Bioorganic & Medicinal Chemistry Letters 20 (2010) 2408–2411

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Identification of GNE-477, a potent and efficacious dual PI3K/mTOR inhibitor

Timothy P. Heffron [a,*], Megan Berry [b], Georgette Castanedo [a], Christine Chang [c], Irina Chuckowree [d], Jennafer Dotson [a], Adrian Folkes [d], Janet Gunzner [a], John D. Lesnick [c], Cristina Lewis [c], Simon Mathieu [a], Jim Nonomiya [c], Alan Olivero [a], Jodie Pang [e], David Peterson [c], Laurent Salphati [e], Deepak Sampath [b], Steve Sideris [c], Daniel P. Sutherlin [a], Vickie Tsui [a], Nan Chi Wan [d], Shumei Wang [a], Susan Wong [e], Bing-yan Zhu [a]

[a] Discovery Chemistry, Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080, USA
[b] Small Molecule Translational Oncology, Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080, USA
[c] Biochemical Pharmacology, Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080, USA
[d] Piramed Pharma, 957 Buckingham Avenue, Slough, Berkshire SL1 4NL, UK
[e] Drug Metabolism and Pharmacokinetics,, Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080, USA

ARTICLE INFO

Article history:
Received 2 February 2010
Revised 8 March 2010
Accepted 9 March 2010
Available online 12 March 2010

Keywords:
PI3K
mTOR
Kinase inhibitors

ABSTRACT

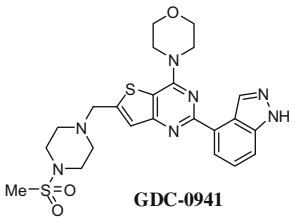
Efforts to identify potent small molecule inhibitors of PI3 kinase and mTOR led to the discovery of the exceptionally potent 6-aryl morpholino thienopyrimidine **6**. In an effort to reduce the melting point in analogs of **6**, the thienopyrimidine was modified by the addition of a methyl group to disrupt planarity. This modification resulted in a general improvement in in vivo clearance. This discovery led to the identification of **GNE-477** (**8**), a potent and efficacious dual PI3K/mTOR inhibitor.

Owing to its common association with oncogenic malignancies, the PI3K/AKT/mTOR signaling pathway is an attractive area of research for the identification of oral small molecule inhibitors.[1] We have previously reported the discovery of the PI3K inhibitor **GDC-0941** and have continued our efforts to identify additional molecules that inhibit PI3K or both PI3K and mTOR.[2]

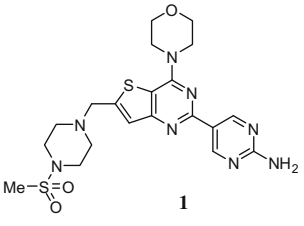
During our investigations, using **GDC-0941** as a starting point, we discovered that the inhibition of PI3K-α was maintained when the indazole moiety was replaced by 2-aminopyrimidine (**1**, Table 1). Furthermore, compound **1** was found to be a potent inhibitor of mTOR kinase activity and had improved potency in the MCF7.1 cell proliferation assay.[3] This replacement group proved to be an entry into the fruitful identification of many attractive dual PI3K/mTOR inhibitors.[4]

By holding constant the morpholino thienopyrimidine core along with the 2-aminopyrimidine, we were able to consistently generate molecules with attractive inhibitory activities against PI3K-α and mTOR. A representative set of compounds from our SAR development at the 6-position of the thienopyrimidine core is presented in Table 2. Compound **1** has excellent potency against both PI3K-α and mTOR and exhibits good cell-based activity in a proliferation assay using MCF7.1 cells. In fact, compound **2**, which lacks any substitution at the 6-position, still maintains reasonable potency, demonstrating that the potency is largely driven by the interactions of the morpholine and aminopyrimidine with the enzyme.[5] Compounds **3**–**5** are of comparable potency in each assay, demonstrating that a wide range of substituents is tolerated at the 6-position of the thiophene.

Table 1
Comparison of relevant potency data for **GDC-0941** and **1**



GDC-0941



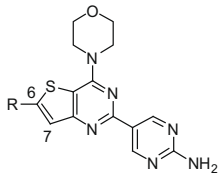
1

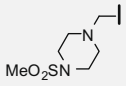
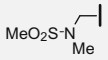
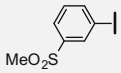
Compd	PI3K-α IC_{50} (nM)	mTOR K_{iapp} (nM)	MCF7.1 Proliferation EC_{50} (nM)
GDC-0941	3	570	720
1	2	29	130

* Corresponding author. Tel.: +1 650 467 3214; fax: +1 650 225 2061.
E-mail address: theffron@gene.com (T.P. Heffron).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.03.046

Table 2
SAR of 6-position substitution on the thienopyrimidine core

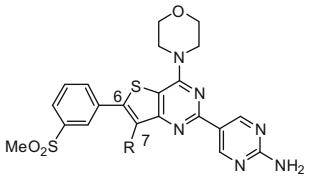


Compd	R	P13K-α IC_{50} (nM)	mTOR K_{iapp} (nM)	MCF7.1 proliferation EC_{50} (nM)	Thermodynamic solubility, pH 6.5 (μg/mL)
1		2	29	0.13	62
2		5	42	0.43	170
3		1	10	0.17	15
4		5	50	0.19	770
5		7	59	0.29	1100
6		<1	5	0.04	1

While the first five examples in Table 2 each have PI3K and mTOR potencies similar to each other (1–7 nM, and 29–59 nM, respectively) resulting in similar cell proliferation activities, an exception in this series was the aryl analog **6** which was significantly more potent in each of the biochemical and cell-based assays.

Given the superior potency of **6** in both the biochemical and cell-based assays we evaluated the pharmacokinetic profile of this compound. Despite moderate stability in liver microsomes, compound **6** had high clearance in vivo as well as low oral bioavailability (Table 3). While the low oral bioavailability could be attributed to first-pass metabolism, because the thermodynamic solubility of **6** was so low we remained concerned that related analogs, even with reduced clearance, would have limited oral bioavailability due to solubility-limited absorption.

Table 3
Rat pharmacokinetic data and key physicochemical properties for **6** and **7**



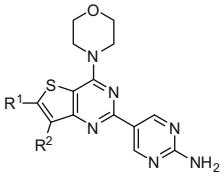
Compd	R_1	Rat LM, Cl_{hep} (mL/min/kg)	Rat Cl (mL/min/kg)	*F* (%)	TD Sol, pH 6.5 (μg/mL)	Melting point[a] (°C)
6[b]	H	7	56	3	1	276
7[c]	Me	12	13	4	3	234

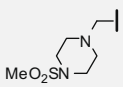
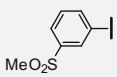
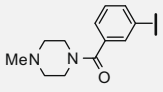
[a] Melting points obtained using crystalline material using the same lot used for rat PK studies.
[b] Male rats were dosed with the TFA salt as a solution intraveinously (1 mg/kg) in 7% DMSO/5% cremophor and dosed orally as a solution in 5% DMSO/76% PEG (5 mg/kg).
[c] Male rats were dosed with the HCl salt as a solution intraveinously (1 mg/kg) in 10% DMSO/60% PEG and dosed orally as a solution in 10% DMSO/80% PEG (5 mg/kg).

The aryl group at the 6-position resulted in superior potency and we sought to improve solubility while maintaining this motif. In doing so, we sought to improve oral bioavailability of further analogs of compound **6** by increased rate of dissolution. The thermodynamic solubility of small molecules is often inversely proportional to the compound melting point.[6] We believed that the high melting point of **6** was due to efficient crystal packing of the highly planar molecule and so developed a strategy to disrupt the co-planarity of the aryl group and the thienopyrimidine core.[7] To this end, we modified the core by introducing a methyl group at the 7-position (Table 3). Quantum mechanics calculations predicted that the methylated thiophene (as in **7**) preferred to be 45° out of plane with the aryl sulfone, with an energy difference of 2.14 kcal/mol relative to the planar conformation.[8]

Compound **7**, the direct analog of **6**, was first prepared to assess the promise of this approach. The melting point of **7** was reduced by more than 40 °C relative to compound **6** resulting in a modest

Table 4
Comparison of analogs with and without methyl at R^2



Compd	R^1	R^2	P13K-α IC_{50} (nM)	mTOR K_{iapp} (nM)	MCF7.1 proliferation EC_{50} (nM)	Rat LM Cl_{hep} (mL/min/kg)	Rat Cl (mL/min/kg)	Rat PPB (%)	Rat Cl_u (mL/min/kg)
1		H	2	29	130	16	24	52	50
8		Me	4	21	143	15	13	75	52
4		H	5	50	187	2	50	47	94
9		Me	3	30	164	20	21	51	43
6		H	<1	5	33	7	56	93	800
7		Me	<1	4	68	12	13	97	433
10		H	1	10	78	20	52	ND	ND
11		Me	2	4	65	38	15	ND	ND

solubility improvement. This reduction in melting point, however, did not result in an improvement in oral bioavailability. The poor oral bioavailability observed for **7** suggested that solubility was still insufficient despite the reduction in melting point. Furthermore, in vivo clearance and passive cell permeability were favorable and hence did not appear to be compromising factors (Table 3).[9]

While the introduction of the methyl group to the 7-position of the thiophene core did not lead to the desired improvement in oral bioavailability, we observed that **7** had reduced rat clearance in vivo relative to the des-methyl analog **6**. This reduction in clearance occurred despite in vitro studies suggesting that the des-methyl analog was of comparable metabolic stability (Table 3). In addition, it did not appear that methyl substitution was blocking a site of metabolism as numerous in vitro metabolite identification studies did not indicate significant metabolic activation of the thiophene ring. Encouraged that the remarkable potency of **6** was unchanged on the modified core of **7**, we extended our studies to determine whether the desirable impact on clearance with maintained potency was a general trend. To this end, we synthesized several pairs of analogs both with and without the methyl group at the 7-position of the thienopyrimidine core. A representative summary of these efforts is contained in Table 4.

In each pair, the potency of the molecules does remain comparable in each assay. Also in each case, the tetrasubstituted thienopyrimidine core has reduced in vivo clearance in rats relative to the trisubstituted analog despite being consistently more labile in rat liver microsomes. The general improvement in in vivo clearance of the tetrasubstituted core is likely a combination of multiple contributing factors. One significant contribution to the reduced clearance is the increase in plasma protein binding observed for the analogs on the core with the methyl group (Table 4). Nevertheless, it does not account for the magnitude of the difference in each case as in two pairs the modified core has reduced unbound clearance (**4**/**9**, and **6**/**7**). Evidently, the additional thiophene substitution reduces metabolism of the molecule not represented in liver microsomes. The other factors contributing to reduced clearance are not well understood. Regardless, the improvement in in vivo clearance of this core was general and while **7** exhibited poor oral bioavailability, other analogs with improved physicochemical properties were identified. These maintained very attractive potency and reduced plasma protein binding. Of these, compound **8** (**GNE-477**) was selected for further evaluation.

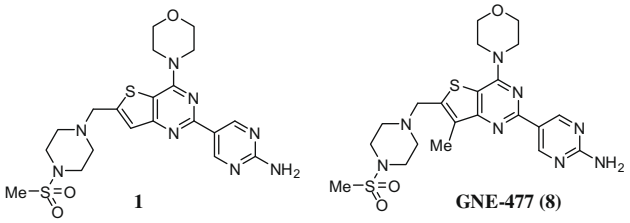
A direct comparison of **8** with its des-methyl analog (**1**) reveals that the trend of reduced in vivo clearance in rats is also observed in mice and dogs (Table 5). The improvement in clearance was particularly significant in dogs where **1** was cleared at two-thirds the rate of hepatic blood flow whereas **8** has low clearance.

The appealing pharmacokinetic profile of inhibitor **8** justified further evaluation of this compound. In an experiment evaluating the tumor growth inhibition of a PC3 tumor xenograft[10] over 14 days, stasis was achieved at a 20 mg/kg QD dose and significant inhibition was observed with doses as low as 1 mg/kg QD. The administered drug was generally well tolerated during this study as demonstrated by acceptable levels of weight loss comparable to that observed with the animals in the vehicle cohort (Fig. 1).[11]

The synthetic route to **GNE-477**, as well as to other analogs depicted within, is described in Scheme 1.[12] Commencing with aminoester **12**, chloropyrimidine intermediate **13** was formed by reaction with molten urea followed by $POCl_3$ mediated chlorination and subsequent nucleophilic aromatic substitution by morpholine. Next, an aldehyde intermediate useful in targeting compounds **1**–**3**, **9**, **14** and **GNE-477** was produced by lithiation of the thiophene core followed by reaction with DMF. Finally, reductive amination with *N*-sulfonylpiperazine followed by Suzuki coupling afforded **GNE-477**.

Each of the 6-aryl compounds was also synthesized by a general route shown in Scheme 1. From the common intermediate **13**, the 6-iodo compound **16** was prepared by lithiation and iodine quench. Iterative regioselective Suzuki couplings were then used

Table 5
Comparison of in vivo pharmacokinetics for **GNE-477** (**8**) and **1**



Compd	Species	Cl (mL/min/kg)	*F* (%)
1	Mouse[a]	49	81
GNE-477		15	98
1	Rat[b]	24	75
GNE-477		13	52
1	Dog[c]	22	35
GNE-477		5	90

[a] Female nu/nu mice were dosed with the HCl salt as a solution intraveinously (1 mg/kg) in 5% DMSO/5% cremophor and dosed orally as a solution in 80% PEG (5 mg/kg).
[b] Male rats were dosed with the TFA salt as a solution intraveinously (1 mg/kg) in 5% DMSO/5% cremophor and dosed orally as a solution in 80% PEG (5 mg/kg).
[c] Male beagle dogs were dosed with the HCl salt as a solution intraveinously (1 mg/kg) in 10% HP-β-CD and dosed orally as a suspension in MCT (2 mg/kg).

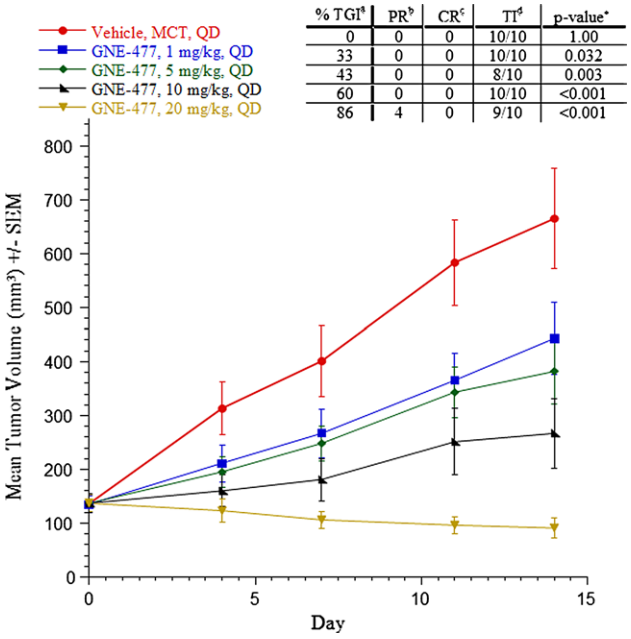
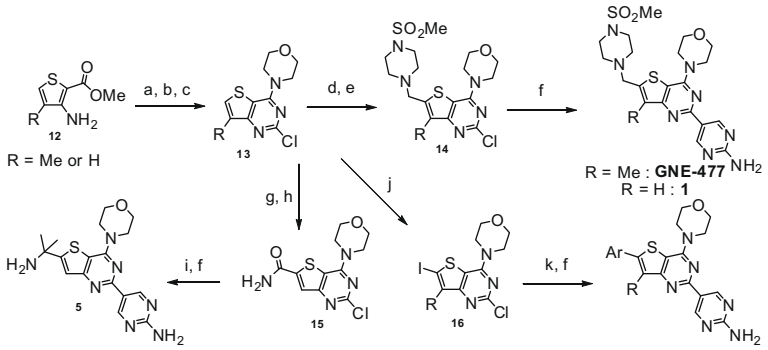


Figure 1. Efficacy study of **GNE-477** (**8**) in the PC3-NCI tumor xenograft model. The plot shows tumor volumes over time including a vehicle cohort. Vehicle = MCT = 0.5% methylcellulose/0.2% Tween-80; QD = every day. [a]% TGI = percent of tumor growth inhibition at the end of study (day 14) compared with the vehicle control group. [b]PR = partial response (reduction of >50% but <100% in tumor volume, compared with the starting tumor volume, observed on any day of the study. [c]CR = complete response (reduction of 100% in tumor volume, compared with the initial tumor volume, observed on any day of the study. [d]TI = tumor incidence (ratio of number of animals with measurable tumors remaining in each group at the end of the study (day 14) compared to the number of animals with measurable tumors at the beginning of the study. *p-Values comparing treated groups with the vehicle group were calculated at day 14 using the Students t-test.



Scheme 1. Reagents and conditions: (a) Urea, 200 °C, 4 h; (b) $POCl_3$, CH_3CN, reflux, 24 h; (c) morpholine, MeOH, 1 h; (d) *n*BuLi, THF, −78 °C, DMF; (e) *N*-sulfonylpiperazine, 1,2-DCE, $HC(OCH_3)_3$, $Na(OAc)_3BH$; (f) 2-aminopyrimidine-5-boronic acid pinacol ester, $PdCl_2(PPh_3)_2$, 1 M aq KOAc, CH_3CN, microwave, 150 °C, 15 min; (g) *n*BuLi, THF, −78 °C, CO_2; (h) HOAT, HATU, *i*Pr_2NEt, DMF, NH_4Cl; (i) $ZrCl_4$, THF, CH_3MgBr, −10 °C to rt; (j) *n*BuLi, THF, −78 °C, I_2; (k) aryl boronate, $PdCl_2(PPh_3)_2$, 1 M aq Na_2CO_3, CH_3CN, microwave, 100 °C, 10 min.

to install the remaining aromatic rings. The carbinamine **4** was produced via primary amide **15** using $ZrCl_4$ and excess methyl Grignard followed by a Suzuki coupling.[13]

In summary, an effort to improve rate of dissolution of high melting point compounds resulted in an improved central core for morpholino thienopyrimidine PI3K inhibitors. The tetrasubstituted thiophene generally has identical potency to compounds of the des-methyl core but benefits from improved clearance. This observation led to the identification of **GNE-477**, a potent dual PI3K/mTOR inhibitor that displays desirable pharmacokinetic properties in each of three species studied.[14] **GNE-477** also exhibited stasis in a PC3 tumor growth inhibition study. Our efforts with these and additional PI3K inhibitors is ongoing.

Acknowledgments

The authors wish to thank Mengling Wong, Martin Struble, and Wen Chiu for compound purification and determination of purity by HPLC, mass spectroscopy, and ^1H NMR. We thank Krista K. Bowman, Alberto Estevez, Kyle Mortara, and Jiansheng Wu for technical assistance of protein expression and purification. We thank Emil Plise for plasma protein binding data.

References and notes

1. (a) Vivanco, I.; Sawyers, C. L. *Nat. Rev. Cancer* **2002**, *2*, 489; (b) Cantley, L. C. *Science* **2002**, *296*, 1655; (c) Guertin, D. A.; Sabatini, D. M. *Cancer Cell* **2007**, *12*, 9; (d) Fasolo, A.; Sessa, C. *Expert Opin. Invest. Drugs* **2008**, *17*, 1717; (e) Bellacosa, A.; Kumar, C. C.; Di Cristofano, A., et al *Adv. Cancer Res.* **2005**, *94*, 29; (f) Ihle, N. T.; Powis, G. *Mol. Cancer Ther.* **2009**, *8*, 1; (g) Maira, S.-M.; Stauffer, F.; Schnell, C.; García-Echeverría, C. *Biochem. Soc. Trans.* **2009**, *37*, 265.
2. Folkes, A. J.; Ahmadi, K.; Alderton, W. K.; Alix, S.; Baker, S. J.; Box, G.; Chuckowree, I. S.; Clarke, P. A.; Depledge, P.; Eccles, S. A.; Friedman, L. S.; Hayes, A.; Hancox, T. C.; Kugendradas, A.; Lensun, L.; Moore, P.; Olivero, A. G.; Pang, J.; Patel, S.; Pergl-Wilson, G. H.; Raynaud, F. I.; Robson, A.; Saghir, N.; Salphati, L.; Sohal, S.; Ultsch, M. H.; Valenti, M.; Wallweber, H. J. A.; Wan, N. C.; Wiesmann, C.; Workman, P.; Zhyvoloup, P.; Zvelebil, M. J.; Shuttleworth, S. J. *J. Med. Chem.* **2008**, *51*, 5522.
3. MCF7.1 is a HER2 transfected clone of the original MCF7 cell line (ATCC, Manassas, VA) that expresses elevated levels of HER2 and contains the PI3K E545K activating mutation found in the parental line.
4. Sutherlin, D. P.; Sampath, D.; Berry, M.; Castanedo, G.; Chang, Z.; Chuckowree, I.; Dotson, J.; Folkes, A.; Friedman, L.; Goldsmith, R.; Heffron, T.; Lee, L.; Lesnick, J.; Lewis, C.; Mathieu, S.; Nonomiya, J.; Olivero, A.; Pang, J.; Prior, W. W.; Salphati, L.; Sideris, S.; Tian, Q.; Tsui, V.; Wan, N. C.; Wang, S.; Wiesmann, C.; Wong, S.; Zhu, B.-Y. *J. Med. Chem.* **2010**, *53*, 1086.
5. The interactions with the enzyme gained by the aminopyrimidine are significant. The PI3K-α IC_{50} for the analog of **2** in which the aminopyrimidine is replaced by the 4-indazole present in GDC-0941 is 40 nM. For a description of these inhibitors and their key interactions within the binding site, and detailed methods, see Ref. 4.
6. Yalkowsky, S. H.; Valvani, S. C. *J. Pharm. Sci.* **1980**, *69*, 912.
7. Quantum mechanics calculations suggest that the lowest energy conformation of **6** positions the aryl sulfone 30° out of plane, but with the co-planar conformation accessible at just 0.4 kcal/mol penalty.
8. Quantum mechanical energies were computed using Jaguar version 7.5 by Schrodinger (Schrodinger, Inc.), at the 6-31G**+/B3LYP level of theory. The 'Relaxed Coordinate Scan' method was used to vary the dihedral angle between the thiophene/methylthiophene and the aryl ring between 0° and 90° at 30° intervals.
9. For **7** P_{app} A − B = 14×10^{-6} cm/s and P_{app} B − A = 23×10^{-6} cm/s in a permeability assay using MDCK cells.
10. PC3 is a cancer cell line that is PTEN(−). Cell proliferation EC_{50} using this cell line was 174 nM.
11. Each group, including vehicle, exhibited similar and acceptable weight loss of less than 10% relative to starting mass.
12. (a) Bayliss, T.; Chuchowree, I.; Folkes, A.; Oxenford, S.; Wan, N. C.; Castanedo, G.; Goldsmith, R.; Gunzner, J.; Heffron, T.; Mathieu, S.; Olivero, A.; Staben, S.; Sutherlin, D. P.; Zhu, B.-Y. WO2008/070740 A1.; (b) Castanedo, G.; Dotson, J.; Goldsmith, R.; Gunzner, J.; Heffron, T.; Mathieu, S.; Olivero, A.; Staben, S.; Sutherlin, D. P.; Tsui, V.; Wang, S.; Zhu, B.-Y.; Bayliss, T.; Chuckowree, I.; Folkes, A.; Wan, N. C. WO2008/073785 A2.
13. Denton, S. M.; Wood, A. *Synlett* **1999**, 55.
14. GNE-477 (**8**) inhibits PI3K-α, β, δ, and γ with IC_{50}'s of 4, 86, 6, and 15 nM, respectively.