An Efficient, Three-Component Synthesis of Isoindolin-1-One-3-Phosphonates Under Mild and Solvent-Free Conditions and Their Biological Activities

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Original Article

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Abstract

In this work, the synthesis 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 was reported. ^{1}H NMR, ^{13}C NMR, FT-IR and elemental analysis techniques, characterized the obtained compounds. The isoindolin-1-one-3-phosphonates were screened for their antimicrobial activities against bacteria and a fungus (*Candida albicans*). They were additionally also investigated for their anti-parasitical activities against *Leishmania major* promastigotes and amastigotes and *Toxoplasma gondii in vitro*. Cytotoxicity investigations of the isoindolin-1-one-3-phosphonates were led conducted in two human cancer cell lines, *MDA-MB-231* and *MCF-7*and vero cells.

Introduction

Isoindolin-1-one moiety is an important scaffold that has attracted great attention in organic synthesis [1-4]. (Fig. 1). Isoindolin-1-one moiety may be a critical framework that need pulled in incredible consideration. 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 differents 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].

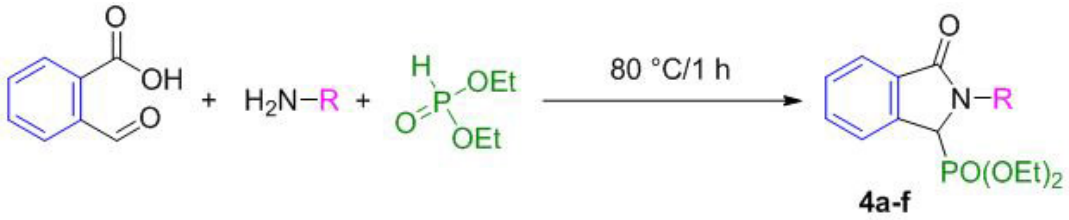
Reduction of phthalimides to 3-hydroxy-isoindolin-1-one followed by a reaction with trifluoroacetic anhydride and triethylphosphite afford the corresponding phosphonates in moderate yields [36-37]. The synthesis were carried out in toluene at reflux or under microwave irradiation [38-44].

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**. The process involves a ‘one-pot’ three-component reaction of 2-formylbenzoic acid, a primary amine and dimethyl phosphite that yields compounds **4a-f**. The 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 *Toxoplasam gondii*. Additionally, cytotoxicity were tested using MTT assay.

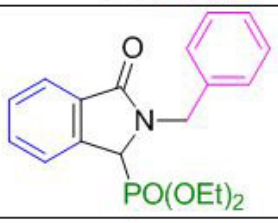
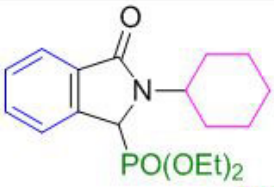
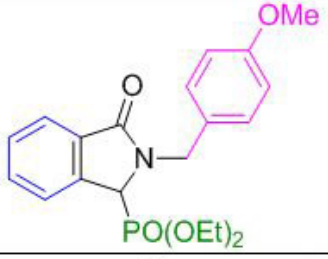
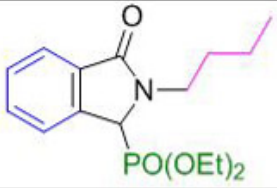
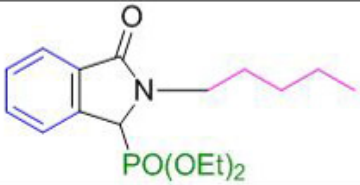
Results And Discussion

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].

Table 1: Isoindolin-1-one-3-phosphonantes 4a-f prepared



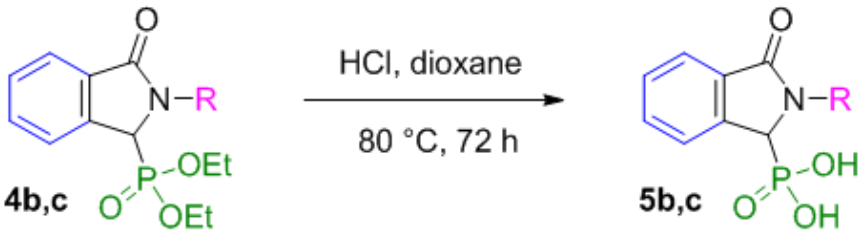
4a-f

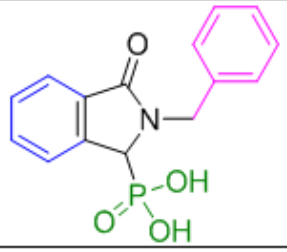
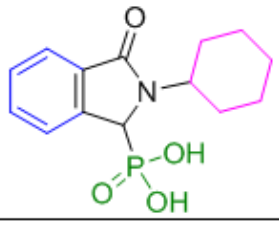
Entry	Structure	Compound	Yield (%)
1		4a	70
2		4b	72
3		4c	71
4		4d	84
5		4e	88
6		4f	78

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 **2a** and (4-methoxyphenyl) methanamine **2d** were obtained in yields of 70 and 84%, respectively (Table 1, entries 2,4). 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** and cyclohexylmethanamine **2c**, which generated the corresponding products in 72 and 71% yields respectively (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, ^{1}H, ^{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 ^{1}H-NMR spectrum of **4b**, the disappearance of one OH peak of **1** and the presence of a signal for the PC*H*N 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 N*C*H_2 (C_{10}) and P*C*HN (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. ^{1}H, ^{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. Further studies for structure biology relation is recommended to indicate the reason for these varying biological activity.

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

Microorganisms Compounds	*M. luteus* LB 141107	*L. monocytogenes* ATCC 1911	*S. aureus* ATCC 6538	*S. Typhimurium* ATCC 14028	*B. cereus*
4a	35±0.13	22±0.14	-	-	-
4b	24±26	18±0.32	-	-	18±0.11
4c	-	-	-	-	-
4d	18±0.22	18±0.11	-	15±0.17	-
4e	-	-	-	15±0.15	18±0.12
4f	-	-	18±0.12	18±0.53	18±0.15

The results obtained by these tests showed that **4a** and **4b** are the most active compounds against *M. luteus* with inhibition zones of 35 and 24 mm, respectively. 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 **4c** with inhibition zones of 35, 25 and 22 mm, respectively. The results obtained show that the different molecules have antimicrobial activity. Isoindoline derivatives were found to have good and potent antimicrobial activity for a long time [60]. This supports our findings.

Table 4. Antifungal inhibition zones of isoindolin-1-one-3- phosphonate compound 4a,b,d in mm

Microorganisms Compounds	*C. albicans*
4a	**38±0.5**
4b	**25±0.6**
4d	**22±0.7**

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 μM; two of them (**4b** and **4a**) had EC_{50} less than 1 μM, and their EC_{50} values were 0.4 and 0.8 μM 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 μM 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. The variation observed in these results may be due to the structure biology relation which is recommended for further evaluation. 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

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

Table 6 indicates that all compounds possess different levels of anti-toxoplasmal activity *in vitro*. Only **4b** and **4a** had EC_{50} values of less than 4 μM (3.2 and 3.8 μM, respectively). The others had less potent anti-toxoplasmal activity with EC_{50} 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 not suitable candidates for anti-toxoplasmal drug discovery.

Table 6. Anti-toxoplasmal activity of isoindolin-1-one-3-phpshonates 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

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.

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 EC_{50} 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 EC_{50} 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)	
	MCF7	*MDA-MB-231*
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
Tetracycline[a]	NT	NT

The cytotoxicity of isoindolin-1-one-3-phosphonate compounds **4c** and **4f** against *MDA-MB-231* cells was strongest with EC_{50} of 0.8 μM, followed by **4a** and **4d** with EC_{50} of 0.9 μM. These results agree with previous results that showed that isoindoline derivatives have activity against cancer cells [65].

Conclusion

In this paper we have designed an efficient three-component synthesis of isoindolin-1-one-3-phosphonates with no undesirable side reactions observed. 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 (IC_{50}) 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.

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 ^{1} H, 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 ± 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-oxoisoindolin-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 ^{1}H, ^{13}C and ^{31}P NMR spectroscopy [46].

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

Yield = 70%;oil; ^{1}H 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

$C_{16}H_{24}NO_6P$ (%): C, 53.78 %; H, 6.77 %; N, 3.92 %. Found (%): C, 53.7; H, 6.7; N, 4.1.

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

Yield = 72%;oil; ^{1}H 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 $C_{19}H_{22}NO_4P$ (%): C, 63.50 %; H, 6.17 %; N, 3.90 % %. Found (%): C, 63.6; H, 6.2; N, 3.9.

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

Yield= 71%; oil; ^{1}H 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 C18H26NO4P (%): C, 61.53 %; H, 7.46 %; N, 3.99 %. Found (%): C, 61.6; H, 7.5; N, 4.1.

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

Yield = 84%;oil; ^{1}H 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 $C_{20}H_{24}NO_5P$ (%): C, 61.69 %; H, 6.21 %; N, 3.60 % .Found (%): C, 61.7; H, 6.3; N, 3.7.

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

Yield = 88%;oil; ^{1}H 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 $C_{16}H_{24}NO_4P$ (%): C, 59.07 %; H, 7.44 %; N, 4.31 %. Found (%): C, 59.1; H, 7.5; N, 4.4.

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

Yield=78%; oil; ^{1}H 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 C17H26NO4P (%): C, 60.17 %; H, 7.72%; N, 4.13 %. Found (%): C, 60.2; H, 7.8; N, 4.2.

Synthesis of isoindolin-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 ^{1}H (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 ^{13}C (75 MHz, DMSO) δ(ppm): 44.1 (CH_2), 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 ^{31}P (121 MHz, DMSO) δ(ppm) : 11.5. Anal. Calc. for $C_{15}H_{14}NO_4P$ (%): 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 ^{1}H (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 ^{13}C (75 MHz, DMSO) δ(ppm): 25.2 (CH_2), 25.8 (CH_2), 26.0 (CH_2), 28.7 (CH_2), 29.1 (CH_2), 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 ^{31}P (121 MHz, DMSO) δ(ppm): 14.0. Anal. Calc. for $C_{18}H_{26}NO_4P$ (%): 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 (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 [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 $1x10^6$. 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].

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 µg/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 µg/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 x 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 µg/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 µg/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 [67].

T. gondii cell line, culture conditions, and assay

Vero cells (ATCC® CCL81™, USA) were cultured in complete RPMI 1640 medium (5 X 10^3 cell/ well in 200 µl), then incubated at 37 °C in with 5% CO_2 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 µg/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.

Inhibition (%) = (I Control - I Experimental)/(I Control) × 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 EC_{50} (effective concentration at 50%) values. EC_{50} 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 µl) for one day in complete RPMI 1640 medium containing 10% FBS and then kept in 5% CO_2 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 µg/ml). Medium in 2% FBS was used as a negative control. Thereafter, the supernatant was removed, 50 µl of RPMI 1640 medium containing 14 µl of MTT (5 mg/ml) was added, and the cells were incubated for 4 h. After that, the supernatant was removed, and 200 µl 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 IC_{50} values (concentration that caused a 50% reduction in viable cells). IC_{50} values were obtained from three independent experiments [70-72].

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Compliance with ethical standards

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Figures

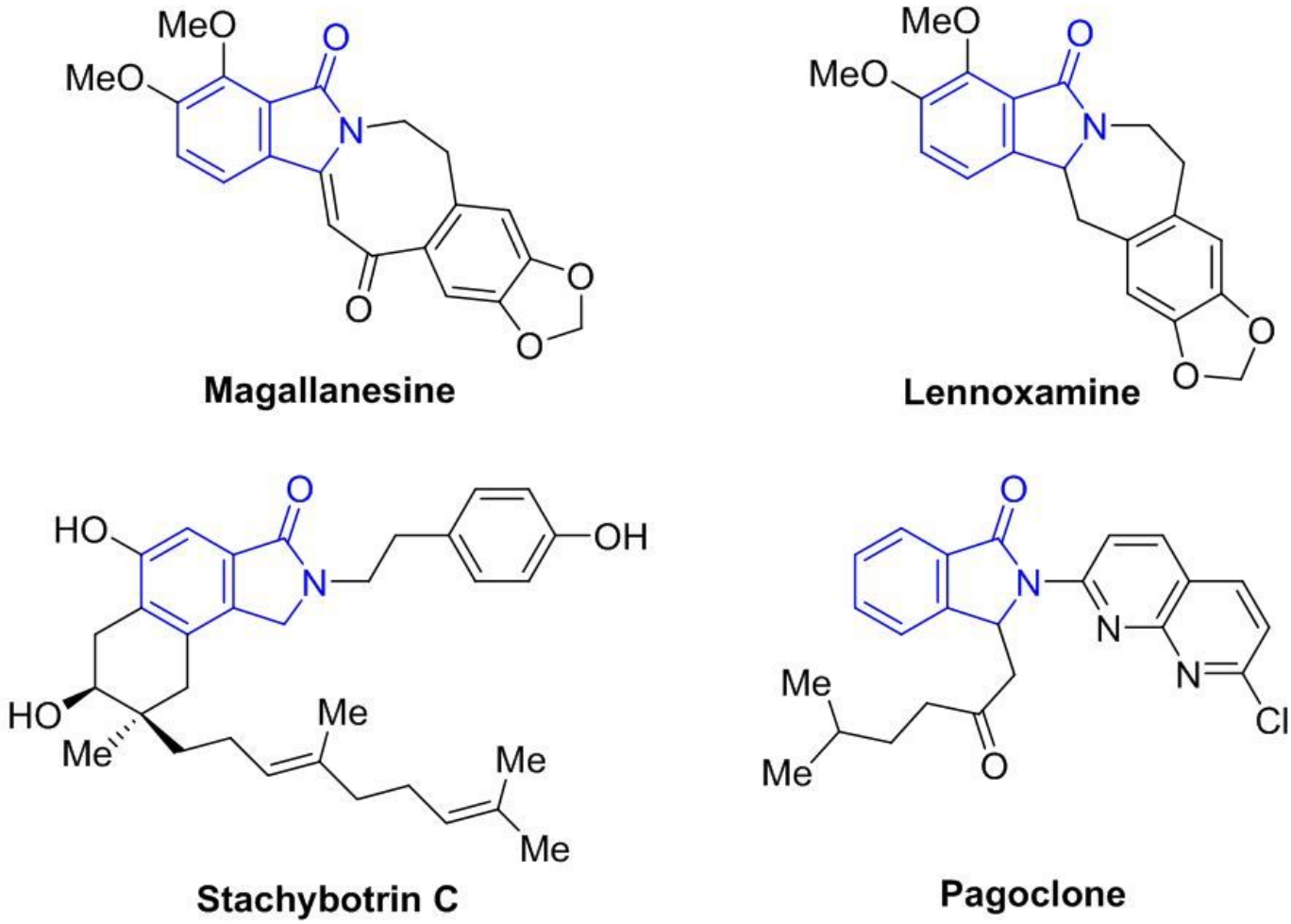

Figure 1

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