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Chemiluminescence detection of piperazine designer drugs and related compounds using tris(2,2′-bipyridine)ruthenium(III)

Rebecca J. Waite, Gregory J. Barbante, Neil W. Barnett, Elizabeth M. Zammit, Paul S. Francis*

Centre for Chemistry and Biotechnology, Deakin University, Geelong, Victoria 3216, Australia

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ABSTRACT

We present an exploration of the chemiluminescence from reactions of benzylpiperazines and phenylpiperazines with tris(2,2′-bipyridine)ruthenium(III). The selectivity of the reagent towards these compounds was found to be highly dependent upon the pH of the solution, and the relative emission intensity was strongly influenced by electron donating or withdrawing substituents on the phenyl or benzyl ring. In spite of previous investigations showing poor responses for aromatic-substituted amines (compared to their aliphatic amine counterparts), intense emissions were observed with phenylpiperazines under acidic conditions, particularly those with halogen or trifluoromethyl substituents on the aromatic ring. Buffered alkaline conditions provided much broader selectivity for the detection of both phenylpiperazine and benzylpiperazine compounds, which we have applied to a rapid HPLC procedure for the determination of piperazines of forensic interest in 'party pill' samples.

1. Introduction

Piperazine functional groups are present in a wide variety of pharmacologically active compounds, such as antidepressants (e.g. mirtazapine, trazodone), antihistamines (levocetirizine, meclozine) antipsychotics (prochlorperazine, quetiapine), and antibiotics (ofloxacin, prulifloxacin). In recent years, several simple benzyl- and phenylpiperazines have emerged as ingredients of 'party pills', which mimic the physiological effects of illicit drugs [1–3]. Although several countries have moved to ban these and other 'legal highs', various piperazine derivatives are readily available on-line [4]. Due to the novelty of these compounds as recreational drugs, strategies for the rapid screening of seized pills are quite limited [3,5,6]. As yet, there are no specific colour spot-tests for piperazines; various colours are formed by piperazine derivatives with Simon's and Marquis reagents [5], some of which are similar to those of amphetamines. A microcrystalline test for 1-benzylpiperazine using mercury chloride has recently appeared in the literature [6], but this reagent may not be suitable for all common piperazine derivatives; 3-trifluoromethylphenylpiperazine was found to give a white precipitate with no crystal formation. A few separation-based procedures have been used to analyse capsules/tablets containing piperazine derivatives, including GC with nitrogen–phosphorus detection (NPD) [1] or mass spectrometry [1,7], and HPLC [7] or CE [8] with UV absorbance detection. Herein we present an examination of the chemiluminescence detection of piperazines with tris(2,2′-bipyridine)ruthenium(III) ($[Ru(bipy)_3]^{3+}$). This reagent has previously been used for highly sensitive detection of certain aliphatic amines (and various other compounds), including controlled substances such as heroin, cocaine, phencyclidine and methamphetamine [9,10], for chromatographic or electrophoretic separations [11–13] and rapid drug screening tests [14,15]. In general, the emission intensity with amines increases in the order primary < secondary < tertiary, but subtle differences in chemical structure can have a dramatic effect on the chemiluminescence response [16–18]. Noffsinger and Danielson noted that the chemiluminescence response for unsubstituted piperazine with $[Ru(bipy)_3]^{3+}$ was similar to that of dialkylamines [16]. The presence of both secondary and tertiary aliphatic amines within the piperazine-based designer drugs and observations of intense emissions with the piperazine-containing pharmaceuticals ofloxacin [19] and quetiapine [20] (Fig. 1) suggests that $[Ru(bipy)_3]^{3+}$ chemiluminescence may provide a more sensitive and selective alternative to UV-absorbance detection for forensic and pharmacological applications. Moreover, the broad range of commercially available piperazine standards provides an excellent opportunity to explore relationship between analyte structure and emission intensity, to derive a greater understanding of this widely used chemiluminescence reagent.

2. Experimental

2.1. Chemicals and reagents

Chemicals were obtained from the following sources: sodium perchlorate from Ajax Finechem (NSW, Australia); sulphuric acid from Merck (Victoria, Australia); perchloric acid (70% w/v) from Univar

* Corresponding author. Tel.: +61 3 5227 1294; fax: +61 3 5227 1040.
E-mail address: paul.francis@deakin.edu.au (P.S. Francis).

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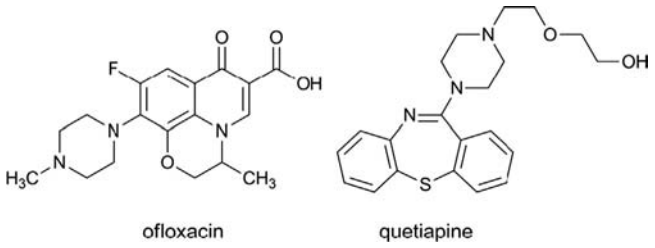

Fig. 1. Pharmaceuticals containing piperazine groups that have previously been shown to produce an intense chemiluminescence with $[Ru(bipy)_3]^{3+}$ under acidic conditions.

(NSW, Australia); acetonitrile from Burdick & Jackson (Michigan, USA) and tris(2,2′-bipyridine)ruthenium(II) dichloride hexahydrate from Strem Chemicals (Minnesota, USA). All other chemicals were purchased from Sigma-Aldrich (NSW, Australia). The aqueous oxidised $[Ru(bipy)_3]^{3+}$ reagent (***Reagent A***) was prepared by reacting the required amount of $[Ru(bipy)_3]^{2+}$ with 0.2 g/100 mL PbO_2 in 0.02 M sulphuric acid. Following the change in colour from orange to emerald green (~5 min with stirring), the excess oxidant was filtered from the solution using a 0.45 μm syringe-tip filter immediately prior to use. The 'stabilised' $[Ru(bipy)_3]^{3+}$ reagent (***Reagent B***) was prepared as follows: $[Ru(bipy)_3]Cl_2$ was treated with sodium perchlorate in aqueous solution to yield a $[Ru(bipy)_3](ClO_4)_2$ precipitate, which was collected by vacuum filtration, washed twice with ice water, and dried over phosphorus pentoxide for 24 h. The reagent solution was prepared by dissolving the crystals in acetonitrile containing 0.02 M or 0.05 M perchloric acid. Lead dioxide (0.2 g/100 mL) was added to form the blue-green $[Ru(bipy)_3]^{3+}$ [21]. The aqueous $[Ru(bipy)_3]^{2+}$ reagent (***Reagent C***) was prepared by dissolving $[Ru(bipy)_3]Cl_2 \cdot 6H_2O$ in 0.05 M sulphuric acid. This reagent was oxidised to $[Ru(bipy)_3]^{3+}$ within the FIA manifold ('on-line') using a cerium(IV) solution. The 'party pill' samples were completely dissolved in 100 mL water with sonication and then diluted 100-fold for analysis.

2.2. Instrumentation

Flow injection analysis manifolds were assembled from a Gilson Minipuls 3 peristaltic pump (John Morris Scientific, NSW, Australia) and a 6-port injection valve (Vici 04W-0192L; Valco Instruments, TX, USA) with a 70 μL sample loop. The chemiluminescence detector consisted of a coil of transparent PTFE-PFA tubing mounted against a photomultiplier tube (Electron Tubes model 9828SB, ETP; NSW, Australia) within a light-tight housing [22]. A T-piece connected to the central inlet of the coil served as the final confluence point of the flow system. All tubing entering and exiting the detectors was black PTFE (0.76 mm i.d.; Global FIA). The output signal from the photomultiplier module was recorded with a chart recorder or an e-corder 410 data acquisition system (eDAQ, NSW, Australia).

Chromatographic separations were carried out on an Agilent Technologies 1200 series HPLC (Agilent Technologies, Vic., Australia), using a Chromolith SpeedROD RP-18 monolithic column fitted with a 5 mm monolithic guard column (Merck, Kilsyth, Vic., Australia), with an injection volume of 20 μL and mobile phase flow rate of 2 mL min^{-1}. An analogue to digital interface box (Agilent Technologies) was used to convert the signal from the chemiluminescence detector. The post-column chemiluminescence detection was performed by merging the column eluate stream with a pH adjustment stream containing either 0.01 M H_2SO_4 or 100 mM borate buffer (adjusted to the desired pH), before mixing with a $[Ru(bipy)_3]^{3+}$ reagent stream (50 μM in acetonitrile with 0.02 M $HClO_4$) in a dual-inlet serpentine flow-cell of a GloCel chemiluminescence detector (Global FIA, WA, USA) [23,24] with an extended range photomultiplier module (Electron Tubes model P30A-05; ETP). The reagents were pumped at 2 mL min^{-1} using Dual Piston Pumps (Series 12x6, model D05PFD01; Scientific Systems, PA, USA).

3. Results and discussion

3.1. Preliminary screening of pharmaceuticals

We initially examined the relative chemiluminescence responses for over 60 pharmaceutical preparations and standards with $[Ru(bipy)_3]^{3+}$, under the acidic chemical conditions previously employed in our laboratory for the detection of various amines [21,25], using FIA methodology (Table S1). Although some interference from the non-active components of the preparations may have occurred, several observations can be made from these data. The greatest signals were generally obtained from compounds with aliphatic tertiary amines (in agreement with previous findings [16]), including some piperazine compounds (e.g. quetiapine, 1-(3-trifluoromethylphenyl)piperazine, and prochlorperazine). All eight tested piperazine derivatives gave measureable responses, although their intensities ranged over three orders of magnitude. The presence of phenols is known to quench the emission with this reagent [17,18,26], and most phenolic compounds gave little or no response, but doxycycline (containing tertiary and primary amines in addition to phenolic functionality) gave a large signal, and interestingly, the anilinic phenylpiperazine derivatives generally gave greater signals than the benzylpiperazines. Moreover, a comparison of piperazine-containing fluoroquinolone pharmaceuticals (including ofloxacin, which has previously been shown to produce an intense emission with $[Ru(bipy)_3]^{3+}$ under these conditions [19]) showed again that subtle changes in chemical structure can have a dramatic effect on chemiluminescence intensity with this reagent (Table S2).

3.2. Response of piperazine derivatives under acidic conditions

During the course of this investigation, our research group developed a new off-line approach to prepare $[Ru(bipy)_3]^{3+}$, involving the PbO_2 oxidation of $[Ru(bipy)_3](ClO_4)_2$ in acetonitrile containing 0.05 M perchloric acid [21], which provided a considerably more stable reagent (***B***) than that obtained by oxidising $[Ru(bipy)_3]Cl_2$ in acidic aqueous solution (***A***). As some differences in the relative chemiluminescence intensity of amines with these two reagents has previously been noted, we initially compared the response of five piperazines with each reagent. The responses for the piperazines with ***Reagent B*** were between 87% and 466% those with ***Reagent A***, and therefore ***B*** was used for all subsequent experiments in which off-line oxidation of $[Ru(bipy)_3]^{2+}$ was employed (Table S3).

For the on-line oxidation of $[Ru(bipy)_3]^{2+}$ with cerium(IV), we compared injecting the reagent (***C***) into the analyte stream (which merged with cerium(IV)) against injecting the reagent into a cerium(IV) stream (which merged with the analyte). The responses for the piperazines when the reagent was injected into the oxidant stream were between 192% and 297% of those obtained when the reagent was injected into the analyte stream (Table S4), which can be attributed to the greater period of time for $[Ru(bipy)_3]^{2+}$ oxidation within the flow manifold.

The effect of acid in these systems was examined by adding different concentrations of sulphuric acid to the analyte solutions. The optimum concentration was found to be dependent on both the analyte and the mode of $[Ru(bipy)_3]^{2+}$ oxidation. When the off-line method of oxidation was employed, the greatest signal-to-blank ratios were observed between 0.02 M and 0.05 M sulphuric acid. When the reagent was oxidised on-line with cerium(IV), the signal-to-blank ratios increased until approximately 1 M sulphuric acid. However, for the purpose of direct comparison, an acid

concentration of 0.05 M was selected for both approaches. The two FIA manifolds are shown in Fig. S1.

Under these conditions, forty-one benzylpiperazine and phenylpiperazine compounds were screened for their chemiluminescence response (Table 1). This comparison revealed: (i) In general the phenylpiperazines gave much greater emission intensities than their benzylpiperazine counterparts. The response for 1-phenylpiperazine (Fig. 2) for example, was over 30-fold greater than that of 1-benzylpiperazine, with either reagent system. This was surprising, considering the previous reports of intense signals from many aliphatic tertiary amines and relatively poor responses for various aromatic amines and aromatic-substituted amines [16,17,27,28]. (ii) Within the phenylpiperazine group, the presence of electron withdrawing substituents such as halogens and the trifluoromethyl group increased the emission intensity, often by over an order of magnitude. The strongest electron withdrawing effect, however, would be expected from the nitro group, but the presence of this substituent decreased the emission intensity.

Table 1
Relative chemiluminescence responses (blank subtracted) of piperazine compounds at 1×10^{-5} M upon the reaction with $[Ru(bipy)_3]^{3+}$ (1×10^{-3} M) in 0.05 M H_2SO_4. Reagent B: $[Ru(bipy)_3]^{3+}$ (prepared off-line) was injected into a 0.05 M H_2SO_4 carrier stream, which merged with the analyte stream shortly prior to entering the detector. Reagent C: The $[Ru(bipy)_3]^{3+}$ was prepared on-line by injecting $[Ru(bipy)_3]^{2+}$ into a cerium(IV) carrier stream, which merged with the analyte stream (FIA manifolds shown in Fig. S1).

Compound	Reagent B	Reagent C
1-phenylpiperazine (PP)	**100**	**100**
1-(4-aminophenyl)piperazine	5	3
1-(2-hydroxyphenyl)piperazine	47	21
1-(3-hydroxyphenyl)piperazine	168	23
1-(4-hydroxyphenyl)piperazine	0	6
1-(3-methoxyphenyl)piperazine	722	15
1-(4-methoxyphenyl)piperazine	9	7
1-(2-ethoxyphenyl)piperazine	124	74
1-(4-fluorophenyl)piperazine	1625	1712
1-(2-chlorophenyl)piperazine	3935	3370
1-(3-chlorophenyl)piperazine	1924	2556
1-(4-chlorophenyl)piperazine	2267	2998
1-(2,3-dichlorophenyl)piperazine	1802	498
1-(3,4-dichlorophenyl)piperazine	3292	2006
1-(4-iodophenyl)piperazine	449	162
1-(3-trifluoromethylphenyl)piperazine	5754	4028
1-(4-trifluoromethylphenyl)piperazine	6412	3898
1-(4-methylphenyl)piperazine	181	1806
1-(4-nitrophenyl)piperazine	44	8
1-(2-pyridyl)piperazine	422	2
1-(4-pyridyl)piperazine	8	1
1-benzylpiperazine (BZP)	3	2
1-(4-methoxybenzyl)piperazine	37	3
1-(2-fluorobenzyl)piperazine	6	−1*
1-(3-fluorobenzyl)piperazine	4	3
1-(4-fluorobenzyl)piperazine	9	3
1-(2,5-difluorobenzyl)piperazine	7	4
1-(4-bromo-2-fluorobenzyl)piperazine	3	0
1-(2-chloro-6-fluorobenzyl)piperazine	7	−1*
1-bis(4-fluorophenyl)methyl piperazine	0	13
1-(3-chlorobenzyl)piperazine	5	4
1-(4-chlorobenzyl)piperazine	46	4
1-(2,4-dichlorobenzyl)piperazine	5	5
1-(2,6-dichlorobenzyl)piperazine	3	5
1-(3,4-dichlorobenzyl)piperazine	5	4
1-(4-bromobenzyl)piperazine	8	5
1-(4-trifluoromethylbenzyl)piperazine	2	4
1-(2-methylbenzyl)piperazine	4	4
1-(3-methylbenzyl)piperazine	2	4
1-(4-methylbenzyl)piperazine	2	4
1-(4-tert-butylbenzyl)piperazine	4	2

* The signal obtained with these two analytes was slightly lower than the blank signal. The blank was obtained by replacing the analyte solution with deionised water and conducting the same injection of the reagent.

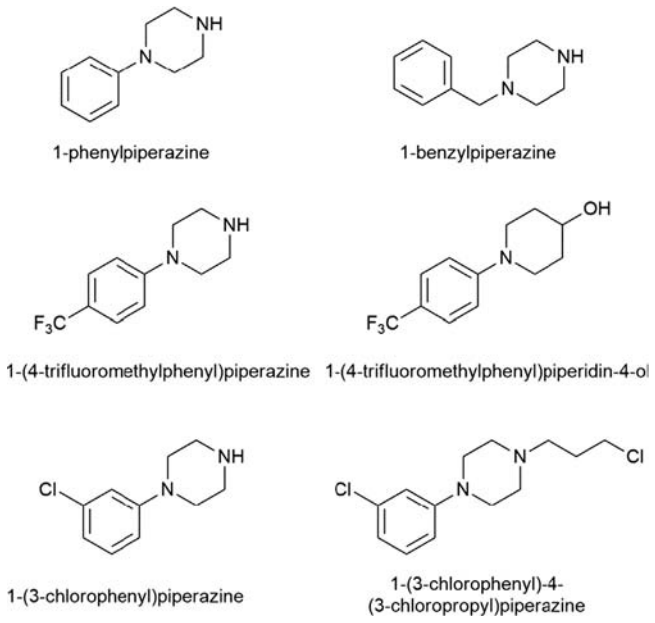

Fig. 2. Piperazine and piperidine derivatives.

This may be attributed to electron-transfer quenching of the ($[Ru(bipy)_3]^{2+}$)* excited state [29]. (iii) Differences in the chemiluminescence intensity for positional isomers of phenylpiperazine derivatives can be partially accounted for by inductive and resonance considerations. For example, a methoxy group in the *meta* position increased the intensity to 722 (compared to 1-phenylpiperazine = 100, using $[Ru(bipy)_3]^{3+}$ reagent B), whereas the same group in the *para* position reduced the relative response to only 9. Similar effects were observed for hydroxy and amino substituents (Table 1). An examination of Hammett constants [30] for these groups reveals that although the electron-donating resonance effect dominates in the *para*-position, the counteracting electron-withdrawing inductive effect prevails in the *meta*-position (Table 2), in which case their influence is more akin to that of the halogens and significant enhancement in the chemiluminescence response is observed. From another standpoint, phenylpiperazine derivatives with hydroxy, amino and perhaps even methoxy substituents in *ortho* or *para* positions may inhibit the chemiluminescence by preferential oxidation through non-radiative pathways [18] to form quinonimine products.

3.3. Response of piperazine derivatives under buffered alkaline conditions

Although a range of tertiary amines can be sensitively detected by $[Ru(bipy)_3]^{3+}$ chemiluminescence under acidic conditions, the optimum pH for secondary amines can be much higher [27,28,31,32]. As the above piperazine compounds possess both tertiary and secondary amines (Fig. 2), we also examined their emission intensity under buffered alkaline conditions. Selected benzylpiperazines and phenylpiperazines were first screened using the conditions previously optimised for the detection of the secondary amine acids proline and hydroxyproline [31,33]. Using FIA methodology, the piperazine standard solutions were merged with sodium tetraborate buffer (100 mM, pH 10), and then a deionised water carrier stream, into which the $[Ru(bipy)_3]^{3+}$ reagent (0.05 mM in acetonitrile containing 0.02 M $HClO_4$) was injected (Fig. S2). For comparison purposes, the experiment was repeated after replacing the buffer solution with deionised water. In contrast to the reactions performed in acidic solution, the greatest emission intensities were now obtained with the benzylpiperazine compounds (Table 3). Moreover, substituents that exerted an electron

withdrawing effect on the α-carbon of the tertiary amine were found to decrease the emission intensity, as observed in the electrogenerated chemiluminescence reactions of alkylamines with $[Ru(bipy)_3]^{3+}$ [17]. An examination of the chemiluminescence intensity of these compounds across a wide pH range revealed maxima (after subtraction of the blank signal [34]) from pH 7 to pH 9 (Fig. 3a). However, the greatest signal-to-blank ratios for the phenylpiperazines occurred at the lowest pH (Fig. 3b).

The influence of the secondary and tertiary amine groups within the phenylpiperazine compounds on the emission under acidic and alkaline conditions was examined by comparing the responses from 1-(4-trifluoromethylphenyl)piperazine with piperidine-4-one and piperidine-4-ol analogues, in which the secondary amine was replaced by a carbon with oxo or hydroxyl substituent, respectively, and the responses of 1-(3-chlorophenyl)piperazine against those of 1-(3-chlorophenyl)-4-(3-chloropropyl)piperazine (Fig. 2), in which both amines are tertiary. Converting the secondary amine to a tertiary amine led to a large *increase* in the emission under acidic conditions, but a large *decrease* in the emission under alkaline conditions. The piperidine analogues, containing only the phenyl substituted (tertiary) amine, also produced a much greater signal under acidic conditions, and a slightly lower signal (with shifted pH optimum) in alkaline conditions. It was concluded that the phenyl substituted tertiary amine of the piperazine was largely responsible for the chemiluminescence under acidic conditions and the secondary amine was most important to the observed emission in buffered alkaline solution.

3.4. HPLC separation of piperazines with $[Ru(bipy)_3]^{3+}$ chemiluminescence detection

The $[Ru(bipy)_3]^{3+}$ chemiluminescence reagent was applied to the post-column detection of several forensically relevant piperazines (Fig. 4). The use of a monolithic column enabled rapid separation of seven analytes within 4 min. By simple modification of pH, the selectivity of the reagent was directed towards different analytes. At pH 1.2, large peaks for the fluoro-, chloro- and trifluoromethyl-phenylpiperazines were observed. At pH 6.0, only the chloro- and trifluoromethyl-phenylpiperazines remained (data not shown), but at pH 8.0, all seven phenylpiperazines and benzylpiperazines were present in the chromatogram (Fig. 4c): six as peaks and one (1-(4-methoxyphenyl)piperazine) as a dip in the baseline signal.

We applied this approach to the determination of piperazine compounds in party pills, with a modified gradient that reduced

Table 2
Relative chemiluminescence intensities (from Table 1) and Hammett substituent data [30] for various phenylpiperazines.

Substituent	CL Intensity (Reagent B)		Hammett substituent constant		Field/inductive parameter	Resonance effect parameter
	meta-	*para-*	σ_m	σ_p	*F*	*R*
NH_3		5	−0.16	−0.66	0.08	−0.74
OH	168	0	0.12	−0.37	0.33	−0.70
OCH_3	722	9	0.12	−0.27	0.29	−0.56
CH_3		181	−0.07	−0.17	0.01	−0.18
H	100	100	0.00	0.00	0.03	0.00
F		1625	0.34	0.06	0.45	−0.39
I		449	0.35	0.18	0.42	−0.24
Cl	1924	2267	0.37	0.23	0.42	−0.19
CF_3	5754	6412	0.43	0.54	0.38	0.16
NO_2		44	0.71	0.78	0.65	0.13

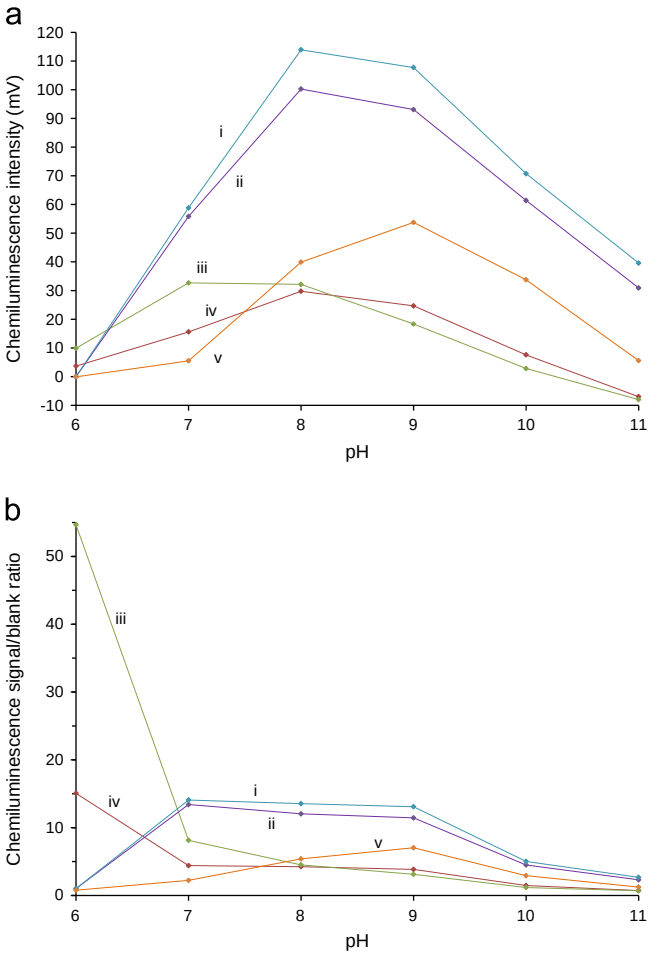

Fig. 3. (a) Chemiluminescence intensity (mV, blank subtracted) and (b) signal-to-blank ratios for: (i) 1-(4-methoxybenzyl)piperazine, (ii) 1-benzylpiperazine, (iii) 1-(4-trifluoromethylphenyl)piperazine, (iv) 1-(4-chlorophenyl)piperazine, and (v) 1-(4-chlorobenzyl)piperazine, with $[Ru(bipy)_3]^{3+}$ at pH 6 to11 (FIA manifold shown in Fig. S2).

Table 3
Analytical figures of merit for the determination of piperazine compounds in party pills by HPLC with $[Ru(bipy)_3]^{3+}$ chemiluminescence detection. Typical chromatograms are shown in Fig. 5.

Analyte	Retention time (min)	% R.S.D. ($n=5$)		Calibration[a] R^2	Limit of detection (M)[b]
		Peak area	Retention time		
1-benzylpiperazine	0.80	3.40	0.20	0.9988	2×10^{-7}
1-(4-methoxyphenyl)piperazine	1.64	2.33[c]	0.19	0.9980	1×10^{-6}
1-(3-fluorophenyl)piperazine	1.74	3.03	0.15	0.9935	3×10^{-6}
1-(3-chlorophenyl)piperazine	2.37	2.92	0.24	0.9990	1×10^{-6}
1-(3-trifluoromethylphenyl)piperazine	2.65	2.74	0.10	0.9760	4×10^{-7}

[a] Using 6–8 standards.
[b] $S/N=3$.
[c] The response for 1-(4-methoxyphenyl)piperazine was observed as a dip rather than a peak in the chromatogram (see Fig. 5b).

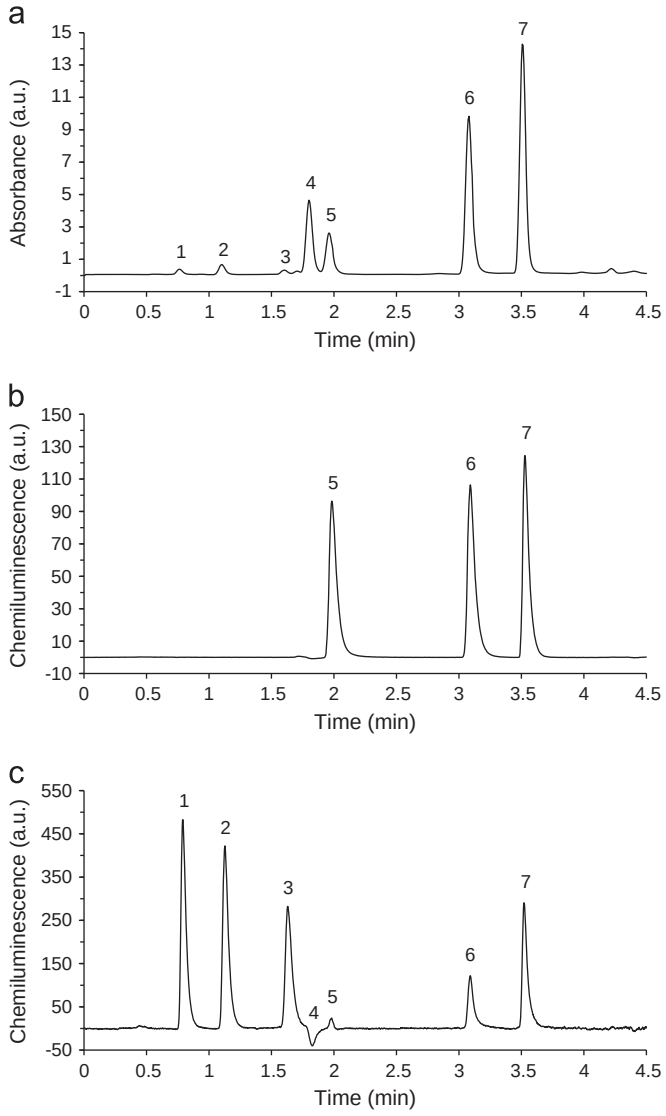

Fig. 4. HPLC separation of seven piperazine compounds (baseline corrected) with (a) UV-absorbance detection, and $[Ru(bipy)_3]^{3+}$ chemiluminescence detection at (b) pH 1.2, and (c) pH 8.0 (these pH values refer to the chemiluminescence detection conditions, not the separation conditions). The separation was performed by gradient elution with deionised water adjusted to pH 2.5 with trifluoroacetic acid (solvent A) and methanol (solvent B) as follows: 0–2 min: 10–30% B, 2–4 min: 30–80% B, 4–4.1 min 80-10% B, 4.1–8 min: 10%. Peaks: (1) 1-benzylpiperazine, (2) 1-(4-methoxybenzyl)piperazine, (3) 1-(4-chlorobenzyl)piperazine, (4) 1-(4-methoxyphenyl)piperazine, (5) 1-(3-fluorophenyl)piperazine, (6) 1-(3-chlorophenyl)piperazine, and (7) 1-(3-trifluoromethylphenyl)piperazine. In the case of compound 4 in Fig. 4c, a dip was observed instead of a peak, due to its quenching effect on the constant background response of the chemiluminescence reagent continuously merging with the column eluate in the detector.

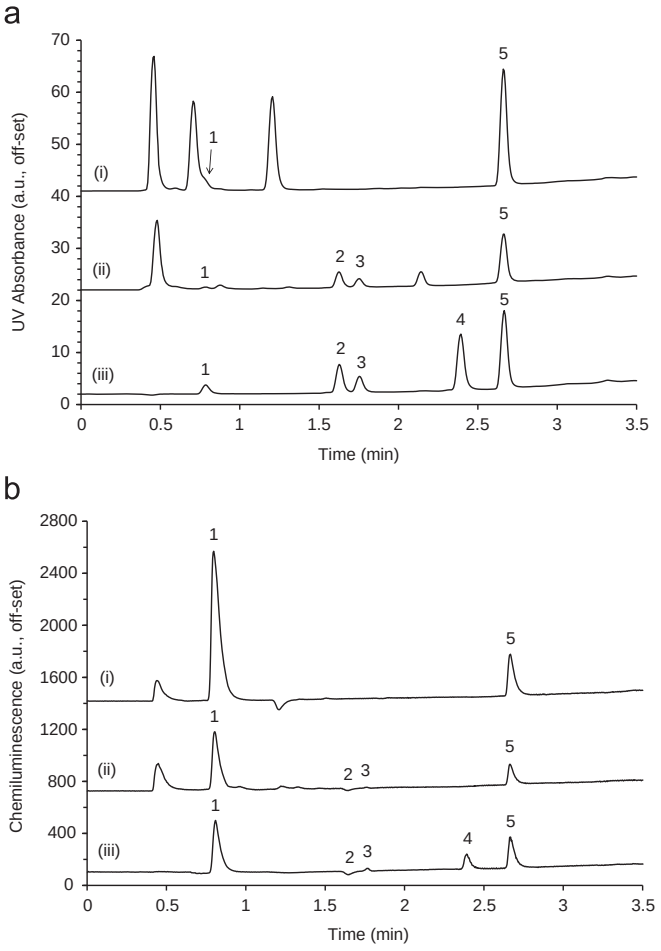

Fig. 5. Piperazine compounds in 'party pill' samples (plots i and ii) with (a) UV-absorbance detection and (b) $[Ru(bipy)_3]^{3+}$ chemiluminescence detection at pH 8.0. Plot iii is a standard piperazine mixture at 1×10^{-5} M under the same separation conditions. Peaks: (1) 1-benzylpiperazine, (2) 1-(4-methoxyphenyl) piperazine, (3) 1-(3-fluorophenyl)piperazine, (4) 1-(3-chlorophenyl)piperazine, and (5) 1-(3-trifluoromethylphenyl)piperazine. The separation was performed by gradient elution with deionised water adjusted to pH 2.5 with trifluoroacetic acid (solvent A) and methanol (solvent B) as follows: 0–1.5 min: 10–40% B, 1.5–2.5 min: 40–80% B, 2.5–3 min 80%, 3–3.1 min 80–10% B, 3.1–7 min: 10%.

the separation time to under 3 min (Fig. 5). Calibrations were prepared using eight standards between 5×10^{-7} M to 2.5×10^{-5} M. Analytical figures of merit for the procedure are shown in Table 3. The piperazine derivatives most commonly identified in party pills are 1-benzylpiperzaine (BZP) and 1-(3-trifluoromethylphenyl)piperazine (TFMPP) [1–6,35], and these compounds were found in both samples. Using UV-absorbance detection, the response for BZP was relatively low, and obscured by a large interfering peak in one sample (Fig. 5a: plot i). In contrast, BZP produced the largest peaks in the chemiluminescence chromatograms. The quantities of piperazines found in the samples were significantly lower than those indicated on the product packaging. Sample 1 was stated to contain 100 mg BZP and 50 mg TFMPP, whereas we found 25.7 mg and 12.3 mg, respectively. Sample 2 was stated to contain 45 mg BZP and 80 mg of "additional piperazine and antioxidant boosters"; we found 17.2 mg BZP, 9.7 mg TFMPP, 6.8 mg 1-(4-methoxyphenyl)piperazine and 9.4 mg 1-(3-fluorophenyl)piperazine. The differences between the stated and measured quantities in these products are similar to those reported by Kenyon et al. [35], who examined 23 different tablets and capsules containing piperazine derivatives (using GC–MS) and found 28–133 mg BZP (mean: 65 mg) and 4–72 mg TFMPP (mean: 22 mg) in products claiming 105–200 mg BZP and 50–75 mg TFMPP.

4. Conclusions

$[Ru(bipy)_3]^{3+}$ is an effective chemiluminescence reagent for the detection of substituted piperazines of pharmaceutical and forensic importance. The ability to tune the selectivity of the reagent towards particular groups of compounds within this class (through the adjustment of pH) may be particularly useful in the determination of piperazines in designer drugs and related samples. At a more fundamental level, this study shows the potential to expand this widely used mode of chemiluminescence

detection to a range of other aromatically-substituted amines, which may have not been considered due to the poor response from related compounds and the general focus on aliphatic tertiary amines.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.08.029.

References

[1] D. de Boer, I.J. Bosman, E. Hidvegi, C. Manzoni, A.A. Benko, L.J.A.L. dos Reys, R.A.A. Maes, Forensic Sci. Int. 121 (2001) 47.
[2] S. Elliott, Drug Test. Anal. 3 (2011) 430.
[3] M.D. Arbo, M.L. Bastos, H.F. Carmo, Drug Alcohol Depend. 122 (2012) 174.
[4] M. Baron, M. Elie, L. Elie, Drug Test. Anal. 3 (2011) 576.
[5] H. Inoue, Y.T. Iwata, T. Kanamori, H. Miyaguchi, K. Tsujikawa, K. Kuwayama, H. Tsutsumi, M. Katagi, H. Tsuchihashi, T. Kishi, Jpn. J. Sci. Technol. Iden 9 (2004) 165.
[6] L. Elie, M. Baron, R. Croxton, M. Elie, Forensic Sci. Int. 214 (2012) 182.
[7] M. Takahashi, M. Nagashima, J. Suzuki, T. Seto, I. Yasuda, T. Yoshida, Talanta 77 (2009) 1245.
[8] S.C. Bishop, B.R. McCord, S.R. Gratz, J.R. Loeliger, M.R. Witkowski, J. Forensic Sci. 50 (2005) 326.
[9] J.L. Adcock, C.J. Barrow, N.W. Barnett, X.A. Conlan, C.F. Hogan, P.S. Francis, Drug Test. Anal. 3 (2011) 145.
[10] B.A. Gorman, P.S. Francis, N.W. Barnett, Analyst 131 (2006) 616.
[11] R. Chiba, M. Fukushi, A. Tanaka, Anal. Sci. 14 (1998) 979.
[12] J. Sun, X. Xu, C. Wang, T. You, Electrophoresis 29 (2008) 3999.
[13] Y. Du, E. Wang, Electroanalysis 20 (2008) 643.
[14] K.M. Agg, A.F. Craddock, R. Bos, P.S. Francis, S.W. Lewis, N.W. Barnett, J. Forensic Sci. 51 (2006) 1080.
[15] Y. Xu, Y. Gao, H. Wei, Y. Du, E. Wang, J. Chromatogr. A 1115 (2006) 260.
[16] J.B. Noffsinger, N.D. Danielson, Anal. Chem. 59 (1987) 865.
[17] A.W. Knight, G.M. Greenway, Analyst 121 (1996) 101R.
[18] C.M. Hindson, G.R. Hanson, P.S. Francis, J.L. Adcock, N.W. Barnett, Chem. Eur. J 17 (2011) 8018.
[19] P.S. Francis, J.L. Adcock, Anal. Chim. Acta 541 (2005) 3.
[20] S.A. Bellomarino, A.J. Brown, X.A. Conlan, N.W. Barnett, Talanta 77 (2009) 1873.
[21] G.P. McDermott, P. Jones, N.W. Barnett, D.N. Donaldson, P.S. Francis, Anal. Chem. 83 (2011) 5453.
[22] J.L. Adcock, J.M. Terry, C.J. Barrow, N.W. Barnett, D.C. Olson, P.S. Francis, Drug Test. Anal. 3 (2011) 139.
[23] J.M. Terry, J.L. Adcock, D.C. Olson, D.K. Wolcott, C. Schwanger, L.A. Hill, N.W. Barnett, P.S. Francis, Anal. Chem. 80 (2008) 9817.
[24] J.M. Terry, S. Mohr, P.R. Fielden, N.J. Goddard, N.W. Barnett, D.C. Olson, D.K. Wolcott, P.S. Francis, Anal. Bioanal. Chem. 403 (2012) 2353.
[25] L.A. Hill, C.E. Lenehan, P.S. Francis, J.L. Adcock, M.E. Gange, F.M. Pfeffer, N.W. Barnett, Talanta 76 (2008) 674.
[26] J. McCall, M.M. Richter, Analyst 125 (2000) 545.
[27] S.N. Brune, D.R. Bobbitt, Anal. Chem. 64 (1992) 166.
[28] G.M. Greenway, S.J.L. Dolman, Analyst 124 (1999) 759.
[29] C.R. Bock, J.A. Connor, A.R. Gutierrez, T.J. Meyer, D.G. Whitten, B.P. Sullivan, J.K. Nagle, J. Am. Chem. Soc. 101 (1979) 4815.
[30] C. Hansch, A. Leo, R.W. Taft, Chem. Rev. 91 (1991) 165.
[31] J.W. Costin, P.S. Francis, S.W. Lewis, Anal. Chim. Acta 480 (2003) 67.
[32] J.L. Adcock, N.W. Barnett, R.D. Gerardi, C.E. Lenehan, S.W. Lewis, Talanta 64 (2004) 534.
[33] J.M. Terry, Z.M. Smith, G.P. McDermott, R.J. Waite, N.W. Barnett, L.C. Henderson, J.M. Altimari, P.S. Francis, Talanta 99 (2012) 1051.
[34] G.M. Greenway, A.W. Knight, P.J. Knight, Analyst 120 (1995) 2549.
[35] S. Kenyon, J. Button, J. Ramsey, D.W. Holt, Ther. Drug Monit., vol. no.29 (207), p. 495.