Cytotoxic and Antitumor Potentialities of Aporphinoid Alkaloids

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Abstract: Aporphinoids form an important group of plant secondary metabolites. Some of these compounds are used for a long time in traditional medicine for the treatment of various diseases, from benign syndromes to more severe illnesses. More than 500 aporphine alkaloids have been isolated from various plant families and many of these compounds display potent cytotoxic activities which may be exploited for the design of anticancer agents. Here we review the origin, biosynthesis, structure and cytotoxic properties of the prominent members of this class of compounds. Simple aporphinoids (boldine, dicentrine) as well as oxo-, pro- and dehydro-aporphines, and dimeric forms such as thalicarpine, are discussed here. Their mechanisms of action are not well known but DNA-manipulating enzymes such as polymerases and topoisomerases are among the most frequently cited targets for these benzylisoquinoline compounds. This review presents an updated view of the cytotoxic properties of the aporphinoids and their potential contribution to the development of anticancer agents.

Key Words: aporphine alkaloids, aporphinoids, anticancer agents, natural products, cytotoxicity.

INTRODUCTION

The use of plants as medicines goes back to early man. For a long time plants have been the almost exclusive therapy accessible to humans. Today most of the population in developing countries still rely on traditional medicine practitioners and local medicinal plants for primary health care [1, 2]. The diversity of the biosynthetic pathways in plants has provided a variety of lead structures that have been used in drug development and account for more than 50 % of our current medicines. Nowadays, the plant kingdom remains an essential source of new molecules with therapeutic potential [2].

During the last decades, works on natural compounds have been particularly successful in the field of anticancer drug research. In the US, between 1983 and 1994, more than 60% of the approved anticancer drugs were from natural origin [3]. A typical example is that of camptothecin, a monoterpenoid alkaloid isolated from the Chinese ornamental tree *Camptotheca acuminata* in the sixties from which two derivatives irinotecan (a pro-drug) and topotecan, less toxic and more stable than camptothecin itself, were developed and are currently used as anticancer agents, for the treatment of colorectal and ovarian cancers in particular. Taxols (paclitaxel and the semi-synthetic derivative docetaxel), *Vinca* alkaloids (vinblastine, vincristine, and the semi-synthetic derivative vinorelbine), podophyllotoxins (the semi-synthetic derivatives: etoposide and teniposide), are also among the most frequently used anticancer drugs [4-6].

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Alkaloids (generally defined as nitrogen-containing natural molecules independently of the basic character of the nitrogen) are abundant secondary metabolites in plants and represent one of the most widespread class of compounds endowed with multiple, varied pharmacological properties. Among alkaloids, the aporphinoids constitute a broad subgroup of benzylisoquinoline compounds, with more than 500 alkaloids isolated up to now. They are widely distributed in a large number of plant families including Annonaceae, Lauraceae, Monimiaceae, Menispermaceae, Hernandiaceae, Ranunculaceae, to cite a few [7]. Since 1975 a few reviews on chemical structures, spectral data, botanical sources and pharmacological activities have been published on aporphinoids, including proaporphines, aporphines, and related naturally occurring derivatives [8-14]. The present review presents an updated view of this family of alkaloids, with specific emphasis on their potential development as anticancer agents.

Chemically speaking, aporphines (*sensu stricto*) are tetracyclic bases formed by direct bonding of the A and D aromatic rings of the typical benzylisoquinoline nucleus. The structures of a variety of aporphines (together with the most commonly used numbering system for the skeleton) are presented in Fig. (**1**) and Fig. (**2**). The nitrogen atom at position 6 is usually tertiary in the base form but may also be quaternary, less frequently acetylated or formylated. *N*-oxide compounds have also been described. The alkaloid is named noraporphine when the nitrogen is secondary. In natural aporphines, positions 1 and 2 are always substituted by hydroxyl, methoxy, or methylenedioxy groups. The tetracyclic core can be substituted in different places, at positions 9, 10 and 11, and less frequently at positions 3 and 8, and in a few cases, the position 7 (or 4) is oxygenated. Aporphines are optically active, possessing either the R-(-) or S-(+)

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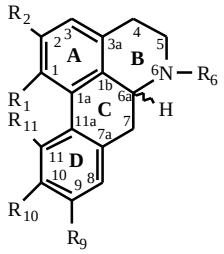
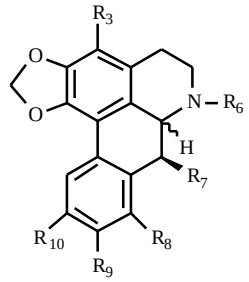


N°	Compound	R_1	R_2	R_6	R_9	R_{10}	R_{11}
1	Actinodaphnine	O-CH_2-O		H	OH	OCH_3	H
2	Anonaine	O-CH_2-O		H	H	H	H
3	Apomorphine	H	H	CH_3	H	OH	OH
4	Boldine	OCH_3	OH	CH_3	OH	OCH_3	H
5	Bulbocapnine	O-CH_2-O		CH_3	H	OCH_3	OH
6	Cassythicine	O-CH_2-O		CH_3	OH	OCH_3	H
7	Corydine	OH	OCH_3	CH_3	H	OCH_3	OCH_3
8	Cryptodorine	O-CH_2-O		H	O-CH_2-O		H
9	Dicentrine	O-CH_2-O		CH_3	OCH_3	OCH_3	H
10	Glaucine	OCH_3	OCH_3	CH_3	OCH_3	OCH_3	H
11	Hernovine	OCH_3	OH	H	H	OH	OCH_3
12	Isocorydine	OCH_3	OCH_3	CH_3	H	OCH_3	OH
13	Isolaureline	O-CH_2-O		CH_3	OCH_3	H	H
14	Laurotetanine	OCH_3	OCH_3	H	OH	OCH_3	H
15	Magnoflorine	OH	OCH_3	$(CH_3)_2$	H	OCH_3	OH
16	Neolitsine	O-CH_2-O		CH_3	O-CH_2-O		H
17	*N*-hydroxyovigerine	O-CH_2-O		OH	H	O-CH_2-O	
18	*N*-methylhernangerine	O-CH_2-O		CH_3	H	OH	OCH_3
19	*N*-methylhernovine	OCH_3	OH	CH_3	H	OH	OCH_3
20	*N*-methyllaurotetanine	OCH_3	OCH_3	CH_3	OH	OCH_3	H
21	*N*-methylovigerine	O-CH_2-O		CH_3	H	O-CH_2-O	
22	Nordicentrine	O-CH_2-O		H	OCH_3	OCH_3	H
23	Nornantenine	OCH_3	OCH_3	H	O-CH_2-O		H
24	Nornuciferine	OCH_3	OCH_3	H	H	H	H
25	*O*-methylbulbocapnine	O-CH_2-O		CH_3	H	OCH_3	OCH_3
26	Ovigerine	O-CH_2-O		H	H	O-CH_2-O	
27	Phanostenine	O-CH_2-O		CH_3	OCH_3	H	H
28	Roemerine	O-CH_2-O		CH_3	H	H	H
29	Roemeroline	O-CH_2-O		CH_3	OH	H	H
30	Roemrefidine	O-CH_2-O		$(CH_3)_2$	H	H	H
31	Xylopine	O-CH_2-O		H	OCH_3	H	H

Fig. (1). Structures of selected aporphine alkaloids.

absolute configuration, depending on the stereochemistry of C6a.

Aporphinoids include aporphines and biogenetically related alkaloids such as proaporphines and catabolic derivatives, such as oxoaporphines and also phenanthrenes which are the most common degradation products for these compounds. Dimeric forms and dehydroaporphines, characterized by an additional unsaturation at C6a, are included in this group. Different examples of each category are presented here (Fig. (**3**) to Fig. (**9**)), but before that, we shall refer briefly to the biosynthetic pathways leading to aporphines.



N°	Compound	R_3	R_6	R_7	R_8	R_9	R_{10}
32	Norannuradhapurine	H	H	H	OH	OCH_3	H
33	Cassythine	OCH_3	H	H	H	OH	OCH_3
34	Oliveridine	H	CH_3	OH	H	OCH_3	H
35	Stephalagine	OCH_3	CH_3	H	H	H	H

Fig. (2). Structures of four other aporphine alkaloids.

BIOSYNTHESIS OF APORPHINES

The precursor of the majority of the isoquinoline alkaloids is *S*-reticuline, a tetrahydrobenzylisoquinoline deriving from the skeleton formed from two tyrosine units. The condensation of these two units, catalyzed by *S*-norcoclaurine synthetase, gives *S*-(+)-norcoclaurine. *O*-Methylation at position 6 and *N*-methylation yield *N*-methylcoclaurine. 3'-and 4' Hydroxylations and 4'-*O*-methylation lead to the tetraoxygenated *S*-(+)-reticuline, the key intermediate and the common building block for the aporphine alkaloids. Aporphines are formed in plants by direct intramolecular oxidative coupling (*ortho-ortho* or *ortho-para*) of *S*-(+)-reticuline from the bisdienone radical form.

The substitution pattern of the tetrahydrobenzylisoquinoline precursor gives rise to the corresponding aporphines, although certain positions of *O*-substitution, such as C-3 or C-7, arise by oxidation of the aporphinoid nucleus. Methylation at C-7 can be induced by the action of *S*-adenosyl methionine. Alternatively, aporphines can also originate from a proaporphine intermediate by the cyclization of an *ortho-para* tetrahydroisoquinoline diradical, direct protonation and subsequent dienone-phenol rearrangement [7, 15].

PHARMACOLOGICAL PROPERTIES

Two aporphines are available on the market as pharmaceutical products [7]. One is boldine (**4**), isolated from the leaves and bark of the South American tree *Peumus boldus* [7, 16], which possesses well-established free radical scavenger properties (antioxidant) and increases of bile secretion (choleretic). The indications in human medicine are minor hepatobiliary dysfunction, symptomatic treatment of mild digestive disturbance and as an adjuvant in constipation [7, 14, 16]. The other is apomorphine, a synthetic alkaloid (**3**), which is considered to be a classical mixed type dopamine D_1 and D_2 receptor agonist. It has been used in the therapy of Parkinson's disease and, more recently, for the treatment of erectile dysfunction more commonly known as impotence in men, by enhancing the natural erection process [17]. Apomorphine is a synthetic product obtained from morphine after treatment in hot acidic medium [7].

Aporphinoids exhibit a wide range of biological properties [13, 14]. Some of them are good dopaminergic agents and may have activity on adrenergic and serotonergic transmissions. Some aporphinoids also possess a vasodilator effect (by inhibition of extracellular calcium entry and sometimes tissue-specific modifications of intracellular calcium movements) and anti-platelet activities. Anti-oxidative properties of phenolic and non-phenolic aporphines have also been described as well as antimicrobial, antiviral and cytotoxic activities. Here we have focused our attention on the cytotoxicity and the antitumor potentialities of this type of molecules. We reviewed the cytotoxic and antitumor data available in the literature and we analyzed the potential modes of action of these aporphine compounds, which as we shall see, remain poorly defined. Whenever possible, the structure-activity relationships have also been investigated. On purpose we have restricted our survey to aporphinoids and closely related compounds. Bisbenzylisoquinolines, another subgroup of isoquinoline alkaloids which also possess antitumor potentialities, are not covered here nor phenanthrenes, as their structures are clearly different from the other aporphinoids.

CYTOTOXIC AND ANTICANCER ACTIVITIES

Aporphines

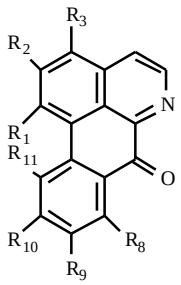
The cytotoxic effects of almost 50 compounds, alkaloids and lignans, isolated from the trunk bark of *Hernandia nymphaeifolia* (Hernandiaceae) were tested against four tumor cell lines *in vitro*: P388 leukemia, human mouth epidermoid KB16 cells, A549 lung and HT-29 colon cells [18, 19]. Among the compounds evaluated, the two aporphines *S*-ovigerine (**26**), *S*-*N*-methylovigerine (**21**) (Fig. (**1**)) showed general cytotoxic activities (IC_{50} values < 4 μM) against the four cell lines. *S*-magnoflorine (**15**) and *S*-hernovine (**11**) both showed selective cytotoxicities against

the P388 cell line (IC_{50}: 0.7 µM). Additionally, some compounds exhibited cytotoxicities close to that of mithramycin, a reference anticancer agent, on one cell line or the other, such as *S*-*N*-hydroxyovigerine (**17**) on KB16 cells and *S*-*N*-methylhernangerine (**18**) and *S*-laurotetanine (**14**) against P388 cells. The authors also showed that the noraporphine *S*-hernovine (**11**) exhibited more potent cytotoxic activity (IC_{50}: 0.7 µM on P-388 cells, 20 µM on HT-29 cells and around 45 µM on KB16 and A549 cells) than its methylated analogue *S*-*N*-methylhernovine (**19**) which is inactive on all tested cells (IC_{50} > 153 µM) [18, 19]. This is in accordance with the results of Munoz *et al.* [20] who showed that *S*-roemrefidine (**30**), a quaternary alkaloid isolated from the Bolivian vine S*parattanthelium amazonum* (Hernandiaceae) showed no cytotoxicity against three cell lines (KB, Hep-2 and HeLa). It is therefore suspected that the quaternization of the ring nitrogen is detrimental to the cytotoxic activity. This is also supported by the results of Tzeng *et al.* [21]. They screened a number of benzylisoquinoline alkaloids, either isolated from plants indigenous to Formosan or obtained by partial synthesis, for their cytotoxic activities on a murine (L1210) and two human (CCRF-CEM and HL-60) leukemia cell lines [21]. In parallel, all compounds were evaluated for their ability to inhibit the incorporation of radioactive precursors into DNA, RNA and proteins at a fixed concentration (30 µg/ml). The tertiary alkaloid dicentrine (**9**) displayed moderate cytotoxicity against the three cell lines (IC_{50} around 30 µM) while the quaternary derivative dicentrine MeI, chemically obtained, was found to be totally inactive on the cell growth and on the inhibition of macromolecule biosynthesis. The most active aporphine tested was *R*-norannuradhapurine HBr (**32**) (Fig. (**2**)) which exhibited a broad spectrum of growth inhibitory activities against the three aforementioned murine and human leukemic cells, with IC_{50} values around 3 µM and had strong inhibitory effects on DNA, RNA and proteins biosynthesis. However, no precise mechanism of action was described [21]. Glaucine HBr (**10**) and *O*-methylbulbocapnine (**25**) also showed little inhibitory activity on the radioactive precursor incorporation into DNA, RNA and proteins and did not inhibit cell growth (IC_{50} > 85 µM on the three tested cell lines). But no clear structure-activity relationships could be extracted from the study.

The *in vitro* antiplasmodial, antiamoebic, and cytotoxic activities of some monomeric isoquinoline alkaloids have been reported [22]. Among the different aporphine alkaloids tested, *S*-corydine (**7**) and *S*-isocorydine (**12**) proved to be non toxic to KB cells (human carcinoma of the nasopharynx) [22]. This is consistent with a previous report in which these two alkaloids were found to be inactive *in vivo* against the Walker 256 tumor [23] and in another study which also indicated that *S*-corydine (**7**) was inactive on KB cells [24]. In contrast, the results differ from those published in another study [25] on the antiproliferative effects of the three *S*-aporphines dicentrine (**9**), glaucine (**10**), corydine (**7**) and *R*-apomorphine (**3**) using five mouse tumor cell lines. Corydine (**7**) showed some activities while apomorphine (**3**) strongly inhibited the proliferation of all five cell lines tested and its inhibitory activity (IC_{50} values from 3 to 10 µM) was at least 4 times higher than the other three aporphine alkaloids except for the growth inhibition of Colon 26 by glaucine (**10**). In an *in vivo* situation, the *i.p.* treatment of mice bearing P388 tumors with apomorphine (**3**) resulted in a slight prolongation of the survival time. These four aporphines inhibit the mitogen-induced lymphocyte proliferation as well as the growth of IL-2 dependent $CTLL_2$ line in a dose-dependent way [25].

The cytotoxic (*in vitro*) and anti-tumor (*in vivo*) effects of *S*-dicentrine (**9**), isolated from *Lindera megaphylla* (Lauraceae), have been evaluated with human tumor cells [26]. This compound significantly inhibits the growth of the human hepatoma cell line HuH-7, inducing a marked delay in its doubling time in tissue culture. At a concentration of 14.7 µM, *S*-dicentrine (**9**) decreases the colony formation efficiency in HuH-7 and MS-G2 hepatoma cells. The biosynthesis of DNA and RNA was also strongly inhibited in a dose-dependent manner. The *in vitro* cytotoxicity of **9** towards 21 human tumor cell lines originating from seven different tissues showed that this molecule exhibits a cytotoxic effect in all tested cell lines, with IC_{50} values ranging from 0.4 µM on the oesophageal carcinoma cell line HCE-6 to up to 29 µM on the hepatoma cell line HA22T. The *in vivo* antitumor evaluation revealed that **9**, intraperitoneally injected twice a week for 4 weeks at a dose of 100 µg/mice, significantly inhibits the growth of K562 cells in SCID mice. All these findings provide direct evidences that this compound exhibits cytostatic effects and has potential antitumor applications [26]. Several other studies have dealt with the cytotoxic evaluation of dicentrine (**9**). Shen and co-workers evaluated its cytotoxic potential on one human non-cancerous cell line and three human cancer cell lines. Dicentrine (**9**) showed IC_{50} values from 4.6 to 21.8 µM on the different tested cell lines [27]. In another study, seventy-four alkaloids isolated from several families of Formosan plants were evaluated for their antimicrobial and antitumor properties [28]. Among the aporphines tested anonaine (**2**), actinodaphnine (**1**), *N*-methylactinodaphnine (cassythicine) (**6**), dicentrine (**9**) and glaucine (**10**) showed cytotoxic effects on P3HR-1, MK-2, and HEP-2 cells. Actinodaphnine (**1**) moderately inhibited the growth of sarcoma-180 implanted sub-cutaneously into mice. It has been proposed that this compound acts on a common site (as yet unidentified) in bacterial, mammalian, and tumor cells [28].

For our part, we showed recently that the alkaloid extract of *Cassytha filiformis* (Lauraceae) and the isolated *S*-aporphines dicentrine (**9**), neolitsine (**16**), actinodaphnine (**1**) and cassythine (**33**) (Fig. (**2**)) possess *in vitro* cytotoxic properties on HeLa, Mel-5, HL60 cancer cell lines and on NIH3T3 non cancer cells [29]. Unsurprisingly, like the majority of cytotoxic agents, these molecules do not display a selective action on cancer cells but mainly inhibit the growth of rapidly proliferating cells whatever their tumorigenic status. Compound **16** was the most active molecule against HeLa and NIH3T3 cells (IC_{50} of 21.6 µM and 21.4 µM, respectively) while cassythine (**33**) and actinodaphnine (**1**) showed the highest activity against Mel-5 (IC_{50} of 24.3 µM and 25.7 µM, respectively) and HL-60 (IC_{50} of 19.9 µM and 15.4 µM, respectively) [29]. We also compared the *in vitro* cytotoxic (on HeLa cells) and antitrypanosomal (on *Trypanosoma brucei brucei*) activities of three *S*-alkaloids isolated from *C. filiformis*, dicentrine (**9**), cassythine (**33**) and actinodaphnine (**1**) and four commercially available *S*-



N°	Compound	R_1	R_2	R_3	R_8	R_9	R_{10}	R_{11}
36	Atherospermidine	O-CH_2-O		CH_3	H	H	H	H
37	Dicentrinone	O-CH_2-O		H	H	OCH_3	OCH_3	H
38	Hernandonine	O-CH_2-O		H	H	H	O-CH_2-O	
39	Hernanymphine	H	OCH_3	H	H	O-CH_2-O		H
40	10-Hydroxyliriodenine	O-CH_2-O		H	H	H	OH	H
41	Kuafumine	O-CH_2-O		OCH_3	OCH_3	OCH_3	H	H
42	Lauterine	O-CH_2-O		H	H	H	OCH_3	H
43	Liriodenine	O-CH_2-O		H	H	H	H	H
44	Lysicamine	OCH_3	OCH_3	H	H	H	H	H
45	*O*-methylatheroline	OCH_3	OCH_3	H	H	OCH_3	OCH_3	H
46	*O*-methylmoschatoline	OCH_3	OCH_3	OCH_3	H	H	H	H
47	Oxoglaucine	OCH_3	OCH_3	H	H	OCH_3	OCH_3	H
48	Oxophoebine	OCH_3	OCH_3	OCH_3	H	O-CH_2-O		H
49	Oxopurpureine	OCH_3	OCH_3	OCH_3	H	OCH_3	OCH_3	H
50	Oxoxylopine	O-CH_2-O		H	H	OCH_3	H	H

Fig. (3). Structures of selected oxoaporphines.

aporphines, bulbocapnine (**5**), glaucine (**10**), isocorydine (**12**) and boldine (**4**) [30]. In order to elucidate their mechanism of action, the binding mode of these molecules to DNA was studied by UV absorption, circular and linear dichroism spectroscopies. The results of the optical measurements indicated that all seven aporphines effectively bind to DNA and behave as typical intercalating agents. Biochemical experiments showed that among the series, only the most trypanocidal compounds (actinodaphnine (**1**), cassythine (**33**) and dicentrine (**9**)) interfere with the catalytic activity of topoisomerases, but not glaucine (**10**) which was the most cytotoxic compound in this series on HeLa cells (IC_{50}: 8.2μM) [30].

In addition, *S*-dicentrine (**9**) was shown by another team to have a significant activity against DNA topoisomerase II. It inhibits the catalytic activity of the enzyme, most likely because of its binding to the DNA target of the enzyme, but it does not stabilize topoisomerase II-DNA covalent complexes, as it is the case with the typical "poisons" such as the antitumor drug etoposide. DNA unwinding assays suggested that this compound intercalates into DNA [31]. *S*-bulbocapnine (**5**), which differs from *S*-dicentrine (**9**) only by the presence of a hydroxyl group at position 11 and the absence of a methoxy group at position 9, was inactive in all the assays. These two aporphines are non-planar molecules, lacking features normally associated with DNA interaction. Molecular modeling showed that dicentrine (**9**) can take a relatively planar conformation, whereas bulbocapnine (**5**) cannot, due to steric interactions between the 11-hydroxyl group and an oxygen of the methylenedioxy ring. The requirement for a sub-optimal conformation to achieve DNA binding appears to make **9** a weaker topoisomerase II inhibitor than the very planar oxoaporphine liriodenine (**43**) (Fig. (**3**)). These results suggest that it may be possible to modulate the DNA binding and the biological activity of these aporphinoids by affecting their ability to adopt planar conformations [31]. Nevertheless, Zhou *et al.* showed that *S*-dicentrine (**9**) isolated from *Ocotea leucoxylon* (Lauraceae), was inactive in a yeast assay for DNA-damaging agents [32]. The mechanism of action of this compound remains largely unknown at present.

While evaluating the inhibitory effects of *S*-bulbocapnine HCl (**5**) on dopamine biosynthesis in PC12 cells, Shin *et al.* showed it had no cytotoxic effects on PC12 cells at concentrations up to 80 μM [33].

Different aporphines isolated from *Stephania dinklagei* (Menispermaceae) were evaluated for their DNA damaging activities by using a yeast-based microtiter assay on several mutant strains lacking some DNA repair mechanisms. *S*-

corydine (**7**) had DNA-damaging properties whereas the other compounds tested *S*-isocorydine (**12**) and *S*-stephalagine (**35**) (Fig. (**2**)) did not show any activity in the yeast bioassay [34].

The bioactivity-directed fractionation of the ethanol extract of *Stephania pierrei* (Menispermaceae) led to the isolation of 23 isoquinoline alkaloids among which some aporphines [35]. The antimalarial property and cytotoxic activities of these compounds against a large variety of mammalian cancer cell lines were evaluated. The cytotoxicity of *S. pierrei* extracts was mainly attributed to the presence of aporphine alkaloids, especially those containing a 1, 2-methylenedioxy group such as *R*-dicentrine (**9**), *R*-roemeroline (**29**), *R*-xylopine (**31**), *R*-isolaureline (**13**), *R*-anonaine (**2**), *R*-nordicentrine (**22**), *R*-phanostenine (**27**), *R*-cassythicine (**6**), which displayed general cytotoxicity in nearly all cancer cell lines tested (IC_{50} around 15 µM). Those lacking this group showed almost no inhibitory effect [35] suggesting therefore that the methylenedioxy ring is a key element of the pharmacophore.

In the course of a study aimed at evaluating the antiviral potential of a series of aporphinoids against herpetic viruses [36] and the polio virus [37, 38], the authors evaluated their cytotoxicity on uninfected viral host Vero cells. The IC_{50} values varied from 7 µM for *S*-oliveridine (**34**) (Fig. (**2**)) to 500 µM for lysicamine (**44**), an oxoaporphine (Fig. (**3**)). The 1, 2-methylenedioxy substitution seemed to contribute importantly to the host cell cytotoxicity because cassythicine (**6**) was 5-fold more toxic than *N*-methyllaurotetanine (**20**) and boldine (**4**). In contrast to the substitution of the A-ring, the *N*-methylation or *N*-acetylation on the B-ring of aporphines had an insignificant effect on host cell cytotoxicity. Nevertheless presence of a methylenedioxy substitution is important but not sufficient to confer cytotoxic properties : aporphine alkaloids from the genus *Guatteria* (Annonaceae) were evaluated for their leishmanicidal properties together with their activity on human foreskin fibroblasts and murine macrophages. *R*-xylopine (**31**), *S*-cryptodorine (**8**), *S*-nornantenine (**23**) (which all 3 possess a methylenedioxy) and *S*-nornuciferine (**24**) (which lacks a methylenedioxy substituent) had all IC_{50} > 40 µM on the two cell lines tested [39]. *R*-xylopine (**31**) was also found to be inactive when tested against five different cell lines (IC_{50} > 13.5 µM) [40]. Although researches are essentially directed toward the discovery of cytotoxic agents, aporphines exhibiting little or no cytotoxicity might be interesting as adjuvant for conventional anticancer agents to reduce their cell efflux for example (see below) or as chemopreventive agents to protect against tumor-inducing compounds. This latter possibility is illustrated by the study of *S*-glaucine (**10**) isolated from plants from the *Corydalis* species (*C. bulbosa* and *C. pallida*) (Fumariaceae). This compound was tested for its inhibitory effects against the Epstein-Barr virus early antigen activation induced by 12-*O*-tetradecanoylphorbol 13-acetate (TPA) in Raji cells [41]. It showed some cytotoxicity but an interesting inhibitory biochemical activity: the molar ratio of alkaloid to TPA needed for inhibiting 50% of positive cells activated with 32 pmol TPA was 320, consistent with a cancer chemopreventive action [41]. The adjuvant effect was revealed in a study of *R*-roemerine (**28**), an aporphine isolated from the ethyl acetate extract of *Annona senegalensis* (Annonaceae) [42]. This compound was tested on a series of human tumor cell lines and showed a general weak cytotoxicity but was found to enhance the cytotoxic response mediated by vinblastine in multidrug-resistant KB-V1 cells. The related compound *R*-isocorydine (**12**) was also isolated from the same plant but was inactive on this test. According to this study, *R*-roemerine (**28**) appears to interact with a P-glycoprotein leading to intracellular drug accumulation [42].

Oxoaporphines

Several studies have dealt with the cell growth inhibitory activity of oxoaporphines. Investigation of the cytotoxic principles of the ethanol extract of *Annona purpurea* (Annonaceae) resulted in the isolation of several alkaloids, among which two oxoaporphines oxopurpureine (**49**) and *O*-methylatheroline (**45**) (Fig. (**3**)) [43]. They showed an *in vitro* activity against the 9-KB tumor test system (IC_{50} of 15.2 µM and 14.5 µM, respectively) [43]. Kuafumine (**41**), a new oxoaporphine isolated from *Fissistigma glaucescens* (Annonaceae), has shown a potent cytotoxicity against KB tissue culture cells *in vitro* (IC_{50}: 0.5 µM) [44]. Three known oxoaporphine alkaloids, among other compounds, were isolated from the whole plant of *Aquilegia ecalcarata* (Ranunculaceae) [45]. Only hernandonine (**38**) exhibited cytotoxicity towards the tested cancer cell lines (IC_{50}: 7.6 µM and 8.2 µM on GLC-82 and HCT cells, respectively). Oxoglaucine (**47**) and oxophoebine (**48**) were inactive against these two cancer cell lines [45], but, in another study aimed at evaluating the cytotoxicity of 74 alkaloids isolated from several families of Formosan plants, a cytotoxic activity was reported for oxoglaucine (**47**) [28].

From the trunk bark of *Hernandia nymphaeifolia*, three oxoaporphines hernandonine (**38**), hernanymphine (**39**) (Fig. (**3**)) and 4-methoxyoxohernandaline (**51**) (Fig. (**4**)) showed general cytotoxic activities (IC_{50} values <4 µM) *in vitro* against P-388, KB16, A549 and HT-29 cell lines. Furthermore, hernandonine (**38**) exhibited a cytotoxicity close to that of the known anticancer agent mithramycin on A-549 and was 60 times more potent than mithramycin on P388 (IC_{50} < 0.004 µM) [18, 19].

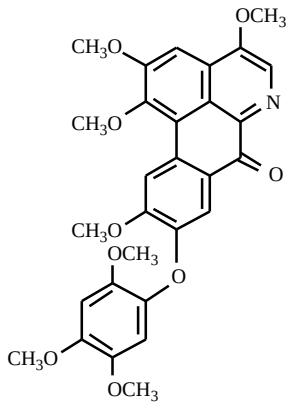


Fig. (4). Structure of 4-methoxyoxohernandaline (**51**).

The alcoholic extract of *Thalictrum sessile* (Ranunculaceae) has revealed a significant cytotoxicity against different cell lines *in vitro* [46]. Bioassay-directed fraction-

ation led to the isolation of liriodenine (**43**) (as well as a dimeric form) which demonstrated a potent cytotoxicity against KB, A-549, HCT-8, P-388 and L-1210 cells with IC_{50} values of 3.6, 2.6, 2.5, 2.1, 8.5 μM, respectively. This compound is up to now the most widely distributed oxoaporphine and has been isolated from different families [12]. It is the main cytotoxic alkaloid found in *Polyalthia longifolia* (Annonaceae) [47], *Annona montana* [48] and *Artabotrys uncinatus* [49]. From this last species, another cytotoxic oxoaporphine, atherospermidine (**36**), has also been identified and was shown to be cytotoxic to KB cells ($IC_{50:}$ 8.2 μM) [49]. The same team screened a series of isoquinoline alkaloids, isolated from various Formosan plants or chemically derived, for their *in vitro* cytotoxicity on the same five cell lines as mentioned above to point out structure-activity relationships [40]. Among these molecules they tested six aporphines, five oxoaporphines, nine aporphine *N*-oxides, seven phenanthrenes and four phenanthrene *N*-oxides. Among all the structural types investigated, the most active compounds were the 3 oxoaporphines liriodenine (**43**), oxodicentrine (dicentrinone) (**37**) and oxoxylopine (**50**), and one phenanthrene. They also showed that removing the oxo function reduces the activity on specific cell lines. These results are consistent with another study by Tzeng *et al*. [21] who also pointed out the importance of the oxo function for cytotoxicity and inhibitory effects on precursor incorporation into DNA, RNA and proteins when comparing aporphines (glaucine HBr (**10**) and *O*-methylbulbocapnine (**25**)) with their corresponding 7-oxo derivatives. This may be explained by the extension of the conjugation of the aporphine ring system due to the presence of the oxo function. As explained above, liriodenine (**43**) was also shown to have a planar conformation more compatible with an activity mediated by binding to DNA and topoisomerase II inhibition [31]. This was confirmed by Woo *et al*. who showed that liriodenine (**43**) was a potent catalytic inhibitor of mammalian topoisomerase II and a weak topoisomerase II poison [50]. On the other hand Goeren *et al*. as already described above, evaluated the DNA damaging activity of some aporphinoids. Atherospermidine (**36**) possessed DNA-damaging properties while liriodenine (**43**) did not show selective DNA damaging activity in the yeast assay but it inhibited the growth of all tested yeast strains [34].

Some oxoaporphines have been isolated from *Xylopia aethiopica* and *Miliusa cf. banacea* (Annonaceae). Oxophoebine (**48**), liriodenine (**43**) and derivatives such as 10-methoxyliriodenine (known as lauterine) (**42**) and 10-hydroxyliriodenine (**40**), proved to be selectively toxic for *Saccharomyces cerevisiae* mutants deficient in DNA repair and recombination enzymes [51]. Three related compounds lacking a methylene dioxy moiety oxoglaucine (**47**), *O*-methylmoschatoline (**46**) and lysicamine (**44**) were inactive showing, as for aporphines, the importance of the presence of a methylenedioxy group. This fact is also confirmed by other studies on aporphinoids [35-37]. It is also interesting to note that substitution of the C-10 position of liriodenine (**43**) (10-methoxy/hydroxy derivatives) reduces the toxicity against specific mutant yeasts. These two derivatives were further evaluated for inhibition of purified mammalian DNA topoisomerase II and were shown to be active. This observation leads the authors to conclude that oxoaporphinoids, particularly those bearing a methylenedioxy substituent, may present a novel class of DNA topoisomerase II inhibitors [51]. There is no doubt that this hypothesis warrants further experimental testing. Inhibition of DNA topoisomerase II and, more generally interference with DNA manipulating enzymes, is certainly one (but certainly not a unique) mechanism of action common to a variety of aporphinoids.

Antiprotozoal and cytotoxic activities against Vero cell lines of aporphinoid alkaloids isolated from *Unonopsis buchtienii* (Annonaceae) have been evaluated [52]. Lysicamine (**44**), liriodenine (**43**) and *O*-methylmoschatoline (**46**) showed IC_{50} values of 27, 3.6 and 22 μM, respectively [52]. The disparity between the cytotoxic activities of **44** and **43** against Vero cells was subsequently confirmed in other studies [36, 37]. Liriodenine (**43**) was found to be 10-fold more toxic to Vero cells than lysicamine (**44**) (IC_{50} was 48 and 500 μM, respectively) but it appeared less active than dicentrinone (**37**) on KB cells (IC_{50} of 26.9 and 10.4 μM, respectively) [24].

In a yeast assay for DNA-damaging agents, Zhou *et al.* showed that a crude extract from *Ocotea leucoxylon* had an activity typical for inhibitors of topoisomerase I [32]. By a bio-guided fractionation, dicentrinone (**37**) was isolated as the major bioactive compound and demonstrated the same selective activity in the yeast assay as the crude extract. But subsequent assays with human topoisomerase I showed that **37** had very little effects on enzyme-mediated DNA relaxation and only weakly stabilized the topoisomerase-DNA covalent binary complex. The authors suggested that either topoisomerase I-DNA interaction was not the sole locus of action of this compound or that the molecule was a specific inhibitor of the yeast enzyme, or that **37** could affect topoisomerase I function in other ways. Topoisomerase I "poisons", typified by the well known plant alkaloid camptothecin, exert their cytotoxic action by stabilizing a covalent intermediate between topoisomerase I and DNA, promoting thus the formation of single strand breaks in DNA but this is not the only route to interfere with topoisomerase I functions. The enzyme activity can also be altered by preventing the binding to DNA [53]. The cytotoxic potency of **37** was determined against wild-type and camptothecin-resistant P-388 mouse leukemia cells using a soft-agar colony-formation assay. The results suggested that dicentrinone (**37**) is weakly cytotoxic and that its mechanism of toxicity differs from that of camptothecin [32].

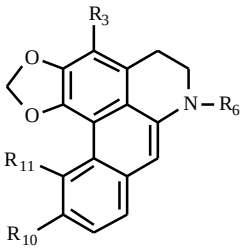
N-Oxides

A series of isoquinolines alkaloids were screened for their cytotoxicity on five different cell lines, among which nine aporphine *N*-oxides and four phenanthrene *N*-oxides. The authors pointed out that the active *N*-oxides were only active on the KB cells (among the 5 tested cell lines), the others were totally inactive. The *N*-oxide moiety is not a critical element to maintain an optimal activity [40].

Dehydroaporphines

Among 50 alkaloids and lignans tested *in vitro* against P-388, KB16, A549 and HT-29 cell lines, two dehydroaporphines, demethylsonodione (**54**) (Fig. (**6**)) and *N*-formyl-

dehydroovigerine (**52**) (Fig. (**5**)), showed overall cytotoxic activities (IC_{50} values <4 µM) against the four cell lines and compound **52** was equally potent to mithramycin on KB16 cells [18, 19]. In contrast, the dehydroaporphine derivative dehydrostephalagine (**53**) (Fig. (**5**)) showed no cytotoxic activity and no DNA damaging potential when tested in the aforementioned yeast bioassay [34].



N°	Compound	R_3	R_6	R_{10}	R_{11}
52	*N*-formyldehydroovigerine	H	CHO	O-CH_2-O	
53	Dehydrostephalagine	CH_3	CH_3	H	H

Fig. (5). *N*-formyldehydroovigerine (**52**) and dehydrostephalagine (**53**).

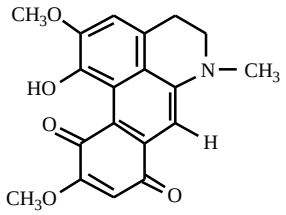


Fig. (6). Demethylsonodione (**54**).

Dimeric Forms

Thaliblastine (also known as thalicarpine) (**55**) (Fig. (**7**)) is a complex dimeric aporphine benzylisoquinoline alkaloid which was initially studied in the late sixties as an antitumor agent [54]. It showed potent *in vivo* activity and was selected for a clinical development but a few years later, the clinical trials were halted due to a lack of efficacy [6, 55]. To our knowledge, the mechanism of action of this dimer remains unknown but it may be considered as a modulator of drug efflux. Indeed, thaliblastine was shown to overcome multidrug resistance in several tumor cell lines, possibly by direct interaction with the P-glycoprotein. Its capacity to increase cellular drug retention [56-58] is an important property of this compound but further studies are warranted to identify its primary target responsible for its antitumor activity.

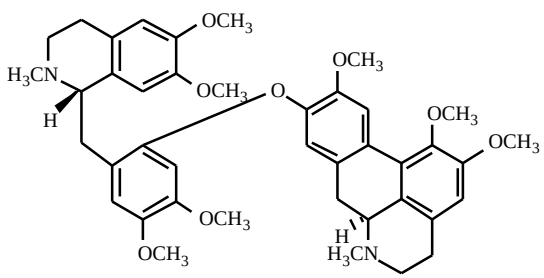


Fig. (7). Thalicarpine (thaliblastine) (**55**).

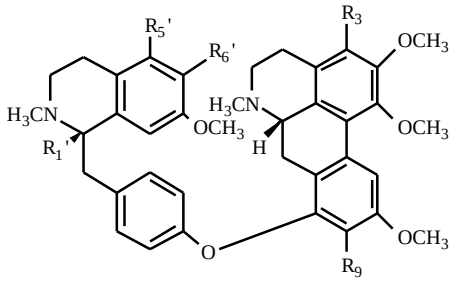
Other dimeric aporphines have been described. The alcoholic extract of *Thalictrum sessile* (Ranunculaceae) revealed significant cytotoxicity against different cell lines *in vitro*. Bioassay-directed fractionation provided an oxoaporphine and a dimeric form: (+)-thalifarazine (**58**) (Fig. (**8**)). This compound is active against KB, A-549, HCT-8, P-388 and L-1210 with IC_{50} values ranging from 2.2 to 9.6 µM [46]. In the same vein, a study of the root constituents of *Thalictrum faberi* (Ranunculaceae) led to the isolation of several alkaloids among which thalifaberidine (**56**), a new aporphine-benzylisoquinoline alkaloid, along with thalifaberine (**57**) and thalifasine (**59**). The three compounds showed similar cytotoxic activities against the several cell lines tested with IC_{50} from 1 to 25 µM [59]. Six additional new aporphine-benzylisoquinoline alkaloids were isolated from *Thalictrum faberi*. Their cytotoxicity toward cancer cell lines is equivalent to that of compounds **56**-**59**. No structure-activity relationships were defined in this dimeric series [60].

Proaporphines

S-Glaziovine (also known as *N*-methylcrotsparine, **60**, Fig. (**9**)) is a proaporphine isolated from *Annona purpurea* endowed with modest cytotoxic activities (IC_{50} of 8.7µM against 9-KB tumor cells) [43]. In the course of a screening of Chinese medicinal plant extracts active against the herpes simplex virus, Nawawi *et al*. tested 49 alkaloids from *Stephania cepharantha* (Menispermaceae). Only the *S*-proaporphine compound *N*-methylcrotsparine (**60**) revealed a limited cytotoxic activity against host Vero cells (IC_{50} of 27.9 µM) [61].

CONCLUSION

The number of aporphinoid compounds isolated from plants or obtained by total synthesis is rapidly growing. Numerous molecules in this family display interesting cytotoxic activities against tumor cell lines *in vitro* and hints of anticancer activities *in vivo* have been reported in a few cases. So far, only one compound of this category, the dimeric molecule thaliblastine has been advanced to human clinical trials but no significant therapeutic activity was observed. Although it is clear that aporphines represent an interesting, potentially useful category of anticancer agents, their development is seriously limited by a lack of solid knowledge of their mechanism(s) of action. Different molecular activities have been described, mostly at the nucleic acids level, but overall, the exact targets of these compounds remain elusive. Some of them, like thaliblastine, may be considered as multidrug resistance reversal agents interfering with the P-glycoprotein. Other compounds rather function as topoisomerase inhibitors. This is the case for dicentrinone and dicentrine for examples, but this inhibitory activity is not at the origin of the potent cytotoxicity measured with numerous aporphines. Effort should be directed toward a more complete elucidation of the mechanism of action of these



N°	Compound	R_3	R_9	R_1'	R_5'	R_6'
56	Thalifaberidine	OCH_3	OH	H	H	OH
57	Thalifaberine	OCH_3	OCH_3	H	H	OCH_3
58	Thalifarazine	OCH_3	OCH_3	CH_3	H	OH
59	Thalifasine	OH	OCH_3	H	OH	OCH_3

Fig. (8). Studied dimeric aporphines.

molecules. This is certainly a prerequisite for the rational development of tumor active compounds.

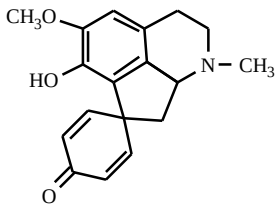


Fig. (9). Structure of the proaporphine *N*-methylcrotsparine (glaziovine) (**60**).

The oxoaporphines appear particularly attractive on the basis of their potent cytotoxic potential. The 7-oxo function, together with the presence of a methylenedioxy functionality, is an important molecular determinant for optimal antiproliferative activities. In this sub-group, liriodenine can be considered as a lead structure from which more potent analogues targeting DNA and topoisomerase II could be built.

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