Canadian Chemical Transactions Year 2015 | Volume 3 | Issue 2 | Page 199-206

Research Article

DOI:10.13179/canchemtrans.2015.03.02.0194

Investigation of Antioxidant Properties of Phthalimide Derivatives

Chimatahalli S. Karthik[1], Lingappa Mallesha[2], Puttaswamappa Mallu[1]*

[1]*Department of Chemistry, Sri Jayachamarajendra College of Engineering, Mysore 570 006, India.*
[2]*PG Department of Chemistry, JSS College of Arts, Commerce and Science, Ooty Road, Mysore 570 025, India.*

[*]*Corresponding Author: Email: drmallu66@gmail.com*

Received: *April 1, 2015* ***Revised:*** *May 6, 2015* ***Accepted:*** *May 10, 2015* ***Published:*** *May 12, 2015*

Abstract: The present work was conducted to study the antioxidant property of phthalimide derivatives **3(a-g)** and were synthesized by the reaction of isoindoline-1,3-dione (**1**) with various amines **2(a-g)**. Newly synthesized compounds were characterized by UV-visible, FT-IR, mass and ^{1}H NMR spectral studies. The free radical scavenging properties of synthesized compounds were evaluated employing different methodologies, including the bleaching of a stable free radical (2,2-diphenyl-1-picrylhydrazyl, DPPH) and the metal chelating assay (Ferrous ion chelating assay). Compounds **3b**, **3d** and **3e** exhibited good antioxidant activity when compared with other compounds in the series.

Keywords: Amine, DPPH, Ferrous ion chelating assay, Phthalimide.

1. INTRODUCTION

Antioxidant is a chemical that suppress the activity of free radical. Free radicals are formed naturally in the body and some environmental toxins may contain free radicals or stimulate the body's cells to produce free radicals. Free radicals are hazardous to the body and damage all major components of cells, including DNA, proteins and cell membranes, may play a role in the development of cancer and other health conditions [1]. They affect living cells and mediate the pathogenesis of many chronic diseases, such as Parkinson's and Alzheimer's diseases, atherosclerosis, arthritis and stroke acting by various mechanisms. A present trend in the field of anti-oxidant development focuses on multipotent antioxidant agents that can prevent biological substrates from radical induced oxidative damage [2]. Antioxidants deactivate and scavenge free radicals and inhibit the effect of oxidants by donating hydrogen atom or chelating metals. Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are used as additives in foods to prevent oxidation of lipids. Besides, BHA and BHT are restricted by legislative rules because of doubts over their toxic and carcinogenic effects [3]. Therefore, there is a growing request and interest for safer antioxidants in food and pharmaceutical applications[4].

The structural diversity and biological importance of nitrogen containing heterocycles have made them attractive targets for synthesis over many years. Among heterocyclic scaffolds, phthalimides are of

particular biological interest and have been reported as herbicides, insecticides [5] and antiinflammatory agents [6]. Phthalimide is a very important subunit for organic synthetic chemists for preparing diverse biologically active molecules. Phthalimide are an important class of drugs exhibiting anxiolytic [7], antimicrobial [8], antibacterial [9], antituberculosis [10], anticancer [11], hypolipidemic [12], analgesic [13], antiproliferative [14], acetylcholinesterase inhibitors [15] and inhibitor of human neuronal nitric oxide synthase [16]. As a result, we have synthesized and characterized by spectral methods [17]. Here, we focused on the effect of these Heck compounds on the antioxidant activity. Using different chemical reaction-based assays, new synthesized phthalimide molecules have been screened for scavenging ability against the free radical 2,2-diphenyl-1-picryl-hydrazyl (DPPH•) and chelating activity on ferrous ions. The results were compared with the synthetic antioxidants Ascorbic acid (Vit-C) and EDTA. The antioxidant activity of new phthalimides and interestingly, some of them have shown significant antioxidant activity.

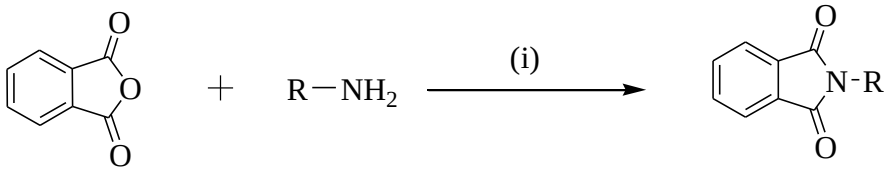
2. EXPERIMENTAL

2.1. Chemistry

All solvents and reagents were purchased from Sigma Aldrich Chemicals Pvt Ltd. Melting range was determined by Veego Melting Point VMP III apparatus. The UV-Visible spectrum was recorded on UV-1800 SHIMADZU UV spectrometer with quartz cell of 1.0 cm path length. The FT-IR spectra was recorded using KBr discs on FT-IR Jasco 4100 infrared spectrophotometer and were quoted in cm^{-1}.^{1}H NMR spectra was recorded on Bruker DMX 300, 400MHz spectrometer using DMSO-d_6 as solvent and TMS as an internal standard. Mass spectral data were obtained by LC-MSD Trap XCT. Silica gel column chromatography was performed using Merck 7734 silica gel (60–120 mesh) and Merck-made TLC plates.

2.2. General procedure for the synthesis of isoindoline-1,3-dione derivatives (3a-g)

Equimolar concentrations of isoindoline-1,3-dione (3.28 mmol, **1**) and different amines (3.28 mmol, **2a-g**) were refluxed at 70-80 ^{0}C for 7-8 hr using methanol (25 ml) and 2-3 drops of conc. sulfuric acid was added to the mixture. The progress of the reaction was followed by TLC until the reaction was complete. Upon completion, the solvent was removed under reduced pressure and residue was taken in water and extracted with ethyl acetate. The organic layer was washed with brine solution and finally water wash was given to organic layer and dried with anhydrous sodium sulphate. The residue was recrystallized from ethyl acetate. Compounds **3(a-g)** were prepared by the method summarized in **Scheme 1**.



(1) **2(a-g)** **3(a-g)**

Scheme 1 Reagents and conditions: (i) Methanol, 70 - 80 ^{o}C, H^+, 7-8 hr.

2.2.1. 2-(3-(4-(Pyridin-4-yl)pyrimidin-2-ylamino)-4-methylphenyl)isoindoline-1,3-dione (3a)

FT-IR (KBr, cm^{-1}): 3168 (N-H), 3077 (Ar-H), 1456 (C=C), 1150 (C-N). ^{1}H NMR (DMSO-d_6) δ ppm: 2.38 (s, 3H, CH_3), 6.58 (d, 1H, Ar-H), 6.98 (d, 1H, Ar-H), 7.11 (s, 1H, Ar-H), 7.29-7.48 (d, 2H, pyrimidine-H), 7.55 (t, 1H, pyridine-H), 7.75 (d, 2H, Ar-H), 7.88 (d, 2H, Ar-H), 8.49 (d, 1H, pyridine-H), 8.70 (d, 1H, pyridine-H), 8.77 (s, 1H, pyridine-H), 9.23 (s, 1H, N-H). MS (ESI) *m/z*: 407.37.

2.2.2. 2-(3-Fluoro-5-(trifluoromethyl)benzyl)isoindoline-1,3-dione (3b)

FT-IR (KBr, cm^{-1}): 3057 (Ar-H), 1451 (C=C), 1138 (C-N), 1109 (C-F). ^{1}H NMR (DMSO-d_6) δ ppm: 4.68 (s, 2H, CH_2), 6.71-7.12 (s, 3H, Ar-H), 7.75 (d, 2H, Ar-H), 7.88 (t, 2H, Ar-H). MS (ESI) *m/z*: 326.66.

2.2.3. 2-(4-Bromo-2-chloro-6-methylphenyl)isoindoline-1,3-dione (3c)

FT-IR (KBr, cm^{-1}): 3061 (Ar-H), 1468 (C=C), 1158 (C-N), 729 (C-Cl), 529 (C-Br). ^{1}H NMR (DMSO-d_6) δ ppm: 2.34 (s, 3H, CH_3), 7.18 (s, 1H, Ar-H), 7.59 (s, 1H, Ar-H), 7.75 (d, 2H, Ar-H), 7.88 (d, 2H, Ar-H).

2.2.4. 2-(3-Chloro-5-(trifluoromethyl)pyridin-2-yl)isoindoline-1,3-dione (3d)

FT-IR (KBr, cm^{-1}): 3083 (Ar-H), 1474 (C=C), 1151 (C-N), 1103 (C-F) 728 (C-Cl). ^{1}H NMR (DMSO-d_6) δ ppm: 7.69 (d, 2H, Ar-H), 7.85 (d, 2H, Ar-H), 8.19 (s, 1H, Py-H), 8.48 (s, 1H, Py-H). MS (ESI) *m/z*: 326.66.

2.2.5. 2-(3-Methyl-5-nitropyridin-2-yl)isoindoline-1,3-dione (3e)

FT-IR (KBr, cm^{-1}): 3051 (Ar-H), 1449 (C=C), 1148 (C-N). ^{1}H NMR (DMSO-d_6) δ ppm: 2.49 (s, 3H, CH_3), 7.60 (d, 2H, Ar-H), 7.85 (d, 2H, Ar-H), 8.31 (s, 1H, Py-H), 8.91 (s, 1H, Py-H). MS (ESI) *m/z*: 283.24.

2.2.6. 2-(3,5-Dibromopyrazin-2-yl)isoindoline-1,3-dione (3f)

FT-IR (KBr, cm^{-1}): 3040 (Ar-H), 1440 (C=C), 1141 (C-N), 514 (C-Br). ^{1}H NMR (DMSO-d_6) δ ppm: 7.79 (d, 2H, Ar-H), 7.88 (d, 2H, Ar-H), 8.81 (s, 1H, Pyrazine-H). MS (ESI) *m/z*: 383.

2.2.7. 2-(5-Mercapto-1,3,4-thiadiazol-2-yl)isoindoline-1,3-dione (3g)

FT-IR (KBr, cm^{-1}): 3064 (Ar-H), 2589 (S-H), 1453 (C=C), 1328 (C-S), 1145 (C-N). ^{1}H NMR (DMSO-d_6) δ ppm: 3.12 (s, 1H, S-H), 7.76 (d, 2H, Ar-H), 7.81 (d, 2H, Ar-H). MS (ESI) *m/z*: 263.3.

2.3. Antioxidant activity

DPPH radical scavenging assay

The free radical scavenging activity was measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay according to the method described earlier [18-19]. The stock solution was prepared by dissolving 24 mg DPPH with 100 ml methanol and stored at 20 °C until required. The working solution was obtained by diluting DPPH solution with methanol to attain an absorbance of about 0.98±0.02 at 517 nm using the spectrophotometer. A 3 ml aliquot of this solution was mixed with 100 µl of the sample at various concentrations (20 - 100 µg/ml). The reaction mixture was shaken well and incubated in the dark for 15 min at room temperature. Then the absorbance was taken at 517 nm. The control was prepared as above without any sample. Ascorbic acid (Vit-C) was used as positive control. All the experiments were done in triplicates and the values are averaged. A dose responsive curve was plotted to determine the IC_{50} values. IC_{50} is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity [20]. All

the tests were run in triplicate and averaged. Scavenging effect (%) = [(control absorbance - sample absorbance) / control absorbance] * 100

Table 1 Chemical structure of the synthesized compounds **3(a-g)**

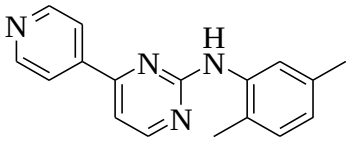
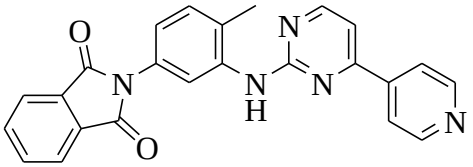
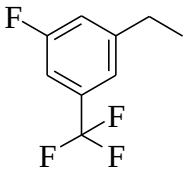
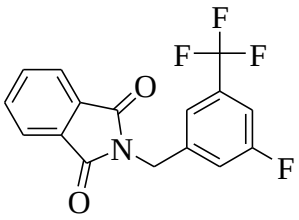
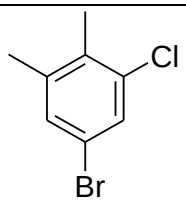
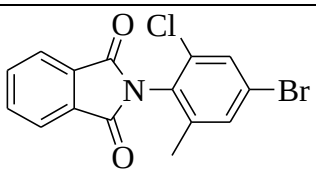
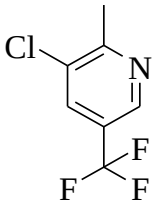
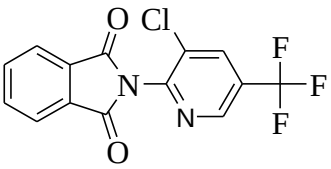
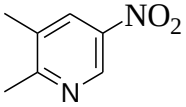
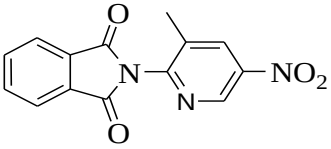
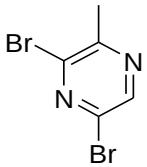
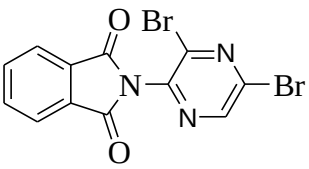
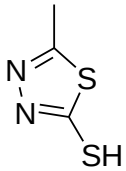
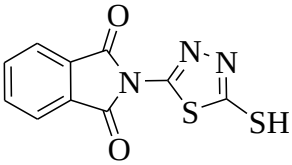
Compound	R	Structure
3a		
3b		
3c		
3d		
3e		
3f		
3g		

Table 2 Physical data of the synthesized compounds **3**(**a-g**)

Compound	UV-visible (nm)	M.R. (°C)	Solubility	Yield (%)
3a	290	180-182	Ethyl acetate	77.60
3b	235	123-125	Ethyl acetate	72.88
3c	233	150-151	Ethyl acetate	78.16
3d	241	162-163	Ethyl acetate	64.29
3e	239	168-169	Ethyl acetate	75.98
3f	274	149-150	Ethyl acetate	82.25
3g	271	170-172	Ethyl acetate	69.29

Ferrous ion chelating assay

The chelating activity of the Schiff base derivatives for ferrous ions (Fe^{2+}) was measured according to the method [21]. Briefly, 0.5 mL different concentration of synthesized compounds was added to a solution of 2 mM $FeCl_2$ (0.05 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL). The mixture was shaken vigorously and left at room temperature for 10 min. Ferrozine reacted with the divalent iron to form stable magenta complex species that were very soluble in water. Absorbance of the solution was then measured spectrophotometrically at 562 nm. EDTA was used as a positive control. All the experiments were done in triplicates and the values are averaged. A dose responsive curve was plotted to determine the IC_{50} values. IC_{50} is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity. All the tests were run in triplicate and averaged. The percentage inhibition of ferrozine-Fe^{2+} complex formation by the compounds was calculated as:

$$\text{Percentage of inhibition (\%)} = [(A_0 - A_1)/A_0] \times 100$$

Where A_0 was the absorbance of the control, and A_1 was the absorbance of the test sample.

3. RESULTS AND DISCUSSION

3.1. Chemistry

The isoindoline-1,3-dione derivatives **3**(**a-g**) were synthesized according to Scheme 1. Formation of isoindoline-1,3-dione derivatives **3**(**a-g**) was confirmed by ^{1}H NMR, LC-MS, FT-IR and UV-visible spectra. The chemical structures and physical data of all the synthesized compounds are tabulated in Table 1 and Table 2, respectively.

The Isoindoline-1,3-dione (**1**) was reacted with various amines (R-NH_2, **2a-g**) in methanol to obtain Isoindoline-1,3-dione derivatives **3**(**a-g**) in good yield (64-82 %). The UV spectra of **3**(**a-g**) were recorded using ethyl acetate solvent in the range of 200 - 800 nm. The electronic absorption spectra of compounds show new bands and appearance of wavelength absorption band in the UV region in UV-visible spectrum owing to confirms the formation of new compounds. The FT-IR spectra of **3**(**a-g**) were recorded using KBr pellets in the range of 4000 - 400 cm^{-1}. The absence of NH_2 and five member anhydride absorption bands in the IR spectra confirmed that the synthesized compounds. The absorption bands at 3040 - 3083 cm^{-1} are assigned to the aromatic C-H stretch. The absorption band at 1796 cm^{-1} is due to the five membered anhydride stretch in compound **1**. The appearance of a medium to strong absorption bands at 1138-1158 cm^{-1} due to a stretching vibration of the C-N bond formation in the new

compounds. The strong bands at 529 cm^{-1} and 514 cm^{-1} are assigned to the C-Br stretch in **3c** and **3f**, respectively. New bands appeared at 1109 cm^{-1} and 1103 cm^{-1} corresponding to C-F stretching frequency in **3b** and **3d**, respectively. The strong bands at 729 cm^{-1} and 728 cm^{-1} are assigned to the C-Cl stretch in **3c** and **3d**, respectively. The characteristic resonance peaks in ^{1}H NMR for the new compounds were reported using DMSO-d_6. The proton spectral data agree with respect to the number of protons and their chemical shifts with the proposed structures. The proton NMR spectral data of NH in **2(a-g)** show single resonances around δ 9.21 ppm. In all the synthesized compounds **3(a-g)** the above resonances disappeared thus, the products are confirmed. The mass spectra of **3a** showed molecular ion peak at m/z 407.37 which is in agreement with the molecular formula $C_{24}H_{17}N_5O_2$.

3.2. Biology

All the investigated substances were capable of chelating Fe^{2+} ions. Fe^{2+} ions initiate free radicals through the Fenton and Haber-Weiss reaction. Fenton Weiss reaction is a reaction between ferrous ion and hydrogen peroxide which produces highly reactive hydroxyl radicals implicated in many diseases [22]. The metal chelating effects of the samples were dependent on concentration and linearly increased with the sample concentration increased. The affinity of **3(a-g)** for ferrous ions was relatively low comparison to EDTA. However the activity of **3b** and **3e** were nearer to standard. Thiazole group in **3d** and thiadiazole group in **3e** are found to be similar antioxidant activity. The aromatic ring system with halogens like chlorine or fluorine in **3a**, **3c**, **3f** and **3g** were found to be less active than other compounds in the series. Percentage of ferrous ion chelating activity was depicted in Table 3.

Table 3. Ferrous Ion Chelating activity of the tested compounds

Compounds	% of Ferrous ion chelating activity					IC_{50} (µg/mL)
	Concentrations (µg)					
	20	40	60	80	100	
3a	40	46	56	62	66	60.43
3b	60	70	80	86	95	14.12
3c	45	48	50	55	68	64.97
3d	43	47	53	58	67	61.24
3e	60	70	80	86	95	14.12
3f	41	45	94	94	94	44.11
3g	45	48	50	55	68	64.97
Standard	72	79	81	83	88	13.88

The DPPH radical scavenging test is a standard and widely used assay for in vitro antioxidant capacity of compounds and it is based on their ability of scavenging of stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) [23].The results of *in vitro* antioxidant activity (IC_{50} values) of the synthesized compounds in comparison with the reference antioxidant Ascorbic acid (Vit-C) were depicted in Table 4. The compound **3b** showed higher radical inhibition activity in the aromatic ring. The nature of the functional groups is crucial for biological activity.

Table 4. DPPH radical scavenging activity of the tested compounds

Compounds	% of Scavenging activity					IC_{50} (µg/mL)
	Concentrations (µg)					
	20	40	60	80	100	
3a	45	48	50	55	58	60.00
3b	63	65	70	72	75	15.40
3c	40	44	46	48	50	100.00
3d	40	65	70	75	80	25.00
3e	41	64	72	76	84	27.00
3f	43	44	45	48	50	100.00
3g	48	59	67	74	88	28.00
Standard	59	61	64	69	74	16.94

4. CONCLUSION

In conclusion, a series of isoindoline-1,3-dione derivatives **3(a-g)** were synthesized in good yield, characterized by different spectral studies and their biological activity has been evaluated. The synthesis employs readily available starting materials and simple procedures making this method very attractive and convenient for the synthesis of various phthalimide compounds.Compounds **3b**, **3d** and **3e** exhibited a significant antioxidant activitywhen compared to other compounds in the series.

ACKNOWLEDGEMENTS

One of the authors (Karthik C. S.) is grateful to University Grants Commission (UGC), New Delhi for financial support under UGC-JRF (F.No.42-366/2013 (SR) dated 25.03.13).

REFERENCES

[1] Valko, M.; Leibfritz, D.; Moncol, J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem & Cell Bio.*, **2007**, 39(1), 44-84.

[2] Zhang, H. Y.; Yang D. P.; Tang, G. Y. Multipotent antioxidants: from screening to design. *Dru.g Disc. Today.*, **2006**, 11, 749–754

[3] Sun, L.; Zhang, J.; Lu, X. .; Zhang, L.; Zhang, Y. Evaluation to the antioxidant activity of total flavonoids extract from persimmon (Diospyros kaki L.) leaves. *Food and Chemical Toxicology.*, **2011**, 49(10), 2689-2696.

[4] Gul, M.; Kulu, I.; Peksel, A.; Ocal, N. Evaluation of the Antioxidative Properties of N-Acylamino-Substituted Tricyclic Imides. *J. Chem.*, **2013**, http://dx.doi.org/10.1155/2013/920130.

[5] Liu, M.; Wang, Y.; Wangyang, W. Z.; Liu, F.; Cui, Y. L.; Duan, Y. S.; Rui, C. H. Design, synthesis, and insecticidal activities of phthalamides containing a hydrazone substructure. *J. agricultural food chem.*, **2010**, 58, 6858-6863.

[6] Nagarapu, L., Mateti, J., Gaikwad, H. K., Bantu, R., Rani, M. S., & Subhashini, N. P. Synthesis and anti-inflammatory activity of some novel 3-phenyl-N-[3-(4-phenylpiperazin-1yl) propyl]-1H-pyrazole-5-carboxamide derivatives. *Bioorg. Med. Chem lett.*, **2011**, 21, 4138-4140.

[7] Hassazadeh, F.; Rabbani, M.; Khodarahmi, G. A.; Fasihi A.; Hakimelahi G. H.; Mohajeri, M. Synthesis of phthalimide derivatives and evaluation of their anxiolytic activity. *Res. Pharm. Sci.*, **2007**, 2, 35-41.

[8] Khalil, A. M.; Berghot, M. A.; Gouda M. A. Synthesis and study of some new 1,3-isoindoledione derivatives as potential antibacterial agents. *Eur. J. of Med. Chem.*, **2010**, 45(4), 1552-1559,

[9] Lima, M.; Lídia.; Paulo, C.; Machado, A. L.; M. Fraga, C. A.; Lugnier, C.; Moraes, V. L. G.; Barreiro, E. J. Synthesis and anti-inflammatory activity of phthalimide derivatives, designed as new thalidomide analogues. *Bioorg. Med. Chem*., **2002**, 10(9), 3067-3073,.

[10] Kok.; Lung, S. H.; Gambari, R.; Chui, C. H.; Yuen, M. C. W.; Lin, E.; Wong, R. S. M.; Lau, F. Y. Synthesis and anti-cancer activity of benzothiazole containing phthalimide on human carcinoma cell lines. *Bioorg. Med. Chem*., **2008**, 16, 3626-3631.

[11] Santos, J. L.; Yamasaki, P. R.; Chin, C. M.; Takashi, C. H.; Pavan, F. R.; Leite, C. Q. (). Synthesis and in vitro antiMycobacterium tuberculosis activity of a series of phthalimide derivatives. *Bioorg. Med. Chem*., **2009**, 17(11), 3795-3799.

[12] Sena, V. L. M.; Srivastava, R. M.; Silva, R. O.; Lima, V. L M. Synthesis and hypolipidemic activity of N-substituted phthalimides. *Part V, Farmaco*., **2003**, 58, 1283.

[13] Jagadish, S.; Tanushree, S.; Naskar, A.; Kundu, M.; Ranjit, K. Synthesis and anti-proliferative activity of some isoindoline-1, 3-dione derivatives against ehrlich's ascites carcinoma bearing mice model. *Pharmacologyonline*, **2011**, 2, 976-987.

[14] Alireza, A.; Foroumadi, A.; Mohammadi-Farani A.; Mahvar M G. Synthesis and Evaluation of Anti-acetylcholinesterase Activity of 2-(2-(4-(2-Oxo-2-phenylethyl) piperazin-1-yl) ethyl) Isoindoline-1, 3-dione Derivatives with Potential Anti-Alzheimer Effects. *Iran. J. Basic. Med. Sci*., **2013**, 16, 1049-54.

[15] Annedi, C.; Subhash.; Ramnauth, J.; Maddaford, S. P.; Renton, P.; Rakhit, S.; Mladenova, Peter Dove G. Discovery of cis-N-(1-(4-(methylamino) cyclohexyl) indolin-6-yl) thiophene-2-carboximidamide: a 1, 6-disubstituted indoline derivative as a highly selective inhibitor of human neuronal nitric oxide synthase (nNOS) without any cardiovascular liabilities. *J. Med Chem*., **2012**, 55, 943-955.

[16] Appelbaum, P. C.; Hunter P. A. The fluoroquinolone antibacterials: past, present and future perspectives." International Journal of Antimicrobial Agents, **2000**, 16, 5-15.

[17] Mallesha, L.; Karthik, C. S.; Mallu, P.; Patil, V. Synthesis, characterization and antibacterial activity of isoindoline-1,3-dione derivatives. *SOP Trans. Org. Chem*., **2014**, 1, 21-28.

[18] Bursal, E.; Gulcin, I. Polyphenol contents and *in vitro* antioxidant activities of lyophilized aqueous extract of kiwifruit (*Actinidia deliciosa*). *Food Res Int*., **2011,** 44, 1482–1489.

[19] Saeed, N.; Khan M. R.; Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts torilis leptophylla L. *BMC Compl. Alternat. Med.*, **2012,** 12(1), 221-225.

[20] Kumaraswamy, M. V.; Satish, S. Antioxidant and anti-lipoxygenase activity of thespesia lampas dalz and gibs. *Adv. Bio. Res*., **2008,** 2(3), 56-59.

[21] Molnar, M.; Sarkanj, B.; Cacic, M.; Gille, L; Strelec, I. Antioxidant properties and growth-inhibitory activity of coumarin Schiff bases against common foodborne fungi. *Der Pharma Chem.*, **2014**, 6, 313-320.

[22] Kehrer, J. P. The Haber–Weiss reaction and mechanisms of toxicity. *Toxicol.*, **2000**, 149, 43–50.

[23] Lai, L. S.; Chou, S. T.; Chao, W. W. Studies on the antioxidant activities of Hsian-tsao **(**Mesona procumbens Hemsl**)** leaf gum. *J. Agric. Food. Chem*., **2001**, 49, 963–968

The authors declare no conflict of interest

© 2015 By the Authors; Licensee Borderless Science Publishing, Canada. This is an open access article distributed under the terms and conditions of the Creative Commons Attribution license http://creativecommons.org/licenses/by/3.0