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Synthesis of new and potent analogues of anti-tuberculosis agent 5-nitro-furan-2-carboxylic acid 4-(4-benzyl-piperazin-1-yl)-benzylamide with improved bioavailability

Rajendra P. Tangallapally,[a] Robin E. B. Lee,[a] Anne J. M. Lenaerts[b] and Richard E. Lee[a,*]

[a]*Department of Pharmaceutical Sciences, College of Pharmacy, University of Tennessee Health Science Center, 847 Monroe Ave., Rm 327, Memphis, TN 38163, USA*
[b]*Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO 80523, USA*

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Abstract—Previously, the lead compound 5-nitro-furan-2-carboxylic acid 4-(4-benzyl-piperazin-1-yl)-benzylamide was identified in our anti-tuberculosis drug discovery program. Although this compound demonstrated excellent in vitro activity, it did not meet the expected in vivo profiles due to structural features that resulted in rapid metabolic cleavage and poor absorption, which therefore limited its bioavailability. In efforts to increase the bioavailability, a new series of analogues was successfully synthesized using three modification schemes: replacement of the benzyl group on the piperazine C-ring with carbamate and urea functional groups; introduction of a nitrogen atom into the aromatic ring-B; and expansion of the ring-B to a bicyclic tetrahydroisoquinoline moiety. These modifications retained strong activity and in some case gained superior anti-tuberculosis activity, increased absorption, and serum half life.

Mycobacterium tuberculosis is a deadly obligate human pathogen. The global effect of tuberculosis is immense. According to the World Health Organization, in 2003 there were 8.8 million new cases reported, killing 1.7 million people.[1] Based on the trend over the past few years, a total of 225 million new cases and 79 million deaths are expected from tuberculosis between 1998 and 2030.[2] According to the Global Burden of Disease Study, TB is the seventh leading cause of global premature mortality and disability, and this figure is estimated to remain the same even in the year 2020.[3] Further complicating this already adverse situation is the increase in multi-drug resistant tuberculosis strains,[4] and the synergistic pathological effects of tuberculosis and HIV co-infection. Hence, there is an urgent need to develop new, potent, and fast acting anti-tuberculosis drugs with low toxicity profiles.

In our ongoing efforts to develop new anti-tuberculosis agents, we previously discovered a series of nitrofuranyl amide compounds with inhibitory activity against *M. tuberculosis*.[5,6] Among them, 5-nitro-furan-2-carboxylic acid 4-(4-benzyl-piperazin-1-yl)-benzylamide [**I**] was identified with good in vitro activity (MIC_{90} 0.0125 μg/mL) (Fig. 1).[6] However, this compound did not perform well during in vivo studies due to a short biological half life and rapid elimination. Analysis of the structure **I** led us to attribute this behavior to two sites that are likely candidates for rapid metabolic cleavage: the furanyl amide bond and the benzyl-piperazine bond. Hence, in this study these features were modified in an attempt to achieve increased bioavailability. Ring-A has been retained intact, as previous attempts to change this part of the molecule to nitrothiophene and nitroimidazole resulted in loss of activity against

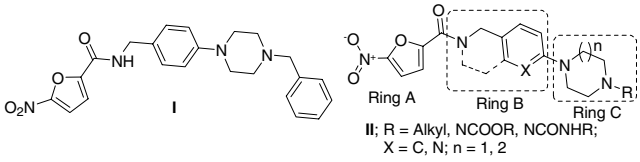

Figure 1. Lead compound from the previous library and general structures of the target compounds.

Keywords: Tuberculosis; Antibiotic; Bioavailability; Nitrofuran.
* Corresponding author. Tel.: +1 9014486018; e-mail: relee@utmem.edu

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M. tuberculosis. However, previous modifications at ring-B and ring-C were well tolerated lending hope that the proposed modifications would not significantly diminish the anti-tuberculosis activity while increasing the potential bioavailability.[6]

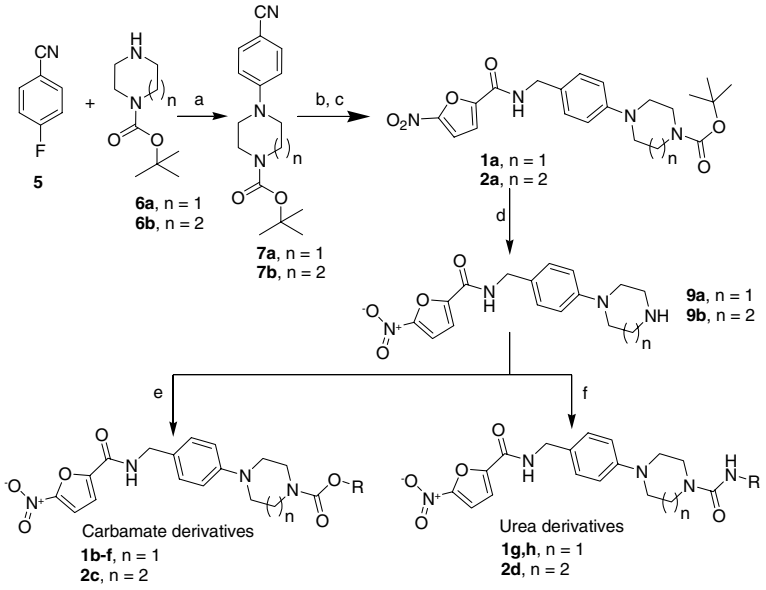
In this study, ring-C analogues were explored by replacement of the benzyl group with more metabolically stable functional groups. During synthesis of intermediate **9a**, it was discovered that the biological activity of the boc-protected **1a** was several fold more active than the lead compound **I**. Therefore, the functionalization of the terminal nitrogen on the piperazine ring was focused using carbamate and urea groups. Two modifications to ring-B were explored. The first modification was the introduction of a nitrogen atom into the ring to make the molecule more polar, in order to facilitate solubility and increase absorption. In the second modification, the ring was expanded to a bicyclic, tetrahydroisoquinoline moiety to make a more rigid tertiary amide, which was expected to be more resistant to proteolysis. The synthesis of the target molecules in these three schemes, their in vitro activities against *M. tuberculosis*, and bioactivities are discussed in this paper.

The synthesis of **9a** (Scheme 1) started from 4-fluoro benzonitrile [**5**], which was subjected to aromatic nucleophilic substitution with 1-boc-piperazine [**6a**] in the presence of K_2CO_3 at 90 °C to give the corresponding nitrile **7a** in 89% yield.[7] **7a** was subjected to reduction with catalytic hydrogenation using Raney-Ni in the presence of palladium on carbon and lithium hydroxide to give the corresponding benzyl amine,[8] which was immediately subjected to acylation using 5-nitro-furan-2-carbonyl chloride [**8**] to afford amide **1a** in 94% yield. The boc-protection group on **1a** was removed using trifluoroacetic acid to give the piperazinyl amine **9a**. Compound **9a** was then treated with alkyl chloroformates in the presence of triethyl amine to afford the corresponding carbamate derivatives **1b–f**, or with alkyl isocyanates[9] to afford the corresponding urea derivatives **1g** and **1h**. The same approach was employed to make the diazepane (seven-membered ring) analogues, starting with 1-boc-(1,4) diazepane [**6b**] instead of **6a**.

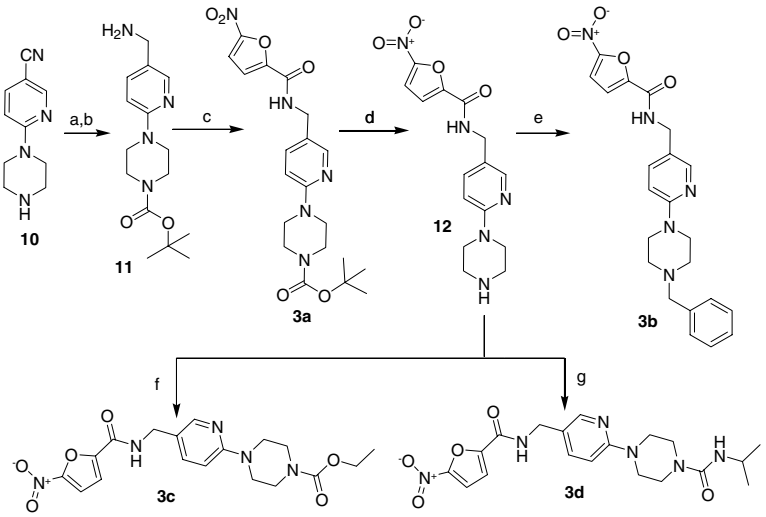
For the synthesis of analogues with a nitrogen atom incorporated in ring-B, commercially available 6-piperazinonicotinonitrile [**10**] was used as the starting material (Scheme 2). The piperazinyl amine **10** was protected with a boc functional group, followed by the reduction of the nitrile functional group by catalytic hydrogenation using Raney-Ni in the presence of palladium carbon and lithium hydroxide to give the corresponding benzyl amine **11** in 93% yield. Amine **11** was then treated with 5-nitro-furan-2-carbonyl chloride [**8**] to give amide **3a** in 86% yield. The boc-deprotection of **3a** was achieved using trifluoroacetic acid leaving the secondary amine functional group open for a variety of functionalization.

Compound **12** was first treated with benzyl bromide in the presence of K_2CO_3 to give **3b** (71% yield), a close analogue of **I**. Analogous compounds to **1b** and **1h** were also synthesized from **12** by treatment with ethyl chloroformate in the presence of triethyl amine to afford the carbamate **3c** in 79% yield. Similarly, compound **12** was treated with 2-isocyanato-propane in the presence of triethyl amine to afford the urea derivative **3d** in 82% yield.

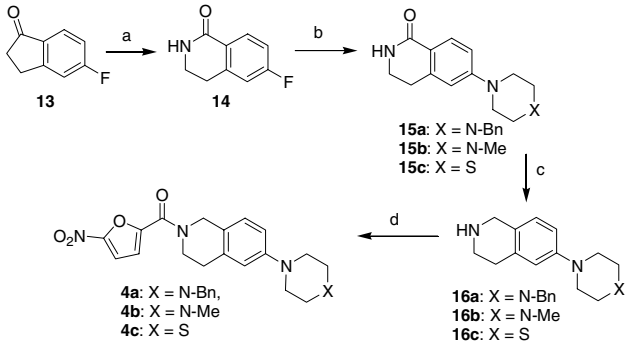
For the synthesis of bicyclic tetrahydroisoquinoline analogues in the ring-B, an efficient and facile strategy was developed by employing Schmidt rearrangement[10] on commercially available 5-fluoroindanone [**13**]. Compound **13** was treated with sodium azide in an acidic solution to afford lactam **14** in 55% yield[11] (Scheme 3). The amide functionality was then exploited as an electron-withdrawing group to activate the fluorine on intermediate **14** for nucleophilic aromatic substitution.



Scheme 1. Reagents and conditions: (a) K_2CO_3, DMSO, 90 °C, 8 h; (b) Raney-Ni, H_2, Pd/C, EtOAc, LiOH; (c) **8**, CH_2Cl_2, Et_3N, rt, 6 h; (d) dil aq HCl, THF, 0 °C to rt, 1 h; (e) ROCOCl, Et_3N, THF, rt, 6 h; (f) RNCO, Et_3N, THF, rt, 6 h.



Scheme 2. Reagents and conditions: (a) K_2CO_3, DMSO, 90 °C, 8 h; (b) Raney-Ni, H_2, Pd/C, EtOAc, LiOH; (c) **8**, CH_2Cl_2, Et_3N, rt; (d) CF_3COOH–H_2O, THF; (e) BnBr, K_2CO_3, DMF, rt, 6 h; (f) EtOCOCl, Et_3N, THF, rt, 6 h; (g) ^{i}PrNCO, Et_3N, THF, rt, 6 h.



Scheme 3. Reagents and conditions: (a) NaN_3, $MeSO_3H$–CH_2Cl_2; (b) *sec*-amine, K_2CO_3, DMSO, 140 °C; (c) BH_3–THF, rt; (d) **8**, CH_2Cl_2, Et_3N, rt.

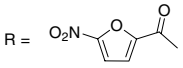
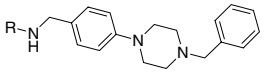
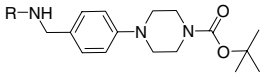
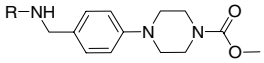
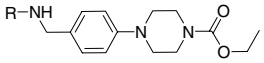
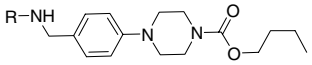
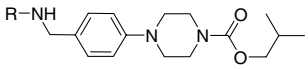
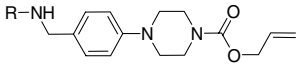
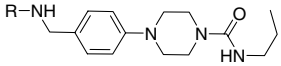
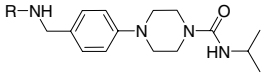
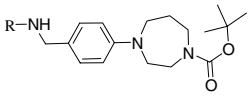
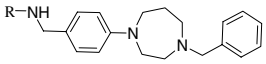
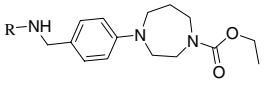
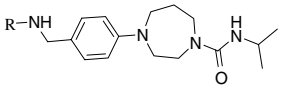
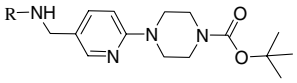
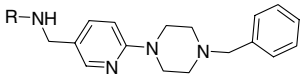
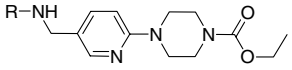
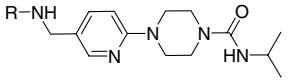
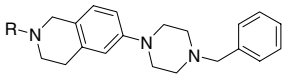
Compound **14** upon reaction with benzyl-piperazine in the presence of K_2CO_3 at 140 °C for 24 hours afforded the substituted lactam **15a** in 75% yield. Compound **15a** was reduced to the tetrahydroisoquinoline derivative **16a** (81% yield) using borane–THF. Compound **16a** was then treated with 5-nitro-furan-2-carbonyl chloride [**8**] to give the desired target compound **4a** in 91% yield. Similarly, the fluoro group on lactam **14** was substituted with *N*-methyl-piperazine and thiomorpholine separately to give the corresponding intermediates **15b** (83% yield) and **15c** (69% yield), respectively. The subsequent reduction of the lactam to tetrahydroisoquinoline derivative **16b** and **16c** followed by acylation with 5-nitro-furan-2-carbonyl chloride [**8**] afforded compound **4b** (82% yield) and **4c** (79% yield).

Determination of the anti-tuberculosis activity was carried out using microbroth dilution and visual inspection as described previously.[6] The MIC values of compounds in this study range from 0.0062 to 1.56 μg/mL and are therefore commensurate with current therapeutic anti-tuberculosis agents (Table 1). Benzyl-piperazine C-ring substitutions **3b** and **4a** demonstrated the most potent MIC activity. Ethyl carbonate **1c** had the best activity of the carbonate/urea series with carbonates **1a**–**f** and **3c** having generally better MIC activity than the ureas **1g**, **1h**, and **3d**. The introduction of a diazepane C-ring **2a**–**d** reduced MIC activity compared with the corresponding piperazines **I**, **1c**, and **1h**. The introduction of pyridyl **3a**–**d** or tetrahydroisoquinoline **4a**–**c** B-ring had little effect on the MIC activity of the series and was well tolerated.

Preliminary bioavailability of a selected set of compounds was assessed using a bioactivity assay. Briefly, mice were dosed via oral gavage at 300 mg/kg in 0.5% methylcellulose. Mice were subsequently bled at set intervals (at 0.5, 2.5, 4 and 8 h after dosing) with three mice per time point. The serum collected from these mice was then serially diluted and tested for anti-tuberculosis activity in a bioassay using *M. tuberculosis*.[12,13] The results from the bioassay reflect approximate concentrations of unbound bioactive product in the serum rather than providing total drug levels (Table 1). The bioactivity results indicate that compounds **1g**, **2d**, **3c**, and **3d** had the best C_{max} values. The longest $T_{1/2}$ values were obtained for **2a**, **3a**, **4a**, and **4b**. Within these data, general trends were observed. The pyridinyl series **3a**–**d** achieved the best absorption, the benzyl B-ring series resulted in short half lives, and the tetrahydroisoquinolines **4a** and **4b** had longer half lives. The diazepane C-ring compounds **2a** and **2d** also had better half lives.

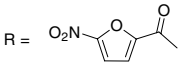
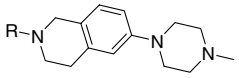
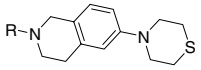
This series of compounds has been designed to increase the bioavailability of lead compound 5-nitro-furan-2-carboxylic acid 4-(4-benzyl-piperazin-1-yl)-benzylamide [**I**] by addressing the metabolic instability of the molecule and increasing absorption. Three modification schemes were explored, 20 compounds were synthesized,[14] tested in vitro for MIC activity, and the bioactivity of nine compounds was tested in mice (Table 1). Modifications in each of these schemes affected the anti-tuberculosis activity, presenting the challenge of selecting compounds from

Table 1. Structures of advanced hit analogues and their anti-tuberculosis activity

Compound	Structure (R = )	MIC_{90} (μg/ml)	C_{max}[b] (μg/ml)	T_{max} (h)	$T_{1/2}$[c] (h)
I		0.0125	2.24[a]	0.5[a]	1.1[a]
1a		0.025			
1b		0.1			
1c		0.0062			
1d		0.05			
1e		0.05			
1f		0.5			
1g		0.2	4.8	2.5	1.5
1h		0.8			
2a		0.1	0.28	0.5	7.5
2b		0.8			
2c		0.1			
2d		0.8	7.4	0.5	2
3a		0.05	1.23	0.5	2.3
3b		0.0062	1.47	0.5	1.3
3c		0.05	6.56	2.5	0.7
3d		0.56	22	2.5	0.5
4a		0.0062	0.736[a]	0.5[a]	3.5[a]

(*continued on next page*)

Table 1 (*continued*)

Compound	R =  Structure	MIC_{90} (μg/ml)	C_{max}[b] (μg/ml)	T_{max} (h)	$T_{1/2}$[c] (h)
4b		0.2	0.28[a]	0.5[a]	2[a]
4c		0.4			

[a] Determined using the HCl salt of the compound.
[b] Drug levels in the mouse serum are estimated by multiplying the dilution factor by the MIC value of the drug in the presence of 10% serum.
[c] Estimated based on graph of concentration versus time curve.

the series that have significant anti-tuberculosis activity, but are also likely to be well absorbed with a long half life in serum. Compounds selected under these criteria can then enter lengthy testing in animal models of tuberculosis infection with an increased chance of success. The first modification explored was the replacement of the benzylpiperazine C-ring substituent of **I** with carbamates and ureas. Compounds in this series showed good MIC activity, increased absorption, and lower serum binding but were just as rapidly eliminated as **I**. The second modification explored was the introduction of the pyridinyl B-ring, which had little affect on MIC activity or serum half life, but significantly boosted absorption, most likely due to increased solubility. The final modification evaluated was introduction of tetrahydroisoquinoline B-ring which significantly increased the serum half life while maintaining or improving MIC activity. This suggests that rapid metabolism of the furanyl amide bond is the limiting factor for bioavailability of this series and that bioisosteric replacement may be a fruitful future strategy for the development of this series. In the course of this study it emerged that serum binding and tissue distribution may also be important factors that need to be addressed as this series progresses. As it was noted during the bioavailability assay the MIC activity of most of the compounds in this series decreased in the presence of serum, for example the MIC value for **4a** was tenfold worse in the presence of 10% mouse serum. The best compounds in this series are currently undergoing a detailed microbiological assessment and are being tested in in vivo infection models. The results from these studies will be reported in due course. In conclusion, we have successfully further elaborated this series of anti-tuberculosis compounds, developed compounds with highly potent anti-tuberculosis activities, and improved bioactivity.

Acknowledgment

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11. Prepartion of 6-fluoro-3,4-dihydro-2*H*-isoquinolin-1-one [**14**]: sodium azide (865 mg, 13.32 mmol) was added portionwise to a stirred solution of 5-fluoroindanone [**13**] (1.0 g, 6.66 mmol) in 1:1 methanesulfonic acid/dichloromethane (10 mL), while the temperature was maintained between 22 °C and 29 °C. Once addition was complete, the mixture was stirred at room temperature for 16 h. The mixture was cooled to 0 °C and neutralized by the addition of 5 N NaOH solution and the organic layer separated. The aqueous layer was extracted with dichloromethane (3× 25 mL) and the combined organic fractions were washed with water (50 mL), brine (50 mL) and dried over anhydrous sodium sulfate. Filtration and concentration followed by flash silica gel chromatography (pet. ether/ethyl acetate) gave 605 mg of lactam **14** (55% yield). TLC 0.6 R_f in 100% ethyl acetate; ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.02 (t, J = 6.59 Hz, 2H), 3.60 (dt, J = 2.29, 6.83 Hz, 2H), 6.08–6.15 (br s, 1H), 6.93 (dd, J = 2.68, 9.03 Hz, 1H), 7.04 (dt, J = 2.68, 8.54 Hz, 1H), 8.1 (dd, J = 5.85, 8.54 Hz, 1H); ESI-MASS: 188.3 (M+23).

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14. Selected spectral data for target compounds. Compound **1c**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.35 (t, J = 7.1 Hz, 3H), 3.17 (t, J = 4.9, 5.2 Hz, 4H), 3.65 (t, J = 5.3, 4.9 Hz, 4H), 4.2 (q, J = 7.1 Hz, 2H), 4.57 (d, J = 5.7 Hz, 2H), 6.76–6.84 (br s, 1H), 6.94 (d, J = 8.7 Hz, 2H), 7.26–7.36 (m, 3H), 7.37 (d, J = 3.7 Hz, 1H); ^{13}C NMR (300 MHz, $CDCl_3$): ppm 14.11, 42.56, 43.0, 48.66, 60.95, 111.85, 115.42, 116.2, 127.96, 128.68, 147.58, 150.42, 154.93, 155.51; ESI-MS: 401.8 (M−1); Anal. Calcd for $C_{19}H_{22}N_4O_6$: C, 56.71; H, 5.51; N, 13.92. Found: C, 56.27; H, 5.51; N, 13.56.
Compound **1g**: ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.86 (t, J = 7.3 Hz, 3H), 1.45 (sextet, J = 7.3 Hz, 2H), 3.01 (q, J = 6.1 Hz, 2H), 3.07 (t, J = 5.1 Hz, 4H), 3.45 (t, J = 4.8, 5.3 Hz, 4H), 4.39 (d, J = 6.1 Hz, 2H), 6.6 (t, J = Hz, 1H), 6.96 (d, J = 8.7 Hz, 2H), 7.22 (d, J = 8.7 Hz, 2H), 7.46 (d, J = 3.9 Hz, 1H), 7.79 (d, J = 3.9 Hz, 1H), 9.36 (t, J = 6.1 Hz, 1H); ^{13}C NMR (300 MHz, DMSO-d_6): ppm 11.3, 22.92, 41.85, 43.2, 48.44, 113.35, 115.43, 115.69, 128.38, 129.25, 148.29, 150.18, 155.88, 157.37; ESI-MS: 438.1 (M+23); Anal. Calcd for $C_{20}H_{25}N_5O_5$: C, 57.82; H, 6.07; N, 16.86. Found: C, 57.72; H, 6.36; N, 16.73.
Compound **3b**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.57 (t, J = 5.0 Hz, 4H), 3.57 (t, J = 4.7, 5.32 Hz, 4H), 4.51 (d, J = 5.8 Hz, 2H), 6.62 (d, J = 8.7 Hz, 2H), 6.7–6.8 (br s, 1H), 7.27–7.42 (m, 7H), 7.51 (dd, J = 2.4, 8.7 Hz, 1H), 8.18 (d, J = 2.2 Hz, 1H); ^{13}C NMR (300 MHz, DMSO-d_6): ppm 39.62, 44.81, 52.25, 62.04, 106.74, 113.34, 115.5, 122.9, 126.88, 128.1, 128.8, 137.5, 137.96, 147.04, 148.18, 151.35, 155.92, 158.4; Anal. Calcd for $C_{22}H_{23}N_5O_4$: C, 62.70; H, 5.50; N, 16.62. Found: C, 61.57; H, 5.56; N, 16.07.
Compound **4b**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.35 (s, 3H), 2.52–2.62 (br s, 4H), 2.88–3.06 (m, 2H), 3.16–3.24 (m, 4H), 3.9–4.05 (m, 2H), 4.75–4.95 (m, 2H), 6.71–6.75 (m, 1H), 6.79–6.87 (m, 1H), 7.0–7.11 (m, 1H), 7.18–7.23 (m, 1H), 7.36 (d, J = 3.9 Hz, 1H); ^{13}C NMR (300 MHz, DMSO-d_6): ppm 27.87, 29.19, 44.29, 45.65, 47.23, 48.14, 54.49, 112.8,113.94, 114.77, 116.82, 122.88, 126.92, 134.84, 147.84, 149.82, 151.18, 157.03; ESI-MS: 393.5 (M+23); Anal. Calcd for $C_{19}H_{22}N_4O_4$: C, 61.61; H, 5.99; N, 15.13. Found: C, 61.47; H, 5.99; N, 15.03.