Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2015, 7 (6):31-36
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Synthesis, characterization and biological evaluation of indole-2-carboxamides fused with imidazolinone moiety

Gollapalli Naga Raju[1]*, Rohini Tejaswi Myneni[1], Medarametla Poornima[2], Sk. Sahera Raksha[2], Sk. Suhana[2] and Rama Rao Nadendla[1]

[1]Department of Pharmaceutical Analysis, Chalapathi Institute of Pharmaceutical Sciences, Guntur, India
[2]Department of Pharmacy Practice, Chalapathi Institute of Pharmaceutical Sciences, Guntur, India

---

ABSTRACT

A novel series of indole-2-carboxamides fused with imidazolinone derivatives were synthesized from 1H-indole-2-carbohydrazide and different 4-arylidene-2-phenyl-5-oxazolinones in pyridine. All the synthesized compounds have been characterized by using elemental analysis, FT-IR, ^{1}H NMR, ^{13}C NMR spectroscopy and further supported by mass spectroscopy. Purity of all the compounds has been checked on thin layer chromatographic plate and HPLC technique. All the synthesized compounds were tested for their antibacterial and antifungal activity (MIC) in vitro by broth dilution method with two Gram-positive bacteria, two Gram-negative bacteria and two fungal strains. The biological activities of the synthesized compounds have been compared with standard drugs Ampicillin and Greseofulvin. The compounds exhibited significant antibacterial and moderate antifungal activities. These compounds can be further exploited to get the potent lead compounds. The detailed synthesis and the antimicrobial screening of the new compounds are reported.

Keywords: Indole, Imidazolinone, Antibacterial activity, Antifungal activity.

INTRODUCTION

The synthesis of heterocyclic compounds has always drawn the attention of chemists over the years mainly because of their important biological properties. The indole skeleton is one of the most attractive frameworks with a wide range of biological and pharmacological activities. This physiologically important nucleus is abundantly found in therapeutic agents as well as in natural products. Many researchers have described synthesis of indole and its derivatives along with its applications in literature.

A large number of heterocyclic compounds containing the indole ring are associated with diverse pharmacological properties such as Analgesic [1], Antiallergic [2], Antibacterial [3], Anticonvulsant [4,5], Antifungal [6], Antihistaminic [7], Anti-inflammatory [8-10], Anticancer [11, 12], Antiviral [13], Anthelminthic [14], Antihypertensive [15], Cardiovascular [16], Antioxidant [17].

Considering the above observations and in connection to previous publications involving the synthesis of new biologically active heterocycles. Thus the efficient synthesis novel series of indole-2-carboxamides fused with imidazolinone derivatives still represent highly pursued target.

MATERIALS AND METHODS

Material and Methods: Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine and UV light. All compounds were purified by recrystallization with suitable organic solvents. IR spectra were recorded on Brooker-ALPHA FT-IR instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using direct inlet probe technique. ^{1}H NMR and ^{13}C NMR was determined in $CDCl_3$ solution on a Bruker Ac 400 MHz spectrometer. Purity of the synthesized compounds was checked by HPLC Agilent. The results are in agreements with the structures assigned. Elemental analysis of the all the synthesized compounds was carried out on Euro EA 3000 elemental analyzer and the results are in agreements with the structures assigned.

Synthesis of 1*H*-Indole-2-carbohydrazide:
Methyl 1*H*-indole-2-carboxylate (1.75 g, 0.01 mol) in absolute ethanol (25 ml) was refluxed with 1.0 ml of hydrazine hydrate for 2 hour. After the completion of the reaction checked by TLC, the reaction mixture was cooled to room temperature. The separated solid was filtered, washed with cold ethanol and recrystallized from ethanol. Yield 98%, M.P 247-248^{0}C.

Synthesis of 4-Arylidene-2-phenyl-5-oxazolinones: These were prepared by the condensation of different substituted benzaldehyde with benzoyl glycine in presence of sodium acetate and acetic anhydride.

General procedure for the preparation of novel series of indole-2-carboxamides fused with imidazolinone derivatives (IMD-1 to IMD-10):
To a mixture of 1*H*-indole-2-carbohydrazide (1.75 g, 0.01 mol) and different 4- arylidene-2-phenyl-5-oxazolinones (0.01 mol) in a pyridine (15 ml) was refluxed for 8-10 hour (monitoring by TLC). The excess of solvent was removed under reduced pressure and reaction mixture was poured on crushed ice. The product was isolated and crystallized from methanol to give analytical pure product. The physical constants of the product are recorded in Table-1.

***N*-[(4*Z*)-4-(2-Methoxybenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1*H*-imidazol-1-yl]-1*H*-indole-2-carboxamide (IMD-1):** Purity by HPLC: 99 %; IR (KBr): 3338 (N-H str), 3059 (Ar, C-H str), 2926 (C-H str), 2847 (C-H str), 1647 (amide, C=O str), 1591 (C=N str), 1570 (Ar, C=C str), 1508 (Ar, C=C str), 1271 (C-H ban), 1026 (C-N str), 810 (C-H, o.p. ban) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 3.92 (s, 3H, OCH_3), 6.84- 6.98 (m, 2H, ArH), 6.99-7.09 (m, 2H, ArH), 7.14-7.21 (m, 3H, ArH), 7.39-7.50 (m, 3H, ArH), 7.52-7.60 (m, 1H, ArH), 7.74-7.79 (m, 1H, ArH), 7.98 (s, 1H, C=CH), 8.05-8.07 (d, *J*=7.12 Hz, 2H, ArH), 8.54 (s, 1H, NH), 10.75 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 55.66, 115.20, 124.06, 124.21, 124.42, 126.10, 127.20, 127.29, 127.95, 128.10, 128.95, 129.27, 129.40, 129.99, 130.08, 130.74, 137.27, 141.20, 157.35, 159.57, 189.06; MS: *m/z* = 437 $[M+1]^+$; Anal. Calcd for $C_{26}H_{20}N_4O_3$: C, 71.55; H, 4.62; N, 12.84. Found: C, 71.29; H, 4.47; N, 12.61%.

***N*-[(4*Z*)-4-(4-Nitrobenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1*H*-imidazol-1-yl]-1*H*indole-2-carboxamide (IMD-2):** Purity by HPLC: 96 %; IR (KBr): 3443, 3068, 1697, 1591, 1522, 1445, 1260, 1040, 820 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 7.42-7.52 (m, 3H, ArH), 7.54-7.63 (m, 3H, ArH), 7.65-7.75 (m, 1H, ArH), 7.84-7.85 (d, *J*=7.08 Hz, 1H, ArH), 8.09-8.13 (m, 2H, ArH), 8.24-8.27 (d, *J*=11.84 Hz, 2H, ArH), 8.35 (s, 1H, C=CH), 8.52 (s, 1H, NH), 8.78-8.86 (m, 2H, ArH), 10.73 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 115.72, 127.60, 127.80, 128.00, 129.74, 130.20, 130.41, 130.65, 131.02, 131.72, 132.10, 132.40, 133.27, 133.62, 134.10, 137.60, 141.10, 157.48, 160.12, 191.16; MS: *m/z* = 451 $[M]^+$; Anal. Calcd for $C_{25}H_{17}N_5O_4$: C, 66.51; H, 3.80; N, 15.51. Found: C, 66.25; H, 3.76; N, 15.42%.

***N*-[(4*Z*)-4-(4-Methylbenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1*H*-imidazol-1-yl]-1*H*indole-2-carboxamide (IMD-3):** IR (KBr): 3466, 3075, 1681, 1613, 1499, 1402, 1261, 1025, 840 cm^{-1}; MS: *m/z* = 420 $[M]^+$; Anal. Calcd for $C_{26}H_{20}N_4O_2$: C, 74.27; H, 4.79; N, 13.33. Found: C, 73.95; H, 4.70; N, 13.21%.

***N*-[(4*Z*)-4-Benzylidene-5-oxo-2-phenyl-4,5-dihydro-1*H*-imidazol-1-yl]-1*H*-indole-2-carboxamide (IMD-4):** IR (KBr): 3469, 3076, 1706, 1646, 1576, 1466, 1281, 1021, 746 cm^{-1}; MS: *m/z* = 406 $[M]^+$; Anal. Calcd for $C_{25}H_{18}N_4O_2$: C, 73.88; H, 4.46; N, 13.78. Found: C, 73.58; H, 4.40; N, 13.67%.

***N*-[(4*Z*)-4-(4-Chlorobenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1*H*-imidazol-1-yl]-1*H*indole-2-carboxamide (IMD-5):** IR (KBr): 3459, 3077, 1694, 1634, 1597, 1464, 1261, 1013, 866 cm^{-1}; MS: *m/z* = 440 $[M]^+$; Anal. Calcd for $C_{25}H_{17}ClN_4O_2$: C, 68.11; H, 3.89; N, 12.71. Found: C, 67.82; H, 3.81; N, 12.54%.

***N*-[(4*Z*)-4-(3-Nitrobenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1*H*-imidazol-1-yl]-1*H*indole-2-carboxamide (IMD-6):** IR (KBr): 3492, 3064, 1705, 1625, 1599, 1406, 1242, 1039, 723 cm^{-1}; MS: *m/z* = 452 $[M+1]^+$; Anal. Calcd for $C_{25}H_{17}N_5O_4$: C, 66.51; H, 3.80; N, 15.51. Found: C, 66.18; H, 3.75; N, 15.39%.

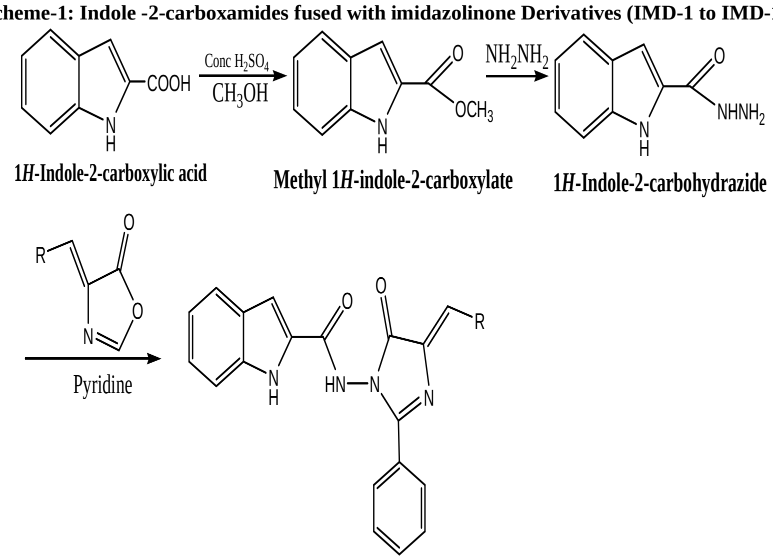
***N*-[(4*Z*)-4-(4-Hydroxybenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1*H*-imidazol-1-yl]-1*H*-indole-2-carboxamide (IMD-7):** IR (KBr): 3469, 3003, 1691, 1589, 1565, 1482, 1250, 1044, 716 cm^{-1}; MS: *m/z* = 422 $[M]^+$; Anal. Calcd for $C_{25}H_{18}N_4O_3$: C, 71.08; H, 4.29; N, 13.26. Found: C, 70.84; H, 4.18; N, 13.08%.

***N*-[(4*Z*)-4-(4-Fluorobenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1*H*-imidazol-1-yl]-1*H*indole-2-carboxamide (IMD-8):** IR (KBr): 3454, 3086, 1712, 1602, 1546, 1445, 1016, 747 cm^{-1}; MS: *m/z* = 425 $[M+1]^+$; Anal. Calcd for $C_{25}H_{17}FN_4O_2$: C, 70.75; H, 4.04; N, 13.20. Found: C, 70.21; H, 3.98; N, 13.07%.

***N*-[(4*Z*)-4-(4-Methoxybenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1*H*-imidazol-1-yl]-1*H*-indole-2-carboxamide (IMD-9):** IR (KBr): 3394, 3074, 1688, 1592, 1572, 1465, 1028, 727 cm^{-1}; MS: *m/z* = 436 $[M]^+$; Anal. Calcd for $C_{26}H_{20}N_4O_3$: C, 71.55; H, 4.62; N, 12.84. Found: C, 71.16; H, 4.51; N, 12.63%.

***N*-[(4*Z*)-4-(2,5-Dimethoxybenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1*H*-imidazol-1-yl]-1*H*-indole-2-carboxamide (IMD-10):** IR (KBr): 3386, 3056, 1690, 1587, 1585, 1464, 1024, 723 cm^{-1}; MS: *m/z* = 467 $[M+1]^+$; Anal. Calcd for $C_{27}H_{22}N_4O_4$: C, 69.52; H, 4.75; N, 12.01. Found: C, 69.27; H, 4.63; N, 11.94%.

Scheme-1: Indole -2-carboxamides fused with imidazolinone Derivatives (IMD-1 to IMD-10)



Indole -2-carboxamides fused with imidazolinone Derivatives (IMD-1 to IMD-10)

Table-1: Physical constant of synthesized indole-2-carboxamides fused with imidazolinone derivatives

Compound	Substitution (R)	M.F	M.W	M.P (^{0}C)
IMD-1	2-Methoxyphenyl	$C_{26}H_{20}N_4O_3$	436.36	236-238
IMD-2	4-Nitrophenyl	$C_{25}H_{17}N_5O_4$	451.43	213-215
IMD-3	4-Methylphenyl	$C_{26}H_{20}N_4O_2$	420.46	179-180
IMD-4	Phenyl	$C_{25}H_{18}N_4O_2$	406.43	163-165
IMD-5	4-Chlorophenyl	$C_{25}H_{17}ClN_4O_2$	440.88	225-224
IMD-6	3-Nitrophenyl	$C_{25}H_{17}N_5O_4$	451.43	183-184
IMD-7	4-Hydroxyphenyl	$C_{25}H_{18}N_4O_3$	422.43	151-153
IMD-8	4-Fluorophenyl	$C_{25}H_{17}FN_4O_2$	424.42	138-139
IMD-9	4-Methoxyphenyl	$C_{26}H_{20}N_4O_3$	436.46	173-174
IMD-10	2,5-Dimethoxyphenyl	$C_{27}H_{22}N_4O_4$	466.48	201-202

BIOLOGICAL EVALUATION:

Preparation of Culture Media

Nutrient broth was used as growth medium for bacteria and Saubouraud dextrose broth for fungi. Nutrient broth was prepared by dissolving 13gm of dehydrated powder (HI-media) in 100ml of distilled water. Saubouraud dextrose broth was prepared by dissolving 4gm of dextrose and 1gm of peptone in 100ml of distilled water. The media were sterilized by autoclaving at 15lbs pressure for 20 minutes.

Preparation of Stock Culture

Stock cultures were obtained by aseptically transferring a loopful of test organisms to 100ml of sterile broth and incubated for 24 hours at 37^0C.

Standardization of Stock Culture

Stock cultures were placed in the incubator (37^0C for bacteria and 24^0C for fungi) and shaken well. One ml of stock cultures was aseptically transferred to 9 ml of sterile water containing 0.05% tween 80. This was mixed with using a cyclomixer and serially diluted from 10^{-1} to 10^{-10}. From each dilution, 0.2ml was taken and spread on sterile nutrient agar plates for bacteria and Sabouraud dextrose agar plates for fungi, which were incubated for 18 hours. After incubation, the numbers of colonies in the plate were counted. The number of colonies for a plate that was formed from the maximum dilute tube was noted. The number of microorganisms in stock were then calculated and expressed as colony forming units per ml (cfu/ml). By back calculation the stock culture was found to contain 15×10^8 cfu/ml.

Preparation of Working Stock Culture

Stock culture (0.1ml) was diluted with nutrient broth (100ml) and Sabouraud dextrose broth (100ml) respectively to obtain 10^5 cfu/ml. This was then used for further *in vitro* screening.

Preparation of Drug Dilutions

Solutions of the title compounds in DMSO (1mg/ml) were prepared and used for screening their antimicrobial activity.

Antimicrobial Screening:

Synthesized compounds were subjected to antimicrobial screening by estimating the minimum inhibitory concentration (MIC) by adopting serial dilution technique. Test was carried out on four bacterial strains, namely *Staphylococcus aureus* (MTCC 96), *Staphylococcus pyogenus*, *Pseudomonas aeruginosa* (MTCC 1688), *Escherichia coli* (MTCC 443) and two fungal strains, namely *Candida albicans* (MTCC 227) and *Aspergilla niger* (MTCC 282).

Table-2: Antimicrobial activity of indole-2-carboxamides fused with imidazolinone derivatives

Compound	Minimal Inhibitory Concentration (µg/ml)					
	Antibacterial Activity				Antifungal activity	
	S.aureus	***S.pyogenus***	***E.coli***	***P.aeruginosa***	***C.albicans***	***A.niger***
IMD-1	100	50	62.5	100	200	500
IMD-2	200	200	100	250	500	200
IMD-3	100	100	250	250	500	1000
IMD-4	500	250	500	100	250	250
IMD-5	500	500	100	100	500	1000
IMD-6	250	500	100	500	1000	250
IMD-7	250	100	200	200	1000	200
IMD-8	200	250	100	500	500	1000
IMD-9	500	500	500	250	500	500
IMD-10	200	500	250	250	250	500
Ampicillin	250	100	100	100	NT	NT
Greseofulvin	NT	NT	NT	NT	500	100

Determination of MIC: The study involved a series of six assay tubes for each title compound against each microorganism. The entire test was done in duplicate. To the first assay tube, 1.8ml of seeded broth and 0.2ml of title compound (1mg/ml) was added and mixed thoroughly and the two fold serial dilution was done up to the sixth tube containing 1 ml of seeded broth. The additions of the drug solution and serial dilution were done under strict aseptic conditions. Solvent control, negative control (growth control) and drug control were maintained during the experiment. The assay tubes were incubated at 37^0C and 25^0C respectively for 24 hours for bacteriae and fungi. The lowest concentration, which apparently caused complete inhibition of growth of microorganisms, was considered as the minimum inhibitory concentration (MIC).The MIC values of the test compounds are recorded in Table-2.

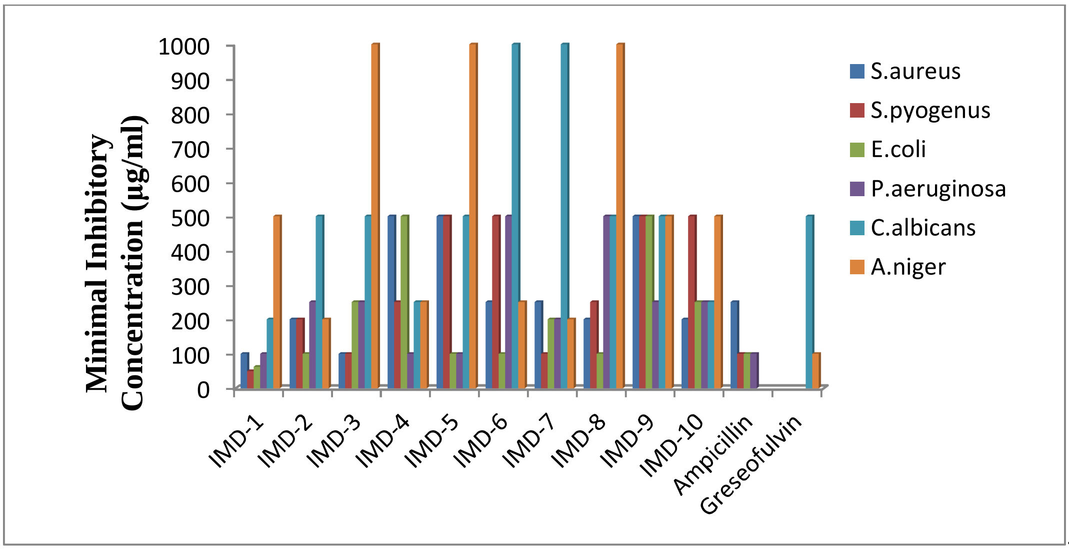


Figure 1: Antimicrobial Activity of novel series of indole-2-carboxamides fused with imidazolinone derivatives

RESULTS AND DISCUSSION

Methyl 1*H*-indole-2-carboxylate in absolute ethanol was refluxed with hydrazine hydrate forms 1*H*-Indole-2-carbohydrazide. Different substituted benzaldehydes with benzoyl glycine in presence of sodium acetate and acetic anhydride to form 4-Arylidene-2-phenyl-5-oxazolinones. The mixture of 1*H*-indole-2-carbohydrazide and different 4- arylidene-2-phenyl-5-oxazolinones in a pyridine forms titled compounds **(IMD-1 TO IMD-10)**. All the synthesized compounds were subjected to antimicrobial screening by estimating the minimum inhibitory concentration (MIC) by adopting serial dilution technique.

The data recorded in Table 2 indicated that compounds IMD-1 and IMD-3 are more potent towards *Staphylococcus aureus.* The compounds IMD-2, IMD-8 and IMD-10 are moderately potent towards the *Staphylococcus aureus.* Compound IMD-1 is more potent towards the *Streptococcus pyogenes.* Compounds IMD-3 and IMD-7 are moderately potent towards the *Streptococcus pyogenes.* Compound IMD-1 is more potent towards the *Escherichia coli* and compounds IMD-2, IMD-5, IMD-6 and IMD-8 were moderately potent towards the *Escherichia coli.* Compounds IMD-1, IMD-4 and IMD-5 are moderately potent towards the *Pseudomonas aeruginosa*. All these compounds are compared with the standard reference (Ampicillin) for their antibacterial activities. The compounds IMD-2, IMD-4 and IMD-10 are potent towards the *Candida albicans.* All these compounds are compared with the standard reference (Greseofulvin) for their antifungal activities.

CONCLUSION

In this study, certain some indole-2-carboxamides fused with imidazolinone moieties were synthesized and evaluated for their antimicrobial activities. Results revealed that the compounds exhibited significant *in-vitro* activity. Compound IMD-1, IMD-2, IMD-3, and IMD-5 are more potent. Remaining compounds also showed moderate to weak antimicrobial activities. The study would be a fruitful matrix for the development of indole-2-carboxamides fused with imidazolinone moieties for further bio-evaluation.

Acknowledgement
The authors are grateful to Department of Pharmaceutical Analysis, Chalapathi Institute of Pharmaceutical Sciences, Guntur for providing facilities to perform this research work.

REFERENCES

[1] Rajashree S Chavan *Tropical Journal of Pharmaceutical Research*, August **2011**; 10 (4): 463-473.
[2] Mahadevan, *Int J Pharm Pharm Sci*, Vol 6, **2012**, Issue 4, 475-482.
[3] Olga N. Burchak, *Bioorganic & Medicinal Chemistry*, Volume 19, Issue 10, 15 May **2011**, Pages 3204–3215.
[4] Priya Ahuja and Nadeem Siddiqui, *EJMC*, **2014**.04.043.
[5] Anil Kumar, *IJPBA*, **2011**; 2(2):744-750.
[6] Mhaske, WJPPS, Vol 3, Issue 8, **2014**, 825-840.
[7] M. Sarangapani, *J. Chem. Pharm. Res.*, **2010,** 2(2): 220-225.
[8] Ashok Kumar, *Int.J. ChemTech Res.***2010**,2(2).
[9] Patil, *WJPR*, Volume 3, **2013** Issue 5, 1292-1306.
[10] R.S. Chavan, *Int J Pharm Biomed Res*, **2010**, 1(4), 135-143
[11] Hardik M. Patel, *International Journal of Institutional Pharmacy and Life Sciences* 2(4): July-August **2012**.
[12] Saundane Anand Raghunath and Kirankumar Nandibeoor Mathada, *International Journal of Medicinal Chemistry*, Volume **2014.**
[13] Adel A.-H. Abdel-Rahman, *Bull. Korean Chem. Soc.* ***2012**, Vol. 33, No. 10.*
[14] K. S. Nataraj, *Int. J. Chem. Sci.:* 8(2), **2010**, 1269-1277.
[15] A. Monge Vega, *Journal of Pharmaceutical Sciences*, 21 SEP **2006**.
[16] Navneet Singh, *International Journal of Pharmaceutical Sciences and Drug Research*, **2013**; 5(1): 14-17.
[17] Hamid Khaledi, *Arch. Pharm. Chem. Life Sci*. **2011**, 344 , 703 – 709.