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# Design and synthesis of low molecular weight compounds with complement inhibition activity

Hoshang E. Master,[a,*] Shabana I. Khan[b,†] and Krishna A. Poojari[a]

[a]*Nadkarni Sakasa Research Laboratory, Department of Chemistry, St. Xavier's College, Mumbai 400 001, India*
[b]*National Centre for Natural Products Research, School of Pharmacy, University of Mississippi, MS 38677, USA*

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**Abstract**—An attempt was made to synthesize a series of non-cytotoxic low molecular weight compounds of varying substitutions and functionalities having pharmacophore activity like carbonyl compounds, carboxylic acid and bioisosteres like tetrazole and phenyl acrylic acid. The in vitro assay of these analogues for the inhibition of complement activity revealed significant inhibitory activity for varying substituents and, particularly, for bioisosteres, that is, tetrazole and phenyl acrylic acid derivatives.


## 1. Introduction

The human body is equipped with defence mechanisms that respond aggressively to infection or injury. This response is uniquely designed for such challenges whether caused by viruses, bacteria or any other matter harmful to the body. Once, the immune system recognizes a foreign invader, complement is activated to destroy or remove it.

The complement system is a potent mechanism for initiating and amplifying the process of inflammation in the human body. Along with the clotting, fibrinolysis and kallikrein–kinin systems, complement represents one of the complex enzyme systems of blood, which can be activated in specific cascade reactions upon a triggering stimulation. The ability to trigger a powerful, coordinated repertoire of antimicrobial reactions, including inflammation, opsonization and direct haemolysis is the best-known function of complement systems. It has been observed that complement-induced alterations in the cell surfaces as well as the interaction of receptors, including complement receptors, adhering to the vascular endothelium enhance inflammation.[1]

However, in some ways the body's first line of defence can also be its worst enemy. The complement system, unfortunately, when activated at the wrong time, can be responsible for unwanted physiological effects causing consequences like organ transplant rejection.

The complement system has been implicated as a factor in the exacerbation and propagation of tissue injury in numerous diseases including neurodegenerative disorders.[2] The involvement of complement in the early recognition phases of inflammatory response, as well as the wide array of proinflammatory consequences of complement activation, makes the complement system an attractive target for therapeutic intervention and has led to the isolation, design and synthesis of a variety of complement inhibitors[3,4] as well as the identification of small molecule compounds from the compound library.[5] The greater understanding of the role of the complement system in the pathogenesis of several diseases has increased the need for more specific and more potent complement inhibitors.

The complement mediated damage can be specifically prevented by specific inhibition of the classical complement pathway without affecting the antimicrobial functions of the complement system via the alternative pathway and the lectin pathway.[6]

Our interest in the development of complement inhibitors has emerged from a greater understanding of the involvement of the complement system in several disease processes.[7–11] Most of the work in this area has been

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* Corresponding author. Tel.: +91 22 2262 0661; fax: +91 22 2232 1912; e-mail: kpoojari@rediffmail.com
† Tel.: +1 662 915 1041; fax: +1 662 915 7062; e-mail: skhan@olemiss.edu

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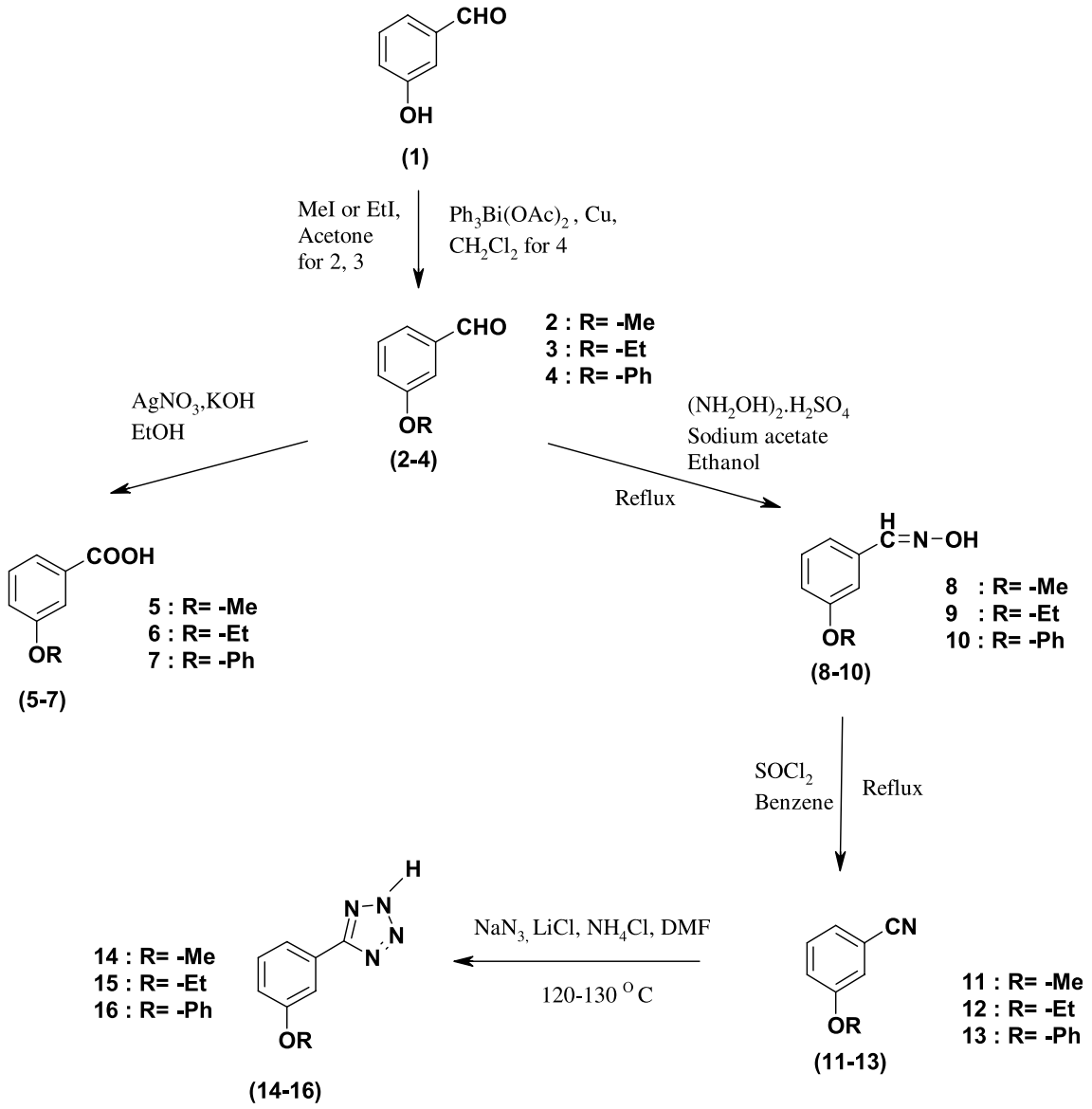
initiated from the identification of a natural product exhibiting complement inhibition activity[9–15] and further development of low molecular weight analogues to understand their structure–activity relationship (SAR) towards complement inhibition.

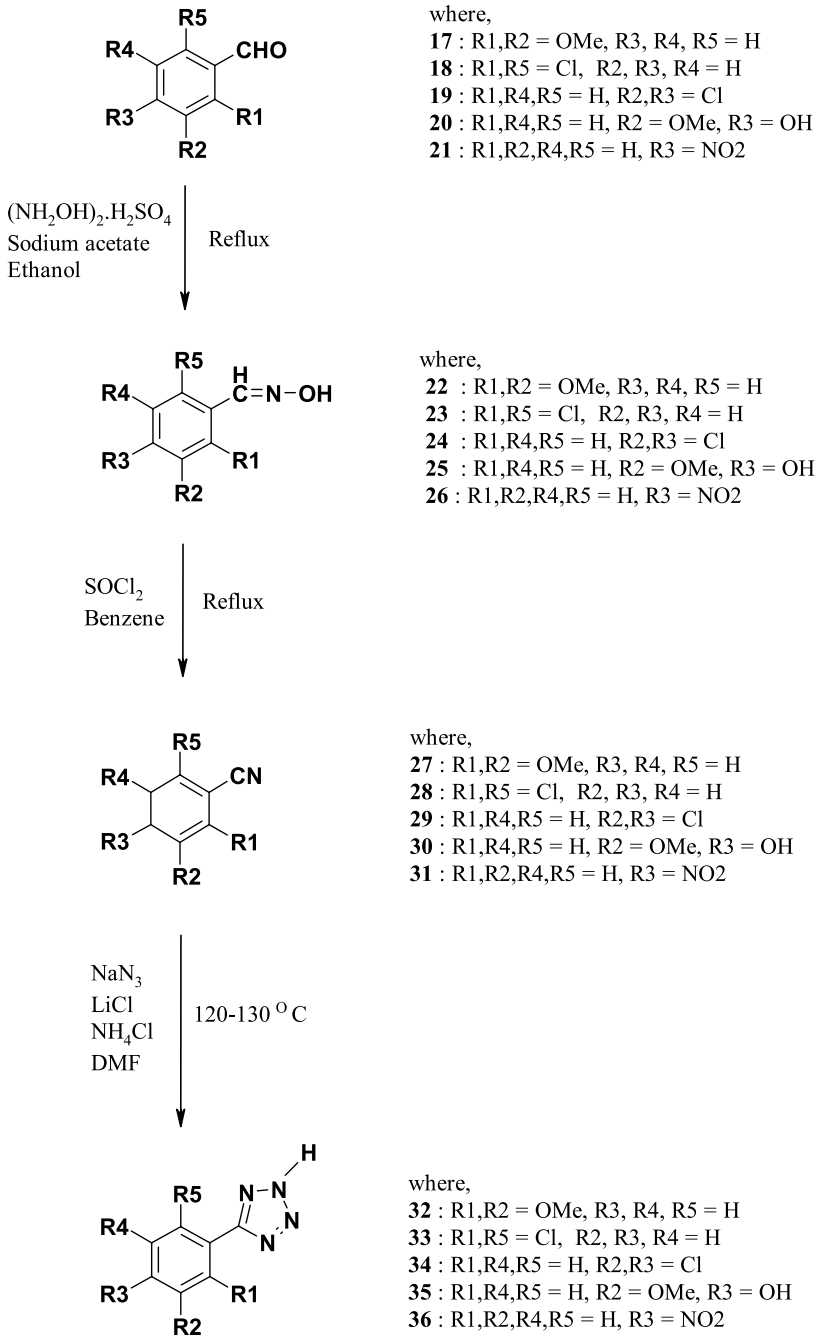
In our ongoing research programme, efforts were made to design and develop low molecular weight compounds, which can block the reactions involved in a complement response.[16] This earlier study was focussed on generating low molecular weight analogues with suitable functional groups exhibiting significant inhibition of complement activity.

In the present study, an attempt has been made to synthesize low molecular weight substituted aromatic ethers (**2**–**4**), carboxylic acids (**5**–**7**) and their bioisosteres (**14**–**16**) in an effort to develop more potent compounds. The observation that the tetrazoles act as excellent carboxylic acid bioisosteres[17,18] prompted us to further develop and design aromatic tetrazoles with varying substitutions. As a result, a series of substituted aromatic tetrazoles (**32**–**36** and **40**) were synthesized. The carbonyl functionality of the substituted aromatic compound was further replaced by an α,β-unsaturated carboxylic acid group resulting in cinnamic acid derivatives (**41**–**45**).

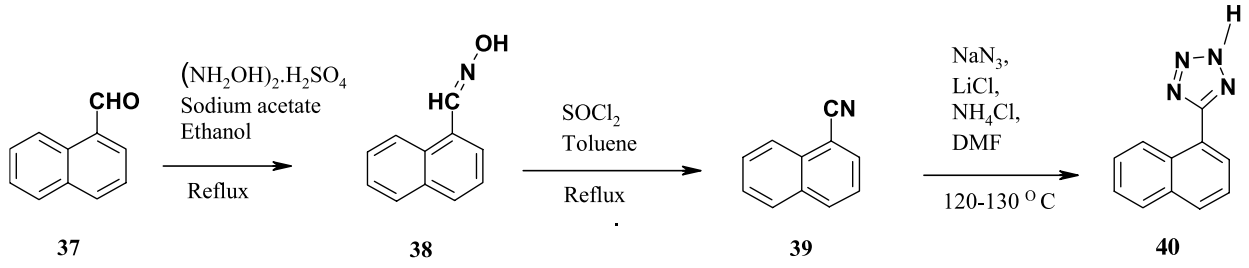
The synthetic strategy is outlined in Schemes 1–4.[19] As described in Scheme 1,[16] **2** and **3**, methoxy, ethoxy and phenoxy derivatives of **1** were successfully prepared by the reaction of **1** with iodomethane, iodoethane/potassium carbonate and triphenylbismuth diacetate/Cu,[20] furnishing **2**, **3** and **4** in quantitative yield (80%). In the next step, compounds **2**–**4** were oxidized by silver nitrate and potassium hydroxide in ethanol to yield the corresponding acid (**5**–**7**) as white crystalline solid in moderate yield (60%). Since tetrazole acts as an excellent bioisostere,[17,18] we introduced this ring into the target com-




**Scheme 1.** Synthesis of aromatic ethers, carboxylic acid and its bioisosteres.[16]

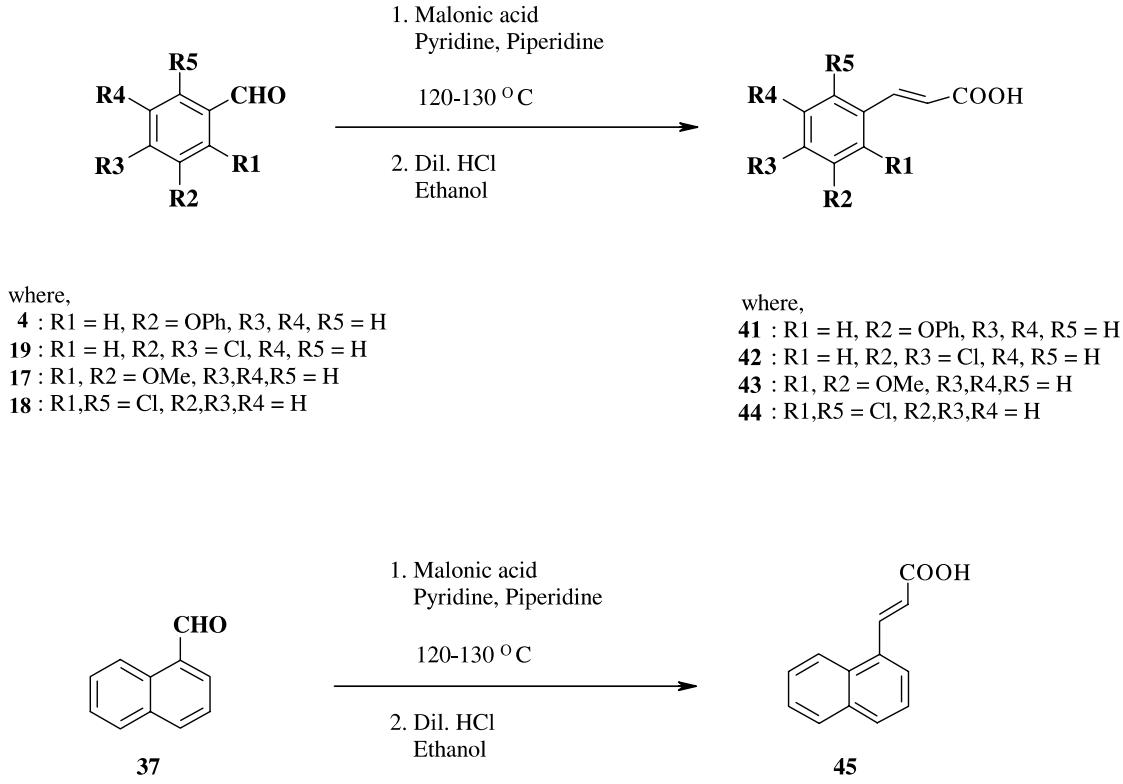



**Scheme 2.** Synthesis of aromatic tetrazole.




**Scheme 3.** Synthesis of naphthalene-1-tetrazole.

pounds. This was accomplished by three sequential reactions. Compounds **2**–**4**, **17**–**21** and **37** were treated with hydroxyl ammonium sulfate and sodium acetate in ethanol to afford the oxime (**8**–**10**, **22**–**26** and **38**) in quantitative yield. Then, the reaction of oxime (**8**–**10**, **22**–**26** and **38**) with thionyl chloride in benzene under reflux




**Scheme 4.** Synthesis of phenyl acrylic acid derivatives.

gave nitrile (**11**–**13**, **27**–**31** and **39**) in good yield. Finally, nitrile (**11**–**13**, **27**–**31** and **39**) was heated at 120–130 °C with ammonium azide (prepared in situ by the decomposition reaction of sodium azide and ammonium chloride) and lithium chloride in DMF to yield the corresponding tetrazoles (**14**–**16**, **32**–**36** and **40**) in low yield (40%).

Similarly, as described in Scheme 4, the corresponding cinnamic acid derivatives were prepared employing the Knoevenagel condensation.[21] Compounds **4**, **19**, **17**, **18** and **37** when heated with malonic acid in pyridine in the presence of piperidine yielded the corresponding substituted phenyl acrylic acid (cinnamic acid derivatives) (**41**–**45**) in good yield (85–90%).

The target compounds (**2**–**7**, **14**–**16**, **32**–**36**, **40** and **41**–**45**) were evaluated for their ability to inhibit the in vitro activation of human complement (classical pathway) as described earlier.[16,22] The intrinsic haemolytic activity of compounds was also determined by incubating them with sensitized sheep erythrocytes (RBCs) in the absence of complement and determining the complement independent lysis.

Compounds **2**–**7** and **14**–**16**, as reported earlier, were tested up to a maximum concentration of 4000 μM,[16] while the next series of compounds **32**–**36**, **40** and **41**–**45** were tested up to a maximum concentration of 1500 μM using a stock solution of 25 mM in DMSO.

Ursolic acid and oleanolic acid were used as positive controls. DMSO was used as the vehicle control.

The compounds were also tested for their in vitro cytotoxicity against mammalian cells (LLC-$PK_1$: kidney epithelial and VERO: kidney fibroblasts) using Neutral Red Assay[23] in a similar way as described earlier.[12]

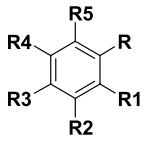
The results of complement inhibition activity are reported as $IC_{50}$, and the intrinsic haemolytic activity of each compound (RBC lysis) is reported as an $EC_{50}$ value in Table 1.

This study was aimed at designing and obtaining low molecular weight compounds analogous to the naturally occurring molecules exhibiting complement inhibitory activity.[13,16]

Functional groups like carbonyl groups were explored. An initial study was carried out with phenolic ethers having a meta-substituted carbonyl functionality. As a result, compounds **2**–**7** were synthesized and their in vitro complement inhibition activity was reported.[16] Based on the observation that tetrazole acts as an excellent carboxylic acid bioisostere,[17,18] compounds **14**–**16** were synthesized where the carbonyl functionality was replaced by a tetrazole ring, which subsequently improved the complement inhibiting activity of the compounds. Interestingly, all these compounds **2**–**7** and **14**–**16** were non-cytotoxic in vitro towards mammalian kidney cell lines.[16]

These initial results prompted us to extend our study to design a more specific inhibitor of complement activity. Compounds **32**–**36** and **40** were synthesized where the carbonyl group was replaced by tetrazole and varied substitutions on the phenyl ring were used to see their effects on complement activity.

**Table 1.** Inhibition of complement activity (classical pathway) and haemolytic activity of target molecules




| Compound[a] | R | Substitution[b] | Inhibition of complement $IC_{50}$ (μM)[d] | Haemolytic activity $EC_{50}$ (μM)[e] |
|---|---|---|---|---|
| **2** | CHO | R2: OMe | 919 | >4411 |
| **3** | CHO | R2: OEt | 1000 | >4000 |
| **4** | CHO | R2: OPh | 1388 | >3030 |
| **5** | COOH | R2: OMe | NA | 2960 |
| **6** | COOH | R2: OEt | NA | 2530 |
| **7** | COOH | R2: OPh | 1401 | >2803 |
| **14** | Tetrazole-5-yl | R2: OMe | 1562 | >3409 |
| **15** | Tetrazole-5-yl | R2: OEt | 631 | 2210 |
| **16** | Tetrazole-5-yl | R2: OPh | 798 | 954 |
| **32** | Tetrazole-5-yl | R1,R2: OMe | 61.9 | 338 |
| **33** | Tetrazole-5-yl | R1,R5: Cl | NA | NA |
| **34** | Tetrazole-5-yl | R2,R3: Cl | 247.6 | 681 |
| **35** | Tetrazole-5-yl | R2: OMe, R3: OH | 657.9 | 1500 |
| **36** | Tetrazole-5-yl | R3: NO2 | 236.5 | 752 |
| **40**[c] | Tetrazole-5-yl | 1-Naphthyl | 204.1 | 729.6 |
| **41** | Acrylic acid | R2: OPh | 23.96 | 595.8 |
| **42** | Acrylic acid | R2,R3: Cl | 21.6 | 650 |
| **43** | Acrylic acid | R1,R2: OMe | 57.14 | 338 |
| **44** | Acrylic acid | R1,R5: Cl | 545.5 | NA |
| **45**[c] | Acrylic acid | 1-Naphthyl | 75 | NA |
| Ursolic acid | | | 54.75 | >300 |
| Oleanolic acid | | | 79.95 | >300 |

[a] Data for **2**–**16** are from Ref. 16 and are included for comparative study.
[b] Those other than designated substituents are protons/hydrogen.
[c] In the case of the naphthyl derivative, the principal substituent functionality (R) is α- or 1-position. NA, not active.
[d] The concentration of the compound required to inhibit complement-mediated haemolysis of sensitized sheep RBCs by 50% compared to vehicle control. $IC_{50}$ values were obtained from dose–response curves of percent inhibition.
[e] The concentration of compound effective to cause 50% haemolysis in the absence of complement. Values were obtained from dose–response curves of percent haemolysis.

A significant improvement in complement inhibition activity was observed with $IC_{50}$ values much lower than the positive controls in some cases.

This significant development led us to explore another functional group that replaces the carbonyl group, that is, the acrylic acid (derivatives of cinnamic acid). Similar substitutions were employed while synthesizing these compounds, that is, **41**–**45**.

Further improvement in the activity of these compounds was observed, some superior to the bioisosteres (refer to Table 1).

Thus, in this study, it was revealed that apart from bioisosteres of carbonyl compounds, that is, tetrazoles, even acrylic acid substitutions significantly improve the activity of compounds in comparison to that of unsubstituted carbonyl compounds (see Fig. 1).

It is noteworthy that most of these molecules (**32**–**36**, **40** and **41**–**45**) are also not cytotoxic in vitro towards mammalian cell lines (kidney cells). Among the 20 compounds synthesized, compounds **41** and **42** were the most potent in inhibiting complement activity with $IC_{50}$ values as low as 23.96 and 21.6 μM, respectively.

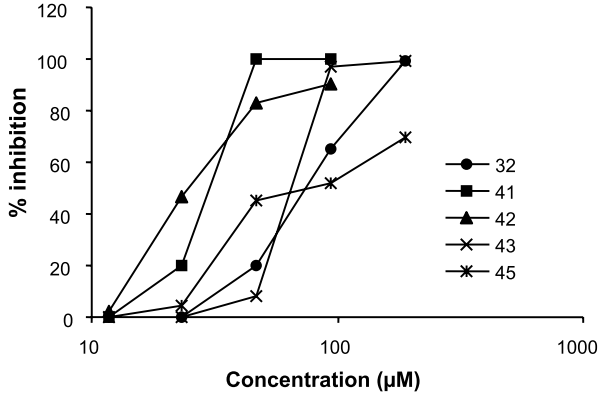



**Figure 1.** Inhibition of complement activity: dose–response curves of representative compounds.

Corresponding analogues of **41**, that is, aldehyde (**4**), carboxylic acid (**7**) and bioisosteres tetrazole (**16**) showed complement activity at higher concentrations (refer to Table 1).

Also, in comparison to compound **42**, its tetrazole (**34**) equivalent exhibited complement activity at 247.6 μM. In the above two cases (compounds **41** and **42**), it is clear that acrylic acid substituent significantly improves inhi-

bition activity. However, haemolytic activity was observed at much higher concentrations (refer to Table 1).

The disubstituted molecules (2,3-dimethoxy) **32** and **43** inhibited complement activity to a similar extent with $IC_{50}$ values of 61.9 and 57.14 μM, respectively, and were much more potent than the monosubstituted analogues **2** and **14** with $IC_{50}$ values of 919 and 1562 μM, respectively.

Compound **35**, having meta-substituted ether along with *para*-hydroxy substitution, was more effective ($IC_{50}$ 657.9 μM) as compared to monosubstituted ether **14** ($IC_{50}$ 1562 μM).

Owing to superior complement activity by compound **42**, other disubstituted derivatives were explored. Compounds with 2,6-dichloro-substituted tetrazoles **33** did not show any complement activity as against 3,4-dichloro-substituted tetrazoles **42**. Replacement of tetrazole with acrylic acid functionality in compound **44** gained activity ($IC_{50}$ 545.5 μM), while no haemolytic activity was observed.

This result further confirms the improved effects of acrylic acid over tetrazoles in exhibiting complement activity.

In one of the cases, a randomly selected molecule was synthesized having an electron-withdrawing group in the para position. Compound **36**, having *p*-nitro substitution, also exhibited complement inhibitory activity with an $IC_{50}$ of 236.8 μM and haemolytic activity with an $EC_{50}$ of 752 μM. This compound also exhibited mild cytotoxicity in kidney fibroblasts with an $IC_{50}$ of 88.4 μM and was not cytotoxic to kidney epithelial cells up to 125 μM. This example introduces one more substituent exhibiting a pharmacophoric effect, which can be further explored.

Finally, the effect of the increase in the number of rings was studied the to understand the effect of natural products (terpenes, steroids having polycyclic ring system) on the complement activity.

Carbonyl functionality of naphthaldehyde was replaced by its bioisosteres, that is, converted to tetrazoles (**40**) and acrylic acid (**45**). Naphthalene-1-acrylic acid (**45**) showed better activity than tetrazole (**40**) with $IC_{50}$ values of 75 and 204.1 μM, respectively.

Although tetrazole (**40**) showed haemolytic activity at a higher concentration ($EC_{50}$ = 729.6 μM), no haemolytic activity was observed for acrylic acid (**45**) up to a concentration of 1500 μM. Based on this study it can be concluded that the tetrazoles significantly improve the activity of aromatic substituted compounds, which is further enhanced by replacement of carbonyl functionality with acrylic acid.

Overall, after the initial study with meta-substituted ethers, a set of randomly selected molecules were synthesized and the results obtained clearly justify the exercise of screening the target compounds to obtain some lead molecules with desired functionality and exhibiting significant inhibition of complement activity.

**Table 2.** Cytotoxicity of compounds to mammalian kidney cells

| Compound[a] | $IC_{50}$ (μM) | |
|---|---|---|
| | Vero cells | LLC-$PK_1$ cells |
| **32** | 120 | 125 |
| **33** | NC | NC |
| **34** | >125 | >125 |
| **35** | NC | NC |
| **36** | 88.4 | >125 |
| **40** | >125 | >125 |
| **41** | NC | NC |
| **42** | NC | NC |
| **43** | NC | NC |
| **44** | NC | NC |
| **45** | NC | NC |
| Ursolic acid | NC | 20 |
| Oleanolic acid | NC | 100 |

The highest concentration tested was 125 μM. Vero, monkey kidney fibroblasts. LLC-$PK_1$, pig kidney epithelial cells. NC, non-cytotoxic up to 125 μM.

[a] Data for **2**–**16** were already discussed in Ref. 16.

In earlier studies, most of the compounds reported to inhibit complement activity have also been reported to have cytotoxic properties and their therapeutic indices (TI: ratio of $IC_{50}$ for cytotoxicity/$IC_{50}$ for complement inhibition) have been very low.[7–9,12,24]

It is noteworthy that the molecules reported in this study were non-cytotoxic in vitro towards mammalian kidney cells up to a fairly high concentration (125 μM) (see Table 2). These molecules, thus, show better therapeutic indices (compound **32**, **43**, **41** and **42** with TI values of 2.0, 2.2, 5.2 and 5.8, respectively) and seem to be promising as complement inhibitors and safer against the human system in vitro.

In view of the above findings, these low molecular weight compounds seem to be potential candidates for further screening to study their effects on complement system pathways in more detail.

## 2. Experimental

All chemicals, reagents and solvents were purchased from authentic commercial sources where available and were used without further purification.

Intermediates were characterized by FTIR, $^1$H NMR and mass spectrometry (MS).

### 2.1. 3-Methoxybenzaldehyde (2)[16]

3-Hydroxy benzaldehyde (**1**) (1 g, 8.196 mmol) was dissolved in 5 ml acetone and cooled to below 5 °C. Methyl iodide (2.3 g, 16.4 mmol) was dissolved in 5 ml chilled acetone and was added dropwise maintaining the temperature below 5 °C. The reaction was refluxed by circulating chilled water through a reflux condenser. The crude product obtained was distilled under reduced pressure to obtain a quantitative yield of the desired product (1.0 g, 90%). (GC: 100%) IR ($cm^{-1}$) (neat): 1710 (C=O), 1270–

1260 (unsym C–O–C); $^1$H NMR ($CDCl_3$, $\delta$ ppm): 3.86 (s, 3H), 7.65 (m, 4H), 8.45 (s, 1H); MS: *m/z* 136 ($M^+$).

### 2.2. 3-Ethoxybenzaldehyde (3)[16]

The procedure followed was the same as that for compound **2**.

3-Hydroxy benzaldehyde (**1**) (1 g, 8.196 mmol) was reacted with ethyl iodide (2.55 g, 16.392 mmol) to yield a crude product. The crude product was distilled to obtain the desired product in quantitative yield (1.094 g, 89%). (GC: 100%) IR ($cm^{-1}$) (neat): 1710–1700 (C=O), 1270–1260 (unsym C–O–C); $^1$H NMR ($CDCl_3$, $\delta$ ppm): 1.35 (t, 3H), 4.1 (q, 2H), 7.2–7.8 (m, 3H), 8.7 (s, 1H); MS: *m/z* 150 ($M^+$).

### 2.3. 3-Phenoxybenzaldehyde (4)[16,20]

1.5 g (12.3 mmol) of 3-hydroxy benzaldehyde (**1**) dissolved in 10 ml dichloromethane was added to the mixture of $Ph_3Bi(OAc)_2$ (8.23 g, 14.76 mmol) (synthesized as described in Ref. 20) and Cu powder (0.078 g, 1.23 mmol) in 5 ml dichloromethane under $N_2$ atmosphere. The mixture was stirred at room temperature for 24 h without $N_2$ atmosphere. Upon completion of the reaction, the crude product was filtered and the organic layer was given an aqueous wash. The organic layer was dried over $MgSO_4$ and concentrated to obtain a crude product, which was distilled under reduced pressure to obtain a pure product (1.8 g, 75%). (GC: 100%) IR ($cm^{-1}$) (neat): 1712 (C=O), 1585, 1453 (C=C, aromatic), 1262 (unsym C–O–C); $^1$H NMR ($CDCl_3$, $\delta$ ppm): 7.2–8.2 (m, 9H), 9.2 (s, 1H); MS: *m/z* 198 ($M^+$).

### 2.4. 3-Methoxy benzoic acid (5)[16]

3-Methoxybenzaldehyde (**2**) (2 g, 0.014 mol) is oxidized with silver nitrate (5.78 g, 0.03426 mol) in the presence of potassium hydroxide (4.4 g, 0.0785 mol) as base in 20 ml ethanol at room temperature to obtain the desired acid in moderate yield. The product was further purified in ether (1.45 g, 65%). IR ($cm^{-1}$) (KBr pellet): 3415 (O–H), 1639 (COOH), 1463 (C=C), 1381 (unsym C–O–C). $^1$H NMR ($CDCl_3$, $\delta$ ppm): 3.86 (s, 3H), 7.38 (m, 4H), 9.45 (s, 1H); MS: *m/z* 152 ($M^+$).

### 2.5. 3-Ethoxy benzoic acid (6)[16]

The procedure followed was the same as that given in example **4**. 3-Ethoxybenzaldehyde (**3**) (2 g, 0.0133 mol) was oxidized to yield the corresponding acid in moderate yield (1.328 g, 60%). IR ($cm^{-1}$) (KBr pellet): 3550 (O–H), 1695 (C=O), 1598, 1453 (C=C), 1259 (unsym C–O–C); $^1$H NMR ($CDCl_3$, $\delta$ ppm): 1.58 (t, 3H), 3.88 (q, 2H), 7.305 (m, 4H), 9.6 (s, 1H); MS: *m/z* 166 ($M^+$).

### 2.6. 3-Phenoxy benzoic acid (7)[16]

The procedure followed was the same as that given in example **4**. 3-Phenoxybenzaldehyde (**4**) (2 g, 0.0101 mol) was oxidized to yield the corresponding acid in moderate yield (1.34 g, 62%). IR ($cm^{-1}$) (KBr pellet): 3545–3480 (O–H), 1680 (C=O), 1585, 1460 (C=C), 1280 (unsym C–O–C); $^1$H NMR ($CDCl_3$, $\delta$ ppm): 6.8–8.2 (m, 9H), 9.5 (s, 1H); MS: *m/z* 214 ($M^+$).

### 2.7. 3-Methoxyphenyltetrazole (14)[16]

Following a sequence of reactions starting with the synthesis of 3-methoxybenzaldoxime (**8**) by reacting 3-methoxybenzaldehyde (**2**) (1.5 g, 0.011 mol) with hydroxyl ammonium sulfate (2.16 g, 0.0132 mol) in the presence of sodium acetate (1.08 g, 0.0132 mol) in 10 ml ethanol. Followed by dehydration of oxime (**8**) in thionyl chloride (1.47 g, 12.42 mmol) and 10 ml benzene to yield the corresponding 3-methoxyphenylcarbonitrile (**11**) in quantitative yield. Nitrile (**11**) is further heated at 120–130 °C with ammonium azide (prepared in situ by the decomposition reaction of sodium azide (0.564 g, 8.68 mmol) and ammonium chloride (0.422 g, 7.89 mmol)) and lithium chloride (0.334 g, 7.89 mmol) in 10 ml DMF for 24 h. Following a workup of the reaction mixture, a crude product was obtained, which on further purification yielded the desired tetrazole (**14**) in low yield (0.776 g, 40%). IR ($cm^{-1}$) (KBr pellet): 2370 (–N=N–N–), 1573, 1455 (C=C), 1255 (unsym C–O–C); $^1$H NMR ($CD_3OD$, $\delta$ ppm): 3.78 (s, 3H), 7.4–7.8 (m, 4H); MS: *m/z* 176 ($M^+$).

### 2.8. 3-Ethoxyphenyltetrazole (15)[16]

Following a similar sequence of reactions as given in example **7**, a series of reactions starting with 3-ethoxybenzaldehyde (**3**) (1.5 g, 0.01 mol) reacting with hydroxyl ammonium sulfate (1.96 g, 0.012 mol) in the presence of sodium acetate (0.984 g, 0.0112 mol) in 10 ml ethanol to yield 3-ethoxybenzaldoxime (**9**) in quantitative yield. Oxime (**9**) is further treated with thionyl chloride (1.4 g, 0.0117 mol) in 10 ml benzene to yield 3-ethoxybenzonitrile (**12**) quantitatively. Nitrile (**12**) is further heated at 120–130 °C with ammonium azide (prepared in situ by the decomposition reaction of sodium azide (0.535 g, 8.23 mmol) and ammonium chloride (0.4 g, 7.48 mmol)) and lithium chloride (0.317 g, 7.48 mmol) in 10 ml DMF for 24 h. Following workup of the reaction mixture, a crude product was obtained, which on further purification yielded the desired tetrazole (**15**) in low yield (0.703 g, 37%). IR ($cm^{-1}$) (KBr pellet): 2365 (–N=N–N–), 1585, 1466 (C=C), 1263 (unsym C–O–C); $^1$H NMR ($CD_3OD$, $\delta$ ppm): 1.445 (t, 3H), 4.0819 (q, 2H), 7–7.8 (m, 4H); MS: *m/z* 190 ($M^+$).

### 2.9. 3-Phenoxyphenyltetrazole (16)[16]

Following a similar sequence of reactions as given in example **7**, a series of reactions starting with 3-phenoxybenzaldehyde (**4**) (1.5 g, 7.57 mmol) reacting with hydroxyl ammonium sulfate (1.49 g, 9.084 mmol) in the presence of sodium acetate (0.745 g, 9.084 mmol) in 10 ml ethanol to yield 3-phenoxybenzaldoxime (**10**) in quantitative yield. Oxime (**10**) is further treated with thionyl chloride (0.837 g, 7.0375 mmol) in 10 ml benzene to yield 3-phenoxybenzonitrile (**13**) quantitatively. Nitrile (**13**) is further heated at 120–130 °C with ammo-

nium azide (prepared in situ by the decomposition reaction of sodium azide (0.348 g, 5.357 mmol) and ammonium chloride (0.26 g, 4.87 mmol)) and lithium chloride (0.206 g, 4.87 mmol) in 10 ml DMF for 24 h. Following workup of the reaction mixture, a crude product was obtained, which on further purification yielded the desired tetrazole (**16**) in low yield (0.703 g, 39%). IR ($cm^{-1}$) (KBr pellet): 2375 (–N=N–N–), 1595, 1465 (C=C), 1257 (unsym C–O–C); $^1H$ NMR ($CD_3OD$, $\delta$ ppm): 6.8–8.1 (m, 9H); MS: *m/z* 238 ($M^+$).

### 2.10. 2,3-Dimethoxyphenyltetrazole (32)

Following a similar sequence of reactions as given in example **7**, a series of reactions starting with 2,3-dimethoxybenzaldehyde (**17**) (1.0 g, 6.024 mmol) reacting with hydroxyl ammonium sulfate (1.18 g, 7.23 mmol) in the presence of sodium acetate (0.592 g, 7.23 mmol) in 10 ml ethanol to yield 2,3-dimethoxybenzaldoxime (**22**) in quantitative yield. Oxime (**22**) is further treated with thionyl chloride (0.788 g, 6.625 mmol) in 10 ml benzene to yield 2,3-dimethoxybenzonitrile (**27**) quantitatively. Nitrile (**27**) is further heated at 120–130 °C with ammonium azide (prepared in situ by the decomposition reaction of sodium azide (0.35 g, 5.39 mmol) and ammonium chloride (0.262 g, 4.9 mmol)) and lithium chloride (0.207 g, 4.9 mmol) in 10 ml DMF for 24 h. Following workup of the reaction mixture, a crude product was obtained, which on further purification yielded the desired tetrazole (**32**) in low yield (0.409 g, 33%). IR ($cm^{-1}$) (KBr pellet): 3202 (N–H), 2345 (N=N–N–), 1597, 1472 (C=C), 1257 (unsym C–O–C); $^1H$ NMR ($CD_3OD$, $\delta$ ppm): 3.8–3.9 (s, 3H), 4.8 (s, 3H), 6.8–7.8 (m, 3H); MS: *m/z* 206 ($M^+$).

### 2.11. 3,4-Dichlorophenyltetrazole (34)

Following a similar sequence of reactions as given in example **7**, a series of reactions starting with 3,4-dichlorobenzaldehyde (**19**) (1.0 g, 5.714 mmol) reacting with hydroxyl ammonium sulfate (1.125 g, 6.85 mmol) in the presence of sodium acetate (0.56 g, 6.85 mmol) in 10 ml ethanol to yield 3,4-dichlorobenzaldoxime (**24**) in quantitative yield. Oxime (**24**) is further treated with thionyl chloride (0.73 g, 6.118 mmol) in 10 ml benzene to yield 3,4-dichlorobenzonitrile (**29**) quantitatively. Nitrile (**29**) is further heated at 120–130 °C with ammonium azide (prepared in situ by the decomposition reaction of sodium azide (0.324 g, 4.98 mmol) and ammonium chloride (0.2426 g, 4.535 mmol)) and lithium chloride (0.192 g, 4.535 mmol) in 10 ml DMF for 24 h. Following workup of the reaction mixture, a crude product was obtained, which on further purification yielded the desired tetrazole (**34**) in low yield (0.5 g, 41%). IR ($cm^{-1}$) (KBr pellet): 2474 (N=N–N–), 1575, 1475 (C=C); $^1H$ NMR ($CD_3OD$, $\delta$ ppm): 7.4–8.4 (m, 3H); MS: *m/z* 215 ($M^+$).

### 2.12. 4-Hydroxy-3-methoxyphenyltetrazole (35)

Following a similar sequence of reactions as given in example **7**, a series of reactions starting with 4-hydroxy-3-methoxybenzaldehyde (vanillin) (**20**) (1.5 g, 9.868 mmol) reacting with hydroxyl ammonium sulfate (1.94 g, 0.0118 mol) in the presence of sodium acetate (0.9676 g, 9.868 mmol) in 10 ml ethanol to yield 4-hydroxy-3-methoxybenzaldoxime (**25**) in quantitative yield. Oxime (**25**) is further treated with thionyl chloride (1.2 g, 0.101 mol) in 10 ml benzene to yield 4-hydroxy-3-methoxybenzonitrile (**30**) quantitatively. Nitrile (**30**) is further heated at 120–130 °C with ammonium azide (prepared in situ by the decomposition reaction of sodium azide (0.505 g, 7.7777 mmol) and ammonium chloride (0.378 g, 7.07 mmol)) and lithium chloride (0.299 g, 7.07 mmol) in 10 ml DMF for 24 h. Following workup of the reaction mixture, a crude product was obtained, which on further purification yielded the desired tetrazole (**35**) in low yield (0.75 g, 40%). IR ($cm^{-1}$) (KBr pellet): 3398 (O–H), 2368 (N=N–N–), 1255 (unsym C–O–C). $^1H$ NMR ($CD_3OD$, $\delta$ ppm): 3.86 (s, 3H), 6.8–7.8 (m, 3H); MS: *m/z* 192 ($M^+$).

### 2.13. 4-Nitrophenyltetrazole (36)

Following a similar sequence of reactions as given in example **7**, a series of reactions starting with 4-nitrobenzaldehyde (**21**) (1.2 g, 7.947 mmol) reacting with hydroxyl ammonium sulfate (1.505 g, 9.536 mmol) in the presence of sodium acetate (0.782 g, 9.536 mmol) in 10 ml ethanol to yield 4-nitrobenzaldoxime (**26**) in quantitative yield. Oxime (**26**) is further treated with thionyl chloride (1.093 g, 9.186 mmol) in 10 ml benzene to yield 4-nitrobenzonitrile (**31**) quantitatively. Nitrile (**31**) is further heated at 120–130 °C with ammonium azide (prepared in situ by the decomposition reaction of sodium azide (0.525 g, 8.08 mmol) and ammonium chloride (0.392 g, 7.3446 mmol)) and lithium chloride (0.311 g, 7.3446 mmol) in 10 ml DMF for 24 h. Following workup of the reaction mixture, a crude product was obtained, which on further purification yielded the desired tetrazole (**36**) in low yield (0.62 g, 41%). IR ($cm^{-1}$): 2345 (N=N–N–), 1570, 1451 (C=C); $^1H$ NMR ($CD_3OD$, $\delta$ ppm): 7.2–8.4 (m, 4H); MS: *m/z* 191 ($M^+$).

### 2.14. α-Naphthyltetrazole (40)

Following a similar sequence of reactions as given in example **7**, a series of reactions starting with α-naphthaldehyde (**37**) (1.0 g, 6.41 mmol) reacting with hydroxyl ammonium sulfate (1.26 g, 7.69 mmol) in the presence of sodium acetate (0.603 g, 7.69 mmol) in 10 ml ethanol to yield α-naphthaldoxime (**38**) in quantitative yield. Oxime (**38**) is further treated with thionyl chloride (0.809 g, 6.798 mmol) in 10 ml benzene to yield naphthalene-1-carbonitrile (**39**) quantitatively. Nitrile (**39**) is further heated at 120–130 °C with ammonium azide (prepared in situ by the decomposition reaction of sodium azide (0.355 g, 5.464 mmol) and ammonium chloride (0.265 g, 4.967 mmol)) and lithium chloride (0.21 g, 4.967 mmol) in 10 ml DMF for 24 h. Following workup of the reaction mixture, a crude product was obtained, which on further purification yielded the desired tetrazole (**40**) in low yield (0.47 g, 38%). IR ($cm^{-1}$) (KBr pellet): 2380 (N=N–N–), 1570, 1451 (C=C); $^1H$ NMR ($CD_3OD$, $\delta$ ppm): 6.7–7.8 (m, 7H); MS: *m/z* 196 ($M^+$).

### 2.15. 3-Phenoxycinnamic acid (41)

1.95 g (7.575 mmol) 3-phenoxybenzaldehyde (**4**) and malonic acid (0.945 g, 9.09 mmol) was heated to 80–90 °C in pyridine (1.212 g, 15.15 mmol) in the presence of piperidine (0.0774 g, 0.909 mmol). The resulting mixture was poured over ice-cold 3 ml concentrated hydrochloric acid and was stirred to obtain the desired cinnamic acid derivative (**41**) as a white solid (1.45 g, 80%). IR ($cm^{-1}$) (KBr pellet): 1694 (C=O), 1633 (C=C, alkene), 1566, 1471 (C=C, aromatic), 1221 (unsym C–O–C). $^1H$ NMR ($CDCl_3$, $\delta$ ppm): 6.4–6.6 (d, 2H), 7.2–8.0 (m, 9H); MS: *m*/*z* 240 ($M^+$).

### 2.16. 3,4-Dichlorocinnamic acid (42)

Following the same procedure as given in example **16**; 3,4-dichlorobenzaldehyde (**19**) (1.0 g, 5.7143 mmol) was treated with malonic acid (0.713 g, 6.857 mmol) in pyridine (0.914 g, 11.43 mmol) and in the presence of piperidine (0.058 g, 0.6857 mmol) yielding compound **42** in quantitative yield (1.24 g, 85%). IR ($cm^{-1}$) (KBr pellet): 1694 (C=O), 1622 (C=C, alkenes), 1553, 1472 (C=C, aromatic). $^1H$ NMR ($CDCl_3$, $\delta$ ppm): 6.2–6.6 (d, 2H), 7.6 (m, 3H); MS: *m*/*z* 217 ($M^+$).s

### 2.17. 2,3-Dimethoxycinnamic acid (43)

Following the same procedure as given in example **16**; 2,3-dichlorobenzaldehyde (**17**) (1.3 g, 7.83 mmol) was treated with malonic acid (0.977 g, 9.396 mmol) in pyridine (1.253 g, 15.66 mmol) and in the presence of piperidine (0.08 g, 0.9396 mmol) yielding compound **43** in quantitative yield (1.44 g, 89%). IR ($cm^{-1}$) (KBr pellet): 1684 (C=O), 1634 (C=C, alkenes), 1576, 1484 (C=C, aromatic), 1266 (unsym C–O–C), 950 (O–H bend); $^1H$ NMR ($CDCl_3$, $\delta$ ppm): 3.8–4.0 (s, 6H), 6.4–6.8 (d, 2H), 7.0–7.2 (m, 3H); MS: *m*/*z* 207 ($M^+$).

### 2.18. 2,6-Dichlorocinnamic acid (44)

Following the same procedure as given in example **16**; 2,5-dichlorobenzaldehyde (**18**) (0.85 g, 4.857 mmol) was treated with malonic acid (0.6 g, 5.828 mmol) in pyridine (0.777 g, 9.714 mmol) and in the presence of piperidine (0.0496 g, 0.5828 mmol) yielding compound **44** in quantitative yield (0.97 g, 92%). IR ($cm^{-1}$) (KBr pellet): 1690 (C=O), 1625 (C=C, alkene), 1555, 1465 (C=C, aromatic); $^1H$ NMR ($CDCl_3$, $\delta$ ppm): 6.6–6.8 (d, 2H), 7.0–7.6 (m, 3H); MS: *m*/*z* 217 ($M^+$).

### 2.19. α-Naphthylacrylic acid (45)

Following the same procedure as given in example **16**; α-naphthaldehyde (**37**) (1.0 g, 6.41 mmol) was treated with malonic acid (0.8 g, 7.69 mmol) in pyridine (1.025 g, 12.82 mmol) and in the presence of piperidine (0.0655 g, 0.7692 mmol) yielding compound **45** in quantitative yield (1.2 g, 95%). IR ($cm^{-1}$) (KBr pellet): 1685 (C=O), 1616 (C=C, alkenes), 1507, 1424 (C=C, aromatic); $^1H$ NMR ($CDCl_3$, $\delta$ ppm): 6.4–6.8 (d, 2H), 7.6–8.4 (m, 7H); MS: *m*/*z* 198 ($M^+$).

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