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G protein-coupled receptor binding and pharmacological evaluation of indole-derived thiourea compounds

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Abstract

Four 2-(1*H*-indol-3-yl)ethylthiourea derivatives were prepared by condensation of 2-(1*H*-indol-3-yl)ethanamine with the corresponding aryl/alkylisothiocyanates in a medium-polarity solvent. Their structures were confirmed by spectral techniques, and the molecular structure of **3** was determined by X-ray crystal analysis. For all derivatives, the binding affinities at the 5-HT_{2A} and 5-HT_{2C} receptors, as well as their functional activities at the 5-HT_{1A} and D_2 receptors, were determined. The arylthioureas **1** and **4** were the most active at the 5-HT_{1A} receptor, showing, at the same time, significant selectivity over the studied 5-HT_2 and D_2 receptor subtypes. The compounds were tested for their pharmacological activities within the central nervous system in relevant mouse models. The involvement of the serotonergic system in the activity of **1** and **4** was indicated. The antinociceptive action of **4** was linked to its anti-inflammatory activity.

KEYWORDS
dopamine receptors, inverse agonists, serotonin antagonism, substituent effect

1 | INTRODUCTION

A literature survey shows that the indole nucleus is an important component of natural and synthetic molecules with significant biological properties. Among the group of neurotransmitters are indole-containing monoamines, such as serotonin (5-HT), that play a regulatory role in the modulation of various cognitive and behavioral functions, such as mood, emotion, and sleep. For instance, serotonin depletion was evidenced as the factor contributing to the development of appetite disorders, anxiety, depression, or schizophrenia.[1–4] *N*- and *O*-methylated products of serotonin biotransformation, for example, bufotenine (5-HO-DMT), also exert central pharmacological activities. Within the central nervous system (CNS), they act as psychostimulants and by increasing dopamine neurotransmission may induce hallucinations. On the contrary, simultaneous *O*-methylation and *N*-acetylation of serotonin gives melatonin (MLT), a hormone secreted by the pineal gland. Its deficiency may lead to circadian rhythm disturbances and sleep disorders. It also modifies immunity, stress response and is involved in aging processes.[5] Tryptamine and its derivative dimethyltryptamine (DMT), the trace amines derived from L-tryptophan, act as a neuromodulator and an endogenous hallucinogen, respectively.[4,6] Many CNS agents, bearing an indole ring, are avaliable in the market as antipsychotics, antidepressants, anticonvulsants, analgesics, and anxiolytic drugs.[3,7] A number of recently synthesized serotonin receptor ligands are also characterized by the presence of the tryptamine ring. They are known as 5-HT_3 receptor antagonists,[8] dual M_4/5-HT_7 receptor ligands[9] and 5-HT_6 receptor agonists,[10] that are associated with antidepressant[8] or antipsychotic-like[9,10] activities, respectively. The affinity of piperazinylpropylindole derivatives for both the 5-HT transporter (SERT) and the 5-HT_{1A} receptor was recently described.[11] In addition, linear indole-derived alkaloids exerting neurotrophin-like

properties on primary dopaminergic neurons[12] and the azecine-type dopamine receptor antagonists[13] represent a novel classes of ligands for the aforementioned targets. Multifunctional agents for Alzheimer's disease (AD) within the indole ring-containing berberine conjugates exhibited reasonable AChE/BuChE inhibiting activity.[14] The anticonvulsant[15,16] and antinociceptive[17] properties of indole derivatives were described as well.

The thiourea branch represents an important synthon, which is responsible for numerous biological activities, such as antimicrobial,[18–23] antiviral,[24–26] antiproliferative,[27] and cytotoxic properties.[19,28] In addition, the class of (thio)urea derivatives were shown to mediate psychotropic-like effects in rodents. Because they reduce the number of the drug-elicited "head-twitch" episodes in mice, they are supposed to exert 5-HT_2 antagonist activity.[29,30] This profile of bioactivity was assigned by our team to 1,3-disubstituted thiourea derivatives, bearing alkylaryl and halogen-containing terminal fragments.[31] Moreover, antipsychotic pimavanserin, the substituted dibenzylurea, is described as a potent 5-HT_{2A} antagonist/inverse agonist with remarkable 5-HT_{2C} antagonistic profile and no appreciable affinity to D_2 or any other neurotransmitter receptors.[30,32–34] Thus, it is the only approved treatment for Parkinson's disease psychosis[33,34] that does not worsen the motor symptoms. It also reverses psychosis-like behaviors in rodent models of AD.[30] Inverse agonists/antagonists of the 5-HT_{2A} receptor, belonging to the diarylthiourea class, have been shown to enhance slow wave sleep as well.[33,35] Currently, a difluorophenylurea compound from this group is under development for the treatment of insomnia[35] and, independently, Lewy body disease.[36] In addition to their impact on the CNS, the 5-HT_{2A} receptor is engaged in the regulation of cardiovascular system function. Therefore, phenyl-pyrazole ureas, selective inverse agonists of this receptor, have found application as antiplatelet agents.[37] Derivatives of butanoyl-3-arylthiourea were also examined biochemically as potential cholinesterase (AChE/BuChE) inhibitors, as these two enzymes are involved in the pathological processes of AD.[38] Among the agomelatine analogs, derived from urea, serotonin 5-HT_{2C} antagonists and MLT MT_1/MT_2 agonists were described.[39] The aforementioned MT receptors are implicated in sleep regulation, circadian rhythms, and neurodegenerative diseases. Moreover, a new potent thiazolopyrimidinurea adenosine A_{2A} receptor antagonist with a low side-effect profile was selected as a promising drug for alleviating Parkinsonian motor deficits.[40] The antidepressant action of heterocyclic derivatives of thiourea has also been reported.[41] Both *N*-aryl and *N*-alkylthiourea analogs have displayed anticonvulsant activity.[31,42,43] Recently, piperazine–phenylurea derivatives were shown to exert a marked antiepileptic potency in both pentylenetetrazol and maximal electroshock models.[44,45] In addition, their antidepressant profile was simultaneously evidenced in vivo.[45] The antinociceptive activity of these compounds indicates their role in endogenous opioid system modulation.[29,31]

Previously, we identified a novel series of 1,3-disubstituted thiourea-based derivatives with a 5-HT_{2A} antagonistic profile.[31] In this paper, four analogs bearing a tryptamine nucleus were prepared and tested for binding to selected G protein-coupled receptors (GPCRs), as well as for their in vivo antipsychotic-like and antinociceptive properties. Their effect on motor function and body temperature in mice was additionally studied.

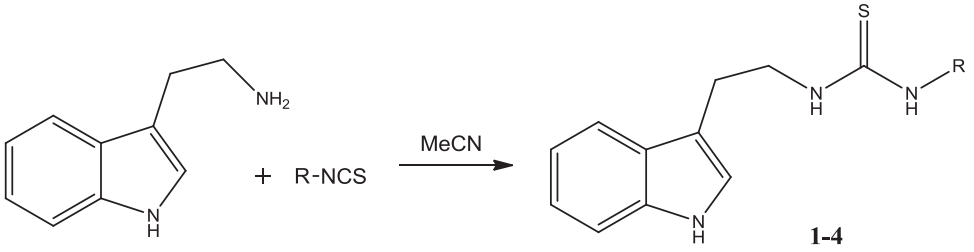
2 | RESULTS AND DISCUSSION

2.1 | Chemistry

The route for the preparation of disubstituted thiourea derivatives is presented in Scheme 1. The starting 2-(1*H*-indol-3-yl)ethanamine was condensed with appropriate isothiocyanates to form thioureas **1–4** (Scheme 1). The compounds obtained were purified by column chromatography and/or crystallized from acetonitrile. Mass spectrometry (MS), ^{1}H nuclear magnetic resonance (NMR), and ^{13}C NMR spectra confirmed the structures of the products. The molecular structure of **3** was determined by X-ray crystallography (Figure 1).

2.2 | In vitro tests

As a continuation of our previous studies,[31] the binding profile of the indole-derived thiourea derivatives toward a series of GPCRs (5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C}, D_2) was established. Compounds **1** and **2** belong to a class of *N*-carbonylthiourea derivatives, whereas in the structure of products **3** and **4**, the methylene group forms a link between the thiourea branch and the terminal part of the molecule. As it was proved,[31] the presence of such an alkyl element could be responsible for the CNS activity of (thio)ureas. The chemical character of the substituent at the carbonyl and methylene groups is either aromatic (**1** and **4**) or aliphatic (**2** and **3**). In this arrangement, the tested compounds formed a good comparative series. In addition, the calculated logP values of the tested thioureas are in the range of 3–3.5, which ensures their bioavailability—the ability to penetrate biological barriers that intend them for possible oral administration.



SCHEME 1 Synthesis of thiourea derivatives **1–4**. R = benzoyl (**1**), ethoxycarbonyl (**2**), ethyl (**3**), benzyl (**4**)

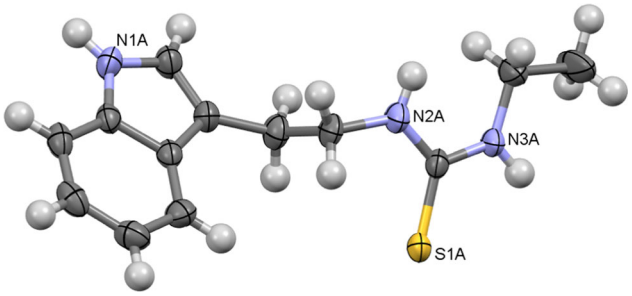


FIGURE 1 Perspective view of the molecule **3**

All compounds exerted weak affinity towards the studied 5-HT_2 receptor subtypes (Table 1). Derivatives **3** and **4**, bearing the $-CH_2-R$ substituent at the terminal part of the molecule, were found to be mixed 5-HT_{2A}/5-HT_{2C} receptor ligands, whereas thioureas with –C(=O)R group (**1** and **2**) bound more selectively to the 5-HT_{2C} receptor subtype (SI > 10). The benzylthiourea **4** simultaneously showed the highest affinity for the 5-HT_{2A} (K_i = 5.9 μM) and 5-HT_{2C} (K_i = 1.9 μM) receptors. This compound also possessed the highest lipophilicity, expressed as logP, within the whole series. The same level of the 5-HT_{2C} receptor binding affinity was also observed for the selective carbonyl-containing analogs **1** and **2**: 4.2 and 2.8 μM, respectively. Functional tests revealed that compounds with aromatic terminal moieties (**1** and **4**) behave as inverse 5-HT_{1A} receptor agonists (Table 2). One of them, the thiourea derivative **4**, was more potent and showed functional activity at the 5-HT_{1A} receptor at 81.8 nM, while remaining selective over both 5-H_{2A}/5-HT_{2C} receptors. Similarly, the benzoyl derivative **1** also preferred the 5-HT_{1A} receptor as its main molecular target. The ethylthiourea **3** and its close structural analog **2** exerted no affinity for the 5-HT_{1A} receptor. On the contrary, these two derivatives were described as weak antagonists of the dopaminergic D_2 receptor (Table 3), with K_i values in the range of 15–22 μM. However, when compared with the model D_2 receptor antagonist, domperidone, their functional activities were inconsiderable.

2.3 | In vivo tests

Rodent models, even those that only outline few aspects of human disease, can be helpful in estimating the potential therapeutic effectiveness of novel bioactive compounds. Therefore, in addition to in vitro tests, the central activity of thiourea derivatives **1–4** was investigated in relevant mouse models. Importantly, the CNS activity of thiourea compounds bearing an indole ring has not been tested before.

Spontaneous activity and amphetamine hyperactivity were evaluated. The effect of the synthesized compounds on body temperature and behavior of animals caused by L-5-hydroxytryptophan (L-5-HTP) administration, motor coordination, as well as nociceptive and anticonvulsant activities, were tested.

Compound **1**, used at doses equivalent to 0.1, 0.05, and 0.025 ED_{50}, significantly decreased the spontaneous motor activity of mice (Figure 2). Derivatives **2** and **4** also tended to reduce visibly motor activity in mice treated in a similar manner, however, without any

TABLE 1 Binding affinity of a series of thiourea compounds **1–4** to 5-HT_{2A} and 5-HT_{2C} receptors

			5-HT_{2A} binding affinity		5-HT_{2C} binding affinity		
Compound	**R**	**logP**[a]	**pK_i**	**K_i (μM ± SEM)**	**pK_i**	**K_i (μM ± SEM)**	**SI**
1	Benzoyl	3.59	4.37 ± 0.08	42.3 ± 4.4	5.37 ± 0.4	4.2 ± 0.78	10.1
2	Ethoxycarbonyl	3.00	4.46 ± 0.08	34.9 ± 1.2	5.5 ± 0.3	2.8 ± 0.38	12.5
3	Ethyl	2.93	4.54 ± 0.11	28.9 ± 1.3	5.0 ± 0.4	10.7 ± 3.22	2.7
4	Benzyl	3.62	5.23 ± 0.14	5.9 ± 0.99	5.7 ± 0.4	1.9 ± 0.43	3.1
Ketanserin		–	8.27 ± 0.06	0.0053 ± 0.001	–	–	–
RS-102221		–	–	–	8.34 ± 0.12	0.0053 ± 0.001	–

Abbreviations: SEM, standard error of the means; SI, selectivity index.
[a]Calculated using online software www.molispiration.com.

TABLE 2 Activity of thiourea compounds **1–4** at the 5-HT_{1A} receptor

Compound	**pEC_{50}**	**EC_{50} (nM ± SEM)**	**E_{max} (%) ± SEM**	**Activity**
1	6.4 ± 0.18	418 ± 97.2	49.7 ± 4.2	Inverse agonist
2	NA	NA	NA	NA
3	NA	NA	NA	NA
4	7.1 ± 0.23*	81.8 ± 28.5*	57.9 ± 3.9	Weak inverse agonist
8-OH-DPAT	7.5 ± 0.11	27.2 ± 0.13	154 ± 2.3	Agonist
WAY-100635	8.4 ± 0.12	4.3 ± 1.4	99.8 ± 1.8	Antagonist

Abbreviations: NA, no activity; SEM, standard error of the means.
*$p < 0.05$ versus compound **1**.

TABLE 3 Antagonistic properties of thiourea compounds **1–4** at the D_2 receptor

Compound	pEC_{50}	EC_{50} (nM ± SEM)	E_{max} (%) ± SEM
1	NA	NA	NA
2	4.6 ± 0.14	22,270 ± 2,947	118 ± 2.8
3	4.8 ± 0.27	15,050 ± 4,063	120 ± 1.8[a]
4	NA	NA	NA
Domperidone	8.9 ± 0.25	1.58 ± 0.18	103 ± 1.36

Abbreviations: NA, no activity; SEM, standard error of the means.
[a]Versus domperidone.

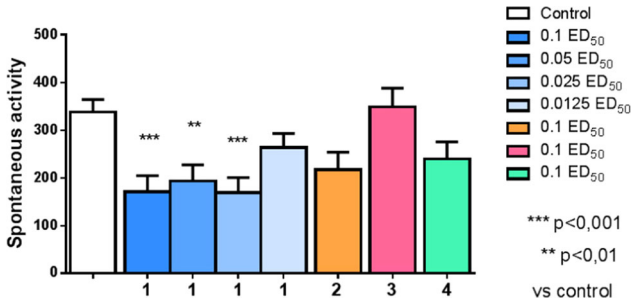


FIGURE 2 The effect of tryptamine derivatives on spontaneous motor activity of mice

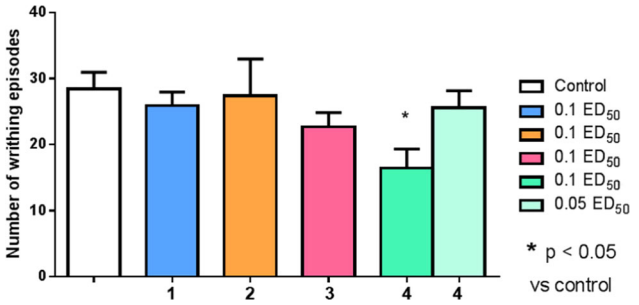


FIGURE 4 The antinociceptive effects of the tested compounds in mice. The results are expressed as mean ± standard error of the means (n = 10)

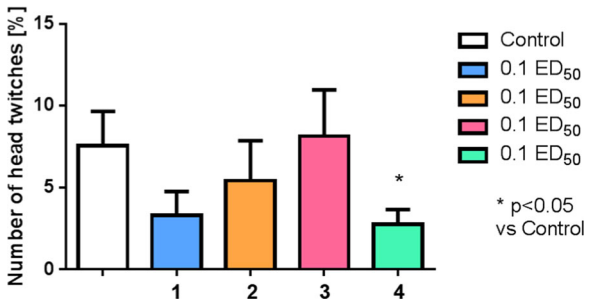


FIGURE 5 The impact of indole derivatives on head-twitch response induced by 5-hydroxytryptophan in mice. The results are expressed as mean ± standard error of the means (n = 10)

statistical importance. None of the tested thioureas, given at a dose equivalent to 0.1 ED_{50}, affected amphetamine-induced hyperactivity, which seems to rule out the impact of the catecholamine system in their action (Figure 3).

The investigated derivatives, used at a dose of 0.1 ED_{50}, caused no coordination deficits, as they did not change the behavior of mice in both the rota-rod and chimney tests (data not shown). The results obtained in all groups were similar to the values in the control group, indicating a lack of a depressive effect on coordination.

The thiourea **4**, at a dose equivalent to 0.1 ED_{50}, considerably decreased the number of writhing episodes ($p < 0.05$; Figure 4), that confirmed its anti-inflammatory activity.

Because the synthesized thioureas are 5-HT_{2A}/5-HT_{2C} ligands, tests were carried out to evaluate their effect on the "head-twitch" response (HTR) in mice, caused by administration of a serotonin precursor, L-5-HTP, which may indicate the involvement of the serotonergic system in the observed effects (Figure 5). The drug-elicited HTR [46,47] is a selective behavioral model for 5-HT_2 agonist activity in rodents, and several previous studies have established that direct and indirect 5-HT agonists induce this effect.[48–50] In addition, 5-HT_2 receptor antagonists selectively block HTR,[50–52] and their potency is highly correlated with the antagonistic affinity at 5-HT_2 receptors.[48,53] Among the compounds tested, only the benzylthiourea **4** significantly ($p < 0.05$) reduced the number of the "head-twitch" episodes, which corresponded with the strength of its binding to 5-HT_{2A}/5-HT_{2C} receptors estimated in vitro. The activities of **1** and **2** were also evident, but the results did not reach statistical significance. As 5-HT_2 receptors mediate the occurrence of a typical HTR caused by L-5-HTP, the data obtained confirm interactions with these receptors in the brain.

The tested derivatives, when administered at 0.1 ED_{50} doses in the pentetrazole seizure test, failed to reduce the severity of clonic or tonic seizures in comparison with the control group.

The results of investigations of the influence of thiourea derivatives on body temperature in normothermic mice also

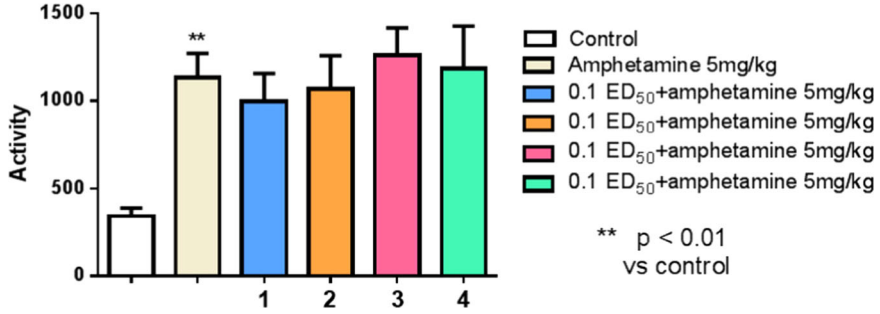


FIGURE 3 The influence of tested compounds on amphetamine-induced hyperactivity of mice

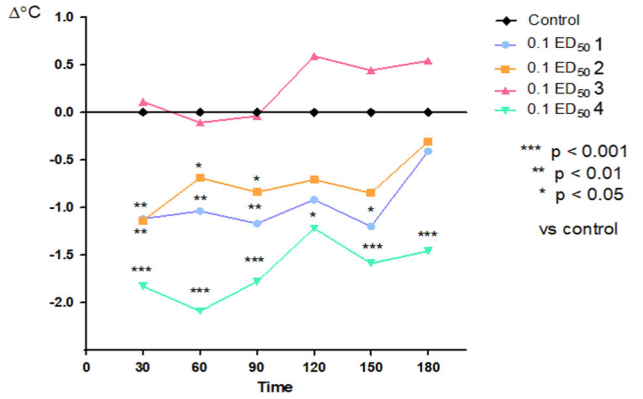


FIGURE 6 The influence of thiourea derivatives **1–4**, used at doses equivalent to 0.1 ED_{50}, on body temperature of mice. Note that each point represents the mean for a group of 10 mice

confirmed the modulatory effect of these compounds on the serotonergic system (Figures 6 and 7). According to literature data, 5-HT_2 receptor agonists and 5-HT_{1A} antagonists may induce hyperthermia; however, 5-HT_2 receptor antagonists and 5-HT_{1A} agonists were also shown to reduce body temperature.[31] The most evident and long-lasting activity was observed for derivative **4**, which at a dose equivalent to 0.1 ED_{50} reduced body temperature between 30–180 min after injection ($p < 0.001$). This activity, at half that dose, that is 0.05 ED_{50}, persisted from 30 to 60 min (Figure 6). The aforementioned compound transiently reduced body temperature at 30 min ($p < 0.01$) and 60 min after injection ($p < 0.05$). In addition, the long-term hypothermic activity was proved for derivative **1**, at a dose equivalent to 0.1 ED_{50}, at 30 ($p < 0.01$), 60 ($p < 0.01$), 90 ($p < 0.01$), and 150 min ($p < 0.05$). The statistically significant ($p < 0.01$) reduction of body temperature between 30 and 60 min was also denoted for that compound at a dose equivalent to 0.05 ED_{50}. Derivative **2** (0.1 ED_{50}) expressed considerable hypothermic activity between 30 and 90 min after administration. The results of body temperature measurements proved that the hypothermic action of *N*-arylthiourea derivatives **1** and **4** could be related to their agonistic properties toward the 5-HT_{1A} receptor as estimated in vitro.

The in vitro and in vivo investigations presented in this study indicate the possible involvement of the serotonergic system in the pharmacological activity of the indole-derived compounds. The CNS

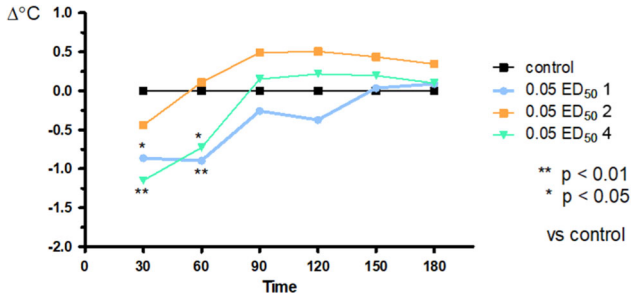


FIGURE 7 The effect of thiourea derivatives **1**, **2**, and **4**, used at doses equivalent to 0.05 ED_{50}, on body temperature of mice. Note that each point represents the mean for a group of 10 mice

activity of these compounds evaluated in animal behavioral tests were a part of a larger research project. Previously, the activity of several new compounds bearing the urea or thiourea moieties was tested, namely, those equipped with an imide,[46,54] 1,2,4-triazole,[29] and diphenyl[31] connections, as well as derivatives in which the thiourea branch was inbuilt into the 1,3-thiazepine ring.[55] In the present study, the cyclic terminal part of the molecule was replaced by an indole ring. On the basis of our previous research on the CNS effects of urea, thiourea, and thiazepine derivatives, along with findings described in this study, we could conclude that these groups of compounds possess significant CNS activity in mice. A comparison of urea and thiourea derivatives showed that urea had only influenced the "HTR," suggesting their potential antipsychotic properties. However, the activity of thiourea derivatives containing active heterocyclic rings, such as triazole or indole, is broader. That group, additionally, decreased body temperature of normothermic mice and significantly reduced the number of writing episodes, thereby confirming their antinociceptive activity.

To sum up, replacement of the phenyl ring bound to thiourea by a methylene or carbonyl linker (compounds **1** and **4**) with an alkyl substituent (compounds **2** and **3**) results in a decrease in CNS activity. The most active benzoyl **1** and benzyl **4** derivatives significantly reduced the number of HTRs and the body temperature of normothermic mice, which seems to indicate the involvement of the serotoninergic system in their action. What is more, the benzyl moiety of the thiourea derivative **4** is responsible for its anti-inflammatory action. In comparison to other compounds from the tested group, only the benzoylthiourea connection was able to limit the spontaneous activity of laboratory animals in doses from 0.1 to 0.0125 ED_{50}. The derivative with the ethoxycarbonyl moiety (**2**) influenced the body temperature in mice, whereas no test confirmed the CNS activity of the ethylthiourea compound (**3**).

3 | CONCLUSIONS

This study, presenting a short series of indole-thiourea derivatives **1–4**, provided new data concerning important structural properties needed for the in vitro binding and selectivity toward the subfamilies of serotonergic and dopaminergic receptors. The most active benzylthiourea **4** showed an interesting mixed 5-HT_{1A}/5-HT_{2A}/5-HT_{2C} activity, with significant selectivity over the studied 5-HT_2 receptor subtypes. Its benzoyl analog **1** kept the same pharmacological profile. Both these derivatives bearing an aromatic terminal moiety exerted an inverse agonistic profile at the 5-HT_{1A} receptor. *N*-Alkylthiourea compound **3**, similarly as its carbonyl derivative **2**, acted as a 5-HT_{2C}/5-HT_{2A} receptor ligand. The synthesized compounds had weak antagonistic effects (**2** and **3**) or exerted no functional activity (**1** and **4**) toward the D_2 receptor.

A correlation between functional receptor assays and in vivo behavioral tests was observed, especially for the most active and multitarget ligands **1** and **4**. Both compounds produced a hypothermic effect in mice, which confirmed their agonistic properties toward

the 5-HT_{1A} receptor. The thiourea derivative **4**, and **1** and **2**, significantly diminished the number of 5-HTP-elicated "head-twitch" shakes, which corresponded with their affinity for 5-HT_{2A}/5-HT_{2C} receptor subtypes. Compound **1** considerably reduced spontaneous motor activity of rodents, whereas **4** influenced the total number of writhing episodes, which identified its antinociceptive properties.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

All reagents and solvents were commercially available (Alfa Aesar, POC—Polskie Odczynniki Chemiczne). The NMR spectra were recorded on a Bruker AVANCE DMX400 spectrometer, operating at 300 MHz (^{1}H NMR) and 75 MHz (^{13}C NMR). The chemical shift values are expressed in ppm relative to tetramethylsilane as an internal standard. Mass spectral electrospray ionization (ESI) measurements were carried out on Waters ZQ Micromass instruments with quadrupol mass analyzer. The spectra were performed in the positive ion mode at a declustering potential of 40–60 V. The sample was previously separated on an ultra-performance liquid chromatography (UPLC) column (C18) using UPLC ACQUITY^{TM} system by Waters connected with DPA detector. Flash chromatography was performed on Merck silica gel 60 (200–400 mesh) using chloroform/methanol (19:1 vol) mixture as eluent. Analytical thin-layer chromatography was carried out on silica gel F254 (Merck) plates (0.25 mm thickness). The diffraction data for **3** were collected at 100(2) K on an Xcalibur diffractometer (Oxford Diffraction) equipped with the Cu Kα X-ray source (λ = 1.54184 Å) and charge-coupled device detector. The CrysAlis program system[56] was used for data collection, cell refinement, and data reduction. The data were corrected for Lorentz and polarization effects and a multiscan absorption correction was applied. The structure was solved using direct methods implemented in the SHELXS, and refined by the full-matrix least-squares on F^2 with the SHELXL-2018/3 program.[57] All non-H-atoms were refined with anisotropic displacement parameters. The H-atoms were positioned geometrically and refined using the riding model with Uiso(H) = 1.2 Ueq(C/N) or 1.5 for CH_3 groups.

The InChI codes of the investigated compounds are provided as Supporting Information.

4.1.2 | General procedure of synthesis of thiourea derivatives of 2-(1*H*-indol-3-yl)ethanamine

To a solution of 2-(1*H*-indol-3-yl)ethanamine (0.0038 mol, 0.61 g) in anhydrous acetonitrile (15 ml), an appropriate isothiocyanate (0.0042 mol) was added. The mixture was heated for 8 hr under a reflux condenser. Next the solvent was evaporated and the solid residue was purified by column chromatography (chloroform/methanol, 9.8:0.2 vol) or crystallized from acetonitrile.

The synthesis of thiourea derivatives **1** and **3** was described previously.[58–60] The molecular structure of **3** has been determined.

*1-(2-(1H-Indol-3-yl)ethyl)-3-ethoxycarbonylthiourea (**2**)*

Yield 89%. Melting point (Mp) 150–152°C. ^{1}H NMR (dimethyl sulfoxide [DMSO]-d_6) δ (ppm): 1.17–1.22 (t, 3H, CH_3, J = 7.2 Hz), 2.98–3.02 (t, 2H, CH_2, J = 7.2 Hz), 3.80–3.87 (q, 2H, CH_2, J = 6.9 Hz), 4.07–4.14 (q, 2H, CH_2, J = 7.2 Hz), 6.95–6.99 (t, 1H, CHarom., J = 6.9 Hz), 7.04–7.09 (t, 1H, CHarom., J = 7.8 Hz), 7.17–7.18 (d, 1H, CHarom., J = 2.4 Hz), 7.32–7.35 (d, 1H, CHarom., J = 8.1 Hz), 7.61–7.64 (d, 1H, CHarom., J = 7.8 Hz), 9.91–9.95 (t, 1H, NH, J = 5.4 Hz), 10.84 (s, 1H, NH), and 10.91 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ (ppm): 14.11, 23.75, 45.23, 61.59, 111.04, 111.38, 118.27, 118.42, 121.02, 122.88, 127.06, 136.30, 153.41, and 179.27. ESI MS: m/z = 314.1 $[M+Na]^+$ (100%).

*1-(2-(1H-Indol-3-yl)ethyl)-3-ethylthiourea (**3**)*

Crystal data: crystal system triclinic, space group P-1, unit cel dimensions at 200 K: a = 7.879(2) Å, b = 8.748(2) Å, c = 19.720(4) Å; α = 77.78(2)°, β = 88.39(2)°, γ = 83.45(2)°; V = 1,319.7(5) Å^3; Z = 4; D_{calc} = 1.245 g/cm^3; F(000) = 528; μ = 2.021 mm^{-1}; θ range = 2.29 to 68.17°; reflections collected/independent/observed 11,264/4,771/3,665, max. and min. transmission 1 and 0.8996; goodness-of-fit on F^2, 1.004; final R indices [I > 2σ (I)], R_1 = 0.0404, wR_2 = 0.0962; R indices (all data), R_1 = 0.0604, wR_2 = 0.1063; residual electron density max./min. 0.27 and −0.20 e/Å^3.

The experimental details and final atomic parameters have been deposited with the Cambridge Crystallographic Data Centre as the Supporting Information material (CCDC ID: 1919556). Copies of the data can be obtained free of charge on request via www.ccdc.cam.ac.uk/structures/.

*1-(2-(1H-Indol-3-yl)ethyl)-3-benzylthiourea (**4**)*

Yield 83%. Mp, 157–159°C. ^{1}H NMR (DMSO-d_6) δ (ppm): 2.89–2.94 (t, 2H, CH_2, J = 7.8 Hz), 3.68 (bs, 2H, CH_2), 4.64–4.65 (d, 1H, CHarom., J = 3.9 Hz), 6.94–6.99 (t, 1H, CHarom., J = 7.8 Hz), 7.04–7.09 (t, 1H, CHarom., J = 6.9 Hz), 7.14–7.14 (d, 1H, CHarom., J = 2.1 Hz), 7.20–7.34 (m, 6H, CHarom.), 7.49 (bs, 1H, NH), 7.58–7.61 (d, 1H, CHarom., J = 7.8 Hz), 7.85 (bs, 1H, NH), and 10.82 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ (ppm): 24.85, 44.31, 46.88, 111.30, 111.58, 118.18, 118.43, 120.89, 122.74, 126.76, 127.24 (3C), 128.21 (2C), 136.21, 139.36, and 182.39. ESI MS: m/z = 332.3 $[M+Na]^+$ (100%).

4.2 | Biological studies

4.2.1 | In vitro tests

Membrane preparation for 5-HT_{2A} and 5-HT_{2C} receptor assays

Male Sprague–Dawley rats were decapitated, their brains removed and placed on ice. Frontal cortices were homogenized with a glass homogenizer in 30 vol of ice-cold homogenization buffer (50 mM Tris-HCl, 1 mM ethylenediaminetetraacetic acid [EDTA], 5 mM $MgCl_2$, pH 7.4). Next, the homogenate was centrifuged at 20,000*g* for 15 min at 4°C. The pellet was suspended in 30 vol of 50 mM

Tris-HCl (pH 7.4) and incubated in a water bath for 15 min at 37°C to remove endogenous serotonin. The suspension was again centrifuged at 20,000*g* for 15 min at 4°C. The pellet was resuspended in 10 vol of 50 mM Tris-HCl (pH 7.4) and the centrifugation step was repeated. The final pellet was suspended in 10 vol of 50 mM Tris-HCl (pH 7.4) and stored at −80°C.

5-HT$_{2A}$ competition binding assay

For the 5-HT$_{2A}$ assay, frontal cortex homogenates (160 µg protein/ml) were incubated in triplicate with 1 nM [^{3}H]ketanserin for 60 min at 36°C in a 50-mM Tris-HCl (pH 7.4) buffer containing 0.1% ascorbate, 3 mM $CaCl_2$, and 10 µM pargyline, and increasing concentrations (10^{-11}–10^{-5}M) of the compound of interest. Nonspecific binding was determined in the presence of 10 µM mianserin. After incubation, the reaction mixture was deposited onto UniFilter-96 GF/B plates with the aid of a FilterMate-96 Harvester. Filter plates were presoaked beforehand with 0.4% PEI for 1 hr. Next, each filter well was washed with 1.75 ml of 50 mM Tris-HCl (pH 7.4) and left to dry on a heating block set to 50°C for 2 hr. Then, 45 µl of Microscint-20 scintillation fluid was added to each filter well and left to equilibrate overnight. Filter-bound radioactivity was counted in a MicroBeta^2 Microplate Counter. Binding curves were fitted with one-site nonlinear regression. Affinity was presented as the inhibitory constant (pK_i and K_i ± SEM [standard error of the means]) from two or three separate experiments.

5-HT$_{2C}$ competition binding assay

For the 5-HT$_{2C}$ assay, frontal cortex homogenates (250 µg protein/ml) were incubated in triplicate with 1 nM [^{3}H]mesulergine for 60 min at 36°C in a 50 mM Tris-HCl (pH 7.4) buffer containing 0.1% ascorbate, 10 mM $MgCl_2$, 10 µM pargyline, 100 nM spiperone and increasing concentrations (10^{-10}–10^{-5}M) of the compound tested. Nonspecific binding was determined in the presence of 10 µM mianserin. After incubation, the reaction mixture was deposited onto UniFilter-96 GF/B plates with the aid of a FilterMate-96 Harvester. Filter plates were presoaked beforehand with 0.4% PEI for 1 hr. Next, each filter well was washed with 1.75 ml of 50 mM Tris-HCl (pH 7.4) and left to dry for 2 hr on a heating block set to 50°C. Then, 45 µl of Microscint-20 scintillation fluid was added to each filter well and left to equilibrate overnight. Filter-bound radioactivity was counted in a MicroBeta^2 Microplate Counter. Binding curves were fitted with one-site nonlinear regression. Affinity was presented as the inhibitory constant (pK_i and K_i ± SEM) from two or three separate experiments.

Membrane preparation for 5-HT$_{1A}$ and D$_2$ receptor assay

Male Sprague–Dawley rats were decapitated, their brains removed and placed on ice. Hippocampi were dissected and homogenized with a glass homogenizer in 30 vol of ice-cold TED buffer (50 mM Tris-HCl, 1 mM EDTA, 1 mM dithiothreitol, pH 7.4). Next, the homogenate was centrifuged at 21,000*g* for 30 min at 4°C. The pellet was suspended in 30 vol of TED buffer (pH 7.4) and incubated in a water bath for 10 min at 37°C to remove endogenous ligands. The suspension was centrifuged again at 21,000*g* for 30 min at 4°C. The pellet was resuspended in 30 vol of TED buffer (pH 7.4) and the centrifugation step was repeated. The final pellet was suspended in 10 vol of 50 mM Tris-HCl (pH 7.4) and stored at −80°C until use.

Functional 5-HT$_{1A}$ receptor assay

In the agonist mode, 15 µg/ml of hippocampus homogenate was incubated in triplicate with 0.8 nM [^{35}S]GTPγS in the assay buffer (50 mM Tris-HCl, pH 7.4, 1 mM EGTA, 3 mM $MgCl_2$, 100 mM NaCl, 30 µM guanosine diphosphate [GDP]) in the presence of increasing concentrations of the tested compounds (10^{-10}–10^{-5}M). In the antagonist mode, compounds were additionally incubated with 100 nM 8-OH-DPAT. Nonspecific binding was determined with 100 µM of unlabeled GTPγS. The reaction mixture was incubated for 90 min at 37°C in a volume of 250 µl. Next, 96-well Unifilter® plates (Perkin Elmer) were presoaked for 1 hr with 50 mM Tris-HCl (pH 7.4) before harvesting. The reaction was terminated by vacuum filtration onto filter plates with the FilterMate Harvester® (Perkin Elmer). The samples were then rapidly washed with 2 ml of 50 mM Tris-HCl (pH 7.4) buffer. Filter plates were dried for 2 hr at 50°C. After drying, 45 µl of EcoScint-20 scintillant (Perkin Elmer) was added to every well. Radioactivity was counted in a Trilux MicroBeta^2 counter (Perkin Elmer). Data were analyzed with GraphPad Prism 5.0 software (www.graphpad.com; GraphPad Software, San Diego, CA). Curves were fitted with a one-site nonlinear regression model. Efficacy (E_{max}) and potency (EC_{50}) were calculated from the Cheng-Prusoff equation and expressed as means ± SEM. Differences in the compound potency and efficacy were evaluated with the extra-sum-of-squares *F* test. One, two, or three symbols represent statistical significance of 0.05, 0.01 and 0.001, respectively.

Functional D$_2$ receptor assay

For the D$_2$ receptor antagonist [^{35}S]GTPγS assay, 15 µg/ml of striatal homogenate was incubated in triplicate with 0.8 nM [^{35}S]GTPγS in assay buffer (50 mM Tris-HCl, pH 7.4, 1 mM EGTA, 3 mM $MgCl_2$, 100 mM NaCl, 0.1 mM dithiotheritol, 500 µM ascorbic acid, 20 µM GDP and 100 µM dopamine) in the presence of increasing concentrations of the tested compounds (10^{-10}–10^{-5}M). The effect on basal G protein activation threshold was determined in assay buffer deprived of dopamine. The final DMSO concentration in the assay was 5%. Dopamine was dissolved in 50 mM Tris buffer (pH 7.4) supplemented with 500 µM ascorbic acid to prevent oxidation. Nonspecific binding was determined with 100 µM of unlabeled GTPγS. The reaction mixture was incubated for 60 min at 30°C at a volume of 250 µl. Next, 96-well Unifilter® Plates (Perkin Elmer) were presoaked for 1 hr with 50 mM Tris-HCl (pH 7.4) before harvesting. The reaction was terminated by vacuum filtration onto filter plates with the FilterMate Harvester® (Perkin Elmer). The samples were then rapidly washed with 2 ml of 50 mM Tris-HCl (pH 7.4) buffer. Filter plates were dried for 2 hr at 50°C. After drying, 45 µl of EcoScint-20 scintillant (Perkin Elmer) was added to the wells. Radioactivity was counted in a Trilux MicroBeta^2 counter (Perkin Elmer). Data were analyzed with GraphPad Prism 5.0 software (www.graphpad.com; GraphPad Software). Curves were fitted with a

one-site nonlinear regression model. Potency (EC_{50}) and efficacy (E_{max}) were calculated from the Cheng-Prusoff equation and expressed as means ± SEM. Differences in compound potency and efficacy were evaluated with the extra-sum-of-squares *F* test. One, two, or three symbols represent statistical significance of 0.05, 0.01 and 0.001, respectively.

4.2.2 | In vivo tests

The experiments were carried out on male Albino Swiss mice (18–30 g). The animals were kept 8–10 to a cage under standard laboratory conditions (at a temperature of 20 ± 1°C and a 12-hr light/dark cycle) with free access to food (LSM, Motycz, Poland) and water. All experiments were performed between 9:00 a.m. and 4:00 p.m. The experiments were performed in accordance with the opinion of local ethics committee for animal experimentation.

For behavioral testing, all the substances investigated (marked as **1–4**) were administered intraperitoneally (ip), as suspensions in aqueous solution of 0.5% methylcellulose (tylose) and injected 60 min before testing. All substances were administered in a volume of 10 ml/kg. Control animals received an equivalent volume of the solvent at the respective time before the test. All tests performed, as suggested by Vogel et al.,[61] are generally accepted as basic for investigation of the central activity by behavioral methods. Acute toxicity of the compound was assessed in mice according to Litchfield and Wilcoxon method,[62] as the ED_{50} calculated as "the loss of righting reflex" within 48 hr. The compounds were injected in doses equivalent to 0.1 LD_{50}. In addition, the activity of compounds was assessed in the following tests:

- Locomotor activity was measured for single mice in photoresistor actometers (circular cages, 25 cm in diameter, two light beams; the number of crossed light beams by the mice was recorded) for 30 min as:
  - Spontaneous activity
  - Amphetamine-induced hyperactivity: Mice received 5 mg/kg of amphetamine subcutaneously (sc) 30 min before the test
- Nociceptive reactions were studied in the acetic acid (0.6%)-induced "writhing" test. The number of writhing episodes was measured for 10 min starting 5 min after ip administration of acid solution
- Motor coordination was evaluated in rota-rod test and chimney test[63]
- Body temperature in normothermic mice was measured in the rectum by thermistor thermometer
- Pentylenetetrazole (110 mg/kg, sc)-induced convulsions were evaluated as the number of mice with clonic seizures, tonic convulsions, and dead animals
- HTRs after 5-HTP, according to Corne et al. [46,47] The mice received 5-HTP (180 mg/kg, ip) and the number of head twitches was recorded in six 2-min intervals (4–6, 14–16, 24–26, 34–36, 44–46, 54–56 min)

4.2.3 | Statistical analysis

Data obtained were calculated by χ^2 test with Yates correction (pentylenetetrazole-induced seizures) and one-way analysis of variance (other tests). Subsequent comparisons between treatment and control groups were carried out using a post hoc Dunnett's test, when $p < 0.05$.

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CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interests.

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