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Research paper

Synthesis and antitumor activities of 1,2,3-triazines and their benzo- and heterofused derivatives

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ABSTRACT

1,2,3-Triazines are a class of biologically active compounds that exhibit a broad spectrum of activities, including antibacterial, antifungal, antiviral, antiproliferative, analgesic and anti-inflammatory properties. This review, which covers the literature from the end of last century to 2016, treats, through a comprehensive, systematic approach, the 1,2,3-triazine and related benzo- and hetero-fused derivatives possessing antitumor activity. Their efficacy, combined with a simple synthesis confers to these molecules a great potential as scaffold for the development of antitumor compounds.

1. Introduction

Triazines are six membered ring heterocyclic compounds containing three nitrogen atoms.

All three isomers of the triazine nucleus, 1,2,3-triazines **1**, 1,2,4-triazines **2** and 1,3,5-triazines **3** (Fig. 1) are frequently focus of interest in medicinal chemistry because of their broad spectrum of activities: anticancer [1], antimicrobial [2], antiviral [3], analgesic [4], anti-inflammatory [5,6], antimicrobial [7,8], antihistaminic [9], antiangiogenic [10] etc..

This paper deals with the recent development of the benzo- and hetero-fused derivatives of 1,2,3-triazines with antiproliferative activity. In the latest decade some reviews on the chemistry and the biological properties of this class of compounds have been published [11,12,13]. We describe the synthesis, the Structure Activity Relationship (SAR), the antitumor activity and, whenever reported, the possible mechanism of action of the most promising compounds belonging to this class.

Among the three isomers, 1,2,3-triazines, also referred to as *vic*-triazines or *v*-triazines, are the least explored ones so far, probably because the ring system is the least stable of the three. As far as we know no biological data are reported for monocyclic 1,2,3-triazines. Benzo- and hetero-fused 1,2,3-triazine derivatives, instead, have attracted interest due to their potent biological properties which, beside the antitumor one, discussed in detail herein, spans, as already mentioned, from antimicrobial [8,14], to antiviral [3], analgesic [4], antiinflammatory [6], antihistaminic [9], anti-angiogenic [10] and antifungal [15].

2. Benzo[1,2,3]triazines

Ullman et al. synthesized, a series of heterocyclic hetero-methyl ketone derivatives of the 1-naphthyloxyacetyl-Val-Asp backbone **4** (Fig. 2) as caspase inhibitors [16].

Caspases are cysteinyl aspartate-specific proteases involved in the regulation of cytokine maturation, inflammation and apoptosis [17]. Among the benzofused 1,2,3-triazine analogues, the benzotriazinone derivative **4** (Fig. 2) showed the best enzyme activity, although the cellular activity in the Jurkat assay, a Fas-induced model of apoptosis, was low (IC_{50} = 137 μM).

Anderson et al. patented the synthesis and biological activity of 4-arylamino-benzo[*d*][1,2,3]triazine derivatives able to activate the

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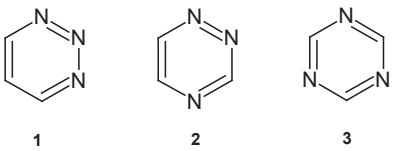

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Fig. 1. Triazine systems.

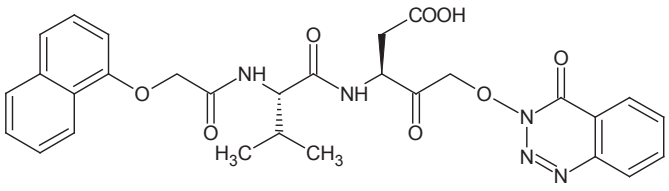

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Fig. 2. Benzotriazinone derivative of 1-naphthyloxyacetyl-Val-Asp backbone **4**.

caspase cascade, in particular caspase-3 activities, inducing apoptosis in cells *in vitro* or *in vivo* in warm-blood animals and able to inhibit tubulin polymerization [18].

In particular, benzo[*d*][1,2,3]triazin-4-yl-(4-methoxy-phenyl)-methyl-amine **7** was identified as potent anticancer compounds and its ability to activate caspase cascade was evaluated on the human breast cancer cell lines T-47D; it showed an EC_{50} of 16 nM and *In vitro* antiproliferative evaluation was performed against the cell lines T-47D, HT-29, H1299, MX-1 and MDAMB435 with IC_{50} in the range 7–50 nM. Derivative **7** was obtained as reported in Scheme 1. Reaction of 3*H*-benzo[*d*][1,2,3]triazin-4-one **5** with phosphorus oxychloride led to 4-chlorobenzo[*d*][1,2,3]triazine **6** which was reacted with *N*-methyl-4-methoxy-aniline, to give the desired compound **7** (Scheme 1) [18].

A series of eighteen 1,2,3-benzotriazines **10a-r**, closely related to **7**, endowed with antiproliferative activity were reported by Lv et al. (Scheme 2) [19]. Cytotoxicity studies of compounds **10a-r** were performed in microvascular endothelial cells (MVECs), they exhibited activity with IC_{50} values in the range between 7.98 and 42.64 μM. Compounds with a methoxy group at C6 position and an alkoxy group at C7 resulted more active compared to the unsubstituted compound **10a**. Within the 6 methoxy derivatives, those having a 3-chloropropoxy group at the C7 position, and derivatives substituted at the phenyl group of C4 anilino moiety showed an increased antiproliferative activity against MVECs with respect to unsubstituted derivative **10r**. The antiproliferative effect of the most active compound, 4-(3-chloro-4-fluoroanilino)-7-(3-chloropropoxy)-6-methoxy-1,2,3-benzotriazine (**10m**), which was 4–10 fold more potent than vatalanib in inhibiting MVECs growth, was further investigated against the breast cancer cell line T47D, the prostate cancer cells DU145 and PC-3, the murine Lewis lung cancer cells LL/2 and the melanoma cell line B16F0. Compound **10m** was able to inhibit cell growth in all the tested cell lines with IC_{50} values ranging from 3.79 to 6.91 μM.

The 7-alkoxy-6-methoxy-4-substituted-1,2,3-benzotriazines **10a-r** were obtained by the cyclization of triazenes **8a-r** in 70% ethanol followed by a Dimroth rearrangement of the intermediates **9a-r** in refluxing acetic acid (Scheme 2).

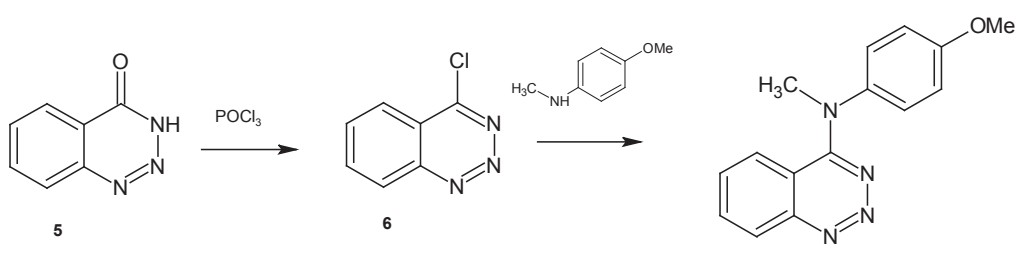
The intermediates **8a-r** were prepared from reaction of 4-cyano-2-methoxyphenol with various alkyl halides in presence of potassium carbonate in DMF; the 4-alkoxy substituted-3-methoxybenzonitrile derivatives thus obtained, were nitrated, reduced to the corresponding amines with Pd/C, diazotized and coupled with the proper anilines.

With the aim of further explore the SAR of this class of compounds and to improve the anticancer properties of derivative **10m** the same authors synthesized a new series of 6-methoxy-1,2,3-benzotriazines, **11a-z** (Fig. 3), following the same synthetic pathway described above (Scheme 2) [20].

Compounds **11a-z** were tested for their antiproliferative activity on MVECs and for their abilities of inhibiting VEGFR-2 (vascular endothelial growth factor receptor-2) kinase activity. VEGF signaling pathway is an interesting therapeutic target in the inhibition of angiogenesis and for the treatment of several types of cancer since its role in both pathological and physiological angiogenesis by binding its receptor VEGFR is well known [21,22].

The inhibition rates of compounds **11a-z** against VEGFR-2 were evaluated at the concentration of 10 μg/ml and they ranged from <10% (**11j,l,p,q,y**) to 49.20% (**11d**). Replacement of the chloropropoxy group at C7 position of **10m** with an ethoxy group (**11a-i**) increased the ability of inhibiting VEGFR-2 activity (24.66%–49.20%), with respect to the lead compound 4-(3-chloro-4-fluoroanilino)-7-(3-chloropropoxy)-6-methoxy-1,2,3-benzotriazine (**10m**), which showed an inhibition rate at 10 μg/ml lower than 10%. Whereas, replacement of the groups 3-chloro-4-fluoro of **10m** with a trifluoromethyl or methyl group at the C4 of the anilino group (**11y, 11z**) had no effects on the inhibitory activity. However these two compounds were less active than **10m** in inhibiting MVECs growth. The most potent antiproliferative compounds were **11k,n,p,z** with IC_{50} values in the range 15.5–17 μM, whilst **10m** showed an IC_{50} of 7.98 μM.

A docking analysis was employed to compare the binding modes of derivatives **10m** and **11d** with VEGFR-2 (PDB code 1YWN) [23]. The anilino group at position C4 occupies the hydrophobic pocket formed by Lys 866, Val 897, Leu 887, Val 912, Val 914, Cys 1043 and Phe 1045 residues while the 1,2,3-benzotriazine moiety made hydrophobic contacts with the aminoacids Leu 838, Gly 920 and Leu 1033. None of the two compounds formed hydrogen bonds with Glu 915 and Cys 917 that are residues of the hinge region in the ATP active site of VEGFR-2, this might explain the weak activity of the two compounds against the enzyme (Fig. 4).



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Scheme 1. Synthetic route to the target compound **7**.

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