Journal of Molecular Structure 1156 (2018) 43–50

Contents lists available at ScienceDirect

Journal of Molecular Structure

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

Synthesis, spectroscopic characterization, antimicrobial evaluation and molecular docking study of novel triazine-quinazolinone based hybrids

Mohammad Dinari [a, *], Fateme Gharahi [a], Parvin Asadi [b]

[a] Department of Chemistry, Isfahan University of Technology, Isfahan, 84156-83111, Islamic Republic of Iran
[b] Department of Medicinal Chemistry, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, 81746-73461, Islamic Republic of Iran

ARTICLE INFO

Article history:
Received 15 June 2017
Received in revised form
27 October 2017
Accepted 21 November 2017
Available online 21 November 2017

Keywords:
s-Triazine
Antimicrobial agents
Docking simulation
Quinazolinone
Hybrids

ABSTRACT

A new series of 1,3,5-triazine incorporating aromatic quinazolinone moieties as a potential antimicrobial agents is reported. The first chlorine group of the cyanuric chloride (**1**) was replaced by aniline and the second one was replaced by various aromatic amines. The prepared monochlorotriazine was allowed to react with hydrazine and subsequently it was reacted with 2-methyl-4H-benzo[1,3]oxazin-4-one to obtain novel triazine-quinazolinone based hybrids (**9a-f**). The chemical structure and purity of the hybrid compounds were evaluated by different techniques such as thin layer chromatography, melting point, Fourier-transform infrared (FTIR), ^{1}H and ^{13}C NMR spectra and elemental analysis. Antimicrobial activity of the hybrid compounds were study by three Gram-negative bacteria (*Salmonella entritidis*, *Escherichia coli*, *Pseudomonas aeruginosa*) and three Gram-positive bacteria (*Staphylococcus aureus*, Listeria monocitogenes, Bacillus subtilis) as well as Candida albicansas a yeast-like fungus using the serial broth dilution method. Among them, compound **9d** with benzenesulfonamide group showed higher antimicrobial activity with a minimum inhibitory concentration (MIC) value of 16 μg/mL. Furthermore, compounds **5d**, **9a** and **9b** showed good activity against several tested strains. In addition, docking simulation was perform to position best antibacterial compounds in to the *S. aureus* dihydrofolate reductase (DHFR) active site to determine the probable binding conformations.

1. Introduction

Heterocyclic compounds have a critical role in synthetic drugs and biological processes. The tri-substituted 1,3,5-triazine heterocyclic analogs is a very important pharmacophore due to its presence in an enormous amount of bioactive compounds [1]. Biologically active tri-substituted triazines showed different potential such as anti malarial [2,3], antibacterial [4–6], anticancer [7,8], antitubercular [9] and etc. Previous studies show that s-triazine core molecules display excellent antimicrobial activity in terms of antibacterial and antifungal [10].

On the other hand, quinazolinone scaffold and their derivatives constitute an important class of biologically active compound. Various classes of quinazolinones with different structures have been already achieved significant attention because of their antimicrobial [11,12], antiviral [13], antitubercular [14,15], anti-inflammatory [16], and anticancer activities [17,18]. Study on structure activity relationship of quinazolinone derivatives have showed that substitution at positions 2 and 3 of the quinazolinone ring can improve their antimicrobial activities [19]. In addition, the presence of substituted aromatic ring at position 3 and amine, methyl or thiol groups at position 2 are helpful for antimicrobial activities [20–22].

Joining two or more biologically active pharmacophores in a single molecular framework might result in pharmaceutically important hybrid molecules which could address the active site of different targets and/or offer the possibility of overcoming drug resistance or reducing the unwanted side effects [23]. Recently, novel hybrid compounds based on heterocyclic pharmacophores have been synthesized and also proved to show different biological activities [24,25]. Previously, a series of hybrid quinazoline–-triazine derivatives were designed and synthesized from cyanuric

* Corresponding author.
E-mail addresses: dinari@cc.iut.ac.ir, mdinary@gmail.com (M. Dinari).

https://doi.org/10.1016/j.molstruc.2017.11.087
0022-2860/

chloride and anthranilic acid through sequential reactions, which contain different pharmacophores like quinazoline and substituted diaryltriazine linked with ethylene diamine [26]. *In vitro* antimicrobial activity was evaluated against four bacteria (*Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa, Klebsiella pneumoniae*) and two fungi (*Aspergillus clavatus, Candida albicans*). From the bioassay results, it can be stated that all derivatives showed appreciable antimicrobial activities. Generally the incorporation of quinazoline moiety linked to triazine via ethylene diamine is beneficial to antimicrobial activity [26]. Another s-triazine based hybrid compounds have also been proved to show inhibitory activity on Dihydrofolate Reductase (DHFR) metabolism [27]. This enzyme has profound impact on the biosynthesis of biomolecules that are essential for cell proliferation. It catalyzes the reduction of dihydrofolate (DHF) to tetrahydrofolates (THF), using nicotinamide adenine dinucleotide phosphate (NADPH) as a cofactor [28]. Thus, the inhibition of DHFR can result in the depletion of the intracellular THF pool and prevents biosynthesis of thymidine, DNA, RNA and protein. Fortunately, differ structurally DHFR from species to species have allowed the expansion of very DHFR inhibitors for some bacteria and some parasitic [29].

Considering the antibacterial activities of *s*-triazine and quinazolinone derivatives, in this study we have combined these two heterocyclic moieties in a novel chemical framework. The resulting hybrid compounds were characterized by different techniques and their antimicrobial activities against three *Gram-positive bacteria*, three *Gram-negative bacteria* and also one *yeast-like fungi* was investigated. To achieve insight of necessary key structural requirements for antimicrobial activity, molecular docking study was consummated.

2. Experimental

2.1. Materials

All chemicals and solvents used in this work were purchased commercially from the Merck Chemical Co. and Aldrich Chemical Co. 2-Aminobenzoic acid or anthranilic acid, cyanuric chloride, 4-chlorobenzenamine, 4-aminobenzonitrile, 4-methoxybenzenamine, sulfanilamide, aniline, 4-nitrobenzenamine and hydrazine hydroxide ($NH_2NH_2 \cdot H_2O$) were used without any extra purification.

2.2. Characterization techniques

A JASCO 680 (Japan) Fourier transform infrared (FT-IR) spectrophotometer was used to study the chemical structure of the materials. Proton nuclear magnetic resonance (^{1}H NMR; 500 MHz) and carbon nuclear magnetic resonance (^{13}C NMR; 125 MHz) spectra were recorded in DMSO-d_6 on a BrukerAvance spectrometer. The chemical shifts are reported in the δ scale in ppm. Electrothermal 9200 melting point apparatus was used to determine the melting points (m.p.) of the compounds. Pre-coated silica gel 60 F254 aluminum plates (Merck, Germany) was performed on analytical thin-layer chromatography.

2.3. Synthesis of 4,6-dichloro-1,3,5-triazin-2-ylphenylamine (**2**)

The first chlorine group of the cyanuric chloride (**1**) was replaced by aniline according to the reported producer [30,31]. In brief, to a solution of cyanuric chloride (9.2 g, 0.05 mol) in 150 mL of acetic acid, 4.65 g (0.05 mol) of aniline was added slowly and 7.2 mL (0.05 mol) of triethylamine (Et_3N) was added to the mixture at 5 °C. Then, it was stirred for 1 h and diluted with 500 mL of brine. The solid product was filtered, and recrystallized from 400 mL of n-heptane. Yield: 67%; m.p. = 136–137 °C. FT-IR (KBr, cm^{-1}) ν: 3363 (NH), 1563 (C=C), 1552 (C=N).

2.4. General procedure for the synthesis of compounds ***4a-f***

The second chlorine group of the cyanuric chloride was replaced by different aromatic amine such as 4-chlorobenzenamine (**3a**), 4-aminobenzonitrile (**3b**), 4-methoxybenzenamine (**c**), sulfanilamide (**3d**), aniline (**3e**), and 4-nitrobenzenamine (**3f**) to formed compounds **4a-f**. First, 5.5 mmol of compounds **3a-f** were added to a stirred solution of compound **2** in the mixture of 50 mL of AcOH and 15 mL of H_2O in the presence of sodium acetate (0.81 g; 6 mmol). Then, the reaction mixture was stirred until the whole solids were dissolved and it was contentious for 12 h. The progress of the reaction was monitored by TLC. Upon completion, the solid product were obtained by filtration, washed with boiling water and recrystallized from isopropanol.

*6-Chloro-N2-(4-chlorophenyl)-N4-phenyl-1,3,5-triazine-2,4-diamine (**4a**):* Yield: 82%, white solid, m.p. = 210 °C; IR (KBr, cm^{-1}) ν: 3397, 3259 (NH), 3179 (Ar–H), 1610 (C=C), 1570 (C=N); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.1–10.4 (br, 2H, NH), 7.66 (m, 4H, Ar–CH), 7.37 (m, 4H, Ar–CH) and 6.9 (m, 1H, Ar–CH).

*4-((4-Chloro-6-(phenylamino)-1,3,5-triazin-2-yl)amino)benzonitrile (**4b**):* Yield: 83%, white solid, m.p. = 217 °C; IR (KBr, cm^{-1}) ν: 3329 (NH), 3065 (Ar–H), 2218 (CN), 1615 (C=C), and 1571 (C=N).

*6-Chloro-N2-(4-methoxyphenyl)-N4-phenyl-1,3,5-triazine-2,4-diamine (**4c**):* Yield: 91%, white solid, m.p. = 167 °C; IR (KBr, cm^{-1}) ν: 3370, 3262 (NH), 3113 (Ar–H), 2839 (CH_3), 1612 (C=C), 1577 (C=N) and 1178 (C–O).

*4-((4-Chloro-6-(phenylamino)-1,3,5-triazin-2-yl)amino)benzenesulfonamide (**4d**):* Yield: 92%, white solid, m.p. = 268 °C; IR (KBr, cm^{-1}) ν: 3398, 3209 (NH), 3358–3282 (NH_2), 3060 (Ar–H), 1612 (C=C), 1571 (C=N), and 1326, 1152 (SO_2).

*6-Chloro-N2,N4-diphenyl-1,3,5-triazine-2,4-diamine (**4e**):* Yield: 90%, white solid, m.p. = 200 °C; IR (KBr, cm^{-1}) ν: 3267 (NH), 3107 (Ar–H), 1615 (C=C), and 1574 (C=N).

*6-Chloro-N2-(4-nitrophenyl)-N4-phenyl-1,3,5-triazine-2,4-diamine (**4f**):* Yield: 79%, yellow solid, mp. = 221 °C; IR (KBr, cm^{-1}) ν: 3374–3338 (NH), 3065, 3159 (Ar–H), 1619 (C=C), 1588 (C=N), and 1533, 1331 (NO_2).

2.5. General procedure for the synthesis of compounds ***5a-f***

The last chlorine group of the **1** was replaced by hydrazine molecule. In this regards, a suspension of compounds **4a-f** in hydrazine hydrate was refluxed for 10–12 h. The excess hydrazine hydrate was evaporated under vacuum and the residue was washed with water to give compounds **5a-f**.

*N2-(4-Chlorophenyl)-6-hydrazinyl-N4-phenyl-1,3,5-triazine-2,4-diamine (**5a**):* Yield: 87%, white solid, m.p. = 130 °C; FT-IR (KBr, cm^{-1}) ν: 3038–3400 (NH, NH_2, CH Aromatic); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 9.0–9.55 (br, 2H, NH), 8.2 (s, 1H, NH), 7.7 (m, 4H, Ar–H), 7.24 (m, 4H, Ar–H), 6.9 (m, 1H, Ar–CH), 4.25–4.7 (s, 2H, NH_2); Anal. Calcd. For $C_{15}H_{14}ClN_7$: C, 54.97; H, 4.31; N, 29.91. Found: C, 54.28; H, 4.20; N, 29.47.

*4-((4-Hydrazinyl-6-(phenylamino)-1,3,5-triazin-2-yl)amino)benzonitrile (**5b**):* Yield: 83%, orange solid, m.p. = 102 °C; FT-IR (KBr, cm^{-1}) ν: 3050–3390 (NH, NH_2, CH Aromatic), 2214 (CN); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 8.9–9.5 (br, 2H, NH), 8.3 (s, 1H, NH), 7.3–7.9 (m, 8H, Ar–H), 6.97 (m, 1H, Ar–CH), 4.1–4.5 (s, 2H, NH_2); Anal. Calcd. For $C_{16}H_{14}N_8$: C, 60.37; H, 4.43; N, 35.20. Found: C, 59.71; H, 4.69; N, 35.07.

*6-Hydrazinyl-N2-(4-methoxyphenyl)-N4-phenyl-1,3,5-triazine-2,4-diamine (**5c**):* Yield: 89%, white solid, m.p. = 126 °C; FT-IR (KBr, cm^{-1}) ν: 3034–3480 (NH, NH_2, CH-Aromatic), 2830 (CH-Aliphatic), 1073 (O–CH_3); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 8.7–9.35 (br, 2H,