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Inhibition of Src Kinase Activity by 4-Anilino-7-thienyl-3-quinolinecarbonitriles

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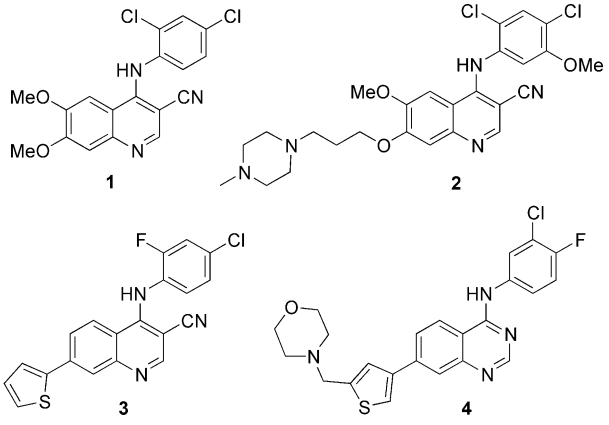
Abstract—Based on a screening lead from a yeast-based assay to identify Src family kinase inhibitors, a series of 4-anilino-7-thienyl-3-quinolinecarbonitriles was prepared. When the thiophene ring was substituted with a water-solubilizing group in a 2,5-, 3,5- or 2,4-pattern, potent inhibition of Src kinase activity was observed. © 2002 Elsevier Science Ltd. All rights reserved.

Protein tyrosine kinases (TKs) are enzymes that catalyze the transfer of a phosphate group from ATP to a tyrosine residue on a protein, resulting in a variety of cell signaling events. The TK Src is a member of the Src family of kinases (SFK) that includes among its members Fyn and Yes.[1a,b] Since the activation and/or overexpression of Src has been implicated in cancer,[1c,d] osteoporosis[1e,f] and stroke,[1g] a small molecule inhibitor of Src activity might be beneficial for the treatment of various disease states. Several classes of SFK inhibitors have been reported in the literature, including pyrazolo[3,4-*d*]pyrimidines,[2a] pyrrolo[2,3-*d*]pyrimidines,[2b–f] pyrido[2,3-*d*]pyrimidin-7(8*H*)-ones,[2g] 1,6-naphthyridin-2(1*H*)-ones,[2h] aminopyrido[2,3-*d*]pyrimidin-7-yl ureas[2i] and 4-anilinoquinazolines.[2j]

We recently reported that a yeast-based screen for Src inhibitors provided 3-quinolinecarbonitrile **1** as a lead compound.[3] It was determined that **1** was an ATP competitive inhibitor of Src kinase activity. Optimization of the C-4 aniline substituents and the addition of a water-solubilizing group at C-7 provided **2**, a very potent inhibitor of Src cellular activity.[4] 3-Quinolinecarbonitriles have been used as templates for the inhibition of kinases other than Src,[5] including epidermal growth factor receptor (EGFr)[6a,b] and MEK.[7a,b]

In yeast-based screens for inhibitors of Fyn and Yes, **3**, a 3-quinolinecarbonitrile with a thiophene substituent at C-7, was identified. We envisioned that **3** could be converted to an Src inhibitor if the 2-fluoro-4-chloroaniline group was replaced by the preferred Src aniline. Furthermore, the addition of a water-solubilizing group to the thiophene should provide cellular activity. Interestingly, a series of related 4-anilinoquinazolines with thiophene substituents at C-7, including **4**, have been reported to be EGFr kinase inhibitors.[8a,b]



The 3-quinolinecarbonitrile analogue of **4** with the preferred 4-anilino group for optimal Src activity was prepared as shown in Scheme 1. Treatment of 3-bromoaniline or 3-iodoaniline with ethyl ethoxymethylenecyanoacetate followed by thermal cyclization resulted in **5a** or **5b**. Chlorination provided **6a** or **6b** with subsequent addition of the aniline giving **7a** and **7b**. Treatment of

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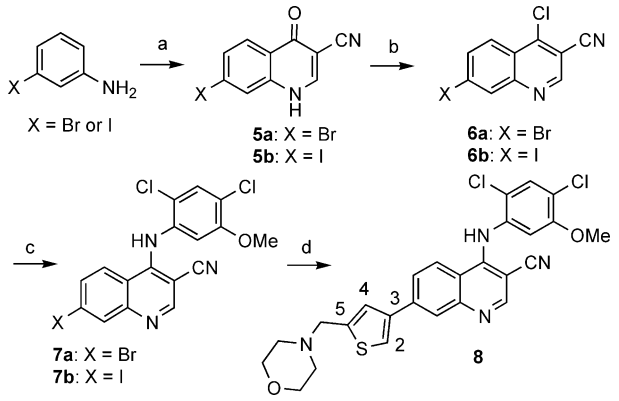
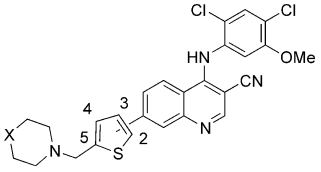


Scheme 1. (a) (1) Ethyl ethoxymethylenecyanoacetate; (2) Dowtherm; (b) $POCl_3$; (c) aniline, pyridine–HCl, 2-ethoxyethanol; (d) diisopropyl [5-(morpholinomethyl)thien-3-yl]boronate, $Pd(PPh_3)_4$, $NaHCO_3$, DME.

Table 1. Inhibition of Src enzymatic and Src cellular activity for compounds **8**–**12** and **14**–**20**

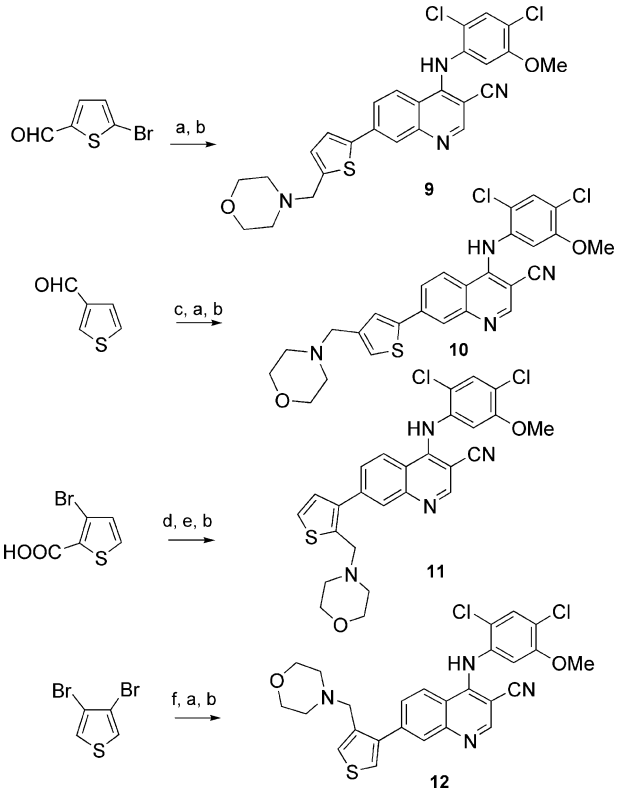


Compd	Thiophene isomer	X	Src IC_{50} (nM)	Cell IC_{50} (nM)
8	3	O	2.7	200
9	2	O	2.5	220
10	2,4 isomer of **8**		5.7	240
11	3,2 isomer of **8**		440	>10,000
12	3,4 isomer of **8**		240	>10,000
14	3	NMe	3.8	64
15	3	CH(OH)	1.4	120
16	2	NMe	3.8	69
17	2	CH(OH)	2.0	120
18	2	CH_2	4.2	190
19	2	S	4.4	630
20	C-6 isomer of **8**		280	3600
2			1.2	100

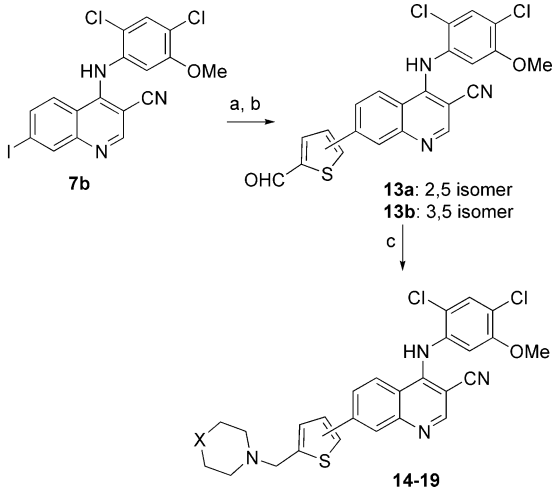
7a with diisopropyl [5-(morpholinomethyl)thien-3-yl]-boronate[8a] under Suzuki coupling conditions provided **8**.[9,10] As shown in Table 1, **8** was a potent inhibitor of Src activity in both the enzymatic and cell assays, showing comparable activity to **2**.[11]

In compound **8**, the thiophene ring has a 3,5-substitution pattern. Four of the five possible thiophene regioisomers of **8**, namely **9**–**12**, were prepared by the routes depicted in Scheme 2.

As shown in Table 1, compounds **9** and **10** wherein the thiophene is substituted in a 2,5- or 2,4-fashion showed Src inhibitory activity comparable to that of **8**, the 3,5-regioisomer, in both the enzymatic and cell assays. These compounds have the solubilizing group pointing away from the 3-quinolinecarbonitrile core and can be considered to be 'linear' analogues as opposed to the 'angular' analogues **11** and **12**. The decreased activity



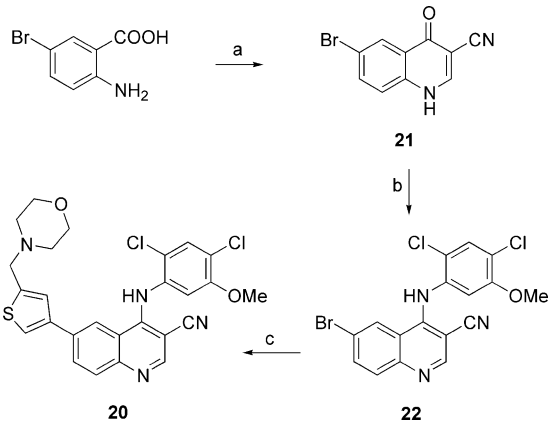
Scheme 2. (a) Morpholine, $NaCNBH_3$, EtOH, AcOH; (b) **7a**, $Pd(PPh_3)_4$, $NaHCO_3$, DME; (c) Br_2, $AlCl_3$; (d) morpholine, EDCI, DMAP; (e) BH_3–Me_2S; (f) *n*-BuLi, DMF.



Scheme 3. (a) Tributyl[5-(1,3-dioxolan-2-yl)-2-thienyl]stannane or tributyl[5-(1,3-dioxolan-2-yl)-3-thienyl]stannane, $(PPh_3)_2PdCl_2$, dioxane; (b) 1 N HCl, THF; (c) amine, $NaB(OAc)_3H$, CH_2Cl_2, DMF, AcOH.

observed with **11** and **12** is not surprising since we previously reported that analogues of **1** with a mono methoxy group at either the C-5 or C-8 position had greatly reduced Src inhibitory activity compared to the mono C-6 or C-7 methoxy analogues.[3]

We next turned our attention to replacing the morpholine group of **8** and **9** with other basic amines. In order to facilitate the preparation of these additional



Scheme 4. (a) (1) DMF–DMA; (2) *n*-BuLi, CH_3CN, THF; (b) (1) $POCl_3$; (2) aniline; (c) (1) tributyl[5-(1,3-dioxolan-2-yl)-3-thienyl]stannane, $(PPh_3)_2PdCl_2$, dioxane; (2) 1 N HCl, THF; (3) morpholine, $NaB(OAc)_3H$, CH_2Cl_2, DMF, AcOH.

analogues, we used the thiophene carboxaldehydes **13a** and **13b** as common intermediates. Palladium catalyzed coupling of tributyl[5-(1,3-dioxolan-2-yl)-3-thienyl]-stannane and tributyl[5-(1,3-dioxolan-2-yl)-2-thienyl]-stannane[12] with **7b** followed by acid hydrolysis of the acetal group provided **13a** and **13b**. Reductive amination with various heterocyclic amines resulted in **14–19** (Scheme 3).[13]

As shown in Table 1, analogues **14–19** had IC_{50}s of 1.4–4.4 nM in the Src enzymatic assay. When tested in the Src cell assay, two compounds with an *N*-methylpiperazine group, namely **14** and **16**, had IC_{50}s that were 3-fold lower than those of the corresponding morpholine analogues **8** and **9**. In addition, **14** and **16** were more potent inhibitors of Src-dependent cell proliferation (IC_{50}s of 64 and 69 nM, respectively) than of Fyn-dependent cell proliferation (IC_{50}s of 580 and 630 nM, respectively).[3]

In our earlier work on 4-anilino-6,7-dialkoxy-3-quinolinecarbonitriles, it was shown that while addition of a water solubilizing group at C-7 was advantageous, addition of the same group at C-6 was detrimental.[3] To determine if this same effect is observed in this series, the C-6 thiophene isomer of **8**, namely **20**, was prepared from 5-bromoanthranilic acid as shown in Scheme 4. As predicted, **20** had greatly reduced activity compared to **8** (see Table 1).

In conclusion, as shown previously with a series of 6,7-dialkoxy-3-quinolinecarbonitriles, 7-thienyl-3-quinolinecarbonitriles are also potent Src inhibitors. The best cell activity was observed with compounds containing an *N*-methylpiperazine group. We are currently extending this work to include other aryl and heteroaryl groups at C-7.

References and Notes

1. (a) Abram, C. L.; Courtneidge, S. A. *Exp. Cell Res.* **2000**, *254*, 1. (b) Boschelli, D. H.; Boschelli, F. *Drug. Fut.* **2000**, *25*, 717. (c) Bjorge, J. D.; Jakymiw, A.; Fujita, D. J. *Oncogene* **2000**, *19*, 5620. (d) Irby, R. B.; Yeatman, T. J. *Oncogene* **2000**, *19*, 5636. (e) Missbach, M.; Altmann, E.; Susa, M. *Curr. Opin. Drug Discov. Dev.* **2000**, *3*, 541. (f) Susa, M.; Teti, A. *Drug News Perspect.* **2000**, *13*, 169. (g) Paul, R.; Zhang, Z. G.; Eliceiri, B. P.; Jiang, Q.; Boccia, A. D.; Zhang, R. L.; Chopp, M.; Cheresh, D. A. *Nat. Med.* **2001**, *7*, 222.

2. (a) Hanke, J. H.; Gardner, J. P.; Dow, R. L.; Changelian, P. S.; Brissette, W. H.; Weringer, E. J.; Pollok, B. A.; Connelly, P. A. *J. Biol. Chem.* **1996**, *271*, 695. (b) Missbach, M.; Altmann, E.; Widler, L.; Susa, M.; Buchdunger, E.; Mett, H.; Meyer, T.; Green, J. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 945. (c) Widler, L.; Green, J.; Missbach, M.; Susa, M.; Altmann, E. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 849. (d) Altmann, E.; Missbach, M.; Green, J.; Susa, M.; Wagenknecht, H.-A.; Widler, L. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 853. (e) Arnold, L. D.; Calderwood, D. J.; Dixon, R. W.; Johnston, D. N.; Kamens, J. S.; Munschauer, R.; Rafferty, P.; Ratnofsky, S. E. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2167. (f) Burchat, A. F.; Calderwood, D. J.; Hirst, G. C.; Holman, N. J.; Johnston, D. N.; Munschauer, R.; Rafferty, P.; Tometzki, G. B. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2171. (g) Klutchko, S. R.; Hamby, J. M.; Boschelli, D. H.; Wu, Z. P.; Kraker, A. J.; Amar, A. M.; Hartl, B. G.; Shen, C.; Klohs, W. D.; Steinkampf, R. W.; Driscoll, D. L.; Nelson, J. M.; Elliott, W. L.; Roberts, B. J.; Stoner, C. L.; Vincent, P. W.; Dykes, D. J.; Panek, R. L.; Lu, G. H.; Major, T. C.; Dahring, T. K.; Hallak, H.; Bradford, L. A.; Showalter, H. D. H.; Doherty, A. M. *J. Med. Chem.* **1998**, *41*, 3276. (h) Thompson, A. M.; Rewcastle, G. W.; Boushelle, S. L.; Hartl, B. G.; Kraker, A. J.; Lu, G. H.; Batley, B. L.; Panek, R. L.; Showalter, H. D. H.; Denny, W. A. *J. Med. Chem.* **2000**, *43*, 3134. (i) Schroeder, M. C.; Hamby, J. M.; Connolly, C. J. C.; Grohar, P. J.; Winters, R. T.; Barvian, M. R.; Moore, C. W.; Boushelle, S. L.; Crean, S. M.; Kraker, A. J.; Driscoll, D. L.; Vincent, P. W.; Elliott, W. L.; Lu, G. H.; Batley, B. L.; Dahring, T. K.; Major, T. C.; Panek, R. L.; Doherty, A. M.; Showalter, H. D. H. *J. Med. Chem.* **2001**, *44*, 1915. (j) Myers, M. R.; Setzer, N. N.; Spada, A. P.; Zulli, A. L.; Hsu, C.-Y. J.; Zilberstein, A.; Johnson, S. E.; Hook, L. E.; Jacoski, M. V. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 417.

3. Boschelli, D. H.; Wang, D. Y.; Ye, F.; Wu, B.; Zhang, N.; Dutia, M.; Powell, D. W.; Wissner, A.; Arndt, K.; Weber, J. M.; Boschelli, F. *J. Med. Chem.* **2001**, *44*, 822.

4. Boschelli, D. H.; Ye, F.; Wang, D. Y.; Dutia, M.; Johnson, S.; Wu, B.; Miller, K.; Powell, D. W.; Arndt, K.; Discafani, C.; Etienne, C.; Gibbons, J.; Grod, J.; Lucas, J.; Weber, J. M.; Boschelli, F. *J. Med. Chem.* **2001**, *44*, 3965.

5. Wang, Y. D.; Miller, K.; Boschelli, D. H.; Ye, F.; Wu, B.; Floyd, M. B.; Powell, D. W.; Wissner, A.; Weber, J. M.; Boschelli, F. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2477.

6. (a) Wissner, A.; Berger, D. M.; Boschelli, D. H.; Floyd, M. B.; Greenberger, L. M.; Gruber, B. C.; Johnson, B. D.; Mamuya, N.; Nilakatan, R.; Reich, M. F.; Shen, R.; Tsou, H. R.; Upeslacis, E.; Wang, Y. F.; Wu, B.; Ye, F.; Zhang, N. *J. Med. Chem.* **2000**, *43*, 3244. (b) Torrance, C. J.; Jackson, P. E.; Montgomery, E.; Kinzler, K. W.; Vogelstein, B.; Wissner, A.; Nunes, M.; Frost, P.; Discafani, C. M. *Nat. Med.* **2000**, *6*, 1024.

7. (a) Zhang, N.; Wu, B.; Powell, D.; Wissner, A.; Floyd, M. B.; Kovacs, E. D.; Toral-Barza, L.; Kohler, C. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2825. (b) Zhang, N.; Wu, B.; Eudy, N.; Wang, Y.; Ye, F.; Powell, D.; Wissner, A.; Feldberg, L. R.; Kim, S. C.; Mallon, R.; Kovacs, E. D.; Toral-Barza, L.; Kohler, C. A. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1407.

8. (a) Barker, A. J.; Johnstone, C. US Patent 5,814,630, Sept 29, 1998. (b) Reviewed in: Bridges, A. J. *Chem. Rev.* **2001**, *10*, 2541.

9. Preparation of **8**: 4-(2,4-Dichloro-5-methoxyanilino)-7-[5-(4-morpholinylmethyl) - 3 - thienyl] - 3 - quinoline - carbonitrile. 1-[(4-Bromo-2-thienyl)methyl]-morpholine (248 mg, 0.95 mmol)[8a] was dissolved in 20 mL of THF and the solution was cooled to −78 °C. Tri-isopropylborate (196 mg, 1.04 mmol) was added followed by 2.5 M *n*-BuLi in hexane (0.39 mL, 97 mmol). The mixture was stirred at −78 °C for 30 min and then allowed to warm to room temperature. The solvent was removed in vacuo to provide the intermediate boronate. A mixture of this boronate, **7a** (200 mg, 0.47 mmol) and $(Ph_3P)_4Pd$ (96 mg, 0.08 mmol) was heated at reflux in 8 mL of DME and 5 mL of saturated $NaHCO_3$ for 4.5 h. The reaction was cooled to room temperature, quenched with 5 mL of 1.0 N NaOH, and partitioned between ethyl acetate and brine. The layers were separated and the ethyl acetate layer was dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by flash column chromatography eluting with 20% MeOH in CH_2Cl_2 to provide 98 mg (41% yield) of **8**: mp 170 °C; ^{1}H NMR (DMSO-d_6) δ 2.35–2.50 (m, 4H), 3.55–3.65 (m, 4H), 3.75 (s, 2H), 3.87 (s, 3H), 7.42 (s, 1H), 7.68 (s, 1H), 7.77 (s, 1H), 8.08 (d, $J = 9$ Hz, 1H), 8.17 (s, 1H), 8.22 (s, 1H), 8.54 (d, $J = 9$ Hz, 1H), 8.57 (s, 1H), 9.99 (s, 1H); MS (ES) 525.1 (M + H). Analysis for $C_{26}H_{22}Cl_2N_4O_2S$: Calcd: C, 59.43; H, 4.22; N, 10.66. Found: C, 59.28; H, 3.93; N, 10.61.

10. All compounds were characterized by MS, NMR and CHN combustion analysis.

11. Compounds were tested in a modified format of the enzymatic assay previously reported.[3–5] Peptide was bound to the streptavidin plate prior to the kinase reaction, and peptide phosphorylation reaction was monitored by europium fluorescence as recommended by the manufacturer (Perkin–Elmer). The IC_{50} values reported represent the means of at least two separate determinations with typical variations of less than 40% between replicate values. For cell assays, Costar ultra-low binding plates were coated with Sigma-Cote to block residual cell attachment.

12. Tributyl[5-(1,3-dioxolan-2-yl)-2-thienyl]stannane was prepared from 2-thiophenecarboxaldehyde according to the procedure used to prepare tributyl[5-(1,3-dioxolan-2-yl)-2-thienyl]stannane from furancarboxaldehyde. Yamamoto, M.; Izukawa, H.; Saiki, M.; Yamada, K. *J. Chem. Soc. Chem. Commun.* **1988**, *8*, 560. Tributyl[5-(1,3-dioxolan-2-yl)-3-thienyl]stannane was prepared from 4-bromo-2-thiophene-carboxaldehyde by conversion of the aldehyde to the acetal with ethylene glycol, followed by the addition of *n*-BuLi to a −78 °C solution of the acetal and $(n\text{-Bu})_3SnCl$ in THF.

13. Preparation of **14**: 4-(2,4-Dichloro-5-methoxyanilino)-7-{5-[(4-methyl-1-piperazinyl)methyl]-3-thienyl}-3-quinolinecarbonitrile. A mixture of **7c** (2.5 g, 5.32 mmol), tributyl[5-(1,3-dioxolan-2-yl)-3-thienyl]stannane (2.98 g, 6.69 mmol) and a catalytic amount of $(Ph_3P)_2PdCl_2$ in 60 mL of dioxane was heated at reflux for 4.5 h. The reaction mixture was concentrated in vacuo and partitioned between ethyl acetate and water. The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was suspended in a mixture of 100 mL of THF and 50 mL of 1 N HCl and stirred at room temperature overnight. The reaction mixture was slowly added to saturated $NaHCO_3$ and then extracted into ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated in vacuo. Ethyl acetate was added to the residue and the solid was collected by filtration to provide 1.20 g of **13b**. To a 0 °C suspension of **13b** (220 mg, 0.48 mmol) in 3 mL of CH_2Cl_2 and 1 mL of DMF were added *N*-methylpiperazine (80 μL, 0.72 mmol) and $NaB(OAc)_3H$ (500 mg, 2.36 mmol) followed by 1 drop of AcOH. The reaction mixture was stirred at room temperature for 3 h then partitioned between ethyl acetate and saturated $NaHCO_3$. The organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by flash column chromatography eluting with 20% MeOH in CH_2Cl_2 to provide 152 mg (59% yield) of **14**: mp 206–209 °C; ^{1}H NMR (DMSO-d_6) δ 2.16 (s, 3H), 2.35 (br m, 4H), 2.49 (br m, 4H), 3.73 (s, 2H), 3.85 (s, 3H), 7.30 (s, 1H), 7.62 (s, 1H), 7.71 (s, 1H), 8.01 (d, $J = 7$ Hz, 1H), 8.11 (s, 2H), 8.46–8.53 (m, 2H); MS (ES) 538.2, 540.2 (M + H). Analysis for $C_{27}H_{25}Cl_2N_5OS$: Calcd: C, 60.22; H, 4.68; N, 13.01. Found: C, 59.85; H, 4.60; N, 13.23.