WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 9, 744-755. Research Article ISSN 2277– 7105

SYNTHESIS AND ANTIOXIDANT ACTIVITY OF UREA AND THIOUREA DERIVATIVES OF GLY/PRO CONJUGATED 2, 3-DICHLOROPHENYL PIPERAZINE

D. M. Suyoga Vardhan[1], H. K. Kumara[1], M. B. Sridhara[2] and D. Channe Gowda[1]*

[1]Department of Studies in Chemistry, University of Mysore, Manasagangotri, Mysore – 570 006, Karnataka, India

[2]Department of Chemistry, Ranichannamma University, Vidya Sangama, Belagavi-591156, Karnataka, India.

Article Received on
20 June 2016,

Revised on 11 July 2016,
Accepted on 02 Aug. 2016

DOI: 10.20959/wjpr20169-6873

***Corresponding Author**
Prof. Dr. D. Channe Gowda
Department of Studies in Chemistry, University of Mysore, Manasagangotri, Mysore – 570 006, Karnataka, India

ABSTRACT

The present research work involves the synthesis and antioxidant studies of urea and thiourea derivatives of glycine and proline conjugated 2,3-dichlorophenyl piperazine. The structures were confirmed by physical and spectroscopical measurements and the molecules were subjected to *in vitro* antioxidant studies by three different methods *viz.*, DPPH, DMPD and ABTS radical scavenging methods. Interestingly compounds **1g, 1h, 2g** and **2h** bearing OCH_3 particularly at *para* position found to be most active. On the basis of results obtained a few interesting structure activity relationships were ascertained.

KEYWORDS: Amino acids, Piperazine, Conjugation, Urea/thiourea, Antioxidant.

INTRODUCTION

The human body has a complex system of natural enzymatic and non-enzymatic antioxidant defenses which counteract the harmful effects of free radicals and other oxidants. Free radicals are responsible for causing a large number of diseases including cancer,[1] cardiovascular disease,[2] neural disorders,[3] Alzheimer's disease,[4] mild cognitive impairment,[5] Parkinson's disease,[6] alcohol induced liver disease,[7] ulcerative colitis,[8] aging,[9] and atherosclerosis.[10] Protection against free radicals can be enhanced by ample

intake of dietary antioxidants. Substantial evidence indicates that foods containing antioxidants and possibly in particular the antioxidant nutrients may be of major importance in disease prevention. There is, however, a growing consensus among scientists that a combination of antioxidants, rather than single entities, may be more effective over the long term. Antioxidants may be of great benefit in improving the quality of life by preventing or postponing the onset of degenerative diseases. In addition, they have a potential for substantial savings in the cost of health care delivery.

In the past few decades, the synthesis of new heterocyclic compounds has been a subject of great interest due to their wide applicability. Heterocyclic compounds are widely occurring in nature and are significantly essential to life. Among a large variety of heterocyclic nuclei is piperazine which is interesting because its derivatives show various pharmacological and biological activities. A number of substituted piperazines exhibit significant pharmacological action such as antidiabetic,[11] antimicrobial,[12] acetyl cholinesterase inhibitors,[13] antimalarial,[14] dopamine transporter,[15] D_2/D_4 antagonist,[16] MC_4 receptor,[17] and HIV-proteinase inhibitor.[18] Indeed, amino acids and their metabolic and physiological ramifications are among the most investigated topics in biomedical science. The synthesis of compounds containing amino acids has attracted the attention of chemists due to their interesting biological activities with low toxicity and ample bioavailability.[19]

Urea and thiourea are important functional groups in numerous natural products and drug intermediates, and are used as neutral receptor for various anions (anion complexation),[20] and building blocks for various heterocycles. Urea and thiourea derivatives possess many promising biological activities, such as herbicidal,[21] antimicrobial,[22] antioxidant,[23] antiviral,[24] anti-HIV[25] and antitumor activity,[26] while urea derivatives exhibit anti-inflammatory[27], antimalarial[28] and antidiabetic activities.[29] These compounds could also be used for the detoxification of super antigens from body fluids and for the treatment of hemoglobinopathies in the cases of sickle cell anemia and Beta (β) thalassemia,[30] and thiourea derivatives were reported to be non-nucleoside inhibitors (NNIs) of the reverse transcriptase (RT) enzyme of the human immunodeficiency virus (HIV).[31] Thiocarlide is a pharmacologically important thiourea drug used as a therapeutic agent in the treatment of tuberculosis.[32] Thiourea inhibitors of plant viruses have also given rise to widespread interest in both the biological and chemical sectors.[33]

Hence with this background and previous investigations from our group,[34-37] the present work was undertaken with a view to evaluate the antioxidant efficacy of the title compounds. Synthesis of these compounds was carried out as reported in literature.[38]

EXPERIMENTAL

MATERIALS AND METHODS

Amino acids used were of *L*-configuration unless otherwise mentioned. TFA was purchased from Advanced Chem. Tech. (Louisville, KY, USA). NMM, phenyl isocyanates/isothiocyanates, DPPH, ABTS and DMPD were purchased from Sigma Chemical Co. (St. Louis, MO). Melting points were determined on a Superfit melting point apparatus (India) and are uncorrected. TLC was performed on pre-coated silica gel plates (Kieselgel 60 F254, E. Merck, Germany) with the solvent system comprising chloroform/methanol/acetic acid in the ratio 98:2:3 (Rfa) and 95:5:3 (Rfb) and the compounds on TLC were detected by iodine vapors. Solvents used were of reagent grade. IR spectra of the compounds were recorded on Jasco Spectrometer (USA). ^{1}H NMR spectra were obtained on VARIAN 400 MHz instrument (USA) using DMSO-d6 and the chemical shifts are reported as parts per million (d ppm) using TMS as an internal standard. Mass spectra were obtained on Bruker (model HP-1100) (USA) electrospray mass spectrometer. Elemental analysis was performed by using VARIO EL III Elementar (Germany). All other chemicals used were of analytical grade.

Chemistry

General procedure for the conjugation of Gly/Pro to 1-(2,3-dichlorophenyl)piperazine[38]

1-(2,3-Dichlorophenyl)piperazine.HCl was synthesized as previously reported.[39] To Boc-Gly/Pro-OH (0.002 moles) dissolved in acetonitrile (10 mL/g of compound) and cooled to 0 °C was added NMM (0.21 mL, 0.002 moles). To this EDCI (0.383 g, 0.002 moles) dissolved in acetonitrile (4 mL) was added and stirred while maintaining the temperature at 0 °C. After stirring the reaction mixture for 10 min at this temperature, HOBt (0.306 g, 0.002 moles) in DMF (3 mL) was added slowly. The reaction mixture was stirred for an additional 10 min and a pre-cooled solution of 2,3-dichlorophenyl piperazine.HCl (0.536 g, 0.002 moles) and NMM (0.21 mL, 0.002 moles) in DMF (5 mL) was added slowly. After 20 min the pH of the solution was adjusted to 8 by addition of NMM and the reaction mixture was stirred over night at room temperature. Acetonitrile was removed under reduced pressure and the residual

DMF was poured into about 100 mL ice-cold 90% saturated $KHCO_3$ solution and stirred for 30 min. The precipitate was extracted into chloroform and washed sequentially with 5% $NaHCO_3$ solution (3 × 20 mL), water (3 × 20 mL), 0.1N cold HCl (3 × 20 mL) followed by brine solution. The organic layer was dried over anhydrous Na_2SO_4, the solvent was removed under reduced pressure, triturated with ether and dried.

General procedure for the synthesis of ureido and thioureido derivatives (1a-1h/2a-2h)

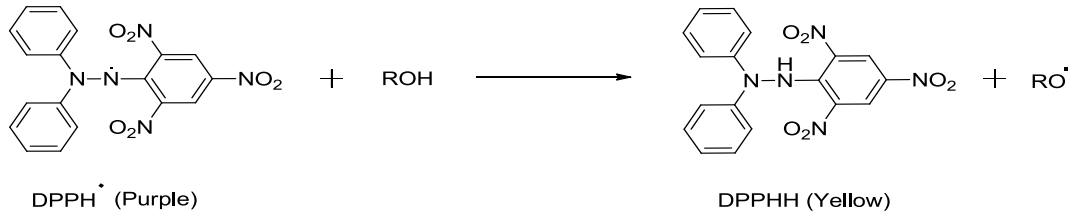
Boc-Xaa-PZN (0.150 g) [where Xaa = Gly or Pro] was stirred with 1.5 mL of TFA for 45 min at room temperature. After completion of the reaction (monitored by TLC), TFA was removed under vacuum, triturated with dry ether, filtered, washed with ether and dried to obtain TFA.H-Xaa-PZN.

Further, TFA.H-Xaa-PZN (0.001 moles) was dissolved in DMF (10 mL/g of compound), cooled to 0 °C and NMM (0.10 mL, 0.001 moles) was added. To this solution respective substituted phenyl isocyanates and isothiocyanates (0.0012 moles) was added dropwise while maintaining the temperature at 0 °C. The reaction mixture was stirred for 8 h slowly warming to room temperature. DMF was evaporated under high vacuum and the residue was poured into about 50 mL ice-cold 90% saturated $KHCO_3$ solution and stirred for 30 min. The precipitate was extracted into chloroform and washed sequentially with 5% $NaHCO_3$ solution (2 × 10 mL), water (2 × 10 mL), 0.1N citric acid (2 × 10 mL) followed by brine solution. The organic layer was dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure, triturated with hexane and dried under vacuum. The analytic and spectroscopic data of all the synthesized compounds are reported.[37]

PHARMACOLOGY

DPPH (1,1-diphenyl-2-picryl-hydrazyl) assay: The radical scavenging activities of DPPH free radicals by synthesized compounds were determined according to the reported method.[40] Briefly, 50 µL of test compounds was mixed at different concentrations (25, 50, 100, 200 and 300 µM/mL) with 1 mL of 0.1 mM DPPH in methanol solution and 450 µL of 50 mM Tris HCl buffer (pH 7.4). Methanol (50 µL) only was used as the experimental control. After 30 min of incubation at room temperature, the reduction in the number of DPPH free radicals was measured by reading the absorbance at 517 nm. BHT (Butylatedhydroxyltoluene) was used as control similar to test concentrations. Percent inhibition was calculated from the following equation:

$$\% \text{ Inhibition} = \left[\frac{\text{Absorbance of control - Absorbance of test sample}}{\text{Absorbance of control}} \right] \times 100$$

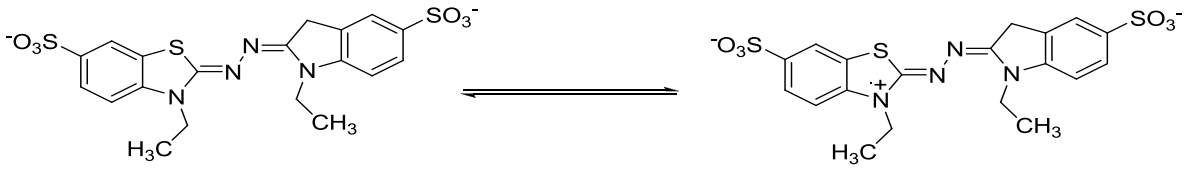


Proposed reaction mechanism of DPPH with antioxidants.

ABTS (2,2-azinobis-(3-ethylbenzothiazoline-6-sufonic acid) assay: The ability of the test sample to scavenge $ABTS^{\cdot+}$ radical cation was determined according to the literature method[41] with slight modifications. The $ABTS^{\cdot+}$ radical cation was pregenerated by mixing 7 mM $ABTS^{\cdot+}$ stock solution with 2.45 mM potassium persulfate (final concentration) and incubating for 12–16 hrs in the dark at room temperature until the reaction was complete and the absorbance was stable. The absorbance of the $ABTS^{\cdot+}$ solution was equilibrated to 0.70 (± 0.02) by diluting with distilled water at room temperature, then 2 mL was mixed with different concentration of the test sample (25, 50, 100, 200, and 300µM/mL) and the absorbance was measured at 734 nm after 6 min. The scavenging capability of $ABTS^{\cdot+}$ radical was calculated using the following equation

$ABTS^{\cdot+}$ scavenging effect (%) = $[(A_c - A_s) / A_c] \times 100$

Where, A_c is the initial concentration of the $ABTS^{\cdot+}$ and A_s is the absorbance of the remaining concentration of $ABTS^{\cdot+}$ in the presence of compounds.



ABTS **$ABTS^{\cdot+}$**

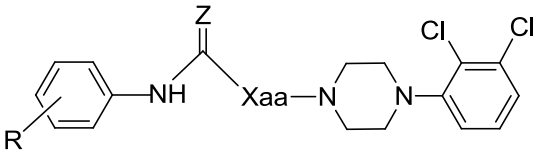
DMPD (*N, N*-dimethyl-*p*-phenylenediamine) assay: The DMPD radical scavenging ability of synthesized compounds was determined by the Fogliano *et al.*, method[42] with slight modifications by Gulcin.[42] This assay is based on the capacity of the extract to inhibit $DMPD^{\cdot+}$ cation radical formation. Briefly, 105 mg of DMPD was dissolved in 5 mL of

distilled water. Then, 1 mL of this solution was added to 100 mL of 0.1 M acetate buffer (pH 5.3). $DMPD^{\cdot+}$ was produced by adding 0.3 mL ferric chloride (0.05 M) to this solution. Different concentrations of standard antioxidants or synthesized compounds (25-300 µM/mL) were added, and the total volume was adjusted to 1 mL with distilled water. One millilitre of the $DMPD^{\cdot+}$ solution was directly added to the reaction mixture. The reaction mixtures were incubated in the dark for 15 min. The absorbance was measured at 505 nm.

$$\% \text{ Inhibition} = \left[\frac{\text{Absorbance of control - Absorbance of test sample}}{\text{Absorbance of control}}\right] \times 100$$

RESULTS AND DISCUSSION

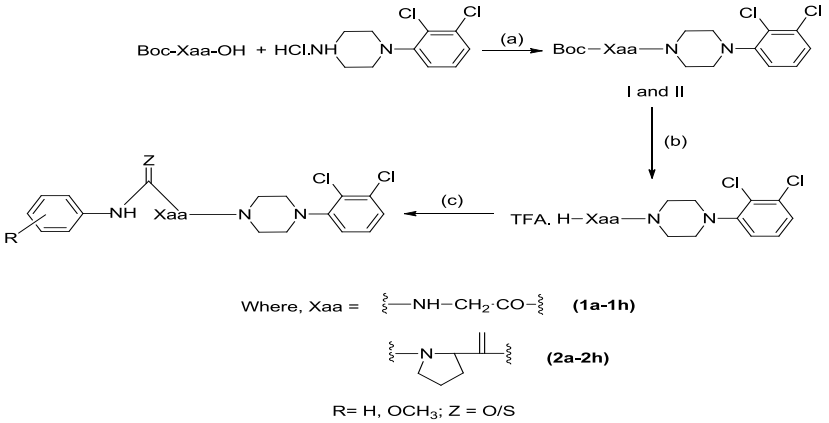
Table 1: Antioxidant activities of synthesized urea and thiourea derivatives



Where Xaa = Gly (1a-1h) and Pro (2a-2h)

Entry	Antioxidant activity IC_{50} (µg/ml)[a]				
			DPPH	ABTS	DMPD
	R	Z			
1a	H	O	>300	270 ± 3.25	285 ± 2.89
1b	H	S	230 ± 1.85	240 ± 2.98	300 ± 2.85
1c	2-OCH_3	O	68 ± 1.62	95 ± 1.50	120 ± 1.22
1d	2-OCH_3	S	43 ± 0.98	50 ± 1.11	50 ± 0.83
1e	3-OCH_3	O	125 ± 1.48	93 ± 1.65	115 ± 1.18
1f	3-OCH_3	S	82 ± 1.95	78 ± 0.93	112 ± 1.98
1g	4-OCH_3	O	42 ± 0.65	38 ± 0.32	55 ± 0.94
1h	4-OCH_3	S	34 ± 0.35	20 ± 0.11	27 ± 0.09
2a	H	O	290 ± 2.89	255 ± 2.67	300 ± 3.45
2b	H	S	265 ± 2.55	250 ± 2.50	285 ± 2.63
2c	2-OCH_3	O	80 ± 1.25	80 ± 1.71	156 ± 1.85
2d	2-OCH_3	S	45 ± 1.02	60 ± 0.96	85 ± 1.18
2e	3-OCH_3	O	95 ± 1.26	83 ± 0.65	160 ± 1.89
2f	3-OCH_3	S	65 ± 0.85	64 ± 0.52	115 ± 1.23
2g	4-OCH_3	O	40 ±0.73	37 ± 0.22	40 ± 0.89
2h	4-OCH_3	S	32 ± 0.45	25 ± 0.12	28 ± 0.64
AA			50 ± 0.20	57 ± 0.10	60 ± 0.12
GA			45 ± 0.37	68 ± 0.09	50 ± 0.09

[a]Values are mean of three determinations, the range of which are < 5% of mean in all cases



Reagents and Conditions: (a) EDCI, HOBt, NMM, 0 °C, overnight at rt, (b) TFA, 45 mins, (c) R-C_6H_4-N=C=Z, NMM

Scheme 1: Synthesis of uriedo/thiouriedo derivatives of Gly/Pro conjugated 2,3-dichlorophenyl piperazine

In vitro antioxidant activities of all the synthesized compounds were evaluated by (i) 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay which is a rapid and convenient technique for screening the antioxidant activities of the antioxidants, (ii) 2,2-azinobis-3-ethylbenzothiazoline-6-sufonic acid (ABTS) cation radical assay which is a conventional and excellent model for assessing the antioxidant activities of hydrogen donating and chain breaking antioxidants and (iii) *N*,*N*-dimethyl-*p*-phenylenediamine dihydrochloride (DMPD) cation radical assay which is similar to the DPPH radical scavenging assay. The values of IC_{50}, the effective concentration at which 50% of the radicals were scavenged, were calculated to evaluate the antioxidant activities. A lower IC_{50} value indicated greater antioxidant activity. IC_{50} values of lower than 10 mg/mL usually implied effective activities in antioxidant properties. The IC_{50} of ascorbic acid (AA) and gallic acid (GA) was also determined for comparison. The results were shown in **Table 1**.

Based on the activity profile, it was noticed that the compounds without $–OCH_3$ derivatives have shown poor antioxidant activity whereas the compounds bearing $–OCH_3$ substitutions at different positions on the phenyl ring found be very active antioxidants. This is in accordance with our earlier observation and also with literature data[43] Initially, synthesized urea and thiourea compounds with no substitution on the phenyl ring showed weak potency whereas derivatives **1d, 1g, 1h, 2d, 2g** and **2h** showed excellent potency toward radical scavenging activities with IC_{50} values 43, 42, 34, 45, 40 and 32 µg/ml, respectively in DPPH assay much better than the standard AA (IC_{50} = 50 µg/ml) and GA (IC_{50} = 45 µg/ml). In $ABTS^+$ radical

scavenging assay, the compounds **1d, 1g, 1h, 2d, 2g and 2h** showed potent antioxidant activity with IC_{50} values 50, 38, 20, 60, 37 and 25 µg/ml respectively which is much better than commercial standards AA (IC_{50} = 57 µg/ml) and GA (IC_{50} = 25 µg/ml). The compounds **1d, 1h, 2g and 2h** also exhibited excellent antioxidant activity with IC_{50} values 50, 27, 40 and 28 µg/ml, respectively which is better than the standards AA (IC_{50} = 60 µg/ml) and GA (IC_{50} = 50 µg/ml) in DMPD assay. In all the three assays, it can be noticed that compounds with ortho and para methoxy substituent showed impressive antioxidant activities with IC_{50} values much lower than the standards. On the basis of above observation, compounds having $–OCH_3$ group in the phenyl ring were found to be the most potent antioxidants. The compounds without substituent (**1a, 1b, 2a and 2b**) showed low antioxidants activity. Considering the activity profile of all the three types of antioxidant activities it was noticed that dominant radical scavenging activity was observed for thiourea compounds which bears methoxy substituents on the *para* position of the phenyl ring.

CONCLUSION

In conclusion, we have described the synthesis and structure-antioxidant activity relationships of urea and thiourea analogues with electron donating methoxy group at different positions on phenyl ring derived from Gly/Pro conjugated 2,3-dichlorophenyl piperazine in reasonably good yields. All the synthesized analogues were evaluated for their in vitro antioxidant activity. Among the analogues compounds **1h** and **2h** exhibited 2 fold antioxidant activities in comparison with standard drug. This study extends the knowledge of different electron donating substituent at the phenyl ring which might be of interest for the identification of novel class of antioxidants.

ACKNOWLEDGEMENT

The authors gratefully acknowledge University Grants Commission (UGC), New Delhi for awarding UGC - Post Doctoral Fellowship (PDFSS), BSR faculty fellowship and DST Inspire fellowship.

ABBREVIATIONS

Boc: *t*-Butoxycarbonyl; EDCI: 1-(3-Dimethylaminopropyl)-3-ethyl-carbodiimide.HCl; HOBt: 1-Hydroxybenzotriazole; NMM: *N*-Methyl morpholine; PBS: Phosphate buffer saline; PZN: 1-(2,3-Dichlorophenyl)piperazine; TFA: Trifluoroacetic acid; DPPH: 2,2-diphenyl-1-picrylhydrazyl; DMPD: (N,N-dimethyl-p-Phenylenediamine); ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)

REFERENCES

1. Kinnula VL, Crapo JD. Superoxide dismutases in malignant cells and human tumors. Free Radic. Biol. Med, 2004; 36: 718-44.
2. Singh U, Jialal I. Oxidative stress and atherosclerosis. Pathophysiology, 2006; 13: 129-42.
3. Sas K, Robotka H, Toldi J, Vecsei L. Mitochondria, metabolic disturbances, oxidative stress and the kynurenine system, with focus on neurodegenerative disorders. J. Neurol. Sci., 2007; 257: 221-39.
4. Smith M.A, Rottkamp C.A, Nunomura A, Raina A.K, Perry G, Oxidative stress in Alzheimer's disease. Biochim. Biophys. Acta, 2000; 1502 (1): 139-44.
5. Guidi I, Galimberti D, Lonati S, Novembrino C, Bamonti F, Tiriticco M, Fenoglio C, Venturelli E, Baron P, Bresolin N, Oxidative imbalance in patients with mild cognitive impairment and Alzheimer's disease. Neurobiol. Aging, 2006; 27: 262-9.
6. Bolton JL, Trush MA, Penning TM, Dryhurst G, Monks TJ. Role of quinones in toxicology. Chem. Res. Toxicol., 2000; 13: 135-60.
7. Arteel GE. Oxidants and antioxidants in alcohol-induced liver disease. Gastroenterol, 2003; 12: 778-90.
8. Ramakrishna BS, Varghese R, Jayakumar S, Mathan M, Balasubramanian KA. Circulating antioxidants in ulcerative colitis and their relationship to disease severity and activity. J. Gastroenterol. Hepatol. 1997; 12: 490-4.
9. Hyun DH, Hernandez JO, Mattson MP, de Cabo R. The plasma membrane redox system in aging. Aging Res. Rev. 2006; 5: 209.
10. Upston JM, Kritharides L, Stocker R. The plasma membrane redox system in aging. Prog. Lipid Res. 2003; 42: 405-22.
11. Frederic R, Bihan GL, Xuan W, Aazdine L, Estera T, Georges D. Design and synthesis of imidazoline derivatives active on glucose homeostasis in a rat model of type II diabetes. 1. Synthesis and biological activities of N-benzyl-N'-(arylalkyl)-2-(4',5'-dihydro-1'H-imidazol-2'-yl)piperazines. J. Med. Chem. 1997; 40: 3793-803.
12. Chaudhary P, Kumar R, Verma AK, Singh. D. Synthesis and antimicrobial activity of N-alkyl and N-aryl piperazine derivatives, Bioorg. Med. Chem. 2006; 14: 1819-26.
13. Hachiro S, Hiroo O, Yasuo A, Youichi I, Yoshiharu Y. Research and Development of Donepezil Hydrochloride, a New Type of Acetylcholinesterase Inhibitor. Japanese J. Pharmacol. 2002; 89: 7-20.

14. Rebecca DP, Patricia. 1,4-Bis(3-Aminopropyl)Piperazine Libraries: From the Discovery of Classical Chloroquine-Like Antimalarials to the Identification of New Targets. Com. Chem. High. T. Scr. 2005; 8: 39-48.
15. Makoto K, Tomoko M, Koji Y, Masaki M, Nobuo K, Nobuyuki K. Novel diphenylalkyl piperazine derivatives with high affinities for the dopamine transpoter. Bioorg. Med. Chem. 2003; 11: 3953-963.
16. He Zhao, Xiaoshu He, Andrew T, Diane H, Andrzej K, Robbin B. Indoline and piperazine containing derivatives as a novel class of mixed D2/D4 receptor antagonists. Part 2: Asymmetric synthesis and biological evaluation. Bioorg. Med. Chem. Lett. 2002; 12: 3111-3115.
17. Brian D, Jessica P, Teresa P, Lee C, Brian M, Robin S. Aryl piperazine melanocortin MC4 receptor agonists, Bioorg. Med. Chem. Lett. 2003; 13: 3793-796.
18. Rossen K, Steven AW, Sager J, Reamer RA, Askin D, Volante RP. Asymmetric hydrogenation of tetrahydropyrazines: Synthesis of (S)-piperazine-2-tert-butylcarboxamide, an intermediate in the preparation of the HIV protease inhibitor. Tetrahedron Lett. 1995; 36: 6419-422.
19. Lee LF, Schleppnik FM, Schneider RW, Campbell DH. Synthesis and 13C NMR of (trifluoromethyl)hydroxypyrazoles. J. Heterocycl. Chem. 1990; 27: 243-45.
20. Tobe Y, Sasaki SI, Mizuno M, Hirose K, Neamura K. Novel Self-Assembly of m-Xylylene Type Dithioureas by Head-to-Tail Hydrogen Bonding. J. Org Chem. 1998; 63: 7481.
21. Yonova PA, Stoilkova GM. Synthesis and biological activity of urea and thiourea derivatives from 2-aminoheterocyclic compounds. J. Plant Growth Regul. 2004; 23: 280.
22. Abdel-Rahman HM, Morsy MA. Novel benzothiazolyl urea and thiourea derivatives with potential cytotoxic and antimicrobial activities. J. Enzyme Inhib. Med. Chem. 2007; 22: 57-64.
23. Adeoye O, Ayandele AA, Odunola OA. J. Agric. Biol. Sci. 2007; 2: 4.
24. Bloom JD, Dushin RG, Curran KJ, Donahue F, Norton EB, Terefenko E, Jonas TR, Ross AA, Feld B, Lang SA, Di-Grandi M. Thiourea inhibitors of herpes viruses. Part 2: N-Benzyl-N'-arylthiourea inhibitors of CMV. J. Bioorg. Med. Chem. 2004; 14: 3401-6.
25. Venkatachalam TK, Sudbeck EA, Mao C, Uckun FM. Anti-HIV activity of aromatic and heterocyclic thiazolyl thiourea compounds. Bioorg. Med. Chem. Lett. 2001; 11: 523-8.

26. Shusheng Z, Tianrong Z, Kun C, Youfeng X, Bo Y. Simple and efficient synthesis of novel glycosyl thiourea derivatives as potential antitumor agents. Eur. J. Med. Chem. 2008; 43: 2778-83.
27. Dominguez JN, Leon C, Rodrigues J, Gamboa de Dominguez N. Gut J, Rosenthal PJ. Synthesis and evaluation of new antimalarial phenylurenyl chalcone derivatives. J. Med. Chem. 2005; 48: 3654-8.
28. Audia JE, Evrard DA, Murdoch GR, Droste JJ, Nissen JS, Schenck KW, Fludzinski P, Lucaites VL, Nelson DL, Cohen ML. Potent, selective tetrahydro-beta-carboline antagonists of the serotonin 2B (5HT2B) contractile receptor in the rat stomach fundus. J. Med. Chem. 1996; 39: 2773-80.
29. Pluempe H, Pulls W. Chemical Abstracts, 1971; 74: 1251154.
30. Longo LD. Physiologic assessment of fetal compromise: biomarkers of toxic exposure. Environ. Health Perspect. 1987; 74: 93-101.
31. Liav A, Angala SK, Brennan PJ, Jackson M. N-D-aldopentofuranosyl-N'-[p-(isoamyloxy)phenyl]-thiourea derivatives: potential anti-TB therapeutic agents. Bioorg Med Chem Lett. 2008; 8: 2649-51.
32. Zou XJ, Jin GY, Yang Z. Synthesis of the thiosemicarbazides of 1-Aryl-1,4-dihydro-3-Carbox-6-methyl-4-pyridazinone and their antiviral activity against TMV. Chem. J. Chin. Univ. 2002; 23: 403-406.
33. Nakano H, Haroda H, Funaoka T, Akashi K. Chemical Abstracts, 1973; 78: 43086.
34. Rakesh KP, Manukumar HM, Channe Gowda D, Schiff's bases of quinazolinone derivatives: Synthesis and SAR studies of a novel series of potential anti-inflammatory and antioxidants, Bioorg. Med. Chem. Lett. 2015; 25: 1072–1077.
35. Suresha, GP, Suhas R, Wethroe K, Gowda DC. Urea/thiourea derivatives of quinazolinone-lysine conjugates: Synthesis and structure activity relationships of a new series of antimicrobials. Eur. J. Med. Chem, 2011; 46: 2530-2540.
36. Shantharam CS, Suyoga Vardhan DM, Suhas R, Sridhara MB, Channe Gowda D. Inhibition of protein glycation by urea and thiourea derivatives of glycine/proline conjugated benzisoxazole analogue – Synthesis and structure–activity studies. Eur. J. Med. Chem, 2013; 60: 325-332.
37. Suhas R, Chandrashekar S, Gowda DC. Synthesis of uriedo and thiouriedo derivatives of peptides conjugated heterocycles – A new class of promising antimicrobials. Eur. J. Med. Chem., 2012; 48: 179-191.

38. Suyoga Vardhan DM, Shantharam CS, Suhas R, Sridhra MB, Channe Gowda D. Protein and Peptde Lett. Synthesis and SAR studies of urea and thiourea derivatives of Gly/Pro conjugated to piperazine analogue as potential AGE inhibitors. 2013; 20: 888-897.
39. Oshiro Y, Tokushima S, Sato and Itana. US Patent, 1991; 5, 006, 528.
40. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958; 181: 1199-200.
41. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free. Radical. Bio. Med. 1999; 26: 1231-7.
42. Fogliano V, Verde V, Randazzo G, Ritieni A. Method for measuring antioxidant activity and its application to monitoring the antioxidant capacity of wines. J. Agric. Food Chem. 1999; 47: 1040-40.
43. Shih MH, Ke FY. Bioorg. Med. Chem. Syntheses and evaluation of antioxidant activity of sydnonyl substituted thiazolidinone and thiazoline derivatives. 2004; 12: 4633-43.