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Research paper

Combinatorial synthesis, *in silico*, molecular and biochemical studies of tetrazole-derived organic selenides with increased selectivity against hepatocellular carcinoma

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ABSTRACT

Novel tetrazole-based diselenides and selenoquinones were synthesized *via* azido-Ugi and sequential nucleophilic substitution (S_N) strategy. Molecular docking study into mammalian TrxR1 was used to predict the anticancer potential of the newly synthesized compounds. The cytotoxic activity of the compounds was evaluated using hepatocellular carcinoma (HepG2) and breast adenocarcinoma (MCF-7) cancer cells and compared with their cytotoxicity in normal fibroblast (WI-38) cells. The corresponding redox properties of the synthesized compounds were assessed employing 2,2-diphenyl-1-picrylhydrazyl (DPPH), glutathione peroxidase (GPx)-like activity and bleomycin dependent DNA damage. In general, diselenides showed preferential cytotoxicity to HepG2 compared to MCF-7 cells. These compounds exhibited also good GPx catalytic activity compared to ebselen (up to 5 fold). Selenoquinones **18**, **21**, **22** and **23** were selected to monitor the expression levels of caspase-8, Bcl-2 and Ki-67 molecular biomarkers. Interestingly, these compounds downregulated the Bcl-2 and Ki-67 expression levels and activated the expression of caspase-8 in HepG2 cells compared to untreated cells. These results indicate that some of the newly synthesized compounds possess anti-HepG2 activity.

1. Introduction

Diversity-oriented synthesis (DOS) has emerged as a powerful tool in pharmaceutical industry and now it is routinely used, for drug discovery, but mostly for improvement of desired properties using focused libraries [1]. Lead candidates in this context can be identified and optimized *via* screening of skeletally diverse small molecules for specific biological target(s) [2]. Recent years have witnessed the developments of new DOS pathways; however, multicomponent reactions (MCRs) are among the extensively investigated synthetic strategy for the efficient generation of small chemical entities with increased molecular diversity and complexity. Fortunately, isocyanide-based multicomponent reactions (IMCRs), the most common subclass of MCR, offer a straightforward way to introduce structural diversity and molecular complexity in a single step [3]. Amongst all known IMCRs, Passerini and Ugi reactions have emerged as a robust mean in the construction of peptide-like compounds by giving access to depsipeptides [4,5], *N*-methyl peptides [6], and peptoids [7–9], including dimeric ones.

Abbreviations: DOS, diversity-oriented synthesis; OS, oxidative stress; HCC, hepatocellular carcinoma; MCF-7, breast adenocarcinoma; S_N, nucleophilic substitution; GPx, glutathione peroxidase; ROS, reactive oxygen species; RNS, reactive nitrogen species; IMCRs, isocyanide-based multicomponent reactions; DPPH, 2,2-diphenyl-1-picrylhydrazyl.

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Despite the potential of the classical, basic form of Passerini and Ugi products, they are limited by their nature; that is, ester and/amide based structures. These compounds are therefore; prone to metabolism, i.e. *in vivo* they might be cleaved or modified to potentially more or less active or desirable metabolites. The potential of Passerini and Ugi reaction was accordingly extended to attain more constrained scaffolds i.e. heterocyclic moieties. This is where Groebke and azido-Ugi reactions come to play [10]. These reactions give access to bioactive imidazole- and tetrazole-based, constrained motifs [11,12].

Another alternative approach to attain further diversity and complexity is to tag the Ugi reaction with subsequent post-condensation modifications, often *via* attaching orthogonal functional group(s) at the backbone of the initial Ugi product [12–14]. The incorporation of bifunctional building block allows subsequent secondary transformations (*e.g.*, nucleophilic substitution (S_N) reactions, cyclocondensation and cycladdition) and attains the second level of diversity [15]. In view of the former, sequential coupling of Ugi reaction to other reactions have been reported with different post-modification reactions such as Heck, Diels-Alder, Pictet–Spengler, Petasis, Mannich, Wittig and Click reactions for the efficient synthesis of a number of therapeutically important heterocyclic scaffolds with dense structural features and functionalities [12,15–20]. This double-layered approach has gained vast importance, and now it is routinely used for drug discovery.

Recently, we employed a multicomponent strategy (e.g., Passerini, Ugi and Ugi/S_N, Michael reactions) for the synthesis of hybrid structures containing diverse organoselenium libraries coupled to bioactive pharmacophores (e.g., quinones, naphthalene, cyclic imides) or pharmacologically relevant heterocycles (e.g., thiazolidinone, pyrazole and thiazolopyrimidine) [21–31]. Some of these compounds exhibit cytotoxicity at sub-micromolar concentrations against various types of cancer cells, such as hepatocellular carcinoma (HepG2), breast adenocarcinoma (MCF-7), A-498 (human kidney carcinoma) and A-431 (human epidermoid carcinoma) cell lines. It is worthwhile to mention that the toxicity was more pronounced in case of HepG2 and MCF-7 cells. Furthermore, some of these compounds show lower cytotoxicity when tested against normal cells such as HUVEC (human umbilical vein endothelial), WI-38 (human lung fibroblast) and HF (primary human fibroblast) cell lines. The underlying cytotoxicity and selectivity mechanisms are quite interesting since these compounds can either function as antioxidant or pro-oxidants relying on their redox properties and the intracellular redox environment in which they are placed [21,25,27].

In normal cells, organoselenium compounds act as antioxidants and thus protect cells form oxidative damage. On the other hand, these compounds become pro-oxidants in oxidatively stressed cells (e.g., MCF-7 and HepG2) [30,32,33]. This bimodal function proposes organoselenium compounds not only as chemopreventive agents, but also as selective chemotherapeutics [34–36].

Although one can only speculate about the organoselenium possible mode(s) of action, their cytotoxicity has been attributed primarily to caspase 3/7 activation and subsequent induction of apoptosis. Additionally, different phenotypical changes were observed and these included endoplasmic reticulum, actin cytoskeleton and cellular morphology alterations as well as cell cycle arrest and various biochemical changes (e.g., ROS and GSH levels) [21,22,26,27,29]. Interestingly, we found that selenium based quinones were among the most active compounds exhibiting increased anticancer activity [22,29,31].

In continuation of our program directed towards the development of therapeutically promising organoselenium agents, we herein report the development of a facile route towards symmetrical diselenide and selenoquinone based-tetrazoles *via* azido-Ugi and azido-Ugi/S_N methodology. The respective mode-of-action(s) of the newly synthesized compounds are assessed in twofold: a) addressing their corresponding cytotoxicity in cell assays using HepG2, MCF-7 and normal cells (WI-38) as well as estimating their corresponding effect on the expression levels of caspase-8, Bcl-2 and Ki-67 molecular biomarkers; b) exploration of the redox modulation activities of the synthesized compounds employing DPPH, GPx-like activity and bleomycin dependent DNA damage assays. Furthermore, i*n silico* molecular modeling studies, including field alignment and docking studies, will be applied as a preliminary prediction tool to estimate the antioxidant and cytotoxic properties of the compounds.

2. Results and discussions

2.1. Design and synthesis

Until recently, the synthesis of organic selenides was not an easy task and included the use of expensive/toxic starting materials. Recent years have witnessed significant progress in the synthesis of different classes of organoselenium compounds such as selenaheterocyclic, selenocyanates, selenides and diselenides [25,37–42].

As a part of our project aimed towards the development of organoselenium-based chemotherapeutic agents, we herein report the synthesis of tetrazole-based symmetrical diselenide and selenoquinone compounds (**6**–**22**) synthesized *via* azido-Ugi and sequential S_N strategy (Fig. 1).

Based on recent findings reported by our group, we envisioned that tetrazole-based organoselenium scaffolds thus might be more efficient anticancer agents. Tetrazoles have received considerable attention from the pharmaceutical market as they constitute the core scaffold of several bioactive compounds and many marketed drugs (e.g., pentylenetetrazol, cilostazol, ceftezole, irbesartan, losartan) [43]. Furthermore, these compounds are of particular interest because tetrazole is a bioisostere for carboxylic acid group (COOH) but yet with better potency, favorable physicochemical properties, improved pharmacokinetic profiles and metabolic stability. This is mainly due to the tetrazole larger size and superior lipophilicity ($\approx$10 times more) which *in turn* leads to an increase of the substrate receptor interaction [44,45].

There are many methods for the construction of tetrazole ring system; however, the azido-Ugi reaction is superior to classical methods in terms of automation, reaction time and overall yields [46]. In order to guarantee a second level of diversity, 4,4′-diselanediyldianiline (**4**) was used as a bifunctional key synthon as it possesses an amino group convenient for the azido-Ugi reaction and a readily liberated selenolate nucleophile (formed *in situ via* reduction of the diselenide using $NaBH_4$) suitable for subsequent S_N reactions.

With regard to library construction, six structurally diverse isonitriles (e.g., *tert*-butylisocyanide (**2a**), cyclohexyl isocyanide (**2b**), 4-isocyanopermethylbutane-1,1,3-triol (IPB, **2c**) [47], benzylisocyanide **(2d)**, 4-methoxybenzylisocyanide (**2e)** and 2,4-dimethoxybenzylisocyanide (**2f**) were included for library validation purposes, and partly their potential for additional post-Ugi reactions. As oxo component, four different aldehydes were used including both aliphatic (paraformaldehyde (**1a**), isobutyraldehyde (**1b**)) and aromatic (4-methylbenzaldehyde (**1d**) and furfuraldehyde (**1c)**)) ones, whereas trimethylsilyl azide ($TMSN_3$) **3** was used as the acid component (see Fig. 2).

Tetrazole-based symmetric diselenides (**6**–**19**) were synthesized by the addition of two equivalents of aldehyde **1** to a methanolic solution of **4** (one equivalent), followed by the subsequent addition of 2.5 equivalents of $TMSN_3$ and isocyanide **2**. After completion of the reactions, reduction of diselenides (**6**–**19**) with