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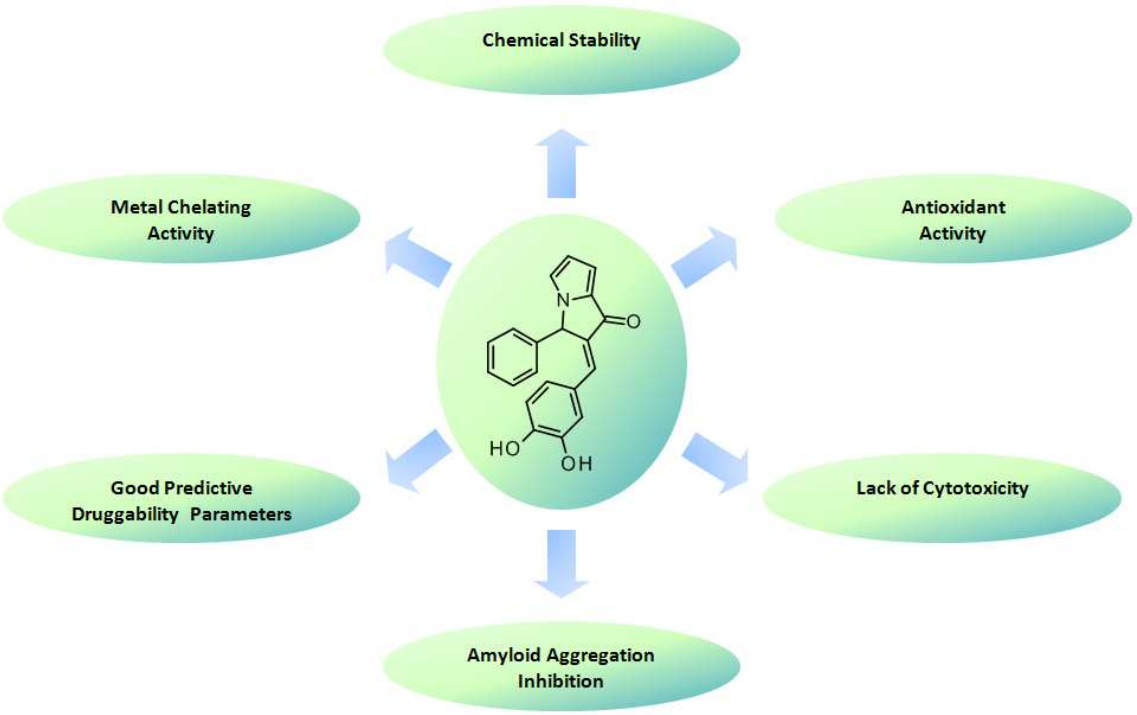

Novel benzylidenephenylpyrrolizinones with pleiotropic activities potentially useful in Alzheimer's disease treatment.

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Highlights :

- Novel benzylidenephenylpyrrolizinones were synthesized.
- Some of them acted as antioxidant, metal chelating and amyloid aggregation inhibitors.
- Benzylidenephenylpyrrolizinones appeared devoid of cytotoxicity.
- They further possess good predictive druggability parameters.

Abstract : This work describes the synthesis and the biological evaluation of novel benzylidenephenylpyrrolizinones as potential antioxidant, metal chelating or amyloid β (βA) aggregation inhibitors. Some derivatives exhibited interesting results in regard to several of the performed evaluations and appear as valuable Multi-Target Directed Ligands with potential therapeutic interest in Alzheimer's disease. Among them, compound **29** particularly appears as a valuable radical and NO scavenger, a Cu(II) and Fe(II) chelating agent and exhibits moderate βA aggregation inhibition

properties. These activities, associated to a good predictive bioavailability and a lack of cytotoxicity, design it as a promising hit for further in vivo investigation.

Key words : Alzheimer; pleiotrope; MTDL; pyrrolizinone; antioxidant; antichelating; anti βA aggregation

1. Introduction

Due to the relative ineffectiveness of the current treatments against Alzheimer's Disease (AD), which mostly all target the cholinergic deficiency,[1] numerous clinical trials during the past decade focused on the inhibition of amyloid or TAU aggregation. Many of these trials however failed and some authors attributed these failures to the extreme selectivity of the tested drugs for one target and their clinical inefficiency towards neurodegenerative syndromes involving multiple pathogenic factors. [2,3] In order to circumvent these problems, drug cocktails have been proposed and today donepezil, one of the marketed acetylcholinesterase (AChE) inhibitors, is frequently added to numerous new clinical candidates.[4] Another paradigm, however, is emerging. It concerns drugs, called Multi-Target Directed Ligands (MTDL), which appear effective in treating complex diseases because of their capacity to interact with several targets implied in the pathogenesis of the disease.[5-18] Besides this synergic effectiveness, these MTDL should furthermore avoid the compliance or drug-drug interaction problems met with drug cocktails.[19,20] Recently some MTDL with a dual mode of action against AD have been reported.[21-32] Most of them are AChE inhibitors (AChEI) with additional biological activities useful for treating AD, like M2 receptor antagonism,[33] direct inhibition of amyloid beta peptide (Aβ) aggregation,[34-39] antioxidant properties,[40-43] in particular linked to MAO-B inhibition,[44-47] calcium channel blockade,[48,49] control oxidative damage properties,[50] or SAPPα secretion improvement.[51,52] Another class of MTDL, potentially useful against AD, lies in polyphenolic compounds. Some of them are obtained from nature or synthetic works such as flavonoids (e.g. EGCG

1),[53] aurones (e.g. Maritimetin **2**),[54] chalcones (e.g. compound **3**),[55] or miscellaneous compounds such as curcumin **4** (Figure 1).[56-58] They share common biological properties such as antioxidant, metal chelation [59,60] and anti Aβ aggregation properties[61-63] which all theoretically account for a therapeutic benefit in AD.

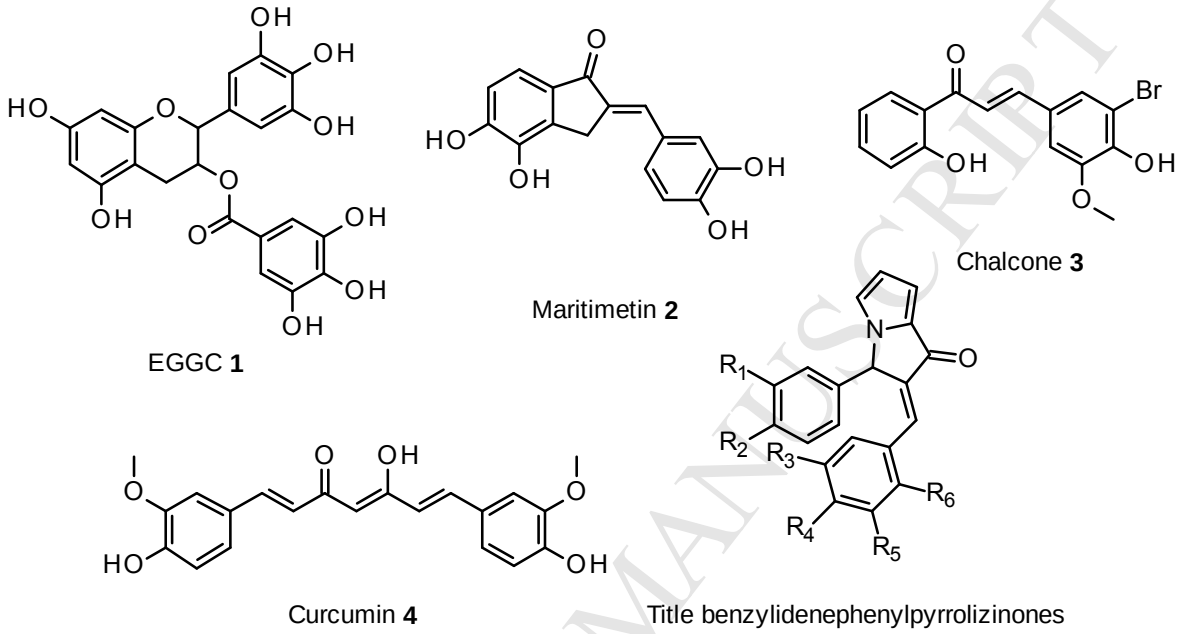


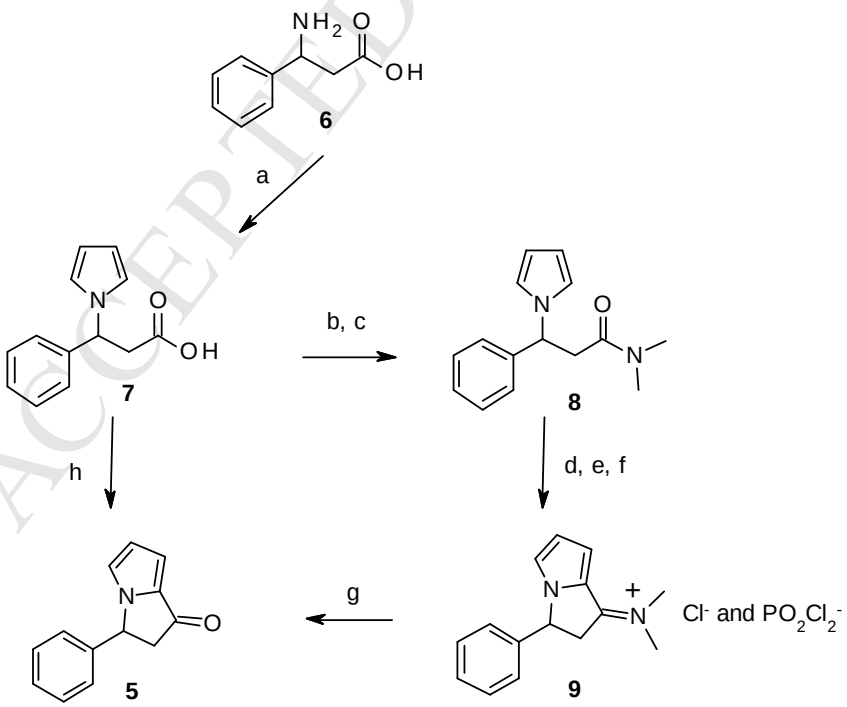
Figure 1. Structure of various naturally occurring polyphenolic derivatives (**1-4**) and title benzylidenephenylpyrrolizinones.

Some common features, important for these activities, have been established. The anti Aβ aggregation potential appears especially linked to the presence of two polyphenolic rings, otherwise needed for the antioxidant and metal chelation activities, but specifically linked by a rigid 8-16 Å long carbon chain.[64] We postulate that the aldolisation of the phenylpyrrolizinone structural system with aromatic benzaldehydes [65, 66] could constitute an appropriate scaffold able to match with these structural requirements. It is the reason why we undertook the synthesis, according to an optimized method, of such benzylidenephenylpyrrolizinones, as well as the in vitro evaluation of their potential antioxidant, metal chelating and anti Aβ aggregation activities. Finally, in view of in vivo

investigations, their cytotoxicity, capacity to cross the gastrointestinal and blood brain barriers and chemical stability were also assessed.

2. Chemistry

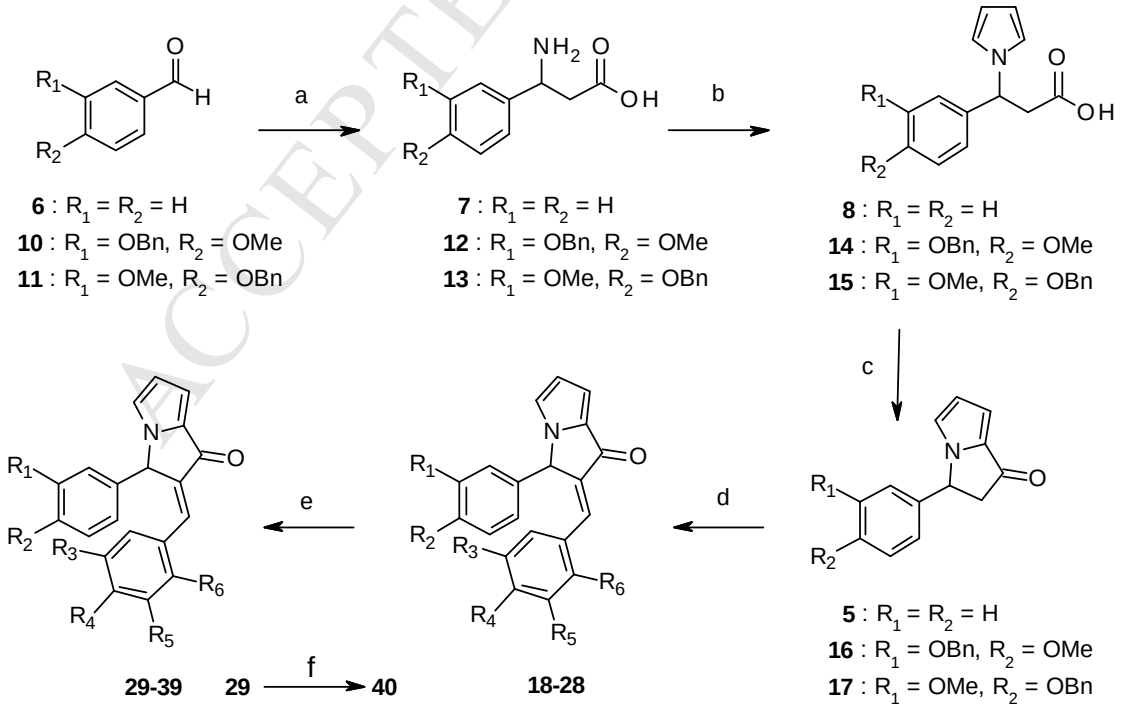
The phenylpyrrolizinone scaffold **5** can be prepared according to a multi-step sequence we previously reported. The latter involved 3-amino-3-phenylpropionic acid **6** in a Clauson-Kaas reaction, then proceeded through the activation of the resulting pyrrolyl derivative **7** under a carboxamide form **8** and then through a ring closure reaction using a Vilsmeier-Haack procedure (Scheme 1). The latter afforded, after alkaline hydrolysis of the iminium salt **9**, the expected phenylpyrrolizinone **5**. An improvement had been already brought to the sequence through the use of BBr_3 for the direct intramolecular cyclization of **7**, but the methodology can't be applied to alkoxyphenyl derivatives without partial *O*-dealkylation. We pursued the study of the sequence and we investigated the use of triphosgen as ring closure reagent for the cyclization of **7** according to a methodology recently reported.[67] It allowed the one-step synthesis of **5** in 52% yield.



Reagents and conditions: a) 2,5-diOMeTHF, AcOH, 60°C, 1h30, 80%; b) TEA, $ClCO_2Et$, CH_3COCH_3, 0°C, 30 min; (c) Me_2NH, Et_2O, room temperature, 15 min, 71%; (d) $POCl_3$, reflux, 1 h; (e) $NaHCO_3$, H_2O; (f) $HClO_4$, H_2O, 0°C, 75%; (g) NaOH, H_2O, room temperature, 10 min, 95%; (h) triphosgen, toluene, 110°C, 5 days (sealed tube), 52%.

Scheme 1. Synthesis of phenylpyrrolizinone **5**.

This procedure was used for the cyclization of the alkoxyphenylpyrrolylpropionic acids **14** and **15**, obtained in two steps from *O*-benzyloxyisovanillin **10** and vanillin **11** respectively, and leading to the synthesis of the phenylpyrrolizinones **16**, **17** (Scheme 2). The latter, as well as the unsubstituted compound **5** were then involved in an aldolisation step with various benzaldehydes yielding compounds **18-28**. Their benzyloxy groups (except **18**) were cleaved using HBr in acetic acid to give the hydroxy derivatives **29-38**. Compound **39** was obtained from the *O*-demethylation of **38** which subsequently took place after the *O*-debenzylation of **28**, the reaction leading to a mixture of the two compounds which had to be separated. Finally the tetrahydroxy derivative **40** was obtained from the *O*-demethylation of **34** under treatment with BBr_3 in refluxing dichloromethane.



Compd	Starting material	R_1	R_2	R_3	R_4	R_5	R_6	Yield (%)
18	**5**	H	H	H	H	H	H	80
19	**5**	H	H	H	OBn	OBn	H	84
20	**5**	H	H	H	OBn	OMe	H	92
21	**16**	OBn	OMe	H	OBn	OBn	H	70
22	**16**	OBn	OMe	H	OMe	OBn	H	64
23	**16**	OBn	OMe	H	OBn	OMe	H	94
24	**17**	OMe	OBn	H	OBn	OBn	H	91
25	**17**	OMe	OBn	H	OMe	OBn	H	90
26	**17**	OMe	OBn	H	OBn	OMe	H	84
27	**17**	OMe	OBn	H	OMe	H	OMe	87
28	**17**	OMe	OBn	OMe	OMe	OMe	H	50
29	**19**	H	H	H	OH	OH	H	53
30	**20**	H	H	H	OH	OMe	H	80
31	**21**	OH	OMe	H	OH	OH	H	80
32	**22**	OH	OMe	H	OMe	OH	H	55
33	**23**	OH	OMe	H	OH	OMe	H	76
34	**24**	OMe	OH	H	OH	OH	H	56
35	**25**	OMe	OH	H	OMe	OH	H	60
36	**26**	OMe	OH	H	OH	OMe	H	53
37	**27**	OMe	OH	H	OMe	H	OMe	47
38	**28**	OMe	OH	OMe	OMe	OMe	H	47
39	**28**	OMe	OH	OMe	OH	OMe	H	15
40	**34**	OH	OH	H	OH	OH	H	71

Reagents and conditions: a) $(CH_2)_2CO_2H$, $AcONH_4$, EtOH, reflux 8-24 h, 23-70%; b) 2,5-diOMeTHF, AcOH, 60°C, 1h30, 56-68%; c) triphosgen, toluene, 110°C, 5 days (sealed tube), 40-60%; d) Benzaldehyde, NaOH, H_2O, MeOH, room temperature, 1 h, 75-95%; e) HBr, AcOH, room temperature, 1 h, 70-80%; f) BBr_3, DCM, reflux, 30 mn, 71%.

Scheme 2. Synthesis of phenylpyrrolizinones **5**,**16**,**17** and benzylidenephenylpyrrolizinones **18-40**.

All the benzylidene derivatives were selectively obtained as *E*-isomers. The latter was attested by NOE experiments and confirmed by X-ray diffraction analyses (Figure 2).

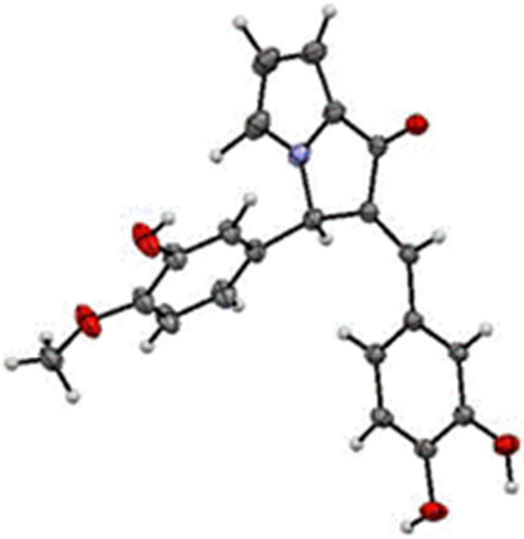


Figure 2. ORTEP diagram of compound **31**.

3. Biology

All the *O*-deprotected benzylidenephenylpyrrolizinones **29-40** as well as the unsubstituted derivative **18** and the tribenzyloxy compound **24** were tested in vitro in order to evaluate their potential antioxidant, metal chelator and anti β-amyloid aggregation properties.

3.1. Antioxidant activities

The antioxidant properties of the tested compounds were evaluated by their capacity to exert high radical scavenging activities and to inhibit $FeCl_2$-induced lipoperoxidation (Table 1).

The radical scavenging activity of the tested compounds was first established using 2,2-diphenyl-1-picrylhydrazyl (DPPH) with curcumin, trolox and ferulic acid as references.[68] Some of the tested derivatives interacted with DPPH with EC_{50} values ranging from 9.6 to 101.3 μM. The best results were obtained for compounds **29**, **31**, **34** and **40** whose EC_{50} are close to 10 μM and therefore are more active than the reference compounds. They all possess a catechol-type benzylidene pattern which seems to play an important role in the activity. Conversely, the sustituents on the phenyl rings do not appear as crucial for the activity.

The NO scavenging activity of the tested compounds was then evaluated through the inhibition of the production of NO generated by the interaction of sodium nitroprusside in aqueous solution at physiological pH with oxygen and measured by the Greiss reagent.[69] Curcumin, ferulic acid and quercetin were used as references. The most active compound was **29** with a NO scavenging activity (65%) close to quercetin (70%).

The lipoperoxidation phenomena exert important influences on the pathogenesis of AD. Aβ causes lipoperoxidation of membranes and induces lipid peroxidation products. Lipids are modified by reactive oxygen species (ROS) and the correlations among lipid peroxides, antioxidant enzymes, senile plaques and neurofibrillary tangles (NFT) in AD brains are very strong. It was furthermore shown that lipid peroxidation is a major cause of depletion of membrane phospholipids in AD.[70] On the other hand, excess concentration of radicals, such as nitric oxide (NO), shows neurotoxicity and acts as a pathological mediator in pathophysiological process in AD. Indeed, oxygen reacts with the excess NO to generate nitrite and peroxynitrite anions, which act as free radicals.[71]

Lipid peroxidation assay was carried out by using the linoleic system according to the ferric thiocyanate method.[72] The best result was obtained with compound **40** which exerted a lipid peroxidation protection similar to those of ferulic acid used as a reference (47%). This result appeared correlated with the radical scavenging activity of this catechol-type compound.

Compd	**Radical scavenging activity (DPPH test) EC_{50} [µM] n = 3**	**NO scavenging activity (%) [40µM] n = 3**	**Lipid peroxidation (%) after 2 h [40µM] n = 3**
Curcumin	19.2 ± 3.3	60.4 ± 4.2	-
Trolox	18.2 ± 1.6	-	-
Ferulic acid	43.2 ± 3.3	46.4 ± 3.9	47.0 ± 1.7
Quercetin	-	70.7 ± 4.5	-
18	nd	34.4 ± 3.2	55.1 ± 5.2
24	> 250	30.7 ± 1.5	92.8 ± 4.5
29	12.6 ± 1.6	65.6 ± 2.2	57.0 ± 2.5
30	> 250	51.7 ± 5.8	67.4 ± 7.2
31	12.8 ± 2.8	57.5 ± 3.0	51.3 ± 4.1
32	> 250	53.7 ± 1.8	71.0 ± 6.5

33	99.5 ± 13.1	55.1 ± 5.1	56.4 ± 3.8
34	12.0 ± 0.9	58.0 ± 0.9	50.6 ± 6.6
35	> 250	57.7 ± 3.2	58.2 ± 6.8
36	101.3 ± 23.2	57.0 ± 0.6	48.5 ± 6.9
37	> 250	40.3 ± 2.7	67.5 ± 7.5
38	> 250	32.6 ± 4.6	67.1 ± 4.0
39	246 ± 9.7	35.3 ± 2.5	70.4 ± 3.3
40	9.6 ± 1.0	47.1 ± 2.4	47.0 ± 6.7

Table 1. Antioxidant activities of curcumin, trolox, ferulic acid, quercetin and compounds **18**, **24**, **29-40** (nd : not determined).

3.2. Metal chelation

The increased level of oxidative stress in AD brain is reflected by the increased brain content of iron (Fe) and copper (Cu) both able to stimulate free radical formation (e.g. hydroxyl radicals *via* Fenton reaction), to enhance protein and DNA oxidation and lipid peroxidation.[73] Moreover, Cu(II) was detected in vivo at elevated concentrations in amyloid plaques, suggesting that it was bound to Aβ peptides.[74] From that, development of new chemical entities able to chelate in the brain Cu(II) or other metal dications as well as Fe(II) or Zn(II) appears to be a valuable therapeutic target against AD.[75] Previous studies showed the possibility to investigate the complexation reactivity of drugs using UV-visible spectral changes of an organic compound incubated with a metal dication. Some of these studies allowed to obtain only qualitative results, [76] whereas some others delivered quantitative data through the determination of dissociation constant.[77,78] Thus, the chelating activity of our novel compounds was explored using these two approaches. First, a qualitative assay was performed on all compounds in order to select active chelator compounds among all newly synthesized derivatives. On the basis of the first results, four derivatives were selected and their reactivity with Cu(II) were deeply explored to determine the stoichiometry and dissociation constant of the reaction.

Thus, the UV-Visible spectral changes of compounds **29-40** at 50 µM in presence of Cu(II) or Fe(II) (50 µM in methanol) were established (Table 2).

Compd	Cu (II)	Fe (II)
curcumine	+	+
29	+	+
30	-	nd
31	+	+
32	+	+
33	+	+
34	+	+
35	-	-
36	+	+
37	-	-
38	-	-
39	-	-
40	+	+

Table 2 : Chelating ability screening of curcumine and compounds **29-40** using UV-Visible spectrophometry (nd : not determined).

Significant spectral changes were measured for curcumin as already reported by Baum [79] and also for compounds **29**, **31**- **34**, **36** and **40**. As an illustration of positive and negative results, figure 3 shows spectral curves obtained for **29** and **38** respectively.

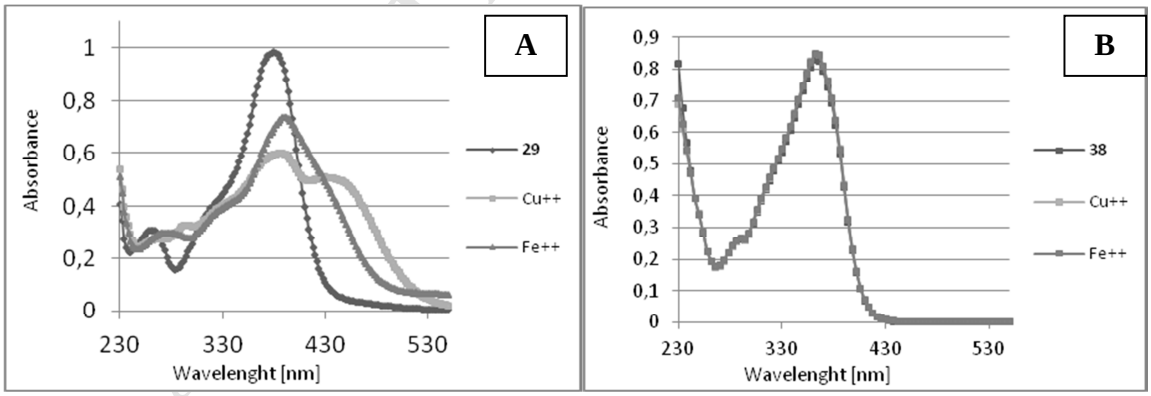


Figure 3 : UV-visible spectra of compounds **29** (A) and **38** (B) alone and in presence of Cu and Fe dications.

Considering the antioxidant activity expressed by the catechol-type derivatives **31**, **34**, **40** and **29**, they were deeply investigated to determine their stoichiometric coefficient and their dissociation

constant with Cu(II) in Methanol-Tris pH 7.4 buffer. A first assay was conducted at a fixed concentration of compound (50 µM) with increasing concentration (0 to 200 µM) of Cu(II) (see for example **29** in Figure 4). For all the considered compounds, an hypochromic effect at 384 nm and a new absorbance band at around 448 nm appeared and grew up when Cu(II) concentration increased. Such a band appeared to be very specific of Cu(II)-compound complex formation. This was illustrated for compound **29** in Figure 4A. This test allowed us to determine a specific wavelength for each complex formation (**29**: 448 nm; **31**: 476 nm; **34**: 500 nm; **40**: 476 nm). Considering that at these specific wavelengths it was possible to study the influence of Cu(II) concentration on the complex formation, these compounds were considered for Job plots assay and for dissociation constant determination.

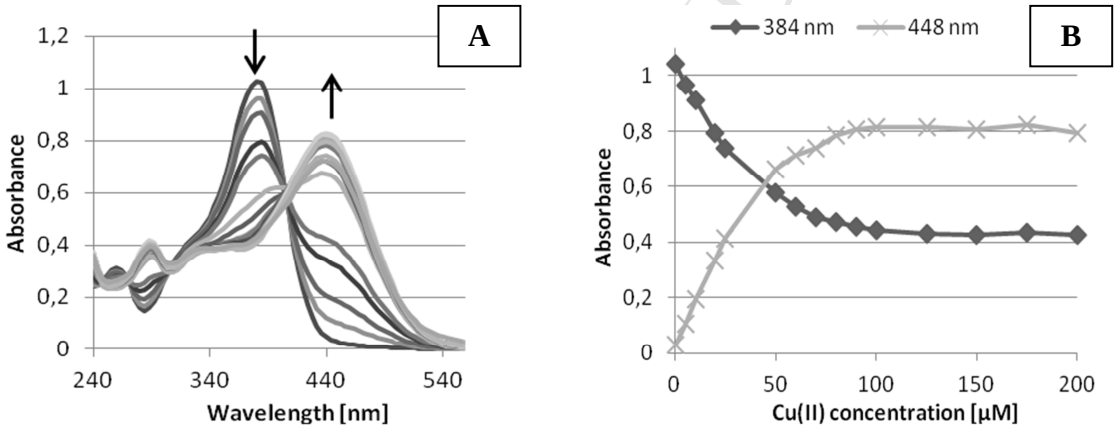


Figure 4 : A) UV-visible spectra of **29** at 50 µM with rising concentration (0 to 200 µM) of Cu(II); B) Variation of absorbance at 384 and 448 nm versus Cu(II) concentration.

For all the tested compounds, as illustrated for **29** in Figure 4B, above 100 µM of Cu(II) added, the signal became stable and remains also stable at higher concentrations (500, 1000, 5000 and 10 000 µM of Cu (II), results not shown). It appears from this result that, above 100 µM Cu(II), 100 % of compound **29** was implied in complex formation, and from the presence of an isobestic point at 404 nm, only one type of complex product is present. Similar results were obtained with **31**, **34** and **40**.

Using method of continuous variation (MCV, Job plots) it was then possible to determine the stoichiometry of the reaction (Figure 5, Table 2).

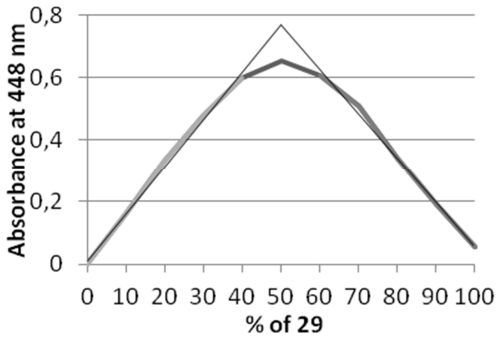


Figure 5 : Job plots of compound **29**.

Contrarily to saturation curve, in MCV method, the addition of ligand (i.e., studied compound) and receptor (i.e., Cu(II)) concentration was fixed (100 µM) and the relative mole fraction of the two reagents varied).[80] From results, **31** revealed a stoichiometry of 2:1 with Cu(II) whereas for compounds **29**, **34** and **40**, reactions appeared equimolar as illustrated for **29** in Figure 5.

A fixed concentration (50 µM) of Cu(II), considered as receptor, was then incubated with growing concentrations of the tested compounds (0 to 200 µM). Difference of absorbance monitored in absence versus presence of Cu(II) was determined at the previously fixed wavelength to extract specific signal of the Cu(II)-compound complex. Scatchard analysis of these data provided same results for stoichiometry than Job plots (data not shown). The dissociation constant (Kd) and the logarithm of the association constant (log K_f) were established using non-linear regression (Table 3). From the results, it appears that in comparison with other compounds, the Cu(II)-**29** bond was significantly stronger.

Compd	**Job's plots stoichiometry X : Cu^{2+}**	**Kd (µM)**	**Log Kf**
29	1 : 1	2.2	5.65
31	2 : 1	70.5	4.15
34	1 : 1	22.1	4.65
40	1 : 1	19.9	4.70

Table 3 : Stoichiometry, dissociation constant and logarithm of association constant of complexation reaction between compounds **29**, **31**, **34**, **40** and Cu(II).

3.3. Self-mediated $A\beta_{1-42}$ aggregation inhibition

The ability of the pyrrolizinones to inhibit the self-mediated Aβ1-42 aggregation was assessed using the thioflavin (ThT) fluorescence assay with curcumin as standard (Table 4).[39] The results showed that some tested compounds exhibited moderate potencies compared to that of curcumin (68.9%). Concerning the SAR, the analysis of these results allows some assertions. The nature of the substituents on the benzylidene ring appears more critical for the activities than those of the phenyl ring. Indeed, compound **29**, devoid of substituents on the phenyl ring, remains active. Conversely the absence of substituents on the benzylidene ring (**18**) or the only presence of *O*-benzyl protecting groups (**24**), without hydroxyl one, dramatically decreases the activity. Curiously, the presence of either a vanillyl- (**36**) or isovanillyl-type (**30**, **32**, **35**) catechol benzylidene pattern such as in curcumine structure, did not afford the best results, the most active compounds remaining the catechol- (**29**, **34**, **40**), syringyl- (**31**, **39**) or trimethoxyphenyl-type (**38**) catechol benzylidene derivatives whatever the substituents on their pending phenyl ring. The best results were obtained with **39**, **29** and **40** (% inhibition = 30.1, 28.7 and 27.0 respectively).

Compd	**inhibition of $A\beta_{1-42}$ aggregation (%) [10 µM] n = 1 triplicate**
Curcumin	68.9 ± 4.9
18	1.4 ± 1.4
24	4.2 ± 3.7
29	28.7 ± 3.3
30	14.5 ± 1.7
31	24.9 ± 4.2
32	8.4 ± 3.1
33	12.8 ± 1.2
34	26.1 ± 7.1
35	15.9 ± 1.3

36	10.4 ± 2.1
37	6.0 ± 10.3
38	23.6 ± 0.6
39	30.1 ± 0.1
40	27.0 ± 3.1

Table 4. Aggregation inhibition activities.

4. Drugability

In view of performing additional in vivo tests upon the synthesized compounds, we undertook the study of their drugability and especially the evaluation of their cytoxicity, their capacity to cross the gastrointestinal and blood-brain barriers and their chemical stability.

4.1. Cytotoxicity

The cytotoxicity of the synthesized compounds was evaluated against KB cells, using curcumin as a reference. None of the tested derivatives exhibited a significant cytotoxicity in this model, the more cytotoxic derivatives (**18**, **37**) remaining two time less active (near 40% inhibition at 10^{-5} M) in regard to curcumin (83%) (Table 5).

4.2. PAMPA assays

Parallel artificial membrane permeability assay (PAMPA) is a high-throughput technique commonly used in drug discovery to predict passive permeability through biological membranes.[82,83] Thus, the ability of the new compounds to cross the gastrointestinal tract (GIT), and to penetrate the Blood-Brain-Barrier (BBB) was evaluated by PAMPA-GIT and PAMPA-BBB assays, respectively. From results (Table 5), it appears that, except **24** and to a lesser degree **40**, all compounds could be well absorbed after oral administration, and could penetrate into the CNS and reach their biological targets located in the CNS.

Compd	**Cytotoxicity Inhibition % at 10^{-5}M (KB cells) n = 3**	**-log Pe (cm s^{-1}) for PAMPA-BBB assay**	**-log Pe (cm s^{-1}) for PAMPA-GIT assay**

Curcumin	83 ± 1	4.6 ± 0.0	4.3 ± 0.2
18	41 ± 9	4.5 ± 0.1	4.0 ± 0.0
24	0 ± 18	10.0 ± 0.0	10.0 ± 0.0
29	0 ± 3	4.1 ± 0.0	3.9 ± 0.0
30	24 ± 6	4.2 ± 0.1	3.9 ± 0.0
31	0 ± 3	4.4 ± 0.1	4.4 ± 0.0
32	24 ± 3	4.3 ± 0.1	3.9 ± 0.0
33	29 ± 4	4.3 ± 0.1	3.9 ± 0.0
34	0 ± 24	4.8 ± 0.1	4.9 ± 0.1
35	0 ± 9	4.7 ± 0.0	4.4 ± 0.3
36	0 ± 4	4.3 ± 0.0	3.9 ± 0.0
37	38 ± 14	4.6 ± 0.1	3.9 ± 0.1
38	nd	4.7 ± 0.0	3.9 ± 0.0
39	0 ± 7	4.8 ± 0.0	3.9 ± 0.0
40	0 ± 14	5.4 ± 0.1	5.7 ± 0.1

Table 5. Cytotoxicity towards KB cells and PAMPA BBB and GIT assays results for curcumin and compounds **18**, **24**, **29-40**.

4.3. Chemical stability

Considering chemical stability in physiological condition could be a discriminant parameter for our compounds. Then the chemical stability of the four catechol-type benzylidene derivatives (**29**, **31**, **34**, **40**) was investigated. A 20 µM solution was incubated at 25°C in a 10 mM phosphate buffer at pH 7.4 with ionic strength fixed at 150 mM. Solutions was analysed using UHPLC with PDA detector. Product disappearance was plotted versus time during 5 - 8 hours, and half-life was calculated from exponential regression (Table 6). Only derivative **29** was stable after a 5 hours incubation.

Compd	**Calculated half-life (hour)**	**R^2**
31	11.9	> 0.99
34	6.8	> 0.99
40	6.4	> 0.99
29	no degradation	-

Table 6: Chemical stability of compounds **31**, **34**, **40**, **29** in pH 7.4 buffer.

5. Discussion and conclusion

We have performed, during this work, the synthesis of fourteen novel benzylidene-phenylpyrrolizinones whose antioxidant, metal chelating and anti Aβ aggregation activities were evaluated in various tests to potentially attest their therapeutic interest in AD treatment. Some of these compounds exerted interesting antioxidant and chelating activities and promising self-mediated Aβ1-42 aggregation inhibitory activity.

If we consider the compounds exhibiting the best results in each test, it seems possible to establish some global structure-activity relationships on the basis of the overall results. Indeed some compounds were found exhibiting the best results in several tests, accounting for common pharmacophoric features for the various considered activities. The analysis of these results allows some assertions. The nature of the substituents on the benzylidene ring appears more critical for the activities than those of the phenyl ring. Indeed, compound **29**, without substituents on the phenyl ring, remains active as radical and NO scavenger and Aβ aggregation inhibitor. Conversely, the absence of substituents on the benzylidene ring (**18**) or the only presence of benzyloxy groups (**24**) or methoxy groups (**37**, **38**), without hydroxyl one, dramatically decreases all the activities, except for the Fe chelating activity of **24** and **38** which remains high. A vanillyl (**36**), isovanillyl (**35**) or syringyl-type benzylidene group (**39**), consequently, appears important for the activities, on the condition that a vanillyl-type phenyl group was associated to the structure. Indeed, compounds bearing such a benzylidene group but with an additional unsubstituted (**30**) or isovanillyl-type phenyl group (**32**, **33**) are almost devoid of activities. Finally, the best results were globally obtained with the compounds bearing a catechol-type benzylidene group (**29**, **31**, **34**, **40**) whose activities are preserved whatever the nature of the pending phenyl group.

Among them, compound **29** further exhibits good predictive drugability parameters in term of permeability, lack of cytotoxicity and chemical stability which design it as a promising hit for in vivo investigations.

7. Experimental section

7.1. General

Melting points were determined on a Köfler melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin Elmer BX FT-IR apparatus using KBr pellets. The ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were obtained on a Jeol Lambda 400 spectrometer using DMSO-d6 or $CDCl_3$ as solvent and TMS as internal standard. The chemical shifts (δ) are reported in ppm, and the coupling constants are in Hertz. Reactions were monitored by LC/MS and stopped when all starting material had disappeared. LC/MS (ESI) analyses were realized with a Waters alliance 2695 as separating module using the following gradient: A (95%)/B (5%) to A (5%)/B (95%) in 10 min. This ratio was hold for 3 min before return to initial conditions in 1 min. Initial conditions were then maintained for 5 min (A: H_2O, B: CH_3CN; each containing HCOOH: 0.1%; Column: C18 Xterra MSC118/2.1_50 mm). MS detection was performed with a SQ Detector by positive ESI. High-resolution mass spectra (EIMS) were obtained using a Jeol JMS GCMate spectrometer. High-resolution mass spectra were performed at 70 eV by electronic impact. Reactions were monitored by thin-layer chromatography (TLC) using 0.2 mm Polygram Sil silica gel G/UV 254 precoated plates with visualisation by irradiation with a short-wavelength UV light. Silica gel flash chromatography was performed using 63-200 mM Kieselgel Merck 60 silica gel.

PAMPA, DPPH and chelating activity assays were performed from a single DMSO (Fisher Scientific, Illkirch, France) stock solution at 50 mM of newly synthesized compounds in order to consume the least amount of product.

All tested compounds for their antioxidant activity were solubilized in ethanol at 100 µM. The analyses were performed in triplicate and the results were averaged.

7.2. Chemistry

7.2.1. General procedure (A) to obtain benzylated products

Unprotected starting material (1 Eq), benzyl bromide (1.2 Eq) and dipotassic carbonate (1,2 Eq) in MeOH (5 mL.mmol^{-1}) were stirred at reflux for 2 hours. The reaction mixture was filtered and evaporated to vacuo, taken up with DCM (15 mL.mmol^{-1}) and washed by water (3 × 1 mL.mmol^{-1}). Organic layer was dried over $MgSO_4$ and evaporated in vacuo to give the expected protected product.

7.2.2. General procedure (B) to obtain beta-aminopropionic acids

Protected aldehyde (1 Eq) was suspended in hot EtOH (2.5 mL.mmol^{-1}). After return to room temperature, malonic acid (1 Eq) and ammonium acetate (2 Eq) were added and the mixture was stirred at reflux for 24 hours before being immediately filtered and washed by hot EtOH (5 mL.mmol^{-1}).[84]

7.2.3. General procedure (C) to obtain pyrrolyl propanoic acids

Appropriate β-amino propionic acid (1 Eq) and 2,5-dimethoxytetrahydrofuran (1 Eq) were suspended in acetic acid (8 mL.mmol^{-1}) and heated at 60 °C for 1.5 hour. After return to room temperature, the mixture was evaporated *in vacuo*, taken up with DCM (8 mL.mmol^{-1}) and washed by HCl 2M (3 × 1 mL.mmol^{-1}). Organic layer was dried under $MgSO_4$ and evaporated *in vacuo*. The crude was purified on silica gel column (Cyclohexane/EtOAc; 9:1).

7.2.4. General procedure (D) to obtain phenylpyrrolizinones

Pyrrolyl propanoic acid (1 Eq.) was dissolved in toluene (25 mL.mmol^{-1}) and stirred at 0 °C under N_2 flux then we added triphosgen (1/3 Eq.) and the tube was sealed and stirred at 110 °C for 5 days. After return to room temperature, the mixture was evaporated to dryness, taken up with DCM (50 mL.mmol^{-1}) and washed by a solution of saturated K_2CO_3 (3 × 20 mL.mmol^{-1}). Organic layer was dried under $MgSO_4$ then evaporated in vacuo and was purified on silica gel column (cyclohexane/EtOAc; 8:2).

7.2.5. General procedure (E) for aldolisation

Phenylpyrrolizinone (1 Eq) and appropriated aldehyde (1 Eq) were suspended in a mixture of MeOH (8 mL.mmol^{-1}) and 5N aqueous NaOH (2Eq) and heated at 60 °C for 1.5 hour. After return to room temperature, the mixture was evaporated *in vacuo*, taken up with DCM (8 mL.mmol^{-1}) and washed by HCl 2M (3×1 mL.mmol^{-1}). Organic layer was dried under $MgSO_4$ and evaporated *in vacuo*. The crude was purified on silica gel column (cyclohexane/EtOAc; 9:1).

7.2.6. General procedure (F) to obtain unprotected benzylidenephenylpyrrolizinones

Benzylated product (1 Eq) was stirred in bromhydric acid 33 % diluted in acetic acid (8 mL.mmol^{-1}) at room temperature for 1.5 hour before ice was introduced in the flask. The mixture was extracted by EtOAc (8 mL.mmol^{-1}). Organic layer was dried under $MgSO_4$ and evaporated *in vacuo*. The crude was purified on silica gel column (cyclohexane/EtOAc; 1:1).

7.2.7. 3-Phenyl-3-pyrrol-1-yl-propanoic acid (7) [65]

Using general procedure (**C**), starting from commercial 3-amino-3-phenylpropanoic acid **6** (1.0 g) to afford **7** (1.1 g, 80 %) as a white solid. *Rf* = 0.60 (1:1/cyclohexane:EtOAc); mp = 120 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.32 (m, 3 H), 7.16 (m, 2 H), 6.75 (m, 2 H), 6.19 (t, *J* = 2 Hz, 2 H), 5.65 (m, 1

H), 3.24 (m, 2 H) ppm; IR (KBr): ν = 2959, 2927, 1712, 1518, 1490, 1190, 1083, 848, 724 cm^{-1}; Anal. calc. for $C_{12}H_{12}N_2O_4$: C, 72.54; H, 6.09; N, 6.51. Found: C, 72.67; H, 6.04; N, 6.47.

7.2.8. 3-Phenyl-2,3-dihydropyrrolizin-1-one (5)

Using general procedure (**D**), starting from **7** (100 mg) to afford **5** (47.6 mg, 52 %) as a white solid. [65] mp = 104 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.35 (m, 3 H), 7.12 (m, 2 H), 6.85 (d, *J* = 2 Hz, 1 H), 6.80 (dd, *J* = 4; 1 Hz, 1 H), 6.55 (dd, *J* = 4; 2 Hz, 1 H), 5.54 (dd, *J* = 8; 4 Hz, 1 H), 3.54 (dd, *J* = 18; 8 Hz, 1 H), 2.96 (dd, *J* = 18; 4 Hz, 1 H) ppm; IR (KBr): ν = 2924, 1698, 1527, 1370, 1309, 1065, 747 cm^{-1}; Anal. Calc. for $C_{13}H_{11}NO$: C, 79.17; H, 5.62; N, 7.10. Found : C, 79.11; H, 5.70; N, 7.13.

7.2.9. 3-Benzyloxy-4-methoxy-benzaldehyde (10)

Using general procedure (A), starting from commercial iso-vanilline to afford 10 in quantitative yield as a pale yellow powder. Rf = 0.57 (1:1/cyclohexane:EtOAc); mp = 67 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.81 (s, 1 H), 7.39 (m, 7H), 6.98 (dd, *J*= 8; 1 Hz, 1 H), 5.18 (s, 2 H), 3.95 (s, 3 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 190.8, 154.9, 148.6, 136.2, 129.9, 128.5 (2 C), 128.0, 127.4 (2 C), 126.8, 111.2, 110.7, 70.7, 56.1 ppm; IR (KBr): ν = 3342, 2933, 2837, 2748, 1677, 1583, 1506, 1431, 1235, 1009, 735 cm^{-1}; HREIMS [$M+H^+$] m/z 243.101444 (calcd for $C_{15}H_{14}O_3$ 243.101571).

7.2.10. 4-Benzyloxy-3-methoxy-benzaldehyde (11)

Using general procedure (A), starting from commercial vanillin to afford 11 in quantitative yield as pale yellow powder. Rf = 0.57 (1:1/cyclohexane:EtOAc); mp = 63 °C; ^{1}H NMR (400 MHz, $CDCl_3$) : δ = 9.84 (s, 1 H), 7.40 (m, 7H), 6.99 (d, *J* = 8 Hz, 1 H), 5.25 (s, 2 H), 3.95 (s, 3 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 190.9, 153.6, 150.1, 136.0, 130.3, 128.7 (2 C), 128.2, 127.3 (2 C), 126.6, 112.4, 109.4, 70.9, 56.0 ppm; IR (KBr): ν = 3412, 2839, 1676, 1538, 1260, 1133, 989, 812, 747, 561 cm^{-1}.

7.2.11. 3-Amino-3-(3-benzyloxy-4-methoxy-phenyl)propanoic acid (12)

Using general procedure (**B**), starting from **10** (33.0 g) to afford 12 (23.8 g, yield 58 %) as a white solid. mp = 266 °C; ^{1}H NMR (400 MHz, CD_3OD): δ = 7.39 (m, 5 H), 7.14 (s, 1 H), 7.04 (m, 2 H), 5.12 (s, 2 H), 4.60 (dd, *J* = 8; 6 Hz, 1 H), 3.86 (s, 3 H), 2.96 (m, 2 H) ppm; ^{13}C NMR (100 MHz, CD_3OD): δ = 173.2, 152.1, 150.0, 138.3, 129.7, 129.5 (2 C), 129.1, 128.8 (2 C), 121.6, 114.6, 113.5, 72.3, 57.6, 52.9, 39.1 ppm; IR (KBr): ν = 3436, 3031, 2930, 2834, 1571, 1522, 1395, 1271, 1144, 1021, 694 cm^{-1}; HREIMS [M+H$^+$] m/z 302.138747 (calc. for $C_{17}H_{20}NO_4$ 302.138685).

7.2.12 3-amino-3-(4-benzyloxy-3-methoxy-phenyl)propanoic acid (13)

Using general procedure (B), starting from **11** (9.90 g) to afford **13** (9.30 g, 75 %) as a white solid. mp = 258 °C; ^{1}H NMR (400 MHz, DMSO-*d6*, 383 K): δ = 7.37 (m, 5 H), 7.06 (d, *J* = 2 Hz, 1 H), 6.97 (d, *J* = 8 Hz, 1 H), 6.87 (dd, *J* = 8; 2 Hz, 1 H), 5.07 (s, 2 H), 4.17 (t, *J* = 8 Hz, 1 H), 3.81 (s, 3 H), 2.45 (d, 2 H) ppm; ^{13}C NMR (100 MHz, DMSO-d6, 383 K): δ = 172.9, 150.2, 147.9, 138.0, 137.9, 128.7 (2 C), 128.1, 127.9 (2 C), 119.2, 115.5, 112.5, 79.6, 71.4, 56.7, 52.2 ppm; IR (KBr): ν = 2937, 1630, 1580, 1539, 1520, 1272, 1147, 1026, 693 cm^{-1}; HREIMS [M+H$^+$] m/z 302.138159 (calc. for $C_{17}H_{20}NO_4$ 302.138685).

7.2.13 3-(3-Benzyloxy-4-methoxy-phenyl)-3-pyrrol-1-yl-propanoic acid (14)

Using general procedure (**C**), starting from **12** (3.47 g), to afford 14 (2.27 g, 56 %) as a white solid. Rf = 0.57 (1:1/cyclohexane:EtOAc); mp = 105 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34 (m, 5 H), 6.82 (d, *J* = 8 Hz, 1 H), 6.74 (dd, *J* = 8; 2 Hz, 1 H), 6.65 (d, *J* = 2 Hz, 1H), 6.64 (t, *J* = 2 Hz, 2 H), 6.13 (t, *J* = 2 Hz, 2 H), 5.51 (m, 1 H), 5.06 (s, 2 H), 3.85 (s, 3 H), 3.11 (m, 2 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 175.5, 149.4, 148.0, 136.6, 132.5, 128.5 (2 C), 127.9, 127.5 (2 C), 119.4, 118.9, 112.5, 111.6, 108.5, 71.0, 70.9, 58.5, 56.0, 55.9, 40.7 ppm; IR (KBr): ν = 3468, 2046, 1638, 1516, 1427, 1260, 1139, 728, 697 cm^{-1}; HREIMS [M+H$^+$] m/z 352.154187 (calc. for $C_{21}H_{22}NO_4$ 352.154335).

7.2.14. 3-(4-Benzyloxy-3-methoxy-phenyl)-3-pyrrol-1-yl-propanoic acid (15)

Using general procedure (**C**), starting from **14** (2.64 g) to afford **15** (2.59 g, 85 %) as a white solid. *Rf* = 0.60 (1:1/cyclohexane:EtOAc); mp = 100 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.35 (m, 5 H), 6.82 (m, 1 H), 6.73 (t, *J* = 2 Hz, 1 H), 6.68 (m, 2 H), 6.17 (t, *J* = 2 Hz, 2 H), 5.58 (m, 1 H), 5.13 (s, 2 H), 3.82 (s, 3 H), 3.20 (m, 2 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 175.9, 149.8, 148.0, 136.9, 133.2, 128.6 (2 C), 127.9, 127.2 (2 C), 119.6 (2 C), 118.3, 113.8, 110.4, 108.7 (2 C), 71.0, 58.7, 56.0, 40.9 ppm; IR (KBr): ν = 3037, 2924, 1701, 1518, 1421, 1230, 1143, 1005, 728 cm^{-1}; HREIMS [$M+H^+$] m/z 352.154171 (calc. for $C_{21}H_{22}NO_4$ 352.154335).

7.2.15. 3-(3-Benzyloxy-4-methoxy-phenyl)-2,3-dihydropyrrolizin-1-one (16)

Using general procedure (**D**), starting from **14** (500 mg) to afford 16 (220 mg, 46 %) as a white solid. mp = 127 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.30 (m, 5 H), 6.85 (d, *J* = 8 Hz, 1 H), 6.78 (d, *J* = 4 Hz, 1 H), 6.72 (m, 2 H), 6.51 (d, *J* = 2 Hz, 1 H), 6.50 (m, 1 H), 5.40 (dd, *J* = 8; 4 Hz, 1 H), 5.06 (m, 2 H), 3.88 (s, 3 H), 3.46 (dd, *J* = 18; 8 Hz, 1 H), 2.85 (dd, *J* = 18; 4 Hz, 1 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 188.3, 149.9, 148.5, 136.4, 133.0, 132.7, 128.5 (2 C), 127.9, 127.4 (2 C), 122.5, 119.1, 117.3, 111.8, 111.5, 107.5, 71.0, 57.8, 55.9, 49.6 ppm; IR (KBr): ν = 3479, 1682, 1519, 1368, 1258, 1230, 1067, 741 cm^{-1}; HREIMS [$M+H^+$] m/z 334.143544 (calc. for $C_{21}H_{20}NO_3$ 334.143770).

7.2.16. 3-(4-Benzyloxy-3-methoxy-phenyl)-2,3-dihydropyrrolizin-1-one (17)

Using general procedure (**D**), starting **15** (0.50 g) to afford 17 (240 mg, 50 %) as a white solid. mp = 121 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.37 (m, 5 H), 6.85 (d, *J* = 8 Hz, 1 H), 6.83 (m, 1 H), 6.72 (dd, *J* = 4; 1 Hz, 1 H), 6.56 (dd, *J* = 8; 2 Hz, 1 H), 6.48 (d, *J* = 2 Hz, 1 H), 6.46 (dd, *J* = 4; 2 Hz, 1 H), 5.40 (dd, *J* = 8; 4 Hz, 1 H), 5.08 (s, 2 H), 3.75 (s, 3 H), 3.46 (dd, *J* = 18; 8 Hz, 1 H), 2.85 (dd, *J* = 18; 4 Hz, 1 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 188.4, 150.3, 148.4, 136.8, 133.4, 133.2, 128.6 (2 C), 127.9, 127.2 (2 C), 122.6, 118.6, 117.4, 114.0, 109.1, 107.6, 71.0, 58.5, 56.1, 49.7 ppm; IR

(KBr): ν 3345, 3111, 1695, 1517, 1370, 1258, 1267, 1070, 747 cm^{-1}; HREIMS [M+H^+] m/z 334.143504 (calc. for $C_{21}H_{20}NO_3$ 334.143770).

7.2.17. (2E)-2-Benzylidene-3-phenyl-3H-pyrrolizin-1-one (18)

Using general procedure (E), starting from 5 (135 mg) and benzaldehyde (73 mg) to afford 18 as a yellow solid (170 mg, 80 %). mp = 178 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.64 (d, *J*= 2 Hz, 1 H), 7.22 (m, 10 H), 6.85 (m, 2 H), 6.40 (m, 1 H), 6.28 (d, *J* = 2 Hz, 1 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 179.3, 138.8, 137.8, 133.4, 133.3, 133.2, 130.6, 129.5 (2 C), 129.1, 128.9 (2 C), 128.4 (2 C), 127.5 (2 C), 121.2, 117.2, 108.9, 62.1 ppm; IR (KBr): ν = 3434, 2924, 1688, 1630,1524, 1366, 1306, 1119, 744, 697 cm^{-1}; HREIMS [M+H^+] m/z 286.122461 (calc. for $C_{20}H_{16}NO$ 286.122641).

7.2.18. (2E)-2-[(3,4-Dibenzyloxyphenyl)methylene]-3-phenyl-3H-pyrrolizin-1-one (19)

Using general procedure (E), starting from 5 (100 mg) and 3,4-dibenzyloxybenzaldehyde synthesized from commercially available 3,4-dihydroxybenzaldehyde[85] (161 mg) to afford 19 as a yellow solid (210 mg, 84 %). mp = 115 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.62 (d, *J*= 2 Hz, 1 H), 7.34 (m, 15 H), 6.99 (dd, *J* = 8; 2 Hz, 1 H), 6.91 (dd, *J* = 4; 1 Hz, 1 H), 6.86 (m, 2 H), 6.82 (d, *J* = 8 Hz, 1 H), 6.45 (dd, *J* = 4; 2 Hz, 1 H), 6.23 (d, *J* = 2 Hz, 1 H), 5.12 (m, 2 H), 4.88 (d, *J* = 12 Hz, 1 H), 4.71 (d, *J* = 12 Hz, 1 H), ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 179.6, 150.5, 148.6, 138.1, 136.7, 136.6, 135.5, 133.6, 133.2, 129.5 (2 C), 129.1, 128.6 (4 C), 128.0, 127.9, 127.6 (2 C), 127.2 (2 C), 127.1 (2 C), 126.8, 126.3, 120.8, 116.9, 116.4, 113.9, 108.6, 71.2, 70.8, 62.2 ppm; IR (KBr): ν = 3435, 1678, 1620, 1513, 1453, 1367, 1268, 1132, 734, 695 cm^{-1}; HREIMS [M+H^+] m/z 498.206378 (calc. for $C_{34}H_{28}NO_3$ 498.206370).

7.2.19. (2E)-2-[(4-Benzyloxy-3-methoxy-phenyl)methylene]-3-phenyl-3H-pyrrolizin-1-one (20)

Using general procedure (**E**), starting from **5** (133 mg) and **11** (163 mg) to afford **20** (261 mg, 92 %) as a yellow powder. mp = 140 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.65 (d, *J* = 1 Hz, 1 H), 7.34 (m, 10 H), 6.96 (dd, *J* = 8; 2 Hz, 1 H), 6.93 (d, *J* = 2 Hz, 1 H), 6.86 (m, 1 H), 6.77 (d, *J* = 8 Hz, 1 H), 6.75 (d, *J* = 1 Hz, 1 H), 6.45 (dd, *J* = 4; 2 Hz, 1 H), 6.29 (d, *J* = 1 Hz, 1 H), 5.12 (s, 2 H), 3.60 (s, 3 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 179.6, 149.7, 149.1, 138.0, 136.4, 135.6, 133.8, 133.1, 129.4 (2 C), 129.1, 128.6 (2 C), 128.0, 127.5 (2 C), 127.1 (2 C), 126.6, 125.8, 120.8, 116.9, 113.2, 112.9, 108.6, 70.6, 62.2, 55.9 ppm; IR (KBr): ν = 3005, 2946, 2849, 1681, 1521, 1266, 1115, 999, 738, 698 cm^{-1}; HREIMS [$M+H^+$] m/z 422.174942 (calc. for $C_{28}H_{24}NO_3$ 422.175070).

7.2.20. (2E)-3-(3-Benzyloxy-4-methoxy-phenyl)-2-[(3,4-dibenzyloxyphenyl) methylene]-3H-pyrrolizin-1-one (21)

Using general procedure (**E**), starting from 16 (94 mg) and 3,4-dibenzyloxybenzaldehyde synthesized from commercially available 3,4-dihydroxybenzaldehyde (90 mg) to afford 21 yellow solid (126 mg, 70 %). mp = 167 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.56 (d, *J*= 2 Hz, 1 H), 7.20 (m, 15 H), 6.97 (dd, *J* = 8; 2 Hz, 1 H), 6.94 (dd, *J* = 8; 2 Hz, 1 H), 6.87 (d, *J* = 2 Hz, 1 H), 6.83 (dd, *J* = 4; 1 Hz, 1 H), 6.79 (m, 2 H), 6.76 (d, *J* = 2 Hz, 1 H), 6.70 (m, 1 H), 6.40 (dd, *J* = 4; 2 Hz, 1 H), 6.10 (d, *J* = 2 Hz, 1 H), 5.13 (m, 2 H), 5.03 (d, *J* = 12 Hz, 1 H), 4.95 (d, *J* = 12 Hz, 1 H), 4.86 (d, *J* = 12 Hz, 1 H), 4.67 (d, *J* = 12 Hz, 1 H), 3.80 (s, 3 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 179.6, 150.5, 150.3, 148.6, 148.5, 136.8, 136.7, 136.4, 135.8, 133.6, 133.0, 130.3, 128.6 (2 C), 128.6 (2 C), 128.5 (2 C), 128.0 (2 C), 127.9, 127.3 (2 C), 127.2 (2 C), 127.1 (2 C), 126.8, 126.3, 120.7, 120.6, 116.8, 116.6, 113.9, 113.1, 111.9, 108.5, 71.2, 70.9, 70.8, 61.8, 56.0 ppm; IR (KBr): ν = 3435, 2926, 1691, 1511, 1360, 1278, 1140, 1014, 729, 696 cm^{-1}; HREIMS [$M+H^+$] m/z 634.258839 (calc. for $C_{42}H_{36}NO_5$ 634.258800).

7.2.21. (2E)-3-(3-Benzyloxy-4-methoxy-phenyl)-2-[(3-benzyloxy-4-methoxy-phenyl)methylene]-3H-pyrrolizin-1-one (22)

Using general procedure (E), starting from **16** (100 mg) and 10 (72 mg) to afford **22** as a yellow solid (106 mg, 64 %). mp = 162 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.58 (d, *J*= 2 Hz, 1 H), 7.29 (m, 10 H), 6.98 (m, 2 H), 6.83 (dd, *J* = 4; 1 Hz, 1 H), 6.79 (m, 4 H), 6.71 (dd, *J* = 2; 1 Hz, 1 H), 6.40 (dd, *J* = 4; 2 Hz, 1 H), 6.10 (d, *J* = 2 Hz, 1 H), 5.02 (d, *J* = 12 Hz, 1 H), 4.94 (d, *J* = 12 Hz, 1 H), 4.84 (d, *J* = 12 Hz, 1 H), 4.64 (d, *J* = 12 Hz, 1 H), 3.85 (s, 3 H), 3.80 (s, 3 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 179.8, 151.3, 150.4, 148.6, 148.1, 136.6, 136.5, 135.6, 133.9, 133.1, 130.4, 128.8 (2 C), 128.7 (2 C), 128.2, 128.0, 127.6 (2 C), 127.5 (2 C), 126.5, 126.4, 120.9, 120.8, 116.9, 115.6, 113.2, 112.0, 111.4, 108.6, 71.1, 71.0, 61.9, 56.1, 56.0 ppm; IR (KBr): ν = 3431, 2915, 1688, 1522, 1332, 1266, 1117, 1014, 733 cm^{-1}; HREIMS [M+H^{+}] m/z 558.228392 (calc. for$C_{36}H_{32}NO_5$ 558.227500).

7.2.22. (2E)-3-(3-Benzyloxy-4-methoxy-phenyl)-2-[(4-benzyloxy-3-methoxy-phenyl)methylene]-3H-pyrrolizin-1-one (23)

Using general procedure (E), starting from **16** (114 mg) and **11** (83 mg) to afford **23** as a yellow solid (180 mg, 94 %). mp = 146 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.58 (d, *J* = 2 Hz, 1 H), 7.30 (m, 10 H), 6.98 (dd, *J* = 8; 2 Hz, 1 H), 6.91 (dd, *J* = 8; 2 Hz, 1 H), 6.83 (m, 2 H), 6.79 (d, *J* = 2 Hz, 1 H), 6.71 (m, 2 H), 6.69 (m, 1 H), 6.40 (dd, *J* = 4; 2 Hz, 1 H), 6.15 (d, *J* = 2 Hz, 1 H), 5.12 (m, 2 H), 5.06 (d, *J* = 12 Hz, 1 H), 4.97 (d, *J* = 12 Hz, 1 H), 3.83 (s, 3 H), 3.60 (s, 3 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 179.8, 151.3, 150.4, 148.6, 148.1, 136.6, 136.5, 135.6, 133.9, 133.1, 130.4, 128.8 (2 C), 128.7 (2 C), 128.2, 128.0, 127.6 (2 C), 127.5 (2 C), 126.5, 126.4, 120.9, 120.8, 116.9, 115.6, 113.2, 112.0, 111.4, 108.6, 71.1, 71.0, 61.9, 56.1, 56.0 ppm; IR (KBr): ν = 3431, 2932, 1680, 1513, 1306, 1260, 1139, 1005, 742, 693 cm^{-1}; HREIMS [M+H^{+}] m/z 558.227078 (calc. for $C_{36}H_{32}NO_5$ 558.227500).

7.2.23. (2E)-3-(4-Benzyloxy-3-methoxy-phenyl)-2-[(3,4-dibenzyloxyphenyl) methylene]-3H-pyrrolizin-1-one (24)

Using general procedure (E), starting from 17 (200 mg) and 3,4-dibenzyloxybenzaldehyde synthesized from commercially available 3,4-dihydroxybenzaldehyde (210 mg) to afford **24** as a yellow solid (345 mg, 91 %). mp = 64 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.58 (d, *J* = 2 Hz, 1 H), 7.29 (m, 15 H), 6.97 (dd, *J* = 8; 2 Hz, 1 H), 6.92 (dd, *J* = 8; 2 Hz, 1 H), 6.88 (dd, *J* = 2; 1 Hz, 1 H), 6.87 (d, *J* = 2 Hz, 1 H), 6.83 (dd, *J* = 4; 1 Hz, 1 H), 6.79 (m, 2 H), 6.68 (d, *J* = 2 Hz, 1 H), 6.43 (dd, *J* = 4; 2 Hz, 1 H), 6.13 (d, *J* = 2 Hz, 1 H), 5.11 (m, 2 H), 5.08 (s, 2 H), 4.84 (d, *J* = 12 Hz, 1 H), 4.71 (d, *J* = 12 Hz, 1 H), 3.67 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 179.6, 150.6, 150.4, 148.7, 148.6, 136.9, 136.8, 136.7, 136.6, 135.9, 133.7, 133.1, 130.9, 128.6 (2 C), 128.6 (2 C), 128.5 (2 C), 128.0 (2 C), 127.3 (2 C), 127.2 (2 C), 127.1 (2 C), 126.8, 126.5, 120.9, 120.4, 116.9, 116.6, 113.8 (2 C), 110.1, 108.5, 71.3, 70.9, 70.8, 61.9, 56.1 ppm; IR (KBr): ν = 3434, 2923, 1685, 1624, 1511,1453, 1269, 1142, 1024, 735 cm^{-1}; HREIMS [$M+H^+$] m/z 634.258067 (calc. for $C_{42}H_{36}NO_5$ 634.258800).

7.2.24. (2E)-3-(4-Benzyloxy-3-methoxy-phenyl)-2-[(3-benzyloxy-4-methoxy-phenyl)methylene]-3H-pyrrolizin-1-one (25)

Using general procedure (E), starting from **17** (100 mg) and 10 (80 mg) to afford 25 (153 mg, 90 %) as a yellow powder. mp = 87 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.62 (d, *J* = 2 Hz, 1 H), 7.29 (m, 10 H), 7.03 (dd, *J* = 8; 2 Hz, 1 H), 6.93 (dd, *J* = 8; 2 Hz, 1 H), 6.90 (dd, *J* = 2; 1 Hz, 1 H), 6.86 (m, 1 H), 6.84 (dd, *J* = 4; 1 Hz, 1 H), 6.82 (d, *J* = 8 Hz, 1 H), 6.79 (d, *J* = 9 Hz, 1 H), 6.70 (d, *J* = 2 Hz, 1 H), 6.45 (dd, *J* = 4; 2 Hz, 1 H), 6.15 (d, *J* = 2 Hz, 1 H), 5.05 (s, 2 H), 4.86 (d, *J* = 12 Hz, 1 H), 4.71 (d, *J* = 12 Hz, 1 H), 3.86 (s, 3 H), 3.68 (s, 3 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 179.8, 151.3, 150.4, 148.6, 148.1, 136.6, 136.5, 135.6, 133.9, 133.1, 130.4, 128.8 (2 C), 128.7 (2 C), 128.2, 128.0, 127.6 (2 C), 127.5 (2 C), 126.5, 126.4, 120.9, 120.8, 116.9, 115.6, 113.2, 112.0, 111.4, 108.6, 71.1, 71.0, 61.9, 56.1, 56.0 ppm; IR (KBr): ν = 2925, 2849, 1686, 1513, 1366, 1272, 1236, 1025, 735 $cm^{-1;}$ HREIMS [$M+H^+$] m/z 558.227446 (calc. for $C_{36}H_{32}NO_5$ 558.227500).

7.2.25. (2E)-3-(4-Benzyloxy-3-methoxy-phenyl)-2-[(4-benzyloxy-3-methoxy-phenyl)methylene]-3H-pyrrolizin-1-one (26)

Using general procedure (E), starting from **17** (90 mg) and 11 (72 mg) to afford 26 (127 mg, 84 %) as a yellow powder. mp = 77 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.64 (d, *J* = 2 Hz, 1 H), 7.35 (m, 10 H), 7.00 (dd, *J* = 8; 2 Hz, 1 H), 6.96 (dd, *J* = 8; 2 Hz, 1 H), 6.92 (dd, *J* = 2; 1 Hz, 1 H), 6.86 (m, 1 H, 1 H), 6.84 (d, *J* = 8 Hz, 1 H), 6.77 (d, *J* = 4 Hz, 1 H), 6.76 (m, 2 H), 6.45 (dd, *J* = 4; 2 Hz, 1 H), 6.21 (d, *J* = 2 Hz, 1 H), 5.12 (m, 4 H), 3.71 (s, 3 H), 3.55 (s, 3 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 179.8, 151.3, 150.4, 148.6, 148.1, 136.6, 136.5, 135.6, 133.9, 133.1, 130.4, 128.8 (2 C), 128.7 (2 C), 128.2, 128.0, 127.6 (2 C), 127.5 (2 C), 126.5, 126.4, 120.9, 120.8, 116.9, 115.6, 113.2, 112.0, 111.4, 108.6, 71.1, 71.0, 61.9, 56.1, 56.0 ppm; IR (KBr): ν = 3438, 2927,1684, 1511, 1366, 1268, 1236, 1021, 732 cm^{-1}; HREIMS [$M+H^+$] m/z 558.228555 (calc. for $C_{36}H_{32}NO_5$ 558.227500).

7.2.26. (2E)-3-(4-Benzyloxy-3-methoxy-phenyl)-2-[(2,4-dimethoxyphenyl) methylene]-3H-pyrrolizin-1-one (27)

Using general procedure (E), starting from 17 (100 mg) and commercial 2,4-dimethoxybenzaldehyde (50 mg) to afford 27 (yellow solid (125 mg, 87 %). mp = 72 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.99 (d, *J* = 2 Hz, 1 H), 7.33 (m, 5 H), 7.09 (d, *J* = 8 Hz, 1 H), 6.85 (m, 2 H), 6.80 (d, *J* = 2 Hz, 1 H), 6.76 (d, *J* = 8 Hz, 1 H), 6.61 (d, *J* = 2 Hz, 1 H), 6.43 (dd, *J* = 4; 2 Hz, 1 H), 6.32 (d, *J* = 2 Hz, 1 H), 6.26 (dd, *J* = 8; 2 Hz, 1 H), 6.18 (d, *J* = 2 Hz, 1 H), 5.06 (s, 2 H), 3.79 (s, 3 H), 3.76 (s, 3 H), 3.69 (s, 3 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 178.9, 161.4, 158.9, 148.9, 147.3, 135.8, 135.6, 132.6, 130.7, 130.2, 127.6, 127.5 (2 C), 126.9, 126.2 (2 C), 119.7, 119.4, 115.5, 114.7, 112.7, 109.3, 107.1, 103.6, 97.0, 69.9, 61.2, 54.9, 54.5, 54.4 ppm; IR (KBr): ν = 3434, 2926, 2850, 1685, 1603, 1512, 1462, 1263, 1140, 1024, 742 cm^{-1}; HREIMS [$M+H^+$] m/z 482.196603 (calc. for $C_{30}H_{28}NO_5$ 482.196199).

7.2.27. (2E)-3-(4-Benzyloxy-3-methoxy-phenyl)-2-[(3,4,5-trimethoxyphenyl) methylene]-3H-pyrrolizin-1-one (28)

Using general procedure (E), starting from 17 (50 mg) and commercial 3,4,5-trimethoxybenzaldehyde (30 mg) to afford 28 yellow solid (38 mg, 50 %). mp = 68 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.63 (d, *J* = 2 Hz, 1 H), 7.35 (m, 5 H), 7.01 (dd, *J* = 8; 2 Hz, 1 H), 6.93 (dd, *J* = 2; 1 Hz, 1 H), 6.87 (dd, *J* = 4; 1 Hz, 1 H), 6.85 (d, *J* = 8 Hz, 1 H), 6.74 (d, *J* = 2 Hz, 1 H), 6.55 (s, 2 H), 6.47 (dd, *J* = 4; 2 Hz, 1 H), 6.21 (d, *J* = 2 Hz, 1 H), 5.15 (m, 2 H), 3.83 (s, 3 H), 3.74 (s, 3 H), 3.61 (s, 6 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 179.1, 153.0 (2 C), 150.5, 148.7, 139.6, 136.8, 136.6, 133.9, 132.9, 131.0, 128.9, 128.6 (2 C), 128.0, 127.1 (2 C), 121.1, 120.3, 117.1, 113.9, 109.9, 108.8, 108.5 (2 C), 70.8, 61.9, 60.9, 56.1 (3 C) ppm; IR (KBr): ν = 3435, 2933, 2837, 1688, 1626, 1505, 1461, 1332, 1239, 1123, 1025, 744 cm^{-1}; HREIMS [$M+H^+$] m/z 512.207468 (calc. for $C_{31}H_{30}NO_6$ 512.206764).

7.2.28. (2E)-2-[(3,4-Dihydroxyphenyl)methylene]-3-phenyl-3H-pyrrolizin-1-one (29)

Using general procedure (**F**), starting from **19** (180 mg) to afford 29 (60 mg, 53 %) as a yellow powder. mp > 260 °C; ^{1}H NMR (400 MHz, CD_3OD): δ = 7.49 (d, *J* = 2 Hz, 1 H), 7.32 (m, 5 H), 7.08 (dd, *J* = 2; 1 Hz, 1 H), 6.90 (d, *J* = 2 Hz, 1 H), 6.83 (m, 2 H), 6.64 (d, *J* = 8 Hz, 1 H), 6.55 (d, *J* = 2 Hz, 1 H), 6.49 (dd, *J* = 4; 2 Hz, 1 H) ppm; ^{13}C NMR (100 MHz, CD_3OD): δ = 180.7, 147.8, 144.9, 138.2, 135.7, 134.1, 132.9, 128.7 (2 C), 128.4, 127.4 (2 C), 125.0, 124.2, 121.9, 117.8, 116.8, 114.9, 108.2, 61.8 ppm; IR (KBr): ν = 3467, 1671, 1592, 1521, 1461, 1362, 1283, 1108, 1032, 735 cm^{-1}; HREIMS [$M+H^+$] m/z 318.112239 (calc. for $C_{20}H_{16}NO_3$ 318.112470).

7.2.29. (2E)-2-[(4-hydroxy-3-methoxy-phenyl)methylene]-3-phenyl-3H-pyrrolizin-1-one (30)

Using general procedure (F), starting from 20 (40 mg) to afford 30 as a pale yellow solid (25 mg, 80 %). mp = 208 °C; ^{1}H NMR (400 MHz, $CDCl_3$) : δ = 7.65 (d, *J* = 1 Hz, 1 H), 7.34 (m, 5 H), 6.96 (dd, *J* = 8; 2 Hz, 1 H), 6.93 (d, *J* = 2 Hz, 1 H), 6.86 (m, 1 H), 6.80 (d, *J* = 8 Hz, 1 H), 6.73 (d, *J* = 1 Hz, 1 H),

6.45 (dd, *J* = 4; 2 Hz, 1 H), 6.29 (d, *J* = 1 Hz, 1 H), 5.80 (ls, 1 H), 3.63 (s, 3 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 179.7, 147.4, 146.3, 138.0, 135.2, 133.9, 133.1, 129.5 (2 C), 129.1, 127.5 (2 C), 126.4, 125.8, 120.8, 116.9, 114.5, 112.5, 108.5, 62.2, 29.7 ppm; IR (KBr): ν = 3366, 2961, 1674, 1585, 1513, 1364, 1289, 1130, 1029, 734, 699 cm^{-1}; HREIMS [M+H$^+$] m/z 332.127968 (calc. for $C_{21}H_{18}NO_3$ 332.128120).

7.2.30. (2E)-2-[(3,4-Dihydroxyphenyl)methylene]-3-(3-hydroxy-4-methoxy-phenyl)-3H-pyrrolizin-1-one (31)

Using general procedure (F), starting from 21 (60 mg) to afford 31 (27 mg, 80 %) as a yellow powder. mp = 128 °C; ^{1}H NMR (400 MHz, CD_3OD): δ = 7.37 (d, *J* = 2 Hz, 1 H), 6.98 (m, 1 H), 6.82 (d, *J* = 2 Hz, 1 H), 6.77 (m, 2 H), 6.70 (dd, *J* = 4; 1 Hz, 1 H), 6.63 (d, *J* = 2 Hz, 1 H), 6.58 (d, *J* = 8 Hz, 1 H), 6.38 (dd, *J* = 4; 2 Hz, 1 H), 6.31 (d, *J* = 2 Hz, 1 H), 3.82 (s, 3 H), 3.70 (s, 3 H) ppm; ^{13}C NMR (100 MHz, CD_3OD): δ = 182.2, 149.4, 148.1, 146.3, 137.0, 135.4, 134.1, 132.3, 126.5, 125.8, 123.3, 120.6, 119.4, 118.1, 116.3, 115.1, 112.7, 109.5, 62.9, 56.2, 30.8 ppm; IR (KBr): ν = 3426, 1669, 1596, 1512, 1363, 1290, 1026, 759, 613 cm^{-1}; HREIMS [M+H$^+$] m/z 364.117530 (calc. for $C_{21}H_{18}NO_5$ 364.117949).

7.2.31. (2E)-3-(3-Hydroxy-4-methoxy-phenyl)-2-[(3-hydroxy-4-methoxy-phenyl)methylene]-3H-pyrrolizin-1-one (32)

Using general procedure (F), starting from **22** (80 mg) to afford 32 (30 mg, 55 %) as a yellow powder. mp = 254 °C; ^{1}H NMR (400 MHz, CD_3OD): δ = 7.48 (d, *J* = 2 Hz, 1 H), 7.07 (dd, *J* = 2; 1 Hz, 1 H), 6.94 (m, 2 H), 6.81 (m, 3 H), 6.72 (d, *J* = 2 Hz, 1 H), 6.48 (dd, *J* = 4; 2 Hz, 1 H), 6.41 (d, *J* = 2 Hz, 1 H), 3.82 (s, 3 H), 3.78 (s, 3 H) ppm; ^{13}C NMR (100 MHz, CD_3OD): δ = 182.0, 150.9, 149.5, 148.1, 147.5, 138.0, 134.9, 134.1, 132.2, 127.7, 125.6, 123.5, 120.6, 118.8, 118.1, 115.0, 112.7, 112.1, 109.6, 62.9, 56.3 (2 C) ppm; IR (KBr): ν = 3429, 1674, 1625, 1510, 1273, 1108, 1027, 760 et 614 cm^{-1}; HREIMS [M+H$^+$] m/z 378.133420 (calc. for $C_{22}H_{19}NO_5$ 378.133599).

7.2.32. (2E)-3-(3-Hydroxy-4-methoxy-phenyl)-2-[(4-hydroxy-3-methoxy-phenyl)methylene]-3H-pyrrolizin-1-one (33)

Using general procedure (F), starting from **23** (100 mg) to afford 33 (52 mg, 76 %) as a yellow powder. mp = 128 °C; ^{1}H NMR (400 MHz, CD_3OD): δ = 7.55 (d, *J* = 2 Hz, 1 H), 7.10 (dd, *J* = 2; 1 Hz, 1 H), 7.02 (dd, *J* = 8; 2 Hz, 1 H), 6.89 (m, 4 H), 6.80 (d, *J* = 4 Hz, 1 H), 6.74 (d, *J* = 8 Hz, 1 H), 6.48 (dd, *J* = 4; 2 Hz, 1 H), 6.45 (d, *J* = 2 Hz, 1 H), 3.80 (s, 3 H), 3.67 (s, 3 H) ppm; ^{13}C NMR (100 MHz, CD_3OD): 180.7, 148.9, 148.2, 147.5, 146.9, 135.1, 134.0, 132.6, 130.9, 126.9, 125.1, 121.9, 118.9, 116.7, 114.9, 114.1, 113.1, 111.7, 108.1, 61.4, 55.2, 54.9 ppm; IR (KBr): ν = 3426, 1669, 1596, 1512, 1363, 1290, 1026, 759, 613 cm^{-1}; HREIMS [M+H^{+}] m/z 378.133894 (calc. for $C_{22}H_{20}NO_5$ 378.133599).

7.2.33. (2E)-2-[(3,4-Dihydroxyphenyl)methylene]-3-(4-hydroxy-3-methoxy-phenyl)-3H-pyrrolizin-1-one (34)

Using general procedure (F), starting from **24** (300 mg) to afford 24 (96 mg, 56 %) as a yellow powder. mp = 192 °C; ^{1}H NMR (400 MHz, CD_3OD): δ = 7.44 (d, *J* = 2 Hz, 1 H), 7.07 (dd, *J* = 2; 1 Hz, 1 H), 6.90 (d, *J* = 2 Hz, 1 H), 6.86 (d, *J* = 2 Hz, 1 H), 6.83 (dd, J = 8; 2 Hz, 1 H), 6.78 (m, 2 H), 6.66 (d, *J* = 8 Hz, 1 H), 6.63 (d, *J* = 8 Hz, 1 H), 6.46 (dd, *J* = 4; 2 Hz, 1 H), 6.42 (d, *J* = 2 Hz, 1 H), 3.72 (s, 3 H) ppm; ^{13}C NMR (100 MHz, CD_3OD): δ = 152.1, 122.9, 122.7, 121.1, 120.9, 109.1, 108.9, 106.1, 105.5, 100.9, 100.3, 99.6, 99.4, 93.2, 92.7, 89.2, 88.9, 86.1, 82.3, 80.9, 34.2 ppm; IR (KBr): ν = 3447, 1677, 1593, 1522, 1295, 1116, 1029, 737 cm^{-1}; HREIMS [M+H^{+}] m/z 364.117813 (calc. for $C_{21}H_{18}NO_5$ 364.117949).

7.2.34. (2E)-3-(4-Hydroxy-3-methoxy-phenyl)-2-[(3-hydroxy-4-methoxy-phenyl)methylene]-3H-pyrrolizin-1-one (35)

Using general procedure (F), starting from **25** (50 mg) to afford 35 (20 mg, 60 %) as a yellow powder. mp = 202 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.62 (d, *J* = 2 Hz, 1 H), 7.01 (dd, *J* = 8; 2 Hz, 1

H), 6.98 (d, *J* = 2 Hz, 1 H), 6.93 (m, 1 H), 6.90 (m, 2 H), 6.86 (dd, *J* = 4; 1 Hz, 1 H), 6.73 (d, *J* = 8 Hz, 1 H), 6.63 (d, *J* = 2 Hz, 1 H), 6.46 (dd, *J* = 4; 2 Hz, 1 H), 6.24 (d, *J* = 2 Hz, 1 H), 5.60 (ls, 1 H), 5.52 (s, 1 H), 3.86 (s, 3 H), 3.73 (s, 3 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 178.7, 146.9, 146.1, 145.1, 144.3, 135.9, 132.4, 132.3, 128.9, 125.9, 123.8, 120.4, 119.9, 115.9, 115.3, 113.7, 109.3, 108.1, 107.5, 61.1, 54.9, 54.9 ppm; IR (KBr): ν = 3361, 2923, 2852, 1666, 1604, 1512, 1463, 1279, 1031, 736 cm^{-1}; HREIMS [M+H$^+$] m/z 378.133610 (calc. for $C_{22}H_{20}NO_5$ 378.133599).

7.2.35. (2E)-3-(4-Hydroxy-3-methoxy-phenyl)-2-[(4-hydroxy-3-methoxy-phenyl)methylene]-3H-pyrrolizin-1-one (36)

Using general procedure (F), starting from 26 (160 mg) to afford 36 (60 mg, 53 %) as a yellow powder. mp = 182 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.64 (d, *J* = 2 Hz, 1 H), 7.11 (dd, *J* = 8; 2 Hz, 1 H), 7.02 (dd, *J* = 8; 2 Hz, 1 H), 6.93 (m, 2 H), 6.86 (dd, *J* = 4; 1 Hz, 1 H), 6.83 (d, *J* = 8 Hz, 1 H), 6.79 (d, *J* = 4 Hz, 1 H), 6.62 (d, *J* = 2 Hz, 1 H), 6.46 (dd, *J* = 4; 2 Hz, 1 H), 6.23 (d, *J* = 2 Hz, 1 H), 5.83 (ls, 1 H), 5.65 (ls, 1 H), 3.69 (s, 3 H), 3.67 (s, 3 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 178.7, 146.5, 146.4, 145.3, 145.4, 134.4, 133.0, 132.1, 129.0, 125.6, 124.9, 120.4, 119.8, 115.9, 113.6, 113.5, 111.6, 107.6, 107.5, 61.2, 54.9 (2 C) ppm; IR (KBr): ν = 3419, 2924, 2856, 1669, 1513, 1463, 1363, 1280, 1028, 732 cm^{-1}; HREIMS [M+H$^+$] m/z 378.133638 (calc. for $C_{22}H_{20}NO_5$ 378.133599).

7.2.36. (2E)-2-[(2,4-Dimethoxyphenyl)methylene]-3-(4-hydroxy-3-methoxy-phenyl)-3H-pyrrolizin-1-one (37)

Using general procedure (F), starting from **27** (109 mg) to afford 37 (40 mg, 47 %) as a yellow powder. mp = 114 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.83 (d, *J* = 2 Hz, 1 H), 7.20 (d, *J* = 8 Hz, 1 H), 6.96 (dd, *J* = 2; 1 Hz, 1 H), 6.71 (dd, *J* = 4; 1 Hz, 1 H), 6.65 (m, 2 H), 6.58 (m, 1 H), 6.39 (dd, *J* = 4; 2 Hz, 1 H), 6.35 (m, 2 H), 6.28 (dd, *J* = 8; 2 Hz, 1 H), 3.79 (s, 3 H), 3.73 (s, 3 H), 3.67 (s, 3 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 182.4, 164.5, 161.6, 149.2, 138.2, 134.5, 133.3, 133.1, 130.8, 129.7,

123.4, 121.9, 118.0, 116.5, 116.4, 111.9, 109.4, 106.2, 98.9, 63.2, 56.4, 56.2, 55.9 ppm; IR (KBr): ν = 3435, 2926, 2850, 1675, 1602, 1523, 1272, 1024, 751 cm^{-1}; HREIMS [M+H^+] m/z 392.149135 (calc. for $C_{23}H_{22}NO_5$ 392.14924).

7.2.37. (2E)-3-(4-Hydroxy-3-methoxy-phenyl)-2-[(3,4,5-trimethoxyphenyl) methylene]-3H-pyrrolizin-1-one (38)

Using general procedure (F), starting from **28** (60 mg) to afford 38 (20 mg, 47 %) as a yellow powder and 39, unexpected product, as a white powder (7 mg). mp = 102 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.62 (d, *J* = 2 Hz, 1 H), 7.13 (dd, *J* = 8; 2 Hz, 1 H), 6.93 (m, 2 H), 6.85 (m, 1 H), 6.65 (d, *J* = 2 Hz, 1 H), 6.58 (s, 2 H), 6.46 (dd, *J* = 4; 2 Hz, 1 H), 6.24 (d, *J* = 2 Hz, 1 H), 5.80 (ls, 1 H), 3.82 (s, 6 H), 3.70 (s, 3 H), 3.68 (s, 3 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 179.7, 153.0 (2 C), 147.5, 146.4, 139.2, 136.5, 134.2, 132.7, 139.9, 128.9, 121.4, 121.3, 117.4, 114.7, 109.1, 108.2, 108.1 (2 C), 62.2, 61.1, 56.1 (2 C), 56.0 ppm; IR (KBr): ν = 3444, 1623, 1507, 1421, 1126 et 624 cm^{-1}; HREIMS [M+H^+] m/z 422.159974 (calc. for $C_{24}H_{24}NO_6$ 422.159814).

7.2.38. (2E)-2-[(4-Hydroxy-3,5-dimethoxy-phenyl)methylene]-3-(4-hydroxy-3-methoxy-phenyl)-3H-pyrrolizin-1-one (39)

mp = 112 °C; ^{1}H NMR (400 MHz, CD_3OD): δ = 7.62 (d, *J* = 2 Hz, 1 H), 7.13 (dd, *J* = 8; 2 Hz, 1 H), 6.93 (m, 2 H), 6.85 (m, 1 H), 6.65 (d, *J* = 2 Hz, 1 H), 6.60 (s, 2 H), 6.46 (dd, *J* = 4; 2 Hz, 1 H), 6.21 (d, *J* = 2 Hz, 1 H), 5.78 (ls, 1 H), 5.74 (ls, 1 H), 3.72 (s, 6 H), 3.70 (s, 3 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 179.7, 147.6, 146.9, 146.5, 136.8, 135.4, 134.4, 132.9, 130.0, 124.8, 121.3, 120.9, 116.9, 114.7, 108.7, 108.6, 108.5 (2 C), 62.2, 60.4, 56.3 (2 C), 55.9 ppm; IR (KBr): ν = 3435, 2933, 2853, 1669, 1621, 1513, 1189, 1116 et 732 cm^{-1}; HREIMS [M+H^+] m/z 408.144141 (calc. for $C_{23}H_{22}NO_6$ 408.144164).

7.2.39. (2E)-2-[(3,4-Dihydroxyphenyl)methylene]-3-phenyl-3H-pyrrolizin-1-one (40)

To a solution of compound 34 (30 mg, 1 Eq) stirred in DCM (8 mL.mmol^{-1}) at 0 °C, BBr_3 (2 Eq) is added and the reaction mixture was put at reflux for 30 mn before it was quenched with ice water (2 mL). After EtOAc extraction (3 × 10 mL), organic layer was dried over $MgSO_4$, evaporated *in vacuo* and purified on silica gel column (DCM/MeOH; 98:2) to afford 40 (18 mg, 71 %) as a yellow powder. mp = 176 °C; ^{1}H NMR (400 MHz, CD_3OD): δ = 7.46 (d, *J* = 2 Hz, 1 H), 7.09 (m, 1 H), 6.93 (m, 1 H), 6.86 (m, 2 H), 6.80 (dd, *J* = 4; 1 Hz, 1 H), 6.72 (d, *J* = 8 Hz, 1 H), 6.67 (dd, *J* = 5; 3 Hz, 2 H), 6.48 (dd, *J* = 4; 2 Hz, 1 H), 6.35 (d, *J* = 2 Hz, 1 H) ppm; ^{13}C NMR (100 MHz, CD_3OD): δ = 184.8, 151.7, 149.5, 149.4, 148.9, 139.7, 137.9, 136.6, 133.6, 129.2, 128.4, 126.0, 123.5, 122.1, 120.6, 119.0, 118.8, 117.6, 112.0, 65.6; IR (KBr): ν = 3435, 1659, 1599, 1517, 1443, 1291, 1110, 1024, 751 cm^{-1}; HREIMS [M+H$^+$] m/z 350.102119 (calc. for $C_{20}H_{16}NO_5$ 350.102299).

7.3. Biology

7.3.1. DPPH radical-scavenging activity. [69]

The antiradical activity of the tested compounds was assessed using a miniaturized DPPH (2,2-diphenyl-1-picrylhydrazyl radical) assay [86]. The tested compounds were diluted from stock solution in absolute ethanol and incubated in the presence of 100 µM DPPH (Sigma-Aldrich, Saint-Quentin Fallavier, France) solution for 30 min at room temperature in a 96-wells microplate (Greiner Bio-One®, Courtaboeuf, France) in the dark. The absorbance at 517 nm was then measured using Synergy 2® microplate reader controlled by Gen 5® software (Biotek, Colmar, France) and used to calculate their DPPH scavenging activity according to the following formula:

$$\%\ \text{DPPH radical scavenging activity} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where $A_{control}$ refers to the absorbance of the negative control (vehicle) and A_{sample} refers to the absorbance of the tested compound.

Assays were conduct in triplicate at 7 concentration levels (0, 5, 10, 50, 100, 150, 250 µM) of the tested compound. The EC_{50} values (concentrations required to obtain a 50% scavenging effect) were calculated using the software Origin 6.1 (OriginLab,Northampton, MA, USA). Trolox, ferulic acid from Sigma-Aldrich (Saint-Quentin Fallavier, France) and curcumin from Alfa Aesar (Schiltigheim, France) were tested as positive control.

7.3.2. Nitric oxide radical scavenging effect.

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions which were measured by the Greiss reagent. Scavengers of free radicals result in the reduced production of NO. Briefly, the reaction mixture (1 mL) containing sodium nitroprusside (200 µL, 50 mM in phosphate buffer pH 7.4), tested compound (400µL) and phosphate buffer (400 µL) was incubated at 37.4°C for 3h. Samples (250 µL) of the incubation was removed and diluted with Greiss Reagent (100 µL). The absorbance of chromophore formed was read after 20 min at 546 nm. Quercetin was used as standard reference. NO inhibitory activity was calculated following the formula:

$$\text{NO inhibitory activity} = ((A_{control} - A_{test})/A_{control}) \times 100$$

where $A_{control}$ is the absorbance of the control reaction and A_{test} represents the absorbance of the test reaction.

7.3.3. Lipid peroxidation assay.

Lipid peroxidation assay was carried out by using the linoleic system according to the ferric thiocyanate method.[72,87] Each sample (120 µL) was added to a reaction solution containing linoleic acid emulsion (1 mL, (500 µL of linoleic acid 2.5 % and 500 µL of Tween 20 in phosphate buffer pH 7.4)), 2,2'-azobis(2-amidino-propane) dihydrochloride (180 µL, (2 mM in phosphate buffer), and phosphate buffer (1.7 mL). The reaction mixture was incubated at 37.4 °C in dark for 2h. The degree of oxidation was measured according to the thiocyanate method. 150 µL of mixture reaction was taken and

mixed with equal volume of $FeCl_2$ (0.02 M) and thiocyanate ammonium (30%, W/V). The absorbance was read at 500 nm. Curcumin and ferulic acid were used as reference. The peroxidation of linoleic acid was calculated as peroxidation percent :

$$\text{Peroxidation (\%)} = (A_{control}/A_{test}) \times 100$$

where $A_{control}$ was the absorption of the control reaction and A_{test} was the absorption in the presence of sample. All analyses were run in triplicate and mean values were calculated. A low peroxidation (%) indicated a high antioxidant activity.

7.3.4. UV-Visible spectrometry study of metal chelation ability

All compounds were assayed as Cu and Fe dication chelators after spectral scanning of solutions in methanol at wavelengths ranging from 230 to 600 nm, at 298°K, and using a Synergy 2 microplate reader Each stock compound solution (50 mM in DMSO) was first diluted in methanol to 50 µM, and mixed with an equimolar concentration of $CuCl_2$ or $FeCl_2$ from Sigma-Aldrich (Saint-Quentin Fallavier, France). Mixtures were homogenized by pipetting, and incubated at room temperature for 10 min before measurement. Difference UV–Vis spectra were obtained after numerical subtraction of the spectra monitored for the considered metal dication from the spectra of each mixture.

For studies carried out with increasing Cu(II) concentrations, methanol - 10 mM Tris pH 7.4 buffer (8:2, v/v) was used as incubation medium, and the same protocol as described above was applied. For the Job plots assays, studies were performed by using also methanol - 10 mM Tris pH 7.4 buffer (8:2, v/v) as incubation medium, and absorbance changes were measured for each tested compound at its specific wavelength with concomitant variations of Cu (II) and compound concentrations from 0 to 100 µM.

For assays performed with increasing concentrations of compounds **29**, **31**, **34**, **40** (from 0 to 200 µM), the considered drug was incubated in methanol - 10 mM Tris pH 7.4 buffer (8:2, v/v) as incubation medium in a 96-wells microplate in absence or in presence of $CuCl_2$ (50 µM). Spectral

scanning was carried out from 230 to 600 nm after 10 and 60 min. Difference spectra were obtained by subtracting that of each compound at each concentration level from that of corresponding compound-$CuCl_2$ mixtures. Variation of the absorbance difference at the specific wavelength for each compound was then used to calculate concentration of Cu(II)-complex [B] and the free ligand [L] according to equation (1) and (2):

$$[B] = \Delta Abs / \Delta Abs_{max} \,.\, C_{Cu} \qquad (1)$$

$$[L] = C_{Ligand} - [B].n \qquad (2)$$

where ΔAbs was the absorbance difference at the specific wavelength, ΔAbs_{max} the maximum absorbance difference at in saturation condition, C_{Cu}, the total Cu(II) concentration (50 µM), C_{Ligand}, the total Cu(II) concentration and n was the stoichiometric coefficient (n=1 for **29**, **34**, **40**; n=2 for **31**). [B] was plotted *versus* [L] and non-linear regression was performed using one site binding model equation (3) with Origin 6.1 software (OriginLab,Northampton, MA, USA) leading to the determination of Kd value.

$$[B] = \frac{[B]max.[L]}{Kd+[L]} \qquad (3)$$

7.3.5. Self-mediated $A\beta_{1-42}$ aggregation inhibition

The self-mediated $A\beta_{1-42}$ aggregation inhibition measurements were conducted using the Sensolyte Thioflavine β amyloid aggregation kit (Anaspec 72214) according to manufacturer's protocol.

The tested compounds were diluted in DMSO at 20mM. In non binding black plate (Greiner 655900) add, in each well, 10 µl Thioflavine T 2mM , 85 µl Aβ42 250 µg/ml and 1 µl test compound 1 mM. In the same time, the positive control was realized without test compound and a blank for all test compounds. The total volume for all samples was completed to 100 µl with buffer provided in the kit.

The fluorescence was measured at 37°C every 5 minutes with a shake for 15s between reads using Tecan infinit M200. The wavelength were Ex/Em = 440 nm/484 nm.

The percent inhibition was calculated by 100 – (IFi/IFo x 100) where IFi was the fluorescence obtained with the test compounds and IFo without the test compounds.

7.4. Drugability

7.4.1. Cytoxicity

Cell culture and cell proliferation assay. The human cell line KB (nasopharyngeal epidermis carcinoma) was originated from the NCI and grown in D-MEM medium supplemented with 10% fetal calf serum, in the presence of penicillin, streptomycin and fungizone in 75 cm^2 flasks under 5% CO_2. 1000 Cells were plated in 96-well tissue culture plates in 200 µL medium and treated 24 h later with compounds dissolved in DMSO using a Biomek 2000 (Beckman-Coulter). Controls received the same volume of DMSO (1% final volume). After 72 h of exposure, the MTS reagent (Celltiter 96Aqueous One Solution; Promega) was added and incubated at 37 °C: the absorbance was monitored at 490 nm, and results were expressed as the inhibition of cell proliferation calculated as the ratio [(1−(OD490 treated/OD490 control))×100] in triplicate experiments. For IC_{50} determinations (50% inhibition of cell proliferation) experiments were performed in duplicate with concentrations ranged 0.5 nM to 100 µM.

7.4.2. Parallel Artificial Membrane Permeability Assay (PAMPA). [82,83]

The PAMPA-BBB and the PAMPA-GIT experiments were conducted using the Pampa Explorer Kit (Pion Inc) according to manufacturer's protocol.

In the case of the PAMPA-BBB, each stock compound solution (20 mM in DMSO) were diluted in Prisma HT buffer pH 7.4 (pION) to 100 µM. 200 µL of this solution (n=6) was added to donor plate (P/N 110243). 5 µL of the BBB-1 Lipid (P/N 110672) was used to coat the membrane filter of the

acceptor plate (P/N 110243). 200 µL of the Brain Sink Buffer (P/N 110674) was added to each well of the acceptor plate.

For the PAMPA-GIT experiments, each stock compound solution (10 mM in DMSO) was diluted in Prisma HT buffer pH 7.4 (pION) to 50 µM. 200 µL of these solutions (n=4) were added to the donor plate (P/N 110243). 5 µL of the GIT-0 Lipid (P/N 110669) was used to coat the membrane filter of the acceptor plate (P/N 110243). 200 µL of Acceptor Sink Buffer (P/N 110139) was added to each well of the acceptor plate.

In both PAMPA-BBB and PAMPA-GIT assays, the sandwiches were incubated at room temperature for 4 h, without stirring. After incubation, the UV-visible spectra were measured with the microplate reader (Tecan infinite M200) and the –logPe were calculated for each compound by using the PAMPA Explorer software v. 3.7 (pION). Quality control standards with known –logPe values were used as references: the highly permeable corticosterone (–logPe = 4.6) and the low permeable theophylline (–logPe <6.0) for the PAMPA-BBB experiments, and the low/moderately permeable ketoprofen and antipyrine (–logPe = 5.8 at pH 7.4) for the PAMPA-GIT assays.

7.4.3. Chemical stability

2 µL of **29**, **31**, **34**, **40** solution at 10 mM in DMSO was added to 998 µL of 7.4 phosphate buffer (10 mM, ionic strength adjusted with KCl to 150 mM) in a HPLC vial and vortexed to make the mixture homogeneous (n=3). Each vial was analyzed at 6 different times from 0 to 5 or 8 h using UHPLC Agilent 1290 Infinity system (Agilent Technologies, Santa Clara, California, USA) equipped with a PDA detector Agilent 1260 Infinity. The chromatographic system was controlled by Open LAB CDS LC Chemstation® software (revision C01.05). Runs were performed with a flow rate 0.8 $mL.min^{-1}$ using a Restek Ultra C18 column (50 mm x 2,1, 5 µm) (Restek Corporation, Bellefonte, PA, USA). Mobile phase was composed by gradient method with water and acetonitrile, both with 0.01% formic acid (v/v). Percentages of initial area were plotted *versus* time, exponential regressions were performed and half-life were calculated.

7.5. Crystallographic data.

Data for crystal structure analysis were first collected at 150 K with a Bruker–Nonius Kappa CCD area detector diffractometer with graphite–monochromatized Mo K_λ radiation (λ=0.71073 Å). The structure was solved using direct methods and refined by full-matrix least-squares analysis on F^2. Program(s) used to solve structure: SHELXS–97.[88] Program(s) used to refine structure: SHELXL–2014.[89] All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were located in Fourier difference maps and refined isotropically. Software used to prepare material for publication: SHELXL–2014. Crystallographic data for compound **31** have been deposited at the Cambridge Crystallographic Data Centre, CCDC N°1428014. Copies of this information may be obtained free of charge from the Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (+44-1223-336408; E-mail: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk).

Crystallographic data of **31**: The suitable crystals of **31** for X-ray diffraction studies were prepared by slow evaporation from mixture methanol (1)/dichloromethane (9). Crystal size: 0.16×0.41×0.52 mm. Formula $C_{21}H_{17}NO_5$, H_2O, formula weight 381.37, crystal system orthorhombic, space group *P*bcn, a = 18.8620(4) Å, b = 9.2475(2) Å, c = 20.3811(5) Å, α = 90°, β = 90°, γ = 90°, V = 3555.00(14) $Å^3$, Z = 8, calculated density = 1.425 g/cm^3, μ = 0.105 mm^{-1}, R_{int} = 0.044, R[F^2>2σ(F^2)] = 0.051, wR(F^2) = 0.145, S=1.02.

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Figure 1. Structure of various naturally occurring polyphenolic derivatives (**1-4**) and title benzylidenephenylpyrrolizinones.

Reagents and conditions: a) 2,5-diOMeTHF, AcOH, 60°C, 1h30, 80%; b) TEA, $ClCO_2Et$, CH_3COCH_3, 0°C, 30 min; (c) Me_2NH, Et_2O, room temperature, 15 min, 71%; (d) $POCl_3$, reflux, 1 h; (e) $NaHCO_3$, H_2O; (f) $HClO_4$, H_2O, 0°C, 75%; (g) NaOH, H_2O, room temperature, 10 min, 95%; (h) triphosgen, toluene, 110°C, 5 days (sealed tube), 52%.

Scheme 1. Synthesis of phenylpyrrolizinone **5**.

Reagents and conditions: a) $(CH_2)_2CO_2H$, $AcONH_4$, EtOH, reflux 8-24 h, 23-70%; b) 2,5-diOMeTHF, AcOH, 60°C, 1h30, 56-68%; c) triphosgen, toluene, 110°C, 5 days (sealed tube), 40-60%; d) Benzaldehyde, NaOH, H_2O, MeOH, room temperature, 1 h, 75-95%; e) HBr, AcOH, room temperature, 1 h, 70-80%; f) BBr_3, DCM, reflux, 30 mn, 71%.

Scheme 2. Synthesis of phenylpyrrolizinones **5**,**16**,**17** and benzylidenephenylpyrrolizinones **18-40**.

Figure 2. ORTEP diagram of compound **31**.

Table 1. Antioxidant activities of curcumin, trolox, ferulic acid, quercetin and compounds **18**, **24**, **29-40** (nd : not determined).

Table 2 : Chelating ability screening of curcumine and compounds **29-40** using UV-Visible spectrophometry (nd : not determined).

Figure 3 : UV-visible spectra of compounds **29** (A) and **38** (B) alone and in presence of Cu and Fe dications.

Figure 4 : A) UV-visible spectra of **29** at 50 µM with rising concentration (0 to 200 µM) of Cu(II); B) Variation of absorbance at 384 and 448 nm versus Cu(II) concentration.

Figure 5 : Job plots of compound **29**.

Table 3 : Stoichiometry, dissociation constant and logarithm of association constant of complexation reaction between compounds **29**, **31**, **34**, **40** and Cu(II).

Table 4. Aggregation inhibition activities.

Table 5. Cytotoxicity towards KB cells and PAMPA BBB and GIT assays results for curcumin and compounds **18**, **24**, **29-40**.

Table 6: Chemical stability of compounds **31**, **34**, **40**, **29** in pH 7.4 buffer.