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Cantharidin-based small molecules as potential therapeutic agents

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Chemical and pharmacological information on cantharidin-based SMs was analyzed. The review summarizes new facts about blister beetles metabolites for the period 2006-2012. General synthetic approaches to cantharidin-based small molecules as well as its chemical transformations and the biological activities related with cantharidin, norcantharidin, cantharidimide and norcantharimide analogues, specially their inhibitory activity of phosphoprotein phosphatases in cancer treatment, were discussed in this mini review that could help to design new small molecules modulators for other biological models.

Key words: Cantharidin analogues, Blister beetles, PP inhibitory activity, Anticancer activity, Diels-Alder cycloaddition reactions, Drug design and development.

Short running title: Cantharidin-based small molecules

INTRODUCTION

With the development of the natural product chemistry, giving many interesting objects to study from the structural, synthetic and pharmacological standpoints, the organic synthesis had evolved at the same rate with the generation of molecules/molecular assemblies with well-defined biological functions, and within this challenging task in the biology-oriented synthesis field, the design and develop of more efficient chemical reactions and methodologies that would revolutionize the next-generation of chemical and biological research. Secondary metabolites of plant and animal worlds are the main objects to study for phytochemistry and organic chemistry; demonstrating with their centennial history that has successfully promoted the discovery and drug development. According to a recent analysis, in the last 25 years nearly

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half of the drugs, currently in clinical use, belong to drugs of natural origin (1,2). Therefore, the success of drug discovery based on nature's secondary metabolites depends on how these natural substances are considered, mainly by medicinal and organic chemists, as source of inspiration rather than target molecules (1,6).

For centuries, it has well known that many plants (shrubs, trees and grasses) possess a wide spectrum of medicinal properties and that bacterium and fungi are also capable to generate toxic constituents that inhibit the growth of other organisms in their proximities. These kingdoms have provided us more information, regarding to the biological interactions of small molecules (SMs), to understand and encourage our investigations in the discovery and development of new SMs with marked physiological activities (7,8). In addition, these SMs are also useful for investigating biological systems as effective tools to elucidate the mechanism of important cellular processes that are based purely on *i*) the performance of enzymatic reactions and *ii*) protein-protein interactions, where the bioactive SMs are called bioprobes, and more important, *iii*) they allow the rapid and conditional modulation of biological functions often in a reversible, dose-dependent manner (9,10).

In contrast to plants and bacteria worlds, the invertebrate animal kingdom, to which belong different insects (about a million species) including beetles, is less studied. However, particular medicinal properties of some beetles stimulated (bio)chemical researches to study and discover new SM inhibitors with an extremely important protein affinity, that are responsible for cell proliferation, and within this group: cantharidin, norcantharidin and cantharimides and its analogues represent one of the most simplest natural model that could play an important role in the search for new effective and selective anticancer drugs (11-13).

The vast volume of information concerned with these natural derivatives prevent us from prepare an exhaustive review; rather, in this mini review we aim to present the key background information related with the chemical and pharmacological information on the SMs that metabolize blister beetles of family Meloidae for the period 2006-2012. We will focus on the biological and synthetic approaches to cantharidin-based small molecules as well as its more recent chemical transformations, hopping that this chemical modification tactics on the structure of these natural products could be a real and rapid way in developing new drug candidates and could help to design new SMs modulators from other biological models.

In the section 1 we will survey a minor scientific classification of the Meloidae family; this will provide a sense of the origin, history and the biological role of cantharidin, norcantharidin and cantharimides.

Section 2 outlines the strategies for the synthesis of cantharidin and norcantharidin analogues while section 3 highlights the current strategies for the synthesis of cantharidinimides and norcantharidinimides. In section 4, the biological role of cantharidin, norcantharidin and cantharimides is discussed, standing out their interaction with the protein phosphatases: PP1 and PP2A. Finally, in section five we present some concluding remarks.

Blister beetles of the Meloidae family

Blister beetles are insects of the order Coleoptera (beetles) of the family Meloidae that contains about 2500 species, divided among 120 genera and four subfamilies: Eleticinae, Meloinae, Nemognathinae and Tetraonycinae (14). Since some of these numerous species contain a chemical secretion of a blistering agent, they are known as blister insects, and despite the approximately 7,500 species that are widespread throughout the world in warmer and drier areas, they are not considered as a pest. Being that in zones as New Zealand, Antarctic and temperate and arid regions, as well as the sub-tropical and tropical savannas, are not present and cannot survive (15).

According to their life cycle, the blister beetles undergo hypermetamorphosis, which first larva stages take the form of a triungulin, highly mobile in order to search out a host, while the following stages are more sedentary and remain on or within the occupied host. The adult beetles are phytophagous that means that they dietary are based on plants of the families Amaranthaceae, Compositae, Leguminosae and Solanaceae, and they are easily recognized by morphological characteristics such as soft body, bright coloration, rather elongate, head deflexed with narrow neck, pronotum not carinate at sides, heteromerous tarsi, and smooth integument (16).

Many of the species of the Meloidae family are blister beetles whose metabolism provides a poisonous substance, comparable to cyanide and strychnine in toxicity, that is used by the beetles as a defensive chemical weapon to protect them from predators and that displayed severe effects on the gastrointestinal tract, kidney, and ureter in mammals and humans. Nevertheless, this toxin has rich history and a wide range of biological activities that have affected human health for centuries.

Cantharidin and norcantharidin: origin and history

Since the year 1264, China began to use the extracts from the wretched insect *Mylabris caragnae* to remove wart, time in which the high incidences of deaths in the treated patients revealed the first evidence of the toxicity in humans and limited the general use of these extracts. Posterior studies revealed that the

dried bodies of the *Mylabris caragnae* possesses antitumor properties and increases the number of leucocytes, but once again the irritant effects on the urinary system of the treated patients reduce its uses (16). In the Middle Ages in Europe, the dried bodies of the "Spanish fly" (*Lytta vesicatoria*), a green 11 - 21 mm long beetle that can be found in certain areas of Europe and some eastern regions such as Vietnam, Taiwan, Thailand, Korea and China, began to be used as an alleged aphrodisiac, abortifacient and in the treatment of malignant tumors as an extended application in the Chinese medicine, but with the poisoning side effects in humans as a result of its use (17).

In order to investigate and explain the mode of action, as well as the acute toxicity, of the extracts of the Spanish fly and based on the observation that when these beetle was pressed, rubbed or squashed by any external agents, they expel a hemolymph "blood" from the joints on the legs as a chemical defense, giving any would-be predators a foul-tasting appetizer. In 1810 the French pharmacist Pierre Jean Robiquet, by grinding and drying the bodies of the Spanish fly, isolated a crystalline compound, known today as cantharidin (CTD) 1, whose correct structure was later proposed by Gadamer and his students (18,19). CTD or *exo*,*exo*-2,3-dimethyl-7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid anhydride **1**, is a monoterpene stored in the insects' blood and that is stable and remains toxic even in beetle carcasses for long periods of time, been classified as a strong vesicant substance that causes blisters on contact with the human skin, giving them the name of blister beetles. The CTD concentration in the blister beetles can varied within all species: The highest reported cantharidin content for a blister beetle is 5.4% in the dry weight bodies of *Epicauta vittata*, while in the "Spanish fly" the CTD content is about 5% (20) (Figure 1).

Unlike CTD, the closer demethyl analog, (±)-palasonin is the only CTD analogue found in both plants and beetles. (S)-(-)-Palasonin **2** has been isolated from the seeds of an Himalayan plant, *Butea frondosa* (Leguminosae), while the (R)-(+)-palasonin **3** is present in the dried bodies of the *Hycleus oculatus* and *H. tinctus* beetles, a southern African Meloid species (21-25). Nonetheless, both natural sources provide very small quantity of this molecule (26) (Figure 1).

CTD is found in the blister beetle's blood of both sexes, but oddly enough, is only "bio-synthesized" by the male specimens, which use it as a male courtship pheromone (27). It was reported that CTD can act as an excitatory pheromone for male fire-colored beetle *Neopyrochroa flabellata,* which consumes this molecule and produces a cantharidin-rich secretion from a cephalic gland during courtship. Thus, CTD has important function in an aphrodisiac-like capacity in this particular insect and then, during the mating (28). CTD is transmitted to females for protection of the beetles' eggs, in an intended to cover and preserve the life of the larvae during their early stages of development (29,30). However, not only

protection is the primary role of CTD in the life cycle of the beetles, this chemical weapon with odor and bad taste is released in small doses when the adult specimens are endangered and are swayed by their predators. As was described CTD is exclusively biosynthesized by male beetles of the family Meloidae, although detailed mechanism of its biosynthesis is unknown at present, this fact can conclude that the production of cantharidin may be a more complicated process and seem to even cast some doubts on the mevalonoid origin of this compound, however specialized researches on the subject have suggested that the production of CTD on insect, instead of the two obvious possible routes: the tail-to-tail condensation of two isoprene units and the head-to-tail condensation of two isoprene units followed by a methyl shift, several unknown oxidation processes from farnesol **4** would be involved in CTD biosynthesis (31,32) (Figure 2).

Once the pharmacological and toxicological properties of CTD become known by other cultures around the world, the responsibility of development more selective and bioactive cantharidin derivatives with lower toxicological profiles lied on organic chemistry. Using the tactic of structural modification of CTD, as a real and rapid way to develop new chemotherapic agents, new cantharidin derivatives: norcantharidin (NCTD) **5** and 5,6-dehydronorcantharidin **6** were prepared. To date, so far any of these derivatives have been isolated or are present in any natural source, but they have been synthesized easily from commercial reagents with the advantage that these analogues have reduced the intrinsic toxicity of CTD, while its biological activities (especially, the anti-cancer activity) are retained (33,34) (Figure 3).

Cantharimides: origin and history

At the beginning of the last decade, all the studies performed in other species of the Meloidae family had as the main objective the isolation of CTD and other oxygen analogues (e.g. NCTD). However, when these studies were extended to the Chinese beetles, whose habitats principally are Korea and China, in the dried bodies of *Mylabris phalerate* PALLAS, besides the presence of CTD and NCTD, a new class of compounds were discovered: the cantharimide **7** and the 5-substituted cantharimide **8**, nonetheless these kind of substances were found in very low concentrations $\leq 0.002\%$ (35) (Figure 4).

The main structural difference between CTD and cantharimides, is that the oxygen atom in the anhydride core of CTD, is replaced by a nitrogen atom, which may or may not be substituted; this fact improved the solubility and the cytotoxicity of these derivatives against human hepatocellular carcinoma cell lines (36). In contrast to the known biological role of CTD, it still unknown the role of cantharimides in the life cycle of the beetle *M. phalerata* and the process, by which is biosynthesized in the insect; probably CTD

undergoes a chemical transformation, in which oxygen is replaced by nitrogen through some kind of biochemical transformation or by an enzymatic source of nitrogen, although these hypothesis are unconfirmed and will encourage further investigations related with these issues (Figure 5).

Strategies for the synthesis of cantharidin and norcantharidin analogues

Being anhydrides of the 7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid core, the CTD and NCTD molecules can be formally considered as Diels-Alder cycloaddition adducts from furan derivatives and maleic anhydride, presented a well-defined *exo*-stereochemistry of oxygenated ring and in the 7-oxa-norborn-2-ene system. Once the synthetic protocol for the preparation of these derivatives had been established, the further investigations focused their attention in the structural transformations on the CTD and NCTD cores in order to increase the bioactivities and reduce the toxicity of these natural products.

Cantharidin and norcantharidin synthesis via Diels-Alder reaction

The structural analysis of the natural CTD **1** and (±)-palasonin **2** molecules indicated that the most, obvious and current protocol that could be adopted for the synthesis of this derivatives, is the direct cycloaddition of furan, dimethylmaleic and/or citraconic anhydrides **9** and **10**, followed by the hydrogenation (H_2, 10% Pd/C, THF) of the respective adducts **11** and **12**, the unsaturated CTD and palasonin analogues. In general, the reduction of the double bond, besides provide the desired natural products, have to be done because the high their instability, since the predominant aromaticity of furan make it a poor Diels-Alder diene, the Diels-Alder adducts are not very thermally stable and it is known that they undergo a retro-Diels-Alder reaction, regenerating the original starting materials. Like most of the subsequent steps are intended to modify the derivatives **11** and **12**, these procedures usually require prolonged reaction times and high reaction temperatures, so the double bond needs to be removed in order to prevent the retro-Diels-Alder reaction (Figure 6). As interesting fact, the dimethylmaleic anhydride **12** cannot react itself with another molecule of furan in a subsequent cascade reaction, even at pressures up to 40 kbars, so some authors have suggested that the failure of this is a result of both electronic and steric factors. Nevertheless, this reaction failed in the terms of the "classic conditions" of the Diels-Alder cycloaddition (the use of solvents like: Et_2O, $CHCl_3$, benzene, toluene, etc. and at room temperature or reflux) (37,38).

The total synthesis of cantharidin was performed in the 50's of the last century by Stork (39,40) and Shenck (41). Both groups used a linear synthetic tactic, using the Diels-Alder reaction between different dienes and dienophiles. But the breadth and complexity of these synthetic efforts contrast with the simple structure of these molecules. But with the development of high-pressure Diels-Alder reaction (8-15 kbar, 138 h) of furan and anhydride **10**, the large scale synthesis of (±)-palasonin were achieve and the racemic mixture was resolved with the preparation of amides using 2 equiv of (S)-(-)-α-methylbenzylamine (42).

Nowadays and with the same approach, a straightforward preparation of CTD **1** with high-pressure Diels-Alder reactions between furan and 2,5-dihydrothiphene-3,4-dicarboxylic anhydride **13**, in which the dihydrothiophene ring induced the selective formation of the *exo*-cycloadduct **14** that is then reduced under catalytic conditions with Raney Ni, leading the CTD **1** in 72 % yield is a the process that had been employed to obtain **1** in large scale (43) (Figure 7).

It should be noted that the Diels-Alder reaction of furans with dienophiles usually results in the formation of the thermodynamically more stable *exo* product, while the kinetic *endo* product, once is formed, undergoes a facile retro reaction to form the starting materials and is rarely observed in synthetic procedures. However, a dramatic acceleration rate of the Diels-Alder reaction of furans with anhydrides as a dienophiles, generates the *endo* products when a 5 M lithium perchlorate-diethyl ether medium is employed (44).

Structural modifications performed on the cantharidin and norcantharidin

The acute toxicity exhibited by CTD and NCTD has motivated the organic chemistry to modify the chemical structure of these natural derivatives, and in that way improve their pharmacological profiles and reduce their toxicological properties. The retro synthetic analysis of the CTD and NCTD structures revealed in first place that intrinsic modifications on the furan and in the anhydride rings could generate diverse libraries of new cantharidin analogues that have been the focus of a wide range of bioassays in which have been discovered novel pharmacological agents.

Substitutions on the 7-oxabicyclo[2.2.1]heptane core

With the hypothesis that fluorine substitution in several SMs often leads to modified and improve the biological activities and/or reduce the toxicity in comparison to the corresponding non-fluorinated parent compounds (45). In 2001, Essers *et al.* reported the synthesis of fluorinated maleic anhydrides **15** and **16** and studied their behaviour as a dienophile in the Diels-Alder reaction with furan. In case of anhydride **15** the reaction occurred at 50 ^{o}C within 42 h and without any solvent. However, the expected adduct *exo*-**17**

was not observed, suggesting a rapid hydrolysis of the cycloadduct when this product enter in contact with moisture, leading to the formation of endothall derivative **18** (Figue 8a). The second fluorinated anhydride **16** reacted with furan at 55 °C within 52 h, and once again without use any solvent, to give the fluorinated dehydropalasonin analogue **19** with an *exo,exo* stereochemistry (46) (Figure 8b).

Besides furan, another heterocyclic compound commonly used as a diene for the synthesis of CTD analogues is thiophen, but usually this compound does not undergo the Diels-Alder reaction with maleic anhydride under the same conditions depicted in Figure 8 because thiophen is a very poor diene, more than furan, due to its aromatic nature, however, the reaction of thiophen with maleic anhydride had been promoted by high pressure in dichloromethane at 40 °C for 71 h to yield the *exo*-7-thiabicyclo[2.2.1]heptane cycloadduct **20** in 60 % (47) (Figure 9).

2-Substituted furan rings have also been used as a dienes in the Diels-Alder reaction with maleic anhydride. Unfortunately, it seems to be that the length of the chain, as well as the presence of withdrawing groups in this chain has a negative impact during the reaction due to the inductive effect of these groups. This statement can be evidenced when 2-cyanomethyl furan **21** and N-acetyl furfurylamine **22** are used as a dienes, giving the corresponding adducts **23** (19 %) and **24** (23 %) in very poor yields (48) (Figure 10).

Based on computational docking experiments, Tatlock *et al.* modified the C-5 position of the 7-oxabicyclo ring system using 3-substituted furan rings. When ten different carboxylic acids **26a-j** were esterified with 3-hydroxymethyl furan **25**, different type of dienes were generated **27a-j** and those compounds undergo the Diels-Alder reaction with maleic anhydride in diethyl ether at 23 °C. The reactivity of the dienes **27a-j** can be compared with the reactivity of dienes **21** and **22**, in which products **23** and **24** were obtained in much lower yields than the adducts **28a-j** are produced, so it can be induced that in those cases the presence of substituents in C-2 position of the furan ring, besides the electronic effects, could generate an steric impediment during the reaction that affect the final yields, while if the substitution is the C-3 position of the furan ring this effect not represent any negative impact (49) (Figure 11).

It was reported that the reaction between maleic and furfuryl alcohol at 69 bar and 35 ^{o}C proceeded 10 times faster in sc-CO_2 compared to reactions carried out in diethyl ether; this obviously illustrates the extraordinary potential of sc-CO_2 as synthetic media to access molecules unobtainable using conventional synthetic methodologies (50).

The avoidance of use organic solvents is one of the goals in current efforts toward the desgn of more environmentally benign chemical processes. Thus, sc-CO_2 is non-toxic alternative solvent in organic chemistry. However, its use requires more sophisticated equipment than standard laboratory apparatus. The scope of reaction media for Diels-Alder reactions is rather large, including water (51), ionic liquids (52), polyethylene glycols (53) and mixtures of simple carbohydrates (fructose, sorbitol, glucose etc.), urea and inorganic salts (54).

Reduction and substitutions on the carbonyl function on the anhydride core

Tarleton *et al.* modified the structure of NCTD by reducing one of their carbonyl functions to produce the (3S,3aR,4S,7R,7aS)-3-hydroxyhexahydro-4,7-epoxyisobenzofuran-1(3H)-one **29**, and promoted by the structure of this cyclic lactone, different alkylation reactions of the hydroxyl group with short and large chains were performed under microwave irradiation yielding 18 compounds (55) (Figure 12).

In the same study and with the hypothesis that the phosphate group of natural fostriecin **31** plays an important role on the broad spectrum of anticancer activities displayed by this molecule (56), a new series of compounds **32a-d** were synthesized with a terminal phosphate moiety employing various chlorophosphates and in the presence of dibutyltin oxide to obtain the desired products in good yields (Figure 13).

Strategies for the synthesis of cantharidimide and norcantharidimide analogues

Being cyclic imides of the 7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid core, cantharidimides and norcantharidimides, as well as CTD and NCTD, can be formally considered as Diels-Alder cycloaddition adducts from furan derivatives and substituted maleimides. However, this methodology has not been useful for the preparation of these derivatives due to the low selectivity to obtain the *exo*-adduct and to the limited structural diversity of the synthetic maleimides (most of them have to be prepared in a two-step methodology). With the discovery of the condensation of CTD and NCTD with primary amines a wide range of compounds have been synthesize, setting this methodology as the most powerful strategy for the preparation of cantharidimide and norcantharidimide derivatives, less toxic, and with similar bioactivities exhibit by CTD and NCTD.

Norcantharidimide synthesis via Diels-Alder reaction

While the preparation of exo-5,6-dehydrocantharidin **6** and its saturated analogue, norcantharidin **5** is more easily to achieve using classic reaction conditions (THF or Et_2O, 16 h, rt) for Diels-Alder cycloaddition of furan and maleic anhydride. Similar results were obtained when the same reaction conditions were successfully applied to the reaction of furan with N-methylmaleimide **33** to give both diastereomers, N-methyl-7-oxabicyclo[2.2.1]hept-5-ene-2-*endo*,3-*endo*-dicarboximide **34** and N-methyl-7-oxabicyclo[2.2.1]hept-5-ene-2-*exo*,3-*exo*-dicarboximide **35** in good yields after chromatographic separation (57) (Figure 14).

Structural modifications performed on the cantharidimide and norcantharidimide analogues

With the discovery of cantharimides and norcantharimides, once again the world found in nature the solution related to the problem of the acute toxicity exhibited by CTD and NCTD. These compounds disclose a major solubility, proved to be less toxic and due to the presence of heterocyclic nitrogen, many structural modifications can be done on this atom. As well as in the case of CTD and NCTD, intrinsic modifications on the furan ring could generate diverse libraries of new cantharidimide analogues in addition to those generated by the N-substitution.

Cantharidimides and norcantharidimides analogues synthesized by a dehydrative condensation of CTD and NCTD with primary amines

One of the first reports related with the synthesis of cantharimides was published by McCluskey *et al.* in 2001. As the early modification of CTD with primary amines, the reaction of NCTD with different amino acids was performed with triethylamine and anhydrous toluene at 200 °C for 24 h. As a result, sixteen new norcantharimides **36a-p** were obtained in moderated yield and the used of both D- and L-amino acids allowed the examination of the effect of these different stereoisomers (58) (Figure 15).

In a similar procedure for the synthesis of the related amino acid substituted N-derivatives **36a-p**, thirteen analogues of norcantharidimide were prepared using the same reaction conditions described in Figure 15. For the first library, twelve N-alkyl amines were employed having short and long chains, cycloalkanes, and unsaturated groups, for the N-alkylation reaction of NCTD **5** to yield the desired products in moderate to excellent yields (31-98 %) compared with the ones exhibit by the series of amino acids. In the second library of nine compounds, different N-alkylated NCTDs were prepared containing in the alkyl chain hydroxyl groups and terminal carboxylic acid groups with moderate to good yields (22-80 %). The morpholine moiety was included as well in three derivatives when it is bonded to the nitrogen directly and

by an alkyl chain of two and three carbons, unfortunately in very low to moderate yields (7-43 %). A fourth library of seven compounds were prepared from substituted anilines and benzylamines, due to the less reactivity of anilines, these derivatives were obtained in moderate to good (36-79 %) in comparison to the benzyl products (43-91 %), in which the substrate is considered more as a primary amine, similar to the ones described for the first library.

Finally, the remarkable compounds synthesized in this study performed by Hill *et al.*, are the bis-NCTDs **39** and **40** prepared with 1,3-diaminopropane **37** and 1,12-diaminododecane **38** under the standard conditions, described in Scheme 15, afforded the rest of the N-alkylated NCTDs (59) (Figure 16).

With the idea to provide a new direction for novel drug discovery in experimental cancer therapy, it has been established that cantharidin can be combine with basic hetero-molecules in a single molecule trough chemical modifications. Indeed, cantharidin-containing N-hetero-molecules **41** and **42**, were easily prepared via a one-pot condensation reaction from cantharidin **1** and 2-aminobenzothiazole derivatives **43** and 5-amino-1,3,4-thiadiazole-2-thiol **44** (60-62) (Figure 17).

Just how was performed for bis-NCTDs **39** and **40**, and promoted by the structural diversity and the high yield in which compound **42** was obtained, efforts to prepare the dimer of cantharimide **42** lead the formation of bis-norcantharimide **45** using piperazinium dichromate as a promoter at room temperature, unfortunately the low yield in which **45** was obtained did not encourage the extension of this methodology to other derivatives (60) (Figure 18).

Previous synthetic efforts have shown that NCTDs can be synthesized using high temperatures and using toxic and corrosive solvents. Recently, it was investigated an alternative approaches to access to a new NCTD libraries, from this study the most notably protocol so far involves the use of microwaves to accelerate the reactions with the use of alternative solvents. With this approach Thaqui *et al.* reported the synthesis of three new libraries of NCTD analogues in which, besides the N-substitution with amino acids and alkyl chains containing or not hydroxyl groups, the 7-oxabicyclo[2.2.1]heptane core was replaced by the hetero-substituted 5,6-ethyl bridge, modification that intended to improve the biological activities of these derivatives, through a reaction carried on water or dimethylacetamide (DMA) at 170 °C and assisted by microwaves (63) (Figure 19).

One tactic to combine the NCTD ring with potentially bioactive hetero-molecules, similar to chloroquine, was developed in a two-step methodology that involves the first amidation and open anhydride ring follow by the dehydratation (ring closure) of this intermediate to yield the novel 5,6-

dehydronorcantharimide-chloroquine hybrids **56** and **57** in excellent yields, compounds that are being investigated for their antitumoral activity (64) (Figure 20).

Another study on chemical modifications on the scaffold **5**, started with the Wittig reaction between NCTD **5** and the phosphorous ylide **58** in order to modified one of the carbonyl functions **59** and the later rapid nucleophilic ring opening of the ester, promoted by the amine, lead to the formation of a new library of twenty two compounds **60**, which contain the ethereal bridge (7-O) of cantharimide and a wide range of substituents bonded to the nitrogen atom (65). Within which are: aliphatic, branched aliphatic cycloaliphatic and aromatic, basically the same substituents used by the same authors in works (55,63) (Figure 21).

To complement the previous work related with the synthesis of cantharimide analogues from amino acids (58,63) Cheng et al. highlights in their study that the reaction of NCTD **5** with L-histidine **61** resulted in the formation of L-histidine norcantharimide **62** in 97 % yield, avoiding the use of toxic solvents like toluene and triethylamine as a catalyst, improving the reaction conditions with the use of ethanol and under low ranges of temperatures. However, compound **62**, due to the proximity of the carboxylic function with one of the nitrogen of the imidazole ring, form zwitter-ionic specie in an equilibrium with derivative **62** (66) (Figure 22).

The same authors that reported the inclusion the phosphate group of fostriecin **31** in the chemical structure of NCTD derivatives with the intention or increase the biological properties of these compounds, explored the incorporation of the phosphate group in the terminal free -OH moiety of some of the derivatives that they introduced in their work (59). With similar methods described above, six N-alkyl substituted cantharimides **63a-f** were treated with different diethylchlorophosphates in the presence of n-butyltin oxide and triethylamine to give the desired terminally substituted phosphate analogues, 18 examples: **64a-f**, **65a-f** and **66a-f** (67) (Figure 23).

The dehydrative condensation of CTD and NCTD with primary amines had been the main methodology for the synthesis of cantharidimide derivatives. In addition, two recent reports have published the synthesis of thirty-four derivatives with the same conditions depicted in Figure 15 in poor to good yields (5-82 %) (36). The problem of the low reactivity of anilines during this reaction mentioned in Figure 16 was overcome by changing the reaction conditions by Deng et al.; this approach involves the use of triethylamine, acetic anhydride and manganese (II) acetate as a catalyst. With this methodology twenty N-phenylnorcantharidimides were prepared in good to excellent yields using anilines with both donating and withdrawing groups (68,69) (Figure 24).

Substitutions on the 7-oxabicyclo[2.2.1]heptane core

Substitutions and modifications on the 7-oxabicyclo[2.2.1]heptane core have been achieved by making react the double bond of NCTD derivatives obtained through the Diels-Alder reaction, as well with the use of substituted dienes in this reaction. As an example for the first case, after the synthesis of compound **35** was reported, in 2010 Goksu *et al.* converted this adduct in the interesting *exo*-arylated product through the Heck arylation, generating 5-substituted NCTDs **67a-c** in good to excellent yields (70) (Figure 25).

With the use of thiophen for the preparation of CTD analogues with the tioetheral bridge (7-S), the methodology developed in that opportunity was extended to the reaction of thiophen with maleimide **68** under the same conditions depicted in Figure 9, high pressures were required and dichloromethane was also used at 40 °C for 71 h to yield the *exo*-7-thiabicyclo[2.2.1]heptane cycloadduct **69** in 60 % (47) (Figure 26).

One strategy to combine reactive 5,6-dehydronorcantharidin derivatives depicted in Figure 23 with nitrile oximes **70** was developed to generate the 5,6-dehydronorcantharidin-isooxazolidine adducts **71**. Through a 1,3-dipolar cycloaddition reaction between those molecules in the presence of *tert*-butyl hypochloride (68) or chloramine-T (69) twenty of these tetracyclic compounds were prepared in good yields with the important pharmacophore group, the 3,4,5-trimetoxyphenyl moiety, responsible for several anticancer activities (Figure 27).

One of the used 2-substituted furan rings that have generated the corresponding adducts **23** and **24** in very poor yields according to Figure 10, was recently used once again as a diene for the Diels-Alder reaction with different N-substituted maleimides **72a-c.** This reaction was performed using boric acid as a catalyst with the hypothesis that the yield and the stereoselectivity of the obtain product will be increase through the possible interaction of the boron atom with the carbonyl functions of the maleimides **72a-c**. In this order, and also employing a green and mild solvent like polyethylene glycol 400 (PEG-400), the new series of *N*-(10-oxa-4-azatricyclo[5.2.1.$0^{2,6}$]decan-8-ene-3,5-dione-7-ylmethyl)acetamides **73a-c** were obtained in excellent yields (71) (Figure 28).

Biological activities related with cantharidin, norcantharidin, cantharidimide and norcantharimide analogues

Despite many studies on the physiological and biochemical effects of cantharidin and its analogs, their mechanism of action only could be elucidated until 1992, when Li and Casida demonstrated that cantharidin and their anhydride analogs can bind, with high affinity, to a specific cantharidin-binding protein (CBP), whose isolation was reported from the cystol of mouse liver (72). With the identification of that CBP, a series of studies begun to investigate the nature of this enzyme and the relationship between it and the chemical structure of the cantharidin and its analogues, natural and synthetic. The acute toxicity exhibit by these SMs delayed their use in the pharmaceutical industry; however the organic chemistry has provided new and more potent derivatives with high activity against protein phosphatase enzyme and with less toxicity profiles. Although this bioactivity has been deep explored during the last five decades, CTD analogues has been used in the treatment of other important diseases due to the development of new strategies for the oral and intravenous administration of these derivatives.

The phospho protein phosphatases: PP1 and PP2A. The main biological targets of cantharidin and cantharidimide derivatives in cancer treatment

Cantharidin and its derivatives act by causing cell death. To explain this fact, many mechanism of action have been proposed and the most accepted of them involves a response to DNA damage and apoptosis through the inhibition of protein phosphatases. With the knowledge that most of these relevant cellular processes involve the interaction: protein-protein, protein-nucleic acid, protein-SM ligand and the enzyme-SM interactions. The latter has been recognized as one of the most important relationships for metabolism regulation and plays a key role in initial stages of signal transduction. This pharmacological approach, in which enzymes function (as protein-based catalysts for chemical reactions in living organisms) is modulated by these SMs, represents a milestone in our understanding of life and in our development of cures. Therefore, the study of enzymes is still a central focus of modern biochemical research, and among the enzyme superfamilies, protein kinases (PK) and protein phosphatases (PP) have occupied a preeminent position in this research (73-76).

Nowadays, it is well established that the reversible phosphorylation of proteins is a key regulatory mechanism in animals and higher plants cell division, and the level of phosphorylation of any protein depends on the relative activities of PK and PP (77). Structurally, PK can be divided into three groups: histidine kinases, cAMP-dependent protein kinase like kinases (cAPK), and other protein kinases. While,

there are three major classes of PP: tyrosine-specific, serine/threonine-specific, and dual-specificity phosphatases. The serine-threonine family has the ability to influence in the cell cycle control and growth process, in addition to be the enzyme to which CTD and it analogues are bonded. Traditionally, the serine-threonine, family has been classified into several groups, with numerous members in each, based on their biological characteristics, sensitivities to specific inhibitors, and substrate specificity: PPP1, PPP2, PPP3, PPP2C, PPP4, PPP5 and PPP6 (11).

The protein phosphorylation-dephosphorylation process consists of two thermodynamically favorable reactions; the transfer of phosphates from a high-energy adenosine triphosphate molecule (ATP) onto the proteins is catalyzed by PK. While PP, on the other hand, remove the phosphate group by the water-driven hydrolysis of phosphoester bonds. The phosphoryl group transfer is addressed to a nucleophilic acceptor group located on the amino acid side-chain(s) of a protein. Typically, the principal acceptor sites are the hydroxyl groups of serine, threonine, and tyrosine, the carboxylate groups of aspartic and glutamic acids, and the nitrogen atoms on the side-chains of histidine, lysine, and arginine (58) (Figure 29).

This reversible phosphorylation-dephosphorylation reaction is a rapid and efficient way to change the properties of proteins in a desired manner in the cellular environment and any derailment in the action of these enzymatic reactions can lead to numerous diseases. As this switch mechanism plays an important role in signaling pathways that control cell proliferation and carcinogenesis, the design and development of a SM modulator of the PK and PP functions are cornerstones in understanding cancer, Alzheimer's disease, and others. Thus, each new potent SM modulator on these proteins may provide potential drug for cancer (or cystic fibrosis, immunosuppression and, cardiac and neurological disorders) therapy since inhibition of any one of these proteins stops the signaling cascade (78,80).

In their report, Li and Casida (72) concluded that the enzyme in which cantharidin is bonded were phosphoprotein phosphatases 1 (PP1) and 2A (PP2A, AC type, EC 3.1.3.16). The special attention that the world has putting on study these phosphatases are that they might influence secretory processes, phosphatase-linked mechanisms that have been implicated extensively in several endocrine tissues, including islets of langerhans and chromaffin and pituitary cells (81). PP1 and PP2A exert also their effects by modulating the activity of cyclin-dependent kinases (cdk) and the retinoblastoma protein (pRb), and knowing that the activation of cdk/cyclin complexes requires the phosphorylation of a conserved threonine residue, as well as the removal of inhibitory phosphorylations, these enzymes play a crucial role in other biological process (48).

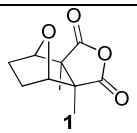
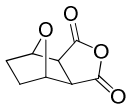
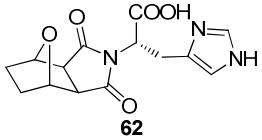
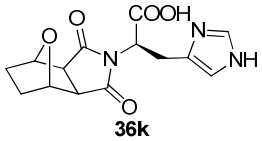
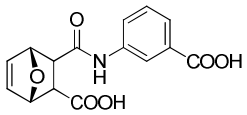
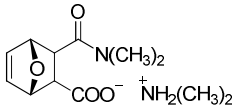
The PP2A is one of the most studied PP enzymes and its inhibition is associated with the toxicity to most organisms, but this property has been useful in cancer chemotherapy and CTD has demonstrated that can be used as an inexpensive and readily available probe for analysis the regulatory phosphorylation-dephosphorylation events mediated by PP2A and other protein phosphatases. The core enzyme of PP2A consists of a catalytic subunit (PP2Ac) and a regulatory subunit known as A subunit (PP2Aa). Actually, four different families of B subunits have been identified and they modulate the substrate specificity of PP2A (82). Interestingly, although PP2A is generally considered to be a cancer repressor, sometimes the inhibition of PP2A has been thought to be a cancer promoting by induction of phosphorylation and activation of several substrate kinases (83,84). However, some kinase-dependent growth inhibition pathways that are induced by PP2A inhibitors have recently been reported, suggesting that the activation of kinase pathways may not always be a cancer promoting (85). Besides their presence in mammals, the enzyme PP2A is also expressed in plants, where CTD possess an herbicidal activity, but its mode of action is not yet clarified and it is presumed that the inhibition of plant PP2A activity is accompanied by a decreased light-induced activation of nitrate reductase (86).

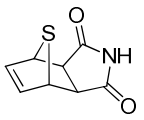
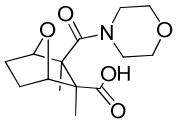
After the nature of PP2A was established, many synthetic CTD analogs have been prepared and evaluated by McCluskey and his group, which published some excellent reviews on this theme (11-13). The straightforwardness of various (nor)cantharidin analogues prepared via Diels-Alder cycloaddition, allowed them to study the relationship between structure and the anticancer activity trough the enzymatic inhibitory activity of PP1 and PP2A. The mode of action of CTD and its derivatives is based on causing cell death and many of the proposed mechanisms involve response to DNA damage and apoptosis through the inhibition of protein phosphatases (87,88).

Recently, it was shown that the anhydride rings of CTD and NCTD are hydrolyzed when they bound to the catalytic domain of the human serine/threonine protein phosphatases 5 (PP5c), and the high-resolution crystal structures of PP5c complexed with the corresponding dicarboxylic acid derivatives of the two molecules. However, NCTD shows a unique binding conformation with the catalytically active Mn2PP5c, while CTD is characterized by a double conformation in its binding mode to the enzyme. Different binding modes of NCTD are observed depending on whether the starting ligand is in the anhydride or in the dicarboxylic acid form. All these structures have been provided the basis for the rational design of the cantharidin-based drugs known so far, and it will be the bases for future design of novel inhibitory agents of PP1 and PP2A (89,90).

Each synthetic work related with the synthesis of CTD, NCTD and cantharidimide derivatives is strictly accompanied and complemented with the biological evaluation of these derivatives, more exactly their PP1 and PP2A inhibitory activity. In the following table, for each of the synthetic approaches described in sections 2 and 3, the representative cantharidin-based SMs with the 7-oxabicyclo[2.2.1]heptane core, which have present the highest values of their enzyme inhibitory activity, against the enzyme PP1 and PP2A, is shown (Table 1).

Table 1: The more potent inhibitors of protein phosphatases 1 and 2A among the known CTD, NCTD and cantharidimide derivatives reported so far.

Active molecule	Cantharidin derivative	PP1 inhibition (IC_{50} µM)	PP2A inhibition (IC_{50} µM)	Ref.
1	 **1** Cantharidin	3.6 ± 0.42	0.36 ± 0.08	(58)
2	 **5** Norcantharidin	5.31 ± 0.36	2.9 ± 1.04	(58)
3	 **62**	3.22 ± 0.7	0.81 ± 0.1	(58)
4	 **36k**	2.82 ± 0.6	1.35 ± 0.3	(58)
5		13 ± 5	7.0 ± 3.0	(48)
6		18 ± 8	3.2 ± 0.4	(48)

7	 **69**	12.5	426	(47)
8		5.9 ± 2.2	0.79 ± 0.1	(91,92)

From the results derived in Table 1, we can establishing a preliminary structural analysis based on the chemical structure and the inhibitory activity exhibit by active molecules **1-8**, six exchangeable points for studying the structure-activity relationship are marked with arrows in Figure 30, accordingly to what we believe that could be influential in future researches related with the synthetic and evaluation of CTD, NCTD and cantharidimide analogues, it can be observed that: 1. Molecules that lack substituents at positions C-1 or C-3 are more active in both types of activity, 2. Saturation type C5-C6 bond appears to affect, but not as much as the first factor, 3. The type of heteroatom in the bridge (X_1) (position C-7) defines much the power of studied molecules, 4. The type of heteroatom (X_2) fused cycle is also important, 5. Molecules with methyl groups at positions C-2 and C-3 (just one example, CTD itself) are more potent, but usually more toxic, 6. The nature of fragment **B** defines the type of activity considerably.

To validate the statements described above, a similar relationship between the structures of the derivatives depicted in sections 2 and 3 and their anticancer activity, through the comparison of their growth inhibition (GI_{50}, μM) values in various tumor cell lines, once again the more potent analogues were selected and they are shown in Table 2. In conclusion, the same relation described in Figure 30 can be applied in future designs for the synthesis of CTD derivatives with potent and selective anticancer activities against several human cancer cell lines. In general, due to the possibility to incorporate any kind of substituents in the nitrogen of cantharidimides, these derivatives shown more anticancer activities and lead the construction of versatile derivatives.

Table 2: Lowest GI_{50} values in μM of CTD, NCTD and cantharidimide derivatives reported so far against a panel of human cancer cell lines

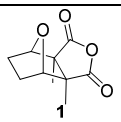
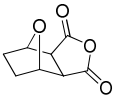
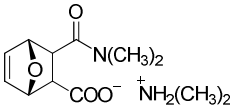
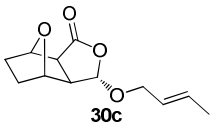
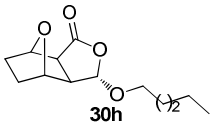
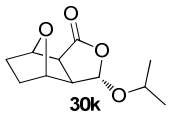
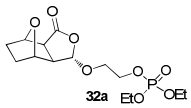
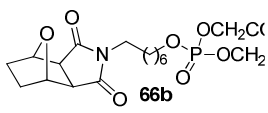
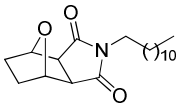
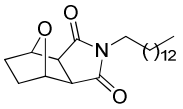
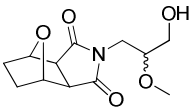
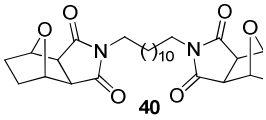
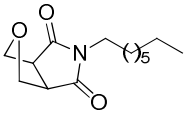
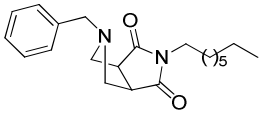
Active molecule	Cantharidin derivative	Tumour cell line										Ref.
		A2780	G401	H460	L1210	HT29	SW480	MCF-7	A431	DU145	BE2-C	SJ-G2	
1	 **1** Cantharidin	10 ± 2	3.5 ± 0.3	4.3 ± 0.9	15 ± 2	6.4 ± 0.7	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	(48)
2	 **5** Norcantharidin	39 ± 5	35 ± 2.3	50 ± 4	13 ± 0.3	33 ± 7	41 ± 4	34 ± 3	72 ± 3	90 ± 10	36 ± 0	23 ± 5	(48) (55)
3		40 ± 10	25 ± 1.5	41 ± 9.2	48 ± 1.5	49 ± 15	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	(48)
4	 **30c**	57 ± 5	N.D.	> 100	N.D.	42 ± 5	93 ± 8	77 ± 10	> 100	> 100	84 ± 5	55 ± 3	(55) (59)
5	 **30h**	20 ± 1	N.D.	> 100	N.D.	32 ± 3	39 ± 4	27 ± 6	68 ± 5	> 100	38 ± 2	37 ± 6	(55)
6	 **30k**	> 100	N.D.	> 100	N.D.	19 ± 1	94 ± 1	> 100	> 100	> 100	44 ± 5	21 ± 3	(55)
7	 **32a**	22 ± 2	N.D.	> 100	N.D.	17 ± 1	9 ± 7	20 ± 1	87 ± 12	84 ± 3	9 ± 0.2	43 ± 3.4	(55)

Table 2: Continued

Active molecule	Cantharidin derivative	Tumour cell line											Ref.
		A2780	G401	H460	L1210	HT29	SW480	MCF-7	A431	DU145	BE2-C	SJ-G2	
8	 **66b**	7.7 ± 0.3	N.D.	13 ± 0.3	N.D.	11 ± 0.3	10 ± 0.3	12 ± 0.9	12 ± 0.3	13 ± 0.9	9.3 ± 0.1	14 ± 0.3	(63)

9		40 ± 4	N.D.	53 ± 4	N.D.	25 ± 4	55 ± 2	52 ± 8	35 ± 4	66 ± 4	40 ± 6	58 ± 2	(55)
10		35 ± 4	N.D.	66 ± 7	N.D.	19 ± 0	56 ± 4	43 ± 7	47 ± 3	73 ± 2	50 ± 4	49 ± 5	(55)
11		< 10	N.D.	< 10	N.D.	12 ± 4	< 10	33 ± 3	< 10	< 10	< 10	< 10	(55)
12	 **40**	19 ± 1	N.D.	31 ± 7	N.D.	8.3 ± 0.7	24 ± 4	18 ± 0	18 ± 4	60 ± 6	17 ± 4	43 ± 10	(55)
13		30 ± 0	N.D.	71 ± 1	N.D.	21 ± 1	29 ± 2	22 ± 4	36 ± 1	33 ± 2	59 ± 4	28 ± 0	(59)
14		58 ± 0	N.D.	81 ± 8	N.D.	59 ± 1	68 ± 2	41 ± 1	61 ± 2	70 ± 2	68 ± 2	65 ± 5	(59)

A2780:Human ovarian carcinoma
G401: Human kidney carcinoma
H460: Human lung carcinoma
L1210: Murine leukemia
HT29: Human colorectal carcinoma
SW480: Human colon carcinoma
MCF-7: Human breast carcinoma
A431: Human skin carcinoma
DU145: Human prostate carcinoma
BE2-C: Human neuroblastoma
SJ-G2: Human Gliobastoma
N.D.: Not determined

Because CTD represents the simplest synthetic target among all known naturally occurring toxins (okadaic acid **74**, tautomycin **75**, microcystin LR **76**, fostriecin **31** among others) (Figure 31), the synthesis of numerous cantharidin-like molecules has been performed to improve PP1 and PP2A binding properties (PP1/PP2A inhibition selectivities) as a strategy for the discovery of novel potential anticancer agent (11-13). However, CTD and NCTD are the archetypal small molecule protein phosphatase inhibitors (11,36), the simple and rigid cantharidin (7-oxabicyclo [2.2.1] heptane) structure gives a little possibility to modify structurally this tricyclic molecule, although all synthetized cantharidin analogues have, at best, maintained similar levels of PP2A selectivity and potency.

Further developments towards a new class of cantharidin analogues allowed to discover more potent enzymatic inhibitors, for example, cantharimides with a basic amino acid residue that facilitates inhibition of PP1 and PP2A or ring-opened cantharidin analogues with only one free carboxylate group, that not only retains inhibitory activity against PP1 and PP2A, but also increases slightly the selectivity towards

PP2A, which mediated most cellular processes and was recognized as a potential target for medical research, especially for cancer treatment (Tables 1,2).

Cantharidin and cantharidimide poisoning and toxicity

The cases of CTD poisoning have been documented in humans due to the ingestion of the blister beetles (specially the Spanish fly, *Lytta vesicatoria*). Poisoning usually results after CTD has been ingested as aphrodisiac, diuretic, to induce abortions or for topical uses for dermatologic treatments, and this goes back to the ancient medicine (93). In addition to this occurrence of self-poisoning, several cases of poisoning has been also described in horses, sheep and chickens due to the ingestion of wild blister beetles, interestingly in wild birds who eats this beetles, the CTD poisoning was only described recently in a male great bustard (*Otis tarda*) (94).

Some studies have concluded unequivocally that these derivatives constitute a potential risk for the population that lives around this beetle and/or consume drugs where the active ingredient is a CTD derivative, whose effect goes from mild poisoning to death. The main symptoms of the population that is poisoned by these beetles are cramping, abdominal pain, vomit and dysphagia, and recent studies intend to identify the main metabolites, in rat's liver, of this compound using gas chromatography/mass spectrometry (95). Unfortunately, to date any kind of antidote to treat the poisoning of this toxin has not been discovered (96).

One of the most relevant cases of CTD poisoning was reported in a 4 year old girl from Zimbabwe when she ingested the blister beetle *Mylabris dicincta. This little girl* presented many of the classic signs and symptoms of CTD poisoning including haematuria, diarrhea and abdominal pains, and after nine days in the hospital she was managed conservatively and got recovered during this period of time (97).

CTD is extremely toxic for mammals, fact that counter its potent anticancer activity, the fatal dose is estimated in a range from 10 to 65 mg, while its median lethal dose (LC_{50}) is less than 0.5 mg/Kg. The death of the poisoned individual results from renal failure and several injuries in the gastrointestinal tract, surprisingly NCTD and some cantharidimides, which not have been involved in any cases of human poisoning, does not have this renal toxicity and this fact has driven the design and synthesis of more NCTD and cantharidimide derivatives than CTD-based molecules (93,98).

Additional bioactivities and pharmacological uses of canthardin and cantharidimides

Besides the inhibition of PP1 and PP2A, as well as their anticancer activity, CTD, NCTD and cantharidimide derivatives might possess additional activities in which these SMs can have a positive biological response through other metabolic ways and/or might interact with other proteins or enzymes and in that order, lead the discovery of new biological targets or roles of these natural molecules. Current studies (two so far), which intend to redirect the pharmacological uses of CTD, NCTD and cantharidimide derivatives and contribute to the little known bioactivities of these natural molecules, besides their anti-cancer effects, have been published and the immunomodulatory activity of CTD against dendritic cells and the antiplasmodial activities against different *Plasmodium falciparum* Malaria strains, would be the activities in which future studies will focus their attention (99,100). However, another preliminary studies have been performed in some others diseases where the effect of different doses of cantharidin on *Leishmania major* (MRHO/IR/75/ER) were investigated both *in vitro* (promastigote and amastigote viability) and *in vivo* in experimentally-infected BALB/c mice (skin lesions) using ointment or soluble cantharidin (101). This report showed that cantharidin at concentrations of 0.5, 1, 2, 5, 10, 20 and 50 µg/mL inhibited the growth of *L. major* promastigotes after 24 h and the resultant inhibition levels were 39.22%, 41.95%, 49.88%, 54.78%, 58.01%, 68.30% and 80.04%, respectively, and reported that two weeks of topical treatment with 0.1% cantharidin ointment was an effective method for treating cutaneous leishmaniasis in infected BALB/c mice.

Exhibiting exquisite PP-inhibitor activity, some cantharidin analogues could be designed and tailored to specifically inhibit selected STPs of nematodes (102). Preliminary investigation has shown that some cantharidin analogues **76a-c** (Figure 32), which have no toxic effect on human cell lines, kill larvae of the trichostrongylid nematodes *Haemonchus contortus*, displaying 99–100% lethality to this parasite in the larval development assay, with LD_{50} values in the range of 25–40 µM (103).

Current strategies developed to increase the bioavailability of cantharidin derivatives

Toxicity is not the only problem to reject the biological benefits of CTD and its derivatives. As many of the natural and synthetic molecules tested in different bioassays and clinical trials, CTD presents problems of administration, solubility, bioavailability and metabolism that have also boosted the study and synthesis of other derivatives.

The pH in mammal's blood and tissues are usually maintained within a narrow pH range around 7.4 and this slightly alkaline pH is mainly maintained through the regulation of respiration and renal acid

extrusion. Nevertheless, as a consequence of lactate accumulation, in the central regions of solid tumors, the extracellular pH decreases at values below 6.5, and this is one of the major problems in the cancer treatment because the activity of many of the chemotherapeutic agents fails under this conditions, nonetheless this might be one of the reasons why CTD is an excellent anticancer agent since recently was found that CTD has higher efficacy under acidic conditions (104).

For that the CTD reach the tumor tissue from its application site (oral or injected), it has to be administered in high-doses because of its poor solubility, and this leads to an intense irritation at the injection site and is responsible for the renal toxicity. Moreover, CTD has displayed poor intestinal absorption, with a bioavailability of 26.7 %, being one of the main factors that must be solved in order to use CTD as a chemotherapeutic agent without affect the life of the treated patient.

One of the first intends to increase the biological activities and the bioavailability of CTD and its derivatives, were done through the structural modification, mainly on cantharidimides, by preparing a series of organoantimony (V) derivatives **77**, based on the 5,6-dehydrocantharidimide structure with the hypothesis that of some organoantimony (III) derivatives have shown significant antitumor activities and good bioavailability (105), and the preparation of several norcantharimide alkylammonium salts **78**, that besides their varying levels of dynamin GTPase inhibition, they are been using as a room temperature ionic liquids (RTILs) (106) (Figure 33).

With the hypothesis that rare earth ions can cleave pBR322 plasmid DNA and with the idea that complexes of these ions can increase the solubility of CTD derivatives, three novel complexes of rare earth ions with NCTD were synthesized. Complexes with lanthanum (Ln), erbium (Er) and ytterbium (Yb) demonstrated, by UV-spectra, fluorescence spectra, viscosity and gel electrophoresis, that they could bind to DNA through nonclassical intercalation and that they could cleave pBR322 plasmid DNA effectively (107).

Despite the less toxicity, NTCD has a short half-life and has to be administrated in high doses in order to maintain the high level of NCTD in the circulatory system. With this idea, Zeng and Sun, to avoid the side-effects of NCTD, proposed an alternative dosage form for this compound using biodegradable polymeric nanoparticles, incorporating NCTD into poly (lactide-co-glycolide) (PLGA) to form nanospheres with 150 nm of diameter. The authors determinate that NCTD entrapment efficiency reaches up to 95% in PLGA nanoparticles and that this delivery system shown a biphasic profile with an initial rapid and a following slower release from this phase for more than 10 days. In addition, this system not only exhibit low toxicity, but also they present high efficacy in anti-tumor growth assays (108).

Perhaps, one of the most relevant works in this field was reported by Yun-jie and Chun-yan in 2012. In their research, they were faced with one of the common pharmaceutics challenges related with the water solubility of CTD. The author's work was based on the concept of solid dispersion, in which CTD will disperse in an inert hydrophilic carrier, to enhance the dissolution rate, solubility and oral absorption of poorly water-soluble CTD. The solid dispersion with CTD was prepared in polyethylene glycol-4000 (CA-PEG4000) in order to study the permeability behavior of this dispersion with the one related with CTD itself. After evaluate the oral bioavailability of both systems in rats, the *in vivo* results showed that the solid dispersion CA-PEG4000 had a higher bioavailability (295.4 %) than when free CTD was administrated via oral (26.7%). This improvement obviously established a new start-point for future tests in mammals due that the new CTD, NCTD and cantharidimide derivatives could be well absorbed by the gastrointestinal tract and more realistic ADME-Tox results can be obtained (109).

Concluding remarks

The many varied and new chemical and pharmacological information on cantharidin-based SMs discussed herein provide substantial evidence related with the synthetic approaches of cantharidin-based small molecules, and their chemical transformations and potential biological applications were also conferred. Furthermore, the advances in the field have provide strong evidence that cantharidin analogues, as metabolites of blister beetles (fam. Meloidae), are still promising, simple biomodels and that their potent PP inhibitor activities will be the main objective for future pharmacological research. Therefore, the design and development of cantharidin analogues could generate other SMs that will stimulate the progress of specific and selective inhibitors of these targets enzymes. Highlighting the synthetic power of the Diels-Alder reactions, as well as simplicity of synthetic green procedures, to obtain these novel and diverse cantharidin-based SMs should be taken into consideration for the development of potential therapeutic agents. Finally, we hope that this mini-review will aid the organic chemistry community to start new designs of novel molecules that could contribute to medicinal and biological chemistry and in this way contribute to the knowledge in this interestingly field.

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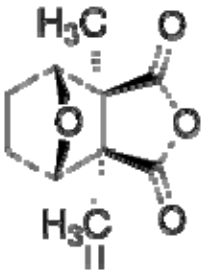
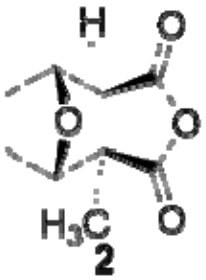


1
Cantharidin



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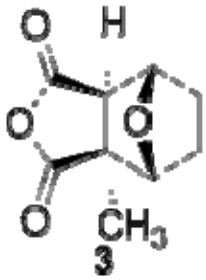

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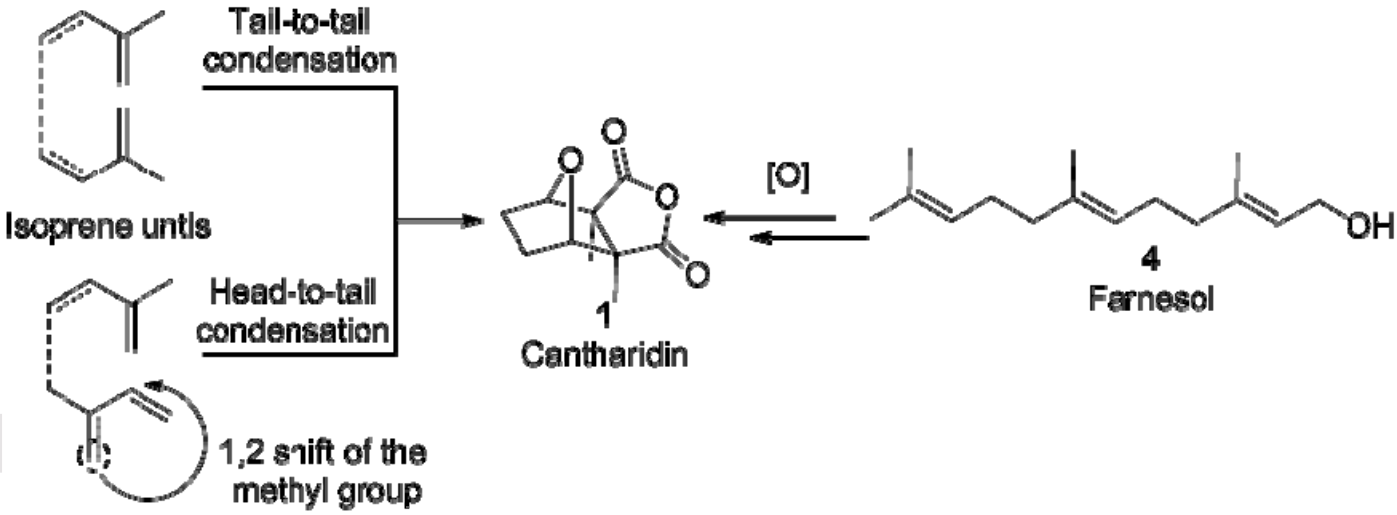

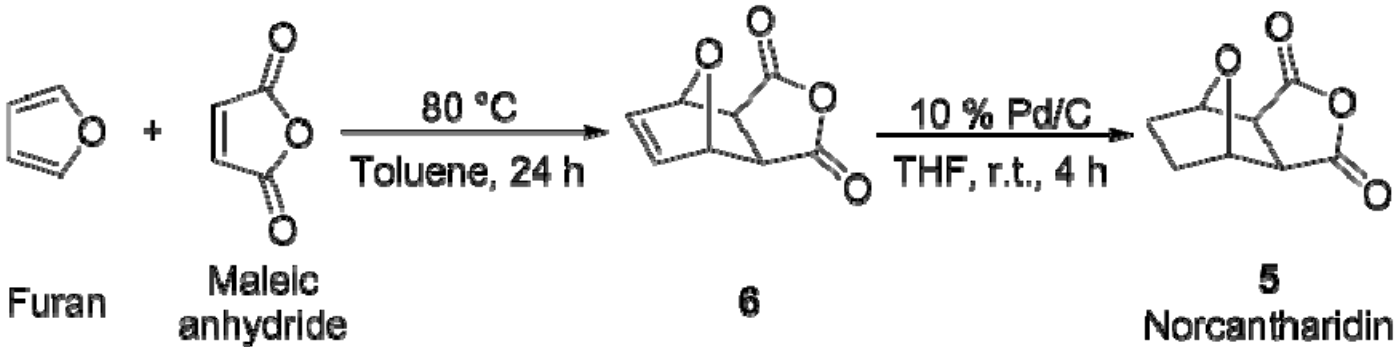

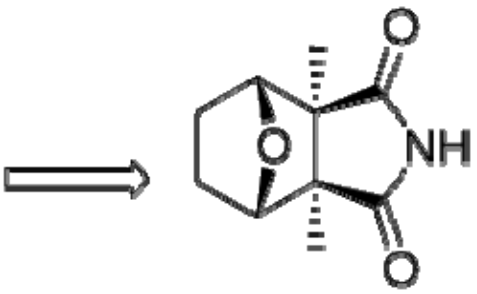

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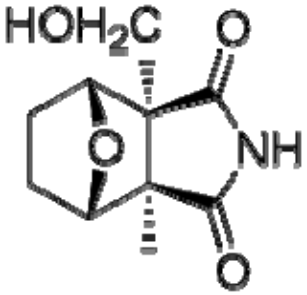

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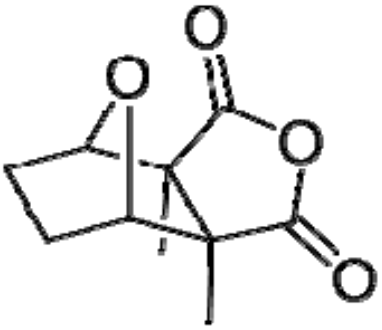
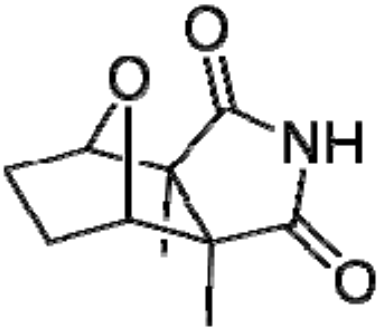


1
Cantharidin

Biochemical transformation

Enzymatic source of nitrogen



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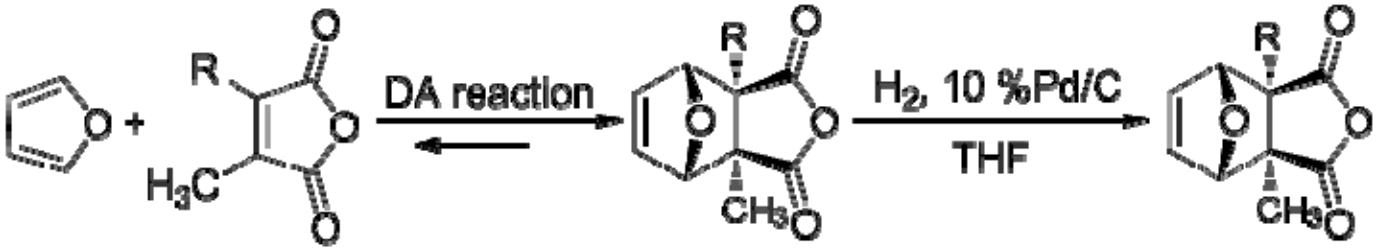
Cantharimide

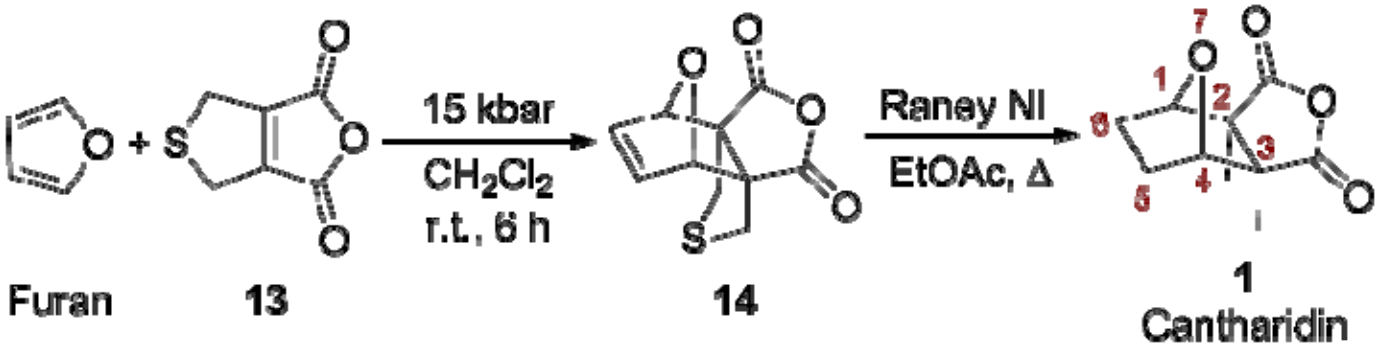


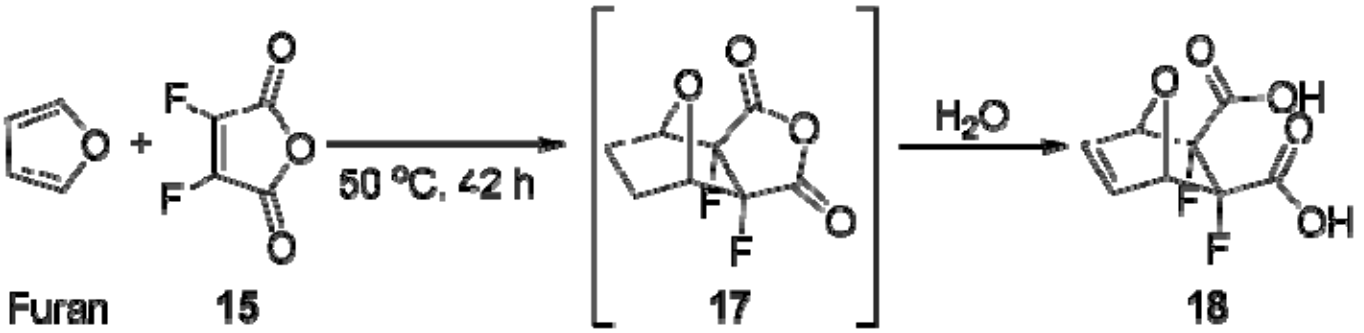
Furan

9 R = CH_3
10 R = H

11 R = CH_3
12 R = H

1 R = CH_3
2 R = H





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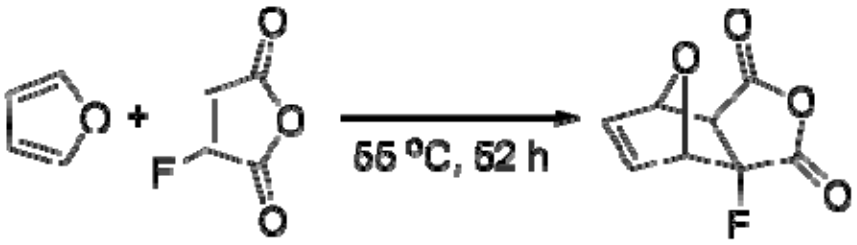

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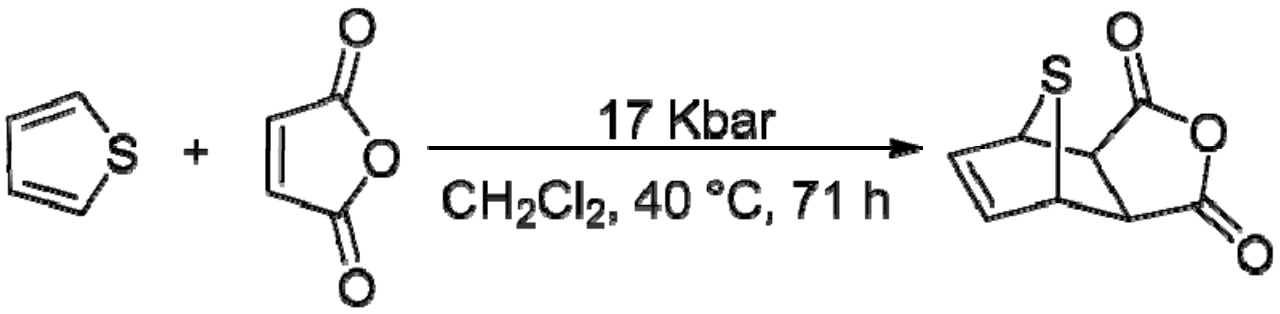
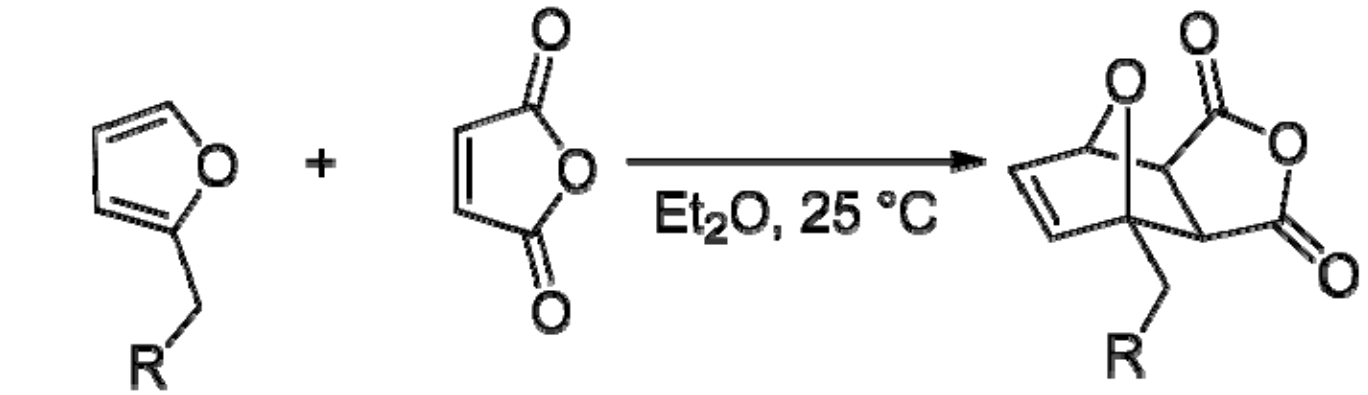
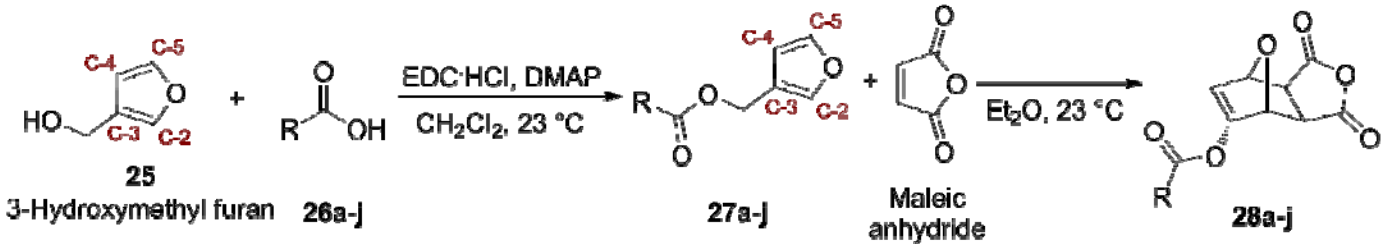
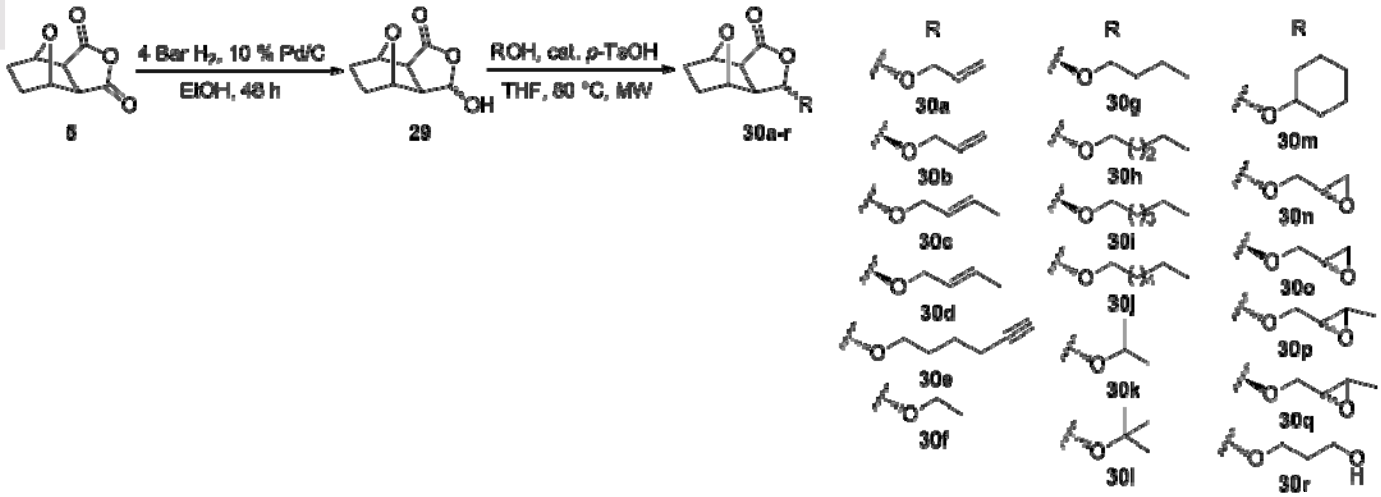
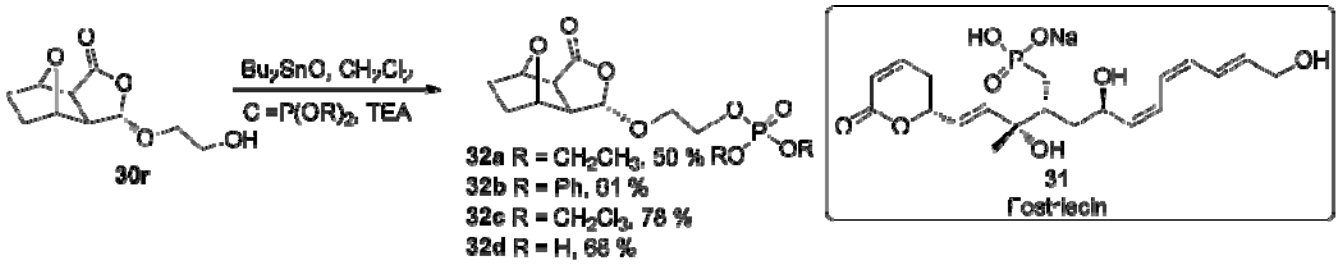
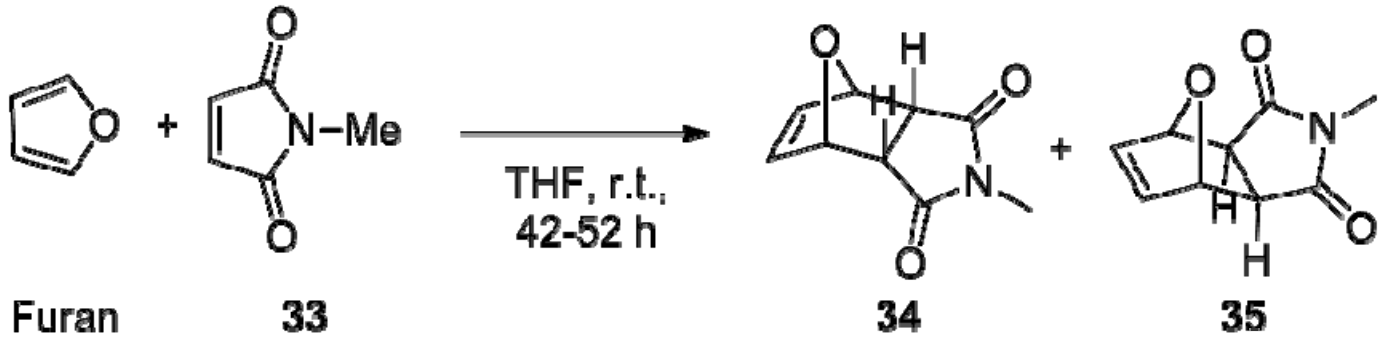


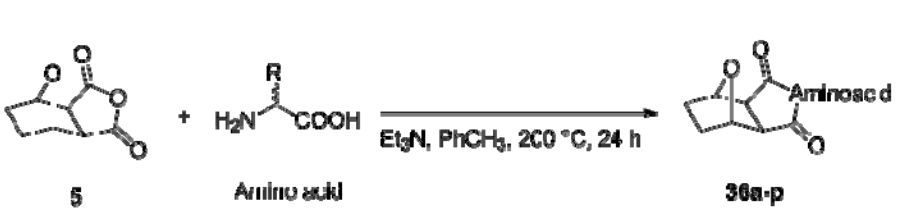




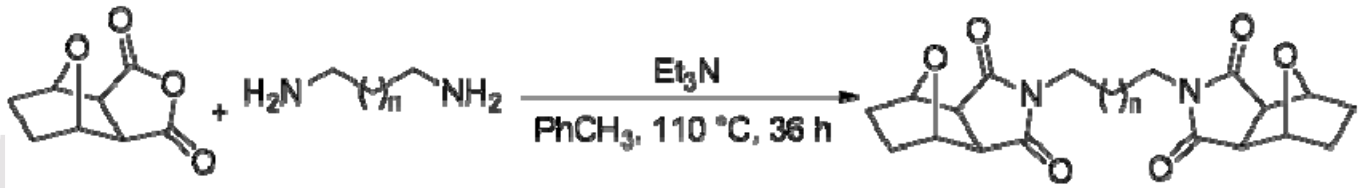


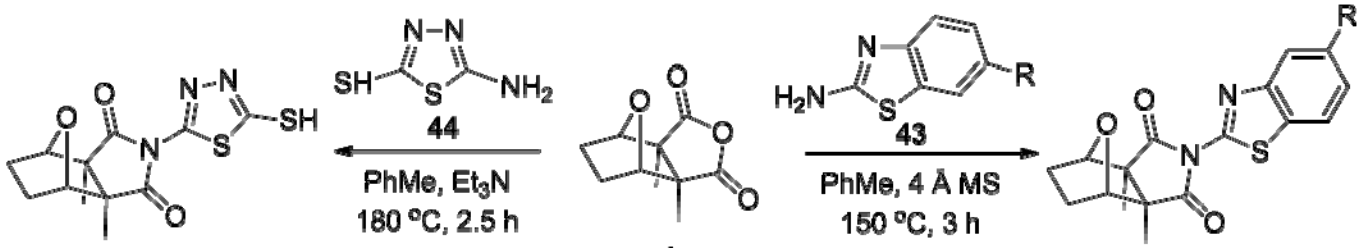


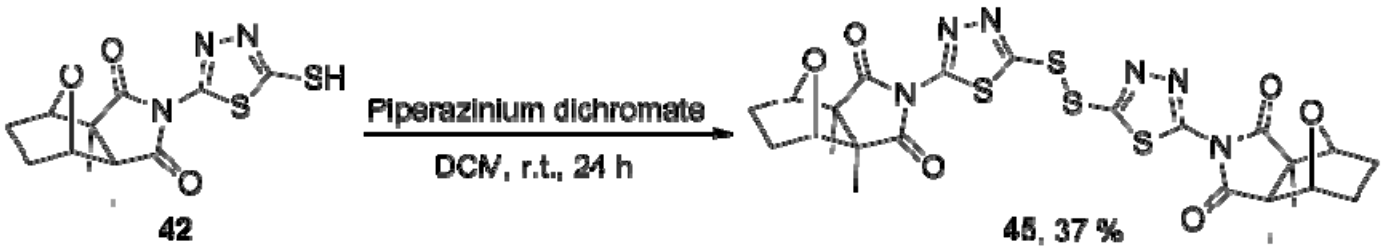




Amino acid, yield %	Amino acid, yield %
D-Ala, 48	D-Try, 31
L-Ala, 53	L-Try, 57
D-Phe, 50	D-His, 8
L-Phe, 57	L-His, 14
D-Leu, 76	D-Tyr, 21
L-Leu, 70	L-Tyr, 74
D-Ile, 66	D-Glu, 16
L-Ile, 64	L-Met 32

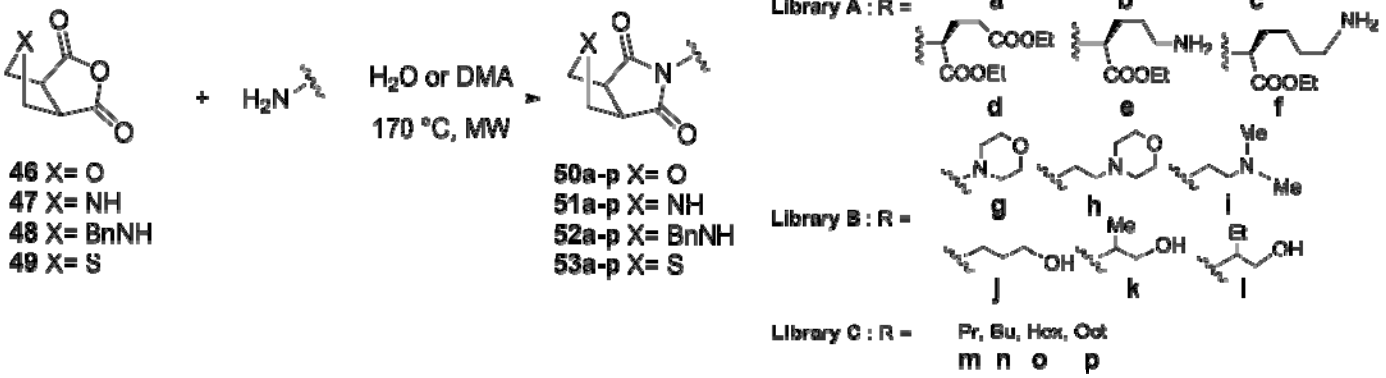






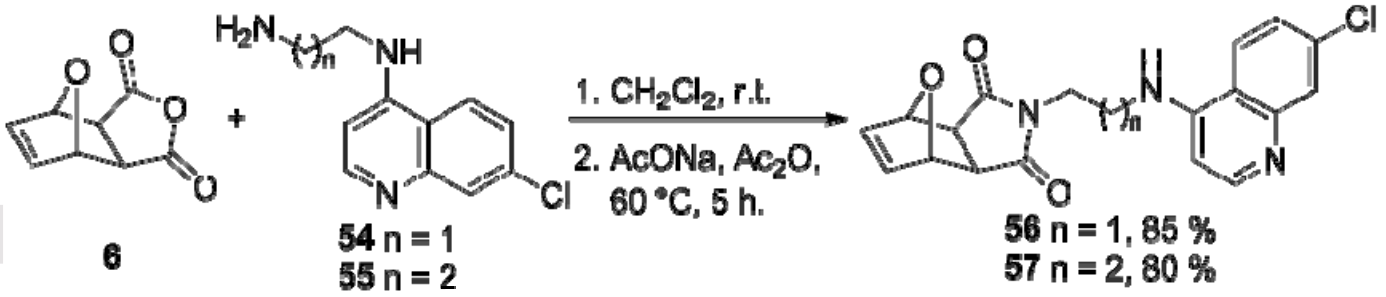
42

45, 37 %



46 X= O
47 X= NH
48 X= BnNH
49 X= S

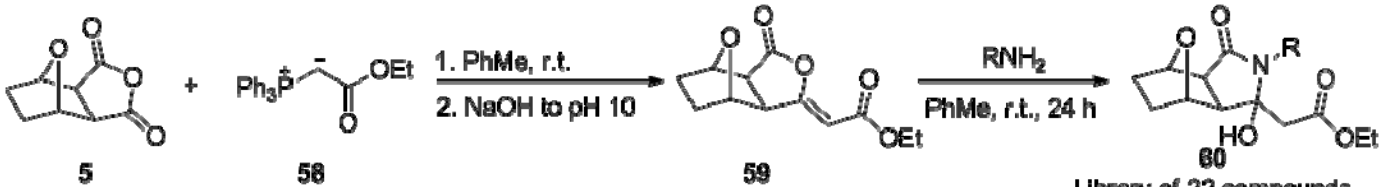
50a-p X= O
51a-p X= NH
52a-p X= BnNH
53a-p X= S



6

54 n = 1
55 n = 2

56 n = 1, 85 %
57 n = 2, 80 %



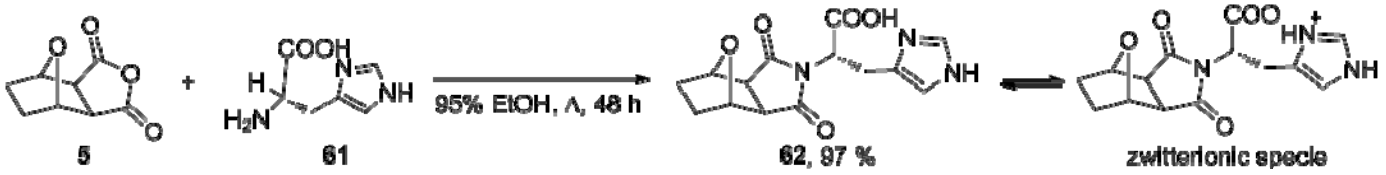
5

58

59

60

Library of 22 compounds

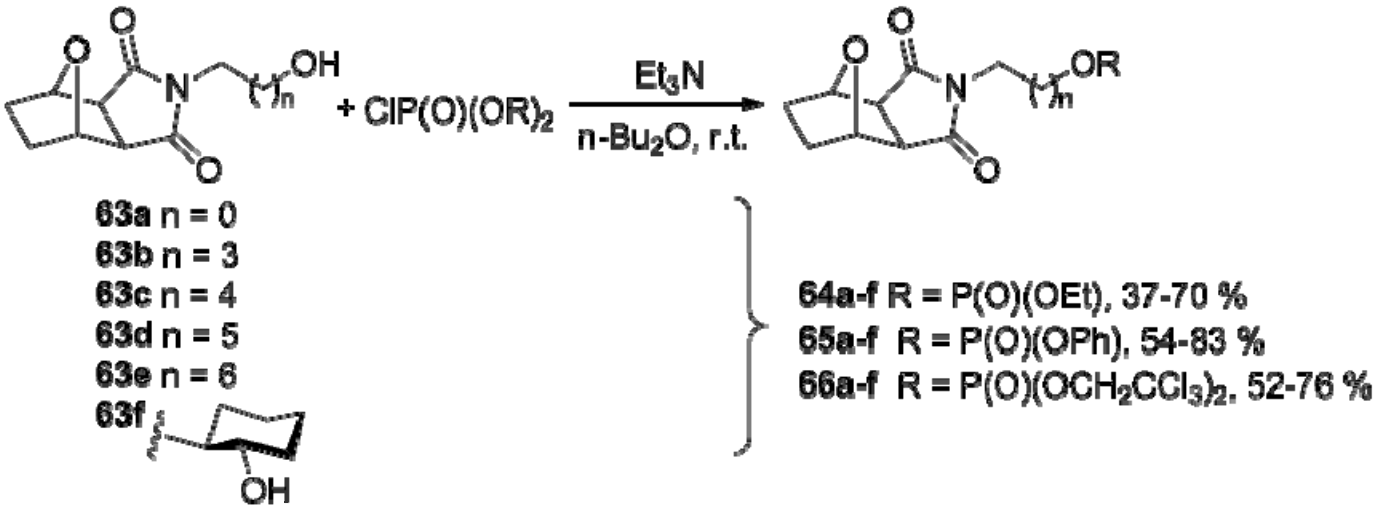


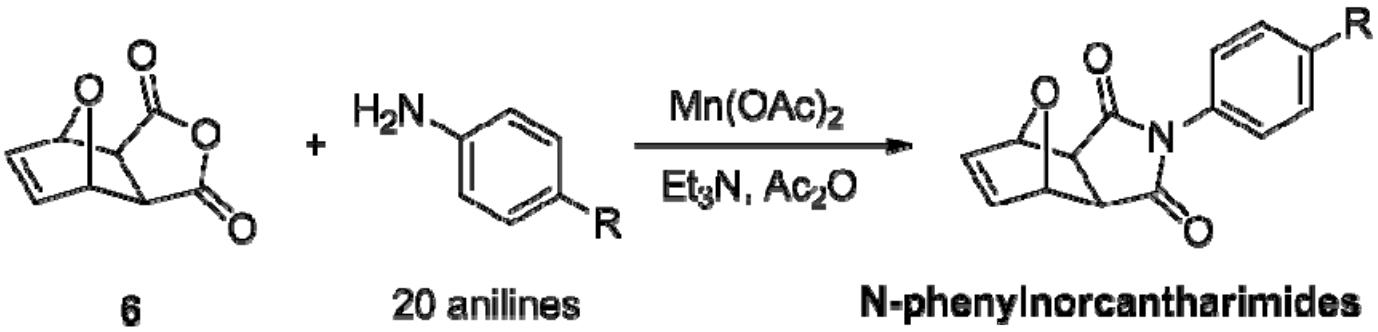
5

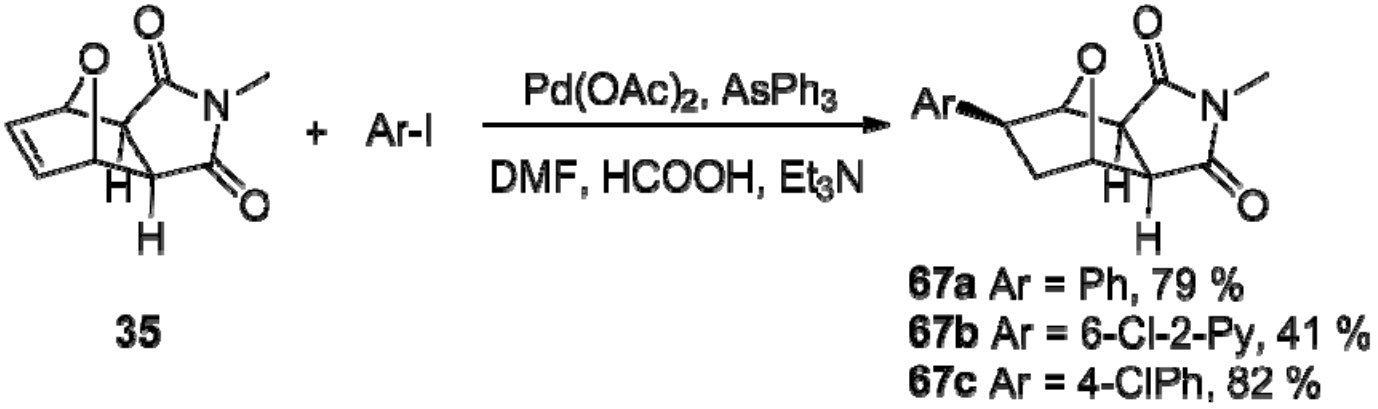
61

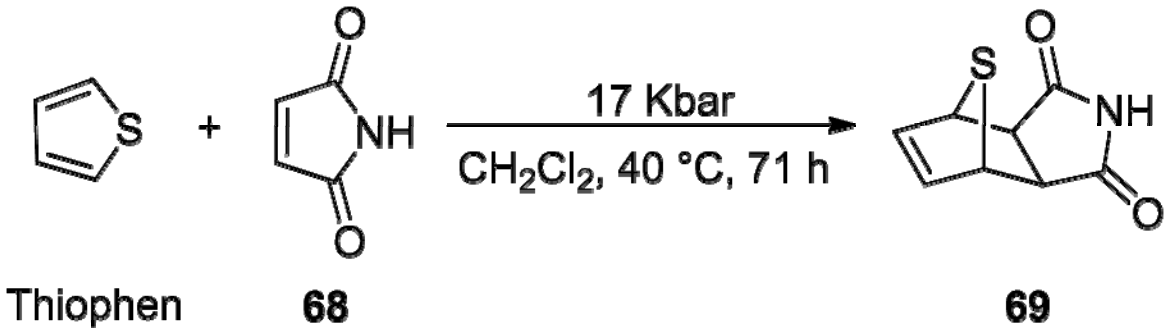
62, 97 %

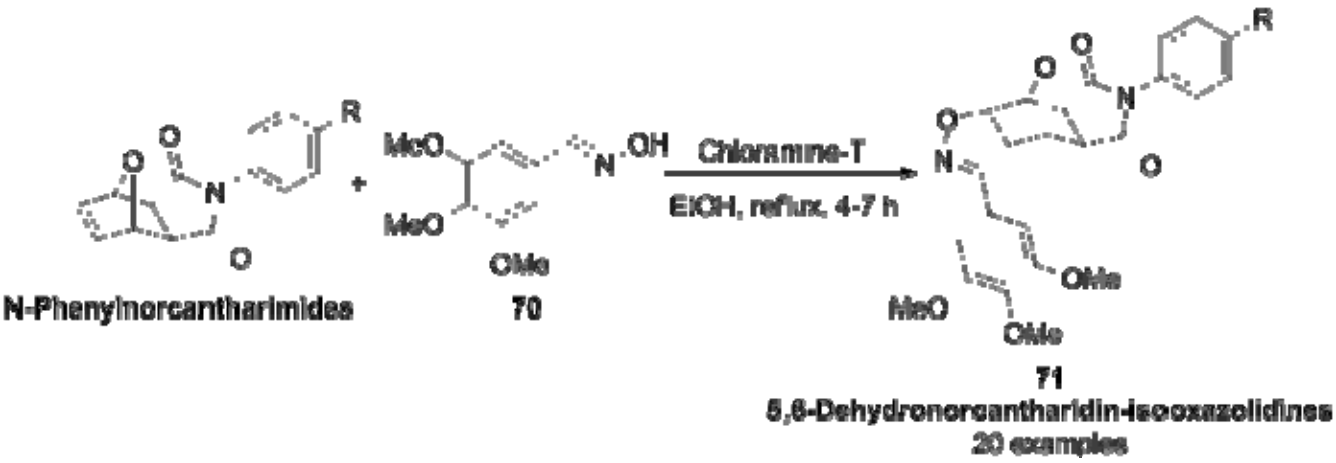
zwitterionic specie

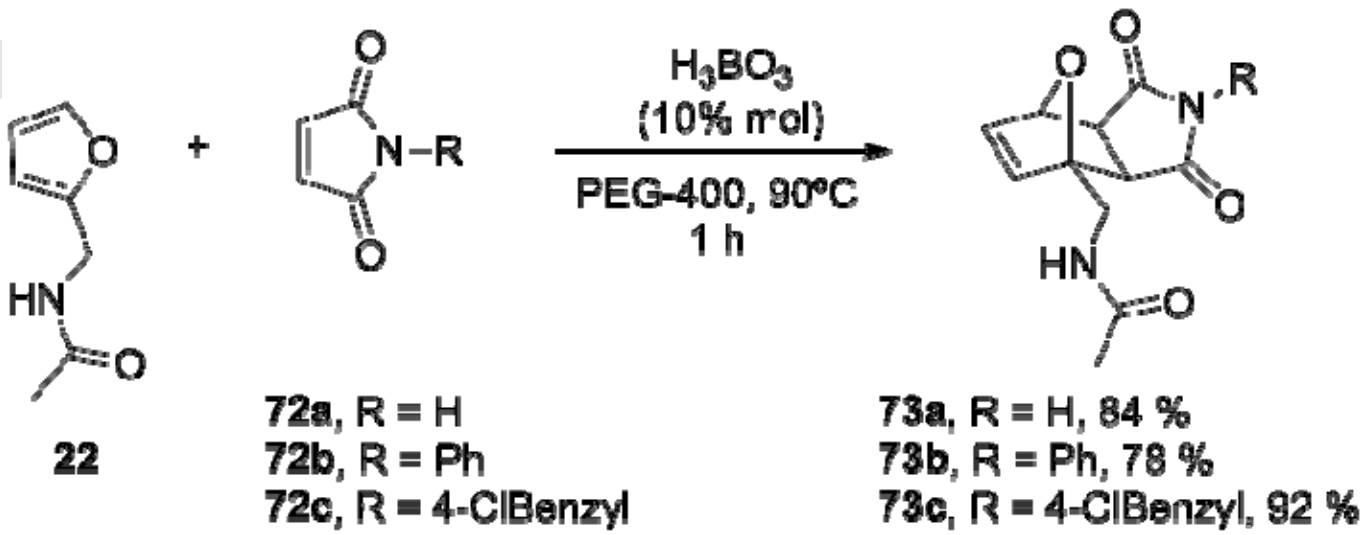


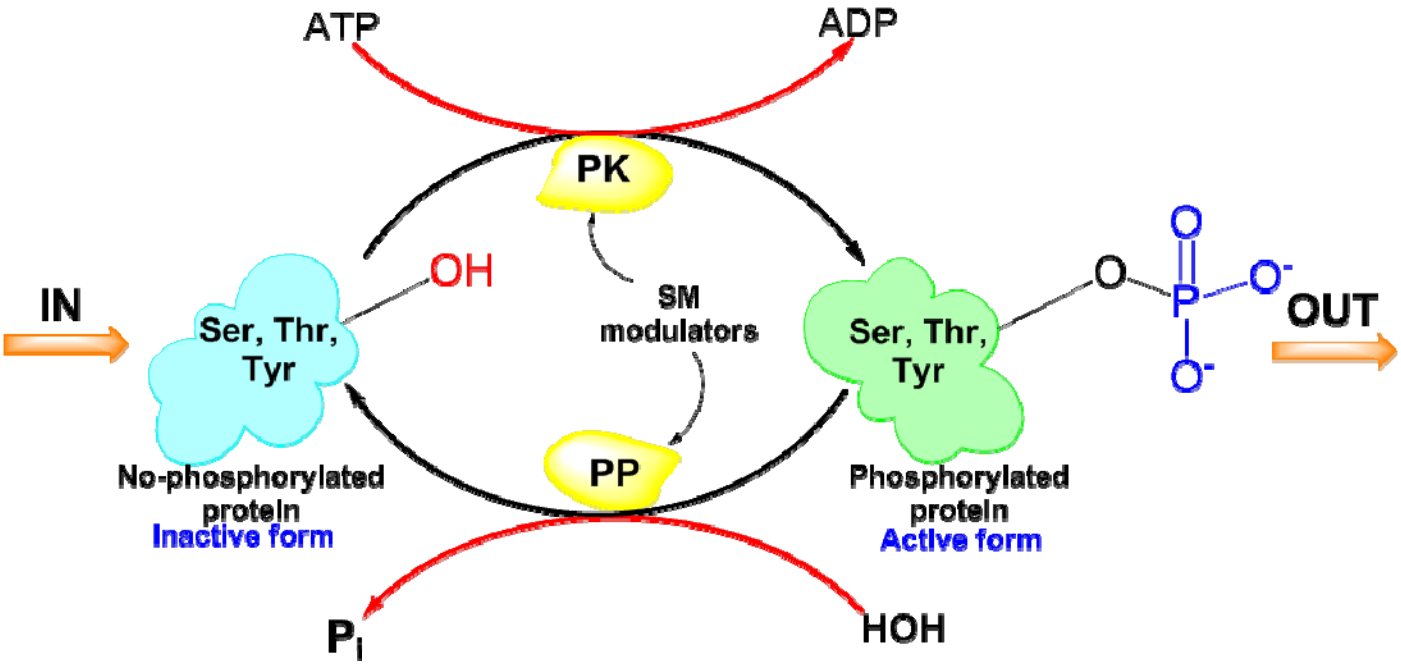


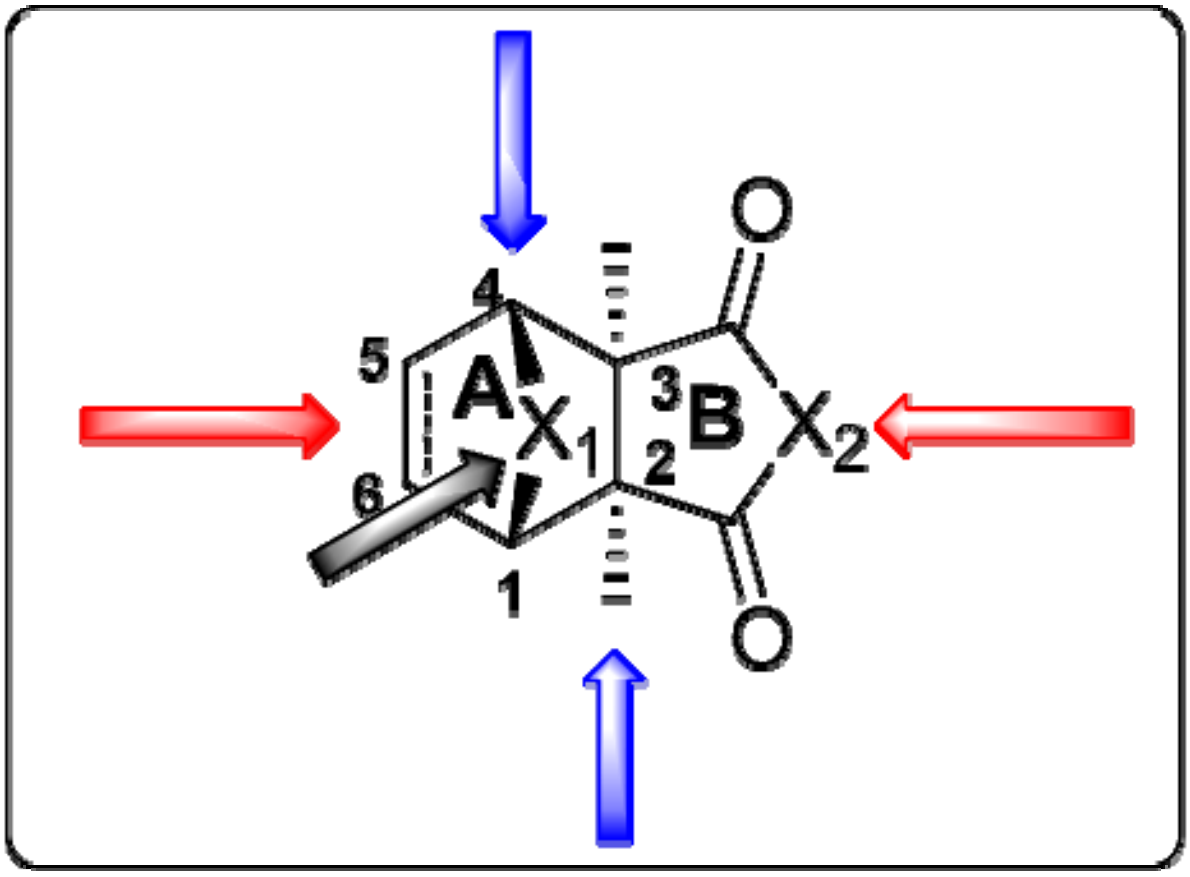


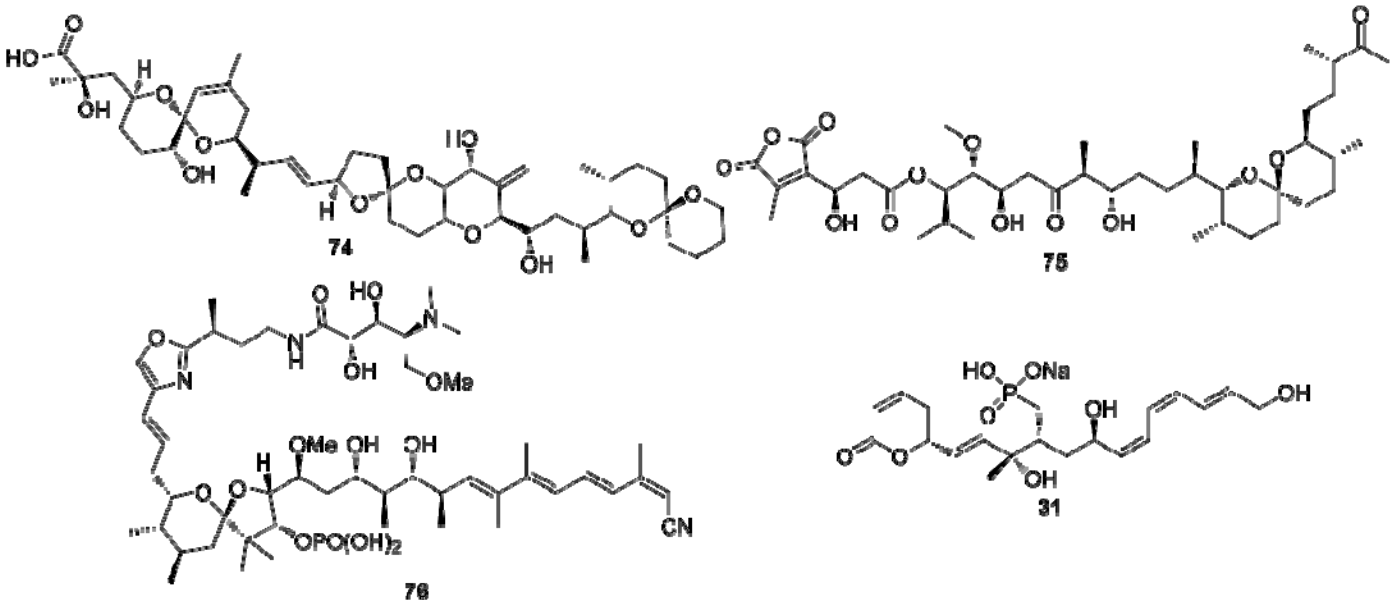












74

75

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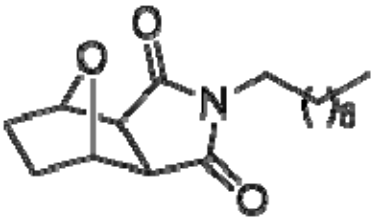
31



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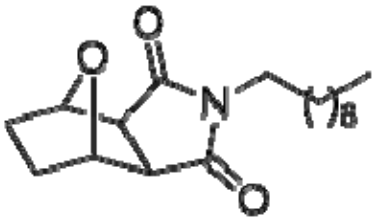

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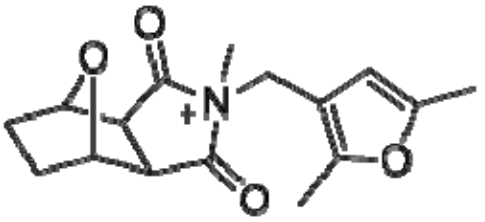

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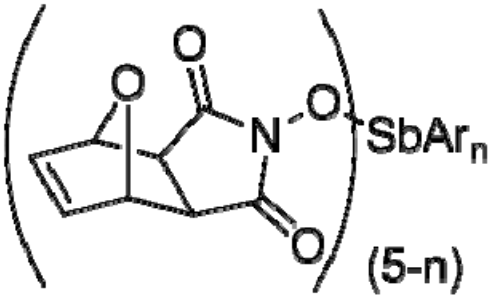

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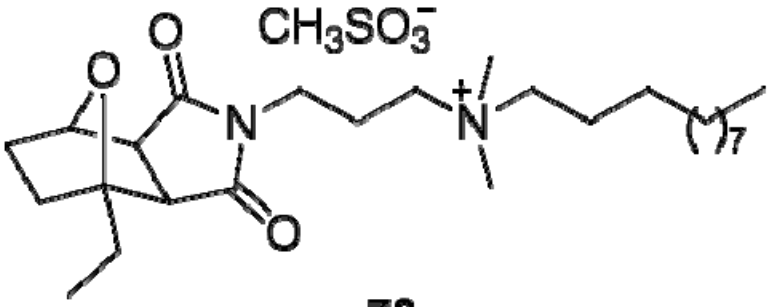

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