Recent Progress in the Development of Synthetic Hybrids of Natural or Unnatural Bioactive Compounds for Medicinal Chemistry

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Abstract: The present mini-review highlights the recent developments on different classes of synthetic hybrids of natural and/or unnatural bioactive compounds, the utilization of which is very promising, as distinct features of each component can be hybridized and their properties leveraged. Particular stress is put on the respective mode of action and the corresponding rationale behind covalent combinations of various bioactive agents to increase their therapeutic potential, facilitate their administration, to reduce harmful side effects and/or to overcome the problem of multi-drug resistance. This rather recent approach has already found applications in the development of new anti-cancer, anti-Alzheimer, anti-malaria, anti-microbial therapeutics and other novel compounds with unprecedented bioactivity.

Keywords: Drug design, natural product hybrids, anti-cancer, anti-Alzheimer, anti-malaria drugs, prodrugs, pharmacophore.

INTRODUCTION

The hybridization of bioactive natural and unnatural compounds is one of the most promising and fundamentally novel approaches for the design of new lead structures and the discovery of new and potent drugs in the field of medicinal chemistry [1]. A natural-product hybrid (sometimes also referred to as conjugate or chimera) is a synthetic compound having two or more than two natural products derived fragments joined by at least one carbon–carbon bond. This idea is inspired by nature itself, since many of the known natural products are built of such fragments - arising from different biosynthetic pathways [2], one example is the hybrid vincristine, a dimeric indole alkaloid having a vindoline and catharanthine moieties, and being the preferred drug for lymphatic leukemia [3]. Neither monomeric compounds show any useful activity. In many cases, artificial hybrid molecules of partial structures of natural compounds have been demonstrated to show more potent activity than the parent compounds themselves [1, 4]. The concept based on the combination of fragments of bioactive compounds seems to have advantages because a sheer inexhaustible variety of such hybrid structures can be designed and are potentially accessible in the light of recent advances of molecular biology and contemporaneous synthetic organic chemistry [5].

The pharmacodynamics of the drugs is a further rationale to be considered in the design of hybrids: the passage through the cell membrane or the absorption of the drug when administered orally should not be impaired.

Another approach in the application of natural product hybrids in the fight against diseases exploits the sometimes complementary properties of the monomers, exemplified by the combination of different or the same, but not fully overlapping, modes of action, for example in the "information-reading" lexitropsins like distamycin [6], a minor B-DNA groove binder to A-T rich regions and that can be covalently coupled to an appropriate cytotoxic payload like radical producing enediyne containing calicheamicin or CC-1065, one of the most potent anticancer antibiotic agents with an IC_{50} value of 30 pm [7]. This example also demonstrates the further potential of the hybridization approach, as the identified pharmocophore in CC-1065, a spirocyclopropanepyrroloindoline (CPI) moiety - which derives its potency from the ability to alkylate the DNA, has been found to be distinct from the part of the molecule that causes the hepatotoxic side effects [8].

Because of the pre-eminent challenge the different forms of cancer, as well as Alzheimer and malaria pose to modern medicine, the overwhelming majority of the more recent literature on synthetic hybrids of natural or unnatural bioactive compounds puts a high focus on these subjects. The present review, therefore, highlights the recent developments in potential applications of new hybrid molecules in the treatment of cancer, Alzheimer's disease, malaria and various other afflictions.

1. New Synthetic Anti-Cancer Hybrids

Cancer is still the second leading disease-related cause of death worldwide [9]. As no curative therapy for many types of cancer is presently available, an ongoing need for novel lead structures for chemotherapy exists.

Among the best known mechanisms for the cancer chemotherapy, the targeting of microtubules is one of the most important [10-12]. Microtubules are proteins comprised of heterodimers of α- and β-tubulin, which bind the taxanes, vinca alkaloids, and colchicines in three distinct tubulin binding sites. These proteins are polymerized during cell proliferation. Several peptide and depsipeptide natural product inhibitors of tubulin polymerization have been shown,

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through competitive binding experiments, to block a site close to the *vinca binding site* [13, 14]. These include dolastatin 10, hemiasterlin, phomopsin, and cryptophycin 1 [15]. For example, taltobulin (HTI-286), a synthetic analog of the naturally occurring tripeptide hemiasterlin (Fig. (**1**)), is a promising anticancer agent that has advanced to clinical trials [16].

The extensive structure-activity relationship (SAR) studies on taltobulin carried out by Zask *et al.* [17] revealed several groups that were critical for the activity and that correlated with moieties on the dolastatin 10 amino terminus tripeptide, dolavaline-valine-dolaisoleuine (Dov-Val-Dil) [15].

To establish a connection between the hemiasterlin tripeptides and the more complex dolastatins, the Zask group synthesized hybrids **1**-**4** (Fig. (**1**)) composing of taltobulin coupled to the carboxy terminus dipeptides (e. g. Dap-Doe) of the dolastatins. The resulting hybrid compounds were potent antimicrotubule agents, thus establishing a structural relationship between the hemiasterlins and the dolastatins [15].

Compounds shown in Fig. (**1**) inhibited tubulin polymerization by 56-69% at a concentration of 3 μM tubulin. In KB-3-1 cell lines, hybrid **4** exhibited the highest potency among these compounds (IC_{50} value of 0.25 nM) [15].

Zask and coworkers found that hybrids **1** and **2** showed improved resistance to P-glycoprotein binding as compared to dolastatin 10. Compounds **3** and **4** showed however higher susceptibility to the P-glycoprotein transporter as compared to taltobulin. This relationship may turn out fruitful for the design of hemiasterlin analogs having improved activity in resistant cell lines expressing the P-glycoprotein transporter.

Natural products of the *polyketide family* have attracted a great deal of attention in recent years because of their high levels of activity against various human tumor cell lines [18].

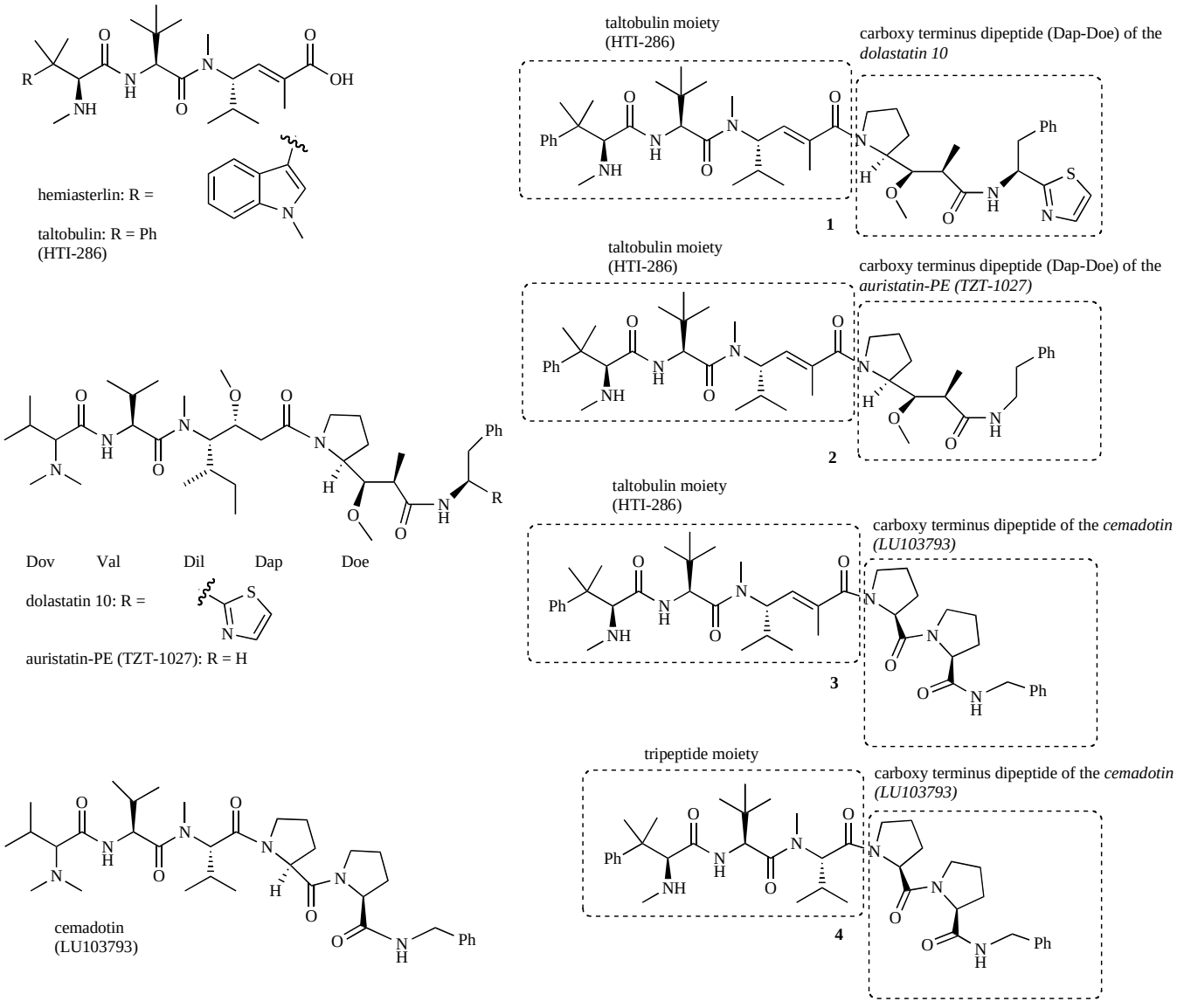


Fig. (1).

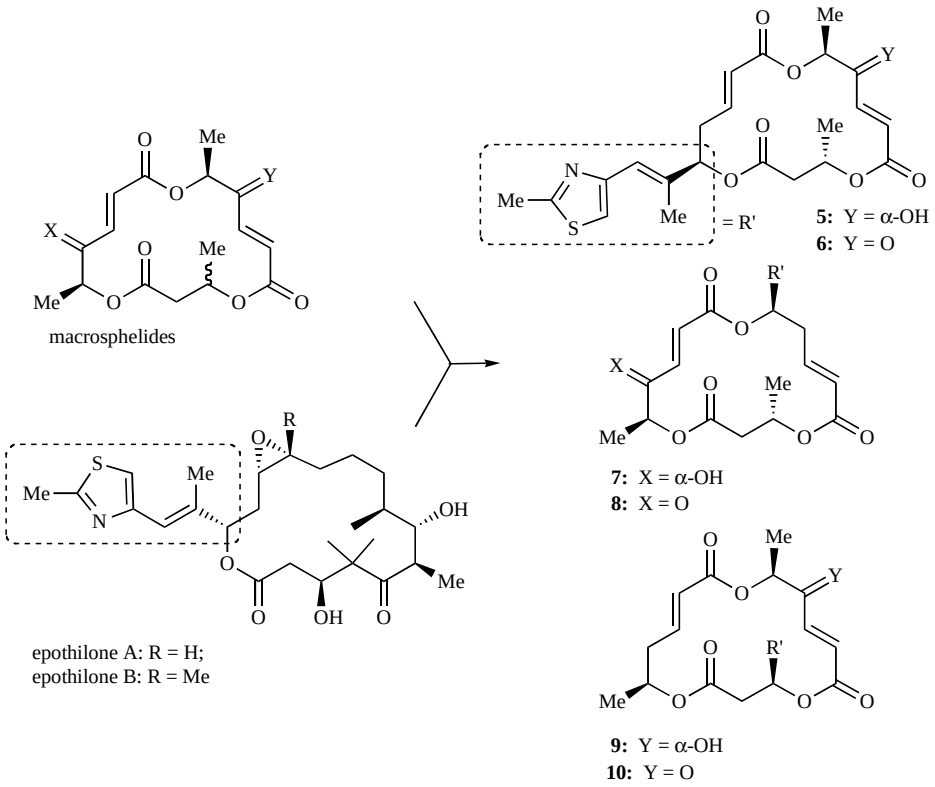


Fig. (2).

Tricyclic polyketides with antitumor activity are minor DNA groove binders which are produced by different streptomycete species. Mithramycin and chromomycins are representative examples, which are discussed in a recent review [18c].

Nemoto and co-workers [19] have recently noticed that some natural macrosphelides and analogs can activate the *apoptotic program* in human lymphoma U937 cells, albeit with rather weak potency. This preliminary result was the first observation on the apoptosis inducing potential of macrosphelides. These authors have further noted that epothilones (16-membered cyclic natural polyketides), which have been reported to exhibit extraordinarily potent cytotoxicity in a broad range of human cancer cell lines through a paclitaxel (Taxol)-like mechanism of action, i.e. by stabilization of the microtubule framework thus leading to mitotic arrest [20, 21]. Epothilones are known to induce mitotic arrest at the G2/M transition as a result of aberrant spindle formation, leading to apoptotic cell death, [22] which is suggested to have close correlation with the tumor cell growth inhibitory effects. One of the structural features of epothilones is the side-chain, containing a thiazole ring, which is supposed to play an important role for their bioactivity [23].

Nemoto and co-workers designed and synthesized novel macrosphelide-epothilone hybrids (**5**-**10**, Fig. (**2**)) and revealed their high potential as a new artificial antitumor agent having an apoptosis inducing ability [19]. They found that introduction of the thiazole substituent significantly enhanced the activity as compared with the parent natural macrosphelides. The assay was performed using a human lymphoma cell line (U937). The hybrid **6** exhibited the most potent activity with negligible secondary necrosis at 1 μM concentration after 6 h incubation, and the hybrids **8** and **10** also induced apoptosis to a lesser extent. The parent macrosphelides did not display any apoptosis-inducing ability at the same concentration even after 12 h incubation [19].

These results of Nemoto and co-workers indicate that the thiazole-containing substituent installed at an appropriate position can give rise to significant intervention with a mechanism controlling an apoptotic program.

As a next possible antitumor drug candidate, acyclic polyketides callystatin A and leptomycin B present a particularly attractive profile in consideration of their more modest structural complexity.

The α-pyranone terminus is a significant recurrent feature among callystatin A and leptomycin B, essential to their high levels of bioactivity. Several studies support the notion that the α,β-unsaturated lactone is the pharmacophore for callystatin A and leptomycin B (Fig. (**3**)). Kobayashi and co-workers performed structure-activity relationship studies on callystatin A and found that analogs lacking the pyranone double bond showed a dramatic decrease in activity against KB cells [24]. In addition, a saturated lactone derivative of leptomycin showed greatly diminished activity against HeLa cells [25].

Recently, Marshall's group prepared four stereoisomeric hybrids of the polyketide natural products callystatin A and leptomycin B (**11**) [26]. Like their natural counterparts, these

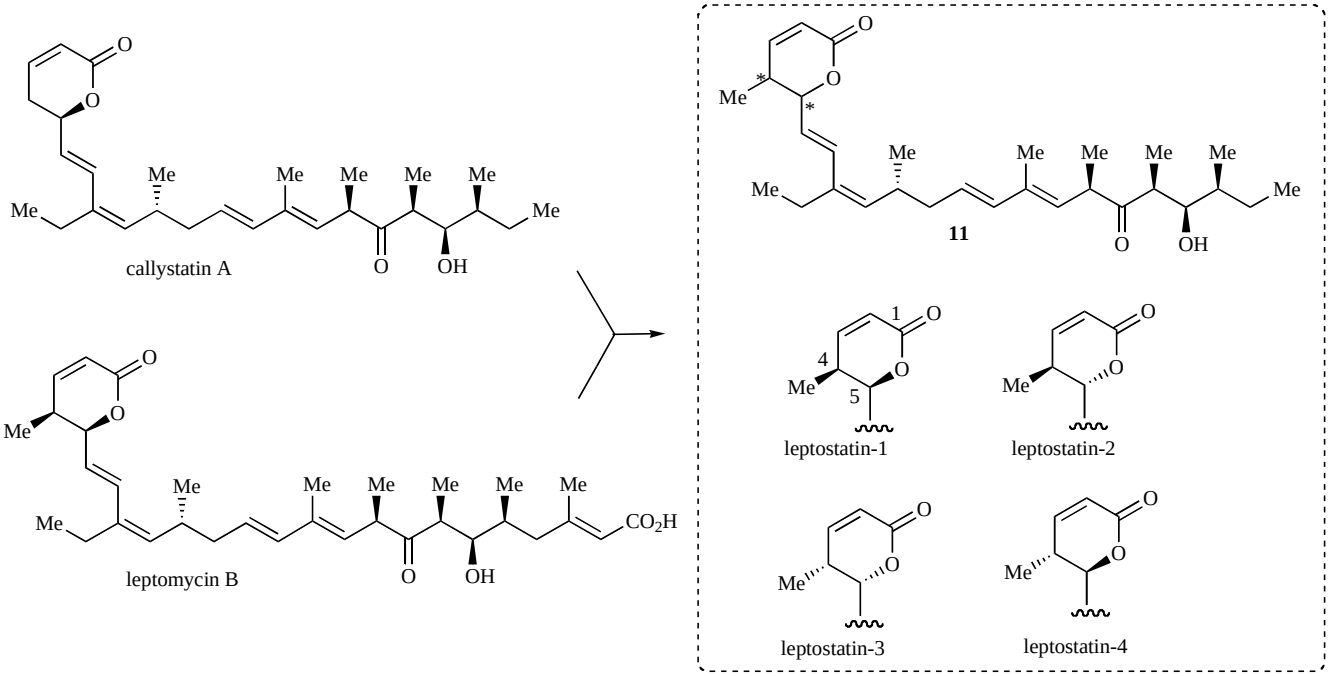


Fig. (3).

hybrids exhibit nanomolar levels of cytotoxicity (IC_{50} = 0.2-30 nM) toward HCT-116 human colon cancer cells.

Discodermolide [27] (**12**, Fig. (**4**)) and dictyostatin [28, 29] (**13**, Fig. (**4**)) are antimitotic *polyketides* isolated from marine sponges, which exhibit potent growth inhibition against a wide range of human cancer cell lines, including multidrug-resistant cancer cells [30]. Their mode of action consists of the same microtubule-stabilising mechanism as for Taxol, causing mitotic arrest by accumulation of cells in the G2/M phase and subsequent apoptosis [20, 21].

Recently, Paterson's group designed and synthesized two potent dictyostatin–discodermolide hybrids (**14** and **15**, Fig. (**4**)), which showed encouraging cell growth inhibitory activity in a range of human cancer cell lines [31, 32].

The authors attributed the antiproliferative activity of analogs **14** and **15** to their constrained (dictyostatin-like)

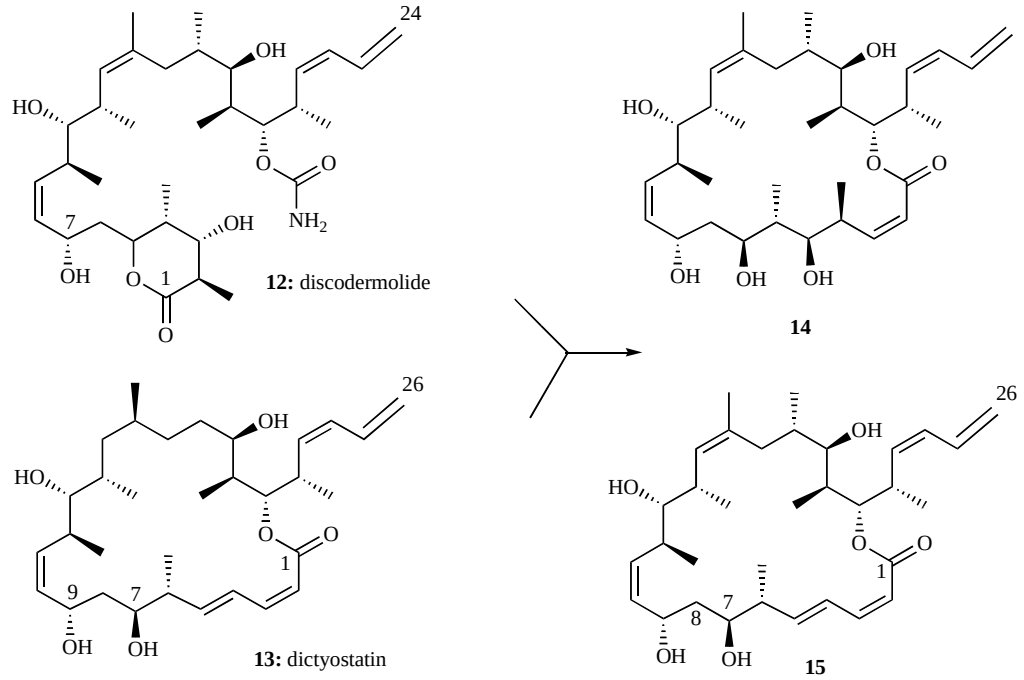


Fig. (4).

macrocyclic structures, which bear a strong resemblance to the bioactive conformation of discodermolide [33].

While hybrid **14**, designed by the authors earlier [31], showed only promising cell growth inhibitory activity with GI_{50} (MDA-MB231 (breast)) = 0.208 μM (reduced with respect to that of discodermolide (GI_{50} (MDA-MB231 (breast)) = 0.029 μM), hybrid **15** demonstrated low nanomolar cell growth inhibitory activity (IC_{50} (PANC-1 cells) = 12.9 ± 2.0 nM; IC_{50} (AsPC-1 cells) = 33.9 ± 6.4 nM; IC_{50} (DLD-1 cells) = 5.9 ± 1.1 nM) that was intermediate between that measured for discodermolide (IC_{50} (PANC-1 cells) = 59 ± 34 nM; IC_{50} (AsPC-1 cells) = 98 ± 34 nM; IC_{50} (DLD-1 cells) = 29 ± 8 nM; NCI/ADR-Res 160 ± 34 nM) and dictyostatin (IC_{50} (PANC-1 cells) = 4.2 ± 0.5 nM; IC_{50} (AsPC-1 cells) = 6.2 ± 0.6 nM; IC_{50} (DLD-1 cells) = 2.2 ± 0.5 nM; NCI/ADR-Res 6.6 ± 0.4 nM) [34a] and similarly maintained this potent activity against the NCI/ADR-Res (Taxol-resistant ovarian) cell line (IC_{50} = 66.4 ± 15.2 nM), where the overexpression of a P-glycoprotein drug efflux pump in the cell membrane gives rise to Taxol resistance. The mode of action of hybrid **15** is the same as that of dictyostatin and discodermolide.

In the structure of **15**, the stereochemistry and substitution from C8 to C26 are identical to those of discodermolide (lacking the carbamate), while the C1 to C7 region, incorporating the dienoate moiety, and 22-membered macrolactone is dictyostatin derived [34]. In contrast, the corresponding C1 to C7 region in **14** does not match exactly that in dictyostatin.

Recently, De Brabander's group [35] described the synthesis of "psympederin", a chimera between the novel antitumor natural product psymberin and the blister beetle toxin pederin. Evaluation of antiproliferative activity revealed that the dihydroisocoumarin fragment is important for psymberin toxicity and the cyclic pederate fragment is important for pederin/mycalamide toxicity. On the basis of these results obtained, they surmised that despite their structural resemblance, psymberin and pederin/mycalamide induce toxicity through different mechanisms. Furthermore, these authors have initiated the first structure-function analysis of psymberin. Although psymberin shows a structural partial resemblance to the pederin/mycalamide family of antitumor compounds, De Brabander and co-workers demonstrated that psymberin's mode of cytotoxicity is distinct to that induced by the pederin/mycalamide natural products. They synthesized a psymberin-pederin conjugate **16**, a compound that is at the same time a psymberin analog lacking its characteristic dihydroisocoumarin fragment and a pederin analog with an acyclic "psymberate" (C1-C6) side chain substituting for the "pederate" cyclic acetal fragment (Fig. (**5**)). The resulting hybrid **16** showed drastically reduced cytotoxicity (~1000 fold) with respect to psymberin and markedly reduced antiproliferative activity (>300 fold) with respect to mycalamide A.

A major contemporary challenge in anticancer therapy is the usually low differentiation between normal and tumor cells by many known antiproliferating or cytotoxic agents, frequently causing severe and sometimes unexpected harmful side effects. A novel approach to overcome this problem is the antibody-directed enzyme prodrug therapy (ADEPT) [36] and the targeted prodrug monotherapy (PMT) [37]. Both approaches exploit specific structural or metabolic features of tumor cells. Whereas in ADEPT artificial conjugates of antibodies and enzymes are needed in targeting malignant cells, PMT employs the specificity of certain endogenous

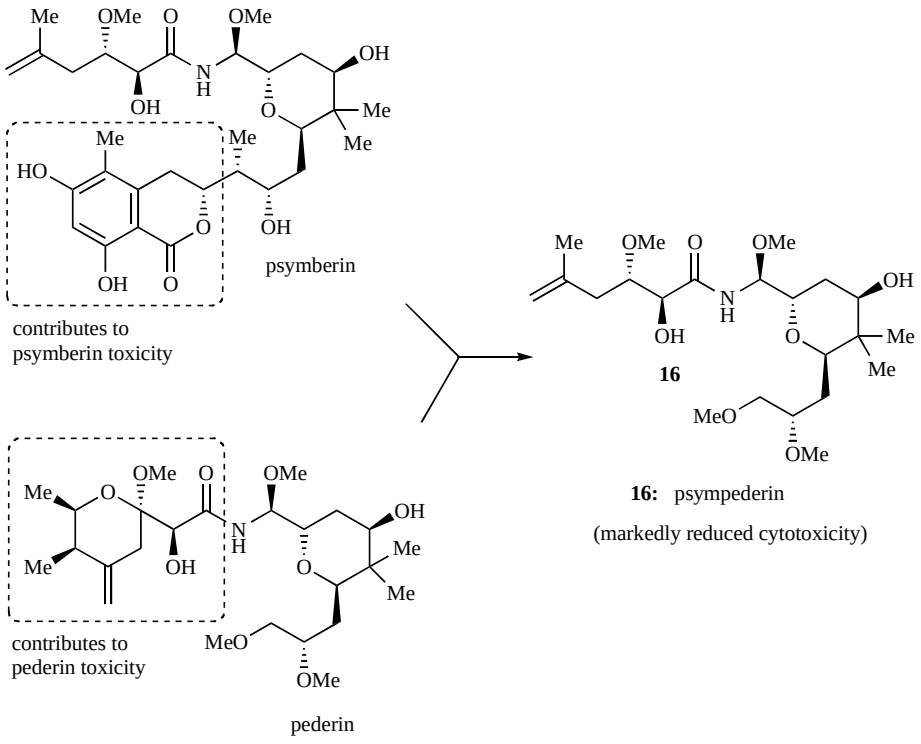


Fig. (5).

enzymes or receptors overexpressed in cancerous tissue, to permit a selective destruction of degenerate cells [36, 37].

Both approaches share the use of a comparably nontoxic prodrug, which is then selectively converted into the corresponding cytotoxic drug in the targeted cancer tissue [36]; however, for PMT, the prodrug is linked to a ligand which allows a specific targeting of cancer cells.

Recently, Tietze and co-workers [38-41] have designed and synthesized novel prodrugs **17**-**20** (Fig. (**6**)) for a selective treatment of cancer based on the naturally occurring antibiotic (+)-duocarmycin SA, which is a particularly potent cytotoxic compound with an IC_{50} value of 10 pM against a murine leukemia cell line (L1210) [42]. The antiproliferative effect of (+)-duocarmycin SA derives most probably from a selective alkylation of the N-3 atom of adenine in DNA by nucleophilic attack at the spirocyclopropyl–cyclohexadienone moiety as the pharmacophoric group [42a, 43].

The design of two gastrin conjugates **17** and **18** for a targeted tumor therapy was based on the assumption that the pentagastrin moiety allows their selective incorporation into cancer cells which overexpresses the CCK-B/gastrin receptor [38, 39]. The advantage of such conjugates over normal gastrin-toxin conjugates is that a less toxic prodrug is used instead of a more toxic drug. In this concept, the pentagastrin moiety should serve not only as a targeting ligand for CCK-B/gastrin receptors, but also as a detoxifying unit. Thus, the corresponding drugs possessing the spirocyclopropyl–cyclohexadienone moiety as the pharmacophor should be formed inside the tumor cells [38, 39].

Novel β-D-galactosidic prodrugs (+)-**19** and (+)-**20** of the duocarmycins were prepared by Tietze *et al*. for an ADEPT [40, 41]. These only slightly toxic compounds can be toxified enzymatically by an antibody– β-D-galactosidase conjugate at the surface of malignant cells to give the cytotoxic drugs, containing the pharmacophoric spirocyclopropyl–cyclohexadienone moiety, which then alkylate DNA. The new prodrugs were tested in *in vitro* cytotoxicity assays showing excellent QIC_{50} (QIC_{50} = IC_{50} (prodrug)/IC_{50} (prodrug + corresponding enzyme)) [44] values of 4800 and 4300 for (+)-**19** and (+)-**20**, respectively. Owing to their excellent QIC_{50} values, their good water solubility, and easy synthesis, β-D-galactosidic prodrugs (+)-**19** and (+)-**20** are superior to all compounds described so far for the use in ADEPT [40, 41].

It is well documented that oxidative stress, caused by unstable radicals or species in high oxidation states can act cytotoxic. For example, the production of ·NO by macro-

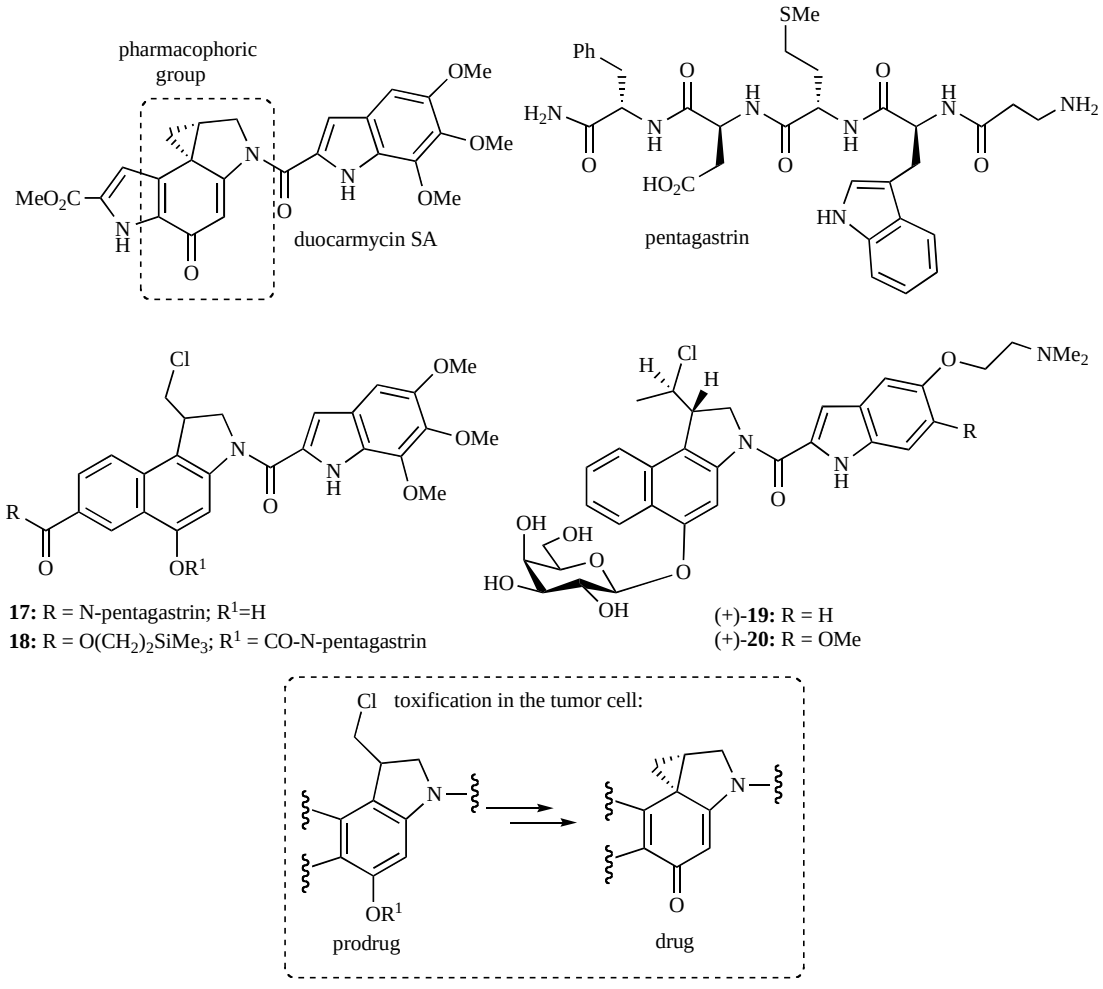


Fig. (6).

phages, Kupfer cells, natural killer T-cells, and endothelial cells can induce cytotoxicity against a variety of tumor cells [45, 46]. Fiorucci and co-workers have shown that NO-releasing derivative of ursodeoxycholic acid inhibits liver inflammation and protects against development of portal hypertension [47a,b]. It is also known that the 1,2,5-oxadiazole *N*-oxide ring (1,2,5-oxadiazole 2-oxide, furoxan ring) and derivatives thereof are able to release ·NO in the presence of thiol cofactors [48].

Recent studies have demonstrated *in vitro* cytotoxic activity exhibited by certain 1,2,5-oxadiazole *N*-oxides possessing a C-3 moiety with a good leaving group that can be displaced by nucleophiles present in biological environments [49, 50]. Knaus and co-workers were inspired by these results to attach the 3-benzenesulfonylfuroxan-4-yl ·NO donor moiety to the 3'-*O*- and 5'-*O*-positions of 2'-deoxyuridine to find out whether this new type of hybrid ·NO donor-nucleoside prodrug combination would result in a synergistic cytotoxic effect [51].

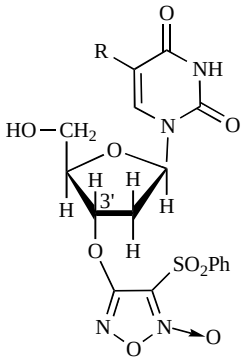
These authors synthesized a group of 3'-*O*- (**21**) and 5'-*O*-(3-benzenesulfonylfuroxan-4-yl)-2'-deoxyuridines (**22**) possessing a variety of substituents (H, Me, I, F, CF_3) at the C-5 position of the nucleoside moiety and evaluated their ability to release cytotoxic nitric oxide (·NO) (Fig. (**7**)). Incubation of these nitric oxide donor-nucleoside conjugates in the presence of 18 mM L-cysteine released a high percentage of ·NO (21-48% in 1 h; 37-86% in 16 h), whereas the release of ·NO in the absence of the thiol cofactor was found to be negligible. These hybrid ·NO donor-nucleosides exhibited high cellular toxicity (CC_{50} = 10^{-6}-10^{-8} M range) against a broad variety of tumor cell lines (143B-LTK, 143B, EMT-6, KBALB-STK, and KBALB) and normal human fibroblasts (Hs578Bst) [51].

The high amount of nitrosyl radical release and potent cytotoxicities shown by these furoxanyl deoxynucleosides prompted Knaus and co-workers to further probe the cytotoxic effect of the furoxan moiety. They found that 3,4-bis(benzenesulfonyl)furoxan (**23a**) released ·NO readily in the presence of 18 mM L-cysteine (23% in 1 h; 39% in 16 h) and that **23a** was highly cytotoxic (CC_{50} = 10^{-7}-10^{-8} M range). Similarly, 3-benzenesulfonyl-4-methoxyfuroxan (**23b**), considered to be closely structurally related to the furoxany-4-yl moiety present in compounds **21a-e** and **22a-d**, was also highly cytotoxic (CC_{50}) in the same range.

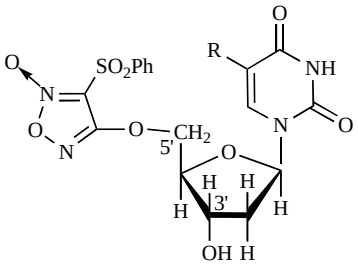
A plausible explanation for these results is that the high cytotoxicity exhibited by the hybrid nucleoside-·*NO* donor compounds **21** and **22** is due predominantly to the furoxan moiety, with only a minimal contribution from the nucleoside part. It is expected that the furoxans **23a** and **23b** exert their cytotoxic effect by an alternative mechanism such as DNA alkylation, since **23a**,**b** cannot serve as nucleotide substrates for incorporation into DNA catalyzed DNA polymerase. In this context, alkylation at the N^7- or O^6-atom of a guanine base in DNA could result in the covalent attachment of DNA to a furoxan-4-yl ring carbon by nucleophilic displacement of the 3-benzenesulfonyl moiety present in compounds **21a-e**, **22a-d**, and **23a**,**b** [51].

Very recently, Zhang, Tian and co-workers synthesized several novel furoxan-based nitric oxide releasing derivatives **24** and **25** (Fig. (**8**)) of oleanolic acid (OA) for potential therapy of liver cancers [52]. Compounds **24a-e** and **25a** exhibited strong selective cytotoxicity against HCC (human hepatocellular carcinoma) cells, which appeared to be dose-dependent and was assumed to be associated with high levels of NO production in HCC cells *in vitro*. For example, **24a**, at a dose of 1 μM promoted 100% cell death and 0.01 μM of it induced 13.34% cell death. Treatment with **24a** or **25a** greatly inhibited the growth of HCC tumors inoculated but did not cause obvious morphological changes in mouse liver. These data demonstrate that high levels of NO are toxic to HCC cells and provide also a proof-of-principle that furoxan/OA hybrids may be used for therapeutic intervention of human liver cancers.

Hybrid drug **26** (NO-ASA) continues to attract intense research from chemists and biologists alike. It consists of Aspirin (ASA) and a -ONO_2 group connected through a spacer and is in preclinical development as an antitumor drug (Fig. (**8**)). Wijtmans and co-workers recently reported

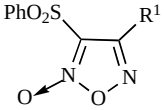


21a: R = H,
21b: R = CH_3,
21c: R = I,
21d: R = F,
21e: R = CF_3



22a: R = H,
22b: R = CH_3,
22c: R = I,
22d: R = F

1,2,5-oxadiazole 2-oxide
(furoxan ring)



23a: R^1 = SO_2Ph,
23b: R^1 = OCH_3

Fig. (7).

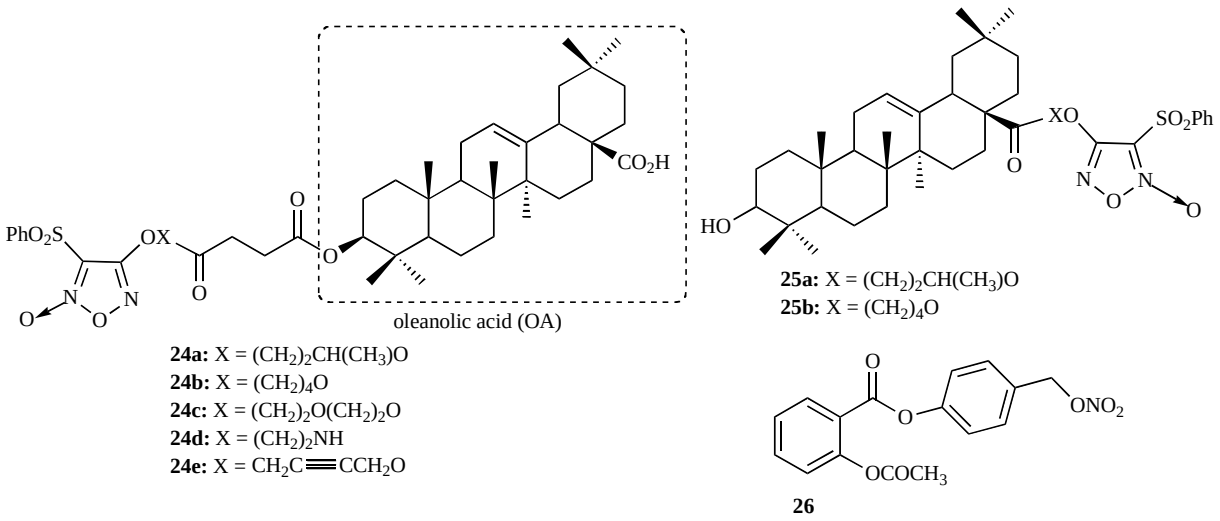


Fig. (8).

[53] that, contrary to current beliefs, neither ASA nor NO contributes to this antitumor effect. Rather, an unsubstituted quinone methide (QM) was identified as the sole cytotoxic agent. QM forms from **26** after carboxylic ester hydrolysis and, in accordance with the hard-soft-acid-base (HSAB) theory, selectively reacts with cellular glutathione (GSH), which in turn triggers cell death. Remarkably, a derivative lacking ASA and the -ONO_2 group was 10 times more effective than **26**. Thus, the data of Wijtmans and co-workers provided a conclusive molecular mechanism for the antitumor activity of **26**. Equally important, they show for the first time that a "presumed invisible" linker in a hybrid drug is not so invisible after all and is in fact solely responsible for the biological effect [53].

Recently, the Lee group prepared fifteen different taxoid conjugates by linking various anticancer compounds, including camptothecin (CPT), epipodophyllotoxin (EP), colchicine (COL), and glycyrrhetinic acid (GA), at the 2'- or 7-position on paclitaxel (TXL) through an ester, imine, amine, or amide bond [54]. Newly synthesized conjugates were evaluated for cytotoxic activity against replication of several human tumor cell lines. Among them, TXL-CPT conjugates, **27**–**29,** were more potent than TXL itself against the human prostate carcinoma cell line PC-3 (ED_{50} = 14.8, 3.1, 19.4 nM compared with 55.5 nM), and conjugate **29** was also eight-fold more active than TXL against the LN-CAP prostate cancer cell line (Fig. (**9**)). These compounds also possessed anti-angiogenesis ability as well as lower inhibitory effects against a normal cell line (MRC-5). Thus, conjugates **27**–**29** are possible antitumor drug candidates, particularly for prostate cancer.

Recently, Wang and co-workers have demonstrated that DC-81-indole conjugate agents **30**-**33** (Fig. (**10**)) have potent antitumor activity [55]. The cytotoxic studies of the hybrid agents on human melanoma A2058 cells indicate most of the hybrids induced higher cytotoxicity, better DNA-binding ability, an increase in the apoptotic sub-G1 population relative to compound DC-81. Moreover, DNA flow cytometric analysis shows that hybrids actively induce a marked loss of cells from the G2/M phase of the cell cycle, which progresses to early apoptosis. The hybrid agents were, thus, suggested being potent inducers of cell apoptosis in A2058 cells [55]. Very recently, again Wang and co-workers described a series of novel pyrrolo[2,1-c][1,4]benzodiazepine (PBD) hybrids linked with enediyne (**34**-**40**, Fig. (**10**)). Most of the hybrids on human cancer cell lines exhibited higher cytotoxicity, and an increase in the sub-G1population as compared to DC-81 [56]. The authors investigated whether DC-81-enediyne agents possess higher cytotoxicity than **33** on human 293T cells. Their data revealed that treatment of 293T cells with DC-81-enediyne resulted in a significant increase of annexin V binding, caspase-3 degradation, and p53 arrest and identified thereby more apoptotic cells than by application of **33** [56].

Following the discovery of the anti-malarial activity of artemisinin, researchers found that this drug and its synthetic dimers does not only show anti-malarial activity, but is also

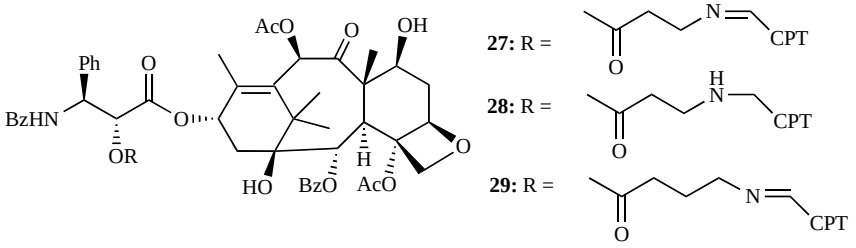


Fig. (9).

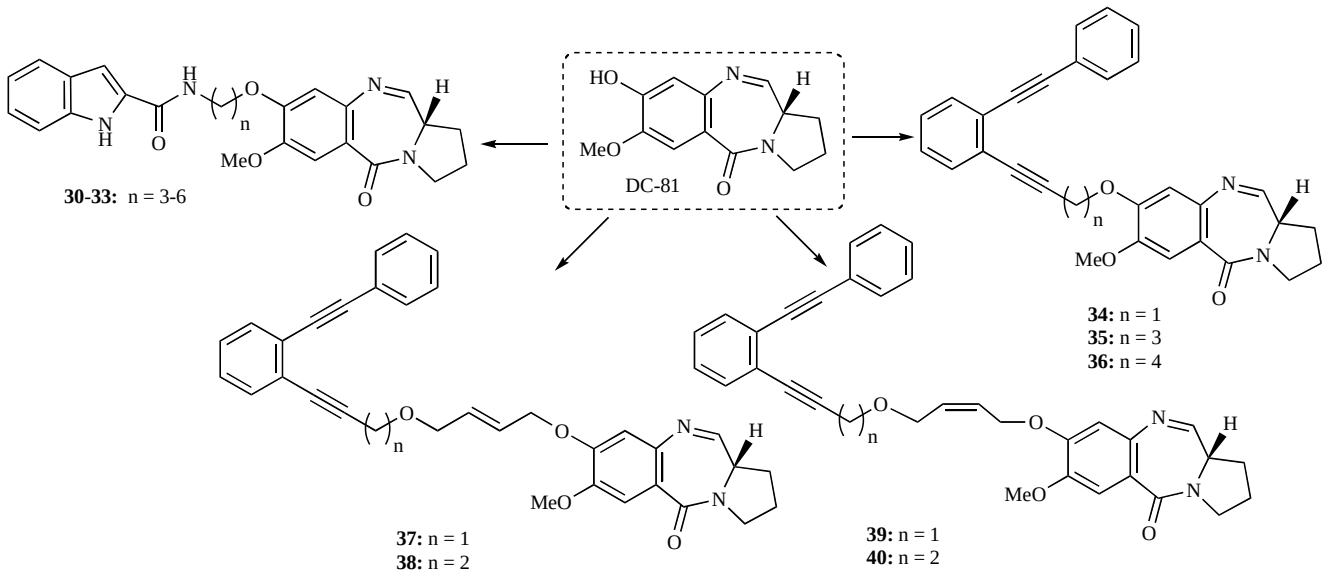


Fig. (10).

active against tumor cells [57, 58]. It has been reported that the anti-tumor activity of artemisinins is mediated by several pathways including inhibition of angiogenesis and induction of apoptosis [59].

Very recently, Efferth's team and our group in collaboration reported the synthesis of novel hybrids of artesunic acid and another cytotoxic natural product - betulin [60], as well as a homodimer of two artesunic acid molecules (**41**-**44** Fig. (**11**)) [61].

The activity of these compounds has been analyzed in a CCRF-CEM human leukemia cell line, as well as in a multidrug-resistant CEM/ADR5000 cells [61]. While artesunic acid-betulin hybrids **41** (IC_{50} (CCRF-CEM) = 31.9 ± 8.6 μM; IC_{50} (CEM/ADR5000) = 20.8 ± 3.5 μM) and **42**

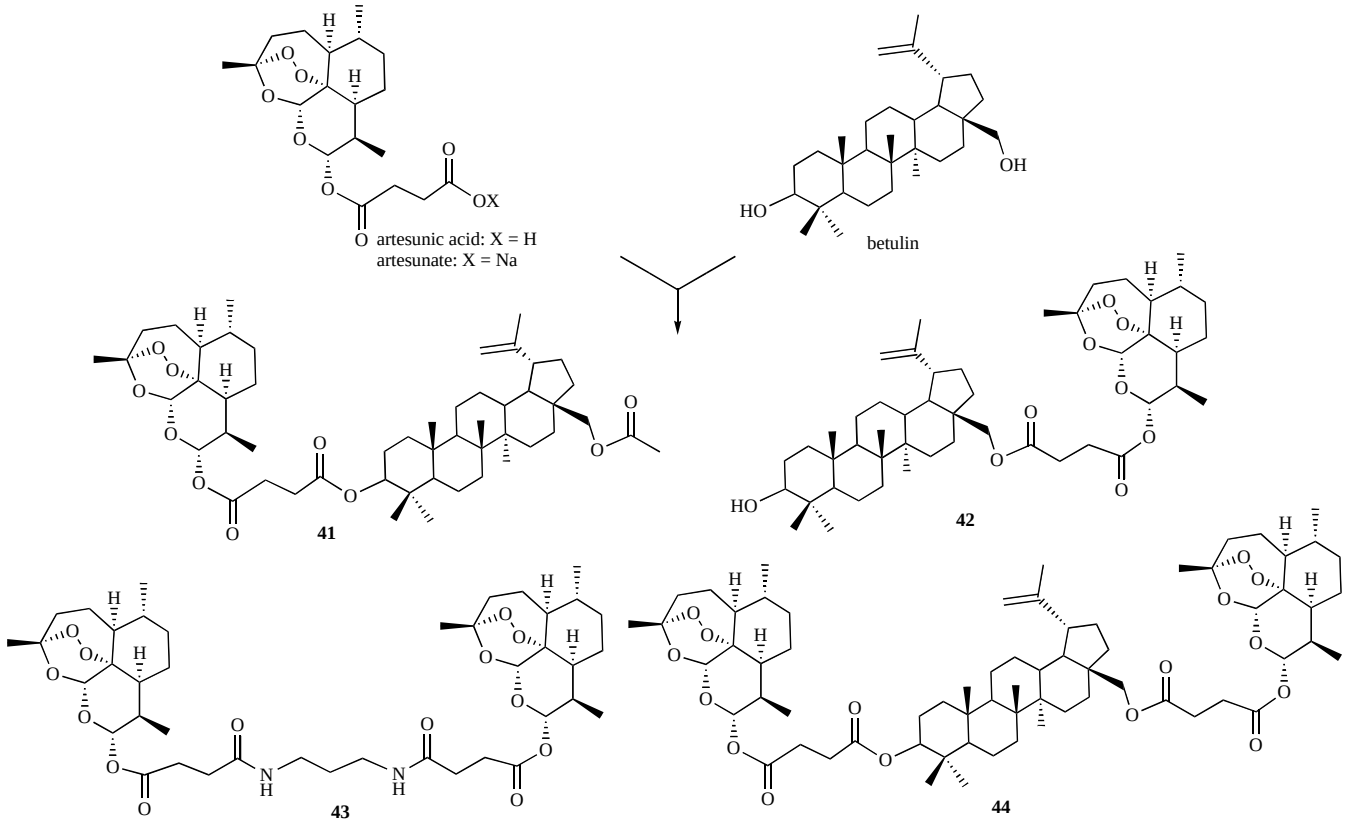


Fig. (11).

(IC_{50} (CCRF-CEM) = 9.7 ± 1.3 μM; IC_{50} (CEM/ADR5000) = 11.9 ± 4.1 μM) showed only promising cell growth inhibitory activity (reduced with respect to that of artesunic acid (IC_{50} (CCRF-CEM) = 1.8 ± 1.2 μM; IC_{50} (CEM/ADR5000) = 1.2 ± 0.7 μM), albeit improved as compared to betulin (IC_{50} (CCRF-CEM) = 50.8 ± 16.0 μM; IC_{50} (CEM/ADR5000) = 56.5 ± 8.6 μM), artesunic acid homodimer **43** showed encouraging cell growth inhibitory activity (IC_{50} (CCRF-CEM) = 1.2 ± 0.1 μM; IC_{50} (CEM/ADR5000) = 0.2 ± 0.03 μM) relative to artesunic acid alone. On the other hand, hybrid **44**, with two artesunic acid moieties grafted onto the betulin fragment, was shown to be less active (IC_{50} (CCRF-CEM) = 42.9 ± 6.9 μM; IC_{50} (CEM/ADR5000) = 42.1 ± 5.9 μM) than homodimer **43**, emphasizing the detrimental role of betulin itself, that acts as a mere rigid spacer, impairing consequently the interaction of the individual endoperoxide moiety with its respective target [61].

It was found that multidrug-resistant cells were not cross-resistant to the novel compounds, some of which were even collateral sensitive. The highest degree of collateral sensitivity was observed for artesunic acid homodimer **43**. Furthermore, induction of G0/G1 cell cycle arrest and apoptosis as well as the formation of reactive oxygen species were observed for artesunic acid homodimer **43**. These results constitute a promising new approach to generate novel active substances against sensitive and multidrug-resistant leukemia cells.

In recent years, several hybrid compounds, in which known antitumor compounds or simple active moieties of known antitumor agents been tethered to the DNA minor groove binder distamycin and netropsin frames to deliver alkylating functions to DNA targets, have been designed, synthesized and tested and discussed already in excellent general reviews. [1a, 62]

2. New Synthetic Anti-Alzheimer Hybrids

Alzheimer's disease (AD), the most common cause of dementia, is a complex neurological affliction that is clinically characterized by loss of memory and progressive deficits in different cognitive domains. The treatment of Alzheimer's disease involves mostly blocking the post-synaptic cleavage of acetylcholine in the cells signal transduction process [63]. One palliative drug is e.g. huprine X, a hybrid of huperzine A, isolated from club moss, and tacrine [64]. Because of its hepatotoxic side effects the use of this drug was discontinued, however, underlining the need to find further lead structures [65].

The consistent neuropathologic hallmark of the disorder in AD, generally noted on postmortem brain examination, is a massive deposit of aggregated protein breakdown products, amyloid-β (Aβ) plaques and neurofibrillary tangles [66]. Even if the primary cause of AD is still speculative, Aβ aggregates are thought to be mainly responsible for the devastating clinical effects of the disease.

Recently, Melchiorre and co-workers [67] demonstrated that the coupling of two different pharmacophores, each endowed with different biological properties, affords the hybrid compound lipocrine (**45**), whose biological profile was markedly improved (IC_{50} (AChE) = 0.253 ± 0.016 nM; IC_{50} (BChE) = 10.8 ± 2.5 nM) relative to those of prototypes tacrine (IC_{50} (AChE) = 424 ± 21 nM; IC_{50} (BChE) = 45.8 ± 3.0 nM) and lipoic acid (IC_{50} (AChE) = >1000000 nM; IC_{50} (BChE) = >1000000 nM) (Fig. (**12**)). Lipocrine is the first compound that inhibits the catalytic activity of acetylcholinesterase (AChE) and AChE-induced amyloid-β aggregation and protects against reactive oxygen species (ROS), which are supposed to be involved in CNS damaging effects [67]. Thus, it emerged as a valuable pharmacological tool to investigate Alzheimer's disease and as a promising lead compound for new anti-Alzheimer drugs.

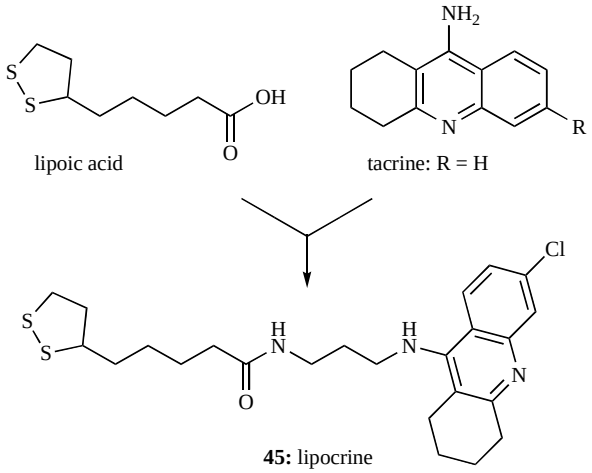


Fig. (12).

Tacrine and melatonin are well-known drugs with activities as an AChE inhibitor and free radical scavenger, respectively. Recently, Rodríguez-Franco and co-workers [68] have developed new tacrine-melatonin hybrids, which were potent inhibitors of human AChE and also showed high capacity for oxygen radical absorbance. Compound **46** (Fig. (**13**)) was the most potent inhibitor of human AChE (IC_{50} = 0.008 nM) described by these authors and that was 40000-fold more potent than tacrine (IC_{50} = 350 nM). It also displayed a remarkable selectivity, being about 1000-fold less

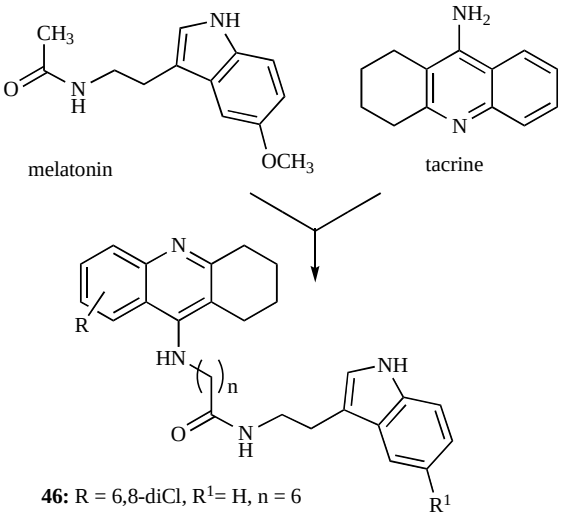


Fig. (13).

active toward human butyrylcholinesterase (BChE) with an IC_{50} of 7.8 nM. In the oxygen radical absorbance capacity assay, this compound performed 2.5-fold better than trolox (used as a standard), a vitamin E analog. Moreover, these tacrine-melatonin hybrids are also predicted to be able to enter the central nervous system. Although there were differences in potencies for AChE inhibition and antioxidant properties, these new compounds can be considered promising in the search for new lead structures of drugs for potential application in AD.

Whereas the activity of the less specific BChE stays the same or is increased, the AChE activity decreases progressively in certain brain regions during the course of AD to reach only 10–15% of the initial value [69]. Decker and coworkers have hence focused on the development of BChE selective inhibitors and identified quinazolinimines as a novel class of ChE inhibitors, which can either inhibit both cholinesterases or are highly selective for BChE with low micromolar or higher inhibitory activities [70, 71]. Very recently, Decker *et al*. succeeded in combining two distinct pharmacologically active moieties, that is, antioxidant lipoic acid and a micromolar unselective ChE-inhibiting [2,1-*b*]quinazolinimine, to hybrid molecules that retain their respective pharmacological behavior [72].

Depending on the length of the alkylene spacer, the amide hybrids **47** (Fig. (**14**)) are inhibitors of AChE with inhibitory activities of 0.5–4.6 μM and inhibitors of BChE with activities down to 5.7 nM, exceeding the inhibitory activities of the parent quinazolinimines by factors of up to 1000, respectively [73]. Due to the increasing activity with respect to BChE with increasing length of the alkylene spacer, an approximately 100-fold higher level of selectivity toward BChE is reached with a hepta- and an octamethylene spacer, as compared to the compound with a hexamethylene spacer. Kinetic measurements revealed competitive and reversible inhibition of both ChEs by the hybrids. Furthermore, cell viability and antioxidant activity of several hybrids were evaluated, showing cytotoxicity at concentrations from 3.7 to 10.2 μM and antioxidant properties in the range of 0.4–0.8 trolox equivalents (lipoic acid = 0.6). The concentration range to observe antioxidant properties *in vitro* (also for the parent compound lipoic acid as well) is though much higher

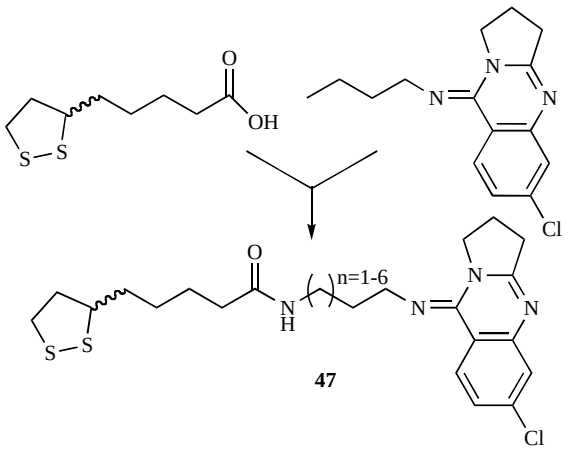


Fig. (14).

than for the ones necessary for inhibition of the enzymes, a problem often encountered with the design of hybrid molecules [73].

Very recently, Zhang, Decker and co-workers have designed and synthesized five novel hybrid compounds (**48a–e**) with an antioxidant ferulic acid moiety connected to the tacrine template *via* an alkylenediamine-type spacer (Fig. (**15**)) [74]. All compounds showed effective inhibition of ChEs *in vitro*. Two target compounds (**48c** and **48d**), in particular, showed 6- and 10- fold higher AChE inhibitory activity as compared to tacrine, respectively. The inhibitory mechanism of **48d** was analyzed by determining the Lineweaver–Burk plots. Most notably, the results indicated that the hybrid exerts a reversible and noncompetitive inhibitory action for AChE, whereas a reversible but competitive inhibitory action for BChE was observed. The former result strongly suggests the inhibition of the peripheral anionic site (PAS) of AChE and therefore the possibility to inhibit A*β*-peptide fibril formation also, but this has still to be unambiguously proven in additional investigations, the authors concede. All the target compounds were screened for their antioxidant activity using the ORAC-Fluorescein assay. Compared to trolox, most of the target compounds, except **48c**, showed a high ability to absorb reactive oxygen species. The results suggest that these tacrine-ferulic acid hybrids may be considered candidates of novel anti-Alzheimer's drugs, which are multi-target-directed ligands which possess not only ChE inhibitory (both acting at the active centre and the PAS of AChE), but also antioxidant activity [74].

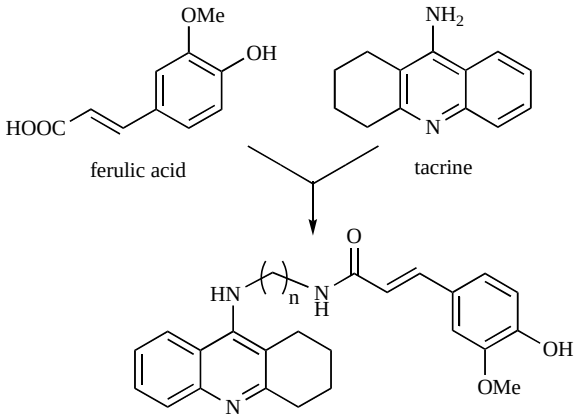


Fig. (15).

Recently, Zhang, Lehmann and co-workers [75] have prepared novel tacrine hybrid compounds with NO-donating nitrate and NONOate moieties connected to the tacrine template *via* - again - an alkylenediaminetype spacer (Fig. (**16**)). Three compounds (**50a-b**), in particular, showed 7 ~ 8-fold higher AChE inhibitory activity compared to tacrine, while compound **53** was found to be selective toward BChE rather than AChE. Compounds **49**, **50c**, **52** and **53** moderately relaxed the porcine pulmonary arteries in *in vitro* vasorelaxation experiments (organ bath), aided by the NO donor part of the molecule. While it was established in the *in vivo* studies

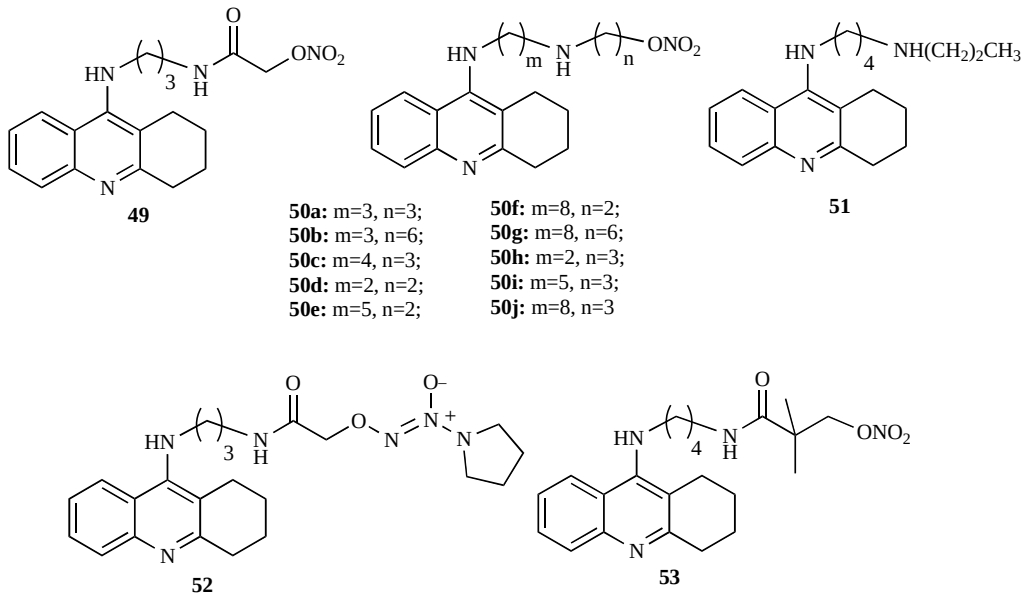


Fig. (16).

that tacrine possesses of serious hepatotoxicity, compound **50c** was found to be significantly less harmful. The results suggest that these NO-donor-tacrine hybrids, especially compound **50c**, may be considered to be novel more potent and safer anti-Alzheimer's drug candidates.

To investigate the influence of the alkylenediamine side chain of the molecules on their activity, Zhang and Lehmann groups [76] have further designed and synthesized new nitrate-tacrine hybrid compounds **50d-j** (Fig. (**16**)) with shorter and longer diamine side chains and evaluated for cholinesterase inhibitory activity, cognition improving activity, and hepatotoxicity. The pharmacological results indicated that hybrid compounds **49**, **50c** and **50d** potently inhibit cholinesterase *in vitro* and significantly improve the scopolamine-induced cognition impairment, whereas an analog (**51**) of **50c** without the NO donor moiety does not. Compared to tacrine, **49** and **50c** show much less hepatotoxicity. Molecular modeling studies suggest that **50c** may interact with the catalytic and the peripheral anionic site of acetylcholinesterase.

3. New Synthetic Anti-Malarial Hybrids

Approximately 40% of the world's population live in areas where they are at risk of malaria infection, causing more than one million deaths worldwide, mostly in children [77]. Due to the availability of several efficient "monomeric" synthetic or natural antimalaria drugs [78] like rifampicin, which blocks the apicoplastic protein synthesis in the parasite or of fosmidomycin, which interferes with the parasite-typical pathway for the biosynthesis of isoprenoids [79], the motivation to develop new hybrid drugs against malaria is not so pronounced as compared to other diseases. Hence, the efforts concentrate on the hybridization or simplification of the artemisinin framework, dimers of artemisinin derivatives or the combination of known drugs like in the combination of the 4-aminocholin lumefantrin with artemether (with an overlapping target spectrum) or the combination of the antifolat agent chlorproguanil with non-natural dapson (targeting the cytoplasm), a drug that has been earlier employed in the treatment of lepra [78a]. This demonstrates a further rationale behind the choice of appropriate monomer combinations: if the tethering of the monomers is not designed as being labile under metabolic conditions, the "point of attack" in the cell (cytoplasm, mitochrondrium, nucleus) or in specific organs must naturally be the same for both monomers, while the targeted reaction or process could be different (but could also be the same, e.g. because of different pharmacokinetic properties of the individual components).

Artemisinin is a relatively recent antimalarial drug, extracted from sweet wormwood (*Artemisia annua*), and that is effective even against multidrug-resistant strains of *Plasmodium falciparum* [80]. This sesquiterpene contains a 1,2,4-trioxane ring, and its endoperoxide function plays a key role in its biological activity. However, as artemisinin exhibits only poor solubility in both water and oil, vehicles are commonly used for drug administration, hemisynthetic derivatives obtained by reduction and functionalization of the lactone function of artemisinin are usually preferred (Fig. (**17**)). These compounds have been used for more than 20 years without reported cases of resistance [80a]. Among them, artesunic acid (Fig. (**11**)), which is the hemiester of succinic acid and dihydroartemisinin (or its sodium salt, artesunate), is used most widely [80b].

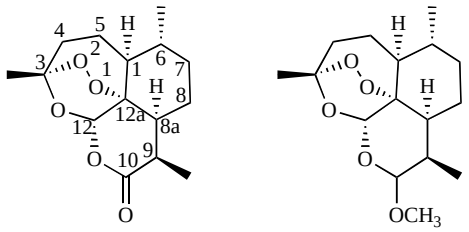


Fig. (17).

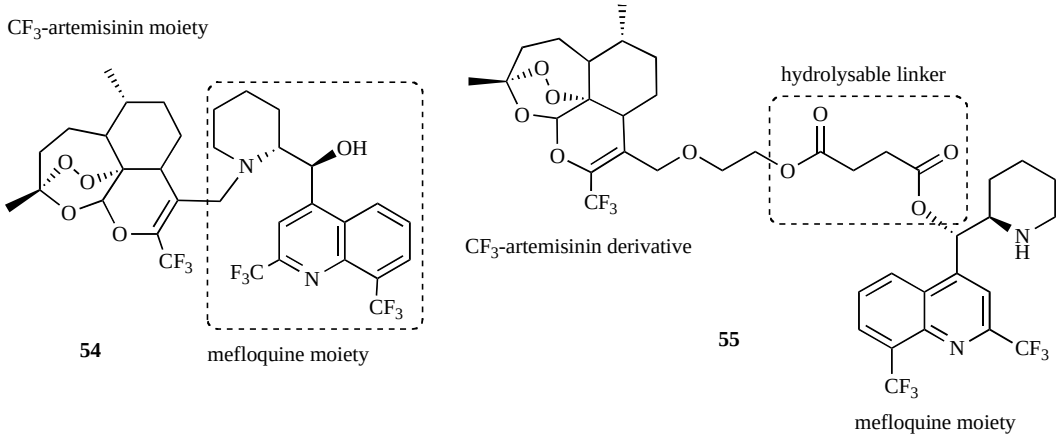


Fig. (18).

The currently available evidence [81-83] suggests that artemisinin and related peroxidic antimalarial drugs exert their parasiticidal activity following the reductive activation by heme, released as a result of hemoglobin digestion by the malaria-causing parasite. Free heme is known to be a powerful oxidant [84]. The irreversible redox reaction produces carbon-centered free radicals, leading to alkylation of heme [85] and certain proteins (enzymes) [86], one of which - the sarcoplasmic/endoplasmic reticulum ATPase PfATP6 [87] - may be critical to the survival of the parasite.

Unprecedented ways of combination therapy have been made possible by the appearance of a new class of drugs, artemisinin derivatives, in the "therapeutic arsenal" against malaria [88]. Indeed, artemisinin and its derivatives are not only the fastest-acting antimalarials known to date, but their tolerance and safety have been amply reported and, most importantly, their mode of action is unrelated to that of any other antimalarials [87, 89].

Recently, Bonnet-Delpon and co-workers designed and synthesized two new types of CF_3-artemisinin-mefloquine dual molecules **54** and **55** (Fig. (**18**)). They expected that these new dual molecules would reduce the risk of drug resistance by the non-overlapping mode of action of each moiety. In the indivisible chimera **54**, the CF_3-artemisinin moiety was covalently linked to the piperidinyl amine of the mefloquine [90].

In another hybrid, the divisible chimera **55**, was the CF_3-artemisinin derivative bound to the mefloquine *via* a diester linker, which was expected to be easily hydrolyzed *in vivo*. *In vitro*, compounds **54** and **55** showed an efficacy against four different strains of Plasmodium falciparum exhibiting different degrees of resistance to mefloquine and chloroquine in the low nanomolar range (IC_{50} values ranging from 2.4 to 17.2 nM) [90]. *In vivo*, both chimera **55** and to a lesser extent chimera **54**, were highly active, even more efficient in inhibition of parasite growth than artemether, used as reference drug.

Recently, Meunier, Benoit-Vical and co-workers prepared and investigated new hybrid antimalarial agents, trioxaquines, which are dual molecules that contain a trioxane linked to an aminoquinoline entity (DU1302, **56**) or to primaquine (DU2303, **57**) (Fig. (**19**)) [91, 92]. Trioxaquine derivative DU1302 showed efficient antimalarial activity *in vitro* on both sensitive and resistant strains of Plasmodium falciparum (IC_{50} = 5-19 nM).

The doses required to decrease parasitemia by 50% (ED_{50}) were 5 and 18 mg $kg^{-1}d^{-1}$ after intraperitoneal and oral administration, respectively. Parasitemia clearance was complete without recrudescence at an intraperitoneal dose of 20 mg $kg^{-1}d^{-1}$ [92].

It was also shown that the trioxane motif of these hybrid molecules was responsible for the artemisinin-like activity,

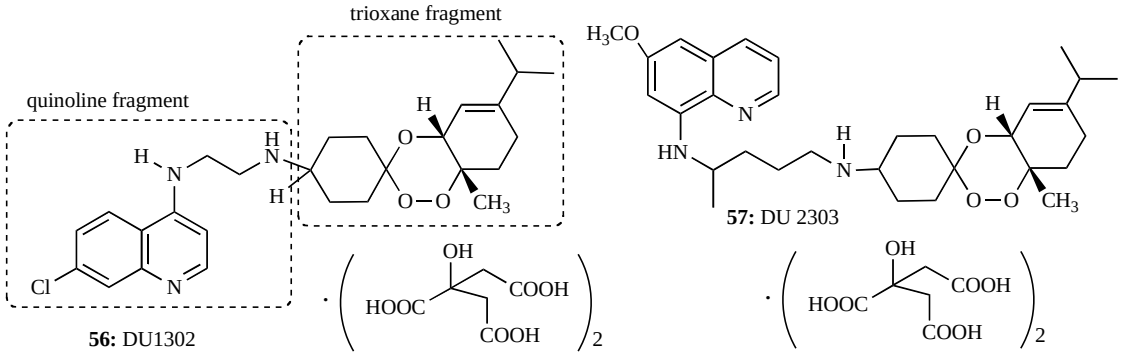


Fig. (19).

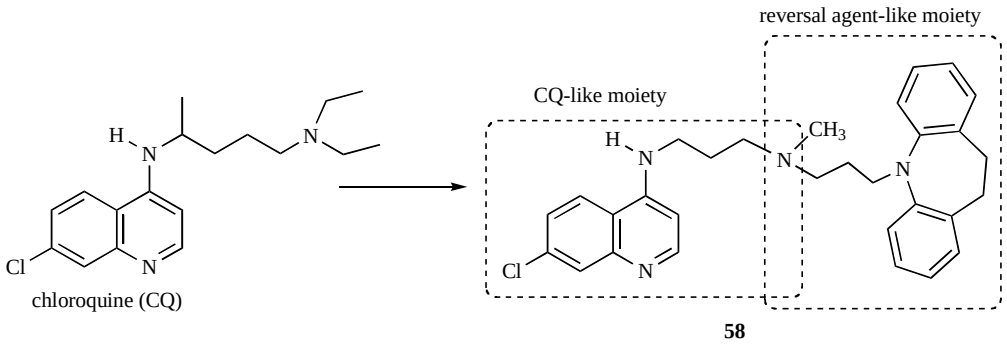


Fig. (20).

since a heme-trioxaquine adduct has been characterized which is similar to that obtained with artemisinin derivatives [93-95].

Moreover, Meunier and co-workers [91] have shown that DU1302 exhibits potent activity against gametocytes, the form transmitted by mosquitoes, as killing of the gametocytes is essential to limit the spread of malaria. Both hybrid molecules, DU1302 and DU2303 were shown to inhibit β-hematin formation [96].With such a biological profile, trioxaquines could be considered to be a model for new candidates in the arsenal of drugs able to fight malaria by treating individual symptoms and limiting parasite transmission.

Peyton and co-workers [97] designed a class of hybrid molecules which are termed "reversed chloroquines" (RCQs), and synthesized a prototype molecule **58** (Fig. (**20**)). They tested the hybrid against both chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum*. An *in vitro* assay against the two strains indicated that **58** was effective at low-nM concentrations against both strains (IC_{50} (D6) = 2.9 nM; IC_{50} (Dd2) = 5.3 nM). A preliminary study in mice demonstrated oral efficacy against *P. chabaudi* and the absence of obvious toxicity. The RCQ approach therefore appears to be feasible.

4. Miscellaneous

Hybrids of natural or unnatural bioactive compounds could be applied to the development of pharmaceuticals for the treatment of various other diseases. In a number of patho-physiological processes and in particular, in Parkinson's diseases, head trauma, as well as in peripheral pathologies like muscular dystrophy, cataract, cardiac ischemia, restenosis or arthritis, the involvement of calpains, members of the thiol protease superfamily, is implicated [98]. Most of these pathologies are associated with inflammatory processes and the production of free radical species such as reactive oxygen species (ROS). Recently, Auvin *et al.* [99] synthesized a series of molecules **59a-d, 60** (Fig. (**21**)) with dual inhibitory activities on calpain and lipid peroxidation. These hybrid compounds were built on the calpain pharmacophore 2-hydroxytetrahydrofuran linked to a set of antioxidants *via* a L-leucine linker [99]. Their studies demonstrated the synthetic feasibility of obtaining dual calpain/lipid peroxidation (LPO) inhibitors in spite of the sterically demanding antioxidant moiety. Calpain has been found to be very sensitive to the nature of the antioxidant group and 2-substituted phenothiazines proved to be superior to the other antioxidants in the calpain and LPO tests. Compound **59b**, not only a potent inhibitor of isolated calpain (IC_{50} = 22.5 nM) turned out to

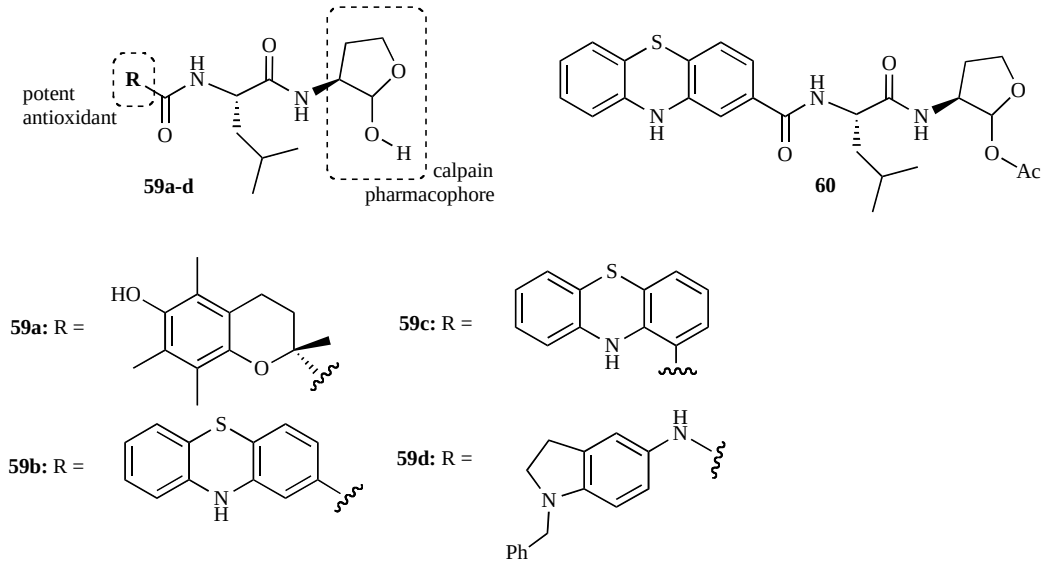


Fig. (21).

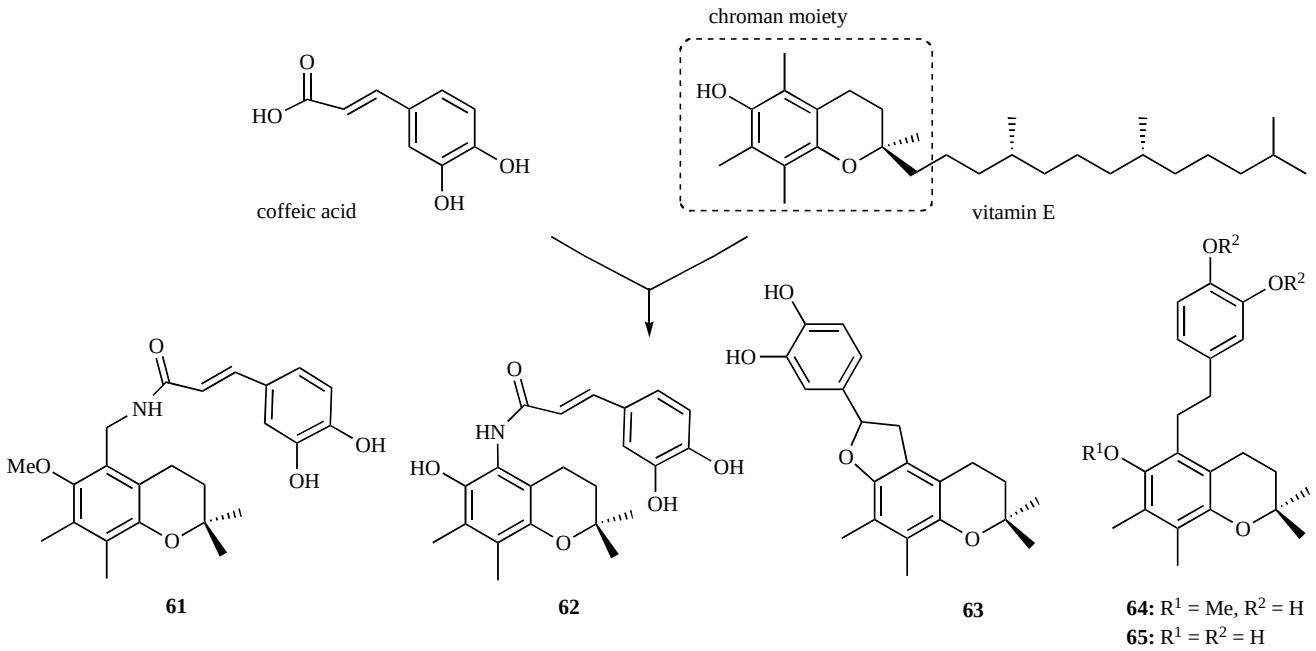


Fig. (22).

be also a powerful free radical scavenger (IC_{50} = 70 nM), with comparable activities in both tests. Compound **60**, a prodrug of **59b**, was shown to be superior to **59b** in cellular assays, and, in addition, these hybrid compounds were much more active than Z-LL-H (a calpain inhibitor) [100] in the protection from C6 glial cell death.

The progression of disease or oxidative damage may be retarded by the administration of exogenous protective compounds, which can act in several different ways, e.g. as chain breaking antioxidants, inhibitors of ROS formation, transition metal chelators or as free radical scavengers. Recently, Koufaki and co-workers synthesized new chroman/catechol hybrids **61**-**65** (Fig. (**22**)) possessing cytoprotective activity against oxidative stress [101]. The 5-substituted chromans **61**, **62** and **65** are very potent against H_2O_2- and glutamate-induced cellular damage with IC_{50} (Jurkat cells) = 1.0 ± 0.1, 5.0 ± 2.0, 5.0 ± 2.0 μM, respectively. Compounds **63** and **64** are less potent against DNA damage (IC_{50} (HT-22 cells) = 10.0 ± 2.0 μM each) than the above three analogs, but more active than the 2-substituted chromans, and they exhibit strong neuroprotective activity with EC_{50} = 1.00 ± 0.21 and 1.17 ± 0.15 μM, respectively. The authors anticipated that the activity of the chromans against DNA damage may be largely attributed to their iron-chelating properties [101].

Human disorders caused by prions, such as the most prevalent Creutzfeldt-Jakob disease (CJD) are rare neurodegenerative diseases that can be of sporadic, genetic, or infectious origin. Recently, Gmeiner and co-workers have introduced a novel family of structural hybrids including the iminodibenzyl derivative **66** (Fig. (**23**)) which demonstrates an unprecedented antiprion potency (EC_{50} = 20 nM). Even though the exact mechanism of antiprion action still remains unclear, SARs are in support of the specific mode of action of this family of compounds. The redistribution of cellular cholesterol from conversion-mandatory lipid rafts on the plasma membrane has been proposed by Gmeiner and co-workers to account for the antiprion actions of the heterocyclic compounds, including test compound **67** [102]. The best compound of this class found by the authors so far, **66**, has a 15-fold increased antiprion potency when compared to quinacrine, rendering it a new lead compound.

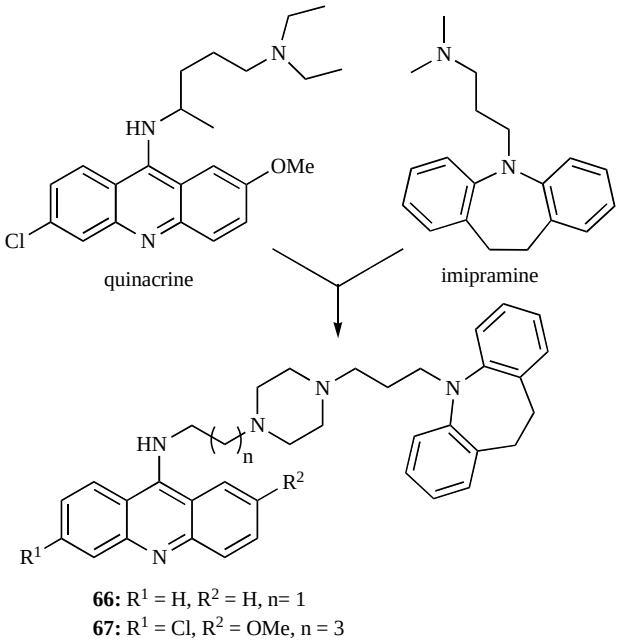


Fig. (23).

Steroids represent a valuable class of natural products, owing to their ability to penetrate cell membranes and bind to specific receptors. Tietze, Schneider and co-workers focused on the synthesis of a natural-product hybrid **68**, possessing the structural features of steroid estrone and myco-

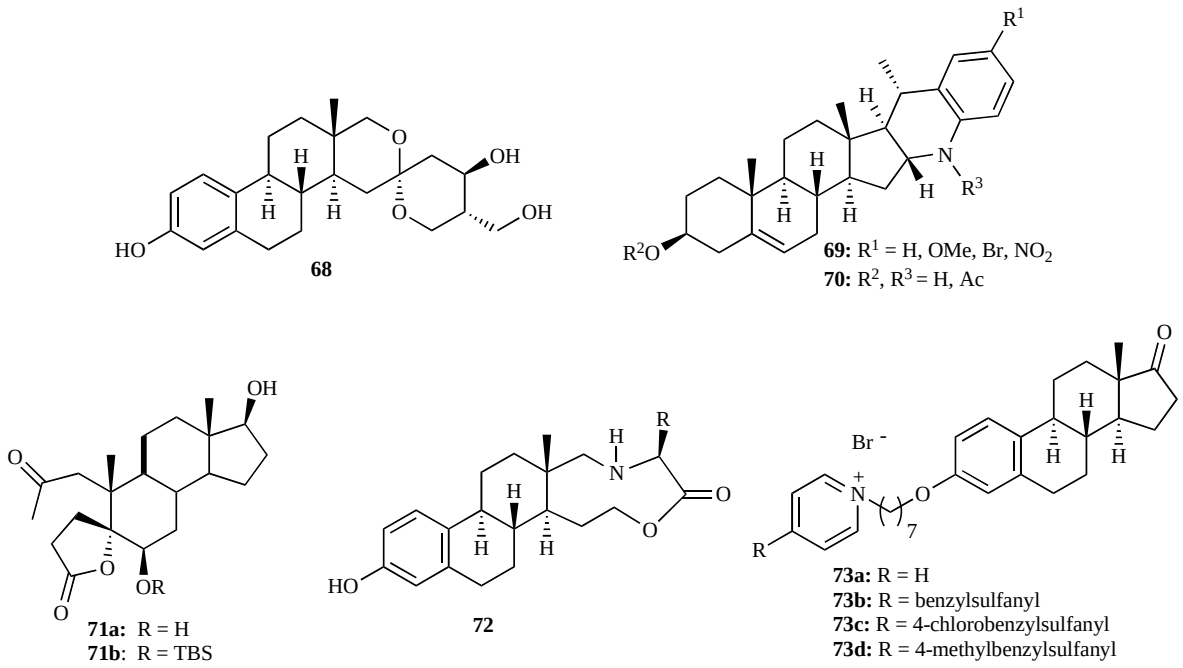


Fig. (24).

toxin talaromycin to design a new class of cytotoxic compounds (Fig. (**24**)) [103]. Schneider and co-workers have further synthesized novel steroid-tertrahydroquinoline hybrid molecules **69** and **70**, motivated by the insight that tetrahydroquinolines are important scaffolds in different natural compounds [104]. Very recently, Arseniyadis and co-workers focused on the construction of the hybrid framework **71**, being a synthetic hybrid of partial structures of natural products [105]. Its synthesis relied on an environmentally friendly domino reaction [106], developed to achieve practical access to a number of analogs for their biological evaluation [105].

A new prototype of steroid-amino acid hybrid **72** has been reported by Panda's group [107] derived from easily available estrone and amino acids, e.g. alanine, phenyl alanine and isoleucine, to give elaborate macrocyclic steroid-amino acid hybrids possessing interesting biological functions.

Recently, a series of new hybrid molecules of estrone with various heterocycles was prepared in the group of Adamec [108]. The heterocyclic moiety involved pyridine- or 4-benzylsulfanylpyridine derivatives (**73**, Fig. (**24**)). Their biological activity against various bacterial, mycobacterial, and fungal strains as well as the antiproliferative and cytotoxic activities were evaluated. For comparison, the precursors of the studied hybrid molecules were screened, too [108]. Adamec and co-workers found that the most active compounds are salts, indicating that the presence of a charge on the molecule was important for all the studied activities.

Not many biological tests of hybrids with antimicrobial activity have been reported so far. Very recently, Gademann and co-workers [109] have presented the synthesis, immobilization, and biological evaluation of the natural product hybrid **74** (Fig. (**25**)) for the generation of antimicrobial surfaces. This compound combines the properties of the two component natural products, namely that of the anachelin H, enabling strong binding to TiO_2 surfaces, and of vancomycin, responsible for the antimicrobial activity. Furthermore, the PEG linker contributes to cell resistance. In addition, the attachment of dead cells and cell material is suppressed. The advantages of the modification of surfaces with **74** include the simplicity of preparation by way of a dip-and-rinse procedure, its expressive antimicrobial activity against B. *subtilis* and cell-resistant properties, causing the suppression of attachment of dead cells and cell materials. Furthermore, there is strong surface binding through the anachelin chromophore and thus high activity even after several cycles.

CONCLUSIONS

Natural products are major sources for the development of new drugs. The sheer unlimited possibilities in combinations of different natural products or conjugations of unnatural bioactive compounds with naturally occurring substances can be exploited to generate molecules with unprecedented bioactivity, thus enabling the development of new lead compounds and discovery of novel drugs in the field of medicinal chemistry. Considerable progress in this field has been made in the last few years and this rather recent approach has already found applications in the development of new anticancer, anti-Alzheimer, anti-malaria and other therapeutics.

The concept based on the combination of pharmocophore fragments of natural compounds seems to have advantages, because numerous numbers of such hybrid structures can be designed and synthesized. The ever expanding contributions to the development of bioactive synthetic hybrids confirm that this field of research continues to be very attractive for drug development and further exciting discoveries of novel hybrid molecules are to be expected in the near future.

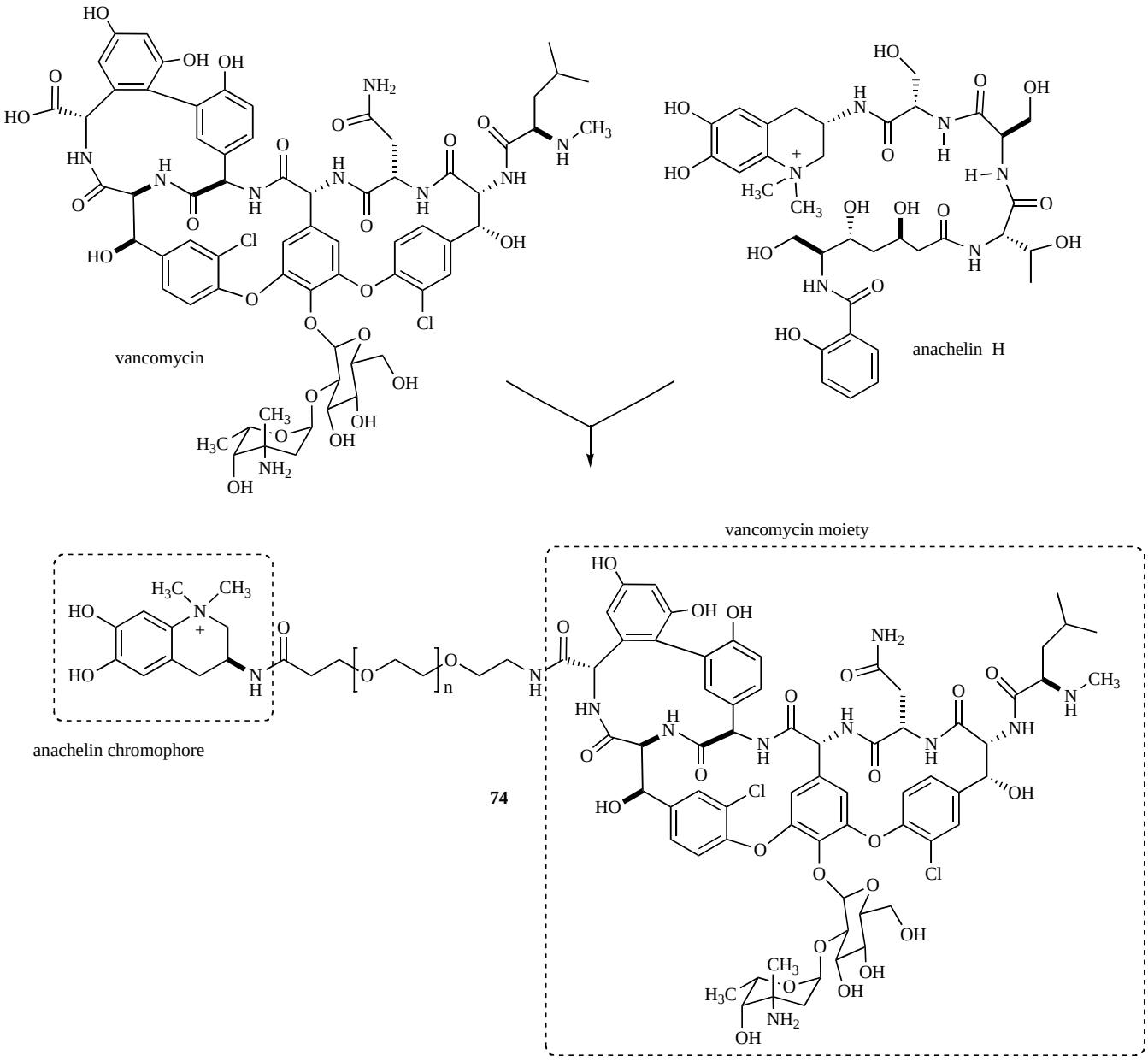


Fig. (25).

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