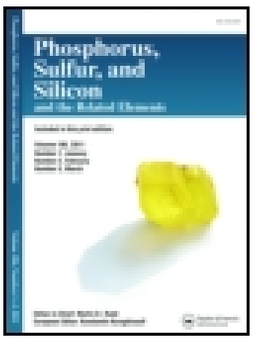

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Running Head: Synthesis of *N,N*-disubstituted *P*-[2-(1*H*-indol-3-yl)acetyl]phosphoramidates.

SYNTHESIS OF NOVEL *N,N*-DISUBSTITUTED ETHYL *P*-[2-(1*H*-INDOL-3-YL)ACETYL]PHOSPHORAMIDATES AND THEIR BIOLOGICAL ACTIVITY

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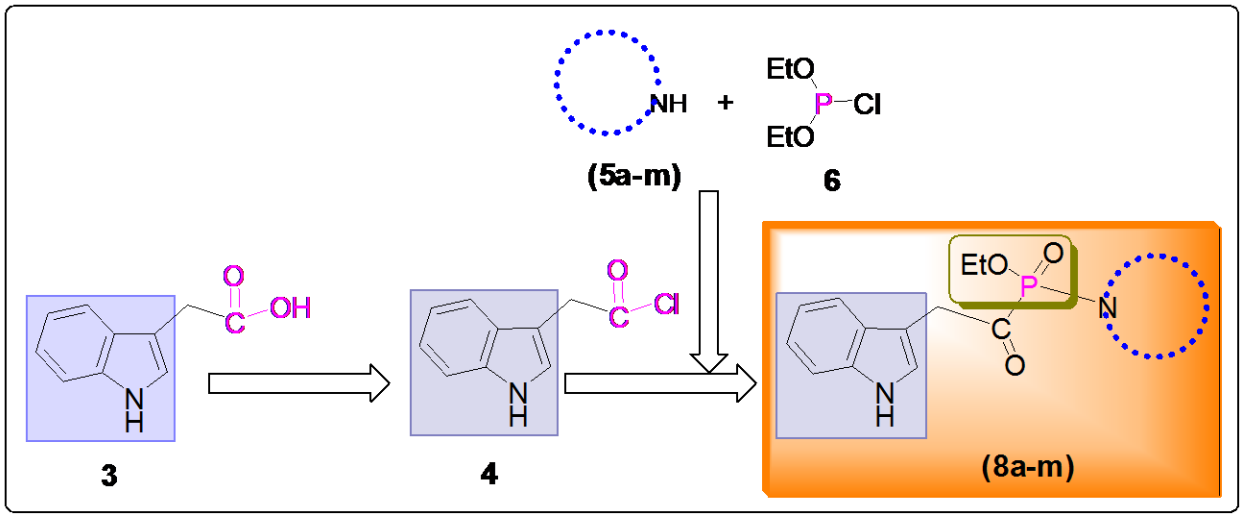
Abstract

A series of novel *N,N*-disubstituted ethyl *P*-[2-(1*H*-indol-3-yl)acetyl]phosphoramidates were synthesized. All title compounds were characterized by IR, NMR (^{1}H, ^{13}C, ^{31}P) spectra, mass spectra and C, H, N analysis. The anticancer activity of the title compounds was evaluated in two human cell lines MCF-7 (breast cancer) and HeLa (Cervical cancer) cell lines by use of the MTT assay, and the IC_{50} values were determined. The derivatives with substituted piperazines exhibited significant cytotoxicity against the tested cell lines at 5 μM and displayed low IC_{50}

values in the region of 5.8-8.8 µM for MCF-7 cell lines and 14.8-16.4 µM for HeLa cell lines when compared with the standard doxorubicin (5.4 and 14.2 µM).



Keywords: Indole-3-acetic acid (IAA); ketophosponates; cytotoxicity; MCF-7 cell lines; HeLa cell lines; MTT assay.

INTRODUCTION

Cancer is an undoubtedly serious and life threatening disease with a complex pathogenesis. A number of natural products like taxol or vinca alkaloids have been used for the treatment of cancer that includes DNA-alkylating agents and antimitotic agents. However, the complex synthesis, difficult formulation, lack of oral availability makes these drugs suboptimum for clinical treatment of cancer.[1] A new era has entered into molecularly targeted therapeutics, which is highly selective and not associated with serious toxicities of conventional cytotoxic drugs.[2] Hence, the considerable interest to search for potential anticancer drugs has led to the discovery of small synthetic molecules with anti-carcinogenic activity and limited harmful side effects.

Indole-based derivatives are commonly used heterocycles and have been identified in various biological products as potential antibacterial, anticancer, antiviral agents and protein inhibitors [3-5] and play a crucial role in medicinal chemistry and agrochemistry fields. Many natural and synthetic bioactive indolyl heterocycles like 5-(3-indolyl)-oxazoles (**1a**), indolylthiazoles (**1b**) are known for their cytotoxic activities and Meridianin (**2**) (Fig. 1) has shown good anticancer activity against MCF-7 breast cancer cell lines.[6]

Phosphoramidates play an important role in a wide variety of structurally diverse natural and biologically active compounds, from glycolipids to nucleic acids.[7] In nucleotide and oligonucleotide chemistry, phosphoramidites and phosphoramidates occupy a well established position. Nucleoside phosphoramidates [8] are commonly used as basic building blocks in modern chemical synthesis of oligonucleotides, modified oligonucleotides bearing either 3^1-5^1-P-N bonds.[9] Phosphoramidates are often used as prodrugs to increase the water solubility and hence increase the bio-availability of the drug.[10] A series of nucleoside 5^1-phosphoramidates and

nucleoside 5[1]-thiophosphoramidates were designed to act as membrane-soluble prodrugs of bioactive free nucleotides and showed selective anti-HIV activity in MT-4 cells.[11] Based on the above facts and extensive biological nature of the indole nucleus and phosphoramidates and in continuation of our research on the design of biologically active new phosphorus molecules,[12] we are interested in the design of some new hybrid molecules that comprise two pharmacophores in a single molecule with an intention to enhance the biological activity. We synthesized thus a series of novel *N,N′*-disubstituted ethyl *P*-[2-(1*H*-indol-3-yl)acetyl]phosphoramidates with moderate to high yields from indoleacetic acid and diethyl substituted cyclic/phenyl/heterocyclic phosphoramidites (**7a-m**). The newly synthesized molecules were tested for their anticancer activity in two human cell lines MCF-7 (breast cancer) and HeLa (Cervical cancer) cell lines using the MTT assay.

RESULTS AND DISCUSSION

Chemistry

A series of 13 novel *N,N′*-disubstituted acylphosphoramidate derivatives containing an indole nucleus were synthesized. The synthetic route is shown in Scheme 1. The target molecules, *N,N′*-disubstituted ethyl *P*-[2-(1*H*-indol-3-yl)acetyl]benzyl, -aryl, cyclic, or heterocyclic phosphonamidates (**8a-m**) were synthesized by the Michaelis-Arbuzov reaction. The title compounds were prepared in three-steps. In the first-step, indole-3-acetic acid (**3**) was reacted with thionyl chloride in anhydrous THF at 0-10 °C followed by 40 °C to convert the acid into the acid chloride (**4**).[13] Diethyl substituted cyclic, phenyl, or heterocyclic phosphoramidites (**7a-m**) were prepared by the reaction of various cyclic, phenyl, and heterocyclic amines (**5a-m**)

with diethyl chlorophosphite (**6**) in the presence of TEA at 20-40 °C under N_2 atmosphere. Finally, the intermediates (**7a-m**) were reacted (Michaelis-Arbuzov reaction) with indole-3-acetyl chloride (**4**) at 40-50 °C to afford the title compounds (**8a-m**). The keto phosphoramidates (**8a-m**) were obtained in moderate to high yields. The reason might be that these molecules enhance the nucleophilicity of the trivalent phosphorus atom which led to enhanced reactivity in the Michaelis-Arbuzov reaction. The chemical structures of the title compounds were established by elemental analysis, IR, NMR (^{1}H, ^{13} C, ^{31}P) spectroscopy and mass spectral data.

Spectroscopic Data

The appearance of bands at 1212-1234 cm^{-1} of the IR spectra confirmed the formation of a P=O function during the Michaelis-Arbuzov reaction. The bands at 3330-3390 cm^{-1} (NH) and 1610-1660 cm^{-1} (C=O), confirmed the presence of these functional groups in the title compounds.[14] In the ^{1}H NMR spectra, the chemical shifts of one ethoxy group linked to phosphorus were observed at 1.30-1.60 ppm for 3H (t), 3.50-4.80 ppm for 2H (q), 6.85-7.60 ppm for aromatic protons, 9.90-10.80 ppm for NH (s). The ^{13}C NMR signals at 189.0-181.2 ppm for C=O confirmed the formation of the title compounds. In the ^{31}P NMR spectra, the appearance of signals at δ 21.4 to 26.8 confirms the nature of pentavalent phosphorus.[15] In addition, the expected ^{13}C NMR chemical shift values, the molecular ion peaks and fragmented daughter ion peaks in their mass spectra and the composition correlation of C, H, N elements in their elemental analysis provided further evidence for the structure of title compounds (**8a-m**).

Anticancer activity

The newly synthesized *N,N′*-disubstituted ethyl P-[2-(1*H*-indol-3-yl)acetyl] benzyl/aryl/cyclic/hetrocyclic phosphonamidates (**8a-m**) were evaluated for their anticancer activity against two human cell lines MCF-7 (breast cancer) and HeLa (Cervical cancer) cell lines by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay[24] using doxorubicin as a positive control. The results are presented in Tables S 1 and S 2 (Supplemental Materials). The IC_{50} value represents the drug concentration required to inhibit the cell growth by 50%. Most of the title compounds exhibited good anticancer activity against breast and cervical cancer cell lines at 5 µM concentration and reasonably low IC_{50} values (Table 2). Among the title compounds, **8i**, **8j**, **8k** and **8l** exhibited good inhibitory activity against the two human cell lines (MCF-7 and HeLa) when compared with doxorubicin. The compound **8i** exhibited better inhibitory activity compared with the remaining title compounds and nearly equal activity at 5 µM concentration when compared (Table S 1) with the positive control. The title compounds **8i**, **8j**, **8k** and **8l** showed an inhibitory effect against MCF-7 with IC_{50} values 5.8±0.82, 6.2±2.20, 6.6±1.28 and 8.8±2.14 comparable with doxorubicin (IC_{50} = 5.4±1.26). The compounds **8i**, **8j**, **8k** and **8l** showed 50% inhibitory activities against HeLa cell lines with IC_{50} values 14.8±1.42, 15.2±1.68, 15.6±2.12 and 16.4±3.20, compared with doxorubicin (14.2±1.52). The compound **8i** has nearly the same IC_{50} value (14.8) when compared with doxorubicin (14.2). Activity results proved that the aromatic, heterocyclic, or acyclic amines substituents at the indole ring of the ketophosphoramidates play a crucial role in imparting the anticancer activity. Hence, **8i** might be considered as a lead molecule among the title compounds.

CONCLUSIONS

In summary, a synthesis of a series of new hybrid molecules, *N,N′*-disubstituted ethyl P-[2-(1*H*-indol-3-yl)acetyl]phosphoramidates derivatives (**8a-m**) was accomplished for biological interest that encompasses two pharmacophoric units such as the indolyl and the phosphoramidite motif with incorporation of biologically active molecules/functional groups in single compound. Cytotoxicity was evaluated for the title compounds (**8a-m**) against two human cell lines, MCF-7 (breast cancer) and HeLa (Cervical cancer) cell lines using MTT assay and IC_{50} values were also determined. The structure-activity studies demonstrated that molecules such as fluorophenylpiperazinyl, pyrimidyl, nitrophenylpiperazinyl and chlorophenylpiperazinyl linked to basic indolylphosphoramidates were mainly influenced the anticancer activity. The majority of the title compounds exhibited good anticancer activity, where as the compounds **8i**, **8j**, **8k** and **8l** displayed promising activity. The results endowed with a foundation for future design and development of anticancer agents and excellent contribution for the phosphorus chemistry.

EXPERIMENTAL

All chemicals and reagents used for the synthesis were purchased from Sigma-Aldrich. All solvents used for spectroscopic and other physical studies were reagent grade and were further purified by literature methods.[16] Melting points (m. p.) were obtained with a digital Guna melting apparatus and are uncorrected. IR spectra (ν_{max}/cm^{-1}) were recorded on a Perkin Elmer 283 unit as KBr discs. ^{1}H, ^{13}C, ^{31}P NMR spectra were recorded on a Bruker 400 NMR spectrometer operating at 400 MHz for ^{1}H, 100 MHz for ^{13}C and 161.9 MHz for ^{31}P NMR using DMSO-d_6 as solvent and TMS as internal standard for ^{1}H and ^{13}C, and 85% H_3PO_4 as external

standard for ^{31}P. Chemical shifts (δ) and coupling constants (J) are reported in ppm and Hz respectively. Standard abbreviations indicating multiplicity are used as follows: 's' for singlet, 'd' for doublet, 'dd' for doublet of doublets, 't' for triplet, 'q' for quartet, 'm' for multiplet and 'br' for broad. LC-MS spectra were recorded on a Jeol SX 102 DA/600 Mass spectrometer. Elemental analyses were performed on a Thermo Finnigan Instrument at University of Hyderabad, Hyderabad, India. Analytical thin-layer chromatography (TLC) was carried out on precoated plates and spots were visualized with UV light.

General procedure for the synthesis of the title compounds (8a-m)

Indole-3-acetic acid (IAA) (**3**) (1 mmol, 0.18 g) was taken in a 50 mL round-bottomed flask containing 10 mL of THF. $SOCl_2$ (1.2 mmol, 0.1 mL) was added drop-wise with stirring at 0-5 °C. The reaction mixture was stirred for 2 h at 10-40 °C to afford 2-(1*H*-indol-3-yl)acetyl chloride (**4**) and the completion of the reaction was checked by TLC. Diethyl phosphorochloridite (**6**) (1.2 mmol, 0.12 mL) was dissolved in 5 mL of THF and Et_3N (1.2 mmol, 0.17 mL) was added and the contents were flushed with N_2. Thiophen-2-ylmethanamine (**5a**) (1 mmol, 0.12 g) in 5 mL of THF was added drop-wise to **6** under a N_2 atmosphere at 20-40 °C. The completion of the reaction was monitored by TLC. The precipitated $Et_3N{\cdot}HCl$ was filtered to get diethyl thiophen-2-ylmethylphosphoramidite (**7a**). Finally, to this filtrate (**7a**) a solution of 2-(1*H*-indol-3-yl)acetyl chloride (**4**) was added at 20 °C and the reaction mixture was stirred at 40-50 °C for 3 h under a N_2 atmosphere. After completion of the reaction as indicated by TLC, the reaction mixture was concentrated under reduced pressure to afford crude ethyl *P*-[2-(1*H*-indol-3-yl)ethyl]-*N*-(thiophen-2-ylmethyl)phosphoramidate (**8a**). The crude product was

purified by column chromatography using 4:1 ethyl acetate: hexane as eluent. The same successful procedure was employed for the preparation of the remaining title compounds **8b-m**. Representative 1H, 13C and 31P NMR spectra for compound 8a are presented in the Supplemental Materials (Figures S 1 – S 3)

Ethyl *P*-[2-(1*H*-indol-3-yl)acetyl]-*N*-(thiophen-2-ylmethyl)phosphoramidate (8a). Yield: 80%; Brown solid, mp. 192-194 °C, IR: 3380 ($NH_{Aromatic}$), 1624 (C=O), 1218 (P=O), 914 (P-O-$C_{aliphatic}$), 872 (P-$NH_{aliphatic}$); ^{1}H NMR: δ 10.24 (1H, NH), 7.54 (d, 1H, J = 5.2 Hz, ArH), 7.45 (d, 1H, J = 6.6 Hz, ArH), 7.37-7.16 (m, 3H, ArH), 7.05-6.85 (m, 3H, Ar'H), 4.56 (qt, 2H, J = 6.4 Hz, P-O$\underline{CH_2}CH_3$), 3.83 (d, 2H, J = 5.4 Hz, NH$\underline{CH_2}$), 3.54 (s, 2H, CH_2), 2.26 (t, 1H, J = 6.2 Hz, $\underline{NH}CH_2$), 1.53 (t, 3H, J = 4.2 Hz, P-OCH$_2\underline{CH_3}$); ^{13}C NMR: δ 186.0 (C-11), 136.2 (C-8), 128.2 (C-4'), 127.2 (C-3'), 126.6 (C-9), 126.2 (C-5'), 125.6 (C-2'), 123.2 (C-2), 121.5 (C-6), 119.4 (C-5), 118.8 (C-4), 112.7 (C-7), 108.2 (C-3), 64.2 (C-12), 38.4 (C-14), 17.4 (C-10), 16.1 (C-13); ^{31}P NMR: δ 22.6; LC-MS *m/z* (%): 360 (100) [M^+]; Anal. Calcd. for $C_{17}H_{19}N_2O_3PS$: C, 56.66; H, 5.87; N, 15.55. Found: C, 56.61, H, 5.84; N, 15.52.

Ethyl *P*-[2-(1*H*-indol-3-yl)acetyl]-*N*-(pyridin-3-ylmethyl)phosphoramidate (8b)

Yield: 79%; Black solid, mp. 201-203 ºC, IR (υ_{max}, KBr, cm^{-1}): 3360 ($NH_{Aromatic}$), 1632 (C=O), 1222 (P=O), 916 (P-O-$C_{aliphatic}$), 872 (P-$NH_{aliphatic}$); ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.34 (s, 1H, NH), 7.51 (d, 1H, J = 4.4 Hz, ArH), 7.38 (d, 1H, J = 4.6 Hz, ArH), 7.48-7.25 (m, 3H, ArH), 7.02-6.88 (4H, m, Ar'H), 4.61 (qt, 2H, J = 4.2 Hz, P-O$\underline{CH_2}CH_3$), 3.78 (d, 2H, J = 5.2 Hz, NH$\underline{CH_2}$), 3.62 (s, 2H, ArCH_2), 2.24 (t, 1H, J = 5.4 Hz, $\underline{NH}CH_2$), 1.60 (3H, t, J = 6.4 Hz, P-OCH$_2\underline{CH_3}$); ^{13}C NMR (100 MHz, DMSO-d_6): 188.4 (C-11), 146.7 (C-6'), 136.5 (C-5'), 136.4 (C-

8), 128.6 (C-4'), 127.5 (C-3'), 127.2 (C-9), 126.2 (C-2'), 123.4 (C-2), 122.1 (C-6), 119.7 (C-5), 118.6 (C-4), 113.2 (C-7), 109.4 (C-3), 64.8 (C-12), 39.2 (C-14), 17.6 (C-10), 16.7 (C-13); ^{31}P NMR (161.9 MHz, DMSO-d_6): δ 21.4; LC-MS m/z (%): 357 (100) [$M^{+\cdot}$]; Anal. Calcd. for $C_{18}H_{20}N_3O_3P$: C, 60.50; H, 5.64; N, 11.76. Found: C, 60.48, H, 5.62; N, 11.73.

Ethyl *N*-[2-(1*H*-imidazol-4-yl)ethyl-*P*-[(2-(1H-indol-3-yl)acetyl]phosphoramidate (8c)

Yield: 78%; Light brown solid, mp. 195-197 ºC, IR (υ_{max}, KBr, cm^{-1}): 3358 ($NH_{aromatic}$), 1652 (C=O), 1224 (P=O), 918 (P-O-$C_{aliphatic}$), 881 (P-$NH_{aliphatic}$); ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.24 (s, 1H, $NH_{imidazole}$), 10.37 (s, 1H, NH), 7.56 (d, 1H, *J* = 6.1 Hz, ArH), 7.52 (d, 1H, *J* = 5.6 Hz, Ar'H), 7.42 (d, 1H, *J* = 4.4 Hz, Ar'H), 7.36 (d, 1H, *J* = 5.2 Hz, Ar-H), 7.30-7.12 (m, 3H, ArH), 4.54 (qt, 2H, *J* = 6.4 Hz, P-O$\underline{CH_2}CH_3$), 3.72 (s, 2H, $ArCH_2$), 3.62-3.51 (m, 2H, NH$\underline{CH_2}CH_2$), 3.01 (t, 2H, *J* =5.8 Hz, $NHCH_2\underline{CH_2}$), 2.54 (t, 1H, *J* = 4.0 Hz, $\underline{NH}CH_2$), 1.57 (t, 3H, *J* = 5.2 Hz, P-$OCH_2\underline{CH_3}$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 189.0 (C-11), 136.4 (C-8), 136.2 (C-2'), 132.5 (C-4'), 127.2 (C-9), 123.4 (C-2), 122.1 (C-6), 119.7 (C-5), 118.6 (C-4), 116.2 (C-5'), 113.2 (C-7), 109.4 (C-3), 64.8 (C-12), 42.5 (C-14), 32.6 (C-15), 17.6 (C-10), 16.7 (C-13); ^{31}P NMR (161.9 MHz, DMSO-d_6): δ 25.2; LC-MS m/z (%): 360 (100) [$M^{+\cdot}$]; Anal. Calcd. for $C_{17}H_{21}N_4O_3P$: C, 56.66; H, 5.87; N, 15.55. Found: C, 56.62, H, 5.85; N, 15.52.

Ethyl *P*-[2-(1*H*-indol-3-yl)acetyl]-*N*-(4-fluorobenzyl)phosphoramidate (8d)

Yield: 82%; Reddish brown solid, mp. 166-168 ºC, IR (υ_{max}, KBr, cm^{-1}): 3338 ($NH_{aromatic}$), 1654 (C=O), 1228 (P=O), 914 (P-O-$C_{aliphatic}$), 882 (P-$NH_{aliphatic}$); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 10.72 (s, 1H, NH), 7.52 (d, 2H, *J* = 5.8 Hz, Ar'H), 7.48 (d, 1H, *J* = 5.8 Hz, ArH), 7.42 (d, 2H, *J* = 5.4 Hz, Ar'H), 7.38 (d, 1H, *J* = 6.2 Hz, ArH), 7.28-6.84 (m, 3H, ArH),

4.52 (qt, 2H, *J* = 4.4 Hz, P-O$\underline{CH_2}CH_3$), 3.64 (s, 2H, ArCH_2), 3.56 (d, 2H, *J* = 4.4 Hz, NH$\underline{CH_2}$), 2.62 (t, 1H, *J* = 4.6 Hz, $\underline{NH}CH_2$), 1.59 (3H, t, *J* = 3.6 Hz, P-OCH$_2\underline{CH_3}$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 185.0 (C-11), 160.2 (C-1'), 137.2 (C-8), 136.2 (C-4'), 127.5 (C-5'), 127.3 (C-9), 126.2 (C-3'), 123.6 (C-2), 122.3 (C-6), 119.6 (C-5), 118.9 (C-4), 116.4 (C-6'), 115.6 (C-2'), 114.5 (C-7), 109.6 (C-3), 64.4 (C-12), 42.5 (C-14), 42.4 (C-15), 17.2 (C-10), 16.6 (C-13); ^{31}P NMR (161.9 MHz, DMSO-d_6): δ 25.6; LC-MS m/z (%): 374 (100) [$M^{+\cdot}$]; Anal. Calcd. for $C_{19}H_{20}FN_2O_3P$: C, 60.96; H, 5.39; N, 7.48. Found: C, 60.92, H, 5.37; N, 7.45.

Ethyl *P*-[2-(1*H*-indol-3-yl)acetyl]-*N*-(3-chlorobenzyl)phosphoramidate (8e)

Yield: 84%; Black solid, mp. 151-153 ºC, IR (υ_{max}, KBr, cm^{-1}): 3332 ($NH_{aromatic}$), 1610 (C=O), 1212 (P=O), 910 (P-O-$C_{aliphatic}$), 862 (P-$NH_{aliphatic}$); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.72 (s, 1H, NH), 7.48 (d, 1H, *J* = 5.4 Hz, ArH), 7.36 (d, 1H, *J* = 4.4 Hz, ArH), 7.34 (d, 2H, *J* = 5.2 Hz, Ar'H), 7.32-7.15 (m, 3H, ArH), 7.12 (d, 2H, *J* = 5.4 Hz, Ar'H), 4.48 (qt, 2H, *J* = 6.4 Hz, P-O$\underline{CH_2}CH_3$), 3.68 (s, 2H, ArCH_2), 3.48 (d, 2H, *J* = 5.6 Hz, NH$\underline{CH_2}$), 2.49 (t, 1H, *J* = 5.8 Hz, $\underline{NH}CH_2$), 1.47 (3H, t, *J* = 5.6 Hz, P-OCH$_2\underline{CH_3}$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 187.0 (C-11), 143.5 (C-5'), 137.7 (C-8), 134.2 (C-1'), 130.4 (C-6'), 127.8 (C-9), 126.6 (C-2'), 126.2 (C-3'), 124.4 (C-4'), 123.2 (C-2), 122.5 (C-6), 120.2 (C-5), 118.4 (C-4), 114.6 (C-7), 109.3 (C-3), 65.2 (C-12), 43.6 (C-14), 42.8 (C-15), 17.5 (C-10), 16.5 (C-13); ^{31}P NMR (161.9 MHz, DMSO-d_6): δ 23.0; LC-MS m/z (%): 390 (100) [$M^{+\cdot}$], 392 (33) [M+2]; Anal. Calcd. for $C_{19}H_{20}ClN_2O_3P$: C, 58.39; H, 5.16; N, 7.17. Found: C, 58.37, H, 5.13; N, 7.14.

Ethyl *P*-[2-(1*H*-indol-3-yl)acetyl-*N*-(3,4-dihydroxyphenethyl)phosphoramidate (8f)

Yield: 77%; Brown solid, mp. 195-197 ºC, IR (υ_{max}, KBr, cm^{-1}): 3347 ($NH_{aromatic}$), 1628 (C=O), 1216 (P=O), 919 (P-O-$C_{aliphatic}$), 874 (P-$NH_{aliphatic}$); ^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 10.62 (s, 1H, NH), 9.43 (s, 1H, Ar'OH), 9.32 (s, 1H, Ar'OH), 7.55 (d, 1H, *J* = 6.4 Hz, ArH), 7.37 (d, 1H, *J* = 6.2 Hz, ArH), 7.35-7.14 (m, 3H, ArH), 6.94 (d, 1H, *J* = 5.6Hz, Ar'H), 6.87 (d, 1H, *J* = 6.4 Hz, Ar'H), 6.82 (s, 1H, Ar'H), 4.51 (qt, 2H, *J* = 5.2 Hz, P-O$\underline{CH_2}CH_3$), 3.46 (s, 2H, ArCH_2), 2.72 (s, 2H, HN$\underline{CH_2}$), 2.68 (t, 2H, *J* =4.8 Hz, N$CH_2\underline{CH_2}$), 2.45 (t, 1H, *J* = 5.8 Hz, $\underline{NH}CH_2$), 1.37 (3H, t, *J* = 5.4 Hz, P-O$CH_2\underline{CH_3}$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 182.4 (C-11), 145.2 (C-3'), 143.5 (C-4'), 137.7 (C-8), 135.4 (C-1'), 126.5 (C-9), 124.2 (C-6), 122.5 (C-2), 122.3 (C-6'); 121.6 (C-5), 118.6 (C-4), 117.4 (C-7), 116.4 (C-2'), 116.2 (C-5'), 107.4 (C-3), 64.5 (C-12), 47.4 (C-14), 39.2 (C-15), 17.8 (C-10), 17.7 (C-13); ^{31}P NMR (161.9 MHz, DMSO-d_6): δ 22.8; LC-MS m/z (%): 402 (100) [$M^{+\cdot}$]; Anal. Calcd. for $C_{20}H_{23}N_2O_5P$: C, 59.70; H, 5.76; N, 6.96. Found: C, 59.67, H, 5.74; N, 6.95.

Ethyl 2-(1*H*-indol-3-yl)acetyl(4-methylpiperazin-1-yl)phosphinate (8g)

Yield: 83%; Black solid, mp. 146-148 ºC, IR (υ_{max}, KBr, cm^{-1}): 3330 ($NH_{aromatic}$), 1642 (C=O), 1220 (P=O), 914 (P-O-$C_{aliphatic}$), 856 (P-$N_{aliphatic}$); ^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 9.90 (s, 1H, NH), 7.45 (d, 1H, *J* = 6.2 Hz, ArH), 7.42 (d, 1H, *J* = 5.6 Hz, ArH), 7.32-6.88 (m, 3H, ArH), 3.68 (s, 2H, ArCH_2), 3.50 (qt, 2H, *J* = 5.2 Hz, P-O$\underline{CH_2}CH_3$), 2.48 (s, 2H, N$\underline{CH_2}$), 2.38 (t, 2H, *J* = 4.4 Hz, N$\underline{CH_2}CH_2$), 2.35 (t, 2H, *J* = 4.2 Hz, N$\underline{CH_2}CH_2$), 2.31 (s, 2H, N$\underline{CH_2}$), 1.82 (s, 3H, NCH_3), 1.30 (3H, t, *J* = 4.8 Hz, P-O$CH_2\underline{CH_3}$); ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ 182.0 (C-11), 138.1 (C-8), 125.2 (C-9), 123.7 (C-2), 122.2 (C-6), 120.5 (C-5), 118.7 (C-4), 115.2 (C-7), 108.5 (C-3), 65.3 (C-12), 56.6 (C-3'), 56.3 (C-5'), 47.2 (C-2'), 45.7 (C-6'), 21.4 (C-4'), 17.9 (C-10), 16.1

(C-13); ^{31}P NMR (161.9 MHz, DMSO-d_6): δ 23.5; LC-MS m/z (%): 349 (100) [$M^{+\cdot}$]; Anal. Calcd. for $C_{17}H_{24}N_3O_3P$: C, 58.44; H, 6.92; N, 12.03. Found: C, 58.40, H, 6.89; N, 12.01.

Ethyl 2-(1*H*-indol-3-yl)acetyl(thiomorpholino)phosphinate (8h)

Yield: 85%; Brown solid, mp. 166-168 ºC, IR (υ_{max}, KBr, cm^{-1}): 3342 ($NH_{aromatic}$), 1639 (C=O), 1216 (P=O), 918 (P-O-$C_{aliphatic}$), 851 (P-$N_{aliphatic}$); ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.41 (s, 1H, NH), 7.45 (d, 1H, *J* = 5.7 Hz, ArH), 7.42 (d, 1H, *J* = 5.2 Hz, ArH), 7.36-7.12 (m, 3H, ArH), 3.68 (qt, 2H, *J* = 5.4 Hz, P-O$\underline{CH_2}CH_3$), 3.36 (s, 2H, $ArCH_2$), 2.42 (s, 2H, N$\underline{CH_2}$), 2.37 (s, 2H, N$\underline{CH_2}$), 2.34 (t, 2H, *J* = 4.0 Hz, N$\underline{CH_2}CH_2$), 2.28 (t, 2H, *J* = 3.8 Hz, N$\underline{CH_2}CH_2$), 1.50 (3H, t, *J* = 4.6 Hz, P-$OCH_2\underline{CH_3}$); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 183.0 (C-11), 138.4 (C-8), 125.6 (C-9), 122.8 (C-2), 122.6 (C-6), 121.2 (C-5), 118.6 (C-4), 115.5 (C-7), 108.9 (C-3), 65.6 (C-12), 46.5 (C-6'), 46.2 (C-2'), 29.6 (C-3'), 29.3 (C-5'), 17.5 (C-10), 16.4 (C-13); ^{31}P NMR (161.9 MHz, DMSO-d_6): δ 24.2; m/z (%): 352 (100) [$M^{+\cdot}$]; Anal. Calcd. for $C_{16}H_{21}N_2O_3PS$: C, 54.53; H, 6.01; N, 7.95. Found: C, 54.51, H, 5.97; N, 7.92.

Ethyl 2-(*1*H-indol-3-yl)acetyl[4-(4-fluorophenyl)piperazin-1-yl]phosphinate (8i)

Yield: 86%; Dark brown solid, mp. 175-177 ºC, IR (υ_{max}, KBr, cm^{-1}): 3390 ($NH_{aromatic}$), 1635 (C=O), 1232 (P=O), 912 (P-O-$C_{aliphatic}$), 847 (P-$N_{aliphatic}$); ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.80 (s, 1H, NH), 7.38 (d, 1H, *J* = 5.8 Hz, ArH), 7.32 (d, 1H, *J* = 5.4 Hz, ArH), 7.30-7.18 (m, 3H, ArH), 7.12 (d, 2H, *J* = 6.4 Hz, Ar'H), 6.78 (d, 2H, *J* = 6.2 Hz, Ar'H), 4.32 (qt, 2H, *J* = 5.2 Hz, P-O$\underline{CH_2}CH_3$), 3.18 (s, 2H, $ArCH_2$), 2.32 (s, 2H, N$\underline{CH_2}$), 2.27 (s, 2H, N$\underline{CH_2}$), 2.18 (t, 2H, *J* = 4.4 Hz,N$\underline{CH_2}CH_2$), 2.14 (t, 2H, *J* = 4.6 Hz, N$\underline{CH_2}CH_2$), 1.44 (3H, t, *J* = 5.4 Hz, P-$OCH_2\underline{CH_3}$);

^{13}C NMR (100 MHz, DMSO-d_6): δ 181.4 (C-11), 156.5 (C-10'), 145.6 (C-7'), 138.1 (C-8), 125.7 (C-9), 123.4 (C-2), 122.2 (C-6), 121.6 (C-5), 118.4 (C-4), 117.4 (C-9'), 116.4 (C-11'), 116.2 (C-8'), 115.7 (C-7), 115.4 (C-12'), 108.2 (C-3), 65.4 (C-12), 46.8 (C-6'), 46.7 (C-2'), 29.5 (C-5'), 28.6 (C-3'), 17.2 (C-10), 16.2 (C-13); ^{31}P NMR (161.9 MHz, DMSO-d_6): δ 26.8; LC-MS m/z (%): 429 (100) [$M^{+\cdot}$]; Anal. Calcd. for $C_{22}H_{25}FN_3O3P$: C, 61.53; H, 5.87; N, 9.79. Found: C, 61.48, H, 5.83; N, 9.74.

Ethyl 2-(1*H*-indol-3-yl)acetyl[4-(pyrimidin-2-yl)piperazin-1-yl]phosphinate (8j)

Yield: 87%; Reddish brown solid, mp. 144-146 ºC, IR (υ_{max}, KBr, cm^{-1}): 3342 ($NH_{aromatic}$), 1630 (C=O), 1230 (P=O), 920 (P-O-$C_{aliphatic}$), 836 (P-$N_{aliphatic}$); ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.28 (s, 1H, NH), 7.56 (d, 2H, *J* = 5.8 Hz, Ar'H), 7.34 (d, 1H, *J* = 6.2 Hz, ArH), 7.25 (d, 1H, *J* = 5.8 Hz, ArH), 7.32-7.18 (m, 3H, ArH), 7.12 (t, 1H, *J* = 4.4 Hz, Ar'H), 4.38 (qt, 2H, *J* = 3.6 Hz, P-O$\underline{CH_2}CH_3$), 3.26 (s, 2H, $ArCH_2$), 2.32 (s, 2H, N$\underline{CH_2}$), 2.28 (s, 2H, N$\underline{CH_2}$), 2.16 (t, 2H, *J* = 4.4 Hz, N$\underline{CH_2}CH_2$), 2.12 (t, 2H, *J* = 4.6 Hz, N$\underline{CH_2}CH_2$), 1.48 (3H, t, *J* = 4.2 Hz, P-O$CH_2\underline{CH_3}$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 181.2 (C-11), 161.6 (C-7'), 156.8 (C-11'), 156.2 (C-9'), 138.5 (C-8), 125.4 (C-9), 125.2 (C-2), 122.6 (C-6), 121.5 (C-5), 117.5 (C-4), 116.5 (C-10'), 115.8 (C-7), 108.4 (C-3), 64.6 (C-12), 49.8 (C-5'), 49.4 (C-3'), 47.4 (C-2'), 47.0 (C-6'), 17.6 (C-10), 16.5 (C-13); ^{31}P NMR (161.9 MHz, DMSO-d_6): δ 22.8; LC-MS m/z (%): 413 (100) [$M^{+\cdot}$]; Anal. Calcd. for $C_{20}H_{24}N_5O3P$: C, 58.11; H, 5.85; N, 16.94. Found: C, 58.09, H, 5.82; N, 16.92.

Ethyl 2-(1*H*-indol-3-yl)acetyl[4-(4-nitrophenyl)piperazin-1-yl]phosphinate (8k)

Yield: 74%; Brown solid, mp. 174-178 ºC, IR (υ_{max}, KBr, cm^{-1}): 3336 ($NH_{aromatic}$), 1660 (C=O), 1234 (P=O), 924 (P-O-$C_{aliphatic}$), 845 (P-$N_{aliphatic}$); ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.54 (s, 1H, NH), 7.54 (d, 2H, *J* = 6.4 Hz, Ar'H), 7.14 (d, 2H, *J* = 6.6 Hz, Ar'H), 7.42 (d, 1H, *J* = 6.4 Hz, ArH), 7.32 (d, 1H, *J* = 6.2 Hz, ArH), 7.28-7.11 (m, 3H, ArH), 4.80 (qt, 2H, *J* = 4.0 Hz, P-O$\underline{CH_2}CH_3$), 3.14 (s, 2H, ArCH_2), 2.28 (s, 2H, N$\underline{CH_2}$), 2.24 (s, 2H, N$\underline{CH_2}$), 2.14 (t, 2H, *J* = 4.2 Hz, N$\underline{CH_2}CH_2$), 2.12 (t, 2H, *J* = 4.4 Hz, N$\underline{CH_2}CH_2$), 1.56 (3H, t, *J* = 3.6 Hz, P-O$CH_2\underline{CH_3}$), ^{13}C NMR (100 MHz, DMSO-d_6): δ 182.7 (C-11), 155.2 (C-7'), 138.4 (C-8), 136.2 (C-10'), 126.2 (C-11'), 125.3 (C-9), 124.5 (C-9'), 123.5 (C-2), 122.5 (C-6), 121.4 (C-5), 118.7 (C-4), 116.0 (C-7), 112.4 (C-8'), 112.2 (C-12'), 107.6 (C-3), 64.2 (C-12), 46.9 (C-2'), 46.5 (C-6'), 29.7 (C-5'), 28.4 (C-3'), 17.6 (C-10), 16.8 (C-13); ^{31}P NMR (161.9 MHz, DMSO-d_6): δ 24.6; LC-MS m/z (%): 456 (100) [$M^{+\cdot}$]; Anal. Calcd. for $C_{22}H_{25}N_4O5P$: C, 57.89; H, 5.52; N, 12.27. Found: C, 57.84, H, 5.49; N, 12.22.

Ethyl 2-(1*H*-indol-3-yl)acetyl[4-(4-chlorophenyl)piperazin-1-yl]phosphinate (8l)

Yield: 75%; Dark red solid, mp. 184-186 ºC. IR (υ_{max}, KBr, cm^{-1}) 3346 (N-$H_{aromatic}$), 1642 (C=O), 1226 (P=O), 921 (P-O-$C_{aliphatic}$), 864 (P-$N_{aliphatic}$), cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.62 (s, 1H, NH), 7.52 (d, 1H, *J* = 6.4 Hz, ArH), 7.44 (d, 1H, *J* = 6.2 Hz, ArH), 7.36 (d, 2H, *J* = 6.8 Hz, Ar'H), 7.34-7.16 (m, 3H, ArH), 6.74 (d, 2H, *J* = 7.2 Hz, Ar'H), 4.54 (qt, 2H, *J* = 3.8 Hz, P-O$\underline{CH_2}CH_3$), 3.12 (s, 2H, ArCH_2), 2.34 (s, 2H, N$\underline{CH_2}$), 2.26 (s, 2H, N$\underline{CH_2}$), 2.19 (t, 2H, *J* = 4.0 Hz, N$\underline{CH_2}CH_2$), 2.17 (t, 2H, *J* = 4.2 Hz, N$\underline{CH_2}CH_2$), 1.58 (3H, t, *J* = 4.0 Hz, P-O$CH_2\underline{CH_3}$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 185.7 (C-11), 148.2 (C-7'), 138.7 (C-8), 129.5 (C-9'), 129.4 (C-10'), 128.2 (C-11'), 125.6 (C-9), 123.6 (C-2), 122.8 (C-6), 121.5 (C-5), 118.9

(C-4), 116.4 (C-7), 115.6 (C-12'), 106.4 (C-8') 107.3 (C-3), 64.6 (C-12), 47.2 (C-2'), 46.8 (C-6'), 30.2 (C-5'), 29.5 (C-3'), 17.3 (C-13), 17.2 (C-10); ^{31}P NMR (161.9 MHz, DMSO-d_6): δ 23.8; LC-MS m/z (%): 445 (100) [$M^{+\cdot}$], 447 (33) [M+2]; Anal. Calcd. for $C_{22}H_{25}ClN_3O_3P$: C, 59.26; H, 5.65; N, 9.42. Found: C, 59.20, H, 5.62; N, 9.38.

Ethyl 2-(1*H*-indol-3-yl)acetyl[4-(pyridin-2-yl)piperazin-1-yl]phosphinate (8m)

Yield: 76%; Brown, mp. 174-178 ºC, IR (υ_{max}, KBr, cm^{-1}): 3352 (N-H Aromatic), 1648 (C=O), 1226 (P=O), 926 (P-O-$C_{aliphatic}$), 847 (P-$N_{aliphatic}$); ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.54 (s, 1H, NH), 8.06 (t, 1H, *J* = 5.4 Hz, Ar'H), 7.58 (t, 1H, *J* = 5.8 Hz, Ar'H), 7.52 (d, 1H, *J* = 6.2 Hz, Ar'H), 7.38 (d, 1H, *J* = 6.2 Hz, ArH), 7.36 (d, 1H, *J* = 5.8 Hz, ArH), 7.35-7.15 (m, 3H, ArH), 6.74 (d, 1H, *J* = 6.6 Hz, Ar'H), 4.61 (qt, 2H, *J* = 4.2 Hz, P-O$\underline{CH_2}CH_3$), 3.25 (s, 2H, $ArCH_2$), 2.37 (s, 2H, N$\underline{CH_2}$), 2.28 (s, 2H, N$\underline{CH_2}$), 2.21 (t, 2H, *J* = 4.2 Hz, N$\underline{CH_2}CH_2$), 2.19 (t, 2H, *J* = 4.4 Hz, N$\underline{CH_2}CH_2$), 1.64 (3H, t, *J* = 3.8 Hz, P-O$CH_2\underline{CH_3}$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 188.2 (C-11), 158.4 (C-7'), 148.4 (C-11'), 139.4 (C-9'), 138.4 (C-8), 125.8 (C-9), 123.2 (C-6), 122.8 (C-2), 121.8 (C-5), 119.2 (C-4), 117.6 (C-10'), 116.7 (C-7), 107.6 (C-3), 106.4 (C-8'), 64.8 (C-12), 47.4 (C-2'), 46.9 (C-6'), 30.4 (C-5'), 29.8 (C-3'), 17.5 (C-13), 17.4 (C-10); ^{31}P NMR (161.9 MHz, DMSO-d_6): δ 25.2; LC-MS m/z (%): 412 (100) [$M^{+\cdot}$]; Anal. Calcd. for $C_{21}H_{25}N_4O_3P$: C, 59.06; H, 6.00; N, 14.50. Found: C, 59.01, H, 5.97; N, 14.45.

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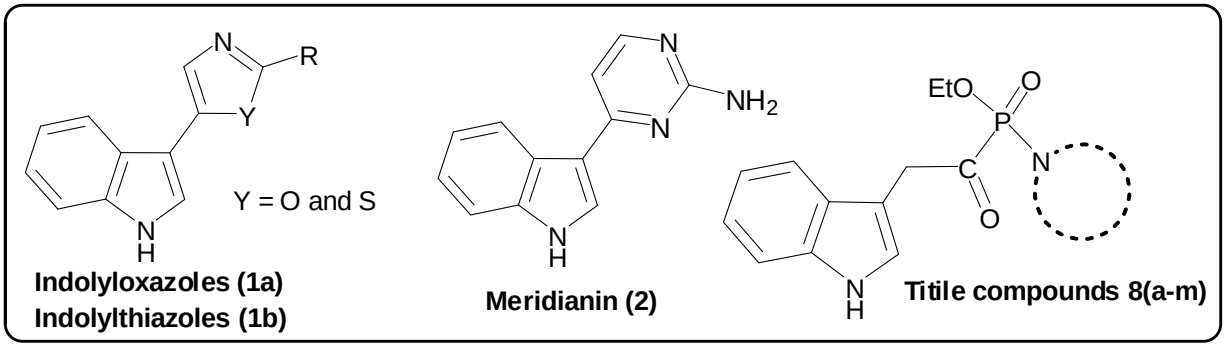
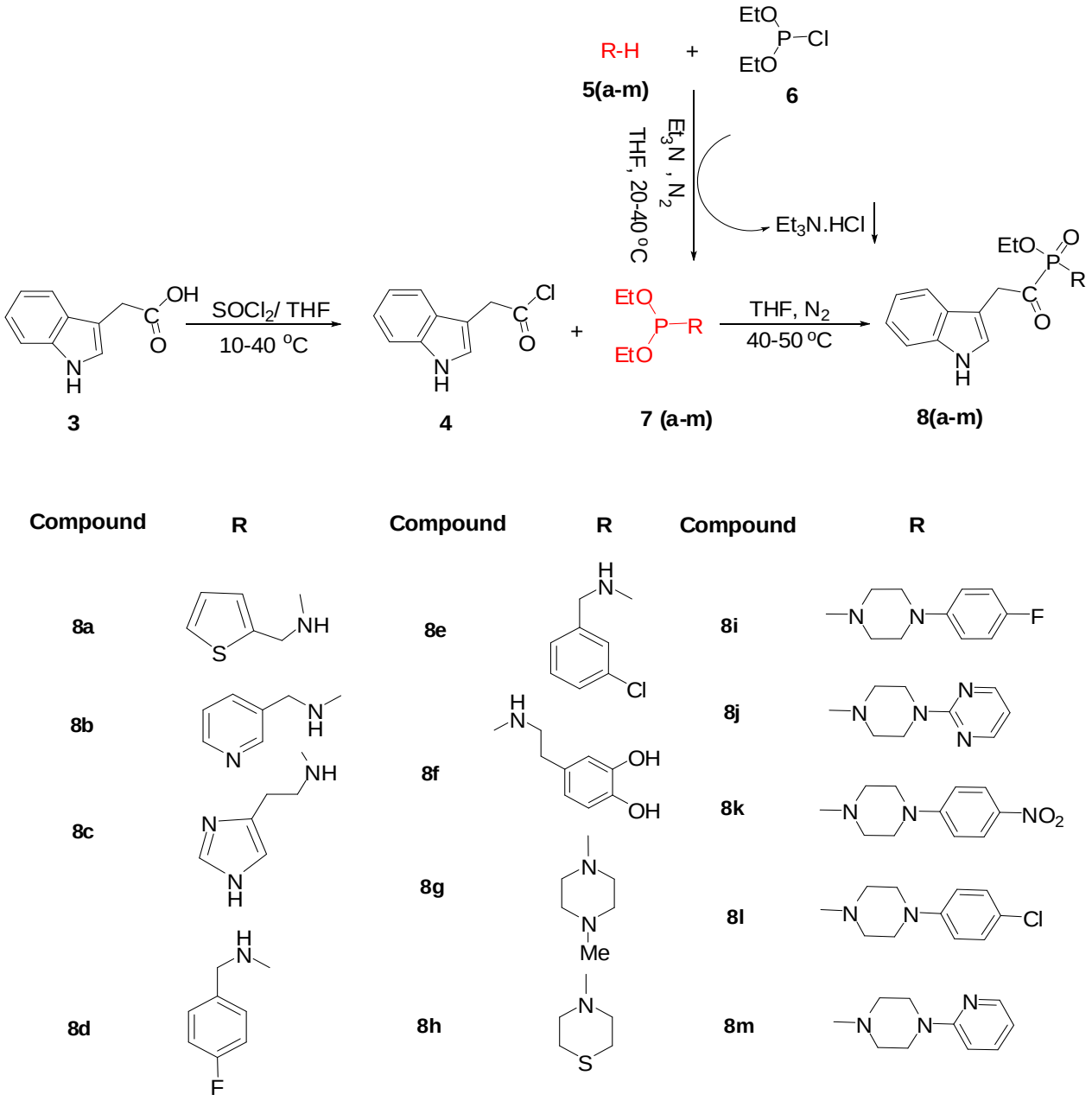


Figure 1. Two biologically active anticancer agents having indole nucleus



Scheme 1. Synthesis of ethyl P-2-(1*H*-indol-3-yl)acetyl-*N*-substituted phosphoramidates **(8a-m).**