



Design, synthesis and cytotoxicity of chimeric erlotinib-alkylphospholipid hybrids

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ABSTRACT

Two series of erlotinib-alkylphospholipid hybrids were prepared and evaluated for their antiproliferative activities against a panel of four cell lines representing lung, breast, liver and skin cancers using erlotinib and miltefosine as reference standards. Amide analogs elicited more enhanced cytotoxic activity than analogous esters. Amide derivatives **8d** and **8e** exhibited promising broad-spectrum antiproliferative activity and higher efficacy than reference erlotinib and miltefosine. Their cellular GI₅₀ values were in the ranges of 24.7–46.9 μM and 26.8–43.1 μM for **8e** and **8d** respectively. Assay results of the inhibitory activity of the prepared compounds on EGFR kinase reaction and Akt phosphorylation in conjugation with statistical correlation analysis indicated that other mechanisms might contribute to their elicited cytotoxicities. In addition, statistical correlation analysis revealed that mechanisms of elicited cytotoxicities for amide series might be different from ester series. In addition, correlation analysis indicated variations in the mechanisms according to the types of cell line.

1. Introduction

Cancer is an arduous obstacle and a major cause for mortality in both developed and developing countries [1,2]. Over the past decades, considerable understanding and development of anticancer therapies were achieved. Nevertheless, statistical analysis of the global burden of disease (GBD) demonstrates that cancer is still the second leading cause of death [3]. Alarming, the lifetime risk of cancer has increased to 53.5% for men and 47.5% for women in some populations [4]. Consequently, there is an urgent need for development of more effective antineoplastic therapies.

Epidermal-growth factor receptor (known as EGFR, HER1, or ErbB1) is a member of tyrosine kinase family involved in several signal transduction pathways impacting cells' proliferation and survival including RAS/RAF/MAPK, and PI3k/PIP3/AKT pathways [5–7]. In fact, many efforts to develop EGFR targeted anticancer therapies were reported [7–12]. Erlotinib 1 (Tarceva®, OSI-744) is a FDA-approved EGFR inhibitor for treatment and maintenance treatment of non-small cell lung cancer, treatment of pancreatic cancer, and first-line treatment of

metastatic non-small cell lung cancer [13]. Unfortunately, tumors heterogeneity and evolving resistance limit its effectiveness [14–19]. Combination and multitarget therapies are two approaches to overcome resistant tumors via simultaneous inhibition of multiple pathological pathways [20,21]. In addition, this would produce higher efficacy and reduce side effects. A single multitarget molecule offers pharmacokinetic and pharmacodynamics advantages over combination therapy [22]. The clinical agent CUDC-101 (**2**, Fig. 1) inhibits EGFR and histone deacetylase (HDAC) simultaneously [23,24]. However, HDAC inhibitors are known to induce multidrug resistance (MDR), via up-regulation of adenosine triphosphate binding cassette (ABC) transporters [25]. It seems that CUDC-101 designed to overcome resistance could induce resistance [26]. An alternative design of a multitarget agent might be needed.

Alkylphospholipids (APLs) are known to possess antitumor activities [27–31]. They inhibit Akt signaling pathway, which is a downstream of EGFR signaling pathway. In addition, they integrate into cell membrane changing its properties, trafficking and signaling. Furthermore, they interfere with lipids metabolism and lipids-dependent signaling

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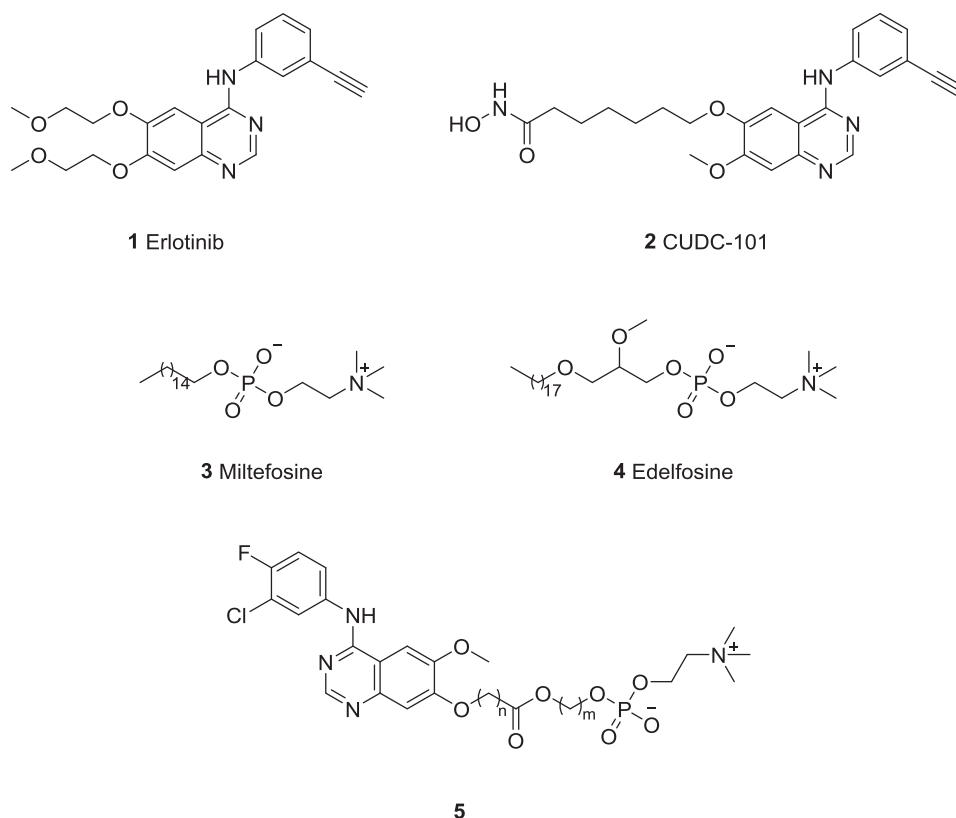


Fig. 1. Structures of reported compounds.

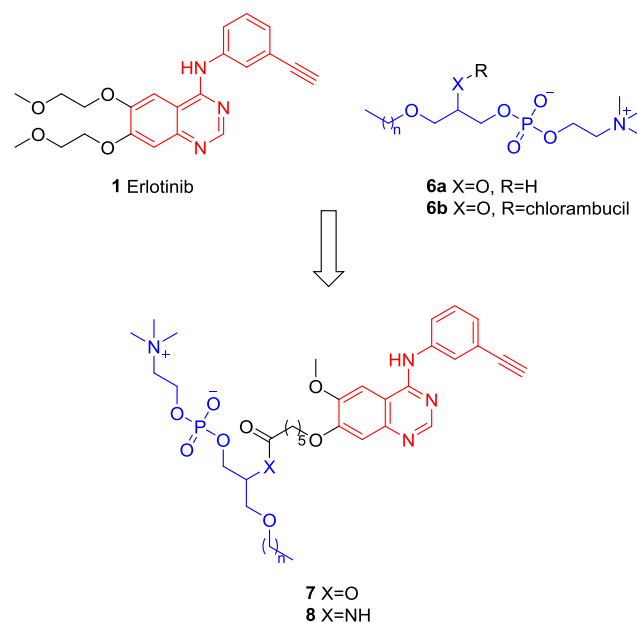
cascades [30–33]. Miltefosine **3** (Miltex®) and edelfosine **4** (ET-18-OCH₃) are prominent antitumor lipids. Unfortunately, miltefosine **3** was approved only as a topical therapy [34] while edelfosine **4** is limited to eradication of bone marrow in acute leukemia [32]. Recently, we have reported compounds (**5**) in which the EGFR inhibitor gefitinib was conjugated to the terminus of the alkyl chain of alkylphosphocholines in attempt to develop multitarget anticancer agents [35]. However, the investigated molecules showed no improved antiproliferative activity. Hence, different design approach might be required to access more effective conjugates.

The literature report the antitumor **6b** (Fig. 2) in which the antitumor alkylphospholipids **6a** were conjugated with chlorambucil at *sn*-2 position of the glycerol moiety [36]. These conjugates were claimed to undergo enzymatic hydrolysis releasing two anticancer agents. To recruit the unique properties of EGFR inhibitors and APLs in a single chemical entity, hybrid compounds **7** and **8**, in which APLs **6a** were conjugated with the pharmacophoric moiety of erlotinib **1** via an ester or amide functionality at *sn*-2 position were designed and synthesized. The idea is that chimeric compounds **7** and **8** might combine EGFR inhibitors and APLs mechanisms of actions. As a multitarget agent, if one mechanism fails, other mechanisms would work. This might result in a broader activity spectrum and a lower resistance liability. Moreover, if hydrolysis takes place at the *sn*-2 position, two mechanistically distinct antitumor drugs would be released. The designed hybrid molecules would have APL moieties of variable alkyl chain length to investigate its impact on the elicited biological activity. Herein, we would like to report our results.

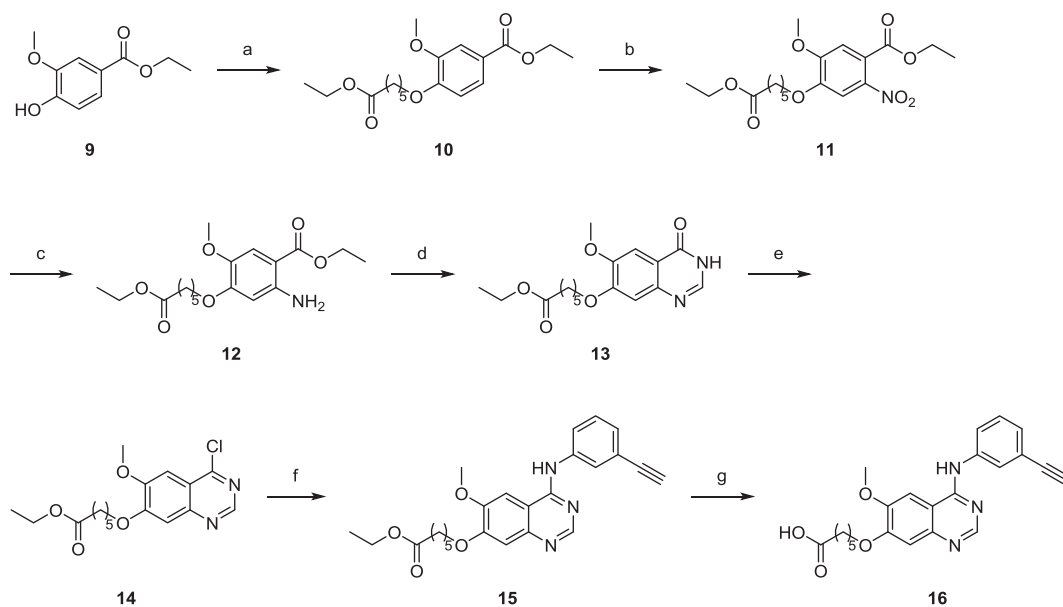
2. Results and discussion

2.1. Chemistry

Hybrid molecules **7a–g**, **8a**, **8b** and **8d–g** were prepared convergently as shown in Schemes 1 and 2. The unreported spacer-attached

Fig. 2. Design of Chimeric molecules **7** and **8** via linking the pharmacophoric moiety of erlotinib **1** with antitumor phospholipids **6a**.

pharmacophoric moiety of erlotinib (**16**) was prepared by synthetic steps outlined in Scheme 1. First, alkylation of ethyl 4-hydroxy-3-methoxybenzoate **9** with ethyl 6-bromohexanoate installed the spacer moiety to the benzoate derivative **10** which was then converted to quinazoline derivative **13** via sequential reactions of nitration to nitro-derivative **11**, reduction to amino-derivative **12**, then condensation/cyclization to quinazoline derivative **13**. The cyclized quinazoline derivative **13** was converted to chloro-derivative **14** then aromatic

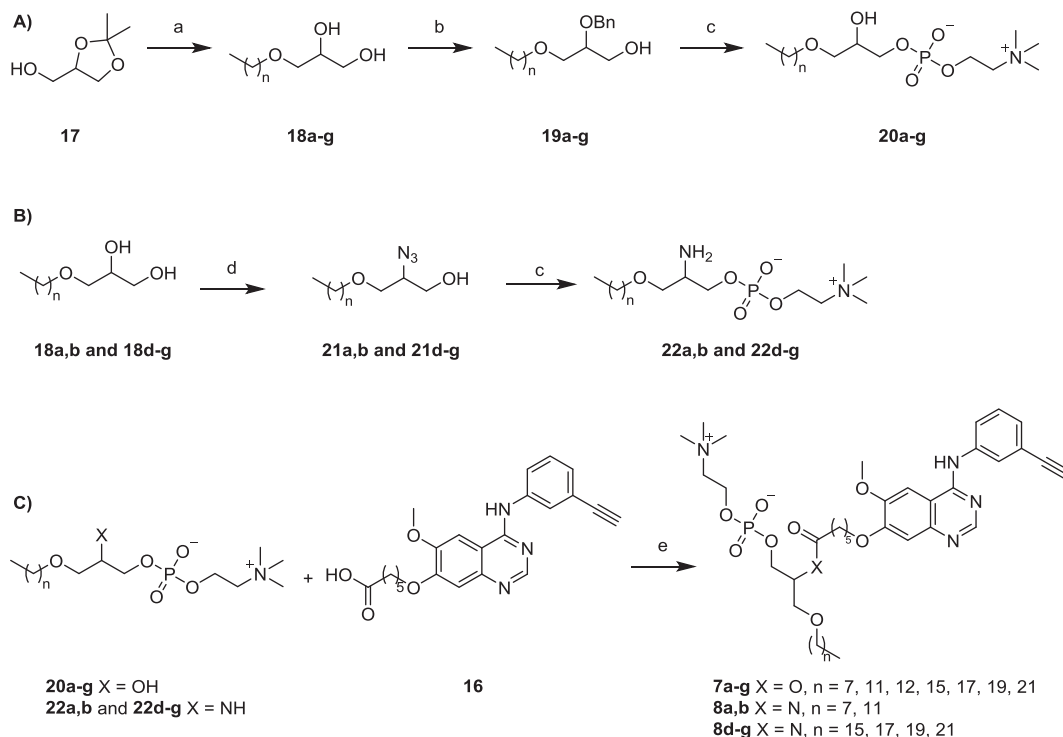


Scheme 1. Reagents and conditions: (a) Ethyl 6-bromohexanoate, K_2CO_3 , rt, 99%; (b) HNO_3 , AcOH, 20 °C, 99%; (c) Fe, HCl, EtOH, reflux, 85%; (d) $HCONH_2$, $HCOONH_4$, 180 °C, 85%; (e) $POCl_3$, reflux, 93%; (f) 3-Ethynylaniline, *i*-PrOH, reflux, 95%; (g) LiOH, THF/ H_2O , rt, 96%.

nucleophilic substitution afforded quinazoline derivative **15** having erlotinib's pharmacophore. Alkaline hydrolysis of ester derivative **15** afforded carboxylic acid derivative **16** ready for conjugation with *sn*-2-hydroxy-alkylphospholipid derivatives **20a–g** and *sn*-2-amino-alkylphospholipid **22a, 22b** and **22d–g**.

Synthesis of *sn*-2-hydroxy-alkylphospholipid derivatives **20a–g** is illustrated in Scheme 2A. Analogous to literature reports [37,38], DL-1,2-isopropylidene glycerol **17** was converted to 3-alkoxypropane-1,2-diol derivatives **18a–g** via *O*-alkylation with different chain lengths

alkyl bromides followed by acid-catalyzed ring cleavage to yield the intermediate diol derivatives **18a–g**. Similar to literature report [39], trityl protection of the primary hydroxyl group followed by benzylation then trityl deprotection afforded 2-(benzyloxy)-3-alkoxypropan-1-ol derivatives **19a–g**. Following reported procedure [38], esterification of alcohol derivatives **19a–g** with 2-chloro-1,3,2-dioxaphospholane-2-oxide then ring opening with trimethylamine followed by palladium catalyzed hydrogenation afforded *sn*-2-hydroxy-alkylphospholipid derivatives **20a–g**.



Scheme 2. Reagents and conditions: (a) (i) NaH, Bromoalkane, Toulene, reflux; (ii) 2 N HCl, MeOH, reflux, 88–95% over 2 steps; (b) (i) TrCl, Py, 50 °C; (ii) BnBr, NaH, DMF, 0 °C to rt; (iii) *p*-Toluenesulfonic acid, CH_2Cl_2 /MeOH, rt, 50–57% over 3 steps; (c) (i) 2-Chloro-1,3,2-dioxaphospholane-2-oxide, NEt_3 , benzene, rt; (ii) TMA, CH_3CN , 65 °C; (iii) H_2 , Pd/C, MeOH, rt, 70–88% over 3 steps; (d) (i) TrCl, Py, 50 °C; (ii) MsCl, NEt_3 , CH_2Cl_2 , 0 °C to rt; (iii) NaN_3 , DMF, 120 °C; (iv) 1 N HCl, Acetone, reflux, 42–50% over 4 steps; (e) DCC, DMAP, $CHCl_3$, reflux, 60–80%.

Adopting the reported procedure [38], *sn*-2-amino-alkylphospholipid derivatives **22a**, **22b** and **22d–g** were synthesized as illustrated in Scheme 2B. Thus, 3-alkoxypropane-1,2-diol derivatives **18a**, **18b** and **18d–g** prepared in Scheme 2A, were subjected to trityl protection of the primary hydroxyl group, activation of the *sn*-2-hydroxy group as mesylate ester, displacement by azide followed by acid induced trityl deprotection to afford 3-alkoxy-2-azidopropan-1-ol derivatives **21a**, **21b** and **21d–g**. Esterification of azido-alcohol derivatives **21a**, **21b** and **21d–g** with 2-chloro-1,3,2-dioxaphospholane-2-oxide then ring opening with trimethylamine followed by palladium catalyzed hydrogenation afforded *sn*-2-amino-alkylphospholipid derivatives **22a**, **22b** and **22d–g**.

Scheme 2C shows the final coupling step of hydroxy **20a–g** and amino **22a**, **22b** and **22d–g** intermediates with carboxylic acid derivative **16** obtained in Scheme 1 using *N,N'*-dicyclohexylcarbodiimide as a coupling agent in presence of stoichiometric amounts of 4-dimethylaminopyridine to yield the coupled compounds **7a–g**, **8a**, **8b** and **8d–g**.

2.2. Biological evaluation

2.2.1. Inhibition of cell line proliferation:

MTT cell proliferation assay (conducted in triplicates) was used to evaluate the antiproliferative activities of the newly synthesized erlotinib-APL hybrids against four human cancer cells (non-small-cell lung adenocarcinoma cells line A-549; breast adenocarcinoma cell line MCF-7, liver hepatocellular carcinoma cell line HepG2, and epidermoid squamous carcinoma cell line A-431). Erlotinib **1** and miltefosine **4** were used as reference standards. All of the employed cell lines elicited limited response to erlotinib **1** (maximum percent growth inhibition at 100 μ M concentration was not more than 42.3% for the best inhibited cell line; Table 1).

Among all cancers, lung cancer is the leading cause of death and has the highest premature cancer-related mortality cost [3,40–42]. Non-small-cell lung cancer is the most frequent type of lung cancer (85% of lung cancer cases). Erlotinib was approved for treatment of non-small cell lung cancer and first-line treatment of metastatic non-small cell lung cancer [13,43]. Nonetheless, erlotinib-resistance frequently encountered [43]. Additionally, insensitivity of non-small-cell lung adenocarcinoma cell line A-549 to APL edelfosine was reported [44]. Therefore, cell line A-549 was used to assess efficacies of prepared

erlotinib-APL hybrids. As shown in Table 1 and outlined in Fig. 3, at 100 μ M concentration, erlotinib and miltefosine produced limited inhibition (39.4% and 36.6% respectively). Amide-linked erlotinib-APL hybrids with long alkyl chain at the *sn*-3 position effectively inhibited A-549 cells proliferation (analogs **8d**, **8e**, **8f**, **8g** with *sn*-3 alkyl chain of 16, 18, 20, and 22 carbons elicited 94.4%, 94.1%, 84.8% and 92.8% growth inhibition respectively). The corresponding ester analogs, was much less active, except for analog **7e** having 18-carbon length alkyl chain substitution at *sn*-3. The latter elicited remarkable, but inferior inhibitory efficiency than the corresponding amide analog (77.1% and 94.1% inhibition for ester and amide analog respectively). Derivatives with *sn*-3 alkyl chain equal to or less than 13 carbons inhibited A-549 growth less effectively. Nevertheless, their efficacy was comparable to reference standards. Concentrations producing 50% growth inhibition (GI_{50}) for the effective amide analogs were in the range of 43.1–55.8 μ M.

Breast Cancer is the top cancer afflicting females and has the second highest premature-mortality costs [40–42]. Incongruous with expression of EGFR in numerous breast cancers, erlotinib, as well as, other EGFR inhibitors are not effective for breast cancers [45,46]. Interestingly, elevated lipid rafts levels are found in breast malignancies [47]. Miltefosine has been approved topically for cutaneous metastatic breast cancer [34]. Therefore, the prepared compounds and reference standards were evaluated at 100 μ M concentrations against a breast cancer cell line model. As shown in Table 1, erlotinib produced modest efficacy against the employed breast cancer MCF-7 cell line (28.7% inhibition). In contrast, miltefosine was more active producing average inhibition of growth of MCF-7 cell line (69.4% inhibition). With exception of ester derivatives **7a**, **7f** and **7g**, all other tested compounds elicited activities comparable to or higher than miltefosine. As illustrated in Fig. 4, amide analogs having long alkyl *sn*-3 substituent were highly effective (93.4%, 93.1%, 89.0%, and 90.7% inhibition for **8d**, **8e**, **8f**, and **8g** respectively). Amide derivatives **8e** and **8d** were the most potent among tested derivatives having GI_{50} values of 24.7 and 26.8 μ M respectively. Amide derivatives **8f** and **8g** having longer alkyl chains were less potent eliciting GI_{50} values of 38.6 and 46.6 μ M respectively. Among ester analogs, only compound **7e** showed significantly superior inhibition to miltefosine (93.1% inhibition and GI_{50} = 53.19 μ M).

Hepatocellular carcinoma is the first primary liver cancer and the third leading cause of death among cancer mortalities [48]. Patients

Table 1

In vitro cytotoxicity of erlotinib-APL hybrid compounds **7** and **8** against selected human cancer cells.

Comp	X	n	A-549 ^a		MCF-7 ^b		HepG2 ^c		A-431 ^d	
			% Inhibition ^e	GI_{50} ^f	% Inhibition ^e	GI_{50} ^f	% Inhibition ^e	GI_{50} ^f	% Inhibition ^e	GI_{50} ^f
7a	O	7	34.8	ND	48.4	ND	46.4	ND	58.1	ND
7b	O	11	33.5	ND	69.0	ND	62.4	ND	76.4	71.9
7c	O	12	26.5	ND	68.6	ND	62.6	ND	78.6	65.5
7d	O	15	15.5	ND	75.4	ND	28.8	ND	70.5	55.5
7e	O	17	77.1	ND	93.1	53.2	85.4	58.3	58.1	ND
7f	O	19	28.1	ND	27.5	ND	54.5	ND	42.7	ND
7g	O	21	31.4	ND	35.3	ND	34.4	ND	24.7	ND
8a	NH	7	29.1	ND	68.9	ND	42.8	ND	76.9	60.7
8b	NH	11	38.5	ND	66.0	ND	51.7	ND	74.9	68.3
8d	NH	15	94.4	43.1	93.4	26.8	92.9	35.4	92.9	32.1
8e	NH	17	94.1	46.9	93.1	24.7	92.8	31.3	ND	ND
8f	NH	19	84.8	55.8	89.0	38.6	57.1	ND	87.9	46.1
8g	NH	21	92.8	53.6	90.7	46.6	91.7	ND	93.2	44.0
Erlotinib			39.4	ND	28.7	ND	31.1	ND	42.3	ND
Miltefosine			36.6	ND	69.4	ND	31.3	ND	35.4	ND

^a Human lung cancer cells.

^b Human hepatocellular carcinoma cells.

^c Human breast carcinoma cells.

^d Human epidermoid carcinoma cells.

^e % inhibition at 100 μ M.

^f GI_{50} was defined as the concentration resulting in 50% inhibition of cell proliferation.

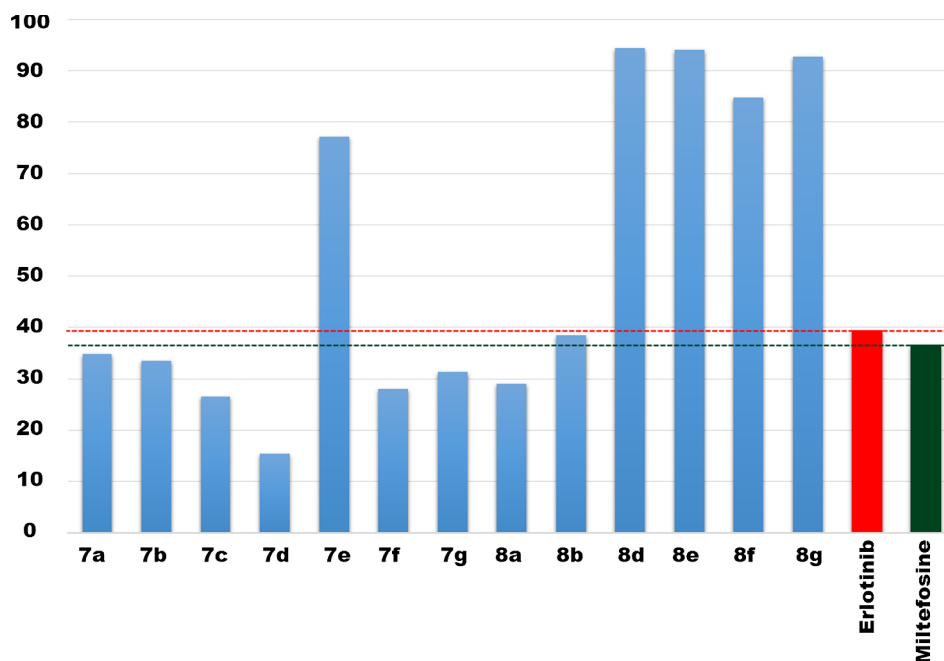


Fig. 3. Percentage inhibition of A-549 cell line proliferation after treatment with erlotinib-APL hybrid compounds **7** and **8**, erlotinib and miltefosine at 100 μ M. Dotted red-line represents inhibition percentage of erlotinib treated cell line while dashed green-line represents inhibition percentage of miltefosine treated cell line.

have average survival time of 6–20 months with less than 5% probability of 5-years survival. According to the clinical study of Thomas et al., 71% of the patients showed high EGFR expression [49]. Paradoxically, erlotinib treatment did not show significant difference between high and non-high EGFR expressing patients. Role of lipids in hepatocellular carcinoma and up-regulation of Akt signals have been confirmed [50]. At high concentrations, miltefosine elicited antiproliferative activity on hepatocellular carcinoma HepG2 cell line [51]. Therefore, the growth-inhibitory activity of the synthesized erlotinib-APL hybrids was assessed using hepatic cancer HepG2 cell line model. At 100 μ M concentrations both of erlotinib and miltefosine produced

almost similar low efficacy level (31.1% and 31.3% inhibition respectively). As shown in Fig. 5, four derivatives exhibited high efficacy (85.4%, 92.9%, 92.8% and 91.7% inhibition for ester analog **7e** and amide analogs **8d**, **8e** and **8g** respectively). These four derivatives were also the most effective derivatives against A-549 and MCF-7 cell lines. Measured GI_{50} values were 35.4, 31.3 and 58.3 μ M for **8d**, **8e** and **7e** respectively. The remaining tested amide and ester derivatives were less effective as antiproliferative agents, but elicited higher efficacy than the reference standards. The only exception was derivative **7d** which elicited efficacy slightly lower than erlotinib and miltefosine.

Epidermal squamous cells carcinoma; subclass of non-melanoma

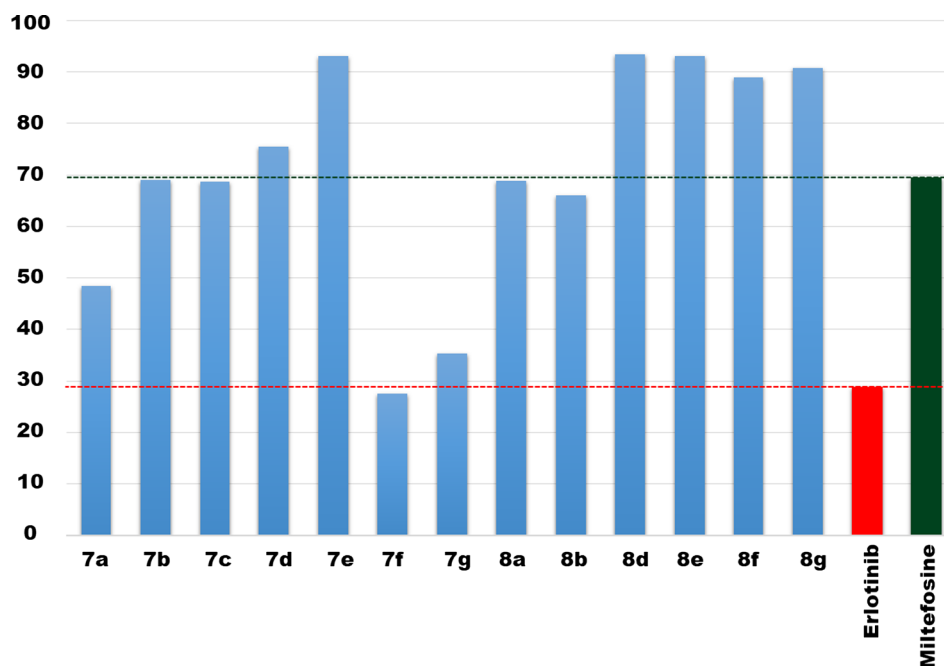


Fig. 4. Percentage inhibition of MCF-7 cell line proliferation after treatment with erlotinib-APL hybrid compounds **7** and **8**, erlotinib and miltefosine at 100 μ M. Dotted red-line represents inhibition percentage of erlotinib treated cell line while dashed green-line represents inhibition percentage of miltefosine treated cell line.

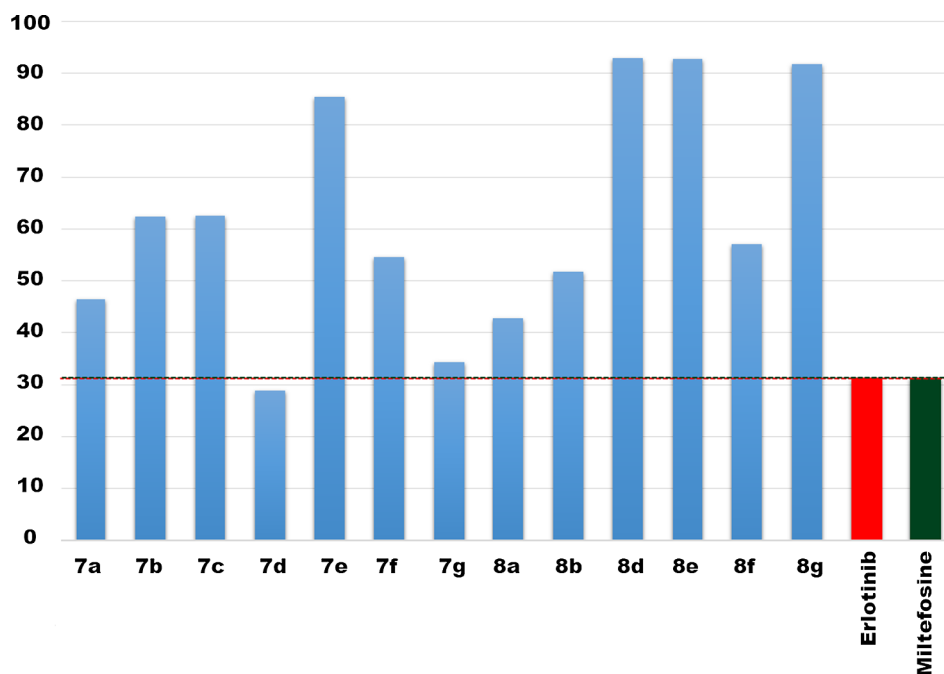


Fig. 5. Percentage inhibition of HepG2 cell line proliferation after treatment with erlotinib-APL hybrid compounds 7 and 8, erlotinib and miltefosine at 100 μ M. Dotted red-line represents inhibition percentage of erlotinib treated cell line while dashed green-line represents inhibition percentage of miltefosine treated cell line.

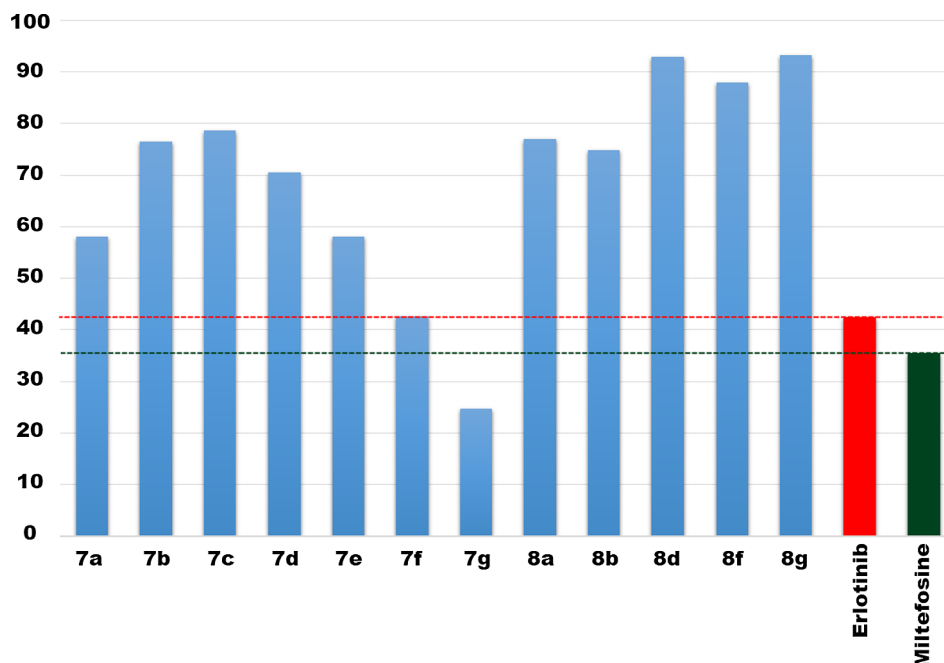


Fig. 6. Percentage inhibition of A-431 cell line proliferation after treatment with erlotinib-APL hybrid compounds 7 and 8, erlotinib and miltefosine at 100 μ M. Dotted red-line represents inhibition percentage of erlotinib treated cell line while dashed green-line represents inhibition percentage of miltefosine treated cell line.

skin cancer (NMSC) is a widespread skin cancer among white people [52]. Over the last fifty years, there was no achievement in decreasing the mortality rate of skin cancer [53]. Targeted therapies hold promise for increasing cure rates and diminishing excision of adjoined healthy tissue [52]. Despite A-431 cells (a cell line model of epidermal squamous cells carcinoma) overexpresses EGFR and responds to erlotinib, exposure over 6 months results in erlotinib-resistant cells. This was attributed to evolution of constitutively active Akt pathway [54]. As Akt signaling pathway is linked with lipid rafts, the efficacy of the prepared derivatives was assessed using A-431 cell line. Interestingly, the pattern of growth inhibition of A-431 cells differed from that of

other three employed cell lines. As illustrated in Fig. 6, at 100 μ M concentration, both erlotinib and miltefosine produced average low inhibition responses of 42.3% and 35.4%. All tested derivatives other than ester analogs 7g and 7f were more effective than erlotinib and miltefosine. While the most effective ester analog among tested ester analogs against A-549, MCF-7 and HepG2 cell lines was derivative 7e, it was less active against A-431 cell line than ester analogs 7c, 7b, 7d and 7a which have shorter alkyl. Ester analog 7c elicited the highest efficiency among ester derivatives producing (78.6% inhibition), while 7d was the most potent among ester analogs (GI_{50} = 55.5 μ M). Accordingly, increasing alkyl chain length at *sn*-3 position of ester analogs

Table 2

The result of erlotinib-APL hybrid compounds **7** and **8** inhibitory assays using enzymatic EGFR kinase assay and cellular Akt phosphorylation inhibition assay in MCF-7 cell line.

Compound	X	n	% EGFR inhibition ^a	IC ₅₀ (nM)	% AKT inhibition ^b	IC ₅₀ (μM)
7a	O	7	91.3	48.0	33.0	ND
7b	O	11	78.1	ND	9.4	ND
7c	O	12	56.9	ND	41.8	47.4
7d	O	15	77.5	ND	16.2	ND
7e	O	17	61.8	ND	11.6	ND
7f	O	19	89.0	ND	14.6	ND
7g	O	21	40.9	ND	12.4	ND
8a	N	7	93.5	21.0	13.3	ND
8b	N	11	87.2	ND	27.8	ND
8d	N	15	63.6	ND	35.5	ND
8e	N	17	ND	ND	56.8	36.2
8f	N	19	76.4	ND	26.1	ND
8g	N	21	50.2	ND	20.2	ND
Miltefosine			ND	ND	81.4	20.0
Erlotinib			97.6	3.0	35.2	ND

^a % EGFR at a single dose concentration of 1 μM.

^b % AKT phosphorylation inhibition at a single dose concentration of 20 μM.

decreases the efficacy in A-431 cell line. In contrast, the higher alkyl chain length did not impair antiproliferative effectiveness of amide analogs. In fact, the most effective amide analogs were derivatives **8g** and **8d** where *sn*-3 was substituted with 22 and 16 carbon chains respectively (92.9% and 93.2% inhibition and GI₅₀ = 32.1 and 44.0 μM for **8d** and **8g** respectively). Other tested amide analogs were also remarkably more efficient (74.9–87.9% inhibition) than reference standards (42.3% and 35.4 for erlotinib and miltefosine respectively).

2.2.2. EGFR assay

The remaining EGFR kinase activity after treatment with tested compounds and erlotinib was determined in triplicates using ADP-Glo™ kinase assay kit. The mean percentage inhibition was calculated relative to the measured kinase activity of untreated EGFR and presented in Table 2. For compounds elicited more than 90% inhibition of the kinase reaction at 1 μM concentration, IC₅₀ were determined and are presented in Table 2. The standard reference erlotinib exhibited 97.6% inhibition at 1 μM concentration and IC₅₀ value of 3.0 nM. At 1 μM concentration, all prepared compounds were less effective than erlotinib. Generally, the amide analogs were more active. The inhibition percentage decreased upon increasing length of the alkyl chain substituent at *sn*-3 position. Only two compounds, **7a** and **8a**, with the smaller alkyl chain substituent of 8 carbons, exhibited inhibition percentage more than 90%. However, their determined IC₅₀ was much higher than erlotinib (48 and 21 nM for ester derivative **7a** and amide

analog **8a** respectively). Although active as EGFR inhibitors, it was surprisingly to note that analogs **7e**, **8d**, **8f** and **8g** which were characterized by pronounced activities in performed cell line proliferation assays, in comparison with other compounds, demonstrated lower inhibitory activities of EGFR kinase reaction. This might indicate a limited contribution of their EGFR inhibitory activities to their overall elicited growth inhibitory activities.

2.2.3. Inhibition of Akt phosphorylation assay

Alkylphospholipids (APLs) were reported to inhibit Akt phosphorylation [55,56]. Consequently, the Akt phosphorylation inhibitory activities of the prepared erlotinib-APL hybrid derivatives **7a–g**, **8a**, **8b**, and **8d–g** were evaluated in triplicates by cell based assay using miltefosine and erlotinib as reference compounds. Thus, cells pretreated with each compound be tested at 20 μM concentration were stimulated for Akt phosphorylation followed by assay of phosphorylated Akt in cell lysate. For compounds that demonstrated more than 40% inhibition, IC₅₀ was determined. As Table 2 indicates, standard reference miltefosine showed 81.4% inhibition while erlotinib elicited only 35.2% downstream inhibition of Akt phosphorylation. Most of the tested compounds showed inhibition of Akt phosphorylation lower than erlotinib and miltefosine. However, compounds **7c** and **8e** (IC₅₀ values of 47.4, 36.2 μM, respectively) were more effective than erlotinib, but less potent than miltefosine as Akt phosphorylation inhibitors. Generally, amide derivatives elicited relatively higher efficiency than analogous ester derivatives. Notably, derivatives **8d** and **8e** which have remarkable activities in performed cell line proliferation assays, showed relatively higher Akt phosphorylation inhibition, however, much lower than that of miltefosine. In addition, derivative **7c** which had Akt-phosphorylation inhibitory activity relatively higher than derivative **8d**, elicited antiproliferative activity lower than that of derivative **8d** in all tested four cell lines. This might indicate a limited contribution of Akt phosphorylation inhibitory activity of these compounds to their overall elicited antiproliferative activity.

2.2.4. Statistical and computational study

For better understanding of relationship between inhibition of EGFR and Akt-phosphorylation to the elicited antiproliferative activity, statistical analysis was performed. Thus, single and multiple correlation coefficients, in addition to regression coefficients for multiple correlation of EGFR kinase inhibition and Akt-phosphorylation inhibition assays as two independent variables, with elicited antiproliferative activities as a dependent variable, were calculated. Correlations were calculated overall the series of erlotinib-APL series, the ester derivatives only, and the amide analogs only to detect differences between ester and amide analogs (if any).

As presented in Table 3, the calculation results revealed profile differences between cell lines, in addition to difference between ester

Table 3

Calculated multiple, single correlation coefficients and partial regression coefficients of EGFR kinase inhibition and/or Akt-phosphorylation inhibition assays with elicited antiproliferative activities of erlotinib-APL hybrid compounds.

Cell line		Multiple Correlation Coefficient	Regression Coefficient of EGFR	Regression Coefficient of Akt	Single EGFR Correlation Coefficient	Single Akt Correlation Coefficient
A-549	Overall Series	0.39918	−0.60502	0.459928	−0.36091	0.18763
	Ester analogs	0.32351	−0.19635	−0.40371	−0.19665	−0.26595
	Amide analogs	0.91196	−1.39974	0.99983	−0.87365	0.54747
MCF-7	Overall Series	0.27152	−0.28273	0.31924	−0.22303	0.16534
	Ester analogs	0.13219	−0.16724	−0.02466	−0.13154	−0.01933
	Amide analogs	0.76801	−0.62079	0.17237	−0.76210	0.35273
HepG2	Overall Series	0.41590	−0.51216	0.28996	−0.39206	0.15738
	Ester analogs	0.03608	−0.03380	−0.02246	−0.03302	−0.01611
	Amide analogs	0.88420	−1.45247	0.07243	−0.88396	0.32494
A-431	Overall Series	0.45200	0.15264	0.85060	0.10308	0.43465
	Ester analogs	0.47258	0.30527	0.56365	0.30544	0.37474
	Amide analogs	0.97280	−0.35882	0.11543	−0.94009	0.55974

and amide analogs. These might be a reflection of variation of the underlying mechanisms responsible for activities of ester and amide analogs, as well as, differences of the biological machinery of different cell line. For A-549, MCF-7, and HepG2 cell lines; both of EGFR kinase and Akt-phosphorylation inhibitory activities did not show a significantly indicative correlation to the elicited antiproliferative activity. In these three cell lines, ester analogs had small negative correlation coefficient and small negative regression coefficients for both of their EGFR and Akt-phosphorylation inhibitory activities. In contrast, for A-431 cell line, ester analogs showed significant, however low, positive correlation coefficient and positive regression coefficient for both of their EGFR inhibitory and Akt-phosphorylation activities. Accordingly, inhibition of EGFR and Akt-phosphorylation might contribute to the elicited antiproliferative activity of ester analogs in A-431 cell line, but ruled out in the other three cell lines. For amide analogs, statistical analysis predicted negative correlation to EGFR inhibition. In contrast, inhibition of Akt-phosphorylation showed significant, however average to low positive correlation. Therefore, in the four cell lines, inhibition of EGFR by amide analogs does not contribute to their antiproliferative activity while inhibition of Akt-phosphorylation does. The calculated average to low correlations for ester and amide analogs indicate that, in addition to inhibition of EGFR and Akt phosphorylation, other mechanisms might also contribute to the elicited cytotoxicities.

3. Conclusion

In this study, two series of erlotinib-APL hybrids have been prepared and biologically evaluated as cytotoxic agents. In comparison with ester derivatives, the amide series was more effective in inhibiting the growth of the tested cell lines. Among them, analogs **8d**, **8e**, **8f** and **8g** had the best efficacy in most of the employed cell lines. Particularly, **8d** and **8e** were the most potent with cellular GI_{50} ranges of 24.7–46.9 μ M and 26.8–43.1 μ M for **8e** and **8d** respectively. Compound **7e** elicited the best efficacy among ester analogs in three cell lines out of four employed cell lines. However, in A-431 cell line, **7b**, **7c**, and **7d** were more effective. Assay of the prepared compounds' inhibitory activity for EGFR kinase reaction and Akt-phosphorylation did not fully explain the measured cytotoxicities. Statistical analysis of the results revealed low contribution for inhibition of EGFR kinase reaction and Akt-phosphorylation to the elicited activities, as well as, the possibility of the presence of other mechanisms mediating activities of ester and amide analogs with variation of employed cell line.

4. Experimental

4.1. Chemistry

Reactions and manipulations were performed under nitrogen atmosphere using standard Schlenk techniques. The reactions solvents were purchased from Aldrich Co., TCI and Alfa and were used without any other purification. Column chromatography was performed on Merck Kiesegel 60 Art 9385 (230–400 mesh). TLC was carried out using glass sheets precoated with silica gel (E. Merck Kiesegel 60 F₂₅₄, layer thickness 0.25 mm). NMR spectra were obtained on Bruker AC 400 or Agilent 500 spectrometer. High-resolution spectra were performed on Jeol accuTOF (JMS-T100TD) equipped with a DART (direct analysis in real time) ion source from Ionsense, Tokyo, Japan in the positive modes. Samples purities were determined using HPLC Agilent 1100 series equipped with SHISEIDO CAPCELL PAK C18 column (250 mm $\times$ 4.6 mm, 5 μ m) at 25 °C. HPLC was performed in isocratic elution mode using MeOH:ACN:DW (85:15: 5 = v:v) with flow a rate of 1.0 mL/min. Compounds **18a-g**, **19a-g**, **20a-g**, **21a**, **21b**, **21d-g** and **22a**, **22b**, **22d-g** were prepared according to literature reports [37–39,57–59].

4.1.1. Ethyl 4-(6-ethoxy-6-oxohexyloxy)-3-methoxybenzoate (**10**)

A mixture of ethyl 4-hydroxy-3-methoxybenzoate **9** (5.0 g, 25.51 mmol), potassium carbonate (10.5 g, 76.5 mmol) and ethyl 6-bromohexanoate (**6.8 g**, 30.6 mmol) in DMF (20 mL) was stirred overnight at rt. The reaction mixture was filtered and concentrated in vacuum. The residue was dissolved in dichloromethane, and washed with brine. The organic layer was dried over magnesium sulfate, filtered and concentrated to yield derivative **10**. Yield 8.6 g, 99%; ¹H NMR (400 MHz, DMSO-*d*₆) δ 1.18 (t, 3H, *J* = 7.0 Hz), 1.31 (t, 3H, *J* = 7.0 Hz), 1.44–1.40 (m, 2H), 1.63–1.56 (m, 2H), 1.78–1.71 (m, 2H), 2.31 (t, 3H, *J* = 7.4 Hz), 3.82 (s, 3H), 4.08–4.00 (m, 4H), 4.28 (q, 2H, *J* = 7.2 Hz), 7.04 (d, 1H, *J* = 8.4 Hz), 7.44 (d, 1H, *J* = 1.8), 7.56 (dd, 1H, *J*₁ = 8.5 Hz, *J*₂ = 2.0 Hz); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 172.8, 165.4, 152.3, 148.5, 123.0, 121.9, 111.9 (2C), 68.1, 60.3, 59.6, 55.5, 33.4, 28.2, 25.0, 24.2, 14.2, 14.0; HR-MS *m/z* 339.1797 (calcd for C₁₈H₂₇O₆ [M + H]⁺, 339.1802).

4.1.2. Ethyl 4-(6-Ethoxy-6-oxohexyloxy)-5-methoxy-2-nitrobenzoate (**11**)

To a stirred solution of compound **10** (8.5 g, 25.14 mmol) in acetic acid (25 mL) at 20 °C, fuming nitric acid (15.8 g, 251.4 mmol) was added dropwise. The reaction mixture was stirred at 20 °C for 1 h, poured into ice-water, and extracted with dichloromethane. The combined organic layer was washed with water, aqueous NaHCO₃, brine; dried over sodium sulfate; filtered and concentrated to give derivative **11**. Yield 9.6 g, 99%; ¹H NMR (400 MHz, DMSO-*d*₆) δ 1.17 (t, 3H, *J* = 7.2 Hz), 1.27 (t, 3H, *J* = 7.0 Hz), 1.44–1.39 (m, 2H), 1.61–1.59 (m, 2H), 1.79–1.72 (m, 2H), 2.31 (t, 3H, *J* = 7.2 Hz), 3.92 (s, 3H), 4.11–4.02 (m, 4H), 4.30 (q, 2H, *J* = 7.2 Hz), 7.29 (s, 1H), 7.62 (s, 1H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 172.8, 164.9, 152.3, 149.5, 140.8, 120.5, 111.1, 108.0, 68.9, 61.8, 59.6, 33.4, 28.0, 24.9, 24.1, 14.1, 13.6; HR-MS *m/z* 384.1696 (calcd for C₁₈H₂₆NO₈ [M + H]⁺, 384.1653).

4.1.3. Ethyl 2-amino-4-(6-ethoxy-6-oxohexyloxy)-5-methoxybenzoate (**12**)

To a solution of compound **11** (6.0 g, 15.6 mmol) in a mixture of ethanol (90 mL), water (30 mL) and conc. hydrochloric acid (4 mL); powdered iron (8.5 g, 156.0 mmol) was added portionwise. The mixture was stirred at reflux for 30 min, cooled to rt, then neutralized with 10% sodium hydroxide till pH = 8. The formed solids were removed by filtration and the filtrate was concentrated in vacuo. The residue was extracted with dichloromethane, washed with brine, dried over sodium sulfate, filtered and concentrated to give compound **12**. Yield 4.6 g, 85%; ¹H NMR (400 MHz, DMSO-*d*₆) δ 1.17 (t, 3H, *J* = 7.2 Hz), 1.28 (t, 3H, *J* = 7.0 Hz), 1.43–1.38 (m, 2H), 1.64–1.57 (m, 2H), 1.79–1.72 (m, 2H), 2.31 (t, 3H, *J* = 7.2 Hz), 3.63 (s, 3H), 3.9 (t, 3H, *J* = 6.5 Hz), 4.05 (q, 2H, *J* = 7.0 Hz), 4.21 (q, 2H, *J* = 7.0 Hz), 6.35 (s, 1H), 6.41 (s, 1H), 7.13 (s, 1H), 7.63 (s, 1H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 173.3, 167.4, 154.8, 148.8, 139.8, 113.6, 100.3 (2C), 68.1, 60.1, 59.9, 56.8, 33.9, 28.6, 25.5, 24.6, 14.8, 14.6; HR-MS *m/z* 354.1952 (calcd for C₁₈H₂₈NO₆ [M + H]⁺, 354.1917).

4.1.4. Ethyl 6-(6-methoxy-4-oxo-3,4-dihydroquinazolin-7-yloxy)hexanoate (**13**)

A mixture of compound **12** (4.5 g, 12.7 mmol), ammonium formate (1.2 g, 19.1 mmol) and formamide (30 mL) was heated with stirring at 180 °C for 4 h. The reaction mixture was cooled, diluted with dichloromethane, washed with brine, dried over sodium sulfate, filtered and concentrated to give compound **13**. Yield 3.6 g, 85%; ¹H NMR (400 MHz, DMSO-*d*₆) δ 1.18 (t, 3H, *J* = 7.8 Hz), 1.46–1.42 (m, 2H), 1.63–1.57 (m, 2H), 1.80–1.76 (m, 2H), 2.32 (t, 3H, *J* = 7.3 Hz), 3.87 (s, 3H), 4.11–4.02 (m, 4H), 4.30 (q, 2H, *J* = 7.2 Hz), 7.12 (s, 1H), 7.44 (s, 1H), 7.99 (d, 1H, *J* = 2.9 Hz), 12.09 (s, 1H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 172.8, 160.0, 154.5, 147.8, 144.7, 143.7, 115.5, 108.0, 105.7, 68.2, 59.6, 55.9, 33.4, 28.2, 25.0, 24.2, 14.1; HR-MS *m/z* 335.1614 (calcd for C₁₇H₂₃N₂O₅ [M + H]⁺, 335.1607).

4.1.5. Ethyl 6-(4-chloro-6-methoxyquinazolin-7-yloxy)hexanoate (**14**)

A mixture of compound **13** (3.0 g, 8.9 mmol) and phosphoryl trichloride (30 mL) was stirred at reflux for 4 h. Excess phosphoryl trichloride was removed in vacuo. The residue was dissolved in dichloromethane, extracted with water and aqueous NaHCO₃, dried over sodium sulfate, filtered and concentrated to give compound **14**. Yield 2.6 g, 93%; ¹H NMR (400 MHz, DMSO-*d*₆) δ 1.18 (t, 3H, *J* = 7.0 Hz), 1.50–1.44 (m, 2H), 1.64–1.59 (m, 2H), 1.84–1.80 (m, 2H), 2.33 (t, 2H, *J* = 7.3 Hz), 3.99 (s, 3H), 4.05 (q, 2H, *J* = 7.0 Hz), 4.22 (t, 2H, *J* = 6.4 Hz), 7.39 (s, 1H), 7.44 (s, 1H), 8.87 (s, 1H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 172.8, 157.8, 156.0, 152.1, 151.4, 148.5, 118.4, 107.3, 102.2, 69.0, 59.6, 56.1, 33.4, 27.9, 25.0, 24.1, 14.1; HR-MS *m/z* 353.1282 (calcd for C₁₇H₂₂ClN₂O₄ [M + H]⁺, 353.1263).

4.1.6. Ethyl 6-(4-(3-ethynylphenylamino)-6-methoxyquinazolin-7-yloxy)hexanoate (**15**)

A mixture of compound **14** (1.0 g, 2.8 mmol) and 3-ethynylaniline (489 mg, 3.3 mmol) in isopropanol (20 mL) was stirred at reflux for 4 h then allowed to cool to rt. The solid was filtered, washed with isopropanol and ether and dried to give compound **15**. Yield 1.16 g, 95%; ¹H NMR (400 MHz, DMSO-*d*₆) δ 1.18 (t, 3H, *J* = 7.2 Hz), 1.50–1.44 (m, 2H), 1.64–1.58 (m, 2H), 1.84–1.81 (m, 2H), 2.32 (t, 3H, *J* = 7.2 Hz), 4.02–4.08 (m, 5H), 4.16 (t, 2H, *J* = 6.2 Hz), 4.31 (s, 1H), 7.37 (s, 1H), 7.41 (dd, 1H, *J*₁ = 6.7 Hz, *J*₂ = 0.76 Hz), 7.50 (t, 1H, *J* = 9.0 Hz), 7.80 (dd, 1H, *J*₁ = 7.2 Hz, *J*₂ = 0.9 Hz), 7.89 (s, 1H), 8.39 (s, 1H), 8.86 (s, 1H), 11.55 (s, 1H).

4.1.7. 6-(4-(3-ethynylphenylamino)-6-methoxyquinazolin-7-yloxy)hexanoic acid (**16**)

Lithium hydroxide monohydrate (869 mg, 20.5 mmol) was added to a stirred solution of compound **15** (3.0 g, 6.9 mmol) in a mixture of THF/water (3:1, 125 mL). Stirring was continued at rt for 24 h. The ice-bath cooled reaction mixture was acidified to pH = 2 with HCl (1 N aqueous solution). The precipitated solid was collected by filtration, washed with water, and dried under vacuum to afford compound **16**. Yield 2.5 g, 96%; ¹H NMR (400 MHz, DMSO-*d*₆) δ 1.48–1.44 (m, 2H), 1.61–1.56 (m, 2H), 1.85–1.82 (m, 2H), 2.26 (t, 2H, *J* = 7.2 Hz), 4.03 (s, 3H), 4.17 (t, 2H, *J* = 6.4 Hz), 4.31 (s, 1H), 7.36 (s, 1H), 7.41 (d, 1H, *J* = 8.0 Hz), 7.50 (t, 1H, *J* = 7.8 Hz), 7.79 (d, 1H, *J* = 8.4 Hz), 7.89 (s, 1H), 8.37 (s, 1H), 8.85 (s, 1H), 11.500 (s, 1H); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 174.9, 158.5, 156.1, 150.7, 149.4 (2C), 137.9, 129.7, 129.6, 127.9, 125.6, 122.5, 107.8, 104.3, 83.4, 81.8, 69.5, 57.4, 34.0, 28.4, 25.5, 24.6; HR-MS *m/z* 406.1815 (calcd for C₂₃H₂₄N₃O₄ [M + H]⁺, 406.1767).

4.1.8. General procedure for the synthesis of compounds **7a–g**, **8a**, **8b**, and **8d–g**

To a solution of each of compounds **20a–g**, **22a**, **22b**, or **22d–g** (1 equivalent) in a mixture of dry chloroform (20 mL per mmol) and dry DMF (2 mL per mmol), DMAP (3 equivalents), compound **16** (3 equivalents), and DCC (3 equivalents) were added. The reaction mixture was refluxed for 24 h, cooled to rt, and evaporated in vacuo. The residue was purified by silica gel chromatography, first eluting with CHCl₃/MeOH (9:1) to wash out the less polar compounds, followed by CHCl₃/MeOH/H₂O (65:20:4).

4.1.8.1. 2-(6-(4-(3-Ethynylphenylamino)-6-methoxyquinazolin-7-yloxy)hexanoyloxy)-3-(octyloxy)propyl 2-(trimethylammonio)ethyl phosphate (7a**).** Compound **7a** was obtained using compound **20a** (37.0 mg, 0.10 mmol). Yield 56.8 mg, 75%; HPLC purity: 99.15% (*R*_t = 3.21 min); ¹H NMR (400 MHz, CD₃OD) δ 0.86 (t, 3H, *J* = 6.8 Hz), 1.23 (s, 10H), 1.53–1.50 (m, 2H), 1.63–1.58 (m, 2H), 1.80–1.73 (m, 2H), 1.96–1.89 (m, 2H), 2.45 (t, 2H, *J* = 7.2 Hz), 3.23 (s, 9H), 3.48–3.40 (m, 2H), 3.66–3.60 (m, 4H), 4.02–3.99 (m, 1H), 4.05 (s, 3H), 4.16 (t, 2H, *J* = 6.2 Hz), 4.30–4.27 (m, 2H), 5.20–5.17 (m, 1H), 7.13 (s, 1H), 7.28 (d, 1H, *J* = 7.6 Hz), 7.39 (t, 1H, *J* = 7.8 Hz), 7.74 (s,

1H), 7.79 (d, 1H, *J* = 8.0 Hz), 7.94 (t, 1H, *J* = 1.4 Hz), 8.44 (s, 1H); ¹³C NMR (100 MHz, CD₃OD) δ 173.6, 157.3, 155.0, 152.5, 150.3, 146.2, 139.5, 128.7, 127.6, 125.9, 123.2, 123.0, 109.2, 106.4, 101.4, 83.2, 78.3, 72.0, 71.5, 69.1, 66.3, 66.2, 64.3, 59.3, 55.8, 53.6, 53.4, 53.3, 33.9, 31.8, 29.6, 29.4, 29.3, 28.5, 26.0, 25.6, 24.6, 22.5, 13.3; ³¹P NMR (202.5 MHz, CD₃OD) δ -0.56; HR-MS *m/z* 757.3956 (calcd for C₃₉H₅₈N₄O₉P [M + H]⁺, 757.3941).

4.1.8.2. 3-(Dodecyloxy)-2-(6-(4-(3-ethynylphenylamino)-6-methoxyquinazolin-7-yloxy)hexanoyloxy)propyl 2-(trimethylammonio)ethyl phosphate (7b**).** Compound **7b** was obtained using compound **20b** (42.6 mg, 0.10 mmol). Yield 54.5 mg, 67%; HPLC purity: 96.79% (*R*_t = 4.67 min); ¹H NMR (400 MHz, CD₃OD) δ 0.88 (t, 3H, *J* = 6.8 Hz), 1.21 (s, 18H), 1.54–1.48 (m, 2H), 1.65–1.57 (m, 2H), 1.81–1.74 (m, 2H), 1.97–1.90 (m, 2H), 2.45 (t, 2H, *J* = 7.2 Hz), 3.23 (s, 9H), 3.50–3.37 (m, 2H), 3.67–3.61 (m, 4H), 4.04–3.99 (m, 1H), 4.05 (s, 3H), 4.18 (t, 2H, *J* = 6.2 Hz), 4.30–4.26 (m, 2H), 5.22–5.17 (m, 1H), 7.16 (s, 1H), 7.28 (d, 1H, *J* = 7.7 Hz), 7.39 (t, 1H, *J* = 8.0 Hz), 7.77 (s, 1H), 7.80 (d, 1H, *J* = 8.3 Hz), 7.94 (m, 1H), 8.45 (s, 1H); HR-MS *m/z* 813.4620 (calcd for C₄₃H₆₆N₄O₉P [M + H]⁺, 813.4567).

4.1.8.3. 2-(6-(4-(3-Ethynylphenylamino)-6-methoxyquinazolin-7-yloxy)hexanoyloxy)-3-(tridecyloxy)propyl 2-(trimethylammonio)ethyl phosphate (7c**).** Compound **7c** was obtained using compound **20c** (44.0 mg, 0.10 mmol). Yield 58.7 mg, 71%; HPLC purity: 97.76% (*R*_t = 5.13 min); ¹H NMR (400 MHz, CD₃OD) δ 0.88 (t, 3H, *J* = 6.8 Hz), 1.20 (s, 20H), 1.52–1.47 (m, 2H), 1.63–1.57 (m, 2H), 1.80–1.72 (m, 2H), 1.95–1.88 (m, 2H), 2.45 (t, 2H, *J* = 7.2 Hz), 3.24 (s, 9H), 3.49–3.39 (m, 2H), 3.62–3.61 (m, 2H), 3.67–3.65 (m, 2H), 4.03–3.99 (m, 1H), 4.04 (s, 3H), 4.15 (t, 2H, *J* = 6.2 Hz), 4.30–4.26 (m, 2H), 5.22–5.17 (m, 1H), 7.12 (s, 1H), 7.27 (d, 1H, *J* = 7.7 Hz), 7.39 (t, 1H, *J* = 7.8 Hz), 7.73 (s, 1H), 7.82 (d, 1H, *J* = 8.3 Hz), 7.96 (t, 1H, *J* = 1.4 Hz), 8.43 (s, 1H); ¹³C NMR (100 MHz, CD₃OD) δ 174.8, 158.4, 156.1, 153.7, 151.1, 147.5, 140.7, 129.9, 128.7, 127.1, 124.3, 124.2, 110.5, 102.5, 90.2, 84.4, 78.8, 73.2, 73.1, 72.6, 70.3, 70.0, 67.5, 65.4 (2C), 60.5, 60.4, 57.0, 54.7 (3C), 35.1, 33.1, 30.8, (4C), 30.7, 30.5, 29.7, 27.2, 26.7, 25.9, 23.8, 18.2, 14.5; HR-MS *m/z* 827.4777 (calcd for C₄₄H₆₈N₄O₉P [M + H]⁺, 827.4722).

4.1.8.4. 2-(6-(4-(3-Ethynylphenylamino)-6-methoxyquinazolin-7-yloxy)hexanoyloxy)-3-(hexadecyloxy)propyl 2-(trimethylammonio)ethyl phosphate (7d**).** Compound **7d** was obtained using compound **20d** (48.2 mg, 0.10 mmol). Yield 60.0 mg, 69%; HPLC purity: 99.62% (*R*_t = 7.90 min); ¹H NMR (400 MHz, CD₃OD) δ 0.88 (t, 3H, *J* = 6.8 Hz), 1.20 (s, 20H), 1.52–1.47 (m, 2H), 1.63–1.57 (m, 2H), 1.78–1.72 (m, 2H), 1.94–1.87 (m, 2H), 2.43 (t, 2H, *J* = 7.2 Hz), 3.23 (s, 9H), 3.47–3.39 (m, 2H), 3.61–3.60 (m, 2H), 3.65–3.63 (m, 2H), 4.02–3.96 (m, 1H), 4.03 (s, 3H), 4.16 (t, 2H, *J* = 6.2 Hz), 4.30–4.25 (m, 2H), 5.20–5.15 (m, 1H), 7.13 (s, 1H), 7.27 (d, 1H, *J* = 7.7 Hz), 7.38 (t, 1H, *J* = 7.8 Hz), 7.75 (s, 1H), 7.78 (d, 1H, *J* = 8.2 Hz), 7.93 (t, 1H, *J* = 1.4 Hz), 8.46 (s, 1H); ¹³C NMR (100 MHz, CD₃OD) δ 173.3, 157.5, 155.4, 150.5, 138.5, 128.6, 128.1, 126.7, 126.1, 82.8, 77.8, 71.3, 68.5, 66.1, 64.1, 63.0, 59.5, 55.7, 53.4, 53.3 (2C), 33.7, 31.6, 29.4 (3C), 29.3, 29.2, 29.0, 25.8 (3C), 25.3, 25.2 (2C), 25.1, 24.4, 24.3 (2C), 22.3, 13.1; HR-MS *m/z* 869.5299 (calcd for C₄₇H₇₄N₄O₉P [M + H]⁺, 869.5193).

4.1.8.5. 2-(6-(4-(3-Ethynylphenylamino)-6-methoxyquinazolin-7-yloxy)hexanoyloxy)-3-(octadecyloxy)propyl 2-(trimethylammonio)ethyl phosphate (7e**).** Compound **7e** was obtained using compound **20e** (51.0 mg, 0.10 mmol). Yield 62.8 mg, 70%; HPLC purity: 98.94% (*R*_t = 11.08 min); ¹H NMR (400 MHz, CD₃OD) δ 0.90 (t, 3H, *J* = 6.8 Hz), 1.21 (s, 30H), 1.53–1.49 (m, 2H), 1.65–1.59 (m, 2H), 1.81–1.73 (m, 2H), 1.97–1.90 (m, 2H), 2.45 (t, 2H, *J* = 7.2 Hz), 3.24 (s, 9H), 3.48–3.40 (m, 2H), 3.62–3.61 (m, 2H), 3.67–3.65 (m, 2H), 4.03–3.99 (m, 1H), 4.05 (s, 3H), 4.18 (t, 2H, *J* = 6.2 Hz), 4.29–4.27 (m, 2H), 5.22–5.17 (m, 1H), 7.16 (s, 1H), 7.29 (d, 1H, *J* = 7.6 Hz), 7.40 (t, 1H,

$J = 8.0$ Hz), 7.78 (s, 1H), 7.80 (d, 1H, $J = 8.4$ Hz), 7.95 (m, 1H), 8.47 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 173.3, 157.0, 154.6, 154.3, 150.0, 146.1, 139.3, 128.5, 127.2, 125.6, 122.8, 122.7, 109.1, 106.2, 101.1, 83.0, 77.4, 71.8, 68.9, 66.1, 66.0, 61.0, 59.1, 55.6, 53.3 (3C), 33.7, 31.7, 29.4 (4C), 29.3 (4C), 29.2 (3C), 29.1, 28.3 (2C), 25.4, 25.3, 24.5, 24.4, 22.4, 13.1; ^{31}P NMR (202.5 MHz, CD_3OD) δ -0.60; HR-MS m/z 897.5490 (calcd for $\text{C}_{49}\text{H}_{78}\text{N}_4\text{O}_9\text{P}$ $[\text{M} + \text{H}]^+$, 897.5506).

4.1.8.6. 2-(6-(4-(3-Ethynylphenylamino)-6-methoxyquinazolin-7-yl)oxy)hexanoyloxy)-3-(icosyloxy) propyl 2-(trimethylammonio)ethyl phosphate (7f). Compound **7f** was obtained using compound **20f** (53.8 mg, 0.10 mmol), Yield 61.1 mg, 66%; HPLC purity: 97.67% ($R_t = 15.89$ min); ^1H NMR (400 MHz, CD_3OD) δ 0.88 (t, 3H, $J = 6.8$ Hz), 1.18 (s, 34H), 1.51–1.46 (m, 2H), 1.60–1.56 (m, 2H), 1.76–1.71 (m, 2H), 1.92–1.87 (m, 2H), 2.43 (t, 2H, $J = 7.2$ Hz), 3.21 (s, 9H), 3.46–3.38 (m, 2H), 3.60–3.58 (m, 2H), 3.64–3.62 (m, 2H), 4.00–3.95 (m, 1H), 4.02 (s, 3H), 4.13 (t, 2H, $J = 6.2$ Hz), 4.28–4.24 (m, 2H), 5.20–5.14 (m, 1H), 7.11 (s, 1H), 7.25 (d, 1H, $J = 7.7$ Hz), 7.36 (t, 1H, $J = 7.8$ Hz), 7.72 (s, 1H), 7.78 (d, 1H, $J = 8.2$ Hz), 7.93 (m, 1H), 8.42 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 173.3, 157.0, 154.6, 154.3, 150.0, 146.1, 139.3, 128.5, 127.2, 125.6, 122.8, 122.7, 109.1, 106.2, 101.1, 83.0, 77.4, 71.8, 68.9, 66.1, 66.0, 61.0, 59.1, 55.6, 53.3 (3C), 33.7, 31.7, 29.4 (4C), 29.3 (4C), 29.2 (3C), 29.1, 28.3 (2C), 25.8, 25.4, 25.3, 24.5, 24.4, 22.4, 13.1; ^{31}P NMR (202.5 MHz, CD_3OD) δ -0.59; HR-MS m/z 925.5820 (calcd for $\text{C}_{51}\text{H}_{82}\text{N}_4\text{O}_9\text{P}$ $[\text{M} + \text{H}]^+$, 925.5819).

4.1.8.7. 3-(Docosyloxy)-2-(6-(4-(3-ethynylphenylamino)-6-methoxyquinazolin-7-yl)oxy)hexanoyloxy) propyl 2-(trimethylammonio)ethyl phosphate (7g). Compound **7g** was obtained using compound **20g** (56.6 mg, 0.10 mmol), Yield 67.7 mg, 71%; HPLC purity: 93.04% ($R_t = 21.68$ min); ^1H NMR (400 MHz, CD_3OD) δ 0.88 (t, 3H, $J = 6.8$ Hz), 1.21 (s, 38H), 1.53–1.48 (m, 2H), 1.63–1.57 (m, 2H), 1.78–1.72 (m, 2H), 1.97–1.90 (m, 2H), 2.34–2.26 (m, 2H), 3.23 (s, 9H), 3.44–3.40 (m, 2H), 3.57–3.50 (m, 2H), 3.67–3.64 (m, 2H), 3.96–3.93 (m, 1H), 4.04 (s, 3H), 4.17 (t, 2H, $J = 6.5$ Hz), 4.30–4.26 (m, 2H), 5.20–5.14 (m, 1H), 7.14 (s, 1H), 7.27 (d, 1H, $J = 7.7$ Hz), 7.39 (t, 1H, $J = 7.8$ Hz), 7.77 (s, 1H), 7.81 (d, 1H, $J = 8.2$ Hz), 7.96 (m, 1H), 8.45 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 174.3, 158.0, 154.6, 153.3, 150.0, 146.1, 139.3, 128.5, 127.2, 125.6, 122.8, 122.7, 109.1, 106.2, 101.1, 83.0, 77.4, 71.8, 68.9, 66.1, 66.0, 61.0, 59.1, 55.6, 53.3 (3C), 33.7, 31.7, 29.4 (4C), 29.3 (4C), 29.2 (3C), 29.1, 28.3 (2C), 25.8, 25.4, 25.3, 24.5, 24.4, 22.4, 13.1; ^{31}P NMR (202.5 MHz, CD_3OD) δ -0.31; HR-MS m/z 953.6090 (calcd for $\text{C}_{51}\text{H}_{82}\text{N}_4\text{O}_9\text{P}$ $[\text{M} + \text{H}]^+$, 953.6127).

4.1.8.8. 2-(6-(4-(3-Ethynylphenylamino)-6-methoxyquinazolin-7-yl)oxy)hexanamido)-3-(octyloxy) propyl 2-(trimethylammonio)ethyl phosphate (8a). Compound **8a** was obtained using compound **22a** (36.8 mg, 0.10 mmol), Yield 55.9 mg, 74%; HPLC purity: 98.14% ($R_t = 3.11$ min); ^1H NMR (400 MHz, CD_3OD) δ 0.86 (t, 3H, $J = 6.8$ Hz), 1.21 (s, 10H), 1.58–1.46 (m, 4H), 1.75–1.70 (m, 2H), 1.91–1.84 (m, 2H), 2.31–2.27 (m, 2H), 3.24 (s, 9H), 3.42–3.39 (m, 2H), 3.52–3.50 (m, 2H), 3.66–3.64 (m, 2H), 3.96–3.93 (t, 2H, $J = 5.7$ Hz), 4.00 (s, 3H), 4.07 (t, 2H, $J = 6.2$ Hz), 4.20 (t, 1H, $J = 5.5$ Hz), 4.30–4.27 (m, 2H), 7.02 (s, 1H), 7.25 (d, 1H, $J = 7.6$ Hz), 7.36 (t, 1H, $J = 6.0$ Hz), 7.64 (s, 1H), 7.80 (d, 1H, $J = 8.1$ Hz), 7.96 (s, 1H), 8.39 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 176.0, 158.3, 155.9, 153.7, 151.3, 147.5, 140.8, 129.9, 128.6, 126.9, 124.2, 124.1, 110.4, 107.7, 102.5, 84.5, 78.9, 72.5, 70.4, 69.9, 67.5 (2C), 67.4 (2C), 65.7 (2C), 60.5, 60.4, 57.0, 54.8, 54.7 (2C), 51.0 (2C), 37.1, 33.0, 30.8, 30.6, 30.5, 29.8, 27.3, 26.8 (2C), 23.8, 14.5; HR-MS m/z 756.4062 (calcd for $\text{C}_{39}\text{H}_{59}\text{N}_5\text{O}_8\text{P}$ $[\text{M} + \text{H}]^+$, 756.4101).

4.1.8.9. 3-(Dodecyloxy)-2-(6-(4-(3-ethynylphenylamino)-6-methoxyquinazolin-7-yl)oxy)hexanamido) propyl 2-(trimethylammonio)ethyl phosphate (8b). Compound **8b** was obtained using compound **22b** (42.6 mg, 0.23 mmol), Yield 60.9 mg, 75%; HPLC purity: 92.68%

($R_t = 4.57$ min); ^1H NMR (400 MHz, CD_3OD) δ 0.86 (t, 3H, $J = 6.8$ Hz), 1.22 (s, 18H), 1.64–1.48 (m, 4H), 1.79–1.73 (m, 2H), 1.95–1.88 (m, 2H), 2.46–2.42 (m, 2H), 3.24 (s, 9H), 3.46–3.39 (m, 2H), 3.63–3.60 (m, 2H), 3.66–3.64 (m, 2H), 4.03–3.98 (m, 2H), 4.06 (s, 3H), 4.20 (t, 1H, $J = 5.5$ Hz), 4.30–4.27 (m, 2H), 7.17 (s, 1H), 7.33–7.30 (m, 1H), 7.41 (t, 1H, $J = 7.9$ Hz), 7.78–7.77 (m, 1H), 7.80 (s, 1H), 7.94–7.93 (m, 1H), 8.49 (s, 1H); HR-MS m/z 812.4703 (calcd for $\text{C}_{43}\text{H}_{67}\text{N}_5\text{O}_8\text{P}$ $[\text{M} + \text{H}]^+$, 812.4727).

4.1.8.10. 2-(6-(4-(3-Ethynylphenylamino)-6-methoxyquinazolin-7-yl)oxy)hexanamido)-3-(hexadecyloxy)propyl 2-(trimethylammonio)ethyl phosphate (8d). Compound **8d** was obtained using compound **22d** (48.1 mg, 0.10 mmol), Yield 59.9 mg, 69%; HPLC purity: 98.11% ($R_t = 7.50$ min); ^1H NMR (400 MHz, CD_3OD) δ 0.88 (t, 3H, $J = 6.8$ Hz), 1.20 (s, 26H), 1.52–1.44 (m, 2H), 1.62–1.56 (m, 2H), 1.76–1.71 (m, 2H), 1.93–1.89 (m, 2H), 2.31–2.26 (m, 2H), 3.21 (s, 9H), 3.40 (td, 2H, $J_1 = 6.6$ Hz, $J_2 = 1.9$ Hz), 3.52–3.49 (m, 2H), 3.64–3.61 (m, 2H), 3.95–3.90 (m, 2H), 4.02 (s, 3H), 4.20–4.14 (m, 3H), 4.28–4.25 (m, 2H), 7.13 (s, 1H), 7.25 (d, 1H, $J = 7.6$ Hz), 7.36 (t, 1H, $J = 6.0$ Hz), 7.74 (s, 1H), 7.78 (d, 1H, $J = 8.2$ Hz), 7.92 (s, 1H), 8.42 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 176.0, 158.3, 156.0, 153.8, 151.3, 147.6, 140.8, 129.9, 128.6, 126.9, 124.2, 124.1, 110.5, 107.7, 102.5, 84.5, 78.8, 72.5, 70.4, 69.9, 67.5 (2C), 67.4 (2C), 65.8, 65.7, 60.5, 60.4, 57.0, 54.7 (3C), 51.1, 51.0, 37.1, 33.1, 30.9, 30.8, 30.7, 30.5, 29.8, 27.3, 26.9, 26.8, 23.8, 14.5; HR-MS m/z 868.5393 (calcd for $\text{C}_{47}\text{H}_{75}\text{N}_5\text{O}_8\text{P}$ $[\text{M} + \text{H}]^+$, 868.5353).

4.1.8.11. 2-(6-(4-(3-Ethynylphenylamino)-6-methoxyquinazolin-7-yl)oxy)hexanamido)-3-(octadecyloxy)propyl 2-(trimethylammonio)ethyl phosphate (8e). Compound **8e** was obtained using compound **22e** (50.9 mg, 0.10 mmol), Yield 62.7 mg, 70%; HPLC purity: 99.86% ($R_t = 23.03$ min); ^1H NMR (400 MHz, CD_3OD) δ 0.87 (t, 3H, $J = 6.8$ Hz), 1.17 (s, 30H), 1.51–1.44 (m, 2H), 1.60–1.52 (m, 2H), 1.75–1.69 (m, 2H), 1.91–1.85 (m, 2H), 2.41 (t, 2H, $J = 7.2$ Hz), 3.20 (s, 9H), 3.46–3.36 (m, 2H), 3.59–3.56 (m, 2H), 3.65–3.61 (m, 2H), 3.99–3.94 (m, 2H), 4.01 (s, 3H), 4.11 (t, 1H, $J = 6.3$ Hz), 4.29–4.23 (m, 2H), 5.16 (t, 1H, $J = 4.7$ Hz), 7.08 (s, 1H), 7.23 (d, 1H, $J = 7.7$ Hz), 7.34 (t, 1H, $J = 7.9$ Hz), 7.70 (s, 1H), 7.78 (d, 1H, $J = 8.2$ Hz), 7.93 (s, 1H), 8.40 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 174.7, 158.4, 156.1, 153.8, 151.4, 147.6, 140.8, 129.9, 128.7, 127.0, 124.2 (2C), 107.7, 102.5, 84.4, 78.8, 73.2, 73.1, 72.6, 70.4, 70.0, 67.5 (3C), 67.4, 65.4, (2C), 60.5, 60.4, 57.0, 54.8, 54.7 (3C), 35.2, 33.1, 30.9, 30.8, 30.7 (2C), 30.6, 30.5, 29.8, 27.3, 26.7, 25.9, 23.8, 14.5; ^{31}P NMR (202.5 MHz, CD_3OD) δ -0.54; HR-MS m/z 896.5603 (calcd for $\text{C}_{49}\text{H}_{79}\text{N}_5\text{O}_8\text{P}$ $[\text{M} + \text{H}]^+$, 896.5666).

4.1.8.12. 2-(6-(4-(3-Ethynylphenylamino)-6-methoxyquinazolin-7-yl)oxy)hexanamido)-3-(icosyloxy) propyl 2-(trimethylammonio)ethyl phosphate (8f). Compound **8f** was obtained using compound **22f** (53.7 mg, 0.10 mmol), Yield 56.4 mg, 61%; HPLC purity: 97.16% ($R_t = 14.82$ min); ^1H NMR (400 MHz, CD_3OD) δ 0.88 (t, 3H, $J = 6.8$ Hz), 1.19 (s, 34H), 1.59–1.46 (m, 4H), 1.75–1.70 (m, 2H), 1.92–1.85 (m, 2H), 2.31–2.25 (m, 2H), 3.22 (s, 9H), 3.40 (td, 2H, $J_1 = 6.6$ Hz, $J_2 = 1.8$ Hz), 3.52–3.48 (m, 2H), 3.65–3.63 (m, 2H), 3.93 (t, 2H, $J = 5.9$ Hz), 4.00 (s, 3H), 4.10 (t, 2H, $J = 6.2$ Hz), 4.23–4.17 (m, 1H), 4.27 (brs, 2H), 7.05 (s, 1H), 7.24 (d, 1H, $J = 7.7$ Hz), 7.35 (t, 1H, $J = 7.8$ Hz), 7.68 (s, 1H), 7.80 (d, 1H, $J = 8.2$ Hz), 7.96 (s, 1H), 8.40 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 176.0, 158.3, 156.0, 153.7, 151.4, 147.6, 140.8, 129.9, 128.6, 126.9, 124.1 (2C), 110.5, 107.7, 102.5, 84.5, 78.8, 72.5, 70.4, 69.9, 67.5 (2C), 67.4, 65.8, 65.7, 60.5, 60.4, 57.0, 54.7 (3C), 51.1, 51.0, 37.1, 33.1, 30.9, 30.7, 30.6, 29.8, 27.3, 26.9, 26.8, 23.8, 14.6; HR-MS m/z 924.5958 (calcd for $\text{C}_{51}\text{H}_{83}\text{N}_5\text{O}_8\text{P}$ $[\text{M} + \text{H}]^+$, 924.5979).

4.1.8.13. 3-(Docosyloxy)-2-(6-(4-(3-ethynylphenylamino)-6-methoxyquinazolin-7-yl)oxy)hexanamido) propyl 2-(trimethylammonio)ethyl phosphate (8g). Compound **8g** was obtained using compound **22g** (56.5 mg, 0.10 mmol), Yield 64.7 mg, 68%; HPLC purity: 92.11%

(R_t = 10.54 min); ^1H NMR (400 MHz, CD_3OD) δ 0.88 (t, 3H, J = 6.8 Hz), 1.29 (s, 38H), 1.59–1.46 (m, 4H), 1.75–1.70 (m, 2H), 1.92–1.85 (m, 2H), 2.31–2.25 (m, 2H), 3.21 (s, 9H), 3.46–3.36 (m, 2H), 3.52–3.48 (m, 2H), 3.65–3.63 (m, 2H), 3.93 (t, 2H, J = 5.9 Hz), 4.05 (s, 3H), 4.10 (t, 2H, J = 6.2 Hz), 4.23–4.17 (m, 1H), 4.27 (brs, 2H), 7.13 (s, 1H), 7.24 (d, 1H, J = 7.7 Hz), 7.35 (t, 1H, J = 7.8 Hz), 7.68 (s, 1H), 7.80 (d, 1H, J = 8.2 Hz), 7.92 (s, 1H), 8.42 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 174.9, 157.3, 155.1, 152.3, 152.1, 150.4, 139.4, 128.8, 128.7, 127.7, 126.0, 123.2, 123.0 (2C), 106.2, 101.4, 83.2, 77.6, 71.3 (2C), 69.2, 68.9, 66.3, 64.6, 59.2, 55.3, 53.6, 53.4, 53.3, 35.8, 31.9, 29.4 (12C), 28.6, 28.5, 26.1 (2C), 25.6 (3C), 25.5, 22.6, 13.3; HR-MS m/z 952.6261 (calcd for $\text{C}_{53}\text{H}_{87}\text{N}_5\text{O}_8$ $[\text{M} + \text{H}]^+$, 952.6295).

4.2. Biological evaluation

4.2.1. Cell line MTT assay

The assay was performed in triplicates as described in the supporting information according to literature reports [35].

4.2.2. Inhibition of EGFR kinase reaction assay

The assay was conducted in triplicates as described in the supporting information according to literature reports [35].

4.2.3. Inhibition of Akt phosphorylation cellular assay

The assay was conducted in triplicates employing MCF-7 human breast cancer cell line as described in the supporting information according to literature reports [35].

4.3. Statistical analysis

The data analysis for this paper was calculated using the Excel Add Ins., Real Statistics Resource Pack Software (Release 4.3). Copyright (2013–2015) by Zaiontz C.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bioorg.2018.11.021>.

References

- [1] M.P. Coleman, C. Allemani, Cancer: the elephant in the room, *Lancet* 385 (2015) 1047–1048.
- [2] B.M. Ong, J. Ngbea, K. Malu, Cancer: an emerging health problem in North Central Nigeria, *IJHSR* 5 (2015) 73–78.
- [3] R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2016, *Cancer J. Clin.* 66 (2016) 7–30.
- [4] A.S. Ahmad, N. Ormiston-Smith, P.D. Sasieni, Trends in the lifetime risk of developing cancer in Great Britain: comparison of risk for those born from 1930 to 1960, *Br. J. Cancer* 112 (2015) 943–947.
- [5] K. Oda, Y. Matsuoka, A. Funahashi, H. Kitano, A comprehensive pathway map of epidermal growth factor receptor signaling, *Mol. Syst. Biol.* 1 (2005), <https://doi.org/10.1038/msb4100014>.
- [6] M. Whirl-Carrillo, E.M. McDonagh, J.M. Hebert, L. Gong, K. Sangkuhl, C.F. Thorn, R.B. Altman, T.E. Klein, Pharmacogenomics knowledge for personalized medicine, *Clin. Pharmacol. Ther.* 92 (2012) 414–417.
- [7] M. Scaltriti, J. Baselga, The epidermal growth factor receptor pathway: a model for targeted therapy, *Clin. Cancer Res.* 12 (2006) 5268–5272.
- [8] H. Zhang, A. Berezov, Q. Wang, G. Zhang, J. Drebin, R. Murali, M.I. Greene, ErbB receptors: from oncogenes to targeted cancer therapies, *J. Clin. Invest.* 117 (2007) 2051–2058.
- [9] C. Yewale, D. Baradia, I. Vhora, S. Patil, A. Misra, Epidermal growth factor receptor targeting in cancer: a review of trends and strategies, *Biomaterials* 34 (2013) 8690–8707.
- [10] S. Davinder, A. Bhupinder Kumar, G. Rupinder Kaur, B. Jitender, Review on EGFR inhibitors: critical updates, *Mini Rev. Med. Chem.* 16 (2016) 1134–1166.
- [11] D. Killock, Lung cancer: a new generation of EGFR inhibition, *Nat. Rev. Clin. Oncol.* 12 (2015) 373, <https://doi.org/10.1038/nrclinonc.2015.93>.
- [12] P.M. Harari, Epidermal growth factor receptor inhibition strategies in oncology, *Endocr. Relat. Cancer* 11 (2004) 689–708.
- [13] W. Pao, J. Chmielecki, Rational, biologically based treatment of EGFR-mutant non-small-cell lung cancer, *Nat. Rev. Cancer* 10 (2010) 760–774.
- [14] X. Cheng, H. Chen, Tumor heterogeneity and resistance to EGFR-targeted therapy in advanced nonsmall cell lung cancer: challenges and perspectives, *Onco. Targets Ther.* 7 (2014) 1689–1704.
- [15] F.B. Furnari, T.F. Cloughesy, W.K. Cavenee, P.S. Mischel, Heterogeneity of epidermal growth factor receptor signalling networks in glioblastoma, *Nat. Rev. Cancer* 15 (2015) 302–310.
- [16] J. Remon, M. Majem, EGFR mutation heterogeneity and mixed response to EGFR tyrosine kinase inhibitors of non small cell lung cancer: a clue to overcoming resistance, *Transl. Lung Cancer Res.* 2 (2013) 445–448.
- [17] M. Majem, J. Remon, Tumor heterogeneity: evolution through space and time in EGFR mutant non small cell lung cancer patients, *Transl. Lung Cancer Res.* 2 (2013) 226–237.
- [18] L.V. Sequist, B.A. Waltman, D. Dias-Santagata, S. Digumarthy, A.B. Turke, P. Fidias, K. Bergeth, A.T. Shaw, S. Gettinger, A.K. Cosper, S. Akhavanfar, R.S. Heist, J. Temel, J.G. Christensen, J.C. Wain, T.J. Lynch, K. Vernovsky, E.J. Mark, M. Lanuti, A.J. Iafrate, M. Mino-Kenudson, J.A. Engelman, Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors, *Sci. Transl. Med.* 3 (2011), <https://doi.org/10.1126/scitranslmed.3002003> 75ra26–75ra26.
- [19] C. Holohan, S. Van Schaeybroeck, D.B. Longley, P.G. Johnston, Cancer drug resistance: an evolving paradigm, *Nat. Rev. Cancer* 13 (2013) 714–726.
- [20] B.P. Rubin, A. Duensing, Mechanisms of resistance to small molecule kinase inhibition in the treatment of solid tumors, *Lab. Invest.* 86 (2006) 981–986.
- [21] C.-S. Tan, D. Gilligan, S. Pacey, Treatment approaches for EGFR-inhibitor-resistant patients with non-small-cell lung cancer, *Lancet Oncol.* 16 (2015) e447–e459.
- [22] Z. Chen, L. Han, M. Xu, Y. Xu, X. Qian, Rationally designed multitarget anticancer agents, *Curr. Med. Chem.* 20 (2013) 1694–1714.
- [23] X. Cai, H.-X. Zhai, J. Wang, J. Forrester, H. Qu, L. Yin, C.-J. Lai, R. Bao, C. Qian, Discovery of 7-(4-(3-Ethynylphenylamino)-7-methoxyquinazolin-6-yloxy)-N-hydroxyheptanamide (CUDC-101) as a potent multi-acting HDAC, EGFR, and HER2 inhibitor for the treatment of cancer, *J. Med. Chem.* 53 (2010) 2000–2009.
- [24] T. Shimizu, P.M. LoRusso, K.P. Papadopoulos, A. Patnaik, M. Bearam, L.S. Smith, D.W. Rasco, T.A. Mays, G. Chambers, A. Ma, J. Wang, R. Laliberte, M. Voi, A.W. Tolcher, Phase I first-in-human study of CUDC-101, a multitargeted inhibitor of HDACs, EGFR, and HER2 in patients with advanced solid tumors, *Clin. Cancer Res.* 20 (2014) 5032–5040.
- [25] X. Ni, L. Li, G. Pan, HDAC inhibitor-induced drug resistance involving ATP-binding cassette transporters (Review), *Oncol. Lett.* 9 (2015) 515–521.
- [26] C.-P. Wu, S.-H. Hsiao, C.-Y. Su, S.-Y. Luo, Y.-Q. Li, Y.-H. Huang, C.-H. Hsieh, C.-W. Huang, Human ATP-Binding Cassette transporters ABCB1 and ABCG2 confer resistance to CUDC-101, a multi-acting inhibitor of histone deacetylase, epidermal growth factor receptor and human epidermal growth factor receptor 2, *Biochem. Pharmacol.* 92 (2014) 567–576.
- [27] M. Murray, A. Hraiki, M. Beaway, C. Pazderka, T. Rawling, Anti-tumor activities of lipids and lipid analogues and their development as potential anticancer drugs, *Pharmacol. Ther.* 150 (2015) 109–128.
- [28] A. Kostadinova, T. Topouzova-Hristova, A. Momchilova, R. Tzoneva, M.R. Berger, Chapter two – antitumor lipids—structure, functions, and medical applications, in: D. Rossen (Ed.), *Advances in Protein Chemistry and Structural Biology*, Academic Press, 2015, pp. 27–66.
- [29] A. Pachioni Jde, J.G. Magalhaes, E.J. Lima, M. Bueno Lde, J.F. Barbosa, M.M. de Sa, C.O. Rangel-Yagui, Alkylphospholipids – a promising class of chemotherapeutic agents with a broad pharmacological spectrum, *J. Pharm. Pharm. Sci.* 16 (2013) 742–759.
- [30] W.J. van Blitterswijk, M. Verheij, Anticancer alkylphospholipids: mechanisms of action, cellular sensitivity and resistance, and clinical prospects, *Curr. Pharm. Des.* 14 (2008) 2061–2074.
- [31] M. Faustino, Antitumor Alkylphospholipid analogs: a promising and growing family of synthetic cell membrane-targeting molecules for cancer treatment, *Anticancer Agents Med. Chem.* 14 (2014) 495–498.
- [32] W.J. van Blitterswijk, M. Verheij, Anticancer mechanisms and clinical application of alkylphospholipids, *Biochim. Biophys. Acta.* 2013 (1831) 663–674.
- [33] K. Danker, W. Reutter, G. Semini, Glycosidated phospholipids: uncoupling of signalling pathways at the plasma membrane, *Br. J. Pharmacol.* 160 (2010) 36–47.
- [34] S. Clive, J. Gardiner, R.C. Leonard, Miltefosine as a topical treatment for cutaneous metastases in breast carcinoma, *Cancer Chemother. Pharmacol.* 44 (Suppl) (1999) S29–S30.
- [35] M.M. Alam, A.H.E. Hassan, Y.H. Kwon, H.J. Lee, N.Y. Kim, K.H. Min, S.Y. Lee, D.H. Kim, Y.S. Lee, Design, synthesis and evaluation of alkylphosphocholine-gefitinib conjugates as multitarget anticancer agents, *Arch. Pharm. Res.* 41 (2018) 35–45.
- [36] P.J. Pedersen, M.S. Christensen, T. Ruysschaert, L. Linderth, T.L. Andresen, F. Melander, O.G. Mouritsen, R. Madsen, M.H. Clausen, Synthesis and biophysical characterization of chlorambucil anticancer ether lipid prodrugs, *J. Med. Chem.* 52 (2009) 3408–3415.
- [37] Z. Huang, F.C. Szoka, Sterol-modified phospholipids: cholesterol and phospholipid chimeras with improved biomembrane properties, *J. Am. Chem. Soc.* 130 (2008) 15702–15712.
- [38] M.M. Ponpimol, R.L. Bugianesi, Synthesis of azide and amide analogs of platelet-activating factor and related derivatives, *Chem. Phys. Lipids* 35 (1984) 29–37.
- [39] J.J. Godfroid, C. Broquet, S. Jouquey, M. Lebbar, F. Heymans, C. Redeuilh,

- E. Steiner, E. Michel, E. Coeffier, Structure-activity relationship in PAF-acether. 3. Hydrophobic contribution to agonistic activity, *J. Med. Chem.* 30 (1987) 792–797.
- [40] P. Hanly, I. Soerjomataram, L. Sharp, Measuring the societal burden of cancer: The cost of lost productivity due to premature cancer-related mortality in Europe, *Int. J. Cancer* 136 (2015) E136–E145.
- [41] P.A. Hanly, L. Sharp, The cost of lost productivity due to premature cancer-related mortality: an economic measure of the cancer burden, *BMC Cancer* 14 (2014) 224.
- [42] A. Jemal, M.M. Center, C. DeSantis, E.M. Ward, Global patterns of cancer incidence and mortality rates and trends, *Cancer Epidemiol. Biomarkers Prev.* 19 (2010) 1893–1907.
- [43] J. Tang, R. Salama, S.M. Gadgeel, F.H. Sarkar, A. Ahmad, Erlotinib resistance in lung cancer: current progress and future perspectives, *Front. Pharmacol.* 4 (2013) 15, <https://doi.org/10.3389/fphar.2013.00015>.
- [44] X. Lu, G. Arthur, The differential susceptibility of A427 and A549 cell lines to the growth-inhibitory effects of ET-18- OCH_3 does not correlate with the relative effects of the alkyl-lysophospholipid on the incorporation of fatty acids into cellular phospholipids, *Cancer Res.* 52 (1992) 2813–2817.
- [45] H. Masuda, D. Zhang, C. Bartholomeusz, H. Doihara, G.N. Hortobagyi, N.T. Ueno, Role of epidermal growth factor receptor in breast cancer, *Breast Cancer Res. Treat.* 136 (2012) 331–345.
- [46] F. Yamasaki, D. Zhang, C. Bartholomeusz, T. Sudo, G.N. Hortobagyi, K. Kurisu, N.T. Ueno, Sensitivity of breast cancer cells to erlotinib depends on cyclin-dependent kinase 2 activity, *Mol. Cancer Ther.* 6 (2007) 2168–2177.
- [47] Y.C. Li, M.J. Park, S.-K. Ye, C.-W. Kim, Y.-N. Kim, Elevated levels of cholesterol-rich lipid rafts in cancer cells are correlated with apoptosis sensitivity induced by cholesterol-depleting agents, *Am. J. Pathol.* 168 (2006) 1107–1118.
- [48] S. Mikhail, D. Cosgrove, A. Zeidan, Hepatocellular carcinoma: systemic therapies and future perspectives, *Expert. Rev. Anticancer. Ther.* 14 (2014) 1205–1218.
- [49] M.B. Thomas, R. Chadha, K. Glover, X. Wang, J. Morris, T. Brown, A. Rashid, J. Dancey, J.L. Abbruzzese, Phase 2 study of erlotinib in patients with unresectable hepatocellular carcinoma, *Cancer* 110 (2007) 1059–1067.
- [50] D.F. Calvisi, C. Wang, C. Ho, S. Ladu, S.A. Lee, S. Mattu, G. Destefanis, S. Delogu, A. Zimmermann, J. Ericsson, S. Brozzetti, T. Staniscia, X. Chen, F. Dombrowski, M. Evert, Increased lipogenesis, induced by AKT-mTORC1-RPS6 signaling, promotes development of human hepatocellular carcinoma, *Gastroenterology* 140 (2011) 1071–1083.
- [51] J.M. Jiménez-López, M.P. Carrasco, J.L. Segovia, C. Marco, Hexadecylphosphocholine inhibits phosphatidylcholine biosynthesis and the proliferation of HepG2 cells, *Eur. J. Biochem.* 269 (2002) 4649–4655.
- [52] G. Spallone, E. Botti, A. Costanzo, Targeted therapy in nonmelanoma skin cancers, *Cancers* 3 (2011) 2255–2273.
- [53] T.E. Røsbjerg, P. Helsing, M.B. Veierød, Cutaneous squamous cell carcinoma in Norway 1963–2011: increasing incidence and stable mortality, *Cancer Med.* 4 (2015) 472–480.
- [54] F. Yamasaki, M.J. Johansen, D. Zhang, S. Krishnamurthy, E. Felix, C. Bartholomeusz, R.J. Aguilar, K. Kurisu, G.B. Mills, G.N. Hortobagyi, N.T. Ueno, Acquired resistance to erlotinib in A-431 epidermoid cancer cells requires down-regulation of MMAC1/PTEN and up-regulation of phosphorylated Akt, *Cancer Res.* 67 (2007) 5779–5788.
- [55] M.M. Alam, E.-H. Joh, Y. Kim, Y.I. Oh, J. Hong, B. Kim, D.-H. Kim, Y.S. Lee, Synthesis and biological evaluation of cyclopentane-linked alkyl phosphocholines as potential anticancer agents that act by inhibiting Akt phosphorylation, *Eur. J. Med. Chem.* 47 (2012) 485–492.
- [56] M.M. Alam, E.-H. Joh, H. Park, B. Kim, D.-H. Kim, Y.S. Lee, Synthesis, characterization and Akt phosphorylation inhibitory activity of cyclopentanecarboxylate-substituted alkylphosphocholines, *Bioorg. Med. Chem.* 21 (2013) 2018–2024.
- [57] E. Kovalevski-Ishai, Z. Ziniuk, G. Halperin, I. Mendel, E. Feige, N. Yacov, E. Breitbart, Oxidized Lipid Compounds and Uses Thereof, WO2010052718, May 14 Vascular Biogenics Ltd., 2010.
- [58] M. Deshpande, J.A. Wiles, A. Hashimoto, A. Phadke, Stabilized Nucleotides for Medical Treatment, WO2016014527, January 28 Achillion Pharmaceuticals Inc., 2016.
- [59] Z. Huang, W. Li, F.C. Szoka Jr, Asymmetric 1-alkyl-2-acyl phosphatidylcholine: a helper lipid for enhanced non-viral gene delivery, *Int. J. Pharm.* 427 (2012) 64–70.