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SYNTHESIS AND BIOLOGICAL EVALUATION OF TRIAZOLYL MONASTROL ANALOGUES USING Cu-CATALYZED CLICK CHEMISTRY

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Abstract – The synthesis of triazolyl monastrol analogues through a Cu(I)-catalyzed [3+2] azide-alkyne cycloaddition (CuAAC) reactions and their biological evaluation against cancer cell lines is described.

INTRODUCTION

Monastrol **1** (mixture of (±)-enantiomers) is a cell-permeable molecule whose discovery during a campaign of screening large chemical library of compounds attracted interest from the medicinal chemistry community due to its potential to be a promising anticancer agent (Figure 1).[1] It has been recognized that **1** acts by specifically blocking mitotic kinesin Eg5, a motor protein that is important for bipolar spindle formation during cell division (mitosis).[2] Monastrol **1** operates by a different mechanism of action which does not involve tubulin targeting compared with current drugs such as the taxanes (e.g. paclitaxel, Taxol®) and therefore is considered a lead compound for the development of new antimitotic drugs. Following its discovery, a number of research groups embarked on the synthesis of monastrol derivatives for the discovery of more potent analogues.[3] Monastrol possesses a dihydropyrimidinethione core which can be assembled by the Biginelli reaction.[4] Since its disclosure by Sharpless and co-workers,[5] the click chemistry concept has received a considerable use in various arenas of chemical science,[6] particularly in drug design for the discovery of inhibitors and chemical probes.[7] The 1,2,3-triazole motif has received extensive use in medicinal chemistry campaigns because it can enhance biological activity,[8] lead to the discovery of highly potent enzyme inhibitors,[9] capable to resist metabolic degradation,[10] and

can participate in favorable H-bonding with bimolecular targets.[11] Inspired by monastrol's bioactivity and the presence of the triazole motif in biologically active molecules, herein we describe the chemical synthesis and biological evaluation of a series of novel monastrol analogues bearing the triazole heterocyclic ring in a four-step synthetic strategy involving click chemistry to assess their anticancer properties *in vitro*. We envisioned the use of click chemistry approach to "click" a monastrol derivative bearing an azide group (i.e. compound **7**) with a variety of alkynes *via* the Cu-catalyzed[12] regioselective maneuver for the generation of 1,4-disubstituted 1,2,3-triazolyl monastrol analogues **17-25** exclusively (Scheme 1). To the best of our knowledge, no report has described the synthesis of monastrol analogues through the functionalization of monastrol's aromatic ring using click chemistry.

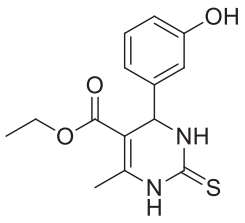

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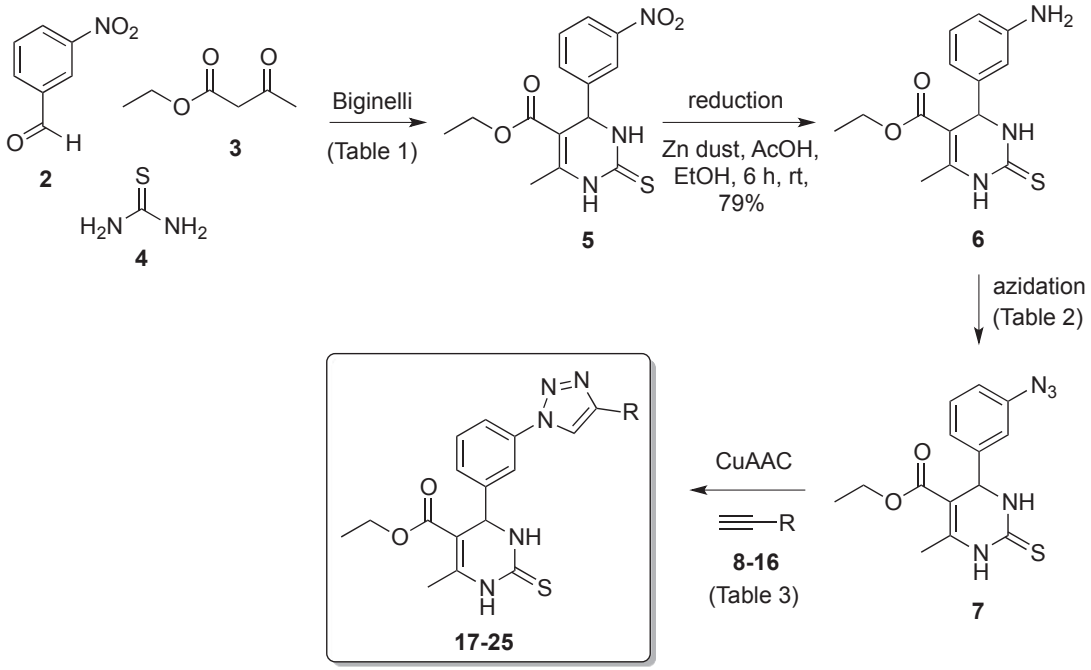
Figure 1. Structure of monastrol **1**

RESULTS AND DISCUSSION

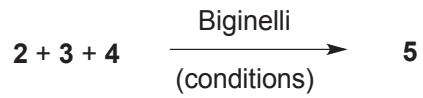
The synthetic strategy to obtain the target triazolyl monastrol analogues **17-25** is depicted in Scheme 1. We commenced the synthesis by performing a Biginelli multi-component reaction (BMCR) which can assemble the 3,4-dihydropyrimidine-2(1*H*)-thione framework of nitro-monastrol **5** in an expeditious way.[13] Motivated by conducting the reaction under solvent- and catalyst-free conditions,[14] we first performed the synthesis of nitro-monastrol **5** in a one-pot BMCR between 3-nitrobenzaldehyde **2**, ethyl acetoacetae **3**, and thiourea **4** at 100 °C which provided **5** in a low yield (19%) (Table 1, entry 1). We then performed the synthesis of **5** using the original Biginelli conditions through the use of catalytic amount of conc. HCl which furnished the desired compound **5** in an improved 39% yield (Table 1, entry 2). Since the Biginelli reaction is an acid-promoted cyclocondensation reaction, the importance of acid catalysis and the observed improvement in yield is evident. In our quest for an improved yield of **5**, we further tested various acid catalysts to enhance its synthesis. The synthesis of **5** using the relatively cheap acids *p*-TsOH[15] or citric acid[16] was examined which furnished **5** in 48% and 43% yields, respectively (Table 1, entries 3 and 4). In each reaction case, using *p*-TsOH or citric acid as catalyst, a number of byproducts were observed.



Scheme 1. Synthetic plan towards monastrol analogues employing click chemistry

Committed to improve the yield of **5** and suppress byproducts formation, we were then prompted to use a Lewis acid catalyst for the Biginelli reaction. It is well documented in the literature that Lewis acid catalysts provide the Biginelli products more efficiently.[17] Indeed, when we conducted the reaction using $AlCl_3$ (10 mol%), compound **5** was delivered in an excellent 92% yield and no side-products were observed (Table 1, entry 5). With compound **5** in hand, we converted its nitro group to an amine group (to give **6**) using zinc dust and acetic acid in EtOH to smoothly afford **6** in 79% yield. The best result was obtained when five equivalent of zinc dust was used to fully transform the nitro group of **5** to its amine equivalent **6** in 6 h at rt.

Table 1. Synthesis of nitro-monastrol **5** under various conditions

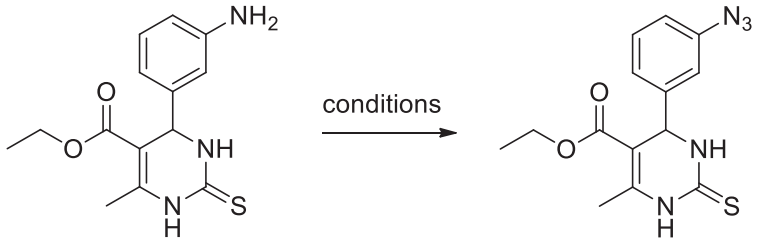


Entry	Acid catalyst	Yield (%)[a]
1	no catalyst (neat)	19[b]
2	conc. HCl (few drops)	39[c]
3	*p*-TsOH (10 mol%)	48[c]
4	citric acid (10 mol%)	43[c]
5	$AlCl_3$ (10 mol%)	92[d]

[a]All reactions were carried out using 3-nitrobenzaldehyde **2** (1 equiv), ethyl acetoacetate (1 equiv), thiourea (1.2 equiv), and catalyst (where applicable). [b]100 °C, neat, 24 h. [c]EtOH, reflux, 24 h. [d]EtOH, reflux, 6 h.

With amino-monastrol **6** prepared, we moved forward in our synthetic endeavor and performed an azidation reaction to convert the amino group to an azido group to provide azido-monastrol **7** for click reactions. Initially, we treated compound **6** with $NaNO_2$ and NaN_3 in aqueous acidic conditions (Table 2, entry 1); however, no product was formed under these conditions, possibly because of the observed poor solubility of the reaction mixture. We then decided to investigate a reaction condition that could potentially increase the solubility of the reaction mixture to give azide **7** by evaluating some solubilizing solvents. We carried out the reaction in EtOH and as a result azido product **7** was delivered in 53% (Table 2, entry 2). With optimization of the reaction in mind, we carried out the azidation reaction to afford azide **7** using THF and obtained the azido-monastrol **7** in a slightly improved yield of 58% (Table 2, entry 3). Pleasingly, switching the solvent choice further from THF to acetone rendered the reaction to proceed smoothly and gave the desired key intermediate azido-monastrol **7** in quantitative yield (98%), requiring only a simple aqueous workup and no purification (Table 2, entry 4).

Table 2. Optimization of the azidation reaction by screening different solvents



6 **7**

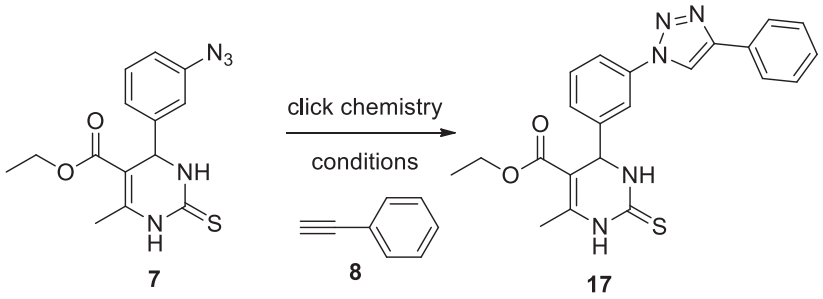
Entry	Solvent	Yield (%)[a]
1	no organic solvent; aqueous HCl only	0
2	EtOH	53
3	THF	58
4	acetone	98

[a] The reactions were carried out using amino-monastrol **6** (1 equiv), $NaNO_2$ (2 equiv), NaN_3 (2 equiv), aqueous HCl, and solvent (where applicable) at -5 °C for 1 h.

Having secured the synthesis of key azido-monastrol **7**, we were in a stage to perform the Huisgen 1,3-dipolar cycloaddition "click" reactions between **7** and the alkyne substrates **8-16** to manufacture our desired monastrol analogues **17-25**. We began by first screening conditions for the click reaction. We chose the combination of azido-monastrol **7** and phenylacetylene **8** as the model reaction to establish reaction conditions that could be applied to our click chemistry small molecule library synthesis. From the outset, we carried out the click reaction of azido-monastrol **7** with phenylacetylene **8** using

$CuSO_4 \cdot 5H_2O$ and sodium ascorbate (NaAsc) in *tert*-BuOH:H_2O mixture;[18] however, no product was formed after 24 h at rt and a further 24 h at 50 °C (Table 3, entry 1). A reaction performed in a three-solvent reaction medium (*tert*-BuOH:H_2O:THF; 1:1:1) did not lead to a fruitful outcome (Table 3, entry 2). We then opted to facilitate the click reaction by incorporating EtOH or THF as co-solvent with H_2O in independent reactions; however, only trace amount of product was formed under these conditions (Table 3, entries 3 and 4). We then shifted our attention to find a suitable method that could provide the triazole product **17** without the use of H_2O as the medium in the click reaction system. Pleasingly, we found the use of CuI in dry THF very efficient in generating triazolyl monastrol **17** in 81% yield (Table 3, entry 5).[19] For further optimization of the click reaction, we performed a reaction to afford **17** by adding NaAsc and base (Et_3N) to the reaction mixture for the purpose to convert any possible oxidized Cu(II) back to the catalytically active Cu(I) as well as accelerate the generation of the active Cu-acetylide[20] intermediate, respectively. The motive was to establish a click reaction catalytic system that can proficiently provide the desired triazole product **17** in a further improved yield. We were pleased to find an improved yield of triazolyl monastrol **17** to 92% (from 81%) which was influenced by these further reagent additions (Table 3, entry 6; Table 4, entry 1).

Table 3. Screening of click chemistry conditions for the synthesis of monastrol analogue **17**



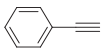
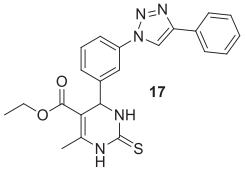
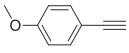
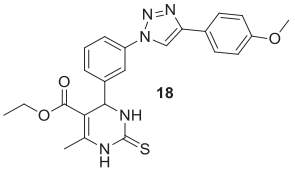
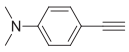
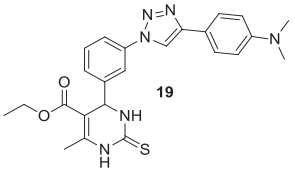
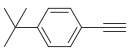
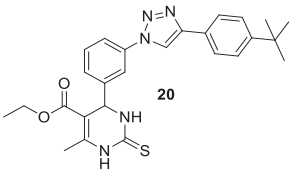
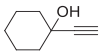
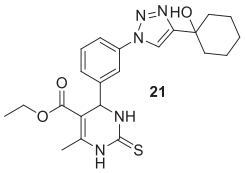
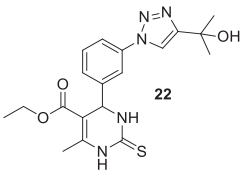
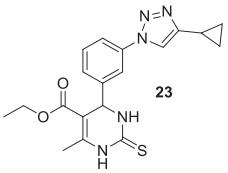
7 **8** **17**

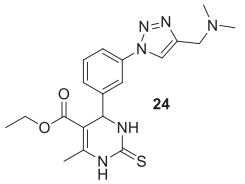
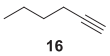
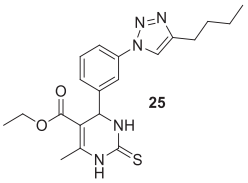
Entry	Cu-catalyzed click chemistry system	Solvent(s)	Reaction outcome[a]
1	$CuSO_4 \cdot 5H_2O$/NaAsc	*t*BuOH/H_2O (1:1)	no product[b]
2	$CuSO_4 \cdot 5H_2O$/NaAsc	*t*BuOH/H_2O/THF (1:1:1)	no product[b]
3	$CuSO_4 \cdot 5H_2O$/NaAsc	THF/H_2O (1:1)	trace[b]
4	$CuSO_4 \cdot 5H_2O$/NaAsc	EtOH/H_2O (1:1)	trace[b]
5	CuI	THF	81%[c]
6	CuI/NaAsc/Et_3N	THF	92%[d]

[a]The reactions were carried out using azide **7** (1 equiv), alkyne **8** (1.5 equiv), Cu catalyst with/without additive, and solvent (s) (see Table). [b]$CuSO_4 \cdot 5H_2O$ (10 mol%) and NaAsc (20 mol%) at rt for 24 h and then at 50 °C for further 24 h. [c]CuI (10 mol%) at 50 °C for 16 h. [d]CuI (10 mol%), NaAsc (20 mol%), and Et_3N (2 equiv) at 50 °C for 16 h.

Having established our click reaction conditions through experimentation with azido-monastrol **7** and phenylacetylene **8**, we then applied our established reaction conditions to *click* azido-monastrol **7** with alkynes **9-16** using the CuI/NaAsc/Et_3N/THF system which accomplished the synthesis of 1,4-disubstituted 1,2,3-triazolyl monastrol analogues **18-25** in excellent yields (Table 4, entries 2-9).

Table 4. Structures of alkynes **8-16** and triazolyl monastrol analogues **17-25**

Entry	Alkyne	Product[a]	Yield (%)
1	 **8**	 **17**	92
2	 **9**	 **18**	94
3	 **10**	 **19**	96
4	 **11**	 **20**	95
5	 **12**	 **21**	86
6	 **13**	 **22**	95
7	 **14**	 **23**	83[b]

8	 15	 24	88
9	 16	 25	97

[a]All reactions were carried out using azido-monastrol **7** (1 equiv), terminal alkyne (1.5 equiv), CuI (10 mol%), NaAsc (20 mol%), Et_3N (2 equiv) in anhydrous THF at 50 °C for 16 h. [b]Reaction temperature = 35 °C, additional portion of alkyne (1.5 equiv) was required, 22 h.

The synthesized triazolyl monastrol analogues **17**-**25** were evaluated for their anticancer activities in three human cancer cell lines: MCF-7 (breast), OVCAR-3 (ovarian) and SKOV-3 (ovarian) (Table 5).

Table 5. Inhibitory activity of triazolyl monastrol analogues **17**-**25** against cancer cell lines

Compound	IC_{50} values (μM)[a]		
	MCF-7[b]	OVCAR-3[c]	SKOV-3[c]
17	22.0	24.2	29.1
18	29.3	45.1	56.3
19	60.6	>100	41.1
20	>100	69.0	23.2
21	>100	51.1	28.5
22	90.7	>100	91.8
23	>100	73.8	>100
24	61.7	29.8	54.4
25	83.9	25.9	40.7
Monastrol	58.5	26.4	19.4

[a]IC_{50} = concentration that elicits 50% inhibition and the values are the average of two independent experiments. [b] Breast cancer. [c] Ovarian cancer.

In general, most of the monastrol analogues were effective in the micromolar range against at least two cell lines. In the breast cell line (MCF-7), monastrol analogues **17** (22 μM) and **18** (29.3 μM) possessed higher potency than monastrol by 62.39% and 49.91%, respectively. Analogues **19** (60.6 μM) and **24** (61.7 μM) were less potent than monastrol by 3.59% and 5.47%, respectively, whereas the other analogues (**20**-**23** and **25**) displayed very weak to no activity. In the ovarian cancer cell line (OVCAR-3),

analogues **17** (24.2 μM) and **25** (25.9 μM) were more potent than monastrol (26.4 μM) by 8.33% and 1.89%, respectively. Analogue **24** (29.8 μM) was less potent than monastrol by 12.88% and the other analogues were least effective. In the SKOV-3 cell line, all monastrol analogues showed less activity compared to monastrol. Only analogue **20** (23.2 μM) was closely comparable to monastrol (19.4 μM), although it was less potent by 19.59%. The efficacy of the monastrol analogues against breast and ovarian cancer cell lines when compared against monastrol indicates the nature of the resistance of the different cell lines against these compounds. Interestingly, some of the analogues were identified to be more potent than monastrol.

CONCLUSIONS

In summary, we have reported a Cu(I)-catalyzed click chemistry method towards the synthesis of triazolyl monastrol analogues and their biological screening. The triazolyl monastrol analogues **17**, **18**, and **24** showed interesting anticancer activities when compared with monastrol. In particular, monastrol analogue **17** was the most promising compound identified in this work. The synthesis of other analogues and their biological evaluation against various cancer cell lines warrant further investigation.

EXPERIMENTAL

General Chemistry Experimental

Chemical reactions were carried out under a positive pressure of nitrogen atmosphere in oven-dried glassware, unless otherwise noted. All reagents, solvents, thin-layer chromatography (TLC) plates, and silica gel were purchased from Sigma Aldrich. Reactions were magnetically stirred and monitored by TLC with fluorescent indicator (254 nm wavelength) using UV light as visualizing agent and aqueous solution of potassium permanganate as developing agent. Silica gel (60Å, 200-425 mesh) was used for flash column chromatography. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker instrument using DMSO-d_6 as solvent. Chemical shifts are reported in parts per million and coupling constants are recorded in Hertz (Hz). Multiplicities are expressed as follows: singlet (s), doublet (d), triplet (t), multiplet (m), and broad (br).

Experimental Procedures for Chemical Synthesis

Synthesis of ethyl 4-(3-nitrophenyl)-6-methyl-2-sulfanylidene-1,2,3,4-tetrahydropyrimidine-5-carboxylate (5):

A mixture of 3-nitrobenzaldehyde **2** (3.00 g, 0.01985 mol), ethyl acetoacetate (2.51 mL, 0.01985 mol), thiourea (1.81 g, 0.02382 mol), anhydrous $AlCl_3$ (265 mg, 0.001985 mol), and dry EtOH (20 mL) was stirred under refluxing conditions for 6 h. The reaction mixture was then allowed to cool to rt and the

solvent was evaporated under reduced pressure. Ice (30 g) was then added to the residue and the resulting mixture was stirred vigorously for 2 h. The precipitate thus formed was filtered, washed with ice-cold H_2O (~ 5 mL), and triturated with Et_2O to obtain **5** (5.86 g, 92%) as an off-white solid. Analytical data for the title compound were consistent with the literature.[21]

Synthesis of ethyl 4-(3-aminophenyl)-6-methyl-2-sulfanylidene-1,2,3,4-tetrahydropyrimidine-5-carboxylate (6):

To a suspension of nitro-monastrol **5** (5.00 g, 0.01558 mol) and fresh zinc dust (5.09 g, 0.07790 mol) in dry EtOH (100 mL) was added acetic acid (5 mL) dropwise slowly and cautiously (reaction is exothermic). The reaction mixture was then stirred at rt for 6 h. After the completion of the reaction, the reaction mixture was filtered through a pad of Celite® and the filtrate was evaporated under reduced pressure. Water was added to the residue which was then neutralized by the careful addition of solid $NaHCO_3$ to adjust the pH to 7−8. The crude product was then extracted with EtOAc (three times). The combined organic layers were washed with saturated aqueous $NaHCO_3$, brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by recrystallization from EtOH to obtain **6** as a white solid (3.58 g, 79% yield). Analytical data for the title compound were consistent with the literature.[22]

Synthesis of ethyl 4-(3-azidophenyl)-6-methyl-2-sulfanylidene-1,2,3,4-tetrahydropyrimidine-5-carboxylate (7):

Caution!: organic azides are potentially explosive, particularly low molecular weight azides. After taking the necessary precautions, we did not encounter any issues with this compound at 2 g reaction scale. To a solution of amino-monastrol **6** (2.00 g, 0.006873 mol) in $(Me)_2CO$ (30 mL) was added conc. HCl (6 M, 5 mL) at 0 °C. The reaction mixture was cooled further to -5 °C using an ice/salt bath and maintained at that temperature throughout the entire synthesis. A chilled solution of $NaNO_2$ (949 mg, 0.01375 mol) dissolved in H_2O (2 mL) was added dropwise over 10−15 min and the reaction mixture was then stirred for a further 30 min. Next, an ice-cold solution of NaN_3 (894 mg, 0.01375 mol) dissolved in H_2O (2 mL) was added dropwise over a period of 15−20 min. The reaction mixture was then stirred for 1 h in the dark. After the consumption of the starting material (TLC), H_2O was added and the crude product was extracted with EtOAc (three times). The combined organic phases were washed with saturated aqueous $NaHCO_3$, brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure in batches (water bath not exceeding 40 °C) to give **7** as a pale yellow solid (2.14 g, 98% yield) which was used in the next step for click reactions without the need of further purification. Analytical data for the title compound were consistent with the literature.[23]

General Procedure for the Synthesis of Triazolyl Monastrol Analogues 17-25 *via* Click Chemistry:

A mixture of alkyne substrate (1.5 equiv), CuI (10 mol%) and triethylamine (2 equiv) in anhydrous THF (4 mL) was stirred at rt for 15 min after of which the reaction mixture turned yellow. Azido-monastrol **7** (200 mg, 0.6309 mmol, 1 equiv) and sodium ascorbate (20 mol%) were then added. The reaction mixture was purged with nitrogen and stirred vigorously at 50 °C for 16 h in the absence of light. After the reaction was completed, the mixture was cooled to rt, filtered through a pad of Celite®, and the filterate was evaporated under reduced pressure. The crude product was then purified by silica gel column chromatography to afford the triazole products.

Synthesis of ethyl 4-[3-(4-phenyl-1*H*-1,2,3-triazol-1-yl)phenyl]-6-methyl-2-sulfanylidene-1,2,3,4-tetrahydropyrimidine-5-carboxylate (17):

Yellow solid, 92% yield. FT-IR (KBr, cm^{-1}): 3242, 3062, 2958, 1721, 1637; ^1H NMR: δ 10.60 (br s, 1H), 10.06 (br s, 1H), 9.32 (s, 1H), 8.00-7.98 (m, 2H), 7.93-7.89 (m, 2H), 7.68 (t, J = 7.9 Hz, 1H), 7.54 (t, J = 7.8 Hz, 1H), 7.44-7.40 (m, 2H), 5.44 (s, 1H), 4.12-4.03 (m, 2H), 2.39 (s, 3H), 1.14 (t, J = 7.1 Hz, 3H); HRMS (ESI) calcd. for $C_{22}H_{22}N_5O_2S$ $[M+H]^+$ 420.1494, found 420.1492. Anal. Calcd for $C_{22}H_{21}N_5O_2S$: C, 62.99; H, 5.05; N, 16.69; S, 7.64. Found: C, 62.92; H, 5.07; N, 16.61; S, 7.65.

Synthesis of ethyl 4-{3-[4-(4-methoxyphenyl)-1*H*-1,2,3-triazol-1-yl]phenyl}-6-methyl-2-sulfanylidene-1,2,3,4-tetrahydropyrimidine-5-carboxylate (18):

Yellow solid, 94% yield. FT-IR (KBr, cm^{-1}): 3252, 3067, 2955, 1724, 1641; ^1H NMR: δ 10.49 (br s, 1H), 10.00 (br s, 1H), 9.21 (s, 1H), 7.92-7.87 (m, 4H), 7.67 (t, J = 7.9 Hz, 1H), 7.40 (d, J = 7.7 Hz, 1H), 7.10 (d, J = 8.8 Hz, 2H), 5.45 (s, 1H), 4.12-4.02 (m, 2H), 3.85 (s, 3H), 2.40 (s, 3H), 1.14 (t, J = 7.1 Hz, 3H); HRMS (ESI) calcd. for $C_{23}H_{24}N_5O_3S$ $[M+H]^+$ 450.1599, found 450.1594. Anal. Calcd for $C_{23}H_{23}N_5O_3S$: C, 61.45; H, 5.16; N, 15.58; S, 7.13. Found: C, 61.51; H, 5.13; N, 15.51; S, 7.12.

Synthesis of ethyl 4-(3-{4-[4-(dimethylamino)phenyl]-1*H*-1,2,3-triazol-1-yl}phenyl)-6-methyl-2-sulfanylidene-1,2,3,4-tetrahydropyrimidine-5-carboxylate (19):

Yellow solid, 96% yield. FT-IR (KBr, cm^{-1}): 3245, 3061, 2959, 1719, 1639; ^1H NMR: δ 10.49 (br s, 1H), 10.06 (br s, 1H), 9.10 (s, 1H), 7.92-7.88 (m, 2H), 7.80 (d, J = 8.7, 2H), 7.67 (t, J = 7.8 Hz, 1H), 7.40 (d, J = 7.7 Hz, 1H), 6.85 (d, J = 8.7 Hz, 2H), 5.48 (s, 1H), 4.12-4.02 (m , 2H), 2.96 (s, 6H), 2.42 (s, 3H), 1.14 (t, J = 7.1 Hz, 3H); HRMS (ESI) calcd. for $C_{24}H_{27}N_6O_2S$ $[M+H]^+$ 463.1916, found 463.1910. Anal. Calcd for $C_{24}H_{26}N_6O_2S$: C, 62.32; H, 5.67; N, 18.17; S, 6.93. Found: C, 62.39; H, 5.63; N, 18.21; S, 6.91.

Synthesis of ethyl 4-{3-[4-(4-*tert*-butylphenyl)-1*H*-1,2,3-triazol-1-yl]phenyl}-6-methyl-2-sulfanylidene-1,2,3,4-tetrahydropyrimidine-5-carboxylate (20):

Yellow solid, 95% yield. FT-IR (KBr, cm^{-1}): 3251, 3065, 2952, 1727, 1644; ^1H NMR: δ 10.61 (br s, 1H), 10.07 (br s, 1H), 9.28 (s, 1H), 7.93-7.89 (m, 4H), 7.68 (t, *J* = 7.9 Hz, 1H), 7.56 (d, *J* = 8.4 Hz, 2H), 7.40 (d, *J* = 7.8 Hz, 1H), 5.45 (s, 1H), 4.11-4.02 (m, 2H), 2.39 (s, 3H), 1.36 (s, 9H), 1.14 (t, *J* = 7.1 Hz, 3H); HRMS (ESI) calcd. for $C_{26}H_{30}N_5O_2S$ $[M+H]^+$ 476.2120, found 476.2116. Anal. Calcd for $C_{26}H_{29}N_5O_2S$: C, 65.66; H, 6.15; N, 14.73; S, 6.74. Found: C, 65.70; H, 6.11; N, 14.79; S, 6.73.

Synthesis of ethyl 4-{3-[4-(1-hydroxycyclohexyl)-1*H*-1,2,3-triazol-1-yl]phenyl}-6-methyl-2-sulfanylidene-1,2,3,4-tetrahydropyrimidine-5-carboxylate (21):

Yellow solid, 86% yield. 3339, 3255, 3059, 2949, 1723, 1649; ^1H NMR: δ 10.52 (br s, 1H), 10.07 (br s, 1H), 8.59 (s, 1H), 7.87-7.84 (m, 2H), 7.63 (d, *J* = 7.9 Hz, 1H), 7.35-7.30 (m, 1H), 5.49 (s, 1H), 4.10-4.02 (m, 2H), 2.38 (s, 3H), 2.02-1.30 (m, 10H), 1.14 (t, *J* = 7.2 Hz, 3H) [OH proton not observed]; HRMS (ESI) calcd. for $C_{22}H_{28}N_5O_3S$ $[M+H]^+$ 442.1913, found 442.1909. Anal. Calcd for $C_{22}H_{27}N_5O_3S$: C, 59.84; H, 6.16; N, 15.86; S, 7.26. Found: C, 59.86; H, 6.13; N, 15.89; S, 7.25.

Synthesis of ethyl 4-{3-[4-(2-hydroxypropan-2-yl)-1*H*-1,2,3-triazol-1-yl]phenyl}-6-methyl-2-sulfanylidene-1,2,3,4-tetrahydropyrimidine-5-carboxylate (22):

Yellow solid, 95% yield. FT-IR (KBr, cm^{-1}): 3353, 3265, 3055, 2944, 1725, 1651; ^1H NMR: δ 10.49 (br s, 1H), 10.08 (br s, 1H), 8.60 (s, 1H), 7.87-7.85 (m, 2H), 7.62 (t, *J* = 7.7 Hz, 1H) 7.38 (d, *J* = 7.7 Hz, 1H), 5.48 (s, 1H), 4.10-4.02 (m, 2H), 2.41 (s, 3H), 1.57 (s, 6H), 1.13 (t, *J* = 7.1 Hz, 3H) [OH proton not observed]; HRMS (ESI) calcd. for $C_{19}H_{24}N_5O_3S$ $[M+H]^+$ 402.1599, found 402.1593. Anal. Calcd for $C_{19}H_{23}N_5O_3S$: C, 56.84; H, 5.77; N, 17.44; S, 7.99. Found: C, 56.80; H, 5.74; N, 17.39; S, 7.97.

Synthesis of ethyl 4-[3-(4-cyclopropyl-1*H*-1,2,3-triazol-1-yl)phenyl]-6-methyl-2-sulfanylidene-1,2,3,4-tetrahydropyrimidine-5-carboxylate (23):

This reaction was stirred at 35 °C instead of 50 °C due to low boiling point of the alkyne. After 16 h, an extra portion of cyclopropylacetylene (1.5 equiv) was added and the reaction mixture was stirred at the same temperature for a further 6 h. Yellow solid, 83% yield. FT-IR (KBr, cm^{-1}): 3239, 3048, 2948, 1720, 1641; ^1H NMR: δ 10.47 (br s, 1H), 10.07 (br s, 1H), 8.59 (s, 1H), 7.82 (m, 2H), 7.63 (m, 1H), 7.38 (d, *J* = 7.8 Hz, 1H), 5.46 (s, 1H), 4.06 (m, 2H), 2.41 (s, 3H), 2.08 (m, 1H), 1.13 (t, *J* = 7.2 Hz, 3H), 0.99 (m, 2H), 0.84 (m, 2H); HRMS (ESI) calcd. for $C_{19}H_{22}N_5O_2S$ $[M+H]^+$ 384.1494, found 384.1492. Anal. Calcd for $C_{19}H_{21}N_5O_2S$: C, 59.51; H, 5.52; N, 18.26; S, 8.36. Found: C, 59.54; H, 5.49; N, 18.30; S, 8.33.

Synthesis of ethyl 4-(3-{4-[(dimethylamino)methyl]-1*H*-1,2,3-triazol-1-yl}phenyl)-6-methyl-2-sulfanylidene-1,2,3,4-tetrahydropyrimidine-5-carboxylate (24):

Yellow solid, 88% yield. FT-IR (KBr, cm^{-1}): 3234, 3049, 2941, 1728, 1643; ^1H NMR: δ 10.46 (br s, 1H), 10.07 (br s, 1H), 7.86-7.82 (m, 2H), 7.62 (d, *J* = 7.8, 1H), 7.36 (d, *J* = 7.7 Hz, 1H), 5.44 (s, 1H), 4.12-4.02 (m, 2H), 3.57 (s, 2H), 2.41 (s, 3H), 2.26 (s, 6H), 1.13 (t, *J* = 7.1 Hz, 3H); HRMS (ESI) calcd. for $C_{19}H_{25}N_6O_2S$ $[M+H]^+$ 401.1760, found 401.1758. Anal. Calcd for $C_{19}H_{24}N_6O_2S$: C, 56.98; H, 6.04; N, 20.98; S, 8.01. Found: C, 57.04; H, 6.07; N, 20.91; S, 8.00.

Synthesis of ethyl 4-[3-(4-butyl-1*H*-1,2,3-triazol-1-yl)phenyl]-6-methyl-2-sulfanylidene-1,2,3,4-tetrahydropyrimidine-5-carboxylate (25):

Yellow solid, 97% yield. FT-IR (KBr, cm^{-1}): 3247, 3044, 2954, 1722, 1645; ^1H NMR: δ 10.47 (br s, 1H), 10.04 (br s, 1H), 8.60 (s, 1H), 7.84-7.81 (m, 2H), 7.62 (t, *J* = 7.8, 1H), 7.37 (d, *J* = 7.7 Hz, 1H), 5.45 (s, 1H), 4.10-4.01 (m, 2H), 2.75 (t, *J* = 7.4 Hz, 2H), 2.41 (s, 3H), 1.71-1.66 (m, 2H), 1.43-1.37 (m, 2H), 1.12 (t, *J* = 7.0 Hz, 3H), 0.95 (t, *J* = 7.3 Hz, 3H); HRMS (ESI) calcd. for $C_{20}H_{26}N_5O_2S$ $[M+H]^+$ 400.1807, found 400.1802. Anal. Calcd for $C_{20}H_{25}N_5O_2S$: C, 60.13; H, 6.31; N, 17.53; S, 8.03. Found: C, 60.20; H, 6.26; N, 17.49; S, 7.99.

General Biology Experimental

The human breast adenocarcinoma cell line (MCF-7) and the ovarian adenocarcinoma cell lines (OVCAR-3, SKOV-3) were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). Dulbecco's minimal essential medium containing low glucose (DMEM-LG), Roswell Park Memorial Institute (RPMI) 1640 Medium, fetal bovine serum (FBS), antibiotic solution (Penicillin/Streptomycin) and Glutamax were all purchased from Invitrogen (Invitrogen, Carlsbad, CA). Bovine insulin, DMSO, and MTT reagent [3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide] were purchased from Sigma (St Louis, MO, USA).

The MCF-7 cells and SKOV-3 cells were cultured in DMEM-LG medium. The OVCAR-3 cells were cultured in RPMI-1640 Medium. Both media were supplemented with 10% FBS, 2 mM Glutamax and antibiotic solution of penicillin (50 IU) and streptomycin (50 μg/mL). Additionally, 0.01 mg/mL bovine insulin was added to the media used for MCF-7. All cancer cell lines were cultured in a humidified CO_2 incubator under standard culture conditions of 37 ºC and 5% CO_2 in air atmosphere.

Experimental Procedure for *in vitro* Biological Evaluation

Cell Inhibition Assay

The effect of the synthesized compounds (monastrol analogues **17-25**) on inhibition of human cancer cell lines (MCF-7, SKOV-3, OVCAR-3) was analyzed using the MTT reagent. The compounds to be screened were dissolved using culture grade DMSO to make a stock solution of 20 mM. Serial dilutions of individual compound were prepared in respective cell culture media. Briefly, the human cancer cell lines, MCF-7 (3×103 cells/well), SKOV-3 (3×104 cells/well) and OVCAR-3 (5×103 cells/well) were seeded in 96-well plates and allowed to attach overnight. The cells were then treated with different concentrations of each compound (1, 3, 10, 30, 100, 300 nm, 1, 3, 10, 30, 100, 300 μM) and incubated at 37 °C in a humidified atmosphere with 5% CO_2 for 24 h, 48 h and 72 h. Following the treatment period, MTT reagent (5 mg/mL) in 100 μL of fresh culture medium was added to each well and incubated for 4 h. The formazan crystals formed with MTT treatment were solubilized with DMSO and the optical density was measured at 570 nm with a reference wavelength of 630 nm using a microplate ELISA reader (SpectraMax® i3x, Molecular Devices, Sunnyvale, CA). Percentage of inhibition was calculated as control-test/control*100 and IC_{50} for each compound was generated.

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