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Short communication

# Evaluation of the anti-inflammatory and anti-nociceptive activities of novel synthesized melatonin analogues

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**Abstract**

This study aimed at evaluation of the reactivity of melatonin (**1**) towards various chemical reagents to produce new melatonin analogues containing heterocyclic moieties which would provide basic pharmacological activities. The pyrrolo[1,2-*a*]indole derivatives **3**, **5**, **12**, **14** and pyrido[1,2-*a*]indole derivatives **9a**, **b** were synthesized via straightforward and efficient methods and their structures were established based on the analytical and spectral data. Also, this work was extended to study the potential role of the novel synthesized melatonin analogues **3**, **5**, **9a** and **12** as anti-inflammatory and anti-nociceptive agents in comparison with melatonin. After s.c. administration all compounds induced significant anti-inflammatory activity, inhibiting the paw oedema response compared with the control group at all time points in the test. Compound **5** has the strongest anti-inflammatory activity which exceeds that of the parent reference, melatonin, followed by compounds **9a** and **12**, at the first 2 h of administration. Effect of melatonin analogues on thermal pain, acetic acid-induced writhing and gastric lesions caused by indomethacin was also investigated. Compounds **5** and **12** were more potent as anti-nociceptive drugs; they are more potent in this respect than melatonin.


*Keywords:* Melatonin; Pyrrole; Indole; Pyridine; Anti-inflammatory; Anti-nociceptive

## 1. Introduction

There is growing evidence for the important role of the pineal hormone melatonin. A number of reports have been published concerning the medicinal importance of melatonin [1,2]. Melatonin has been shown to possess marked anti-inflammatory [3,4], analgesic [5], and anti-nociceptive [6] properties. Prostaglandin levels in the inflammatory exudates and cyclooxygenase-2 (COX-2) expression from carrageenan-treated rats were reported to be completely inhibited by melatonin [7]. Melatonin was recently shown to attenuate acute gastric lesions induced by strong irritants because of the scavenging of free radicals [8,9]. The development of synthetic melatonin analogues capable of mimicking the effects of melatonin has progressed considerably during the past decade [10–13]. Pyrrole and pyridine compounds are a promising starting materials in drug research in view of their various pharmacological activities [14–17]. Derivatives of pyrrole proved as non-steroidal anti-inflammatory drugs (NSAIDs) are tolmetin (CAS 64490-92-2) and zomepirac (CAS 64092-48-4). Other pyrrole and pyridine compounds have been recently reported as potent selective COX-2 inhibitors and antioxidants [18–22].

Based on these considerations, we investigate herein a straightforward and efficient synthesis of novel melatonin analogues containing pyrrole or pyridine nucleus fused to the essential pharmacophoric features of the melatonin molecule. Also the systemic anti-inflammatory and anti-nociceptive activities of the novel synthesized melatonin analogues were investigated in comparison with melatonin.

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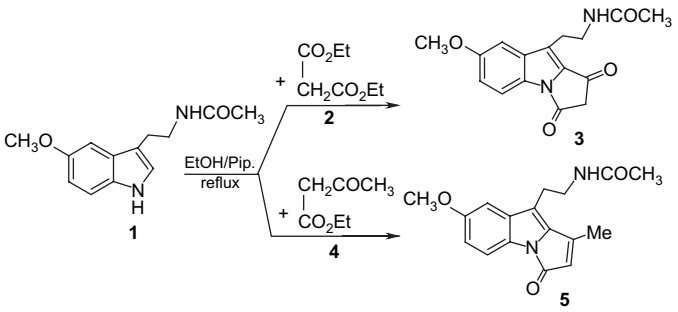
## 2. Results and discussion

### 2.1. Chemistry

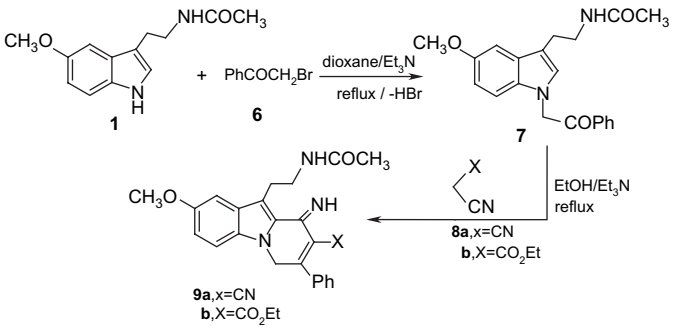
We have attempted a straightforward synthesis of pyrrolo[1,2-*a*]indole derivatives by the reaction of melatonin (**1**) with equimolar amount of diethylmalonate **2** in refluxing absolute ethanol containing piperidine (2 mL) which provided a colorless chromatographically pure product in 82% yield. The 1,3-dioxo-2*H*-pyrrolo[1,2-*a*]indole derivative **3** is assigned to this product through elemental analyses and spectroscopic data (Scheme 1). The mass spectrum (MS) of compound **3** showed molecular ion peak at $m/z = 300$ (35%) which corresponds to the molecular formula $C_{16}H_{16}N_2O_4$. The IR spectrum revealed absorption bands at $\upsilon$ 1745, 1738, and 1685 $cm^{-1}$ for the three carbonyl groups and the $^1H$ NMR spectrum revealed, in addition to the expected signals of melatonin moiety, the presence of singlet (2H) at $\delta$ 6.15 ppm for β-protons of the pyrrole nucleus. Moreover, the $^{13}C$ NMR (ppm) data showed the characteristic signals of the pyrrole ring at 111.6 (C-2) and 118.3 (fused pyrrole-C). Similarly, under the same experimental conditions, melatonin also reacted with ethyl acetoacetate **4** to afford the corresponding 1-oxo-3-methylpyrrolo[1,2-*a*]indole derivative **5** in 78% yield (Scheme 1).

A wide range of pharmacological activities have been attributed to fused pyridines [17,23]. In view of this information, we would like to report here on the formation and characterization of novel pyridomelatonin derivatives. The indole NH group of melatonin reacted readily with phenacyl bromide **6** via elimination of HBr by refluxing in dioxane/triethylamine solution to afford 1-benzoyl methinomelatonin derivative **7** in 78% yield as the only isolated product as confirmed by IR, $^1H$ NMR, MS and elemental analyses (cf. Section 3). Further confirmation for the structure of compound **7** was obtained by studying its reactivity towards some active methylene reagents to give pyrido[1,2-*a*]indole derivatives with potential biological activities. Thus, the reaction of compound **7** with either malononitrile **8a** or ethyl cyanoacetate **8b** in refluxing ethanolic triethylamine solution resulted in the corresponding chromatographically pure products, iminopyrido[1,2-*a*]indole derivatives **9a** and **9b** in 82% and 75% yield, respectively (Scheme 2).

The reaction of melatonin (**1**) with cyanoacetylhydrazide **10** in absolute ethanol under reflux resulted in 1-imino-3-hydrazinopyrrolo[1,2-*a*]indole derivative **12** (Scheme 3) which




Scheme 1.




Scheme 2.

was assigned through the elemental analyses and spectroscopic data. The reaction takes place via simple addition of the cyanoacetylhydrazide to the NH group of melatonin to give the intermediate **11** which readily underwent cyclization via loss of $H_2O$ molecule to afford compound **12** in 80% yield. Confirmation for the structure of compound **12** was obtained by studying its reactivity towards diketones. Therefore, compound **12** reacted with equimolar amount of pentan-2,4-dione (acetyl acetone) **13** in boiling absolute ethanol to afford the corresponding 3-pyrazolylpyrrolo[1,2-*a*]indole derivative **14** in 72% yield (Scheme 3).

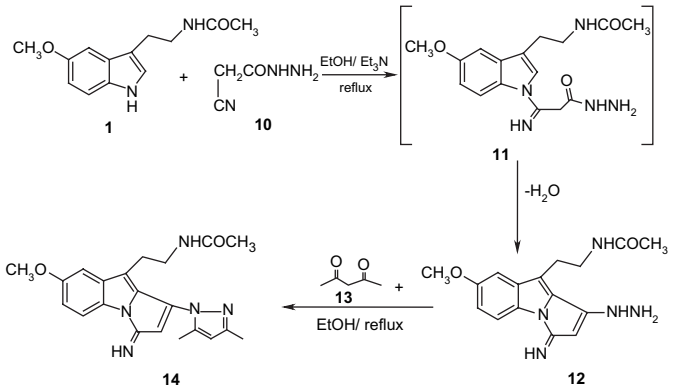
The structures of all the novel synthesized melatonin analogues were assigned based on their spectroscopic and elemental analyses data (cf. Section 3)

### 2.2. Pharmacology

In the present work, the effect of the tricyclic pyrrolo- and pyrido-indole derivatives **3**, **5**, **9a** and **12** was compared with that of melatonin on inflammation, thermal and visceral pain and on the development of gastric mucosal lesions caused by indomethacin.

#### 2.2.1. Tests of inflammation: effect of melatonin analogues on carrageenan-induced paw oedema

The effects of systemic injections of the tested compounds on oedema formation were studied using the carrageenan-induced paw inflammation in comparison with melatonin. A previous study [6] showed that melatonin has comparable




Scheme 3.

potency to indomethacin in inhibiting carrageenan-induced paw oedema response (29.6 vs 27.4% inhibition, respectively, 4 h post-carrageenan). After s.c. administration, all compounds induced significant anti-inflammatory activity, inhibiting the paw oedema response compared with the control group at all time points in the test (two-way ANOVA: treatment effect: $F_{5,120} = 58.4$; $P < 0.001$; time effect: $F_{3,120} = 88.9$; $P < 0.001$) (Fig. 1). The percentages of inhibition were −42.9, −35.7, −30 and −27.1% for compound **3**; −67.3, −59.4, −43, and −28.8% for compound **5**; −52.2, −55.2, −48 and −36.3% for compound **9a**; −54.8, −52.6, −44.2 and −36.8% for compound **12** compared with −50.8, −50.1, −44.4 and −30% for melatonin at 1, 2, 3 and 4 h time points. Post-hoc comparison revealed more significant inhibition of oedema formation by compound **5** compared with compounds **3**, **9a**, **12** or melatonin, at 1 h post-carrageenan. Compound **3** less significantly inhibited oedema formation than compound **9a** at 2–3 h post-carrageenan and also less significantly inhibited oedema formation compared with compounds **5**, **12** and melatonin at 2 h post-carrageenan.

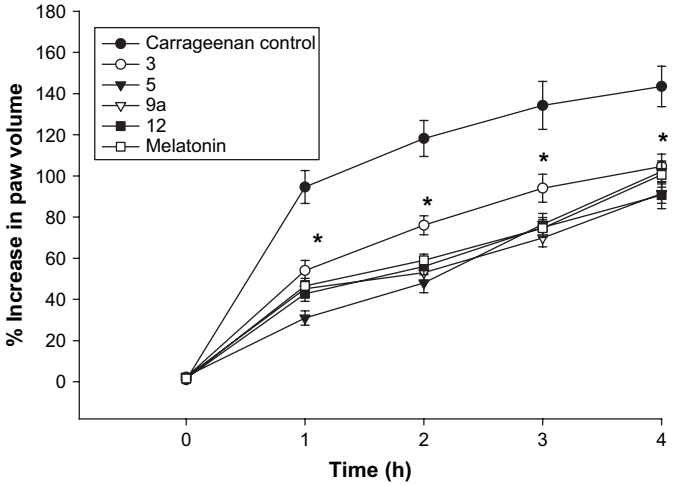



Fig. 1. The anti-inflammatory effect of compounds **3**, **5**, **9a**, **12** and melatonin administered at equimolar doses of 5.16, 5.13, 6.88, 5.40 and 4 mg/kg, respectively. Results are expressed as a percentage change from control (pre-drug) values, each point represents mean ± S.E. of 6 rats/group. Asterisks indicate significant change from control value at respective time points (ANOVA and Duncan's multiple comparison test).

### 2.2.2. Tests of nociception

*2.2.2.1. Effect of melatonin analogues on thermal pain.* Hot-plate latency was increased after the administration of all test compounds (Table 1, Fig. 2). One hour after drug injection, the increase in hot-plate latency by all test compounds and melatonin was significantly less than that induced by indomethacin (IND). Two hours after injection of test compounds, the increase in hot-plate latency by IND and compound **12** was significantly more prolonged than that induced by compounds **3**, **5**, **9a** or melatonin. Compound **9a** induced significantly less prolongation of hot-plate latency than compound **3** or **5**.

*2.2.2.2. Effect of melatonin analogues on acetic acid-induced writhing.* All test compounds significantly reduced the number of abdominal writhes induced by i.p. injection of acetic acid in mice (Fig. 3). Compounds **5** and **12** were the most potent in this respect, inhibiting the number of abdominal writhes by 68.7 ± 4.9 and 81.1 ± 6.1%, respectively, compared with the control group. Meanwhile, compounds **3**, **9a** and melatonin inhibited the number of abdominal writhes by 24.4 ± 1.2, 40.7 ± 3.6 and 55.6 ± 3.9%, respectively. Post-hoc comparison revealed that compounds **5** and **12** inhibited the writhing response more significantly than **3**, **9a** and melatonin, whereas melatonin was more potent than compounds **3** and **9a** in this respect.

*2.2.2.3. Effect of melatonin analogues on gastric lesions caused by indomethacin.* Compounds **5**, **12** or melatonin significantly reduced the number of gastric lesions induced by the administration of indomethacin in rats (Table 2, Fig. 4). Compound **5** was more potent in this respect than melatonin. Meanwhile, all test compounds and melatonin significantly reduced the severity of indomethacin-induced gastric lesions. Compound **5** was the most potent in this respect (Fig. 4).

These results indicated that all compounds exhibited anti-inflammatory and pain alleviating properties. It was noted that compound **5** was more effective in inhibiting inflammation and visceral pain induced by acetic acid injection in mice, whereas compound **12** showed efficacy in inhibiting both thermal and visceral pain. The development of gastric mucosal lesions evoked by the non-steroidal anti-inflammatory drug indomethacin in rats was much reduced by the administration of compound **5** or **12**, indicating safe gastric profile for these two compounds, similar to that of melatonin [24,6]. This is of particular importance in the development of new drugs to target pain conditions, since most of the available NSAIDs are well known for their propensity to evoke gastric mucosal injury [25,26]. Data obtained with compounds **5** and **12** thus encourage the development of melatonin derivatives which could have particular role in the management of pain, especially that of visceral origin.

From the structure–activity relationship point of view, the oxomethylpyrrolo[1,2-*a*]indole derivative **5** has the strongest anti-inflammatory activity. Replacement of the methyl group by either carbonyl group in compound **3** or hydrazone group in compound **12** decreased the anti-inflammatory activity of the indolopyrrole moiety. The presence of imine and

Table 1
Percentage increase in hot-plate latency in rats treated with melatonin analogues and melatonin in comparison with indomethacin, compared with their corresponding pre-drug basal level

| Compound | Hot-plate latency (% increase) | |
|---|---|---|
| | 1 h | 2 h |
| **3** | 103.6 ± 11.3 | 132.3 ± 14.7 |
| **5** | 116.6 ± 10.3 | 144.6 ± 16.4 |
| **9a** | 31.70 ± 4.70 | 56.20 ± 6.6 |
| **12** | 43.70 ± 5.0 | 243.7 ± 27.4 |
| Melatonin | 39.70 ± 3.80 | 96.0 ± 9.2 |
| Indomethacin | 176.7 ± 21.3 | 286.6 ± 29.5 |

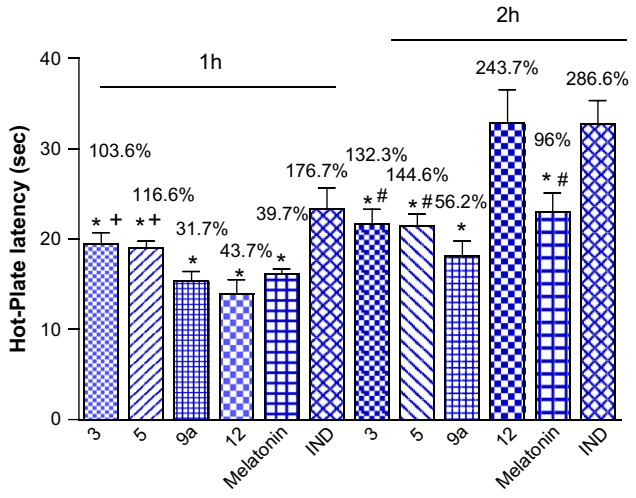



Fig. 2. Reaction time on the hot-plate in seconds after the administration of compounds **3**, **5**, **9a**, **12** and melatonin at equimolar doses of 5.16, 5.13, 6.88, 5.40 and 4 mg/kg, respectively. The percentage change from basal (pre-drug) values is shown ($n = 6$/group). Asterisks indicate significant change from the indomethacin (IND)-treated group at the respective time point. The plus sign (+) indicates significant change from the melatonin-treated group at the respective time point. The sign # indicates significant change from the compound **12**-treated group at the respective time point (ANOVA and Duncan's multiple comparison tests).

hydrazone groups attached to the indolopyrrole moiety in compound **12** increased its activity as anti-nociceptive agent compared to the other tested compounds. The pyrrole moiety in compounds **5** and **12** was more potent as anti-inflammatory and anti-nociceptive agent than the pyridine moiety in compound **9a**. Compounds **5**, **12** are more potent in this respect

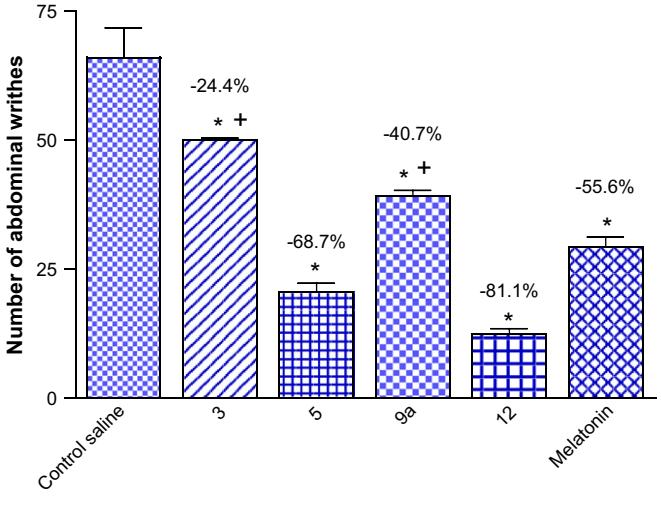



Fig. 3. Effect of compounds **3**, **5**, **9a**, **12** and melatonin, administered at equimolar doses of 5.16, 5.13, 6.88, 5.40 and 4 mg/kg, respectively, on the number of abdominal writhes induced by i.p. injection of acetic acid in mice (mean S.E. of 6–8 mice/group). The percent decrease in the number of writhes from the saline control group is represented above the respective group bar. Asterisks indicate significant change from the saline control group, whereas the plus sign (+) indicates significant change from compound **5**- or **12**-treated group (ANOVA and Duncan's multiple comparison tests).

Table 2
Percentage inhibition of the number and severity of indomethacin-induced gastric lesions in rats treated with melatonin analogues and melatonin

| Compound | Number of lesions (% inhibition) | Severity of lesions (% inhibition) |
|---|---|---|
| **3** | $43.5 \pm 3.1$ | $54.8 \pm 5.3$ |
| **5** | $72.6 \pm 5.7$ | $82.6 \pm 4.2$ |
| **9a** | $30.2 \pm 3.0$ | $53.6 \pm 3.8$ |
| **12** | $53.9 \pm 5.0$ | $57.7 \pm 4.0$ |
| Melatonin | $56.3 \pm 4.1$ | $69.0 \pm 5.2$ |

than melatonin. It indicates that the fusion of pyrrole ring to melatonin moiety is a profitable modification as it increased its anti-inflammatory and anti-nociceptive activities.

In search for NSAIDs, that can relieve pain and at the same time do not harm gastric mucosal, selective COX-2 inhibition have been published [27,28]. Directed by the structure of compounds **3**, **5**, **9a** and **12**, it is probably that these compounds have a dual mechanism for their anti-inflammatory and anti-nociceptive activities: the selective inhibition of COX-2 by melatonin moiety [7] or by the fused pyrrole or pyridine ring [18–20] and the free radical scavenging properties of melatonin moiety [3,29] and the fused pyrrole or pyridine ring [21,22]. Further studies should be made to establish the mechanism of action with the possibility to formulate a potent anti-inflammatory and anti-nociception prescription.

### 2.3. Conclusion

In this study we have described a straightforward and efficient synthesis of novel melatonin analogues containing fused pyrrole or pyridine nucleus in addition to the pharmacophoric features of the melatonin moiety. Melatonin and its novel

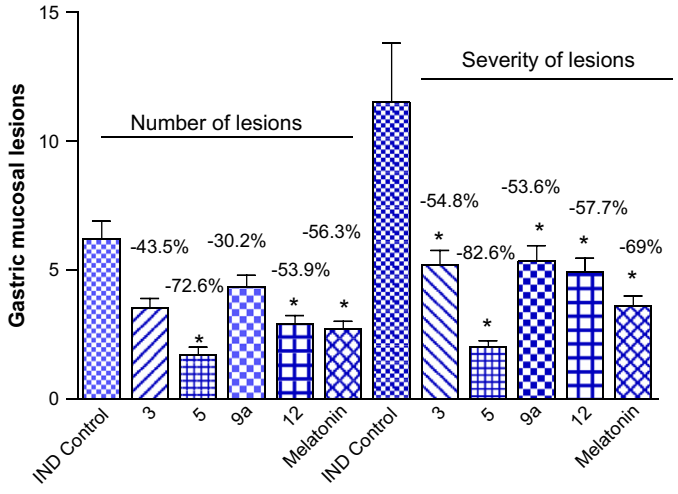



Fig. 4. Effect of compounds **3**, **5**, **9a**, **12** and melatonin, administered at equimolar doses of 5.16, 5.13, 6.88, 5.40 and 4 mg/kg, respectively, on the number and severity of gastric mucosal lesions caused by s.c. injection of indomethacin in rats. The percent decrease in the number or severity of gastric lesions from the indomethacin control group is represented above the respective group bar. Asterisks indicate significant change from the corresponding indomethacin (IND) control group (ANOVA and Duncan's multiple comparison test).

synthesized derivatives **3**, **5**, **9a** and **12** showed anti-inflammatory and anti-nociception activities with various intensities. The oxomethylpyrrolo[1,2-*a*]indole derivative **5** has the strongest anti-inflammatory activity which exceeds that of the parent reference, melatonin, followed by iminopyrido[1,2-*a*]indole derivative **9a** and iminohydrazinopyrrolo[1,2-*a*]indole derivative **12**, at the first 2 h of administration. Compounds **5** and **12** were more potent as anti-nociceptive drugs; they are more potent in this respect than melatonin.

## 3. Experimental section

### *3.1. Synthesis*

All chemicals were purchased from commercial suppliers and used directly. The appropriate precautions in handling moisture-sensitive compounds were undertaken. All melting points were measured using an electrothermal capillary melting point apparatus and are uncorrected. The IR spectra were recorded (KBr discs) on a Shimadzu FT-IR 8201 PC spectrophotometer and expressed in $cm^{-1}$. $^1H$ and $^{13}C$ NMR spectra were recorded on a Varian EM-390 90 MHz spectrometer in DMSO-$d_6$ as solvent, using TMS as internal reference and chemical shifts ($\delta$) are expressed in ppm. Mass spectra were recorded on a GCMS-QP 1000 Ex spectra mass spectrometer operating at 70 eV. Elemental analyses were carried out by Microanalytical Data Unit at National Research Centre, Cairo, Egypt. Analytical thin-layer chromatography (TLC) was carried out using Merck 60 F254 aluminum sheets and visualized by UV light (254 nm).

#### *3.1.1. General method for preparation of compounds **(3)** and **(5)***

To a solution of melatonin (**1**) (1.16 g, 0.005 mol) in ethanol (25 mL) containing an amount of piperidine (2 mL) either diethylmalonate (0.80 g, 0.005 mol) or ethyl acetoacetate (0.65 g, 0.005 mol) was added. The reaction mixture, in each case, was heated under reflux for 5 h until all starting materials had disappeared as indicated by TLC, and then left to cool at room temperature, poured over ice/water mixture, neutralized with dilute hydrochloric and extracted with diethyl ether (3 × 20 mL). The organic layer, in each case, was dried over calcium chloride. Removal of the solvent in vacuo afforded the corresponding product, which was crystallized from the appropriate solvent.

*3.1.1.1. N-[2-(1,3-Dioxo-dihydro-7-methoxy-1H-pyrrolo[1,2-a]indol-9-yl)ethyl]acetamide (**3**).* Yellow crystals, from MeOH, yield 1.23 g (82%), mp 189–190 °C. IR ($\upsilon/cm^{-1}$): 3380 (NH), 3030 (CH-aromatic), 2970 ($CH_3$), 2853 ($CH_2$), 1745, 1738, 1685 (3C=O). $^1H$ NMR ($\delta$ ppm): 1.78 (s, 3H, $COCH_3$), 3.04 (t, $J = 7.2$ Hz, 2H, $CH_2$), 3.43 (t, $J = 7.2$ Hz, 2H, $CH_2$), 3.82 (s, 3H, $OCH_3$), 4.92 (brs, 1H, NH, $D_2O$-exchangeable), 6.15 (s, 2H, pyrrole H-β), 7.45–7.85 (m, 3H, aromatic-H). $^{13}C$ NMR ($\delta$): 22.6 ($COCH_3$), 172.5 ($COCH_3$), 23.5, 38.2 ($2CH_2$), 54.7 ($OCH_3$), 196.7 (C-1, C=O), 111.6 (C-2), 214.8 (C-3, C=O), 109.6 (C-5), 110.7 (C-6), 152.9 (C-7), 100.3 (C-8), 112.8 (C-9), 118.3 (fused pyrrole-C), 127.5, 131.6 (fused-aromatic-C). MS (EI): *m/z* (%): 300 ($M^{+\bullet}$, 35), 269 (22), 242 (100), 225 (50). Anal. Calcd. for $C_{16}H_{16}N_2O_4$ (300.312): C, 63.99; H, 5.37; N, 9.32; found: C, 64.11; H, 5.54; N, 9.08.

*3.1.1.2. N-[2-(3-Oxo-7-methoxy-1-methyl-1H-pyrrolo[1,2-a]indol-9-yl)ethyl]acetamide (**5**).* Pale yellow crystals, from 1,4-dioxane, yield 1.16 g (78%), mp 148–150 °C. IR ($\upsilon/cm^{-1}$): 3369 (NH), 3035 (CH-aromatic), 2978 ($CH_3$), 2850 ($CH_2$), 1740, 1695 (2C=O). $^1H$ NMR ($\delta$ ppm): 1.82 (s, 3H, $COCH_3$), 2.32 (s, 3H, $CH_3$), 3.02 (t, $J = 7.1$ Hz, 2H, $CH_2$), 3.45 (t, $J = 7.1$ Hz, 2H, $CH_2$), 3.85 (s, 3H, $OCH_3$), 4.97 (brs, 1H, NH, $D_2O$-exchangeable), 6.23 (s, 1H, pyrrole H-β), 7.57–7.90 (m, 3H, aromatic-H). $^{13}C$ NMR ($\delta$): 23.0 ($COCH_3$), 173.4 ($COCH_3$), 23.8, 37.2 ($2CH_2$), 27.6 ($CH_3$), 55.6 ($OCH_3$), 134.2 (C-1), 115.0 (C-2), 196.7 (C-3, C=O), 109.7 (C-5), 110.3 (C-6), 150.9 (C-7), 102.0 (C-8), 112.4 (C-9), 118.7 (fused pyrrole-C), 127.2, 130.8 (fused-aromatic-C). MS (EI): *m/z* (%): 298 ($M^{+\bullet}$, 27), 283 (34), 267 (22), 240 (100), 214 (42). Anal. Calcd. for $C_{17}H_{18}N_2O_3$ (298.342): C, 68.44; H, 6.08; N, 9.38; found: C, 68.17; H, 6.27; N, 9.20.

#### *3.1.2. N-[2-(1-Benzoylmethino-5-methoxyindol-3-yl)ethyl]-acetamide (**7**)*

To a mixture of melatonin (**1**) (1.16 g, 0.005 mol) and phenacyl bromide **6** (1 g, 0.005 mol) in 1,4-dioxane (30 mL), a catalytic amount of triethylamine was added. The reaction mixture was heated under reflux for 7 h, cooled at room temperature, poured into ice and acidified with dilute hydrochloric acid. The solution was extracted with chloroform (3 × 20 mL), the organic extract was dried over calcium chloride and concentrated under *vacuum* to dryness to yield 1.36 g (78%) of compound **7** as oil, IR ($\upsilon/cm^{-1}$): 3384 (NH), 3050 (CH-aromatic), 2985 ($CH_3$), 2830 ($CH_2$), 1720, 1690 (2C=O). $^1H$ NMR ($\delta$ ppm): 1.87 (s, 3H, $COCH_3$), 2.98 (t, $J = 7.2$ Hz, 2H, $CH_2$), 3.35 (t, $J = 7.2$ Hz, 2H, $CH_2$), 3.85 (s, 3H, $OCH_3$), 4.43 (s, 2H, $CH_2$), 4.95 (brs, 1H, NH, $D_2O$-exchangeable), 6.75 (s, 1H, indole H-2), 7.46–7.98 (m, 8H, aromatic-H). MS (EI): *m/z* (%): 351 ($MH^{+\bullet}$, 52), 231 (24), 200 (43), 120 (100), 105 (22), 77 (37). Anal. Calcd. for $C_{21}H_{22}N_2O_3$ (350.424): C, 71.98; H, 6.32; N, 7.99; found: C, 72.25; H, 6.12; N, 8.21.

#### *3.1.3. General method for preparation of compounds **(9a)** and **(9b)***

To a solution of compound **7** (0.7 g, 0.002 mol) in absolute ethanol (25 mL) containing a catalytic amount of triethylamine either malononitrile **8a** (0.13 g, 0.002 mol) or ethyl cyanoacetate **8b** (0.23 g, 0.002 mol) was added. The reaction mixture, in each case, was heated under reflux for 4 h until all starting materials had disappeared as indicated by TLC, and then left to cool at room temperature. The solution was concentrated under *vacuum*, whereby the resulted oily product was triturated with ice/water mixture and neutralized with dilute hydrochloric acid. The formed solid product, in each case,

was filtered off, washed several times with water, dried and crystallized from the appropriate solvent.

*3.1.3.1. 10-[2-(Acetylamino)ethyl]-9-imino-2-methoxy-7-phenyl-6,9-dihydropyrido[1,2-a]indole-8-carbonirile (**9a**).* Brown powder, from EtOH, yield 0.65 g (82%), mp 181–182 °C. IR (υ/cm$^{-1}$): 3356–3375 (2NH), 3040 (CH-aromatic), 2974 ($CH_3$), 2853 ($CH_2$), 2225 (CN), 1695 (C=O). $^1$H NMR (δ ppm): 1.87 (s, 3H, $COCH_3$), 3.07 (t, $J = 7.2$ Hz, 2H, $CH_2$), 3.47 (t, $J = 7.2$ Hz, 2H, $CH_2$), 3.79 (s, 3H, $OCH_3$), 4.94 (brs, 1H, NH, $D_2O$-exchangeable), 7.55–8.04 (m, 8H, aromatic-H), 8.35 (s, 2H, pyridine H-α), 8.75 (brs, 1H, NH, $D_2O$-exchangeable). $^{13}$C NMR (δ): 22.7 ($COCH_3$), 173.2 ($COCH_3$), 23.4, 37.8 (2 $CH_2$), 54.8 ($OCH_3$), 122.6 (CN), 101.3 (C-1), 152.5 (C-2), 110.2 (C-3), 109.8 (C-4), 115.0 (C-6), 151.6 (C-7), 114.8 (C-8), 133.5 (C-9), 113.2 (C-10), 149.3 (fused pyridine-C), 128.3, 132.2 (fused-aromatic-C), 148.3, 113.2, 113.8, 129.3, 129.5, 131.4 (phenyl-C). MS (EI): *m/z* (%): 398 ($M^{+\bullet}$, 40), 367 (38), 321 (48), 292 (100), 77 (67). Anal. Calcd. for $C_{24}H_{22}N_4O_2$ (398.462): C, 72.34; H, 5.56; N, 14.06; found: C, 72.52; H, 5.29; N, 13.90.

*3.1.3.2. Ethyl 10-[2-(acetylamino)ethyl]-9-imino-2-methoxy-7-phenyl-6,9-dihydropyrido[1,2-a]indole-8-carboxylate (**9b**).* Yellow powder, from MeOH, yield 0.66 g (75%), mp 217–218 °C. IR (υ/cm$^{-1}$): 3350–3373 (2NH), 3050 (CH-aromatic), 2975 ($CH_3$), 2869 ($CH_2$), 1735, 1698 (2C=O). $^1$H NMR (δ ppm): 1.13 (t, $J = 6.8$ Hz, 3H, ester $CH_3$), 1.79 (s, 3H, $COCH_3$), 2.95 (t, $J = 7.2$ Hz, 2H, $CH_2$), 3.40 (t, $J = 7.2$ Hz, 2H, $CH_2$), 3.85 (s, 3H, $OCH_3$), 4.25 (q, $J = 6.8$ Hz, 2H, ester $CH_2$), 4.95 (brs, 1H, NH, $D_2O$-exchangeable), 7.54–8.10 (m, 8H, aromatic-H), 8.45 (s, 2H, pyridine H-α), 8.82 (s, 1H, NH, $D_2O$-exchangeable). $^{13}$C NMR (δ): 22.1 ($COCH_3$), 174.8 ($COCH_3$), 23.8, 36.3 (2 $CH_2$), 55.3 ($OCH_3$), 15.8 ($CO_2CH_2CH_3$), 24.2 ($CO_2CH_2CH_3$), 172.6 ($CO_2CH_2CH_3$), 102.0 (C-1), 153.7 (C-2), 110.2 (C-3), 109.2 (C-4), 115.7 (C-6), 152.4 (C-7), 114.5 (C-8), 134.5 (C-9), 113.5 (C-10), 148.4 (fused pyridine-C), 128.7, 131.9 (fused-aromatic-C), 148.5, 113.7, 114.3, 129.3, 129.7, 131.4 (phenyl-C). MS (EI): *m/z* (%): 445 ($M^{+\bullet}$, 24), 339 (35), 308 (100), 77 (56). Anal. Calcd. for $C_{26}H_{27}N_3O_4$ (445.523): C, 70.09; H, 6.10; N, 9.43; found: C, 70.25; H, 5.91; N, 9.65.

*3.1.4. N-[2-(3-Imino-1-hydrazino-7-methoxy-1H-pyrrolo[1,2-a]indol-9-yl)ethyl]acetamide (**12**)*

Cyanoacetylhydrazide **10** (0.5 g, 0.005 mol) was added to a solution of melatonin (**1**) (1.16 g, 0.005 mol) in absolute ethanol (30 mL) containing a catalytic amount of triethylamine. The reaction mixture was refluxed for 3 h and then evaporated to dryness under reduced pressure. The residue was dissolved in diethyl ether and the solution was washed with saturated sodium carbonate. The organic layer was separated, dried over anhydrous magnesium sulfate and the solvent was removed under reduced pressure. The resulting solid was recrystalized from 1,4-dioxane to give 1.25 g (80%) of compound **12**, pale brown crystals, mp 219–220 °C. IR (υ/cm$^{-1}$): 3458–3295 (2NH, $NH_2$), 3043 (CH-aromatic), 2970 ($CH_3$), 2865 ($CH_2$), 1692 (C=O). $^1$H NMR (δ ppm): 1.87 (s, 3H, $COCH_3$), 3.05 (t, $J = 7.0$ Hz, 2H, $CH_2$), 3.47 (t, $J = 7.0$ Hz, 2H, $CH_2$), 3.80 (s, 3H, $OCH_3$), 4.95 (brs, H, NH, $D_2O$-exchangeable), 5.25 (s, 2H, $NH_2$, $D_2O$-exchangeable), 6.17 (s, 1H, pyrrole H-β), 7.67–7.88 (m, 3H, aromatic-H), 8.58, 8.72 (2brs, 2H, 2NH, $D_2O$-exchangeable). $^{13}$C NMR (δ): 22.8 ($COCH_3$), 171.5 ($COCH_3$), 23.5, 38.2 (2$CH_2$), 54.9 ($OCH_3$), 134.8 (C-1), 108.7 (C-2), 133.6 (C-3), 109.6 (C-5), 110.7 (C-6), 152.9 (C-7), 100.3 (C-8), 112.8 (C-9), 126.9, 131.4 (fused-aromatic-C), 118.3 (fused pyrrole-C). MS (EI): *m/z* (%): 314 ($MH^{+\bullet}$, 52), 282 (25), 176 (30), 145 (100). Anal. Calcd. for $C_{16}H_{19}N_5O_2$ (313.362): C, 61.32; H, 6.11; N, 22.34; found: C, 61.15; H, 6.31; N, 22.57.

*3.1.5. N-[2-(1-(3,5-Dimethylpyrazol-1-yl)-3-imino-7-methoxy-1H-pyrrolo[1,2-a]indol-9-yl)ethyl]acetamide (**14**)*

A mixture of compound **12** (1.56 g, 0.005 mol) and acetyl acetone **2** (0.5 g, 0.005 mol) in absolute ethanol (25 mL) was boiled under reflux for 3 h until all starting materials had disappeared as indicated by TLC. Then the reaction mixture was concentrated under *vacuum*, whereby the resulted oily product was triturated with petroleum ether (bp 60–80 °C). The formed solid product was filtered off, dried and crystallized from MeOH to yield 1.35 g (72%) of compound **14**, brown crystals, mp 239–241 °C. IR (υ/cm$^{-1}$): 3380–3295 (2NH), 3035 (CH-aromatic), 2977 ($CH_3$), 2875 ($CH_2$), 1695 (C=O). $^1$H NMR (δ ppm): 1.75 (s, 3H, $COCH_3$), 2.26 (s, 6H, 2$CH_3$), 3.03 (t, $J = 7.0$ Hz, 2H, $CH_2$), 3.38 (t, $J = 7.0$ Hz, 2H, $CH_2$), 3.85 (s, 3H, $OCH_3$), 4.78 (brs, 1H, NH, $D_2O$-exchangeable), 6.12 (s, 1H, pyrrole H-β), 6.65 (s, 1H, pyrazole H-4), 7.68–7.92 (m, 3H, aromatic-H), 8.62 (brs, 1H, 1NH, $D_2O$-exchangeable). $^{13}$C NMR (δ): 22.3 ($COCH_3$), 173.5 ($COCH_3$), 23.5, 37.3 (2 $CH_2$), 54.7 ($OCH_3$), 25.3, 26.2 (2$CH_3$), 134.8 (C-1), 108.6 (C-2), 133.6 (C-3), 109.2 (C-5), 110.3 (C-6), 152.8 (C-7), 101.3 (C-8), 112.8 (C-9), 125.3, 132.2 (fused-aromatic-C), 118.7 (fused pyrrole-C), 135.2, 106.3, 136.7 (pyrazole-C). MS (EI): *m/z* (%): 377 ($M^{+\bullet}$, 35), 356 (42), 282 (54), 271 (30), 145 (100), 95 (63). Anal. Calcd. for $C_{21}H_{23}N_5O_2$ (377.452): C, 66.82; H, 6.14; N, 18.55; found: C, 67.05; H, 6.32; N, 18.76.

### 3.2. Pharmacological assay

#### 3.2.1. Animals

Sprague–Dawley strain rats weighing 120–130 g or Swiss albino mice 20–25 g body weight was used throughout the experiments. Food and water were provided *ad libitum*. Experiments were performed between 0900 and 1500 h.

#### 3.2.2. Tests of inflammation: carrageenan-induced paw oedema assay

All tested compounds were screened for anti-inflammatory activity using the carrageenan-induced paw oedema assay in rats. This model is widely used as a screening tool for evaluation of putative anti-inflammatory agents. The activity of the compounds was compared with melatonin at a dose of 4 mg/kg. The dose of melatonin was chosen based on previous studies

[30]. The equimolar doses (0.0172 mol) 5.16, 5.13, 6.88 and 5.40 mg/kg of compounds **3**, **5**, **9a** and **12**, respectively, were used.

Paw oedema was induced by sub-plantar injection of 100 μL of 1% sterile carrageenan lambda in saline into the right hind paw [31]. Contra-lateral paw received an equal volume of saline. Paw volume was determined immediately before carrageenan injection and at selected times thereafter using a plethysmometer (Ugo Basile, Milan, Italy). The oedema component of inflammation was quantified by measuring the increase in paw volume (mL) before carrageenan injection and at 1, 2, 3 and 4 h after carrageenan injection with respect to the pre-injection value for each animal. Oedema was expressed as a percentage of change from control (pre-drug) values. The effect of systemic administration of each of the test compounds **3**, **5**, **9a** or **12** at doses of 5.16, 5.13, 6.88 or 5.40 mg/kg, respectively (0.5 mL, s.c., $n = 6$/group) or melatonin (4 mg/kg, s.c., 0.5 mL) given as a 30 min pretreatment was studied. The control groups received saline (0.5 mL, $n = 6$/group; s.c.) instead.

### 3.2.3. Tests of nociception

*3.2.3.1. Hot-plate assay.* The hot-plate test was performed by using an electronically controlled hot-plate (Ugo Basile, Italy) heated to 52 °C (±0.1 °C). The cut-off time was 30 s. Groups of rats ($n = 6$/group) were given the test compounds **3**, **5**, **9a**, **12** or melatonin at equimolar doses (0.0172 mol) of 5.16, 5.13, 6.88, 5.40 or 4 mg/kg, respectively, s.c., or IND (control), 30 min prior to testing. The experimenter was blind to drugs. Latency to lick a hind paw or jump out of the apparatus was recorded for the control and drug-treated groups.

*3.2.3.2. Acetic acid-induced writhing.* Compounds **3**, **5**, **9a**, **12** or melatonin at equimolar doses (0.0172 mol) of 5.16, 5.13, 6.88, 5.40 or 4 mg/kg, respectively, or saline (control) were given s.c., 1 h before i.p. injection of 0.6% acetic acid (0.4 mL) in mice [32]. The number of writhes (constriction of abdomen, twisting of trunk and extension of hind legs) during 30 min observation period was noted. The experimenter was blind to drugs.

*3.2.3.3. Gastric ulcerogenic studies.* Gastric mucosal damage was evoked in rats by the administration of indomethacin (20 mg/kg, s.c.). The effect of s.c. administration of compounds **3**, **5**, **9a**, **12** or melatonin at equimolar doses (5.16, 5.13, 6.88, 5.40 or 4 mg/kg, respectively), administered at time of indomethacin injection was studied. Food and water were provided *ad libitum*. Rats were sacrificed 24 h after drug administration, stomachs excised, opened along the greater curvature, rinsed with saline, extended on a plastic board and examined for mucosal lesions. The number and severity of mucosal lesions were noted and lesions were scaled as described elsewhere [33].

### 3.2.4. Statistical analyses

Data are expressed as mean ± S.E. The results of carrageenan-induced paw oedema experiments are expressed as a percentage of change from control (pre-drug) values. Differences between vehicle control and treatment groups were tested using one- and two-way ANOVA followed by multiple comparison by the Duncan's multiple comparison test. A probability value less than 0.05 was considered statistically significant.

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