Benzo[*b*]thiophenes, II:

Novel Benzo[*b*]thienylhydrazine and 1,3,4-Oxadiazole Derivatives as Potential Antidepressant Agents

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Three novel series of benzo[*b*]thiophene derivatives bearing various hydrazone, hydrazine and 1,3,4-oxadiazole moieties were synthesized as potential antidepressant agents. 22 Compounds were evaluated for their *in vitro* inhibitory effect on monoamine oxidase enzyme (MAO) type A. Several compounds inhibited MAO stronger than pargyline hydrochloride. Maximum inhibitions of 83% and 90% were observed with 1-benzyl-2-(3-chlorobenzo[*b*]thienyl-2-carbonyl)hydrazine (**24**) and 1-[2-(4-chlorophenyl)ethyl]-2-(3-chlorobenzo[*b*]thienyl-2-carbonyl)hydrazine (**35**), respectively.

Benzo[*b*]thiophene, 2. Mitt.: Neue Benzo [*b*]thienylhydrazine und 1,3,4-oxadiazole als potentiell antidepressive Wirkstoffe

Drei neue Serien von Benzo[*b*]thiophenen mit Hydrazon-, Hydrazin- und 1,3,4-Oxadiazol-Gruppen wurden als potentiell antidepressive Wirkstoffe synthetisiert. 22 Verbindungen wurden auf ihre *in vitro* Hemmung der Monoaminoxidase A hin geprüft. Mehrere Verbindungen hemmten MAO stärker als Pargylin-HCl. Die stärksten Hemmwirkungen von 83 bzw. 90% zeigten die Verbindungen **24** und **35**.

Depression is among the most common mental disorders encountered in clinical practice. This illness is caused by a functional deficit of monoamine transmitters at the post synaptic adrenergic receptor sites, resulting in a faulty transmission of impulses within the central nervous system[1)]. Monoamine oxidase (MAO) is an enzyme located intracellularly mostly associated with the mitochondria and is responsible for the regulation of the free intraneuronal concentration of noradrenaline (NA), dopamine (DA) or serotonin (5-HT) and is important for the inactivation of endogenous and exogenous monoamines[2)].

MAO exists in two distinct molecular forms[3)]: MAO-A has a substrate preference for NA and 5-HT and is the target for the antidepressant monoamine oxidase inhibitors (MAOI). MAO-B has a substrate preference for phenylethylamine and drugs acting on this type of the enzyme are used clinically in the treatment of Parkinson's disease[2)]. Many of the drugs belonging to MAOI are utilized clinically for their antidepressive, anticonvulsant, hypotensive, and analgesic activities specially in the treatment of depressive psychoses[4)]. These effects, particularly the antidepressive action, are due to the *in vivo* inhibition of MAO, therefore enhancing the availability of NA or 5-HT at the post synaptic receptor sites and alleviating the hypothesized neurotransmitter deficiency in depressed patients[1,5)].

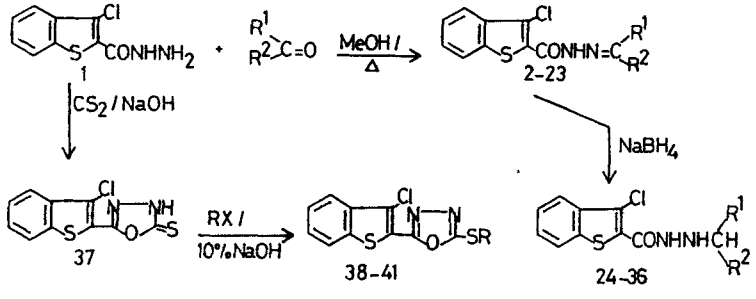
Despite the great number of antidepressants synthesized and studied as MAOI-type A both *in vivo* and *in vitro*[6-10)], hydrazines and 1,3,4-oxadiazoles[11-16)] still remain one of the most versatile classes of compounds possessing high activity and are therefore useful substructures for further molecular exploration. Although the chemistry and biological profile of various pharmacophores attached to hydrazines has been worked out in detail, the synthesis and antidepressant evaluation of derivatives of benzo[*b*]thiophene have received only limited attention despite the fact that various compounds containing this moiety possess psychopharmacological properties[17-21)].

Concurrent with our studies on biologically active heterosulfur compounds[22,23)], we adopted the benzo[*b*]thiophene system, which is an isostere of the biologically active indole nucleus present in 5-HT, as a carrier for the hydrazine moiety. Considering that the N-N-group was implicated in the activity of the hydrazine MAOI, it seemed also interesting to examine the effect of incorporating this group into a heterocyclic ring system as 1,3,4-oxadiazole with the hope that such an arrangement might provide potent inhibitors and new psychopharmacologic agents.

The designed compounds namely: 1-alkylidene (or arylidene)-2-(3-chlorobenzo[*b*]thienyl-2-carbonyl)hydrazines **2-23**, 1-aralkyl-2-(3-chlorobenzo[*b*]thienyl-2-carbonyl)hydrazines **24-36** and 2-substituted thio-5-[2-(3-chlorobenzo[*b*]-thienyl)-1,3,4-oxadiazoles **38-41** were synthesized and evaluated for their antidepressant activity by measuring their *in vitro* inhibitory activity on MAO-type A.

Chemistry

The synthesis of these compounds is illustrated in Scheme 1.



Scheme 1

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3-Chloro-2-hydrazinocarbonylbenzo[*b*]thiophene (**1**), required as starting material, was prepared[23] from 3-chloro-2-chlorocarbonylbenzo[*b*]thiophene[24]. The acid hydrazide **1** easily condensed with different aldehydes and ketones in absolute methanol while heating under reflux for 2 h to give compounds **2-23** in excellent yields (Scheme 1, Table 1).

These hydrazones 2-23 show IR absorption bands due to CO, NH, and C=N. The ^{1}H-NMR spectra of **2** and **23**, as representative examples, were in agreement with the assigned structures. Reduction of the hydrazones with $NaBH_4$ in ethanol/H_2O or dioxane-ethanol-water afforded the 1-aralkyl-2-(3-chlorobenzo[*b*]thienyl-2-carbonyl)hydrazines **24-36** (Scheme 1, Table 2). Compounds **24-36** lacked the C=N absorption band but still showed the C=O and NH IR-bands. Compound **24** showed a doublet at 4.23 ppm asigned for CH_2 while compound **32** showed the methine proton resonating at 4.10 ppm. **37** was prepared by heating the acid hydrazide **1** with CS_2 in ethanolic NaOH under reflux. The IR spectrum of 37 showed NH, C=N, C-O-C, and NCS amide I, II, III, and IV stretching vibrational bands indicating that it exists in the thione rather than in the mercapto form. Its ^{1}H-NMR spectrum showed the oxadiazoline NH signal included whithin the multiplet of the benzo[*b*]thiophene C-5 and C-6 protons. Alkylation of the thione derivative **37** with various alkyl halides produced 2-substituted thio-5-[2-(3-chlorobenzo[*b*]thienyl]-1,3,4-oxadiazoles **38-41** (Scheme 1, Table 3). Their IR spectra lacked NH and NCS amide bands. The ^{1}H-NMR spectrum of compound **41** showed a singlet at 4.23 (S-CH_2). All the synthesized compounds showed the H-4, H-5, H-6, and H-7 of the benzo[*b*]thiophene nucleus[25] resonating at their expected chemical shifts.

Results and Discussion

Selected compounds, as 10^{-4} M solution in propylene glycol, were evaluated *in vitro* for their abilities to inhibit

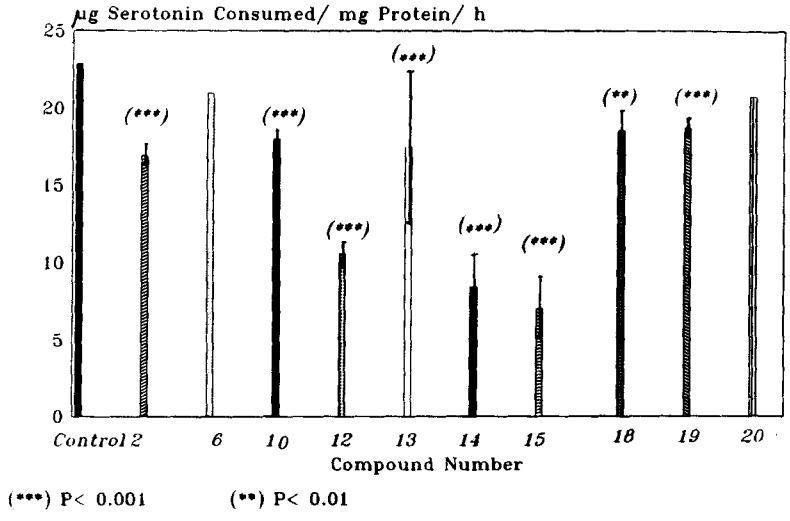

Fig. 1: Effect of Benzo[*b*]thienylhydrazones on the Activity of Monoamine Oxidase Enzyme of Rat Liver Mitochondria

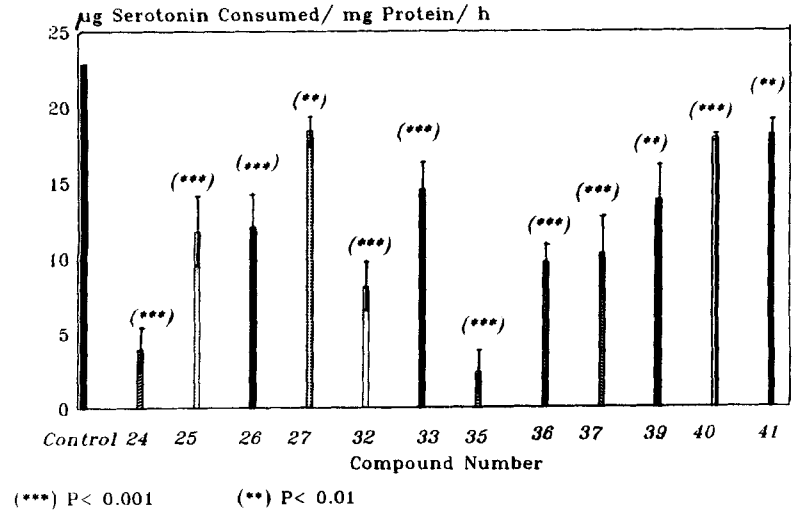

Fig. 2: Effect of Benzo[*b*]thienylhydrazine and 1,3,4-Oxadiazole Derivatives on the Activity of Monoamine Oxidase Enzyme

MAO-type A of rat liver mitochondria by using the method of *Udenfriend* et al.[26]. The results were expressed as μg serotonin (5-HT) consumed/mg protein/h and as percentage inhibition of the activity of MAO-type A (Table 4, Figs. 1, 2).

Compounds **12, 14, 15, 24-26, 32, 33, 35-37** showed inhibition of MAO higher than that exhibited by pargyline hydrochloride suggesting that the high lipophilicity and low toxicity[27] given to the molecule by the benzo[*b*]thiophene nucleus could provide potent inhibitors. The inhibition of MAO by hydrazines was suggested to be the result of the binding of the inhibitor covalently to the enzyme resulting in a non-competitive and long lasting inhibition[2]. In general, 1-aralkyl-2-(3-chlorobenzo[*b*]thienyl-2-carbonyl)-hydrazines were the most active showing MAO inhibition activity ranging from 45-90% (Table 4, Fig. 2). Compound **35** was the most potent inhibitor showing inhibitory activity of 90% compared to 40% inhibition for pargyline hydrochloride while compound **24** elicited 83% MAO inhibition activity. This is in agreement with the results reporting that substitution of a chlorine atom on the *para* position of the benzene ring attached to the hydrazine moiety resulted in an increase in *in vitro* MAO inhibition[28]. The hydrazones were less active than the hydrazines, however inhibitory activities of 53, 70, and 75% were observed for compounds **12, 14,** and **15,** respectively (Table 4, Fig. 1). **37** showed 61% MAO inhibition activity. This compound combines the cyclic hydrazine structure which is believed to be safer in therapy[15] and the 2-thione group which is interconvertible with the thiol group that could be responsible for binding of the inhibitor at the active site of the enzyme[29,30].

Alkylation of the thione group in compounds **39-41** lowered the activity (Table 4, Fig. 2) probably due to the decreased ability of binding of the inhibitor at the enzyme active site.

Experimental Part

Melting points: Griffin melting point apparatus, uncorrected.- UV: Shimadzu double beam spectrophotometer - 200 S.- IR spectra (Nujol): Shimadzu spectrophotometer.- ^{1}H-NMR spectra: Varian EM 360 L, 60 MHz, TMS as internal standard.- Microanalyses: Microanalytical Unit, Faculty of Science, Cairo University, Egypt.

1-Alkylidene (or arylidene)-2-(3-chlorobenzo[b]thienyl-2-carbonyl-hydrazines **2-23**

A solution of the acid hydrazide **1** (0.5 g, 2.2 mmole) in absol. methanol (50 ml) was heated with the appropriate aldehyde or ketone (2.2 mmole) under reflux for 2 h. The products separated either during reflux or upon cooling to room temp. (RT). The mixture was left overnight at RT, filtered and crystallized from the proper solvent. Table 1.- IR: 3350-3150 (NH), 1650-1615 (C=O), 1600 (C=N), 1592 and 1530-1490 cm^{-1} (C=C aromatic).- ^{1}H-NMR of **2** ($DMSO-d_6$): δ (ppm) = 7.00-7.60 (m, 7H, phenyl + H-5 and H-6 of benzothiophene), 7.68-7.99 (m, 2H, H-4 and H-7 of benzothiophene), 8.60 (s, 1H, NH), 8.18 (s, 1H, CH=N).- ^{1}H-NMR of **23** ($DMSO-d_6$): δ (ppm) = 2.38 (s, 3H, CH_3), 7.40-8.20 (m, 9H, 4H phenyl + H-4, H-5, H-6, and H-7 of benzothiophene + NH).

1-Aralkyl-2-(3-chlorobenzo[b]thienyl-2-carbonyl)hydrazines **24-36**

A solution of the arylidene hydrazine derivative (0.4 g) in EtOH (20 ml) or dioxane:EtOH (2:1, v/v) was mixed with H_2O (0.5 ml). $NaBH_4$ (30 molar equivalents) was added in small portions while stirring and keeping the temp. at 60°C for 1 h. The mixture was cooled to RT and treated dropwise with glacial HOAc until pH 7.5. The solvent was removed *in vacuo* and the residue washed with H_2O. The viscous mass obtained was scratched with H_2O until it solidified, filtered, washed several times with H_2O until free from NaOAc and crystallized from the proper solvent. Table 2.- IR: 3150-3050 (NH), 1660-1625 (C=O), 1595 and 1515-1480 cm^{-1} (C=C aromatic).- ^{1}H-NMR of **24** ($CDCl_3$): δ (ppm) = 4.23 (d, J = 5 Hz, 2H, CH_2), 7.13-7.67 (m, 8H, 5 phenyl + H-5 and H-6 of benzothiophene + NH), 7.67-8.00 (m, 2H, H-4 and H-7 of benzothiophene), 8.49 (s, 1H, NHCO).- ^{1}H-NMR of **32** ($DMSO-d_6$): δ (ppm) = 1.38 (s, 3H, CH_3), 2.28 (s, 3H, tolyl CH_3), 4.10 (m, 1H, methine proton), 7.00 (d, 2H, J = 6 Hz, tolyl ar H *ortho* to CH_3), 7.45 (d, 2H, J = 6 Hz tolyl ar H *meta* to CH_3), 7.27-7.65 (m, 3H, overlapping with the doublet at 7.45, H-5 and H-6 of benzothiophene + NH), 7.70-7.98 (m, 2H, H-4 and H-7 of benzothiophene), 8.50 (s, 1H, NHCO).

5-[2-(3-Chlorobenzo[b]thienyl]-3H-1,3,4-oxadiazole-2-thione (**37**)

A solution of the acid hydrazide **1** (380 mg, 1.68 mmole) in EtOH (15 ml) containing NaOH (100 mg, 2.35 mmole) was treated with CS_2 (130 mg, 1.68 mmole). The mixture was stirred, heated under reflux for 2 h and cooled to RT. Acidification with glacial HOAc followed by addition of a few drops of H_2O precipitated **37** as white solid, which was crystallized from aqueous EtOH.- M.p. 235-236°C.- Yield 89%.- IR: 3200 (NH), 1610 (C=N), 1595, 1505 (C=C aromatic), 1560, 1310, 1150, and 950 (NCS amide I, II, III, and IV bands), 1245 and 1055 cm^{-1} (C-O-C ν as and ν s).- ^{1}H-NMR ($DMSO-d_6$): δ (ppm) = 7.38-7.68 (m, 3H, H-5 and H-6 of benzothiophene + NH), 7.70-8.00 (m, 2H, H-4 and H-7 of benzothiophene).- $C_{10}H_5ClN_2OS$ (268.5) Calc. C 44.7 H 1.86 N 10.4 Found C 45.0 H 2.40 N 10.0.

2-Substituted Thio-5-[2-(3-chlorobenzo[b]thienyl]-1,3,4-oxadiazoles **38-41**

An ice-cold solution of **37** (250 mg, 0.93 mmole) in EtOH:10% NaOH (1:1.5, v/v) was treated dropwise with the selected alkyl halide (1 molar equivalent). A yellow solid deposited after 15 min. The mixture was stirred while cooling in ice for 1 h and at RT for another h. It was treated with drops of H_2O to precipitate the remainder of the product. The crude products **38-41** were filtered and crystallized from the proper solvent. Table 3.- IR: 1625-1620 (C=N), 1610-1595, 1520-1490 (C=C aromatic), 1245-1240 and 1065-1045 cm^{-1} (C-O-C ν as and ν s).- ^{1}H-NMR of **41** ($CDCl_3$): δ (ppm) = 4.23 (s, 2H, S-CH_2), 7.18-7.60 (m, 7H, 5 phenyl + H-5 and H-6 of benzothiophene), 7.68-8.00 (m, 2H, H-4 and H-7 of benzothiophene).

Biochemistry

Chemicals

5-Hydroxytryptamine-creatinine sulfate (5-HT): E. Merck, Darmstadt, Germany.- Pargyline hydrochloride (Eutonyl): Abbott Laboratories, North Chicago, USA.- 1-Nitroso-2-naphthol: BDH chemicals Ltd., Pool, England, was used after crystallization from light petroleum 60-80°C.- Bovine serum albumin: Sigma Co., St. Louis, USA.

Mitochondrial Homogenate Preparation

A crude mitochondrial fraction was prepared as described by *Schneider*[31]. Male adult albino rats (Alexandria Faculty of Pharmacy animal house) weighing 130-160 g were decapitated. Livers were removed rapidly, blotted, weighed and pressed through a tissue press. The resulting paste was homogenized in a glass homogenizer with seven volumes of cold 0.25 M sucrose. The homogenate was centrifuged at 600 x g for 10 min at 4°C. The resulting supernatant was centrifuged at 8500 x g for 12 min. The crude mitochondrial pellet obtained was suspended in 0.25 M (5 ml) sucrose solution and centrifuged at 8500 x g for 10 min. The washing

Table 1: 1-Alkylidene (or arylidene)-2-(3-chlorobenzo[*b*]thienyl-2-carbonyl)hydrazines 2-23

Compd No.	R^1	R^2	Yield %	Mp°C (cryst. solvent)[a]	Molecular Formula	Analysis % Calc/F C	H	N
2	H	C_6H_5	93	173-75 (A)	$C_{16}H_{11}ClN_2OS$ (314.5)	61.05 60.90	3.49 3.60	8.90 9.20
3	H	2-OH-C_6H_4	97	213-14 (B)	$C_{16}H_{11}ClN_2O_2S$ (330.5)	58.09 58.30	3.33 3.50	8.47 8.80
4	H	4-OH-C_6H_4	94	248-49 (C)	$C_{16}H_{11}ClN_2O_2S$ (330.5)	58.09 57.80	3.33 3.40	8.47 8.60
5	H	2-NO_2-C_6H_4	86	234-35 (B)	$C_{16}H_{10}ClN_3O_3S$ (359.5)	53.40 53.20	2.78 2.50	11.68 11.70
6	H	4-NO_2-C_6H_4	95	242-43 (B)	$C_{16}H_{10}ClN_3O_3S$ (359.5)	53.40 53.50	2.78 2.60	11.68 12.00
7	H	3-OCH_3-C_6H_4	92	155-56 (A)	$C_{17}H_{13}ClN_2O_2S$ (344.5)	59.21 59.00	3.77 3.50	8.12 8.40
8	H	3-OCH_3-4OH-C_6H_4	86	195-96 (C)	$C_{17}H_{13}ClN_2O_3S$ (360.5)	56.58 56.50	3.60 3.80	7.76 7.50
9	H	4-$N(CH_3)_2$-C_6H_4	87	183-84 (C)	$C_{18}H_{16}ClN_3OS$ (357.5)	60.41 60.80	4.47 4.60	11.74 12.10
10	H	2-Furyl	87	178-79 (A)	$C_{14}H_9ClN_2O_2S$ (304.5)	55.17 55.30	2.95 2.70	9.19 9.00
11	H	2-(5-NO_2-Furyl)	89	225-26 (B)	$C_{14}H_8ClN_3O_3S$ (333.5)	50.37 50.10	2.39 2.50	12.59 12.60
12	H	2-Thienyl	99	185-86 (C)	$C_{14}H_9ClN_2OS_2$ (320.5)	52.41 52.50	2.80 2.50	8.73 9.10
13	H	2-Pyridyl	97	208-209 (C)	$C_{15}H_{10}ClN_3OS$ (315.5)	57.05 57.20	3.17 3.00	13.31 13.50
14	CH_3	CH_3	86	129-130 (D)	$C_{12}H_{11}ClN_2OS$ (266.5)	54.03 54.40	4.12 4.00	10.50 10.60
15	C_5H_{10}(c)		81	165-67 (A)	$C_{15}H_{15}ClN_2OS$ (306.5)	58.72 58.90	4.89 5.00	9.13 9.20
16	CH_3	C_6H_5	92	200-201 (C)	$C_{17}H_{13}ClN_2OS$ (328.5)	62.10 62.00	3.95 3.80	8.52 8.60
17	CH_3	4-OH-C_6H_4	79	276-77 (C)	$C_{17}H_{13}ClN_2O_2S$ (344.5)	59.21 59.30	3.77 3.50	8.12 8.00
18	CH_3	4-$CH_3C_6H_4$	97	203-204 (C)	$C_{18}H_{15}ClN_2OS$ (342.5)	63.00 62.60	4.37 4.50	8.17 8.10
19	CH_3	4-NO_2-C_6H_4	94	167-68 (B)	$C_{17}H_{12}ClN_3O_3S$ (373.5)	54.61 54.50	3.21 3.30	11.24 11.20
20	CH_3	4-OCH_3-C_6H_4	84	187-88 (A)	$C_{18}H_{15}ClN_2O_2S$ (358.5)	60.25 60.10	4.18 4.20	7.81 7.50
21	CH_3	2-NH_2-C_6H_4	73	178-180 (A)	$C_{17}H_{14}ClN_3OS$ (343.5)	59.38 59.50	4.07 3.90	12.22 12.50
22	CH_3	4-Cl-C_6H_4	76	220-21 (C)	$C_{17}H_{12}Cl_2N_2OS$ (363)	56.19 56.00	3.30 3.50	7.71 8.00
23	CH_3	4-Br-C_6H_4	92	223-24 (C)	$C_{17}H_{12}BrClN_2OS$ (407.5)	50.06 50.10	2.94 3.10	6.87 6.50

a) Crystallization solvents : (A) = EtOH , (B) = dioxane, (C) = EtOH-dioxane, (D) = acetone.

procedure was repeated twice to remove microsomal and cellular contaminations. The pellet was finally suspended in 0.25 M sucrose approximately 1 ml per g of original tissue weight, fractionated in vials and stored at -30°C. Before use, mitochondrial suspensions were diluted with 0.05 M phosphate buffer pH 7.4, to give a working suspension equivalent to 2 mg protein/ml.

Table 2: 1-Aralkyl-2-(3-chlorobenzo[*b*]thienyl-2-carbonyl)hydrazines **24-36**

Compd No.	R^1	R^2	Yield %	Mp °C (cryst. solvent)[a]	Molecular Formula	Analysis % Calc/F C	H	N
24	H	C_6H_5	Quanti-tative	155-56 (A)	$C_{16}H_{13}ClN_2OS$ (316.5)	60.66 60.60	4.10 4.20	8.84 9.20
25	H	2-OH-C_6H_4	86	179-81 (B)	$C_{16}H_{13}ClN_2O_2S$ (332.5)	57.74 57.70	3.90 4.00	8.42 8.50
26	H	4-OH-C_6H_4	75	243-44 (B)	$C_{16}H_{13}ClN_2O_2S$ (332.5)	57.74 57.90	3.90 3.90	8.42 8.40
27	H	3-OCH_3-C_6H_4	Quanti-tative	197-98 (B)	$C_{17}H_{15}ClN_2O_2S$ (346.5)	58.87 59.00	4.32 4.20	8.08 8.00
28	H	4-$N(CH_3)_2$-C_6H_4	72	180-82 (B)	$C_{18}H_{18}ClN_3OS$ (359.5)	60.08 60.20	5.00 5.10	11.68 11.70
29	H	2-Furyl	80	160-62 (A)	$C_{14}H_{11}ClN_2O_2S$ (306.5)	54.81 55.00	3.58 3.50	9.13 10.00
30	H	2-Thienyl	82	170-72 (C)	$C_{14}H_{11}ClN_2OS_2$ (322.5)	52.09 52.00	3.41 3.50	8.68 8.50
31	CH_3	C_6H_5	60	190-92 (A)	$C_{17}H_{15}ClN_2OS$ (330.5)	61.72 61.50	4.53 4.50	8.47 8.30
32	CH_3	4-CH_3-C_6H_4	72	197-98 (B)	$C_{18}H_{17}ClN_2OS$ (344.5)	62.69 62.30	4.93 5.00	8.12 8.50
33	CH_3	2-NH_2-C_6H_4	56	197-99 (C)	$C_{17}H_{16}ClN_3OS$ (345.5)	59.04 59.00	4.63 4.50	12.15 12.30
34	CH_3	4-OCH_3-C_6H_4	62	180-82 (B)	$C_{18}H_{17}ClN_2O_2S$ (360.5)	59.91 60.00	4.71 4.50	7.76 7.90
35	CH_3	4-Cl-C_6H_4	67	212-13 (B)	$C_{17}H_{14}Cl_2N_2OS$ (365)	55.89 55.70	3.83 3.50	7.67 7.90
36	CH_3	4-Br-C_6H_4	75	218-19 (B)	$C_{17}H_{14}BrClN_2OS$ (409.5)	49.81 50.00	3.41 3.50	6.83 7.00

a) Crystallization solvents : (A) = EtOH-H_2O, (B) = EtOH, (C) = EtOH-dioxane

Table 3: 2-Substituted Thio-5-[2-(3-chlorobenzo[*b*]thienyl]-1,3,4-oxadiazoles **38-41**

Compd No.	R	Yield %	Mp °C (cryst. solvent)[a]	Molecular Formula	Analysis % Calc/F C	H	N
38	CH_3	69	241-42 (A)	$C_{11}H_7ClN_2OS_2$ (282.5)	46.72 46.00	2.47 2.50	9.91 10.00
39	C_2H_5	50	199-201 (A)	$C_{12}H_9ClN_2OS_2$ (296.5)	48.56 48.50	3.03 3.00	9.44 9.20
40	$CH_2CH{=}CH_2$	70	105-107 (A)	$C_{13}H_9ClN_2OS_2$ (308.5)	50.56 50.70	2.91 3.00	9.07 9.00
41	$CH_2CH_6H_5$	60	127-28 (B)	$C_{17}H_{11}ClN_2OS_2$ (358.5)	56.90 57.00	3.06 3.20	7.81 7.80

a) Crystallization solvents : (A) = EtOH - H_2O, (B) = EtOH

Protein Determination

The protein content of the mitochondrial homogenate was determined according to the method of *Lowry* et al.[32]. The amount of protein present was determined using a pre-constructed standard curve of bovine serum albumin.

Mitochondrial Monoamine Oxidase (MAO) Activity Determination

The method described by *Udenfriend* et al.[26] using serotonin (5-HT) as substrate was used for the determination of MAO-type A activity. 0.05 M Phosphate buffer pH 7.4 (1 ml) containing 5-HT (60 µg) was added to the mitochondrial suspension (1 ml) in 0.05 M phosphate buffer containing 2

Table 4: Effect of benzo[*b*]thienylhydrazone, hydrazine and 1,3,4-oxadiazole derivatives on the activity of MAO of rat liver mitochondria[a)]

Compound No.	ug serotonin consumed/ mg protein/h	(n)[b)]	P	% MAO inhibition
Control	22.92 ± 0.65	(8)	-	-
2	16.92 ± 0.78***	(5)	< 0.001	22.62 ± 3.28
6	20.99 ± 1.87	(4)	> 0.05	7.24 ± 2.04
10	18.045 ± 0.65***	(4)	< 0.001	20.28 ± 1.15
12	10.59 ± 0.84***	(5)	< 0.001	53.14 ± 4.72
13	17.505 ± 5.29***	(6)	< 0.001	22.73 ± 2.93
14	8.47 ± 2.12***	(6)	< 0.001	69.98 ± 7.71
15	7.05 ± 2.07***	(7)	< 0.001	75.00 ± 7.86
18	18.40 ± 1.15**	(4)	< 0.01	18.80 ± 3.64
19	18.55 ± 0.59***	(5)	< 0.001	22.35 ± 3.22
20	20.54 ± 0.96	(5)	> 0.05	12.20 ± 1.82
24	3.94 ± 1.56***	(5)	< 0.001	82.50 ± 6.93
25	11.76 ± 2.30***	(5)	< 0.001	47.12 ± 9.82
26	12.12 ± 2.13***	(6)	< 0.001	45.22 ± 9.54
27	18.44 ± 1.05**	(6)	< 0.01	21.10 ± 2.99
32	8.12 ± 1.64***	(6)	< 0.001	63.93 ± 6.96
33	14.57 ± 1.69***	(5)	< 0.001	38.50 ± 4.90
35	2.42 ± 1.55***	(5)	< 0.001	89.60 ± 6.97
36	9.63 ± 1.40***	(5)	< 0.001	57.56 ± 6.14
37	10.23 ± 2.67***	(5)	< 0.001	61.23 ± 10.87
39	13.72 ± 2.24**	(5)	< 0.01	35.30 ± 9.45
40	17.75 ± 0.13***	(3)	< 0.001	20.30 ± 3.05
41	18.006 ± 1.07**	(5)	< 0.01	20.32 ± 4.80
Pargyline HCl	13.83 ± 0.36***	(4)	< 0.001	39.66 ± 1.57

[a)] The results were expressed as mean ± SEM. Data were analyzed by one way of variance. Student's t test for unpaired observations was used. Differences between means were considered significant if $P < 0.05$.

[b)] n = Number of separate experiments indicated in parentheses

mg protein. Drugs dissolved in propylene glycol were added to give a final concentration of 1 x 10^{-4} M and the mixtures were incubated at 37°C for 60 min. 1-Nitroso-2-naphthol (0.1% in 95% EtOH) (1 ml) and "HNO_2 reagent" (0.2 ml of 2.5% $NaNO_2$ in 5 ml 2 N H_2SO_4) (1 ml) were added. The tubes were shaken, placed in a water bath at 55°C for 5 min and treated with ethylene dichloride (5 ml), shaken to extract the unreacted nitrosonaphthol and then centrifuged at low speed. The violet colour developed in the aqueous layer was measured at 540 nm. The activity was expressed in terms of µg serotonin consumed/mg protein/h (table 4, figs. 1 and 2).

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