International Journal of Drug Development & Research
| October-December 2011 | Vol. 3 | Issue 4 | ISSN 0975-9344 |
Available online http://www.ijddr.in
Covered in Official Product of Elsevier, The Netherlands

In-Vitro antioxidant assay of synthesized 2 & 4 substituted phenyl Urea/Thiourea (Carboxylic Acid/Carboxamide) by using 1, 1, Diphenyl-2-Picrylhydrazyl as a stable oxidant

Viraj Pareshbhai Jatakiya*, Kiran Manubhai Patel, Ravikumar V. Modi, Dr. R. Badmanaban and Dr. Dhrubo Jyoti Sen
Shri Sarvajanik Pharmacy College, Gujarat Technological University, Arvind Baug, Mehsana-384001, Gujarat

Abstract

The structure activity relationship study for the synthesized eight compounds has been divided into two series: 2-substituted and 4-substituted phenyl ureas having variable atoms in (X). X=O (urea) and X=S (thiourea) for carboxylic acid and carboxamide substitutions in phenyl ring produces open chain ureas which have been screened for CNS depression study by using closed chain ureas to identify the correlation analogy between closed chain and open chain ureas on CNS depression and sleeping time potentiation. But in the present study we have taken the part of work related to the "in-vitro antioxidant assay" by using DPPH. This approach is there to support and serve as a tool for doing antioxidant assay at laboratory level to get a basic and preliminary conclusion for the potency of the compounds as an antioxidant. It may be a useful tool for a researcher and scientist to ascertain the antioxidant potential of synthesised compounds in rapid way.

*Corresponding author, Mailing address:
Viraj Pareshbhai Jatakiya
Shri Sarvajanik Pharmacy College, Gujarat Technological University, Arvind Baug, Mehsana-384001, Gujarat
E-mail: virajjatakiya@yahoo.in

Key words:
Electronegativity, DPPH, Antioxidant, Phenyl urea/thiourea (carboxylic acid/carboxamide), IC_{50}

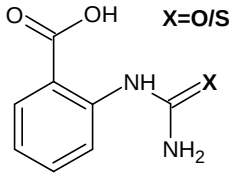
***How to Cite this Paper*:**
Viraj Pareshbhai Jatakiya*, Kiran Manubhai Patel, Ravikumar V. Modi, Dr. R. Badmanaban and Dr. Dhrubo Jyoti Sen "*In-Vitro* antioxidant assay of synthesized 2 & 4 substituted phenyl Urea/Thiourea (Carboxylic Acid/Carboxamide) by using 1, 1, Diphenyl-2-Picrylhydrazyl as a stable oxidant", Int. J. Drug Dev. & Res., Oct-Dec 2011, 3(4): 294-299

***Article History:* ----------**
Date of Submission: 09-10-2011
Date of Acceptance: 31-10-2011
Conflict of Interest: NIL
Source of Support: NONE

Aims and Objectives:

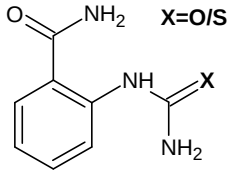
Series of Synthesized Molecules:-

Series: 1



Compound -1: 2-carboxy-phenyl urea
Compound-3: 2-carboxy-phenyl thiourea

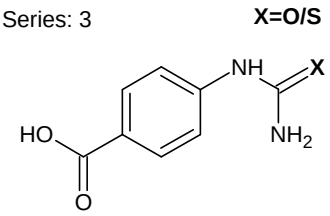
Series: 2



Compound-2: 2-carboxamido-phenyl urea

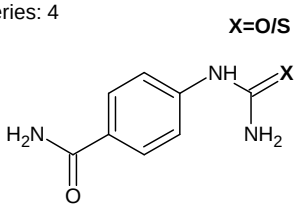
Compound-4: 2-carboxamido-phenyl thiourea

Series: 3



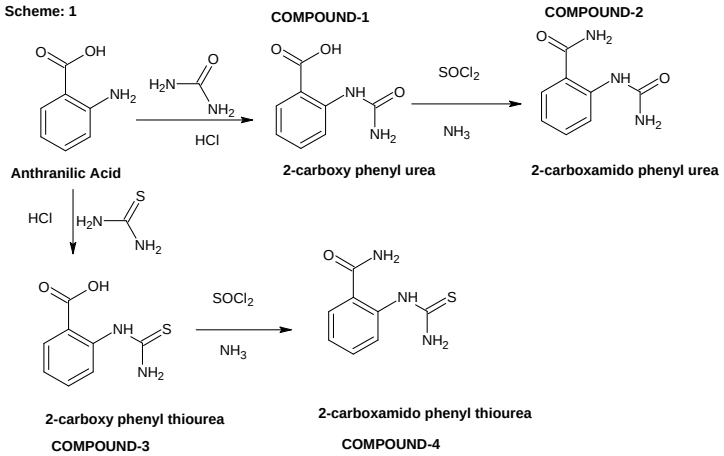
Compound-5: 4-carboxy phenyl urea
Compound-7: 4-carboxy phenyl thiourea

Series: 4



Compound-6: 4-carboxamido phenyl urea
Compound-8: 4-carboxamido phenyl thiourea

Scheme for Synthesis of Compounds (1-4)[1-5]:-



Scheme for Synthesis of Compounds (5-8)[1-5]:-

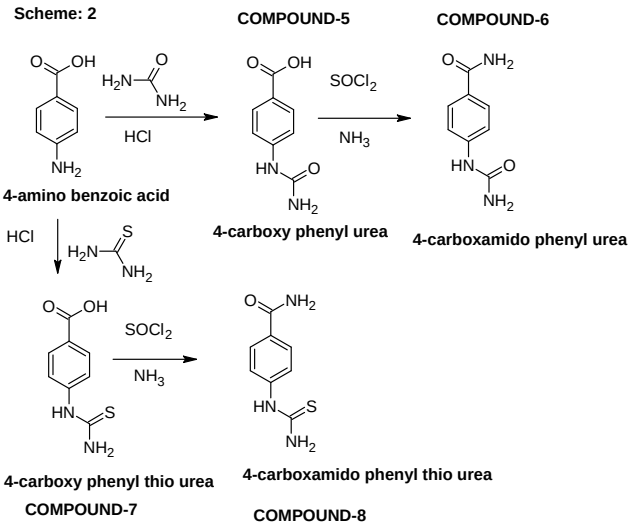


Table 1: Solubility Parameters

Compounds	Solubility	Polarity
2-carboxy phenyl urea	Hot Water (1mg/ml), Methanol (2mg/ml)	Semi polar
2-carboxamido phenyl urea	Hot Water (1mg/ml)	Semi polar
2-carboxy phenyl thiourea	Water (1mg/ml), Methanol (1mg/ml)	Polar
2-carboxamido phenyl thiourea	Hot Water (1mg/ml)	Semi polar
4-carboxy phenyl urea	Water (1mg/ml), Methanol (2mg/ml)	Polar
4-carboxamido phenyl urea	Hot Water (1mg/ml)	Semi polar
4-carboxy phenyl thiourea	Water (1mg/ml), Methanol	Polar
4-carboxamido phenyl thiourea	Hot Water (1mg/ml)	Semi polar

Table 2: U.V. Spectras

Compounds	λ_{max}±SD	Absorbance
2-carboxy phenyl urea	306.2 ±0.97	0.766
2-carboxamido phenyl urea	310.4±0.85	0.960
2-carboxy phenyl thiourea	326.8±0.92	0.939
2-carboxamido phenyl thiourea	306.6±0.71	0.314
4-carboxy phenyl urea	277.6±0.79	0.963
4-carboxamido phenyl urea	266.6±0.82	1.940
4-carboxy phenyl thiourea	279.6±0.90	0.815
4-carboxamido phenyl thiourea	266.4±0.82	1.294

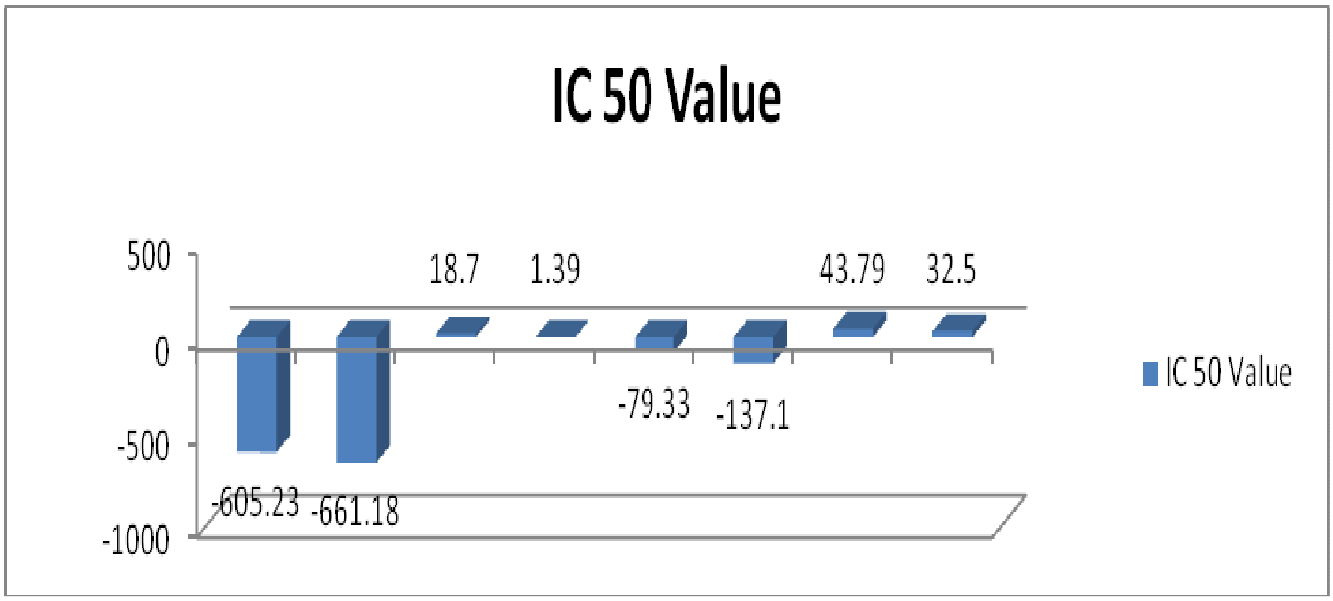
Table 3: Physicochemical Parameters

Compounds	% Yield	M.P. ^{o}C	Polarity	Molecular Formula	N% Calculated	N% Found
2-CARBOXY PHENYL UREA	77.37%	158-162±1.41	Semi Polar	$C_8H_8N_2O_3$	15.555	15.983
2-CARBOXAMIDO PHENYL UREA	68.33%	101-103±0.5	Semi Polar	$C_8H_9N_2O_2$	16.969	17.218
2-CARBOXY PHENYL THIOUREA	82.48%	176-178±0.56	Polar	$C_8H_8N_2O_2S$	14.285	14.574
2-CARBOXAMIDO PHENYL THIOUREA	56.66%	120-122±0.49	Semi Polar	$C_8H_9N_2OS$	15.469	15.872
4-CARBOXY PHENYL UREA	80.29%	260-262 ± 0.53	Polar	$C_8H_8N_2O_3$	15.555	15.886
4-CARBOXAMIDO PHENYL UREA	48.33%	304-306±0.55	Semi Polar	$C_8H_9N_2O_2$	16.969	17.438
4-CARBOXY PHENYL THIOUREA	76.64%	262-264 ± 0.50	Polar	$C_8H_8N_2O_2S$	14.285	14.674
4-CARBOXAMIDO PHENYL THIOUREA	71.66%	295-297± 0.81	Semi Polar	$C_8H_9N_2OS$	15.469	15.787

Histogram of Test Compounds

Table 4: IC_{50} values of Test Compounds

Comp's.	Name of the compound	IC 50 Value
1	2-carboxy phenyl urea	- 605.23 ppm
2	2-carboxamido phenyl urea	-661.18 ppm
3	2-carboxy phenyl thio urea	18.7 ppm
4	2-carboxamido phenyl thio urea	1.39 ppm
5	4-carboxy phenyl urea	-79.33ppm
6	4-carboxamido phenyl urea	-137.10 ppm
7	4-carboxy phenyl thio urea	43.79 ppm
8	4-carboxamido phenyl thio urea	32.50 ppm



Histogram of IC_{50} of Test Compounds

DETERMINATION OF ANTIOXIDANT POTENTIAL OF SYNTHESISED COMPOUNDS BY AN OXIDANT

Antioxidant assay

The antioxidant activity of synthesized compounds was determined by different *in-vitro* methods such as the DPPH free radical scavenging assay and reducing power methods. The different compounds were dissolved in ethanol at the concentration of 2mg/ml. All the assays were carried out in triplicate and average value was considered.

(a) DPPH Radical scavenging activity

DPPH scavenging activity of the synthesized compounds were carried out according to the method of Koleva *et al* 2002; Mathiesen *et al* 1995. 2 ml of ethyl alcohol solution of synthesized compounds at different concentration (20-100 μgml^{-1}) was mixed with 0.8 ml of Tris HCl buffer (100 Mm, pH 7.4). One ml DPPH (500 Mm in ethanol) solution was added to above mixture. The mixture was shaken vigorously and incubated for 30 min in room temperature.[8,9] Absorbance of the resulting solution was measured at 517 nm UV-Visible Spectrophotometer (Shimadzu 1700, INDIA). All the assays were carried out in triplicates with BHA (Butylated Hydroxy Anisole) as a positive control. Blank was prepared without the addition of DPPH and for control 0.2 ml of ethyl alcohol (without synthesized compounds) was added.

Percentage of DPPH scavenging activity determined as follows.

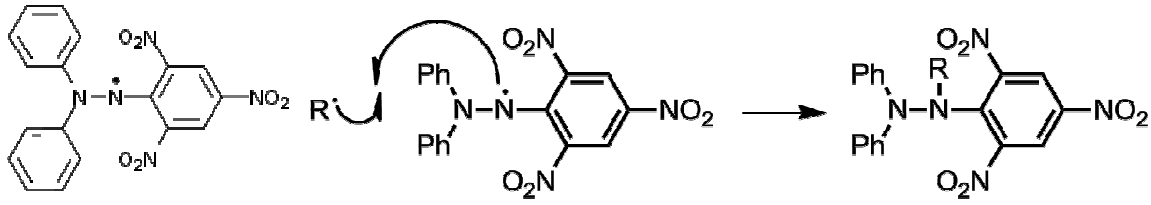
% DPPH radical-scavenging = (Absorbance of control – Absorbance of test sample) ÷ (Absorbance of control) × 100

Where A=absorbance of the extract and DPPH at 517 nm after 30 min of reaction

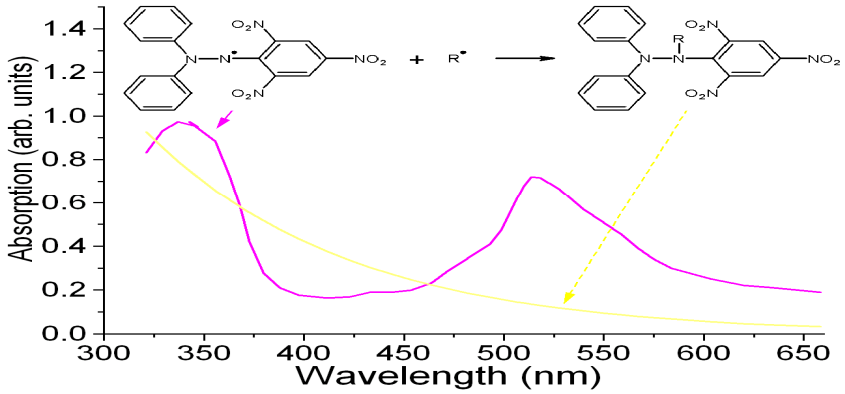
Abs. Cont.=absorbance of DPPH at 517 nm as a control, Abs. test =an absorbance of extract at 517 nm as a blank

Control was the DPPH solution without synthesized compounds. Purified sample 2 mg/ml in ethyl alcohol of synthesized compounds were taken for antioxidant activity with a standard BHA (Butylated Hydroxy Anisole) antioxidant.

Decreased absorbance of the reaction mixture indicates stronger DPPH radical-scavenging activity.[6-10]



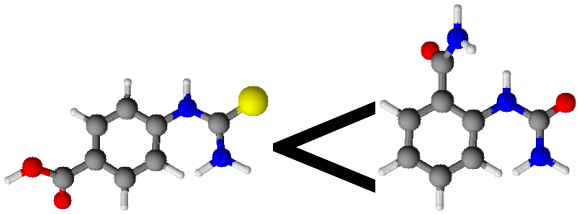
IUPAC Name of DPPH: di (phenyl)-(2, 4, 6-trinitrophenyl) iminoazanium



Conclusion: DPPH is a common abbreviation for an organic Chemical Compound 1, 1-diphenyl-2-picrylhydrazyl radical. It is a dark-colored crystalline powder composed of stable free-radical molecules. DPPH has major applications to calculate antioxidant potential of the compounds at laboratory level.

Potentiality of IC_{50} value:

4-CARBOXY PHENYL THIOUREA (43.79 ppm) < 2-CABOXAMIDO PHENYL UREA (-661.18 ppm)



Electronegative atoms O and S both have two lone pair of electrons but electronegativity for O: 3.44>N: 3.04>S: 2.58, so in $\lambda_{max\ in}$ UV thiourea compound gives maximum absorption.

Brand-Williams *et al* 1995 and Blois 1958 defined the use of IC_{50}, (that is, EC_{50}) to calculate the antioxidant potential of the compounds. According to their work, as the antioxidant potential of the compound increases IC_{50} (that is, EC_{50}) value of the compounds decreases.[11,12] So, 2-carboxamido phenyl urea has highest antioxidant potential while 4-carboxy phenyl thiourea has lower antioxidant potential.

Acknowledgement: The authors are thankful to the Department of Pharmaceutical Chemistry of Shri Sarvajanik Pharmacy College, Mehsana to perform the research work successfully with the expertise of project guides.

References:

1) Prabhu G.V. and Venkappayya D. (1995)**, J.Indian Chem.Soc**., 72, 681-684.
2) Dandiya P.C., Sharma P.K. and Menon M.K. (1962), **Ind. J. Med. Res.,** 50, 752-760.
3) A.I. Vogel, Vogel's Textbook of Practical Organic Chemistry 3rd Edition (1956), Longmans, Green and Co Ltd, London, 280-310.
4) Sen D.J. (2000), PhD thesis, Jadavpur University.
5) Modi R. V. and Sen D. J. (2010); *Structure activity relationship studies of synthesized urea diamides on CNS depression and sleeping time potentiation effect*: **International Journal of Drug Development and Research**: 2(3), 512-528.
6) Kiers, C. T.; De Boer, J. L.; Olthof, R.; Spek, A. L. (1976). *The crystal structure of a 2,2-diphenyl-1-picrylhydrazyl (DPPH) modification*: **Acta Crystallographica Section B Structural Crystallography and Crystal Chemistry** 32, 2297.
7) M.J. Davies (2000). Electron Paramagnetic Resonance. Royal Society of Chemistry. p. 178
8) Koleva I. I., Van Beek T. A., Linssen J.P.H., De Groot A, Evstatieva L.N., (2002), *Screening of plant extracts for antioxidant activity: a comparative study on three testing methods*. **Phytochemical Analysis,** 13, 8-17.
9) Mathiesen, L., Malterud, K.E. and Sund R.B. (1995), *Antioxidant activity of fruit exudate and methylated dihydrochalcones from Myrica gale*. **Planta Med**, 61, 515-518.
10) Gangu R. A., Bontha V. K., Lagishetty V., Garrepally P., (2011) Madhu Babu A, Rizwanulla K *In-Vitro Antioxidant Activity of Curculigo orchioides* **J.Pharm.Pract.,** 1(1), 11-13.
11) Brand-Williams, W., Cuvelier, M.E. and Berset, C. (1995) *Use of a free radical method to evaluate antioxidant activity*, **Lebensmittel-Wissenschaft und-Technologie/Food Science and Technology**, 28, 25-30.
12) Blois, M.S. (1958) *Antioxidant determinations by the use of a stable free radical*, **Nature**, 181, 1199-1200.