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New derivatives bearing alkyl phosphonic acid moieties as a promising scaffold against drug-resistant H69AR small cell lung cancer models

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Small cell lung cancer (SCLC), although less common than non-small cell lung cancer (NSCLC), is an aggressive and lethal form of lung cancer. Even if it was diagnosed and started being treated early, it still contributes to poor survival rates. Standard therapies, including platinum-based chemotherapy and immune checkpoint inhibitors, offer limited and short-lived benefits due to the rapid disease relapse and widespread metastasis. One of the promising strategies for the new drug against SCLC development is the rational design of 9*H*-carbazole or other structurally similar chromophores with an alkyl phosphonic acid moiety, expecting dual-targeting ability, potentially targeting both nuclear and cytoplasmic effectors involved in SCLC progression and resistance. The aim of this work was to synthesise phenothiazine, phenoxazine, anthraquinone and substituted carbazole derivatives, containing an alkyl phosphonic acid moiety and to evaluate their *in vitro* antiproliferative activity using the well-established anthracycline-resistant H69AR small cell lung cancer (SCLC) cell model. The results demonstrate that 9*H*-carbazole derivatives bearing alkyl phosphonic acid groups could be explored as a promising scaffold class for the further development of compounds against drug-resistant SCLC.

Keywords: phosphonic acid derivatives, lung cancer, antiproliferative activity

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INTRODUCTION

Lung cancer remains the leading cause of cancer-related mortality worldwide, with an estimated 1.8 million deaths reported annually [1, 2]. Lung derived tumours are classified into non-small cell lung cancer (NSCLC), accounting for approximately 85% of cases, and small cell lung cancer (SCLC), which comprises the remaining 15% [3, 4]. Despite its lower incidence, SCLC is an exceptionally aggressive neuroendocrine malignancy characterised by rapid proliferation, early metastasis, acquisition of multiple resistance and aggressiveness-associated mutations, and high recurrence rates [4–6]. Even with early diagnosis, aggressive surgical resection, the overall prognosis for SCLC remains poor, with a 5-year overall survival rate of less than 7%, largely due to therapeutic options [4, 7, 8]. The intrinsic biological aggressiveness and genomic instability of SCLC underscore the urgent need for novel therapeutic strategies targeting novel SCLC-specific targets [8].

Current treatment regimens for SCLC typically involve platinum-based chemotherapy, such as cisplatin or carboplatin in combination with etoposide, often administered alongside aggressive thoracic radiotherapy [9–11]. The addition of immune checkpoint inhibitors like atezolizumab and durvalumab to first-line chemotherapy has modestly improved survival in extensive-stage SCLC [10, 11]. However, these improvements are often transient, as the majority of patients experience disease relapse and metastatic multi-organ progression [4, 9–11].

Drug resistance in SCLC comes from complex molecular mutations that confer SCLC survival advantages under therapeutic pressure [12, 13]. One prominent mechanism involves the overexpression of anti-apoptotic proteins, particularly members of the BCL-2 family, which inhibit mitochondrial outer membrane permeabilisation and prevent activation of the intrinsic apoptotic pathway [14–16]. Concurrently, the aberrant activation of the PI3K/AKT/mTOR signalling axis promotes cell proliferation, inhibits apoptosis and enhances metabolic adaptation, facilitating resistance to cytotoxic agents such as cisplatin, and anthracyclines such as doxorubicin [17]. SCLC cells also exhibit the upregulated expression of DNA repair machinery, including components of the homologous recombination and non-homologous end-joining pathways, which mitigate the cytotoxic effects of DNA-damaging chemotherapeutics such as etoposide and platinum compounds [14–17]. Additionally, the disease is marked by pronounced intratumoral heterogeneity, driven by dynamic clonal evolution and phenotypic plasticity, enabling the rapid adaptation and emergence of resistant subpopulations [18]. These multi-drug resistance mechanisms result in the rapid development of treatment-resistant disease following the initial clinical response.

Diverse heterocycles such as indoles, pyrroles, quinazolines, imidazoles and triazoles have shown promise in preclinical studies, acting through mechanisms including kinase inhibition, DNA intercalation, redox modulation and apoptosis induction [19–21]. Among these, the 9*H*-carbazole scaffold has emerged as a particularly attractive pharmacophore due to its planar tricyclic structure, which enables π–π stacking interactions with DNA, as well as its potential to inhibit topoisomerases, kinases, and oxidative stress-related targets [22, 23]. The incorporation of alkyl phosphonic acid moieties enhances aqueous solubility and bioavailability and can facilitate selective interactions with zinc-dependent enzymes and phosphate-recognising domains such as kinases or phosphatases [24. Rational design of hybrid molecules combining substituted 9*H*-carbazole cores or other structurally similar chromophores with alkyl phosphonic acid side chains allows dual-targeting ability leading to targeting both nuclear and cytoplasmic effectors involved in SCLC progression and resistance [19–24].

In this study, we report the synthesis of a series of substituted 9*H*-carbazole and 10*H*-phenothiazine, 10*H*-phenoxazine, 2-hydroxyanthraquinone derivatives bearing alkyl phosphonic acid moieties and evaluate their *in vitro* antiproliferative activity using the well-established anthracycline-resistant H69AR small cell lung cancer (SCLC) cell model.

EXPERIMENTAL

General methods and materials

Chemicals were purchased from Sigma-Aldrich and TCI Europe and used as received without further purification. The ^{1}H and ^{13}C NMR spectra

were taken on a Bruker Avance III (400 MHz) spectrometer at RT. All the data are given as chemical shifts in δ (ppm). The course of the reactions was monitored by TLC on ALUGRAM SIL G/UV254 plates and developed with UV light. Silica gel (grade 9385, 230–400 mesh, 60 Å, Aldrich) was used for column chromatography. Elemental analysis was performed with an Exeter Analytical CE-440 elemental analyzer, Model 440 C/H/N/. An electrothermal A.KRÜSS M3000 capillary melting point apparatus was used for the determination of the melting points.

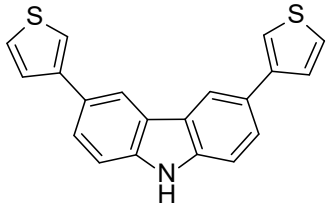
The detailed synthesis procedures

2PACz, **MeO-2PACz**, **Me-4PACz** and **Br-2PACz** were synthesised according to the previously published synthesis procedures [25–27].

9*H*-Carbazole-3,6-dicarbonitrile was synthesised according to the literature from 3,6-dibromocarbazole using copper (I) cyanide; mixture of products used for further alkylation without isolating the desired product [28].

3,6-Difluoro-9*H*-carbazole was synthesised according to the literature from 2'-bromo-4'-fluoroacetanilide, by employing copper(0)-mediated Ullmann homocoupling and acid-mediated intramolecular amination reactions [29].

3,6-Di(thiophen-3-yl)-9*H*-carbazole



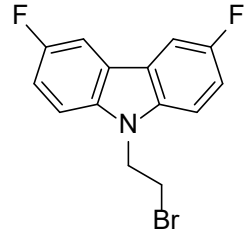
3,6-Dibromo-9*H*-carbazole (0.5 g, 1.53 mmol) was dissolved in anhydrous 1,4-dioxane (15 ml), followed by addition of 3-thienylboronic acid (0.49 g, 3.84 mmol), $Pd(PPh_3)_4$ (0.17 g, 0.15 mmol) and K_2CO_3 2M aqueous solution (2.3 ml, 4.61 mmol). Reaction was conducted at 70°C under inert argon atmosphere for 24 h. After the termination of reaction (TLC, acetone:*n*-hexane, 4:21), the reaction mixture was cooled down and filtered through celite which was washed with THF. Organic solvent was evaporated and the crude product was purified by column chromatography using acetone:*n*-hexane (4:21) as an eluent, resulting in white crystals (0.351 g, 69%) as a product. M.p. 201–202°C.

^{1}H NMR (400 MHz, THF-d_8): δ 10.36 (s, 1H), 8.45 (s, 2H), 7.71 (d, *J* = 8.4 Hz, 2H), 7.61–7.55 (m, 4H), 7.49–7.45 (m, 2H), 7.43 (d, *J* = 8.4 Hz, 2H) ppm. ^{13}C NMR (101 MHz, THF-d_8): δ 144.57, 141.11, 128.42, 127.38, 126.70, 125.51, 124.93, 119.27, 118.84, 111.86 ppm. Anal. calcd. for $C_{20}H_{13}NS_2$: C 72.48; H 3.95; N 4.23; found: C 72.29; H 4.02; N 4.40.

General procedure for the *N*-alkylation using 1,2-dibromoethane

Halogenated carbazole (1 eq.) was dissolved in 1,2-dibromoethane (25 eq.), followed by the addition of 50% KOH aqueous solution (5 eq.) and tetrabutylammonium bromide (0.15 eq.). The reaction mixture was heated to 80°C and stirred for 2 days. After the first 24 h, additional amounts of 50% KOH solution (5 eq.) and tetrabutylammonium bromide (0.15 eq.) were added. After the termination of reaction (TLC, acetone:*n*-hexane, 2:23), organic components were extracted with ethyl acetate, the organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography, using acetone:*n*-hexane (2:23) as an eluent.

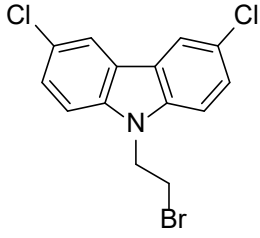
9-(2-Bromoethyl)-3,6-difluoro-9*H*-carbazole (1a)



3,6-Difluoro-9*H*-carbazole (0.5 g, 2.45 mmol), 1,2-dibromoethane (5.32 ml, 61.48 mmol), 50% KOH aqueous solution (1.4 ml, 12.29 mmol) and tetrabutylammonium bromide (0.12 g, 0.36 mmol) were used for the reaction. Product obtained as brownish white crystals (0.36 g, 47% yield). M.p. 132–133.5°C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 (dd, *J* = 8.7, 2.1 Hz, 2H), 7.36 (dd, *J* = 8.9, 4.0 Hz, 2H), 7.26 (td, *J* = 9.1, 2.4 Hz, 2H), 4.69 (t, *J* = 7.3 Hz, 2H), 3.68 (t, *J* = 7.3 Hz, 2H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 158.69, 156.34, 137.29, 123.26, 123.22, 123.17, 123.13, 114.65, 114.40, 109.57, 109.48, 106.69, 106.45, 45.14, 28.24 ppm. Anal. calcd. for $C_{14}H_{10}BrF_2N$: C 54.22, H 3.25, N 4.52; found: C 54.10, H 3.27, N 4.31.

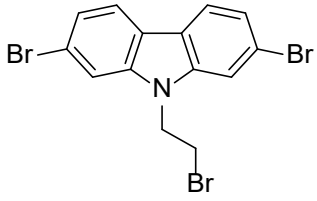
9-(2-Bromoethyl)-3,6-dichloro-9*H*-carbazole (1b)



3,6-Dichloro-9*H*-carbazole (0.5 g, 2.11 mmol), 1,2-dibromoethane (4.58 ml, 52.94 mmol), 50% KOH aqueous solution (1.2 ml, 10.58 mmol) and tetrabutylammonium bromide (0.10 g, 0.32 mmol) were used for the reaction. Product obtained as white crystals (0.51 g, 70% yield). M.p. 131.5–133°C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96 (d, *J* = 1.2 Hz, 2H), 7.44 (dd, *J* = 8.7, 1.6 Hz, 2H), 7.33 (d, *J* = 8.7 Hz, 2H), 4.64 (t, *J* = 7.2 Hz, 2H), 3.65 (t, *J* = 7.2 Hz, 2H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 138.89, 126.85, 125.62, 123.42, 120.56, 109.91, 45.02, 28.10 ppm. Anal. calcd. for $C_{14}H_{10}BrCl_2N$: C 49.02, H 2.94, N 4.08; found: C 49.09, H 3.00, N 4.25.

9-(2-Bromoethyl)-2,7-dibromo-9*H*-carbazole (1c)

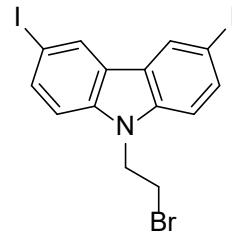


2,7-Dibromo-9*H*-carbazole (0.5 g, 1.53 mmol), 1,2-dibromoethane (3.32 ml, 38.46 mmol), 50% KOH aqueous solution (0.86 ml, 7.69 mmol) and tetrabutylammonium bromide (0.07 g, 0.23 mmol) were used for the reaction. Product obtained as white crystals (0.44 g, 67% yield). M.p. 164–165°C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.87 (d, *J* = 8.3 Hz, 2H), 7.55 (s, 2H), 7.37 (d, *J* = 8.3 Hz, 2H), 4.58 (t, *J* = 7.2 Hz, 2H), 3.65 (t, *J* = 7.2 Hz, 2H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 141.04, 123.50, 121.77, 121.68, 120.12, 112.03, 44.91, 27.82 ppm. Anal. calcd. for $C_{14}H_{10}Br_3N$: C 38.93, H 2.33, N 3.24; found: C 38.82, H 2.40, N 3.06.

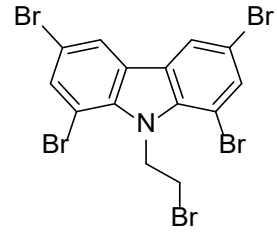
9-(2-Bromoethyl)-3,6-diiodo-9*H*-carbazole (1d)

3,6-Diiodo-9*H*-carbazole (1.5 g, 3.58 mmol), 1,2-dibromoethane (7.74 ml, 89.49 mmol), 50% KOH aqueous solution (2.0 ml, 17.89 mmol) and tetrabutylammonium bromide (0.17 g, 0.53 mmol) were used for the reaction. Product obtained as white crystals (1.79 g, 95% yield).



^{1}H NMR (400 MHz, DMSO-d_6): δ 8.61 (s, 2H), 7.73 (d, *J* = 8.6 Hz, 2H), 7.54 (d, *J* = 8.6 Hz, 2H), 4.82 (t, *J* = 6.1 Hz, 2H), 3.88 (t, *J* = 6.1 Hz, 2H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ 139.14, 134.24, 129.25, 123.59, 112.39, 82.73, 43.96, 31.23 ppm. Anal. calcd. for $C_{14}H_{10}BrI_2N$: C 31.97, H 1.92, N 2.66; found: C 32.08, H 2.02, N 2.41. M.p. 160.5–161.5°C.

1,3,6,8-Tetrabromo-9-(2-bromoethyl)-9*H*-carbazole (1e)



1,3,6,8-Tetrabromo-9*H*-carbazole (0.6 g, 1.24 mmol), 1,2-dibromoethane (2.68 ml, 27.28 mmol), 50% KOH aqueous solution (0.7 ml, 6.2 mmol) and tetrabutylammonium bromide (0.06 g, 0.18 mmol) were used for the reaction. Product obtained as white crystals (0.60 g, 83% yield). M.p. 188.5–190°C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96 (s, 2H), 7.73 (s, 2H), 5.35 (t, *J* = 7.9 Hz, 2H), 3.65 (t, *J* = 8.0 Hz, 2H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 136.83, 135.30, 126.42, 122.45, 113.81, 104.16, 45.32, 30.04 ppm. Anal. calcd. for $C_{14}H_{10}Br_5N$: C 28.51, H 1.37, N 2.38; found: C 28.74, H 1.52, N 2.47.

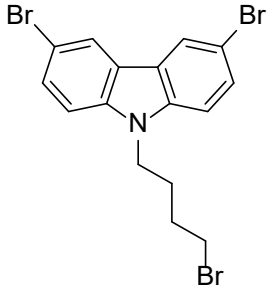
General procedure for the *N*-alkylation with 1,4-dibromobutane

Method A. Starting material (1 eq.) was dissolved in anhydrous DMF under argon atmosphere, followed by the addition of 1,4-dibromobutane (1.5 eq.). The mixture was cooled down in an ice bath to 0°C. Afterwards, NaH (60% dispersion in mineral oil) (1.5 eq.) was added portionwise and 0°C temperature was maintained until the complete

consumption of starting material (TLC, acetone:hexane, 1:24). Organic components were extracted with ethyl acetate, the organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography, using acetone:hexane (1:24) as an eluent.

Method B. Starting material (1 eq.) was dissolved in anhydrous THF under argon atmosphere, followed by the addition of 1,4-dibromobutane (1.5 eq.) and ground KOH (1.5 eq.). The reaction mixture was stirred for 24 h at 25°C temperature. After the termination of reaction (TLC, acetone:*n*-hexane, 1:24), organic components were extracted with ethyl acetate, the organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography, using acetone:*n*-hexane (1:24) as an eluent.

9-(4-Bromobutyl)-3,6-dibromo-9*H*-carbazole (1f)

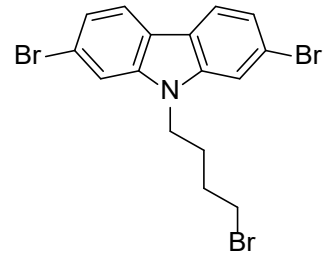


Method A was used. 3,6-Dibromo-9*H*-carbazole (1 g, 3.07 mmol), 1,4-dibromobutane (0.55 ml, 4.61 mmol), NaH (0.18 g, 4.61 mmol) and 15 ml of DMF were used for the reaction. Product obtained as white crystals (1.09 g, 77% yield). M.p. 116.5–118°C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.12 (s, 2H), 7.55 (d, *J* = 8.7 Hz, 2H), 7.25 (d, *J* = 8.7 Hz, 2H), 4.27 (t, *J* = 7.0 Hz, 2H), 3.36 (t, *J* = 6.4 Hz, 2H), 2.07–1.96 (m, 2H), 1.91–1.81 (m, 2H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 139.17, 129.19, 123.54, 123.38, 112.21, 110.27, 42.47, 32.86, 30.04, 27.47 ppm. Anal. calcd. for $C_{16}H_{14}Br_3N$: C 41.78, H 3.07, N 3.04; found: C 41.90, H 3.17, N 3.20.

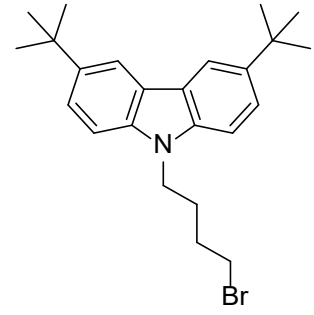
9-(4-Bromobutyl)-2,7-dibromo-9*H*-carbazole (1g)

Method A was used. 3,6-Dibromo-9*H*-carbazole (0.5 g, 1.53 mmol), 1,4-dibromobutane (0.28 ml, 2.30 mmol), NaH (0.09 g, 2.30 mmol) and 10 ml of DMF were used for the reaction. Product obtained as white crystals (0.56 g, 80% yield). M.p. 109.5–110.5°C.



^{1}H NMR (400 MHz, $CDCl_3$): δ 7.89 (d, *J* = 8.3 Hz, 2H), 7.53 (s, 2H), 7.35 (d, *J* = 8.3 Hz, 2H), 4.24 (t, *J* = 7.0 Hz, 2H), 3.41 (t, *J* = 6.3 Hz, 2H), 2.09–1.98 (m, 2H), 1.97–1.86 (m, 2H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 152.79, 141.35, 122.93, 121.72, 121.50, 119.97, 112.04, 42.63, 32.97, 30.17, 27.61 ppm. Anal. calcd. for $C_{16}H_{14}Br_3N$: C 41.78, H 3.07, N 3.04; found: C 42.01, H 3.20, N 3.17.

9-(4-Bromobutyl)-3,6-di-*tert*-butyl-9*H*-carbazole (1h)



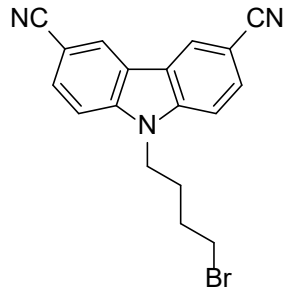
Method A was used. 3,6-Di-tert-butyl-9*H*-carbazole (1 g, 3.57 mmol), 1,4-dibromobutane (0.64 ml, 5.36 mmol), NaH (0.21 g, 5.36 mmol) and 10 ml of THF were used for the reaction. Product obtained as white crystals (1.01 g, 70% yield). M.p. 125–126°C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.12 (s, 2H), 7.52 (d, *J* = 8.5 Hz, 2H), 7.31 (d, *J* = 8.6 Hz, 2H), 4.30 (t, *J* = 6.8 Hz, 2H), 3.39 (t, *J* = 6.4 Hz, 2H), 2.11–2.00 (m, 2H), 1.98–1.88 (m, 2H), 1.48 (s, 18H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 141.84, 138.98, 123.50, 122.91, 116.50, 108.04, 42.38, 34.81, 33.42, 32.20, 30.43, 27.98 ppm. Anal. calcd. for $C_{24}H_{32}BrN$: C 69.56, H 7.78, N 3.38; found: C 69.70, H 7.68, N 3.21.

9-(4-Bromobutyl)-9*H*-carbazole-3,6-dicarbonitrile (1i)

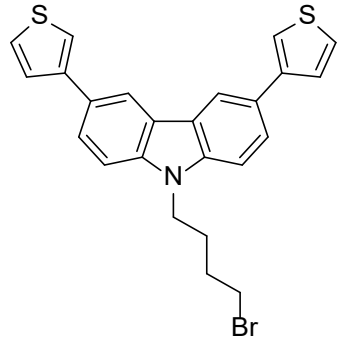
Method A was used. Mixture containing 9*H*-carbazole-3,6-dicarbonitrile (0.8 g, 3.68 mmol) and 1,4-dibromobutane (0.66 ml, 5.52 mmol), NaH (0.44 g, 5.52 mmol) and 25 ml of THF were used for the reaction. Eluent: TLC (acetone:*n*-hexane, 1:4),

column (acetone:*n*-hexane, 1:4). Product obtained as white crystals (0.26 g, 20% yield).



^{1}H NMR (400 MHz, DMSO-d_6): δ 8.80 (s, 2H), 7.96–7.88 (m, 4H), 4.54 (t, *J* = 6.4 Hz, 2H), 3.53 (t, *J* = 6.2 Hz, 2H), 1.94–1.76 (m, 4H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ 142.47, 130.12, 126.35, 121.56, 119.97, 111.35, 102.06, 42.04, 34.53, 29.58, 27.18 ppm. Anal. calcd. for $C_{18}H_{14}BrN_3$: C 61.38, H 4.01, N 11.93; found: C 61.21, H 3.88, N 12.09.

9-(4-Bromobutyl)-3,6-di(thiophen-3-yl)-9*H*-carbazole (1j)

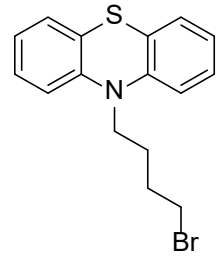


Method B was used. 3,6-Di(thiophen-3-yl)-9*H*-carbazole (0.32 g, 0.96 mmol), 1,4-dibromobutane (0.17 ml, 1.44 mmol), KOH (0.08 g, 1.44 mmol) and 5 ml of THF were used for the reaction. Eluent: TLC (acetone:*n*-hexane, 1:4), column (acetone:*n*-hexane, 1:4). Product obtained as colourless resin (0.28 g, 62% yield).

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.61 (s, 2H), 7.84 (d, *J* = 1.1 Hz, 4H), 7.74–7.60 (m, 6H), 4.46 (t, *J* = 6.3 Hz, 2H), 3.55 (t, *J* = 6.3 Hz, 2H), 1.98–1.79 (m, 4H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ 142.43, 139.68, 126.81, 126.62, 126.40, 124.54, 122.74, 118.99, 118.10, 109.75, 41.57, 34.71, 29.82, 27.34 ppm. Anal. calcd. for $C_{24}H_{20}BrNS_2$: C 61.80, H 4.32, N 3.00; found: C 61.99, H 4.25, N 2.87.

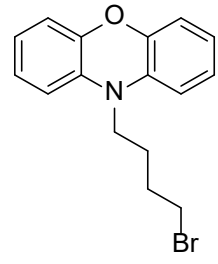
10-(4-Bromobutyl)-10*H*-phenothiazine (1k)

Method B was used. 10*H*-Phenothiazine (0.5 g, 2.51 mmol), 1,4-dibromobutane (0.45 ml, 3.76 mmol), KOH (0.21 g, 3.76 mmol) and 5 ml of THF were used for the reaction. Product obtained as yellowish resin (0.41 g, 49% yield).



^{1}H NMR (400 MHz, DMSO-d_6): δ 7.28–7.11 (m, 4H), 7.11–6.80 (m, 4H), 4.05–3.78 (m, 2H), 3.53 (t, *J* = 6.6 Hz, 2H), 1.98–1.85 (m, 2H), 1.85–1.73 (m, 2H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ 144.73, 127.59, 127.14, 123.82, 122.57, 115.90, 45.55, 34.76, 29.64, 24.98 ppm. Anal. calcd. for $C_{16}H_{16}BrNS$: C 57.49, H 4.82, N 4.19; found: C 57.61, H 4.59, N 4.02.

10-(4-Bromobutyl)-10*H*-phenoxazine (1l)

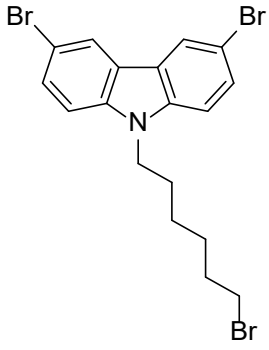


Method B was used. 10*H*-Phenoxazine (0.5 g, 2.72 mmol), 1,4-dibromobutane (0.49 ml, 4.08 mmol), KOH (0.16 g, 4.08 mmol) and 5 ml of THF were used for the reaction. Product obtained as grey crystals (0.3 g, 35% yield). M.p. 60–61°C.

^{1}H NMR (400 MHz, DMSO-d_6): δ 6.86–6.77 (m, 2H), 6.75–6.58 (m, 6H), 3.69–3.48 (m, 4H), 2.00–1.86 (m, 2H), 1.75–1.57 (m, 2H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ 144.03, 132.72, 124.06, 120.80, 115.04, 111.97, 41.92, 34.80, 29.35, 22.91 ppm. Anal. calcd. for $C_{16}H_{16}BrNO$: C 60.39, H 5.07, N 4.40; found: C 60.19, H 5.27, N 4.28.

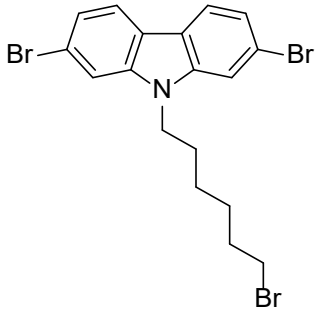
9-(6-Bromohexyl)-3,6-dibromo-9*H*-carbazole (1m)

Method B was used. 3,6-Dibromo-9*H*-carbazole (1 g, 3.07 mmol), 1,4-dibromobutane (1.12 ml, 4.61 mmol), KOH (0.257 g, 4.61 mmol) and 15 ml of THF were used for the reaction. Product obtained as white crystals (0.7 g, 47% yield) ppm.



^{1}H NMR (400 MHz, DMSO-d_6): δ 8.47 (s, 2H), 7.66–7.55 (m, 4H), 4.37 (t, *J* = 6.9 Hz, 2H), 3.45 (t, *J* = 6.6 Hz, 2H), 1.78–1.64 (m, 4H), 1.36 (dt, *J* = 14.3, 7.1 Hz, 2H), 1.26 (dt, *J* = 14.2, 7.2 Hz, 2H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ 139.03, 128.80, 123.43, 122.89, 111.66, 111.23, 42.41, 34.99, 32.07, 28.21, 27.19, 25.44. Anal. calcd. for $C_{18}H_{18}Br_3N$: C 44.30, H 3.72, N 2.87; found: C 44.48, H 3.85, N 2.70.

9-(6-Bromohexyl)-2,7-dibromo-9*H*-carbazole (1n)



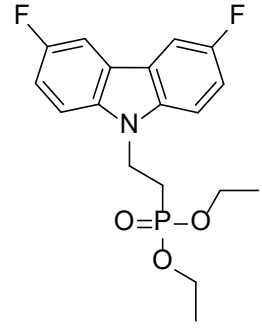
Method B was used. 2,7-Dibromo-9*H*-carbazole (1 g, 3.07 mmol), 1,4-dibromobutane (1.12 ml, 4.61 mmol), KOH (0.08 g, 4.61 mmol) and 15 ml of THF were used for the reaction. Product obtained as white crystals (1.175 g, 79% yield).

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.11 (d, *J* = 8.3 Hz, 2H), 7.90 (s, 2H), 7.35 (dd, *J* = 8.3, 1.2 Hz, 2H), 4.37 (t, *J* = 7.2 Hz, 2H), 3.47 (t, *J* = 6.7 Hz, 2H), 1.72 (tt, *J* = 14.4, 7.0 Hz, 4H), 1.45–1.24 (m, 4H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ 141.08, 122.19, 122.12, 120.68, 119.18, 112.42, 42.31, 34.99, 32.14, 28.16, 27.21, 25.34 ppm. Anal. calcd. for $C_{18}H_{18}Br_3N$: C 44.30, H 3.72, N 2.87; found: C 44.19, H 3.80, N 2.99.

General procedure for the Michaelis–Arbuzov reaction

Alkylated derivative (1 eq.) was dissolved or suspended in triethyl phosphite (20 eq.) and the reaction was refluxed overnight. After the termination of reaction (TLC, acetone:*n*-hexane, 8:17), the solvent was removed under reduced pressure and the crude product was purified by column chromatography, using acetone:*n*-hexane (8:17) as an eluent.

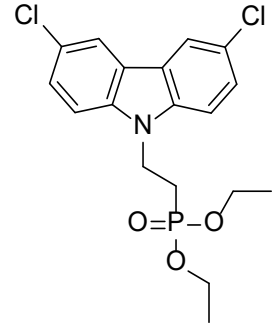
Diethyl [2-(3,6-difluoro-9*H*-carbazol-9-yl) ethyl]phosphonate (2a)



Compound **1a** (0.33 g, 1.06 mmol) and triethyl phosphite (3.6 ml, 21.28 mmol) were used for the reaction. Product obtained as white crystals (0.35 g, 92% yield). M.p. 96–97.5°C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.67 (dd, *J* = 8.7, 2.2 Hz, 2H), 7.35 (dd, *J* = 8.9, 4.1 Hz, 2H), 7.23 (td, *J* = 9.0, 2.3 Hz, 2H), 4.58 (dd, *J* = 15.8, 9.8 Hz, 2H), 4.05 (quint, *J* = 7.2 Hz, 4H), 2.36–2.09 (m, 2H), 1.25 (t, *J* = 7.0 Hz, 6H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 158.58, 156.24, 137.06, 123.21, 123.17, 123.12, 123.07, 114.56, 114.30, 109.64, 109.56, 106.61, 106.37, 62.11, 62.04, 37.48, 37.47, 26.10, 24.72, 16.51, 16.45 ppm. Anal. calcd. for $C_{18}H_{20}F_2NO_3P$: C 58.86, H, 5.49, N, 3.81; found: C 59.01, H 5.55, N 3.98.

Diethyl [2-(3,6-dichloro-9*H*-carbazol-9-yl) ethyl]phosphonate (2b)

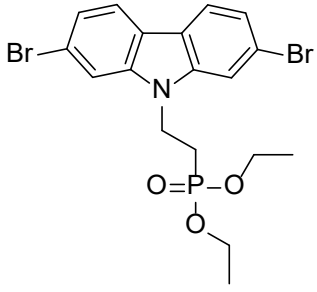


Compound **1b** (0.48 g, 1.39 mmol) and triethyl phosphite (4.8 ml, 27.98 mmol) were used for the reaction. Product obtained as white crystals (0.52 g, 94% yield). M.p. 106.5–108.5°C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96 (s, 2H), 7.43 (d, *J* = 8.6 Hz, 2H), 7.34 (d, *J* = 8.7 Hz, 2H), 4.55 (dd, *J* = 15.2, 9.0 Hz, 2H), 4.08–3.95 (m, 4H), 2.33–2.16 (m, 2H), 1.24 (t, *J* = 7.0 Hz, 6H) ppm. ^{13}C NMR

(101 MHz, $CDCl_3$): δ 138.63, 126.75, 125.33, 123.38, 120.47, 110.00, 62.15, 62.09, 37.43, 26.09, 24.71, 16.30, 16.23 ppm. Anal. calcd. for $C_{18}H_{20}Cl_2NO_3P$: C 54.02, H, 5.04, N, 3.50; found: C 53.93, H 5.15, N 3.68.

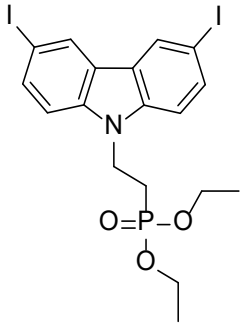
Diethyl [2-(2,7-dibromo-9*H*-carbazol-9-yl) ethyl] phosphonate (2c)



Compound **1c** (0.42 g, 0.97 mmol) and triethyl phosphite (3.3 ml, 19.44 mmol) were used for the reaction. Product obtained as white crystals (0.45 g, 95% yield). M.p. 88.5–90°C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.86 (d, *J* = 8.3 Hz, 2H), 7.55 (s, 2H), 7.35 (d, *J* = 8.3 Hz, 2H), 4.49 (dd, *J* = 15.9, 10.0 Hz, 2H), 4.14–4.02 (m, 4H), 2.28–2.19 (m, 2H), 1.27 (t, *J* = 7.1 Hz, 6H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 140.67, 123.06, 121.58, 121.51, 119.91, 111.98, 62.11, 62.04, 37.24, 37.22, 25.97, 24.59, 16.45, 16.39 ppm. Anal. calcd. for $C_{18}H_{20}Br_2NO_3P$: C 44.20, H, 4.12, N, 2.86; found: C 44.32, H 4.25, N 2.90.

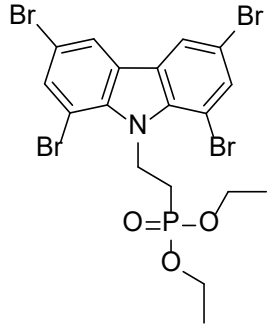
Diethyl [2-(3,6-diiodo-9*H*-carbazol-9-yl)ethyl] phosphonate (2d)



Compound **1d** (1.7 g, 2.91 mmol) and triethyl phosphite (11 ml, 58.30 mmol) were used for the reaction. Product obtained as yellowish resin (1.74 g, 93% yield).

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.60 (s, 2H), 7.74 (d, *J* = 8.6 Hz, 2H), 7.44 (d, *J* = 8.6 Hz, 2H), 4.54 (dt, *J* = 14.3, 7.0 Hz, 2H), 3.85 (quint, *J* = 7.2 Hz, 4H), 2.26 (dt, *J* = 17.8, 6.9 Hz, 2H), 1.04 (t, *J* = 7.0 Hz, 6H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ 138.73, 134.23, 129.26, 123.66, 112.09, 82.50, 61.16, 61.10, 36.83, 36.80, 24.68, 23.32, 16.00, 15.94 ppm. Anal. calcd. for $C_{18}H_{20}I_2NO_3P$: C 37.07, H, 3.46, N, 2.40; found: C 37.01, H 3.65, N 2.53.

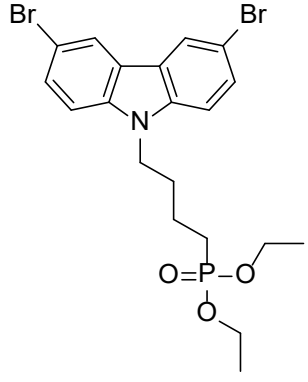
Diethyl [2-(1,3,6,8-tetrabromo-9*H*-carbazol-9-yl)ethyl]phosphonate (2e)



Compound **1e** (0.270 g, 0.45 mmol) and triethyl phosphite (1.6 ml, 9 mmol) were used for the reaction. Product obtained as white crystals (0.1 g, 36% yield). M.p. 211.5–213.5°C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.03 (s, 2H), 7.79 (s, 2H), 5.43 (dt, *J* = 8.7, 5.3 Hz, 2H), 4.18–4.06 (m, 4H), 2.38–2.20 (m, 2H), 1.33 (t, *J* = 7.0 Hz, 6H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 136.91, 135.22, 126.73, 122.32, 113.65, 104.36, 62.00, 61.93, 39.55, 29.40, 28.04, 16.68, 16.62 ppm. Anal. calcd. for $C_{18}H_{18}Br_4NO_3P$: C 33.42, H, 2.80, N, 2.17; found: C 33.28, H 2.71, N 2.25.

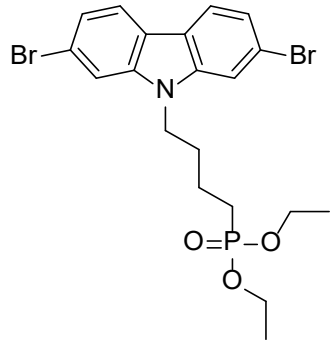
Diethyl [4-(3,6-dibromo-9*H*-carbazol-9-yl) butyl]phosphonate (2f)



Compound **1f** (1.07 g, 2.06 mmol) and triethyl phosphite (7.1 ml, 41.37 mmol) were used for the reaction. Product obtained as yellowish resin (1.109 g, 92% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10 (s, 2H), 7.53 (d, *J* = 8.7 Hz, 2H), 7.24 (d, *J* = 8.7 Hz, 2H), 4.23 (t, *J* = 7.0 Hz, 2H), 4.01 (quint, *J* = 7.2 Hz, 4H), 2.01–1.86 (m, 2H), 1.78–1.50 (m, 4H), 1.24 (t, *J* = 7.0 Hz, 6H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 139.29, 129.21, 123.59, 123.40, 112.22, 110.41, 61.71, 61.64, 42.93, 29.71, 29.56, 26.17, 24.76, 20.52, 20.47, 16.55, 16.50 ppm. Anal. calcd. for $C_{20}H_{24}Br_2NO_3P$: C 46.45, H, 4.68, N, 2.71; found: C 46.60, H 4.79, N 2.55.

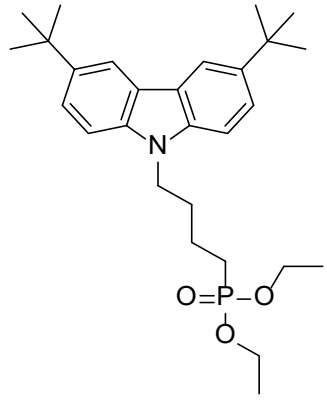
Diethyl [4-(2,7-dibromo-9*H*-carbazol-9-yl) butyl]phosphonate (2g)



Compound **1g** (0.52 g, 1.13 mmol) and triethyl phosphite (3.9 ml, 22.60 mmol) were used for the reaction. Product obtained as lightly yellow resin (0.52 g, 90%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.87 (d, J = 8.3 Hz, 2H), 7.51 (s, 2H), 7.33 (d, J = 8.3 Hz, 2H), 4.20 (t, J = 7.1 Hz, 2H), 4.13–3.98 (m, 4H), 2.03–1.87 (m, 2H), 1.83–1.60 (m, 4H), 1.27 (t, J = 7.0 Hz, 6H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 141.35, 122.83, 121.66, 121.45, 119.89, 112.02, 61.80, 61.73, 42.99, 29.70, 29.55, 26.24, 24.83, 20.60, 20.55, 16.61, 16.55 ppm. Anal. calcd. for $C_{20}H_{24}Br_2NO_3P$: C 46.45, H, 4.68, N, 2.71; found: C 46.58, H 4.50, N 2.84.

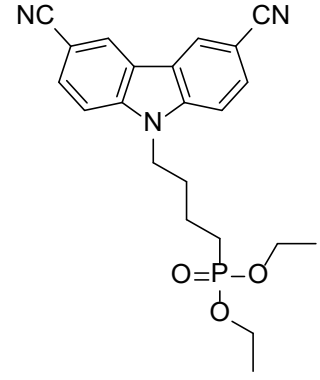
Diethyl [4-(3,6-di-*tert*-butyl-9*H*-carbazol-9-yl)butyl]phosphonate (2h)



Compound **1h** (0.95 g, 2.29 mmol) and triethyl phosphite (7.86 ml, 45.84 mmol) were used for the reaction. Product obtained as slightly yellow resin (1.04 g, 96%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.09 (s, 2H), 7.50 (d, J = 8.3 Hz, 2H), 7.29 (d, J = 8.5 Hz, 2H), 4.27 (t, J = 6.3 Hz, 2H), 4.07–3.96 (m, 4H), 2.03–1.90 (m, 2H), 1.83–1.66 (m, 4H), 1.45 (s, 18H), 1.24 (t, J = 6.5 Hz, 6H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 141.71, 138.96, 123.41, 122.83, 116.39, 108.08, 61.67, 42.67, 34.75, 32.16, 30.14, 30.00, 26.50, 25.04, 20.60, 16.29, 16.23 ppm. Anal. calcd. for $C_{28}H_{42}NO_3P$: C 71.31, H, 8.98, N, 2.97; found: C 71.41, H 9.05, N 2.80.

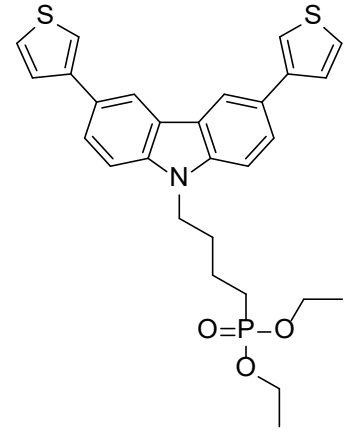
Diethyl [4-(3,6-dicyano-9*H*-carbazol-9-yl)butyl]phosphonate (2i)



Compound **1i** (0.23 g, 0.65 mmol) and triethyl phosphite (2.23 ml, 13.05 mmol) were used for the reaction. Product obtained as yellowish crystals (0.23 g, 86%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.40 (s, 2H), 7.77 (d, J = 8.5 Hz, 2H), 7.53 (d, J = 8.6 Hz, 2H), 4.38 (t, J = 7.2 Hz, 2H), 4.09–3.97 (m, 4H), 2.07–1.94 (m, 2H), 1.80–1.62 (m, 4H), 1.25 (t, J = 7.0 Hz, 6H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 142.70, 130.46, 125.83, 122.26, 119.80, 110.27, 103.68, 61.77, 61.71, 43.37, 29.59, 29.45, 26.05, 24.63, 20.51, 20.46, 16.56, 16.50 ppm. Anal. calcd. for $C_{22}H_{24}N_3O_3P$: C 64.54, H, 5.91, N, 10.26; found: C 64.74, H 5.99, N 10.45.

Diethyl [4-(3,6-di(thiophen-3-yl)-9*H*-carbazol-9-yl)butyl]phosphonate (2j)

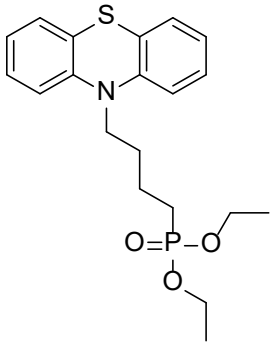


Compound **1j** (0.25 g, 0.53 mmol) and triethyl phosphite (1.83 ml, 10.71 mmol) were used for the reaction. Product obtained as yellowish resin (0.26 g, 93%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.61 (s, 2H), 7.83 (d, J = 7.0 Hz, 4H), 7.73–7.58 (m, 6H), 4.43

(t, *J* = 6.7 Hz, 2H), 3.89 (p, *J* = 7.2 Hz, 4H), 1.94–1.82 (m, 2H), 1.81–1.68 (m, 2H), 1.59–1.44 (m, 2H), 1.13 (t, *J* = 7.0 Hz, 6H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ 142.46, 139.73, 126.80, 126.56, 126.40, 124.46, 122.71, 118.95, 118.05, 109.83, 60.79, 60.73, 41.97, 29.39, 29.23, 24.93, 23.55, 19.76, 19.71, 16.25, 16.19 ppm. Anal. calcd. for $C_{28}H_{30}NO_3PS_2$: C 64.22, H, 5.77, N, 2.67; found: C 64.10, H 5.84, N 2.60.

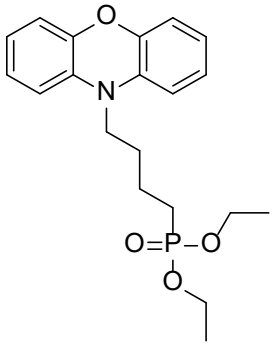
Diethyl [4-(10*H*-phenothiazin-10-yl)butyl] phosphonate (2k)



Compound **1k** (0.38 g, 1.13 mmol) and triethyl phosphite (3.89 ml, 22.73 mmol) were used for the reaction. Product obtained as orange resin (0.42 g, 94%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.19 (t, *J* = 7.7 Hz, 2H), 7.14 (d, *J* = 7.5 Hz, 2H), 7.03 (d, *J* = 8.1 Hz, 2H), 6.94 (t, *J* = 7.4 Hz, 2H), 3.98–3.80 (m, 6H), 1.82–1.66 (m, 4H), 1.65–1.49 (m, 2H), 1.17 (t, *J* = 7.0 Hz, 6H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ 144.77, 127.57, 127.10, 123.70, 122.46, 115.90, 60.76, 60.70, 45.87, 26.99, 26.83, 24.76, 23.39, 19.51, 19.47, 16.27, 16.22 ppm. Anal. calcd. for $C_{20}H_{26}NO_3PS$: C 61.36, H, 6.69, N, 3.58; found: C 61.51, H 6.49, N 3.33.

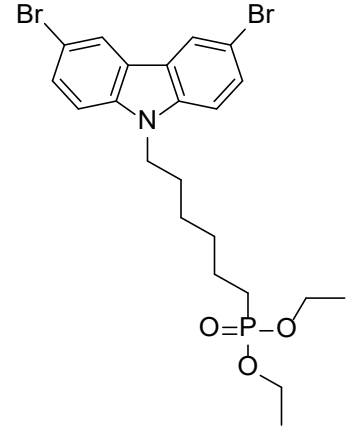
Diethyl [4-(10*H*-phenoxazin-10-yl)butyl] phosphonate (2l)



Compound **1l** (0.25 g, 0.78 mmol) and triethyl phosphite (2.69 ml, 15.71 mmol) were used for the reaction. Product obtained as yellowish resin (0.27 g, 91%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 6.85–6.77 (m, 2H), 6.70 (d, *J* = 8.0 Hz, 2H), 6.68–6.60 (m, 4H), 4.01–3.90 (m, 4H), 3.55 (t, *J* = 6.7 Hz, 2H), 1.87–1.73 (m, 2H), 1.69–1.51 (m, 4H), 1.22 (t, *J* = 7.1 Hz, 6H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ 144.07, 132.83, 124.04, 120.73, 114.99, 112.07, 60.83, 60.76, 42.38, 24.98, 24.90, 24.83, 23.53, 19.37, 19.33, 16.33, 16.27 ppm. Anal. calcd. for $C_{20}H_{26}NO_4P$: C 63.99, H, 6.98, N, 3.73; found: C 64.09, H 6.71, N 3.56.

Diethyl [6-(3,6-dibromo-9*H*-carbazol-9-yl) hexyl]phosphonate (2m)



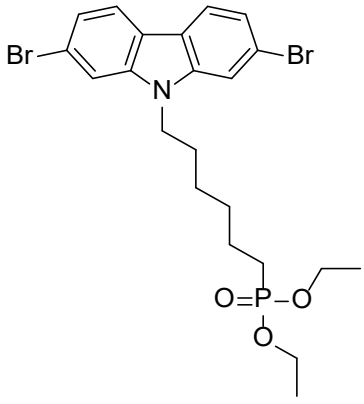
Compound **1m** (0.65 g, 1.33 mmol) and triethyl phosphite (4.5 ml, 26.63 mmol) were used for the reaction. Product obtained as slightly yellow resin (0.7 g, 97%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11 (d, *J* = 1.7 Hz, 2H), 7.53 (dd, *J* = 8.7, 1.7 Hz, 2H), 7.23 (d, *J* = 8.7 Hz, 2H), 4.21 (t, *J* = 7.1 Hz, 2H), 4.13–3.97 (m, 4H), 1.86–1.76 (m, 2H), 1.71–1.60 (m, 2H), 1.60–1.48 (m, 2H), 1.42–1.26 (m, 10H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 139.35, 129.13, 123.53, 123.36, 112.09, 110.45, 61.60, 61.53, 43.29, 30.41, 30.24, 28.74, 26.85, 26.33, 24.93, 22.43, 22.38, 16.62, 16.56 ppm. Anal. calcd. for $C_{22}H_{28}BrNO_3P$: C 48.46, H, 5.18, N, 2.57; found: C 48.35, H 5.32, N 2.63.

Diethyl [6-(2,7-dibromo-9*H*-carbazol-9-yl) hexyl]phosphonate (2n)

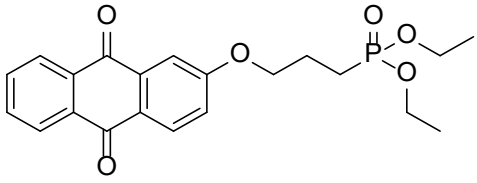
Compound **1n** (1.1 g, 2.25 mmol) and triethyl phosphite (7.7 ml, 45.07 mmol) were used for the reaction. Product obtained as colourless resin (1.16 g, 94%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.86 (d, *J* = 8.3 Hz, 2H), 7.49 (d, *J* = 0.9 Hz, 2H), 7.32 (dd, *J* = 8.3, 1.3 Hz, 2H), 4.16 (t, *J* = 7.2 Hz, 2H), 4.13–4.00 (m, 4H), 1.89–1.78 (m, 2H), 1.75–1.63 (m, 2H), 1.62–1.51 (m, 2H), 1.47–1.34 (m, 4H), 1.30 (t, *J* = 7.1 Hz, 6H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 141.39, 122.68, 121.61, 121.37, 119.81, 112.04, 61.61, 61.55, 43.32,



30.45, 30.29, 28.71, 26.84, 26.35, 24.95, 22.48, 22.43, 16.63, 16.57 ppm. Anal. calcd. for $C_{22}H_{28}BrNO_3P$: C 48.46, H, 5.18, N, 2.57; found: C 48.58, H 5.30, N 2.71.

Diethyl {3-[(9,10-dioxo-9,10-dihydroanthracen-2-yl)oxy]propyl}phosphonate (3)



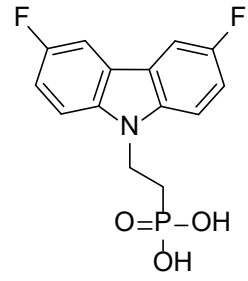
2-Hydroxyanthracene-9,10-dione (1 g, 2.49 mmol) was mixed with anhydrous K_2CO_3 (1.54 g, 11.12 mmol) and diethyl(3-bromopropyl)phosphonate (2.14 ml, 11.11 mmol) in anhydrous DMF (20 ml). The reaction mixture was stirred at 100°C overnight. After the reaction completion (TLC: acetone:*n*-hexane, 8:17), the reaction mixture was extracted with ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography (acetone:*n*-hexane, 8:17) resulting in yellow resin (1.7 g, 95% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.37–8.12 (m, 3H), 7.84–7.73 (m, 2H), 7.70 (d, *J* = 2.4 Hz, 1H), 7.31–7.20 (m, 1H), 4.22 (t, *J* = 6.0 Hz, 2H), 4.18–4.06 (m, 4H), 2.25–2.10 (m, 2H), 2.03–1.90 (m, 2H), 1.34 (t, *J* = 7.1 Hz, 6H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 183.30, 182.22, 163.62, 160.95, 135.69, 134.29, 133.80, 133.67, 129.92, 127.26, 121.44, 110.68, 68.12, 63.69, 61.77, 23.09, 22.65, 21.67, 16.57 ppm. Anal. calcd. for $C_{21}H_{23}O_6P$ C 62.68; H 5.76; found C 62.74; H 5.69.

General procedure for the diethyl phosphonate hydrolysis reaction

Phosphonate derivative (1 eq.) was dissolved in anhydrous 1,4-dioxane under argon atmosphere. Afterwards, bromotrimethylsilane (10 eq.) was added dropwise, and the reaction was stirred overnight at 25°C under argon atmosphere. After the consumption of phosphonate starting material (TLC, acetone:*n*-hexane, 8:17), methanol (10 eq.) was added and stirring continued for 2 h. Next, distilled water was added dropwise until a precipitate was formed and stirring continued overnight. The precipitate was filtered off and washed with distilled water. Product was purified by dissolving in the minimum amount of THF, precipitating into the 20-fold excess of *n*-hexane, filtering, and washing with *n*-hexane.

[2-(3,6-Difluoro-9*H*-carbazol-9-yl)ethyl]phosphonic acid (F-2PACz)

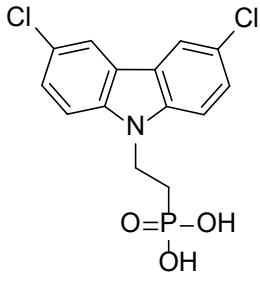


Compound **2a** (0.32 g, 0.87 mmol), bromotrimethylsilane (1.4 ml, 8.7), methanol (1.5 ml, 38.84 mmol) and 10 ml of 1,4-dioxane were used. Product obtained as grey crystals (0.19 g, 73% yield). M.p. 207.5–209.5°C (melting with decomposition).

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.02 (d, *J* = 7.7 Hz, 2H), 7.56 (dd, *J* = 8.8, 4.1 Hz, 2H), 7.35 (t, *J* = 8.3 Hz, 2H), 4.54 (dd, *J* = 15.6, 8.0 Hz, 2H), 2.08–1.93 (m, 2H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ 157.67, 155.35, 136.89, 122.38, 122.34, 122.28, 122.24, 114.31, 114.05, 110.47, 110.38, 106.66, 106.42, 37.81, 27.95, 26.64 ppm. Anal. calcd. for $C_{14}H_{12}F_2NO_3P$: C 54.03, H, 3.89, N, 4.50; found: C 54.28, H 3.97, N 4.69.

[2-(3,6-Dichloro-9*H*-carbazol-9-yl)ethyl]phosphonic acid (Cl-2PACz)

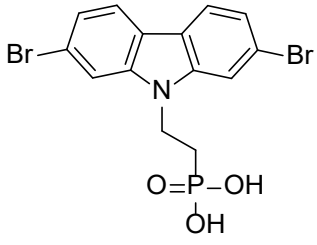
Compound **2b** (0.5 g, 1.25 mmol), bromotrimethylsilane (1.6 ml, 12.49), methanol (2 ml, 49.97 mmol) and 15 ml of 1,4-dioxane were used. Product obtained as white crystals (0.39 g, 91%



yield). M.p. 220–221.5°C (melting with decomposition).

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.32 (s, 2H), 7.59 (d, *J* = 8.7 Hz, 2H), 7.51 (dd, *J* = 8.7, 1.0 Hz, 2H), 4.54 (dd, *J* = 15.8, 8.5 Hz, 2H), 2.13–1.89 (m, 2H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ 138.48, 126.36, 123.74, 122.66, 120.55, 111.01, 37.81, 27.85, 26.55 ppm. Anal. calcd. for $C_{14}H_{12}Cl_2NO_3P$: C 48.86, H, 3.51, N, 4.07; found: C 48.69, H 3.40, N 3.80.

[2-(2,7-Dibromo-9*H*-carbazol-9-yl)ethyl] phosphonic acid (Br'-2PACz)

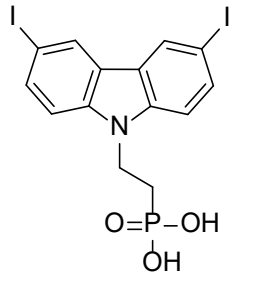


Compound **2c** (0.43 g, 0.88 mmol), bromotrimethylsilane (1.1 ml, 8.79), methanol (1.4 ml, 35.16 mmol) and 15 ml of 1,4-dioxane were used. Product obtained as grey crystals (0.32 g, 86% yield). M.p. 259–261°C (melting with decomposition).

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.12 (d, *J* = 8.3 Hz, 2H), 7.81 (s, 2H), 7.37 (d, *J* = 8.3 Hz, 2H), 4.52 (dd, *J* = 15.7, 8.6 Hz, 2H), 2.12–1.97 (m, 2H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ 140.64, 122.38, 122.30, 120.95, 119.23, 112.30, 37.76, 27.75, 26.44 ppm. Anal. calcd. for $C_{14}H_{12}Br_2NO_3P$: C 38.83, H, 2.79, N, 3.23; found: C 38.61, H 2.90, N 3.35.

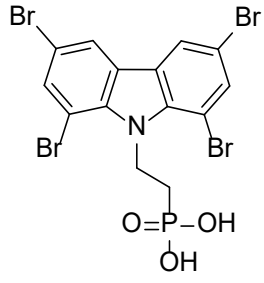
[2-(3,6-Diiodo-9*H*-carbazol-9-yl)ethyl]phosphonic acid (I-2PACz)

Compound **2d** (1.7 g, 2.91 mmol), bromotrimethylsilane (3.8 ml, 29.15), methanol (4.7 ml, 116.61 mmol) and 30 ml of 1,4-dioxane were used. Product obtained as white crystals (1.33 g, 87% yield). M.p. 209.5–211°C (melting with decomposition).



^{1}H NMR (400 MHz, DMSO-d_6): δ 8.61 (s, 2H), 7.75 (dd, *J* = 8.6, 1.3 Hz, 2H), 7.42 (d, *J* = 8.6 Hz, 2H), 4.50 (dd, *J* = 15.9, 8.4 Hz, 2H), 2.06–1.93 (m, 2H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ 138.66, 134.36, 129.41, 123.67, 111.79, 82.50, 37.65, 27.78, 26.47 ppm. Anal. calcd. for $C_{14}H_{12}I_2NO_3P$: C 31.91, H, 2.30, N, 2.66; found: C 31.79, H 2.22, N 2.76.

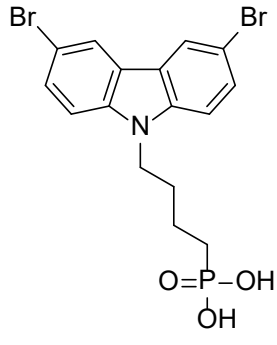
[2-(1,3,6,8-Tetrabromo-9*H*-carbazol-9-yl) ethyl]phosphonic acid (4Br-2PACz)



Compound **2e** (0.09 g, 0.14 mmol), bromotrimethylsilane (0.2 ml, 1.39), methanol (0.22 ml, 5.56 mmol) and 6 ml of 1,4-dioxane:DCM (1:1) were used. Product obtained as white crystals (0.07 g, 87% yield). M.p. 331–332°C (melting and decomposition).

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.58 (s, 2H), 7.90 (s, 2H), 5.29–5.19 (m, 2H), 2.13–2.02 (m, 2H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ 136.70, 134.30, 126.39, 123.14, 112.84, 103.89, 45.12, 30.15 ppm. Anal. calcd. for $C_{14}H_{10}Br_4NO_3P$: C 28.46, H, 1.71, N, 2.37; found: C 28.50, H 1.79, N 2.56.

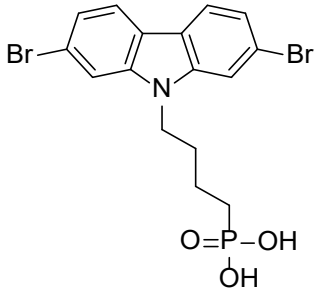
[4-(3,6-Dibromo-9*H*-carbazol-9-yl)butyl] phosphonic acid (Br-4PACz)



Compound **2f** (0.5 g, 0.96 mmol), bromotrimethylsilane (1.3 ml, 9.66), methanol (1.6 ml, 38.66 mmol) and 15 ml of 1,4-dioxane were used. Product obtained as barely yellowish white crystals (0.289 g, 65%). M.p. 203–204.5°C (melting with decomposition).

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.46 (s, 2H), 7.71–7.50 (m, 4H), 4.39 (t, *J* = 6.9 Hz, 2H), 1.90–1.72 (m, 2H), 1.60–1.40 (m, 4H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ 139.05, 128.82, 123.43, 122.91, 111.76, 111.26, 42.29, 29.49, 29.33, 27.96, 26.61, 20.36, 20.32 ppm. Anal. calcd. for $C_{16}H_{16}Br_2NO_3P$: C 41.68, H, 3.50, N, 3.04; found: C 41.49, H 3.35, N 3.11.

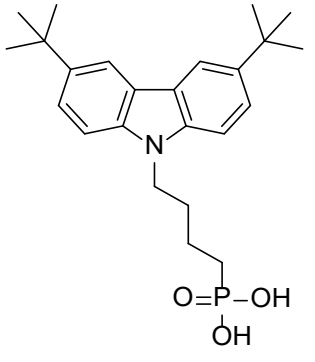
[4-(2,7-Dibromo-9*H*-carbazol-9-yl)butyl] phosphonic acid (Br'-4PACz)



Compound **2g** (0.5 g, 0.96 mmol), bromotrimethylsilane (1.3 ml, 9.66), methanol (1.6 ml, 38.66 mmol) and 15 ml of 1,4-dioxane were used. Product obtained as white crystals (0.254 g, 57% yield). M.p. 189.5–190.5°C (melting with decomposition).

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.11 (d, *J* = 8.3 Hz, 2H), 7.94 (s, 2H), 7.35 (d, *J* = 8.3 Hz, 2H), 4.39 (t, *J* = 7.2 Hz, 2H), 1.87–1.70 (m, 2H), 1.63–1.46 (m, 4H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ 141.09, 122.18, 122.14, 120.68, 119.22, 112.52, 42.26, 29.44, 29.28, 27.99, 26.63, 20.26, 20.21 ppm. Anal. calcd. for $C_{16}H_{16}Br_2NO_3P$: C 41.68, H, 3.50, N, 3.04; found: C 41.82, H 3.39, N 2.98.

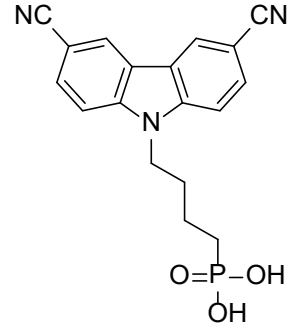
[4-(3,6-Di-*tert*-butyl-9*H*-carbazol-9-yl)butyl] phosphonic acid (*t*-Bu-4PACz)



Compound **2h** (1 g, 2.12 mmol), bromotrimethylsilane (2.75 ml, 21.2 mmol), methanol (3.7 ml, 84.81 mmol) and 10 ml of 1,4-dioxane were used. Product obtained as white crystals (0.81 g, 92% yield). M.p. 184–186°C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.97–9.00 (m, 2H), 8.10 (s, 2H), 7.48 (d, *J* = 8.5 Hz, 2H), 7.31–7.20 (m, 2H), 4.22 (t, *J* = 6.9 Hz, 2H), 2.03–1.91 (m, 2H), 1.89–1.70 (m, 4H), 1.44 (s, 18H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 141.78, 138.94, 123.47, 122.89, 116.46, 108.03, 42.73, 34.78, 32.20, 29.81, 29.66, 25.79, 24.33, 20.17, 20.12 ppm. Anal. calcd. for $C_{24}H_{34}NO_3P$: C 69.38, H, 8.25, N, 3.37; found: C 69.47, H 8.39, N 3.40.

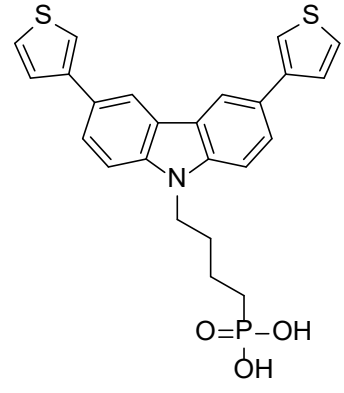
[4-(3,6-Dicyano-9*H*-carbazol-9-yl)butyl] phosphonic acid (CN-4PACz)



Compound **2i** (0.23, 0.56 mmol), bromotrimethylsilane (0.73 ml, 5.61 mmol), methanol (0.9 ml, 22.47 mmol) and 8 ml of 1,4-dioxane:DCM (5:3) were used. Product obtained as brownish white crystals (0.18 g, 91% yield).

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.80 (s, 2H), 7.99–7.84 (m, 4H), 4.59–4.41 (m, 4H), 1.92–1.74 (m, 2H), 1.63–1.41 (m, 4H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ 142.50, 130.06, 126.31, 121.52, 120.01, 111.46, 101.96, 42.63, 29.42, 29.26, 27.90, 26.54, 20.28 ppm. Anal. calcd. for $C_{18}H_{16}N_3O_3P$: C 61.19, H, 4.56, N, 11.89; found: C 61.22, H 4.40, N 11.73.

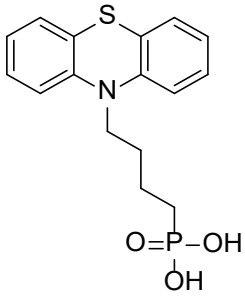
[4-(3,6-Di(thiophen-3-yl)-9*H*-carbazol-9-yl) butyl]phosphonic acid (3TPH-4PACz)



Compound **2j** (1 g, 2.12 mmol), bromotrimethylsilane (2.75 ml, 21.2 mmol), methanol (3.7 ml, 84.81 mmol) and 10 ml of 1,4-dioxane were used. Product obtained as dark grey crystals (0.81 g, 92% yield). M.p. 186.5–188°C.

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.61 (s, 2H), 7.83 (d, *J* = 7.2 Hz, 4H), 7.72–7.58 (m, 6H), 4.42 (t, *J* = 6.5 Hz, 2H), 1.95–1.79 (m, 2H), 1.65–1.46 (m, 4H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ 142.48, 139.74, 126.80, 126.54, 126.42, 124.51, 122.70, 118.96, 118.07, 109.82, 42.25, 29.78, 29.63, 28.08, 26.72, 20.50, 20.46 ppm. Anal. calcd. for $C_{24}H_{22}NO_3PS_2$: C 61.66, H, 4.74, N, 3.00; found: C 61.71, H 4.90, N 3.12.

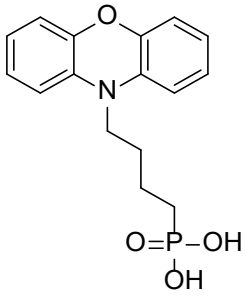
[4-(10*H*-phenothiazin-10-yl)butyl]phosphonic acid (4PAPTZ)



Compound **2k** (0.4 g, 1.02 mmol), bromotrimethylsilane (1.3 ml, 10.21 mmol), methanol (1.7 ml, 40.87 mmol) and 10 ml of 1,4-dioxane were used. Product obtained as dark grey crystals (0.26 g, 76% yield). M.p. 193–195°C (melting with decomposition).

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.19 (t, *J* = 7.7 Hz, 2H), 7.14 (d, *J* = 7.5 Hz, 2H), 7.03 (d, *J* = 8.1 Hz, 2H), 6.93 (t, *J* = 7.4 Hz, 2H), 3.86 (t, *J* = 6.6 Hz, 2H), 1.82–1.70 (m, 2H), 1.67–1.44 (m, 4H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ 144.77, 127.61, 127.12, 123.53, 122.44, 115.80, 46.18, 27.84, 27.29, 27.14, 26.49, 20.34, 20.30 ppm. Anal. calcd. for $C_{16}H_{18}NO_3PS$: C 57.30, H, 5.41, N, 4.18; found: C 57.58, H 5.58, N 4.01.

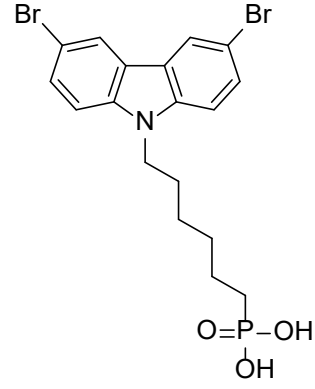
[4-(10*H*-phenoxazin-10-yl)butyl]phosphonic acid (4PAPOZ)



Compound **2l** (0.24 g, 0.64 mmol), bromotrimethylsilane (0.8 ml, 6.39 mmol), methanol (1 ml, 25.57 mmol) and 5 ml of 1,4-dioxane were used. Product obtained as dark grey crystals (0.15 g, 73% yield). M.p. 182–184°C (melting with decomposition).

^{1}H NMR (400 MHz, DMSO-d_6): δ 6.86–6.77 (m, 2H), 6.69 (d, *J* = 8.0 Hz, 2H), 6.67–6.58 (m, 4H), 3.58–3.49 (m, 2H), 1.70–1.50 (m, 6H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ 144.06, 132.88, 124.10, 120.71, 114.99, 112.02, 42.66, 27.93, 26.57, 25.19, 25.04, 20.10, 20.06 ppm. Anal. calcd. for $C_{16}H_{18}NO_4P$: C 60.19, H, 5.68, N, 4.39; found: C 60.01, H 5.73, N 4.20.

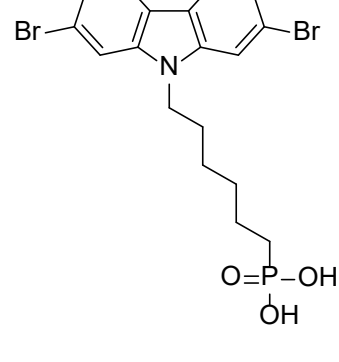
[6-(3,6-Dibromo-9*H*-carbazol-9-yl)hexyl]phosphonic acid (Br-6PACz)



Compound **2m** (0.65 g, 1.19 mmol), bromotrimethylsilane (1.5 ml, 11.92 mmol), methanol (1.9 ml, 47.68 mmol) and 10 ml of 1,4-dioxane were used. Product obtained as white crystals (0.41 g, 70% yield).

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.46 (s, 2H), 7.65–7.54 (m, 4H), 4.37 (t, *J* = 6.7 Hz, 2H), 1.78–1.65 (m, 2H), 1.50–1.22 (m, 8H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ 139.08, 128.85, 123.47, 122.92, 111.69, 111.26, 42.48, 29.74, 29.59, 28.25, 28.14, 26.78, 26.02, 22.73, 22.64 ppm. Anal. calcd. for $C_{18}H_{20}Br_2NO_3P$: C 44.20, H, 4.12, N, 2.86; found: C 44.34, H 4.02, N 2.70.

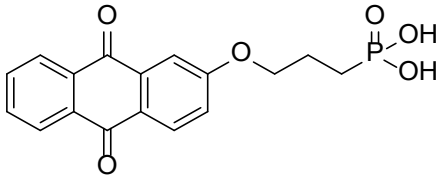
[6-(3,6-Dibromo-9*H*-carbazol-9-yl)hexyl]phosphonic acid (Br'-6PACz)



Compound **2n** (1.1 g, 2.01 mmol), bromotrimethylsilane (2.6 ml, 20.17 mmol), methanol (3.2 ml, 80.69 mmol) and 20 ml of 1,4-dioxane were used. Product obtained as white crystals (0.88 g, 90% yield).

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.10 (d, *J* = 8.3 Hz, 2H), 7.89 (s, 2H), 7.34 (d, *J* = 8.3 Hz, 2H), 4.39–4.34 (m, 2H), 1.72–1.61 (m, 2H), 1.49–1.25 (m, 8H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ 141.12, 122.22, 122.15, 120.70, 119.20, 112.44, 42.43, 29.90, 29.74, 28.33, 28.22, 26.86, 26.02, 22.79, 22.74 ppm. Anal. calcd. for $C_{18}H_{20}Br_2NO_3P$: C 44.20, H, 4.12, N, 2.86; found: C 44.23, H 4.30, N 3.01.

{3-[(9,10-Dioxo-9,10-dihydroanthracen-2-yl) oxy]propyl}phosphonic acid (3PAAQ)



Compound **3** (1.7 g, 4.9 mmol) was dissolved in 35% HCl (50 ml) and refluxed for 24 h under argon atmosphere. After the termination of the reaction (acetone:*n*-hexane, 8:17), the solvent was evaporated and the crude product dissolved in THF (200 ml) and precipitated into *n*-hexane (400 ml). The precipitate was filtered off and washed with *n*-hexane (50 ml) to give 1.38 g (95% yield) of pale-yellow crystals. M.p. 191.5–193°C.

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.19–8.14 (m, 3H), 7.90 (p, J = 7.7 Hz, 2H), 7.56 (s, 1H), 7.43 (d, J = 8.5 Hz, 1H), 4.25 (s, 2H), 1.98 (s, 2H), 1.72 (s, 2H) ppm.^{13}C NMR (101 MHz, DMSO-d_6): δ 182.41, 181.31, 163.32, 135.08, 134.64, 134.19, 133.08, 129.55, 126.74, 126.66, 126.36, 121.15, 110.61, 62.95, 32.30, 22.81 ppm. Anal. calcd. for $C_{17}H_{15}O_6P$ C 58.97; H 4.37; found C 58.89; H 4.45.

Cells and culture conditions

H69AR (CRL-11351) were obtained from the American Type Culture Collection (Rockville, MD, USA). Cells were cultivated in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F-12) (Gibco, Waltham, MA, USA), 10% fetal bovine serum (FBS) (Gibco, Waltham, MA, USA), 100 U/mL penicillin, and 100 μg/mL streptomycin (P/S) (Gibco, Waltham, MA, USA). Culturing conditions were maintained at 37°C with a humidified atmosphere containing 5% CO_2. The culture medium was refreshed every 2–3 days, and the cells were passaged upon reaching 70–80% confluence.

MTT cell viability assay

The antiproliferative activity of the synthesised compounds was evaluated using the MTT assay. H69AR cells were seeded into 96-well plates at a density of 1×10^4 cells per well and allowed to adhere overnight at 37°C in a humidified atmosphere containing 5% CO_2. The following day, the cells were exposed to test compounds at a final concentration of 100 μM, with treatments performed in triplicate. After 20 h of incubation, a MTT reagent was added to each well, and plates were incubated for an additional 4 h to allow for formazan crystal formation. The medium was then removed, and the formazan product was solubilised using anhydrous DMSO. Absorbance was recorded at 570 nm using a microplate reader. Data were analysed using the GraphPad Prism or QuickCalcs statistical software.

STATISTICAL ANALYSIS

Unless otherwise specified, results are presented as the mean ± standard deviation (SD) from three independent biological replicates. Statistical analysis was performed using one-way ANOVA in the GraphPad Prism software. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Synthesis of heterocyclic and anthraquinone derivatives bearing alkyl phosphonic acid moieties

Carbazole-, phenothiazine- and phenoxazine-based materials, containing a phosphonic acid functional group, different substituents and aliphatic chains of varying lengths were synthesised by a 3-step synthesis procedure, applying alkylation, phosphonylation and hydrolysis reactions. Meanwhile, synthesis of anthraquinone-based analogue required two steps overall. The starting materials 3,6-difluoro-9*H*-carbazole and 9*H*-carbazole-3,6-dicarbonitrile were synthesised according to the procedures described in Refs. [28, 29].

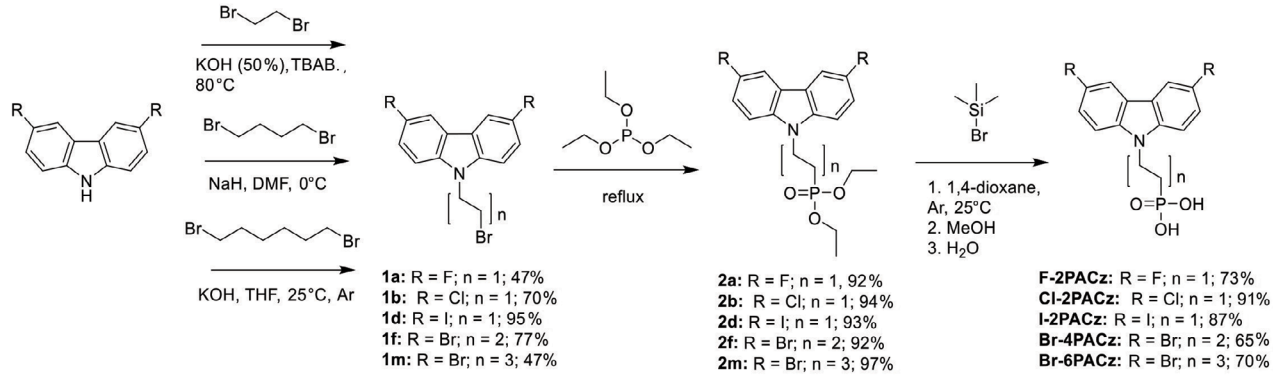
3,6-Halogenated carbazoles were alkylated using 1,2-dibromoethane, 1,4-dibromobutane or 1,6-dibromohexane (Scheme 1). Then alkylated derivatives were used in the Arbuzov reaction with

triethyl phosphite forming the intermediate phosphonates **2a**, **2b**, **2d**, **2f** and **2m**. The final products **F-2PACz**, **Cl-2PACz**, **I-2PACz**, **Br-4PACz** and **Br-6PACz**, containing a phosphonic acid functional group, were obtained by the hydrolysis of obtained intermediates in 1,4-dioxane, using bromotrimethylsilane, methanol and water.

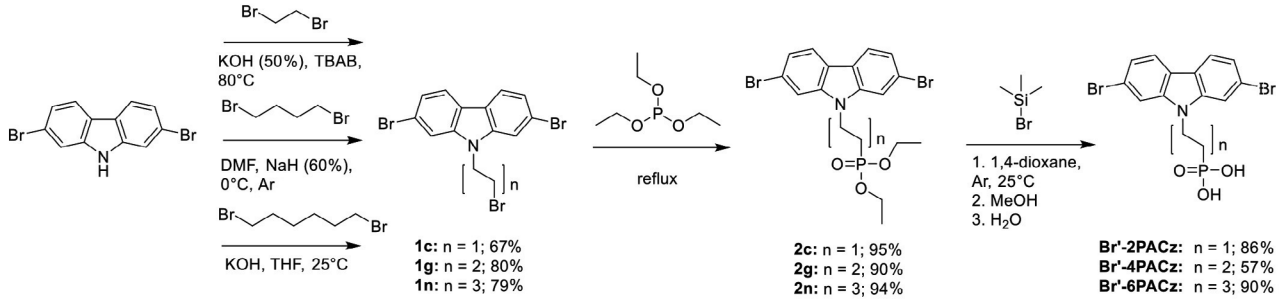
The target compounds **Br'-2PACz**, **Br'-4PACz** and **Br'-6PACz**, containing a 2,7-dibromo-9*H*-carbazole central core, were synthesised via a similar synthesis procedure as 3,6-halogenated carbazole analogues (Scheme 2).

One more halogenated derivative **4Br-2PACz** containing 1,3,6,8-tetrabromo-carbazole chromophore was synthesised using a similar three-step procedure (Scheme 3).

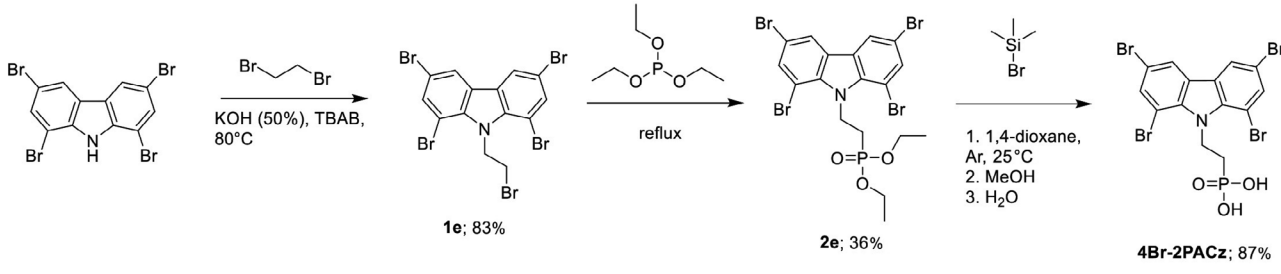
Carbazoles containing *tret*-butyl, CN or 3-thienyl functional groups were alkylated using 1,4-dibromobutane and either NaH (60% dispersion in mineral oil), DMF at 0°C (Method A, to obtain intermediates **1h** and **1i**) or KOH, THF at 25°C (Method B, to obtain intermediate **1j**) (Scheme 4). Further steps for intermediate phosphonates formation and final hydrolysis reaction remained



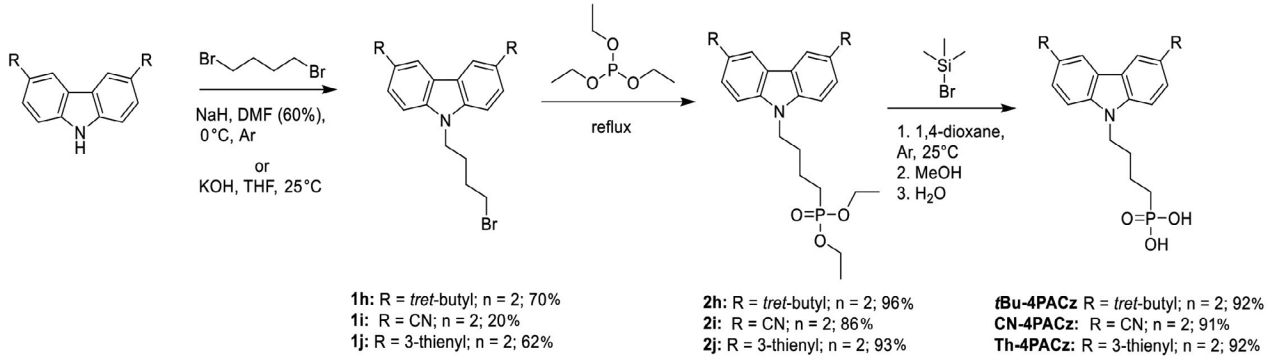
Scheme 1. Synthesis of halogenated 3,6-substituted carbazole-based molecules containing a phosphonic acid group



Scheme 2. Synthesis of 2,7-substituted carbazole-based molecules containing a phosphonic acid group



Scheme 3. Synthesis of 1,3,6,8-tetrabromocarbazole containing a phosphonic acid group

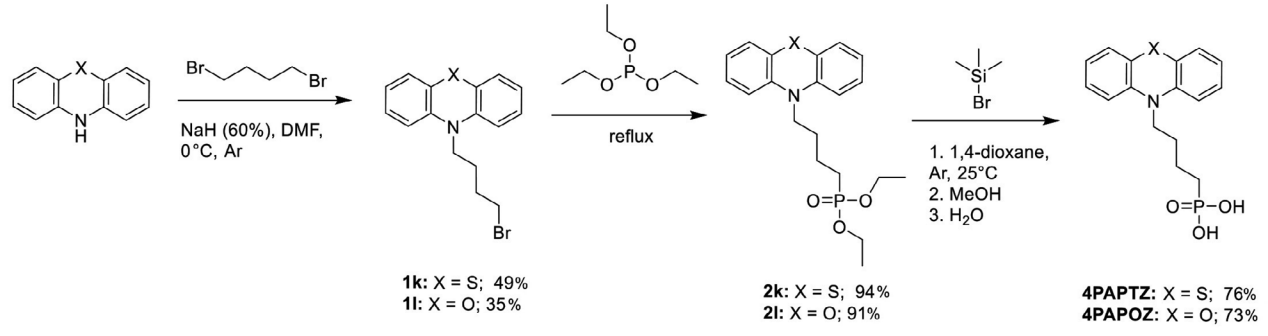


Scheme 4. Synthesis of carbazole-based molecules containing *tret*-butyl, CN or 3-thienyl functional groups and a phosphonic acid group

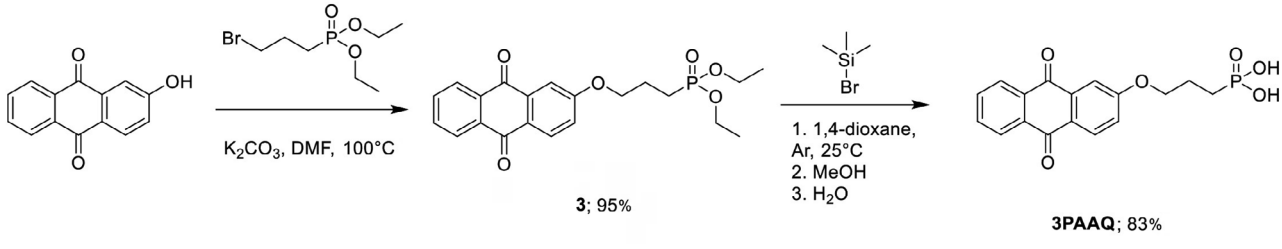
unchanged, resulting in the carbazole derivatives ***t*Bu-4PACz**, **CN-4PACz** and **Th-4PACz**.

Carbazole analogues, containing either phenothiazine or phenoxazine central fragment were synthesised similarly as in previous examples (Scheme 5). Different chromophores were alkylated using 1,4-dibromobutane and NaH (60% dispersion in mineral oil). Then, alkylated derivatives **1k** and **1l** were used in the Arbuzov reaction with triethyl phosphite forming intermediate phosphonates **2k** and **2l**, respectively. The final products **4PAPTZ** and **4PAPOZ**, containing a phosphonic acid functional group, were obtained by hydrolysis, using bromotrimethylsilane, methanol and water.

Finally, anthraquinone-based compound was synthesised by two-step synthesis procedure. 2-Hydroxyanthraquinone was alkylated introducing a diethyl propyl phosphonate moiety (Scheme 6), using diethyl(3-bromopropyl)phosphonate and K_2CO_3. The target product **3PAAQ**, containing a phosphonic acid functional group, was obtained by hydrolysis, using bromotrimethylsilane, methanol and water.



Scheme 5. Synthesis of phenothiazine or phenoxazine-based molecules containing a phosphonic acid group



Scheme 6. Synthesis of anthraquinone-based compound containing a phosphonic acid group

Evaluation of structure-depended antiproliferative activity in H69AR cells

After synthesising and characterising the compounds, we then aimed to characterise their *in vitro* antiproliferative activity using the H69AR anthracycline-resistant SCLC model [30, 31]. In this model, we exposed H69AR cells to the compounds at a fixed 100 μM concentration. To compare compound-mediated cytotoxicity, we included cisplatin (CP) and doxorubicin (DOX), standard chemotherapeutic agents used in the treatment of SCLC.

The synthesised heterocyclic and anthraquinone-based phosphonic acid derivatives demonstrated a statistically significant antiproliferative activity at 100 μM concentration. Among these, six compounds (**F-2PACz**, **Br'-4PACz**, **CN-4PACz**, **4PAPTZ**, **4PAPOZ** and **3PAAQ)** induced a reduction in cell viability compared to the untreated control (UC) ($p < 0.05$), with *in vitro* activity similar to the standard chemotherapeutic agents, cisplatin (CP) and doxorubicin (DOX) (Figure).

The compound **F-2PACz**, a 3,6-difluoro-9*H*-carbazole derivative bearing an ethyl phosphonic acid moiety, resulted in one of the highest antiproliferative effect in H69AR (Figure). The incorporation of fluorine atoms at 3 and 6 positions potentially enhances the lipophilicity and metabolic stability while modulating the electronic distribution of the carbazole ring to potentially favour DNA intercalation or interaction with redox-sensitive cellular pathways [32, 33]. Similarly, **Br'-4PACz**, containing a 2,7-dibromo substitution pattern on the carbazole core and a butyl phosphonic acid moiety, benefits from increased van der Waals interactions and possible halogen bonding with nucleophilic residues in protein targets [34, 35]. The longer butyl linker may also promote optimal spatial orientation for interaction with intracellular targets such as kinases, topoisomerases, or DNA [36, 37]. Carbazoles are well-known pharmacophores in anticancer drug discovery due to their ability to intercalate DNA, inhibit topoisomerases and modulate key signalling pathways, including PI3K/AKT and MAPK cascades [38].

The nitrile-substituted **CN-4PACz**, bearing cyano groups at positions 3 and 6 of the carbazole ring

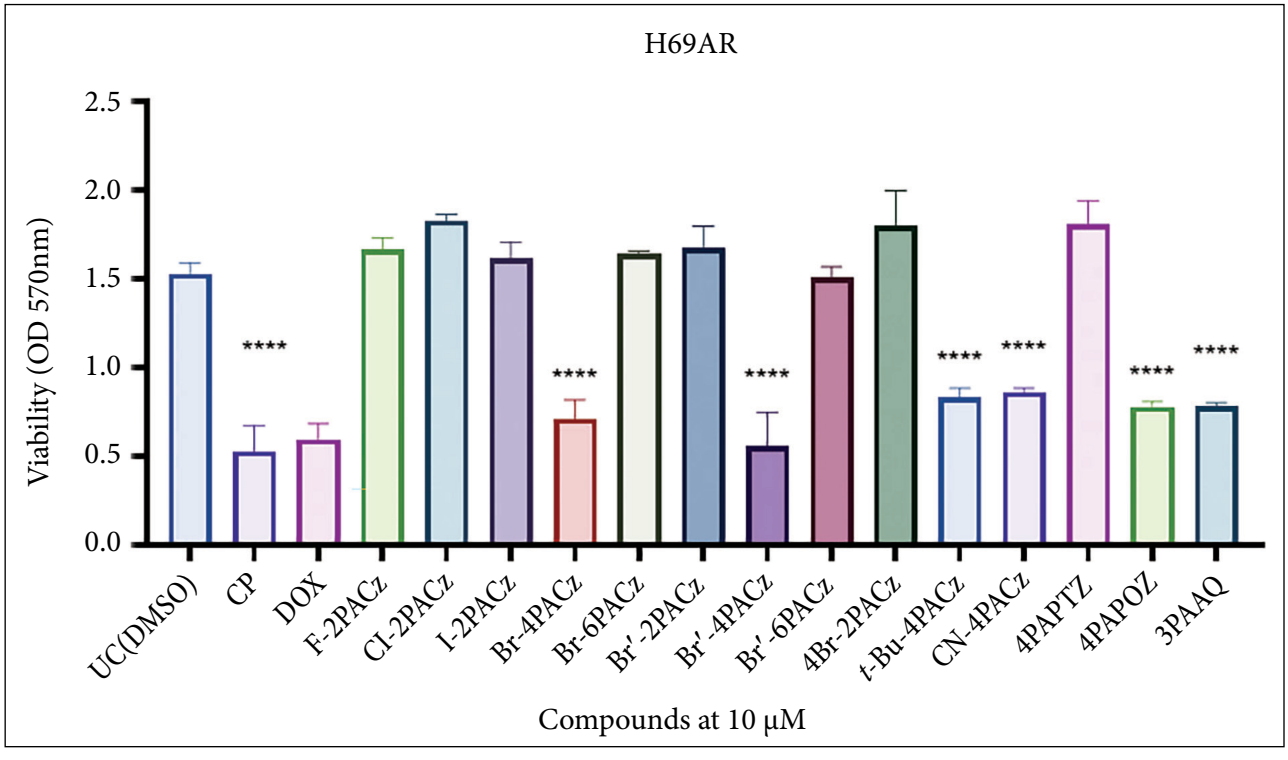


Figure. Tested materials bearing alkyl phosphonic acid moieties show structure-depended antiproliferative activity in the H69AR small cell lung cancer model. The H69AR cells were exposed with compounds or doxorubicin (DOX) and cisplatin (CP) for 24 h and the viability was determined using MTT assay and normalised to untreated control (UC). Data shown are mean ± SD values from three separate experiments for each group. The significance of the data was determined using a one-way ANOVA test. **** $p < 0.0001$

and a butylphosphonic acid moiety, demonstrated a significant cytotoxicity (Figure) likely due to the strong electron-withdrawing nature of the cyano groups, which could influence redox balance, promote ROS generation, or disrupt mitochondrial function [39, 40]. In parallel, the phenothiazine-based **4PAPTZ** and phenoxazine-based **4PAPOZ** compounds showed a potent antiproliferative activity in the H69AR model. These tricyclic heterocycles are well-characterised for their planar aromatic systems, enabling an efficient DNA intercalation, and their intrinsic redox-active properties, which may disrupt mitochondrial respiration or induce oxidative stress-mediated apoptosis [41, 42].

Finally, **3PAAQ**, a phosphonic acid-functionalised anthraquinone derivative, also demonstrated a significant antiproliferative activity (Figure). Anthraquinones are known to participate in redox cycling and generate reactive oxygen species leading to oxidative DNA damage and mitochondrial dysfunction [43, 44]. The presence of a propyl phosphonic acid group in this compound may contribute to increased solubility and cellular bioavailability while also enabling targeting of enzymes involved in nucleotide metabolism or phosphate transport [45–47].

Collectively, these results demonstrate that the 9*H*-carbazole derivatives bearing alkyl phosphonic acid moieties could be further explored as a promising scaffold targeting SCLC.

CONCLUSIONS

This study reports the synthesis and *in vitro* evaluation of a library of substituted heterocyclic and anthraquinone-based compounds bearing alkyl phosphonic acid moieties, with a particular focus on 9*H*-carbazole-based derivatives. Using a modular synthetic approach involving alkylation, Arbuzov phosphonylation, and hydrolysis, we generated a diverse set of molecules featuring various substitution patterns and linker lengths. *In vitro* screening against the anthracycline-resistant H69AR small cell lung cancer (SCLC) model revealed that compounds **F-2PACz, Br'-4PACz, CN-4PACz, 4PAPTZ, 4PAPOZ** and **3PAAQ** exhibited a significant antiproliferative activity at 100 μM, comparable to standard chemotherapeutics. These active compounds contain electron-withdrawing or redox-active groups on the aromatic core, coupled with water-solubilising phosphonic acid functionalities, suggesting mechanisms involving DNA intercalation, oxidative stress induction, and interference with intracellular signalling pathways. Notably, structural features such as halogenation (F, Br), cyano substitution and tricyclic redox-active cores (10*H*-phenothiazine, 10*H*-phenoxazine, anthraquinone) were associated with increased activity, highlighting the importance of electronic and steric modulation.

The results demonstrate that 9*H*-carbazole derivatives bearing alkyl phosphonic acid groups could be explored as a promising scaffold class for further development of compounds against drug-resistant SCLC. These findings provide a rationale for continued optimisation, mechanistic elucidation, and the *in vivo* assessment of this compound series as potential therapeutic agents for one of the most aggressive and treatment-refractory lung cancer subtypes.

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NAUJI ALKILFOSFONO RŪGŠTIES FRAGMENTUS TURINTYS DARINIAI – PERSPEKTYVŪS KARKASAI H69AR VAISTAMS ATSPARAUS SMULKIŲJŲ LĄSTELIŲ PLAUČIO VĖŽIO MODELIAMS KURTI

S a n t r a u k a

Naudojant 10*H*-fentiaziną, 10*H*-fenoksaziną, 2-hidroksiantrachinoną ir įvairius pakaitus turinčius 9*H*-karbazolus kaip skirtingus chromoforus, buvo susintetinta junginių su alkilfosfono rūgšties pakaitais serija. Taikant gerai žinomą antraciklinams atsparių H69AR smulkiųjų ląstelių plaučių vėžio (SCLC) ląstelių modelį, buvo vertinamas gautų junginių *in vitro* antiproliferacinis aktyvumas. Didžiausią antiproliferacinį aktyvumą, esant 100 μM koncentracijoms, parodė junginiai **F-2PACz**, **Br'-4PACz**, **CN-4PACz**, **4PAPTZ**, **4PAPOZ** ir **3PAAQ**. Šių junginių *in vitro* aktyvumas ir sukeltas ląstelių gyvybingumo sumažėjimas, palyginti su kontrole (UC), buvo artimi standartinių chemoterapijoje naudojamų vaistų – cisplatinos (CP) ir doksorubicino (DOX) – aktyvumui.