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Synthesis, Cholesterolmic Activity of Formamidino Isothio Amides

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In the present paper, the synthesis and cholesterolmic activity of formamidino isothio amides has been studied.

Key Words: Formamidino isothioamides, Hyper cholesterolmic activity.

INTRODUCTION

A large number of compounds like guanidines, amidines and isothioronium type of salts are biologically potent. Structure-activity relationship (SAR) of synthetic amidines was reviewed by Fartier in the year 1962 which includes activities such as antihypertensile, CNS depressant, local anaesthetic, muscle relaxant, antifungal and blood sugar lowering activities. Phenformin[1] and metformin[2] are the two drugs currently in use to lower blood sugar level. Guanithidine and guandrine are orally active antihypertensile agents[3]. Several cyclic thiourea derivatives like 2-thiobarbiturates have sedative hypnotic activity. A sulfide containing prodrug of clofibric acid, tiafibrate have shown positive results in lowering chlolesterol levels and have been used as antilepidemic agents[4]. Another drug probucol {4,4′-(isopropylidinedithio)-bis-(2,6-di-tertiary) phenol]} has been used for the reduction of elevated serum cholesterol level in patients with primary hypercholesterolemia[5]. Another sulfur containing series of arylthioalkanoic acids exhibited hypolepidemic activities. It was found that the hydroxyphenyl thiopropanoic acid possessed significant serum cholesterol and triglyceride lowering activities in rats.

Therefore it was thought pertinent to synthesise some new compounds having guanidino and sulfide functionalities in the molecule.

EXPERIMENTAL

The melting points are uncorrected. The purity of compounds was confirmed by TLC using silica gel as stationary phase and benzene-ethanol 90 : 10 as moving phase. NMR spectra were recorded on Jeol FX908 Fourier transform NMR spectrophotometer. IR spectra were recorded on Jasco FTIR 5300 infrared spectrophotometer. Spectral studies of compounds were done by KBr disc method. Elemental analyses were performed by Heraeus CHN analyses.

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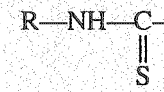
Preparation of arylthioureas: These were prepared by ammonium thiocyanate method[7]. The authenticity of the results is confirmed by literature study.

N-(Alkyl/Aryl thiocarbamoyl) derivatives of heterocyclic secondary amines:

1-(Methyl thiocarbamoyl) piperidine: Piperidine hexahydrate (19.6 g, 0.10 mol) was dissolved in absolute alcohol (50 mL) and methyl isothiocyanate (7.3 g 0.10 mol) in ethanol (25 mL) was added to it slowly. The reaction mixture was refluxed for 2 h and the colourless solid which was separated out on cooling was filtered, washed with aqueous ammonia and ether, to remove unreated materials. It was recrystallized from a mixture of formamide and methanol (50 : 50). Yield: 8.0 g (50%); m.p. 226°C. Using the same procedure other N-alkyl/aryl thiocarbamoyl derivatives having morpholine and piperidine moieties were prepared. The physical data of thiourea prepared by the foregoing methods are given in Table-1.

TABLE-1
PHYSICAL CONSTANTS OF N-(ALKYL/ARYL THIOCARBAMOYL) DERIVATIVES OF HETEROCYCLIC, SECONDARY AMINES



R	R′	m.p. (°C)	Yield (%)	Nitrogen analysis (%)	
				Calcd.	Found
CH_3	4-Morpholine	150	60	17.58	17.48
p-OC_2H_5	4-Morpholine	150	60	17.50	17.46
4-$OCH_3C_6H_4$	4-Morpholine	159	50	–	–
$CH(CH_2)_3$	4-Morpholine	155	60	14.89	14.86
4-ClC_6H_4	1-Piperidine	147	53	–	–
p-OC_2H_5	1-Piperidine	161	57	16.28	16.24
$CH(CH_3)_2$	1-Piperidine	163	53	15.05	15.00

Arylcyanamides: These were prepared using the method of Sahashrabudhey and Nrall[9].

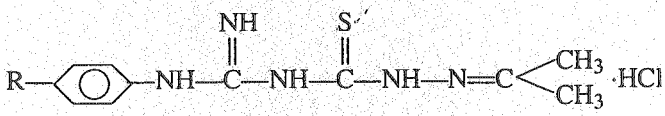
Preparation of aryl formamidine hydrochloride

Interaction of α-chlrotolylformamidine hydrochloride with acetone thiosemicarbazone:

Acetone-4-*p*-tolylformamidino thiosemicarbazone (Compound 1, Table-2A): A solution of α-chloro-*p*-tolylformamidine hydrochloride (4.1 g, 0.02 mol) in acetone was treated with an acetonic solution of acetone thiosemicarbazone (2.9 g, 0.02 mol). The reaction mixture was cooled at 0–5°C. A white microcrystalline product precipitated out, which was filtered and washed with dry acetone to remove unreacted materials. Yield: 4.54 g (72%); m.p. 201°C. Anal. (%) for

$C_{13}H_{20}N_5SCl$: Calcd.: N = 22.32, C = 49.76; Found: N = 22.28, C = 49.58. IR (Nujol) ν_{max} cm^{-1}: 3150 (s) (broad-NH_2 and NH); 1630 (s) (>C═N), 1500 (w) and 1200–1000 (s) (>C—S—C<).

TABLE-2A
PHYSICAL CONSTANTS OF 4-ARYL FORMAMIDINO THIOSEMICARBAZONE HYDROCHLORIDES



Compd. No.	R	Yield (%)	m.p. (°C)	m.f.	Nitrogen Analysis (%)	
					Calcd.	Found
1	*p*-CH_3	73	201	$C_{12}H_{18}N_5SCl$	22.32	22.28
2	Cl	74	201	$C_{11}H_{15}N_5SCl_2$	20.95	20.86
3	*p*-OCH_3	92	200	$C_{12}H_{18}N_5OSCl$	21.60	21.23
4	H	76	203	$C_{11}H_{16}N_5SCl$	24.51	24.23
5	*p*-OC_2H_5	72	211	$C_{13}H_{20}N_5OSCl$	20.39	20.29

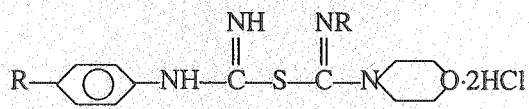
*All the compounds gave satisfactory C, H analyses.

By using the same method other compounds **(2–5)** were also prepared.

Interaction of α-chloroarylformamidinedihydrochlorides with 4-(alkyl/arylthiocarbamoyl) morpholine derivatives:

S-(4-Tolylformamidino)-4(N-isothioamide) morpholine dihydrochloride (Compound 6, Table-2B): α-Chloro-(4-tolylformamidine hydrochloride with 4-isopropylthiocarbamoyl) morpholine in acetone medium at 0–5°C, resulted in the

TABLE-2B
PHYSICAL CONSTANTS OF S-ARYLFORMAMIDINO-4-(N-ALKYL/ARYL ISOTHIOAMIDE) MORPHOLINE DIHYDROCHLORIDES NR



Compd. No.	R	R′	Yield (%)	m.p. (°C)	m.f.	Nitrogen analysis (%)	
						Calcd.	Found
6	4-CH_3	$CH(CH_3)_2$	69	148–49	$C_{16}H_{26}N_4OSCl_2$	14.25	14.29
7	4-OC_2H_5	4-$OCH_3C_6H_4$	81	133	$C_{21}H_{28}N_4O_3SCl_2$	–	–
8	4-OC_2H_5	4-ClC_6H_4	74	131	$C_{20}H_{25}N_4O_2SCl_3$	11.41	11.37
9	4-OCH_3	4-4$OCH_3C_6H_4$	79	135	$C_{20}H_{26}N_4O_3SCl_2$	11.84	11.80
10	4-CH_3	$CH_2(CH_2)_2CH_3$	64	144–46	$C_{17}H_{28}N_4OSCl_2$	13.76	13.69

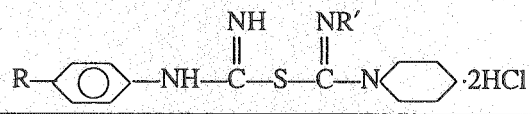
*All the compounds gave satisfactory C, H analyses.

formation of a white microcrystalline product. The product was identified as S-(4-tolylformamidino)-4-(N-isopropyl isothioamide) morpholine dihydrochloride, m.p. 148°C **(IIA–8)** on the basis of elemental analysis, IR spectra and chemical behaviour. Anal. (%) for $C_{16}H_{26}N_4OSCl_2$: Calcd: N = 14.25; Found: N = 14.29. IR (Nujol) ν_{max} cm^{-1}: 3640–3125 (broad-NH_2 and NH), 1650 (s) (>C═N), 1330, 820 and 695 (>C—S—C<). Similarly other compounds of Table-2B were also prepared.

Interaction of α-chloroaryl formamidine hydrochlorides with 1-(alkyl/ arylthiocarbamoyl) piperidine derivatives (IIIA):

S-(4-Ethoxyphenyl formamidine)-1-(N-chlorophenyl isothioamide)-piperidine dihydrochloride: At lower temperature (0–5°C) 1-chloro (phenyl thiocarbamoyl) piperidine was allowed to react with α-chloro-(4-ethoxyphenyl) formamidinehydrochloride in acetone medium. As a result, a microcrystalline solid product was obtained which was soluble in cold water. This product was characterized as S-(4-ethoxy phenyl formamidino)-1-(chlorophenylisothioamide) piperidine dihydrochloride. m.p. 139°C **(IIIA–3)**. On the basis of IR spectra, elemental analysis suggested the presence of two molecules of hydrochloric acid in the compound which corresponded to the molecular formula $C_{21}H_{27}N_4OSCl_3$. Anal. (%) for $C_{21}H_{27}N_4OSCl_3$: Calcd.: N = 11.44; Found: N = 11.41%. IR (Nujol) ν_{max} cm^{-1}: 3600–3125 (broad-NH_2 and NH), 1650 (S) (>C—N), 1305, 815 and 695 (>C—S—C<). Similarly other compounds of Table-2C were also prepared.

TABLE-2C
PHYSICAL CONSTANTS OF S-ARYL FORMAMIDINO-1-(N-ALKYL/ARYL ISOTHIOAMIDE) PIPERIDINE DIHYDROCHLORIDE



Compd. No.	R	R′	Yield (%)	m.p. (°C)	m.f.	Nitrogen analysis (%)	
						Calcd.	Found
11	Cl	4-ClC_6H_4	63	151	$C_{19}H_{22}NSCl_4$	11.67	11.63
12	4-OC_2H_5	4-$OCH_3C_6H_4$	79	143–44	$C_{22}H_{30}N_4O_2SCl_2$	11.55	11.51
13	4-OC_2H_5	4-ClC_6H_4	72	139	$C_{21}H_{27}Cl_3N_4OS$	11.44	11.41
14	4-Br	$CH(CH_3)_2$	71	137–39	$C_{16}H_{25}N_4SBrCl_2$	12.28	12.23
15	4-Cl	$CH_2(CH_2)_2CH_3$	64	149–51	$C_{17}H_{27}N_4SCl_3$	13.16	13.12

*All the compounds gave satisfactory C, H, Analyses.

Biological studies

Effect on serum cholesterol level of albino rats:

Serum cholesterol estimation:

(i) Bloor's mixture (alcohol : ether mixture): Three parts of 95% ethyl alcohol and one part of ether was mixed. Both the alcohol and ether must have been previously distilled.

(ii) Stock solution of cholesterol (0.1%): Weighed 0.1 g recrystallized pure cholesterol and transferred to a 100 mL clean dried volumetric flask. Added chloroform up to the mark, stoppered and stirred to dissolve solid.

(iii) Dilute standard solution in chloroform: By pipette one mL of the stock cholesterol solution was taken in a clean, dried, 10 mL volumetric flask and made to mark with chloroform. Kept the stopper closed. The solution was made afresh each time. This solution contains 0.1 mg of cholesterol per mL. Serum cholesterol estimation was done by the method of Bloor.[10] In a clean, dried, glass stoppered, 10 mL graduated cylinder, about 9 mL of Bloor's alcohol-ether mixture was placed. Then by means of a Mohr pipette, allowed exactly 0.2 mL of serum into the mixture. Made it to 10 mL with Bloor's mixture and stoppered the cylinder immediately and shook it vigorously several times. Allowed it to remain in a horizontal position for 30 min. After this centrifuged it for 5 min and poured the supernatant in a boiling tube. It is evaporated to almost dryness on a water bath. 5 mL of chloroform was then added to the residue in the boiling tube, which was gently rotated during addtion of chloroform.

Standard: In a second boiling tube, 5 mL of a dilute solution of cholesterol was placed. Then to both tubes 2 mL of acetic anhydride and 0.1 mL of concentrated sulphuric acid was added and the contents were shaken several times to mix with each other.

The tubes were placed together in the dark for 2 min, after which the solutions were compared in a colorimeter as rapidly as possible.

Calculation:

$$\text{mg Cholesterol/100 mL of blood serum} = \frac{\text{Reading of test (O.D)}}{\text{Reading of standard (O.D)}} \times 100$$

The results obtained are given in Tables 3A, 3B and 3C.

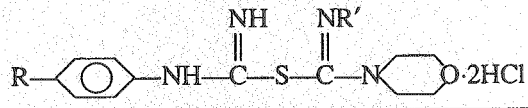
TABLE-3A
EFFECT OF 4-ARYL FORMAMIDINO THIOSEMICARBAZONE HYDROCHLORIDES ON THE SERUM CHOLESTEROL LEVEL OF ALBINO RATS

Compd. No.	R	Serum Cholesterol Level (mg %) Mean ± S.E. (n)			% Change in the Mean Serum Cholesterol level	
		Control	Post Treated			
			1 h	24 h	1 h	24 h
1	H	51.14 ± 2.28	39.62 ± 1.19	50.56 ± 1.72	22.52*↓	1.13
2	Cl	51.14 ± 2.28	35.80 ± 1.05	49.58 ± 1.33	29.99*↓	3.05
3	*o*-CH_3	51.14 ± 2.28	30.52 ± 2.59	44.30 ± 2.11	40.32*↓	13.37†
4	CH_3	51.14 ± 2.28	37.10 ± 1.09	47.38 ± 1.62	27.45*↓	7.54
5	OC_2H_5	51.14 ± 2.28	29.30 ± 1.35	50.84 ± 3.81	42.70*↓	0.58

Dose = 20 mg/kg i.p.; n = 5; ↓ = Hypocholesterolmic.

*$p < 0.001$ (very highly significant); †$p < 0.0025$ (significant).

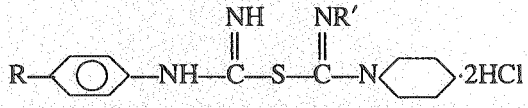
TABLE-3B
EFFECT OF S-(ARYL FORMAMIDINO)-4-N-(ALKYL/ARYL ISOTHIOAMIDE) MORPHOLINE DIHYDROCHLORIDES ON THE CHOLESTEROL LEVEL OF ALBINO RATS



Compd. No.	R	R′	Serum cholesterol level (mg %) Mean ± S.E. (n)			% Change in the mean serum cholesterol level	
			Control	1 h	24 h	1 h	24 h
6	4-OC_2H_5	4-ClC_6H_4	57.90 ± 2.26	28.09 ± 2.09	57.14 ± 1.39	39.55*↓	1.31↓
7	4-OC_2H_5	4-$OCH_3C_6H_4$	57.90 ± 2.26	25.40 ± 2.26	54.92 ± 3.15	40.58*↓	5.14↓
8	4-CH_3	$CH(CH_3)_2$	62.00 ± 1.04	50.56 ± 2.59	39.58 ± 2.57	18.45†↓	36.16*↓
9	4-OCH_3	4-$OCH_3C_6H_4$	58.86 ± 2.06	55.38 ± 1.81	42.70 ± 3.80	22.50**↓	27.48‡↓
10	4-CH_3	$CH_2(CH_2)_2CH_3$	62.00 ± 1.06	53.26 ± 1.75	35.16 ± 1.37	14.09†↓	43.29*↓

Dose = 20 mg/kg i.p.; n = 5; ↓ = hypocholesterolmic.
*$p < 0.001$ (very highly significant); †$p < 0.01$ (highly significant);
‡$p < 0.025$ (significant); **$p < 0.05$ (possibly significant).

TABLE-3C
EFFECT OF S-(ARYL FORMAMIDINO)-4-N-(ALKYL/ARYL ISOTHIOAMIDE) PIPERDINEDIHYDRO CHLORIDES ON THE SERUM CHOLESTEROL LEVEL OF ALBINO RATS



Compd. No.	R	R′	Serum cholesterol level (mg %) Mean ± S.E. (n)			% Change in the mean serum cholesterol level	
			Control	Post Treated		1 h	24 h
				1 h	24 h		
11	Cl	4-ClC_6H_4	57.90 ± 2.26	32.69 ± 2.05	42.84 ± 1.76	43.54*↓	26.01*↓
12	4-OC_2H_5	4-$OCH_3C_6H_4$	58.86 ± 2.06	55.68 ± 1.47	38.12 ± 1.22	5.40↓	35.23*↓
13	4-OC_2H_5	4-ClC_6H_4	58.86 ± 2.06	22.74 ± 1.43	30.40 ± 1.33	59.66*↓	48.36*↓
14	4-Br	$CH(CH_3)_2$	62.00 ± 1.04	47.16 ± 1.99	55.10 ± 2.39	23.93*↓	11.12†↓
15	4-Cl	$CH_2(CH_2)_2CH_3$	62.00 ± 1.04	55.74 ± 1.79	35.98 ± 2.04	10.09†↓	45.19*↓

Dose = 20 mg/kg i.p.; n = 5; ↓ = hypocholesterolmic.
*$p < 0.001$ (very highly significant); †$p < 0.05$ (possibly significant).

RESULTS AND DISCUSSION

The compounds have been estimated for their hypocholesterolmic effect and all the three series of amidines, thioureas and amidine sulfide showed hypocholesterolmic effect. The serum cholesterol levels in the thiosemicarbazone derivatives have shown a decrease. In this the groups with the ethoxy (42.7% decrease) and methoxy

(40.32% decrease) are most significant. Only compound with methoxy substitution shows slight hypocholesterolmic activity even after 24 h of drug administration.

The same hypocholesterolmic effect is true for the piperidine and morpholine series of compounds. In these cases also the *p*-ethoxy and *p*-methoxy (> 40% decrease) derivatives are highly potent. The compound in which both *p*-ethoxy and *p*-methoxy group are present is the most potent in the series (40% decrease). Unexpectedly, the compound with *p*-methyl group initially shows very little decrease in serum cholesterol but after 24 h, the level of decrease becomes more significant. Similarly, in the piperidine series the compounds in which both *p*-ethoxy (50% decrease) and *p*-chloro group is present, shows hypocholesterolmic effect which remains even aften 24 h of drug administration.

Conclusion

In the initial 1 h the activity of compounds containing methoxy and ethoxy groups were very high as compared to the compounds containing methyl group. This is due to the reason that metabolically these compounds are very stable as compared to the methyl group which can metabolise to carboxylic acid group. This trend is also evident in chloro containing compounds which are also very stable and compound number 13 is one of the best hypocholesterolmic agent which has got its effect even after 24 h. Thus from these studies it can be concluded that the piperidine sulfide containing compound with ethoxy chloro substituent can be considered as the lead compound. Further molecular modifications in these structures can lead to even better compounds.

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