Journal of Molecular Structure 1139 (2017) 371–380

Contents lists available at ScienceDirect

Journal of Molecular Structure

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

Spectroscopic, molecular docking and structural activity studies of (*E*)-*N*′-(substituted benzylidene/methylene) isonicotinohydrazide derivatives for DNA binding and their biological screening

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ARTICLE INFO

Article history:
Received 10 January 2017
Received in revised form
9 March 2017
Accepted 14 March 2017

Keywords:
Isonicotinohydrazides
UV spectroscopy
Molecular docking
Binding mode
Binding constant
Antitumor activity

ABSTRACT

Acid catalyzed condensation of isoniazid with a number of suitably substituted aromatic and heterocyclic aldehydes was carried out in dry ethanol to afford the title (*E*)-*N*′-(substituted benzylidene/methylene) isonicotinohydrazides (SF 1 – SF 4) in good yields. These compounds were characterized and further investigated for their binding with ds.DNA using UV– spectroscopy and molecular docking and for antitumor and antimicrobial potentials. A good correlation was found among spectroscopic, theoretical and biological results. UV– spectra in the presence of DNA concentrations and their data interpretation in terms binding constant "K_b" and free energy change (ΔG) provided evidences for the significant and spontaneous binding of the compounds with DNA. Molecular docking studies and structural analysis further supported the UV-findings and indicated that the modes of interactions between bromo- (SF 1) and flouro- (SF 4) substituted isonicotinohydrazides is intercalation while methoxy- (SF 2) and hydroxy- (SF 3) substituted isonicotinohydrazides interact with DNA helix via groove binding. SF 1 exhibited comparatively higher K_b value (UV–; 8.07×10^3 M^{-1}, docking; 8.11×10^3 M^{-1}) which inferred that the respective compound muddles to DNA most powerfully. SF 1 has shown the lowest IC_{50} (345.3 μg/mL) value among all the compounds indicating its comparatively highest activity towards tumor inhibition. None of the compound has shown perceptible antibacterial and antifungal activities.

1. Introduction

Hydrazide, an effective class of organic compounds, is known for therapeutic and biological activities [1,2]. Most of the hydrazides have been reported for their anti-fungal, anti-bacterial and anti-inflammatory activities [1–4]. Isoniazid (isonicotinic hydrazide; INH) is one of the primary drugs used in combination with ethanbutol, rifampin, streptomycin and pyrazinamide to treat tuberculosis [5]. Despite the large number of compounds containing the isoniazid moiety which have already been synthesized and tested, there is still a need for new compounds of this kind, due to the increasing resistance of bacterial strains of certain type of antibiotics [6].

The remarkable biological activity of Schiff bases, or the aroyl hydrazones, R–CO–NH–N=CH-R′ and the dependence of their mode of chelation with transition metal ions present in the living system have been of significant interest in the past [7–10]. Isoniazid was treated with different substituted aromatic aldehydes to produce Schiff bases [11]. The coordination compounds of aroyl hydrazones have been reported to act as enzyme inhibitors [12] and are useful due to their pharmacological applications [13,14]. Isoniazid is a drug of proven therapeutic importance and is used against a wide spectrum of bacterial ailments, e.g., tuberculosis [15]. Hydrazones derived from condensation of isoniazid with pyridine aldehydes have been found to show better antitubercular activity than INH [16].

Unfortunately, the actual formulations show several undesired collateral effects and can even cause irreversible damage in the liver in chronic patients. This fact, together with the resistance that microorganisms develop against these drugs, encouraged the search for

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http://dx.doi.org/10.1016/j.molstruc.2017.03.055
0022-2860/

new compounds with therapeutic effects [17,18]. The combination of INH with some hydroxy aldehydes leads to the formation of stable hydrazones that show conserved activity and less toxicity, due to the inactivation of the NH_2 group of INH [13]. In particular, a group of hydrazones has been reported as more effective and efficient anti-tuberculous agents in macrophages than INH itself [14].

Cancer – a leading cause of premature death in the world – can be cured by preventing the rapid proliferation of cancer cells for which the replication of DNA is to be arrested. Antiviral, anticancer, antitumor and antibiotic drugs can easily target nucleic acids [19–21]. Drugs can bind to DNA both covalently (irreversible) as well as non-covalently (reversible via intercalation, grove binding or electrostatic interactions).

Various platinum(II)-based complexes of like cisplatin, oxalylplatin, nedaplatin and carboplatin has achieved clinical status as well known antitumor agents. These drugs targeted DNA primarily via covalent linkage with the nitrogen atoms on DNA bases, mainly N^7 of guanine [22]. However, despite of their high activity towards killing cancer cells, applications are limited by serious disadvantages like poor water solubility, tolerance by the tumor and irreversible covalent binding which may further lead to toxic side effects like alopecia (hair falls out), kidney failure, allergies, hearing loss etc.

Non-covalent binding is reversible and is typically preferred over covalent adduct formation. Since irreversible binders like cisplatin bind quite strongly to the damaged DNA, it has been difficult to achieve similar affinity using small non-covalent binders, and remains a major challenge among researchers to design drugs that bind reversibly with the DNA. Although lot of research has been carried out, but there is still a quest to gain more insight on different aspects of the association of small molecules with DNA in order to obtain highly selective and efficient drug candidates. A verity of intercalators and groove binders are known for their anticancer, antiviral, antitumor, antibacterial and antifungal activities [23]. Kinetic and thermodynamic studies on compound – DNA interaction by using spectroscopic, electrochemical and variety of other techniques have initially provided important physical parameters that may further lead to investigate a compound as a potential drug candidate [1,24–27]. Modes of interactions of a compound with DNA can also be predicted by molecular docking simulation and implication of this theoretical technique along with the assistance of experimental techniques is very helpful for rational drug design [28].

Antimitotic activity of potato disc tissue, in which inhibition of *Agrobacterium tumefaciens*– induced tumors is monitored, can be used to detect antitumor effects in broader range [29]. Potato disc antitumor assay is based on assumption that similar tumorogenic mechanisms occur in both animals and plants [30]. Antitumor screening assays in animals and potato disc antitumor assay have been reported for their good correlation [29]. Antitumor activity of several compounds has been investigated for *A – tumefaciens* induced tumors in potato discs [1,24,27,31].

The enormous therapeutic properties of hydrazides is a key motivation in this studies to synthesize stable and less toxic compounds of INH with suitably substituted aromatic aldehydes and to investigate their interactions with DNA by using spectroscopic and molecular docking techniques and with *A – tumefaciens* induced tumor in potato disc.

2. Experimental

2.1. Materials and methods

All the chemical and reagents used in synthesis, DNA binding procedures and biological assays were of analytical grade. Monitoring of the synthetic reactions was carried out by the thin-layer chromatography (TLC) using silica gel (aluminum card, layer thickness 0.2 mm, HF-254, Riedal-de-Haen) precoated plates and was visualized under UV-lamp. All the necessary purification and drying of solvents were carried out according to standard methods [32]. The dried solvents were stored over molecular sieves. Falcon method was adopted as a protocol to extract double strand (ds.) DNA from chicken blood [33]. All the glassware, Falcon tubes and water used in extraction procedure were autoclaved.

DNA threads were dissolved in water and concentration of stock DNA solution (phosphate groups' molarity) was determined through UV–visible spectroscopy. Absorbance is measured at λ_{max} of 260 nm. Stock DNA concentration was obtained by substituting molar extinction coefficient, $\varepsilon_{260} = 6600\ cm^{-1}M^{-1}$ and absorbance at maximum wavelength in Lambert-Beer's equation ($A = \varepsilon Cl$) [33,34]. DNA absorbance was measured at another wavelength (280 nm) and absorbance ratio A_{260}/A_{280} was evaluated. The value was found greater than 1.8 which assured that the extracted DNA is sufficiently pure and no ambiguity is there for the presence of protein [35]. The stock solutions of synthesized compounds were prepared by dissolving them in 10% aqueous DMSO. For DNA binding studies, compound's concentration was optimized and kept constant while adding various diluted concentrations of DNA at 37 °C. *Agrobacterium tumefaciens* strain (AT-10) and red skinned potatoes were used in antitumor assay, while four bacterial ((ATCC 6538, ATCC 10240, ATCC 15224, ATCC 14028) and four fungal strains (FCBP 0300, FCBP 0198, FCBP 66, FCBP 0291) were used in antimicrobial assays.

2.2. Instrumentations

Melting points were recorded using a digital Gallenkamp (SANYO) model MPD.BM 3.5 apparatus and are uncorrected. ^1H NMR and ^{13}C NMR spectra were determined at 300 MHz using a Bruker AM-300 spectrophotometer in acetone d_6. FTIR spectra were recorded on Bio-Rad-Excalibur Series Mode FTS 3000 MX spectrophotometer (USA) in range of 4000–400 cm^{-1}. Mass Spectra (EI, 70eV) on a GC-MS, Agilent technologies 6890 N and an inert mass selective detector 5973 mass spectrometer technologies. Thin layer chromatography (TLC) was conducted on 0.25 mm silica gel plates (60 F254, Merck). Visualization of chromatograms was made with UV at 365 and 254 nm.

Shimadzu1800 spectrophotometer (TCC-240A, Japan)) armed with temperature control device was used to record the electronic absorption spectra using 1.0 cm matched quartz cells. Hettich EBA20 Portable Centrifuge C 2002 (Max. speed: 6000 min-1) and vortex machine were used for the extraction of DNA from chicken blood. MOE-dock by Chemical Computing Group Inc was used for molecular docking simulation. Molecular modeling studies were performed on Pentium1.6 GHz workstation, 512 MB memory with the Windows Operating System that applies a two stage scoring process to sort out the best conformations and orientations of the ligand based on its interaction pattern with the DNA.

2.3. Synthesis of N′-(substituted benzylidene/methylene) isonicotinohydrazides

Isoniazid (0.5 g, 3.65 mmol) was dissolved in 15 mL of absolute alcohol. The suitable aldehyde (3.70 mmol) was added dropwise with constant stirring in presence of catalytic amount of acetic acid. The reaction mixture was refluxed for 4–6 h and the completion of reaction was monitored by TLC (Petroleum ether: ethyl acetate 4:1). The reaction mixture was cooled and the resulting solid was filtered washed with cold ethanol and finally recrystallized from absolute ethanol.