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Synthesis of 7-oxabicyclo[2.2.1]hept-5-en-2-yl derivatives and their screening for antimicrobial and antioxidant properties

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ABSTRACT

Novel 7-oxabicyclo[2.2.1]hept-5-en-2-yl derivatives have been synthesized using boron trifluoride diethyl etherate catalyzed Diels–Alder reaction. This method presents considerable synthetic advantages in terms of high atom economy, mild reaction condition and good yields. The synthesized compounds have been screened for their antibacterial and antioxidant activities.

Antimicrobial chemotherapy is one of the important life saving treatments that increased average life expectancy in recent decades. However, many disease-causing microbes gained resistance through gene transfer and plasmid formation. As a result, many new multi-drug resistant microbes are evolving that necessitate the introduction of new antimicrobial agents. In principle, these new drug candidates can block or prevent any specific biological functions such as cell wall growth, protein synthesis, metabolism etc., which are very vital for the bacterial survival. Minor structural modification and functionalization in the existing drugs, otherwise known as improved chemical entities, (ICE) is the method of choice in this regard. Alternatively, a wide range of chemical structures (novel chemical entities, NCE) can also be synthesized and screened for their antimicrobial activities.

In our group, we are exploiting the 4n+2 cycloaddition reactions with different dienes and dienophiles including imino Diels–Alder reactions for the synthesis of variety of carbocycles and heterocycles towards medicinal applications.[1] Recently, we reported the synthesis and their application of quinoline based compounds using aza-Diels–Alder reactions in bacterial detection using fluorescent imaging at micromolar concentration.[2] Interestingly, at a relatively higher concentration, the quinoline based derivatives showed bacteriocidal properties. Herein, we report the synthesis and antimicrobial screening of 7-oxabicyclo[2.2.1]hept-5-en-2-yl derivatives synthesized by reacting substituted chalcones (dienophile) with 2-ethylfuran (dienophile).

Indeed cantharidin and norcantharidin like molecules that have an oxabicyclo structural moiety, are known for their serine/threonine protein phosphate 1 and 2A inhibition properties.[3] Since their introduction, few oxabicyclic compounds were reported and studied for their antitumor activities. One of the key aspects in designing anticancer and related drugs is to account for their cytogenetic effects, more specifically on the antioxidant properties. Since free radicals and reactive oxygen species (ROS) play an important role in pathogenesis of many diseases including aging and tumor, introduction of novel antioxidants is increasingly important.

Reactive oxygen species (ROS) are produced by abnormal metabolic processes as well as sunlight, ultraviolet light, and chemical reactions, and can change the structure of DNA, membrane lipids, and protein, which may result in diseases such as cancer, aging, inflammation and atherosclerosis. Therefore, supplementation of antioxidants, which can overcome oxidation-mediated problems, may prevent the body from a set of diseases by directly quenched ROS. Therefore, there has been a growing interest to develop novel antioxidant compounds.

Based on the experimental findings, it is believed that molecules with high antioxidant potential and low pro-oxidant properties are better candidates for clinical applications. Moreover antioxidants are potential classes of anticancer drugs. In spite of the reports on oxabicycloheptane compounds for various medicinal applications, the antioxidant properties of them are rarely

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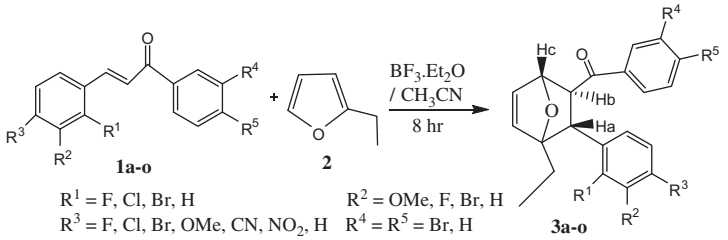


Scheme 1. Synthesis of 7-oxabicyclo[2.2.1]hept-5-en-2-yl derivatives.

Table 1
Synthesis of 7-oxabicyclo[2.2.1]hept-5-en-2-yl derivatives

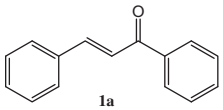
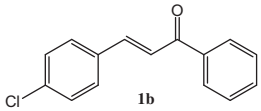
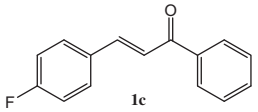
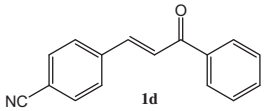
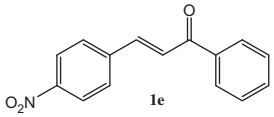
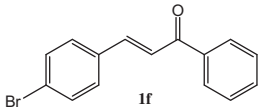
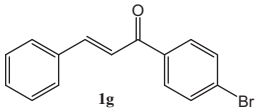
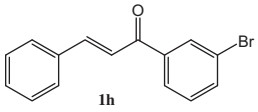
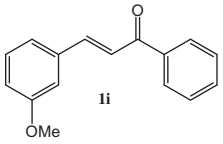
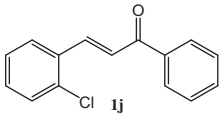
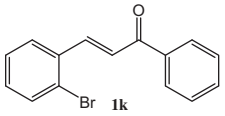
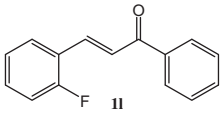
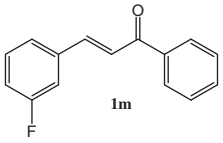
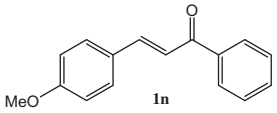
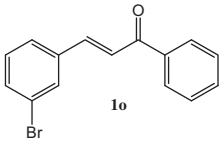
Entry	Chalcone (1)	Product (3)	Yield (%)
1	 1a	**3a**	90
2	 1b	**3b**	89
3	 1c	**3c**	92
4	 1d	**3d**	85
5	 1e	**3e**	91
6	 1f	**3f**	89
7	 1g	**3g**	90
8	 1h	**3h**	85
9	 1i	**3i**	90
10	 1j	**3j**	85
11	 1k	**3k**	90

Table 1 (*continued*)

Entry	Chalcone (1)	Product (3)	Yield (%)
12	 1l	**3l**	82
13	 1m	**3m**	85
14	 1n	**3n**	86
15	 1o	**3o**	90

explored. Hence, we included the studies on the antioxidant properties of the title compounds.

Synthetically, we utilized the Diels–Alder reaction, which is one of the most powerful reactions with a concerted two carbon–carbon bond formation and is widely used for the synthesis of various bioactive natural compounds.[1c,i,j] Particularly it is an important synthetic tool for the construction of various carbocycles and heterocycles. The other significant features of this reaction are excellent chemo- and regio selectivity, and high diastereo and enantio selectivity when chiral auxiliaries and chiral Lewis acids are used. Another favorable aspect of the reaction is its high atom efficiency, thereby allowing the construction of complex ring systems from relatively simple precursors. Intermolecular Diels–Alder reactions employing 2-substituted furans as 4π components, first reported in a synthesis of 3-hydroxy phthalic anhydrides,[4] provide rapid access to oxygen-substituted aromatic and cyclohexene rings.[5,6]

Boron trifluoride diethyl etherate was used for ring opening reaction[7] and has been applied for rearrangement.[8] In connection with our interest in the cycloaddition reaction and development of new methodologies for the synthesis and biological evaluation of diverse heterocyclic compounds,[9] we have successfully synthesized furan derived oxabicyclo compounds from chalcone and 2-ethylfuran via Diels–Alder reaction and evaluated their antibacterial and antioxidant activities.

To begin with, chalcone **1j** was prepared as in the general procedure and treated with 2-ethylfuran **2** in acetonitrile in the presence of boron trifluoride diethyl etherate at room temperature.[10] The reaction took place regioselectively to yield the title compound **3j** (Scheme 1, Table 1, entry 10). To demonstrate the methodology applicable to a variety of substrates having bromo, fluoro, cyano, nitro and alkyl substitutions, synthesis of several furan derived oxabicyclics have also been synthesised and the results are shown in Table 1. For these cycloaddition reactions there are two regioisomers possible, via structures A and B for **3j**. (Fig. 1) The regioisomers can be identified using ^{2}D NMR HMBC experiments. If structure A is favoured, three ^{3}J couplings are possible for the carbonyl carbon, whereas for the structure B, only two such correlations will appear. Experimentally, we observed three correlations (Fig. 3S in Supporting information). This clearly indicated that the regioselectivity is favoured towards the structure A. The structures of compounds **3a–o** were confirmed by IR, ^{1}H and ^{13}C NMR spectroscopy, mass spectrometry and elemental analysis. The ^{1}H NMR spectrum of compound **3j** consist of a characteristic doublet of doublet due to the proton Ha at δ 3.50 (J = 5.35, 16.8 Hz), a doublet due to the proton Hb at δ 3.75 (J = 8.40, 16.8 Hz) and a multiplet Hc at δ 3.27–5.30. To investigate the scope of this reaction, a series of furan derived oxabicyclic compounds were synthesized and characterized (Table 1, entries 1–15). All the prepared compounds gave excellent yields (85–92%).

All the newly synthesised compounds (**3a–o**) were screened for their antibacterial and antioxidant activities. The in vitro antibacterial and antioxidant activities of the synthesized **3a–o** are tabulated (Tables 2 and 3) and represented as a graph in Figure 2.

Antimicrobial studies: The antibacterial tests were conducted against five human bacterial pathogens such as *Vibrio cholera*, *Bacillus subtilus*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Escherichia coli* and the activities of the compounds were determined by means of microdilution broth assay method[11] with modifications reported by Sarker et al.[12] using resazurin as an indicator. In this method instead of resazurin dye, 2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide inner salt (XTT) is used as the indicator for the growth of bacteria or inhibition of bacterial growth.[13] This modification is very easy, simple to execute and sensitive method compare to the normal methods to predict the MIC (minimum inhibitory concentration) values.[14,15] These MIC values (in mg/ml) of bacterial pathogens for the above mentioned oxabicyclo derivatives were compared with a control antibiotic sample, ciprofloxacin. Apart from this microdilution method, well diffusion assay[16] was also carried out in the determination of the antibacterial activity. For the well diffusion assay, 17 h old bacterial cultures were grown over the surface of a dried Muller Hinton agar plates using a sterile cotton swab and allowed to absorb in the agar for 10 min. Then the well was cut using a cork borer and loaded with different concentrations of the synthesized compounds along with a control, DMSO. The plates were then incubated at 37 °C for 24 h, and after incubation the diameter of zone of inhibition in mm was measured.

Through these assay methods, it was found that the oxabicyclo heptenyl derivatives have moderate to good antibacterial activity for various human pathogens. Except for *S. aureus* and *K. pneumoniae*, compound **3g** showed the lowest minimum inhibitory concentration for *B. subtillus, E. coli,* and *V. cholera*. For *K. pneumoniae*, **3a** showed the lowest MIC that is better than the standard ciprofloxacin. With respect *S. aureus*, **3o** found to have an

Table 2
Minimum inhibitory concentration

Entry	Minimum inhibitory concentration (MIC) (mg/ml)				
	B. subtilus	*V. cholera*	*K. pneumonia*	*S. aureus*	*E. coli*
3a	0.312	1.25	0.004	0.625	0.312
3b	0.322	0.625	0.156	0.625	0.312
3c	0.625	0.625	0.625	1.25	0.625
3d	0.156	1.25	0.156	0.156	0.156
3e	1.25	1.25	0.625	1.25	1.25
3f	0.312	0.625	0.156	0.156	0.312
3g	0.078	0.312	0.078	1.25	0.078
3h	0.625	0.625	0.312	0.625	0.625
3i	0.625	0.625	0.312	0.625	0.625
3j	1.25	0.625	0.625	1.25	1.25
3k	1.25	0.625	0.625	1.25	1.25
3l	1.25	0.625	0.625	1.25	1.25
3m	1.25	0.625	0.312	0.625	1.25
3n	0.625	0.625	0.625	1.25	0.625
3o	0.312	0.625	0.156	0.031	0.312
Ciprofloxacin	0.125	0.015	0.007	0.015	0.062

Table 3
DPPH antioxidant scavenging activity

Entry	Compounds	Inhibition of free radicals (%)
1	**3a**	58.9
2	**3b**	35.5
3	**3c**	42.4
4	**3d**	58.9
5	**3e**	21.6
6	**3f**	30.5
7	**3g**	32.6
8	**3h**	36.0
9	**3i**	33.4
10	**3j**	22.2
11	**3k**	17.7
12	**3l**	18.1
13	**3m**	22.3
14	**3n**	15.1
15	**3o**	23.2
16	**BHT**	58.4

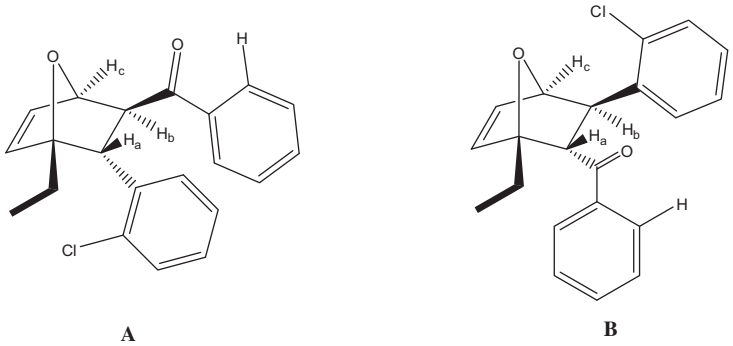


Figure 1. Possible regioisomers in the oxabicyclo compound **3j**.

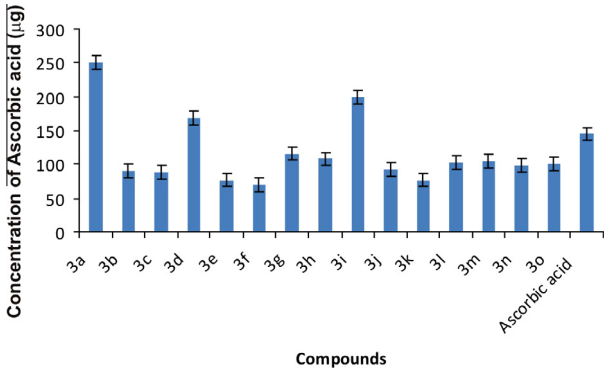


Figure 2. Total antioxidant activity of various compounds.

activity very close to the standard. The antimicrobial activity results clearly indicate that the 7-oxabicyclo[2.2.1]hept-5-en-2-yl derivatives are new class of potent antibacterial agents. Although the derivatives **3a**, **g** and **o** are showing very good activity against individual species that is in par with the standard, their overall activity is moderate. A proper design and further tuning of substituents in these compounds is an interesting perspective in the development of antimicrobial chemotherapy.

Antioxidant studies: In this study, we investigated the antioxidant properties of oxabicyclo compounds using DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity with respect to the standard BHT (butylated hydroxy toluene) and using a spectrometric assay.[17,18]

Based on the experimental results,[19,20] we found out that compounds **3a**, **d**, and **i** showed total antioxidant activities better than ascorbic acid. (Fig. 1 and Table 2) Indeed most of the compounds showed antioxidant properties closer to the standard. However, DPPH scavenging assay predicted that only **3a** and **d** showed a slightly better activity than the control sample BHT. Surprisingly, **3i** showed only a weak scavenging activity.

In summary, we have demonstrated a high yielding approach involving Diels–Alder reaction to synthesize 7-oxabicyclo[2.2.1]hept-5-en-2-yl derivatives. Antimicrobial and antioxidant studies of these compounds showed promising results. The operational simplicity of this method and the high yields of the product make it attractive for the synthesis of this class of potential biologically active molecules.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.04.017.

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10. *Typical experimental procedure for compound **3j**:* Chalcone **1j** (Chalcone **1j** was synthesized by condensing equimolar mixture of *o*-chloro benzaldehyde and acetophenone in methanol with a dropwise addition of 50% NaOH (1 equiv) solution at RT. After completion of the reaction, the reaction mixture was poured into cold water and acidified using 10% HCl solution and the resulting solid product was filtered and dried to offer **1j** with a yield of 90%) was reacted with 2-ethylfuran **2** in acetonitrile followed by drop-wise addition of boron trifluoride diethyl etherate at 0 °C, The resulting mixture was stirred at room temperature for 8 h under nitrogen atmosphere, the reaction proceeded smoothly and the product was formed as indicated by TLC. After complete consumption of the starting materials, acetonitrile was concentrated by vacuum. The crude compound was extracted with ethyl acetated and washed with water. The organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude product was purified by column chromatography using silica gel (230:400 mesh) with hexane/ethyl acetate (99:1) as eluent to yield the title compound **3j**. Isolated yield: 85%, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.15 (t, 3H, J = 7.6 Hz), 2.55 (q, 2H, J = 7.6 Hz), 3.50 (dd, 1H, J = 5.3, 16.8 Hz), 3.79 (dd, 1H, J = 8.4, 16.8 Hz), 5.27–5.30 (m, 1H), 5.85–5.86 (m, 1H), 5.93–5.94 (m, 1H), 7.14–7.25 (m, 3H), 7.37 (d, 1H, J = 7.6 Hz), 7.45 (t, 2H, J = 7.6 Hz), 7.55 (t, 2H, J = 6.9 Hz), 7.96 (d, 2H, J = 8.4 Hz), ^{13}C NMR (100 MHz, $CDCl_3$) δ: 12.0, 21.3, 37.0, 42.4, 104.4, 107.0, 127.0, 127.9, 128.1, 128.6, 128.9, 129.8, 133.1, 133.5, 136.8, 139.8, 153.0, 156.9, 197.2; ESI: 339 [M$^+$+1], 341 [M$^+$+2]. Anal. Calcd for $C_{21}H_{19}ClO_2$: C, 77.44, H 5.65. Found: C, 77.53; H, 5.62.
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14. *Minimum inhibitory concentration:* A sterile 96 well plate was used for this assay. Volume of 100 µL of test material in 10% (v/v) DMSO (concentration of 10 mg/mL) was pipetted into the first row of the plate. To all other wells 50 µL of Muller Hinton Broth was added. Serial dilutions were performed using a multi-channel pipette and the concentration of compound for serial dilution is 10 mg in the first row and tips were discarded after use such that each well had 50 µL of the test material in serially descending concentrations. To each well 10 µL of (sodium 2,3,-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)-carbonyl]-2*H*-tetrazolium) inner salt (XTT) indicator solution was added. Finally, 10 µL of bacterial suspension (5 × 106 cfu/mL) was added to each well to achieve a concentration of 5 × 105 cfu/mL. Each plate was wrapped loosely with cling film to ensure that bacteria did not become dehydrated. Each plate had a positive control (ciprofloxacin in serial dilution) in first column and the last two columns C_1 and C_2, comprising Muller Hinton Broth + indicator, with and without the bacterial suspension, respectively. The plates were prepared in triplicate, and placed in an incubator set at 37 °C for 18–24 h. Then the color change was assessed visually. This (XTT) tetrazolium salt assay measures the cells ability to convert the tetrazolium salt to the formazan product.
15. Any color changes from yellow to red were recorded as positive. The lowest concentration was taken as MIC value.
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19. *Determination of total antioxidant capacity:* Furan derived compounds 0.1 mL (mg/mL) was combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95 °C for 90 min. This solution was allowed to cool at room temperature and the absorbance of the solution was measured at 695 nm against a blank. The antioxidant activity was expressed as the number of equivalents of ascorbic acid. According to assay based on the reduction of Mo (VI)–(V) by the furan derived compounds and subsequent formation of green phosphate/Mo (V) complex at acidic pH.

20. *Scavenging activity of DPPH radical:* A concentrations (mg/ml) of 100 μL of the tested furan derived compounds were added to 2.9 mL of a 0.004% (w/v) ethanol solution of 1,1-dephenyl-2-picrylhydrazyl (DPPH). After 30 min of incubation period at room temperature, the absorbance was measured against a blank at 517 nm. Inhibition of free radical DPPH in percent (%) was calculated by: % scavenging effect = $[(A_{DPPH} - A_S)/A_{DPPH}] \times 100$. Where A_{DPPH} is the absorbance of the control reaction (containing all reagents except tested sample) and A_S is the absorbance of the tested sample reaction. Synthetic antioxidant reagents, butylated hydroxyanisole (BHA) and L-ascorbic acid were used as positive controls.