MONASTROL AND DIHYDROPYRIMIDINES: THE FUTURE OF SMALL MOLECULE KINESIN EG5 INHIBITORS

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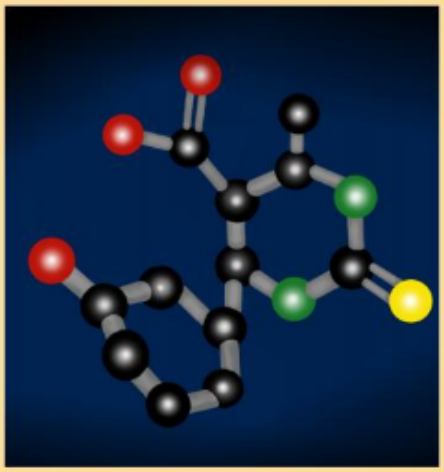
ABSTRACT

Dihydropyrimidines (DHPMs) are a group of privileged heterocycles found to have various biological effects in cells. One specific DHPM, monastrol, inhibits Eg5 kinesin – a key protein involved in cell division- and can be useful in limiting the growth of cancerous tumors. Monastrol, discovered in 2000 by Tim Mitchinson at Harvard Medical School, and related DHPMs are being synthesized and investigated in hopes of creating novel anticancer drugs. DHPMs can be synthesized very efficiently through a multicomponent reaction (MCR), which are extremely important in synthetic chemistry. Herein, we wish to report on the pharmaceutical industry's efforts in engineering different dihydropyrimidines.

INTRODUCTION

Over a third of the world's population has been diagnosed with cancer at some point in their lifetimes. In 2018, cancer and its related effects took the lives of over 9.6 million individuals worldwide [1]. This illness currently has no definite cure, and many cancer treatments are detrimental to the health of noncancerous cells. Some well-known anti-cancer drugs include taxol, doxorubicin, and etoposide (Figure 1). Taxol (1) targets rapidly growing cancer cells by attaching to their microtubules, preventing cancer cells from further dividing [2]. Doxorubicin (2) slows down the spread of cancer cells in the body by inhibiting DNA synthesis, causing tumor growth to slow or halt [3]. Etoposide (3) blocks topoisomerase activity that breaks phosphate backbone in the DNA, leading to cancer cell death [4]. While effective in combination, these drugs exhibit a long list of side effects which can be detrimental to patients' health [5].



DHPMs are biologically important partially saturated pyrimidine ring with two separate functional groups replacing two of the double bonds (Figure 2). By exhibiting potent antiproliferative activity, some DHPMs captured the attention of many researchers with the hopes of creating new cancer therapies [6]. One DHPM, monastrol, is capable of crossing cell membranes and halting mitosis by inhibiting kinesin Eg5 – a motor protein involved in the assembly and separation of the mitotic spindle. After long periods of mitotic arrest, monastrol induces apoptosis, programmed cell death, in cells. In this way, monastrol and its analogs have demonstrated to inhibit uncontrolled cell division and cause pronounced tumor regression. Additionally, the use of monastrol as an anticancer agent has been demonstrated to be less cytotoxic to neighboring cells than taxol. The visualized potency gives promise in the development of anticancer

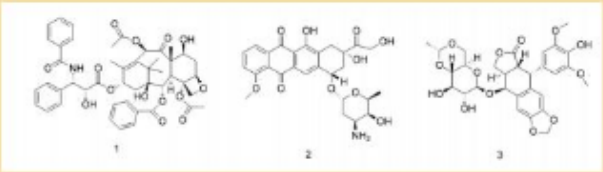


Figure 1: Structure of taxol (1), doxorubicin (2), and etoposide (3)

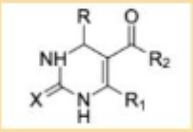


Figure 2: General structure of a dihydropyrimidine

drugs [7].

However, monastrol is a relatively weak anticancer agent and is not completely effective. Other DHPMs structurally similar to monastrol have shown great potential in the creation of anticancer agents capable of treating aggressive cancers such as glioma, renal, and breast cancers [8].

HISTORY & DISCOVERY OF MONASTROL

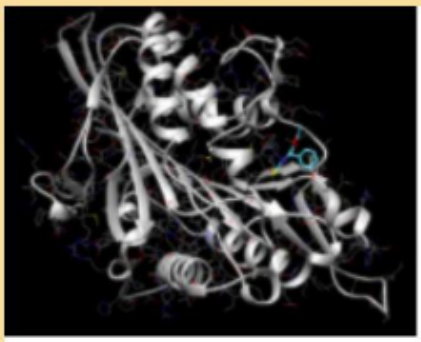
Monastrol was first discovered in 2000 by the Mitchison group at Harvard Medical School in a high-throughput screen (HTS) [9]. It was found that upon allosteric binding of monastrol to its pocket on kinesin Eg5, microtubule assembly is inhibited, and therefore, the basal ATPase activity is inhibited through a slower ATP release. Monastrol changes the conformation of Eg5 kinesin, which decreases the affinity of ATP for microtubules, leading to its misformation. This inhibition of ATPase activity shows a decreased affinity for microtubules, and monastrol stabilizes a conformation, allowing for an easy reversal when ATP is hydrolyzed. The periodical interactions between ATP reversals and microtubules yields a non-productive kinesin Eg5 complex that can establish a monoastral spindle which alters mitotic function. The incorrect formation of the spindle fibers halts mitosis, making monastrol capable of stopping the cell cycle. While monastrol has properties that arrest the cell cycle, in clinical trials, they were only partially successful [7]. Monastrol does not inhibit progression through S and G2 phases of the cell cycle or centrosome duplication. While it is successful in some areas, the goal for the researchers now, is to optimize its functions and make the inhibition more successful. This can be done by modifying starting reagents and creating new analogs [10]. In the last two decades, scientists have begun to conduct various experiments to elucidate the structure-activity relationship of monastrol and related dihydropyrimidines by creating analogues towards the development of more potent compounds against cancer cells. Strategies to improve the efficacy of monastrol-like anticancer compounds are currently in the works [7].

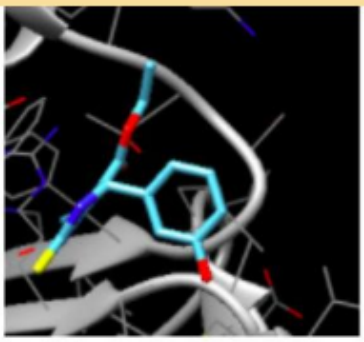
Figure 3: Crystal structure of monastrol bound to kinesin/Eg5 (a); Zoomed in crystal structure of monastrol bound to kinesin Eg5 (b); Chemical structure of Monastrol (c)

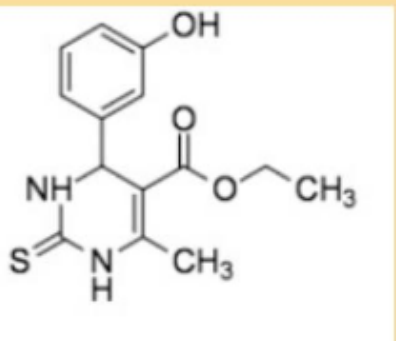
BIOLOGICAL ACTIVITY OF MONASTROL

A combination of two phenotype-based screens, one specific post translational modification and the other illustrating microtubules and chromatin, were used to select compounds affecting mitosis. Monastrol was one of those compounds, arresting mammalian cells in mitosis with monopolar spindles. With an in vitro study, monastrol was discovered to inhibit mitotic kinesin Eg5, which is a motor protein needed for spindle bipolarity.

By experimenting with the effectiveness of monastrol on HeLa cells (most commonly used human cell line), the cytotoxic activity of monastrol was tested [11]. Monastrol inhibited and targeted kinesin Eg5 in the







mitotic spindle to stop the replication of the cell. To study the effectiveness of the monastrol on the HeLa cells, the researchers used time-lapse video microscopy and biochemical analysis. The mitotic result is controlled by the spindle checkpoint in mitosis. This checkpoint remains active until all the kinetochores on the chromosomes are attached to a spindle during metaphase in mitosis. The active checkpoint generates a "wait anaphase signal," which inhibits the anaphase-promoting complex. This complex prevents the degradation of several key mitotic proteins, which must be degraded for anaphase initiation to occur. The presence of unattached chromosomes or a lack of spindle tension that is generated by bipolar chromosome attachment results in continued checkpoint activation, mitotic arrest, and eventually programmed cell death. Compounds that target the mitotic spindle are among the most effective cancer drugs in medical use. In checkpoint compromised HeLa cells, monastrol induced apoptosis following mitotic exit into the next G1 phase, showing that Eg5 inhibition can lead to caspase activation and apoptosis in the absence of critical checkpoint components, such as BubR1 or Mad2.

MOLECULES THAT INHIBIT KINESIN EG5

(Figure 4) shows several molecules that inhibit the protein kinesin Eg5, much like monastrol.

HIGH-THROUGHPUT SCREENING

Extensive computational docking experiments of monastrol to its binding pocket on kinesin Eg5 have been conducted to shed light upon its structure activity relationship (SAR). Monastrol has been previously docked to its (R) and (S) enantiomers onto the active site of a Leishmania donovani PTR1 (LdPTR1) model [16]. Monastrol fits well into the binding pocket with Arg17, Asn109, Ser111, Asp181, Tyr191, Tyr194, Lys198, Leu226 and Ala230 residues, forming key interactions with Arg17, Asn109, Ser111, Asp181, Tyr191, Tyr194, Lys198, Leu226 and Ala230. Results have indicated the necessity of the ethyl ester group of pyridine for tight binding to PTR1. The carbonyl oxygen of this group has been predicted to serve as a hydrogen bond acceptor, interacting with the nitrogen atoms of side chain Arg17 and backbone Ala230. The hydrogen bonding interaction between Tyr194 and the hydroxyl group substituted on the third position of the phenyl ring has also demonstrated the overall stability of the complex. The docking results onto PTR1 also indicated that both monastrol (R)- and (S)- enantiomers (Figure 4) have very

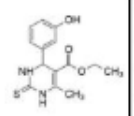
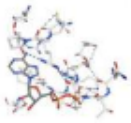
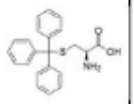
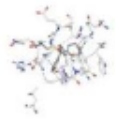
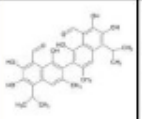
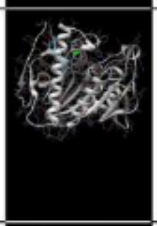
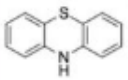
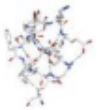
Molecule / Structure	Significance	Crystal Structure	Ligand Interaction
 Monastrol	Monastrol inhibits and targets kinesin Eg5 in the mitotic spindle to stop the replication of the cell, inducing apoptosis[11].		
 S-trityl-Cysteine (STLC)	STLC was found more potent in kinesin Eg5 inhibition compared to monastrol[12].		
 Gossypol	Gossypol is known for its use as a male antifertility drug. It was found that gossypol can suppress prostate cancer by inhibiting kinesin Eg5[13].		
 Phenothiazine	Phenothiazine acts as antimicrobial, antifungal, psychotropic, and antitumor molecules[14].		

Table 1: Molecules that inhibit kinesin Eg5 (crystal structure and ligand interactions obtained from UCSF Chimera)

similar binding affinities. Monastrol (R) exhibited IC50 values of 5.23×10−5 mol/L with binding free energy of −24.92 kJ/mol, while monastrol (S) exhibited values of 6.94×10−5 mol/L with binding free energy of −24.20 kJ/mol. Monastrol (R) and (S) enantiomers were also docked into the active site of human kinesin Eg5. While monastrol (R) showed IC50 values of 3.42×10−3 mol/L with binding free energy of −14.35 kJ/mol, monastrol (S) demonstrated an IC50 value 6.42×10−3 mol/L with binding free energy of −12.76 kJ/mol. This study established how monastrol has superior antileishmanial activity and provides insight on its potential in preclinical studies[16].

MULTICOMPONENT REACTION: BIGINELLI CONDENSATION RELEVANCE

Multicomponent reactions (MCRs), wherein different starting materials that undergo different reactivity are mixed and matched (Figure 5) have always been very important to medicinal chemistry, offering advantages such as to produce large libraries of

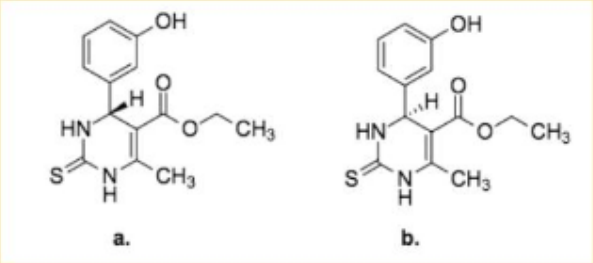


Figure 4: (a) Structure of monastrol (R) enantiomers; (b) Structure of monastrol (S) enantiomers

compounds. These types of reactions mix and match different starting materials that undergo a similar reactive process. The Biginelli reaction17 is a prime example of a multicomponent reaction that has been used and altered to create various analogs of dihydropyrimidines which create different analogs of DHPMS with structural similarities of monastrol. Due to the recently discovered pharmacological properties associated with dihydropyrimidines, the multicomponent Biginelli reaction has been experiencing a resurgence in scientific interest. In the past decades, a range of biological effects such as antitumoral, antibacterial, and anti-inflammatory have been attributed to synthetic dihydropyrimidines. To this day, chemical synthesis of similar compounds has expanded the field's understanding of the structure-activity relationship of such compounds in a variety of biological contexts.

In 1891 Pietro Biginelli reported the synthesis of functionalized 3,4-dihydropyrimidin- 2(1H)- ones (DHPMs) via the three-component condensation reaction, an acid-catalyzed cyclocondensation reaction of thiourea, ethyl acetoacetate, and benzaldehyde. The reaction was carried out by simply refluxing a mixture of the three components dissolved in ethanol with a catalytic amount of HCl. The product of this reaction was identified by Biginelli as 3,4-dihydropyrimidine-2(1H)-one[18]. Monastrol is an example of a DHPM and was synthesized similarly to the Biginelli condensation (Figure 6).

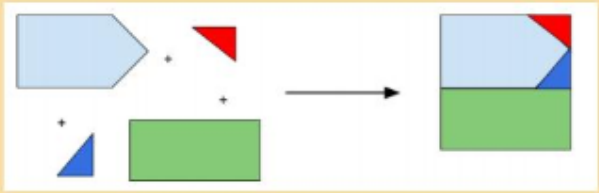


Figure 5: A multicomponent reaction allows one to synthesize complex compounds in a one-step reaction

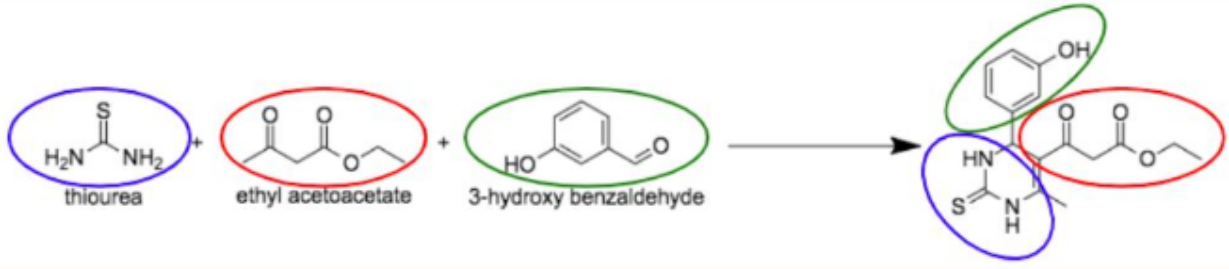
WORK IN DHPM-DERIVED MOLECULES

Scientists have been working on synthesizing DHPM analogs that could potentially serve as anti-cancer drugs. These compounds work through a variety of mechanisms: not all bind to Eg5 kinesin.

CONCLUDING REMARKS

Monastrol is a small, cell-permeable molecule that inhibits Eg-5, a kinesin-related motor protein that is involved in the assembly and maintenance of the mitotic spindles. By inhibiting Eg-5, monastrol is a promising candidate in cancer therapy. Since the discovery of monastrol, there have been further investigations into other small molecule inhibitors of kinesin Eg5. These include several DHPM derivatives that have been tested and show rising leads. Literature provides accurate and important data relating to the structure-activity relationship of a number of kinesin Eg5 inhibitors and other small molecules. In specific, the Biginelli condensation reaction gives rapid access to compounds structurally similar to monastrol. Further investigation into creating more analogs could yield a wide variety of new molecules with various uses within medicine. This continued creation of new dihydropyrimidine and monastrol analogs have a lasting effect on the current and future treatments of cancer, potentially reducing

Figure 6: Biginelli Condensation Reaction of Monastrol



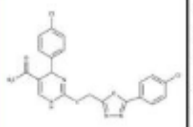
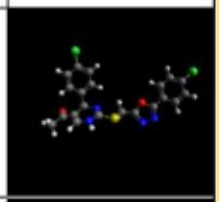
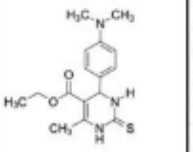
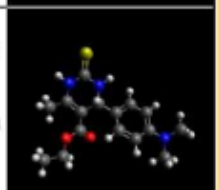
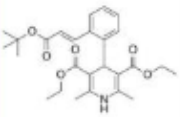
Molecule Structure	Significance & Activity	3D Structure
	After the synthesis of hundreds of compounds as well as biological testing, this compound had the highest cytotoxic activity, specifically against leukemia cell line HL-60(TB)[18].	
	This compound was created as an analog of monastrol and was tested on glioma cells. In contrast to monastrol, this compound doesn't arrest the cell cycle but instead induces apoptosis in glioma cells[19].	
	The compound is an effective oral antihypertensive agent, which can be administered to a wide variety of patients. It has previously shown potentially beneficial antiatherosclerotic effects. Therefore, lacidipine is an attractive therapy for the long-term management of essential hypertension[20].	

Table 2: Structures and relevance of novel DHPM analogs

the mortality rate of the illness through their activity as potent anticancer agents.

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Analogues Bearing 1,3,4-Oxadiazole Moiety." European Journal of Medicinal Chemistry 138 (September 29, 2017): 140–51.

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