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Novel perylene diimide based antiproliferative chromophores

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Abstract

The primary objective of the work was to monitor the antiproliferative activity of two newly synthesized and described perylene diimides. The MTT test was used to investigate the antiproliferative effect against PC3 the prostat cancer cell line, Panc1 pancreatic cancer cell line, Saos2 osteosarcoma cell line, Hep3B hepatoma cell line and HUVEC non-cancerous human umbilical vein endothelial cells. To PC3, Panc1, and Saos 2 cells, both compounds were found to be considerably cytotoxic; however, a comparable impact was not seen in Hep3B cells. Compound 2 had the lowest IC50 value of 40,88 µg/ml and significantly inhibited PC3 cell proliferation when compared to other cell lines.

Keywords: *Perylene diimide, antiproliferative activity, cytotoxicity*

Yeni PDI tabanlı antiproliteratif kromoforlar

Öz

Çalışmanın birincil amacı, yeni sentezlenmiş ve tanımlanmış iki perilen diimidin antiproliferatif aktivitesini bulmaktır. MTT testi, PC3 prostat kanseri hücre dizisi, Panc1 pankreas kanseri hücre dizisi, Saos2 osteosarkoma hücre dizisi, Hep3B hepatoma hücre dizisi ve HUVEC kanserli olmayan insan göbek damarı endotel hücrelerine karşı antiproliteratif etkiyi araştırmak için uygulandı. PC3, Pancl ve Saos 2 hücrelerine göre, her iki bileşiğin de oldukça sitotoksik olduğu bulundu; ancak, Hep3B hücrelerinde karşılaştırılabilir bir etki görülmedi. İkinci molekül, 40,88 g/ml ile en

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düşük IC50 değerine sahip olarak bulundu ve diğer hücre hatlarına kıyasla PC3 hücre çoğalmasını önemli ölçüde inhibe etti.

***Anahtar kelimeler**: Perilen diimid, antiproliteratif aktivite, sitotoksisite*

1. Introduction

Perylene diimides are reddish chromophores which have very high quantum yields. PDIs based on the 3,4:9,10-perylene bis(dicarboximide) pigment have a wide various applications owing to their outstanding thermal, photochemical and chemical stability. There are many applications of PDIs such as organic light-emitting diodes [3-4], reprographic processes [1-2], molecular switches and wires [5-6], light-harvesting arrays [7], photoreactive thin films [8-11], solar cells[12-13], dye lasers [14-15], and photosensitizers [16]. By double or single amine substitution on the perylene core, the absorption maxima in these chromophores can be shifted to 750 nm with further appropriate modification, improving resolution.

Perylene diimides also became criticial field of interest for cancer researchers after discovery of potential therapeutic benefits of hypericin on particular cancer types. Hypericin is an aromatic polycyclic compound synthesized by Hypericum perforatum and it is the perylene derivative that is most frequently studied, causing or facilitating necrosis and apoptosis in a variety of cancer cell types [17]. There are several studies showing antiproliferative activities of perylene derivatives besides their function in the chemical industry.

Therefore mentioned above, it is essential to discover new molecules effective on resistance targets of antiproliferative activity on cancer molecules. For this purpose, antiproliferative activities of two novel perylene diimide molecules were determined by MTT test against different cell lines.

2. Materials and methods

2.1.Chemicals and measurements

All chemicals and solvents obtained from Aldrich and Sigma used without further purification. Column chromatography of all the products was performed using Merck Silica Gel60 (particle size:0.040-0.063mm,230-400 mesh ASTM) penetrated with the eluent. Reactions were monitored by thin layer chromatography using precoated silica gel plates (Merck Silica Gel 60 Kiesel gel F254 TLC Aluminum Sheets 20x20 cm).

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Instruments Avance Series-Spectro spin DPX-400 Ultrashield (400 MHz) High Performance digital FTNMR spectrometer (METU, NMR Laboratory). All chemical shifts are referenced to residual solvent signals previously referenced to peaks and splitting patterns are designated as s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). Electrospray Ionization (ESI) mass spectra were recorded on Agilent 6500 Series LC-MS spectrometer, Agilent instruments, Paolo Alto, CA, USA. UV-Visible spectra were performed with Perkin Elmer Lambda 25 UV/Vis Spectrometer.

2.2.Synthesis of N,N'-O-t-butyl-L-serine-t-butylester-3,4:9,10-perylene diimide (Compound 1)

In 1.5 mL trimethylamine, 10 mL H_20, 10 mL n-butanol, a mixture of 0.646 g ($2.548x10^{-3}$mol) O-*t*-butyl-L-serine-*t*-butyl ester hydrochloride and 0.5 g ($1.274x10^{-3}$ mol) perylene-3,4:9,10-tetracarboxylicacid dianhydride (parent anhydride) was stirred until 48h at 85°C. Under vacuo, the evaporation of solution was done. The fractions were separated from each other sufficiently on column (97:3-$CHCl_3$:CH_3OH). After solvent removal by rotary evaporator, the precipitate of product was dried under vacuo. Yield 29%

(1) $C_{47}H_{53}N_2O_{10,}$ESI-MS:m/z805.37(M^+ + 1).

(2) ^{1}H-NMR (400 MHz, $CDCl_3$), δ[ppm], 1.10 (s, 18H), 1.48 (s, 18H), 4.15-4.20 (m, 4H), 5.88 (t, 2H), 7.26 (d, J= 8Hz, 4H), 8.51 (d, J=8.1 Hz,2H), 8.61 (d, J=8Hz, 2H)

(3) ^{13}C-NMR (100 MHz, $CDCl_3$), δ[ppm], 27.43, 27.97, 38.72, 68.14, 76.98, 82.35, 120.83, 121.91, 128.57, 128.77, 130.24, 130.84, 132.43, 138.18, 167.18, 167.73.

2.3.Synthesis of N,N'-O-t-butyl-L-threonine-t-butylester-3,4:9,10-perylene diimide (Compound 2)

In 1.5 mL triethylamine, 10 mL H_20,10 mL n-butanol, a mixture of 0.742g ($2.548x10^{-3}$ mol) O-*t*-butyl-L-threonine-*t*-butyl ester acetate salt and 0.5 g ($1.274x10^{-3}$ mol) parent anhydride was stirred until 48h at 85°C. Under vacuo, the evaporation of solution was done. The fractions were separated from each other sufficiently on column (97:3-$CHCl_3$:CH_3OH). After solvent removal by rotary evaporator, the precipitate of product was dried under vacuo. Yield 31%

(1) $C_{49}H_{57}N_2O_{10,}$ESI-MS: m/z833.40 (M^+ + 1).

(2) ^{1}H-NMR (400 MHz, $CDCl_3$), δ[ppm], 1.27 (s, 18H), 1.43 (s, 18H), 1.54 (s, 6H), 4.43-4.47 (m, 4H), 5.44 (t, 2H), 7.24 (d, J= 8 Hz, 2H), 8.55 (d, J=8,1 Hz, 4H), 8.66 (d, J=8Hz, 2H)

(3) ^{13}C-NMR (100 MHz, $CDCl_3$), δ[ppm], 23.75, 27.42, 28.448, 29.616, 31.58, 58.78, 59.32, 64.88, 67.38, 73.75, 77.20, 81.68, 122.96, 126.41, 129.07, 131.49, 134.73, 162.84, 167.55.

Prior to the biological evaluation of the synthesized PDI's, the protecting groups were removed after treatment with CF_3COOH:$CHCl_{3.}$ (50:50).

2.4.Cell lines and culture :

Of the human cancer cell lines; PC3 prostatic cancer cell line, Panc1 pancreatic cell line, Saos2 osteosarcoma cell line, and Hep3B hepatoma cell line were grown as adherent monolayers in flasks with DMEM (Sigma Dulbecco's Modified Eagle Medium) culture medium supplemented with 2 mM L-Glutamine (Hyclone) and 10% heat inactivated (56˚C for 1h) Fetal Calf Serum (Invitrogen). The cultures were maintained at 37˚C using a humidified incubator containing 5% (v/v) CO_2 in air.

2.5.MTT assay:

MTT (3-(4,5-dimethylthiazole-2yl)-2,5-biphenyltetrazolium bromide) assay was used for evaluation of cell proliferation [32]. Briefly, the cells were harvested with trypsin and seeded into 96-well multiplates in a final concentration of $5x10^3$ cells/well.

Dimethyl sulfoxide (DMSO) was used to dissolve each component, and the growth medium was then added to dilute it. The final concentration of DMSO was 1%, while the final concentrations of the complexes ranged from 0.01 to 0.4 mg/ml. The cells were treated with varying doses of the chemicals for 48 and 72 hours after adhering to the culture surface. Following the application, 20 µl of MTT (5 mg/mL) per well was added to the cells and then continuing to incubate for an additional 4 h, allowing live cells to transform the yellow MTT into dark-blue formazan crystals. The MTT-containing media was then removed, and 200 µl of acidified isopropanol with 0.04 N HCl was added. Each sample's spectrophotometric absorbance was measured at 550 nm and experiments were carried out in 8 repeats. Using the following equation, the percent inhibition indicated the effects of the tested complexes:

$$\% \text{ inhibition} = [1-(T/C)]x100$$

where C is the mean absorbance of the control cells and T is the mean absorbance of the treated cells. The concentration necessary to inhibit cell viability by 50% (IC50) was estimated using the concentration-percentage inhibition curves.

3. Results and discussion

In this work, we wanted to design two different perylene diimides (Compound 1&2) which are expected to show antiproliferative activity. Target molecules were synthesized in a few steps from commercially available materials (Fig.1). Relative absorption of Compound 1 and Compound 2 was shown using by uv-vis spectrometer in Figure 2. The IC50 values for the Hep3B, Panc1, PC3 and Saos2 cell lines against Cofmpounds 1&2 after 48 and 72 hours of treatment are given in Table 1. Then biological activities as antiproliferative activity of two novel perylene diimides were monitored in Figure 3.

Table 1. IC_{50} values for Hep3B, Panc1, PC3, and Saos2 cell lines against Compound 1&2 following 48 and 72 hour treatment periods (A and B, respectively).

(A) **48h**	**IC_{50}(µg/ml)**			
Compounds	***HEP3B***	***Panc1***	***PC3***	***Saos2***
Compund 1(Serine)	444.59	276.91	ND	185.48
Compound 2 (Threonine)	238.61	100.99	40.88	88.61
(B) 72h				
Compounds	***HEP3B***	***Panc1***	***PC3***	***Saos2***
Compound 1	ND	85.58	121.63	181.70
Compound 2	**375.63**	**134.79**	**98.37**	**92.808**

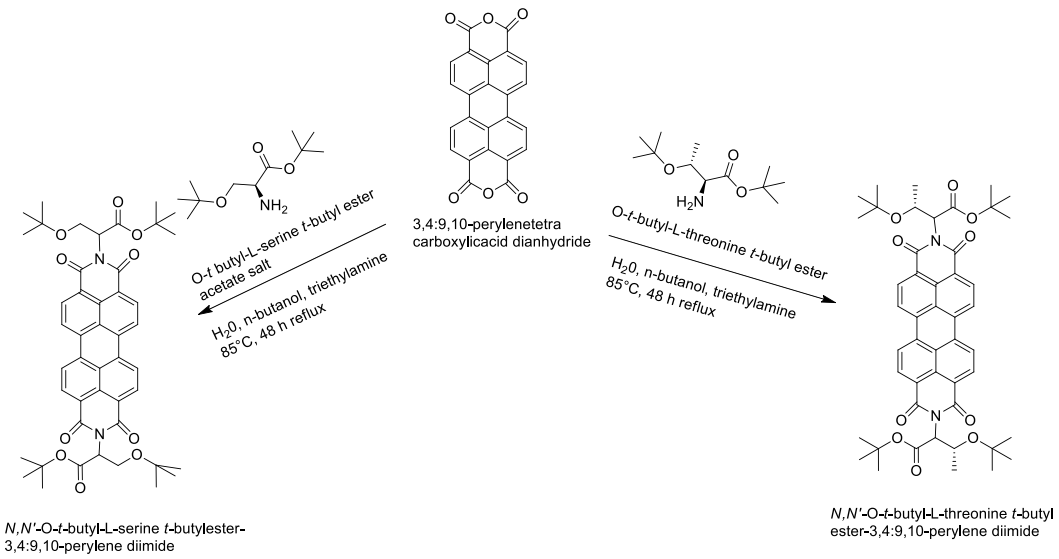

Figure1. Chemical Syntheses of Perylene diimides

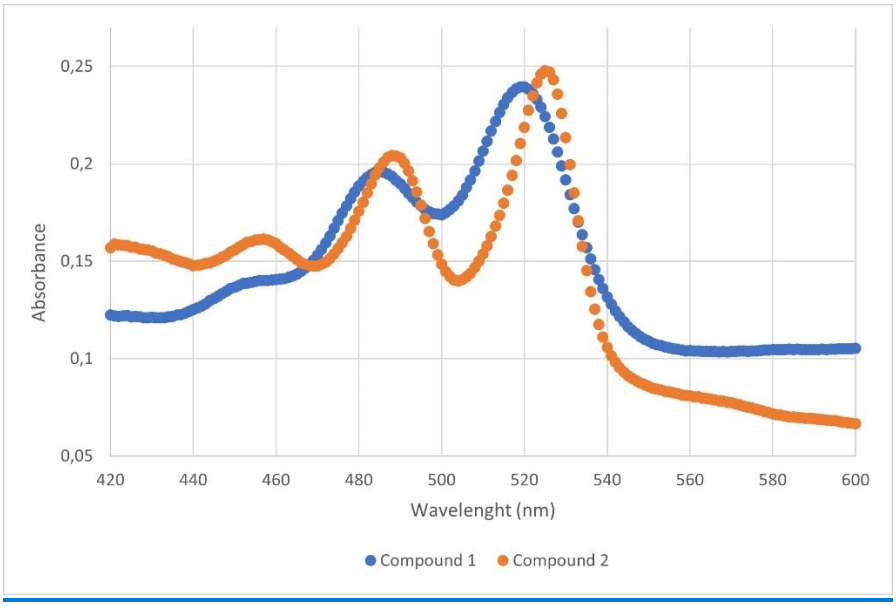

Figure 2. Relative Absorbance Graph of Compound 1 and Compound 2

3.1.The synthesis of two novel compounds

The synthesis was started with double amine substitution on the peylene core to be improved solubility. We synthesized compound 1 and compound 2 (Figure 1). The chemical structures of two compounds were verified analytically.

3.2.Anti-proliferative activity

Two novel perylene dimides (Compound 1&2) were compared in terms of their cytotoxicity toward four different cancer cell lines, Hep3B, PC3, Panc1, Saos2 and non-cancerous human endothelial cell line HUVEC. Research into biological impacts concentrated on the harmful effects of perylene dimide compounds on cell viability,

Fig. 3 shows the survival inhibition percentage of the complexes after 48 h and 72 h treatment. After 48 and 72 hours exposure, the IC_{50} values were determined (Table 1). A dose-dependent decline in relative cell proliferation was observed, by the treatment of compound 1 and 2 for 48 and 72h in Panc1 cells. (Fig.3). The lowest IC_{50} value, 85.58 µg/ml. for Panc1 cells were obtained using Compound 1 for 72 h treatment (Table 1). Treatment with Compound 1 also decreased cell viability of PC3 cells until 48 and 72h in a dose-dependent manner; however, the reduction of 48 h was not greater than 50% for the concentrations used (Table 1). After 48 and 72 hours of administration, compound 2 appeared to significantly reduce cell proliferation of all cancer cell lines used in the study in a dose-dependent manner (Figure 3). Table 1 shows that by the lowest IC_{50} values 40.88 µg/ml for 48h, compound 2 effected the proliferation of PC3 prostate cells more drastically compared to other cell lines. However treatment with two novel perylene dimide compounds for 48 and 72h slightly reduced the proliferation of human hepatoma cell line Hep3B, exhibiting higher IC_{50} values among the cell lines used (Table 1), no remarkable cytotoxic effect was observed for HUVEC cells excluding the highest concentration, 0,4 mg/ml, until 48 h treatment of compound1. Briefly, the cytotoxic effect of Compound 1 appears to be stronger at 72 hours. It showed a significant cytotoxic effect at 72 hours, especially in Panc 1 cells. It is seen that the antiproliferative effect of Compound 2 at 48 hours is higher than its effect at 72 hours. It also showed a stronger cytotoxic effect than compound 1 for 48 hours.

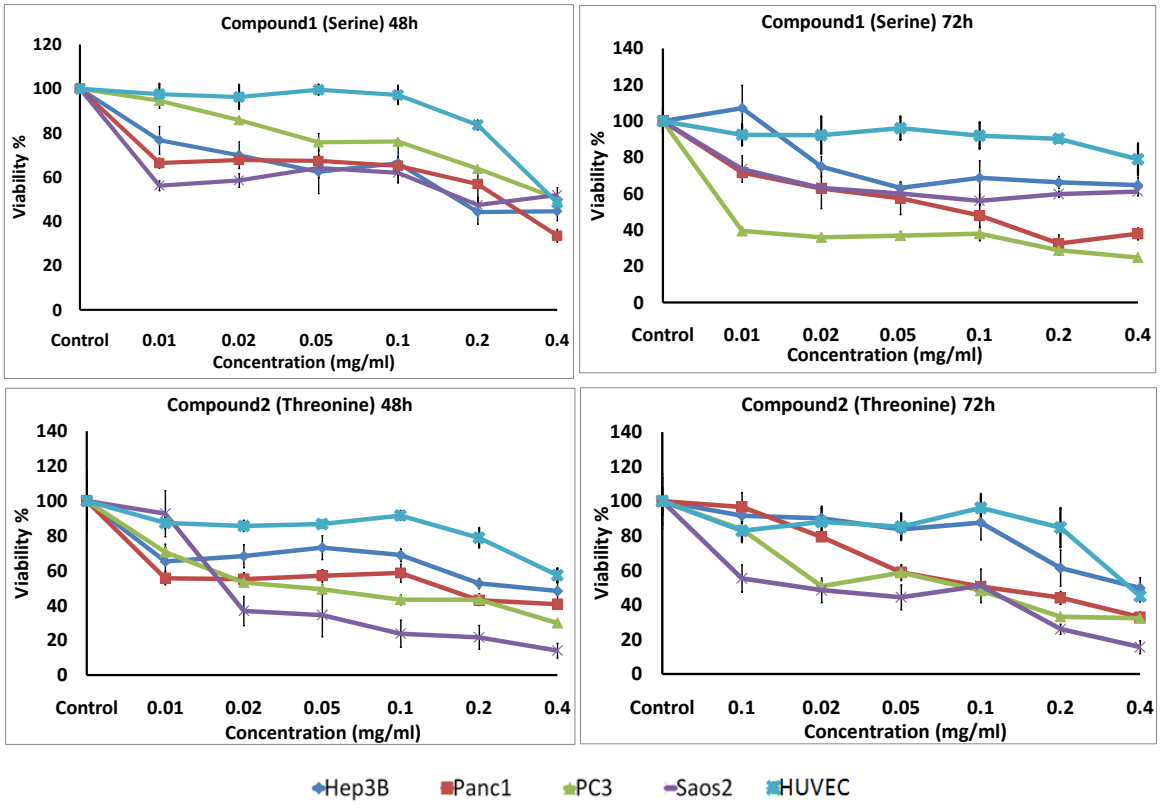

Figure 3. Diagrams showing the effects of the chemicals on the Hep3B, PC3, Saos2, and Panc1 cell lines after 48 and 72 hours are expressed as inhibition%. MTT colorimetric assay was performed and cells were treated with six different concentration of Compound 1-2. DMSO treated cells were used as control.

Recently, perylene diimide derivatives have been attracted great interest and find lots of area to use Yukruk et al, synthesized green water soluble perylene diimide molecules to be photosensitizers for photodynamic therapy [16]. Keskin et al. studied potential therapeutic advantages of perylene diimide derivatives on cancer therapy [19]. Liu et al. reported that the potential usage of perylene diimide derivatives as an efficient DNA delivery transporter and a recyclable specific Hg^{2+}ion sensor [20].

Cytotoxic activities of our novel perylene diimides were evaluated against various cancer cell lines and non cancerous HUVEC cells. For many cancers, chemotherapy is still only systemic treatment, however the effectiveness of chemotherapy is generally limited by toxic side effects or both intrinsic and acquired resistance to chemotherapeutics [21]. Therefore developing less cytotoxic and more effective anticancer drugs are in great interest of researchers. Perylene derivatives are under investigation for their anticancer activity contributed to their potential therapeutic benefits on particular cancer types. As there are several studies evaluating the cytotoxic effects of perylene derivatives on cancer cell lines, great effort was focused on hypericin [19, 22-28]. Limited information is included in the literature about antiproliferative activity of the perylene diimide compounds [19, 27-28]. In this regard, the study's findings represent the first evidence of these novel perylene diimide compounds ability to suppress the proliferation of four different cancer cell lines namely Hep3B, PC3, Panc1 and Saos2. These compounds were found to be significantly cytotoxic to Saos 2, Panc1 and PC3cells in a dose dependent manner however similar effect was not observed on Hep3B cells. Different responses of different cell lines represent the cyto-selectivity of compounds that is the most important properties for the chemotherapeutic drugs. Regarding the dominant non-cytotoxic effect of compounds on HUVEC cells, different responses of different cancer cell lines represent the cyto-selectivity of compounds that is the most important properties for the chemotherapeutic drugs. The findings of this study will help in the development of additional compounds in the future.

4. Conclusion

In this study, two novel perylene diimides were synthesized and characterized. Accumulating evidence has demonstrated the anti-proliferative activities of two novel perylene diimides. They displayed a clear, *in vitro* anti-proliferative activity results showed that both of the compounds reduced the proliferation of PC3, Saos2 and Panc1 cells in dose dependent manner but the proliferation of Hep3B cells were not affected in the same pattern. This evidence offered is evidently describes that it is possible to use novel perylene diimide compounds in anti-proliferative studies. More consideration should be given to the research on perylene diimides that could produce more valuable data to expand on their usage area.

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