TIPS

Design, synthesis and docking study of *3,4-dihydropyrimidine-2-one* derivatives as inhibitors of the Mitotic Kinesin, Eg5

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Abstract

A series of 3,4-dihydropyrimidine-2-one derivatives were designed and synthesized as monastrol analogs by Biginelli cyclocondensation of a β-keto compounds and aromatic aldehyde with urea or thiourea. Biginelli cyclocondensation usually produces racemic mixtures of 3,4-dihydropyrimidine-2-ones. Enantiomers of 3,4-dihydropyrimidine-2-ones (R and S) often show different biological activities and may even have an opposite action profile. Therefore, developing of the stereoselective synthesis of 3,4-dihydropyrimidine-2-ones is one of the priorities of medicinal chemistry. Following observed diverse pharmacological effects of monastrol, 3,4-dihydropyrimidine-2-ones as new anti-cancer agents are the pharmacological category which improve and decrease the side effects of dihydropyrimidines especially resistance to chemotherapeutic drugs. 3,4-Dihydropyrimidine-2-ones followed their anticancer effects by inhibiting the kinesin motor protein. Because of the specific function of these proteins in mitosis, targeting the Kinesin spindle protein (KSP, or Eg5) as the most dramatic target of the mitotic kinesin family open up an opportunity for the progress of more selective antimitotics with an improved side effect profile. These facts prompted us to investigate if the effects of inhibitory enantiomers (R and S) of 3,4-dihydropyrimidine-2-ones could possibly differ from each other. Therefore, molecular docking of both R and S stereoisomers of 3,4-dihydropyrimidine-2-ones and monastrol was performed to study the interaction between inhibitors and the kinesin spindle protein binding site. According to docking studies, S stereoisomer of D_2 (SD_2) has the best score among the synthesized compounds and shows probably more stable binding with the active site of the enzyme and can be considered as a candidate for biological evaluation.

Keywords: 3,4-Dihydropyrimidine-2-one, Biginelli reaction, Kinesin motor protein, Docking

1. Introduction

Cancer is a disorder resulting fundamental abnormalities in the regulations that control cell survival, proliferation and differentiation. Chemotherapy is a frequent treatment of many kinds of cancer; however, by itself, it is able to improve the chances of survival only about 10-15% of all cancer patients.

In addition, development of drug resistance remains the major obstacles in the successful chemotherapy. Kinesins are a diverse two-headed superfamily of motor proteins found in eukaryotic cells that use the energy from hydrolysis of ATP to

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generate force and movement along microtubules (MTs). Microtubules are dynamic linear polymers composed of α and β tubulin dimmers. They are a cytoskeleton component with an important role in a number of cellular process including the movement of secretary vesicles, organelles, and intracellular macromolecular assemblies of kinesins in chromosome separation (mitosis and meiosis), and are the major constituents of mitotic spindles, which are used to pull apart eukaryotic chromosomes (1-7).

A sub-group of kinesins are the kinesin spindle enzyme (KSP) or Homo sapiens Eg5 (HsEg5) that are needed for proper spindle length and sliding microtubules apart through bipolar spindle formation during mitosis phase of cell division (8-10).

Mitotic spindle causes segregation of replicated DNA into two newly formed cells during mitotic phase (11). Any disorders in formation of spindle afford abnormal chromosomal segregation resulting in mitotic arrest (12).

In addition, Eg5 is expressed more in transformed cells than in primary cells and its overexpression is higher in numerous proliferative tissues, such as leukemia, solid tumors including breast, colon, lung, ovary, uterus, bladder and pancreatic cancers, as compared to nonproliferative tissues. Almost, no Eg5 is present in nonproliferative tissues (13-18). Therefore, it could be concluded that Eg5 has been viewed as a potential target for cancer treatment (19).

The first effect of Eg5 inhibition is accumulation of monopolar spindles known as "monoasters" surrounded by condensed chromosomes, leading to prevent centrosomal separation and so mitotic block without disturbing the microtubules. This form of mitotic arrest in a monopolar stage leads to absolute cell death (apoptosis) of many cancer cells without affecting non-proliferating and normal cells (20, 21). Comparing to current antimicrotubule agents such as taxanes, vinca alkaloids, and epothilones, it can be predicted that inhibitors of Eg5 may not have serious unwanted side effects (22). Traditional antimiotic agents interfere with microtubules and affect both normal and proliferating cells. The disruption of microtubule formation can have toxic side effects, includ-

ing hair loss, body weight loss, and neurotoxicity (23-25). There is no risk of neuropathic side effects associated with Eg5 inhibitors because it is not expressed in the adult peripheral nervous system, unlike other tubulin target agents (26, 27).

Eg5 inhibitors are effective against mechanisms by which cancer cells develop resistance to chemotherapeutic drugs such as taxols. They may result from tubulin mutations or P-glycoprotein (Pgp)-mediated cellular efflux. The gene for P-glycoprotein, called MDR-1 is an energy-dependent pump that is capable of actively expelling chemotherapeutic agents from within cells. Overexpression of MDR-1 causes multidrug resistant phenotype exhibited by cells (28).

A variety of Eg5 inhibitors have been studied in two groups, as allosteric binding inhibitors (ATP uncompetitive) and ATP competitive with the majority of the first one (29, 30). The first group of compounds, adociasulfate-2, that inhibited kinesin was extracted from a marine sponge, Haliclona (also known as adosia) species (31). This sponge secretes a poison due to protect itself against aggressive effects. The poison is adociasulfate-2 that inhibits basal and MT-stimulated ATPase activity of Eg5, and is reported as inhibitor of some kinesins which exhibited nonspecific inhibitory activity.

Recently, several varieties of of Eg5 inhibitors have been reported (32-37). Mayer et al. had previously identified a dihydropyrimidine (DHPM) structure, (S)-monastrol, the first specific KSP inhibitor and the first known cell-permeable small molecule that has been shown to be an ATP uncompetitive allosteric inhibitor (38). Due to the potent antitumor activity of DHPM by Eg5 inhibition we decided to synthesize several structural analogues, in part to generate inhibitors with improved potency.

In this project, some new racemic 3,4-dihydropyrimidine-2-one derivatives were designed and synthesized by Biginelli cyclocondensation of a β-keto compound and aromatic aldehyde with urea. Because of different biological activity and side effects of dihydropyrimidine stereoisomers especially resistance to chemotherapeutic medicines, enantiomers of 3,4-dihydropyrimidine-2-ones (R and S) were evaluated by docking studies. Docking analysis was performed to clarify the binding

affinity of compounds against the active site and helped in revealing the potential mode of action through their interactions. Both configuration R and S in the binding site of Eg5 were investigated from the docking method to find out which enantiomer has the most prominent inhibitory activity. This method could potentially uncover the ways in which the studied stereoisomer inhibitors and Eg5 interact, which could lead to the development of Eg5 inhibitors that could be used to treat cancer. The mode of interaction between receptor and ligand and predicted free energy can be effectively used for design of bioactive compounds.

2. Experimental protocols

All solvents and reagents were purchased from Sigma or Merck Chemical Companies. Reactions were monitored by thin-layer chromatography (TLC) using coated silica gel plates (Merck, Germany), and the spots were detected under UV light (254 nm). The melting point was measured on an Electro Thermal 9200 micro melting point apparatus and reported uncorrected. IR spectra (KBr-disc) were recorded using a Perkin Elmer Spectrometer (Germany). ^{1}H and ^{13}C NMR spectra were acquired in DMSO on a Bruker Avance DPX 500 instrument (Germany). Chemical shifts are expressed in δ (ppm) relative to TMS as internal standard. Coupling constants are given in Hz. The splitting patterns are designed as s; singlet, d; doublet, t; triplet, m; multiplet. Mass spectra were recorded on a Hewlett Packarb (HP) Mass Spectrometer.

2.1. General procedure for Synthesis of 3,4-dihydropyrimidine-2-ones

General procedure is consist of combination of 2 mmol of β-keto compound (C_{1-8}) and 2 mmol of aromatic aldehyde (A_{1-8}) with 3 mmol of urea or thiourea (B) in 20 ml of solvent (ethanol or acetonitril) in the presence of an appropriate Lewis acid such as $FeCl_3$ (0.07 g) as a catalyst. The reaction was refluxed during 24 h. After the accomplishment of reaction, solvent was evaporated under vacuum and the pure product obtained by silica gel chromatography with petroleum ether and ethylacetate (75:25) and then crystallized at diethylether to afford D_{1-8} as a pure powder in 19

to 45 % yield. Finally the structures confirmed by spectroscopic methods such as IR, 1H-NMR and Mass spectroscopy. The overall scheme of the reaction brought in Scheme 1. Yield of all products come in Table 1.

2.2. 5-benzoyl-6-methyl-4-(thiophene-2-yl)-1,2,3,4-tetrahydropyrimidine-2-one (D_1)

Compound C1 was treated with A1 and B according to the general cyclization procedure to give the desired product D1 as a yellow powder in 19.44% yield; 1H-NMR (400 MHZ, DMSO): δ (ppm) 1.67 (s, 3H, CH3-DHPM), 5.56 (d, 1H, C4H-DHPM, J=3.2 Hz), 6.86 (dd, 1H, C3′H-thiophen, J=3.2, 1.2 Hz), 6.93 (dd, 1H, C4′H-thiophen, J=5.2, 3.2 Hz), 7.38 (dd, 1H, C5′H-thiophe, J=5.2 , 1.2 Hz), 7.44-7.55 (comp., 5H, H-phenyl), 7.80 (s, 1H, N3H), 9.33 (s, 1H, N1H); MS m/z (%): 298 [M+] (65.5), 283 (13.7), 193 (41.3), 105 (71.2), 77 (100) ; IR(KBr) υ (cm-1) : 3288.81 (N-H, DHPM), 2925.24 (C-H, aromatic), 2854,14 (C-H, aliphatic), 1698.17 (C=O, keton), 1676.12 (C=O, amide).

2.3. 5-benzoyl-6-methyl-4-(naphthalene-2-yl)-1,2,3,4-tetrahydropyrimidine-2-one (D_2)

Compound C_2 was treated with A_1 and B according to the general cyclization procedure to give the desired product D2 as a yellow crystal in 25.16% yield; 1H-NMR (400MHZ, DMSO): δ (ppm) 1.71 (s, 3H, CH_3-DHPM), 5.52 (d, 1H, C_4H-DHPM, J=3.2 Hz), 7.40-7.52 (comp., 8H, CH-naphthalen and CH-phenyl), 7.63 (s, 1H, CH-naphthalen), 7.85-7.91 (comp., 4H, CH-naphthalen and N_3H), 9.24 (bs, 1H, N_1H); MS (m/z, %): 342 [M$^+$] (76.1), 237 (47.7), 215 (45.45), 105 (95.45), 77 (100); IR(KBr) υ (cm^{-1}) : 3239.29 (N-H, DHPM), 3112.96 (C-H, aromatic), 1699.12 (C=O, keton), 1630.11 (C=O, amide).

2.4. 5-benzyloxycarbonyl-6-methyl-4-(naphthalene-2-yl)-1,2,3,4-tetrahydropyrimidine-2-one (D_3)

Compound C_3 was treated with A_3 and B according to the general cyclization procedure to give the desired product D_3 as a white powder in 42.21% yield; 1H-NMR (400MHZ, DMSO): δ (ppm) 2.32 (s, 3H, CH_3-DHPM), 4.99(d, 1H,

CH_2phenyl, J=12.8 Hz), 5.06 (d, 1H, CH_2phenyl, J=12.8 Hz), 5.36 (d, 1H, C_4H-DHPM, J=2.8 Hz), 7.11-7.24 (comp., 5H, CH-phenyl), 7.42-7.52 (comp., 3H, CH-naphthalen), 7.62 (s, 1H, CH-naphthalen), 7.80-7.89 (comp., 4H, CH-naphthalen and N_3H), 9.32 (s, 1H, N1H); MS (m/z, %) : 372 [M^+] , (5.8), 281 (45.88), 245 (15.2), 128 (11.7), 91 (100) ; IR(KBr) υ (cm-1) : 3223.68 (N-H, DHPM), 3113.33 (C-H, aromatic), 2979.95 (C-H, aliphatic), 1709.77 (C=O, keton), 1686.83 (C=O, amide).

2.4. 5-allyloxycarbonyl-6-methyl-4-(naphthalene-2-yl)-1,2,3,4-tetrahydropyrimidine-2-one (D_4)

Compound C_4 was treated with A_4 and B according to the general cyclization procedure to give the desired product D_4 as a white powder in 13.39% yield; 1H-NMR (400MHZ, DMSO): δ (ppm) 2.33 (s, 3H, CH_3-DHPM), 4.49 (d, 2H, COOCH_2, J=5.2 Hz), 5.07 (dd, 1H, CHgeminal-CH=CH_2, J=10.4, 1.6 Hz), 5.11 (dd, 1H, CHgeminal-CH=CH_2, J=17.2, 1.6 Hz), 5.37 (d, 1H, C_4H-DHPM, J=3.2 Hz), 5.78-5.88(ddt, 1H, CH=CH2, J=17.2, 10.4, 5.2 Hz), 7.45-7.52 (comp., 3H, CH-naphthalen), 7.69 (s, 1H, CH-naphthalen), 7.87-7.91 (comp., 4H, CH-naphthalen and N_3H), 9.32 (d, 1H, N1H, J=1.2 Hz); MS (m/z, %) : 322 [M^+] , (24.2), 281 (100), 237 (48), 195 (97.5), 127 (40.2), 41 (15.85) ; IR(KBr) υ (cm^{-1}) : 3232.56 (N-H, DHPM), 3101.29 (C-H, aromatic), 2942.63 (C-H, aliphatic), 1704.33 (C=O, keton), 1650.77 (C=O, amide).

2.5. 5-benzoyl-4-(4-methoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one (D_5).

Compound C_5 was treated with A_5 and B according to the general cyclization procedure to give the desired product D_5 as a yellow crystal in 41.20% yield; 1H-NMR (400MHZ, DMSO): δ (ppm) 1.68 (s, 3H, CH_3-DHPM), 3.71 (s, 3H, CH_3O), 5.25 (d, 1H, C4H-DHPM, J=3.2 Hz), 6.86 (d, 2H, C3′H, C5′H-aromatic, J=8.4 Hz), 7.12 (d, 2H, C2′H, C6′H-aromatic, J=8.4 Hz), 7.41-7.54 (comp., 5H, CH-phenyl), 7.75 (bs, 1H, N_3H), 9.15 (s, 1H, N1H) ; MS (m/z, %) : 322.2[M^+] , (100), 307.2 (35.8), 217 (50.3), 105 (36.6), 77 (39.1) ; IR(KBr) υ (cm^{-1}) : 3282.48 (N-H, DHPM), 2996.16 (C-H, aromatic), 2927.67 (C-H, aliphatic), 1708.8

(C=O, keton), 1674.28 (C=O, amide)

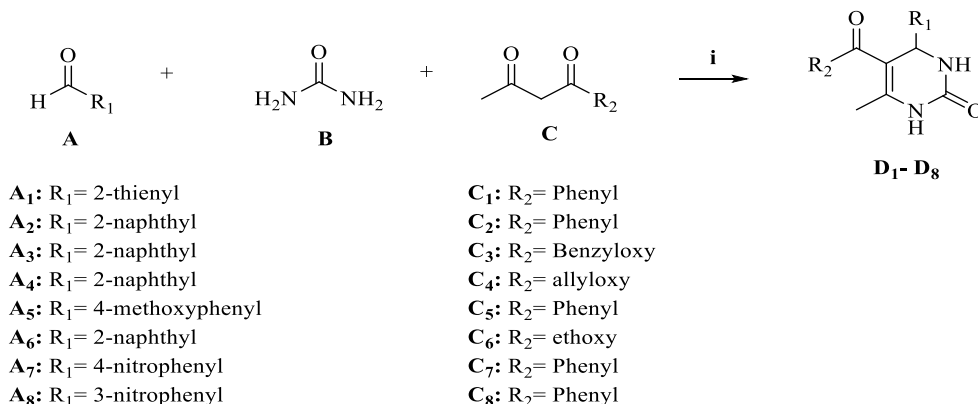### 2.6. 5-ethyloxycarbonyl-6-methyl-4-(naphthalene-2-yl)-1,2,3,4-tetrahydropyrimdine-2-one (D_6)

Compound C_6 was treated with A_6 and B according to the general cyclization procedure to give the desired product D_6 as a white powder in 14.35% yield; 1H-NMR (400MHZ, DMSO): δ (ppm) 1.08 (t, 3H, -OCH_2CH_3, J=7.2 Hz), 2.30 (s, 3H, CH_3-DHPM), 3.97 (q, 2H, -OCH_2CH_3, J=7.2 Hz), 5.33 (d, 1H, C_4H-DHPM, J=3.2 Hz), 7.44-7.50 (comp., 3H, CH-naphthalen), 7.68 (s, 1H, CH-naphthalen), 7.86-7.91 (comp., 4H, CH-naphthalen and N3H), 9.28 (s, 1H, N1H) ; MS (m/z, %) : 310 [M^+] (79.16), 281 (82.5), 264.1 (28.3), 237.2 (61.6), 183.3(100), 155.1 (38.3) ; IR(KBr) υ (cm-1): 3222.83 (NH-DHPM), 3100.3 (C-H, aromatic), 2929.91 (C-H, aliphatic), 1704.54 (C=O, keton), 1651.55 (C=O, amide).

2.7. 5-benzoyl-6-methyl-4-(4-nitrophenyl)-3,4-dihydropyrimidin-2(1H)-one (D_7).

Compound C_7 was treated with A_7 and B according to the general cyclization procedure to give the desired product D_7 as a beige crystal in 25.2% yield; 1H-NMR (400MHZ, DMSO): δ (ppm): 1.66 (s, 3H, CH_3-DHPM), 5.43 (d, 1H, C_4H-DHPM, J=3.2 Hz), 7.42-7.51 (comp., 5H, CH-phenyl), 7.52 (d, 2H, C2′H,C6′H-nitrophenyl, J=8.8 Hz), 8.00 (bs, 1H, N_3H), 8.21 (d, 2H, C_3′H,C_5′H-nitrophenyl, J=8.8 Hz), 9.38 (s, 1H, N_1H) ; MS (m/z, %) : 337.2 [M^+] (7.07), 290 (35.35), 232 (15.15), 215 (54.54), 105 (82.82), 77(100); IR(KBr) υ (cm-1): 3284.61 (N-H,DHPM), 3072.86 (C-H, aromatic), 2936.72 (C-H, aliphatic), 1710.53 (C=O, keton), 1677.09 (C=O, amide), 1533.15, 1346.35 (NO_2).

2.8. 5-benzoyl-6-methyl-4-(3-nitrophenyl)-3,4-dihydropyrimidin-2(1H)-one (D8).

Compound C_8 was treated with A_8 and B according to the general cyclization procedure to give the desired product D_8 as a beige crystal in 12.07% yield; 1H-NMR (400MHZ, DMSO): δ (ppm): 1.68 (s, 3H, CH3-DHPM), 5.45 (d, 1H, C_4H-DHPM, J=3.2 Hz), 7.42-7.54 (comp., 5H, CH-phenyl), 7.65 (dd, 1H, C_5′H-nitrophenyl, J=8, 7.6 Hz), 7.72 (dd, 1H, C_6′H-nitrophenyl, J=7.6,

A₁: R₁= 2-thienyl
A₂: R₁= 2-naphthyl
A₃: R₁= 2-naphthyl
A₄: R₁= 2-naphthyl
A₅: R₁= 4-methoxyphenyl
A₆: R₁= 2-naphthyl
A₇: R₁= 4-nitrophenyl
A₈: R₁= 3-nitrophenyl

C₁: R₂= Phenyl
C₂: R₂= Phenyl
C₃: R₂= Benzyloxy
C₄: R₂= allyloxy
C₅: R₂= Phenyl
C₆: R₂= ethoxy
C₇: R₂= Phenyl
C₈: R₂= Phenyl

Scheme 1. Reagents and conditions: (i) $FeCl_3$, acetonitril, reflux.

2.4 Hz), 8.04 (bs, 1H, N3H), 8.09 (m, 1H, $C_2'H$-nitrophenyl), 8.13 (ddd, 1H, $C_4'H$-nitrophenyl, J=8, 2.4, 1.2 Hz), 9.41 (d, 1H, N1H, J=1.6 Hz); MS (m/z, %): 337 [M$^+$] (48.9), 322 (21.27), 232 (26.59), 215 (52.1), 105 (81.9), 77 (100); IR(KBr) υ (cm-1): 3300.91 (N-H, DHPM), 2925.94 (C-H, aliphatic), 1706.49 (C=O, keton), 1676.18 (C=O, amide), 1574.66 , 1351.41 (NO$_2$).

2.9. Docking simulation method

Since different 3D-structures were released for KSP, docking validation was used to pick a proper x-ray structure for docking simulation. From these complexes, 11 PDB codes for KSP were chosen from Protein Data Bank (http://www.rcsb.org). All the retrieved PDB codes were subjected to self-docking validation test. In this step, the cognate ligands were re-docked on their corresponding 3D structures and the best pose of docking was superimposed with the native conformation of the ligand at crystallographic state. Amongst all the retrieved PDB codes 1Q0B, 1X88, 2IEH, 2X7C, 2X7D, 2X7E and 3K5E were selected based on root-mean-square deviation (RMSD) values as shown in Table 2. PDB code 1X88 complexed with monastrol as a cognate ligand was selected in this investigation. The compound structures were drawn by chem office 3D program and were optimized by AM1 semiempirical calculation method. After that, charge calculation was done and the ligands were saved in pdbqt format. To prepare protein for docking simulation, at first, the amino acid chains were kept and the water molecules and co-crystallized ligand were removed. Afterward, the polar hydrogen was add-

ed to the receptor and the resulting protein was subjected to minimization. The prepared protein was saved in pdbqt format. The true 3D space (grid box) containing active site was considered for KSP enzyme.

All preparation was done using Auto Dock Tools package (1.5.6). Cross-docking simulations were performed using bash scripting in linux operating system. Autodock Vina (1.1.2) (http://vina.scripps.edu/) was used for docking within a box defined by following parameters. The grid box with the size of 30×30×30 was determined and the box was centered on co-crystalized ligand. The center of grid box was determined as [x=42.21, y=26.49, z=112.05] for 1X88. The exhaustiveness was set to 100 and the other docking parameters were set as default. At the end of cross-docking simulations, the best docking poses were selected for further analysis of enzyme-inhibitor interactions (42).

3. Results and discussion
3.1. Chemistry

The Biginelli cyclocondensation (39) of a β-keto compound (C_{1-8}), aromatic aldehyde (A_{1-8}) with urea or thiourea (B) (9) in the presence of FeCl3 afforded target compounds D_{1-8} (Scheme 1). The reaction was refluxed in appropriate solvent (ethanol or acetonitrile) for 24 h. Once the reaction was finished, the solvent was evaporated from the mixture in a vacuum. The crude product was purified by silica gel chromatography using n-hexane/ethylacetate to get the final products. The yield of reactions was 19-45% (Table 1).

The key step of this novel one pot, three-component synthesis involves the acid-catalyzed

Table 1. The results achieved from synthesis of 3,4-dihydropyrimidine-2-one derivatives

| Comp.	R1	R2	Rf	Product	B.P.	Yield	color
D1	2-thienyl	Phenyl	0.22		203-204	19.44%	Yellow powder
D2	2-naphthyl	Phenyl	0.20		269-270	25.16%	Yellow crystal
D3	2-naphthyl	Benzy-loxy	0.33		214-215	42.21%	White Powder
D4	2-naphthyl	Allyloxy	0.32		202-204	13.39%	White Powder
D5	4-methoxyphenyl	Phenyl	0.3		243-245	41.2%	Yellow Crystal
D6	2-naphthyl	Ethoxy	0.26		210-211	14.35%	White Powder
D7	4-nitro phenyl	Phenyl	0.18		249-252	25.2%	Beige cyrstal
D8	3-nitrophenyl	Phenyl	0.20		239-245	12.07%	Beige crystal

formation of an N-acyliminum ion intermediate from the aldehyde and urea precursors (40). In continue, iminium ion interact by β-keto compound through its enol tatomer and produces open-chain ureide. Ureide through an intramolecular cyclization turns into the hexahydropyrimidine. Acid-catalyzed removal of water from hexahydropyrimidine leads to the final product 3,4-dihydropyrimidine-2-one. α -Amidoalkylation, or more specifically α-ureidoalkylation are the mechanism

of these reactions (41).

3.2. Molecular docking study of 3,4-dihydropyrimidine-2-one stereoisomers with KSP enzyme active site

Docking is a method which predicts the most stable form of receptor-ligand by using genetic algorithm. The mode of interaction between receptor and ligand and predicted free energy can be effectively used for design of bioactive
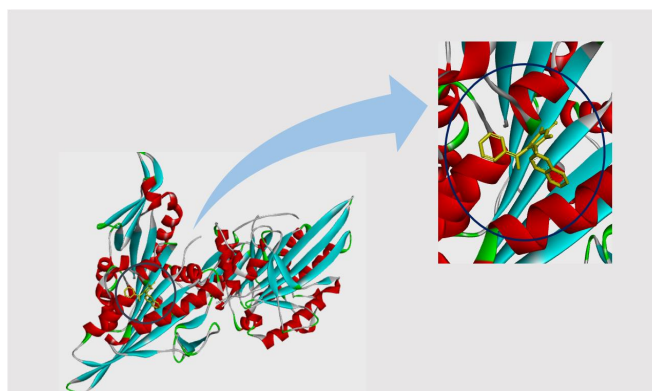
Figure 1. The full structure and magnification of KSP enzyme active site, PDB code: 1X88.

Table 2. RMSD values and predicted binding energies of synthesized 3,4-dihydropyrimidine-2-one stereoisomers derivatives in compare with monastrol as the standard compound.

PDB Code	RMS-Dof self-docking	Binding Energy (Kcal.mol^{-1})															
		RD$_1$	RD$_2$	RD$_3$	RD$_4$	RD$_5$	RD$_6$	RD$_7$	RD$_8$	SD$_1$	SD$_2$	SD$_3$	SD$_4$	SD$_5$	SD$_6$	SD$_7$	SD$_8$	Mon
1q0b	1.21	-9	-10	-10.5	-9.5	-9.4	-9.2	-9.5	-10	-8.9	-11.5	-8.1	-9.6	-9.8	-9.4	-10	-10.5	-7.9
1x88	1.15	-9	-10.1	-10.2	-9.4	-9.5	-9.1	-9.6	-9.5	-9.1	-11.6	-10	-9.7	-9.9	-9.4	-10	-10.5	-8.1
2ieh	0.17	-9.5	-10.6	-10.3	-9.3	-9.2	-9.1	-9.3	-9.1	-8.8	-11.4	-10.5	-9.7	-9.8	-9.5	-10	-10.6	-8
2x7c	0.33	-8.8	-10	-10.4	-9.2	-9.5	-9	-9.6	-9.4	-9	-11.4	-10.2	-9.6	-9.8	-9.3	-10	-10.5	-7.8
2x7d	0.66	-9.2	-11	-10.6	-9	-9.3	-8.7	-9.4	-9.2	-8.8	-11.6	-10.4	-9.7	-9.9	-9.6	-10.1	-10.5	-7.9
2x7e	0.46	-8.9	-10.7	-10.3	-9.3	-8.8	-8.9	-9	-9.5	-8.3	-11.1	-9.8	-9.3	-9.4	-9	-9.3	-9.9	-7.3
3k5e	0.33	-8.8	-9.9	-10.5	-9.1	-9.4	-8.3	-9.5	-9.3	-8.9	-11.6	-10.4	-9.6	-9.9	-9.5	-10.2	-10.5	-7.7

compounds. The overall goal of this docking research project is the discovery of binding affinity of enantiomers of 3,4- dihydropyrimidine-2-one analogues to target enzyme. Binding capability of synthesized 3,4-dihydropyrimidine-2-one stereoisomers and monastrol as a control compound have investigated with KSP enzyme active site. Docking process of designed compounds with receptor is done by means of AutoDock Vina (1.1.2). Therefore, docking simulation of these compounds as anticancer agents with KSP as the target has been done to study potential possibility of binding of both stereoisomers R and S. For this purpose, seven 3D X-ray crystal structures of KSP were retrieved from Protein Data Bank (PDB) as a co-crystallized complex with monastrol and other similar structures. All of them were selected based on optimal self-docking criteria of RMSD <2 Å described in Table 2. To determine the accuracy of docking process, the known co-crystallized inhibitor (monastrol) was excluded from the selected

crystalline 1X88 complex and re-docked into the active site.

The parameters obtained during the validation phase were used to cross-dock the synthesized compounds. Following completion of the cross-docking simulation, the best pose for the most prominent compound was selected for visualization and analysis. The findings of the cross-docking investigation concerning binding energies and RMSD values displays in Table 2 for each PDB codes. According to the predicted free energy by Autodock program, S and R stereoisomers have almost proximate binding affinity into the receptor active site, however, the binding energy of S isomers are less than R isomers. Due to this fact, it could be concluded that possibly S isomers of this group of compounds especially D$_2$ (SD$_2$) have more important role in biological activity (Table 2). The results of this study demonstrate that the docked structures could be fit in an acceptable way with x-ray crystal structure. All interactions
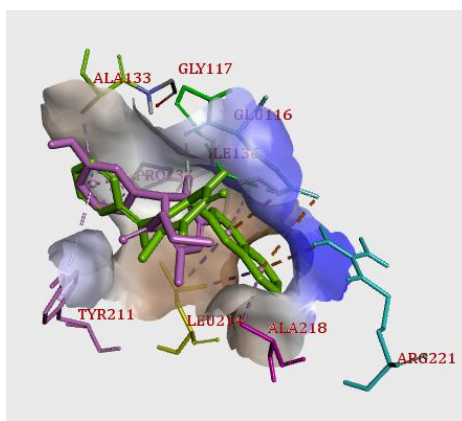
Figure 2. Superimposition of the most favorable docked conformation SD$_2$ on dock pose of cocrystallized ligand, PDB code: 1X88, RMSD=1.15 Å.
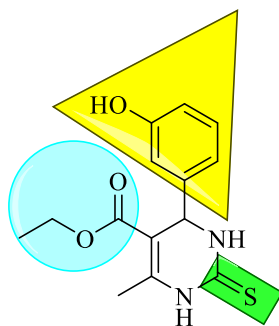
Figure 3. The structure of monastrol as the standard compound and as the basis for designing new analogues with modification sites.

that are used for prediction of binding energy are hydrophobic interactions and hydrogen bond between ligand and receptor. Based on these data, it is possible to design the same structures with more binding potency to enzyme's active site. The full structure of KSP receptor and magnification of the binding pocket of KSP receptor is shown in Figure 1. After docking process and evaluating each docked pose, superimposition of the most favorable docked conformation SD_2 on co-crystallized conformation, monastrol, is depicted in Figure 2. As shown in this figure, the docked structure was fitted in a good agreement way to active site of target enzyme with x-ray crystal structure which demonstrated the reliability of docking process with RMSD of 1.15 Å.

Monastrol was the first compound of 3,4-dihydropyrimidine-2-one derivatives, which was used as the basis for designing new analogues using bioisoster modification in this scalfold as a KSP enzyme inhibitor. In this study monastrol

investigated as a control compound (Figure 3). Monastrol make a hydrogen bond through hydrogen group with amino acid residues Glu116, and have alkyl and pi-alkyl bond through methyl group with Ala133, Pro137, Leu214 and Ala218 (Figure 4). To optimize its interactions with the receptor, KSP, critical hydrophobic interactions and hydrogen bonds were taken into consideration. The modifications were focused on improving these interactions. One of the initial modifications involved replacing the thiourea moiety in monastrol with urea. This was done because urea is known to be more biocompatible and tolerable than thiourea. Additionally, urea has more favorable hydrogen bonding properties. Another modification was made to the aromatic site of the parent compound, monastrol. In this case, more lipophilic moieties were introduced in order to enhance the pi-pi interactions within the active site. Another modification involved replacing the ethyl ester group with other carbonyl substituents. The aim of these modifica-
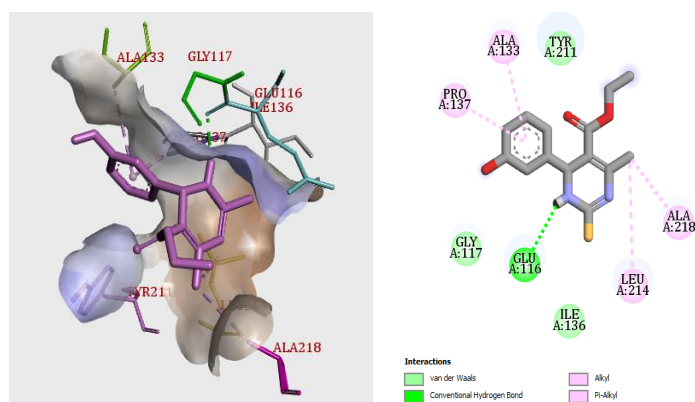
Figure 4. Schematic representation of monastrol interaction with KSP enzyme active site, PDB code: 1X88.
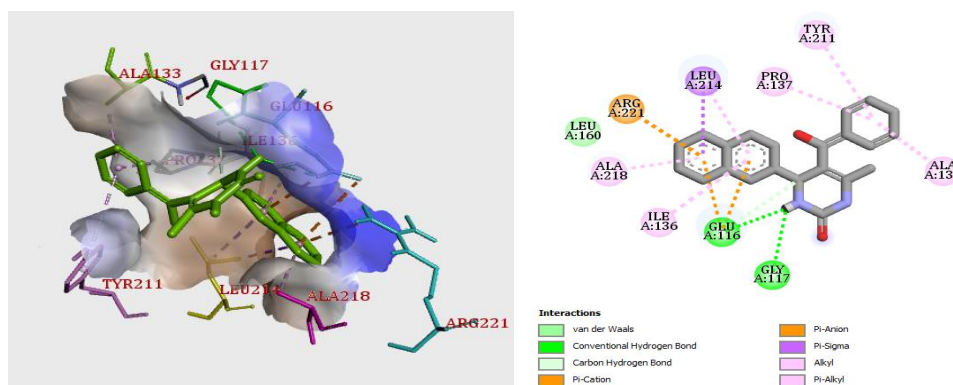
Figure 5. 3D (left) and 2D (right) schematic representation of interactions of stereoisomer SD_2 with KSP enzyme active site, PDB code: 1X88.

tions was to enhance the interactions between the ligand and the receptor. The overall goal of these modifications is to enhance hydrophobic interactions and hydrogen bonding between the modified compounds and the receptor, KSP. According to the obtained results of docking process, interaction energy of D_2 was lower than other compounds. Accordingly, interaction mode of this compound with enzyme active site is depicted in Figure 5. On the other way, S isomer of this compound make hydrophobic bond with active site through 2-naphtyl ring, phenyl ring and dihydropyrimidinone with amino acid residues Ile136, Ala133, Leu214, Glu116, Gly117, Pro137, and Tyr211. On the other hand, SD_2 makes a hydrogen bond through its dihydropyrimidinone ring with residues Glu116. This isomer establish π-cation bond through its 2-naphtyl ring and phenyl ring with residues Arg221 and Glu116, respectively (Figure 5, right). As it can be seen from the results of interaction for 1X88 PDB code, SD_2 showed that this ligand was well oriented towards the active site.

Based on the different substituents on dihydropyrimidinone structure, structure activity relationship of these compounds can be discussed. Amongst D_{1-8}, it can be concluded that SD_2, SD_5, SD_7 and SD_8 are prominent predicted structure due to the lower binding energy. The results revealed that the inhibitory activity for Eg5 could be increased with the phenyl substituent at position 5 especially about D_2 with the naphthyl group at position 4. Binding of these compounds with active site of receptor accomplish by hydrophobic interaction in which the effect has been perceived in studied substituents.

4. Conclusion

In conclusion, a series of 3,4-Dihydropyrimidine-2-one stereoisomer derivatives as a new anti-cancer agents were designed and synthesized in this study. Because of different biological activity and side effects of dihydropyrimidine stereoisomers especially resistance to chemotherapeutic drugs, enantiomers of 3,4-dihydropyrimidine-2-ones (R and S) were investigated by docking studies. Molecular docking were carried out to find the optimal configuration of each inhibitor in the KSP binding site. The molecular modeling studies supported that stereoisomer SD2 as the most prominent inhibitor could be selected to elucidate the interaction binding modes and further detailed analysis. Moreover, the results derived from molecular docking exhibited detailed information of residues in the binding pocket and confirmed that the hydrogen bond and hydrophobic interaction play a significant role in the binding of inhibitors to KSP. On the basis of mentioned interactions, it could be concluded that the interaction of these synthesized inhibitors with amino acid residues of enzyme active site is similar to monastrol as a control compound. Considering the similarity of interaction mode with residues it could be understand that these compounds possibly bind with enzyme active site.

Conflict of Interest

The authors declare no conflict of interest.

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