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MICROWAVE ASSISTED SYNTHESIS OF POTENTIAL ANTI INFECTIVE AND ANTICONVULSANT THIOSEMICARBAZONES

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ABSTRACT: 1,2,4-triazole nucleus is therapeutically interesting drug candidate as anti-inflammatory ,anti-infective and CNS stimulant. Heteroaryl semicarbazones and thiosemicarbazone have emerged as structurally novel anticonvulsants and found to possess antifungal properties and anti-HIV properties. Therefore thiosemicarbazide derivatives of 5-mercapto-3-(3'-pyridyl)-4H-1,2,4-triazole 4 were synthesized. Aryl thiosemicarbazides were reacted with different aromatic aldehydes to yield corresponding thiosemicarbazones 6a-6e. The structures of the synthesized compounds were confirmed by spectral data and elemental analysis. The synthesized compounds were screened for antifungal activity by using cup plate agar diffusion method against *C. albicans*, antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* and anticonvulsant activity by Maximum Electroshock (MES) method. The compounds have shown good antifungal and anticonvulsant activity.
KEYWORDS: Aryl thiosemicarbazones, 1,2,4-triazole, anticonvulsant, anti-infective.

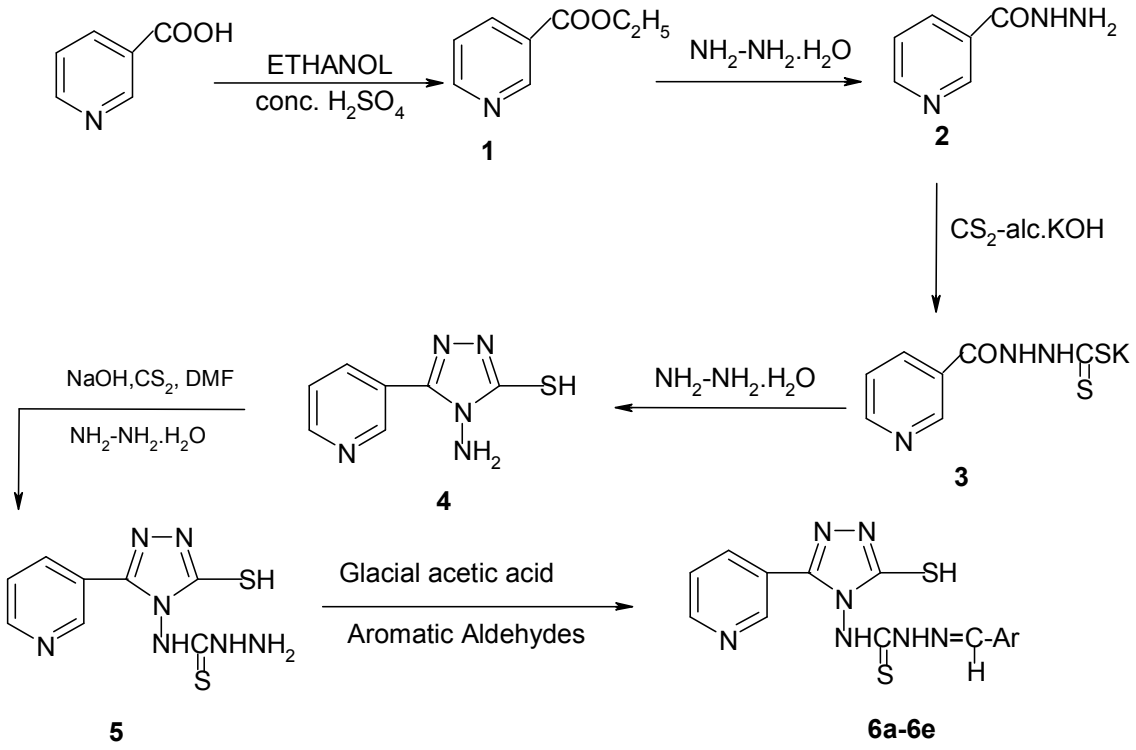
INTRODUCTION

The triazole nucleus shows a broad spectrum of pharmacological properties[1] . Semicarbazones and related compounds have a documented and consistent role in the design of novel anticonvulsant agents.[2] A number of semicarbazones, thiosemicarbazones[3], bis-carbohydrazones, aryl, arylidene, aryloxyaryl semicarbazones, acetyl hydrazones and oxamoylhydrazones are synthesized and evaluated for anticonvulsant activity[4,5] .Moreover a no. of pyridine ring containing compounds are known for their varied biological activities like antibacterial, antitubercular, antihistaminic effect.

Microwave (MW) irradiation, an unconventional energy source, has been used for a variety of applications including organic synthesis , wherein chemical reactions are accelerated because of selective absorption of MW energy by polar molecules.[6] Also we have described microwave assisted synthesis and antifungal evaluation of Schiff's bases of benztriazolyl 4-amino-1,2,4-triazoles.[7] Microwave irradiation produces efficient internal heat transfer (in situ heating), resulting in even heating through out the sample as compared with the wall heat transfer that occurs when an water/oil bath is applied as an energy source[8]. The microwave assisted reactions occur more rapidly, safely and with higher chemical yields.

In view of these observations, we have synthesized Schiff's derivatives for thiosemicarbazides of 5-mercapto-3 (3'-pyridyl)-4-amino substituted-1,2,4-triazole by microwave assisted reaction. The derivatives obtained were pure and in quantitative yield. Therefore the present communication describes synthesis of substituted N-(5-mercapto-3-pyridyl-3-yl-4H-1,2,4-triazol-4-yl)-thiosemicarbazone

SCHEME OF SYNTHESIS



BIOLOGICAL EVALUATION

ANTIFUNGAL ACTIVITY

All the compounds were screened for antifungal activity using cup-plate agar diffusion method [9,10,11] by measuring the zone of inhibition in mm. Fluconazole (100 µg/ml) was used as a standard and was also screened under similar conditions for comparison. A stainless steel borer of 8 mm diameter (pre-sterilized) was used to bore the cavities. Dimethylsulphoxide (DMSO) was used as a solvent for all the compounds and as a control. The compounds were screened for antifungal activity against *Candida albicans* in Sabouraud's Chlororamphenicol Agar medium at concentrations (100and 150 µg/ml). The plates were incubated at 37 ^{0}C for 24 hrs and the zone of inhibition observed and was measured.The results are presented in Table 2.

ANTIBACTERIAL ACTIVITY

All the compounds were screened for antibacterial activity using cup-plate agar diffusion method [12,13] by measuring the inhibition zone in mm. Streptomycine (100 µg/ml) was used as a standard and was also screened under similar conditions for comparison. A stainless steel borer of 8 mm diameter (pre-sterilized) was used to bore the cavities. Dimethylsulphoxide (DMSO) was used as a solvent for all the compounds and as a control. The compounds were screened for antibacterial activity against *E.coli* in medium at concentrations (100and 150 µg/ml). The plates were incubated at 37 ^{0}C for 24 hrs and the zone of inhibition observed and was measured.The results are presented in Table 3

ANTICONVULSANT ACTIVITY

The compounds were screened for their anticonvulsant activity [14,15] against the MES induced convulsions in male Swiss albino mice by literature method. The test compounds were suspended in 4% aq. CMC suspension and were injected intraperitoneally at dose of 300mg/kg. Phenytoin was used as a reference drug at the dose of 30mg/kg which was observed to protect 100% against the induced convulsions.Results are presented in Table-3 and Table-4 & hind limb extension recovery data & total recovery data represented in figure-1 and 2.

EXPERIMENTAL

GENERAL

All the chemicals and solvents were obtained from commercial source and purified using standard procedure whenever required. Melting points were measured in open capillary tube on VEEGO (VMP-D) melting point apparatus and are uncorrected. IR spectra (KBr pellets)

were recorded on a SHIMADZU FTIR 8400S infrared spectrophotometer. The ^{1}H-NMR spectra were determined in DMSO-d_6 at 300 MHz on a BRUKER DP-X 300 NMR spectrophotometer using TMS as an internal standard. The progress of the reaction and the purity of compounds were monitored by TLC using silica gel plates. Microwave assisted reaction were carried out in a "CATALYST SYSTEM" microwave oven.

SYNTHESIS OF ETHYLNICOTINATE (1)

In 1000ml round bottom flask, a mixture of 100gm (0.81mole) pure nicotinic acid, 259gm (310ml, 5.4mole) of absolute ethanol and 243gm (135ml) of concentrated sulphuric acid was taken and refluxed on water bath for 5-6 hours. The excess of ethanol was removed under reduced pressure. The reaction mixture was cooled and poured slowly with stirring on to 540gm of crushed ice. Sufficient ammonia solution was added to render the reaction mixture strongly alkaline (up to 11pH).The mixture was extracted with three times 135ml portion of ether and combined etheral extracts were dried over magnesium sulphate and the ether was removed by flash distillation and the residue was distilled under reduced pressure. The ethyl nicotinate distill at 117-118 ^{o} C.Yield (%) 90.16%,b.p. 222-224^{0}C,IR (KBr, cm^{-1}) 1723(C=O stretching),TLC Ethyl acetate: Hexane (4.5:0.5)

SYNTHESIS OF PYRIDINE-3-CARBOHYDRAZIDE(2)

A mixture of ethyl nicotinate (80gm; 0.53mole) and hydrazine hydrate (30gm; 29.12 ml; 0.614mole) was refluxed with absolute ethanol (136ml) for 6-7 hours. Then ethanol was distilled off, the reaction mixture was cooled , white crystals of nicotinic acid hydrazide was precipitated.Yield (%) 76.32%,m.p. 161-163 ^{0}C,IR(KBr,cm^{-1}) :3015(CH of pyridyl), 1672 (CONH),1595 (C=N).TLC :ethyl acetate: hexane (4:1)

SYNTHESIS OF POTASSIUM SALT OF PYRIDINE-3-CARBOHYDRAZIDE(3)

In a 500ml round bottom flask 16.64gm (13.17ml, 0.21mole) carbon disulphide was added to a solution of 11.76gm (0.21mole) potassium hydroxide, (290ml) absolute ethanol and 20gm pyridine-3-carbohydrazide.This mixture was agitated for 12-18 hour. It was diluted with (207ml) dry ether and the product was filtered off and vacuum dried at 65-70^{0}C. The salts prepared as described above were obtained in nearly quantitative yields and were used without further purification.

PREPARATION OF 5-MERCAPTO- 4-AMINO- 3-(3'-PYRIDYL)-1,2,4-TRIAZOLE(4)

Compound **3** (0.1mole) was dissolved in water. To it, 99% hydrazine hydrate 10gm (28.1ml, 0.2mole) was added. The reaction mixture was refluxed on a water bath until the evolution of H_2S gas ceased. It was then diluted with cold water (20-30ml) and carefully acidified with concentrated hydrochloric acid. The white solid thus separated was filtered, washed with cold water and dried and recrystallized from ethanol. Yield 78% ,m.p. 210-212 ^{0}C ,IR (KBr, cm^{-1}) 3232(NH_2), 3150(C-N), 3051 (-CH), 1534 (C-N), 1620 (C=N),1337 (C=S), 1237,1150, 1026 (C-N-C) TLC :ethyl acetate: hexane (4:1)

GENERAL PROCEDURE FOR SYNTHESIS OF 5-MERCAPTO-3(3'-PYRIDYL)-4H-1,2,4-TRIAZOLE-4-YL- THIOSEMICARBAZIDE (5).

To a solution of **4** (0.01 mole, 2gm)in DMF (10ml) was added, sodium hydroxide (0.015 mole, 0.62gm) and carbon disulphide (0.015 mole, 1.12 ml). The mixture was stirred at 15-20^{0}C for 1 hour, to the stirred mixture was added hydrazine hydrate (0.015 mole) and stirring continued at 60^{0}C for 1 hour, more on adding water, a pale white solid separated out which is recrystallized from DMF-ethanol afforded pale white crystals.Yield45% m.p.205 ^{0}C, IR (KBr, cm^{-1}) 3233(NH_2), 3150(C-N), 1569 (C-N),1648 (C=N), 1337 (C=S),1237,1128, 1050 (C-N-C),1H-NMR 14.3 (S,1H,SH); 9.2 (S,1H,NH); 8.8 (S,2H,α-Pyridyl); 8.4-8.2 ,(M,1H,β Pyridyl); 7.8-7.5 (D,1H,γ-Pyridyl); 2.4 (S, 1H, NH2).TLC ethyl acetate: hexane (4.5:0.5ml)

GENERAL MICROWAVE PROCEDURE [16,17,18] FOR SYNTHESIS OF SCHIFF'S BASES OF 5-MERCAPTO-3-(3'-PYRIDYL)-4H-1, 2, 4 TRIAZOLE-4-YL-THIOSEMICARBAZIDE (6a-6e)

In a 50 ml of RBF (microwave flask) solution of 5-Mercapto-3-(3'-pyridyl)-4H-1,2,4- triazole-4-yl-thiosemicarbazide **5** (0.5gm 1 mol) in glacial acetic acid (pH 3-5)was reacted with various aromatic aldehydes (1.5 mole) in microwave oven at power 7 (455 watt, 65%) for 40 min. The progress of reaction was monitored by TLC. Then the reaction mixture was poured into crushed ice and solid gets precipitated out. The filtered precipitate was washed with sodium bicarbonate solution and recrystallized with ethanol Table 2 summarizes the physical and analytical data of these compounds.

RESULT AND DISCUSSION

Schiff'derivatives of 5-mercapto-3-(3'-pyridyl)-4H-1,2,4-triazole-4-yl- thiosemicarbazide presented herein showed anti-infective and anticonvulsant activity. During determination of anticonvulsant activity all the animals were protected against the MES .Also total recovery time and time for hind limb extension recovery for **6a** was less than the standard .Derivatives 6b&6d have shown more antibacterial activity. Derivatives **6a, 6d** and **6e** exhibited antifungal activity which was more than standard. Hence it can be concluded that thiosemicarbazones of 5-mercapto-3-(3'-pyridyl)-4H-1,2,4-triazole-4-yl-thiosemicarbazide can lead to potential bioactivity.

TABLE 1: PHYSICOCHEMICAL CHARACTERISTICS OF TITLED COMPOUNDS (6a – 6e)

Compounds	Ar	m.f.(m.w.)	m.p(°C) (%yield)	IR(cm^{-1})	^{1}H NMR
6a	Phenyl	$C_{15}H_{13}N_7S_2$ (369g)	220°c (98%)	1512(-N=CH) 1271 (N-C=S),870 (Ar.-H), 3035 (C-H).	14.3 (s,1H,SH); 10.1 (s,1H,NH); 9 (s,2H,α-Pyridyl); 8.3-8.1 (m,1H,β-Pyridyl); 8.8-8.6 (d,1H,γ-Pyridyl); 7.4-7.9 (m,4H,Phenyl); 6.9 (s,1H,N=CH)
6b	2-Furyl	$C_{13}H_{11}N_7S_2$ (345g)	222°c (97%)	1520(-N=CH),1298 (N-C=S),837 (Ar.-H), 3078 (C-H).	14.4 (s,1H,SH); 9.8 (s,1H,NH); 9 (s,2H,α-Pyridyl); 8.4-8.1 (m,1H,β-Pyridyl); 8.8-8.6 (d,1H,γ-Pyridyl); 7.6-7.4 (m,4H,Phenyl); 6.8 (s,1H,N=CH)
6c	2-Hydroxy phenyl	$C_{15}H_{13}N_7S_2$ (383g)	224°c (98%)	1551(N=CH),1264 (N-C=S),831 (Ar.-H), 2834 (C-H), 3400 (OH),	14.4 (s,1H,SH); 9.8 (s,1H,NH); 9.1 (s,2H,α-Pyridyl); 8.3-8.2 (m,1H,β-Pyridyl); 8.7-8.6 (d,1H,γ-Pyridyl); 7.5-8 (m,4H,Phenyl); 6.8 (s,1H,N=CH)
6d	2-Chloro phenyl	$C_{15}H_{12}N_7ClS_2$ (401g)	223°c (99%)	1526(-N=CH),1274 (N-C=S),757 (Ar.-H), 3048 (C-H)	14.3 (s,1H,SH); 10.5 (s,1H,NH); 9 (s,2H,α-Pyridyl); 8.3-8.1 (m,1H,β-Pyridyl); 8.8-8.7 (d,1H,γ-Pyridyl); 7.6-7.5 (m,4H,Phenyl); 6.8 (s,1H,N=CH).
6e	4-Methoxy phenyl	$C_{16}H_{15}N_7OS_2$ (385g)	225°c (97%)	1567(-N=CH),1253 (N-C=S),831 (Ar.-H), 2834 (C-H),	14.3 (s,1H,SH); 9.6 (s,1H,NH); 9 (s,2H,α-Pyridyl); 8.2-8.3 (m,1H,β-Pyridyl); 8.8-8.7 (d,1H,γ-Pyridyl); 7.6-7.5 (m,4H,Phenyl); 7-7.1 (s,1H,N=CH); 3.8 (s, 3H, methox

TABLE 2: IN-VITRO ANTI-FUNGAL ACTIVITY

Compounds	100 µg/ml	150 µg/ml
6a	12	13
6b	6	8
6c	9	11
6d	6	10
6e	6	8

Fluconazole (100 µg/ml)- 11 mm

#Diameter of zone of inhibition expressed in mm

TABLE 3: ANTIBACTERIAL ACTIVITY

Compounds	100 µg/ml	150 µg/ml
6a	3	4
6b	12	18
6c	3	6
6d	11	15
6e	3	5

Inhibition diameter in mm, **Standard Streptomycin (100µg/ml)** = 20 mm

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TABLE 4: ANTICONVULSANT ACTIVITY

Average value of hind limb extension recovery data (in sec.) of compounds

Compounds	Average time (in sec.)
standard	5.83
5	19.83
6a	4.83
6b	6.33
6c	11.66
6d	15.33
6e	21.33

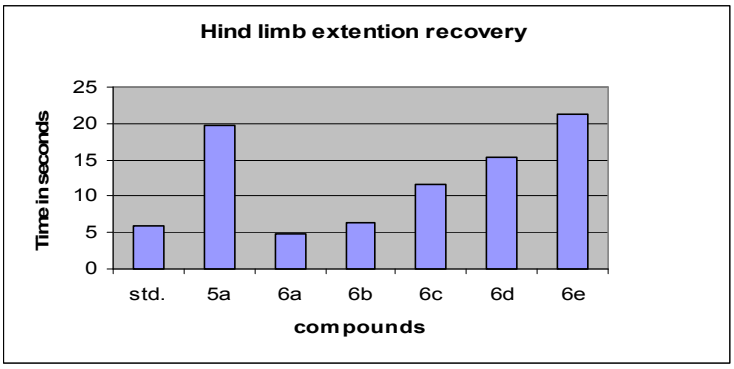

Fig.1 Hind Limb Extension Recovery Data

TABLE 5: AVERAGE VALUE OF TOTAL RECOVERY (IN SEC.) OF COMPOUNDS

Compounds	Average time (in sec.)
Standard	10.33
5	39.66
6a	10.16
6b	12.33
6c	32.5
6d	48.33
6e	49.66

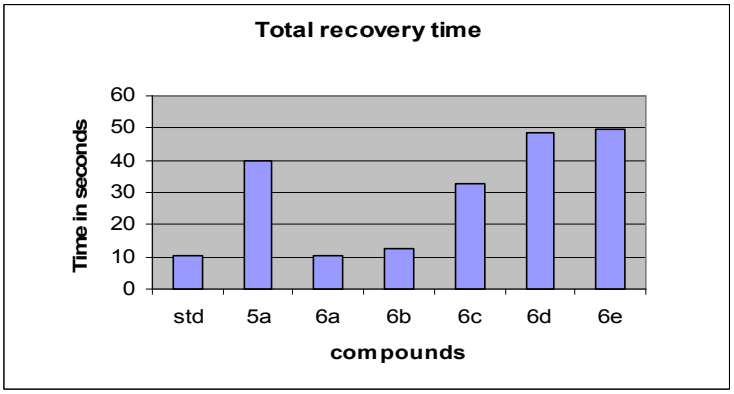

Fig.2 Total Recovery Data

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