Discovery and bioactivity of 4-(2-arylpyrido[3′,2′:3,4]pyrrolo[1,2-f][1,2,4]-triazin-4-yl) morpholine derivatives as novel PI3K inhibitors

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ABSTRACT

PI3K is a promising therapeutic target for cancer. With PI-103 as the lead compound, we designed and synthesized 4-(2-arylpyrido[3′,2′:3,4]pyrrolo[1,2-f][1,2,4]triazin-4-yl)morpholine derivatives. **9**, **10a**, **10d**, **10e** had the IC$_{50}$ against PI3Kα comparable with PI-103. All of the compounds showed selectivity over 15 tested protein kinases and anti-proliferative activity at micromolar concentration against several cancer cell lines.

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Phosphatidylinositol 3-kinases (PI3Ks) are a family of lipid kinases that phosphorylate the hydroxyl group at position 3 on the inositol ring to generate the second messengers phosphatidyl-inositol 3,4,5-trisphosphate (for class I PI3Ks), phosphatidylinositol 3,4-bisphosphate (for class II PI3Ks) or phosphatidylinositol 3-phosphate (for class III PI3K) and then initiate the PI3K mediated signaling.[1] Among the various subtypes of PI3Ks identified to date, class I PI3Ks are known to play critical roles in cell growth and survival mainly through downstream components Akt and mTOR.[2] PI3K signaling is negatively regulated by PTEN.[3] Hyper-activation of PI3Ks and/or inactivation of PTEN occur frequently in human cancers. Therefore targeting PI3Ks is a promising approach for cancer therapy[4] and significant efforts have been made to develop PI3K inhibitors.[5]

Piramed pharma's PI-103 is a dual PI3K/mTOR inhibitor, which exhibited potent antitumor activity in human tumor xenograft models as a single agent or in combination with other anticancer drugs. PI-103 induced cell cycle arrest at G1 phase at submicromolar concentration and its anti-angiogenic activity was also reported.[6] Morpholine, phenol, and pyridine moieties in PI-103 were presumed to play an important role in their interaction with PI3K.[7] Utilizing the structural information available and bioisostere, we designed 3-(4-morpholinopyrido[3′,2′:3,4]pyrrolo[1,2-f][1,2,4]triazin-2-yl)phenol (Fig. 1), which maintained the conformation of PI-103 and changed
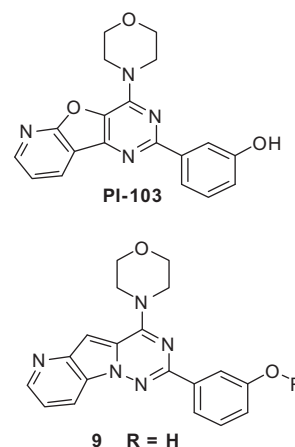
**Figure 1.** The structures of PI-103 and the designed compounds.

furan to pyrrole. Futhermore, phenol moiety was modified to ester, wishing to improve the pharmacological properties.

The designed compound **9** and its derivatives **10**a–g were synthesized as Scheme 1.[8] The intermediate ethyl 1H-pyrrolo[3,2-b]-pyridine-2-carboxylate **4** was synthesized from 2-chloro-3-nitropyridine **1** by three steps according to the literature.[9] Compound **4** was reacted with the fresh solution of NH$_2$Cl in ether. After **4** was consumed completely, the reaction solution was quenched by saturated Na$_2$S$_2$O$_3$ aqueous solution, extracted by AcOEt and concentrated to give the crude product **5**, which was used without purification at once because it was labile. Annulation of **5** with ethyl

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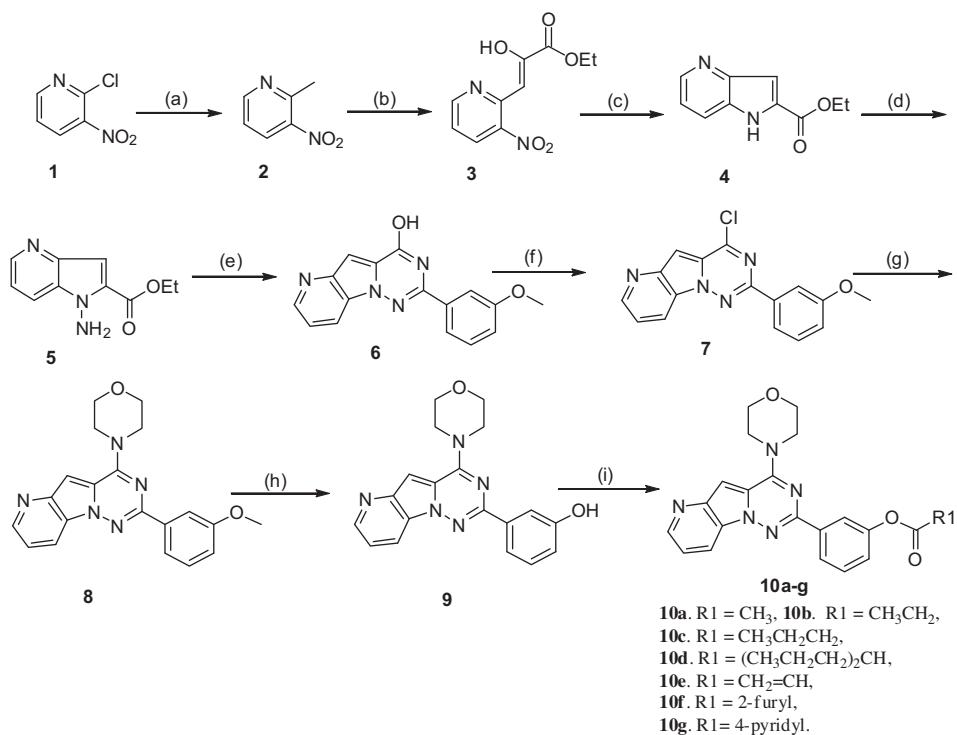
Scheme 1. Reagents and conditions: (a) Na, $CH_2(CO_2Et)_2$, 90 °C, 7 h, then H_2SO_4, 105 °C, 7 h, 80%; (b) Na, $(COOEt)_2$, EtOH, rt, 8 h, 81%; (c) $TiCl_4$, $SnCl_2\ 2H_2O$, EtOH, 80 °C, 8 h, 67%; (d) NH_2Cl, KO^tBu, DMF, 0 °C to rt, 4 h, 83%; (e) ethyl 3-methoxybenzimidate hydrochloride, EtOH, 80 °C, 8 h, 26%; (f) $POCl_3$, 110 °C, 3 h, 88%; (g) morpholine, THF, 70 °C, 5 h, 90%; (h) BBr_3, CH_2Cl_2, rt, 2 h, 62%; (i) R1COOH, DCC, CH_2Cl_2, rt, 8 h, 78–83%.

Table 1
Inhibitory activity against PI3Kα of **9** and **10**

Compound	IC_{50} (nM)	Compound	IC_{50} (nM)
9	33.6	**10e**	47.8
10a	49.7	**10f**	424.7
10b	544.0	**10g**	421.3
10c	510.5	**PI-103**	16.8
10d	60.7		

3-methoxybenzimidate hydrochloride in refluxing EtOH gave intermediate **6**. Chlorination of **6** with phosphorus oxychloride followed by substitution with morpholine afforded **8**. Demethylation of **8** by BBr_3 in CH_2Cl_2 gave the desired product **9**. Condensation of **9** with various carboxylic acids under the condition of DCC/CH_2Cl_2 afforded **10a–g**.

We first detected the effect of the compounds on the kinase activity of PI3Kα using Kinase-Glo® Luminescent Kinase Assays. The results were summarized in Table 1. **9** inhibited PI3Kα with an IC_{50} of 33.6 nM, showing a two-fold decrease in potency compared with PI-103. Acetylation of **9** resulted in almost similar potency (**10a**: IC_{50} = 49.7 nM). Replacement of acetyl with propionyl or butyryl decreased the PI3Kα inhibitory potency by about 10-fold (**10b**: IC_{50} = 544.0 nM, **10c**: IC_{50} = 510.5 nM), whereas 2-propyl-pentanoate and acrylate retained similar PI3Kα inhibitory activity (**10d**: IC_{50} = 60.7 nM, **10e**: IC_{50} = 47.8 nM) with **10a**. Heteroaryl carboxylate such as isonicotinate and furan-2-carboxylate showed unsatisfied inhibitory activity (**10f**: IC_{50} = 424.7 nM, **10g**: IC_{50} = 421.3 nM).

Table 2
The inhibitory activity against 15 protein kinases of **9** and **10**

| Compound	Inhibitory rate at the concentration of 10 μM														
	Flt-1	KDR	c-Kit	PDGFRα	PDGFRβ	RET	EGFR	ErbB2	c-Src	EPH-A2	EPH-B2	c-Met	RON	IGF1R	FGFR1
9	45.7	39.2	49.4	41.2	42.4	37.8	17.4	10.9	65.3	53.2	44.0	43.8	37.0	35.2	39.2
10a	28.1	23.1	24.7	21.8	0	19.0	4.6	7.1	40.0	22.8	37.2	29.0	9.4	33.4	27.2
10b	60.6	25.0	29.0	13.0	11.8	43.0	26.8	12.5	52.8	32.9	48.6	31.7	17.6	23.7	37.4
10c	53.4	25.6	25.4	14.0	5.1	25.8	16.0	12.5	48.2	22.7	53.0	33.9	7.4	15.6	32.9
10d	34.1	29.1	24.9	28.7	13.6	14.4	20.2	25.6	49.8	15.7	49.2	29.0	23.1	32.7	37.2
10e	39.3	28.4	18.9	29.4	6.3	31.9	17.8	21.3	56.2	19.0	53.4	32.8	3.3	41.1	40.8
10f	40.0	24.7	26.9	22.2	13.5	22.8	11.8	23.6	47.9	35.8	32.5	34.8	4.2	29.2	43.0
10g	49.1	35.4	45.2	40.0	34.7	28.9	6.2	23.3	67.2	41.2	53.8	34.8	27.2	39.8	40.3
Su11248	88.0	94.2	84.8	93.0	90.6	84.8	ND[a]	ND	ND	ND	ND	ND	ND	ND	ND
Lapatinib	ND	ND	ND	ND	ND	ND	97.7	78.0	ND	ND	ND	ND	ND	ND	ND
Dasatinib	ND	ND	ND	ND	ND	ND	ND	ND	93.6	94.5	89.2	ND	ND	ND	ND
Su11274	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	61.8	52.2	ND	ND
AG538	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	82.2	ND
Su5402	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	78.6

[a] ND means not determined.
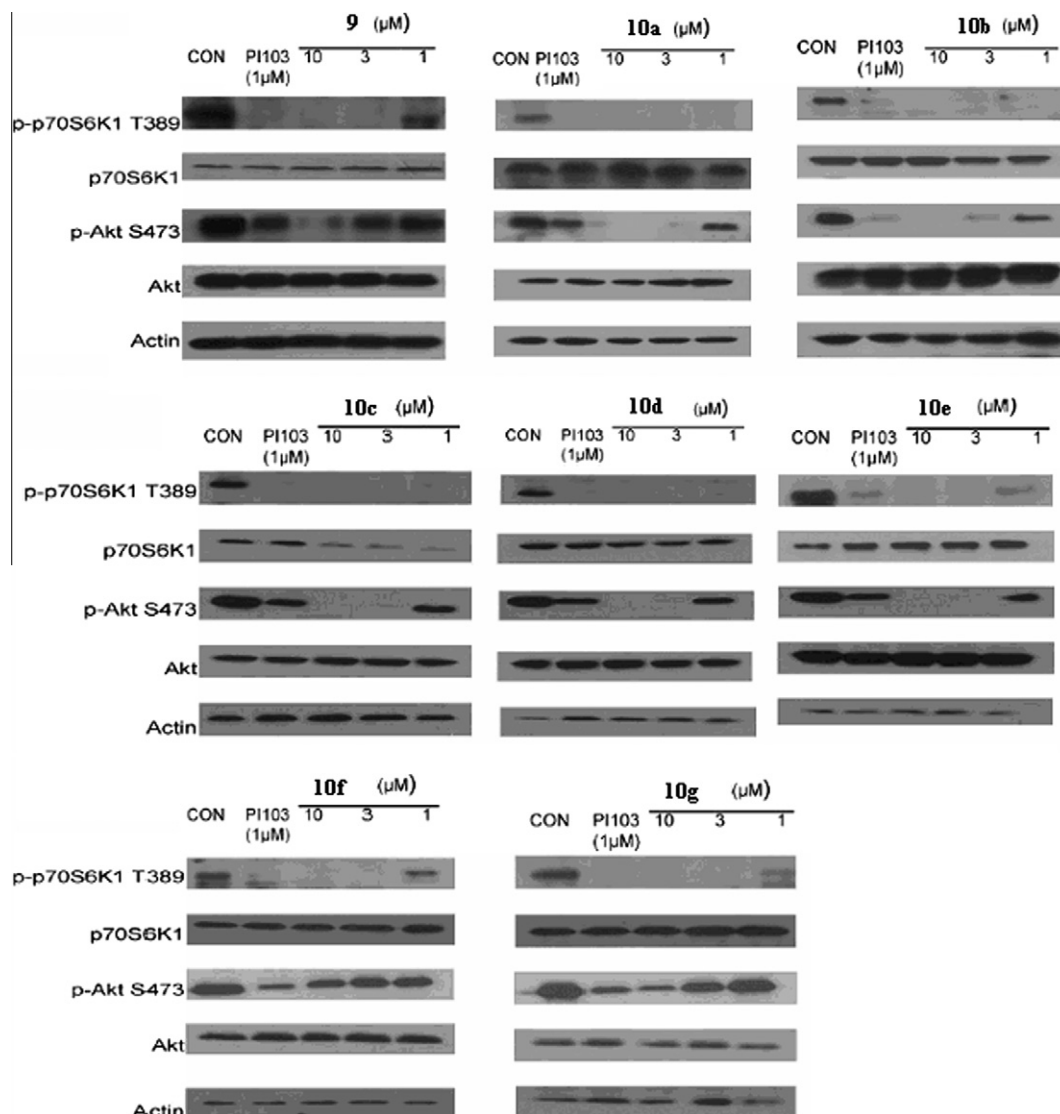
Figure 2. Modulation of p-Akt and p-p70s6k1.

Table 3
The anti-proliferative activity of **9** and **10** in several human tumor lines

Compound	IC$_{50}$ (μM)			
	ECV-304	BEL-7402	MCF-7	HT-29
9	6.7	13.1	22.7	22.3
10a	3.8	12.1	14.4	>50
10b	4.0	8.6	18.7	30.4
10c	1.4	3.6	6.1	16.4
10d	2.5	5.6	7.3	37.5
10e	4.0	7.9	15.3	29.9
10f	6.0	9.3	12.8	21.6
10g	10.0	13.3	20.0	28.0
PI-103	6.3	7.0	22.4	>50

PI103 has been shown previously to be a selective inhibitor towards class I PI3Ks among a panel of 70 protein kinases representing of the human kinome.[10] To examine selectivity for PI3Kα, the inhibitory activity of these compounds was then evaluated against 15 protein tyrosine kinases. As shown in Table 2, most of these compounds showed weak inhibitory activity, with the inhibitory rate lower than 50% at the concentration of 10 μM, indicating selective inhibitory activity against PI3Kα.[1]

Furthermore, the effect of these compounds on the downstream signal was examined by Western blot. The results (shown in Fig. 2) suggested that these compounds inhibited PI3K-Akt-mTOR pathway in a dose-dependent manner. The phosphorylated Akt and p70S6K1 levels were significantly down regulated by all compounds except **10f** and **10g** at the concentration of 3 μM.

The anti-proliferative activity of these derivatives was evaluated in several human tumor cell lines. As shown in Table 3, all of these derivatives, especially **10c** and **10d**, exerted comparable or even stronger anti-proliferative activity than the lead compound PI-103 in human bladder cancer ECV-304 cells, human hepatoma BEL-7402 cells, human breast cancer MCF-7 cells and human colon carcinoma HT-29 cells. It should be notable that **10b**, **10c**, **10f** and **10g** displayed superior anti-proliferative activity than **9**, while they possessed weaker activity against PI3Kα (Table 1). The inconsistency might be due to the hydrolysis of the ester moieties in cells. As different from the purified kinase experiment using kinase-Glo assays, the cancer cell assays provided full cell-machinery to execute such hydrolysis, thus increasing the likelihood of metabolism-driven hydrolysis of the compounds **10a–10g**.

Finally, the effect of the compounds on cell cycle distribution was determined by flow cytometer. As shown in Figure 3, cell
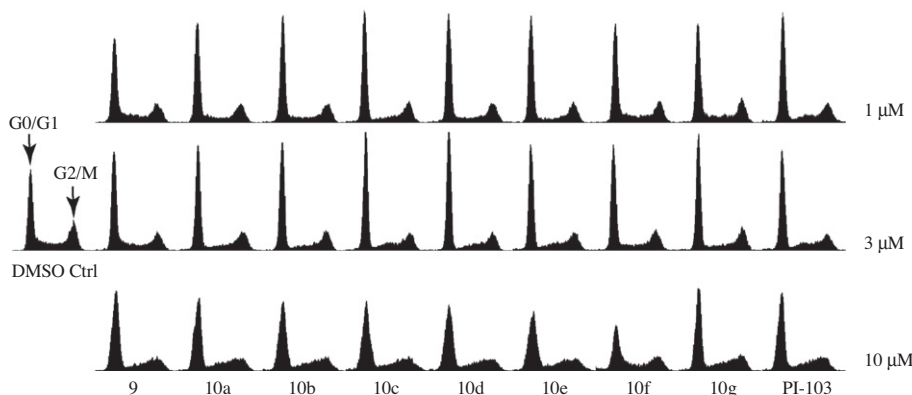
Figure 3. Compounds **9** and **10** induce cell cycle arrest at G1 phase.

population in G1 phase increased after treatment by **9** and **10**, demonstrating that the compounds tested induced cell cycle arrest at G1 phase, which is consistent with results obtained with the lead compound PI103. Additionally, **9** and **10** also slightly induced apoptosis in Rh30 cell at the concentration of 10 µM, as demonstrated by a small sub-G1 peak in Figure 3.

In summary, structural modification of PI-103 led to the discovery of 4-(2-arylpyrido[3′,2′:3,4]pyrrolo[1,2-*f*][1,2,4]triazin-4-yl)morpholine derivatives **9** and **10** as novel PI3K inhibitors. **9**, **10a**, **10d**, **10e** had the IC_{50} against PI3Kα comparable with PI-103. All of the compounds showed selectivity over 15 tested protein kinases and anti-proliferative activity at micromolar concentration against several cancer cell lines. More importantly, this modification may provide a useful scaffold for further optimization of PI3K inhibitors.

Acknowledgments

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8. All compounds were characterized by ¹H NMR and MS. Spectral data of representative compounds **9**, **10a–g**: Compound **9**: ¹H NMR (300 MHz, DMSO-d_6) δ 9.59 (s, 1H), 8.72 (d, *J* = 3.9 Hz, 1H), 8.50 (d, *J* = 8.1 Hz, 1H), 7.82 (m, 1H), 7.76 (m, 1H), 7.50 (s, 1H), 7.44 (dd, *J* = 8.5, 4.4 Hz, 1H), 7.31 (t, *J* = 7.8 Hz, 1H), 6.88 (d, *J* = 6.4 Hz, 1H), 4.22 (t, *J* = 4.2 Hz, 4H), 3.85 (t, *J* = 4.2 Hz, 4H). MS (EI): *m/z* 347(100, M⁺), 290(35). Compound **10a**: ¹H NMR (300 MHz, DMSO-d_6) δ 8.58 (dd, *J* = 4.3, 1.2 Hz, 1H), 8.12 (d, *J* = 8.0 Hz, 1H), 8.02 (d, *J* = 7.8 Hz, 1H), 7.94 (m, 1H), 7.87 (t, *J* = 7.9 Hz, 1H), 7.64 (s, 1H), 7.44 (dd, *J* = 8.4, 4.3 Hz, 1H), 7.26 (dd, *J* = 8.3, 2.1 Hz, 1H), 4.20 (t, *J* = 4.9 Hz, 4H), 3.84 (t, *J* = 4.9 Hz, 4H), 2.28 (s, 3H). MS (EI): *m/z* (%) 389(100, M⁺), 347(40). Compound **10b**: ¹H NMR (300 MHz, DMSO-d_6) δ 8.58 (dd, *J* = 4.3, 1.2 Hz, 1H), 8.14 (d, *J* = 8.0 Hz, 1H), 7.99 (d, *J* = 7.8 Hz, 1H), 7.85 (m, 1H), 7.74 (t, *J* = 7.9 Hz, 1H), 7.64 (s, 1H), 7.43 (dd, *J* = 8.4, 4.3 Hz, 1H), 7.28 (dd, *J* = 8.3, 2.1 Hz, 1H), 4.21 (t, *J* = 4.9 Hz, 4H), 3.84 (t, *J* = 4.9 Hz, 4H), 2.67 (q, *J* = 6.8 Hz, 2H), 1.32 (t, *J* = 6.8 Hz, 3H). MS (EI): *m/z* (%) 403(100, M⁺), 224(50). Compound **10c**: ¹H NMR (300 MHz, DMSO-d_6) δ 8.73 (dd, *J* = 4.3, 1.2 Hz, 1H), 8.55 (d, *J* = 8.0 Hz, 1H), 8.25 (d, *J* = 7.8 Hz, 1H), 8.02 (m, 1H), 7.57 (t, *J* = 7.9 Hz, 1H), 7.53 (s, 1H), 7.44 (dd, *J* = 8.4, 4.3 Hz, 1H), 7.26 (dd, *J* = 8.3, 2.1 Hz, 1H), 4.21 (t, *J* = 4.9 Hz, 4H), 3.84 (t, *J* = 4.9 Hz, 4H), 2.63 (t, *J* = 7.3 Hz, 2H), 1.71 (dt, *J* = 14.7, 7.2 Hz, 2H), 1.01 (t, *J* = 7.4 Hz, 3H). MS (EI): *m/z* (%) 417(100, M⁺), 347(45), 290(30). Compound **10d**: ¹H NMR (300 MHz, DMSO-d_6) δ 8.74 (dd, *J* = 4.3, 1.2 Hz, 1H), 8.23 (d, *J* = 8.0 Hz, 1H), 7.87 (d, *J* = 7.8 Hz, 1H), 7.67 (m, 1H), 7.47 (t, *J* = 7.9 Hz, 1H), 7.44 (s, 1H), 7.41 (dd, *J* = 8.4, 4.3 Hz, 1H), 7.26 (dd, *J* = 8.3, 2.1 Hz, 1H), 4.11 (t, *J* = 4.9 Hz, 4H), 3.84 (t, *J* = 4.9 Hz, 4H), 2.25 (td, *J* = 8.7, 4.1 Hz, 1H), 1.59 (m, 2H), 1.50 (ddd, *J* = 18.7, 10.7, 5.6 Hz, 2H), 1.40 (m, 4H), 0.94 (t, *J* = 7.3 Hz, 6H). MS (EI): *m/z* (%) 473(100, M⁺), 347(35). Compound **10e**: ¹H NMR (300 MHz, DMSO-d_6) δ 8.58 (dd, *J* = 4.3, 1.2 Hz, 1H), 8.02 (d, *J* = 8.0 Hz, 1H), 7.98 (d, *J* = 7.8 Hz, 1H), 7.69 (m, 1H), 7.57 (t, *J* = 7.9 Hz, 1H), 7.53 (s, 1H), 7.44 (dd, *J* = 8.4, 4.3 Hz, 1H), 7.26 (dd, *J* = 8.3, 2.1 Hz, 1H), 6.38 (d, *J* = 4.3 Hz, 2H), 6.08 (t, *J* = 4.0 Hz, 1H), 4.21 (t, *J* = 4.9 Hz, 4H), 3.84 (t, *J* = 4.9 Hz, 4H). MS (EI): *m/z* (%) 401(100, M⁺), 347(40). Compound **10f**: ¹H NMR (300 MHz, DMSO-d_6) δ 8.58 (dd, *J* = 4.3, 1.2 Hz, 1H), 8.02 (d, *J* = 8.0 Hz, 1H), 7.97 (d, *J* = 7.8 Hz, 1H), 7.89 (m, 1H), 7.85 (d, *J* = 4.0 Hz, 1H), 7.65 (t, *J* = 7.9 Hz, 1H), 7.57 (s, 1H), 7.52 (d, *J* = 4.1 Hz, 1H), 7.44 (dd, *J* = 8.4, 4.3 Hz, 1H), 7.26 (dd, *J* = 8.3, 2.1 Hz, 1H), 6.68 (t, *J* = 4.0 Hz, 1H), 4.21 (t, *J* = 4.9 Hz, 4H), 3.84 (t, *J* = 4.9 Hz, 4H). MS (EI): *m/z* (%) 441(20, M⁺), 224(70), 56(100). Compound **10g**: ¹H NMR (300 MHz, DMSO-d_6) δ 8.81 (d, *J* = 4.5 Hz, 2H), 8.63 (dd, *J* = 4.3, 1.2 Hz, 1H), 8.58 (d, *J* = 8.0 Hz, 1H), 8.05 (d, *J* = 7.8 Hz, 1H), 7.91 (m, 4H), 7.62 (t, *J* = 7.9 Hz, 1H), 7.54 (s, 1H), 7.42 (dd, *J* = 8.4, 4.3 Hz, 1H), 7.38 (dd, *J* = 8.3, 2.1 Hz, 1H), 4.21 (t, *J* = 4.9 Hz, 4H), 3.84 (t, *J* = 4.9 Hz, 4H). MS (EI): *m/z* (%) 452(100, M⁺).
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