European Journal of Medicinal Chemistry 151 (2018) 186–198

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Research paper

Repositioning of the antipsychotic trifluoperazine: Synthesis, biological evaluation and *in silico* study of trifluoperazine analogs as anti-glioblastoma agents

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ARTICLE INFO

Article history:
Received 27 November 2017
Received in revised form
18 March 2018
Accepted 19 March 2018
Available online 23 March 2018

Keywords:
Trifluoperazine
Trifluoperazine analogs
Brain cancer
Glioblastoma
Calcium
Orthotopic brain xenograft mouse model

ABSTRACT

Repositioning of the antipsychotic drug trifluoperazine for treatment of glioblastoma, an aggressive brain tumor, has been previously suggested. However, trifluoperazine did not increase the survival time in mice models of glioblastoma. In attempt to identify an effective trifluoperazine analog, fourteen compounds have been synthesized and biologically *in vitro* and *in vivo* assessed. Using MTT assay, compounds **3dc** and **3dd** elicited 4–5 times more potent inhibitory activity than trifluoperazine with $IC_{50} = 2.3$ and 2.2 μM against U87MG glioblastoma cells, as well as, $IC_{50} = 2.2$ and 2.1 μM against GBL28 human glioblastoma patient derived primary cells, respectively. Furthermore, they have shown a reasonable selectivity for glioblastoma cells over NSC normal neural cell. *In vivo* evaluation of analog **3dc** confirmed its advantageous effect on reduction of tumor size and increasing the survival time in brain xenograft mouse model of glioblastoma. Molecular modeling simulation provided a reasonable explanation for the observed variation in the capability of the synthesized analogs to increase the intracellular Ca^{2+} levels. In summary, this study presents compound **3dc** as a proposed new tool for the adjuvant chemotherapy of glioblastoma.

1. Introduction

Glioblastoma is the most prevalent and devastating primary malignant tumor of the brain [1,2]. It is characterized by very poor prognosis with median survival time of 14–16 months in patients, as well as, a very low 5-years survival rate of only 9.8% [3–6]. Because of its location within the CNS, rapid proliferative rate, resistance to apoptosis, and aggressive invasive cellular property, glioblastoma therapy is very challenging [1,7–9]. Up-to-date, temozolomide (TMZ, **1**, Fig. 1) is the major drug of choice for the first line treatment of glioblastoma according to the European Organization for Research and Treatment of Cancer (EORTC) and the Radiation Therapy Oncology Group (RTOG) [10]. TMZ is a prodrug whose core, the imidazotetrazine ring,

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https://doi.org/10.1016/j.ejmech.2018.03.055
0223-5234/

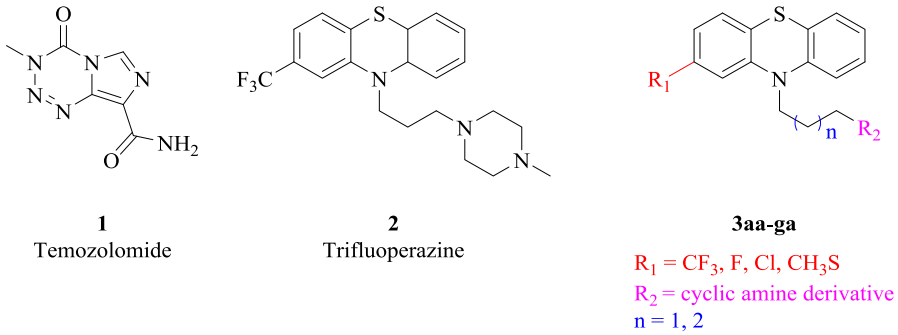

Fig. 1. Structures of TMZ (**1**), TFP (**2**) and the designed ligands (**3**).

undergoes spontaneous hydrolysis at the physiologic pH to produce 5-(3-methyltriazen-1-yl)imidazole-4-carboxamide (MTIC) which acts as an alkylating agent delivering a methyl group to DNA bases [11]. Unfortunately, more than 50% of TMZ-treated glioblastoma patients do not show effective response to TMZ, may be because of TMZ resistant glioblastoma cells [12,13]. In addition, the average increase in the life span of TMZ-treated glioblastoma patients is estimated to be around only 2.5 months with high tumor relapse [5,11,14–16]. Despite the introduction of novel surgical procedures, radiation techniques and adjuvant chemotherapeutic agents, the efficient treatment of glioblastoma is still lacking [1,5,17].

Recently, repositioning or repurposing of the approved drugs for treatment of diseases other than their known indications has gained the attention of the scientific society [18,19]. Because of the proven safety and quality of the drugs already available on the market, the process of drug repositioning could save much time, efforts and costs that would be invested in the discovery and development of novel chemical entities [19,20].

Repositioning of trifluoperazine (TFP, **2**, Fig. 1); a well-known antipsychotic drug for treatment of schizophrenia, has been explored as chemotherapeutic agent against several tumors including lung cancer [21], breast cancer, [22,23] and T-cell lymphoma [24]. In addition, it has been reported that TFP could inhibit glioblastoma cells' proliferation, migration, and invasion [5]. A mechanistic study revealed that the anti-glioblastoma activity of TFP is mediated through increasing the intracellular Ca^{2+} level *via* opening of inositol 1,4,5-trisphosphate receptor (IP_3R) subtype 1 and 2 which is the result of the induced dissociation of calmodulin (CaM) subtype 2 from IP_3R [5]. However, *in vivo* TFP-treated brain xenograft mouse model showed no increase in the survival time [5]. TFP analogs inducing more elevated intracellular Ca^{2+} levels could result in better biological response. The reported crystal structures for TFP bound to CaM show different binding modes within the hydrophobic cleft C-domain of CaM (Protein Databank codes: 1LIN and 1CTR) [25]. In the crystal structure 1LIN, the trifluoromethyl moiety is buried within the hydrophobic pocket which is not the case in the crystal structure 1CTR. Also, the cyclic amine; methylpiperazinyl moiety contributes to the binding by forming favorable interactions with residues within its vicinity. Exploration of the chemical space in the vicinity of TFP through modification of the trifluoromethyl and the cyclic amine moieties, as well as the length of the alkyl chain linker connecting the cyclic amine moiety with the core phenothiazine ring might provide TFP analogs **3aa**–**3ga** (Fig. 1) eliciting more increased intracellular Ca^{2+} level. Thus, these analogs might be more effective chemotherapeutic agents possessing significant *in vivo* increased survival time.

2. Results and discussion

2.1. Chemistry

A concise synthesis of the targeted TFP analogs (**3aa**–**ga**) was performed in two synthetic steps starting from commercially available phenothiazine derivatives **4a**–**d** (Scheme 1). First, deprotonation of phenothiazine derivatives **4a**–**d** with NaH (60% in oil) followed by S_N2 nucleophilic substitution reaction with 1,3- or 1,4-dihaloalkane at 100 °C for 24 h afforded the *N*-alkylated intermediates **5a**–**g** in 95–25.6% isolated yields. In the second step, the chloro group in intermediates **5a**–**g** has been displaced with the appropriate cyclic amine derivative *via* S_N2 reaction using NaI as a reaction promoter and K_2CO_3 as a base at 80 °C for 24 h to yield the desired TFP analogs (**3aa**–**ga**). Accordingly, new fourteen TFP analogs were prepared and submitted for biological evaluation.

2.2. Evaluation of Ca^{2+} imaging

Intracellular Ca^{2+} signaling is important for gene expression, migration, invasion, and survival of glioblastoma cells [4]. TFP has been reported to inhibit the proliferation and invasion of glioblastoma by increasing the intracellular Ca^{2+} release [5]. Consequently, TFP analogs capable of eliciting more intracellular Ca^{2+} increase relative to TFP could possess an enhanced antitumor effect against glioblastoma cells. Accordingly, the ability of the newly synthesized TFP analogs to increase the intracellular Ca^{2+} was evaluated by measuring the intracellular Ca^{2+} in U87MG glioblastoma cells. The results (Table 1) were calculated as percent of the increase of the intracellular Ca^{2+} produced by TFP.

First, TFP analog **3aa** having no *N*-methyl group in the piperazine moiety was synthesized and evaluated. This analog elicited 8% higher increase in the intracellular Ca^{2+} than that of TFP. Replacement of the 4-methylpiperazinyl with 4-(pyrrolidinyl)-piperidinyl (two cyclic amine moities linked together) afforded analog **3ab** which elicited an increase in the intracellular Ca^{2+} lower by 28% relative to that of TFP. Also, replacement of the 4-methylpiperazinyl with 4-methylpiperidinyl which has only one nitrogen atom resulted in analog **3ac** which also showed a less increase of intracellular Ca^{2+} relative to TFP. Modification of the trifluoromethyl and the cyclic amine moieties into chloro group and 4-methylpiperidinyl moiety respectively afforded analog **3ba** which elicited a lower increase of intracellular Ca^{2+} relative to TFP but higher than analog **3ac**. However, analog **3ca** possessing methylthio and 4-methylpiperidinyl moieties elicited even a lower level of intracellular Ca^{2+}. Next, analogs with increased length of the alkyl chain linker connecting the cyclic amine moiety with the phenothiazine scaffold were explored. Analog **3da** possessing 4-methylpiperidinyl moiety elicited a little decrement in the ability