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Synthesis of some urea and thiourea derivatives of 3-phenyl/ethyl-2-thioxo-2,3-dihydrothiazolo[4,5-*d*]pyrimidine and their antagonistic effects on haloperidol-induced catalepsy and oxidative stress in mice

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ABSTRACT

A series of 3-phenyl/ethyl-2-thioxo-2,3-dihydrothiazolo[4,5-*d*]pyrimidin-7-yl urea and thiourea derivatives were designed and synthesized. All the compounds have been evaluated for their antiparkinsonian activity in catalepsy induced by haloperidol in mice. A majority of the compounds exhibited significant antiparkinsonian activity after intraperitoneal administration. The most active compound carries methoxy group at 2-position of the phenyl ring. Some of the potent compounds were selected for biochemical estimations of malondialdehyde, glutathione, superoxide dismutase and glutathione peroxidase from brain homogenate to highlight the neuroprotective properties associated with them. The results obtained in the present study may lead to the development of a suitable approach to the treatment of Parkinson's disease and may be the starting point for the future drug design.

1. Introduction

Parkinson's disease (PD) is considered as one of the major progressive neurologic disorders of the elderly population and about three percent of this age group over 65 years have developed the overt illness [1]. Clinically, PD is characterized by the tetrad of tremor at rest, slowness of voluntary movements, rigidity, and postural instability [2]. The cardinal biochemical abnormality in PD is the profound deficit in brain dopamine level, primarily attributed to the loss of neurons of the nigrostriatal dopaminergic pathway [3]. Over the last two decades, tremendous strides toward acquiring a better knowledge of both the etiology and the pathogenesis of PD revealed the free radical theory as one of the mechanisms involved in the pathogenesis of this disease [4].

There is substantial evidence that the brain, which consumes large amounts of oxygen, is particularly vulnerable to oxidative damage. Free radicals are normal products of cellular metabolism [5]. The predominant cellular free radicals are the superoxide ($O_2^{\bullet-}$) and hydroxyl ($OH^{\bullet}$) species [4,6]. Other molecules, such as hydrogen peroxide (H_2O_2) and peroxynitrite ($ONOO^-$), although not themselves free radicals, can lead to the generation of free radicals through various chemical reactions. Thus H_2O_2, in the presence of reduced metal, forms the highly reactive $OH^{\bullet}$ via the Fenton reaction [6]. $ONOO^-$, formed by the reaction of nitric oxide ($NO^{\bullet}$) with $O_2^{\bullet-}$, is a highly reactive molecule that also breaks down to form $OH^{\bullet}$. Together, these molecules are referred to as reactive oxygen species (ROS) to signify their ability to lead to oxidative changes within the cell [6]. Problems occur when the production of ROS exceeds the ability of cells to defend themselves against these substances. This imbalance between cellular production of ROS and the ability of cells to defend themselves against them is referred to as oxidative stress [6]. Oxidative stress can cause cellular damage and ROS oxidize critical cellular components such as membrane lipids, proteins, and DNA, thereby inducing apoptosis or necrosis [7,8]. There is a large scientific literature regarding the relation between ROS production, the induction of apoptosis or necrosis and the pathogenesis of PD [9,10].

Superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) are among the major antioxidant enzymes present in the

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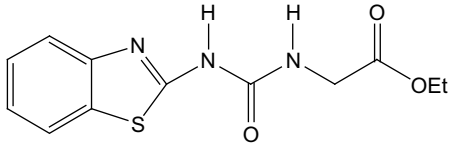
human body that protect against the oxygen toxicity [11]. Nonenzymatic antioxidant system includes glutathione (GSH), an oxyradical scavenger, thereby useful in protecting against oxidative damage by free radicals and inhibiting lipid peroxidation and DNA damage [12]. There are reports suggesting a decrease in SOD, GSH-Px and other antioxidant enzyme activities in substantia nigra of PD patients [13,14]. Consistent with this, a reduction of glutathione [15] and polyunsaturated fatty acid contents, as well as an increase in lipid peroxidation [16] has also been reported.

Dopamine-replacement therapy has dominated the treatment of motor symptoms of PD since the early 1960s. The effects are predictable (as are the side effects) and none of the more recently introduced synthetic analogues has surpassed the clinical benefit derived from levodopa [17]. Although levodopa is the most effective drug for the control of motor symptoms, it also causes a high level of motor complications, particularly dyskinesias [18]. However, despite the many dopaminergic agents currently available, the search for novel approaches based on dopamine-replacement therapy continues. The multiplicity of dopamine receptors in the brain offers a range of potential targets, but so far exploitation of drugs acting on specific receptor subtypes has been disappointing.

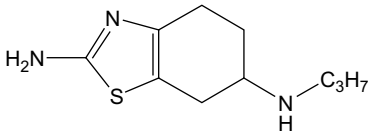
Thiazole derivatives have evoked considerable attention in recent years as these are endowed with wide range of biological activities [19–21] as well as drugs of PD [22,23]. For example, pramipexole (Fig. 1), an aminobenzothiazole analogue is a dopamine D_2/D_3 receptor agonist currently in clinical use for the treatment of PD [24]. It has been reported that pramipexole acts as a scavenger of ROS, based on the findings of an *in vivo* microdialysis study of the rat striatum [25]. Protective effect of pramipexole against H_2O_2-induced PC12 cell death is also reported in literature [26]. Another example of an analogue with an aminothiazole moiety is riluzole (Fig. 1), a Na^+ channel blocker with neuroprotective activity [27] effective against MPTP-induced neurodegeneration of the nigrostriatal dopaminergic neuronal pathway [28]. In addition, cabergoline, a dopamine D_2 receptor agonist which was launched in 1993 as an anti-Parkinson's agent containing urea functionality in its structure [29] while other derivatives such as KHG21834 (Fig. 1) with urea and thiazole moiety were capable of protecting PC12 cells and cortical and mesencephalic neurons from amyloid β-induced degeneration [30]. Likewise, some thiazol-2-yl urea derivatives (e.g. AR-A014418, Fig. 1) are being developed as an inhibitor of glycogen synthase kinase-3β as its dysregulation is implicated in certain psychiatric and neurodegenerative diseases [31,32] and these agents are being considered as a novel strategy to treat PD [33]. Also, a series of aryl/heteroaryl ureas bearing thiazole moiety have emerged as a potent and selective inhibitors of cyclin dependent kinases for the treatment of Alzheimer's disease and other neurodegenerative disorders [34].

Thiazolo[4,5-*d*]pyrimidine derivatives, which can be considered as thia-analogues of the natural purine bases such as adenine and guanine, have acquired a growing importance in the field of medicinal chemistry because of their biological potential while some thiazolo[3,2-*a*]pyrimidines have been demonstrated to be associated with potent immunomodulating properties [35]. Furthermore, the recently demonstrated adenosine A_{2A} receptor antagonistic activities of certain thiazoles with a urea moiety [36] and thiazolopyrimidines (Fig. 1) [37] for the development of a suitable approach to the treatment of PD may be the starting point for the future drug design.

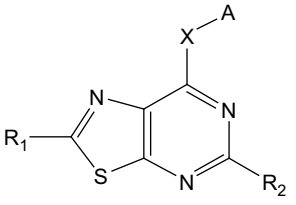
Led by above facts and coupled with our ongoing project aimed at investigating new bioactive heterocycles derived from thiazole nucleus [19–23], we have designed a series of compounds incorporating above moieties together in order to prepare the molecules having enhanced biological activity. The compounds were evaluated for haloperidol-induced catalepsy model of PD. The extent of oxidative stress has been evaluated by measuring MDA level, GSH content, SOD and GSH-Px activities from brain homogenate in



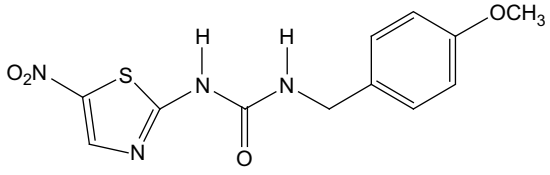
KHG21834 [30]



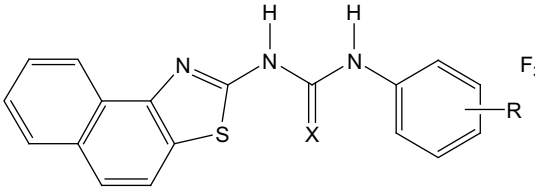
Pramipexole [24-26]



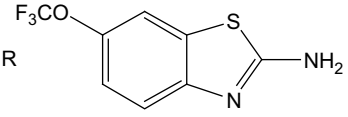
Thiazolopyrimidine derivatives [37]



AR-A014418 [33]



Thiazole derivatives with urea/thiourea moiety [23]



Riluzole [27]

Fig. 1. Structure of some cited molecules.

selected compounds. Part of this work has been presented in an abstract form [38].

2. Synthesis

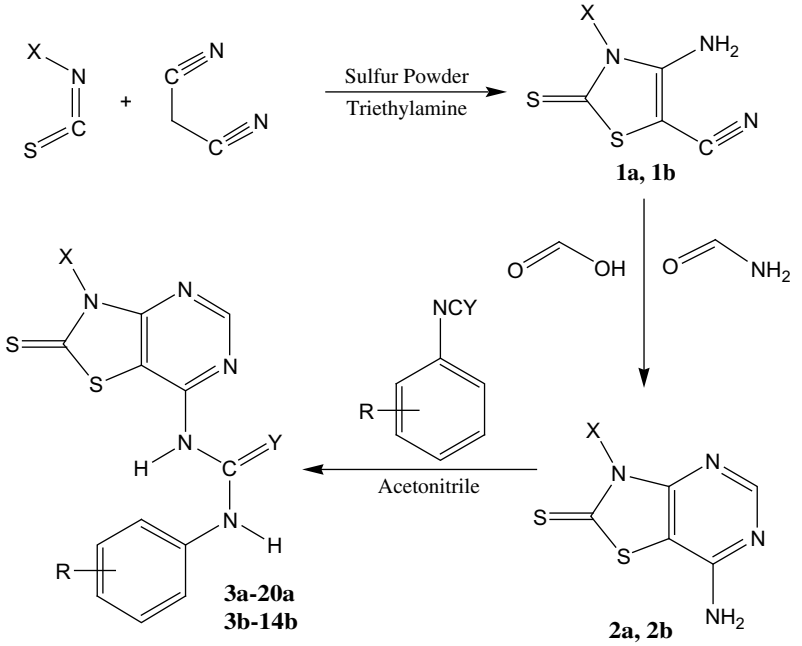
In the present study urea and thiourea derivatives of 3-phenyl/ethyl-2-thioxo-2,3-dihydrothiazolo[4,5-*d*]pyrimidine were synthesized as presented in Scheme 1. 4-Amino-3-phenyl/ethyl-2-thioxo-2,3-dihydrothiazole-5-carbonitrile (**1a**, **1b**) was prepared when to a mixture of malononitrile, phenyl/ethyl isothiocyanate and finely divided sulfur in DMF was added triethylamine very slowly with constant stirring at room temperature. **1a** or **1b** was treated with formamide and formic acid with heating to yield 7-amino-3-phenyl/ethyl thiazolo[4,5-*d*]pyrimidine-2(3*H*)-thione (**2a**, **2b**). The urea and thiourea derivatives (**3a–20a**, **3b–14b**) were obtained when **2a** or **2b** and appropriate aryl isocyanate/isothiocyanate were refluxed with stirring in dry acetonitrile. All compounds had IR, ^{1}H NMR and mass spectra in accord with their anticipated structure. The physical characterization data of the compounds are given in Table 1.

3. Results and discussion

Catalepsy is a behavioral condition characterized by the rigid state of a part or all of the muscles which shares some similarity to human PD [39,40]. The phenomenon of cataleptic immobility induced in rodents by the use of a dopamine antagonist such as haloperidol is a robust behavioral method for studying nigrostriatal function. This is a behavioral condition in which the animal is unable to correct an externally imposed posture. Catalepsy-free mice, i.e., those without haloperidol, should be able to come down from the horizontal bar within certain time period. On the other hand, cataleptic mice, when placed in this awkward position, were unable to come down from the bar over a period of 180 s or more.

Haloperidol (5 mg/kg) produced a profound increase in catalepsy as shown by a progressive increase in the latency to step down the rod over time as compared with controls ($P < 0.001$). Efficacious compounds were defined as those that allowed the mice to come down from the bar within 180 s. The results obtained from acute administration of haloperidol are in agreement with previous studies of our research group [22,23] as well as report from other laboratory [41]. Acute subcutaneous administration of compounds (**3a–20a** and **3b–14b**) at a dose of 100 mg/kg significantly antagonized haloperidol-induced catalepsy as evident from the decrease in mean descent time in treated animals when compared to mice injected with haloperidol alone. The effect of title compounds and standard drug in terms of mean descent time observed at different time intervals, mean CDT [3 h] and % catalepsy are presented in Table 2.

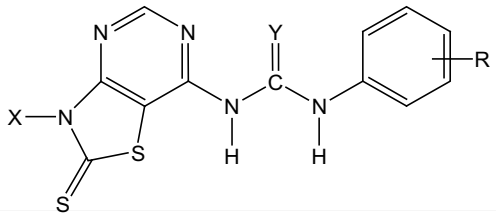
Compounds **5a** and **14b** showed better activity than standard drug levodopa. The least active compound among the series was **10b** while **5a** was most potent. Rest of the compounds exhibited moderate activity and the results obtained are presented in Table 2. When talking about the structure–activity relationship, electron attractive groups decreased the antiparkinsonian activity, while pronounced activity was observed in derivatives having electron donors like –OCH_3 groups especially at position 2 of the phenyl ring. These results buttress the theoretical dopamine D_2 receptor model of Sukalovic et al. [42] where it is reported that methoxy group increases the affinities of ligands if attached to position 2, since one additional hydrogen bond could be formed with Trp 182. Similarly, it is possible that compounds having –OCH_3 group at position 2 may interact with Trp 182 of dopamine D_2 receptor binding site, making the compound potent in terms of observed anticataleptic as well as



a (X = Ph)	b (X = Et)	Y = O or S			
3a,b; 12a,b	(R = H)	**6a,b; 15a**	(R = 2-F)	**9a,b; 18a**	(R = 4-Cl)
4a,b; 13a,b	(R = 4-OCH_3)	**7a,b; 16a**	(R = 4-F)	**10a,b; 19a**	(R = 2-NO_2)
5a,b; 14a,b	(R = 2-OCH_3)	**8a,b; 17a**	(R = 2-Cl)	**11a,b; 20a**	(R = 4-NO_2)

Scheme 1. Synthetic protocol of the title compounds.

Table 1
Characterization data of the urea and thiourea derivatives **3a**–**20a** and **3b**–**14b**.



Compound	X	Y	R	Yield (%)	m.p. (°C)	Mol. formula[a]	Mol. weight	log *P*[b]
3a	Ph	O	H	79	260–262	$C_{18}H_{13}N_5OS_2$	379.46	3.48
4a	Ph	O	4-OCH_3	86	310–312	$C_{19}H_{15}N_5O_2S_2$	409.48	3.43
5a	Ph	O	2-OCH_3	63	306–308	$C_{19}H_{15}N_5O_2S_2$	409.48	3.54
6a	Ph	O	2-F	55	199–200	$C_{18}H_{12}FN_5OS_2$	397.45	3.45
7a	Ph	O	4-F	90	255–256	$C_{18}H_{12}FN_5OS_2$	397.45	3.93
8a	Ph	O	2-Cl	85	242–243	$C_{18}H_{12}ClN_5OS_2$	413.9	3.99
9a	Ph	O	4-Cl	91	234–236	$C_{18}H_{12}ClN_5OS_2$	413.9	4.47
10a	Ph	O	2-NO_2	83	284–286	$C_{18}H_{12}N_6O_3S_2$	424.46	3.73
11a	Ph	O	4-NO_2	76	265–267	$C_{18}H_{12}N_6O_3S_2$	424.46	3.94
12a	Ph	S	H	67	250–252	$C_{18}H_{13}N_5S_3$	395.52	2.96
13a	Ph	S	4-OCH_3	82	304–305	$C_{19}H_{15}N_5OS_3$	425.55	2.91
14a	Ph	S	2-OCH_3	85	287–288	$C_{19}H_{15}N_5OS_3$	425.55	2.86
15a	Ph	S	2-F	83	271–273	$C_{18}H_{12}FN_5S_3$	413.51	2.92
16a	Ph	S	4-F	59	261–263	$C_{18}H_{12}FN_5S_3$	413.51	3.41
17a	Ph	S	2-Cl	88	270–272	$C_{18}H_{12}ClN_5S_3$	429.97	3.47
18a	Ph	S	4-Cl	65	278–280	$C_{18}H_{12}ClN_5S_3$	429.97	3.95
19a	Ph	S	2-NO_2	80	284–286	$C_{18}H_{12}N_6O_2S_3$	440.52	3.21
20a	Ph	S	4-NO_2	76	256–257	$C_{18}H_{12}N_6O_2S_3$	440.52	3.42
3b	Et	O	H	66	265–267	$C_{14}H_{13}N_5OS_2$	331.42	2.26
4b	Et	O	4-OCH_3	78	249–250	$C_{15}H_{15}N_5O_2S_2$	361.44	2.21
5b	Et	O	2-OCH_3	72	258–260	$C_{15}H_{15}N_5O_2S_2$	361.44	2.32
6b	Et	O	2-F	65	271–273	$C_{14}H_{12}FN_5OS_2$	349.41	2.22
7b	Et	O	4-F	73	283–284	$C_{14}H_{12}FN_5OS_2$	349.41	2.71
8b	Et	O	2-Cl	66	225–227	$C_{14}H_{12}ClN_5OS_2$	365.86	2.77
9b	Et	O	4-Cl	58	286–288	$C_{14}H_{12}ClN_5OS_2$	365.86	3.25
10b	Et	O	2-NO_2	63	220–222	$C_{14}H_{12}N_6O_3S_2$	376.41	2.51
11b	Et	O	4-NO_2	77	264–265	$C_{14}H_{12}N_6O_3S_2$	376.41	2.72
12b	Et	S	H	71	268–270	$C_{14}H_{13}N_5S_3$	347.48	1.74
13b	Et	S	4-OCH_3	78	298–300	$C_{15}H_{15}N_5OS_3$	377.51	1.69
14b	Et	S	2-OCH_3	82	282–283	$C_{15}H_{15}N_5OS_3$	377.51	1.64

[a] Elemental analyses for C, H and N were within ±0.4% of the theoretical values.
[b] log *P* was generated using ACD/log *P* 11.0 software (Advanced Chemistry Development Inc., Toronto, Canada).

antioxidant activities. In addition, the electron attractive groups decrease the binding affinity, while electron donors like –OCH_3 increase the affinity for the binding at the dopamine D_2 receptor in comparison with the unsubstituted analogues [43,44]. Also, methoxy group is said to be linked with binding affinity of the drug molecules at dopamine D_2 and D_3 receptor sites [43]. In a report of Teeter and DuRand [45] and Simpson et al. [46] it was revealed that benzimidazole structural motif interacts with the receptor through hydrogen bonds that involve amino acids Ser 122 and Ser 141 in TM II and TM III, that are a part of catechol binding site of the dopamine D_2 receptor. Likewise it can be assumed that one of the nitrogen atoms of pyrimidine ring may interact with the receptor through hydrogen bond in a similar fashion. Based on these observations, the proposed binding interactions of the title compounds at the receptor site are presented in Fig. 2.

Some potent compounds (**5a**, **5b**, **14a** and **14b**), were selected for the measurement of antioxidant parameters from the brain homogenate of mice to investigate their role in the pathophysiology of PD. Oxidative stress to dopaminergic neurons of substantia nigra pars compacta is believed to be one of the leading causes of neurodegeneration in PD. Thus ROS scavenging antioxidants may play an important role in the prevention of PD and combat against oxidative stress-induced progressive neurodegeneration [47–49]. Our results revealed that haloperidol increased the lipid peroxidation and reduced the GSH level, along with reduced activities of antioxidant enzyme (SOD and GSH-Px) in the brain homogenate. Many reports indicate that an excessive production of free radicals is associated with haloperidol treatment [50,51]. This abnormal free radical production often goes hand in hand with GSH and ATP depletion [50,52], and a loss of detoxifying enzyme activity such as SOD, catalase and GSH-Px [53,54]. Also, many preclinical and clinical studies have proposed the production of ROS as causes of haloperidol-induced toxicity [51] and a loss of detoxifying enzymes activities [52,55].

Compounds **5a**, **5b**, **14a** and **14b** administered 30 min before haloperidol significantly suppressed oxidative stress, restoring enzyme activities as well as GSH and MDA contents to almost similar to the controls (Table 3). The present data demonstrate that urea and thiourea derivatives possess antioxidant effect on catalepsy model of PD, offering protection by enhancing GSH content and antioxidant enzyme (SOD and GSH-Px) activities as well as decreasing LPO in mice treated with haloperidol. Such regulation of oxidative stress markers and antioxidant enzymes by the title compounds in the present study may be well correlated with our previous reports, where thiazole derivatives as well as urea/thiourea derivatives were effective in restoring the levels of oxidative stress markers and antioxidative enzymes up to the basal level against oxidative stress induced by haloperidol [22,23] and pentylenetetrazole in mice brain [21].

Table 2
Mean descent time observed at different time intervals.

Compound	Mean descent time (s) ± S.E.M. at time (min)						Mean CDT [3 h] ± SEM	Catalepsy (%)
	30	60	90	120	150	180		
3a	45 ± 0.56[a]	128 ± 2.86[a]	98 ± 1.61[b]	134 ± 1.99[a]	200 ± 1.69[a]	195 ± 2.60[b]	800 ± 5.55[a]	49.5
4a	57 ± 1.1[a]	92 ± 1.34[a]	100 ± 2.01[a]	135 ± 2.32[a]	125 ± 1.67[a]	129 ± 3.19[b]	638 ± 3.68[a]	39
5a	26 ± 2.18[a]	49 ± 0.75[a]	57 ± 1.18[a]	89 ± 1.26[a]	83 ± 2.54[a]	94 ± 1.11[a]	398 ± 4.49[a]	23.4
6a	83 ± 1.25[a]	126 ± 2.17[b]	166 ± 2.19[b]	141 ± 2.13[a]	152 ± 3.14[a]	158 ± 2.56[a]	826 ± 5.12[a]	51.2
7a	85 ± 1.18[a]	114 ± 2.45[a]	115 ± 2.51[a]	158 ± 3.72[a]	153 ± 2.89[a]	228 ± 4.88[a]	853 ± 3.74[a]	52.9
8a	76 ± 1.25[a]	118 ± 2.16[a]	144 ± 2.53[a]	167 ± 2.76[a]	175 ± 2.34[a]	174 ± 2.33[a]	854 ± 4.29[a]	53
9a	76 ± 1.12[b]	177 ± 2.12[a]	139 ± 2.53[b]	205 ± 1.81[a]	206 ± 1.58[b]	242 ± 2.72[a]	1045 ± 5.65[a]	65.3
10a	55 ± 1.12[a]	63 ± 1.53[a]	121 ± 1.57[a]	135 ± 2.65[a]	176 ± 2.19[a]	177 ± 3.61[a]	727 ± 3.93[a]	44.8
11a	53 ± 1.17[a]	79 ± 1.27[a]	119 ± 1.7[a]	125 ± 2.57[a]	191 ± 1.29[b]	236 ± 3.35[b]	803 ± 4.81[a]	49.7
12a	66 ± 0.88[a]	58 ± 1.32[a]	75 ± 1.67[a]	192 ± 2.76[a]	216 ± 4.15[a]	217 ± 3.79[a]	824 ± 3.19[a]	51
13a	70 ± 1.12[a]	66 ± 1.53[a]	103 ± 1.74[a]	158 ± 2.11[a]	172 ± 2.67[a]	133 ± 2.34[a]	702 ± 4.47[a]	43.1
14a	25 ± 0.15[a]	44 ± 0.54[a]	78 ± 0.93[a]	108 ± 1.17[a]	112 ± 1.93[a]	109 ± 1.87[a]	476 ± 3.64[a]	28.5
15a	78 ± 0.81[b]	99 ± 1.43[a]	142 ± 2.81[a]	160 ± 2.11[a]	208 ± 3.61[a]	257 ± 3.45[b]	944 ± 3.44[a]	58.8
16a	75 ± 1.17[a]	129 ± 2.14[b]	165 ± 2.27[b]	195 ± 3.22[a]	218 ± 3.28[a]	200 ± 2.74[a]	982 ± 4.99[a]	61.3
17a	64 ± 1.56[a]	116 ± 3.19[a]	185 ± 2.21[a]	158 ± 1.16[a]	241 ± 2.62[a]	238 ± 2.61[a]	1002 ± 5.29[a]	62.6
18a	58 ± 1.06[b]	147 ± 4.17[a]	153 ± 2.66[a]	168 ± 2.57[a]	209 ± 3.5[b]	268 ± 4.12[a]	1003 ± 3.16[a]	62.6
19a	42 ± 1.01[a]	69 ± 1.36[a]	69 ± 2.34[a]	93 ± 1.14[a]	234 ± 2.82[a]	232 ± 3.23[b]	737 ± 4.15[a]	45.4
20a	58 ± 0.81[a]	113 ± 1.61[b]	131 ± 1.98[a]	147 ± 2.32[a]	161 ± 1.87[a]	159 ± 2.58[a]	769 ± 3.09[a]	47.5
3b	49 ± 1.12[a]	122 ± 1.75[a]	127 ± 1.33[b]	144 ± 2.95[a]	133 ± 3.31[a]	199 ± 2.36[a]	774 ± 5.52[a]	47.8
4b	77 ± 0.17[a]	111 ± 1.32[a]	114 ± 3.15[a]	121 ± 1.44[b]	138 ± 2.37[a]	143 ± 2.0[a]	704 ± 4.61[a]	43.3
5b	35 ± 0.31[a]	31 ± 0.73[a]	40 ± 0.56[a]	90 ± 2.65[a]	135 ± 3.12[a]	157 ± 3.19[a]	488 ± 4.01[a]	29.3
6b	67 ± 1.14[a]	119 ± 1.89[a]	138 ± 1.66[a]	173 ± 2.37[a]	186 ± 2.66[a]	177 ± 2.37[a]	860 ± 5.11[a]	53.4
7b	66 ± 1.02[b]	99 ± 1.71[a]	113 ± 1.58[a]	184 ± 2.31[a]	187 ± 4.14[a]	237 ± 3.9[a]	886 ± 4.36[a]	55.1
8b	127 ± 1.98[a]	138 ± 2.91[b]	131 ± 2.48[a]	157 ± 2.79[a]	196 ± 2.38[b]	189 ± 2.17[a]	938 ± 4.67[a]	58.4
9b	64 ± 1.31[a]	173 ± 4.12[b]	163 ± 2.74[a]	152 ± 2.16[a]	226 ± 4.77[a]	247 ± 2.38[b]	1025 ± 4.38[a]	64.1
10b	102 ± 1.82[a]	168 ± 1.93[a]	209 ± 2.91[a]	198 ± 3.53[a]	225 ± 3.58[a]	228 ± 2.62[a]	1130 ± 5.45[a]	70.9
11b	100 ± 2.37[b]	140 ± 2.55[a]	194 ± 3.12[a]	212 ± 4.11[a]	210 ± 4.88[a]	231 ± 3.34[b]	1087 ± 3.73[a]	68.1
12b	73 ± 0.69[a]	115 ± 1.12[a]	139 ± 2.31[a]	140 ± 2.49[a]	172 ± 1.18[a]	171 ± 2.73[a]	810 ± 3.71[a]	50.1
13b	26 ± 0.19[a]	43 ± 0.33[a]	92 ± 2.11[a]	114 ± 1.86[a]	126 ± 2.16[a]	134 ± 2.24[a]	535 ± 4.56[a]	32.3
14b	18 ± 0.18[a]	15 ± 0.12[a]	53 ± 0.19[a]	75 ± 0.81[a]	97 ± 1.1[a]	144 ± 4.22[a]	402 ± 4.24[a]	23.7
Levodopa	19 ± 0.51[b]	38 ± 1.54[a]	86 ± 1.54[a]	88 ± 0.68[b]	85 ± 1.62[a]	108 ± 1.51[a]	424 ± 3.22[a]	25.1
Haloperidol	183 ± 3.68[c]	224 ± 4.45[c]	273 ± 3.82[c]	300 ± 4.23[c]	300 ± 4.31[c]	300 ± 4.96[c]	1580 ± 5.19[c]	100
Control	8 ± 0.06	5 ± 0.21	5 ± 0.36	5 ± 0.08	6 ± 0.57	7 ± 0.64	36 ± 1.04	–

Data are presented as mean descent time (s) ± S.E.M. Number of animals per group (n) = 5, Haloperidol (5 mg/kg), compounds **3a–20a**, **3b–14b** and levodopa at the dose of 100 mg/kg 30 min prior to haloperidol injection. The sum of the descent time values measured every 30 min during the 3 h after haloperidol or vehicle is defined as the cumulative descent time (CDT [3 h]). Data were analyzed by one-way ANOVA followed by Bonferroni post hoc analysis.

[a] $P < 0.001$ as compared with haloperidol group.

[b] $P < 0.01$ as compared with haloperidol group.

[c] $P < 0.001$ as compared with control group.

Poor solubility and poor permeability are among the main causes for failure during drug development [56,57]. It is therefore important to determine these physicochemical properties associated with a drug, before synthetic work is undertaken. The estimated log P values (P is the partition coefficient of the molecule in the water/octanol system), which can be used as an indicator of passive diffusion across cell membranes and cellular uptake [58–60], were determined for all urea and thiourea derivatives and are presented in Table 1. The lipophilicity study showed that most of compounds possess optimum lipophilicities (log P 1.64–4.47) required for oral absorption and biomembrane penetration, even for BBB penetration according to Lipinski's drug-likeness 'rules of five' [61]. This bioavailability feature makes it possible to use these compounds in treatment of neurodegenerative diseases. A precise correlation between lipophilicity and biological activity was not observed which reflects that the lipophilicity has an influence on the activity, but it does not solely determine the antiparkinsonian activity of these compounds.

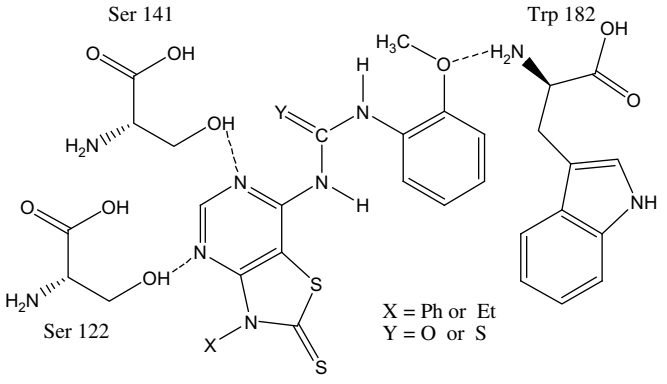


Fig. 2. Proposed binding interactions of the title compounds at the receptor site. Dashed lines indicate H-bond.

Table 3
Biochemical estimation from brain homogenate.

Compound	LPO (nmol MDA/mg protein)	GSH (μg/gm tissue)	GSH-Px (U/g protein)	SOD (U/mg protein)
5a	3.201 ± 0.47[a]	14.362 ± 2.34[c]	1.134 ± 0.1[a]	1.992 ± 0.11[a]
5b	2.806 ± 0.39[a]	13.981 ± 1.71[a]	1.544 ± 0.08[a]	1.815 ± 0.12[a]
14a	2.746 ± 0.14[a]	14.156 ± 1.18[a]	1.597 ± 0.14[a]	2.345 ± 0.09[a]
14b	3.123 ± 0.38[c]	11.672 ± 0.97[a]	1.331 ± 0.24[a]	1.866 ± 0.14[a]
Haloperidol	5.521 ± 0.23[b]	6.753 ± 0.15[b]	0.765 ± 0.13[b]	0.941 ± 0.15[b]
Control	0.958 ± 0.08	24.74 ± 0.29	1.893 ± 0.12	2.327 ± 0.06

The data are expressed as mean ± S.E.M (n = 5).

[a] $P < 0.001$ compared with corresponding value for haloperidol-treated mice.

[b] $P < 0.001$ compared with corresponding value for control mice.

[c] $P < 0.01$ compared with corresponding value for haloperidol-treated mice.

4. Conclusions

Finally, we have recognized a novel series of 3-phenyl/ethyl-2-thioxo-2,3-dihydrothiazolo[4,5-*d*]pyrimidin-7-yl urea/thiourea derivatives that reduced haloperidol-induced catalepsy and oxidative stress in an animal model of PD which could be important for future development of new antiparkinsonian drugs or it could as well be an important treatment armamentarium for PD.

5. Experimental protocols

5.1. Chemistry

Synthetic starting material, reagents and solvents were of analytical reagent grade or of the highest quality commercially available and were purchased from Aldrich Chemical Co., Merck Chemical Co. and were dried when necessary. The progress of the reactions was monitored by thin layer chromatography with F_{254} silica-gel precoated sheets (Merck) using chloroform/methanol 95/5 as eluent; UV light and iodine vapours were used for detection. IR spectra were recorded, as KBr pellets, on a Shimadzu 8201 PC FT-IR spectrophotometer and wave numbers are given in cm^{-1}. The mass spectra were recorded on Jeol SX-102 (FAB). ^1H NMR spectra, in DMSO-d_6 and $CDCl_3$ solutions, were recorded on a Bruker DRX-300 instrument at 298 K. Chemical shifts are reported as ppm relative to TMS as internal standard. Melting points (°C) were determined with an open glass capillary tube and are uncorrected. Elemental analyses were performed on Elementar Vario EL III instrument.

5.1.1. Synthesis of 4-amino-3-phenyl-2-thioxo-2,3-dihydrothiazol-5-carbonitrile (**1a**)

To phenyl isothiocyanate (0.05 mol) in 50 ml DMF was added finely divided sulfur (0.05 mol) and malononitrile (0.05 mol). Triethylamine (0.1 mol) was added dropwise with constant stirring to the above mixture at room temperature and the stirring was continued for 3 h. The deep red thick solution so obtained was poured into a mixture of 90 ml ice-cold water and 10 ml methanol. The colorless solid so obtained was filtered, washed with water, dried and recrystallized from methanol. Yield 46%; m.p. 263 °C; IR (KBr) υ_{max} 3370, 3300, 3229, 3184, 2206, 1637, 1580, 1437, 1244, 1037, 722 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.78 (s, 2H, NH_2, D_2O exchangeable), 6.54–7.28 (m, 5H, ArH); MS (FAB) *m*/*z* 234 $(M+1)^+$.

5.1.2. Synthesis of 4-amino-3-ethyl-2-thioxo-2,3-dihydrothiazol-5-carbonitrile (**1b**)

To ethyl isothiocyanate (0.05 mol) in 50 ml DMF was added finely divided sulfur (0.05 mol) and malononitrile (0.05 mol). Triethylamine (0.1 mol) was added dropwise with constant stirring to the above mixture at room temperature and the stirring was continued for 3 h. The deep red thick solution so obtained was poured into a mixture of 90 ml ice-cold water and 10 ml methanol. The colorless solid so obtained was filtered, washed with water, dried and recrystallized from methanol. Yield 39%; m.p. 168 °C; IR (KBr) υ_{max} 3308, 3225, 3184, 2972, 2207, 1644, 1576, 1466, 1435, 1341, 1238, 1118, 1015, 804 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.76 (t, 3H, CH_3), 2.74 (q, 2H, CH_2), 3.92 (s, 2H, NH_2, D_2O exchangeable); MS (FAB) *m*/*z* 186 $(M+1)^+$.

5.1.3. Synthesis of 7-amino-3-phenylthiazolo[4,5-d]pyrimidin-2(3H)-thione (**2a**)

A mixture of **1a** (0.01 mol), formamide (40 ml) and formic acid (10 ml) was heated at 110 °C for 6 h, cooled, poured onto ice-water to give precipitates, which were filtered off, dried and recrystallized from ethanol to afford **2a**. Yield 72%; m.p. 204–206 °C; IR (KBr) υ_{max} 3435, 3049, 2927, 2842, 1666, 1587, 1539, 1420, 1256, 1090, 882, 716 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.12 (br s, 2H, NH_2, D_2O exchangeable), 6.7–7.56 (m, 5H, ArH), 8.52 (s, 1H, CH pyrimidine); MS (FAB) *m*/*z* 259 $(M+1)^+$.

5.1.4. Synthesis of 7-amino-3-ethylthiazolo[4,5-d]pyrimidin-2(3H)-thione (**2b**)

A mixture of **2a** (0.01 mol), formamide (40 ml) and formic acid (10 ml) was heated at 110 °C for 6 h, cooled, poured onto ice-water to give precipitates, which were filtered off, dried and recrystallized from ethanol to afford **2b**. Yield 64%; m.p. 181–183 °C; IR (KBr) υ_{max} 3403, 3151, 2952, 2834, 1665, 1586, 1540, 1472, 1450, 1256, 1121, 1068, 892, 767, 699 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.34 (t, 3H, CH_3), 3.61 (q, 2H, CH_2), 4.74 (s, 2H, NH_2, D_2O exchangeable), 9.44 (s, 1H, CH pyrimidine); MS (FAB) *m*/*z* 213 $(M+1)^+$.

5.1.5. General method for the synthesis of urea and thiourea derivatives of 3-phenyl/ethyl-2-thioxo-2,3-dihydrothiazolo[4,5-d]pyrimidine (**3a**,**b**–**20a**,**b**)

To a solution of **2a** or **2b** (0.002 mol) in acetonitrile (20 ml), an appropriate isocyanate or isothiocyanate (0.0024 mol) in acetonitrile (5 ml) was added dropwise with stirring at room temperature. The mixture was refluxed with stirring for 1–4 h until the completion of the reaction and the resultant precipitate was filtered, washed with acetonitrile, dried and recrystallized from 95% ethanol. The physical data of the compounds are presented in Table 1. The spectral data of the compounds are as follows.

5.1.5.1. 1-Phenyl-3-(3-phenyl-2-thioxo-2,3-dihydrothiazolo[4,5-d]pyrimidin-7-yl)urea (**3a**).

IR (KBr) υ_{max} 3360, 3170, 3029, 2928, 1700, 1608, 1547, 1188 cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.69–7.13 (m, 10H, ArH), 8.48 (s, 1H, CH pyrimidine), 9.63 (s, 1H, NH), 10.51 (s, 1H, NH); MS (FAB) *m*/*z* 380 $(M+1)^+$.

5.1.5.2. 1-(4-Methoxyphenyl)-3-(3-phenyl-2-thioxo-2,3-dihydrothiazolo[4,5-d]pyrimidin-7-yl)urea (**4a**).

IR (KBr) υ_{max} 3378, 3206, 3092, 2957, 1715, 1602, 1532, 1507, 1389, 1223, 790 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.66 (s, 3H, OCH_3), 6.46–7.64 (m, 9H, ArH), 8.71 (s, 1H, CH pyrimidine), 8.95 (s, 1H, NH), 9.98 (s, 1H, NH); MS (FAB) *m*/*z* 410 $(M+1)^+$.

5.1.5.3. 1-(2-Methoxyphenyl)-3-(3-phenyl-2-thioxo-2,3-dihydrothiazolo[4,5-d]pyrimidin-7-yl)urea (**5a**).

IR (KBr) υ_{max} 3392, 3235, 3082, 3001, 2876, 1701, 1577, 1542, 1376, 1134, 796 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.89 (s, 3H, OCH_3), 6.74–7.54 (m, 8H, ArH), 8.65 (d, 1H, $J = 7.4$ Hz), 8.96 (s, 1H, CH pyrimidine), 9.92 (br s, 1H, NH), 11.13 (s, 1H, NH); MS (FAB) *m*/*z* 410 $(M+1)^+$.

5.1.5.4. 1-(2-Fluorophenyl)-3-(3-phenyl-2-thioxo-2,3-dihydrothiazolo-[4,5-d]pyrimidin-7-yl) urea (**6a**).

IR (KBr) υ_{max} 3355, 3253, 3164, 2985, 1689, 1600, 1532, 1247, 804 cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.72–7.24 (m, 7H, ArH), 8.36 (d, 1H, $J = 7.42$ Hz), 8.74 (d, 1H, $J = 7.66$ Hz), 8.96 (s, 1H, CH pyrimidine), 9.38 (s, 1H, NH), 10.46 (br s, 1H, NH); MS (FAB) *m*/*z* 398 $(M+1)^+$.

5.1.5.5. 1-(4-Fluorophenyl)-3-(3-phenyl-2-thioxo-2,3-dihydrothiazolo-[4,5-d]pyrimidin-7-yl) urea (**7a**).

IR (KBr) υ_{max} 3408, 3251, 3166, 2990, 1699, 1613, 1533, 1248, 804 cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.46–7.44 (m, 9H, ArH), 8.32 (s, 1H, CH pyrimidine), 8.96 (s, 1H, NH), 11.32 (s, 1H, NH); MS (FAB) *m*/*z* 398 $(M+1)^+$.

5.1.5.6. 1-(2-Chlorophenyl)-3-(3-phenyl-2-thioxo-2,3-dihydrothiazolo-[4,5-d]pyrimidin-7-yl) urea (**8a**).

IR (KBr) υ_{max} 3474, 3259, 3130, 2854, 1676, 1396, 790 cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.61–7.58 (m, 9H, ArH), 8.10 (s, 1H, CH pyrimidine), 8.82 (s, 1H, NH), 10.50 (s, 1H, NH); MS (FAB) *m*/*z* 414 $(M+1)^+$.

*5.1.5.7. 1-(4-Chlorophenyl)-3-(3-phenyl-2-thioxo-2,3-dihydrothiazolo-[4,5-*d*]pyrimidin-7-yl) urea (**9a**).* IR (KBr) υ_{max} 3372, 3091, 2983, 1703, 1584, 1541, 1377, 1256, 1142, 1085, 818, 790 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.13–8.12 (m, 9H, ArH), 8.65 (s, 1H, CH pyrimidine), 9.36 (s, 1H, NH), 10.84 (s, 1H, NH); MS (FAB) *m/z* 414 $(M+1)^+$.

*5.1.5.8. 1-(2-Nitrophenyl)-3-(3-phenyl-2-thioxo-2,3-dihydrothiazolo-[4,5-*d*]pyrimidin-7-yl) urea (**10a**).* IR (KBr) υ_{max} 3352, 3231, 1690, 1583, 1344, 1152 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.28–8.36 (m, 9H, ArH), 8.98 (s, 1H, CH pyrimidine), 9.59 (s, 1H, NH), 11.26 (s, 1H, NH); MS (FAB) *m/z* 425 $(M+1)^+$.

*5.1.5.9. 1-(4-Nitrophenyl)-3-(3-phenyl-2-thioxo-2,3-dihydrothiazolo-[4,5-*d*]pyrimidin-7-yl) urea (**11a**).* IR (KBr) υ_{max} 3340, 3215, 3156, 1587, 1363, 1180, 768 cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.4–7.2 (m, 7H, ArH), 8.3 (d, 1H, $J = 7.0$ Hz), 8.76 (d, 1H, $J = 7.31$ Hz), 9.25 (s, 1H, CH pyrimidine), 9.98 (s, 1H, NH), 10.86 (s, 1H, NH); MS (FAB) *m/z* 425 $(M+1)^+$.

*5.1.5.10. 1-Phenyl-3-(3-phenyl-2-thioxo-2,3-dihydrothiazolo[4,5-*d*]-pyrimidin-7-yl)thiourea (**12a**).* IR (KBr) υ_{max} 3406, 3259, 3130, 1674, 1365, 1199 cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.77–7.63 (m, 10H, ArH), 7.81 (s, 1H, CH pyrimidine), 8.24 (s, 1H, NH), 9.33 (s, 1H, NH); MS (FAB) *m/z* 396 $(M+1)^+$.

*5.1.5.11. 1-(4-Methoxyphenyl)-3-(3-phenyl-2-thioxo-2,3-dihydrothiazolo[4,5-*d*]pyrimidin-7-yl)thiourea (**13a**).* IR (KBr) υ_{max} 3360, 3308, 3200, 1605, 1360, 1170 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.26 (s, 3H, OCH_3), 6.35–7.01 (m, 9H, ArH), 7.66 (s, 1H, CH pyrimidine), 8.22 (s, 1H, NH), 8.95 (s, 1H, NH); MS (FAB) *m/z* 426 $(M+1)^+$.

*5.1.5.12. 1-(2-Methoxyphenyl)-3-(3-phenyl-2-thioxo-2,3-dihydrothiazolo[4,5-*d*]pyrimidin-7-yl)thiourea (**14a**).* IR (KBr) υ_{max} 3440, 3325, 3200, 1585, 1360, 1170 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.94 (s, 3H, OCH_3), 6.85–7.81 (m, 9H, ArH), 8.79 (s, 1H, CH pyrimidine), 9.5 (s, 1H, NH), 10.91 (s, 1H, NH); MS (FAB) *m/z* 426 $(M+1)^+$.

*5.1.5.13. 1-(2-Fluorophenyl)-3-(3-phenyl-2-thioxo-2,3-dihydrothiazolo-[4,5-*d*]pyrimidin-7-yl) thiourea (**15a**).* IR (KBr) υ_{max} 3313, 3206, 3081, 1691, 1587, 1480, 1364, 1276, 1218, 953, 820 cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.74 (d, 1H, $J = 7.14$ Hz), 6.96 (d, 1H, $J = 7.22$ Hz), 7.48–7.85 (m, 7H, ArH), 8.84 (s, 1H, CH pyrimidine), 10.52 (s, 1H, NH), 11.23 (s, 1H, NH); MS (FAB) *m/z* 414 $(M+1)^+$.

*5.1.5.14. 1-(4-Fluorophenyl)-3-(3-phenyl-2-thioxo-2,3-dihydrothiazolo-[4,5-*d*]pyrimidin-7-yl) thiourea (**16a**).* IR (KBr) υ_{max} 3300, 3190, 3011, 1590, 1360, 1220, 687 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.42–7.91 (m, 9H, ArH), 9.15 (s, 1H, CH pyrimidine), 10.32 (br s, 1H, NH), 10.88 (s, 1H, NH); MS (FAB) *m/z* 414 $(M+1)^+$.

*5.1.5.15. 1-(2-Chlorophenyl)-3-(3-phenyl-2-thioxo-2,3-dihydrothiazolo-[4,5-*d*]pyrimidin-7-yl) thiourea (**17a**).* IR (KBr) υ_{max} 3235, 3161, 2992, 1621, 1509, 682 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.27–7.88 (m, 9H, ArH), 8.93 (s, 1H, CH pyrimidine), 9.31 (s, 1H, NH), 9.86 (br s, 1H, NH); MS (FAB) *m/z* 431 $(M+1)^+$.

*5.1.5.16. 1-(4-Chlorophenyl)-3-(3-phenyl-2-thioxo-2,3-dihydrothiazolo-[4,5-*d*]pyrimidin-7-yl) thiourea (**18a**).* IR (KBr) υ_{max} 3221, 3023, 2998, 1614, 1522, 691 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.71–7.85 (m, 9H, ArH), 9.01 (s, 1H, CH pyrimidine), 9.55 (s, 1H, NH), 10.22 (s, 1H, NH); MS (FAB) *m/z* 431 $(M+1)^+$.

*5.1.5.17. 1-(2-Nitrophenyl)-3-(3-phenyl-2-thioxo-2,3-dihydrothiazolo-[4,5-*d*]pyrimidin-7-yl) thiourea (**19a**).* IR (KBr) υ_{max} 3288, 3219, 3137, 3009, 1617, 1511, 682 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.22–8.09 (m, 9H, ArH), 8.57 (s, 1H, CH pyrimidine), 8.97 (s, 1H, NH), 11.01 (s, 1H, NH); MS (FAB) *m/z* 441 $(M+1)^+$.

*5.1.5.18. 1-(4-Nitrophenyl)-3-(3-phenyl-2-thioxo-2,3-dihydrothiazolo-[4,5-*d*]pyrimidin-7-yl) thiourea (**20a**).* IR (KBr) υ_{max} 3316, 3203, 2972, 1701, 1588, 1575, 1334, 1160, 805 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.38–7.91 (m, 9H, ArH), 8.80 (s, 1H, CH pyrimidine), 10.11 (br s, 1H, NH), 11.43 (s, 1H, NH); MS (FAB) *m/z* 441 $(M+1)^+$.

*5.1.5.19. 1-(3-Ethyl-2-thioxo-2,3-dihydrothiazolo[4,5-*d*]pyrimidin-7-yl)-3-phenylurea (**3b**).* IR (KBr) υ_{max} 3346, 3252, 2991, 1608, 1521, 692 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.01 (t, 3H, CH_3), 3.12 (q, 2H, CH_2), 6.98–7.66 (m, 5H, ArH), 8.82 (s, 1H, CH pyrimidine), 9.43 (s, 1H, NH), 10.67 (br s, 1H, NH); MS (FAB) *m/z* 332 $(M+1)^+$.

*5.1.5.20. 1-(3-Ethyl-2-thioxo-2,3-dihydrothiazolo[4,5-*d*]pyrimidin-7-yl)-3-(4-methoxyphenyl) urea (**4b**).* IR (KBr) υ_{max} 3279, 3102, 3078, 2998, 2937, 1701, 1547, 1364, 1243, 732 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.39 (t, 3H, CH_3), 2.78 (q, 2H, CH_2), 3.74 (s, 3H, OCH_3), 7.00–7.59 (m, 4H, ArH), 8.48 (s, 1H, CH pyrimidine), 9.39 (br s, 1H, NH), 10.47 (s, 1H, NH); MS (FAB) *m/z* 362 $(M+1)^+$.

*5.1.5.21. 1-(3-Ethyl-2-thioxo-2,3-dihydrothiazolo[4,5-*d*]pyrimidin-7-yl)-3-(2-methoxyphenyl) urea (**5b**).* IR (KBr) υ_{max} 3319, 3207, 3088, 2945, 1699, 1584, 1541, 1377, 1012, 734 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.17 (t, 3H, CH_3), 2.68 (q, 2H, CH_2), 3.89 (s, 3H, OCH_3), 7.34–7.79 (m, 4H, ArH), 8.91 (s, 1H, CH pyrimidine), 9.01 (br s, 1H, NH), 10.33 (s, 1H, NH); MS (FAB) *m/z* 362 $(M+1)^+$.

*5.1.5.22. 1-(3-Ethyl-2-thioxo-2,3-dihydrothiazolo[4,5-*d*]pyrimidin-7-yl)-3-(2-fluorophenyl) urea (**6b**).* IR (KBr) υ_{max} 3220, 3110, 2983, 2894, 1680, 1350, 1050 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.22 (t, 3H, CH_3), 1.46 (q, 2H, CH_2), 6.72 (1H, d, $J = 7.9$ Hz), 7.08 (2H, d, $J = 8.2$ Hz), 7.51 (1H, dd, $J = 1.6$, 8.3 Hz), 7.76 (s, 1H, CH pyrimidine), 7.95 (s, 1H, NH), 9.81 (s, 1H, NH); MS (FAB) *m/z* 350 $(M+1)^+$.

*5.1.5.23. 1-(3-Ethyl-2-thioxo-2,3-dihydrothiazolo[4,5-*d*]pyrimidin-7-yl)-3-(4-fluorophenyl) urea (**7b**).* IR (KBr) υ_{max} 3250, 3120, 3100, 3080, 1245, 1350, 1070 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.39 (t, 3H, CH_3), 1.74 (q, 2H, CH_2), 6.97 (2H, d, $J = 7.7$ Hz), 7.88 (1H, d, $J = 7.6$ Hz), 8.32 (s, 1H, CH pyrimidine), 9.95 (br s, 1H, NH), 11.25 (s, 1H, NH); MS (FAB) *m/z* 350 $(M+1)^+$.

*5.1.5.24. 1-(2-Chlorophenyl)-3-(3-ethyl-2-thioxo-2,3-dihydrothiazolo-[4,5-*d*]pyrimidin-7-yl) urea (**8b**).* IR (KBr) υ_{max} 3367, 3288, 3195, 3071, 2962, 1689, 1585–1277, 1375, 1018, 800 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.01 (t, 3H, CH_3), 1.8 (q, 2H, CH_2), 6.64–7.62 (m, 4H, ArH), 8.93 (s, 1H, CH pyrimidine), 10.68 (s, 1H, NH), 11.56 (s, 1H, NH); MS (FAB) *m/z* 366 $(M+1)^+$.

*5.1.5.25. 1-(4-Chlorophenyl)-3-(3-ethyl-2-thioxo-2,3-dihydrothiazolo-[4,5-*d*]pyrimidin-7-yl) urea (**9b**).* IR (KBr) υ_{max} 3356, 3329, 3189, 3045, 1702, 1541, 1266, 1154, 834, cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.62 (t, 3H, CH_3), 1.95 (q, 2H, CH_2), 6.44–7.12 (m, 4H, ArH), 7.96 (s, 1H, CH pyrimidine), 8.30 (s, 1H, NH), 10.93 (s, 1H, NH); MS (FAB) *m/z* 366 $(M+1)^+$.

*5.1.5.26. 1-(3-Ethyl-2-thioxo-2,3-dihydrothiazolo[4,5-*d*]pyrimidin-7-yl)-3-(2-nitrophenyl) urea (**10b**).* IR (KBr) υ_{max} 3317, 3238, 3136, 1699, 1580, 1540, 1287, 1150, 756 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.27 (t, 3H, CH_3), 2.3 (q, 2H, CH_2), 6.86–7.72 (m, 4H, ArH), 7.89 (1H, d, $J = 7.5$ Hz), 9.79 (s, 1H, NH), 11.85 (s, 1H, NH); MS (FAB) *m/z* 377 $(M+1)^+$.

*5.1.5.27. 1-(3-Ethyl-2-thioxo-2,3-dihydrothiazolo[4,5-*d*]pyrimidin-7-yl)-3-(4-nitrophenyl) urea (**11b**).* IR (KBr) υ_{max} 3330, 3260, 3150,

3150, 1251, 1350, 1071 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.67 (t, 3H, CH_3), 2.94 (q, 2H, CH_2), 7.22–8.03 (m, 5H, ArH + CH pyrimidine), 10.37 (br s, 1H, NH), 11.34 (s, 1H, NH); MS (FAB) m/z 377 $(M+1)^+$.

*5.1.5.28. 1-(3-Ethyl-2-thioxo-2,3-dihydrothiazolo[4,5-*d*]pyrimidin-7-yl)-3-phenylthiourea (**12b**).* IR (KBr) υ_{max} 3298, 3182, 3076, 2960, 1692, 1580, 1363, 1336, 792, 752 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.85 (t, 3H, CH_3), 3.22 (q, 2H, CH_2), 6.98–8.19 (m, 6H, ArH + CH pyrimidine), 9.78 (s, 1H, NH), 10.59 (s, 1H, NH); MS (FAB) m/z 348 $(M+1)^+$.

*5.1.5.29. 1-(3-Ethyl-2-thioxo-2,3-dihydrothiazolo[4,5-*d*]pyrimidin-7-yl)-3-(4-methoxyphenyl) thiourea (**13b**).* IR (KBr) υ_{max} 3372, 3205, 3093, 2928, 1703, 1607, 1542, 1236, 806 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.13 (t, 3H, CH_3), 2.28 (q, 2H, CH_2), 3.37 (s, 3H, OCH_3), 6.83–7.61 (m, 4H, ArH), 7.93 (s, 1H, CH pyrimidine), 8.42 (s, 1H, NH), 10.61 (s, 1H, NH); MS (FAB) m/z 378 $(M+1)^+$.

*5.1.5.30. 1-(3-Ethyl-2-thioxo-2,3-dihydrothiazolo[4,5-*d*]pyrimidin-7-yl)-3-(2-methoxyphenyl) thiourea (**14b**).* IR (KBr) υ_{max} 3378, 3200, 3082, 2974, 1698, 1578, 1362, 1270, 1018, 804, 748 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.27 (t, 3H, CH_3), 2.74 (q, 2H, CH_2), 3.96 (s, 3H, OCH_3), 6.89–7.76 (m, 5H, ArH + CH pyrimidine), 9.36 (s, 1H, NH), 10.65 (s, 1H, NH); MS (FAB) m/z 378 $(M+1)^+$.

5.2. Pharmacological evaluation

All the compounds were screened for antiparkinsonian activity in haloperidol-induced catalepsy test in mice. The results obtained are summarized in Table 2.

5.2.1. Animals and drugs

Adult male pathogen-free Swiss albino mice weighing 18–25 g were used. All animal experimentation was conducted in accordance with the Animal Ethics Committee of the Institute. The procedures adhered to the NIH Guidelines for the Care and Use of Laboratory Animals. Levodopa (Sigma–Aldrich) was injected i.p. in dose of 100 mg/kg. Haloperidol (Sigma–Aldrich) was administered in a dose of 5 mg/kg i.p. Synthesized compounds **3a**–**20a** and **3b**–**14b** were administered at 100 mg/kg i.p. All the drugs and synthesized compounds were suspended in 0.5% gum acacia in redistilled water and administered at a volume of 0.1 ml/100 g.

5.2.2. Haloperidol-induced catalepsy test

Haloperidol-induced catalepsy was measured with the standard bar test [40], in a wooden chamber (length, 23 cm; width, 10.5 cm; height, 9 cm) with a horizontal metal bar (diameter, 0.4 cm; length, 10.5 cm) fixed at 9 cm above the floor, and at 4 cm from the back of the box. All experiments were carried out between 8:00 and 15:30 h in a room with controlled temperature (23 ± 1 °C), and light intensity of 20 lux. Five mice were taken in each group. Control animals received 0.5% gum acacia in redistilled water. Synthesized compounds and levodopa were injected intraperitoneally at 100 mg/kg, 30 min prior to haloperidol injection. Animals were used only once. Catalepsy was measured every 30 min during the whole session that lasted 3 h after haloperidol injection. To assess whether the repeated handling of animals could have any influence on catalepsy intensity over time [40], the bar test was performed in groups of mice that were injected only with the vehicle in which haloperidol was dissolved.

To measure catalepsy, the mouse was gently lifted until its forepaws firmly grasped the metal bar. Then, the mouse body was released and simultaneously a stopwatch was started. The time elapsed until the animal released both forepaws from the bar, up to a maximum of 300 s, was defined as the descent time. The sum of the descent time values measured every 30 min during the 3 h after haloperidol or vehicle was defined as the cumulative descent time (CDT [3 h]). The mean CDTs measured in animals treated by the vehicle in which haloperidol was dissolved were subtracted from the mean CDTs recorded in mice treated with haloperidol. This difference was taken as 100% of catalepsy, and served as a reference value to calculate the percent inhibition of drugs on catalepsy intensity.

5.3. Biochemical evaluation

Mice were sacrificed by decapitation 3 h after the last injection. The brains were quickly removed and were washed twice with ice-cold saline solution, placed into glass bottles, labeled, and stored in a deep freezer (−30 °C) until processing (maximum 10 h). Tissues were homogenized in four volumes of ice-cold Tris–HCl buffer (50 mM, pH 7.4) using a glass Teflon homogenizer (Ultra Turrax IKA T18 Basic, USA) after cutting up the brain into small pieces with scissors (for 2 min at 5000 rpm). Malondialdehyde (MDA) and protein levels were carried out at this stage. The homogenate was then centrifuged at 10,500 × *g* for 20 min to remove nuclear debris. Clear supernatant fluid was taken and GSH-Px activity was carried out in this stage. The supernatant solution was then extracted with an equal volume of an ethanol/chloroform mixture (5:3, v/v). After centrifugation at 5000 × *g* for 30 min, the clear upper layer (the ethanol phase) was taken and used in the SOD activity. All preparation procedures were performed at +4 °C.

5.3.1. LPO assay

The extent of LPO in brain homogenate was determined by measuring the release of thiobarbituric acid reactive substance (TBARS) in terms of MDA equivalent using a molar extinction coefficient of 1.56×10^5/min/cm as described by Ohkawa et al. [62]. Briefly, the homogenate was centrifuged at 3000 × *g* for 15 min and supernatant was used for assay. Samples of 0.1 ml homogenate were mixed with 0.2 ml of 8.1% SDS, 1.5 ml 20% glacial acetic acid and 1.5 ml of 0.8% thiobarbituric acid (TBA). Following these additions, tubes were mixed and heated at 95 °C for 1 h on a water bath and cooled under tap water before mixing 1 ml of distilled water and 5 ml mixture of *n*-butanol and pyridine (15:1). The mixture was centrifuged at 2200 *g* for 10 min. The amount of MDA formed was measured by the absorbance of upper organic layer at a wavelength of 532 nm in Perkin Elmer spectrophotometer using appropriate controls. The results are expressed as nmol MDA/mg protein.

5.3.2. GSH determination

The amount of GSH in brain was measured according to the method of Sedlak and Lindsay [63]. Briefly, brain tissue was deproteinized with an equal volume of 10% TCA and was allowed to stand at 4 °C for 2 h. The contents were centrifuged at 2000 *g* for 15 min. The supernatant was added to 2 ml of 0.4 M Tris buffer (pH 8.9) containing 0.02 M EDTA (pH 8.9) followed by the addition of 0.01 M DTNB {5,5′-dithiobis(2-nitrobenzoic acid)}. Finally, the mixture was diluted with 0.5 ml of distilled water, to make the total mixture to 3 ml and absorbance was read in a spectrophotometer at 412 nm and results are expressed as μg GSH/gm tissue.

5.3.3. SOD activity determination

Cu,Zn-SOD activity was determined according to the method of Sun et al. [64]. In this method, a xanthine–xanthine oxidase complex produces superoxide radicals, which react with nitrobluetetrazolium (NBT) to form the farmazan compound. In brief, a reactive was prepared with 0.1 mM of xanthine, 0.1 mM of EDTA, 50 mg of bovine serum albumin, 25 μM of NBT and 40 mM of

Na_2CO_3 (pH 10.2). To 2.45 ml of reactive was added 0.5 ml of an ethanol/chloroform (5:3, v/v) extract, previously prepared from brain homogenate. Subsequently, 50 μl of 9.9 nM of xanthine oxidase solution was added, the mixture was kept in a water bath of 25 °C for 20 min, and the reaction was terminated using 1 ml of $CuCl_2$. The absorbance of the samples was read at 560 nm. In the control sample the amount of the ethanol supernatant was replaced by equivalent volume of PBS buffer. One unit SOD activity was defined as the amount of enzyme causing 50% inhibition of NBT reduction to formazan. SOD activity was expressed as U/mg protein.

5.3.4. GSH-Px activity determination

The GSH-Px activity was measured by the method of Paglia and Valentine [65]. The enzymatic reaction was conducted in 3 ml quartz cuvettes of 1 cm path length in a Perkin–Elmer spectrophotometer. Each 3 ml assay volume contained 50 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA, 1 mM sodium azide, 0.2 mM NADPH, 1 U glutathione reductase and 1 mM reduced glutathione. The sample (0.2 ml of the tissue homogenate), after its addition, was allowed to equilibrate for 5 min at 25 °C. The reaction was initiated by adding 0.1 ml of 2.5 mM H_2O_2. Changes in absorbance were recorded at 340 nm for 5 min. Values were expressed as units of NADPH oxidized to NADP by using the extinction coefficient of 6.22 mM/cm at 340 nm. All samples were assayed in duplicate. GSH-Px activity was expressed as units per gram protein.

5.3.5. Total protein determinations

Total protein concentration of brain homogenates was determined by folin–phenol reaction as described by Lowry et al. [66]. Bovine serum albumin (BSA) was used as the standard.

5.4. Statistical analysis

Data were expressed as the mean ± standard error (S.E) of the means. For a statistical analysis of the data, group means were compared by one-way analysis of variance (ANOVA) with post hoc analysis. The post hoc Bonferroni multiple comparison test was applied to identify significance among groups. $P < 0.05$ was considered to be statistically significant.

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