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Original article

Synthesis and characterization of novel azo-morphine derivatives for possible use in abused drugs analysis

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Abstract

A new simple and fast spectroscopic method was presented as a new marker for heroin use. Novel azo-morphine derivatives with spectroscopic absorption peaks ranging from 330–470 nm, were synthesized by the coupling of morphine (M) and 6-acetyl morphine (6-AM) with freshly prepared diazonium salt of aniline hydrochloride at 0 °C. However, no reaction was observed with codeine under the same reaction conditions. Separation of azo dyes was performed by TLC using tetrahydrofuran and dichloromethane in the ratio 1:1. The chemical structure of the products was established by their microanalysis, NMR, IR, UV–vis, and mass spectroscopies. Electronic absorption and excitation spectra of the dyes were measured in solvents of different polarities. The dyes exhibited positive solvatochromism, i.e., a bathochromic band shift as the solvent polarity is increased. Also, the fluorescence quantum yield was sensitive to the polarity and the pH of the medium. The UV–vis spectroscopy of spiked compounds in human urine samples was also reported. The drugs (M, 6-AM and mixture of both) were coupled with freshly prepared diazonium salt even at very low concentration of the drugs 10^{-9} M.

Keywords: Heroin; Morphine; Abused drugs; Dyes; Azo coupling; Fluorescence; Determination

1. Introduction

Heroin, which is obtained synthetically from the acetylation of morphine, has an analgesic potency two to three times that of the parent drug and, due to the presence of two acetyl groups, it has better penetration across the blood–brain barrier [1]. Heroin itself is rarely present in detectable quantities in body fluids. The drug hydrolyses rapidly to 6-acetyl morphine (6-AM), which in turn hydrolyses to morphine (M). Therefore, heroin consumption can be confirmed by identifying its two primary metabolites [2,3]. In addition, heroin is different from most other opioids in that it has little or no affinity for opioid receptors in the brain. The analgesic effect of the drug is attributed to the combined effect of 6-AM and M [4].

Among the most common fluorescent derivatization reactions are those which target reactive species such as acids, alcohols, and primary and secondary amines. Unfortunately, many abused drugs (i.e., heroin, 6-AM and morphine) are tertiary amines (Fig. 1) and are not compatible with the most commonly utilized amine reactive fluorescent dyes. These dyes include compounds such as fluorescein isothiocyanate isomer I (FITC) [5], 4-(4,6-dichloro-s-triazin-2-ylamino) fluorescein (DTAF) [6], 4-fluoro-7-nitrobenzofurazan (NBD-F) [7] and 3-(4-carboxybenzoyl)-2-quinolinecarboxaldehyde (CBQCA) [8]. Other fluorogenic reagents specifically made for derivatization of the tertiary amine group such as the malonic acid/acetic anhydride system [9] and the aconitic acid method [10] result in a deteriorating effect on the fluorescence of the reaction product. In addition the products of these reactions are unstable, light sensitive and give many components that seem to be associated with the reagent blank.

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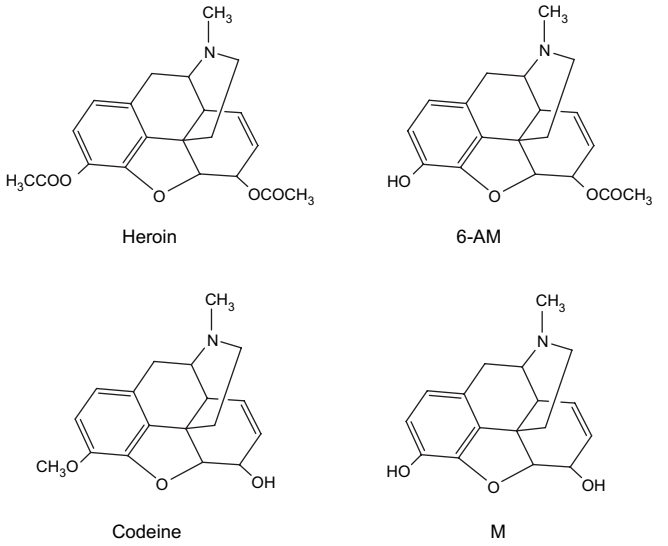


Fig. 1. Structures of some drugs of abuse.

Aryl and heteroaryl dyes have been attracting much attention over the past decade because of their rapidly increasing role in the design of advanced materials and devices [11–19]. The use of dyes in chemistry, biology, and medicine is growing continuously, with many new applications in the diagnosis and treatment of disease [20–23]. The *E*/*Z*-isomerizable N=N double bond within a conducting chain can work as a molecular switcher, making the aryl or heteroaryl systems promising candidates for molecular devices[16].

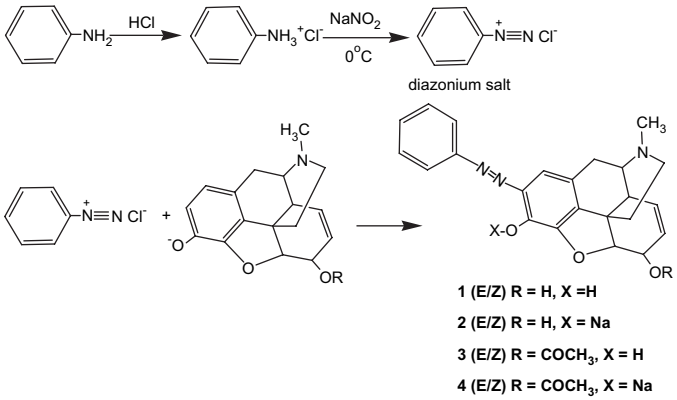
The present work describes the main features of the synthesis of fluorescent azo-morphine derivatives, peculiarities of their structure, and some fundamental properties. The developed method was used for determination of M and 6-AM in spiked human urine sample through simple and fast spectroscopic method.

2. Results and discussion

2.1. Chemistry

The most important problem for the development of new morphine marker compound is tedious and time-consuming reaction steps. To meet the challenge and to introduce such a promising family of dyes to chemistry, material science, forensic science and medicine, we have explored simple approaches to the synthesis of azo-morphine derivatives dyes.

Azo compounds were synthesized by first reacting aniline hydrochloride with nitrous acid (HNO_2) to produce an aryldiazonium ion. This ion can couple with a nucleophilic M or 6-AM in basic solution to produce the azo-M (phenyl-2-azo-morphine **1**, 76% and phenyl-2-azo-morphine sodium salt **2**, 24%) and azo-6-AM (phenyl-2-azo-6-acetyl morphine **3**, 77% and phenyl-2-azo-6-acetyl morphine sodium salt **4**, 23%) in reasonable yields (Scheme 1). All compounds were purified by thin layer chromatography (TLC) using tetrahydrofuran (THF) and dichloromethane (CH_2Cl_2) in the ratio 1:1 as



Scheme 1. Coupling of M and 6-AM to their azo-derivatives.

eluent. The constitution and purity of each of these compounds were established by elemental analysis, NMR, UV–vis, IR, and mass spectroscopies (see Section 4 for details). However, no reaction was found to occur with codeine under the same reaction conditions.

The NMR spectrum of **1** showed clearly that the compound was found in two forms *Z*, 30% and *E*, 70%, which could not be separated. On the other hand, the two forms (**3***Z*, $R_f = 0.72$, 35% and **3***E*, $R_f = 0.85$, 42%) of compound **3** could be separated on TLC, however, both compounds **2** and **4** were found only as sodium salt with $R_f = 0.52$ and 0.55, respectively. NMR spectrum of **1** showed the resonances of the OH protons at $\delta = 5.45$ ppm which was not observed in the spectrum of compound **2**. Also, ^1H and ^{13}C NMR spectra indicated the

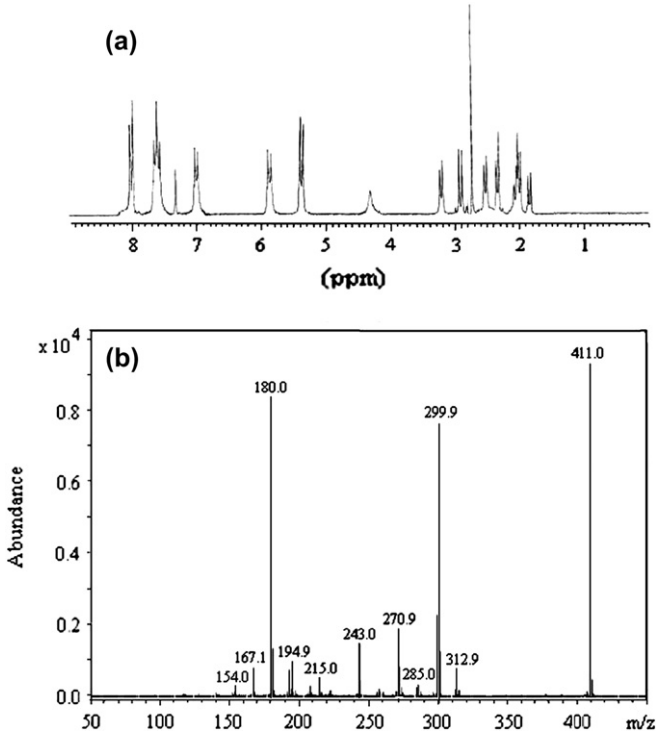


Fig. 2. (a) ^1H NMR spectrum of compound **2** in $CDCl_3$ at 20 °C; (b) ESI mass spectra of compound **2** in MeOH.

appearance of new signals corresponding to the organic moiety of each azo compound (Fig. 2). Mass spectra of all the compounds showed molecular ion peaks corresponding to their expected pattern of abundance ranging from 45–98% (Fig. 2).

The vibrational frequency of unreactive OH band ν(O–H) of M or the $COCH_3$ band ν(C=O) was not found to be sensitive to the connection of the azo moiety with M or 6-AM. For compound **1** ν(O–H) lies in the range of 3374–3376 cm^{-1}, and for compounds **3** and **4** ν(C=O) from 1710 to 1715 cm^{-1}, comparable to the frequencies of M ν(O–H) = 3375–3377 cm^{-1}, and 6-AM ν(C=O) = 1711–1715 cm^{-1}, indicating that the intra bonding of the hetero moiety was not perturbed by substitution on the other phenyl ring. The IR spectra of compounds **1**, **3***Z*, and **3***E* showed absorption bands within the 3510–3515 cm^{-1} region characteristic of the reactive OH group of the M or 6-AM moieties. However, the IR spectra of compounds **2** and **4** clearly indicated the lack of OH group absorptions in the wavenumber range 3500–3515 cm^{-1} which was also confirmed by the disappearance of (C–O) stretching band.

2.2. Optical studies

The electronic absorption spectra of the reaction mixture showed that the reaction was time dependent. In this case, the absorption intensity of the azo band increased slowly with time till precipitation after 15 min (Fig. 3). The electronic absorption spectral characteristics (λ_{max}, ε values) of the investigated azo compounds **1**–**4**, in ethanolic solutions are cited in Table 1. The high ε values pointed to the extended conjugation between the organic moieties through azo group. The compounds comprised two to three bands in the UV region and one band in the visible region (Fig. 4). Band of the shortest wavelength appearing in the range 205, 240, 250 nm was best ascribed to π–π^* transition of the benzenoid system of the compounds (this band is of higher intensity). The second band observed in UV region, in the wavelength range 265–280 nm was attributed to π–π^* transition within the furan heterocyclic moiety of the compounds. The third band observed in the UV region at 285–295 nm was assigned to n–π^* electronic transition of OH groups. This assignment was supported by the disappearance of this band in acid medium when excitation of the n-electrons was expected to be hindered by protonation in acetate buffer. The compounds displayed two main broad visible bands in ethanol within the range 330–350 nm (compounds **1** and **2**) and 440–470 nm (compounds **3** and **4**). These bands were capable of being assigned to π–π^* transition involving the whole electronic system of the compounds with a considerable charge-transfer (CT) character. Such a CT originated mainly from the phenyl moiety (phenyl azo) to the hetero moiety, i.e., this band was due to intramolecular CT transition. Furthermore, the CT nature of this band was also substantiated by the spectral behavior of these compounds in buffer solutions of varying pHs as given hereafter. The effect of solvent on the azo-morphine dyes produced a bathochromic shift with increasing solvent polarity.

Table 1
Yields of azo-M and azo-6-AM dyes (**1**–**4**) and their UV–vis spectral characteristics in ethanol

Compound	Color (yield, %)	λ_{max} (nm)	$\lambda_{emission}$ (nm)	ε (l/mol cm)
1	Yellow (76)	335	405	24 180
2	Yellow (24)	350	410	35 720
3*Z*	Orange (35)	445	515	1707
3*E*	Orange (42)	456	527	1603
4	Brown (23)	470	536	1868

The picks of the fluorescence spectra of the compounds **1**, **2**, **3***Z*, **3***E*, and **4** in ethanol appeared at $\lambda_{emission}$ values of 405 nm, 410 nm, 515 nm, 527 nm, and 536 nm, respectively, (Fig. 5, Table 1). The emission and excitation spectra of the dyes were measured at a concentration of $c = 1 \times 10^{-9}$ mol/dm^3 in solvents of different polarities (Fig. 6).

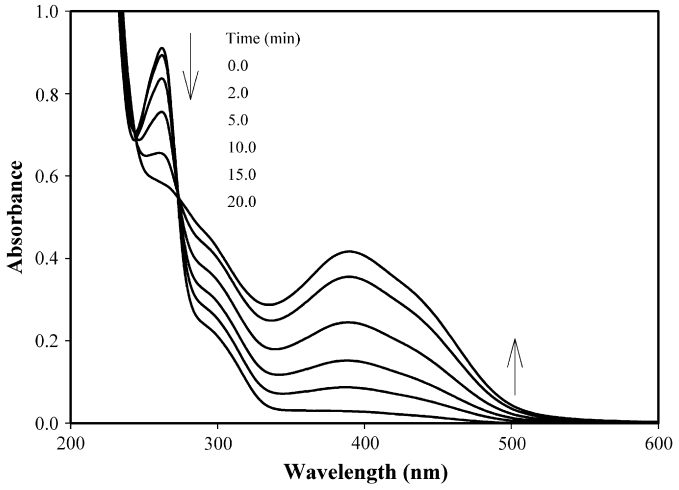


Fig. 3. Visible spectra of the reaction mixture of azo 6-AM in DI water at different reaction times.

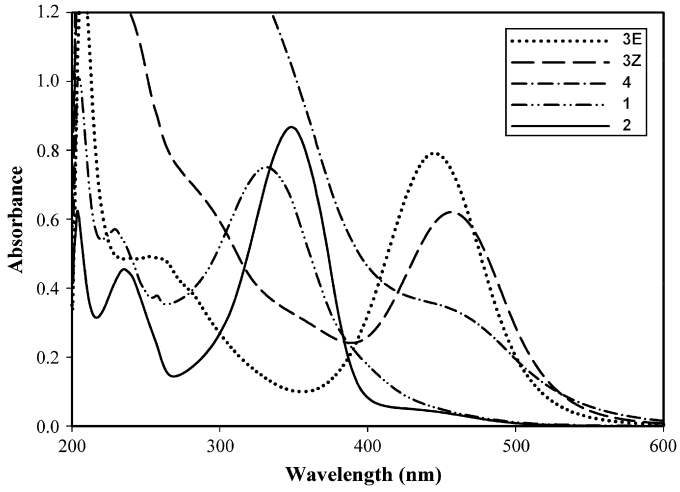


Fig. 4. Electronic absorption spectra of 2.5×10^{-5} M of azo-morphine derivatives (**1**–**4**) in ethanol.

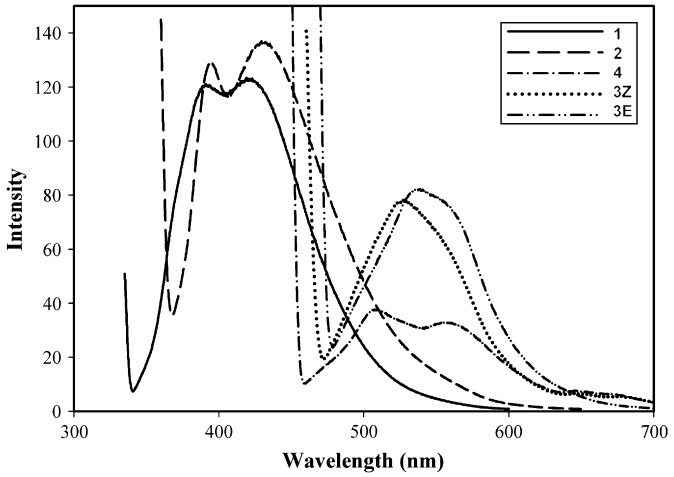


Fig. 5. Fluorescence spectra of 1×10^{-9} M of azo compounds **1**–**4** in ethanol.

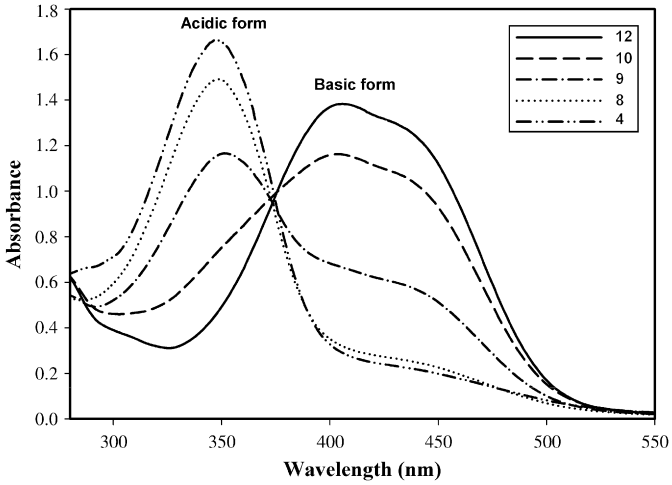


Fig. 7. Electronic absorption spectra of 5.2×10^{-5} M of **2** in phosphate buffer containing 4% (v/v) ethanol.

The new fluorescent dyes were also studied in buffer solutions of different pHs (1–12) containing 4% (v/v) ethanol to affect the solubility of the dyes. The bands due to localized $\pi-\pi^*$ transitions displayed slight changes, while the azo band (CT band) showed interesting changes with pH (for example see Fig. 7). The spectra of dyes exhibited changes in the extinction of the azo band only within the whole range of pH 2–12. For compound **2**, the absorbance of the azo band decreased with rise in pH while a new band was developed at longer wavelength. Clear isosbestic points were observed denoting the existence of an equilibrium of the acid base type (Fig. 7). The pH absorbance curves displayed two clear steps indicating the establishment of two acid base equilibria over the pH range 4–9. Change of the pH values had a remarkable effect on the fluorescence spectra of the dyes. The intensity of the fluorescence decreased with decreasing pH value of the solution due to the protonation of the dyes.

The visible band of these azo compounds acquired a blue shift at very low pH (<4) and a red shift at high pH (>8) values relative to the types of media. This could be explained based on the observation, that in highly acidic media, the protonation occurring on the furan heterocyclic moiety of the compounds enhanced its electron withdrawing ability and hence facilitated the CT transition, i.e., low energy was required for such a transition. On the other hand, the ionization of the OH group which took place at high pH values (>8) increased the electron density on the phenyl moiety of M or 6-AM and consequently increased its electron releasing character which resulted in an easier CT. This behavior confirmed the assignment of the visible band of these compounds to an electronic CT (tautomeric) which took place from the electron donor phenyl coupled moiety, to the electron acceptor heteroaromatic moiety of M or 6-AM in acid media and vice versa.

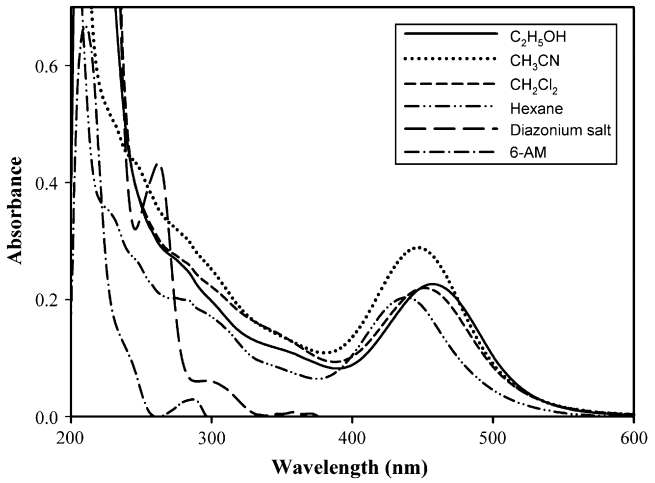


Fig. 6. Absorption spectra of 2.5×10^{-5} M of **3Z** in different organic solvents.

2.3. Analytical parameters

Under the experimental conditions described (Section 4.2), standard calibration curves of M and 6-AM were constructed by plotting absorbance versus concentration. The method was found to be linear in the range of 1×10^{-3}–1×10^{-5} M with a weighed regression described in Eqs. (1) and (2) for M and 6-AM, respectively.

$$A = 5081.2\,C + 0.0022,\ r = 0.9998 \quad (1)$$

$$A = 5267.2\,C - 0.0005,\ r = 0.9996 \quad (2)$$

where C is the concentration in M and r is the correlation coefficient. This indicates wide dynamic range within good linearity. The precision and intermediate precision (within 5 days) of the proposed method were examined by running five experiments. The relative standard deviation (RSD) was found to be <1.12%.

2.4. Biology

The determination of M in biological samples has become almost a routine assay in many toxicology laboratories owing

to the spread of the abuse of heroine, which is mainly biotransformed into M. In this experiment, the coupling reaction was carried out with drug-free urine sample, and with urine sample spiked with M and 6-AM. No remarkable change was observed for the drug-free urine sample as indicated by UV–vis spectroscopy. For urine sample spiked with the M and 6-AM, a brick red color appeared at once which was measured by UV–vis giving two peaks of azo compounds, at λ_{max} 395 nm and 580 nm corresponding to phenyl-azo-M and phenyl-azo-6-AM, respectively, (Fig. 8). The extraction recoveries were found to be >98.8% and RSD values of the recovery did not exceed 2.5% indicating good repeatability of the adopted method.

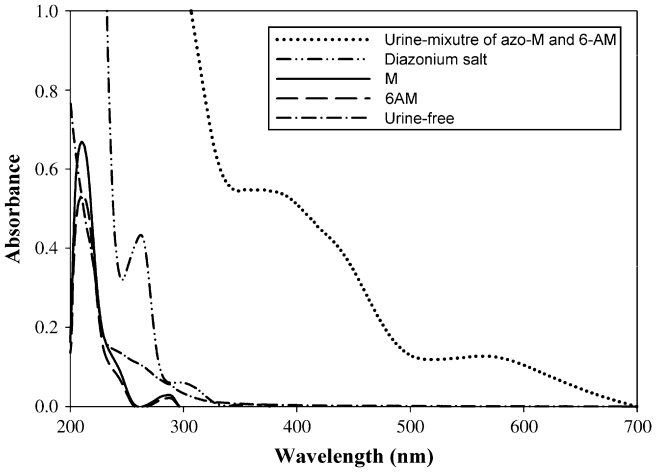


Fig. 8. Absorption spectra of azo reaction mixture of M and 6-AM in human urine sample.

3. Conclusions

A novel and rapid spectrophotometric method for the determination of M and 6-AM is proposed in this paper. A general efficient approach to the synthesis of novel promising reactive azo-morphine derivatives has been developed. The approach is based on the low temperature azo coupling of M or 6-AM with diazonium salt of aniline hydrochloride in a basic medium. It was possible to obtain suitable colored M or 6-AM compounds with maximum absorption peaks ranging from 330 to 470 nm by choosing the appropriate azo chromophore. All azo compounds under investigation displayed two or three bands in the UV region in different organic solvents. The first and second bands attributed to π–π* transition in benzenoid system and furan heterocyclic moiety, respectively, whereas the third band in UV region was assigned to n–π* electronic transition. In the visible region, all the compounds displayed two main broad visible bands, which were attributed to intramolecular CT transition. The resulting azo compounds were highly fluorescent in most organic solvents and water. Thus, our results show that this reaction can be considered as a marker of heroin use.

4. Experimental

4.1. General

All chemicals and solvents used in this study were of analytical grade. M, 6-AM·HCl and codeine were obtained from Lipomed Inc. (One Broaway, Cambridge, MA, USA). Aniline hydrochloride (BDH), sodium nitrite and sodium hydroxide were purchased from chemical sources. Plate chromatography was conducted on Sigma-Aldrich TLC plates silica gel matrix, H × W 20 cm × 20 cm. Elemental analyses were performed by a Perkin-Elmer 2400 automatic elemental analyzer. All compounds gave elemental analysis within ±0.4%. The measurements of NMR (^{1}H and ^{13}C) were carried out on a Bruker DPX 200 spectrometer. The chemical shifts δ are given in ppm relative to $\Xi = 200$ MHz for δ (^{1}H) (nominally $SiMe_4$), and $\Xi = 50$ MHz for δ (^{13}C) (nominally $SiMe_4$). IR (cm^{-1}) spectra were determined as KBr disc on a Shimadzu FTIR 8400 spectrometer. Electron spray ionization (ESI) mass spectra were recorded on a Bruker Esquire in CH_3OH. The pH-meter used for pH adjustments was Thermo Electron Cooperation, Orion 420A+. The UV–vis data were measured on Shimadzu 1601 PC instrument. The fluorescence (excitation and emission) spectra were determined with Shimadzu RF-5301 PC spectrophotometer: excitation slit width = 10 nm, emission slit width = 10 nm. Melting points were recorded on Gallenkamp apparatus.

4.2. General procedure for the synthesis of compounds *1–4*

A 0 °C solution of aniline hydrochloride (0.5 mmol) and 1 N HCl (1 mL) in deionized (DI) water (5 mL) was treated with a 0 °C solution of $NaNO_2$ (0.105 g, 1.5 mmol) in DI water (5 mL), and the mixture was stirred at 0 °C for 5 min. The resulting diazonium salt solution was poured into a 0 °C solution of M or its derivative (0.5 mmol) in NaOH (0.05 g, 1.25 mmol). The mixture was stirred at 0 °C for 15 min. The precipitate was filtered off, washed with NaCl, DI water and dried in vacuo. The products were purified by TLC using THF and CH_2Cl_2 in ratio 1:1 as eluent.

Upon storage of the azo coupling products **1–4** at ambient temperature for several months neither change in their UV–vis spectra nor appearance of foreign signals in their ^{1}H NMR spectra were observed, which provides evidence of their stability.

4.2.1. Phenyl-2-azo-morphine (*1*)

Yield (0.19 g, 76%) as a yellow solid; m.p. 73–75 °C; $R_f = 0.75$ (THF/CH_2Cl_2 1:1); IR ν_{max}(KBr disc, cm^{-1}) 3500s, 3375s (OH), 2503s (N^tH), 1635s (C=C, alkene), 1616, 1510s (C=C, aromatic), 1315–1245 (phenolic, CO), 1282, 1091s (C–O–C); ^{1}H NMR (200 MHz; $CDCl_3$; Me_4Si) δ (ppm) *Z*: 8.2, 7.85, 7.51, 7.44, 7.37, (5H, d, aromatic H, $J = 8.9$), 7.27, 7.19, 7.06 (3H, t, aromatic H, $J = 7.87$), 5.6, (1H, s, OH); *E*: 8.16, 7.57, 7.49, 7.43, 7.37, (5H, d, aromatic H, $J = 9.3$); ^{13}C NMR (50 MHz; $CDCl_3$; Me_4Si) δ (ppm) *Z*: 156.36, 155.83, 152.25, 148.75, 147.19, 135.89, 130.69,

127.31, 122.43, 121.14, 117.76, 111.89; *E*: 154.14, 153.94, 152.12, 148.81, 147.12, 135.68, 130.57, 127.12, 122.16, 121.03, 117.69, 111.45 (aromatic C, CH); (ESI): *m/z*(%) 389 (90) [M^+]; anal. calcd. for $C_{23}H_{23}N_3O_3$: C, 70.95; H, 7.95; N, 10.79; found: C, 70.89; H, 7.92; N, 10.68.

*4.2.2. Phenyl-2-azo-morphine sodium salt (**2**)*

Yield (0.063 g, 24%) as a yellow solid; m.p. 79–81 °C $R_f = 0.52$ (THF/CH_2Cl_2 1:1); IR ν_{max}(KBr disc, cm^{-1}) 3376s (OH), 2505s (N^{t}H), 1639s (C=C, alkene), 1620, 1515s (C=C, aromatic), 1286, 1092s (C–O–C); ^{1}H NMR (200 MHz; $CDCl_3$; Me_4Si) δ (ppm) 8.21, 7.97, 7.86, 7.82, 7.78 (5H, d, aromatic H, $J = 8.9$), 7.72, 7.27, 7.15 (3H, t, aromatic H, $J = 7.95$), 4.35 (1H, s, OH), 3.38–3.25 (1H, m); ^{13}C NMR (50 MHz; $CDCl_3$; Me_4Si) δ (ppm) 156.16, 154.78, 152.89, 148.62, 147.73, 134.97, 130.87, 126.65, 121.54, 121.35, 116.45, 113.08 (aromatic C, CH); (ESI): *m/z*(%) 411 (52) [M^+]; anal. calcd. for $C_{23}H_{22}N_3NaO_3$: C, 67.15; H, 5.35; N, 10.21; found: C, 67.02; H, 4.98; N, 9.94.

*4.2.3. Z-Phenyl-2-azo-6-acetyl morphine (**3Z**)*

Yield (0.1 g, 35%) as an orange solid; m.p. 71–73 °C; $R_f = 0.72$ (THF/CH_2Cl_2 1:1); IR ν_{max}(KBr disc, cm^{-1}) 3512s, 3377s (OH), 2495s (N^{t}H), 1746s (C=O), 1642s (C=C, alkene), 1619, 1510s (C=C, aromatic), 1321–1245s (phenolic, CO), 1278–1086s (C–O–C); ^{1}H NMR (200 MHz; $CDCl_3$; Me_4Si) δ (ppm) 7.88, 7.77, 7.49, 7.38, 7.06 (5H, d, aromatic H, $J = 9.0$), 6.93, 6.81, 6.36, (3H, t, aromatic H, $J = 12.07$), 5.52 (H, s, OH); ^{13}C NMR (50 MHz; $CDCl_3$; Me_4Si) δ 171.21 (C, COCH$_3$), 157.49, 152.85, 152.24, 148.14, 147.36, 134.62, 129.77, 127.51, 122.23, 121.64, 116.25, 111.47 (aromatic C, CH); (ESI): *m/z*(%) 431 (10) [M^+]; anal. calcd. for $C_{25}H_{25}N_3O_4$: C, 69.6; H, 5.8; N, 9.74; found: C, 69.23; H, 5.56; N, 9.37.

*4.2.4. E-Phenyl-2-azo-6-acetyl morphine (**3E**)*

Yield (0.12 g, 42%) as an orange solid; $R_f = 0.85$; m.p. 78–80 °C; (THF/CH_2Cl_2 1:1); IR ν_{max}(KBr disc, cm^{-1}) 3515s, 3375s (OH), 2501s (N^{t}H), 1751s (C=O), 1644s (C=C, alkene), 1616, 1514s (C=C, aromatic), 1325–1247s (phenolic, CO), 1280–1089s (C–O–C); ^{1}H NMR (200 MHz; $CDCl_3$; Me_4Si) δ (ppm) 7.85, 7.77, 7.49, 7.39, 7.22 (5H, d, aromatic H, $J = 10.12$), 7.18, 7.07, 7.03, (3H, t, aromatic H, $J = 10.12$), 5.55 (H, s, OH); ^{13}C NMR (50 MHz; $CDCl_3$; Me_4Si) δ (ppm) 170.47 (C, COCH$_3$), 157.89, 153.11, 151.87, 149.21, 148.12, 133.57, 130.12, 127.05, 121.94, 121.32, 116.41, 111.01 (aromatic C, CH); (ESI): *m/z*(%) 431 (10) [M^+]; anal. calcd. for $C_{25}H_{25}N_3O_4$: C, 69.6; H, 5.8; N, 9.74; found: C, 69.49; H, 5.74; N, 9.62.

*4.2.5. E-Phenyl-2-azo-6-acetyl morphine sodium salt (**4**)*

Yield (0.06 g, 23%) as a brown solid; m.p. 82–85 °C, $R_f = 0.55$ (THF/CH_2Cl_2 1:1); IR ν_{max}(KBr disc, cm^{-1}) 3377s (OH), 2496s (N^{t}H), 1751s (C=O), 1640s (C=C, alkene), 1618, 1512s (C=C, aromatic), 1282–1089s (C–O–C); ^{1}H NMR (200 MHz; $CDCl_3$; Me_4Si) δ (ppm) 7.93, 7.89, 7.7, 7.61, 7.59 (5H, d, aromatic H, $J = 9.72$), 7.51, 7.2, 6.16, (3H, t, aromatic H, $J = 9.72$); ^{13}C NMR (50 MHz; $CDCl_3$; Me_4Si) δ (ppm) 170.34 (C, COCH$_3$), 179.56, 156.17, 153.08, 147.87, 147.41, 133.98, 129.12, 127.04, 121.86, 121.15, 116.42, 111.32 (aromatic C, CH); (ESI): *m/z*(%) 453 (67) [M^+]; anal. calcd. for $C_{25}H_{24}N_3NaO_4$: C, 66.22; H, 5.29; N, 9.27; found: C, 66.11; H, 5.08; N, 9.14.

4.3. Biological studies

A 500 mg Bond Elut SPE column was used for the extraction. The SPE columns were conditioned by the sequential passage of 2×3 mL of methanol, 2×3 mL of water and 2×5 mL of water adjusted to pH 9.5 with NH_4OH. 10 mL of human urine sample adjusted to pH 9.5 with NH_4OH was vortex, centrifuged and applied to the SPE columns at a rate of 1.0 mL/min. The columns were washed with 2×5 mL of distilled water and left to dry for 10 min. The drugs were eluted with a solution consisting of a single phase mixture of dichloromethane/acetone (50:50) and collected in glass tubes. The elution solvent was evaporated to dryness under a nitrogen stream. The dried residues were then reconstituted in slightly warm water, and derivatization was carried out and then the samples were analyzed using UV–vis spectrophotometer.

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References

[1] S. Kerrigan, B.A. Goldberger, in: B. Levine (Ed.), Principles of Forensic Toxicology, second ed. American Association for Clinical Chemistry Inc., Washington, DC, 2003, pp. 187–205.
[2] P. Fernandez, L. Morales, C. Vazquez, A.M. Bermejo, M.J. Tabernero, Forensic Sci. Int. 161 (2006) 31–35.
[3] A. Alnajjar, B. McCord, J. Pharm. Biomed. Anal. 33 (2003) 463–473.
[4] P.E. Levi, in: P.E. Levi (Ed.), Modern Toxicology, Elsevier Science Publishing Co., Inc., Connecticut, 1997, pp. 222–274.
[5] W.J.M. Underberg, J.C.M. Waterval, Electrophoresis 23 (2002) 3922–3933.
[6] S. Hu, P.C.H. Li, J. Chromatogr., A 876 (2000) 183–191.
[7] H. Miyano, T. Toyo'oka, K. Imai, Anal. Chim. Acta 170 (1985) 81–87.
[8] J. Liu, Y.-Z. Hsieh, D. Wiesler, M. Novotny, Anal. Chem. 63 (1991) 408–412.
[9] N.V. Rama Rao, S.N. Tandon, Anal. Lett. 6 (1978) 477–483.
[10] A.B. Groth, G. Wallerberg, Acta Chem. Scand. 20 (1966) 2628–2629.
[11] N. Yokomichi, K. Seki, S. Tada, T. Yamabe, Synth. Met. 69 (1995) 577–578.
[12] G. Zotti, S. Zecchin, G. Schiavon, A. Berlin, G. Pagani, A. Canavesi, G. Asalbore-Miceli, Synth. Met. 78 (1996) 51–57.
[13] A. Chyla, S. Kucharski, J. Sworakowski, M. Bien'kovski, Thin Solid Films 284–285 (1996) 496–499.

[14] J.D. Nero, D. Laks, Synth. Met. 101 (1999) 440–441.
[15] B.A. Trofimov, E.Yu. Schmidt, A.I. Mikhaleva, A.M. Vasil'tsov, A.B. Zaitsev, N.S. Smolyanina, E.Yu. Senotrusova, A.V. Afonin, I.A. Ushakov, K.B. Petrushenko, O.N. Kazheva, O.A. Dyachenko, V.V. Smirnov, A.F. Schmidt, M.V. Markova, L.V. Morozova, Eur. J. Org. Chem. 17 (2006) 4021–4033.
[16] Z. Zhu, Y. Wang, Y. Lu, Macromolecules 36 (2003) 9585–9593.
[17] E. Wagner-Wysiecka, E. Luboch, M. Kowalczyk, J.F. Biernat, Tetrahedron 59 (2003) 4415–4420.
[18] A. Facchetti, A. Abbotti, L. Beverina, M.E. van der Boom, P. Dutta, G. Evmenenko, T.J. Marks, G.A. Pagani, Chem. Mater. 14 (2002) 4996–5005.
[19] Y. Wang, J. Ma, Y. Jiang, J. Phys. Chem. A 109 (2005) 7197–7206.
[20] J.C.V.P. Moura, in: A.N. Chakrabarty, J. Molnar (Eds.), Non-antibiotics, NISCOM, New Delhi, 1998, pp. 327–356.
[21] B. Altenberg-Greulich, G. Vriend, J. Mol. Struct. 598 (2001) 1–8.
[22] P.E. Nelson, J. Chromatogr. 290 (1984) 59–65.
[23] A. Alnajjar, J.A. Butcher, B. McCord, Electrophoresis 25 (2004) 1592–1600.