Bioorg. Med. Chem. Lett. 41 (2021) 128000

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Fiaud's Acid, a novel organocatalyst for diastereoselective bis α-aminophosphonates synthesis with *in-vitro* biological evaluation of antifungal, antioxidant and enzymes inhibition potential

Rim Aissa [a], Samia Guezane-Lakoud [a,*], Martial Toffano [b], Lynda Gali [c], Louisa Aribi-Zouioueche [a]

[a] *Ecocompatible Asymmetric Catalysis Laboratory (LCAE) Badji Mokhtar Annaba-University, B.P 12, 23000 Annaba, Algeria*
[b] *Equipe de Catalyse Moléculaire-ICMMO Bât 420, Université Paris- Saclay, France*
[c] *Research Center in Biotechnology, Ali Mendjli Nouvelle Ville UV 03, BP E73 Constantine, Algeria*

ARTICLE INFO

Keywords:
Fiaud's acid
Chiral organocatalyst
Bis α-aminophosphonates
Cholinesterase
Tyrosinase

ABSTRACT

(*S, S*)-1-hydroxy-1-oxo-2-*c*,5-*t*-diphenylphospholane or Fiaud's acid is used as a novel and effective chiral organocatalyst for bis *α*-aminophosphonates synthesis with excellent diastereoselectivity and yields within short reaction time. All synthesized bis α-aminophosphonates revealed a good to excellent antifungal capacity, where the six compounds **4a, 4b, 4e, 4h, 4k** and **4l** are the best fungicide inhibiting the growth of Fusarium oxysporum and Botrytis cinerea by 65% to 84% with IC_{50} values <0.02 mg/mL. Similarly, these six products exhibited a strong antioxidant effect, whereas a low inhibition activity was obtained with both AChE and BChE. Furthermore, they displayed a very weak inhibitory activity against tyrosinase except for the compound 4l. These findings suggest a possible use of these compounds as synthetic pesticides with less hazardous effects with antioxidant, and anti-tyrosinase properties.

Introduction

Cholinesterase is a category of hydrolysis enzymes found in cholinergic neurons. It plays a major role in the cholinergic transmission by the hydrolysis of the neurotransmitter acetylcholine (ACh) into choline and acetic acid, allowing cholinergic neurons to get back to their resting state. The impaired level of ACh due mainly to neuron death is a prevalent theory that explains the origin of most neurodegenerative disease such as Alzheimer's and Parkinson's disease.[1–3] Acetylcholinesterase enzyme (AChE) is one of the crucial targets of organophosphorus (OPs) causing acute toxicity for the human nervous system inducing irreversible inhibition of acetylcholinesterase.[4] Tyrosinase is an essential enzyme for melanin pigments biosynthesis, some OPs as phosphinic acids derivatives, and aminophosphonates have an inhibitory potential on tyrosinase that could be developed for the treatment of skin cancers such as melanoma.[5]

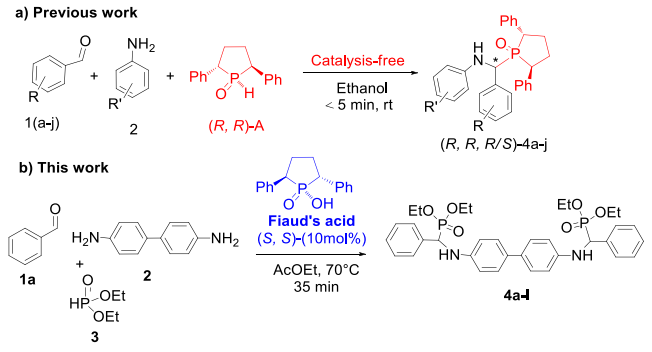
Despite the fact that OPs are recognized as nerve agents,[6] they are widely employed as pesticides due to their environmental weak persistence.[7]

Organophosphorus compounds are gaining continuous attention because of their asymmetric chemical and pharmacological importance.[8] The *α*-aminophosphonates are natural amino acid analogs, and important compounds in medicinal chemistry.[9] These building blocks were first reported in 1967 by *Pudovik*.[10] They have considerable employments in organic synthesis as ligands for liquid extraction of metals,[11] and substrates serving in the construction of different natural products analogs having pharmacological and therapeutic properties.[12] The diverse applications of the *α*-aminophosphonate derivatives include their use as antioxidant, anti-inflammatory,[13] antibacterial, antifungal,[14] and anti-tubercular agents.[15]

It is known that the *Kabachnik-Fields* reaction offers a useful methodology for the α-aminophosphonates synthesis via a multi-component condensation reaction.[16] Various conditions were described using different catalysts for the synthesis of *α*-aminophosphonates derivatives, proceeding with atom economy, reduced steps and reactions time, offering selectivity, ecological and economic benefits.[17] Recently, we reported the new synthesis of a novel chiral cyclic tertiary phosphine oxides *via Kabachnik–Fields* reaction using (*R, R*)-1-oxo-2c,5 *t*-diphenylphospholane as a precursor (scheme1, **a**).[18] This secondary phospholane oxide has been prepared from 1-hydroxy-1-oxo-2c,5*t*-

* Corresponding author.
E-mail address: samia.guezanelakoud@univ-annaba.dz (S. Guezane-Lakoud).

https://doi.org/10.1016/j.bmcl.2021.128000
Received 10 November 2020; Received in revised form 22 March 2021; Accepted 23 March 2021
Available online 30 March 2021



Scheme 1. Advanced synthesis of organophosphorus compounds.

diphenylphospholane, known as Fiaud's acid.[19] This phosphinic acid was demonstrated as an important precursor for the preparation of various chiral organophosphorus compounds, such as ligands,[20] phase transfer agent,[21] or building block.[22] Latterly, Fiaud's acid was described as an effective chiral Brønsted acid catalyst.[23]

In continuation of our ongoing research in this area and to access the diastereoselective bis α-aminophosphonates derivatives,[24] we wish to show the interest of Fiaud's acid as a new chiral organocatalyst for the hydrophosphinylation by double *Kabachnik-Fields* reaction by one-pot condensation of diamine, aromatic aldehyde and diethylphosphite to prepare the bis *α*-aminophosphonates, with excellent diastereoselectivity and chemical yields (Scheme 1, **b**). To the best of our knowledge, the Fiaud's acid has never been used as a chiral Brønsted acid catalyst in the multi-component *Kabachnik-Field* reaction.

Numerous bis *α*-aminophosphonates exhibit significant biological activities: compound **I** showed an anti-oxidant activity,[25] compound **II** is considered as anti-tubercular agents,[15] and compound **III** present optimal anti-proliferative activity against human tumor cells from colon carcinoma[26] (Fig. 1).

They are extremely interesting multidentate ligands that can be used in the extraction of metals, or as monomers for the preparation of macrocyclic compounds, and even polymers carrying phosphonates and amines.[27]

Bis *α*-aminophosphonates have been produced by various synthetic methodologies from dialdehydes,[28] diamines,[29] Schiff bases,[30] using Brønsted and Lewis acids,[31] bases[32] and organocatalysts.[33] As well, with unconventional catalytic systems by way of bicationic acidic ionic liquid (AIL).[34] However, most of these approaches show several disadvantages including long reaction time, stoichiometric amount of catalysts, low diastereoisomers ratios. For these reasons, innovative efficient syntheses are required to designing new bis α-aminophosphonates.

Moreover, to extend understanding about the bis

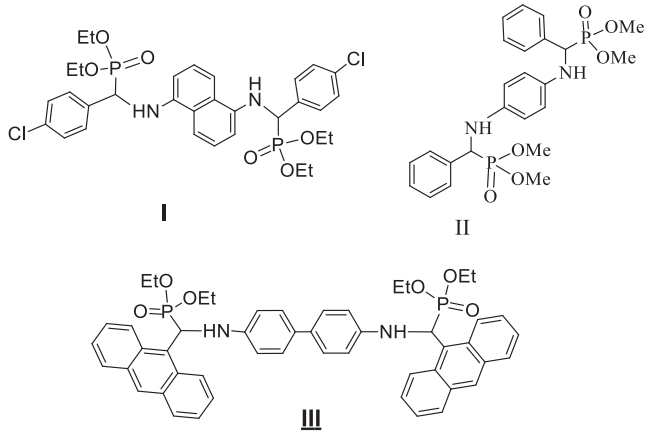


Fig. 1. Bis α-aminophosphonates having biological activities.

α-aminophosphonates biological effects *in vitro* experiments, we have carried out the investigation of their antifungal, antioxidant and enzymatic inhibition potential.

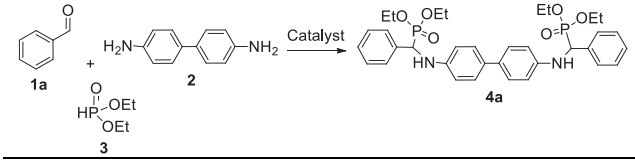
Results and discussion

Chemistry

The synthesis of diastereoselective bis α-aminophosphonates was realized by three components condensation in one pot via *Kabachnik Fields* reaction, using Fiaud's acid or (*S, S*)-1-oxo-1-hydroxy-2-c,5-*t*-diphenylphospholane in ethyl acetate as green solvent, constitutes an important improvement towards the a lower burden for the environment. For that, the condensation of benzaldehyde (2 mmol), benzidine (1 mmol) and diethylphosphite (2 mmol) in 2 mL of organic solvent was chosen as a model reaction. We first examined the condensation without catalyst, in ethyl acetate (AcOEt), no progress of reaction was observed after 24 h and even by increasing the temperature up to 70 °C (Table 1, **Entry 1).** After, we have tested several Brønsted acids such as (*S, S*)-1-oxo-1-hydroxy-2-c,5-*t*-diphenylphospholane (**A**), 1,1′-binaphtyl-2,2′-dihydrogene- phosphonate (**B**), diphenylphosphoric acid (**C**) and diphenylphosphinic acid (**D**) (Fig. 2). The catalysts screening was made with 10 mol% of catalyst in ethyl acetate at 70 °C within 24 h.

Under these conditions only (*S, S*)-1-oxo-1-hydroxy-2-c,5-*t*-diphenylphospholane **A** promoted the multicomponent reaction and allowed obtaining compound **4a** with an excellent chemical yield (93%) and total diastereoselectivity at 70 °C (Table 1, Entry **2**), compared with the use of organocatalysts (**B**), (**C**) and (**D**), which led under the same conditions to the bis α-aminophosphonates with lower yields and diastereomeric ratio (Table 1, entries **4–6**). Decreasing the temperature to 50 °C led to the product with lower yield (31%) (Table 1, entry **3**).The high activity of catalyst (**A**),could be attributed to his low acidity of (dialkylphosphinic acid) (**A**) compared with the diarylphosphinic (**D**) or phosphoric acids (**B**) and (**C**) which played probably an important role in these results. Several parameters were studied, such as; the catalytic amount of the organocatalysts, solvents effect, temperature and reaction time. The product 4a was obtained as one diastereoisomer and purified by crystallization in hexane (Table 1).

Table 1
Optimization Reaction of the synthesis of bis α-aminophosphonates.



Entry [a]	Catalyst (10 mol %)	Solvent	T °C	Time /h	Yield(%)[c]
1[b]	–	AcOEt	70	24	–
2	**A**	AcOEt	70	24	93
3	A	AcOEt	50	24	31
4	B	AcOEt	70	24	55
5	C	AcOEt	70	24	75
6	D	AcOEt	70	24	30
7	A (5mol %)	AcOEt	70	24	65
8	A	THF	70	24	85
9	A	Et_2O	70	24	43
10	A	PhMe	70	24	Traces
11	A	TBME	70	24	Traces
12	**A**	**AcOEt**	**70**	**35min**	**93**
13	A	AcOEt	70	20min	56

at 70 °C. No reaction was observed at 25 °C. [b]Reaction conditions: benzaldehyde (2 mmol), benzidine (1 mmol) and diethylphosphite (2.4 mmol) were stirred with catalyst in solvent (2 mL) at 70 °C. [c] Yield of the pure product purified by crystallization in hexane.

[a] Reaction conditions: benzaldehyde (2 mmol), benzidine (1 mmol) and diethylphosphite (2.4 mmol) were stirred without catalyst in solvent (2 mL).

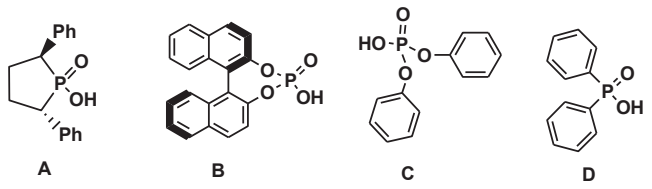


Fig. 2. Organocatalysts tested.

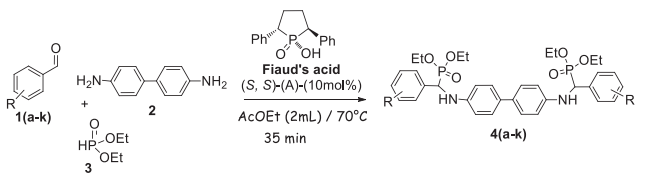
By decreasing the catalytic amount of (*S, S*)-1-oxo-1-hydroxy-2-c,5-*t*-diphenylphospholane from 10% to 5%, the product 4a was obtained in 65% yield (Table 1, entry **7**). The solvent study showed that ethyl acetate was the best solvent. In THF 85% yield was obtained (Table 1, entry **8**) and 43% in Et_2O (Table 1, entry **9**). While no progress was observed in toluene and TBME (Table 1, entries **10** and **11**), this was probably due to the fact that both catalysts (**A**) and (**B**) were insoluble in these solvents. In addition, with ethyl acetate, we found that the yield has reached 93% yield after 35 min (Table 1, entry **12**), but it reduced to 56% after 20 min (Table 1, entry **13**).

To evaluate the synthetic utility of the developed method using (*S, S*)-1-oxo-1-hydroxy-2-c,5-*t*-diphenylphospholane, variety of substituted aryl aldehydes with electron-withdrawing and electron-donating groups were used. The results summarized in Table 2 showed the efficiency of Fiaud's acid used as chiral *Brønsted* catalyst in the MCRs by *Kabachnik-Fields* reaction for bis α-aminophosphonates preparation. The yields in Table 2 correspond to the mixture of diastereomers purified by column chromatography to remove byproducts. All spectral analysis (^{1}H, ^{13}C and ^{31}P NMR) and HRMS were performed on the major diastereoisomer. The diastereomeric ratio was determinated by ^{31}P NMR on the crude product. The chemical yields (63–93%) depend on the nature of electronic effects of the substrates according to our observations and some works of literature,[35] the results show that the increase in electron density on carbonyl and imine groups, caused by electron donating substituents, decreases nucleophilic addition efficiency (Table 2). The best yields (from 82 to 93%) were obtained for **4a**, **4b**, **4c**, **4d**, **4g**, **4j**, **4k** and **4l** while average yields (63–77%) were attained for **4e, 4f,4h** and **4i** compounds. Moreover, in the case of **4a**, **4c**, **4g**, **4h**, **4j**, **4k** and **4l** the *d/l* stereoisomer was obtained as a single compound with a total diastereoselectivity (100% dr) and optical rotation $[\alpha]_D^{20} = -10(c0.002, CH_2Cl_2)$, $[\alpha]_D^{20} = -54.54(c0.001, CH_2Cl_2)$, $[\alpha]_D^{20} = +4(c0.002, CH_2Cl_2)$, $[\alpha]_D^{20} = +13(c0.002, CH_2Cl_2)$, $[\alpha]_D^{20} = -25(c0.002, CH_2Cl_2)$, $[\alpha]_D^{20} = +15(c0.002, CH_2Cl_2)$, $[\alpha]_D^{20} = -10(c0.002, CH_2Cl_2)$ respectively. This suggests a real efficiency of the catalyst A on the diastereoselectivity control. In the other hand, both forms (*d/l* and *meso)* were obtained for the remaining products **4b**, **4d**, **4e**, **4f** and **4i.** Unfortunately, the determination the relative configuration of the chiral centers failed, since we do not succeed to obtain single mono-crystals with sufficient quality for an X-ray analysis.

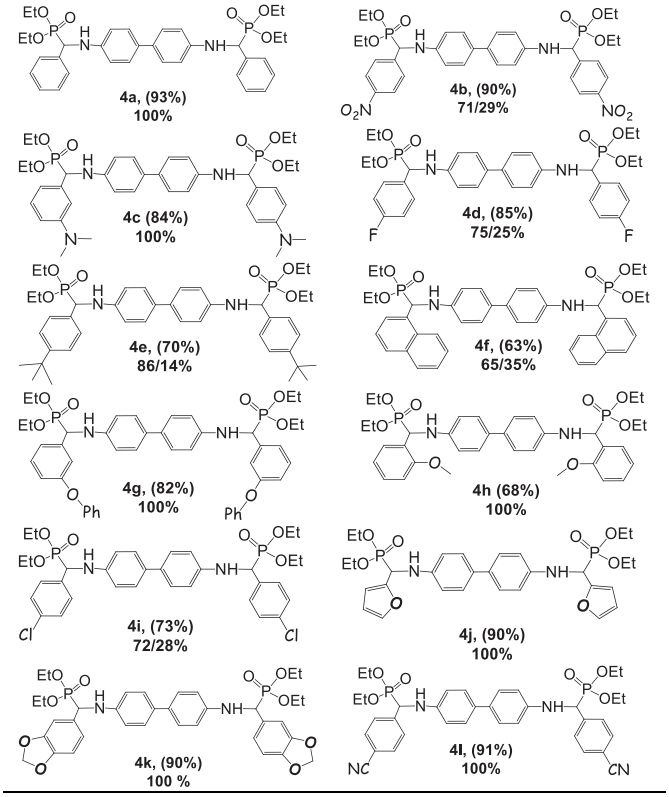
Based on the tests in our previous work and on other works,[24] we find that no traces of mono-phosphonated compound were observed using an equimolar quantity of starting materials (1/1/1 ratio of aldehyde/benzidine/diethylphosphite), and the formation of the C—P bond was performed simultaneously on both sides of benzidine. However, we suppose that the (*S,S*)-1-oxo-1-hydroxy-2-c,5-*t*-diphenylphospholane plays a crucial role in the formation of the P—C bond simultaneously on both sides of benzidine, and the results showed that the bis α-aminophosphonate was the sole compound obtained at the first time. The (*S, S*)-1-oxo-1-hydroxy-2-c,5-*t*-diphenyl-phospholane probably coordinates the nitrogens of di-imine to accelerate the nucleophile attack of the diethylphosphite in their tautomerized form on both sides of di-imine to obtain the desired product (Scheme 2).

We notice that the (S, S)-1-oxo-1-hydroxy-2-c,5-*t*-diphenylphospholane allowed successfully access for seven bis α-aminophosphonates synthesis (4a, 4c, 4g, 4h, 4j, 4k and 4l) with a total diastereoselectivity

Table 2
(S, S)-1-oxo-1-hydroxy-2-c,5-*t*-diphenylphospholane catalysed bis α-aminophosphonates synthesis.[a]



Product **4** (yield)[b], *dr%*[c]

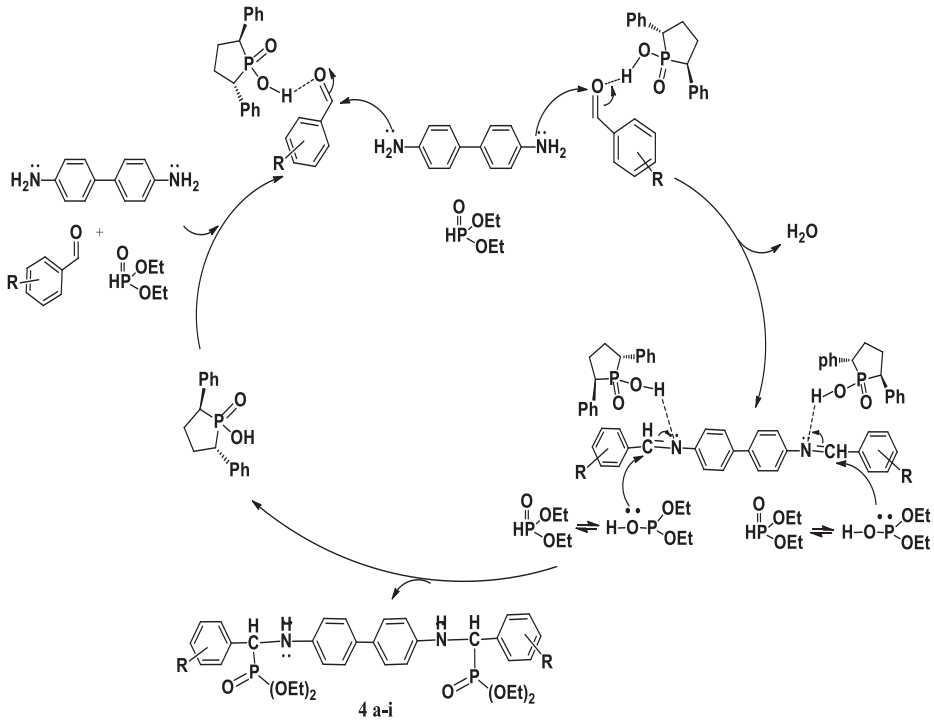


[a] Reaction conditions: benzaldehyde (2 mmol), benzidine (1 mmol) and diethylphosphite(2.4 mmol), AcOEt (2 mL), (*S, S)-1-oxo-1-hydroxy-2-c,5-t-diphenyl- phospholane* (10 mol %), 70 °C, 35 min. [b]Yield of the mixture diastereomers purified by chromatography column or crystallization from hexane.[c] The diastereomeric ratio was determinated by ^{31}P NMR on the mixture diastereomers.

(100% dr) and high yields. This new chiral organocatalyst was introduced in "one pot" reaction in very fast catalytic approach. The observed diastereoselectivities suggest a control by the chiral secondary phospholane oxide during the nucleophilic addition step. The chiral induction appears to be influenced by the nature of the substituents of the initial aldehyde

Biological activity

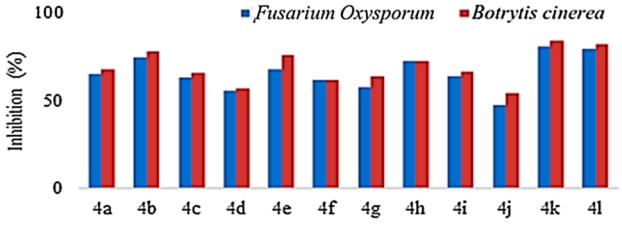
In vitro assays were explored in order to understand the pharmacological effect of the synthesized bis α-aminophosphonates. The antifungal activity evaluation against two plant pathogens *Fusarium oxysporum* and *Botrytis cinerea* showed a great antifungal potential for all tested components. From the results presented in (**Histogram 1**), exposing the inhibition rate (%) at the concentration of 0.14 mg/ml and (**Histogram 2**) revealing outcomes of IC_{50} calculated after accomplishment of antifungal test at diverse concentrations (Results are reported in supplement Tables 1 and 2), we observed that after 7 days of incubation, compounds **4a, 4b, 4e, 4h, 4l,** and **4k** at 0.14 mg/mL had inhibited the growth of both fungi by 67% to 84% with IC_{50} values lower



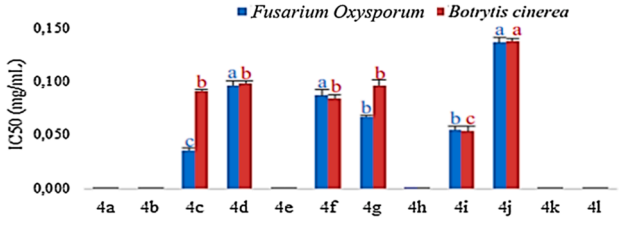
Scheme 2. Proposed mechanism of bis α-aminophosphonates synthesis catalyzed by (S, S)-1-oxo-1-hydroxy-2-c, 5-*t*-diphenylphospholane.

than 0.02 mg/mL, while the other products had exhibited an inhibition varying from 47% to 64% against *Fusarium oxysporum* with IC_{50} values ranging between 0.053 and 0.148 mg/mL, and from 54% to 67% against *Botrytis cinerea* and IC_{50} values varying from 0.054 to 0.136 mg/ml. We noticed that *Botrytis cinerea* fungus was more sensitive than *Fusarium oxysporum*.

Histogram 1. Antifungal inhibition (%) at 0.14 mg/ml concentration of bis α aminophosphonates.



Histogram 2. IC_{50} values of antifungal activity.



Compounds **4a, 4b, 4e, 4h, 4l** and **4k** displayed the highest antifungal activity, therefore, they were selected for investigation of their antioxidant and enzymes inhibitory effects on three enzymes. To study their antioxidant properties, we proceed with five spectrophotometric methods comprising Azinobis-3-ethylbenthiazoline-6-sulphonic acid, radical scavenging (ABTS assay), 1, 1-diphenyl, 2-picrylhydrazy free radical scavenging (DPPH assay), iron reduction by the phenanthroline assay (Phenassay), cupric reducing antioxidant capacity (CUPRAC) assay, and Galvinoxyl radical scavenging (GOR assay). The results were reported as IC_{50} and $A_{0.5}$ values and presented in Table 3 determined from the regression curves at different concentrations (Inhibition percentages at different concentrations are accessible from supplement, Tables 3, 4, 5, 6 and 7), we have observed that all the compounds exhibited a significant reducing effect of iron ions assessed by the phenanthroline assay with $A_{0.5}$ values ranging from 1.14 ± 0.26 μg/mL (4 h) and 6.26 ± 0.05 μg/mL (4b), and they were more efficient than the standards trolox and ascorbic acid ($A_{0.5}$: 5.21 ± 0.27 and 3.08 ± 0.02 μg/mL, respectively). However, the reduction capacity of copper ions determined by CUPRAC was lower compared to iron reduction. The compound **4h** was also found to be the most efficient in copper ions reduction with $A_{0.5}$ value of 3.41 ± 0.19 μg/mL followed by **4l** with $A_{0.5}$ value of 14.47 ± 0.23 μg/mL. The antiradical activity was measured using DPPH, ABTS, and galvinoxyl, as synthetic free radicals. The

Table 3
Antioxidant activities results.

Entry	ABTS IC_{50} (μg/mL)	DPPH IC_{50} (μg/mL)	CUPRAC $A_{0.50}$ (μg/mL)	GOR IC_{50} (μg/mL)	Phenanthroline $A_{0.5}$ (μg/mL)
4a	40.50 ± 7.49^{b}	159.44 ± 2.15^{a}	58.40 ± 0.94^{a}	25.90 ± 0.32^{b}	2.08 ± 0.12^{b}
4b	30.06 ± 0.81^{c}	118.02 ± 1.08^{b}	53.31 ± 1.02^{a}	7.34 ± 0.59^{c}	6.26 ± 0.05^{a}
4e	20.34 ± 1.96^{d}	>800	25.27 ± 1.08^{b}	>200	1.80 ± 0.46^{b}
4h	14.32 ± 1.47^{a}	22.43 ± 0.89^{c}	24.82 ± 1.27^{b}	3.41 ± 0.19^{d}	1.14 ± 0.26^{c}
4k	97.92 ± 0.67^{a}	>800	3.17 ± 0.93^{e}	6.62 ± 0.42^{c}	1.29 ± 0.12^{c}
4l	21.28 ± 0.48^{d}	>800	14.47 ± 0.23^{c}	120.10 ± 0.85^{a}	1.22 ± 0.19^{c}
Trolox*	3.21 ± 0.06^{f}	5.12 ± 0.21^{c}	8.69 ± 0.14^{d}	4.31 ± 0.05^{d}	5.21 ± 0.27^{b}
Ascorbic acid*	3.04 ± 0.05^{f}	4.39 ± 0.01^{c}	8.31 ± 0.15^{d}	5.02 ± 0.02^{d}	3.08 ± 0.02^{e}

Values are expressed as means ± S.D of three parallel measurements. Values with different letters are significantly different (Tukey multiple comparison test, $p < 0.05$). *Reference compounds.

scavenging activity exerted by the compounds differs according to the type of radical. The strongest scavenging activity was against the radical ABTS, displayed by **4k** with an IC_{50} value of 14.32 ± 1.47 μg/mL, whereas **4h** showed the highest effect against DPPH radical, which gave an IC_{50} value of 22.43 ± 0.89 μg/mL. The compounds **4e, 4k,** and **4l** exerted a very weak potential in the reduction of DPPH radical (IC_{50} > 800 μg/mL), while the compounds **4h, 4k,** and **4b** were found to exhibit the best scavenging effect against galvinoxyl radical (IC_{50}: 3.41 ± 0.19, 6.62 ± 0.42, and 7.34 ± 0.59 μg/mL, respectively. It was clear that the radical scavenging potential decreased in the following order: Gor assay > ABTS assay > DPPH assay, indicating that the bis α-aminophosphonates acted the best via an electron transfer mechanism for radical scavenging like in the case of GOR assay, and according to the galvinoxyl scavenging radical mechanism theory reported by *Wang and Zhang*.[36]

Furthermore, it was notable that a steric bulk caused by both tested compounds and free radical molecule sizes affected the antioxidant activity mainly observed in GOR assay conducted with the smallest studied free radical compared to DPPH assay presenting the largest free radical molecule. Otherwise, structural and electronic properties of differently substituted compounds are other main influencing factors. For example, the compound **4h** was the best antioxidant among tested compounds with $A_{0.5}$ of 1.14 ± 0.26 μg/mL in Phen assay and IC_{50} of 3.41 ± 0.19 μg/mL in GOR assay promoting better antioxidant effects than standards. However, compound 4 h showed lower activity against ABTS and DPPH radicals as well as in the reduction of copper ions. Similarly, the compounds 4 k presented a remarkable capacity in reducing iron and copper ions (A0.5 = 1.29 ± 0.12 3.17 ± 0.93 μg/mL, respectively) and in the scavenging of galvinoxyl radical (IC_{50} = 6.62 ± 0.42 μg/mL), while it exhibited a weak scavenging activity against ABTS and DPPH radicals (IC_{50} = 97.92 ± 0.67 μg/mL and IC_{50} < 800 μg/mL, respectively). We can conclude that both the structure of the antioxidant compound and the assay can influence the antioxidant effect.

Organophosphorus neurological toxicity is one of the principal worries of their use as pesticides. For this purpose, we have evaluated the capacity of the selected compounds to inhibit both acetylcholinesterase (AChE), and butyrylcholinesterase (BChE). Tyrosinase inhibition activity was also investigated (Inhibition percentages at different concentrations are available in supplement, Tables 8, 9 and 10). The summarized results of IC_{50} in Table 4, showed that all tested compounds presented a moderate to weak inhibitory activity against AChE. The compounds **4b, 4a** and **4k** exhibited the strongest inhibition with IC_{50} of 34.13 ± 0.65, 85.04 ± 1.35, and 82.18 ± 1.23, respectively but much low compared to the standard Galantamine, which gave an IC_{50} of 6.27 ± 1.15 μg/mL. On behalf of BChE, **4e** was found to be inactive, **4a, 4b** and **4l** exerted week inhibition (IC_{50} = 87.58 ± 3.86 and 62.72 ± 2.16 μg/mL for **4a** and **4b**, respectively, and IC_{50} exceed 200 μg/mL for **4l**), while IC_{50} values of 39.58 ± 3.05 and 39.42 ± 2.98 μg/mL were obtained with **4h** and **4k** respectively, which were close (values are not significantly different, p > 0.05) from the IC_{50} value recorded with the standard Galantamine (34.75 ± 1.99 μg/mL). The inhibitory effect of the selected compounds on tyrosinase showed that all compounds were inactive or have IC_{50} exceeding 200 μg/mL including **4a** and **4h** excepting **4l**, which has IC_{50} of 12.11 ± 0.36 μg/ml and was, therefore, more powerful than the standard kojic acid (IC_{50} = 25.23 ± 0.78 μg/mL). It should be mentioned that the biological effects exhibited by the major diastereomer and the mixture of diastereoisomers for tested compounds are similar in all exanimated activities.

Table 4
Enzymes inhibition assays results.

Entry	AChE IC_{50} (μg/mL)	BChE IC_{50} (μg/mL)	Tyrosinase IC_{50} (μg/mL)
4a	85.04±1.35[a]	87.58±3.86[a]	>200
4b	34.13±0.65[b]	62.72±2.16[b]	–
4e	>200	39.58±3.05[c]	–
4h	>200	–	>200
4k	82.18±1.23[a]	39.42±2.98[c]	–
4l	>200	>200	12.11±0.36[b]
Galantamine*	6.27±1.15[c]	34.75±1.99[c]	–
Kojic acid*	–	–	25.23±0.78[a]

Values are reported as means ± S.D of three parallel measurements. Values with different letters are significantly different (p < 0.05), *Reference compounds.

Conclusion

In summary, we proved that the (*S, S*)-1-oxo-1-hydroxy-2-c,5-*t*-diphenylphospholane could be implicated successfully as an effective new chiral organocatalyst for bis *α*-aminophosphonates synthesis with an efficient one pot catalytic approaches giving nine products **4a-4i** in high yields (63–93%) and with an excellent to good diastereoselectivity within 35 min at 70 °C. All synthesized bis α-aminophosphonates revealed an excellent antifungal capacity, with compounds **4a, 4b, 4e, 4h, 4l** and **4k** having IC_{50} under 0.02 mg/ml. Moreover, these compounds afforded high antioxidant activity, and display a low enzymatic inhibition of acetylcholinesterase and butyrylcholinesterase, whereas for Tyrosinase inhibition they were inactive or have high IC_{50} values except the compound **4i**. These results demonstrated that the biological effect depend on the examination of each analogue which is supported by the structure–activity relationship (SAR)[37]. For that, some organophosphorus could be considered as less hazardous promising pesticides. This last statement should be supported by future *in-vivo* biological investigations, cytotoxicity and *eco-toxicity* studies.

Experimental section

Chemistry

All reagents were purchased from Sigma-Aldrich or Acros Company used without further purification. Reactions were monitored by thin layer chromatography (TLC) carried out on 0.25-mm Merck silica gel plates (60F-254) using ultraviolet light (254 nm) as the visualizing agent and $KMnO_4$ solution as developing agents. NMR spectra were recorded with Bruker spectrometers operating at (360 MHz, 300 MHz and 250 MHz for 1H, 90 MHz, 75 MHz or 63 MHz for ^{13}C and 101 MHz or 121 MHz for ^{31}P). Chemical shift of Solvent reference peaks used were $CDCl_3$ (δ = 7.26 ppm) for 1H and (δ = 77 ppm) for ^{13}C NMR spectra, while H_3PO_4 was used as external standard for chemical shift references for ^{31}P NMR. Couplings constants (J) are given in Hz, with the Following abbreviations multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad signal. Mass spectra were taken by a MicrOTOF-Q Bruker spectrometer using electrospray ionization (ESI) analysis. Melting points were measured using Buchi Melting Point B-545. Optical rotations were measured on an Anton Paar's MCP 150 and Bellingham & Stanley ADS 420 polarimeters and reported as follows: $[\alpha]_D^T$ (concentration (g/mL), solvent).

General procedure for synthesis of bis α-aminophosphonates 4a-l

A mixture of N,N' benzyldiamine (1 mmol, 0.18 g), aromatic aldehyde (2 mmol, 0.2 g) and diethyl phosphate (2.4 mmol, 0.34 g) was well stirred with (*S, S*)-1-oxo-1-hydroxy-2-c,5-*t*-diphenylphospholane (10 mol%) in 2 mL of ethyl acetate at 70 °C for 35 min. The reaction progress was monitored by TLC. The resulting mixture diastereomers was purified by column chromatography on silica gel (ethyl acetate/hexane: 30/70) to afford the major diastereoisomer of products **4a-l** for analysis spectra.

Tetraethyl [(4,1-phenylene) bis(azanediyl)] bis[(phenyl) methylene] bisphosphonate (4a). Yellow solid, 93% Yield, mp: 210 °C, $[\alpha]_D^{20} = -10(c0,002,CH_2Cl_2)$. ^1H NMR (250 MHz, $CDCl_3$) δ 7.54 – 7.41 (m, 4H-Ar), 7.29 (m, 10*H*-Ar), 6.60 (d, *J* = 8.5 Hz, 4H-Ar), 4.77 (d, *J* = 24.3 Hz, 2H, HCP + NH), 4.11 (m, 4H, O-CH_2-CH_3), 4.01 – 3.83 (m, 2H, O-

CH_2-CH_3), 3.77 – 3.55 (m, 2H, O-CH_2-CH_3), 1.28 (t, J = 7.1 Hz, 6H, O-CH_2-CH_3), 1.11 (t, J = 7.1 Hz, 6H, O-CH_2-CH_3). ^{13}C NMR (63 MHz, Chloroform-*d*) δ 144.87 (d, J = 14.7 Hz), 135.88(s), 131.42(s), 128.59 (s), 127.82 (d, J = 5.6 Hz), 127.06(s), 114.09(s), 63.27 (d, J = 6.9 Hz), 57.31 (d, J = 6.2 Hz), 54.97, 16.41 (d, J = 5.4 Hz), 16.16 (d, J = 6.0 Hz). ^{31}P NMR (101 MHz, Acetone-d_6): δ 22.63 ppm. HRMS (ESI) *m/z* calcd for $C_{34}H_{42}N_2O_6NaP_2$ [M + Na^+]: 659.2410; Found 659.2399.

Tetraethyl [(4,1-phenylene)bis(azanediyl)]bis[(4-nitrophenyl) methylene] bisphosphonate(4b). Yellow solid, 90% Yield, mp: 219.5 °C.^{1}H NMR (360 MHz, $CDCl_3$) δ 8.20 (d, J = 8.4 Hz, 4H-Ar), 7.67 (dd, J = 8.8, 2.3 Hz, 4H-Ar), 7.31 – 7.15 (m, 4H-Ar), 6.55 (d, J = 8.6 Hz, 4H-Ar), 4.86 (d, J = 25.1 Hz, 2H, HCP + NH), 4.21 – 4.08 (m, 4H), 4.09 – 3.97 (m, 2H, O-CH_2-CH_3), 3.95 – 3.80 (m, 2H, O-CH_2-CH_3), 1.30 (t, J = 7.1 Hz, 6H, O-CH_2-CH_3), 1.18 (t, J = 7.1 Hz, 6H, O-CH_2-CH_3). ^{13}C NMR (91 MHz, $CDCl_3$) δ 147.68 – 147.55 (m), 144.37, 144.22, 144.00 (d, J = 3.8 Hz), 131.85(s), 128.64 (d, J = 4.8 Hz), 127.30, 123.79, 114.12, 63.78 (d, J = 7.5 Hz), 63.53 (d, J = 6.7 Hz), 56.92, 55.29, 16.36 (dd, J = 15.4, 5.7 Hz). ^{31}P NMR (101 MHz, $CDCl_3$) δ 20.71 ppm. HRMS (ESI) *m/z* calcd for $C_{34}H_{40}N_4O_{10}NaP_2$ [M + Na^+]: 749.2112; Found 749.2127.

Tetraethyl [(4,1-phenylene)bis(azanediyl)]bis[(4-dimethyl-aminophenyl) methylene]bisphosphonate(4c). Orange solid, 84% Yield, mp: 199.3 °C. $[\alpha]_D^{20} = -54,54(c0,001,CH_2Cl_2)$. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.32 (dd, J = 8.9, 2.3 Hz, 4H-Ar), 7.22 (d, J = 8.6 Hz, 4H-Ar), 6.69 (d, J = 8.5 Hz, 4H-Ar), 6.61 (d, J = 8.7 Hz, 4H-Ar), 4.68 (d, J = 23.4 Hz, 2H, HCP + NH), 4.20 – 4.02 (m, 4H, O-CH_2-CH_3), 3.94 (m, 2H, O-CH_2-CH_3), 3.76 – 3.58 (m, 2H, O-CH_2-CH_3), 2.92 (s, 12H, 2$(CH_3)_2$-N-), 1.28 (t, J = 7.1 Hz, 6H, O-CH_2-CH_3), 1.14 (t, J = 7.1 Hz, 6H, O-CH_2-CH_3). ^{13}C NMR (63 MHz, $CDCl_3$) δ 150.10(s), 145.10 (d, J = 14.8 Hz), 139.16(s), 131.29(s), 128.59 (d, J = 5.5 Hz), 126.99(s), 123.11(s), 114.13(s), 112.60(s), 63.10 (t, $J_{C\text{-}P}$ = 6.2 Hz), 56.67(s), 54.24(s), 40.52(s), 16.37 (dd, $J^3_{C\text{-}P}$ = 10.0, 5.8 Hz). ^{31}P NMR (121 MHz, $CDCl_3$, 25 °C): δ 22.30 ppm. HRMS (ESI) *m/z* calcd for $C_{38}H_{52}N_4O_6NaP_2$ [M + Na^+]: 745.3254; Found 745.33257.

Tetraethyl [(4,1-phenylene)bis(azanediyl)]bis[(4-fluoro-phenyl)methylene] bisphosphonate(4d). Brown olid, Yield 85%, mp: 172.3 1C°C. ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.45 (m, 4H, H-Ar), 7.23 (d, J = 8.6 Hz, 4H, H-Ar), 7.02 (t, J = 8.5 Hz, 4H, H-Ar), 6.58 (d, J = 8.7 Hz, 4H, H-Ar), 4.75 (d, J = 24.2 Hz, 3H, HCP + NH), 4.18 – 4.04 (m, 4H, O-CH_2-CH_3), 4.02 – 3.90 (m, 2H, O-CH_2-CH_3), 3.80 – 3.68 (m, 2H, O-CH_2-CH_3), 1.27 (t, J = 7.1 Hz, 6H, O-CH_2-CH_3), 1.14 (t, J = 7.1 Hz, 6H, O-CH_2-CH_3). ^{13}C NMR (91 MHz, $CDCl_3$) δ 163.79 (d, J = 3.0 Hz), 161.07 (d, J = 3.9 Hz), 144.74 (d, J = 14.8 Hz), 131.60 (d, J = 16.7 Hz), 129.72 – 129.20 (m), 127.14, 115.72, 114.13, 63.34 (t, $J_{C\text{-}P}$ = 6.6 Hz), 56.29(s), 54.62(s), 16.46 (d, $J^3_{C\text{-}P}$ = 5.5 Hz), 16.26 (d, $J^3_{C\text{-}P}$ = 5.8 Hz). ^{31}P NMR (101 MHz, $CDCl_3$, 25 °C): δ 22.26 ppm. HRMS (ESI) *m/z* calcd for $C_{34}H_{40}N_2O_6NaP_2F_2$ [M + Na^+]: 695.2222; Found 695.2239.

Tetraethyl [(4,1-phenylene)bis(azanediyl)]bis[4-(tert-butyl)phenyl)methylene]bisphosphonate(4e). Green solid, 77% Yield, mp: 220 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 – 7.31 (m, 8H, H-Ar), 7.30 – 7.21 (m, 4H, H-Ar), 6.66 (d, J = 8.5 Hz, 4H, H-Ar), 4.76 (d, J = 23.0 Hz, 2H, HCP), 4.20 – 4.04 (m, 4H, O-CH_2-CH_3), 3.95 (m, 3H, O-CH_2-CH_3 + NH), 3.77–3.61 (m, 3H, O-CH_2-CH_3 + NH), 1.39 – 1.25 (m, 25H, 9 CH_3 + 2(O-CH_2-CH_3)), 1.10 (t, J = 7.0 Hz, 6H, O-CH_2-CH_3). ^{13}C NMR (75 MHz, Chloroform-*d*) δ 130.01, 127.46 (d, J = 5.5 Hz), 127.03, 126.94 – 126.57 (m), 125.91 – 125.16 (m), 120.52 – 120.25 (m), 114.07, 63.28, 34.51, 31.30, 16.42 (d, $J^3_{C\text{-}P}$ = 5.7 Hz), 16.11 (d, $J^3_{C\text{-}P}$ = 5.6 Hz). ^{31}P NMR (121 MHz, Acetone-d_6): δ 22.52 ppm. HRMS (ESI) *m/z* calcd for $C_{42}H_{58}O_6NaN_2P_2$ [M + Na^+]: 771.3662; Found 771.3682.

Tetraethyl [(4,1-phenylene)bis(azanediyl)]bis[(napht-1-ylmethylene] bisphosphonate(4f). Brown solid, 63% Yield,mp: 222.7 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25 (d, J = 8.5 Hz, 2H-Ar), 7.90 (d, J = 7.8 Hz, 2H-Ar), 7.79 (dd, J = 7.7, 2.8 Hz, 4H-Ar), 7.62 (t, J = 7.6 Hz, 2H-Ar), 7.55 (d, J = 7.0 Hz, 2H-Ar), 7.44 (t, J = 7.7 Hz, 2H-Ar), 7.14 (d, J = 8.2 Hz, 4H-Ar), 6.55 (d, J = 8.2 Hz, 4H-Ar), 5.64 (d, J = 23.8 Hz, 2H), 4.18 (p, J = 7.1 Hz, 4H, O-CH_2-CH_3), 3.82 – 3.65 (m, 2H, O-CH_2-CH_3), 3.31 – 3.14 (m, 2H, O-CH_2-CH_3), 1.32 (t, J = 7.1 Hz, 6H, O-CH_2-CH_3), 0.74 (t, J = 7.1 Hz, 6H, O-CH_2-CH_3). ^{13}C NMR (91 MHz, Chloroform-*d*) δ 145.72, 137.07, 128.69 (d, J = 16.4 Hz), 127.59, 126.65, 126.29 (d, J = 13.9 Hz), 125.70 (d, J = 6.3 Hz), 125.41 (d, J = 6.5 Hz), 120.14, 113.88, 63.58 (d, J = 7.0 Hz), 63.37 (d, J = 6.7 Hz), 24.59, 16.50 (d, $J^3_{C\text{-}P}$ = 5.7 Hz), 15.78 (d, $J^3_{C\text{-}P}$ = 6.1 Hz).^{31}P NMR (101 MHz, $CDCl_3$, 25 °C): δ 22.72 ppm. HRMS (ESI) *m/z* calcd for $C_{30}H_{38}N_2O_8NaP_2$ [M + Na^+]: 759.2723; Found 759.2732.

Tetraethyl[(4,1-phenylene)bis(azanediyl)]bis[(3-phenoxy-phenyl)methylene]bisphosphonate(4g). Yellow solid, 82% Yield, mp: 184.6 °C, $[\alpha]_D^{20} = +4(c0,002,CH_2Cl_2$ ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.35 – 7.30 (m, 2H, H-Ar), 7.28 (d, J = 1.5 Hz, 4H, H-Ar), 7.27 – 7.20 (m, 5H, H-Ar), 7.15 (m, 2H, H-Ar), 7.11–7.05 (m, 2H, H-Ar), 6.99 – 6.87 (m, 6H, H-Ar), 6.60 (d, J = 8.7 Hz, 4H, H-Ar), 4.76 (d, J = 24.5 Hz, 3H, HCP + NH), 4.24 – 4.07 (m, 4H, O-CH_2-CH_3), 4.06 – 3.92 (m, 2H, O-CH_2-CH_3), 3.88 – 3.68 (m, 2H, O-CH_2-CH_3), 1.29 (t, J = 7.1 Hz, 6H, O-CH_2-CH_3), 1.17 (t, J = 7.1 Hz, 6H, O-CH_2-CH_3). ^{13}C NMR (63 MHz, $CDCl_3$) δ 157.33 (d, J = 2.5 Hz), 156.98(s), 144.75 (d, J = 14.6 Hz), 138.11(s), 131.53(s), 129.92(s), 129.69(s), 127.07(s), 123.24(s), 122.70 (d, J = 5.1 Hz), 118.73(s), 118.49 – 118.09 (m), 114.24(s), 63.61 – 63.00 (m), 57.20(s), 54.80(s), 16.33 (dd, $J^3_{C\text{-}P}$ = 11.9, 5.9 Hz). ^{31}P NMR (101 MHz, Acetone-d_6) δ 22.20 ppm. HRMS (ESI) *m/z* calcd for $C_{46}H_{50}N_2O_8NaP_2$ [M + Na^+]: 843.2935; Found 843.2914.

Tetraethyl[(4,1-phenylene)bis(azanediyl)]bis[(2-methoxy-phenyl)methylene] bisphosphonate(4h). Orange solid, 68% Yield, mp: 165.6 °C, $[\alpha]_D^{20} = +13(c0,002,CH_2Cl_2)$ ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.51 – 7.42 (m, 2H-Ar), 7.21 (dt, J = 8.1, 1.8 Hz, 6H-Ar), 6.97 – 6.83 (m, 4H-Ar), 6.61 (s, 3H, O-CH_2-CH_3), 5.40 (d, J = 19.9 Hz, 2H, O-CH_2-CH_3), 4.26 – 4.06 (m, 4H, O-CH_2-CH_3), 3.93 (s, 6H, OCH_3), 3.92 – 3.79 (m, 2H, O-CH_2-CH_3), 3.69 – 3.51 (m, 2H, O-CH_2-CH_3), 1.30 (t, J = 7.1 Hz, 6H, O-CH_2-CH_3), 1.03 (t, J = 7.1 Hz, 6H, O-CH_2-CH_3). ^{13}C NMR (63 MHz, Chloroform-*d*) δ 139.15, 128.90, 128.18, 127.02 (d, J = 7.9 Hz), 121.01, 113.79, 110.46, 63.38 – 62.70 (m), 55.72, 16.43 (d, $J^3_{C\text{-}P}$ = 5.3 Hz), 16.09 (d, $J^3_{C\text{-}P}$ = 5.8 Hz). ^{31}P NMR (101 MHz, Acetone-d_6) δ 23,54 ppm. HRMS (ESI) *m/z* calcd for $C_{36}H_{46}O_8N_2NaP_2$ [M + Na^+]: 719.2649.

Tetraethyl[(4,1-phenylene)bis(azanediyl)]bis[4-chlorophenyl)methylne] bisphosphonate (4i). Green oil, 73% yield. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.43 (m, 4H), 7.31 (d, J = 8.4 Hz, 4H, H–Ar), 7.27–7.19 (m, 4H, H–Ar), 6.58 (d, J = 8.6 Hz, 4H, H–Ar), 4.75 (d, J = 24.4 Hz, 2H, HCP), 4.23–4.03 (m, 5H, O–CH_2–CH_3 + NH), 3.96 (m, 2H, O–CH_2–CH_3), 3.84–3.67 (m, 2H, O–CH_2–CH_3), 1.28 (t, J = 7.1 Hz, 6H, O–CH_2–CH_3), 1.16 (t, J = 7.1 Hz, 6H, O–CH_2–CH_3). ^{13}C NMR (91 MHz, $CDCl_3$) δ 144.65 (d, J = 14.7 Hz), 134.56(s), 133.74 (d, J = 3.5 Hz), 131.59(s), 129.14 (d, J = 5.3 Hz), 129.01 – 128.73 (m), 128.65(s), 127.66(s), 127.17(s), 126.78(s), 114.11(s), 63.74 – 63.25 (m), 56.44, 54.77, 16.44 (d, $J^3_{C\text{-}P}$ = 5.7 Hz), 16.26 (d, $J^3_{C\text{-}P}$ = 6.0 Hz). ^{31}P NMR (101 MHz, Acetone-d_6): δ 21.96 ppm. HRMS (ESI) *m/z* calcd for $C_{34}H_{40}O_6N_2P_2Cl_2$ [M + H^+]: 727.1630; Found 727.1610.

Tetraethyl [(4,1-phenylene)bis(azanediyl)]bis[(furan-2 ylmethylene]bis phosphonate (4j). Brown solid, 90% Yield,mp: 218.9 °C, $[\alpha]_D^{20} = +15(c0,002,CH_2Cl_2)$ ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.39 (t, J = 1.9 Hz, 2H– Ar), 7.33 – 7.29 (m, 4H, H–Ar), 7.26 (s, 4H, H–Ar), 6.69 (d, J = 8.7 Hz, 1H), 6.40 (t, J = 3.2 Hz, 2H, H-furan), 6.36 – 6.31 (m, 2H, H-furan), 4.91 (d, J = 23.8 Hz, 2H, HCP), 4.26 – 4.13 (m, 4H, O-CH_2-CH_3), 4.12 – 4.01 (m, 2H, O-CH_2-CH_3), 3.88 (m, 2H, O-CH_2-CH_3), 1.30 (t, J = 7.1 Hz, 6H, O-CH_2-CH_3), 1.21 (t, J = 7.1 Hz, 6H, O-CH_2-CH_3). ^{13}C NMR (91 MHz, $CDCl_3$) δ 149.38, 144.69 (d, J = 13.2 Hz), 142.51, 131.86, 127.16,

114.22, 110.82, 108.82 (d, $J = 6.7$ Hz), 63.55 (d, $J_{C\text{-}P} = 7.1$ Hz), 63.35 (d, $J = 6.7$ Hz), 51.20, 49.44, 16.47 (d, $J^3_{C\text{-}P} = 5.3$ Hz), 16.31 (d, $J^3_{C\text{-}P} = 5.3$ Hz). ^{31}P NMR (101 MHz, $CDCl_3$): δ 20.14 ppm. HRMS (ESI) *m/z* calcd for $C_{30}H_{38}O_8NaN_2P_2$ [$M + Na^+$]: 639.1996; Found 639.1992.

Tetraethyl[(4,1phenylene)bis(azanediyl)]bis(benzo[1,3]dioxol-5-ylmethylene) bisphosphonate (4k). Green solid,90% Yield, mp: 229,7°C, $[\alpha]_D^{20} = -25(c0,002,CH_2Cl_2)$ ^{1}H NMR (360 MHz, Chloroform-*d*) δ 7.24 (d, $J =$ 8.6 Hz, 4H-Ar), 6.97 (t, $J = 1.8$ Hz, 2H-Ar), 6.96 – 6.93 (m, 1H-Ar), 6.94 – 6.91 (m, 1H-Ar), 6.76 (d, $J = 8.0$ Hz, 2H-Ar), 6.60 (d, $J = 8.6$ Hz, 4H), 5.92 (dd, $J = 6.1$, 1.4 Hz, 4H, O-CH_2-O), 4.67 (d, $J = 24.0$ Hz, 3H, O-CH_2-CH_3), 4.22 – 4.06 (m, 4H, O-CH_2-CH_3), 4.06 – 3.90 (m, 2H, O-CH_2-CH_3), 3.85 – 3.69 (m, 2H, O-CH_2-CH_3), 1.29 (t, $J = 7.1$ Hz, 6H, O-CH_2-CH_3), 1.17 (t, $J = 7.1$ Hz, 6H, O-CH_2-CH_3). ^{13}C NMR (91 MHz, Chloroform-*d*) δ 148.00, 147.36 (d, $J = 2.8$ Hz), 144.92, 144.75, 131.48, 129.68 (d, $J = 2.0$ Hz), 127.10, 121.36 (d, $J = 6.3$ Hz), 114.12, 108.33, 108.15 (d, $J = 4.2$ Hz), 63.33 (d, $J = 6.9$ Hz), 56.65, 54.97, 16.47 (d, $J =$ 5.7 Hz), 16.30 (d, $J = 6.0$ Hz). ^{31}P NMR (101 MHz, $CDCl_3$): δ 22.53 ppm. HRMS (ESI) *m/z* calcd for $C_{30}H_{38}O_8NaN_2P_2$ [$M + Na^+$]: 747.2206; Found 747.2225.

Tetraethyl [(4,1-phenylene)bis(azanediyl)] bis[(4-cyano-phenyl) methylene] bisphosphonate (4 l).[19]. Yellow solid, 91% yield, mp: 203.7 1C, $[\alpha]_D^{20} = -10(c0,002,CH_2Cl_2)$ ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C) δ 7.71–7.58 (m, 8H, H–Ar), 7.31–7.18 (m, 4H, H–Ar), 6.57 (d, $J = 11.0$ Hz, 4H, H–Ar), 4.84 (d, $J = 22.0$ Hz, 4H, HCP + NH), 4.24–4.10 (m, 4H, O–CH_2– CH_3), 4.09–3.96 (m, 2H, O–CH_2–CH_3), 3.95–3.75 (m, 2H, O–CH_2– CH_3), 1.31 (t, $J = 7.1$ Hz, 6H, O–CH_2–CH_3), 1.19 (t, $J = 7.1$ Hz, 6H, O–CH_2–CH_3). NMR ^{13}C (63 MHz, $CDCl_3$, 25 1C): d 144.42 (d, $J^3_{C\text{–}P} = 14.2$ Hz), 142.00, 139.17 (s), 132.38 (s), 131.78 (s), 128.55 (d, $J^2_{C\text{–}P} = 5.1$ Hz), 127.27 (s), 114.09 (s), 111.79 (d, $J = 3.4$ Hz), 111.13 (s), 63.66 (d, $J_{C\text{–}P} = 14.8$ Hz, C—O), 63.42 (d, JC–P = 6.7 Hz, C—O), 57.38 (s, C—N), 55.02(s, C—P), 16.43 (d, $J^3_{C\text{–}P} = 5.7$ Hz, CH_3–CH_2—O—P), 16.24 (d, $J_{C\text{–}P}$ 3 = 5.5 Hz, CH_3–CH_2—O—P). ^{31}P NMR (121 MHz, $CDCl_3$, 25 °C) δ 22.05 ppm. HRMS (ESI) *m/z* calcd for $C_{36}H_{40}N_4NaO_6P_2$ [M + Na +]: 709.2135; found 709.2344.

In vitro biological activities

Antioxidant and enzymatic inhibition activities were performed using a 96-well microplate reader, PerkinElmer Mul-timode Plate Reader EnSpire at National Center of biotech- nology Research. The chemical products and reagents used were: Folin-ciocalteu's reagent (FCR), 1,1-diphenyl-2-pic- rylhydrazyl (DPPH), butylatedFolin-Ciocalteu (FCR), 1,1-diphenyl-2-pic- rylhydrazyl (DPPH), Trolox, Ascorbic acid, Tween-40, Neocuproine, 2,2′-azino- bis(3-ethylbenzothiazoline-6-sulfonicacid) diammonium salt (ABTS), Trichloroacetic acid (TCA), Potassium ferricyanide, 3-(2-Pyridyl)-5,6-di(2-furyl)-1,2,4-triazine-5′,5″-disulfonic acid disodium salt (Ferene), Ethylenediaminetetraacetic acid (EDTA), Nitro blue tetrazolium (NTB), b1000 U/mg solid, Butyrylcholinesterase (BChE) from equine serum 100 U/mg solid, 5,50-dithiobis[2-nitrobenzoic acid] (DTNB), butyrylthiocholine chloride, galanthamine, Tyrosinase from mushroom ≥ 1000U/mg solid , 3-(3,4-Dihydroxyphenyl-2,5,6-d3)-L-alanine (L-DOPA), are from Sigma Aldrich. Aluminum nitrate, Sodium bicarbonate, $CuCl_2$, potassium persulfate, potassium acetate were obtained from BiochemChemopharma. Diméthylsulfoxyde (DMSO), and other solvents were of analytical grade were purchased from Sigma Aldrich. hydroxylanisole (BHA), Butylatedhydroxyltoluene (BHT), α- Tocopherol, polyoxyethylenesorbitanmonopalmitate (Tween-40), Neocuproine, 2,2′-azino- bis(3-ethylbenzothiazoline-6-sulfonicacid) diammoniumsalt (ABTS), Trichloroacetic acid (TCA), Potassium ferricyanide, 3-(2-Pyridyl)-5,6-di(2-furyl)-1,2,4-triazine-5′,5″-disulfonic acid disodium salt (Ferrene), Ethylenediaminetetraacetic acid (EDTA), Nitro blue tetrazolium (NTB), Diméthylsulfoxyde (DMSO), Acetylcholinesterase from electric eel (AChE, Type-VI-S, EC 3.1.1.7, 827,84 U/mg, Sigma), butyl cholinesterase from horse serum (BChE, EC 3.1.1.8,7,8 U/mg, Sigma), Acetylthiocholine iodide, S-Butyrylth- iocholine iodide, 5,5′-Dithiobis (2-nitrobenzoic) acid (DTNB),Galantamine, Sodium Carbonate, Aluminum Nitrate, Iron (III) chloride ($FeCl_3$), Iron (II) chlo- ride, Sodium bicarbonate, Copper (II) chloride, Potassium persulfate, Potassium acetate, were obtained from BiochemChemopharma. All other chemicals and solvents were of analytical grade. Potato-dextrose agar (PDA) for microbiology is perchased from Sigma Aldrich.

Antifungal activity

All synthesized products were tested for determination of their antifungal activity trough mycelial growth inhibition of tow phytopathogenic fungi *Fusarium oxysporum* f. sp *lycopersici* (FOL) strain 4287, and *Botrytis cinerea.* For that, 14 mg of compounds was dissolved in 1 mL of DMSO and added to 100 mL of PDA medium at 60 °C to have a final concentration of 0.14 mg/ml in the mixture which was distributed in 4 petri dishes. A disk of 5 mm diameter was taken from a young fungal culture and placed in the petri dishes center. Development of the phytopathogenic agent was measured at millimetric scale After 7 days of incubation at 28 °C. Three experiments repetitions were performed for all tests. Other petri dishes were prepared with 1 mL of DMSO added to 100 mL of PDA medium as positive control with fungal disks, while negative control was set with PDA medium only, according to Song method.[38] Growth inhibition capacity was calculated through radial growth of the fungal colonies as described in Dennis and Webstert work.[39] Results were presented in (**Histogram 1).**

The tests were repeated for divertive concentration (0.14/ 0.12/ 0.10/ 0.08/ 0.06/ 0.04/ 0.02 mg/ml) in order to identify the IC_{50} of tested compounds by a linear regression; results were presented in (**Histogram 2)**.

The following antioxidant activity and enzymatic inhibition assays are spectrophotometric methods adapted to multimode plate reader.

Antioxidant activity essays

Basing on the complex structures and nature of interactions involved in the antioxidant effect, five of effective different complementary spectrophotometric methods, were implemented for testing synthetized compounds 4a, 4b, 4e, 4 h, 4 k, 4 l.

ABTS radical scavenging activity. ABTS radical was generated by the oxidation of 2 mM of ABTS with 2.45 mM potassium persulfate ($K_2S_2O_8$). The resulted solution was mixed with samples and the absorbance was measured at 734 nm.[40]

DPPH scavenging activity. DPHH scavenging activity was assessed following the method described by Blois.[41] Briefly, 1 mM DPPH solution was added to samples at different concentrations and the absorbance was measured at 517 nm.

Phenanthroline assay. Iron ions reduction was assessed by the phennathroline assay as reported in the literature.[42]The reaction mixture contains sample, 0.2% $FeCl_3$, and 0.5% phenanthroline. The absorbance was read at 510 nm.

Cupric reducing antioxidant capacity (CUPRAC). The reduction of copper ions was investigated out by the reduction of colorless copper (II) neocuproine (2,9-dimethyl-1,10-phenanthroline) complex to the coloured copper(I)-neocuproine complex.[43] 10 mM $CuCl_2$, 7.5 mM neocuproine, and 1 M CH_3COONH_4 were mixed with samples in a microplate and the absorbance was read at 450 nm.

Galvinoxyl radical scavenging activity. The reduction of Galvinoxyl radical was determined as previously reported.[44] 0.1 mM of Galvinoxyl radical was added to the samples and the absorbance was measured at

428 nm.

Enzymes inhibition essays

Cholinesterase inhibition. The anticholinesterase activity of compounds 4a, 4b, 4e, 4 h, 4 k, 4 l, was investigated according to the colorimetric method of *Ellman.*[45] *AChE* (5.32×10^{-3} U) and BChE (6.85×10^{-3} U) in phosphate buffer (0.1 M, pH8) were incubated in a 96-well microplate with different concentrations of the compounds. 0.5 mMof DTNB and 0.71 mM acetylthiocholine iodide or 0.2 mM butyrylthiocholine chloride were added and the absorbance was monitored at 412 nm using a multimode plate reader (Perkin Elmer, EnSpire®, Singapore).

Tyrosinase inhibition. The inhibitory effect of the selected compounds against tyrosinase was determined according to the method described by Deveci *et al.*[46] The reaction medium contains 150U/ml of tyrosinase in 100 mM phosphate buffer (pH 6.8), and 5 mM of L-DOPA, and the samples at different concentrations. Absorbance was read at 475 nm.

Statistical analysis

Results are reported as mean value ± SD of three measurements; the IC_{50} and $A_{0.50}$ values were calculated by linear regression analysis, and one-way analysis of variance ANOVA followed by Tukey's multiple comparison test to detect significant differences ($p < 0.05$) using Graphpad prism software (GraphPad Software Inc, San Diego, CA, USA).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The Algerian Ministry of Education and Scientific Research (DGRSDT, FNR) is gratefully acknowledged for financial support of this work. The technical support provided by Emilie KOLODZIEJ is highly appreciated. Dr. Ali DEBBI is thanked for his helpful in biological activities part.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.128000.

References

1 Cavdar H, Senturk M, Guney M, et al. *Enzyme Inhib Med Chem.* 2019;34(1):429–437.
2 Sharma P, Tripathi MK, Shrivastava SK. Targeting enzymes for pharmaceutical development. In: Labrou N, ed. *Methods in Molecular Biology*. vol. 2089. New York: Humana; 2020.
3 Qiu GL, He SS, Chen SCJ. *Enzyme Inhib Med Chem.* 2018;33:1506–1515.
4 Pohanka M. *Curr Med Chem.* 2019;26:1.
5 Cheung JM, Rudolph J, Burshteyn F, et al. *J Med Chem.* 2012;55:10282–10286.
6 a) Barbuto AF, Chai PR. In: *21st Century Prometheus*. Springer, Cham; 2020:287–313. b) Peplow M. *Chem. Eng. News*. 2018;96:3.
7 Ray DE, Richards PG. *Toxicol. Lett.* 2001;120:343–351.
8 a) Dutartre M, Bayardon J, Juge S. Chem Soc Rev. 2016;45(20):5771–5794; b) Maestro A, de Martinez EM, Palacios F, Vicario J. J Org Chem, 2019;84:1094−1102.
9 a) Phillips MA, Fletterick R, Rutter WJ. *J Biol Chem.* 1990;265:20692–20698.b) Mucha A, Kafarski P, Berlicki L. *J Med Chem.* 2011;54:5955–5980.c) Maier L, Spoerri H. *Phosphorus Sulfur Silicon Relat Elem.* 1991;61:69–75.d) Emsley J. *Hall D. New York: Harper and Row; 1976:494–498.e) Meyer JH, Barlett PA. *J Am Chem Soc.* 1998;120:4600–4609.
10 Pudovik AN, Pudovik DA. *Zhur Obshch Khim.* 1967;36:1467.
11 Cherkasov RA, Garifzyanov AR, Zakharov SV, Vinokurov AV, Galkin VI. *Zhur Obshch Khim.* 2006;76(3):438–441.
12 Damodar K, Jun J-G. *Bull Korean Chem Soc.* 2014;35(12):3618–3622.
13 Damiche R, Chafaa S. *J Mol Struct.* 2017;1130:1009–1017.
14 Devineni SR, Doddaga S, Donka R, Chamarthi NR. *Chinese Chem Lett.* 2013;24:759–763.
15 Mulla ARS, Pathan MY, Chavan SS, Gample SP, Sarkar D. *RSC Adv.* 2014;4:7666–7672.
16 a) Fields E. *J Am Chem Soc.* 1952;74:1528.b) Kabachnik MIT, Medved Y. *Dokl Akad Nauk SSSR.* 1952;83:689–692.
17 a) Guezane-Lakoud, S.; Merabet-Khelassi, M.; Aribi-Zouioueche, L. Res.Chem. Intermed. 2016, 42, 4403–4415; b) Guezane-Lakoud, S.; Toffano, M.; Aribi-Zouioueche, L. Heteroat. Chem. 2017, 28, e21408; c) Kolodiazhnyi, O. I.; Kukhar, V. P.; Kolodiazhna, A. O. Tetrahedron: Asymmetry, 2014, 25, 865; d) Kukhar, V. P.; Solodenko, V. A. Russ. Chem. Rev. 1987, 56, 859; e) Laschat, S.; Kunz, H. Synthesis 1992, 90; f) Kabachnik, M. I.; Medved, T. Y. Dokl. Akad. Nauk. SSSR., 1952, 83, 689–692; g) Cherkasov, R. A.; Galkin, V. I.; Russ. Chem. Rev. 1998, 67, 857 – 882; h) Usp. Khim. 1998, 67, 940 – 968.
18 Guezane Lakoud S, Aissa R, Guillot R, Toffano M, Aribi- Zouioueche L. *ChemistrySelect.* 2020;5:379–383.
19 a) Guillen F, Rivard M, Toffano M, Legros JY, Daran JC, Fiaud JC. *Tetrahedron.* 2002;58:5895.b) Guillen F, Fiaud JC. *Tetrahedron Lett.* 1999;40:2939–2942.
20 a) Galland, A.; Paris, J. M.; Schlama, T.; Guillot, R.; Fiaud,J. C.; Toffano, M. Eur. J. Org. Chem. 2007, 863; b) Galland, A.; Dobrota, C.; Toffano, M.; Fiaud, J. C. Tetrahedron: Asymmetry, 2006, 17, 2354; c) Toffano, M.; Dobrota, M.; Fiaud, J. C. Eur. J. Org. Chem. 2006, 650-656; d) Dobrota, C.; Toffano, M. Fiaud, J. C. Tetrahedron Lett. 2004, 45, 8153; e) Dobrota, C.; Fiaud, J. C. Toffano, M. Chem. Cat. Chem. 2015, 7, 144-148.
21 Dobrota C, Duraud A, Toffano M, Fiaud JC. *Eur J Org Chem.*. 2008:2439.
22 a) Duraud A, Jacquet O, Fiaud JC, Guillot R, Toffano M. *Chem Cat Chem.* 2011;3:883–886.b) Duraud A, Toffano M, Fiaud JC. *Eur J Org Chem.*. 2009:4400–4403.
23 Chatterjee S, Hintermann L, Mandal M, Achari A, Gupta S, Jaisankar P. *Org Lett.* 2017;19:3426–3429.
24 a) Aissa R, Guezane-Lakoud S, Kolodziej E, Toffano M, Aribi- Zouioueche L. *New J Chem.* 2019;43:8153–8159.b) Sadik SM, Santhisudha S, Mohan G, et al. *Res Chem Intermed.* 2019;45:1401–1420.
25 Sadik SM, Santhisudha S, Mohan G, et al. *Res Chem Intermed.* 2019;45(3):1401–1420.
26 Kraicheva I, Vodenicharova E, Shivachev B, et al. *Phosphorus Sulfur Silicon Relat Elem.* 2013;188(11):1535–1547.
27 Rezaei Z, Firouzabadi H, Iranpoor N, et al. *Eur J Med Chem.* 2009;44:4266–4275.
28 a) Kaur T, Saha D, Singh N, Singh UP, Sharma A. *Chem Select.* 2016;1(3):434–439. b) Abdel-Rahman RM, Ali TE. *Monatsh Chem.* 2013;144(8):1243–1252.
29 Milen M, Balogh PA, Kangyal R, Dancso A, Frigyes D, Keglevich G. *Heteroat Chem.* 2014;25(4):245–255.
30 a) Lewkowski J, Karpowicz R, Mazur A. *Phosphorus Sulfur Silicon Relat Elem.* 2010;185:2108–2112.b) Lewkowski J, Tokarz P, Lis T, Slepokura K. *Tetrahedron*. 2014;70:810–816.
31 a) 13.; b) Li, X. C.; Gong, S. S.; Zeng, D. Y.; You, Y. H.; Sun, Q. Tetrahedron Lett. 2016, 57(16), 1782-1785; c) Han, W.; Mayer, P.; Ofiala, R. A. Adv. Synth. Catal. 2010, 352(10), 1667 – 1676.
32 Prishchenko, A. A.; Livantsov, M. V.; Novikova, O. P.; Livantsova,; L. I.; Petrosyan, V. S. Heteroat. Chem. 2010, 21, 430-440.
33 a) Abranyi-Balogh, P.; Kangyal, R.; Dancso, A.; Frigyes, D.; Keglevich. G. Heteroat. Chem. 2014, 4,245-255; b) Malamiri, F.; Khaksar, S.; J. Chem. Sci. 2014, 126, 807-811; c) Yu, Y-Q.; Xu, D-Z. Synthesis. 2015, 47, 1869-1876; d) Xu, W.; Zhang, S. Yang, S.; Jin, L.-H.; Bhadury, P. S.; Hu, D.-Y.; Zhang, Y. Molecules. 2010, 15, 5782-5796.
34 Eshghi H, Mirzaei M, Hasanpour M, Mokaber-Esfahani M. *Sulfur Silicon Relat Elem.* 2015;190(10):1606–1620.
35 Mehraban JA, Jalali MS, Heydari A. *Chem Pap.* 2018;72:2215–2223.
36 Wang L-F, Zhang HY. *Bioorg Chem.* 2005;33:108–115.
37 a) Crum-Brown A. *R Soc Edinb.* 1865;25:1968–1969.b) Varmuza K. Lecture Notes in Chemistry. *Springer, Berlin, Heidelberg*. 1980;21:177–183.
38 Song W, Zhou L, Yang C, Cao X, Zhang L, Liu X. *Crop Protect.* 2004;23:243–247.
39 Dennis C, Webster J. *Br Mycol Soc.* 1971;57(3):363–369.
40 Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Biol Med. 1999, 26- 1231–1237.
41 Blois MS. *Nature*. 1958;4617(181):1119–1200.
42 Szydlowska-Czerniaka A, Dianoczki C, Recser K, Karlovits G, Szlyk E. *Talanta*. 2008;76:899–905.
43 Apak R, Guclu K, Ozyurek M, Karademir SE. *J Agric Food Chem.* 2004;52:7970–7981.
44 Shi H, Noguchi N. *Methods Enzymol.* 2001;335:157–166.
45 Ellman GL, Courtney KD, Andres Jr V, Featherstone RM. *Biochem Pharmacol.* 1961;7:88–95.
46 Deveci E, Tel-Çayan GM, Duru E. *Int J Food Prop.* 2018;21(1):771–783.