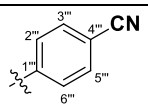
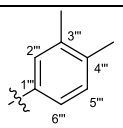
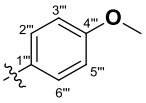
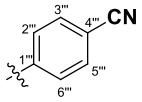
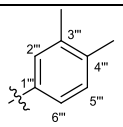
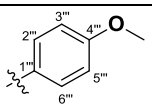
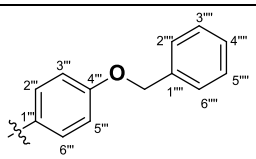
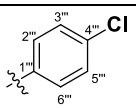
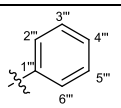
CODE	Y	R_1	R_2	R_3
9a	H	OCH_3	OCH_3	 4’’’-cyanophenyl
9b	H	OCH_3	OCH_3	 4’’’-methoxyphenyl
9c	H	OCH_3	OCH_3	 4’’’-benzyloxyphenyl
9d	H	OCH_3	OH	 4’’’-cyanophenyl
9e	H	OCH_3	OH	 4’’’-benzyloxyphenyl
9f	H	OCH_3	OH	 4’’’-chlorophenyl
9g	CH_3	OCH_3	OCH_3	 4’’’-cyanophenyl
9h	CH_3	OCH_3	OCH_3	 (3’’’,4’’’-dimethyl)phenyl

Table 2 (continued)

9i	CH_3	OCH_3	OCH_3	 4’’’-methoxyphenyl
9j	CH_3	OCH_3	OH	 4’’’-cyanophenyl
9k	CH_3	OCH_3	OH	 (3’’’,4’’’-dimethyl)phenyl
9l	CH_3	OCH_3	OH	 4’’’-methoxyphenyl
9m	CH_3	OCH_3	OH	 4’’’-benzyloxyphenyl
9n	CH_3	OCH_3	OH	 4’’’-chlorophenyl
9o	H	OCH_3	OH	 phenyl

of labile protons, such as phenolic hydroxyl groups, which react directly with the free radical.

Among the four synthesized quinolinone–chalcones, it seems that the presence of a phenolic hydroxyl group in compounds **8b** and **8d** leads to good DPPH radical scavengers (81% and 78% after 20-min incubation at a concentration of 0.1 mM, respectively) and LOX inhibitors (IC_{50} 56.0 μM and 50.0 μM, respectively). The similar values of DPPH scavenging and LOX inhibition ability presented by quinolinone–chalcones **8b** and **8d**, which differ only in the substituent at the heterocyclic N, indicate that the most important structural feature for both activities is the presence of the phenolic OH. Moreover, substitution of the phenolic OH with a OCH_3 group (compounds **8a** and **8c**) leads to complete loss of DPPH and LOX inhibitory activity. On the other hand, the lipid peroxidation ability of compounds **8b** and **8d** is low, but the replacement of the OH by a OCH_3 group (compounds **8a** and **8c**) leads to significant inhibitors

Table 3 In vitro biological evaluation of all the synthesized analogues

CODE	Interaction with the free radical DPPH (%)		Inibition of lipid peroxidation of linoleic acid induced by AAPH radical (%) 0.1 mM	Inhibition of soybean lipoxygenase IC_{50} (μM)
	0.1 mM/20 min	0.1 mM/60 min		
8a	–	–	100.0	(19.2% at 0.1 mM)
8b	81.0	84.0	23.0	56.0
8c	–	–	100.0	(13.0% at 0.1 mM)
8d	78.0	82.0	31.0	50.0
9a	27.3	12.7	74.7	(27.3% at 0.1 mM)
9b	85.3	86.2	82.9	10.0
9c	86.2	98.9	93.3	(43.8% at 0.1 mM)
9d	95.0	95.3	83.8	52.0
9e	76.0	84.0	88.2	63.0
9f	63.0	71.0	71.4	74.0
9g	99.0	44.0	25.0	65.0
9h	60.0	71.0	92.3	67.5
9i	87.0	91.0	100.0	(16% at 0.1 mM)
9j	74.0	8.0	77.0	15.0
9k	77.0	79.0	87.2	57.0
9l	83.0	87.0	97.0	57.5
9m	85.0	100.0	79.0	39.0
9n	67.0	73.0	59.0	50.0
9o	74.5	85.5	59.1	90.0
NDGA	88.0	97.0	–	0.5
Trolox	–	–	88.0	–

of lipid peroxidation (100% inhibition at a concentration of 0.1 mM for both compounds) but inactive DPPH scavengers and LOX inhibitors. From the above observations, it can be postulated that in the case of quinolinone–chalcones, LOX inhibition is related to the antioxidant activity as measured by the DPPH scavenging ability and can probably be correlated with their H atom transfer capacity.

An important observation emerging from the evaluation of the antioxidant activity of all the new analogues is related to the 4-hydroxyl group of the quinolinone moiety. This proton creates a strong hydrogen bond with the adjacent carbonyl group, and for this reason it cannot react with the free DPPH radical, so it does not participate in the enhancement of the antioxidant activity of quinolinone–chalcones. The stability of this proton is easily proven by the ^{1}H NMR analysis, where it appears through a singlet peak at very low fields of the spectrum, 17–18 ppm. In the spectra of pyrazoline analogues this peak is shifted to 13–14 ppm, indicating that the proton in this case creates a weaker hydrogen bond with the nitrogen of the pyrazoline ring. This is a plausible explanation to account for the observation that the majority of the tested pyrazolines show a better DPPH radical scavenger capacity, compared to their quinolinone–chalcone precursors. As a striking example, one can compare the DPPH scavenging ability of pyrazolines **9a**, **9b** and **9c**, which were prepared from the same precursor, quinolinone–chalcone **8a**: **8a** is inactive, whereas **9b** and **9c** are among the most active DPPH scavengers (85.3% and 86.2%, respectively, at a concentration of 0.1 mM and after 20 min incubation) of the series. Pyrazoline **9a** is not a good DPPH scavenger; however, it is not completely inactive as **8a**. The same trend is followed in the case of the very good DPPH scavengers pyrazolines **9g** and **9i** (99% and 87% inhibition, respectively), which are derived from the inactive quinolinone–chalcone **8c**.

In the case of the pyrazoline analogues produced from the good radical scavengers **8b** and **8d**, namely compounds **9d**–**9f** and **9j**–**9o**, respectively, the contribution of the OH group at position 4 of the quinolinone heterocyclic ring in the DPPH scavenging ability does not seem to be as significant as the one of the phenolic OH. Thus, the above pyrazolines were found to possess analogous or even less antioxidant activity compared to their precursors **8b** and **8d**.

As far as the ability of the tested pyrazolines to inhibit lipid peroxidation of linoleic acid induced by a thermal free radical producer (AAPH), five pyrazolines, namely **9c, 9e, 9h, 9i** and **9m**, were found to be potent inhibitors showing activity equal or higher than the reference compound Trolox. Insertion of the pyrazoline moiety at quinolinone–chalcone **8b** (with very low lipid peroxidation ability, 23% at 0.1 mM) resulted in compounds **9d**–**9f** which had remarkably higher activity. The same is true for pyrazolines arising from

quinolinone–chalcone **8d**: the parent compound had very low activity (31% at 0.1 mM), whereas the corresponding pyrazolines **9j–9o** showed moderate to high activity (> 59%). However, pyrazolines **9a–9c** and **9g–9i** which were derived from **8a** and **8c**, respectively, showed analogous or lower activity with the parent quinolinone–chalcones.

Among all the pyrazoline analogues tested, the most potent LOX inhibitor is compound **9b** ($IC_{50} = 10$ μM) which possesses a *p*-methoxy-phenyl substituent attached to the nitrogen of the pyrazoline moiety. In addition, pyrazoline **9b** exhibits good antioxidant activity in both DPPH and lipid peroxidation assays; thus, it can be considered as a promising lead compound for further development. Regarding structural modifications on **9b**, replacement of the *p*-methoxy group by a *p*-cyano (**9a**) or a *p*-benzyloxy (**9c**) group or insertion of a methyl group on the heterocyclic NH of the quinolinone ring (compound **9i**) resulted in loss of activity.

However, the insertion of a methyl group on the nitrogen of the heterocyclic ring of the quinolinone moiety seems to enhance LOX inhibitory activity in the case of pyrazolines that share the *p*-cyano-phenyl or the *p*-benzyloxy-phenyl substituent as a common structural feature: N–H pyrazolines **9a**, **9d** and **9e** ($IC_{50} = 27.3\%$ at 0,1 mM, 52.0 and 63.0 μM, respectively) are less potent than their N-CH_3 analogues **9g**, **9j** and **9m** ($IC_{50} = 65.0$, 15.0 and 39.0 μM, respectively).

The comparison of pyrazoline analogues possessing two methoxy groups at the aromatic ring B (compounds **9a, 9c, 9g, 9h, 9i**) with their analogues which bear one methoxy and one hydroxyl group at ring B (compounds **9d, 9e, 9j, 9k, 9l**) shows that as a general trend, the presence of the hydroxyl group dramatically increases the ability of the compounds to inhibit LOX. In fact, compound **9j** is the second best lipoxygenase inhibitor in this series.

Molecular modeling of the synthesized derivatives in soybean LOX

All the synthesized derivatives have been subjected to in silico docking. The docking results provided useful interpretation of the experimental study. Figure 2 depicts the preferred docking pose for the most potent derivative **9b** in soybean LOX (PDB code: 3PZW) presenting a high AutoDock Vina score (− 10.3 kcal/mol). From the docking results, it can be concluded that the novel synthesized derivatives present allosteric interactions with the enzyme. It seems that **9b** accommodates to an extensively hydrophobic cavity with possible hydrophobic interactions (π–π stacking). It is possible that **9b** extends into the hydrophobic domain and prevents access of substrates to the active site and hence prevents lipoxygenation. It is well known that LOX inhibitory activity is based on a carbon-centered radical on a lipid chain and most LOX inhibitors are antioxidants or free radical scavengers [69]. Pyrazoline **9b** is also a potent antioxidant; therefore, it can serve as the starting point for the development of potential agents with combined anti-inflammatory and antioxidant activity.

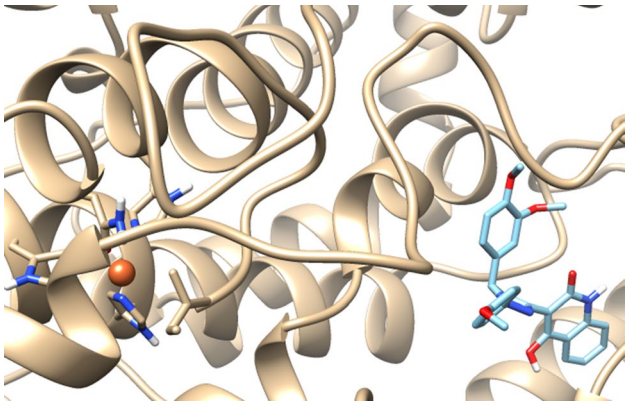


Fig. 2 Docking orientation of **9b** (depicted in turquoise) bound to soybean LOX

Conclusions

In conclusion, this work reports the synthesis of four quinolinone–chalcones and fifteen novel structurally modified pyrazoline analogues, which to our knowledge have not been reported in the literature. In order to classify the new molecules as promising antioxidant and anti-inflammatory agents, we studied how the final biological effect is affected in relation to the different substituents at several positions of the framework, like the nitrogen heteroatom of the quinolinone ring, the aromatic ring B of the chalcone moiety, as well as the phenyl group attached to the pyrazoline ring. The evaluation of all the synthesized compounds showed that pyrazolines **9b** and **9m** possess the best combined activity (IC_{50} 10.0 and 39.0 μM for LOX inhibition, 86% and 100% for DPPH scavenging ability and 83% and 79% in lipid peroxidation inhibition, respectively). The in silico docking results revealed that the pyrazoline analogue **9b** showed high AutoDock Vina score (− 10.3 kcal/mol), while all the tested derivatives presented allosteric interactions with the enzyme.

Experimental

Chemistry

General methods

The reagents and solvents used for synthesis and analysis were commercially available and used without further

purification. NMR spectra were recorded on a Varian 600 MHz spectrometer at the National Hellenic Research Foundation. The HR-MS spectrum was obtained using a UHPLC-MSn Orbitrap Velos-Thermo mass spectrometer at the National Hellenic Research Foundation. Melting points were determined on a Gallenkamp MFB-595 melting point apparatus and are uncorrected.

Synthesis

General procedure (A) for the synthesis of quinolinone–chalcone analogues (8a–8d)

Equimolar amounts of 3-acetyl-4-hydroxy-2-(1H)-quinolinone (**3**, **6**) and the appropriate benzaldehyde (**7**) are dissolved in absolute ethanol (EtOH), and a catalytic amount of piperidine is added. The reaction mixture is stirred for 5–24 h at 78 °C with a reflux condenser, while the reaction is monitored by thin-layer chromatography (TLC). After the reaction is complete, the mixture is cooled in an ice bath and acidified with aq. HCl 10% v/v. Finally, the product is obtained from the acidified aqueous solution in solid form.

(E)-3-(3-(3,4-dimethoxyphenyl)acryloyl)-4-hydroxyquinolin-2(1H)-one (8a)

This compound was prepared following the general procedure A. 3-acetyl-4-hydroxy-2-(1H)-quinolinone (**3**) (1000 mg, 4,92 mmol) and 3,4-dimethoxy benzaldehyde (**7a**) (810 mg, 4.92 mmol) were dissolved in 20 ml EtOH and 40 drops of piperidine were added. The mixture was stirred at 78 °C for 5 h. The solid (chalcone **8a**) was obtained upon acidification as yellow powder. Yield: 680 mg (68%); m.p. > 250 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ ppm 18.32 (s, 1H, O<u>H</u>), 11.44 (s, 1H, N<u>H</u>), 8.54 (d, 1H, J = 15 Hz, COCH = C<u>H</u>Ar), 8.01 (d, 1H, J = 4.2 Hz, Ar–H), 7.94 (d, 1H, J = 15.6 Hz, COC<u>H</u> = CHAr), 7.67 (t, 1H, J = 6.0 Hz, Ar–H), 7.37 (d, 1H, J = 8.4 Hz, Ar–H), 7.27 (m, 3H, Ar–H), 7.08 (d, 1H, J = 8.4 Hz, Ar–H), 3.83 (s, 6H, 2OC<u>H</u>$_3$).

(E)-4-hydroxy-3-(3-(4-hydroxy-3-methoxyphenyl)acryloyl) quinolin-2(1H)-one (8b)

This compound was prepared following the general procedure A. 3-acetyl-4-hydroxy-2-(1H)-quinolinone (**3**) (500 mg, 2.46 mmol) and 4-hydroxy-3-methoxy benzaldehyde (**7b**) (374.3 mg, 2.46 mmol) were dissolved in 10 ml EtOH, and 20 drops of piperidine were added. The mixture was stirred at 78 °C for 5 h. The solid (chalcone **8b**) was obtained upon acidification as orange powder. Yield: 290 mg (58%); m.p. > 250 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ ppm 18.46 (s, 1H, O<u>H</u>), 11.42 (s, 1H, N<u>H</u>), 9.90 (s, 1H, Ar-O<u>H</u>), 8.51 (d, 1H, J = 15.6 Hz, COCH = C<u>H</u>-Ar), 7.00 (d, 1H, J = 8.4 Hz, Ar–H), 7.93 (d, 1H, J = 15.6 Hz, COC<u>H</u> = CH-Ar), 7.67 (t, 1H, J = 7.2 Hz, Ar–H), 7.29 (m, 3H, Ar–H), 7.22 (t, 1H, J = 7.8 Hz, Ar–H), 6.90 (d, 1H, J = 7.8 Hz, Ar–H), 3.85 (s, 3H, 2OC<u>H</u>$_3$);

(E)-3-(3-(3,4-dimethoxyphenyl)acryloyl)-4-hydroxy-1-methylquinolin-2(1H)-one (8c)

This compound was prepared following the general procedure A. 3-acetyl-4-hydroxy-2-(1-CH_3)-quinolinone (**6**) (500 mg, 2.30 mmol) and 3,4-dimethoxy benzaldehyde (**7a**) (382.2 mg, 2.30 mmol) were dissolved in 15 ml EtOH, and 18 drops of piperidine were added. The mixture was stirred overnight at 78 °C. The solid produced upon acidification was triturated from methanol/dichloromethane to afford chalcone **8c** as orange powder. Yield: 357 mg (71%); m.p. > 250 °C ^{1}H NMR ($CDCl_3$, 600 MHz): δ ppm 18.20 (s, 1H, O<u>H</u>), 8.58 (d, 1H, J = 15.6 Hz, COCH = C<u>H</u>-Ar), 8.25 (d, 1H, J = 7.8 Hz, Ar–H), 7.96 (d, 1H, J = 15.6 Hz, COC<u>H</u> = CH-Ar), 7.68 (t, 1H, J = 7.8 Hz, Ar–H), 7.32 (m, 2H, Ar–H), 7.26 (m, 2H, Ar–H), 6.89 (d, 1H, J = 8.4 Hz, Ar–H), 3.97 (s, 3H, 2OC<u>H</u>$_3$), 3.93 (s, 3H, OC<u>H</u>$_3$), 3.67 (s, 3H, NC<u>H</u>$_3$);

(E)-4-hydroxy-3-(3-(4-hydroxy-3-methoxyphenyl) acryloyl)-1-methylquinolin-2(1H)-one (8d)

This compound was prepared following the general procedure A. 3-acetyl-4-hydroxy-2-(1-CH_3)-quinolinone (**6**) (600 mg, 2.76 mmol) and 4-hydroxy-3-methoxy benzaldehyde (**7b**) (419.9 mg, 2.76 mmol) were dissolved in 18 ml EtOH, and 22 drops of piperidine were added. The mixture was stirred overnight at 78 °C. The solid produced upon acidification was triturated from methanol/dichloromethane to afford chalcone **8d** as orange powder. Yield: 286 mg (48%); m.p. > 250 °C;^{1}H NMR (DMSO-d_6, 600 MHz): δ ppm 18.25 (s, 1H, O<u>H</u>), 9.90 (s, 1H, Ar-O<u>H</u>), 8.44 (d, 1H, J = 15.6 Hz, COCH = C<u>H</u>-Ar), 8.09 (d, 1H, J = 7.8 Hz, Ar–H), 7.89 (d, 1H, J = 15.6 Hz, COC<u>H</u> = CH-Ar), 7.77 (t, 1H, J = 7.8 Hz, Ar–H), 7.50 (d, 1H, J = 8.4 Hz, Ar–H), 7.27 (m, 3H, Ar–H), 6.88 (d, 1H, J = 7.8 Hz, Ar–H), 3.85 (s, 3H, OC<u>H</u>$_3$), 3.55 (s, 3H, NC<u>H</u>$_3$); ^{13}C NMR (DMSO-d_6, 600 MHz): δ ppm 193.0 (C, C-11), 175.7 (C, C-4), 160.7 (C, C-2), 150.4 (C, C-3′, C-4′), 148.0 (CH, C-12), 146.1 (C, C-5), 141.4 (C, C-1′), 135.4 (CH, C-8), 126.3 (CH, C-13), 125.3 (CH, C-2′), 123.7 (CH, C-5′), 122.1 (CH, C-6′), 121.1 (CH, C-6), 116.0 (CH, C-7), 115.2 (CH, C-9), 112.3 (C, C-10), 104.9 (C, C-3), 55.7 (CH_3, Ar–O-<u>C</u>H$_3$), 29.1 (CH_3, N-<u>C</u>H$_3$); HR-MS *m/z* (neg): 350.10314 $C_{20}H_{17}NO_5$ (calcd. 351.1107).

General procedure (B) for the synthesis of pyrazoline analogues (9a–9o)

The appropriate hydrazine derivative (2.5 eq.) is dissolved in absolute ethanol. The appropriate quinolinone–chalcone **8** (1 eq.) is dissolved in acetic acid, and it is added dropwise to the ethanolic solution of hydrazine. The resulting mixture is stirred at 120 °C for 24 h. The reaction is monitored by TLC. After completion of the reaction, the mixture is poured in ice and a yellow or orange precipitate is formed. The solid is filtered and washed with ice water. If no solid precipitates, the mixture is extracted with dichloromethane, the organic extracts are dried with Na_2SO_4 and the solvent is evaporated under reduced pressure to give the pyrazoline analogue as a solid product. Final compounds are further purified by trituration with methanol and dichloromethane.

4-(5-(3,4-Dimethoxyphenyl)-3-(4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)benzonitrile (9a)

This compound was prepared following the general procedure B. 4-cyano phenyl-hydrazine hydrochloride (89.1 mg, 0.53 mmol) was dissolved in 0.67 ml of absolute ethanol. Quinolinone-chalcone **8a** (73 mg, 0.21 mmol) was dissolved in 1.3 ml of acetic acid and it was added dropwise to the ethanolic solution hydrazine. The resulting solution was stirred at 120 °C for 24 h. The solid produced upon filtration was triturated from methanol/dichloromethane to afford the pure pyrazoline **9a** as yellow powder. Yield: 35.8 mg (49%); m.p. > 250 °C; ^{1}H NMR (DMSO-d_6, 600 MHz,): δ ppm 13.04 (s, 1H, O<u>H</u>), 11.61 (s, 1H, N<u>H</u>), 7.98 (br, 1H, Ar–H), 7.62 (m, 3H, Ar–H), 7.27 (m, 2H, Ar–H), 6.94 (m, 4H, Ar–H), 6.71 (br, 1H, Ar–H), 5.48 (br, 1H, C_5-<u>H_X</u>), 4.21 (br, 1H, C_4-<u>H_B</u>), 3.72 (br, 6H, 2OC<u>H_3</u>), 3.59 (br, 1H, C_4-<u>H_A</u>); ^{13}C NMR (DMSO-d_6, 600 MHz): δ ppm 163.7 (C, C-4′), 161.7 (C, C-3), 154.2 (C, C-2′), 149.7 (C, C-3″), 148.7 (C, C-4″), 146.1 (C, C-1‴), 139.0 (C, C-10′), 133.9 (CH, C-3‴, C-5‴), 133.8 (CH, C-8′), 132.8 (C, C-1″), 123.9 (CH, C-6′), 122.4 (CH, C-7′), 120.2 (C, C-6″), 117.7 (C, Ar–<u>C</u>≡N), 115.7 (CH, C-9′), 114.3 (C, C-5′) 113.1 (CH, C-2‴, C-6‴), 112.6 (CH, C-5″), 110.0 (C, C-2″), 101.5 (CH, C-4‴), 100.0 (C, C-3′), 60.9 (CH, C-5), 56.0 (CH_3, Ar–O-C<u>H_3</u>, Ar–O-<u>CH_3</u>), 55.9 (CH_3, Ar–O-C<u>H_3</u>, Ar–O-<u>CH_3</u>), 47.2 (CH_2, C-4); HR-MS *m/z* (neg): 465.15554 $C_{27}H_{22}N_4O_4$ (calcd. 466.1641).

3-(5-(3,4-Dimethoxyphenyl)-1-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxyquinolin-2(1H)-one (9b)

This compound was prepared following the general procedure B. 4-methoxy phenyl hydrazine hydrochloride (149.1 mg, 0.85 mmol) was dissolved in 0.94 ml of absolute ethanol. Quinolinone-chalcone **8a** (120 mg, 0.34 mmol) was dissolved in 1.9 ml of acetic acid, and it was added dropwise to the ethanolic solution of hydrazine. The resulting solution was stirred at 120 °C for 24 h. The solid produced upon filtration was triturated from methanol/dichloromethane to afford the pure pyrazoline **9b** as yellow powder. Yield: 82 mg (68.3%); m.p. 230 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ ppm 13.54 (s, 1H, O<u>H</u>), 11.54 (s, 1H, N<u>H</u>), 7.96 (d, 1H, J = 7.8 Hz, Ar–H), 7.55 (t, 1H, J = 7.8 Hz, Ar–H), 7.29 (d, 1H, J = 8.7 Hz, Ar–H), 7.23 (t, 1H, J = 7.8 Hz, Ar–H), 6.99 (s, 1H, Ar–H), 6.86 (m, 6H, Ar–H), 5.16 (dd, 1H, J = 11.4, 9.0 Hz, C_5-<u>H_X</u>), 4.19 (dd,1H, J = 18.6, 12.0 Hz, C_4-<u>H_B</u>), 3.72 (br, 6H, 2OC<u>H_3</u>), 3.67 (s, 3H, OC<u>H_3</u>), 3.44 (dd, 1H, J = 18.0, 8.4 Hz, C_4-<u>H_A</u>); ^{13}C NMR (DMSO-d_6, 600 MHz): δ ppm 162.4 (C, C-4′), 161.3 (C, C-3), 153.4 (C, C-2′), 150.6 (C, C-4‴), 149.1 (C, C-3″), 148.1 (C, C-4″), 138.4 (C, C-10′), 138.2 (C, C-1‴), 134.3 (CH, C-8′), 123.3 (C, C-1″), 123.1 (CH, C-6′), 122.1 (C, C-7′), 121.6 (CH, C-6″), 118.2 (CH, C-3‴, C-5‴), 115.4 (CH, C-9′), 115.2 (CH, C-5″), 114.8 (CH, C-2‴, C-6‴), 114.4 (C, C-5′), 112.2 (CH, C-2″), 101.4 (C, C-3′), 63.2 (CH, C-5), 55.6 (CH_3, Ar–O-<u>CH_3</u>), 55.4 (CH_3, Ar–O-<u>CH_3</u>), 55.1 (CH_3, Ar–O-<u>CH_3</u>), 46.6 (CH_2, C-4); HR-MS *m/z* (pos): 471.17808 $C_{27}H_{25}N_3O_5$ (calcd. 471.1794).

3-(1-(4-(Benzyloxy)phenyl)-5-(3,4-dimethoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxyquinolin-2(1H)-one (9c)

This compound was prepared following the general procedure B. 4-benzyloxy phenyl hydrazine hydrochloride (214.1 mg, 0.85 mmol) was dissolved in 0.9 ml of absolute ethanol. Quinolinone-chalcone **8a** (120 mg, 0.34 mmol) was dissolved in 1.9 ml of acetic acid, and it was added dropwise to the ethanolic solution of hydrazine. The resulting solution was stirred at 120 °C for 24 h. The solid produced upon filtration was triturated from methanol/dichloromethane to afford the pure pyrazoline **9c** as yellow powder. Yield: 73.2 mg (61%); m.p. 245 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ ppm 13.53 (s, 1H, O<u>H</u>), 11.55 (s, 1H, N<u>H</u>), 7.96 (d, 1H, J = 7.2 Hz, Ar–H), 7.55 (t, 1H, J = 7.2 Hz, Ar–H), 7.38 (m, 4H, Ar–H), 7.30 (m, 2H, Ar–H), 7.23 (t, 1H, J = 7.2 Hz, Ar–H), 6.98 (s, 1H, Ar–H), 6.90 (m, 5H, Ar–H), 6.81 (d, 1H, J = 7.2 Hz, Ar–H), 5.17 (br, 1H, C_5-<u>H_X</u>), 5.0 (s, 2H, Ar-C<u>H_2</u>-O-Ar), 4.19 (dd, 1H, J = 18.6, 12.0 Hz, C_4-<u>H_B</u>), 3.72 (s, 6H, OC<u>H_3</u>), 3.44 (dd, 1H, J = 18.0, 8.4 Hz, C_4-<u>H_A</u>); ^{13}C NMR (DMSO-d_6, 600 MHz): δ ppm 162.4 (C, C-4′), 161.3 (C, C-3), 152.4 (C, C-2′), 150.6 (C, C-4‴), 149.1 (C, C-3″), 148.1 (C, C-4″), 138.5 (C, C-10′), 138.2 (C, C-1‴), 137.3 (C, C-1‴), 134.3 (CH, C-8′), 131.8 (C, C-1″), 128.3 (CH, C-3‴, C-5‴), 127.7 (CH, C-4‴), 127.6 (CH, C-2‴, C-6‴), 123.2 (CH, C-6′), 121.8 (CH, C-7′), 118.0 (CH, C-6″), 115.6(CH, C-2‴, C-6‴), 115.1 (CH,

C-9′), 114.8 (CH, C-3‴, C-5‴), 114.0 (CH, C-5″), 112.0 (C, C-5′), 109.8 (CH, C-2″), 101.4 (C, C-3′), 69.5 (CH_2, Ar–O-$\underline{CH_2}$-Ar), 63.3(CH, C-5), 55.4 (CH_3, Ar–O-$\underline{CH_3}$, Ar–O-$\underline{CH_3}$), 30.7 (CH_2, C-4); HR-MS *m/z* (pos): 548.21717 $C_{33}H_{29}N_3O_5$ (calcd. 547.2107).

4-(3-(4-Hydroxy-2-oxo-1,2-dihydroquinolin-3-yl)-5-(4-hydroxy-3-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)benzonitrile (9d)

This compound was prepared following the general procedure B. 4-cyano phenyl hydrazine hydrochloride (150.8 mg, 0.89 mmol) was dissolved in 1 ml of absolute ethanol. Quinolinone-chalcone **8b** (120 mg, 0.36 mmol) was dissolved in 2 ml of acetic acid, and it was added dropwise to the ethanolic solution of hydrazine. The resulting solution was stirred at 120 °C for 24 h. The solid produced upon filtration was triturated from methanol/dichloromethane to afford the pure pyrazoline **9d** as orange powder. Yield: 62 mg (51.7%); m.p. > 250 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ ppm 13.08 (s, 1H, O$\underline{H}$), 11.61 (s, 1H, N$\underline{H}$), 9.01 (s, 1H, Ar-O$\underline{H}$), 7.98 (d, 1H, J = 8.4 Hz, Ar–H), 7.62 (d, 2H, J = 9.0 Hz, Ar–H), 7.58 (t, 1H, J = 8.4 Hz, Ar–H), 7.29 (d, 1H, J = 8.4 Hz, Ar–H), 7.25 (t, 1H, J = 7.8 Hz, Ar–H), 6.98 (d, 2H, J = 9.0 Hz, Ar–H), 6.90 (d, 1H, J = 1.2 Hz, Ar–H), 6.71 (d, 1H, J = 8.4 Hz, Ar–H), 6.59 (dd, 1H, J = 7.8, 1.2 Hz, Ar–H), 5.42 (dd, 1H, J = 11.4, 5.4 Hz, C_5-$\underline{H_X}$), 4.19 (dd, 1H, J = 18.6, 12.0 Hz, C_4-$\underline{H_B}$), 3.73 (s, 3H, OC$\underline{H_3}$), 3.58 (dd, 1H, J = 19.2,6.0 Hz, C_4-$\underline{H_A}$); ^{13}C NMR (DMSO-d_6, 600 MHz): δ ppm 163.5 (C, C-4′), 161.6 (C, C-3), 154.2 (C, C-2′), 148.5 (C, C-3″), 146.5 (C, C-4″), 146.0 (C, C-1‴), 138.9 (C, C-10′), 133.9 (CH, C-3‴, C-5‴), 132.8 (C, C-1″), 132.3 (CH, C-8′), 123.8 (CH, C-6′), 122.4 (CH, C-7′), 120.3 (CH, C-6″), 118.1 (C, Ar–$\underline{C}\equiv$N), 116.3 (CH, C-9′), 115.7 (CH, C-5″), 114.2 (C, C-5′), 113.1 (CH, C-2‴, C-6‴), 110.4 (C, C-2″), 101.5 (C, C-4‴), 99.9 (C, C-3′), 61.0 (CH, C-5), 56.1 (CH_3, Ar–O-$\underline{CH_3}$,), 47.2 (CH_2, C-4); HR-MS *m/z* (neg): 451.13988 $C_{26}H_{20}N_4O_4$ (calcd. 452.1485).

3-(1-(4-(Benzyloxy)phenyl)-5-(4-hydroxy-3-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxyquinolin-2(1H)-one (9e)

This compound was prepared following the general procedure B. 4-benzyloxy phenyl hydrazine hydrochloride (223 mg, 0.89 mmol) was dissolved in 1 ml of absolute ethanol. Quinolinone-chalcone **8b** (120 mg, 0.36 mmol) was dissolved in 2 ml of acetic acid and it was added dropwise to the ethanolic solution of hydrazine. The resulting solution was stirred at 120 °C for 24 h. The solid produced upon filtration was triturated from methanol/dichloromethane to afford the pure pyrazoline **9e** as orange powder. Yield: 68 mg (56.7%); m.p. > 250 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ ppm 13.54 (s, 1H, O$\underline{H}$), 11.54 (s, 1H, N$\underline{H}$), 8.95 (s, 1H, Ar-O$\underline{H}$), 7.95 (d, 1H, J = 7.8 Hz, Ar–H), 7.55 (t, 1H, J = 7.8 Hz, Ar–H), 7.41 (m, 2H, Ar–H), 7.37 (t, 2H, J = 7.2 Hz, Ar–H), 7.30 (m, 2H, Ar–H), 7.23 (t, 1H, J = 7.2 Hz, Ar–H), 6.93 (m, 3H, Ar–H), 6.88 (m, 2H, Ar–H), 6.71 (m, 2H, Ar–H), 5.11 (br, 1H, C_5-$\underline{H_X}$), 5.00 (s, 2H, Ar-C$\underline{H_2}$-O-Ar), 4.17 (dd, 1H, J = 18.6, 12.0 Hz, C_4-$\underline{H_B}$), 3.73 (s, 3H, OC$\underline{H_3}$), 3.43 (dd, 1H, J = 18.6, 8.4 Hz, C_4-$\underline{H_A}$); ^{13}C NMR (DMSO-d_6, 600 MHz): δ ppm 162.3 (C, C-4′), 161.3 (C, C-3), 152.4 (C, C-2′), 150.6 (C, C-4‴), 147.9 (C, C-3″), 145.9 (C, C-4″), 138.6 (C, C-10′), 138.2 (C, C-1‴), 137.3 (C, C-1‴), 132.8 (CH, C-8′), 131.8 (C, C-1″), 128.3 (CH, C-3‴, C-5‴), 127.7 (CH, C-4‴), 127.6 (CH, C-2‴, C-6‴), 123.1 (CH, C-6′), 121.8 (CH, C-7′), 118.4 (CH, C-6″), 115.6 (CH, C-2‴, C-6‴), 115.1 (CH, C-9′), 114.8 (CH, C-3‴, C-5‴), 114.0 (CH, C-5″), 112.9 (C, C-5′), 110.2 (CH, C-2″), 101.4 (C, C-3′), 69.5 (CH_2, Ar–O-$\underline{CH_2}$-Ar), 63.4 (CH, C-5), 55.6 (CH_3, Ar–O-$\underline{CH_3}$,), 46.6 (CH_2, C-4); HR-MS *m/z* (pos): 533.19400 $C_{32}H_{27}N_3O_5$ (calcd. 533.1951).

3-(1-(4-Chlorophenyl)-5-(4-hydroxy-3-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxyquinolin-2(1H)-one (9f)

This compound was prepared following the general procedure B. 4-chloro-phenyl hydrazine hydrochloride (100.9 mg, 0.56 mmol) was dissolved in 0.6 ml of absolute ethanol. Quinolinone-chalcone **8b** (76 mg, 0.2 mmol) was dissolved in 1.2 ml of acetic acid and was added dropwise to the ethanolic solution of hydrazine. The resulting solution was stirred at 120 °C for 24 h. The solid produced upon filtration was triturated from methanol/dichloromethane to afford the pure pyrazoline **9f** as orange powder. Yield: 32 mg (42.1%); m.p. > 250 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ ppm 13.29 (s, 1H, O$\underline{H}$), 11.58 (s, 1H, N$\underline{H}$), 9.0 (s, 1H, Ar-O$\underline{H}$), 7.96 (d, 1H, J = 7.8 Hz, Ar–H), 7.56 (t, 1H, J = 7.2 Hz, Ar–H), 7.25 (m, 4H, Ar–H), 6.91 (m, 3H, Ar–H), 6.71 (d, 1H, J = 8.4 Hz, Ar–H), 6.64 (dd, 1H, J = 7.8, 0.6 Hz, Ar–H), 5.26 (dd, 1H, J = 12.0, 7.2 Hz, C_5-$\underline{H_X}$), 4.18 (dd, 1H, J = 18.6, 12.0 Hz, C_4-$\underline{H_B}$), 3.73 (s, 3H, OC$\underline{H_3}$), 3.51 (dd, 1H, J = 19.2, 7.2 Hz, C_4-$\underline{H_A}$); ^{13}C NMR (DMSO-d_6, 600 MHz): δ ppm 162.6 (C, C-4′), 161.3 (C, C-3), 151.7 (C, C-2′), 148.0 (C, C-3″), 146.0 (C, C-4″), 142.4 (C, C-1‴), 138.3 (C, C-10′), 132.4 (CH, C-8′), 132.1 (C, C-4‴), 130.1 (C, C-1″), 128.9 (CH, C-3‴, C-5‴), 123.2 (CH, C-6′), 122.9 (CH, C-7′), 121.9 (CH, C-6″), 119.6 (CH, C-2‴, C-6‴), 118.0 (CH, C-9′), 115.2 (CH, C-5″), 113.9 (C, C-5′), 111.6 (CH, C-2″), 101.3 (C, C-3′), 61.8 (CH, C-5), 55.6 (CH_3, Ar–O-$\underline{CH_3}$,), 46.8 (CH_2, C-4); HR-MS *m/z* (neg): 460.10629 $C_{25}H_{20}ClN_3O_4$ (calcd. 461.1142).

4-(5-(3,4-Dimethoxyphenyl)-3-(4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)benzonitrile (9g)

This compound was prepared following the general procedure B. 4-cyano phenyl hydrazine hydrochloride (116.1 mg, 0.68 mmol) was dissolved in 0.9 ml of absolute ethanol. Quinolinone–chalcone **8c** (100 mg, 0.27 mmol) was dissolved in 1.7 ml of acetic acid and it was added dropwise to the ethanolic solution of hydrazine. The resulting solution was stirred at 120 °C for 24 h. The solid produced upon filtration was triturated from methanol/dichloromethane to afford the pure pyrazoline **9g** as orange powder. Yield: 73 mg (73%); m.p. > 250 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ ppm 13.07 (s, 1H, O<u>H</u>), 8.07 (br, 1H, Ar–H), 7.69 (br, 1H, Ar–H), 7.62 (d, 2H, J = 7.8 Hz, Ar–H), 7.50 (br, 1H, Ar–H), 7.33 (br, 1H, Ar–H), 6.96 (m, 3H, Ar–H), 6.88 (d, 1H, J = 8.4 Hz, Ar–H), 6.71 (d, 1H, J = 8.4 Hz, Ar–H), 5.46 (br, 1H, C_5-<u>H</u>$_X$), 4.21 (dd, 1H, J = 18.6, 12.6 Hz, C_4-<u>H</u>$_B$), 3.71 (br, 6H, 2OC<u>H</u>$_3$), 3.59 (br, 1H, C_4-<u>H</u>$_A$), 3.57 (s, 3H, NC<u>H</u>$_3$); ^{13}C NMR (DMSO-d_6, 600 MHz): δ ppm 162.2 (C, C-3), 160.8 (C, C-4′), 154.4 (C, C-2′), 149.6 (C, C-3″), 148.7 (C, C-4″), 146.0 (C, C-1‴), 139.8 (C, C-10′), 134.0 (CH, C-3‴, C-5‴), 133.8 (C, C-1″), 133.3 (CH, C-8′), 124.3 (CH, C-6′), 122.6 (CH, C-7′), 117.8 (C, Ar–<u>C</u>≡N), 115.4 (CH, C-6″), 115.0 (CH, C-9′), 113.1 (CH, C-2‴, C-6‴), 112.6 (CH, C-5″), 110.0 (C, C-5′), 101.4 (C, C-4‴), 100.1 (CH, C-2″), 99.5 (C, C-3′), 60.9 (CH, C-5), 55.9 (CH_3, Ar–O-<u>C</u>H$_3$), 55.9 (CH_3, Ar–O-<u>C</u>H$_3$), 47.4 (CH_2, C-4), 29.5 (CH_3, N-<u>C</u>H$_3$); HR-MS *m*/*z* (neg): 479.17102 $C_{28}H_{24}N_4O_4$ (calcd. 480.1798).

3-(5-(3,4-Dimethoxyphenyl)-1-(3,4-dimethylphenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-1-methylquinolin-2(1H)-one (9h)

This compound was prepared following the general procedure B. 1-(3,4-dimethylphenyl) hydrazine hydrochloride (118.2 mg, 0.68 mmol) was dissolved in 0.9 ml of absolute ethanol. Quinolinone-chalcone **8c** (100 mg, 0.27 mmol) was dissolved in 1.7 ml of acetic acid, and it was added dropwise to the ethanolic solution of hydrazine. The resulting solution was stirred at 120 °C for 24 h. The solid produced upon filtration was triturated from methanol/dichloromethane to afford the pure pyrazoline **9h** as orange powder. Yield: 34 mg (34%); m.p. > 250 °C; ^{1}H NMR ($CDCl_3$, 600 MHz): δ ppm 13.74 (br, 1H, O<u>H</u>), 8.17 (d, 1H, J = 7.8 Hz, Ar–H), 7.56 (t, 1H, J = 7.8 Hz, Ar–H), 7.25 (m, 2H, Ar–H), 6.94 (d, 1H, J = 7.8 Hz, Ar–H), 6.81 (m, 4H, Ar–H), 6.64 (d, 1H, J = 7.8 Hz, Ar–H), 5.01 (dd, 1H, J = 12.0, 9.0 Hz, C_5-<u>H</u>$_X$), 4.29 (dd, 1H, J = 18.6, 12.6 Hz, C_4-<u>H</u>$_B$), 3.82 (br, 6H, 2OC<u>H</u>$_3$), 3.76 (br, 1H, C_4-<u>H</u>$_A$), 3.61 (s, 3H, NC<u>H</u>$_3$), 2.18 (s, 3H, Ar-C<u>H</u>$_3$), 2.145 (s, 3H, Ar-C<u>H</u>$_3$); ^{13}C NMR ($CDCl_3$, 600 MHz): δ ppm 162.1 (C, C-3), 160.8 (C, C-4′), 149.5 (C, C-2′), 149.2 (C, C-3″), 148.7 (C, C-4″), 143.0 (C, C-1‴), 139.0 (C, C-10′), 137.7 (C, C-3‴), 137.5 (C, C-4‴), 136.7 (CH, C-5‴), 131.3 (C, C-1″), 130.1 CH, C-8′), 126.4 (CH, C-6′), 124.6 (CH, C-7′), 122.8 (CH, C-6‴), 121.8 (CH, C-2‴), 116.6 (CH, C-6″), 114.0 (CH, C-9′), 112.1 (C, C-5′), 111.0 (CH, C-5″), 108.2 (CH, C-2″), 100.8 (C, C-3′), 70.7 (CH, C-5), 56.0 (CH_2, C-4), 55.9 (CH_3, Ar–O-<u>C</u>H$_3$), 55.9 (CH_3, Ar–O-<u>C</u>H$_3$), 29.4 (CH_3, N-<u>C</u>H$_3$), 20.0 (CH_3, Ar-<u>C</u>H$_3$), 19.6 (CH_3, Ar-<u>C</u>H$_3$); HR-MS *m*/*z* (neg): 482.20754 $C_{29}H_{29}N_3O_4$ (calcd. 483.2158).

3-(5-(3,4-Dimethoxyphenyl)-1-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-1-methylquinolin-2(1H)-one (9i)

This compound was prepared following the general procedure B. 4-methoxy phenyl hydrazine hydrochloride (239 mg, 1.37 mmol) was dissolved in 1.7 ml of absolute ethanol. Quinolinone-chalcone **8c** (200 mg, 0.55 mmol) was dissolved in 3.5 ml of acetic acid, and it was added dropwise to the ethanolic solution of hydrazine. The resulting solution was stirred at 120 °C for 24 h. The solid produced upon filtration was triturated from methanol/dichloromethane to afford the pure pyrazoline **9i** as orange powder. Yield: 152 mg (76%); m.p. > 250 °C; ^{1}H NMR ($CDCl_3$, 600 MHz): δ ppm 13.74 (s, 1H, O<u>H</u>), 8.17 (dd, 1H, J = 7.8, 0.6 Hz, Ar–H), 7.58 (t, 1H, J = 8.4 Hz, Ar–H), 7.283 (m, 2H, Ar–H), 6.92 (m, 2H, Ar–H), 6.87 (m, 2H, Ar–H), 6.81 (m, 3H, Ar–H), 4.96 (dd, 1H, J = 12.0, 9.6 Hz, C_5-<u>H</u>$_X$), 4.33 (dd, 1H, J = 18.6, 12.0 Hz, C_4-<u>H</u>$_B$), 3.86 (s, 3H, OC<u>H</u>$_3$), 3.83 (s, 3H, OC<u>H</u>$_3$), 3.74 (s, 3H, OC<u>H</u>$_3$), 3.64 (s, 3H, NC<u>H</u>$_3$), 3.60 (br, 1H, C_4-<u>H</u>$_A$); ^{13}C NMR ($CDCl_3$, 600 MHz): δ ppm 162.6 (C, C-3), 161.9 (C, C-4′), 154.1 (C, C-2′), 151.4 (C, C-4‴), 149.6 (C, C-3″), 149.5 (C, C-4″), 148.5 (C, C-10′), 139.4 (C, C-1‴), 139.4 (C, C-1″), 134.8 (CH, C-8′), 124.7 (CH, C-6′), 124.6 (CH, C-7′), 121.9 (CH, C-6″), 118.8 (CH, C-9′), 116.1 (C, C-5′), 115.6 (CH, C-5″), 114.8 (CH, C-2‴, C-6‴), 111.7 (CH, C-3‴, C-5‴), 109.3 (CH, C-2″), 101.7 (C, C-3′), 65.3 (CH, C-5), 56.0 (CH_3, Ar–O-<u>C</u>H$_3$), 55.8 (CH_3, Ar–O-<u>C</u>H$_3$), 55.6 (CH_3, Ar–O-<u>C</u>H$_3$), 47.4 (CH_2, C-4), 29.1 (CH_3, N-<u>C</u>H$_3$); HR-MS *m*/*z* (neg): 484.18742 $C_{28}H_{27}N_3O_5$ (calcd. 485.1951).

4-(3-(4-Hydroxy-1-methyl-2-oxo-1,2-dihydroquinolin-3-yl)-5-(4-hydroxy-3-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)benzonitrile (9j)

This compound was prepared following the general procedure B. 4-cyano phenyl hydrazine hydrochloride (120.7 mg, 0.71 mmol) was dissolved in 0.9 ml of absolute ethanol. Quinolinone-chalcone **8d** (100 mg, 0.28 mmol) was dissolved in 1.8 ml of acetic acid, and it was added dropwise to the ethanolic solution of hydrazine. The resulting solution

was stirred at 120 °C for 24 h. The solid produced upon filtration was triturated from methanol/dichloromethane to afford the pure pyrazoline **9j** as orange powder. Yield: 82 mg (82%); m.p. > 250 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ ppm 13.06 (s, 1H, O<u>H</u>), 9.01 (s, 1H, Ar-O<u>H</u>), 8.05 (d, 1H, J = 7.2 Hz, Ar–H), 7.67 (t, 1H, J = 6.6 Hz, Ar–H), 7.60 (d, 2H, J = 7.8 Hz, Ar–H), 7.48 (d, 1H, J = 7.8 Hz, Ar–H), 7.31 (t, 1H, J = 7.2 Hz, Ar–H), 6.95 (d, 2H, J = 7.8 Hz, Ar–H), 6.90 (br, 1H, Ar–H), 6.71 (d, 1H, J = 7.8 Hz, Ar–H), 6.60 (d, 1H, J = 7.2 Hz, Ar–H), 5.38 (dd, 1H, J = 10.8, 4.2 Hz, C_5-<u>H</u>$_X$), 4.18 (dd, 1H, J = 18.6, 12.6 Hz, C_4-<u>H</u>$_B$), 3.73 (s, 3H, OC<u>H</u>$_3$), 3.57 (dd, 1H, J = 19.8, 3.6 Hz, C_4-<u>H</u>$_A$), 3.52 (s, 3H, NC<u>H</u>$_3$); ^{13}C NMR (DMSO-d_6, 600 MHz): δ ppm 162.2 (C, C-3), 160.8 (C, C-4′), 154.4 (C, C-2′), 148.5 (C, C-3″), 146.5 (C, C-4″), 146.0 (C, C-1‴), 139.8 (C, C-10′), 133.9 (CH, C-3‴, C-5‴), 133.1 (C, C-1″), 132.3 (CH, C-8′), 124.3 (CH, C-6′), 122.5 (CH, C-7′), 120.2 (CH, C-5″), 118.2 (C, Ar–<u>C</u>≡N), 116.3 (CH, C-9′), 115.2 (CH, C-6″), 115.0 (C, C-5′), 113.1 (CH, C-2‴, C-6‴), 110.4 (CH, C-2″), 101.3 (C, C-4‴), 100.0 (C, C-3′), 61.0 (CH, C-5), 56.1 (CH_3, Ar–O-<u>C</u>H$_3$), 47.4 (CH_2, C-4), 29.4 (CH_3, N-<u>C</u>H$_3$); HR-MS *m*/*z* (neg): 465.15552 $C_{27}H_{22}N_4O_4$ (calcd. 466.1641).

3-(1-(3,4-Dimethylphenyl)-5-(4-hydroxy-3-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-1-methylquinolin-2(1H)-one (9k)

This compound was prepared following the general procedure B. 1-(3,4-dimethylphenyl) hydrazine hydrochloride (122.85 mg, 0.71 mmol) was dissolved in 0.9 ml of absolute ethanol. Quinolinone-chalcone **8d** (100 mg, 0.28 mmol) was dissolved in 1.8 ml of acetic acid and it was added dropwise to the ethanolic solution of hydrazine. The resulting solution was stirred at 120 °C for 24 h. The solid produced upon filtration was triturated from methanol/dichloromethane to afford the pure pyrazoline **9k** as orange powder. Yield: 67 mg (67%); m.p. > 250 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ ppm 13.55 (s, 1H, O<u>H</u>), 8.95 (s, 1H, Ar-O<u>H</u>), 8.04 (d, 1H, J = 7.2 Hz, Ar–H), 7.65 (t, 1H, J = 7.2 Hz, Ar–H), 7.46 (d, 1H, J = 8.4 Hz, Ar–H), 7.30 (t, 1H, J = 7.2 Hz, Ar–H), 6.95 (d, 1H, J = 7.8 Hz, Ar–H), 6.91 (br, 1H, Ar–H), 6.75 (br, 1H, Ar–H), 6.70 (d, 1H, J = 7.8 Hz, Ar–H), 6.65 (d, 1H, J = 7.8 Hz, Ar–H), 6.60 (d, 1H, J = 7.2 Hz, Ar–H), 5.14 (dd, 1H, J = 10.8, 7.8 Hz, C_5-<u>H</u>$_X$), 4.14 (dd, 1H, J = 18.0, 12.0 Hz, C_4-<u>H</u>$_B$), 3.72 (s, 3H, OC<u>H</u>$_3$), 3.52 (s, 3H, NC<u>H</u>$_3$), 3.46 (dd, 1H, J = 18.6, 7.8 Hz, C_4-<u>H</u>$_A$), 2.11 (br, 6H, 2Ar-C<u>H</u>$_3$); ^{13}C NMR (DMSO-d_6, 600 MHz): δ ppm 161.4 (C, C-3), 160.8 (C, C-4′), 151.0 (C, C-2′), 148.3 (C, C-3″), 146.2 (C, C-4″), 142.3 (C, C-1‴), 139.4 (C, C-10′), 137.1 (C, C-3‴), 133.5 (C, C-4‴), 132.6 (CH, C-5‴), 130.4 (CH, C-8′), 127.7 (C, C-1″), 124.1 (CH, C-6′), 122.4 (CH, C-7′), 118.6 (CH, C-6‴), 116.2 (CH, C-6″), 115.2 (CH, C-2‴), 115.1 (CH, C-9′), 115.0 (CH, C-5″), 111.1 (C, C-5′), 110.5 (CH, C-2″), 101.6 (C, C-3′), 62.7 (CH, C-5), 56.0 (CH_3, Ar–O-<u>C</u>H$_3$), 47.1 (CH_2, C-4), 29.4 (CH_3, N-<u>C</u>H$_3$), 20.3 (CH_3, Ar-<u>C</u>H$_3$), 18.9 (CH_3, Ar-<u>C</u>H$_3$); HR-MS *m*/*z* (neg): 468.19315 $C_{28}H_{27}N_3O_4$ (calcd. 469.2002).

4-Hydroxy-3-(5-(4-hydroxy-3-methoxyphenyl)-1-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)-1-methylquinolin-2(1H)-one (9l)

This compound was prepared following the general procedure B. 4-methoxy phenyl hydrazine hydrochloride (124.3 mg, 0.71 mmol) was dissolved in 0.9 ml of absolute ethanol. Quinolinone-chalcone **8d** (100 mg, 0.28 mmol) was dissolved in 1.8 ml of acetic acid, and it was added dropwise to the ethanolic solution of hydrazine. The resulting solution was stirred at 120 °C for 24 h. The solid produced upon filtration was triturated from methanol/dichloromethane to afford the pure pyrazoline **9l** as orange powder. Yield: 48 mg (48%); m.p. > 250 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ ppm 13.57 (s, 1H, O<u>H</u>), 8.95 (s, 1H, Ar-O<u>H</u>), 8.04 (d, 1H, J = 7.2 Hz, Ar–H), 7.66 (t, 1H, J = 7.2 Hz, Ar–H), 7.48 (d, 1H, J = 8.4 Hz, Ar–H), 7.31 (t, 1H, J = 7.8 Hz, Ar–H), 6.94 (br, 1H, Ar–H), 6.86 (m, 4H, Ar–H), 6.71 (m, 2H, Ar–H), 5.08 (dd, 1H, J = 11.4, 9.0 Hz, C_5-<u>H</u>$_X$), 4.16 (dd, 1H, J = 18.6, 12.0 Hz, C_4-<u>H</u>$_B$), 3.73 (s, 3H, OC<u>H</u>$_3$), 3.67 (s, 3H, OC<u>H</u>$_3$), 3.54 (s, 3H, NC<u>H</u>$_3$), 3.44 (dd, 1H, J = 18.6, 8.4 Hz, C_4-<u>H</u>$_A$); ^{13}C NMR (DMSO-d_6, 600 MHz): δ ppm 161.5 (C, C-3), 160.9 (C, C-4′), 153.8 (C, C-2′), 151.2 (C, C-4‴), 148.4 (C, C-3″), 146.3 (C, C-4″), 139.4 (C, C-10′), 138.8 (C, C-1‴), 133.2 (CH, C-8′), 132.6 (C, C-1″), 124.1 (CH, C-6′), 122.4 (CH, C-7′), 118.9 (CH, C-6″), 116.2 (CH, C-9′), 115.4 (CH, C-2‴, C-6‴), 115.3 (C, C-5′), 115.2 (CH, C-5″), 115.0 (CH, C-3‴, C-5‴), 110.7 (CH, C-2″), 101.6 (C, C-3′), 63.9 (CH, C-5), 56.0 (CH_3, Ar–O-<u>C</u>H$_3$), 55.7 (CH_3, Ar–O-<u>C</u>H$_3$), 47.2 (CH_2, C-4), 29.4 (CH_3, N-<u>C</u>H$_3$); HR-MS *m*/*z* (pos): 472.18606 $C_{27}H_{25}N_3O_5$ (calcd. 471.1794).

3-(1-(4-(Benzyloxy)phenyl)-5-(4-hydroxy-3-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-1-methylquinolin-2(1H)-one (9m)

This compound was prepared following the general procedure B. 4-benzyloxy phenyl hydrazine hydrochloride (178.4 mg, 0.71 mmol) was dissolved in 0.9 ml of absolute ethanol. Quinolinone-chalcone **8d** (100 mg, 0.28 mmol) was dissolved in 1.8 ml of acetic acid, and it was added dropwise to the ethanol solution of hydrazine. The resulting solution was stirred at 120 °C for 24 h. The solid produced upon filtration was triturated from methanol/dichloromethane to afford the pure pyrazoline **9 m** as orange powder. Yield: 38 mg (38%); m.p. > 250 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ ppm 13.51 (s, 1H, O<u>H</u>), 8.93 (br, 1H, Ar-O<u>H</u>), 8.03 (br, 1H, Ar–H), 7.64 (br, 1H, Ar–H), 7.37 (m, 7H, Ar–H), 6.89 (m, 5H, Ar–H), 6.69

(br, 2H, Ar–H), 5.09 (br, 1H, C_5-$\underline{H}_X$), 4.99 (s, 2H, Ar-$C\underline{H}_2$-O-Ar), 4.17 (br, 1H, C_4-$\underline{H}_B$), 3.72 (s, 3H, $OC\underline{H}_3$), 3.55 (s, 3H, $NC\underline{H}_3$), 3.45 (br, 1H, C_4-$\underline{H}_A$); ^{13}C NMR (DMSO-d_6, 600 MHz): δ ppm 161.0 (C, C-3), 160.4 (C, C-4′), 152.4 (C, C-2′), 150.8 (C, C-4‴), 147.9 (C, C-3″), 145.9 (C, C-4″), 138.9 (C, C-10′), 138.5 (C, C-1‴), 137.3 (C, C-1‴), 132.8 (CH, C-8′), 132.2 (C, C-1″), 128.4 (CH, C-3‴, C-5″), 127.7 (CH, C-4‴), 127.6 (CH, C-2‴, C-6‴), 123.6 (CH, C-6′), 122.0 (CH, C-7′), 118.4 (CH, C-6″), 115.7 (CH, C-9′), 115.6 (CH, C-2‴, C-6‴), 114.8 (CH, C-5″), 114.8 (CH, C-3‴, C-5‴), 114.7 (C, C-5′), 110.2 (CH, C-2″), 101.1 (C, C-3′), 69.5 (CH_2, Ar–O-$C\underline{H}_2$-Ar), 63.4 (CH, C-5), 55.6 (CH_3, Ar–O-$C\underline{H}_3$), 46.8 (CH_2, C-4), 28.9 (CH_3, N-$C\underline{H}_3$); HR-MS *m/z* (pos): 548.21901 $C_{33}H_{29}N_3O_5$ (calcd. 547.2107).

3-(1-(4-Chlorophenyl)-5-(4-hydroxy-3-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-1-methylquinolin-2(1H)-one (9n)

This compound was prepared following the general procedure B. 4-chloro phenyl hydrazine hydrochloride (255.2 mg, 1.43 mmol) was dissolved in 1.80 ml of absolute ethanol. Quinolinone-chalcone **8d** (200 mg, 0.57 mmol) was dissolved in 3.60 ml of acetic acid, and it was added dropwise to the ethanolic solution of hydrazine. The resulting solution was stirred at 120 °C for 24 h. The solid produced upon filtration was triturated from methanol/dichloromethane to afford the pure pyrazoline **9n** as yellow powder. Yield: 180 mg (90%); m.p. > 250 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ ppm 13.26 (s, 1H, O$\underline{H}$), 8.99 (s, 1H, Ar-O$\underline{H}$), 8.00 (d, 1H, J = 7.8 Hz, Ar–H), 7.64 (t, 1H, J = 7.2 Hz, Ar–H), 7.43 (d, 1H, J = 8.4 Hz, Ar–H), 7.28 (t, 1H, J = 7.2 Hz, Ar–H), 7.24 (d, 2H, J = 8.4 Hz, Ar–H), 6.89 (br, 1H, Ar–H), 6.87 (d, 2H, J = 8.4 Hz, Ar–H), 6.71 (d, 1H, J = 7.8 Hz, Ar–H), 6.63 (d, 1H, J = 8.4 Hz, Ar–H), 5.18 (dd, 1H, J = 11.4, 7.2 Hz, C_5-$\underline{H}_X$), 4.15 (dd, 1H, J = 18.6, 12.0 Hz, C_4-$\underline{H}_B$), 3.72 (s, 3H, $OC\underline{H}_3$), 3.49 (s, 3H, $NC\underline{H}_3$), 3.47 (br, 1H, C_4-$\underline{H}_A$); ^{13}C NMR (DMSO-d_6, 600 MHz): δ ppm 161.3 (C, C-3), 160.4 (C, C-4′), 151.9 (C, C-2′), 148.0 (C, C-3″), 146.0 (C, C-4″), 142.3 (C, C-1‴), 139.1 (C, C-10′), 132.4 (CH, C-8′), 128.9 (CH, C-3‴, C-5‴), 123.7 (CH, C-6′), 122.9 (CH, C-7′), 122.1 (CH, C-6″), 118.0 (C, C-4‴), 115.8 (CH, C-1″), 114.8 (C, C-9′), 114.7 (C, C-5″), 114.4 (CH, C-2‴, C-6‴), 110.0 (C, C-5′), 101.0 (CH, C-2″), 99.1 (C, C-3′), 61.8 (CH, C-5), 55.6 (CH_3, Ar–O-$C\underline{H}_3$), 47.0 (CH_2, C-4), 29.0 (CH_3, N-$C\underline{H}_3$); HR-MS *m/z* (pos): 476.13721 $C_{26}H_{22}ClN_3O_4$ (calcd. 475.1299).

Experimental procedure for the synthesis of 4-hydroxy-3-(5-(4-hydroxy-3-methoxyphenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one (9o)

In 4.5 ml of acetic acid, 0.15 mmol (50 mg) of quinolinone–chalcone **8b** and 0.30 mmol (32.4 mg) of phenyl hydrazine were dissolved. The resulting solution was stirred at 118 °C for 4 h. The reaction was monitored by thin-layer chromatography (TLC). After completion of the reaction, 2 ml of aqueous solution of absolute ethanol 50% was added to the mixture, and an orange precipitate was formed. The solid was filtered, washed with ice water and further triturated from methanol and dichloromethane. Yield: 17 mg (34%); m.p. > 250 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ ppm 13.48 (s, 1H, O$\underline{H}$), 11.55 (s, 1H, N$\underline{H}$), 8.95 (s, 1H, Ar-O$\underline{H}$), 7.96 (d, 1H, J = 7.8 Hz, Ar–H), 7.55 (t, 1H, J = 7.2 Hz, Ar–H), 7.29 (d, 1H, J = 7.8 Hz, Ar–H), 7.23 (m, 3H, Ar–H), 6.92 (m, 3H, Ar–H), 6.80 (t, 1H, J = 7.2 Hz, Ar–H), 6.72 (d, 1H, J = 8.4 Hz, Ar–H), 6.67 (d, 1H, J = 7.8 Hz, Ar–H), 5.24 (dd, 1H, J = 11.4, 7.2 Hz, C_5-$\underline{H}_X$), 4.18 (dd, 1H, J = 18.6, 12.0 Hz, C_4-$\underline{H}_B$), 3.73 (s, 3H, $OC\underline{H}_3$), 3.49 (dd, 1H, J = 19.2, 7.8 Hz, C_4-$\underline{H}_A$); ^{13}C NMR (DMSO-d_6, 600 MHz): δ ppm 163.0 (C, C-4′), 161.7 (C, C-3), 151.5 (C, C-2′), 148.4 (C, C-3″), 146.3 (C, C-4″), 144.2 (C, C-1‴), 138.7 (C, C-10′), 133.3 (CH, C-8′), 132.3 (C, C-1″), 129.5 (CH, C-3‴, C-5‴), 123.6 (CH, C-6′), 122.3 (CH, C-4‴), 119.8 (CH, C-7′), 118.5 (CH, C-6″), 116.2 (CH, C-9′), 115.6 (CH, C-5″), 114.4 (C, C-5′), 113.4 (CH, C-2‴, C-6‴), 110.5 (CH, C-2″), 101.8 (C, C-3′), 62.4 (CH, C-5), 56.1 (CH_3, Ar–O-$C\underline{H}_3$), 47.1 (CH_2, C-4); HR-MS *m/z* (neg): 426.14538 $C_{25}H_{21}N_3O_4$ (calcd. 427.1532).

Biological evaluation

Determination of the reducing activity of the stable radical 2,2-Diphenyl-1-picrylhydrazyl (DPPH)

To an ethanolic solution of DPPH (100 µM) in absolute ethanol, an equal volume of the compounds dissolved in DMSO was added (100 µM). The mixture was shaken vigorously and allowed to stand for 20 or 60 min; absorbance at 517 nm was determined spectrophotometrically, and the percentage of activity was calculated. All tests were undertaken on three replicates, and the results presented in Table 3 were averaged and compared with the appropriate standard nordihydroguaiaretic acid (NDGA) [66].

Inhibition of linoleic acid lipid peroxidation

Production of conjugated diene hydroperoxide by oxidation of linoleic acid in an aqueous dispersion is monitored at 234 nm. 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) is used as a free radical initiator. Ten microliters of the 16 mM linoleic acid sodium salt solution was added to the UV cuvette containing 0.93 ml of 0.05 M phosphate buffer, pH 7.4 prethermostated at 37 °C. The oxidation reaction was initiated at 37 °C under air by the addition of 50 µl of 40 mM AAPH solution. Oxidation was carried out in the presence of the synthesized compounds (10 µl, from a

stock solution of 10 mM in DMSO). In the assay without antioxidant, lipid oxidation was measured in the presence of the same level of DMSO. The rate of oxidation at 37 °C was monitored by recording the increase in absorption at 234 nm caused by conjugated diene hydroperoxides and compared with the appropriate standard trolox [66].

Soybean LOX inhibition study in vitro

The tested compounds dissolved in DMSO were incubated at room temperature with sodium linoleate (0.1 ml) and 0.2 ml of enzyme solution ($1/9 \times 10^{-4}$ w/v in saline) in Tris buffer pH 9. The conversion of sodium linoleate to 13-hydroperoxylinoleic acid at 234 nm was recorded and compared with the appropriate standard inhibitor NDGA [66].

Molecular docking simulations for soybean LOX

Molecular docking studies of soybean LOX were performed selecting 3PZW from the Protein Data Bank (PDB) and using UCSF Chimera for the visualization of the protein (PDB code: 3PZW) [70]. Water molecules were removed, missing residues were added with Modeller [71], hydrogen atoms and AMBER99SB-ILDN charges were added while the iron charge was set to +2.0, with no restraint applied to the iron atom and the ligands.

OpenBabel was used to generate and minimize Ligand 3D coordinates [72] using the MMFF94 force field [73], while ACPYPE (AnteChamber PYthon Parser interfacE) [74] was used to generate ligand topologies and parameters using Antechamber [75]. Energy minimizations were achieved using the AMBER99SB-ILDN force field [76] with GROMACS 4.6.5 [77] as the molecular dynamics simulation toolkit. Docking studies were carried out with AutoDock Vina (1.1.2) [78] using a grid box of size 100 Å, 70 Å, 70 Å in X, Y, Z dimensions for soybean LOX. UCSF Chimera was used for the generation of docking input files and analysis of the obtained docking results. Docking was carried out with an exhaustiveness value of 10 and a maximum output of 20 docking modes soybean LOX.

Acknowledgements I.K. gratefully acknowledges financial support from the Eugenides Foundation (scholarship for postgraduate studies).

Compliance with ethical standards

Conflict of interest The authors have declared no conflict of interest.

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