Synthesis and biological evaluation of a triazole-based library of pyrido[2,3-*d*]pyrimidines as FGFR3 tyrosine kinase inhibitors†

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A library of pyrido[2,3-*d*]pyrimidines was designed as inhibitors of FGFR3 tyrosine kinase allowing possible interactions with an unexploited region of the ATP binding-site. This library was built-up with an efficient step of click-chemistry giving easy access to triazole-based compounds bearing a large panel of substituents. Among the 27 analogues synthesized, more than half exhibited 55–89% inhibition of *in vitro* FGFR3 kinase activity at 2 μM and one (**19g**) was able to inhibit auto-phosphorylation of mutant FGFR3-K650M in transfected HEK cells.

Introduction

Receptor tyrosine kinases (RTKs) play an important role in proliferation, motility and differentiation, and can therefore become potent oncoproteins when mutated or overexpressed.[1] The family of fibroblast growth factor receptor (FGFR), one of the twenty sub-classes of RTKs, consists of four homologous receptors numbered FGFR1-4.[2] Initially identified at the germinal level, activating mutations within FGFR3 are responsible for human skeletal disorders[3] ranging from hypochondroplasia (the mildest form)[4] to thanatophoric dysplasia (the neonatal lethal form)[5] through achondroplasia (the most frequent form).[6] Interestingly, several mutations involved in chondrodysplasia were also identified somatically in human tumours.[7] It is now well established that FGFR3 can act as an oncogene in bladder cancers,[8] as well as in multiple myeloma,[9] cervical[10] or prostate[11] cancers and benign skin tumours.[12]

Over the years, extensive efforts have been made to develop ATP-competitive inhibitors as target-directed therapeutics for RTK-associated diseases, mostly in the field of cancer.[13] Rational design of such inhibitors usually refers to the general pharmacophore model built up by Traxler and Furet[14] showing that the highly conserved ATP binding site is divided into five subregions: hydrophobic regions I and II, the adenine, the ribose and the phosphate-binding regions (Fig. 1). We observed the same topology in the 3D-structure of the kinase domain of FGFR3 according to a homology-based model.[15] Amongst the plethora of inhibitors reported so far, PD173074, a compound from the pyrido[2,3-*d*]pyrimidine class, was found to exhibit high potency against

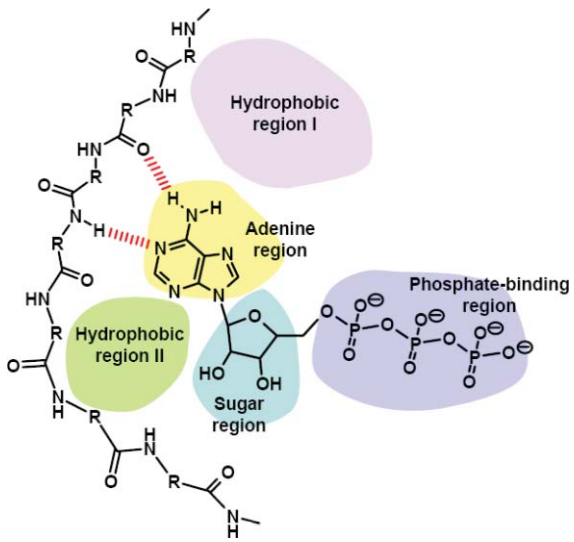

Fig. 1 Pharmacophore model of ATP binding site.

FGFR1 and VEGFR2,[16] and was more recently identified as an FGFR3 inhibitor.[17] The crystal structure of unphosphorylated FGFR1 with PD173074[18] (Fig. 2a) has been determined (2FGI) and the electron density map shows good supporting density for the pyrido[2,3-*d*]pyrimidine ring system, the dimethoxyphenyl (6-position) and *tert*-butyl urea (7-position) groups, but shows weaker density for the diethylaminobutylamino chain (2-position).

Such electron density is consistent with a high contribution to potency of aromatic moieties that occupy the adenine binding site and hydrophobic pocket I with an excellent surface complementarity, and a neglectable contribution for the aliphatic butyl chain that lies in hydrophobic pocket II.[19]

In this context, we became interested in modifying this alkylamino group at position 2 in order to establish new interactions with the large cavity that encompasses hydrophobic and ribose pockets. For example, when co-crystallized with FGFR1 (1FGI),[20] SU5402, which is another FGFR3 inhibitor, exhibits a pyrrole ring in hydrophobic pocket II with the methyl group in Van der Waals contact with Gly 567 and a carboxylate group hydrogen bonded with Asn 568, which is part of the ribose pocket (Fig. 2b). We

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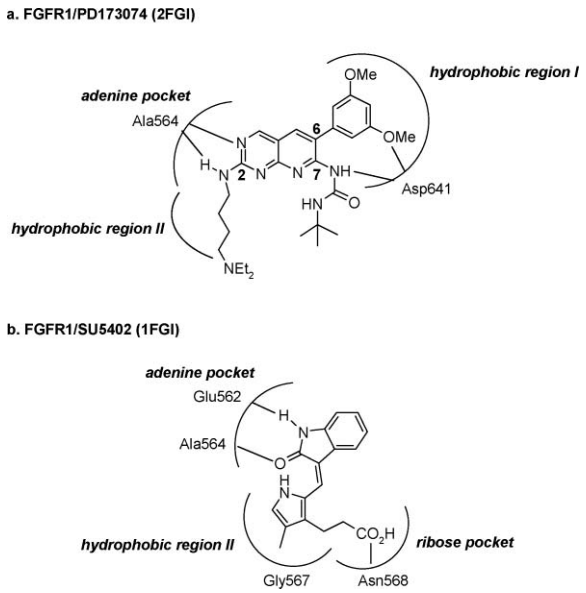
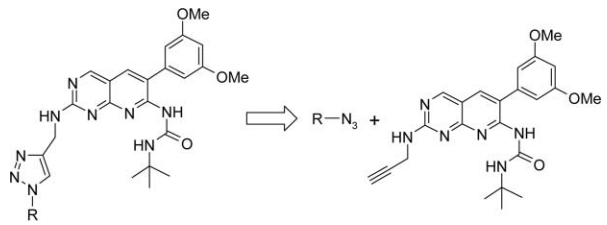


Fig. 2 X-Ray analysis of FGFR1-inhibitors crystals.

therefore aimed at the synthesis of PD173074 analogs bearing in position 2 a large panel of substituents, in terms of size and polarity. Such compounds could lead to enhanced activity and/or selectivity. A complementary study, focusing on heteroaromatic substituents has been recently reported by Sanofi Aventis.[21] In order to quickly obtain a small library of inhibitors with a large structural diversity, we decided to introduce modifications through click reaction, as depicted in Scheme 1. As a matter of fact, since Sharpless introduced the click chemistry concept,[22] the Huisgen [3+2] cycloaddition has proved to be a powerful tool in drug discovery.[23] Beside synthetic interest, the resulting 1,2,3-triazole unit is not just a passive linker but a rather active pharmacophore that may significantly contribute to protein binding.[24] Moreover, 1,2,3-triazole moiety has been used with success to optimize ZD4190, a VEGFR inhibitor from the quinazoline family[25] and very recently to discover a new PfPK7 protein kinase inhibitor.[26]



Scheme 1 Rationale for the synthesis of clicked analogs.

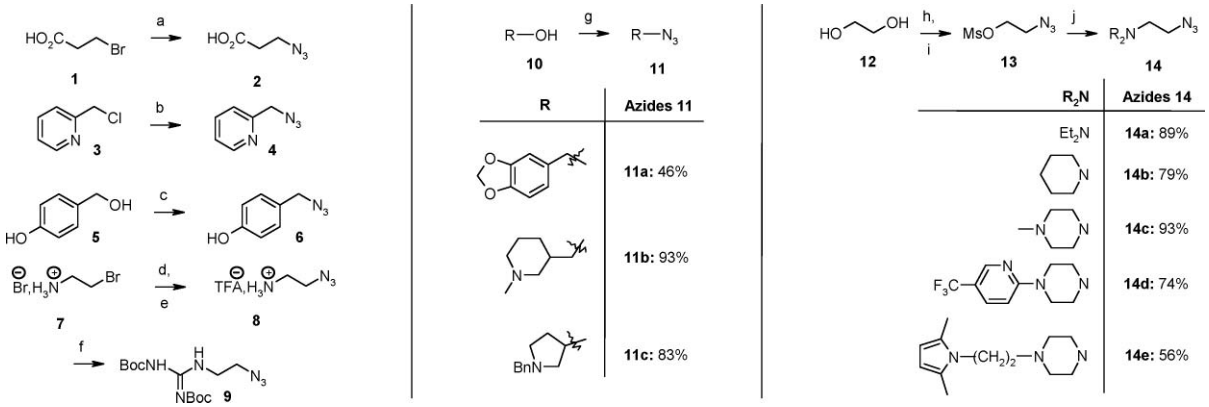
Based on the above considerations and our ongoing efforts to develop FGFR3 inhibitors,[27] we report herein the synthesis of the first "click" library of RTK inhibitors based on the pyrido[2,3-*d*]pyrimidine skeleton of the PD173074 structure and their biological evaluations.

Results and discussion

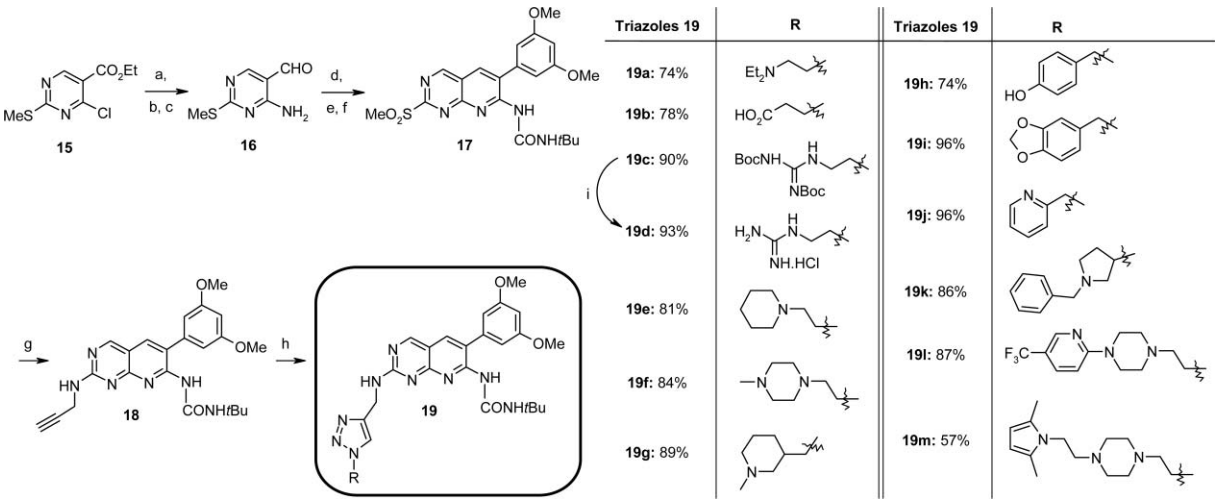
Chemical Synthesis

Our aim was to build a library of triazole-based inhibitors through efficient click-chemistry connection between the core structure of PD173074 and various substituents. For this purpose, we prepared a collection of azide building blocks according to different literature protocols, depending on the commercial availability of the starting material (Scheme 2).

Azides **2**[28] and **4**[29] were prepared by standard nucleophilic substitution from their parent bromide **1** and chloride **3**, respectively, whereas alcohol **5** was converted to azide **6** using a facile one-pot reaction.[30] Guanidino derivative **9** was obtained from azidoamine **8** treated with a guanylation agent.[31] We first attempted to synthesize **8** from ethane-1,2-diamine according to a procedure previously described in the literature.[32] However, we found out that starting from the corresponding bromoamine **7** was a more efficient pathway. Of note, bromine displacement was followed by *N*-Boc protection in order to facilitate the purification step. Moreover, *N*-Boc was cleaved with TFA, which allowed a better storage of azido-amine **8** as its ammonium salt. The synthesis of azides **11** was achieved in two steps *via* standard conversion of alcohols **10** to mesylate or tosylate intermediates,



Scheme 2 *Reagents and conditions*: **(a)** NaN_3, DMF, 60 °C, 48 h, 51%. **(b)** NaN_3, H_2O, reflux, 24 h, 36%. **(c)** NaN_3, PPh_3, CCl_4/DMF, 90 °C, 1 h, 56%. **(d)** *i.* NaN_3, H_2O, 70 °C, 5 h; *ii.* KOH, Boc_2O, *t*BuOH/H_2O, r.t., 24 h, 95%. **(e)** TFA, DCM, r.t., 1 h, 95%. **(f)** DIPEA, BocNH-C(NBoc)-Im, r.t., 24 h, 64%. **(g)** *i.* BuLi, TsCl, THF, 70 °C, 15 h (**11a**) or MsCl, TEA, DCM (**11b**, **c**); *ii.* NaN_3, DMF, 90 °C, 5 h. **(h)** MsCl, TEA, DCM, 0 °C, 1 h then r.t., 2 h, 76%. **(i)** NaN_3, MeCN, reflux, 24 h, 38%. **(j)** R_2NH, TEA, MeCN, reflux, 16–24 h.



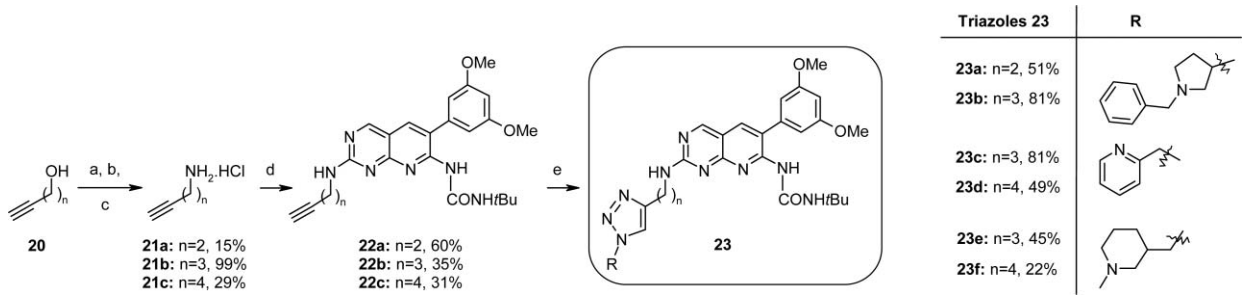
Scheme 3 *Reagents and conditions*: **(a)**. 28% aq NH_4OH, TEA, THF, r.t., 2 h 30, 76%. **(b)** $LiAlH_4$, THF, 25 °C, 45 min, 78%. **(c)** MnO_2, $CHCl_3$, r.t., 16 h, 93%. **(d)** NaH, $ArCH_2CN$, DMF, 5 °C, 5 h then r.t., 18 h, 91%. **(e)** NaH, *t*BuNCO, DMF, 0 °C, 8 h then r.t., 16 h, 90%. **(f)** *m*CPBA, $CHCl_3$, 0 °C, 2h30, 80%. **(g)** propargylamine, dioxane, 50 °C, 24 h, 86%. **(h)** RN_3, $CuSO_4{\cdot}H_2O$, sodium ascorbate, *t*BuOH/H_2O (2 : 1), r.t., 20 h-3 d. **(i)** 3M HCl, dioxane.

which were in turn reacted with sodium azide, affording **11a**[27] in moderate yield compared to **11b**, **c**. The last set of azides **14** was prepared from ethylene glycol **12** *via* a route adapted from the literature.[33] Thus, after bis-mesylation of diol **12**, one of the mesyl groups was displaced by sodium azide to give intermediate **13**, and the other one was substituted with commercially available amines providing **14a–e** in good to excellent yields. We next turned our attention to the synthesis of the PD173074 core structure bearing an alkyne handle in 2-position. Interestingly, the synthetic pathway previously reported for the pyridopyrimidine scaffold allows the introduction of various primary amines in this position,[21] giving us the opportunity to use propargylamine as alkyne carrier. Therefore, key derivative **17** was prepared following a literature procedure, the main steps of which are summarized in Scheme 3.

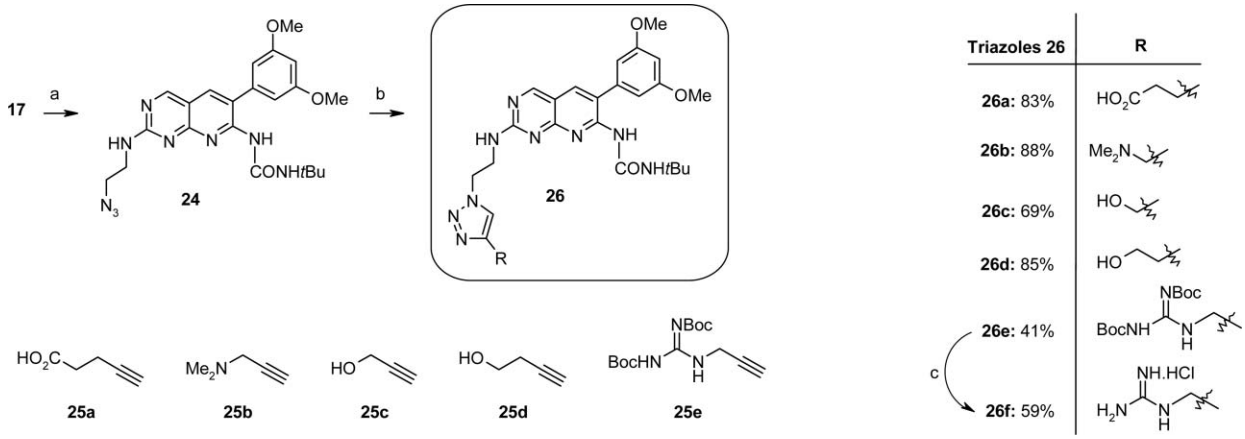
Starting from commercially available 4-chloro-5-carbethoxy-2-methylthiopyrimidine **15**, nucleophilic substitution of chlorine with aqueous ammonia followed by reduction of the ester to alcohol with lithium-aluminium hydride and oxidation with manganese oxide afforded amino-aldehyde **16** in high yield. Condensation of the aldehyde with dimethoxyphenyl-acetonitrile and subsequent protection of the amine were achieved prior to oxidation of methyl sulfide in sulfone, leading to pyridopyrimidine **17**.Attempts to perform the oxidation step with oxone® instead of *m*CPBA did not result in yield increase. The sulfone was in turn reacted with propargylic amine to provide 2-alkynylamino derivative **18**. Having alkyne **18** and azide partners in hand, we investigated the click assembling reaction. According to the standard procedure, we performed 1,3-dipolar cycloaddition with a slight excess of azido reactants, $CuSO_4$ as copper source and sodium ascorbate as reducing agent. Thus, as expected for the Cu(I) catalyzed version of Huisgen reaction, these conditions produced 1,4-disubstituted triazoles **19** as single products. We also found out that *t*BuOH/H_2O was the best solvent system and most of the click reactions were completed within 20 h at room temperature. As can be seen in Scheme 3, the overall range of azides reacted smoothly under these conditions with good to excellent yields. In the particular case of **19c**, a further step of acidolysis allowed the removal of Boc protecting groups, furnishing guanidino derivative **19d** as an hydrochloride salt.

Next, with the aim of studying the influence of chain length, we undertook the synthesis of various pyridopyrimidine derivatives **22a–c** bearing an alkyne portion tethered by 2, 3 or 4 methylene groups as shown in Scheme 4. Indeed, a flexible linker may allow substituents to interact very nicely with surrounding residues in the ATP-binding site. Thus, the homopropargyl alcohol and its higher homologous **20** were converted to their corresponding mesylates which were in turn reacted with sodium azide as described in the literature.[34] In order to facilitate the purification step, the reduction of the azido group was achieved with ethylenebis (diphenylphosphine) instead of triphenylphosphine. In addition, difficulties to isolate free amines were circumvented *via* the formation of their hydrochloride salts. This modified methodology provided aminoalkyne **21b–c** in excellent yields, except for **21a**, probably because of its low molecular weight. Subsequent S_NAr with sulfone **17** was carried out in presence of DIPEA and afforded pyridopyrimidine **22a–c**. Further 1,3-cycloadditions were performed under usual conditions to afford new triazoles **23a–f**.

Finally, we were interested in obtaining the azido-analog of alkyne **18** in order to examine the impact of triazole orientation. Unfortunately, the lack of synthetic method to prepare such azido-methanamine derivative restricted us to the synthesis of homologous **24** bearing a two methylene spacer as shown in Scheme 5. For this purpose, sulfone **17** was reacted with azidoamine **8** in presence of DIPEA to afford pyridopyrimidine **24**. Click experiments were realized using a series of commercially available alkynes **25a–d** whereas **25e** was prepared by guanylation of propargylamine. Cycloadditions were performed under usual conditions, as described above, and readily afforded triazoles **26a–f**. In the particular case of **26e**, a further acidolysis step allowed the removal of Boc protecting groups, yielding the guanidino derivative **26f** as an hydrochloride salt.



Scheme 4 *Reagents and conditions*: **(a)** *i.* MsCl, TEA, DCM, 0 °C, 3 h; 55% ($n = 2$), 100% ($n = 3$), 91% ($n = 4$). **(b)** NaN_3, DMF, 80 °C, 3 h 30; 61% ($n = 2$), 100% ($n = 3$), 52% ($n = 4$). **(c)** *i.* Ph_2P-$(CH_2)_2$-PPh_2, THF, H_2O, r.t., 16h; *ii.* 10% HCl; 44% ($n = 2$), 100% ($n = 3$), 61% ($n = 4$). **(d) 17**, DIPEA, dioxane, 70 °C, 20 h. **(e)** RN_3, $CuSO_4 \cdot H_2O$, sodium ascorbate, *t*BuOH/H_2O (2 : 1), r.t., 20 h-3 d.



Scheme 5 *Reagents and conditions*: **(a)** DIPEA, **8**, dioxane, 50 °C, 24 h, 80%. **(b)** RC≡CH, $CuSO_4 \cdot H_2O$, sodium ascorbate, *t*BuOH/H_2O (2 : 1), r.t., 20 h. **(c)** 3 M HCl, dioxane.

Biological evaluations

The inhibitory potency of all target compounds was examined *in vitro* at 500 nM and 2 μM using the recombinant FGFR3 kinase domain. PD173074 was used as the reference inhibitor and the amount of residual phosphotyrosine products was estimated by measurement of absorbance at 450 nm (see experimental section for details). Ranking of inhibitor activities was similar for 500 nM and 2 μM inhibitor concentration (see ESI, section **4**†). For the sake of clarity, only results observed at 2 μM are shown in Fig. 3. The general trend shows that most of the active compounds belongs to the first series (**19a–19m**, Scheme 3) and the less active compounds are mainly part of the last series (**26a–f**, Scheme 5).

All of the 13 analogues tested within the 1st series were able to inhibit phosphorylation at 2 μM and most of them with similar potency to the parent PD173074.

Therefore, introduction of 1,2,3-triazole moiety in position 2 of the pyridopyrimidine scaffold seems to have no significant negative effect on potency. However, alkyne **18** is unexpectedly one of the most potent inhibitors, which is nevertheless consistent with the hydrophobicity of region II. On the other hand, compound **19b** is the only one within this 1st series to display less than 50% inhibition at 2 μM, indicating that the carboxylic acid function, which is negatively charged at physiological pH, is less tolerated than other functions (phenol, alkoxy, guanidine and amine) which are neutral or positively charged under physiological conditions.

Finally, focusing on the most potent derivatives (*i.e.* residual kinase activity < 25% at 2 μM) highlights that the best triazole substitution is either an aromatic ring (*i.e.* pyridine **19j** or phenol **19h**) or an aza-containing heterocycle (*i.e.* pyrrolidine **19k**, piperidine **19g** or piperazine **19f**). Of note, any further substitution of these moieties resulted in decreased inhibition potency (**19l–m** *versus* **19f** and **19i** *versus* **19h**).

Within this 1st series, pyridine **19j**, pyrrolidine **19k** and piperidine **19g** were chosen as points of reference for the synthesis of the 2nd set of analogues. These compounds bear a spacer, the length of which varies from two to four carbon atoms. All of the 9 analogues (**22a**–**23f**) were less active than their parent compounds, respectively, indicating that increasing the chain length had a negative effect on potency.

Finally, the 3rd group of derivatives, built up to examine the impact of the triazole orientation, was examined. All of the 5 compounds **26a–f** exhibited a complete loss of activity, indicating that triazole geometry may be crucial for biological activity.

Amongst the most promising inhibitors, we next selected **19g** and **19h** for further cellular assays. To examine the FGFR3 tyrosine phosphorylation level after inhibitor treatment, we used HEK cells transfected with FGFR3-K650M construct,[35] K650M being an activating mutation responsible for SADDAN, a severe achondroplasia. Immunoblot analysis reported in Fig. 4 shows that after treatment at 2 μM for one night, the level of phosphorylated FGFR3 was still detected with **19h** (lane 2) whereas

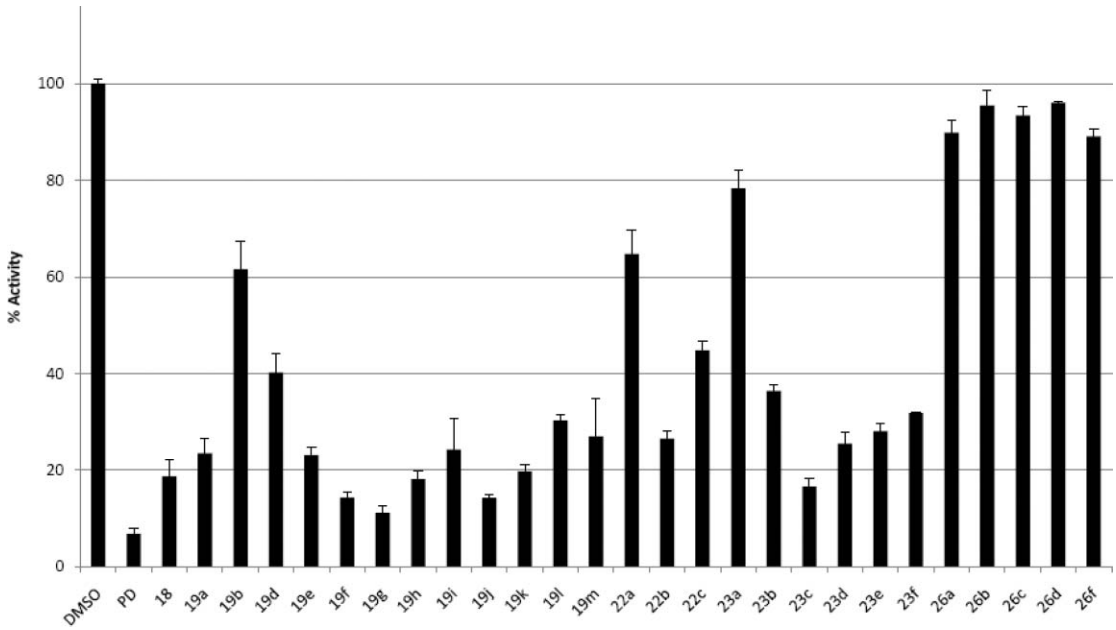


Fig. 3 Percentage of *in vitro* activity of recombinant FGFR3 kinase domain in presence of synthesized compounds at 2 μM; values are presented as means of triplicates, error bars indicate one standard deviation. Percentage was calculated relative to the DMSO control.

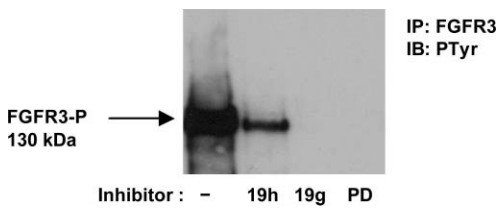


Fig. 4 Immunodetection of phosphorylated FGFR-3 in HEK transfected cells without (lane 1) or with a tyrosine kinase inhibitor treatment (lanes 2, 3 and 4 with **19h**, **19g** and PD173074, respectively). Immunoprecipitation (IP) and immunoblotting (IB) protocols are detailed in the experimental section.

it was strongly reduced with **19g** and PD173074 (lanes 3 and 4, respectively) compared to the level of unphosphorylated FGFR3 which was the same in the four cellular extracts (data not shown).

Discrepancy of inhibition may reveal that **19h**, bearing a phenol substituent, is less able to pass through the cellular membrane than PD173074 and **19g**, that both bear an alkylamino substituent.

Conclusions

The present study identifies a new class of FGFR3 kinase inhibitors, designed from a pyrido[2,3-*d*]pyrimidine central core to examine possible interactions with an unexplored region of the ATP binding-site. The key step of click- chemistry allowed a rapid and efficient synthesis of various analogues bearing a large panel of substituents in terms of size and polarity. Among the 27 analogues synthesized, more than half exhibited 55–89% inhibition of *in vitro* FGFR3 kinase activity at 2 μM and one was able to inhibit auto-phosphorylation of mutant FGFR3-K650M in transfected HEK cells. The present investigation highlights that the targetted region of the ATP binding-site accepts 1,2,3-triazole moiety, with a preference for positively charged substituents.

Experimental

Chemical Synthesis

All non aqueous reactions were carried out under an argon atmosphere. All commercial reagents and anhydrous solvents were purchased from Aldrich or Acros and were used without further purification. Thin layer chromatography (TLC) was performed with Merck 60F-$_{254}$ precoated silica (0.2 mm) on glass. Flash column chromatography was performed with Merck Kieselgel 60 (40–63 μm). NMR spectra were recorded on a Bruker AM250 or a Bruker Avance II 500 instrument in $CDCl_3$ at 298 K (unless indicated) and chemical shifts (δ) are reported in ppm. Mass spectra, electrospray (ESI) and high resolution (HRMS) were recorded by the Service de Spectrométrie de Masse, ICSN, Gif sur Yvette. Elemental analyses were performed by the Service de Microanalyse, ICSN, Gif sur Yvette.

1-*tert*-Butyl-3-(6-(3,5-dimethoxyphenyl)-2-(prop-2-ynylamino)-pyrido[2,3-*d*]pyrimidin-7-yl)urea (18). A mixture of **17**[21] (3.81 g, 8.29 mmol) and propargylamine (1.42 mL, 20.73 mmol) in dry dioxane (65 mL) was heated at 50 °C for 20 h. The solvent was then concentrated *in vacuo* and the crude residue was purified by flash column chromatography (CH_2Cl_2–MeOH 98 : 2) to give alkyne **18** (3.1 g, 86%) as a white solid. R_f 0.26 (CH_2Cl_2–MeOH 98 : 2); ^{1}H NMR: δ 10.23 (br s, 1H, NHCO), 8.78 (s, 1H, H-4), 7.71 (s, 1H, H-5), 7.15 (br s, 1H, NH*t*Bu), 6.56–6.44 (m, 3H, H-2′, H-4′, H-6′), 5.56 (br t, J = 5.6, 1H, N*H*CH_2), 4.39 (dd, J = 5.6, 2.5, 2H, H-1″), 3.83 (s, 6H, OCH_3), 2.27 (t, J = 2.5, 1H, H-3″), 1.53 (s, 9H, CMe_3); ^{13}C NMR: δ 162.1, 161.9, 161.1, 158.6, 155.9, 152.8, 137.2, 136.7, 123.3, 110.3, 107.4, 101.0, 80.5, 71.3, 55.7, 51.1, 31.5, 29.1; HRMS (ESI): Calc. for $C_{23}H_{26}N_6O_3Na$ $[M+Na]^+$ 457.1964, found 457.1945.

General procedure for the cycloaddition reactions

A mixture of alkyne (1 eq.), azide (1 eq.), sodium ascorbate (0.2 eq.), $CuSO_4 \cdot 5H_2O$ (0.1 eq.) in 2 : 1 *t*BuOH/H_2O (26 mL/1 mmol) was vigorously stirred at room temperature for several h (TLC control). Typical reaction times are from 20 h to 3 d. After disappearance of alkyne, the mixture was diluted with CH_2Cl_2 and washed with water, dried ($MgSO_4$) then concentrated *in vacuo*. Purification of the crude residue afforded the corresponding triazoles.

1-*tert*-Butyl-3-(2-((1-(2-(diethylamino)ethyl)-*1H*-1,2,3-triazol-4-yl)methylamino)-6-(3,5-dimethoxyphenyl)pyrido[2,3-*d*]pyrimidin-7-yl)urea (19a). R_f 0.24 (CH_2Cl_2–MeOH 93 : 7); ^{1}H NMR: δ 10.33 (br s, 1H, NHCO), 8.75 (s, 1H, H-4), 7.72 (br s, 1H, H_{tri}), 7.68 (s, 1H, H-5), 7.15 (br s, 1H, NH*t*Bu), 6.56–6.44 (m, 3H, H-2′, H-4′, H-6′), 5.94 (t, J = 5.9, 1H, N*H*CH2), 4.86 (d, J = 5.9, 2H, H-1″), 4.37 (t, J = 6.4, 2H, H-a), 3.89 (s, 6H, OCH_3), 2.85 (t, J = 6.4, 2H, H-b), 2.51 (q, J = 7.1, 4H, CH_2CH$_3$), 1.49 (s, 9H, CMe_3); 1.01 (t, J = 7.1, 6H, CH$_2$CH_3); ^{13}C NMR: δ 162.4, 161.8, 161.1, 158.5, 155.7, 152.8, 145.1, 137.2, 136.7, 123.1, 122.8, 110.0, 107.3, 100.8, 55.7, 55.6, 53.1, 51.0, 49.2, 47.4, 37.2, 29.1, 12.0; Anal. Calc. for $C_{29}H_{40}N_{10}O_3$: C, 60.40; H, 6.99; N, 24.29; found: C, 59.98; H, 7.13; N, 24.16; HRMS (ESI): Calc. for $C_{29}H_{40}N_{10}O_3Na$ [M+Na]$^+$ 599.3183, found 599.3144.

3-(4-((7-(3-*tert*-Butylureido)-6-(3,5-dimethoxyphenyl)pyrido-[2,3-*d*]pyrimidin-2-ylamino) methyl)-*1H*-1,2,3-triazol-1-yl)propanoic acid (19b). R_f 0.17 (CH_2Cl_2–MeOH 90 : 10); ^{1}H NMR: δ 10.18 (br s, 1H, NHCO), 8.84 (br s, 1H, H-4), 7.95 (br s, 1H, H_{tri}), 7.69 (br s, 1H, H-5), 6.55–6.38 (m, 3H, H-2′, H-4′, H-6′), 4.93 (br s, 2H, H-1″), 4.69 (t, J = 5.0, 2H, H-a), 3.80 (s, 6H, OCH_3), 2.85 (m, 2H, H-b), 1.45 (s, 9H, CMe_3); ^{13}C NMR: δ 175.3, 162.5, 161.9, 161.7, 156.2, 152.6, 145.2, 137.3, 136.4, 123.6, 123.2, 109.7, 107.4, 101.0, 55.7, 55.6, 51.5, 45.9, 37.9, 35.1, 29.2; MS (ESI): m/z = 550 [M+H]$^+$ 100%; HRMS (ESI): Calc. for $C_{26}H_{31}N_9O_5Na$ [M+Na]$^+$ 572.2346, found 572.2358.

1-*tert*-Butyl-3-(6-(3,5-dimethoxyphenyl)-2-((1-(2-(*N*,*N*′-bis-(*tert*-butoxycarbonyl)guanidino)ethyl)-*1H*-1,2,3-triazol-4-yl)methylamino)pyrido[2,3-*d*]pyrimidin-7-yl)urea hydrochloride (19c). R_f 0.23 (CH_2Cl_2–MeOH 96 : 4); ^{1}H NMR: δ 11.42 (s, 1H, NHBoc), 10.31 (br s, 1H, NHCO), 8.76 (s, 1H, H-4), 8.53 (t, J = 5.3, NH_{gua}), 7.71 (s, 1H, H_{tri}), 7.69 (s, 1H, H-5), 7.18 (s, 1H, NH*t*Bu), 6.60–6.40 (m, 3H, H-2′, H-4′, H-6′), 5.99 (br s, 1H, N*H*CH$_2$), 4.89 (d, J = 5.5, 2H, H-1″), 4.55 (t, J = 5.3, 2H, H-a), 3.91 (m, 2H, H-b), 3.82 (s, 6H, OCH_3), 1.49 and 1.47 (s, 27H, CMe_3); ^{13}C NMR: δ 163.3, 162.3, 161.7, 161.1, 158.4, 156.4, 155.7, 153.0, 152.7, 145.6, 137.2, 136.6, 122.8, 110.0, 107.3, 100.8, 83.5, 79.6, 55.6, 51.0, 49.3, 40.6, 37.1, 29.1, 28.3, 28.1; MS (ESI): m/z = 785 [M+Na]$^+$ 100%.

1-*tert*-Butyl-3-(6-(3,5-dimethoxyphenyl)-2-((1-(2-guanidinoethyl)-*1H*-1,2,3-triazol-4-yl)methylamino)pyrido[2,3-*d*]pyrimidin-7-yl)urea hydrochloride (19d). To a solution of triazole **19c** (30 mg, 0.039 mmol) in dioxane (3 mL) was added 3 N HCl (1.3 mL) and the mixture was stirred at room temperature for 3 h 30 then concentrated in vacuo. The residue was triturated with Et_2O and the solid was filtered then dried under vacuum to afford guanidine **19d** (22 mg, 93%) as a yellow solid. ^{1}H NMR (CD_3OD): δ 9.09 (s, 1H, H-4), 8.41 (s, 1H, H_{tri}), 8.13 (s, 1H, H-5), 6.80–6.60 (m, 3H, H-2′, H-4′, H-6′), 4.90–4.60 (m, 4H, H-a, H-1″), 3.95–3.75 (m, 8H, OCH_3, H-b), 1.42 (s, 9H, CMe_3); MS (ESI): m/z = 563 [M-HCl +H]$^+$ 100%.

1-*tert*-Butyl-3-(6-(3,5-dimethoxyphenyl)-2-((1-(2-(piperidin-1-yl)ethyl)-*1H*-1,2,3-triazol-4-yl)methylamino)pyrido[2,3-*d*]pyrimidin-7-yl)urea (19e). R_f 0.37 (CH_2Cl_2–MeOH/NEt_3 90 : 10 : 0.5); ^{1}H NMR: δ 10.31 (br s, 1H, NHCO), 8.73 (br s, 1H, H-4), 7.75 (br s, 1H, H_{tri}), 7.68 (s, 1H, H-5), 7.13 (br s, 1H, NH*t*Bu), 6.55–6.40 (m, 3H, H-2′, H-4′, H-6′), 5.96 (br t, J = 6.0, 1H, N*H*CH$_2$), 4.84 (d, J = 6.0, 2H, H-1″), 4.42 (t, J = 6.3, 2H, H-a), 3.80 (s, 6H, OCH_3), 2.73 (t, J = 6.3, 2H, H-b), 2.45–2.30 (m, 4H, H_{pip}), 1.53–1.28 (m, 15H, CMe_3, H_{pip}); ^{13}C NMR: δ 162.4, 161.8, 161.1, 158.6, 155.7, 152.8, 145.3, 137.3, 136.7, 123.1, 122.8, 110.0, 107.4, 100.9, 58.3, 55.7, 55.6, 54.6, 51.0, 47.9, 37.2, 29.1, 25.9, 24.2; Anal. Calc. for $C_{23}H_{26}N_6O_3 \cdot 0.7H_2O$: C, 59.92; H, 6.94; N, 23.29; found: C, 59.90; H, 6.89; N, 23.24; HRMS (ESI): Calc. for $C_{30}H_{40}N_{10}O_3Na$ [M+Na]$^+$ 611.3183, found 611.3181.

1-*tert*-Butyl-3-(6-(3,5-dimethoxyphenyl)-2-((1-(2-(4-methylpiperazin-1-yl)ethyl)-*1H*-1,2,3-triazol-4-yl)methylamino)pyrido[2,3-*d*]pyrimidin-7-yl)urea (19f). R_f 0.12 (CH_2Cl_2–MeOH/NEt_3 90 : 10 : 0.3); ^{1}H NMR: δ 10.31 (br s, 1H, NHCO), 8.79 (s, 1H, H-4), 7.78 (s, 1H, H_{tri}), 7.73 (s, 1H, H-5), 7.19 (s, 1H, NH*t*Bu), 6.60–6.45 (m, 3H, H-2′, H-4′, H-6′), 6.00 (br t, J = 5.5, 1H, N*H*CH$_2$), 4.86 (d, J = 5.5, 2H, H-1″), 4.47 (t, J = 6.5, 2H, H-a), 3.86 (s, 6H, OCH_3), 2.84 (t, J = 6.5, 2H, H-b), 2.65–2.30 (m, 8H, H_{pip}), 2.24 (s, 3H, NCH_3), 1.53 (s, 9H, CMe_3); ^{13}C NMR: δ 162.4, 161.8, 161.2, 158.6, 155.7, 152.8, 145.4, 137.3, 136.7, 123.1, 122.8, 110.1, 107.3, 100.8, 57.6, 55.7, 55.6, 55.0, 53.1, 51.0, 47.9, 46.0, 37.2, 29.2; HRMS (ESI): Calc. for $C_{30}H_{41}N_{11}O_3Na$ [M+Na]$^+$ 626.3292, found 626.3284.

1-*tert*-Butyl-3-(6-(3,5-dimethoxyphenyl)-2-((1-((1-methylpiperidin-3-yl)methyl)-*1H*-1,2,3-triazol-4-yl)methylamino)pyrido[2,3-*d*]pyrimidin-7-yl)urea (19g). R_f 0.17 (CH_2Cl_2–MeOH 90 : 10); ^{1}H NMR: δ 10.30 (br s, 1H, NHCO), 8.75 (br s, 1H, H-4), 7.69 (br s, 1H, H-5), 7.61 (br s, 1H, H_{tri}), 7.16 (br s, 1H, NH*t*Bu), 6.51–6.47 (m, 3H, H-2′, H-4′, H-6′), 6.25 (br s, 1H, N*H*CH$_2$), 4.85 (d, J = 5.7, 2H, H-1″), 4.24 (d, J = 7.1, 2H, H-a), 3.81 (s, 6H, OCH_3), 2.19 (s, 3H, NCH_3), 1.48 (s, 9H, CMe_3), 2.68–1.00 (m, 8H, H_{pip}); ^{13}C NMR: δ 162.6, 162.1, 161.4, 158.8, 156.0, 153.0, 145.8, 137.4, 136.9, 123.2, 122.9, 110.4, 107.7, 101.2, 59.3, 56.2, 56.1, 55.9, 54.0, 51.2, 46.7, 37.5, 37.4, 29.4, 27.8, 24.5; HRMS (ESI): Calc. for $C_{30}H_{40}N_{10}O_3Na$ [M+Na]$^+$ 611.3183, found 611.3165.

1-(2-((1-(4-Hydroxybenzyl)-*1H*-1,2,3-triazol-4-yl)methylamino)-6-(3,5-dimethoxyphenyl)pyrido[2,3-*d*]pyrimidin-7-yl)-3-*tert*-butylurea (19h). R_f 0.17 (CH_2Cl_2–MeOH 95 : 5); ^{1}H NMR (500 MHz, $CDCl_3$+CD_3OD): δ 8.97 (br s, 1H, H-4), 7.66 (s, 1H, H-5), 7.50 (s, 1H, H_{tri}), 7.01 (d, J = 7.0, 2H, H-b′, H-f′), 6.69 (d, J = 7.0, 2H, H-c′, H-e′), 6.55–6.35 (m, 3H, H-2′, H-4′, H-6′), 5.31 (s, 2H, H-a), 4.70 (br s, 2H, H-1″), 3.77 (s, 6H, OCH_3), 1.37 (s, 9H, CMe_3); ^{13}C NMR (125 MHz, $CDCl_3$+CD_3OD): δ 161.9, 161.8, 161.4, 157.5, 155.5, 153.1, 145.0, 137.4, 136.3, 129.7, 125.2, 123.1, 122.4, 115.9, 109.9, 107.3, 101.0, 55.5, 53.9, 51.1, 36.8, 28.8; MS (ESI): m/z = 583 [M]$^+$ 100%.

1-(2-((1-(Benzo[d][1,3]dioxol-5-ylmethyl)-*1H*-1,2,3-triazol-4-yl)methylamino)-6-(3,5-dimethoxyphenyl)pyrido[2,3-*d*]pyrimidin-7-yl)-3-*tert*-butylurea (19i). R_f 0.21 (CH_2Cl_2–MeOH 95 : 5); ^{1}H NMR (DMF): δ 10.41 (br s, 1H, NHCO), 9.02 (br s, 1H, NH*t*Bu),

8.13 (br s, 1H, C-5′), 8.08 (s, 1H, H-5), 8.02 (s, 1H, H-4), 7.29 (br s, 1H, N*H*CH_2), 6.98–6.82 (m, 3H, H-b′, H-e′, H-f′), 6.78–6.64 (m, 3H, H-2′, H-4′, H-6′), 6.05 (s, 2H, OCH_2O), 5.57 (s, 2H, H-a), 4.81 (m, 2H, H-1″), 3.89 (s, 6H, OCH_3), 1.43 (s, 9H, C*Me*$_3$); ^{13}C NMR (DMF): δ 163.6–162.9, 159.2, 156.2, 153.4, 149.0, 148.6, 147.8, 139.2, 138.5, 131.2, 123.8, 122.9, 122,7, 111.1, 109.5, 109.3, 108.3, 102.6, 101.3, 56.4, 56.3, 53.9, 51.3, 37.9, 29.5; HRMS (ESI): Calc. for $C_{31}H_{33}N_9O_5Na$ [M+Na]$^+$ 634.2502, found 634.2488.

1-*tert*-Butyl-3-(6-(3,5-dimethoxyphenyl)-2-((1-(pyridin-2-ylmethyl)-*1H*-1,2,3-triazol-4-yl)methylamino)pyrido[2,3-*d*]pyrimidin-7-yl)urea (19j). ^{1}H NMR: δ 10.26 (br s, 1H, NHCO), 8.72 (s, 1H, H-4), 8.56 (d, J = 4.9, 1H, H-f′), 7.76 (br s, 1H, H_{tri}), 7.67 (s, 1H, H-5), 7.65 (td, J = 7.8, 1.7, 1H, H-d′), 7.28–7.10 (m, 3H, H-e′, H-c′, NH*t*Bu), 6.53–6.43 (m, 3H, H-2′, H-4′, H-6′), 5.93 (t, J = 5.8, 1H, N*H*CH_2), 5.61 (s, 2H, H-a), 4.85 (d, J = 5.8, 2H, H-1″), 3.80 (s, 6H, OCH_3), 1.43 (s, 9H, C*Me*$_3$); ^{13}C NMR: δ 162.4, 161.8, 161.2, 158.6, 155.7, 154.6, 152.8, 149.9, 146.0, 137.4, 137.3, 136.7, 123.5, 123.1, 122.9, 122.4, 110.1, 107.5, 100.9, 55.8, 55.7, 55.6, 51.0, 37.3, 29.1; MS (ESI): m/z = 591 [M+Na]$^+$ 100%; HRMS (ESI): Calc. for $C_{29}H_{32}N_{10}O_3Na$ [M+Na]$^+$ 591.2557, found 591.2554.

1-(2-((1-(1-Benzylpyrrolidin-3-yl)-*1H*-1,2,3-triazol-4-yl)methylamino)-6-(3,5-dimethoxyphenyl)pyrido[2,3-*d*]pyrimidin-7-yl)-3-*tert*-butylurea (19k). R_f 0.70 (CH_2Cl_2–MeOH/NEt_3 90 : 10 : 0.5); ^{1}H NMR: δ 10.35 (br s, 1H, NHCO), 8.77 (br s, 1H, H-4), 7.87 (br s, 1H, H_{tri}), 7.70 (br s, 1H, H-5), 7.26–7.22 (m, 5H, H_{Ph}), 7.16 (br s, 1H, NH*t*Bu), 6.53–6.46 (m, 3H, H-2′, H-4′, H-6′), 6.13 (br s, 1H, N*H*CH_2), 5.23-5.21 (m, 1H, H-c), 4.87 (d, J = 5.2, 2H, H-1″), 3.82 (s, 6 H, OCH_3), 3.70 (AB, J = 11.9, 2H, CH_2Ph), 3.11–2.10 (m, 6H, H-b, H-d, H-e), 1.49 (s, 9H, C*Me*$_3$); ^{13}C NMR: δ 162.7, 162.1, 161.4, 158.8, 156.0, 153.0, 146.0, 138.5, 137.4, 136.9, 128.9, 128.7, 127.7, 123.2, 120.8, 110.4, 107.6, 101.2, 60.3, 59.9, 59.7, 55.9, 55.8, 53.0, 51.2, 37.6, 33.0, 29.3; MS (ESI): m/z = 637 [M+H]$^+$ 100%; HRMS (ESI): Calc. for $C_{34}H_{40}N_{10}O_3Na$ [M+Na]$^+$ 659.3183, found 659.3192.

1-*tert*-Butyl-3-(6-(3,5-dimethoxyphenyl)-2-((1-(2-(4-(5-(trifluoromethyl)pyridin-2-yl)piperazin-1-yl)ethyl)-*1H*-1,2,3-triazol-4-yl)methylamino)pyrido[2,3-*d*]pyrimidin-7-yl)urea (19l). R_f 0.21 (CH_2Cl_2–MeOH 95 : 5); ^{1}H NMR: δ 10.31 (br s, 1 H, NHCO), 8.71 (s, 1H, H-4), 8.33 (s, 1H, H_{pyr}), 7.78 (br s, 1H, H_{tri}), 7.63 (s, 1H, H-5), 7.56 (d, J = 8.9, 1H, H_{pyr}), 7.17 (s, 1H, NH*t*Bu), 6.64 (br s, 1H, N*H*CH_2), 6.54 (d, J = 8.9, 1H, H_{pyr}), 6.50–6.39 (m, 3H, H-2′, H-4′, H-6′), 4.84 (d, J = 5.5, 2H, H-1″), 4.45 (t, J = 5.8, 2H, H-a), 3.80 (s, 6H, OCH_3), 3.49 (m, 4H, H_{pip}), 2.83 (t, J = 5.8, 2H, H-b), 2.51 (m, 4H, H_{pip}), 1.46 (s, 9H, C*Me*$_3$); ^{13}C NMR: δ 162.4, 161.8, 161.2, 160.2, 158.5, 155.7, 152.8, 145.9, 145.5, 137.3, 134.6, 136.6, 134.6, 123.0, 122.5, 115.4, 110.1, 110.5, 105.6, 100.7, 57.6, 55.7, 55.6, 52.8, 51.0, 47.8, 44.6, 37.2, 29.2; MS (ESI): m/z = 736 [M+H]$^+$ 100%; HRMS (ESI): Calc. for $C_{35}H_{41}N_{12}O_3F_3Na$ [M+Na]$^+$ 757.3274, found 757.3278.

1-*tert*-Butyl-3-(6-(3,5-dimethoxyphenyl)-2-((1-(2-(4-(2-(2,5-dimethyl-*1H*-pyrrol-1-yl)ethyl)piperazin-1-yl)ethyl)-1*H*-1,2,3-triazol-4-yl)methylamino)pyrido[2,3-*d*]pyrimidin-7-yl)urea (19m). R_f 0.42 (CH_2Cl_2–MeOH 90 : 10); ^{1}H NMR: δ 10.29 (br s, 1H, NHCO), 8.75 (s, 1H, H-4), 7.70 (s, 1H, H_{tri}), 7.69 (s, 1H, H-5), 7.17 (s, 1H, NH*t*Bu), 6.58–6.42 (m, 3H, H-2′, H-4′, H-6′), 6.00 (t, J = 5.8, 1H, N*H*CH_2), 5.74 (s, 2H, H_{pyr}), 4.86 (d, J = 5.8, 2H, H-1″), 4.45 (t, J = 6.0, 2H, H-a), 3.89 (m, 2H, CH_2-pyrrole), 3.82 (s, 6 H, OCH_3), 2.86 (t, J = 6.0, 2H, H-b), 2.63–2.40 (m, 10H, H_{pip}, CH_2-piperazine), 2.17 (s, 6H, CH_3-pyrrole), 1.49 (s, 9H, C*Me*$_3$); ^{13}C NMR: δ 162.4, 161.8, 161.2, 158.6, 155.7, 152.8, 145.3, 137.2, 136.6, 127.4, 122.9, 122.7, 110.1, 107.4, 105.4, 100.8, 58.4, 57.6, 55.7, 55.6, 53.6, 53.1, 51.0, 47.8, 41.5, 37.3, 29.1, 12.6; MS (ESI): m/z = 712 [M+H]$^+$ 100%; HRMS (ESI): Calc. for $C_{37}H_{50}N_{12}O_3Na$ [M+Na]$^+$ 733.4034, found 733.4027.

1-*tert*-Butyl-3-(6-(3,5-dimethoxyphenyl)-2-(but-3-ynylamino)pyrido[2,3-*d*]pyrimidin-7-yl)urea (22a). To a solution of **17**[21] (414 mg, 0.90 mmol) in dry dioxane (10 mL) was added 3-butyn-1-amine hydrochloride **21a** (95 mg, 0.90 mmol) and DIPEA (0.31 mL, 1.81 mmol). The mixture was heated at 70 °C for 20 h, and then concentrated in vacuo. The crude residue was diluted with CH_2Cl_2, washed with a saturated solution of $NaHCO_3$ (20 mL) and water (20 mL), dried ($MgSO_4$) then concentrated in vacuo. Purification by column chromatography (CH_2Cl_2–MeOH 98 : 2) gave alkyne **22a** (251 mg, 60%) as a yellow solid. R_f 0.40 (CH_2Cl_2–MeOH 95 : 5); ^{1}H NMR: δ 10.32 (br s, 1H, NHCO), 8.74 (s, 1H, H-4), 7.68 (s, 1H, H-5), 7.16 (br s, 1H, NH*t*Bu), 6.52–6.44 (m, 3H, H-2′, H-4′, H-6′), 5.96 (br s, 1H, N*H*CH_2), 3.77 (s, 6H, OCH_3), 3.73 (q, J = 6.8, 2H, H-1″), 2.70–2.50 (m, 2H, H-2″), 1.99 (t, J = 2.6, H-4″), 1.48 (s, 9H, C*Me*$_3$); ^{13}C NMR: δ 162.4, 161.8, 161.2, 158.5, 155.8, 152.8, 137.1, 136.6, 122.9, 110.0, 107.4, 100.9, 81.8, 70.1, 55.7, 55.6, 51.1, 40.5, 29.0, 19.2; MS (ESI): m/z = 449 [M+H]$^+$ 100%.

1-*tert*-Butyl-3-(6-(3,5-dimethoxyphenyl)-2-(pent-4-ynylamino)pyrido[2,3-*d*]pyrimidin-7-yl)urea (22b). This compound was prepared according the procedure outlined for compound **22a** starting from **17** (574 mg, 1.25 mmol), 4-pentyn-1-amine hydrochloride **21b** (299 mg, 2.50 mmol) and DIPEA (0.86 mL, 5 mmol) in dry dioxane (12 mL) at 50 °C for 20 h. Purification by flash column chromatography (CH_2Cl_2–MeOH 98 : 2) furnish alkyne **22b** (411 mg, 35%) as a yellow solid. R_f 0.27 (CH_2Cl_2–MeOH 95 : 5); ^{1}H NMR: δ 10.34 (br s, 1H, NHCO), 8.71 (s, 1H, H-4), 7.66 (s, 1H, H-5), 7.14 (br s, 1H, NH*t*Bu), 6.50–6.46 (m, 3H, H-2′, H-4′, H-6′), 5.73 (m, 1H, N*H*CH_2), 3.81 (s, 6H, OCH_3), 3.70 (q, J = 6.7, 2H, H-1″), 2.34 (td, J = 6.7, 2.7, 2H, H-3″), 1.99 (t, J = 2.7, 1H, H-5″), 1.94 (qt, J = 6.7, 6.7, 2H, H-2″), 1.49 (s, 9H, C*Me*$_3$); ^{13}C NMR: δ 163.1, 162.0, 161.2, 158.6, 155.9, 153.0, 137.4, 137.0, 122.7, 110.1, 107.6, 101.1, 83.9, 69.3, 55.9, 55.8, 51.2, 41.0, 29.2, 28.2, 16.4; MS (ESI): m/z = 485 [M+Na]$^+$ 100%; HRMS (ESI): Calc. for $C_{25}H_{30}N_6O_3Na$ [M+Na]$^+$ 485.2277, found 485.2255.

1-*tert*-Butyl-3-(6-(3,5-dimethoxyphenyl)-2-(hex-5-ynylamino)pyrido[2,3-*d*]pyrimidin-7-yl)urea (22c). This compound was prepared according the procedure outlined for compound **22b** starting from **17** (344 mg, 0.75 mmol), 5-hexyn-1-amine hydrochloride **21c** (200 mg, 1.50 mmol) and DIPEA (0.50 mL, 2.9 mmol) in dry dioxane (12 mL) at 70 °C for 17 h. Purification by flash chromatography (CH_2Cl_2–MeOH 98 : 2) furnish alkyne **22c** (109 mg, 31%) as a yellow solid. R_f 0.28 (CH_2Cl_2–MeOH 99 : 1); ^{1}H NMR (500 MHz) : δ 10.35 (br s, 1H, NHCO), 8.75 (br s, 1H, H-4), 7.69 (br s, 1H, H-5), 7.14 (br s, 1H, NH*t*Bu), 6.58–6.48 (m, 3H, H-2′, H-4′, H-6′), 5.49 (br s, 1H, N*H*CH_2), 3.83 (s, 6H, OCH_3), 3.63 (m, 2H, H-1″), 2.28 (td, J = 7.0, 2.5, 2H, H-4″), 1.96 (t, J = 2.5, 1H, H-6″), 1.84 (qt, J = 7.0, 2H, H-2″), 1.67 (qt, J = 7.0, 2H, H-3″); 1.46 (s, 9H, C*Me*$_3$); ^{13}C NMR (125 MHz): δ 162.7, 161.7,

161.0, 157.0, 155.6, 152.8, 137.1, 136.7, 122.4, 109.7, 107.4, 100.8, 83.9, 68.7, 55.6, 51.0, 40.9, 29.0, 28.6, 25.9, 18.2; MS (ESI): m/z = 477 $[M+H]^+$ 100%.

1-(2-(2-(1-(1-Benzylpyrrolidin-3-yl)-1*H*-1,2,3-triazol-4-yl)-ethylamino)-6-(3,5-dimethoxyphenyl)pyrido[2,3-*d*]pyrimidin-7-yl)-3-*tert*-butylurea (23a). R_f 0.43 (CH_2Cl_2–MeOH 90 : 10); ^1H NMR: δ 10.35 (br s, 1H, NHCO), 8.70 (br s, 1H, H-4), 7.65 (br s, 1H, H-5), 7.63 (br s, 1H, H_{tri}), 7.40–7.19 (m, 5H, H_{Ph}), 7.15 (br s, 1H, NH*t*Bu), 6.50–6.45 (m, 3 H, H-2′, H-4′, H-6′), 6.08 (t, J = 5.5, 1H, N*H*CH_2), 5.22–5.16 (m, 1H, H-c), 4.05–3.82 (m, 2H, H-1″), 3.80 (s, 6H, OCH_3), 3.65 (s, 2H, CH_2Ph), 3.25–2.75 (m, 5H, H-2″, H-b, H-e), 2.65–2.35 (m, 2H, H-d, H-e), 2.15–1.95 (m, 1H, H-d), 1.48 (s, 9H, C*Me*$_3$); ^{13}C NMR: δ 163.0, 162.0, 161.2, 158.9, 155.9, 153.0, 146.0, 138.5, 137.4, 136.9, 128.9, 128.7, 127.6, 122.8, 120.0, 110.1, 107.6, 101.1, 60.4, 59.9, 59.6, 55.9, 55.8, 52.9, 51.2, 41.2, 33.0, 29.3, 25.9; MS (ESI): m/z = 673 $[M+Na]^+$ 100%; HRMS (ESI): Calc. for $C_{35}H_{42}N_{10}O_3Na$ $[M+Na]^+$ 673.3339, found 673.3329.

1-(2-(3-(1-(1-Benzylpyrrolidin-3-yl)-*1H*-1,2,3-triazol-4-yl)propylamino)-6-(3,5-dimethoxyphenyl)pyrido[2,3-*d*]pyrimidin-7-yl)-3-*tert*-butylurea (23b). R_f 0.29 (CH_2Cl_2–MeOH 95 : 5); ^1H NMR: δ 10.33 (br s, 1H, NHCO), 8.68 (br s, 1H, H-4), 7.62 (br s, 1H, H-5), 7.55 (br s, 1H, H_{tri}), 7.40–7.26 (m, 5H, H_{Ph}), 7.14 (br s, 1H, NH*t*Bu), 6.47–6.44 (m, 3H, H-2′, H-4′, H-6′), 5.99 (t, J = 5.2, 1H, N*H*CH_2), 5.19–5.12 (m, 1H, H-c), 3.78 (s, 6H, OCH_3), 3.63–3.58 (m, 4H, H-1″, CH_2Ph), 3.10–2.70 (m, 5H, H-3″, H-b, H-e), 2.65–2.35 (m, 2H, H-d, H-e), 2.20–1.95 (m, 3H, H-2″, H-d), 1.45 (s, 9H, C*Me*$_3$); ^{13}C NMR: δ 163.0, 162.0, 161.2, 158.9, 155.8, 153.0, 147.9, 138.6, 137.4, 137.0, 128.8, 128.7, 127.5, 122.7, 119.3, 110.0, 107.6, 101.0, 60.5, 59.9, 59.5, 55.8, 55.7, 52.9, 51.2, 41.3, 33.0, 29.2, 27.2, 23.5; MS (ESI): m/z = 687 $[M+Na]^+$ 100%; HRMS (ESI): Calc. for $C_{36}H_{44}N_{10}O_3Na$ $[M+Na]^+$ 687.3496, found 687.3472.

1-*tert*-Butyl-3-(6-(3,5-dimethoxyphenyl)-2-(3-(1-(pyridin-2-ylmethyl)-*1H*-1,2,3-triazol-4-yl)propylamino)pyrido[2,3-*d*]pyrimidin-7-yl)urea (23c). R_f 0.28 (CH_2Cl_2–MeOH 95 : 5); ^1H NMR: δ 10.35 (br s, 1H, NHCO), 8.71 (br s, 1H, H-4), 8.64–8.55 (m, 1H, H-f′), 7.71–7.65 (m, 2H, H-5, H-d′), 7.48 (br s, 1H, H_{tri}), 7.25–7.09 (m, 3H, NH*t*Bu, H-c′, H-e′), 6.52–6.47 (m, 3H, H-2′, H-4′, H-6′), 5.86 (t, 1H, J = 7.0, N*H*CH_2), 5.62 (s, 2H, CH_2Pyr), 3.82 (s, 6H, OCH_3), 3.64 (dd, 2H, J = 7.0, 7.0, H-1″), 2.86 (t, 2H, J = 7.0, H-3″), 2.17–2.07 (m, 2H, H-2″), 1.48 (s, 9H, C*Me*$_3$); ^{13}C NMR: δ 163.1, 162.0, 161.2, 158.9, 155.9, 155.0, 153.1, 150.0, 148.1, 137.6, 137.4, 137.0, 123.7, 122.7, 121.7, 110.0, 107.6, 101.0, 55.9, 55.8, 51.2, 41.3, 29.2, 23.4; MS (ESI): m/z = 597 $[M+H]^+$ 100%; HRMS (ESI): Calc. for $C_{31}H_{36}N_{10}O_3Na$ $[M+Na]^+$ 619.2870, found 619.2881.

1-*tert*-Butyl-3-(6-(3,5-dimethoxyphenyl)-2-(4-(1-((pyridine-2-ylmethyl)methyl)-*1H*-1,2,3-triazol-4-yl)butylamino)pyrido[2,3-*d*]-pyrimidin-7-yl)urea (23d). R_f 0.27 (CH_2Cl_2–MeOH 95 : 5); ^1H NMR (500 MHz): δ 10.34 (br s, 1H, NHCO), 8.79 (s, 1H, H-4), 8.56 (d, J = 4.8, H-f′), 7.81–7.61 (m, 2H, H-5, H-d), 7.52 (s, 1H, H_{tri}), 7.35–7.10 (m, 3H, H-c′, H-e′, NH*t*Bu), 6,60–6.40 (m, 3H, H-2′, H-4′, H-6′); 5.83 (br s, 1H, N*H*CH_2), 5.64 (s, 2H, CH_2pyr), 3.80 (s, 6H, OCH_3), 3.66 (br s, 2H, H-1″), 2.83 (t, 3H, J = 6.8, H-4″), 1.95–1.75 (m, 4H, H-2″, H-3″), 1.51 (s, 9H, C*Me*$_3$); ^{13}C NMR (125 MHz): δ 162.7, 161.8, 161.0, 158.6, 155.6, 154.9, 152.8, 149.8, 148.4, 137.3, 137.2, 136.8, 123.4, 122.4, 121.5, 109.7, 107.4, 100.8, 55.6, 51.0, 41.2, 29.0, 26.8, 25.4; MS (ESI): m/z = 633 $[M+Na]^+$ 100%; HRMS (ESI): Calc. for $C_{32}H_{38}N_{10}O_3Na$ $[M+Na]^+$ 633.3026, found 633.3017.

1-*tert*-Butyl-3-(6-(3,5-dimethoxyphenyl)-2-(3-(1-((1-methylpiperidin-3-yl)methyl)-*1H*-1,2,3-triazol-4-yl)propylamino)pyrido-[2,3-*d*]pyrimidin-7-yl)urea (23e). R_f 0.11 (CH_2Cl_2–MeOH 90 : 10); ^1H NMR (500 MHz): δ 10.35 (br s, 1H, NHCO), 8.72 (br s, 1H, H-4), 7.66 (s, 1H, H-5), 7.32 (s, 1H, H_{tri}), 7.13 (s, 1H, NH*t*Bu), 6,51–6.46 (m, 3H, H-2′, H-4′, H-6′), 5.77 (br s, 1H, N*H*CH_2), 4.35–4.18 (m, 2H, H-a), 3.81 (s, 6H, OCH_3), 3.71–3.56 (m, 2H, H-1″), 2.85–2.68 (m, 4H, H-b′, H-f′, H-3″), 2.29–2.20 (m, NCH_3, H-c′), 2.19–2.01 (m, 2H, H-f′, H-2″), 1.99–1.84 (m, 1H, H-b′), 1.81–1.60 (m, 3H, H-d′, H-e′), 1.48 (s, 9H, C*Me*$_3$), 1.15–0.98 (m, 1H, H-d′); ^{13}C NMR (125 MHz): δ 162.9, 161.8, 161.0, 158.7, 155.7, 152.9, 147.6, 137.3, 136.8, 122.6, 121.2, 109.8, 107.5, 100.9, 58.9, 55.8, 55.6, 53.4, 51.0, 46.3, 41.1, 36.9, 29.1, 29.0, 27.3, 24.0, 23.2; MS (ESI): m/z = 639 $[M+Na]^+$ 100%; HRMS (ESI): Calc. for $C_{32}H_{44}N_{10}O_3Na$ $[M+Na]^+$ 639.3496, found 639.3502.

1-*tert*-Butyl-3-(6-(3,5-dimethoxyphenyl)-2-(4-(1-((1-methylpiperidin-3-yl)methyl)-*1H*-1,2,3-triazol-4-yl)butylamino)pyrido-[2,3-*d*]pyrimidin-7-yl)urea (23f). R_f 0.13 (CH_2Cl_2–MeOH 90 : 10); ^1H NMR (500 MHz): δ 10.34 (br s, 1H, NHCO), 8.72 (br s, 1H, H-4), 7.68 (s, 1H, H-5), 7.39 (s, 1H, H_{tri}), 7.51 (s, 1H, NH*t*Bu), 6,70–6.35 (m, 3H, H-2′, H-4′, H-6′), 5.72 (br s, 1H, N*H*CH_2), 4.45–4.15 (m, 2H, H-a), 3.83 (s, 6H, OCH_3), 3.70–3.55 (m, 2H, H-1″), 3.05–2.70 (m, 4H, H-b′, H-f′, H-4″), 2.45–2.30 (m, 4H, NCH_3, H-c′), 2.28–2.12 (m, 1H, H-f′), 2.10–1.99 (m, 1H, H-b′), 1.97–1.64 (m, 7H, H-2″, H-3″, H-d′, H-e′), 1.49 (s, 9H, C*Me*$_3$), 1.20–1.02 (m, 1H, H-d′);^{13}C NMR (125 MHz): δ 162.9, 161.8, 161.0, 158.7, 155.7, 152.9, 148.1, 137.3, 136.8, 122.6, 121.4, 109.8, 107.6, 100.9, 58.9, 55.8, 55.6, 55.2, 53.4, 51.0, 46.2, 41.3, 36.7, 29.1, 29.0, 27.2, 26.9, 25.4, 24.0; MS (ESI): m/z = 631 $[M+H]^+$ 100%; HRMS (ESI): Calc. for $C_{33}H_{46}N_{10}O_3Na$ $[M+Na]^+$ 653.3652, found 653.3647.

1-(2-(2-Azidoethylamino)-6-(3,5-dimethoxyphenyl)pyrido-[2,3-*d*]pyrimidin-7-yl)-3-*tert*-butylurea (24). A mixture of **17**[21] (1.23 g, 2.68 mmol), 2-azidoethylamine salt **8** (1.02 g, 5.08 mmol) and DIPEA (0.92 mL, 5.35 mmol) in dry dioxane (30 mL) was warmed at 50 °C for 24 h; the solvent was removed *in vacuo* and the crude residue was purified by flash column chromatography (CH_2Cl_2–MeOH 99 : 1 to 98 : 2) to give **24** (987 mg, 80%) as a yellow solid. R_f 0.26 (CH_2Cl_2–MeOH 97 : 3); ^1H NMR: δ 10.26 (br s, 1H, NHCO), δ 8.75 (br s, 1H, H-4), 7.69 (br s, 1H, H-5), 7.16 (br s, 1H, NH*t*Bu), 6.55–6.41 (m, 3H, H-2′, H-4′, H-6′), 5.71 (br s, 1H, N*H*CH_2), 3.86–3.71 (m, 8H, OCH_3, H-1″), 3.67–3.57 (t, J = 5.3, 2H, H-2″), 1.48 (s, 9H, C*Me*$_3$); ^{13}C NMR: δ 162.6, 161.9, 161.3, 158.7, 155.9, 152.9, 137.3, 136.7, 123.2, 110.3, 107.5, 101.0, 55.8, 55.7, 51.2, 50.6, 41.2, 29.1; MS (ESI): m/z = 488 $[M+Na]^+$ 100%; HRMS (ESI): Calc. for $C_{22}H_{27}N_9O_3Na$ $[M+Na]^+$ 488.2135, found 488.2139.

3-(1-(2-(7-(3-*tert*-Butylureido)-6-(3,5-dimethoxyphenyl)pyrido-[2,3-*d*]pyrimidin-2-ylamino)ethyl)-*1H*-1,2,3-triazol-4-yl)propanoic acid (26a). R_f 0.14 (CH_2Cl_2–MeOH 9 : 1); ^1H NMR (DMF): δ 12.43 (br s, 1H, CO_2H), 10.47 (br s, 1H, NHCO), 8.99 (s, 1H, H-4), 8.09 (s, 1H, H-5), 7.92 (br s, 1H, H_{tri}), 7.84 (br s, 1H, NH*t*Bu), 6.80–6.60 (m, 3H, H-2′, H-4′, H-6′), 4.83–4.70 (m, 2H, H-2″), 4.08–3.95

(m, 2H, H-1″), 3.88 (s, 6H, OCH_3), 3.00–2.85 (m, 2H, H-a), 2.70–2.55 (m, 2H, H-b), 1.48 (s, 9H, CMe_3); ^{13}C NMR (DMF): δ 174.3, 164.0, 163.3, 162.9, 159.1, 156.2, 153.4, 147.1, 139.3, 138.5, 123.3, 122.9, 111.1, 108.3, 101.3, 56.4, 56.3, 51.4, 49.4, 42.8, 34.3, 29.1, 22.1; MS (ESI): m/z = 564 $[M+H]^+$ 100%; HRMS (ESI): Calc. for $C_{27}H_{33}N_9O_3Na$ $[M+Na]^+$ 586.2502, found 586.2509.

1-*tert*-Butyl-3-(6-(3,5-dimethoxyphenyl)-2-(2-(4-((dimethylamino)methyl)-*1H*-1,2,3-triazol-1-yl)ethylamino)pyrido[2,3-*d*]-pyrimidin-7-yl)urea (26b). R_f 0.30 (CH_2Cl_2–MeOH 80 : 20); ^1H NMR: δ 10.28 (br s, 1H, NHCO), 8.74 (s, 1H, H-4), 7.93 (br s, 1H, H-5″), 7.70 (s, 1H, H-5), 7.15 (br s, 1H, NH*t*Bu), 6.56-6.44 (m, 3H, H-2′, H-4′, H-6′), 5.83 (br s, 1H, N*H*CH_2), 4.75 (t, J = 5.9, 2H, H-2″), 4.15–4.01 (m, 2H, H-1″), 3.94 (br s, 2H, CH_2NMe_2), 3.81 (s, 6H, OCH_3), 2.53 (s, 6H, CH_3), 1.50 (s, 9H, CMe_3); ^{13}C NMR: δ 162.5, 161.9, 161.2, 158.5, 155.9, 152.8, 146.1, 137.2, 136.6, 123.8, 123.3, 110.3, 107.5, 100.9, 55.8, 55.7, 54.3, 51.1, 49.2, 45.0, 42.0, 29.1; MS (ESI): m/z = 549 $[M+H]^+$ 100%; HRMS (ESI): Calc. for $C_{27}H_{36}N_{10}O_3Na$ $[M+Na]^+$ 571.2870, found 571.2875.

1-*tert*-Butyl-3-(6-(3,5-dimethoxyphenyl)-2-(2-(4-(hydroxymethyl)-*1H*-1,2,3-triazol-1-yl)ethylamino)pyrido[2,3-*d*]pyrimidin-7-yl)urea (26c). R_f 0.42 (CH_2Cl_2–MeOH 80 : 20); ^1H NMR (500 MHz, 330 K): δ 9.88 (br s, 1H, NHCO), 8.77 (s, 1H, H-4), 7.72 (br s, 1H, H-5), 7.59 (s, 1H, H-5″), 6.57–6.46 (m, 3H, H-2′, H-4′, H-6′), 4.77 (s, 2H, CH_2OH), 4.72 (t, J = 5.2, 2H, H-2″), 4.17–4.08 (m, 2H, H-1″), 3.84 (s, 6H, OCH_3), 1.52 (s, 9H, CMe_3); ^{13}C NMR (500 MHz): δ 162.2, 161.9, 161.3, 158.5, 156.0, 152.7, 137.3, 136.4, 123.3, 121.9, 110.0, 107.4, 100.9, 55.9, 55.6, 51.1, 48.9, 42.1, 29.0; MS (ESI): m/z = 522 $[M+H]^+$ 100%; HRMS (ESI): Calc. for $C_{25}H_{31}N_9O_4Na$ $[M+Na]^+$ 544.2397, found 544.2391.

1-*tert*-Butyl-3-(6-(3,5-dimethoxyphenyl)-2-(2-(4-(2-hydroxyethyl)-*1H*-1,2,3-triazol-1-yl)ethylamino)pyrido[2,3-*d*]pyrimidin-7-yl)urea (26d). R_f 0.18 (CH_2Cl_2–MeOH 95 : 5); ^1H NMR: δ 10.29 (br s, 1H, NHCO), 8.70 (s, 1H, H-4), 7.70 (br s, 1H, H-5), 7.41 (s, 1H, H_{tri}), 7.20 (br s, 1H, NH*t*Bu), 6.51–6.46 (m, 4H, N*H*CH_2, H-2′, H-4′, H-6′), 4.78–4.62 (m, 2H, H-2″), 4.05 (dd, J = 5.6, 5.6, 2H, H-1″), 3.88 (t, J = 5.8, 2H, H-b), 3.81 (s, 6H, OCH_3), 2.88 (t, J = 5.8, 2H, H-a), 1.48 (s, 9H, CMe_3); ^{13}C NMR: δ 162.3, 161.8, 161.3, 158.4, 155.9, 152.7, 145.6, 137.2, 136.5, 123.3, 122.7, 110.2, 107.4, 100.9, 61.5, 55.9, 55.6, 51.1, 49.3, 42.1, 29.1, 28.8; MS (ESI): m/z = 558 $[M+Na]^+$ 100%; HRMS (ESI): Calc. for $C_{26}H_{33}N_9O_4Na$ $[M+Na]^+$ 558.2553, found 558.2534.

***tert*-Butyl(*tert*-butoxycarbonylamino)((1-(2-(7-(3-*tert*-butylureido)-6-(3,5-dimethoxyphenyl)pyrido[2,3-*d*]pyrimidin-2-ylamino)ethyl)-*1H*-1,2,3-triazol-4-yl)methylamino)methylenecarbamate (26e).** R_f 0.19 (CH_2Cl_2–MeOH 96 : 4); ^1H NMR (500 MHz, 330 K): δ 11.40 (s, 1H, NHBoc), 10.33 (br s, 1H, NHCO), 8.73 (br s, 2H, H-4, NH_{gua}), 7.70 (s, 1H, H-5), 7.55 (s, 1H, H_{tri}), 7.19 (s, 1H, NH*t*Bu), 6.60–6.40 (m, 3H, H-2′, H-4′, H-6′), 6.32 (br s, 1H, N*H*CH_2), 4.80–4.55 (m, 4H, H-2″, CH_2-guanidine), 4.05 (br s, 2H, H-1″), 3.79 (s, 6H, OCH_3), 1.46, 1.45 and 1.44 (s, 27 H, CMe_3); ^{13}C NMR: δ 163.4, 162.4, 161.8, 161.1, 158.4, 156.0, 155.8, 153.0, 152.7, 144.0, 137.2, 136.5, 123.2, 110.3, 107.4, 100.9, 83.3, 79.4, 55.6, 51.0, 49.0, 41.8, 36.5, 29.0, 28.4, 28.1; MS (ESI): m/z = 763 $[M+H]^+$ 100%; HRMS (ESI): Calc. for $C_{36}H_{50}N_{12}O_7Na$ $[M+Na]^+$ 785.3823, found 785.3836.

1-*tert*-Butyl-3-(6-(3,5-dimethoxyphenyl)-2-(2-(4-(guanidinomethyl)-*1H*-1,2,3-triazol-1-yl)ethylamino)pyrido[2,3-d]pyrimidin-7-yl)urea hydrochloride (26f). ^1H NMR (CD_3OD): δ 8.90 (s, 1H, H-4), 8.04 (s, 1H, H_{tri}), 7.86 (s, 1H, H-5), 6.65–6.50 (m, 3H, H-2′, H-4′, H-6′), 4.90–4.70 (m, 2H, H-2″), 4.46 (s, 2H, CH_2-guanidine), 4.05–3.95 (m, 2H, H-1″), 3.84 (s, 6H, OCH_3), 1.42 (s, 9H, CMe_3); MS (ESI): m/z = 563 $[M\text{-HCl} +H]^+$ 100%; HRMS (ESI): Calc. for $C_{26}H_{35}N_{12}O_3$ $[M\text{-HCl}+H]^+$ 563.2955 found 563.2953.

Biological evaluations

***In vitro*.** Recombinant FGFR3 kinase domain (FGFR3 KD, amino acid residues 439–806) was expressed in baculovirus-infected Sf9 cells as hexa-histidine tagged protein. FGFR3 KD was purified by affinity chromatography on Ni-NTA beads for use in the assay. Nunc MaxiSorp 96-well plates were coated by incubation with 100 µL/well of 10 µg mL^{-1} poly(Glu:Ala:Tyr, 6 : 3 : 1), (pGAT, Sigma Aldrich) in PBS overnight at 4 °C. Excess pGAT was removed by aspiration and the plate was washed 2 times with PBS. The kinase reaction was performed in 100 µL of 50 mM TRIS (pH 7.5) containing 0.01% BSA, 5 mM $MgCl_2$, 5 mM $MnCl_2$, 1 mM dithiothreitol, 5 µM ATP and ~8 ng of FGFR3 KD. Inhibitors in DMSO were added; the final DMSO concentration was 2% (v/v). After 15 min preincubation at room temperature, phosphorylation was initiated by addition of ATP and the assay was incubated for 1 h at 37 °C. The kinase reaction was terminated by aspiration of the reaction mixture and washing two times with PBST (0.05% Tween 20 in PBS). Blocking was performed with 300 µL blocking buffer (3% BSA in PBST) for 1 h at room temperature. For detection of substrate phosphorylation, 100 µL PY99 *anti*-phosphotyrosine antibody (Santa Cruz Biotechnology), diluted to 0.2 µg mL^{-1} with 1% BSA in PBST, were incubated for 1 h, removed by aspiration and washed three times with PBST. 100 µL of HRP conjugated anti-mouse antibody were added and incubated for 1 h at room temperature, removed by aspiration and washed three times with PBST. The colorimetric signal was developed by addition of 100 µL TMB substrate (TMB substrate kit, Thermo Fisher Scientific) and stopped by the addition of 100 µL 2M sulfuric acid. The amount of phosphotyrosine product in the wells was estimated by measurement of absorbance at 450 nm.

***In cellulo*.** Human Embryonic Kidney cells (HEK) stably expressing the vitronectin receptor (293 VnR) were incubated in DMEM supplemented with 10% FCS (Invitrogen), and antibiotics. Transfection of constructs (FGFR3-K650M) into cells at 50–80% confluency using Fugene6 (Roche) was performed according to the manufacturer's instructions. The tyrosine kinase inhibitor PD173074, **19g** and **19h** were dissolved in DMSO and used at 2 µM overnight.

Transfected cells were lysed in RIPA buffer (50 mM Tris-HCl pH 7.6, 150 mM NaCl, 0.5% NP40, 0.25% Sodium deoxycholate, supplemented with protease and phosphatase inhibitors). Immunoprecipitation (IP) was performed by incubating 3 µL rabbit *anti* FGFR3 (Sigma)/500 µg protein with protein A-agarose (Roche). Immunoblotting (IB): Rabbit- Anti-FGFR3 (Sigma) was used at 1 : 1000 and the mouse *anti*-phosphotyrosine at 1 : 400 (Cell signaling). Anti-rabbit and anti-mouse HRP (Amersham) secondary antibodies were used at a concentration of 1 : 10000

and were detected by chemiluminescence (ECL, Amersham). Immobilon membranes were stripped in 2% SDS, 100 mM 2-mercaptoethanol, 60 mM Tris, pH 6.8.

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