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N-Substituted homopiperazine barbiturates as gelatinase inhibitors

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ABSTRACT

Matrix metalloproteinases are implicated in a wide range of pathophysiological processes and potent selective inhibitors for these enzymes continue to be eagerly sought. 5,5-Disubstituted barbiturates hold promise as inhibitor types being stable in vivo and relatively selective for the gelatinases (MMP-2 and MMP-9). In this paper we describe the synthesis of 5-piperazine and -homopiperazine substituted barbiturates. The activity of these compounds as gelatinase inhibitors was evaluated using supernatants from 12-*O*-tetradecanoylphorbol-13-acetate (PMA)-stimulated HT-1080 cells as well as using recombinant human MMPs. *N*-Acyl homopiperazine compounds were found to be potent inhibitors of the gelatinases (range in nM) and generally more potent than the corresponding piperazine analogues. The panel of *N*-acyl homopiperazines was enlarged in order to exploit differences between the gelatinases at the S2′ site in order to design MMP-2- or MMP-9-selective inhibitors. Compounds in this group exhibited single digit nano-molar potency and some selectivity between the two enzymes. Representative potent compounds were effective inhibitors of cancer cell migration.

1. Introduction

Matrix metalloproteinases (MMPs) comprise a family of zinc and calcium-dependent endopeptidases that are involved in the remodelling and degradation of the extracellular matrix (ECM) during physiological processes and in pathological conditions such as inflammation, tumour growth and metastasis.[1] MMPs have been classified into the collagenases, gelatinases and stromelysins based on their substrate specificity. These enzymes possess a high degree of sequence similarity (56–64%) in the catalytic domain. The active site is a groove, shallow in places, usually presented horizontally across the protein with three un-primed sub-sites to the left of the co-ordinated zinc and three primed sub-sites to the right (S3-S2-S1-Zn-S1′-S2′-S3′).[2,3] Inhibitors of the MMPs have been intensively pursued for the past thirty years because of their involvement in disease processes. Inhibitor design usually employs a zinc binding group (ZBG) attached to a hydrophobic moiety sometimes based on a collagen fragment. Zinc binding groups include thiols, phosphinic acids, carboxylic acids and perhaps most commonly, hydroxamates. Attempts to introduce selectivity have focussed almost exclusively on the primed side of the catalytic groove and especially the S1′ channel.[3,4]

In 2001, following a random screen for anti-tumour agents, workers from the Hoffman La-Roche company reported the surprising finding that certain 5,5-disubstituted pyrimidine-2,4,6,-triones (barbiturates) are inhibitors of the MMPs with good gelatinase selectivity.[3,5] MMP inhibitory barbiturates are without the sedative effects of the classical agents, but exhibit some of their pharmacokinetic strengths; some other Zn binding groups impart poor in vivo stability (e.g., hydroxamates).[6] The structural basis for barbiturate interactions with the MMPs was revealed in several X-ray studies.[7] The ionised barbiturate ring chelates the zinc atom in a tridentate mode directing the 5-substituents into the S1′ and S2′ substrate binding pockets. The class exhibit broadly similar SAR to non-barbiturate MMP inhibitor families. The 5-aromatic substituents (biphenyl or phenyloxyphenyl) bind in the deep hydrophobic S1′ substrate pocket. The second 5-substitutent is directed in this arrangement into the S2′ pocket towards solvent. Several elegant studies have demonstrate that C5- piperazine or piperidine substitution is favourable for high potency and moreover that the heterocycle imparts flexibility in the design of a range of barbiturate-based pharmacological, clinical and biochemical tools.[8–10]

Our laboratory has research programs investigating the role of MMPs in inflammatory bowel disease, cancer-platelet interactions and heart failure. We have an ongoing need for inhibitor tools with selectivity for MMP-2 or MMP-9 (gelatinases A and B). In designing such molecules we decided to adopt the barbiturate template mainly because of its pharmacokinetic characteristics.

We are currently interested in probing for interactions at the mouth of the S2′ pocket that might impart gelatinase type or sub-type selectivity. We were in this regard attracted to the

Abbreviations: MMP, matrix metalloproteinase; ECM, extracellular matrix; ZBG, zinc binding group; PMA, 12-*O*-tetradecanoylphorbol-13-acetate; TGF-β, transforming growth factor beta; HGF, hepatocyte growth factor; FCS, fetal calf serum; APMA, *p*-aminophenylmercuric acetate.

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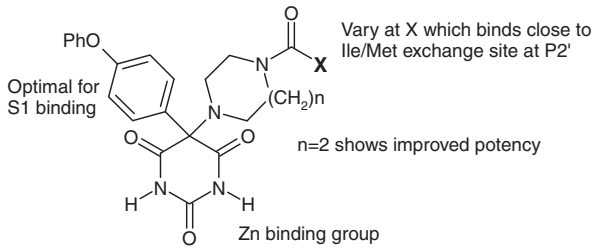


Figure 1. Strategy for exploring the P2′ site on the gelatinases.

piperazine model as these provide the possibility for distal substitution through simple N-acylation or alkylation. However, it seemed to us from inspection of X-ray models of the barbiturate docking into collagenases that the S2′ pocket might be better occluded with a seven membered ring (Fig. 1). We were also interested in differences between the gelatinases at this site that might endow interacting compounds with some sub-type selectivity. This paper describes synthesis and evaluation of acyl 5-homopiperazine barbiturates, their potential to exhibit gelatinase subtype selectivity and their effects on cancer cell migration.

2. Results and discussion

2.1. Chemistry

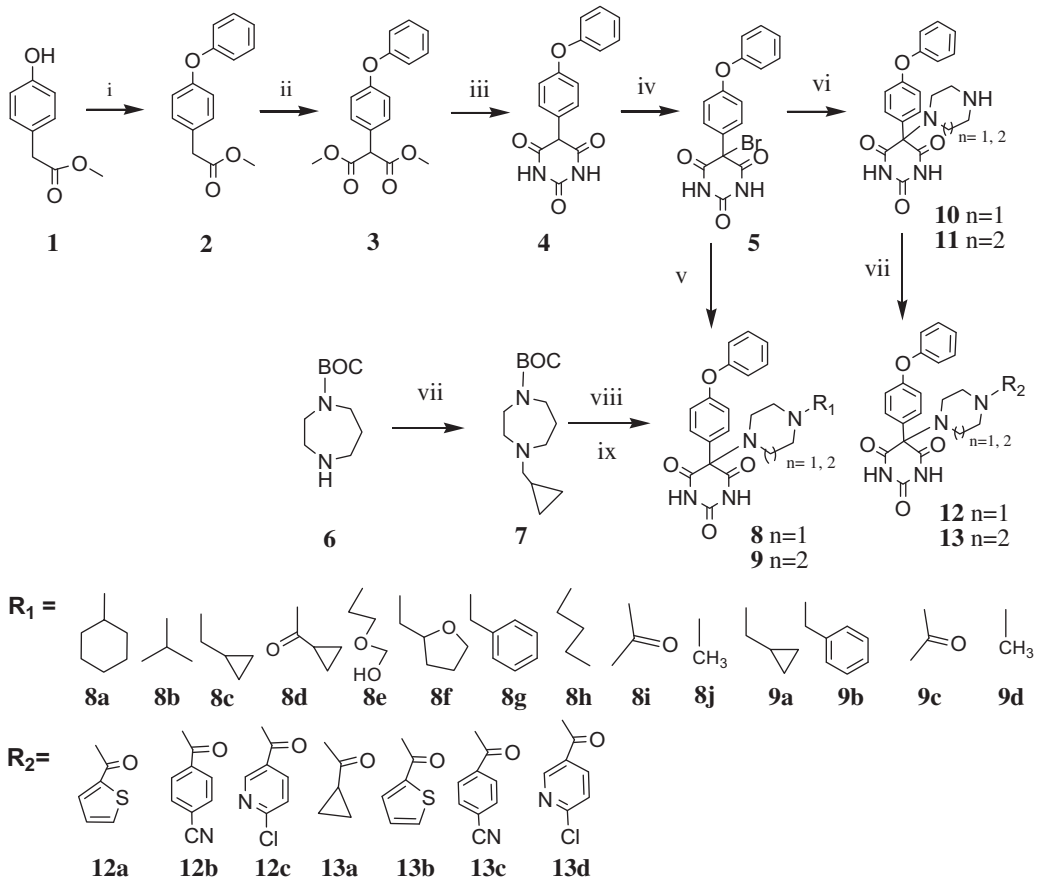
The compounds were prepared based on a standard approach to 5-substituted barbiturates outlined in Scheme 1. We had some initial problems with the incorporation of the phenoxyphenyl group. This remains an occasionally problematic transformation in organic chemistry. A number of different methods were tried such as microwave assisted reactions or the Ullmann coupling. These approaches either gave low yield or presented problems with purification. Eventually it was found that a copper-mediated coupling with phenyl boronic acid in the presence of copper acetate and pyridine worked well producing methyl 2-(4-phenoxyphenyl) acetate (**2**) in good yield.[11]

The key intermediate **5** was either converted directly to the *N*-acyl or *N*-alkyl piperazines or homopiperazine barbiturate derivatives **8a–j** and **9a–d** or reacted with piperazine or homopiperazine to produce the intermediates **10** and **11**, which then yielded **12** and **13** using the appropriate acid chlorides or carboxylic acids (Scheme 1). A wider panel of *N*-acyl homopiperazines was obtained (Scheme 2) by acylation of **11** at low temperature with the appropriate acid chloride or by diimide coupling.

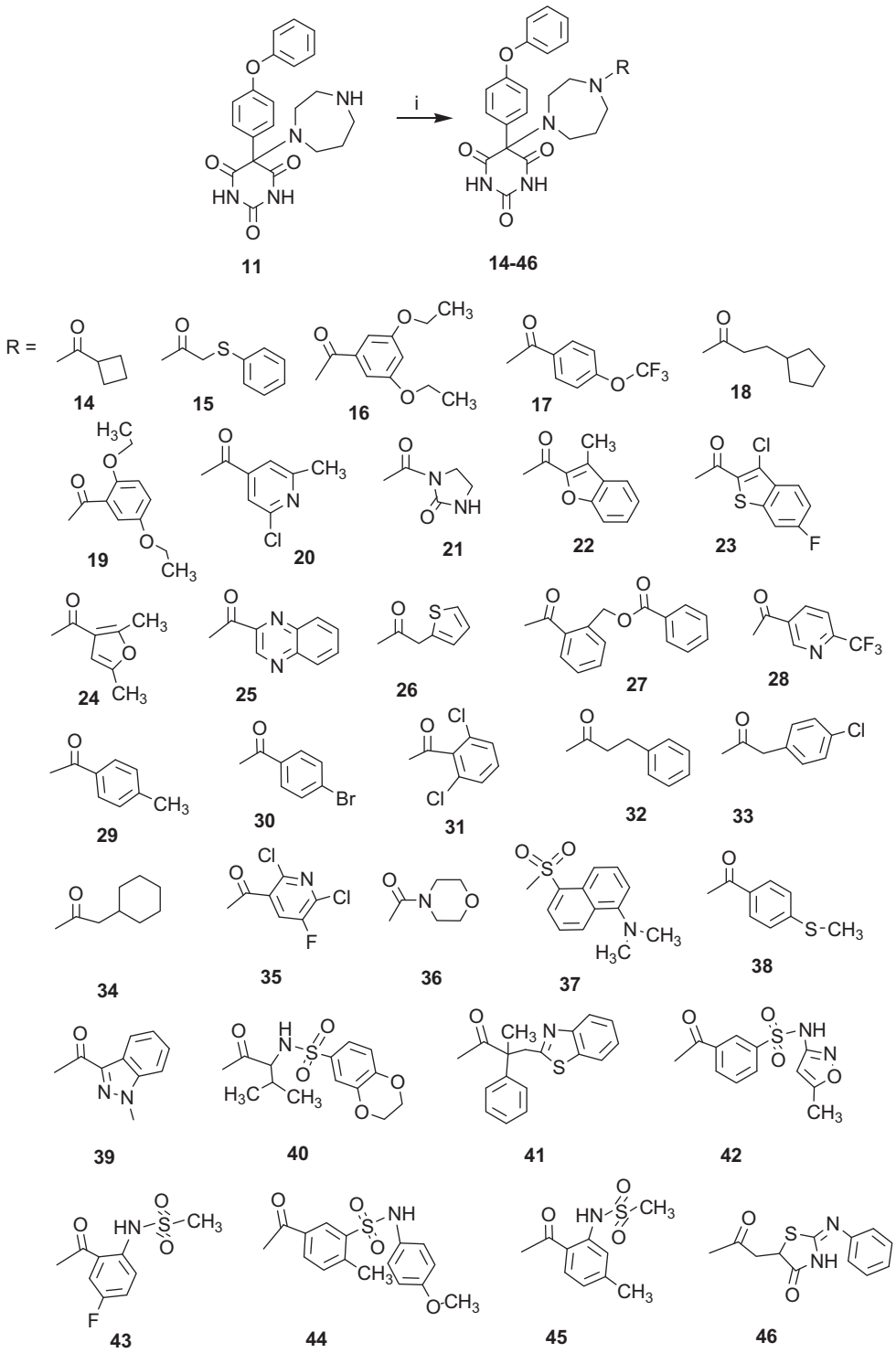
2.2. Enzyme inhibition and modelling

The novel barbiturates were screened by zymography for inhibitory effects on MMP-2 and MMP-9 using the HT1080 fibrosarcoma cell line following stimulation with 12-*O*-tetradecanoylphorbol-13-acetate (PMA). Residual gelatinolytic activity in the presence of the test compounds was measured by densitometry. Representative results of% inhibition of MMP-2 and MMP-9 at 0.5 μM are shown in Table 1.

The zymography results indicated that compound **9d**, a 5-homopiperazine caused notably good inhibition of the gelatinases,



Scheme 1. Preparation of phenoxyphenyl substituted piperazine- and homopiperazine-based barbiturate derivatives. Reagents and conditions: (i) $C_6H_5B(OH)_2$, copper(II) acetate, pyridine, DCM, rt, 24 h, 32%; (ii) $(CH_3O)_2CO$, NaH, dry THF, 105 °C, 5 h, 86%; (iii) Na, urea, EtOH, 100 °C, 7 h, 78%; (iv) Br_2, HBr, H_2O, 0 °C, 5 h, 82%; (v) substituted piperazines or homopiperazines, MeOH, rt, 24 h, 37–63%; (vi) piperazine or homopiperazine, MeOH, rt, 24 h 54–58%; (vii) acid chlorides, THF, −70 °C, 5 h or carboxylic acids, DCC, DMAP, dry THF, rt, 24 h, 31–42%; (vii) $C_3H_5CH_2Br$, MeOH, rt, 12 h, 64%; (viii) CF_3COOH, DCM, 0 °C, overnight; (ix) MeOH, compounds **5**, rt, 24 h, 41%.



Scheme 2. Preparation of 5-*N*-acyl homopiperazine barbiturates. Reagents and conditions: (i) acid chlorides, THF, −70 °C, 5 h, or DCC, DMAP, carboxylic acids, THF, rt, 12 h, 26–52%.

supporting the idea that the larger ring might impart improved affinity/potency. To further investigate this, a series of directly analogous piperazine and homo piperazine compounds **8a**–**j**, **9a**–**c**, **12a**–**c**, and **13a**–**d** were assessed. Inhibitory potency of these modified compounds was measured using human recombinant MMP-2 and MMP-9 and a fluorogenic assay.[12] The results are shown in Table 2.

The barbiturate homopiperazine compounds exhibited greater potency than the corresponding piperazine-substituted compounds apart from compounds **13d** (IC_{50} (MMP-2) = 110 nM, (MMP-9) = 104 nM) with homopiperazine ring, being somewhat less potent than **12c** (IC_{50} (MMP-2) = 45 nM, (MMP-9) = 54 nM) with piperazine ring (Table 2). The MMP-2 and MMP-9 inhibition by cyclopropylcarbonyl substituted barbiturate derivatives slightly increased when the piperazine ring **8d** (IC_{50} (MMP-2) = 27 nM, (MMP-9) = 15 nM) was replaced by the homopiperazine ring **13a** (IC_{50} (MMP-2) = 13 nM, (MMP-9) = 13 nM).

Benzyl homopiperazine **9b** (IC_{50} (MMP-9) = 22 nM) showed fourfold higher potency towards MMP-9 than its piperazine

Table 1
Representative gelatinase inhibition by **8a–i** and **9d** determined by zymographically separating gelatinases from PMA-stimulated human fibrosarcoma HT1080 cells and incubating in the presence of test compounds at 0.5 μM ($n = 3$)

Compd	MMP-9 inhibition (%)	MMP-2 inhibition (%)	Compd	MMP-9 inhibition (%)	MMP-2 inhibition (%)
8a	64 ± 5	74 ± 5	**8e**	25 ± 7	30 ± 11
8b	62 ± 5	71 ± 5	**8f**	55 ± 11	64 ± 7
8c	37 ± 8	55 ± 10	**8g**	53 ± 12	52 ± 11
8d	69 ± 13	76 ± 12	**8h**	42 ± 6	63 ± 7
9d	28 ± 7	77 ± 7	**8i**	49 ± 8	54 ± 11

Table 2
IC_{50} values comparing piperazine and homopiperazine substituted compounds

Piperazines			Homopiperazines		
Compd	MMP-2 IC_{50} (nM)	MMP-9 IC_{50} (nM)	Compd	MMP-2 IC_{50} (nM)	MMP-9 IC_{50} (nM)
8c	5.2 (3.5–7.7)	10 (8–13)	**9a**	1.1 (0.7–1.5)	1.1 (0.8–1.5)
8d	27 (21–34)	15 (12–20)	**13a**	13 (11–16)	13 (8–19)
8g	131 (114–151)	86 (71–104)	**9b**	26 (19–35)	22 (16–30)
8i	14 (14–16)	16 (13–20)	**9c**	1.5 (1.1–2.1)	2.9 (2.1–3.9)
8j	16 (11–23)	9.4 (7.8–11)	**9d**	1.9 (1.6–2.3)	7.5 (6.2–9.1)
12a	43 (31–62)	73 (58–91)	**13b**	19 (13–27)	16 (12–20)
12b	43 (34–56)	45 (36–56)	**13c**	28 (20 – 41)	9.8 (7.7–13)
12c	45 (34–61)	54 (45–65)	**13d**	110 (97–125)	104 (89–123)

analogue **8g** (IC_{50} (MMP-9 = 86). The acetyl substituted homopiperazine inhibitor **9c** (IC_{50} (MMP-2) = 1.50 nM, (MMP-9) = 2.90 nM) had a ninefold increased potency on MMP-2 and fivefold in MMP-9 compared with piperazine-based compound **8i** (IC_{50} (MMP-2) = 14 nM, (MMP-9) = 16 nM). The changing of piperazine substitution in the cyclopropylmethyl compound **8c** (IC_{50} (MMP-2) = 5.20 nM, (MMP-9) = 10 nM), the most potent piperazine-based compound for MMP-2 in the library, to homopiperazine ring **9a** (IC_{50} (MMP-2) = 1.08 nM, (MMP-9) = 1.15 nM) improved the potency fivefold in MMP-2 and 10-fold in MMP-9. Molecular modelling methods were used to investigate initial biochemical findings. Piperazine and homopiperazine compounds were docked into both MMP-2 and MMP-9 using Autodock 4. The docking experiments placed the C5-phenoxyphenyl group deep into the S1′ pocket as expected, with the piperazine/homopiperazine directed into the S2′ site and towards solvent. Docking of several of the piperazine/homopiperazine pairs suggested that the homopiperazine compounds are more potent because of increased occlusion of the S2 with better surface contacts with both MMP2 and MMP9.

A larger panel of compounds was constructed in order to explore the potential of homopiperazines to exhibit gelatinase subtype selectivity (Scheme 2). We deliberately included fragments for conjugation with **11** that could potentially donate a H-bond to the sulfur atom of Met422 of MMP-9 in the expectation that this might impart selectivity over MMP-2, which is substituted with an Ile at the corresponding position. Initial modelling studies indicated that compounds **38**–**46** would position functionality close to Met422 to make favourable contacts for binding.

Results for the homopiperazine panel are presented in Table 3. Compound **16** had the highest IC_{50} value on MMP-2, exhibiting >4-fold selectivity for MMP-9 over MMP-2 (IC_{50}: MMP-2 = 1190 nM, MMP-9 = 269 nM). Compound **31** (IC_{50}: MMP-2 = 87 nM, MMP-9 = 23 nM) was >11-fold more potent than **16** in inhibition of gelatinases and had nearly fourfold selectivity for MMP-9 over MMP-2. Compounds **18** (IC_{50}: (MMP-2) = 155 nM, MMP-9 = 50 nM) and **35** (IC_{50}: MMP-2 = 81 nM, MMP-9 = 25 nM) also exhibited MMP-9 selectivity. Compound **38** (IC_{50}: MMP-2 = 93 nM, MMP-9 = 411 nM) was the weakest MMP-9 inhibitor, but was the most MMP-2 selective inhibitor. Other compounds such as **20** (IC_{50}: MMP-2 = 28 nM, MMP-9 = 86 nM) and **37** (IC_{50}: MMP-2 = 74 nM, MMP-9 = 311 nM) also showed moderate selectivity for MMP-2 over MMP-9.

Table 3
IC_{50} values of C5 homopiperazine-based barbiturate derivatives estimated from sigmoids obtained at five concentration levels in duplicate

Compd	MMP-2 (nM)	MMP-9 (nM)	MMP-9 selectivity
14	14 (12–17)	13 (11–16)	1.08
15	36 (28–47)	29 (24–35)	1.24
16	1190 (974–1453)	269 (220–330)	4.42
17	70 (61–81)	60 (51–71)	1.17
18	155 (121–198)	50 (42–59)	3.10
19	376 (294–480)	329 (286–378)	1.14
20	28 (22–36)	86 (64–115)	0.33
21	191 (156–233)	264 (218–320)	0.72
22	2.88 (2.35–3.52)	2.55 (1.96–3.32)	1.13
23	215 (181–255)	308 (251–377)	0.70
24	25 (20–30)	24 (20–29)	1.04
25	34 (28–43)	30 (25–37)	1.13
26	51 (44–59)	47 (39–57)	1.09
27	120 (96–149)	106 (83–135)	1.13
28	198 (172–228)	264 (213–327)	0.75
29	44 (37–51)	80 (69–93)	0.55
30	282 (232–343)	268 (220–327)	1.05
31	87 (77–97)	23 (20–26)	3.78
32	62 (50–76)	31 (22–43)	2.00
33	173 (127–236)	95 (72–125)	1.82
34	77 (69–86)	100 (82–124)	0.77
35	81 (68–97)	25 (21–30)	3.24
36	24 (19–30)	25 (19–32)	0.96
37	74 (66–84)	311 (232–417)	0.24
38	93 (76–113)	411 (267–635)	0.23
39	214 (169–271)	24 (21–28)	8.92
40	698 (595–818)	706 (524–953)	0.99
41	978 (800–1196)	681 (525–901)	1.44
42	15 (13–18)	20 (15–25)	0.75
43	57 (48–68)	83 (39–101)	0.69
44	28 (22–35)	54 (38–75)	0.52
45	80 (64–99)	142 (118–170)	0.56
46	76 (65–90)	184 (153–221)	0.41

The most potent inhibitor in the homopiperazine library (Table 3) was **22** (IC_{50}: MMP-2 = 2.88 nM, MMP-9 = 2.55 nM), which had very low IC_{50} values on both MMP-2 and MMP-9, but no selectivity. Other inhibitors such as **14** (IC_{50}: MMP-2 = 14 nM, MMP-9 = 13 nM), **24** (IC_{50}: MMP-2 = 25 nM, MMP-9 = 24 nM), and **36** (IC_{50}: MMP-2 = 24 nM, MMP-9 = 25 nM) were potent MMP-2 and MMP-9 inhibitors, but again with poor selectivity. Several

compounds were neither potent nor selective, including **19**, **21**, **23**, **28** and **30**.

Compound **22** is structurally similar to **23** but the inhibitory potencies of the two compounds were markedly different, with **22** (IC_{50}: MMP-2 = 2.88 nM, MMP-9 = 2.55 nM) showing nearly 100-fold greater inhibition of MMP-2 and MMP-9 than **23** (IC_{50}: MMP-2 = 215 nM, MMP-9 = 308 nM). The lower potency of **23** could be due to the size of sulfur and chlorine substitutions on the indene ring, relative to the oxygen and methyl groups in **22**. Compound **23** with its bulky, rigid substituent attached to homopiperazine might be sterically excluded from the mouth of the S2′ pocket resulting in loss of binding affinity. Therefore, the size and rigidity of the subgroup attached to homopiperazine is likely to be important for potency. This would explain why **16** and **19** showed weak inhibition towards MMP-2 and MMP-9 compared with **17** which has a smaller benzene ring substituent (–OCF_3).

The docking indicated that the ethoxyl groups in **16** prevents the homopiperazine substitution from entering the S2′ pocket.

The most MMP-9 selective compound in the library was **39**, showing nearly ninefold selectivity for MMP-9 over MMP-2 (IC_{50}: MMP-2 = 214 nM, MMP-9 = 24 nM). This may be because of the orientation the compound can adopt in the gelatinases (Fig. 2). When the compound was docked with MMP-9, the N-methyl group pointed towards the sulfur on Met422. Although **39** could be docked with a similar orientation in MMP-2, the interaction between the nitrogen and Ile222 was not as strong.

Another reason for selectivity of **39** on MMP-9 might be the size and rigidity of subgroup attached to the homopiperazine ring, that is, methyl indazole. When the nitrogen atom and methyl group in the indazole pointed toward the Met422 residue, it had the best binding affinity because the methyl indazole just fitted into the mouth of the S2′ pocket. If the methyl group turned to other directions, the walls of the S2′ pocket would push the indazole group away from the mouth of the pocket, resulting in loss of affinity.

In most of the models that we studied, the mouth of S2′ pocket of MMP-2 was slightly smaller than of MMP-9.[7] Substituents of appropriate size attached to the homopiperazine ring could go deeper into the S2′ pocket of MMP-9 than MMP-2. This might be the reason why **16**, **31** and **35** exhibited (moderate) selectivity towards MMP-9. These three compounds have substitutions on the benzene ring capable more easily penetrating the mouth of the S2′ pocket in MMP-9 than the narrower MMP-2, resulting in the better binding and inhibition of MMP-9.

2.3. Inhibition of cancer cells invasiveness

To inhibit the ability of cancer cells to undergo migration and invasion is the one of most crucial issues in the cancer research field.[13] The ECM by providing a physical support or barrier for cells plays an important role in maintenance of tissue structure and cell migration.[14] During invasion, neoplastic cells detach from primary tumours enter the lymphatic and blood vessels by crossing the epithelial basement membrane, and undergo metastatic growth at distant sites.[2] MMPs are intimately connected with these processes.[2] The degradation of ECM component is necessary for cell migration.[15] MMPs have been considered to be a major regulator of ECM composition and to be promoters for cell migration due to their ability to degrade the ECM components. For example, MMP-2 and MMP-9 can cleave a major protein component of basement membrane-type IV collagen.[16,17]

Some biologically active modular breakdown products released from the proteolytic remodelling of ECM can promote cell migration. For example, laminin-5 cleaved by MMP-2 produces a gamma2 subunit, which has been shown to induce migration of breast epithelial cells.[18] Recently it has been clearly demonstrated that MMPs are involved in regulation of cytokines, chemokines, growth factors, and a variety of enzymes.[14] Degradation of ECM molecules results in release of various growth factors and cytokines stored in the ECM molecules. For example, decroin, a small proteoglycan, functions as a reservoir for TGF-β, and cleavage of decroin by various MMPs results in release of TGF-β triggering its biological functions.[19]

In addition, MMPs are able to cleave cell-cell adhesion proteins, to release bioactive cell surface molecules, and to degrade signal-transducing molecules.[20] For example, the cleavage of signal-transducing molecule E-cadherin and the soluble E-cadherin fragment by MMP-3 and MMP-7 inhibits cell aggregation and promotes cell invasion.[21]

Therefore, inhibition of MMP activity can counteract cancer cell invasion by inhibiting degradation of ECM and release of cytokines and growth factors. Homopiperazine-base barbiturates with potent inhibition on MMP-2 and MMP-9 were tested in an invasion assay to investigate the potential of inhibition of gelatinase activity on cancer cell invasion. Many types of cells have been used in invasion assays such as HT1080, MCF-7, and Caco-2 cells. Migration of Caco-2 cells was monitored in our assay using a Matrigel membrane with hepatocyte growth factor (HGF) used to stimulate growth and invasiveness of Caco-2 cells[22] (in our hands Caco-2 cells are not so aggressive and quite easy to manipulate pharmacologically in this assay, whereas HT1080 cells produce a better MMP secretion response to PMA). The negative control in this assay consisted of cells untreated with HGF. Cell migration was monitored by microscopy (Figure 3).

Compounds **9d**, **31**, and **39** were assayed at 10 μM and 100 nM; **22** and **37** were tested at 100 nM. The inhibition of Caco-2 cells invasiveness by these compounds was dependent on their ability

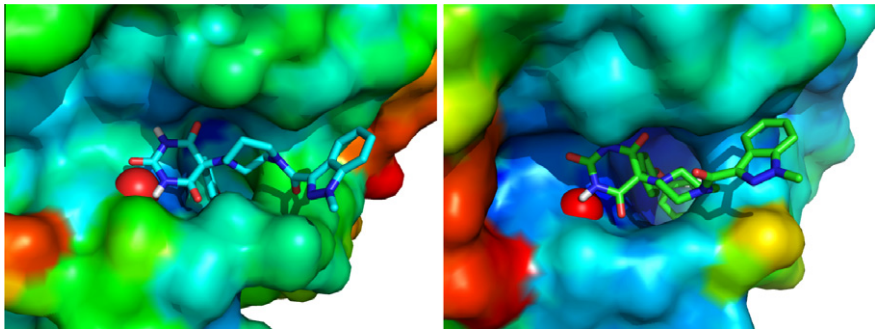


Figure 2. Pictures of **39** docked with Connolly surfaces on MMP-2 (left) and MMP-9 (right). The surfaces were generated by the programme PyMOL based on the crystal structures of MMP-2 (pdb code: 1q1b) and MMP-9 (pdb code: 2ovx). The ligand orientations were used which had the highest predicted affinity. The nitrogen atom and methyl group on the indazole of **39** were close to the amino acid Ile222/Met422 (yellow sulfur) interchange between MMP-2 and MMP-9. The active site zincs are shown as red spheres.

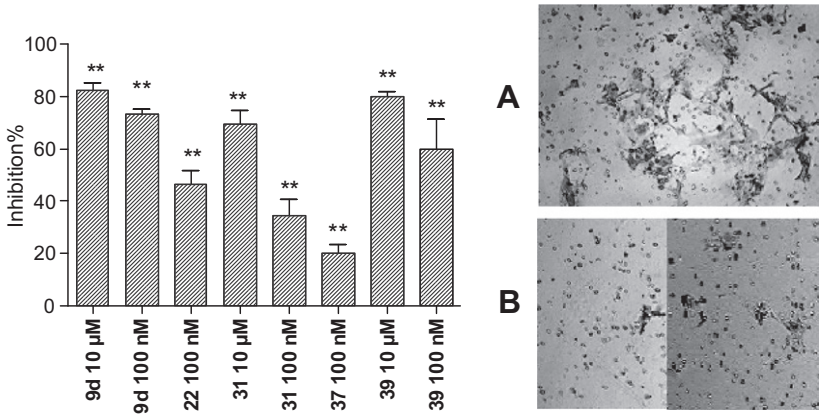


Figure 3. Results from the invasion assay with compounds **9d**, **22**, **31**, **38**, **39** (P <0.05). Representative microscope images are shown from the invasion assays: (A) a positive control insert, incubated with Caco-2 cells and HGF. (B) Insert incubated with Caco-2 cells, HGF, and 10 μM and 100 nM **9d**.

to inhibit activities of MMP-2 and MMP-9. The least potent compound in the recombinant enzyme assay (**37**) exerted weakest inhibition (20.15%) at 100 nM. Compound **39** caused greater inhibition of invasion of the cells than **31** at 10 μM and 100 nM, even though **39** (IC_{50}: MMP-2 = 213.79 nM, MMP-9 = 23.90 nM) had higher IC_{50} values on both MMP-2 and MMP-9 than **31** (IC_{50}: MMP-2 = 86.50 nM, MMP-9 = 22.71 nM). In addition, **39** had better ability to inhibit the invasiveness than **22** (IC_{50}: MMP-2 = 2.88 nM, MMP-9 = 2.55 nM) which possessed over 70-fold potency on MMP-2 and ninefold on MMP-9 than **39**. It is possible that the out of trend inhibitory ability of **39** is due to its MMP-9 selectivity. MMP-9 may play a more important functional role in facilitating cancer cell migration than MMP-2. For example, MMP-2 and MMP-9 can both activate latent TGF-β, but only MMP-9 coordinated with TGF-β could promote cancer cell growth and invasion.[23] It has also been demonstrated that tumour cell invasion is correlated and regulated by MMP-2.[24] Compound **9d** which exerted fourfold selectivity for MMP-2 over MMP-9 had lower potency on MMP-9 than **22**, but showed better inhibition of invasion. It seems that inhibition of only one protein's activity, either MMP-2 or MMP-9, may result in better inhibition of invasion. Because only five compounds were examined in the invasion assay it is hazardous to draw conclusions about relationships nevertheless the percentage of inhibition of migration values at 100 nM exhibited a moderate correlation with MMP-9 IC_{50} values (r^2 0.45). But our results illustrate that inhibition of activity of MMP-2 and MMP-9 could decrease cancer cell invasive ability, affirming that MMP-2 and MMP-9 are important for cancer cell invasion and important targets for cancer therapy.

3. Conclusion

A panel of barbiturate 5-piperazine- and homopiperazine-based compounds were prepared to investigate interactions at the mouth of S2′ pocket of the gelatinases where this group binds. The lower IC_{50} values of homopiperazine ring barbiturates and the docking support our hypothesis that the larger ring could better fill the S2′ pocket. Several compounds exhibited moderate gelatinase subtype selectivity and good potency. However, selectivity in the series was not as good as anticipated. We can speculate on possible contributors to this observation- overlapping physicochemical properties and binding characteristics in the Ile and Met side chains; binding competition with water on the exposed site; failure to properly target the identified amino acid exchange site because of a lack of flexibility in the barbiturate binding mode. Nonetheless, representative compounds from the homopiperazine class were effective inhibitors of cancer cell migration in an in vitro model of metastasis using colon carcinoma cells (CACO-2). We are currently using the inhibitor panel described here to probe pathophysiological contributions of the gelatinases in inflammatory bowel disease.

4. . Methods and materials

4.1. Chemistry

All chemicals were purchased from Sigma–Aldrich (Ireland), except where stated. All the reactions were monitored using TLC. Uncorrected melting points were measured on a Stuart Apparatus. Infra-red (IR) spectra were performed on a Perkin Elmer FT-IR Paragon 1000 spectrometer. ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded at 27 °C on a Brucker DPX 400 spectrometer (400.13 MHz, ^1H; 100.61 MHz, ^{13}C). Coupling constants are reported in Hertz. For ^1H NMR assignments, chemical shifts are reported: shift value (number of protons, description of absorption, coupling constant(s) where applicable). Electrospray ionisation mass spectrometry (ESI-MS) was performed in the positive ion mode on a liquid chromatography time-of-flight mass spectrometer (Micromass LCT, Waters Ltd., Manchester, UK). The samples were introduced into the ion source by an LC system (Waters Alliance 2795, Waters Corporation, USA) in acetonitrile/water (60:40% v/v) at 200 μl/min. The capillary voltage of the mass spectrometer was at 3 kV. The sample cone (de-clustering) voltage was set at 40 V. For exact mass determination, the instrument was externally calibrated for the mass range m/z 100 to m/z 1000. A lock (reference) mass (m/z 556.2771) was used. Mass measurement accuracies of <±5 ppm were obtained. Compound purity/homogeneity was confirmed using a combination of NMR, TLC and HPLC.

4.1.1. Methyl 2-(4-phenoxyphenyl)acetate (2)

Methyl 4-hydroxyphenylacetate (3.32 g, 20 mmol), copper (II) acetate (3.58 g, 20 mmol), phenylboronic acid (4.88 g, 40 mmol), powdered 4 Å molecular sieves and pyridine (8 ml) were added into DCM (100 ml). The reaction mixture was stirred at room temperature for 20 h. The resulting mixture was filtered and diethyl ether (50 ml × 3) was used to extract the compound and purified by column chromatography to afford the pure compounds as the yellow oil (1.55 g, 32%). MS: calcd for $C_{15}H_{14}O_3Na$ = 265.0841. $(M+Na)^+$ = 265.0835 found. ^1H NMR ($CDCl_3$) δ ppm: 3.68 (s, 2H, CH_2), 3.76 (s, 3H, CH_3), 7.03–7.09 (m, 4H, Ar-H), 7.14–7.18 (t, J = 7.53, 1H, Ar-H), 7.30–7.32 (d, J = 8.53, 2H, Ar-H), 7.37–7.41 (m,

J = 7.53, 2H, Ar-H). ^{13}C NMR ($CDCl_3$) δ ppm: 40.2; 51.9; 115.2; 118.7; 123.1; 128.5; 129.6; 130.5; 156.2; 156.9; 172.2; IR (CH_2Cl_2): ν (cm^{-1}): 3063, 1731, 1613, 1589.

4.1.2. Dimethyl 2-(4-phenoxyphenyl)malonate (3)

A suspension of NaH (60% suspension in paraffin oil (paraffin oil was removed by repeated washings with hexane)) (80 mg, 20 mmol) and dimethyl carbonate (5.4 ml, 64 mmol) in dry THF (64 ml) was heated to 100 °C, and a solution of methyl 2-(4-phenoxyphenyl) acetate (2.42 g, 10 mmol) in THF (20 ml) was added dropwise over a period of 1 h. After being refluxed for 5 h, the mixture was poured onto ice water and extracted with DCM (50 ml × 3). The combined organic layers were washed with water and brine, dried (Na_2SO_4), and concentrated. The crude products were purified by flash column chromatography using hexane and ethyl acetate to yield the pure compounds (2.57 g, 86%) as yellow solid. Mp: 43–45 °C. MS: calcd for $C_{17}H_{16}O_5Na$ = 323.0895. $(M+Na)^+$ = 323.0888 found. ^{1}H NMR ($CDCl_3$) δ ppm: 3.79 (s, 6H, CH_3), 4.68 (s, 1H, CH), 7.01–7.07 (m, 4H, Ar-H), 7.13–7.17 (t, J = 7.53, 1H, Ar-H), 7.35–7.41 (m, 4H, Ar-H). ^{13}C NMR ($CDCl_3$) δ ppm: 52.8; 56.6; 118.4; 119.2; 123.5; 126.9; 129.7; 130.6; 156.5; 157.4; 168.5. IR (KBr): ν (cm^{-1}): 3064, 1734, 1611, 1590.

4.1.3. 5-(4-Phenoxyphenyl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (4)

Sodium (460 mg, 2 0 mmol) was dissolved in 60 ml ethanol (HPLC grade), and urea (1.02 g, 17 mmol) was added to this solution. A solution of dimethyl 2-(4-phenoxyphenyl)malonate (3.00 g, 10 mmol) in ethanol was added dropwise, and the reaction mixture was heated to reflux for 7 h. After being cooled to ambient temperature, the mixture was poured onto ice water and adjusted to pH 2, using dilute HCl. The precipitate was collected by suction filtration and dried in vacuo to afford the product off-white solids (2.31 g, 78%). Mp: 250–253 °C. MS: calcd for $C_{16}H_{12}N_2O_4$-Na = 319.0695. $(M+Na)^+$ = 319.0684 found. ^{1}H NMR (DMSO-d_6) δ ppm: 4.84 (s, 1H, CH), 6.95–6.97 (d, 2H, J = 8.56, Ar-H), 7.01–7.03 (m, 2H, Ar-H), 7.13–7.16 (t, 1H, J = 6.85, Ar-H), 7.26–7.28 (d, 2H, J = 8.07, Ar-H), 7.37–7.41 (t, 2H, J = 7.58, Ar-H) 11.4 (s, 2H, NH). ^{13}C NMR (DMSO-d_6) δ ppm: 54.4; 118.5; 119.1; 123.9; 129.3; 130.2; 131.2; 151.1; 156.4; 156.5; 169.4. IR (KBr): ν (cm^{-1}): 3063, 1788, 1761, 1720, 1572.

4.1.4. 5-bromo-5-(4-phenoxyphenyl)pyrimidine-2,4,6(1*H*,3*H*, 5*H*)-trione (5)

A suspension of 5-(4-phenyoxyphenyl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (1.185 g, 4 mmol) in 20 ml water was cooled to 0–5 °C, and a mixture of 48% HBr (764 μl, 7.1 mmol) and bromine (328 μl, 6.4 mmol) was added dropwise.[8,25] After stirring for 4–5 h at 0–10 °C the precipitate was collected by filtration and dried in vacuo to afford off-white solids (1.22 g, 82%). Mp: 111–113 °C. ^{1}H NMR (DMSO-d_6) ppm: 6.98–7.08 (m, 4H, Ar-H), 7.15–7.18 (t, 2H, J =7.53, Ar-H), 7.38–7.45 (m, 3H, Ar-H), 7.55–7.57 (d, 2H, J = 9.04, Ar-H), 11.56(s, 1H, NH), 11.57 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) ppm: 115.6; 118.4; 118.8; 119.1; 121.1; 124.0; 127.1; 127.2; 130.2; 133.0; 149.9; 156.0; 157.3; 170.9. IR (KBr): ν (cm^{-1}): 3078, 1737, 1718, 1607.

4.1.5. *tert*-Butyl 4-(cyclopropylmethyl)-1,4-diazepane-1-carboxylate (7)

A solution of Boc-homopiperazine (400 mg, 2 mmol) and triethylamine (202 mg, 2 mmol) in methanol was treated with (bromomethyl)cyclopropane (270 mg, 2 mmol) and stirred for 24 h at room temperature. After reaction finished, the solvent was removed and the residue was purified by flash column chromatography to yield the pure oil compound (328 mg, 64%). MS: calcd for $C_{14}H_{27}N_2O_2$ = 255.2073. $(M+H)^+$ = 255.2060 found. ^{1}H NMR ($CDCl_3$) ppm: 0.12–0.14 (m, 2H, CH_2), 0.49–0.51 (m, 2H, CH_2), 0.88–0.96 (m, 1H, CH), 1.34 (s, 9H, CH_3), 1.95–2.02 (m, 2H, CH_2), 2.50–2.55 (m, 2H, CH_2), 2.82–2.85 (m, 2H, CH_2), 2.87–2.90 (m, 2H, CH_2), 3.35–3.41 (m, 2H, CH_2), 3.49–3.52 (m, 1H, CH_2), 3.57–3.60 (m, 1H, CH_2). ^{13}C NMR ($CDCl_3$) ppm: 3.8; 7.1; 25.4; 27.8; 42.9; 43.9; 53.7; 55.2; 62.0; 79.2; 154.8. IR (CH_2Cl_2): ν (cm^{-1}): 2974, 1701, 1699, 1590.

4.1.6. General procedure for the preparation of compounds 8a–j and 9a–d

A solution of 5-bromo-5-(4-phenoxyphenyl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (1.0 mmol) in methanol (5 ml) was treated with the substituted piperazines or homopiperazines (2 mmol) and stirred for 24 h at ambient temperature. Compounds **8a**, **8b**, **8c**, **8f**, and **8g** precipitated. The precipitates were collected by filtration and dried in vacuo to afford the solids. In the case of compounds **8d**, **8e**, **8h**, **8i** and **9a–d**, the solvents of reactions were removed and the residues purified by flash column chromatography and dried to yield the pure solids.

4.1.6.1. 5-(4-Cyclohexylpiperazin-1-yl)-5-(4-phenoxyphenyl)-pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (8a). Yield: 63% of off-white solids. Mp: >252 °C. MS: calcd for $C_{26}H_{31}N_4O_4$ = 463.2345. $(M+H)^+$ = 463.2350 found. ^{1}H NMR (DMSO-d_6) ppm: 1.03–1.19 (m, 6H, CH_2), 2.18–2.25 (m, 1H, CH), 2.51–2.57 (m, 8H, CH_2), 7.00–7.06 (m, 4H, Ar-H), 7.16–7.20 (t, J = 7.53, 1H, Ar-H), 7.38–7.43 (m, 3H, Ar-H), 7.55–7.58 (m, 1H, Ar-H),11.61 (s, 2H, NH). ^{13}C NMR (DMSO-d_6) ppm: 25.3; 25.9; 28.2; 47.7; 49.0; 62.5; 73.9; 115.8; 118.0; 118.4; 119.3; 121.3; 124.1; 129.7; 129.8; 130.2; 133.0; 149.5; 155.8; 157.4; 170.0. IR (KBr): ν (cm^{-1}): 2933, 1711, 1737, 1614.

4.1.6.2. 5-(4-Isopropylpiperazin-1-yl)-5-(4-phenoxyphenyl)-pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (8b). Yield: 57% of off-white solids. Mp: >252 °C. MS: calcd for $C_{23}H_{27}N_4O_4$ = 423.2032. $(M+H)^+$ = 423.2032 found. ^{1}H NMR (DMSO-d_6) ppm: 0.95–0.97 (d, J = 6.52, 6H, CH_3), 2.47–2.51 (m, 4H, CH_2), 2.58–2.60 (m, 4H, CH_2), 2.64–2.69 (m, 1H, CH), 7.00–7.06 (m, 4H, Ar-H), 7.16–7.20 (t, J = 7.53, 1H, Ar-H), 7.39–7.43 (m, 3H, Ar-H), 7.55–7.59 (m, 1H, Ar-H),11.61 (s, 2H, NH). ^{13}C NMR (DMSO-d_6) ppm 18.0; 47.5; 48.6; 53.9; 73.9; 115.8; 118.1; 118.4; 119.3; 121.3; 124.1; 129.7; 129.8;130.2; 133.0;149.5; 155.8; 157.4; 170.0. IR (KBr): ν (cm^{-1}): 3066, 1735, 1717, 1608.

4.1.6.3. 5-(4-(Cyclopropylmethyl)piperazin-1-yl)-5-(4-phenoxyphenyl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (8c). Yield: 59% of off-white solids. Mp: 242–246 °C. MS: calcd for $C_{23}H_{27}N_4O_4$ = 435.2032. $(M+H)^+$ = 435.2032 found. ^{1}H NMR (DMSO-d_6) ppm: 0.02–0.05 (m, 2H, CH_2), 0.40–0.44 (m, 2H, CH_2), 0.73–0.83 (m, 1H, CH), 2.14–2.16 (d, J = 6.53, 2H, CH_2), 2.43 (s, br, 2H, CH_2), 2.59 (s, br, 2H, CH_2), 7.00–7.06 (m, 4H, Ar-H), 7.15–7.20 (t, J = 7.53, 1H, Ar-H), 7.38–7.43 (m, 3H, Ar-H), 7.55–7.58 (m, 1H, Ar-H). ^{13}C NMR (DMSO-d_6) ppm: 3.1; 8.1; 47.3; 53.2; 62.8; 73.9; 118.1; 118.4; 119.3; 121.3; 124.1; 129.7; 129.8; 130.2; 129.8; 149.5; 155.8; 157.4; 170.0. IR (KBr): ν (cm^{-1}): 3000, 1735, 1709, 1607.

4.1.6.4. 5-(4-(Cyclopropylcarbonyl)piperazin-1-yl)-5-(4-phenoxyphenyl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (8d). Yield: 38% of off-white solids. Mp: 148–150 °C. MS: calcd for $C_{25}H_{27}N_4O_5$-Na = 471.1644. $(M+Na)^+$ = 471.1651 found. ^{1}H NMR (acetone-d_6) ppm: 0.67–0.72 (m, 2H, CH_2), 0.81–0.85 (m, 2H, CH_2), 1.89–1.93 (m, H, CH), 2.72–2.81 (m, 4H, CH_2); 3.58–3.74 (m, 4H, CH_2); 7.04–7.10 (m, 4H, Ar-H), 7.18–7.12 (t, J = 7.53, 1H, Ar-H), 7.41–7.45 (m, 1H, Ar-H), 7.57–7.64 (m, 3H, Ar-H) 10.49 (s, 2H, NH). ^{13}C NMR (acetone-d_6) ppm: 7.1; 10.7; 43.1; 46.5; 48.4; 48.9; 75.6; 116.5; 118.6; 119.0; 120.0; 121.9; 124.6; 129.9; 130.4; 130.6; 149.1; 156.7; 159.0; 170.2; 171.8. IR (KBr): ν (cm^{-1}): 3010, 1751, 1737, 1708, 1608.

4.1.6.5. 5-(4-2(-2-Hydroxyethoxy)ethyl)piperazin-1-yl)-5-(4-phenoxyphenyl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (8e). Yield: 49% of off-white solids. Mp: 156–160 °C. MS: calcd for $C_{24}H_{29}N_4O_6$ = 469.2087. $(M+H)^+$ = 469.2088 found. ^{1}H NMR (DMSO-d_6) ppm: 2.42–2.47 (m, 6H, CH_2), 2.56–2.59 (m, 4H, CH_2), 3.36–2.50 (m, 6H, CH_2), 7.00–7.06 (m, 4H, Ar-H), 7.15–7.19 (t, *J* = 7.53, 1H, Ar-H), 7.37–7.43 (m, 3H, Ar-H), 7.54–7.57 (m, 1H, Ar-H), 11.62 (s, 2H, NH). ^{13}C NMR (DMSO-d_6) ppm: 47.3; 53.7; 51.2; 62.6; 72.2; 74.0; 115.8; 118.1; 118.4; 119.3; 121.4; 124.1; 129.7; 130.2; 133.0; 149.5; 155.8; 157.4; 170.2. IR (KBr): *v* (cm^{-1}): 3066, 1737, 1708, 1605.

4.1.6.6. 5-(4-((Tetrahydrofuran-2-yl)methyl)piperazin-1-yl)-5-(4-phenoxyphenyl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (8f). Yield: 52% of off-white solids. Mp: 248–250 °C. MS: calcd for $C_{25}H_{29}N_4O_5$ = 465.2138. $(M+H)^+$ = 465.2143 found. ^{1}H NMR (DMSO-d_6) ppm: 1.30–1.39 (m, 1H, CH_2), 1.61–1.71 (m, 2H, CH_2), 1.76–1.82 (m, 1H, CH_2), 1.61–1.71 (d, *J* = 5.52, 2H, CH_2), 2.32–2.48 (m, 6H, CH_2), 3.46–3.51 (m, 2H, CH_2), 3.59–3.64 (m, 1H, CH_2), 3.76–3.82 (m, 1H, CH_2), 6.92–6.98 (m, 4H, Ar-H), 7.07–7.11 (t, *J* = 7.53, 1H, Ar-H), 7.30–7.34 (t, *J* = 7.53, 3H, Ar-H), 7.46–7.49 (m, 1H, Ar-H), 11.50 (s, 2H, NH). ^{13}C NMR (DMSO-d_6) ppm: 25.0; 29.8; 47.3; 54.0; 62.3; 67.1; 73.9; 76.5; 115.7; 118.1; 118.4; 119.3; 121.3; 124.1; 129.7; 130.2; 133.0; 149.5; 155.8; 157.4; 170.0. IR (KBr): *v* (cm^{-1}): 3088, 1750, 1718, 1692.

4.1.6.7. 5-(4-Benzylpiperazin-1-yl-5-(4-phenoxyphenyl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (8g). Yield: 57% of off-white solids. Mp: 206–208 °C. MS: calcd for $C_{27}H_{27}N_4O_4$ = 471.2032. $(M+H)^+$ = 471.2048 found. ^{1}H NMR (DMSO-d_6) ppm: 2.36 (s, br, 4H, CH_2), 2.58–2.61 (m, 4H, CH_2), 7.00–7.06 (m, 4H, Ar-H), 7.16–7.20 (t, *J* = 7.53, 1H, Ar-H), 7.22–7.33 (m, 5H, Ar-H), 7.39–7.43 (t, *J* = 7.53, 3H, Ar-H), 7.55–7.58 (m, 1H, Ar-H), 11.55 (s, 2H, NH). ^{13}C NMR (DMSO-d_6) ppm: 47.4; 53.1; 62.0; 73.9; 115.7; 118.1; 118.4; 119.3; 121.3; 124.1; 126.9; 128.2; 128.9; 129.7; 130.2; 133.0; 132.9; 149.5; 155.8; 157.4; 170.0. IR (KBr): *v* (cm^{-1}): 3064, 1731, 1708, 1609.

4.1.6.8. 5-(4-Butylpiperazin-1-yl)-5-(4-phenoxyphenyl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (8h). Yield: 39% of off-white solids. Mp: 196–198 °C. MS: calcd for $C_{24}H_{29}N_4O_4$ = 437.2189. $(M+H)^+$ = 437.2177 found. ^{1}H NMR (DMSO-d_6) ppm: 0.83–0.87 (t, *J* = 7.28, 3H, CH_3), 1.22–1.29 (m, 2H, CH_2), 1.34–1.41 (m, 2H, CH_2), 2.29–2.32 (t, *J* = 7.03, 2H, CH_2), 2.41 (s, br, 4H, CH_2), 2.58–2.61 (m, 4H, CH_2), 7.00–7.06 (m, 4H, Ar-H), 7.15–7.19 (t, *J* = 7.53, 1H, Ar-H), 7.38–7.43 (m, 3H, Ar-H), 7.55–7.57 (m, 1H, Ar-H). ^{13}C NMR (DMSO-d_6) ppm: 13.9; 20.0; 28.1; 47.1; 53.3; 57.3; 73.9; 76.5; 118.1; 118.4; 119.3; 121.3; 124.1; 129.6; 129.7; 130.2; 133.0; 149.5; 155.8; 157.4; 170.0. IR (KBr): *v* (cm^{-1}): 3057, 1734, 1705, 1605.

4.1.6.9. 5-(4-Acetyl-piperazin-1-yl)-5-(4-phenoxyphenyl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (8i). Yield: 45% of off-white solids. Mp: 176–179 °C. MS: calcd for $C_{22}H_{23}N_4O_5$ = 423.1668; found $(M+H)^+$ = 423.1617. ^{1}H NMR (MeOD) ppm: 2.08 (s, 3H, CH_3), 2.69–2.71 (m, 2H, CH_2), 2.76–2.78 (m, 2H, CH_2), 3.51–3.53 (m, 2H, CH_2), 3.57–3.59 (m, 2H, CH_2), 6.96–7.05 (m, 4H, Ar-H), 7.16–7.20 (t, *J* = 7.53, 1H, Ar-H), 7.37–7.41 (t, *J* = 7.53, 3H, Ar-H), 7.50–7.55 (m, 3H, Ar-H). ^{13}C NMR (MeOD) ppm: 21.1; 43.3; 48.2; 76.3; 119.3; 119.6; 120.8; 122.4; 125.3; 130.1; 131.1; 134.1; 150.7; 156.9; 157.4; 171.6; 171.8. IR (KBr): *v* (cm^{-1}): 2961; 1739; 1709; 1614.

4.1.6.10. 5-(4-Methyl-piperazin-1-yl)-5-(4-phenoxyphenyl)-pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (8j). Yield: 38% of off-white solids. Mp: 270–272 °C. MS: calcd for $C_{21}H_{23}N_4O_4$ = 395.1719; found $(M+H)^+$ = 395.1706. ^{1}H NMR (DMSO-d_6) ppm: 2.16 (s, 3H, CH_3), 2.29–2.35 (m, 4H, CH_2), 2.57–2.61 (m, 4H, CH_2), 7.02–7.08 (m, 4H, Ar-H), 7.17–7.21 (t, *J* = 7.53, 1H, Ar-H), 7.41–7.44 (t, *J* = 7.53, 3H, Ar-H), 7.57–7.59 (m, 3H, Ar-H), 11.65 (s, 2H, NH). ^{13}C NMR (DMSO-d_6) ppm: 45.6; 47.2; 55.2; 73.8; 118.1; 118.4; 119.4; 121.4; 124.0; 129.7; 130.2; 132.9; 149.4; 155.8; 157.4; 169.9. IR (KBr): *v* (cm^{-1}): 2960; 1730; 1715; 1608.

4.1.6.11. 5-(4-Phenoxyphenyl)-5-(cyclopropylmethyl)-1,4-diazepan-1-yl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (9a). Yield: 41% of off-white solids. Mp: 201–204 °C. MS: calcd for $C_{25}H_{29}N_4O_4$ = 449.2189; found $(M+H)^+$ = 449.2186. ^{1}H NMR (MeOD) ppm: 0.47–0.49 (m, 2H, CH_2), 0.77–0.79 (m, 2H, CH_2), 1.16–1.19 (m, 1H, CH), 1.47–1.52 (m, 2H, CH_2), 2.01–2.10 (m, 2H, CH_2), 2.87–2.93 (m, 2H, CH_2), 3.41–3.46 (m, 2H, CH_2), 3.55–3.58 (m, 1H, CH_2), 3.62–3.66 (m, 1H, CH_2), 7.00–7.09 (m, 4H, Ar-H), 7.19–7.23 (t, *J* = 7.53, 1H, Ar-H), 7.40–7.44 (t, *J* = 7.53, 1H, Ar-H), 7.52–7.58 (m, 3H, Ar-H). ^{13}C NMR (MeOD) ppm: 4.9; 9.2; 26.2; 47.8; 51.0; 53.7; 56.8; 63.2; 78.5; 119.4; 119.8; 120.9; 122.6; 125.5; 130.6; 130.7; 131.2; 131.3; 150.6; 156.7; 157.2; 171.9. IR (KBr): *v* (cm^{-1}): 2962; 1768; 1711; 1604.

4.1.6.12. 5-(4-Benzyl-1,4-diazepan-1-yl)-5-(4-phenoxyphenyl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (9b). Yield: 63% of off-white solids. Mp: 123–125 °C. MS: calcd for $C_{28}H_{29}N_4O_4$ = 485.2189, $(M+H)^+$ = 485.2202 found. ^{1}H NMR (acetone-d_6): ppm: 1.80–1.86 (m, 2H, CH_2), 2.07–2.10 (m, 2H, CH_2), 2.75 (s, br, 2H, CH_2), 2.90–2.94 (m,4H, CH_2), 3.86 (s, 2H, CH_2), 7.02–7.08 (m, 4H, Ar-H), 7.18–7.21 (t, 1H, *J* = 7.33, Ar-H), 7.24–7.27 (t, *J* = 7.33,1H, Ar-H), 7.30–7.34 (t, 2H, *J* =7.33, Ar-H), 7.40–7.48 (m, 3H, Ar-H), 7.56–7.62 (m, 3H, Ar-H). ^{13}C NMR (acetone-d_6) ppm: 28.3; 50.3; 51.1; 53.5; 57.6; 61.5; 77.6; 116.5; 118.9; 120.0; 121.9; 124.6; 127.8; 128.7; 129.7; 130.2; 130.4; 130.6; 131.4; 133.5; 149.3; 156.7; 158.9; 171.1. IR (KBr): *v* (cm^{-1}): 3062, 1736, 1708, 1604.

4.1.6.13. 5-(4-Acetyl-1,4-diazepan-1-yl)–5-(4-phenoxyphenyl)-pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (9c). Yield: 51% of off-white solids. Mp: 136–139 °C. MS: calcd for $C_{23}H_{24}N_4O_5$ = 4459.1644. $(M+Na)^+$ = 459.1625 found. ^{1}H NMR (Acetone-d_6) ppm: 1.59–1.65 (m, 1H, CH_2), 1.72–1.78 (m, 1H, CH_2), 2.06–2.10 (m, 5H, CH_2+CH_3), 2.78–2.82 (m, 1H, CH_2), 2.92–2.94 (m, 1H, CH_2), 3.46–3.50 (m, 2H, CH_2), 4.04–4.09 (m, 2H, CH_2), 7.03–7.08 (m, 4H, Ar-H), 7.18–7.21 (t, *J* = 7.53, 1H, Ar-H), 7.40–7.44 (t, *J* = 7.33, 1H, Ar-H), 7.56–7.62 (m, 3H, Ar-H). ^{13}C NMR (acetone-d_6) ppm: 21.2; 28.4; 48.1; 50.4; 52.5; 53.8; 78.1; 116.5; 118.9; 120.0; 121.9; 124.6; 130.0; 130.5; 133.4; 149.2; 156.6; 158.9; 170.1; 170.9. IR (KBr): *v* (cm^{-1}): 3067, 1737, 1706, 1608.

4.1.6.14. 5-(4-Methyl-1,4-diazepan-1-yl)-5-(4-phenoxyphenyl)-pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (9d). Yield: 49% of off-white solids. Mp: 249–251 °C. MS: calcd for $C_{22}H_{25}N_4O_4$ = 409.1876. $(M+H)^+$ = 409.1873 found. ^{1}H NMR (DMSO-d_6) ppm: 1.62–1.68 (m, 2H, CH_2), 2.24 (s, 3H, CH_3), 2.42–2.44 (m, 2H, CH_2), 2.59–2.62 (m, 2H, CH_2), 2.68–2.72 (m, 4H, CH_2), 7.00–7.06 (m, 4H, Ar-H), 7.16–7.20 (t, *J* = 7.53, 1H, Ar-H), 7.38–7.44 (m, 3H, Ar-H), 7.55–7.58 (m, 1H, Ar-H). ^{13}C NMR (DMSO-d_6) ppm: 28.0; 46.1; 46.7; 50.6; 55.4; 59.5; 76.2; 118.1; 118.4; 119.4; 121.4; 124.2; 129.5; 130.2; 131.1; 133.0; 149.8; 155.7; 157.5; 171.1. IR (KBr): *v* (cm^{-1}): 3062, 1732, 1682, 1592.

4.1.7. General procedure for the preparation of compounds 10 and 11

A solution of 5-bromo-5-(4-phenoxyphenyl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (1.0 mmol) in methanol (5 ml) was treated with the piperazine or homopiperazine (2 mmol) and stirred for 24 h at ambient temperature. The precipitates were collected by filtration and dried in vacuo to afford the solids.

4.1.7.1. 5-(4-Phenoxyphenyl)-5-(piperazin-1-yl)pyrimidine-2,4,6 (*1H,3H,5H*)-trione (10). Yield: 54% of off-white solids. Mp: 246–248 °C. MS: calcd for $C_{20}H_{21}N_4O_4$ = 381.1563; $(M+H)^+$ = 381.1582 found. ^{1}H NMR (DMSO-d_6) ppm: 2.54–2.58 (m, 4H, CH_2), 2.70–2.73 (m, 4H, CH_2), 7.16–7.19 (t, J = 7.53, 2H, Ar-H), 7.39–7.43 (m, 3H, Ar-H), 7.56–7.58 (m, 1H, Ar-H). ^{13}C NMR (DMSO-d_6) ppm: 46.1; 48.6; 74.4; 118.2; 119.4; 121.4; 124.2; 130.2; 130.4; 150.6; 156.1; 157.4; 170.8. IR (KBr): v (cm^{-1}): 3065, 1705, 1665, 1631.

4.1.7.2. 5-(1,4-Diazepan-1-yl)-5-(4-phenoxyphenyl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (11). Yield: 58% of off-white solids. Mp: 240–244 °C. MS: calcd for $C_{21}H_{23}N_4O_4$ = 395.1719. $(M+H)^+$ = 395.1704 found. ^{1}H NMR (DMSO-d_6) ppm: 1.52–1.58 (m, 2H, CH_2), 2.66–2.68 (m, 4H, CH_2), 2.69–2.71 (m, 2H, CH_2), 2.87–2.90 (m, 2H, CH_2), 6.99–7.06 (m, 4H, Ar-H), 7.16–7.19 (t, J = 7.53, 1H, Ar-H), 7.38–7.47 (m, 3H, Ar-H), 7.55–7.58 (m, 1H, Ar-H). ^{13}C NMR (DMSO-d_6) ppm: 29.8; 45.7; 49.6; 50.7; 53.0; 76.3; 115.7; 118.0; 119.3; 121.3; 124.0; 129.4; 130.2; 150.6; 155.8; 157.3; 172.1. IR (KBr): v (cm^{-1}): 3065, 1702, 1665, 1625.

4.1.8. General procedure for the preparation of compounds 12a–c and 13a–d

A suspension of compounds **10** or **11** (1 mmol) in 10 ml THF was cooled to −70 °C and triethylamine (1 mmol) was added. A solution of the substituted acid chlorides (1 mmol) was added to the suspension dropwise. After stirring for 3–4 h at −70 °C, the reaction mixture was dried and purified by flash column chromatography to yield the pure compounds.

4.1.8.1. 5-(4-(Thiophene-2-carbonyl)piperazin-1-yl)-5-(4-phenoxyphenyl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (12a). Yield: 41% of off-white solids. Mp: 142–144 °C. MS: calcd for $C_{25}H_{23}N_4O_5$ = 491.1389; found $(M+H)^+$ = 491.1377. ^{1}H NMR ($CDCl_3$) ppm: 2.77–2.82 (m, 4H, CH_2), 3.77–3.79 (m, 4H, CH_2), 6.94–6.96 (d, J = 8.53, 2H, Ar-H), 6.89–7.04 (m, 3H, Ar-H&CH), 7.16–7.19 (t, J = 7.53, 1H, Ar-H), 7.35–7.39 (t, J = 7.53, 2H, Ar-H), 7.42–7.48 (m, 4H, Ar-H&CH), 9.63 (s, 1H, NH), 9.68 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) ppm: 47.6; 67.5; 75.1; 117.9; 119.4; 121.0; 123.9; 128.6; 129.0; 129.3; 129.6; 132.6; 136.1; 148.6; 154.7; 155.4; 158.6; 163.5; 169.1; 169.2. IR (KBr): v (cm^{-1}): 2959, 1738, 1711, 1608.

4.1.8.2. 5-(4-(4-Cyanobenzoyl))piperazin-1-yl)-5-(4-phenoxyphenyl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (12b). Yield 40% of light yellow solids. Mp: 146–148 °C. MS: calcd for $C_{28}H_{24}N_5O_5$ = 510.1777; found $(M+H)^+$ = 510.1765. ^{1}H NMR ($CDCl_3$) ppm: 2.71–2.83 (m, 4H, CH_2), 3.78–3.81 (m, 4H, CH_2), 6.94–6.96 (d, J = 8.53, 2H, Ar-H), 7.00–7.03 (m, 2H, Ar-H), 7.17–7.21 (t, J = 7.53, 1H, Ar-H), 7.36–7.38 (d, J = 8.03, 1H, Ar-H), 7.40–7.46 (m, 3H, Ar-H), 7.51–7.53 (d, J = 7.53H, Ar-H), 7.68–7.70 (d, J = 7.53, 2H, Ar-H), 9.63 (s, 2H, NH). ^{13}C NMR ($CDCl_3$) ppm: 47.5; 67.5; 75.2; 117.6; 117.9; 119.5; 121.1; 124.1; 126.7; 127.5; 129.1; 129.7; 132.0; 132.6; 135.4; 139.2; 148.3; 155.2; 158.8; 168.1; 169.2. IR (KBr): v (cm^{-1}): 2959, 2231, 1738, 1711,1608.

4.1.8.3. 5-(4-(6-Chloropyridine-3-carbonyl)piperazin-1-yl)-5-(4-phenoxyphenyl) pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (12c). Yield 42% of off-white solids. Mp: 203–206 °C. MS: $(M+H)^+$ = 520.1390. calcd for $C_{26}H_{23}ClN_5O_5$ = 520.1388. ^{1}H NMR ($CDCl_3$) ppm: 2.78–2.87 (m, 4H, CH_2), 3.78–3.81 (m, 4H, CH_2), 6.96–7.09 (m, 4H, Ar-H), 7.17–7.21 (t, 1H, J = 7.53, Ar-H), 7.33–7.36 (m, 1H, Ar-H), 7.41–7.47 (m, 3H, Ar-H), 7.75–7.80 (m, 1H, Ar-H), 8.46–8.49 (m, 1H, Ar-H), 8.49–8.50 (d, 1H, J = 5.02, Ar-H), ^{13}C NMR ($CDCl_3$) ppm: 47.5; 67.5; 75.2; 117.6; 117.9; 119.5; 121.1; 124.1; 126.7; 127.5; 129.1; 129.7; 132.0; 132.6; 135.4; 139.2; 148.3; 155.2; 158.8; 168.1; 169.2. IR (KBr): v (cm^{-1}): 2956, 1739, 1706, 1614.

4.1.8.4. 5-(4-Phenoxyphenyl)-5-(4-(cyclopropylcarbonyl)-1,4-diazepan-1-yl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (13a). Yield: 37% of off-white solids. Mp: 147–149 °C. MS: calcd for $C_{25}H_{36}N_4O_5$ = 485.1801. $(M+H)^+$ = 485.1796 found. ^{1}H NMR ($CDCl_3$): ppm: 0.71–0.76 (m, 2H, CH_2), 1.00–1.03 (m, 2H, CH_2), 1.58–1.67 (m, 1H, CH), 1.70–1.77 (m, 2H, CH_2), 2.71–2.82 (m, 4H, CH_2), 3.57–3.63 (m, 2H, CH_2), 3.69–3.72 (m,1H, CH_2), 3.82–3.85 (m, 1H, CH_2), 6.90–7.03 (m, 4H, Ar-H), 7.14–7.17 (t, 1H, J = 7.53, Ar-H), 7.34–7.38 (t, J = 7.53, 1H, Ar-H), 7.44–7.51 (m, 3H, Ar-H), 10.03 (s, 2H, NH). ^{13}C NMR ($CDCl_3$): 7.5; 11.0; 29.1; 46.7; 50.3; 51.1; 53.1; 77.8; 116.6; 118.2; 119.6; 121.2; 124.1; 129.3; 129.8; 132.8; 149.1; 155.5; 158.6; 170.5; 173.0. IR (KBr): v (cm^{-1}): 3000, 1734, 1709, 1607, 1587.

4.1.8.5. 5-(4-Phenoxyphenyl)-5-(4-(thiophene-2-carbonyl)-1,4-diazepan-1-yl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (13b). Yield: 35% of off-white crystalline solids. Mp: 110–113 °C. MS: calcd for $C_{26}H_{25}N_4O_5S$ = 527.1365. $(M+H)^+$ = 527.1342 found. ^{1}H NMR ($CDCl_3$): ppm: 1.76–1.86 (m, 2H, CH_2), 2.79–2.90 (m, 4H, CH_2), 3.60–3.69 (m, 2H, CH_2), 3.80–3.85 (m, 2H, CH_2), 6.87–7.01 (m, 5H, CH), 7.13–7.17 (t, 1H, J = 7.53, Ar-H), 7.33–7.46 (m, 6H, CH), 9.57 (s, 2H, NH). ^{13}C NMR ($CDCl_3$): 30.0; 45.6; 49.2; 50.7; 53.4 77.7; 117.8; 119.4; 121.0; 123.9; 128.6; 129.0; 129.3; 129.6; 132.6; 136.1 148.7; 154.6; 155.3; 158.6; 164.4; 169.9; 171.0. IR (KBr): v (cm^{-1}): 3067, 1736, 1708, 1605, 1587.

4.1.8.6 5-(4-Phenoxyphenyl)-5-(4-(4-cyanobenzoyl)-1,4-diazepan-1-yl)pyrimidine-2,4,6(1***H***,3***H***,5***H***)-trione (**13c**).

Yield: 31% of yellow crystalline solids mp: 220–224 °C. MS: calcd for $C_{29}H_{26}N_5O_5$ = 524.1934, $(M+H)^+$ = 524.1934 found. ^{1}H NMR ($CDCl_3$): ppm: 1.83–1.87 (m, 2H, CH_2), 2.76–2.84 (m, 3H, CH_2), 3.21–3.25 (m, 1H, CH_2), 3.41–3.45 (m, 1H, CH_2), 3.67–3.70 (m, 1H, CH_2), 3.74–3.78 (m, 1H, CH_2), 3.82–3.86 (m, 1H, CH_2), 6.84–7.00 (m, 4H, Ar-H), 7.15–7.19 (t, 1H, J = 7.53, Ar-H), 7.34–7.40 (m, 2H, Ar-H), 7.44–7.48 (m, 2H, Ar-H), 7.53–7.57 (m, 2H, Ar-H), 7.66–7.72 (m, 2H, Ar-H), 10.11 (s, 2H, NH). ^{13}C NMR ($CDCl_3$): 29.7; 44.7; 48.2; 50.6; 51.6; 77.6; 112.9; 116.7; 116.8; 118.2; 119.8; 121.3; 124.2; 127.1; 127.4; 129.2; 130.0; 132.3; 132.8; 135.7; 140.9; 149.0; 154.7; 158.9; 169.8; 170.5. IR (KBr): v (cm $^{-1}$): 3068, 2230, 1737, 1709, 1606, 1582.

4.1.8.7. 5-(4-Phenoxyphenyl)-5-(4-(6-chloropyridine-3-carbonyl)-1,4-diazepan-1-yl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (13d). Yield: 32% of off-white crystalline solids. Mp: 246–248 °C. MS: calcd for $C_{24}H_{25}ClN_5O_5$ = 534.1544, $(M+H)^+$ = 534.1569 found. ^{1}H NMR (DMSO-d_6): ppm: 1.48–1.54 (m, 1H, CH_2), 1.62–1.68 (m, 1H, CH_2), 2.73–2.85 (m, 4H, CH_2), 3.19–3.23 (m, 1H, CH_2), 3.40–3.43 (m, 1H, CH_2), 3.54–3.47 (m, 1H, CH_2), 3.67–3.70 (m, 1H, CH_2), 6.96–7.09 (m, 4H, Ar-H), 7.15–7.19 (t, 1H, J = 7.53, Ar-H), 7.33–7.36 (m, 1H, Ar-H), 7.40–7.47 (m, 3H, Ar-H), 7.57–7.62 (m, 1H, Ar-H), 7.91–7.96 (t, 1H, J = 8.03, Ar-H), 8.49–8.50 (d, 1H, J = 5.02, Ar-H), 11.50 (s, 2H, NH). ^{13}C NMR (DMSO-d_6): 29.5; 43.9; 47.8; 50.7; 52.6; 76.8; 116.0; 118.3; 119.5; 121.6; 124.3; 124.9; 128.0; 129.1; 129.4; 130.2; 132.2; 133.0; 138.2; 139.2; 147.8; 149.4; 150.5; 155.0; 157.7; 170.7; 170.8. IR (KBr): v (cm^{-1}): 3068, 1736, 1708, 1609, 1586.

4.1.9. General procedure for the preparation of compounds 14–38

A suspension of 5-(1,4-diazepan-1-yl)-5-(4-phenoxyphenyl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (394 mg, 1 mmol) in 10 ml THF was cooled to −70 °C and triethylamine (101.2 mg, 1 mmol) was added. A solution of substituted the acid chlorides (1 mmol) was added to the suspension dropwise. After stirring for 2 h at −70 °C, the reaction mixture was dried and purified by flash column chromatography to yield the pure compounds.

4.1.9.1. 5-(4-Phenoxyphenyl)-5-(4-(cyclobutanecarbonyl)-1,4-diazepan-1-yl) pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (14). Yield: 35% as off-white solids. Mp: 142–145 °C. MS: calcd for $C_{26}H_{29}N_4O_5$ = 477.2138. $(M+H)^+$ = 477.2133 found. ^1H NMR ($CDCl_3$): ppm: 1.68–1.74 (m, 2H, CH_2), 1.82–2.01 (m, 2H, CH_2), 2.05–2.15 (m, 2H, CH_2), 2.30–2.45 (m, 2H, CH_2), 2.71–2.84 (m, 4H, CH_2), 3.23–3.31 (m, 1H, CH), 3.52–3.60 (m, 2H, CH_2), 3.69–3.72 (m,1H, CH_2), 3.77–3.80 (m, 1H, CH_2), 6.91–7.05 (m, 4H, Ar-H), 7.15–7.20 (t, 1H, *J* = 7.53, Ar-H), 7.36–7.40 (t, *J* = 7.53,1H, Ar-H), 7.44–7.49 (m, 3H, Ar-H), 9.55 (s, 2H, NH). ^{13}C NMR ($CDCl_3$): 17.7; 24.9; 29.2; 36.7; 45.8; 49.4; 50.2; 53.4; 77.6; 116.5; 118.1; 119.5; 121.0; 124.0; 129.2; 129.6; 132.6; 148.7; 154.7; 158.5; 170.0; 174.5. IR (KBr): *v* (cm^{-1}): 3068, 1754, 1736, 1708, 1608.

4.1.9.2. 5-(4-Phenoxyphenyl)-5-(4-(2-(phenylthio)acetyl)-1,4-diazepan-1-yl) pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (15). Yield: 38% as off-white solids. Mp: 100–103 °C. MS: calcd for $C_{29}H_{29}N_4O_5S$ = 545.1869, $(M+H)^+$ = 545.1840 found. ^1H NMR ($CDCl_3$): ppm: 1.67–1.74 (m, 2H, CH_2), 2.71–2.80 (m, 3H, CH_2), 2.88–2.91 (m, 1H, CH_2), 3.42–3.45 (m, 1H, CH_2), 3.58–3.64 (m, 2H, CH_2), 3.67–3.70 (m, 1H, CH_2), 3.82 (s, 2H, CH_2), 6.88–6.92 (m, 4H, Ar-H), 7.01–7.03 (d, 1H, *J* = 8.53, Ar-H), 7.15–7.20 (m, 2H, Ar-H), 7.23–7.29 (m, 2H, Ar-H), 7.35–7.39 (t, 1H, *J* = 7.53, Ar-H), 7.41–7.49 (m, 5H, Ar-H), 9.75 (s, 2H, NH). ^{13}C NMR ($CDCl_3$): 29.3; 36.7; 47.2; 50.7; 52.2; 53.1; 77.9; 116.8; 118.3; 119.8; 121.4; 124.3; 125.4; 126.9; 128.9; 129.5; 129.9; 130.3; 132.9; 135.0; 149.1; 154.9; 158.3; 170.3; 170.4. IR (KBr): *v* (cm^{-1}): 3064, 1736, 1708, 1611.

4.1.9.3. 5-(4-Phenoxyphenyl)-5-(4-(3,5-diethoxybenzoyl)-1,4-diazepan-1-yl) pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (16). Yield: 33% as off-white solids. Mp: 213–217 °C. MS: calcd for $C_{32}H_{35}N_4O_7$ = 587.2506, $(M+H)^+$ = 587.2488 found. ^1H NMR (DMSO-d_6): ppm: 1.27–1.33 (dt, *J* = 7.53, 6H, CH_3), 1.50–1.56 (m, 1H, CH_2), 1.65–1.71 (m, 1H, CH_2), 2.70–2.73 (m, 3H, CH_2), 2.83–2.86 (m, 1H, CH_2), 3.10–3.13 (m, 1H, CH_2), 3.34–3.40 (m, 1H, CH_2), 3.51–3.64 (m, 1H, CH_2), 3.63–3.66 (m, 1H, CH_2), 3.95–4.06 (m, 4H, CH_2), 6.44–6.50 (m, 3H, Ar-H), 6.87–6.92 (m, 1H, Ar-H), 7.01–7.10 (m, 3H, Ar-H), 7.20–7.23 (m, 1H, Ar-H), 7.35–7.47 (m, 3H, Ar-H), 7.57–7.60 (m, 1H, Ar-H), 11.57 (s, 2H, NH). ^{13}C NMR (DMSO-d_6): 14.6; 29.7; 47.7; 50.7; 52.6; 53.7; 63.3; 76.7; 101.1; 104.6; 116.0; 118.1; 119.5; 121.6; 124.2; 124.9; 129.1; 130.2; 133.0; 139.2; 149.4; 155.4; 157.8; 159.5; 169.9; 170.6. IR (KBr): *v* (cm^{-1}): 3063, 2851, 1736, 1706, 1590.

4.1.9.4. 5-(4-Phenoxyphenyl)-5-(4-(4-(trifluoromethoxy)benzoyl)-1,4-diazepan-1-yl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (17). Yield: 32% as off-white solids. Mp: 183–187 °C. MS: calcd for $C_{29}H_{26}F_3N_4O_6$ = 583.1804, $(M+H)^+$ = 583.1823 found.^1H NMR (MeOD): ppm: 1.61–1.67 (m, 1H, CH_2), 1.81–1.87 (m, 1H, CH_2), 2.84–2.87 (m, 3H, CH_2), 3.00–3.02 (m, 1H, CH_2), 3.27–3.30 (m, 1H, CH_2), 3.52–3.55 (m, 1H, CH_2), 3.68–3.71 (m, 1H, CH_2), 3.81–3.84 (m, 1H, CH_2), 6.84–6.89 (m, 1H, Ar-H), 6.92–6.95 (m, 1H, Ar-H), 6.98–7.02 (m, 1H, Ar-H), 7.03–7.07 (m, 1H, Ar-H), 7.17–7.21 (t, 1H, *J* = 7.53, Ar-H), 7.37–7.46 (m, 4H, Ar-H), 7.51–7.54 (m, 1H, Ar-H), 7.55–7.57 (m, 1H, Ar-H), 7.58–7.63 (m, 2H, Ar-H). ^{13}C NMR (MeOD): ppm: 28.8; 48.4; 51.4; 52.6; 53.7; 78.7; 117.4; 118.8; 119.2; 120.5; 122.0; 122.3; 125.1; 125.9; 129.4; 129.9; 130.9; 136.8; 150.9; 156.7; 159.4; 160.0; 172.1; 172.4. IR (KBr): *v* (cm^{-1}): 3067, 2852, 1752, 1732, 1708, 1603.

4.1.9.5. 5-(4-Phenoxyphenyl)-5-(4-(3-cyclopentylpropanoyl)-1,4-diazepan-1-yl)pyrimidine-2,4,6(1*H,3H,5H*)-trione (18). Yield: 38% as off-white solids. Mp: 139–141 °C. MS: calcd for $C_{29}H_{35}N_4O_5$ = 519.2607, $(M+H)^+$ = 19.2609 found. ^1H NMR ($CDCl_3$) δ ppm: 1.50–1.52 (m, 2H, CH_2), 1.58–1.61 (m, 2H, CH_2), 1.65–1.69 (m, 2H, CH_2), 1.71–1.77 (m, 5H, CH_2 + CH), 1.86–1.90 (m, 2H, CH_2), 2.33–2.39 (m, 2H, CH_2), 2.73–2.80 (m, 3H, CH_2), 3.40–3.43 (m, 1H, CH_2), 3.59–3.62 (m, 1H, CH_2), 3.64–6.67 (m, 1H, CH_2), 3.70–3.73 (m, 1H, CH_2), 3.77–3.80 (m, 1H, CH_2), 6.92–7.01 (m, 4H, Ar-H), 7.04–7.06 (d, *J* = 7.53, 1H, Ar-H), 7.37–7.41 (t, *J* = 8.53, 1H, Ar-H), 7.46–7.49 (m, 3H, Ar-H), 9.36–9.54 (d, 2H, NH). ^{13}C NMR ($CDCl_3$) δ ppm: 25.1; 29.6; 31.5; 32.3; 32.7; 34.1; 39.8; 44.6; 46.9; 50.4; 52.3; 52.7; 77.8; 118.2; 118.4; 121.4; 124.3; 129.5; 129.9; 148.8; 155.0; 158.3; 170.2; 170.3; 173.4. IR (KBr): *v* (cm^{-1}): 3065, 1750, 1731, 1697, 1598.

4.1.9.6. 5-(4-Phenoxyphenyl)-5-(4-(2,5-diethoxybenzoyl)-1,4-diazepan-1-yl) pyrimidine-2,4,6(*1H,3H,5H*)-trione (19). Yield: 35% as off-white solids. Mp: 117–119 °C. MS: calcd for $C_{32}H_{35}N_4O_7$ = 587.2506, $(M+H)^+$ = 587.2507 found. ^1H NMR ($CDCl_3$): ppm: 1.35–1.38 (m, 6H, CH_3), 1.86–1.91 (m, 2H, CH_2), 2.66–2.73(m, 1H, CH_2), 2.82–2.93 (m, 3H, CH_2), 3.14–3.25 (m, 1H, CH_2), 3.43–3.51 (m, 1H, CH_2), 3.79–3.89 (m, 2H, CH_2), 3.96–4.00 (m, 4H, CH_2), 6.78–6.87 (m, 5H, Ar-H), 6.95–6.97 (d, *J* = 8.03, 1H, Ar-H), 7.01–7.05 (m, 2H, Ar-H), 7.17–7.21 (t, 1H, *J* = 7.53, Ar-H), 7.36–7.40 (t, *J* = 7.28, 2H, Ar-H), 7.51–7.54 (4, *J* = 7.03,1H, Ar-H). ^{13}C NMR ($CDCl_3$): 14.9; 29.4; 47.7; 50.9; 52.3; 53.1; 64.0; 64.8; 77.8; 113.7; 113.9; 116.0; 118.2; 118.4; 119.8; 121.4; 124.3; 124.9; 127.4; 129.2; 130.0; 132.9; 148.4; 152.9; 155.6; 158.9; 169.1; 169.9. IR (KBr): *v* (cm^{-1}): 3069, 2845, 1738, 1710, 1606, 1588.

4.1.9.7. 5-(4-Phenoxyphenyl)-5-(4-(2-chloro-6-methylpyridine-4-carbonyl)-1,4-diazepan-1-yl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (20). Yield: 25% as off-white solids. Mp: 116–119 °C. MS: calcd for $C_{28}H_{27}ClN_5O_5$ = 548.1701, $(M+H)^+$ = 548.1712 found. ^1H NMR ($CDCl_3$) ppm: 1.86–1.88 (m, 2H, CH_2), 2.52–2.55 (d, 3H, CH_3), 2.82–2.94 (m, 4H, CH_2), 3.19–3.24 (m, 1H, CH_2), 3.42–3.46 (m, 1H, CH_2), 3.66–3.70 (m, 1H, CH_2), 3.78–3.84 (m, 1H, CH_2), 6.89–6.91 (d, *J* = 8.03, 2H, Ar-H), 6.94–6.96 (d, *J* = 8.03, 1H, Ar-H), 6.99–7.03 (t, *J* = 7.53, 1H, Ar-H), 7.13–7.22 (m, 3H, Ar-H), 7.35–7.39 (t, 2H, *J* = 7.53, Ar-H), 7.43–7.47 (m, 2H, Ar-H), 9.69–9.73 (m, 2H, NH). ^{13}C NMR ($CDCl_3$): 24.0; 29.7; 48.1; 50.8; 52.5; 54.1; 77.8; 116.9; 118.1; 119.0; 119.3; 119.8; 121.4; 124.4; 129.2; 129.9; 132.9; 147.3; 148.9; 154.8; 159.0; 160.2; 168.1; 170.1. IR (KBr): *v* (cm^{-1}): 3074, 2959, 2849, 1738, 1710, 1608.

4.1.9.8. 5-(4-Phenoxyphenyl)-5-(4-(2-oxoimidazolidine-1-carbonyl)-1,4-diazepan-1-yl)pyrimidine-2,4,6(*1H,3H,5H*)-trione (21). Yield: 35% as off-white solids. Mp: 186–190 °C. MS: calcd for $C_{25}H_{27}N_6O_6$ = 529.1838, $(M+H)^+$ = 529.1812 found. ^1H NMR (MeOD) ppm: 1.76–1.82 (m, 2H, CH_2), 2.80–2.83 (m, 2H, CH_2), 2.91–2.94 (m, 2H, CH_2), 3.46–3.50 (m, 4H, CH_2), 3.62–3.68 (m, 2H, CH_2), 3.84–3.87 (m, 2H, CH_2), 5.51 (s, 1H, NH), 6.99–7.01 (d, *J* = 8.04, 2H, Ar-H), 7.05–7.07 (d, *J* = 8.03, 2H, Ar-H), 7.18–7.21 (t, *J* = 7.53, 1H, Ar-H), 7.40–7.43 (t, *J* = 8.03, 2H, Ar-H), 7.50–7.53 (m, 2H, Ar-H). ^{13}C NMR (MeOD) ppm: 29.4; 30.7; 38.9; 48.5; 51.8; 53.4; 54.8; 78.8; 118.5; 119.1; 120.8; 125.3; 130.6; 131.1; 150.9; 155.8; 157.5; 157.8; 160.4; 172.5. IR (KBr): *v* (cm^{-1}): 3068, 2958, 1735, 1641, 1588.

4.1.9.9. 5-(4-Phenoxyphenyl)-5-(4-(3-methylbenzofuran-2-carbonyl)-1,4-diazepan-1-yl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (22). Yield: 29% as off-white solids. Mp: 134–137 °C. MS: calcd for $C_{31}H_{29}N_4O_6$ = 533.2093, $(M+H)^+$ = 533.2087 found. ^1H NMR (MeOD) ppm: 1.76–1.82 (m, 2H, CH_2), 2.43 (s, 3H, CH_3), 2.79–2.83 (m, 1H, CH_2), 2.90–2.94 (m, 1H, CH_2), 3.45–3.48 (m, 1H, CH_2), 3.58–3.63 (m, 1H, CH_2), 3.70–3.73 (m, 1H, CH_2), 3.79–3.85 (m, 2H, CH_2), 3.95–3.98 (m, 2H, CH_2), 6.88–7.02 (m, 3H, Ar-H), 7.14–7.17 (t, *J* = 7.53, 1H, Ar-H), 7.23–7.26 (t, *J* = 7.53, 1H, Ar-H), 7.30–7.37 (m, 3H, Ar-H), 7.41–7.44 (m, 1H, Ar-H), 7.47–7.54 (m,

2H, Ar-H), 7.58–7.60 (m, 1H, Ar-H), 7.63–7.65 (m, 1H, Ar-H). ^{13}C NMR (MeOD) ppm: 8.8; 28.6; 48.7; 51.9; 52.8; 54.3; 78.9; 112.4; 119.0; 119.2; 120.7; 121.3; 121.5; 122.4; 125.3; 130.5; 131.1; 134.1; 145.8; 150.9; 155.0; 157.3; 159.4; 160.3; 172.4. IR (KBr): ν (cm^{-1}): 3065, 2954, 2846, 1737, 1711, 1608, 1588.

4.1.9.10. 5-(4-Phenoxyphenyl)-5-(4-(3-chloro-6-fluorobenzo-[*b*]-thiophene-2-carbonyl)-1,4-diazepan-1-yl)pyrimidine-2,4,6-(1*H*,3*H*,5*H*)-trione (23). Yield: 27% as off-white solids. Mp: 138–142 °C. MS: calcd for $C_{30}H_{24}ClFN_4O_5SNa$ = 629.1609, $(M+Na)^+$ = 629.1601 found. ^{1}H NMR ($CDCl_3$) ppm: 1.70–1.76 (m, 1H, CH_2), 1.92–1.98 (m, 1H, CH_2), 2.85–2.91 (m, 3H, CH_2), 3.00–3.05 (m, 1H, CH_2), 3.43–3.46 (m, 1H, CH_2), 3.68–3.72(m, 1H, CH_2), 3.80–3.85 (m, 1H, CH_2), 3.93–3.98 (m, 1H, CH_2), 6.84–6.90 (t, J = 8.03, 1H, Ar-H), 6.95–7.13 (m, 3H, Ar-H), 7.23–7.34 (m, 2H, Ar-H), 7.40–7.57 (m, 5H, Ar-H), 7.77–7.88 (m, 1H, Ar-H), 9.45–9.57 (m, 2H, NH). ^{13}C NMR ($CDCl_3$): 29.4; 47.8; 50.5; 52.3; 53.4; 77.6; 108.6; 114.5; 116.6; 117.9; 118.3; 119.5; 121.1; 123.5; 124.1; 128.4; 129.4; 131.7; 137.9; 148.7; 154.3; 155.0; 158.1; 158.7; 162.4; 169.9. IR (KBr): ν (cm^{-1}): 3071, 2953, 1736, 1708, 1606, 1588.

4.1.9.11. 5-(4-Phenoxyphenyl)-5-(4-(2,5-dimethylfuran-3-carbonyl)-1,4-diazepan-1-yl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (24). Yield: 37% as off-white solids. Mp: 122–126 °C. MS: calcd for $C_{28}H_{28}N_4O_6Na$ = 539.1923, $(M+Na)^+$ = 539.1907 found. ^{1}H NMR ($CDCl_3$) ppm: 1.78–1.82 (m, 1H, CH_2), 1.93–1.99 (m, 1H, CH_2), 2.28–2.33 (d, 3H, CH_3), 2.39–2.44 (d, 3H, CH_3), 2.86–2.91 (m, 3H, CH_2), 2.99–3.04 (m, 1H, CH_2), 3.51–3.55 (m, 1H, CH_2), 3.72–3.78(m, 2H, CH_2), 3.85–3.89 (m, 1H, CH_2), 6.03–6.06 (d, 1H, CH), 7.01–7.03 (m, 4H, Ar-H), 7.26–7.31 (t, J = 7.53, 1H, Ar-H), 7.49–7.58 (m, 4H, Ar-H), 9.33–9.48 (m, 2H, NH). ^{13}C NMR ($CDCl_3$): 12.8; 13.2; 27.5; 47.8; 51.4; 52.7; 53.5; 77.7; 105.8; 116.6; 118.2; 119.8; 121.4; 124.3; 129.4; 130.0; 149.6; 150.9; 155.6;158.0; 158.9; 166.9; 170.1. IR (KBr): ν (cm^{-1}): 3068, 2952, 1737, 1708, 1588.

4.1.9.12. 5-(4-Phenoxyphenyl)-5-(4-(quinoxaline-2-carbonyl)-1,4-diazepan-1-yl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (25). Yield: 42% as yellow solids. Mp: 186–188 °C. MS: calcd for $C_{30}H_{26}N_6O_5Na$ = 573.1862, $(M+Na)^+$ = 578.7877 found. ^{1}H NMR ($CDCl_3$) δ ppm: 1.86–1.89 (m, 2H, CH_2), 2.89–2.92 (m, 2H, CH_2), 2.93–2.96 (m, 1H, CH_2), 3.01–3.04 (m, 1H, CH_2), 3.60–3.63 (m, 1H, CH_2), 3.80–3.83 (m, 2H, CH_2), 3.96–3.99 (m, 1H, CH_2), 6.85–6.87 (d, J = 8.53, 1H, Ar-H), 6.93–6.98 (m, 2H, Ar-H), 7.03–7.05 (d, J = 8.53, 1H, Ar-H), 7.15–7.18 (t, J = 8.03, 1H, Ar-H), 7.34–7.37 (m, 1H, Ar-H), 7.42–7.45 (m, 2H, Ar-H), 7.50–7.52 (d, J = 8.03, 1H, Ar-H), 7.79–7.85 (m, 2H, Ar-H), 8.05–8.09 (t, J = 7.53, 1H, Ar-H), 8.17–8.22 (t, J = 8.03, 1H, Ar-H), 9.27 (s, 1H, Ar-H), 9.38 (s, 1H, NH), 9.46 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ ppm: 29.7; 48.8; 51.1; 51.9; 53.3; 77.7; 117.8; 117.9. 119.6. 121.1; 124.0; 125.2; 128.6; 128.8; 129.1; 129.3; 129.6; 130.3; 130.8; 132.6; 140.0; 141.8; 144.6; 148.5; 155.3; 158.7; 166.7; 169.8; 169.9. IR (KBr): ν (cm $^{-1}$): 3066, 2953, 1729, 169, 1578.

4.1.9.13. 5-(4-Phenoxyphenyl)-5-(4-(2-(thiophen-2-yl)acetyl)-1,4-diazepan-1-yl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (26). Yield: 32% as off-white solids. Mp: 107–109 °C. MS: calcd for $C_{27}H_{27}N_4O_5S$ = 519.1702, $(M+H)^+$ = 519.1721 found. ^{1}H NMR ($CDCl_3$) δ ppm: 1.63–1.68 (m, 1H, CH_2), 1.73–1.77 (m, 1H, CH_2), 2.70–2.73 (m, 1H, CH_2), 2.74–2.77 (m, 1H, CH_2), 2.80–2.84 (m, 2H, CH_2), 3.40–3.43 (m, 1H, CH_2), 3.61–3.63 (m, 1H, CH_2), 3.67–3.70 (m, 1H, CH_2), 3.72–3.75 (m, 1H, CH_2), 3.93 (s, 1H, CH_2), 3.95 (s, 1H, CH_2), 6.90–6.95 (m, 4H, Ar-H), 7.01–7.05 (t, J = 7.53, 2H, Ar-H), 7.136–7.19 (m, 2H, Ar-H), 7.36–7.40 (m, 2H, Ar-H), 7.42–7.48 (m, 2H, Ar-H), 9.44 (s, 1H, NH), 9.51 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ ppm: 29.8; 36.5; 45.8; 49.2; 51.0; 53.2; 77.8; 117.8; 119.4; 121.2; 123.9; 124.3; 125.4; 126.1; 129.0; 129.3; 129.6; 130.3; 139.1; 148.7; 155.3; 158.6; 159.3; 169.9. IR (KBr): ν (cm^{-1}): 3068, 2957, 2766, 1730, 1706, 1601.

4.1.9.14. 2-(4-(2,4,6-Trioxo-5-(4-phenoxyphenyl)hexanhydropyrimidin-5-yl)-1,4-diazepane-1-carbonyl)benzyl benzoate (27). Yield: 36% as off-white solids. Mp: 120–123 °C. MS: calcd for $C_{36}H_{33}N_4O_7$ = 633.2349, $(M+H)^+$ = 633.2344 found. ^{1}H NMR ($CDCl_3$) δ ppm: 1.87–1.90 (m, 2H, CH_2), 2.71–2.74 (m, 2H, CH_2), 2.78–2.81 (m, 1H, CH_2), 2.92–2.96 (m, 1H, CH_2), 3.18–3.27 (m, 1H, CH_2), 3.35–3.40 (m, 1H, CH_2), 3.55–3.61 (m, 2H, CH_2), 3.78–3.81 (m, 1H, CH_2), 5.47 (s, 2H, CH_2), 6.83–6.85 (d, J = 8.53, 1H, Ar-H), 6.89–6.94 (t, J = 8.53, 1H, Ar-H), 6.99–7.03 (t, J = 8.53, 2H, Ar-H), 7.17–7.21 (m, 2H, Ar-H), 7.35–7.46 (m, 9H, Ar-H), 7.52–7.56 (m, 2H, Ar-H), 8.04–8.06 (d, J = 8.33, 2H, Ar-H), 9.49–9.54 (d, 2H, NH). ^{13}C NMR ($CDCl_3$): 29.6; 47.8; 50.9; 52.5; 53.4; 64.1; 77.8; 118.0; 118.2; 119.8; 121.4; 124.3; 125.5; 126.5; 128.0; 128.3; 128.4; 128.9; 129.0; 129.2; 129.4; 129.6; 129.8; 129.9; 132.9; 133.1; 136.0; 148.9; 155.8; 158.9; 166.2; 170.2; 170.4; 170.6.. IR (KBr): ν (cm^{-1}): 3065, 2953, 1738, 1708, 1607, 1588.

4.1.9.15. 5-(4-Phenoxyphenyl)-5-(4-(6-(trifluoromethyl)nicotinoyl)-1,4-diazepan-1-yl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (28). Yield: 31% as light yellow solids. Mp: 162–165 °C. MS: calcd for $C_{28}H_{24}F_3N_5O_5Na$ = 590.1627, $(M+Na)^+$ = 590.1622 found. ^{1}H NMR ($CDCl_3$) δ ppm: 1.87–1.90 (m, 2H, CH_2), 2.83–2.90 (m, 2H, CH_2), 3.03–3.05 (m, 1H, CH_2), 3.31–3.35 (m, 1H, CH_2), 3.50–3.54 (m, 1H, CH_2), 3.77–3.81 (m, 2H, CH_2), 3.88–3.92 (m, 1H, CH_2), 6.92–6.94 (d, J = 8.53, 2H, Ar-H), 6.96–7.06 (m, 2H, Ar-H), 7.20–7.23 (t, J = 7.53, 1H, Ar-H), 7.39–7.43 (m, 2H, Ar-H), 7.48–7.51 (t, J = 7.53, 1H, Ar-H), 7.75–7.81(t, J = 7.53, 1H, Ar-H), 8.01–8.03 (d, J = 7.53, 2H, Ar-H), 8.34–8.36 (d, J = 7.53, 2H, Ar-H), 8.84 (s, 1H,Ar-H), ^{13}C NMR ($CDCl_3$): 29.6; 48.5; 51.6.52.3; 52.9; 78.1; 118.2; 118.4; 119.8; 120.0; 120.3; 121.6; 124.6; 125.5; 128.2; 129.1; 129.5; 130.0; 133.0; 138.8; 143.3; 147.9; 151.5; 155.5; 159.4; 167.7; 169.9. IR (KBr): ν (cm^{-1}): 3090, 2954, 1730, 1702, 1610, 1588.

4.1.9.16. 5-(4-Phenoxyphenyl)-5-(4-(4-methylbenzoyl)-1,4-diazepan-1-yl) pyrimidine-2,4,6(*1H,3H,5H*)-trione (29). Yield: 38% as off-white solids. Mp: 206–210 °C. MS: calcd for $C_{29}H_{29}N_4O_5$ = 513.2137, $(M+H)^+$ = 513.2138 found. ^{1}H NMR (DMSO-d_6) ppm: 1.48–1.53 (m, 1H, CH_2) 1.67–1.71 (m, 1H, CH_2), 2.27 (s, 3H, CH_3), 2.69–2.74 (m, 3H, CH_2), 2.83–2.87 (m, 1H, CH_2), 3.17–3.20 (m, 1H, CH_2), 3.37–3.43 (m, 1H, CH_2), 3.52–3.55 (m, 1H, CH_2), 3.65–3.69 (m, 1H, CH_2), 6.84–6.88 (m, 1H, Ar-H), 7.00–7.09 (m, 3H, Ar-H), 7.21–7.28 (m, 5H, Ar-H), 7.33–7.35 (d, J = 7.35, 1H, Ar-H), 7.40–7.47 (m, 2H, Ar-H), 7.57–7.59 (d, J = 7.53, 1H, Ar-H), 11.56 (s, 1H, NH), 11.62 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) ppm: 20.8; 29.7; 47.6; 50.9; 52.8; 53.6; 76.7; 117.9; 119.5; 121.6; 124.2; 124.9; 126.3; 128.7; 129.1; 130.2; 133.0; 138.4; 149.4; 155.5; 157.7; 170.5; 170.7. IR (KBr): ν (cm^{-1}): 3068, 2952, 3068, 2952, 1736, 1709, 1597.

4.1.9.17. 5-(4-Phenoxyphenyl)-5-(4-(4-bromobenzoyl)-1,4-diazepan-1-yl) pyrimidine-2,4,6(*1H,3H,5H*)-trione (30). Yield: 43% as off-white solids. Mp: 258–260 °C. MS: calcd for $C_{28}H_{25}BrN_4O_5$-Na = 599.0906, $(M+Na)^+$ = 599.0880 found. ^{1}H NMR (DMSO-d_6) ppm: 1.49–1.53 (m, 1H, CH_2) 1.68–1.72 (m, 1H, CH_2), 2.68–2.74 (m, 3H, CH_2), 2.82–2.87 (m, 1H, CH_2), 3.17–3.20 (m, 1H, CH_2), 3.35–3.41 (m, 1H, CH_2), 3.50–3.54 (m, 1H, CH_2), 3.65–3.69 (m, 1H, CH_2), 6.91–6.94 (m, 2H, Ar-H), 7.00–7.09 (m, 2H, Ar-H), 7.19–7.23 (t, J = 7.53, 1H, Ar-H), 7.27–7.32 (m, 4H, Ar-H), 7.45–7.49 (m, 2H, Ar-H), 7.59–7.61 (d, J = 7.53, 2H, Ar-H). ^{13}C NMR (DMSO-d_6) ppm: 29.6; 47.7; 50.9; 52.7; 53.8; 76.9; 118.0; 118.4; 119.3;

121.3; 124.5; 128.5; 128.9; 129.1; 129.6; 129.8; 130.2; 131.5; 131.6; 135.4; 150.3; 155.7; 168.9; 170.7. IR (KBr): ν (cm^{-1}): 3086, 2956, 1738, 1709, 1588.

4.1.9.18. 5-(4-Phenoxyphenyl)-5-(4-(2,6-dichlorobenzoyl)-1,4-diazepan-1-yl) pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (31). Yield: 40% as off-white solids. Mp: 158–160 °C. MS: calcd for $C_{28}H_{25}Cl_2N_4O_5Na$ = 589.1021, $(M+Na)^+$ = 589.1019 found. ^1H NMR ($CDCl_3$) δ ppm: 1.88–1.92 (m, 2H, CH_2), 2.86–2.90 (m, 2H, CH_2), 2.96–2.98 (m, 1H, CH_2), 3.15–3.18 (m, 1H, CH_2), 3.41–3.44 (m, 1H, CH_2), 3.78–3.81 (m, 2H, CH_2), 3.97–3.99 (m, 1H, CH_2), 6.89–6.91 (d, J = 8.03, 2H, Ar-H), 6.95–6.97 (d, J = 8.03, 1H, Ar-H), 7.00–7.05 (t, J = 8.03, 1H, Ar-H), 7.16–7.25 (m, 2H, Ar-H), 7.27–7.33 (m, 2H, Ar-H), 7.36–7.39 (m, 2H, Ar-H), 7.46–7.49 (m, 2H, Ar-H), 9.49–9.54 (d, 2H, NH). ^{13}C NMR ($CDCl_3$): 28.9; 47.3; 50.3; 52.1;52.8; 77.9; 116.8; 118.4; 119.8; 121.4; 124.3; 127.9; 128.1; 128.2; 129.0; 129.3; 131.6; 132.9; 135.3; 135.6; 148.9; 154.9; 155.6; 158.9; 165.3; 170.2. IR (KBr): ν (cm^{-1}): 3105, 2958, 1728, 1702, 1579.

4.1.9.19. 5-(4-Phenoxyphenyl)-5-(4-(3-phenylpropanoyl)-1,4-diazepan-1-yl) pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (32). Yield: 45% as off-white solids. Mp: 121–124 °C. MS: calcd for $C_{30}H_{30}N_4O_5$-Na = 549.2114, $(M+Na)^+$ = 549.2110 found. ^1H NMR ($CDCl_3$) δ ppm: 1.61–1.67 (m, 1H, CH_2), 1.70–1.76 (m, 1H, CH_2), 2.71–2.76 (m, 2H, CH_2), 2.80–2.84 (m, 2H, CH_2), 2.99–3.04 (m, 2H, CH_2), 3.35–3.38 (m, 1H, CH_2), 3.57–3.60 (m, 1H, CH_2), 3.61–3.64 (m, 1H, CH_2), 3.71–3.74 (m, 1H, CH_2), 6.92–6.96 (m, 2H, Ar-H), 7.02–7.05 (m, 2H, Ar-H), 7.19–7.29 (m, 6H, Ar-H), 7.36–7.40 (t, J = 7.53, 2H, Ar-H), 7.44–7.48 (m, 2H, CH_2), 9.36 (s, 1H, NH), 9.50 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ ppm: 29.0; 31.4; 34.1; 48.3; 50.7; 52.2; 53.4; 77.8; 118.2; 119.8; 121.5; 124.3; 126.1; 128.4; 128.6; 129.1; 129.3; 129.9; 130.0; 141.4; 148.9; 155.6; 158.9; 170.2; 172.5. IR (KBr): ν (cm^{-1}): 3068, 2955, 1730, 1711, 1590.

4.1.9.20. 5-(4-Phenoxyphenyl)-5-(4-(2-(4-chlorophenyl)acetyl)-1,4-diazepan-1-yl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (33). Yield: 32% as off-white solids. Mp: 138–140 °C. MS: calcd for $C_{29}H_{27}ClN_4O_5Na$ = 569.1568, $(M+Na)^+$ = 569.1571 found. ^1H NMR (MeOD) δ ppm: 1.55–1.59 (m, 1H, CH_2), 1.66–1.72 (m, 1H, CH_2), 2.67–2.73 (m, 2H, CH_2), 2.85–2.89 (m, 1H, CH_2), 2.94–2.98 (m, 1H, CH_2), 3.33–3.39 (m, 1H, CH_2), 3.52–3.55 (m, 1H, CH_2), 3.60–3.63 (m, 1H, CH_2), 3.71–3.74 (m, 1H, CH_2), 3.89–3.93 (m, 1H, CH_2), 3.99–4.02(m, 1H, CH_2), 6.85–6.91 (m, 1H, Ar-H), 6.93–6.97(m, 2H, Ar-H), 7.01–7.05 (m, 1 H, Ar-H), 7.18–7.25 (m, 2H, Ar-H), 7.28–7.3 (m, 1H, Ar-H), 7.33–7.38 (m, 3H, Ar-H), 7.41–7.45 (m, 1H, Ar-H), 7.49–7.57 (m, 2H, Ar-H). ^{13}C NMR (MeOD) δ ppm: 30.8; 45.4; 51.4; 52.3; 53.7; 54.8; 78.7; 119.0; 119.5; 120.7; 121.0 l 122.7; 125.5; 129.4; 129.9; 130.4; 130.6; 130.7; 130.9; 131.1; 131.2; 134.0; 134.4; 150.8; 156.1; 159.2; 160.4; 170.3. IR (KBr): ν (cm^{-1}): 3096, 2956, 1738, 1710, 1588.

4.1.9.21. 5-(4-Phenoxyphenyl)-5-(4-(2-cyclohexylacetyl)-1,4-diazepan-1-yl) pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (34). Yield: 34% as off-white solids. Mp: 121–124 °C. MS: calcd for $C_{29}H_{35}N_4O_5$ = 519.2607, $(M+H)^+$ = 519.2597 found. ^1H NMR ($CDCl_3$) δ ppm: 1.22–1.25 (m, 2H, CH_2), 1.28–1.32 (m, 2H, CH_2), 1.66–1.70 (m, 4H, CH_2), 1.74–1.79 (m, 3H, CH_2 + CH), 1.86–1.91 (m, 2H, CH_2), 2.20–2.22 (d, J = 6.84, 1H, CH_2), 2.24–2.26 (d, J = 6.84, 1H, CH_2), 2.73–2.76 (m, 1H, CH_2), 2.79–2.82 (m, 2H, CH_2), 2.86–2.89 (m, 1H, CH_2), 3.41–3.44 (m, 1H, CH_2), 3.60–3.63 (m, 1H, CH_2), 3.64–3.67 (m, 1H, CH_2), 3.71–3.74 (m, 1H, CH_2), 3.78–3.81 (m, 1H, CH_2), 6.94–6.97 (m, 2H, Ar-H), 7.04–7.06 (d, J = 8.03, 2H, Ar-H), 7.16–7.20 (t, J = 7.53, 1H, Ar-H), 7.37–7.40 (t, J = 7.53, 1H, Ar-H), 7.47–7.51 (m, 3H, Ar-H), 9.60 (s, 2H, NH). ^{13}C NMR ($CDCl_3$) δ ppm: 25.8; 27.4; 29.0; 33.0; 34.6; 40.5; 48.0; 50.3; 52.4; 53.1; 77.5; 117.9. 118.1; 119.4; 119.5; 121.0; 127.9; 128.9; 129.0; 129.2; 129.6; 148.8; 151.1; 155.4; 158.5; 169.9; 170.0. IR (KBr): ν (cm^{-1}): 3066, 2956, 1730, 1711, 1590.

4.1.9.22. 5-(4-Phenoxyphenyl)-5-(4-(2,6-dichloro-5-fluoronicotinoyl)-1,4-diazepan-1-yl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (35). Yield: 32% as off-white solids. Mp: 154–156 °C. MS: calcd for $C_{27}H_{22}ClN_5O_5Na$ = 608.0880, $(M+Na)^+$ = 608.0881 found. ^1H NMR ($CDCl_3$) δ ppm: 1.87–1.90 (m, 2H, CH_2), 2.75–2.81 (m, 1H, CH_2), 2.83–2.87 (m, 1H, CH_2), 2.96–2.98 (m, 1H, CH_2), 3.18–3.23 (m, 1H, CH_2), 3.43–3.46 (m, 1H, CH_2), 3.78–3.81 (m, 1H, CH_2), 3.95–3.99 (m, 1H, CH_2), 6.91–6.94 (m, 2H, Ar–H), 6.96–6.98 (m, 1H, Ar-H), 7.04–7.06 (m, 1H, Ar-H), 7.19–7.23 (t, J = 7.53, 1H, Ar-H), 7.37–7.41 (m, 2H, Ar-H), 7.43–7.45 (d, J = 8.03, 1H, Ar-H), 7.48–7.51 (m, 1H, Ar-H), 9.13 (s, 1H, Ar–H), 9.24 (s, 1H, NH), 9.28 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ ppm: 29.5; 48.0; 50.5; 50.7; 52.6; 78.1; 118.1; 118.2; 120.0; 121.6; 124.6; 125.7; 128.2; 129.1; 129.3; 130.0; 132.6; 133.0; 148.5; 148.7; 152.7; 155.3; 159.2; 164.6; 170.2. IR (KBr): ν (cm^{-1}): 3066, 2958, 1735, 1640, 1588.

4.1.9.23. 5-(4-Phenoxyphenyl)-5-(4-(morpholine-4-carbonyl)-1,4-diazepan-1-yl) pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (36). Yield: 41% as off-white solids. Mp: 135–138 °C. MS: calcd for $C_{26}H_{29}N_5O_6$-Na = 530.2016, $(M+Na)^+$ = 530.1994 found. ^1H NMR ($CDCl_3$) δ ppm: 1.79–1.83 (m, 1H, CH_2), 1.86–1.89 (m, 1H, CH_2), 2.76–2.78 (m, 2H, CH_2), 2.87–2.90 (m, 1H, CH_2), 3.22–3.24 (m, 4H, Ar–H), 3.36–3.39 (m, 2H, CH_2), 3.48–3.52 (m, 2H, CH_2), 3.3.67–3.69 (m, 4H CH_2), 3.78–3.80 (m, 1H, CH_2), 6.91–6.96 (m, 3H, Ar-H), 7.00–7.04 (m, 1H, Ar-H), 7.15–7.19 (t, J = 7.53, 1H, Ar-H), 7.35–7.39 (m, 1H, Ar-H), 7.46–7.50 (m, 3H, Ar-H) 9.81 (s, 1H, NH), 9.87 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ ppm: 29.2; 47.4; 47.6; 50.6; 51.1; 52.2; 66.6; 77.2; 118.1; 118.3; 119.7; 121.3; 124.4; 128.2; 129.3; 129.4; 130.0; 149.2; 155.7; 158.7; 164.2; 170.3; 170.4. IR (KBr): ν (cm^{-1}): 3065, 2978, 1711, 1610, 1580.

4.1.9.24. 5-(4-Phenoxyphenyl)-5-(4-((8-(dimethylamino)naphthalen-4-yl)sulfonyl)-1,4-diazepan-1-yl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (37). Yield: 52% as yellow solids. Mp: 138–140 °C. MS: calcd for $C_{33}H_{34}N_5O_6S$ = 628.2230, $(M+H)^+$ = 628.2249 found. ^1H NMR ($CDCl_3$) ppm: 1.75–1.78 (m, 2H, CH_2), 2.86–2.89 (m, 4H, CH_2), 2.91 (s, 6H, CH_3), 3.34–3.38 (m, 2H, CH_2), 3.63–3.66 (m, 2H, CH_2), 6.89–6.92 (m, 3H, Ar-H), 7.02–7.04 (d, J = 7.53, 1H, Ar-H), 7.16–7.22 (t, J = 7.53, 2H, Ar-H), 7.36–7.43 (m, 4H, Ar-H), 7.51–7.59 (m, 2H, Ar-H), 8.16–8.18 (d, J = 7.53, 1H, Ar-H), 8.34–8.36 (d, J = 7.03, 1H, Ar-H), 8.54–8.56 (m, 1H, Ar-H), 8.86 (s, 1H, N–H), 8.90 (s, 1H, N–H). ^{13}C NMR ($CDCl_3$) ppm: 29.6; 46.7; 50.0; 50.8; 51.6; 53.8; 78.1; 118.2; 118.3; 119.9; 121.4; 123.2. 123.4; 128.0; 128.8; 129.0; 129.2; 129.4; 130.0; 132.9; 144.3; 148.5; 151.3; 155.5; 159.1; 169.9; 170.0. IR (KBr): ν (cm^{-1}): 3170, 1736, 1713, 1609, 1588.

4.1.9.25. 5-(4-Phenoxyphenyl)-5-(4-(4-(methylthio)benzoyl)-1,4-diazepan-1-yl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (38). Yield: 32% as off-white solids. Mp: 240–244 °C. MS: calcd for $C_{29}H_{29}N_4O_5S$ = 545.1841, $(M+H)^+$ = 545.1859 found. ^1H NMR (DMSO-d_6) ppm: 1.48–1.55 (m, 1H, CH_2) 1.64–1.70 (m, 1H, CH_2), 2.46 (s, 3H, CH_3), 2.68–2.73 (m, 3H, CH_2), 3.19–3.23 (m, 1H, CH_2), 3.41–3.41 (m, 1H, CH_2), 3.51–3.54 (m, 1H, CH_2), 3.64–3.68 (m, 1H, CH_2), 6.90–6.95 (m, 1H, Ar-H), 7.00–7.03 (m, 3H, Ar-H), 7.18–7.21 (t, J = 7.53, 1H, Ar-H), 7.27–7.35 (m, 5H, Ar-H), 7.39–7.47 (m, 2H, Ar-H), 7.56–7.58 (m, 1H, Ar-H), 11.57 (s, 2H, NH). ^{13}C NMR (DMSO-d_6) ppm: 14.3; 29.8; 47.7; 50.8; 52.9; 53.5; 76.7; 118.0; 119.4; 121.5; 124.2; 125.1; 127.0; 127.4; 129.0; 129.4; 130.0; 133.0; 133.3; 139.6; 149.5; 155.5; 157.6; 170.0; 170.7. IR (KBr): ν (cm^{-1}): 3058, 2951, 2763, 1729, 1708, 1589.

4.1.10. General procedure for the preparation of compounds 39–46

To a solution of 5-(1,4-diazepan-1-yl)-5-(4-phenoxyphenyl)pyrimidine-2,4,6(1*H*,3*H*, 5*H*)-trione (394 mg, 1 mmol) in 10 ml THF was added DCC (206 mg, 1 mmol), DMAP (132 mg, 1 mmol) and the substituted carboxylic acids (1 mmol). The mixture was stirred at room temperature for 24 h before filtering. The solvents of the filtrate were removed in vacuo and the resulting crude products purified by flash column chromatography to yield pure products.

4.1.10.1. 5-(4-Phenoxyphenyl)-5-(4-(1-methyl-1*H*-indazole-3-carbonyl)-1,4-diazepan-1-yl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (39). Yield: 49% as off-white solids. Mp: 185–187 °C. MS: calcd for $C_{30}H_{29}N_6O_6$ = 553.2199. $(M+H)^+$ = 553.2193 found. ^{1}H NMR (DMSO-d_6) δ ppm: 1.76–1.78 (m, 2H, CH_2), 2.71–2.75 (m, 2H, CH_2), 2.82–2.88 (m, 2H, CH_2), 3.60–3.63 (m, 2H, CH_2), 3.77–3.80 (m, 2H, CH_2), 4.11 (s, 3H, CH_2), 7.03–7.10 (m, 3H, Ar-H), 7.21–7.26 (m, 2H, Ar-H), 7.30–7.34 (t, *J* = 7.53, 1H, Ar-H), 7.41–7.49 (m, 4H, Ar-H), 7.59–7.62 (m, 1H, Ar-H), 7.70–7.72 (d, *J* = 8.53, 1H, Ar-H), 8.00–8.03 (d, *J* = 8.03, 1H, Ar-H), 11.55 (s, 2H, NH). ^{13}C NMR (DMSO-d_6) δ ppm: 29.9; 35.8; 48.4; 51.2; 52.1; 53.3; 76.7; 110.0; 117.6; 118.5; 119.6; 121.4; 123.4; 124.9; 126.4; 128.0; 129.1; 129.3; 130.2; 133.0; 139.2; 140.0; 149.4; 155.4; 157.7; 162.8; 170.7. IR (KBr): ν (cm^{-1}): 2929, 1737, 1710, 1625.

4.1.10.2. 5-(4-Phenoxyphenyl)-5-(4-(2-(2,3-dihydrobenzo[*b*][1,4]dioxine-6-sulfonamido)3-methylbutyryl)-1,4-diazepan-1-yl)-pyrimidine-2,4,6(1H,3H,5H)-trione (40). Yield: 31% as off-white solids. Mp: 143–146 °C. MS: calcd for $C_{34}H_{38}N_5O_9S$ = 692.2390, $(M+H)^+$ = 692.2383 found. ^{1}H NMR ($CDCl_3$) δ ppm: 0.84–0.89 (m, 3H, CH_3), 1.01–1.04 (m, 3H, CH_3), 1.88–1.93 (m, 2H, CH_2), 2.39–2.43 (m, 1H, CH), 2.68–2.74 (m, 2H, CH_2), 2.82–2.84 (m, 1H, CH_2), 3.24–3.26 (m, 1H, CH_2), 3.51–3.54 (m, 2H, CH_2 + CH), 3.76–3.80 (m, 1H, CH_2), 3.84–3.87 (m, 1H, CH_2), 3.92–3.94 (m, 1H, CH_2), 4.27–4.31 (m, 4H, CH_2), 6.91–7.00 (m, 6H, Ar-H), 7.04–7.07 (t, *J* = 7.53, 1H, Ar-H), 7.18–7.22 (m, 1H, CH), 7.29–7.32 (m, 3H, Ar–H), 7.37–7.47 (m, 1H, Ar–H), 7.47–7.51 (m, 2H, Ar–H), 8.87 (s, 1H, NH), 9.07 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ ppm: 19.5; 29.0; 30.8; 46.1; 49.6; 50.1; 51.5; 53.2; 57.5; 77.7; 116.4; 116.8; 117.4; 117.9; 118.1; 119.6; 120.6; 121.2; 124.2; 128.9; 129.1; 129.7; 132.0; 146.9; 151.2; 154.5; 155.2; 158.3; 169.4; 170.5. IR (KBr): ν (cm^{-1}): 2929, 1736, 1710, 1615.

4.1.10.3. 5-(4-Phenoxyphenyl)-5-(4-(3-(benzo[*d*]thiazol-2-yl)-2-methyl-2-phenylpropanoyl)-1,4-diazepan-1-yl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (41). Yield: 33% as off-white solids. Mp: 141–144 °C. MS: calcd for $C_{38}H_{36}N_5O_5S$ = 674.2437, $(M+H)^+$ = 674.2426 found. ^{1}H NMR (MeOD) δ ppm: 1.82–7.89 (m, 2H, CH_2), 2.24 (s, 3H, CH_3), 2.93–2.96 (m, 3H, CH_2), 3.32–3.35 (m, 2H, CH_2), 3.45–3.51 (m, 2H, CH_2), 3.73–3.76 (m, 2H, CH_2), 6.93–6.97 (m, 6H, Ar–H), 6.99–7.02 (m, 2H, Ar-H), 7.14–7.18 (t, *J* = 7.53, 1H, Ar-H), 7.35–7.39 (m, 2H, Ar-H), 7.48–7.52 (m, 3H, Ar-H), 7.54–7.57 (m, 2H, Ar-H), 7.80–7.82 (d, *J* = 8.53, 1H, Ar-H), 7.87–7.89 (d, *J* = 8.53, 1H, Ar-H). ^{13}C NMR (MeOD) δ ppm: 21.4; 29.0; 39.7; 47.3; 48.4; 52.3; 55.7; 78.7; 117.4; 119.1; 119.4; 120.7; 122.3; 122.5; 122.9; 125.2; 126.1; 127.0; 129.5; 130.3; 130.4; 130.7; 130.9; 131.1; 133.0; 139.1; 150.9; 154.0; 157.0; 159.2; 170.4; 172.6; 178.7. IR (KBr): ν (cm^{-1}): 2929, 1736, 1710, 1615.

4.1.10.4. 5-(4-Phenoxyphenyl)-5-(4-(3-(*N*-(*5-methylisoxazol-3-yl)sulfamoyl) benzoyl)-1,4-diazepan-1-yl)pyrimidine-2,4,6(1H, 3H,5H)-trione (42). Yield: 36% as off-white solids. Mp: 145–147 °C. MS: calcd for $C_{32}H_{31}N_6O_8S$ = 659.1924, $(M+H)^+$ = 659.1931 found. ^{1}H NMR (MeOD) δ ppm: 1.83–1.88 (m, 2H, CH_2), 2.24 (s, 3H, CH_3), 2.81–2.85 (m, 3H, CH_2), 2.98–3.00 (m, 1H, CH_2), 3.18–3.21 (m, 1H, CH_2), 3.42–3.45 (m, 1H, CH_2), 3.68–3.71(m, 1H, CH_2), 3.81–3.83 (m, 1H, CH_2), 6.2 (s, 1H, CH), 6.80–6.83 (d, *J* = 8.53, 1H, Ar-H), 6.94–6.95 (m, 1H, Ar-H), 6.97–7.00 (m, 1H, Ar-H), 7.03–7.06 (m, 1H, Ar-H), 7.18–7.21 (t, *J* = 7.03, 1H, Ar-H), 7.36–7.41 (m, 1H, Ar-H), 7.51–7.55 (m, 1H, Ar-H), 7.56–7.59 (m, 1H, Ar-H), 7.63–7.68 (m, 1H, Ar-H), 7.74–7.76 (m, 1H, Ar-H), 7.96–7.98 (m, 1H, Ar-H), 8.01–8.03 (m, 2H, Ar-H). ^{13}C NMR (MeOD) δ ppm: 12.3; 28.9; 45.6; 51.6; 52.4; 54.3; 78.8; 96.5; 117.7; 119.0; 119.3; 119.6; 120.9; 122.6; 125.4; 129.2; 129.5; 130.3; 130.5; 130.8; 131.1; 132.2; 136.2; 141.3; 150.8; 152.8; 156.7; 158.9; 171.8; 172.1; 172.5. IR (KBr): ν (cm^{-1}): 2929, 1738, 1712, 1613.

4.1.10.5. 5-(4-Phenoxyphenyl)-5-(4-(5-fluoro-2-(methylsulfonamido) benzoyl)-1,4-diazepan-1-yl)pyrimidine-2,4,6(1*H*,3*H*, 5*H*)-trione (43). Yield: 38% as off-white solids. Mp: 200–204 °C. MS: calcd for $C_{29}H_{29}N_5O_7S$ = 610.1772, $(M+H)^+$ = 610.1799 found. ^{1}H NMR (MeOD) δ ppm: 1.82–1.89 (m, 2H, CH_2), 2.85–2.90 (m, 3H, CH_2), 3.00–3.03 (m, 1H, CH_2), 3.06 (s, 3H, CH_3), 3.21–3.24 (m, 1H, CH_2), 3.44–3.50 (m, 1H, CH_2), 3.68–3.71 (m, 1H, CH_2), 3.81–3.85 (m, 1H, CH_2), 6.87–6.91 (m, 1H, Ar-H), 6.93–6.96 (m, 2H, Ar-H), 7.00–7.06 (m, 2H, Ar-H), 7.17–7.22 (m, 1H, Ar-H), 7.25–7.28 (m, 1H, Ar-H), 7.34–7.39 (m, 1H, Ar-H), 7.74–7.44 (m, 1H, Ar-H), 7.45–7.50 (m, 1H, Ar-H), 7.51–7.55 (m, 2H, Ar-H). ^{13}C NMR (MeOD) δ ppm: 29.4; 41.0; 45.7; 51.1; 52.5; 53.6; 79.0; 113.5; 115.6; 116.3; 117.9; 118.1; 119.2; 119.6; 120.8; 122.5; 129.5; 130.4; 130.6; 130.9; 131.1; 139.2; 150.8; 152.3; 156.8; 159.6; 169.8; 172.4. IR (KBr): ν (cm^{-1}): 2931, 1738, 1712, 1613.

4.1.10.6. 5-(4-Phenoxyphenyl)-5-(4-(3-(*N*-(4-methoxyphenyl)-sulfamoyl)-4-methylbenzoyl)-1,4-diazepan-1-yl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (44). Yield: 26% as off-white solids. Mp: 147–149 °C. MS: calcd for $C_{36}H_{26}N_5O_8S$ = 698.2285, $(M+H)^+$ = 698.2290 found. ^{1}H NMR (MeOD) δ ppm: 1.70–1.76 (m, 2H, CH_2), 2.67 (s, 3H, CH_3) 2.70–2.73 (m, 1H, CH_2), 2.75–2.78 (m, 2H, CH_2), 2.91–2.96 (m, 1H, CH_2), 3.33 (s, 3H, CH_3), 3.47–3.50 (m, 1H, CH_2), 3.56–3.58 (m, 1H, CH_2), 3.63–3.65 (m, 1H, CH_2), 3.70–3.73 (m, 1H, CH_2), 6.53–6.55 (d, *J* = 8.53, 1H, Ar-H), 6.70–6.73 (d, *J* = 8.53, 1H, Ar-H), 6.77–6.80 (m, 2H, Ar-H), 6.90–6.94 (m, 2H, Ar-H), 6.97–7.03 (m, 2H, Ar-H), 7.20–7.23 (t, *J* = 7.03, 1H, Ar-H), 7.32–7.37 (m, 1H, Ar-H), 7.40–7.46 (m, 2H, Ar-H), 7.52–7.55 (m, 1H, Ar-H), 7.59–7.61 (d, *J* = 8.03, 1H, Ar-H), 7.66–7.68 (d, *J* = 8.03, 1H, Ar-H), 8.11 (s, 1H, Ar-H). ^{13}C NMR (MeOD) δ ppm: 20.5; 28.9; 45.5; 51.2; 52.7; 53.8; 55.9; 78.9; 115.3; 115.4; 118.8; 119.1; 119.5; 119.7; 120.8; 121.0; 122.7; 124.3; 125.5; 125.7; 129.5; 129.6; 130.4; 130.5; 130.6; 130.7; 130.8; 135.4; 138.5; 139.2; 150.9; 152.9; 155.8; 157.2; 169.4; 172.2. IR (KBr): ν (cm^{-1}): 2932, 1731, 1708, 1607.

4.1.10.7. 5-(4-Phenoxyphenyl)-5-(4-(4-methyl-2-(methylsulfonamido)benzoyl)-1,4-diazepan-1-yl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (45). Yield: 40% as off-white solids. Mp: 203–206 °C. MS: calcd for $C_{30}H_{31}N_5O_7SNa$ = 628.1842, $(M+Na)^+$ = 628.1821 found. ^{1}H NMR (DMSO-d_6) δ ppm: 1.64–1.70 (m, 2H, CH_2), 2.27 (s, 3H, CH_3), 2.70–2.75 (m, 2H, CH_2), 2.84–2.87 (m, 1H, CH_2), 2.98 (s, 3H, CH_3), 3.09–3.13(m, 1H, CH_2), 3.31–3.36 (m, 2H, CH_2), 3.70–3.73 (m, 1H, CH_2), 6.88 (s, 1H, Ar-H), 6.94–6.96 (d, *J* = 8.03, 1H, Ar-H), 7.02–7.04 (d, *J* = 8.53, 1H, Ar-H), 7.06–7.09 (m, 1H, Ar-H), 7.16–7.23 (m, 2H, Ar-H), 7.23–7.31 (m, 2H, Ar-H), 7.34–7.39 (m, 2H, Ar-H), 7.42–7.44 (m, 1H, Ar-H), 7.57–7.59 (d, *J* = 7.53, 1H, Ar-H). ^{13}C NMR (DMSO-d_6) δ ppm: 21.0; 29.4; 42.3; 47.3; 50.5; 50.9; 52.3; 76.7; 116.0; 117.7; 118.2; 118.5; 119.5; 119.6; 121.7; 127.9; 128.4; 129.4; 129.9; 130.1; 130.6; 139.2; 141.1; 149.4; 151.5; 155.2; 157.3; 168.1; 170.7. IR (KBr): ν (cm^{-1}): 2933, 1744, 1706, 1602.

4.1.10.8. 5-(4-Phenoxyphenyl)-5-(4-(2-(4-oxo-2-(phenylimino)thizolidin-5-yl)acetyl)-)-1,4-diazepan-1-yl)pyrimidine-2,4,6-(1*H*,3*H*,5*H*)-trione (46). Yield: 34% as off-white solids. Mp: 167–169 °C. MS: calcd for $C_{32}H_{30}N_6O_6SNa$ = 649.1845, $(M+Na)^+$ = 649.1827 found.^{1}H NMR (MeOD) δ ppm: 1.82–7.89 (m, 2H, CH_2), 2.75–2.79 (m, 2H, CH_2), 2.83–2.86 (m, 1H, CH_2), 2.90–2.94 (m, 2H, CH_2), 3.42–3.47 (m, 2H, CH_2), 3.50–3.55 (m, 1H, CH_2), 3.61–3.67 (m, 2H, CH_2), 4.53–4.55 (m, 1H, CH_2), 6.91–6.97 (m, 3H, Ar-H), 7.01–7.02 (d, *J* = 7.53, 2H, Ar-H), 7.15–7.19 (t, *J* = 7.03, 2H, Ar-H), 7.30–7.32 (m, 1H, Ar-H), 7.34–7.37 (t, *J* = 7.03, 2H, Ar-H), 7.46–7.54 (m, 4H, Ar-H). ^{13}C NMR (MeOD) δ ppm: 29.0; 35.7; 46.3; 47.7; 50.4; 52.5; 53.6; 79.0; 117.5; 119.3; 119.8; 120.7; 122.2; 122.4; 122.5; 127.2; 129.5; 130.0; 130.5; 130.8; 131.1; 150.8; 152.3; 156.9; 157.4; 159.5; 171.6; 172.4; 178.0. IR (KBr): ν (cm^{-1}): 2930, 1737, 1710, 1618.

4.2. Biological methods

4.2.1. Cell culture and treatment

Human fibrosarcoma HT-1080 cells were cultivated in Eagle's minimal essential medium (MEM) containing 10% fetal bovine serum (FBS), 100 IU/ml penicillin, and 50 μg/ml streptomycin. The cultures were incubated at 37 °C in a humidified 5% CO_2 atmosphere until confluence. Before experiments, cultures were washed twice with serum-free medium and then incubated under serum-free conditions for 6 h. 12-*O*-Tetradecanoylphorbol-13-acetate (PMA) was added into the cell and final concentration of PMA was 5 nM. After incubation for 24 h, the supernatants from the cell cultures were taken out for assay.[26] Caco-2 cells (Sigma–Aldrich, Ireland) were cultivated in Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS, 1 × nonessential amino acids, 2 mM L-glutamine, 100 IU/ml penicillin, and 50 μg/ml streptomycin.

4.2.2. Zymography

The enzymatic activities of MMP-2 and MMP-9 were assayed by gelatin zymography in the absence of serum.[27] Cell supernatants were electrophoresed on an 8% SDS–PAGE containing 2% gelatin. The gels were washed with 0.25% triton three times. Then the gels were cut and incubated in the zymography buffer (0.15 M NaCl, 5 mM $CaCl_2$, 0.05% NaN_3 and 50 mM Tris–HCl buffer, pH 7.5) at 37 °C for 48 h, which contained the compounds at the final concentration of 0.5–10 μM. After incubation, the gels were stained with 0.25% Coomassie Brilliant Blue R250, and destained with acetic acid, methanol and water. MMP activity was represented by a white band of gelatin digestion. Remaining enzymatic activity in the presence of test inhibitor was quantitated by densitometry on the gel reader (Bio-RAD, Universal hood II).

4.2.3. MMP-2 and MMP-9 fluorogenic assay

Recombinant MMP-2 and MMP-9 (R&D Systems, Ireland) were activated by treatment with APMA (*p*-aminophenylmercuric acetate) at 37 °C for 1 h and 24 h, respectively.[1] The synthetic broad-spectrum fluorogenic substrate (7-methoxycoumarin-4-yl)-acetyl-pro-Leu-Gly-Leu-(3-(2,4-dinitrophenyl)-L-2,3-diaminopropionyl)-Ala-Arg-NH_2 (R&D Systems, UK) was used to assay MMP-2 and MMP-9 activity. The inhibition of human active MMP-2 and MMP-9 was assayed by preincubating MMP-2 (2 nM) or MMP-9 (2 nM) and the inhibitory compounds at varying concentrations (10 pM to 10 μM) in 50 mM Tris–HCl, pH 7.5, containing 0.2 M NaCl, 5 mM $CaCl_2$, 20 μM $ZnSO_4$, and 0.05% Brij 35, at 37 °C for 30–45 min. An aliquot of substrate (10 μl of a 50 μM solution) was then added to 90 μl of the preincubated MMP/inhibitor mixture, and the activity was determined at 37 °C by following product release with time. The fluorescence changes were monitored using a plate reader (Fluostar Optima, BMG Labtech) with excitation and emission wavelengths set to 330 and 405 nm, respectively. Reaction rates were measured from the initial 10 min of the reaction profile where product release was linear with time and plotted as a function of inhibitor dose. From the resulting inhibition curves, the IC_{50} value for each inhibitor was calculated by nonlinear regression analysis using GraphPad Prism 4.0 (La Jolla, CA, USA).

4.2.4. Invasion assay

Caco-2 cells were cultured until 80% confluence and removed from the flask using DPBS-EDTA. The cells were collected in serum-free medium and counted in a cell-counting machine (Z1 coulter® Particle counter). The inserts containing matrigel membranes with 8.0 μm pore size (BD Bioscience, UK) were treated with 500 μl serum-free medium. After incubation for 2 h, the blank inserts were treated with 250,000 Caco-2 cells and the positive control inserts contained 250,000 Caco-2 cells and 75 ng HGF.[15] All the remaining inserts were treated with 250,000 Caco-2 cells, 75 ng HGF, and the tested inhibitors (with final concentrations at 10 μM and/or 100 nM). Medium with 2% FCS was added to each inserts to make the total volume 1 ml. The inserts were then transferred to the assay chambers containing 750 ml of 20% FCS medium and left in cell culture incubator for 48 h. After incubation, supernatants were taken out and the non-migratory cells on the upper surface of the membranes were removed with serum-free medium using cotton swabs. The inserts were treated with the Diff-Quik kit (BD Bioscience, USA) for fixing and staining the invade cells on the membranes. The inserts were left to dry and the migrated cells were counted under using microscopy (Zeiss Axiovert 200 M). The experiments were carried out in duplicate and repeated three times for each concentration inhibitor.

4.3. Molecular modelling

The crystal structure of MMP-2 (PDB code 1QIB) was used for the study. Since this is a co-crystallised structure complexed with a hydroxamate inhibitor, the hydroxamate inhibitor was removed. The structure of MMP-9 (PDB code 2VOX) used was an MMP-9 active site mutant with barbiturate inhibitor, so the barbiturate inhibitor was removed. In addition water molecules were removed. Docking calculations were carried out using AutoDock version 4.0, with the Lamarckian genetic algorithm (LGA).[28] The molecular models of each inhibitor were built using the builder function of MOE and minimised with MOPAC 7 (AM1 method) interfaced to MOE. The zinc parameters were changed to zinc radius: 0.87 Å; well depth: 0.35 kcal/mol; and zinc charges: +0.95*e*.[29] The 3D affinity grid box was designed to include the full active site and possible residues. The setting of the centre of grid boxes was based on the value of active zinc atom. Docking calculations were set to 20 runs. Energy (250,00,000) evaluations were allowed as a maximum in each run. At the end of the calculation, AutoDock performed cluster analysis. Docking solutions with ligand all-atom root mean square deviation (RMSD) within 2.0 Å of each other were clustered together and ranked by the lowest energy representative.

4.4. Statistical analysis

All date are presented as group of means with standard error of the mean of $n \geqslant 3$. Statistical analysis of the mean difference between multiple groups was determined by one-way ANOVA followed by Tukey-Kramer multiple comparison post test. A *P* value <0.05 was considered to be statistically significant. All statistical analyses were performed using GraphPad Prism 4.0.

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