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Dimeric Approaches to Anti-Cancer Chemotherapeutics

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Abstract

Numerous proteins responsible for cell proliferation and differentiation exist either as hetero or homodimers or become activated through dimerization as a key step in their respective signaling cascade. Many of these proteins have been identified as major components in oncogenic signaling pathways and have become popular targets for the development of anti-tumor agents. For this reason, bivalent anti-cancer drugs that could potentially interact with each monomer of a dimeric protein target have been developed. This review provides a brief background on prevalent dimeric drug targets within the anti-cancer field and focuses mainly on dimeric natural product and synthetic cancer chemotherapeutics.

Keywords

Anti-cancer; dimer; natural product dimers; synthetic dimers; bisintercalators

INTRODUCTION

Advantages associated with the inhibition of a homodimeric protein structure were first postulated by Monod *et al*. in 1965 [1]. Their argument emphasized that isologous associations usually result in a ‘closed structure’ that maintains intrinsic symmetry and enhanced stability. In addition, they suggested that a rapid organization of monomeric subunits into an oligomeric species could potentially prevent the random association of its subunits with other cellular proteins. A more recent review into the dimeric nature of proteins found that of all the oligomeric proteins identified (as of 2004) approximately 50% exist as homodimers [2]. Several reasons for the potential advantage of dimeric proteins were discussed: genetic saving, functional gain, and structural advantage. These authors agreed that an essential advantage for the dimeric proteins is their rapid assembly within the cell. In addition, several oncogenic signaling pathways are mediated by the formation of dimeric proteins that ultimately lead to cell proliferation.

DIMERIC PROTEIN TARGETS

The human genome contains 58 receptor-type tyrosine kinases (RTKs) and 32 non-receptor types as essential components of cellular signal transduction pathways [3]. These enzymes catalyze the transfer of a single phosphoryl group from ATP to a tyrosine phenol located on their protein substrate and play a key role in cellular growth, differentiation, metabolism, cell proliferation and differentiation. In general, extracellular ligand binding either induces or stabilizes receptor dimerization, resulting in RTK kinase activity [4]. In nonmalignant cells, cellular signaling through RTK mediation is tightly controlled and coordinated. Deregulation

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of the RTK signaling system causes aberations in kinase activity, which generally results in enhanced signaling capacity and malignant transformation. The overexpression of RTKs in many cancer cells can increase the response of these cells to normal growth factor levels and increase the potential of dimerization even in the absence of ligand, leading to uncontrolled cell growth [5]. The growth factor families of RTKs; including epidermal growth factor (EGF), fibroblast growth factor (FGF), *RET*, insulin-like growth factor (IGF), and vascular endothelial growth factor are particularly important due to their role in maintaining controlled cell growth [reviewed in 5].

The 90 kDa heat shock proteins (Hsp90) are molecular chaperones responsible for the conformational maturation of nascent polypeptides and the refolding of denatured proteins into biologically active tertiary and quaternary structures [6]. Hsp90 is a homodimer that binds nascent polypeptides, various co-chaperones and immunophilins to form a heteroprotein complex. This activated multiprotein complex binds ATP, “clamps” around the client protein, and uses subsequent ATP hydrolysis to promote folding of the client protein substrate [7]. Client proteins dependent upon Hsp90 have been implicated in all six hallmarks of cancer and inhibitors of Hsp90 provide a combinatorial attack on multiple signaling pathways responsible for malignant cell growth [8,9]. Many of these Hsp90 client proteins are independently sought after chemotherapeutic targets, such as Bcr-Abl kinase, telomerase, Src family kinases, Raf, ErbB2 (Her-2), PLK, protein kinase B (AKT), MET (kinase), focal adhesion kinase, death domain kinases, hypoxia inducible factor 1α(Hif), centrosomal proteins, and the steroid hormone receptors [8]. As a consequence of Hsp90 inhibition, these oncogenic proteins are uniformly degraded and may result in the administration of one drug for the treatment of cancer instead of cocktails consisting of multiple drugs to inhibit one or more of these individual targets. Hsp90 inhibitors are currently being tested in more than 20 phase I clinical trials for the treatment of a wide range of cancers [10].

DNA topoisomerases are ubiquitous enzymes responsible for relieving torsional stress in the supercoiled DNA helix, which is generated through the normal processes of replication, transcription, and recombination [11]. Type I enzymes (monomers) cleave a single strand of duplex DNA, whereas type II enzymes (homodimers) cleave both strands. The mechanism of action for topoisomerase II inhibitors relies upon their ability to enhance double-stranded DNA breaks by stabilization of the intermediate topoisomerase II-DNA covalent complex during the normal catalytic cycle. This shifts the cleavage/religation equilibrium towards strand lesions, resulting in an increased number of DNA breaks and eventual cell death [12]. Compounds that inhibit the enzyme *via* this mechanism are commonly referred to as topoisomerase poisons. Several well-known, clinically effective anti-cancer chemotherapeutics are topoisomerase II inhibitors, including the podophyllotoxins, etoposide and teniposide, and the anthracycline, doxorubicin.

The signal transducer and activator of transcription (STAT) family of proteins is an emerging target for the development of novel anti-tumor agents. These transcription factors mediate numerous physiological processes including cell growth and differentiation [13]. Extracellular ligand binding to cytokine or growth factor receptors promotes activation of the STATs through phosphorylation of a critical tyrosine residue in the SH2 domain of the monomer, resulting in dimerization and translocation to the nucleus [13]. Under normal conditions, STAT activition is tightly controlled. In malignant cells, however, constitutive STAT3 activity results in deregulated growth and angiogenesis [14,15]. Direct disruption of dimerization in the STAT signaling cascade has been demonstrated to induce transformed cell death and tumor regression [16,17].

Microtubules are heterodimeric polymers of α and β tubulin that are arranged along a cylindrical axis. They are a major structural component of the cytoskeleton and are critical for

the maintenance of cell shape and polarity. During the cell cycle, microtubules form the mitotic spindle to align replicated chromosomes and mediate segregation of chromosomes into daughter cells [18]. In malignant cells, microtubule-interacting drugs act as spindle poisons, blocking the cell cycle in the M phase and inducing apoptosis. There are three well-established drug binding sites on β tubulin, the vinca domain, the taxane site, and the colchicine site [18]. The dynamic heterodimeric nature of microtubules and the clinical success of microtubule stabilizers/destabilizers suggest they will continue to be an important target for the development of anti-cancer therapeutics [19].

NATURAL PRODUCT DIMERS

Rationale behind the development of dimeric compounds as drug candidates stems from their potential to bind two distinct individual binding sites on a single receptor or a defined site on two separate monomers of a dimeric protein. Confining the free pharmacophore of a univalently bound dimeric ligand to a fixed position in space could serve to constrain the molecule in an optimal orientation for binding of the second ligand. In addition, when the target is a dimeric species, the ability to effectively inhibit two receptors with a single molecule would allow for increases in potency and selectivity.

Quinone Dimers

Torreyanic acid (**1a**), a dimeric quinone, was isolated and characterized as a secondary metabolite of the endophytic fungi *P. microspora* [20]. After testing in 25 distinct cancer cell lines, **1a** was found to be 5–10 times more potent against cell lines that are sensitive to protein kinase C agonists. This molecule demonstrated an average IC_{50} value of 9.4 μg/mL and caused G1 arrest in G0 synchronized cells at 1–5 μg/mL [20]. While initial studies have suggested the eukaryotic translation initiation factor EIF-4a as a putative target, conclusive evidence for its binding to this target has not been described [21]. Several total syntheses of both racemic [22,23] and chiral [24] **1a** have been reported, however, no analogues have been prepared to date, and no further structure–activity relationship (SAR) studies have been performed.

Jesterone (**2**), was isolated as a monomeric epoxyquinol from the endophytic fungi *P. jesteri* [25]. Originally evaluated for its antifungal properties, its structural similarity to torreyanic acid suggested its use as a potential anti-cancer agent. This similarity led Porco and colleagues to design a total synthesis of (−)-jesterone and a jesterone dimer (JD, **3**) which was produced by a tandem, oxidation-6π electrocyclization-dimerization cascade sequence [26]. In preliminary studies against three distinct human cancer cell lines, JD exhibited low micromolar activity (IC_{50} values 1.5-19 μM) and was 10-200 fold more active than the jesterone monomer. Initial investigation of the mechanism of action suggests JD induces the apoptosis of cancer cells through inhibition of Rel/NF-κB activity [27]. Interestingly, isotorreyanic acid (**1b**), also inhibited activation of NF-κB, while torreyanic acid and several related epoxyquinoids were significantly less active in this assay.

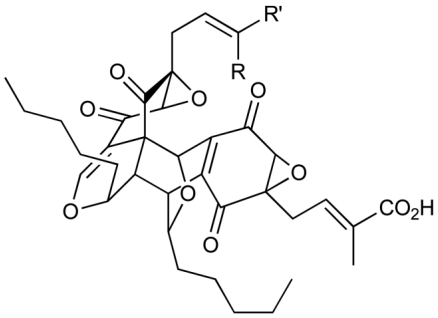
Coumermycin A1

Novobiocin, chlorobiocin, and coumermycin A1 are members of the coumarin family of antibiotics that inhibit the supercoiling of DNA through binding to the ATP-binding site of the bacterial DNA gyrase B protein. The atypical ATP-binding domain of gyrase B shares homology with the ATP-binding site located at the N-terminus of Hsp90, leading Neckers and colleagues to investigate whether the coumarin antibiotics could bind Hsp90 and subsequently exhibit inhibitory activity [28]. Surprisingly, while the three coumarin antibiotics did bind Hsp90, they bound to a previously unrecognized second nucleotide-binding region in the carboxy terminus that overlaps with the dimerization domain [29]. In addition, coumermycin A1 exhibited approximately 10-fold greater activity for the degradation of Hsp90 client

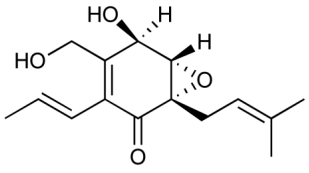
proteins p185^{erbB2} and Raf-1 in SKBr3 human breast cancer cells (IC_{50} ~ 75 μM) compared with novobiocin. Recently, coumermycin A1 was shown to interfere with dimerization of the C-terminal domain, suggesting a novel mechanism of action for which destabilization of the Hsp90 dimer promotes subsequent release of the protein substrate [30].

Until recently, extensive SAR for coumermycin A1 and Hsp90 had not been investigated. Burlison and coworkers utilized two separate approaches toward preparing coumermycin analogues: (1) linking a modified novobiocin scaffold through *meta*- and *para*-phthalic acid and (2) using cross metathesis to generate a series of flexible tethers containing various methylene spacers between the coumarin rings [31]. These analogues were evaluated for their anti-proliferative activity and their ability to induce the degradation of Her2, a well-documented Hsp90 client protein.

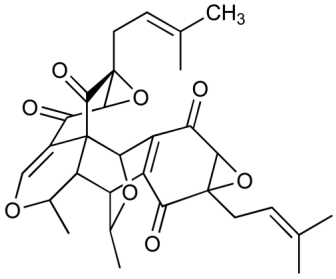
The phthalic acid derivatives were originally proposed to mimic the pyrrole linker of coumermycin A1, however, neither the *meta* (**4**) nor *para* (**5**) analogues exhibited activity in either the anti-proliferation or the anti-Her2 assays (IC_{50} > 100 μM). Subsequently, a series of analogues were prepared that contained varying methylene linkers tethered through a *trans* olefin, which established eight carbons as the optimal tether length (**6**, IC_{50} = 1.5 – 6.5 μM in various assays) [31] (Table **1**). In addition, the stereochemistry of the linker was examined and the saturated compound (**7**, IC_{50} = 2.7 ± 1.0 μM) was found to be more active than the alkyne (**8**, IC_{50} = 16.2 ± 0.2 μM) and *cis* (**9**, IC_{50} = 23.9 ± 5.4 μM) linkers, and comparable to the *trans* compound **6**, suggesting that conformation is important for Hsp90 inhibitory activity. In addition, **7** induced the degradation of two Hsp90 clients (Her2 and Raf) in a dose-dependent manner as measured by western blot analyses. Continued research into the optimization of compound **7** and its mechanism of action remains underway.



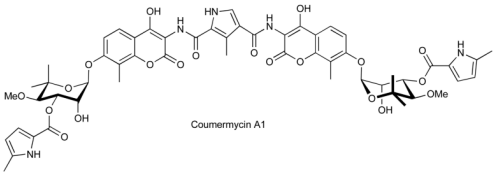
1a, R = CH_3, R' = CO_2H
1b, R = CO_2H, R' = CH_3

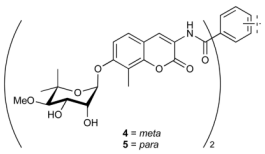


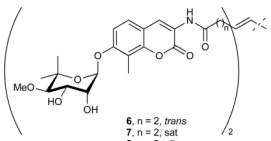
Jesterone, **2**

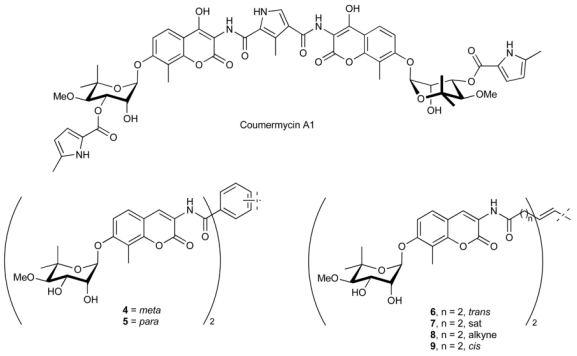


Jesterone dimer, **3**

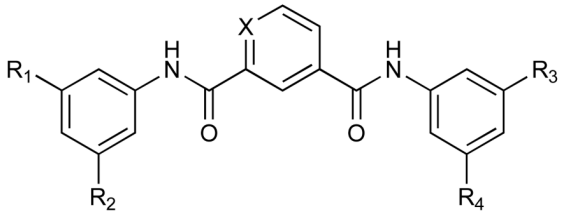








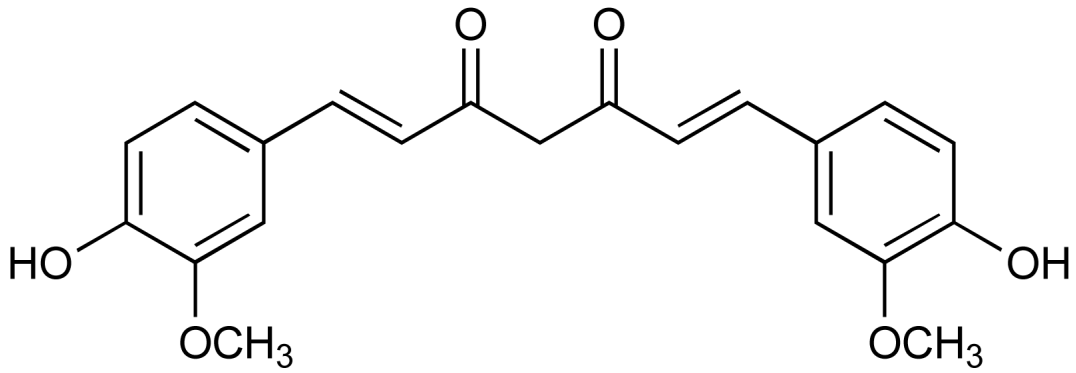
In an attempt to identify and optimize a simple dimeric scaffold that exhibits anti-proliferative activity against human breast cancer cells, a series of small molecule dimers structurally related to the coumermycin A1 aglycon were designed and prepared [32]. These dimeric compounds consisted of simplified amines and anilines mimicking the coumarin ring linked through 5- or 6-membered aryl rings closely related to the central pyrrole. An iterative process designed to optimize each portion of the scaffold resulted in several compounds with low micromolar activity comparable to coumermycin A1 (**10a**-**10d**, Table **2**). SAR for this series of compounds indicated that a central isophthalic core linking anilines containing 3,5-disubstitutions is important for anti-proliferative activity. These dimers were found to be more selective for the MCF-7 cell line, suggesting the potential for development of this scaffold as a cell-type specific inhibitor.



10a; X = N, $R_1 = R_2 = R_3 = R_4 = Cl$
10b; X = C, $R_1 = R_2 = R_3 = R_4 = Cl$
10c; X = N, $R_1 = R_3 = Cl$, $R_2 = R_4 = H$
10d; X = C; $R_1 = R_2 = R_3 = R_4 = CH_3$

Curcumin

Curcumin is a dimeric polyphenol isolated from *Curcuma longa* with broad biological and pharmacological activities. Its chemotherapeutic potential as an anti-cancer agent has been extensively reviewed elsewhere and therefore will not be presented herein [33-35].



Curcumin

SYNTHETIC DIMERS

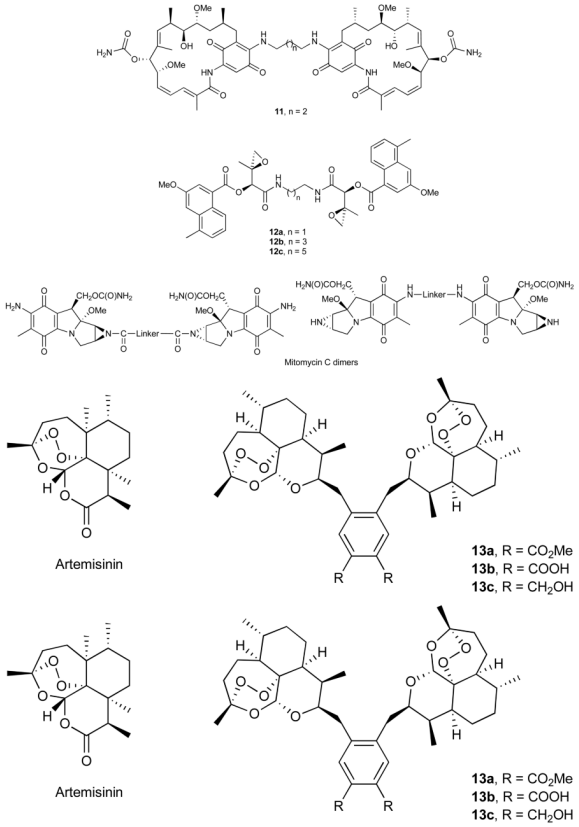
Dimerized Natural Products

Geldanamycin (GM) is a potent benzoquinoid ansamycin Hsp90 inhibitor that binds to the Hsp90 N-terminal ATP-binding site and prevents ATP hydrolysis and the subsequent folding of client protein substrates [7]. Based upon the homodimeric nature of Hsp90 and several of its client proteins, a series of GM dimers containing varying linkers was prepared and evaluated for its inhibitory activity. GM-dimers with linkers ≤7 carbons retained Hsp90 inhibitory activity comparable to GM (IC_{50}s = 60-70 nM and 45 nM, respectively) [36]. Interestingly, one compound, **11**, demonstrated selective degradation of Her2 compared to Raf-1, two Hsp90 clients involved in oncogenic signaling pathways. Additional studies demonstrated that **11** induced selective degradation of Her-family kinases and showed significantly increased anti-proliferative activity against tumor cell lines that overexpress Her2 [36].

The azinomycins were isolated from *S. griseofuscus* in 1986 and shown to possess significant *in vivo* anti-tumor activity [37]. Their mechanism of action was determined to be the result of DNA intrastrand cross-links (ISCs) formed by binding to the major groove of DNA [38]. Not surprisingly, the electrophilic epoxide has been shown to be important for cytotoxic activity [39] and simplified compounds containing the epoxide moiety have maintained similar cytotoxic activity compared to the natural product [40,41]. This led to evaluation of a series of dimeric epoxides containing flexible methylene linkers of varying length [42]. Compound **12b**, with a four-carbon linker, was idenitifed as the most active dimer in the DNA cross-linking assay, causing 100% DNA ISCs at 1 μM. In addition, all three compounds were cytotoxic against a wide-range of human cancer cell lines and manifested IC_{50} values ranging from 0.5 – 1.4 μM. Mitomycin C, another well-known natural product that promotes DNA ISCs, has also been prepared as a dimer and evaluated for its cytotoxic activity [43, reviewed in 44].

Artemisinin, a clinically efficacious antimalarial agent isolated from *Artemisia annua* L. (Asteraceae), is a sesquiterpene lactone containing a unique endoperoxide bridge system that is necessary for its antimalarial activity [45,46]. A synthetic campaign designed to improve the chemical stability of the parent natural product resulted in the identification of dimeric compounds that exhibited potent anti-tumor activity, many of which have been reviewed elsewhere [44]. More recent advances in the development of dimeric artemisinin analogues led to a series of hydrolytically stable derivatives linked through a series of phthalate acids [47]. In the NCI screen of 60 human cancer cell lines, **13a** demonstrated high potency and selectivity against non-small cell lung carcinoma HOP-92 cells, melanoma SK-MEL-5 cells, and breast cancer BT-549 cells. In addition, dimer **13c** exhibited potent activity against the

human cervical cancer cell line, HeLa (IC_{50} = 46.5 nM), while being nontoxic towards normal cervical cells.



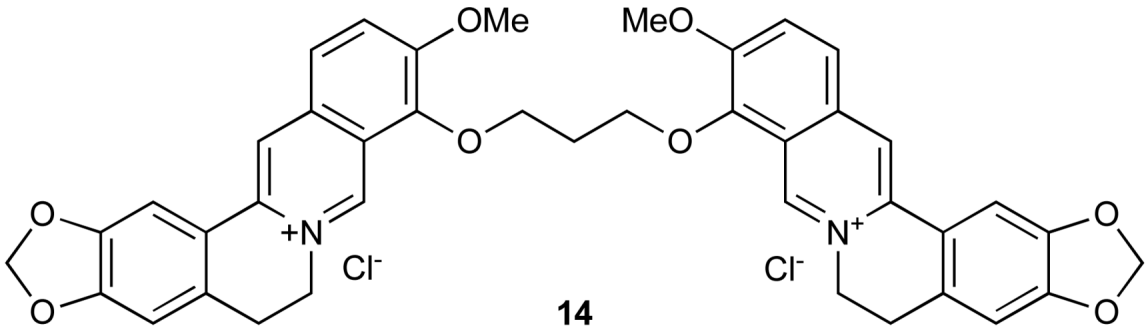
The biochemical and pharmacological activities of the protoberberine alkaloids have been extensively studied [48]. This class of compounds exhibits a dual topoisomerase I and II poisoning activity that is directly linked to their observed cytotoxicity. In addition, the DNA binding properties of the protoberberine analogues were shown to play an important role in their topoisomerase I inhibitory activity [49]. A series of protoberberine dimers was synthesized and evaluated for their DNA-binding affinities and topoisomerase I inhibition [50,51]. The dimeric analogues showed significantly higher DNA-binding affinities than the corresponding monomers, and affinity was found to be dependent on linker length and position [50]. Compounds **14** and **15** inhibited toposiomerase I by stabilizing the enzyme-mediated DNA ‘cleavable complex’ similar to the mechanism by which the known inhibitor camptothecin [51] inhibits this enzyme. Interestingly, at high concentrations (>200 μM), the ability to stabilize the complex sharply decreased with these compounds, suggesting their DNA binding may compete with topoisomerase binding to DNA. The identification of other natural protoberberine dimers provide similar scaffolds on which new compounds can be pursued [52,53].

Bisintercalators

The naphthalimide class of anti-tumor compounds has been extensively studied for its anti-tumor activity [54]. These compounds are DNA intercalators that also act as topoisomerase II poisons by stabilizing the drug/DNA/enzyme complex [55]. The ability of certain dimeric anitibiotics (such as echinomycin) to intercalate twice into DNA led numerous groups to develop bisnaphthalimides that exhibited increased intercalating and anti-tumor activity [54]. Elinafide (LU 79553) was shown to be highly effective against tumor xenografts *in vivo* [56]

and was evaluated in clinical trials [57]. **DMP 840** is also a potent DNA binder that has entered the clinic; however, it appears to be a monointercalator with its cytotoxicity resulting primarily from topoisomerase II inhibition [55,58,59].

A series of elifanide-related analogues that incorporated either a π-excedent furan or thiophene ring fused in differing positions relative to the naphthalimide moiety was synthesized and evaluated [60]. SAR for this series of compounds demonstrated that when the heteroatom of the furan ring was orientated towards the inside of the naphthalimide, an increase in anti-proliferative activity was observed. The most active compound, **16**, was shown to be ~2.5-fold more potent than elifanide against HT-29 human colon cancer cells (IC_{50} = 6.8 nM) and caused 80% reduction in tumor volume of HT-29 xenografts. Molecular modeling of this set of analogues suggests their improved anti-tumor activity (compared to imidazonaphthalimides and pyrazinonaphthalimides) is related to their ability to form stable DNA-drug complexes. A similar analogue, **MCI3335**, was prepared and evaluated in various biochemical and biophysical assays [61]. Qualitative and quantitative binding studies demonstrated that the dimer bound up to 1000 times more tightly than the corresponding monomer and exhibited sequence selectivity for GC-rich domains. While **MCI3335** lacked any topoisomerase poisoning effects, it maintained potent cytotoxicity against human leukemia cells (IC_{50} = 4.9 nM), which was attributed to its enhanced ability to bind DNA.



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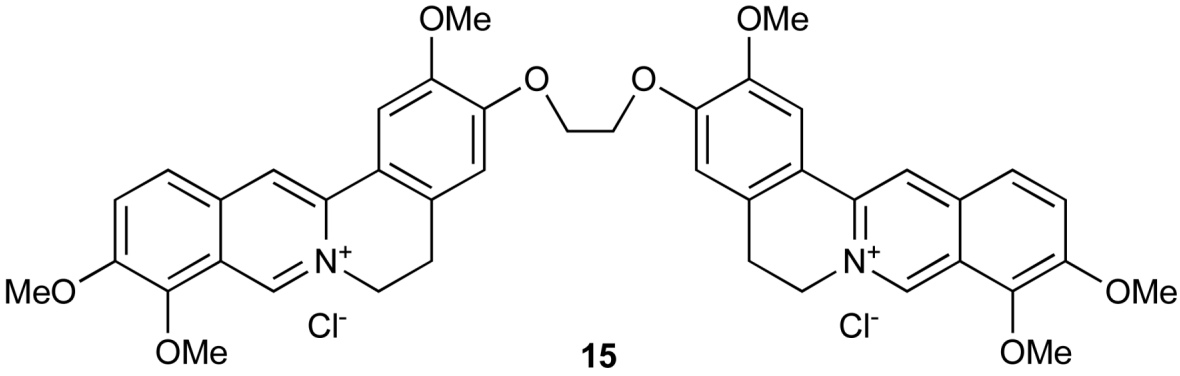

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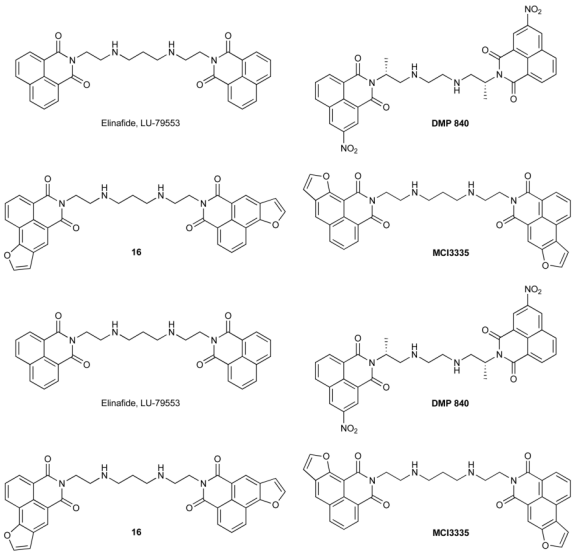
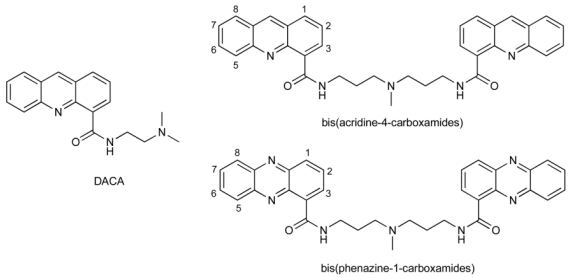


The DNA intercalating properties of *N*-[(2-dimethylamino) ethyl]acridine-4-carboxamide (DACA) and the success of the bisnaphthalimides led Denny and colleagues to develop the bis (acridine-4-carboxamide) series of anti-tumor agents [62,63]. The bis (DACA) analogue demonstrated a 5-fold increase in cytotoxic potency compared to the monomer [62]. Extensive SAR studies for substituents at various positions of the acridine ring showed that small substituents (Me, Cl) at the 5-position were optimal for activity and produced IC_{50} values in the low nanomolar range (2-11 nM) [63]. Replacement of the acridine ring with a phenazine gave a series of bis(phenazine-1-carboxamides) that exhibited SAR similar to the acridine derivatives with small lipophilic substituents peri to the pyridine nitrogen demonstrating the highest cytotoxic activity [41]. The most active analogue, containing a 5-OMe substituent, was identified as a dual topoisomerase I/II inhibitor and showed significant growth delays of xenograft tumor models *in vivo*.

The bisimidazoacridone WMC-26 demonstrated selective activity against colon cancers in the NCI *in vitro* screen and was similarly active against colon adenocarcinoma xenografts in nude mice [65]. Current evidence suggests that WMC-26 does not bind DNA *via* bisintercalation. In contrast to the naphthalimides and carboxamides, it appears to bind in an unsymmetrical fashion, where one aromatic monomer intercalates into DNA and the other binds to the minor groove in a GC-rich region [66]. A series of acridone carboxamides based on WMC-26 focused on linker length and linker orientation [67]. The most active compounds identified from this study resulted in highly cytotoxic molecules that exhibited IC_{50} values against HT-29 cells at low or subnanomolar concentrations (**17**, **18a**, and **18b**). Second generation ‘cyclized’ acridone carboxamides (**19**, **20a**, and **20b**) were generally poorer DNA binders than the corresponding ‘open’ analogues and possessed a preference for AT-rich sequences; however, they continued to maintain low to subnanomolar cytotoxic activity [68]. Preliminary *in vivo* results from a hollow fiber assay demonstrated that **19** exhibited *in vivo* activity worthy of further testing in xenograft models, which are currently underway. Reports suggesting that WMC-26 binds unsymmetrically to DNA has prompted the development of asymmetrical bisintercalators that attempt to take advantage of both putative mechanisms of action [69-72]. To date, these compounds have shown cytotoxicity and DNA binding affinities comparable to their symmetrical counterparts.

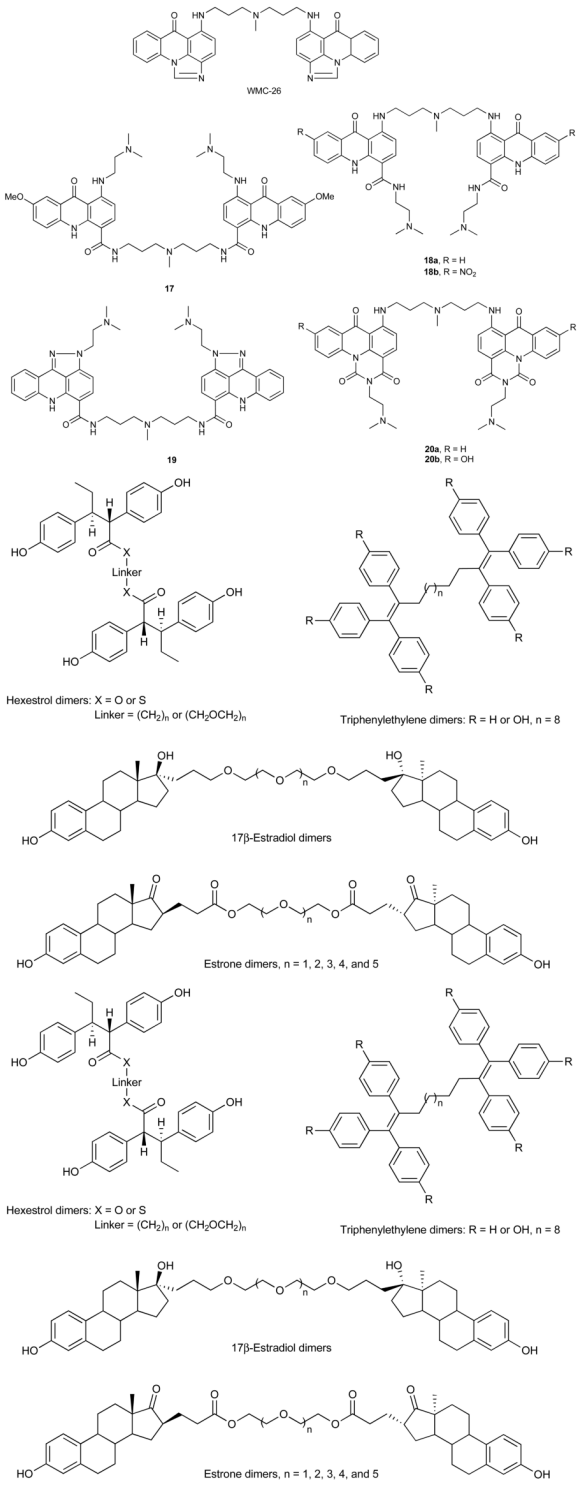


Estrogen Dimers

Bivalent estrogenic ligands were first synthesized as simple hexestrol analogues designed to better understand structural requirements for estrogen receptor (ER) dimerization [73]. Several symmetrical dimers exhibited interesting antiestrogenic activity and prompted further exploration for this class of compounds. A series of nonsteroidal homo- and heterobifunctional-estrogenic dimers consisting of two triphenylethylene moieties designed to mimic tamoxifen linked through an aliphatic chain were evaluated [74]. While these compounds exhibited cytotoxic activity comparable to tamoxifen, they exhibited no inherent selectivity towards ER^+ breast cancer cells. More recently, the synthesis and evaluation of estrogenic and 17β-estradiol dimers linked through ether bonds was described [75,76,77]. While some of the dimers were selectively cytotoxic against the ER^+ cancer cells, their overall activity was greatly reduced compared to tamoxifen and they demonstrated only weak affinity for ERα and no affinity for ERβ. Interestingly, all of the analogues tested imparted cytotoxic effects towards murine skin cancer cells (B16-F10) suggesting the potential for an alternative target [76].

Pyrrolo[2,1-c][1,4]benzodiazepines

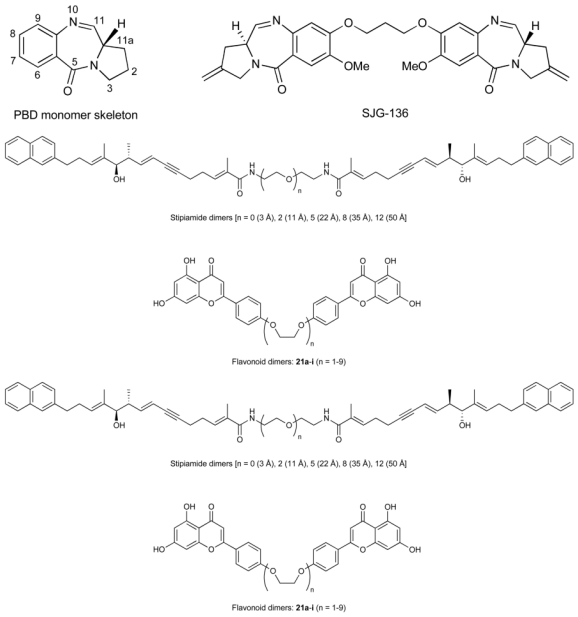
The pyrrolo[2,1-*c*][1,4]benzodiazepine (PBD) anti-tumor agents have been extensively studied and reviewed for their ability to bind the minor groove of DNA and form interstrand crosslinks [44,78]. This class of compounds exerts its anti-cancer activity through covalent binding *via* the N10–C11 imine-carbinolamine moiety to the C2-position of guanine. The ability of PBD monomers to bind short sequences of DNA led to the development of PBD dimers designed to better recognize DNA sequences and potentially form interstrand crosslinks [79]. The early success of these compounds as efficient irreversible DNA crosslinkers led to continued SAR development [reviewed in 44,78]. To date, the most potent member of this class of compounds is SJG-136, which exhibits subnanomolar cytotoxicity against the cisplatin resistant A2780 human ovarian carcinoma (IC_{50} = 0.024 nM) [80] as well as potent anti-tumor activity in mouse xenograft models [81]. SJG-136 is currently in several phase I clinical trials for the treatment of a variety of human tumors. The development, SAR, and DNA binding properties of similar PBD dimeric compounds has been previously summarized [44,82].



Bivalent P-Glycoprotein Modulaters

A common form of multidrug resistance (MDR) is related to overexpression of the efflux transporter, P-glycoprotein (Pgp), and has become a major hurdle for the development of cancer chemotherapeutics. Evidence for at least two distinct drug binding sites associated with Pgp

[83,84] prompted the design, synthesis, and evaluation of a class of bivalent dimeric polyenes based upon the natural product Pgp modulator, (−)-stipiamide [85,86]. These analogues were designed to contain tethers ranging in size from 3–50 Å in an effort to determine the optimal length for dual Pgp modulation. A minimal spacer of 11 Å was found to be necessary for Pgp inhibition. Activity increased exponentially as the spacer was increased to 50 Å (IC_{50} = 100 nM) [86]. The homodimers with medium length spacers (22 or 35 Å) also inhibited Pgp-mediated drug efflux in intact cells that overexpressed the protein, confirming that tether link is important for MDR modulation. More recently, a series of apigenin-based flavonoid dimers was synthesized and evaluated for Pgp modulatory activity [87]. Dimeric compounds with shorter ethylene glycol linkages (2–4 spacers) were identified to be the most effective at modulating Pgp activity. In particular, compound **21d** (4 spacer units) increased cytotoxicity and drug accumulation in drug-resistant human breast cancer and leukemia cells when co-administered with several clinically efficacious anti-tumor agents.



CONCLUSION

This review presents a general overview of recent developments in the field of dimeric compounds as anti-cancer agents. Numerous well-known cancer chemotherapeutic targets, including signaling proteins, microtubules, topoisomerases, and DNA are dimeric in nature or require dimerization for activation. Their bivalent nature has made the development of dimeric and multivalent inhibitors an emerging field in anti-cancer drug research. The potential to bind two individual binding sites on a single receptor or a defined site on two separate monomers of a dimeric protein could increase drug potency and efficacy. To date, research in this area has mainly focused on two types of compounds: (1) natural product dimers and their analogues, and (2) dimerized derivatives of monomers with anti-cancer activity. The relevance of these approaches to drug development is evident as several phase I clinical trials have been initiated. This field will continue to expand as more natural and synthetic dimers are identified as anti-cancer chemotherapeutics.

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ABBREVIATIONS

DACA, *N*-[(2-dimethylamino)ethyl]acridine-4-carboxamide
EGF, Epidermal growth factor
ER, Estrogen receptor
FGF, Fibroblast growth factor
GM, Geldanamycin
Hsp90, 90 kDa family of heat shock proteins
IGF, Insulin-like growth factor
ISC, Intrastrand cross-links
JD, Jesterone dimer
MDR, Multidrug resistance
PBD, Pyrrolo[2,1-*c*][1,4]benzodiazepine
Pgp, P-glycoprotein
RTKs, Receptor-type tyrosine kinase
SAR, Structure-activity relationships
STAT, Signal transducer and activator of transcription

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Table 1

Hsp90 Inhibitory Activity of Coumermycin A1 Analogues [a]

Compound ID	MCF-7	SKBr3	Her2 ELISA
Coumermycin A1	5.0 ± 0.1[b]	8.8 ± 0.1	1.6 ± 1.2
4	>100	>100	>100
5	>100	>100	>100
6	3.9 ± 0.7	1.5 ± 0.1	5.6 ± 1.3
7	2.7 ± 1.0	1.9 ± 0.2	6.7 ± 1.3
8	16.2 ± 0.2	82.2 ± 0.7	95.2 ± 1.6
9	23.9 ± 5.4	27.6 ± 2.9	86.9 ± 7.8

[a] Adapted from Burlison *et al.* [31].

[b] All values presented in μM.

Table 2

[a] Anti-Proliferative Activity of Small Molecule Dimers

Compound ID	MCF-7	SKBr3
10a	4.2 ± 1.3	10.5 ± 2.3
10b	2.7 ± 0.2	4.5 ± 0.2
10c	2.4 ± 0.4	9.0 ± 5.3
10d	2.1 ± 0.8	1.9 ± 0.2

[a] Adapted from Hadden, *et al.*

[b] All values presented in μM.