Advances in the development of hybrid anticancer drugs

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Abstract

Introduction: Hybrid anticancer drugs are of great therapeutic interests as they can potentially overcome most of the pharmacokinetic drawbacks encountered when using conventional anticancer drugs. In fact, the future of hybrid anticancer drugs is very bright for the discovery of highly potent and selective molecules that triggers 2 or more cytocidal pharmacological mechanisms of action acting in synergy to inhibit cancer tumor growth.

Area covered: This review represents the most advanced and recent data in the field of hybrid anticancer agents covering mainly the last 5 years of research. It also accounts for other significant reviews already published on the topic of anticancer hybrids. The review showcases the research that is at the leading edge of hybrid anticancer drug discovery. The main areas covered by the present review are: DNA alkylating agent hybrids (e.g. Pt(II), nitrogen mustard, etc.), vitamin-D receptor, agonist-histone deacetylase inhibitors, combi-molecule therapies and other types of hybrid anticancer agents.

Expert opinion: The current development in the field describes strategies never used before for the design of hybrid anticancer drugs. The information currently available and described in this section allows us to identify the main parameters required to design such molecules. It also provides a clear view of the future directions that must be explored for the successful development and discovery of useful hybrid anticancer drugs.

Article highlights

- The review covers the most advanced development of hybrid anticancer drugs.
- The usual parental drugs used to build the hybrids and their mechanism(s) of action are listed in two tables.
- The review takes into account the various ways a hybrid can be fashioned and the general strategies for the design of hybrid anticancer drugs.
- The potential advantages for the patient using hybrid anticancer drugs are presented.
- The review discusses the parameters involved in the design of hybrid anticancer drugs for clinical usages.

This box summarizes key points contained in the article.

Keywords: Drug design; Drug-targeting; hybrids; anticancer agents; combi-molecules.

List of abbreviations: Histone deacetylase, HDAC; absorption, distribution, metabolism, and excretion, ADME; Philadelphia chromosome, Bcr-Abl; multidrug resistance, MDR; National Cancer Institute, NCI; epidermal growth factor receptor, EGFR.

1. Introduction

This review presents the most recent advances in the design and development of hybrid anticancer agents. This type of design was comprehensively reviewed in 2009 by Gediya *et al.* [1] and, in 2007 by Viegas-Junior *et al.* [2]. As a general rule, the review showcases the most current work in the field. Consequently, molecular structures already described in the previous reviews will not be addressed and only hybrids recently published in the literature will be discussed at the exception of hybrids required to illustrated significant elements of the discussion. Moreover, for sake of clarity and conciseness, only the most promising and potent hybrids will be described. The goal of this review is also to assist and guide the scientific community to design even better hybrid anticancer drugs for the next generation of cancer treatments.

Hybrid drug design is in constant evolution and remains essential for the discovery of innovative and potent anticancer drugs. To that end, several research groups are devising and testing new chemical and biochemical strategies for their development. This review is divided into sections that take into account two main ways by which hybrid anticancer molecules can be designed and prepared: (i) merge and blend haptophoric moieties of different drugs and (ii) combine two or several entire drugs together. Some hybrids use both design approach and are therefore difficult to classify in one or the other category.

The first approach that merges and blends haptophoric moieties of different drugs used to design new anticancer hybrids is based on the ability of a combination of haptophoric moieties on a new molecular structure to retain their affinity and activity for the biological targets. This concept is achieved using two strategies: (i) merging of two haptophoric groups from two different drugs acting through the same mechanism of action and (ii)

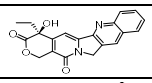
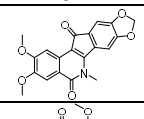
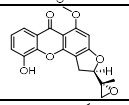
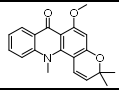
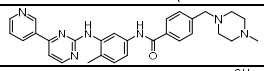
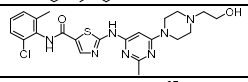
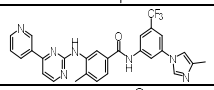
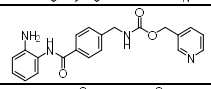
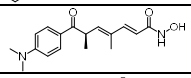
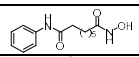
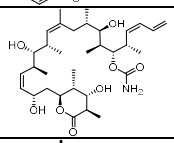
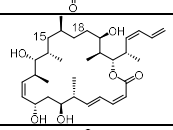
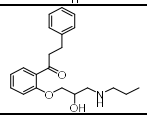
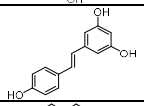
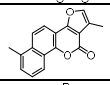
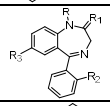
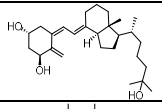
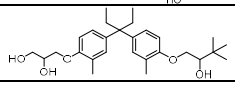
merging of haptophoric groups from two drugs acting through different mechanisms of action.

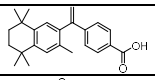
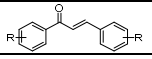
The second approach combines two or several entire drugs together (combi-molecules) directly or connected through a linking arm. Moreover, the connection can be achieved using cleavable or non-cleavable linkages. The connection of two molecular entities through non-cleavable linking arms is also based on the ability of the different molecules to retain their biological activity and their specific and respective affinity for their biological targets. On the opposite, the approach using cleavable bond is based on the release of two parental molecular structures under physiological or the enzymatic conditions that prevail at site of activity aims: to either improve poor pharmacokinetic properties of the anticancer drugs to slowly deliver the two therapeutic entities in the body (e.g. ester, amide or carbamate) or to improve the selectivity and the antineoplastic activity of the drugs and to release the two drugs directly in the targeted tissues (e.g. phosphorylated DES prodrugs for prostate cancer). In this review, we chose to classify the different hybrids by the mechanism(s) of action of their constituting drugs: (i) connection of two drugs with the same mechanism of action, (ii) connecting two drugs with different mechanisms of action and (iii) connecting two drugs in the aim to target specific biological tissues.

2. Merging of the haptophoric moieties of different drugs

Merging haptophoric moiety of different drugs is the first strategy that has been used to design new anticancer hybrids. Table 1 shows the parental anticancer drugs and their mechanism of action used for the design of potent hybrids.

Table 1. Parental anticancer drugs and their mechanism of action used in the design of merged haptophoric moiety hybrid of different drugs.

#	Names	Structures	Main mechanism(s) of action
1	Camptothecin		Topoisomerase I inhibitor
2	5,11-Diketoindenoisoquinoline (NSC 314622)		Topoisomerase I inhibitor
3	Psorospermin		DNA strand breaks
4	Acronycine		DNA alkylation
5	Imatinib		Tyrosine-kinase Bcr-Abl and Src inhibitors
6	Dasatinib		Tyrosine-kinase Bcr-Abl and Src inhibitors
7	Nilotinib		Tyrosine-kinase Bcr-Abl inhibitors
8	MS-275		HDAC inhibitors
9	Trichostatin A (TSA)		HDAC inhibitor
10	Vorinostat (SAHA)		HDAC inhibitor
11	Discodermolide		Microtubule-stabilizing agent
12	Disctyostatin		Microtubule-stabilizing agent
13	5-Fluorouracil		Thymidylate synthase inhibitor
14	Propafenone		Anti-arrhythmic
15	Resveratrol		Cancer prevention
16	Coumarin		N.D.*
17	Neo-tanshinlactone		N.D.
18	Benzodiazepines		Allosteric modulator of $GABA_A$
19	1α,25-Vitamin D3 (1,25D)		Vitamin D receptor agonist
20	3,3-diarylpentanes (LG190178)		Vitamin D receptor agonist

21	Bexarotene		Retinoid X receptors agonist
22	Chalcone		Cancer prevention

* N.D.: Not fully determined

2.1 Hybrid anticancer molecules aiming to an identical biological target

The first strategy approach is the synthesis of the merging of two haptophoric groups selected from two drugs exhibiting the same cytocidal mechanism of action. This design aims to improve activity, selectivity and biopharmaceutical properties (absorption, distribution, metabolism and excretion (ADME properties)) of both parental anticancer drugs. Several anticancer hybrids designed following this concept are shown in Figure 1.

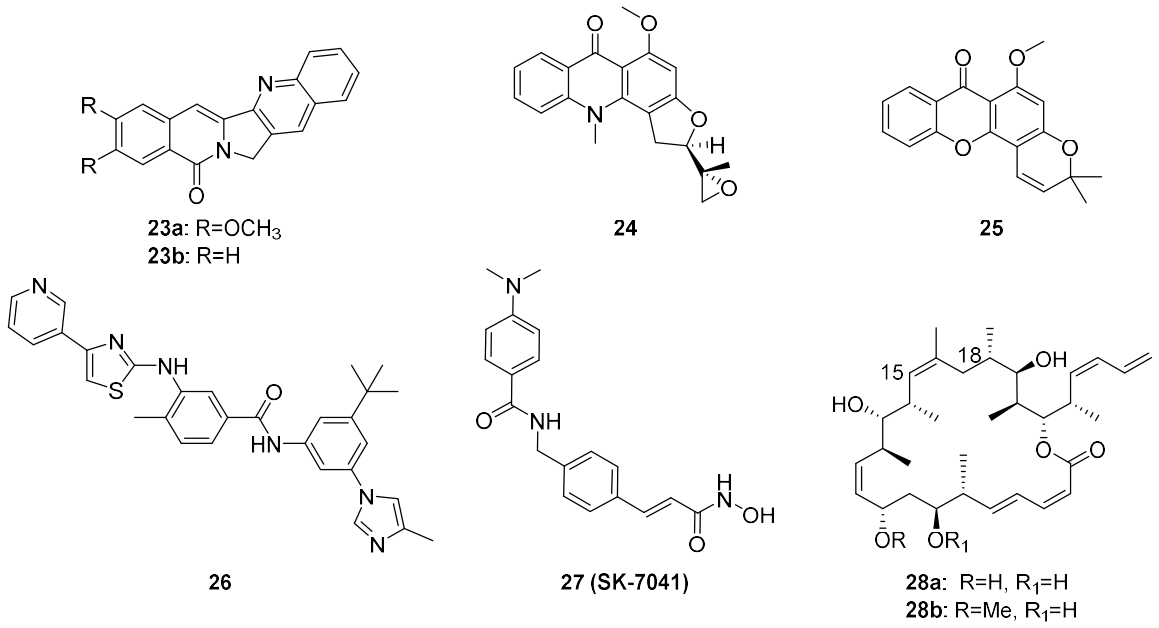


Figure 1. Molecular structures of anticancer drug hybrids merging two haptophoric groups from two different drugs acting through the same mechanism of action.

The indenoisoquinoline-camptothecin hybrids (**23a** and **b**) result from the merging of two topoisomerase I inhibitors: camptothecin (**1**) and 5,11-diketoindenoisoquinoline (NSC 314622, **2**), respectively. The hybrids combine the 2,3-dihydro-1*H*-pyrrolo[3,4-*b*]quinoline haptophoric moiety of **1** at position 6 and 7 of the isoquinolone moiety of **2** [3]. Compounds

23a, **b** exhibit antiproliferative activity in the μM range and possess significant topoisomerase I inhibitory activity but showed lower biological activity than their parental compounds alone.

Nguyen *et al.* thoroughly reviewed the antitumor activity of psoropermum xanthones and sarcomelicope acridones [4]. Their review discusses also of the biological properties of several hybrids such as epoxyfuroacridone (**24**) and pyranoxanthone (**25**). The latter are merging the haptophore of psorospermin (**3**) and acronycine (**4**), two anticancer drugs targeting DNA. The epoxyfuroacridone **24** was prepared by amalgamating the acridone moiety of **4** and the epoxyfuran of **3** whereas pyranoxanthone **25** was designed using the xanthone moiety of **3** and the pyran group of **4**. Compound **24** exhibits antiproliferative activity in the nM range while compound **25** show only marginal antiproliferative activities.

Compound **26** is a new Philadelphia chromosome (Bcr-Abl) inhibitor hybrid that has been designed using molecular fragments found in FDA approved imatinib (**5**), dasatinib (**6**) and nilotinib (**7**) [5] namely the pyridine group of **5,** the thiazol-2-amine and the 4-methyl-*N*-phenylbenzamide moieties of **6** and **7**, respectively. Compound **26** exhibits antiproliferative activity in the nM range, which is in the same order of magnitude as nilotinib on the cancer cell lines assessed. In addition, **26** displayed similar inhibitory potency on Bcr-Abl kinase than that of nilotinib, arrested the cell cycle progression in G0/G1-phase and induced apoptosis of K562 cancer cells.

Combination of the benzamidyl moiety of MS-275 (**8**) in the aliphatic chain of trichostatin A (TSA, **9**), two histone deacetylase (HDAC) inhibitors, lead to the generation of SK-7041 (**27**) [6]. Compound **27** exhibits antiproliferative activity in the low μM range and is 5-

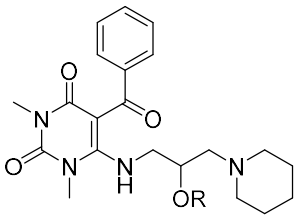
folds more potent than vorinostat (SAHA, **10**) on lung and breast cancer cell lines. This hybrid shows also time-dependent histone hyperacetylation leading successively to G2/M-phase, G1-phase arrest and ultimately to apoptosis.

Florence *et al.* nicely reviewed in 2008 the marine anticancer agents discodermolide (e.g **11**) and dictyostatin (e.g. **12**) and discussed of the preparation and the evaluation of several hybrids deriving from these antimitotics [7]. In recent years, Paterson group has synthesized several dictyostatin–discodermolide hybrids exemplified by compounds **28a**, **b** [8, 9]. This type of hybrid consists to the addition of an alkene bond and a α-methyl group at positions 15 and 18 of **11** on the molecular structure of **12**. These hybrids are based on the overlapping molecular structure of **12** and the semi-cyclic conformation of **11**. Compounds **28a**, **b** show antiproliferative activity in the low nM range on several cell lines, which is in the same order of magnitude than **11**, **12** and paclitaxel. Dictyostatin–discodermolide hybrids are microtubule-stabilizing agents that do not interact with P-glycoprotein and still highly cytocidal against paclitaxel-resistant cancer cell lines.

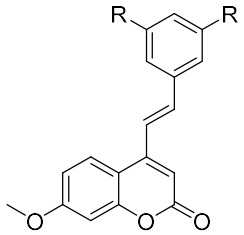
2.2 Hybrid anticancer molecules aiming to multiple biological targets

The second strategy used to merge haptophoric moieties of different drugs is to integrate 2 different drugs that are addressing separately two or multiple biological targets. Again this strategy is used to design new families of drugs to improve the pharmacokinetic and pharmacodymamic properties of the parent components as well as to synergize their mechanisms of action in a single molecular entity structure. This approach seems to disobey to conventional strategies used in medicinal chemistry where the selectivity of a molecule for a specific biological target is the cornerstone for the development of new drugs. However it is in agreement with the logic of using “chemotherapeutic cocktails” in

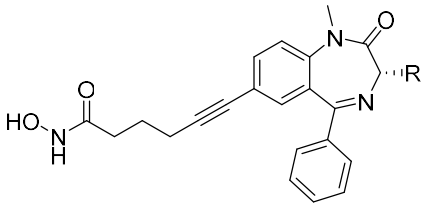
clinic that are combining chemotherapeutic agents exhibiting different mechanisms of action (e.g. 5-fluorouracil, epirubicin, cyclophosphamide for the treatment of breast cancer) that are more effective than the same agents used alone. In addition, that approach was found to prevent chemoresistance. Therefore, polychemotherapeutic approach was translated in recent years into the design of hybrid molecules aiming to several cytocidal targets at once [10]. Consequently, several new anticancer agent hybrids were recently designed using this strategy and are shown in Figure 2.



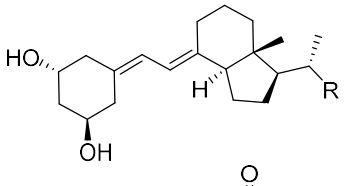
29a: R=H
29b: R=COPh

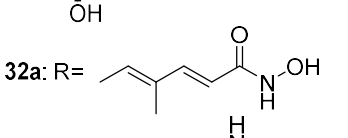


30a: R=OMe
30b: R=Me

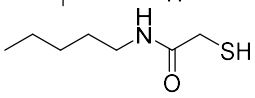


31a: R=Me
31b: R=H

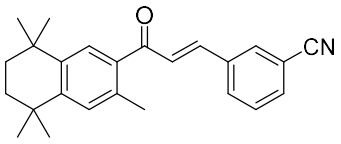




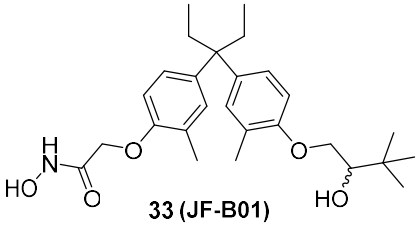
32a: R=



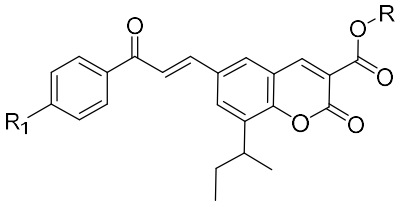
32b: R=



34



33 (JF-B01)



35a: R=Me, R_1=H
35b: R=Et, R_1=H
35c: R=Et, R_1=Me

Figure 2. Molecular structures of anticancer drug hybrids merging haptophoric groups from two drugs acting through different mechanisms of action.

Multidrug resistance (MDR) is plaguing chemotherapies of numerous cancer tumors via several mechanisms of action including the increase of efflux of drug (e.g. P-glycoprotein (P-gp), multidrug resistance-associated protein). To circumvent chemoresistance potent multidrug resistance modulators are required. To that end, 5-fluorouracil (**13**), a potent thymidylate synthase inhibitor enzyme was linked to propafenone (**14**) that is an anti-arrhythmic drug known also as a potent multidrug resistance (MDR) modulator to generate the uracil-based hybrid molecules (**29a**, **b**) [11]. Compounds **29a**, **b** interact with P-gp at 0.5 μM and they could be potential candidates for new MDR modulators.

Stilbene-coumarin hybrids (compounds **30a**, **b**) are composed of the phenylethenyl moiety of resveratrol (**15**), an agent recognized for its cancer prevention properties and the coumarinic ring system (e.g. compounds **16** and **17**). The latter family of anticancer agent acts through a variety of mechanisms of action on the cells and are recognized to exhibit potent proapoptic effects [12]. Compounds **30a**, **b** exhibit antiproliferative activity in the low μM range on cell lines studied. They arrest the cell cycle progression in the G2/M-phase and induce apoptosis.

Benzodiazepines (**18**) are a family of psychotropic drugs producing sedative, hypnotic and anxiolytic effects including cytostatic and differentiative effects in a variety of transformed cell types [13]. Guandalini *et al.* used the latter property of benzodiazepines to design new hydroxamate histone deacetylase inhibitors (HDACi, compounds **31a**, **b**) [14]. These hybrids are designed by substituting the aromatic ring of HDACi **9** and **10** by the benzodiazepine ring and merging the hydroxamate function (terminal zinc-binding groups)

by an ethynyl and an alkyl chain. Compounds **31a**, **b** exhibit antiproliferative activity in the hundreds of nM range in human acute promyelocytic leukemia NB4 cell line and they induce also histone H3/H4 acetylation.

1α,25-Vitamin D3 (1,25D, **19**) plays a pivotal role in controlling calcium homeostasis and has also antiproliferative and cancer chemopreventive properties. Moreover, the antiproliferative effects of the combination of 1,25D and HDACi have been confirmed on 1,25D-resistant cancer cells. Therefore, combination of analogs of 1,25D and a variety of terminal zinc-binding groups including hydroxamate present in classic HDACi **9** and **10** (triciferol, **32a**) [15] and 2-mercaptoacetamide (compound **32b**) [16] to design vitamin D receptor agonist-histone deacetylase inhibitor hybrids (compounds **32a**, **b**) has been natural. Compounds **32a**, **b** exhibit antiproliferative activity in AT84 squamous carcinoma cells, bind to the vitamin D receptor and act as HDACi while they do not trigger hypercalcemic effects, a deleterious effect induced by 1,25D.

High-throughput screening recently identified non-secosteroidal 3,3-diarylpentane **20** (LG190178) as a new vitamin D receptor agonist and a more easily synthesizable chemical structures with pro-differentiation and antiproliferative activities without triggering hypercalcemia effects. Fisher *et al.* merged **20** and the terminal zinc-binding groups (hydroxamate function) of **9** and **10** to design the non-secosteroidal vitamin D receptor agonist-HDACi hybrids (JF-B01, **33**) [17]. Compound **33** shows antiproliferative activity in the μM range on both 1,25D-sensitive (SCC25, AT84) and 1,25D-resistant (SCC4) squamous carcinoma cell lines. Moreover, compound **33** acts as full vitamin D receptor agonist and as a HDACi.

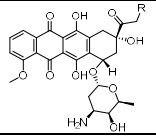
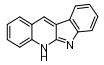
The retinoid-chalcone hybrids (**34**) are composed of the retinoid moiety of bexarotene (**21**) combined to the chalcone moiety of compound **22**, two potent anticancer molecular entities [18]. Compound **34** shows antiproliferative activity in low μM range on colon adenocarcinoma HT-29 cell line.

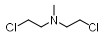
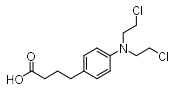
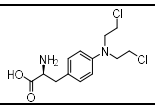
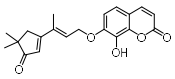
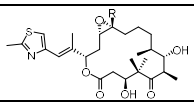
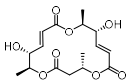
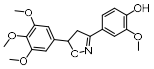
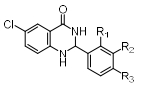
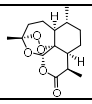
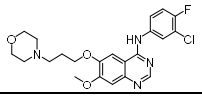
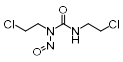
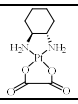
Chalcone (**22**) were also used by Sashidhara *et al.* and fuses with the coumarin ring systems (**16** and **17**) to generate new coumarin–chalcone hybrids (**35a-c**) [19] that showed antiproliferative activity in the μM range on KB, C33A, MCF-7, A549 and NIH3T3 cell lines, with a superior activity on a cervical carcinoma C33A cell line.

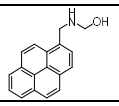
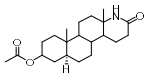
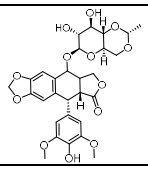
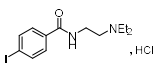
3. Combination of two or several entire drugs (combi-molecules)

The amalgamation of two or several entire drugs in the same molecular structure, also termed combi-molecule is the second approach used to design new anticancer hybrids. Several anticancer drugs used for this application and their respective mechanisms of action are described in Tables 1 and 2.

Table 2. Parental anticancer drugs and their mechanism of action used in the design of combi-molecules.

#	Names	Structures	Main mechanism(s) of action
36	a: Doxorubicin: R = OH b: Daunorubicin: R = H		DNA intercalation, topoisomerase II inhibitor, and generation of iron-mediated free oxygen radicals damaging DNA and cell membranes
37	Indolo[2,3-b]quinoline		DNA intercalation and topoisomerase II inhibitor
38	Tetrahydro-β-carboline		DNA intercalation, CDK, topoisomerase I and/or II and monoamine oxidase inhibitor

39	1,3,5-Triazine		N.D.*
40	Mechlorethamine		DNA alkylation
41	Chlorambucil		DNA alkylation
42	Melphalan		DNA alkylation
43	Pyrrolo[2,1-c][1,4]benzodiazepines (PBDs)		DNA alkylation
44	Coumarin (Geiparvarin)		N.D.
45	Epothilone A: R = H Epothilone B: R = CH_3		Microtubule-stabilizing agent
46	Macrosphelide A		Cell-cell adhesion inhibitor
47	1H-Indole-2,3-dione (Isatin)		Tyrosine kinase, cyclin-dependent kinases and carbonic anhydrase isozyme inhibitor
48	Diaryl substituted isoxazoline analog of CA-4		Microtubule polymerization inhibitor
49	2,3-Dihydro-2-aryl-4-quinazolinones (DHQZ)		Microtubule polymerization inhibitor
50	Artemisinin		N.D.
51	Gefitinib		EGFR tyrosine kinase inhibitor
52	Carmustine (BCNU)		DNA alkylation
53	1,2,3-Triazene		DNA alkylation
54	Cisplatin		DNA alkylation
55	Carboplatin		DNA alkylation
56	Oxaliplatin		DNA alkylation

57	Acridine		DNA intercalation
58	β-Lactam		N.D.
59	Pyrene derivatives		DNA intercalation
60	Acridone		DNA intercalation
61	Homo-azasteroids		N.D.
62	Fluorodeoxyglucose (^{18}F)		Distributed in high-glucose-consuming cells
63	Etoposide		Topoisomerase II inhibitor
64	Aziridine		N.D.
65	Nitroxyl compounds (e.g. PROXYL)		Accumulation in melanomas
66	4-Iodobenzamide (BZA)		High affinity for melanin

* N.D.: Not fully determined

3.1 Combi-molecules with the same mechanism of action

The first strategy to design new anticancer hybrids is the connection of two drugs with the same mechanism of action. The drugs can be composed of different drugs and moieties recognized to have enhanced anticancer properties or composed of the same drug (dimer). Several anticancer hybrids designed following this concept are shown in Figures 3a (different drugs hybrids) and 3b (dimer hybrids).

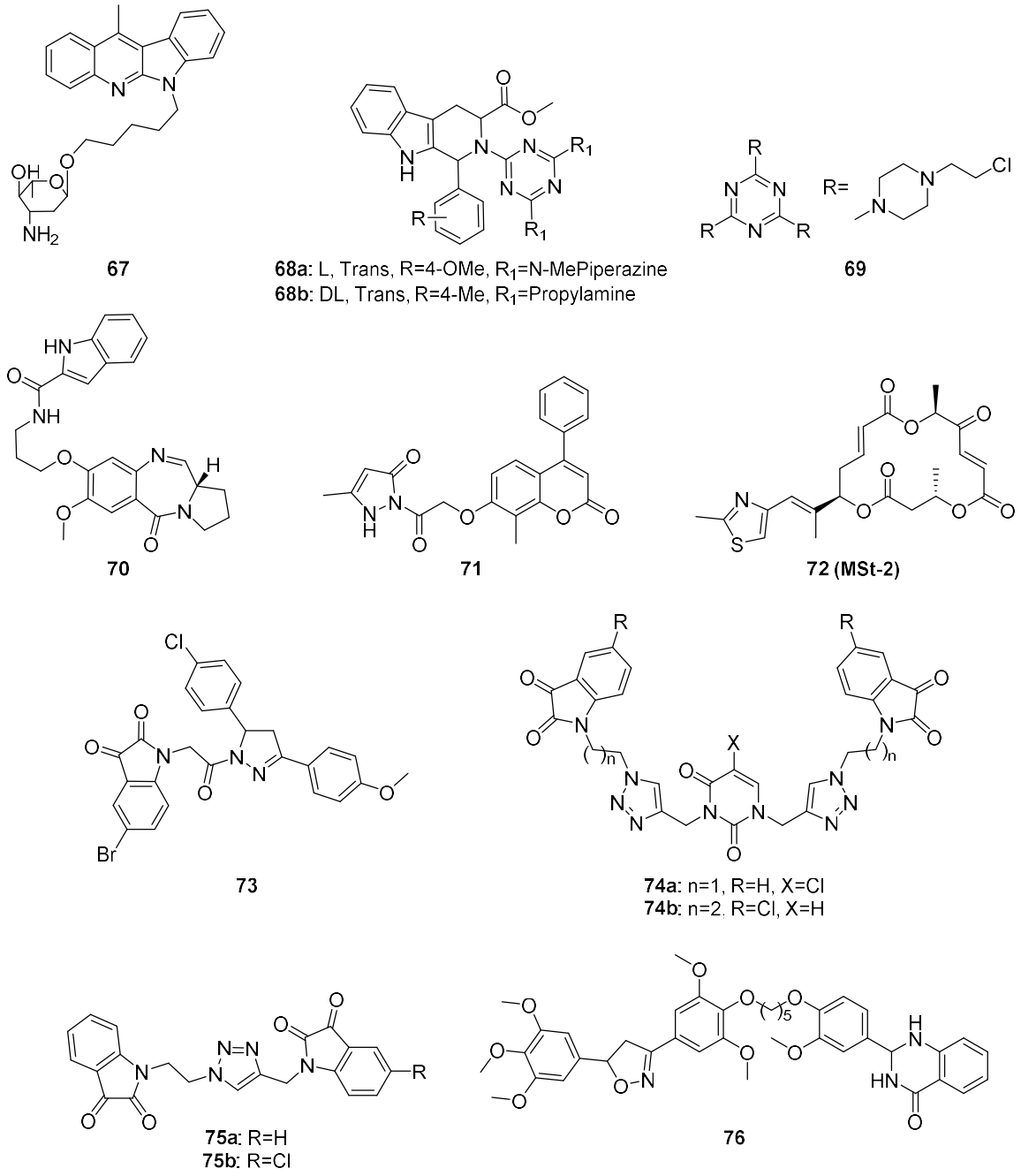


Figure 3a. Molecular structures of combi-molecules with the same mechanism of action.

It is known that antitumor activity of anthracycline antibiotics such as doxorubicin (**36a**) and daunorubicin (**36b**) is strongly dependent on the presence of the daunosamine (aminocarbohydrate) moiety. Bednarek *et al.* used that observation to improve the properties of indolo[2,3-b]quinolones (e.g. **37**) which are analog of neocryptolepine DNA

topoisomerase II inhibitors. They have linked **37** to aminoglycoside to produce new indolo[2,3-b]quinoline–hybrid (**67**) that are describe as a new family of potent anticancer drugs [20].

Kumar *et al.* designed (tetrahydro-β-carboline)-1,3,5-triazine hybrids (compounds **68a**, **b**) connecting tetrahydro-β-carboline (**38**) and triazine (**39**), two molecular structure showing potent antiproliferative activities [21]. Compounds **68a**, **b** show antiproliferative activity in the μM range on several cancer cell lines and arrest the cell cycle progression in G0/G1-phase.

Triazine (**39**) has also been connected at position 2, 4 and 6 of the 1-(2-chloroethyl)piperazine moiety which bears a 2-chloroethylamino group raising similitudes with nitrogen mustards (**40**-**42**) to give compound **69** [22] that exhibits antiproliferative activity in μM range on MCF-7 cells and forms DNA crosslinks, and induces apoptosis and necrosis.

Pyrrolo[2,1-c][1,4]benzodiazepines (PBD, **43**) are natural antitumor antibiotics known for their cytotoxic and antitumor effects through DNA alkylation and nucleic acid synthesis inhibition. These compounds were reviewed by Gerratana in 2012 [23]. In the literature, several manuscripts reported researches that incorporate an indole moiety in a synthetic anticancer agent showed potent cytotoxicity. Wang *et al.* have prepared also the pyrrolo[2,1-c][1,4]benzodiazepine-indole hybrids such as compound **70** [24] that displays antiproliferative activity in the nM range, induces apoptosis in A2058 cells, forms stable complexes, binds to DNA, and exhibits *in vivo* antitumor activity in the hollow fiber assay.

7-Substituted-benzopyran-2-ones-heterocyclic hybrids (e.g. compound **71**) were prepared by merging the pyrazolin-5-one heterocyclic ring to a coumarin moiety (**16** and **17**) using

an acetoxy linker in the aim to mimic geiparvarin (**44**), a coumarinic analog showing potent antiproliferative activity on various cancer cell lines [25]. Compound **71** displays median antiproliferative activity in the low μM range when tested using the National Cancer Institute (NCI) anticancer cell line panel.

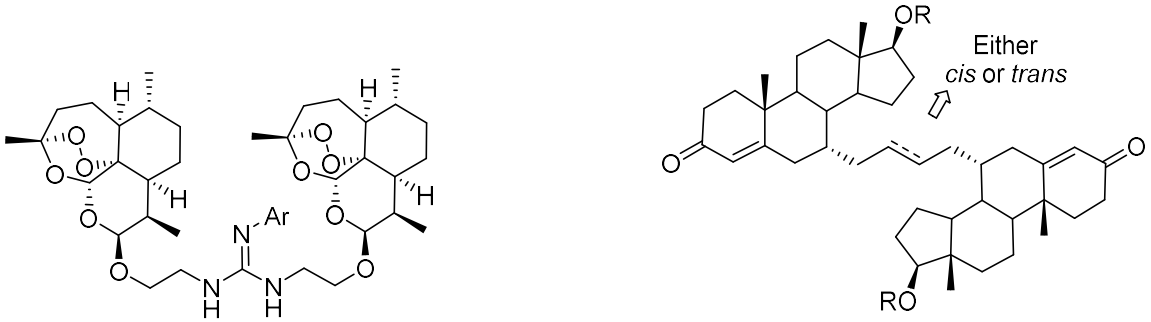
Macrosphelide-epothilone hybrids (e.g. MSt-2, **72**) are the results of a combination of the 2-methyl-4-(prop-1-en-1-yl)thiazole present in the epothilone (**45**) and macrosphelide A (**46**). Compound **72** displays antiproliferative potency on human colon carcinoma (HCT116) and human gastric cancer (AGS) cells while it shows no effects on human normal dermal fibroblast [26]. It induces also the formation of reactive oxygen species (ROS), activation of Jun N-terminal kinase (JNK) and apoptosis in human lymphoma (U937) [27].

Isatin (**47**) is an anticancer agent showing high affinity to tyrosine and cyclin-dependent kinases, and carbonic anhydrase isozymes. Moreover, diazoles (pyrazoles and pyrazolines) are privileged structures in the design of new anticancer agents. In this context, Havrylyuk *et al.* have designed and prepared isatin-pyrazoline hybrids (compound **73**) [28]. That exhibits an antiproliferative activity in the μM range and a selectivity index at the GI_{50} level of 15 toward leukemia subpanel tumor cell lines.

1*H*-1,2,3-triazole tethered C-5 substituted uracile-isatin conjugates (compounds **74a**, **b**) is another class of anticancer hybrids designed by connecting isatin, uracile and 1,2,3-triazole, three scaffolds considered as privileged structures in antineoplasics discovery [29]. Compounds **74a**, **b** exhibit GI_{50} of 18.21 and 13.90 μM, respectively on DU145 cell line while they are inactive on MCF-7 cells.

Singh *et al.* have also used isatin and 1,2,3-triazole to design new 1*H*-1,2,3-triazole tethered isatin hybrids (compounds **75a**, **b**) [30]. Compounds **75a**, **b** are the most active compounds of the series prepared and show antiproliferative in the nM range on A-549, PC-3 and THP-1 cell lines whereas they are inactive on Caco-2 cells.

3,5-Diaryl isoxazoline linked 2,3-dihydroquinazolinone hybrids (compound **76**) were designed by connecting a five-membered heterocycles analog of combretastatin-A4 namely diaryl substituted isoxazoline derivatives (**48**) and 2,3-dihydro-2-aryl-4-quinazolinones (e.g. DHQZ, **49**) [31]. Compound **76** shows antiproliferative activity in the μM range, arrests the cell cycle progression in G2/M-phase, inhibits cyclin B1 and CDK1 and disrupts the cytoskeleton.



77a: Ar=2,4-Dichlorophenyl
77b: Ar=4-Trifluoromethylphenyl

78a: R=Ac
78b: R=H

Figure 3b. Molecular structures of combi-molecules with the same mechanism of action (dimer).

Another approach to design anticancer hybrids is the formation of homodimers of anticancer drugs. This strategy is also used to improve biopharmaceutical properties such as low solubility or short plasma half-life of drugs such as artemisinin (**50**). To overcome those limitations, artemisinin–guanidine hybrids (compounds **77a**, **b**) were designed by

connecting two molecules of compound **50** on a guanidine water-soluble backbone [32]. Compounds **77a**, **b** show antiproliferative activity in the ten nM range on HT-29 cell line. Likewise, compounds **78a**, **b** are dimers of testosterone and have been designed to act as an "bidentate" antiandrogen that binds simultaneously to two androgen receptors, resulting in the disruption of the androgen receptor signaling pathway [33]. The dimers were linked together either by a *trans* or a *cis* but-2-enyl tether chain. The *cis* dimer (*cis*-**78b**) was more active than the *trans* counterpart (*trans*-**78b**). Compound *cis*-**78b** exhibits antiproliferative activity in the μM range on prostate LNCap and PC3 cell lines and was slightly more active than cyproterone acetate, a known antiandrogen used in clinics. However, no selectivity on androgen-dependent prostate cancer cells was observed for these dimers.

3.2 Combi-molecules with different mechanisms of action

The combi-molecules were developed in the aim to exploit multi-biological targets. It is one of the most promising approaches to design highly potent, effective and useful therapeutical hybrids. Tumors cells possess numerous proteins, enzymes, signaling pathways or other biological entities to bypass, overcome the mechanisms of action of anticancer agents. These protective molecules and salvation mechanisms abrogate the antiproliferative activity and the induction of apoptosis normally triggered by anticancer agents and, this often induce chemoresistance of the tumor cancer cells. Consequently, that concept aims to target different unrelated or related mechanisms of action to synergize their efficacy and circumvent the rescue mechanisms employed by tumor cells. Several anticancer hybrids designed according to this concept are shown in Figures 4a and 4b.

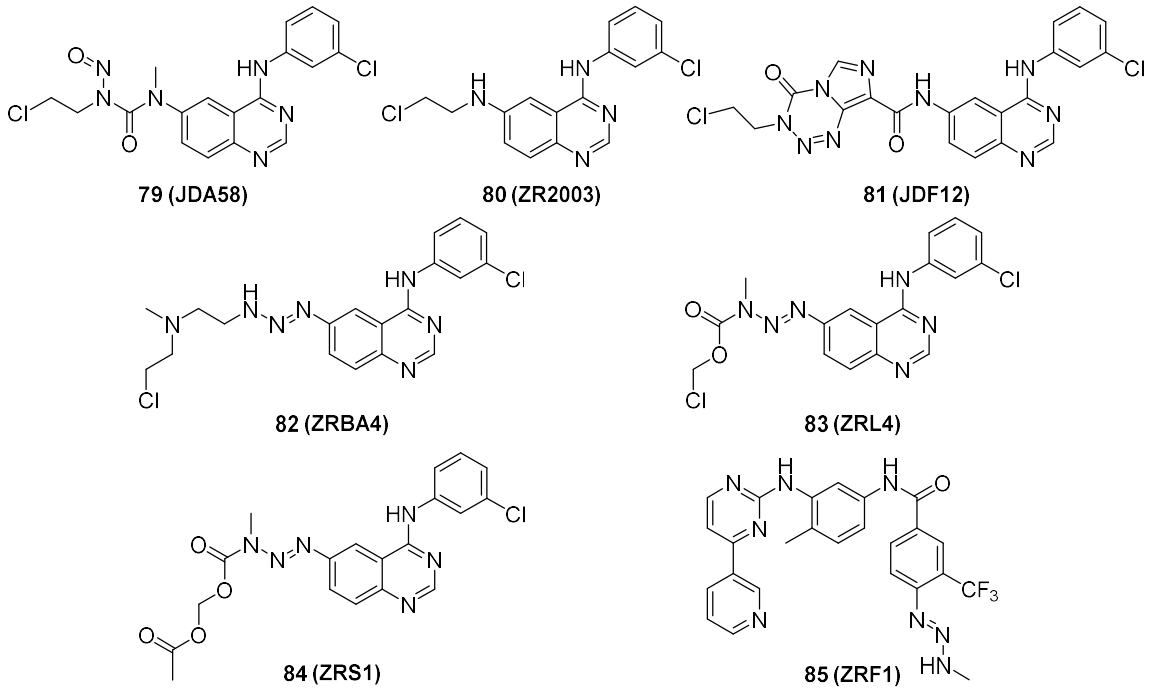


Figure 4a. Molecular structures of combi-molecules with EGFR tyrosine kinase inhibitor (gefitinib, **51**) and tyrosine-kinase Bcr-Abl inhibitor (imatinib, **5**) mechanisms of action.

Jean-Claude group's has extensively studied the combi-molecules concept by merging the pharmacophore moiety of a gefitinib analog (**51**), an epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor, with different alkylating groups to generate combi-molecules designated as TZ-I. Chloroethylnitrosourea (compound **79**, JDA58) [34], 2-chloroethylamino, (compound **80**, ZR2003) [35, 36] and mitozolomide moieties (compound **81**, JDF12)[37] were linked with **51** together since they are important pharmacophore functions responsible for the anticancer properties of nitrosoureas (e.g. carmustine (BCNU, **52**)), nitrogen mustards (compounds **40**-**42**) and mitozolomide, respectively. Compounds **79**, **80** and **81** exhibit antiproliferative activity in the low μM range, potent anti-EGFR activity as well as potent DNA damaging effects.

1,2,3-Triazene (**53**) is a relatively new alkylating group entity and when connected to **51** (compounds **82**, **83** and **84** corresponding to ZRBA4, ZRL4 and ZRS1, respectively) it

generates methyldiazyne a powerful DNA methylating and damaging agent [38-41]. The first generation of this type of combi-molecules was physiologically unstable. Compounds **82**, **83** and **84** were designed to circumvent the unstable triazene moiety when linked to the quinazoline ring. They exhibit antiproliferative activity in the low μM range, and show EGFR inhibition and DNA damage effects.

Imatinib (**5**) is used in clinic to inhibit tyrosine-kinase Bcr-Abl in the treatment of several cancers notably the chronic myelogenous leukemia. Jean-Claude group's also used a similar approach to design new combi-molecules Bcr-Abl/DNA (compound **85**, ZRF1) by connecting the pharmacophore of **5** to 1,2,3-triazene (**53**) [42]. Compound **85** exhibits antiproliferative activity in the nM range and was slightly more active than imatinib alone. It shows well-balanced activity between strong Abl tyrosine kinase (TK) inhibitory activity and high levels of DNA damages.

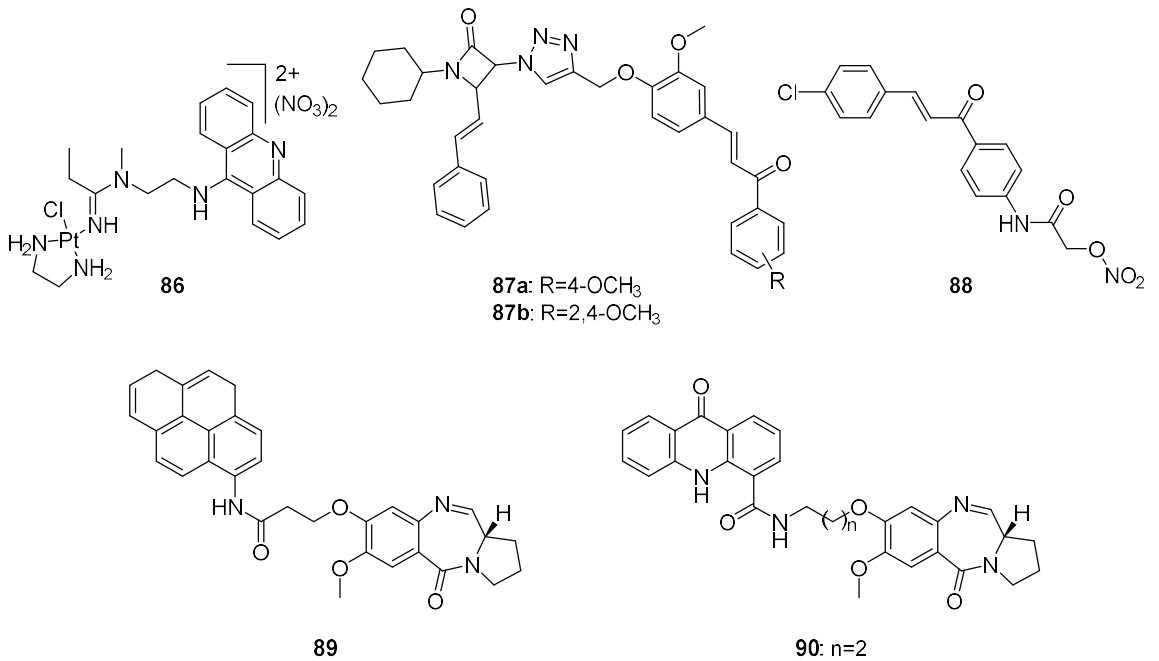


Figure 4b. Molecular structures of combi-molecules with different mechanisms of action.

Platinium complexes such as cisplatin (**54**), carboplatin (**55**) and oxaliplatin (**56**) are members of a class of platinum(II)-containing anticancer drugs and are widely used against various cancer types including ovarian carcinoma, lung, head and neck cancers. This class of anticancer drugs generates reactive platinum species that mainly crosslink DNA and inhibits ATM/ATR reparation pathways of cancer cells. Acridine (**57**) is another privileged molecular structure that is often used in the design of anticancer agents. Drugs designed using this planar molecular structure is recognized as potent DNA intercalating agents (e.g. amsacrine) or to inhibit topoisomerase (e.g. acridine carboxamide, DACA). The platinum–acridine anticancer agent **86** was prepared by linking the acridine moiety to a platinum(II) complex [43]. Compound **86** exhibits an antiproliferative activity in the low nM range and was approximately 100-fold more efficiently than cisplatin on chemoresistant non-small cell lung cancer (NCI-H460 and NCI-H522 cell lines). In addition, it arrests the cell cycle in S-phase, produces DNA adducts and displays important inhibition of DNA replication similarly as other known platinum(II) complexes.

1,2,3-Triazole tethered β-lactam-chalcone heterofunctional hybrids (compounds **87a**, **b**) were prepared using a click chemistry approach to merge the β-lactam **58** and the chalcone **22**, two molecular structures recognized as potent antiproliferative agents against various cancer cell lines [44]. Compounds **87a**, **b** show cytocidal activity in the μM range on A-549, THP-1 and Caco-2 cell lines.

Nitric oxide (NO) is an important endogen signaling mediator involved in a variety of biological processes including vasodilatation and vessel homeostasis. Moreover, evidence show that NO released via metabolic pathways also mediates anticancer activity and may prevent metastasis. Hence, NO donating-chalcone hybrids such as compound **88** were

designed by conjugating the nitric oxide (NO)-releasing properties of nitrate ester (ONO_2) moiety of chalcones (**22**) [45]. Compound **88** exhibits antiproliferative activity in the low μM range on NCI anticancer cell line panel.

The planar and semi-planar ring system of pyrene (**59**) and acridone (**60**) are known to intercalate in DNA and to show potent anticancer activity. Kamal *et al.* have conjugated both structures with PBD (**43**) to create new pyrene-linked pyrrolo [2,1-c][1,4]benzodiazepine (compound **89)** [46] and C8-linked pyrrolo[2,1-c][1,4]benzodiazepine-acridone (e.g. compounds **90**) hybrids [47]. Compounds **89** and **90** exhibit median lethal dose (LC_{50}) in the nM range on NCI anticancer cell line panel and display good DNA-binding property.

3.3 Combi-molecules in the aim to target specific biological tissues

The combi-molecule strategy aims to the vectorization of anticancer drugs to target specific tissues is also a promising method to design highly effective, non-toxic and useful hybrids. Unfavorable biopharmaceutical properties such as inadequate biodistribution, low tissue penetration, and poor tropism for cancer cells are major limitations that prevent most drugs to reach freely the cancer tissues or to be effective, despite high *in vitro* potency. This concept addresses this problematic by adding to an anticancer drug unable to biodistribute itself into the cancer tissue to be targeted, a second molecular fragment able to bioaccumulate into that specific tissue. Vectorized anticancer drugs are divided into three different categories: (i) combi-molecule based on the conjugation of steroids to an anticancer drug (Figure 5a), (ii) other combi-molecule targeting specific biological tissue

(Figure 5b), and (iii) combi-molecule targeting specific biological tissue and carrying a radionuclide (Figure 5c).

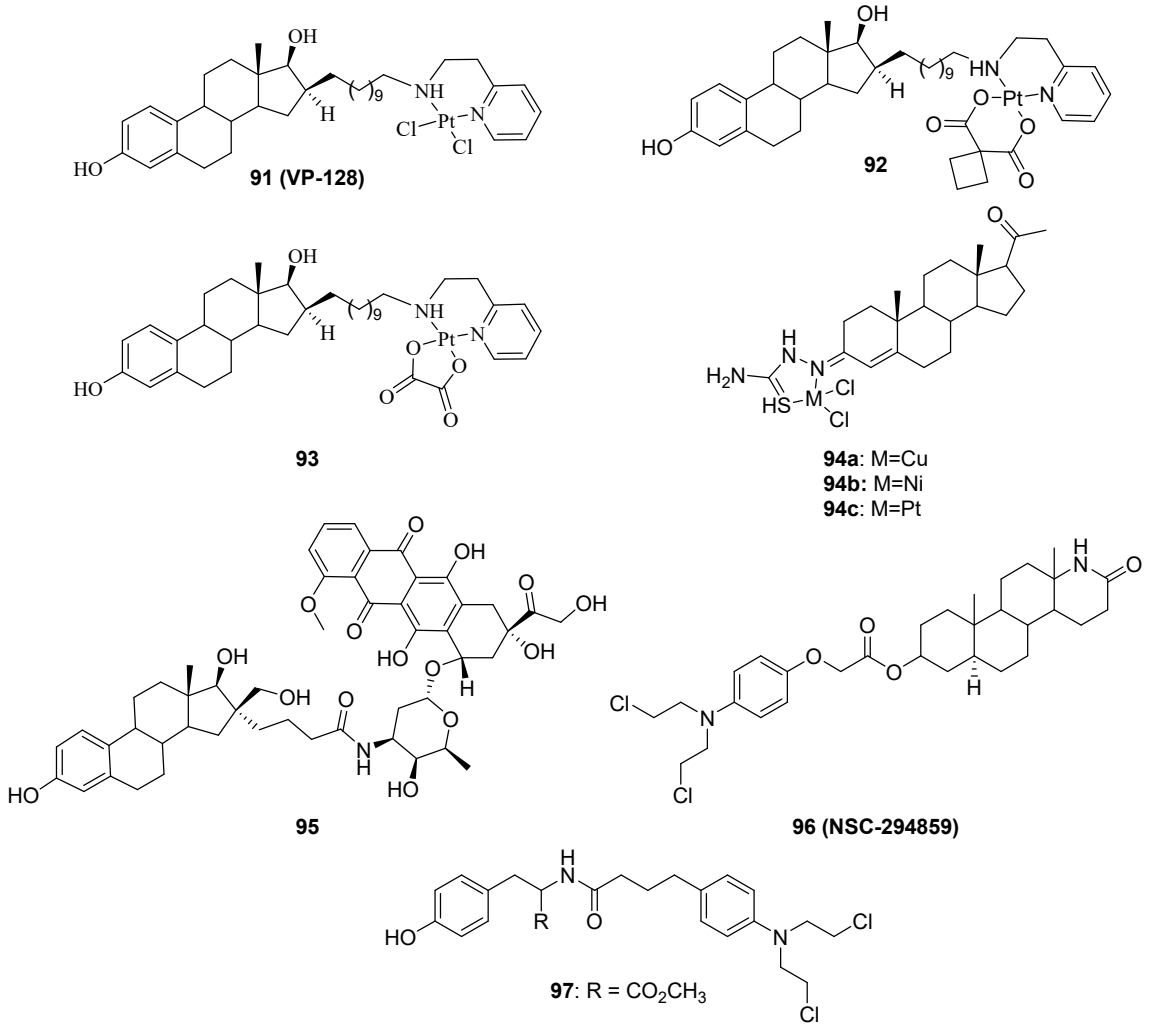


Figure 5a. Molecular structures of combi-molecules based on the conjugation of steroids to an anticancer drug.

Despite that combi-molecules of steroid-anticancer hybrids were thoroughly reviewed in 2012 by Dao *et al.* [48], we found that this subset of hybrids has been in recent years in constant evolution, regularly using new approaches, new combinations of molecules to produce molecular hybrids exhibiting pharmacokinetic and pharmacodynamic properties always closer to those required in clinic clearly justifying their presentation.

Over the years, Bérubé's group developed several anticancer hybrids where 17β-estradiol was linked to anticancer agents such as platinum-containing anticancer drugs and anthracyclines. Among all compounds they have prepared and biologically evaluated, compound **91** (VP-128) is one of their most promising hit compound [49]. Compound **91** exhibits antiproliferative activity in the low μM range and is approximately 2 to 8-fold more potent than cisplatin on estrogen-dependent and estrogen-independent breast cancer cell lines such as MCF-7 and MDA-MB-231, respectively. Moreover, it shows high affinity towards the estrogen receptor α, RNA and DNA and better tumor regression properties than cisplatin on the MCF-7 human breast cancer tumors mice model [50]. They also studied different platinum(II) complexes of **91** by replacing the dichloroplatinum(II) moiety by a cyclobutane-1,1-dicarboxylateplatinum(II) (carboplatin moiety, **55**) and oxalateplatinum(II) (oxaliplatin moiety, **56**) groups leading to compounds **92** and **93**, respectively [51]. Compound **93** shows 3- to 5-fold higher activity than **92** that is similar to cisplatin (**54**) and oxaliplatin (**56**). Interestingly, despite their closely related structures, **93** retains an excellent affinity for the estrogen receptor α while compound **92** loses it completely.

Progesterone is another essential steroidal hormone involved in various biological processes including control of mammary homeostasis (e.g. female menstrual cycle, decrease the maternal immune response), effect on the nervous system (e.g. affect synaptic functioning and myelination) and effect on several other systems. Adsule *et al.* prepared isothiocyanate–progesterone metal complexes (compounds **94a**-**c**) by merging isothiocyanate metal complexes to progesterone [52]. Compound **94a** bearing a copper complex showed antiproliferative activity in the low μM range on five cell lines whereas

the platinum(II) complex (**94c**) was 2- to 4-fold less active. The nickel complex (compound **94b**) shows also activity in the low μM range on breast cancer cell lines (MCF-7 and MDA-MB-231) and was inactive on BT20 and PC3 cancer cells through the inhibition of the Akt signaling pathway.

In the aim to reduce the cardiotoxicity of anthracyclines such as doxorubicin (**36a**), Bérubé's research group attempted to conjugate this anticancer agent to position 16α of the estrogen skeleton to generate compounds **95** [53]. Compound **95** shows antiproliferative activity in the μM range on HT-29 and MCF-7 cell lines and is inactive on M21 and MDA-MB-231 cells, and exhibits affinity for the estrogen receptor α in the nanomolar range. However, the antiproliferative activity of this compound is about 100-fold lower than **36a**, suggesting that the molecular conformation is not optimal to keep the affinity for the estrogen receptor and the cytocidal activity at the same time.

Trafalis *et al.* used homo-azasteroids (**61**), which contain an amide group inside the A or the D ring of steroid scaffold, to link aromatic nitrogen mustards (**40**-**42**) leading to homo-aza-steroidal alkylating esters such as compound **96**, NSC-294859 [54]. Compound **96** shows antiproliferative activity in the μM range on malignant melanoma cell lines and induces significant cytostatic and antineoplastic effects on B16 melanoma-bearing mice.

Compound **97** is a hybrid connecting also aromatic nitrogen mustards (**40**-**42**) and tyrosine that was designed to mimic the estradiol nucleus [55]. Compound **97** exhibits antiproliferative activity in the μM range on prostate, breast, ovarian and uterine cancer cell lines and was slightly more active than chlorambucil (**41**).

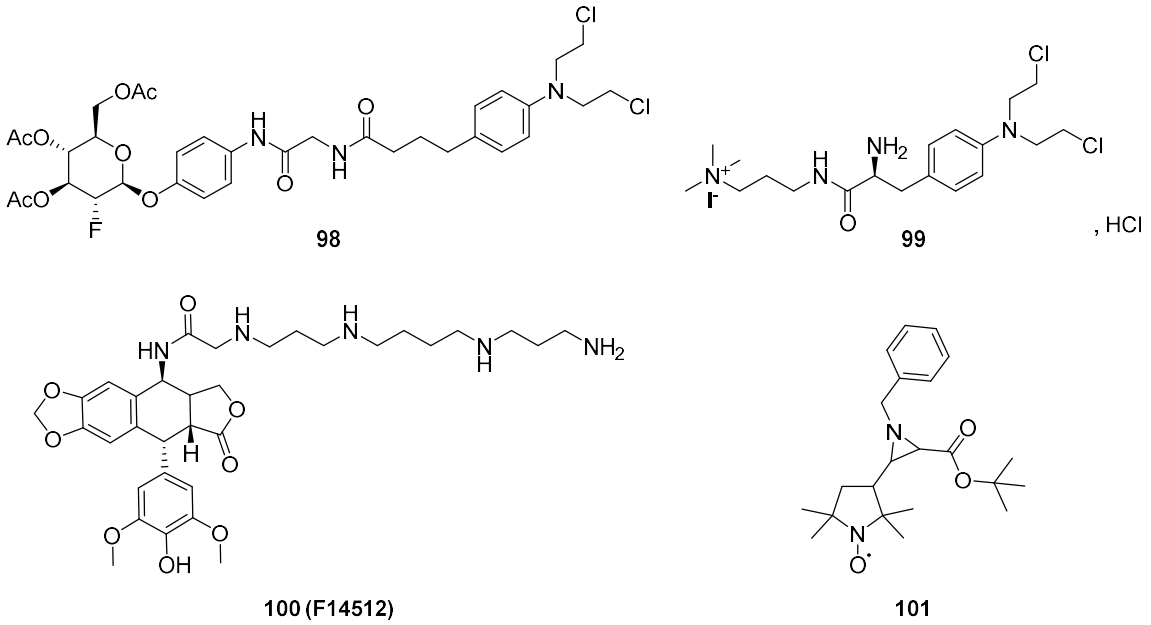


Figure 5b. Molecular structures of other combi-molecules targeting specific biological tissue.

^{18}F-Fluorodeoxyglucose (compound **62**) is a radiopharmaceutical used for medical imaging based on its accumulation in high-glucose-using cells such as brain, kidney, and cancer cells. Fluorodeoxyglucose-chlorambucil hybrid (compound **98**) was designed by connecting fluorodeoxyglucose (**62**) and chlorambucil (**41**) [56]. Compound **98** shows antiproliferative activity in μM range and was approximately 10-fold more active than chlorambucil (**41**) on B16F0 and CT-26 cell lines. It arrests the cell cycle progression in G2-phase and was highly active *in vivo* in the CT-26 colon carcinoma and B16F0 melanoma mice models, with log cell kill values of l.52 and 1.81, respectively.

The design of the quaternary ammonium-melphalan hybrid (compound **99**) is based on the high affinity of positively-charged quaternary ammonium function for proteoglycans (PG), a component of the chondrogenic extracellular matrix of cartilages was designed to transport melphalan selectively (**42**) to cartilage tumor tissues [57]. Compound **99** shows

antiproliferative activity in the μM range on Saos-2 human osteosarcoma and HEMCSS human chondrosarcoma cell lines while it is inactive against chondrocytes and M4Beu human melanoma cell lines. It arrests the cell cycle progression in S-phase and it is well tolerated in animals. Compound **99** also inhibits tumor cell growth *in vivo* in orthotopic model of primary Swarm rat chondrosarcoma.

On one hand, etoposide (**63**) is a potent semi-synthetic topoisomerase II inhibitor deriving from podophyllotoxin. On the other hand, the polyamines transport system is frequently expressed in cancer cells. The polyamine-etoposide hybrid **100** (F14512) was designed by replacing the C4 glycosidic moiety of etoposide by a sperminyl chain in the aim to exploit the polyamines transport system to favor its accumulation into the cancer cells [58]. Compound **100** displays antiproliferative activity on a large panel of tumor cell lines and are more active than **63** [59, 60]. Moreover, compound **100** is more active on cells expressing polyamines transport system and induces cell death by non-apoptotic and senescence-type pathways.

Aziridine (**64**) is another privileged cytocidal molecular structure found in mitomycin C and tiotepa and exhibiting interesting anticancer properties. Nitroxyl–aziridine hybrid (compound **101**) was designed by linking the aziridinyl to nitroxyl moiety (compound **65**), a stable radical species recognized for its selective accumulation in mice melanotic melanomas [61]. Despite that the authors claimed that the cytotoxicity of this hybrid is not observed, no data are shown to support this statement.

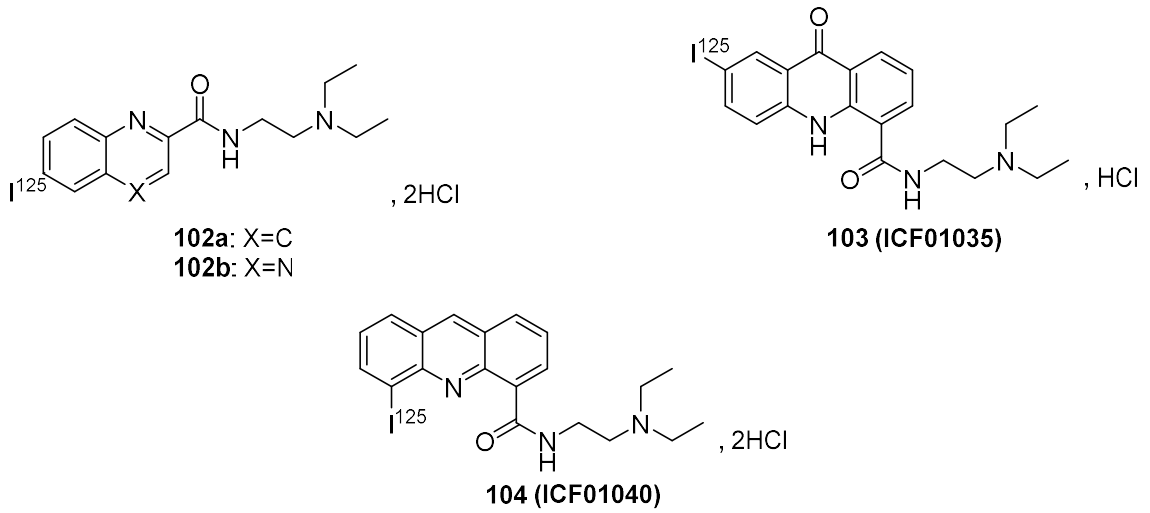


Figure 5c. Molecular structures of combi-molecules targeting specific biological tissue and carrying radionuclide ^{125}I.

4-Iodobenzamide (BZA, **66**) is a family of anticancer agents that interacts with melanin and shows specific affinity primary and metastasis malignant melanoma tumors. Unit 484 at the INSERM in Clermont-Ferrand (France) has developed several anticancer hybrids that target malignant melanoma tumors (compounds **102**-**104**) by connecting analogs of **66** to iodine-125 (^{125}I-**66**). ^{125}I emits Auger electron that causes double-strand breaks in DNA and has a half-life around 59 days and its high-energy emission results in tissue low penetration (nm). Consequently, this radionuclide must be located within the cancer cells for radiotherapeutic effectiveness and suggests low toxicity for the surrounding healthy tissues. Commercial availability, easy labelling, and good physical properties select ^{125}I as a promising radioisotope for vectorized radionuclide therapy.

Several polycyclic heteroaromatic compounds such as chloroquine and acridine orange have been previously shown to bind strongly to melanin. Compounds **102a**, **b** were designed by connecting molecular structure of **66** merged with heteroaromatic molecular structure and ^{125}I [62]. Compounds **102a**, **b** bind to melanin, exhibit specific affinity for

melanoma tumors in mice bearing melanoma tumors and show significantly higher tumoral uptakes than ^{125}I-**66** 72 h post administration.

Acridone-radionuclide (ICF01035, **103**) and acridine-radionuclide hybrids (ICF01040, **104**) were designed by connecting acridone (**60**) and acridine (**57**) to ^{125}I [63, 64]. Acridone (**60**) and acridine (**57**) are known to intercalate into DNA strains and to show potent anticancer activity. Moreover, some acridines and acridones such as DACA (NSC 601316) bearing a basic side chain similar to BZA (**66**) bind strongly to synthetic melanins and show antiproliferative activity on melanoma cell lines, and exhibit antitumor activity on tumor xenografts in mice. Consequently, this design appears suitable for application in vectorized radionuclide therapy. Compounds **103** and **104** bind to melanin and biodistribution studies on B16F0 murine melanoma tumour-bearing mice show specific, high and sustained *in vivo* tumor concentrations which is more than 10-fold longer than ^{125}I-**66**.

4. Conclusion

This review shows the various fashions by which a hybrid anticancer agent can be designed. We classified the hybrids into several categories according to their construct, same or different drugs and their intended targets (or mechanism(s) of action) being single, multiple or simply as vectorized hybrids. Hence, there are numerous ways to make hybrids. This area of research presents great interest to the scientific community and is in constant expansion. We have focused only on the most recent advances in the field. It is obvious that interesting results are obtained by the construction of hybrids. In many cases, not only the biological activity is enhanced but also the selectivity is improved and the toxicities diminished. It is also clear that the hybrid themselves, its entire structure, can provide

additional biological properties that only the combination of drugs can do. This review shows the feasibility and great potential of the hybrid approach. The final goal is to further improve the design of the hybrid anticancer agents. This goal is in our reach. To conclude, imagination and creativity are key elements to construct a successful hybrid anticancer agent.

5. Expert Opinion

This review presents the latest development in the design of hybrid and combi-molecule anticancer drugs. The overall objective underlying their development is to build molecules merging two molecular fragments recognized for their individual anticancer activity towards definite targets in the cancer cells to achieve improved activity, selectivity and toxicity of anticancer agents in the aim to reduce the incidence, the mortality and morbidity outcomes of that disease. It is clear that a single agent having the capacity of interacting with several key biological targets in the cell might show significant and even synergetic anticancer properties than targeting single biological target.

The general strategy for the design of such hybrid anticancer drugs is relatively straightforward. Firstly, it involves the structure of known anticancer agent by blending it or simply linking it to a carrier molecule (or another anticancer drug) that target cancer cells more efficiently via a receptor or specific cellular processes leading to improved and even synergized biological properties. There are a number of potential advantages of hybrid anticancer drugs for the patient. Firstly, they have the ability to increase the specificity and strengthen the potency of the anticancer agents. Furthermore, they allow a reduction of the dose and the toxic side effects due to the treatment and offer a synergy between multiple anticancer mechanisms in the cells leading to apoptosis. Additionally, hybrid anticancer

drugs allow for a reduction of the induction of chemoresistance mechanisms in tumor and increase the chance of successful treatment.

It should be said that the requirements to design such a hybrid drug are quite evident as not only do they have the potential for high affinity towards the targeted receptor(s) or targeted molecular mechanism(s) but they also have the potential for *in vitro* and *in vivo* selectivity on cancer cells. There is also an easiness of synthesis (few and efficient chemical steps are crucial for future commercial development as well as the potential for large-scale production. It should also be said that there is, as always, the potential for intellectual property (IP) protection.

In addition, it must be borne in mind that the future success for the drug targeting approaches will be the use of "blended" or "fully-integrated" chemical structures such as those described for example by Gleason [15-17] *et al.* as well as Jean-Claude *et al.* [36, 37, 39-41, 65-68] and many others. The concept of "fully-integrated" molecules has great potential because it can be constructed with an appropriate molecular size and physicochemical properties and thus, it can be more easily accessible and "druggable" substances.

However, the use of "vectorized" anticancer hybrids shows interesting potential applications. Recent successes were obtained by several research teams. In the future, the best results should arise from compounds that can release the biological active anticancer component at the appropriate location in the tissue and in the cell, as well as the carrier molecule that can act simultaneously by triggering either agonistic or antagonistic actions and thereby enhancing the overall biological effect(s) of the anticancer component.

So, the future directions for the construct of hybrid anticancer molecules can be divided into two main categories: “fully-integrated” and “vectorized” hybrids. The goal of these hybrid anticancer molecules is to obtain derivatives that will act synergistically on cancer cells. Clearly, the “fully-intagrated” hybrids present great potential as they often lead to relatively smaller hybrids and thus more drug-like compounds [69]. However, the amalgamation of two (or more) different components retaining their respective biological properties can be challenging. This strategy is at its infancy and needs to be further investigated. On the other hand, the synthesis of “vectorized” hybrids is relatively easier. But, yet again the retention of the properties of the carrier moiety as well as the anticancer moiety can be difficult. In the future, we will see refined design where the carrier molecule has high affinity for its cognate receptor and where the anticancer moiety possesses higher biological activity than the parent drug itself. Such combination will lead to new, more selective and more efficient anticancer therapies devoid of side effects. Finally, successful development of a hybrid drug will also be achieved following the requirements of design described above. Obviously, work is in progress in this field and the future is bright. Only imagination is the limit to drug development.

Declaration of Interest

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References

Papers of special note have been highlighted as either of interest (*) or of considerable interest (**) to readers.

1. Gediya LK, Njar VC. Promise and challenges in drug discovery and development of hybrid anticancer drugs. Expert Opin Drug Discov 2009;4(11):1099-111.

** Important review on hybrid anticancer drugs

2. Viegas-Junior C, Danuello A, da Silva Bolzani V, *et al.* Molecular hybridization: a useful tool in the design of new drug prototypes. Curr Med Chem 2007;14(17):1829-52.

** Relevant review on hybrid anticancer drugs

3. Fox BM, Xiao X, Antony S, *et al.* Design, synthesis, and biological evaluation of cytotoxic 11-alkenylindenoisoquinoline topoisomerase I inhibitors and indenoisoquinoline-camptothecin hybrids. J Med Chem 2003;46(15):3275-82.

4. Nguyen HT, Lallemand MC, Boutefnouchet S, *et al.* Antitumor psoropermum xanthones and sarcomelicope acridones: Privileged structures implied in DNA alkylation. J Nat Prod 2009;72(3):527-39.

* Review on psoropermum xanthones and sarcomelicope acridones

5. Wang D, Zhang Z, Lu X, *et al.* Hybrid compounds as new Bcr/Abl inhibitors. Bioorg Med Chem Lett 2011;21(7):1965-68.

6. Lee KW, Kim JH, Park JH, *et al.* Antitumor activity of SK-7041, a novel histone deacetylase inhibitor, in human lung and breast cancer cells. Anticancer Res 2006;26(5 A):3429-38.

7. Florence GJ, Gardner NM, Paterson I. Development of practical syntheses of the marine anticancer agents discodermolide and dictyostatin. Nat Prod Rep 2008;25(2):342-75.

* Review on discodermolide and dictyostatin

8. Paterson I, Naylor GJ, Fujita T, *et al.* Total synthesis of a library of designed hybrids of the microtubule-stabilising anticancer agents taxol, discodermolide and dictyostatin. Chem Commun 2010;46(2):261-63.

9. Paterson I, Naylor GJ, Wright AE. Total synthesis of a potent hybrid of the anticancer natural products dictyostatin and discodermolide. Chem Commun 2008;(38):4628-30.

10. Meunier B. Hybrid molecules with a dual mode of action: dream or reality? Acc Chem Res 2008;41(1):69-77.

11. Singh P, Paul K. Studies of interactions between uracil-based hybrid molecules and P-glycoprotein-Search for multidrug resistance modulators. Bioorg Med Chem 2006;14(21):7183-86.

12. Belluti F, Fontana G, Bo LD, *et al.* Design, synthesis and anticancer activities of stilbene-coumarin hybrid compounds: Identification of novel proapoptotic agents. Bioorg Med Chem 2010;18(10):3543-50.

13. Nordenberg J, Fenig E, Landau M, *et al.* Effects of psychotropic drugs on cell proliferation and differentiation. Biochem Pharmacol 1999;58(8):1229-36.

14. Guandalini L, Cellai C, Laurenzana A, *et al.* Design, synthesis and preliminary biological evaluation of new hydroxamate histone deacetylase inhibitors as potential antileukemic agents. Bioorg Med Chem Lett 2008;18(18):5071-74.

15. Tavera-Mendoza LE, Quach TD, Dabbas B, *et al.* Incorporation of histone deacetylase inhibition into the structure of a nuclear receptor agonist. Proc Natl Acad Sci USA 2008;105(24):8250-5.

16. Lamblin M, Dabbas B, Spingarn R, *et al.* Vitamin D receptor agonist/histone deacetylase inhibitor molecular hybrids. Bioorg Med Chem 2010;18(11):4119-37.

* Vitamin D-HDAC hybrids with interesting activity

17. Fischer J, Wang TT, Kaldre D, *et al.* Synthetically accessible non-secosteroidal hybrid molecules combining vitamin D receptor agonism and histone deacetylase inhibition. Chem Biol 2012;19(8):963-71.

18. Mizuno CS, Paul S, Suh N, *et al.* Synthesis and biological evaluation of retinoid-chalcones as inhibitors of colon cancer cell growth. Bioorg Med Chem Lett 2010;20(24):7385-87.

19. Sashidhara KV, Kumar A, Kumar M, *et al.* Synthesis and in vitro evaluation of novel coumarin-chalcone hybrids as potential anticancer agents. Bioorg Med Chem Lett 2010;20(24):7205-11.

20. Bednarek E, Bocian W, Sitkowski J, *et al.* 1H and 13C NMR data for indolo[2,3-b]quinoline- aminoglycoside hybrids, a novel potent anticancer drug family. Magn Reson Chem 2006;44(4):459-62.

21. Kumar R, Gupta L, Pal P, *et al.* Synthesis and cytotoxicity evaluation of (tetrahydro-β-carboline)-1,3,5-triazine hybrids as anticancer agents. Eur J Med Chem 2010;45(6):2265-76.

22. Kolesinska B, Barszcz K, Kaminski ZJ, *et al.* Synthesis and cytotoxicity studies of bifunctional hybrids of nitrogen mustards with potential enzymes inhibitors based on melamine framework. J Enzyme Inhib Med Chem 2012;27(5):619-27.

23. Gerratana B. Biosynthesis, synthesis, and biological activities of pyrrolobenzodiazepines. Med Res Rev 2012;32(2):254-93.

* Relevant review on pyrrolobenzodiazepines

24. Wang JJ, Shen YK, Hu WP, *et al.* Design, synthesis, and biological evaluation of pyrrolo[2,1-c][1,4] benzodiazepine and indole conjugates as anticancer agents. J Med Chem 2006;49(4):1442-49.

25. Ismail MMF, Rateb HS, Hussein MMM. Synthesis and docking studies of novel benzopyran-2-ones with anticancer activity. Eur J Med Chem 2010;45(9):3950-59.

26. Ahmed K, Matsuya Y, Nemoto H, *et al.* Mechanism of apoptosis induced by a newly synthesized derivative of macrosphelides with a thiazole side chain. Chem-Biol Interact 2009;177(3):218-26.

27. Matsuya Y, Kobayashi Y, Kawaguchi T, *et al.* Design, synthesis, and biological evaluation of artificial macrosphelides in the search for new apoptosis-inducing agents. Chem-Eur J 2009;15(23):5799-813.

* Artificial macrosphelide hybrids with interesting biological properties

28. Havrylyuk D, Kovach N, Zimenkovsky B, *et al.* Synthesis and anticancer activity of isatin-based pyrazolines and thiazolidines conjugates. Arch Pharm 2011;344(8):514-22.

29. Kumar K, Sagar S, Esau L, *et al.* Synthesis of novel 1H-1,2,3-triazole tethered C-5 substituted uracil-isatin conjugates and their cytotoxic evaluation. Eur J Med Chem 2012;58:153-159.

30. Singh P, Sharma P, Anand A, *et al.* Azide-alkyne cycloaddition en route to novel 1H-1,2,3-triazole tethered isatin conjugates with in vitro cytotoxic evaluation, Eur J Med Chem 2012;55:455-61.

31. Kamal A, Bharathi EV, Reddy JS, *et al.* Synthesis and biological evaluation of 3,5-diaryl isoxazoline/isoxazole linked 2,3-dihydroquinazolinone hybrids as anticancer agents. Eur J Med Chem 2011;46(2):691-703.

32. Xie L, Zhao Y, Zhai X, *et al.* The application of tandem aza-wittig reaction to synthesize artemisinin-guanidine hybrids and their anti-tumor activity. Arch Pharm 2011;344(10):631-38.

33. Bastien D, Leblanc V, Asselin E, *et al.* First synthesis of separable isomeric testosterone dimers showing differential activities on prostate cancer cells. Bioorg Med Chem Lett 2010;20(7):2078-81.

34. Domarkas J, Dudouit F, Williams C, *et al.* The combi-targeting concept: synthesis of stable nitrosoureas designed to inhibit the epidermal growth factor receptor (EGFR). J Med Chem 2006;49(12):3544-52.

35. Golabi N, Brahimi F, Huang Y, *et al.* A bioanalytical investigation on the exquisitely strong in vitro potency of the EGFR-DNA targeting type II combi-molecule ZR2003 and its mitigated in vivo antitumour activity. J Pharm Biomed Anal 2011;56(3):592-9.

36 Watt HL, Rachid Z, Jean-Claude BJ. The concept of divergent targeting through the activation and inhibition of receptors as a novel chemotherapeutic strategy: signaling responses to strong DNA-reactive combinatorial mimicries. J Signal Transduction 2012;2012:282050.

37. Fang Y, Qiu Q, Domarkas J, *et al.* "Combi-targeting" mitozolomide: conferring novel signaling inhibitory properties to an abandoned DNA alkylating agent in the treatment of advanced prostate cancer. The Prostate 2012;72(12):1273-85.

38. Rachid Z, Brahimi F, Qiu Q, *et al.* Novel nitrogen mustard-armed combi-molecules for the selective targeting of epidermal growth factor receptor overexperessing solid tumors: discovery of an unusual structure-activity relationship. J Med Chem 2007;50(11):2605-8.

39. MacPhee M, Rachid Z, Todorova M, *et al.* Characterization of the potency of epidermal growth factor (EGFR)-DNA targeting combi-molecules containing a hydrolabile carbamate at the 3-position of the triazene chain. Invest New Drugs 2011;29(5):833-45.

40. Golabi N, Rachid Z, Qiu Q, *et al.* In vitro and in vivo biodistribution of ZRS1, a stabilized type I N-acetoxymethyl carbamate-containing combi-molecule. Drug Metab Lett 2011;5(2):141-9.

41. Huang Y, Rachid Z, Jean-Claude BJ. MGMT is a molecular determinant for potency of the DNA-EGFR-combi-molecule ZRS1. Mol Cancer Res 2011;9(3):320-31.

* DNA-EGFR-combi-molecule showing important biological activity

42. Rachid Z, Katsoulas A, Williams C, *et al.* Optimization of novel combi-molecules: identification of balanced and mixed bcr-abl/DNA targeting properties. Bioorg Med Chem Lett 2007;17(15):4248-53.

43. Smyre CL, Saluta G, Kute TE, *et al.* Inhibition of DNA synthesis by a platinum-acridine hybrid agent leads to potent cell kill in nonsmall cell lung cancer. ACS Med Chem Lett 2011;2(11):870-74.

44. Singh P, Raj R, Kumar V, *et al.* 1,2,3-Triazole tethered β-lactam-Chalcone bifunctional hybrids: Synthesis and anticancer evaluation. Eur J Med Chem 2012;47(1):594-600.

45. Mourad MAE, Abdel-Aziz M, Abuo-Rahma GEDAA, *et al.* Design, synthesis and anticancer activity of nitric oxide donating/chalcone hybrids. Eur J Med Chem 2012;54:907-13.

46. Kamal A, Ramesh G, Srinivas O, *et al.* Synthesis and antitumour activity of pyrene-linked pyrrolo [2,1-c][1,4]benzodiazepine hybrids. Bioorg Med Chem Lett 2004;14(2):471-74.

47. Kamal A, Srinivas O, Ramulu P, *et al.* Synthesis of C8-linked pyrrolo[2,1-c][1,4]benzodiazepine-acridone/acridine hybrids as potential DNA-binding agents. Bioorg Med Chem Lett 2004;14(15):4107-11.

* Hybrids with biological activity in the nM range and displaying good DNA-binding properties

48. Dao KL, Hanson RN. Targeting the estrogen receptor using steroid-therapeutic drug conjugates (hybrids). Bioconjug Chem 2012;23(11):2139-58.

** Very important review on steroid-based hybrids

49. Descôteaux C, Leblanc V, Bélanger G, *et al.* Improved synthesis of unique estradiol-linked platinum(II) complexes showing potent cytocidal activity and affinity for the estrogen receptor alpha and beta. Steroids 2008;73(11):1077-89.

50. Van Themsche C, Parent S, Leblanc V, *et al.* VP-128, a novel oestradiol-platinum(II) hybrid with selective anti-tumour activity towards hormone-dependent breast cancer cells *in vivo*. Endocr-relat cancer 2009;16(4):1185-95.

* Estradiol-Pt(II) hybrid showing selective *in vivo* biological activity

51. Saha P, Descoteaux C, Brasseur K, *et al.* Synthesis, antiproliferative activity and estrogen receptor α affinity of novel estradiol-linked platinum(II) complex analogs to

carboplatin and oxaliplatin. Potential vector complexes to target estrogen-dependent tissues. Eur J Med Chem 2012;48:385-90.

* A rare example of estradiol-oxaliplatin analogs with good biological activity

52. Adsule S, Banerjee S, Ahmed F, *et al.* Hybrid anticancer agents: Isothiocyanate-progesterone conjugates as chemotherapeutic agents and insights into their cytotoxicities. Bioorg Med Chem Lett 2010;20(3):1247-51.

53. Saha P, Fortin S, Leblanc V, *et al.* Design, synthesis, cytocidal activity and estrogen receptor α affinity of doxorubicin conjugates at 16α-position of estrogen for site-specific treatment of estrogen receptor positive breast cancer. Steroids 2012;77(11):1113-22.

54. Trafalis DTP, Camoutsis C, Papageorgiou A. Research on the anti-tumour effect of steroid lactam alkylator (NSC-294859) in comparison with conventional chemotherapeutics in malignant melanoma. Melanoma Res 2005;15:273-81.

55. Descôteaux C, Brasseur K, Leblanc V, *et al.* Design of novel tyrosine-nitrogen mustard hybrid molecules active against uterine, ovarian and breast cancer cell lines. Steroids 2012;77(5):403-12.

56. Miot-Noirault E, Reux B, Debiton E, *et al.* Preclinical investigation of tolerance and antitumour activity of new fluorodeoxyglucose-coupled chlorambucil alkylating agents. Invest New Drugs 2011;29(3):424-33.

57. Peyrode C, Weber V, David E, *et al.* Quaternary ammonium-melphalan conjugate for anticancer therapy of chondrosarcoma: in vitro and in vivo preclinical studies. Invest New Drugs 2012;30(4):1782-90.

** Important quaternary ammonium-melphalan conjugate targeting extracellular matrix of cartilage exhibiting good *in vitro* and *in vivo* activities

58. Brel V, Annereau JP, Vispe S, *et al.* Cytotoxicity and cell death mechanisms induced by the polyamine-vectorized anti-cancer drug F14512 targeting topoisomerase II. Biochem Pharmacol 2011;82(12):1843-52.

59. Barret JM, Kruczynski A, Vispe S, *et al.* F14512, a potent antitumor agent targeting topoisomerase II vectored into cancer cells via the polyamine transport system. Cancer Res 2008;68(23):9845-53.

60. Kruczynski A, Vandenberghe I, Pillon A, *et al.* Preclinical activity of F14512, designed to target tumors expressing an active polyamine transport system. Invest New Drugs 2011;29(1):9-21.

61. Kumamoto T, Suzuki K, Kim SK, *et al.* Synthesis, physical properties, and cytotoxicity of Nitroxyl-Aziridine hybrid. Helv Chim Acta 2010;93(11):2109-14.

62. Chezal JM, Papon J, Labarre P, *et al.* Evaluation of radiolabeled (hetero)aromatic analogues of N-(2-diethylaminoethyl)-4-iodobenzamide for imaging and targeted radionuclide therapy of melanoma. J Med Chem 2008;51(11):3133-44.

63. Desbois N, Gardette M, Papon J, *et al.* Design, synthesis and preliminary biological evaluation of acridine compounds as potential agents for a combined targeted chemo-radionuclide therapy approach to melanoma. Bioorg Med Chem 2008;16(16):7671-90.

* Potent acridine-radionuclide hybrid showing long-lasting effects in the tumor and favorable pharmacokinetic profile

64. Gardette M, Papon J, Bonnet M, *et al.* Evaluation of new iodinated acridine derivatives for targeted radionuclide therapy of melanoma using 125I, an Auger electron emitter. Invest New Drugs 2011;29(6):1253-63.

65. Banerjee R, Huang Y, Qiu Q, *et al.* The combi-targeting concept: mechanism of action of the pleiotropic combi-molecule RB24 and discovery of a novel cell signaling-based combination principle. Cellular Signalling 2011;23(4):630-40.

66. Khan H, Badshah A, Murtaz G, *et al.* Synthesis, characterization and anticancer studies of mixed ligand dithiocarbamate palladium(II) complexes. Eur J Med Chem 2011;46(9):4071-7.

67. Al-Safadi S, Domarkas J, Han Y, *et al.* Enhancement of the cytotoxic potential of the mixed EGFR and DNA-targeting 'combi-molecule' ZRBA1 against human solid tumour cells by a bis-quinazoline-based drug design approach. Anti-Cancer Drugs 2012;23(5):483-93.

68. Larroque-Lombard AL, Ning N, Rao S, *et al.* Biological effects of AL622, a molecule rationally designed to release an EGFR and a c-Src kinase inhibitor. Chem Biol Drug Des 2012;80(6):981-91.

69. Keller TH, Pichota A, Yin Z. A practical view of 'druggability'. Curr Opin Chem Biol 2006;10:357-61.