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5-Non-amino aromatic substituted naphthalimides as potential antitumor agents: Synthesis via Suzuki reaction, antiproliferative activity, and DNA-binding behavior

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ABSTRACT

Amonafide is a naphthalimide derivative with antitumor activity and has failed to enter clinical phase III, because of its high-variable and unpredictable toxicity. In order to develop selective, efficient, and safe drugs, applying the 'nonfused' aromatic system strategy, a series of 5-non-amino aromatic substituted naphthalimides as replacement for amonafide were designed and were synthesized from naphthalic anhydride by three steps including bromination, amination, and $Pd(PPh_3)_4$ catalyzed Suzuki reaction. These new naphthalimide derivatives, except **4b**, not only exhibited better activity than amonafide against HeLa and P388D1 cell lines in vitro under the same experimental conditions, but also could avoid the side effect of amonafide due to their structure, which lacks an easy acetylated arylamine at the 5 position. The DNA-binding behavior of the naphthalimide derivatives was also investigated, and the results suggested that they bind to DNA via intercalation and **4a** and **4g** intercalated into DNA in different fashion.

1. Introduction

Naphthalimide (benz[*de*]isoquinoline-1,3-dione) derivatives have been extensively investigated as antitumor agents for many years.[1–4] Amonafide (see Fig. 1), a DNA-intercalator, was the first compound that reached the clinical trial stage in this family and showed good antitumor activity against advanced breast cancer. However, amonafide has so far failed to enter clinical phase III because its amino group was easily metabolized to *N*-acetyl-amonafide (Fig. 1) by enzyme *N*-acetyltransferase 2 (NAT2), which caused a high-variable, unpredictable toxicity (Fig. 1) among individuals.[5,6] As a strategy to modify amonafide, the N-substituted amonafide derivatives were synthesized by Kiss and co-workers[7] and us[8] (Fig. 1A and B), respectively. These derivatives not only revealed similar or better antitumor activity than amonafide in vitro or in vivo and could avoid the side effect of amonafide due to their structure, which lacks an easy acetylated arylamine. Whereas, the above-mentioned amonafide derivatives had shortcoming, on the one hand the numbers of the derivatives was limited according to the Kiss's synthetic method, on the other hand the yield was low according to our synthetic method.

The 'minimal intercalation' was brought forward by Denny et al.[9] which was hypothesized that an anticancer DNA-intercalator with lowest possible association constants may be better distribution in vivo and generally present a broader spectrum of activity than structurally related compounds with higher binding constants, and was applied to design some tricyclic carboxamides presenting potent cytotoxicity where the third phenyl ring is appended but not fused to the chromophore. Based on the results obtained by Denny et al.[9] Braña et al. designed and reported five 'nonfused' arylnaphthalimides[10] (Fig. 1) with good antitumor activity. These 'nonfused' arylnaphthalimides are in apparent contrast with an accepted concept that an efficient approach to enhance the antitumor activity of naphthalimides was to fuse one or more aromatic heterocycles with the naphthalene core.[3] Meanwhile, in the Braña's report it aroused our attention that among the arylnaphthalimides, except the 5-phenyl substituted dimeric compound, the others 5-aromatic substituted compounds were not studied in detail, while the 5-phenyl substituted compound **4a** exhibited similar antitumor activity to amonafide and may be seen as the 'nonfused' azonafide (Fig. 1). Moreover, existing research results confirmed that the substituent in 5-position of the naphthalene ring is key to antitumor activity of the naphthalimides.[1,3] Thus, on the one hand as a part of our continuing search for safe and potential anticancer naphthalimides, on the other hand as an attempt to explore whether the 'nonfused' strategy was an efficient measure to design anticancer naphthalimides, we aimed at the development of 5-non-amino aromatic substituted naphthalimides as replacement for amonafide.

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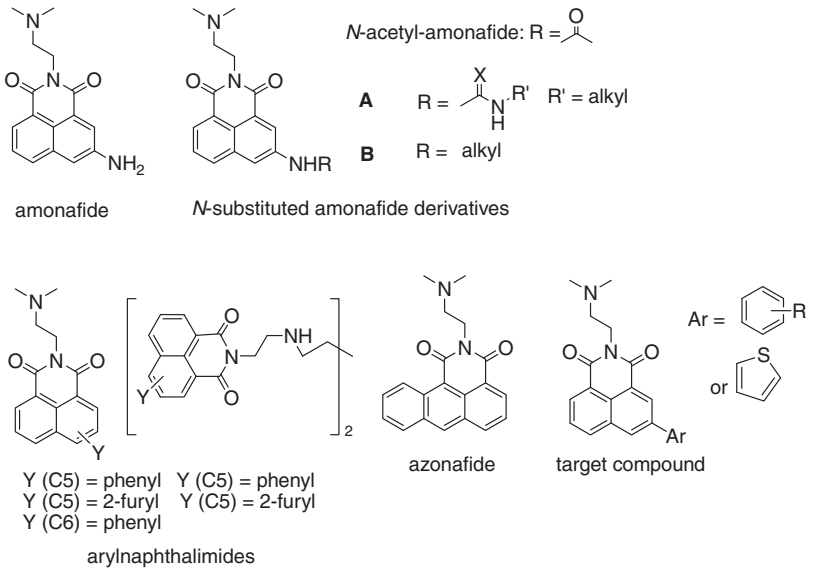


Figure 1.

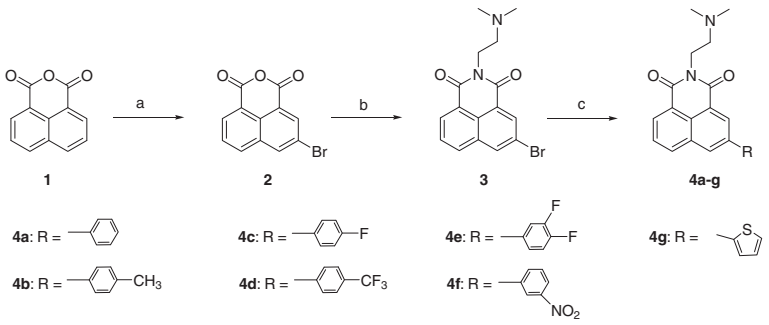
According to the Braña's method, the 5-phenyl substituted naphthalimide **4a** was synthesized from naphthalic anhydride by three steps including bromination, Stille coupling reaction and amination. In Stille coupling procedure, the expensive and poisonous phenyl tributiltin was used as raw material, and was excessive compared with naphthalic anhydride, moreover, the $Pd(PPh_3)_4$ was also used as catalyst. The fact that 5-alkylamino substituted amonafide derivatives[8] (Fig. 1A) were successfully synthesized inspired us to improve the synthesis method for the target compounds. The Suzuki–Miyaura cross-coupling reaction (also named Suzuki reaction) has evolved into a most effective strategy for carbon–carbon (C–C) bond formation,[11–13] and is less expensive and less toxic compared with Stille reaction. In recent years, Suzuki reaction has been greatly developed, and some previously inaccessible compounds were synthesized, nevertheless the scope of substrate was mainly limited to simple aryl.[11–13] In this paper, the synthesis of 5-non-amino aromatic substituted naphthalimides (Fig. 1) by Pd-catalyst Suzuki reaction, antiproliferative activity in vitro and the DNA-binding are reported.

2. Results and discussion

2.1. Synthesis

The target compounds **4a–g** were synthesized starting from naphthalic anhydride by three steps including bromination, amination and Suzuki reaction by Pd-catalyst, and the synthetic route was illustrated in the Scheme 1.

In the synthesis procedure for target compounds, the third step reaction was the most crucial and difficult, thus the coupling reaction of **3** with phenylboric acid was chosen as the model reaction to optimize the reaction conditions and detailed in Table 1. Initially, the catalyst was evaluated. The cheaper Ni catalyst $Ni(PPh_3)_2Cl_2$ has been explored for the model reaction (entry 1). The result revealed that $Ni(PPh_3)_2Cl_2$ failed to give the target compound **4a** and suggested that the Ni-based catalysts or the chosen catalyst were not suitable for the substrate. For the Pd-catalyst have been most widely used in the Suzuki reaction of organoboron compounds with organic halides,[11,12] $PdCl_2$, $Pd(PPh_3)_2Cl_2$, and $Pd(PPh_3)_4$ were assayed respectively (entries 2–4). The yield was the highest when $Pd(PPh_3)_4$ was used as catalyst. The results could be related to the phenylboronic acid are inert to $PdC1_2$ and $Pd(PPh_3)_2Cl_2$, or the dissociation of the PPh_3 is the easiest among the three Pd-catalyst in reductive elimination procedure of the Suzuki reaction mechanism.[11] Next, in order to determine suitable reaction time, the yield was analyzed by HPLC detection during different reaction time (entries 4–6). According to the experimental data, the yield approached a constant as the increase of reaction time and 28 h was a suitable reaction time. This phenomenon could be attributed to that the reaction involved Suzuki reaction between **3** and phenylboronic acid, self-coupling reaction and debrominated reaction of **3** reached the equilibrium along with the reaction time. The debrominated product of **3** was found, which gave evidence to the above explanation. It is well known that base also play an important role in the Suzuki reaction, however, at present, the choice of base is still empirical, and no general



Scheme 1. Reagents and conditions: (a) Br_2, concd HNO_3, 70 °C, 2 h, room temperature overnight; (b) $NH_2(CH_2)_2N(CH_3)_2$, C_2H_5OH, reflux, 2 h; (c) aromatic boric acid with substituted groups, $Pd(PPh_3)_4$, toluene reflux, 28 h.

Table 1
The optimization of the reaction conditions in coupling reaction of **3** with phenylboronic acid

Entry	Catalyst	Base	Solvent	*t* (h)	Yield (%)
1[a]	$Ni(PPh_3)_2Cl_2$	K_3PO_4	Toluene	48	0
2[b]	$PdCl_2/PPh_3$	K_3PO_4	Toluene	48	35
3[b]	$Pd(PPh_3)_2Cl_2$	K_3PO_4	Toluene	48	37
4[b]	$Pd(PPh_3)_4$	K_3PO_4	Toluene	48	46
5[b]	$Pd(PPh_3)_4$	K_3PO_4	Toluene	36	48
6[b]	$Pd(PPh_3)_4$	K_3PO_4	Toluene	28	48
7[b]	$Pd(PPh_3)_4$	K_3PO_4	1,4-Dioxane	28	45
8[b]	$Pd(PPh_3)_4$	K_2CO_3	Toluene	28	45
9[b]	$Pd(PPh_3)_4$	t-BuOK	Toluene	28	40

[a] Reaction conditions: **3** (0.5 mmol) $Ni(PPh_3)_2Cl_2$ (0.015 mmol), base (0.75 mmol), solvent (3 mL).
[b] Reaction conditions: **3** (0.5 mmol), $Pd(PPh_3)_4$ (0.015 mmol), base (0.75 mmol), solvent (3 mL).

rule for their selection has been established.[14] The K_3PO_4, K_2CO_3, and *t*-BuOK have been investigated as base. The K_3PO_4 gave a better yield (entries 6, 8 and 9). Last, as proper selection of solvent is also essential for the reaction, the toluene and 1,4-dioxane were also tested (entries 7 and 8). The results indicated that the effect of toluene and 1,4-dioxane on the yield was similar, and toluene was used as solvent due to its lower toxicity. On the basis of above studies, it can be concluded that the Suzuki reaction carry out using the $Pd(PPh_3)_4$ as catalyst and K_3PO_4 as the base in toluene under reflux for 28 h. The desired compounds were synthesized by the optimized conditions, and the results were described in Table 2. To our delight, the reaction times of 5-pehnyl naphthalimide derivative were dramatically reduced as much as 42 h and the yield was unchanged in comparison with the report by Braña et al.[10]

To the best of our knowledge, so far no studies on the use of the Pd-based catalyst Suzuki reaction into the naphthalimide system were reported. Several papers[13,15,16] described perylene bisimides derivatives which are a similar structure to naphthalimide, have been reported, while our study was conducting. The yields in our experiment are similar or lower than reported perylene bisimides derivatives. This difference implied that the Suzuki reaction of the perylene bisimides derivatives easily process compared with the 5-position naphthalimide or our reaction condition would deserved to be optimized.

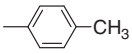
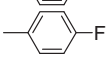
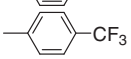
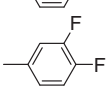
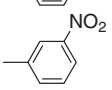
2.2. Antiproliferative activity in vitro

The in vitro antiproliferative activity of the 5-non-amino aromatic substituted naphthalimides against **HeLa** (human cervical carcinoma cells) and P388D1 (murine lymphoid neoplasm cells) were assessed by **MTT** tetrazolium dye assay[17] and summarized in Table 2, while amonafide was used as positive control. The IC_{50} value of **4a** and amonafide against HeLa cell in our study is in agreement with reported data by Braña et al.[10] Based on the IC_{50} value of **4a**, **4b**, **4e**, and **4f**, we hypothesized that electro-withdrawing group residing on the substituted phenyl could improve the antitumor activity of the new naphthalimides. The hypothesis was strengthened by synthesis and assay IC_{50} value of compound **4c** and **4d** against HeLa cell. It is well known that thiophene is a bioisostere for benzene. **4g**, a 'nonfused' thiophene substituted naphthalimide, exhibited stronger antiproliferative activity than amonafide. Referring to the Braña's report that the furyl substituted naphthalimide[10] is better bioactivity than amonafide, this seems to indicate that the aromatic heterocycle substituted naphthalimide could possess better bioactivity. The experimental results displayed that all new naphthalimides except **4b** exhibited similar or stronger antiproliferative activity than amonafide against HeLa cell and P388D1, moreover, the antiproliferative activity against P388D1 cell was stronger than that against HeLa under the same experimental conditions. Therefore, it can be deduced that the 'nonfused' strategy can be applied to develop the anticancer naphthalimides.

2.3. DNA-binding studies

amonafide, therefore, the DNA-binding of the 5-aromatic substituted naphthalimides derivatives was also studied. **4a** bear-

Table 2
Preparation of **4a–g** by Suzuki reaction of **3** with various substituted phenylboric acid and the antiproliferative activity against HeLa and P388D1 cell line in vitro

Compound	R	Yield (%)	Cytotoxicity (IC_{50}[b], μM)			K_b[b] (10^5 M^{-1})	K_{app}[c]
			HeLa	P388D1	HeLa		
4a		48	5.70 ± 0.09	0.68 ± 0.08	6.04 ± 0.09	10.84 ± 0.12	0.3175
4b		51	45.81 ± 0.13	2.19 ± 0.10	36.82 ± 0.11	10.76 ± 0.11	0.3134
4c		42	Not determined	Not determined	5.09 ± 0.08	12.36 ± 0.11	0.4335
4d		40	Not determined	Not determined	4.51 ± 0.08	11.76 ± 0.10	0.4324
4r		43	5.30 ± 0.11	0.98 ± 0.12	5.54 ± 0.09	12.78 ± 0.09	0.4341
4f		40	4.70 ± 0.08	0.38 ± 0.09	4.72 ± 0.08	14.85 ± 0.11	1.0402
4g		55	3.10 ± 0.09	0.68 ± 0.11	3.70 ± 0.08	13.21 ± 0.07	0.5323
Amonafide			6.02 ± 0.09	0.68 ± 0.08	6.45 ± 0.09	1.05 ± 0.07	0.0559

[a] Reaction conditions: compound **3** (0.5 mmol), $Pd(PPh_3)_4$ (0.025 mmol), K_3PO_4 (0.75 mmol), and toluene (3 mL).
[b] K_b Scatchard binding constants which was calculated according to the fluorescence quenching technique.
[c] K_{app} apparent binding constants which was calculated according to the equation $K_{app} = ([EB]/[compound]_{50\%}) \times K_{EB}$. where K_{app} is the apparent binding constant of the naphthalimides, K_{EB} is the DNA-binding constant of EB, [EB] is the EB concentrations of the EB–DNA system and $[compound]_{50\%}$ are the naphthalimides concentrations at 50% fluorescence.

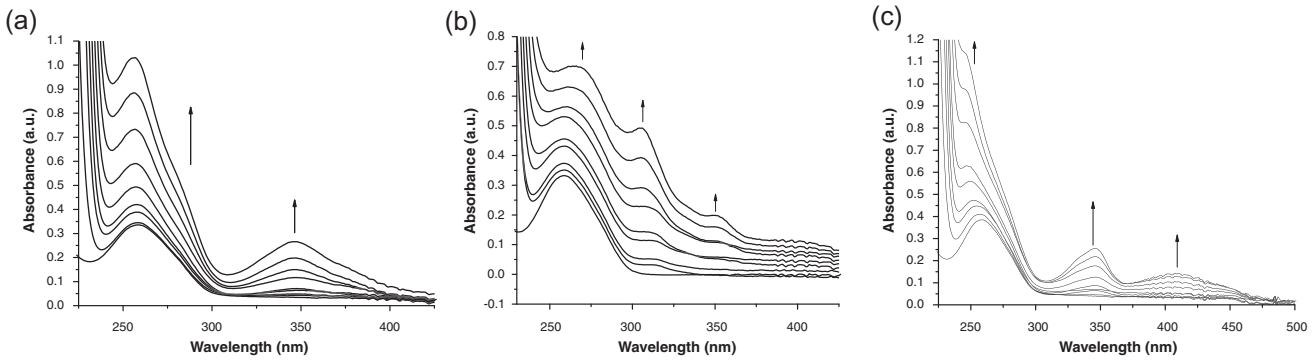


Figure 2. Absorption spectral changes of CT-DNA ([DNA] = 50 μM) in the presence of **4a** (a), **4g** (b) or amonafide (c), which was added in ratio R (R = [compound]/[DNA]) was 0, 0.02, 0.06, 0.1, 0.15, 0.2, 0.3, 0.4, and 0.5 in Tris–HCl buffer (30 mM, pH 7.5), respectively.

ing phenyl which is a typical substituted group and **4g** was only heteroacryl substituted derivative in the new naphthalimides, thus **4a** and **4g** were selected as study object.

Primarily, the absorption spectra of CT-DNA in the absence and presence of **4a** were measured and showed in Figure 2a. As the concentration of **4a** was increased, the hyperchromic phenomenon of the DNA solution was observed, which was attributed to absorption superposition of CT-DNA and **4a**, meanwhile no significant wavelength shift was seen. The absorption spectra of CT-DNA in the absence and presence of **4g** or amonafide (Fig. 2b or c) are similar to the absorption spectra of CT-DNA-**4a**. These spectral characteristics suggested that there are some similar interactions between the compounds and DNA.

Next, the fluorescent properties (Fig. 3) were performed and Scatchard binding constants (K_b) were calculated according to the fluorescence quenching technique[18,19] (Table 2). The emission intensities of **4a** and **4g** decreased with increasing the amount of CT-DNA as most of the intercalators did[20–23] and the wavelength of **4g** appeared blue shift (Fig. 3b). The blue shift would tend to indicating that the **4g** enters CT-DNA-stacking region with lower polarity rather than the bulk solution of CT-DNA[24] and implying that the **4g** more easily inserts into CT-DNA than **4a**. Based on the Scatchard method,[18,19] two following Eqs. (1) and (2) are generated, where C_o is the total concentration of bond and free drug, C_{DNA} is the free DNA concentration, and F_o and F_1 are the fluorescence intensity before and after the intercalation of the drug into DNA.

$$r_b = C_o(F_o - F_1)/(C_{DNA}F_o) \quad (1)$$

$$r_b/r_f = C_o(F_o - F_1)/(C_{DNA}F_1) \quad (2)$$

A plot of r_b/r_f versus r_b was constructed using linear regression at the origin and the Scatchard binding constant was evaluated from the slope of the plot (Fig. 3a and b, Table 2). In most cases, compounds strongly binding to DNA are high cytotoxic agent, whereas, the results revealed that there is no same relationship between DNA-binding and cytotoxicity. Because the K_b value of azonafide has not been reported, the DNA-binding between the new 5-aromatic substituted naphthalimides and the azonafide, a fused phenylnaphthalimides, can not be compared. Typical binding constant between organic compound and DNA usually range from 10^4 to 10^6 M^{-1}, in contrary to the guess that minimal intercalation mode, these compounds may be strong DNA-intercalator, thus the detailed action between DNA and the naphthalimides need further study.

In order to obtain further information regarding the DNA-binding properties, the competitive binding between ethidium bromide (EB) and these naphthalimides were carried out and 'apparent' equilibrium constants (K_{app})[25,26] were calculated, respectively (Table 2). EB emits weak fluorescence in aqueous or buffer solution, whereas, displays a dramatic enhancement of its emission intensity when it intercalate into DNA. If a molecule intercalates into DNA, it leads to a decrease in the binding sites of DNA available for EB, which in turn decreases the fluorescence intensity of the EB–DNA system. The addition of these naphthalimides to EB–DNA system caused appreciable reduction in emission intensity and indicated that these naphthalimides compete with ethidium bromide in binding to DNA (no show) by intercalation. The apparent equilibrium constants (K_{app})[25,26] was calculated from the competition experiments using Eq. (3) and presented in Table 2.

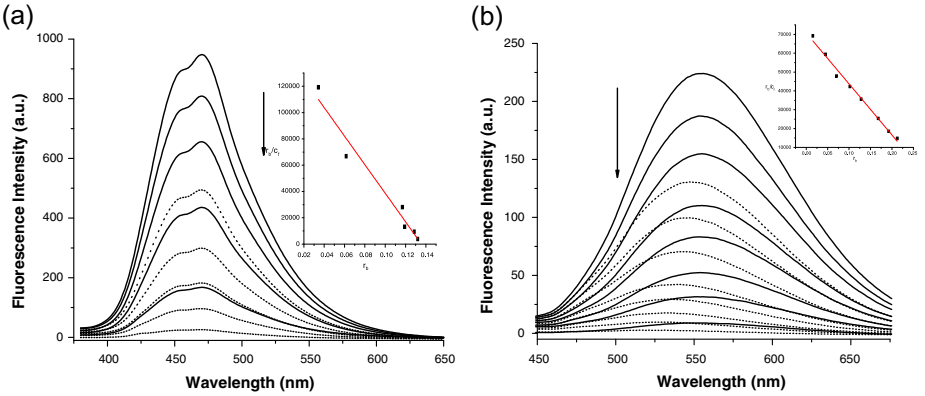


Figure 3. Fluorescence spectrum before and after interaction of **4a** (a) or **4g** (b) and CT-DNA (calf thymus DNA) in Tris–HCl buffer (30 mM, pH 7.5), and inserts show Scatchard plot of binding of **4a** or **4g**. The concentration of **4a** is 15, 10, 5, 3 and 1 μM contained 50 μM DNA, respectively. The concentration of 4 g is 25, 20, 15, 10, 7.5, 5, 3, and 1 μM contained 50 μM DNA, respectively.

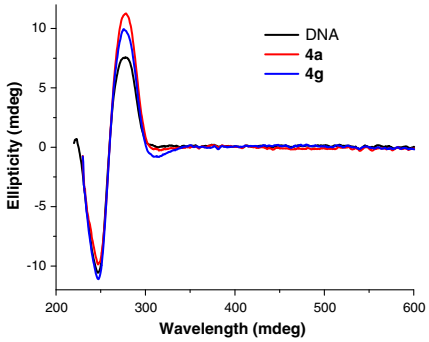


Figure 4. CD spectra of CT-DNA in the absence and presence of **4a** and **4g** at concentration of DNA 100 μM, the concentration of **4a** or **4g** is 10 μM, in Tris–HCl buffer (pH 7.0).

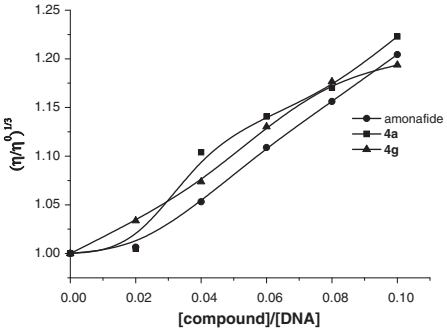


Figure 5. Effect of **4a** (■), **4g** (▲), and amonafide (●) on the relative viscosities of CT-DNA at 25 (±0.1) °C. [DNA] = 100 μM. η is the viscosity of DNA in the presence of the compounds and η^0 is the viscosity of DNA in the absence of the compounds.

$$K_{app} = ([EB]/[compound]_{50\%}) \times K_{EB} \quad (3)$$

Where K_{app} is the apparent binding constant of the naphthalimides, K_{EB} is the DNA-binding constant of EB, [EB] is the EB concentrations of the EB–DNA system and $[compound]_{50\%}$ are the naphthalimides concentrations at 50% fluorescence. The DNA-binding constants of EB reported vary considerably due to the experimental condition, thereby, the K_{app} of the naphthalimides were showed in a number $\times K_{EB}$. While fluorescence intensity of EB–DNA decrease 50% in the presence of a fluorescent molecule, the ratio of concentration between the molecule and DNA is still less than 100 which is supposed that the molecule possesses the same interaction model like EB does.[27] In our experiment, EB and DNA concentrations are 1.26 and 50 μM, respectively, according to the K_{app} equation, the all ratio of $[compound]_{50\%}$/[DNA] were less than 1, thus the naphthalimides bind to DNA via intercalation. Usually, the K_{EB} is 10^7,[28] and the Scatchard binding constant K_b and the apparent binding constant K_{app} are parallel.

CD (circular dichroism) is a very powerful technique to monitor the conformational state of the DNA double helix in solution. Later, the CD spectra of **4a**-DNA and **4g**-DNA were recorded and displayed in Figure 4, where the 275 nm band due to base stacking and 248 nm band due to right-handed helicity. The increases in the intensity of the positive band and the decrease in the intensity of the negative band of **4a** were observed, which was consistent with the B to A-like conformational change,[29] while the increase in intensity of both the positive and negative bands of **4g** were exhibited, which was typical of intercalation involving π-stacking and stabilization of the right-handed B form of CT-DNA,[30] after the compounds were incubated with CT-DNA. A negative induced CD (ICD) signal of **4g** was found, which was ascribed to that **4g** intercalated into DNA with its long axis parallel to the base-pair,[29] while ICD signal was not detected in **4a**. ICD of the intercalated compound is dependent on the orientation of the transition moment inside the intercalation site, their lateral displacement relative to the helix axis and the type of base pairs forming the intercalation site. The difference of ICD signals between **4a** and **4g** additionally supported the notion that the compound **4a** and **4g** intercalated into DNA in different fashion.

Spectroscopic data are necessary, but not sufficient to support a binding mode. Viscosity measurement is regarded as a reliable tool to determine the binding model in solution in the absence of crystallographic structural data and NMR data.[20] Therefore, the viscosity of DNA solution versus the concentration of **4a**, **4g**, and amonafide were studied and were illustrated in Figure 5. Intercalation of a molecule into DNA results in a lengthening, unwinding and stiffening of the helix which increases the viscosity of the solution.[20,22] The increase in viscosity of DNA solution was observed versus the increase in concentration of **4a**, **4g**, and amonafide, especially, the plot of **4g** approach to a line, which predicated these compounds bind to DNA via intercalation.

3. Conclusion

It is generally accepted that an efficient approach to enhance the antitumor activity of naphthalimides was to fuse one or more aromatic heterocycles with the naphthalene core. Therefore, this research may provide some new suggestions for the design of novel antitumor agents based on naphthalimides. The study demonstrated that the Pd-catalyzed Suzuki coupling reaction can serve as a valuable tool for the functionalization of naphthalimide system and provided the replacements for amonafide. The replacements not only exhibited improved antitumor activity over amonafide, but also can avoid the side effects.

4. Experimental

All the solvents are of analytic grade. ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker AV-400 spectrometer with chemical shifts reported in ppm (in $CDCl_3$, TMS as internal standard). Melting points were determined by using an X-6 micro-melting point apparatus and are uncorrected. Column chromatography was performed using silica gel 200–300 mesh. IR spectra were obtained using a Nicolet 470 FT-IR instrument. High-resolution mass spectra were obtained on a HP 1100 LC–MS spectrometer.

4.1. Synthesis

4.1.1. 5-Bromo-2-[2-(dimethylamino)ethyl]-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (3)

The compound **2** was prepared according to the report.[31] The compound **2** (277 mg, 1.0 mmol) and *N,N*-dimethylethyldiamine (92 mg, 1.0 mmol) were refluxed in EtOH (15 mL) for 2 h to give intermediate **3**.

Yield 92%, an light brown solid, mp 77.2–78.6 °C; ^{1}H NMR (400 MHz $CDCl_3$), δ (ppm), 8.65 (s, 1H), 8.59 (dd, 1H, J = 1.0 and 7.2 Hz), 8.35 (s, 1H), 8.11 (dd, 1H, J = 0.7 and 8.3 Hz), 7.77 (t, 1H, J = 7.8 Hz), 4.34 (t, 2H, J = 6.9 Hz), 2.70 (t, 2H, J = 6.9 Hz), 2.39 (s, 6H); MS (APCI) m/z 348 (M+1).

4.1.2. General procedure for the new naphthalimides (4a–g)

The intermediate **3** (174 mg, 0.5 mmol), $Pd(PPh_3)_4$ (12.5 mg, 0.01 mmol), K_3PO_4 (230.3 mg, 0.75 mmol) and aromatic boronic acid with substituted groups (0.75 mmol) in dry toluene (3 mL) were mixed, stirred and reflux for 28 h under nitrogen. The crude

products were concentrated under vacuum, and then were purified by chromatography on silica gel with a solution of CH_2Cl_2 and MeOH as eluent to give desired product.[10]

4.1.3. 5-Phenyl-2-[2-(dimethylamino)ethyl]-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (4a)

Yield 48%, a white solid, mp 126.4–128.2 °C; ^{1}H NMR (400 MHz $CDCl_3$), δ (ppm), 8.87 (s, 1H), 8.58 (dd, 1H, J = 0.8 and 6.0 Hz), 8.38 (s, 1H), 8.25 (dd, 1H, J = 0.8 and 6.0 Hz), 7.78–7.75 (m, 3H), 7.53 (t, 2H, J = 6.0 Hz), 7.47–7.44 (m, 1H), 4.38 (t, 2H, J = 7.0 Hz), 2.73 (t, 2H, J = 7.0 Hz), 2.41 (s, 6H); ^{13}C NMR (100 MHz $CDCl_3$), δ (ppm), 164.2, 140.1, 139.3, 134.1, 132.2, 131.3, 131.0, 130.8, 129.2, 128.4, 127.5, 127.4, 127.3, 123.2, 122.6, 57.0, 45.6, 38.0; IR (KBr cm^{-1}), 3444, 2948, 1688, 1654; HRMS (EI) m/z $(M+H)^+$ calcd for $C_{22}H_{20}N_2O_2$ 344.1525; found 344.1526.

4.1.4. 5-(4′-Methyl-phenyl)-2-[2-(dimethylamino)ethyl]-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (4b)

Yield 51%, a white solid, mp 219.8–220.9 °C; ^{1}H NMR (400 MHz $CDCl_3$), δ (ppm), 8.87 (s, 1H), 8.57 (d, 1H, J = 8.0 Hz), 8.37 (s, 1H), 8.25 (d, 1H, J = 8.0 Hz), 7.79 (t, 1H, J = 7.2 Hz), 7.68 (d, 2H, J = 8.0 Hz), 7.35 (d, 2H, J = 8.0 Hz), 4.41 (t, 2H, J = 6.8 Hz), 2.79 (t, 2H, J = 6.8 Hz), 2.45 (s, 9H); ^{13}C NMR (100 MHz $CDCl_3$), δ (ppm), 164.3, 140.0, 138.4, 136.3, 134.1, 132.2, 130.9, 130.8, 130.0, 129.9, 127.2, 123.0, 122.5, 56.8, 45.4, 37.8, 21.2; IR (KBr cm^{-1}), 3370, 2940, 1694, 1663; HRMS (ESI) m/z $(M+H)^+$ calcd for $C_{23}H_{23}N_2O_2$ 359.1735, found: 359.1736.

4.1.5. 5-(4′-1F-Phenyl)-2-[2-(dimethylamino)ethyl]-1*H*-benzo-[*de*]isoquinoline-1,3(2*H*)-dione (4c)

Yield 42%, a white solid, mp 115.1–116.3 °C; ^{1}H NMR (400 MHz $CDCl_3$), δ (ppm), 8.82 (d, 1H, J = 1.6 Hz), 8.59 (t, 1H, J = 6.8 Hz), 8.34 (d, 1H, J = 1.6 Hz), 8.27–8.25 (m, 1H), 7.80–7.72 (m, 3H), 7.25–7.21 (m, 2H), 4.43 (t, 2H, J = 6.8 Hz), 2.80 (t, 2H, J = 6.8 Hz), 2.51 (s, 6H); ^{13}C NMR (100 MHz $CDCl_3$), δ (ppm), 164.5, 162.7, 162.1, 139.3, 135.6, 134.3, 132.4, 131.6, 130.8, 129.4, 129.3, 127.5, 123.4, 122.8, 116.5, 116.3, 56.8, 45.4, 36.6; ^{19}F NMR δ (ppm), −114.2; IR (KBr cm^{-1}), 2925, 2854, 1747, 1697, 1662, 1172; HRMS (ESI) m/z $(M+H)^+$ calcd for $C_{22}H_{20}N_2O_2F$ 363.1509, found: 363.1522.

4.1.6. 5-(4′-Trifluomethyl-phenyl)-2-[2-(dimethylamino)ethyl]-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (4d)

Yield 40%, a white solid, mp 144.7–146.1 °C; ^{1}H NMR (400 MHz $CDCl_3$), δ (ppm), 8.79 (d, 1H, J = 6.4 Hz), 8.52 (t, 1H, J = 8.0 Hz), 8.32 (d, 1H, J = 7.2 Hz), 8.22–8.14 (m, 1H), 7.74–7.67 (m, 2H), 7.47 (t, 1H, J = 7.2 Hz), 7.39 (t, 1H, J = 7.2 Hz), 7.20 (s, 1H), 4.38 (t, 2H, J = 6.8 Hz), 2.82 (t, 2H, J = 6.8 Hz), 2.45 (s, 6H); ^{13}C NMR (100 MHz $CDCl_3$), δ (ppm), 173.4, 173.0, 164.4, 139.4, 134.3, 132.3, 131.9, 131.3, 129.4, 128.6, 127.9, 127.9, 126.3, 123.3, 122.8, 56.7, 45.2, 37.6; ^{19}F NMR δ (ppm), −63.0; IR (KBr cm^{-1}), 2925, 2854, 1746, 1697, 1663, 1171; HRMS (ESI) m/z $(M+H)^+$ calcd for $C_{23}H_{20}N_2O_2F_3$, 413.1477 found: 413.1469.

4.1.7. 5-(3′,4′-2F-Phenyl)-2-[2-(dimethylamino)ethyl]-1*H*-benzo-[*de*]isoquinoline-1,3(2*H*)-dione (4e)

Yield 43%, a white solid, mp 115.1–116.3 °C; ^{1}H NMR (400 MHz $CDCl_3$), δ (ppm), 8.78 (d, 1H, J = 2.0 Hz), 8.61 (d, 1H, J = 7.2 Hz), 8.32 (d, 1H, J = 2.0 Hz), 8.26 (d, 1H, J = 8.0 Hz), 7.80 (t, 1H, J = 8.0 Hz), 7.61–7.56 (m, 1H), 7.53–7.49 (m, 1H), 7.37–7.30 (m, 1H), 4.37 (t, 2H, J = 6.8 Hz), 2.69 (t, 2H, J = 6.8 Hz), 2.37 (s, 6H); ^{13}C NMR (100 MHz $CDCl_3$), δ (ppm), 164.0, 151.9, 149.4, 137.9, 136.4, 136.3, 134.0, 132.1, 131.3, 131.1, 130.1, 127.5, 123.5,122.7, 118.2, 118.0, 116.5, 116.4, 57.0, 45.8, 38.3; ^{19}F NMR δ (ppm), −136.4, −138.1; IR (KBr cm^{-1}), 2940, 1703, 1654, 1142; HRMS (ESI) m/z $(M+H)^+$ calcd for $C_{22}H_{19}N_2O_2F_2$ 381.1415, found: 381.1396.

4.1.8. 5-(3′-Nitro-phenyl)-2-[2-(dimethylamino)ethyl]-1*H*-benzo-[*de*]isoquinoline-1,3(2*H*)-dione (4f)

Yield 40%, a pale yellow solid, mp 188.2–183.6 °C; ^{1}H NMR (400 MHz $CDCl_3$), δ (ppm), 8.87 (s, 1H), 8.64–8.63 (m, 2H), 8.45 (s, 1H), 8.33–8.30 (m, 2H), 8.11 (d, 1H, J = 8.0 Hz), 7.83 (t, 1H, J = 8.0 Hz), 7.74 (t, 1H, J = 8.0 Hz), 4.39 (t, 2H, J = 6.8 Hz), 2.74 (t, 2H, J = 6.8 Hz), 2.41 (s, 6H); ^{13}C NMR (100 MHz $CDCl_3$), δ (ppm), 165.2, 149.0, 141.0, 137.4, 134.2, 133.3, 132.1, 131.7, 130.3, 130.0, 127.8, 123.7, 123.1, 122.7, 122.2, 56.9, 49.9, 45.6, 38.1, 29.7, 22.7; IR (KBr cm^{-1}), 3333, 2933, 1695, 1658, 1385; HRMS (ESI) m/z $(M+H)^+$ calcd for $C_{22}H_{20}N_3O_4$ 390.1430, found: 390.1434.

4.1.9. 5-Thiophene-2-[2-(dimethylamino)ethyl]-1*H*-benzo[*de*]-isoquinoline-1,3(2*H*)-dione (4g)

Yield 55%, a yellow solid, mp 110.5–112.2 °C; ^{1}H NMR (400 MHz $CDCl_3$), δ (ppm), 8.87 (s, 1H), 8.55 (d, 1H, J = 7.2 Hz), 8.36 (s, 1H), 8.21 (d, 1H, J = 7.2 Hz), 7.76 (t, 1H, J = 7.6 Hz), 7.58 (dd, 1H, J = 0.8 and J = 4.0 Hz), 7.43 (dd, 1H, J = 0.8 and J = 4.0 Hz), 7.20–7.18 (m, 1H), 4.40 (t, 2H, J = 6.8 Hz), 2.78 (t, 2H, J = 6.8 Hz), 2.45 (s, 6H); ^{13}C NMR (100 MHz $CDCl_3$), δ (ppm), 164.1, 142.3, 140.6, 136.9, 133.9, 133.4, 132.2, 130.9, 129.4, 129.3, 128.6, 127.6, 127.3, 126.4, 124.9, 123.2, 122.6, 56.8, 45.5, 37.8; IR (KBr cm^{-1}), 3348, 3103, 2370, 1691, 1654; HRMS (ESI) m/z $(M+H)^+$ calcd for $C_{20}H_{19}N_2O_2S$ 351.1123, found: 351.1144.

4.2. Evaluation of in vitro cell proliferation by means of the MTT colorimetric assay

HeLa (human cervical carcinoma) and P388D1 (murine macrophage) cells were seeded into 96-well microculture plates at a density of 2.5×10^4 cells/well in 180 μL, maintained in 1640 complete medium and incubated at 37 °C in a 5% CO_2 atmosphere for 24 h. The D-hank's buffer was used as a negative control and amonafide as a positive control. After cells were exposed to compounds in 20 μL/well at concentrations from 100 to 0.01 μM for 48 h, the cells were washed twice with PBS (phosphate-buffered saline, pH 7.4), and then 100 μL/well of MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, 0.5 μg/μL in PBS; Sigma) in 1640 complete medium were added. The plates were returned to the incubator for 4 h. Subsequently, DMSO was added as solvent. Absorbance was determined at 492 nm (with a reference at 630 nm) with a microplate reader. All experiments were performed at least three times, and the average of the percentage absorbance was plotted against concentration. The concentration of drug required to inhibit 50% of cell growth (IC_{50}) was then calculated for each compound.

4.3. DNA-binding studies

UV–vis absorption spectra were recorded on a PGENERAL TU-1901 UV–vis spectrophotometer and fluorescent spectra were measured on a Hitachi F-4500 luminescence spectrophotometer. Calf-thymus (CT) DNA was purchased from the Sino–American Biotechnology Company. Solution of CT-DNA in Tris–HCl buffer (30 mM, pH 7.5) gave a ratio of UV absorbance at 260 and 280 nm of 1.8–1.9:1, indicating that the DNA was sufficiently free from protein. The concentration of CT-DNA was determined by its absorption intensity at 260 nm with a known molar absorption coefficient value of 6600 M^{-1} cm^{-1}.

4.3.1. UV–vis absorption spectra studies

The titration absorption spectra studies were performed by keeping constant the concentration of DNA while varying the concentration of compound at room temperature. Initially, solutions of the blank buffer were placed in the reference and sample cuvettes (1 cm path length), respectively, and then the first

spectrum was recorded in the range 200–600 nm. During the titration, aliquots of buffered compound solution were added and the solutions were mixed by repeated inversion. After mixing for 10 min, the absorption spectra were recorded. The titration processes were repeated until there was no change in the spectra for at least four titrations indicating binding saturation had been achieved.

4.3.2. Fluorescent spectra and EB competitive studies

The two groups of samples for experiments were prepared, one at a constant DNA concentration of 50 μM and at concentrations compounds ranging from 1 μM to 25 μM in Tris–HCl (30 mM, pH 7.5), and the other having the same concentration of compound but absence of DNA as control. All the above solutions were ultrasonic shaken for 1 day at 25 °C in the dark. Fluorescence wavelength and intensity area of the samples were measured by the excitation wavelength at 350 nm.

DNA and EB were dissolved in buffer Tris–HCl (30 mM, pH 7.5) at the concentrations of 50 and 1.26 μM, respectively, and then the fluorescent spectra was performed. Different amounts of naphthalimides were added to EB bound with CT-DNA, mixed and emission spectra of EB were measured using excitation wavelength 518 nm. Before examining the fluorescent properties of EB, it was checked if the used naphthalimides did not quench the EB fluorescence. The plot of fluorescent intensity of EB–DNA system versus concentration of the naphthalimides were constructed at the Origin software, the $[\text{compound}]_{50\%}$ were obtained and the apparent binding K_{app} constant were calculated from Eq. (3).

$$K_{app} = ([EB]/[\text{compound}]_{50\%}) \times K_{EB} \quad (3)$$

4.3.3. Circular dichroism spectra studies

The CD (circular dichroism) spectra were scanned with a J-810 spectrophotometer (Jasco, Japan) using a 1-cm path quartz cell and subtracted from the spectrum of Tris–HCl buffer alone. The CD spectra were recorded at the compound concentration of 10 μM and DNA concentration of 100 μM, in the region 200–600 nm.

4.3.4. Viscosity experiments

Calf-thymus DNA was dissolved in Tris–HCl buffer (30 mM, pH 7.5) and left at 4 °C overnight. It was treated in an ultrasonic bath for 10 min, and the solution was filtered through a PVDF membrane filter (pore size of 0.45 μm) to remove insoluble material, the concentration of CT-DNA was 100 μM.[31] Viscometric titrations were performed with an Ubbelodhe viscometer immersed in a thermostated bath maintained 25 (±0.1) °C. The flow times were measured with a digital stopwatch, each sample was measured three times, and an average flow time was calculated. Data are presented as $(\eta/\eta^0)^{1/3}$ versus [complex]/[DNA], where η is the viscosity of DNA in the presence of complex and η^0 is the viscosity of DNA alone. Viscosity values were calculated from the observed flowing time of DNA-containing solutions (t) corrected for that of the buffer alone (t_0), $\eta = (t - t_0)$.[32,33]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.11.055. These data include MOL files and InChiKeys of the most important compounds described in this article.

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