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Syntheses and EGFR and HER-2 Kinase Inhibitory Activities of 4-Anilinoquinoline-3-carbonitriles: Analogues of Three Important 4-Anilinoquinazolines Currently Undergoing Clinical Evaluation as Therapeutic Antitumor Agents

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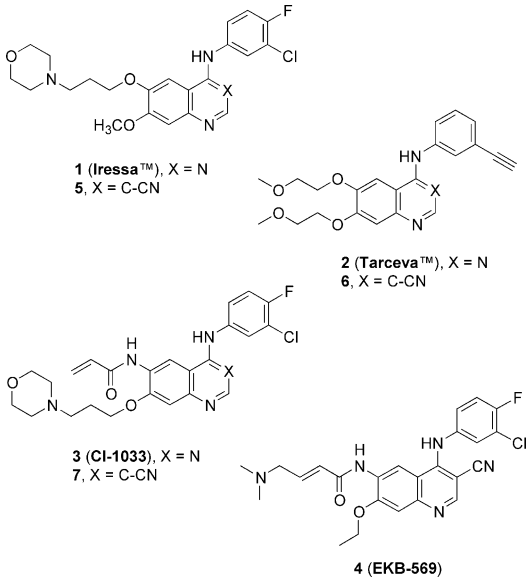
Abstract—The syntheses and biological evaluations of 4-anilinoquinoline-3-carbonitrile analogues of the three clinical lead 4-anilinoquinazolines Iressa™, Tarceva™, and CI-1033 are described. The EGFR and HER-2 kinase inhibitory activities and the cell growth inhibition of the two series are compared with each other and with the clinical lead EKB-569. Similar activities are observed between these two series.

Receptor protein tyrosine kinases play a key role in signal transduction pathways that regulate cell division and differentiation. Overexpression of certain growth factor receptor kinases such as Epidermal Growth Factor Receptor (EGFR) as well as the related Human Epidermal Growth Factor Receptor (HER-2, also known as erbB-2) are markers for poor prognosis in many human cancers.[1,2] Compounds which inhibit the kinase activity of EGFR and/or HER-2 after binding of its cognate ligand, are of potential interest as new therapeutic antitumor agents.[3] The middle of the past decade was marked by the discovery of the 4-anilinoquinazolines, a series of potent and selective ATP-competitive inhibitors of EGFR and HER-2 kinases.[4,5] A number of these inhibitors have, by now, entered clinical trial.[6] One of the first to do so was the compound Iressa™, **1**, developed by the group at AstraZeneca.[7] This compound is currently in Phase III trial. A second inhibitor, currently under development by Oncogene Sciences, is Tarceva™ (OSI-774), **2**.[8] This compound is also in Phase III trial.



While the above two compounds function as conventional reversible binding inhibitors of the target enzymes, other groups,[9] including ourselves,[10] have been working with irreversible binding inhibitors. These irreversible inhibitors are believed to function in this

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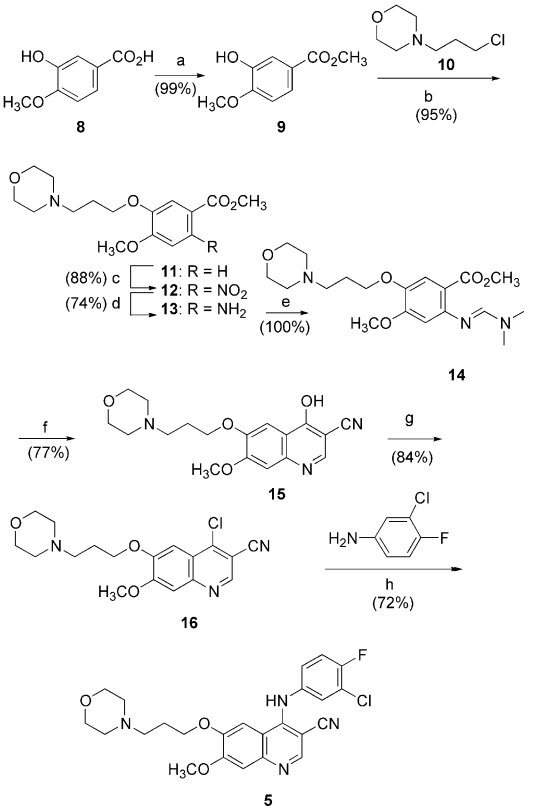
manner by virtue of the fact that they form a covalent bond to a Cys residue (Cys 773 in EGFR and Cys 805 in HER-2) located in the ATP binding pocket of these enzymes. The Parke Davis group, now part of Pfizer, is studying CI-1033, **3**.[11] This compound is now in early clinical trial.

We recently described the discovery and EGFR kinase inhibitory activity of a class of 4-anilinoquinoline-3-carbonitriles.[12] These inhibitors were derived, based on modeling studies, from the 4-anilinoquinazolines. In subsequent reports, we also described the inhibition of Src[13] and MAP[14] kinases by members of this series. Ultimately, this series was optimized resulting in the irreversible binding EGFR kinase inhibitor EKB-569, **4**.[15] We recently initiated a Phase I clinical trial with this compound.

Given the obvious importance of the quinazolines **1**–**3**, we prepared the corresponding quinoline-3-carbonitrile analogues of these compounds **5**–**7**. This paper describes these syntheses and presents the results of a comparison of the in vitro activities associated with these compounds.

The synthesis of **5**, the cyanoquinoline analogue of Iressa™, is shown in Scheme 1. Fisher esterification of **8** gave **9** in good yield. Alkylation of the hydroxyl group of **9** with the halide **10** using K_2CO_3 in refluxing 2-butanone in the presence of the phase transfer catalyst tri-*n*-butyl ammonium iodide (TBAI) gave **11**. This could be nitrated in acetic acid selectively to give **12**. Reduction of the nitro group to give **13** followed by amidine formation using DMF acetal gave intermediate **14**. Cyclization to the hydroxyquinoline was accomplished by treating **14** with the lithium anion of CH_3CN in THF at −78 °C, quenching with HOAc, and warming to room temperature. The chloroquinoline **16** was prepared by refluxing **15** in an excess of $SOCl_2$ followed by an extractive basic workup. Finally, the aniline group was introduced giving **5** by refluxing 3-chloro-4-fluoroaniline and **16** in ethoxyethanol using a catalytic amount of pyridine hydrochloride.



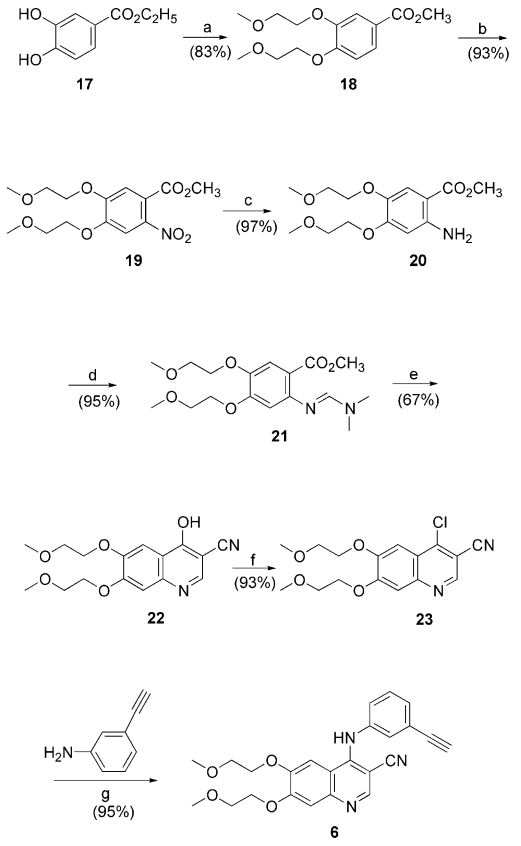
Scheme 1. Synthesis of the Iressa™ analogue **5**: (a) H_2SO_4, CH_3OH, reflux; (b) TBAI, K_2CO_3, 2-butanone, reflux; (c) HNO_3, HOAc; (d) H_2, Pd/C, EtOAc, EtOH; (e) DMF-DMA; (f) *n*-BuLi, CH_3CN, THF, −78 °C then HOAc, 25 °C; (g) $SOCl_2$, reflux; (h) $EtOCH_2CH_2OH$, pyridine-HCl (cat), reflux.

A similar strategy was used to prepare **6**, the cyanoquinoline analogue of Tarceva™. This is shown in Scheme 2. The diol **17** was bis-alkylated with 1-bromo-2-methoxy-ethane using K_2CO_3 and a catalytic amount of TBAI in refluxing acetone. Nitration of **18** in acetic acid was accomplished selectively to give **19**. Reduction of the nitro group using iron powder and HOAc in refluxing EtOH furnished **20**. In this case, the amidine **21** was prepared using DMF and $POCl_3$. This compound was cyclized to the hydroxyquinoline **22** using the lithium anion of CH_3CN as described above. Chlorinating of **22** was accomplished in refluxing $POCl_3$. The chloro derivative **23** was then reacted with 3-ethynylaniline in refluxing ethoxyethanol in the presence of a catalytic amount of pyridine-HCl to give **6** in good yield.

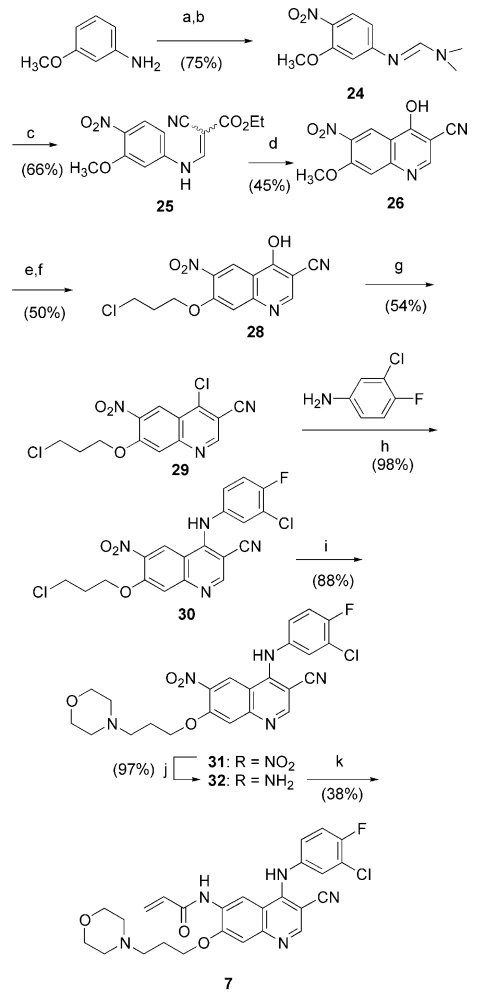
A different method, that of Bredereck,[16] was used to construct the quinoline-3-carbonitrile ring system of **7**, the analogue of the irreversible binding inhibitor CI-1033 as shown in Scheme 3. The dimethylformamidine derivative of 3-methoxyaniline was nitrated under anhydrous conditions in HOAc to give the intermediate **24**. Reaction of **24** with $NCCH_2CO_2Et$ gave **25** as a mixture of isomers which on thermal cyclization in refluxing Dowtherm gave the 4-hydroxyquinoline **26**. The intermediate **26** was then demethylated by heating in molten pyridine hydrochloride and then realkylated with tosylate **27** using K_2CO_3 in DMF at 75 °C to introduce the 3-carbon side chain and give **28**. The 4-chloro substituent was introduced by refluxing in an excess of $POCl_3$. The 4-anilino group was incorporated by refluxing 3-chloro-4-fluoroaniline and **29** in isopropanol. The aliphatic chloro group of **30** was replaced with the morpholine group by heating a toluene solution of the compound with excess morpholine and catalytic quantities of NaI and the phase transfer catalyst TBAI.

The nitro group was reduced with iron powder and HOAc in refluxing methanol. Finally, the amino group of **32** was acylated with acryloyl chloride and Hunig's base in THF to introduce the Michael acceptor functional group to give desired inhibitor **7**.

The biological results for the quinazoline inhibitors **1**–**3** and the respective quinoline-3-carbonitrile inhibitors **5**–**7** are shown in Table 1. For comparison we also include data for our own inhibitor **4** (EKB-569). These com-



Scheme 2. Synthesis of the Tarceva™ analogue **6**: (a) $CH_3OCH_2CH_2Br$, K_2CO_3, TBAI, acetone, reflux; (b) HNO_3, HOAc, 40 °C; (c) Fe, HOAc, EtOH, reflux; (d) $POCl_3$, DMF, 55 °C (e) *n*-BuLi, CH_3CN, THF, −78 °C then HOAc, 25 °C; (f) $POCl_3$, reflux; (g) pyridine-HCl (cat), $EtOCH_2CH_2OH$, reflux.



Scheme 3. Synthesis of the CI-1033 analogue **7**: (a) DMF–DMA; (b) HNO_3, HOAc, Ac_2O; (c) $NCCH_2CO_2Et$, HOAc, reflux; (d) Dowtherm, reflux; (e) pyridine-HCl, 210 °C; (f) $Cl(CH_2)_3OTs$ (**27**), K_2CO_3, DMF, 75 °C; (g) $POCl_3$, reflux; (h) *i*-PrOH, reflux; (i) morpholine, NaI (cat), TBAI (cat), toluene, reflux; (j) Fe, HOAc, MeOH, reflux; (k) acryloyl chloride, *i*-Pr_2NEt, THF, 0 °C.

pounds were evaluated for their ability to inhibit the autophosphorylation of EGFR and HER-2 kinases using a solid-phase ELISA assay. We previously pointed out that the IC_{50} values that we measure in these kinase assays are routinely higher than determinations made by other workers and the reasons for this are discussed in an earlier publication.[10a] The compounds were also evaluated for their ability to inhibit the growth of several cell lines; these data are also shown in Table 1. Three human carcinoma cell lines were used: A431 (epidermoid) which over-expresses EGFR, SKBR3 (breast) which over-expresses HER-2 and to a lesser extent EGFR, and SW620 (colon) which is believed not to express either EGFR or HER-2 to a significant extent. Full experimental details for these assays have already been reported.[10a]

Table 1. EGFR and HER-2 kinase and cell growth inhibition assay results

Compd[a]	Enzyme assays		Cell-based assays		
	EGFR[b] (μM)	HER-2[b] (μM)	A431[c] (μg/mL)	SKBR3[c] (μg/mL)	SW620[c] (μg/mL)
1	0.515	1.604	0.276	0.174	>5
5	1.04	14.70	0.250	0.332	>5
2	1.45	18.52	0.608	0.868	>5
6	0.850	11.75	0.173	0.071	0.680
3	0.074	0.529	0.083	0.102	1.450
7	0.080	1.692	0.120	0.030	0.770
4	0.083	1.229	0.030	0.007	0.317

[a]All new compounds were fully characterized by NMR, MS, and elemental analyses; see ref 17.
[b]Concentration in μM needed to inhibit the autophosphorylation of the cytoplasmic domain of the enzyme by 50% as determined from the dose–response curve. Values are averages of duplicate determinations.
[c]Dose–response curves were determined at five concentrations. The IC_{50} values are the concentrations in μg/mL needed to inhibit cell growth by 50% as determined from these curves. Reported IC_{50} values are averages of at least two determinations.

With respect to the cell assays, it is evident that each compound is a better inhibitor of the A431 and SKBR3 cell lines than the SW620 line. This is consistent with the mechanism of cell growth inhibition being reliant, to some degree, on the target kinases. The fact that some of these compounds do inhibit the growth of the SW620 line at higher concentrations could suggest that this line has some dependence on EGFR or HER-2 even though these receptors are not expressed to a significant degree or that these compounds inhibit cell growth by an undefined mechanism at these concentrations.

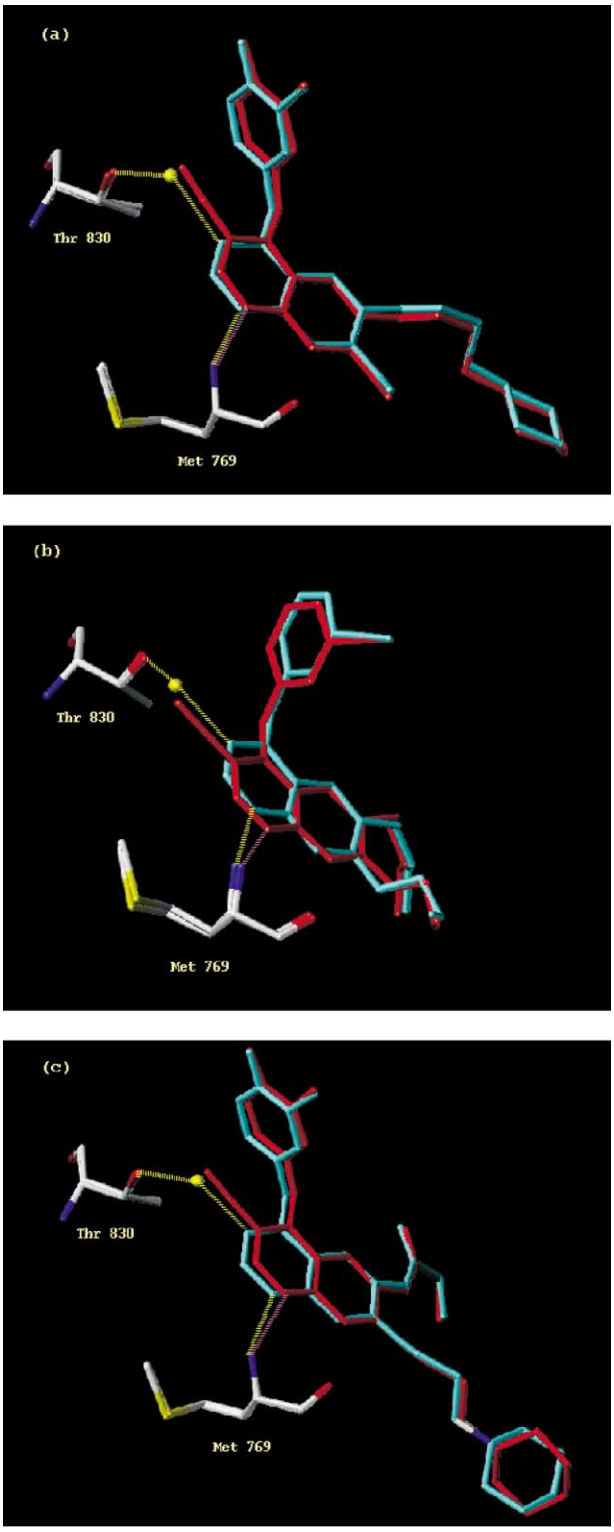


Figure 1. Binding models of **1**–**3** and the quinoline-3-carbonitrile counterparts **5**–**7**. (a) Overlap of **1** (cyan), and **5** (red). (b) Overlap of **2** (cyan), and **6** (red). (c) Overlap of **3** (cyan) and **7** (red). In each case, note the H-bond to Met 769 and the water-bridged H-bond to Thr 830. The hydrogen-bonds between the quinoline-3-carbonitrile N1 and Met 769 are shown in magenta, while the other hydrogen bonds are in yellow.

On the whole, the activities of the quinazoline inhibitors compared to those of the respective quinoline-3-carbonitrile inhibitors are similar in both the enzyme and cell assays. With respect to EGFR kinase inhibition, activities between related pairs of compounds differ by no more than 2-fold. A similar situation is evident with respect to HER-2 inhibition except for the pair **1** and **5** where the quinazoline appears to be a significantly better inhibitor of this enzyme than the quinoline-3-carbonitrile. However, this large difference in potency is not evident in the cell assays. With respect to the cell assays, again, there is no major difference in activities when comparing related pairs with IC_{50}'s differing no more than 3-fold with the exception of the pair **2** and **6** where, in this case, the quinoline-3-carbonitrile **6** is significantly more potent than the related quinazoline **2**. There is a trend suggesting that the irreversible binding inhibitors **3**, **4**, and **7** appear to be somewhat more potent inhibitors of the enzymes and of cell growth compared to the reversible binding inhibitors **1**, **2**, **5**, and **6**.

In our earlier work,[12] we proposed binding models for both the quinazoline and quinoline-3-carbonitrile class of inhibitors at the ATP binding pocket of EGFR using a homology model of the enzyme which was constructed based on two other kinases, FGF Receptor-1 for the N-terminal lobe and Hematopoietic Cell Kinase (HcK) for the C-terminal lobe. With these binding models, we suggested that these two types of inhibitors bind in a very similar manner where the N3 atom of the quinazoline inhibitor interacts with a bridging water molecule which, in turn, interacts with a residue on the protein backbone (Thr 830). This is similar to the situation that has been observed for a 4-anilinoquinazoline complexed to P38-MAP kinase.[18] For the quinoline-3-carbonitrile based inhibitors, we proposed that this water molecule is displaced and the nitrogen atom of the cyano group interacts with this same residue. In essence, we are suggesting that the carbocyano group of the cyanoquinoline is bioisosteric with the azomethine group of the quinazoline hydrogen bonded to the water molecule. Such a situation has actually been demonstrated with an unrelated enzyme.[19] In addition, given the electron withdrawing properties of a cyano group, the charge distribution within the quinoline-3-carbonitrile ring system was shown to be similar to that of the quinazoline ring system with its H-bonded water molecule. Continuing with the same theme, we built models for the quinazolines **1**–**3** and their respective cyanoquinoline counterparts **5**–**7** complexed to EGFR kinase. We docked each compound into the ATP-binding site in a manner similar to our previous work. The complexes were energy minimized using the CharmM forcefield as implemented in Quanta™ software.[20] As in our previous work, we allowed the entire

system of protein, ligand, and water molecules to move during minimization. As one might expect, the binding models of the quinazoline and quinoline-3-carbonitrile versions were very similar in each case. Figure 1 shows the three pairs of models. In each case, the N1 atom of the quinazoline is hydrogen-bonded to the backbone nitrogen of Met 769, while the N3 atom is hydrogen-bonded to the sidechain hydroxyl of Thr 830 via a water molecule. In the quinoline-3-carbonitrile counterparts, the N1 atom is similarly hydrogen-bonded to Met 769 while the water molecule is displaced by the cyano group with the nitrogen atom of that group within a distance where it can interact with the Thr residue.

Given the similar binding modes predicted by the models for these two classes of inhibitors, the conclusion of the present study, namely, that quinazoline-based compounds **1**–**3** have similar inhibitory activities compared to the respective quinoline-3-carbonitrile compounds **5**–**7**, is not surprising.

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17. (a) For **5**: ^{1}H NMR (DMSO-d_6) δ 9.47 (s, 1H), 8.51 (s, 1H), 7.73 (s, 1H), 7.4–7.3 (m, 1H), 7.39 (s, 1H), 7.30 (s, 1H), 7.3–7.2 (m, 2H), 4.41 (m, 2H), 4.23 (m, 2H), 4.22 (s, 1H), 3.75 (m, 4H), 3.36 (s, 3H), 3.34 (s, 3H); MS (ESI) *m*/*z* 418.1 $(M+H)^+$. Anal. calcd for $C_{24}H_{23}N_3O_4$: C, 69.05; H, 5.55; N, 10.07. Found: C, 68.91; H, 5.40; N, 9.98. (b) For **6**: ^{1}H NMR (DMSO-d_6) δ 9.53 (s, 1H), 8.48 (s, 1H), 7.70 (s, 1H), 7.52 (m, 1H), 7.44 (d, $J=9.0$ Hz), 7.34 (s, 1H), 7.28 (m, 1H), 4.14 (t, $J=6.3$ Hz, 2H), 3.96 (s, 3H), 3.57 (m, 4H), 2.45 (t, $J=7.0$ Hz, 2H), 2.37 (m, 4H), 1.97 (m, 2H); MS (ESI) *m*/*z* 470.9, 473.0 $(M+H)^+$. Anal. calcd for $C_{24}H_{24}ClFN_4O_3$ $(0.5H_2O)$: C, 60.06; H, 5.25; N, 11.67. Found: C, 60.43; H, 5.23; N, 11.69. (c) For **7**: ^{1}H NMR (DMSO-d_6) δ 9.73 (s, 1H), 9.57 (s, 1H), 8.93 (s, 1H), 7.47 (m, 1H), 7.45 (s, 1H), 7.40 (d, $J=9.1$ Hz, 1H), 7.25 (m, 1H), 6.73 (dd, $J=17.0$, 10.1 *Hz*, 1H), 6.29 (dd, $J=17.0$, 1.9 Hz, 1H), 5.81 (dd, $J=10.1$, 1.9 Hz, 1H), 4.31 (t, $J=6$ Hz, 2H), 3.58 (m, 4H), 2.5–2.4 (m, 6H), 2.01 (m, 2H); MS (ESI) *m*/*z* 510.3, 512.2 $(M+H)^+$. Anal. calcd for $C_{26}H_{25}ClFN_5O_3$ $(0.5H_2O)$: C, 60.17; H, 5.05; N, 13.49. Found: C, 60.07; H, 5.20; N, 13.20.
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