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Original article

Ultrasound-assisted bismuth nitrate-induced green synthesis of novel pyrrole derivatives and their biological evaluation as anticancer agents

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ABSTRACT

A series of novel *N*-substituted pyrrole derivatives have been designed and synthesized following ultrasound-assisted and bismuth nitrate-catalyzed eco-friendly route. This reaction has also provided a general method to prepare diverse varieties of *N*-substituted pyrroles with less nucleophilic polyaromatic amines. Based on ^{1}H NMR spectroscopy, a plausible mechanistic pathway has been advanced. Cytotoxicity of some selected *N*-substituted pyrrole derivatives has been evaluated *in vitro* in a panel of mammalian cancer cell lines which includes liver cancer cell lines (HepG2 and Hepa1-6), colon cancer cell lines (HT-29 and Caco-2), a cervical cancer cell line (HeLa) and NIH3T3 cells. Two compounds, 5-(1*H*-pyrrol-1-yl)-1,10-phenanthroline (**9**) and 1-(phenanthren-2-yl)-1*H*-pyrrole (**10**) have shown good cytotoxicity against some cancer cell lines. Furthermore, these compounds have exhibited cytotoxic specificity against liver cancer cell lines *in vitro* when compared with normal cultured primary hepatocytes.

1. Introduction

According to the World Health Organization, cancer is a leading cause of death worldwide that accounted for 7.6 million deaths in 2008 [1]. In addition to the extensive use of irradiation and surgical treatment, chemotherapy still plays an important role for the treatment of cancer. In this context, the search for novel chemotherapeutic agents is an interesting and continually evolving field of cancer research.

Pyrroles are found to be as the fundamental structural motifs in various classes of natural and biologically important molecules such as porphyrins, bile pigments, coenzymes, and alkaloids [2–7]. This moiety is also present in several synthetic pharmaceuticals as well as electrically conducting polymers [8–10]. Consequently, many methods for the synthesis of diversely substituted pyrroles have been developed [11–24]. However, the most reliable method for the synthesis of pyrroles is the Paal–Knorr reaction [25,26]. Previous studies from our group have focused on the synthesis of polyaromatic compounds and their biological activities as anticancer agents [27–32]. On this basis, we have become interested in the synthesis of pyrroles bound to polyaromatic skeletons and their potential use as anticancer agents.

Herein we report a simple ultrasound-assisted eco-friendly practical method for the synthesis of *N*-substituted pyrroles by reacting 2,5-dimethoxytetrahydrofuran (**1**) with various amines in the presence of catalytic amounts (5 mol%) of bismuth nitrate pentahydrate under solvent-free conditions. This reaction produces pyrroles in excellent yield and within a short period of time. Two of these pyrrole conjugated derivates have caused cytotoxicity in multiple cancer cell lines *in vitro*, and have demonstrated cytotoxic specificity against liver cancer cell lines *in vitro* when compared with normal cultured primary hepatocytes.

2. Results and discussion

2.1. Chemistry

In recent years organic reactions in solvent-free condition have received tremendous priority in view of green methodology

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[33,34]. In view of the fact that Paal–Knorr procedure requires a 1,4-dicarbonyl compound and Lewis acid as catalyst, we predict that our work on bismuth nitrate-catalyzed [35,36] reactions on acetal and glycosylation may prove useful for the success of the reaction if 2,5-dimethoxytetrahydrofuran (**1**) is selected as one of the reactants. This concept has been tested and we disclose herein the preparation of a number of new pyrroles using ultrasound irradiation. Ultrasonic exposure of amine (**2**) with 2,5-dimethoxytetrahydrofuran (**1**) in the presence of catalytic amount (5 mol%) of bismuth nitrate pentahydrate produced excellent yield of the corresponding pyrroles (**3**) (Scheme 1).

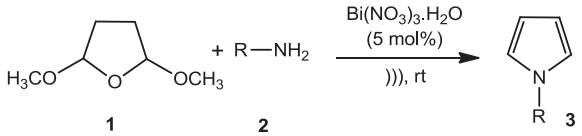
The methodology has also been successfully examined with a number of amines. First of all, the amount of the catalyst *viz*. $Bi(NO_3)_3\cdot 5H_2O$ was optimized by reacting aniline (1 mmol) with 2,5-dimethoxytetrahydrofuran (1.2 mmol) under ultrasound irradiation at room temperature for 5 min. The results are presented in Table 1. This data clearly shows that the presence of 5 mol% of $Bi(NO_3)_3\cdot 5H_2O$ is necessary for a successful reaction in excellent (99%) yield.

Diverse amines (for example, aromatic, aliphatic, polyaromatic and heteropolyaromatic amines) react with 2,5-dimethoxytetrahydrofuran (**1**) in presence of catalytic amount (5 mol%) of bismuth nitrate pentahydrate under ultrasound irradiation to give excellent yield of the corresponding pyrroles. The results are depicted in Table 2.

Notably, our current method is better than the existing methods because we have not used any solvent. Our catalyst $Bi(NO_3)_3\cdot 5H_2O$ is eco-friendly and non-toxic. According to our scientific knowledge, this solvent-free method is superior to the method where acetic acid was used as solvent. Most importantly, we could not prepare pyrroles by reacting polyaromatic amines and **1** in the presence of acetic acid. These amines are not nucleophilic enough to undergo condensation with a carbonyl group unless there is appropriate activation. Acetic acid was used for the preparation of pyrroles under drastic conditions with amines that have much higher basicity and nucleophilicity. In contrast, our method with non-toxic bismuth nitrate worked very well with different types of amines, even with amines that are very weakly nucleophilic in nature.

Based on the nature of the substrate, catalyst and condition of the experiment, a most probable mechanism is advanced. The methoxy groups in **1** can undergo a deprotection reaction under mild acidic conditions and this process can be highly facilitated by ultrasound irradiation. The intermediate can easily form the reactive dialdehyde **5**. The reactive dialdehyde **5** on reaction with amines can lead to pyrroles **3** following a nucleophilic addition and subsequent dehydration–aromatization route. This reaction suggests the capability of bismuth nitrate to serve as a Lewis activator. Proton NMR spectroscopy has been used to strengthen the proposed mechanism as in Scheme 2.

Upon irradiating a $CDCl_3$ solution of **1** for 5 min, ^1H NMR has been taken. A downfield signal due to the –CHO group is observed. The intensity of the –CHO group becomes more predominant in the ^1H NMR when **1** was irradiated in $CDCl_3$ in the presence of catalytic amounts of bismuth nitrate. This suggests the formation of **5** in the reaction media in the presence of bismuth nitrate under ultrasonic irradiation. Demethylation should take place in compound **1** only because electrophilic reagent bismuth nitrate should attack compound **1**. There is sufficient scope to use this reaction for the synthesis of several biologically active compounds as described herein.



Scheme 1. $Bi(NO_3)_3.5H_2O$ catalyzed ultrasound-induced synthesis of *N*-substituted pyrroles.

Our method has been tested with a wide range of amines and no side products are identified in any of these examples. In the case of adamantanyl amine (Entry 6, Table 2), there was a possibility of molecular rearrangement in the presence of Lewis acid but interestingly no rearranged product was observed. The corresponding product *viz*. 1-(adamantan-1-yl)-1*H*-pyrrole was isolated in good (79%) yield. The spectral and m.p. data were identical with our previous reports [15–17]. The structure is confirmed by X-ray crystallographic analysis [37]. The ORTEP projection is shown in Fig. 1.

The reaction between **1** and **2** can give pyrrole (about 55%) in the absence of bismuth nitrate by irradiating the reaction mixture for a long time (approximately 5 h). Multicyclic aromatic amines failed to yield the products in the absence of catalyst. However, the reaction gives products when ultrasound was used (Table 2). The presence of small amounts of bismuth nitrate (5 mol%) is necessary for the success of the reaction. This method has also been applied with other bismuth-based reagents like bismuth chloride, bismuth subnitrate, bismuth oxide and bismuth bromide in catalytic proportion (5 mol%). Stannic chloride, zinc chloride and boron trifluoride etherate failed to improve the yield of the products. The best results have been observed with bismuth nitrate as the catalyst.

2.2. Biology

As a part of our research to synthesize novel polyaromatic anticancer agents, biological assays for the four pyrrole containing polyaromatic compounds *viz*. 1-(chrysen-6-yl)-1*H*-pyrrole (**7**) (Entry 7, Table 2), 1-(pyren-1-yl)-1*H*-pyrrole (**8**) (Entry 8, Table 2), 5-(1*H*-pyrrol-1-yl)-1,10-phenanthroline (**9**) (Entry 9, Table 2) and 1-(phenanthren-2-yl)-1*H*-pyrrole **10** (Entry 10, Table 2) have been carried out. Due to its fused ring structure as well as puckered configuration 1-(adamantan-1-yl)-1*H*-pyrrole (**6**) (Entry 6, Table 2) was also included in that series.

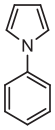
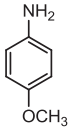
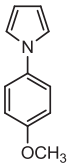
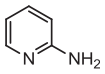
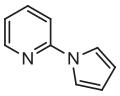
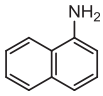
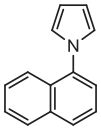
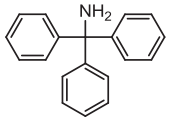
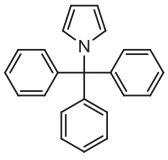
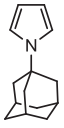
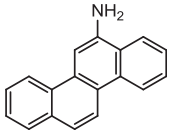
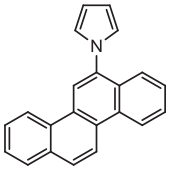
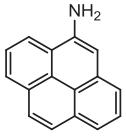
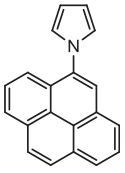
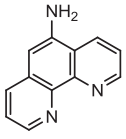
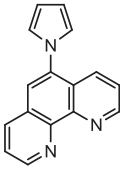
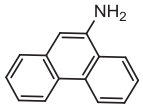
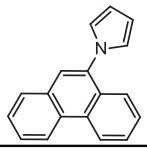
The effect of pyrrole conjugated compounds (**6**–**10**) on spectral properties has been studied. Previous reports [38–40] have identified that the peak emission wavelength of both pyrene and chrysene is less than 400 nM. Therefore, we sought to determine the effects of the addition of a pyrrole to these PAH molecules would affect the absorption and spectral emission patterns of these molecules. In addition, we sought to determine the absorbance and spectral emission patterns after conjugation of a pyrrole moiety to either polyaromatic or adamantyl scaffold. No significant absorbance could be detected for any of the pyrrole conjugated compounds, and no spectral emission could be detected for the

Table 1
$Bi(NO_3)_3\cdot 5H_2O$ catalyzed ultrasound-assisted synthesis of pyrrole using aniline (1 mmol) and 2,5-dimethoxytetrahydrofuran (1.2 mmol) for 5 min at room temperature: optimization of the amount of catalyst.

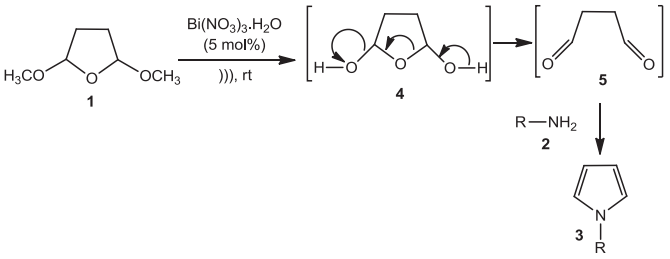
Entry	Bi $(NO_3)_3\cdot 5H_2O$ (mol %)	Yield (%)[a]
1	30	89
2	25	91
3	20	91
4	15	94
5	10	97
6	5	99
7	2	57
8	1	33

[a] Isolated yield.

Table 2
Ultrasound-induced $Bi(NO_3)_3 \cdot 5H_2O$ catalyzed synthesis of pyrroles following Scheme 1.

Entry	Amine	Product	Time (min)	Yield(%)[a]
1			5	99
2			5	95
3			5	92
4			5	95
5			30	87
6			35	79
7			10	92
8			15	87
9			60	76
10			5	86

[a] Isolated yield.



Scheme 2. Ultrasound-assisted bismuth nitrate-catalyzed synthesis of pyrroles: plausible mechanism of the reaction.

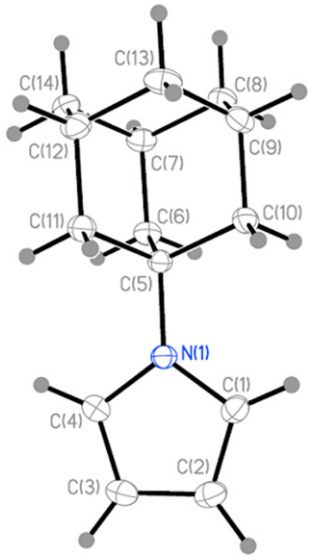


Fig. 1. X-ray crystallographic structure (ORTEP projection) of 1-(adamantan-1-yl)-1*H*-pyrrole (**6**).

pyrrole conjugated adamantyl derivative (**6**), the pyrrole conjugated heteropolyaromatic compound (**9**), or most of the pyrrole conjugated PAH derivatives including chrysene (**7**) and phenanthrene (**10**) in this study.

However, we were able to detect a fluorescence emission spectrum for the pyrrole conjugated pyrene compound (**8**), with a peak emission occurring between 460 nM and 470 nM (Fig. 1). The shoulders for this emission peak were at 420 nM and 550 nM (Fig. 2). This data suggests that the addition of pyrrole to pyrene

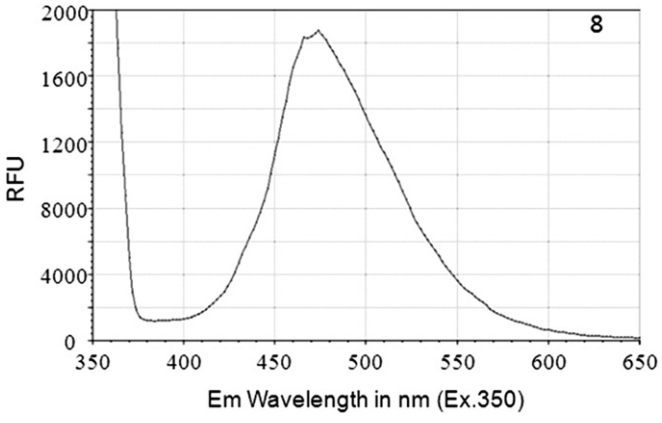


Fig. 2. Emission spectrum of the pyrene derivative **8**: The compound **8** (50 μM) was diluted in PBS and excited at a wavelength of 350 nM.

Table 3
Estimated IC_{50} values (μM) for the compounds (**6**–**10**) in a small panel of mammalian cell lines.

Compounds	HepG2	Hepa1-6	Caco-2	HT-29	HeLa	NIH3T3
6	38.6 ± 11.5	19.9 ± 6.1	>50	11.9 ± 1.0	12.9 ± 5.9	24.0 ± 18.5
7	>50	0.7 ± 0.8[a]	>50	>50	>50	>50
8	>50	10.7 ± 0.4	>50	24.3 ± 0.7	17.7 ± 9.9	ND[b]
9	3.0 ± 1.6[a]	3.4 ± 0.4	ND	4.2 ± 0.5	27.9 ± 20.7	1.9 ± 1.5
10	13.4 ± 4.8	3.9 ± 0.3	>50	>50	>50	2.1 ± 1.3
Cisplatin	7.0	4.0	10.8[a]	16.8	11.7	8.5

[a] Only reduced cell viability to a minimum of ~45–50%.
[b] Not determined.

causes a red-shift in its fluorescent emission spectrum but ablates the fluorescent emission when conjugated to a chrysene molecule.

It has been found [30,41,42] that certain derivatives of poly-aromatic hydrocarbons (PAHs), including pyrene and chrysene derivatives, reduce the viability of transformed cell lines, and some of these PAH derivatives have been reported to reduce cell viability by induction of apoptosis [42,43]. Therefore, we tested the effects of compounds **6**–**10** on the viability of a small panel of human and mouse cell lines, which included liver cancer cell lines (HepG2 and Hepa1-6), colon cancer cell lines (HT-29 and Caco-2), a cervical cancer cell line (HeLa), and NIH3T3 cells. Although Caco-2 cells were resistant to the effects of all of these pyrrole conjugated compounds, each pyrrole conjugated compound was capable of reducing viability of the other cell types (Table 3).

The addition of pyrrole to a chrysene or pyrene molecule, **7** and **8**, respectively, were the least effective in reducing cell viability in this small panel of cell lines as the majority of cell lines had an estimated IC_{50} value greater than 50 μM in most cell lines when treated with these compounds. Although compound **7** had a calculated IC_{50} value of less than 1 μM when used to treat Hepa1-6 cells, the maximum reduction in Hepa1-6 cell viability for this compound was only ~50%, even when used at a dosage of 50 μM, suggesting that this compound may be inducing cytostasis but not cytotoxicity in this cell line.

In contrast, other pyrrole derived compounds did cause cytotoxicity in multiple cell lines *in vitro*. Compound **8** effectively decreased the viability in Hepa1-6, HT-29, and HeLa cells with an estimated IC_{50} values of 24 μM or lower, and the pyrrole conjugated adamantane molecule, **6**, reduced the viability of all cell lines tested, other than Caco-2 and HepG2, with an estimated IC_{50} value less than 24 μM (Table 3). Most importantly, compounds with addition of a pyrrole to heteropolyaromatic and phenanthrene molecules (**9** and **10**, respectively) showed the most significant potency for reduction of HepG2, Hepa1-6, and NIH3T3 cell viability, with an estimated IC_{50} value of 13 μM or lower (Table 3). Furthermore, compound **9** and compound **10** mediated reduction in viability occurred between 24 and 48 h after treatment, since compounds **9** and **10** (when used at twice the calculated IC_{50} concentrations) caused a >50% decrease in viable HepG2 cells after 48 and 72 h treatments but only about a 20% decrease after a 24 h treatment (Fig. 3).

Since compounds **9** and **10** were the most potent compounds for reducing cell viability *in vitro*, we sought to monitor their effects on anchorage-independent growth of HepG2 cells. Both of these compounds inhibited anchorage-independent growth greater than 60% when cells were treated with 25 μM of compound and greater than 80% when used at a lower concentration of 12.5 μM (Fig. 4).

We also sought to determine whether compounds **9** and **10** reduced cell viability by inducing apoptosis. We examined whether these two compounds would lead to increased Caspase 3/7 activity or increased DNA fragmentation (as measured by a TUNEL assay) in HepG2 cells. However, we did not detect an increase in either of these features of apoptosis (data not shown). Taken together, these data suggest that the ability of compounds **9** and **10** to reduce cell viability occurs through an apoptosis-independent mechanism.

Since compounds **9** and **10** were the most potent pyrrole conjugated compounds for reducing cell viability *in vitro* and in anchorage-independent growth assays, we next asked whether these compounds would be less effective at reducing the viability of non-cancerous cells *in vitro* (i.e., exhibit tumor cell specificity). We examined the effects of increasing dosages of compounds **9** and **10** on the viability of Hepa1-6 cells and normal primary hepatocytes. As stated in Table 3, compounds **9** and **10** were both capable of reducing Hepa1-6 cell viability at doses 2.5 μM and 5 μM (Fig. 4). However, neither compound **9** nor compound **10** was capable of reducing viability of normal primary hepatocytes, even when used at dosages of 10 μM as shown in Fig. 5 (A & B). Therefore, compounds **9** and **10** showed distinct inhibitory effects on viability of cancer cells when compared with normal cells.

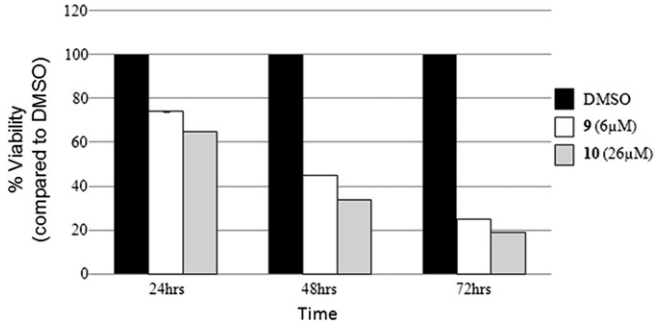


Fig. 3. The effects of pyrrole conjugated compounds (**9** and **10**) on the viability of HepG2 cells in a time-dependent manner. HepG2 cells were treated with compounds **9** and **10** at 2× IC_{50} concentrations for 24, 48, and 72 h, and then cell viability was analyzed using SRB assay. A representative experiment of three independent experiments performed in duplicates is shown. Each data point represents the average ± standard deviation value.

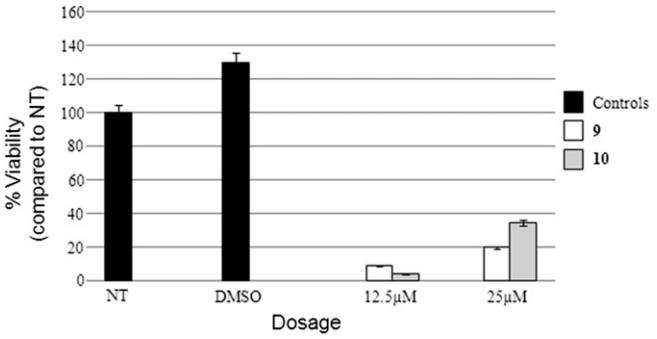


Fig. 4. Effect of pyrrole conjugated compounds (**9** and **10**) on anchorage-independent growth of HepG2 cells. HepG2 cells, grown in soft agar, were treated with the indicated doses of pyrrole conjugated compounds for 7 day as described in Materials and Methods. A representative experiment is shown and each dose of compound was administered in duplicate – the standard deviation bars are shown.

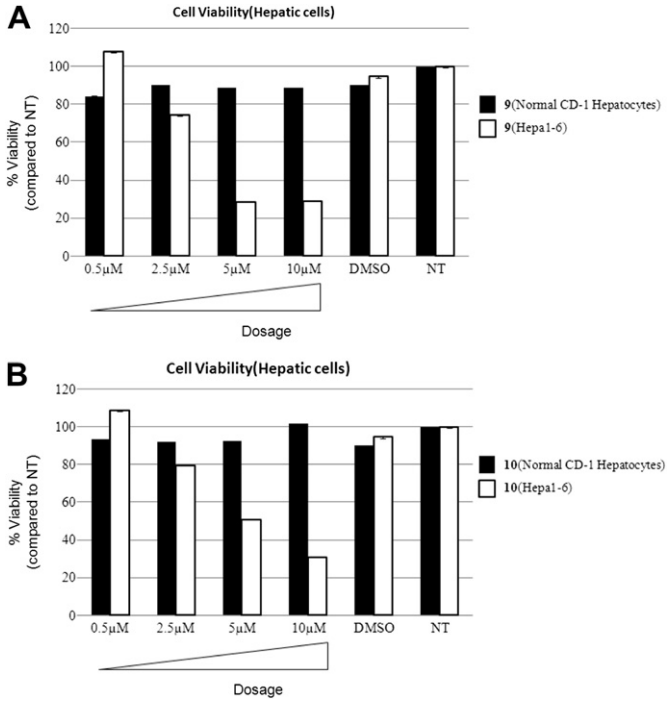


Fig. 5. Inhibitory effects of pyrrole conjugated compounds (**9** and **10**) on the viability of normal mouse hepatocytes (Mouse CD-1 cells) and mouse hepatoma cells (Hepa1-6). Normal (Mouse CD-1) and cancer (Hepa1-6) cells were treated with the indicated concentrations of compounds **9** (A) and **10** (B) for 48 h, and then cell viability (normalized to non-treated cells) was measured using MTS assay. Date denotes the average ± standard deviation value of a representative experiment of two independent experiments performed in duplicates.

Although many of the pyrrole conjugated polycyclic compounds did not demonstrate low IC_{50} values in this study, we clearly show that two compounds (compounds **9** and **10** in this paper) were cytotoxic to some cell lines at doses less than 5 μM, a dosage range that is similar to that of drugs such as Cisplatin and 5-FU in certain cell lines. It is very crucial to note that these compounds are new and no synthesis and biological studies of these molecules have been investigated. Our data has confirmed selective cytotoxic activity in important cancer cell lines without destroying the normal cell. Despite significant progress in the development of anticancer agents, selective and non-toxic new cytotoxic molecules are in demand.

3. Conclusion

In conclusion, the present synthetic protocol allows the preparation of a variety of *N*-substituted pyrroles in high yields without the use of expensive or sensitive reagents/instruments. Due to simplicity of the procedure, products can be isolated very easily. The novelty of the reaction is that it gives good yield (76%, Compound **9**) even if the substrate is a potential metal chelator. A plausible mechanistic route has been suggested and cytotoxicity of some selected compounds was evaluated *in vitro* in a panel of mammalian cancer cell lines which included liver cancer cell lines (HepG2 and Hepa1-6), colon cancer cell lines (HT-29 and Caco-2), a cervical cancer cell line (HeLa) and NIH3T3 cells. Compounds **9** and **10** showed good cytotoxicity against some cancer cell lines. Furthermore, these compounds exhibited selective cytotoxicity of hepatic cancer cell lines when compared with normal hepatocytes *in vitro*. Interestingly, previous studies have shown that polyaromatic compounds cause apoptosis in certain cell types *in vitro*, as indicated by Caspase 3 activation and DNA fragmentation [42,43]; however, features of apoptosis were not observed upon treatment of liver cancer cell lines with either compound **9** or **10** as part of this study. These data suggest that *N*-substituted pyrroles may have a different mechanism of cytotoxicity when compared with other polyaromatic derivatives. In summary, some promising anticancer pyrrole derivatives have been identified, and the method as reported herein may find applications in other areas of research. The mechanism of action of these new compounds is being evaluated and will be reported in due course.

4. Experimental protocols

4.1. Chemistry

4.1.1. General

Melting points were determined in a Fisher Scientific electrochemical Mel-Temp* manual melting point apparatus (Model 1001) equipped with a 300 °C thermometer. Elemental analyses (C, H, N) were conducted using the Perkin–Elmer 2400 series II elemental analyzer, their results were found to be in good agreement (±0.2%) with the calculated values for C, H, N. Sonication was performed in B5510-DTH, Branson ultrasonic cleaner (Model-5510, frequency of 42 kHz and an output power of 135 Watts) with digital timer, heater, temperature monitor and degas. Inside tank dimensions are 11.5″ × 9.5″ × 6″ (length × width × height) with a fluid capacity of 2.5 gallons. FT-IR spectra were registered on a Bruker IFS 55 Equinox FT-IR spectrophotometer as KBr discs. ^{1}H NMR (300 MHz) and ^{13}C NMR (75.4 MHz) spectra were obtained at room temperature with JEOL Eclipse-300 equipment using TMS as internal standard and $CDCl_3$ as solvent. Bismuth nitrate pentahydrate (reagent grade) 98% (Cat # 248592-500G, Batch # MKBC6772) purchased from Sigma–Aldrich was used. All other chemicals were purchased from Sigma–Aldrich Corporation (analytical grade). Throughout the project solvents were purchased from Fisher Scientific. Deionized water was used for the preparation of all aqueous solutions.

4.1.2. General procedure for the synthesis of pyrroles (*3*)

Amine **2** (1.0 mmol), 2,5-dimethoxytetrahydrofuran (**1**, 1.2 mmol) and bismuth nitrate pentahydrate (24 mg, 5 mol%) was irradiated in a B5510-DTH (Branson ultrasonic cleaner; Model-5510, frequency 42 kHz with an output power 135 Watts), as specified in Table 2. After completion of the reaction (monitored by TLC) diethyl ether (10 mL) was added to the reaction mixture and filtered. Pure product was isolated from the reaction mixture after evaporation of ether.

4.1.3. Spectral data

Spectroscopic data for representative compounds, e.g. monoaromatic (Entries 1 and 2, Table 2), benzylic (Entry 5, Table 2), alicyclic (Entry 6, Table 2), polyaromatic (Entries 7 and 8, Table 2) as well as heteropolyaromatic (Entry 9, Table 2) are as follows:

4.1.3.1. 1-Phenyl-1H-pyrrole. (Entry 1, Table 2): Brown sticky oil. IR: 2923, 1312, 1106, 782, 611 cm^{-1}; ^{1}H NMR δ (ppm): 6.39 (m, 2H, pyrrole), 7.24 (d, 2H, J = 3.12 Hz, pyrrole), 7.42–7.53 (m, 5H, Ar–H); ^{13}C NMR δ (ppm): 109.07 (2C), 115.51 (2C), 122.96 (2C), 124.57, 128.62 (2C), 137.08. Anal. Calcd. for $C_{10}H_9N$: C, 83.88; H, 6.34; N, 9.78. Experimental: C, 83.79; H, 6.31; N, 8.72.

4.1.3.2. 1-(4-Methoxyphenyl)-1H-pyrrole. (Entry 2, Table 2): Black amorphous solid. Solidified from dichloromethane/hexane mixture; M.p.: 89 °C; IR: 2954, 1301, 1191, 850, 748 cm^{-1}; ^{1}H NMR δ (ppm): 3.87 (s, 3H, OCH_3), 6.33 (m, 2H, pyrrole), 6.67 (d, 2H, J = 3.30 Hz), 6.96–7.73(m, 4H, Ar–H); ^{13}C NMR δ (ppm): 55.66

(OCH_3), 109.93 (2C), 114.71 (2C), 120.15 (2C), 126.07 (2C), 132.56, 158.21. Anal. Calcd. for $C_{11}H_{11}NO$: C, 76.28; H, 6.40; N, 8.09. Experimental: C, 76.20; H, 6.27; N, 7.97.

4.1.3.3. 1-Trityl-1H-pyrrole. (Entry 5, Table 2): This compound was obtained according to above general procedure; yellowish brown amorphous solid. M.p.: 238–240 °C; IR (KBr disc. ν cm^{-1}): 2599, 2337, 1594, 1476, 1423, 1322, 1261, 1222, 1182, 1073, 1033, 1001, 971, 861, 738, 718, 622; ^{1}H NMR δ (ppm): 6.16 (t, 2H, $J = 2.19$ Hz, pyrrole), 6.63 (t, 2H, $J = 2.19$ Hz, pyrrole), 7.17–7.20 (m, 6H, Ar–H), 7.29–7.32 (m, 9H, Ar–H); ^{13}C NMR δ (ppm): 92.82, 107.45 (2C), 123.60 (2C), 127.58 (3C), 127.79 (6C), 130.13 (6C), 143.86 (3C). Anal. Calcd. for $C_{23}H_{19}N$: C, 89.28; H, 6.19; N, 4.53. Experimental: C, 89.03; H, 6.08; N, 4.41.

4.1.3.4. 1-(Adamntan-1-yl)-1H-pyrrole. (Entry 6, Table 2): Yellow crystals. Crystallized from diethyl ether/hexane mixture; M.p.: 71 °C; IR: 2923, 2855, 1479, 1450, 1219, 713, 619 cm^{-1}; ^{1}H NMR δ (ppm): 1.77 (m, 3H), 2.12 (d, 6H, $J = 3.00$), 2.23 (m, 6H), 6.19 (t, 2H, $J = 2.19$ Hz, pyrrole), 6.91(m, 2H, pyrrole); ^{13}C NMR δ (ppm): 29.79 (3C), 36.35 (3C), 44.04 (3C), 55.00, 107.24 (2C), 116.55 (2C). Anal. Calcd. for $C_{14}H_{19}N$: C, 83.53; H, 9.51; N, 6.96. Experimental: C, 83.41; H, 9.50; N, 6.89.

4.1.3.5. 1-(Chrysen-6-yl)-1H-pyrrole. (Entry 7, Table 2): Brown crystals. Crystallized from ethyl acetate/hexane mixture; M.p.: 139 °C; IR: 2947, 2924, 1513, 1461, 1071, 812 cm^{-1}; ^{1}H NMR δ (ppm): 6.49 (t, 2H, $J = 2.21$ Hz, pyrrole), 7.12 (m, 2H, pyrrole), 7.70–8.72 (m, 11H, Ar–H); ^{13}C NMR δ (ppm): 109.27 (2C), 120.98, 121.29 (2C), 123.23, 123.49 (2C), 123.64 (2C), 124.05, 126.45 (2C), 126.75 (2C), 126.88 (2C), 127.42, 128.64 (2C), 132.26, 137.94. Anal. Calcd. for $C_{22}H_{15}N$: C, 90.08; H, 5.15; N, 4.77. Experimental: C, 89.91; H, 5.04; N, 4.76.

4.1.3.6. 1-(Pyren-1-yl)-1H-pyrrole. (Entry 8, Table 2): Pale yellow crystals. Crystallized from diethyl ether/hexane mixture; M.p.: 78 °C; IR: 2956, 2927,1598, 1511, 1304, 1072, 848 cm^{-1}; ^{1}H NMR δ (ppm): 6.53 (m, 2H, pyrrole), 7.15 (m, 2H, pyrrole), 8.01–8.19 (m, 9H, Ar–H); ^{13}C NMR δ (ppm): 109.45 (2C), 122.36, 123.83 (2C), 124.89, 125.42, 126.51, 126.72, 127.19, 127.89 (2C), 128.63 (2C), 128.92, 130.62, 131.01, 131.39, 133.43, 135.97. Anal. Calcd. for $C_{20}H_{13}N$: C, 89.86; H, 4.90; N, 5.24. Experimental: C, 89.77; H, 4.81; N, 5.17.

4.1.3.7. 5-(1H-Pyrrol-1-yl)-1,10-phenanthroline. (Entry 9, Table 2): Yellowish brown amorphous solid. Solidified from methanol/hexane mixture; M.p.: 192 °C; IR: 3186, 2362, 2336, 1591, 1539, 1074, 739 cm^{-1}; ^{1}H NMR δ (ppm): 6.44 (broad s, 2H, pyrrole), 7.02 (broad s, 2H, pyrrole), 7.62–9.20 (m, 7H, Ar–H); ^{13}C NMR δ (ppm): 110.14 (2C), 122.79, 123.24 (2C), 123.55 (2C), 123.74 (2C), 129.04, 132.26, 136.17, 143.91, 144.09, 150.80, 150.91. Anal. Calcd. for $C_{16}H_{11}N_3$: C, 78.35; H, 4.52; N, 17.13. Experimental: C, 78.23; H, 4.43; N, 17.02.

4.2. Biology

*4.2.1. Spectral analyses of pyrrole conjugated compounds (**6**–**10**)*

Compounds (**6**–**10**) were dissolved in DMSO at a concentration of 20 mM. For spectral analyses, compounds were then diluted to 50 μM in either DMSO or phosphate-buffered saline (PBS). The absorbance of each compound, dissolved in either DMSO or PBS, was then analyzed between the wavelengths of 350 nM–750 nM using a SpectraMax M5 plate reader (Molecular Devices, Sunnyvale, CA). In addition, diluted compounds were excited with light at a wavelength of 350 nM, and the subsequent light emission was analyzed between the wavelengths of 350 nM–750 nM using a SpectraMax M5 plate reader.

4.2.2. Cell culture

HepG2, Hepa1-6, NIH3T3 and HeLa cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Invitrogen, Carlsbad, CA) containing 10% Fetal Bovine Serum (FBS, Invitrogen); Caco-2 cells were cultured in DMEM containing 20% FBS, and HT-29 cells were cultured in McCoy's media (Invitrogen) containing 10% FBS. Mouse (CD-1) fresh hepatocytes were purchased and maintained in Williams' E medium according to manufactures instructions (Invitrogen). HepG2, Hepa1-6, NIH3T3, HeLa, Caco-2, and HT-29 cell lines were purchased from American Type Culture Collection (ATCC, Manassas, VA), and all cells were incubated at 37 °C with 5% CO_2.

4.2.3. Cell viability assays

Cells were plated (5000 cells/well) onto a 96-well dish and incubated overnight at 37 °C. The following day, cells were treated with increasing dosages (0.1 μM–100 μM) of each pyrrole conjugated compound, which had been dissolved in DMSO. The DMSO concentration of treatments was limited to 0.25%, and cells were treated with DMSO alone (0.25%) or 10 μM Doxorubicin as negative and positive controls for cytotoxicity, respectively. After 48-h, cells were fixed and cell viability was analyzed using the Sulforhodamine B colorimetric assay [44]. Absorbance of SRB was measured utilizing a SpectraMax M5 plate reader and absorbance values were normalized to non-treated cells. Normalized cell viabilities, with increasing drug doses, were plotted on a 4-parameter logistical curve, and the IC_{50} of each compound in each cell line was calculated using SigmaPlot software (Systat Software, Inc.). Each compound was used in two independent cell viability assays to generated dose–response curves. The mean IC_{50}, with the corresponding standard deviation, of the two independent treatments were then calculated. Reduction of cell viability in a time-dependent manner was assessed by treating HepG2 cells (5000 cells/well) with compounds at twice the calculated IC_{50} value (concentrations) of each compound for 24, 48, and 72 h. After cells were treated at the indicated times and doses, cells were fixed and cell viability was analyzed using the SRB assay as described above.

The comparison of the effects of compounds on the viability of normal mouse hepatocytes (Mouse CD-1 cells) and Hepa1-6 cells was determined by the colorimetric CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS) (Promega, Madison, WI) according to the manufacturer's instructions. Briefly, cells were plated onto a 96-well dish (200 μL media/well) and incubated overnight at 37 °C. After 24 h wells were replaced with fresh media and cells were treated with compounds **9** and **10** (0.5, 2.5, 5, and 10 μM) for 48 hrs, then MTS solution (20 μL per 100 μL of culture media) was added to each well and each plate was incubated at 37 °C with 5% CO_2 for 4 h. The absorbance was then read at 490 nm using a SpectraMax M5 plate reader, and absorbance values were normalized to non-treated cells.

4.2.4. Anchorage-independent cell growth assays

Anchorage-independent cell growth assays were performed using the CytoSelect™ 96-well Cell Transformation (Soft Agar Colony Formation) Assay according to the manufacturer's instructions. Briefly, HepG2 cells were plated at 5000 cells/well in top agar. Cells were then exposed to the indicated dose of each compound for seven days – the compound and fresh media was replaced once, on day 4 of the 7-day assay. On day 7, the agar was solubilized and cells were quantified using a SpectraMax M5 plate reader with filters set to capture light between 485 nM and 520 nM.

Authors' contributions

DB and SM conducted the sonochemical reactions, isolated and characterized the compounds by spectral analyses. JCG, DB and JDS carried the biological evaluation of the compounds on cancer cell lines.

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[37] X-ray crystallographic data were recorded with an automatic single crystal X-ray diffractometer consists of a 3 kw X-ray generator, Saturn 724 CCD detector, AFC12K goniometer, and X-stream 2000 (for nitrogen generation). The compound crystallizes in the $P2_1/n$ space group with only one molecule in the asymmetric unit, situated on a general position. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were located in a difference Fourier map and were refined with $U_{iso}(H) = 1.2U_{eq}(C)$. Crystals were selected from the original sample. An X-ray quality colorless chip-shaped crystal of dimensions 0.22 × 0.10 × 0.17 mm was selected for structural analysis. The ORTEP projection is shown in Fig. 1. Crystallographic data for 1-(Adamntan-1-yl)-1*H*-pyrrole have been deposited as DBSMPy-5 with Cambridge Crystallographic Data Centre as CCDC 787708. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 IEZ, UK. (fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).
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