European Journal of Medicinal Chemistry 45 (2010) 2473–2479

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and study of antiproliferative activity of novel thienopyrimidines on glioblastoma cells

Stéphane Pédeboscq [a,c,*], Denis Gravier [b], Françoise Casadebaig [b], Geneviève Hou [b], Arnaud Gissot [b], Francesca De Giorgi [c], François Ichas [c], Jean Cambar [d], Jean-Paul Pometan [a,b]

[a] Service Pharmacie, Hôpital Saint-André, CHU de Bordeaux, 1 rue Jean Burguet, 33 075 Bordeaux, France
[b] Laboratoire de Chimie Organique, UFR de Pharmacie, Université Victor Segalen Bordeaux 2, 146 rue Léo Saignat, 33 076 Bordeaux, France
[c] INSERM U916, Institut Bergonié, Université Victor Segalen Bordeaux 2, 229 cours de l'Argonne, 33 076 Bordeaux, France
[d] Laboratoire de Biologie Cellulaire, Laboratoire Santé, Travail, Environnement EA 3672, Université Victor Segalen Bordeaux 2, 146 rue Léo Saignat, 33 076 Bordeaux, France

ARTICLE INFO

Article history:
Received 1 April 2009
Received in revised form
10 February 2010
Accepted 12 February 2010
Available online 18 February 2010

Keywords:
Thienopyrimidine
Epidermal growth factor receptor
Tyrosine kinase
Cytotoxicity
Apoptosis

ABSTRACT

The receptor tyrosine kinases (for example EGFR, PDGFR, VEGFR) are a transmembrane protein family which plays a crucial role in tumor growth, survival, metastasis dissemination and angiogenesis. During the past 10 years, many tyrosine kinase inhibitors (TKIs) have been approved for cancer treatment (imatinib, gefitinib, erlotinib, sunitinib, sorafenib). These compounds generally possess a pyrrolo- or pyrimido- pyrimidine scaffold or approaching molecular structure. We synthesized 10 thienopyrimidine compounds (including 5 newly synthesized) whose scaffold is very similar to the agents cited above. The cytotoxicity of these agents was evaluated using a MTT assay and a flow cytometry technique on glioblastoma cell lines. Two compounds showed a similar cytotoxicity to the standard anti-EGFR gefitinib (IC50: gefitinib = 51.9 μM, **6b** = 61.8 μM, **6c** = 41.2 μM), suggesting a blockade of the EGFR pathway by binding to the TK receptor.

1. Introduction

The receptor tyrosine kinases (RTK) are a transmembrane protein family which plays a crucial role in tumor growth, survival, metastasis dissemination and angiogenesis. They are overexpressed in several types of tumors (in particular EGFR (epidermal growth factor receptor), PDGFR (platelet-derived growth factor receptor), FGFR (fibroblast growth factor receptor) and VEGFR (vascular endothelial growth factor receptor)) and they mediate the cellular transduction pathway consecutively to the binding of growth factors to their extracellular domain. Proliferation signaling is mediated after this binding by phosphorylation of the TK domain, which leads to a cascade of downstream signaling pathways. Abnormal signaling via these TK is linked to cancerous pathologies and to pathologies other than cancer, for example, cardiovascular and immunoinflammatory pathologies [1,2].

The ATP binding site of tyrosine kinase (TK) receptors could constitute a viable target for drug design [3,4] in these pathologies. Novel targeted therapies against these receptors have been synthesized and are now commonly used in clinical practice in various cancerous pathologies. Among these new agents, the TKIs (TK inhibitors) are small molecules that bind to the intracellular domain of growth factor receptors, inhibit autophosphorylation and consequently block the proliferation signaling pathway. These agents then prevent the proliferation of cells which have amplification or dysregulation of growth factor pathways. Compounds based on quinazoline, indolin or pyridopyrimidine scaffolds [5–11] have been synthesized and allow ATP-competitive binding on the TK domain of the growth factor receptors.

During the past 10 years, many TKIs have been approved for cancer treatment. Imatinib (anti Bcr/Abl kinase, c-kit, PDGFR) was the first TKI commercialized and became the standard therapy for chronic myelogenous leukemia (CML) with very impressive results, and for gastrointestinal stromal tumors (GIST). Erlotinib and gefitinib (anti-EGFR) are currently used in non-small cell lung cancer (NSCLC), sunitinib (anti VEGFR-2, PDGFR-β, c-kit and FLT-3 (FMS-like tyrosine kinase 3)) in renal cancer and GIST, sorafenib (anti Raf-kinase, VEGFR-2, PDGFR-β and c-kit) in renal cancer and

* Corresponding author. Service Pharmacie, Hôpital Saint-André, CHU de Bordeaux, 1 rue Jean Burguet, 33 075 Bordeaux, France. Tel.: +33 557 82 06 12; fax: +33 556 79 58 98.
E-mail address: stephane.pedeboscq@chu-bordeaux.fr (S. Pédeboscq).

0223-5234/$ – see front matter © 2010 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2010.02.032

hepatocellular carcinoma, and lapatinib (anti-EGFR and ErbB-2 (erythroblastic leukemia viral oncogene homolog 2)) in breast cancer. These compounds generally possess a pyrrolo- or pyrimidopyrimidine scaffold or approaching molecular structure.

In a previous work we synthesized a series of 3-substituted 1,2,3,4-tetrahydroquinazoline and 3-substituted thieno[2,3-d]pyrimidin-4-one compounds which presented a noticeable platelet antiaggregating power [12,13]. The most potent activity was exhibited by the thienopyrimidinone derivatives. These thienopyrimidine compounds present a similar scaffold to the agents cited above. Several studies have shown that the presence of a quinazoline skeleton substituted in position 4 by various substituted anilino groups potentially increased the EGFR inhibitory effect [14,15]. According to the results observed with the former thienopyrimidinone derivatives as platelet antiaggregating agents, the substitution of these compounds at the 4 position could lead to new PDGFR or EGFR pathway inhibitors. Moreover, authors recently show that compounds based on this thienopyrimidine scaffold could present an interest in the inhibition of VEGFR and PDGFR kinase domains [16]. This study describes the chemical synthesis of 10 thienopyrimidine derivatives and evaluates their activity on the epidermal growth factor receptor pathway.

2. Chemistry

The derivatives of thieno[2,3-d]pyrimidin-4-one were prepared according to a method described by Gewald [17,18], which involves the cyclization of the appropriate derivative of ethyl-2-aminothiophene-3-carboxylate with formamide. The use of phosphorus oxychloride affords the corresponding chlorosubstituted compounds which, by refluxing in dimethylformamide with the appropriate aniline, lead to the final derivatives of 4-anilinothieno [2,3-d]pyrimidine according to Scheme 1. Compounds synthesized are summarized in Table 1 with the different substitution groups.

3. Results

3.1. Cell characterization

Expression of EGF receptor was assessed on DBTRG.05-MG and U87-MG glioblastoma cell lines by immunocytochemistry and immunoblotting. Both techniques gave very similar results. The immunoblots in Fig. 1 show a 170 kDa band corresponding to EGFR for the DBTRG.05-MG cell line, demonstrating that DBTRG.05-MG displays high constitutive EGFR expression. This band was not present for the U87-MG cell line, representing a negative control of EGFR expression.

Table 1
Synthesized thieno[2,3-d]pyrimidine derivatives.

Compound	X	R^1	R^2	Reference
3a	Anilino	H	H	[25]
3b	Anilino	Methyl	H	[22]
3c	Anilino	Methoxy	H	[26]
3d	Anilino	H	Methoxy	[25]
3e	Dimethylamino	–	–	[27]
6a[a]	Anilino	H	H	–
6b[a]	Anilino	Methyl	H	–
6c[a]	Anilino	Methoxy	H	–
6d[a]	Anilino	H	Methoxy	–
6e[a]	Dimethylamino	–	–	–

[a] The newly synthesized compounds were characterized by spectroscopic data.

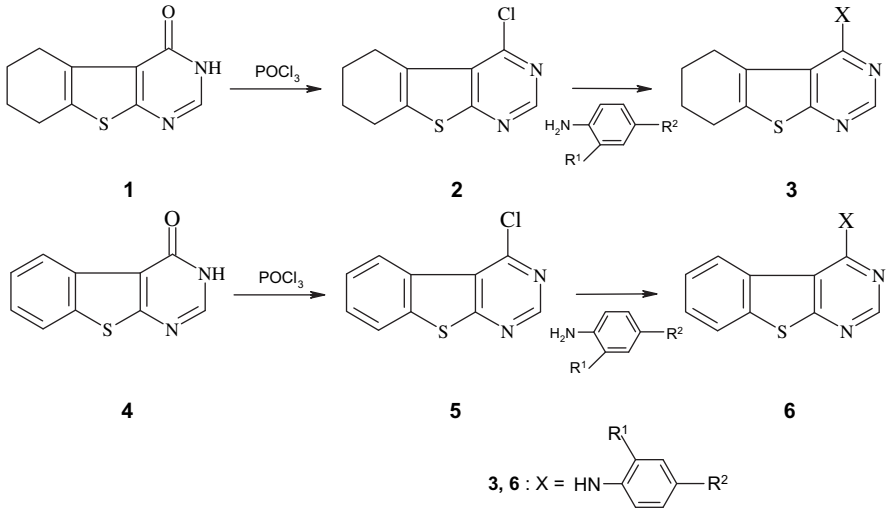
3.2. Cytotoxicity of the synthesized compounds

We assessed the cytotoxicity of DMSO, which was used as a dilution vehicle for the agents tested. DMSO showed very weak cytotoxicity (<10%) for concentrations ≤1% of DMSO. These DMSO concentrations corresponded to tested agent concentrations ≤200 μM. Consequently, concentrations ≤200 μM can be considered specific of the cytotoxicity of the tested agent itself and can be taken into account.

Gefitinib, which was used as a reference, was very cytotoxic on DBTRG.05-MG cells in a dose-dependent manner. IC_{50} was 51.9 ± 3.7 μM. Fig. 2A shows that two synthesized compounds (**6b** and **6c**) exerted a comparable cytotoxicity to gefitinib with IC_{50} of about 50 μM (IC_{50} **6b** = 61.8 ± 0.9 μM, IC_{50} **6c** = 41.2 ± 1.2 μM). Another group of synthesized agents (**6a**, **6d**, **6e**, **3d** and **3e**) was less cytotoxic (Fig. 2A and B) with IC_{50} around 100–150 μM (IC_{50} **6a** = 109.1 ± 9.3 μM, IC_{50} **6d** = 117.2 ± 8.3 μM, IC_{50} **6e** = 138.5 ± 1.9 μM, IC_{50} **3d** = 151.7 ± 11.7 μM, IC_{50} **3e** = 149.4 ± 13.9 μM). Finally, three compounds (**3a**, **3b** and **3c**) had very low cytotoxicity (IC_{50} **3a** = 584.7 ± 179.9 μM, IC_{50} **3b** = 474.1 ± 8.7 μM, IC_{50} **3c** = 571.0 ± 61.1 μM).

3.3. Apoptosis induced on two glioblastoma cell lines

Cell lines of glioblastoma (U87-MG and DBTRG.05-MG) were treated with increasing concentrations of **6b** (which shows



Scheme 1. Synthesis of the 4-anilinothieno[2,3-d]pyrimidine derivatives.