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Synthesis and Antibacterial Activity of Thymyl Ethers †

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Abstract: In this paper, we report herein a simple and efficient synthesis method of thymyl ethers for structural modifications of natural products such as monoterpenoids and studies of ether derivatives of thymol in biological importance. Our investigations showed that thymol reacts very smoothly with different types of substituted acetanilides. The synthesized compounds were tested for their bacterial potency against four bacterial species. Such a structural modification will be beneficial to designing active molecules for pest management.

Keywords: thymol; monoterpenoids; green chemistry; microwave irradiation and antibacterial activity

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1. Introduction

Thymol is an important phenolic monoterpenoid obtained from *Thymus Vulgare*. It exerts a cool influence on muscle. Like phenol, it does not irritate the skin and may be taken internally. It is twenty times more antiseptic than phenol. Thymol resembles phenols in chemical properties, but its hydroxyl groups are more reactive than phenol [1,2]. Thymol is effective against Gram-positive, Gram-negative bacteria, fungi, and Candida albicans yeast [3–8]. Thymus stimulates the appetite, aids in sluggish digestion, and improves liver function.

Structural modifications of phenolic monoterpenoids were obtained by reacting thymol with various substituted α-chloro acetanilidesto improve biological activities, which give a product with better yield and higher purity under mild reaction conditions with the help of microwave irradiation techniques [9,10].

We report herein a rapid, simple, and efficient method for synthesizing thymyl ethers that could be useful to introduce new groups of pest-management agents through the bio-rational design of the derivatives.

2. Materials and Methods

Various aromatic amines (aniline, *p*–toludine, *m*–nitro aniline, *m*–chloro aniline, *m*, *p*-dichloro aniline and α-napthyl amine), chloro acetyl chloride, thymol, potassium carbonate, sodium hydroxide, and solvents were of analytical grade (s. d. fine chemicals, Qualigens, etc.) and distilled before use.

Melting points were determined using open capillary method in the paraffin liquid. I. R. spectra (cm^{-1}) were recorded on a Perkin Elmer RX1 FTIR spectrophotometer (PerkinElmer, Waltham, MA, USA). ^{1}H NMR spectra were recorded on a Bruker DRX-300 MHz: FT NMR spectrometer (chemical shift inδ, ppm; Bruker, Billerica, MA, USA). MS were recorded on a Jeol SX 102/Da mass spectrometer (Jeol, AKISHIMA, Japan), and elemental analyses were performed on a Perkin-Elmer Series II CHNS analyzer 2400 (PerkinElmer, Waltham, MA, USA). A Samsung (Model No. 9 OM 9925 E) domestic Microwave oven

(2450 MHz, 800 W, Samsung, Suwon Si, Korea) was used for all experiments. The purity of compounds was checked by TLC.

3. Experimental Section

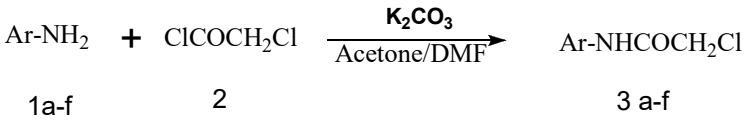
3.1. Synthesis of N-chloro Acetyl Aryl Amines (α-Chloro Acetanilides)

We added potassium carbonate (5.87 mg, 0.0425 mole) in substituted anilines **1a–f** (4 g, 0.0425 moles), which was dissolved in 30 mL solvent, Acetone: DMF (9:1), then added (dropwise) chloro acetyl chloride **(2)** (4.765 mg, 0.0425 moles) with constant stirring. The reaction temperature (0–5 °C) was maintained by ice–salt mixture and refluxed for 2–3 h. The progress of reaction was monitored by TLC system (Pet. Ether:$CHCl_3$, 8:2) Then, we poured the reaction mixture into cold water to obtain the product. The product was filtered, dried, and recrystallized in ethanol solvent. Physical data of N-chloro acetyl aryl amines **3a–f** are given in Table 1. The N-chloro acetyl aryl amines **3a–f** were identified by comparing their spectral data with reported values in the literature [11–14] or their melting points (Scheme 1).

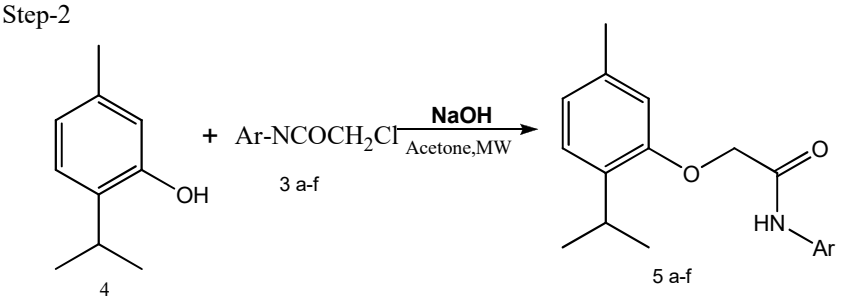
Table 1. Characterization data of the compounds **3a–f**.

Compounds	Ar	Molecular Formula	M.P. (°C)	Reaction Time (h)	Yield (%)
3a	$-C_6H_5$	C_8H_8NOCl	87–91	2.0	88
3b	$-p-CH_3C_6H_4$	$C_9H_{10}NOCl$	163–93	3.0	91
3c	$-m-NO_2C_6H_4$	$C_8H_7N_2O_3Cl$	90–93	2.0	82
3d	$-m-ClC_6H_4$	$C_8H_7NOCl_2$	87–92	2.5	86
3e	$-m,p-ClC_6H_3$	$C_8H_6NOCl_3$	97–101	2.0	84
3f	$-C_{10}H_7$	$C_{12}H_{10}NOCl$	155–157	3.0	80

Step-1



Step-2



Where Ar is

a) $-C_6H_5$ b) $-p-CH_3-C_6H_4$ c) $-m-NO_2\,C_6H_4$ d) $-m-Cl-C_6H_4$ e) $-m,p-Cl-C_6H_4$ f) $C_{10}H_7$

Scheme 1. Synthesis of α-Chloro Acetanilides and Thymyl Ethers.

3.2. Synthesis of Thymyl Ethers Using Microwave Method

In synthesis of thymyl ethers by conventional methods, e.g., compounds **5a–f** (Table 2), practical yield is lower, more time is required, the isolation procedure is difficult, and product obtained requires purification by either column chromatography or TLC. Due to these problems, we synthesized same compounds using microwave irradiation technique.

Table 2. Characterization data of the compounds **5a–f**.

Compounds [a]	Ar	Molecular Formula	M.P. (°C)	Reaction Time		Yields [b]	
				Conventional (h)	M W (min)	Conventional (%)	MW (%)
5a	-C_6H_5	$C_{18}H_{21}NO_2$	80	4.0	1.5	50	91
5b	-*p*-CH_3 C_6H_4	$C_{19}H_{23}NO_2$	77	4.5	1.0	47	90
5c	-*m*-NO_2 C_6H_4	$C_{18}H_{20}N_2O_4$	107	5.0	2.0	48	94
5d	-*m*-Cl C_6H_4	$C_{18}H_{20}NO_2Cl$	72	4.5	2.5	57	91
5e	-*m*,*p*-Cl_2 C_6H_3	$C_{18}H_{19}NO_2Cl_2$	65	5.0	1.5	60	89
5f	-$C_{10}H_7$	$C_{22}H_{23}NO_2$	115	4.5	1.5	61	91

Notes: [a] All compounds were identified using comparison of their physical and spectral data (IR, NMR, and Mass). [b] Isolated yields.

Microwave (MW) irradiation technique has opened new prospects in synthetic organic chemistry due to high reaction rates, ease of experimental procedures, and reaction selectivity and cleanliness. The use of MW technique reduces the reaction time and improves the yield and purity of the products [15–17].

3.3. General Procedure

A mixture of thymol **(4)** (2 mg, 0.013 moles), 1–2 mL 1% solution of NaOH, and 0.013 moles of *α*-chloro acetanilide solution **3a–f** in acetone (2 mL) was placed in an Erlenmeyer flask. This was subjected to microwave irradiation for sufficient interval of time using resting intervals of 1 min after every 30 s of irradiation. The progress of reaction was monitored by TLC (Pet. Ether: $CHCl_3$ 9:1). The product was extracted into ether (2 × 20 mL), and then the extract was washed with water (20 mL) and dried over sodium sulfate. After removal of the solvent, needle-shaped crystals of thymyl ethers (**5a–f**) were obtained.

3.4. Compounds and Their Spectral Data

3a: IRν_{max} (cm^{-1}):3400 (N-H stretching), 2854 (-CH_2 stretching), 1672 (C=0 acyclic stretching, 1292, 1252 (C-N stretching of aromatic primary amine), 557, 502 (C-Cl stretching).

3b: IRν_{max} (cm^{-1}):3237 (N-H stretching, 2923 (-CH_2- stretching), 3203, 3134 (weak extra band due to N-H stretching), 1673 (C=0 stretching of amide), 864 (*p*-di substituted aromatic).(ES/MS): *m*/*z* (297) (M^+) 298, 284, 256, 179, 163, 149, and 133.

3c: IRν_{max} (cm^{-1}):3401 (N-H stretching), 2924, 2853 (-CH_2- stretching), 1673 (C=0 stretching of amide), 738 (*m*-di substituted aromatic).(ES/MS): *m*/*z* (317.5) (M^+) 318, 317, 289, 276, 177, 163, 149, 136, 121, 105, and 95.

3e: (ES/MS): *m*/*z* (352) (M-H)351, 336, 310, 298, 273, 190, 174, 163, 149, and 133.

3f: IRν_{max} (cm^{-1}):3410 (N-H stretching), 2924, 2854 (-CH_2- stretching), 1664 (C=0 stretching of amide), 1406, 1464 (1-naphthyl ring).

5a: IRν_{max} (cm^{-1}):3405 (-NH stretching), 2854 (Ar-H stretching), 1700 (>C=O stretching of amides), 1464 (-C-O stretching), 1463–1500 cm^{-1}(multiple bond -CH stretching).H^1 NMR spectral data ($CDCl_3$,300 MHZ): δ 8.280 (1H, s, N-H), δ 6.679 to 7.473 (8H, m, Ar-H), δ 4.604 (2H, s, -O-CH_2), δ 3.310 to 3.376 (1H, m, -CH<), δ 2.332 (3H, s, Ar-CH_3), δ 1.295 to 1.317 (6H, d, 2-CH_3 gem.).

5b: IRν_{max} (cm^{-1}):3405 (-NH stretching), 2854(Ar-H stretching), 1701 (>C=O stretching of amides), 1378–1062 (Ar-O-CH_2 stretching), 1463–1595 (multiple bond-CH stretching). H^1 NMR spectral data ($CDCl_3$,300 MHZ): δ 8.286 (1H, s, N-H), δ 6.583 to 7.473 (7H, m, Ar-H), δ 4.603 (2H, s, -O-CH_2), δ 3.310 to 3.377 (1H, m, -CH<), δ 2.332 (3H, s, Ar-CH_3), δ 1.390 (6H, d, 2-CH_3 gem.).

5c: IRν_{max} (cm^{-1}):3430 (-NH stretching), 2854 (Ar-H stretching), 1623 (>C=O stretching of amides), 1524 (-NO2 group), 1463–1500 (multiple bond -CH stretching). H^1 NMR spectral data ($CDCl_3$, 300 MHZ):

5d: IRν_{max} (cm^{-1}):3411 (-NH stretching), 2854 (Ar-H stretching), 1595 (>C=O stretching of amides), 1254–1378 (Ar-O-CH_2 stretching), 1459–1523 (multiple bond -CH stretching). (ES/MS): *m*/*z* (318) (M-H)318, 317, 289, 276, 177, 163, 149, 136, 121, 105, and 95.

5e: IRν_{max} (cm^{-1}):3393 (-NH stretching), 2854 (Ar-H stretching), 1691 (>C=O stretching of amides), 1253–1378 (Ar-O-CH_2 stretching), 1463–1523 (multiple bond -CH stretching). H^1 NMR spectral data ($CDCl_3$, 300 MHZ): δ 8.340 (1H, s, N-H), δ 6.493 to 7.845 (6H, m, Ar-H), δ 4.655 (2H, s, -O-CH_2), δ 3.285 to 3.717 (1H, m, -CH<), δ 2.331 (3H, s, Ar-CH_3), δ 1.296 to 1.319 (6H, d, 2-CH_3 gem.).

5f: IRν_{max} (cm^{-1}):3427 (-NH stretching), 2854 (Ar-H stretching), 1707 (>C=O stretching of amides), 1251–1378 (Ar-O-CH_2 stretching), 1464–1547 (multiple bond -CH stretching). NMR spectral data ($CDCl_3$, 300 MHZ): δ 8.874 (1H, s, N-H), δ 6.772 to 8.186 (10H, m, Ar-H), δ 4.774 (2H, s, -O-CH_2), δ 3.463 to 3.529 (1H, m, -CH<), δ 2.354 (3H, s, Ar-CH_3), δ 1.254 to 1.355 (6H, d, 2-CH_3 gem).

3.5. Antibacterial Activity

In the present work, all the synthesized compounds were tested for their bacterial potency against different bacteria (*Bacillus subtilis*, *Escherichia coli*, *Proteus vulgaris,* and *Staphylococcus aureus*) species. The results are summarized in Table 3.

Table 3. Antibacterial activities of compounds **3a–f** and **5a–f**.

Compounds	Zone of Inhibition in mm at Concentration of 20 mg/mL			
	P. vulgaries	***S. aureus***	***E. coli***	***B. subtilis***
Aniline	—-	25	—-	25
3a	07	25	10	18
3b	09	28	—-	12
3c	14	—-	07	13
3d	18	20	—-	25
3e	14	34	—-	20
3f	—-	23	—-	20
Thymol	—-	—-	—-	10
5a	06	—-	—-	—-
5b	09	—-	05	—-
5c	14	31	—-	—-
5d	06	—-	—-	10
5e	—-	—-	—-	06
5f	05	05	—-	05

The overall antibacterial study data showed that the synthesized *N*–chloro acetyl aryl amines **1a–f** reflected better antibacterial potency than the same compounds when coupled with thymol, e.g., **3a–f**. Such a structural modification will be beneficial to designing active molecules for pest management.

4. Results and Discussion

In the present work, all the synthesized compounds were tested for their bacterial potency against four bacterial species, viz. *Proteus vulgaris*, *staphyloccocuus aureus*, *Escherichia coli*, and *Bacillus subtilis* species.

Incase of *Proteus vulgaris*, compound **3d** shows the highest antibacterial potency. The parent compound aniline, thymol, as well as compound **5e,** does not show antibacterial activity.

In comparison with aniline, N-chloro acetyl aryl amines compounds **3a**–**f** reflected much higher antibacterial activity, except compound **3f**. However, when the same compound, **3f**, is coupled with thymol, its antibacterial activity increases.

The compound **3e** shows the highest antibacterial potency against *Staphylococcus aureus* as compared to all synthesized compounds. The compound **3c** does not possess antibacterial activity. It shows high potency when coupled with a thymol moiety, e.g., compound **5c**. This enhancement in activity is attributed to the introduction of the thymol moiety. The starting compounds thymol and the synthesized compounds **5a**, **5b**, **5d** and **5e** do not possess antibacterial activity.

In the case of *Escherichia coli,* all the synthesized compounds do not show remarkable antibacterial activity. Similarly, the starting compounds, e.g., thymol and aniline, do not show antibacterial activity. Compound **3a** reflects the highest antibacterial activity against *E. coli,* and compounds **3c** and **5b** show good antibacterial activity.

In the case of *Bacillus subtilis* species, all the compounds of the series **3a**–**f** show very good antibacterial activities. The parent compound aniline and **3d** compound exhibited the highest antibacterial activity compared to the other synthesized compounds. Additionally, thymyl ether derivatives such as **5d**, **5e** and **5f** are remarkable at a 2% concentration. The synthesized compounds**5a**, **5b** and **5c** do not show antibacterial activity.

From overall antibacterial data, it is evident that aniline and compounds **3a**–**f** show better antibacterial potency against all test bacteria species at a 2% concentration than the synthesized compounds **5a**–**f**. The activity order of the compounds of these series is **3a**–**f** $\geq$ **5a**–**f**.

5. Conclusions

Microwave synthesis prevents waste, compared to treating waste after it is formed. This approach will require new environmentally benign synthesis catalytic methods and chemical products that are benign by design and utilize renewable resources wherever possible [8–12].

A simple, efficient, and cost-effective method is described for the synthesis of thymol and carvacrol derivatives. This simple, quick, and environmentally benign safe procedure is advantageous in terms of the experimentation yield of the product, short reaction time, and avoidance of toxic solvents. This is a very useful method for the synthesis and generation of potentially biologically active thymol and carvacrol compounds.

6. Future Prospects

A structure–activity relationship can be established on the basis of structural modification and bioassay. The MW irradiation technique was successfully applied in synthetic organic chemistry to remove the drawbacks of conventional methodologies and reaction conditions. These are important aspects of the Green Chemistry approach because they occur more quickly, safely, and in an environmentally friendly manner. The use of microwave irradiation technique without solvents or to avoid toxic solvents is beneficial to the environment. In the future, structural modification via this method will be beneficial for designing active molecules for pest management.

Monoterpenoids and their derivatives are completely biodegradable and do not cause environmental pollution. Extensive research is ongoing for the derivatization of natural products isolated from essential oils of higher plants. The monoterpenoid group is a promising product for pest management efficacy.

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