Synthesis and biological activity of functionalized phosphorus derivatives of isatin imines

Emtithal Ahmed El-Sawi*, Tahia Bayoumy Mostafa and Hyam Ali Radwan

Chemistry Department, Faculty of Girls for Arts, Science and Education, Ain Shams University, Helipolis, Cairo, 11757, Egypt

*Corresponding author at: Chemistry Department, Faculty of Girls for Arts, Science and Education, Ain Shams University, Helipolis, Cairo, 11757, Egypt.
Tel.: +2.0224196561; fax: +2.0224157895; E-mail address: elsawi_e@yahoo.com (E.A. El-Sawi).

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ABSTRACT
Isatin-3-imine derivatives (1a-d) have been synthesized. These compounds were then converted into phosphorylated products 2a 2c with triethylphosphate and 3a-d with triphenylphosphate. The structures of the new compounds were confirmed by elemental analyses, IR, UV/VIS, 1H NMR, 13C NMR and MS studies. The structure of compound 1a was also confirmed by single crystal X-ray diffraction studies. Compounds 1b-d and 3c-d exhibited potent antibacterial activity against Bacillus subtilis and Escherichia Coli. Compound 3a was found to exhibit antifungal activity against the tested organisms.

1. Introduction
The chemistry of isatin and its derivatives is particularly interesting because of their potential applications in medicinal chemistry. The synthetic versatility of isatin has led to the extensive use in organic synthesis. 3-Imino-2-amino-isatins were obtained by a one-pot reaction of an excess of aniline (or its derivatives) with 1,2-bis(dimethylamino)-1,2-dichloro-ethene followed by hydrolysis to yield the corresponding isatin derivatives [1]. The construction of spiroxindole via imino Diels-Alder reaction of in-situ generated isatin imine with dihydropyran [2].

Isatins are known to exhibit variety of biological and pharmaceutical properties [3-5]. Schiff and Mannich bases of isatin derivatives are also reported to show a variety of biological activities [6-11]. Some isatin Schiff bases derivatives have been synthesized and realized as anti-HTV activity [12]. Some organometallic Schiff bases containing Ni(II), Co(II) and Cu(II), were found to be killing agents for Biomphalaria Alexandrina snail without affecting the surrounding environment [13]. Other synthesized Schiff bases from the reaction of isatin with primary aromatic amines were randomly screened for their in vitro anti-leishmanial potential [14]. 1-(Substituted phenylamimethyl)-3-(coumarin-3-yl-carboxyhydrate) isatins were synthesized and exhibited potential anti-covulants [15]. The isatin core structure was found to be a novel chemical scaffold in trans-thyretin fibrillogenesis inhibitor design [16].

Organophosphorus derivatives containing isatin-3-hydrazones were detected as chemotherapeutants against fungal pathogens of sugarcane [17]. Isatin derivatives bearing 1,2,4-triazole ring were also synthesized [18], where N-bridged heterocyclic derivatives derived from 1,2,4-triazoles showed varied biological activities such as: antimicrobial [19], anticonvulsant [20], anticancer [21], analgesic [22], anti-HIV [23], and anti-inflammatory [24]. These biological and the other data prompted us to synthesize new isatin derivatives with potential of interesting biological activities.

2. Experimental

2.1. Instrumentation

Melting points were determined with Gallen Kamp melting point apparatus and were not corrected.

Elemental analyses were performed by the Microanalytical Center, Cairo University, Giza.

FT-IR spectra were recorded on Mattson 1000 spectrophotometer, Microanalytical Center, Cairo University, Giza.

UV/VIS spectra were recorded using Shimadzu UV 1601 Spectrophotometer.

Mass spectra were measured on GCMS-QP 1000 EX Gas Chromatography-Mass spectrometer, Cairo University, Giza and on Gas chromatography-Mass spectrometer in National Research Center, Egypt.

1H NMR spectra were recorded on Gemini 200, spectrometer in DMSO-d6 solution with TMS as internal standard in Cairo University, Giza.

X-ray single crystal diffraction studies were performed in National Research Center, Egypt. A suitable crystal (size 0.50 × 0.50 × 0.10 mm) was selected from batch of crystals of compound 1a obtained by crystallization from alcohol. All diagrams and calculations were performed using maxus (Bruker Nonius, Delft & MacScience, Japan), using graphite monochromated MoKα, radiation (λ=0.71073 Å).

Biological activity was performed in Micro Analytical Center, Cairo University, Giza, Egypt.
2.2. Synthesis

2.2.1. Compounds 1a-d

A mixture of (0.001 mol, 0.166 g) of isatin and amine derivatives namely 2,6-disopropylaniline, 4-aminosalicylic acid, 4-aminodiphenylamine and 4-amino-N-[5-methoxy-2-pyrimidinyl] benzene sulphonamide in ethyl alcohol (20 mL) was refluxed for 3h. The reaction mixture was cooled and the resulting precipitate was filtered, dried and crystallized from the proper solvent (Scheme 1).

(Z)-3-((2, 6-disopropylphenyl)imino)indolin-2-one (1a): Orange crystals from ethanol in 86% yield. M.p.: 264-265 °C. IR (KBr, νmax, cm⁻¹): 3163 (N-H), 2962-2866 (C-H aliph.), 1733 (C=O), 1662 (C=N). UV/Vis (λmax nm, ε (L/mol/cm)): 459, 11.29x10³, 7.2x10³; 298, 9.6x10³; 314, 10.5x10³; 415, 1.8x10³. 1H NMR (DMSO-d₆, δ, ppm): 8.00 (s, 2H, 2NHCO), 7.6-6.5 (m, 13H, Arom.), 3.6 (s, 1H, NH). Anal. calcd. for C₁₅H₁₀N₂O₄: C, 76.67; H, 4.79; N, 13.41. Found: C, 76.62; H, 4.79; N, 13.20%.

(Z)-N-[5-methoxypyrimidin-2-yl]-4-((2-oxoindolin-3-ylidene)amino)benzenesulphonamide (1d): Orange crystals from ethanol in 64% yield. M.p.: 188-189 °C. IR (KBr, νmax, cm⁻¹): 3163 (N-H), 2962-2866 (C-H aliph.), 1733 (C=O), 1647 (C=N). UV/Vis (λmax nm, ε (L/mol/cm)): 407, 2.7x10³. 1H NMR (DMSO-d₆, δ, ppm): 8.2 (s, 1H, NH), 8.0-7.2 (m, 10H, Arom.), 3.9 (s, 1H, SO₂NH), 3.6 (s, 3H, OCH₃). Anal. calcd. for C₁₅H₁₀N₂O₄S: C, 55.74; H, 3.66; N, 17.11. Found: C, 55.69; H, 3.54; N, 17.23%.

2.2.2. Compounds 2a, c

A mixture of (0.001 mol, 0.166 g) triethylphosphite and 3-(2, 6-disopropylphenyl-imino)indolin-2-one (1a) or 3-(4-(phenylamino)phenyl-imino)indolin-2-one (1c) in 10 mL THF was stirred for 3 hours at room temperature, then left to stand overnight. The resulting precipitate was filtered and crystallized from petroleum ether (40-60 °C). It gave one spot (on TLC Scheme 2).

Scheme 2: Black crystals from THF in 89.47% yield. M.p.: 270-271 °C. IR (KBr, νmax, cm⁻¹): 1758, 1734 (C=O), 1032 (P=O). 1H NMR (DMSO, δ, ppm): 8.00 (s, 2H, 2NHCO), 7.6-6.5 (m, 14H, Arom.), 3.6 (q, 6H, 3CH₃), 3.2 (m, 4H, 4CH for isopropyl), 1.3 (d, 2H, 8 CH₃), 1.0 (t, 9H, 3(OCH₃CH₃)). 13C NMR (DMSO, δ, ppm): 163 (C=O), 155.9-111.5 (aromatic carbons), 40.33-22.81 (aliphatic carbons). Anal. calcd. for C₂₅H₂₅N₃O₆P: C, 70.95; H, 7.58; N, 7.19. Found: C, 70.51; H, 7.56; N, 7.12%.
**Compound 2c:** Deep violet crystals from THF in 77% yield, M.p.: 98-99 °C (IR (KBr, cm⁻¹): 1727 (C=O [NH-CO]), 1020 (P-O). The ¹H NMR (DMSO, δ, ppm): 8.1 (s, 1H, NH0), 7.6-6.2 (m, 13H, Arom.), 4.1 (s, 1H, NH). 1.5 (q, 6H, 3CH₃). 1.1 (t, 9H, 3 CH₃). Anal. calcd. for C₃₈H₃₇N₂O₆P: C, 80.28; H, 6.51; N, 4.92%. Found: C, 79.88; H, 6.46; N, 4.86%. 2.2.3. **Compounds 3a-d**

A mixture of (0.001 mol, 0.262 g) triphenylphosphine and imine derivatives namely (2, 6-dioisopropoxyphenyl-imino)indolin-2-one (1a), 2-hydroxy-4-(2-oxoindoylidene)benzoic acid (1b), 3-(4-(phenylaminophenyl-imino)indolin-2-one (1c) and N-[5-(methoxyxypirimidin-2-yl)-4-(2oxoindolin-3-ylideneamino)-benzenesulphonamide (1d) in 30 mL THF was stirred for 3 h at room temperature, then left to stand overnight. The resulting precipitate was filtered and crystallized from the proper solvent (Scheme 3).

**Compound 3a:** Yellow crystals from THF in 92% yield, M.p.: 190-191°C (IR (KBr, cm⁻¹): 1758, 1734 (C=O), 1432 (P=Ph) [25]). UV/Vis (λ max (nm), ε (L/mol/cm)): 403, 4.65x10⁴; 294, 10.5x10³. ¹H NMR (DMSO, δ, ppm): 11.01 (s, 1H, NH), 7.6-6.70 (m, 22H, Arom.), 2.67 (m, 2H, 2CH₂). 1.09 (d, 12H, 4CH₃). UQC NMR (DMSO, δ, ppm): 163 (30.05), 108 (100), 107 (98), 77 (55). Anal. calcd. for C₂₆H₃₀N₃O₄P: C, 65.13; H, 6.26; N, 8.76. Found: C, 65.20; H, 6.19; N, 8.64%. **Compound 3b:** Pale brown crystals from THF in 85% yield, M.p.: 111-112°C (IR (KBr, cm⁻¹): 1435 (P=Ph) [25]). UV/Vis (λ max (nm), ε (L/mol/cm)): 254, 8.4x10³; 298, 10.06x10³; 315, 10.9x10³. ¹H NMR (DMSO, δ, ppm): 11.2 (s, 1H, COOH), 10 (s, 1H, NH), 7.8-6.2 (m, 22 H, Arom.), 4.9 (s, 1H, NH), 7.8 (101), H, 4.58; N, 5.14. Found: C, 72.45; H, 4.56; N, 5.34%. **Compound 3c:** Black crystals from THF in 81.48% yield, M.p.: 156-157°C. IR (KBr, cm⁻¹): 1725 (C=O (NH-CO)), 1432 (P=Ph). UV/Vis (λ max (nm), ε (L/mol/cm)): 493, 6.36x10⁴; 309, 12x10³. ¹H NMR (DMSO, δ, ppm): 8.2 (s, 1H, NH0), 7.7-6.2 (m, 28H, Arom.) and 4.2 (s, 1H, NH). MS (m/z %): 512 (9), 360 (96), 283 (C₃H₅NO₂P), 100, 277 (6), 179 (34), 164 (50), 77 (13), 51 (4). Anal. calcd. for C₃₇H₃₇N₂O₆P: C, 79.30; H, 5.21; N, 7.30. Found: C, 79.20; H, 5.40; N, 7.25%. **Compound 3d:** Reddish brown crystals from THF in 50% yield, M.p.: 76-77°C (IR (KBr, cm⁻¹): 1725 (C=O (NH-CO)), 1431 (P=Ph). UV/Vis (λ max (nm), ε (L/mol/cm)): 395, 1.35x10⁴. ¹H NMR (DMSO, δ, ppm): 11.00 (s, 1H, (N=C-OH)), 8.00-6.57 (m, 25H, Arom.), 4.00 (s, 1H, SO₂NH), 3.77 (s, 3H, CH₃). ¹C NMR (DMSO, δ, ppm): 153 (C=O), 151 (-SO₂NH), 150 (-N=O), 149 (benzene-C-NH). 144 (pyrimidine-C-O); 138-128 (triphenyl phosphine), 125-112 (indolyl), 109 (indolyl C-P), 56 (aliphatic C-O). MS (m/z %): 479 (1.22), 279 (9), 278 (32), 277 (100), 262 (14), 201 (11), 147 (2), 118 (4), 77 (6). Anal. calcd. for C₁₅H₁₄N₃O₄P: C, 66.14; H, 4.47; N, 10.43. Found: C, 66.54; H, 4.45; N, 10.32%. **2.3. In vitro antimicrobial activity (Disc diffusion method)**

A filter paper sterilized disc saturated with measured quantity of the sample is placed on plate containing solid bacterial medium (nutrient agar broth) or fungal medium (Doxs medium) which has been heavily seeded with the spore suspension of the tested organism. After inoculation, the diameter of the clear zone of inhibition surrounding the sample is taken as a measure of the inhibitory power of the sample against the particular test organism [26-29].

3. Results and discussion

Isatin reacted with 2,6-dioisopropoxyaniline, 4-aminoalacetic acid, 4-amidophenylamine and 4-amino-N-[5-methoxy-2-pyrimidinyl] benzene sulphonamide in ethyl alcohol under reflux to afford 3-imine derivatives; 3-(2,6-dioisopropoxyphenyl imino)indolin-2-one (1a), 2-hydroxy-4-(2-oxoindolin-3-ylidene amino)benzoic acid (1b), 3-(4-(phenylaminophenyl) imino) indolin-2-one (1c) and N-[5-methoxyxypirimidin-2-yl)-4-(2oxoindolin-3-ylideneamino)-benzenesulphonamide (1d). The structures of these compounds were confirmed by elemental analyses, IR, UV/Vis spectra. All characterization data is given in experimental section.

In addition, the structure of the compound 1a is confirmed by X-ray single crystal diffraction studies. The perspective view of the molecular structure of compound 1a is shown in Figure 1. X-ray single crystal diffraction data for compound 1a: C₂₁₂H₁₇₂N₄O₄S; tetragonal; 4/1/a; unit cell dimensions a = 28.860(9) Å, b = 28.860(9) Å, c = 0.7587(5) Å; V = 7295.1(5) Å³; Z = 16; Dx = 1.116 Mg m⁻³; 1721 independent reflections; 0max = 19.57; 1158 observed reflections. Refinement method was full matrix least squares refinement, R(all)= 0.087, R(ge)=0.063, wR(geal)=0.128; wR(all)=0.130. wR(ge)=0.128, δ(ref)= 4.068, S(all)= 3.632, S(ge)= 4.067. Selected geometrical parameters are given in Table 1.
Table 1. Selected geometrical parameters (Å, °)

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<th>Bond distance, Å</th>
<th>Value</th>
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<tr>
<td>C1-C2</td>
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<tr>
<td>C1-N1</td>
<td>1.35(1)</td>
</tr>
<tr>
<td>C1-C2</td>
<td>1.411(3)</td>
</tr>
<tr>
<td>C2-C7</td>
<td>1.376(3)</td>
</tr>
<tr>
<td>C7-C8</td>
<td>1.461(3)</td>
</tr>
<tr>
<td>N2-C1</td>
<td>1.260(9)</td>
</tr>
<tr>
<td>N2-C9</td>
<td>1.434(3)</td>
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</table>

<table>
<thead>
<tr>
<th>Bond angles, °</th>
<th>Value</th>
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<tr>
<td>C1-N1-C2</td>
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</tr>
<tr>
<td>C8-N2-C9</td>
<td>121.1(2)</td>
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</table>

Table 2. Antibacterial and antifungal activities of compounds.

<table>
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<tr>
<th>Compound No</th>
<th>Inhibition zone diameter (mm/mm sample)</th>
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<tr>
<td></td>
<td>Bacillus Subtilis</td>
</tr>
<tr>
<td>1a</td>
<td>0 0 0</td>
</tr>
<tr>
<td>1b</td>
<td>14 14 0</td>
</tr>
<tr>
<td>1c</td>
<td>13 14 0</td>
</tr>
<tr>
<td>1d</td>
<td>15 13 0</td>
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<tr>
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<td>0 0 0</td>
</tr>
<tr>
<td>3c</td>
<td>14 14 0</td>
</tr>
<tr>
<td>3d</td>
<td>16 15 0</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>32 35 0</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>0 0</td>
</tr>
</tbody>
</table>

Triphenylphosphate reacted with 2-hydroxy-4-(2-oxoindolin-3-ylidene amino) benzoic acid (1b) in tetrahydrofuran to form five membered hetero ring of compound 3b. The IR spectrum showed the absence of one νC=O (NH-CO) and νC=O in the presence of a new absorption band at 1435 cm⁻¹ due to νP=Ph [25].

The reaction of 3-(4-(phenylamino)phenylamino)indolin-2-one (1c) with triethylphosphate and triphenylphosphate yielded compounds 2c and 3c, respectively, with three-membered hetero rings.

The reaction of N-(5-methoxypyrimidin-2-yl)-4-(2-oxoindolin-3-ylideneamino)-benzenesulphonamide (1d) with triphenylphosphate yielded compound 3d with three membered hetero ring. IR spectrum showed a new absorption band at 1431 cm⁻¹ due to νP=Ph. The presence of νC=O (NH-CO) at 1727 cm⁻¹ confirmed the three membered heterophosphorus form. The UV/Vis spectrum indicated λmax at 395 nm due to n→π transition.

3.2. In vitro antimicrobial activity

The synthesized compounds were screened for their antimicrobial activity against *Bacillus subtilis* (G) and *Escherichia Coli* (G). Control experiment was carried out under similar condition by using tetracycline as standard. The inhibition zone measure in mm showed that compounds 1a, 2a and 3a were inactive towards bacteria. The antifungal activity was tested against the fungal species *Aspergillus flavus* and *Candida albicans* at 100 µg concentration. Amphotericin B was used as standard under the same condition. The antifungal data revealed that the compounds 1a-d, 2a, 3c and 3d showed no effect towards fungus, while the compounds 3a were effective towards the above fungus (Table 2).

**Supplementary material**

CCDC-766320 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif or by e-mailing data_request@ccdc.cam.ac.uk or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44(0)1223-336033.

**References**