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SYNTHESIS OF CERTAIN NITROPYRIDINE DERIVATIVES BEARING 2-THIAZOLYL HYDRAZINES WITH EXPECTED MONOAMINE OXIDASE INHIBITORY ACTIVITY

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تم تحضير سلسلتين من مركبات النيتروبيريدين المحملة ب٢- ثيازوليل هيدرازين و قد تم اختبار فعالية المركبات المحضرة كمثبطات لمؤكسدات الأمين الأحادية من الخارج على ميتاكوندريا الكبد بواسطة التحليل الفلوروميترى للكينورامين.

تم تحضير المركبات ن-(٦- ميثيل-٥ -نيتروبيريدين-٢-ايل ميثيليدين)-ن- (٤- محتل ثيازول-٢-ايل) هيدرازين (٣أ-و) و ٦-ميثيل-٥- نيتروبيريدين-٢- حمض كربوكسيليك ن- (٤- محتل ثيازول-٢ أيل) هيدرازيدات (٨أ-هـ) بتكاثف الوسيطين ٢ و ٧ مع ألفا هالوجينوكيتون ، كلوروأسيتون أو بروميدات الفينيسيل.

وقد أظهرت كل المركبات فعالية كمثبطات لمؤكسدات الأمين الأحادية من الخارج عند التركيز ٠,٣٣ - ١,٦٦ x ١٠ $^{-٥}$ عيارى.

Two series of nitropyridine derivatives bearing 2-thiazolyl hydrazines were synthesized and evaluated for their monoamine oxidase (MAO) inhibitory activity by in vitro tests to assay their effect on rat liver mitochondria by a kynuramine fluorimetric assay.

The two key intermediates, 6-methyl-5-nitropyridine-2-carboxaldehyde thiosemicarbazone (2) and 1-(6-methyl-5-nitropicolinoyl) thiosemicarbazide (7), were prepared by conventional methods. The target compounds, N-(6-methyl-5-nitropyridin-2-ylmethylidene)-N′-(4-substituted thiazol-2-yl) hydrazines (3a-f) and 6-methyl-5-nitropyridine-2-carboxylic acid N′-(4-substituted thiazol-2-yl) hydrazides (8a-e), were prepared by condensation of 2 or 7 with the appropriate α-halogeno-ketone, chloroacetone or Phenacyl bromides. All the assayed compound posses in vitro monoamine oxidase inhibitory activity at a concentration of 0.33 - 1.66 x 10^{-5} M.

INTRODUCTION

Depression is a very widespread mental disease in the adult population. The first antidepressant candidates for clinical testing appeared around the middle of this century in the form of monoamine oxidase inhibitors (MAOIs). Monoamine oxidase (MAO) is a flavoenzyme located on the outer wall of mitochondria and it is known to be the enzyme responsible, within the central nervous system, for the metabolic inactivation of some neurotransmitters, such as serotonin, norepinehrine and dopamine.[1,2] In the last decades, the synthesis of a new generation of MAOIs has been developed to obtain more selective molecules with a reversible and a more rapid onset of activity and, at the same time, having a greater therapeutic safety and a lower toxicity against liver and cardiovascular system (hypertensive crisis).[3,4] The role of MAOIs in hypertensive crisis is due to an indirect potentiation of sympathomimetic amines, such as tyramine.[5] Hydrazino compounds and hydrazones[6,7] have been extensively studied for their potentiality as therapeutic agents for the treatment of hypertension and CNS depression. Several researches were directed to the preparation of heterocyclic hydrazines and hydrazides, since iproniazide had demonstrated *in vitro* activity.[8-10] It is also known that the nature of the heterocyclic residue present could increase the potency of the inhibitor.[11]

As a continuation of our interest for the

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preparation of biologically active nitropyridines,[12-14] as well as pyridines with MAO inhibitory activity.[15] The present investigation is concerned with the synthesis of two series of nitropyridine derivatives incorporating the 2-thiazolyl hydrazines with MAO inhibitory activity[8,9] in the hope that the resulting compounds have more potent MAO inhibitory activity. The prepared compounds were evaluated for their MAO inhibitory activity by *in vitro* tests to assay their effect on rat liver mitochondria by a kynuramine fluorimetric assay and their structure-activity-relationship (SAR) were discussed.

EXPERIMENTAL

A- Chemistry

All melting points were determined in an open capillary tube apparatus and are uncorrected. Elemental microanalysis was performed by the Microanalysis Unit, Faculty of Science, Assiut University and the Microanalysis Unit, Faculty of Science, Cairo University. IR spectra were recorded on a Shimadzu 740 spectrometer as KBr discs. ^{1}H-NMR spectra were recorded on an EM-360 60 MHz Varian NMR spectrometer and JEOL JNM-EX 270 MHz spectrometer, with tetramethylsilane (TMS) as an internal standard, and the chemical shift values are given in δ ppm. Dimethyl sulfoxide-d_6 (DMSO) was used as the solvent, unless otherwise noted. The purity of the compounds was confirmed by TLC. Mass spectra (MS) were obtained on a JEOL/JMS-HX/HX 110 A spectrometer, Hewlett Packard MS-5988 at 70 ev and JEOL JMS 600.

The starting material 6-methyl-5-nitropyridine-2-carboxaldehyde (1)[16,17] was obtained by the conventional nitration of 2,6-dimethyl pyridine followed by oxidation with SeO_2 of the resulting 3-nitro-2, 6-dimethyl pyridine.[18]

6-Methyl-5-nitropyridine-2-carboxaldehyde thiosemicarbazone (2). A mixture of 1[16] (8.3 g, 0.05 mol), thiosemicarbazide (4.55 g, 0.05 mol) in absolute ethanol (100 ml), was heated under reflux, with stirring for one hour. The crystalline product separated, was filtered and recrystallized from ethanol to yield compound 2 (10.4 g, 87 %), mp 222-223°C.

Anal. Calcd for $C_8H_9N_5O_2S$ (239.25): C, 40.16; H, 3.79; N, 29.27 Found: C, 39.81; H, 3.46; N, 28.89. IR (KBr): 3350, 3330, 3150, 2980, 1622, 1574, 1531, 1338 cm^{-1}. ^{1}H-NMR (DMSO-d_6) (270 MHz), δ: 11.88 (1H, s, NHCS, D_2O exchangeable), 8.51 (2H, brs, NH_2, D_2O exchangeable), 8.42 (1H, d, J= 8.43 Hz, pyridine H-4), 8.33 (1H, d, J= 8.43 Hz, pyridine H-3), 8.07 (1H, s, CH=N), 2.75 (3H, s, 6-CH_3). ^{13}C-NMR (DMSO-d_6) δ: 178.404, 156.14, 152.484, 143.955, 139.873, 132.467, 118.080, 23.490. MS, m/z (%): 240 (M^++1, 20.8), 239 (M^+, 100), 179 (54.17), 105 (16.66), 93 (29.17).

N-(6-Methyl-5-nitropyridin-2-yl methylidene)-N´-(4-methyl thiazol-2-yl) hydrazine (3a). To a mixture of 2 (1.2 g, 0.005 mol) and fused sodium acetate (0.41 g, 0.005 mol) in 25 ml DMF/acetone (1:9), excess chloroacetone (2.5 ml) in acetone 10 ml was added with stirring. The mixture was refluxed with continuous stirring for 3 h and the solvent was concentrated under vacuum. The residue was collected by filtration, washed with water and crystallized (Table 1). MS, m/z (%): 278 (M^++1, 11.8), 277 (M^+, 80.39), 164 (2.3), 141 (6.99); 140 (100); 138 (11.81), 69 (10.51).

N-(6-Methyl-5-nitropyridin-2-yl methylidene)-N´-(4-substituted thiazol-2-yl) hydrazines (3b-f). To a suspension of 2 (1.2 g, 0.005 mol) in ethanol (20 ml), was added an ethanolic solution (20 ml) of the appropriate phenacyl bromide (0.005 mol). The mixture was heated under reflux for 2 hours, with continuous stirring and left to cool. The crystalline product was collected by filtration, washed with ethanol and crystallized from the appropriate solvent (Table 1). MS data; Compound 3b, m/z (%): 341 (M^++2, 11.8); 340 (M^++1, 27.2); 339(M^+, 100), 202 (70.3), 176 (19). Compound 3c, m/z (%): 419(M^++2, 29.47), 418 (M^++1, 6.73), 417 (M^+, 29.56), 282 (72.16), 280 (72.79), 256

(19.56), 174 (100), 138 (41.58). Compound **3d**, m/z (%): 375 (M^++2, 55.1), 374 (M^++1, 65.8), 373 (M^+, 100), 372 (86.5), 236 (61.5), 210 (20.6), 138 (18.6). Compound **3e**, m/z (%): 371 (M^++2, 6.97), 370 (M^++1, 20.82), 369 (M^+, 100), 339 (7.63), 232 (59.59), 164 (24.49). Compound **3f**, m/z (%): 386 (M^++2, 7.1), 385 (M^++1, 29.5), 384 (M^+, 100), 338 (11.7), 247 (89.7), 221 (17.9), 138 (25.3).

1-(6-Methyl-5-nitropicolinoyl) thiosemicarbazide (7). A mixture of 6-methyl-5-nitropyridine-2-carboxylic acid hydrazide (**6**)[19] (9.8 g, 0.05 mol) and ammonium thiocyanate (11.4 g, 0.15 mol), in 20% hydrochloric acid (100 ml), was heated under reflux for 2 hours, then cooled. The resulting precipitate, was collected by filtration and crystallized from acetonitrile/ethanol (1:2) to give 9.5 g of **7** (74.5%), mp. 205-206°C as reported.[14] IR: 3410, 3275, 1680, 1611, 1519, 1344 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 10.66 (1H, s, CONH, D_2O exchangeable), 9.46 (1H, s, NH, D_2O exchangeable), 8.56 (1H, d, J= 8.5 Hz, pyridine H-4), 8.07 (1H, d, J= 8.5 Hz, pyridine H-3), 7.69 (2H, br s, NH_2, D_2O exchangeable), 2.82 (3H, s, 6-CH_3). MS, m/z (%): 257 (M^++2, 4.65), 256 (M^++1, 10.14), 255 (M^+, 83.04), 238 (45.78), 165 (37.74), 138 (100), 121 (10.14), 91 (39.56).

6-Methyl-5-nitropyridine-2-carboxylic acid N´-(4-methyl thiazol-2-yl) hydrazide (8a). A suspension of **7** (1.28 g, 0.005 mol) in 10 ml DMF/acetone (2:8), was added with stirring to a mixture of chloroacetone (2.5 ml) and fused sodium acetate (0.41 g, 0.005 mol) in 20 ml acetone. The mixture was heated under reflux for 6 hours and the solvent was concentrated under vacuum. The residue was triturated with water, the solid was collected and crystallized (Table 3). MS, m/z (%): 294 (M^++1, 1.9); 293 (M^+, 9.94), 181 (58.34), 165 (6.34), 138 (100), 137 (13.52), 128 (5.92), 121 (2.52), 114 (4.8), 65 (24.94).

6-Methyl-5-nitropyridine-2-carboxylic acid N´-(4-substituted thiazol-2-yl) hydrazides (8b-e). A mixture of **7** (1.28 g, 0.005 mol) and the appropriate phenacyl bromide (0.005 mol) in 30 ml DMF/ethanol mixture (1:9), was heated under reflux for 6 hours. The solvent was concentrated under vacuum, the residue was triturated with sodium bicarbonate solution (20%). The solid was collected, washed with water and crystallized from appropriate solvent (Table 3). MS data: Compound **8b**, m/z (%): 357 (M^++2, 6.92), 356 (M^++1, 20.05), 355 (M^+, 100), 190 (80.85), 181 (13.38), 165 (3.78), 138 (55.46). Compound **8c**, m/z (%): 435 (M^++2, 99.1), 434 (M^++1, 21.73), 433 (M^+, 100), 270 (56.82), 268 (62.56), 254 (6.52), 165 (14.47), 138 (59.85). Compound **8d**, m/z (%): 391 (M^++2, 21.5), 390 (M^++1, 76.6), 389 (M^+, 51.2), 388 (56), 224 (91.8), 210 (95.1), 181 (100), 165 (65.5), 138 (85.50), 121 (17.9). Compound **8e**, m/z (%): 386 (M^++1, 4.5), 385 (M^+, 4.3), 384 (38.6), 247 (75.8), 233 (34.3), 220 (31.3), 180 (13.4), 175 (49.6), 165 (28.0), 138 (41.1), 121 (31.3), 105 (100), 91 (22.8).

B- Biochemical assay

MAO enzyme was prepared using tissue homogenizer and centrifuged (Minifuge-2-Heraeus-type 4123). Protein concentration was estimated using Spekol II. Assay of MAO inhibitory activity was performed using SFM 23/B Kontron, Switzer land spectrofluorometer.

Mitochondrial preparations

Crude mitochondrial fractions were obtained from the liver of male albino rats weighing 200-250 g. The animals were decapitated and the organs rapidly removed and homogenized in cold phosphate buffer (0.11 M, pH 7.4) using tissue grinder. The homogenate containing 20% w/v of fresh liver was centrifuged at -4°C for 10 minutes at 4000 rpm. The supernatant was decanted and centrifuged at -4°C for 30 minutes at 4000 rpm to sediment the mitochondrial pellets, which were then suspended in the least amount of phosphate buffer and stored at -20°C. Protein content was determined by the method of Lowry *et al.*[20] using bovine serum albumin (BSA) as the standard.

MAO inhibitory activity determination

Test compounds were dissolved in

DMSO/ethanol (1:4, v/v) and different inhibitor concentrations ($0.33x10^{-5}$ - $1.66x10^{-5}$ M) were used.

Into six to seven incubation tubes, different aliquots of standard solution of the tested compound and phenelzine sulphate was added to 0.5 ml of enzyme preparation (300 μg/ml) then incubated at 37°C for 10 minutes. To each tube 0.4 ml of kynuramine solution (100 μg/ml) and 0.5 ml of phosphate buffer was added and the volume was then completed to 3 ml with water. The mixture was vortexed and incubated at 37°C for 30 minutes. The reaction was then stopped by addition of 2 ml of trichloroacetic acid (TCA, 10%) and the precipitated protein was spundown by centrifugation at 3000 rpm for 10 minutes, 1 ml of the supernatant was pipetted into 2 ml of 1N NaOH and mixed. A control experiment was prepared for each compound at the same time and condition except replacing the compound with water. The solution was activated at 315 nm and measuring the fluorescence intensity at 380 nm against a blank prepared in the same manner but replacing the enzyme preparation with phosphate buffer. MAO inhibiting activity of samples was expressed as % of the control where

$$\% \text{ of inhibition} = \frac{\text{Control - Experiment}}{\text{Control}} \times 100$$

RESULTS AND DISCUSSION

A- Chemistry

The designed compounds **3a-f** and **8a-e** were synthesized via routes shown in Schemes 1 and 2 respectively.

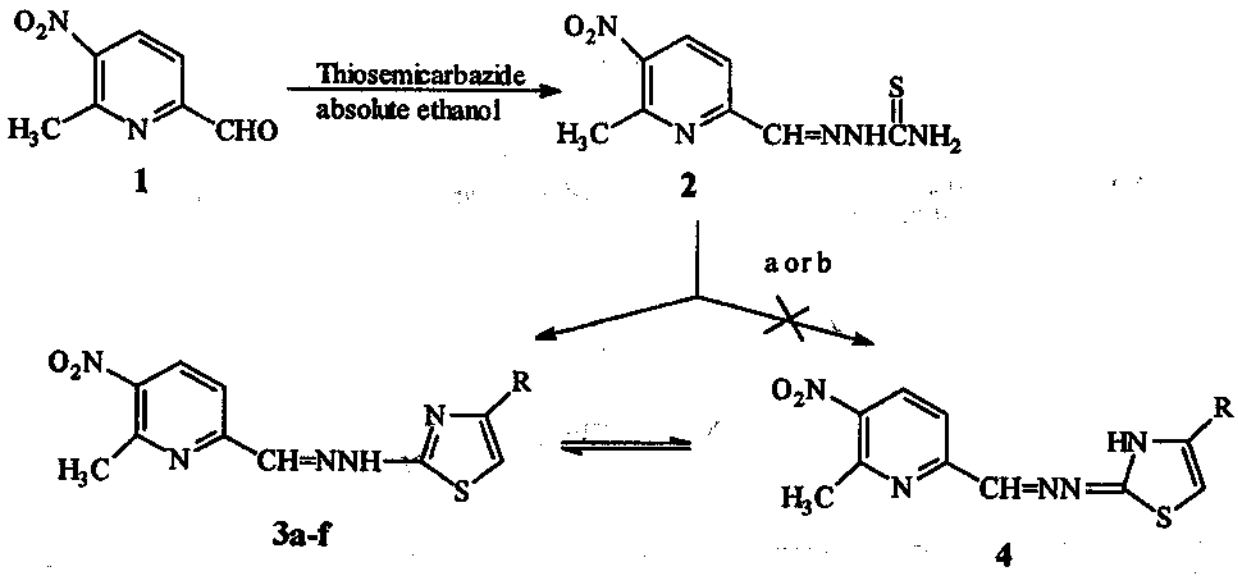
Reaction of the starting material 6-methyl-5-nitropyridine-2-carboxaldehyde (**1**)[16,17] with thiosemicarbazide in absolute ethanol gave the key intermediate **2** in high yield.

6-Methyl-5-nitropyridine-2-carboxylic acid hydrazide (**6**) was prepared from **1** by oxidation with H_2O_2 to give the carboxylic acid **5**,[12] followed by esterification and the ethyl ester was reacted with hydrazine hydrate in absolute ethanol.[19] Reaction of **6** with ammonium thiocyanate in 20% hydrochloric acid afforded the key intermediate **7**. The structure of the two key intermediates **2&7** were performed by spectral data, which are in good agreement with their structures (Experimental section).

Formation of the corresponding thiazolyl hydrazines **3a-f** was accomplished via reaction of **2** with chloroacetone in boiling DMF/acetone in the presence of fused sodium acetate or an appropriately substituted phenacyl bromide in boiling ethanol. The structures of the synthesized compounds were confirmed by elemental analyses, IR, mass and ^{1}H-NMR spectroscopy (Tables 1&2).

It is known that the condensation products of α-haloketones with the thiosemicarbazones are the tautomeric forms of 2-substituted thiazoles[21] i.e. 2-arylidene hydrazinothiazole **3** and/or thiazolone-2-arylidenehydrazones **4**, but the tautomeric form **3** should be preferred according to the literature.[8,22] The IR spectra could not be used to prove the structure is either **3** or **4**. C=N stretching of thiazole ring, hydrazone (or imino) group and -N=N- stretching are characterized by strong absorption band at ν= 1596-1580 cm^{-1}. NH band is detected at ν= 3415-3275 cm^{-1} and the bands for the NO_2 group are detected at the expected range (1557-1549, 1341-1320 cm^{-1}). The ^{1}H-NMR spectra of **3a-f** revealed the disappearance of the NH_2 signal of their precursor **2** and appearance of new singlet signal for H-5 (δ 7.46-6.39), of the thiazole ring for compounds **3a-e**. Compound **3f** showed signal for H-5 at δ 8.8 ppm, due to the deshileding effect of the nitro group and the solvent effect (Table 2). The NH singlet signal at about 12.79-12.3 ppm strongly and definitively indicated that this proton (H) is carried by the nitrogen atom attached to 2-position, of thiazole ring, to give the -CH=N-NH- group rather than to give the NH moiety of thiazolone ring.[21,22] This structure is also supported by the presence of a one-proton singlet at δ= 8.07-7.8 ppm corresponding to the -CH= proton.

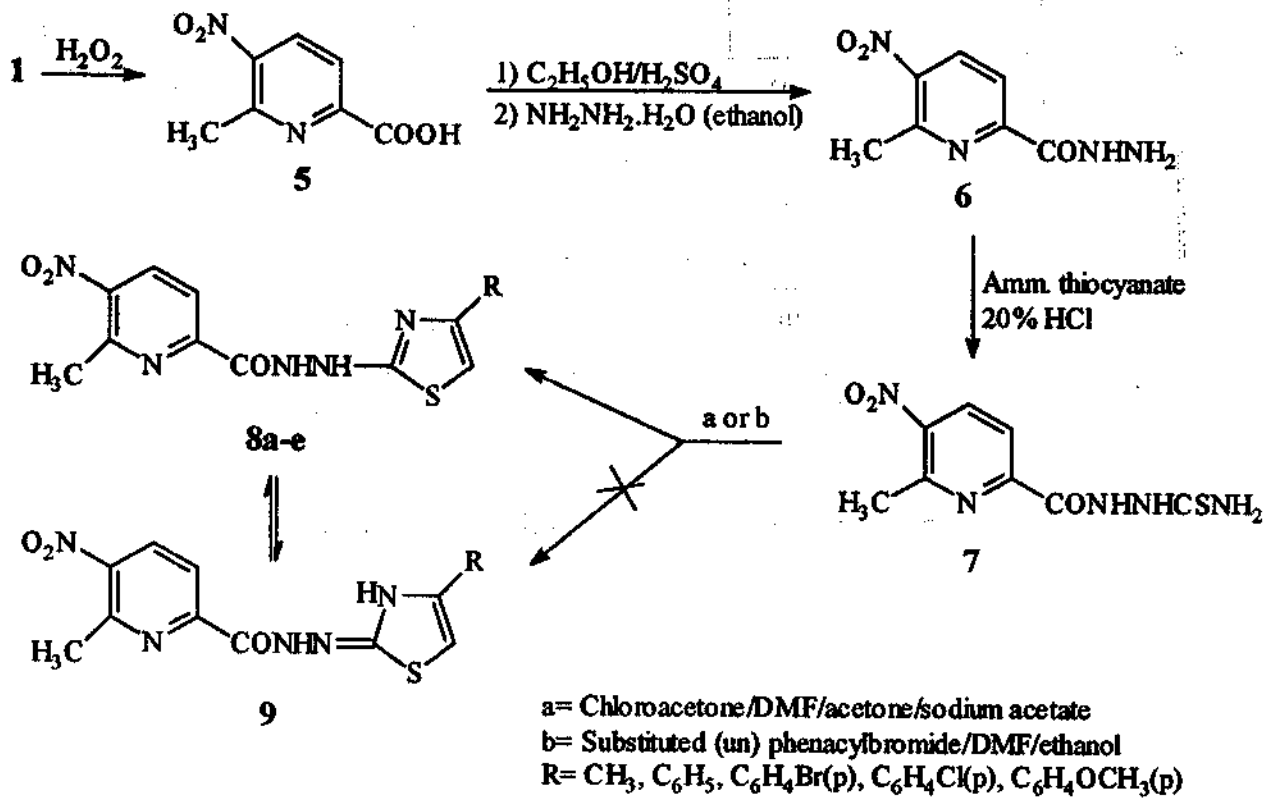
Further reaction of **7** with chloroacetone in boiling DMF/acetone containing fused sodium acetate or with an appropriately substituted



1 2 3a-f 4

a= Chloroacetone/DMF/acetone/sodium acetate
b= Substituted (un) phenacylbromide/absolute ethanol
R= CH_3, C_6H_5, $C_6H_4Br(p)$, $C_6H_4Cl(p)$, $C_6H_4OCH_3(p)$, $C_6H_4NO_2(p)$

Scheme 1

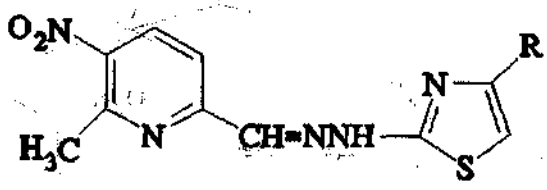


1 5 6 7 8a-e 9

a= Chloroacetone/DMF/acetone/sodium acetate
b= Substituted (un) phenacylbromide/DMF/ethanol
R= CH_3, C_6H_5, $C_6H_4Br(p)$, $C_6H_4Cl(p)$, $C_6H_4OCH_3(p)$

Scheme 2

Table 1 Physical constants of N-(6-methyl-5-nitropyridin-2-yl methylidene)-N´-(4-substituted thiazol-2-yl) hydrazines (3a-f).



Compd. No	R	Yield (%)	mp. (°C) Cryst. Sol.	Mol. Formula (M.W.)	Analysis (%) Calc./Found		
					C	H	N
3a	CH_3	75	235-237 DMF/EtOH	$C_{11}H_{11}N_5O_2S$ 277.30	47.65 48.10	4.00 4.17	25.26 25.09
3b	C_6H_5	95	239-240 DMF/EtOH	$C_{16}H_{13}N_5O_2S$ 339.37	56.63 56.70	3.86 4.10	20.64 21.03
3c	$C_6H_4Br(p)$	93	242-244 CH_3CN/EtOH	$C_{16}H_{12}BrN_5O_2S$ 418.27	45.95 46.35	2.89 3.18	16.74 16.79
3d	$C_6H_4Cl(p)$	90	231-232 CH_3CN/EtOH	$C_{16}H_{12}ClN_5O_2S$ 373.82	51.41 51.30	3.24 3.20	18.73 18.80
3e	$C_6H_4OCH_3(p)$	98	233-234 DMF/EtOH	$C_{17}H_{15}N_5O_3S$ 369.40	55.28 54.90	4.09 4.00	18.96 18.90
3f	$C_6H_4NO_2(p)$	98	269-271 DMF/EtOH	$C_{16}H_{12}N_6O_4S$ 384.37	50.00 50.40	3.15 3.60	21.86 21.50

Table 2: IR & ^{1}H-NMR spectral data of N-(6-methyl-5-nitropyridin-2-yl methyllidene)-N´-(4-substituted thiazol-2-yl) hydrazines (3a-f).

Compd. No	IR (KBr. cm^{-1})	^{1}H-NMR (δ ppm. DMSO-d_6)
3a	3415, 1592, 1557, 1341	12.33 (1H, br s, NH)[a], 8.39 (1H, d, J= 8.5 Hz, pyridine H-4), 8.07 (1H, s, CH= N), 7.84 (1H, d, J= 8.5 Hz pyridine H-3), 6.39 (1H, s, C5-H pf thiazole), 2.79 (3H, s, 6-CH_3 of pyridine), 2.2 (3H, s, 4-CH_3 of thiazole).
3b	3290, 1580, 1549, 1320	12.69 (1H, s, NH)[a], 8.33 (1H,d, J= 8.5 Hz, pyridine H-4), 7.9 (1H, s, CH= N), 7.7 (1H, d, J= 8.5 Hz pyridine H-3), 7.4-7.2 (5H, m, Ar-H), 7.29 (1H, s, C_5-H of thiazole), 2.67 (3H, s, 6-CH_3).
3c	3280, 1584, 1550, 1321	12.79 (1H, s, NH)[a], 8.44 (1H, d, J= 8.9 Hz, pyridine H-4), 8.06 (1H, s, CH= N), 7.92 (1H, d, J= 8.6 Hz pyridine H-3), 7.81 (2H, d, J= 8.58, 3´, 5´ Ar-H), 7.58 (2H, d, J= 8.58, 2´, 6´ Ar-H), 7.46 (1H, s, C_5-H of thiazole), 2.79 (3H, s, 6-CH_3).
3d	3275, 1594, 1551, 1324	12.5 (1H, s, NH)[a], 8.2 (1H,d, J= 8.5 Hz, pyridine H-4), 7.85 (1H, s, CH= N), 7.65 (3H, d, pyridine H-3, 3´, 5´ Ar-H), 7.2 (3H, d, 2´, 6´ Ar-H, C_5-H of thiazole), 2.68 (3H, s, 6-CH_3).
3e	3290, 1582, 1550, 1323	12.5 (1H, s, NH)[a], 8.15 (1H,d, J= 8.5 Hz, pyridine H-4), 7.8 (lH, s, CH= N), 7.7 (1H, d, J= 8.5 Hz pyridine H-3), 7.6 (2H, d, 2´, 6´ Ar-H), 7.06 (1H, s, C_5-H of thiazole), 6.8 (2H, d, 3´, 5´ Ar-H), 3.8 (3H, s, OCH_3), 2.77 (3H, s, 6-CH_3).
3f	1596, 1561, 1338	TFA) 9.27 (1H, d, J= 8.5 Hz, pyridine H-4), 8.95 (1H, s, CH= N), 8.62 (1H, d, J= 8.5 Hz pyridine H-3), 8.55 (2H, d, 3´, 5´ Ar-H), 7.94 (2H, d, 2´, 6´ Ar-H), 7.55 (1H, s, C_5-H of thiazole), 3.26 (3H, s, 6-CH_3).

[a]: Exchangeable in D_2O.

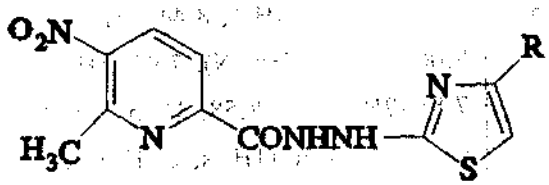
phenacyl bromide in boiling DMF/ethanol gave the target compounds **8a-e** and not their tautomeric isomers **9**, as preferred according to the literature[8,9] (Table 3). IR spectra of the compounds **8a-e** showed two NH bands at ν= 3345-3315, 3225-3155 cm^{-1} in addition to the C=O stretching band at ν= 1696-1671 cm^{-1}. The ^{1}H-NMR spectra of **8a-e** lacked the NH_2 signal of their precursor **7**. The spectra revealed the appearance of a new singlet signal for H-5 (δ 7.26-6.39), of the thiazole ring, and for the -CONHNH- group as two singlets for the amide and hydrazide-NH at δ 11.13-10.98 and δ 10.1-9.8 (D_2O exchange), respectively (Table 4).

The mass spectral fragmentation pattern of all new compounds conforms with the proposed structure.

In compounds **3a-f**, the molecular ion peak (M^+) is always the base peak, except compounds **3a&3c**.

In compounds **8a-e**, the MS of this series showed that the breakage can occur at multiple sites along the hydrazine chain, giving different base peaks (Experimental Section).

Table 3: Physical constants of N-(6-methyl-5-nitropyridin-2-carboxylic acid-N´-(4- substituted thiazol-2-yl) hydrazides (**8a-e**).



Compd. No	R	Yield (%)	mp. (°C) Cryst. Sol.	Mol. Formula (M.W.)	Analysis (%) Calc./Found		
					C	H	N
8a	CH_3	73	221-223 DMF/EtOH	$C_{11}H_{11}N_5O_3S$ (293.30)	45.05 45.22	3.75 3.45	23.88 24.30
8b	C_6H_5	76	214-216 DMF/EtOH	$C_{16}H_{13}N_5O_3S$ (355.37)	54.08 53.60	3.69 4.04	19.71 19.80
8c	C_6H_4Br(p)	78	219-221 DMF/EtOH	$C_{16}H_{12}BrN_5O_3S$ (434.27)	44.25 44.42	2.78 2.76	16.13 16.33
8d	C_6H_4Cl(p)	75	213-214 DMF/acetone	$C_{16}H_{12}ClN_5O_3S$ (389.82)	49.30 49.65	3.10 3.25	17.97 18.23
8e	$C_6H_4OCH_3$(p)	75	225-226 DMF/EtOH	$C_{17}H_{15}N_5O_4S$ (385.40)	52.98 53.15	3.92 3.72	18.17 18.45

Table 4: IR & ^{1}H-NMR spectral data of 6-methyl-5-nitropyridine-2-carboxylic acid-N´-(4-substituted thiazol-2-yl) hydrazides (8a-e).

Compd. No	IR (KBr. cm^{-1})	^{1}H-NMR (δ ppm. DMSO-d_6)
8a	3315, 3185, 1696, 1594, 1523, 1341	10.98 (1H, s, NH)[a], 10.35 (1H, br s, NH)[a], 8.6 (1H, d, J= 8.5 Hz, pyridine H-4), 8.2 (1H, d, J= 8.5 Hz pyridine H-3), 6.39 (1H, s, C_5-H of thiazole), 2.89 (3H, s, 6-CH_3 of pyridine), 2.29 (3H, s, 4-CH_3 of thiazole).
8b	3345, 3195, 1671, 1524, 1327	11.2 (1H, s, NH)[a], 9.8 (1H, br s, NH)[a], 8.56 (1H, d, J= 8.5 Hz, pyridine H-4), 8.09 (1H, d, J= 8.5 Hz pyridine H-3), 7.8 (2H, m, Ar-H), 7.35 (3H, m, Ar-H), 7.13 (1H, s, C_5-H of thiazole). 2.83 (3H, s, 6-CH_3).
8c	3400, 3155, 1679, 1597, 1540, 1331	11.13 (1H, s, NH)[a], 9.88 (1H, br s, NH)[a], 8.63 (1H, d, J= 8.5 Hz, pyridine H-4), 8.2 (1H, d, J= 8.5 Hz pyridine H-3), 7.8 (2H, d, 3´, 5´ Ar-H), 7.6 (2H, d, 2´, 6´ Ar-H), 7.26 (1H, s, C_5-H of thiazole), 2.9 (3H, s, 6-CH_3).
8d	3400, 3175, 1668, 1597, 1520, 1323	11.19 (1H, s, NH)[a], 9.85 (1H, br s, NH)[a], 8.67 (1H, d, J= 8.5 Hz, pyridine H-4), 8.2 (1H, d, J= 8.5 Hz pyridine H-3), 7.9 (2H, d, 3´, 5´ Ar-H), 7.45 (2H, d, 2´, 6´ Ar-H), 7.25 (1H, s, C_5-H of thiazole), 2.92 (3H, s, 6-CH_3).
8e	3440, 3225, 1695, 1595, 1522, 1335	11.1 (1H, s, NH)[a], 9.8 (1H, br s, NH)[a], 8.65 (1H, d, J= 8.5 Hz, pyridine H-4), 8.15 (1H, d, J= 8.5 Hz pyridine H-3), 7.8 (2H, d, 2´, 6´ Ar-H), 7.1-6.8 (3H, m, C_5-H of thiazole, 3´, 5´ Ar-H), 3.85 (3H, s, OCH3) 2.82 (3H, s, 6-CH_3).

[a]: Exchangeable in D_2 O.

B- *In vitro* MAO inhibition

The fluorimetric method of Krajel[23] for *in vitro* determination of MAO inhibitory activity were selected to test the prepared compounds, as well as the reference compound, phenelzine sulphate. The test depends on oxidative deamination of kynuramine substrate by the MAO enzyme to 4-hydroxy quinoline.

The amount of the product formed can be measured spectrofluorimetrically at 315 nm for excitation and 380 nm for emission. Under controlled standard conditions the fluorescence intensity is directly proportional to enzyme activity. Mitochondrial MAO was obtained by homogenizing liver rat. Protein concentration of the enzyme preparation was estimated by the method of Lowry *et al.*,[20] using crystalline bovine serum albumin (BSA) to construct a standard calibration curve. Standard conditions were selected after investigating the effect of protein concentration, substrate concentration and incubation time on the enzyme activity. Optimal conditions were found to be 300 μg protein concentration, 100 μg kynuramine concentration and 30 minutes incubation time. All the examined compounds displayed a MAOI

activity at a concentration of 0.33-1.66 x 10^{-5} M. The results of *in vitro* MAOI activity of the tested compounds at concentration (1.33 x 10^{-5} M) are listed in Table 5. From a comparison of the two series of the synthesized derivatives, it is possible to outline as follows. Within the series **3a-f**, with, CH=NNH grouping, compound **3a** bearing methyl group in the 4-position of the thiazole nucleus showed the highest activity (93%). The 4-methoxy phenyl derivative **3e** is more active than the 4-phenyl derivative **3b**, while the 4-bromo phenyl derivative **3c** is lightly less active. On the contrary, in, the series **8a-e**, with, CONHNH grouping, compound **8b**, bearing phenyl group in the 4-position of the thiazole nucleus, showed the highest activity (96%), while the 4-methyl derivative **8a** showed the lowest activity.

On the basis of these results, it can be stated that all the assayed compounds posses *in vitro* monoamine oxidase inhibitory activity.

Table 5: *In vitro* MAOI activity on rat liver mitochondria of 2- thiazolyl hydrazines **3** and **8** (1.33x 10^{-5} M) by kynuramine fluorimetric assay.

Compd. No.	Inhibition (%)	Compd. No.	Inhibition (%)
3a	93	8a	69
3b	85	8b	96
3c	80	8c	82
3e	88	8e	85
3f	83		
Ref. st*.	99		

* Phenelzine sulfate

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