Synthesis, Biological Evaluation, Docking and QSAR Studies of Some Novel Naphthalimide Dithiocarbamate Analogs as Antitumor and Anti-Inflammatory Agents

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Abstract

A series of novel naphthalimide dithiocarbamate 4a-f, 5a-f were efficiently synthesized via introduce dithiocarbamate and dithioate side chain onto the naphthalic anhydride core. The structures of the synthesized analogs were elucidated by spectroscopic methods, including IR, 1H and 13C NMR, and (ESIHRMS) techniques. The anti-cancer activities of the generated naphthalimide derivatives 4c, 4d, 4e, 4f, and 5d were evaluated against 21 tumour cell lines; including 10 tumor subpanels using MTT assay. Analogue 4c offer antitumor activity with an IC50 of 10.54 µM against SKBR3 breast cancer cells. Compound 4d showed varying degrees of antitumor activities towards several tumour cell lines ranging from 21.1 to 71.7 µM. In addition to the antitumor activities; the synthesized compounds were evaluated for their in vitro anti-inflammatory activity. Compounds 4c and 4d revealed potent anti-inflammatory properties in comparison with the reference drug celecoxib. Molecular docking studies provided complementary theoretical support for experimental biological data.

Keywords: Naphthalimide; Dithiocarbamates; Dithioates; Anticancer; Anti-inflammatory

Introduction

The development of new classes of chemotherapeutic agents to treat cancer patients remains an important goal of the medical community worldwide. The International Agency for Research on Cancer reported that the global cancer burden rose to 14.1 million new cases with 8.2 million cancer deaths in 2012, and the annual incidence of new cancer cases is estimated to increase to 19.3 million by 2050 [1]. During the past few years, cyclooxygenases (COX-1 and COX-2) were introduced as novel targets for cancer treatment [2], COX-2 is an effective approach for the prevention or treatment of various types of cancers such as colon, prostate, and breast [3]. Anticancer drugs that target DNA are one of the most effective agents for cancer therapy [4]. DNA intercalating agents are characterized by the presence of a tricyclic or tetracyclic annealed, planar, aromatic ring that intercalates between nucleic acid bases and one or two flexible amino side chains that enhance DNA binding affinity via electrostatic or hydrophobic interactions [5]. Among the agents directly intercalating with DNA; naphthalimides which belong to the cyclic imide class represent an important moiety in the antitumor drug design concept [6]. Several biological effects suggest a potential pharmacological use of cyclic imides, such as anti-inflammatory [7], anti-inflammatory [8], antimicrobial [9], and potential antitumor agents against different cancer cell lines [10,11]. Historically naphthalimide [1H-benz [de] isoquinoline-1, 3-(2H)-diones] is considered as one of the simplest poly cyclic amides from a famous class of intercalating agents consisting of a flat, generally π -deficient aromatic or heteroaromatic system which bind to DNA by insertion between base pairs of the double helix [12-14]. The cytotoxic properties of several N-substituted naphthalimides are well documented to possess significant anticanancer activity [15,16]. Naphthalimide derivatives exhibit their cytostatic activity through DNA intercalation, which causes enzymatic blockade and reading errors during the replication process, inhibiting both RNA and DNA synthesis and generating a multitude of reactive intermediates that result in DNA photocleavage [17,18]. The role of the sulfur atom in improving antitumor activities has been reported. Previous studies showed that especially sulfur-containing derivatives exhibited promising anti-proliferative efficiency towards several tumor cell lines [19,20]. Dithiocarbamates have been attracting considerable interest because of their diverse activities [21-24] and in the treatment of cancer [25,26]. Our group recently reported the synthesis of a novel thalidomide-containing dithiocarbamate moiety; several derivatives exhibited potent anti-cancer activity [27,28]. Based on the research for potential antitumor agents, the introduction of alkylamino groups into some pharmaceophores can enhance their antitumor activity [29]. In view of this data; this study was carried out to discover novel antitumor agents based on naphthalimide skeleton.

Here we designed, synthesized and characterized a series of novel naphthalimide analogs by conjugating various dithiocarbamate and dithioate moieties to DNA- intercalating naphthalimide ring system (Figure 1). We subsequently evaluated their antitumor activities; fluorescence quenching assays were employed to determine their DNA-binding activity in addition to anti-inflammatory properties.

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Experimental Section

Chemistry

All chemicals and reagents are commercially available and were used directly without further purification. The progress of the synthesis reactions were monitored using analytical gel silica gel TLC plates 60 F254 (Merck) and visualized using UV light. The 1H and 13C NMR spectra associated with the synthesized compounds were determined using a Bruker (spectrometer and 101 MHz for 1H) at 400 MHz and 101 MHz, respectively. TMS was used as an internal standard for 1H NMR. To evaluate ion mass, the [M]+ or [M]+ ion was calibrated with NaI (University of Southern Denmark, Denmark). Infrared (IR) spectra (KBr) were recorded using a Pye-Unicam Sp-883 Perkins-Elmer spectrometer (Micro-analytical Laboratory, Faculty of Science, Cairo University). Melting points were determined with an Electro thermal melting point apparatus, and are uncorrected.

Preparation of naphthalimide dithiocarbamates and dithioate derivatives (4a-f and 5a-f)

Appropriate amine (1 mmol) use in this study was added to an ethanolic solution of KOH (1 mmol/10 mL). The mixture was cooled in ice bath and CS2 (10 mmol) was added drop wise with stirring. Further agitation of the reaction mixture thus obtained for 1 h at room temperature then, the solvent was evaporated under reduced pressure, followed by consequent addition of dry ether until precipitation reached completion. Then the precipitate was filtered, washed with dry ether and dried to afford dithiocarbamate and dithioate derivatives with high purity. Then, a solution of the appropriate dithiocarbamates and dithioates (2 mmol) in DMPF (5 mL) was added to compounds 4 and 5 (1 mmol). The mixture was stirred and heated at 70–80°C for 2 h. After cooling the solution was poured into cooled distilled water (50 mL). The precipitate was collected by filtration, washed with distilled water and dried. The crude product was purified by crystallization from ethanol (30 mL). The structures of amines applied in this reaction protocol and the newly synthesized compounds are given in Table 1 and see Supplementary File.

2-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)ethyl methycarbamodithioate (4a): Yellow solid; mp: 190–192°C, Yield 76%. IR (KBr) 3265, 2958, 2936, 1696, 1650, 1587, 1338, 1233 cm⁻¹. 1H NMR (DMSO-d6) δ 2.97 (d, 3H, CH3), 3.55 (t, 2H, NCH2CH3), 4.34 (t, 2H, NCH2CH3), 7.87 (t, 2H, CH=), 8.44–8.51 (m, 4H, H), 9.91 (br s, 1H, NH). 13C NMR (DMSO-d6) δ 32.17 (CH3), 33.78 (NCH2CH3), 38.74 (NCH2CH3), 122.06 (C6), 123.35 (C5), 124.77 (C4), 130.96 (C3), 131.39 (C13), 134.52 (C9), 163.52 (2 × C = O), 194.82 (C = S). HRMS (ESI) m/z Calc for C18H14N2O2SNa+ [M + Na]+: 353.0839. Found: 353.0401.

2-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)ethyl dimethycarbamodithioate (4b): Pale yellow solid; mp: 189–190°C, Yield 69%. IR (KBr) 2966, 2920, 1698, 1655, 1587, 1378, 1251 cm⁻¹. 1H NMR (DMSO-d6) δ 3.31 (s, 3H, CH3), 3.42 (s, 3H, CH3), 3.60 (t, 2H, NCH2CH3), 4.38 (t, 2H, NCH2CH3), 7.88 (t, 2H, CH=), 8.45–8.51 (m, 4H, H), 1.3C NMR (DMSO-d6) δ 34.48 (NCH2CH3), 38.43 (NCH2CH3), 41.37, 44.96 (2 × CH2), 122.02 (C6), 127.23 (C4), 127.43 (C3), 130.79 (C5), 131.29 (C13), 134.37 (C7), 163.39 (2 × C = O), 194.57 (C = S). HRMS (ESI) m/z Calc for C18H14N2O2SNa+ [M + Na]+: 367.0545. Found: 367.0530.


Figure 1: The design strategy of naphthalimide-dithiocarbamates.
Table 1: Appropriate amine and corresponding naphthalimide dithiocarbamate analogs.
3-[(1,3-dioxo-1H-benzo[d]isoquinolin-2(3H)-yl)propylidene]carbamothioate (5b): Yellow crystal; mp. 173–174°C. Yield 49%. IR (KBr) 2929, 1697, 1656, 1590, 1339, 1232 cm⁻¹. H NMR (DMSO-d₆) δ 2.00 (2H, NCH₂CH₂S), 3.28 (2H, NCH₂CH₂S), 3.30 (s, 3H, CH₃), 4.13 (t, 2H, NCH₂CH₂S), 7.86 (2H, H-3), 8.42–8.48 (m, 4H, H-2). ¹³C NMR (DMSO-d₆) δ 27.49 (NCH₂CH₂S), 34.22 (NCH₂CH₂S), 38.76 (NCH₂CH₂S), 41.42, 43.88 (2 × CH₂), 120.99 (C₆), 127.56 (C₆), 127.38 (C₆), 130.66 (C₈), 131.24 (C₂), 132.30 (C₃), 163.53 (2 × C = O), 195.20 (C = S). HRMS (ESI) m/z Calcd for C₁₈H₁₂N₂O₄SNa [M + Na]^⁺: 367.0545. Found: 367.0529.

The cytotoxic activity of the naphthalimide analogs (4c, 4d, 4e, 4f, and 5f) was evaluated using a 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) (Sigma–Aldrich, St. Louis, MO, USA) cleavage assay; against human breast cancer (MCF-7, T-47D, SKBR3, BT474, MBB31 and BT474), pancreas (KLM-1, PK8 and PK39) hepatocellular (HepG2, Huh-7 and PLC/PRF/5), glioma (A127 and U251-MG), lung (L929), colon carcinoma (HT-29 and CW-2), spleen (PMF-k014), lymphocyte (MY), Ovarian (SKOV3 and OVK16), and T-cell leukemia (MOLT-4). The compounds that exhibited bad solubility or precipitated in cell culture media were omitted from biological evaluation experiments. The cells were seeded in 96-well plates (5 × 10⁴ cells/well) in DMEM or RPMI supplemented with 10% FBS. After 24 h in culture, the analogs were added to the cells at concentrations ranging from 6.25 to 100 μM for 72 h. The cells were then exposed to MTT (5 mg/mL in PBS) at a final concentration of 1 mg/mL for 4 h. Formazan crystals that had formed during the incubation period were dissolved overnight at 37°C by adding 10% SDS supplemented with 0.02 N HCl. The absorbance at 570 nm was subsequently measured. The experiments were performed in triplicate; and the results were reported in Table 2.

Cycloxygenase inhibition assay

The ability of the test compounds to inhibit ovine COX-1 and COX-2 was determined using an enzyme immunooassay (ELISA) (kit catalog number 560101, Cayman Chemical, Ann Arbor, MI, USA), according to the manufacturer’s instructions for cycloxygenase inhibition assay [30]. The experiments were performed in triplicate and the results were given in Table 3.

Intercalation studies to ct-DNA

A solution of compounds 4c, 4d, 4e, 4f, and 5f (0.25 mM) in DMSO (10⁻⁴ M) was added to 20 mM Tris–HCl (pH 7.5) to a final volume of 5 mL. The control group of samples was treated with the compound (100 μM) in the absence of calf thymus DNA. The other samples were incubated with DNA (25, 50, 100, or 200 μM). The reactions were shaken for 3 days at 25°C in the dark. Fluorescence intensities were measured using the following parameters: excitation: 405 nm, emission: 460 nm.

Docking methodology

The five promising synthesized compounds (4c, 4d, 4e, 4f, and 5f) as well as Celecoxib were exported to Discovery Studio 2.5 and prepared for docking using Prepare Ligands protocol. The Prepare Ligands protocol helps to prepare ligands for input into other protocols, performing tasks such as removing duplicates, enumerating isomers and tautomers, and generating 3D conformations. The pdb codes used for COX-2 is (3LN1), and for COX-1 is (3KK6), loaded from the Protein Data Bank. These proteins were optimized and prepared through a Prepare Protein protocol, which is one of the general

purpose protocols. The Prepare Protein protocol prepares proteins for input into other protocols, performing tasks such as inserting missing atoms in incomplete residues, modeling missing loop regions, deleting alternate conformations, removing un-needed waters, standardizing atom names, and protonating titratable residues using predicted pKs.

Results and Discussion

Chemistry

As a further extension of our research for promising antitumor compounds [31,32] and in this endeavor a series of novel-dithiocarbamate-naphthalimide hybrids have been prepared (4a-4f, 5a-f) by merging the bridgeheads of various dithiocarbamate and dithioate moieties to naphthalimide core. The novel naphthalimide derivatives were synthesized according to the procedures depicted in Scheme 1. Hydroxyl derivatives (2.3) were generated by refluxing commercially available 1,8-naphthalic anhydride (1) with a corresponding amino alcohol [33]. Compounds 2 and 3 were converted to the corresponding chloro derivatives (4, 5) via our modified methodology using thionyl chloride under ice cooling in DMF [34,35]. The target compounds (4a-f, 5a-f) were obtained by nucleophilic substitution of the chloro group with RCS,K in DMF under thermal conditions 70–80°C. The structures of amines applied in this reaction protocol are given in Table 1. The newly synthesized compounds were identified using IR, 1H NMR and 13C, and electrospray ionization with high resolution mass spectra (ESI/HRMS). We then evaluated the antitumor activity of the resulting derivatives as well as their anti-inflammatory and DNA binding properties.

Biological evaluation

In vitro cytotoxic activity: The synthetic derivatives 4c, 4d, 4e, 4f, and 5d were evaluated for their in vitro cytotoxic activity against 21 cell lines by MTT assay and the results are given in Table 2. From the preliminary screening we concluded that, compound 4c was found to be sensitive to SKBR3 and MCF-7 with IC50 values 26.57, 38.58, 46.68, and 28.65 µM/ml respectively. Breast cancer cell lines BT474 and MB231 proved to be sensitive to SKBR3 and MCF-7 with IC50 values 26.57, 38.58, 46.68, and 28.65 µM/ml respectively.
sensitivity toward breast cancer cell lines T47D with IC\textsubscript{50} values of 21.1 and 29.13 µM/ml respectively. Among the pancreatic cancer cell lines, PK93 the order of cytotoxicity exhibited by these derivatives (IC\textsubscript{50}) was 4c > 4d > 5d > 4e; while for PK8 compound 4d showed good activities with IC\textsubscript{50} value 27.36 µM/ml. Hepatocellular cancer cell lines HepG2 and Huh-7 proved to be sensitive toward compounds 4d with IC\textsubscript{50} values 35.36 and 25.21 µM/ml respectively. Glioblastoma U251-MG showed sensitivity only towards compound 4d with IC\textsubscript{50} values 22.19 µM/ml. Colon cancer cell lines HT-29 were highly affected by all tested compounds (4c, 4d, 4e, 4f, and 5d) with IC\textsubscript{50} values 25.09, 33.05, 46.76, 35.62 and 38.77 µM/ml respectively. Among the evaluated compounds, 4d and 4f exhibited sensitivity towards spleen cancer cell line PMF-k014 with IC\textsubscript{50} values 37.89 and 31.03 µM/ml respectively. Lymphocyte cancer cell line MY displayed good activity towards compound 4d with IC\textsubscript{50} value 29.86 µM/ml. Ovarian cancer cell lines SKOV3 and OVK18 exhibited sensitivity toward Compound 4c with IC\textsubscript{50} values 47.27 and 38.97 µM/ml respectively. Based on the reported data, the stabilization of DNA-drug complexes may be caused by the formation of hydrogen bonds between the amino group and sugar phosphate chain [36]. Compounds 4c, 4d, 4e and 4f could form similar hydrogen bonds which further block DNA replication; thus exerting their activity. Although compounds 4d and 5d do not have a hydrogen bond donor group; during the experimental situation the protonation of the terminal nitrogen in the pipridine group greatly facilitates the DNA intercalation by an initial electrostatic contact with the anionic DNA polymer and this might be the reason for the activity [13,37,38]. It is noteworthy that the selective cytotoxicity was possibly due to different membrane crossing ability of the compounds for different cell lines in addition to; the nature of the amino alkyl side chain connected with the chromosome [39]. The preliminary evaluation highlighted compound 4c as the most potent in vitro cytotoxic agent. Consequently, analog 4c was selected as a prospective candidate for in vivo studies. Prior to that, with the purpose to enhance the efficiency of the anticancer effect, a drug-delivery system (DDS) based on liposomal encapsulation is currently under development following our previous publication [40]. Once the optimization of the DDS is achieved, further experimental analysis for the cytotoxicity in normal cell lines will be required in order to assess the possible side effects resulting from the chemotherapeutic effect of this analog. Since this is the first reported paper about the naphthalimide-dithiocarbamate and their antitumor activities further structure optimization is required. To that end, the introduction of various enantiomERICALLY pure amino acids should enhance the potency of the naphthalimides analogs; since recent studies have reported that various drug amino acid conjugates, such as 9-hydroxyellipticinium, anthraquinone, doxorubicin, vinblastin, hydroxymethylacylulvene, imidazotetrazines, methionine-enkephalin and camptothecins improve the antitumor activity [41].

**In vitro cyclooxygenase inhibition assay:** It is well known that COX enzymes especially COX-2 are overexpressed in many types of tumor; this strong association make it a valid target for treatment of cancer [41]. In an attempt to investigate the molecular mechanism of the synthesized compound, COX inhibition assay was performed. The ability of compounds 4c-4f and 5d to inhibit both COX-1 and COX-2 was determined and the results are given in Table 3. According to the IC\textsubscript{50} data, compounds 4c and 4d showed potent activity against both COX subtypes. The results obtained from in vitro cytoxicity studies were consistent with cyclooxygenase inhibition data. From these results; we could infer that COX inhibition play a key role in the modulation of cytoxicity potencies of Naphthalimide analogs.

**Molecular docking study**

The docking study was performed using the *Dock Ligands* (*CDOCKER*) protocol, which is one of the Receiver-Ligand Interactions protocols in Accelrys Discovery Studio 2.5. The docking protocol was chosen and validated after comparing results from different docking protocols with the co-crystallized Celecoxib in COX-2 (pdb code 3LN1), and COX-1 (pdb code 3K66), through alignment of the docked Celecoxib with the co-crystallized one and calculating the RMSD value, which was 0.90, as well as comparing the binding interactions formed with the receptor’s amino acids [42–44]. The docking results are presented in Table 4 and Figure 2. It was found that most of the synthesized compounds and Celecoxib interact with the same binding orientations inside COX-2 active site.

**QSAR study**

The QSAR study was performed using Discovery Studio 2.5 Software. The training set for the present QSAR modelling was composed of the 21 compounds with reported COX-2 activity [45] while the test set was composed of 4c, 4d, 4e, 4f, and 5d. The validation for the QSAR model employed was leave one-out cross-validation (internal validation), external validation using Celecoxib, as well as residuals between the predicted and experimental activity of the test set. The “Calculate Molecular Properties” module was used for calculating the 2D molecular properties such as AlogP, fingerprints, molecular properties, surface area, volume and the topological descriptors. The partial Least Squares (PLS) model was employed to search for optimal QSAR models that combine high quality binding pharmacophores with other molecular descriptors and being capable of correlating bioactivity variation across the used training set collection. The trials were held while changing the independent properties until the best model with the least variables was obtained. QSAR models were validated employing leave one-out cross-validation, \( r^2 \) (squared correlation coefficient value), and external validation using Celecoxib, as well as residuals between the predicted and experimental activity of the test set; the results are displayed in Table 4.

![Scheme 1: Reagents and reaction conditions: (i) H\textsubscript{2}N(CH\textsubscript{2})\textsubscript{n}OH, EtOH, reflux; (ii) thionyl chloride, 0°C, 2 h; (iii) RCS,K, DMF, 70–80°C, 2 h.](image-url)
QSAR validation

QSAR models were validated employing leave one-out cross-validation (internal validation), calculating the residuals between experimental activities and those predicted by the QSAR model, as well as calculating the predicted activity for Celecoxib through running as an external test the compound on the constructed QSAR model using “Calculate Molecular Properties” protocol and selecting the model from the “Other” set. The regression values were as follows: $r=0.956$, $r^2=0.911$, $r^2$ (adjusted)=1.064 and Least-squared error=0.062. The experimental activities and those predicted by QSAR studies. Equation 1 represents the best performing QSAR model (Table 5).

Predicted $pIC_{50}=0.311 \times \text{[AlogP]}+0.061\times \text{[Molecular_FractionalPolarSurfaceArea]}+0.389$ (1)

It should be noted that the predicted activities by our QSAR models were very close to those experimentally observed, indicating that these models can be safely applied for prediction of more effective hits having the same skeletal framework. Scatter plots of the experimental versus the predicted activity values ($pIC_{50}$) according to Equation 1 are presented in Figure 3.

DNA binding study

The antitumor activity of any compound can be determined by many factors, including cell membrane crossing ability, protein transport, DNA binding ability and drug metabolism [20]. Previous studies have demonstrated that compounds with strong DNA-binding affinity are highly cytotoxic [37]. The fluorescence spectra for compounds 4c, 4d, 4e, 4f, and 5d are presented in Figure 1. As the compound intercalate DNA, the fluorescence emission intensities decrease as the concentration of DNA increases [46,47]. For all the compounds, fluorescence quenching was decreased with increasing concentrations of DNA (up to 100 µM), and subsequently remained constant. These compounds have the ability to intercalate the DNA helix; forming complex which further blocks DNA replication thus exerting their cytotoxic activities. Our results are consistent with previous studies, which suggested that naphthalimides have DNA intercalating properties [48] (Figure 4).

Conclusion

Here, we report the synthesis and biological evaluation of...
naphtalimides analogues with flexible side chains (dithiocarbamate as well as dithioate). In this study compound 4d showed a broad spectrum antitumor activity and exhibited potent anti-inflammatory effect; the experimental as well as the theoretical studies clearly predicts that bipiperidine group is essential for the activities. Moreover the result; revealed the direct correlation between the COX inhibition and anticancer activity. In addition to derivative 4c, with amino alkyl chain; inhibited the growth of SKBR-3 by 84.13% (IC$_{50}$: 10.54 µM); this analogue could be used as a promising target for future development of new anti-breast agents. Further investigation to gain more insight the molecular mechanism is currently under planning in the Laboratory of Nano-Biotechnology, Department of Medical Bioengineering Science,Okayama University, Kita-ku, Okayama, Japan.

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