Asian Journal of Chemistry; Vol. 30, No. 1 (2018), 126-128

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2018.20933

Synthesis and Antibacterial Study of Thymol Derivatives

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Received: 22 July 2017; *Accepted*: 17 October 2017; *Published online*: 30 November 2017; AJC-18663

Five thymol derivatives (**2-6**) were synthesized and evaluated for their antibacterial activity against four bacterial strains *i.e.*, *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Escherichia coli* using standard well-diffusion method.

Keywords: Thymol derivatives, Antibacterial activity, Well-diffusion method.

INTRODUCTION

Thymol (2-isopropyl-5-methylphenol) (**1**) is naturally occurring phenolic monoterpene derivative of cymene, which is found in essential oils extracted from plants belonging to the Lamiaceae family [1]. Since 16th century, thymol-rich essential oils have been evaluated for their benefits in medicinal application [2,3] as well as for their antimicrobial properties [1,4]. Thymol (**1**) itself exhibits a large number of biological activities, such as antibacterial [5], antileishmanial [6], anti-inflammatory [7], antitumor [8] and aedes aegypti larvicidal [9] properties. Thymol also exhibited insecticidal and genotoxic activities on *Drosophila melanogaster* [10].

In continuation of our interest in searching for potential antibacterial compounds derived from natural products [11], herein we report the synthesis, characterization and antibacterial evaluation of thymol esters and ethers (**2-6**) using well-diffusion method.

EXPERIMENTAL

Thymol is commercially available and purchased from Sigma-Aldrich. All of other reagents were obtained from Sigma-Aldrich, Merck or Acros Organics and used without additional purification. All the reactions were performed under nitrogen atmosphere. The reactions were monitored by thin layer chromatography (TLC) using plastic precoated sheets (Silica gel 60 F_{254}, 0.25 mm thick). Plates were visualized under UV 365 nm and UV 254 nm without treatment. Column chromatography was performed on silica gel 60 (230-400 mesh, Merck). NMR data were recorded in $CDCl_3$ on Bruker FT-400 (400 MHz) or Jeol (500 MHz) Spectrometer. ^1H and ^{13}C NMR chemical shifts (δ) are given in ppm. Infrared spectra were recorded in KBr disc on Perkin Elmer 100 FT-IR Spectrometer. UV-visible spectra were recorded on Shimadzu UV-1601PC Spectrophotometer. HREIMS were recorded on LTQ Orbitrap mass spectrometer (Thermo Scientific).

General method for the synthesis of thymol ethers (2,3): Thymol ethers were synthesized according to the procedure described previously [11]. K_2CO_3 (9.99 mmol) was added to a solution of thymol (6.66 mmol) and benzyl halide (9.99 mmol) in acetone (7 mL) under an inert atmosphere. The mixture was refluxed upon completion *via* TLC monitoring. After the completion, the reaction crude was diluted with 30 mL of distilled water and then extracted with CH_2Cl_2 (3 × 30 mL). The organic extracts were dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography (hexane:chloroform) to give compounds (**2,3**).

General method for the synthesis of thymol esters (4-6): Triethylamine (5 mL) was added to a solution of thymol (6.60 mmol) in dichloromethane (15 mL). The mixture was stirred for 0.5 h at 0 ºC. Then, an excess of acyl chloride (26.62 mmol) was added dropwise. The solution was stirred for another 30 min at 0 ºC before it slowly warmed to room temperature and continued stirring for another 24 h. The progress of the reaction was monitored by thin layer chromatography (TLC). After the completion of reaction, the solvent was removed *via in vacuo*. The residue was purified by column chromatography (hexane: chloroform) to yield compounds **4-6** in good yields.

2-(Benzyloxy)-1-isopropyl-4-methylbenzene (2) [12]**:** Yield: 84.71 %; IR (KBr, ν_{max}, cm^{-1}): 3032, 2960, 1612, 1455, 1256; UV (MeOH) λ_{max} (log ε) 281.0 (3.5), 274.5 (3.5) nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (d *J* = 7.2 Hz, 6H, $2CH_3$, H-6), 2.31 (s, 3H, CH_3, H-1), 3.33-3.40 (m, 1H, H-5), 5.06 (s, 2H, CH_2, H-7), 6.77 (d, *J* = 8.0 Hz, 1H, CH_{ar}), 6.74 (s, 1H, CH_{ar}), 7.13 (d, *J* = 7.6 Hz, 1H, CH_{ar}), 7.31-7.46 (m, 5H,CH_{ar}); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.3, 22.8, 26.6, 9.9, 112.6, 121.4, 125.9, 127.1, 127.6, 128.5, 134.0, 136.3, 137.6, 155.8 ppm. EIMS $[M]^+$ *m/z* = 240.

2-Isopropyl-4-methyl-2-[(4-nitrobenzyl)oxy]benzene (3): Yield: 84.59 %; IR (KBr, ν_{max}, cm^{-1}): 3071, 2962, 1606, 1517, 1453, 1342, 1262; UV (MeOH) λ_{max} (log ε) 269.5 (4.0), 211.5 (4.2) nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25 (d *J* = 6.8 Hz, 6H, $2CH_3$, H-6), 2.31 (s, 3H, CH_3, H-1), 3.33-3.40 (m, 1H, H-5), 5.17 (s, 2H, CH_2, H-7), 6.68 (s, 1H, CH_{ar}) 6.81 (d, *J* = 76 Hz, 1H, CH_{ar}), 7.16 (d, *J* = 7.6 Hz, 1H, CH_{ar}), 7.63 (d, *J* = 8.8 Hz, 2H, CH_{ar}), 8.27 (d, *J* = 8.8 Hz, 2H, CH_{ar}) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.3, 22.8, 26.6, 68.7, 112.5, 122.0, 123.8, 126.2, 127.3, 134.3, 126.5, 145.1, 147.4, 155.1 ppm. EIMS $[M]^+$ *m/z* = 285.

2-Isopropyl-5-methylphenyl 4-chlorobenzoate (4): Yield: 66.44 %; IR (KBr, ν_{max}, cm^{-1}): 3029, 2963, 1738, 1594, 1487, 123, 753; UV (MeOH) λ_{max} (log ε) 242.5 (4.4) nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ1.21 (d *J* = 7.2 Hz, 6H, $2CH_3$, H-6), 2.34 (s, 3H, CH_3, H-1), 2.98-3.05 (m, 1H, H-5), 6.93 (s, 1H, CH_{ar}), 7.08 (d, *J* = 7.6 Hz, 1H, CH_{ar}), 7.25 (d, *J* = 7.2 Hz, 1H, CH_{ar}), 7.50 (d, *J* = 8.4 Hz, 2H, CH_{ar}), 8.16 (d, *J* = 8.4 Hz, 2H, CH_{ar}) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.8, 23.0, 27.3, 122.7, 126.5, 127.3, 128.0, 129.0, 131.5 136.7, 140.1, 147.9, 164.5 ppm. EIMS $[M]^+$ *m/z* = 288.

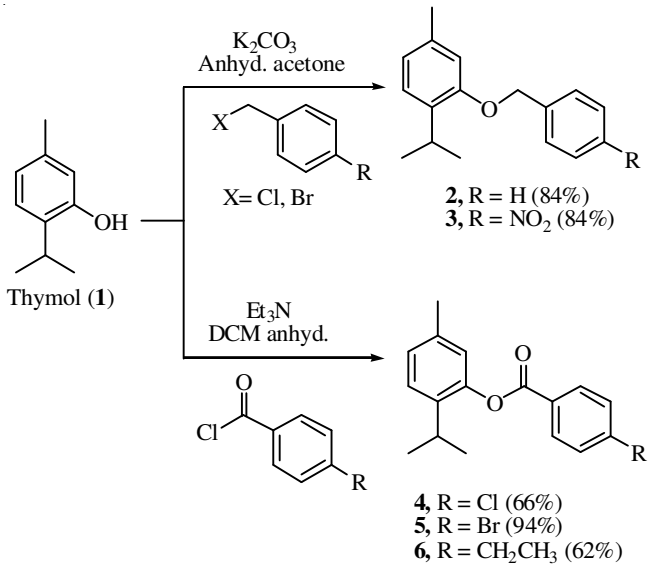
2-Isopropyl-5-methylphenyl 4-bromobenzoate (5): Yield: 94.21 %; IR (KBr, ν_{max}, cm^{-1}): 3029, 2962, 1743, 1590, 1485, 1264; UV (MeOH) λ_{max} (log ε) 247.0 (4.2) nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.21 (d *J* = 7.2 Hz, 6H, $2CH_3$, H-6), 2.34 (s, 3H, CH_3, H-1), 2.97-3.04 (m, 1H, H-5), 6.92 (s, 1H, CH_{ar}), 7.08 (d, *J* = 7.6 Hz, 1H, CH_{ar}), 7.25 (d, *J* = 8.0 Hz, 1H, CH_{ar}), 7.67 (d, *J* = 8.4 Hz, 2H, CH_{ar}), 8.08 (d, *J* = 8.7 Hz, 2H, CH_{ar}) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.8, 23.0, 27.3, 122.7, 126.5, 127.3, 128.5, 128.8, 131.6, 132.0, 136.7, 137.0, 147.9, 164.7 ppm. EIMS $[M]^+$ *m/z* = 332.

2-Isopropyl-5-methylphenyl 4-ethylbenzoate (6): Yield: 62.35 %; IR (KBr, ν_{max}, cm^{-1}): 3032, 2965, 1737, 1611, 1456, 1237; UV (MeOH) λ_{max} (log ε) 240.5 (4.2) nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.21 (d *J* = 6.8 Hz, 6H, $2CH_3$, H-6), 1.29 (t, *J* = 7.6 Hz, 3H, CH_3, H-10), 2.34 (s, 3H, CH_3, H-1), 2.72-2.77 (m, 2H, CH_2, H-9), 3.00-3.10 (m, 1H, H-5), 6.93 (s, 1H, CH_{ar}), 7.06 (d, *J* = 8.0 Hz, 1H, CH_{ar}), 7.24 (d, *J* = 8.4 Hz, 1H, CH_{ar}), 7.35 (d, *J* = 8.4 Hz, 2H, CH_{ar}), 8.14 (d, *J* = 8.0 Hz, 2H, CH_{ar}) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.2, 20.8, 23.0, 27.3, 29.0, 122.9, 126.4, 127.6, 127.9, 128.1, 130.3, 136.6, 137.2, 148.2, 150.5, 165.4 ppm. EIMS $[M]^+$ *m/z* = 282.

Antibacterial assay: All the synthesized compounds were evaluated for their antibacterial activity against four bacterial strains of Gram-positive and Gram-negative bacteria; three Gram-positive bacteria (*Bacillus subtilis* ATCC11774, *Staphylococcus aureus* ATCC25923 and *Staphylococcus epidermidis* ATCC13518) and one Gram-negative bacterium (*Escherichia coli* ATCC11775) using standard well-diffusion method. The Mueller-Hinton agar (MHA) plates were inoculated with cultured bacterial strains using cotton swab. By using sterile cork borer, wells of 6.0 mm diameter were cut on the media and loaded with 60 µL of diluted compounds. 1 mg/mL of synthesized compounds were prepared in methanol. Streptomycin (Abtek Biologicals Ltd.) was used as positive control and methanol as negative control. All plates were incubated at 37 ºC for overnight before evaluating the antibacterial activity by measuring the diameter of inhibition zones against bacteria.

RESULTS AND DISCUSSION

The synthetic route for thymol derivatives **2-6** is illustrated in **Scheme-I**. By employing the previous published method [11], thymol ethers (**2,3**) have been synthesized by the reaction of thymol (**1**) with benzyl halide in the presence of K_2CO_3. Whilst, the treatment of thymol (**1**) with acyl chloride in CH_2Cl_2 in the presence of Et_3N [11] furnished thymol esters (**4-6**) in 66-94% yields.



Scheme-I: Synthesis of derivatives (**2-6**)

All the derivatives (**2-6**) were screened for their antibacterial activity [11-13]. Unfortunately, when compared with standard drug as streptomycin, all the derivatives (**2-6**) do not show any antibacterial activity against all four types of bacteria.

Conclusion

In the present work, five thymol derivatives were synthesized and characterized by spectral studies. All the synthesized compounds were evaluated for their antibacterial activities against *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Escherichia coli* using standard well-diffusion method. However, all the derivatives (**2-6**) did not show any antibacterial activity against all the tested strains.

ACKNOWLEDGEMENTS

The authors acknowledge Ministry of Higher Education Malaysia for financial support (RACE - 50068).

REFERENCES

1. A. Marchese, I.E. Orhan, M. Daglia, R. Barbieri, A. Di Lorenzo, S.F. Nabavi, O. Gortzi, M. Izadi and S.M. Nabavi, *Food Chem.*, **210**, 402 (2016); https://doi.org/10.1016/j.foodchem.2016.04.111.
2. A.A.E. Ibrahim, *Am. J. Biol. Life Sci.*, **2**, 154 (2014).
3. A. Özkan and A. Erdogan, *Turk. J. Biol.*, **35**, 735 (2011) https://doi.org/10.3906/biy-1011-170.
4. M. Boskovic, N. Zdravkovic, J. Ivanovic, J. Janjic, J. Djordjevic, M. Starcevic and M.Z. Baltic, *Procedia Food Sci.*, **5**, 18 (2015); https://doi.org/10.1016/j.profoo.2015.09.005.
5. N. Didry, L. Dubreuil and M. Pinkas, *Pharm. Acta Helv.*, **69**, 25 (1994); https://doi.org/10.1016/0031-6865(94)90027-2.
6. S.M. de Morais, N.S. Vila-Nova, C.M.L. Bevilaqua, F.C. Rondon, C.H. Lobo, A. de Alencar Araripe Noronha Moura, A.D. Sales, A.P.R. Rodrigues, J.R. de Figuereido, C.C. Campello, M.E. Wilson and H.F. de Andrade Jr., *Bioorg. Med. Chem.*, **22**, 6250 (2014); https://doi.org/10.1016/j.bmc.2014.08.020.
7. K.R. Riella, R.R. Marinho, J.S. Santos, R.N. Pereira-Filho, J.C. Cardoso, R.L.C. Albuquerque-Junior and S.M. Thomazzi, *J. Ethnopharmacol.*, **143**, 656 (2012); https://doi.org/10.1016/j.jep.2012.07.028.
8. A. Anderson, *Int. J. Toxicol.*, **25**, 29 (2006); https://doi.org/10.1080/10915810600716653.
9. V.B. Silva, D.L. Travassos, A. Nepel, A. Barison, E.V. Costa, L. Scotti, M.T. Scotti, R.J.B. Mendonca-Junior, R.C. Santos and S.C. Cavalcanti, *J. Arthropod Borne Dis.*, **11**, 315 (2017);
10. I. Karpouhtsis, E. Pardali, E. Feggou, S. Kokkini, Z.G. Scouras and P. Mavragani-Tsipidou, *J. Agric. Food Chem.*, **46**, 1111 (1998); https://doi.org/10.1021/jf970822o.
11. N.H.C. Abdul Rahim, A. Asari, N. Ismail and H. Osman, *Asian J. Chem.*, **29**, 22 (2017); https://doi.org/10.14233/ajchem.2017.20100.
12. P.P. Kumbhar and P.M. Dewang, *J. Sci. Ind. Res. (India)*, **60**, 645 (2001).
13. N. Raman, S. Thalamuthu, J.D. Raja, M.A. Neelakandan and S. Banerjee, *J. Chil. Chem. Soc.*, **53**, 1450 (2008); https://doi.org/10.4067/S0717-97072008000100025.