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ORIGINAL PAPER

Synthesis and antiproliferative activity of new hybrids bearing neocryptolepine, acridine and α-aminophosphonate scaffolds

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Abstract

Synthesis of novel α-aminophosphonate hybrids **8a–f** was accomplished by the reaction of 11-phenoxy-4-formylneocryptolepine **3**, aminoalkylamino acridines **6a–f** and diphenyl phosphite **7** in the presence of lithium perchlorate as Lewis acid catalyst in methanol. The starting aldehyde **3** was obtained by reaction of 11-chloroneocryptolpine **1** with 4-hydroxybenzaldehyde **2** in the presence of potassium carbonate in DMF, whereas 9-aminoalkylamino acridines **6a–f** were synthesized by reaction of 9-chloroacridine **4** with appropriate diamines **5a–f**. The structure of all synthesized hybrids was confirmed by spectroscopic methods and showed spectra in consistence with the expected structures. The antiproliferative activity of all synthesized hybrids was evaluated against HCT-116, MCF-7, HepG2 and A549 human cancer cell lines. The screened results proved that most of the reported compounds are potent, especially hybrid **8a**, which displayed the highest activity against MCF-7, HepG2 and A549 cancer cell lines with IC_{50} 8.2, 23.1 and 19.4 μM, respectively. This activity is significantly higher than the standard drug doxorubicin. Moreover, hybrid **8f** showed most potent activity against HCT-116 cancer cell line with IC_{50} 2.4 μM higher than the reference drug doxorubicin (IC_{50}:10.90 μM).

Keywords Hybrid · Neocryptolpine · Acridine · α-Aminophosphonate · Antiproliferative activity

Introduction

Natural and synthetic neocryptolepine derivatives are a series of heterocyclic compounds that are of considerable interest for medicinal chemists and are widely used as antimalarial and antitumor agents [1–5]. Neocryptolepine is a potential compound for developing new anticancer drugs due to its planar structure that can bind to DNA by intercalation and inhibition of the DNA-topoisomerases I or II that form the basis of its anticancer activity [6, 7]. Acridine and α-aminophosphonate derivatives have gained attention of medicinal chemists due to their wide range of biological activities, which include antitumor, antiviral, antimicrobial, antiparasitic and fungicidal effects [8–15]. Unfortunately, the use of these classes of biologically important compounds in clinical use has been limited due to adverse effect and drug resistance [16–18]. There is a rising need for development of novel neocryptolepine analogues possessing potent antitumor activities, but with reduced side effects. A new approach to reduce drug resistance is the synthesis of hybrid molecules with enhanced activities [19]. Hybridization of two or three bioactive molecules often leads to increase activity due to synergistic effects. In light of the above-mentioned considerations for anticancer agents with enhanced activity and reduced side effects, the objective of this study was to synthesize potential anticancer compounds that are hybrids of neocryptolepine, acridine and α-aminophosphonate scaffolds (Fig. 1). To examine this proposal, modifications were made to the side chain at the 11-position of neocryptolepine skeleton with the installation of α-aminophosphonate and acridine pharmacophores. The objective was to synthesize neocryptolepine hybrids based on the lead compound reported in the literature, with the aim to fulfill the structure activity relationships as well as to establish how these modifications affected anticancer activity.

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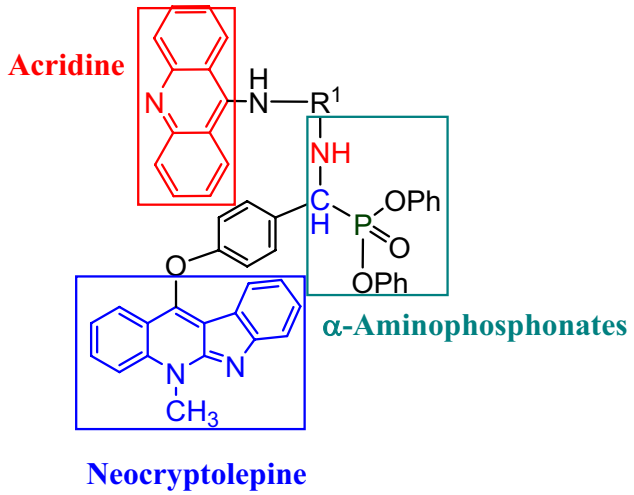


Fig. 1 Structure of hybrids of neocryptolepine, acridine and α-aminophosphonate scaffolds

Materials and methods

Chemistry

General procedure

All ^{1}H-NMR and ^{31}P-NMR experiments were carried out with 500 MHz Jeol and 100 MHz Jeol for ^{13}C-NMR at NMR unit, Mansoura University. In addition, some of ^{1}H-NMR samples were measured with 400 MHz Varian at the main chemical warfare laboratories, Ministry of Defense. Chemical shifts were reported in part per million (ppm) relative to the respective solvent. The mass spectrometry experiments were recorded on thermos scientific trace 1310 gas chromatograph at Fungi National Centre, Al-Azhar University, and IR spectroscopy was performed at Cairo University. Melting points (m.p) were recorded on Stuart scientific melting point apparatus and are uncorrected. The anticancer activity was executed at the National Research Center, Cairo, Egypt. All reactions were followed by thin-layer chromatography (TLC) on silica gel F254 precoated plates (Merck). Solvents were used as received without further purification. The starting materials were either commercially available or synthesized as 11-chloroneocryptolepine [1], 9-chloro acridine [9] and 9-hydrazinylacridine [20] according to the literature procedures.

Synthesis of 11-phenoxy-4-formylneocryptolepine (3)

To 11-chloroneocryptolpine **1** (1 g, 3.75 mmol) were added 4-hydroxybenzaldehyde **2** (0.92 g, 7.50 mmol) and potassium carbonate (1.04 g, 7.50 mmol) in DMF (5 mL). The reaction mixture was refluxed and monitored by TLC until it finished, after 24 h. The mixture was cooled, then poured into ice water, filtered off and dried to yield yellow solid of 11-phenoxy–4–formyl neocryptolepine **3.**

4-[(5-Methyl-5H indolo[2,3-b]quinolin-11-yl)oxy] benzaldehyde (3)

Yellow solid, Yield: (0.93 g, 70%), m.p: 108–110 °C, **IR** (KBr) cm^{-1} υ: 2850 (CH), 1678 (C=O), 1635 (C=C, Ar) 1570 (C=N). **^{1}H-NMR** (DMSO-d$_6$ 400 MHz) δ: 9.64 (s, 1H, CHO), 8.55–7.33 (m, 12H, CH$_{Ar}$), 4.34 (s, 3H, CH$_3$), **^{13}C-NMR**(DMSO-d$_6$,100 MHz)δ:192.11, 172.58,157.41,147.78,154.20,152.37,138.83,131.26,127.52,126.53,124.16, 121.62,120.86, 119.37,117.37,116.02, 43.96. **EIMS**, m/z ($C_{23}H_{16}N_2O_2$) calcd, 352.39 [M] $^{+}$; found, 352.12.

Synthesis of aminoalkylamino acridine derivatives (6a-f)

To 9-chloroacridine **4** (0.12 g, 1 mmol) was added an excess amount of appropriate amines **5a–f** (3 mmol); then, the reaction mixture was refluxed for 1–2 h, until the complete consumption of the starting materials occurred as monitored by TLC. The mixture was cooled and poured into ice water, filtered off and dried to yield the desired product **6a–f.**

N^{1}-(acridin-9-yl)ethane-1,2-diamine (6b)

Yellow solid, yield: (0.15 g, 63%), m.p: 82–84 °C, **IR** (KBr) cm^{-1} υ: 3402 (NH), 3340, 3286(NH$_2$), 2823(C–H), 1635 (C=C, Ar), 1558(C=N). **^{1}H-NMR** (DMSO d$_6$ 500 MHz) δ: 8.40–7.18 (m, 8H, CH$_{Ar}$), 3.97 (br.s, 2H, CH$_2$), 3.02 (m, 2H, CH$_2$). **^{13}C-NMR**(DMSO-d$_6$,100 MHz) δ:153.36, 149.21, 131.61, 130.09, 126.59, 123.88, 121.65, 46.24, 40.52. **EIMS**, m/z ($C_{15}H_{15}N_3$) calcd, 237.31 [M]$^{+}$; found, 237.18.

N^{1}-(acridin-9-yl)propane-1,3-diamine (6c)

Yellow solid, yield: (0.15 g, 60%), m.p: 88–90 °C, **IR** (KBr) cm^{-1} υ: 3417 (NH, NH$_2$ overlap), 1643 2924(C–H), 1643(C=C, Ar), 1604(C=N). **^{1}H-NMR** ($CDCl_3$, 500 MHz), δ ppm: 11.62(s, 1H, NH), 11.45 (s, 2H, NH$_2$), 8.00–7.42 (m, 8H, CH$_{Ar}$), 3.64 (br.s, 2H, CH$_2$), 2.42 (m, 2H, CH$_2$), 1.61 (m, 2H, CH$_2$). **^{13}C-NMR**(DMSO-d$_6$,100 MHz)δ:161.53, 142.30, 129.75, 127.82, 126.38, 120.81, 114.84, 41.32, 39.42, 31.54. **EIMS**, m/z ($C_{16}H_{17}N_3$) calcd, 251.33 [M] $^{+}$; found 251.02.

N^1-(acridin-9-yl)-N^2-(2-aminoethyl) ethane-1,2-diamine. (6d)

Brown solid, yield: (0.15 g, 54%), m.p: 160–162 °C, **IR** (KBr) cm^{-1} υ: 3363(NH, NH_2 overlap), 2939 (C–H), 1620 (C=C, Ar), 1566(C=N). **^{1}H-NMR** ($CDCl_3$, 500 MHz) δ ppm: 7.80–7.29 (m, 8H, CH_{Ar}), 4.24 (br.m, 2H, CH_2), 3.90 (br.m, 4H, CH_2), 2.92 (br.m, 2H, CH_2).**^{13}C-NMR**(DMSO-d_6,100 MHz) δ:154.34, 148.23, 132.41, 130.16, 127.59, 121.88, 120.52, 52.37, 50.31, 43.91, 40.71. **EIMS**, m/z ($C_{17}H_{20}N_4$) calcd, 280.38 $[M]^+$; found 280.03.

N^1-(acridin-9-yl)-N^3-(3-aminopropyl) propane-1,3-diamine (6e)

Brown solid, yield: (0.17 g, 51%), m.p: 148–150 °C, **IR** (KBr) cm^{-1} υ: 3436 + 3286(NH, NH_2 overlap), 2931 (C–H).1627 (C=C, Ar), 1573(C=N). **^{1}H-NMR** ($CDCl_3$, 500 MHz) δ: 11.97 (s, 1H, NH), 11.43 (s, 1H, NH), 11.09 (s, 2H, NH_2), 8.01–7.32 (m, 8H, CH_{Ar}), 4.22 (br.m, 2H, CH_2), 3.36 (br.m, 6H, CH_2), 2.28 (br. m, 4H, CH_2). **^{13}C-NMR** (DMSO-d_6,100 MHz)δ:162.43, 141.34, 130.03, 129.82, 125.38, 121.81, 115.74,46.35,41.62,39.44,31.96, 29.25. **EIMS**, m/z ($C_{19}H_{24}N_4$) calcd, 308.43 $[M]^+$; found 308.25.

N-(3-(4-(3-aminopropyl)piperazin-1-yl)propyl) acridin-9-amine (6f)

Yellow solid, yield: (0.19 g, 50%), m.p: 166–168 °C, IR (KBr) cm^{-1} υ: 3294(NH,NH_2 overlap), 2931 (C–H).1635 (C=C, Ar), 1596(C=N). **^{1}H-NMR** ($CDCl_3$, 500 MHz) δ: 9.13 (br.s, 1H, NH), 8.27–7.34(m, 8H, CH_{Ar}), 4.08 (br.s, 2H, CH_2), 2.66 (br.s, 14H, CH_2), 1.95 (br.s, 4H, CH_2).**^{13}C-NMR**(DMSO-d_6,100 MHz) δ:164.31, 148.19, 134.21, 131.36, 125.95, 120.82, 120.22, 58.26, 56.34, 51.83, 39.77, 27.11, 23.58. **EIMS**, m/z ($C_{23}H_{31}N_5$) calcd, 377.54 $[M]^+$; found 377.30.

Synthesis of α-aminophosphonate derivatives using solid of 11-phenoxy-4-formyl neocryptolepine and aminoalkylamino acridine derivatives (8a-f)

To 11-phenoxy-4-formyl neocryptolepine **3** (0.3 g, 0.84 mmol) and aminoalkylamino acridine derivatives **6a–f** (0.84 mmol), diphenyl phosphite **7** (0.75 mL, 0.92 mmol), lithium perchlorate (10 mol %) in methanol (5 mL) were added and refluxed for 48–72 h. TLC monitored the reaction completion until all reactants are consumed. The desired product filtered off and crystallized by diethyl ether to yield the corresponding products **8a–f** in good yields.

Diphenyl((2-(acridin-9-yl)hydrazineyl) (4-((5-methyl-5H-indolo[2,3-b]quinolin-11-yl) oxy) phenyl)methyl)phosphonate (8a)

Brown solid, yield: (0.47 g, 72%), m.p: 162–164 °C, **IR** (KBr) cm^{-1} υ: 3263 (NH), 2870 (C–H).1620 (C=C, Ar), 1527(C=N), 1234 (P=O). **^{1}H-NMR** (DMSO-d_6, 400 MHz) δ: 12.07 (s, 1H, NH), 11.72 (s, 1H, NH), 8.22–7.08 (m, 30H, CH_{Ar}), 6.73 (d, 1H, C<u>H</u>–P, $J = 8$ MHz), 4.36 (s, 3H, CH_3). **^{13}C-NMR** (DMSO-d_6,100 MHz) δ:177.85, 143.16, 141.55, 140.02, 135.30, 134.28, 132.92, 132.66, 131.14, 130.17, 129.52, 127.99, 127.57, 126.78, 124.41, 124.01, 123.32, 121.75, 121.21, 118.07, 117,36, 166.55, 116.09, 115.92, 67.60, 36.49. **^{31}P-NMR** (DMSO-d_6, 500 MHz) δ: 24.64. **EIMS**, m/z ($C_{48}H_{36}N_5O_4P$) calcd, 777.82 $[M]^+$ found 779.30.

Diphenyl(((2-(acridin-9-ylamino)ethyl)amino) (4-((5-methyl-5H-indolo[2,3-b]quinolin-11yl)oxy) phenyl) methyl) phosphonate (8b)

Pale yellow solid, yield: (0.46 g, 68%), m.p: 160–162 °C, **IR** (KBr) cm^{-1} υ: 3410(NH), 2978 (C–H), 1620 (C=C, Ar), 1527(C=N), 1257 (P=O). **^{1}H- NMR** (DMSO-d_6, 400 MHz) δ: 12.10(s, 1H, NH), 11.74(s, 1H, NH), 8.22–7.20 (m, 30H, CH_{Ar}), 6.73 (d, 1H, C<u>H</u>–P, $J = 8$ MHz), 4.23 (m, 2H, CH_2), 3.97 (s, 3H, CH_3), 3.82 (br.s, 2H, CH_2). **^{13}C-NMR** (DMSO-d_6,100 MHz) δ:159.56, 156.08, 153.36, 151.47, 151.35, 149.20, 148.02, 139.07, 131.61, 130.08, 129.26, 128.81, 126.59, 125.68, 123.88, 122.98, 122.96, 122.82, 122.06, 121.64, 121.57, 119.38, 118.17, 113.76, 66.10, 48.77, 44.68, 35.41. **^{31}P-NMR** (DMSO-d_6, 500 MHz) δ: 16.50. **EIMS**, m/z ($C_{50}H_{40}N_5O_4P$) calcd, 805.87 $[M]^+$ found 807.27.

Diphenyl(((3-(acridin-9-ylamino)propyl)amino) (4-((5-methyl-5H-indolo[2,3-b]quinolin-11-yl) oxy) phenyl)methyl)phosphonate (8c)

Pale yellow solid, yield: (0.35 g, 51%), m.p: 185–187 °C, **IR** (KBr) cm^{-1} υ: 3425(NH), 2985 (C–H), 1635(C=C, Ar), 1597, 1527(C=N),1257(P=O). **^{1}H-NMR**(DMSO-d_6, 400 MHz) δ: 12.08 (s, 1H, NH), 11.73 (s, 1H, NH), 8.38–7.20 (m, 30H, CH_{Ar}), 6.73 (d, 1H, C<u>H</u>–P, $J = 8$ MHz), 4.17(m, 2H, CH_2), 3.97(s, 3H, CH_3), 3.40 (br.m, 2H, CH_2), 2.45 (br.m, 2H, CH_2). **^{13}C-NMR** (DMSO-d_6,100 MHz) δ:177.87, 172.61, 147.80, 141.76, 140.02, 134.27, 131.98, 131.93, 127.99, 127.71, 126.78, 125.43, 124.82, 124.26, 123.56, 122.41, 121.92, 121.74, 122.21, 120.87, 118.07, 115.92, 70.10, 46.78, 42.44, 35.41, 29.33. **^{31}P-NMR** (DMSO- d_6, 500 MHz) δ: 16.12. **EIMS**, m/z ($C_{51}H_{42}N_5O_4P$) calcd, 819.90 $[M]^+$ found 822.38.

Diphenyl(((2-((2-(acridin-9-ylamino)ethyl)amino) ethyl)amino)(4-((5-methyl-5H-indolo[2,3-b] quinolin-11-yl)oxy)phenyl)methyl)phosphonate (8d)

Reddish brown solid, yield: (0.44 g, 61%), m.p: 180–182 °C, **IR** (KBr) cm^{-1} υ: 3433(NH), 2939(C–H), 1627 (C=C, Ar), 1597, 1543(C=N), 1249 (P=O). **^{1}H- NMR** (DMSO - d_6, 400 MHz) δ: 12.19 (s, 1H, NH), 11.80 (s, 2H, 2NH), 8.22–7.23 (m, 30H, CH_{Ar}), 6.73(d, 1H, C<u>H</u>–P, $J = 8$ MHz), 4.31(s, 3H, CH_3), 3.96 (br.s, 2H, CH_2), 3.50 (br,s, 6H, $3CH_2$). **^{13}C-NMR** (DMSO-d_6,100 MHz) δ:177.81, 172.54, 147.81, 141.77, 138.41, 134.07, 133.04, 131.93, 130.11, 128.47, 128.05, 127.84, 126.71, 126.50, 125.40, 124.77, 124.48, 123.46, 122.34, 121.80, 121.65, 121.16, 119.27, 117.64, 115.92, 70.04, 50.03, 45.18, 35.52, 33.50. **^{31}P-NMR** (DMSO-d_6, 500 MHz) δ: 2.87. **EIMS**, m/z ($C_{52}H_{45}N_6O_4P$) calcd, 848.94 $[M]^+$ found 850.23.

Diphenyl(((3-((3-(acridin-9-ylamino)propyl)amino) propyl)amino)(4-((5-methyl-5H-indolo[2,3-b] quinolin-11-yl)oxy)phenyl)methyl)phosphonate (8e)

Reddish brown solid, yield: 0.35 g, 48%, m.p: 210–212 °C, **IR** (KBr) cm^{-1} υ: 3425(NH), 2985 (C–H), 1635 (C=C, Ar), 1597, 1535(C=N), 1257 (P=O). **^{1}H-NMR** (DMSO-d_6, 400 MHz) δ: 12.19 (s, 1H, NH), 11.80 (s, 2H, NH), 8.47–7.32 (m, 30 H, CH_{Ar}), 6.73 (d, 1H, C<u>H</u>–P, $J = 8$ MHz), 4.27 (s, 3H, CH_3), 3.97 (s, 2H, CH_2), 3.49 (br.s, 2H, CH_2), 3.07(m, 4H, $2CH_2$), 1.72(m, 2H, CH_2), 1.58 (m, 2H, CH_2). **^{13}C-NMR** (DMSO-d_6,100 MHz) δ:177.96, 172.75, 156.46, 153.19, 149.96, 147.88, 141.80, 139.75, 138.49, 135.70, 134.39, 133.36, 132.13, 130.27, 128.70, 128.05, 126.60, 124.48, 122.90, 122.52, 121.99, 121.23, 118.15, 116.01, 113.29, 70.08, 52.00, 45.42, 42.69, 35.84, 33.74, 26.94, 18.20.**^{31}P-NMR** (DMSO-d_6, 500 MHz) δ: 2.31, 10.23. **EIMS**, m/z ($C_{54}H_{49}N_6O_4P$) calcd, 877.00 $[M]^+$ found 880.30.

Diphenyl(((3-(4-(3-(acridin-9-ylamino) propyl)piperazin-1-yl)propyl)amino) (4-((5-methyl-5H-indolo[2,3-b]quinolin-11-yl)oxy) phenyl)methyl)phosphonate (8f)

Brown solid, yield: (0.37 g, 46%), mp: 150–152 °C, **IR** (KBr) cm^{-1} υ: 3425(NH), 2947 (C–H), 1635 (C=C, Ar), 1597, 1527(C=N), 1257 (P=O). **^{1}H-NMR** (DMSO-d_6, 400 MHz) δ: 12.09 (s, 1H, NH), 11.74 (s, 1H, NH), 8.22–6.62 (m, 30 H, CH_{Ar}), 6.73(d, 1H, C<u>H</u>–P, $J = 8$ MHz), 4.21 (m, 4H, $2CH_2$), 3.97(s, 3H, CH_3), 3.44 (m, 6H, CH_2), 3.01 (m, 6H, CH_2), 2.53 (br.s, 2H, CH_2), 2.40 (br.s, 2H, CH_2). **^{13}C-NMR** (DMSO-d_6,100 MHz) δ:177.85, 172.25, 147.82, 141.75, 140.04, 135.57, 134.26, 131.99, 130.15, 126.76, 126.54, 125.53, 124.85, 123.55, 122.41, 121.91, 121.73, 121.20, 120.86, 118.06, 115.93, 70.09, 45.83, 44.69, 40.95, 42.69, 34.50, 33.49. **^{31}P-NMR** (DMSO-d_6, 500 MHz) δ: -0.66. **EIMS**, m/z ($C_{58}H_{56}N_7O_4P$); calcd, 946.10 $[M]^+$ found: 947.10.

Biological activity

In vitro cytotoxic activity

Cell culture of human colorectal carcinoma (HCT-116), hormone-dependent human breast adenocarcinoma (MCF-7), human hepatocellular carcinoma (HepG2) and human lung carcinoma (A549) cell lines was purchased from the American Type Culture Collection (Rockville, MD) and maintained in DMEM which was supplemented with 10% heat-inactivated FBS (fetal bovine serum), 100 U/ml penicillin and 100 U/mL streptomycin. The cells were grown at 37 °C in a humidified atmosphere of 5% CO_2.

MTT cytotoxicity assay

The cytotoxicity activities on HCT-116, MCF-7, HepG2 and A549 human cancer cell lines were estimated using the 3-[4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay, which is based on the reduction of the tetrazolium salt by mitochondrial dehydrogenases in viable cells [21–23]. Cells were dispensed in a 96-well sterile microplate (5×10^4 cells/well) and incubated at 37 °C with series of different concentrations, in DMSO, of each tested compound or doxorubicin (positive control) for 48 h in a serum-free medium prior to the MTT assay. After incubation, media were carefully removed; 40 µL of MTT (2.5 mg/mL) was added to each well and then incubated for an additional 4 h. The purple formazan dye crystals were solubilized by the addition of 200 µL of DMSO. The absorbance was measured at 570 nm using a Spectra Max Paradigm Multi-Mode microplate reader. The relative cell viability was expressed as the mean percentage of viable cells compared to the untreated control cells. All experiments were conducted in triplicate and repeated on three different days. All the values were represented as mean ± SD. IC_{50}s were determined by probit analysis by SPSS Inc. probit analysis (IBM Corp., Armonk, NY, USA).

Results and discussion

Chemistry

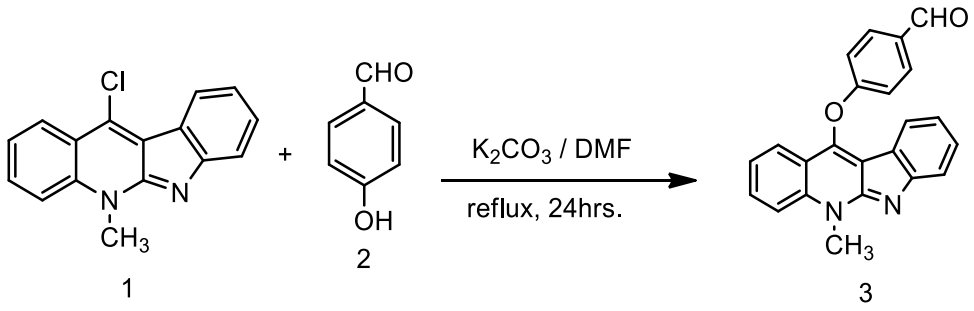
The starting 11-phenoxy-4-formyl neocryptolepine **3** was synthesized in good yield through reaction of 11-chloro-neocryptolpine **1** with 4-hydroxybenzaldehyde **2** in the

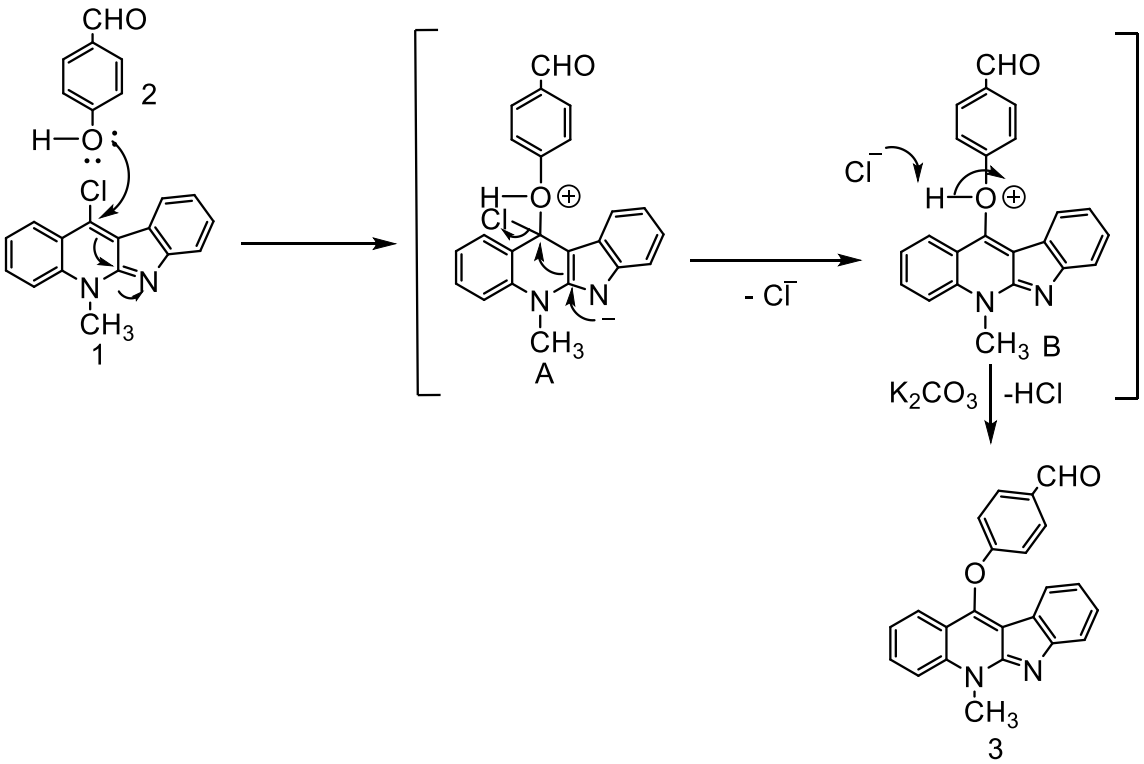
presence of potassium carbonate in DMF under reflux for 24 h as illustrated in Scheme 1.

In the structure elucidation of **3**, the IR spectrum showed a strong absorption band for the (C=O) of (CHO) group at υ: 1678 cm^{-1} which confirmed the presence of the formyl (CHO) group. In addition, ^{1}H-NMR spectrum for **3** showed a singlet with chemical shift at δ: 9.64 ppm, which corresponds to the aldehydic proton. Further confirmation of present aldehydic group was observed in ^{13}C-NMR spectrum, which was showed a peak at 192.11 ppm for the aldehydic carbon atom of **3**. Furthermore, the EI mass spectrum showed a molecular ion peak which corresponds to **3**. A proposed mechanism for formation of **3** involved displacement of chlorine atom by the nucleophilic oxygen of the hydroxy group of **2** throughout nucleophile aromatic substitution (S_{NAr}) type **A**, followed by elimination of chlorine atom as HCl, aided by potassium carbonate as a base catalyst as given in Scheme 2.

Moreover, the requested 9-aminoalkylamino acridines **6a–f** were synthesized by reaction of 9-chloroacridine **4** with excess appropriate diamines **5a–f** with different carbon spacers to afford aminoalkylamino acridines **6a–f** in good yields as depicted in Scheme 3. In the structure characterization of 9-aminoalkylamino acridine derivatives **6a–f**, the IR analysis showed an absorption band ranging from υ: 3417 to 3232 cm^{-1} that represent the (NH and NH_2) groups, respectively. Moreover, ^{1}H-NMR spectra showed characteristic exchangeable peaks ranged from δ: 9.31 to 12.64 ppm, which are characteristic to (NH_2 and NH) groups. For the mass spectroscopy, the presence of the expected molecular

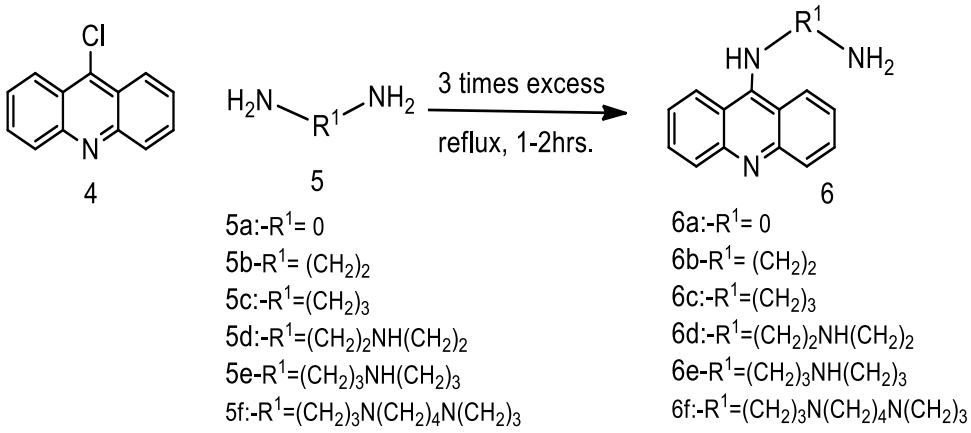
Scheme 1 Synthesis of 11-phenoxy-4-formylneocryptolepine **3**





Scheme 2 Suggested mechanism for formation of **3**

Scheme 3 Synthesis of aminoalkylamino acridine derivatives

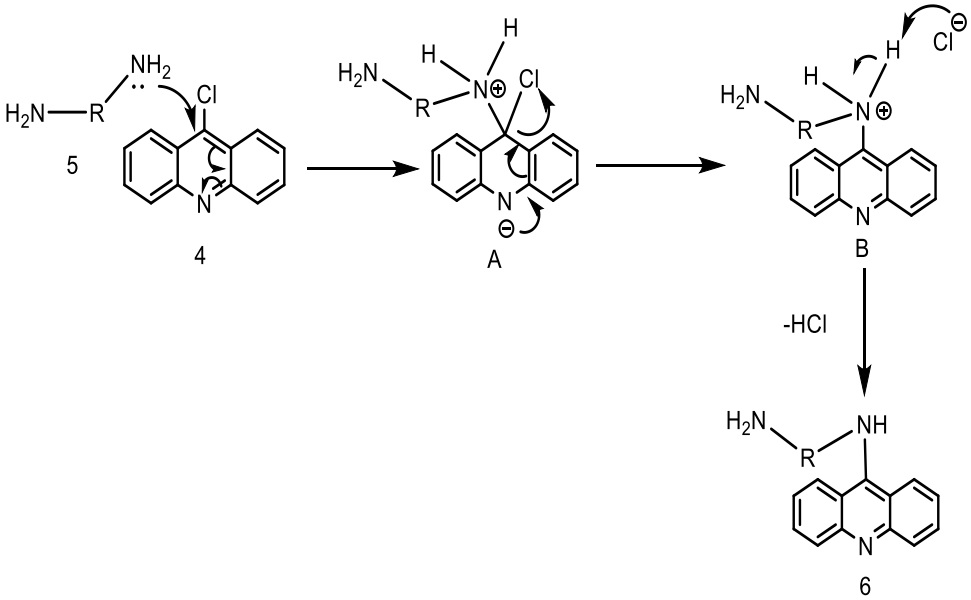


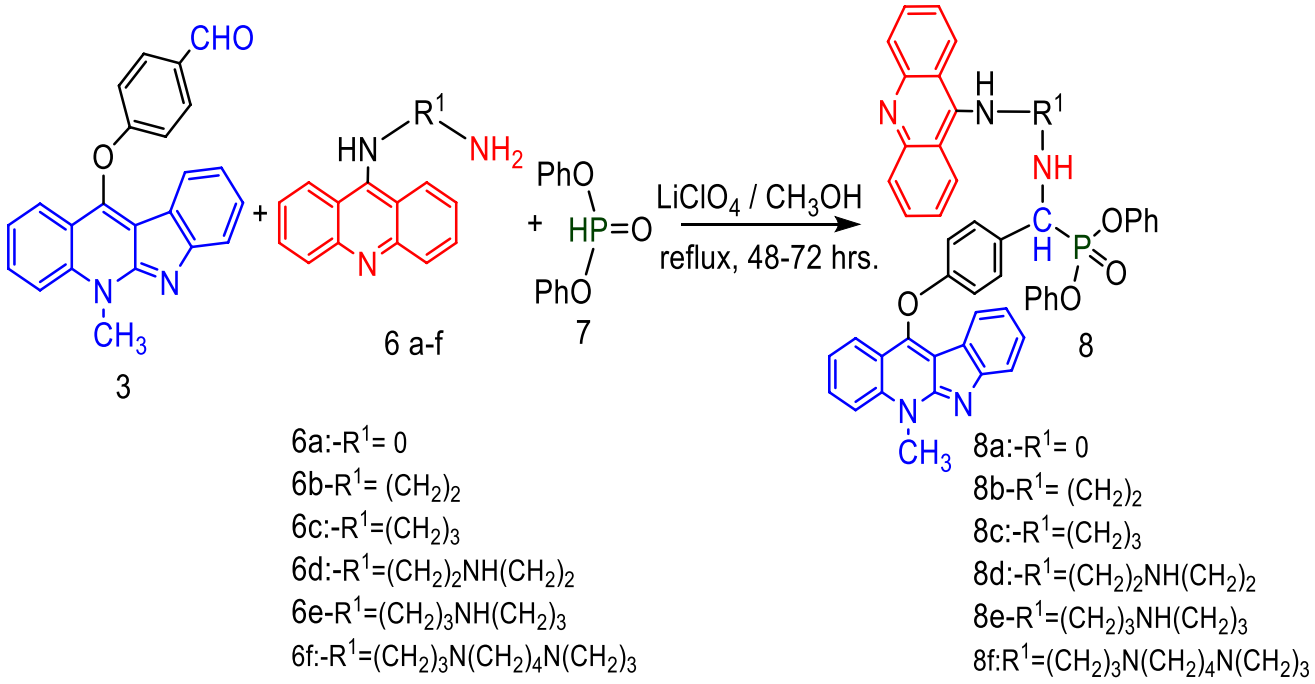
ion peaks of the target structures confirms the formation of the desired compounds. The plausible mechanism for the formation of **6** is nucleophilic aromatic substitution (S_{NAr}) of the amine nitrogen to the chloride ion at position C-9 of the acridine core through the formation of a resonance-stabilized anion with a new C– N bond **A** as shown in Scheme 4.

Finally the hybrids of neocryptolepine, acridine and α-aminophosphonate scaffolds **8a–f** were synthesized by installation of α-aminophosphonate moiety by three-component one-pot reaction of 11-phenoxy-4-formylneocryptolepine **3,** 9-aminoalkayamino acridines **6a–f** and diphenyl phosphite **7** with heating in methanol and in the presence of $LiClO_4$ as Lewis acid catalyst as illustrated in Scheme 5.

The newly synthesized hybrids **8** were characterized by ^{1}H-NMR which revealed the presence of (C<u>H</u>–P) proton with characteristic signals at δ: 6.72–6.74 ppm appeared as doublet which confirms the installation of α-aminophosphonate moiety, while ^{13}C-NMR spectra showed the characteristic peak of (<u>C</u>H–P) ranging from 66.1 to 70.10 ppm which prove the installation of aminophosphonate moiety on the expense of the disappearance of the aldehydic carbon at 192.11. Moreover, ^{31}P-NMR confirmed the formation of α-aminophosphonate moiety with characteristic (CH–<u>P</u>) ranging from − 0.66 to 24.64 ppm and in consistence with reported range [24]. Furthermore, the presence of (NH) was confirmed by the presence of broad singlet ranging from δ: 11.72 to 12.19 ppm. On the other hand, the IR spectra of these compounds showed a characteristic absorption bands for the NH group ranged from υ: 3433 to 3410 cm^{-1}. In addition, the presence of absorption band ranging from υ: 1257 to 1249 cm^{-1} is characteristic to the (P=O) group. Finally, the mass spectra of all synthesized compounds showed molecular ion peaks, which are in consistence with the expected molecular weight of the desired compounds. A plausible mechanism for preparation of α-aminophosphonates using $LiClO_4$ as a catalyst is shown

Scheme 4 Suggested mechanism for synthesis of aminoalkyl amino acridine derivatives





Scheme 5 Synthesis of hybrids of neocryptolepine, acridine and α-aminophosphonates

in Scheme 6. First, the activation of carbonyl group of the formyl group was achieved by Lewis acid catalyst, $LiClO_4$, followed by condensation of the carbonyl group of **3** with aminoalkylamino acridine derivatives **6** to afford the intermediate Schiff base **C**. Then the nitrogen of Schiff base was activated by $LiClO_4$ followed by the addition of phosphorus–hydrogen bond across the imine double bond to afford the target hybrids **8**.

Biological evaluation

Cytotoxicity screening

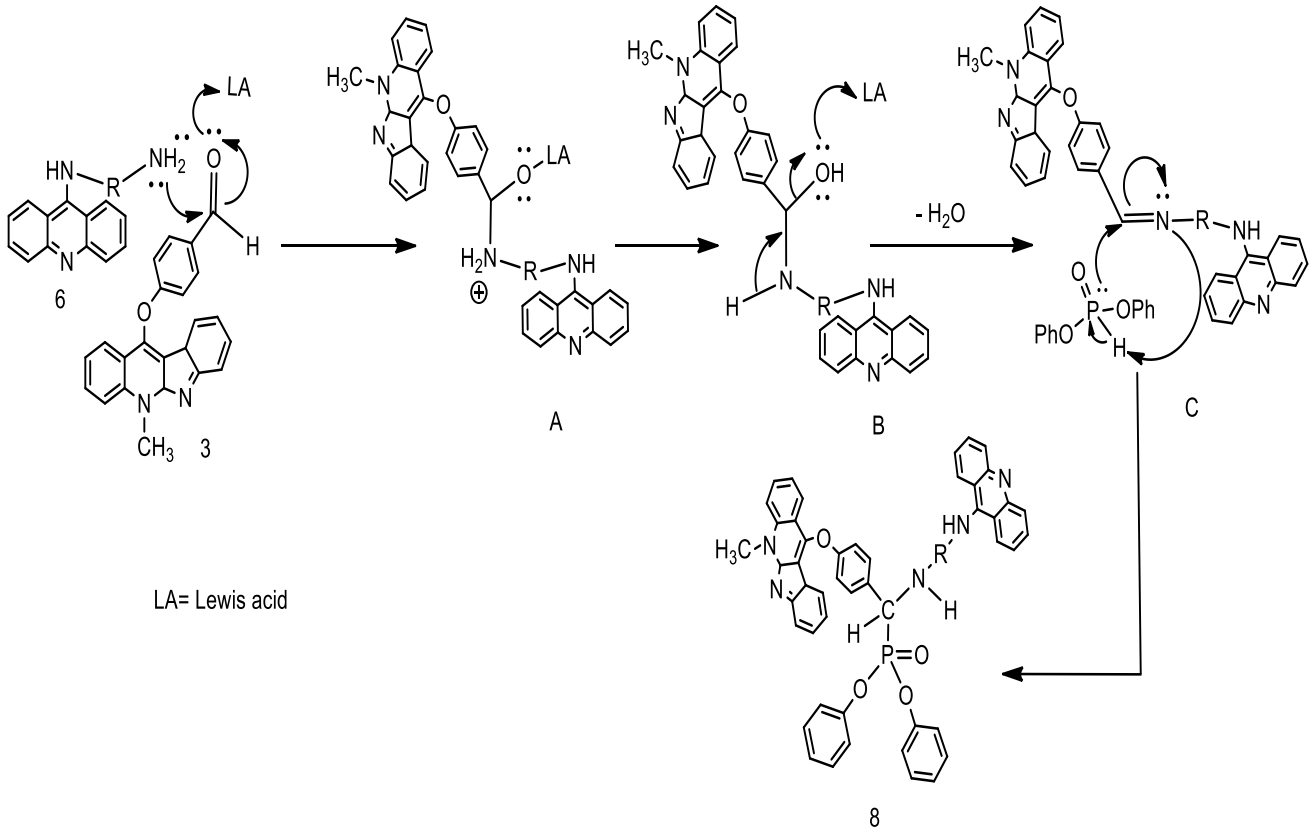
Sixteen compounds were examined in vitro for their activities against HCT-116, MCF-7, HepG2 and A549 human cancer cells using the MTT assay. The percentages of viable cells were calculated and compared to those of the control. Activities of these compounds against the four cancer cell lines were compared to the activity of doxorubicin as reference drug as well. All compounds suppressed the four cancer human cells in a dose-dependent manner (Figs. 2, 3, 4, 5). In case of HCT-116 human colorectal carcinoma cells, both Fig. 2 and Table 1 show that all compounds had significantly more potent cytotoxic activities except for two compounds (**6e and 6d,** respectively) which had slightly less activities compared to doxorubicin.

In case of MCF-7 human breast cancer cells, all compounds had significantly more potent cytotoxic activities except for three compounds (**6e, 2 and 6b,** respectively) which had equipotent activities compared to doxorubicin (Fig. 3 and Table 1). In case of HepG2 human liver cancer, all compounds had significantly more potent anticancer effect compared to that of the reference drug doxorubicin (Fig. 4 and Table 1). In case of A549 cancer cells, twelve compounds (**8a, 1, 2, 6a, 6d, 6b, 8f, 3, 6c, 8c, 8b and 6e,** respectively) had significantly more potent cytotoxic activities; two compounds (**7 and 8f,** respectively) had comparable cytotoxic effect; the rest of the compounds had slightly less anticancer activities compared to that of doxorubicin (Fig. 5 and Table 1).

From the above-mentioned results, one can conclude that compounds **3, 7, 6a, 6c, 6e, 6f, 8a–c, 8f, 1 and 2** are potent anticancer candidate drugs on all the four human cancer types; compound **6d** is specifically good anticancer candidate drug on human breast, liver and lung cancer types rather than on human colon cancer type; compound **6b** is potent anticancer candidate drug on human liver, colon and lung cancer types but less active on breast cancer type; compound **8d** is potent anticancer candidate drug on human liver, breast and colon cancer types but not active on human lung cancer type; finally, compound **8e** has better anticancer activity on human liver, colon and breast cancer types than on human lung cancer type.

In vitro antiproliferative activity

The results of the cytotoxic activity in vitro were expressed as the IC_{50}–concentration of the compound (in μM) that



Scheme 6 A plausible mechanism for synthesis of hybrids **8**

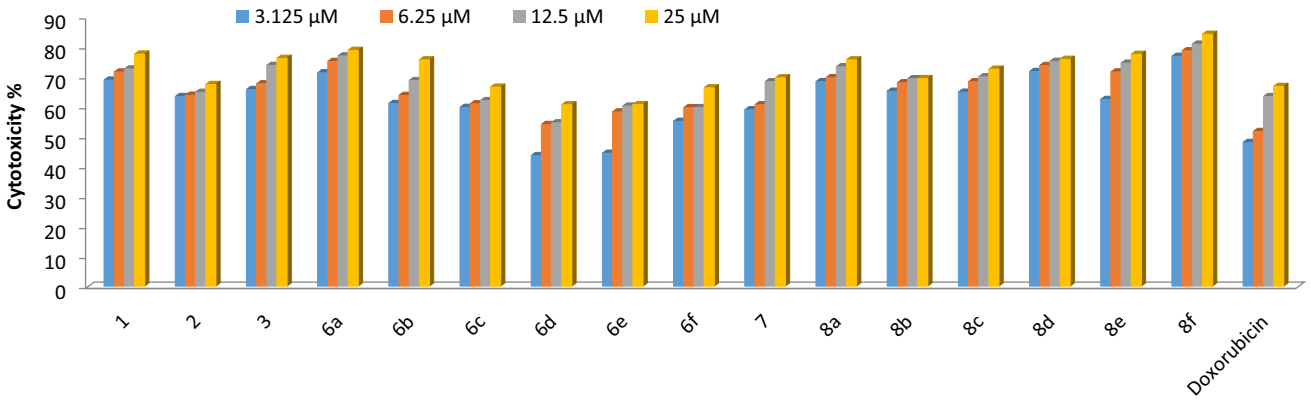


Fig. 2 Dose-dependent cytotoxic activities of sixteen compounds on HCT-116 cancer cells according to the MTT assay

inhibits proliferation of the cells by 50% as compared to the untreated control cells. The inhibition concentration (IC) values were separately calculated for each experiment, and the mean values ± SD were recorded from at least 3 independent experiments. The results on the antiproliferative activity of α-aminophosphonate derivatives are summarized in Table 1, along with the data of the anticancer drug, doxorubicin. The cytotoxicity test of the combined structure of neocryptolepine, acridine and α-aminophosphonate scaffolds as a chemically modified hybrids indicated a synergetic effect on anticancer activity. It is worth to note that the anticancer activity of the modified analogues is found to be higher than the reference drug doxorubicin as well as the starting 11-phenoxy-4-formyl neocryptolepine **3**. Based on the antiproliferative activities shown in Table 1, the most potent compounds with very strong and selective anticancer activity among all tested derivatives are **8a–f** with IC_{50}: 5.8, 7.3, 7.0, 4.7, 5.7 and 2.4 µM against colorectal

Table 1 IC_{50} of the compounds against the four cancer types according to the MTT assay

Compound code	IC_{50} (μM) ± SD			
	HCT-116	MCF-7	HepG2	A549
1	5.2 ± 1.3	13.1 ± 3.2	25.2 ± 2.7	19.6 ± 2.9
2	5.7 ± 1.2	26.6 ± 3.5	28.1 ± 3.5	20.6 ± 3.1
3	6.2 ± 1.5	20.6 ± 2.9	21.6 ± 2.7	21.5 ± 2.3
6a	4.5 ± 1.3	13.9 ± 2.1	22.2 ± 2.3	20.7 ± 2.2
6b	7.6 ± 1.8	27.5 ± 3.1	22.2 ± 2.2	20.8 ± 2.1
6c	9.3 ± 1.7	25.2 ± 2.7	21.9 ± 2.1	23.5 ± 2.5
6d	12.5 ± 2.1	18.6 ± 2.1	21.4 ± 2.3	20.7 ± 2.2
6e	11.6 ± 1.9	26.2 ± 2.5	24.7 ± 2.5	26.0 ± 2.7
6f	10.0 ± 1.9	19.6 ± 2.1	23.6 ± 2.5	28.0 ± 3.1
7	8.5 ± 1.9	18.0 ± 2.2	27.2 ± 3.7	27.7 ± 3.2
8a	5.8 ± 1.4	8.2 ± 1.7	23.1 ± 2.4	19.4 ± 2.3
8b	7.3 ± 1.6	15.9 ± 2.1	24.6 ± 2.7	25.6 ± 3.1
8c	7.0 ± 1.7	18.3 ± 2.1	27.8 ± 2.9	25.2 ± 3.2
8d	4.7 ± 1.3	18.4 ± 2.1	28.0 ± 3.1	33.4 ± 3.7
8e	5.7 ± 1.5	18.8 ± 2.3	26.8 ± 2.9	29.0 ± 3.5
8f	2.4 ± 0.4	16.0 ± 2.1	27.1 ± 3.1	20.9 ± 2.9
Doxorubicin	10.9 ± 2.3	26.5 ± 3.7	32.8 ± 3.6	27.9 ± 4.1

carcinoma (HCT-116), respectively, while they exhibited the same strong activity against breast carcinoma (MCF-7) with IC_{50}: 8.2, 15.9, 18.3, 18.4, 18.8 and 16.0 μM, respectively. On the other hand, the same trend is seen against the hepatocellular carcinoma (HepG2) with IC_{50}: 23.1, 24.6, 27.8, 28.0, 26.8 and 27.1 μM, respectively. Moreover, compounds **8a–c**, **8e** and **8f** exhibited a very potent activity with IC_{50}: 19.4, 25.6, 25.2, 29.0 and 20.9 μM against (A549), respectively. Based on the above-mentioned results most of the compounds showed anticancer activity significantly higher than the reference drug doxorubicin (*cf.* table 1). However, compound **8d** is the only one that showed antiproliferative activity less than the reference drug doxorubicin with IC_{50} 33.4 μM against human lung carcinoma cell line (A549). It is worth to note that the hybrid structures **8a, 8d**, **8e** and **8f** showed better antiproliferative activity against colorectal carcinoma (HCT-116) comparing with each starting scaffold independently as well as reference drug doxorubicin. The same result and trend were observed with the hybrids **8a**, **8b** and **8f** when evaluated with human breast carcinoma cell line (MCF7), while in case of human lung carcinoma cell line (A549) only hybrids **8a** and **8f** exhibited synergistic activity than each scaffold alone.

Study of structure activity relationships (SARs)

The study of structure activity relationship was investigated in order to correlate between the structure and the pharmacological activity in view of searching for lead compound for further optimization. The variety of all synthesized hybrids specified in the presence of carbon spacer incorporated with neocryptolepine, acridine and α-aminophosphonate scaffolds.

It is noticeable from above-mentioned results that hybrid **8f** containing piperazine spacer between two amino groups gave the highest antiproliferative activity against HCT-116 cell line, revealing the impact of this pharmacophore group on enhancement of antitumor activity of **8f** [25], while the linkage of four- and six-carbon spacers included internal NH group illustrated in hybrids **8d and 8e,** respectively, showed high anticancer activity but less than **8f** containing piperazine spacer. Moreover, hybrids **8a–c** containing hydrazinyl, two- and three-carbon spacers, respectively, showed high antitumor activity but less than **8f** containing piperazine spacer.

It also reported that hybrids **8a, 8d, 8e** and **8f** have synergistic activity comparing with their single scaffolds, 11-phenoxy-4-formylneocryptolepine **3**, diphenyl phosphite **7** and substituted acridines at position 9 with hydrazinyl, four- and

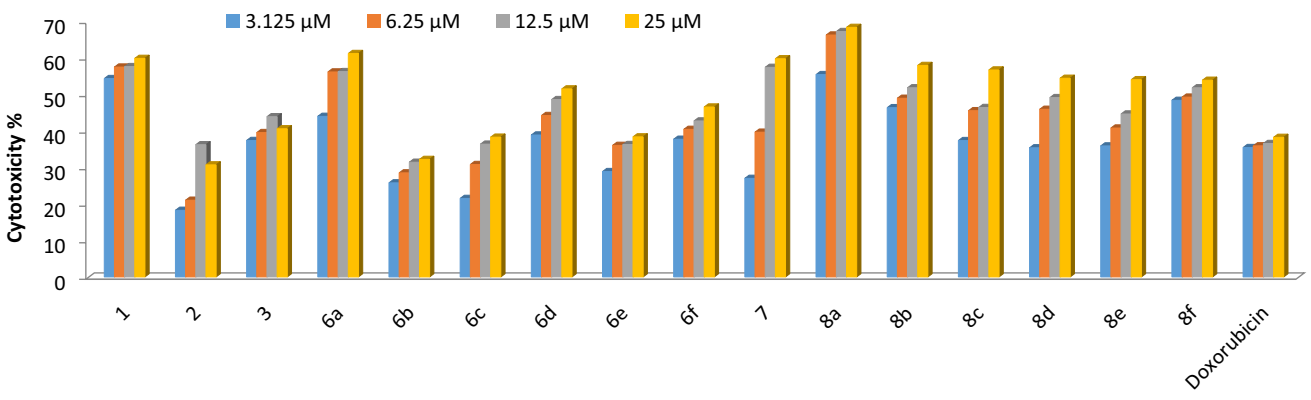


Fig. 3 Dose-dependent cytotoxic activities of sixteen compounds on MCF-7 cancer cells according to the MTT assay

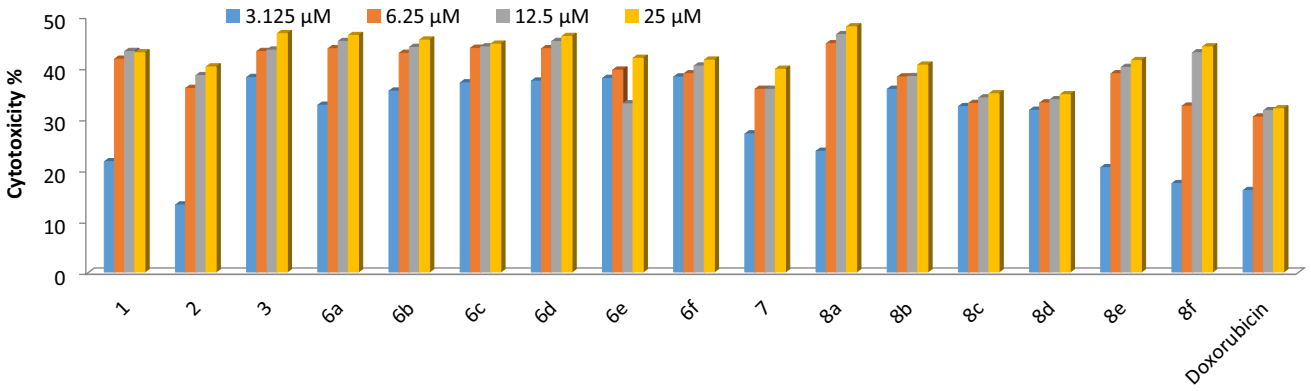


Fig. 4 Dose-dependent cytotoxic activities of sixteen compounds on HepG2 cancer cells according to the MTT assay

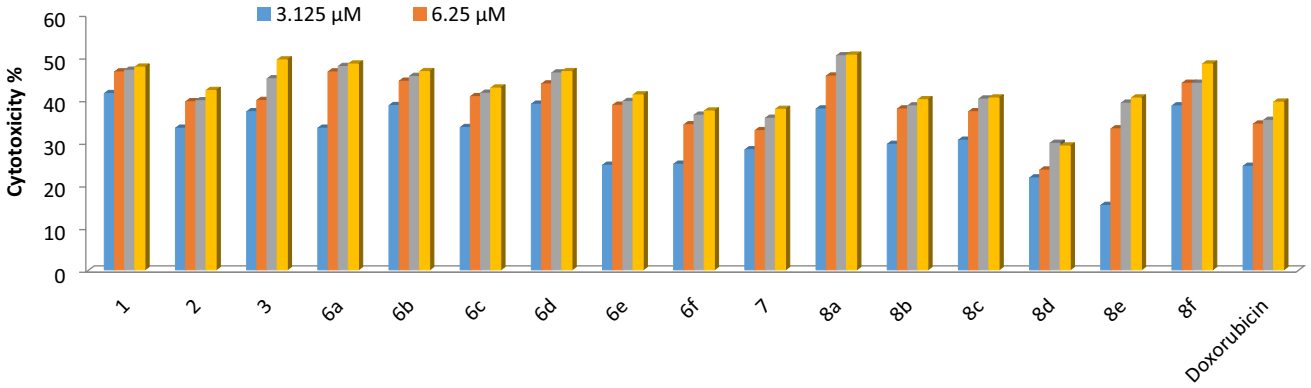


Fig. 5 Dose-dependent cytotoxic activities of sixteen compounds on A549 cancer cells according to the MTT assay

six-carbon spacers including internal NH group in addition to piperazine spacer **6a, 6d, 6e** and **6f,** respectively. On the other hand, hybrid **8a** containing hydrazinyl linkage showed the highest antiproliferative activity against MCF-7 comparing with other hybrids containing two- or three-carbon spacer, four- or six-carbon spacer with internal amino group and piperazine spacer. The synergistic activity was reported in hybrids containing hydrazinyl, two-carbon spacers and piperazine spacer, revealing the importance of the presence of these linkages among the three scaffolds. In addition, hybrid **8a** containing hydrazinyl linkage gave highest antitumor activity against HepG2 followed by hybrids two-carbon spacer, six-carbon spacer with internal amino group, piperazine spacer three-carbon spacer and four spacer with internal amino group, respectively. Unfortunately, there is no synergistic effect reported for synthesized hybrids. Finally, for A549 cell line, it is illustrated that **8a** containing hydrazinyl linkage has the best antitumor activity comparing with piperazine spacer, three-carbon spacers, two-carbon spacers, six-carbon spacers with internal amino group except **8d** containing four carbon spacers with internal amino group, showing lower activity than hybrids and reference drug. Moreover, hybrids containing hydrazinyl and piperazine spacer **8a** and **8f** also have synergistic effect comparing with 11-phenoxy-4-formylneocryptolepine **3**, diphenyl phosphite **7** and substituted acridines at position C-9 with hydrazinyl, piperazine spacer **6a** and **6f** scaffolds.

In conclusion, our initial goal to make hybrids of neocryptolepine, acridine and α-aminophosphonate scaffolds was achieved. This led to have a small series of modified structure with synergistic antiproliferative activity against HCT-116, MCF-7, HepG2 and A549 human cancer cell lines.

Conclusion

Novel strong antiproliferative hybrids **8a–f** were successfully synthesized based on neocryptolepine scaffold through three-component one-pot reaction of **3**, with **6a–f** and **7** in the presence of lithium perchlorate as Lewis acid catalyst and were elucidated by spectroscopic methods. All synthesized hybrids were screened against HCT-116, MCF-7,

HepG2 and A549 human cancer cell lines and revealed that most of hybrids showed strong antiproliferative activity against reference drug doxorubicin with synergistic effect.

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