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A new therapeutic approach in Alzheimer disease: Some novel pyrazole derivatives as dual MAO-B inhibitors and antiinflammatory analgesics

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Abstract—The increasing life expectancy in our population makes Alzheimer's disease (AD) a growing public health problem. There is a great need to find a way to prevent and delay the disease. It was shown that monoamine oxidase-B (MAO-B) inhibitors and antiinflammatory agents might be effective in treating AD. Therefore, a novel series of 1-thiocarbamoyl-3-substituted phenyl-5-(2-pyrrolyl)-4,5-dihydro-(1*H*)-pyrazole derivatives as promising MAO-B inhibitors was synthesized and investigated for the ability to inhibit selectively the activity of the A and B isoforms of monoamine oxidase (MAO). Most of the synthesized compounds showed high activity against both the MAO-A (compounds **3e**–**3h**) and the MAO-B (compounds **3i**–**3l**) isoforms. All the synthesized compounds were also tested for their in vivo antiinflammatory activity by two different bioassays namely, carrageenan-induced oedema and acetic acid-induced increase in capillary permeability in mice. In addition, analgesic and ulcerogenic activities were determined. The combined antiinflammatory data from in vivo animal models showed that compound **3k** exhibited anti-inflammatory activity comparable to that of indomethacin with no ulcerogenic effects. Since compound **3k** exhibits both antiinflammatory-analgesic activity and MAO-B inhibition, it needs further detailed studies.

1. Introduction

Alzheimer's disease (AD) is a progressive, neurodegenerative disease accounting for 60–70% of dementia cases in the elderly with no current-modifying treatment.[1] AD disease accounts for most cases of dementia that are diagnosed after the age of 60 years in life. It affects 20–30 million individuals worldwide.[2] In the US, the number reaches roughly 4 million patients. Because the prevalence of AD increases with age and life expectancy is constantly increasing in civilized countries, it has been predicted that the incidence of AD will increase threefold over the next 50 years. Epidemiological and molecular studies suggest that AD has multiple aetiologies, including genetic mutations, susceptibility genes and environmental factors such as aluminium that promotes formation and accumulation of insoluble amyloid-beta (Aβ) and hyperphosphorylated tau.[3]

Even though significant advances have been made in delineating the molecular and cellular factors contributing to the aetiology of AD, satisfactory approaches to ameliorate the symptoms or retard the degenerative course of the disease have not been developed. Currently acetylcholinesterase inhibitors are the only drugs approved for treatment of cognitive dysfunction in AD.[4,5] But acetylcholine-enhancing drugs can compensate for only part of the neuronal dysfunction in AD. Among the drugs now moving into clinical trials are several compounds that may modify the progression of

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AD. These are antiinflammatory agents, antioxidants, estrogen, antagonists of APO E4, inhibitors of amyloid-β production and aggregation and MAO-B inhibitors.[6–8] Antiinflammatory agents are being tried in AD, as the disease lesions are characterized by the presence of numerous inflammatory proteins. The extent by which inflammation contributes to the neurodegenerative process, however, is an open question. The available studies in AD are of limited value and statistical significance because of small size and insufficient design. However, the data suggest that anti-inflammatory agents might exert protective effects against AD, but only in low doses.

Nonetheless, given the failure of recent clinical trials, the potential of NSAIDs for the treatment of AD is still a matter of debate.[9] This failure could be due to a number of factors, for example, the selection of patients which already had mild to moderate AD, the pharmacological characteristics of the drugs, their brain penetration properties and the dosing schedule.

Some epidemiological studies have shown that chronic use of NSAIDs may delay or even prevent the onset of AD.[10,11] Recent studies testing non-selective NSAIDs in murine models of AD neuropathology have indicated that the frequency of Aβ plaque deposits in the brains of animals can be significantly reduced by treatment with the non-selective cyclooxygenase inhibitors.[12–16] But the long term use of NSAIDs is associated with undesirable side effects, and so novel antiinflammatory agents devoid of these effects are currently being explored.

As mentioned above, another potential therapy for AD is the use of monoamine oxidase-B (MAO-B) inhibitors.[17] Favourable results have been documented in short-term studies, but have not yet been confirmed in long-term-studies. The evaluation of selegiline and other MAO inhibitors was led by the finding that MAO-B type activity is increased in patients with AD. There are different accepted mechanisms by which MAO-B inhibitors could prevent neurodegeneration. First, they may decrease the free radical formation,[18] which causes membrane and DNA destruction, from normal metabolism of the biogenic amines by inhibition of MAO-B in the central nervous systems. Second, they exhibit protective effects against neuronal apoptosis in cell culture.[19] Third, MAO-B inhibitors were shown to reduce the secretion of neurotoxic products.[20] These are only some of the underlying mechanisms of MAO-B inhibitors in AD. Therefore, it is challenging to find an agent that has a dual effect both analgesic-antiinflammatory and MAO-B inhibition.

In the last decades, research has been directed to the preparation of heterocyclic hydrazines and hydrazides and their potentiality as therapeutic agent for the treatment of CNS depression, since iproniazide demonstrated in vitro activity.[21,22] Some authors investigated MAO inhibitory properties of 1,3,5-triphenyl-2-pyrazolines which bear a cyclic hydrazine moiety and found high activity.[23–26] These findings have pushed us to synthesize various pyrazole derivatives and examine for their different amine oxidase inhibition activities (bovine serum amine oxidase (BSAO), MAO and semicarbazide sensitive amine oxidase (SSAO)) (Fig. 1). A considerable number of the prepared compounds were found to have BSAO, SSAO and MAO inhibitory activity comparable to or higher than the reference compounds.[27–29] At the same time, some of the pyrazoles, which were presented as selective MAO-B inhibitors,[28] were found to inhibit AChE activities of human erythrocyte and plasma, approved for treatment of cognitive dysfunction in AD, selectively and non-competitively.[30]

On the other hand, during the last decades a variety of non-steroidal antiinflammatory drugs, belonging to different structural classes, were discovered and proved useful in the treatment of inflammatory diseases. Among them metamizol bearing pyrazole moiety has shown good analgesic-antiinflammatory effect. Celecoxib comprising the pyrazole nucleus has occupied a unique posi-

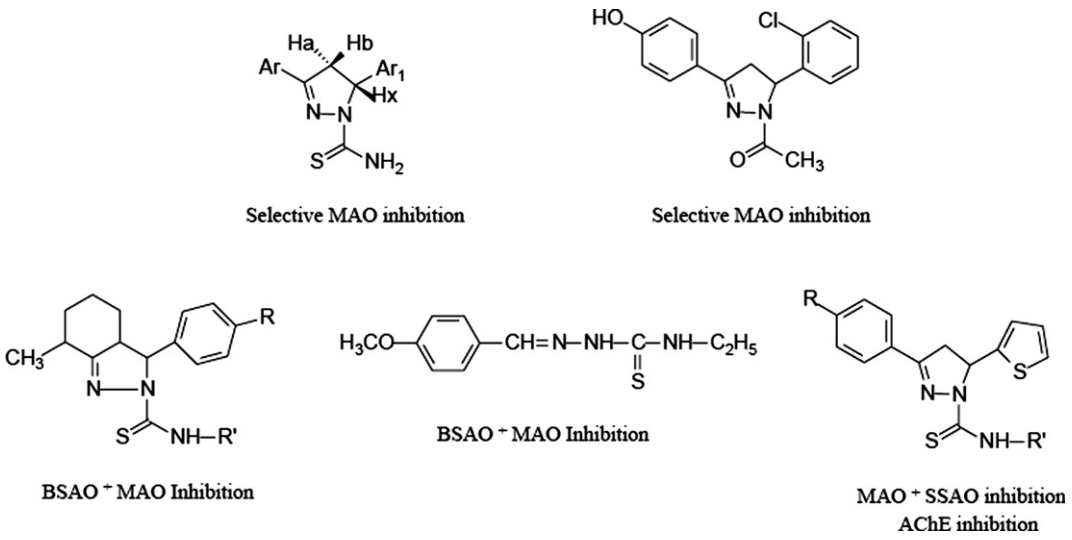


Figure 1. Pyrazole derivatives having MAO, SSAO and BSAO inhibition.

tion as a potent and gastrointestinal safe antiinflammatory and analgesic agent.

Since multiple factors contribute to the pathology of AD, a recent approach in the treatment of this disease as well as other diseases such as cancer, AIDS, cardiovascular diseases is the development of drugs with multiple actions.[31] In this regard, compounds such as pyrazoles, which possess different biochemical and pharmacological actions, may be hopeful to treat and suppress the progress of the disease.

Considering that pyrazoles are promising compounds for antiinflammatory analgesic[32–36] and MAO-B inhibitors[23–28] and in the light of afore-mentioned findings, we aimed to synthesize novel series of 1-N-substituted thiocarbamoyl-3-phenyl-5-(pyrrol-2-yl)-4,5-dihydro-(1*H*)-pyrazole derivatives in order to evaluate them as novel potential MAO-B inhibitors/antiinflammatory agents which might have promising features in the treatment of AD.

2. Results and discussion

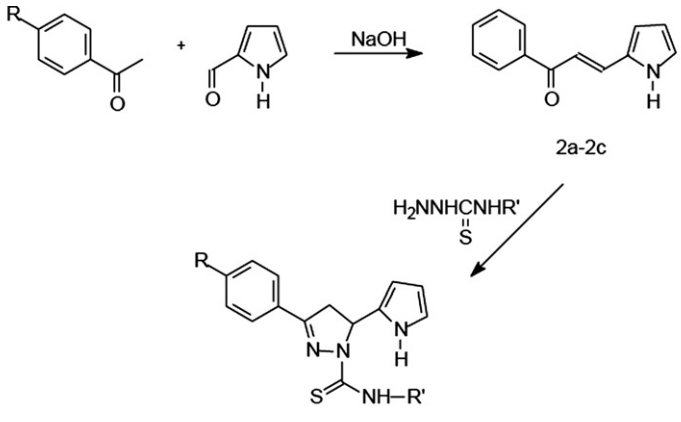
To verify the effects of structural modifications on both inhibition and selectivity towards MAO-A/MAO-B, and antiinflammatory analgesic activity, twelve new 1-N-substituted thiocarbamoyl-3-phenyl-5-(pyrrole-2-yl)-2-pyrazoline derivatives have been synthesized. In particular, the influence, on the biological behaviour such as MAO inhibition, analgesic-antiinflammatory activities, of the introduction of different aromatic rings in the 3 and 5 positions of the 4,5-dihydro-(1H)-pyrazole nucleus has been investigated.

2.1. Chemistry

The chalcones were synthesized by condensing the appropriate pyrrole-2-carboxaldehyde and 4-substituted acetophenone derivatives in alcohol in ice bath using sodium hydroxide catalyst.[37,38] This procedure gave high yields of the desired α,β-unsaturated ketone with no undesirable side reactions. The products precipitated from the reaction mixture in a relatively pure state and were easily recrystallized. 1-Phenyl-3-(pyrrole-2-yl)-2-propen-1-ones (**2a**–**2c**) were treated with 4-substituted thiosemicarbazides to obtain 1-(N-substituted thiocarbamoyl)-3-(substituted phenyl)-5-(pyrrole-2-yl)-4,5-dihydro-(1H)-pyrazole (Scheme 1).

This reaction mechanism involved in the intermediate formation of hydrazones and subsequent addition of N–H on the olephinic bond of the propenone moiety and condensation of chalcones with 4-substituted thiosemicarbazide was discussed rigorously, previously.[19]

The structure of **3a**–**3l** is inferred from spectroscopic as well as elemental analyses data. The IR spectra of the compounds **3a**–**3l** reveal absorption bands in the regions 1509–1610 cm^{-1} corresponding to C=N stretching bands because of ring closure. In the 200 MHz, ^1H NMR spectra of compounds exhibit the presence of two unequivalent protons of a methylene group (H_A/H_B) at δ 3.00–3.40 ppm, 3,72–3.80 coupled with each other and in turn with the vicinal methine proton (H-5) at δ 6.38–6.5. It has also been noticed that, the upfield shifted proton of methylene residue coupled with the vicinal methine one (H-5) with a coupling constant value J = 2.96–3.08 Hz, indicating the presence of *trans*-configuration. In other words, this mentioned proton is located *cis* to the aryl group attached to the pyrazole C-5. Single crystal X-ray diffraction of compound **3k** supports this situation. Also, the coupling constant value between the downfield shifted proton of methylene function and the vicinal methine proton (H-5) J = 10.96–11.12 Hz supports this assumption.[39,40] The difference in the chemical shift of H_A/H_B indicates that the aromatic group has a quasi-equatorial orientation in the *trans* isomer; thus the H_A lies in the diamagnetic zone



3a, R= CH_3, R'=CH_3; **3b**, R= CH_3, R'=C_2H_5; **3c**, R= CH_3, R'=C_3H_5; **3d**, R= CH_3, R'=C_6H_5; **3e**, R=Cl, R'=CH_3; **3f**, R= Cl, R'=C_2H_5; **3g**, R= Cl, R'=C_3H_5; **3h**, R= CH_3, R'=C_6H_5; **3i**, R= OCH_3, R'=CH_3; **3j**, R= OCH_3, R'=C_2H_5; **3k**, R= OCH_3, R'=C_3H_5; **3l**, R=OCH_3, R'=C_6H_5.

Scheme 1. Synthetic pathway of compounds.

of the hindered pyrrole ring, in contrast with the *cis* isomer in which H_B and the phenyl group are well separated. The protons belonging to the aromatic ring and the other aliphatic groups are observed with the expected chemical shift and integral values. In MS spectra, the fragment peaks which correspond to loss of SH, NHR′, CSNHR′ from the molecular ion are consistent with the postulated structure. Characteristic M+2 isotop peaks are observed in the mass spectra of the compounds having a halogen or a sulfur. All compounds give satisfactory elemental analysis. The view of the compound **3k** performed using ORTEP is given in Figure 2.[41] The 2-pyrazoline ring deviates significantly from planarity with the torsion angle N1–C3–C2–C1 of 18.7(2)°. The pyrazoline ring is in a distorted envelope conformation. Atoms N1, N2, C1 and C2 are almost coplanar, the C3 atom deviates from this plane with the distance of −0.316(2) Å. The bond lengths and angles of the pyrazoline ring are in agreement well with the values reported in 2-[3-phenyl-5-(*m*-chlorophenyl)-2-pyrazoline-1-yl]-3-methyl-4(3H)-quinazolinone and in 1-acetyl-5-(2-methoxyphenyl)-3-(2-methoxy-styryl)-2-pyrazoline.[42,43]

The allylthiocarbamoyl group attached to the N1 atom and the N1–N2 bond [1.400(2) Å] show a single bond character. The bond length is nearly similar to the values of 1.411(7) and 1.413(7) Å reported for 2-[3-phenyl-5-(*m*-chlorophenyl)-2-pyrazoline-1-yl]-3-methyl-4(3H)-quinazolinone.[42] In 5-benzylidene-8-ethylthiocarbamoyl-9-phenyl-7,8-diazabicyclo-[4.3.0]non-6-ene, the N1 atom also attached to the ethylthiocarbamoyl group and N1–N2 bond length is 1.408(2) Å.[44]

The methoxyphenyl and the pyrazoline ring planes are almost coplanar. The dihedral angle between two ring planes is 7.2(1)°. The pyrrole ring deviates slightly from planarity. The dihedral angle between the pyrazoline ring and the pyrrole ring is 73.4(1)°. The allyl group is perpendicular to the thiocarbamoyl group with the torsion angle C15–N4–C16–C17 of −97.8(3)°. The crystal structure is stabilized by N4–H4···N2 and N3–H5···S1 intramolecular hydrogen bonds. The crystal packing of the molecule is illustrated in Figure 3. Single crystal X-ray diffraction analysis shows that compound **3k** has the absolute (*S*)-configuration.

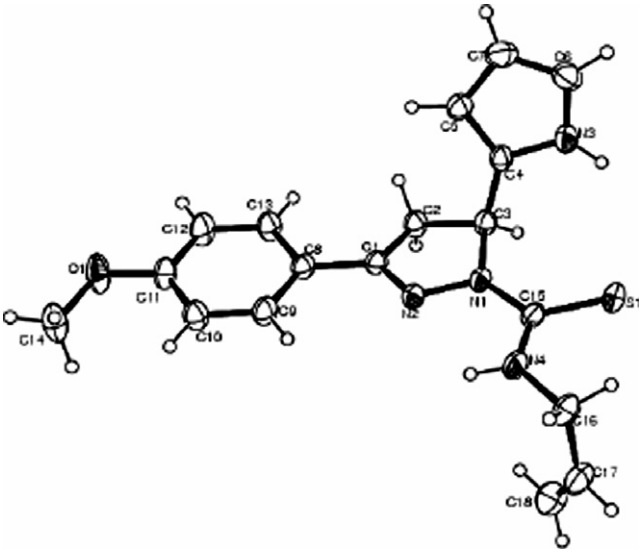


Figure 2. ORTEP drawing of the compound **3k** indicating atom numbering scheme with 30% probability. Selected intramolecular bond lengths (Å), bond angles (°) and torsion angles (°) of **3a**. S1–C15 = 1.686(2), O1–C11 = 1.366(2), C14–O1 = 1.416(3), N2–N1 = 1.400(2), N1–C3 = 1.487(2), N1–C15 = 1.347(2), N2–C1 = 1.292(2), C1–C2 = 1.497(3), C2–C3 = 1.526(3), N4–C15 = 1.341(3), C4–C3 = 1.491(3), N4–C16 = 1.450(3), C8–C1 = 1.462(3), C17–C16 = 1.478(3), C17–C18 = 1.290(4), C19–C10 = 1.466(9), C4–N3–C67 = 100.2(2), N2–C1–C2 = 113.6(2), N2–C1–C8 = 122.6(2), N4–C16–C17 = 114.4(2), C1–N2–N1 = 107.2(2), C5–C4–N3 = 106.5(2), C7–C5–C4 = 107.8(2), C5–C7–C6 = 107.6(2), C16–C17–C18 = 126.9(2), N2–N1–C15–S1 = 177.6(2), C16–N4–C15–S1 = 3.7(3), C14–O1–C11–C12 = 175.0(2), C5–C4–C3–N1 = −94.6(2), C16–N4–C15–N1 = −176.8(2), C15–N4–C16–C17 = −97.8(3).

2.2. Biochemistry

Owing to the presence of a chiral centre at the C-5 position of the pyrazole moiety and knowing that stereochemistry may be important modulator of biological activity, we intend to perform the semipreparative chromatographic enantioseparation of the most potent, selective and active chiral compounds and explain the selectivity of these enantiomers in the future.

All of the compounds (**3a**–**3l**) tested inhibited rat liver total MAO (Table 1). Compounds **3e**–**3h** inhibited rat liver MAO-A selectively while compounds **3i**–**3l** inhibited rat liver MAO-B selectively. It is worth noting that the main difference in the MAO inhibitory activities of these compounds was found when the methyl of the first aro-

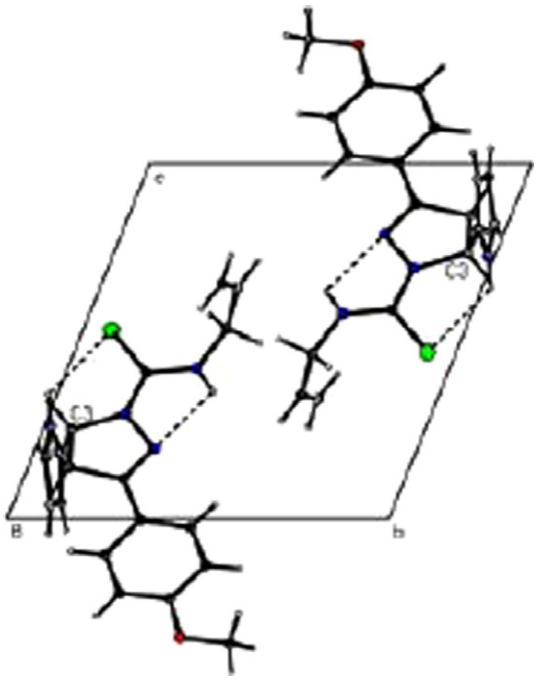


Figure 3. The hydrogen bonding geometry of the compound viewed down the *a* axis.

Table 1. IC_{50} values corresponding to the inhibition of rat liver MAO isoforms by the newly synthesized pyrazoline derivatives[a]

Compound	IC_{50} for MAO-A [b] (l M)		IC_{50} for MAO-B [b] (l M)		MAO inhibitory
	Preincubation 0	Preincubation 60 min	Preincubation 0	Preincubation 60 min	Selectivity
3a	302.19 ± 25.51	295.18 ± 19.58	349.12 ± 28.23	349.30 ± 21.25	Non-sele ctive
3b	330.35 ± 30.55	329.16 ± 30.78	380.32 ± 29.29	369.25 ± 30.26	Non-sele ctive
3c	366.12 ± 28.05	369.16 ± 30.78	390.32 ± 29.29	389.25 ± 31.55	Non-sele ctive
3d	323.19 ± 29.90	300.18 ± 28.13	345.12 ± 30.23	351.30 ± 29.04	Non-sele ctive
3e	30.10 ± 2.80	11.23 ± 1.06	450.90 ± 33.24	415.70 ± 35.44	Selective for MAO-A
3f	44.48 ± 3.23	20.18 ± 2.03	444.20 ± 38.11	450.00 ± 39.11	Selective for MAO-A
3g	67.93 ± 5.55	29.18 ± 2.17	423.19 ± 35.70	420.67 ± 37.91	Selective for MAO-A
3h	98.22 ± 8.13	59.34 ± 4.80	456.49 ± 39.22	440.60 ± 40.00	Selective for MAO-A
3i	433.56 ± 30.00	430.23 ± 29.00	29.11 9 ± 2.90	12.50 ± 1.76	Selective for MAO-B
3j	407.16 ± 30.13	400.00 ± 33.20	50.78 ± 3.60	22.13 ± 2.77	Selective for MAO-B
3k	411.70 ± 30.18	409.00 ± 30.20	58.55 ± 4.27	29.13 ± 2.56	Selective for MAO-B
3l	424.33 ± 20.15	430.93 ± 30.18	66.097 ± 5.45	30.12 ± 2.80	Selective for MAO-B
Pargyline	432.26 ± 18.60	390.20 ± 17.23	4.05 ± 0.95	2.85 ± 0.83	Selective for MAO-B
Clorgyline	4.16 ± 0.93	2.72 ± 0.98	410.15 ± 16.78	400.90 ± 15.54	Selective for MAO-A

[a] The IC_{50} values were determined from the kinetic experiments in which *p*-tyramine was used at 500 μM to measure MAO-A and 2.5 mM to measure MAO-B. Pargyline or clorgyline was added at 0.50 μM to determine the isoenzymes A and B.
[b] Each value represents the mean ± SEM of three independent experiments.

matic moiety is replaced by a chlorine atom or a methoxy group. This substitution appeared to be responsible for the remarkable differences in the inhibition activity mainly against MAO-A/MAO-B isoforms, respectively.

Compounds **3a**–**3d**, which carry a methyl group at phenyl ring, inhibited the rat liver MAO non-selectively, whereas compounds **3e**–**3h**, which carry a chloride at their phenyl ring, inhibited MAO-A selectively with the K_i values of 13.22 ± 1.28, 26.88 ± 2.30, 33.25 ± 2.80 and 60.77 ± 5.13 μM, respectively. Inhibition of liver MAO-A by these compounds was found to be non-competitive, irreversible and time-dependent. It was suggested that chlorine substitution at phenyl ring caused a selective MAO-A inhibitory activity.

Compounds **3i**–**3l**, which carry a *p*-methoxy group at their phenyl ring, appeared as selective MAO-B inhibitors with K_i values of 12.50 ± 1.76, 24.00 ± 2.18, 33.70 ± 2.98 and 37.12 ± 3.16 μM, respectively. Inhibition of liver MAO-B by these compounds was found to be non-competitive, irreversible and time-dependent. It was suggested that *p*-methoxy group on the phenyl ring increases the inhibitory effect and selectivity of the compounds towards MAO-B, probably by its electron donating effect.

In this study, we evaluated inhibitory activities of novel compounds by comparing their IC_{50} values with IC_{50} values of these known inhibitors (Table 1) (not with their K_i values) just for screening the MAO-inhibitory activities of the novel compounds. MAO was partially purified from rat liver and IC_{50} values we calculated might be different from the ones reported earlier for this reason.

However, for IC_{50} determination, the same assay procedure was applied both for known inhibitors and the novel inhibitors and IC_{50} values of newly synthesized compounds were compared with those of pargyline and clorgyline (All IC_{50} values were expressed as μM). We think that comparing the IC_{50} values of novel inhibitors with those of the known inhibitors would be meaningful for indicating their MAO-inhibitory actions.

We also determined the K_i values corresponding to the inhibitory activities of the new compounds on MAO-A and -B (K_i values were varied in 13.22–60.77 μM for MAO-A and 12.50–37.12 μM for MAO-B, whereas K_i value for pargyline was calculated as 5.3 ± 0.22 μM; and 6.1 ± 0.34 μM for clorgyline). We believe that further studies with pure enzyme will let us to determine the actual K_i values (should be lower, we believe) for the newly synthesized compounds.

These compounds were found as time-dependent inhibitors of MAO isoforms since their inhibitory activities were significantly increased parallel to the increased incubation time. The most potent inhibitory activities of these compounds were found by incubation at 37 °C for 60 min.

Non-competitive and irreversible inhibition of rat liver MAO by these derivatives suggested that these compounds cannot enter the small active site cavity of the enzyme and may interact tightly with another binding site or with some other reactive groups present in the molecule.

In this study, the use of pyrrole ring instead of thiophen moiety has given us oppurtunity to discuss ring effect which brings about sterical and electronic differences in the molecules. It seems that pyrrole ring does not contribute to the MAO inhibition because of the similar activity results published concerned with thiophen ring.[28]

This study was designed as a preliminary investigation to screen the MAO inhibitory activities of newly synthesized drugs as candidates for novel MAO inhibitors. More detailed studies supported by the pharmacological methods are needed.

Liver tissue was used to screen the MAO-inhibitory actions of these novel compounds since liver was reported to be a good source for both isoforms of the enzyme. Further studies with brain MAO isoforms (which are reported to have almost the same substrate specificities compared to liver-origined ones) by comparing their dual activities with those of rasagiline may be planned.

2.3. Pharmacology

2.3.1. Analgesic activity. The analgesic activity of the prepared pyrazolines **3a**–**3l** was determined by the *p*-benzoquinone-induced writhing in mice.[45] From the obtained results (Table 2, Fig. 4), it was noticed that most of the pyrazole derivatives showed considerable analgesic activities. Moreover, all compounds showed a remarkable gastric tolerance.

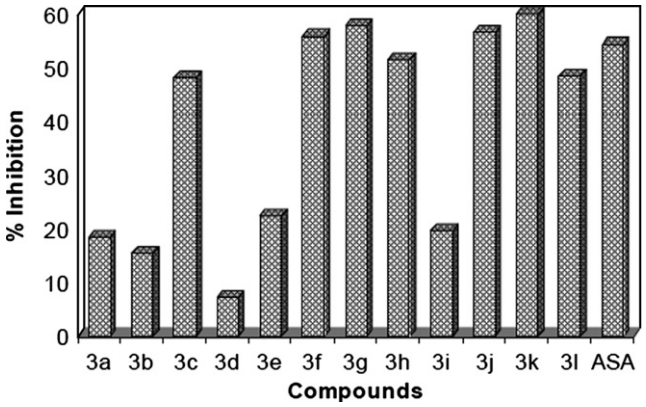


Figure 4. Analgesic effect of compounds against PBQ-induced writhings.

Some preliminary conclusions can be drawn as follows: A significant difference in analgesic activity was observed depending on the substituents on the thiocarbamoyl at the first position of the 2-pyrazoline ring. In general, the compounds bearing ethyl and especially allyl groups, except **3b**, exhibited higher activity than the others and ASA. While a notable decrease in analgesic activity was seen in compounds **3a**, **3b**, **3d** which bear methyl group on the phenyl ring at the third position of the 2-pyrazoline ring, the introduction of *p*-OCH_3 and *p*-Cl (**3f**, **3g**, **3h**, **3j**, **3k**, **3l**) into the phenyl ring dramatically increased the activity. In addition, the experimental data showed that compounds **3a**, **3e**, **3i**, which carry a methyl group on thiocarbamoyl moiety, possessed a marked drop in analgesic activity. The highest analgesic activity was observed for derivatives **3g**, **3k**, with a allyl group on thiocarbamoyl moiety; derivative **3k** is the most effective, displaying 60% inhibition value.

2.3.2. Antiinflammatory activity. The anti-inflammatory activity of the synthesized compounds was studied at 100 mg/kg dose by carrageenan-induced hind paw oedema model (Table 2, Fig. 5).[46] A quite similar pattern of activity was observed with that of analgesic activity. Although not significant, inhibitory ratios for **3c**, **3e**, **3f**, **3g**, **3h**, **3j**, **3k**, **3l** were above 15% for the third measurement. However, the inhibitory effects of these compounds reached significant values after 360 min. It was found that, 4-methoxyphenyl analogues **3i**–**3l** revealed slightly enhanced antiinflammatory properties than those of the corresponding 4-chlorophenyl derivatives **3e**–**3h** at 100 mg/kg dose level. Contrary to this, *p*-methyl series, except **3c**, showed a marked drop in antiinflammatory activity compared to the other derivatives and indomethacin. Moreover, it was also observed that ethyl, allyl derivatives linked thiocarbamoyl moiety **3f**, **3g**, **3j**, **3k** exhibited better activities than methyl, phenyl analogues linked thiocarbamoyl moiety **3e**, **3h**, **3i**, **3l**.

Table 2. Analgesic effect of compounds against PBQ-induced writhings in mice and effect of compounds against carrageenan-induced hind paw oedema model in mice

Compound	Dose (mg/kg) per os	Number of writhing ± SEM Inhibition (%)	Ratio of ulceration	Swelling in thickness (×10^{-2} mm) ± SEM (Inhibition %)			
				90 min	180 min	270 min	360 min
Control	100	48.8 ± 3.2	0/6	49.2 ± 4.6	57.2 ± 3.9	60.7 ± 2.1	69.5 ± 3.1
3a	100	39.7 ± 2.2* (18.6)	0/6	42.7 ± 4.3 (13.2)	50.8 ± 5.5 (11.2)	53.3 ± 7.3 (12.2)	58.2 ± 6.2 (16.3)
3b	100	41.3 ± 3.6 (15.4)	0/6	46.8 ± 4.2 (4.9)	51.2 ± 2.9 (10.5)	53.2 ± 3.5 (12.4)	58.2 ± 2.8*(16.3)
3c	100	25.3 ± 2.5*** (48.2)	0/6	42.0 ± 5.2 (14.6)	45.2 ± 3.5* (20.9)	51.2 ± 3.5* (15.7)	53.7 ± 3.4** (22.7)
3d	100	45.2 ± 3.6 (7.4)	0/6	44.7 ± 4.1 (9.1)	49.7 ± 6.6 (13.1)	57.0 ± 5.7 (6.1)	61.3 ± 4.6 (11.8)
3e	100	37.8 ± 4.3 (22.5)	1/6	57 ± 3.2 —	56.5 ± 1.3 (6.6)	57.8 ± 2.7 (7.2)	59.5 ± 2.9 (1.9)
3f	100	21.5 ± 1.7*** (55.9)	0/6	41.3 ± 4.0 (16.1)	45.0 ± 3.4* (21.3)	48.2 ± 4.3* (20.6)	52.8 ± 4.3* (24.0)
3g	100	20.5 ± 2.1*** (57.9)	0/6	39.8 ± 4.0 (19.8)	42.8 ± 4.2* (25.2)	46.2 ± 2.2*** (23.9)	50.0 ± 2.7*** (28.1)
3h	100	23.7 ± 1.4*** (51.4)	0/6	41.3 ± 3.9 (16.1)	44.0 ± 3.2* (23.1)	51.3 ± 3.3* (15.5)	53.5 ± 4.0** (23.0)
3i	100	39.2 ± 4.7 (19.7)	0/6	42.2 ± 3.4 (14.2)	48.8 ± 3.0 (14.7)	54.3 ± 2.7 (10.5)	57.2 ± 3.0* (17.7)
3j	100	21.2 ± 2.7*** (56.6)	0/6	37.8 ± 5.1 (23.2)	44.2 ± 4.3* (22.7)	49.2 ± 4.7* (18.9)	50.3 ± 4.9** (27.6)
3k	100	19.5 ± 1.6*** (60.0)	0/6	39.2 ± 4.0 (20.3)	43.7 ± 3.5* (23.6)	47.0 ± 2.2** (22.6)	46.7 ± 3.2*** (32.8)
3l	100	25.2 ± 2.0*** (48.4)	0/6	39.0 ± 4.4 (20.7)	44.3 ± 3.0* (22.6)	50.7 ± 2.7 (16.5)	54.5 ± 3.6* (21.6)
ASA	100	22.3 ± 2.8*** (54.3)					
Indomethacin e	100	—	2/6	33.3 ± 3.6* (32.3)	36.0 ± 2.8** (37.1)	40.3 ± 3.4*** (33.6)	40.2 ± 1.9*** (42.2)

$^{*}p < 0.05$.
$^{**}p < 0.01$.
$^{***}p < 0.001$ significant from control.

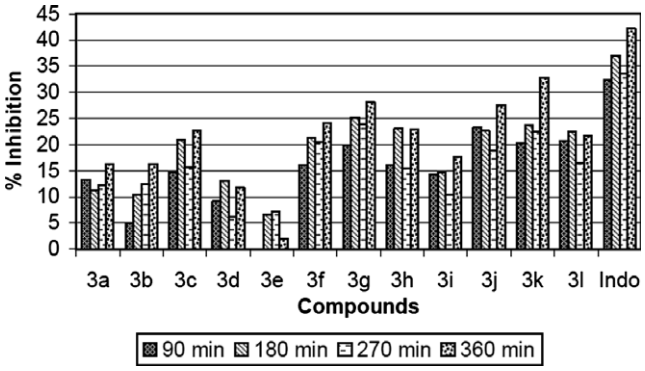


Figure 5. Effect of compounds against carrageenan-induced hind paw oedema model.

Table 3. Effect of some compounds on increased capillary permeability induced by acetic acid in mice

Compound	Dose (mg/kg) per os	Evans blue concentration ± SEM	Inhibitory ratio (%)
Control	100	7.91 ± 0.74	
3c	100	6.27 ± 0.76	20.7
3f	100	6.05 ± 0.43*	23.5
3g	100	5.68 ± 0.77	28.3
3h	100	5.82 ± 0.43*	26.4
3j	100	5.86 ± 0.84	25.9
3k	100	5.16 ± 0.54**	34.8
3l	100	6.15 ± 0.71	22.3
Indomethacine	10	4.10 ± 0.25***	48.1

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$ significant from control.

In order to confirm the anti-inflammatory activity, inhibitory effect of the compounds **3c**, **3f**, **3g**, **3h**, **3j**, **3k**, **3l**, which showed the highest inhibitory rates on PBQ-induced writhing, was further studied by using acetic acid-induced increased capillary permeability method in mice.[47] As shown in Table 3 and Figure 6, the substituents attached to the phenyl group were found to be of great importance, enhancing the observed biological activity. The best result was obtained of compound **3k**, where the phenyl residue was substituted with a methoxy moiety and allyl group on the thiocarbamoyl moiety. Therefore it deserves further attention in order to develop new leads.

2.4. Ulcerogenic liability

Antiinflammatory compounds exhibit significant ulcerogenic potential that can be demonstrated in animal models using indomethacin as a positive control. We therefore screened all compounds for ulcerogenic adverse effect. Ulceration risk was very low in our compounds on microscopic examination.

3. Conclusion

Multiple factors contribute to the pathology of AD and the disease process may involve several cellular and molecular aberrations. Therefore, the challenge is to identify compounds that will enhance cognitive function as well as will prevent, retard or halt the progression of AD. In this regard, compounds such as pyrazoles, which possess different biochemical and pharmacological actions, need further consideration in the treatment and prevention of degenerative disease. This prospect gives hope to the possibility to treat and suppress the progress of the disease. As a result, we suggest that these N-substituted pyrazole derivatives can be evaluated as both MAO-B inhibitors and antiinflammatory analgesics which may have promising features in the treatment of AD. Among the synthesized compounds, compound **3k** has possessed the most potent MAO-B inhibition and antiinflammatory activity. Therefore, compound **3k** deserves further attention in order to develop new leads in this series.

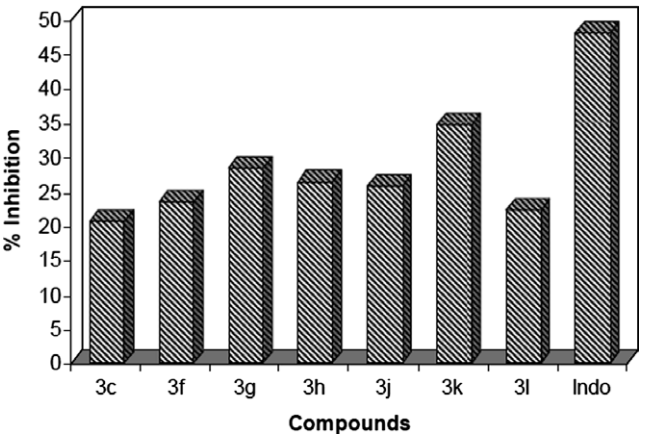


Figure 6. Effect of compounds on increased capillary permeability induced by acetic acid.

4. Experimental

4.1. Chemistry

All chemicals were supplied by E. Merck (Darmstadt, Germany), Sigma and Aldrich Chemical Co. (Steinheim, Germany). Melting points were determined on a Thomas Hoover Capillary melting point apparatus and are uncorrected. IR spectra were obtained on a Perkin-Elmer 1720X FT-IR spectrometer (KBr pellets). ^{1}H NMR spectra were recorded on a Bruker AC 200 MHz spectrometer using TMS as internal standard in $CDCl_3$. Mass spectra were measured on a Agillent 5973 with MSD detector. The $[\alpha]_D^{20}$ values (at 405 nm) were determined and calculated using a Rudolph Autopol IV Automatic Polarimeter. The purity of the compounds was checked by thin layer chromatography on silica gel coated aliminium sheets. Microanalyses of the compounds were performed on a Leco CHNS 932 analyzer at the Scientific and Technical Research Council of Turkey. All compounds gave satisfactory elemental analysis.

4.2. Preparation of 1,3-diphenyl-2-propen-1-ones (chalcones) (2a–2c) (general procedure)

Chalcone derivatives were synthesized by condensing acetophenone or 4-substituted acetophenones with

thiophen aldehyde according to the methods given before.[48,49]

4.3. Preparation of 1-thiocarbamoyl-3,5-diphenyl-2-pyrazolines (3a–3l) (general procedure)

To a solution of chalcone derivatives (0.01 mol) and substituted thiosemicarbazide (0.012 mol) in 25 ml of ethanol, a solution of sodium hydroxide (0.025 mol) in 5 ml of water was added and refluxed for 8 h. The products were poured into crushed ice and the solid mass which separated out was filtered, dried and crystallized from appropriate solvents affording the corresponding **3a**–**3l**. Yields, melting points, spectral and analytical data of synthesized compounds are given below.

4.3.1. 1-*N*-Methylthiocarbamoyl-3-(4-methylphenyl)-5-(2-pyrrolyl)-4,5-dihydro-(1H)-pyrazole. Yield 59%; mp 228–230 °C (chloroform–methanol); $[\alpha]_D^{20}$ +20 (*c* 0.2, $CHCl_3$); IR (KBr): 3366 (N–H), 2908 (C–H), 1537 (C=N), 1415 (C=C), 1353 (C=S) cm^{-1}; ^1H NMR δ (ppm): 2.41 (s; 3H; Ph–CH_3), 3.20 (d; 3H; *J*: 4.83 Hz, NH–CH_3), 3.34 (dd; 1H; H_A, J_{AB}: 17.44 Hz, J_{AX}: 3.08 Hz), 3.73 (dd; 1H; H_B, J_{AB}: 17.44 Hz, J_{BX}: 11.12 Hz), 6.40 (dd; 1H; H_X, J_{AX}: 3.08 Hz, J_{BX}: 11.12 Hz), 6.92 (t; 1H; pyrrole H^4), 7.05 (d; 1H; pyrrole H^3), 7.17 (d; 1H; pyrrole H^5), 7.26 (d; 2H; phenyl H^3,H^5), 7.45 (d; 1H; *J*: 4.83 Hz, NH), 7.66 (d; 2H; phenyl H^2,H^6), 10.45 (br; 1H; NH); MS (70 eV, EI): *m*/*z* (%): 298 ($M^{+\cdot}$, 100%), 300 ($M+2^{+\cdot}$, 10.34%), 265 (M^+–SH, 39.02%), 224 (M^+–$CSNHCH_3$, 35.83%), 166 (M^+–$C_{10}H_{11}$, 92.05%), 93 (M^+–$C_{11}H_{12}N_2S$, 16.89%). Analysis calculated for ($C_{16}H_{17}N_4S$).

4.3.2. 1-*N*-Ethylthiocarbamoyl-3-(4-methylphenyl)-5-(2-pyrrolyl)-4,5-dihydro-(1H)-pyrazole. Yield 60%; mp 163–164 °C (chloroform–methanol); $[\alpha]_D^{20}$ +23 (*c* 0.2, $CHCl_3$); IR (KBr): 3363 (N–H), 2960 (C–H), 1524 (C=N), 1459 (C=C), 1336 (C=S); ^1H NMR δ (ppm): 1.26 (t; 3H; CH_2–CH_3), 2.39 (s; 3H; Ph–CH_3), 3.30 (dd; 1H; H_A, J_{AB}: 17.44 Hz, J_{AX}: 3 Hz), 3.70 (m; 3H; H_B ve CH_2–CH_3), 6.40 (dd; 1H; H_X, J_{AX}: 3 Hz, J_{BX}: 11.04 Hz), 6.92 (dd; 1H; pyrrole H^4), 7.02 (d; 1H; pyrrole H^3), 7.16 (dd; 1H; pyrrole H^5), 7.24 (d; 2H; phenyl H^3, H^5), 7.35 (s; 1H; NH), 7.64 (d; 2H; phenyl H^2,H^6). 10.3 (br; 1H; NH); MS (70 eV, EI): *m*/*z* (%): 312 ($M^{+\cdot}$, 100%), 314 ($M+2^{+\cdot}$, 8.72%), 279 (M^+–SH, 26.33%), 224 (M^+–$CSNHC_2H_5$, 27.39%), 180 (M^+–C_9H_{10}, 57.17%), 152 (M^+–$C_{11}H_{15}N$, 24.42%), 93 (M^+–$C_{12}H_{14}N_2S$, 17.57%), 44 (M^+–$C_{15}H_{13}N_3S$, 21.98%); Analysis calculated for $C_{17}H_{19}N_4S$.

4.3.3. 1-*N*-Allylthiocarbamoyl-3-(4-methylphenyl)-5-(2-pyrrolyl)-4,5-dihydro-(1H)-pyrazole. Yield 37%; mp 164 °C (chloroform–methanol); $[\alpha]_D^{20}$ +30 (*c* 0.2, $CHCl_3$); IR (KBr): 3329 (N–H), 1509 (C=N), 1460 (C=C), 1337 (C=S); ^1H NMR δ (ppm): 2.39 (s; 3H; Ph–CH_3), 3.32 (dd; 1H; H_A, J_{AB}: 17.44 Hz, J_{AX}: 2.96 Hz), 3.73 (dd; 1H; H_B, J_{AB}: 17.44 Hz, J_{BX}: 11.04 Hz), 4.34 (m; 2H; NH–CH_2), 5.25 (dd; 2H; J_{AX}: 17.08 Hz, J_{BX}: 10.24 Hz, CH=CH_2), 5.98 (m; 1H; C*H*=CH_2), 6.38 (dd; 1H; H_X, J_{AX}: 2.96 Hz, J_{BX}: 11.04 Hz), 6.90 (dd; 1H; pyrrole H^4), 7.02 (d; 1H; pyrrole H^3), 7.16–7.15 (dd; 1H; pyrrole H^5), 7.24 (d; 2H; phenyl H^3,H^5), 7.45 (s; 1H; NH), 7.63 (d; 2H; phenyl H^2,H^6), 10.4 (br; 1H; NH); MS (70 eV, EI): 324 ($M^{+\cdot}$, 94.31%), ($M+2^{+\cdot}$, 8.27%), 291 (M^+–SH, 15.37%), 268 (M^+–NHC_3H_5, 88.88%), 224 (M^+–$CSNHC_6H_5$, 27.05%), 151 (M^+–$C_{12}H_{14}N$ 100%), 93 (M^+–$C_{13}H_{14}N_2S$, 25.51%), 41 (M^+–$C_{15}H_{14}N_4S$, 13.83%); Analysis calculated for $C_{18}H_{19}N_4S$.

4.3.4. 1-*N*-Phenylthiocarbamoyl-3-(4-methylphenyl)-5-(2-pyrrolyl)-4,5-dihydro-(1H)-pyrazole. Yield 38%; mp 170–172 °C (chloroform–methanol); $[\alpha]_D^{20}$ +28 (*c* 0.2, $CHCl_3$); IR (KBr): 3355 (N–H), 1524 (C=N), 1451 (C=C), 1346 (C=S); ^1H NMR δ (ppm): 2.38 (s; 3H; Ph–CH_3), 3.40 (dd; 1H; H_A, J_{AB}: 17.52 Hz, J_{AX}: 2.96 Hz), 3.80 (m; 3H; H_B, J_{AB}: 17.52 Hz, J_{BX}: 10.96 Hz), 6.50 (dd; 1H; H_X, J_{AX}: 2.96 Hz, J_{BX}: 10.96 Hz), 6.91 (dd; 1H; pyrrole H^4), 7.06 (d; 1H; pyrrole H^3), 7.69–7.16 (m; 10H; pyrrole H^5 ve phenyl H), 9.19 (s; 1H; NH), 10.6 (br; 1H; NH); MS (70 eV, EI): *m*/*z* (%): 360 ($M^{+\cdot}$, 70.19%), ($M+2^{+\cdot}$, 4.87%), 327 (M^+–SH, 35.07%), 268 (M^+–NHC_6H_5, 33.32%), 151 (M^+–$C_{15}H_{14}N$, 100%), 77 (M^+–$C_{15}H_{14}N_4S$, 38.63%); Analysis calculated for $C_{21}H_{19}N_4S$.

4.3.5. 1-*N*-Methylthiocarbamoyl-3-(4-chlorophenyl)-5-(2-pyrrolyl)-4,5-dihydro-(1H)-pyrazole. Yield 55%; mp 219–220 °C (chloroform–methanol); $[\alpha]_D^{20}$ +25 (*c* 0.2, $CHCl_3$); IR (KBr): 3370 (N–H), 2922 (C–H), 1528 (C=N), 1352 (C=S); ^1H NMR δ (ppm): 3.17 (d; 3H; *J*: 4.78 Hz, NH–CH_3), 3.30 (dd; 1H; H_A, J_{AB}: 17.48 Hz, J_{AX}: 3.12 Hz), 3.72 (dd; 1H; H_B, J_{AB}: 17.48 Hz, J_{BX}: 11.14 Hz), 6.41 (dd; 1H; H_X, J_{AX}: 3.12 Hz, J_{BX}: 11.14 Hz), 6.90 (dd; 1 H; pyrrol H^4), 7.02 (d; 1H; pyrrole H^3), 7.16 (d;1H; pyrrole H^5), 7.40 (d; 2H; phenyl H^3,H^5), 7.66 (d; 2H; phenyl H^2,H^6), 10.4 (br; 1H; NH); MS (70 eV, EI): 318 ($M^{+\cdot}$, 79.34%), ($M+2^{+\cdot}$, 33.65%), 285 (M^+–SH, 26.81%), 244 (M^+–$CSNHCH_3$, 26.91%), 166 (M^+–C_9H_8Cl, 100%), 93 (M^+–$C_{10}H_9ClN_2S$, 22.66%); Analysis calculated for $C_{15}H_{14}ClN_4S$.

4.3.6. 1-*N*-Ethylthiocarbamoyl-3-(4-chlorophenyl)-5-(2-pyrrolyl)-4,5-dihydro-(1H)-pyrazole. Yield 51%; mp 219–220 °C (chloroform–methanol); $[\alpha]_D^{20}$ +26 (*c* 0.2, $CHCl_3$); IR (KBr): 3358 (N–H), 2930 (C–H), 1529 (C=N), 1412 (C=C), 1341 (C=S); ^1H NMR δ (ppm): 1.28 (t; 3H; CH_2–CH_3), 3.29 (dd; 1H; H_A, J_{AB}: 17.48 Hz, J_{AX}: 3.08 Hz), 3.74 (m; 3H; H_B ve CH_2-CH_3), 6.40 (dd; 1H; H_X, J_{AX}: 3.08 Hz, J_{BX}: 11.12 Hz), 6.92 (dd; 1H; pyrrole H^4), 7.02 (d; 1H; pyrrole H^3), 7.15 (d; 1H; pyrrole H^5), 7.39–7.34 (s; 1H; NH), 7.40 (d; 2H; phenyl H^3,H^5), 7.67 (d; 2H; phenyl H^2,H^6), 10.5 (br; 1H; NH); MS (70 eV, EI): 332 ($M^{+\cdot}$, 100%), 334 ($M+2^{+\cdot}$, 39.74%), 299 (M^+–SH, 24.24%), 244 (M^+–$CSNHC_2H_5$, 27.03%), 180 (M^+–C_9H_8Cl, 73.21%), 93 (M^+–$C_{11}H_{11}ClN_2S$, 28.02%), 44 (M^+–$C_{14}H_{10}ClN_3S$, 27.34); Analysis calculated for $C_{16}H_{16}ClN_4S$.

4.3.7. 1-*N*-Allylthiocarbamoyl-3-(4-chlorophenyl)-5-(2-pyrrolyl)-4,5-dihydro-(1H)-pyrazole. Yield 40%; mp 142–143 °C (chloroform–methanol); $[\alpha]_D^{20}$ +24 (*c* 0.2, $CHCl_3$); IR (KBr): 3358 (N–H), 2919 (C–H), 1523 (C=N), 1411 (C=C), 1342 (C=S); ^1H NMR δ (ppm): 3.32 (d; 1H; H_A, J_{AB}: 17 Hz, J_{AX}: 3 Hz), 3.75 (dd; 1H;

H_B, J_{AB}: 17 Hz, J_{BX}: 10.96 Hz), 4.35 (m; 2H; NH–CH_2), 5.26 (dd; 2H; J_{AX}: 17.08 Hz, J_{BX}: 10.24 Hz, CH=CH_2), 6.05–5.93 (m; 1H; CH=CH_2), 6.42 (m; 1H; H_X), 6.94 (dd; 1H; pyrrole H^4), 7.04 (d; 1H; pyrrole H^3), 7.19 (d; 1H; pyrrole H^5), 7.42 (d; 2H; phenyl H^3, H^5), 7.45 (s; 1H; NH), 7.69 (d; 2H; phenyl H^2,H^6), 10.5 (br; 1H; NH); MS (70 eV, EI): 344 ($M^{+\cdot}$, 79.35%), 346 (M+2$^{+\cdot}$, 34.63%), 311 (M^+–SH, 15.78%), 288 (M^+–NHC_2H_5, 85.54%), 151 (M^+–$C_{11}H_{11}ClN$, 100%), 57 (M^+–$C_{14}H_9ClN_3S$, 30.20%); Analysis calculated for $C_{17}H_{16}ClN_4S$.

4.3.8. 1-*N*-Phenylthiocarbamoyl-3-(4-chlorophenyl)-5-(2-pyrrolyl)-4,5-dihydro-(1H)-pyrazole. Yield 35%; mp 202–204 °C (chloroform–methanol); $[\alpha]_D^{20}$ +28 (*c* 0.2, $CHCl_3$); IR (KBr): 3336 (N–H), 1519 (C=N), 1413 (C=C), 1346 (C=S); ^{1}H NMR δ (ppm): 3.38 (dd; 1H; H_A, J_{AB}: 17.56 Hz, J_{AX}: 3 Hz), 3.81 (m; 3H; H_B, J_{AB}: 17.56 Hz, J_{BX}: 11.04 Hz), 6.54 (dd; 1H; H_X, J_{AX}: 3 Hz, J_{BX}: 11.04 Hz), 6.93 (dd; 1H; pyrrole H^4), 7.07 (d; 1H; pyrrole H^3), 7.21 (m; 2H; pyrrole H^5, NH–phenyl H_4), 7.34 (m; 8H; phenyl prot), 10.15 (s; 1H; NH); MS (70 eV, EI): 380 ($M^{+\cdot}$, 55.02%), 382 (M+2$^{+\cdot}$, 22.58%), 347 (M^+–SH, 22.03%), 288 (M^+–NHC_6H_5, 26.15%), 244 (M^+–$CSNHC_6H_5$, 19.97%), 151 (M^+–$C_{14}H_{11}ClN$ 100%), 93 (M^+–$C_{15}H_{11}ClN_2S$, 24.73%); Analysis calculated for $C_{20}H_{16}ClN_4S$.

4.3.9. 1-*N*-Methylthiocarbamoyl-3-(4-methoxyphenyl)-5-(2-pyrrolyl)-4,5-dihydro-(1H)-pyrazole. Yield 56%; mp 215–216 °C (chloroform–methanol); $[\alpha]_D^{20}$ +18 (*c* 0.2, $CHCl_3$); IR (KBr): 3327 (N–H), 1610 (C=N), 1425 (C=C), 1354 (C=S); ^{1}H NMR δ (ppm): 3.2 (d; 3H; *J*: 4.81 Hz, NH–CH_3), 3.30 (dd; 1H; H_A, J_{AB}: 17.32 Hz, J_{AX}: 3 Hz), 3.70 (dd; 1H; H_B, J_{AB}: 17.32 Hz, J_{BX}: 11.04 Hz), 3.87 (s; 3H; OCH_3), 6.36 (dd; 1H; H_X, J_{AX}: 3 Hz, J_{BX}: 11.04 Hz), 6.92 (m; 3H; pyrrole H^4, phenyl H^2,H^6), 7.02 (d; 1H; pyrrole H^3), 7.15 (d; 1H; pyrrole H^5), 7.40 (s; 1H; NH), 7.68 (d; 2H; phenyl H^3,H^5), 10.3 (s; 1H; NH); MS (70 eV, EI): 314 ($M^{+\cdot}$, 100%), 316 (M+2$^{+\cdot}$, 8.12%) 281 (M^+–SH, 33.60%), 240 (M^+–$CSNHCH_3$, 24.25%), 180 (M^+–C_9H_9O, 28.10%), 166 (M^+–$C_{10}H_{11}O$, 83.49%), 93 (M^+–$C_{11}H_{12}N_2OS$, 14.08%); Analysis calculated for $C_{16}H_{17}N_4OS$.

4.3.10. 1-*N*-Ethylthiocarbamoyl-3-(4-methoxyphenyl)-5-(2-pyrrolyl)-4,5-dihydro-(1H)-pyrazole. Yield 60%; mp 174–176 °C (chloroform–methanol); $[\alpha]_D^{20}$ +22 (*c* 0.2, $CHCl_3$); IR (KBr): 3364 (N–H), 1564 (C=N), 1453 (C=C), 1369 (C=S); ^{1}H NMR δ (ppm): 1.27 (t; 3H; CH_2–CH_3), 3.29 (dd; 1H; H_A, J_{AB}: 17.32 Hz, J_{AX}: 2.96 Hz), 3.72 (m; 3H; H_B ve CH_2–CH_3), 3.85 (s; 3H; OCH_3), 6.39 (dd; 1H; H_X, J_{AX}: 2.96 Hz, J_{BX}: 11 Hz), 6.93 (m; 3H; pyrrole H^4 phenyl H^2, H^6), 7.02 (d; 1H; pyrrole H^3), 7.15 (d; 1H; pyrrole H^5), 7.32 (s; 1H; NH), 7.68 (d; 2H; phenyl H^3,H^5), 10.3 (s; 1H; NH); MS (70 eV, EI): 328 ($M^{+\cdot}$, 100%), 330 (M+2$^{+\cdot}$, 9.06%), 295 (M^+–SH, 32.37%), 240 (M^+–$CSNHC_2H_5$, 29.54%), 180 (M^+–$C_{10}H_{11}O$, 57.78%), 152 (M^+–$C_{11}H_{15}O$, 27.54%); Analysis calculated for $C_{17}H_{19}N_4OS$.

4.3.11. 1-*N*-Allylthiocarbamoyl-3-(4-methoxyphenyl)-5-(2-pyrrolyl)-4,5-dihydro-(1H)-pyrazole. Yield 53%; mp 147–148 °C (chloroform–methanol); $[\alpha]_D^{20}$ +16 (*c* 0.2, $CHCl_3$); IR (KBr): 3379 (N–H), 1530 (C=N), 1509 (C=C), 1336 (C=S); ^{1}H NMR δ (ppm): 3.30 (dd; 1H; H_A, J_{AB}: 17.36 Hz, J_{AX}: 2.96 Hz), 3.72 (dd; 1H; H_B, J_{AB}: 17.36 Hz, J_{BX}: 11 Hz), 3.87 (s; 3H; OCH_3), 4.32 (m; 2H; NH–CH_2), 5.24 (dd; 2H; J_{AX}: 17.08 Hz, J_{BX}: 10.24 Hz, CH=CH_2), 5.96 (m; 1H; CH=CH_2), 6.39 (dd; 1H; H_X, J_{AX}: 3 Hz, J_{BX}: 10.96Hz), 6.92 (m; 3H; pyrrole H^4 phenyl H^2, H^6), 7.02 (dd; 1H; pyrrole H^3), 7.15 (dd; 1H; pyrrole H^5), 7.44–7.42 (s; 1H; NH), 7.68 (d; 2H; phenyl H^3,H^5), 10.4 (s; 1H; NH); MS (70 eV, EI): 340 ($M^{+\cdot}$, 98.09%), 342 (M+2$^{+\cdot}$, 8.79%), 307 (M^+–SH, 18.69%), 284 (M^+–NHC_3H_5, 100%), 240 (M^+–$CSNHC_3H_5$, 22.57%), 151 (M^+–$C_{12}H_{14}NO$, 91.77%), 93 (M^+–$C_{13}H_{14}N_2OS$, 22.35%), 41 (M^+–$C_{15}H_{14}N_4OS$, 11.10%); Analysis calculated for $C_{18}H_{19}N_4OS$.

4.3.12. 1-*N*-Phenylthiocarbamoyl-3-(4-methoxyphenyl)-5-(2-pyrrolyl)-4,5-dihydro-(1H)-pyrazole. Yield 32%; mp 179–182 (dec) (chloroform–methanol); IR (KBr): 3347 (N–H), 1558 (C=N), 1429 (C=C), 1341 (C=S); ^{1}H NMR δ (ppm): 3.30 (dd; 1H; H_A, J_{AB}: 17.32 Hz, J_{AX}: 3 Hz), 3.80 (d; 1H; H_B, J_{AB}: 17.32 Hz, J_{BX}: 11.0 Hz), 3.85 (s; 3H; OCH_3), 6.50 (dd; 1H; H_X, J_{AX}: 3 Hz, J_{BX}: 11.00 Hz), 6.95 (m; 2H; pyrrole H^3,H^4), 7.10 (s; 1H; pyrrole H^5), 7.2–7.8 (m; 9H; phenyl prot), 9.25 (s; 1H; NH), 10.5 (s; 1H; NH); MS (70 eV, EI): 376 ($M^{+\cdot}$, 36.93%), 378 (M+2$^{+\cdot}$, 5.32%), 343 (M^+–SH, 15.90%), 284 (M^+–NHC_6H_5, 19.47%), 240 (M^+–$CSNHC_6H_5$, 18.67%), 151 (M^+–$C_{15}H_{14}NO$, 100%), 77 (M^+–$C_{15}H_{14}N_4OS$, 27.23%); Analysis calculated for $C_{21}H_{19}N_4OS$.

4.4. Single crystal X-ray crystallographic data of 3k

The data collection was performed on a CAD-4 diffractometer employing graphite-monochromated MoK_α radiation (λ = 0.71073 Å). Crystallographic and refinement parameters are summarized in Table 4. Three standard reflections were measured every 2 h. The structure was solved by direct methods. The refinement was made with anisotropic temperature factors for all non-hydrogen atoms. The hydrogen atoms were generated geometrically expect N–H bonds. An empirical Ψ scan absorption correction was applied. Some selected bond lengths, angles and torsion angles are listed in Figure 2. Crystallographic data (excluding structure factors) for compound **3k** reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC-632691. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk). Some selected bond lengths, angles and torsion angles are listed in Figure 2.

4.5. Biochemistry

4.5.1. Purification of MAO from the liver homogenates. Ethics Committee of Laboratory Animals in Hacettepe University, Turkey (2001/25-4), approved the animal experimentation. MAO was purified from the rat liver

Table 4. Crystallographic and refinement parameters of the molecule

Molecular formula	$C_{18}H_{19}ON_4S$
Formula weight	339.43
Temperature	293 (2) K
Wavelength	0.71073 Å
Crystal system	Triclinic
Space group	$P\bar{1}$
Cell dimensions	a = 9.0380 (9) Å
	b = 10.1367 (12) Å
	c = 10.3644 (10) Å
	α = 68.029 (9)°
	β = 80.377 (8)°
	γ = 86.771 (9)°
Volume	868.2 (2) Å^3
Z	2
Density (calculated)	1.298 mg m^{-3}
Absorption coefficient	0.199 mm^{-1}
F_{000}	358
Crystal size	0.21 × 0.42 × 0.42 mm
Crystal colour	Yellow
θ range for data collection	2.17 to 26.29°
Index ranges	$-11 \leqslant h \leqslant 0$
	$-12 \leqslant k \leqslant 12$
	$-12 \leqslant l \leqslant 12$
Reflections collected	3656
Independent reflections	3436 [R (int) = 0.0209]
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	3436/0/225
Goodness-of-fit on F^2	1.031
R indices [$I > 2\sigma$ (I)]	R_1 = 0.046, wR_2 = 0.129
(Δ/σ)	0
$\Delta\rho_{max}$	0.228 e Å^{-3}
$\Delta\rho_{min}$	−0.278 e Å^{-3}

according to the method of Holt with some modifications.[50] Liver tissue was homogenized 1:40 (w/v) in 0.3 M sucrose. Following centrifugation at 1000*g* for 10 min, the supernatant was centrifuged at 10,000*g* for 30 min to obtain crude mitochondrial pellet. The pellet was incubated with CHAPS of 1% at 37 °C for 60 min and centrifuged at 1000*g* for 15 min. Pellet was resuspended in 0.3 M sucrose and was layered onto 1.2 M sucrose, centrifuged at 53,000*g* for 2 h and resuspended in potassium phosphate buffer, pH 7.4, kept at −70 °C until used.[50]

4.5.2. Measurement of MAO activity. Total MAO activity was measured spectrophotometrically according to the method of Holt.[50] Assay mixture contained a chromogenic solution consisted of 1 mM vanillic acid, 500 μM 4-aminoantipyrine and 4 U ml^{-1} peroxidase type II in 0.2 M potassium phosphate buffer, pH 7.6. Assay mixture contained 167 μl chromogenic solution, 667 μl substrate solution (500 μM *p*-tyramine) and 133 μl potassium phosphate buffer, pH 7.6. The mixture was preincubated at 37 °C for 10 min. before the addition of enzyme. Reaction was initiated by adding the homogenate (100 μl), and increase in absorbance was monitored at 498 nm at 37 °C for 60 min. Molar absorption coefficient of 4654 M^{-1} cm^{-1} was used to calculate the initial velocity of the reaction. Results were expressed as nmol h^{-1} mg^{-1}.

4.5.3. Selective measurement of MAO-A and MAO-B activities. Homogenates were incubated with the substrate *p*-tyramine (500 μM to measure MAO-A and 2.5 mM to measure MAO-B) following the inhibition of one of the MAO isoforms with selective inhibitors. Aqueous solutions of clorgyline or pargyline (50 μM), as selective MAO-A and -B inhibitor, were added to homogenates at the ratio of 1:100 (v/v), yielding the final inhibitor concentrations of 0.50 μM. Homogenates were incubated with these inhibitors at 37 °C for 60 min. prior to activity measurement. After incubation of homogenates with selective inhibitors, total MAO activity was determined by the method described above.

Reversibility of the inhibiton of MAO by these compounds was assessed by dilution.

4.5.4. Analysis of the kinetic data. Newly synthesized compounds were dissolved in dimethyl sulfoxide (DMSO) and used in the concentration range of 1–1000 μM. Inhibitors were incubated with the purified MAO at 37 °C for 0–60 min prior to adding to the assay mixture. Semicarbazide (1 mM) was used as SSAO inhibitor when the compounds were assayed as MAO inhibitors (by 60 min. of incubation at 37 °C). Reversibility of the inhibition of the enzyme by this compound was assessed by dilution.

Kinetic data for interaction of the enzyme with this compound was determined using Microsoft Excel package program. IC_{50} values were determined from plots of residual activity percentage, calculated in relation to a sample of the enzyme treated under the same conditions without inhibitor, versus inhibitor concentration [I].

4.5.5. Protein determination. Protein was determined according to the method of Bradford,[51] in which bovine serum albumin was used as standard.

4.6. Pharmacology

4.6.1. Animals. Male Swiss albino mice (20–25 g) were purchased from the animal breeding laboratories of Refik Saydam Hıfzısıhha Institute (Ankara, Turkey). The animals were left for 2 days for acclimatization to animal room conditions and were maintained on standard pellet diet and water ad libitum. The food was withdrawn on the day before the experiment, but allowed free access of water. A minimum of six animals was used in each group, otherwise described in procedure. Mice used in the present study were cared in accordance with the directory of Refiksaydam Hıfzıssıhha Institute's Animal Care Unit, which applies the guidelines of National Institutes of Health on laboratory animal welfare.

4.6.2. Preparation of test samples for bioassay. Test samples were given orally to test animals after suspending in 0.5% sodium carboxymethyl cellulose (CMC) and distilled water. The control group animals received the same experimental handling as that of the test groups except that the drug treatment was replaced with appropriate volumes of the dosing vehicle. Either indometha-

cin (10 mg/kg) or acetyl salicylic acid (ASA) (200 mg/kg) in 0.5% CMC was used as reference drug.

4.6.3. Analgesic activity

4.6.3.1. *p*-Benzoquinone-induced abdominal constriction test in mice.[45] Sixty minutes after the oral administration of test samples, the mice were intraperitoneally injected with 0.1 ml/10 g body weight of 2.5% (v/v) *p*-benzoquinone (PBQ; Merck) solution in distilled water. Control animals received an appropriate volume of dosing vehicle. The mice were then kept individually for observation and the total number of abdominal contractions (writhing movements) was counted for the next 15 min, starting on the 5th min after the PBQ injection. The data represent average of the total number of writhes observed. The antinociceptive activity was expressed as percentage change from writhing controls.

4.6.4. Anti-inflammatory activity

4.6.4.1. Carrageenan-induced oedema.[46] For the determination of the effects on carrageenan-induced paw oedema the modified method of Kasahara et al. was employed.[46] 60 min after the oral administration of either test sample or dosing vehicle, each mouse was injected with freshly prepared (0.5 mg/25 μl) suspension of carrageenan (Sigma, St. Louis, Missouri, U.S.A.) in physiological saline (154 mM NaCl) into subplantar tissue of the right hind paw. As the control, 25 μl saline solution was injected into that of the left hind paw. Paw oedema was measured in every 90 min during 6 h after induction of inflammation. The difference in footpad thickness between the right and left foot was measured with a pair of dial thickness gauge callipers (Ozaki Co., Tokyo, Japan). Mean values of treated groups were compared with mean values of a control group and analyzed using statistical methods.

4.6.4.2. Acetic acid-induced increase in capillary permeability.[47] Effect of the test samples on the increased vascular permeability induced by acetic acid in mice was determined according to Whittle method with some modifications.[42] Each test sample was administered orally to a group of 10 mice in 0.2 ml per 20 g body weight. Thirty minutes after the administration each mouse was injected with 0.1 ml of 4% Evans blue (Sigma, St. Louis, Missouri, U.S.A.) in saline solution (iv) at the tail. Then, 10 min after the iv injection of the dye solution, 0.4 ml of 0.5% (v/v) AcOH was injected ip. After 20 min, the mice were killed by dislocation of the neck, and the viscera were exposed and irrigated with distilled water, which was then poured into 10 ml volumetric flasks through glass wool. Each flask was made up to 10 ml with distilled water, 0.1 of 0.1 N NaOH solution was added to the flask, and the absorption of the final solution was measured at 590 nm (Beckmann Dual Spectrometer). In control animals, a mixture of distilled water and 0.5% CMC was given orally, and they were treated in the same manner as described above.

4.6.5. Ulcerogenic adverse effect. Three hours after the analgesic activity experiment, mice were killed under deep ether anaesthesia and stomachs were removed. Then the abdomen of each mouse was opened through great curvature and examined under dissecting microscope for lesions or bleedings.

4.6.6. Statistical analysis of data. Data obtained from animal experiments were expressed as means ± standard deviation (SD). Statistical differences between the treatments and the control were tested by one-way analysis of variance (ANOVA) and Student–Newman–Keuls post hoc test. A value of $p < 0.05$ was considered to be significant.

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