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One pot synthesis of substituted 1*H*-benzo[f]chromen-3-yl-2*H*-chromen-2-one derivatives

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ABSTRACT

The title compounds, substituted 1*H*-benzo[f]chromen-3-yl-2*H*-chromen-2-ones were obtained by reacting 3-aryl-1-(3-coumarinyl)propen-1-ones with 2-napthol catalyzed by DBU (1,8-diazabicyclo[5,4,0]undec-7-ene) and concentrated H_2SO_4 in ample yields. Their structures were characterized by IR, ^{1}H NMR, ^{13}C NMR, mass spectral and elemental analysis. All the synthesized compounds have been evaluated for their *in-vitro* antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* and antifungal activity against *Aspergillus Niger* and *Candida albicans* by using serial broth dilution method. Among those compounds 3 band 3c exhibits prominent results.

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1. Introduction

Chromene derivatives are known to exhibit a wide range of biological activities, such as antifungal, antibacterial [1-5], antioxidative [6], antileishmanial [7], antitumor [8,9], hypotensive [9], antiproliferation [10,11], local anesthetic [12], antiallergenic [13,14], central nervous system activities and effects [15], as well as efficacious in the treatment of Alzheimer's disease [16] and schizophrenia disorder [17]. Coumarins are of scientific interest as anti-HIV agents [18], antituberculosis agents [19], cholinesterase and monoamine oxidase inhibitors [20], antioxidants and anti-inflammatory [21,22].

In continuation of our work on the synthesis of tetrazolo and triazolo pyrimidin-yl-*2H*-chromen-2-ones [23], we have developed a new route to synthesis of substituted 1*H*-benzo [f]chromen-3-yl-2*H*-chromen-2-ones from 3-aryl-1-(3-coumarinyl)propen-1-ones with 2-napthol catalyzed by 1,8-diaza bicyclo[5,4,0]undec-7-ene (DBU) and concentrated H_2SO_4 in high yields and also studied their antimicrobial activity.

2. Experimental

2.1. Instrumentations

Melting points were recorded in open capillary and were uncorrected. Column chromatography was performed using silicagel (100-200 mesh size) purchased from Thomas Baker and TLC was carried out using aluminum sheets pre-coated with silica gel $60F_{254}$ purchased from Merck. IR spectra (KBr) were recorded on a Bruker WM-4(X) spectrometer (577 model). ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on Bruker AC-300 spectrometer in DMSO-d_6 with TMS as an internal standard. Mass spectra (ESI) were recorded on JEOL SX-102 spectrometer. CHN analysis was done by Carlo Erba EA 1108 automatic elemental analyzer.

2.2. Materials

The chemicals and solvents used were of commercial grade and were used without further purification unless, otherwise, stated.

2.3. Synthesis

2.3.1. Synthesis of substituted-1H-benzo[f]chromen-3-yl-2H-chromen-2-ones (3a-e)

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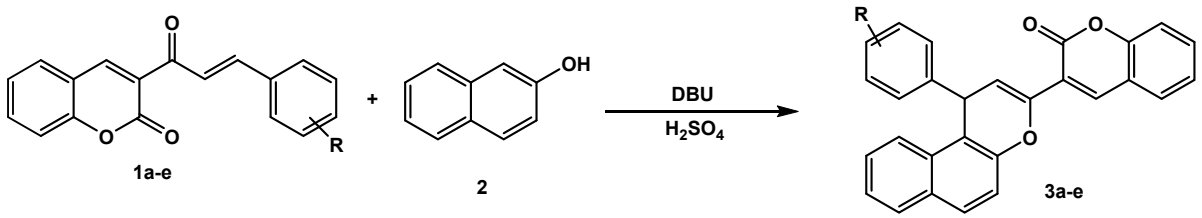

R = a) -C_6H_4-Cl; b) -C_6H_4-OMe; c)-C_6H_3-Cl_2; d) -C_6H_4-OH; e) -C_5H_4N

Scheme 1

To a stirred solution of compound **1a-e** (0.12 mmol), 2-naphthol (**2**) (0.12 mmol) and DBU (0.02 mmol) in DCM (2.0 mL) was reacted at room temperature for 12 h, the reaction was monitored by TLC. Then a drop of concentrated H_2SO_4 was added directly and stirring was continued for 3 h at room temperature. The crude reaction mixture was purified by column chromatography by using ethyl acetate and petroleum ether (1:9, *v*:*v*) to give the corresponding products (Scheme 1).

3-(1-(2-Chlorophenyl)- 1H-benzo[f]chromen-3-yl)-2H-chromen-2-one (**3a**): Color: White. Yield: 78%. M.p.: 172-173 °C. FT-IR (KBr, ν, cm^{-1}): 1742 (CO), 1645 (cyclic CO), 1592 (C=C), 1056 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 4.84 (d, 1H, CH), 5.78 (d, 1H, CH), 7.19-7.26 (m,3H, Ar-H), 7.48-7.50 (d, 2H, Ar-H), 7.54-7.58 (m, 3H, Ar-H), 7.72-7.74 (d, 2H,Ar-H), 7.80-7.84 (m, 2H, Ar-H), 8.14-8.18 (m, 2H, Ar-H), 8.52 (s, 1H, coumarin-H). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 38.4 (1C, C-4′), 98.4 (1C, C-3′), 117.4 (1C, C-8), 119.3 (1C, C-9), 119.8 (1C, C-7′), 123.4 (1C, C-10′),124.5 (1C, C-5′), 125.1 (1C, C-6), 125.9 (1C, C-4), 127.2 (1C, C-7), 127.6 (1C, C-4″), 128.0 (1C, C-6″), 128.7(1C, C-12′), 129.1 (1C, C-11′), 129.5 (1C, C-5), 129.9(1C, C-9′), 130.4 (1C, C-13′), 130.9 (1C, C-5″), 131.4 (1C, C-14′), 132.0 (1C, C-8′), 132.4 (1C, C-7), 133.8 (1C, C-3), 150.3 (1C, C-1″), 150.8 (1C, C-6′), 151.8 (1C, C-10), 157.4 (1C, -O-C-2′), 159.5 (1C, CO), 160.4 (1C, C-3″). MS (EI, *m/z*): 437 (M+1)$^+$. Anal. calcd. for $C_{28}H_{17}ClO_3$: C, 76.98; H, 3.92. Found: C, 76.92; H, 3.89%.

3-(1-(3-Methoxyphenyl)- 1H-benzo[f]chromen-3-yl)-2H-chromen-2-one (**3b**): Color: White. Yield: 69%. M.p.: 201-203 °C. FT-IR (KBr, ν, cm^{-1}): 1746 (CO), 1648 (cyclic CO),1590 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 3.84 (s, 3H, OCH_3), 5.10 (d, 1H, CH), 6.19 (d, 1H, CH), 6.67-6.76 (m,3H, Ar-H), 7.02-7.05 (d, 4H, Ar-H), 7.41-7.50 (m, 3H, Ar-H), 7.69-7.76 (m, 2H, Ar-H), 7.92-7.94 (d, 2H, Ar-H), 8.63 (s, 1H, coumarin-H). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 42.4 (1C, OCH_3, C-7″), 56.2 (1C, C-4′), 96.2 (1C, C-3′), 113.4 (1C, C-4′), 114.2 (1C, C-2′), 117.2 (1C, C-9), 120.4 (1C, C-5), 121.2 (1C, C-6″), 121.7 (1C, C-5′), 123.5 (1C, C-12′), 124.3 (1C, C-10′), 124.9 (1C, C-7), 126.2 (1C, C-6), 126.9 (1C, C-4), 127.5 (1C, C-9′), 128.4 (1C, C-8′), 129.8 (1C, C-11′), 130.2 (1C, C-3), 131.3 (1C, C-5″), 131.9 (1C, C-14′), 132.4 (1C, C-13′), 144.6 (1C, C-1′), 152.4 (1C, C-6′), 152.9 (1C, C-10), 157.2 (1C, C-2′), 167.2 (1C, -CO, C-2), 171.4 (1C, -C-CH_3, C-3″). MS (EI, *m/z*): 433 (M+1)$^+$. Anal. calcd. for $C_{29}H_{20}O_4$: C, 80.54; H, 4.66. Found: C, 80.48; H, 4.63%.

3-(1-(2, 3-Dichlorophenyl)-1H-benzo[f]chromen-3-yl)-2H-chromen-2-one (**3c**): Color: Grey. Yield: 65%. M.p.: 186-187 °C. FT-IR (KBr, ν, cm^{-1}): 1742 (CO), 1652 (cyclic CO), 1585 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 5.76 (s, 1H, CH), 6.73 (d, 1H, CH), 7.15-7.28 (m, 2H, Ar-H), 7.34-7.39 (m, 3H, Ar-H), 7.43-7.53 (m, 3H, Ar-H), 7.57-7.63 (m, 3H, Ar-H), 7.96-8.00 (d, 2H, Ar-H), 8.58 (s, 1H, coumarin-H). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 41.4 (1C, C-4′), 98.4 (1C, C-3′), 117.5 (1C, C-8), 119.2 (1C, C-9), 119.9 (1C, C-7′), 124.6, (1C, C-10′), 125.3 (1C, C-12′), 125.9 (1C, C-11′), 126.8 (1C, C-6″), 127.6 (1C, C-4″), 128.2 (1C, C-5″), 128.7 (1C, C-9′), 129.3 (1C, C-5), 129.9 (1C, C-14′), 130.4 (1C, C-2′), 130.9 (1C, C-3), 131.8 (1C, C-3′), 132.0 (1C, C-13′), 132.4 (1C, C-3′), 135.2 (1C, C-13″), 153.2 (1C, C-2′), 154.3 (1C, C-1′), 157.9 (1C, C-10), 160.7 (1C, -O-C-2′), 162.8 (1C,-OC,-C-2). MS (EI, *m/z*): 472 (M+1)$^+$. Anal. calcd. for $C_{28}H_{16}Cl_2O_3$: C, 71.35; H, 3.42. Found: C, 71.32; H, 3.39%.

3-(1-(2-Hydroxyphenyl)-1H-benzo[f]chromen-3-yl)-2H-chromen-2-one (**3d**): Color: Brown. Yields: 71%. M.p.: 197-199 °C. FT-IR (KBr, ν, cm^{-1}): 1740 (CO), 1657 (cyclic CO), 1585 (C=C), 3345(OH). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 4.82 (d, 1H, CH), 5.48 (s, 1H, CH), 6.92-7.08 (m, 2H, Ar-H), 7.09-7.10 (m, 2H, Ar-H), 7.35-7.39 (m, 4H, Ar-H), 7.65-7.67 (m, 2H, Ar-H), 7.73-7.75 (m, 2H, Ar-H), 8.20-8.32 (m, 2H, Ar-H), 8.61 (s, 1H, coumarin-H), 9.71 (br, 1H, OH). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 42.2 (1C, C-4′), 98.4 (1C, C-3′), 117.7 (1C, C-8), 119.9 (1C, C-9), 119.9 (1C, C-7′), 120.4 (1C, C-3″), 124.3 (1C, C-1″), 124.6 (1C, C-5′), 124.8 (1C, C-12′), 125.9 (1C, C-10′), 126.8 (1C, C-11′), 127.3 (1C, C-4), 128.4 (1C, C-9′), 128.7 (1C, C-4″), 129.2 (1C, C-6), 129.8 (1C, C-8′), 129.9 (1C, C-5), 130.6 (1C, C-7), 130.9 (1C, C-14′), 131.0 (1C, C-6′), 132.4 (1C, C-13′), 135.2 (1C, C-3), 153.4 (1C, C-10), 154.3 (1C, C-6′), 157.9 (1C, -C-OH, C-2′), 161.4 (1C, -O-C-2′), 162.5 (1C, CO, -C-2). MS (EI, *m/z*): 419 (M+1)$^+$. Anal. calcd. for $C_{28}H_{18}O_4$: C, 80.37; H, 4.34. Found: C, 80.30; H, 4.32%.

3-(1-(Pyridine-2-yl)-1H-benzo[f]chromen-3-yl)-2H-chromen-2-one (**3e**): Color: Grey. Yield: 58%. M.p.: 161-163 °C. FT-IR (KBr, ν, cm^{-1}): 1744 (CO), 1652 (cyclic CO), 1588 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 4.92 (d, 1H, CH), 5.82 (s, 1H, CH), 6.96-7.04 (d, 2H, Ar-H), 7.10-7.14 (d, 2H, Ar-H), 7.37-7.42 (m, 4H, Ar-H), 7.58-7.62 (m, 2H, Ar-H), 7.73-7.76 (m, 2H, Ar-H), 8.20-8.27 (d, 2H, Ar-H), 8.68 (s, 1H, coumarin-H). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 41.2 (1C, C-4′), 98.4 (1C, C-3′), 118.1 (1C, C-8), 119.4 (1C, C-7′), 119.8 (1C, C-9), 121.4 (1C, C-5″), 121.5 (1C, C-5″), 123.2 (1C, C-10′), 124.2 (1C, C-12′), 124.6 (1C, C-6), 126.8 (1C, C-4), 127.4 (1C, C-5), 128.9 (1C, C-3″), 129.0 (1C, C-7), 129.4 (1C, C-14′), 129.9 (1C, C-11″), 130.4 (1C, C-8′), 131.6 (1C, C-13′), 132.5 (1C, C-9′), 134.5 (1C, C-3), 135.2 (1C, C-4″), 149.6 (1C, C-6″), 154.2 (1C, C-6′), 154.8 (1C, C-10), 156.4 (1C, -O-C-2′), 160.2 (1C, C-2″), 164.2 (1C, CO, C-2). MS (EI, *m/z*): 404 (M+1)$^+$. Anal. calcd. for $C_{27}H_{17}NO_3$: C, 80.38; H, 4.25, N, 3.47. Found: C, 80.30; H, 4.21, N, 3.45%.

2.4. Antibacterial activity

The antibacterial susceptibility test was done by determining the zone of inhibition by using disc diffusion method [24]. The substituted 1*H*-benzo[f]chromen-3-yl-2*H*-chromen-2-ones (**3a-e**) was dissolved in dimethyl sulfoxide solvent to make a solution of 120 µM/mL. From this stock solution, serial dilutions have been done to 20, 10, 5, and 1.25 µM/mL with dimethyl sulfoxide in sterile test tubes. Sterilized filter discs were dipped in these solutions and subsequently dried to remove the dimethyl sulfoxide. Nutrient agar medium plates were prepared using Muller-Hinton agar and were allowed to solidify. The three different bacteria like *E. coli*, *S. aureus*, *P. aeruginosa* were selected, and 1 mL of each bacteria and culture broth were added to the plate and spread with the help of a sterile spreader.

Table 1. Zone of inhibition in mm of 1*H*-benzo[f]chromen-3-yl-2*H*-chromen-2-ones, (**3a-e**).

Compound	3a	3b	3c	3d	3e
Escherichia coli	10	14	16	10	08
Staphylococcus aureus	12	16	15	08	10
Pseudomonas aeruginosa	08	15	14	08	10
Norfloxacin standard drug	14	14	14	14	14

Table 2. MIC of 1*H*-benzo[f]chromen-3-yl-2*H*-chromen-2-ones, (**3a-e**).

Compound	3a	3b	3c	3d	3e
Escherichia coli	17	08	10	19	15
Staphylococcus aureus	19	08	06	16	17
Pseudomonas aeruginosa	20	09	08	22	19
Norfloxacin standard drug	14	14	14	14	14

Table 3. Zone of inhibition in mm of 1*H*-benzo[f]chromen-3-yl-2*H*-chromen-2-ones (**3a-e**) against fungi.

Compound	3a	3b	3c	3d	3e
A. niger	09	18	14	10	08
C. albicans	08	20	15	08	10
Fluconazole standard drug	16	16	16	16	16

Table 4. MIC of 1*H*-benzo[f]chromen-3-yl-2*H*-chromen-2-ones (**3a-e**) against fungi.

Compound	3a	3b	3c	3d	3e
A. niger	18	08	10	22	20
C. albicans	20	10	09	20	18
Fluconazole standard drug	12	12	12	12	12

The filter paper discs soaked in solution of 1*H*-benzo[f]chromen-3-yl-2*H*-chromen-2-ones (**3a-e**) derivatives were placed aseptically over the inoculated plates using sterile forceps. The plates were incubated at 37 °C for 24 h with respect to standard drug Norfloxacin. The zone of inhibition was measured.

2.5. Antifungal activity

The antifungal susceptibility test was done by using disc diffusion method. PDA (Potato Dextrose Agar) plates were prepared and the standardized suspension of fungal spores was poured and uniformly spread. All the synthesized compounds 1*H*-benzo[f]chromen-3-yl-2*H*-chromen-2-ones (**3a-e**) were dissolved in dimethyl sulfoxide to make a concentration of 120 µM/mL and serially diluted to different concentrations of 20, 10, and 5 µM/mL. Sterile discs with 150 mm diameter were further sterilized and loaded with synthesized compounds and after drying these discs were stored at 4 °C. The fungi strains such as *C. albicans* and *A. niger* was incubated in PDA (Potato Dextrose Agar) at 25 °C for 5 days with respect to standard drug Fluconozole. The zone of inhibition was measured.

3. Results and discussion

3.1. Chemistry

To develop a new method for the synthesis of substituted 1*H*-benzo[f]chromen-3-yl-2*H*-chromen-2-ones (**3a-e**), experiments were conducted by reacting 3-aryl-1-(3-coumarinyl) propen-1-ones(**1a-e**) with 2-napthol (**2**) catalyzed by DBU and concentrated H_2SO_4 for 5 h in ample yields (Scheme 1). The new molecules (**3a-e**) were confirmed on the basis of IR, ^{1}H NMR, ^{13}C NMR, mass spectral data, and elemental analysis.

3.2. Antibacterial activity

The antibacterial activity of 1*H*-benzo[f]chromen-3-yl-2*H*-chromen-2-ones (**3a-e**) were tested on bacterial resistant's like *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* by micro dilution broth method. The results are tabulated in Table 1 and 2. The zone of inhibition of Norfloxacin was 14 mm while the synthesized molecule **3b** and **3c** shown 14-16 mm against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Derivatives of 1*H*-benzo*[f]*chromen-3-yl-2*H*-chromen-2-one (**3a-e**) exhibited prominent results. The most encouraging results were obtained in the case of compound **3b** and **3c**having MIC value 8-10 µM/mL against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, while norfloxacin as standard, shows MIC of 14 µM/mL.

3.3. Antifungal activity

Antifungal activity of synthesized1*H*-benzo[f]chromen-3-yl-2*H*-chromen-2-ones (**3a-e**) tested against fungi like *Candida albicans* and *Aspergillus niger* using Fluconazole as a standard drug. All the synthesized compounds **3a-e** shows admirable antifungal results against Fluconazole as a standard drug. Among these, compounds **3b** and **3c** shows most encouraging results against *A. niger* and *C. albicans.* The result of zone of inhibition of Fluconazole was 16 mm while the synthesized molecules **3b** and **3c** exhibits 18-20 mm. The results are shown in Table 3 and 4.

4. Conclusions

Using a concise synthetic method, we successfully designed substituted 1*H*-benzo[*f*]chromen-3-yl-2*H*-chromen-2-ones (**3a-e**) scaffolds and obtained by treating 3-aryl-1-(3-coumarinyl)propen-1-ones with 2-napthol in the presence of DBU as catalyst and concentrated H_2SO_4in ample yields. All the synthesized compounds have been evaluated for their *in vitro* antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* and antifungal activity against *Aspergillus niger and Candida albicans, among* those compounds **3b** and **3c** exhibits prominent results.

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References

[1]. Okumura, K.; Ashino, K.; Okuda, T. *J. Pharm. Soc.* **1961**, *81*, 1482-1488.
[2]. Cingolani, G. M.; Gualtieri, F.; Pigini, M. *J. Med. Chem.* **1969**, *12,* 531-532.

[3]. Rao, B. R.; Mouli, G. V. P. C.; Reddy, Y. D. *Indian J. Chem. B* **1983**, *22*, 176-177.
[4]. El-Naggar, A. M.; Ahmed, F. S. M.; Abd El-Salam, A. M.; Haroun, B. M.; Latif, M. S. A. *Int. J. Pept. Prot. Res.* **1982**, *19,* 408-412.
[5]. Moustafa, M. A. A. *Sci. Pharm.* **1991**, *59*, 213-220.
[6]. Alvey, L.; Prado, S.; Huteau, V.; Saint-Joanis, B.; Michel, S.; Koch, M.; Cole, S. T.; Tillequin, F.; Janin, Y. L. *Bioorg. Med. Chem.* **2008**, *16*, 8264-8272.
[7]. Narender, T.; Shweta, Gupta, S. *Bioorg. Med. Chem. Lett.* **2004**, *14,* 3913-3916.
[8]. Mohr, S. J.; Chirigos, M. A.; Fuhrman, F. S.; Pryor, J. W. *Cancer Res.* **1975**, *35*, 3750-3754.
[9]. Tandon, V. K.; Vaish, M.; Jain, S.; Bhakuni, D. S.; Srimal, R. C. *Indian J. Pharm. Sci.* **1991**, *53*, 22-23.
[10]. Brunavs, M.; Dell, C. P.; Gallagher, P. T.; Owton, W. M.; Smith, C. W. *Eur. Pat. Appl.* EP557075 A1 19930825, 1993. Chem. Abstr., 1994, *120,* 106768t.
[11]. Abha, K.; Sarah, J.; Rakesh, T.; Amir, N. S.; Shilpi, G.; Shiv, K.; Keykavous, P.; Sunil, K. S. *Chem. Biol. Int.* **2011**, *1(2),* 279-296.
[12]. Longobardi, M.; Bargagna, A.; Mariani, E.; Schenone, P.; Vitagliano, S.; Stella, L.; Di Sarno, A.; Marmo, E. *Farmaco* **1990**, *45,* 399-404.
[13]. Martinez-Grau, A.; Marco, L. J. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 3165-3170.
[14]. Coudert, P.; Couquelet, J. M.; Bastide, J.; Marion, Y.; Fialip, J. *Ann. Pharm. Fr*. **1988**, *46,* 91-96.
[15]. Eiden, F.; Denk, F. *Arch. Pharm.* (Weinheim, Ger.) **1991**, *324,* 353-354.
[16]. Brühlmann, C.; Ooms, F.; Carrupt, P. A.; Testa, B.; Catto, M.; Leonetti, F.; Altomare, C.; Carotti, A. *J. Med. Chem.* **2001**, *44,* 3195-3198.
[17]. Kesten, S. R.; Heffner, T. G.; Johnson, S. J.; Pugsley, T. A.; Wright, J. L.; Wise, D. L. *J. Med. Chem.* **1999**, *42*, 3718-3725.
[18]. Kostova, I.; Raleva, S.; Genova, P.; Argirova, R. *Bioinorg. Chem . App.* **2006**, *2006,* 1-9.
[19]. Keri, R. S.; Sasidhar, B. S.; Nagaraja, B. M.; Santos, M. A. *Eur. J. Med. Chem.* **2015**, *100*, 257-269.
[20]. Orhan, I. E.; Gulcan, H. O. *Curr. Top. Med. Chem.* **2015**, *15(17),* 1673-1682.
[21]. Fylaktakidou, K. C.; Hadjipavlou-Litina, D. J.; Litinas, K. E.; Nicolaides, D. N. *Curr. Pharm. Des.* **2004**, *10(30)*, 3813-3833.
[22]. Najmanova, I.; Dosede l. M.; Hrdina, R.; Anzenbacher, P.; Filipsky, T.; R ıha, M.; Mladenka, P. *Curr. Top. Med. Chem.* **2015**, *15(9)*, 830-849.
[23]. Prasanna, B.; Jagannatham, Y.; Rateesh, V.; Ramadevi, B. *Int. J. Adv. Res.* **2014**, *2(12),* 125-131.
[24]. Collin CH. Microbiology methods, Butterworth-Heinemann, London. 1964, pp. 92.