Acrylamide Functional Group Incorporation Improves Drug-like Properties: An Example with EGFR Inhibitors

Kuen-Da Wu,[†] Grace Shiahuy Chen,[‡] Jia-Rong Liu,[†] Chen-En Hsieh,[‡] and Ji-Wang Chern^{*,†}[†]School of Pharmacy and Center for Innovative Therapeutics Discovery, National Taiwan University, Taipei 10055, Taiwan[‡]Department of Applied Chemistry, Providence University, Taichung 43301, Taiwan

Supporting Information

ABSTRACT: We demonstrate that the acrylamide group can be used to improve the drug-like properties of potential drug candidates. In the EGFR inhibitor development, both the solubility and membrane permeability properties of compounds **6a** and **7**, each containing an acrylamide group, were substantially better than those of gefitinib (**1**) and AZD3759 (**2**), respectively. We demonstrated that incorporation of an acrylamide moiety could serve as a good strategy for improving drug-like properties.

KEYWORDS: Acrylamide, EGFR inhibitors, hydrophilicity, lipophilicity

Molecules containing an acryloyl functional group constitute an important class of biologically active compounds, characterized by the formation of a covalent bond with the target enzyme through Michael addition. Over the past decades, this approach has had limited success in clinical applications since it will induce unwanted side effects by reacting with various off-targets.^{1,2} Recently, the advancement of targeted therapy has led to a better understanding of the balances between unavoidable side effects and efficacy in the treatment oncology diseases.^{3,4} Kinase inhibitors afatinib and ibrutinib, recently approved by the U.S. Food & Drug Administration (FDA), belong to the class of acryloyl compounds. This type of drug has advantages in terms of lengthening the residence time through the formation of a covalent bond with the targeted enzymes (Figure 1).⁵

Figure 1. FDA-approved irreversible inhibitors.

Gefitinib (**1**) came to the market as the first-generation epidermal growth factor receptor (EGFR) inhibitor for the treatment of nonsmall cell lung cancer (NSCLC).⁶ Due to drug resistance, scientists have developed the fourth-generation EGFR tyrosine kinase inhibitors (TKIs) that enable comparatively prolonged survival rates in patients.⁷ However, metastasis to the CNS is observed in about 50% of treated lung cancer patients. Unfortunately, no drug has yet been developed to overcome this issue.⁸ AZD3759 (**2**), as an alternative to EGFR-TKIs, is in Phase 1 clinical trials, and it may have a potential use advantage since it penetrates the blood–brain barrier (BBB).⁹ Designing drug candidates by functional group modification to penetrate the BBB remains a major challenge in medicinal chemistry.

Poor aqueous solubility and high lipophilicity are both obstacles to drug absorption, increasing the risk of the patient experiencing microsomal clearance, toxicity, and other side effects.¹⁰ To increase either cell permeability or solubility of lead compounds without detriment to the potency is a challenging task in drug development. Previous approaches to overcome solubility and permeability problems have been the

Received: June 15, 2018

Accepted: December 6, 2018

Published: December 6, 2018

introduction of functional groups such as methyl, phenyl, piperazine, morpholine, sugar, and amino acid.^{11,12}

Log *P* is a tool to evaluate lipophilicity of the compound. Generally speaking, the cLog *P* value for a potential drug candidate should range between 0 to 5. When this value is over 5, however, it is considered a high-risk compound for development because of anticipated poor pharmacokinetics profile for its difficulty in crossing cell membrane. The ideal value for a drug candidate is ~2 to 3 (BBB-penetrating drugs have values close to 2).¹³ A previous study has shown that more than 50% of high-potency compounds possess cLog *P* values of over 4.25.¹⁴ A widely used approach for decreasing the cLog *P* value of a compound involves the addition of a basic group, but this also increases the number of hydrogen bond acceptors, which is not beneficial for BBB penetration.¹⁵

As a general rule, a balance between potency and lipophilicity in molecules results in reasonable efficacy and pharmacokinetics clinical drug candidates. The enzymatic interactions between afatinib and EGFR have been described.¹⁶ The acryloyl group at the 6-position demonstrated a covalent bond with Cys₇₉₇.⁵ Replacement of the *N*-hydrogen atom at the 4-position with a methyl group decreased the binding activity. Thus, the NH group at the 4-position has been considered to form critical hydrogen bond in the active site of enzyme. In this study, we chose EGFR inhibitors gefitinib (1) and AZD3759 (2) as lead compounds because of their well-documented interactions with the active sites of the enzymes. Compounds 6 and 7 were designed by introducing an acryloyl group to the 4-anilinoquinazoline to study the effects of the physicochemical properties of acrylamide derivatives on the lead compounds gefitinib (1) and AZD3759 (2), respectively (Figure 2). We assumed that the

Figure 2. Design of EGFR inhibitors.

N-acryloyl group at the 4-position of the quinazoline core would not form covalent bond with the target enzyme, acryloyl group, situated away from residue Cys₇₉₇, but it might provide better physicochemical properties. The solubility and lipophilicity of the designed target compounds are also investigated in this study.

At the outset, commercially available 7-methoxy-6-(3-morpholinopropoxy)-3,4-dihydroquinazolin-4-one (3) was treated with phosphorus oxychloride at 80 °C to provide key intermediate 4 according to the reported method¹⁷ (Scheme 1). Then, compound 4 was reacted with a variety of anilines in the presence of acetic acid to obtain 4-anilinoquinazoline derivatives 5b–l. Subsequent treatment with acryloyl chloride

Scheme 1. Synthetic Route to Compounds 6a–l and 7^a

^aReagents and conditions: (a) POCl₃, Et₃N, 80 °C, 6 h; (b) substituted aniline, AcOH, 80 °C, 7 h; (c) acryloyl chloride, Et₃N, CH₂Cl₂, 8 h.

furnished the acrylamide derivatives 6b–l. Similarly, commercially available gefitinib (1) and AZD3759 (2) were reacted with acryloyl chloride to give compounds 6a and 7, respectively.

Two types of acryloyl quinazoline compounds 6a–l and 7 were subjected to EGFR enzyme kinetic assay (Table 1). Gefitinib derivative 6a exhibited an IC₅₀ value of 65.2 nM, less potent by over 100-fold than that of gefitinib (1) (IC₅₀ < 0.5 nM). When the 3'-chlorine atom of 6a (3'-chloro-4'-fluoro derivative) was replaced with either a methyl or trifluoromethyl

Table 1. Structure–Activity Relationships for EGFR Inhibitors Obtained by Enzyme Assay

compd	Structure		EGFR IC ₅₀ (nM)
	R ¹	R ²	
1	H	3-Cl, 4-F	<0.5
6a	acryloyl	3-Cl, 4-F	65.2
6b	acryloyl	3-Me, 4-F	499
6c	acryloyl	3-CF ₃ , 4-F	>1000
6d	acryloyl	3-CF ₃	844
6e	acryloyl	3-F, 4-F	368
5f	H	3-Cl, 4-Cl	1.0
6f	acryloyl	3-Cl, 4-Cl	31.7
5g	H	2-F, 3-Cl	<0.5
6g	acryloyl	2-F, 3-Cl	52.6
6h	acryloyl	2-F, 4-Cl	234
6i	acryloyl	2-F, 5-Cl	133
6j	acryloyl	2-F, 4-OMe	>1000
6k	acryloyl	2-F, 4-F	302
6l	acryloyl	2-F, 4-F, 5-F	500
2	H		<0.5
7	acryloyl		8.7

group, the resulting compounds **6b** and **6c** lost activity with IC_{50} values of 499 nM and $>1.0 \mu M$, respectively. We assumed that the decreased inhibitory activity might be due to the steric effect.

Next, 3',4'-difluoro (**6e**) and 3',4'-dichloro (**6f**) derivatives were prepared, and they exhibited enzyme inhibitory activity with IC_{50} values of 368 and 31.7 nM, respectively. Replacement of the 3'-trifluoromethyl of **6c** by a fluorine atom (**6e**, IC_{50} = 368 nM) enhanced the potency against EGFR kinase. Surprisingly, 3',4'-dichloro derivative **6f** exhibited an increased enzyme inhibitory activity with an IC_{50} value of 31.7 nM.

It should be pointed out that there is a slight difference from 4'-fluoro to 4'-chloro between compound **1** and **5f**: the activity of **1** (IC_{50} < 0.5 nM) against the enzyme is better than that of **5f** (IC_{50} = 1.0 nM). However, when the acryloyl group was onto the 4-*N*-position of **1** and **5f**, the enzyme inhibitory activity of **6f** is 2-fold better than that of **6a**. This indicates acryloyl moiety might play an important role in changing the physicochemical properties related to biological activity.

It is known that a fluorine atom at the 2'-position of aniline was beneficial to form an intramolecular halogen bond with the amide group.^{18,19} We introduced a fluorine at the 2'-position to investigate the effect of the anilino orientation. Hence, variously substituted 2'-fluoroanilines were used to synthesize the corresponding acrylamide derivatives **6g–i**, and they were subjected to enzyme assays. Compound **6g** with 3'-chloro-2'-fluoro substituent showed an IC_{50} value of 52.6 nM; meanwhile, 4'-chloro-2'-fluoro (**6h**, IC_{50} = 234 nM) and 5'-chloro-2'-fluoro (**6i**, IC_{50} = 133 nM) derivatives were less potent. When the 4'-chlorine atom of **6h** was replaced by 4'-methoxy (**6j**, IC_{50} > $1.0 \mu M$), markedly reduced activity was observed, indicating that an electron donating group at the 4'-position reduces potency in this series of compounds. Neither replacement of 4'-chlorine with a fluorine atom (difluoro **6k**, IC_{50} = 302 nM) nor trifluoro compound **6l** (with one more fluorine atom at the 5'-position, IC_{50} = 500 nM) improved activity.

Based on the results described *in supra*, we observed that the active compounds **6a**, **6f**, and **6g** all have the 3'-chloroaniline substitution in common, indicating that the chlorine atom at the 3'-position is important for efficient binding. An SAR analysis of this series of compounds allowed us to assume that the pronounced enzyme inhibition activity might arise from the 3'-chloroaniline. In addition, we were motivated to investigate the effect of the acryloyl group at the 4-position of AZD3759 (**2**). Notably, compound **7** showed the greatest activity with an IC_{50} value of 8.7 nM, and this pronounced activity might be due to the fluorine atom at the 2'-position allowing coplanarity through internal halogen bonding.

Potent EGFR inhibitors were subjected to antiproliferative assays against EGFR inhibitors sensitive A549 nonsmall lung carcinoma cells.²⁰ The antiproliferative effects of compounds containing the acryloyl moiety, such as **6a**, **6f**, **6g**, and **7**, had lower IC_{50} values than their corresponding precursors **1**, **5f**, **5g**, and **2**, respectively (Table 2). Notably, compounds **6a** and **6g** were the most potent with IC_{50} values of 0.15 and 0.45 μM , respectively. However, **6a** and **6g** were 100-fold less potent than their corresponding aniline derivatives **1** and **5g** in the enzymatic assay. Surprisingly, we did not find correlation between the antiproliferative effect and EGFR enzyme inhibitory activity. The acryloyl containing compounds **6a**,

Table 2. *In Vitro* Cytotoxicity Assay against A549 Cancer Cell Lines

compd	R ¹	R ²	IC ₅₀ (μM)
1	H	3-Cl, 4-F	5.74 $\pm$ 0.40
6a	acryloyl	3-Cl, 4-F	0.15 $\pm$ 0.01
5f	H	3-Cl, 4-Cl	24.67 $\pm$ 2.08
6f	acryloyl	3-Cl, 4-Cl	5.72 $\pm$ 0.16
5g	H	2-F, 3-Cl	7.51 $\pm$ 0.28
6g	acryloyl	2-F, 3-Cl	0.45 $\pm$ 0.02
2	H	2-F, 3-Cl	28.67 $\pm$ 5.69
7	acryloyl	2-F, 3-Cl	6.10 $\pm$ 0.13

6f, **6g**, and **7** exhibited better antiproliferative activity than their precursors **1**, **5f**, **5g**, and **2**.

To investigate the underlying cause of the noncorrelation between cellular cytotoxicity and enzymatic inhibition, the physicochemical properties of the studied compounds were determined (Table 3). It should be noted that AZD3759 (**2**)

Table 3. Assay Results for Physicochemical Properties

compd	solubility (mM)		aLog P ^c	Log D _{7.4} ^d	t _{1/2} ^e (min)
	pH _{7.4} aqueous solution ^a	1-octanol ^b			
1	<0.01	1.40	4.02	2.41	NA ^g
6a	1.65	4.94	3.23	2.01	85.1
5f	ND ^f	4.57	4.63	ND ^f	NA ^g
6f	0.63	4.47	3.80	3.57	17.0
5g	0.07	4.34	4.08	ND ^f	NA ^g
6g	1.20	5.60	3.32	2.64	49.0
2	0.02	4.54	3.66	2.84	NA ^g
7	0.03	5.02	3.83	2.46	27.5

^aThermodynamic solubility in pH_{7.4} buffer solution. ^bThermodynamic solubility in 1-octanol. ^cALOGPS 2.1, molecular property prediction.²² ^dShake flask assay (solubility in 1-octanol/pH 7.4 aqueous solution = 1/2, v/v).²³ ^eHalf-life (t_{1/2}) with glutathione in pH 7.4 and 37 °C (compound/glutathione = 1/10, c/c).²⁴ ^fND, not detectable. ^gNA, not applicable.

has better solubility in 1-octanol than gefitinib (**1**), indicating that it crosses the BBB more easily (Sol_{1-octanol} = 4.54 and 1.40 mM, respectively).⁹ Interestingly, an introduction of the acryloyl group to **1**, leading to **6a**, significantly enhanced the solubility in both aqueous solution (from <0.01 to 1.65 mM) and 1-octanol (~3.5-fold; from 1.40 to 4.94 mM). The enhanced solubility was also observed in acrylamide-containing compounds **6f**, **6g**, and **7**. However, compound **7** showed only slightly improved solubility relative to **2** (~1.7-fold in aqueous solution, 0.02 mM for **2**, 0.03 mM for **7**; 1.1-fold in 1-octanol, 4.54 mM for **2**, 5.02 mM for **7**). This observation might be due to the intramolecular interaction between the 2'-fluorine atom and the oxygen atom of amide group leading to either a reduced acrylamide hydration²¹ or an aggregation of planar structure.

The thermodynamic solubility assay demonstrated that the acrylamide derivative was more soluble than its precursor in both aqueous solution and 1-octanol. In general, enhancing the aqueous solubility of one molecule would lead to reduced lipophilicity. Surprisingly, we found out that acrylamide modification not only significantly enhanced aqueous solubility but also slightly promoted 1-octanol solubility. This might lead to improved intracellular drug concentrations as well.

The Log *P* values (where *P* is the partition coefficient) were then predicted using the ALOGPS 2.1 of each compound listed in Table 3; the Log *D* (where *D* is the distribution coefficient) values were obtained by the shake flask assay to measure the two-phase distributions.^{22,23} Both of these values suggest that the inclusion of an acrylamide group decreased the lipophilicity of the compounds (such as 6a, 6f, 6g, and 7), resulting in an ideal balance between solubility and permeability. Our results demonstrate that acrylamide modification can be applied for reducing the Log *P* (partition-coefficient) or Log *D* (distribution-coefficient) values.

It is of our interest to study the stability of this type of compounds in aqueous solution. Glutathione should add to the acryloyl moiety easily by Michael addition.²⁴ Thus, glutathione was used to test stability. Among four acryloyl compounds (Table 3), the most antiproliferative compound 6a demonstrated a half-life value of 85.1 min, even in the presence of high concentration of glutathione.

The toxicity assays to FHS and Vero normal cell lines and hERG were carried out to exclude off-target effect with acryloyl group (Table 4). Further, compound 6a was screened against a

Table 4. Evaluation of Drug Toxicity

cmpd	IC ₅₀ (μM)		
	FHS ^a	Vero ^b	hERG
1	>10 (34%)	>10 (30%)	ND ^c
6a	9.17 ± 0.09	>10 (47%)	>10 (20%)

^aFHS: normal cell of human small intestine. ^bVero: normal cell of *Cercopithecus aethiops* kidney. ^cND: not determined.

panel of 22 kinases, and the results showed that the compound 6a was a highly selective target therapy agent (Supporting Information).

To investigate whether a covalent bond exists or not, computation and molecular modeling were performed. First, the structure of 6a was optimized by DFT, and the optimized structure showed a dihedral angle of 40° between aniline and quinazoline rings, which was close to that of 1 (45°) in the cocrystal structure to EGFR kinase. Further, the optimized 6a was docked into EGFR kinase (pdb ID: 2ITY), and the results illustrated no covalent bond formation between 6a and kinase (Supporting Information). Indeed, molecular modeling demonstrated that compound 6a would have a hydrogen bond to the hinge residue Met₇₉₃ as that in the crystal structure. This result provided further evidence for the potency of compound 6a. The residue Cys₇₉₇ was far away from the acryloyl moiety of 6a, and thus, there is no possibility to form covalent bond in the binding model (Supporting Information).

In summary, acrylamide compound 6a was synthesized by introducing an acryloyl functional group to the EGFR inhibitor 1. The acrylamide group significantly improved both water and 1-octanol solubility. The improved hydrophilicity should arise from the oxygen atom of the acryloyl group hydrogen bonded to water, and the improved lipophilicity came from the change of secondary amine to a tertiary amide. Both improved physicochemical properties contributed to the improved cell permeability. Our results suggest that acrylamide could serve as a potential functional group for the optimization of desirable physicochemical properties in drug design.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmmedchemlett.8b00270.

Experimental details for preparation of compound 6a–1 and 7, including characterization data (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +886 2 33668697. E-mail: jwchern@ntu.edu.tw.

ORCID

Ji-Wang Chern: 0000-0002-4310-8834

Author Contributions

K.D.W. and J.W.C. conceived the project and designed the experiments. K.D.W. synthesized the compounds and analyzed the physicochemical properties. J.R.L. performed the *in vitro* cell assay. C.E.H. and G.S.C. carried out the molecular modeling and computational calculation, respectively. K.D.W. and J.W.C. analyzed the data and wrote the manuscript.

Funding

This study was supported by grants from the Aiming for Top University Project, Ministry of Education, Taiwan.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This study was supported by grants from the Aiming for Top University Project, Ministry of Education, Taiwan.

■ ABBREVIATIONS

EGFR, epidermal growth factor receptor; NSCLC, nonsmall cell lung cancer; TKI, tyrosine kinase inhibitor; CNS, central nervous system; SAR, structure–activity relationship; BBB, blood–brain barrier

■ REFERENCES

- (1) Singh, J.; Petter, R. C.; Baillie, T. A.; Whitty, A. The Resurgence of Covalent Drugs. *Nat. Rev. Drug Discovery* **2011**, *10*, 307–317.
- (2) Scheiber, J.; Chen, B.; Milik, M.; Sukuru, S. C. K.; Bender, A.; Mikhailov, D.; Whitebread, S.; Hamon, J.; Azzaoui, K.; Urban, L.; Glick, M.; Davies, J. W.; Jenkins, J. L. Gaining Insight into Off-Target Mediated Effects of Drug Candidates with a Comprehensive Systems Chemical Biology Analysis. *J. Chem. Inf. Model.* **2009**, *49*, 308–317.
- (3) Potashman, M. H.; Duggan, M. E. Covalent Modifiers: An Orthogonal Approach to Drug Design. *J. Med. Chem.* **2009**, *52*, 1231–1246.
- (4) Barf, T.; Kaptein, A. Irreversible protein kinase inhibitors: balancing the benefits and risks. *J. Med. Chem.* **2012**, *55*, 6243–6262.
- (5) Shibata, Y.; Chiba, M. The Role of Extrahepatic Metabolism in the Pharmacokinetics of the Targeted Covalent Inhibitors Afatinib, Ibrutinib, and Neratinib. *Drug Metab. Dispos.* **2015**, *43*, 375–384.
- (6) Fukuoka, M.; Yano, S.; Giaccone, G.; Tamura, T.; Nakagawa, K.; Douillard, J. Y.; Nishiwaki, Y.; Vansteenkiste, J.; Kudoh, S.; Rischin, D.; Eek, R.; Horai, T.; Noda, K.; Takata, I.; Smit, E.; Averbuch, S.; Macleod, A.; Feyereislova, A.; Dong, R. P.; Baselga, J. Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer. *J. Clin. Oncol.* **2003**, *21*, 2237–2246.
- (7) Jia, Y.; Yun, C. H.; Park, E.; Ercan, D.; Manuia, M.; Juarez, J.; Xu, C.; Rhee, K.; Chen, T.; Zhang, H.; Palakurthi, S.; Jang, J.; Lelais, G.; DiDonato, M.; Bursulaya, B.; Michellys, P. Y.; Eppe, R.; Marsilje, T. H.; McNeill, M.; Lu, W.; Harris, J.; Bender, S.; Wong, K. K.; Janne, P.

- A.; Eck, M. J. Overcoming EGFR(T790M) and EGFR(C797S) resistance with mutant-selective allosteric inhibitors. *Nature* **2016**, *534*, 129–132.
- (8) Nayak, L.; Lee, E. Q.; Wen, P. Y. Epidemiology of brain metastases. *Curr. Oncol. Rep.* **2012**, *14*, 48–54.
- (9) Zeng, Q.; Wang, J.; Cheng, Z.; Chen, K.; Johnstrom, P.; Varnas, K.; Li, D. Y.; Yang, Z. F.; Zhang, X. Discovery and Evaluation of Clinical Candidate AZD3759, a Potent, Oral Active, Central Nervous System-Penetrant, Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitor. *J. Med. Chem.* **2015**, *58*, 8200–8215.
- (10) Hughes, J. D.; Blagg, J.; Price, D. A.; Bailey, S.; Decrescenzo, G. A.; Devraj, R. V.; Ellsworth, E.; Fobian, Y. M.; Gibbs, M. E.; Gilles, R. W.; Greene, N.; Huang, E.; Krieger-Burke, T.; Loesel, J.; Wager, T.; Whiteley, L.; Zhang, Y. Physicochemical drug properties associated with in vivo toxicological outcomes. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4872–4875.
- (11) Gorham, R. D., Jr.; Forest, D. L.; Khoury, G. A.; Smadbeck, J.; Beecher, C. N.; Healy, E. D.; Tamamis, P.; Archontis, G.; Larive, C. K.; Floudas, C. A.; Radeke, M. J.; Johnson, L. V.; Morikis, D. New compstatin peptides containing N-terminal extensions and non-natural amino acids exhibit potent complement inhibition and improved solubility characteristics. *J. Med. Chem.* **2015**, *58*, 814–826.
- (12) Leu, Y.-L.; Chen, C.-S.; Wu, Y.-J.; Chern, J.-W. Benzyl Ether-Linked Glucuronide Derivative of 10-Hydroxycamptothecin Designed for Selective Camptothecin-Based Anticancer Therapy. *J. Med. Chem.* **2008**, *51*, 1740–1746.
- (13) Morphy, R. The Influence of Target Family and Functional Activity on the Physicochemical Properties of Pre-Clinical Compounds. *J. Med. Chem.* **2006**, *49*, 2969–2978.
- (14) Oprea, T. I. Current trends in lead discovery: Are we looking for the appropriate properties? *Mol. Diversity* **2000**, *5*, 199–208.
- (15) Tehler, U.; Fagerberg, J. H.; Svensson, R.; Larhed, M.; Artursson, P.; Bergstrom, C. A. Optimizing solubility and permeability of a biopharmaceutics classification system (BCS) class 4 antibiotic drug using lipophilic fragments disturbing the crystal lattice. *J. Med. Chem.* **2013**, *56*, 2690–2694.
- (16) 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. The preparation and SAR of 4-(anilino), 4-(phenoxy), and 4-(thiophenoxy)-quinazolines: Inhibitors of p56lck and EGF-R tyrosine kinase activity. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 417–420.
- (17) Marzaro, G.; Guiotto, A.; Pastorini, G.; Chilin, A. A novel approach to quinazolin-4(3H)-one via quinazoline oxidation: an improved synthesis of 4-anilinoquinazolines. *Tetrahedron* **2010**, *66*, 962–968.
- (18) Jablonski, M. Energetic and geometrical evidence of non-bonding character of some intramolecular halogen...oxygen and other Y...Y interactions. *J. Phys. Chem. A* **2012**, *116*, 3753–3764.
- (19) Gillis, E. P.; Eastman, K. J.; Hill, M. D.; Donnelly, D. J.; Meanwell, N. A. Applications of Fluorine in Medicinal Chemistry. *J. Med. Chem.* **2015**, *58*, 8315–8359.
- (20) Jaramillo, M. L.; Banville, M.; Collins, C.; Paul-Roc, B.; Bourget, L.; O'Connor-McCourt, M. Differential sensitivity of A549 non-small lung carcinoma cell responses to epidermal growth factor receptor pathway inhibitors. *Cancer Biol. Ther.* **2008**, *7*, 557–568.
- (21) Cavallo, G.; Metrangolo, P.; Milani, R.; Pilati, T.; Priimagi, A.; Resnati, G.; Terraneo, G. The Halogen Bond. *Chem. Rev.* **2016**, *116*, 2478–2601.
- (22) Tetko, I. V.; Gasteiger, J.; Todeschini, R.; Mauri, A.; Livingstone, D.; Ertl, P.; Palyulin, V. A.; Radchenko, E. V.; Zefirov, N. S.; Makarenko, A. S.; Tanchuk, V. Y.; Prokopenko, V. V. Virtual computational chemistry laboratory - design and description. *J. Comput.-Aided Mol. Des.* **2005**, *19*, 453–463.
- (23) Wenlock, M. C.; Potter, T.; Barton, P.; Austin, R. P. A method for measuring the lipophilicity of compounds in mixtures of 10. *J. Biomol. Screening* **2011**, *16*, 348–355.
- (24) Flanagan, M. E.; Abramite, J. A.; Anderson, D. P.; Aulabaugh, A.; Dahal, U. P.; Gilbert, A. M.; Li, C.; Montgomery, J.; Oppenheimer, S. R.; Ryder, T.; Schuff, B. P.; Uccello, D. P.; Walker, G. S.; Wu, Y.; Brown, M. F.; Chen, J. M.; Hayward, M. M.; Noe, M. C.; Obach, R. S.; Philippe, L.; Shanmugasundaram, V.; Shapiro, M. J.; Starr, J.; Stroh, J.; Che, Y. Chemical and computational methods for the characterization of covalent reactive groups for the prospective design of irreversible inhibitors. *J. Med. Chem.* **2014**, *57*, 10072–10079.