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Synthesis and evaluation of 4-anilinoquinazoline bioisosteres as potential anti-breast cancer agents

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ABSTRACT

Based on one of the four major categories of scaffold hopping theory namely hetrocycle replacements, a series of 5-arylthieno[2,3-*d*]pyrimidines had been prepared and evaluated as anti-breast cancer agents. Optimization by combination of different pharmacophores with the thienopyrimidine scaffold led to discovery of biologically active compounds.

1. Introduction

Breast cancer is a leading cause of morbidity and mortality worldwide with over a million cases yearly [1].

In recent years, a number of tyrosine kinase inhibitors (TKIs) targeting epidermal growth factor receptor (EGFR) family had been synthesized and some have been approved for clinical treatment of cancer by the FDA e.g. ATP competitive inhibitors (gefitinib, erlotinib, lapatinib and vandetanib). They all are based on a central quinazoline core (Fig. 1) [2].

Lapatinib ditosylate (GW2016; GW572016; Tykerb®), a member of the quinazoline family with a 4-anilinoquinazoline core is a reversible, small molecule tyrosine kinase dual inhibitor of EGFR and human epidermal growth factor receptor 2 (HER2). Lapatinib exerts its activity intracellularly by competing with ATP for the ATP-binding domain in the cytoplasmic tail of the tyrosine kinase receptor. Inhibition of tyrosine kinase phosphorylation is its major mechanism of action [2].

X-ray crystal structures indicate that the inhibitors affinity for the binding site originates from hydrogen bonding between N-1 of the quinazoline and the Met769 NH, also N-3of quinazoline interact with the side chain of Thre766 through a water bridge. The C2 and the C8 of the quinazoline ring are positioned close to the backbone carbonyls of Gln 767 and Met769 respectively [3–7]. The anilino group is oriented such that the meta-substituent is directed towards the kinase specificity pocket [8]. The large anilino substituent induces a dramatic conformational change in the ATP-binding site, the position of the C-helix and the hydrogen binding pattern with quinazoline ring [9]. The structure Activity Relationship (SAR) of the 4-anilinoquinazolines indicated that, the replacement of N1 and N3 with carbon results in 3700 and 200 fold decreases in inhibitory activity respectively [10–11]. The 4-anilino group plays an important role in the binding as well as selectivity of these inhibitors. The presence of electron withdrawing lipophilic groups at the meta-position of aniline is favored for activity [12]. Replacing the quinazoline core with its bioisostere thieno[2,3-*d*]pyrimidine is the goal of this work to evaluate the effect of this replacement on the anticancer activity, a strategy known as scaffold hopping [13].

Thienopyrimidines in general have become an interesting structural element in development of pharmaceutical compounds especially as potential anticancer agents *via* cGMP phosphodiesterase inhibition and protein kinase inhibition mechanisms [14–23]. Moreover, different spacers between thieno[2,3-*d*]

Abbreviations: TKIs, tyrosine kinase inhibitors; HER2, human epidermal growth factor receptor 2; EGFR TK, epidermal growth factor receptor tyrosine kinase; EGFR PTK, epidermal growth factor receptor protein tyrosine kinase; cGMP, cyclic guanosine monophosphate; MOE, molecular operating environment; EGFR, epidermal growth factor receptor; ATP, adenosine triphosphate; SAR, structure activity relationship.

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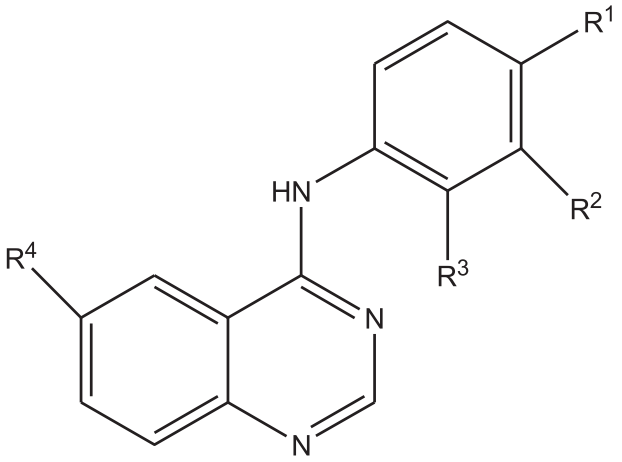

Fig. 1. 4-Anilinoquinazolines.

pyrimidine core and 4-anilino group were introduced to explore the effect of such spacer on position and interaction of aromatic ring with hydrophobic pocket present in EGFR PTK. Total replacement of the aniline moiety with substituted piperazine, piperidine or other heterocyclic rings was to assess if such dramatic change in the pharmacophoric model will have appreciable effect on the inhibitory activity. Synthesis of the target thieno[2,3-*d*]pyrimidine derivatives was represented in Schemes 1–3.

2. Results and discussion

2.1. Designing the target compounds

By using the above findings as guidance for the molecular modeling study and by varying the substitution on the aromatic rings to explore their effect on activity, and also by comparing the compounds to be synthesized to the reference compound, ninety derivatives were docked in EGFR-TK. Only fifty six derivatives showed good binding to the receptor, good superposition and good docking scores. From these fifty six derivatives only twenty two compounds were synthesized and seventeen derivatives out of these twenty two compounds subjected to biological screening for their antitumor activity.

Docking study showed that, the docked compounds bind to the binding site with moderate to good binding score. Moreover, They fit to the active site pocket with moderate to good interactions with the amino acids (Table 1) suggesting moderate to good biological activity as anti-cancer agents.

Thieno[2,3-*d*]pyrimidine ring was sandwiched from the top and bottom by the side chains of Ala743 and Leu844, respectively, binds in the adenine binding pocket and mimics the quinazoline ring of lapatinib. Also the benzyl group of compound **5b** for example projected into a hydrophobic region, which is formed by the side chains of Lys745, Leu788, Thr854, and Asp855 (Fig. 2), which verified the potent inhibitory activity of **5b**.

Compound **6b** demonstrated hydrogen bond formation between N-1 and Met793 and N-3 and a water molecule. The CH_2 spacer between piperidine and aromatic ring positioned the aromatic ring to the back of ATP binding site and made predominant interaction with the hydrophobic pocket at back of ATP binding site mimicking the 3-fluorobenzyloxy group of lapatinib. Also (Fig. 3) represents the superposition of the binding of lapatinib and **6b** which confirmed that **6b** bound in a similar way to lapatinib. This superposition also showed a near-perfect alignment between both compounds with the exception of the orientation of the aromatic ring at 5-position of compound **6b** compared to the furan of lapatinib which was positioned to the solvent interface (Fig. 3).

Compounds **7c** and **7d** were bound to the ATP catalytic domain of the EGFR with two hydrogen bonds. One was formed between N-1 of pyrimidine ring and main chain NH of Met793. The other one was formed between N-3 and the hydroxyl group at the side chain of Thr854 through a water molecule. (3,4 Dimethoxybenzylidene) hydrazine moiety of compound **7c** and the dimethylamino group of compound **7d** are deeply oriented inside the hydrophobic pocket and made a predominant hydrophobic interaction with the adjacent pocket lined by Met766, Leu777,Thr790, and Phe856 mimicking the 3-chloro-4-[(3-fluorobenzyl) oxy] aniline moiety of lapatinib (Fig. 4). The aromatic ring at C-5 of compounds **7c** and **7d** were extended to the solvent-exposed region. Compounds **7c** and **7d** interacted deeply in the back of ATP binding site similar to lapatinib. The additional binding energy provided by the presence of the p-, m-dimethoxy groups of compound **7c** and the extra hydrophobic interaction provided by p-dimethylamino group of compound **7d** in this deep pocket might be the reason for the high binding affinity of these compounds and suggested that these compounds may reveal strong anticancer activity.

2.2. Synthesis of the target compounds

Knoevenagel condensation of acetophenone with malononitrile afforded 2-[1-(4-aryl)-ethylidene]-malononitrile which upon base-promoted cyclization with elemental sulfur gave 2-aminothiophene-3- carbonitrile **1a**–**b** [24] in excellent yield. Reaction of compounds **1a**–**b** with formic acid 85% gave 5-aryl-3*H*-thieno[2,3-*d*]pyrimidin-4-ones **2a**–**b**. The structure of compound **2b** was confirmed by element analysis, IR, ^{1}H NMR, ^{13}C NMR and mass spectral data. The IR spectrum showed absorption band at 1681 cm^{-1} due to C=O group and showed the disappearance of C≡N and NH_2 absorption bands of its precursor **1b**. ^{1}H NMR spectrum revealed the presence of a singlet signal at δ 8.18 ppm, due to C2–H. In addition, an exchangeable singlet signal appeared at δ 12.59 ppm corresponding to NH/OH proton. Moreover, the mass spectrum of **2b** showed molecular ion peaks at *m*/*z* 273 (M^+, 55%), 227 ($M-NO_2^+$, 17.8%). The synthesis of the 4-chloro derivatives namely, 4-chloro-5-(4-chlorophenyl)-thieno[2,3-*d*]pyrimidine (**3a**), 4-chloro-5-(4-nitrophenyl)-thieno[2,3-*d*]pyrimidine (**3b**) was achieved *via* chlorination of compounds **2a**–**b** using thionyl chloride in only a catalytic amount of dry DMF to accelerate the reaction rate (Scheme 1).

The structure of compounds **3a**–**b** was confirmed by element analyses, IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopy. The IR spectra of **3a**–**b** lacked the presence of absorption bands due to NH and C=O groups. Moreover, ^{1}H NMR spectra showed the disappearance of the signal corresponded to NH/OH proton and displayed the characteristic signal corresponding to C–2H. Hydrazinolysis of compounds **3a**–**b** using hydrazine hydrate in ethanol resulted in the key intermediate 4-hyrazinothienopyrimidines **4a**–**b**. The IR, ^{1}H NMR, ^{13}C NMR mass spectroscopy and element analysis were used to identify compounds **4a**–**b**.

IR spectra of **4a**–**b** revealed two absorption bands at the 3240 and 3340 cm^{-1} indicating the presence of NH and NH_2 groups. ^{1}H NMR spectra of **4a**–**b** revealed the presence of two exchangeable singlet signals at δ 4.54, 4.57 ppm and δ 6.64, 7.10 ppm corresponding to NH and NH_2 protons. Further evidence stemmed from the mass spectrum of **4a** giving molecular ion peaks at *m*/*z* 278