European Journal of Medicinal Chemistry 122 (2016) 436–441

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Short communication

Synthesis and cytotoxicity evaluation of aryl triazolic derivatives and their hydroxymethine homologues against B16 melanoma cell line

Shiva Kalhor-Monfared [a], Claire Beauvineau [a], Daniel Scherman [b], Christian Girard [a, *]

[a] CNRS UMR8258, INSERM U1022, Unité de Technologies Chimiques et Biologiques pour la Santé, Equipe SEISAD, Ecole Nationale Supérieure de Chimie de Paris (Chimie ParisTech), PSL Research University, 11 rue Pierre & Marie Curie, Paris, 75005, France
[b] CNRS UMR8258, INSERM U1022, Faculté de Pharmacie, Université Paris Descartes, Sorbonne Paris Cité, 4 avenue de l'Observatoire, 75006, Paris, France

ARTICLE INFO

Article history:
Received 22 March 2016
Received in revised form
29 June 2016
Accepted 30 June 2016
Available online 4 July 2016

Keywords:
Alkyne
Triazole
Click chemistry
Anticancer
B16 melanoma

ABSTRACT

In this manuscript we describe synthesis and cytotoxicity evaluation of some triazolic derivatives against B16 melanoma cell line. For this purpose, we transformed a set of aromatic aldehydes into terminal alkynes, using Besthmann-Ohira reagent, and we made the corresponding hydroxymethyl homologated alkynes by an acetylene Grignard reagent. These generated two sets of alkynes were then subjected to a copper(I)-catalyzed alkyne-azide cycloaddition reaction (CuAAC) using a solid-supported catalyst (Amberlyst A-21 CuI), with a third set composed of organic azides. Synthesized triazoles were then tested *in vitro* against B16 melanoma cell line. Amongst them, compounds **a1b1** (R^1 = *p*-nitrophenyl, R^2 = benzyl), **a4b1** (R^1 = naphthyl, R^2 = benzyl) and **a4b5** (R^1 = naphthyl, R^2 = (*R*/*S*)- dioxolane) showed the best activity against B16 melanoma cells, with IC_{50} of 5.12, 3.89 and 6.60 μM respectively.

1. Introduction

Melanin pigments are produced by cells in the most layer of the epidermis which determines the color of skin [1]. Unrepaired DNA damage to skin cells caused usually by ultraviolet radiation triggers genetic defects that leads rapidly multiplication of skin cells forming malignant tumors. Recent studies showed that the incidence of melanoma is increasing in US and Europe [2].

Despite of the use of a wide variety of anticancer drugs, their deficiency and side effects such as normal tissue damage due to the lack of the specificity against cancer cells made researchers to develop other compounds to overcome resistance in chemothe-rapy [3].

There are some reported chemical compounds in literature with biological activity against B16 melanoma cells [4–11]. Our group previously reported *cis*-constrained analogs of Combretastatin A4 containing triazolic core with a cyto-toxic effect against B16 melanoma cells in μM range [12]. We also presented some mono- and bis-triazoles with activity against B16 melanoma cell lines [13].

In this article, we present our findings after the synthesis of two libraries of triazolic derivatives, and their *in vitro* evaluation against murine B16 melanoma cell line. The selection of the B16 melanoma as a model was stimulated by the need of identifying quickly new active molecules that can be used especially in the case of chemoresistant metastatic melanoma in Humans [14]. The B16 melanoma is recognized as an excellent model of Human cell line because of its high invasive property, capacity to metastasize by grafting into syngeneic mice and its resistance to chemotherapy, like in Human case [15,16]. B16 melanoma is also a widely used model in comparison with many other studies worldwide, including preclinical studies by the National Cancer Institute and others. Furthermore, this cell line gives the opportunity to identify hits *in vitro* and readily test them *in vivo* for further evaluation. Our group reported also discovery of cells express $\alpha_v\beta_3$ integrin and E-selectine, which are important targets for antiangiogenic cancer therapy [17].

Two different libraries of mono-triazoles were prepared; 4-aryltriazoles and their 4-arylhydroxymethyl homologues. They were prepared based on common aromatic aldehyde precursors, which could be transformed into aryl alkynes and arylhydroxymethyl alkynes. Classical Seyferth-Gilbert homolo-gation and addition of acetylene Grignard reagent were used. By reaction with a third set composed of azides, using the copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC), seventy-two compounds were prepared in high yields. Both libraries were then tested against B16 melanoma for their biological activity *in vitro* using the

* Corresponding author.
E-mail address: christian.girard@enscp.fr (C. Girard).

http://dx.doi.org/10.1016/j.ejmech.2016.06.057
0223-5234/

MTT (3-(4,5-dimethylthiazol -2-yl)-2,5-diphenyltetrazolium bromide) assay. From these results, we could find a few triazolic derivatives with cytotoxicity effect in micromolar range.

2. Chemistry

In order to prepare a library of triazolic derivatives, an approach using common aldehyde precursors has been selected to prepare aryl alkynes and arylhydroxylmethyl alkynes.

Aryl alkynes were prepared from aldehydes by Seyferth-Gilbert homologation using the Bestmann-Ohira reagent as shown in Table 1 [18,19]. Aldehydes were treated with 1.5 equivalents of this reagent in the presence of 2 equivalents of potassium carbonate in methanol at room temperature. Three alkynes **a1**, **a4** and **a6** were prepared using this reaction, and alkynes **a2-3** and **a5** were commercial. Good yields were obtained except for **a6** (45% yield).

In order to synthesize hydroxymethyl alkyne analogues, alkynes **a7-12** were prepared through a reaction with 1.3 equivalents of ethynyl magnesium bromide (0.5 M in THF) in THF under argon atmosphere at 0 °C (Table 2). The final products obtained in more or less good yields (97-40%).

All of these terminal alkynes bear either an electron donating (EDG) or electron-withdrawing group (EWG) on their aromatic ring to study their effect on biological activity of the final compounds.

Finally, the azides required for the CuAAC preparation of triazoles were prepared by azide anion S_N2 displacement of a bromide, chloride or mesylate, as a function of the starting material (Table 3). All azide derivatives **b1-6** were obtained in very good yields.

Triazolic libraries were then synthesized by CuAAC using A-21 CuI in methylene chloride overnight (Schemes 1 and 2). For the C-4 aryl compounds **a1b1** to **a6b6**, the library was obtained with an average yield of 90%. The majority of the triazoles were isolated with a yield between 71 and 99%. Only compounds **a1b3** (38%) and **a3b6** (57%) were obtained in a low yield. It is interesting to note that very few compounds needed a purification after the CuAAC reaction.

For the synthesis of arylhydroxymethyl derivatives **a7b1** to **a12b6**, the average yield was 86%. In this case, the obtained yields were lower, but still between 74 and 99% for most of the triazoles. Yields were often lower in these series for triazoles with the "(*S*)- or (*R*,*S*) dioxolane" substituent [(*4S*)-2,2-dimethyl-1,3-dioxolan-4-yl) methyl] due to the use of the azide **b6**. Thus yields for derivatives **a8b6** (65%), **a9b6** (64%), **a11b5** (42%), **a11b6** (46%) and **a12b6** (59%) were much lower. Furthermore, the reaction was incomplete under the same condition used for the other derivatives. We observed formation of precipitates that prevented the completion of the reaction even with incubation for a longer time. Our hypothesis is that the dioxolane system can form stable complexes with reacting species, and that these complexes prevent further reaction under these conditions.

3. Biological activity

The triazole libraries were then tested for their biological activity against B16 melanoma cells, and cytotoxicities are reported as IC_{50} in Tables 4 and 5. Unfortunately, some compounds could not be evaluated in the standard assay due to solubility problems. Thus results only show a partial view of the activity of these two libraries.

For the first library of triazoles **a1b1** to **a6b6** (Table 4), compound **a1b1** (R^1 = 4-NO_2Ph, R^2 = $PhCH_2$) gave an IC_{50} = 5.12 ± 0.39 μM. Changing R^2 to 4-MeOPhCH_2 in **a1b4**, decreased the activity to 19.05 ± 0.73 μM. Other compounds resulted from **a1** gave cytotoxcities >100 μM. When R^1 = 4-CF_3Ph, compound with R^2 = $PhCH_2$ (**a2b1**) was not active. However, where R^2 is 4-MeOPhCH_2 for compound **a2b4**, an IC_{50} similar to that one of **a1b4** was observed (17.37 ± 0.35 μM). For **a2b5**, with R^2 = " (*R*/*S*)-dioxolane", a slightly better activity was obtained (13.48 ± 1.26 μM). Other 4-trifluoromethylphenyl derivatives were beyond 100 μM. Changing the 4-CF_3 to a methyl group in the **a3** series gave again two compounds with R^2 = 4-MeOPhCH_2 (**a3b4**) and R^2 = " (*R*/*S*)-dioxolane" (**a3b5**) with IC_{50} of 19.49 ± 1.53 and 41.68 ± 6.89 μM respectively, being at the same level as **a2b4** for **a3b4**, and less active for **a3b5** when compared to **a2b5**. The best activity was obtained for **a4b1** (R^1 = 2-naphthyl, R^2 = $PhCH_2$) with an IC_{50} = 3.89 ± 0.31 μM. Replacing R^2 by "(*R*/*S*)-dioxolane" (**a4b5**) and (*S*)-dioxola-ne (**a4b6**) gave a lower IC_{50} of 6.60 ± 5.61 and 12.02 ± 1.5 μM respectively. Finally, when R^1 = 2,3,4-$(MeO)_3$Ph and R^2 = $PhCH_2$ (**a6b1**), an IC_{50} of 27.54 ± 2.06 μM was measured.

For the second series of triazoles presented in Scheme 2 having a hydroxymethyl between the triazole and the R^1 group, solubility problems were an issue. Most of the compounds cannot be tested or showed an IC_{50} > 100 μM. Only the compounds **a10b1** (R^1 = 2-naphthyl, R^2 = $PhCH_2$) and **a10b1** (R^1 = 2-naphthyl, R^2 = (*R*/*S*)-dioxolane) gave moderate values of 28.84 ± 1.33 and 26.91 ± 7.00 μM respectively.

Table 1
Synthesis of aryl alkynes **a1**, **a4** and **a6** using Bestmann-Ohira reagent.

N°	R	Yield[a]	N°	R	Yield[a]
a1	4-NO_2Ph	99	**a4**	2-naphthyl	83
a2	4-CF_3Ph	–[b]	**a5**	4-MeOPh	–[b]
a3	4-CH_3Ph	–[b]	**a6**	2,3,4-$(MeO)_3$Ph	45

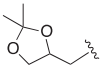
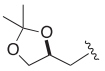
[a] Isolated yield.
[b] Commercial alkyne.

Table 2
Synthesis of arylhydroxymethyl alkynes **a7-12** by reaction of aldehydes with ethynyl Grignard reagent.

N°	R	Yield[a]	N°	R	Yield[a]
a7	4-NO_2Ph	40	**a10**	2-naphthyl	74
a8	4-CF_3Ph	80	**a11**	4-MeOPh	78
a9	4-CH_3Ph	97	**a12**	2,3,4-$(MeO)_3$Ph	57

[a] Isolated yield.

Table 3
Synthesis of azides **b1-7**.

N°	R	Yield[a]	N°	R	Yield[a]
b1	$PhCH_2$	91	**b4**	4-MeOPhCH_2	81
b2	EtO_2CCH_2	99	**b5**		99
b3	$HO(CH_2)_2$	99	**b6**		99

[a] Isolated yield.

4. Discussion

For the active compounds, while looking at the various substituents, some similarities could be found (Fig. 1).

When the triazole had an N_1-benzyl resulted from **b1** in the presence of a 2-naphthyl on C-4 (**a4b1**) gave the most active compound (3.89 μM). Replacing the C-4 substituent by a 4-nitrophenyl (**a1b1**) or a 2,3,4-trimethoxyphenyl (**a6b1**) progressively reduced the activity to 5.12 and 27.54 μM.

The C-4 2-naphthyl is also found in derivatives in the series of N_1-[2,2-dimethyl-1,3-dioxolan-4-yl)methyl] ("dioxolane")