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Discovery of a novel family of polycyclic aromatic molecules with unique reactivity and members valuable for fluorescent sensing and medicinal chemistry

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A novel polycyclic aromatic molecule, *i.e.* 1-oxo-1*H*-phenalene-2,3-dicarbonitrile (compound **1**, initially misidentified as 8-oxo-8*H*-acenaphtho[1,2-*b*]pyrrolecarbonitrile) was discovered by our group in 2005. This parent compound is highlighted for its unique oxidative S_NAr^H (nucleophilic substitution of aromatic hydrogen) reactivity that provides easy approaches to diverse derivatives with different long-wavelength fluorescence and important biological activities. To date, a large number of derivatives have been synthesized and evaluated by several international research groups, indicating the formation of a new and valuable family of functional chemicals. Some members have been functionalized for molecular or nanoparticle-based probes applicable in chemical and environmental sensing, biomolecule imaging and tumor diagnosis. Others have qualified as high potency anticancer agents specifically targeting different functional proteins in tumor cells. With regard to the increasing attention paid to this new chemical family, it is a good time to review major achievements in order to promote further and deeper investigation.

1. Introduction

Novel polycyclic aromatic compounds are easily obtained from the petroleum or coal tar chemicals and are important precursors for further modification to develop a variety of functional materials, *e.g.* dyes, sensors and medicines. The reasons are easily understandable: on the one hand, they have extended π-conjugation skeletons that are critical structural bases for chromophores and fluorophores; on the other hand, polycyclic materials are rigid, planar and hydrophobic, which allows them to insert into the cavities of biomacromolecules adapted to these small molecules in terms of size, hydrophobicity and other supramolecuar interactions. If these polycyclic precursors can undergo chemical

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Yi Xiao

Yi Xiao was born in Sichuan, in 1973. He received his BS and MS degrees from Tianjin University in 1996 and 1999, respectively. Then he moved to Dalian University of Technology (DLUT) where he received his PhD in 2003 with Prof. Xuhong Qian. After 10 months working as a postdoctoral researcher in AIST, Tsukuba, Japan, he returned DLUT. Since 2010, he is a professor of applied chemistry. He is interested in sensors and semiconductors based on fluorescent dyes.

Fengyu Liu

Fengyu Liu was born in Liaoning, in 1973. She received her BS and MS degrees from East China University of Science and Technology in 1995 and 2002, and PhD degree from Dalian University of Technology in 2006. She became associate professor (2013) in Dalian University of Technology. Her research interest focuses on electrogenerated chemiluminescence (ECL) and its applications.

modification under mild conditions, this will strengthen their importance as universal scaffolds, or leads toward practically applicable products.

In 2005, a new polycyclic compound **1** (initially misidentified to be 8-oxo-8*H*-acenaphtho[1,2-*b*]pyrrolecarbonitrile) was discovered.[1] From then on, compound **1** exhibited great attraction for researchers in different fields, not only because its planar and rigid chemical structure qualified it as desirable precursor to develop novel derivatives with diversified spectral properties or biological activities, but also because of its unique chemical reactivity, favorable for versatile and simplified derivations. To this date, a large number of compound **1**'s derivatives, as well as their promising applications as chemosensors or antitumor therapy agents, have been reported by several different research groups. Considering the growing interest in this new chemical family, it is necessary to summarize the important progress made from their discovery until now.

2. Discovery of the parent compound 1

During our research project to develop efficient fluorescent dyes for biological imaging, we noticed the unsatisfactory quantum yields of some fluorophores. It had been known that introducing electron-withdrawing cyano to the conjugated systems was favorable for fluorescence emission.[2] Previously, quite a few fluorophores had been obtained by the Knoevenagel condensations between various aromatic aldehydes/ketones and malononitrile, as shown in Scheme 1.[3] While these fluorophores demonstrated some advantages, such as long and tunable emission wavelength and environmental sensitivities, their fluorescence was not very strong in common solvents. This could be ascribed to the flexible conjugation structures; obviously, the free rotation and vibration of the carbon–carbon double bonds connecting aromatic planes to cyanos dissipated the excitation energy and thus quenched the fluorescence. Additionally, according to the experiences on

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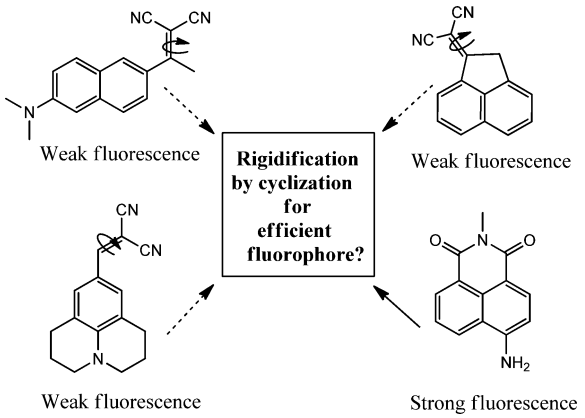
Weiping Zhu received his Bachelor's degree from the National University of Defense and Technology in 1989, and Master's degree from East China University of Science and Technology (ECUST) in 1992. After working at Yangzi Petrochemical Institute of SINOPEC for seven years, he returned and received his PhD degree at ECUST (2004). He spent one year as a visiting scholar at UCLA (2009), and now is a professor at ECUST. His research interests focus on the design and synthesis of fluorescent sensors and smart drug delivery systems.

Yufang Xu

Yufang Xu was born in Jiangsu, in 1965. She received her PhD degree in Chemistry from East China University of Science and Technology (ECUST) in 1994. Then she joined in ECUST. From 1999 to 2001 she worked as postdoctoral fellow in Tokyo Medical and Dental University. Her research interests focus on small organic molecules to regulate bioprocesses, including the design and synthesis of fluorescent probes to respond to hypoxia and related enzymes to diagnose and treat related diseases especially in solid tumours.

Xuhong Qian

Xuhong Qian was born in Jiangsu, in 1962. He received his BS, MS and PhD degrees from East China University of Science and Technology (ECUST) in 1982, 1985 and 1988, respectively. He became an associate professor (1992) and professor (1995–2000, 2004–present) in ECUST. He was a Chongkong professor in DLUT (2000–2004). He was FRSC (2009) and academician of Chinese Academy of Engineering (2011). His research interest covers certain aspects of bioorganic chemistry & engineering related to dyes and pesticides, e.g. fluorescent sensors and anti-tumor agents derived from dyes, as well as green insecticides and plant-activators.



Scheme 1 Comparison of the structures of three Knoevenagel adducts with weak fluorescence and that of strong fluorescent 4-amino-1,8-naphthalimide, indicating the strategy to obtain efficient fluorophores.

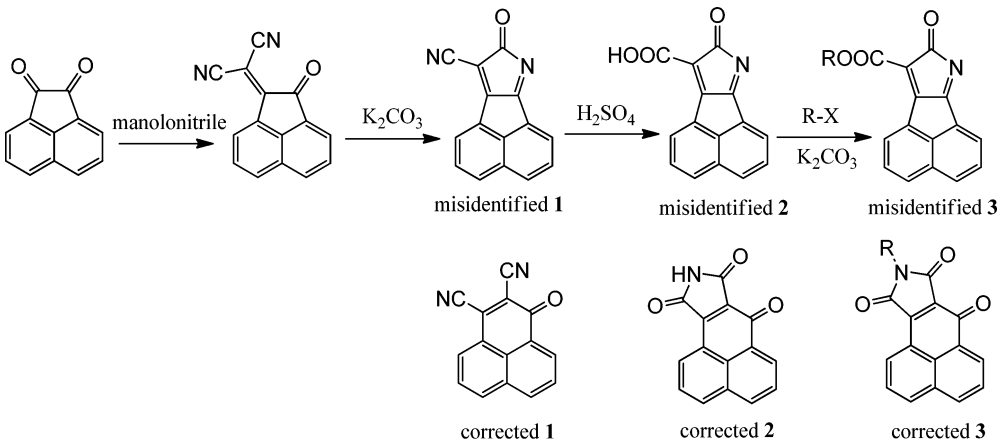
another type of fluorophores, 4-amino-1,8-naphthalimides that had fluorescence quantum yields up to 100%,[4] it was easy to conclude that the rigid polycyclic conjugation structures were essential for strong fluorescence. Thus, in order to construct novel and strong fluorophores, it became a noteworthy idea to search for a ring-formation strategy to fix the double bonds formed by Knoevenagel condensation.

Acenaphthenequinone was chosen as starting material because it was a common and cheap coal chemical product. Knoevenagel condensation of acenaphthenequinone with malononitrile was carried out, as shown in Scheme 2. When there was no basic catalyst, the monoadduct with two cyano groups was the only product. We predicted that, under basic conditions, the remaining ketone group should be activated to attack the adjacent electron-deficient double bond, which might promote a further ring-closing transformation. So, this strategy was attempted by adding anhydrous K_2CO_3 to the solution of the monoadduct intermediate, which immediately generated a yellow precipitate, *i.e.* compound **1** with high yield up to 88% and high purity. Through characterization with HRMS, ^{1}H NMR, ^{13}C NMR, IR and so on, compound **1** was identified as 8-oxo-8*H*-acenaphtho[1,2-*b*]pyrrolecarbonitrile, which has very recently been proved an error. The correct structure of compound **1** should be 1-oxo-1*H*-phenalene-2,3-dicarbonitrile (phenalene dicarbonitrile in the rest of this review). The hydrolysis of cyano groups in compound **1** was carried out to facilely get another important compound **2** (naphtho[1,8-*ef*]isoindole-7,8,10(9*H*)-trione, abbreviated as phenalene imide).[5] This molecule was also misidentified, as a result of the incorrect structure of the parent. Thankfully, by two international teams, *i.e.* Wang and Qian *et al.*,[6] and Lebreton *et al.*,[7] these misidentifications had been corrected through the analyses of X-ray crystallographic structures and 2D NMR spectra.

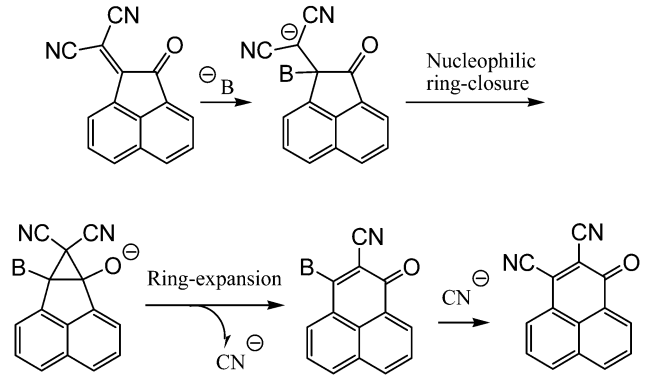
In 2014, Wang *et al.* tried to optimize the reaction *via* using a different basic catalyst, and found that an organic base was best, DMAP, and it could improve the yield of compound **1** to 92%, which was a little higher than K_2CO_3 catalysis. They also proposed a reasonable mechanism of the base-promoted ring expansion, as shown Scheme 3.[6b] Several months later, Lebreton *et al.*,[7] also proposed a very similar mechanism and confirmed it through using K^{13}CN to exchange the cyano group on position 3. Understanding this mechanism proved to be very helpful to develop new phenalene-containing molecules analogous to compound **1**, as Lebreton *et al.* had used 4-nitrophenylacetonitrile, instead of malononitrile to react with acenaphthenequinone in the presence of K_2CO_3 and obtained a novel nitrophenyl substituted phenalene derivative.[7]

3. Unique oxidative nucleophilic substitution of aromatic hydrogen (S_NAr^H): versatile and easy derivations

Looking at the structure of compound **1** and considering its features, it could be classified as an electron-deficient arene, because of the strong electron-withdrawing carbonyl and cyano groups located directly on the conjugated skeleton. According to previous studies, nucleophilic substitutions of very few electron-deficient arenes took place on the aromatic hydrogen atoms, which could be classified as a kind of green chemistry because it was the hydrogen but not a conventional



Scheme 2 The syntheses and structure identifications of novel polycyclic parent molecule **1** and its hydrolysis derivatives **2** and **3**.



Scheme 3 Mechanism of the base-catalyzed ring expansion proposed by Wang *et al.*,[6b] and Lebreton *et al.*[7]

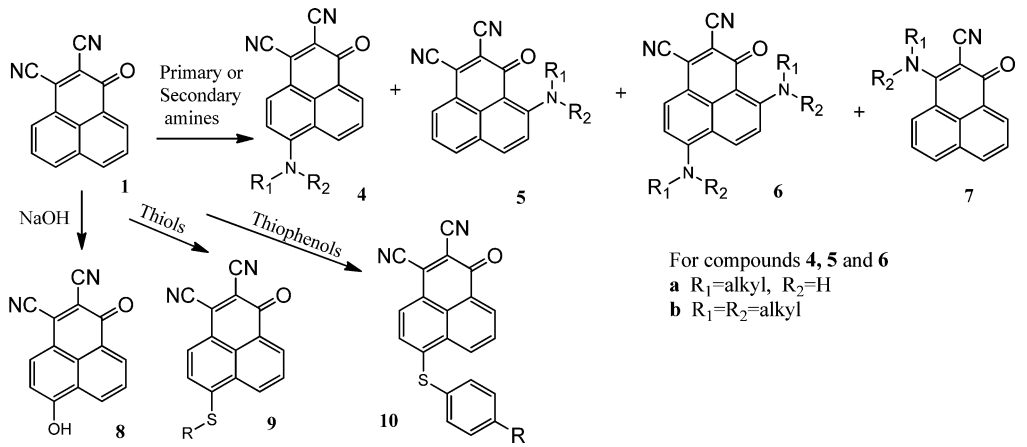
leaving group that was substituted.[8] Such a rare reaction was called oxidative nucleophilic substitution of aromatic hydrogen (S_NAr^H). With these considerations in mind, we decided to find out if it was feasible for compound **1** to undergo oxidative S_NAr^H.

Fortunately, compound **1** proved to be highly reactive to common N, S and O type nucleophiles, as shown in Scheme 4.[1,6,7] Consequently, the oxidative S_NAr^H reactions can produce variety of derivatives. Generally, the reaction conditions were very mild. For example, in acetonitrile, S_NAr^H reactions with the amines and hydroxide processed rapidly at room temperature. And it was found that both H-6 and H-9 on the phenalene ring were readily substituted by amines, which not only produced the regioisomeric derivatives **4** and **5**, but also the bis-substituted compound **6**. The yield distributions of the amine-substitution products were complicated and highly dependent on the types of amines, the reaction conditions *e.g.* mass ratios and reaction times. If reaction times were short, 6-amino derivatives would be the dominant products (yields 20–50%) when equivalent amines were used; this seemed to indicate that H-6 was the most activated aromatic hydrogen. However, if excess amounts of amines were added and the reaction time was extended, the yields of **6** would increase and it would then become the major product. For instance, when a 4–6 equivalence of piperidine reacted with compound **1** in acetonitrile solution at room temperature for 2 hours, the 6-piperidino product was up to 49%, while the 9-piperidino isomer was 30%, but no bis-substituted product was observed; when the reaction time was extended to 12 h, the yield of the 6,9-bispiperidino derivative increased remarkably to 48%. In acetonitrile, the S_NAr^H reactivity of thiols was not as high as amines: after 72 h heating, the yield of 6-dodecylsulfanyl derivative was 42%. As for hydroxide, its reaction with compound **1** could proceed smoothly in DMSO–H_2O at room temperature overnight to give compound **6** with a moderate yield (38%).

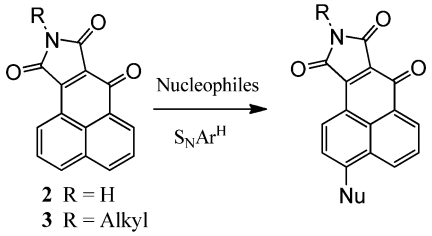
The above results confirmed that the compound **1** was a new and highly efficient platform molecule for oxidative S_NAr^H reactions and it was feasible to get different substituted derivatives by choosing nucleophiles or tuning the reaction conditions.

Other groups were inspired to try S_NAr^H reactions on compound **1** by using other kinds of nucleophiles not initially attempted by us, and also obtained positive results. For example, Wang *et al.*[6b] had successfully achieved the reactions of compound **1** with thiophenols and obtained the corresponding 6-substituted derivatives **10**. Besides S_NAr^H reactions, Lebreton *et al.* also found that the cyano-3 of compound **1** could also be substituted by amines to produce small amount of compounds **7** (*e.g.* butylamine substitution yield 3%).[7] Although this conventional nucleophilic substitution needing a leaving group was not as efficient as oxidative S_NAr^H reactions, it helped to expand the diversity of derivatives from parent **1**.

We reasoned that compound **2** and **3** should inherit high S_NAr^H reactivity from its parent **1** because their conjugation structures remained highly electron-deficient. As expected, compound **2** and **3** could also be readily substituted by various nucleophiles to produce the corresponding 6-substituted derivatives, as shown in Scheme 5.[5] As their unique S_NAr^H reactions with a variety of nucleophiles provided versatile and easy approaches to a large number of derivatives, compounds **1**, **2** and **3** became valuable scaffolds for the development of new functional chemicals.



Scheme 4 Nucleophilic substitution reactions of compound **1** with different types of nucleophiles.



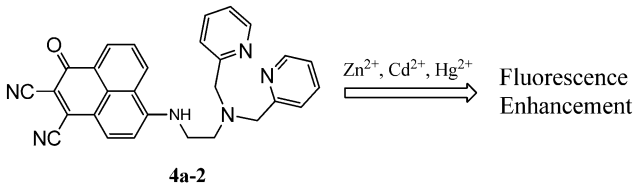
Scheme 5 Oxidative S_NAr^H reactions of compounds **2** and **3**.

4. Favourable fluorescence characteristics for the development of fluorescent sensors

While the parent compound **1** is nonfluorescent, most of the 6-substituted derivatives presented strong long-wavelength fluorescence.[1,6b] For instance, the fluorescence quantum yields of compound **4a-1** (butylamine substituted), **8** and **9** (dodecanethiol substituted) were 0.81 (in dichloromethane), 0.87 (in chloroform) and 0.43 (in chloroform), respectively. Firstly, this fluorogenic phenomenon should be ascribed to these derivatives' ICT (intramolecular charge transfer) nature: with strong electron-withdrawing cyano and carbonyl groups on one side of the conjugation skeleton, the introduction of electron-donating groups (nucleophiles) to the other opposite side (position-6) constructed the donor–π–acceptor structure. That was the origin of their ICT processes. The 9-substituted derivatives had been reported to emit much weaker fluorescence, due to the improper location of the donating group, unfavorable for ICT. The ICT fluorophores are usually sensitive to environmental polarity. This is especially true for 6-piperidino compound **4b-1** (on behalf of all the 6-secondary-amine substituted derivatives) whose fluorescence quantum yield is up to 0.38 in toluene but it is almost nonfluorescent in acetonitrile. Another advantage of these new ICT fluorophores was their high photostability, because the electron-deficiency inherited from their parent made them more resistant against photooxidation (one of the major reasons for light-induced decomposition). More interestingly, different 6-substituted derivatives exhibited tunable fluorescence in the long wavelength range of visible light, because nucleophiles with different electron donating capability strengthened the ICT to different extents. As a result, the emission maxima of compounds **4a-1**, **9** and **8** were at around 595, 567 and 548 nm, respectively, and so, their fluorescences were in different colors (orange, yellow and green). Owing to these advantageous fluorescence properties *e.g.* longer and tunable wavelength, high photostability, environmental sensitivity and the convenient synthesis *etc.*, the fluorophores from this phenalene dicarbonitrile family have been adopted for the construction of various small-molecule probes and nanoparticle-based multifunctional chemosensors.

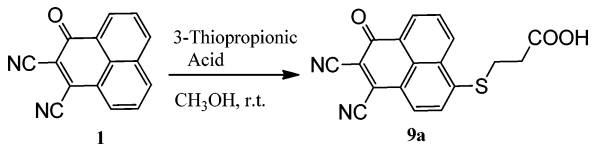
4.1 Molecular probes

As 6-amino phenalene dicarbonitrile fluorophores for the long wavelength emission and convenient synthesis through S_NAr^H, Akkaya *et al.*[9] employed this fluorophore for the first time to develop a fluorescent chemosensor for metal ions (compound **4a-2** in Scheme 6). In their design, they adopted aminoethyl dipicolylamine as the receptor of the cations, and facilely introduced this unit to the 6-position of the phenalene dicarbonitrile through S_NAr^H under the same reaction conditions previously provided by us. Compound **4a-2** responded selectively to group IIB cations by remarkable fluorescence enhancement through inhibiting the fluorescence quenching process of PET (photoinduced electron transfer) from the dipicolylamine to the excited fluorophore. For example, the emission intensity at the peak emission wavelength of 588 nm increased 7-fold upon Zn(II) binding. Benesi–Hildebrand analysis of the binding data revealed strong association constants: 2.3×10^6 for Zn(II), 5.4×10^5 for Cd(II) and 2.9×10^6 for Hg(II), all in M^{-1}.



Scheme 6 A fluorescent molecular probe of group IIB cations.

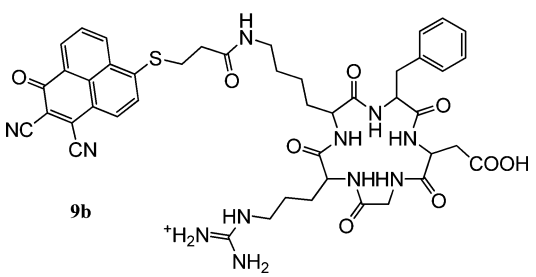
While our group reported that the S_NAr^H reaction of the parent **1** with *n*-dodecyl thiol required heating in acetonitrile,[1b] Li *et al.* optimized the conditions by using protic solvents. As shown in Scheme 7, they found that in methanol, the reaction of **1** with 3-thiopropionic acid can efficiently occur at room temperature to produce the corresponding 6-thiol substituted compound **9a**. Because Cys and Hcy, are similar in chemical structure to 3-thiopropionic acid, Li *et al.* then reasoned that the reaction of **1** with Cys or Hcy would readily promote a fluorescent recognition of these thiol-containing amino acids critical for cell function. As they had expected, the parent **1** proved to be an ideal 'naked-eye' visible and fluorescent probe for Cys or Hcy. In a mixture of methanol and HEPES solution at pH 7, upon addition of Cys or Hcy, the absorption band of **1** centered at 430 nm gradually decreased and a new absorption band centered at 580 nm appeared, and an emission peak at 588 nm increased rapidly. The observed spectral changes indicated that the oxidative S_NAr^H reaction took place. The final fluorescence enhancement was up to 75 fold in the presence of 40 equivalents of Hcy, which revealed a high sensitivity. Subsequently, compound **1** was successfully applied for the imaging of Cys/Hcy under confocal laser scanning microscopy and two-photon laser scanning microscopy. Since this new sensor's excitation and emission were in longer wavelength ranges than the previous reported Cys/Hcy probes, compound **1** was of great benefit for studying the effects of Cys/Hcy in biological systems.[10]



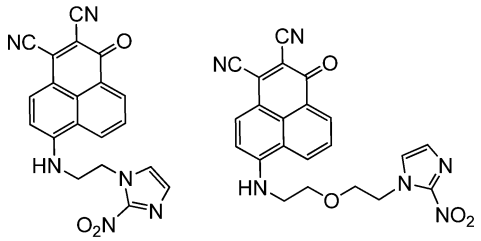
Scheme 7 S_NAr^H reaction under optimized conditions.

In order to achieve specific tumor imaging, Li *et al.*[11] developed a probe (compound **9b** in Scheme 8) composed of a 6-thiol phenalene dicarbonitrile as fluorophore and a cyclic peptide, c(RGDFK) as the targeting unit, based on the knowledge that an arginine–glycine–aspartic acid tripeptide sequence could specifically bind integrin $\alpha_v\beta_3$ of tumor cells. Although there had been some other probes constructed by the same strategy, the major limitation was their intolerance of photobleaching. Li *et al.* carried out photodecomposition test in cells under the confocal microscope and found that probe **9b** had much better photostability than Acridine Orange, a commonly used cellular fluorescent label. Moreover, probe **9b** possessed high solubility and exhibited very low cytotoxicity. These features were favorable for cell biology applications. Two different cell lines were chosen: one was human cervical carcinoma HeLa cells overexpressing integrin $\alpha_v\beta_3$, and the other was human hepatocellular carcinoma SMMC-7721 cells as a control. A strong fluorescence signal was observed in the HeLa cells, whereas the SMMC-7721 cells (expressing low amounts of $\alpha_v\beta_3$) showed much less fluorescence. This comparison confirmed that probe **9b** specifically targeted $\alpha_v\beta_3$, and thus qualified it as a practical diagnostic tool for detecting certain tumor cells expressing $\alpha_v\beta_3$.

Solid tumor cells are hypoxic and these hypoxic cells are more resistant to radiation than cells under well-oxygenated conditions. Thus, to predict better treatment outcomes and select appropriate therapies, it is very meaningful to differentiate accurately the hypoxic cells from cells under normal oxygenation status. In 2006, our group attached the novel 6-amino phenalene dicarbonitrile fluorophore to the classical hypoxic-environment-targeting molecule nitroimidazole, and developed long wavelength (maximum emission at 592 nm) hypoxic fluorescent indicators, *e.g.* **4a-3** and **4a-4**, as shown in Scheme 9.[12] This work was carried out because a number of previously developed intracellular hypoxic fluorescent markers had unsatisfactory detection accuracy or sensitivity, as biological background fluorescence can interfere with their emissions at relatively short wavelength ranges. The molecular design utilized two properties of nitroimidazole: it had a strong fluorescence quenching effect, and it could undergo bioreduction in hypoxic cells to generate a product with reduced quenching ability. This was why the markers could release 'turn-on' fluorescent signals in hypoxic cells while in normal cells no fluorescence enhancement was observable. After 3 h incubation of V79 379A Chinese hamster cells, **4a-4** and **4a-3** showed remarkable 15- and 11-times fluorescence enhancement, respectively, in the hypoxic cells compared to the cells at normal oxygenation status. The reason that **4a-4** outperformed



Scheme 8 A fluorescent probe for targeted imaging of tumor cells.



Scheme 9 Two fluorescent probes for the hypoxic environment of solid tumors.

4a-3 could be partly ascribed to the better solubility and higher cell uptake brought by the ester-type spacer between the fluorophore and the nitroimdazole.

4.2 Nanoparticle/polymer-based fluorescent chemosensors

Small molecular probes have some limitations: they have a relatively low sensitivity, due to the relatively low fluorescence intensity of a single dye molecule; they cannot be reused; and usually a molecular probe has a single function. Nanoparticle-based fluorescent sensors can overcome these major problems. In order to develop silica nanoparticles as multifunctional sensing materials, we introduced a siloxane unit to the 6-amino phenalene dicarbonitrile fluorophore.[13] This fluorescent siloxane derivative underwent hydrolysis to form a core for the core–shell nanoparticles; and this core emitted a stable long-wavelength fluorescence (peaking at 595 nm) from the 6-amino phenalene dicarbonitrile derivative, which did not experience interference from environmental factors, *e.g.* pH; and thus this fluorescence signal could act as an inner reference. Outside the core, a silica shell loaded with fluorescent probe was formed to sense selectively Zn^{2+} with a 'turn-on' signal. As shown in Fig. 1, since the shell's short-wavelength fluorescence (centered at 480 nm) from the zinc probe could be clearly differentiated from that of the core, this created an ideal ratiometric sensing platform (**DSSN**) to quantify accurately zinc ions, not only in aqueous solution but also in live cells. After **DSSN** binding of Zn^{2+} to form the new nanoparticle named **DSSN@Zn^{2+}**, its regeneration was simple and the recovered **DSSN** could be reused again. It was proved that **DSSN** could be recycled at least four times without losing Zn^{2+} sensitivity. It was more interesting that **DSSN@Zn^{2+}** could be used to recognize selectively $H_2PO_4^-$ against other anions. This was because $H_2PO_4^-$ was an efficient Zn^{2+} binder, and such binding decreased the probe's fluorescence intensity and changed the intensity ratio of the zinc probe to the inner reference fluorophore. In a neutral aqueous solution, **DSSN@Zn^{2+}** exhibited a detection limit lower than 6×10^{-6} M, indicating a high sensitivity to $H_2PO_4^-$.

Different from our strategy to synthesize a siloxane-substituted fluorophore for direct modification of silica nanoparticles, Spange *et al.*[14] established a novel and convenient two-step approach toward fluorophore-functionalized silica particles. As demonstrated in Scheme 10, they used silica as a 'solubilizer' which could absorb the water insoluble precursor **1** and water soluble poly(vinyl amine) (PVAm) and simultaneously provided them a large specific surface area to undergo the S_NAr^H reaction. If without silica, such a reaction

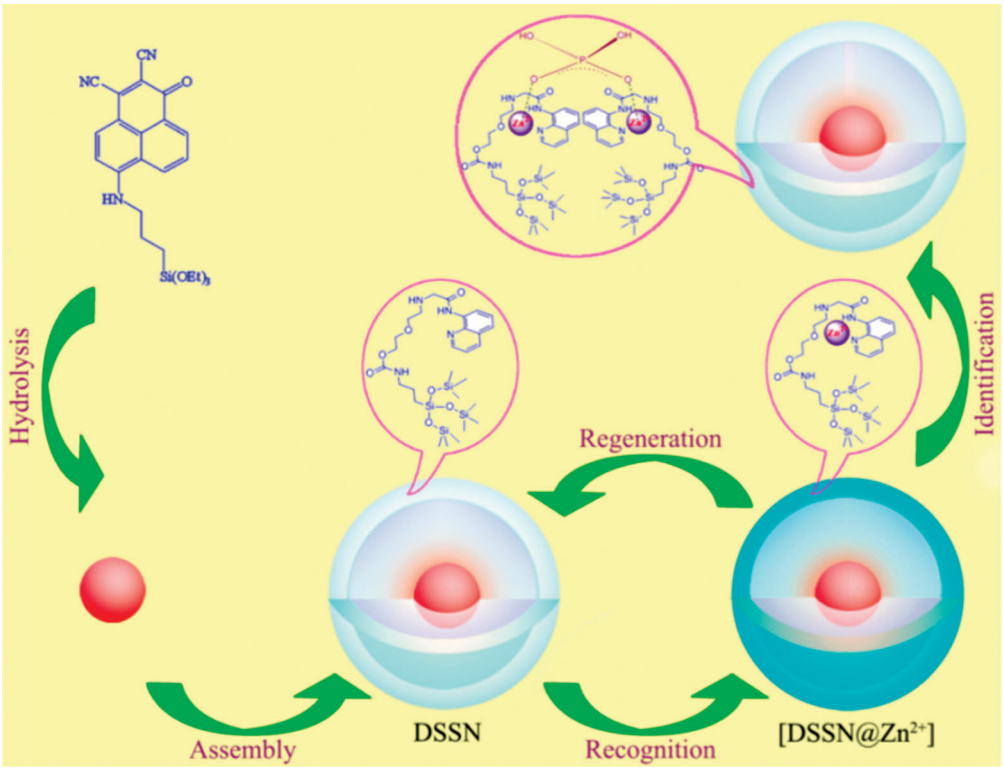
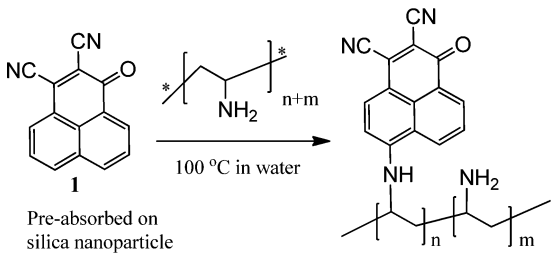


Fig. 1 The structure of a siloxane-modified fluorophore and the design concept of nanoparticle-based ratiometric chemosensors for Zn^{2+} and $H_2PO_4^-$.

between the two incompatible reactants needed the aid of cyclodextrins. They also found that the fluorophore substituted PVAm exhibited pH-sensitive fluorescence spectral shifts: in the alkaline aqueous solution the fluorescent polymer possessed two emission bands peaked at 515 and 579 nm, respectively; with decreasing pH value, the intensity of the short wavelength emission band decreases, whereas for the emission band at the longer wavelength a slightly bathochromic shift was observed. These phenomena should be associated with the characteristics of the PVAm: under acidic conditions, the amino groups were protonated and the mutual exclusion between the positive charged ammoniums resulted in stretching of the polymer chains, and thus the interactions between fluorophores were slight; but in basic solution, the curling chains of PVAm brought fluorophores closer to each other and thus strengthened their intramolecular interactions. Therefore, Spange *et al.* successfully developed a multifunctional hybrid material possessing the advantages of silica nanoparticles, the 'smart' pH-response of PVAm and the environment-sensitive fluorescence of the new fluorophore.



Scheme 10 The synthesis strategy of polymer–silica hybrid fluorescent particles by nucleophilic aromatic substitution of **1** adsorbed onto silica particles.

5. Phenalene dicarbonitrile and imide derivatives as anticancer drug leads

5.1 Discovery of anticancer activities: DNA intercalators or protein inhibitors

Some electron-deficient polycyclic aromatic molecules, *e.g.* naphthalimides, anthracyclines exhibit antitumor activities and have become important drug leads. For long time, our group has been investigating anticancer agents based on naphthalimide as DNA intercalators.[15] It was no surprise that we immediately began experimental evaluation of the biological activities of the new phenalene dicarbonitrile and imide derivatives, taking into account their similarity in terms of chemical structure and electron deficiency to naphthalimides. As was expected, quite a few derivatives exhibited strong cytotoxicity to cancer cells, with submicromolar IC_{50} values, which qualified their status as drug leads. Scheme 11 lists three representative examples of higher activity: compound **4b-2** showed an IC_{50} of 0.17 μM against the human cervical carcinoma (HeLa) cell line; compound **3a** showed IC_{50} values of 0.45 and 0.80 μM against A549 and P388 cell lines; and the IC_{50} values of compound **10** against the A549 and P388 cell lines were 0.14 and 0.019 μM, respectively.

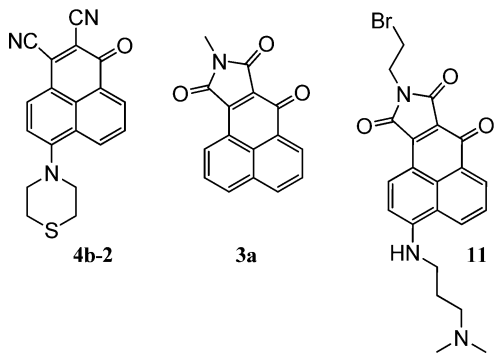
As the naphthalimide-based drugs were well-known DNA intercalators,[16] we initially attempted to explain the antitumor activities of these new compounds by their DNA interacting abilities.

Actually, they indeed demonstrated strong DNA binding in solution, as we had evaluated in DNA melt curves and circular dichroism experiments.[17] However, during the preliminary investigation of the mechanism of the compound **4b-2**, it was discovered that this compound could inhibit the anti-apoptotic B-cell lymphoma 2 (Bcl-2) interacting protein and thus induce tumor-cell apoptosis.[18] This result indicated that some new phenalene dicarbonitrile and imide derivatives, or at least compound **4b-2**, may bind specifically to and influence certain functional proteins, instead of DNA. Therefore, subsequent studies should be focused on the identification of the targeted proteins to lay the foundation to achieve higher specificity and stronger inhibition of tumors.

5.2 Anticancer mechanism 1: dual inhibitors of Mcl-1 and Bcl-2

Zhang, one of the former members at our group, independently carried out further research on the antitumor mechanisms of compound **4b-2**. Subsequently it was found that **4b-2** inactivated not only Bcl-2, but also Mcl-1, indicating that this compound was an inhibitor of *pan*-BCl-2 proteins. Zhang successfully clarified that compound **4b-2** could insert into and occupy the hydrophobic BH3 groove, the critical domain on the surface of some prosurvival Bcl-2-like proteins including Bcl-2 and Mcl-1. Nanomolar compound **4b-2** could inhibit them (K_i = 58 and 310 nM against Bcl-2 and Mcl-1, respectively) and thus induced tumor cell apoptosis efficiently.[19]

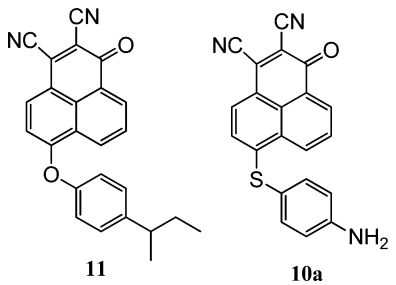
Based on their verification of compound **4b-2** as a novel molecular BH3 mimetic and a potent drug lead, Zhang *et al.* focused on the search for more efficient *pan*-BCl-2 inhibitors derived from the family of phenalene dicarbonitrile.[20,21] They designed and synthesized two series of 6-phenoxy and 6-phenylthio substituted phenalene dicarbonitrile derivatives. Among them, the *p*-sBu phenoxy compound **11**, and the *p*-amino phenylthio compound **10a** (Scheme 12) were screened to exhibit nanomolar affinities toward Mcl-1 and Bcl-2, as well as nanomolar cytotoxicity activity against multiple cancer cell lines. The affinity for **11** toward Mcl-1 and Bcl-2 (K_i = 24 nM and 158 nM, respectively) was enhanced by about 2–3 times compared to the lead compound **4b-2**. The IC_{50} of compound **10a** to Mcl-1 (5 nm) was significantly better than that of compound **4b-2**, while it maintained a similar affinity to Bcl-2. It was also exciting that compound **10a**'s cytotoxicity against tumor cells (IC_{50} = 12 nM and 16 nM against MCF-7 and MMC-7721, respectively) was 10-fold stronger than that of the compound **4b-2**.



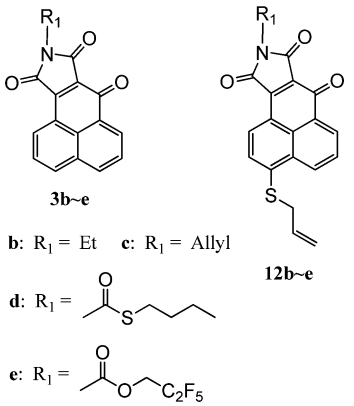
Scheme 11 Three early discovered compounds with high antitumor activities.

5.3 Anticancer mechanism 2: FGFR1 inhibitors

Inhibition of FGFRs (the fibroblast growth factor receptors 1–4) represents an attractive tumor therapeutic strategy. However, on account of the structural homology of the catalytic domains of various kinases, most existing FGFRs inhibitors inhibit different tyrosine kinases nonspecifically, which might cause side effects. In 2011, we reported for the first time potent and selective FGFR1 inhibitors based on phenalene imide derivatives.[22] After verifying the great *in vitro* antiproliferative effects of a simple phenalene imide (compound **3b**, in Scheme 13), we continued the mechanistic investigation and successfully identified FGFR1 as the molecular target. Compound **3b** not only demonstrated potent inhibition (IC_{50} = 74 nM) of FGFR1 *in vitro*, and exhibited remarkable selectivity toward FGFR1 compared to other tested tyrosine kinases, including other FGFRs and highly homologous enzymes PDGFRs and VEGFRs. Subsequently, a number of phenalene imides and 6-thiol substituted phenalene imides were designed and synthesized for the systematic SAR investigation. In our design, we were trying to use the unsaturated hydrocarbon, fluorine or sulfur-containing side chains to modify the precursor, because, based on our research experience, these were beneficial factors to improve biological activities. It was found that for the phenalene imides, the introduction of different side chains to the imide nitrogen was critical for FGFR1 inhibition. Especially, compound **3c**, possessing an allyl group on the imide, presented the strongest inhibition with a much lower IC_{50} (19 nM) than compound **3b**. While the 6-thiol substituted phenalene imides demonstrated weaker inhibition than corresponding non substituted phenalene imides, it was interesting that



Scheme 12 Two highly efficient dual inhibitors of Mcl-1 and Bcl-2.



Scheme 13 Highly efficient FGFR1 inhibitors.

the groups on the thiol sulfur atom affected the activities remarkably. Again, the allyl thiol substituted molecule (compound **12c**) showed the lowest IC_{50} of 216 nM. *In vitro* antiproliferative assays were also carried out and the results confirmed that these new synthesized FGFR1 inhibitors inhibited the proliferation of human cancer cell lines much efficiently than normal cells. The above discovery of phenalene imides as novel FGFR1 inhibitors provided a promising starting point for further drug optimization and development as novel anticancer agents.

6. Conclusion and prospect

Starting from acenaphthenequinone, through the base-promoted ring expansion, we synthesized a novel rigid polycylic molecule **1**, 1-oxo-1*H*-phenalene-2,3-dicarbonitrile. We discovered that compound **1**'s aromatic hydrogen atoms on position 6 and 9 were highly activate for the oxidative S_NAr^H reaction by the N, O and S types of nucleophiles. By choosing different nucleophiles and by adjusting the reaction conditions, a large number of 6- or 9-substituted derivatives and 6,9-bis-substituted derivatives could be conveniently prepared. Additionally, hydrolyzing the two cyano groups of compound **1** would result in another 5-membered dicarboxylic acid imide ring fused to the phenalene plane. More importantly, aromatic hydrogen-6 of the NH imide **2** and the alkylated imides **3** inherited the oxidative S_NAr^H reactivity from the parent **1**.

Since the discovery of the polycyclic aromatic parent **1** as well as its unique oxidative S_NAr^H reactions, the family of phenalene dicarbonitriles has already attracted much attention for their rigid and planar structures, convenient syntheses and modifications, diverse fluorescence properties, biological activities and so on. As typical ICT fluorophores with long wavelength and high photostability, they have proved to be good candidates for the construction of molecular or nanoparticle probes/sensors. As drug leads, at least two types of highly potent inhibitors specifically targeting different tumor regulating proteins, *i.e. pan*-BCl-2 and FGFR1, have been developed rationally.

It is believed that the interest in this promising chemical family will last for a long time and investigations to promote their applications will go on. From the viewpoint of fluorescence imaging, currently, the important fields at the forefront are single molecular imaging and supraresolution imaging which require the fluorophore to be highly photostable and intensivelt fluorescent; these are exactly the characteristics of the members of this family; thus, they are highly recommended for these sophisticated imaging applications. From the viewpoint of drug leads, identification of the targeted biological macromolecules, especially the functional proteins, are extremely important; to date, the work to find the potential targets of this new chemical family is still very limited; the combination of modern bioinformatics and high-throughput drug screening technology may help to identify more targets other than *pan*-BCl-2 and FGFR1, and thus open new avenues to efficient antitumor agents; and combinatorial syntheses based on oxidative S_NAr^H of compound **1**, **2** or **3** should be utilized to provide large libraries of compounds for SAR study and structure optimization.

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Notes and references

1 (*a*) Y. Xiao, F. Liu, X. Qian and J. Cui, *Chem. Commun.*, 2005, 239; (*b*) F. Liu, Y. Xiao, X. Qian, Z. Zhang, J. Cui and R. Zhang, *Tetrahedron*, 2005, **61**, 11264.
2 X. Qian and Y. Xiao, *Tetrahedron Lett.*, 2002, **43**, 2991.
3 (*a*) M. A. Haidekker, T. Ling, M. Anglo, H. Y. Stevens, J. A. Frangos and E. A. Theodorakis, *Chem. Biol.*, 2001, **8**, 123; (*b*) A. Jacobson, A. Petric, D. Hogenkamp, A. Sinur and J. R. Barrio, *J. Am. Chem. Soc.*, 1996, **118**, 5572.
4 X. Qian, Y. Xiao, Y. Xu, X. Guo, J. Qian and W. Zhu, *Chem. Commun.*, 2010, **46**, 6418.
5 (*a*) F. Liu, X. Qian, J. Cui, Y. Xiao, R. Zhang and G. Li, *Bioorg. Med. Chem.*, 2006, **14**, 4639; (*b*) L. Xie, Y. Xiao, F. Wang, Y. Xu, X. Qian, R. Zhang, J. Cui and J. Liu, *Bioorg. Med. Chem.*, 2009, **17**, 7615.
6 (*a*) H. L. Li, P. J. Pellechia, Y. Xiao, M. D. Smith, G. R. Wang, X. Qian and Q. Wang, *Chem. Commun.*, 2014, **50**, 9596; (*b*) H. L. Li, F. Liu, Y. Xiao, P. J. Pellechia, M. D. Smith, X. Qian, G. R. Wang and Q. Wang, *Tetrahedron*, 2014, **70**, 5872.
7 R. Lenk, A. Tessier, P. Lefranc, V. Silvestre, A. Planchat, V. Blot, D. Dubreuil and J. Lebreton, *J. Org. Chem.*, 2014, **79**, 9754.
8 M. Mąkosza and K. Wojciechowski, *Chem. Rev.*, 2004, **104**, 2631.
9 A. Coskun, M. D. Yilmaz and E. U. Akkaya, *Tetrahedron Lett.*, 2006, **47**, 3689.
10 M. Zhang, M. X. Yu, F. Y. Li, M. W. Zhu, M. Y. Li, Y. H. Gao, L. Li, Z. Q. Liu, J. P. Zhang, D. Q. Zhang, T. Yi and C. H. Huang, *J. Am. Chem. Soc.*, 2007, **129**, 10322.
11 L. Q. Xiong, M. X. Yu, M. J. Cheng, M. Zhang, X. Y. Zhang, C. J. Xu and F. Y. Li, *Mol. BioSyst.*, 2009, **5**, 241.
12 Y. Liu, Y. Xu, X. Qian, Y. Xiao, J. Liu, L. Shen, J. Li and Y. Zhang, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 1562.
13 C. He, W. Zhu, Y. Xu, Y. Zhong, J. Zhou and X. Qian, *J. Mater. Chem.*, 2010, **20**, 10755.
14 K. Hofmann, I. Kahle, F. Simon and S. Spange, *Beilstein J. Org. Chem.*, 2010, **6**, 79, DOI: 10.3762/bjoc.6.79.
15 Z. Chen, X. Liang, H. Zhang, H. Xie, J. Liu, Y. Xu, W. Zhu, Y. Wang, X. Wang, S. Tan, D. Kuang and X. Qian, *J. Med. Chem.*, 2010, **53**, 2589.
16 S. Banerjee, E. Veale, C. Phelan, S. Murphy, G. Tocci, L. Gillespie, D. Frimannsson, J. Kelly and T. Gunnlaugsson, *Chem. Soc. Rev.*, 2013, **42**, 1601.
17 Z. Zhang, Y. Yang, D. Zhang, Y. Wang, X. Qian and F. Liu, *Bioorg. Med. Chem.*, 2006, **14**, 6962.
18 Z. Zhang, L. Jin, X. Qian, M. Wei, Y. Wang, J. Wang, Y. Yang, Q. Xu, Y. Xu and F. Liu, *ChemBioChem*, 2007, **8**, 113.
19 (*a*) Z. Zhang, Y. Liu, T. Song, Z. Xue, X. Shen, F. Liang, Y. Zhao, Z. Li and H. Sheng, *Br. J. Cancer*, 2013, **108**, 1870; (*b*) Z. Zhang, T. Song, T. Zhang, J. Gao, G. Wu, L. An and G. Du, *Int. J. Cancer*, 2011, **128**, 1724; (*c*) Z. Zhang, G. Wu, J. Gao and T. Song, *Mol. Pharmaceutics*, 2010, **7**, 1348; (*d*) T. Song, X. Chang, Z. Zhang, Y. Liu and X. Shen, *J. Pharmacol. Sci.*, 2012, **119**, 330.
20 Z. Zhang, H. Yang, G. Wu, Z. Li, T. Song and X. Li, *Eur. J. Med. Chem.*, 2011, **46**, 3909.
21 (*a*) Z. Zhang, G. Wu, F. Xie, T. Song and X. Chang, *J. Med. Chem.*, 2011, **54**, 1101; (*b*) T. Song, Q. Chen, X. Li, G. Chai and Z. Zhang, *J. Med. Chem.*, 2013, **56**, 9366.
22 (*a*) Z. Chen, X. Wang, W. Zhu, X. Cao, L. Tong, H. Li, H. Xie, Y. Xu, S. Tan, D. Kuang, J. Ding and X. Qian, *J. Med. Chem.*, 2011, **54**, 3732; (*b*) Z. Chen, X. Wang, W. Zhu, X. Cao, L. Tong, H. Li, H. Xie, Y. Xu, S. Tan, D. Kuang, J. Ding and X. Qian, *J. Med. Chem.*, 2014, **57**, 1621.

