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Synthesis and biological evaluation of conformationally flexible as well as restricted dimers of monastrol and related dihydropyrimidones

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ABSTRACT

A series of conformationally flexible and restricted dimers of monastrol as well as related dihydropyrimidones have been synthesized by employing one-pot Biginelli multicomponent reaction. These dimers have been evaluated for cytotoxic potency against selected human cancer cell lines and some of the compounds have exhibited more cytotoxic potency than the parent monastrol. Further, the DNA binding ability by thermal denaturation studies and antimicrobial activities of these compounds are also discussed.

1. Introduction

With the discovery of multicomponent reaction, the dihydropyrimidin-(1*H*)-ones (DHPM's) were reported for the first time by Biginelli over a century ago [1]. The multifunctionalized dihydropyrimidinones scaffold represents a class of heterocylic compounds with significant pharmacological efficiency and are receiving considerable amount of interest. They exhibit a diverse pharmacological profile like calcium channel blockade, α_{1a}-adrenoreceptor antagonism, antibacterial, antifungal and other related properties [2,3]. From natural marine sources, several alkaloids containing the dihydropyrimidine core unit have been isolated such as batzelladine alkaloids, which are found to be potent HIV gp-120-CD4 inhibitors [4]. With the advent of combinatorial synthesis, which is particularly useful for multicomponent reactions like Biginelli condensation, diverse DHMPs libraries have been synthesized and subjected to high throughtput screening for biological activity [5].

By the end of last century a structurally simple DHPM, monastrol (**1**) has been identified on screening a large library of diverse small molecules, as a novel cell permeable molecule that causes mitotic arrest by blocking bipolar mitotic spindle in mammalian cells (Fig. 1) [6]. Monastrol is the first Eg5 inhibitor to be identified with an IC_{50} value of 14 μM causing a specific and reversible cell cycle block. It is considered as a new lead in anticancer drug development as it specifically inhibits mitotic kinesin Eg5 motor protein. The antimitotic activity of monastrol itself is not very high and this does not warrant it as a drug candidate [7]. Therefore the development of more potent, specific, cell permeable monastrol analogues with enhanced kinesin inhibitory and anti-proliferative properties has been carried out in recent years with encouraging results [8] and necessitates more studies in this potential cancer drug development area.

In continuation of our research endeavors on the development of new agents based on heterocyclic scaffolds with enhanced biological effects [9], it is considered of interest to synthesize the dimers of dihydropyrimidone scaffold and evaluate their biological properties. Moreover as the DHPMs exhibits a diverse pharmacological profile, dimers of monastrol and related dihydropyrimidones with flexible alkyl chain spacers of varying length and conformationally restricted spacers with unsaturation (*cis* and

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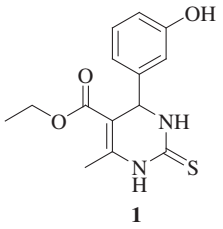

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Fig. 1. Structure of Monastrol.

trans) have been designed and synthesized. The dimers with flexible alkyl chain bridge are intended to assume different interconvertible conformations, whereas the dimers with unsaturated spacers shows reduced conformational flexibility. Further the cytotoxic potency, DNA binding ability and antimicrobial properties of these dimers have been evaluated.

2. Chemistry

The key intermediates (**4a**–**e, 5a**–**e**) required for the synthesis of dimers of monastrol and related dihydropyrimidones have been prepared by the condensation of the corresponding substituted aromatic benzaldehyde with dibromo aliphatic compounds. The condensation of the 3-hydroxy aldehyde **2** with dibromo compounds (1, 3-dibromo propane, 1, 4-dibromobutane, 1, 5-dibromo pentane, *cis*-1, 4-dichlorobutane and *trans*-1, 4-dibromobutane) provides the substituted dibenzaldehydes **4a**–**e** (Scheme 1). The condensation is carried out under reflux condition employing anhydrous potassium carbonate as base in acetonitrile. The use of potassium iodide salt as catalyst has provided the intermediates **4a**–**e** in good yields. The synthesis of the intermediates **5a**–**e** is carried out with vanillin (**3**) under similar conditions in good yields.

The substituted dibenzaldehydes (**4a**–**e** and **5a**–**e)** have been utilized to synthesize the dimers by employing Biginelli reaction. The Biginelli condensation is an important multicomponent reaction (MCR), in which the product is assembled according to a cascade of elementary chemical reactions [10]. The MCR approach is considered as a powerful synthetic tool for preparing target molecules of biological relevance in an efficient manner. The MCRs can provide products with the diversity needed for the discovery of new lead compounds or lead optimization employing combinatorial chemistry techniques [11]. This aspect of multicomponent approach for the synthesis of dimers of monastrol and related dihydropyrimidone employing Biginelli condensation protocol is exploited in the present study. The substituted dibenzaldehydes **4a**–**e** and **5a**–**e** are subjected to Bignelli condensation with thiourea **6** and 1,3-dicarbonyl ester (ethyl acetoacetate **7**) as well as β-ketoamide **8**.

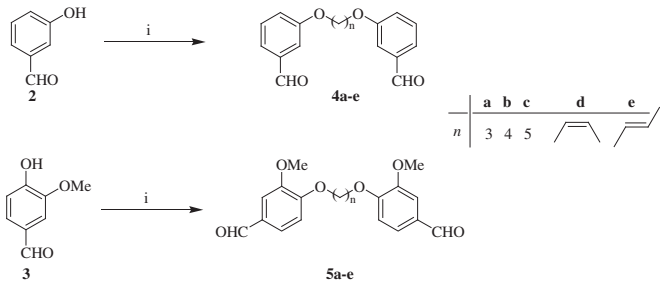
In the present investigation, structural modification on the monastrol backbone has been carried out at the ester moiety (C-5 position) by introducing amide functionality. The naturally occurring amino acid proline has been utilized in the introduction of amide functionality as it is present in many natural and synthetic compounds which exhibits anti-proliferative activity. The required β-ketoamide intermediate has been prepared starting from L-proline by *trans*-acetoacetylation of methylated proline with *tert*-butyl acetoacetate. The symmetric dimers of monastrol **9a**–**e** are synthesized by cyclocondensation of the substituted dibenzaldehyde **4a**–**e**, thiourea **6** and ethyl acetoacetate **7** by employing cerium chloride in acetonitrile under reflux condition in good yields. The use of β-ketoamide **8** in the Biginelli condensation provides the asymmetric dimers **10a**–**e** (Scheme 2).

The symmetric dimers of dihydropyrimidones **11a**–**e** are also synthesized by cerium chloride catalyzed condensation of substituted dibenzaldehydes **5a**–**e**, ethyl acetoacetate **7** and thiourea **6** under refluxing acetonitrile in good yields. Similarly, the asymmetric dimers of monastrol related dihydropyrimidones (**12a**–**e**) are synthesized by the same Biginelli protocol with β-ketoamide **8** in good to acceptable yields (Scheme 3).

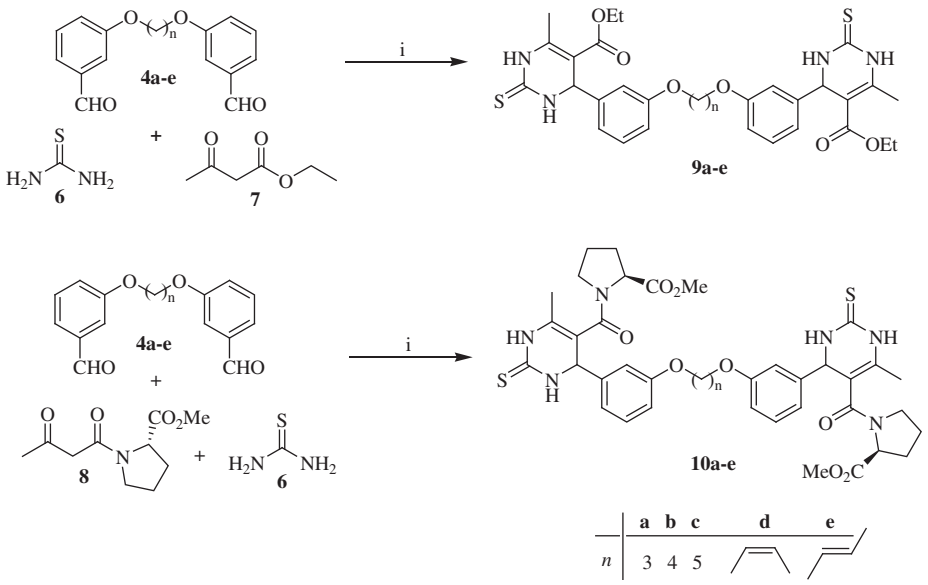
3. Pharmacology

3.1. Cytotoxicity assay

The synthesized dimers (**9**–**12a**–**e**) have been evaluated for their *in vitro* cytotoxicity in selected human cancer cell lines of breast (MCF-7), colon (Colo-205, HT-29), skin (A431), and lung cancer (A549). The *in vitro* cytotoxic activity of the compounds has been tested in triplicates and determined by MTT cell proliferative assay based on mitochondrial reduction of yellow MTT tetrazolium to a highly coloured blue formazan product [12]. The human tumor cell lines of the cancer screening panel are grown in DMEM medium (for A431, Colo-205), RPMI medium (for A549) and MEM medium (for MCF-7) supplemented with 10% fetal bovine serum and 2 mM L-glutamine. The cultures are incubated at 37 °C, 5% CO2, 95% air and 100% relative humidity for 24 h prior to addition of experimental compounds. For a typical screening experiment, 1×10^4 cells counted by Tryptan blue exclusion dye method are incubated with a series of concentration (200, 100, 50, 25 and 10 μg/mL) of the compounds in a 96 well micro titer plates for 48 h at 37 °C in RPMI/DMEM/MEM with 10% FBS medium. The viability of the cells is measured by adding 10 μl of MTT reagent (5 mg/mL in PBS) to each well containing 90 μl of fresh serum free media. The plates are incubated at 37 °C for 4 h, followed by replacing the media with 200 μl of DMSO and again incubated for 10 min at 37 °C. The absorbance is measured on a spectrophotometer (SpectraMax, Molecular devices) at 570 nm wavelength. The mean percent of cell viability relative to that of untreated cells is estimated from data of three individual experiments. Finally, the IC_{50} value of each compound is calculated by curve fitting method.



Scheme 1. Synthesis of substituted dibenzaldehydes (**4a**–**e, 5a**–**e**). *Reagents and conditions*: (i) Dibromo compound, and. K_2CO_3, cat. KI, CH_3CN.



Scheme 2. Synthesis of dimers of monastrol (**9a**–**e**, **10a**–**e**). *Reagents and conditions*: (i) $CeCl_3.7H_2O$, CH_3CN.

3.2. Thermal denaturation studies

The thermal denaturation studies for determining the DNA binding ability of dimers with dihydropyrimidine ring system have been investigated with duplex form of DNA from calf thymus (CT-DNA) following reported procedure [13]. Working solutions in aqueous buffer (10 mM NaH_2PO_4/Na_2HPO_4, 1 mM Na_2EDTA, pH 7.00) containing CT-DNA (100 μM in phosphate) and the dimer compounds (20 μM) are prepared by addition of concentrated compound solutions in DMSO to obtain a fixed [Compd]/[DNA] molar ratio of 1:5. The resulting DNA-ligand solutions are incubated for 0 and 18 h at 37 °C, followed by monitoring at 260 nm using a Beckman–Coulter DU 800 spectrophotometer fitted with high performance temperature controller by heating applied at 1 °C min^{-1} in the 40–90 °C range.

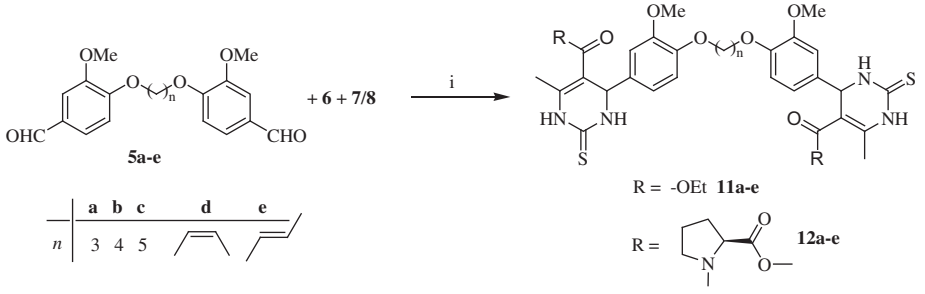
3.3. Antimicrobial assay

The antibacterial screening of the synthesized dimers have been determined by the well diffusion method. First, three to five identical colonies from cultured agar plates are inoculated into a 5 mL of nutrient medium in culture tube. The culture tubes are incubated and the turbidity of each bacterial suspension is adjusted to reach an optical comparison to that of a 0.5 McFarland standard; resulting in a suspension containing approximately 1×10^8 CFU/mL. Then, Mueller-Hinton agar plates are inoculated by streaking with a sterilized swab over the entire sterile agar surface twice to ensure even distribution of the inoculums and the rim of the agar is also swabbed. The plates are allowed to dry at room temperature and on the dry inoculums 6 mm diameter wells are prepared with the help of sterilized cork borer. Finally, different concentration of test compounds (50–100 μg/mL) are prepared by dissolving in dimethyl sulfoxide (DMSO) and introduced into duplicate wells. The plates are incubated at 37 °C for 18 h. Subsequently, the plates are examined for bacterial growth inhibition and the inhibition zone diameter (IZD) is measured to the nearest millimeter. The standard antibiotic, ciprofloxacin is used as positive control and DMSO is used as negative control.

4. Results and discussion

4.1. In vitro cytotoxic activity

The dimers of monastrol (**9a**–**e**, **10a**–**e**) and related dihydropyrimidones (**11a**–**e**, **12a**–**e**) have been evaluated for their *in vitro* cytotoxicity in selected human cancer cell lines of breast (MCF-7), colon (Colo-205, HT-29), skin (A431), and lung cancer (A549) by using MTT cell proliferative assay. Table 1 reveals the cytotoxic activity of symmetric (**9a**–**e**) and asymmetric dimers (**10a**–**e**) of monastrol expressed in half maximal inhibitory concentration (IC_{50}) in microgram per milliliter. Almost all the dimers of monastrol did not show any significant activity against the HT-29 cancer cell line (IC_{50} value >200 μg/mL). Some encouraging activities are shown by the dimers particularly for the cell lines Colo-205 and A431. The most cytotoxic effect is shown on MCF-7 cell line and the monastrol dimer **9d** and **10e** exhibit moderate cytotoxic activity against this cell line with an IC_{50} value around 50 μg/mL, whereas the dimer **10c** exhibited IC_{50} value of



Scheme 3. Synthesis of dimers of monastrol related dihydropyrimidones (**11a**–**e**, **12a**–**e**). *Reagents and conditions*: (i) $CeCl_3.7H_2O$, CH_3CN.

Table 1
IC_{50} values[a] (in μg/mL) for dimers **9a**–**e** and **10a**–**e** in selected human cancer cell lines.

Compound	MCF-7[b]	A431[c]	Colo-205[d]	A549[e]
9a	16	76	144	120
9b	186	>200	>200	>200
9c	178	74	190	>200
9d	49	190	64	154
9e	175	185	>200	>200
10a	175	190	>200	175
10b	128	148	154	>200
10c	34	159	71	184
10d	156	183	180	>200
10e	54	96	127	148
Monastrol	**45**	**53**	**164**	**118**

[a] 50% Inhibitory concentrations and the values are mean of three determinations.
[b] Breast cancer.
[c] Skin cancer.
[d] Colon cancer.
[e] Lung cancer.

34 μg/mL. Amongst the series of dimers of monastrol the compound **9a,** with three carbon alkyl spacing and an ester group at the C5 position of the pyrimidine ring, is found to be the most active displaying an IC_{50} value of 16 μg/mL against MCF-7 cell line. It is interesting to note that some of the compounds in the series are more potent than monastrol, the parent structure and the dimer **9a** is almost 3 times more potent than monastrol against MCF cell line. During the completion of this present investigation, bis(dihydropyrimidinone)benzenes have been reported to possess weak to moderate cytotoxic potency [14].

The cytotoxic activity of symmetric (**11a**–**e**) and asymmetric dimers (**12a**–**e**) of monastrol related dihydropyrimidones is shown in Table 2. Similar to the dimers of monastrol, these dimers have shown negligible activity against the tested cell lines of colon (HT-29). The dimers **11e** and **12a** have shown an IC_{50} value of 90 μg/mL against colo-205 cell line. Amongst the series, the dimers with *trans* unsaturated spacer (**11e** and **12e**) are found to be active as they exhibit an IC_{50} value of 35 μg/mL and 34 μg/mL against MCF-7 and A431 cell lines, respectively. Similar to dimers of monastrol, some compounds in this series have also shown more cytotoxic potency than monastrol. Compound **11a** is found to be 2 times more potent than monastrol against MCF cell line exhibiting an IC_{50} value of 22 μg/mL.

4.2. Thermal denaturation studies

The DNA binding ability of these conformationally flexible and restricted dimers has been investigated by thermal denaturation

Table 2
IC_{50} values[a] (in μg/mL) for dimers **11a**–**e** and **12a**–**e** in selected human cancer cell lines.

Compound	MCF-7[b]	A431[c]	Colo-205[d]	A549[e]
11a	22	66	49	116
11b	178	185	>200	>200
11c	180	124	113	>200
11d	84	170	120	>200
11e	35	185	90	165
12a	140	165	92	180
12b	169	190	154	>200
12c	55	125	142	150
12d	175	183	>200	>200
12e	60	34	69	158
Monastrol	**45**	**53**	**164**	**118**

[a] 50% Inhibitory concentrations and the values are mean of three determinations.
[b] Breast cancer.
[c] Skin cancer.
[d] Colon cancer.
[e] Lung cancer.

Table 3
Thermal denaturation data with CT-DNA.

Compound	[Compd.]:[DNA] Molar ratio[a]	ΔT_m (°C)[b] after incubation at 37 °C	
		0 h	18 h
9a	1:5	0.2	0.1
9e	1:5	2.5	2.5
10d	1:5	3.0	3.1
10e	1:5	2.1	2.5
11e	1:5	2.8	3.2
12d	1:5	3.8	4.1
12e	1:5	2.5	2.8
Monastrol	1:5	0.1	0.0

[a] For CT-DNA alone at pH 7.00 ± 0.01, T_m = 69.2°C ± 0.01 (mean value from 10 separation determinations), all ΔT_m values are ± 0.01–0.02.
[b] For a 1:5 M ratio of [Compd.]/[DNA], where CT-DNA concentration = 100 μM and ligand concentration = 20 μM in aqueous sodium phosphate buffer [10 mM sodium phosphate + 1 mM EDTA, pH 7.00 ± 0.01].

studies with CT-DNA and the increase in melting temperature (ΔT_m) for each compound was examined at 0 and 18 h incubation at 37 °C. Among the compounds investigated, some of the dimers elevated the helix melting temperature of CT-DNA and are shown in Table 3. It is interesting to note that the conformationally restricted dimers with *cis* and *trans* double bond have specifically exhibited an increase in helix melting temperature of CT-DNA. The compound **12d** has shown more affinity to DNA with a ΔT_m of 3.8 °C at 0 h and 4.1 °C after 18 h of incubation. Interestingly, the parent compound monastrol showed no increase in DNA melting temperature.

4.3. Antibacterial and antifungal activity

The multi-drug resistance to clinically available antibiotics against bacterial and fungal pathogens is a frequently emerging challenge resulting in efforts to design new chemical entities with improved properties. The dihydropyrimidinones scaffold is well known to possess antimicrobial activity [2], therefore these new conformationally varying dimers of DHPM are also tested for antibacterial activity against a panel of Gram-positive bacteria like *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis* and Gram-negative bacteria such as *Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa.* Whereas the antifungal activity is evaluated against *Candida albicans* (MTCC 227). The inhibitory zones (in mm) have been determined by using agar well method (cup plate method) and the tests are performed in duplicates. Most of the compounds have exhibited poor antibacterial activity and some of the dimers which have exhibited moderate activity are shown in Table 4. Further, the fungus *C. albicans* (MTCC 227) have been found to be resistant and the dimers have shown no zone of inhibitions.

5. Conclusion

In summary, we have synthesized a series of conformationally flexible and restricted dimers of monastrol as well as related dihydropyrimidones by employing Biginelli cyclocondensation reaction. These dimers have been evaluated for different biological properties such as cytotoxic potency, antimicrobial activity and DNA binding ability. The dimers showed moderate to good cytotoxic potency against selected human cancer cell lines and compounds **9a**, **10c**, **11a**, **11e** and **12e** are more potent than monastrol. In addition, few of the compounds have exhibited moderate DNA binding affinity, however no significant antimicrobial activity has been observed.

Table 4
Preliminary antibacterial activity of selected dimers against Gram-positive and negative bacteria at 100 μg/mL concentration (zone of inhibition in mm).

Compound[a]	*S. aureus*	*S. epidermidis*	*B. substilis*	*E. Coli*	*K. pneumonia*	*P. aeroginosa*
9a	10	15	8	20	5	12
9d	15	12	10	8	8	18
9e	8	18	12	10	8	15
10d	15	17	12	12	3	12
11b	9	12	15	10	5	13
11c	19 (10)[b]	10	8	10	10	20 (12)[b]
11d	12	18	13	12	-	15
CIP[c]	25	35	33	35	12	38

[a] Bacteria are resistant to the compound at 50 mg/mL concentration.
[b] Zone of inhibition 50 mg/mL concentration.
[c] Ciprofloxacin used as standard.

6. Experimental protocols

6.1. Material and methods

Reactions were monitored by TLC, performed on silica gel glass plates containing 60 F-254 and visualization on TLC was achieved by UV light or iodine indicator. Column chromatography was performed with Merck (Navi Mumbai, India) 60–120 mesh silica gel. Infrared (IR) spectra are recorded on Perkin–Elmer model 683 or 1310 spectrometers with sodium chloride optics. ^{1}H NMR spectra were recorded on Gemini (200 MHz) (Varian Inc, Palo Alto, CA, USA) or [Avance (300 MHz); Bruker, Fallanden, Switzerland] instruments. Chemical shifts (d) are reported in ppm, downfield from internal TMS standard. ESI spectra were recorded on Micro mass, Quattro LC using ESIþ software with capillary voltage of 3.98 kV and ESI mode positive ion trap detector. Elemental analyses were performed on an elemental analyzer (Model: VARIO EL, Elementar,Hanau, Germany). Melting points were determined with an Electrothermal melting point apparatus and are uncorrected. Starting materials and reagents were commercially available, purchased from SigmaAldrich (St Louis, MO, USA), Lancaster (Alfa Aesar, Johnson Matthey Co, Ward Hill, MA, USA) and Spectrochem Pvt Ltd (Mumbai, India).

6.2. General procedure for the synthesis of substituted dibenzaldehydes (*4a–e and 5a–e*)

To a stirred solution of 3-hydroxy benzaldehyde or vanillin (20 mmol) in 25–30 mL of acetonitrile, anhydrous potassium carbonate (60 mmol), dibromo compound (6.5 mmol) and potassium iodide (2 mmol) were added. The resulting reaction mixture was stirred under reflux at 90 °C for 48 h, followed by filtration of potassium carbonate over celite pad and washed with ethyl acetate. The filtrate was removed under reduced pressure and the residue obtained was re-dissolved in ethyl acetate. The organic layer was washed with 5% NaOH solution, followed by brine solution and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by column chromatography to give substituted dibenzaldehydes **4a–e** and **5a–e** in 65–74% yields.

6.2.1. 3-[3-(3-Formylphenoxy)propoxy]benzaldehyde (*4a*)

Prepared from 3-hydroxy benzaldehyde **2** (2.44 g, 20 mmol), 1,3 dibromo propane (0.66 mL, 6.5 mmol), anhydrous potassium carbonate (8.29 g, 60 mmol) and potassium iodide (0.3 g, 2 mmol) by following the above general procedure to provide 2.05 g of the substituted dibenzaldehyde **4a** as white coloured solid. Yield: 72%; mp 48–50 °C; IR (KBr): 2927, 1696, 1587, 1264 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.16–2.30 (2H, m), 4.06–4.17 (4H, m), 7.09–7.16 (2H, m), 7.31–7.46 (6H, m), 9.95 (2H, s); ESIMS (*m*/*z*): 285 $[M + H]^+$, 307 $[M + Na]^+$; Anal. Calcd for $C_{17}H_{16}O_4$: C, 71.82; H, 5.67. Found: C, 71.80; H, 5.65%.

6.2.2. 3-[4-(3-Formylphenoxy)butoxy]benzaldehyde (*4b*)

Yield: 74%; mp 49–50 °C; IR (KBr): 2926, 1692, 1586, 1263 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.96–2.08 (4H, m), 4.05–4.16 (4H, m), 7.08–7.18 (2H, m), 7.32–7.47 (6H, m), 9.95 (2H, s); ESIMS (*m*/*z*): 299 $[M + H]^+$, 321 $[M + Na]^+$; Anal. Calcd for $C_{18}H_{18}O_4$: C, 72.47; H, 6.08. Found: C, 72.43; H, 6.10%.

6.2.3. 3-[5-(3-Formylphenoxy)pentyl]oxybenzaldehyde (*4c*)

Yield: 75%; mp 55–57 °C; IR (KBr): 2927, 1688, 1581, 1266 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.56–1.78 (2H, m), 1.82–2.00 (4H, m), 4.06 (4H, t, J = 6.6 Hz), 7.10–7.21 (2H, m), 7.33–7.48 (6H, m), 9.96 (2H, s); ESIMS (*m*/*z*): 313 $[M + H]^+$, 335 $[M + Na]^+$; Anal. Calcd for $C_{19}H_{20}O_4$: C, 73.06; H, 6.45. Found: C, 73.01; H, 6.42%.

6.2.4. 3-[(Z)-4-(3-Formylphenoxy)-2-butenyl]oxybenzaldehyde (*4d*)

Yield: 68%; mp 57–59 °C; IR (KBr): 2954, 1703, 1589, 1269 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.69–4.80 (4H, m), 5.91–6.01 (2H, m), 7.12–7.20 (2H, m), 7.33–7.48 (6H, m), 9.95 (2H, s); ESIMS (*m*/*z*): 297 $[M + H]^+$, 319 $[M + Na]^+$; Anal. Calcd for $C_{18}H_{16}O_4$: C, 72.96; H, 5.44. Found: C, 72.95; H, 5.43%.

6.2.5. 3-[(E)-4-(3-Formylphenoxy)-2-butenyl]oxybenzaldehyde (*4e*)

Yield: 65%; mp 89–91 °C; IR (KBr): 2949, 1701, 1586, 1266 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.58–4.69 (4H, m), 6.03–6.13 (2H, m), 7.10–7.21 (2H, m), 7.31–7.49 (6H, m), 9.94 (2H, s); ESIMS (m/z): 297 $[M + H]^+$, 319 $[M + Na]^+$; Anal. Calcd for $C_{18}H_{16}O_4$: C, 72.96; H, 5.44. Found: C, 72.93; H, 5.46%.

6.2.6. 4-[3-(4-Formyl-2-methoxyphenoxy)propoxy]-3-methoxybenzaldehyde (*5a*)

Yield: 70%; mp 140–142 °C; IR (KBr): 2925, 1680, 1587, 1263, 1133 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.41–2.51 (2H, m, J = 6.0 Hz), 3.94 (6H, s), 4.36 (4H, t, J = 6.0 Hz), 7.05 (2H, d, J = 8.3 Hz), 7.41–7.48 (4H, m), 9.87 (2H, s); ESIMS (*m*/*z*): 345 $[M + H]^+$, 367 $[M + Na]^+$; Anal. Calcd for $C_{19}H_{20}O_6$: C, 66.27; H, 5.85. Found: C, 66.26; H, 5.81%.

6.2.7. 4-[4-(4-Formyl-2-methoxyphenoxy)butoxy]-3-methoxybenzaldehyde (*5b*)

Yield: 71%; mp 150–153 °C; IR (KBr): 2929, 1685, 1586, 1261, 1130 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.88–2.02 (4H, m), 3.93 (6H, s), 4.26 (4H, t, J = 6.6 Hz), 7.01 (2H, d, J = 7.3 Hz), 7.40–7.47 (4H, m), 9.86 (2H, s); ESIMS (*m*/*z*): 359 $[M + H]^+$, 381 $[M + Na]^+$; Anal. Calcd for $C_{20}H_{22}O_6$: C, 67.03; H, 6.19. Found: C, 67.05; H, 6.15%.

6.2.8. 4-[5-(4-Formyl-2-methoxyphenoxy)pentyl]oxy-3-methoxybenzaldehyde (*5c*)

Yield: 73%; mp 107–110 °C; IR (KBr): 2930, 1681, 1586, 1265, 1129 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.69–1.79 (2H, m),

1.90–2.08 (4H, m), 3.92 (6H, s), 4.15 (4H, t, $J = 6.6$ Hz), 6.97 (2H, d, $J = 7.3$ Hz), 7.38–7.49 (4H, m), 9.85 (2H, s); ESIMS (*m/z*): 373 $[M + H]^+$, 395 $[M + Na]^+$;Anal. Calcd for $C_{21}H_{24}O_6$: C, 67.73; H, 6.50. Found: C, 67.71; H, 6.48%.

*6.2.9. 4-[(Z)-4-(4-Formyl-2-methoxyphenoxy)-2-butenyl]oxy-3-methoxy benzaldehyde (**5d**)*

Yield: 65%; mp 113–114 °C; IR (KBr): 2942, 1688, 1581, 1265, 1132 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 3.93 (6H, s), 4.88 (4H, d, $J = 2.9$ Hz), 5.98 (2H, t, $J = 2.9$ Hz), 6.98 (2H, d, $J = 8.05$), 7.38–7.45 (4H, m), 9.85 (2H, s); ESIMS (*m/z*): 379 $[M + Na]^+$; Anal. Calcd for $C_{20}H_{20}O_6$: C, 67.41; H, 5.66. Found: C, 67.42; H, 5.64%.

*6.2.10. (E)-4-(4-Formyl-2-methoxyphenoxy)-2-butenyl]oxy-3-methoxy benzaldehyde (**5e**)*

Yield: 63%; mp 160–162 °C; IR (KBr): 2940, 1686, 1582, 1266, 1130 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 3.87 (6H, s), 4.69–4.75 (4H, m), 6.11–6.19 (2H, m), 7.12 (2H, d, $J = 8.0$ Hz), 7.36–7.52 (4H, m), 9.82 (2H, s); ESIMS (*m/z*): 357 $[M + H]^+$, 379 $[M + Na]^+$; Anal. Calcd for $C_{20}H_{20}O_6$: C, 67.41; H, 5.66. Found: C, 67.43; H, 5.62%.

*6.3. General procedure for the synthesis of dimers of monastrol (**9a–e and 10a–e**)*

A solution of substituted dibenzaldehydes **4a–e** (1 mmol), 1,3-dicarbonyl ester or amide (**7** or **8**) (2 mmol), and thiourea **6** (4 mmol) in acetonitrile (10 mL) was heated under reflux (85–90 °C) overnight in the presence of cerium (III) chloride heptahydrate (35 mol %) under nitrogen. The reaction mixture after being cooled to room temperature was poured into crushed ice and stirred for 5–10 min. The solid separated was filtered under suction, washed with ice-cold water (20 mL) and some of the products were purified on column chromatography to afford dimers of monastrol **9a-e** and **10a-e** in 60–70% yields.

*6.3.1. Ethyl 4-[3-(3-3-[5-(ethoxycarbonyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro-4-pyrimidinyl]phenoxypropoxy)phenyl]-6-methyl-2-thioxo-1,2,3,4-tetrahydro-5-pyrimidinecarboxylate (**9a**)*

Prepared from substituted dibenzaldehyde **4a** (0.28 g, 1 mmol), ethyl acetoacetate **7** (0.26 mL, 2 mmol) and thiourea **6** (0.11 g, 2 mmol) by following the above general procedure to provide 0.43 g of the dimer **9a** as white coloured solid. Yield: 70%; mp 250–252 °C; IR (KBr): 3175, 2986, 1709, 1596, 1472 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 1.17 (6H, t, $J = 7.3$ Hz), 2.16–2.30 (2H, m), 2.32 (6H, s), 3.97–4.21 (8H, m), 5.21 (2H, d, $J = 2.9$ Hz), 6.78–6.89 (6H, m), 7.16–7.26 (2H, m), 9.54 (2H, s), 10.17 (2H, s); ^{13}C NMR (100 MHz; DMSO-d_6): 14.21, 15.58, 29.78, 57.42, 60.33, 65.08, 107.96, 112.11, 115.60, 118.52, 128.35, 142.79, 158.73, 166.45, 172.41, 174.29; ESIMS (*m/z*): 625 $[M + H]^+$, 647 $[M + Na]^+$; Anal. Calcd for $C_{31}H_{36}N_4O_6S_2$: C, 59.60; H, 5.81; N, 8.97. Found: C, 59.62; H, 5.80; N, 8.94%.

*6.3.2. Ethyl 4-[3-(4-3-[5-(ethoxycarbonyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro-4-pyrimidinyl]phenoxybutoxy)phenyl]-6-methyl-2-thioxo-1,2,3,4-tetrahydro-5-pyrimidinecarboxylate (**9b**)*

Yield: 72%; mp 257–258 °C; IR (KBr): 3195, 2982, 1714, 1589, 1485 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 1.18 (6H, t, $J = 7.4$ Hz, $J = 6.6$ Hz), 1.90–2.01 (4H, m), 2.33 (6H, s), 3.96–4.13 (8H, m), 5.25 (2H, d, $J = 3.7$ Hz), 6.75–6.89 (6H, m), 7.15–7.25 (2H, m), 9.45 (2H, d, $J = 3.7$ Hz), 10.08 (2H, s); ^{13}C NMR (100 MHz; DMSO-d_6): 14.20, 15.55, 26.08, 57.47, 60.31, 68.25, 107.92, 112.17, 115.58, 118.55, 128.31, 142.75, 158.72, 166.47, 172.44, 174.25; ESIMS (*m/z*): 639 $[M + H]^+$, 661 $[M + Na]^+$; Anal. Calcd for $C_{32}H_{38}N_4O_6S_2$: C, 60.17; H, 6.00; N, 8.77. Found: C, 60.12; H, 5.98; N, 8.74%.

*6.3.3. Ethyl 4-3-[(5-3-[5-(ethoxycarbonyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro-4-pyrimidinyl]phenoxypentyl)oxy]phenyl-6-methyl-2-thioxo-1,2,3,4-tetrahydro-5-pyrimidinecarboxylate (**9c**)*

Yield: 72%; mp 258–260 °C; IR (KBr): 3324, 3189, 2968, 1668, 159, 1492 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 1.18 (6H, t, $J = 6.9$ Hz, $J = 6.6$ Hz), 1.56–1.94 (6H, m), 2.35 (6H, s), 3.97 (4H, t, $J = 6.1$ Hz, $J = 6.9$ Hz), 4.07 (4H, q, $J = 7.6$ Hz, $J = 6.9$ Hz), 5.27 (2H, d, $J = 3.0$ Hz), 6.74–6.91 (6H, m), 7.14–7.25 (2H, m), 9.34 (2H, d, $J = 3.0$ Hz), 9.97 (2H, s); ^{13}C NMR (100 MHz; DMSO-d_6): 14.21, 15.55, 22.29, 26.06, 57.45, 60.29, 68.26, 107.94, 112.16, 115.55, 118.56, 128.34, 142.73, 158.71, 166.45, 172.42, 174.23; ESIMS (*m/z*): 653 $[M + H]^+$, 675 $[M + Na]^+$; Anal. Calcd for $C_{33}H_{40}N_4O_6S_2$: C, 60.72; H, 6.18; N, 8.58. Found: C, 60.70; H, 6.15; N, 8.54%.

*6.3.4. Ethyl 4-3-[((Z)-4-3-[5-(ethoxycarbonyl)-6-methyl-2-thioxo-1,2,3,4-tetra hydro-4-pyrimidinyl]phenoxy-2-butenyl)oxy]phenyl-6-methyl-2-thioxo-1,2,3,4-tetrahydro-5-pyrimidinecarboxylate (**9d**)*

Yield: 64%; mp 256–258 °C; IR (KBr): 3308, 3175, 2984, 1665, 1575, 1482 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 1.16 (6H, t, $J = 7.4$ Hz), 2.32 (6H, s), 4.06 (4H, q, $J = 7.4$ Hz, $J = 6.6$ Hz), 4.64–4.73 (4H,m), 5.22 (2H, d, $J = 3.0$ Hz), 5.86–5.94 (2H, m), 6.80–6.90 (6H, m), 7.15–7.22 (2H, m), 9.53 (2H, d, $J = 3.0$ Hz), 10.18 (2H, s); ^{13}C NMR (100 MHz; DMSO-d_6): 14.11, 15.59, 57.39, 58.02, 60.34, 67.87, 108.01, 112.01, 115.24, 118.49, 128. 42, 129. 90, 142.97, 158.75, 166.58, 172.42, 174.26; ESIMS (*m/z*): 637 $[M + H]^+$, 659 $[M + Na]^+$;Anal. Calcd for $C_{32}H_{36}N_4O_6S_2$: C, 60.36; H, 5.70; N, 8.80. Found: C, 60.34; H, 5.67; N, 8.78%.

*6.3.5. Ethyl 4-3-[((E)-4-3-[5-(ethoxycarbonyl)-6-methyl-2-thioxo-1,2,3,4-tetra hydro-4-pyrimidinyl]phenoxy-2-butenyl)oxy]phenyl-6-methyl-2-thioxo-1,2,3,4-tetrahydro-5-pyrimidinecarboxylate (**9e**)*

Yield: 65%; mp 238–240 °C; IR (KBr): 3193, 2983, 1713, 1587, 1485 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 1.17 (6H, t, $J = 7.4$ Hz, $J = 6.6$ Hz), 2.33 (6H, s), 4.05 (4H, q, $J = 7.4$ Hz, $J = 6.6$ Hz), 4.55–4.61 (4H,m), 5.12 (2H, d, $J = 3.6$ Hz), 6.02–6.10 (2H, m), 6.78–6.89 (6H, m), 7.16–7.24 (2H, m), 9.53 (2H, d, $J = 3.7$ Hz), 10.19 (2H, s); ESIMS (*m/z*): 637 $[M + H]^+$, 659 $[M + Na]^+$; Anal. Calcd for $C_{32}H_{36}N_4O_6S_2$: C, 60.36; H, 5.70; N, 8.80. Found: C, 60.33; H, 5.66; N, 8.81%.

*6.3.6. Methyl (2S)-1-[4-(3-3-[3-(5-[(2S)-2-(methoxycarbonyl) tetrahydro-1H-1-pyrrolyl] carbonyl-6-methyl-2-thioxo-1,2,3,4-tetrahydro-4-pyrimidinyl) phenoxy]propoxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro-5-pyrimidinyl] carbonyltetrahydro-1H-2-pyrrolecarboxylate (**10a**)*

Yield: 69%; mp 186–188 °C; IR (KBr): 3201, 2953, 1743, 1605, 1440 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.85–2.16 (10H, m), 1.96 and 1.97 (6H, s), 3.10–3.19 (4H, m), 3.71 and 3.72 (6H, s), 4.01–4.11 (4H, m), 4.31–4.45 (2H, m), 5.24 and 5.32 (2H, s), 6.70–6.96 (6H, m), 7.15–7.23 (2H, m), 7.53 and 7.62 (2H, s), 8.18 and 8.23 (2H, s); HRMS (ESI) *m/z* calcd for $C_{39}H_{46}N_6O_8NaS_2$ $[M + Na]^+$, 813.2716; found, 813.2697; Anal. Calcd for for $C_{39}H_{46}N_6O_8S_2$: C, 59.22; H, 5.86; N, 10.63. Found: C, 59.20; H, 5.85; N, 10.60%.

*6.3.7. Methyl(2S)-1-[4-(3-4-[3-(5-[(2S)-2-(methoxycarbonyl) tetrahydro-1H-1-pyrrolyl]carbonyl-6-methyl-2-thioxo-1,2,3,4-tetrahydro-4-pyrimidinyl) phenoxy]butoxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro-5-pyrimidinyl] carbonyltetrahydro-1H-2-pyrrolecarboxylate (**10b**)*

Yield: 65%; mp 150–151 °C; IR (KBr): 3195, 2952, 1744, 1605, 1440 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.54–1.86 (4H, m), 1.93–2.10 (8H, m), 1.96 and 1.97 (6H, s), 3.11–3.20 (4H, m), 3.71 and 3.72 (6H, s), 3.97–4.08 (4H, m), 4.32–4.44 (2H, m), 5.23 and 5.31 (2H, s), 6.72–6.96 (6H, m), 7.16–7.24 (2H, m), 7.50 and 7.62 (2H, s), 8.15 and 8.22 (2H, s); ^{13}C NMR (100 MHz; DMSO-d_6): 15.22, 22.71,

26.12, 29.01, 45.92, 51.21, 56.45, 59.52, 68.09, 107.62, 112.29, 115.75, 118.54, 129.48, 143.12, 157.52, 159.03, 171.28, 172.48, 175.03; ESIMS (m/z): 806 $[M + H]^+$, 828 $[M + Na]^+$; Anal. Calcd for $C_{40}H_{48}N_6O_8S_2$: C, 59.68; H, 6.01; N, 10.44. Found: C, 59.65; H, 6.03; N, 10.41%.

*6.3.8. Methyl (2S)-1-(4-[3-(5-[3-(5-[(2S)-2-(methoxycarbonyl) tetrahydro-1H-1-pyrrolyl]carbonyl-6-methyl-2-thioxo-1,2,3,4-tetrahydro-4-pyrimidinyl) phenoxy]pentyloxy)phenyl]-6-methyl-2-thioxo-1,2,3,4-tetrahydro-5-pyrimidinylcarbonyl) tetrahydro-1H-2-pyrrolecarboxylate (**10c**)*

Yield: 70%; mp 168–170 °C; IR (KBr): 3197, 2952, 1743, 1603, 1440 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.68–2.17 (14H, m), 1.95 and 1.97 (6H, s), 3.12–3.20 (4H, m), 3.69 and 3.70 (6H, s), 3.98–4.09 (4H, m), 4.29–4.42 (2H, m), 5.21 and 5.30 (2H, s), 6.71–6.96 (6H, m), 7.14–7.23 (2H, m), 7.51 and 7.61 (2H, s), 8.16 and 8.21 (2H, s); ^{13}C NMR (100 MHz; DMSO-d_6): 15.21, 22.28, 22.70, 26.12, 29.04, 45.91, 51.22, 56.45, 59.54, 68.11, 107.61, 112.30, 115.76, 118.53, 129.48, 143.13, 157.54, 159.01, 171.25, 172.45, 175.05; ESIMS (m/z): 820 $[M + H]^+$, 842 $[M + Na]^+$; Anal. Calcd for $C_{41}H_{50}N_6O_8S_2$: C, 60.13; H, 6.15; N, 10.26. Found: C, 60.11; H, 6.12; N, 10.22%.

*6.3.9. Methyl-(2S)-1-(4-[3-((Z)-4-[3-(5-[(2S)-2-(methoxycarbonyl) tetrahydro-1H-1-pyrrolyl]carbonyl-6-methyl-2-thioxo-1,2,3,4-tetrahydro-4-pyrimidinyl) phenoxy]-2-butenyloxy)phenyl]-6-methyl-2-thioxo-1,2,3,4-tetrahydro-5-pyrimidinylcarbonyl) tetrahydro-1H-2-pyrrolecarboxylate (**10d**)*

Yield: 67%; mp 182–184 °C; IR (KBr): 3196, 2928, 1744, 1604, 1440 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.87 and 1.98 (6H, s), 1.98–2.10 (8H, m), 3.10–3.22 (4H, m), 3.71 and 3.73 (6H, s), 4.32–4.44 (2H, m), 4.56–4.68 (4H, m), 5.25 and 5.33 (2H, s), 6.01–6.12 (2H, m), 6.80–6.97 (6H, m), 7.20–7.26 (2H, m), 7.53 and 7.63 (2H, s), 8.20 and 8.21 (2H, s); HRMS (ESI) m/z calcd for $C_{40}H_{46}N_6O_8NaS_2$ $[M + Na]^+$, 825.2716; found, 825.2693; Anal. Calcd for $C_{40}H_{46}N_6O_8S_2$: C, 59.83; H, 5.77; N, 10.47. Found: C, 59.85; H, 5.75; N, 10.45%.

*6.3.10. Methyl-(2S)-1-(4-[3-((E)-4-[3-(5-[(2S)-2-(methoxycarbonyl)tetrahydro-1H-1-pyrrolyl]carbonyl-6-methyl-2-thioxo-1,2,3,4-tetrahydro-4-pyrimidinyl) phenoxy]-2-butenyloxy)phenyl]-6-methyl-2-thioxo-1,2,3,4-tetrahydro-5-pyrimidinylcarbonyl) tetrahydro-1H-2-pyrrolecarboxylate (**10e**)*

Yield: 60%; mp 155–157 °C; IR (KBr): 3195, 2930, 1746, 1604, 1440 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.88 and 1.96 (6H, s), 1.95–2.18 (8H, m), 3.09–3.21 (4H, m), 3.70 and 3.71 (6H, s), 4.34–4.43 (2H, m), 4.57–4.67 (4H, m), 5.21 and 5.31 (2H, s), 6.01–6.09 (2H, m), 6.79–6.97 (6H, m), 7.19–7.24 (2H, m), 7.51 and 7.66 (2H, s), 8.19 and 8.23 (2H, s); ^{13}C NMR (100 MHz; DMSO-d_6): 15.25, 22.69, 29.04, 45.94, 51.19, 56.44, 59.53, 71.65, 107.66, 112.32, 115.73, 118.52, 129.45, 143.13, 157.55, 159.01, 171.34, 172.50, 175.07; ESIMS (m/z): 804 $[M + H]^+$, 826 $[M + Na]^+$; Anal. Calcd for $C_{40}H_{46}N_6O_8S_2$: C, 59.83; H, 5.77; N, 10.47. Found: C, 59.85; H, 5.72; N, 10.43%.

*6.4. General procedure for the synthesis of dimers of monastrol related dihydropyrimidone (**11a–e and 12a–e**)*

A solution of substituted dibenzaldehydes **5a–e** (1 mmol), 1,3-dicarbonyl ester or amide (**7** or **8**) (2 mmol), and thiourea **6** (4 mmol) in acetonitrile (10 mL) was heated under reflux (85–90 °C) overnight in the presence of cerium (III) chloride heptahydrate (35 mol %) under nitrogen. The reaction mixture after being cooled to room temperature was poured into crushed ice and stirred for 5–10 min. The solid separated was filtered under suction, washed with ice-cold water (20 mL) and some of the products were purified on column chromatography to afford dimers of monastrol **11a–e** and **12a–e** in 58–70% yields.

*6.4.1. Ethyl-4-[4-(3-4-[5-(ethoxycarbonyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro-4-pyrimidinyl]-2-methoxyphenoxypropoxy)-3-methoxyphenyl]-6-methyl-2-thioxo-1,2,3,4-tetrahydro-5-pyrimidinecarboxylate (**11a**)*

Prepared from substituted dibenzaldehyde **5a** (0.34 g, 1 mmol), ethyl acetoacetate **7** (0.26 mL, 2 mmol) and thiourea **6** (0.30 g, 4 mmol) by following the above general procedure to provide 0.46 g of the dimer **11a** as light yellow coloured solid. Yield: 68%; mp 180–182 °C; IR (KBr): 3198, 2954, 1716, 1570, 1464 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.19 (6H, t, J = 7.2 Hz), 2.18–2.29 (2H, m), 2.38 (6H, s), 3.80 (6H, s), 4.01–4.39 (8H, m), 5.28–5.40 (2H, m), 6.50–6.89 (6H, m), 8.08 (2H, s), 8.42 (2H, s); ^{13}C NMR (100 MHz; DMSO-d_6): 14.22, 15.52, 29.74, 56.21, 57.44, 60.37, 65.10, 107.94, 112.14, 115.62, 118.49, 128.34, 142.81, 158.74, 166.44, 172.40, 174.31; ESIMS (m/z): 686 $[M + H]^+$, 708 $[M + Na]^+$; Anal. Calcd for $C_{33}H_{40}N_4O_8S_2$: C, 57.88; H, 5.89; N, 8.18. Found: C, 57.85; H, 5.87; N, 8.15%.

*6.4.2. Ethyl-4-[4-(4-4-[5-(ethoxycarbonyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro-4-pyrimidinyl]-2-methoxyphenoxybutoxy)-3-methoxyphenyl]-6-methyl-2-thioxo-1,2,3,4-tetrahydro-5-pyrimidinecarboxylate (**11b**)*

Yield: 70%; mp 145–147 °C; IR (KBr): 3195, 2941, 1710, 1566, 1465 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.18 (6H, t, J = 7.3 Hz), 1.91–2.13 (4H, m), 2.37 (6H, s), 3.76 (6H, s), 3.98–4.29 (8H, m), 5.29–5.39 (2H, m), 6.56–6.87 (6H, m), 8.01 (2H, s), 8.26 (2H, s); ^{13}C NMR (100 MHz; DMSO-d_6): 14.22, 15.52, 26.12, 56.23, 57.42, 60.35, 68.28, 107.93, 112.15, 115.60, 118.52, 128.32, 142.83, 158.74, 166.42, 172.39, 174.33; ESIMS (m/z): 700 $[M + H]^+$; Anal. Calcd for $C_{34}H_{42}N_4O_8S_2$: C, 58.44; H, 6.06; N, 8.02. Found: C, 58.41; H, 6.04; N, 8.00%.

*6.4.3. Ethyl-4-4-[(5-4-[5-(ethoxycarbonyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro-4-pyrimidinyl]-2-methoxyphenoxypentyl)oxy]-3-methoxyphenyl-6-methyl-2-thioxo-1,2,3,4-tetrahydro-5-pyrimidinecarboxylate (**11c**)*

Yield: 68%; mp 137–139 °C; IR (KBr): 3196, 2952, 1712, 1572, 1463 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 1.11–1.26 (6H, m), 1.63–1.99 (6H, m), 2.37 (6H, s), 3.73 (6H, s). 3.96–4.21 (8H, m), 5.34–5.41 (2H, m), 6.71–6.91 (6H, m), 7.86 (2H, s), 8.13 (2H, s); HRMS (ESI) m/z calcd for $C_{35}H_{44}N_4O_8NaS_2$ $[M + Na]^+$, 735.2498; found, 735.2527; Anal. Calcd for $C_{35}H_{44}N_4O_8S_2$: C, 58.97; H, 6.22; N, 7.86. Found: C, 58.95; H, 6.19; N, 7.83%.

*6.4.4. Ethyl 4-4-[((Z)-4-4-[5-(ethoxycarbonyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro-4-pyrimidinyl]-2-methoxyphenoxy-2-butenyl) oxy]-3-methoxy phenyl-6-methyl-2-thioxo-1,2,3,4-tetrahydro-5-pyrimidinecarboxylate (**11d**)*

Yield: 65%; mp 135–137 °C; IR (KBr): 3187, 2975, 1698, 1565, 1458 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.19–1.29 (6H, m), 3.36 and 3.37 (6H, s), 3.77 and 3.81 (6H, s), 4.08–4.19 (4H, m), 4.73–4.89 (4H, m), 5.39 (1H, d, J = 3.21 Hz), 5.42 (1H, d, J = 3.77 Hz), 5.83–5.90 (2H, m), 6.64–6.77 (6H, m), 8.04 and 8.21 (2H, s), 8.53 and 8.78 (2H, s); ESIMS (m/z): 698 $[M + H]^+$; Anal. Calcd for $C_{34}H_{40}N_4O_8S_2$: C, 58.60; H, 5.79; N, 8.04. Found: C, 58.55; H, 5.75; N, 8.01%.

*6.4.5. Ethyl 4-4-[((E)-4-4-[5-(ethoxycarbonyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro-4-pyrimidinyl]-2-methoxyphenoxy-2-butenyl) oxy]-3-methoxy phenyl-6-methyl-2-thioxo-1,2,3,4-tetrahydro-5-pyrimidinecarboxylate (**11e**)*

Yield: 64%; mp 145–147 °C; IR (KBr): 3188, 2973, 1701, 1568, 1460 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.19–1.29 (6H, m), 3.25 and 3.38 (6H, s), 3.83 and 3.84 (6H, s), 4.08–4.21 (4H, m), 4.61–4.77 (4H, m), 5.39 (1H, d, J = 3.02 Hz), 5.43 (1H, d, J = 3.77 Hz), 5.93–5.99 (2H, m), 6.69–6.86 (6H, m), 7.84 and 7.95 (2H, s), 8.31 and 8.75 (2H,

s); ^{13}C NMR (100 MHz; DMSO-d_6): 14.22, 15.54, 56.25, 57.42, 60.33, 71.65, 107.95, 112.13, 115.62, 118.54, 128.29, 142.79, 158.71, 166.40, 172.41, 174.29; ESIMS (*m*/*z*): 698 $[M + H]^+$, 719 $[M + Na]^+$; Anal. Calcd for $C_{34}H_{40}N_4O_8S_2$: C, 58.60; H, 5.79; N, 8.04. Found: C, 58.57; H, 5.74; N, 8.02%.

*6.4.6. Methyl (2S)-1-[4-(3-methoxy-4-3-[2-methoxy-4-(5-[(2S)-2-(methoxy carbonyl) tetrahydro-1H-1-pyrrolyl]carbonyl-6-methyl-2-thioxo-1,2,3,4-tetra hydro-4-pyrimidinyl)phenoxy]propoxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetra hydro-5-pyrimidinyl] carbonyltetrahydro-1H-2-pyrrolecarboxylate (**12a**)*

Yield: 65%; mp 148–150 °C; IR (KBr): 3210, 2960, 1745, 1612, 1442 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.80–2.20 (10H, m), 1.94 and 1.99 (6H, s), 2.99–3.29 (4H, m), 3.69 and 3.70 (6H, s), 3.81 and 3.82 (6H, s), 3.99–4.10 (4H, m), 4.33–4.44 (2H, m), 5.24 and 5.31 (2H, s), 6.74–6.94 (6H, m), 7.49 and 7.56 (2H, s), 7.74 and 7.86 (2H, s); ^{13}C NMR (100 MHz; DMSO-d_6): 15.29, 22.54, 29.23, 29.69, 46.11, 51.76, 56.07, 58.36, 65.16, 107.53, 112.32, 115.81, 118.26, 143.56, 158.09, 159.12, 171.69, 172.31, 175.15; ESIMS (*m*/*z*): 852 $[M + H]^+$, 874 $[M + Na]^+$; Anal. Calcd for for $C_{41}H_{50}N_6O_{10}S_2$: C, 57.87; H, 5.92; N, 9.88. Found: C, 57.85; H, 5.91; N, 9.87%.

*6.4.7. Methyl (2S)-1-[4-(3-methoxy-4-4-[2-methoxy-4-(5-[(2S)-2-(methoxy carbonyl)tetrahydro-1H-1-pyrrolyl]carbonyl-6-methyl-2-thioxo-1,2,3,4-tetra hydro-4-pyrimidinyl)phenoxy]butoxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetra hydro-5-pyrimidinyl] carbonyltetrahydro-1H-2-pyrrolecarboxylate (**12b**)*

Yield: 68%; mp 145–147 °C; IR (KBr): 3215, 2955, 1744, 1610, 1442 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.68–2.18 (12H, m), 1.92 and 1.98 (6H, s), 2.96–3.27 (4H, m), 3.68 and 3.69 (6H, s), 3.80 and 3.82 (6H, s), 4.03–4.11 (4H, m), 4.32–4.44 (2H, m), 5.23 and 5.32 (2H, s), 6.72–6.92 (6H, m), 7.47 and 7.54 (2H, s), 7.73 and 7.85 (2H, s); HRMS (ESI) *m*/*z* calcd for $C_{42}H_{52}N_6O_{10}NaS_2$ $[M + Na]^+$, 887.3084; found, 887.3106; Anal. Calcd for for $C_{42}H_{52}N_6O_{10}S_2$: C, 58.32; H, 6.06; N, 9.72. Found: C, 58.31; H, 6.04; N, 9.70%.

*6.4.8. Methyl (2S)-1-((4-(3-methoxy-4-((5-(2-methoxy-4-(5-(((2S)-2-(methoxy carbonyl)tetrahydro-1H-1-pyrrolyl)carbonyl)-6-methyl-2-thioxo-1,2,3,4-tetra hydro-4-pyrimidinyl)phenoxy)pentyl) oxy)phenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro-5-pyrimidinyl) carbonyltetrahydro-1H-2-pyrrolecarboxylate (**12c**)*

Yield: 67%; mp 168–170 °C; IR (KBr): 3217, 2953, 1743, 1609, 1441 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.620–2.19 (14H, m), 1.91 and 1.99 (6H, s), 2.96–3.29 (4H, m), 3.69 and 3.71 (6H, s), 3.80 and 3.81 (6H, s), 4.00–4.09 (4H, m), 4.33–4.42 (2H, m), 5.21 and 5.37 (2H, s), 6.72–6.93 (6H, m), 7.48 and 7.57 (2H, s), 7.74 and 7.89 (2H, s); ESIMS (*m*/*z*): 880 $[M + H]^+$, 902 $[M + Na]^+$; Anal. Calcd for for $C_{43}H_{54}N_6O_{10}S_2$: C, 58.75; H, 6.19; N, 9.56. Found: C, 58.73; H, 6.20; N, 9.58%.

*6.4.9. Methyl (2S)-1-((4-(3-methoxy-4-(((Z)-4-(2-methoxy-4-(5-(((2S)-2-(methoxycarbonyl)tetrahydro-1H-1-pyrrolyl)carbonyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro-4-pyrimidinyl)phenoxy)-2-butenyl)oxy)phenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro-5-pyrimidinyl) carbonyltetrahydro-1H-2-pyrrolecarboxylate (**12d**)*

Yield: 58%; mp 146–148 °C; IR (KBr): 3202, 2952, 1742, 1606, 1441 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.92 and 1.99 (6H, s), 1.97–2.18 (8H, m), 3.14–3.34 (4H, m), 3.71 and 3.80 (6H, s), 3.82 and 3.88 (6H, s), 4.28–4.44 (2H, m), 4.69–4.87 (4H, m), 5.22 and 5.44 (2H, s), 5.79–5.91 (2H, m), 6.68–6.98 (6H, m), 7.84 and 7.90 (2H, s), 8.11 and 8.17 (2H, s); HRMS (ESI) *m*/*z* calcd for $C_{42}H_{50}N_6O_{10}NaS_2$ $[M + Na]^+$, 885.2927; found, 885.2957; Anal. Calcd for for $C_{42}H_{50}N_6O_{10}S_2$: C, 58.45; H, 5.84; N, 9.74. Found: C, 58.46; H, 5.83; N, 9.74%.

*6.4.10. Methyl (2S)-1-((4-(3-methoxy-4-(((E)-4-(2-methoxy-4-(5-(((2S)-2-(methoxycarbonyl)tetrahydro-1H-1-pyrrolyl)carbonyl)-6-methyl-2-thioxo-1,2,3,4-tetra hydro-4-pyrimidinyl)phenoxy)-2-butenyl)oxy)phenyl)-6-methyl-2-thioxo-1,2, 3,4-tetrahydro-5-pyrimidinyl) carbonyltetrahydro-1H-2-pyrrolecarboxylate (**12e**)*

Yield: 60%; mp 168–170 °C; IR (KBr): 3205, 2948, 1738, 1610, 1444 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.90 and 1.96 (6H, s), 1.96–2.19 (8H, m), 3.13–3.32 (4H, m), 3.69 and 3.78 (6H, s), 3.82 and 3.88 (6H, s), 4.28–4.44 (2H, m), 4.70–4.85 (4H, m), 5.25 and 5.48 (2H, s), 5.88–5.12 (2H, m), 6.65–6.94 (6H, m), 7.81 and 7.91 (2H, s), 8.13 and 8.21 (2H, s); ^{13}C NMR (100 MHz; DMSO-d_6): 15.32, 22.59, 29.21, 46.14, 51.80, 56.01, 58.37, 71.71, 107.55, 112.36, 115.85, 118.31, 129.17, 143.51, 158.01, 159.09, 171.72, 172.34, 175.13; ESI-MS (*m*/*z*): 864 $[M + H]^+$, 886 $[M + Na]^+$;Anal. Calcd for for $C_{42}H_{50}N_6O_{10}S_2$: C, 58.45; H, 5.84; N, 9.74. Found: C, 58.46; H, 5.83; N, 9.74%.

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References

[1] P. Biginelli, Gazz. Chim. Ital 23 (1893) 360.
[2] (a) C.O. Kappe, Eur. J. Med. Chem. 35 (2000) 1043;
(b) C.O. Kappe, Tetrahedron 49 (1993) 6937;
(c) J.C. Barrow, P.G. Nantermet, H.G. Selnick, K.L. Glass, K.E. Rittle, K.F. Gilbert, R.S.L. Chang, S.S.O. 'Malley, T.V. Olah, J.D. Ellis, A. Barrish, K. Kassahun, D. Nagarathnam, C.J. Forray, J. Med.Chem 43 (2000) 2703;
(d) T. Matsuda, I. Hirao, Nippon Kag. Zass 86 (1965) 1195.
[3] (a) K.S. Atwal, B.N. Swanson, S.E. Unger, D.M. Floyd, S. Moreland, A. Hedberg, B.C. O'Reilly, J. Med. Chem 34 (1991) 806;
(b) G.C. Rovnyak, K.S. Atwal, A. Hedberg, S.D. Kimball, S. Moreland, J.Z. Gougoutas, B.C. O'Reilly, J. Schwartz, M.F. Malley, J. Med. Chem 35 (1992) 3254;
(c) G.C. Rovnyak, S.D. Kimball, B. Beyer, G. Cucinotta, J.D. Di-Marco, J. Gougoutas, A. Hedberg, M. Malley, J.P. McCarthy, R. Zhang, S. Moreland, J. Med. Chem 38 (1995) 119;
(d) J.C. Barrow, P.G. Nantermet, H.G. Selnick, K.L. Glass, K.E. Rittle, K. Kassahun, P. Leppert, D. Nagarathnam, C. Forray, J. Med. Chem 43 (2000) 2703;
(e) D. Nagarathnam, S.W. Miao, C.M. Harrel, C. Gluchowski, J. Med.Chem 42 (1999) 4764.
[4] (a) L. Heys, C.G. Moore, P.J. Murphy, Chem. Soc. Rev. 29 (2000) 57;
(b) A.D. Patil, N.V. Kumar, W.C. Kokke, M.F. Bean, A.J. Freyer, C. De Brosse, S. Mai, A. Truneh, D.J. Faulkner, B. Carte, A.L. Breen, R.P. Hertzberg, R.K. Johnson, J.W. Westley, B.C. Potts, J. Org. Chem 60 (1995) 1182.
[5] C.O. Kappe, Acc. Chem. Res. 33 (2000) 879.
[6] (a) T.U. Mayer, T.M. Kapoor, S.J. Haggarty, R.W. King, S.L. Schreiber, T.J. Mitchison, Science 286 (1999) 971;
(b) T.M. Kapoor, T.U. Mayer, M.H. Coughlin, T.J.J. Mitchison, Cell. Biol. 150 (2000) 975.
[7] R. Leizerman, M. Avunie-Masala, M. Elkabets, A. Fich, L. Gheber, Cell. Mol. Life Sci. 61 (2004) 2060.
[8] (a) M. Gartner, N. Sunder-Plassmann, J. Seiler, M. Utz, I. Vernos, T. Surrey, A. Giannis, ChemBioChem 6 (2005) 1173;
(b) E. Klein, S. DeBonis, B. Thiede, D.A. Skoufias, F. Kozielskib, L. Lebeaua, Bioorg. Med. Chem. 15 (2007) 6474.
[9] (a) A. Kamal, D. Dastagiri, M.J. Ramaiah, E.V. Bharathi, C. Srinivas, S.N.C.V.L. Pushpavalli, D. Pal, M.P. Bhadra, ChemMedChem 5 (2010) 1937;
(b) A. Kamal, R.V.C.R.N.C. Shetti, S. Azeeza, S.A. Ahmed, P. Swapna, A.M. Reddy, I.A. Khan, S. Sharma, S.T. Abdullah, Eur. J. Med. Chem. 45 (2010) 4545;
(c) A. Kamal, Y.V.V. Srikanth, M.N.A. Khan, T.B. Shaik, M. Ashraf, Bioorg. Med. Chem. Lett. 20 (2010) 5229;
(d) A. Kamal, G. Ramakrishna, P. Raju, A. Viswanath, M.J. Ramaiah, G. Balakishan, M.P. Bhadra, Bioorg. Med. Chem. Lett. 20 (2010) 4865;
(e) A. Kamal, S.F. Adil, R.T. Jaki, B. Siddardha, U.S.N. Murthy, Lett. Drug Des. Discov 6 (2009) 201.
[10] C.O. Kappe, A. Stadler, A.Org. React 63 (2004) 1.
[11] J. Zhu, H. Bienaymé, Multicomponent Reactions, first ed. Wiley-VCH, Weinheim, 2005.
[12] http://www.atcc.org/Portals/1/Pdf/30-1010k.pdf
[13] G.B. Jones, C.L. Davey, T.C. Jenkins, A. Kamal, G.G. Kneale, S. Neidle, G.D. Webster, D.E. Thurston, Anti-Cancer Drug Des 5 (1990) 249.
[14] J. Azizian, M.K. Mohammadi, O. Firuzi, B. Mirza, R. Miri, Chem. Biol. Drug Des 75 (2010) 375.