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Synthesis, Characterization and Biological Activities of *N*-(4-(3,4-Dichlorophenoxy)-phenyl)-4-alkoxybenzamide Derivatives

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Asian Journal of Organic & Medicinal Chemistry

Volume: 6 Year: 2021
Issue: 2 Month: April–June
pp: 73–78
DOI: https://doi.org/10.14233/ajomc.2021.AJOMC-P309

Received: 15 January 2021
Accepted: 5 May 2021
Published: 24 July 2021

ABSTRACT

An alkoxy benzamide derivatives are have been synthesized in four steps. Alkylation, halo phenol coupling, nitro group reduction and acid amine coupling gave in decent yield. Likewise, these targets were synthesized by coupling of 4-(3,4-dichlorophenoxy) aniline with *N*-(4-(3,4-dichlorophenoxy)phenyl)-4-alkoxybenzamide by using (1-[*bis*(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexa fluorophosphate, hexa fluorophosphate azabenzotriazole tetramethyl uronium) (HATU), *N*,*N*-diisopropylethylamine (DIPEA) in dimethylformamide (DMF) at 0 °C to room temperature. Reduction of nitro group in the presence of 10% Pd/C, H_2 (g) in MeOH at room temperature. Obtained in decent to excellent yield. Anti-tuberculosis activity of all synthesized derivatives (**7a-l**) was complete against the H_{37}RV strain as per reported broth dilution method mentioned in experimental section. Bio-assay results showing that derivatives **7c**, **7e** and **7i** exhibited exceptional activity against the H_{37}RV strain with MIC value 62.5 µg/mL. Furthermore, other derivatives were showed poor potency against same strain when compared with standard drugs isoniazid and rifampicin.

KEYWORDS

Antibiotics, 4-(Octadecyloxy)benzamide, Antimicrobial, Esters.

INTRODUCTION

New antibiotics are instantly desired to treat the growing number of life-threatening bacterial infections that are resistant to current remedies. In particular, the emergence and spread of drug-resistant staphylococci is of serious anxiety. Here we report the discovery and characterization of an innovative class of small synthetic antibacterial that have potent activity against staphylococci and that has been accepted as an pretty but as yet under exploited target for antibacterial drug discovery [1,2]. Local anesthetic, adrenergic blocking, antispasmodic, sympathomimetic, analgesic and antiserotonin activities [3-5]. Furthermore, this development of molecules has recently shown selective nano-molar activity against human prostanoid DP receptor antagonists, which are supposed to be an important receptor aim in the treatment of allergic rhinitis [6-8]. In a new antimalarial technique, *in vivo* studies have presented that the U.S. Food and Drug Administration accepted antihistamine astemizole inhibits the malaria parasite *Plasmodium falciparum*, possibly permitting a streamlined treatment for the most lethal

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disease worldwide [9]. This inclusive ranging pharmaceutical activity is believed to develop from alkoxybenzamide-mediated physico-chemical properties; in particular, the relative acidity alkoxybenzamide facilitates promising pharmacodynamics and pharmacokinetics, thereby making them ideal components of drug applicants [10].

Literature reveals that the compound with amide derivatives of biphenyl ether containing alkoxy side chain and their biological activities are not reported. Herein, we report the newly functionalized 4-(3,4-dichlorophenoxy)aniline as a core substituted by the variable phenyl alkoxy side chain and studied their biological activities.

EXPERIMENTAL

All chemicals of the utmost purity available were purchased from commercial sources and used as received. The development of the reaction was scrutinized by thin layer chromatography (TLC) analysis on Merck pre-coated silica gel 60 F_{254} aluminum sheets, visualized by UV light. Melting points were measured on an Optimelt MPA 100 melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on Bruker AV 400 MHz spectrometer using $CDCl_3$ as solvent and TMS as the internal reference. ^{13}C NMR was recorded on a Bruker AV 100 MHz spectrometer using $CDCl_3$ as solvent. Mass spectra were recorded at Advion expression CMS, USA. Acetone was used as the mobile phase and electron spray ionization (ESI) was used as the ion source. Elemental analysis was performed on a CHNS elemental analyzer.

Synthesis of 4-alkyloxybenzoic acid (2a-l): To a stirred solution of 1-alkylbromide (90 mmol) and 4-hydroxybenzoic acid (**1**) (36.20 mmol) in ethanol (200 mL) was added KOH (72.40 mmol) followed by potassium iodide (1.5 mmol). Heat the reaction mixture under reflux for 12 h. After completion of the reaction, cool it to room temperature the suspension was treated with aqueous HCl up to pH 1. The white precipitation was filtered off and dried *in vacuo* at 40 ºC to give 4-alkyloxybenzoic acid (**2a-l**), which was used without further purification in the next step. All the products were confirmed by ESI-MS analysis.

Synthesis of 1,2-dichloro-4-(4-nitrophenoxy)benzene (5): In a stirred solution of 1-fluoro-4-nitrobenzene (**3**) (71 mmol) in *N,N*-dimethylformamide (DMF) (1 L) was added 3,4-dichlorophenol (**4**) (78 mol) followed by K_2CO_3 (142 mol) and the mixture was stirred at room temperature for 12 h and then cooled to room temperature. Poured the reaction mixture to ice-cold water and obtained precipitate was collected by filtration and dried under reduced pressure to give 1,2-dichloro-4-(4-nitrophenoxy)benzene (**5**) as light yellow solid.

Synthesis of 4-(3,4-dichlorophenoxy)aniline (6): To a stirred solution of 1,2-dichloro-4-(4-nitrophenoxy)benzene (**5**) (35.20 mmol) in methanol (900 mL) and water (100 mL) was added acetic acid (100 mL) and allowed to stir for 10 min at room temperature . Then, Fe powder (246.4 mmol) was added to the reaction in a portionwise and further stirred at 60 ºC for 4 h. After completion of the reaction, solvent was removed under vacuum and the reaction mixture was poured in ice-cold water, basify with aqueous $NaHCO_3$ solution and extracted with ethyl acetate. Organic layer dried over Na_2SO_4 and concentrated under reduce pressure to give 4-(3,4-dichlorophenoxy)aniline (**6**) as light brown solid, which was used for next step without further purification.

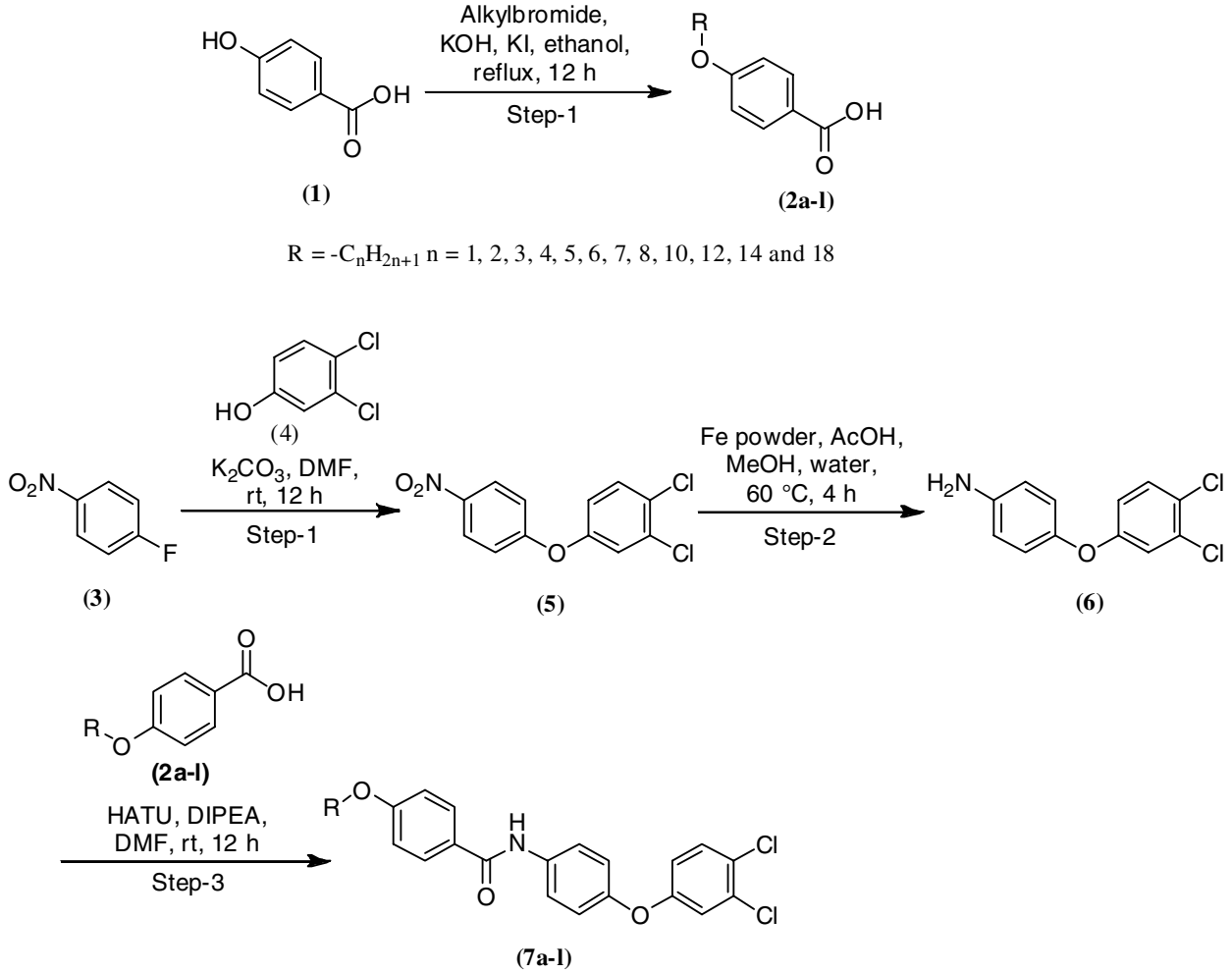
Synthesis of *N*-(4-(3,4-dichlorophenoxy)phenyl)-4-alkoxybenzamide (7a-l): To a stirred solution of 4-alkyloxybenzoic acid (**2a-l**) (10 mmol) in DMF (5 mL) was added HATU (17 mmol) at room temperature and stirred for 1 h. After that, 4-(3,4-dichlorophenoxy)aniline (**6**) (12 mmol) was added to the reaction mixture followed by *N,N*-diisopropyl ethylamine (DIPEA) (30 mmol). Resulting solution was stirred for 12 h at room temperature. After completion of the reaction mixture, reaction mixture was poured into ice-cold water and obtained solid was filtered, dried under vacuum to give crude product which was purified column chromatography to give *N*-(4-(3,4-dichlorophenoxy)phenyl)-4-alkoxybenzamide (**7a-l**) as white solid (**Scheme-I**).

***N*-(4-(3,4-Dichlorophenoxy)phenyl)-4-methoxybenzamide (7a):** White solid. ^{1}H NMR (400 MHz, $CDCl_3$, δ_H ppm): 7.89-7.86 (d, Ar, 2H, *J* = 8.8 Hz), 7.76 (s, -CONH, 1H), 7.65-7.63 (d, Ar, 2H, *J* = 9.2 Hz), 7.50 (s, Ar, 1H), 7.23-7.20 (m, Ar, 1H), 7.03-7.00 (d, Ar, 4H, *J* = 8.0 Hz), 6.94-6.92 (d, Ar, 1H, *J* = 8.8 Hz), 3.91 (s, -OCH_3, 1H). LC-MS (ESI) *m/z* for (387.04): 388.1 $(M+1)^+$, 390.2 $(M+2)^+$, R_t = 2.051 min, Purity = 97.75%. Elemental analysis of calcd. (found) % for $C_{20}H_{15}NO_3Cl_2$ (*m.w.* 388.24): C, 61.87 (61.86); H, 3.89 (3.88); N, 3.61 (3.60).

***N*-(4-(3,4-Dichlorophenoxy)phenyl)-4-ethoxybenzamide (7b):** White solid. ^{1}H NMR (400 MHz, $CDCl_3$, δ_H ppm): 7.87-7.85 (d, Ar, 2H, *J* = 8.8 Hz), 7.79 (s, -CONH, 1H), 7.65-7.62 (d, Ar, 2H, *J* = 8.8 Hz), 7.50 (s, Ar, 1H), 7.23-7.20 (m, Ar, 1H), 7.02-6.98 (m, Ar, 4H), 6.93-6.91 (d, Ar, 1H, *J* = 8.8 Hz), 4.16-4.11 (q, -OCH_2, 2H, *J* = 14 Hz), 1.50-1.47 (t, CH_3, 3H, *J* = 7 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, δ_C ppm): 18.45, 52.18, 52.40, 52.61, 52.82, 53.04, 53.25, 53.47, 67.64, 80.80, 81.12, 81.44, 118.15, 122.56, 124.64, 126.40, 129.95, 130.55, 131.91, 132.75, 133.11, 134.27, 138.34, 155.70, 156.68, 165.79, 170.38 LC-MS (ESI) *m/z* for (401.06): 402.2 $(M+1)^+$, 404.2 $(M+2)^+$, R_t = 2.130 min, Purity = 99.35%. Elemental analysis of calcd. (found) % for $C_{21}H_{17}NO_3Cl_2$ (*m.w.* 402.27): C, 62.70 (62.69); H, 4.26 (4.25); N, 3.48 (3.47).

***N*-(4-(3,4-Dichlorophenoxy)phenyl)-4-propoxybenzamide (7c):** White solid. ^{1}H NMR (400 MHz, $CDCl_3$, δ_H ppm): 7.87-7.85 (d, Ar, 2H, *J* = 8.8), 7.76 (s, -CONH, 1H), 7.65-7.63 (d, Ar, 2H, *J* = 9.2 Hz), 7.50 (s, Ar, 1H), 7.23-7.21 (m, Ar, 1H), 7.03-7.00 (m, Ar, 4H), 6.94-6.92 (d, Ar, 1H, *J* = 8.8 Hz), 4.04-4.01 (t, -OCH_2, 2H, *J* = 6.4 Hz), 1.91-1.86 (q, -CH_2, 2H, *J* = 14 Hz), 1.12- 1.08 (t, CH_3, 3H, *J* = 7.6 Hz). LC-MS (ESI) *m/z* for (415.07): 416.4 $(M+1)^+$, 418.4 $(M+2)^+$, R_t = 2.216 min, Purity = 100%. Elemental analysis of calcd. (found) % for $C_{22}H_{19}NO_3Cl_2$ (*m.w.* 416.30): C, 63.47 (63.46), H, 4.60 (4.58), N, 3.36 (3.35).

***N*-(4-(3,4-Dichlorophenoxy)phenyl)-4-butoxybenzamide (7d):** White solid. ^{1}H NMR (400 MHz, $CDCl_3$, δ_H ppm): 7.85-7.83 (d, Ar, 2H, *J* = 8.8 Hz), 7.73 (s, -CONH, 1H), 7.63-7.61 (d, Ar, 2H, *J* = 8.8 Hz), 7.48 (s, Ar, 1H), 7.21-7.19 (m, Ar, 1H), 7.01-6.98 (m, Ar, 4H), 6.92-6.90 (d, Ar, 1H, *J* = 8.8 Hz), 4.07-4.03 (t, -OCH_2, 2H, *J* = 6.4 Hz), 1.86-1.79 (m, -CH_2, 2H), 1.56-1.49 (m, -CH_2, 2H), 1.03-0.99 (t, CH_3, 3H, *J* = 7.6 Hz).



Scheme-I: Synthesis scheme of *N*-(4-(3,4-dichlorophenoxy)phenyl)-4-alkoxy benzamide

^{13}C NMR (100 MHz, $CDCl_3$) δ_C ppm: 14.02, 21.45, 28.15, 28.83, 68.28, 76.71, 77.03, 77.35, 114.48, 118.91, 120.75, 122.04, 126.15, 126.57, 128.02, 128.88, 130.47, 134.15, 151.79, 152.82, 162.19, 165.29. LC-MS (ESI) *m/z* for (429.09): 430.2 $(M+1)^+$, 432.2 $(M+2)^+$, R_t = 2.262 min, Purity = 99.68%. Elemental analysis of calcd. (found) % for $C_{23}H_{21}NO_3Cl_2$ (*m.w.* 430.33): C, 64.20 (64.19); H, 4.92 (4.90); N, 3.25 (3.24).

***N*-(4-(3,4-Dichlorophenoxy)phenyl)-4-(pentyloxy)-benzamide (7e):** White solid.^{1}H NMR (400 MHz, $CDCl_3$, δ_H ppm): 7.85-7.83 (d, Ar, 2H, *J* = 8.8 Hz), 7.73 (s, -CONH, 1H), 7.63-7.61 (d, Ar, 2H, *J* = 8.8v), 7.48 (s, Ar, 1H), 7.21-7.19 (m, Ar, 1H), 7.01-6.98 (m, Ar, 4H), 6.92-6.90 (d, Ar, 1H, *J* = 8.8 Hz), 4.06-4.02 (t, -OCH_2, 2H, *J* = 6.4 Hz), 1.87-1.80 (m, -CH_2, 2H), 1.52-1.39 (m, 2 × -CH_2, 4H), 0.99-0.96 (t, CH_3, 3H, *J* = 7.6 Hz). LC-MS (ESI) *m/z* for (443.11): 444.2 $(M+1)^+$, 446.2 $(M+2)^+$, R_t = 2.346 min, Purity = 99.43%. Elemental analysis of calcd. (found) % for $C_{24}H_{23}NO_3Cl_2$ (*m.w.* 444.35): C, 64.87 (64.85); H, 5.22 (5.21); N, 3.15 (3.13).

***N*-(4-(3,4-Dichlorophenoxy)phenyl)-4-(hexyloxy)-benzamide (7f):** White solid. ^{1}H NMR (400 MHz, $CDCl_3$, δ_H ppm): 7.85-7.83 (d, Ar, 2H, *J* = 8.8 Hz), 7.74 (s, -CONH, 1H), 7.63-7.61 (d, Ar, 2H, *J* = 8.8 Hz), 7.48 (s, Ar, 1H), 7.21-7.18 (m, Ar, 1H), 7.01-6.97 (m, Ar, 4H), 6.92-6.90 (d, Ar, 1H, *J* = 8.4 Hz), 4.06-4.02 (t, -OCH_2, 2H, *J* = 6.8 Hz), 1.85-1.79 (m, -CH_2, 2H), 1.51-1.37 (m, 3 × -CH_2, 6H), 0.95-0.92 (t, CH_3, 3H, *J* = 6.8 Hz). LC-MS (ESI) *m/z* for (457.12): 458.2 $(M+1)^+$, 460.2 $(M+2)^+$, R_t = 2.426 min, Purity = 99.51%. Elemental analysis of calcd. (found) % for $C_{25}H_{25}NO_3Cl_2$ (*m.w.* 458.38): C, 65.51 (65.10); H, 5.50 (5.49); N, 3.06 (3.05).

***N*-(4-(3,4-Dichlorophenoxy)phenyl)-4-(heptyloxy)-benzamide (7g):** White solid. ^{1}H NMR (400 MHz, $CDCl_3$, δ_H ppm): 7.87-7.85 (d, Ar, 2H, *J* = 8.8 Hz), 7.77 (s, -CONH, 1H), 7.65-7.63 (d, Ar, 2H, *J* = 8.8 Hz), 7.50 (s, Ar, 1H), 7.23-7.20 (m, Ar, 1H), 7.03-6.99 (m, Ar, 4H), 6.94-6.92 (d, Ar, 1H, *J* = 8.8 Hz), 4.07-4.04 (t, -OCH_2, 2H, *J* = 6.6 Hz), 1.88-1.81 (m, -CH_2, 2H), 1.54-1.36 (m, 4 × -CH_2, 8H), 0.95-0.92 (t, CH_3, 3H, *J* = 6.8 Hz). LC-MS (ESI) *m/z* for (471.14): 472.5 $(M+1)^+$, 474.5 $(M+2)^+$, R_t = 2.990 min, Purity = 99.51%. Elemental analysis of calcd. (found) % for $C_{26}H_{27}NO_3Cl_2$ (*m.w.* 472.41): C, 66.11 (66.10); H, 5.76 (5.75); N, 2.97 (2.96).

***N*-(4-(3,4-Dichlorophenoxy)phenyl)-4-(octyloxy)-benzamide (7h):** White solid. ^{1}H NMR (400 MHz, $CDCl_3$, δ_H ppm): 7.87-7.85 (d, Ar, 2H, *J* = 6.8 Hz), 7.77 (s, -CONH, 1H), 7.65-7.63 (d, Ar, 2H, *J* = 7.6 Hz), 7.50 (s, Ar, 1H), 7.23-7.21 (m, Ar, 1H), 7.03-6.99 (m, Ar, 4H), 6.94-6.92 (d, Ar, 1H, *J* = 8.4 Hz), 4.07-4.04 (t, -OCH_2, 2H, *J* = 6.8 Hz), 1.88-1.80 (m,

-CH_2, 2H), 1.53-1.37 (m, 6 × -CH_2, 12H), 0.95-0.92 (t, CH_3, 3H, *J* = 6.8 Hz). LC-MS (ESI) *m/z* for (499.17): 500.6 $(M+1)^+$, 502.6 $(M+2)^+$, R_t = 3.138 min, Purity = 99.52%. Elemental analysis of calcd. (found) % for $C_{28}H_{31}NO_3Cl_2$ (*m.w.* 500.46): C, 67.20 (67.18); H, 6.24 (6.23); N, 2.80 (2.79).

***N*-(4-(3,4-Dichlorophenoxy)phenyl)-4-(decyloxy)-benzamide (7i):** White solid. ^{1}H NMR (400 MHz, $CDCl_3$, δ_H ppm): 7.87-7.85 (d, Ar, 2H, *J* = 8.4 Hz), 7.78 (s, -CONH, 1H), 7.65-7.63 (d, Ar, 2H, *J* = 8.8 Hz), 7.50 (s, Ar, 1H), 7.23-7.20 (m, Ar, 1H), 7.03-6.99 (m, Ar, 4H), 6.94-6.92 (d, Ar, 1H, *J* = 8.8 Hz), 4.07-4.04 (t, -OCH_2, 2H, *J* = 6.4 Hz), 1.88-1.81 (m, -CH_2, 2H), 1.51-1.32 (m, 7 × -CH_2, 14H), 0.94-0.91 (t, CH_3, 3H, *J* = 6.4 Hz). LC-MS (ESI) *m/z* for (513.18): 514.6 $(M+1)^+$, 516.6 $(M+2)^+$, R_t = 2.657 min, Purity = 99.50%. Elemental analysis of calcd. (found) % for $C_{29}H_{33}NO_3Cl_2$ (*m.w.* 514.49): C, 67.70 (67.68); H, 6.47 (6.45); N, 2.72 (2.71).

***N*-(4-(3,4-Dichlorophenoxy)phenyl)-4-(dodecyloxy)-benzamide (7j):** White solid. ^{1}H NMR (400 MHz, $CDCl_3$, δ_H ppm): 7.87-7.85 (d, Ar, 2H, *J* = 8.8 Hz), 7.76 (s, -CONH, 1H), 7.65-7.63 (d, Ar, 2H, *J* = 8.8 Hz), 7.50 (s, Ar, 1H), 7.23-7.20 (m, Ar, 1H), 7.03-6.99 (m, Ar, 4H), 6.94-6.92 (d, Ar, 1H, *J* = 8.8), 4.07-4.04 (t, -OCH_2, 2H, *J* = 6.4 Hz), 1.88-1.81 (m, -CH_2, 2H), 1.54-1.31 (m, 9 × -CH_2, 18H), 0.94-0.91 (t, CH_3, 3H, *J* = 6.4 Hz). LC-MS (ESI) *m/z* for (541.22): 542.7 $(M+1)^+$, 544.7 $(M+2)^+$, R_t = 1.243 min, Purity = 99.68%. Elemental analysis of calcd. (found) % for $C_{31}H_{37}NO_3Cl_2$ (*m.w.* 542.54): C, 68.63 (68.62); H, 6.87 (6.86); N, 2.58 (2.58).

***N*-(4-(3,4-Dichlorophenoxy)phenyl)-4-(tetradecyloxy)-benzamide (7k):** White solid. ^{1}H NMR (400 MHz, $CDCl_3$, δ_H ppm): 7.87-7.85 (d, Ar, 2H, *J* = 8.4 Hz), 7.77 (s, -CONH, 1H), 7.65-7.63 (d, Ar, 2H, *J* = 8.8 Hz), 7.50 (s, Ar, 1H), 7.23-7.20 (m, Ar, 1H), 7.03- 6.99 (m, Ar, 4H), 6.94-6.92 (d, Ar, 1H, *J* = 8.8 Hz), 4.06-4.02 (t, -OCH_2, 2H, *J* = 6.4 Hz), 1.86-1.78 (m, -CH_2, 2H), 1.50-1.30 (m, 11 × -CH_2, 22H), 0.93-0.90 (t, CH_3, 3H, *J* = 6.4). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C ppm: 14.13, 22.70, 26.00, 26.07, 28.79, 29.14, 29.31, 29.37, 29.58, 29.60, 29.67, 31.94, 64.83, 68.21, 68.31,114.02, 114.49, 118.92, 120.76, 122.00, 122.69, 126.16, 126.58, 128.02, 128.85, 128.91, 130.47, 131.51, 134.15, 151.79, 152.83, 162.21, 162.88, 165.24, 166.55. LC-MS (ESI) *m/z* for (569.25): 570.7 $(M+1)^+$, 572.8 $(M+2)^+$, R_t = 1.781 min, Purity = 99.54%. Elemental analysis of calcd. (found) % for $C_{33}H_{41}NO_3Cl_2$ (*m.w.* 570.60): C, 69.46 (69.45); H, 7.24 (7.23); N, 2.45 (2.43).

***N*-(4-(3,4-Dichlorophenoxy)phenyl)-4-(octadecyloxy)-benzamide (7l):** White solid. ^{1}H NMR (400 MHz, $CDCl_3$, δ_H ppm): 7.87-7.85 (d, Ar, 2H, *J* = 8.8 Hz), 7.76 (s, -CONH, 1H), 7.65-7.63 (d, Ar, 2H, *J* = 8.8 Hz), 7.50 (s, Ar, 1H), 7.23-7.20 (m, Ar, 1H), 7.03-6.99 (m, Ar, 4H), 6.94-6.92 (d, Ar, 1H, *J* = 8.8 Hz), 4.07-4.04 (t, -OCH_2, 2H, *J* = 6.4 Hz), 1.87-1.83 (m, -CH_2, 2H), 1.50-1.23 (m, 15 × -CH_2, 30H), 0.93-0.90 (t, CH_3, 3H, *J* = 6.8 Hz). LC-MS (ESI) *m/z* for (625.31): 626.8 $(M+1)^+$, 628.8 $(M+2)^+$, R_t = 2.921 min, Purity = 100%. Elemental analysis of calcd. (found) % for $C_{37}H_{49}NO_3Cl_2$ (*m.w.* 626.70): C, 70.91 (70.90); H, 7.88 (7.87); N, 2.24 (2.23).

Biological assay

***in vitro* Antibacterial and antifungal activity:** All freshly synthesized compounds (**7a-l**) were evaluated for their antibacterial activity using the Gram-positive and Gram-negative strains. Two strains of Gram-positive bacteria (*Staphylococcus aureus* MTCC 96 and *Streptococcus pyogenes* MTCC 442) and two strains of Gram-negative bacteria (*Escherichia coli* MTCC 443 and *Pseudomonas aeruginosa* MTCC 1688) as well as three fungal strains (*Aspergillus clavatus* MTCC 1323, *Candida albicans* MTCC 227 and *Aspergillus niger* MTCC 282) by using the reported agar dilution method [11]. Ciprofloxacin, ampicillin and chloramphenicol were used as a standard drugs for the antibacterial activity and greseofulvin and nystatin were used as a standard drugs for the antifungal activity.

***in vitro* Antimalarial and antituberculosis activity:** All the synthesized compounds (**7a-l**) were evaluated in *in vitro* antimalarial activity as per the reported method against the *Plasmodium falciparum* strain [12] and *in vitro* antituberculosis activity as per the reported method against the H_{37}RV strain [13].

RESULTS AND DISCUSSION

Initially, we have synthesized 4-alkoxybenzoic acid (**2a-l**) through the reaction of 4-hydroxybenzoic acid with 1-alkylbromide in presence of KOH and catalytic amount of KI under refluxing ethanol for 12 h. In this reaction, we have demonstrated different 1-alkylbromide having varied long chain from 1 to 18 carbon chain. Simultaneously, we have synthesized 3,4-dichloro-1-(4-nitrophenoxy)benzene (**5**) *via* reaction of 1-fluoro-4-nitrobenzene (**3**) with 3,4-dichlorophenol (**4**) in the presence of K_2CO_3 in DMF at room temperature for 12 h. After that 4-(3,4-dichlorophenoxy)aniline (**6**) was prepared from the reduction of derivatives (**5**) in presence of Fe powder and acetic acid in methanol: water at 65 ºC for 4 h. Finally, *N*-(4-(3,4-dichlorophenoxy)phenyl)-4-alkoxybenzamide (**7a-l**) were synthesized from the reaction of 4-(3,4-dichlorophenoxy)-aniline (**6**) with derivatives (**2a-l**) in the presence of HATU and DIPEA in DMF at room temperature for 12 h. All the newly synthesized derivatives (**7a-l**) were purified either by column chromatography.

The reaction proceeded smoothly and provided excellent yields in all cases (Table-1). Various long carbon chain alkyl bromides reacted smoothly with this protocol to afford the excellent yields of the products. The purity of the synthesized compounds was confirmed by TLC and elemental analysis. The structure of the final products was well characterized by ^{1}H & ^{13}C NMR and ESI-MS.

TABLE-1
N-(4-(3,4-DICHLOROPHENOXY)PHENYL)-4-ALKOXYBENZAMIDE

Compd. No.	R	Yield[a] (%)	m.p. (°C)
7a	CH_3	89	178
7b	C_2H_5	81	172
7c	C_3H_7	80	169
7d	C_4H_9	82	159
7e	C_5H_{11}	81	152
7f	C_6H_{13}	88	148
7g	C_7H_{15}	84	145
7h	C_9H_{19}	81	141
7i	$C_{10}H_{21}$	80	137
7j	$C_{12}H_{25}$	82	129
7k	$C_{14}H_{29}$	85	122
7l	$C_{18}H_{37}$	90	116

[a]Isolated yields

The ^{1}H NMR spectrum showed triplet nearer δ 7.74-7.78 ppm indicates a proton of the -CONH- group, while triplet nearer δ 4.06-4.00 ppm indicates a proton of the -CH_2O group. In addition, peaks between δ 7.8 and 6.8 ppm were observed for respective aromatic protons. The ^{13}C NMR spectrum displayed peak nearer 165 to 166 ppm, which indicated C=O group of -CONH. The LC-MS spectra of compounds (**7a-l**) showed corresponding $(M+1)^+$ peak with purity.

***in vitro* Antibacterial and antifungal activity:** The biological investigation of novel diphenyl ether derivatives revealed that most of tested derivatives were found to be more potent antimicrobial agents as compared to standard reference drugs (Table-2). In general, most of the screened molecules displayed excellent potency against Gram-positive bacterial strains as compared to Gram-negative bacterial strains. Most of prepared molecules derivatives (**7a-l**) disclosed comparatively higher potency against Gram-positive bacterial strains *S. aureus* as compared to *S. pyogenes*. Specifically, derivatives **7f**, **7h** and **7i** (MIC = 62.5 µg/mL) having medium length of alkyl chain were found to be the most active against the *S. aureus* as compared to standard drug ampicillin (MIC = 250 µg/mL). Although derivatives **7d**, **7g**, **7j** and **7l** (MIC = 100 µg/mL) displayed extraordinary activity against the *S. aureus* as compared to standard drug ampicillin (MIC = 250 µg/mL), but 50% less potent then ciprofloxacin (MIC = 50 µg/mL) and chloramphenicol (MIC = 50 µg/mL). Moreover, investigated molecules **7c**, **7e** and **7k** comprising excellent activity against *S. aureus* with MIC = 125 µg/mL. Other compounds displayed equipotent or moderate activity against *S. aureus* as compared to ampicillin. On other hand, compounds **7f** and **7l** (MIC = 62.5 µg/mL) exhibited excellent potency against *S. pyogenes* when compared to the standard drug ampicillin. Moreover, screened molecules **7d**, **7g**, **7h**, **7i** and **7j** exhibited equipotent activity against *S. pyogenes* with MIC = 100 µg/mL when compared with standard drug ampicillin, but 50% less active then ciprofloxacin (MIC = 50 µg/mL) and chloramphenicol (MIC = 50 µg/mL). Rest of molecules displayed moderate potency against *S. pyogenes*.

The bio-assay results of Gram-negative bacterial strains revealed that compounds **7d**, **7g**, **7j** and **7k** displayed excellent activity with MIC = 62.5 µg/mL against *E. coli* bacterial strain as compared to standard drug ampicillin (MIC = 100 µg/mL). Furthermore, compounds **7f**, **7h** and **7i** (MIC = 100 µg/mL) possessed equipotent activity against the *E. coli* as compared to standard drug ampicillin. Rest of the derivatives displayed moderate potency against Gram-negative *E. coli*. Moreover, all investigated molecules (**7a-l**) displayed poor potency against Gram-negative *P. aeruginosa* bacterial strain.

TABLE-2
in vitro BIOLOGICAL ACTIVITY OF TARGETED COMPOUNDS (**7a-l**)

Compd. No.	Antibacterial activity				Antifungal activity			Antimalarial activity[d]	Antituberculosis activity[e]
	MIC (µg/mL)				MIC (µg/mL)			Mean IC_{50} (mg/mL)	Mean IC_{50} (mg/mL)
	Ec[a]	*Pa*[a]	*Sa*[b]	*Sp*[b]	*Ca*[c]	*An*[c]	*Ac*[c]	*Pf*[d]	$H_{37}RV$[e]
7a	500	250	250	125	250	250	1000	1.03	125
7b	100	200	250	250	500	1000	1000	1.52	250
7c	250	100	125	125	250	500	500	0.97	62.5
7d	62.5	250	100	100	1000	250	500	1.23	100
7e	250	250	125	250	250	250	>1000	1.18	62.5
7f	100	100	62.5	62.5	500	1000	1000	0.89	250
7g	62.5	62.5	100	100	1000	500	500	1.24	125
7h	100	62.5	62.5	100	500	250	500	0.77	100
7i	100	100	62.5	100	250	500	1000	1.02	62.5
7j	62.5	62.5	100	100	1000	250	>1000	0.18	250
7k	125	250	125	250	500	500	500	0.23	100
7l	62.5	250	100	62.5	1000	500	500	0.08	250
INH[f]	–	–	–	–	–	–	–	–	0.2
CHL[f]	50	50	50	50	–	–	–	–	–
AMP[f]	100	–	250	100	–	–	–	–	–
CIP[f]	25	25	50	50	–	–	–	–	–
NOR[f]	10	10	10	10	–	–	–	–	–
GEN[f]	0.05	1	0.25	0.5	–	–	–	–	–
NYS[f]	–	–	–	–	100	100	100	–	
GRE[f]	–	–	–	–	500	100	100	–	–
QUIS[f]	–	–	–	–	–	–	–	0.268	–
CHLO[f]	–	–	–	–	–	–	–	0.02	–
RIFA[f]	–	–	–	–	–	–	–	–	0.25

Note: –, not tested.
[a]Values expressed in µg/mL, *Escherichia coli* MTCC 443 (*Ec*), *Pseudomonas aeruginosa* MTCC 1688 (*Pa*).
[b]Values expressed in µg/mL, *Staphylococcus aureus* MTCC 96 (*Sa*), *Streptococcus pyogenes* MTCC 442 (*Sp*).
[c]Values expressed in µg/mL, *Candida albicans* MTCC 227 (*Ca*), *Aspergillus niger* MTCC 282 (*An*), *Aspergillus clavatus* MTCC 1323 (*Ac*).
[d]Values expressed in IC_{50} µg/mL, *Plasmodium falciparum.*
[e]Values expressed in MIC µg/mL, $H_{37}RV$ strain.
[f]Standard drugs (INH = Isoniazid, CHL = Chloramphenicol, AMP = Ampicillin, CIP = Ciprofloxacin, NOR = Norfloxacin, GEN = Gentamycin, NYS = Nystain, GRE = Greseofulvin, Qui = Quinine, Chlo = Chloroquine, Rif = Rifampicin).

About the antifungal activity of all screened molecules (**7a-l**), only one strain, *i.e. C. albicans*, displayed certain sensitivity towards some of the tested molecules, whereas the other two fungal strains were remain insensitive towards the same compounds (Table-2). The derivatives **7a**, **7c**, **7e** and **7i** (MIC = 250 µg/mL) displayed excellent activity against *C. albicans* when compared with standard reference drug greseofulvin (MIC = 500 µg/mL). Whereas derivatives **7b**, **7f**, **7h** and **7k** (MIC = 500 µg/mL) displayed equipotent activity against *C. albicans*, when compared with standard reference drug greseofulvin (MIC = 500 µg/mL). Rest of the molecules displayed moderate to good potency against Gram-negative *C. albicans*.

***in vitro* Antimalarial activity:** All the synthesized derivatives were also investigated for their *in vitro* antimalarial activity against *Plasmodium falciparum* (Pf) 3D7 chloroquine-sensitive strain as per reported method. All experiments were performed in duplicate and the mean IC_{50} values are displayed in Table-2. From the investigated molecules (**7a-l**), derivatives **7j**, **7k** and **7l** displayed outstanding activity against *Plasmodium falciparum* strain with IC_{50} values between 0.08 to 0.23 µg/mL when compared to standard reference drug quinine. However, derivative **7l** having longest alkyl chain displayed excellent potency with IC_{50} values 0.08 µg/mL. Other remaining derivatives also displayed a moderated activity as compared to standard reference drugs quinine and chloroquine.

***in vitro* Antituberculosis activity:** Antituberculosis activity of all synthesized derivatives (**7a-l**) was consummate against the $H_{37}RV$ strain as per broth dilution method. The bioassay results exposed that derivatives **7c**, **7e** and **7i** displayed exceptional activity against the $H_{37}RV$ strain with MIC value 62.5 µg/mL. Furthermore, other derivatives were showed poor potency against same strain when compared with standard drugs isoniazid and rifampicin.

Conclusion

In conclusion, we have efficaciously synthesized a novel amide derivatives based homologues series (**7a-l**) and well characterized through different spectroscopic technique like ^{1}H NMR, ^{13}C NMR and LC-MS. All synthesized molecules were demonstrated for their antimicrobial and antimalarial activity. In addition, all the compounds exhibited excellent potency against *S. aureus* bacterial as well as *C. albicans* microorganism strain. While some molecules having alkyl chain possess brilliant antimicrobial activity. Moreover, among the synthesized compounds, derivative **7l** having longest alkyl chain exhibited highly potent antimalarial activity.

ACKNOWLEDGEMENTS

The authors acknowledge to Department of Chemistry, Shri M.M Patel Institute of Sciences and Research, Kadi Sarva Kadi Sarva Vishwavidyalaya, Gandhinagar, India for provding the research and spectral facilities.

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