An expedient approach for the synthesis and the anti-acetylcholinesterase activity evaluation of 6H-benzimidazol[1,2-c][1,3,2]benzoxazaphosphorine 2-(4-methoxyphenyl)-2-sulfide derivatives from 4-mercapto-2H-chromene-2-thione

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Abstract: A new efficient route for the synthesis of substituted 6H-benzimidazol[1,2-c][1,3,2]benzoxazaphosphorine 2-(4-methoxyphenyl)-2-sulfide from 4-mercapto-2H-chromene-2-thione is described. The anti-acetylcholinesterase activity of the most important synthons is considered. Ways of constructing six-membered phosphorus-heterocycles via cyclization of Lawesson’s reagent with bifunctional substrates are discussed.

Keywords: 4-Mercapto-2H-chromene-2-thione, Lawesson’s reagent, six-membered phosphorus-heterocycles, 2-thio-benzoxazaphosphine ring, anti-acetylcholinesterase activity.

Introduction

Thione-containing coumarin has remained poorly studied until recently. We have developed methods for the synthesis of phosphorus-containing heterocyclic compounds. The interest in the synthesis of new phosphorus heterocycles is due to their unique biological and pharmaceutical properties1-3. These compounds exert their biological action on arthropods by attacking the system of neural transmission and inhibiting the function of acetylcholinesterase4-6. Introduction of phosphorus and sulfur atoms in organic molecules, especially in those fragments which are responsible for their biological activities (herbicides, insecticides and fungicides), becomes an important aspect of pharmaceutical investigations, which in turn stimulates research for new methods for synthetic of diverse classes of phosphorus or sulfur-containing compounds.

Previous works on the synthesis of (4-methoxyphenyl) phosphinothioyleno moiety7,8, describe the preparation of phosphorus heterocycles by cyclization reactions of Lawesson’s reagent (LR) with some bifunctional derivatives and Schiff’s bases from 4-mercapto-2H-

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chromene-2-thione. The anti-acetylcholinesterase activity of the synthesized phosphorus containing heterocycles was also evaluated and discussed.

**Results and Discussion**

The starting material 4-mercapto-2H-chromene-2-thione\(^9\) 2 was originally prepared by condensing the commercially available 4-hydroxycoumarin 1 with 2,4-bis-(4-methoxyphenyl)-1,3,2,4-dithiaprophetane-2,4-disulfide, known as Lawesson’s reagent (LR), refluxing in anhydrous toluene for 4 hours under nitrogen. It must be noted that compound 2 possesses several reactive sites which may be employed as a simple synthetic intermediate in routes to many heterocycles, but this work investigates their behavior towards some nitrogen binucleophiles.

The reaction as shown in Scheme 1, was performed by allowing compound 2 to react with various o-phenylenediamine\(^10\) in refluxing butanol for 8 hours. The progress of the reaction was followed by thin-layer chromatography analysis, which revealed the formation of a new spot featuring a characteristic blue fluorescence.

**Scheme 1.** Synthetic pathway of substituted 2-(2-hydroxyphenyl)benzimidazole 6 a-d

**Table 1.** Synthesis of 2-(2-hydroxyphenyl)benzimidazoles 6 a-d with substituted o-phenylenediamines in butanol

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>Yield(^a) (%)</th>
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<tbody>
<tr>
<td>6a</td>
<td>H</td>
<td>60</td>
</tr>
<tr>
<td>6b</td>
<td>CH(_3)</td>
<td>50</td>
</tr>
<tr>
<td>6c</td>
<td>Cl</td>
<td>30</td>
</tr>
<tr>
<td>6d</td>
<td>NO(_2)</td>
<td>33</td>
</tr>
</tbody>
</table>

\(^a\) Yields correspond to isolated chromatographed products.

The reaction afforded exclusively the expected 2-(2-hydroxyphenyl)benzimidazoles 6 in good yield (Table 1), and their structure was elucidated by IR, mass spectrometry and NMR spectroscopy (1D and 2D).

Their IR spectra revealed the disappearance of the SH absorption band around 2362 cm\(^{-1}\) and also of the C=S band around 1155 cm\(^{-1}\). New stretching vibration bands appeared at 3328 cm\(^{-1}\) and at 3448 cm\(^{-1}\) corresponding to the N-H and O-H bonds respectively. In the mass spectrum (ESI\(^+\)) of compound 6a, the molecular ion peak appeared at \(m/z\) 211.

Inspection of the \(^1\)H NMR spectrum (600 MHz, DMSO-d\(_6\) with a few drops of TFA-d) of 6a showed the presence of two doublets relative to the proton resonances H-6' and H-5' at 8.10 ppm and 7.26 ppm, respectively. Moreover, among the set of aromatic signals of the
proton resonances H-5,6 (m, 7.50-7.55 ppm) and H-7,4 (m, 7.85-7.90 ppm), the particularly deshielded signal of the proton resonances H-4' (m, 7.56-7.60 ppm) and H-3' at 7.14 ppm was easily located.

In addition, a whole set of linkages confirming the molecular structure of compounds 6 was deduced from the HMBC spectrum. Thus, we detected correlations between the aromatic proton H-6' and carbons atoms C-2' (157.2 ppm), C-2 (146.7 ppm) and C-4' (135.1 ppm). H-3’ also correlates with the quaternary carbon C-1’ (109.1 ppm). By contrast, we found that the proton H-7 correlates simultaneously with C-7 (114.1 ppm), C-7a (131.2 ppm) and C-3a (131.2 ppm). Thus, the use of 2D NMR technique permits unambiguous and complete assignment for protons and carbons of the compounds 6.

A plausible mechanism explaining the formation of 2-(2-hydroxyphenyl)benzimidazole 6 are shown in Scheme 2. One involves the intermediate 3. This mechanism results in the non-isolable 4-(2-hydroxyphenyl)-1,5-benzodiazepine-2-thione 4 intermediate, develops by protonation and consequent aromatization to the intermediate 5, which in turn undergoes an intramolecular cyclization releasing a C2H2S molecule.

Scheme 2. Mechanism of action of o-phenylenediamines on 4-mercapto-2H-chromene-2-thione

The 2-(2-hydroxyphenyl)benzimidazoles are an important class of heterocyclic compounds that exhibit a range of pharmaceutical and biological activities\textsuperscript{12}. In order to contribute to their exploration, we used them in the present study as precursors for the synthesis of bioactive P-S heterocycle systems\textsuperscript{13}. Compounds 6a-d were converted into new fused phosphorus-heterocycles 7a-d (Scheme 3) by treatment for 10 hours under dry nitrogen with one equivalent of LR\textsuperscript{20} in anhydrous toluene at 100°C.
Scheme 3. Synthetic pathway of substituted $6H$-benzimidazol[1,2-c][1,3,2]benzoxazaphosphorine 2-(4-methoxyphenyl)-2-sulfide 7a-d

Table 2. Synthesis of $6H$-benzimidazol[1,2-c][1,3,2]benzoxazaphosphorine 2-(4-methoxyphenyl)-2-sulfide 7

<table>
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<tr>
<th>Entry</th>
<th>R</th>
<th>Yield$^a$ (%)</th>
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<tbody>
<tr>
<td>7a</td>
<td>H</td>
<td>61</td>
</tr>
<tr>
<td>7b</td>
<td>CH₃</td>
<td>50</td>
</tr>
<tr>
<td>7c</td>
<td>Cl</td>
<td>45</td>
</tr>
<tr>
<td>7d</td>
<td>NO₂</td>
<td>25</td>
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</table>

$^a$ Yields correspond to isolated chromatographed products.

The structure of the isolated products 7a-d (table 2) was in agreement with their molecular weight determined by mass spectrometry (MS) and with their spectroscopic properties. Thus, the $^{31}$P NMR signal (in CDCl₃, 120 MHz) appeared at 68.06 ppm in concordance with the phosphorous oxidation state in the 2-thio-benzoxazaphosphine moiety. The IR spectrum (in KBr) of compound 7a showed the presence of an absorption band at 645 cm$^{-1}$ corresponding to (P=S) bond.

The $^1$H NMR spectrum of 7a (in CDCl₃, 300 MHz) indicates the presence of a singlet at 3.83 ppm corresponding to methoxy protons. In addition, the AA’BB’ parasubstituted system appeared as two sets of doublet of doublets at 7.37 (dd, $J = 9.9$ Hz, $J = 5.1$ Hz, 2H, H-3’, 5’) and 7.85 (dd, $J = 15.3$ Hz, $J = 8.7$ Hz, 2H, H-2’, 6’), whereas the other aromatic protons appeared as a multiplet at ($\delta$: 6.93-8.44), compared with those of the starting compound 6 ($\delta$: 7.14-7.87), owing to the deshielding effect of the 2-thio-benzoxazaphosphinine ring.

Observation of the $^{13}$C NMR spectrum revealed duplication of signals relative to the aromatic carbons (C1’, C2’,6’ and C3’,5’) and even of the quaternary carbons (C3a, C7a, C8a, C8b and C12a), indicating their non-equivalence, by coupling with phosphorus in the six-membered chair conformation of the 2-thio-benzoxazaphosphinine system. Thus the combined spectral data conclusively agree with the proposed structures.
Biological properties

Acetylcholinesterase-inhibition. It is explained in the literature that the toxicity of organophosphorus esters to animals is attributed to their ability to inhibit acetylcholinesterase (AChE, choline hydrolase), which is a class of enzymes that catalyze the hydrolysis of the neurotransmitting agent acetylcholine (ACh). The inhibition of AChE has been clearly demonstrated to be the result of an actual chemical reaction between the enzyme and the organophosphate. The phosphorylated enzyme is no longer capable of effecting the hydrolysis of ACh; this results in a build-up of the neurotransmitter at a nerve synapse, so the concentration of the ACh in the synaptic junction remains high, and continuous stimulation of the nerve fiber occurs\(^1\).

The inhibition of AChE by an organophosphorus ester takes place via a chemical reaction in which the serine hydroxyl moiety in the enzyme active site is phosphorylated in a manner analogous to the acetylation of AChE. In contrast to the acetylated enzyme, which rapidly breaks down to give acetic acid and the regenerated enzyme, the phosphorylated enzyme is highly stable, and in some cases, depending on the groups attached to the phosphorus atom\(^2\), the serine hydroxyl group, blocked by the phosphorylated moiety, is no longer able to participate in the hydrolysis of ACh. With our phosphorylated compounds 7a-d, the inhibition reaction may take place as indicated by the following equation (Scheme 4). In this equation, En-OH represents AChE, in which the serine hydroxyl moiety (-OH) is emphasized.

\[
\text{HN-CHC-NH} + \text{R} \rightarrow \text{HN-CHC-NH} + \text{R}
\]

Scheme 4. Blocking-up reaction of serine hydroxyl by the phosphorylated moiety

The anti-AChE activity of compounds 7a-d was evaluated by the colorimetric method\(^3\). The results indicated in Table 3 showed that all the tested heterocycles gave significant activity, which is explained as shown in Scheme 4 by the presence of the benzoxazaphosphorine sulfide moiety. Compared to those given in the literature as malathion\(^4\), we can say that the prepared derivatives are considered good acetylcholinesterase inhibitors. These results showed that compound 7d with the NO\(_2\) group was the more active (IC\(_{50}\) = 10µg/mL). This activity decreases in the same direction as the attractive effect of the groups (NO\(_2\), Cl, H and CH\(_3\)) fixed at C6 of the benzimidazole system.

This study discloses a part of the structure activity relationship of 6H-benzimidazo[1,2-c][1,3,2]benzoxazaphosphorine 2-(4-methoxyphenyl)-2-sulfide derivatives 7a-d toward AChE and will be of value for their development to treat certain pathologies, such as Alzheimer’s disease.
Table 3. Acetylcholinesterase inhibition capacity, represented by IC$_{50}$ (µg/ mL), of 6H-benzimidazo[1,2-c][1,3,2]benzoxazaphosphorine 2-(4-methoxyphenyl)-2-sulfide derivatives 7a-d and standard compound (malathion)

<table>
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<th>Entry</th>
<th>R</th>
<th>Acetylcholinesterase inhibition capacity, IC$_{50}$ (µg/mL)</th>
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<tbody>
<tr>
<td>7a</td>
<td>H</td>
<td>12±0.01</td>
</tr>
<tr>
<td>7b</td>
<td>CH$_3$</td>
<td>18±0.02</td>
</tr>
<tr>
<td>7c</td>
<td>Cl</td>
<td>14±0.02</td>
</tr>
<tr>
<td>7d</td>
<td>NO$_2$</td>
<td>10±0.1</td>
</tr>
<tr>
<td>Malathion$^{19}$</td>
<td></td>
<td>49.5 ±0.01</td>
</tr>
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</table>

$^a$ Averages ± SD were obtained from three different experiments.

IC$_{50}$ values represent the concentration of inhibitor required to decrease enzyme activity by 50% and are the mean of two independent measurements, each performed in triplicate.

Conclusion

Thus we have demonstrated that the 4-mercapto-2H-chromene-2-thione can be a convenient precursor for the preparation of 6H-benzimidazo[1,2-c][1,3,2]benzoxazaphosphorine 2-(4-methoxyphenyl)-2-sulfide, although some yields need to be optimized. We believe that the reported method offers a simple route for the preparation of these derivatives. The anti-acetylcholinesterase activity of some of the newly synthesized compounds was evaluated and seems to be significant.

Experimental

Melting points were taken on a Büchi-510 capillary melting point apparatus. IR spectra were recorded with a Perkin-Elmer Spectrum BX FT-IR apparatus, as films on KBr. $^{1}$H and $^{13}$C spectra were recorded with AC-300 AMX-400 and AVANCE DRX-600 Bruker spectrometers. HRMS spectra were either performed on a PerSeptive Voyager DE STR MALDI TOF mass spectrometer (AB Sciex) or on an ESI-TOF (LCT, Waters) using the reflectron mode in both cases. For MALDI experiments, 2,5-dihydroxybenzoic acid was used as a matrix and spectra were internally calibrated using matrix peaks. For ESI experiments, leucine-enkephaline peptide was employed as the LockSpray lockmass. Commercial TLC plates (Silica gel 60, F254, SDS) were used to monitor the progress of the reaction. Column chromatography was performed with silica gel 60 (particle size 40-63 µm, SDS). The starting materials 2 were prepared according to the literature$^9$. Almost all the products described here showed a very low degree of solubility on recrystallization in most solvents.

Synthesis of 4-mercapto-2H-chromene-2-thione (2)

4-Hydroxycoumarin 1 (1.62 g, 1 mmol) and Lawesson’s reagent (4.04 g, 1 mmol) in anhydrous toluene (90 mL) were heated under reflux for 4 hours. The dark brown solution was evaporated under reduced pressure to give a dark red solid, which was filtered, washed with ethanol and dried to yield pure 2.

(2) Yield: 77.2%; mp 217 °C.

IR (KBr): 2362, 1155 cm$^{-1}$. $^{1}$H NMR (300 MHz, CDCl$_3$) δ (ppm): 7.16 (s, 1H, H-3), 7.37 (t, J = 7.5 Hz, 1H, H-6), 7.52 (d, J = 8.4 Hz, 1H, H-5), 7.69 (t, J = 8.4 Hz, 1H, H-7), 7.79 (d, J = 7.8 Hz, 1H, H-8). $^{13}$C NMR (75 MHz, CDCl$_3$) δ 117.5 (C3), 119.1 (C8b), 124.8 (C7), 125.9
ESI-HRMS: \( m/z \ [M - H]^+ \) calcd for (C\(_9\)H\(_2\)O\(_3\))\(^+\): 192.9782; found: 192.9777.

**General procedure for the synthesis of 2-(2-hydroxyphenyl)benzimidazole derivatives 6a-d**

An equimolar amount of substituted \( o \)-phenylenediamines was added to a solution of compound 2 (500 mg, 0.0025 mmol) in n-BuOH (30 mL), and the mixture was refluxed for 8 hours. The residue obtained after removing the solvent *in vacuo*, was chromatographed on silica gel, employing CH\(_2\)Cl\(_2\) as eluent. Crystallization from CH\(_2\)Cl\(_2\) give 6a-d.

**2-(2-hydroxyphenyl)benzimidazole (6a):** Yield: 60%; mp 239-240°C.

IR (KBr): 3448-3328, 1630, 1457, 754 cm\(^{-1}\). \(^1\)H NMR (600 MHz, DMSO-d\(_6\)) \( \delta \) (ppm): 7.11-7.15 (m, 1H, H-3'), 7.26 (d, \( J = 6.7 \) Hz, 1H, H-5'), 7.50-7.55 (m, 2H, H-5, 6), 7.56-7.60 (m, 1H, H-4'), 7.85-7.90 (m, 2H, H-7, 4), 8.10 (d, \( J = 6.7 \) Hz, 1H, H-6'), 14.50 (s, 2H, OH, NH). \(^{13}\)C NMR (150 MHz, DMSO-d\(_6\)) \( \delta \) 109.1 (C1'), 111.4 (C7, C4), 117.3 (C3'), 120.1 (C5'), 125.8 (C6 C5), 129.3 (C6'), 131.2 (C3a, 7a), 135.1 (C4'), 146.7 (C2), 157.2 (C2'). ESI-HRMS: \( m/z \ [M + H]^+ \) calcd for (C\(_{13}\)H\(_{11}\)N\(_2\)O\(_3\))^+ : 211.0871; found: 211.0878.

**6-Methyl-2-(2-hydroxyphenyl)benzimidazole (6b):** Yield: 50%; mp 240-242°C.

IR (KBr): 3448-3235, 1600, 1458, 747 cm\(^{-1}\). \(^1\)H NMR (300 MHz, DMSO-d\(_6\)) \( \delta \) (ppm): 2.43 (s, 3H, CH\(_3\)), 6.96-7.03 (m, 2H, H-3', 5'), 7.08 (d, \( J = 8.1 \) Hz, 1H, H-5), 7.32-7.37 (m, 1H, H-4'), 7.42 (s, 1H, H-7), 7.52 (d, \( J = 6 \) Hz, 1H, H-4), 8.02 (d, \( J = 7.8 \) Hz, 1H, H-6'), 13.02 (s, 2H, OH, NH). \(^{13}\)C NMR (75MHz, DMSO-d\(_6\)) \( \delta \) 21.7 (CH\(_3\)), 110.1 (C1'), 113.2 (C7, C4), 117.6 (C3'), 119.5 (C5'), 125 (C5), 126.5 (C6'), 132 (C4'), 132.7 (C6), 135.9 (C3a), 138.8 (C7a), 151.9 (C2), 158.4 (C2'). ESI-HRMS: \( m/z \ [M + H]^+ \) calcd for (C\(_{14}\)H\(_{13}\)N\(_2\)O\(_3\))^+ : 225.1028; found: 225.1020.

**6-Chloro-2-(2-hydroxyphenyl)benzimidazole (6c):** Yield: 30%; mp 279-281°C.

IR (KBr): 3447-3329, 1617, 1466, 807 cm\(^{-1}\). \(^1\)H NMR (300 MHz, DMSO-d\(_6\)) \( \delta \) (ppm): 7.0-7.06 (m, 2H, H-3', 5'), 7.30 (d, \( J = 8.4 \) Hz, 1H, H-4), 7.37-7.42 (m, 1H, H-4'), 7.67 (d, \( J = 8.7 \) Hz, 1H, H-5), 7.72 (s, 1H, H-7), 8.05 (d, \( J = 7.2 \) Hz, 1H, H-6'), 13.01 (s, 2H, OH, NH). \(^{13}\)C NMR (75MHz, DMSO-d\(_6\)) \( \delta \) 110.1 (C1'), 111 (C7), 112.8 (C4), 117.5 (C3'), 119.6 (C5'), 123.3 (C5), 126.9 (C6'), 127.4 (C6), 132.4 (C4'), 137 (C3a), 140.3 (C7a), 153.1 (C2), 158.1 (C2'). ESI-HRMS: \( m/z \ [M + H]^+ \) calcd for (C\(_{14}\)H\(_{13}\)N\(_2\)O\(_3\))\(^+\): 245.0482; found: 245.0481.

**6-Nitro-2-(2-hydroxyphenyl)benzimidazole (6d):** Yield: 33%; mp 298-299°C.

IR (KBr): 3481-3371, 1615, 1490, 1458 cm\(^{-1}\). \(^1\)H NMR (300 MHz, DMSO-d\(_6\)) \( \delta \) (ppm): 6.96-7.06 (m, 2H, H-3', 5'), 7.61-7.64 (m, 1H, H-5), 7.77-7.80 (m, 1H, H-4'), 8.21 (d, \( J = 7.2 \) Hz, 1H, H-6'), 8.35 (s, 1H, H-7), 13.01 (s, 2H, OH, NH). \(^{13}\)C NMR (75MHz, DMSO-d\(_6\)) \( \delta \) 110 (C1'), 111.4 (C7), 115.3 (C4), 117.3 (C3'), 119.7 (C5'), 121.5 (C5), 126.9 (C6'), 128.9 (C6), 132.5 (C4'), 137 (C3a), 143.1 (C7a), 149.3 (C2), 158.1 (C2'). MALDI-HRMS: \( m/z \ [M - H]^- \) calcd for (C\(_{13}\)H\(_{8}\)N\(_2\)O\(_3\))\(^-\): 256.0600; found: 256.1200.

**General procedure for the synthesis of the fused phosphorus-heterocycles 7a-d**

A mixture of 6 (200 mg, 1 mmol) and Lawesson’s reagent (1 eq, 1 mmol) in anhydrous toluene (30 mL) was heated under dry nitrogen for 10 hours. The solvent was removed in vacuo, and the residue was chromatographed on silica gel. Elution with chloroform gave a white solid. Crystallization from (CHCl\(_3\)-petroleum-ether, 2:8) furnished pure 7a-d.
6H-benzimidazol[1,2-c][1,3,2]benzoxazaphosphorine 2-(4-methoxyphenyl)-2-sulfide (7a): Yield: 61%; mp 160 °C.
IR (KBr): 1237, 645 cm⁻¹. ¹H NMR (300 MHz, CDCl₃) δ (ppm): 3.83 (s, 3H, OCH₃), 6.93-6.97 (m, 2H, H-12, 10), 7.18-7.27 (m, 3H, H-5, 7), 7.27-7.32 (m, 1H, H-6), 7.37 (dd, J= 9.9 Hz, J= 5.1 Hz, 2H, H-3', 5'), 7.52 (td, J= 7.8 Hz, J= 1.5 Hz, 1H, H-11), 7.80-7.83 (m, 1H, H-4), 7.85 (dd, J= 15.3 Hz, J= 8.7 Hz, 2H, H-2', 6'), 8.44 (dd, J= 7.8 Hz, J= 1.8 Hz, 1H, H-9). ³¹C NMR (75MHz, CDCl₃) δ 55.6 (OCH₃), 112.8 (C4), 114.4-114.6 (d, C3', 5', J_C,P= 17.2 Hz), 115.9 (d, C8b, J_C,P= 4.8 Hz), 119.5-119.6 (d, C1, J_C,P= 7.8 Hz), 120 (C12), 124.5-124.6 (d, C6, 5), 125.4 (C10), 127 (C9), 132.8 (C11), 133.2-133.3 (d, C7a, J_C,P= 6.5 Hz), 134-134.2 (d, C2', 6', J_C,P= 15.5 Hz), 144.5-144.7 (d, C3a, J_C,P= 11.4 Hz), 148.2 (C4'), 149.5-149.6 (d, C8a, J_C,P= 11.2 Hz), 164.3 (d, C12a, J_C,P= 3.2 Hz). ³¹P NMR (120 MHz, CDCl₃) δp 68.06 ppm. ESI-HRMS: m/z [M + H]⁺ calcd for (C₂₀H₁₆N₂O₂PScI)⁺: 379.0670; found: 379.0682.

6-Methyl-6H-benzimidazol[1,2-c][1,3,2]benzoxazaphosphorine 2-(4-methoxyphenyl)-2-sulfide (7b): Yield: 50%; mp 150.3°C.
IR (KBr): 1253, 689 cm⁻¹. ¹H NMR (300 MHz, CDCl₃) δ (ppm): 2.25 (s, 3H, CH₃), 3.77 (s, 3H, OCH₃), 6.72-7.41 (m, 9H, H_arom), 7.95-8.03 (m, 2H, H_arom). ¹³C NMR (75MHz, CDCl₃) δ 23 (CH₃), 55.7 (OCH₃), 113.8-113.9 (d, C3', 5', J_C,P= 17.3 Hz), 114.7 (C4), 115.1 (d, C8b, J_C,P= 4.8 Hz), 119.9-119.8 (d, C1, J_C,P= 8 Hz), 120.1 (C12), 125.6 (C10), 127.8 (C5), 129.6 (C9), 130.2 (C11), 132-132.4 (d, C2', 6', J_C,P= 15.4 Hz), 132.9 (C6), 134-134.1 (d, C3a, J_C,P= 10.1 Hz), 136.5-136.7 (d, C7a, J_C,P= 6.6 Hz), 145.7 (C4'), 151-151.1 (d, C8a, J_C,P= 11.2 Hz), 162.4-162.3 (d, C12a, J_C,P= 3.15 Hz). ³¹P NMR (120 MHz, CDCl₃) δp 73.95 ppm. ESI-HRMS: m/z [M + H + H₂O]⁺ calcd for (C₂₁H₂₂N₂O₃PScI)⁺: 411.0932; found: 411.0935.

6-Chloro-6H-benzimidazol[1,2-c][1,3,2]benzoxazaphosphorine 2-(4-methoxyphenyl)-2-sulfide (7c): Yield: 45%; mp 153.7°C.
IR (KBr): 1247, 714 cm⁻¹. ¹H NMR (300 MHz, CDCl₃) δ (ppm): 3.78 (s, 3H, OCH₃), 6.88-6.93 (m, 2H, H-12, 10), 7.18-7.27 (dd, J= 8.7 Hz, J= 1.8 Hz, 1H, H-5), 7.23 (dd, J= 8.4 Hz, J= 5.1 Hz, 2H, H-3', 5'), 7.29-7.34 (m, 1H, H-4), 7.47 (td, J= 6.9 Hz, J= 1.2 Hz, 1H, H-11), 7.71 (s, 1H, H-7), 7.77 (dd, J= 15.3 Hz, J= 8.7 Hz,2H, H-2', 6'), 8.36 (dd, J= 7.8 Hz, J= 1.5 Hz, 1H, H-9). ¹³C NMR (75MHz, CDCl₃) δ 55.5 (OCH₃), 113.4 (C4), 114.4-114.6 (d, C3', 5', J_C,P= 17.3 Hz), 115.6 (d, C8b, J_C,P= 4.8 Hz), 119.4-119.6 (d, C1, J_C,P= 8 Hz), 119.8 (C12), 124.8 (C5), 125.4 (C10), 127.1 (C9), 130.2 (C6), 131.8-131.9 (d, C7a, J_C,P= 6.6 Hz), 133 (C11), 133.8-134 (d, C2', 6', J_C,P= 15.4 Hz), 145.5-145.6 (d, C3a, J_C,P= 11.3 Hz), 149.4 (C4'), 149.5-149.6 (d, C8a, J_C,P= 11.4 Hz), 164.4-164.3 (d, C12a, J_C,P= 3.2 Hz). ³¹P NMR (120 MHz, CDCl₃) δp 67 ppm. ESI-HRMS: m/z [M + H]⁺ calcd for (C₂₁H₁₉N₂O₂PScI)⁺: 413.0280; found: 413.0285.

6-Nitro-6H-benzimidazol[1,2-c][1,3,2]benzoxazaphosphorine 2-(4-methoxyphenyl)-2-sulfide (7d): Yield: 25%; mp 154 °C.
IR (KBr): 1260, 687 cm⁻¹. ¹H NMR (300 MHz, CDCl₃) δ (ppm): 3.98 (s, 3H, OCH₃), 7.05-7.09 (m, 2H, H-12, 10), 7.14-7.17 (m, 1H, H-5), 7.21 (dd, J= 8.4 Hz, J= 5.4 Hz, 2H, H-3', 5'), 7.54-7.59 (m, 1H, H-4), 7.66-7.70 (m, 1H, H-11), 7.80 (s, 1H, H-7), 7.92 (dd, J= 12.6 Hz, J= 9 Hz, 2H, H-2', 6'), 8.28 (dd, J= 9 Hz, J= 2.1 Hz,1H, H-9). ¹³C NMR (75MHz, CDCl₃) δ 55.8 (OCH₃), 113.4 (C4), 114.3-114.6 (d, J_C,P= 17.3 Hz, C3', 5'), 118.4 (d, J_C,P= 4.8 Hz, C8b), 121.4-121.6 (d, J_C,P= 8 Hz, C1'), 122 (C12), 124.8 (C5), 125.4 (C10), 128.9 (C9), 130.2 (C11), 132.5-133 (d, J_C,P= 15.4 Hz, C2', 6'), 139.8-139.9 (d, J_C,P= 6.6 Hz, C7a), 142 (C6), 144.5-144.6 (d, J_C,P= 11.3 Hz, C3a), 149.4 (C4'), 149.5-149.6 (d, J_C,P= 11.4 Hz,
C8a), 164.4-164.3 (d, J_C,C= 3.2 Hz, C12a). \(^{31}\)P NMR (120 MHz, CDCl\(_3\)) \(\delta\) 71 ppm. ESI-HRMS: \(m/z\) [M + H + H\(_2\)O]\(^+\) calcd for (C\(_{20}\)H\(_{17}\)N\(_3\)O\(_3\)PS)\(^+\): 442.0627; found: 442.0623.

**Biological methods:**

**Acetylcholinesterase inhibition**

Inhibition of AChE by plant extracts was evaluated as described by Ellman et al. (1961)\(^{18}\) with some modifications as detailed by Moyo et al. (2010)\(^{20}\). The assay is based on the spectrophotometric measurement of the increase in yellow color produced by thiocholine when it reacts with the dithiobisnitrobenzoate ion. The increase in absorbance value due to the spontaneous hydrolysis of the substrate was corrected by subtracting the rate of the reaction before adding the enzyme from the rate after the enzyme addition. The percentage inhibition of the best samples was calculated using the formula:

\[
\text{Inhibition} \ (%) = (1 - \text{Sample reaction rate/Blank reaction rate}) \times 100
\]

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