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Review on Monastrol: A Novel Kinesin-5 Inhibitor

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Abstract: Dihydropyrimidines are pharmacologically active compounds and monastrol is one of the potential drug like molecule having dihydropyrimidine scaffold with potent Kinesin-5 inhibitory activity. In this article, we are reporting pharmacological profile of monastrol and its derivatives as inhibitors of Kinesin-5. In addition to pharmacological profile, chemistry of monastrol, resolution of racemic mixture and interaction of Kinesin-5 with monastrol have been discussed in this review. In addition, we have covered the most important aspects of the monastrol that may facilitate the development of novel dihydropyrimidines as selective inhibitors of Kinesin-5.

Keywords: Monastrol, Kinesin-5, Dihydropyrimidines, ADMET, Topological parameters, Enantiomeric excess (ee)

1. INTRODUCTION

Monastrol **1** is a dihydropyrimidine derivative and is a cell permeable molecule discovered by Thomas U. Mayer which reversibly arrests cells in mitosis phase by specifically inhibiting Eg5(which is highly expressed in neurons during development) and has been used to investigate the dynamic organization of mitotic spindles [1]. Dihydropyrimidines (DHPM) are the products of Bignelli's reaction and are well reported for their anticancer [2], anti-inflammatory [3], antitubecular [4], α-glucosidase inhibitor [5] and its Ca^{2+} channel blocking activities [6]. DHPMs are also reported for their anti-bacterial [7], anti-fungal [8] and pteridine reductase inhibiting activity [9]. Some natural alkaloids containing DHPM core unit like Batzeladine B**2** from *Batzella* species has been reported as potent HIV-gp-120-CD4 inhibitor. Some DHPM derivatives like bromo analogue of monastrol **3**, DHPM SQ32926 **4** and SQ32547 **5** have shown potent antihypertensive activity [10] (Fig. 1). The pyrimidine ring is the building block of nucleic acids, DNA and RNA molecules which provoked the scientists around the globe to develop new molecules for various biological activities.

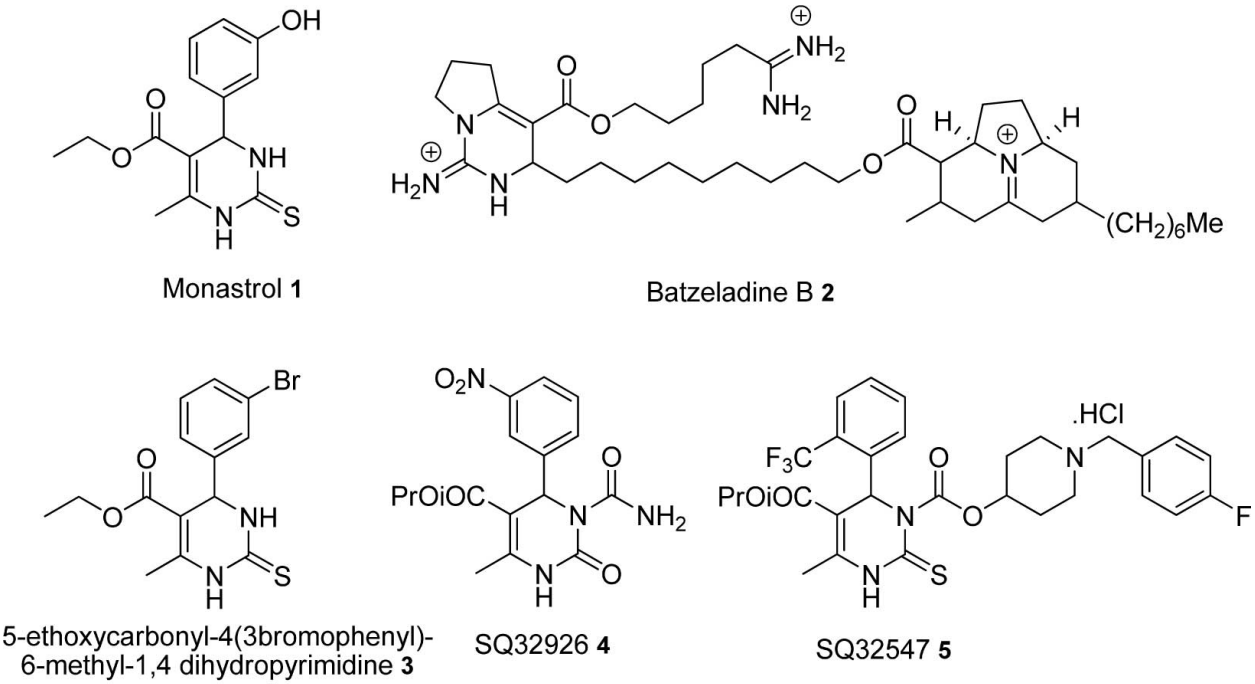


Figure-1

1.1 Kinesins

Kinesins were discovered in 1985 and they are classified into fourteen classes according to standardized nomenclature of kinesins [11-12]. They are also called 'hub' proteins and conventional proteins that are involved in many cellular functions like mitosis, meiosis and transport of macromolecules. They are conserved classes of microtubules associated with motor proteins that has ATPase activity and carry cargoes like organelles, vesicles, protein complexes along with microtubules [13-14]. They convert chemical energy of ATP into mechanical and helps in transportation of different molecules to perform various cellular functions [15]. The head of kinesins consists of 360 amino acids with ATP binding site and functions to hydrolyze the ATP.

1.2 Classification of Kinesins

Kinesins are classified into 3 categories i.e. N-kinesins, C-kinesins, M-kinesins. The N-kinesin has motor domain at *N*-terminus and move toward plus end of microtubules. The C-kinesin has motor domain at C-terminus and moves toward negative end of microtubules and M-kinesins depolymerize the microtubules [16-17]. The classification of Kinesins is shown in table 1 with the functions they perform.

TABLE 1: Classification of Kinesins according to their function

S. No.	Kinesin	MEMBERS	FUNCTIONS
1.	Kinesin-1	KIF5A, KIF5B,KIF5C	Mitochondrial transportation
2.	Kinesin-2	KIF3A, KIF3B, KIF17	Spermatogenesis
3.	Kinesin-3	KIF1A, KIF1B, KIF13A	Mitochondria transportation,
4.	Kinesin-4	KIF4A, KIF4A, KIF4B	Organelle transportation, mitosis

5.	Kinesin-5	KIF11, Eg-5, KLP61F	Bipolar spindle formation
6.	Kinesin-6	KIF20, KIF23, MKLP1	Cytokinesis, Bipolar spindle formation
7.	Kinesin-7	KIF10, CMET, CANA	Kinetochore microtubule capture
8.	Kinesin-8	KIF18B, KIF19A, KIP3	Nuclear migration
9.	Kinesin-9	KRP3, KIF6, KIF9	Spermatogenesis
10.	Kinesin-10	KID/KIF22	Chromosome congression
11.	Kinesin-11	KIF26A, KIF26B	Signal transduction
12.	Kinesin-12	KIF12/15	Organelle transportation
13.	Kinesin-13	MCAK, KIF2A	Microtubule depolymerization
14.	Kinesin-14	KIFC1	Chromosome segregation

2. Kinesin-5

Mitotic cell division in the living cells is regulated by a core protein known as kinesin which maintains the spindle formation within the nucleus. It is also known as Eg-5 protein that cross-links anti-parallel microtubules which is required for spindle bipolarity. It also plays a major role in mitosis where it slow down the rate of separation of half spindles and is a plus end directed motor protein that hydrolyses ATP or has ATPase activity [18-19]. Kinesin-5 is encoded by KIF11 gene and is a homotetrameric motor protein [20-21].

2.1 Structure of Kinesin-5

Kinesin-5 consists of motor head, neck linker, stalk and tail [22]. The motor domain or head is homotetrameric which is located at *N*-terminus and perform ATP hydrolysis and cross-links anti-parallel microtubules. The neck domain is essential for motility of proteins. The stalk is important for interwine of subunits of holoenzymes to form kinesin dimer and tail which is located at *C*-terminus plays important role in transportation of large molecules like lipids and proteins [23]. It consists of six β-sheets surrounded by six α-helics. The core part consists of nucleotide-binding cleft containing Mg-ADP complex and it is observed that loop-5 (L5) undergoes conformational changes when kinesin-5 is bound to microtubules [24]. Kinesin-5 differs from other kinesin family member proteins due to the presence of loop-5. The structure of kinesin-5 is shown in figure-2.

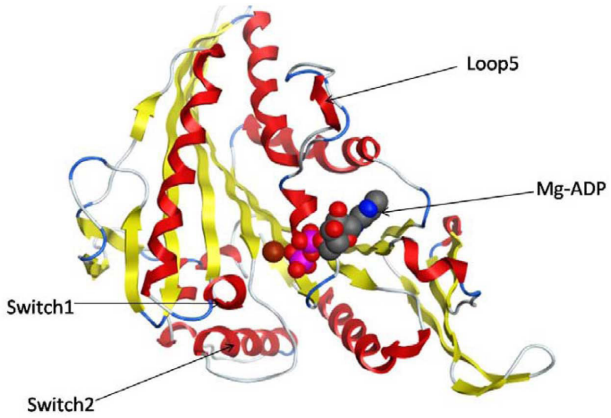


Figure 2: Binding of motor domain to Mg-ADP complex (PDB: 1OQB)

Figure 2 is showing the structure of kinesin-5 in which loop-5 is an important binding site for allosteric inhibitors like *S*-trityl-*L*-cysteine and monastrol [25-26]. Kinesin-5 consists of two switches, Switch-1 is located at end of α-3 helix and Switch-2 (also known as "relay helix") is made up of α-4 helix [27-28]. In presence of ATP, both switches come in contact with γ-phosphate and in this confirmation Switch-2 is in upper side and after conversion of ATP to ADP release of γ-phosphate occurs and the contact of these two switches lost and in doing so, Switch-2 moves to down position. When Switch-2 leads to down position, it induces steric interference that prevents association between head and neck linker that allows neck linker to attain perpendicular position to head linker and allows transportation of cargoes along with microtubules track [29].

2.2 Inhibitors of Kinesin-5

Kinesin-5 is involved in different type of cancers [30]. Monastrol is the prototype and potent inhibitor of Eg5. Other inhibitors like Ispinesib **6** [31], EMD534085 **7** [32], MK-0731 **8** have been reported as potent Kinesin-5 inhibitors [33]. These compounds are under clinical trials and structures of these compounds are shown in figure 3.

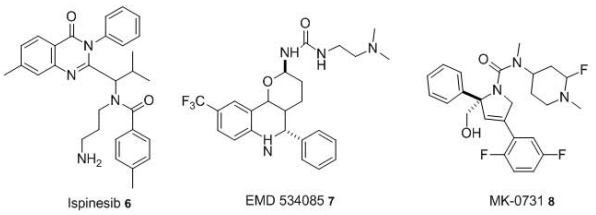


Figure-3

Apart from dihydropyrimidine/dihydropyridine derivatives, there are some other inhibitors that inhibit kinesin-5. Various kinesin-5 inhibitors from other chemical families are discussed here in this section.

1. **S-trityl-L-cysteine (STLC):** It targets human Eg5 which is responsible for the formation of bipolar mitotic spindle and acts as potential chemotherapeutic agent. *S*-Trityl-l-cysteine **9** is a tight binding inhibitor binds more tightly than monastrol. It has no steriospecificity as both enantiomers of it are equally potent. Other analogues of STLC have been synthesized having IC_{50} value 0.15μM [23, 34]. The structures of STLC and their analogues (**10-12**) are shown in figure 4.

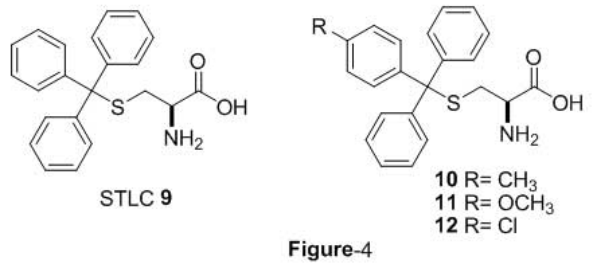


Figure-4

2. **Quinazolines:** Ispinesib **6** contains quinazoline core unit that has undergone in Phase II clinical trials for variety of both solid and hematologic cancers. It shows antiproliferative activity against breast and prostatic cancer cell lines also. The preclinical data of ispinesib indicate that it has additive effect when given in combination with other chemotherapeutic drugs. Other inhibitors like SB 743921 **13**, 2-benzyl-3-(4-hydroxyphenyl)-6-morpholino-2,3-dihydroquinazolin-4(1*H*)-one **14** and 2-benzyl-3-(4-hydroxyphenyl)-6-(piperidin-1-yl)-2,3-dihydroquinazolin-4(1*H*)-one **15** have shown potent inhibitory action against Eg-5 [23, 35-36]. The structures of these quinazolines derivatives are shown in figure 5.

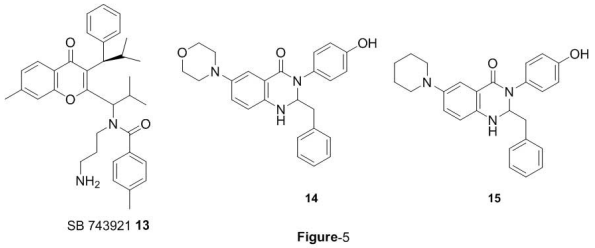


Figure-5

3. **β-Carbolines:** HR22C16 **16** and its derivatives are potent inhibitors of kinesin-5 (figure 6). These derivatives were discovered from chemical screening of 16000 compounds. The 50 analogues of the HR222C16 were synthesized and tested in cell based assay. A *trans*-tetrahydro-β-carboline (**17)** bearing *N*-benzyl substitution was found to be potent inhibitor of Eg-5 (IC_{50}=0.65) [37].

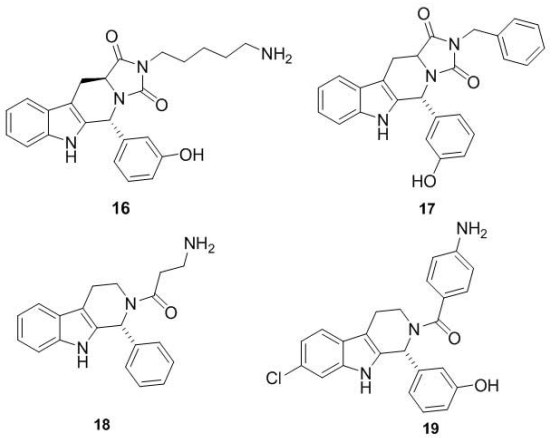


Figure-6: HR22C16 and its derivatives

4. **Hexahydro-2*H*-Pyrano[3,2-*c*] quinolines (HHPQs):** EMD-534085 **7** and its derivatives has been reported as potent inhibitors of kinesin-5 as they allosterically bind to kinesin-5, distant from nucleotide and microtubule binding sites [38]. EMD-534085 **7** is a potent inhibitor of kinesin-5 and shows antiproliferative activity against HL60 cancer cell lines (IC_{50}= 8.0nM) [23, 39]. The structure of EMD-534085 and its derivatives **20, 21, 22** are shown in figure 7.

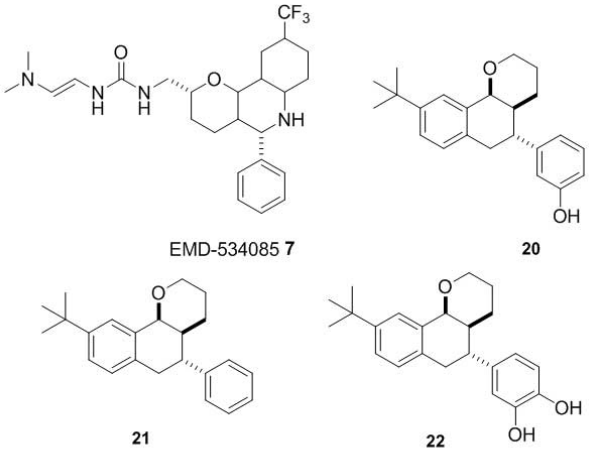


Figure-7: EMD-534085 and its derivatives

5. **Pyrazolopyridines and oxygen-bridged azolopyrimidines:** It is well reported that pyrazoles have kinesin spindle protein inhibiting activity. Therefore, researchers have discovered a new series of fused monastrol hetero analogues by integrating monastrol, pyrimidines with five membered pyrazole ring to yield a single heterocyclic nucleus. Here are some derivatives of monastrol which are potent kinesin-5 inhibitors with IC_{50} values ranging within 100-200nM [40]. Their structures are shown in figure 8.

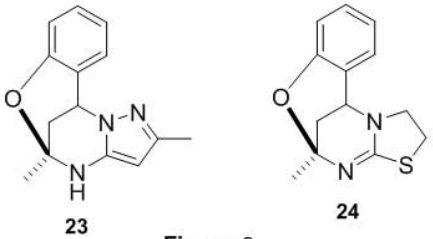


Figure-8

6. **Naturally occurring kinesin-5 inhibitors:** Some natural products like Terpendol **25**, Gossypol **26** also showed inhibitory activity against kinesin-5 [41]. The structures of these compounds are shown in figure 9.

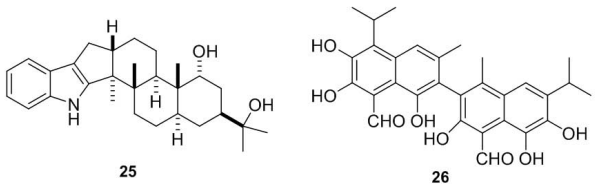


Figure-9

3. MONASTROL:

It is a non-tubulin interacting inhibitor that inhibits spindle bipolarity and impairs centrosome separation [42]. The reported IC_{50} value of monastrol and its derivatives lies between 100-200nM.

3.1 Mechanism of Action of monastrol

Kinesin-5 (Eg-5) is a motor protein that is responsible for spindle bipolarity [41]. From crystal structure (PDB:1OQB), it has been observed that loop-5, Switch-1 and Switch-2 undergoes conformational changes when Kinesin-5 attaches to microtubules that hydrolyses ATP to ADP and generates a force to move along cellular tracks [43-44] as shown in figure 10.

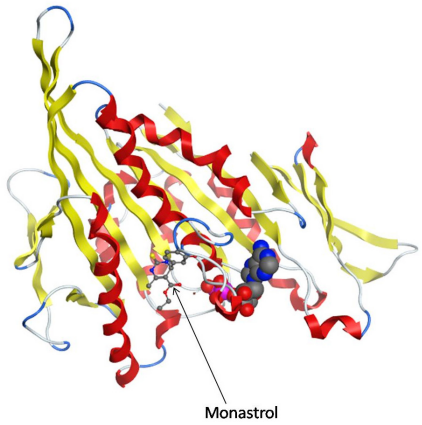


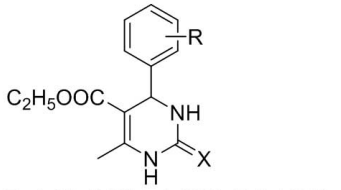
Figure 10: Allosterically inhibition of kinesin-5 (red and yellow) with monastrol.

Monastrol allosterically inhibits microtubule stimulated ADP release but does not inhibit ATPase activity and does not compete with microtubule binding. This suggests that it binds allosterically to Eg-5 protein[45]. Monastrol binds to loop-5 and α3-helix, bringing a little movement of α3-helix towards α2-helix that causes local and distal changes which allows ATP binding but prevent ADP release [46]. Monastrol binds allosterically to Eg5-ADP complex as shown in figure 10 and thus prevent ATP hydrolysis by making Eg5-ADP-monastrol ternary complex [47].

3.2 Anti-proliferative activity of monastrol and its derivatives

Russowsky *et al.* [48] reported the antiproliferative activity of monastrol, oxo-monastrol and their derivatives **27** against seven human cancer cell lines i.e. UACC62 (melanoma), MCF-7 (breast), OVCAR03 (ovarian), PC0 3 (prostate), HT-29 (colon), 786-0 (renal) and NCI-ADR (breast expressing phenotype multiple drugs resistance) Figure 11. All thio-derivatives showed significant antiproliferative activity against seven human cancer cell lines as compared to its oxo-derivatives due to the presence of sulphur atom in monastrol and its derivatives.

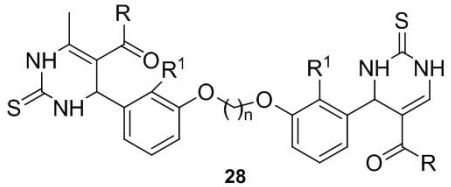
Russowsky *et al*. had reported the biological activity of some new DHPMs on various



27

Figure-11

cancer cell lines at concentration of 0.25, 2.5, 25 and 250μg/mL. Monastrol was found to be active at 25 μg/mL against PCO3, MCF-7, 786-0, UACC.62 and NCI-ADR cell lines. At same concentration (25μg/mL) oxo-moanstrol showed much lower cytostatic activity against all cancer cell lines except OVCAR03. At 250 μg/mL monastrol and its derivatives showed good cytotoxicity against UACC.62 whereas, oxo-monastrol and its derivatives showed very low cytostatic activity. Kamal *et al.* [49] had reported conformationally flexible as well as restricted symmetrical and asymmetrical dimers of monastrol and screened these derivatives against four cancer cell lines *i.e.* MCF7 (breast), A431 (skin), Colo (colon) and A549 (lung). The structures of various these derivatives are shown in figure 12. The compound **28** and its derivatives (>IC_{50}value200 μg/mL) were less active against colon cancer cell line whereas compound **28a** having IC_{50} value16 μg/mL with three carbon spacing [n= $(CH_2)_3$] and ester linkage (R= OC_2H_5, R^1= H) showed potent antiproliferative activity against MCF7 cancer cell line *i.e*. 3 times more potent than monastrol (45 μg/mL).



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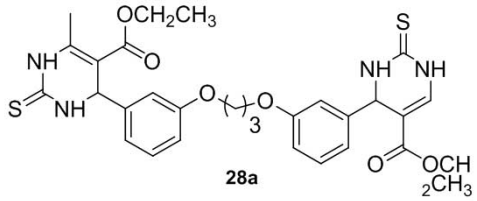
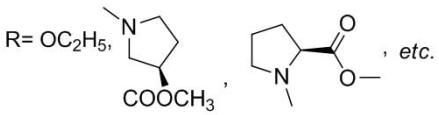


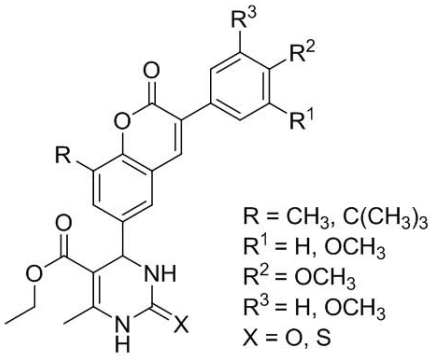
28a



R^1= H, OCH_3
n= 3, 4, 5

Figure-12

Sashidhara *et al.* [50] had reported coumarin-monastrol derivatives **29** that have better antiproliferative activity against breast cancer cell lines (MCF-7, T47D and MDA-MB-231), lung cancer cell line (A549), Human prostate lines (PC-3 and DU-145), Human hepatocellular liver carcinoma cancer cell line (HepG2). The structures of these derivatives are shown in figure 13.



29

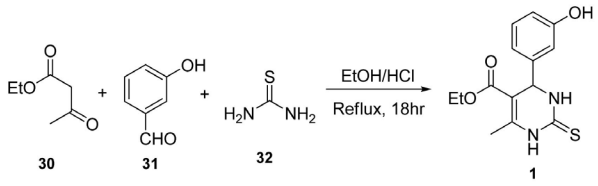
Figure-13

4. Chemistry of monastrol

Monastrol is chemically known as ethyl-4-(3-hydroxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetra-hydropyrimidine-5-carboxylate.

4.1 Synthesis of monastrol

Pietro Bignelli's in 1893 had reported the synthesis of monastrol **1** by condensation of ethyl acetoacetate **30**, 3-hydroxybenzaldehyde **31** and thiourea **32** under slightly acidic condition using conc. HCl as catalyst in appropriate solvent such as ethanol [51] as shown in Scheme-1.

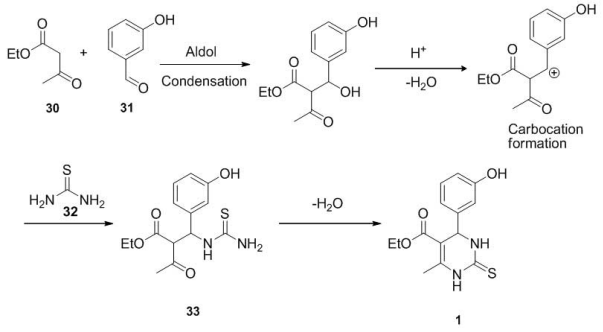


30 **31** **32** **1**

Scheme-1: Synthesis of monastrol

4.2 Mechanism of Bignelli's reaction

Mechanism of Bignelli's reaction has been extensively studied and reported by number of authors such as Kappe *et al.,* [52] Folkers, Sweet and Fissekis *et al.,* [53] Sweet and Fissekis, mechanism of Bignelli's reaction involves the Aldol condensation of ethyl acetoacetate **30** and 3-hydroxybenzaldehyde **31** resulting in the formation of carbocation which after nucleophilic addition of thiourea **32** gave the intermediate **33** that subsequently undergo dehydration to give monastrol **1** as shown in Scheme 2.

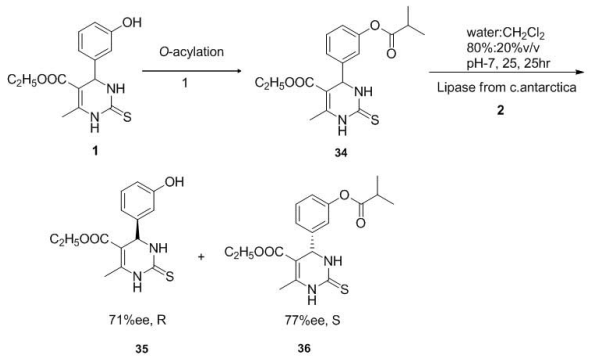


30 **31** **32** **33** **1**

Scheme-2: Mechanism of bignelli's reaction

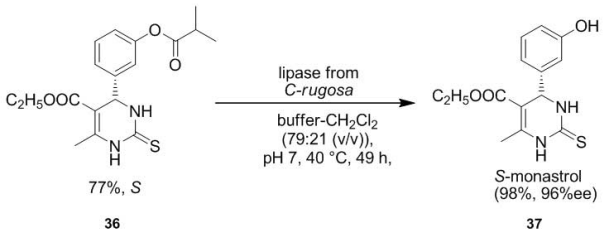
5. Resolution of chiral monastrol

Blasco *et al.* had reported racemic Monastrol as the first molecule to inhibit mitotic kinesin-5 [54]. According to Blasco *et al.* the *S*-enantiomer of monastrol has fifteen times more potency than *R*-enantiomer. Preparation of *S*-monastrol involves *O*-acylation at phenolic hydroxyl group of racemic monastrol resulting in formation of **(±)-**Ethyl-4(3-(isobutryloxy)phenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate **34** as shown in Scheme-3. Further, enzymatic hydrolysis of **34** was done in the presence of enzyme lipase, obtained from "*Candida antarctica*-B" (CAL-B) which ultimately give *R*-enantiomer (*R*-monastrol, **35**) in 71% ee and *S*-enantiomer (*S*-Ethyl-4(3-(isobutryloxy)phenyl)-6methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate, **36**) in 77% ee.



Scheme-3: 1) conversion of (±)-Monastrol to *o*-acylated (±)-Monastrol **34**
2) enzymatic hydrolysis of *o*-acylated (±)-Monastrol to *o*-acylated *S*-monastrol **36**

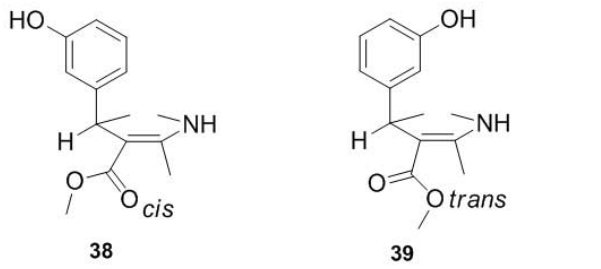
The hydrolysis of compound **36** was not possible under the same conditions to obtain the desired product *i.e. S*-monastrol **37**. Thus, non-selective biocatalytic hydrolysis was done based on the use of enzyme lipase from *Candida rugosa* (C-rugosa) that showed high activity (98% yield with 96% ee) [55-56].



Scheme-4: Enzymatic hydrolysis of **36** to *S*-Monastrol **37**

6. Geometry and conformations of monastrol:

Kappe *et al.* had reported the conformational studies of monastrol with Eg-5 receptor. It is important to understand the geometry and conformational studies of monastrol. The geometry optimization and conformational studies of monastrol was carried out through *ab-initio* (HF/3-.21G), semiempirical (AM1), X-rays diffraction methods and NMR studies. Four distinct local minima were found which includes, a) ester moiety of monastrol was found to be coplanar with respect to double bond at C5 and C6, *i.e.* carbonyl group of ester moiety have *cis* orientation with respect to double bond of C5 and C6 of dihydropyrimidine ring b) carbonyl group of ester moiety also have *trans* orientation with respect to double bond of C5 and C6 of dihydropyrimidine ring c) hydroxyl group of phenyl ring at C4 is synperiplaner (*sp*) with respect to hydrogen of C4 and d) hydroxyl group of phenyl ring at C4 is antiperiplaner (*ap*) with respect to hydrogen of C4 as shown in figure 14.



Schematic representation of conformations (side view)

Figure-14

In all the four conformations, 3-hydroxyphenyl ring is positioned axially (perpendicular and bisecting the boat like conformation of dihydropyrimidine ring). The lower energy conformer was one where ester group is *cis* oriented with respect to double bond at C5 and C6 and hydroxyl group of phenyl ring is synperiplaner with respect to hydrogen at C4 as in **38** and higher energy conformer was predicted with one where ester group is *trans* oriented with respect to double bond at C5 and C6 and hydroxyl group is antiperiplaner with respect to hydrogen at C4 as in **39**.

In the biological system all four conformations were accessible. In order to prove it, NMR experiment in solution was carried out. In this experiment it has been observed that hydrogen at C4 of dihydropyrimidine ring showed NOE (nuclear overhauser effect) with respect to hydrogens of C2' and C6' of 3-hydroxyphenyl ring and it was proved that, in solution both conformers *i.e. sp* and *ap* were present as shown in figure 14. Also, it was observed that conformation of ester moiety was difficult to find and dependent on the solvent interactions. In polar solvents (DMSO-d_6, DMF-d_7) one

distinct quartet (J= 7.5 Hz) for the OCH_2 group at 4.0 ppm was observed and in other solvents (acetone-d_6, $CDBr_3$, benzened_6) more composite splitting patterns were found, indicating the solvent interactions and/or hindered rotation. Thus, it was proved that all four conformations were accessible in the biological system with no clear first choice for one picky conformer [56-58].

7. Interaction study of monastrol with kinesin-5

Docking is the method used to predict binding orientation of small drug molecule to another protein targets in order to predict affinity and activity of small molecule [59-60]. Docking studies of *S*-Monastrol with Eg-5 has been reported by Saez *et al*. [61] that showed interactions of *S*-Monastrol with residues like Glu116, Gly117, Glu118, Arg119, Trp127, Asp130, Ala133, Ile136, Pro137, Tyr211, Glu215 and also showed interaction with water like HOH609, HOH695 and HOH 701 as shown in Figure 15. Some of important interactions of monastrol with the residues of kinesin-5 are:

1. One of nitrogen (*N*1) showed interaction with one lone pair of oxygen of water609 (HOH609) and other lone pair of oxygen

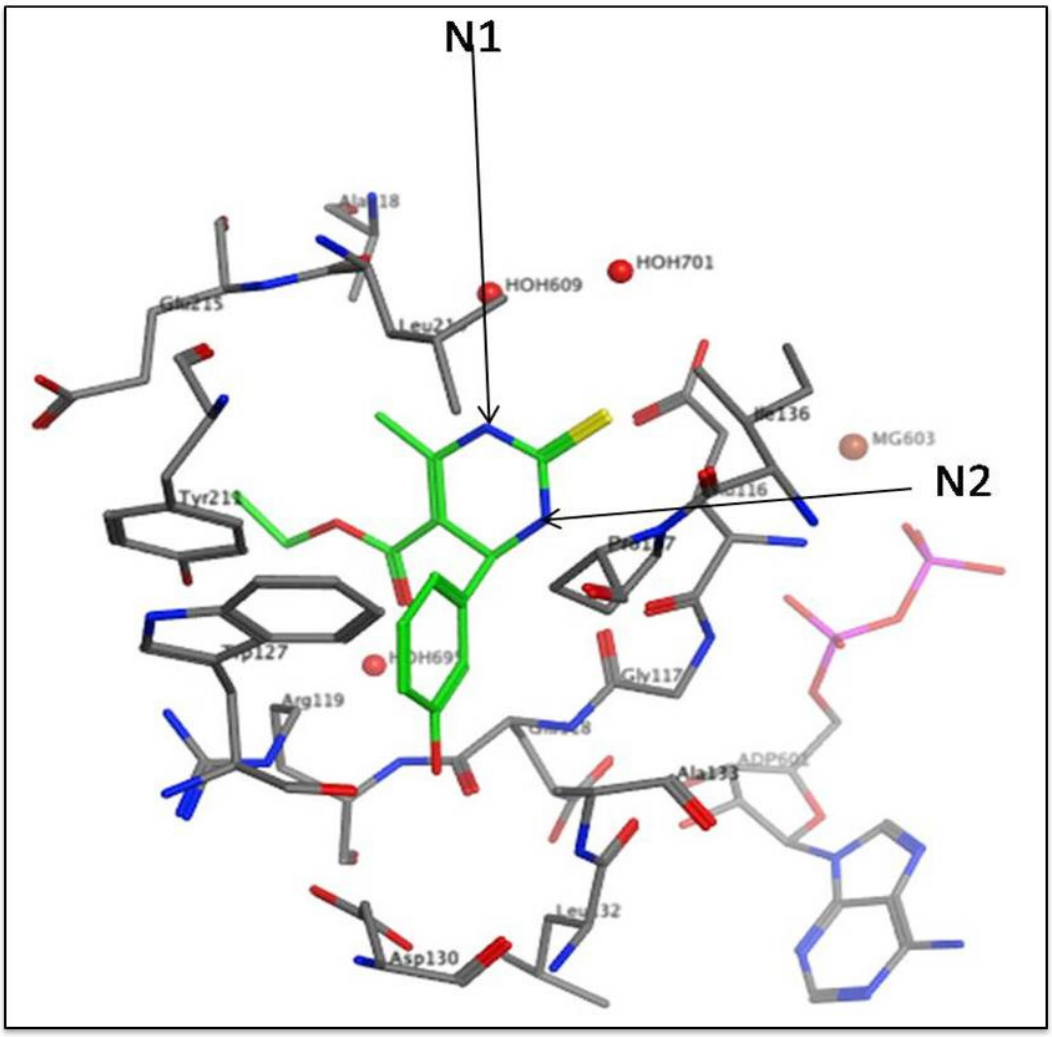


Figure 15: Interactions of Monastrol (green) with binding residues

of HOH609 showed interaction with lone pair of oxygen of leucine214 (Leu214) and distance were found to be 3.013 Å and 2.703 Å, respectively.

2. Sulphur atom showed interaction with oxygen of water701 (HOH701) and distance was found to be 3.267 Å.
3. Other nitrogen (*N*2) showed interaction with oxygen of glutamic acid116 (Glu116) and distance was found to be 2.819 Å.
4. Oxygen of ester group showed interaction with one lone pair of water695 (HOH695) and lone pair of HOH695 showed interaction with arginine119 (Arg119) and distance was found to be 3.203 Å and 2.910 Å, respectively.
5. Oxygen of phenol ring was found to be interacted with glutamic acid118 (Glu118) and distance was found to be 2.728 Å.

8. Pharmacokinetics study of monastrol

8.1 *In vitro* pharmacokinetics

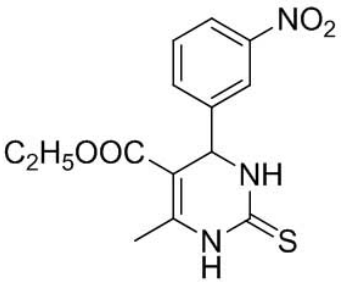
The pharmacokinetic study of monastrol was carried out by using ADMET software (version6.0). ADMET is accurate and useful program to predict physico-chemical and biological properties of drug. It also helps the researchers to develop better drug candidates and to eliminate those having low drug like properties [62] Hassan *et al.* has reported various topological parameters that affect the absorption, distribution, metabolism, elimination and toxicity of moanstrol as shown in Table-2 by using ADMET software [63].

TABLE 2: Reported observation of various processes affected by different topological parameters

PROCESSES	TOPOLOGICAL PARAMETERS	OBSERVATION
Absorption	Molecular weight (MW), hydrophobicity (logP), logD, hydrogenBonding descriptors (HBD), polarsurface area (PSA)	Reported human jejunal permeability (P_{eff}) and Madin–Darby Canine Kidney (MDCK) was found to be 0.1 cm/s×$10^4$and 166.5 cm/s×10^4respectively. The solubility of monastrol in intestinal fluid was found to be 0.37mg/mL.
Distribution	Volume of distribution (V_D), Molecular weight (MW), hydrophobicity (logP), logD, hydrogenBonding descriptors (HBD), polar surface area (PSA), pKa, BBB (Blood Brain Barrier) filtering, BBB ratio, %unbound, logK, logBBB	Reported volume of distribution was found to be 0.89L/kg. Reported plasma protein binding was found to be 77.98%. logk and logD values were found to be -0.55 and 1.82. The percentage unbound is predicted to be 22.02 for monastrol. Also logBBB(-0.26) and BBB (Blood Brain Barrier) filtering for monastrol is predicted to be low. Due to low volume of distribution, it may not be used for solid tumors.
Metabolism	Monastrol was found to be metabolized through CYP2C9, CYP2C19 and CYP2D6 via hepatic microsomes. Also log p and log D are the parameters that may affect the metabolism of monastrol	Metabolic stability is due to lower log D (1.82). It was predicted to have good efficacy in melanoma cancer due to high log P value (1.83).
Elimination	Molecular weight, polar surface area and active secretion of p-glycoprotein (P-gp) efflux transporters.	Due to reduced molecular weight of monastrol (<450kDa), its elimination is predicted to be through renal corpuscle of kidney in urine and also active secretion of monastrol.
Toxicity	Phospholipidosis estimations were done using ADMET™ software	Monastrol is nontoxic and no chance of phospholipidosis has been observed.

8.2 *In vivo* pharmacokinetics

The *in vivo* pharmacokinetic study had been carried out by using one of the derivative of monastrol, named LaSOM 65 (**40)** (ethyl 6-methyl-4-(3-nitrophenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-carboxylate) in Wistar rats.



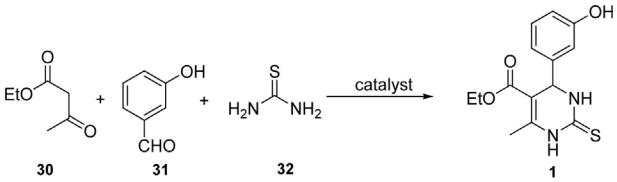
LaSOM 65 **40**

It has been observed that LaSOM 65 **40** is a potent anticancer agent against sarcoma 180 cell lines and glioma cell line. The study of LaSOM 65 has been reported on three groups of rats. The first group received intravenous dose of 1mg/kg, second and third group received oral dose of 10mg/kg and 30mg/kg. After dosing, the blood samples were withdrawn from three rat groups and the plasma was separated by centrifugation and deproteinized using acetonitrile which is a protein precipitant.

The total clearances were found to be (0.82 ± 0.12 L/h/kg; 0.81 ± 0.09 L/h/kg and 0.94 ± 0.41 L/h/kg).The volumes of distribution was found to be (1.76 ± 0.33 L/kg, 1.74 ± 0.39 L/kg, 2.92 ± 1. 04 L/kg). The terminal half-lives were found to be (1.7 ± 0.39 h; 1.48 ± 0.26 h and 2.33 ± 0.83 h) after *i.v* and *p.o.* dosing (10 and 30 mg/kg). The oral bioavailability was found to be 58.6% for the 10 mg/kg oral dosing and 49.2% for the 30 mg/kg. The absorption rate constant for the 10 mg/kg oral dosing (0.43 ± 0.11 min^{-1}) was higher than that observed for the 30 mg/kg dosing (0.26 ± 0.04 min^{-1}) [64-65].

9. Recently used synthetic methodologies for the synthesis of monastrol:

Generally, synthesis of monastrol **1** was done using ethylacetoacetate **30**, aldehyde **31** and urea **32** in boiling ethanol in the presence of conc. HCl (Scheme 1). However, it was found to suffer from long reaction time, harsh conditions and low product yield. Therefore, to improve the efficacy and yield of the reaction, the new methodologies has been developed using various catalysts, solvents, solvent free environments (neat conditions) and using microwave assisted synthesis (MW). To sum up, the most important methods for the synthesis of dihydropyrimidines are presented in Table-3:



Scheme-1: Synthesis of monastrol via various catalyst

TABLE 3: Synthesis of Monastrol *via* various catalysts

S.No.	Catalyst	Reaction condition	Reference
1	Al-M41	MW	[66]
2	$H_3PMo_{12}O_{40}$	MW	[67]
3	$CaCl_2$	MW	[68]
4	TTSA	MW	[69]
5	NiF_2	Refluxing	[70]
6	EPZ10	Refluxing	[71]
7	$BMImBF_4$ or $BMImBF_6$	Refluxing	[72]
8	P4VPy-CuI	Refluxing	[73]
9	PPE	Refluxing	[74]
10	Sr.Cl_2	Refluxing	[75]

Al-M41 = aluminium-planted mesoporous silica, **$H_3PMo_{12}O_{40}$** = 12-molybdophosphoric acid, **$CaCl_2$**= calcium chloride, **TTSA**= 1,3,5-triazine-2,4,6-triyltrisulfamic acid, **NiF_2** = nickel fluoride, **EPZ10** = clay supported $ZnCl_2$, **$BMImBF_4$ or $BMImBF_6$** = 1-*n*-Butyl-3-methylimidazolium tetrafluoroborate or 1-*n*-Butyl-3-methylimidazolium

hexafluorophosphorate, **P4VPy-CuI** = poly(4-vinylpyridine)-Supported Copper Iodide Nanoparticles, **PPE**= polyphosphate ester, **$SrCl_2$** = strontium chloride

10. Conclusion:

In this article, reported pharmacological study of monastrol and its various derivatives as inhibitor of Eg-5 is discussed. Being specific in inhibition of the enzyme Eg-5, it acts as a promising target for cancer therapy. Many of DHPMs are under clinical trials (Ispinesib, EMD534085, MK-731). In addition, study of several catalysts is discussed to make improvements in the synthetic applicability. Eg-5 represents a proficient target for designing new Eg-5 inhibitors and hence it would offer a novel approach to develop potent Eg-5 inhibitors.

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