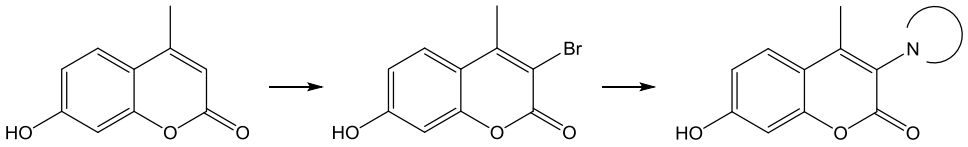
ONE-POT SYNTHESIS OF 3-AMINO-CHROMEN-2-ONES AND THEIR ANTIMICROBIAL STUDY

Tirth Thaker[1*], Sweta Maurya[2], Om Trivedi[3], Bhumika Soni[4], Riya Parmar[5]

Article History: Received: 05.02.2023 **Revised:** 20.03.2023 **Accepted:** 05.05.2023

Abstract

Bromination of 7-Hydroxy-4-methyl-2H-chromen-2-one (3) gives 3-Bromo-7-hydroxy-4-methyl-2H-chromen-2-one (4) which on condensation with different amines, gives 3-Amino analogs of 7-hydroxy-4-methyl-chromen-2-ones (6a-d). These title compounds are confirmed by spectral Analysis as FTIR and NMR. These compounds were subjected to microbial activity against Escherichia coli (gm^{-ve}), Staphylococcus aureus (gm^{+ve}) bacteria, and antifungal activity against Candida sp fungi and showed good results.



Keywords: Antibacterial activity, antifungal activity, bromination, 3-bromo-7-hydroxy-4-methyl-2H-chromen-2-one.

[1*,2,3,4,5]Department of Chemistry, Parul Institute of Applied Sciences, Parul University, Vadodara-391760, Gujarat, India.
***Corresponding author:** Dr. Tirth. H. Thaker**,** Department of Chemistry, Parul Institute of Applied Sciences, Parul University, Waghodia, Vadodara – 391760, Gujarat, India
Mobile Number: 9974030992

Email: [1*]tirth6582@gmail.com

DOI: 10.31838/ecb/2023.12.s3.616

1. Introduction

Novel Amino benzopyrone derivatives possess high anti-viral activity. Methyl amino derivatives inhibited the replication of the H1N1 influenza A virus [1]. Benzopyrone Oxime Ethers shows anti-tubercular activities and gives low toxicity [2]. Novel Thiazolyl benzopyrones inhibit HDAC activity and decrease profibrotic effects on Cardiac fibroblasts [3]. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide gives anti-proliferative activity in stomach cancer which is less toxic comparably other drugs [4]. The In-vitro study of Some Nitro containing benzopyrone derivatives against Renal Carcinoma, Gastric Carcinoma, Colon Carcinoma, Hepatoma, Lymphoblastic cell line and other Cancer cell showed potent cytotoxic and cytostatic effects [5]. Benzopyrone-tethered Isooxazoline derivatives are also effective on Cancerous cells [4-5]. Nitrogen-containing benzopyrones with thiazole ring gives better performance against micro-organisms [6]. It shows that the anti-microbial activity increased with the rising amount of the Coumarin moieties. Derivatives of Amino and Nitrogen containing Coumarins have a good Electrophilic substrate and showed a wide range of antimicrobial activities [7].

Benzopyrones are naturally found in many plants [8]. It may serve as a chemical predator. By inhibiting the synthesis of vitamin K, a related compound is used as the prescription drug Warfarin – an anticoagulant [8-10]. The biosynthesis of coumarin in plants via Hydroxylation, Glycolysis & Cyclization of Cinnamic acid [11-12]. In humans, the enzyme encoded by the gene UGT1A8 has Glucuronidase activity with many substrates, including coumarins [12]. It has different types of family includes, Brodifacoum [13-14], Bromadiolone [15], Difenacoum [16], Auraptene [17], Phenprocoumon (Marcoumar) [17], Scopoletin can be isolated from the bark of Shorea pinanga [17], Warfarin (coumadin) [18]. Microorganisms are also a source of coumarins are Novobiocin and Coumermycin extracted from Streptomyces and Aflatoxins from Aspergillus species [19-20]. Aflatoxins are a very toxic fungus antibiotic [21]. Novobiocin and Coumermycin are the Antibiotics features of 3-amino-4-hydroxy coumarin moiety and substituted of Deoxy sugar [22]. Some of the derivatives are used for Alzheimer's disease treatment due to their ability to inhibit the Acetylcholinesterase (AchE) [23-25]. It may be used to prevent recurrent blood clot formation from atrial fibrillation, thrombotic stroke and transient ischemia attacks [26]. Synthesis of coumarin is from different methods includes: Pechmann condensation, Perkin condensation, Knoevenagal condensation, Witting reaction, Baylis-Hillmann, Claisen & Vilsmeier Haack or Suzuki reaction which is published a review in year 2020 by Loncaric et.al [24]. Some show anticorrosive activity [27]. Some problems concerning the mechanism of the uricosuric action are discussed. The possibility that the uricosuric action of dicoumarol might be ascribed to a metabolite of this drug is mentioned [28]. Coumarin dyes are used as laser dye and their some of the types are active medium in coherent OLED emitters and as a sensitizer in older photovoltaic technologies [29]. Not only in bioactivity but also in their photoreativity. In 1902, Ciamician and Silber investigated the photodimerization of coumarin under UV light exposure (>300), in ethanol, or aqueous solution [30].

2. Result and Discussion

IR, NMR, Mass and elemental studies were used to characterized the target structures of the synthesized compounds. The title

compounds **[6a– 6d]** were prepared according to the method outlined in Figure 1. 7-Hydroxy-4-methyl coumarin was prepared according to the literature [24]. 7-Hydroxy-4-methyl coumarin **[3]** brominated with bromine liquid in distilled water to give 7-hydroxy-4-methyl-3-bromo coumarin **[4].** Condensation of 7-hydroxy-4-methyl-3-bromo coumarin different 2.1 mole aromatic & aliphatic amines to give title compounds **[6a – 6d]** in the presence of solvent DMF as shown in table 1. Substitution at the 3rd position of benzopyrone has never been observed before which has OH group at the 7th position and brotherhood of vinylic ketone with support of electron releasing -OH gp. Makes the -Br position more stable. Aprotic solvent DMF made a high impact to polarize ketone gp, reflux temp. plays a supportive role to substitute the -Br with at 3rd position never observed before.

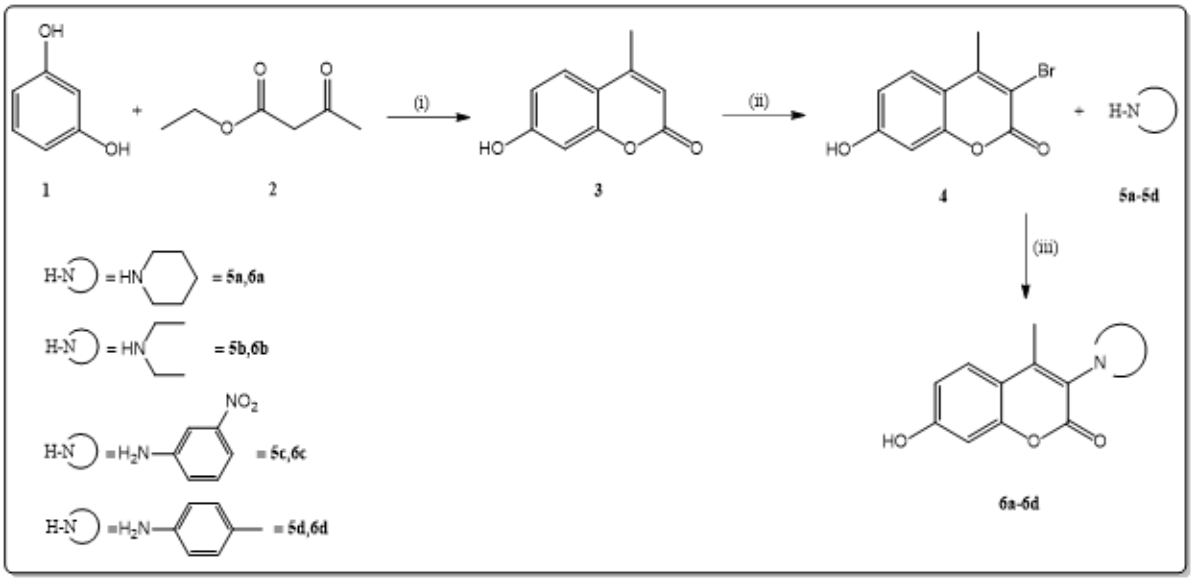


Figure 1: Synthesis of 3-Amino analogs of 7-hydroxy-4-methyl-2H-chromen-2-one derivatives: (i) Conc. H_2SO_4, 24h, room temp (ii) Br_2/H_2O, stir for 15min, reflux 2h (iii) DMF reflux, 3 Hrs.

Pharmacological Screening:

3-bromo-7-hydroxy-4-methyl-chromen-2-one **[4]** and 3-amino-7-hydroxy-4-methyl-chromen-2-one **[6a – 6d]** derivatives were screened for their antibacterial and antifungal activities.

Methodology for Antibacterial and Antifungal screening

3-bromo-7-hydroxy-4-methyl-chromen-2-one **[4]** and 3-amino-7-hydroxy-4-methyl-chromen-2-one **[6a – 6d]** derivatives were tested for their antibacterial activity against E.coli (gm -ve),and Staphylococcus aureus (gm+ve) using corc borer plate method [31] at 100 ppm (10mg/ml) concentration in DMSO(dimethyl sulfoxide) solvent. The synthesized compounds were cultured in nutrient agar containing plates (10mm) and each synthesized compound dissolved in DMSO (Dimethyl sulfoxide) (0.2ml of 10mg/ml) and added into the plates under aseptic condition. Then the dishes were incubated at 37℃ for 24 hrs. Then the zone of inhibition of growth of the bacterial which were produced by diffusion of the compounds from the well into the surrounding medium was measured to evaluate the antimicrobial activity.

3-bromo-7-hydroxy-4-methyl-chromen-2-one **[4]** and 3-amino-7-hydroxy-4-methyl-chromen-2-one **[6a – 6d]** were tested for their antifungal activity against candida sp. using cork borer plate method [31] at 100 ppm (10mg/ml) concentration in DMSO (dimethyl sulfoxide) solvent. Ampicillin was used as standard drug. All compounds showed excellent activity against candida sp. fungus.

All the synthesized compound was tested for their antibacterial and antifungal activities against Escherichia coli (gm^{-ve}), Staphylococcus aureus (gm^{+ve}) and fungi Candida sp. Ampicillin was used as standard drug. All compounds showed excellent activity compare to standard as shown in table.

Table 1. Zone of inhibition of title compounds for Antibacterial & Antifungal Activity

Zone of inhibition in mm					
Compound	Escherichia coli (gm^{-ve})	Enterobacter (gm^{-ve})	Staphylococcus aureus (gm^{+ve})	Bacillus(gm^{+ve})	Candida. sp. fungi
4	11mm	16mm	19mm	20mm	15mm
6a	18mm	20mm	32mm	15mm	13mm
6b	22mm	17mm	33mm	19mm	14mm
6c	29mm	22mm	16mm	15mm	13mm
6d	19mm	15mm	13mm	12mm	14mm
Control **(Ampicillin)**	11mm	10mm	10mm	10mm	11mm

3. Conclusion

7-hydroxy-4-methyl-3-amino coumarins **[6a -6d]** are synthesized by condensation of 3-Bromo-7-hydroxy-4-methyl-chromen-2-one **[4]** with different amines in DMF. Synthesis of title compounds confirmed by IR & ^{1}H NMR. So, the important conclusion is the removal of any halogen to any organic compound results in diminished values of halogen region that is 800-400 Cm-1 and shrank in values and shrunk values of other functional gps. frequencies. ^{1}H NMR of the synthesized compounds shows the peak of –NH proton which means the replacement of –Br with amines is confirmed. Synthesized 3-amino analogs possess good results at biological screening also. The process is novel as the substitution at the 3rd position has never been observed before in coumarin moiety which has a 7th position unprotected -OH proton due to the brotherhood of vinylic ketone with support of electron releasing -OH gp. which makes the -Br position more stable. Aprotic solvent DMF made a high impact to polarize ketone gp. Moreover, reflux temperature plays a supportive role to substitute the -Br with amines. Title amino coumarins were screened for antimicrobial activity against Escherichia coli (gm^{-ve}), Enterobacter (gm^{-ve}), Staphylococcus aureus (gm^{+ve}), Bacillus(gm^{+ve}), bacteria and Candida sp. Fungi and synthesized compounds **4** and **[6a – 6d]** showed better antimicrobial activity compare to standard (Ampicillin).

Experimental section:

The melting points were determined in scientific open capillaries and are uncorrected. The IR spectra were determined as KBr pellets on a Schimadzu model IR-408 spectrophotometer. The ^{1}H-NMR spectra were recorded using Bruker DRX 400 MHz in CDCl3 or DMSO-d6 with tetramethylsilane as the internal standard. Mass spectra were recorded on a GC–MS spectrometer. Elemental analyses were performed on Carlo Erba-1108

elemental analyzer. Acme's Silica gel (mesh size 60–120) was used for column chromatography.

7-hydroxy-4-methyl-2H-chromen-2-one [3]:

In order to synthesize coumarin derivatives, a simple protocol using Pechmann condensation of Resorcinol **[1]** with Ethyl acetoacetate **[2]** to synthesis is 7-hydroxy-4-methyl-chromen-2-one [24].

Yield 91.19 %, (Lit [24]. M.P. 141-143 °C) IR (KBr) cm^{-1}: 3502.88, 2897.32, 1755.03, 1671.92, 16.07.27, 1502.76, 1371.90, 1226.19. ^1H NMR (DMSO-d_6) ppm^{-1}: δ 2.36 (s, 3H, C_4-CH_3), 6.15 (s, 1H, C_3-H), 6.50 (s, 1H, (C_8-H), 6.72 (d, 1H C_6-H),7.42 (d, 1H, C_5-H), 10.23 (s, 1H, C_7-OH). Mass (m/z): 176 M^+ (100%). Elemental Analysis for $C_{10}H_8O_3$: C, 68.08; H, 4.52; O, 27.19.

3-bromo-7-hydroxy-4methyl-2H-chromen-2-one [4]:

7-hydroxy-4-methyl-chromen-2-one **[3]** (2gm, 0.0113 mole) and 20ml distilled water kept for 15min under stirring then started addition of pure Bromine (4.148gm, 0.0226mole) at room temperature then slowly raised the temperature 55℃ to 60℃ up to 2hr. Charged in ice-cold water, the light creamy-colored compound was filtered and recrystallized by petroleum ether.

Yield 92.15%, M.P. 225-228ºC IR (KBr) cm^{-1}: 3303.88, 2948.32, 1756.19, 1631.70, 1590.27, 1502.82, 1353.61, 1196.01, 666.09, 760.45, 848.33. ^1H NMR (DMSO-d_6) ppm^{-1}: δ 2.40 (s, 3H, C_4-CH_3), 6.50 (s, 1H, C_8-H), 7.42 (d, 1H, C_5-H), 6.72 (d, 1H, C_6-H), 10.23 (s, 1H, C_7-OH). Mass (m/z): 253 M^+ (100%). Elemental Analysis for $C_{10}H_7BrO_3$: C, 47.19; H, 2.77; Br, 31.30; O, 18.76.

General procedure for 7-hydroxy-4-mehtyl-3-(amino)-2H-chromen-2-one [6a-d]:

3-bromo-7-hydroxy-4-methyl-2H-chromen-2-one **[4]** (1gm, 0.00392 mole), amines **[5a – 5d]** (0.0082 mole) and 15 ml DMF added in a 100 ml RBF. The reaction mixture refluxed for 3 Hr. Then pour it in crushed ice and filter out the precipitate and recrystallized it by petroleum ether: ethyl acetate by column chromatography to give title compounds **[6a – 6d]**.

7-hydroxy-4-methy-3-(piperidin-1-yl)-2H-chromen2-one [6a]:

Yield 70.82%, M.P. 118ºC IR (KBr) cm^{-1}: 3589, 2879.02, 3011.21, 2878.02, 1712.07, 1645.42, 1498.5, 1352.75, 1165.92. ^1H NMR (DMSO-d_6) ppm^{-1} : δ 2.46 (s, 3H, C_4-CH_3), 7.75 (d, 1H, C_5-H), 7.72 (d, 1H, C_6-H), 8.42 (s, 1H, C_8-H), 8.58 (s, 1H, C_7-OH), 3.04-3.02 (t, 2H, C_2, C_6-CH_2), 1.65 (m, 2H, C_3, C_5-CH_2), 1.56-1.55 (m, 2H, C_4-CH_2). Mass (m/z): 259 M^+ (100%). Elemental Analysis for $C_{15}H_{17}NO_3$: C, 69.38; H, 6.65; N, 5.46; O, 18.54.

3-(diethylamino)-7-hydroxy-4-methyl-chromen-2-one [6b]:

Yield 68.04%, M.P. 101ºC, IR (KBr) cm^{-1}: 3700, 2878.99, 3011.64, 1723.87, 1645.12, 1607.20, 1498.22, 1349.73, 1059.75. ^1H NMR (DMSO-d_6) ppm^{-1} : δ 2.42 (s, 3H, C_4-CH_3), 7.54 (d, 1H, C_5-H), 7.76 (d, 1H, C_6-H), 6.62 (s, 1H, C_8-H), 10.30 (s, 1H, C_7-OH), 3.22 (m, 2H, C_2, C_4-CH_2), 1.15 (t, 3H, C_1, C_5-CH_3). Mass (m/z): 247 M^+ (100%). Elemental Analysis for $C_{14}H_{17}NO_3$: C, 68.00; H, 6.93; N, 5.66; O, 19.41.

7-hydroxy-4-methyl-3-((3-nitrophenyl)amino)-2H-chromen-2-one [6c]:

Yield 86.06%, M.P. 166ºC, IR (KBr) cm^{-1}: 3400, 2870.93, 3188, 1707.97, 1631.70, 1596.33, 1516.89, 1308.61, 1164.96. ^1H NMR (DMSO-d_6) ppm^{-1} : δ 2.57 (s, 3H, C_4-CH_3), 7.74 (d, 1H, C_5-H), 7.23 (d, 1H, C_6-H), 7.01 (s, 1H, C_8-H), 11.17 (s, 1H, C_7-OH), 11.51 (s, 1H, C_3-NH),

8.07 (s, 1H, C_2-H), 6.87 (d, 1H, C_4-H), 7.72 (t, 1H, C_5-H), 6.99 (d, 1H, C_6-H). Mass (m/z): 312 M^+ (100%). Elemental Analysis for $C_{16}H_{12}N_2O_5$: C, 61.54; H, 3.87; N, 8.97; O, 25.62.

7-hydroxy-4-methyl-3-(p-tolylamino)-2H-chromen-2-one [6d]:
Yield 79.09%, M.P.127ºC, IR (KBr) cm^{-1}: 3500, 2878.27, 3012.52, 1727.40, 1645.59, 1587.43, 1499.03, 1351.27, 1063.81. ^{1}H NMR (DMSO-d_6) ppm^{-1} : δ 2.52 (s, 3H, C_4-CH_3), 7.64 (d, 1H, C_5-H), 6.73 (d, 1H, C_6-H), 7.05 (s, 1H, C_8-H), 10.48 (s, 1H, C_7-OH), 10.64 (s, 1H, C_3-NH), 6.65 (d, 1H, C_2, C_6-H), 7.05 (d, 1H, C_3, C_5-H), 2.36 (s, 3H, C_4-CH_3). Mass (m/z): 281 M^+ (100%). Elemental Analysis for $C_{17}H_{15}NO_3$: C, 72.58; H, 5.37; N, 4.98; O, 17.06.

Acknowledgements
The authors are thankful to Dr. Devanshu Patel, President of Parul University for providing the necessary facilities. The authors are thankful to Dr. Anupama Shrivastav, Asst. Professor, Dept. of Life Sciences, Parul Institute of Applied Sciences for valuable help in biological activity.

4. Reference

1. H. Osman, S.K. Yusufzai, M.S. Khan, B.M. Abd Razik, O. Sulaiman, S. Mohamad, J.A. Gansau, M.O. Ezzat, T. Parumasivam, M.Z. Hassan. Journal of Molecular Structure., 2018, 1166, 147-154.
2. D.S. Reddy, M. Kongot, S.P. Netalkar, M.M. Kurjogi, R. Kumar, F. Avecilla, A. Kumar. European Journal of Medicinal Chemistry., 2018, 150, 864-875.
3. Viviana Pardo-Jiménez, Patricio Navarrete-Encina, Guillermo Díaz-Araya. MDPI., 2019, 24, 739.
4. Haribalan Perumalsamy, Karuppasamy Sankarapandian, Karpagam Veerapan, Sathishkumar Natarajan, Narendran Kandaswamy , Lakshmi Thangavelu , Sri Renukadevi Balsam. Phytomedicine., 2018, 46, 119-130.
5. Gejjalagere S. Lingarajua, Kyathegowdanadoddi S. Balajib, Shankar Jayaramab, Seegehalli M. Anila, Kuppalli R. Kirana, Maralinganadoddi P. Sadashivaa. Bioorganic & Medicinal Chemistry letters., 2018, 28, 3606-3612.
6. McFadden, P.D.; Frederick, K.; Argüello, L.A.; Zhang, Y.; Vandiver, P.; Odegaard, N.; Loy, D.A. ACS Appl. Mater. Interfaces., 2017, 9, 10061–10068.
7. Biljana R. Dekić, Niko S. Radulović, Vidoslav S. Dekić, Rastko D. Vukićević 3 and Radosav M. Palić. Molecules., 2010, 15, 2246-2256.
8. Boisde PM, Meuly WC. NY: Wiley Interscience., 1993, 7, 647-58.
9. Jan Koch-Weser, Edward M. Sellers. N Engl J Med., 1971, 285, 547-558.
10. Yan-Hong Wang, Bharathi Avula, N. P. Dhammika Nanayakkara, Jianping Zhao, and Ikhlas A. Khan. Journal of Agricultural and Food Chemistry., 2013, 61, 4470–4476.
11. Link K P. Circulation., 1959, 19, 97–107.
12. Joseph K. Ritter, Fan Chen, Yhun Y. Sheen, Huu M. Tran, Shioko KimuraO, Matthew T. Yeatman, and Ida S. Owensq. J. Biol. Chem., 1992, 267, 3257–3261.
13. K. G. Koubek et al. J. Assoc. Off. Anal. Chem., 1979, 62(6), 1297.
14. Michael Laposata, M.D, Elizabeth M. Van Cott, Michael H. Lev. New England Journal of Medicine., 2007, 356, 174–82.
15. Hunter K. J Chromatog., 1985, 321(2), 255-72.
16. Widdershoven J, van Munster P, De Abreu R, Bosman H, van Lith Th, vander Putten-van Meyel M,

Motohara K, & Matsuda I. Clin Chem., 1987, 33(11), 2074-2078.

17. Yana M. Syah, Euis H. Hakim, Emilio L. Ghisalberti, Afghani Jayuska, Didin Mujahidin, Sjamsul A. Achmad. Natural Product Research., 2009, 23, 591–594.
18. Venugopala, K. N.; Rashmi, V; Odhav, B. BioMed Research International., 2013, 10, 1155.
19. Cooke, D.; Fitzpatrick, B.; O'Kennedy, R.; Mc Cormack, T.; Egan, D. John Wiley & Sons: Hoboken; NJ, USA, 1997
20. Cooke, D. Ph.D. Thesis, Dublin City University, Dublin, Ireland, 1999.
21. O'Kennedy, R.; Thornes, R.D. Coumarins: Wiley: Hoboken, NJ, USA., 1997; ISBN 978-0-471-96997-6.
22. Huawei, C.; Walsh, C.T. Chem. Biol., 2001, 8, 301–312.
23. Katsori, A.M.; Hadjipavlou-Litina, D. Expert Opin. Pat., 2014, 24, 1323–1347.
24. Lonˇcari´c, M.; Gašo-Sokaˇc, D.; Joki´c, S, Molnar, M. Biomolecules., 2020, 10, 151.
25. Stefanachi, A.; Leonetti, F.; Pisani, L.; Catto, M.; Carotti, A. Molecules., 2018, 23, 250.
26. Leal, L. K. A. M.; Ferreira, A. A. G.; Bezerra, G. A.; Matos, F. J. A.; Viana, G. S. B., 2000, 70, 151–159.
27. Xiaoyun Liu, Ruhong Zhang, Tianquan Li, Pengfei Zhu, and Qixin Zhuang. ACS Sustainable Chemistry & Engineering., 2017, 5 (11), 10682-10692. DOI:
28. Flemming Christen., Acta Medica Scandinavica. Vol. 175, fasc. 4, 1964
29. F. J. Duarte, L. S. Liao, K. M. Vaeth., Optics Letters., November 15, 2005, 22.
30. Yun Chen, Cheng-Fu Chou., Journal of Polymer Science: Part A Polymer Chemistry., (1995), 33, 2705-2714.
31. Pati, U.S. & Kurade, N.P., Antibacterial Screening method for evaluation of natural products, 2012.