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Der Chemica Sinica, 2015, 6(1):1-6

Der Chemica Sinica
Pelagia Research Library
ISSN: 0976-8505
CODEN (USA) CSHIA5

Synthesis of novel click coumarins and biological evaluation

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ABSTRACT

Owing to great importance of coumarins in their versatile biological behaviour, novel series of coumarinswere synthesized and cytotoxic study was conducted. Comparative antibacterial screening was performed with the existing series of coumarins which were synthesised from traditional method. Propargyloxy coumarins reacted with different azide derivatives in acid catalysis to yield triazolated coumarins. Reaction progress was monitored by TLC. The synthesised compounds were characterized by IR, H^1 NMR, C^{13} NMR, ESIMS and Elemental analytical data.

Key words: Propargylation, triazolated coumarins, cytotoxic screening, antibacterial activity.

INTRODUCTION

Coumarins are biologically active class of the flavonoids. These were first isolated by Vogel from the extraction of 'Tonka beans' (*Dipteryx Odorata/ Coumarona odorata*) in 1820. The term '*Coumarin*' was generated from its first origin plant i.e., *Coumarona odorata*. It was further identified in a large number families of plants. The coumarin nucleus corresponds to benzo-α-pyrone or 2*H*-1-benzopiran-2-one whose systematic nomenclature was established by IUPAC[1].They have been showing versatility in effective biological activities including anti-bacterial activity[2-7]; anti-cancer activity [8-13]; anti-HIV activity [14-17]; anti-inflammatory and antioxidant activities[18-21]. Such importance of coumarins led to the development of many synthetic methodologies include Perkin reaction [22, 23]; Knoevenagel reaction[24-29]; Reformatsky reaction [30]; Wittig reaction[31-34].

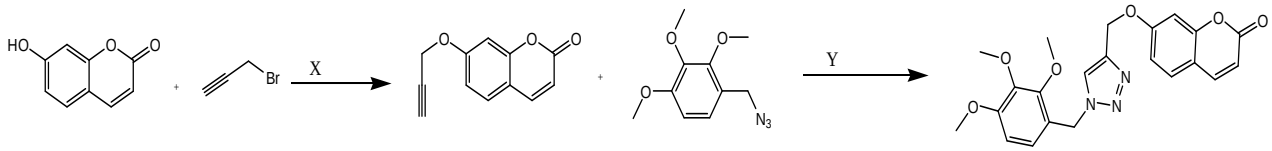
MATERIALS AND METHODS

4.1. Chemicals and Instruments:
All the melting points were determined in open capillaries, using Boitus melting point apparatus, expressed in ^{0}C. The IR spectra of the compounds were recorded on Shimadzu IR Affinity FTIR Spectrophotometer using KBr discs and the values are expressed in cm^{-1}. The H^1 NMR and C^{13}NMR spectra of compounds were recorded on Bruker AMX 400 NMR spectrophotometer using TMSand DMSO as an internal standards respectively and the values are expressed in ppm. The elemental analyses of the compounds were recorded on Elemental VARIO EL III.

Cytotoxic analysis was conducted by MTT-micro cultured tetrazolium assay method. Antibacterial activity was conducted by Cup Plate agar diffusion method.

4.2. Synthetic procedures:
4.2.1. ***Synthesis of '7-propargyloxy coumarin':*** Equimolar quantities of 7-hydroxy coumarin, propargyl bromide and potassium carbonate were dissolved in 10 ml of acetone. The reaction mixture was refluxed for 6 hrs and poured in crushed ice. Precipitate was filtered, dried and recrystallized from absolute alcohol.

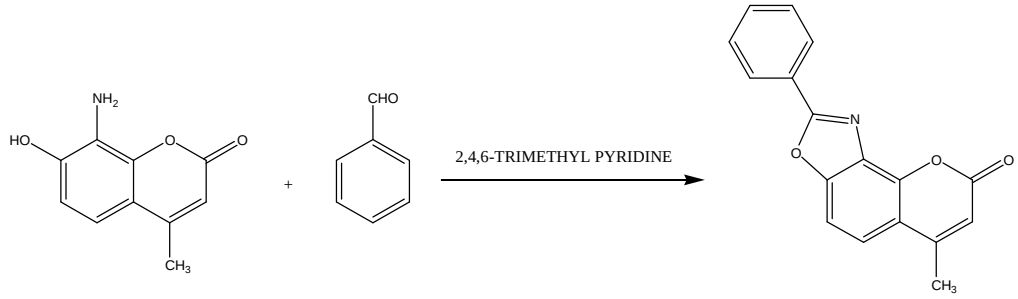
4.2.2. *Synthesis of '7-((1- (2, 3, 4-trimethoxybenzyl)-1H-1, 2, 3-triazol-4-yl) methoxy)-2H-chromen-2-one':* Equimolar quantities of 7-propargyloxy coumarin, 1-(azidomethyl)-2,3,4-trimethoxybenzene, $CuSO_4$ (0.1 mmol) and Sodium ascarbate (0.15 mmol) were charged in the solution of t-Bu.OH/H_2O (8 ml 1:1). Reaction mixture was stirred well for 12 hrs at 55-60 ^{0}C. The progress of reaction was monitored by TLC, using n-hexane and ethyl acetate with the ration of 7:3. Then mixture was poured crushed ice. PPT was filtered, dried and purified by column chromatography.



1 2 3 4 5 a

X= K2CO3, Acetone, 5hrs reflux. Y= t-butanol, per chloric acid, stirring 12 hrs at r.t.

Second set of coumarins were synthesised from traditional procedure [36].



6 7 8 a

4.2.3. Spectral Studies:

5a) 7-((1- (2, 3, 4-trimethoxybenzyl)-1H-1, 2, 3-triazol-4-yl) methoxy)-2H-chromen-2-one

Solid,**m.p.**185–187°C. **IR (neat):** ν_{max} , 1613, 1463, 1214, 1127, 1004, 836, 742, 666, 629; **H^1 NMR** δ: 7.63(d, 1H, *J* = 9.06, Hz), 7.60 (s, 1H), 7.35(d, 1H, *J* = 9.06, Hz), 6.95- 6.9(m, 2H), 6.50 (s, 2H), 6.27 (d, 1H, *J* = 9.8, Hz)5.47 (s, 2H), 5.26 (s, 2H), 3.84(s, 3H), 3.82 (s, 6H); **C^{13}NMR**: 50.5, 50.6, 60.7, 60.5, 102.3, 104.7, 112.5, 113.0, 113.5, 125.5, 129.2, 130.1, 140.5, 150.5, 160.3; Elemental analysis: C22H21N3O6; Calculated: C, 62.41; H, 5.00; N, 9.92; O, 22.67; Found: C, 62.44; H, 5.03; N, 9.90; O, 22.64; **ESIMS**: *m/z* 423(M)$^+$.

5b) 7-((1-(4-isopropylbenzyl)-1H-1, 2, 3-triazol-4-yl) methoxy)-2H-chromen-2-one

Solid, **m.p.**190–192°C **IR (neat):**ν_{max}3853, 3610, 3140, 2925, 1638, 1605, 1511, 1443, 1230, 1178, 1011, 771, 667. **H^1 NMR** δ: 7.62 (d, 1H, *J* = 10.05, Hz), 7.57 (s, 1H), 7.327 (d, 1H, *J* = 8.22. Hz), 7.28-7.20 (m, 4H), 6.91 (s, 2H), 6.26 (d, 1H, *J* = 10.05, Hz), 5.51 (s, 2H), 5.23 (s, 2H), 3.84(s, 3H), 2.97-2.8 (m, 1H), 1.24 (d, 6H, *J* = 6.40, Hz); **^{13}C NMR** 160.7, 160.5, 155.1, 149.2, 142.8, 131.0, 128.4, 127.7, 126.7, 112.8, 112.4, 112.2, 101.5, 61.8, 53.7, 33.3, 23.3; Elemental analysis: C_{22} H_{21} N_3 O_3; Calculated: C, 70.38; H, 5.64; N, 11.19; O, 12.79; Found: C, 70.36; H, 5.62; N, 11.16; O, 12.81; **ESIMS**: *m/z* 375(M)$^+$.

5c)4-phenyl-7-((1-(2,3,4-trimethoxybenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-2H-chromen-2-one

Solid, **m.p.**195–197°C**IR (neat):**ν_{max}2923, 1726, 1629, 1442, 1255, 1220, 1169, 1011, 772, 701, 603; **H^1 NMR** δ: 7.76 (s, 1H), 7.75-7.71 (m, 1H), 7.73 (dd, 1H, *J* = 8.31, 1.51 Hz), 7.6 (s, 1H), 7.50 (m, 1H), 7.48-7.37 (m, 3H), 6.97-6.92 (m, 2H), 6.51 (s, 2H), 5.47 (s, 2H), 5.26 (s, 2H), 3.84(s, 3H), 3.82 (s, 6H); **^{13}C NMR:** 46.8, 52.5, 73.2, 113.4, 120.6, 121.3, 126.3, 128.6, 140.8, 142.8, 143.5, 149.3, 152.9, 161.7; **Elemental analysis:**C_{28} H_{28} N_3 O_6; Calculated: C, 67.33; H, 5.04; N, 8.41; O, 19.22; Found: C, 67.34; H, 5.06; N, 8.43; O, 19.25; **ESIMS**: *m/z 498* (M)$^+$, **ESIMS**: *m/z* 499(M)$^+$.

5d): 7-((1-(2, 4-dichlorobenzyl)-1H-1, 2, 3-triazol-4-yl) methoxy)-3-chloro -4-methyl-2H-chromen-2-one.

Solid; m.p.- 180-183^{0}C**IR (cm^{-1}):**3745, 2924, 1633, 1608, 1444, 1242,1182, 1125, 1001, 752, 666.**H^{1}NMRδ:** 2.37(S,3H), 6.26(S, 2H), 6.44(S,2H), 6.13(S,1H), 6.51(S,2H), 6.90(S,1H), 6.94(d,1H, J= 9.1), 7.49(d, 1H, J= 9.14), 7.61(S,1H); **C^{13} NMR:** 23.8, 54.8, 62.2, 101.9, 112.6, 113.3, 127.7, 128.2, 128.8, 131.4, 143.2, 149.7, 155.5, 161.0, 161.20; **Elemental analysis:**C_{20} H_{14} Cl_3 N_3 O_3; Calculated: C, 53.30; H, 3.13; Cl, 23.60; N, 9.32; O, 10.65; Found: C, 53.32; H, 3.11; Cl, 23.63; N, 9.33; O, 10.62; **ESIMS: *m/z***451 (M)$^+$.

5e:7-((1-((furan-2-yl) methyl)-1H-1, 2, 3-triazol-4-yl) methoxy) -4-phenyl-2H-chromen-2-one.
Solid, m.p.193-195 ^{0}C**IR (cm^{-1}):** 3744, 3017, 1629, 1441, 1397, 1255, 1215, 1011, 751, 667; **H^1 NMR**δ: 7.64(d, 1H, *J* = 9.06, Hz), 7.62 (s, 1H), 7.31(d, 1H, *J* = 9.06, Hz), 6.95- 6.9(m, 2H), 6.52 (s, 2H), 6.25 (S, 1H), 5.47 (s, 2H), 5.26 (s, 2H), 3.84(s, 3H), 3.82 (s, 6H), 2.13 (S, 3H); **C^{13}NMR**: 20.7, 50.2, 50.5, 60.3, 61.3, 102.7, 104.4, 112.6, 113.5, 113.8, 125.5, 129.2, 130.2, 140.4, 150.5, 160.4; **Elemental analysis:** C_{23} H_{23} N_3 O_6; Calculated: C, 63.15; H, 5.30; N, 9.61; O, 21.94; Found: C, 63.13; H, 5.32; N, 5.58; O, 21.96; **ESIMS**: *m/z*: 398 $(M)^+$.

Spectral data of second series (8a-8d) of the compounds was concordance with the literature [36].

5. Biological evaluation:
5.1. Cytotoxic analysis:
Cellular viability in the presence of test compounds was determined by MTT-micro cultured tetrazolium assay. The cells seeded to flat bottom 96(10000cells/100ul) well plates &cultured in the medium containing 10% serum and allowed to attach and recover for 24 hours in a humid chamber containing 5% CO2.

MTT (3-(4, 5-dimethylthiazol-2yl)-2,5diphenyl tetrazolium bromide; sigma Catalog noM2128) was dissolved in PBS at 5mg/ml and filtered to sterilize and remove a small amount of insoluble residue present in MTT.

Different concentrations of compounds were added to the cells. After 48 hours, stock MTT solution (10ul) was added to the culture plate .Cells were again kept in CO2 incubator for 2 hours. After incubation 100ulof DMSO was added and mixed.

The absorbance was read at 562nm in a plate reader. The results were represented as percentage of cytotoxicity/viability. All the experiments were carried out in triplicates. From the percentage of cytotoxicity the IC -50 value calculated. Media used was MEM Catalog No M0643.

DPBS Catalog No D5652.
1X antibiotic solution of 100X Catalog No A5955.
1% Sodium pyruvate Catalog No.S8636.
1% Non-essential amino acids Catalog No M7145.
10% Fetal bovine serum Catalog No F2442.
DMSO Catalog No D5879.
Trypsin-EDTA solution (0.25%, 2.5 g porcine trypsin and 0.2 g EDTA) Catalog NoT4049.
Trypsin-EDTA solution used for detaching cells during sub culturing process.

5.2. Anti-bacterial activity:
All the prepared compounds were tested for the anti-bacterial activity against Staphylococcus aureus (G +Ve), Bacillus Subtilis (G +Ve) and Escherichia Coli (G –Ve). The activity was done by Cup Plate agar diffusion method [35]. The cups of 10 mm diameter in the agar media spread with the microorganisms were prepared using a sterile borer.

0.1 mL of inoculums (of 10 CFU / mL population prepared from standardized culture, adjusted with peptone water) were spread on the agar plate by spread plate technique.
0.1 ml of sample solutions and standard were added to the cups with micro pippete.
Effective diffusion conditions were maintained to the test compounds and standards by keeping all plates at 3-8^{0}C in refrigerator. Then they were incubated at 37^{0}C +/-1^{0}C for 24 hrs. It was found that the presence of definite zones of inhibitions around the cup which indicates anti-bacterial activity. The DMSO was run as control which was used as solvent for the sample compounds.

Preparation of nutrient medium:
The all ingredients-Nutrient agar 2%; Beef extract 1%; Peptone 1%; NaCl 0.5 % - were weighed accurately and added to the 10 ml of distilled and sterilized water. This mixture was heated on water bath for 30 mnts, up to clear solution was found. This nutrient media was sterilized in autoclave at 120 ^{0}C at 15 psi.
The zone of inhibitions in mm were found for all compounds and compared.

RESULTS AND DISCUSSION

The complete synthetic work was well performed with very good to excellent yield i.e., 75-90%. IR signal at around 1214 cm-1, confirms ether linkage in benzpyrone ring. Signal at around 1125 cm-1 is due to -N=N- stretching vibration in triazole ring. Signal at around 1620 cm^{-1}confirms the carbonyl group in ring system.

H^1 NMR chemical shift shows signal at around 5.5 due to methylene protons; signal around at 7.5 due to aromatic proton of triazole ring. C^{13} NMR chemical shifts at 140 due to aromatic 'C'; 50 due to methylene bridged 'C' s of triazole ring and at 160 due to carbonyl carbon.

4th and 5th compounds show poor cytotoxic activity. 1st, 2nd and 3rd compounds show moderate to good activity in different doses. Compound 1 is found to be more active at its higher concentration.

Antibacterial activities of new set of compounds are moderate to good. New set of compounds showed almost equal potency with the existing set of compounds. However, 4th compound showed highest potency among all the synthesized compounds.

Table 1*Data of synthesized compounds:*

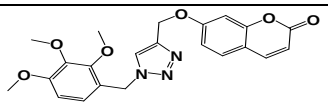
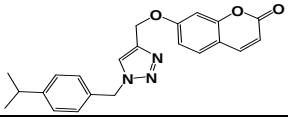
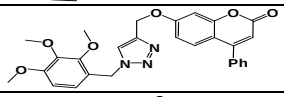
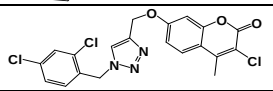
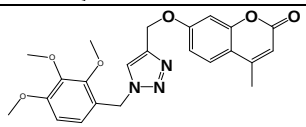
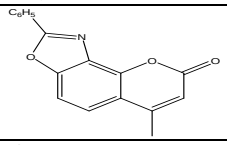
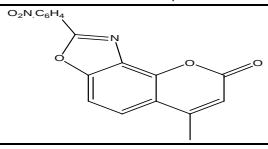
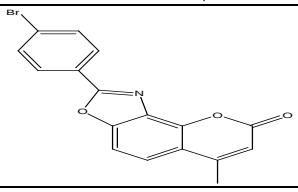
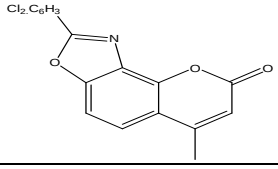
S. No.	Structure of Compound	IUPAC Name	M.Wt.	m.p. ^{0}C	Yield %
5a		7-((1- (2, 3, 4-trimethoxybenzyl)-1H-1,2, 3-triazol-4-yl) methoxy)-2H-chromen-2-one	243	185–187	85
5b		7-((1-(4-isopropylbenzyl)-1H-1, 2, 3-triazol-4-yl) methoxy)-2H-chromen-2-one	375	190–192	82
5c		4-phenyl-7-((1-(2,3,4-trimethoxybenzyl) 1H-1,2,3-triazol-4-yl)methoxy)-2H-chromen- 2-one	499	195–197	78
5d		7-((1-(2, 4-dichlorobenzyl)-1H-1, 2, 3-triazol-4-yl) methoxy)-3-chloro -4-methyl-2H-chromen-2-one.	450	180-183	85
5e		7-((1-(2,3,4-trimethoxybenzyl)-1H-1,2,3-triazol-4-yl) methoxy)-4-methyl-2H-chromen-2-one	399	193-195	76
8a		6-methyl-2-phenyl-8H-chromeno[8,7-d]oxazol-8-one	277	230-234	63
8b		2-(4'-nitrophenyl)-6-methyl-8H-chromeno[8,7-d]oxazol-8-one	200	246-248	75
8c		2-(4'-bromophenyl)-6-methyl-8H-chromeno[8,7-d]oxazol-8-one	356	253-255	62
8d		2-(3', 4'-chlorophenyl)-6-methyl-8H-chromeno[8,7-d]oxazol-8-one	201	220-223	70

Table 2 *Cytotoxic Activity*

Compound	% Growth of Inhibition		
	10 µM	50 µM	100 µM
1	25.72	37.27	43.25
2	19.48	21.33	23.53
3	21.93	32.55	39.46
4	-	2.78	7.32
5	-	5.65	8.36

Table 3 ***Anti-bacterial activity***

Compound	Concentration µg/ml	Zone of inhibition(mm)		
		S. aureus	*B. subtilis*	*E. coli*
1	500	8	6	7
	1000	12	11	15
2	500	9	7	5
	1000	15	13	17
3	500	10	9	10
	1000	14	17	12
4	500	19	16	18
	1000	27	21	23
5	500	11	8	9
	1000	19	15	14
6	500	9	11	13
	1000	17	15	18
7	500	16	16	18
	1000	18	17	19.5
8	500	14	10	16
	1000	20	20	21
9	500	15	12	16
	1000	21.75	22.28	24.43
Amoxicillin	500	27	24	28
	1000	30	28	36

CONCLUSION

In conclusion, Click chemistry is more applicable to establish triazole at alkynic bond. We applied this method to Propargyloxy coumarins. Yields are very good. Some of the compounds are very good at anti-bacterial and cytotoxic activities.

Acknowledgement
We are thankful to our mentor Dr. V. Madhavarao, Bapatla Engineering College, Department of Chemistry; for his great guide lines.

REFERENCES

[1] IUPAC, *Nomenclature of Organic Chemistry*, Pergamon Press, **1979**.
[2] Chimenti. F.; Bizzarri, B.; Bolasco, A.; Daniela Secci, D.; Chimenti, P.; Carradori, S.; Granese, A.; Rivanera, D.; Daniela Lilli, D.; Scaltrito, M. M.; Brenciaglia, I. M. *Eur. J. Med. Chem*. **2006**, *41*, 208.
[3] Creaven, S. B.; Egan, D. A.; Kavanagh, K.; McCann, M.; Noble, A.; Bhumika T. B.; Walsh, M. *InorganicaChim. Octa*. **2006**, *359*, 3976.
[4] Abd-El-Aziz, A. S.; Hany, M. M.; Shawka, M.; Shamsulhaq, Z.; Athar, A.; Ahmed H. B.; Ahmed M. E.; Pierre D. H. *J. Heterocyclic Chem*. **2007**, *44*, 1287.
[5] Biradar, J. S.; Anekal, D. *Indian J. Heterocyclic Chem*. **2007**, *16*, 375.
[6] Yamamoto, Y.; Kurazono, M. *Bioorg. Med. Chem. Lett*. **2007**, *17*, 1626.
[7] Shilabin,A. B.; Kasanah, N.; Wedge, D. E.; Hamann, M. T. *J. Med. Chem*. **2007**, *50*, 4340.
[8] Thornes, R. D.; Edlow, D. W.; Wood, S. J.; John H. *Med. J.***1968**, *123*, 305.
[9] Valenti, P. *Fitoterapia*, **1996**, *68*, 115.
[10] Reddy, N. S.; Kiranmai, G.; Muralidhar, R.; Malli, R.; Stephen C. C.; Padmavathi, V.; Stanley, Bell. C. E.; Reddy, E. P.; Reddy, M. V. R.; *Bioorg. Med. Chem*. **2005**, *13*, 3141.
[11] Chen, Y-L.; Lu, C-M.; Lee, S-J.; Kuo, D-H.; Chen, I-L.; Wang, T-C.; Tzeng, C-C.*Bioorg. Med. Chem*. **2005**, *13*, 5710.
[12] Kumar, S.; Singh, B. K.; Neerja, K.; Vineet, K.; Ajit, K.; Prasad, A. K.;. Raj, G. H.; Parmar, V. S.; Balaram G. *Bioorg. Med. Chem*. **2005**, *13*, 1605.
[13] Kostova, I.; Momekov, G.; Zaharieva, M.; Karaivanova, M.; *Eur. J. Med. Chem.* **2005**, *40*, 542.
[14] McKee, T. C.; Covington, C. D.; Fuller, R. W.; Bokesch, H. R.; Young, S.; Cardellina, J. H.; Kadushin, M. R.; Soejarto, D. D.; Stevens, P. F.; Cragg, G. M.; Boyd, M. R. *J. Nat. Prod*. **1998**, *61*, 1252.
[15] Kostova, I. *Cur. HIV Res*. **2006**, *4*, 347.
[16] Lee, K. H.; Morris-Natschke, S. L. *Pure and Applied Chemistry*, **1999**, *71*, 53.
[17] Lee, T. T.; Kashiwada, Y.; Huang, L.; Snider, J.; Cosentino, M.; Lee, K. H. *Bioorg. Med. Chem*. **1994**, *2*, 1051.
[18] Kontogiorgis, C. A.; Hadjipavlou-Litina, D. J. *Bioorg. Med. Chem. Lett*. **2004**, *14*, 611.
[19] Kontogiorgis, C. A.; Hadjipavlou-Litina, D. *J. Med. Chem*. **2005**, *48*, 6400.
[20] Kontogiorgis, C. A.; Savvoglou, K.; Hadjipavlou-Litina, D. J. *J. Enzyme Inhibition Med. Chem.***2006**, *21*, 21.

[21] Hadjipavlou-Litina, D.; Kontogiorgis, C. A.; Pontiki, E.; Dakanali, M.; Akoumianaki, A.; Katerinopoulos, H. E. *J. Enzyme Inhibition Med. Chem.* **2007**, *22*, 287.
[22] Trkovnik, M.; Ivezi, Z. *J. Heterocyclic Chem*. **2000**, *37*, 137.
[23] Erk, C.; Bulut, M.; Gocmen, A. *J. Incl. Phenom. Supramol. Chem*. **2000**, *37*, 441.
[24] Trkovnik, M.; Ivezi, Z. *J. Heterocyclic Chem*. **2000**, *37*, 137.
[25] Hormi, O. E. O.; Peltonen, C.; Bergström, R. *J. Chem. Soc., Perkin Trans. 1*, **1991**, 219.
[26] Alonso, M. T.; Brunet, E.; Hernandez, C.; Rodríguez-Ubis, J. C. *Tetrahedron Lett.* **1993**, *34*, 7465.
[27] Takadate, A.; Masuda, T.; Murata, C.; Tanaka, T.; Goya, S. *Bull. Chem. Soc. Jpn*., **1995**, *68*, 3105.
[28] Pochet, L.; Doucet, C.; Schynts, M.; Thierry, N.; Boggetto, N.; Pirotte, B.; Jiang, K.Y.; Masereel, B.; Tulio, P.; Delarge, J.; Reboud-Ravaux, M. *J. Med. Chem*. **1996**,*39*, 2579.
[29] Takechi, H.; Kamada, S.; Machida, M. *Chem. Pharm. Bull*. **1996**, *44*, 793.
[30] Fall, Y.; Teran, C.; Teijeira, M.; Santana, L.; Uriarte, E. *Synthesis*, **2000**, *5*, 643.
[31] Murray, R. D. H.; *Nat. Prod. Rep*. **1995**, *12*, 477.
[32] 32. Ferroud, D.; Collard, J.; Klich, M.; Dupuis-Hamelin, C.; Mauvais, P.; Lassaigne, P.; Bonnefoy A.; Musicki, B. *Bioorg. Med. Chem. Lett*. **1999**, *9*, 2881.
[33] Takechi, H.; Kamada, S.; Machida, M. *Chem. Pharm. Bull*. **1996**, *44*, 793.
[34] Ishii, H.; Ishikawa, T.; Wada, H.; Miyazaki, Y.; Kaneko, Y.; Harayama, T. *Chem. Pharm. Bull*. **1992**, *40*, 2614.
[35] Barry A.L, The Antimicrobial Susceptibility Test, *Principle and Practices*, **1999**, 4th Edition, ELBS: London, 180.
[36] SwayamSouravSahoo, Smita Shukla, SubhangankarNandy and HimanshuBhusan Sahoo; *European Journal of Experimental Biology*,**2012**, 2 (4):899.