ACTA SCIENTIFIC PHARMACEUTICAL SCIENCES (ISSN: 2581-5423)

Volume 3 Issue 12 December 2019

Research Article

New 6-Formyl-oxazolo[3,2-*a*]Pyrimidine Derivatives: Synthesis and Evaluation as Potential Src Kinase Inhibitors

Jean Guillon[1]*, François Hallé[1], Solène Savrimoutou[1], Stéphane Moreau[1], Daniel-Henri Caignard[2] and Pascal Sonnet[3]

[1]*Université de Bordeaux, INSERM U1212 / UMR CNRS 5320, UFR des Sciences Pharmaceutiques, Laboratoire ARNA, France*

[2]*Institut de Recherches Servier, Centre de Recherches de Croissy, France*

[3]*Université de Picardie Jules Verne, AGIR - EA4294, UFR des Sciences Pharmaceutiques, France*

***Corresponding Author:** Jean Guillon, Université de Bordeaux, UFR des Sciences Pharmaceutiques, Laboratoire ARNA, France.

Received: November 15, 2019; **Published:** November 19, 2019

DOI: 10.31080/ASPS.2019.03.0440

Abstract

The proto-oncogene Src kinase is a protein-tyrosine kinase that is defined to be involved in cell growth, migration, division, and also survival signaling pathways. The Src kinase activation has been described to be linked to various cancers, such as breast, colon, lung, pancreas, or brain. Thus, Src kinase is a promising and interesting target for different cancer therapies. In this work, we have designed and prepared an original series of substituted 6-formyl-oxazolo[3,2-*a*]pyrimidines **1a-k** that have been then screened on the Src kinase in a drug discovery approach in order to identify new chemical scaffolds.

Keywords: *Src Kinase; 6-formyl-oxazolo[3,2-a]Pyrimidine; Synthesis.*

Introduction

The Src tyrosine-protein kinase, which is also known as proto-oncogene c-Src, is a non-receptor tyrosine kinase protein that has been described to be involved in the regulation of important cellular processes including migration, survival and proliferation [1]. In fact, the Src activation has been associated with multiple cancers, such as breast, colon, lung, pancreas, or brain [2,3]. As Src kinase's prominent roles is involved in the invasion and the tumor progression, the epithelial-to-mesenchymal transition, the angiogenesis, and the development of metastasis, this kinase is a promising and interesting target for different cancer therapies. There are only few Src inhibitors in clinical development (Bosutinib, Dasatinib, Saracatinib and KX-01), therefore, there is an urgent need to identify new original therapeutics able to inhibit the Src kinase and, thus, to modulate aberrant pathways leading to malignant transformation of cells [4].

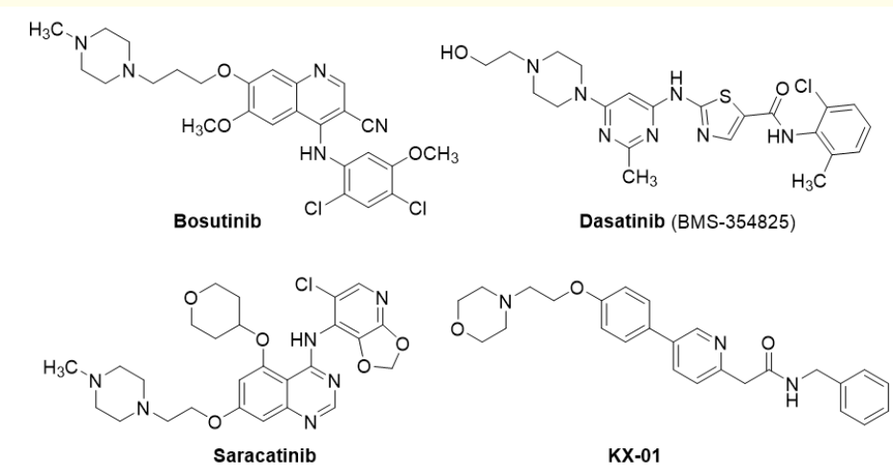


Figure 1: Chemical structures of Src inhibitors Bosutinib, Dasatinib, Saracatinib and KX-01.

Heterocyclic derivatives attracted a lot of attention because of their wide spread pharmacological activities. Thus, we have previously reported the synthesis of bio-active heterocyclic compounds based on the reactivity of the amidine moiety of 2-amino-2-oxazolines **2** with various bis-electrophiles [5]. By following this chemical strategy, we have designed and synthesized a novel series of various substituted 6-formyl-oxazolo[3,2-*a*]pyrimidine derivatives **1a-k** that have been screened in a drug discovery approach in order to identify new chemical entities on this Src kinase.

Materials and Methods

Chemistry

The received commercial reagents were used without additional purification. Melting points were determined with a SM-LUX-POL Leitz hot-stage microscope and are uncorrected. IR spectra were recorded on a NICOLET 380FT-IR spectrophotometer. NMR spectra were recorded with tetramethylsilane as an internal standard using a BRUKER AVANCE 300 spectrometer. Splitting patterns have been designated as follows: s = singlet; d = doublet; t = triplet; q = quartet; dd = double doublet; m = multiplet. Analytical TLC were carried out on 0.25 precoated silica gel plates (POLYGRAM SIL G/UV254) and visualization of compounds after UV light irradiation. Silica gel 60 (70-230 mesh) was used for column chromatography. High resolution mass spectra (electrospray in positive mode, ESI MS) were recorded on a Waters Q-TOF Ultima apparatus. Elemental analyses were found within ±0.4% of the theoretical values.

Synthesis of *N'*-[5-(4-methoxyphenoxy)methyl-4,5-dihydrooxazol-2-yl]-*N*,*N*-dimethylformamidine **3d**. A solution of 5-(4-methoxyphenoxy)methyl-2-amino-2-oxazoline **2d** (10 g, 0.045 mol.) and *N*,*N*-dimethylformamide dimethyl acetal (5.47 g, 0.046 mol., 1.02 equiv.) in dry toluene (160 mL) was refuxed for 6 h. After evaporation of the solvent, the residue was triturated in diethyl ether to give **1d**. White crystals from diethyl ether (66%), mp 43°C; IR (KBr) λ_{max} 1630 (C=N); ^{1}H NMR ($CDCl_3$) δ 8.20 (s, 1H, N=CH), 6.77 (d, *J*=9.50 Hz, 2H, H-2' and H-6'), 6.74 (d, *J*=9.50 Hz, 2H, H-3' and H-5'), 4.80 (m, 1H, H-5), 4.02 (dd, *J*=9.85 and 5.90 Hz, 1H, OCH_{2a}), 3.92-3.85 (m, 2H, OCH_{2b} and H-4_a), 3.69 (s, 3H, OCH_3), 3.62 (dd, *J*=13.30 and 6.90 Hz, 1H, H-4_b), 2.99 (s, 3H, CH_3), 2.96 (s, 3H, CH_3); Anal. Calcd for $C_{14}H_{19}N_3O_3$: C, 60.63; H, 6.90; N, 15.15. Found: C, 60.74; H, 6.96; N, 15.26.

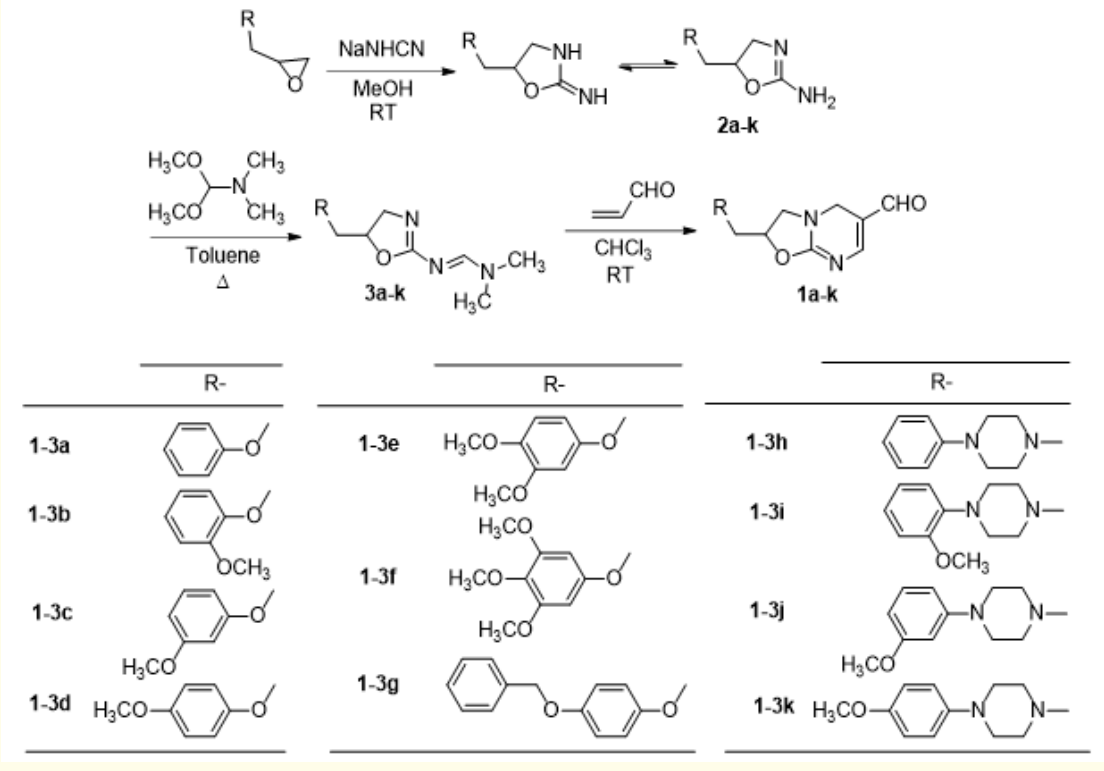
Synthesis of 6-Formyl-2-(4-methoxyphenoxy)methyl-2,3-dihydro-5*H*-oxazolo[3,2-*a*]pyrimidine **1d**. A mixture of amidine **3d** (1.0 g, 3.62 mmol) and acrolein (0.51 g, 10 mmol., 2.5 equiv.) in chloroform (12 mL) was stirred for 20 h at room temperature, then evaporated under reduced pressure and chromatographed on silica gel using EtOAc/Acetone (7/3, v/v) as eluant to afford **1d**. Beige crystals (53%), mp 142°C; IR (KBr) λ_{max} 1704 (C=O), 1648 (C=C); ^{1}H NMR (DMSO-d_6) δ 9.21 (s, 1H, CHO), 7.33 (s, 1H, H-7), 6.91 (d, *J*=9.50 Hz, 2H, H-2' and H-6'), 6.84 (d, *J*=9.50 Hz, 2H, H-3' and H-5'), 5.14 (m, 1H, H-2), 4.21 (m, 2H, OCH_2), 4.19 (s, 2H, H-5), 3.77 (m, 2H, H-3_a), 3.68 (s, 3H, OCH_3), 3.43 (dd, *J*=13.30 and 6.80 Hz, 1H, H-3_b); ^{13}C NMR (DMSO-d_6) δ 189.6 (CO), 164.2 (C-8a), 162.3 (C-7), 155.6 (C-4'), 153.8 (C-1'), 117.5 (C-2' and C-6'), 116.5 (C-3' and C-5'), 115.7 (C-6), 77.9 (C-2), 70.4 (OCH_2), 57.2 (OCH_3), 50.1 (C-3), 45.0 (C-5); Anal. Calcd for $C_{15}H_{16}N_2O_4$: C, 62.49; H, 5.59; N, 9.72. Found: C, 62.55; H, 5.63; N, 9.61. HR-MS m/z $[M + H]^+$ Calcd for $C_{15}H_{17}N_2O_4$: 289.1188, Found: 289.1193.

Biology

Src inhibitory assays: inhibitors were diluted with a Tecan Evo150 robot. The kinase assay was performed with 4 µL of inhibitor (10%DMSO), 10 µL of kinase assay buffer 4x concentrated (80 mM $MgCl_2$, 200 mM HEPES, 0.4 mM EDTA, 2 mM DTT), 10 µL substrate peptide (KVEKIGEGYYGVVYK, 370 nM) and 6 µL Src kinase (stock GTP purified diluted with 1x kinase assay buffer to 200 nM). 10 µL co-substrate (40 µM ATP with 0.2 µCi P^{33}-g-ATP) was added with a Precision 2000 (Biotek Robotic). The assay was incubated 20 minutes at 30°C then stopped by adding 200 µL 0.85% orthophosphoric acid, then transferred to a phosphocellulose filter microplate (Whatman - P81). The plate was washed 3 times with 200 µL 0.85% orthophosphoric acid dried with 200 µL acetone. The remaining activity is measured on a Topcount with 25 µL scintillation solution (Packard UltimaGold).

Results and Discussion

These original oxazolo[3,2-*a*]pyrimidine derivatives **1a-k** were synthesized through a Diels-Alder cycloaddition of alkylidene derivatives of substituted 2-amino-2-oxazolines **3a-k** with acrolein, used as an electron-poor dienophile, and followed by spontaneous elimination of dimethylamine [6]. The heterocyclic diazodienes **3a-k** were synthesized according to a general literature method by heating a toluene solution of substituted 2-amino-2-oxazolines **2a-k** and *N*,*N*-dimethylformamide-dimethyl acetal [7,8]. The starting racemic 2-amino-2-oxazolines **2a-k** were easily prepared from the corresponding epoxides [9,10]. The structure of these new substituted oxazolo[3,2-*a*]pyrimidine derivatives **1** has been confirmed by FTIR, ^{1}H and ^{13}C-NMR, and also ESI-MS analysis.



Scheme 1: Synthesis of new 6-formyl-oxazolo[3,2-*a*]pyrimidine derivatives **1a-k**.

These original 6-formyl-oxazolo[3,2-*a*]pyrimidine derivatives **1a-k** were then submitted to a preliminary screening on various biological targets. Therefore, this new series was evaluated on the Src kinase. Among the data, compound **1d** has been identified as a new Src kinase inhibitor "hit" with an IC_{50} of 4 µM (Table 1). Unfortunately, all the other derivatives were found inactive toward this Src target kinase (IC_{50} > 20 µM). However, compound **1d** showing interesting Src kinase inhibition may constitute an interesting scaffold for further pharmacomodulations and pharmacological studies.

Compound	IC_{50} (µM)	Compound	IC_{50} (µM)	Compound	IC_{50} (µM)
1a	> 20	**1e**	> 20	**1i**	> 20
1b	> 20	**1f**	> 20	**1j**	> 20
1c	> 20	**1g**	> 20	**1k**	> 20
1d	4	**1h**	> 20		

Table 1: IC_{50} Src kinase of 6-formyl-oxazolo[3,2-*a*]pyrimidine derivatives **1a-k**.

Conclusion

Versatility given by this reaction allowed us to access a promising family of diversely substituted 6-formyl-oxazolo[3,2-*a*]pyrimidines with inhibitory effect on Src kinase. Moreover, this promising Src kinase inhibitor **1d** will be then tested for its antiproliferative activity against various cancer cell lines. Its potency on different isolated enzymes will be also evaluated to determine its specificity. In addition, further studies could be required to know the specific mechanism of action of this new bioactive 6-formyl-oxazolo[3,2-*a*] pyrimidine derivative. These biological studies are currently under progress.

Acknowledgements

This work was supported by a grant from Ligue Contre le Cancer (Gironde, Bordeaux, France).

Conflict of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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