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Novel 3-alkoxy-1*H*-pyrazolo[3,4-*d*]pyrimidines as EGFR and erbB2 receptor tyrosine kinase inhibitors

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Abstract—Novel 4-anilino-1*H*-pyrazolo[3,4-*d*]pyrimidines have been synthesized and evaluated in vitro for erbB2 and EGFR kinase inhibition. A representative compound displaying oral bioavailability in rat and dog illustrates the potential of this series to provide orally active erbB2 inhibitors.

The erbB2 receptor tyrosine kinase (also known as HER2 or neu) belongs to the erbB family which also includes the epidermal growth factor receptor (EGFR), erbB3 and erbB4.[1] Overexpression of EGFR and erbB2 has been implicated in the development of various types of cancers. Since the introduction of the monoclonal antibody trastuzumab (Herceptin™) and the dual EGFR/erbB2 kinase inhibitor lapatinib (**1**, Tykerb™) in clinical practice, inhibition of the erbB2 pathway has been an increasingly attractive approach for the treatment of advanced metastatic breast cancer.[1,2] Other erbB2 kinase inhibitors are in ongoing clinical trials such as the dual EGFR/erbB2 inhibitors AEE788[3] (**2**) and BMS-599626[4] (**3**), the irreversible dual inhibitors HKI-272[5] and BIBW-2992,[6] and the erbB2 selective inhibitor CP-724714[7] (**4**) (See Fig. 1).

We recently published the discovery of 5-alkoxy-4-anilino-quinazolines (e.g., compound **5**, Fig. 2) as selective erbB2 inhibitors.[8] In the course of these studies, we were interested in utilizing 1*H*-pyrazolo[3,4-*d*]pyrimidine as the central core to replace the well-known 4-anilino-quinazoline scaffold. Although 4-anilino-1*H*-pyrazolo[3,4-*d*]pyrimidines incorporating either an amino or an aryl group at the 3 position have been described as kinase inhibitors,[9] such compounds with a 3-alkoxy substitution (structure **6**, Fig. 2) were so far unknown. In this paper, we describe the synthesis and in vitro evaluation of 3-alkoxy-4-anilino-1*H*-pyrazolo[3,4-*d*]pyrimidines as erbB2 inhibitors.

The synthesis of compounds **12a**–**q** (Scheme 1) starts with the known pyrazole **8**[10] obtained by reaction of hydrazine with 2-dicyanomethylene-1,3-dioxolane **7**.[11] Reaction of **8** with DMF dimethyl acetal provided amidine **9** which underwent a cyclo-condensation with the appropriate aniline in acetic acid presumably via a Dimroth rearrangement[12] to give intermediates **10a**–**i**. The hydroxyl group was then transformed into a chloride or a mesylate, which was displaced by a range of primary and secondary amines providing the desired products.[13] Compounds **16a**–**c** have been obtained through the same synthetic route starting from dicyanoketene trimethylene acetal **13**.[14]

Compounds listed in Tables 1 and 2 were tested in erbB2 and EGFR isolated enzyme kinase assays and in a cellular erbB2 autophosphorylation assay in an MCF-7 cell line engineered to overexpress the erbB2 receptor (Clone 24). Table 1 summarizes the SAR of different anilines at C4. Our most potent compound **12a**, which incorporates a fluoro-benzyloxy group, showed excellent activity in the kinase and cellular assays. Whereas most of these compounds displayed good activity against erbB2 (IC_{50} 1–17 nM), EGFR activity could be modulated by the *para* substitution (R^2) of the aniline. As shown with compound **12f**, a 2-methylpyridin-5-yl ether was

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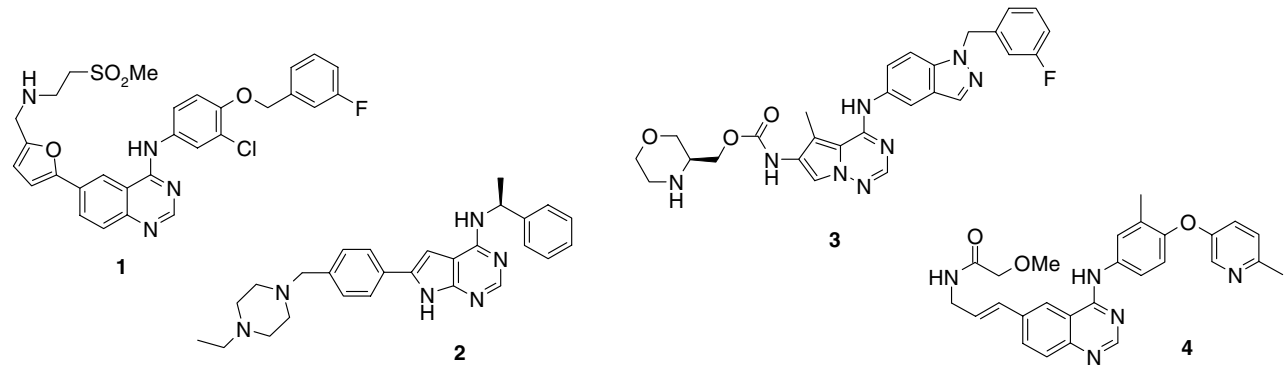


Figure 1. Representative erbB2 kinase inhibitors in clinical trials.

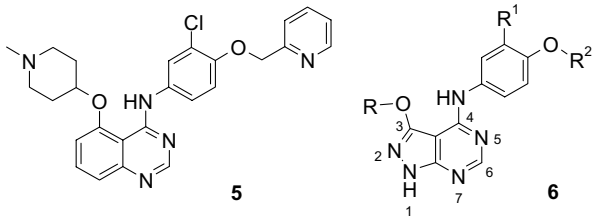
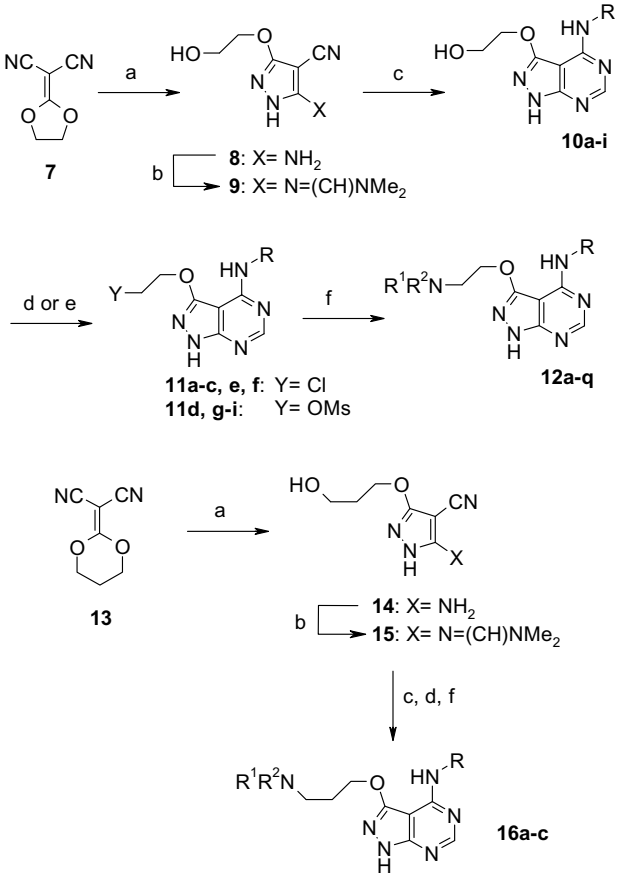


Figure 2. Quinazoline and pyrazolopyrimidine based erbB2 inhibitors.

found to significantly remove EGFR activity compared to the pyridin-2-yl-methyl and fluorobenzyl groups. This effect is even more pronounced with a fluorine on the *meta* position (R^1) whereas a *meta*-chloro maintains the EGFR activity (see **12g** and **12c**, respectively). The optimal *meta* substitution for erbB2 activity was found to be chloro or methyl, the chloro derivatives being generally more active in the cell based assay (compare **12a** with **12d** and **12b** with **12e** for example). The methoxy derivatives **12h** and **12i** were found to be less active than their methyl counterparts.

Influence of the side chain on the C3 position is illustrated in Table 2. We carried out this exploration using the 4-pyridin-2-yl-methoxy-3-chloroaniline at the C4 position because it provided the best pharmacokinetic properties. We also postulated that the superior in vitro activity of the lipophilic 3-fluorobenzyl derivatives would not translate into a better in vivo efficacy because of their higher protein binding.[15] Furthermore, their lower aqueous solubility (data not shown) might increase the risk of a limited oral absorption. Several amino-ethoxy (**12b**, **12j–q**) or amino-propoxy side chains (**16a–c**) provided good kinase inhibition with cellular IC_{50} values typically ranging from 0.1 to 1.0 μM. As illustrated by compound **10b**, a basic amine is not necessary for the erbB2 activity.

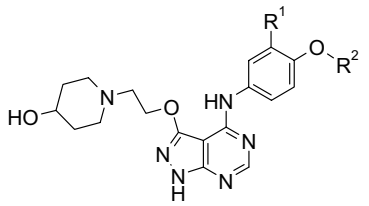
Further assessment of compound **12k** has been carried out in anti-proliferative cellular assays (Table 3). Very good activity was seen against the BT474C cell line, which is sensitive to inhibition of either erbB2 or EGFR (unstimulated assay). The moderate activity of **12k** in the EGF stimulated KB cell proliferation assay shows that **12k** is less selective against EGFR compared to the quinazoline **5**.[16]



Scheme 1. Synthesis of compounds **12a–q** and **16a–c**. Reagents and conditions: (a) hydrazine hydrate, water, room temp, 30 min. (b) DMF dimethylacetal, acetonitrile, 50 °C, 1 h; (c) aniline, AcOH, microwave irradiation at 150 °C for 2 min. (d) $SOCl_2$, cat. DMF, 80 °C, 2 h. (e) MsCl, pyridine, room temp, 1 h. (f) R^1R^2NH, KI (when Y=Cl), DMA, microwave irradiation at 150 °C for 5 min. For intermediates **10** and **11**, the letter refers to the definitions in Table 1.

Pharmacokinetic properties of **12k** were evaluated in rat and dog and the results are shown in Table 4. Acceptable oral bioavailability is observed, associated with a moderate half-life. The limitation in bioavailability can be explained by a moderate to high plasma clearance, respectively, 55% and 130% of the hepatic blood flow in rat and dog. Indeed good intestinal absorption was

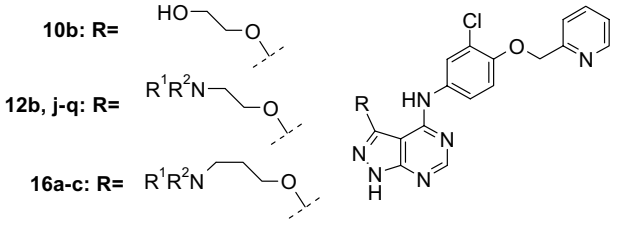
Table 1. Modification of the aniline



Compound	R^1	R^2	Kinase inhibition[a]		Clone 24[a]
			erbB2	EGFR	
12a	Cl	3-Fluorobenzyl	<0.001	<0.001	0.019
12b	Cl	CH_2-(2-pyridyl)	0.005	0.013	0.135
12c	Cl	2-Me-pyridin-5-yl	<0.001	0.007	0.184
12d	Me	3-Fluorobenzyl	<0.001	<0.001	0.107
12e	Me	CH_2-(2-pyridyl)	0.005	0.018	0.695
12f	Me	2-Me-pyridin-5-yl	0.011	0.216	0.415
12g	F	2-Me-pyridin-5-yl	0.005	1.43	—
12h	OMe	3-Fluorobenzyl	0.003	0.013	0.613
12i	OMe	CH_2-(2-pyridyl)	0.017	0.104	1.48

[a] IC_{50} (μM). Values are means of at least two experiments.

Table 2. Modification of the C3 side chain



Compound	NR^1R^2	Kinase inhibition[a]		Clone 24[a]
		erbB2	EGFR	
12b	4-OH-piperidinyl	0.005	0.013	0.135
12j	NHMe	0.003	0.017	1.0
12k	*N*-Me-piperazinyl	0.001	0.005	0.166
12l	*N*-Ac-piperazinyl	<0.001	0.003	0.129
12m	Pyrrolidinyl	0.002	0.008	0.818
12n	Piperazinyl	0.003	0.008	0.128
12o	Oxazepanyl	<0.001	<0.001	0.148
12p	4-OMe-piperidinyl	0.004	0.028	0.082
12q	Morpholinyl	<0.001	0.001	—
10b	—	0.004	0.016	—
16a	Morpholinyl	<0.001	0.016	0.440
16b	4-OH-piperidinyl	0.006	0.009	—
16c	*N*-Me-piperazinyl	0.004	0.014	—

[a] IC_{50} (μM). Values are means of at least two experiments.

Table 3. Cellular anti-proliferative data for **12k** and **5**

Compound	BT474C[a]	KB (EGF)[a]
12k	0.017	0.22
5	0.041	2.60

[a] IC_{50} (μM). Values are means of two experiments.

anticipated from the MDCK-MDR1 assay in which **12k** showed a good permeability (Papp A-B 9.5×10^{-6} cm s^{-1}) and limited efflux (efflux ratio 3.6).

Table 4. Rat and dog pharmacokinetic parameters for **12k**

Species	Plasma protein binding (%free)	Vdss (l/kg)	Cl (ml/min/kg)	Half-life (h)	F%
Rat[a]	10	4.5	41	1.4	23
Dog[b]	21	9.0	46	2.4	30

[a] Female rats dosed at 0.4 mg/kg i.v. and 1 mg/kg p.o.

[b] Mean values for male and female beagle dogs dosed at 0.2 mg/kg i.v. and 0.4 mg/kg p.o.

No significant activity against P450 enzymes (IC_{50} > 10 μM on five isoforms) was observed with **12k**.

In conclusion, we have discovered a new class of erbB2 tyrosine kinase inhibitors based on the 1*H*-pyrazolo[3,4-*d*]pyrimidine scaffold. Encouraging results with compound **12k** highlight the potential of this series to provide potent, orally active erbB2 kinase inhibitors.

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15. Replacement of the 2-pyridyl group in compound **12k** by a 3-fluorophenyl led to a significant decrease of the unbound fraction in rat plasma (from 10% to 0.9% free drug).
16. IC_{50} values of **5** in erbB2 and EGFR kinase inhibition assays are, respectively, 0.002 and 0.14 μM (see Ref. 8).