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ORIGINAL PAPER

Synthesis and serotonin antagonist and antianexity activities of pyrrolidine derivatives from 4-hydrazinyl-1-*p*-substituted phenyl-2,5-dihydro-1*H*-pyrrole-3-carbonitriles

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Abstract *N*-(*p*-substituted phenyl)-4-cyanopyrrolidin-3-ones and their corresponding hydrazines were prepared and used as starting materials to synthesize heterocyclic candidates as serotonin antagonist and antianexity agents. Condensation of hydrazines with selected aromatic aldehydes afforded the corresponding Schiff bases. The hydrazines were treated with phenyl isothiocyanate to afford the corresponding thiosemicarbazides, which were cyclized with ethyl bromoacetate to *N*-phenylthiazolidinones. The hydrazine was reacted with 1,2,4,5-tetrachlorophthalic anhydride to give the tetrachloroimide derivative. It was reacted with benzoyl acetonitrile, 2-(bismethylsulfanyl-methylene)malononitrile, 2-ethoxymethylenemalononitrile, or 2-cyano-3-ethoxyacrylic acid ethyl ester to afford the corresponding pyrazoline derivatives. Schiff bases were obtained by simple condensation of the hydrazine with different carbonyl compounds. All the compounds were screened for their serotonin antagonistic and antianexity activities, and they showed high activities compared to buspirone and diazepam as controls.

Keywords Pyrrolidinone · Thiosemicarbazide · Schiff bases · Pharmacological activities

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Introduction

A literature survey reveals that the pyrrolidine ring is important for antimycobacterial activity [1]. In addition, many hetero-nitrogen derivatives exhibit a wide variety of biological activities, such as antimicrobial [2], anti-inflammatory [3], antihistaminic [4], antihypertensive [5], hypnotic [6] and anticonvulsant [7] activity. From previous work, Schiff bases are known to possess antimicrobial [8–11] and anti-inflammatory [12, 13] activities. Also, thiosemicarbazide and triazole derivatives have been found to be of interest with potential activities including antimicrobial, analgesic and anticonvulsant [14–17]. In addition, we have reported that certain of our new heterocyclic compounds exhibited antiparkinsonian [18], antitumor [19–21], antimicrobial [22] and anti-inflammatory [23] activities. In view of these observations and in continuation of our previous work in heterocyclic chemistry, we herein synthesized some new substituted pyrrolidine derivatives for their pharmacological screening.

Results and discussion

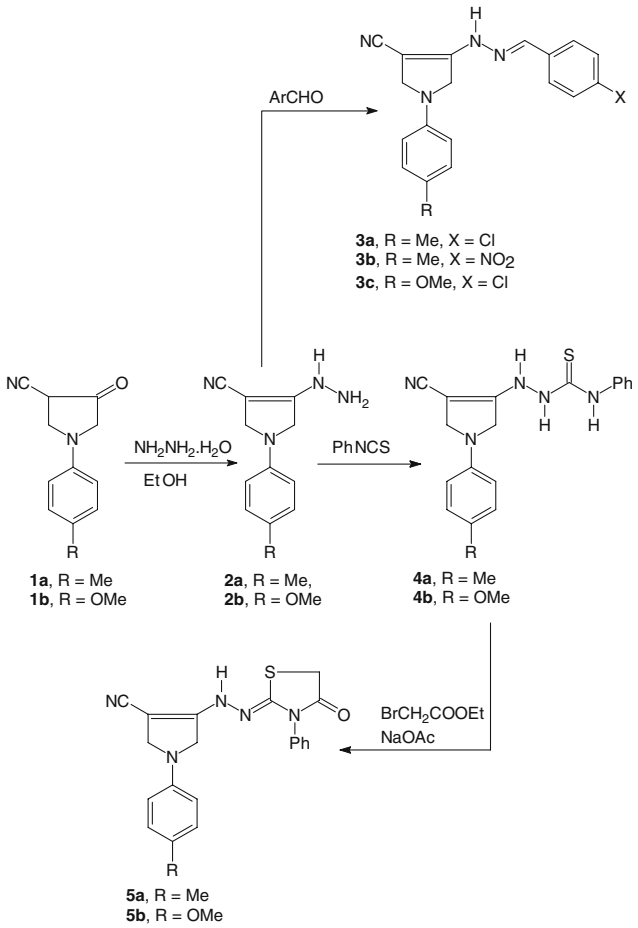
Chemistry

N-(*p*-substituted phenyl)-4-cyano-pyrrolidin-3-ones **1a** and **1b** were synthesized according to the reported procedure [24] and used as starting materials. They were treated with hydrazine hydrate to afford the corresponding hydrazine derivatives **2a** and **2b** [25], which were reacted with *p*-chlorobenzaldehyde or *p*-nitrobenzaldehyde in refuxing ethanol to afford the corresponding Schiff bases **3a–3c**. Condensation of hydrazines **2a** and **2b** with phenyl isothiocyanate afforded the corresponding thiosemicarbazide

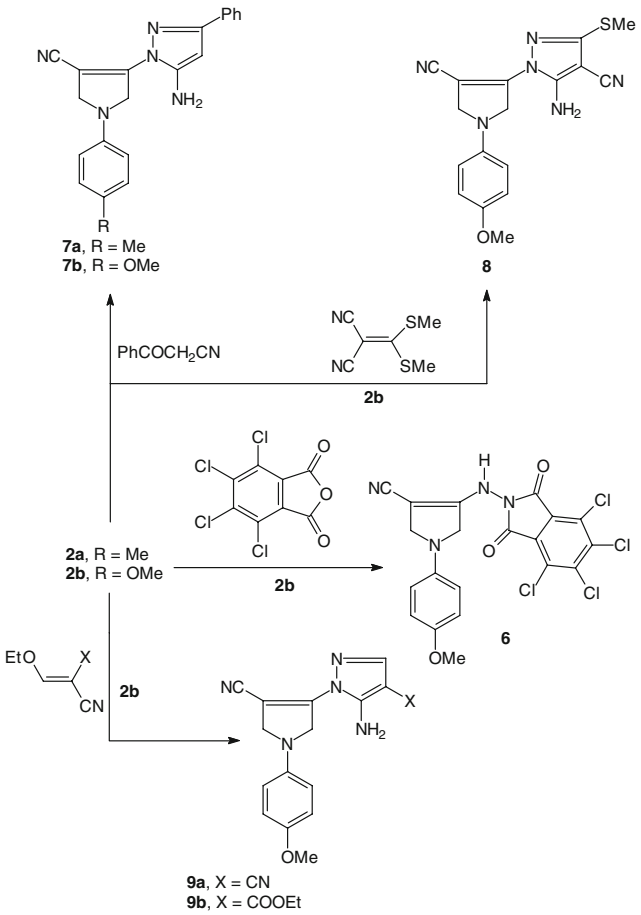
derivatives **4a** and **4b**, which were cyclized with ethyl bromoacetate in the presence of sodium acetate to afford the corresponding *N*-phenyl thiazolidinone derivatives **5a** and **5b** (Scheme 1).

The hydrazine **2b** was reacted with 1,2,4,5-tetrachlorophthalic anhydride in refluxing glacial acetic acid to give the corresponding imide **6**. Compounds **2a** and **2b** were treated with benzoyl acetonitrile to afford the corresponding 3,5-disubstituted pyrazole derivatives **7a** and **7b**. Compound **2b** was reacted with 2-(bis-methylsulfanyl-methylene)malononitrile, 2-ethoxy-methylenemalononitrile, or 2-cyano-3-ethoxyacrylic acid ethyl ester to afford the corresponding 3,4,5-tri- and 4,5-disubstituted pyrazolines **8**, **9a** and **9b** (Scheme 2).

Schiff bases **10–13** were obtained via simple condensation of the hydrazine **1b** with 1-(4-methoxyphenyl)-4-oxopyrrolidine-3-carbonitrile, indan-1,3-dione, acetyl derivatives, or cycloalkanones in refluxing ethanol containing a catalytic amount of acetic acid, thus affording the Schiff bases **10–13** (Scheme 3).



Scheme 1



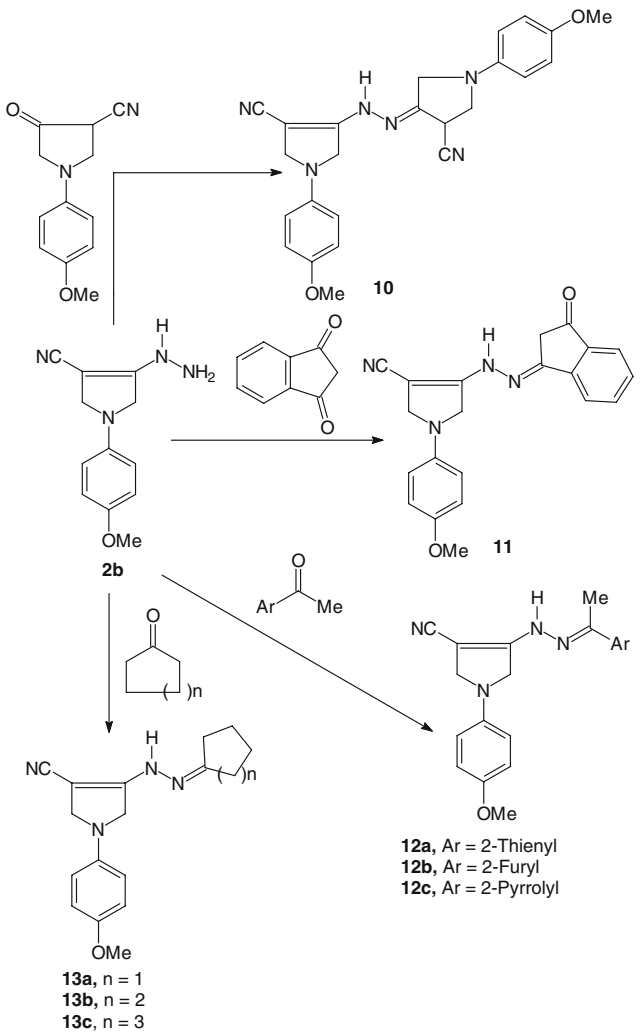
Scheme 2

Pharmacological screening

Serotonin antagonist activity

Determination of the affinity of tested compounds for the $5HT_{1A}$ receptor in brain may be useful for predicating compounds with novel anxiolytic or atypical antipsychotic profiles. The existence of at least two populations of $5HT_1$ receptors in rat brain was shown by differential sensitivity to spiroperdiol. The spiroperdiol-sensitive receptors were designed as the $5HT_{1A}$ subtype and the insensitive receptors were referred as the $5HT_{1B}$ subtype. Schlegel and Peroutka identified [3H] DPAT as a selective ligand for $5HT_1$ receptors [26].

Specific binding is defined as the difference between total binding and binding in the presence of 10 μ*M* 5HT [26, 27]. IC_{50} values are calculated from the percent specific binding at each drug concentration (Table 1). Serotonin may play a role in anxiety, since drugs which reduce serotoninergic function have anxiolytic effects in animal models. Since buspirone and its analogs have relatively higher affinity for the $5HT_{1A}$ receptor than other receptors and no effect on the benzodiazepine site, their



Scheme 3

anxiolytic properties are attributed to activity at the $5HT_{1A}$ receptor. So all derivatives were subjected to anxiolytic screening. From the results in Table 1, all the tested compounds showed potent serotonin antagonist activities; the order of descending activities is **13c**, **12c**, (**5a**, **4c** and Buspirone), **7a**, **7b**, **3b**, **12b**, (**1**, **3b** and **6**), **3a**, (**11**, **4b** and **4a**), **9a**, **9b**, **12a**, **5b**, **8** and **10**.

Structure activity relationship

- Schiff's bases with alicyclic compounds give the highest activity (as the carbon atoms of the alicyclic ring system increase the activity increases).
- The *p*-methoxy group sharply increases the activities.
- Steric hindrance tend to diminish the activity to moderate levels.
- 5-Amino-3-phenyl pyrazol is more active than its bioisosteric methylthiol.

Table 1 Serotonin antagonist activities of the new compounds

Compound	Concentration/mg kg^{-1}	IC_{50}/mg kg^{-1}
3a	2×10^{-7}	63
	2×10^{-8}	77
3b	2×10^{-7}	58
	2×10^{-8}	80
4a	2×10^{-7}	62
	2×10^{-8}	75
4b	2×10^{-7}	62
	2×10^{-8}	75
4c	2×10^{-7}	63
	2×10^{-8}	87
5a	2×10^{-7}	63
	2×10^{-8}	87
5b	2×10^{-7}	39
	2×10^{-8}	67
6	2×10^{-7}	66
	2×10^{-8}	77
7a	2×10^{-7}	66
	2×10^{-8}	87
7b	2×10^{-7}	63
	2×10^{-8}	86
8	2×10^{-7}	39
	2×10^{-8}	65
9a	2×10^{-7}	61
	2×10^{-8}	75
9b	2×10^{-7}	60
	2×10^{-8}	73
10	2×10^{-7}	47
	2×10^{-8}	56
11	2×10^{-7}	62
	2×10^{-8}	75
12a	2×10^{-7}	38
	2×10^{-8}	67
12b	2×10^{-7}	68
	2×10^{-8}	79
12c	2×10^{-7}	83
	2×10^{-8}	89
13b	2×10^{-7}	66
	2×10^{-8}	77
13c	2×10^{-7}	77
	2×10^{-8}	96
Buspirone	2×10^{-7}	63
	2×10^{-8}	87

Antianexity test in mice

Crawley and colleagues have described a simple behavioral model in mice to detect compounds with anxiolytic effects. Mice tend to explore a novel environment, but to retreat

Table 2 Antianexity activities of new compounds

Compound	Relative potencies to diazepam
3a	2
3b	4
4a	3
4b	2
4c	1
5a	12
5b	3
6	4
7a	4
7b	24
8	6
9a	7
9b	8
10	3
11	5
12a	22
12b	5
12c	8
13a	6
13b	9
13c	5
Diazepam	1

Table 3 Acute toxicity (LD_{50}) of the synthesized compounds

Compound	LD_{50}/mg/kg
3a	321 ± 0.10
3b	294 ± 0.19
4a	243 ± 0.18
4b	376 ± 0.18
4c	344 ± 0.11
5a	235 ± 0.17
5b	252 ± 0.12
6	124 ± 0.18
7a	324 ± 0.18
7b	136 ± 0.16
8	497 ± 0.15
9a	221 ± 0.67
9b	342 ± 0.18
10	524 ± 0.18
11	308 ± 0.19
12a	387 ± 0.18
12b	303 ± 0.18
12c	236 ± 0.15
13a	346 ± 0.16
13b	434 ± 0.17
13c	274 ± 0.18
Buspirone	113 ± 0.18
Diazepam	102 ± 0.10

from the aversive properties of a brightly-lit open field. In a two-chambered system, where mice can freely move between a brightly-lit open field and a dark corner, animals show more crossings between the two chambers and more locomotor activity after treatment with anxiolyties [28–30].

Dose response curves were obtained and the number of crossings through the partition between the light and the dark chambers are compound with total activity compound during 10 min. Dose-made decreases in total activity, such as that made by chlorpromazine 10 mg/kg s.c., and the relative potencies to chlorpromazine were determined.

From the results in Table 2, all the tested compounds showed potent antianexity activities; the order of descending activities is **7b**, **12a**, **5a**, **13b**, (**12c** and **9b**), **9a**, (**8** and **13a**), (**13c**, **12b** and **11**), (**6**, **7a** and **3b**), (**5b**, **4a** and **10**), **3a** and **3b**.

Structure activity relationship

- The pyrazol ring is essential for high antianexity activity.
- (5-Amino-3-phenyl-1*H*-pyrazol-1-yl) provides higher activity than the (4-cyano-1-(4-methoxyphenyl)-2,5-dihydro-1*H*-pyrrol-3-yl), while the later is more active than the [4-cyano-1-(4-methoxyphenyl)-2,5-dihydro-1*H*-pyrrol-3-yl] residue.
- *Schiff's* bases with an alicyclic are lees active than those containing heteroatoms or aromatic systems.
- Steric hindrance tend to diminish the activity to moderate levels

Determination of acute toxicity (LD_{50})

The LD_{50} was determined by using rats. They were injected with a range of increasing doses of the synthesized compounds. The dose that killed 50% of the animals was calculated according to Austen and Brocklehurst [31] (Table 3).

Experimental

Melting points were determined on open glass capillaries using an Electrothermal IA 9000 digital melting point apparatus. Elemental analyses were performed on Elementar Vario EL (Microanalytical Unit, National Research Center, Cairo, Egypt), and were found within ±0.4% of the theoretical values. Infrared spectra were recorded on a Carl Zeiss Spectrophotometer (model UR 10) using the KBr disc technique. ^{1}H-NMR spectra were recorded on a Varian Gemini 270 MHz spectrometer (DMSO-d_6) and the

chemical shifts are given in δ (ppm) downfield from tetramethylsilane as an internal standard. The mass spectra were measured using a Finnigan SSQ 7000 mass spectrometer. Follow up of the reactions and checking the purity of the compounds was made by TLC on silica gel-precoated aluminum sheets (Type 60 F_{254}; Merck, Darmstadt, Germany). The starting materials **1**, **2a**, and **2b** were prepared according to Southwich et al. [24] and Amer et al. [25].

4-(2-Arylidenehydrazinyl)-2,5-dihydro-1-(4-substituted phenyl)-1H-pyrrole-3-carbonitriles ***(3a–3c)***

A mixture of 5 mmol **2a** or **2b** and 5 mmol *p*-chlorobenzaldehyde or *p*-nitrobenzaldehyde in 30 cm^3 absolute ethanol in the presence of few drops of *Ac*OH was refluxed for 1 h. The reaction mixture was cooled and the formed solid was filtered off, dried and crystallized to give 1.48 g **3a** (88%), 1.6 g **3b** (94%) and 1.37 g **3c** (78%).

4-(2-(4-Chlorobenzylidene)hydrazinyl)-1-p-tolyl-2,5-dihydro-1H-pyrrole-3-carbonitrile (**3a**, $C_{19}H_{17}ClN_4$)

Mp 210–211 °C (DMF/EtOH); IR (film): $\bar{\nu} = 1{,}660$ (C=O) cm^{-1}, 2,224 (CN) cm^{-1}, 3,213 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6): $\delta = 2.21$ (s, CH_3), 4.08, 4.20 (2s, 2 CH_2), 7.49 (d, $J = 7.90$ Hz *Ar*-H), 7.56 (d, $J = 7.90$ Hz *Ar*-H), 7.72 (d, $J = 7.92$ Hz *Ar*-H), 7.86 (d, $J = 7.92$ Hz *Ar*-H), 8.10 (s, olefinic CH), 8.72 (s, NH, exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 338$ ($M^+ + 1$, 12) and at 336 (100, base peak).

4-(2-(4-Nitrobenzylidene)hydrazinyl)-1-p-tolyl-2,5-dihydro-1H-pyrrole-3-carbo-nitrile (**3b**, $C_{19}H_{17}N_5O_2$)

Mp 229–230 °C (DMF/EtOH); IR (film): $\bar{\nu} = 1{,}673$ (C=N) cm^{-1}, 2,210 (CN) cm^{-1}, 3,198 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6): $\delta = 2.19$ (s, CH_3), 4.06, 4.19 (2s, 2 CH_2), 6.89 (d, $J = 7.92$ Hz *Ar*-H), 7.12 (d, $J = 7.92$ Hz *Ar*-H), 7.41 (d, $J = 7.90$ Hz *Ar*-H), 7.75 (d, $J = 7.90$ Hz *Ar*-H), 8.12 (s, olefinic-CH), 9.13 (s, NH, exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 348$ ($M^+ + 1$, 10) and at 347 (100, base peak).

4-(2-(4-Chlorobenzylidene)hydrazinyl)-1-(4-methoxyphenyl)-2,5-dihydro-1H-pyrrole-3-carbonitrile
(**3c**, $C_{19}H_{17}ClN_4O$)

Mp 200–202 °C (DMF/EtOH); IR (film): $\bar{\nu} = 1{,}641$ (C=N) cm^{-1}, 2,195 (CN) cm^{-1}, 3,196 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6): $\delta = 3.53$ (s, OCH_3), 4.11, 4.23 (2s, 2 CH_2), 7.11 (d, $J = 7.94$ Hz *Ar*-H), 7.43 (d, $J = 7.94$ Hz *Ar*-H), 7.51 (d, $J = 7.92$ Hz *Ar*-H), 7.60 (d, $J = 7.92$ Hz *Ar*-H), 8.13 (s, olefinic-CH), 8.99 (s, NH, exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 353$ (M^+, 5) and at 108 (100, base peak).

1-(4-Cyano-2,5-dihydro-1-p-substitued phenyl-1H-pyrrol-3-yl)-4-phenylthiosemicarbazide ***(4a and 4b)***

A mixture of 5 mmol **2a** or **2b** and 0.67 g phenylisothiocyanate (5 mmol) in 30 cm^3 absolute ethanol was refluxed for 1.5 h. The formed solid was filtered off, dried and crystallized to give 1.46 g **4a** (84%) and 1.48 g **4b** (81%).

2-(4-Cyano-1-p-tolyl-2,5-dihydro-1H-pyrrol-3-yl)-N-phenylhydrazinecarbothioamide (**4a**, $C_{19}H_{19}N_5S$)

Mp 195–196 °C (DMF/EtOH); IR (film): $\bar{\nu} = 1{,}257$ (C=S) cm^{-1}, 2,249 (CN) cm^{-1}, 3,205 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6): $\delta = 2.11$ (s, CH_3), 4.08, 4.15 (2s, 2CH_2), 6.39–7.11 (m, *Ar*-H), 8.01, 9.11, 10.01 (3s, 3 NH, exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 350$ ($M^+ + 1$, 2) and at 108 (100, base peak).

2-(4-Cyano-1-(4-methoxyphenyl)-2,5-dihydro-1H-pyrrol-3-yl)-N-phenylhydrazine-carbothioamide
(**4b**, $C_{19}H_{19}N_5OS$)

Mp 186–188 °C (DMF/EtOH); IR (film): $\bar{\nu} = 1{,}268$ (C=S) cm^{-1}, 2,251 (CN) cm^{-1}, 3,169–3,343 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6): $\delta = 3.61$ (s, OCH_3), 4.10, 4.18 (2s, 2CH_2), 6.42–7.19 (m, *Ar*-H), 8.12, 9.03, 10.12 (3s, 3 NH, exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 366$ ($M^+ + 1$, 5) and at 108 (100, base peak).

4-(2-(4-Oxo-3-phenylthiazolidin-2-ylidene)hydrazinyl)-1-(p-substituted phenyl)-2,5-dihydro-1H-pyrrole-3-carbonitrile ***(5a and 5b)***

A mixture of 1 mmol **3a** or **3b** and 0.17 g ethyl bromoacetate (1 mmol) in 30 cm^3 absolute ethanol in the presence of 0.33 g anhydrous sodium acetate (4 mmol) was refluxed for 6 h. The reaction mixture was cooled, diluted with water and allowed to stand overnight, and the obtained solid was filtered off, washed with water, dried and crystallized to give 0.25 g **5a** (64%) and 0.24 g **5b** (61%).

4-(2-(4-Oxo-3-phenylthiazolidin-2-ylidene)hydrazinyl)-1-p-tolyl-2,5-dihydro-1H-pyrrole-3-carbonitrile
(**5a**, $C_{21}H_{19}N_5OS$)

Mp 185–186 °C (*EtOH*); IR (film): $\bar{\nu} = 1{,}605$ (C=N) cm^{-1}, 1,735 (C=O) cm^{-1}, 2,224 (CN), 3,206 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6): $\delta = 2.13$ (s, CH_3), 3.91 (s, SCH_2), 4.12, 4.19 (2s, 2CH_2), 6.51, 6.87 (2d, $J = 7.95$ Hz *Ar*-H), 11.12 (s, NH exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 389$ (M^+, 11) and at 77 (100, base peak).

1-(4-Methoxyphenyl)-4-(2-(4-oxo-3-phenylthiazolidin-2-ylidene)hydrazinyl)-2,5-dihydro-1H-pyrrole-3-carbonitrile
(**5b**, $C_{21}H_{19}N_5O_2S$)

Mp 209–210 °C (*EtOH*); IR (film): $\bar{\nu} = 1{,}601$ (C=N) cm^{-1}, 1,733 (C=O) cm^{-1}, 2,219 (CN) cm^{-1}, 3,198 (NH)

cm^{-1}; ^1H NMR (DMSO-d_6): $\delta = 3.49$ (s, OCH_3), 4.01 (s, SCH_2), 4.11, 4.20 (2s, $2CH_2$), 6.53, 6.88 (2d, $J = 7.93$ Hz *Ar*-H), 11.25 (s, NH, exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 405$ (M^+, 10) and at 77 (100, base peak).

1-(4-Methoxyphenyl)-4-(4,5,6,7-tetrachloro-1,3-dioxoisoindolin-2-yl-amino)-2,5-dihydro-1H-pyrrole-3-carbonitrile (**6**, $C_{20}H_{12}Cl_4N_4O_3$)

A mixture of 0.23 g **2b** (1 mmol) and 0.29 g 3,4,5,6-tetrachlorophthalic anhydride (1 mmol) in 50 cm^3 *Ac*OH was heated under reflux for 6 h. The reaction mixture was concentrated under reduced pressure, the obtained solid was filtered off and crystallized to yield 0.41 g **6** (83%). Mp > 300 °C (*Ac*OH/H_2O); IR (film): $\bar{\nu} = 1{,}749$, 1,779 (2 C=O) cm^{-1}, 2,206 (CN) cm^{-1}; ^1H NMR (DMSO-d_6): $\delta = 3.65$ (s, OCH_3), 4.10 (s, CH_2), 4.14 (s, CH_2), 6.56, 6.86 (2d, $J = 7.95$ Hz *Ar*-H), 10.25 (s, NH, exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 500$ ($M^+ + 2$, 12) and at 277 (100, base peak).

*4-(5-Amino-3-phenyl-1H-pyrazol-1-yl)-2,5-dihydro-1-p-substitued phenyl-1H-pyrrole-3-carbonitrile (**7a** and **7b**)*

A mixture of 5 mmol **2a** or **2b** and 0.73 g benzoylaceteonitrile (5 mmol) in 20 cm^3 absolute ethanol was refluxed for 4 h. The reaction mixture was cooled, the formed solid was filtered off, dried and crystallized to afford 0.75 g **7a** (44%) and 0.86 g **7b** (48%).

4-(5-Amino-3-phenyl-1H-pyrazol-1-yl)-1-p-tolyl-2,5-dihydro-1H-pyrrole-3-carbonitrile (**7a**, $C_{21}H_{19}N_5$)

Mp 150–151 °C (*EtOH*); IR (film): $\bar{\nu} = 1{,}598$ (C=N) cm^{-1}, 2,198 (CN) cm^{-1}, 3,198, 3,218 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): $\delta = 2.15$ (s, CH_3), 3.92, 4.25 (2s, 2 CH_2), 6.38 (s, pyrazole-CH), 6.61–7.08 (m, *Ar*-H), 8.31 (s, NH_2, exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 341$ (M^+, 6) and at 321 (100, base peak).

4-(5-Amino-3-phenyl-1H-pyrazol-1-yl)-1-(4-methoxyphenyl)-2,5-dihydro-1H-pyrrole-3-carbonitrile
(**7b**, $C_{21}H_{19}N_5O$)

Mp 177–179 °C (*EtOH*); IR (film): $\bar{\nu} = 1{,}619$ (C=N) cm^{-1}, 2,203 (CN) cm^{-1}, 3,218, 3,201 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): $\delta = 3.54$ (s, OCH_3), 3.92, 4.25 (2s, 2 CH_2), 6.38 (s, pyrazole-CH), 6.61–7.08 (m, *Ar*-H), 8.31 (s, NH_2, exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 357$ (M^+, 4) and at 321 (100, base peak).

5-Amino-1-(4-cyano-1-(4-methoxyphenyl)-2,5-dihydro-1H-pyrrol-3-yl)-3-(methylthio)-1H-pyrazole-4-carbonitrile
(**8**, $C_{17}H_{16}N_6OS$)

A mixture of 0.46 g **2b** (2 mmol) and 0.34 g 2-[bis(methylthio)methylene]malononitrile (2 mmol) in 50 cm^3 absolute ethanol was refluxed for 3 h. The formed solid was filtered off, dried and crystallized to give 0.46 g **8** (65%). Mp 230–231 °C (*Me*OH); IR (film): $\bar{\nu} = 1{,}653$ (C=N) cm^{-1}, 2,214, 2,249 (2CN) cm^{-1}, 3,263, 3,220 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): $\delta = 2.41$ (s, SCH_3), 3.62 (s, OCH_3), 4.15, 4.23 (2s, 2 CH_2), 6.51, 6.83 (2d, $J = 7.95$ Hz *Ar*-H), 8.21 (s, NH_2, exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 352$ (M^+, 2) and at 134 (100, base peak).

*Synthesis of pyrazolocarbonitrile derivatives (**9a** and **9b**)*

A mixture of 0.23 g **2b** (5 mmol) and 2-(ethoxymethylene)malononitrile or ethyl 2-cyano-3-ethoxyacrylate (5 mmol) in 25 cm^3 absolute ethanol was refluxed for 4 h. After cooling, the formed solid was filtered off, dried and crystallized to give 0.88 g **9a** (58%) and 0.92 g **9b** (52%).

5-Amino-1-(4-cyano-1-(4-methoxyphenyl)-2,5-dihydro-1H-pyrrol-3-yl)-1H-pyrazole-4-carbonitrile
(**9a**, $C_{16}H_{14}N_6O$)

Mp 193–195 °C (*EtOH*); IR (film): $\bar{\nu} = 1{,}660$ (C=N) cm^{-1}, 2,213, 2,252 (2 CN) cm^{-1}, 3,281, 3,240 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): $\delta = 3.39$ (s, OCH_3), 4.48 (s, CH_2), 4.85 (s, CH_2), 6.30 (s, pyrazole-CH), 6.62, 6.92 (2d, $J = 7.90$ Hz *Ar*-H), 8.52 (s, NH_2, exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 306$ (M^+, 100, base peak).

Ethyl 5-amino-1-(4-cyano-1-(4-methoxyphenyl)-2,5-dihydro-1H-pyrrol-3-yl)-1H-pyrazole-4-carboxylate
(**9b**, $C_{18}H_{19}N_5O_3$)

Mp 166–167 °C (*EtOH*); IR (film): $\bar{\nu} = 1{,}652$ (C=N) cm^{-1}, 1,686 (C=O) cm^{-1}, 2,215 (CN) cm^{-1}, 3,235, 3,219 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): $\delta = 1.41$ (t, CH_3), 3.39 (s, OCH_3), 3.96 (q, CH_2), 4.27 (s, CH_2), 4.41 (s, CH_2), 6.19 (s, pyrazole-CH), 6.70, 6.91 (2d, $J = 7.92$ Hz *Ar*-H), 8.63 (s, NH_2, exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 353$ (M^+, 100, base peak).

*Synthesis of Schiff bases (**10** and **11**)*

A mixture of 0.46 g **2b** (2 mmol) and 1-(4-methoxyphenyl)-4-oxopyrrolidine-3-carbonitrile or 1, 3-indanedione (5 mmol) in 50 cm^3 absolute ethanol in the presence of drops of acetic acid was refluxed for 1 h. The formed solid was filtered off, dried and recrystallized to afford 0.75 g **10** (88%) and 0.60 g **11** (84%).

4-(2-(4-Cyano-1-(4-methoxyphenyl)pyrrolidin-3-ylidene)hydrazinyl)-1-(4-methoxy-phenyl)-2,5-dihydro-1H-pyrrole-3-carbonitrile (**10**, $C_{24}H_{24}N_6O_2$)

Mp 249–50 °C (DMF/EtOH); IR (film): $\bar{\nu} = 1{,}656$ (C=N) cm^{-1}, 2,205, 2,254 (2CN) cm^{-1}, 3,198 (NH) cm^{-1}; ^1H NMR (DMSO-d_6): $\delta = 3.31$, 3.52 (s, 2 OCH_3), 3.97- 4.10 (m, CHCN and CH_2), 4.21, 4.28, 4.32 (3s, $3CH_2$), 6.46–

6.88 (m, *Ar*-H), 9.67 (s, NH, exchangeable with D_2O) ppm; MS (EI, 70 eV): *m/z* = 428 (M^+, 15) and at 316 (100, base peak).

1-(4-Methoxyphenyl)-4-(2-(3-oxo-2,3-dihydroinden-1-ylidene)hydrazinyl)-2,5-dihydro-1H-pyrrole-3-carbonitrile (**11**, $C_{21}H_{18}N_4O_2$)
Mp 230–31 °C (DMF/EtOH); IR (film): $\bar{\nu}$ = 1,648 (C=N) cm^{-1}, 1,713 (C=O) cm^{-1}, 2,197 (CN) cm^{-1}, 3,220 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ = 2.39 (s, CH_2), 3.35 (s, OCH_3), 4.12 (s, CH_2), 4.22 (s, CH_2), 6.52–7.80 (m, *Ar*-H), 10.42 (s, NH, exchangeable with D_2O) ppm; MS (EI, 70 eV): *m/z* = 359 (M^+ +1, 18) and at 212 (100, base peak).

Synthesis of 1-(4-methoxyphenyl)-4-(2-(1-arylethylidene)hydrazinyl)-2,5-dihydro-1H-pyrrole-3-carbonitrile ***(12a–12c)***

A mixture of 0.46 g **2b** (2 mmol) and appropriate methyl ketone, namely, 2-acetylthiophene, 2-acetylfurane or 2-acetylpyrrole (2 mmol) in 20 cm^3 absolute ethanol containing a few drops of *Ac*OH was heated under reflux for 1 h. The formed solid was filtered off, dried and recrystallized to afford 0.58 g **12a** (86%), 0.56 g **12b** (87%) and 0.52 g **12c** (82%).

1-(4-Methoxyphenyl)-4-(2-(1-(thiophen-2-yl)ethylidene)hydrazinyl)-2,5-dihydro-1H-pyrrole-3-carbonitrile
(**12a**, $C_{18}H_{18}N_4OS$)
Mp 226–227 °C (DMF/EtOH); IR (film): $\bar{\nu}$ = 1,644 (C=N) cm^{-1}, 2,193 (CN) cm^{-1}, ^{1}H NMR (DMSO-d_6): δ = 2.13 (s, CH_3), 3.65 (s, OCH_3), 4.12, 4.24 (2s, 2 CH_2), 6.51–6.98 (m, *Ar*-H), 10.28 (s, NH, exchangeable with D_2O) ppm; MS (EI, 70 eV): *m/z* = 338 (M^+, 100, base peak).

4-(2-(1-(Furan-2-yl)ethylidene)hydrazinyl)-1-(4-methoxyphenyl)-2,5-dihydro-1H-pyrrole-3-carbonitrile
(**12b**, $C_{18}H_{18}N_4O_2$)
Mp 228–230 °C (DMF/EtOH); IR (film): $\bar{\nu}$ = 1,631 (C=N) cm^{-1}, 2,197 (CN) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ = 2.08 (s, CH_3), 3.57 (s, OCH_3), 4.14, 4.27 (2s, 2 CH_2), 6.53–7.11 (m, *Ar*-H), 10.21 (s, NH, exchangeable with D_2O) ppm; MS (EI, 70 eV): *m/z* = 323 (M^+ +1, 3) and at 322 (M^+, 100, base peak).

4-(2-(1-(1H-Pyrrol-2-yl)ethylidene)hydrazinyl)-1-(4-methoxyphenyl)-2,5-dihydro-1H-pyrrole-3-carbonitrile
(**12c**, $C_{18}H_{19}N_5O$)
Mp 236–238 °C (DMF/EtOH); IR (film): $\bar{\nu}$ = 1,637 (C=N) cm^{-1}, 2,203 (CN) cm^{-1}, ^{1}H NMR (DMSO-d_6): δ = 2.11 (s, CH_3), 3.62 (s, OCH_3), 4.10, 4.22 (2s, 2 CH_2), 6.11 (s, NH), 6.52–6.95 (m, *Ar*-H), 10.28 (s, NH, exchangeable with D_2O) ppm; MS (EI, 70 eV): *m/z* = 321 (M^+, 88) and at 160 (100, base peak).

Synthesis of 4-(2-cycloalkylidenehydrazinyl)-1-(4-methoxyphenyl)-2,5-dihydro-1H-pyrrole-3-carbonitrile ***(13a–13c)***

A mixture of 0.46 g **2b** (2 mmol) and cycloalkanone derivative, namely, cyclopentanone, cyclohexanone, or cycloheptanone (2 mmol) in 20 cm^3 absolute ethanol in the presence of *Ac*OH as a catalyst was refluxed for 1 h. The formed solid was filtered off, dried and recrystallized to afford 0.5 g **13a** (85%), 0.54 g **13b** (88%) and 0.54 g **13c** (83%).

4-(2-Cyclopentylidenehydrazinyl)-1-(4-methoxyphenyl)-2,5-dihydro-1H-pyrrole-3-carbonitrile
(**13a**, $C_{17}H_{20}N_4O$)
Mp 209–210 °C (DMF/EtOH); IR (film): $\bar{\nu}$ = 1,634 (C=N) cm^{-1}, 2,209 (CN) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ = 1.38 (s, cyclopentane), 2.10 (s, cyclopentane), 2.27 (s, cyclopentane), 3.35 (s, OCH_3), 4.12 (s, CH_2), 4.23 (s, CH_2), 6.57, 6.82 (2d, *J* = 7.95 Hz *Ar*-H), 10.12 (s, NH, exchangeable with D_2O) ppm; MS (EI, 70 eV): *m/z* = 296 (M^+, 28) and at 160 (100, base peak).

4-(2-Cyclohexylidenehydrazinyl)-1-(4-methoxyphenyl)-2,5-dihydro-1H-pyrrole-3-carbonitrile (**13b**, $C_{18}H_{22}N_4O$)
Mp 204–205 °C (DMF/EtOH); IR (film): $\bar{\nu}$ = 1,643 (C=N) cm^{-1}, 2,198 (CN) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ = 1.50–2.40 (m, cyclohexane), 3.29 (s, OCH_3), 4.08 (s, CH_2), 4.11 (s, CH_2), 6.55, 6.88 (2d, *J* = 7.95 Hz *Ar*-H), 10.03 (s, NH, exchangeable with D_2O) ppm; MS (EI, 70 eV): *m/z* = 310 (M^+, 30) and at 160 (100, base peak).

4-(2-Cycloheptylidenehydrazinyl)-1-(4-methoxyphenyl)-2,5-dihydro-1H-pyrrole-3-carbonitrile
(**13c**, $C_{19}H_{24}N_4O$)
Mp 242–243 °C (DMF/EtOH); IR (film): $\bar{\nu}$ = 1,628 (C=N) cm^{-1}, 2,231(CN) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ = 1.50–2.38 (m, cycloheptane), 3.33 (s, OCH_3), 4.11 (s, CH_2), 4.19 (s, CH_2), 6.61, 6.95 (2d, *J* = 7.94 Hz *Ar*-H), 10.13 (s, NH, exchangeable with D_2O) ppm; MS (EI, 70 eV): *m/z* = 324 (M^+, 41) and at 160 (100, base peak).

Pharmacological screening

Serotonin antagonist

Procedure:

1. Tris buffers pH 7.7
   a. 57.29 Tris–HCl, 16.2 g Tris base, q. s. 1 dm^3 with distilled water (0.5 *M* Tris buffer, pH 7.7)
   b. Make a 1:10 dilution in deionized H_2O (0.05 *M* Tris buffer, pH 7.7)
   c. 0.05 *M* Tris buffer, pH 7.7 containing 10 μM paragyline 4 mM $CaCl_2$ and 0.1% ascorbic acid.

0.49 mg paragyline HCl, 111 mg $CaCl_2$, 20 mg vitamin C, q. s. to 250 ml with 0.05 *M* Tris buffer, pH 7.7 (reagent 1b)

2. [^{3}H]-DPAT(2-*N,N*-Di[2,3(n) $^{-3}$propylamino)-8-hydroxy-1,2,3,4-tetrahydronaphthalene) (160–206 ci/mmol) was obtained from Amersham. For IC_{50} determination = a, 10 nM stock solution is made up and 50 mm^3 are added to each tube (final concentration = 0.5 nM).
3. Serotonin sulphate. 0.5 nM stock solution is made up in 0.01 N HCl and 20 mm^3 added to three tubes for determination of non-specific binding (final concentration = 10 μM).
4. Test compound

One millimolar stock solution is made up in a suitable solvent and serially diluted, such that the final concentrations in the assay range from 2×10^{-5} to 2×10^{-8} M. Seven concentrations are used for each assay. Higher or lower concentrations may be used based on the potency of the drug

Tissue preparation

Male Wister rats are sacrificed by decapitation. Hippocampus are removed, weighed and homogenized in 20 volumes of 0.05 *M* Tris buffer, pH 7.7. The homogenate is centrifuged at 48,000*g* for 10 min and the supernatant is discarded. The pellet is re-suspended in an equal volume of 0.05 *M* Tris buffer, incubated at 37 °C for 10 min and re-centrifuged at 48,000*g* for 10 min. The final membrane pellet is re-suspended in 0.05 *M* Tris buffer containing 4 mM $CaCl_2$, 0.1% vitamin C and 10 μM pargyline.

Assay

- 800 mm^3 Tissue
- 130 mm^3 0.05 *M* Tris + $CaCl_2$ + pargyline + vitamin C
- 20 mm^3 Vehicle/5HT/drug
- 50 mm^3 [^{3}H] DPAT

Tubes are incubated for 15 min at 25 °C. The assay is stopped by vacuum filtration through Whatman GF/B filters, which are then washed twice with 5 cm^3 of ice-cold 0.05 *M* Tris buffer. The filters are then placed into scintillation vials with 10 cm^3 of liquescent scintillation cocktail and counted.

Antianexity test in mice

Procedure

The testing apparatus consists of a light and a dark chamber divided by a photo cell-equipped zone, A polypropylene animal cage, 44 cm × 21 cm, is darkened with black spray over one-third. A partition containing a 13-cm long × 5-cm high opening separates the dark one-third from the bright two-thirds of the cage. The cage rests on an animex activity monitor, which counts total locomotor activity. An electronic system using four sets of photocells across the partition automatically counts movements through the partition and clocks the time spent in the light and dark compartments. Naive male albino mice with a body weight of 18–25 g are placed into the cage. The animals are treated 30 min before the experiment with the test drugs or the vehicle intraperitoneally and are then observed for 10 min.

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