Chapter

Bozepinib: A Promising Selective Derivative Targeting Breast Cancer Stem Cells

Joaquín M. Campos, Ana Conejo-García
and Olga Cruz-López

Abstract

Bozepinib is a potent antitumour compound that shows an IC_{50} of 0.166 μM against MDA-MB-231 human breast cancer cell line. It is also a very selective drug that presents a therapeutic index (TI) of 11.0 against MDA-MB-231 in relation to the normal MCF-10A. It is important to identify new cancer stem-like cells (CSCs) anticancer drugs to struggle against the resistance and the high risk of relapse in patients. In the present chapter, we show how bozepinib demonstrated selectivity on cancer cells and showed an inhibitory effect over kinases involved in carcinogenesis, proliferation and angiogenesis. Bozepinib inhibits HER-2 signaling pathway and JNK and ERK kinases. In addition, it has an inhibitory effect on AKT and VEGF together with anti-angiogenic and anti-migratory activities. Interestingly, bozepinib suppresses the formation of both mammo- and colonospheres and eliminated ALDH+ CSC subpopulations at a low micromolar range similar to salinomycin. It also induces the downregulation of SOX2, c-MYC and β-CATENIN and upregulation of the GLI-3 Hedgehog signaling repressor. Finally, bozepinib shows in vivo antitumor and anti-metastatic efficacy in xenotransplanted nude mice without presenting subacute toxicity. However, further studies in cancer patients are needed to confirm the therapeutic potential of bozepinib.

Keywords: benzoxazepine, bozepinib, cancer stem-like cells, MDA-MB-231, MCF-7, MCF-10A, protein kinases, seven-membered ring

1. Introduction

Expansion of cancer keeps going on as an important health problem in the developed, undeveloped and developing countries. Although major advances have been made in the chemotherapeutic management of some patients, the continued commitment to the laborious task of discovering new anticancer agents remains critically important, in the course of identifying various chemical substances, which may serve as leads for designing novel antitumor agents.

1.1 Cancer stem cells

Currently, one of the most interesting concepts being explored in cancer research is the theory of cancer stem cells. CSCs can be defined as the
subpopulations of cells within tumors that possess the ability to self-renew and differentiate into the different lines of cancer cells that make up the tumor. This type of cell is proposed as the promoter of resistance against antitumor therapies, being able to maintain benign and malignant tumors, as well as causing relapses [1]. Compared to normal stem cells, CSCs are thought to have no control over their proliferation. These CSCs, present in tumors in small numbers, are characterized by their ability to remain quiescent for long periods of time, capacity for self-renewal, maintenance of growth and heterogeneity of the tumor, affinity for environment resistance to chemotherapy and development of metastases [2].

CSCs are characterized by their ability to form spherical colonies when cultivated in suspension [3]. Al-Hajj et al. managed to demonstrate that the injection of 200 tumoral cells expressing characteristic markers of CSCs was more effective in generating tumors in immunodepressed mice than the injection of 50,000 tumor cells with differentiated cell markers of the same histological lineage [4]. It has been found that multiple molecules related to the characteristic properties of stem cells such as self-renewal and pluripotency and certain enzymatic activities are largely expressed in CSCs, including c-MYC, β-CATENIN [5], SOX-2 [6] and aldehyde dehydrogenase activity (ALDH1) [7]. New strategies for selective and effective cancer therapy can be provided by selectively acting on overregulated pathways or molecules in differentiated cancer cells and/or in CSC populations, but not in normal cells.

1.2 Therapies targeted against cancer stem cells

Cancer treatment currently targets its proliferation potential, and therefore most treatments target rapidly dividing cells. The presence of CSCs may explain the failure of treatments to eradicate the disease or the recurrence of cancer [1]. CSCs have to remain in a state of quiescence, a state in which the cell does not divide staying in the G0 phase of the cell cycle [8] allows them to survive most anticancer treatments. This characteristic makes relapses possible, even decades after initial treatment, such as in colon or breast cancers [1, 9]. Although current treatments may reduce the tumor size, these effects are transient and generally do not improve patients’ survival. For tumors in which CSCs play a role, there are three possibilities. First, the mutation of normal stem cells or CSC progenitor cells can lead to the development of the primary tumor. Second, during chemotherapy, most cells in the primary tumor can be destroyed, but if CSCs are not eradicated, they become refractory CSCs and can lead to the recurrence of the tumor [10]. Third, CSCs can migrate to distant sites of the primary tumor and cause metastasis [11–13]. Theoretically, the identification of CSCs can allow the development of treatment modalities that target these cells rather than rapidly dividing cells [14].

One of the therapeutic approaches currently being studied to combat CSCs is related to the signaling pathways involved in the processes of their self-renewal, proliferation and differentiation. This is because the loss of regulation of pathways such as Hedgehog (Hh), Notch and Wnt/β-catenin results in the key processes involved in the characteristics of CSCs. Currently, the therapy directed against these routes represents one of the most promising mechanisms of action against these initiation cells of the tumor [15, 16].

Herein we will discuss the search and biological activity of small synthetic derivatives: racemate 2, named as bozepinib (isomer of 1), was selected for anti-CSC studies (Figure 1). The information presented in this chapter should be of interest to medicinal chemists and represents an effort to summarize the experimental research and advances in the field of CSCs [17, 18].
A better understanding of the molecular mechanisms responsible for CSC formation probably lead to the design and synthesis of new anticancer drugs which will be able to eliminate CSC or to halt tumor growth by interfering with important intracellular signaling pathways related with CSC stemness or CSC differentiation or both.

2. (RS)-2,6-Dichloro-9-[1-(o- or p-nitrobenzenesulfonyl)-1,2,3,5-tetrahydro-4,1-benzoxazepine-3-yl]-9H-purines 1 and 2

2.1 Synthesis of tetrahydrobenzoxazepine O,O-acetals with electron withdrawing groups on the nitrogen atom

As part of an Anticancer Drug Programme, we were interested in the preparation of the heterocycles 3a-b (Figure 2) that could be useful intermediates for the synthesis of novel bioactive compounds. Figure 2 shows the synthesis of derivatives 3a-b [19]. The Mitsunobu reaction is a versatile method for the transformation of aliphatic alcohols into alkylating agents in situ and under mild conditions [20]. A successful Mitsunobu displacement depends on the pKₐ associated with the N–H bond [21]. Thus, a powerful electron withdrawing group for the amino moiety was needed such as the p- or o-nitrobenzenesulfonyl fragment.

The synthesis of compounds 3a-b begins with the protection of the hydroxyl group of anthranilic alcohol by the tert-butyldimethylsilanyl group to give 4; the synthesis of sulfonamides 5a and 5b was accomplished under the conditions of Fukuyama et al. [22]. Compound 6a was obtained in a 70% yield under Mitsunobu conditions from 5a and glycolaldehyde dimethyl acetal. The yield of this reaction depends greatly on the temperature: at rt., it is 19%, increasing up to 70% at 30°C and falling to 40% when the temperature is 40°C (Figure 2). When the optimized temperature conditions were applied to 5b, 6b was obtained in an 80% yield. After deprotection of the silanyl group of 6a (and 6b) with tetra-n-butyllammonium fluoride (TBAF) in tetrahydrofuran (THF), 7a was obtained in an 83% yield (and 7b in 100% yield). Compounds 7a and 7b quantitatively afforded the benzo-fused seven-membered O,O-acetals 3a and 3b, respectively, using boron trifluoride diethyl etherate as previously reported [23].
Until compounds 3a and 3b were obtained under the optimal conditions described above, other conditions were studied giving rise to unwanted products. Product 5b was obtained using a twofold excess of the o-aminobenzyl silanyl ether 4, and these conditions were most important for the preparation of this compound (5b, Figure 2). Actually, the derivative 8 was isolated when the reaction was carried out using triethylamine (TEA) as a hydrochloride acid scavenger and 1.1 equiv. of the sulfonyl chloride was added (Figure 3) [19].

A reasonable explanation implied the previous ionization of the sulfonamide hydrogen atom of 5b to give a \( \text{NSO}_2 \) anion which reacts more rapidly than the starting amine to give 8. Disulfonimides have been stated [24] to be by-products in reactions of sulfonyl halides with primary amines and ammonia. The o-nitro group might sterically hinder the \( \text{NSO}_2 \) anion, and therefore the analogous side product was not isolated.

We also investigated other conditions to synthesize 6b (Figure 4) [19]. The isopropyl alkylated derivative 9 (38%) was obtained together with the expected acetal 6b (31%) when an excess of glycolaldehyde dimethyl acetal (4.3 equiv) was added. Such a compound could be interpreted by the transesterification reaction of glycolaldehyde dimethyl acetal and diisopropyl azodicarboxylate (DIAD), with the concomitant leaving of isopropanol. A similar process was previously reported for diethyl azodicarboxylate (DEAD) but not when DIAD was used [25].

The \( O,O \)-acetals 3a and 3b were formed after treatment of the \( p \)-toluenesulfonic acid-mediated cyclization from the acyclic acetals 7a and 7b, using anhydrous

---

**Figure 2.**
Reagents and conditions: (a) \( o-O,N-C_6H_4-SO_2Cl \) (1.1 equiv), TEA (1.5 equiv), \( CH_2Cl_2 \), reflux, 24 h, for 5a; \( p-O,N-C_6H_4-SO_2Cl \) (0.5 equiv), \( CH_2Cl_2 \), rt., 3 h, for 5b; (b) \( HOCH_2CH(OMe)_2 \) (1 equiv), diisopropyl azodicarboxylate (DIAD, 1.1 equiv), PPh_3 (1.2 equiv), anhydrous THF, 21 h; (c) TBAF (1 equiv), THF, rt., 1 h; (d) BF_3·OEt_2 (2 equiv), anhydrous Et_2O, rt., 7 days for 3a; when these conditions were used to obtain 3b, the yield was 69%; \( p-H,C-C_6H_4-SO_3H \) (0.03 equiv), anhydrous toluene, 110°C, 2 h under argon for 3a and 3b [19].
toluene as solvent. When neutral and mild conditions using triphenylphosphine/carbon tetrachloride were employed, the substitution of the hydroxyl group by the chlorine took place, and compounds 10a and 10b were obtained instead of the cyclic derivatives 7a and 7b (Figure 5) [26]. Compounds 7a and 7b present bulky groups that limit their conformational motions, making them rigid structures. The oxygen atoms of the acetalic groups cannot act as nucleophiles against the benzylic position (with the triphenylphosphine ether) due to steric hindering. Hydrogen atoms of the methylene groups of both compounds (7a and 7b) are diastereotopic protons \( J_{\text{gem}} = 12.6-14.1 \text{ Hz} \) [19].

![Figure 3](image)

**Figure 3.**
Reagents and conditions: (a) p-O\( \text{N-C}_6\text{H}_4\)-SO\( \text{Cl} \) (1.1 equiv), TEA (1.5 equiv) and anhydrous CH\( \text{Cl}_2 \), rt., 5 h when 0.5 equiv. of p-O\( \text{N-C}_6\text{H}_4\)-SO\( \text{Cl} \) was used, see Figure 2 (conversion 4 \( \rightarrow \) 5b) [19].

![Figure 4](image)

**Figure 4.**
Reagents and conditions: (a) HO\( \text{CH}_2\text{CH(OMe)}_2 \) (4.3 equiv), DIAD (1.2 equiv), PPh\(_3\) (1.2 equiv) and anhydrous THF, rt., 18 h; when 1.2 equiv. of HO\( \text{CH}_2\text{CH(OMe)}_2 \) were used, see Figure 2 (conversion 5b \( \rightarrow \) 6b) [19].

![Figure 5](image)

**Figure 5.**
Reagents and conditions: (a) Ph\(_3\)P (1 equiv), CCl\(_4\), 110°C, 30 min [19].
2.2 Synthesis of tetrahydrobenzoxazepine 2,6-dichloropurine $O,N$-acetals with nitrosulfonyl groups on the nitrogen atom

The preparation of the $O,N$-acetals 1 and 2 was achieved by the microwave-assisted Vorbrüggen one-pot condensation of the cyclic acetals 3a and 3b [27] and the commercially available purine base 2,6-dichloropurine, using trimethylsilyl chloride (TMSCl), 1,1,1,3,3,3-hexamethyldisilazane (HMDS) and tin(IV) chloride as the Lewis acid in anhydrous acetonitrile. The reaction mixture was microwave-irradiated at a temperature of 140°C for 5 min (Figure 6).

Compounds 11 and 12 were obtained along with the cyclic 2,6-dichloropurine $O,N$-acetals (1 and 2, bozepinib) and the acyclic one 13 (when starting from 3b) in the reaction of purines with 3a and 3b, respectively. The mechanism of the reaction of these compounds is important as none of them were previously isolated in the

![Diagram](image)

Figure 6. Reagents and conditions: (a) 3a (1 equiv), 2,6-dichloropurine (1.5 equiv), trimethylsilyl chloride (TMSCl, 1.5 equiv), 1,1,1,3,3,3-hexamethyldisilazane (HMDS, 1.5 equiv) and SnCl$_4$ (1 M solution in CH$_2$Cl$_2$, 1.5 equiv), 140°C, microwave, 5 min; (b) 3b (1 equiv), 2,6-dichloropurine (2.5 equiv), TMSCl (4.0 equiv), HMDS (4.0 equiv) and SnCl$_4$ (1 M solution in CH$_2$Cl$_2$, 4.0 equiv), 140°C, microwave, 5 min.
corresponding reactions with uracil or 5-FU [27]. We have previously explained the mechanism of the reaction leading to the benzo-fused derivatives 1, 2, 11 and 12 [28].

2.3 Homochiral drugs

A better understanding of the molecular recognition of the therapeutic targets in many diseases highlights the issue of drug in the design and development of new drugs. The separation of racemates, chiral pool and asymmetric synthesis are the three most used methods for the production of a chiral drug. Since the 1980s there has been a significant increase in the development of chiral pharmaceutical drugs. When patents of racemic drugs expire, pharmaceutical companies can extend patents by developing the synthesis of enantiomers that exhibit the desired biological activity [29].

Compound 2 was resolved into its two enantiomers: [(R)-2: [α]$_D^{25}$ = −43.6 (c = 0.22, THF) and (S)-2: [α]$_D^{25}$ = +41.0 (c = 0.23, THF)]; using a semipreparative column CHIRALPAK® and a mixture of hexane/t-BuOMe/iPrOH as eluant [28]. From this moment on, the racemic 2 [(RS)-2] will be named only as bozepinib.

2.4 Biological studies

Table 1 shows the anti-proliferative activity (IC$_{50}$ values) for 1, bozepinib and 5-fluorouracil (5-FU) as a reference drug. Compounds were first assayed as anti-proliferative agents against the human breast adenocarcinoma cell line MCF-7 (p53 wild type and ras mutated). Compounds 1 and bozepinib were further assayed against the human breast cancer cell line MDA-MB-231 which presents high levels of mutant p53 [28, 30]. The IC$_{50}$ = 0.166 μM for bozepinib against the human cancerous cell line MDA-MB-231 stands out.

In order to determine the in vitro therapeutic index of the compounds, they were assayed against the non-cancerous human mammary epithelial cell line MCF-10A. The TI of a drug is defined as the ratio of the toxic dose to the therapeutic dose (in vitro TI = IC$_{50}$ non-tumor cell line/IC$_{50}$ tumor cell line) [28]. Bozepinib is more selective against both human breast adenocarcinoma MCF-7 and MDA-MB-231 cancer cell lines (TIs = 5.14 and 11.0, respectively) in relation to the normal one (Table 2).

As bozepinib is more active and more selective and more active than its isomer 1, we decided to carry out the separation of bozepinib into their component enantiomers (resolution). (S)-2 shows higher anti-proliferative effect that of (R)-2 in the MCF-7 cell line. However no differences against the MDA-MB-231 cell line were observed (Table 3). The enantioselective cytotoxicity indicates that the

| Compound | IC$_{50}$ MCF-7 (μM) | IC$_{50}$ MDA-MB-231 (μM) | IC$_{50}$ MCF-10A (μM) |
|----------|----------------------|---------------------------|------------------------|
| 1        | 0.383 ± 0.027        | 0.280 ± 0.006             | 1.530 ± 0.198          |
| Bozepinib| 0.355 ± 0.011        | 0.166 ± 0.063             | 1.825 ± 0.503          |
| 5-FU$^c$ | 4.32 ± 0.020         | N.D.$^b$                  | N.D.$^b$               |

$^a$All experiments were conducted in duplicate and gave similar results. Data are means ± SEM of three independent determinations. The treatment time was 48 h.

$^b$N.D. = not determined.

$^c$Taken from Ref. [31].

Table 1.
Anti-proliferative activities$^a$ for compounds 1 and bozepinib against the cancerous cell lines MCF-7 and MDA-MB-231 and the non-cancerous cell line MCF-10A.
enantiomers of some chiral drugs may differ both quantitatively and qualitatively in their biological activity [32, 33]. Moreover, enantiomers can show minimal in vitro but a dramatic in vivo chiral dependency in their antitumor activities [34, 35].

2.4.1 Effect of bozepinib against CSC subpopulations

The cytotoxic effect of bozepinib was determined on SKBR-3 and MDA-MB 468 breast and HCT-116 colon CSC-enriched subpopulations by growing them into low attachment plates with sphere-forming medium for 72 h. Salinomycin is a selective and potent drug used in the treatment of CSCs, but its use is limited in humans due to considerable toxicity [36]. Thus, we decided to include salinomycin in our studies as reference drug. Bozepinib displayed IC50 values in a similar range to salinomycin in SKBR-3 and MDA-MB 468 cells [30].

CSCs were separated using ALDH activity by FACS. IC50 values were determined in both ALDH positive cells (called ALDH+), ALDH negative cells (called ALDH−) and cells growing in sphere-forming medium without sorter enrichment process [30]. Since there is controversy about the efficacy of sphere-forming cultures to select CSCs [37, 38], we used ALDH activity to enrich cell cultures with clonal ability and stem-like properties. Bozepinib substantially diminished the number and size of spheres at 5 μM and abolished the formation of spheres at 20 μM [30] in subpopulations isolated by ALDH activity. High ALDH activity is associated with metastasis, resistance to chemotherapies and poor prognosis in human cancers, and it has been identified as one of the most specific markers of human CSCs [39].

Apoptosis is also an important factor in the onset of a tumor. It has been reported than CSCs are resistant to apoptosis to ensure succeeding generation [40] and may be on the causes to chemo- or radiotherapy survival. Reactivating death programmes in CSCs may increase efficacy of the treatment. Bozepinib induced a significant level of cell death by apoptosis in the resistant ALDH+ subpopulations from both SKBR-3 and HCT-116 cell lines. Higher levels of apoptosis were detected in the ALDH− subpopulations.

Bozepinib induced the downregulation of important genes involved in Notch and Wnt signaling. These genes contribute to the CSC phenotype when they are deregulated [41]. A downregulation was detected of the co-activator

| Compound | Therapeutic index (TI) |
|----------|------------------------|
|          | MCF-7 | MDA-MB-231 |
| 1        | 4.00  | 5.50       |
| Bozepinib| 5.14  | 11.0       |

Table 2. Therapeutic indexes for 1 and bozepinib.

| Compound | MCF-7 (μM)a | MDA-MB-231 (μM)a |
|----------|-------------|-----------------|
| Bozepinib| 0.355 ± 0.011 | 0.166 ± 0.063 |
| (R)-2    | 0.19 ± 0.001 | 0.11 ± 0.001   |
| (S)-2    | 0.10 ± 0.001 | 0.11 ± 0.001   |

a All experiments were conducted in duplicate and gave similar results. The data are means ± SEM of three independent determinations.

Table 3. Anti-proliferative activities of bozepinib and its enantiomers against the cancerous cell lines MCF-7 and MDA-MB-231.
Mastermind-like MAML-2 protein, which decreases Notch signaling, reducing the primary tumor sphere formation and side population in MCF-7 cell line, contributing to the decrease in the number of CSC subpopulations [42]. We also found an evident downregulation of the NOTCH 3 gene, which has recently been involved in the proliferation of both HER2 positive and negative breast cancer cells, suggesting that targeted suppression of this signaling pathway may be a promising strategy for the treatment of determined HER2-related breast cancers [43, 44].

The modification of proteins related to the CSC phenotype such as β-catenin, GLI-3, c-MYC and SOX-2 was additionally analysed. This study was carried out in both ALDH+ and ALDH− SKBR-3 breast and HCT-116 colon cancer subpopulations.

β-Catenin is a key mediator of the Wnt signaling which has a crucial role in CSCs [45]. β-Catenin expression was detected in ALDH+ SKBR-3 but not in ALDH− SKBR-3 cells. A high level of expression in HCT-116 cell lines with an ALDH+ subpopulations in comparison with the ALDH− in HCT-116 subpopulations was also detected. After bozepinib treatment, β-catenin expression was reduced in ALDH+ HCT-116 subpopulation. Its inhibition in ALDH− HCT-116 cells was also observed.

GLI-3, a described target gene transcription repressor of Hedgehog signaling pathway [16], was detected in ALDH− HCT-116 isolated cells and was not present in the ALDH+ cells, denoting that the Hedgehog signaling pathway is involved in the CSC phenotype as previously reported [16]. Bozepinib greatly induced the expression of GLI-3 in both ALDH+ and ALDH− HCT-116 cells. However, no changes at protein level were observed in ALDH+/− subpopulations isolated from the SKBR-3 cell line. Furthermore, the activation of GLI-3 protein corresponded with an inactivation of β-catenin expression in CSC subpopulations after bozepinib treatment in accordance with the previous published studies [46]. Considering that GLI-3 overexpression decreased tumor cell proliferation and induced apoptosis in colon CSCs [47], the GLI-3 induction by bozepinib could be one of the mechanisms by which this drug exerts its antitumor activity in colon CSCs. This hypothesis will be deeply study the future.

The c-MYC oncoprotein was observed in SKBR-3 and HCT-116 cell lines with a high level of expression in ALDH+ subpopulations in comparison with the ALDH− subpopulations. Bozepinib induced a significant decrease of c-MYC level in ALDH+ HCT-116 cells and inhibited its expression in ALDH+ SKBR-3-treated cells. It also inhibited ALDH− subpopulations of both HCT-116 and SKBR-3 cells [30]. In accordance with the high level of stem signaling proteins described for CSCs, higher levels of these proteins in the ALDH+ subpopulations in comparison with ALDH− cells were detected. The downregulation of c-MYC activates the inhibition of cancer cell proliferation, invasion and migration [48].

The transcription factor SOX2 was detected in HCT-116 ALDH+ subpopulation. However SOX2 was not observed in breast cancer cells, and the expression of SOX2 was very weak in HCT-116 ALDH− subpopulation [30]. Its expression practically disappeared after treatment with bozepinib. SOX2 is involved in the induction and maintenance of pluripotent stem cells and has also been associated with metastases and poor prognosis in colon cancer [49].

2.4.2 In vivo studies of bozepinib

The acute toxicity profile of bozepinib was determined in BALB/c mice. After 2 weeks, bozepinib was nontoxic to BALB/c mice even at the highest intraperitoneal bolus dose of 200 mg/kg and orally bolus dose of 50 mg/kg. Control mice were treated with vehicle alone. All 50 bozepinib-treated mice remained healthy and gained weight throughout the 15-day observation period, with no evidence of morbidity [28].
We then evaluated the subacute toxicity after 29 days of intraperitoneal treatment with 100 mg/kg twice a week. Bozepinib-treated mice presented no weight loss or unusual behaviour, and the histopathologic examination did not find any detectable toxicity in the liver or kidneys. These data indicate that, at the concentration used, bozepinib did not cause any systemic damage [30].

3. Conclusion

Although we show promising data proving the efficacy of bozepinib over CSCs, the mechanism by which bozepinib inhibits the CSC growth requires further detailed investigation. Moreover, the in vivo antitumor and anti-metastatic effect and the non-systemic toxicity of bozepinib encourage further studies on the therapeutic potential of this synthetic compound in breast and colon cancer patients.

The detection of CSCs in multiple solid tumors over the past years represents a paradigm shift in oncology and will probably change our understanding of the tumourigenic process. The impressive number of publications that have appeared in this field in recent years bears testimony to its enormous current interest.

We expect this chapter to stimulate fresh activity within the medicinal chemistry community and to result in the enlargement of chemical structures active against CSCs.

Abbreviations

| Abbreviation | Definition |
|--------------|------------|
| ALDH         | aldehyde dehydrogenase activity |
| CSCs         | cancer stem cells |
| DEAD         | diethyl azodicarboxylate |
| DIAD         | diisopropyl azodicarboxylate |
| FACS         | fluorescence-activated cell sorter |
| 5-FU         | 5-fluorouracil |
| Hh           | Hedgehog |
| HMDS         | 1,1,1,3,3,3-hexamethyldisilazane |
| PKR          | protein kinase R |
| TBAF         | tetra-$n$-butylammonium fluoride |
| TEA          | triethylamine |
| THF          | tetrahydrofuran |
| TI           | therapeutic index |
| TMSCl        | trimethylsilyl chloride |
| VEGF         | vascular endothelial growth factor |
Bozepinib: A Promising Selective Derivative Targeting Breast Cancer Stem Cells
DOI: http://dx.doi.org/10.5772/intechopen.91423

Author details
Joaquín M. Campos1,2*, Ana Conejo-García1,2 and Olga Cruz-López1,2

1 Department of Pharmaceutical and Organic Chemistry, Faculty of Pharmacy, University of Granada, Granada, Spain

2 Granada Biosanitary Institute (ibs. GRANADA), Andalusian Health Service, University of Granada, Granada, Spain

*Address all correspondence to: jmcampos@ugr.es

IntechOpen
© 2020 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
References

[1] Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. Nature. 2001;414:105-111. DOI: 10.1038/35102167

[2] Tirino V, Desiderio V, Paino F, De Rosa A, Papaccio F, La Noce M, et al. Cancer stem cells in solid Tumours: An overview and new approaches for their isolation and characterization. The FASEB Journal. 2013;27:13-24. DOI: 10.1096/fj.12-218222

[3] Dontu G, Abdallah WM, Foley JM, Jackson KW, Clarke MF, Kawamura MJ, et al. In vitro propagation and transcriptional profiling of human mammary stem/progenitor cells. Genes & Development. 2003;17:1253-1270. DOI: 10.1101/gad.1061803

[4] Al-Hajj M, Whicha MS, Benito-Hernández A, Morrison SJ, Clarke MF. Prospective identification of tumourigenic breast cancer cells. Proceedings of the National Academy of Sciences of the United States of America. 2003;100:3983-3988. DOI: 10.1073/pnas.0530291100

[5] Moumen M, Chiche A, Decraene C, Petit V, Gandarillas A, Deugnier MA, et al. Myc is required for β-catenin-mediated mammary stem cell amplification and tumourigenesis. Molecular Cancer. 2013;12:132-140. DOI: 10.1186/1476-4598-12-132

[6] Leis O, Eguiar A, López-Arribillaga E, Alberdi MJ, Hernández-García S, Elorriaga K, et al. Sox2 expression in breast tumours and activation in breast cancer stem cells. Oncogene. 2012;31:1354-1365. DOI: 10.1038/onc.2011.338

[7] Deng S, Yang X, Lassus H, Liang S, Kaur S, Ye Q, et al. Distinct expression levels and patterns of stem cell marker, aldehyde dehydrogenase isofrom 1 (ALDH1), in human epithelial cancers. PLoS One. 2010;5:e10277. DOI: 10.1371/journal.pone.0010277

[8] Arai F, Hirao A, Ohmura M, Sato H, Matsuoka S, Takubo K, et al. Tie2/angiopoietin-1 signalling regulates hematopoietic stem cell quiescence in the bone marrow niche. Cell. 2004;118:149-161. DOI: 10.1016/j.cell.2004.07.004

[9] Dick JE. Stem cell concepts renew cancer research. Blood. 2008;112:4793-4807. DOI: 10.1182/blood-2008-08-077941

[10] Stockler M, Wilcken N, Ghersi D, Simes RJ. Systematic reviews of chemotherapy and endocrine therapy in metastatic breast cancer. Cancer Treatment Reviews. 2000;26:151-168. DOI: 10.1053/ctrv.1999.0161

[11] Jordan CT, Guzman ML. Mechanisms controlling pathogenesis and survival of leukemic stem cells. Oncogene. 2004;23:7178-7187. DOI: 10.1038/sj.onc.1207935

[12] Jordan CT, Guzman ML, Noble M. Cancer stem cells. New England Journal of Medicine. 2006;355:1253-1261. DOI: 10.1056/NEJMra061808

[13] Liu HG, Chen C, Yang H, Pan YF, Zhang XH. Cancer stem cell subsets and their relationships. Journal of Translational Medicine. 2011;9:50-58. DOI: 10.1186/1479-5876-9-50

[14] Sagar J, Chaib B, Sales K, Winslet M, Seifalian A. Role of stem cells in cancer therapy and cancer stem cells: A review. Cancer Cell International. 2007;7:9-14. DOI: 10.1186/1475-2867-7-9

[15] Maugeri-Saccà M, Zeuner A, De Maria R. Therapeutic targeting of cancer stem cells. Frontiers in Oncology. 2011;1:10. DOI: 10.3389/fonc.2011.00010
[16] Takebe N, Harris PJ, Warren RQ, Ivy SP. Targeting cancer stem cells by inhibiting Wnt, Notch, and Hedgehog pathways. Nature Reviews. Clinical Oncology. 2011;8:97-106. DOI: 10.1038/nrclinonc.2010.196

[17] Oreste P, García-Rubiño ME, Franchini C, Campos J. Cancer stem cells: The new objective for the eradication of tumours. In: Watanabe HS, editor. Horizons in Cancer Research. Vol. 56. 2015. pp. 41-81. ISSN: 2159-1326. ISBN: 978-1-63482-248-0 (eBook)

[18] García-Rubiño ME, Lozano-López C, Campos J.M. Inhibitors of cancer stem cells. Anti-Cancer Agents in Medicinal Chemistry. 2016;16:1230-1239. DOI: 10.2174/18715206166661603104715

[19] Díaz-Gavilán M, Rodríguez-Serrano F, Gómez-Vidal JA, Marchal JA, Aránega A, Gallo MA, et al. Synthesis of tetrahydrobenzoxazepine acetals with electron-withdrawing groups on the nitrogen atom. Novel scaffolds endowed with anticancer activity against breast cancer cells. Tetrahedron. 2004;60:11547-11557. DOI: 10.1016/j.tet.2004.09.072

[20] Fletcher S. The Mitsunobu reaction in the 21st century. Organic Chemistry Frontiers. 2015;2:739-752. DOI: 10.1039/CSQ000016E

[21] Koppel I, Kippel J, Degerbeck F, Grehn L, Ragnarsson V. Acidity of imidodicarbonates and tosylcarbamates in dimethyl sulfoxide. Correlation with yields in the Mitsunobu reaction. The Journal of Organic Chemistry. 1991;56:7172-7174. DOI: 10.1021/jo00025a041

[22] Fukuyama T, Jow CK, Cheung M. 2,4-Dinitrobenzenesulphonamides: A simple and practical method for the preparation of a variety of secondary amines and diamine. Tetrahedron Letters. 1995;36:6373-6374. DOI: 10.1016/S0040-4099(97)01334-8

[23] Campos J, Pineda MJ, Gómez JA, Entrena A, Trujillo MA, Gallo MA, et al. 5-fluorouracil derivatives. 1. Acyclonucleosides through a Tin (IV) chloride-mediated regiospecific ring opening of alkoxy-1,4-diheteroepanes. Tetrahedron. 1996;52:8907-8924. DOI: 10.1016/0040-4020(96)00439-5

[24] Andersen KK, Jones DN, editors. Comprehensive Organic Chemistry. Vol. 3. Pergamon: Oxford; 1979. pp. 339-341. https://doi.org/10.1002/jps.260090651

[25] Rew Y, Goodman M. Solid-phase synthesis of amine-bridged cyclic enkephalin analogues via on-resin cyclization utilizing the Fukuyama–Mitsunobu reaction. The Journal of Organic Chemistry. 2002;67:8820-8826. https://doi.org/10.1021/jo0204471

[26] Espinosa A, Gallo MA, Campos J, Gómez JA. Diastereoselective intramolecular cyclization through the triphenylphosphine/carbon tetrachloride system: Synthesis of saturated 1,4-dihetero seven-membered Cycloacetals. Synlett. 1995;11:1119-1120. DOI: 10.1055/s-1995-5207

[27] Díaz-Gavilán M, Gómez-Vidal JA, Entrena A, Gallo MA, Espinosa A, Campos JM. Study of the factors that control the ratio of the products between 5-fluorouracil, uracil, and tetrahydrobenzoxazepine O,O-acetals bearing electron-withdrawing groups on the nitrogen atom. The Journal of Organic Chemistry. 2006;71:1043-1054. DOI: 10.1021/jo052167m Ref. 12 del EJMC 2011

[28] López-Cara LC, Conejo-García A, Marchal JA, Macchione G, Cruz-López O, Boulaiz H, et al. New (RS)-benzoxazepin-purines with antitumour activity: The chiral switch from (RS)-2,6-dichloro-9-([1-(p-nitrobenzenesulfonyl)-1,2,3,5-tetrahydro-4,1-benzoxazepin-3-yl]-9H-purine. European Journal of Medicinal Chemistry. 2011;46:249-258. DOI: 10.1016/j.ejmech.2010.11.011
[29] Núñez MC, García-Rubiño ME, Conejo-García A, Cruz-López O, Kimatrai M, Gallo MA, et al. Homochiral drugs: A demanding tendency of the pharmaceutical industry. Current Medicinal Chemistry. 2009;16:2064-2074. DOI: 10.2174/092986709788682173

[30] Ramírez A, Boulaiz H, Morata-Tarifa C, Perán M, Jiménez G, Picón-Ruiz M, et al. HER2-signalling pathway, JNK and ERKs kinases, and cancer stem-like cells are targets of bozepinib. Oncotarget. 2014;5:3590-3606. DOI: 10.18632/oncotarget.1962

[31] Núñez MC, Díaz-Gavilán M, Conejo-García A, Cruz-López O, Gallo MA, Espinosa A, et al. Design, synthesis and anticancer activity against the MCF-7 cell line of benzo-fused 1,4-dihetero seven- and six-membered tethered pyrimidines and purines. Current Medicinal Chemistry. 2008;15:2614-2631. DOI: 10.2174/092986708785909021

[32] Liu H, Xu L, Zhao M, Liu W, Zhang C, Zhou S. Enantiomer-specific, bifenthrin-induced apoptosis mediated by MAPK signalling pathway in Hep G2 cells. Toxicology. 2009;261:119-125. DOI: 10.1016/j.tox.2009.05.002

[33] Shelley MD, Hartley L, Fish RG, Groundwater P, Morgan JG, Mort D, et al. Stereo-specific cytotoxic effects of gossypol enantiomers and gossypolone in tumour cell lines. Cancer Letters. 1999;135:171-180. DOI: 10.1016/S0304-3835(98)00302-4

[34] Lai JC, Brown BD, Voskresenskiy AM, Vonhoff S, Klussman S, Tan W, et al. Comparison of D-G3139 and its enantiomer L-G3139 in melanoma cells demonstrates minimal in vitro but dramatic in vivo chiral dependency. Molecular Therapy. 2007;15:270-278. DOI: 10.1038/sj.mt.6300037

[35] Brown NM, Belles CA, Lindley SL, Zimmer-Nechemias LD, Zhao X, Witte DP, et al. The chemopreventive action of equol enantiomers in a chemically induced animal model of breast cancer. Carcinogenesis. 2010;31:886-893. DOI: 10.1093/carcin/bgq025

[36] Naujokat C, Steinhart R. Salinomycin as a drug for targeting human cancer stem cells. Journal of Biomedicine & Biotechnology. 2012;2012:950658. DOI: 10.1155/2012/950658

[37] Smart CE, Morrison BJ, Saunus JM, Vargas AC, Keith P, Reid L, et al. In vitro analysis of breast cancer cell line tumourspheres and primary human breast epithelia mammospheres demonstrates inter- and intrasphere heterogeneity. PLoS One. 2013;8:e64388. DOI: 10.1371/journal.pone.0064388

[38] Ma L, Lai D, Liu T, Cheng W, Guo L. Cancer stemlike cells can be isolated with drug selection in human ovarian cancer cell line SKOV3. Acta Biochimica et Biophysica Sinica. 2010;42(9):593-602. DOI: 10.1093/abbs/gmq067

[39] Charafe-Jauffret E, Ginestier C, Iovino F, Tarpin C, Diebel M, Esterni B, et al. Aldehyde dehydrogenase 1-positive cancer stem cells mediate metastasis and poor clinical outcome in inflammatory breast cancer. Clinical Cancer Research. 2010;16:45-55. DOI: 10.1158/1078-0432.CCR-09-1630

[40] Kruyt FA, Schuringa JJ. Apoptosis and cancer stem cells: Implications for apoptosis targeted therapy. Biochemical Pharmacology. 2010;80:423-430. DOI: 10.1016/j.bcp.2010.04.010

[41] Dontu G, Jackson KW, McNicholas E, Kawamura MJ, Abdallah WM, Wicha MS. Role of Notch signalling in cell-fate
determination of human mammary stem/progenitor cells. Breast Cancer Research. 2004;6:R605-R615. DOI: 10.1186/bcr920

[42] Wong NK, Fuller M, Sung S, Wong F, Karsan A. Heterogeneity of breast cancer stem cells as evidenced with Notch-dependent and Notch-independent populations. Cancer Medicine. 2012;1:105-113. DOI: 10.1002/cam4.18

[43] Pradeep CR, Kostler WJ, Lauriola M, Granit RZ, Zhang F, Jacob-Hirsch J, et al. Modeling ductal carcinoma in situ: A HER2-Notch3 collaboration enables luminal filling. Oncogene. 2012;31:907-917. DOI: 10.1038/onc.2011.279

[44] Yamaguchi N, Oyama T, Ito E, Satoh H, Azuma S, Hayashi M, et al. NOTCH3 signalling pathway plays crucial roles in the proliferation of ErbB2-negative human breast cancer cells. Cancer Research. 2008;68:1881-1888. DOI: 10.1158/0008-5472.CAN-07-1597

[45] Reya T, Clevers H. Wnt signalling in stem cells and cancer. Nature. 2005;434(7035):843-850. DOI: 10.1038/nature03319

[46] Ulloa F, Itasaki N, Briscoe J. Inhibitory Gli3 activity negatively regulates Wnt/beta-catenin signalling. Current Biology. 2007;17:545-550. DOI: 10.1016/j.cub.2007.01.062

[47] Merchant AA, Matsui W. Targeting Hedgehog—A cancer stem cell pathway. Clinical Cancer Research. 2010;16:3130-3140. DOI: 10.1158/1078-0432.CCR-09-2846

[48] Zhao Y, Jian W, Gao W, Zheng YX, Wang YK, Zhou ZQ, et al. RNAi silencing of c-Myc inhibits cell migration, invasion, and proliferation in HepG2 human hepatocellular carcinoma cell line: c-Myc silencing in hepatocellular carcinoma cell. Cancer Cell International. 2013;13:23. DOI: 10.1186/1475-2867-13-23

[49] Neumann J, Bahr F, Horst D, Kriegl L, Engel J, Luque RM, et al. SOX2 expression correlates with lymph-node metastases and distant spread in right-sided colon cancer. BMC Cancer. 2011;11:518. DOI: 10.1186/1471-2407-11-518