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Synthesis, anticancer activity and DNA-binding properties of novel 4-pyrazolyl-1,8-naphthalimide derivatives

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ABSTRACT

A novel series of 4-pyrazolyl-1,8-naphthalimide derivatives have been designed and facilely synthesized. For anticancer activity in vitro, most of the compounds were found to be more toxic against human mammary cancer cells (MCF-7) than human cervical carcinoma cells (Hela) and human lung cancer cells (A549). Compounds **4i**, **4h**, **4b** and **4a** showed improved cytotoxic activity against MCF-7 cells over amonafide, in particular compounds **4i** and **4h**, the IC_{50} values of which against cell lines of MCF-7 were 0.51 μM and 0.79 μM, respectively. The DNA-binding properties of **4i** were investigated by UV–vis, fluorescence, and Circular Dichroism (CD) spectroscopies and thermal denaturation. The results indicated that compound **4i** as the DNA-intercalating agent exhibited middle binding affinity with CT-DNA.

Cancer is well-known as one of the leading causes of death worldwide, and it is estimated that about 10 million new cancer cases are diagnosed every year which represents a real crisis for public health in the world.[1] Therefore, the discovery of selective, efficient, and safe drugs for cancer chemotherapy remains an urgency and high priority for medicinal research.

Now DNA is still an important target for cancer chemotherapy. Generally, DNA interactive drugs include DNA-alkylating agents, DNA groove binders and DNA intercalators.[2] Naphthalimide derivatives, known as DNA intercalators, showed high anticancer activities against a broad spectrum of cell lines.[3] Some compounds, such as amonafide, elinafide and bisnafide (Fig. 1) have reached clinical trials. However, heretofore no naphthalimide derivative has ever entered the antineoplastic market because of dose-limiting toxicity.[4] To improve anticancer activities and/or decrease side effects, great efforts have been made to fuse aromatic rings, such as benzene, imidazole, pyrazine, furan or thiophene, and others, to naphthalene nucleus, some of the obtained compounds showed significant improvement in cellular cytotoxic activity over amonafide.[3,5] Another series of naphthalimides, where an unfused benzene or furan ring was introduced, were reported by Brana and co-workers exhibited extraordinary cytotoxicity for cancer cell lines.[6] More recently, Qian and co-workers reported a series of modified naphthalimide derivatives by linking 1,2,3-triazole ring

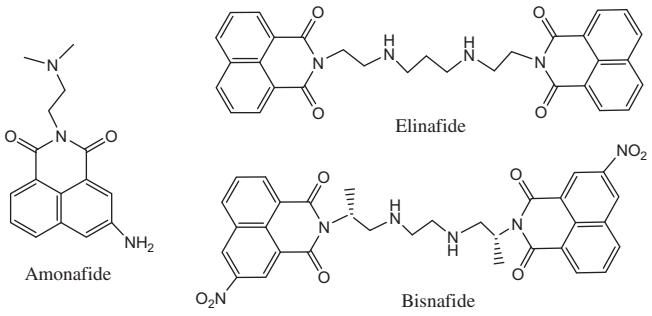

Figure 1. Representative naphthalimide derivatives in clinical trial.

to 4- or 3-position of naphthalimide skeleton and found that the majority of these compounds showed better cytotoxic activity against MCF-7 cells than amonafide.[7,8]

Pyrazoles are considered as extremely versatile building blocks in organic chemistry,[9,10] which is also an important class of compounds in medicinal chemistry.[11] Pyrazole derivatives possess a broad spectrum of pharmacological activities such as anti-inflammatory,[12] antibacterial,[13] antifungal,[14] hypoglycemic,[15,16] anti-hyperlipidemic,[17] etc. A variety of pyrazole derivatives have also been tested for their antitumor activity in vivo, which often result in promising lead compounds.[18–22] This gave us a great impetus to the search for potential pharmacologically active drugs carrying pyrazole substituent. Herein, we described the design,

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synthesis, anticancer activity and DNA-binding properties of novel 4-pyrazolyl-1,8-naphthalimide derivatives, expecting the incorporation of a pyrazole heterocyclic unit into naphthalimide could improve the biological activities while eliminating the *N*-acetyl transferase-2 (NAT2) acetylation of amonafide.[23]

As shown in Scheme 1, 4-pyrazolyl-1,8-naphthalimide derivatives **4a**–**4l** were synthesized by two steps starting from 4-bromo-1,8-naphthalic anhydride through condensation with *N*,*N*-dimethylethylenediamine in ethanol, followed by aromatic substitution reaction with various 4-aryl-1*H*-pyrazoles or 1*H*-pyrazole.[24] Structures of all the final products were confirmed by using ^{1}H and ^{13}C NMR, IR, MS and elemental analysis.[25]

The anticancer activity of the compounds **4a**–**4l**, was evaluated in vitro against MCF-7, Hela and A549 cells by MTT tetrazolium assay.[26] The IC_{50} values were listed in Table 1 and the activities of amonafide were as a control. The preliminary results showed that most of the obtained compounds were more toxic against MCF-7 than Hela and A549 cells. Toward MCF-7 and Hela cells, compounds **4b**, **4h** and **4i** were found to be more toxic than amonafide. Importantly, compound **4i**, bearing 3,4,5-trimethoxy group on its phenyl ring, was the most effective one, its inhibition abilities was 3.3-fold and 2.2-fold higher than that of amonafide under the same experimental conditions. Toward A549 cells, compounds **4a**, **4e**–**4i** exhibited higher activity than amonafide, especially, compound **4e**, decorating with a 4-methoxy group on its phenyl ring, was the most effective, its inhibition ability was 2.6-fold higher than that of amonafide.

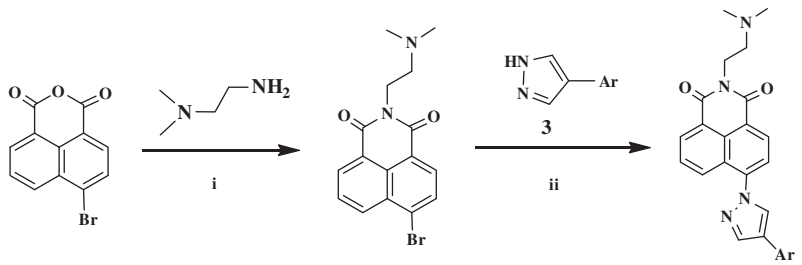
For these novel naphthalimide derivatives functionalized with pyrazole moiety, the structure–activity relationships (SAR) are summarized as follows: (1) Phenyl pyrazole-substitution makes the obtained naphthalimide derivatives more toxic than simple pyrazole-substitution. Compound **4a** exhibited better activity than compound **4l** against MCF-7, Hela and A549 cells. (2) The introduction of different substituent on the phenyl pyrazole unit could obviously affect the cytotoxicity against A549 cells. Generally, electron-donating group on the position-4 of the phenyl ring is essential for optimal anti-tumor activity. For example, compounds **4e** and **4f** with electron-donating groups on the benzene ring displayed higher cytotoxic activity than compounds **4b**, **4c**, **4d** and **4j** with electron-withdrawing groups. (3) The number, position and nature of electron-donating groups on substituted phenyl ring also played a profound role on cytotoxic activity against MCF-7 and Hela cells. Firstly, the cytotoxicity increases with the number of electron-donor group present on phenyl ring. A comparison between the cytotoxic activities of compounds **4f**, **4g**, **4h** and **4i** illustrated the cytotoxic SAR of the number of methoxy groups substituted on the phenyl ring (3 > 2 > 1). Secondly, the position of electron-donating group seems to have great effect on cytotoxic activity. Comparing compounds **4f**, **4g** and **4h**, it could be concluded that the substituted C-3 position is essential for cytotoxicity against MCF-7 cell line. Thirdly, the nature of the electron-donating group seems to be essential too. Compound **4e**, bearing a weakly electron-donating methyl group in C-4 position, is weakly cytotoxic against MCF-7 cells than **4f** with a relative strongly electron-donating methoxy group. For Hela and A549 cells, exactly different observation was obtained and this may be related to the tumor cell species. These results will be very helpful for designing new anticancer drugs in the future.

Table 1
The cytotoxicity of compounds against MCF-7, Hela and A549 cells

Compound	Cytotoxicity (IC_{50}, μM)		
	MCF-7	Hela	A549
4a	1.58	6.50	8.52
4b	1.56	3.10	24.02
4c	4.24	10.64	17.16
4d	10.66	3.47	>50
4e	17.01	4.60	5.09
4f	2.62	4.99	7.69
4g	1.99	14.60	11.95
4h	0.79	3.50	11.84
4i	0.51	3.09	5.14
4j	4.26	13.32	25.03
4k	3.39	16.60	25.36
4l	2.72	9.47	>50
Amonafide	1.68	6.71	13.00

In order to understand the cytotoxicity of the amonafide-analogues, the DNA-binding properties of the most active **4i** with calf thymus DNA (CT-DNA) were investigated by UV–vis absorption spectra, fluorescent spectra, Circular Dichroism spectra (CD), and thermal denaturation experiment.

It is widely accepted that if the compound can intercalate DNA, the UV–vis curve of their complex will be induced bathochromic shift and hypochromicity.[27] The UV–vis spectra of **4i**, the efficient cytotoxic among compounds, was measured (Fig. 2). As DNA concentration was increased, the curve showed significant hypochromicities and obvious bathochromic shifts. The spectral characteristic implies that compound **4i** can insert into the base pairs of DNA.[28] Its binding constant was calculated with the following equation:[29]



1 **2** **4a-4l**

4a: Ar = Ph; **4b**: Ar = 4-FC_6H_4; **4c**: Ar = 4-ClC_6H_4; **4d**: Ar = 4-BrC_6H_4; **4e**: Ar = 4-$CH_3C_6H_4$;
4f: Ar = 4-$CH_3OC_6H_4$; **4g**: Ar = 3-$CH_3OC_6H_4$; **4h**: Ar = 3,4-$(CH_3O)_2C_6H_3$;
4i: Ar = 3,4,5-$(CH_3O)_3C_6H_2$; **4j**: Ar = 4-$CF_3C_6H_4$; **4k**: Ar = 3,5-$(CF_3)_2C_6H_3$; **4l**: Ar = H

Scheme 1. Synthesis of 4-pyrazolyl-1, 8-naphthalimide derivatives. Reagents and conditions: (i) ethanol, reflux, 2 h; (ii) *t*-BuOK, DMSO, 72 °C, 1.5 h.

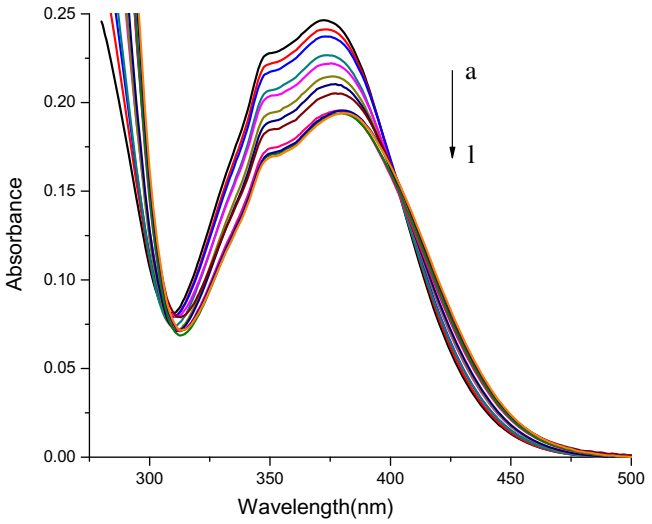


Figure 2. UV–vis spectral of compound **4i** at the concentration of 20 μM upon addition of CT-DNA ((a) 0 μM, (b) 10 μM, (c) 20 μM, (d) 30 μM, (e) 40 μM, (f) 60 μM, (g) 70 μM, (h) 80 μM, (i) 110 μM, (j) 120 μM, (k) 130 μM, (l) 140 μM) in 0.1 M CH_3COONa–CH_3COOH buffer (pH 4.0).

$$[DNA]/(\epsilon_a - \epsilon_f) = [DNA]/(\epsilon_b - \epsilon_f) + 1/K_b(\epsilon_b - \epsilon_f)$$

where [DNA] is the concentration of DNA in base pairs, ε_a, ε_f and ε_b correspond to average molar extinction coefficient of the solution ($A_{obsd}/_{[compound\ 4i]}$), molar extinction coefficient of free **4i**, and molar extinction coefficient of totally binding **4i**, respectively, and K_b is the intrinsic binding constant. A linear fit of the plot of $[DNA]/(\varepsilon_a-\varepsilon_f)$ versus [DNA] gives a slope of $1/(\varepsilon_b-\varepsilon_f)$ and an intercept of $1/K_b(\varepsilon_b-\varepsilon_f)$. A value of $K_b = 1.01 \times 10^4\ M^{-1}$ was determined by this method, however, it is lower than that of amonafide (K_b, $1.05 \times 10^5\ M^{-1}$ in 30 mM Tris–HCl, pH 7.5),[28] which implies that **4i** bind to DNA relatively less strongly. The decreased intercalation of **4i** compared with amonafide may be due to the incorporation of a pyrazole heterocyclic unit into naphthalimide.

To further investigate the interactions of compound **4i** with CT-DNA, the fluorescence quenching technique was employed to measure the Scatchard binding constants (K_b) for compound **4i** and the apparent binding constant (K_b) to CT-DNA was determined to be $1.04 \times 10^4\ M^{-1}$. As shown in Figure 3, the emission intensities of compound **4i** decreased with increasing the amount of CT-DNA and the wavelength showed slightly blue shift (Fig. 3). The observed quenching may be due to the binding of compound **4i** with CT DNA, while the blue shift is probably consistent with intercalation.[30]

Circular Dichroism (CD) is a valuable technique for examining the structure of DNA. From the changes in DNA's CD and the Induced Circular Dichroism (ICD) of compounds, the binding mode and the mechanism of interaction can be deduced. Compound **4i** was chosen to illustrate the CD spectra at different ratio (Fig. 4). Both the changes of DNA and ICD of **4i** were obtained. The CD spectrum of free CT-DNA shows bands at 279 nm (positive) and 247 nm (negative) typical of the B-form.[31] When $R_{(4i/DNA)} \leqslant 0.1$ (Fig. 4A), the increase of positive band and the decrease of the negative band were observed, and without significant wavelength change, which was consistent with the B to A-like conformational change.[32] This may be explained by the intercalation of **4i** into stacked DNA base pairs, which resulted in the separation of the base pairs and distortion of the sugarphosphate backbone.[7] Additionally, significant Induced Circular Dichroisms (ICD) signals was observed in the region of the characteristic absorption of the naphthalimides (325–475 nm). The weak and negative signal at low R values ($0.1 < R_{(4i/DNA)} \leqslant 0.5$, Fig. 4A) further indicated that **4i** intercalated DNA with a vertical orientation in the intercalation pocket.[33,34]

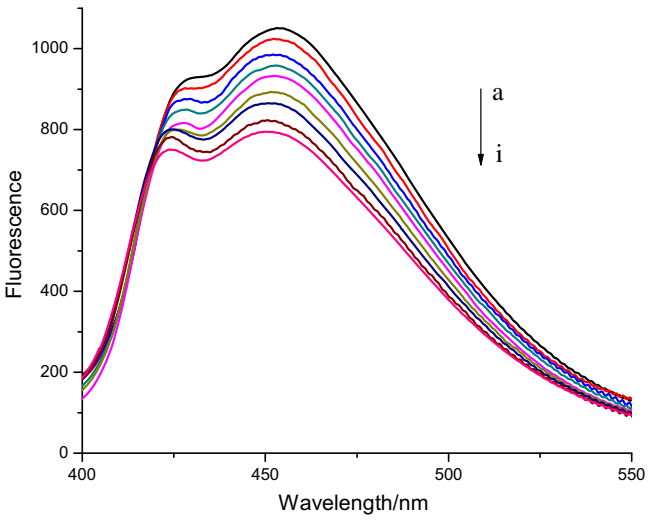


Figure 3. Fluorescence spectral of compound **4i** at the concentration of 20 μM upon addition of CT-DNA((a) 0 μM, (b) 10 μM, (c) 20 μM, (d) 30 μM, (e) 50 μM, (f) 70 μM, (g) 90 μM, (h) 110 μM, (i) 130 μM) in 0.1 M CH_3COONa–CH_3COOH buffer (pH 4.0).

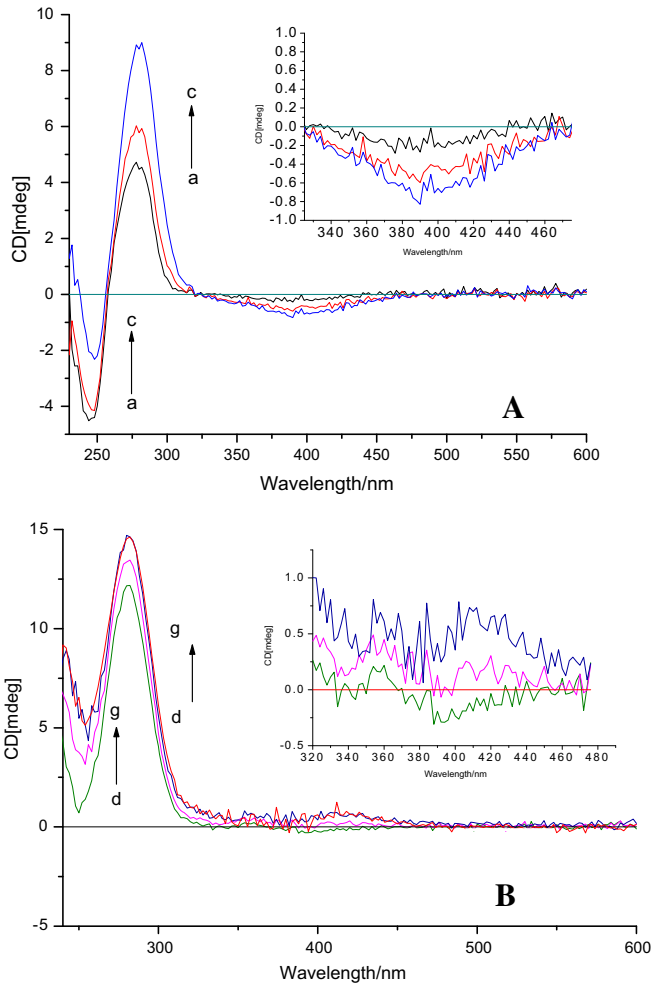


Figure 4. Titration CD spectra of CT-DNA by **4i** at different R ratio in 0.1 M CH_3COONa–CH_3COOH buffer (pH 4.0). The CT-DNA concentration is 60 μM. The value of R (**4i**/DNA) is (a) 0 (b) 0.02 (c) 0.1 (d) 0.5 (e) 1.0 (f) 2.0 (g) 4.0.

When $0.5 < R_{(\mathbf{4i}/DNA)} < 2.0$ (Fig. 4B), change trends of CD spectra were continued, but the negative ICD signals disappeared, and a new positive ICD signals appeared. The ICD behaviour at high R values is presumably a consequence of external stacking.[33,34] When $R_{(\mathbf{4i}/DNA)} \geqslant 2.0$, the positive band reached the highest peak, which suggested that the DNA had been saturated with **4i**.

Thermal denaturation studies of CT-DNA are useful in determining the ability of the present compound to stabilize the double stranded DNA. The intercalation of small molecules into the double helix was known to increase the DNA melting temperature (T_m), which characterizes the transition of DNA from double-stranded to single standard nucleic acid.[35] The melting curve of CT-DNA in the absence and presence of **4i** are illustrated in Figure 5 and Table 2, respectively. The T_m value for the free CT-DNA is 59.7 °C. Upon addition of **4i**, obvious changes in the DNA melting temperature were observed. The T_m value increased to 61.5 °C. The level of the increased melting temperature (ΔT_m) induced by DNA-compound interactions is 1.8 °C. Compound **4i** possessed lower DNA melting temperature than some other amonafide analogues reported in related literatures. These results illuminated that compounds **4i**, as the DNA intercalator, exhibited middle affinity with CT-DNA, this is consistent with the results from the UV–Vis titration data.[36]

The present work describes the design, synthesis and biological activity evaluation of the 4-pyrazolyl-1,8-naphthalimide derivatives as DNA-intercalating agents. The convenient method that introduce pyrazole ring to the 4 site of naphthalimide was offered. This reconstruction improved the cytotoxicity of the compounds over the lead compound amonafide. Based on the testing of antitumor activity in vitro, most of the obtained compounds were found to be more cytotoxic against MCF-7 than Hela and A549 cells. In particular, compound **4i**, bearing 3,4,5-trimethoxy group on its phenyl ring, with the values of IC_{50} against three cell lines were 3.3-fold, 2.2-fold, and 2.5-fold lower than that of amonafide. Compound **4e**, decorating with a 4-methoxy group on its phenyl ring, with the cytotoxicity against A549 cells was 2.6-fold higher than that of amonafide. The preliminary SAR revealed that 1) by linking a pyrazole ring onto the naphthalene core can significantly improve cytotoxic activity and 2) activity would be enhanced if the phenyl pyrazole moiety possessed electron-donating groups. The DNA binding properties were investigated by UV–vis, fluorescence and CD spectra and thermal denaturation experiment. The results showed that compound **4i** as the DNA intercalator exhibited middle binding affinity with CT-DNA. These results will be helpful for designing naphthalimide-based lead compounds with improved anticancer activity. Further structural optimization and detailed biological studies on the mechanism of action about the designed naphthalimide derivatives were under way.

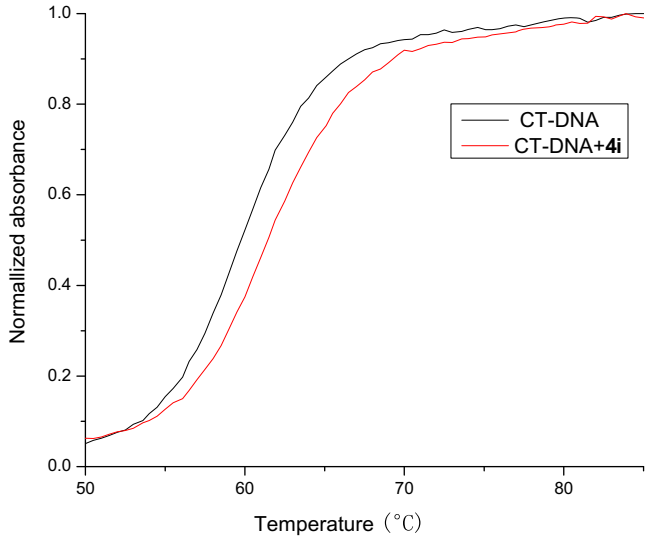


Figure 5. DNA melting curves for CT-DNA (50 μM) in the absence and presence of **4i** with concentration of 5 μM in 0.1 M CH_3COONa–CH_3COOH buffer (pH 4.0).

Table 2
Average T_m and ΔT_m for CT-DNA in the absence and presence of **4i**

Compound	T_m (°C)	ΔT_m (°C)
CT-DNA	59.7	0
CT-DNA+**4i**	61.5	1.8

Acknowledgments

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Supplementary data

Supplementary data (such as synthetic procedure, cytotoxicity analysis, and DNA binding experiment) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl. 2013.12.014.

References and notes

1. Garcia, M.; Jemal, A.; Ward, E. M.; Center, M. M.; Hao, Y.; Siegel, R. L.; Thun, M. J. *Global Cancer Facts & Figures 2007*; American Cancer Society: Atlanta, GA, 2007.
2. Martinez, R.; Chacon-Garcia, L. *Curr. Med. Chem.* **2005**, *12*, 127.
3. (a) Banerjee, S.; Veale, E. B.; Phelan, C. M.; Murphy, S. A.; Tocci, G. M.; Gillespie, L. J.; Frimannsson, D. O.; Kelly, J. M.; Gunnlaugsson, T. *Chem. Soc. Rev.* **2013**, *42*, 1601; (b) Li, S.; Zhong, W.; Li, Z.; Meng, X. *Eur. J. Med. Chem.* **2012**, *47*, 546.
4. Ingrassia, L.; Lefranc, F.; Kiss, R.; Mijatovic, T. *Curr. Med. Chem.* **2009**, *16*, 1192.
5. (a) Sami, S. M.; Dorr, R. T.; Solyom, A. M.; Alberts, D. S.; Iyengar, B. S.; Remers, W. A. *J. Med. Chem.* **1996**, *39*, 1609; (b) Li, F.; Cui, J.; Guo, L.; Qian, X.; Ren, W.; Wang, K.; Liu, F. *Bioorg. Med. Chem.* **2007**, *15*, 5114; (c) Brana, M. F.; Cacho, M.; Ramos, A.; Dominguez, M. T.; Pozuelo, J. M.; Abradelo, C.; Rey-Stolle, M. F.; Yuste, M.; Carrasco, C.; Bailly, C. *Org. Biomol. Chem.* **2003**, *1*, 648; (d) Brana, M. F.; Cacho, M.; Garcia, M. A.; Pascual-Teresa, B.; Ramos, A.; Dominguez, M. T.; Pozuelo, J. M.; Abradelo, C.; Rey-Stolle, M. F.; Yuste, M.; Banez-Coronel, M.; Lacal, J. C. *J. Med. Chem.* **2004**, *47*, 1391; (e) Qian, X.; Li, Y.; Xu, Y.; Liu, Y.; Qu, B. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2665.
6. Brana, F. M.; Gradillas, A.; Gomez, A.; Acero, N.; Llinares, F.; Munoz-Mingarro, D.; Abradelo, C.; Rey-Stolle, M. F.; Yuste, M.; Campos, J.; Gallo, M. A.; Espinosa, A. *J. Med. Chem.* **2004**, *47*, 2236.
7. Li, X.; Lin, Y.; Wang, Q.; Yuan, Y.; Zhang, H.; Qian, X. *Eur. J. Med. Chem.* **2011**, *46*, 1274.
8. Li, X.; Lin, Y.; Yuan, Y.; Liu, K.; Qian, X. *Tetrahedron* **2011**, *67*, 2299.
9. Sachse, A.; Penkova, L.; Noel, G.; Dechert, S.; Varzatskii, O. A.; Fritsky, I. O.; Meyer, F. *Synthesis* **2008**, *5*, 800.
10. Dvorak, C. A.; Rudolph, D. A.; Ma, S.; Carruthers, N. I. *J. Org. Chem.* **2005**, *70*, 4188.
11. Kumar, G. G.; Vikas, K.; Vinod, K. *Res. J. Chem. Environ.* **2011**, *15*, 90.
12. Szabo, G.; Fischer, J.; Kis-Varga, A.; Gyires, K. *J. Med. Chem.* **2008**, *51*, 142.
13. Tanitame, A.; Oyamada, Y.; Ofuji, K.; Terauchi, H.; Kawasaki, M.; Wachi, M.; Yamagishi, J. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4299.
14. Tanitame, A.; Oyamada, Y.; Ofuji, K.; Fujimoto, M.; Suzuki, K.; Ueda, T.; Terauchi, H.; Kawasaki, M.; Nagai, K.; Wachi, M.; Yamagishi, J. *Bioorg. Med. Chem.* **2004**, *12*, 5515.
15. Cho, N.; Kamaura, M.; Yogo, T.; Imoto, H. *PCT Int. Appl.* WO 2009139340, 2009; *Chem. Abstr.* **2009**, *151*, 550563.
16. Dugi, K.; Mark, M.; Himmelsbach, F. *PCT Int. Appl.* WO 2009022009, 2009; *Chem. Abstr.* **2009**, *156*, 268010.
17. Momose, Y.; Maekawa, T.; Odaka, H.; Kimura, H. *PCT Int. Appl.* WO 2001038325, 2001; *Chem. Abstr.* **2001**, *135*, 19632.

18. Balbi, A.; Anzaldi, M.; Macciò, C.; Aiello, C.; Mazzei, M.; Gangemi, R.; Castagnola, P.; Miele, M.; Rosano, C.; Viale, M. *Eur. J. Med. Chem.* **2011**, *46*, 5293.
19. Insuasty, B.; Tigreros, A.; Orozco, F.; Quiroga, J.; Abonia, R.; Nogueras, M.; Sanchez, A.; Cobo, J. *Bioorg. Med. Chem.* **2010**, *18*, 4965.
20. Labbozzetta, M.; Baruchello, R.; Marchetti, P.; Gueli, M. C.; Poma, P.; Notarbartolo, M.; Simoni, D.; D'Alessandro, N. *Chem. Biol. Interact* **2009**, *181*, 29.
21. Perchellet, E. M.; Ward, M. M.; Skaltsounis; Alexios, L.; Kostakis, I. K.; Pouli, N.; Marakos, P.; Perchellet, J. H. *Anticancer Res.* **2006**, *26*, 2791.
22. Pevarello, P.; Brasca, M. G.; Amici, R.; Orsini, P.; Traquandi, G.; Corti, L.; Piutti, C.; Sansonna, P.; Villa, M.; Pierce, B. S.; Pulici, M.; Giordano, P.; Martina, K.; Fritzen, E. L.; Nugent, R. A.; Casale, E.; Cameron, A.; Ciomei, M.; Roletto, F.; Isacchi, A.; Fogliatto, G. P.; Pesenti, E.; Pastori, W.; Marsiglio, A.; Leach, K. L.; Clare, P. M.; Fiorentini, F.; Varasi, M.; Vulpetti, A.; Warpehoski, M. A. *J. Med. Chem.* **2004**, *47*, 3367.
23. Norton, J. T.; Witschi, M. A.; Luong, L.; Kawamura, A.; Ghosh, S.; Stack, M. S.; Sim, E.; Avram, M. J.; Appella, D. H.; Huang, S. *Anti-Cancer Drugs* **2008**, *19*, 23.
24. Yang, P.; Yang, Q.; Qian, X. *Tetrahedron* **2005**, *61*, 11895.
25. To a solution of 4-aryl-1*H*-pyrazole or 1*H*-pyrazole (1.25 mmol) in dimethyl sulfoxide (DMSO) (1.5 mL), solid potassium *tert*-butoxide (1.38 mmol) was added, the mixture was stirred at room temperature for 15 min, then a solution of *N*-(2-(dimethylamino)ethyl)-4-bromo-naphthalimide **2** (1.31 mmol) in DMSO (0.5 mL) was added through a syringe. The mixture was heated to 72 °C and kept at this temperature for 1.5 h. Then the mixture was cooled to room temperature and quenched with ice water (10 mL), the precipitate was collected by filtration and oven-dried in vacuum. The crude product was further purified by recrystallization from ethanol to give pure products **4a**–**4l** in excellent yields. Compound **4a** (92%), light yellow solid, mp 138.3–139.0 °C. IR (KBr, cm^{-1}): 3100, 2958, 2763, 1697, 1652, 1585, 1425, 1398, 1376, 1344, 1242, 1118, 952, 758, 694; ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 2.39 (s, 6H), 2.71(t, J = 7.2 Hz, 2H), 4.38 (t, J = 7.2 Hz, 2H), 7.35 (t, J = 7.8 Hz, 1H), 7.47 (t, J = 7.8 Hz, 2H), 7.64 (d, J = 7.2 Hz, 2H), 7.86 (m, 3H), 8.18 (s, 1H), 8.22 (s, 1H), 8.67 (d, J = 8.4 Hz, 1H), 8.71 (d, J = 7.8 Hz, 2H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm): 38.37, 45.74, 55.77, 56.99, 122.47, 122.89, 125.45, 125.86, 126.37, 127.32, 127.79, 127.99, 129.10, 129.44, 130.68, 131.05, 131.39, 131.92, 140.19, 141.77, 163.43, 163.96. Anal. Calcd for $C_{25}H_{22}N_4O2{\cdot}0.25H_2O$: C, 72.36; H, 5.47; N, 13.50. Found: C, 72.49; H, 5.44; N, 13.35. ESI-MS m/z: 411.4 $[M+H]^+$; Compound **4b** (93%). Golden yellow solid, mp 155.1–155.6 °C. IR (KBr, cm^{-1}): 3124, 2958, 1700, 1654, 1583, 1513, 1473, 1427, 1398, 1367, 1232, 1168, 970, 827, 781, 750. ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 2.39 (s, 6H), 2.71(t, J = 7.2 Hz, 2H), 4.38 (t, J = 7.2 Hz, 2H), 7.16 (t, J = 8.4 Hz, 2H), 7.59 (td, J1 = 7.2 Hz, J1 = 3.6 Hz, 2H), 7.85 (m, 2H), 8.13 (s, 1H), 8.17 (s, 1H), 8.66 (d, J = 8.4 Hz, 1H), 8.71 (d, J = 7.8 Hz, 2H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm): 38.38, 45.77, 57.00, 115.98 116.12, 122.35, 122.48, 122.92, 124.55, 126.38, 127.45 127.50, 127.57 127.59, 127.82 127.85, 129.45, 130.59, 131.03, 131.94, 140.04, 141.68, 161.32, 162.95, 163.41, 163.94. Anal. Calcd for $C_{25}H_{21}N_4O_2F$: C, 70.08; H, 4.94; N, 13.08. Found: C, 69.70; H, 5.02; N, 12.84. ESI-MS m/z: 429.4 $[M+H]^+$; Compound **4c** (95%), light yellow solid, mp 174.8–175.4 °C. IR (KBr, cm^{-1}): 3108, 2971, 1700, 1658, 1583, 1471, 1432, 1396, 1240, 1097, 971, 856, 786, 754; ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 2.39 (s, 6H), 2.71(t, J = 7.2 Hz, 2H), 4.38 (t, J = 7.2 Hz, 2H), 7.44 (d, J = 8.4 Hz, 2H), 7.56 (d, J = 8.4 Hz, 2H), 7.85 (m, 2H), 8.17 (s, 1H), 8.19 (s, 1H), 8.64 (d, J = 8.4 Hz, 1H), 8.71 (d, J = 7.8 Hz, 2H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm): 38.39, 45.77, 57.00, 122.43, 122.53, 122.94, 124.34, 126.38, 127.06, 127.85, 128.05, 129.27, 129.45, 129.94, 130.53, 131.00, 131.95, 133.04, 140.01, 141.61, 163.39, 163.91. Anal. Calcd for $C_{25}H_{21}N_4O_2Cl{\cdot}0.25H_2O$: C, 66.81; H, 4.82; N, 12.47. Found: C, 66.97 H, 4.92; N, 12.29. ESI-MS m/z: 445.6 $[M+H]^+$; 4d (96%), light yellow solid, mp 177.8–180.3 °C. IR (KBr, cm^{-1}): 3106, 2960, 1702, 1658, 1583, 1471, 1432, 1396, 1365, 1240, 1062, 971, 856, 786, 754. ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 2.82 (s, 6H), 3.28 (t, J = 7.2 Hz, 2H), 4.59 (t, J = 7.2 Hz, 2H), 7.50 (d, J = 8.4 Hz, 2H), 7.59 (d, J = 8.4 Hz, 2H), 7.85 (m, 2H), 8.18 (s, 1H), 8.19 (s, 1H), 8.65 (d, J = 7.8 Hz, 1H), 8.71 (d, J = 7.2 Hz, 2H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm): 38.38, 45.79, 56.99, 121.04, 122.43, 122.56, 122.92, 124.35, 126.37, 127.37, 127.89, 128.05, 129.44, 130.38, 130.56, 131.03, 131.99, 132.22, 139.99, 141.60, 163.60, 163.95. Anal. Calcd for $C_{25}H_{21}N_4O_2Br{\cdot}0.25H_2O$: C, 60.80; H, 4.39; N, 11.34. Found: C, 60.79; H, 4.40; N, 11.19. ESI-MS m/z: 491.1 $[M+H]^+$. Compound **4e** (95%), light yellow solid, mp 180.5–181.3 °C. IR (KBr, cm^{-1}): 3112, 2973, 2821, 1698, 1654, 1396, 1241, 1174, 1118, 975, 779; ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 2.39 (s, 6H), 2.42(s, 3H), 2.71 (t, J = 7.2 Hz, 2H), 4.38 (t, J = 7.2 Hz, 2H), 7.27 (d, J = 8.4 Hz, 2H), 7.53 (d, J = 8.4 Hz, 2H), 7.85 (m, 2H), 8.15 (s, 1H), 8.20 (s, 1H), 8.69 (d, J = 7.8 Hz, 1H), 8.71 (d, J = 7.8 Hz, 2H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm): 21.18, 38.36, 45.77, 56.99, 122.16, 122.39, 122.88, 125.45, 125.76, 126.35, 127.69, 127.75, 128.46, 129.46, 129.77, 130.77, 131.07, 131.91, 137.12, 140.18, 141.84, 163.46, 163.99. Anal. Calcd for $C_{26}H_{24}N_4O_2{\cdot}0.5H_2O$: C, 72.04; H, 5.81; N, 12.92. Found: C, 72.18; H, 5.59; N, 12.70. ESI-MS m/z: 425.5 $[M+H]^+$. Compound **4f** (90%); light yellow solid, mp 156.7–157.8 °C. IR (KBr, cm^{-1}): 3108, 2964, 2765, 1698, 1660, 1583, 1508, 1471, 1243, 1180, 1027, 833, 779. ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 2.39 (s, 6H), 2.71(t, J = 7.2 Hz, 2H), 3.88 (s, 3H), 4.38 (t, J = 7.2 Hz, 2H), 7.00 (d, J = 8.4 Hz, 2H), 7.55 (d, J = 8.4 Hz, 2H), 7.84 (m, 2H), 8.10 (s, 1H), 8.16 (s, 1H), 8.69 (m, 3H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm): 38.33, 45.74, 55.37, 56.96, 114.55, 122.09, 122.36, 122.86, 123.94, 125.22, 126.33, 127.06, 127.34, 127.74, 129.46, 130.82, 131.10, 131.92, 140.07, 141.86, 159.02, 163.49, 164.02. Anal. Calcd for $C_{26}H_{24}N_4O_3{\cdot}0.5H_2O$: C, 69.47; H, 5.61; N, 12.46. Found: C, 69.51; H, 5.37; N, 12.29. ESI-MS m/z: 441.5 $[M+H]^+$. Compound **4g** (84%), light yellow solid, mp 145.2–145.8 °C. IR (KBr, cm^{-1}): 3112, 2944, 2777, 1697, 1658, 1519, 1473, 1396, 1240, 1176, 937, 779; ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 2.56 (s, 6H), 2.93(t, J = 7.2 Hz, 2H), 3.90(s, 3H), 4.46 (t, J = 7.2 Hz, 2H), 6.90 (dd, J_1 = 8.4 Hz, J_2 = 1.8 Hz, 1H), 7.16 (s, 1H), 7.22 (d, J = 7.8 Hz, 1H), 7.38 (t, J = 7.8 Hz, 1H), 7.84 (m, 2H), 8.18 (s, 1H), 8.20 (s, 1H), 8.67 (d, J = 8.4 Hz, 1H), 8.70 (d, J = 7.8 Hz, 2H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm): 38.37, 45.78, 55.33, 57.00, 111.83, 112.47, 118.37, 122.29, 122.48, 122.90, 125.33, 126.38, 127.79, 128.14, 129.45, 130.15, 130.67, 131.04, 131.93, 132.74, 140.26, 141.75, 160.21, 163.44, 163.96. Anal. Calcd for $C_{26}H_{24}N_4O_3$: C, 70.89; H, 5.49; N, 12.72. Found: C, 70.68; H, 5.40; N, 12.51. ESI-MS m/z: 441.7 $[M+H]^+$. Compound **4h** (88%), light yellow solid, mp 171.2–172.1 °C. IR (KBr, cm^{-1}): 3116, 2948, 2763, 1695, 1652, 1583, 1508, 1475, 1398, 1247, 1025, 970, 782; ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 2.79 (s, 6H), 3.24(t, J = 7.2 Hz, 2H), 3.96 (s, 3H), 3.99 (s, 3H), 4.57 (t, J = 7.2 Hz, 2H), 6.97 (d, J = 7.8 Hz, 1H), 7.11 (d, J = 1.8 Hz, 1H), 7.19 (dd, J_1 = 8.4 Hz, J_2 = 1.8 Hz, 1H), 7.85 (m, 2H), 8.14 (s, 1H), 8.16 (s, 1H), 8.70 (m, 3H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm): 38.35, 45.76, 56.03, 56.99, 109.48, 111.89, 118.40, 122.15, 122.36, 122.87, 124.34, 125.41, 126.34, 127.51, 127.74, 129.45, 130.76, 131.04, 131.90, 140.11, 141.80, 148.63, 149.51, 163.44, 163.96. Anal. Calcd for $C_{27}H_{26}N_4O_4{\cdot}0.25H_2O$: C, 68.27; H, 5.62; N, 11.79. Found: C, 68.06; H, 5.32; N, 11.64. ESI-MS m/z: 471.5 $[M+H]^+$. Compound **4i** (91%), golden yellow solid, mp 184.6–186.8 °C. IR (KBr, cm^{-1}): 2937, 2817, 2767, 1698, 1664, 1587, 1506, 1457, 1398, 1365, 1243, 1029, 998, 971, 836, 782. ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 2.72 (s, 6H), 3.15 (t, J = 7.2 Hz, 2H), 3.92 (s, 3H), 3.97 (s, 6H), 4.54 (t, J = 7.2 Hz, 2H), 6.82 (s, 2H), 7.85 (m, 2H), 8.16 (s, 1H), 8.17 (s, 1H), 8.67 (d, J = 7.8 Hz, 2H), 8.71 (d, J = 7.8 Hz, 2H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm): 38.39, 45.78, 56.32, 57.01, 61.00, 103.46, 122.31, 122.48, 122.90, 125.59, 126.41, 127.15, 127.82, 127.97, 129.45, 130.68, 131.03, 131.95, 137.77, 140.19, 141.73, 153.85, 163.44, 163.96. Anal. Calcd for $C_{28}H_{28}N_4O_5$: C, 67.19; H, 5.64; N, 11.19. Found: C, 66.76; H, 5.47; N, 11.00. ESI-MS m/z: 501.5 $[M+H]^+$. Compound **4j** (91%), yellow solid, mp 190.7–192.1 °C. IR (KBr, cm^{-1}): 3118, 2950, 2777, 1697, 1650, 1585, 1519, 1471, 1396, 1328, 1166, 1124, 1066, 838, 779. ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 2.55 (s, 6H), 2.92 (t, J = 7.2 Hz, 2H), 4.46 (t, J = 7.2 Hz, 2H), 7.73 (m, 4H), 7.86 (m, 2H), 8.25 (s, 1H), 8.26 (s, 1H), 8.63 (d, J = 8.4 Hz, 2H), 8.71 (d, J = 7.8 Hz, 2H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm): 38.41, 45.78, 57.00, 122.67, 122.97, 123.23, 124.08, 125.03, 125.94, 126.09, 126.43, 127.95, 128.63, 129.16, 129.43, 130.42, 130.99, 132.01, 135.06, 140.12, 141.49, 163.38, 163.90. Anal. Calcd for $C_{26}H_{21}N_4O_2F_3{\cdot}0.25H_2O$: C, 64.66; H, 4.49; N, 11.60. Found: C, 64.70; H, 4.30; N, 11.49. ESI-MS m/z: 479.4 $[M+H]^+$. Compound **4k** (86%), white solid, mp 180.5–181.1 °C. IR (KBr, cm^{-1}): 3102, 2962, 2796, 1695, 1656, 1587, 1382, 1351, 1276, 1176, 1122, 941, 781, 703. ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 2.58 (s, 6H), 2.96 (t, J = 7.2 Hz, 2H), 4.47 (t, J = 7.2 Hz, 2H), 7.29 (s, 1H), 7.87 (m, 2H), 8.04 (s, 2H), 8.29 (s, 1H), 8.32 (s, 1H), 8.58 (d, J = 8.4 Hz, 2H), 8.72 (d, J = 7.8 Hz, 2H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm): 38.39, 45.75, 56.99, 120.71, 122.33, 122.86, 123.02, 124.14, 125.69, 126.46, 128.11, 128.98, 129.41, 130.19, 130.92, 132.08, 132.46, 132.68, 133.83, 139.87, 141.22, 163.34, 163.86. Anal. Calcd for $C_{27}H_{20}N_4O_2F_6{\cdot}0.25H_2O$: C, 58.38; H, 3.81; N, 10.09. Found: C, 58.62; H, 3.50; N, 9.97. ESI-MS m/z: 547.4 $[M+H]^+$. Compound **4l** (96%), light yellow solid, mp 141.1–142.0 °C. IR (KBr, cm^{-1}):3118, 2971, 2771, 1697, 1654, 1587, 1515, 1396, 1371, 1238, 1043, 790, 755; ^{1}H NMR (d_6-$CDCl_3$, 600 MHz) δ (ppm): 2.37 (s, 6H), 2.68 (t, J = 7.2 Hz, 2H), 4.35 (t, J = 7.2 Hz, 2H), 6.64 (t, J = 1.8 Hz, 1H), 7.78 (m, 2H), 7.94 (s, 2H), 8.55 (d, J = 8.4 Hz, 2H), 8.66 (d, J = 7.8 Hz, 2H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm): 38.35, 45.76, 56.98, 108.05, 122.21, 122.64, 122.85, 126.55, 127.73, 129.40, 130.70, 131.06, 131.52, 131.88, 141.99, 142.37, 163.47, 163.99. $C_{19}H_{18}N_4O_2{\cdot}0.25H_2O$: C, 67.34; H, 5.50; N, 16.53. Found: C, 67.65; H, 5.28; N, 16.26. ESI-MS m/z: 335.3 $[M+H]^+$.
26. Mosmann, T. J. *Immun. Meth.* **1983**, *65*, 55.
27. Selvi, P. T.; Palaniandavar, M. *Inorg. Chim. Acta* **2002**, *337*, 420.
28. Xie, L.; Xu, Y.; Wang, F.; Liu, J.; Qian, X.; Cui, J. *Bioorg. Med. Chem.* **2009**, *17*, 804.
29. Pyle, A. M.; Rehmann, J. P.; Meshoyrer, R.; Kumar, C. V.; Turro, N. J.; Barton, J. K. *J. Am. Chem. Soc.* **1989**, *111*, 3051.
30. (a) Suh, D.; Chaires, J. B. *Bioorg. Med. Chem.* **1995**, *3*, 723; (b) Yang, X.; Liu, W.; Jin, W.; Shen, G.; Yu, R. *Spectrochim. Acta. A* **1999**, *55*, 2719; (c) Banerjee, S.; Kitchen, J. A.; Gunnlaugsson, T.; Kelly, J. M. *Org. Biomol. Chem.* **2012**, *10*, 3033; (d) Banerjee, S.; Kitchen, J. A.; Gunnlaugsson, T.; Kelly, J. M. *Org. Biomol. Chem.* **2013**, *11*, 5642.
31. Jiang, X.; Shang, L.; Wang, Z.; Dong, S. *Biophys. Chem.* **2005**, *118*, 42.
32. Maheswari, P. U.; Palaniandavar, M. *J. Inorg. Biochem.* **2004**, *98*, 219.
33. Norden, B.; Kurucsev, T. *J. Mol. Recognit.* **1994**, *7*, 141.
34. Schipper, P. E.; Norden, B.; Tjerneld, F. *Chem. Phys. Lett.* **1980**, *70*, 17.
35. Cao, Y.; He, X. *Spectrochim. Acta Part A* **1998**, *54*, 883.
36. Wang, K.; Wang, Y.; Yan, X.; Chen, H.; Ma, G.; Zhang, P.; Li, J.; Li, X.; Zhang, J. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 937.