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Article

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Opioid receptor modulators with a cinnamyl group

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Abstract

To obtain selective and potent opioid receptor ligands, we synthesized dehydro derivatives of alvimopan and found compound (**28f),** a selective but modest affinity MOR antagonist, weaker than alvimopan **(1)**. We replaced the aryl piperidine unit by an aryl piperazine to obtain the 1-(α-carboxycinnamyl)-4-arylpiperazines like **(13h),** which to our surprise had no MOR or DOR activity but was a KOR agonist with moderate affinity. In contrast, literature examples of aryl piperazines **(4)** and **(5)** were reported to be pan opioid receptor antagonists, while **(6)** was a MOR agonist. Two compounds **(13l)** and **(11b)** showed analgesic response in tail flick test which was blocked by pretreatment with norbinaltorphimine (norBNI). Among ten 1-(α-carboxycinnamyl)-4-arylpiperidines, compound **(28g)** and five others were specific MOR antagonists. Interestingly, Compound **(26b)** of this series was found to be more potent than naloxone, but weaker than **(1)**. Docking studies have explained differential activities of the above piperazines and piperidines.

Introduction

Opioid receptors are member of G-protein coupled receptors (GPCRs) that couple to inhibitory G protein (Gi/o) isoforms and therefore cause overall inhibitory response upon agonist binding. These receptors are broadly classified into three subtypes: mu (MOR), kappa (KOR) and delta (DOR).[1-3]Being involved in several biological functions, selective opioid receptor modulators (both agonists and antagonists) are proposed to have beneficial therapeutic effects in certain disease conditions like pain and depression. Hence a large number of opioid compounds have been synthesized and studied.[4] Among these a subclass of molecules with a core of 4-arylpiperazine or 4-arylpiperidine has been found to have interesting opioid properties relevant to this study and are shown below **(Figure 1).**

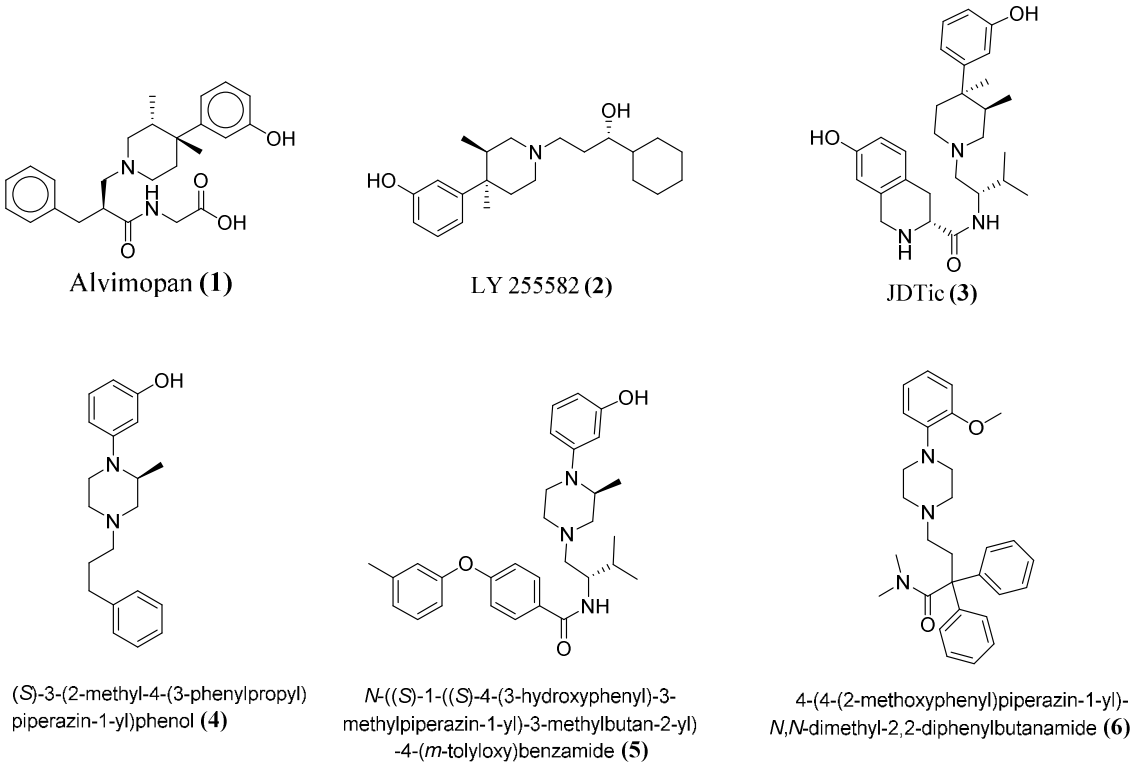


Figure 1. Piperidines and piperazines with opioid receptor activities

N-(3-Hydroxy-4-phenyl) piperidines: **1** (Alvimopan), a selective MOR antagonist[5, 6] was approved in 2008 by USFDA for the treatment of post operative ileus. **2** (LY255582), [7, 8] a MOR,

KOR and DOR antagonist with potent anorectic properties was discontinued in phase-III clinical trials. **3** (JDTic), [9] a selective KOR antagonist with potential antidepressant properties[10-12] was discarded during phase-I in cocaine abuse clinical trials due to unfavorable ADME properties.

N-(3-Hydroxyphenyl) piperazines: Compound **(4)** with a phenylpropyl side chain and similar compounds were found to be pure antagonists of MOR, KOR and DOR.[13] Compound **(5)** inspired by **(3)** was the most potent in a class of compounds which inhibited agonist stimulated [35S] GTPγS binding in cloned human MOR, KOR and DOR. Compound **(5)** had a high selectivity for MOR over KOR and DOR.[14, 15]

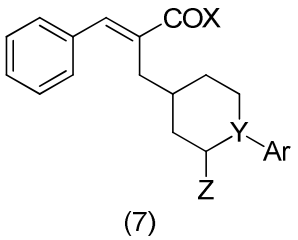
Much earlier to the discovery of **(4)** and **(5)** T. Komoto et al had published MOR agonist activity for a series of aryl piperazines replacing the aryl piperidines in loperamide.[16] Interestingly in this series, the 2-methoxyphenyl compound **(6)** was more active than the 2-, 3- and 4- hydroxy phenyl compounds.

Like other opioid receptor (MOR and DOR) agonists, KOR agonists are known to produce analgesia without having dependence and abuse liability, but are not approved for clinical use as most of them induce sedation, hallucinations and dysphoria in human as well as in preclinical models. Therefore, various groups worldwide are still trying to obtain KOR agonists based on distinct templates in a hope to get new molecules with analgesic properties but devoid of adverse effects. Multiple lines of evidence have also revealed that local application of synthetic opioid receptor agonists could produce analgesic effect by activating peripheral opioid receptors particularly in inflamed tissues.[17] The peripheral opioid agonists bind to opioid receptors expressed on sensory nerve terminals and produce analgesia by modulating the excitability of sensory nerves and the release of pro-inflammatory neuropeptides (e.g. substance P, calcitonin gene related peptide). Since such analgesic activity occurs by engaging the opioid receptor

locally in peripheral tissues, adverse effects such as respiratory depression and addiction are absent. Thus, such observations have opened new frontiers of research on developing peripherally restricted opioid agonists that lack CNS effects. [18] Our studies were aimed at using these concepts to find a useful analgesic.

Chemistry

During the course of our engagement in the chemistry of **(1)**, we observed that in all the above publications, a cinnamyl group had not been studied in place of a phenyl propyl or a benzamide group. On the other hand, we had developed a high yielding synthesis of N-aryl piperazines from anilines.[19] Most of these piperazines are significant components of important drugs and were available to us. Therefore, we decided to fill the vacant chemical space by constructing molecules with the template **(7)**, where in X was OR, OH, NHC(R)COOR. The aryl piperidine was chosen from the structure of **(1)** or loperamide and the aryl piperazines were chosen from medicinal chemistry literature. e.g. **9b** (2, 3-dichlorophenyl piperazine) present in antipsychotic aripiprazole,[20] **9e** (4-flurophenyl piperazine) present in sedative niaprazine,[21] **9h** (2-hydroxyphenyl piperazine) present in antipsychotic bifeprunox,[22] **9j (**3-chlorophenyl piperazine) present in antidepressant trazodone[23] and **14** (benisothiazolyl piperazine) present in antipsychotic ziprasidone.[24] We were aware of a class of antiallergic drugs like cinnarizine and flunarizine which are 1-cinnamyl piperazines with a benzyhydryl group at position 4. Some examples of **7** (**figure 2**) were also hydrogenated to give new phenylpropyl compounds.



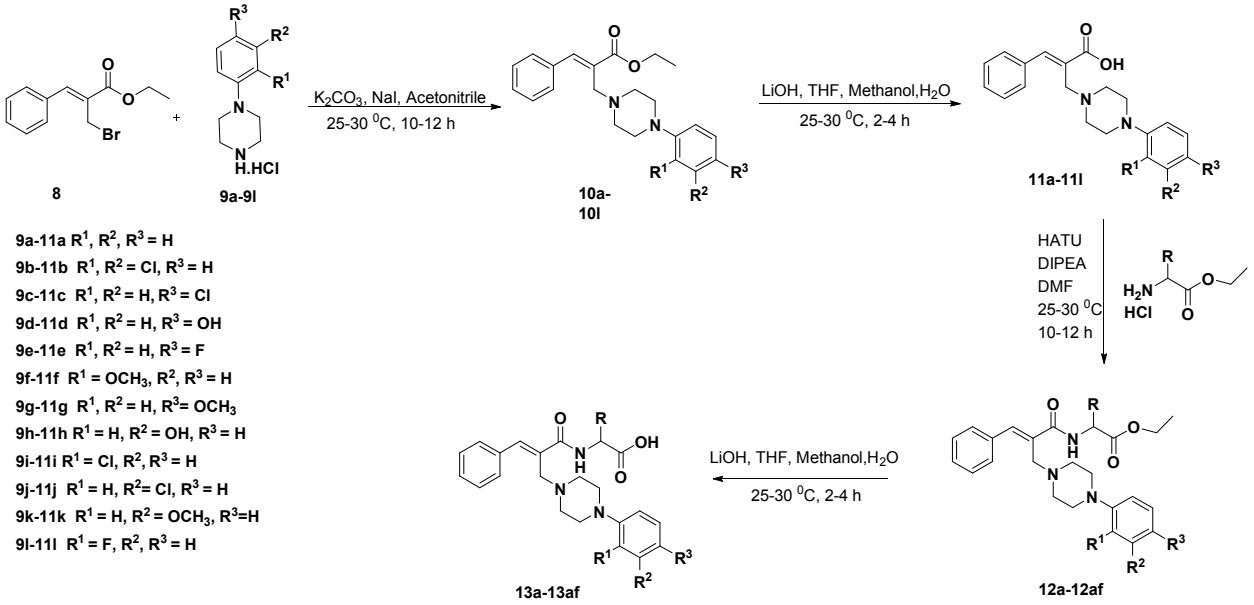
(7)

X = OR, NHC(R)COOR
Y = CH(OH), Z = H
Y = C(Me), Z = Me
Y = N-Ar, Z =H

Figure 2. General structure of synthesized molecules

Their synthesis and assay for MOR, KOR and DOR modulatory activities are discussed below. The study yielded new molecules with moderate KOR and MOR activity *in vitro*. Interestingly, a couple of piperazines- **(13l)** and **(11b)** were found to have KOR agonist activities apparently unknown earlier and exhibited potent analgesic activity. It was also observed that a cinnamyl piperidine **(26b)** was equipotent with naloxone as a MOR antagonist.

Scheme 1. Synthesis of phenyl piperazine molecules



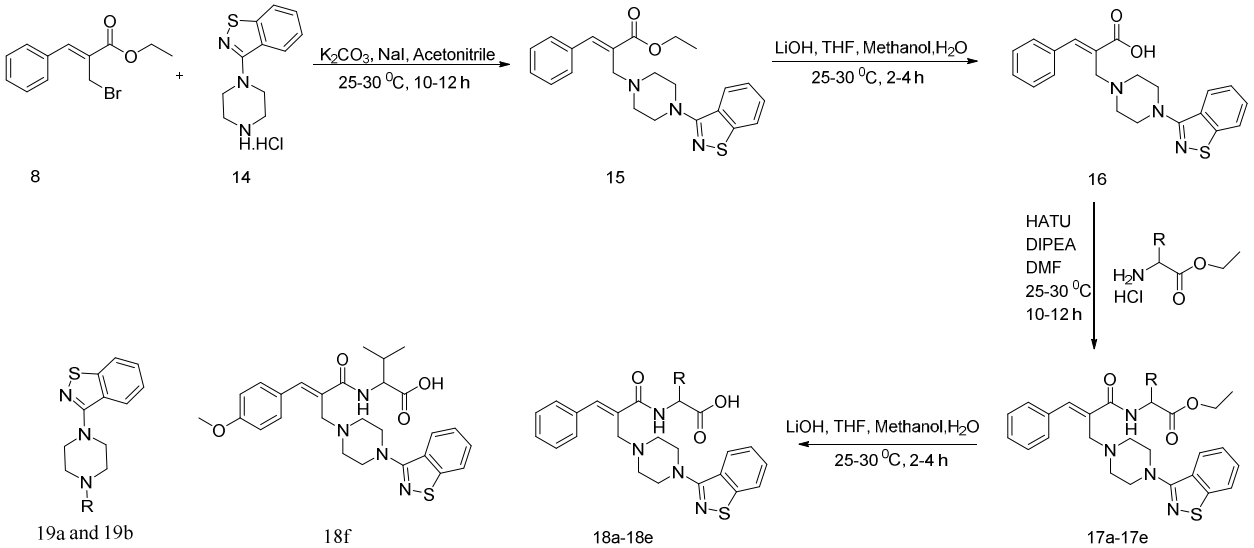
9a-11a R^1, R^2, R^3 = H
9b-11b R^1, R^2 = Cl, R^3 = H
9c-11c R^1, R^2 = H, R^3 = Cl
9d-11d R^1, R^2 = H, R^3 = OH
9e-11e R^1, R^2 = H, R^3 = F
9f-11f $R^1 = OCH_3$, R^2, R^3 = H
9g-11g R^1, R^2 = H, $R^3 = OCH_3$
9h-11h R^1 = H, R^2 = OH, R^3 = H
9i-11i R^1 = Cl, R^2, R^3 = H
9j-11j R^1 = H, R^2= Cl, R^3 = H
9k-11k R^1 = H, $R^2 = OCH_3$, R^3=H
9l-11l R^1 = F, R^2, R^3 = H

12a-13a R, R^1, R^2, R^3 = H
12b-13b R = $CH(CH_3)_2$, R^1, R^2, R^3 = H
12c-13c R = CH_2-$CH(CH_3)_2$, R^1, R^2, R^3 = H
12d-13d R = $CH(CH_3)CH_2CH_3$, R^1, R^2, R^3 = H
12e-13e R = $(CH_2)_2CH_3$, R^1, R^2, R^3 = H
12f-13f R = H, R^1, R^2 = Cl, R^3 = H
12g-13g R = $CH(CH_3)_2$, R^1, R^2 = Cl, R^3 = H
12h-13h R = CH_2-$CH(CH_3)_2$, R^1, R^2 = Cl, R^3 = H
12i-13f i = $CH(CH_3)CH_2CH_3$, R^1, R^2 = Cl, R^3 = H
12j-13j R = $(CH_2)_2CH_3$, R^1, R^2 = Cl, R^3 = H
12k-13k R, R^1, R^2 = H, R^3 = Cl
12l-13l R = $CH(CH_3)_2$, R^1, R^2 = H, R^3 = Cl
12m-13m R = CH_2-$CH(CH_3)_2$, R^1, R^2 = H, R^3 = Cl
12n-13n R = $CH(CH_3)CH_2CH_3$, R^1, R^2 = H, R^3 = Cl
12o-13o R = $(CH_2)_2CH_3$, R^1, R^2,= H, R^3 = Cl
12p-13p R, R^1, R^2 = H, R^3 = OH
12q-13q R = $CH(CH_3)_2$, R^1, R^2 = H, R^3 = OH
12r-13r R= CH_2-$CH(CH_3)_2$, R^1, R^2 = H, R^3 = OH
12s-13s R= $CH(CH_3)CH_2CH_3$, R^1, R^2 = H, R^3 = OH
12t-13t R= $(CH_2)_2CH_3$, R^1, R^2 = H, R^3 = OH
12u-13u R, R^1, R^2 = H, R^3 = F
12v-13v R = $CH(CH_3)_2$, R^1, R^2 = H, R^3 = F
12w-13w R = CH_2-$CH(CH_3)_2$, R^1, R^2 = H, R^3 = F
12x-13x R = $CH(CH_3)CH_2CH_3$, R^1, R^2 = H, R^3 = F
12y-13y R = $(CH_2)_2CH_3$, R^1, R^2 = H, R^3 = F
12z-13z R = H, $R^1 = OCH_3$, R^2, R^3 = H
12aa-13aa R, R^1,R^2 = H, $R^3 = OCH_3$
12ab-13ab R, R^1 = H, R^2 = OH, R^3 = H
12ac-13ac R = H, R^1= Cl, R^2, R^3 = H
12ad-13ad R, R^1 = H, R^2 = Cl, R^3 = H
12ae-13ae R, R^1 = H, $R^2 = OCH_3$, R^3 = H
12af-13af R = H, R^1= F, R^2, R^3 = H
11b R^1, R^2 = Cl, R^3 = H
11c R^1, R^2 = H, R^3 = Cl

Ethyl 2-bromomethylcinnamate **(8)** was condensed with aryl/hetaryl piperazines (**schemes 1, 2**) and piperidines (**scheme 4**) at 25-30^{o} C to give **(10), (15) and (25)**. The retention of E-geometry during displacement of bromine was demonstrated as follows in the case of **(10b)**. When the reaction was conducted at 70^{o} C two products were formed; one was **(10b)** and the other was the Z-isomer as shown by NMR studies (see supplementary information).

Compounds **(10)**, **(15)** and **(25)** were hydrolyzed to carboxylic acids **(11)**, **(16)** and **(26)** respectively which were coupled to ethyl/methyl esters of α-aminoacids (L-glycine, L-valine, L-leucine, L-isoleucine, L-norvaline) to offer **(12)**, **(17)** and **(27)**. A second hydrolysis offered the target compounds **(13)**, **(18)** and **(28)**. **(28f)** is the dehydro analogue of alvimopan and on catalytic hydrogenation yielded a diasterioisomeric mixture from which **(1)** was isolated in modest yield. **(28f)** has been recently synthesized by another route but its activity has not been reported.[25] Palladium-catalyzed hydrogenation of **(10a)**, **(10f-10h)** led to the reduced products, **(20a), (20f-20h)** with minimal concurrent hydrogenolysis of the cinnamyl group (which happened when the reaction was done in an agitated hydrogenator). Hydrolysis to **(21)** followed by coupling with ethyl glycinate using HATU provided amides **(22a)**, **(22f-22h)** (**Scheme 3**) which were transformed to target molecules **(23a)**, **(23f-23h)** which are piperazine analogues of **(1)**. Apart from them, compounds **(19a)** and **(19b)** were also synthesized for SAR studies. **(19a)** was obtained by alkylation of **(14)** with benzyl chloride and **(19b)** by alkylation with ethyl-3-chloro propionate and hydrolysis to the acid followed by coupling with L-valine.

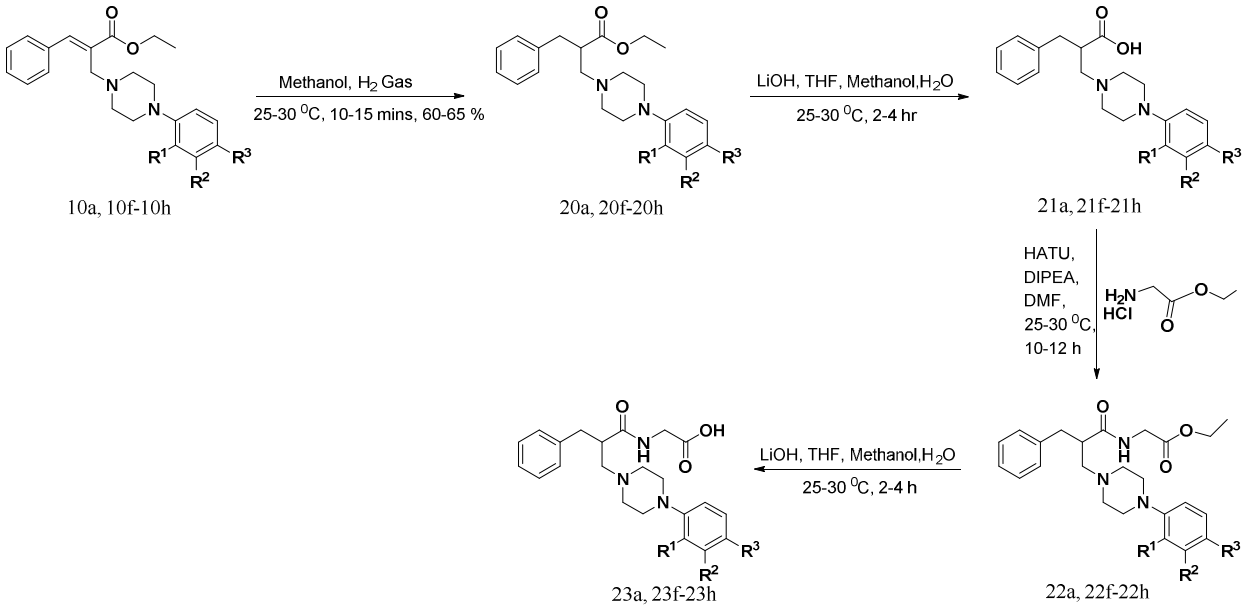
Scheme 2. Synthesis of benisothiazolyl piperazine derivatives



17a-18a R= H **17b-18b R= $CH(CH_3)_2$** **17c-18c R=CH_2-$CH(CH_3)_2$** **17d-18d R=$CH(CH_3)CH_2CH_3$**

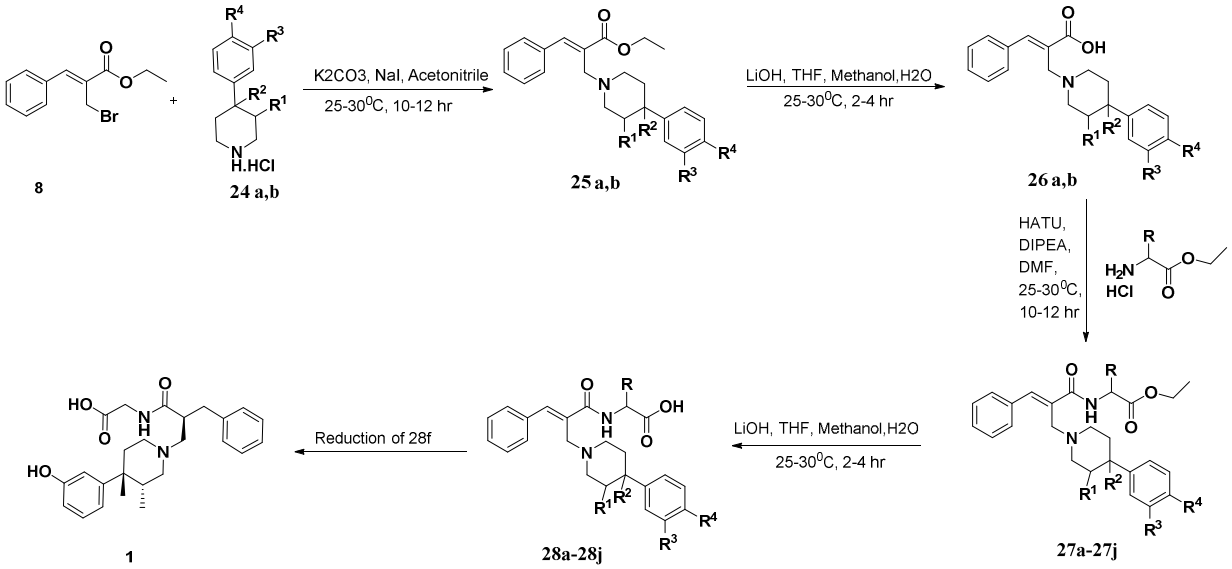
17e-18e R= $(CH_2)_2CH_3$ **19a R = Ph** **19b R = $(CH_2)_2CONHC(R)COOH$**

Scheme 3: Synthesis of reduced phenyl piperazine analogues



22a-23a R, R^1, R^2 = H **22f-23f R = OCH_3, R^1, R^2 = H** **22g-23g R , R^1 = H, R^2 = OCH_3** **22h-23h R = OH, R^1, R^2 = H**

Scheme 4. Synthesis of phenyl piperidine molecules



24-26 a R^1 = H, R^2 = OH, R^3 = H, R^4 = Cl

27a - 28a R = H, R^1 = H, R^2 = OH, R^3 = H, R^4 = Cl

27c - 28c R = CH_2-$CH(CH_3)_2$, R^1 = H, R^2 = OH, R^3 = H, R^4 = Cl

27e - 28e R = $(CH_2)_2CH_3$, R^1 = H, R^2 = OH, R^3 = H, R^4 = Cl

27g - 28g R = $CH(CH_3)_2$, R^1 , R^2 = CH_3, R^3 = OH, R^4 = H

27i - 28i R = $CH(CH_3)CH_2CH_3$, R^1 , R^2 = CH_3, R^3 = OH, R^4 = H

24-26 b R^1 , R^2 = CH_3, R^3 = OH, R^4 = H

27b - 28b R = $CH(CH_3)_2$, R^1 = H, R^2 = OH, R^3 = H, R^4 = Cl

27d - 28d R = $CH(CH_3)CH_2CH_3$, R^1 = H, R^2 = OH, R^3 = H, R^4 = Cl

27f - 28f R = H, R^1 , R^2 = CH_3, R^3 = OH, R^4 = H

27h - 28h R = CH_2-$CH(CH_3)_2$, R^1 , R^2 = CH_3, R^3 = OH, R^4 = H

27j - 28j R = $(CH_2)_2CH_3$, R^1 , R^2 = CH_3, R^3 = OH, R^4 = H

Results and discussion

A total of 44 piperazines listed in **Scheme 1-3** and 11 piperidines listed in **Scheme 4** were evaluated on a panel of 11 GPCRS (Opioid receptors: KOR, DOR, MOR; Dopamine receptors: D_1R, D_2R and D_5R; Serotonin receptor 5-HT6; Histamine Receptors: H_1R, H_2R and H_3R; Orphan Receptor GPR40) for agonist and antagonist activity. In the first stage, all the compounds were evaluated at only 10 μM concentration in triplicate for agonist or antagonist activity at these receptors individually, and if any compound exhibited minimum 50% of inhibition or stimulation in comparison to control then that particular compound was further evaluated in a concentration dependent (10 μM to 10 pM) response to determine the EC_{50}/IC_{50} values. The following eleven compounds **(Table 1 and 2)** were active by the above definition in these tests.

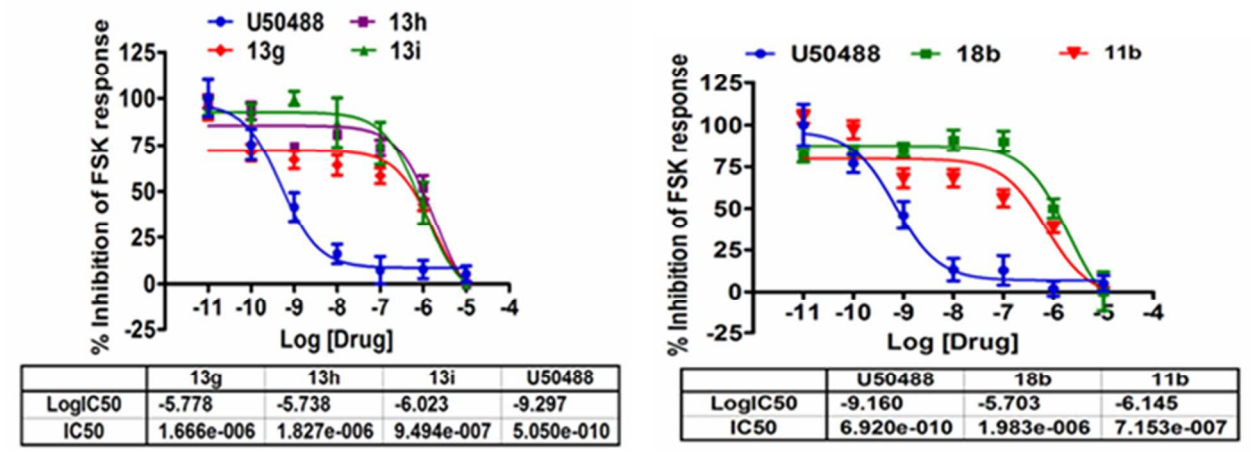
Table 1. IC_{50} values of cinnamyl piperazine derivatives at KOR. These compounds did not exhibit any activity at MOR or DOR.

Compounds Codes	$logIC_{50}$ (IC_{50}: μM)
11b	-6.09 ± 0.27 (0.8)
13g	-5.83 ±0.16 (1.4)
13h	-5.73 ±0.15 (1.8)
13l	-6.041± 0.17 (0.91)
18b	-5.72±0.18 (1.8)
U50488	9.42±0.18 (0.0003)

Compounds with a cinnamyl piperazine template 11b, 13g, 13h, 13l and 18b (table 1) showed agonist activity at KOR without any activity at MOR or DOR in contrast to piperidines. To determine the IC_{50} of these cinnamyl piperazine template compounds, we measured inhibition of forskolin (10 μM, FSK) induced cAMP formation using GloSensor assay in HEK293T cells transiently transfected with hKOR. **29** (U50488)[26]was used as standard KOR agonist for cAMP assay. Results presented in **table 1** show that five analogues of **28f** (dehydroalvimopan) with an aryl piperazine instead of aryl piperidine were modest KOR agonists with about 3 orders of magnitude less potency than the standard **(29)**. (IC_{50}: 0.3 nM, **Figure 3**). The aryl piperazines involved have 2,3-dichlorophenyl, 4-chlorophenyl or 3-benisothiazolyl groups present in the antipsychotics aripiprazole, haloperidol and ziprasidone, respectively. It is interesting to note that our piperazines with an α-carboxycinnamyl substituent did not exhibit Dopamine D2 receptor (known to have antipsychotic activity) and histamine H1 receptor activities like 1-benzhydryl-4-cinnamyl piperazine (cinnarizine). Compound **(11b)** that showed KOR agonist activity equivalent to the earlier three is quite interesting as it lacks the peptide bond which seems to

have no special contribution. Surprisingly though, the 3-hydroxy phenyl piperazine derivative **(13ab)** was found inactive unlike the literature compound **(5)**. Further, none of the other phenyl piperazine derivatives containing an oxygen function in the phenyl residue such as 3-methoxy **(13ae)**, 4-hydroxy **(13p-13t)**, 2-methoxy **(13z)** and 4-methoxy **(13aa)** had any activity in KOR and MOR assays in contrast to the MOR agonist activity exhibited when they were present in structures like **(6)**. Four dihydro derivatives **(23a), (23f-23h)** were also devoid of activity on KOR and MOR.

Similar to MOR and DOR agonists, KOR agonists are also widely known to have analgesic properties and are devoid of gastrointestinal associated adverse effects. Therefore, we sought to determine the analgesic effects of two of our best KOR agonists that is **(13l)** and **(11b)** in tail flick assay, a standard test to measure analgesic response.[27] Although compounds **(13l)** and **(11b)** had modest affinity at KOR ($IC_{50} \leq 1$ μM)**,** we observed significant and time dependent analgesic response at 10 mg/kg dose, in comparison to the standard KOR agonist **(29)** at 5 mg/kg dose. This observation clearly indicates that both compounds readily cross the blood brain barrier. While **(13l)** exhibited modest analgesic response, **(11b)** showed a strong analgesic response equivalent to **(29)** (**Figure 4**). This activity was blocked by the KOR selective antagonist norBNI. Further work is warranted to fully characterize the biological properties of **(13l**) and **(11b)** and to improve their activity.



	13g	13h	13i	U50488
LogIC50	-5.778	-5.738	-6.023	-9.297
IC50	1.666e-006	1.827e-006	9.494e-007	5.050e-010

	U50488	18b	11b
LogIC50	-9.160	-5.703	-6.145
IC50	6.920e-010	1.983e-006	7.153e-007

Figure 3. Agonist activity of **11b, 13g, 13h, 13l** and **18b** at KOR. HEK293T cells transiently transfected with KOR were stimulated with varying concentrations of **(29)** and test compounds (10 µM-10pM) followed by forskolin (FSK, 10 µM) to determine the inhibition of FSK induced cAMP formation in live cells via GloSensor assay. The relative luminescence units for cAMP with test compounds or **(29)** were normalized to % control (FSK alone) and IC_{50} values were generated by non-linear regression analysis using Graphpad Prism 5.0. For presentation purpose, normalized values of two independent experiments (performed in triplicates) were pooled and presented as % mean ± standard error of mean (SEM).

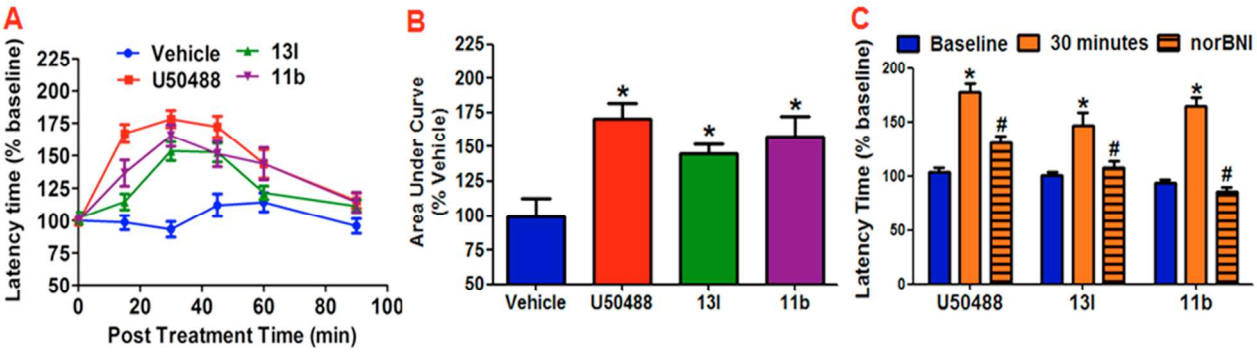


Figure 4: Analgesic effect of **(29)** and two novel KOR agonists: **13l** and **11b**. **(A)** Time dependent analgesic effect of **(29)** (5 mg/kg), **13l** and **11b** (10 mg/kg, *i.p.*). Tail withdrawal latency was determined and presented as % baseline (tail flick measured at 15 min prior to

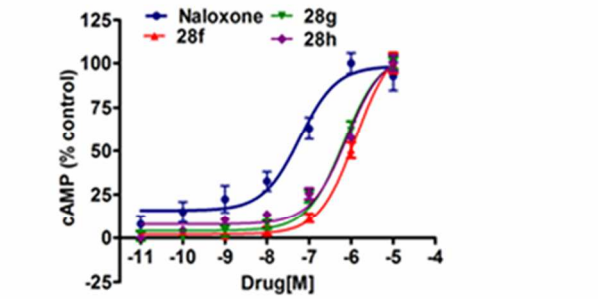
vehicle or drug administration). **(B)** Bar graph showing area under the curve of tail flick test graph.*$p < .01$ by unpaired student's t-test (n=5-6 mice/group). (**C)** KOR selective antagonist norBNI (10 mg/kg, *i.p.*) blocked the analgesic effect of **(29)** as well as of two novel compounds **13l** and **11b**. A separate set of animals (5-6/group) were administered norBNI (10 mg/kg, *i.p.*) 30 min prior to **(29)** or **13l** or **11b** administration, and tail withdrawal latency was determined at 30 min time point.

Table 2. IC_{50} values of cinnamyl piperidine derivatives at MOR. These compounds did not exhibit any activity at KOR or DOR.

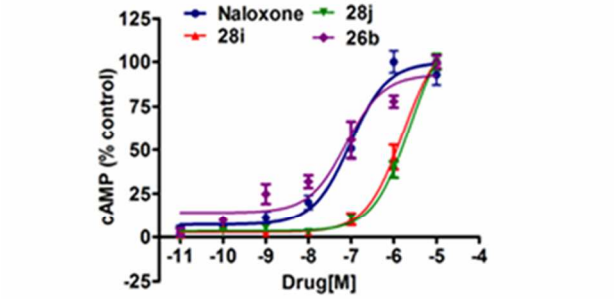
Compounds Codes	**logIC_{50} (IC_{50}: µM)**
26b	-7.73 ± 0.27 (0.018)
28f	-5.89 ± 0.07 (1.26)
28g	-6.18 ±0.07 (0.65)
28h	-6.10 ±0.09 (0.78)
28i	-5.79 ± 0.07 (1.60)
28j	-5.63 ± 0.07 (2.32)
Alvimopan (1)	-8.50 ± 0.22 (0.003)
Naloxone	-7.34 ± 0.26 (0.045)

Among the piperidines screened, besides **(1),** the reference compound, **(26b)** and **(28f-28j)** showed antagonist activity to MOR **(table 2)**, but lack any activity at KOR and DOR in GloSensor assay (**figure 5**). Interestingly, all these have the framework of **(1)**, characterized by 3-m-hydroxyphenyl-3,4-dimethyl piperidine residue. The other set of analogues having the 4-(4-chlorophenyl)-4-hydroxy piperidine **(28a-28e)** moiety present in loperamide were inactive. Hence, the former group was taken up for IC_{50} determination. The activity was evaluated in

HEK293T cells transiently expressing MOR for suppression of DAMGO (1 μM) induced inhibition of cAMP production using GloSensor assay.[28] Results presented in **table 2** show that **(28f)** and **(28g-28j)** wherein the glycine residue in **(28f)** has been replaced by higher homologues L-valine, L-leucine, L-isoleucine, L-norvaline were less active than **(1)** by a factor of 2.5 to 3 log units. Interestingly, compound **(26b)** which is dehydroalvimopan truncated by the removal of glycine is more potent than the others **($IC_{50} \leq 20$ nM**) which is about one sixth of the activity of **(1)** and slightly surpasses the potency of naloxone ($IC_{50} \leq 50$ nM). These observations suggest that manipulation of structure **(26b)** could be a fruitful strategy for newer MOR antagonists.



	Naloxone	28f	28g	28h
LogIC50	-7.228	-5.899	-6.182	-6.107
IC50	5.922e-008	1.263e-006	6.574e-007	7.817e-007



	Naloxone	28i	28j	26b
LogIC50	-7.023	-5.795	-5.634	-7.116
IC50	9.475e-008	1.604e-006	2.324e-006	7.658e-008

Figure 5. Antagonist activity of **28f, 28g, 28h, 28i, 28j** and **26b** at MOR. HEK293T cells transiently transfected with MOR were stimulated with Naloxone and test compounds (10 μM-10 pM) followed by DAMGO (1 μM) and forskolin to determine the inhibition of DAMGO response on cAMP formation in live cells via GloSensor assay. The relative luminescence units for cAMP with test compounds or Naloxone were normalized to % control (DAMGO + forskolin) and IC_{50} values were generated by non-linear regression analysis using Graphpad Prism 5.0. For presentation purpose, normalized values of two independent experiments (performed in triplicates) were pooled and presented % mean ± standard error of mean (SEM).

Methods of Pharmacological studies:

Animals: The *in vivo* analgesia experiments with mice in this study were approved by the institutional animal ethics committee (IAEC) of CSIR- Central Drug Research Institute, Lucknow, India. Male C57BL/6J mice (6-8 weeks old) weighing 22-25 g were used in this study. Animals were housed on a 12-h light/dark cycle (lights on at 8.00 am). Food and water were provided *ad libitum*.

GloSensor Assay for cAMP measurement: HEK293T cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 25 mM Glucose and 20 mM HEPES supplemented with 10% FBS at 37^0C and 5% CO_2 in a humidified incubator. The cultured HEK293T cells were transfected with KOR or DOR or MOR, and pGloSensor™-22F plasmid (Promega corp.) using calcium phosphate method of transfection. Briefly, 5 µg of receptor cDNA plasmid and 5µg of pGloSensor™-22F plasmid DNA were added to 61.3 µl of 2 M $CaCl_2$ and water to final volume of 500 µl. This mixture was added to 500 µl of 2X HBS slowly and was incubated at room temperature for ten minutes. After incubation, the mix was added drop wise to the cells in dish. After overnight incubation, the cells were dislodged from the dish by multiple pipetting and resuspended in drug buffer (1X HBSS with 20 mM HEPES, pH7.4) comprising sodium-luciferin solution (10 µg/µl) and were plated in 96 well plate for 90 minutes. Thereafter, cells were treated with various concentrations (10000nM to 0.01nM) of reference compound (**(29)** for KOR, DAMGO for MOR and DADLE for DOR) and test compounds in triplicates and were incubated in a humidified tissue culture incubator at 37°C with 5% CO_2 for 15-20 minutes to get a steady state condition. After 15 minutes, cells were again treated either with final 10 µM of forskolin (FSK) (to test compounds for agonistic activity) or agonist (1µM) followed by addition of FSK (10 µM; to test compounds for antagonistic activity) and luminescence per well was measured

using luminescence plate reader (BMG Labtech). The relative luminescence units (RLU) were plotted as percent inhibition of FSK response using non linear regression fit analysis equation of GraphPad prism-5.0.

Tail flick test for analgesia: For assessment of thermal analgesia, tail flick test was done as described previously.[29] Briefly, for each testing session, mice were individually acclimatized into the restrainers for one minute without tail immersion. After acclimatization, each mouse was gently introduced into the restrainers and the distal one-third of the tail was dipped into the hot water bath (temp 52° ± 0.5°C) and tail withdrawal latencies were recorded thrice at a time point by an observer unknown to the drug treatment in the mice.

***In silico* analysis:**

The crystal structures of MOR and KOR are available in the ligand-bound forms with morphinan antagonist (PDB ID 4DKL) [30]and with JDTic antagonist (PDB ID 4DJH),[31] respectively. These two protein structures were selected for docking simulations. In an attempt to understand the moderate MOR antagonistic activity of **(28f)** and the KOR agonist activity of **(18b)**, docking simulations were performed, after removing the existing ligands (β-FNA and **(3)**) of the proteins. Further, *in silico* analysis of the binding site and the interacting residues revealed the following findings.

β-FNA is the ligand that binds with the MOR (PDB ID 4DKL) and among the interacting residues, Asp147 and Tyr326 are known to be conserved in MORs. Asp147 is involved in a charge-charge interaction with the amine group of the ligand and also forms hydrogen bond with Tyr326. His297 is found to interact with the aromatic ring of β-FNA (not hydrogen bonds). After docking of **(28f)** with MOR, the conformation of the docked protein molecule was selected based on low Binding Energy (BE) and conserved interactions. The residues that contribute to the

different interactions of the ligand are listed in **Table 3** (see supplementary information) and can be viewed in **Figure 6**. It is seen in **Table 3**, that most of the interacting residues are conserved even in the docked structure of the MOR with **(28f)**. Conserved His297 forms hydrogen bonds with **(28f)** and conserved Asp147 along with Lys233 forms salt bridges in the docked structure. Such conserved and important interactions are the contributing factors for the high Binding Energy and favorable binding of the docked structure. It is also interesting to note that when **(28f)** binds to MOR, two antiparallel β-strands get extended to a region which was earlier a loop.

A similar docking of **(1)** was done with MOR and the interactions are presented in **Table 3** (see supplementary information) and represented in **Figure 8**.The binding energies of **(28f)** and **(1)** are -8.87 kcal/mol and -9.38 kcal/mol, respectively. This agrees with the observed IC_{50} only qualitatively, thus exposing a need for further tweaking the docking model. It is noteworthy, that **(28f)** fits exactly in the existing pocket and in a similar orientation to that of β-FNA. Thus, considering all factors, **(28f)** is clearly a suitable choice for MOR.

In the available structure of KOR (PDB ID 4DJH), the residue Asp138 is crucial in binding of the ligand (**3**) in the active site of protein. It is also suggested that the characteristic V-shape bend of **(3)** is due to the interaction of the two aromatic rings with conserved residue Asp138. Four other residues (Val108, Val118, Ile294 and Tyr312) interacting with **(3)** are thought to be specific to KOR binding with ligands. The desirable conformation of the docked protein molecule, **(18b)** with KOR, was selected based on the optimal BE. **Table 3** (see supplementary information) lists the interacting residues and **Figure 7** represents the graphical view. Apart from the interacting residues being conserved in the docked structure, it is interesting and important to note that the ligand **(18b)** adopts a similar V-shape like **(3)**. This bend in the ligand is probably due to the charged interaction of conserved Asp138. A study involving site-directed mutagenesis

of Asp138 in KOR (D138A and D138N) resulted in reduced binding affinities to the respective agonists[32]. This undeniably manifests the significance of residue Asp138, which forms a salt bridge with 18b in the docked structure. In addition, one of the hydrogen bonds formed by Tyr312 is conserved in the binding site of the protein bound to **(3)**, as well as to **(18b)**. It is due to the interactions of these conserved residues that the Binding Energy of the docked structure is high, explaining the observed KOR activity of **(18b)**. It is worth mentioning that **(3)** and **(18b)**, both, interact strongly with Cys210 (β-strand). We were unable to efficiently dock 18b in MOR and 28f in KOR.

Thus, the *in silico* studies provide favorable binding energies for the synthesized small molecules in the respective sites and support the results from the biological studies. Favorable BE indicates proper binding of the ligands in the pocket of the protein molecule and the interaction of the residues in the active site.

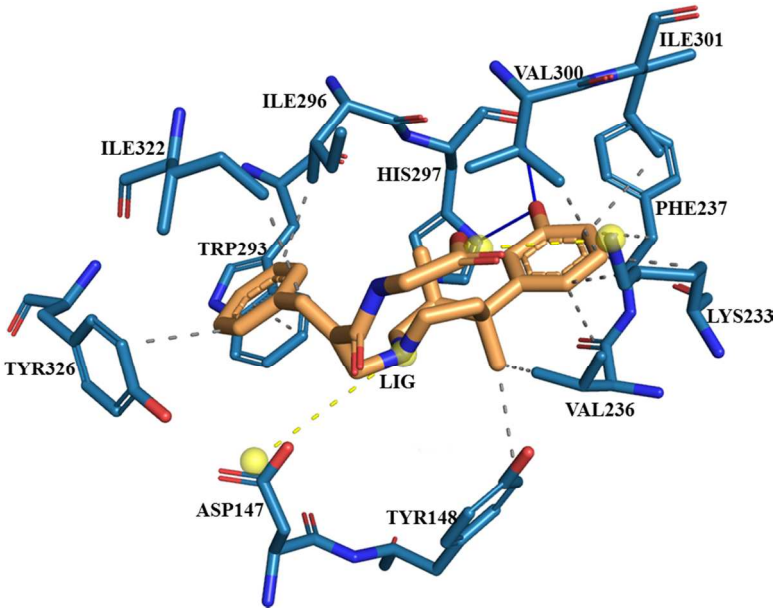


Figure 6. Image of all the interactions of the MOR - **(28f)** docked protein structure, obtained from PLIP.[33]The blue lines represent the hydrogen bonds, the grey dotted lines represent hydrophobic interactions and the yellow dotted lines represent salt bridges.

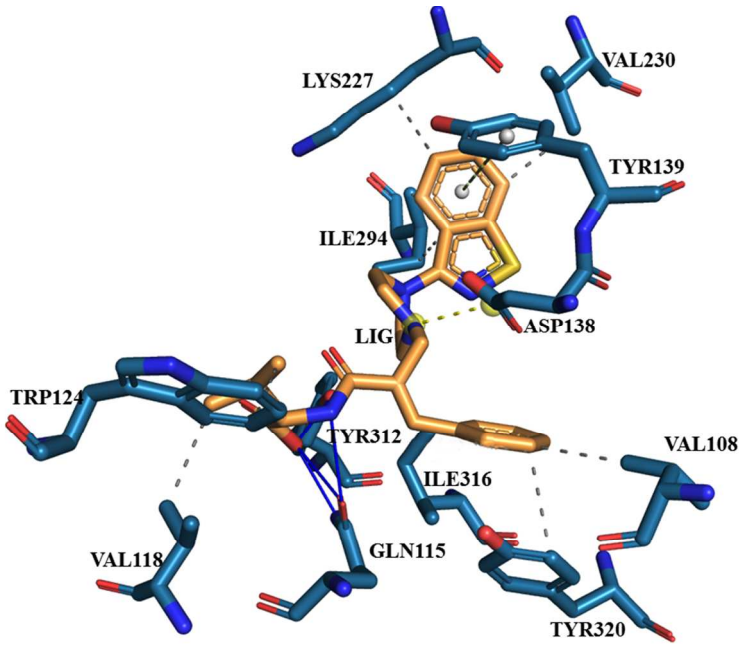


Figure 7. Image of all the interactions of the KOR - **(18b)** docked protein structure, obtained from PLIP. The blue lines represent the hydrogen bonds, the grey dotted lines represent hydrophobic interactions the yellow dotted lines represent salt bridges and black dotted lines represent pi-interactions.

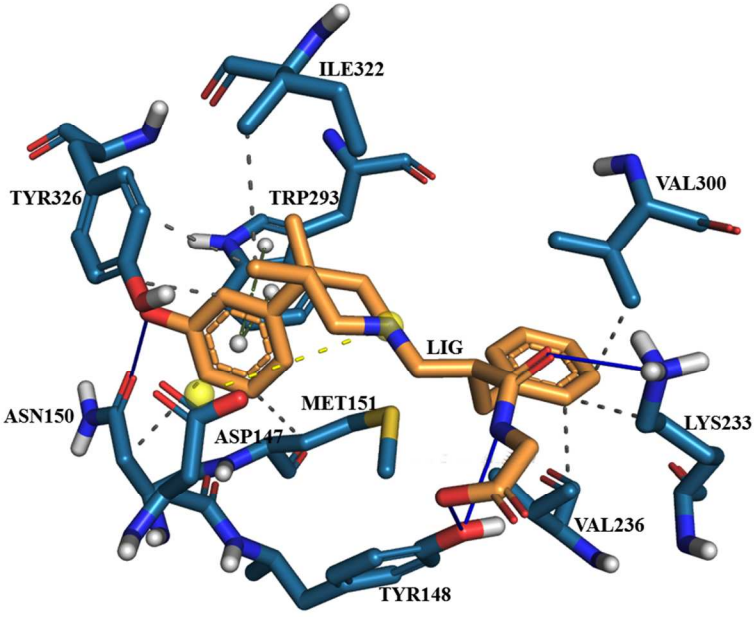


Figure 8. Image of all the interactions of the MOR - **(1)** docked protein structure (obtained from PLIP). The blue lines represent the hydrogen bonds, the grey dotted lines represent hydrophobic interactions and the yellow dotted lines represent salt bridges.

Conclusion:

Our studies have shown that replacement of the piperidine moiety by a piperazine in structures like **(28f)** results in a dramatic change in the opioid properties of the molecules from MOR antagonism to KOR agonism (**28f** vs. **13l**). This also reveal that replacing a phenyl propyl group in substituted piperazines like **(4)** by a α-carboxy cinnamyl group (**13g**) changes from KOR antagonist property to agonist. Further the antipsychotic properties of aryl piperazines incorporated in drugs disappear when the side chain is replaced by α-carboxy cinnamyl group leading to KOR agonist properties. Among the piperazines studied **(13l)** and **(11b)** had moderate *in vitro* but potent *in vivo* analgesic activity comparable to **(29)**. Among the piperidines with MOR antagonist activity **(28f)** was less potent than **(1)**. Compound **(26b)** lacking the peptide chain was more active than **(28f)** and was slightly more potent than naloxone as MOR antagonist. Thus, observations made in this study provides valuable insight for designing and synthesis of novel and selective opioid receptor ligands that can further optimized as lead candidates for the treatment of pain disorders. *In silico* docking studies offered explanations for the KOR activity of piperazine **(18b)** as against the MOR activity of **(1)**

Experimental Section

All chemicals and solvents were commercially purchased and used as such without further purification. The progress of all reactions was monitored by thin layer chromatography using Merck TLC silica gel 60 F_{254} plates with ethyl acetate/n-hexane solvent systems as mobile phase. Column purification was carried on Globe chem silica gel (100-200 mesh); spots were identified

with UV light of wavelength 254 nm. Purity assessment and mass spectra (MS) data were obtained using Shimadzu lcms2020 using electrospray ionization (ESI) for detection. ^{1}H and ^{13}C NMR spectra were measured on a Bruker 300 and 400 MHz instrument. Chemical shifts are reported in δ ppm relative to TMS. (^{1}H NMR: TMS δ = 0.00 ppm, $CDCl_3$ δ = 7.26 ppm, DMSO-d_6 δ = 2.50 ppm; ^{13}C NMR (APT): TMS δ = 0.00 ppm, $CDCl_3$ δ = 77.16 ppm, DMSO-d_6 δ = 39.52 ppm). Coupling constants (*J*) are given in hertz (Hz). The following abbreviations were used to express the multiplicities: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; dd = doublet of doublets; dt = doublet of triplet; td = triplet of doublet; bs = broad singlet.

General procedure for synthesis of 10a-10l, 15 and 25a-25b (General procedure A)

To a solution of ethyl 2-(bromomethyl)-3-phenylacrylate **8** (3.7 mmol) in 15 mL of acetonitrile were added potassium carbonate (7.4 mmol), sodium iodide (1.8 mmol) and N-aryl piperazine **9a-9l, 14** (4.1 mmol) or aryl piperidine **24a-24b** (4.1 mmol) and the reaction mass was stirred at 25-30^{o} C for 10 - 12 h. Completion of reaction was monitored by TLC. The reaction mass was filtered through celite bed and the bed was washed with 10 mL of acetonitrile. The solution was removed of its solvent by rotary evaporation. The remaining residue was partitioned between water (40 mL) and DCM (30 mL). The aqueous layer was extracted twice with DCM (20 mL). The pooled DCM layer was dried over anhydrous sodium sulphate and concentrated to get crude product, which was purified by silica gel column chromatography, elution being done with 10% ethyl acetate in n-hexane.

Ethyl (*E*)-3-phenyl-2-((4-phenylpiperazin-1-yl)methyl)acrylate (10a)

Following the above general procedure A, reaction of **8** and **9a** resulted in **10a** as a syrup (Yield: 90%); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm : 1.34 - 1.37 (t, *J* = 7.12 Hz, 3 H), 2.62 (s, 4 H), 3.14 (s, 4 H), 3.46 (s, 2 H), 4.27 - 4.32 (q, *J* = 7.08 Hz, 2 H), 6.73 - 6.83 (m, 1 H), 6.87 - 6.98 (m, 2

H), 7.16 - 7.27 (m, 2 H), 7.44 (d, *J* = 7.61 Hz, 3 H), 7.66 (d, *J* = 6.97 Hz, 2 H), 7.80 (s, 1 H); MS (ESI): found: $[M + H]^+$, 351.25.

Ethyl (*E*)-2-((4-(2,3-dichlorophenyl)piperazin-1-yl)methyl)-3-phenylacrylate (10b)

Following the above general procedure A, reaction of **8** and **9b** resulted in **10b** as a gummy material (Yield: 89%); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm : 1.35 - 1.38 (t, *J* = 7.12 Hz, 3 H), 2.67 (s, 4 H), 3.04 (s, 4 H), 3.44 (s, 2 H), 4.28 - 4.33 (q, *J* = 7.12 Hz, 2 H), 6.92 - 6.95 (dd, *J* = 6.76, 2.76 Hz, 1 H), 7.10 - 7.15 (dd, *J* = 6.92, 2.52 Hz, 2 H), 7.33 - 7.42 (m, *J* = 7.28 Hz, 3 H), 7.66 - 7.68 (d, *J* = 7.12 Hz, 2 H), 7.86 (s, 1 H); MS (ESI): found: $[M + H]^+$, 420.25.

Ethyl (*E*)-2-((4-(4-chlorophenyl)piperazin-1-yl)methyl)-3-phenylacrylate (10c)

Following the above general procedure A, reaction of **8** and **9c** resulted in **10c** a viscous oil (Yield: 92%); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm : 1.34 - 1.37 (t, *J* = 7.12 Hz, 3 H), 2.61 - 2.64 (t, *J* = 7.12 Hz, 4 H), 3.12 - 3.14 (t, *J* = 7.12 Hz, 4 H), 3.42 (s, 2 H), 4.27 - 4.32 (q, *J* = 7.12 Hz, 2 H), 6.80 - 6.83 (dd, *J* = 7.12 Hz, 2 H), 7.17 - 7.19 (dd, *J* = 7.12 Hz, 2 H), 7.35 - 7.41 (m, *J* = 7.12 Hz, 3 H), 7.66 - 7.68 (d, *J* = 7.12 Hz, 2 H), 7.87 (s, 1 H); MS (ESI): found: $[M + H]^+$, 385.20.

Ethyl (*E*)-2-((4-(4-hydroxyphenyl)piperazin-1-yl)methyl)-3-phenylacrylate (10d)

Following the above general procedure A, reaction of **8** and **9d** resulted in **10d** a syrup (Yield: 96%); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm : 1.34 - 1.37 (t, *J* = 3.63 Hz, 3 H), 2.65 (s, 4 H), 3.05 (s, 4 H), 3.49 (s, 2 H), 4.27 - 4.32 (q, *J* = 7.08 Hz, 2 H), 4.92 (s, 1 H), 6.73 - 6.75 (d, *J* = 8.84 Hz, 2 H), 6.81 - 6.83 (d, *J* = 8.76 Hz, 2 H), 7.33 - 7.41 (m, *J* = 7.04 Hz, 3 H), 7.64 - 7.66 (d, *J* = 7.24 Hz, 2 H), 7.87(s, 1 H); MS (ESI): found: $[M + H]^+$, 367.30.

Ethyl (*E*)-2-((4-(4-fluorophenyl)piperazin-1-yl)methyl)-3-phenylacrylate (10e)

Following the above general procedure A, reaction of **8** and **9e** resulted in **10e** a viscous oil (Yield: 92%); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm : 1.26 - 1.31 (t, *J* = 7.12 Hz, 3 H), 2.65 (s, 4

H), 3.15 - 3.18 (t, *J* = 6.0 Hz, 4 H), 3.53 (s, 2 H), 4.15 - 4.22 (q, *J* = 7.08 Hz, 2 H), 6.83 - 6.88 (m, 2 H), 6.92 - 6.98 (t, *J* = 8.76 Hz, 2 H), 7.26 - 7.32 (t, *J* = 9.04 Hz, 2 H), 7.34 - 7.41 (m, 3 H), 7.99 (s, 1 H), 10.18 (s, 1 H); MS (ESI): found: $[M + H]^+$, 369.15.

Ethyl (*E*)-2-((4-(3-hydroxyphenyl)piperazin-1-yl)methyl)-3-phenylacrylate (10h)

Following the above general procedure A, reaction of **8** and **9h** resulted in **10h** as a gummy mass (Yield: 92%); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm : 1.34 - 1.37 (t, *J* = 7.12 Hz, 3 H), 2.61 - 2.63 (t, *J* = 4.92 Hz, 4 H), 3.14 - 3.17 (t, *J* = 4.84 Hz, 4 H), 3.42 (s, 2 H), 4.27 - 4.30 (q, *J* = 7.12 Hz, 2 H), 4.75 (s, 1 H), 6.28 - 6.30 (dd, *J* = 7.92, 2.08 Hz, 1 H), 6.37 - 6.38 (t, *J* = 2.20 Hz, 1 H), 6.47 - 6.50 (dd, *J* = 7.72, 2.18 Hz, 1 H), 7.06 - 7.12 (t, *J* = 8.08 Hz, 1 H), 7.34 - 7.41 (m, *J* = 9.04 Hz, 4 H), 7.66 - 7.68 (d, *J* = 6.84 Hz, 2 H), 7.87 (s, 1 H); MS (ESI): found: $[M + H]^+$, 367.2.

Ethyl (*E*)-2-((4-(3-chlorophenyl)piperazin-1-yl)methyl)-3-phenylacrylate (10j)

Following the above general procedure A, reaction of **8** and **9j** resulted in **10j** a syrup (Yield: 92%); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm : 1.36 - 1.39 (t, *J* = 7.12 Hz, 3 H), 2.66 (s, 4 H), 2.87 (s, 4 H), 3.45 (s, 2 H), 3.70 (s, 1 H), 4.28 - 4.34 (q, *J* = 7.08 Hz, 2 H), 6.82 - 6.86 (m, 1 H), 6.92 - 6.94 (d, *J* = 8.00 Hz, 1 H), 7.04 - 7.08 (t, *J* = 9.04 Hz, 1 H), 7.14 - 7.16 (d, *J* = 7.60 Hz, 1 H), 7.35 - 7.44 (m, 3 H), 7.66 - 7.68 (d, *J* = 6.88 Hz, 2 H), 7.88 (s, 1 H); MS (ESI): found: $[M + H]^+$, 385.2.

Ethyl (*E*)-2-((4-(2-fluorophenyl)piperazin-1-yl)methyl)-3-phenylacrylate (10l)

Following the above general procedure A, reaction of **8** and **9l** resulted in **10l** a viscous oil (Yield: 87%); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm : 1.34 - 1.39 (t, *J* = 7.11 Hz, 3H), 2.66 - 2.69 (t, *J* = 6.00 Hz, 4 H), 3.07 - 3.10 (t, *J* = 6.00 Hz, 4 H), 3.44 (s, 2 H), 4.27 - 4.34 (q, *J* = 7.09 Hz, 2 H), 6.89 - 7.07 (m, 4 H), 7.32 - 7.45 (m, 3 H), 7.64 - 7.68 (m, 2 H), 7.70 (s, 1 H); MS (ESI): found: $[M + H]^+$, 369.15.

General procedure for synthesis of 11a-11l, 16, 21a, 21f-21h and 26a-26b (General procedure B)

To a solution of **10a-10l, 15, 20a, 20f-20h or 25a-25b** (3.2 mmol) in 14 mL (14 v) 1:1 mixture of methanol and THF was added pre dissolved $LiOH.H_2O$ (9.7 mmol) in minimum quantity of water. The reaction mass was stirred at 25-30^o C for 2 - 4 h. Completion of reaction was monitored by TLC. THF was distilled under reduced pressure, 20v water was added and the pH was adjusted up to 7 using 1N HCl. The product was extracted twice with ethyl acetate. The total ethyl acetate layer was dried with anhydrous sodium sulphate and concentrated to get an acid as white color solid which was used as such without purification for next step.

(*E*)-2-((4-(2,3-Dichlorophenyl)piperazin-1-yl)methyl)-3-phenylacrylic acid (11b)

11b was synthesized from **10b** by following the general procedure B as a white solid (Yield: 89%); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm : 2.61 (s, 4 H), 2.97 (s, 4 H), 3.42 (s, 2 H), 7.11 - 7.14 (dd, *J* = 5.48, 3.96 Hz, 1 H), 7.27 - 7.30 (dd, *J* = 10.08, 2.00 Hz, 2 H), 7.33 - 7.36 (q, *J* = 7.2 Hz, 1 H), 7.39 - 7.43 (q, *J* = 7.56 Hz, 2 H), 7.63 - 7.65 (d, *J* = 7.40 Hz, 2 H), 7.72 (s, 1 H); MS (ESI): found: $[M + H]^+$, 391.05.

(*E*)-2-((4-(4-Chlorophenyl)piperazin-1-yl)methyl)-3-phenylacrylic acid (11c)

11c was synthesized from **10c** by following the general procedure B as a white solid (Yield: 92%); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm : 2.69 - 2.74 (t, 4 H), 3.15 - 3.19 (t, 4 H), 3.66 (s, 2 H), 6.78 - 6.85 (t, 2 H), 7.18 - 7.19 (d, 2 H), 7.26 (s, 1 H), 7.38 (m, 4 H), 8.04 (s, 1 H); ^{13}C NMR (DMSO-d_6) □□ppm : 167.78, 150.12, 141.90, 135.48, 130.71, 129.36, 129.07, 128.96, 122.90, 117.41, 53.71, 52.33, 48.49; MS (ESI): found: $[M + H]^+$, 357.10.

(*E*)-2-((4-(4-Hydroxyphenyl)piperazin-1-yl)methyl)-3-phenylacrylic acid (11d)

11d was synthesized from **10d** by following the general procedure B as a white solid (Yield: 93%); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm : 2.96 (s, 4 H), 3.23 (s, 4 H), 3.99 (s, 2 H), 6.68 - 6.70 (d, *J* = 8.6 Hz, 2 H), 6.81 (s, 2 H), 7.30 - 7.39 (m, *J* = 7.96 Hz, 5 H), 8.11 (s, 1 H), 8.31 (s, 1 H); MS (ESI): found: $[M + H]^+$, 339.20.

(*E*)-2-((4-(2-Methoxyphenyl)piperazin-1-yl)methyl)-3-phenylacrylic acid (11f)

11f was synthesized from **10f** by following the general procedure B as a white solid (Yield: 72%); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm : 2.97 (s, 8 H), 3.75 - 3.76 (d, *J* = 1.38 Hz, 2 H), 3.85 (s, 3 H), 6.87 - 6.95 (m, 3 H), 7.00 - 7.13 (m, 1 H), 7.29 (dd, *J* = 5.41, 2.11 Hz, 2 H), 7.36 - 7.50 (m, 3 H), 8.07 (s, 1 H); MS (ESI): found: $[M + H]^+$, 353.20.

(*E*)-2-((4-(4-Methoxyphenyl)piperazin-1-yl)methyl)-3-phenylacrylic acid (11g)

11g was synthesized from **10g** by following the general procedure B as a white solid (Yield: 80%); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm : 2.83 (s, 4 H), 3.17 (s, 4 H), 3.72 - 3.73 (d, *J* = 1.38 Hz, 2 H), 3.78 (s, 3 H), 6.80 - 6.92 (m, 4 H), 7.25 -7.33 (m, 2 H), 7.35 - 7.49 (m, 3 H), 8.07 (s, 1 H); MS (ESI): found: $[M + H]^+$, 353.20.

(*E*)-2-((4-(3-Hydroxyphenyl)piperazin-1-yl)methyl)-3-phenylacrylic acid (11h)

11h was synthesized from **10h** by following the general procedure B as a white solid (Yield: 86%); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm : 2.81 (s, 4 H), 3.25 (s, 4 H), 3.82 (s, 2 H), 6.28 - 6.35 (m, *J* = 8.6 Hz, 3 H), 6.96 - 7.00 (t, *J* = 7.92 Hz, 1 H), 7.27 (s, 1 H), 7.30 - 7.39 (m, *J* = 6.72 Hz , 3 H), 8.06 (s, 1 H); MS (ESI): found: $[M + H]^+$, 339.20.

(*E*)-2-((4-(2-Chlorophenyl)piperazin-1-yl)methyl)-3-phenylacrylic acid (11i)

11i was synthesized from **10i** by following the general procedure B as a white solid (Yield: 72%); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm : 2.62 (s, 4 H), 3.73 (s, 4 H), 3.50 (s, 2 H), 6.73 - 6.81

(d, 1 H), 6.84 - 6.94 (t, 2 H), 7.12 – 7.24 (t, 1 H), 7.36 - 7.50 (m, 3 H), 7.63 - 7.70 (d, 2 H), 7.80 (s, 1 H), 13.50 (s, 1 H); MS (ESI): found: $[M + H]^+$, 357.10.

(*E*)-2-((4-(3-Chlorophenyl)piperazin-1-yl)methyl)-3-phenylacrylic acid (11j)

11j was synthesized from **10j** by following the general procedure B as a white solid (Yield: 86%); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm : 2.61 - 2.63 (s, 4 H), 2.90 - 3.10 (s, 4 H), 3.5 (s, 2 H), 6.90 - 7.10 (t, 1 H), 7.10 - 7.20 (t, 1 H), 7.20 - 7.30 (t, 1 H), 7.40 - 7.50 (m, 4 H), 7.66 - 7.70 (d, 2 H), 7.87 (s, 1H); MS (ESI): found: $[M + H]^+$, 357.10.

General procedure for synthesis of 12a-12af, 17a-17e, 22a, 22f-22h and 27a-27j (General procedure C)

To **11a-11l, 16, 21a, 21f-21h** or **26a, 26b** (0.6 mmol) in 3 mL (3 v) of DMF were added ethyl/methyl ester of α-aminoacid hydrochloride (0.9 mmol), HATU (0.9 mmol) and DIPEA (1.86 mmol), the reaction mass was stirred at 25-30^o C for 10-12 h. Reaction completion was checked by TLC. DMF was concentrated under reduces pressure. To the residue ice water was added and the mixture was extracted three times with DCM. The combined DCM layers were washed with ice water and brine, dried with anhydrous sodium sulphate and concentrated to get crude product. This was then subjected to silica gel column chromatography, elution being done with ethyl acetate in n-hexane. The fraction containing desired product was evaporated to dryness to get an oily mass.

Methyl (*E*)-(3-phenyl-2-((4-phenylpiperazin-1-yl)methyl)acryloyl)glycinate (12a)

Following above general procedure C, reaction of **11a** and methyl glycinate hydrochloride resulted in **12a** as a syrup (Yield: 87%); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 1.20 (s, 3 H), 2.52 - 2.60 (m, 4 H), 3.17 (s, 4 H), 3.46 (s, 2 H), 4.02 (d, J = 5.59 Hz, 2 H), 4.07 - 4.17 (m, 2 H),

6.71 - 6.82 (m, 1 H), 6.88 - 7.00 (m, 2 H), 7.16 - 7.25 (m, 2 H), 7.31 - 7.39 (m, 1 H), 7.45 (d, *J* = 4.31 Hz, 4 H), 7.63 - 7.69 (m, 1 H), 9.61 - 9.71 (m, 1 H).

Methyl (*E*)-(3-phenyl-2-((4-phenylpiperazin-1-yl)methyl)acryloyl)valinate (12b)

Following above general procedure C, reaction of **11a** and methyl valinate hydrochloride resulted in **12b** as a viscous oil (Yield: 70%); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 0.93 (t, *J* = 6.92 Hz, 6 H), 2.10 - 2.22 (m, 1 H), 2.53 - 2.63 (m, 4 H), 3.12 - 3.25 (m, 4 H), 3.50 - 3.55 (m, 2 H), 3.66 (s, 3 H), 4.37 - 4.47 (m, 1 H), 6.73 - 6.83 (m, 1 H), 6.92 (d, *J* = 8.07 Hz, 2 H), 7.20 (s, 2 H), 7.32 - 7.52 (m, 5 H), 7.71 (s, 1 H), 9.95 - 10.05 (m, 1 H).

Methyl (*E*)-(3-phenyl-2-((4-phenylpiperazin-1-yl)methyl)acryloyl)leucinate (12c)

Following above general procedure C, reaction of **11a** and methyl leucinate hydrochloride resulted in **12c** as a syrup (Yield: 70%); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 0.90 (dd, *J* = 6.37, 2.43 Hz, 6 H), 1.48 - 1.66 (m, 2 H), 1.67 - 1.83 (m, 1 H), 2.52 - 2.60 (m, 4 H), 3.15 (s, 4 H), 3.48 (s, 2 H), 3.65 (s, 3 H), 4.40 - 4.53 (m, 1 H), 6.78 (s, 1 H), 6.93 (d, *J* = 8.07 Hz, 2 H), 7.21 (s, 2 H), 7.33 - 7.40 (m, 1 H), 7.43 (s, 4 H), 7.61 (s, 1 H), 9.69 - 9.77 (m, 1 H).

Methyl (*E*)-3-methyl-2-(3-phenyl-2-((4-phenylpiperazin-1-yl)methyl)acrylamido) pentanoate (12d)

Following above general procedure C, reaction of **11a** and methyl 2-amino-3-methylpentanoate hydrochloride resulted in **12d** as a viscous oil (Yield: 75%); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 0.79 - 0.99 (m, 1 H), 1.24 (s, 1 H), 1.12 - 1.30 (m, 1 H), 1.32 - 1.51 (m, 1 H), 1.81 - 1.94 (m, 1 H), 2.55 (d, *J* = 4.49 Hz, 1 H), 2.52 - 2.64 (m, 1 H), 3.17 (s, 1 H), 3.49 - 3.56 (m, 1 H), 3.65 (s, 1 H), 4.39 - 4.53 (m, 1 H), 6.78 (s, 1 H), 6.92 (d, *J* = 8.07 Hz, 1 H), 7.14 - 7.28 (m, 1 H), 7.32 - 7.52 (m, 1 H), 7.70 (s, 1 H), 10.00 (d, *J* = 8.25 Hz, 1 H).

Ethyl (*E*)-2-(3-phenyl-2-((4-phenylpiperazin-1-yl)methyl)acrylamido)pentanoate (12e)

Following above general procedure C, reaction of **11a** and ethyl 2-aminopentanoate hydrochloride resulted in **12e** as a syrup (Yield: 78%); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm : 0.88 (s, 3 H), 1.19 (t, *J* = 7.11 Hz, 3 H), 1.32 - 1.42 (m, 2 H), 1.64 - 1.79 (m, 2 H), 2.53 - 2.63 (m, 4 H), 3.11 - 3.24 (m, 4 H), 3.49 (s, 2 H), 4.07 - 4.19 (m, 2 H), 4.36 - 4.48 (m, 1 H), 6.69 - 6.82 (m, 1 H), 6.88 - 7.01 (m, 2 H), 7.13 - 7.27 (m, 2 H), 7.42 (s, 5 H), 7.62- 7.68 (m, 1 H), 9.80 - 9.92 (m, 1 H).

Ethyl (*E*)-(2-((4-(2,3-dichlorophenyl)piperazin-1-yl)methyl)-3-phenylacryloyl)glycinate (12f)

Following above general procedure C, reaction of **11b** and ethyl glycinate hydrochloride resulted in **12f** as a viscous oil (Yield: 61%, purity by LC-MS: >99%); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm : 1.18 - 1.33 (t, *J* = 7.15 Hz, 3 H), 2.68 (s, 4 H), 3.11 (s, 4 H), 3.56 (s, 2 H), 4.19 (d, *J* = 4.95 Hz, 2 H), 4.21 - 4.29 (q, *J* = 5.95 Hz ,2 H), 6.96 - 6.99 (dd, *J* = 6.33, 3.30 Hz, 1 H), 7.10 - 7.18 (m, 2 H), 7.27 - 7.29 (d, *J* = 1.19 Hz, 2 H), 7.31 - 7.45 (m, 3 H), 7.99 (s, 1 H), 10.28 - 10.30 (t, *J* = 3.05 Hz,1 H); MS (ESI): found: $[M + H]^+$, 476.20.

Methyl (*E*)-(2-((4-(2,3-dichlorophenyl)piperazin-1-yl)methyl)-3-phenylacryloyl)valinate (12g)

Following above general procedure C, reaction of **11b** and methyl valinate hydrochloride resulted in **12g** as a thick liquid (Yield: 73%, purity by LC-MS: >99%); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm : 0.98 - 1.04 (dd, *J* = 11.28, 6.88 Hz, 6 H), 2.25 - 2.31 (m, *J* = 6.85, 4.72 Hz, 1 H), 2.67 - 2.74 (m, 4 H), 3.09 - 3.14 (d, *J* = 13.94 Hz, 4 H), 3.49 - 3.63 (m, 2 H), 3.75 (s, 3 H), 4.69 - 4.73 (dd, *J* = 8.76, 4.63 Hz, 1 H), 6.94 - 6.98 (dd, *J* = 6.46, 3.16 Hz, 1 H), 7.14 - 7.18 (m, 2 H),

7.26 - 7.30 (m, 3 H), 7.31 - 7.44 (m, 3 H), 7.98 (s, 1 H), 10.22 - 10.25 (d, *J* = 8.80 Hz, 1 H); MS (ESI): found: $[M + H]^+$, 504.25.

Ethyl (*E*)-(2-((4-(4-chlorophenyl)piperazin-1-yl)methyl)-3-phenylacryloyl)glycinate (12k)

Following above general procedure C, reaction of **11c** and ethyl glycinate hydrochloride resulted in **12k** as a thick liquid (Yield: 68%, purity by LC-MS: >99%); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm : 1.19 - 1.23 (t, *J* = 6.00 Hz, 3 H), 2.54-2.57 (t, *J* = 4.54 Hz, 4 H), 3.12 - 3.16 (t, *J* = 6.24 Hz, 4 H), 3.45 (s, 2 H), 4.08 - 4.17 (m, 4 H), 6.73 - 6.76 (m, 2 H), 7.09 - 7.13 (m, 2 H), 7.18 - 7.21 (m, 2 H), 7.25 - 7.34 (m, 3 H), 7.93 (s, 1 H), 10.09 - 10.12 (t, *J* = 4.36 Hz, 3 H); found: $[M + H]^+$, 442.20.

Methyl (*E*)-(2-((4-(4-chlorophenyl)piperazin-1-yl)methyl)-3-phenylacryloyl)valinate (12l)

Following above general procedure C, reaction of **11c** and methyl valinate hydrochloride resulted in **12l** as a thick liquid (Yield: 72%, purity by LC-MS: >99%); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm : 0.93 - 0.96 (d, *J* = 6.88 Hz, 3 H), 0.99 - 1.01 (d, *J* = 6.88 Hz, 3 H), 2.22 - 2.28 (m, *J* = 6.83, 4.68 Hz, 1 H), 2.60 - 2.62 (d, *J* = 4.95 Hz, 2 H), 2.66 - 2.72 (m, 2 H), 3.18 - 3.24 (m, 4 H), 3.47 - 3.60 (q, *J* = 10.50, 8.00 Hz, 2 H), 3.73 (s, 1 H), 4.66 - 4.71 (q, *J* = 8.71, 4.58 Hz, 1 H), 6.78 - 6.86 (m, 2 H), 7.16 - 7.23 (m, 2 H), 7.25 - 7.29 (m, 2 H), 7.30 - 7.43 (m, 3 H), 7.98 (s, 1 H), 10.09 - 10.12 (d, *J* = 7.89 Hz, 1 H); found: $[M + H]^+$, 470.25.

Ethyl (*E*)-(2-((4-(4-chlorophenyl)piperazin-1-yl)methyl)-3-phenylacryloyl)leucinate (12m)

Following above general procedure C, reaction of **11c** and ethyl leucinate hydrochloride resulted in **12m** as a thick liquid (Yield: 57%, purity by LC-MS: >99%); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm : 0.97 - 1.01 (t, *J* = 6.14 Hz, 6 H), 1.28 - 1.32 (t, *J* = 7.15 Hz, 4 H), 1.59 - 1.80 (m, 3 H), 2.60 - 2.65 (m, 2 H), 2.67 - 2.72 (m, 2 H), 3.18 - 3.28 (m, 4 H), 3.49 - 3.60 (q, *J* = 6.00 Hz, 2 H), 4.17 - 4.24 (q, *J* = 7.15 Hz, 2 H), 4.68 - 4.75 (m, *J* = 8.23, 5.00 Hz, 1 H), 6.81 - 6.86 (m, 2 H),

7.19 - 7.24 (m, 2 H), 7.27 - 7.31 (m, 2 H), 7.33 - 7.43 (m, 3 H), 8.00 (s, 1 H), 10.13 - 10.16 (d, *J* = 7.79 Hz, 1 H); found: $[M + H]^+$, 498.25.

Methyl (*E*)-2-(2-((4-(4-chlorophenyl)piperazin-1-yl)methyl)-3-phenylacrylamido)-3-methylpentanoate (12n)

Following above general procedure C, reaction of **11c** and methyl 2-amino-3-methylpentanoate hydrochloride resulted in **12n** as a thick liquid (Yield: 62%); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm : (Yield: 62%, purity by LC-MS: >99%); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 0.83 - 0.90 (m, 6 H), 1.07 - 1.23 (m, 2 H), 1.34 - 1.49 (m, 1 H), 1.84 - 1.97 (m, 1 H), 2.48 - 2.57 (m, 2 H), 2.57 - 2.68 (m, 2 H), 3.04 - 3.22 (m, 4 H), 3.38 - 3.53 (m, 2 H), 3.65 (s, 3 H), 4.62 - 4.66 (q, *J* = 8.48, 4.72 Hz, 1 H), 6.70 - 6.78 (m, 2 H), 7.09 - 7.15 (m, 2 H), 7.16 - 7.22 (m, 2 H), 7.23 - 7.35 (m, 3 H), 7.91 (s, 1 H), 10.04 - 10.07 (d, *J* = 8.53 Hz, 1 H); found: $[M + H]^+$, 484.25.

Ethyl (*E*)-2-(2-((4-(4-chlorophenyl)piperazin-1-yl)methyl)-3-phenylacrylamido)pentanoate (12o)

Following above general procedure C, reaction of **11c** and ethyl 2-aminopentanoate hydrochloride resulted in **12o** as a thick liquid (Yield: 66%, purity by LC-MS: >99%); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm : 0.92 - 0.96 (t, *J* = 7.34 Hz, 3 H), 1.26 - 1.30 (t, *J* = 6.34 Hz, 3 H), 1.36 - 1.44 (m, 2 H), 1.66 - 1.77 (m, 2 H), 1.81 - 1.91 (m, 1 H), 2.58 - 2.69 (m, 4 H), 3.14 - 3.25 (m, 4 H), 3.47 - 3.58 (m, 2 H), 4.15 - 4.22 (q, *J* = 7.15 Hz, 2 H), 4.67 - 4.73 (m, *J* = 7.31, 5.46 Hz, 1 H), 6.79 - 6.84 (m, 2 H), 7.17 - 7.21 (m, 2 H), 7.24 - 7.26 (m, 2 H), 7.33 - 7.41 (m, 3 H), 7.98 (s, 1 H), 10.17 - 10.19 (d, *J* = 7.79 Hz, 1 H); found: $[M + H]^+$, 484.25.

Ethyl (*E*)-(2-((4-(4-hydroxyphenyl)piperazin-1-yl)methyl)-3-phenylacryloyl)glycine (12p)

Following above general procedure C, reaction of **11d** and ethyl glycinate hydrochloride resulted in **12p** as a thick liquid (Yield: 47%, purity by LC-MS: >99%); ^{1}H NMR (300 MHz, $CDCl_3$) δ

ppm : 1.26 - 1.31 (t, *J* = 7.34 Hz, 3 H), 2.65 (s, 4 H), 2.81 (s, 1 H), 3.10 - 3.13 (t, *J* = 4.58 Hz, 4 H), 3.53 (s, 2 H), 4.15 - 4.17 (d, *J* = 5.14 Hz, 2 H), 4.19 - 4.24 (t, *J* = 6. 14 Hz, 2 H), 6.75 - 6.79 (m, 2 H), 6.81 - 6.84 (m, 2 H), 7.25 (s, 1 H), 7.28 (d, *J* = 1.19 Hz, 1 H), 7.32 - 7.41 (m, 3 H), 7.98 (s, 1 H); MS (ESI): found: $[M + H]^+$, 424.15.

Methyl (*E*)-(2-((4-(4-hydroxyphenyl)piperazin-1-yl)methyl)-3-phenylacryloyl)valinate (12q)

Following above general procedure C, reaction of **11d** and methyl valinate hydrochloride resulted in **12q** as a thick liquid (Yield: 52%, purity by LC-MS: >99%); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm : 0.87 - 0.94 (q, *J* = 13.43, 6.83 Hz, 6 H), 2.11 - 2.24 (m, *J* = 11.63, 6.85 Hz, 1 H), 2.57 (s, 2 H), 2.63 (s, 2 H), 3.04 - 3.05 (d, *J* = 3.94 Hz, 4 H), 3.40 - 3.53 (q, *J* = 6.83 Hz, 2 H), 3.65 (s, 3 H), 4.58 - 4.62 (q, *J* = 8.57, 4.63 Hz, 1 H), 6.68 - 6.71 (m, 2 H), 6.74 - 6.77 (m, 2 H), 7.18 (s,1 H), 7.20 (d, *J* = 1.28 Hz, 1 H), 7.23 - 7.33 (m, 3 H), 7.89 (s, 1 H), 10.14 (s, 1 H); MS (ESI): found: $[M + H]^+$, 452.30.

Ethyl (*E*)-(2-((4-(4-hydroxyphenyl)piperazin-1-yl)methyl)-3-phenylacryloyl)leucinate (12r)

Following above general procedure C, reaction of **11d** and ethyl leucinate hydrochloride resulted in **12r** as a thick liquid (Yield: 49%, purity by LC-MS: >99%); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm : 0.95 - 0.98 (dd, *J* = 6.05, 4.22 Hz, 6 H), 1.25 - 1.30 (t, *J* = 7.34 Hz, 3 H), 1.61 - 1.77 (m, 2 H), 2.55 - 2.74 (m, 4 H), 3.08 (s, 4 H), 3.46 - 3.58 (q, *J* = 15.34 Hz, 2 H), 4.15 - 4.22 (q, *J* = 7.15 Hz, 2 H), 4.64 - 4.72 (td, *J* = 8.28, 5.09 Hz, 1 H), 6.75 - 6.78 (m, 2 H), 6.81 - 6.84 (m, 2 H), 7.24 (s, 2 H), 7.31- 7.40 (m, 3 H), 7.97 (s, 1 H), 10.23 - 10.25 (d, *J* = 7.79 Hz, 1 H); MS (ESI): found: $[M + H]^+$, 480.30.

Methyl (*E*)-2-(2-((4-(4-hydroxyphenyl)piperazin-1-yl)methyl)-3-phenylacrylamido)-3-methylpentanoate (12s)

Following above general procedure C, reaction of **11d** and methyl 2-amino-3-methylpentanoate hydrochloride resulted in **12s** as a thick liquid (Yield: 61%, purity by LC-MS: >99%); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm : 0.90 - 0.98 (m, 6 H), 1.20 - 1.28 (m, 2 H), 1.40 - 1.54 (m, 1 H), 1.98 (m, 1 H), 2.63 - 2.68 (d, *J* = 15.41 Hz, 4 H), 3.09 -3.10 (d, *J* = 3.76 Hz, 4 H), 3.45 - 3.58 (m, 2 H), 3.71 (s, 3 H), 4.68 - 4.72 (q, *J* = 8.39, 4.72 Hz, 1 H), 6.73 - 6.85 (m, 4 H), 7.22 - 7.27 (m, 2 H), 7.30 - 7.41 (m, 3 H), 7.96 (s, 1 H), 10.29 - 10.31 (d, *J* = 8.34 Hz, 1 H); MS (ESI): found: $[M + H]^+$, 466.30.

Ethyl (*E*)-2-(2-((4-(4-hydroxyphenyl)piperazin-1-yl)methyl)-3-phenylacrylamido) pentanoate (12t)

Following above general procedure C, reaction of **11d** and ethyl 2-aminopentanoate hydrochloride resulted in **12t** as a thick liquid (Yield: 58%, purity by LC-MS: 97%); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm : 0.91 - 0.96 (t, *J* = 7.34 Hz, 3 H), 1.26 - 1.29 (t, *J* = 6.01 Hz, 3 H), 1.35 - 1.46 (m, 2 H), 1.67 - 1.77 (m, 1 H), 1.78 - 1.94 (m, 2 H), 2.65 (s, 4 H), 3.00 - 3.20 (m, 4 H), 3.47 - 3.57 (m, 2 H), 4.15 - 4.22 (q, *J* = 7.15 Hz, 2 H), 4.65 - 4.72 (m, 1 H), 6.75 - 6.83 (m, 4 H), 7.24 - 7.26 (m, 2 H), 7.31 - 7.40 (m, 3 H), 7.96 (s, 1 H), 10.33 - 10.35 (d, *J* = 7.79 Hz, 1 H); MS (ESI): found: $[M + H]^+$, 466.30.

Ethyl (*E*)-(2-((4-(4-fluorophenyl)piperazin-1-yl)methyl)-3-phenylacryloyl)glycinate (12u)

Following above general procedure C, reaction of **11e** and ethyl glycinate hydrochloride resulted in **12u** as a thick liquid (Yield: 76%, purity by LC-MS: >99%); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm : 1.26 - 1.31 (t, *J* = 7.15 Hz, 3 H), 2.65 (s, 4 H), 3.15 - 3.28 (t, *J* = 6.14 Hz, 4 H), 3.53 (s, 2 H), 4.15 - 4.17 (d, *J* = 5.14 Hz, 2 H), 4.20 - 4.24 (t, *J* = 7.15 Hz, 2 H), 6.81 - 6.90 (m, 2 H), 6.90 -

7.01 (m, 2 H), 7.27 (d, *J* = 6.24 Hz, 2 H), 7.31 - 7.43 (m, 3 H), 7.99 (s, 1 H), 10.18 (s, 1 H); MS (ESI): found: $[M + H]^+$, 426.20.

Methyl (*E*)-(2-((4-(4-fluorophenyl)piperazin-1-yl)methyl)-3-phenylacryloyl)valinate (12v)

Following above general procedure C, reaction of **11e** and methyl valinate hydrochloride resulted in **12v** as a thick liquid (Yield: 63%, purity by LC-MS: >99%); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm : 0.94 - 1.02 (q, *J* = 14.40, 6.88 Hz, 6 H), 2.22 -2.09 (m, *J* = 6.83, 4.77 Hz, 1 H), 2.57 - 2.65 (m, 2 H), 2.67 - 2.76 (m, 2 H), 3.10 - 3.21 (m, 4 H), 3.47 - 3.59 (q, *J* = 12.40, 8.88 Hz, 2 H), 3.73 (s, 3 H), 4.66 - 4.71 (q, *J* = 8.71, 4.58 Hz, 1 H), 6.82 - 6.89 (m, 2 H), 6.91 - 7.00 (m, 2 H), 7.27 (dd, *J* = 6.19, 1.88 Hz, 2 H), 7.31 - 7.42 (m, 3 H), 7.98 (s, 1 H), 10.11 - 10.13 (d, *J* = 8.53 Hz, 1 H); MS (ESI): found: $[M + H]^+$, 454.30.

Ethyl (*E*)-(2-((4-(4-fluorophenyl)piperazin-1-yl)methyl)-3-phenylacryloyl)leucinate (12w)

Following above general procedure C, reaction of **11e** and ethyl leucinate hydrochloride resulted in **12w** as a thick liquid (Yield: 70%, purity by LC-MS: >99%); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm : 0.95 - 0.99 (t, *J* = 5.73 Hz, 6 H), 1.26 - 1.31 (t, *J* = 7.11 Hz, 4 H), 1.55 - 1.64 (m, 1 H), 1.66 - 1.76 (m, 2 H), 2.55 - 2.65 (m, 2 H), 2.66 - 2.75 (m, 2 H), 3.06 - 3.22 (m, 4 H), 3.47 - 3.58 (q, *J* = 12.03 Hz, 2 H), 4.15 - 4.22 (q, *J* = 6.00, 9.00 Hz, 2 H), 4.66 - 4.73 (m, *J* = 8.25, 5.04 Hz, 1 H), 6.82 - 6.90 (m, 2 H), 6.91 - 7.00 (m, 2 H), 7.24 - 7.29 (m, 3 H), 7.30 - 7.42 (m, 3 H), 7.98 (s, 1 H), 10.12 - 10.14 (d, *J* = 7.79 Hz, 1 H); MS (ESI): found: $[M + H]^+$, 482.35.

Methyl (*E*)-2-(2-((4-(4-fluorophenyl)piperazin-1-yl)methyl)-3-phenylacrylamido)-3-methyl pentanoate (12x)

Following above general procedure C, reaction of **11e** and methyl 2-amino-3-methylpentanoate hydrochloride resulted in **12x** as a thick liquid (Yield: 76%, purity by LC-MS: >99%); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm : 0.91 - 0.98 (q, *J* = 6.04 Hz, 6 H), 1.15 - 1.30 (m, 2 H), 1.43 - 1.57

(m, 1 H), 1.91 - 2.04 (m, 1 H), 2.55 - 2.65 (m, 2H), 2.66 - 2.77 (m, 2 H), 3.09 - 3.23 (m, 4 H), 3.46 - 3.59 (q, *J* = 12.00, 9.02 Hz, 2 H), 3.73 (s, 3 H), 4.69 - 4.73 (q, *J* = 8.53, 4.77 Hz, 1 H), 6.82 - 6.90 (m, 2 H), 6.91 - 7.01 (m, 2 H), 7.24 - 7.29 (m, 2 H), 7.31 - 7.42 (m, 3 H), 7.98 (s, 1 H), 10.13 - 10.16 (d, *J* = 8.44 Hz, 1 H); MS (ESI): found: $[M + H]^+$, 468.30.

Ethyl (*E*)-2-(2-((4-(4-fluorophenyl)piperazin-1-yl)methyl)-3-phenylacrylamido)pentanoate (12y)

Following above general procedure C, reaction of **11e** and ethyl 2-aminopentanoate hydrochloride resulted in **12y** as a thick liquid (Yield: 72%, purity by LC-MS: >99%); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm : 0.85 - 0.90 (t, *J* = 7.34 Hz, 3 H), 1.19 - 1.23 (t, *J* = 7.15 Hz, 3 H), 1.27 - 1.41 (m, 2 H), 1.65 - 1.86 (m, 2 H), 2.49 - 2.67 (m, 4 H), 3.00 - 3.16 (m, 4 H), 3.40 - 3.51 (q, *J* = 15.15, 3.00 Hz, 2 H), 4.08 - 4.16 (q, *J* = 7.15 Hz, 2 H), 4.59 - 4.66 (m, 1 H), 6.74 - 6.82 (m, 2 H), 6.84 - 6.94 (m, 2 H), 7.18 (s, 1 H), 7.20 (s, 1 H), 7.23 - 7.35 (m, 3 H), 7.90 (s, 1 H), 10.11 - 10.13 (d, *J* = 7.79 Hz, 1 H); MS (ESI): found: $[M + H]^+$, 468.30.

Ethyl (*E*)-(2-((4-(2-chlorophenyl)piperazin-1-yl)methyl)-3-phenylacryloyl)glycinate (12ac)

Following above general procedure C, reaction of **11i** and ethyl glycinate hydrochloride resulted in **12ac** as a thick liquid (Yield: 64%); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 1.28 - 1.33 (t, *J* = 7.15 Hz, 3 H), 2.69 (s, 4 H), 3.12 (s, 4 H), 3.56 (s, 2 H), 4.16 - 4.20 (d, *J* = 5.14 Hz, 2 H), 4.22 - 4.27 (m, 2 H), 6.94 - 6.99 (td, *J* = 7.61, 1.56 Hz, 1 H), 7.04 - 7.08 (dd, *J* = 8.07, 1.47 Hz, 1 H), 7.19 - 7.24 (td, *J* = 7.68, 1.51 Hz, 1 H), 7.29 (d, *J* = 1.19 Hz, 2 H), 7.30 - 7.37 (m, 3 H), 7.38 - 7.42 (m, 1 H), 7.99 (s, 1 H), 10.32 - 10.34 (t, *J* = 3.14 Hz 1 H).

Ethyl (*E*)-(2-((4-(3-chlorophenyl)piperazin-1-yl)methyl)-3-phenylacryloyl)glycinate (12ad)

Following above general procedure C, reaction of **11j** and ethyl glycinate hydrochloride resulted in **12ad** as a thick liquid (Yield: 59%); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 1.26 - 1.31 (t, *J*

= 7.15 Hz, 3 H), 2.61 - 2.64 (t, *J* = 6.00 Hz, 3 H), 2.67 - 2.70 (t, *J* = 4.63 Hz, 1 H), 3.23 - 3.27 (t, *J* = 6.00 Hz, 4 H), 3.53 (s, 2 H), 4.16 - 4.17 (d, *J* = 5.04 Hz, 2 H), 4.20 - 4.27 (q, *J* = 6.04 Hz, 2 H), 6.72 - 6.89 (m, 3 H), 7.10 - 7.20 (m, 1 H), 7.22 - 7.26 (m, 1 H), 7.27 - 7.30 (m, 1 H), 7.30 - 7.42 (m, 3 H), 8.00 (s, 1 H), 10.16 -10.19 (t, *J* = 4.54 Hz, 1 H).

General procedure for synthesis of 13a-af, 18a-e, 23a, 23f-h, 28a-j (General procedure D)

To a solution of 12a-12af, 17a-17e, 22a, 22f-22h or 27a-27j (0.49 mmol) in 5.0 mL 1:1 mixture of methanol and THF was added $LiOH.H_2O$ (1.47 mmol) pre dissolved in 0.5 mL of water, the reaction mass was stirred at 25-30^o C for 2-4 h. Completion of reaction was monitored by TLC. THF was removed from the reaction mass under reduced pressure, water was added and the pH was adjusted up to 7 using 1N HCl. The product was extracted twice with ethyl acetate. Ethyl acetate layers were dried with anhydrous sodium sulphate, filtered and concentrated to get product as white color solid.

(*E*)-(3-Phenyl-2-((4-phenylpiperazin-1-yl)methyl)acryloyl)Glycine (13a)

Following the above general procedure D, **13a** was synthesized from **12a** as a white solid (Yield: 46%, purity by LC-MS: 98%); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 3.18 (s, 4 H), 3.35 (s, 4 H), 3.46 (s, 2 H), 3.94 - 3.96 (d, *J* = 5.32 Hz, 2 H), 6.74 - 6.79 (t, *J* = 7.20 Hz, 1 H), 6.90 - 6.93 (d, *J* = 8.07 Hz, 2 H), 7.17 - 7.22 (t, *J* = 1.00 Hz, 2 H), 7.33 - 7.40 (m, 1 H), 7.41 - 7.48 (m, 4 H), 7.65 (s, 1 H), 9.67 - 9.70 (t, *J* = 5.23 Hz, 1 H), 12.65 (s, 1 H); MS (ESI): found: $[M + H]^+$, 380.15.

(*E*)-(3-Phenyl-2-((4-phenylpiperazin-1-yl)methyl)acryloyl)valine (13b)

Following the above general procedure D, **13b** was synthesized from **12b** as a white solid (Yield: 51%, purity by LC-MS: >99%); M.P: 188 - 192^o C; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 0.90 - 0.95 (t, *J* = 6.20 Hz, 6 H), 2.14 - 2.20 (dd, *J* = 11.60, 6.74 Hz, 1 H), 2.54 - 2.55 (d, *J* = 4.58 Hz,

4 H), 3.17 - 3.18 (d, *J* = 3.67 Hz, 4 H), 3.45 - 3.59 (m, 2 H), 4.34 - 4.39 (dd, *J* = 8.53, 4.58 Hz, 1 H), 6.74 - 6.79 (t, *J* = 7.20 Hz, 1 H), 6.89 - 6.92 (d, *J* = 8.07 Hz, 2 H), 7.17 - 7.22 (t, *J* = 6.20 Hz, 2 H), 7.30 - 7.44 (m, 5 H), 7.71 (s, 1 H), 9.92 - 9.94 (d, *J* = 8.53 Hz, 1 H), 12.75 (s, 1 H); MS (ESI): found: $[M + H]^+$, 422.25.

(*E*)-(3-Phenyl-2-((4-phenylpiperazin-1-yl)methyl)acryloyl)leucine (13c)

Following the above general procedure D, **13c** was synthesized from **12c** as a white solid (Yield: 53%, purity by LC-MS: >99%); M.P: 82 - 85° C; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 0.84 - 0.91 (q, *J* = 8.10 Hz, 6 H), 1.13 - 1.29 (m, 1 H), 1.43 - 1.50 (m, 1 H), 1.85 - 1.92 (m, 1 H), 2.55 (s, 4 H), 3.16 - 3.18 (d, *J* = 3.58Hz, 4 H), 3.50 (s, 2 H), 4.37 - 4.41 (dd, *J* = 8.34, 4.77 Hz, 1 H), 6.74 - 6.79 (t, *J* = 7.24 Hz, 1 H), 6.89 - 6.92 (d, *J* = 8.10 Hz, 2 H), 7.17 - 7.22 (t, *J* = 9.10 Hz, 2 H), 7.36 - 7.46 (m, 5 H), 7.71 (s, 1 H), 9.93 - 9.96 (d, *J* = 8.44 Hz, 1 H), 12.77 (s, 1 H); ^{13}C NMR (DMSO- d_6) δ ppm : 173.39, 167.33, 151.28, 139.19, 135.66, 130.99, 129.59, 129.41, 128.92, 128.54, 119.43, 115.86, 56.89, 54.73, 52.54, 48.44, 37.37, 25.41, 16.45 and 12.04; MS (ESI): found: $[M + H]^+$, 436.25.

(*E*)-3-Methyl-2-(3-phenyl-2-((4-phenylpiperazin-1-yl)methyl)acrylamido)pentanoic acid (13d)

Following the above general procedure D, **13d** was synthesized from **12d** as a white solid (Yield: 64%, purity by LC-MS: >99%); M.P: 87 - 91° C; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 0.88 - 0.91 (t, *J* = 7.34 Hz, 3 H), 1.33 - 1.40 (m, 2 H), 1.66 - 1.78 (m, 2 H), 2.54 (s, 4 H), 3.15 (s, 4 H), 3.48 (s, 2 H), 4.35 - 4.39 (m, 1 H), 6.74 - 6.79 (t, *J* = 7.24 Hz, 1 H), 6.90 - 6.93 (d, *J* = 8.07 Hz, 2 H), 7.20 - 7.22 (t, *J* = 6.10 Hz, 2 H), 7.36 - 7.49 (m, 5 H), 7.66 (s, 1 H), 9.83 - 9.85 (d, *J* = 7.61 Hz, 1 H), 12.73 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm : 174.58, 167.56, 151.33, 138.52, 135.72,

131.71, 129.69, 129.40, 128.91, 128.54, 119.41, 115.89, 54.54, 52.52, 51.21, 48.61, 25.00, 23.38 and 22.15; MS (ESI): found: $[M + H]^+$, 436.30.

(*E*)-2-(3-Phenyl-2-((4-phenylpiperazin-1-yl)methyl)acrylamido)pentanoic acid (13e)

Following the above general procedure D, **13e** was synthesized from **12e** as a white solid (Yield: 59%, purity by LC-MS: >99%); M.P: 90-92^o C; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 0.89 - 0.91 (d, *J* = 5.50 Hz, 6 H), 1.57 - 1.62 (m, 2 H), 1.68 - 1.75 (m, 1 H), 2.51 (s, 4 H), 3.15 (s, 4 H), 3.47 (s, 2 H), 4.34 - 4.41 (q, *J* = 7.55 Hz, 1 H), 6.75 - 6.80 (t, *J* = 7.24 Hz, 1 H), 6.90 - 6.93 (d, *J* = 8.16 Hz, 2 H), 7.17 - 7.23 (t, *J* = 8.30 Hz, 2 H), 7.36 - 7.44 (m, 5 H), 7.63 (s, 1 H), 9.68 - 9.69 (d, *J* = 7.79 Hz, 1 H), 12.73 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm : 174.08, 167.41, 151.33, 138.71, 135.70, 131.44, 129.67, 129.40, 128.91, 128.56, 119.40, 115.88, 54.58, 52.50, 52.33, 48.55, 34.05, 18.85 and 14.09; MS (ESI): found: $[M + H]^+$, 422.30.

(*E*)-(2-((4-(2,3-Dichlorophenyl)piperazin-1-yl)methyl)-3-phenylacryloyl)glycine (13f)

Following the above general procedure D, **13f** was synthesized from **12f** as a white solid (Yield: 63%, purity by LC-MS: >99%); M.P: 119 - 122^o C; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 2.50 - 2.51 (s, 4 H), 3.04 (s, 4 H), 3.47 (s, 2 H), 3.83 - 3.85 (d, *J* = 4.68 Hz, 2 H), 7.10 - 7.13 (dd, *J* = 5.55, 4.08 Hz, 1 H), 7.28 - 7.30 (m, 2 H), 7.38 - 7.44 (m, 5 H), 7.64 (s, 1 H), 9.59 - 9.60 (t, *J* = 4.40 Hz, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm : 171.86, 167.57, 151.55, 138.44, 135.83, 133.04, 131.83, 129.72, 128.90, 128.51, 126.49, 124.84, 120.07, 54.38, 52.68, 51.08 and 42.81; MS (ESI): found: $[M + H]^+$, 448.10.

(*E*)-(2-((4-(2,3-Dichlorophenyl)piperazin-1-yl)methyl)-3-phenylacryloyl)valine (13g)

Following the above general procedure D, **13g** was synthesized from **12g** as a white solid (Yield: 59%, purity by LC-MS: >99%); M.P: 116 - 120^o C; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 0.88 - 0.90 (d, *J* = 6.60 Hz, 6 H), 2.15 - 2.21 (m, 1 H), 2.51 (s, 4 H), 3.01 - 3.07 (d, 4 H), 3.47 (s, 2 H),

4.16 - 4.20 (q, *J* = 7.52, 3.39 Hz, 1 H), 7.07 - 7.10 (m, 1 H), 7.26 - 7.28 (d, *J* = 4.95 Hz, 2 H), 7.32 - 7.46 (m, 5 H), 7.65 (s, 1 H), 9.60 - 9.62 (d, *J* = 8.34 Hz, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm : 174.31, 166.73, 151.54, 138.11, 136.05, 133.03, 132.24, 129.55, 128.86, 128.28, 126.45, 124.79, 120.02, 59.31, 54.72, 52.72, 51.06, 31.38, 20.33 and 18.61; MS (ESI): found: $[M + H]^+$, 490.20.

(*E*)-(2-((4-(2,3-Dichlorophenyl)piperazin-1-yl)methyl)-3-phenylacryloyl)leucine (13h)

Following the above general procedure D, **13h** was synthesized from **12h** as a white solid (Yield: 60%, purity by LC-MS: >99%); M.P: 129 - 134^o C; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 0.90 - 0.94 (t, *J* = 6.01 Hz, 6 H), 1.60 - 1.66 (m, 2 H), 1.70 - 1.77 (m, 1 H), 2.55 (s, 4 H), 3.00 (s, 4 H), 3.48 (s, 2 H), 4.35 - 4.42 (q, *J* = 6.00 Hz, 1 H), 7.09 - 7.12 (dd, *J* = 5.87, 3.76 Hz, 1 H), 7.30 - 7.32 (m, 2 H), 7.36 - 7.45 (m, 5 H), 7.61 (s, 1 H), 9.58 - 9.61 (d, *J* = 7.70 Hz, 1 H), 12.16 (s, 1H); ^{13}C NMR (DMSO- d_6) δ ppm : 174.66, 167.57, 151.41, 138.47, 135.74, 133.11, 131.81, 129.69, 128.95, 128.91, 128.54, 126.55, 124.97, 119.92, 54.47, 52.64, 51.26, 41.20, 25.02, 23.43 and 22.17; MS (ESI): found: $[M + H]^+$, 504.25.

(*E*)-2-(2-((4-(2,3-Dichlorophenyl)piperazin-1-yl)methyl)-3-phenylacrylamido)-3-methyl pentanoicacid (13i)

Following the above general procedure D, **13i** was synthesized from **12i** as a white solid (Yield: 61%, purity by LC-MS: >99%); M.P: 149 - 154^o C; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 0.88 - 0.94 (q, *J* = 9.01 Hz, 6 H), 1.20 - 1.24 (m, 1 H), 1.46 - 1.54 (q, *J* = 12.65, 5.41 Hz, 1 H), 1.91 (m, 1 H), 2.69 (s, 4 H), 3.03 (s, 4 H), 3.51 (s, 2 H), 4.34 - 4.38 (q, *J* = 7.93, 4.91 Hz, 1 H), 7.07 - 7.11 (dd, *J* = 5.64, 3.99 Hz, 1 H), 7.30 - 7.31 (d, *J* = 6.00 Hz, 2 H), 7.33 - 7.49 (m, 6 H), 7.69 (s, 1 H), 9.84 - 9.87 (d, *J* = 8.62 Hz, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm : 173.54, 167.16,

151.39, 138.99, 129.59, 128.91, 119.89, 57.22, 54.66, 52.66, 51.09, 37.58, 25.46, 16.48 and 12.13; MS (ESI): found: $[M + H]^+$, 504.20.

(*E*)-2-(2-((4-(2,3-Dichlorophenyl)piperazin-1-yl)methyl)-3-phenylacrylamido) pentanoic acid (13j)

Following the above general procedure D, **13j** was synthesized from **12j** as a white solid (Yield: 72%, purity by LC-MS: >99%); M.P: 88 - 93^o C; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 0.89 - 0.93 (t, *J* = 7.29 Hz, 3 H), 1.37 - 1.42 (m, 2 H), 1.67 - 1.80 (m, 2 H), 2.56 (s, 4 H), 3.01 (s, 4 H), 3.50 (s, 2 H), 4.35 - 4.42 (m, *J* = 8.69 Hz, 1 H), 7.09 - 7.12 (dd, *J* = 5.69, 3.94 Hz, 1 H), 7.30 - 7.32 (m, 2 H), 7.35 - 7.45 (m, 5 H), 7.64 (s, 1 H), 9.76 - 9.79 (d, *J* = 7.52 Hz, 1 H), 12.91 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm : 174.20, 167.35, 151.41, 138.65, 135.74, 133.11, 131.56, 129.67, 128.90, 128.53, 126.55, 124.96, 119.33, 54.52, 52.64, 52.51, 51.21, 34.19, 18.88 and 14.16; MS (ESI): found: $[M + H]^+$, 490.20.

(*E*)-(2-((4-(4-Chlorophenyl)piperazin-1-yl)methyl)-3-phenylacryloyl)glycine (13k)

Following the above general procedure D, **13k** was synthesized from **12k** as a white solid (Yield: 53%, purity by LC-MS: >99%); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 2.56 (s, 4 H), 3.23 (s, 4 H), 3.50 (s, 2 H), 3.96 (s, 2 H), 6.96 - 6.98 (d, *J* = 7.70 Hz, 2 H), 7.25 - 7.27 (d, *J* = 7.43 Hz, 2 H), 7.41 (s, 1 H), 7.48 (s, 4 H), 7.70 (s, 1 H), 9.67 (s, 1 H); MS (ESI): found: $[M + H]^+$, 414.15.

(*E*)-(2-((4-(4-Chlorophenyl)piperazin-1-yl)methyl)-3-phenylacryloyl)valine (13l)

Following the above general procedure D, **13l** was synthesized from **12l** as a white solid (Yield: 51%, purity by LC-MS: >99%); M.P: 104 - 107^o C; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 0.92 - 0.94 (d, *J* = 6.06 Hz, 6 H), 2.16 (s, 1 H), 2.51 (s, 4 H), 3.18 (s, 4 H), 3.50 (s, 2 H), 4.34 (s, 1 H), 6.90 - 6.93 (d, *J* = 8.07 Hz, 2 H), 7.20 -7 .22 (d, *J* = 7.52 Hz, 2 H), 7.38 - 7.43 (m, 5 H), 7.70 (s, 1 H), 9.83 - 9.85 (d, *J* = 7.70 Hz, 1 H), 12.13 (m, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm :

173.46, 167.39, 150.06, 139.10, 135.68, 131.11, 129.59, 129.09, 128.91, 128.52, 122.87, 117.28, 57.74, 54.69, 52.36, 48.25, 30.74, 19.87 and 18.31; MS (ESI): found: $[M + H]^+$, 456.25.

(*E*)-(2-((4-(4-Chlorophenyl)piperazin-1-yl)methyl)-3-phenylacryloyl)leucine (13m)

Following the above general procedure D, **13m** was synthesized from **12m** as a white solid (Yield: 60%, purity by LC-MS: 98%); M.P: 150 - 152^o C; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 0.88 - 0.90 (d, *J* = 5.59 Hz, 6 H), 1.58 - 1.62 (m, 2 H), 1.68 - 1.74 (m, 1 H), 2.55 (s, 4 H), 3.15 (s, 4 H), 3.46 (s, 2 H), 4.34 - 4.42 (q, *J* = 9.00 Hz, 1 H), 6.91 - 6.94 (d, *J* = 9.59 Hz, 2 H), 7.20 - 7.23 (d, *J* = 9.00, 2 H), 7.35 - 7.46 (m, 5 H), 7.62 (s, 1 H), 9.61 - 9.64 (d, *J* = 6.0 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ ppm : 174.54, 167.55, 150.12, 138.50, 135.72, 131.73, 129.68, 129.08, 128.90, 128.54, 122.91, 117.32, 54.48, 52.34, 51.22, 48.43, 25.00, 23.37 and 22.16; MS (ESI): found: $[M + H]^+$, 470.20.

(*E*)-2-(2-((4-(4-Chlorophenyl)piperazin-1-yl)methyl)-3-phenylacrylamido)-3-methyl pentanoic acid (13n)

Following the above general procedure D, **13n** was synthesized from **12n** as a white solid (Yield: 79%, purity by LC-MS: >99%); M.P: 162 - 164^o C; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 0.86 - 0.88 (d, *J* = 3.76 Hz, 6 H), 1.17 - 1.19 (m, 1 H), 1.20 - 1.23 (m, 1 H), 1.45 - 1.47 (d, *J* = 5.04 Hz, 1 H), 1.88 (s, 1 H), 2.51 (s, 4 H), 3.16 - 3.19 (d, *J* = 8.34 Hz, 4 H), 3.47 (s, 2 H), 4.29 - 4.30 (d, *J* = 3.76 Hz, 1 H), 6.90 - 6.92 (d, *J* = 6.79 Hz, 2 H), 7.19 - 7.21 (d, *J* = 6.60 Hz, 2 H), 7.38 - 7.42 (m, 5 H), 7.68 (s, 1 H), 9.74 - 9.76 (d, *J* = 7.61 Hz, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm : 173.71, 166.80, 150.12, 138.49, 135.90, 131.76, 129.56, 129.05, 128.87, 128.37, 122.72, 117.35, 58.02, 54.71, 52.37, 48.21, 37.80, 25.44, 16.60 and 12.27; MS (ESI): found: $[M + H]^+$, 470.00.

(*E*)-2-(2-((4-(4-Chlorophenyl)piperazin-1-yl)methyl)-3-phenylacrylamido)pentanoic acid (13o)

Following the above general procedure D, **13o** was synthesized from **12o** as a white solid (Yield: 56%, purity by LC-MS: >99%); M.P: 124 - 129° C; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 0.76 - 0.91 (t, *J* = 8.52 Hz, 3 H), 1.31 - 1.33 (d, *J* = 7.52 Hz, 2 H), 1.65 - 1.73 (m, 2 H), 2.51 (s, 4 H), 3.17 (s, 4 H), 3.44 (s, 2 H), 4.27 (s, 1 H), 6.89 - 6.92 (d, *J* = 7.15 Hz, 2 H), 7.18 - 7.21 (d, *J* = 6.97 Hz, 2 H), 7.35 - 7.42 (m, 5 H), 7.65 (s, 1 H), 9.67 - 7.69 (d, *J* = 5.69 Hz, 1 H); MS (ESI): found: $[M + H]^+$,456.20.

(*E*)-(2-((4-(4-Hydroxyphenyl)piperazin-1-yl)methyl)-3-phenylacryloyl)glycine (13p)

Following the above general procedure D, **13p** was synthesized from **12p** as a white solid (Yield: 56%, purity by LC-MS: 99%); M.P: 133 - 135° C; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 3.00 (s, 4 H), 3.45 (s, 6 H), 3.92 - 3.94 (d, *J* = 5.23 Hz, 2 H), 6.62 - 6.65 (d, *J* = 9.00 Hz, 2 H), 6.74 - 6.77 (d, *J* = 9.00 Hz, 2 H), 7.34 - 7.39 (m, 1 H), 7.40 - 7.44 (m, 4 H), 7.64 (s, 1 H), 8.83 (s, 1 H), 9.66 - 9.70 (t, *J* = 5.32 Hz, 1 H); MS (ESI): found: $[M + H]^+$, 396.15.

(*E*)-(2-((4-(4-Hydroxyphenyl)piperazin-1-yl)methyl)-3-phenylacryloyl)valine (13q)

Following the above general procedure D, **13q** was synthesized from **12q** as a white solid (Yield: 58%, purity by LC-MS: >99%); M.P: 147 - 151° C; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 0.90 - 0.95 (t, *J* = 7.38 Hz, 6 H), 2.14 - 2.20 (m, *J* = 6.72, 5.00 Hz, 1 H), 3.00 (s, 4 H), 2.36 (s, 4 H), 3.50 (s, 2 H), 4.33 - 4.37 (q, *J* = 8.53, 4.58 Hz, 1 H), 6.62 - 6.65 (d, *J* = 9.00 Hz, 2 H), 6.73 - 6.76 (d, *J* = 9.00 Hz, 2 H), 7.36 - 7.45 (m, 5 H), 7.70 (s, 1 H), 8.84 (s, 1 H), 9.92 - 9.95 (d, *J* = 8.44 Hz, 1 H), 12.71 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm : 173.41, 167.47, 151.43, 144.39, 139.10, 135.69, 131.11, 129.58, 128.91, 128.51, 118.16, 115.91, 57.69, 54.79, 52.78, 50.16, 30.67, 19.85 and 18.34; MS (ESI): found: $[M + H]^+$, 438.20.

(*E*)-(2-((4-(4-Hydroxyphenyl)piperazin-1-yl)methyl)-3-phenylacryloyl)leucine (13r)

Following the above general procedure D, **13r** was synthesized from **12r** as a white solid (Yield: 47%, purity by LC-MS: >99%); M.P: 125 - 131° C; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 0.89 - 0.92 (q, *J* = 6.46 Hz, 6 H), 1.57 - 1.62 (m, 2 H), 1.68 - 1.75 (m, *J* = 13.16, 6.92 Hz, 1 H), 2.97 (s, 4 H), 3.36 (m, 4 H), 3.46 (s, 2 H), 4.36 - 4.38 (q, *J* = 7.49 Hz, 1 H), 6.62 - 6.65 (d, *J* = 9.00 Hz, 2 H), 6.74 - 6.77 (d, *J* = 9.00 Hz, 2 H), 7.36 - 7.44 (m, 5 H), 7.62 (s, 1 H), 8.85 (s, 1 H), 9.70 - 9.73 (d, *J* = 7.70 Hz, 1 H), 12.72 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm : 174.53, 167.58, 151.45, 144.45, 138.52, 135.71, 131.68, 129.68, 128.90, 128.54, 118.24, 115.90, 54.57, 52.73, 51.16, 50.38, 24.99, 23.38 and 22.11; MS (ESI): found: $[M + H]^+$, 452.30.

(*E*)-2-(2-((4-(4-Hydroxyphenyl)piperazin-1-yl)methyl)-3-phenylacrylamido)-3-methyl pentanoic acid (13s)

Following the above general procedure D, **13s** was synthesized from **12s** as a white solid (Yield: 54%, purity by LC-MS: >99%); M.P: 123 - 127° C; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 0.86 - 0.93 (m, 6 H), 1.23 - 1.27 (m, 2 H), 1.43 - 1.50 (m, 1 H), 1.85 - 1.94 (m, 1 H), 2.51 (s, 4 H), 2.99 - 3.00 (d, *J* = 3.30 Hz, 4 H), 3.49 (s, 2 H), 4.36 - 3.41 (q, *J* = 8.39, 4.81 Hz, 1 H), 6.62 - 6.65 (d, *J* = 9.00 Hz, 2 H), 6.74 - 6.77 (d, *J* = 9.00 Hz, 2 H), 7.34 - 7.40 (m, 5 H), 7.70 (s, 1 H), 8.82 (s, 1 H), 9.92 - 9.95 (d, *J* = 8.44 Hz, 1 H), 12.67 (s, 1 H); MS (ESI): found: $[M + H]^+$, 452.25.

(*E*)-2-(2-((4-(4-Hydroxyphenyl)piperazin-1-yl)methyl)-3-phenylacrylamido)pentanoic acid (13t)

Following the above general procedure D, **13t** was synthesized from **12t** as a white solid (Yield: 56%, purity by LC-MS: >99%); M.P: 128 - 131° C; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 0.86 - 0.91 (t, *J* = 7.29 Hz, 3 H), 1.33 - 1.40 (m, 2 H), 1.66 - 1.74 (m, 2 H), 2.51 (s, 4 H), 2.99 (s, 4 H), 3.47 (s, 2 H), 4.34 - 4.40 (q, *J* = 6.00 Hz, 1 H), 6.62 - 6.65 (d, *J* = 9.00 Hz, 2 H), 6.74 - 6.77 (d, *J*

= 9.00 Hz, 2 H), 7.34 - 7.47 (m, 5 H), 7.65 (s, 1 H), 8.81 (s, 1 H), 9.83 - 9.85 (d, *J* = 7.61 Hz, 1 H), 12.65 (m, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm : 174.06, 167.41, 151.43, 144.45, 138.69, 135.70, 131.45, 129.67, 128.90, 128.54, 118.23, 115.90, 54.60, 52.72, 52.33, 50.31, 34.05, 18.87 and 14.10; MS (ESI): found: $[M + H]^+$, 438.25.

(*E*)-(2-((4-(4-Fluorophenyl)piperazin-1-yl)methyl)-3-phenylacryloyl)glycine (13u)

Following the above general procedure D, **13u** was synthesized from **12u** as a white solid (Yield: 68%, purity by LC-MS: >99%); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 2.51 (s, 4 H), 3.12 (s, 4 H), 3.46 (s, 2 H), 3.93 - 3.94 (d, *J* = 5.23 Hz, 2 H), 6.90 - 6.94 (m, 2 H), 7.00 - 7.06 (t, *J* = 9.00Hz, 2 H), 7.34 - 7.39 (m, 1 H), 7.40 - 7.44 (m, 4 H), 7.65 (s, 1 H), 9.65 - 9.68 (t, *J* = 5.18 Hz, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm : 171.77, 167.96, 157.98, 154.86, 148.32, 148.29, 138.59, 135.74, 131.70, 129.75, 128.91, 128.59, 117.62, 117.52, 115.84, 115.55, 54.35, 52.50, 49.20 and 41.95; MS (ESI): found: $[M + H]^+$, 398.15.

(*E*)-(2-((4-(4-Fluorophenyl)piperazin-1-yl)methyl)-3-phenylacryloyl)valine (13v)

Following the above general procedure D, **13v** was synthesized from **12v** as a white solid (Yield: 62%, purity by LC-MS: >99%); M.P: 98 - 102^o C; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 0.86 - 0.91 (t, *J* = 7.20 Hz, 6 H), 2.12 - 2.22 (m, 1 H), 2.51 (s, 4 H), 3.09 - 3.15 (t, *J* = 6.0, 12 Hz, 4H), 3.47 (s, 2 H), 4.23 - 4.28 (q, *J* = 8.57, 4.17 Hz, 1 H), 6.88 - 6.92 (m, 2 H), 6.98 - 7.04 (t, *J* = 9.00 Hz, 2 H), 7.32 - 7.46 (m, 5 H), 7.68 (s, 1 H), 9.69 - 9.71 (d, *J* = 8.62 Hz, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm : 173.83, 167.06, 157.97, 154.85, 148.22, 148.20, 138.58, 135.88, 131.70, 129.56, 128.88, 128.39, 117.57, 117.47, 115.84, 115.55, 58.45, 54.75, 52.56, 49.15, 31.00, 20.11 and 18.40; MS (ESI): found: $[M + H]^+$, 440.20.

(*E*)-(2-((4-(4-Fluorophenyl)piperazin-1-yl)methyl)-3-phenylacryloyl)leucine (13w)

Following the above general procedure D, **13w** was synthesized from **12w** as a white solid (Yield: 67%, purity by LC-MS: >99%); M.P: 88 - 95° C; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 0.87 - 0.89 (d, *J* = 5.59 Hz, 6 H), 1.17 - 1.24 (m, 1 H), 1.54 - 1.62 (m, 2 H), 1.64 - 1.73 (m, 1 H), 2.51 (s, 4 H), 3.09 - 3.11 (d, *J* = 3.85 Hz, 4 H), 3.44 (s, 2 H), 4.25 - 4.33 (m, 1 H), 6.89 - 6.94 (m, 2 H), 7.00 - 7.05 (t, *J* = 9.00, 6.00 Hz, 2 H), 7.32 - 7.46 (m, 5 H), 7.62 (s, 1 H), 9.56 - 9.58 (d, *J* = 7.79 Hz, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm : 174.84, 167.07, 158.00, 154.88, 148.27, 148.25, 138.14, 135.89, 132.09, 129.64, 128.87, 128.42, 117.63, 117.53, 115.85, 115.56, 54.53, 52.52, 52.09, 49.31, 41.80, 25.03, 23.54 and 22.46; MS (ESI): found: $[M + H]^+$, 454.25.

(*E*)-2-(2-((4-(4-Fluorophenyl)piperazin-1-yl)methyl)-3-phenylacrylamido)-3-methyl pentanoic acid (13x)

Following the above general procedure D, **13x** was synthesized from **12x** as a white solid (Yield: 70%, purity by LC-MS: >99%); M.P: 139 - 142° C; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 0.84 - 0.93 (m, 6 H), 1.09 - 1.30 (m, 2 H), 1.37 - 1.53 (m, 1 H), 1.83 - 1.95 (m, 1 H), 2.57 (s, 4 H), 3.11 - 3.12 (d, *J* = 3.58 Hz, 4 H), 3.50 (s, 2 H), 4.37 - 4.41 (q, *J* = 8.39, 4.81 Hz, 1 H), 6.89 - 6.94 (m, 2 H), 7.01 - 7.07 (t, *J* = 9.00 Hz, 2 H), 7.36 - 7.44 (m, 5 H), 7.70 (s, 1 H), 9.91 - 9.94 (d, *J* = 8.34 Hz, 1 H), 12.75 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm : 173.38, 167.33, 158.07, 154.95, 148.16, 139.20, 135.65, 130.98, 129.59, 128.91, 128.54, 117.63, 117.54, 115.90, 115.61, 56.87, 54.68, 52.52, 49.21, 37.37, 25.41, 16.44 and 12.03; MS (ESI): found: $[M + H]^+$, 454.25.

(*E*)-2-(2-((4-(4-Fluorophenyl)piperazin-1-yl)methyl)-3-phenylacrylamido)pentanoic acid (13y)

Following the above general procedure D, **13y** was synthesized from **12y** as a white solid (Yield: 70%, purity by LC-MS: >99%); M.P: 81 - 86° C; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 0.83 -

0.91 (t, *J* = 7.34 Hz, 3 H), 1.17 - 1.24 (m, 1 H), 1.27 - 1.43 (m, 2 H), 1.59 - 1.85 (m, 2 H), 2.58 (2, 4 H), 3.10 (s, 4 H), 3.48 (s, 2 H), 4.35 - 4.41 (m, 1 H), 6.90 - 6.94 (m, 2 H), 7.01 - 7.07 (t, *J* = 9.00 Hz, 2 H), 7.35 - 7.44 (m, 5 H), 7.65 (s, 1 H), 9.81 - 9.83 (d, *J* = 7.61 Hz, 1 H), 12.72 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm : 174.08, 167.42, 158.06, 154.94, 148.23, 138.72, 135.69, 131.44, 129.67, 128.91, 128.56, 117.65, 117.55, 115.88, 115.60, 54.53, 52.49, 52.31, 49.32, 34.04 and 14.09; MS (ESI): found: $[M + H]^+$, 440.25.

(*E*)-(2-((4-(2-Methoxyphenyl)piperazin-1-yl)methyl)-3-phenylacryloyl)Glycine (13z)

Following the above general procedure D, **13z** was synthesized from **12z** as a white solid (Yield: 44%, purity by LC-MS: >99%); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 2.52 (s, 4 H), 3.00 (s, 4 H), 3.46 (s, 3 H), 3.75 (s, 2 H), 3.95 - 3.97 (d, *J* = 5.32 Hz, 2 H), 6.85 - 6.89 (d, *J* = 2.75 Hz, 2 H), 6.92 - 6.94 (m, 2 H), 7.35 - 7.39 (m, 1 H), 7.41 - 7.45 (m, 4 H), 7.64 (s, 1 H), 9.68 - 9.71 (t, *J* = 6.00,3.00 Hz, 1 H), 12.66 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm : 171.76, 168.09, 152.41, 141.57, 138.57, 135.75, 131.76, 129.78, 128.91, 128.59, 122.93, 121.25, 118.40, 112.38, 55.78, 54.48, 52.87, 50.27 and 41.82; MS (ESI): found: $[M + H]^+$, 410.20.

(*E*)-(2-((4-(4-Methoxyphenyl)piperazin-1-yl)methyl)-3-phenylacryloyl)Glycine (13aa)

Following the above general procedure D, **13aa** was synthesized from **12aa** as a white solid (Yield: 43%, purity by LC-MS: >99%); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 2.51 (s, 4 H), 3.06 (s, 4 H), 3.45 (s, 2 H), 3.67 (s, 3 H), 3.92 - 3.94 (d, *J* = 5.23 Hz, 2 H), 6.78 - 6.81 (d, *J* = 9.00 Hz, 2 H), 6.85 - 6.88 (t, *J* = 9.00 Hz, 2 H), 7.36 - 7.38 (m, 1 H), 7.42 - 7.44 (m, 4 H), 7.65 (s, 1 H), 9.66 - 9.69 (t, *J* = 5.23 Hz, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm : 171.73, 167.97, 153.32, 145.77, 138.53, 135.76, 131.77, 129.76, 128.91, 128.58, 117.84, 114.66, 55.62, 54.40, 52.65, 49.82 and 41.97; MS (ESI): found: $[M + H]^+$, 410.20.

(*E*)-(2-((4-(3-Hydroxyphenyl)piperazin-1-yl)methyl)-3-phenylacryloyl)glycine (13ab)

Following the above general procedure D, **13ab** was synthesized from **12ab** as a white solid (Yield: 33%, purity by LC-MS: >91%); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 2.51 (s, 4 H), 3.12 (s, 4 H), 3.46 (s, 2 H), 3.94 - 3.95 (d, *J* = 4.95 Hz, 2 H), 6.19 - 6.21 (d, *J* = 7.79 Hz, 1 H), 6.28 (s, 1 H), 6.35 - 6.36 (d, *J* = 8.44 Hz, 1 H), 6.94 - 6.99 (t, *J* = 8.07 Hz, 1 H), 7.25 - 7.38 (m, 2 H), 7.39 - 7.54 (m, 4 H), 7.65 (s, 1 H), 9.38 (s, 1 H), 9.70 - 9.71 (t, *J* = 4.63 Hz, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm : 171.7, 167.99, 158.51, 152.80, 138.60, 135.71, 131.67, 129.96, 129.77, 128.91, 128.76, 128.60, 107.08, 106.59, 102.90, 54.39, 52.51, 48.44, 41.86 and 38.71; MS (ESI): found: $[M + H]^+$, 396.15.

(*E*)-(2-((4-(2-Chlorophenyl)piperazin-1-yl)methyl)-3-phenylacryloyl)glycine (13ac)

Following the above general procedure D, **13ac** was synthesized from **12ac** as a white solid (Yield: 37%, purity by LC-MS: >99%); M.P: 80 - 87^{o} C; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 2.51 (s, 4 H), 3.02 (s, 4 H), 3.47 (s, 2 H), 3.94 (s, 2 H), 7.03 - 7.05 (t, *J* = 6.88 Hz, 1 H), 7.12 - 7.14 (d, *J* = 6.33 Hz, 1 H), 7.27 - 7.29 (d, *J* = 5.87 Hz, 1 H), 7.38 - 7.44 (m, 6 H), 7.64 (s, 1 H), 9.64 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm : 171.81, 167.94, 149.37, 138.55, 135.77, 131.79, 130.78, 129.76, 128.91, 128.58, 128.50, 128.10, 124.38, 121.31, 54.39, 52.75, 51.04 and 42.11; MS (ESI): found: $[M + H]^+$, 414.15.

(*E*)-(2-((4-(3-Chlorophenyl)piperazin-1-yl)methyl)-3-phenylacryloyl)glycine (13ad)

Following the above general procedure D, **13ad** was synthesized from **12ad** as a white solid (Yield: 37%, purity by LC-MS: >99%); M.P: 93 - 98^{o} C; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 2.51 (s, 4 H), 3.22 (s, 4 H), 3.46 (s, 2 H), 3.95 (s, 2 H), 6.76 - 6.79 (m, 1 H), 6.86 – 6.92 (m, 2 H), 7.17 - 7.20 (m, 1 H), 7.28 - 7.43 (m, 5 H), 7.65 (s, 1 H), 9.65 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm : 171.77, 171.71, 167.99, 166.09, 152.63, 143.80, 139.72, 138.65, 135.70, 134.25, 131.63,

130.66, 129.77, 128.92, 128.76, 128.62, 127.84, 123.18, 118.50, 115.00, 114.85, 114.13, 113.99, 72.48, 54.31, 52.28, 51.39, 47.92, 41.83 and 41.49; MS (ESI): found: $[M + H]^+$, 414.15.

(*E*)-(2-((4-(3-Methoxyphenyl)piperazin-1-yl)methyl)-3-phenylacryloyl)glycine (13ae)

Following the above general procedure D, **13ae** was synthesized from **12ae** as a white solid (Yield: 42%, purity by LC-MS: >99%); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 3.17 (s, 4 H), 3.45 (s, 6 H), 3.70 (s, 3 H), 3.92 - 3.94 (d, *J* = 5.23 Hz, 2 H), 6.34 - 6.37 (dd, *J* = 8.02, 1.97 Hz, 1 H), 6.42 (s, 1 H), 6.48 - 6.51 (dd, *J* = 8.30, 1.70 Hz, 1 H), 7.06 - 7.12 (t, *J* = 8.16 Hz, 1 H), 7.33 - 7.39 (m, 1 H), 7.40 - 7.44 (m, 4 H), 7.65 (s, 1 H), 9.65 - 9.68 (t, *J* = 5.18 Hz, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm : 171.76, 168.09, 152.41, 141.57, 138.57, 135.75, 131.76, 129.78, 128.91, 128.59, 122.93, 121.25, 118.40, 112.38, 55.78, 54.48, 52.87, 50.27 and 41.82; MS (ESI): found: $[M + H]^+$, 410.25.

(*E*)-(2-((4-(2-Fluorophenyl)piperazin-1-yl)methyl)-3-phenylacryloyl)glycine (13af)

Following the above general procedure D, **13af** was synthesized from **12af** as a white solid (Yield: 53%, purity by LC-MS: >99%); M.P: 117 - 120^o C; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 1.74 (s, 4 H), 3.08 (s, 4 H), 3.43 (s, 2 H), 3.58 - 3.59 (d, *J* = 4.03 Hz, 2 H), 6.90 - 7.12 (m, 4 H), 7.33 - 7.42 (m, 5 H), 7.64 (s, 1 H), 9.37 - 9.40 (t, *J* = 3.94 Hz, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm : 175.79, 172.44, 166.79, 156.93, 153.70, 140.38, 140.28, 137.84, 136.09, 132.28, 129.63, 128.85, 128.31, 125.22, 122.60, 122.50, 119.76, 119.72, 116.44, 116.17, 54.51, 52.71, 50.20, 45.15 and 24.93; MS (ESI): found: $[M + H]^+$, 398.20.

Ethyl (*E*)-2-((4-(benzo[d]isothiazol-3-yl)piperazin-1-yl)methyl)-3-phenylacrylate (15)

Following the above general procedure A, reaction of **8** and **14** resulted in **15** as a gummy material (Yield: 83%); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm : 1.36 - 1.39 (t, *J* = 7.12 Hz, 3 H), 2.72 (s, 4 H), 3.46 (s, 2 H), 3.53 (s, 4 H), 4.28 - 4.34 (q, *J* = 7.15 Hz, 2 H), 7.25 - 7.47 (m, 5 H),

7.69 - 7.71 (d, *J* = 7.16 Hz, 2 H), 7.79 - 7.81 (d, *J* = 8.12 Hz, 1H), 7.88 - 7.91 (d, *J* = 9.32 Hz, 2 H); MS (ESI): found: $[M + H]^+$, 408.2.

(*E*)-2-((4-(Benzo[d]isothiazol-3-yl)piperazin-1-yl)methyl)-3-phenylacrylic acid (16)

16 was synthesized from **15** by following the general procedure B as a white solid (Yield: 88%); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm : 2.90 (s, 4 H), 3.66 (s, 2 H), 3.72 (s, 3 H), 3.89 (s, 1 H), 7.26 - 7.27 (d, *J* = 5.48 Hz, 1 H), 7.32 - 7.49 (m, 6 H), 7.80 - 7.82 (d, *J* = 7.92 Hz, 1 H), 8.07 (s, 1 H); ^{13}C NMR (DMSO-d_6) □□ppm : 163.84, 152.44, 130.73, 129.44, 129.00, 128.36, 127.79, 124.90, 124.64, 121.54, 52.31 and 50.06; MS (ESI): found: $[M + H]^+$, 380.20.

Ethyl (*E*)-(2-((4-(benzo[d]isothiazol-3-yl)piperazin-1-yl)methyl)-3-phenylacryloyl)glycinate (17a)

Following above general procedure C, reaction of **16** and ethyl glycinate hydrochloride resulted in **17a** as a thick liquid (Yield: 49%, purity by LC-MS: 99%); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm : 1.28 - 1.33 (t, *J* = 7.15 Hz, 3 H), 2.74 (s, 4 H) 2.81 (s, 2 H), 3.64 (s, 4 H), 4.18 - 4.20 (d, *J* = 5.04 Hz, 2 H), 4.21 - 4.29 (m, 2 H), 7.26 - 7.32 (m, 2 H), 7.33 - 7.43 (m, 4 H), 7.44 - 7.51 (m, 1 H), 7.84 (dd, *J* = 15.96, 8.16 Hz, 2 H), 8.02 (s, 1 H) 10.29 (s, 1 H); MS (ESI): found: $[M + H]^+$, 465.20.

Methyl (*E*)-(2-((4-(benzo[d]isothiazol-3-yl)piperazin-1-yl)methyl)-3-phenylacryloyl)valinate (17b)

Following above general procedure C, reaction of **16** and methyl valinate hydrochloride resulted in **17b** as a thick liquid (Yield: 57%, purity by LC-MS: 98%); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm : 0.90 - 0.96 (q, *J* = 10.27, 6.88 Hz, 6 H), 2.16 - 2.24 (q, *J* = 11.60, 6.74 Hz, 1 H) 2.60 - 2.63 (m, 2 H) 2.69 - 2.72 (m, 2 H), 3.44 - 3.55 (m, 6 H), 3.66 (s, 3 H), 4.61 - 4.65 (q, *J* = 8.67, 4.54 Hz, 1 H), 7.19 - 7.22 (d, *J* = 7.70 Hz, 2 H), 7.24 - 7.35 (m, 4 H), 7.36 - 7.43 (m, 1 H), 7.72 -

7.79 (dd, *J* = 14.95, 8.16 Hz, 2 H), 7.92 (s, 1 H), 10.11 - 10.14 (d, *J*=8.62 Hz, 1 H); MS (ESI): found: $[M + H]^+$, 493.22.

Ethyl (*E*)-(2-((4-(benzo[d]isothiazol-3-yl)piperazin-1-yl)methyl)-3-phenylacryloyl)leucinate (17c)

Following above general procedure C, reaction of **16** and ethyl leucinate hydrochloride resulted in **17c** as a thick liquid (Yield: 58%, purity by LC-MS: 99%); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm : 0.86 - 0.93 (q, *J* = 6.03 Hz, 6 H), 1.13 - 1.23 (m, 2 H), 1.38 - 1.54 (m, 1 H), 1.89 - 1.97 (m, 1 H), 2.55 - 2.65 (m, 2 H), 2.65 - 2.76 (m, 2 H), 3.40 - 3.51 (m, 2 H), 3.52 - 3.60 (m, 4 H), 3.66 (s, 3 H), 4.65 - 4.69 (q, *J*=8.53, 4.68 Hz, 1 H), 7.19 - 7.21 (d, *J* = 6.97 Hz, 2 H), 7.23 - 7.35 (m, 4 H), 7.39 (t, *J* = 7.43 Hz, 1 H), 7.76 (dd, *J* = 14.95, 8.16 Hz, 2 H), 7.92 (s, 1 H), 10.13 - 10.16 (d, *J* = 8.53 Hz, 1 H); MS (ESI): found: $[M + H]^+$, 421.30.

Methyl (*E*)-2-(2-((4-(benzo[d]isothiazol-3-yl)piperazin-1-yl)methyl)-3-phenylacrylamido)-3-methylpentanoate (17d)

Following above general procedure C, reaction of **16** and methyl 2-amino-3-methylpentanoate hydrochloride resulted in **17d** as a thick liquid (Yield: 60%, purity by LC-MS: 97%); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm : 0.91 - 0.93 (d, *J* = 5.41 Hz, 7 H), 1.19 - 1.23 (t, *J* = 7.11 Hz, 3 H), 1.52 - 1.64 (m, 2 H), 1.65 - 1.76 (m, 2 H), 2.62 - 2.67 (d, *J* = 15.04 Hz, 4 H), 3.44 - 3.52 (m, 6 H), 4.08 - 4.15 (q, *J* = 7.15 Hz, 2 H), 4.60 - 4.67 (m, 1 H), 7.19 - 7.21 (d, *J* = 6.51 Hz, 2 H), 7.24 - 7.33 (m, 4 H), 7.35 - 7.43 (m, 1 H), 7.71 - 7.79 (m, 2 H), 7.92 (s, 1 H), 10.12 - 10.14 (d, *J* = 7.61 Hz, 1 H); MS (ESI): found: $[M + H]^+$, 507.30.

Ethyl (*E*)-2-(2-((4-(benzo[d]isothiazol-3-yl)piperazin-1-yl)methyl)-3-phenylacrylamido) pentanoate (17e)

Following above general procedure C, reaction of **16** and ethyl 2-aminopentanoate hydrochloride resulted in **17e** as a thick liquid (Yield: 67%, purity by LC-MS: 98%); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm : 0.87 - 0.92 (t, *J* = 7.34 Hz, 3 H), 1.19 - 1.24 (t, *J* = 7.15 Hz, 4 H), 1.31 - 1.40 (m, 2 H), 1.62 - 1.75 (m, 2 H), 1.76 -1.89 (m, 1 H), 2.63 - 2.66 (m, 4 H), 3.43 - 3.61 (m, 6 H), 4.09 - 4.16 (q, *J* = 7.15 Hz, 2 H), 4.61 - 4.68 (m, 1 H), 7.19 - 7.21 (d, *J* = 6.79 Hz, 2 H), 7.24 - 7.35 (m, 4 H), 7.36 - 7.44 (m, 1 H), 7.72 -7.79 (dd, *J* = 14.44, 8.12 Hz, 2 H), 7.92 (s, 1 H), 10.17 - 10.20 (d, *J* = 7.61 Hz, 1 H); MS (ESI): found: $[M + H]^+$, 507.30.

(E)-(2-((4-(Benzo[d]isothiazol-3-yl)piperazin-1-yl)methyl)-3-phenylacryloyl)glycine (18a)

Following the above general procedure D, **18a** was synthesized from **17a** as a white solid (Yield: 60%, purity by LC-MS: >99%); M.P: 110 - 114^o C; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 2.62 (s, 4 H), 3.51 (s, 6 H), 3.94 - 3.96 (d, *J* = 5.04 Hz, 2 H), 7.34 - 7.45 (m, 6 H), 7.52 - 7.57 (t, *J* = 7.52 Hz, 1 H), 7.67 (s, 1 H), 8.02 - 8.06 (dd, *J* = 7.93, 4.63 Hz, 2 H), 9.71 - 9.74 (t, *J* = 5.04 Hz, 1 H), 12.51 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm : 171.84, 167.85, 163.95, 152.42, 138.65, 135.77, 131.65, 129.75, 128.91, 128.57, 128.32, 127.81, 124.85, 124.63, 121.50, 54.48, 52.36, 4.87 and 42.11; MS (ESI): found: $[M + H]^+$, 437.20.

(*E*)-(2-((4-(Benzo[d]isothiazol-3-yl)piperazin-1-yl)methyl)-3-phenylacryloyl)valine (18b)

Following the above general procedure D, **18b** was synthesized from **17b** as a white solid (Yield: 53%, purity by LC-MS: >99%); M.P: 113 - 119^o C; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 0.93 - 0.97 (t, *J* = 5.78 Hz, 6 H), 2.14 - 2.22 (m, 1 H), 2.64 - 2.65 (d, *J* = 4.13 Hz, 4 H), 3.49 (s, 4 H), 3.56 (s, 2 H), 4.36 - 4.40 (q, *J* = 8.44, 1 H), 7.34 - 7.49 (m, 6 H), 7.48 - 7.58 (t, *J* = 7.52 Hz, 1 H), 7.73 (s, 1 H), 8.04 - 8.07 (d, *J* = 8.16 Hz, 2 H), 9.94 - 9.97 (d, *J* = 8.62 Hz, 1 H), 12.81 (s, 1 H);

^{13}C NMR (DMSO-d_6) δ ppm : 173.47, 167.42, 163.85, 152.41, 139.22, 135.69, 131.02, 129.61, 128.92, 128.53, 128.36, 127.76, 124.88, 124.64, 121.52, 57.68, 54.82, 52.38, 49.88, 30.75, 19.84 and 18.32; MS (ESI): found: $[M + H]^+$, 479.30.

(*E*)-(2-((4-(Benzo[d]isothiazol-3-yl)piperazin-1-yl)methyl)-3-phenylacryloyl)leucine (18c)

Following the above general procedure D, **18c** was synthesized from **17c** as a white solid (Yield: 41%, purity by LC-MS: >99%); M.P: 102 - 107^o C; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 0.90 - 0.94 (t, *J* = 6.10, 4.54 Hz, 6 H), 1.61 - 1.65 (t, *J* = 6.00 Hz, 2 H), 1.72 - 1.76 (q, *J* = 12.93, 6.60 Hz, 1 H), 2.62 - 2.64 (d, *J* = 4.68 Hz, 4 H), 3.46 (s, 4 H), 3.52 (s, 2 H), 4.36 - 4.44 (m, 1 H), 7.37 - 7.39 (m, 1 H), 7.42 - 7.45 (m, 5 H), 7.53 - 7.58 (t, *J* = 7.52 Hz, 1 H), 7.64 (s, 1 H), 8.03 - 8.07 (dd, *J* = 7.93, 5.00 Hz, 2 H), 9.69 - 9.72 (d, *J* = 7.79 Hz, 1 H), 12.67 (s, 1 H); MS (ESI): found: $[M + H]^+$, 493.25.

(*E*)-2-(2-((4-(Benzo[d]isothiazol-3-yl)piperazin-1-yl)methyl)-3-phenylacrylamido)-3-methyl pentanoic acid (18d)

Following the above general procedure D, **18d** was synthesized from **17d** as a white solid (Yield: 60%, purity by LC-MS: >99%); M.P: 101 - 105^o C; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 0.89 - 0.96 (t, 6 H), 1.24 (s, 1 H), 1.45 - 1.51 (q, *J* = 13.02, 5.41 Hz, 1 H), 1.92 (s, 1 H), 2.63 (s, 4 H), 3.49 (s, 4 H), 3.55 (s, 2 H), 4.39 - 4.43 (q, *J* = 8.02, 4.81 Hz, 1 H), 7.37 - 7.48 (m, 6 H), 7.53 - 7.58 (t, *J* = 9.0, 6.0 Hz, 1 H), 7.73 (s, 1 H), 8.04 - 8.07 (dd, *J* = 7.98, 2.38 Hz, 2 H), 9.97 - 10.00 (d, *J* = 8.34 Hz, 1 H), 12.80 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm : 173.42, 167.29, 163.86, 152.41, 139.23, 135.67, 130.97, 129.61, 128.92, 128.54, 128.36, 127.77, 124.89, 124.64, 121.52, 56.92, 54.79, 52.38, 49.87, 37.45, 25.44, 16.45 and 12.07; MS (ESI): found: $[M + H]^+$, 493.25.

(*E*)-2-(2-((4-(Benzo[d]isothiazol-3-yl)piperazin-1-yl)methyl)-3-phenylacrylamido) pentanoic acid (18e)

Following the above general procedure D, **18e** was synthesized from **17e** as a white solid (Yield: 41%, purity by LC-MS: >99%); M.P: 160 - 165^{o} C; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 0.88 - 0.93 (t, *J* = 7.24 Hz, 3 H), 1.34 - 1.42 (m, 2 H), 1.67 - 1.80 (m, 2 H), 2.63 - 2.64 (d, *J* = 3.21 Hz, 4 H), 3.47 (s, 4 H), 3.53 (s, 2 H), 4.33 - 4.46 (m, 1 H), 7.32 - 7.50 (m, 6 H), 7.53 - 7.58 (t, *J* = 7.47 Hz, 1 H), 7.67 (s, 1 H), 8.03 - 8.07 (dd, *J* = 7.79, 3.94 Hz, 2 H), 9.85 - 9.88 (d, *J* = 7.52 Hz, 1 H); MS (ESI): found: $[M + H]^+$, 479.25.

(E)-(2-((4-(Benzo[d]isothiazol-3-yl)piperazin-1-yl)methyl)-3-(4-methoxyphenyl)acryloyl)valine (18f)

(Yield: 53%, purity by LC-MS: >99%); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 0.86 - 0.95 (t, *J* = 9.00 Hz, 6 H), 2.18 - 2.19 (m, 1 H), 2.69 (s, 4 H), 3.50 (s, 4 H), 3.59 (s, 2 H), 3.79 (s, 3 H), 4.37 - 4.38 (q, *J* = 8.44, 1 H), 6.99 - 7.02 (d, *J* = 9.00 Hz, 2 H), 7.38 - 7.44 (t, *J* = 9.00 Hz, 3 H), 7.53 - 7.58 (t, *J* = 6.00, 1 H), 7.66 (s, 1 H), 8.04 - 8.07 (d, *J* = 8.16 Hz, 2 H), 9.88 - 9.90 (d, *J* = 6.62 Hz, 1 H), 12.71 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm : 173.51, 167.84, 163.87, 159.63, 152.41, 139.05, 131.46, 129.19, 128.38, 127.80, 124.91, 124.65, 121.55, 114.40, 57.60, 55.62, 52.37, 49.92, 30.68, 30.20, 22.80, 19.84 and 18.32; MS (ESI): found: $[M + H]^+$, 509.20.

3-(4-Benzylpiperazin-1-yl)benzo[d]isothiazole (19a)

(Yield: 48%, purity by LC-MS: >99%); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 2.66 (s, 4 H), 3.54 (s, 4 H), 3.59 (s, 2 H), 7.24 - 7.34 (m, 6 H), 7.39 - 7.44 (t, *J* = 6.00 Hz, 1 H), 7.75 - 7.77 (d, *J* = 6.00, 1 H), 7.85 - 7.88 (d, *J* = 9.00 Hz, 1 H); MS (ESI): found: $[M + H]^+$, 310.10.

(3-(4-(Benzo[d]isothiazol-3-yl)piperazin-1-yl)propanoyl)valine (19b)

(Yield: 42%, purity by LC-MS: >99%); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 0.88 - 0.90 (t, *J* = 3.00 Hz, 6 H), 2.05 - 2.07 (m, 1 H), 2.35 - 2.44 (m, 3 H), 2.63 - 2.65 (m, 6 H), 3.44 (s, 4 H), 4.18 - 4.22 (t, *J* = 6.00 Hz, 1 H), 7.41 - 7.46 (t, *J* = 6.00 Hz, 1 H), 7.53 - 7.58 (t, *J* = 6.00, 1 H), 8.04 - 8.07 (d, *J* = 9.00 Hz, 2 H), 8.32 - 8.35 (d, *J* = 9.00 Hz, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm : 171.78, 163.99, 152.44, 128.35, 127.80, 124.90, 124.62, 121.55, 57.37, 54.47, 52.59, 50.11, 33.06 and 30.55; MS (ESI): found: $[M + H]^+$, 391.15.

General procedure for synthesis of 20a, 20f-20h (General procedure E)

To a solution of **10a or 10f-10h** (3.72 mmol) in 30 mL methanol, after purging with nitrogen gas, 10% Pd/C (50% wet, 0.95 mmol) was added and applied hydrogen atmosphere through bladder for 10 minutes at 25-30^{o} C, completion of the reduction was confirmed by TLC. Pd/C was filtered through celite bed, methanol was concentrated under reduced pressure and the residue was subjected to silica gel column chromatography, fractions containing product were evaporated to dryness to get product as an oily mass which was subjected to general procedure-B, C and D to produce **23a, 23f-23h** respectively.

(2-Benzyl-3-(4-phenylpiperazin-1-yl)propanoyl)glycine (23a)

By following the above general procedure E **23a** was synthesized by reduction of **20a** with Pd/C in methanol (Yield: 39%, purity by LC-MS: >99%); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 2.60 - 2.67 (t, *J* = 6.32 Hz, 1 H), 2.78 - 2.91 (m, 2 H), 3.09 (s, 4 H), 3.23 (s, 4 H), 3.63 - 3.79 (m, 3 H), 6.73 - 6.78 (t, *J* = 6.20 Hz, 1 H), 6.86 - 6.89 (d, *J* = 9.20 Hz, 2 H), 7.17 - 7.22 (t, *J* = 6.20 Hz, 8 H), 8.52 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm : 172.78, 171.84,150.20, 138.47, 129.56, 129.49, 128.80, 126.99, 120.28, 116.25, 57.39, 52.00, 46.36, 42.90, 41.33, 38.71, 37.10 and 29.48; MS (ESI): found: $[M + H]^+$, 382.25.

(2-Benzyl-3-(4-(2-methoxyphenyl)piperazin-1-yl)propanoyl)glycine (23f)

By following the above general procedure E **23f** was synthesized by reduction of **20f** with Pd/C in methanol (Yield: 60%, purity by LC-MS: >99%); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 2.70 - 2.76 (m, 1 H), 2.89 - 2.90 (m, 1 H), 3.00 (s, 1 H), 3.17 (s, 8 H), 3.75 (s, 2 H), 3.70 - 3.82 (m, 5 H), 6.90 (s, 2 H), 6.95 - 6.98 (m, 2 H), 7.26 - 7.31 (m, 5 H), 8.60 (m,1 H); ^{13}C NMR (DMSO-d_6) δ ppm : 172.81, 171.82, 152.29, 151.54, 140.04, 138.47, 129.50, 129.31, 128.80, 126.98, 123.78, 121.32, 121.18, 118.64, 112.43, 57.43, 55.84, 52.46, 47.85, 42.87, 41.33, 37.17 and 27.88; MS (ESI): found: $[M + H]^+$, 412.20.

(2-Benzyl-3-(4-(4-methoxyphenyl)piperazin-1-yl)propanoyl)glycine (23g)

By following the above general procedure E **23g** was synthesized by reduction of **20g** with Pd/C in methanol (Yield: 42%, purity by LC-MS: >99%); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 2.69 - 2.73 (m, 2 H), 2.89 - 2.99 (m, 2 H), 3.18 (s, 8 H), 3.68 (s, 3 H), 3.76 - 3.82 (m, 2 H), 4.24 - 4.28 (m, 1 H), 6.83 - 6.85 (d, *J* = 6.00 Hz, 2 H), 6.90 - 6.93 (d, *J* = 6.00 Hz, 2 H), 7.26 - 7.31 (m, 5 H), 8.60 (m,1 H); ^{13}C NMR (DMSO-d_6) δ ppm : 172.78, 171.83,154.10, 144.45, 138.48, 129.49, 128.80, 126.99, 118.98, 114.81, 68.74,57.37, 55.66, 52.16, 4770, 42.89, 4132, 37.10, 27.88, 22.21; MS (ESI): found: $[M + H]^+$, 412.20.

Ethyl (*E*)-2-((4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl)methyl)-3-phenylacrylate (25a)

Following the above general procedure A, reaction of **8** and **24a** resulted in **25a** as a gummy material (Yield: 83%); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm : 1.33-1.38 (t, *J* = 7.11 Hz, 3 H), 1.69 - 1.71 (d, *J* = 12.01 Hz, 2 H), 1.93 (s, 1 H), 2.02 - 2.09 (m, 2 H), 2.47 - 2.55 (m, 2.11 Hz, 2 H), 2.73 - 2.77 (d, *J* = 11.19 Hz, 2 H), 3.43 - 3.44 (d, *J* = 5.04 Hz, 2 H), 4.24 - 4.32 (q, *J* = 6.0 Hz, 2 H), 7.30 (s, 2 H), 7.34 - 7.43 (m, 5 H), 7.65 -7.67 (m, 2 H), 7.84 (s, 1 H); MS (ESI): found: $[M + H]^+$, 400.20.

Ethyl (*E*)-2-(((3R,4R)-4-(3-hydroxyphenyl)-3,4-dimethylpiperidin-1-yl)methyl)-3-phenyl acrylate (25b)

Following the above general procedure A, reaction of **8** and **24b** resulted in **25b** as a gummy material (Yield: 89%); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm : 0.77 - 0.78 (d, *J* = 6.8 Hz, 3 H), 1.24 - 1.26 (d, *J* = 10.08 Hz, 1 H), 1.30 (s, 3 H), 1.35 - 1.38 (t, *J* = 7.04 Hz, 3 H), 1.54 - 1.57 (d, *J* = 12.36 Hz,1 H), 1.97 (s, 1 H), 2.25 - 2.28 (t, *J* = 3.76 Hz, 1 H), 2.41 - 2.44 (t, *J* = 10.96 Hz, 1 H), 2.54 - 2.57 (d, *J* = 11.04 Hz,1 H), 2.67 - 2.69 (d, *J* = 8.08 Hz, 1 H), 2.81 - 2.83 (d, *J* = 10.48 Hz, 1 H), 3.28 - 3.31 (d, *J* = 11.96 Hz, 1 H), 3.38 - 3.41 (d, *J* = 11.96 Hz, 1 H), 4.27 - 4.32 (q, *J* = 6.92 Hz, 2 H), 5.07 - 5.55 (m, 1 H), 6.64 - 6.66 (d, *J* = 6.64 Hz, 1 H), 6.77 (s, 1 H), 6.82 - 6.84 (d, *J* = 7.64 Hz, 1 H), 7.14 - 7.17 (t, *J* = 7.84 Hz, 1 H), 7.33 - 7.37 (m, 3 H), 7.74 - 7.75 (d, *J* = 6.88 Hz, 2 H), 7.84 (s, 1 H); MS (ESI): found: $[M + H]^+$, 394.30.

(*E*)-2-(((3R,4R)-4-(3-Hydroxyphenyl)-3,4-dimethylpiperidin-1-yl)methyl)-3-phenylacrylic acid (26b)

26b was synthesized from **25b** by following the general procedure B as a white solid (Yield: 90%); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm : 0.74 (s, 3 H), 1.27 (s, 3 H), 1.59 (m, 1 H), 1.68 - 1.71 (m, 2 H), 1.93 (m, 1 H), 2.09 - 2.11 (m, 1 H), 2.35 - 2.41 (t, *J* = 12.72 Hz, 1 H), 2.57 (s, 1 H), 2.89 (s, 2 H), 3.19 (s, 1 H), 3.72 - 3.81 (q, *J* = 14.12 Hz, 2 H), 5.29 (s, 1 H), 6.67 - 6.74 (m, 2 H), 6.86 (s, 1 H), 7.10 - 7.14 (t, *J* = 7.96 Hz, 1 H), 7.22 - 7.25 (t, *J* = 7.04 Hz, 2 H), 7.36 - 7.43 (m, 3 H), 8.06 (s, 1 H); MS (ESI): found: $[M + H]^+$, 366.20.

Ethyl (*E*)-(2-((4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl)methyl)-3-phenylacryloyl) glycinate (27a)

Following above general procedure C, reaction of **26a** and ethyl glycinate hydrochloride resulted in **27a** as a thick liquid (Yield: 40%, purity by LC-MS: >99%); ^{1}H NMR (300 MHz, $CDCl_3$) δ

ppm : 1.30 - 1.35 (t, *J* = 7.15 Hz, 4 H), 1.73 (s, 2 H), 2.27 (m, *J* = 12.95, 4.08 Hz, 2 H), 2.41 - 2.52 (m, 2 H), 2.85 - 2.88 (d, *J* = 8.07 Hz, 2 H), 3.54 (s, 2 H), 4.19 - 4.20 (d, *J* = 2.84 Hz, 2 H), 4.22 - 4.33 (q, *J* = 6.15 Hz ,2 H), 7.25 (s, 1 H), 7.27 - 7.28 (d, *J* = 1.56 Hz, 1 H), 7.29 (s, 1 H), 7.31 - 7.34 (m, 2 H), 7.36 (s, 1 H), 7.37 - 7.42 (m, 1 H), 7.48 - 7.51 (m, 1 H), 7.51 - 7.54 (m, 1 H), 7.95 (s, 1 H), 10.75 (s, 1 H); MS (ESI): found: $[M + H]^+$, 457.20.

Methyl (*E*)-(2-((4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl)methyl)-3-phenylacryloyl) valinate (27b)

Following above general procedure C, reaction of **26a** and methyl valinate hydrochloride resulted in **27b** as a thick liquid (Yield: 61%, purity by LC-MS: >99%); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm : 0.97 - 1.03 (q, *J* = 12.01, 6.88 Hz, 6 H), 1.72 - 1.80 (m, 2 H), 2.15 (m, *J* = 13.02, 4.58 Hz, 1 H), 2.23 - 2.43 (m, 3 H), 2.49 - 2.62 (m, 1 H), 2.80 - 2.97 (m, 2 H), 3.43 - 3.50 (m, 1 H), 3.58 - 3.65 (m, 1 H), 3.76 (s, 3 H), 4.72 - 4.76 (q, *J* = 8.67, 4.45 Hz, 1 H), 7.25 (s,1 H), 7.28 - 7.28 (d, *J* = 1.28 Hz, 1 H), 7.29 - 7.31 (m, 1 H), 7.31 - 7.34 (m, 2 H), 7.36 (s, 1 H), 7.37 - 7.42 (m, 1 H), 7.44 - 7.46 (m, 1 H), 7.46 - 7.50 (m, 1 H), 7.95 (s, 1 H), 10.60 - 10.63 (d, *J* = 8.62 Hz, 1 H); MS (ESI): found: $[M + H]^+$, 485.30.

Ethyl (*E*)-(2-((4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl)methyl)-3-phenylacryloyl) leucinate (27c)

Following above general procedure C, reaction of **26a** and ethyl leucinate hydrochloride resulted in **27c** as a thick liquid (Yield: 57%, purity by LC-MS: >99%); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm : 0.96 - 1.01 (t, *J* = 6.37 Hz, 6 H), 1.28 - 1.33 (t, *J* = 7.15 Hz, 4 H), 1.61 - 1.71 (m, 4 H), 1.77 (s, 1 H), 2.06 - 2.31 (m, 2 H), 2.33 - 2.45 (m, 1 H), 2.47 - 2.58 (m, 1 H), 2.78 - 2.94 (m, 2 H), 3.40 - 3.50 (m, 1 H), 3.53 - 3.68 (m, 1 H), 4.18 - 4.25 (q, *J* = 7.09 Hz, 2 H), 4.75 (m, *J* = 7.79, 5.14 Hz, 1 H), 7.25 (s, 1 H), 7.27 - 7.28 (m, 1 H), 7.29 - 7.30 (m, 1 H), 7.32 (dt, *J* = 3.53,

1.54 Hz, 2 H), 7.35 (s, 1 H), 7.37 - 7.41 (m, 1 H), 7.44 -7.47 (m, 1 H), 7.47 - 7.50 (m, 1 H), 7.95 (s, 1 H), 10.62 - 10.65 (d, *J* = 7.70 Hz, 1 H); MS (ESI): found: $[M + H]^+$, 513.35.

Methyl (*E*)-2-(2-((4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl)methyl)-3-phenyl acrylamido)-3-methylpentanoate (27d)

Following above general procedure C, reaction of **26a** and methyl 2-amino-3-methylpentanoate hydrochloride resulted in **27d** as a thick liquid (Yield: 56%, purity by LC-MS: >99%); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm : 0.94 - 1.00 (m, 6 H), 1.17 - 1.24 (m, 1 H), 1.48 - 1.57 (m, 1 H), 1.73 - 1.78 (m, 2 H), 1.96 - 2.05 (m, 1 H), 2.14 - 2.23 (m, 1 H), 2.26 - 2.32 (q, *J* = 13.07, 3.81 Hz, 1 H), 2.34 - 2.42 (m, 1 H), 2.49 - 2.57 (m, 1 H), 2.83 - 2.93 (m, 2 H), 3.44 - 3.49 (m, 1 H), 3.58 - 3.62 (m, 1 H), 3.75 (s, 3 H), 4.75 - 4.79 (q, *J* = 8.44, 4.49 Hz, 1 H), 7.25 (s, 1 H), 7.28 (d, *J* = 1.19 Hz, 1 H), 7.29 - 7.31 (m, 1 H), 7.31 - 7.34 (m, 2 H), 7.35- 7.42 (m, 2 H), 7.44 - 7.46 (m, 1 H), 7.46 - 7.50 (m, 1 H), 7.95 (s, 1 H), 10.60 - 10.63 (d, *J* = 8.34 Hz, 1 H); MS (ESI): found: $[M + H]^+$, 499.30.

Ethyl (*E*)-2-(2-((4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl)methyl)-3-phenylacrylamido) pentanoate (27e)

Following above general procedure C, reaction of **26a** and ethyl 2-aminopentanoate hydrochloride resulted in **27e** as a thick liquid (Yield: 48%, purity by LC-MS: >99%); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm : 0.94 - 0.99 (t, *J* = 7.34 Hz, 3 H), 1.29 - 1.04 (t, *J* = 7.11 Hz, 4 H), 1.36 - 1.49 (m, 2 H), 1.72 - 1.79 (m, 3 H), 1.84 - 1.97 (m, 1 H), 2.11 - 2.33 (m, 2 H), 2.34 - 2.45 (m, 1 H), 2.48 - 2.56 (t, *J* = 11.10 Hz, 1 H), 2.83 - 2.91 (t, *J* = 12.10 Hz, 2 H), 3.45 - 3.62 (t, *J* = 15.21 Hz, 2 H), 4.19 - 4.26 (q, *J* = 7.15 Hz, 2 H), 4.72 - 4.79 (q, *J* = 6.54 Hz, 1 H), 7.25 (s, 1 H), 7.28 - 7.42 (m, 6 H), 7.45 - 7.48 (m, 1 H), 7.48 - 7.52 (m, 1 H), 7.95 (s, 1 H), 10.71 - 10.73 (d, *J* = 7.15 Hz, 1 H); MS (ESI): found: $[M + H]^+$, 499.30.

(*E*)-(2-((4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl)methyl)-3-phenylacryloyl)glycine (28a)

Following the above general procedure D, **28a** was synthesized from **27a** as a white solid (Yield: 35%, purity by LC-MS: >99%); M.P: 172 - 179^{o} C; ^{1}H NMR (300 MHz, DMSO-d_6) δ vppm : 1.29 - 1.04 (t, *J* = 7.11 Hz, 4 H), 1.36 - 1.49 (m, 2 H), 1.72 - 1.79 (m, 3 H), 1.84 -1.97 (m, 1 H), 2.11 - 2.33 (m, 2 H), 2.34 - 2.45 (m, 1 H), 2.48 - 2.56 (t, *J* = 11.10 Hz, 1H), 2.83 - 2.91 (t, *J* = 12.10 Hz, 2 H), 4.19 - 4.26 (q, *J* = 7.15 Hz, 2 H), 4.72 - 4.79 (q, *J* = 6.54 Hz, 1 H), 7.25 (s, 1 H), 7.28 - 7.42 (m, 6 H), 7.45 - 7.48 (m, 1 H), 7.48 - 7.52 (m, 1 H), 7.95 (s, 1 H), 10.71 - 10.73 (d, *J* = 7.15 Hz, 1 H). MS (ESI): found: $[M + H]^{+}$, 429.20.

(*E*)-(2-((4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl)methyl)-3-phenylacryloyl)valine (28b)

Following the above general procedure D, **28b** was synthesized from **27b** as a white solid (Yield: 60%, purity by LC-MS: >99%); M.P: 168 - 171^{o} C; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 0.96 - 0.98 (q, *J* = 6.33, 3.21 Hz, 6 H), 1.17 - 1.24 (m, 1 H), 1.54 - 1.59 (m, 2 H), 1.92 (s, 1 H), 2.05 - 2.09 (d, *J* = 11.92 Hz, 1 H), 2.18 - 2.30 (m, 2 H), 2.73 (s, 2 H), 3.49 (s, 2 H), 4.37 - 4.47 (m, 1 H), 4.99 (s, 1 H), 7.36 - 7.44 (m, 9 H), 7.71 (s, 1 H), 10.38 - 10.40 (s, 1 H), 12.82 (s, 1 H); MS (ESI): found: $[M + H]^{+}$, 471.20.

(*E*)-(2-((4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl)methyl)-3-phenylacryloyl)leucine (28c)

Following the above general procedure D, **28c** was synthesized from **27c** as a white solid (Yield: 57%, purity by LC-MS: >99%); M.P: 148 - 152^{o} C; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 0.91 - 0.95 (t, *J* = 6.69 Hz, 6 H), 1.65 - 1.69 (m, 4 H), 1.70 - 1.79 (m, 2 H), 2.00 (s, 2 H), 2.28 (s, 2 H), 2.73 (s, 2 H), 3.49 (s, 2 H), 4.40 - 4.47 (q, *J* = 6.00 Hz, 1 H), 5.02 (s, 1 H), 7.39 - 7.46 (m, 9 H), 7.67 (s, 1 H), 10.17 (s, 1 H); MS (ESI): found: $[M + H]^{+}$, 485.25.

(*E*)-2-(2-((4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl)methyl)-3-phenylacrylamido)-3-methylpentanoic acid (28d)

Following the above general procedure D, **28d** was synthesized from **27d** as a white solid (Yield: 68%, purity by LC-MS: >99%); M.P: 123 - 128° C; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 0.90 - 0.95 (m, 6 H), 1.16 - 1.30 (m, 2 H), 1.52 - 1.67 (m, 2 H), 1.87 - 2.00 (m, 2 H), 2.04 - 2.06 (d, *J* = 11.92 Hz, 1 H), 2.22 - 2.28 (d, *J* = 7.24 Hz, 1 H), 2.73 (s, 2 H), 3.47 (s, 2 H), 4.42 - 4.46 (q, *J* = 8.30 Hz, 1 H), 4.98 (s, 1 H), 7.34 - 7.39 (m, 5 H), 7.42 - 7.45 (m, 4H), 7.69 (s, 1 H), 10.36 - 10.39 (d, *J* = 8.53 Hz, 1 H); MS (ESI): found: $[M + H]^+$, 485.25.

(*E*)-2-(2-((4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl)methyl)-3-phenylacrylamido) pentanoic acid (28e)

Following the above general procedure D, **28e** was synthesized from **27e** as a white solid (Yield: 53%, purity by LC-MS: >99%); M.P: 152 - 157° C; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 0.90 - 0.95 (t, *J* = 6.92 Hz, 3 H), 1.24 - 1.43 (m, 2 H), 1.50 - 1.55 (t, *J* = 4.44 Hz, 2 H), 1.71 - 1.73 (dd, *J* = 14.90, 6.37 Hz, 2 H), 1.93 - 2.13 (m, 2 H), 2.21 - 2.36 (m, 2 H), 2.69 - 2.71 (d, *J* = 11.37 Hz, 2 H), 3.45 (s, 2 H), 4.36 - 4.37 (d, *J* = 5.32 Hz, 1 H), 4.95 (s, 1 H), 7.35 - 7.42 (m, 5 H), 7.44 - 7.50 (m, 4 H), 7.66 (s, 1 H), 10.32 - 10.33 (d, *J* = 6.79 Hz, 1 H); MS (ESI): found: $[M + H]^+$, 471.20.

((*E*)-2-(((3R,4R)-4-(3-Hydroxyphenyl)-3,4-dimethylpiperidin-1-yl)methyl)-3-phenyl acryloyl)glycine (28f)

Following the above general procedure D, **28f** was synthesized from **27f** as a white solid (Yield: 68%, purity by LC-MS: >99%); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 0.70 - 0.72 (d, *J* = 5.50 Hz, 3 H), 1.24 - 1.29 (m, 6 H), 1.54 - 1.56 (d, *J* = 8.16 Hz, 1 H), 1.97- 2.01 (m, 1 H), 2.30 (s, 2 H), 2.63 (s, 1 H), 2.91 (s, 1 H), 3.84 - 3.90 (m, 1 H), 4.03 - 4.08 (m, 1 H), 6.59 - 6.61 (d, *J* = 7.61

Hz, 1 H), 6.70 - 6.75 (m, 2 H), 7.10 - 7.15 (t, *J* = 7.11 Hz, 1 H), 7.42 (s, 1 H), 7.49 (s, 4 H), 7.68 (s, 1 H), 9.25 - 9.26 (s, 1 H), 9.74 (s, 1 H); MS (ESI): found: $[M + H]^+$, 435.25.

((*E*)-2-(((3R,4R)-4-(3-Hydroxyphenyl)-3,4-dimethylpiperidin-1-yl)methyl)-3-phenylacryloyl) valine (28g)

Following the above general procedure D, **28g** was synthesized from **27g** as a white solid (Yield: 64%, purity by LC-MS: >99%); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 0.72 - 0.73 (d, *J* = 5.23 Hz, 3 H), 0.87 - 0.91 (t, *J* = 6.33 Hz, 6 H), 1.18 - 1.24 (m, 3 H), 1.46 - 1.50 (d, *J* = 12.01 Hz, 1 H), 1.99 - 2.09 (m, 3 H), 2.29 - 2.33 (d, *J* = 13.66 Hz, 1 H), 2.57 (s, 1 H), 2.81 - 2.84 (d, *J* = 10.27 Hz, 1 H), 3.41 (s, 2 H), 4.38 - 4.42 (t, *J* = 6.01 Hz, 1 H), 6.53 - 6.56 (d, *J* = 8.07 Hz, 1 H), 6.65 - 6.70 (m, 2 H), 7.06 - 7.10 (t, *J* = 7.02 Hz, 1 H), 7.41 - 7.44 (t, *J* = 8.53 Hz, 5 H), 7.68 (s, 1 H), 9.20 (s, 1 H), 9.70 - 9.73 (d, *J* = 8.25 Hz, 1 H), 12.81 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm : 173.69, 167.72, 157.53, 151.79, 139.02, 135.83, 131.52, 129.66, 129.38, 128.89, 128.47, 116.62, 112.99, 112.65, 57.63, 56.82, 55.33, 49.05, 38.42, 38.28, 30.97, 27.68, 19.73, 18.69 and 16.92; MS (ESI): found: $[M + H]^+$, 465.30.

((*E*)-2-(((3R,4R)-4-(3-Hydroxyphenyl)-3,4-dimethylpiperidin-1-yl)methyl)-3-phenylacryloyl) leucine (28h)

Following the above general procedure D, **28h** was synthesized from **27h** as a white solid (Yield: 59%, purity by LC-MS: >99%); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 0.69 - 0.71(d, *J* = 5.14 Hz, 3 H), 0.86 (s, 6 H), 1.18 - 1.24 (m, 4 H), 1.46 - 1.50 (m, 3 H), 1.67 (s, 1 H), 1.97-1.20 (d, *J* = 9.63 Hz, 1 H), 2.09 - 2.16 (m, 1 H), 2.24 - 2.28 (d, *J* = 11.19 Hz, 1 H), 2.84 - 2.87 (d, *J* = 8.71 Hz, 1 H), 3.22 (s, 1 H), 3.44 (s, 1 H), 4.40 - 4.42 (d, *J* = 6.00 Hz, 1 H), 6.54 - 6.56 (d, *J* = 7.34 Hz, 1H), 6.65 - 6.70 (m, 2 H), 7.05 - 7.10 (t, *J* = 6.69 Hz, 1 H), 7.36 - 7.42 (m, 5 H), 7.63 (s, 1 H), 9.24 (s, 1 H), 9.71 - 9.74 (d, *J* = 8.25 Hz, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm : 174.89,

167.64, 157.61, 151.91, 138.52, 135.87, 131.84, 129.70, 129.41, 128.87, 128.45, 116.44, 112.88, 112.68, 56.31, 55.13, 51.02, 49.02, 38.22, 29.79, 27.56, 24.90, 23.37, 21.89 and 16.87; MS (ESI): found: $[M + H]^+$, 479.30.

2-((*E*)-2-(((3R,4R)-4-(3-Hydroxyphenyl)-3,4-dimethylpiperidin-1-yl)methyl)-3-phenyl acrylamido)-3-methylpentanoic acid (28i)

Following the above general procedure D, **28i** was synthesized from **27i** as a white solid (Yield: 75%, purity by LC-MS: >99%); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 0.71 - 0.73 (d, *J* = 4.86 Hz, 3 H), 0.83 - 0.89 (m, 6 H), 1.18 - 1.24 (m, 4 H), 1.46 - 1.50 (d, *J* = 12.75 Hz, 2 H), 1.80 (s, 1H), 1.97 (s, 1 H), 2.10 - 2.14 (d, *J* = 13.02 Hz, 1 H), 2.28 - 2.36 (m, 1 H), 2.55 - 2.59 (m, 1 H), 2.80 - 2.83 (d, *J* = 10.09 Hz, 1 H), 3.21 (s, 1 H), 3.40 (s, 2 H), 4.39 - 3.41 (d, *J* = 5.96 Hz, 1 H), 6.54 - 6.56 (d, *J* = 7.43 Hz, 1 H), 6.65 - 6.70 (m, 2 H), 7.06 - 7.10 (t, *J* = 7.15 Hz, 1 H), 7.38 - 7.44 (t, *J* = 8.80 Hz, 5 H), 7.67 (s, 1 H), 9.21 (s, 1 H), 9.71 - 9.74 (d, *J* = 8.07 Hz, 1 H), 12.75 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm : 173.72, 167.63, 157.54, 151.77, 138.99, 135.81, 131.50, 129.65, 129.39, 128.89, 128.47, 116.61, 112.99, 112.66, 56.89, 56.70, 55.32, 49.15, 38.38, 38.27, 37.43, 27.68, 25.41, 16.92, 16.11 and 11.69; MS (ESI): found: $[M + H]^+$, 479.25.

2-((*E*)-2-(((3R,4R)-4-(3-Hydroxyphenyl)-3,4-dimethylpiperidin-1-yl)methyl)-3-phenyl acrylamido)pentanoic acid (28j)

Following the above general procedure D, **28j** was synthesized from **27j** as a white solid (Yield: 66%, purity by LC-MS: >99%); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 0.69 - 0.70 (d, *J* = 5.23 Hz, 3 H), 0.83 - 0.87 (t, *J* = 6.28 Hz, 3 H), 1.18 - 1.24 (m, 4 H), 1.33 - 1.35 (d, *J* = 5.96 Hz, 2 H), 1.47 - 1.57 (m, 2 H), 1.71 - 1.73 (d, *J* = 5.87 Hz, 1 H), 1.98 (s, 1 H), 2.10 - 2.18 (m, 1 H), 2.25 - 2.28 (d, *J* = 10.82 Hz, 1 H), 2.56 - 2.60 (m, 1 H), 2.83 - 2.86 (d, *J* = 9.35 Hz, 1 H), 3.40 - 3.48 (m, 2 H), 4.38 - 4.44 (t, *J* = 9.6.00 Hz, 1 H), 6.54 - 6.56 (d, *J* = 7.24 Hz, 1 H), 6.65 - 6.70 (m, 2

H), 7.06 -7.10 (t, J = 7.15 Hz, 1 H), 7.38 - 7.42 (m, 5 H), 7.64 (s, 1 H), 9.22 (s, 1 H), 9.75 - 9.78 (d, J = 7.61 Hz, 1 H), 12.68 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm : 174.38, 167.66, 157.58, 151.90, 138.63, 135.83, 131.72, 129.70, 129.42, 128.88, 128.48, 116.48, 112.88, 112.68, 56.27, 55.16, 52.18, 49.17, 38.23, 29.83 27.58, 19.13, 16.78 and 13.99; MS (ESI): found: $[M + H]^+$, 465.25.

Supporting Information

Table 3, geometric discussion of *E* and *Z* isomers, NMR (**^{1}**H, ^{13}C, NOE and NOESY) and LC-MS spectral data of compounds

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Notes

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Abbreviation

MOR, Mu-opioid receptor; KOR, Kappa-opioid receptor; DOR, Delta-opioid receptor; Ors, opioid receptors; norBNI, Norbinaltorphimine; ADME, Absorption, distribution, metabolism and excretion; CNS, Central nervous system; Pd/C, Palladium on carbon; GPCRS, G-protein-coupled receptors; PLIP, Protein Ligand Interaction Profiler; FSK, Forskolin; BE, Binding energy; μM, micromolar; pM, picomolar; nM, nanomolar; kg, kilo gram; mg, milligram; M.P., melting point; NA, not active; DMSO-*d6*, deuterated dimethyl sulfoxide; $CDCl_3$, deuterated chloroform; HCl, hydrochloric acid;.

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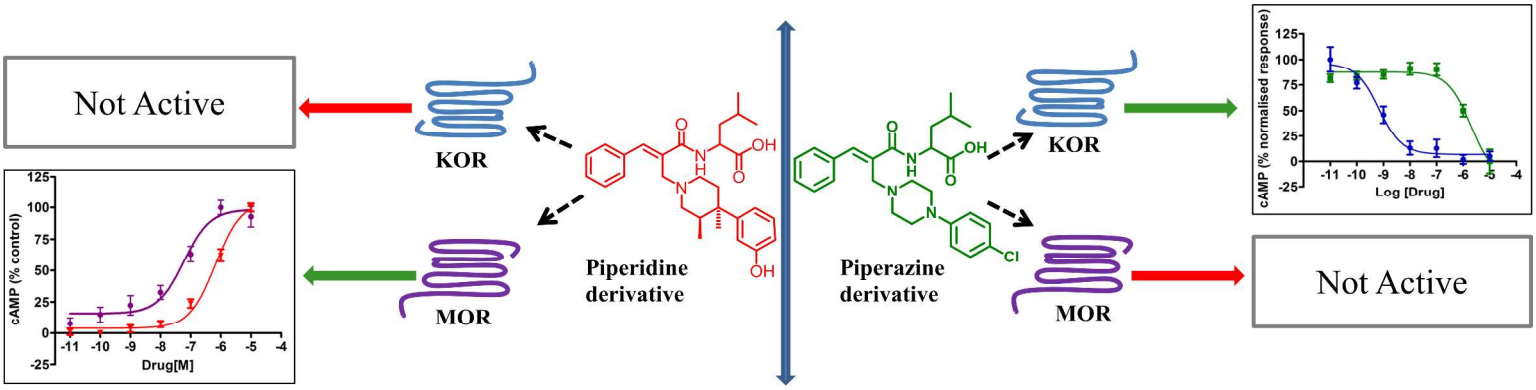
Table of Contents graphic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