# Lycorine and its Derivatives for Anticancer Drug Design

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**Abstract:** Amaryllidaceae alkaloids are extensively studied for their biological activities in several pharmaceutical areas, including, for example, Alzheimer's disease for which galanthamine has already reached the market. Among this chemical family, lycorine displays very promising anti-tumor properties. This review first focuses on the chemical diversity of natural and synthetic analogues of lycorine and their metabolites, and then on mechanisms of action and biological targets through which lycorine and its derivatives display their anti-tumor activity. Our analysis of the structure-activity relationships of this family of compounds highlights the existence of various potential leads for the development of novel anticancer agents.

**Key Words:** Lycorine, Amaryllidaceae, Cancer, Design, Discovery, Structure-Activity relationship.

## INTRODUCTION

Cancer is a worldwide disease, which is responsible for millions of deaths every year. The standard cancer treatment protocols include surgery, radiotherapy and chemotherapy. Unfortunately, chemotherapy is not effective in treating cancers associated with either natural (innate) resistance to apoptosis (e.g. gliomas [1], melanomas [2], esophageal cancers [3], pancreatic cancers [4], non-small-cell-lung cancers [5], and above all metastatic cancers [6, 7]), and/or acquired resistance to drugs during treatment [8-10]. In this connection, three major points must be emphasized: (i) > 80% of the drugs currently used to combat cancers are pro-apoptotic, (ii) pro-apoptotic drugs are inefficient against naturally apoptosis-resistant cancers (see above) and/or effluxed by various types of pumps during the process of acquired resistance [11, 12], and (iii) > 90% of cancer patients die from their metastases. Discovery of new cellular and/or molecular pathways enabling innate or acquired resistance of cancers to current chemotherapeutics to be overcome is therefore of crucial importance if one wants to efficiently combat those cancers associated with dismal prognoses as those mentioned above. Within the oncology area, natural products and their derivatives accounted for up to 60% of approved drugs during the period from 1983 to 1994 [13]. In the current review, we will report on the toxicity versus pharmacological properties of compounds isolated from *Amaryllidaceae* plants, known for their medicinal properties over millenaries. Indeed, the useful anticancer properties of oil extracted from the daffodil *Narcissus poeticus* L. were already known to Hippocrates of Cos, who used it to treat uterine tumors in the fourth century B.C. [14]. Pliny the elder also reported the topical anticancer use of different *Amaryllidaceae* in the first century A.D. [14]. Recently, an important breakthrough was achieved with alkaloid galanthamine that has reached the market for the treatment of Alzheimer's disease (Razadyne™ formerly Reminyl ®; [15]).

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## CHEMICAL DIVERSITY

*Amaryllidaceae* provide a great diversity of alkaloids which have been extensively studied due to their challenging chemistry and promising activities [14, 16-21]. Biosynthetically, these natural products have been shown to arise from a common intermediate, norbelladine (Fig. **1**). This intermediate undergoes different cyclizations, possibly followed by rearrangements and/or elimination of two carbons, ring opening, and/or recyclization to provide a variety of skeletons [14, 22-27]. As detailed below, the tetracyclic alkaloid lycorine [**1**] and related phenanthridines display very interesting anti-tumor properties among all the various skeletons available [28-37]. This lycorine family of compounds comprises analogues possessing a double bond in the C-ring as found in lycorine itself, 1-*O*-acetylnorpluviine **2** [38], pseudolycorine **3** [35], galanthine **4** [35], sternbergine **5** [35, 39], diacetyllycorine **6** [36], 2-*O*-acetylpseudolycorine **7** [35], diacetyllycorinone **8** [37], 1-*O*-acetyllycorine **9** [29], lycorine-2-one **10** [29], *N*-methyllycorine iodide **11** [29], norpluviine **12** [29], amarbellisine **13** [29, 40], and lycorene **14** [29]. The C-ring can be totally saturated as in 1,2-di-*O*-

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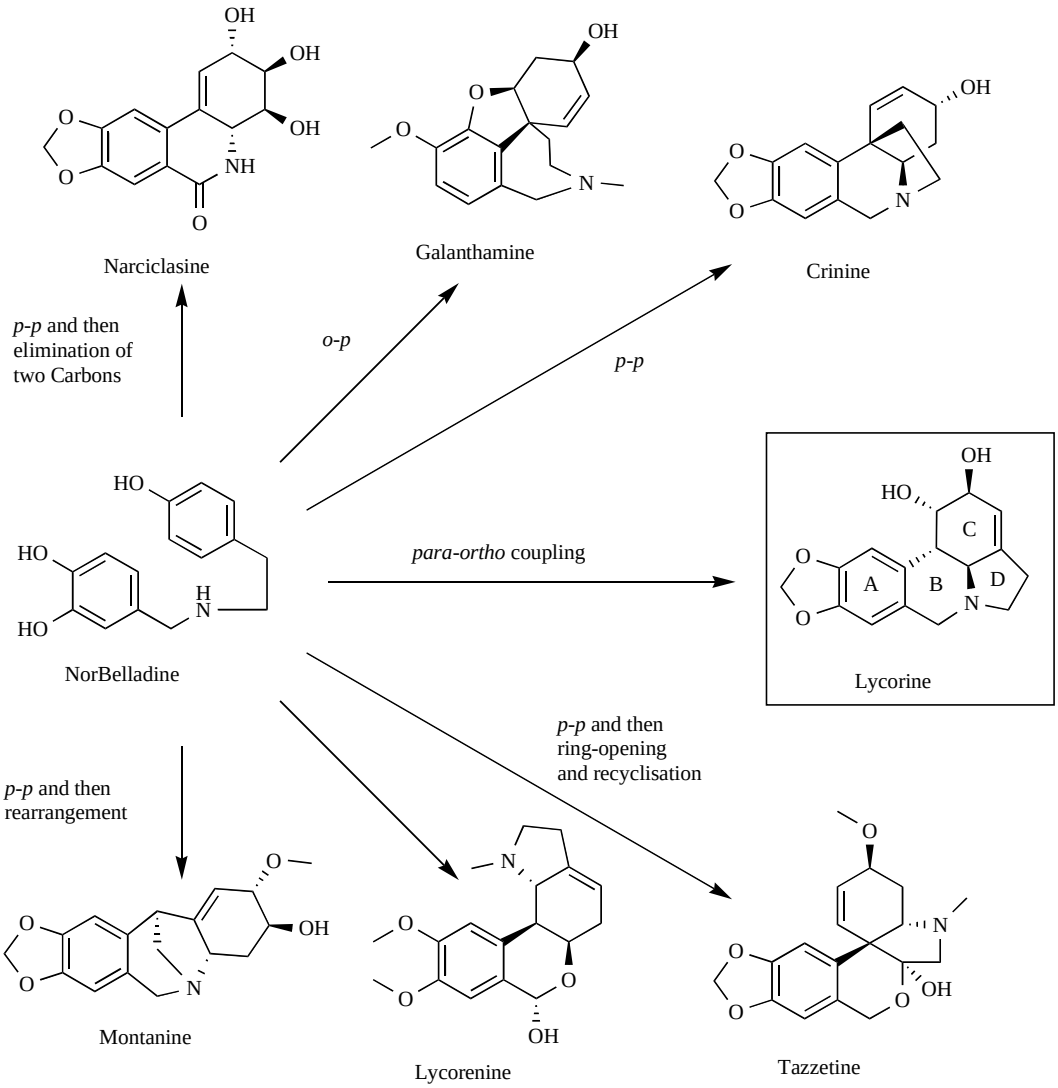



**Fig. (1).** Different phenol oxidizing coupling patterns for norbelladine.

acetyl-α-dihydrolycorine **15** [29, 41] and compounds **16**-**18** [41]. Other compounds have a fully aromatized C-ring and an olefin in the D-ring as found in crisiaticidine A **19** [42] and pratorimine **20** [42, 43]. In addition, some analogues have an aromatic B-ring but no double bond in the ring D as found in anhydrolycorinium chloride **21** [37, 44, 45], compounds **22**-**23** [46], anhydrolycorinone **24** [38], the nitro-functionalized compound **25** [41] and ungeremine (lycobetaine) **26** [44, 47-49]. Among the aromatic lycorine analogues, some are neutral, some bear a positive charge and one is zwitterionic. And finally, among all these compounds, other differences occur, such as an amide function in lieu of the amine, dioxole ring versus no dioxole ring, variations of the stereochemistry of the asymmetric carbons, and last but not least, the number of free hydroxyl residues (see Fig. **1** and Table **1**).

## DIVERSITY IN BIOLOGICAL MECHANISMS OF ACTION

Various biological mechanisms have been invoked to explain the antitumor activities of lycorine and its congeners. It was first postulated that lycorine inhibits protein biosynthesis [50]. However, some experiments showed that it is not an initiation inhibitor [51] and others showed that it could not inhibit the peptide bond formation though it did link to ribosome under certain conditions [52]. Lycorine interferes with vitamin C biosynthesis [53]. While some studies report pro-apoptotic effects for lycorine [30, 33, 54], others point to anti-apoptotic activity in cells challenged with polymorphonuclear leukocyte-derived calprotectin [55]. Several groups observed cell cycle arrest after treatment with lycorine [33, 55] or with ungeremine [54]. In this last case, the effect was attributed to ungeremine's ability to inhibit specifically topoisomerase IIβ [49]. Besides this biomolecular target, caspases 3, 7 and 9 (proapoptotic proteins) have been shown to be up-regulated by lycorine while Mcl-1 and Bcl-xl (antiapoptotic proteins) were down regulated [30]. Finally, lycorine also proved to be able to interact with DNA [56].

## STRUCTURE-ACTIVITY RELATIONSHIP (SAR) ANALYSES WITH RESPECT TO ANTI-TUMOR ACTIVITY

To the best of our knowledge, there is only one literature report offering a rationalization of the differences in antican-

cer activities in this chemical series [46]. This study compares *in vivo* activities of ungeremine **26**, compounds **21** and **22**, and other analogues lacking the D-ring of lycorine, or those having one more carbon in this ring, for their activity against mouse leukemia cells [46]. Four conclusions resulted from the SAR analyses in the above-mentioned study:

1. The presence of a positive charge on the nitrogen is important for the antineoplastic activity.
2. The planarity of molecules is important, may be because of the DNA-interaction possibility.
3. The quaternized nitrogen should not be sterically hindered.
4. The presence of alkoxy functions at proper positions is important for the activity, may be because of metabolism-related issues [46].

Among approximately three hundred molecules sharing the ABCD tetracycle of lycorine (natural, synthetic or hemisynthetic compounds), only twenty-six have been tested for their activity against different cancer cell lines. Among these twenty-six compounds, twenty were found active against at least one cell line and six were found inactive against every cell lines tested. We detail in Table **1** all the reported data with the corresponding references. The importance of some structural features cannot be evaluated since the reported compounds show no diversity for those structural elements. For example the stereochemistry of the asymmetric carbons is the same for all the tested compounds or is not determined. With the exception of ungeremine **26** Fig. (**2**), which displays nanomolar activity against one cell line [49], the active compounds usually display $IC_{50}$ *in vitro* growth inhibitory activity ranging between 0.1 and 50 μM (Table **1**). When considering these mean growth inhibitory activities within the various compound categories, we failed to identify clear SAR trends. Therefore, we instead focused our analyses on nine structural elements as detailed below. Furthermore, we carried out statistical analyses to determine whether each of these structural elements (parameters) provides a significant contribution to the SAR data. To this end, the exact Fisher test was used to evaluate the difference in proportions of active compounds between those characterized by the presence *vs*. the absence of a specific structural element. A p-value < 0.05 is considered as indicative of a significant contribution. However, it should be kept in mind that the obtained p-values depend on the number of compounds available for computing the proportions.

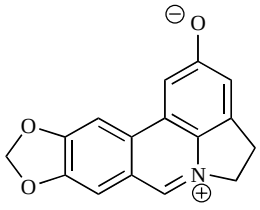


**Fig. (2).** Ungeremine **26**, the most active compound in the series.

**The presence of at least one charge:** Among the molecules that do have a charge, three are active out of four, whereas among neutral products, seventeen are active out of twenty-two ($p > 0.05$). Thus, in contrast to what is emphasized in ref. [46], the presence of at least one charge does not contribute to the SAR data significantly. This feature could be explained, at least partly, by the fact that neutral compounds may be transformed into charged active metabolites (e.g. protonation of uncharged alkaloids or oxidation to iminium or N-oxide forms). So if there is really a difference, the metabolism would provide a large bias, thus preventing us from observing a statistically relevant discrimination. Two possibilities may therefore explain the difference between our current analysis and the conclusions drawn in ref. [46], namely, (i) this study has been carried out on murine leukemia cell lines, which can differ from human cancer cell lines in terms of activity and/or metabolism, and (ii) it includes compounds lacking the D-ring of lycorine as well as those having one more carbon in this ring.

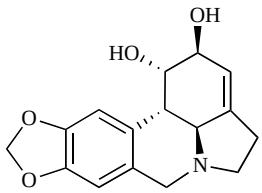
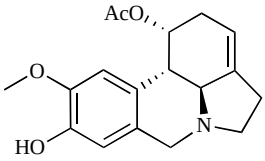
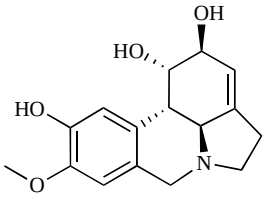
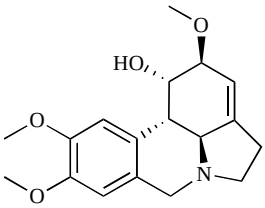
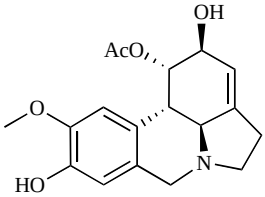
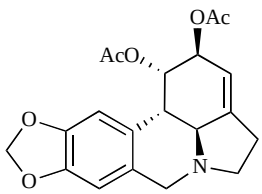
**The presence of an additional olefin in the ring D**. Among the molecules that have an additional double bond, two are active out of two, whereas among compounds with the saturated ring D, eighteen are active out of twenty four ($p > 0.05$). This parameter seems to be not discriminative, but the very biased repartition should lead to cautious interpretation. It will be possible to conclude about this structural variation only when more compounds having a double bond in the ring D will have been tested for their antitumor activity.

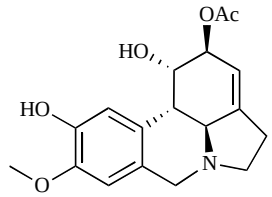
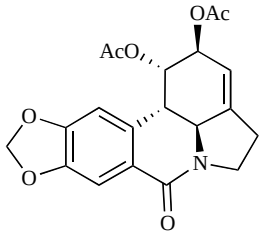
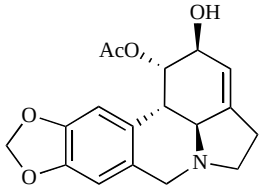
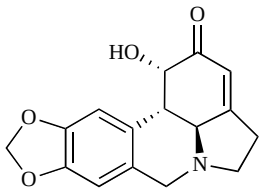
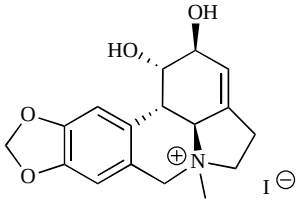
**Planarity**. Among the planar molecules, five are active out of eight while among the non-planar compounds, fifteen are active out of eighteen ($p > 0.05$). Again, in contrast to what is emphasized in ref. [46] this parameter seems to be not discriminative in this series of compounds, but this is probably due to the same reason(s) as the ones we advocated for the presence of a charge.

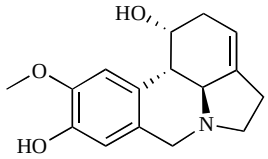
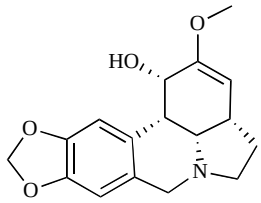
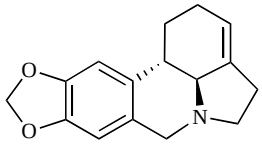
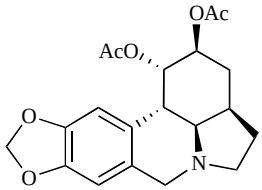
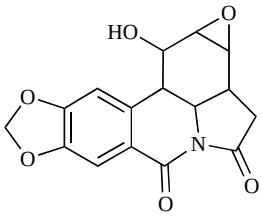
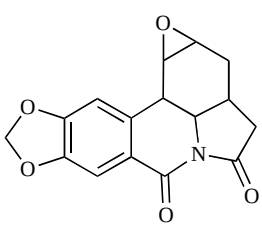
**The presence of the dioxole ring**. Among the molecules that have the dioxole ring, eleven out of sixteen are active, whereas among those that do not, nine are active out of ten ($p > 0.05$). The statistical analysis thus reveals that this parameter does not provide discriminent SAR information. The fact remains that when the dioxole ring is open, it may unmask a phenol function or even two. As the presence of at least one hydroxyl function is one of the most significant parameters (see below), there is a strong bias in the interpretation of the importance of this structural variation. The caution that we emphasized here is supported by the fact that compounds **21** and **22**, differing only by the presence or absence of the dioxole ring (no unmasking of a phenol), have in fact a significant difference in activity. Compound **21** with a dioxole is active, while compound **22** without a dioxole is not. Only studies with a larger selection of compounds having no dioxole (but no phenol) on the A-ring will allow one to draw definitive conclusions as whether this structural element is an important SAR contributor.

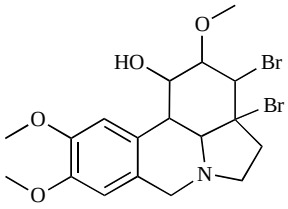
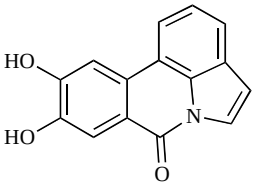
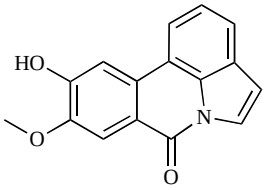
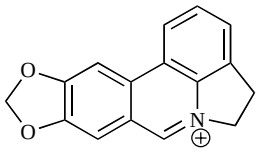
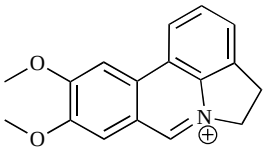
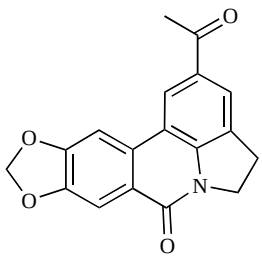
**The presence of at least two hydroxyl functions** (either alcohol or phenol). Among the molecules that have at least two hydroxyl groups, seven are active out of seven, whereas among molecules without hydroxyl functions, thirteen are active out of nineteen ($p > 0.05$). Thus, this parameter should not be considered as being discriminent.

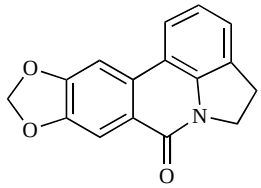
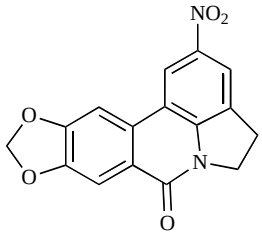
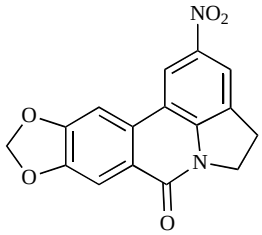
**Table 1.** ***In vitro*** **and** ***in vivo*** **Antitumor Activities of Lycorine Analogues.**

| Structure | $IC_{50}$ *in vitro* Growth Inhibitory Concentration | *T/C index (%) to Characterize *in vivo* Antitumor Activity |
|---|---|---|
|  Lycorine **1** | 0.5 < $IC_{50}$ < 10 μM on various types of leukemia and lymphoma cell lines [30]<br><br>3 < $IC_{50}$ < 10 μM on various types of carcinoma [32], multiple myeloma [33] and melanoma [38] cell lines | T/C = 134% in HL-60 leukemia-bearing mice treated with 10 mg/kg [31] |
|  1-*O*-Acetylnorpluviine **2** | 6 μM on BL-6 mouse melanoma cells [38] | |
|  Pseudolycorine **3** | 2 μM on MOLT-4 (acute lymphoblastic leukemia) to 35 μM on HEPG2 (hepatocellular carcinoma) cells [35] | |
|  Galanthine **4** | 12 μM on MOLT-4 (acute lymphoblastic leukemia) to > 150 μM on HEPG2 (hepatocellular carcinoma) cells [35] | |
|  Sternbergine **5** | 39 μM on MOLT-4 (acute lymphoblastic leukemia) to > 150 μM on HEPG2 (hepatocellular carcinoma) cells [35, 39] | |
|  Diacetyl-lycorine **6** | 24 μM on BL-6 mouse melanoma cells [36] | |

| **Structure** | **$IC_{50}$ *in vitro* Growth Inhibitory Concentration** | ***T/C index (%) to Characterize *in vivo* Antitumor Activity** |
|---|---|---|
|  2-*O*-acetylpseudolycorine **7** | 2 μM on MOLT-4 (acute lymphoblastic leukemia) to 79 μM on HEPG2 (hepatocellular carcinoma) cells [35] | |
|  diacetyl-lycorinone **8** | Inactive on 50 cancer cell lines [41] | |
|  1-*O*-acetyl-lycorine **9** | <5 μM on HeLa (adenocarcinoma) cells [29] | |
|  Lycorine-2-one **10** | 5 < $IC_{50}$ <25 μM on HeLa (adenocarcinoma) cells [29] | |
|  N-methyl-lycorine iodide **11** | 25 μM on HeLa (adenocarcinoma) cells [29] | |

| Structure | $IC_{50}$ *in vitro* Growth Inhibitory Concentration | *T/C index (%) to Characterize *in vivo* Antitumor Activity |
|---|---|---|
|  Norpluviine **12** | > 25 μM on HeLa (adenocarcinoma) cells [29] | |
|  Amarbellisine **13** | 5 μM on HeLa (adenocarcinoma) cells [29, 40] | |
|  Lycorene **14** | Inactive on HeLa (adenocarcinoma) cells [29] | |
|  1,2-di-*O*-acetyl-α-dihydrolycorine **15** | > 25 μM on HeLa (adenocarcinoma) cells [29] | |
|  **16** | Inactive on 48 cancer cell lines [41] | |
|  **17** | Inactive on 48 cancer cell lines [41] | |

| **Structure** | **$IC_{50}$ *in vitro* Growth Inhibitory Concentration** | ***T/C index (%) to Characterize *in vivo* Antitumor Activity** |
|---|---|---|
|  **18** | Inactive on 57 cancer cell lines [41] | |
|  Crisiaticidine A **19** | 13 μM on Meth-A (mouse sarcoma) to 17 μM on LLC (mouse lung carcinoma) cells [42] | |
|  Pratorimine **20** | 16 μM on Meth-A (mouse sarcoma) cells [42] Low activity on MOLT-4 (acute lymphoblastic leukemia) cells (no $IC_{50}$ is provided) [43] | |
|  anhydrolycorinium **21** | | T/C = 153% in P388 lymphocytic leukemia-bearing mice treated with 25 mg/kg [46] |
|  **22** | | Inactive (T/C < 125%) in P388 lymphocytic leukemia-bearing mice treated with 25 mg/kg [46] |
|  **23** | Inactive on 39 cancer cell lines [41] | |

| Structure | $IC_{50}$ *in vitro* Growth Inhibitory Concentration | *T/C index (%) to Characterize *in vivo* Antitumor Activity |
|---|---|---|
|  Anhydrolycorinone **24** | 12 μM on BL-6 mouse melanoma cells [38] | |
|  **25** | | Inactive (T/C < 125%) in P388 lymphocytic leukemia-bearing mice treated with 25 mg/kg [41] |
|  **25** | 5 μM on P388 mouse lymphocytic leukemia cells [37]<br>4.5 μM on P388 (mouse lymphocytic leukemia).<br>$0.1 < IC_{50} < 3$ μM on various types of carcinoma cells [48, 49, 44, 47] and IC50 = 2 nM on GXF251 [49] | T/C = 152% in P388 lymphocytic leukemia-bearing mice treated with 25 mg/kg [46] |

Numbers in parentheses refer to references.

*the potential survival gain obtained using lycorine derivatives was evaluated by means of the %T/C index, which is the ratio of the median survival time of the treated animal group (T) and that of the control group (C). Toxic effects are indicated by %T/C indices < 75%. The benefits of chemotherapy are considered to be significant when associated with %T/C indices > 130%.

**The presence of at least one phenolic function**. Among the molecules that have at least one phenolic function, eight are active out of eight, whereas among molecules without a phenol, twelve are active out of eighteen ($p = 0.08$). Although this parameter cannot strictly be considered as discriminative in terms of SAR analysis, a slight tendency for phenolic compounds as being better candidates for anticancer activity seems nevertheless to emerge.

**The insaturation pattern of the C-ring.** This structural element has three possible variants: fully saturated, one double bond or aromatic. There are only four examples of molecules with a fully saturated C-ring. We thus first compared only compounds containing an aromatic ring or a single olefin moiety. Among the molecules that do have a double bond, thirteen are active out of fourteen, whereas among molecules with an aromatic ring C, five are active out of eight ($p > 0.05$). This parameter cannot be considered as significant. But once again, this should not be surprising because of a possible metabolic transformation of compounds with one double bond into aromatic products. Indeed, keeping this possible metabolism in mind, it would not have been logical to find a strong discrimination for this parameter. Then we wanted to check the significance of each value versus the two others. When considering the aromatic compounds versus all the others, the result is the same as when considering the saturated compounds *versus* all the others. The *p*-value is 0.25 in both cases, thus pointing at non significant repartitions. But if one considers the compounds bearing a double bond in the C-ring (thirteen active out of fourteen) *versus* all the others (seven active out of twelve), then the *p* value is 0.052. While this value is just above 5%, we can nevertheless consider that it reflects an actual tendency in terms of SAR analysis due to the small number of compounds that have been taken into consideration. The presence of one double bond and only one in the C-ring gives better chances to have an active compound against at least one cancer cell line. We believe this feature to be also associated with metabolic transformations. A compound with no double bound at all might be metabolized too slowly while a compound already aromatic might not reach its target as easily. Therefore, only one double bond would be just the good compromise. In order to prove such a hypothesis, it would be necessary to know for sure the activities of all the final metabolites in this series.

**The presence of at least one hydroxyl function** (either alcohol or phenol). Among the molecules that have at least one hydroxyl group, fifteen are active out of sixteen, whereas among molecules with no hydroxyl at all, five are

active out of ten ($p$ = 0.02). This parameter thus allows a statistically significant discrimination, emphasizing that the research for novel lycorine derivatives should lead to the generation of molecules with at least one hydroxyl group. When comparing this discrimination to the one obtained with "at least two hydroxyls", which cannot be considered significant, it is interesting to notice that the first hydroxyl improves the value of the candidates much more pronouncedly than do the additional hydroxyls. As secondary and tertiary alcohols are commonly linked to a stereogenic center, this observation is highly significant for the synthesis of compounds bearing only one asymmetric carbon compared to compounds bearing two or more asymmetric carbons. And because the hydroxyls may be phenolic, one might design only symmetrical compounds for efficient synthesis, facile purification, and for easier eventual scale-up.

**The presence of a basic amine** (*versus* a non-basic amide). Only twenty two molecules were taken into account here because the iminium ions and the ammonium salts (or at maximum ions) are not suitable for this comparison. Among the compounds that have a basic amine, fourteen are active out of fourteen, whereas among compounds with an amide function, three are active out of eight ($p$ = 0.002). This parameter thus contributes to actual statistical significance in terms of SAR analysis. In other words, the amide function is highly detrimental for anticancer activity. Synthetic chemists should therefore focus on amine entities instead of amides in order to generate novel lycorine derivatives with improved anti-tumor activities.

## CONCLUSION

Although only twenty-six out of more than three hundred lycorine analogues have been evaluated for antitumoral activity, the reported results are already highly encouraging and attest to a high potential of this family of compounds to provide excellent leads for anticancer drug discovery. The current review offers chemists some rational guidelines for the design and synthesis of compounds. In this series, improved anticancer activities might be reached by focusing on the importance of phenol and alcohol functions, the insaturation pattern, and the presence of an amine.

## ACKNOWLEDGEMENTS

**Grant support**: Fonds Yvonne Boël (Brussels, Belgium). R. Kiss is a director of research, C. Decaestecker is a senior research associate and V. Mathieu is a senior research assistant with the Fonds National de la Recherche Scientifique (FNRS, Belgium).

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Received: August 20, 2009 Revised: November 20, 2009 Accepted: November 21, 2009