

In Vitro Bioactivities of Isoindolin-1-3-Phosphonate Compounds

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Abstract

In this work an efficient synthesis of isoindolin-1-one-3-phosphonates under catalyst- and solvent-free conditions was reported to afford the desired compounds in excellent yields with potent pharmacological properties. The synthetic method involves the preparation of isoindolin-1-one-3-phosphonates by a 'one-pot' three-component reaction of 2-formylbenzoic acid with primary amines and dimethyl phosphite under solvent and catalyst free-conditions. All new compounds were characterized by ^1H NMR, ^{13}C NMR, FT-IR and elemental analysis techniques. The synthesized compounds were screened for their antimicrobial activities against gram-positive bacterial strains (*Micrococcus luteus*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Bacillus cereus*), a gram-negative bacterial strain (*Salmonella typhimurium*) and a fungus (*Candida albicans*). Compound **4a** was found to be the most active against antimicrobial against *L. M. luteus*, *L. monocytogenes* and *C. albicans* with an inhibition zone of 35, 22 and 38 mm respectively. They were additionally also investigated for their anti-parasitical activities against *Leishmania major* promastigotes and amastigotes and *Toxoplasma gondii in vitro*. The compounds 4a,b are the most active against *L. major* amastigotes and promastigotes with $\text{EC}_{50} < 1 \mu\text{M}$. Cytotoxicity investigations of the isoindolin-1-one-3-phosphonates were conducted in two human cancer cell lines, *MDA-MB-231* and *MCF-7* all the compounds gave anticancer activity $< 1.5 \mu\text{M}$. We can conclude that 4a is a good drug candidate for all the biological assays, further studies for SAR detection and *in vivo* evaluation are highly recommended.

Introduction

Isoindolin-1-one moiety is an important scaffold that has attracted great attention in organic synthesis [1-4]. (Fig. 1). This motif is in reality found over regularly compounds, for example, magallanesine [1], lennoxamine [2], Also stachybotrin C [3-4]. (Fig. 1).

Compounds containing isoindolin-1-one moiety have different biological activities such as antimicrobial [5], anti-viral [6], HIV-1 inhibitory [7-8]. Some isoindolin-1-one compounds also have been reported to be effective for treating diabetes [9-10], cancer [11-13], and CNS diseases [14-18]. Moreover, the derivatives isoindoline especially those come from isoindolin-1-one reduction suggested for the inhibition of dipeptidyl peptidase DPP8/9 [19-20].

In non-symmetrical preparation of some compounds from isoindolin-1-one they can introduced as building blocks for a Diels–Alder reaction [21-25]. Because of their multi biological properties of isoindoline derivatives, there are many chemical pathways have been reported for the preparation of these heterocycles [26-35].

Reduction of phthalimides to 3-hydroxy-isoindolin-1-one followed by a reaction with trifluoroacetic anhydride and triethylphosphite afford the corresponding phosphonates in moderate yields [36-37]. The synthesis were carried out in toluene at reflux or under microwave irradiation [38-44].

As part of our interest to develop a practical and efficient synthesis method in an environmentally friendly manner [45-47], we herein report an alternative, simple route for the synthesis of isoindolin-1-one-3-phosphonates **4**. Furthermore, these compounds were investigated for their antimicrobial activity against *Micrococcus luteus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhimurium* and *Candida albicans* and for their anti-parasitical activity against *Leishmania major* and *Toxoplasma gondii*. Additionally, cytotoxicity were tested using MTT assay.

2. Results And Discussion

For the synthesis of target compounds **4**, we initially decided to explore the use of 2-formylbenzoic acid as starting material, considering its recent application in the synthesis of *N*-substituted isoindolin-1-one compounds [48-58]. The process involves a 'one-pot' three-component reaction of 2-formylbenzoic acid, a primary amine and dimethyl phosphite under solvent-free for 1h at 80°C that yields compounds **4a-f**.

Scheme 1

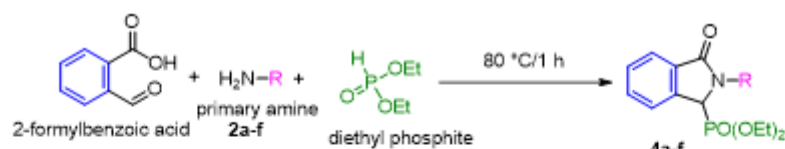


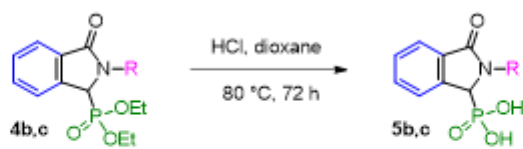
Table 1: Structure of Isoindolin-1-one-3-phosphonates **4a-f**

Entry	Structure	Compound	Yield (%)
1		4a	70
2		4b	72
3		4c	71
4		4d	84
5		4e	88
6		4f	78

Thus, the 'one-pot' three-component reaction of 2-formylbenzoic acid with a primary amine and diethylphosphite was stirred at 80 °C under solvent free-conditions, which generated the corresponding isoindolin-1-one-3- phosphonate **4a-f** in 70-88% yield after 1 h (Table 1). The scope and limitations of the reaction were studied by using various types of amines. Products starting from cyclohexylamine **2c** and (4-methoxyphenyl) methanamine **2d** were obtained in yields of 71 and 84%, respectively (Table 1, entries 3,4). The electron-donating substrates in the phenyl ring were found to promote the reaction. The different reactivities of butan-1-amine **2e** and pentan-1-amine **2f** can be explained similarly. Finally, the reaction was extended using phenylmethanamine **2b** which generated the corresponding products in 72 % yield (Table 1, entries 2,3).

Treatment of isoindolin-1-one-3-phosphonate **4b,c** with concentrated HCl in dioxane at 80 °C for 72 h led to production of isoindoline-1-one-3-yl phosphonic acid **5b,c** (Table 2).

Table 2: Isoindolin-1-one-3-yl-phosphonic acids 5b-c prepared



Entry	Structure	Compound	Yield (%)
1		5b	60
2		5c	68

The structures of the synthesized products were deduced by IR, ^1H , ^{13}C , and ^{31}P NMR spectroscopy and elemental analyses. The IR spectrum of compound **4b** exhibited strong absorption bands at 1681 (C=O), 1588 (C=C, acyclic), 1248 (P=O), and 1046 (P-O-C). In the ^1H -NMR spectrum of **4b**, the disappearance of one OH peak of **1** and the presence of a signal for the PCHN proton that appears as a doublet at δ 5.3 ppm with a strong coupling constant with the close phosphorous atom indicated that the synthesis of compound **4b** was accomplished. The signals of aromatic protons were observed between δ 7.08-7.68 ppm. In the ^{13}C NMR spectrum of compound **4b**, the new peaks at 44.4 ppm and 54.8 ppm belonging to the NCH_2 (C_{10}) and PCHN (C_7) carbons, respectively, also confirmed that the cyclisation had occurred. The presence of the phosphonate group is clearly evidenced by the ^{31}P NMR spectrum that exhibits a singlet at 18 ppm. ^1H , ^{13}C and ^{31}P NMR spectra of derivatives **4a-f** show very similar patterns of signals for the PCHN group which are very characteristic of the isoindolin-1-one-3-phosphonate structure.

3. Biological Activities

a-Antimicrobial activity of isoindolin-1-one-3-phosphonates 4a-f

The *in vitro* antimicrobial activities of the isoindolin-1-one-3-phosphonate compounds **4a–f** were evaluated for *in vitro* antimicrobial activity by the well diffusion method [59]. All products were screened for activity against gram-positive bacteria (*M. luteus*, *L. monocytogenes*, *S. aureus* and *B. cereus*) and gram-negative bacteria (*S. typhimurium*). As shown in Table 3, all compounds exhibit considerable activity against the tested microorganisms except **4c**. These results differ according to the compound structure and type of bacteria tested.

Table 3. Antibacterial inhibition zones of isoindolin-1-one-3- phosphonate compounds 4a-f in mm

Microorganisms	<i>M. luteus</i> LB 141107	<i>L. monocytogenes</i> ATCC 1911	<i>S. aureus</i> ATCC 6538	<i>S. Typhimurium</i> ATCC 14028	<i>B. cereus</i>
Compounds					
4a	35±3.13	22±2.14	-	-	-
4b	24±2.6	18±1.32	-	-	18±1.11
4c	-	-	-	-	-
4d	18±1.22	18±1.11	-	15±1.17	-
4e	-	-	-	15±1.15	18±2.12
4f	-	-	18±2.12	20±2.53	18±2.15
Tetracycline	22±3.1	23±3.3	20±1.6	18±3.5	18±2.9

This table indicates the antibacterial activities of tested compounds in inhibition zones by mm. The values are means ± S.D. Tetracycline was used as control positive drug.

A simple inspection of Table 3 indicates that the highest antibacterial activity against *M. luteus* LB 141107 was reported by **4a** and **4b** with inhibition zone (IZ) 35 mm and 24 mm respectively followed by **4d** with IZ 18 mm. Also, **4f** is the most active against *S. Typhimurium* ATCC 14028 with IZ 20 mm. Additionally, **4a** was found to be the most active against *L. monocytogenes* with an inhibition zone of 22 mm. The other antibacterial results showed inhibition zones of less than 20 mm. As shown in Table 4, only 3 compounds possess antifungal activity against *C. albicans*—**4a**, **4b** and **4d** with inhibition zones of 38, 25 and 22 mm, respectively. The results obtained show that the different molecules have antimicrobial activity. **4a** and **4b** can be considered as good antimicrobial drug candidates. Isoindoline derivatives were found to have good and potent antimicrobial activity for a long time [60]. This supports

our findings. Further studies for structure activity relation (SAR) is recommended to indicate the reason for these varying biological activity.

Table 4. Antifungal inhibition zones of isoindolin-1-one-3- phosphonate

compound 4a,b,d in mm

Microorganisms	<i>C. albicans</i>
Compounds	
4a	38±3.5
4b	25±3.6
4c	-
4d	22±2.7
4e	-
4f	-
Fluconazole	24±3.7

This table indicates the antifungal activities of tested compounds in inhibition zones by mm. The values are means \pm S.D. Fluconazole was used as control positive drug.

b-Anti-leishmanial activities of isoindolin-1-one-3- phosphonate compounds 4a-f

Table 5 shows that all compounds except **4e** had anti-leishmanial activity against *L. major* promastigotes *in vitro* with half maximal effective concentration (EC_{50}) less than 2.5 μ M; two of them (**4b** and **4a**) had EC_{50} less than 1 μ M, and their EC_{50} values were 0.4 and 0.8 μ M with Selectivity index (SI) of 12.9 and 11.9, respectively. Additionally, **4a** and **4b** were the most active against *L. major* amastigotes with EC_{50} values of 0.7 and 0.8 μ M and SI of 13.5 and 6.4, respectively. All compounds had a very good SI in the range of 3.5 to 41.2 with the exception of **4c** against *L. major* amastigote. According to its SI value 4a can be considered a good antileishmanial drug candidate. The variation observed in these results may be due to the structure biology relation. Further studies for structure activity relation (SAR) is recommended to indicate the reason for these varying biological activity. However, in general, synthesized isoindoline derivatives exhibited potent biological activities [61]. Additionally, phosphonium compounds were found to have potent anti-leishmanial activity [62]. These results can agree with these suggestions.

Table 5. Anti-leishmanial activity of isoindolin-1-one-3-phosphonate compounds 4a-f against *L. major* promastigotes and amastigotes

Isoindolin-1-one-3-phosphonates 4	IC ₅₀ of Vero cells , (μM)	Amastigote EC ₅₀ (μM)	promastigotes EC ₅₀ (μM)	Amastigote SI	promastigote SI
4a	9.5 ± 3.1	0.7 ± 0.23	0.8 ± 0.26	13.5	11.9
4b	5.2 ± 1.8	0.8 ± 0.19	0.4 ± 0.08	6.4	12.9
4c	44.9 ± 8.6	> 50	2.3 ± 0.84	-	19.5
4d	48.7 ± 9.2	3.9 ± 0.82	1.8 ± 0.41	12.49	27.1
4e	50 ± 9.5	2.9 ± 0.75	14.2 ± 3.8	17.2	3.5
4f	45.3 ± 7.7	3.4 ± 1.1	1.1 ± 0.27	13.3	41.2
AmB	7.4 ± 2.64	0.46 ± 0.07	0.78 ± 0.09	16.09	9.49

This table indicates the antileishmanial activities of tested compounds in EC₅₀ of μM. The values are means ± S.D. Amphotericin B (AmB) was used as control positive drug. SI values was calculated by CC₅₀ (from Vero cell) over EC₅₀.

c-Anti-toxoplasma activities of isoindolin-1-one-3- phosphonate compounds 4a-f

Table 6 indicates that all compounds possess different levels of anti-toxoplasma activity *in vitro*. Only **4b** and **4a** had EC₅₀ values of less than 4 μM (3.2 and 3.8 μM, respectively). The others had less potent anti-toxoplasma activity with EC₅₀ values in the range of 11.9 to 33.9 μM and SI in the range of 1.4 to 3.8. The following results indicate that isoindolin-1-one-3-phosphonates are suitable candidates for anti-toxoplasma drug discovery.

Table 6. Anti-toxoplasma activity of isoindolin-1-one-3-phosphonates compounds 4a-f against *T. gondii*

Isoindolin-1-one-3- phosphonates 4	IC ₅₀ of Vero cells , EC ₅₀ (μM)	Antitoxoplasma EC ₅₀ (μM)	SI
4a	9.5 ± 3.1	3.8 ± 0.88	2.5
4b	5.2 ± 1.8	3.2 ± 0.67	1.6
4c	44.9 ± 8.6	13.4 ± 3.4	3.3
4d	48.7 ± 9.2	33.9 ± 6.1	1.4
4e	50 ± 9.5	20.7 ± 4.8	2.4
4f	45.3 ± 7.7	11.9 ± 2.6	3.8
ATO	9.3 ± 2.08	0.09 ± 0.02	103.33

This table indicates the antitoxoplasma activities of tested compounds in EC₅₀ of μM. The values are means ± S.D. Atovaquone (ATO) was used as control positive drug. SI values was calculated by CC₅₀ (from Vero cell) over EC₅₀.

There have not been previous investigations of isoindoline against *T. gondii*, but previous investigations found that some isoindoline derivatives have less potent activity against *Plasmodium* [63-64]. These published results agree with our finding. Further studies for structure activity relation (SAR) is recommended to indicate the reason for these varying biological activity.

D-Anti-cancer activity

As shown in Table 7, all tested compounds significantly affected the viability of malignant cell lines, showing a promising level of cytotoxicity with EC₅₀ of < 1.5 μM. However, the cytotoxicities of isoindolin-1-one-3- phosphonate compounds **4d**, **4c** and **4d** were much stronger in *MCF7* cells with EC₅₀ values 0.6, 0.7 and 0.7 μM, respectively.

Table 7. Anticancer profile of synthesized of isoindolin-1-one-3-phosphonate compounds **4a-f**.

Compounds	Anticancer activity	
	EC ₅₀ (μM)	
	<i>MCF7</i>	<i>MDA-MB-231</i>
4a	0.9 ± 0.6	0.9± 1.3
4b	0.8± 0.7	1.5± 0.9
4c	0.7± 0.6	0.8± 1.2
4d	0.6 ± 1.1	0.9 ±0.3
4e	0.68± 1.2	1.1± 0.6
4f	1.3± 1.1	0.8± 0.6
Dox	0.7±0.2	1.6±0.4

This table indicates the anticancer activities of tested compounds in EC₅₀ of μM. The values are means ± S.D. Doxorubicin (Dox) was used as control positive drug.

The cytotoxicity of isoindolin-1-one-3-phosphonate compounds **4c** and **4f** against *MDA-MB-231* cells was strongest with EC₅₀ of 0.8 μM, followed by **4a** and **4d** with EC₅₀ of 0.9 μM. These results agree with previous results that showed that isoindoline derivatives have activity against cancer cells [65]. Further studies for structure activity relation (SAR) is recommended to indicate the reason for these varying biological activity.

Conclusion

In conclusion, we have developed an efficient three-component synthesis of isoindolin-1-one-3-phosphonates. The reaction proceeds quickly and with no undesirable side reactions observed. This procedure may find wide application in the large-scale synthesis of cyclic α-aminophosphonates. Further investigations are currently in progress to demonstrate the potential of this methodology in the diastereoselective preparation of isoindolin-1-ones bearing phosphonate functionality of biological interest. The biological activities revealed that **4a** and **4b** are good drug candidates for antimicrobial (better than standard antibiotic) and anti-leishmanial agents and have potent anticancer activity against both *MCF7* and *MDA-MB-123*. However, the activity of the above compounds against normal vero cells indicates their safety (half maximal inhibitory concentration (IC₅₀) in the range between 5 – 50 μg/ml) that can support and enhance our suggestion about the uses of these compounds in future as drug candidates. Further studies for structure activity relation (SAR) is recommended to indicate the reason for these varying biological activity as well as in vivo evaluation.

Experimental

General information

Chemicals were purchased from Sigma Aldrich and used without further purification. All solvents were purified and dried with the MBraun SPS 800 solvent purification system. NMR spectra were recorded with a Varian System instrument (400 MHz for ^1H , and 100 MHz for ^{13}C) with CDCl_3 as the solvent and TMS as the internal standard signal. NMR multiplicities are abbreviated as follows: s = singlet, d = doublet, t = triplet, and m = multiplet signal. IR spectra were recorded on a 398 spectrophotometer (Perkin-Elmer). Elemental microanalysis was performed on an ElementarVario El III Carlo Erba 1108 elemental analyzer, and the values found were within $\pm 0.3\%$ of the theoretical values. Melting points were determined with Kofler bench at Isste of Borj cedria (Hammam Lif, University of Carthage, Borj Cedria, Tunisia) [46].

General procedure for the synthesis of Diethyl 3-oxoisindolin-1-yl phosphonate compounds (4a-f)

2-Formylbenzoic acid (2.5 g, 17 mmol), amine (18 mmol) and diethylphosphite (2.9 g, 21 mmol) were stirred at 80°C for 1.0 h, and the progress of the reaction was monitored by TLC. The mixture was extracted with dichloromethane, dried with MgSO_4 , filtered and evaporated. Finally, the crude product was purified by column chromatography. The crude product was analyzed by ^1H , ^{13}C and ^{31}P NMR spectroscopy [46].

Diethyl 2-(2,2-dimethoxyethyl)-3-oxoisindolin-1-yl phosphonate (4a)

Yield = 70%;oil; ^1H NMR (300 MHz, CDCl_3) δ (ppm): 1.02 (t, $J = 7.1$ Hz, 3H), 1.17 (t, $J = 7.1$ Hz, 3H), 3.27 (d, $J = 9.6$ Hz, 6H), 3.62-3.91 (m, 3H), 3.99-4.09 (m, 2H), 4.22 (dd, $J = 4.0, 14.4$ Hz, 1H), 4.50 (dd, $J = 4.0, 6.5$ Hz, 1H), 5.11 (d, $J = 13.1$ Hz, 1H), 7.39-7.52 (m, 2H), 7.70 (dd, $J = 3.4, 7.6$ Hz, 2H); ^{13}C NMR (75 MHz, CDCl_3) δ (ppm): 16.0 (d, $J = 5.5$ Hz, CH_3), 16.2 (d, $J = 5.7$ Hz, CH_3), 42.4 (CH_2), 53.5 (CH_3), 54.4 (CH_3), 57.0 (CH_2), 59.0 (CH_2), 63.05(CH_2), 102.31(CH); 123.59(CH); 124.32(CH); 128.56(CH); 131.50(CH); 131.69(C); 139.03(C); 168.80(C); ^{31}P NMR (121 MHz, CDCl_3) δ (ppm): 18.3. Anal. Calc. for

$\text{C}_{16}\text{H}_{24}\text{NO}_6\text{P}$ (%): C, 53.78 %; H, 6.77 %; N, 3.92 %. Found (%): C, 53.7; H, 6.7; N, 4.1.

Diethyl 2-benzyl-3-oxoisindolin-1-yl phosphonate (4b)

Yield = 72%;oil; ^1H NMR (300 MHz, CDCl_3) δ (ppm): 0.90 (t, $J = 7.1$ Hz, 3H), 1.00 (t, $J = 7.1$ Hz, 3H), 3.56-3.91 (m, 4H), 4.43 (dd, $J = 14.1, 18.9$ Hz, 2H), 5.32 (d, $J = 14.9$ Hz, 1H), 6.94-7.09 (m, 5H), 7.22-7.32 (m, 2H), 7.47 (d, $J = 7.2$ Hz, 1H), 7.67 (d, $J = 8.2$ Hz, 1H); ^{13}C NMR (75 MHz, CDCl_3) δ (ppm): 15.7 (d, $J = 5.4$ Hz, CH_3), 15.9 (d, $J = 5.4$ Hz, CH_3), 44.4 (CH_2), 55.9 (d, $J = 156$ Hz, CH), 62.7 (d, $J = 6.9$ Hz, 2CH_2), 123.3 (CH), 124.0 (CH), 127.1 (CH), 127.7 (2CH), 128.2 (2CH), 128.3 (CH), 131.1 (CH), 131.4 (C), 136.3 (C), 138.2 (C), 168.2 (C); ^{31}P NMR (121 MHz, CDCl_3) δ (ppm) : 18.0. Anal. Calc. for $\text{C}_{19}\text{H}_{22}\text{NO}_4\text{P}$ (%): C, 63.50 %; H, 6.17 %; N, 3.90 % %. Found (%): C, 63.6; H, 6.2; N, 3.9.

Diethyl (2-cyclohexyl-2-oxoisindolin-1-yl) phosphonate (4c)

Yield= 71%; oil; ^1H NMR (300 MHz, CDCl_3) δ (ppm) : 1.11 (t, J = 7.1 Hz, 3H), 1.19-1.40 (m, 6H), 1.65-1.93 (m, 5H), 2.14-2.49 (m, 2H), 3.71-4.13 (m, 5H), 4.82 (d, J = 13.2 Hz, 1H), 7.43-7.54 (m, 2H), 7.71 (d, J = 7.6 Hz, 1H), 7.79 (d, J = 8.1 Hz, 1H). ^{13}C NMR (75 MHz, CDCl_3) δ (ppm): 16.35 (d, J = 4.0 Hz, CH_3), 16.42 (d, J = 4.5 Hz, CH_3), 25.4 (CH_2), 26.2 (CH_2), 26.5 (CH_2), 29.5 (CH_2), 29.8 (CH_2), 56.8 (CH), 58.7 (d, J = 156 Hz, CH), 63.3 (d, J = 7.4 Hz, CH_2), 63.5 (d, J = 7.1 Hz, CH_2), 123.5 (CH), 124.6 (CH), 128.8 (CH), 131.3 (CH), 133.7 (C), 138.9 (C), 169.1 (C); ^{31}P NMR (121 MHz, CDCl_3) δ (ppm) :18.6. Anal. Calc. for $\text{C}_{18}\text{H}_{26}\text{NO}_4\text{P}$ (%): C, 61.53 %; H, 7.46 %; N, 3.99 %. Found (%): C, 61.6; H, 7.5; N, 4.1.

Diethyl (2-(4-methoxybenzyl)-3-oxoisindolin-1-yl) phosphonate (4d)

Yield = 84%;oil; ^1H NMR (300 MHz, CDCl_3) δ (ppm) :1.03 (t, J = 7.1 Hz, 3H), 1.15 (t, J = 7.1 Hz, 3H), 3.62 (s, 3H), 3.70-4.05 (m, 4H), 4.43 (d, J = 14.7 Hz, 1H), 4.57 (d, J = 13.4 Hz, 1H), 5.39 (d, J = 14.7 Hz, 1H), 6.70 (d, J = 8.7 Hz, 2H), 7.14 (d, J = 8.6 Hz, 2H), 7.36-7.45 (m, 2H), 7.58 (d, J = 7.2 Hz, 1H), 7.78 (d, J = 8.2 Hz, 1H). ^{13}C NMR (75 MHz, CDCl_3) δ (ppm): 16.0 (d, J = 5.5 Hz, CH_3), 16.2 (d, J = 5.5 Hz, CH_3), 44.1(CH_2), 54.9(CH_3), 55.9 (d, J = 157 Hz, CH), 63.1 (d, J = 6.9, 2CH_2), 113.8 (2CH), 123.6 (CH), 124.2 (CH), 128.55 (CH), 128.60 (C), 129.5 (2CH), 131.4 (CH), 131.8 (C), 138.6 (C), 158.7 (C), 168.5 (C); ^{31}P NMR (121 MHz, CDCl_3) δ (ppm) :18.1. Anal. Calc. for $\text{C}_{20}\text{H}_{24}\text{NO}_5\text{P}$ (%): C, 61.69 %; H, 6.21 %; N, 3.60 % .Found (%): C, 61.7; H, 6.3; N, 3.7.

Diethyl (2-butyl-3-oxoisindolin-1-yl) phosphonate (4e)

Yield = 88%;oil; ^1H NMR (300 MHz, CDCl_3) δ (ppm) : 0.66-1.40 (m, 13H), 3.30-3.87 (m, 6H), 4.66-4.74 (m, 1H), 7.27-7.55 (m, 4H). ^{13}C NMR (75 MHz, CDCl_3) δ (ppm): 13.3 (CH_3), 15.8 (2CH_3), 19.5 (CH_2), 29.5 (CH_2), 41.0 (CH_2), 56.6 (d, J = 155 Hz, CH), 62.8 (d, J = 14.8 Hz, 2CH_2), 123.1 (CH), 124.0 (CH), 128.3 (CH), 130.9 (CH), 131.9 (C), 138.1 (C), 168.1 (C); ^{31}P NMR (121 MHz, CDCl_3) δ (ppm) : 17.9. Anal. Calc. for $\text{C}_{16}\text{H}_{24}\text{NO}_4\text{P}$ (%): C, 59.07 %; H, 7.44 %; N, 4.31 %. Found (%): C, 59.1; H, 7.5; N, 4.4.

Diethyl (3-oxo-2-pentylisindolin-1-yl) phosphonate (4f)

Yield=78%; oil; ^1H NMR (300 MHz, CDCl_3) δ (ppm): 0.76 (t, J = 7.0 Hz, 3H), 1.00 (t, J = 7.1 Hz, 3H), 1.12-1.25 (m, 7H), 1.46-1.65 (m, 2H), 3.40-3.49 (m, 1H), 3.66-4.06 (m, 5H), 4.79 (d, J = 13.6 Hz, 1H), 7.36-7.48 (m, 2H), 7.70 (dd, J = 2.3, 7.5 Hz, 2H). ^{13}C NMR (75MHz, CDCl_3) δ (ppm): 13.8 (CH_3), 16.1 (d, J = 5.5 Hz, CH_3), 16.2 (d, J = 5.7 Hz, CH_3), 22.2 (CH_2), 27.4 (CH_2), 28.8 (CH_2), 41.6 (CH_2), 57.0 (d, J = 155 Hz, CH), 63.0 (d, J = 7.2 Hz, CH_2), 63.3 (d, J = 7.1 Hz, CH_2), 123.5 (CH), 124.3 (CH), 128.6 (CH), 131.3 (CH), 132.3 (C), 138.4 (C), 168.5 (C); ^{31}P NMR (121 MHz, CDCl_3) δ (ppm) : 17.9. Anal. Calc. for $\text{C}_{17}\text{H}_{26}\text{NO}_4\text{P}$ (%): C, 60.17 %; H, 7.72%; N, 4.13 %. Found (%): C, 60.2; H, 7.8; N, 4.2.

Synthesis of isindolin-1-one-3-yl phosphonic acids (5a-b)

A solution of isoindolin-1-one-3-yl phosphonate **4a-b** (3.01 mmol) in a mixture of concentrated HCl and dioxane (1:1) (100 ml) was heated at 80 °C for 72 h, cooled and the solvent was evaporated. The crystalline residue was treated with ethanol (10 ml), filtered off, washed and dried to give compounds **5a-b** as solids.

(2-Benzyl-3-oxoisoindolin-1-yl) phosphonic acid (5a)

Yield=60%; mp: 119-120 °C; NMR ¹H (300 MHz, DMSO) δ(ppm) 4.50 (d, *J* = 16.3 Hz, 1H), 4.78 (d, *J* = 15.0 Hz, 1H), 5.25 (d, *J* = 15.0 Hz, 1H), 7.21(s, 5H), 7.45-7.57 (m, 2H), 7.71 (d, *J* = 7.3 Hz, 1H), 7.82 (d, *J* = 6.7 Hz, 1H); NMR ¹³C (75 MHz, DMSO) δ(ppm): 44.1 (CH₂), 57.9 (d, *J* = 149 Hz, CH), 122.8 (2CH), 125.1 (CH), 127.3 (CH), 127.9 (2CH), 128.6 (2CH), 131.1 (CH), 131.7 (C), 137.8 (C), 141.5 (C), 168.1 (C); NMR ³¹P (121 MHz, DMSO) δ(ppm) : 11.5. Anal. Calc. for C₁₅H₁₄NO₄P (%): C, 59.41; H, 4.65; N, 4.62. Found (%): C, 59.5; H, 4.7; N, 4.5.

(2-Cyclohexyl-3-oxoisoindolin-1-yl) phosphonic acid (5b)

Yield=68%; mp: 259-260 °C; NMR ¹H (300 MHz, DMSO) δ(ppm) : 1.15-1.25 (m, 3H), 1.59-2.26 (m, 7H), 3.74 (t, *J* = 11.9 Hz, 1H), 4.78-4.92 (d, *J* = 15.3 Hz, 1H), 7.43-7.67 (m, 4H); NMR ¹³C (75 MHz, DMSO) δ(ppm): 25.2 (CH₂), 25.8 (CH₂), 26.0 (CH₂), 28.7 (CH₂), 29.1 (CH₂), 55.4 (CH), 59.7 (d, *J* = 148 Hz, CH), 122.2 (CH), 124.5 (CH), 127.8 (CH), 130.8 (CH), 133.3 (C), 141.0 (C), 167.8 (C). NMR ³¹P (121 MHz, DMSO) δ(ppm): 14.0. Anal. Calc. for C₁₈H₂₆NO₄P (%): C, 61.53 %; H, 7.46 %; N, 3.99 %. Found (%): C, 61.6; H, 7.5; N, 4.1.

Bioassays

Antimicrobial activity by the disk diffusion method

M.luteus (LB 141107), *L. monocytogenes* (ATCC 1911), *S. aureus* (ATCC 6538), *B. cereus*, *S. typhimurium* (ATCC 14028) and *B. cereus* were grown on nutrient agar plates (HiMedia, India), and *C. albicans* (ATCC 90028) was grown on potato dextrose agar (HiMedia, India) for 24 h at 35 °C. All cultures were obtained from the American Type Culture Collection (ATCC). The disc diffusion method was used to assess the antimicrobial activities [11,59] (Breytenbach et al. 2000). Microbes were suspended in sterile saline solution (0.9%), and the turbidity was adjusted to 0.5 OD values using a spectrophotometer (Labomed Inc., USA). The inoculum was swabbed on the surface of agar plates used using a sterilized cotton swab. Sterile blank disks (6-mm) were loaded with 10 µl of compound stock solution (10 mg/mL), giving a concentration of 50 µg/disc. Commercial tetracycline discs (30 µg per disc) were used as positive controls, and methanol as a negative control for comparison. The petri-plates were incubated at 35 °C for 24 h. The diameters of the zones of inhibition produced by the compounds on the test isolates were measured in mm [46,64].

L. major cell isolation, culture conditions, and assays

Promastigotes of *L. major* were isolated from a Saudi male patient in February 2016 and maintained at 26 °C in Schneider's Drosophila medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) and antibiotics in a tissue culture flask with weekly transfers. Promastigotes were cryopreserved in liquid nitrogen at concentrations of 3×10^6 parasites/mL. The virulence of *L. major* parasites was maintained by passing in female BALB/c mice by injecting hind footpads with 1×10^6 . Fifty-six days later, the amastigotes of *L. major* were collected from the infected animals. Then transformed to promastigote stages via culturing at room temperature using complete Schneider's medium containing FBS 10% with antimicrobial. For infection, *in vitro* subculturing was used for maintaining different pathogen stages [65,66].

For the evaluation of the compounds for their activity against *L. major* promastigotes, complete RPMI 1640 medium containing 10% FBS without phenol red-free was used for culturing the parasite in 96-wells plates to yield 10^6 organisms mL^{-1} (200 μL /well), the counting was conducted by hemocytometer. Then the compounds were added in concentrations of (50, 25, 12.5, 6.25, 3.13, 1.65, and 0.75 $\mu\text{g}/\text{mL}$). DMSO with (1%) was used as negative control, while Amphotericin (AmB) was used as positive control with the same compounds concentrations (50, 25, 12.5, 6.25, 3.13, 1.65, and 0.75 $\mu\text{g}/\text{mL}$). After that they were allowed to stay at room temperature for 3 days. Tetrazolium salt colorimetric assay (MTT) was used for assessing the viable organisms. The samples were analyzed with an spectrophotometer at 570 nm. EC_{50} values were derived from three independent experiments [66].

For the assessing the compounds activities against intramacrophages amastigotes, a group of 6 mice of 56 days age were used for macrophages collection from peritoneum cavity by aspiration. Ninety-six well ELISA plates were used for culturing the cells at concentration 5×10^4 cells/well supplied by complete RPMI 1640 medium with 10% FBS for 4 h at 37 °C and 5% CO_2 for enhancing the adhesion of the cell. Followed by the discarding of the medium and washing with phosphate buffered saline (PBS). Promastigotes in a complete RPMI 1640 medium with 10% FBS solution of 200 μL containing promastigotes to ration of 10 promastigotes: 1 macrophage for each well. Then followed by overnight incubation at 37 °C in a humidified 5% CO_2 atmosphere for enhancing differentiation and amastigote infection. PBS was used for washing the infected macrophages and removal of free promastigotes. For compound assessing against amastigotes, the final concentrations of (50, 25, 12.5, 6.25, 3.13, 1.65, and 0.75 $\mu\text{g}/\text{mL}$) in complete RPMI 1640 medium were added and cells were added to each well and then incubated at 37 °C in with 5% CO_2 atmosphere for 3 days. DMSO with (1%) was used as negative control, while AmB was used as positive control with the same compounds concentrations (50, 25, 12.5, 6.25, 3.13, 1.65, and 0.75 $\mu\text{g}/\text{mL}$). The evaluation of macrophages percentage infection was carried out microscopically after removing medium, washing, fixation, and Giemsa staining. EC_{50} values were obtained from three independent experiments [66,67].

***T. gondii* cell line, culture conditions, and assay**

Vero cells (ATCC® CCL81™, USA) were cultured in complete RPMI 1640 medium (5×10^3 cell/ well in 200 μ l), then incubated at 37 °C in with 5% CO₂ atmosphere for 24 hours. After washing cells using PBS, cells were infected by *T. gondii* (Rh strain). Then compounds were applied at concentrations (50, 25, 12.5, 6.25, 3.13, 1.65, and 0.75 μ g/ml). DMSO with (1%) was used as negative control, while atovaquone (ATO) was used as positive control with the same compounds concentrations. After that, toluidine blue of 1% was used for staining and then PBS used for washing and the fixation took place by 10% formalin. Inverted photomicroscope was used for the examination of cells and the determination of the infection index (number of infected cells out of 200 examined cells) of *T. gondii*. For the calculation inhibition% the following equation was applied.

$$\text{Inhibition (\%)} = (\text{I Control} - \text{I Experimental}) / (\text{I Control}) \times 100$$

where “I Control” refers to the infection index of untreated cells, and “I Experimental” refers to the infection index of cells treated with test compounds.

Then, the effects of test compounds on parasite growth were expressed as EC₅₀ (effective concentration at 50%) values. EC₅₀ values were obtained from three independent experiments [68,69].

***In vitro* cytotoxicity assay**

For the assessing the compound cytotoxicity MTT colorimetric assay was carried out according to the method described previously [67]. Ninety six well plates were used for culturing vero cells and two human cancer cell lines—MCF7 and MDA-MB-231—at concentration of (5×10^3 cells/well/200 μ l) for one day in complete RPMI 1640 medium containing 10% FBS and then kept in 5% CO₂ with temperature of 37 °C. followed by washing with PBS and then incubated with the compounds for 3 days at varying concentrations (100, 33, 11, 3.7, 1.2, 0.4, 0.14 and 0.04 μ g/ml). Medium in 2% FBS was used as a negative control. Thereafter, the supernatant was removed, 50 μ l of RPMI 1640 medium containing 14 μ l of MTT (5 mg/ml) was added, and the cells were incubated for 4 h. After that, the supernatant was removed, and 200 μ l of DMSO was added to dissolve the formazan. A FLUOstar OPTIMA spectrophotometer was applied for colorimetric analysis (λ = 540 nm). Cytotoxic effects were expressed as IC₅₀ values (concentration that caused a 50% reduction in viable cells). IC₅₀ values were obtained from three independent experiments [70-72].

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Figures

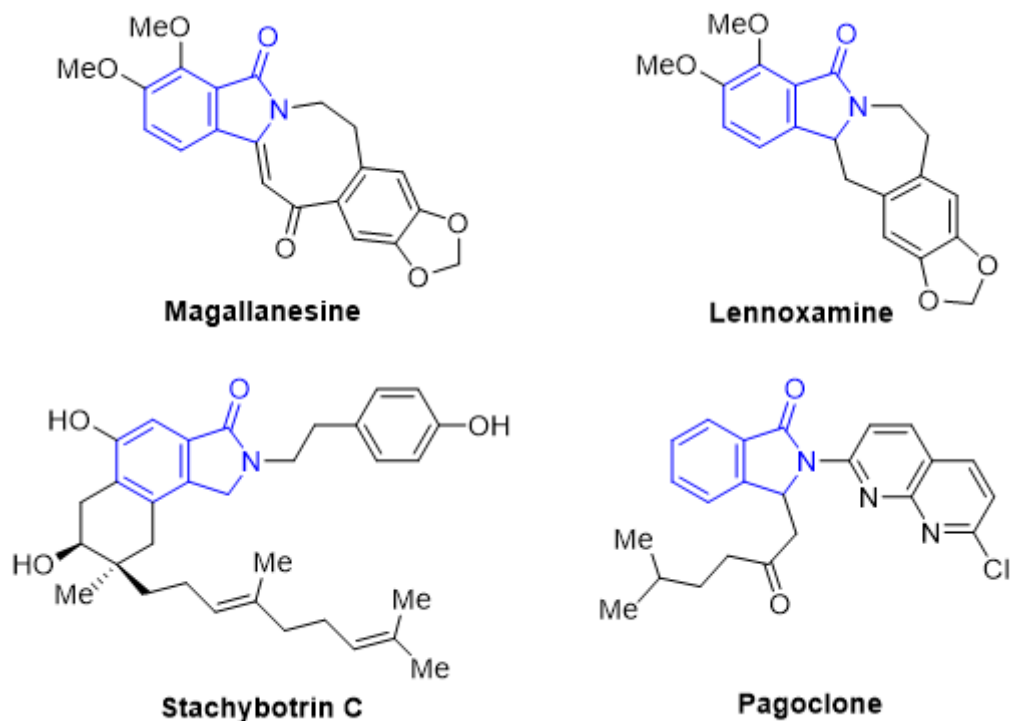


Figure 1

Structure of pharmacologically compounds that contain isoindolin-1-one core structure.

Scheme 1

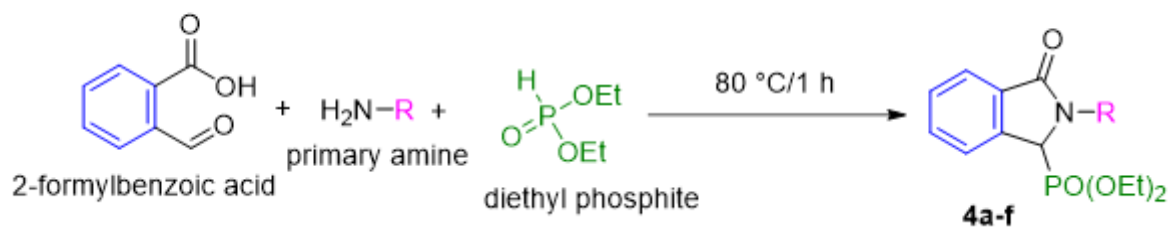


Figure 2

Protocol synthesis of Isoindolin-1-one-3-phosphonates 4a-f

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