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SYNTHESIS AND ANTI-OXIDANT ACTIVITY OF SOME N-(ANILINOCARBONOTHIOYL) BENZAMIDE AND HETEROCYCLIC BASED THIOUREA DERIVATIVES

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Abstract: A series of some novel n-(anilinocarbonothioyl) benzamide and heterocyclic based thiourea derivatives were synthesized and evaluated for anti-oxidant activity. The purity of the synthesized compounds were judged by their C, H and N analysis and the structure was analyzed on the basis of IR, NMR and Mass spectral data. The anti-oxidant activity of new compounds were determined on carbon tetrachloride challenged rats using Vit-E as a standard (Evion,E-merck). Among the compounds tested two compounds, A8 *N*-{[(4-hydroxyphenyl) amino] carbonothioyl} benzamide (% inhibition86.6), H10 *N*-{[(4-methoxyphenyl) amino] carbonothioyl} benzamide (% inhibition 87.7) were the most active compounds in benzamide series. B3 N-(2-phenylethyl) piperidine-1-carbothioamide (% inhibition 84.4) and B4 N-(2-phenylethyl) morpholine-4-carbothioamide (% inhibition 86.7) were the more active compounds in heterocycle based thioureas. As expected the compounds with phenolic hydroxyl and methoxy substituted compounds in benzamide series and in hereocylcle based thioureas morpholine and piperidine nucleus containing compounds showed more percentage protection than the other substituents containing compounds.

Key words: guanidines, thiobarbituric acid, thiuourea, lipid peroxidation

Introduction

Aryl and hetero aryl compounds with thioureas moiety exhibits the unprecedented biological activity ranging from potential anti-cancer[1], Vanilloid receptor (VR1) antagonist[2] (analgesic), Antitubercular[3] and anti-trypanosomal[4] and Antioxidant activity[5]. Dual actions like Anti HIV and anti TB[6], anti-HIV and spermicidal [7] have also been reported

The hydroxyl radical is highly reactive and can damage biological molecules, when moieties of cell membrane phospholipids, lipid hydro-peroxides interact with polyunsaturated fatty acid and can decompose to produce alkoxy and peroxy radical which eventually yield numerous carbonyl products such as malondialdehyde (MDA).

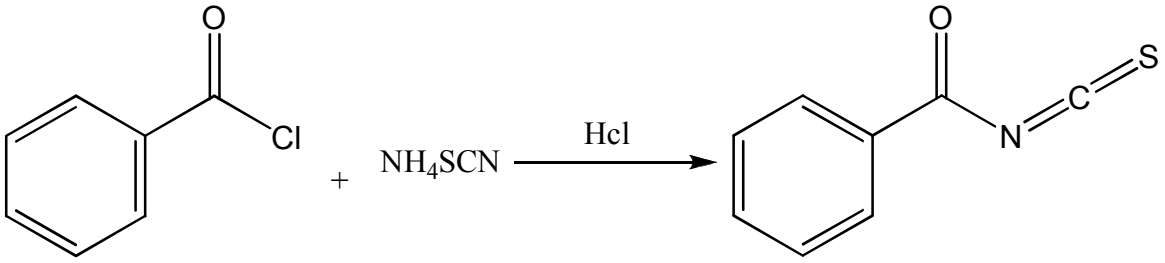
During the last twenty years, the studies of the biological activities of thioureas derivatives have been the aim of many researchers. Also, the structure activity relationships of compounds have revealed that the presence of substituted diaryl derivatives showed antitubercular properties and dihetroaryl derivatives showed anti-HIV activities and based on these findings, we describe the synthesis of some compounds featuring thioureas with diaryl and heteroaryl thioureas derivatives

In the present experiment, thiobarbituric acid (TBA) assay was used for the estimation of peroxidation of rat brain phospholipids. TBA reacts with malondialdehyde (MDA; one of the products of interaction of lipids on hydroxyl radical) with or without the sample under acidic conditions and the amount of pink colored MDA-TBA-adduct formed were measured at λ 532nm.

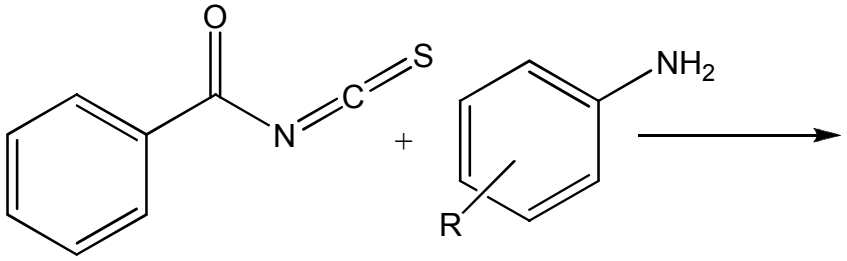
Experimental work

The melting points of the compounds were determined in open capillary tubes on a Thomas hoover melting point apparatus (Perfit) and are uncorrected. IR spectra were recorded in KBr pellets on JASCO FT IR-5300 infrared spectrophotometer (Japan). [1] H-NMR spectra were determined at 300.40 MHZ JEOL-AL 300 (Fourier Transformer, Japan) and mercury plus Varian (400MHZ) spectrometers with tetramethyl silane as

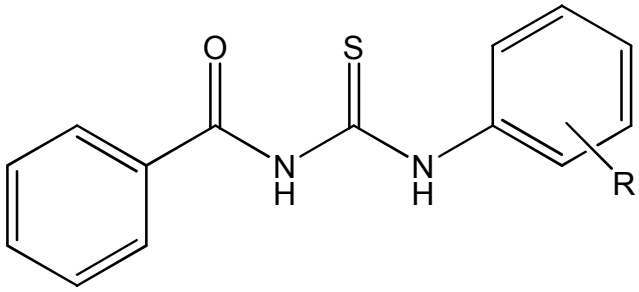
internal standard. The FT 13C NMR recorded in $CDCl_3$ at 25.2MHZ. Mass spectra were recorded on JOEL SX 102/DA -6000 Mass spectrometer (Japan). U.V/Visible spectra were taken in the region of 200-600nm, on Jasco UV-Visible spectrophotometer (Japan). The elemental analysis of the compounds was performed by Perkin Elmer model 240C analyzer (U.S.A).



benzoyl chloroide benzoyl isothiocyanate



Substituted aromatic amine



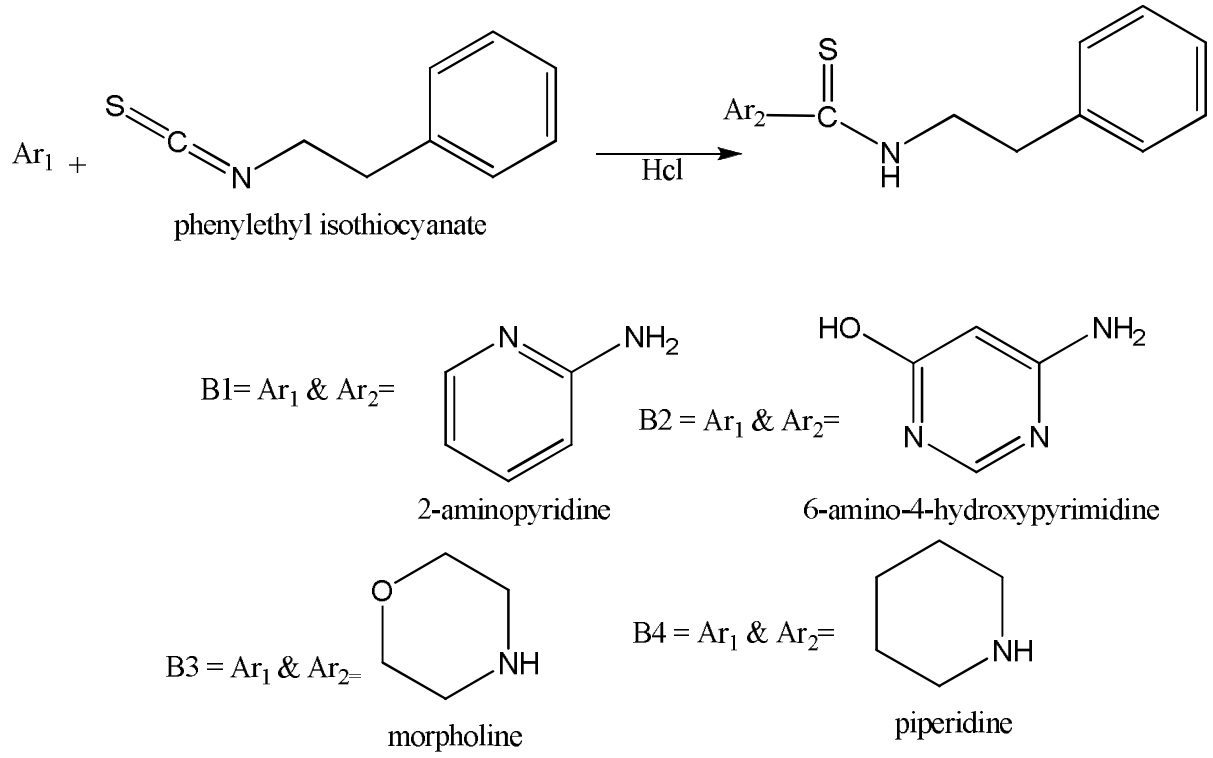
Substituted N-(anilinocarbonothioyl)benzamide

A1= R= H A2 = R = m-Cl A3 = R = p-Cl A4 = o-NO2 A5 = m-NO2 A6 = p-Br

A7 = o-OH A8 = p-OH A9 = o-OCH3 A10 = p-OCH3

Scheme:1

Scheme: 2 Synthesis of Hetero cycle based thioureas



Scheme:2

***N*-(anilinocarbonothioyl) benzamide (A1)**

Method

General method for the synthesis of *N*-(anilinocarbonothioyl)benzamide erivatives (Scheme-1)

1.5g (0.02mol) ammonium thiocyanate was dissolved in 25 ml of methanol to which benzoyl chloride (2.4ml, 0.02mol) was added slowly in a fume hood with stirring. The mixture was cooled intermittently during the addition of benzoyl chloride. The white precipitate of ammonium chloride formed was filtered and the filtrate was treated with 0.02mol of substituted aromatic amines. The reaction mixture was heated on a steam bath till it started to solidify. Nitro substituted compounds solidified in 50minutes, methoxy substituted compounds took 1.5hrs to solidify, unsubstituted and p-chloro substituted compounds solidified approximately in 2hrs. Then, it was removed from the steam bath and cooled; washed with water, drained well and dried.

Physical constant of the compounds (A1-A10) are given in the table 1.

Synthesis of Heterocycle based thioureas (Scheme-2)

Synthesis of 1-pyridin-2yl-thiourea (B1)

Equimolar quantities 2-aminopyridine (3.06g,0.02mol), and ammonium thiocyanate (1.5g, 0.02mol) were dissolved in methanol containing 2ml of Conc.Hydrochloric acid .The reaction mixture was heated on a steam bath for 30 minutes. The contents were allowed to cool and poured in to 100 ml of cold water and the resultant product obtained was filtered, washed and dried. The brown precipitate obtained was recrystallised from rectified sprit.

Synthesis of 1-(4-hydroxypyrimidin-2yl)-3-(2-phenylethyl) thiourea (B2)

Dissolved the equal quantities of 2-amino-4-hydroxypyrimidine (B2), and 2-phenylethylisothiocyanate in a small amount of rectified spirit. The reaction mixture was refluxed for 30 minutes on a steam bath and then cooled in a ice bath. The white crystals of thiourea were separated. The pure product was obtained upon recrystallisation from rectified spirit

Synthesis of N-(2-phenylethyl) piperidine-1-carbothioamide (B3) and N-(2-phenylethyl) morpholine-4-carbothioamide (B4)

Dissolved piperidine (3.4ml, 0.04mol) for B3, morpholine (3.5ml, 0.04mol) for B4, triethylamine (0.6ml, 0.005mol) dissolved in 10ml of tetrahydofuran. To this phenylethyl isothiocyanate (3.3ml, 0.02mol) and stirred well. The temperature was raised during the

addition of phenylethyl isothiocyanate. Then, the mixture was cooled in ice-water with stirring, upon cooling and scratching with a glass rod the white scales were separated. It was filtered at vacuum pump and washed withcold water. Reccrystallised it from rectified spirit.

Physical constant of the compounds (B1-B4) are given in the table 2

Synthesis of *N*-(anilinocarbonothioyl) benzamide derivatives

UV (λ_{max}) in ethanol: 352nm, **(IR) υ_{max} (KBr/cm^{-1}):** 3296(NH), 3041(Ar=CH), 2359 (C-N of –C(O) NH), 1703(C=O), 1598 (N-C=S),1469(C-N), 1274 (C=S), 748, 667 (Ar-H bending viberation). **^{1}H-NMR (δ-ppm):** 2.794NH (s,br,1H,NH-Ar), 3.764-4.096 (s, br,1H,-CO-NH), 7.21 (d, 1H, para to phenyl), 7.187-7.264 (d, 1H, para to phenyl of NH-Ph), 7.335 (dd, 2H, meta phenyl hydrogens of –NH-Ph). 7.63 (d, 2H, orthohydrogens of –NH-Ph), 7.28(d, 1H, of -CO-Ph), 7.41(dd, 2H, meta phenyl hydrogens of –CO-Ph). 7.81 (d, 2H, orthohydrogens of –CO-Ph),

N-{[(3-chlorophenyl)amino]carbonothioyl}benzamide (A2)

UV (λ_{max}) in ethanol: 360nm, **(IR) υ_{max} (KBr/cm^{-1}):** 3291 (NH), 3039 (Ar=CH), 2362 (C-N of –C(O) NH), 1702 (C=O), 1596 (N-C=S), 1426 (C-N), 1273(C=S), 782(C-Cl), 749, 669 (Ar-H bending viberation), **1H-NMR (δ-ppm):** 2.92NH (s,br,1H,NH-Ar), 3.884-4.105 (s, br,1H,-CO-NH), 7.194-7.254 (m, 4H, Ar-H's of Ar-Cl), 7.31(d, 1H, of -CO-Ph), 7.52 (dd, 2H, meta phenyl hydrogens of –CO-Ph). 7.88 (d, 2H, orthohydrogens of –CO-Ph),

N-{[(4-chlorophenyl)amino]carbonothioyl}benzamide (A3)

UV (λ_{max}) in ethanol: 372nm, **(IR) υ_{max} (KBr/cm^{-1}):** 3294(NH), 3036 (Ar=CH), 2363 (C-N of –C(O) NH), 1701 (C=O), 1421 (C-N),1594 (N-C=S), 1271 (C=S), 783 (C-Cl), 747, 668 (Ar-H bending viberation), **^{1}H-NMR (δ-ppm):** 2.91NH (s,br,1H,NH-Ar), 3.882-4.08 (s, br,1H,-CO-NH), 7.284 (d,2H, ortho hydrogens to –NH of (–NH-Ph-Cl), 7.31(d, 2H, ortho hydrogens to –Cl of (–NH-Ph-Cl), 7.36 (d, 1H, of -CO-Ph), 7.48 (dd, 2H, meta phenyl hydrogens of –CO-Ph). 7.79 (d, 2H, orthohydrogens of –CO-Ph),

***N*-{[(2-nitrophenyl) amino] carbonothioyl}benzamide (A4)**

UV (λ_{max}) in ethanol : 373nm, **(IR) υ_{max} (KBr/cm^{-1}):** 3296 (NH), 3033 (Ar=CH), 2360 (C-N of –C(O) NH), 1702 (C=O), 1593 (N-C=S), 1458(C-N), 1352 (C-NO2), 1269 (C=S), 746, 662 (Ar-H bending viberation, **^{1}H-NMR (δ-ppm):** 2.581 NH (s,br,1H,NH-Ar), 3.855-4.128 (s, br,1H,-CO-NH), 6.931-6.964 (dd, para hydrogen of Ar-NO_2 J= 1.66) 7.253-7.307 (m, 3H, Ar-H's of Ar-NO_2), 7.41 (d, 1H, of -CO-Ph), 7.54 (dd, 2H, meta phenyl hydrogens of –CO-Ph). 7.91 (d, 2H, orthohydrogens of –CO-Ph).

***N*-{[(3-nitrophenyl) amino] carbonothioyl} benzamide (A5)**

(IR) υ_{max} (KBr/cm^{-1}): 3298 (NH), 3032 (Ar=CH), 2359 (C-N of –C(O) NH), 1703 (C=O), 1591 (N-C=S), 1445(C-N), 1354 (C-NO2), 1267 (C=S), 743,664 (Ar-H bending viberation).

1H-NMR (δ-ppm): 2.54NH (s,br,1H,NH-Ar), 3.851-4.106 (s, br,1H,-CO-NH) , 6.82-6.86 [dd, para hydrogen of Ar-NO_2 (para to NH) J= 1.54)] 7.232-7.295 (m, Ar-H's of Ar-NO_2),), 7.39 (d, 1H, of -CO-Ph), 7.49 (dd, 2H, meta phenyl hydrogens of –CO-Ph). 7.89 (d, 2H, orthohydrogens of –CO-Ph)

***N*-{[(4-bromophenyl)amino]carbonothioyl}benzamide (A6)**

UV (λ_{max}) in ethanol: 374nm, **(IR) υ_{max} (KBr/cm^{-1}):** 3294(NH), 3036 (Ar=CH), 2360 (C-N of –C(O) NH), 1701(C=O), 1594 (N-C=S), 1468(C-N), 1270 (C=S), 749, 669 (Ar-H bending viberation), 502 (C-Br), **^{1}H-NMR (δ-ppm):** 2.94NH (s,br,1H,NH-Ar), 3.886-4.23 (s, br,1H,-CO-NH), 7.234-7.384 (d,2H, ortho to NH of –NH-Ar-Br),), 7.29 (d, 2H, rtho to Br of –NH-Ar-Br), 7.31 (d, 1H, of -CO-Ph), 7.46 (dd, 2H, meta phenyl hydrogens of –CO-Ph). 7.87 (d, 2H, orthohydrogens of –CO-Ph)

***N*-{[(2-hydroxyphenyl) amino] carbonothioyl} benzamide (A7)**

UV (λ_{max}) in ethanol: 403nm, **(IR) υ_{max} (KBr/cm^{-1}):** 3294(NH), 3038 (Ar=CH), 2360 (C-N of –C(O) NH), 1703 (C=O), 1594 (N-C=S), 1271(C=S), 1462(C-N), 13282(C-O) 742.662 (Ar-H bending viberation), **^{1}H-NMR (δ-ppm):** 2.54NH (s,br,1H,NH-Ar), 4.10-4.42s, br,1H,-CO-NH), 7.234-7.384 (m, Ar-H's of Ar-OH), 7.38(d, 1H, of -CO-Ph), 7.51 (dd, 2H, meta phenyl hydrogens of –CO-Ph). 7.89 (d, 2H, orthohydrogens of –CO-Ph), 9.64 (s, proton of ph-OH), 3432 (-OH)

***N*-{[(4-hydroxyphenyl) amino] carbonothioyl}benzamide (A8)**

UV (λ_{max}) in ethanol: 396nm, **(IR) υ_{max} (KBr/cm^{-1}):** 3291 (NH), 3034 (Ar=CH), 2362 (C-N of –C(O) NH), 1702(C=O), 1422(C-N), 1596(N-C=S), 1273 (C=S), 1339(C-O) 747.662 (Ar-H bending viberation), **^{1}H-NMR (δ-ppm):** 2.49NH (s,br,1H,NH-Ar), 4.08-4.217(s, br,1H,-CO-NH), 7.189-7.264 (m, Ar-H's of Ar-OH),), 7.34 (d, 1H, of -CO-Ph), 7.48 (dd, 2H, meta phenyl hydrogens

of –CO-Ph). 7.86 (d, 2H, orthohydrogens of –CO-Ph), 9.31(s, proton of ph-OH), 3392 (-OH)

***N*-{[(2-ethoxyphenyl)amino]carbonothioyl}benzamide (A9)**

UV (λ_{max}) in ethanol: 403nm, **(IR) υ_{max} (KBr/cm^{-1}):** 3294 (NH), 3035 (Ar=CH), 2905 (CH_3) 2362 (C-N of –C(O) NH), 1701(C=O), 1592 (N-C=S), 1421 (C-N), 1274 (C=S), 1267(C-O),1076(C-O-C), 749, 669 (Ar-H bending viberation), **^{1}H-NMR (δ-ppm):** 1.58 [s,(3H),-CH_3], 2.34 [s,1H,NH(s,br,1H,NH-Ar], 3.823-4.096(s, br,1H,-CO-NH), 7.186-7.264(m, Ar-H's of Ar-OCH_3),), 7.33(d, 1H, of -CO-Ph), 7.49 (dd, 2H, meta phenyl hydrogens of –CO-Ph). 7.85 (d, 2H, orthohydrogens of –CO-Ph)

***N*-{[(4-methoxyphenyl)amino]carbonothioyl}benzamide (H10)**

UV (λ_{max}) in ethanol: 412nm, **(IR) υ_{max} (KBr/cm^{-1}):** 3298 (NH), 3031(Ar=CH), 2949 (CH_3) 2366 (C-N of –C(O) NH), 1698 (C=O), 1426(C-N), 1590 (N-C=S), 1271 (C=S), 1263 (C-O), 1053 (C-O-C) 745,667 (Ar-H bending viberation), **^{1}H-NMR (δ-ppm):** 1.46 [s,(3H),-CH_3], 2.30 (s,1H,NH (s,br,1H,NH-Ar), 3.81-4.065 (s, br,1H,-CO-NH), 7.182 (d, Ar-H's ortho to NH of –NH-Ar-OCH_3), 7.29 (d, Ar-H's ortho to –OCH_3 of –NH-Ar-OCH_3) 7.31 (d, 1H, of -CO-Ph), 7.46(dd, 2H, meta phenyl hydrogens of –CO-Ph). 7.82 (d, 2H, orthohydrogens of –CO-Ph).

The 13C–NMR spectrum of *N*-(anilinocarbonothioyl) benzamide (A1) shown in exhibits a singlet at δ 216.1 for thethiocarbonyl carbon.the thiocarbonyl carbon appeared in the 13C–spectrum at δ 202.8 as a doublet. The spectrum also exhibits unusual long range couplings between the N and C-1 of the phenyl ring, which appears as a doublet (J = 8.40 Hz) at δ 143.0 and between 15N andthe two equivalent ortho carbon of the phenyl ring, which appear as a doublet (J = 3.10 Hz) at δ 128.8.

The mass spectrum of exhibited the molecular ion peak which was consistent with the molecular weight of molecular formula and base peak M/Z= 135 (M-59) for *N*-(anilinocarbonothioyl) benzamide due to cleavage of N=C= S from the molecular ion and the other intense peaks with relative intensity of 42% due to cleavage of –CO and m/z=64 due to pentylium were commonly observed in all the mass spectrum.

Biological investigation
Materials and methods

Experimental protocols and procedures used in this study were approved by the Animal Ethics Committee of the Allahabad Agricultural Institute, Deemed University, Allahabad and confirm to the "Guide to the Care and Use of Animals in Research and Teaching" [published by the

Ethics Committee of the Allahabad Agricultural Institute, Deemed University, Allahabad

Animals

Wister rats of both sexes weighing 250–300g were used. The animals were kept and maintained under laboratory conditions of temperature, humidity, and light; and were allowed free access to food (standard pellet diet) and water ad libitum. The animals were divided into – Guanidine treated, reference drug treated 'test', and distilled water-treated 'control' groups of six animals per group.

***In vivo* antioxidant activity[8]**

In the present experiment, thiobarbituric acid (TBA) assay was used for the estimation of peroxidation of rat brain phospholipids. TBA reacts with malondialdehyde (MDA; one of the products of interaction of lipids on hydroxyl radical) with or without the sample under acidic conditions and the amount of pink colored MDA-TBA-adduct formed were measured at λ 532nm

Data analysis

Experimental data obtained from 'test' rats treated synthesized compounds Guanidines (B1-B7), Vitamin- E treated group alone, as well as those obtained from sodium carboxy methyl cellulose-treated (Na CMC)'control' mice and rats, were pooled and expressed as means (±S.E.M.). The differences standard drug treated - or synthesized compounds - treated 'test' rats means, and sodium carboxy methyl cellulose (NaCMC) treated' control' rats means, statistical comparisons were performed using Students ' t' test, to assess the level of significance of the differences between the 'test' and 'control' group data means. Values of $P \leq 0.05$ were taken to imply statistical significance.

Method

The rats used were divided into three broad (A, B and C) experimental groups of six rats per group. Group 'A' rats were used as control and each animal in this group received sodium carboxy methyl cellulose (0.1% 3ml / kg i.p.) only. Group B 'test rats received the n-(anilinocarbonothioyl) benzamide and heterocyclic based thiourea derivatives and Group C 'test' rats received vitamin-E at the dose of (100mg/kg i.p.).

Treatment

The treatment schedule is given in table no.3 and 4. All the drugs were given at the dose mentioned above in 0.1% NaCMC for seven consecutive days. On fifth day except group (A) all other groups were given CCl_4 (2ml/Kg body wt). All the animals were sacrificed on seventh day (i.e. after one hour of drug treatment and 48

hrs of CCl_4 challenge by dislocation of cervical vertebrae) and the following estimations were done.

Preparation of tissue homogenate[9]

Rat brain was removed, immediately washed with ice cold 1.15% w/v Potassium chloride solution, briefly dried between filter papers (to remove excess blood), weighed and brain homogenate was prepared in the ratio of wet brain tissue (0.75g) to potassium chloride solution (1.15%, 9.25ml) using Teflon homogenizer. The homogenate was centrifuged at 1500 rpm for 10 min to remove nuclear fraction.
The supernatant was used for the estimation of lipid peroxide level (MDA) content.

Estimation of Malonodialdehyde[10]
Procedure
The incubation mixture was prepared as shown in the table 3 and the incubation mixture was made up to 5ml with triple distilled water and then boiled on a water bath at 80^0 C for 30 min. After cooling the mixture was centrifuged at 1600 rpm for 10min. The supernatant was taken ant its absorbance at 532 nm was measured. The free radical scavenging activity was calculated according to the following equation: % Inhibition = ((A0-A1) / A0 x 100). Where A0 was the absorbance of the carbon tetrachloride administered rats and A1 was the absorbance in the presence of the compounds whose activity to be determined

The extent of lipid peroxidation in tissues was assessed by measuring the level of malondialdehyde (MDA) in the brain tissue.

The incubation mixture was made up to 5ml with triple distilled water and then boiled on a water bath at 80^0 C for 30 min. After cooling the mixture was centrifuged at 1600 rpm for 10min. The supernatant was taken ant its absorbance at 532 nm was measured. The free radical scavenging activity was calculated according to the following equation: % Inhibition = ((A0-A1) / A0 x 100).Where A0 was the absorbance of the carbontetrachloride administered rats and A1 was the absorbance in the presence of the compounds whose activity to be determined.

Results and discussion

N-(anilinocarbonothioyl)benzamides and heterocycle based thioureas were synthesized as shown in the scheme-1 and scheme-2. All the compounds obtained were good yield ranging from 64-85%. The homogeneity of the compounds was monitored by performing TLC by which Rf and Rm values were calculated. A2, A4, A5 and A10 and were found to be more lipophilic indicated by their higher Rm values. Compounds B2, B3 and b4 were found to be more lipophilic.

The IR spectrum of the titled compounds shown the presence of stretching viberations in theregion of 1703 -1701 due to C=O stretch and the stretching vibrations at 3296-3291 by 2^0 NH stretch confirmed the (-C=O and NH) in benzoyl thioureas.

1-(4-hydroxypyrimidin-2yl)-3-(2-phenylethyl) thiourea (B2), exhibited the O-H stretch at 3464, C-H stretch of (CH_2-CH_2) at 2587-2341, absorption at 1146 by the C-N,aliphatic sretch, broader stretch at 808-750 confirmed the pyrimidyl C-H bending viberation1054 and 1072 (C-O-C deformation)1046, 668, 636 (Ar-H bending vibration) and I4 along with the characteristic peaks of thioureas viberation1054 and 1072 due to C-O-C deformation of morpholine moiety.

The formation of benzoyl thioureas confirmed by the presence of two singlets one at δ 1.794 (1H), broad signal due to NH of (-NH-Ph) and another singlet at δ 3.764-4.096, broad, (1H), which could be assigned to the –NH of (CO-NH) .

1-(4-hydroxypyrimidin-2yl)-3-(2-phenylethyl) thiourea formation is confirmed by multiplet at 1.08-1.56 , 2H of Ar-CH2-(Ar-CH2CH2-NH-)], triplet at 5.591-5.692 by –NH of -NH-CH2-, 5.861(s (br)(1H),-NH-Ar), multiplet at 7.547-7.597 by 2H of pyrimidyl 10.811-10.849 and the singlet, 1H of -OH attached to pyrimidine.

N-(anilinocarbonothioyl)benzamides, heterocycle based thioureas, and Vit-E (Evion, E-Merck) were screened for its antioxidant activity on carbon tetrachloride challenged rat brain phospholipids. Free radical scavenging activity was given in terms of the percentage inhibition which was calculated by % Inhibition = ((A0-A1) / A0 x 100). Where A0 was the absorbance of the carbon tetrachloride administered rats and A1 was the absorbance of the compounds whose activity to be determined. The % Inhibition was related with the amount of malonodialdehyde. The higher amount of absorption at 532nm is directly proportional to the malonodialdehyde.

The free radical was generated by the administration of carbon tetrachloride. The extensive generation of free radicals was observed in the control and vehicle treated groups. It is further observed that administration of *N*-(anilinocarbonothioyl)benzamides , heterocycle based thioureas and guanidine derivatives reduced the amount of carbon tetrachloride induced free radical which was observed through reduction in the quantity of malonodialdehyde formation using Vit-E as a standard

All the *N*-(anilinocarbonothioyl)benzamide derivatives exhibited inhibition of lipid peroxidation.The % protection against the free radical formation is given in table no 4 for *N*-(anilinocarbonothioyl)benzamides and

for heterocycle based thioureas given in table No 5 Among the chloro substituted compounds the parachloro substituted compound (A3 (% inhibition 78.8) showed slightly higher inhibition than the m-chloro substituted compound. In the nitro substituted compounds meta nitro substituted A5 exhibited higher % inhibition (78.3) than ortho substituted compound A4. Similiarly, the para bromo substituted compound also showed significant inhibition of lipid peroxidation. In case of phenoic hydroxyl the ortho substituted (A7) and para substituted (A8) compounds showed the inhibition 83.8 and 86.6 respectively. Methoxy substituted compounds, ortho substituted A9 and para substituted A10 showed the % inhibition of 84.4 and 87.7 respectively. The % inhibition of A10 (87.7) was comparable with the activity of the standard Vit-E (90.5). The *N*-(anilinocarbonothioyl)benzamides derivatives possess the electro negative groups at the para position on the phenyl ring of benzoyl thiourea derivatives showing the high degree of inhibition than the un substituted and substitution in the other positions indicating substitution in this position might be favouring for the receptor binding.

Heterocycle based thioureas also showed the inhibition in the lipid peroxidation when compared with the standard Vit-E. When the activities of these compounds compared thiourea with morpholine (% inhibition 86.7) and piperidine (% inhibition 84.4%) was comparable with the standard Vit-E (% inhibition 90.5)

The carbonyl products are responsible for DNA damage, generation of cancer and aging related diseases (Green DR et al 1998).Thus the decrease in the MDA level in young and aged rat with the administration of benzoyl thioureas, heterocycle based thioureas, guanidine derivatives indicating the antioxidant activity of these compounds through scavanging of the free radical which participate in various pathophysiologies of diseases including ageing. N,N'-substituted thioureas have been employed as potent hydroxyl radical scavengers and also inhibit production of oxygen free radicals (Sang Geon Kim.A, et al., 1999)

Table:1 PHYSICAL DATA OF *N*-(ANILINOCARBONOTHIOYL) BENZAMIDE

S.No	Com pound	R	X	%Yield	M.P (^{0}C)	Mol.Formula	Mol.Wt	Rf[a]	Rm[b]
1	H1	H	H	82.5	155	$C_{14}H_{12}N_2OS$	256.32288	0.728	-0.4276
2	H2	3-Cl	H	77.5	121	$C_{14}H_{11}ClN_2OS$	290.76794	0.80	-0.602
3	H3	4-Cl	H	81	143	$C_{14}H_{11}ClN_2OS$	290.76794	0.733	-0.438
4	H4	2-NO2	H	85	102	$C_{14}H_{11}N_3O_3S$	301.32044	0.831	-0.6917
5	H5	3-NO2	H	83	113	$C_{14}H_{11}N_3O_3S$	301.32044	0.842	-0.726
6	H6	4-Br	H	65.5	136	$C_{14}H_{11}BrN_2OS$	335.21894	0.766	-0.515
7	H7	2-OH	H	63.5	167	$C_{14}H_{12}N_2O_2S$	272.32228	0.714	-0.397
8	H8	4-OH	H	66	154	$C_{14}H_{12}N_2O_2S$	272.32228	0.731	-0.434
9	H9	2-OCH3	H	76	181	$C_{15}H_{14}N_2O_2S$	286.34886	0.716	-0.401
10	H10	4-OCH3	H	74	167	$C_{15}H_{14}N_2O_2S$	286.34886	0.860	-0.788

Table 2 Physical data of Hetero Cycle based Thioureas

S.No	Compound	Yield (%)	M.P (^{0}C)	Mol.Formula	Mol.Wt	Rf[a]	Rm[b]
1	I1	74.5	65	$C_6H_7N_3S$	153.20488	0.776	-0.539
2	I2	83	212	$C_{13}H_{14}N_4OS$	274.34146	0.902	-0.963
3	I3	80.5	94	$C_{14}H_{20}N_2S$	248.388	0.865	-0.806
4	I4	63.5	81	$C_{13}H_{18}N_2OS$	250.361	0.880	-0.865

[a]The solvent system used for TLC was Chloroform: Methanol (8:2) for all the compounds
[b] Rm = log [(1/Rf)-1]

Table 3

Sl.No	*Ingredients*	*Volume*
1	Tissue homogenate (supernatant)	0.5ml
2	Acetate buffer	1.5ml
3	Sodium lauryl sulphate (4%)	0.2 ml
4	0.8% aqueous solution of thiobarbituric acid (pH7.4)	1.5ml
5	Triple distilled water	1.3ml

Table 4 Effect of synthesized *N*-(anilinocarbonothioyl)benzamide derivatives (A1-A10) (100mg/Kg i.p) and Vitamin-E (E.Merck) (100 mg/kg i.p.) on lipid peroxide level. Each value represents the mean (± S.E.M) of six observations.

Experimental group	*Dose (i.p.)*	*Absorbance at 532nm*	*% inhibition*
Control Group A (untreated)	***0***	***0.12 ±2.79*****	***93.3***
Group B (sodium CMC treated)	3ml/kg	1.72 ±3.12**	0.04(NS)
Carbon tetra chloride treated group	2ml/kg	1.8 ±3.28*	-
A1	100mg/kg	0.52 ±2.78**	71.1
A2	100mg/kg	0.44 ±2.65**	75.5
A3	100mg/kg	0.38 ±2.45**	78.8
A4	100mg/kg	0.41 ±3.12**	77.2
A5	100mg/kg	0.39 ±1.12***	78.3
A6	100mg/kg	0.32 ±2.75**	82.2
A7	100mg/kg	0.29 ±3.11**	83.8
A8	100mg/kg	0.24 ±2.89**	86.6
A9	100mg/kg	0.28 ±1.17***	84.4
A10	100mg/kg	0.22 ±2.65**	87.7
Vit-E	100mg/kg	0.17 ±2.89**	90.5

* $P < 0.05$; **$P < 0.01$; ***$P < 0.001$ vs. control. N S: No significant activity

Table 5
Effect of synthesized Hetercycle based thioureas derivatives (B1-B4) (100mg/Kg i.p) and Vitamin-E (E.Merck) (100 mg/kg i.p.) on lipid peroxide level. Each value represents the mean (± S.E.M) of six observations.

Experimental group	*Dose (i.p, oral)*	*Absorbance at 532nm*	*% inhibition*
Control Group A (untreated)	***0***	***0.12 ±2.79*****	***93.3***
Group B (sodium CMC treated)	***3ml/kg***	***1.72 ±3.12*****	***0.04(NS)***
Carbon tetra chloride treated group	2ml/kg	1.8 ±2.54*	
B1	100mg/kg	0.42 ±3.11**	76.7
B2	100mg/kg	0.39 ±2.12**	78.3
B3	100mg/kg	0.28 ±3.81**	84.4
B4	100mg/kg	0.24 ±1.14***	86.7
Vit-E	100mg/kg	0.17 ±2.89**	90.5

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ vs. control. N S: No significant activity

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