Chapter-1

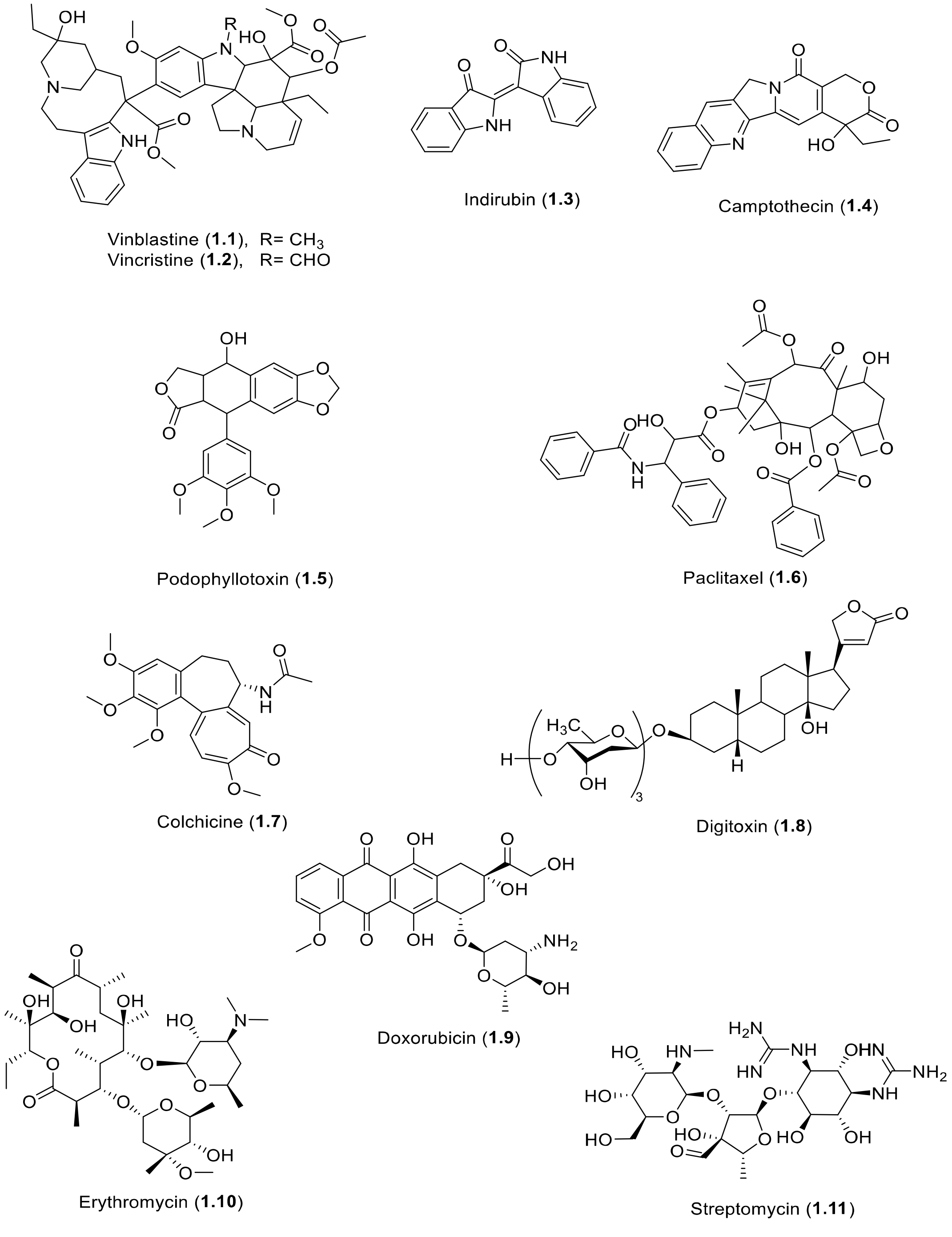
Introduction

1. Introduction

A natural product (NP) can be an entire organism (animals, plants, and microbes), a part of an organism, an extract of the organism or its part, or pure compounds (e.g., alkaloids, coumarins, flavonoids, glycosides, steroids, and terpenoids) isolated from them (Sarker and Nahar, 2012; Samuelson and Bohlin, 1999). However, Natural products refer to the secondary metabolites produced by any living organism, which may have pharmacological or biological activity that can be clinically useful either as such in the form of crude (Traditional medicines) or isolated/modified form (Modern System) (Samuelson and Bohlin, 1999; Cutler and Cutler, 1999). Approximately 80% of medicinal products till 1996 were either directly derived from naturally occurring compounds or were inspired by a natural product. A total of 1881 new drugs were approved between 1 Jan 1981 to 30 Sep 2019, 22.7 % (427) of them were either natural products or derived from natural products, and another 25.7 % (489) were inspired by a natural product (Newman and Cragg, 2020).

1.1. Natural products as potential sources of drugs

Plants are considered a classical source of drug discovery, around 3,00,000 to 5,00,000 higher plant species have been estimated, and only 6% of that has been investigated pharmacologically and 15% phytochemically (Cragg et al., 2009). Some of the natural products used as drugs include vinblastine (**1.1**) and vincristine (**1.2**) from *Vinca rosea*, indirubin (**1.3**) from *Indigofera* spp., camptothecin (**1.4**) from *camptotheca accuminata*, podophyllotoxin (**1.5**) from *Podophyllum peltatum* Linn., paclitaxel (**1.6**, Taxol) from *Taxus brevifolia*, colchicine (**1.7**) from *Colchicum autumnale*, digitoxin (**1.8**) from *Digitalis purpurea*, doxorubicin (**1.9**) from *Streptomyces peucetius*, antibiotics like erythromycin (**1.10**), streptomycin (**1.11**) (Figure 1.1) (Newman and Cragg, 2007; Newman et al., 2003; Cragg et al., 2009). They are used against a wide range of therapeutic indications like cancer, various types of infections, diabetes, hypertension, metabolic disorders, multiple sclerosis, and others (Newman and Cragg, 2020).



Vinblastine (**1.1**), R= CH_3
Vincristine (**1.2**), R= CHO

Indirubin (**1.3**)

Camptothecin (**1.4**)

Podophyllotoxin (**1.5**)

Paclitaxel (**1.6**)

Colchicine (**1.7**)

Digitoxin (**1.8**)

Doxorubicin (**1.9**)

Erythromycin (**1.10**)

Streptomycin (**1.11**)

Figure 1.1. Chemical structures of plant- and microbial-derived natural products used as drugs.

Although natural products itself have been used as drug, there has been other disadvantages of natural products as follows:

(i) Supply issue: Natural products are often biosynthesized in small quantities. Obtaining sufficient quantities for preclinical development requires large-scale reacquisition or fermentation that would substantially impact the development timeline.

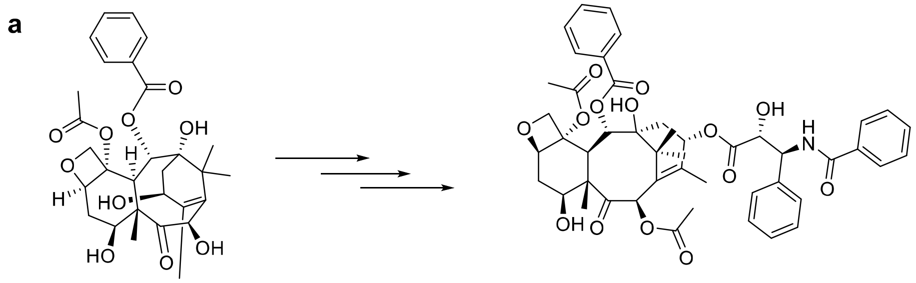
(ii) Rediscovery: When screening natural product libraries, some natural products were discovered more frequently than others. Rediscovery of known compounds is caused by a lack of efficient dereplication methodologies.

(iii) Suboptimal biological and pharmacokinetic properties: NPs may show suboptimal biological activities and/or pharmacokinetic (ADMET) properties.

To meet the above challenges, various concepts have emerged in natural product-based drug discovery. Along with the search for new natural products, continuous research is going on to optimize the established natural products by using semi-synthetic strategies and total synthesis. So, in natural product-based drug discovery, chemical modifications may be required to optimize the natural product to become a lead compound and reach the market.

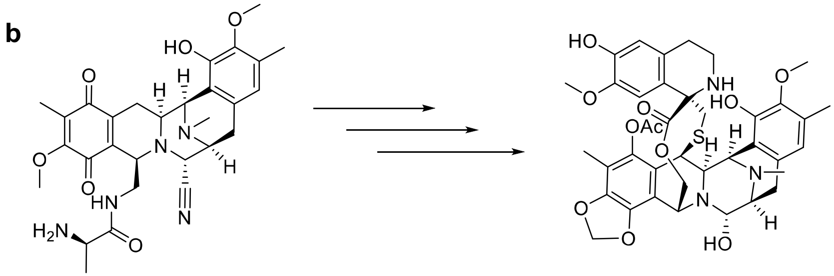
1.2. Semi-synthesis as an important tool in natural products-based drug discovery

In this approach, the parent natural products are modified by chemical means to improve the biological activity and/or pharmacokinetic properties. Using semi-synthesis, a large number of diverse analogues can be synthesized quickly to optimize the pharmacological as well as pharmacokinetic profile of the parent natural product lead molecule. This approach also offers a quick dip into structure-activity relationship (SAR) studies. Following are the classical examples of semi-synthetic drugs (Figure 1.2).



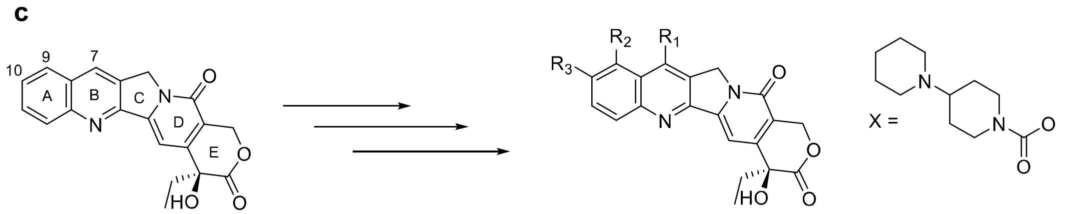
10-deacetylbaccatine III (**1.12**)
Taxus baccata needles contain relatively
large amount of 10-deacetylbaccatine III

Paclitaxel (**1.6**)
The supply problem of paclitaxel was solved by
its semisynthesis from 10-deacetylbaccatine III



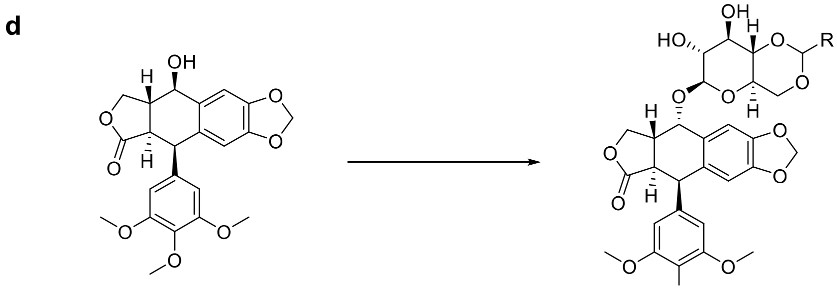
Cyanosafracin B (**1.13**)
Fermentation product of bacteria
Pseudomonas fluorescens

Trabectedin (**1.14**)
Originally isolated from *Ecteinascidia turbinata*



Camptothecin (**1.4**)
Water insoluble

Water soluble semi-synthetic derivatives
Topotecan (**1.15**), R_1=H, R_2=$CH_2N(CH_3)_2$, R_3=OH
Irinotecan (**1.16**), R_1=CH_2CH_3, R_2=H, R_3= X



Podophyllotoxin (**1.5**)
Potentially toxic adverse effects

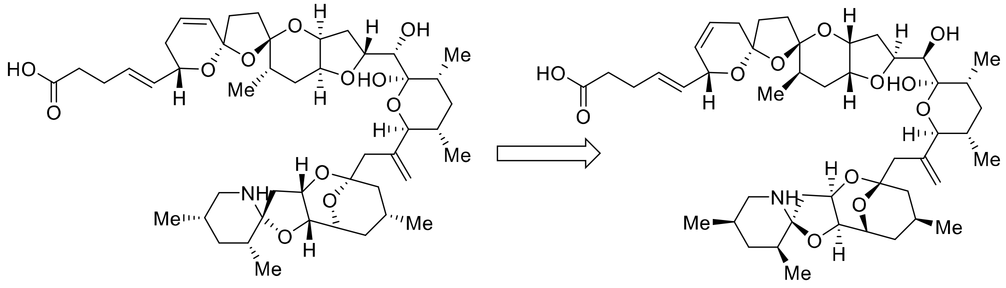
Etoposide (**1.17**), R = CH_3
Teniposie (**1.18**), R = 2-Thienyl
Potential antitumor activity as well as lower side effects

Figure 1.2. Typical examples of semisynthetic anticancer drugs. The supply issue of paclitaxel and trabectedin is solved by their semi-synthesis (a-b). Semi-synthesis of compounds superior to their parent molecule (c-d).

Cholesterol-lowering semi-synthetic simvastatin is more potent than parent lovastatin (Alberts, 1990). Paclitaxel (**1.6**) is one of the most effective anticancer drugs ever developed. It accumulates at a low concentration in the inner bark of *Taxus* species. The growing demand for paclitaxel greatly exceeds the supply that can be sustained by isolation from its natural source, and alternative sources of the drug are being sought (Exposito et al., 2009; Cragg et al., 1993). In 1992, Holton patented an improved semi-synthetic process for paclitaxel (**1.6**) from 10-deacetylbaccatin (**1.12**) isolated from the needles of the *Taxus baccata* (Holton, 1993). However, the current supply of paclitaxel is based on plant cell fermentation (PCF) technology developed by BMS, in which a specific *Taxus* cell line propagated in aqueous medium in large fermentation tanks with the endophytic fungus *Penicillium raistrickii*. Paclitaxel is then extracted directly, and purified. Similarly, camptothecin (**1.4**) was identified as a potent anticancer; later, the solubility issue was resolved by semi-synthesizing potent anticancer topotecan (**1.15**) and irinotecan (**1.16**), widely used for cancer treatment. Supply of starting material **1.4** depends on its biological source; *Camptotheca acuminata* (Nyssaceae), *Nothapodytes foetida*, *Pyrenacantha klaineana*, *Merrilliodendron megacarpum* (Icacinaceae), *Ophiorrhiza pumila* (Rubiaceae), *Ervatamia heyneana* (Apocynaceae) and *Mostue abrunonis* (Gelsemiaceae) (Lorence and Nessler, 2004). Another example is clinically useful anticancer etoposide (**1.17**), a semi-synthetic derivative of podophyllotoxin (**1.5**), which is isolated from *Podophyllum hexandrum* (Kingston, 2008). There are a number of potential candidates of natural origin used for semi-synthesis based natural product libraries.

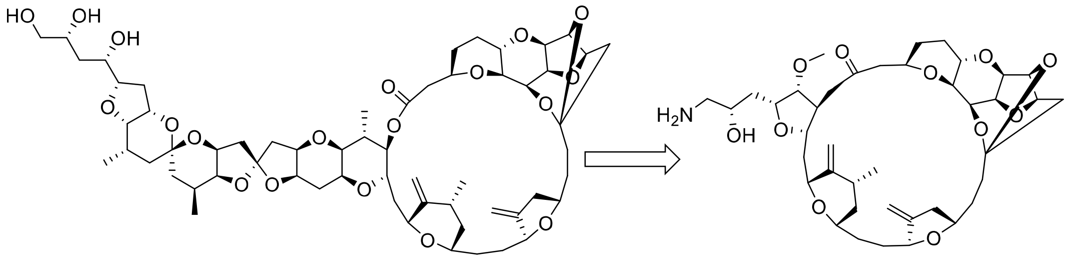
1.3. Total synthesis

The total synthesis of natural products has always been a topic of interest for medicinal chemists. Total synthesis helped in the structure determination of complex natural products, for example, azaspiracid-1 (**1.20**), such type of case studies has been reviewed by Nicolaou (Nicolaou and Snyder, 2005; Paul et al., 2021). In many cases, the quantities of natural products available from natural sources are so small that total synthesis is required to provide material for further biological characterization, which is required to determine whether the natural product merits further investigation as a lead structure for drug development. Many of the complex natural products have been synthesized in laboratories, meeting the demand of the time (Nicolaou et al., 2000). For example, total synthesis was employed to access the required quantity of anticancer lead discodermolide for clinical development. One of the excellent example of natural product inspired total synthesis is the discovery of eribulin (**1.22**, Figure 1.3).



1.19
Originally proposed structure of azaspiracid-1

1.20
Revised structure of azaspiracid-1
after total synthesis



Halichondrin B (**1.21**)
(Isolated from marine sponge *Halichondria okadai* Kadota)

Eribulin (**1.22**)
Approved for the treatment of
breast cancer and liposarcoma

Figure 1.3. Examples of total synthesis.

While addressing the limited material supply of halichondrin B (**1.21**), Kishi *et al.* carried out the first total synthesis. At the same time, SAR studies were performed with polyether and macrolactone subunits and it was found that macrolactone subunit was equipotent to halichondrin B (**1.21**) *in-vitro* but not active *in-vivo*. Further, the optimization of the macrolactone subunit analogue led to the discovery of eribulin (**1.22**) that was approved by the FDA in 2010 to treat breast cancer and later in 2016 for the treatment of liposarcoma.

1.4. Diverted total synthesis (DTS)

This strategy was introduced by Danishefsky, for the synthesis of natural product analogues with the aim of synthesizing modified NPs for elucidating mechanism of action (Wilson and Danishefsky, 2006; Szpilman and Carreira, 2010). Some of the deep-seated structural modifications are not possible in semi-synthesis, however, this can be done through total synthesis by using modified building blocks. One of the classical examples of DTS is the synthesis of epothilone B (**1.23**) analogue, ixabepilone (**1.24**), which is used clinically (Figure 1.4) (Maier, 2015).

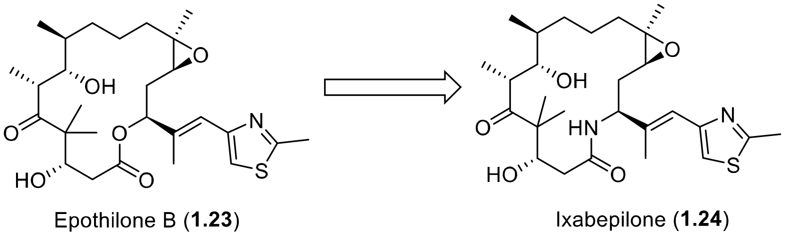
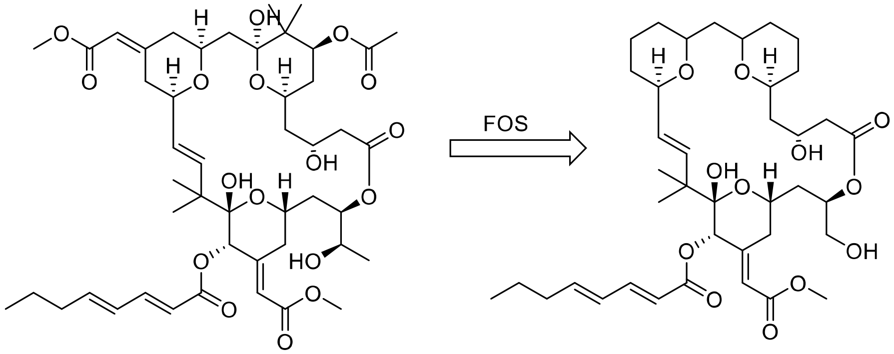


Figure 1.4. Example of diverted total synthesis (DTS)

1.5. Function-oriented synthesis (FOS)

In this approach, the simpler analogues of complex and scarcely available NPs are designed and synthesized, provided straightforward synthesis in a step-economic way and retained biological activity. One of the leading example of FOS is the synthesis of bryostatin analogues. Bryostatins are the complex molecules possessing anticancer, immune-modulating, and cognition enhancing properties. However, bryostatin is obtained in a very low yield of only 0.00014% from marine bryozoa. Since the activity resided only in a fraction of the molecule, Wender *et al.* (2008) synthesized the simpler analogues of bryostatin having superior biological activities (Figure 1.5).



Bryostatin 1 (**1.25**, Ki = 1.4 nM)
Total synthesis > 70 steps

(**1.26**), Ki = 0.3 nM
29 steps

Figure 1.5. Example of function-oriented synthesis (FOS)

1.6. Biology-oriented synthesis (BIOS)

In the BIOS approach, the selection of compound classes and scaffolds for the synthesis of bioactivity-enriched compound libraries is based on biological relevance. The basic scaffolds present in NPs are used as the starting material for the synthesis of compound libraries, which are functionalized by applying elaborate chemistry methods that offer relevant compounds. This strategy has been involved in the synthesis of large number of natural product-inspired libraries. For example, the synthesis of the iridoid-inspired compound library consisting of 115 derivatives, yielded nine compounds with Wnt signalling inhibitory activity with $IC_{50} < 10\ \mu M$ (Takayama et al., 2013).

1.7. Biosynthesis inspired synthesis

In case of microorganisms like bacteria and fungi producing NPs, their biosynthetic machinery and genetic makeup is well-known. Thus, by altering their genes to block the biosynthesis of metabolites and simultaneously feeding with unnatural building blocks, novel NP analogues can be obtained. This approach is also known as 'mutasynthesis' (Weissman, 2007). Using this approach, various derivatives of geldanamycin (**1.27**) have been synthesized (Figure 1.6). The blocked mutant of *S. hygroscopicus* K390-61-1 was fed with different 3-aminobenzoic acids to produce various novel geldanamycin derivatives (Eichner et al., 2009). Semisynthesis can be combined with mutasynthesis to produce compound libraries when mutasynthetic derivative allows for subsequent derivatization.

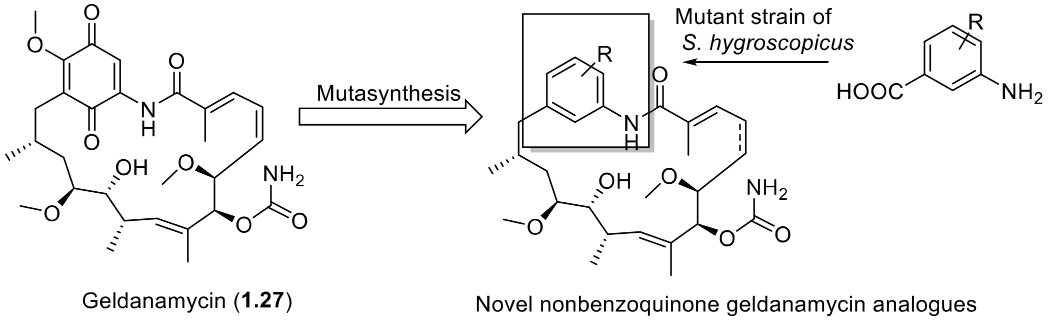


Figure 1.6. Biosynthesis inspired synthesis

In conclusion, various examples of natural products used as drugs are discussed. The different strategies used for optimization of suboptimal properties of natural products in order to yield valuable new drugs are discussed with the help of examples. In the present work, natural products were isolated from plants. The identified lead was further subjected to semi-synthetic modifications to generate diverse derivatives.

1.8. Aims and objectives

In the present work, two medicinal plants *Ipomoea nil* and *Gloriosa superba* were selected. *Ipomoea nil* seeds were selected on the basis of the chemotaxonomy, being rich source of medicinally important diterpenoids; gibberellins. The seeds of *I. nil* are also very less explored for phytochemistry as well as pharmacology. The phytochemical investigation of *I. nil* seeds, in order to discover possible anticancer leads, has been planned.

Another plant for the study was chosen *Gloriosa superba.* The roots of *G. superba* are a rich source of the medicinally important alkaloid colchicine. A review of the literature revealed that *G. superba* is a rich source of several tropolone-containing colchinoids also. At the same time, the pharmacology of gloriosine (*N*-deacetyl-*N*-formylcolchicine), another colchinoid, has not been investigated. In search for anticancer lead, G. *superba* roots were planned to explore the phytochemistry and medicinal chemistry of gloriosine.

Our objectives are:

1. Isolation, total synthesis, and cytotoxic activity of compounds of *Ipomoea nil* seeds
    1.1. Phytochemical investigation of *I. nil* seeds
    1.2. *In-vitro* cytotoxic activity of ipomone
    1.3. Total Synthesis of ipomone
2. Isolation, semi-synthetic modification, and cytotoxic evaluation of compounds of *Gloriosa superba* roots
    2.1. Phytochemistry of *G. superba* roots
    2.2. *In-vitro* cytotoxic activity of gloriosine
    2.3. Synthetic modifications of gloriosine

1.9. Plan of work

1.9.1. Isolation, total synthesis, and cytotoxic activity of compounds of *Ipomoea nil* seeds

For the study of *I. nil* seeds, bioassay-guided fractionation of extract, followed by the purification of compounds *via* repeated column chromatography of the bioactive fraction was planned. Initially, an acidified hydromethanolic solvent was planned to prepare the extract as hypothesized to separate alkaloids from non-alkaloids. The HP20 resin-based fractionation of the prepared extract was scheduled. Targeted bioactive fractions and isolated compounds were planned for *in-vitro* cytotoxicity evaluation (Figure 1.7). Furthermore, it was intended to subject the isolated compounds to synthetic or semi-synthetic modification to create a diverse compound library around the identified lead for biological evaluation.

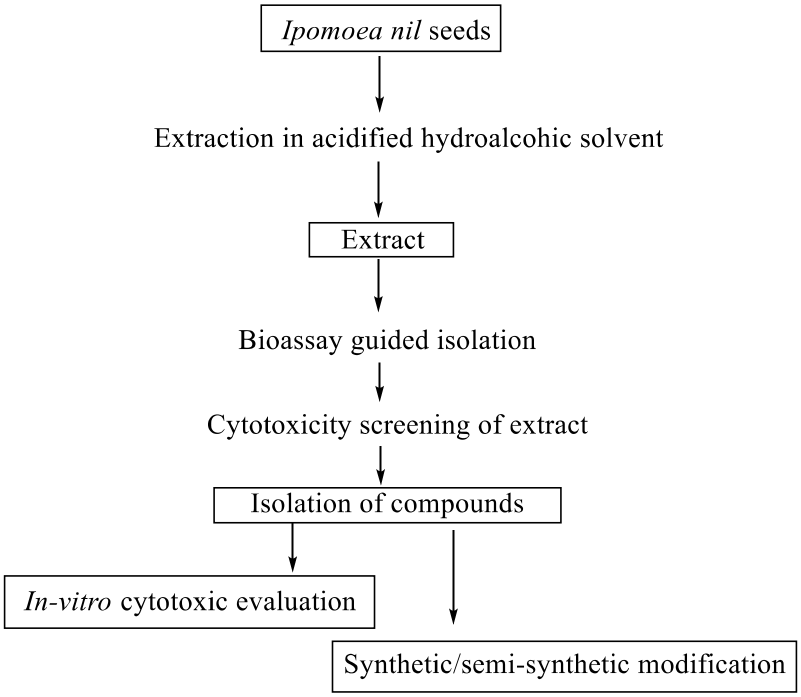


Figure 1.7. Flow chart showing plan of work of *Ipomoea nil*

1.9.2. Isolation, semi-synthetic modification, and cytotoxic evaluation of compounds of *Gloriosa superba* roots

A typical procedure for the extraction of roots of *G. superba* was planned to prepare the crude extract. As usual, the extract was planned for phytochemical investigation by repeated column chromatography. Many tropolone-containing colchinoids were scheduled to be isolated. Gloriosine had never been explored for its pharmacology, so it was targeted to scale up in sufficient amount to investigate its cytotoxic potential. Further, semi-synthetic modification of gloriosine was planned to explore the medicinal chemistry around the lead (Figure 1.8).

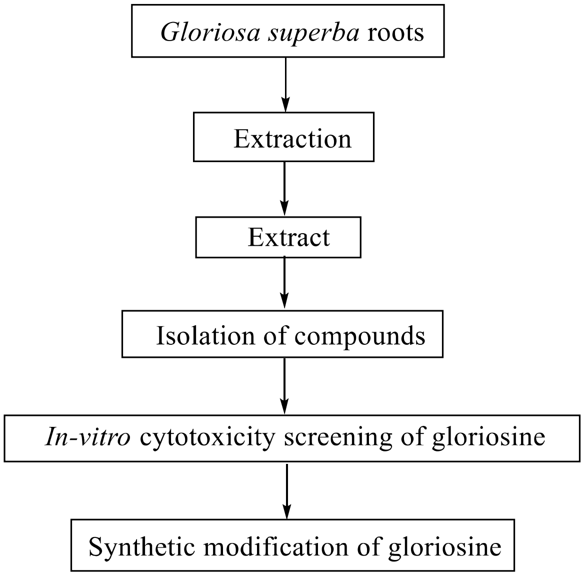


Figure 1.8. Flow chart showing plan of work of *Gloriosa superba*