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Original article

# Inhibition of protein glycation by urea and thiourea derivatives of glycine/proline conjugated benzisoxazole analogue – Synthesis and structure–activity studies

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ABSTRACT

Synthesis of a new series of urea/thiourea derivatives of Gly/Pro conjugated benzisoxazole has been reported. Structure of the compounds was characterized by physical and spectroscopical data and has been screened for their *in vitro* antiglycation activity. Several compounds showed promising activity with $IC_{50} < 5$ μM compared to standard rutin ($IC_{50} = 41.9$ μM). Further, it was found that compounds containing methoxy and bromine substituents have exerted highly potent activity. Thus, the title compounds represent novel class of potent antiglycating agents.



## 1. Introduction

Diabetes is a very common endocrine disorder, which results from an absolute or relative deficiency of insulin or insulin resistance and is characterized by hyperglycemia. Diabetes predisposes patients to chronic complications, and affects multiple parts of the body. Life expectancy of diabetic patients is only two thirds of that of the normal population. This chronic disorder affects 1–2% of the world population [1].

Glycation (non-enzymatic glycosylation) results in Amadori product which accumulates in living organisms that leads to structural and functional modifications of tissue proteins [2]. Many studies have shown a significant role of glycation in the progress of normal ageing and the pathogenesis of age-related diseases, such as diabetes, atherosclerosis, end-stage renal disease, rheumatoid arthritis, and neurodegenerative diseases. Therefore, targeting glycation should have a broad and beneficial effect on ageing and age-related diseases [3]. One can consider that AGEs inhibitors are the compounds which are able to cleave the α-dicarbonyl species and act as potent chelators of $Cu^{2+}$ and inhibit the oxidation process [4].

Urea is a functional moiety that is commonly found in natural products and often displays a wide range of biological activities [5]. Urea and thiourea compounds are known to possess antidiabetic and antimicrobial activities [6]. Ureas and thioureas have been studied for the systematic control of tuberculosis also [7]. A few compounds of thiourea derivatives bearing benzothiazole moiety have shown antimicrobial and anticancer activities [8]. A series of diaryl substituted heterocyclic urea which have been reported to inhibit cholesterol *O*-acy1 transferase (ACAT) as hypocholestrolemic [9]. *N*,*N*-Disubstituted cyclic urea-3-benzamide was found to be HIV protease inhibitor; which may be useful in the treatment of AIDS [10]. Some of the urea compounds are multistage glycation inhibitors with the highest post-Amadori activities. Some of these inhibited AGE–protein cross-linking including AGE–collagen cross-linking from the reaction of bovine serum albumin with glucose [11].

The therapeutic applications of amino acids have received a considerable attention in respiratory physiology, cardiology, renal failure, neurological disorders, and congenital defects. Moreover some of the amino acids are used as antiglycating agents also [12,13]. One of the most frequently encountered heterocycle in medicinal chemistry is benzisoxazole and its derivatives which are reported to have diverse biological applications including antihistaminic, antifungal, antitumour, antiulcer, anticonvulsant, hypoglycemic, anti-inflammatory and antitubercular activities [14,15].

*Abbreviations:* Boc, *t*-butoxycarbonyl; EDCI, 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide; HOBt, 1-hydroxybenzotriazole; NMM, *N*-methyl morpholine; TFA, trifluoroacetic acid.

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Furthermore, benzisoxazole and its analogues constitute an active class of compounds possessing antidiabetic activity [16].

Jung et al. have synthesized methoxy derivatives of benzoquinones and naphthoquinones and identified them as inhibitors of glycation [17]. Later Khan et al. [18,19] have synthesized various urea and isatin derivatives and evaluated their antiglycation activity. Recently the same research group [20] has synthesized benzoxazole derivatives and found that they act as possible antiglycating agents. It is evident from the results obtained by them that the synthesized compounds have exhibited moderate or equipotent activity as that of the standard. Prompted by the above observations and in continuation of our research programme [21–26], we envisioned our approach towards the synthesis of a new series of ureido and thioureido derivatives of Gly/Pro conjugated benzisoxazole followed by the antiglycation evaluation.

## 2. Results and discussion

### 2.1. Chemistry

The heterocycle, [3-(4-piperidyl)-6-fluoro-1,2-benzisoxazole]·HCl was synthesized by following the previously reported method [27]. This was further conjugated to Boc-Gly/Pro-OH using EDCI/HOBt as coupling agent and NMM as base. Boc group of the conjugates was removed using TFA and reacted with various substituted phenyl isocyanates/isothiocyanates to obtain urea/thiourea derivatives respectively (Scheme 1). Synthesis was confirmed by IR, $^1$H NMR, HRMS and elemental analysis. In IR spectra stretching frequencies appeared at 1615–1644 cm$^{-1}$ (CO), ~2040 cm$^{-1}$ (CS) and ~3300 cm$^{-1}$ (NH) and in $^1$H NMR spectra signals appeared at $\delta$ ~9.95 (1H, s, –NH) and ~10.36 (1H, s, –NH) for thiourea compounds whereas for the urea derivatives signals were observed at $\delta$ ~8.05 (1H, t, –NH) and ~8.95 (1H, s, –NH). Further, the presence of all requisite peaks and the absence of extraneous peaks confirm the synthesis.

### 2.2. Antiglycation activity (in vitro)

Evaluation of antiglycation activity of the synthesized compounds was examined by the method of Nakagawa et al. [28]. The $IC_{50}$ values are shown in Table 1. It is clear from the results that all the synthesized ureido and thioureido derivatives of the heterocyclic conjugates have shown enhanced activity than their counter parts. Most of them are more potent than the standard, rutin. Further, to the best of our knowledge, our synthesized compounds have exhibited high activity than the compounds documented in the literature [17–20].

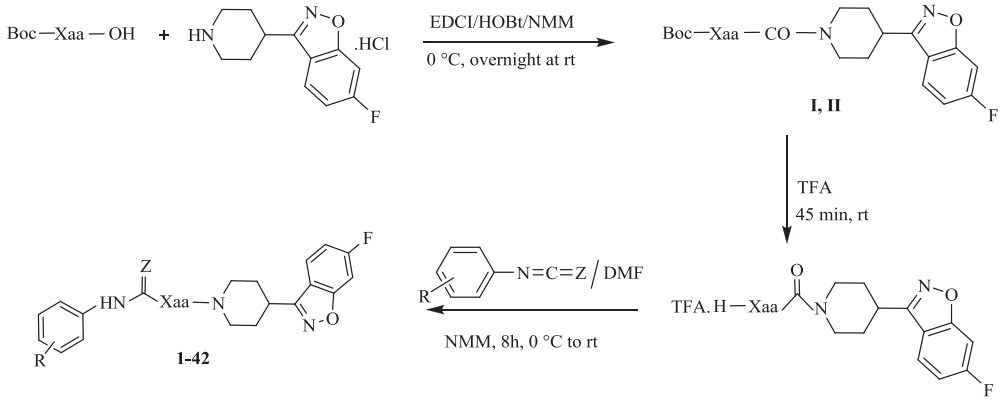
Among the series, methoxy (**3**–**8**, **24**–**29**) and bromo (**18**–**21**, **39**–**42**) containing compounds have exhibited high activity. The high activity of methoxy derivatives may be due to the electron donating nature of methoxy group and that of bromo derivatives may be due to less electron withdrawing (F > Cl > Br) and also due to inductive and resonance effect. This may also be due to the fact that the halogens particularly bromine is not involved in resonance stabilization.

Among the two series of compounds tested, Gly containing analogues have exhibited increased activity over the Pro containing compounds. This may be due to the small size and simple nature of Gly which would enhance the binding abilities. Between urea and thiourea analogues, compounds with thiourea moiety have shown increased activity over the former. This may probably be due to more nucleophilic character of sulphur compared to oxygen. The plausible mechanism of action involves the trapping of dicarbonyl species and preventing oxidation using transition metal chelators ($Cu^{2+}$) or free radical scavengers [4].

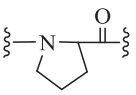
For the first time we have replaced 'O' by 'S' and succeeded in obtaining highly potent molecules. Initially, we have chosen simple amino acids like glycine and proline. Further, the work with other amino acids and substituents is in progress.

## 3. Conclusion

We have successfully synthesized a series of urea/thiourea derivatives of Gly/Pro conjugated benzisoxazole with different functionalities. Some of the representatives of the series were identified as highly potent antiglycating agents. The antiglycating activity of the synthesized compounds showed that urea and thiourea moieties play a major role in enhancing the activity. Further, it is interesting to note that $OCH_3$ and Br act as active moieties in inhibiting the glycation. Thus, nature of the substituent



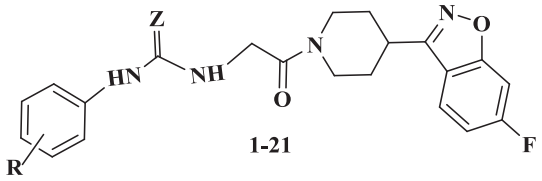

**Scheme 1.** Synthesis of urea/thiourea derivatives of amino acids conjugated heterocycle.

where, Xaa = -NH-$CH_2$-CO- ; 

R = H, $OCH_3$, F, Cl, Br

Z = O/S

**Table 1**
Antiglycation activity of the synthesized compounds.

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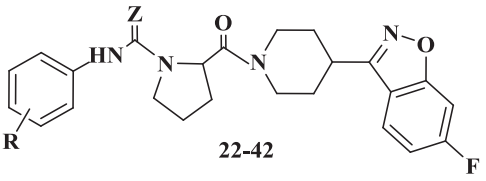


22-42

| Entry | Compounds | | $IC_{50} \pm SEM$ (μM)[a] | Entry | Compounds | | $IC_{50} \pm SEM$(μM)[a] |
|---|---|---|---|---|---|---|---|
| | R | Z | | | R | Z | |
| 1 | H | O | 390.4 ± 0.4 | 22 | H | O | 340.1 ± 0.5 |
| 2 | H | S | 380.5 ± 0.8 | 23 | H | S | 295.1 ± 0.3 |
| 3 | 2-$OCH_3$ | O | 20.2 ± 0.3 | 24 | 2-$OCH_3$ | O | 30.6 ± 0.7 |
| 4 | 2-$OCH_3$ | S | 4.1 ± 0.6 | 25 | 2-$OCH_3$ | S | 5.2 ± 0.4 |
| 5 | 3-$OCH_3$ | O | 21.1 ± 0.1 | 26 | 3-$OCH_3$ | O | 33.4 ± 0.2 |
| 6 | 3-$OCH_3$ | S | 15.3 ± 0.8 | 27 | 3-$OCH_3$ | S | 20.5 ± 0.1 |
| 7 | 4-$OCH_3$ | O | 19.5 ± 0.4 | 28 | 4-$OCH_3$ | O | 22.1 ± 0.7 |
| 8 | 4-$OCH_3$ | S | 3.6 ± 0.3 | 29 | 4-$OCH_3$ | S | 3.9 ± 0.4 |
| 9 | 2-F | O | 55.5 ± 0.7 | 30 | 2-F | O | 54.8 ± 0.9 |
| 10 | 2-F | S | 9.1 ± 0.5 | 31 | 2-F | S | 7.8 ± 0.8 |
| 11 | 3-F | O | 100.2 ± 0.6 | 32 | 3-F | O | 116.7 ± 0.1 |
| 12 | 4-F | O | 75.8 ± 0.2 | 33 | 4-F | O | 76.1 ± 0.4 |
| 13 | 4-F | S | 70.3 ± 0.7 | 34 | 4-F | S | 70.9 ± 0.6 |
| 14 | 3-Cl | O | 143.5 ± 0.5 | 35 | 3-Cl | O | 145.4 ± 0.7 |
| 15 | 3-Cl | S | Inactive | 36 | 3-Cl | S | 125.7 ± 0.8 |
| 16 | 4-Cl | O | 83.9 ± 0.9 | 37 | 4-Cl | O | 60.3 ± 0.3 |
| 17 | 4-Cl | S | Inactive | 38 | 4-Cl | S | 59.4 ± 0.8 |
| 18 | 2-Br | S | 6.3 ± 0.8 | 39 | 2-Br | S | 7.8 ± 0.2 |
| 19 | 3-Br | O | 9.3 ± 0.4 | 40 | 3-Br | O | 6.9 ± 0.4 |
| 20 | 3-Br | S | 4.9 ± 0.7 | 41 | 3-Br | S | 4.7 ± 0.1 |
| 21 | 4-Br | O | 8.5 ± 0.5 | 42 | 4-Br | O | 6.8 ± 0.6 |
| I | Boc-Gly-Heterocycle | | Inactive | II | Boc-Pro-Heterocycle | | Inactive |
| | Heterocycle | | Inactive | | Rutin (Std) | | 41.9 ± 0.7 |

[a] Values are mean of three determinations, the ranges of which are <5% of the mean in all cases.

was found to be crucial to improve the activity. This study extends the knowledge of different substituents at phenyl ring and also various amino acids which might be of interest for the identification of more antiglycation agents.

## 4. Experimental

### 4.1. Materials

Boc-amino acids, EDCI, HOBt and TFA were purchased from Advanced Chem. Tech. (Louisville, Kentucky, USA). NMM and substituted phenyl isocyanates/isothiocyanates were purchased from Sigma–Aldrich Co., USA. All solvents and reagents used for the synthesis were of analytical grade. Silica gel for TLC was purchased from Sisco Research Laboratories Pvt. Ltd. (Mumbai, India). Progress of the reaction was monitored by TLC using silica gel coated on glass plates with the solvent system comprising chloroform/methanol/acetic acid in the ratio 98:2:3 ($R_f^a$) and 95:5:3 ($R_f^b$) and the compounds on TLC plates were detected by iodine vapours. Melting points were determined on Superfit (India) melting point apparatus and are uncorrected. Elemental analysis was performed by using VARIO EL III CHNS Elementar. $^1$H NMR spectra were obtained on VARIAN 400 MHz instrument using DMSO-$d_6$ and the chemical shifts are reported as parts per million ($\delta$ ppm) using TMS as an internal standard. Electrospray ionization mass spectrometry (ESI-MS) was performed on a Bruker electrospray mass spectrometer. FT-IR was performed in Jasco spectrometer. Bovine serum albumin (BSA) was purchased from Research Organics, Cleveland, while other chemicals used for inhibition studies were purchased from Sigma Aldrich.

### 4.2. General procedure for the conjugation of Boc-Gly/Pro-OH to heterocycle (*I* and *II*)

Boc-Gly/Pro-OH (0.002 mol) and HOBt (0.306 g, 0.002 mol) dissolved in DMF (10 mL/g of compound) and cooled to 0 °C was added NMM (0.22 mL, 0.002 mol). EDCI (0.458 g, 0.002 mol) was added under stirring while maintaining the temperature at 0 °C and stirred for 15 min. To this a pre-cooled solution of [3-(4-piperidinyl)-6-fluoro-1,2-benzisoxazole]·HCl (0.514 g, 0.002 mol) and NMM (0.22 mL, 0.002 mol) in DMF (5 mL) was added slowly. After 20 min, pH of the solution was adjusted to 8 by the addition of NMM and the reaction mixture was stirred overnight at room temperature. DMF was removed under reduced pressure and the residue was poured into about 100 mL ice-cold 90% saturated $KHCO_3$ solution and stirred for 30 min. The precipitated product was taken into $CHCl_3$ and washed sequentially with 5% $NaHCO_3$ solution (2 × 20 mL), water (2 × 20 mL), 0.1 N cold HCl solution (2 × 20 mL) and finally brine solution (2 × 20 mL). The $CHCl_3$ layer was dried over anhydrous $Na_2SO_4$ and the solvent was removed under reduced pressure. The products so obtained were recrystallized from ether/petroleum ether to get desired products, **I** and **II**.

### 4.3. General procedure for the synthesis of ureido and thioureido derivatives *1–42*

Boc-Gly/Pro-Heterocycle (0.150 g) was deprotected by stirring in TFA (1.5 mL) for 45 min. TFA was removed under reduced pressure, triturated with ether, filtered, washed with ether and dried to obtain TFA·H-Gly/Pro-Heterocycle (100%).

A solution of TFA·H-Gly/Pro-Heterocycle (0.001 mol) in DMF (10 mL/g of compound) was cooled to 0 °C and added NMM

(0.10 mL, 0.001 mol). To this solution respective substituted phenyl isocyanates/isothiocyanates (0.0012 mol) was added dropwise while maintaining the temperature at 0 °C. The reaction mixture was stirred for 8 h slowly warming to room temperature. DMF was evaporated under high vacuum and the residue was poured into about 50 mL ice-cold 90% saturated $KHCO_3$ solution and stirred for 15 min. The precipitated compound was extracted with ethyl acetate and washed with 5% $NaHCO_3$ solution (2 × 10 mL), water (2 × 10 mL), 0.1 N HCl (2 × 10 mL) followed by brine solution. The organic layer was dried over anhydrous $Na_2SO_4$ and the solvent was removed under reduced pressure, triturated with hexane, filtered and dried.

*4.3.1. tert-Butyl 2-(4-(6-fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)-2-oxoethylcarbamate (**I**)*

$R_f^a = 0.46$; $R_f^b = 0.63$; Yield = 85%; M.P. = Gum; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1629 (CO), 3275 (NH); $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm): Boc = 1.43 (9H, s); Gly = 4.27 (2H, d, $-^{\alpha}CH_2$), 8.01–8.05 (1H, t, NH); Heterocycle = 1.67–2.08 (4H, m, $-CH_2$), 2.08–2.92 (1H, m, −CH), 3.18–3.56 (4H, m, $-CH_2$), 7.25–7.67 (3H, m, Ar-H); Calculated HRMS = 377.4090; Found HRMS = 400.1532 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{19}H_{24}FN_3O_4$: C: 60.47; H: 6.41; N: 11.13; Found: C: 60.42; H: 6.38; N: 11.16.

*4.3.2. tert-Butyl 2-(4-(6-fluorobenzo[d]isoxazol-3-yl)piperidin-1-carbonyl)pyrrolidine-1-carboxylate (**II**)*

$R_f^a = 0.47$; $R_f^b = 0.65$; Yield = 82%; M.P. = 140 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1625 (CO), 3273 (NH); $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm): Boc = 1.43 (9H, s); Pro = 4.07 (1H, t, $-^{\alpha}CH$), 1.84–1.89 (2H, m, $-^{\beta,\gamma}CH_2$), 3.71–3.75 (2H, m, $-^{\delta}CH_2$); Heterocycle = 1.84–1.89 (4H, m, $-CH_2$), 2.5 (1H, m, −CH), 3.22–3.61 (4H, m, $-CH_2$), 7.02–7.62 (3H, m, Ar-H); Calculated HRMS = 417.4727; Found HRMS = 440.2054 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{22}H_{28}FN_3O_4$: C: 63.29; H: 6.76; N: 10.07; Found: C: 63.24; H: 6.79; N: 10.03.

*4.3.3. 1-(2-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)-2-oxoethyl)-3-phenylurea (**1**)*

$R_f^a = 0.47$; $R_f^b = 0.68$; Yield = 89%; M.P. = 160 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1622 (CO), 3283 (NH); $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm): Urea = 6.54–7.46 (5H, m, Ar-H), 8.19 (1H, s, −NH), 8.08 (1H, m, −NH); Gly = 4.45 (2H, d, $-^{\alpha}CH_2$); Heterocycle = 1.64–2.07 (4H, m, $-CH_2$), 2.93 (1H, m, −CH), 3.21–3.52 (4H, m, $-CH_2$), 6.54–7.46 (3H, m, Ar-H); Calculated HRMS = 397.4219; Found HRMS = 419.1432 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{21}H_{21}FN_4O_3$: C: 63.63; H: 5.34; N: 14.13; Found: C: 63.58; H: 5.41; N: 14.17.

*4.3.4. 1-(2-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)-2-oxoethyl)-3-phenylthiourea (**2**)*

$R_f^a = 0.46$; $R_f^b = 0.66$; Yield = 85%; M.P. = 165 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1628 (CO), 2036 (CS), 3277 (NH); $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm): Thiourea = 6.73–7.68 (5H, m, Ar-H), 9.92 (1H, s, −NH), 9.87 (1H, s, −NH); Gly = 4.44 (2H, d, $-^{\alpha}CH_2$); Heterocycle = 1.69–2.27 (4H, m, $-CH_2$), 2.94 (1H, m, −CH), 3.13–3.47 (4H, m, $-CH_2$), 6.73–7.68 (3H, m, Ar-H); Calculated HRMS = 412.4776; Found HRMS = 435.1398 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{21}H_{21}FN_4O_2S$: C: 61.15; H: 5.13; N: 13.58; S: 7.77; Found: C: 61.21; H: 5.18; N: 13.51; S: 7.69.

*4.3.5. 1-(2-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)-2-oxoethyl)-3-(2-methoxy phenyl)urea (**3**)*

$R_f^a = 0.47$; $R_f^b = 0.69$; Yield = 90%; M.P. = 65 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1617 (CO), 3293 (NH); $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm): Urea = 6.62–7.42 (5H, m, Ar-H), 7.98 (1H, s, −NH), 7.03–7.09 (1H, m, −NH), 3.79 (3H, s, $OCH_3$); Gly = 4.42–4.44 (2H, d, $-^{\alpha}CH_2$); Heterocycle = 1.67–2.09 (4H, m, $-CH_2$), 2.92 (1H, m, −CH), 3.21–3.53 (4H, m, $-CH_2$), 6.62–7.42 (3H, m, Ar-H); Calculated HRMS = 426.4379; Found HRMS = 449.1775 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{22}H_{23}FN_4O_4$: C: 61.96; H: 5.44; N: 13.14; Found: C: 61.93; H: 5.42; N: 13.10.

*4.3.6. 1-(2-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)-2-oxoethyl)-3-(2 methoxyphenyl)thiourea (**4**)*

$R_f^a = 0.46$; $R_f^b = 0.68$; Yield = 89%; M.P. = 100 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1644 (CO), 2032 (CS), 3277 (NH); $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm): Thiourea = 6.82–7.64 (4H, m, Ar-H), 8.00 (1H, s, −NH), 7.06–7.10 (1H, m, −NH), 3.81 (3H, s, $-OCH_3$); Gly = 4.47–4.48 (2H, d, $-^{\alpha}CH_2$); Heterocycle = 1.88–2.20 (4H, m, $-CH_2$), 2.29–3.04 (1H, m, −CH), 3.28–3.95 (4H, m, $-CH_2$), 6.82–7.64 (3H, m, Ar-H); Calculated HRMS = 442.5055; Found HRMS = 465.1528 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{22}H_{23}FN_4O_3S$: C: 59.71; H: 5.24; N: 12.66; S: 7.25; Found: C: 59.75; H: 5.21; N: 12.62; S: 7.21.

*4.3.7. 1-(2-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)-2-oxoethyl)-3-(3-methoxyphenyl)urea (**5**)*

$R_f^a = 0.43$; $R_f^b = 0.66$; Yield = 90%; M.P. = 143 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1636 (CO), 3305 (NH); $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm): Urea = 6.54–7.46 (4H, m, Ar-H), 7.96 (1H, s, −NH), 7.02–7.08 (1H, m, −NH), 3.78 (3H, s, $-OCH_3$); Gly = 4.43–4.46 (2H, d, $-^{\alpha}CH_2$); Heterocycle = 1.67–2.09 (4H, m, $-CH_2$), 2.92 (1H, m, −CH), 3.19–3.51 (4H, m, $-CH_2$), 6.54–7.46 (3H, m, Ar-H); Calculated HRMS = 426.4379; Found HRMS = 449.1782 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{22}H_{23}FN_4O_4$: C: 61.96; H: 5.44; N: 13.14; Found: C: 61.91; H: 5.46; N: 13.13.

*4.3.8. 1-(2-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)-2-oxoethyl)-3-(3-methoxyphenyl)thiourea (**6**)*

$R_f^a = 0.45$; $R_f^b = 0.67$; Yield = 92%; M.P. = 160 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1627 (CO), 2040 (CS), 3272 (NH); $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm): Thiourea = 6.71–7.64 (4H, m, Ar-H), 8.04 (1H, s, −NH), 7.09–7.15 (1H, m, −NH), 3.82 (3H, s, $-OCH_3$); Gly = 4.41–4.43 (2H, d, $-^{\alpha}CH_2$); Heterocycle = 1.66–2.23 (4H, m, $-CH_2$), 2.91 (1H, m, −CH), 3.12–3.46 (4H, m, $-CH_2$), 6.71–7.64 (3H, m, Ar-H); Calculated HRMS = 442.5055; Found HRMS = 465.1536 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{22}H_{23}FN_4O_3S$: C: 59.71; H: 5.24; N: 12.66; S: 7.25; Found: C: 59.74; H: 5.21; N: 12.61; S: 7.29.

*4.3.9. 1-(2-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)-2-oxoethyl)-3-(4-methoxyphenyl)urea (**7**)*

$R_f^a = 0.45$; $R_f^b = 0.67$; Yield = 89%; M.P. = 147–150 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1638 (CO), 3304 (NH); $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm): Urea = 6.69–7.35 (4H, m, Ar-H), 7.98 (1H, s, −NH), 7.01–7.07 (1H, m, −NH), 3.81 (3H, s, $-OCH_3$); Gly = 4.39–4.42 (2H, d, $-^{\alpha}CH_2$); Heterocycle = 1.67–2.09 (4H, m, $-CH_2$), 2.92 (1H, m, −CH), 3.22–3.53 (4H, m, $-CH_2$), 6.69–7.35 (3H, m, Ar-H); Calculated HRMS = 426.4379; Found HRMS = 449.1763 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{22}H_{23}FN_4O_4$: C: 61.96; H: 5.44; N: 13.14; Found: C: 61.85; H: 5.40; N: 13.17.

*4.3.10. 1-(2-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)-2-oxoethyl)-3-(4-methoxyphenyl)thiourea (**8**)*

$R_f^a = 0.43$; $R_f^b = 0.66$; Yield = 91%; M.P. = 123 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1625 (CO), 2041 (CS), 3275 (NH); $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm): Thiourea = 6.79–7.61 (4H, m, Ar-H), 8.04 (1H, s, −NH), 7.04–7.12 (1H, m, −NH), 3.83 (3H, s, $-OCH_3$); Gly = 4.41–4.43 (2H, d, $-^{\alpha}CH_2$); Heterocycle = 1.66–2.23 (4H, m, $-CH_2$), 2.91 (1H, m, −CH), 3.11–3.48 (4H, m, $-CH_2$), 6.79–7.61 (3H, m, Ar-H); Calculated HRMS = 442.5055; Found HRMS = 465.1565 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{22}H_{23}FN_4O_3S$: C: 59.71; H: 5.24; N: 12.66; S: 7.25; Found: C: 59.77; H: 5.20; N: 12.60; S: 7.28.

*4.3.11. 1-(2-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)-2-oxoethyl)-3-(2-fluorophenyl)urea (**9**)*

$R_f^a = 0.38$; $R_f^b = 0.53$; Yield = 90%; M.P. = 195 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1617 (CO), 3299 (NH); $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm): Urea = 7.04–7.71 (4H, m, Ar-H), 8.91 (1H, s, –NH), 8.09 (1H, m, –NH); Gly = 4.41 (2H, d, $-^{\alpha}CH_2$); Heterocycle = 1.67–2.09 (4H, m, $-CH_2$), 2.92 (1H, m, –CH), 3.21–3.47 (4H, m, $-CH_2$), 7.04–7.71 (3H, m, Ar-H); Calculated HRMS = 414.4045; Found HRMS = 437.1525 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{21}H_{20}F_2N_4O_3$: C: 60.86; H: 4.86; N: 13.52; Found: C: 60.81; H: 4.82; N: 13.56.

*4.3.12. 1-(2-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)-2-oxoethyl)-3-(2-fluorophenyl)thiourea (**10**)*

$R_f^a = 0.45$; $R_f^b = 0.67$; Yield = 88%; M.P. = 158 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1627 (CO), 2028 (CS), 3276 (NH); $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm): Thiourea = 7.19–8.21 (4H, m, Ar-H), 10.25 (1H, s, –NH), 9.91 (1H, m, –NH); Gly = 4.48 (2H, d, $-^{\alpha}CH_2$); Heterocycle = 1.66–2.23 (4H, m, $-CH_2$), 2.91 (1H, m, –CH), 3.09–3.51 (4H, m, $-CH_2$), 7.19–8.21 (3H, m, Ar-H); Calculated HRMS = 430.4701; Found HRMS = 453.1365 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{21}H_{20}F_2N_4O_2S$: C: 58.59; H: 4.68; N: 13.02; S: 7.45; Found: C: 58.53; H: 4.61; N: 13.11; S: 7.49.

*4.3.13. 1-(2-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)-2-oxoethyl)-3-(3-fluorophenyl)urea (**11**)*

$R_f^a = 0.46$; $R_f^b = 0.68$; Yield = 89%; M.P. = 105 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1623 (CO), 3272 (NH); $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm): Urea = 7.04–7.73 (4H, m, Ar-H), 8.85 (1H, s, –NH), 8.15 (1H, m, –NH); Gly = 4.36 (2H, d, $-^{\alpha}CH_2$); Heterocycle = 1.67–2.09 (4H, m, $-CH_2$), 2.92 (1H, m, –CH), 3.22–3.49 (4H, m, $-CH_2$), 7.04–7.73 (3H, m, Ar-H); Calculated HRMS = 414.4045; Found HRMS = 437.1536 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{21}H_{20}F_2N_4O_3$: C: 60.86; H: 4.86; N: 13.52; Found: C: 60.89; H: 4.80; N: 13.49.

*4.3.14. 1-(2-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)-2-oxoethyl)-3-(4-fluorophenyl)urea (**12**)*

$R_f^a = 0.43$; $R_f^b = 0.66$; Yield = 91%; M.P. = 110 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1629 (CO), 3279 (NH); $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm): Urea = 7.04–7.67 (4H, m, Ar-H), 8.95 (1H, s, –NH), 8.05 (1H, m, –NH); Gly = 4.42 (2H, d, $-^{\alpha}CH_2$); Heterocycle = 1.67–2.09 (4H, m, $-CH_2$), 2.92 (1H, m, –CH), 3.23–3.51 (4H, m, $-CH_2$), 7.04–7.67 (3H, m, Ar-H); Calculated HRMS = 414.4045; Found HRMS = 437.0951 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{21}H_{20}F_2N_4O_3$: C: 60.86; H: 4.86; N: 13.52; Found: C: 60.79; H: 4.82; N: 13.61.

*4.3.15. 1-(2-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)-2-oxoethyl)-3-(4-fluorophenyl)thiourea (**13**)*

$R_f^a = 0.49$; $R_f^b = 0.69$; Yield = 85%; M.P. = 145 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1639 (CO), 2032 (CS), 3285 (NH); $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm): Thiourea = 7.14–8.05 (4H, s, Ar-H), 10.36 (1H, s, –NH), 9.95 (1H, m, –NH); Gly = 4.41 (2H, d, $-^{\alpha}CH_2$); Heterocycle = 1.66–2.23 (4H, m, $-CH_2$), 2.91 (1H, m, –CH), 3.13–3.49 (4H, m, $-CH_2$), 7.14–8.05 (3H, m, Ar-H); Calculated HRMS = 430.4701; Found HRMS = 453.0305 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{21}H_{20}F_2N_4O_2S$: C: 58.59; H: 4.68; N: 13.02; S: 7.45; Found: C: 58.51; H: 4.59; N: 13.08; S: 7.53.

*4.3.16. 1-(3-Chlorophenyl)-3-(2-(4-(6-fluorobenzo[d]isoxazol-3-yl) piperidin-1-yl)-2-oxoethyl)urea (**14**)*

$R_f^a = 0.42$; $R_f^b = 0.65$; Yield = 82%; M.P. = 93 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1634 (CO), 3291 (NH); $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm): Urea = 6.72–7.58 (4H, m, Ar-H), 8.95 (1H, s, –NH), 8.03 (1H, m, –NH); Gly = 4.42 (2H, d, $-^{\alpha}CH_2$); Heterocycle = 1.67–2.09 (4H, m, $-CH_2$), 2.92 (1H, m, –CH), 3.19–3.50 (4H, m, $-CH_2$), 6.72–7.58 (3H, m, Ar-H); Calculated HRMS = 430.8591; Found HRMS = 453.1291 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{21}H_{20}ClFN_4O_3$: C: 58.54; H: 4.68; N: 13.00; Found: C: 58.46; H: 4.61; N: 13.07.

*4.3.17. 1-(3-Chlorophenyl)-3-(2-(4-(6-fluorobenzo[d]isoxazol-3-yl) piperidin-1-yl)-2-oxoethyl)thiourea (**15**)*

$R_f^a = 0.46$; $R_f^b = 0.67$; Yield = 87%; M.P. = 147 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1615 (CO), 2034 (CS), 3286 (NH); $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm): Thiourea = 6.92–7.95 (4H, m, Ar-H), 10.28 (1H, s, –NH), 9.91 (1H, m, –NH); Gly = 4.41 (2H, d, $-^{\alpha}CH_2$); Heterocycle = 1.66–2.23 (4H, m, $-CH_2$), 2.91 (1H, m, –CH), 3.09–3.45 (4H, m, $-CH_2$), 6.92–7.95 (3H, m, Ar-H); Calculated HRMS = 446.9247; Found HRMS = 469.1021 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{21}H_{20}ClFN_4O_2S$: C: 56.44; H: 4.51; N: 12.54; S: 7.17; Found: C: 56.38; H: 4.56; N: 12.49; S: 7.23.

*4.3.18. 1-(4-Chlorophenyl)-3-(2-(4-(6-fluorobenzo[d]isoxazol-3-yl) piperidin-1-yl)-2-oxoethyl)urea (**16**)*

$R_f^a = 0.43$; $R_f^b = 0.63$; Yield = 88%; M.P. = 88–90 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1636 (CO), 3294 (NH); $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm): Urea = 6.76–7.63 (4H, m, Ar-H), 8.89 (1H, s, –NH), 8.07 (1H, m, –NH); Gly = 4.47 (2H, d, $-^{\alpha}CH_2$); Heterocycle = 1.67–2.09 (4H, m, $-CH_2$), 2.92 (1H, m, –CH), 3.20–3.50 (4H, m, $-CH_2$), 6.76–7.63 (3H, m, Ar-H); Calculated HRMS = 430.8591; Found HRMS = 453.1258 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{21}H_{20}ClFN_4O_3$: C: 58.54; H: 4.68; N: 13.00; Found: C: 58.61; H: 4.63; N: 13.09.

*4.3.19. 1-(4-Chlorophenyl)-3-(2-(4-(6-fluorobenzo[d]isoxazol-3-yl) piperidin-1-yl)-2-oxoethyl)thiourea (**17**)*

$R_f^a = 0.43$; $R_f^b = 0.62$; Yield = 82%; M.P. = 125 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1618 (CO), 2039 (CS), 3280 (NH); $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm): Thiourea = 6.97–7.83 (4H, m, Ar-H), 10.31 (1H, s, –NH), 9.85 (1H, m, –NH); Gly = 4.46 (2H, d, $-^{\alpha}CH_2$); Heterocycle = 1.66–2.23 (4H, m, $-CH_2$), 2.91 (1H, m, –CH), 3.10–3.45 (4H, m, $-CH_2$), 6.97–7.83 (3H, m, Ar-H); Calculated HRMS = 446.9247; Found HRMS = 469.1041 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{21}H_{20}ClFN_4O_2S$: C: 56.44; H: 4.51; N: 12.54; S: 7.17; Found: C: 56.49; H: 4.46; N: 12.58; S: 7.27.

*4.3.20. 1-(2-Bromophenyl)-3-(2-(4-(6-fluorobenzo[d]isoxazol-3-yl) piperidin-1-yl)-2-oxoethyl)thiourea (**18**)*

$R_f^a = 0.45$; $R_f^b = 0.65$; Yield = 86%; M.P. = 145 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1634 (CO), 2043 (CS), 3276 (NH); $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm): Thiourea = 6.92–7.95 (4H, m, Ar-H), 10.28 (1H, s, –NH), 9.91 (1H, m, –NH); Gly = 4.41 (2H, d, $-^{\alpha}CH_2$); Heterocycle = 1.66–2.23 (4H, m, $-CH_2$), 2.91 (1H, m, –CH), 3.10–3.45 (4H, m, $-CH_2$), 6.92–7.95 (3H, m, Ar-H); Calculated HRMS = 491.3757; Found HRMS = 513.0498, 515.0611 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{21}H_{20}BrFN_4O_2S$: C: 51.33; H: 4.10; N: 11.40; S: 6.53; Found: C: 51.39; H: 4.17; N: 11.46; S: 6.59.

*4.3.21. 1-(3-Bromophenyl)-3-(2-(4-(6-fluorobenzo[d]isoxazol-3-yl) piperidin-1-yl)-2-oxoethyl)urea (**19**)*

$R_f^a = 0.44$; $R_f^b = 0.63$; Yield = 87%; M.P. = 134 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1633 (CO), 3271 (NH); $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm): Urea = 6.74–7.61 (4H, m, Ar-H), 8.45 (1H, s, –NH), 8.07 (1H, m, –NH); Gly = 4.41 (2H, d, $-^{\alpha}CH_2$); Heterocycle = 1.63–2.23 (4H, m, $-CH_2$), 2.95 (1H, m, –CH), 3.21–3.54 (4H, m, $-CH_2$), 6.74–7.61 (3H, m, Ar-H); Calculated HRMS = 475.3101; Found HRMS = 497.0681, 499.0732 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{21}H_{20}BrFN_4O_3$: C: 53.07; H: 4.24; N: 11.79; Found: C: 53.01; H: 4.29; N: 11.83.

*4.3.22. 1-(3-Bromophenyl)-3-(2-(4-(6-fluorobenzo[d]isoxazol-3-yl) piperidin-1-yl)-2-oxoethyl)thiourea (**20**)*

$R_f^a = 0.46$; $R_f^b = 0.67$; Yield = 91%; M.P. = 140 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1625 (CO), 2031 (CS), 3268 (NH); $^1H$ NMR (DMSO-$d_6$,

δ ppm): Urea = 6.75–7.54 (4H, m, Ar-H), 8.85 (1H, s, –NH), 8.09 (1H, m, –NH); Gly = 4.39 (2H, d, $-^{\alpha}CH_2$); Heterocycle = 1.64–2.03 (4H, m, $-CH_2$), 2.96 (1H, m, –CH), 3.24–3.55 (4H, m, $-CH_2$), 6.75–7.54 (3H, m, Ar-H); Calculated HRMS = 491.3757; Found HRMS = 513.0498, 515.0611 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{21}H_{20}BrFN_4O_2S$: C: 51.33; H: 4.10; N: 11.40; S: 6.53; Found: C: 51.39; H: 4.04; N: 11.46; S: 6.58.

#### 4.3.23. 1-(4-Bromophenyl)-3-(2-(4-(6-fluorobenzo[d]isoxazol-3-yl) piperidin-1-yl)-2-oxoethyl)urea (**21**)

$R_f^a$ = 0.44; $R_f^b$ = 0.63; Yield = 88%; M.P. = 118 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1629 (CO), 3276 (NH); $^1H$ NMR (DMSO-$d_6$, δ ppm): Urea = 6.81–7.78 (5H, m, Ar-H), 8.79 (1H, s, –NH), 8.17 (1H, m, –NH); Gly = 4.36 (2H, d, $-^{\alpha}CH_2$); Heterocycle = 1.67–2.09 (4H, m, $-CH_2$), 2.89 (1H, m, –CH), 3.24–3.53 (4H, m, $-CH_2$), 6.81–7.78 (3H, m, Ar-H); Calculated HRMS = 475.3101; Found HRMS = 497.0672, 499.0742 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{21}H_{20}BrFN_4O_3$: C: 53.07; H: 4.24; N: 11.79; Found: C: 53.02; H: 4.29; N: 11.83.

#### 4.3.24. 2-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidine-1-carbonyl)-N-phenylpyrrolidine-1-carboxamide (**22**)

$R_f^a$ = 0.47; $R_f^b$ = 0.68; Yield = 83%; M.P. = 126 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1629 (CO), 3284 (NH); $^1H$ NMR (DMSO-$d_6$, δ ppm): Urea = 6.86–7.41 (5H, m, Ar-H), 7.89 (1H, s, –NH); Pro = 4.31–4.44 (1H, t, $-^{\alpha}CH$), 1.78–2.21 (4H, m, $-^{\beta,\ \gamma}CH_2$), 3.75–3.78 (2H, m, $-^{\delta}CH_2$); Heterocycle = 1.98–2.19 (4H, m, $-CH_2$), 2.58 (1H, m, –CH), 3.23–3.57 (4H, m, $-CH_2$), 6.86–7.41 (3H, m, Ar-H); Calculated HRMS = 436.4777; Found HRMS = 459.1872 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{24}H_{25}FN_4O_3$: C: 66.04; H: 5.77; N: 12.84; Found: C: 66.09; H: 5.71; N: 12.89.

#### 4.3.25. 2-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidine-1-carbonyl)-N-phenylpyrrolidine-1-carbothioamide (**23**)

$R_f^a$ = 0.44; $R_f^b$ = 0.65; Yield = 86%; M.P. = 137 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1623 (CO), 2039 (CS), 3281 (NH); $^1H$ NMR (DMSO-$d_6$, δ ppm): Thiourea = 6.71–7.63 (5H, m, Ar-H), 7.87 (1H, s, –NH); Pro = 4.19 (1H, t, $-^{\alpha}CH$), 1.91–2.26 (4H, m, $-^{\beta,\ \gamma}CH_2$), 3.74–3.85 (2H, m, $-^{\delta}CH_2$); Heterocycle = 2.07–2.26 (4H, m, $-CH_2$), 2.82 (1H, m, –CH), 3.33–3.47 (4H, m, $-CH_2$), 6.71–7.63 (3H, m, Ar-H); Calculated HRMS = 452.5433; Found HRMS = 475.1605 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{24}H_{25}FN_4O_2S$: C: 63.70; H: 5.57; N: 12.38; S: 7.09; Found: C: 63.77; H: 5.63; N: 12.44; S: 7.14.

#### 4.3.26. 2-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidine-1-carbonyl)-N-(2-methoxyphenyl)pyrrolidine-1-carboxamide (**24**)

$R_f^a$ = 0.47; $R_f^b$ = 0.68; Yield = 92%; M.P. = 68 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1634 (CO), 3296 (NH); $^1H$ NMR (DMSO-$d_6$, δ ppm): Urea = 6.94–7.35 (4H, m, Ar-H), 7.86 (1H, s, –NH), 3.77 (3H, s, $-OCH_3$); Pro = 4.30–4.40 (1H, t, $-^{\alpha}CH$), 1.74–2.17 (4H, m, $-^{\beta,\ \gamma}CH_2$), 3.71–3.72 (2H, m, $-^{\delta}CH_2$); Heterocycle = 1.96–2.16 (4H, m, $-CH_2$), 2.55 (1H, m, –CH), 3.21–3.57 (4H, m, $-CH_2$), 6.94–7.35 (3H, m, Ar-H); Calculated HRMS = 466.5036; Found HRMS = 489.1947 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{25}H_{27}FN_4O_4$: C: 64.37; H: 5.83; N: 12.01; Found: C: 64.31; H: 5.89; N: 12.08.

#### 4.3.27. 2-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidine-1-carbonyl)-N-(2-methoxyphenyl)pyrrolidine-1-carbothioamide (**25**)

$R_f^a$ = 0.49; $R_f^b$ = 0.71; Yield = 86%; M.P. = 104–107 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1644 (CO), 2031 (CS), 3294 (NH); $^1H$ NMR (DMSO-$d_6$, δ ppm): Thiourea = 6.63–7.58 (4H, m, Ar-H), 7.92 (1H, s, –NH), 3.83 (3H, s, $-OCH_3$); Pro = 4.16 (1H, t, $-^{\alpha}CH$), 1.95–2.21 (4H, m, $-^{\beta,\ \gamma}CH_2$), 3.77–3.82 (2H, m, $-^{\delta}CH_2$); Heterocycle = 2.04–2.21 (4H, m, $-CH_2$), 2.84 (1H, m, –CH), 3.30–3.41 (4H, m, $-CH_2$), 6.63–7.58 (3H, m, Ar-H); Calculated HRMS = 482.5692; Found HRMS = 505.1702 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{25}H_{27}FN_4O_3S$: C: 62.22; H: 5.64; N: 11.61; S: 6.64; Found: C: 62.13; H: 5.69; N: 11.67; S: 6.58.

#### 4.3.28. 2-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidine-1-carbonyl)-N-(3-methoxyphenyl)pyrrolidine-1-carboxamide (**26**)

$R_f^a$ = 0.43; $R_f^b$ = 0.67; Yield = 89%; M.P. = 80 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1631 (CO), 3319 (NH); $^1H$ NMR (DMSO-$d_6$, δ ppm): Urea = 6.86–7.46 (4H, m, Ar-H), 7.87 (1H, s, –NH), 3.76 (3H, s, $-OCH_3$); Pro = 4.32–4.44 (1H, t, $-^{\alpha}CH$), 1.78–2.20 (4H, m, $-^{\beta,\ \gamma}CH_2$), 3.75–3.79 (2H, m, $-^{\delta}CH_2$); Heterocycle = 1.96–2.16 (4H, m, $-CH_2$), 2.55 (1H, m, –CH), 3.16–3.53 (4H, m, $-CH_2$), 6.86–7.46 (3H, m, Ar-H); Calculated HRMS = 466.5036; Found HRMS = 489.1856 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{25}H_{27}FN_4O_4$: C: 64.37; H: 5.83; N: 12.01; Found: C: 64.29; H: 5.87; N: 12.05.

#### 4.3.29. 2-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidine-1-carbonyl)-N-(3-methoxyphenyl)pyrrolidine-1-carbothioamide (**27**)

$R_f^a$ = 0.45; $R_f^b$ = 0.65; Yield = 88%; M.P. = 140 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1626 (CO), 2034 (CS), 3285 (NH); $^1H$ NMR (DMSO-$d_6$, δ ppm): Thiourea = 6.69–7.61 (4H, m, Ar-H), 7.95 (1H, s, –NH), 3.82 (3H, s, $-OCH_3$); Pro = 4.16 (1H, t, $-^{\alpha}CH$), 1.95–2.21 (4H, m, $-^{\beta,\ \gamma}CH_2$), 3.70–3.73 (2H, m, $-^{\delta}CH_2$); Heterocycle = 2.04–2.21 (4H, m, $-CH_2$), 2.84 (1H, m, –CH), 3.30–3.41 (4H, m, $-CH_2$), 6.69–7.61 (3H, m, Ar-H); Calculated HRMS = 482.5692; Found HRMS = 505.1756 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{25}H_{27}FN_4O_3S$: C: 62.22; H: 5.64; N: 11.61; S: 6.64; Found: C: 62.14; H: 5.73; N: 11.65; S: 6.56.

#### 4.3.30. 2-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidine-1-carbonyl)-N-(4-methoxyphenyl)pyrrolidine-1-carboxamide (**28**)

$R_f^a$ = 0.45; $R_f^b$ = 0.66; Yield = 84%; M.P. = 163 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1638 (CO), 3304 (NH); $^1H$ NMR (DMSO-$d_6$, δ ppm): Urea = 6.81–7.31 (4H, m, Ar-H), 7.86 (1H, s, –NH), 3.77 (3H, s, $-OCH_3$); Pro = 4.35–4.47 (1H, t, $-^{\alpha}CH$), 1.76–2.16 (4H, m, $-^{\beta,\ \gamma}CH_2$), 3.71–3.76 (2H, m, $-^{\delta}CH_2$); Heterocycle = 1.96–2.16 (4H, m, $-CH_2$), 2.55 (1H, m, –CH), 3.16–3.54 (4H, m, $-CH_2$), 6.81–7.31 (3H, m, Ar-H); Calculated HRMS = 466.5036; Found HRMS = 489.1874 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{25}H_{27}FN_4O_4$: C: 64.37; H: 5.83; N: 12.01; Found: C: 64.42; H: 5.91; N: 12.13.

#### 4.3.31. 2-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidine-1-carbonyl)-N-(4-methoxyphenyl)pyrrolidine-1-carbothioamide (**29**)

$R_f^a$ = 0.45; $R_f^b$ = 0.68; Yield = 90%; M.P. = 163 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1625 (CO), 2041 (CS), 3275 (NH); $^1H$ NMR (DMSO-$d_6$, δ ppm): Thiourea = 6.73–7.68 (4H, m, Ar-H), 7.89 (1H, s, –NH), 3.84 (3H, s, $-OCH_3$); Pro = 4.26 (1H, t, $-^{\alpha}CH$), 1.95–2.21 (4H, m, $-^{\beta,\ \gamma}CH_2$), 3.77–3.83 (2H, m, $-^{\delta}CH_2$); Heterocycle = 2.04–2.21 (4H, m, $-CH_2$), 2.84 (1H, m, –CH), 3.30–3.41 (4H, m, $-CH_2$), 6.73–7.68 (3H, m, Ar-H); Calculated HRMS = 482.5692; Found HRMS = 505.1707 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{25}H_{27}FN_4O_3S$: C: 62.22; H: 5.64; N: 11.61; S: 6.64; Found: C: 62.24; H: 5.68; N: 11.67; S: 6.54.

#### 4.3.32. 2-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidine-1-carbonyl)-N-(2-fluorophenyl)pyrrolidine-1-carboxamide (**30**)

$R_f^a$ = 0.47; $R_f^b$ = 0.68; Yield = 89%; M.P. = 138 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1627 (CO), 3278 (NH); $^1H$ NMR (DMSO-$d_6$, δ ppm): Urea = 6.88–7.35 (4H, m, Ar-H), 7.87 (1H, s, –NH); Pro = 4.28–4.42 (1H, t, $-^{\alpha}CH$), 1.77–2.16 (4H, m, $-^{\beta,\ \gamma}CH_2$), 3.78–3.82 (2H, m, $-^{\delta}CH_2$); Heterocycle = 1.96–2.16 (4H, m, $-CH_2$), 2.55 (1H, m, –CH), 3.18–3.53 (4H, m, $-CH_2$), 6.88–7.35 (3H, m, Ar-H); Calculated HRMS = 454.4682; Found HRMS = 477.1738 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{24}H_{24}F_2N_4O_3$: C: 63.43; H: 5.32; N: 12.33; Found: C: 63.52; H: 5.26; N: 12.42.

*4.3.33. 2-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidine-1-carbonyl)-N-(2-fluorophenyl)pyrrolidine-1-carbothioamide (**31**)*

$R_f^a$ = 0.48; $R_f^b$ = 0.66; Yield = 88%; M.P. = 83 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1641 (CO), 2027 (CS), 3281 (NH); $^1$H NMR (DMSO-$d_6$, $\delta$ ppm): Thiourea = 6.79–7.81 (4H, m, Ar-H), 7.85 (1H, s, –NH); Pro = 4.26 (1H, t, $-^{\alpha}$CH), 1.89–2.21 (4H, m, $-^{\beta,\ \gamma}CH_2$), 3.60–3.73 (2H, m, $-^{\delta}CH_2$); Heterocycle = 2.04–2.21 (4H, m, $-CH_2$), 2.84 (1H, m, –CH), 3.30–3.41 (4H, m, $-CH_2$), 6.79–7.81 (3H, m, Ar-H); Calculated HRMS = 470.5338; Found HRMS = 493.1618 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{24}H_{24}F_2N_4O_2S$: C: 61.26; H: 5.14; N: 11.91; S: 6.81; Found: C: 61.21; H: 5.22; N: 11.77; S: 6.74.

*4.3.34. 2-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidine-1-carbonyl)-N-(3-fluorophenyl)pyrrolidine-1-carboxamide (**32**)*

$R_f^a$ = 0.48; $R_f^b$ = 0.66; Yield = 91%; M.P. = 72 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1629 (CO), 3274 (NH); $^1$H NMR (DMSO-$d_6$, $\delta$ ppm): Urea = 6.88–7.51 (4H, m, Ar-H), 7.91 (1H, s, –NH); Pro = 4.27–4.40 (1H, t, $-^{\alpha}$CH), 1.81–2.16 (4H, m, $-^{\beta,\ \gamma}CH_2$), 3.63–3.71 (2H, m, $-^{\delta}CH_2$); Heterocycle = 1.96–2.16 (4H, m, $-CH_2$), 2.55 (1H, m, –CH), 3.16–3.57 (4H, m, $-CH_2$), 6.88–7.51 (3H, m, Ar-H); Calculated HRMS = 454.4682; Found HRMS = 477.1743 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{24}H_{24}F_2N_4O_3$: C: 63.43; H: 5.32; N: 12.33; Found: C: 63.49; H: 5.22; N: 12.22.

*4.3.35. 2-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidine-1-carbonyl)-N-(4-fluorophenyl)pyrrolidine-1-carboxamide (**33**)*

$R_f^a$ = 0.44; $R_f^b$ = 0.65; Yield = 92%; M.P. = 63 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1631 (CO), 3271 (NH); $^1$H NMR (DMSO-$d_6$, $\delta$ ppm): Urea = 6.98–7.41 (4H, m, Ar-H), 7.81 (1H, s, –NH); Pro = 4.30–4.40 (1H, t, $-^{\alpha}$CH), 1.76–2.16 (4H, m, $-^{\beta,\ \gamma}CH_2$), 3.73–3.76 (2H, m, $-^{\delta}CH_2$); Heterocycle = 1.96–2.16 (4H, m, $-CH_2$), 2.55 (1H, m, –CH), 3.16–3.53 (4H, m, $-CH_2$), 6.98–7.41 (3H, m, Ar-H); Calculated HRMS = 454.4682; Found HRMS = 477.2639 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{24}H_{24}F_2N_4O_3$: C: 63.43; H: 5.32; N: 12.33; Found: C: 63.49; H: 5.22; N: 12.22.

*4.3.36. 2-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidine-1-carbonyl)-N-(4-fluorophenyl)pyrrolidine-1-carbothioamide (**34**)*

$R_f^a$ = 0.47; $R_f^b$ = 0.69; Yield = 89%; M.P. = 167 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1638 (CO), 2023 (CS), 3275 (NH); $^1$H NMR (DMSO-$d_6$, $\delta$ ppm): Thiourea = 6.69–7.61 (4H, m, Ar-H), 7.95 (1H, s, –NH); Pro = 4.16 (1H, t, $-^{\alpha}$CH), 1.95–2.21 (4H, m, $-^{\beta,\ \gamma}CH_2$), 3.70–3.73 (2H, m, $-^{\delta}CH_2$); Heterocycle = 2.04–2.21 (4H, m, $-CH_2$), 2.84 (1H, m, –CH), 3.30–3.41 (4H, m, $-CH_2$), 6.69–7.61 (3H, m, Ar-H); Calculated HRMS = 470.5338; Found HRMS = 493.1546 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{24}H_{24}F_2N_4O_2S$: C: 61.26; H: 5.14; N: 11.91; S: 6.81; Found: C: 61.17; H: 5.26; N: 11.83; S: 6.69.

*4.3.37. N-(3-Chlorophenyl)-2-(4-(6-fluorobenzo[d]isoxazol-3-yl) piperidine-1-carbonyl)pyrrolidine-1-carboxamide (**35**)*

$R_f^a$ = 0.47; $R_f^b$ = 0.65; Yield = 90%; M.P. = 89 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1633 (CO), 3290 (NH); $^1$H NMR (DMSO-$d_6$, $\delta$ ppm): Urea = 6.86–7.47 (4H, m, Ar-H), 7.71 (1H, s, –NH); Pro = 4.25–4.38 (1H, t, $-^{\alpha}$CH), 1.67–2.19 (4H, m, $-^{\beta,\ \gamma}CH_2$), 3.67–3.72 (2H, m, $-^{\delta}CH_2$); Heterocycle = 1.96–2.16 (4H, m, $-CH_2$), 2.55 (1H, m, –CH), 3.17–3.59 (4H, m, $-CH_2$), 6.86–7.47 (3H, m, Ar-H); Calculated HRMS = 470.9228; Found HRMS = 493.8182 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{24}H_{24}ClFN_4O_3$: C: 61.21; H: 5.14; N: 11.90; Found: C: 61.28; H: 5.23; N: 11.82.

*4.3.38. N-(3-Chlorophenyl)-2-(4-(6-fluorobenzo[d]isoxazol-3-yl) piperidine-1-carbonyl)pyrrolidine-1-carbothioamide (**36**)*

$R_f^a$ = 0.46; $R_f^b$ = 0.67; Yield = 88%; M.P. = 150 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1635 (CO), 2019 (CS), 3279 (NH); $^1$H NMR (DMSO-$d_6$, $\delta$ ppm): Thiourea = 6.65–7.56 (4H, m, Ar-H), 7.84 (1H, s, –NH); Pro = 4.19 (1H, t, $-^{\alpha}$CH), 1.89–2.26 (4H, m, $-^{\beta,\ \gamma}CH_2$), 3.69–3.75 (2H, m, $-^{\delta}CH_2$); Heterocycle = 2.04–2.21 (4H, m, $-CH_2$), 2.84 (1H, m, –CH), 3.30–3.41 (4H, m, $-CH_2$), 6.65–7.56 (3H, m, Ar-H); Calculated HRMS = 486.9884; Found HRMS = 509.1287 ($M^+$ + Na); Elem. Anal.: Calcd. For $C_{24}H_{24}ClFN_4O_2S$: C: 59.19; H: 4.97; N: 11.50; S: 6.58; Found: C: 59.11; H: 4.86; N: 11.55; S: 6.51.

*4.3.39. N-(4-Chlorophenyl)-2-(4-(6-fluorobenzo[d]isoxazol-3-yl) piperidine-1-carbonyl)pyrrolidine-1-carboxamide (**37**)*

$R_f^a$ = 0.51; $R_f^b$ = 0.70; Yield = 89%; M.P. = 110 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1633 (CO), 3294 (NH); $^1$H NMR (DMSO-$d_6$, $\delta$ ppm): Urea = 6.91–7.51 (4H, m, Ar-H), 7.86 (1H, s, –NH); Pro = 4.34–4.43 (1H, t, $-^{\alpha}$CH), 1.66–2.15 (4H, m, $-^{\beta,\ \gamma}CH_2$), 3.73–3.76 (2H, m, $-^{\delta}CH_2$); Heterocycle = 1.96–2.16 (4H, m, $-CH_2$), 2.55 (1H, m, –CH), 3.17–3.55 (4H, m, $-CH_2$), 6.91–7.51 (3H, m, Ar-H); Calculated HRMS = 470.9288; Found HRMS = 493.1457 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{24}H_{24}ClFN_4O_3$: C: 61.21; H: 5.14; N: 11.90; Found: C: 61.26; H: 5.09; N: 11.94.

*4.3.40. N-(4-Chlorophenyl)-2-(4-(6-fluorobenzo[d]isoxazol-3-yl) piperidine-1-carbonyl)pyrrolidine-1-carbothioamide (**38**)*

$R_f^a$ = 0.47; $R_f^b$ = 0.68; Yield = 86%; M.P. = 93 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1644 (CO), 2027 (CS), 3282 (NH); $^1$H NMR (DMSO-$d_6$, $\delta$ ppm): Thiourea = 6.64–7.71 (4H, m, Ar-H), 7.89 (1H, s, –NH); Pro = 4.26 (1H, t, $-^{\alpha}$CH), 1.91–2.21 (4H, m, $-^{\beta,\ \gamma}CH_2$), 3.63–3.71 (2H, m, $-^{\delta}CH_2$); Heterocycle = 2.04–2.21 (4H, m, $-CH_2$), 2.84 (1H, m, –CH), 3.30–3.41 (4H, m, $-CH_2$), 6.64–7.71 (3H, m, Ar-H); Calculated HRMS = 486.9884; Found HRMS = 509.1286 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{24}H_{24}ClFN_4O_2S$: C: 59.19; H: 4.97; N: 11.50; S: 6.58; Found: C: 59.07; H: 4.91; N: 11.49; S: 6.69.

*4.3.41. N-(2-Bromophenyl)-2-(4-(6-fluorobenzo[d]isoxazol-3-yl) piperidine-1-carbonyl)pyrrolidine-1-carbothioamide (**39**)*

$R_f^a$ = 0.44; $R_f^b$ = 0.67; Yield = 78%; M.P. = 108 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1625 (CO), 2037 (CS), 3281 (NH); $^1$H NMR (DMSO-$d_6$, $\delta$ ppm): Thiourea = 6.59–7.63 (4H, m, Ar-H), 7.81 (1H, s, –NH); Pro = 4.23 (1H, t, $-^{\alpha}$CH), 1.91–2.29 (4H, m, $-^{\beta,\ \gamma}CH_2$), 3.71–3.83 (2H, m, $-^{\delta}CH_2$); Heterocycle = 2.07–2.24 (4H, m, $-CH_2$), 2.74 (1H, m, –CH), 3.34–3.47 (4H, m, $-CH_2$), 6.55–7.67 (3H, m, Ar-H); Calculated HRMS = 531.4394; Found HRMS = 553.0762, 555.0698 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{24}H_{24}BrFN_4O_2S$: C: 54.24; H: 4.55; N: 10.54; S: 6.03; Found: C: 54.29; H: 4.59; N: 10.47; S: 6.07.

*4.3.42. N-(3-Bromophenyl)-2-(4-(6-fluorobenzo[d]isoxazol-3-yl) piperidine-1-carbonyl)pyrrolidine-1-carboxamide (**40**)*

$R_f^a$ = 0.45; $R_f^b$ = 0.63; Yield = 83%; M.P. = 135 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1629 (CO), 3284 (NH); $^1$H NMR (DMSO-$d_6$, $\delta$ ppm): Urea = 6.83–7.51 (4H, m, Ar-H), 7.74 (1H, s, –NH); Pro = 4.27–4.34 (1H, t, $-^{\alpha}$CH), 1.62–2.23 (4H, m, $-^{\beta,\ \gamma}CH_2$), 3.73–3.81 (2H, m, $-^{\delta}CH_2$); Heterocycle = 1.89–2.21 (4H, m, $-CH_2$), 2.59 (1H, m, –CH), 3.19–3.52 (4H, m, $-CH_2$), 6.83–7.51 (3H, m, Ar-H); Calculated HRMS = 515.3738; Found HRMS = 537.0982, 539.0876 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{24}H_{24}BrFN_4O_3$: C: 55.93; H: 4.69; N: 10.87; Found: C: 55.99; H: 4.75; N: 10.83.

*4.3.43. N-(3-Bromophenyl)-2-(4-(6-fluorobenzo[d]isoxazol-3-yl) piperidine-1-carbonyl)pyrrolidine-1-carbothioamide (**41**)*

$R_f^a$ = 0.42; $R_f^b$ = 0.61; Yield = 91%; M.P. = 113 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1634 (CO), 2041 (CS), 3282 (NH); $^1$H NMR (DMSO-$d_6$, $\delta$ ppm): Thiourea = 6.68–7.75 (4H, m, Ar-H), 7.86 (1H, s, –NH); Pro = 4.29 (1H, t, $-^{\alpha}$CH), 1.94–2.26 (4H, m, $-^{\beta,\ \gamma}CH_2$), 3.67–3.74 (2H, m, $-^{\delta}CH_2$); Heterocycle = 2.09–2.24 (4H, m, $-CH_2$), 2.87 (1H, m, –CH), 3.36–3.453 (4H, m, $-CH_2$), 6.68–7.75 (3H, m, Ar-H); Calculated HRMS = 531.4394; Found HRMS = 553.0746, 555.0712 ($M^+$ + Na); Elem. Anal.: Calcd. for $C_{24}H_{24}BrFN_4O_2S$: C: 54.24; H: 4.55; N: 10.54; S: 6.03; Found: C: 54.28; H: 4.61; N: 10.59; S: 6.09.

*4.3.44. N-(4-Bromophenyl)-2-(4-(6-fluorobenzo[d]isoxazol-3-yl) piperidine-1-carbonyl)pyrrolidine-1-carboxamide (**42**)*

$R_f^a = 0.47$; $R_f^b = 0.65$; Yield = 89%; M.P. = 167 °C; IR (Nujol, $\nu_{max}$, $cm^{-1}$) = 1624 (CO), 3286 (NH); $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm): Urea = 6.91–7.42 (4H, m, Ar-H), 7.75 (1H, s, –NH); Pro = 4.29–4.48 (1H, t, $-^{\alpha}CH$), 1.63–2.15 (4H, m, $-^{\beta,\ \gamma}CH_2$), 3.67–3.72 (2H, m, $-^{\delta}CH_2$); Heterocycle = 1.94–2.19 (4H, m, $-CH_2$), 2.61 (1H, m, –CH), 3.18–3.55 (4H, m, $-CH_2$), 6.91–7.42 (3H, m, Ar-H); Calculated HRMS = 515.3738; Found HRMS = 537.0972, 539.0864 ($M^+ + Na$); Elem. Anal.: Calcd. for $C_{24}H_{24}BrFN_4O_3$: C: 55.93; H: 4.69; N: 10.87; Found: C: 55.97; H: 4.62; N: 10.93.

*4.4. Antiglycation assay (in vitro) [28]*

Sodium phosphate buffer (pH 7.4) was prepared by mixing $Na_2HPO_4$ and $NaH_2PO_4$ (67 mM) containing sodium azide (3 mM); phosphate buffer saline (PBS) was prepared by mixing NaCl (137 mM) + $Na_2HPO_4$ (8.1 mM) + KCl (2.68 mM) + $KH_2PO_4$ (1.47 mM) and pH 10 was adjusted with NaOH (0.25 mM), while BSA (10 mg/mL) and anhydrous glucose (50 mg/mL) solutions were prepared in sodium phosphate buffer.

Bovine serum albumin (10 mg/mL) was incubated with glucose anhydrous (50 mg/mL) in sodium phosphate buffer (pH 7.4). DMSO used for dissolving the test compounds was found to have no effect on the reaction at <2% (v/v). Glycated control contains 20 μL BSA + 20 μL glucose + 20 μL sodium phosphate buffer, while blank control contains 20 μL BSA and 40 μL sodium phosphate buffer. The mixture was incubated at 37 °C for 7 days. After incubation, 6 μL (100%) of trichloroacetic acid (TCA) was added into each well and centrifuged (15,000 rpm) for 4 min at 4 °C. After centrifugation, the pellets were rewashed with 60 μL (10%) of TCA. The supernatant containing glucose, inhibitor and interfering substance was removed and pellet containing advanced glycated end product (AGE)–BSA were dissolved in 60 μL phosphate buffer solution. Evaluation of fluorescence spectrum (excitation 370 nm) and change in fluorescence intensity (excitation 370 nm to emission 440 nm), based on AGEs were monitored by using spectrofluorimeter (Varioskan, Germany). % Inhibition was calculated using the formula:

$$\%\text{Inhibition} = [1 - (\text{Fluorescence of sample}/\text{Fluorescence of glycated sample})] \times 100$$

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