Synthesis and Antitumor Activity of New Thiosemicarbazones of 2-Acetylimidazo[4,5-*b*]pyridine

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A number of thiosemicarbazones of 2-acetyl-imidazo[4,5-*b*]pyridine were prepared in order to investigate their *in vitro* antineoplastic activities. Three compounds: (i) 2-acetylimidazo[4,5-*b*]pyridin-4-*sec*-butyl-3-thiosemicarbazone [(A_7), NSC674098], (ii) 2-acetylimidazo[4,5-*b*]pyridin-4-*tert*-butyl-3-thiosemicarbazone [(A_9), NSC674099], (iii) 2-acetylimidazo[4,5-*b*]pyridin-4-cyclohexyl-3-thiosemicarbozone [(A_{11}), NSC674101] showed remarkable activity against some of the cell lines tested. The Biological Evaluation Committee of N.C.I. determined that further secondary testing should be carried out (these compounds were tested against prostate cancer).

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Introduction.

There is a strong degree of correlation between tumor growth rate and the enzyme ribonucleoside diphosphate reductase (RDR) [1]. In sharp contrast, two other enzymes involved in deoxy-ribonucleotide synthesis, namely thymidilate synthetase and thymidine kinase did not exhibit such a close degree of correlation. Hence, it has been suggested that an inhibitor of RDR would be an important chemotherapeutic substance for the treatment of rapidly growing cancers.

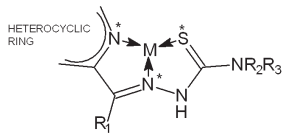
In 1956, Brockman *et al*. [2] demonstrated that administration of 2-formyl-pyridine thiosemicarbazone increased the life span of mice inoculated with 1210 Leukemia. Later other investigators [3] confirmed the anticancer properties of a-(N)-heterocyclic carboxaldehydes thiosemicarbazones with different types of cancer.

Therefore, thiosemicarbazones belong to a class of compounds that occupy a wide spectrum of medicinal properties and have been studied for their activity against tuberculosis [4], bacterial [5] and viral infections [6,7].

Investigation of this interesting class of compounds still continues. Structure-activity relationship studies showed that a large number of thiosemicarbazones of a-(N)-heterocyclic carboxaldehydes have the following characteristics: (a) π-electron density at the point of attachment in the formal thiosemicarbazone side chain should be low and, (b) the ring nitrogen atom should be reasonably a good electron pair donor to transitions metals for the formation of coordination compounds. Their anticancer activity studies have shown that the carbonyl group must be in a position α to the heteroaromatic nitrogen atom.

Inhibition of DNA synthesis, in mammalian cells, by thiosemicarbazones is due to coordination of iron by these agents though their N*-N*-S* thiodentate ligand system, either by a preformed iron complex binding to the enzyme or by the free ligand complexing with the iron-charged enzyme [8-10].

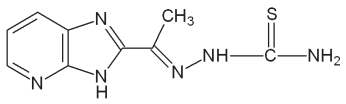
Scheme 1



where R_1, R_2, R_3 : H, alkyl, etc

It has been found that complexes of several thiosemicarbazones with iron (II), isolated by Pettering *et al*. [11], were 3-6 times more active as inhibitors of RDR than the free ligands. Thiosemicarbazones and their complexes are usually insoluble in water.

Our new derivatives of thiosemicarbazones of 2-acetylimidazo[4,5-*b*]pyridine contain one more heterocyclic nitrogen atom, which gives the advantage that this molecule, or its complexes with divalent metals, form hydrogen bonds with water resulting in more hydrophilicity and likely better biological action. So, we were encouraged to explore the synthesis of new derivatives of thiosemicarbazones of 2-acetylimidazo[4,5-*b*]pyridine. Our strategy is to modify molecule (A) by replacing the hydrogen atoms of the $–NH_2$ with different groups and to correlate these structural differences with their biological action.

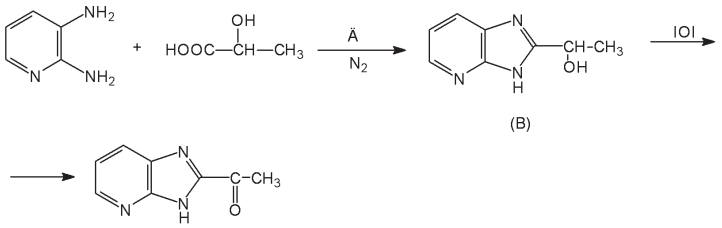


(A)

Chemistry.

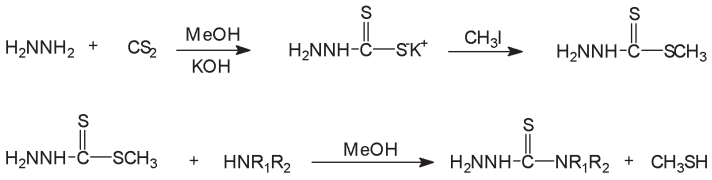
The 2-acetylimidazo[4,5-*b*]pyridine (C) was prepared by the condensation reaction of 2,3-diaminopyridine with lactic acid and then oxidation of the *sec*-alcohol (B) to the corresponding ketone (C).

Scheme 2



(B)

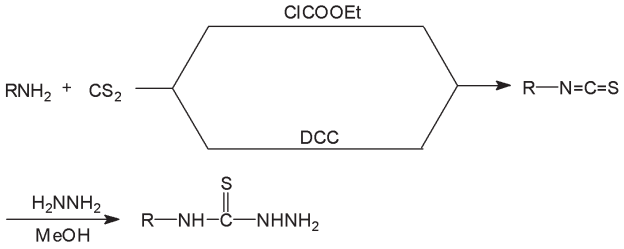
Scheme 3



where R_1R_2 = H, alkyl etc.

N^4-Monosubstituted or N^4,N^4-disubstituted thiosemicarbazides were prepared by one of the two different methods: i) by aminolysis of *S*-methyl-dithiocarbazate, CH_3-S-(C=S)-$NHNH_2$, according to the reactions in references [12,13], and ii) from hydrazine and isothiocyanates according to reactions in references [15,16].

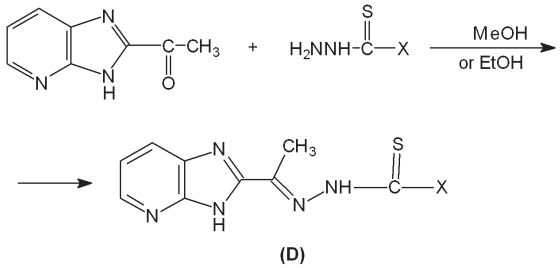
Scheme 4



where R = alkyl, phenyl etc.

Condensation of (C) with the appropriate thiosemicarbazide gave the corresponding thiosemicarbazone.

Scheme 5



(D)

where X could be : see table I

Results and Discussion.

Out of the compounds prepared sixteen (Table IV) were selected by N.C.I. to be screened for their *in vitro* antitumor activity against 60 human cell lines derived from nine clinically isolated cancer types. (leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate and breast) according to a standard protocol (conducted at the National Cancer Institute, Bethesda, MD, USA).

From the sixteen compounds in Table IV six were selected for secondary evaluation against the same 60 human cell lines and 3 of them (NSC674098, NSC674099, NSC 674101 were sent to the Biological Evaluation Committee who determined that further secondary testing should be carried out, these compounds were tested against prostate cancer (Table V).

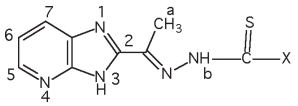
The compounds NSC674098, NSC674101 tested in the *in vitro* disease – oriented antitumor screen have been further evaluated in an investigational AIDS related lymphoma (ARL) screen *in vitro*. The ARL screen utilizes 5 human lymphoma cell lines (2 established from AIDS patients), which grow as cell suspensions and CCRF-CEM a leukemia cell line included in the antitumor screen.

Like the antitumor screen, agents are tested at five and tenfold dilutions from a maximum concentration of $1x10^{-4}$ *M* to $1x10^{-8}$ *M* for 48 hours continuous exposure.

From the compounds that were exposed to the test, compound A_1 exhibited a weak antineoplastic activity. The tests show a growth inhibition of around 50% at concentrations 10^{-4} *M*. This compound also showed activity against numerous cell lines of Leukemia and ovarian cancer. However the sensitivity of these cell lines was not enough above the average.

The same activity was observed for compound A_2 with the difference being that the cell lines OVCAR-4 (ovarian

Table I Thiosemicarbazones 2-acetyl-imidazo[4,5-b]pyridine



	Thiosemicarbazones	X
A_1	2-acetyl-imidazo[4,5-b]pyridine-3-thiosemicarbazone	
A_2	2-acetyl-imidazo[4,5-b]pyridine-4-methyl-3-thiosemicarbazone	
A_3	2-acetyl-imidazo[4,5-b]pyridine-4-ethyl-3-thiosemicarbazone	
A_4	2-acetyl-imidazo[4,5-b]pyridine-4-n-propyl-3-thiosemicarbazone	
A_5	2-acetyl-imidazo[4,5-b]pyridine-4-isopropyl-3-thiosemicarbazone	
A_6	2-acetyl-imidazo[4,5-b]pyridine-4-n-butyl-3-thiosemicarbazone	
A_7	2-acetyl-imidazo[4,5-b]pyridine-4-sec-butyl-3-thiosemicarbazone	
A_8	2-acetyl-imidazo[4,5-b]pyridine-4-isobutyl-3-thiosemicarbazone	
A_9	2-acetyl-imidazo[4,5-b]pyridine-4-tert-butyl-3-thiosemicarbazone	
A_{10}	2-acetyl-imidazo[4,5-b]pyridine-4-allyl-3-thiosemicarbazone	
A_{11}	2-acetyl-imidazo[4,5-b]pyridine-4-cyclohexyl-3-thiosemicarbazone	
A_{12}	2-acetyl-imidazo[4,5-b]pyridine-4,4-dimethyl-3-thiosemicarbazone	
A_{13}	2-acetyl-imidazo[4,5-b]pyridine-4-pyrolidine-3-thiosemicarbazone	

Table I. (continued)

	Thiosemicarbazones	X
A_{14}	2-acetyl-imidazo[4,5-b]pyridine-4-piperidyl-3-thiosemicarbazone	
A_{15}	2-acetyl-imidazo[4,5-b]pyridine-4-morpholyn-3-thiosemicarbazone	
A_{16}	2-acetyl-imidazo[4,5-b]pyridine-4-benzyl-3-thiosemicarbazone	
A_{17}	2-acetyl-imidazo[4,5-b]pyridine-4-phenyl-3-thiosemicarbazone	
A_{18}	2-acetyl-imidazo[4,5-b]pyridine-4-(p-methyl)phenyl-3-thiosemicarbazone	
A_{19}	2-acetyl-imidazo[4,5-b]pyridine-4-(p-methoxy)phenyl-3-thiosemicarbazone	
A_{20}	2-acetyl-imidazo[4,5-b]pyridine-4-(p-chloro)phenyl-3-thiosemicarbazone	
A_{21}	2-acetyl-imidazo[4,5-b]pyridine-4-(p-nitro)phenyl-3-thiosemicarbazone	

cancer) and VO-31 (renal cancer) were tenfold more sensitive for this agent.

The activity of the compound A_5 was at the same level of the above agents but also this compound showed clear activity against numerous cell lines of Leukemia and colon cancer. While this agent showed remarkable activity against the cell line T-47D (breast cancer). This activity corresponds to a sensitivity 100 fold more than the average value of the above agent. Compound A_7 belongs to the agents that were sent to the Biological Evaluation Committee (B.E.C.) who determined what further secondary testing should be carried out.

Compound A_9 exhibited higher activity than the above agents and also remarkable activity against cell lines of ovarian cancer. This agent indicated, at a concentration $10^{-5.33}$ *M* for the cell line OVCAR-5, a growth inhibition 10 fold more than the average value.

Compound A_{12}, a disubstituted agent with methyl groups, was one of the molecules of this row that showed more dras-

Table II. Substituted thiosemicarbazides by aminolysis of S-methyl-dithio-carbazate

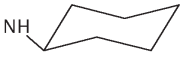
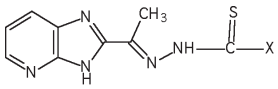
R	Yield: %
$NHCH_3$	30
$NHCH_2CH_2CH_3$	34
$N(CH_3)_2$	26
$NHCH_2Ph$	25
	60
	64
	40
	38

Table III. Substituted thiosemicarbazides, by the reaction hydrazine and isothiocyanates

R	Yield: %
$(CH_3)_2CH$-	90
$CH_3CH_2C(CH_3)H$-	70
$(CH_3)_3C$-	75
Ph-	70
p-CH_3-Ph-	81
p-CH_3O-Ph-	91
p-Cl-Ph-	83

Table IV. Thiosemicarbazones of the 2-acetyl-imidazo[4,5-b]pyridine



a/a	NSC Number	-X	MG-MID(Log_{10}) GI_{50}	TGI	LC_{50}
A_1	674095	-NH_2	-4,26	-4,01	>-4,00
A_2	674096	-NHMe	-4,41	-4,02	>-4,00
A_5	674097	-NHisoPr	-4,93	-4,07	>-4,00
A_7	674098	-NHsecBut	-5,05	-4,11	>-4,00
A_9	674099	-NHtertBut	-5,56	-4,14	>-4,00
A_{12}	674100	-NMe_2	-6,17	-4,47	>-4,00
A_{11}	674101	-NHcyclohexyl	-5,18	-4,03	>-4,00
A_{17}	674102	-NHPh	-5,59	-4,45	>-4,00
A_{20}	674103	-NHPh(p-Cl)	-5,72	-4,68	-4,18
A_{21}	674104	-NHPh(p-NO_2)	-6,13	-5,02	-4,29
A_{19}	674105	-NHPh(p-OMe)	-5,32	-4,19	>-4,00
A_{18}	674106	-NHPh(p-Me)	-5,69	-4,67	-4,15
A_{16}	674107	-$NHCH_2$Ph	-5,13	-4,43	>-4,00
A_{13}	674108		-6,40	-4,59	>-4,00
A_{14}	674109		-5,08	-4,22	>-4,00
A_{15}	674110		-4,46	-4,03	>-4,00

Table V. Antineoplastic activity against prostate cancer

Cell lines	GI_{50} NSC-674098	NSC-674099	NSC-674101
WIS	<1,00E-08	<1,00E-08	3,32E-08
WBW	<1,00E-08	<1,00E-08	1,57E-08
RVP1	<1,00E-08	<1,00E-08	2,60E-08

tic changes in activity. This agent shows remarkable activity against the cell line Non-Small Cell lung Cancer.

Table VI. Testing Results AIDS-related lymphoma (ARL) screen in vitro

Cell lines	GI_{50} NSC-674098	NSC-674101
Leukemia		
CCRF-CEM	3,52E-0_6	1,79E-0_6
AIDS-related lymphoma		
RL	7,18E-0_6	2,31E-0_6
KD488	2,92E-0_6	6,15E-0_6
AS283	2,91E-0_6	4,10E-0_6
PA682	2,64E-0_6	2,05E-0_6
SV-DHL-7	1,86E-0_6	8,78E-0_6

Compound A_{11} showed analogous activity with the agents A_7 and A_9. The introduction of an aromatic ring (A_{17}) in the position of the saturated ring (A_{11}) improves, a little, the activity of this compound but causes a little confusion about the sensitivity of different cell lines.

Compound A_{20} indicated more activity but now there is a new element, that is a remarkable activity against the cell of Melanoma where there is an integral growth inhibition at concentrations $10^{-4.65}$ *M* to $10^{-5.18}$ *M*.

Compound A_{21} was from the compounds with more drastic changes in activity. The cell lines Non-Small cell lung, CNS, Melanoma and ovarian cancer were more sensitive under the effect of this agent.

The compound A_{16} indicated analogous activity with the agent A_{17} while the compound A_{19} showed more activity. This compound showed almost the same activity against all the cell lines, which is an unusual observation.

The "removal" of the aromatic ring from the chain of the thiosemicarbazone with the intercession of a methylene group (compound A_{16}) caused no change to the activity of the molecule and remained almost the same as that of compound A_{17}.

The comprisal of the final nitrogen into a saturated, five membered, ring (A_{13}) gave a compound with remarkable activity, the cell lines of Leukemia continues to be sensitive while the cell lines SF-539 (CNS-cancer) VO-3 (renal cancer) are more sensitive by 100 fold than that of the average value.

The expansion of the size of the ring and the incorporation of a second nitrogen atom gave compounds (B_{14}, B_{15}) with lower activity. (These compounds caused more activity against cell lines of Leukemia in comparison with the remainder of cell lines).

EXPERIMENTAL PROTOCOLS.

Chemistry.

Melting points were determined on a Büchi 535 melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin Elmer 841 using samples in potassium bromide discs.

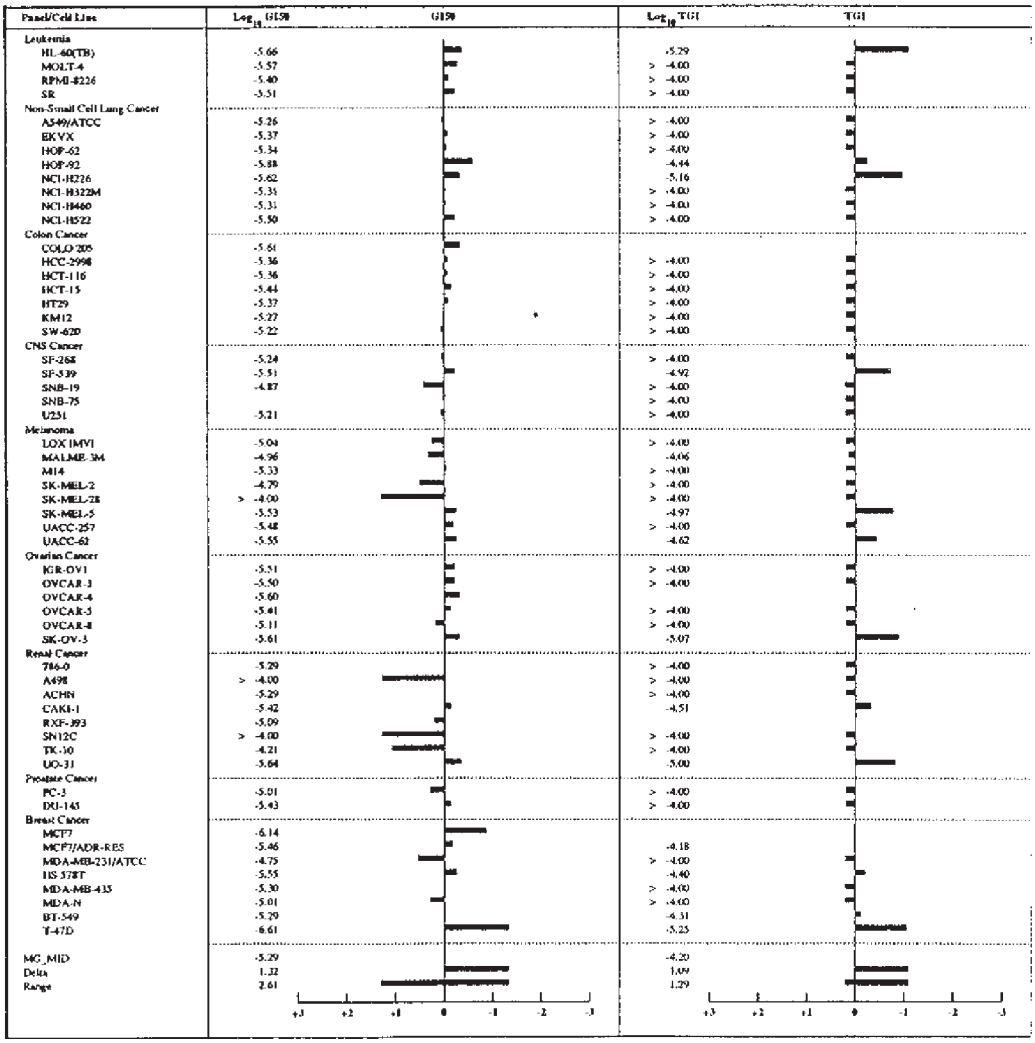


Figure 1. GI_{50} and TGI mean graph from the initial testing of 2-acetylimidazo[4,5-*b*]pyridine-4-cyclohexyl-3-thiosemicarbazone (A_{11} NSC 674101) in the NCI *in vitro* screen.

The ^{1}H-NMR spectra were recorded on a Bruker AC 300 (300 MHz) Hellenic National Research Foundation, Athens, and to a Varian Unity Plus (300 MHz) Laboratory of Inorganic Chemistry Department of Chemistry of University of Athens, using $CDCl_3$, CD_3OD or DMSO-d_6 with tetramethylsilane (TMS) as the internal standard. Elemental analyses (C, H, N) were carried out at: i) Lab. of Inorg. Chemistry Dept. of Chem. Of University of Athens, ii) Lab. of Org. Chemistry Dept. of Chemistry. Aristotle University of Thessaloniki, and iii) Lab. of microanalysis of the Hellenic National Research Foundation Athens.

The R_f values were determined by thin layer chromatography. Rf_A: chloroform-methanol (90:10), Rf_B: chloroform-methanol (80:20), Rf_Γ: chloroform-methanol-acetic acid (90:10:3), Rf_Δ: hexane-ethylacetate-acetic acid (3:3:0.2), Rf_E: chloroform-ethylacetate (9:1).

Synthesis of 2-(1-Hydroxyethyl)imidazo[4,5-*b*]pyridine.

2,3-Diaminopyridine 4.36 g (0.04 mole) and D,L-lactic acid (88%±2) 8.20 g (0.08 mole) with vigorous stirring and under nitrogen atmosphere were heated to 140-150 °C for 2.5 hours. The heating was extended for another 1.5 h to the temperature of 140-150 °C. On cooling, the reaction mixture is treated with a saturated solution of sodium hydrogen carbonate until alkaline reaction (pH =8). The aqueous solution was filtered and concentrated *in vacuo* almost to dryness. To the residue, methanol was added (3x50 ml) and evaporated again until all the water was removed. Then 100 ml of methanol was added and the methanolic solution was filtered and dried over sodium sulfate. The dark solution was treated with charcoal, and then was added to a multifold volume of ether and then was filtrated. The filtrate was concentrated to dryness. The residue was triturated repeatedly with acetone and collected by filtration as yellow pure solid. The yield was 3.11 g (48%). After recrystallization from dioxane the melting point was 166-168 °C; Rf_B: 0.52 Rf_Γ: O.24; GC-MS: m/2 163 (M^+); IR (cm^{-1}, KBr): NH 3360 VS-b, CH(benzene) 3086 VS, CH 2987m, C=C & C=N 1595, C-O 1113; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.49-1.51 (d, 3H, b), 4.93 (q, 1H, a), 5.70-5.85 (d, 1H, -OH), 7.13-7.8 (q, 1H, 6), 7.80-7.91 (dd, 1H, 7), 8.24-8.29 (dd, 1H, 5), 13.9 (s, 1H, 3).

Synthesis of 2-Acetylimidazo[4,5-*b*]pyridine.

Chromium trioxide 3.75 mmol (0.38 g) in water (1.25 ml) was added, dropwise, at a temperature 87-92 °C in a solution of 2-(1-hydroxyethyl)imidazo[4,5-*b*]pyridin 5 mmol (0.82 g) in glacial acetic acid (3.75 ml). The reaction mixture was heated five minutes more at a temperature of 100-106 °C and then was decanted in water (50 ml). The yellow precipitate was collected by filtration washed with cold water and dried. The yield was 0.71 g (88%) after recrystallization from 1-propanol. The substance has no definite mp, it starts decomposing at *ca*. 235-236°C; Rf_A=0.56, Rf_B=0.70, Rf_Γ=0.71; IR (cm^{-1}; KBr): NH 3365m, CH (benzene) 3060s, CH 2970m 2916m, C=O 1694vs; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.70 (s, H, α), 7.35-7.39 (q, 1H, 6), 8.14 (s, 1H, 7), 8.52-8.53 (d, 1H, 5), 13.9 (s, 1H, 3); ^{13}C-NMR (300MHz, DMSO-d_6): δ 26.00 (s, 1C, -CH_3), 119.89 (s, 2C, 6&7), 146.99 (s, 2C, 5&8), 191.62 (-CO-).

Anal. Calcd. for $C_8H_7N_3O$: C, 59.62; H, 4.38; N, 26.07. Found: C, 59.48; H, 4.28; N 25.80.

Synthesis of Alkyl isothiocyanates.

The compounds: methyl, isopropyl, *tert*-butyl-isothiocyanates were prepared according to the method described by Maurice L. Moore and Frank S. Crossley [15]. The compounds: *n*-Butyl, *sec*-butyl, phenyl isothiocyanates were prepared according to the method described by Jochims J C *et al.* [16].

Synthesis of 4-*N*-alkyl-Thiosemicarbazides and 4,4-*N*-dialky-thiosemicarbazides.

These compounds were prepared by the following two general methods:

Method A.

By the reaction of the appropriate amine and S-methyldithiocarbazate: *S*-methyldithiocarbazate (0.01 mol) and the appropriate amine (0.03 mol) in water, were heated under reflux for 4.5 hr, MeSH was evolved during the course of the reaction. The reaction mixture was allowed to reach ambient temperature and then cooled by pouring the mixture into an ice bath. The precipitate that formed was collected washed with cold water and dried. The compound was used some times without further purification and some times after recrystallization from the appropriate solvent.

By this method were prepared 4-methyl-, 4-isobutyl, 4-cyclohexyl-, 4-benzyl-, 4-pyrolidin-, 4-piperidin-, 4-morpholin-, and 4,4-dimethyl-thiosemicarbazides.

For the 4-n-propyl-thiosemicarbazide there is a differentiation, so, this procedure was as follows:

4-*n*-Propyl-thiosemicarbazide.

S-methyldithiocarbazate 1.22 g (0.01 mole) and *n*-propylamine 1.77 g (0.03 mol) in water (5 ml) were heated at the reflux for 4.5 hrs, MeSH was evolved during the course of the reaction. The reaction mixture was allowed to reach ambient temperature and then cooled by pouring the solution into an ice bath but precipitate did not form. So, the water solution was extracted with $CHCl_3$ (10x10 ml). The combined $CHCl_3$ extracts were dried over sodium sulfate and evaporated to dryness. The residue was purified with column chromatography (gradient from $CHCl_3$ 100% to $CHCl_3$/CH_3OH 98:2). The appropriated fractions were combined and concentrated. This concentration was brought, dropwise under cooling and agitation, into petroleum ether. The yield was 0.45 g (34%), mp 60.5-62 °C, Rf_A= 0.4

Method B.

By the reaction of the appropriate alkyl isothiocyanate with hydrazine: The appropriate alkylisothiocyanate (30 mmol) was added, dropwise, over a period 1 hr to a stirred solution of hydrazine (30 mmol) in methanol (8 ml) at 65-80 °C. The stirring of the reaction mixture was continued for twenty minutes more at the same temperature. The solvent was removed by evaporation *in vacuo* and the crude product was dissolved in chloroform (5 ml). The solution was added, dropwise, with continued stirring, to a multifold volume of petroleum ether 60-80 °C. The precipitate was collected by filtration and washed with petroleum ether. The compound was used some times without further purification and same times after recrystallization from the appropriate solvent.

By this method were prepared: 4-ethyl-, 4-isopropyl-, 4-*n*-butyl-, 4-*sec*-butyl-, 4-*tert*-butyl-, 4-phenyl-, 4-(*p*-methyl)-phenyl-, 4-(*p*-methoxy)phenyl-, 4-(*p*-chloro)phenyl-, 4-(*p*-nitro)phenyl-thiosemicarbazides.

Thiosemicarbazones of the 2-acetyl-imidazo[4,5-*b*] pyridine.

The 2-acetyl-imidazo[4,5-*b*]pyridine derivatives were synthesised by the following general method.

The appropriate 4-*N*-alkylthiosemicarbazide (1 mmol) and 10 drops acetic acid was added to a warm solution of 2-acetylimidazo[4,5-*b*]pyridine (1 mmol) in 1-propanol (6 ml). The reaction mixture was heated under reflux for 2-4 hrs (Thin layer chromatography is needed for determination of the precise time). Upon cooling the product precipitate was collected by filtration, washed with cold aqueous ethanol and dried.

2-Acetylimidazo[4,5-*b*]pyridine-3-thiosemicarbazone (**A_1**).

This compound was prepared by the general method given above and recrystallised from methanol to give the title compound in 57% yield; mp 212.5- 213.5°C; Rf_A: 0.52, Rf_Γ: 0.3; IR (cm^{-1}, KBr): NH 3490S, SH 2620vw, C=C and C=N 1615S 1601w, C=S 1121S; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.43 (s, 3H, a), 7.23-7.28 (q, 1H, 6) 8.02-8.05 (d, 1H, 7), 8.35-8.37 (d, 1H, 5), 8.57 (s, 1H, c), 8.66 (s, 1H, b) 10.75 (s, 1H, 3).

Anal. Calcd. for $C_9H_{10}N_6S$ (M.W. 234.28): C, 46.14; H, 4.30; N, 35.87. Found: C, 46.01; H, 4.22; N, 35.68.

2-Acetylimidazo[4,5-*b*]pyridine-4-methyl-3-thiosemicarbazone (**A_2**).

This compound was prepared by the general method given above to give the title compound in 44% yield; mp 237.3-237.6°C; Rf_A: 0.38; IR (cm^{-1}, KBr): NH 3332VS, CH(CH_3) 2981m, C=N and C=C 1616S 1587w, C-N 1280VS, C=S 1229S; ^{1}H-NMR (300MHz, DMSO-d_6): δ 2.42 (s, 3H, a), 3.09-3.11 (d, 3H, d) 7.23-7.27 (q, 1H, 6), 8.04-8.06 (d, 1H, 7) 8.35-8.37 (d, 1H, 5), 9.10 (s, 1H, c) 10.81 (s, 1H, b).

Anal. Calcd. for $C_{10}H_{12}N_6S$ (M.W. 248.31): C, 48.37; H, 4.87; N, 33.85. Found: C, 48.21; H, 4.58; N, 33.68.

2-Acetylimidazo[4,5-*b*]pyridine-4-ethyl-3-thiosemicarbazone (**A_3**).

This compound was prepared by the general method given above to give the title compound in 65% yield; mp 223.5-224.46°C; Rf_A: 0.53, Rf_Γ=0.72, Rf_Δ=0.19; IR (cm^{-1}, KBr): CH(-CH_3) 2975S, SH 2640w, C=N and C=C 1589S, C=S 1214S; ^{1}H-NMR (300MHz, DMSO-d_6): δ 1.18-1.23 (t, 3H, e), 2.42 (s, 3H, a),

3.65-3.69 (t, 2H, d), 7.23-7.27 (q, 1H, 6) 8.06-8.09 (d, 1H, 7), 8.35-8.36 (d, 1H, 5), 9.18 (t, 1H, c), 10.69 (s, 1H, b), 13.41 (s, 1H, 3).

Anal. Calcd. for $C_{11}H_{14}N_6S$ (M.W. 262.33): C, 50.36; H, 5.38; N, 32.04. Found: C, 50.04; H, 5.33; N, 31.34.

2-Acetylimidazo[4,5-*b*]pyridine-4-*n*-propyl-3-thiosemicarbazone (**A_4**).

This compound was prepared by the general method given above to give the title compound in 74.5% yield; mp 213.8-214.4 °C; Rf_A: 0.61; IR (cm^{-1}, KBr): CH(-CH_3) 2965S, C=N and C=C 1619S 1587s, C=S 1208S; ^{1}H-NMR (300MHz, DMSO-d_6): δ 0.90-0.95 (t, 3H, f), 1.61-1.69 (m, 2H, e), 2.42 (s, 3H, a), 3.56-3.63 (q, 2H, d), 7.23-7.27 (q, 1H, 6) 8.06-8.09 (d, 1H, 7), 8.35-8.36 (d, 1H, 5), 9.19 (t, 1H, c) 10.68 (s, 1H, b), 12.95 and 13.43 (s, 1H, 3).

Anal. Calcd. for $C_{12}H_{16}N_6S$ (M.W. 276.36): C, 52.15; H, 5.84; N, 30.41. Found: C, 52.39; H, 5.83; N, 29.89.

2-Acetylimidazo[4,5-*b*]pyridine-4-isopropyl-3-thiosemicarbazone (**A_5**).

This compound was prepared by the general method given above and purified with column chromatography (gradient from $CHCl_3$ 100% to $CHCl_3$/CH_3OH 95:5) to give the title compound in 72% yield; mp 223.5-224 °C; Rf_A: 0.61, Rf_E: 0.16; IR (cm^{-1}, KBr): CH(-CH_3) 2973S, SH 2640vw, C=N and C=C 1599S 1585s, C=S 1213S; ^{1}H-NMR (300MHz, DMSO-d_6): δ 1.27-1.29 (d, 6H, e), 2.43 (s, 3H, a), 4.62-4.69 (m, 1H, d), 7.24-7.28 (q, 1H, 6), 8.06-8.08 (d, 1H, 7), 8.36-8.38 (d, 1H, 5), 8.66 (s, 1H, c), 10.54 (s, 1H, b), 13.64 (s, 1H, 3).

Anal. Calcd. for $C_{12}H_{16}N_6S$ (M.W. 276.36): C, 52.15; H, 5.84; N, 30.41. Found: C, 52.37; H, 5.85; N, 29.51.

2-Acetylimidazo[4,5-*b*]pyridine-4-*n*-butyl-3-thiosemicarbazone (**A_6**).

This compound was prepared by the general method given above and purified with column chromatography (gradient from $CHCl_3$ 100% to $CHCl_3$/CH_3OH 95:5) to give the title compound in 79% yield; mp 206-207 °C; Rf_A: 0.23, Rf_E: 0.71; IR (cm-1, KBr): CH 2959S, SH 2635w, C=N and C=C 1612S 1590s, C=S 1196S; ^{1}H-NMR (300MHz, DMSO-d_6): δ 0.91-0.96 (t, 3H, g), 1.32-1.40 (s, 2H, f), 1.57-1.67 (m, 2H, e), 2.42 (s, 3H, a), 3.60-3.67 (q, 2H, d), 7.23-7.28 (q, 1H, 6), 8.05-8.07 (d, 1H, 7), 8.36-8.37 (d, 1H, 5), 9.14 (s, 1H, c), 10.68 (s, 1H, d), 13.42 (s, 1H, 3).

Anal. Calcd. for $C_{13}H_{18}N_6S$ (M.W. 290.39): C, 53.77; H, 6.25; N, 28.94. Found: C, 53.15; H, 6.17; N, 28.17.

2-Acetylimidazo[4,5-*b*]pyridine-4-*sec*-butyl-3-thiosemicarbazone (**A_7**).

This compound was prepared by the general method given above and purified with column chromatography (gradient from $CHCl_3$ 100% to $CHCl_3$/CH_3OH 95:5) to give the title compound in 65% yield; mp 230 °C; Rf_A: 0.64, Rf_B: 0.82, Rf_Γ: 0.68; IR (cm^{-1}, KBr): NH 3313vs, CH(CH_3) as 2970S, CH(CH_2) as 2930, CH(-CH) (sym) 2875, SH 2635w, C=N and C=C 1579S, C=S 1205S; ^{1}H-NMR (300MHz, DMSO-d_6): δ 0.88-0.93 (t, 3H, g), 1.23-1.25 (d, 3H, e), 1.65 (m, 2H, f), 2.44 (s, 3H, a), 4.49 (m, 1H, d), 7.27 (q, 1H, 6), 8.06-8.09 (d, 1H, 7), 8.36 (d, 1H, 5), 8.62 (s, 1H, c), 10.52 (s, 1H,b), 13.61 (s, 1H, 3).

Anal. Calcd. for $C_{13}H_{18}N_6S$ (M.W. 290.39): C, 53.77, H, 6.25, N, 28.94. Found: C, 52.60, H, 6.09, N, 28.38.

2-Acetylimidazo[4,5-*b*]pyridine-4-isobutyl-3-thiosemicarbazone (**A_8**).

This compound was prepared by the general method given above and purified with column chromatography (gradient from $CHCl_3$ 100% to $CHCl_3$/CH_3OH 95:5) to give the title compound in 75% yield; mp 209-209.7 °C; Rf_A: 0.41, Rf_Γ: 0.76, Rf_E: 0.31; IR (cm^{-1}, KBr): CH(-CH_3) as 2961S, CH(-CH_2) as 2930, SH 2650vw C=N and C=C 1613S 1591s, C=S 1203S; ^{1}H-NMR (300MHz, DMSO-d_6): δ 0.92-0.94 (d, 6H, f), 2.03-2.12 (m, 1H, e), 2.43 (s, 1H, a), 3.44-3.49 (t, 2H, d), 7.23-7.28 (q, 1H, 6), 8.06 (d, 1H, 7), 8.35-8.37 (d, 1H, 5), 9.19 (s, 1H,c), 10.67 (s, 1H, b), 13.48 (s, 1H, 3).

Anal. Calcd. for $C_{13}H_{18}N_6S$ (M.W. 290.39): C, 53.77; H, 6.25; N, 28.94. Found: C, 53.46; H, 6.19; N, 28.44.

2-Acetylimidazo[4,5-*b*]pyridine-4-*tert*-butyl-3-thiosemicarbazone (**A_9**).

This compound was prepared by the general method given above and purified with column chromatography (gradient from $CHCl_3$ 100% to $CHCl_3$/CH_3OH 95:5) to give the title compound in 55% yield; mp 211.5-213 °C; Rf_B: 0.58; IR (cm^{-1}, KBr): NH 3298vs and 1369vs, C=S 1179S; ^{1}H-NMR (300MHz, DMSO-d_6): δ 1.60 (s, 9H, d), 2.45 (s, 3H, a), 7.24-7.28 (q, 1H, 6), 8.04, (s, 1H, c), 8.06-8.08 (d, 1H, 7), 8.36-8.38 (d, 1H, 5), 10.21 (s, 1H, b).

Anal. Calcd. for $C_{13}H_{18}N_6S$ (M.W. 290.39): C, 53.77; H, 6.25; N, 28.94. Found: C, 53.59; H, 6.30; N, 29.20.

2-Acetylimidazo[4,5-*b*]pyridine-4-allyl-3-thiosemicarbazone (**A_{10}**).

This compound was prepared by the general method given above and purified with column chromatography (gradient from $CHCl_3$ 100% to $CHCl_3$/CH_3OH 90:10) to give the title compound in 95% yield; mp 202-202.5 °C; Rf_A: 0.53, Rf_B: 0.78, Rf_Γ: 0.61; IR (cm^{-1}, KBr): NH 3304, C=C(alkene) 1680m, C=N and C=C 1594s, 1541s, CH(-CH=CH), C=S 1197S; ^{1}H-NMR (300MHz, DMSO-d_6): δ 2.44 (s, 3H, a), 4.31-4.34 (t, 2H, d), 5.13-5.24 (m, 2H, g-f), 5.90-6.00 (m, 1H, e), 7.23-7.28 (q, 1H, 6), 8.04-8.07 (d, 1H, 7), 8.35-8.37 (d, 1H, 5), 9.29 (s, 1H, c), 10.82 (s, 1H, b), 11.95 and 13.42 (s, 1H, 3).

Anal. Calcd. for $C_{12}H_{14}N_6S{\cdot}\frac{1}{2}H_2O$ (M.W. 283.35): C, 50.87; H, 5.34; N, 29.66. Found: C, 51.18; H, 5.32; N, 29.20.

2-Acetylimidazo[4,5-*b*]pyridine-4-cyclohexyl-3-thiosemicarbazone (**A_{11}**).

This compound was prepared by the general method given above and recrystallised from 1-propanol to give the title compound in 76% yield; mp 218.6 °C; Rf_A: 0.58, Rf_E: 0.22; IR (cm^{-1}, KBr): CH (ring)(sym) 2934vs, 2857s, SH 2635w, C=N and C=C 1613s, 1594s, C=S 1203S (deformation vibrations of cyclohexylo ring) 1026m and 1005m; ^{1}H-NMR (300MHz, DMSO-d_6): δ 1.13-1.92 (m, 11H, ring), 2.43 (s, 3H, a), 7.24-7.28 (q, 1H, 6), 8.06-8.08 (d, 1H, 7), 8.36-8.38 (d, 1H, 5), 8.65 (s, 1H, c), 10.53 (s, 1H, b), 13.63 (s, 1H, 3).

Anal. Calcd. for $C_{15}H_{20}N_6S{\cdot}H_2O$ (M.W. 334.44): C, 53.87; H, 6.63; N, 25.13. Found: C, 54.22; H, 6.27; N, 24.83.

2-Acetylimidazo[4,5-*b*]pyridine-4,4-dimethyl-3-thiosemicarbazone (**A_{12}**).

This compound was prepared by the general method given above and recrystallised from methanol to give the title

compound in 59% yield; mp 177.7-179 °C; Rf_A: 0.24; IR (cm^{-1}, KBr): SH 2640vw, C=N and C=C 1614s 1582s, C=S 1218S; ^{1}H-NMR (300MHz, DMSO-d_6): δ 2.43 (s, 3H, a), 3.09 (s, 6H, c), 9.9 (s, 1H, b), 7.40-7.37 (q, 1H, 6), 8.20-8.15 (d, 1H, 7), 8.50-8.37 (d, 1H, 5), 14.5 (s, 1H, 3).

Anal. Calcd. for $C_{11}H_{14}N_6S$ (M.W. 262.33): C, 50.36; H, 5.38; N, 32.04. Found: C, 50.20; H, 5.28; N, 31.88.

2-Acetylimidazo[4,5-*b*]pyridine-4-pyrolidine-3-thiosemicarbazone (**A_{13}**).

This compound was prepared by the general method given above and recrystallised from methanol to give the title compound in 59% yield; mp 212.5 °C; Rf_Γ: 0.63; IR (cm^{-1}, KBr): SH 2640vw, C=N and C=C 1610s 1593s, C=S 1189S; ^{1}H-NMR (300MHz, DMSO-d_6): δ 1.89 (s, 4H, a), 2.46 (s, 3H, a), 3.75-3.79 (t, 4H, c), 7.24-7.28 (q, 1H, 6), 13.64 (s, 1H, 3).

Anal. Calcd. for $C_{13}H_{16}N_6S$ (M.W. 288.16): C, 54.14; H, 5.60; N, 29.16. Found: C, 53.88; H, 5.68; N, 28.88.

2-Acetylimidazo[4,5-*b*]pyridine-4-piperidyl-3-thiosemicarbazone (**A_{14}**).

This compound was prepared by the general method given above and purified with column chromatography (gradient from $CHCl_3$ 100% to $CHCl_3/CH_3OH$ 90:10) to give the title compound in 23% yield; mp 212.5 °C; Rf_A: 0.21; IR (cm^{-1}, KBr): CH 2938s, C=N and C=C 1587s, C=S 1252S, C-Cd(piperidine) 1020m. 952m γCHd 899m; ^{1}H-NMR (300MHz, DMSO-d_6): δ 1.59 (s, 6H,d-e), 2.39 (s, 3H, a), 7.19-7.24 (m, 3H, 6), 7.91 (d, 1H, 7), 8.32-8.34 (d, 1H, 5), 11.68 (s, 1H, b) 8.32-8.34 (d, 1H, 5), 11.68 (s, 1H, b), 13.61 (s, 1H, 3).

Anal. Calcd. for $C_{14}H_{18}N_6S$ (M.W. 302.40): C, 55.61; H, 6.00; N, 27.79. Found: C, 55.34; H, 6.12; N, 27.32.

2-Acetylimidazo[4,5-*b*]pyridine-4-morpholyn-3-thiosemicarbazone (**A_{15}**).

This compound was prepared by the general method given above to give the title compound in 55% yield; mp 180.5-181.5 °C; Rf_A: 0.31, Rf_B: 0.75, Rf_Γ: 0.49; IR (cm^{-1}, KBr): C=N and C=C 1579s, C=S 1237S, C-O-C 1119s; ^{1}H-NMR (300MHz, DMSO-d_6): δ 2.40 (s, 3H, a), 3.72 (s, 4H, d), 7.20-7.24 (q, 1H, 6), 7.94 (d, 1H, 7), 8.33-8.34 (d, 1H, 5), 11.79 (s, 1H, b), 13.48 (s, 1H, 3).

Anal. Calcd. for $C_{13}H_{16}N_6OS$ (M.W. 304.5): C, 51.30; H, 5.30; N, 27.61. Found: C, 51.45; H, 5.18; N, 27.29.

2-Acetylimidazo[4,5-*b*]pyridine-4-benzyl-3-thiosemicarbazone (**A_{16}**).

This compound was prepared by the general method given above to give the title compound in 46% yield; mp 218.7-219 °C; Rf_A: 0.48, Rf_Γ: 0.75, Rf_Γ: 0.57; IR (cm^{-1}, KBr): NH 3315s, CH(aromatic) 3191s, C=N and C=C 1613s, 1590s, C=S 1186s; ^{1}H-NMR (300MHz, DMSO-d_6): δ 2.47 (s, 3H, a), 4.95-4.97 (d, 2H, d), 7.25-7.33 (m, 4H, e&f), 7.36-7.37 (d, 2H, 6&g), 8.06-8.08 (d, 1H, 7), 8.36-8.37 (d, 1H, 5), 9.64-9.68 (t, 1H, c), 10.95 (s, 1H, b), 13.63 (s, 1H, 3).

Anal. Calcd. for $C_{16}H_{16}N_6S$ (M.W. 324.40): C, 59.24; H, 4.97; N, 25.91. Found: C, 59.20; H, 4.91; N, 25.80.

2-Acetylimidazo[4,5-*b*]pyridine-4-phenyl-3-thiosemicarbazone (**A_{17}**).

This compound was prepared by the general method given above and recrystallised from methanol to give the title compound in 22.5% yield; mp 159.5-161 °C; Rf_A: 0.41; IR (cm^{-1}, KBr): SH 2640w, C=N and C=C 1594s, C=S 1187s; ^{1}H-NMR (300MHz, DMSO-d_6): δ 7.24-7.30 (m, 2H, 6-f), 7.41-7.46 (m, 2H, e), 7.51-7.54 (m, 2H, d), 8.06-8.08 (d, 1H, 7), 8.36-8.37 (d, 1H, 5), 10.56 (s, 1H, c), 11.09 (s, 1H, b), 13.64 (s, 1H, 3).

Anal. Calcd. for $C_{15}H_{14}N_6S{\cdot}H_2O$ (M.W. 328.29): C, 54.86; H, 4.91; N, 25.59. Found: C, 54.91; H, 4.43; N, 25.68.

2-Acetylimidazo[4,5-*b*]pyridine-4-(*p*-methyl)phenyl-3-thiosemicarbazone (**A_{18}**).

This compound was prepared by the general method given above and recrystallised from acetonitrile to give the title compound in 65% yield; mp 216.6 °C; Rf_A: 0.32, Rf_Γ: 0.64; IR (cm^{-1}, KBr): C=N and C=C 1610 and 1590s, C=C(phenyl) 1521 vs, C=S 1187s; ^{1}H-NMR (300MHz, DMSO-d_6): δ 2.34 (s, 3H, f), 7.22-7.28 (m, 3H, 6), 7.37-7.40 (d, 1H, d), 8.05-8.08 (d, 1H, 7), 8.36-8.37 (d, 1H, 5), 10.51 (s, 1H, c), 11.03 (s, 1H, b), 13.00 (s, 1H, 3), 13.61 (s, 1H, 3).

Anal. Calcd. for $C_{16}H_{16}N_6S{\cdot}½H_2O$ (M.W. 333.41): C, 57.64; H, 5.14; N, 25.21. Found: C, 57.02; H, 5.12; N, 25.11.

2-Acetylimidazo[4,5-*b*]pyridine-4-(*p*-methoxy)phenyl-3-thiosemicarbazone (**A_{19}**).

This compound was prepared by the general method given above and recrystallised from acetonitrile to give the title compound in 82% yield; mp 193-194.5 °C; Rf_A: 0.58, Rf_Γ: 0.60; IR (cm^{-1}, KBr): NH 3281vs, CH(aromatic) 3008s, CH(Me) 2956m, C=N and C=C 1594s, C-O-C- 1274 and 1104s, C=S 1186s; ^{1}H-NMR (300MHz, DMSO-d_6): δ 3.79 (s, 3H, f), 6.97-7.00 (d, 1H, e), 7.27-7.29 (q, 1H, 6), 7.37-7.40 (d, 2H, d), 8.05-8.08 (d, 1H, 7), 8.36-8.37 (d, 1H, 5), 10.48 (s, 1H, c), 11.03 (s, 1H, b), 13.62 (s, 1H, 3).

Anal. Calcd. for $C_{16}H_{16}N_6OS{\cdot}H_2O$ (M.W. 358.41): C, 53.62; H, 5.06; N, 23.45. Found: C, 52.85; H, 4.79; N, 23.03.

2-Acetylimidazo[4,5-*b*]pyridine-4-(*p*-chloro)phenyl-3-thiosemicarbazone (**A_{20}**).

This compound was prepared by the general method given above and recrystallised from acetonitrile/ethanol to give the title compound in 57% yield; mp 178°C; Rf_A: 0.52, Rf_Γ: 0.58; IR (cm^{-1}, KBr): NH 3197vs, C=N and C=C 1591s, C=C(phenyl) 1546 and 1503s, C=S 1170s; ^{1}H-NMR (300MHz, DMSO-d_6): δ 2.58 (s, 3H, a), 7.26-7.60 (m, 5H,d,e & 6), 8.08-8.10 (d, 1H, 7), 8.38-8.39 (d, 1H, 5), 13.60 (s, 1H, 3).

Anal. Calcd. for $C_{15}H_{13}N_6SCl{\cdot}½H_2O$ (M.W. 353.83): C, 50.92; H, 3.99; N, 23.75. Found: C, 51.01; H, 3.76; N, 24.03.

2-Acetylimidazo[4,5-*b*]pyridine-4-(*p*-nitro)phenyl-3-thiosemicarbazone (**A_{21}**).

This compound was prepared by the general method given above and recrystallised from acetonitrile to give the title compound in 45% yield; mp 209 °C; Rf_A: 0.22, Rf_Γ: 0.28; IR (cm^{-1}, KBr): NH 3273vs, CH(aromatic) 3059s, C=N and C=C 1600 and 1551vs, NO 1507vs(anti) 1330vs(symm.) and 847, C=S 1177s; ^{1}H-NMR (300MHz, DMSO-d_6): δ 2.55 (s, 3H, a), 7.27-7.31 (q, 1H, 6), 7.99-8.03 (d, 1H, 7), 8.31-8.33 (d, 1H, 5), 10.78 (s, 1H, c), 11.46 (s, 1H, b), 13.58 (s, 1H, 3).

Anal. Calcd. for $C_{15}H_{13}N_7O_2S$ (M.W. 355.37): C, 50.70; H, 3.69; N, 27.59. Found: C, 50.72; H, 3.59; N, 26.76.

Protocol for Biological Activity.

Antineoplastic Activity.

A total of 60 human tumor cell lines, derived from seven clinically isolated cancer types (lung, colon, melanoma, renal, ovarian, brain and leukemia), that adequately meet minimal quality assurance criteria, which are adaptable to a single growth medium and which have reproducible profile for growth and drug sensitivity, were used in pilot-scale screening operation. Each compound was tested at five tenfold dilutions, a 48 h continuous drug exposure protocol was used and a sulforodamine B (SRB) protein assay was used to estimate cell viability or growth. The cytotoxic effects are evaluated and the assay results are reported as both dose response curves or pictographically as mean graphs (Figure 1). For more detailed descriptions of the screening assay in use [17-21].

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REFERENCES AND NOTES

[1] H. L. Elford, M. Freege E. Passamani and H. P. Morris, *J. Biol. Chem*., **245**, 5228 (1970).

[2] R. W. Brockman, J. R. Thomson, M. J. Beel and H. E. Skipper, *Cancer Res.*, **6**, 167 (1956).

[3] F. A. French B. L. Freedlander *Cancer Res*., **18**, 1290 (1958).

[4] D. L. Klayman, J. P. Scovill, J. F. Barosevich and C. J. Mason, *J. Med. Chem.*, **22**, 1367-1373 (1979).

[5] A. S. Dobek, D. L. Klayman, E. T. Dickson, J. P. Scovill and C. N. Oster, *Arzneim-Forsch*, **33(II)**, 1583 (1983).

[6] C. Shipman, Jr., S. H. Smith, J. C. Drach and D. J. Klayman *Antimicrob Agents Chemother*, **19**, 682 (1981).

[7] J. Easmon, G. Heinisch, W. Holzer and B. Rosenwirth *J. Med. Chem*., **35**, 3288 (1992).

[8] B. R. Singh *Talanta*, **25**, 619 (1978).

[9] S. M. M. H. Majumder, Ali M. Akbar, F. Smith and M. A. U. Mridha *Polyedron*, **7**, 2183 (1988).

[10] T. Bamgboye and O. A. Bamgboye, *Inorg Chim Acta*, **105**, 223 (1985).

[11] H. G. Petering, H. H. Bustirk and J. A. Crim, *Cancer Res.*, **27**, 1115 (1967).

[12] R. S. McElhinney *J. Chem. Soc. (C)*, 950 (1966).

[13] D. M. Livinstone *Inorg Chim Acta*, **19**, 5 (1976).

[14] J. A. Kiritsy and D. K. Yung *J. Med. Chem.*, **21**, 1301 (1978).

[15] M. L. Moore and F. S. Crossley *Organic Synthesis*, Collective Vol. **3**, 599.

[16] J. C. Jochims *Chem. Ber*., **101**, 1746 (1968).

[17] M. R. Boyd, in Cancer: Principles and Practice of Oncology; V.T. De Vita Jr. S. Hellman and S.A. Rosenberg, Eds.; Lippincott: Philadelphia, PA, (1989); Vol. **3**, pp 1-12.

[18] M. R. Boyd, and K. D. Paul., *Drug Development Research*, **34**, 91 (1995).

[19] A. Monks, D. Scudiero, P. Skelian, R. Shoemaker, K. Paull, D. Vistico, D. Hose, J. Langler, P. Cronise, A. Vaigro-Wolf, M. Gray-Goodrich, H. Campoell and M. R. Boyd, *J. Natl. Cancer Inst.*, **83**, 757 (1991).

[20] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. Warren, H. Bokesh, S. Kenney and M. Boyd, *J. Natl. Cancer Inst.*, **82**, 1107 (1990).

[21] K. D. Paul, Cancer Chemotherapeutic Agents. Chapter 2: Prediction of Biochemical Mechanism of Action from the in vitro Antitumour Screen of the National Cancer Institute; W. D. Foye ed. American Chemical Society, Washington D.C. 1995.