Two-Step Macrocycle Synthesis by Classical Ugi Reaction

Eman M. M. Abdelraheem,†‡ Samad Khaksar,† Katarzyna Kurpielska,§ Justyna Kalinowska-Tluścił,§ Shabnam Shaabani,‖ and Alexander Dömling*,†

†Department of Drug Design, University of Groningen, A. Deusinglaan 1, 9713 AV Groningen, The Netherlands
‡Chemistry Department, Faculty of Science, Sohag University, Sohag, 82524, Egypt
§Faculty of Chemistry, Jagiellonian University, Gronostajowa 2, 30-387 Krakow, Poland
‖Chemistry Department, Faculty of Science, Sohag University, Sohag, 82524, Egypt

ABSTRACT: The direct nonpeptidic macrocycle synthesis of α-isocynano-ω-amines via the classical Ugi four-component reaction (U-4CR) is introduced. Herein an efficient and flexible two-step procedure to complex macrocycles is reported. In the first step, the reaction between unprotected diamines and isocyanocarboxylic acids gives high diversity of unprecedented building blocks in high yield. In the next step, the α-isocynano-ω-amines undergo a U-4CR with high diversity of aldehydes and carboxylic acids in a one-pot procedure. This synthetic approach is short and efficient and leads to a wide range of macrocycles with different ring sizes.

INTRODUCTION

The Ugi four-component reaction (U-4CR) is a widely used multicomponent reaction (MCR) to provide a general route to diverse peptides, macrocycles, and other complex small molecules.1,2 This reaction has emerged as a powerful synthetic method for organic and pharmaceutical targets. Among MCRs, isocyanide-based multicomponent reactions (IMCRs) play an important role in pharmaceutical and drug discovery research3−7 and provide access to more diverse, complex, and novel scaffolds including small molecules and macrocycles. Macrocycles as intermediates between small molecules and biologics are useful to target flat, large, and featureless protein−protein interfaces.8,9 Artificial macrocycles promise to provide better control over synthetizability and over their physicochemical properties resulting in drug-like properties. However, there are only very few general and short synthetic routes toward macrocycles. Therefore, we report here such a general and short two-step synthesis of macrocycles using the Ugi reaction.

Macrocycles can be synthesized through MCRs by using bifunctional substrates. Faiil et al. first used N,C-unprotected tri- and hexaepitides to synthesize bioactive cyclic hexapeptides.10 Wessjohann et al. used homobifunctional starting materials to synthesize macrocycles using Ugi reactions.11 Yudin et al. introduced formylaziridines as bifunctional Ugi starting materials to synthesize spectacular macrocycles.12,13 Recently, Dömling et al. has shown the great impact of the direct use of bifunctional substrates such as α-isocynano-ω-carboxylic acids14 and α-carboxylic acid-ω-amines15 in macrocycle synthesis via the Ugi reaction (Figure 1). Of all six possible permutations of bifunctional substrates for macrocyclizations via the Ugi reaction, three have been already realized, while the last three still deserve validation: α-isocynano-ω-carboxylic acids, α-carboxylic acid-ω-amines, α-isocynano-ω-amines, α-carboxylic acid-ω-aldehydes, α-isocynano-ω-aldehyde, and α-amino-ω-aldehydes. In light of our extended research interest in MCRs and our previous experience in the chemistry of macrocycles, herein we report the use of α-isocynano-ω-amine for the synthesis of macrocycles via the Ugi-macrocyclization reaction.

RESULTS AND DISCUSSION

The first step of our current work is an extension of our recent report on using α-isocynano-ω-amines as building blocks in the cyclization reaction.16 We started our study by the synthesis of amino isocyanides via coupling of diamines with isocyanide esters under protecting group free conditions. Their synthesis and isolation is demanding due to the highly polar nature of α,ω-amino isocyanides. Therefore, various solvents such as chloroform, dichloromethane (DCM), methanol, water, tetrahydrofuran, ethanol, and trifluoroethanol were tested at room temperature (Table 1). Screening of different solvents revealed that dioxane was the best solvent for this process. Purification was performed by preparative column chromatography on silica (60−200 μm) using 1:1 dichloromethane:ethyl acetate as eluent A and ammonia in methanol 5% as eluent B in a gradient method. Under the optimized conditions, ten α-isocynano-ω-amines of different lengths were synthesized from commercially available diamines in good purity and yields, each on a gram scale (Scheme 1).
In the next step, the macrocyclic ring closure was carried out by an U-4CR under optimized conditions using 1 equiv of an oxo component and an acid (Scheme 2). The optimization was performed by using N-(5-aminopentyl)-5-isocyanopentanamide, paraformaldehyde, and 2-phenylacetic acid as a model reaction. The reaction did not proceed in 1.0 M methanol solution. The same reaction was carried out in different dilutions of methanol, and it was found that a highly diluted 0.01 M equimolar mixture of reactants in methanol gives the 15-membered macrocycle 6a in good yields (60%). Although trifluoroethanol (65% yield) was slightly superior to MeOH, we chose MeOH for further scope and limitation studies due to the higher price of TFE. Polar aprotic solvents such as THF and CH₃CN gave the product in moderate yields of 30% and 22%, respectively, at room temperature. Next, different Lewis acids such as ZnCl₂ in MeOH and TFE as a solvent were screened. It was found that ZnCl₂ in MeOH affords product in good yield (43%). Under sonication conditions, however, the reaction led to low yield of the product (Table 1).

With the optimized reaction conditions in hand, the scope and limitations of the Ugi-macrocyclization reaction were further investigated by synthesizing 15 different macrocycles (12–17 membered ring size) which are shown in Scheme 2. In this reaction, several commercially available carboxylic acids, aliphatic and aromatic aldehydes, and ketones as oxo-components assemble to afford macrocyclic derivatives in good yields of 33–74% after purification by column chromatography. With aliphatic aldehydes, product was obtained in good yields, up to 50%; however, aliphatic carboxylic acids such as isobutyric acid, butyric acid, and pivalic acid resulted in only trace amounts of product. To investigate potential intramolecular hydrogen bonds of our compounds, a sulfur-containing macrocycle was treated with m-chloroperbenzoic acid (mCPBA) in DCM to afford sulfoxide and sulfone. As an example, the reaction of macrocycle 6m with 1 equiv and 4 equiv of mCPBA in DCM afforded sulfoxide 7a and sulfone 7b in good yields of 65% and 38%, respectively.

**Figure 1.** Six theoretical possibilities for macrocycle synthesis by classical Ugi 4-CR.

**Table 1. Optimization of Ugi-4CR**

<table>
<thead>
<tr>
<th>entry</th>
<th>solvent (M)</th>
<th>time (h)</th>
<th>catalyst/conditions</th>
<th>yield (%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MeOH (1.0)</td>
<td>12</td>
<td>rt</td>
<td>traces</td>
</tr>
<tr>
<td>2</td>
<td>MeOH (0.1)</td>
<td>12</td>
<td>rt</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>MeOH (0.01)</td>
<td>12</td>
<td>rt</td>
<td>48</td>
</tr>
<tr>
<td>4</td>
<td>TFE (0.01)</td>
<td>12</td>
<td>rt</td>
<td>65</td>
</tr>
<tr>
<td>5</td>
<td>CH₃CN (0.01)</td>
<td>12</td>
<td>rt</td>
<td>22</td>
</tr>
<tr>
<td>6</td>
<td>THF (0.01)</td>
<td>12</td>
<td>rt</td>
<td>30</td>
</tr>
<tr>
<td>7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>MeOH (0.01)</td>
<td>12</td>
<td>ZnCl₂</td>
<td>43</td>
</tr>
<tr>
<td>8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>TFE (0.01)</td>
<td>12</td>
<td>ZnCl₂</td>
<td>25</td>
</tr>
<tr>
<td>9</td>
<td>MeOH (0.01)</td>
<td>24</td>
<td>rt</td>
<td>60</td>
</tr>
<tr>
<td>10</td>
<td>MeOH (0.01)</td>
<td>12</td>
<td>sonication</td>
<td>20</td>
</tr>
</tbody>
</table>

<sup>a</sup>The reaction was carried out with N-(5-aminopentyl)-5-isocyanopentanamide (1.0 mmol), paraformaldehyde (1.0 mmol), and 2-phenylacetic acid (1.0 mmol). 10 mol% catalyst used. *Yield of isolated product.
Scheme 2. Synthesized Macrocycles with Corresponding Yields

77%, respectively, after 4 h. As shown in Scheme 3, these sulfoxide and sulfone functional groups are potentially capable to form amide−sulfoxide and amide−sulfone intramolecular hydrogen bonds leading to lower energy conformations of the corresponding macrocycles with interlocked structures which could have a significant impact on biological membrane permeability.

X-ray crystal structures of several macrocycles with different sizes and substituents can further provide some first insight into possible solid-state conformations (Figure 2). For instance, compound 6l shows an intramolecular hydrogen bonding.

Scheme 3. Selective Oxidative Modifications of a Sulfur-Containing Macrocycle

Figure 2. X-ray crystal structure of some synthesized macrocycles.

Physicochemical properties are of high importance for the development of drug-like compounds. What is the property profile of our macrocycles? To answer this question, we constructed a random virtual 1000 macrocycle library (SI). We calculated some properties of the library related to drug-likeliness including molecular weight, lipophilicity, number of hydrogen bond donors and acceptors, number of rotatable bonds, polar surface area, and moment of inertia (Figure 3). Interestingly, analysis of the library shows that 21% obey the Lipinski rule of 5 (RO5). The cLogP vs MW distribution of a considerable fraction of the chemical space is favorable drug-like with an average MW and cLogP of 572 and 4.1, respectively.

Moreover, punctual analysis of 3D modeled representatives and X-ray structures underline the nonflat shapes of the medium sized rings. Overall, a considerable fraction of our macrocyclic space is predicted to have drug-like properties. This is in accordance with the recent proposal that the chemical space from 500 to 1000 Da remains virtually unexplored and represents a vast opportunity for those prepared to venture into new territories of drug discovery.17,18

■ CONCLUSIONS

A very mild, straightforward, two-step, rapid, and highly diverse macrocycle (12−17 membered) synthesis pathway via MCRs was introduced. In this strategy, macrocyclic ring closure was performed through Ugi-4CR to afford novel complex compounds with potentially biological and pharmaceutical importance. Moreover, our strategy will allow a unique simple route for the synthesis of nonpeptidic macrocycles. Other macrocyclic scaffolds obtained from different combinations of MCRs and their applications as inhibitors for protein−protein interactions are currently being investigated in our laboratory and will be reported shortly.

■ EXPERIMENTAL SECTION

General Methods. All chemicals were purchased from commercial suppliers and used without any purification unless otherwise noted. Nuclear magnetic resonance spectra were recorded. Chemical shifts for 1H NMR are reported as δ values, and coupling constants are in hertz (Hz). The following abbreviations are used for spin multiplicity: s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, quint = quintet, dd = double of doublets, ddd = double of doublet of doublets, m = multiplet. Chemical shifts for 13C NMR reported in ppm relative to the solvent peak. Thin layer chromatography was performed on silica gel plates (0.20 mm thick, particle size 25 μm). Flash chromatography was performed using RediSep Rf normal-phase silica.
flash columns (silica gel 60 Å, 230–400 mesh). Electrospray ionization mass spectra (ESI-MS) were recorded.

Procedure and Analytical Data for Synthesis of α-isocyanoo-ω-amine. A round-bottom flask was charged with a magnet stirrer, the diamine (6.0 equiv), and the α-isocyanoo-ω-methyl ester (5.0 equiv), and 1,4-dioxane (0.1 M) was added. The reaction mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure, and the residue was purified by column chromatography on silica (eluent: 0–100% AB; A 1:1 mixture of EtOAc:DCM, B: methanol, next with C: methanol containing 5% concd aq ammonia, particle size: 40–63 μm).

**N-(5-Aminopentyl)-5-isocyanopentanamide 3a.** The product was obtained as an oil (55%, 0.580 g).

1H NMR (500 MHz, CDCl3) δ 6.58 (t, J = 5.8 Hz, 1H), 3.41–3.34 (m, 2H), 3.15 (q, J = 6.7 Hz, 2H), 2.65 (t, J = 7.1 Hz, 2H), 2.16 (t, J = 7.0 Hz, 2H), 1.74–1.61 (m, 4H), 1.49–1.38 (m, 4H), 1.33–1.24 (m, 2H).

13C NMR (126 MHz, CDCl3) δ 172.3, 155.6, 41.4, 39.2, 35.2, 32.1, 29.2, 28.5, 24.0, 22.5. HRMS (ESI-TOF) m/z: [M+H]1+ Calcd for C11H22N3O 212.1758; found 212.1757.

**N-(3-Aminopropyl)-6-isocyanohexanamide 3b.** The product was obtained as an oil (60%, 0.591 g).

1H NMR (500 MHz, CDCl3) δ 6.62 (bs, 1H), 3.42–3.36 (m, 2H), 3.36–3.29 (m, 2H), 2.77 (t, J = 6.4 Hz, 2H), 2.18 (t, J = 7.5 Hz, 2H), 1.72–1.59 (m, 6H), 1.51–1.42 (m, 2H); 13C NMR (126 MHz, CDCl3) δ 172.8, 155.6, 50.2, 41.5, 39.8, 37.7, 36.3, 32.2, 28.8, 25.9.

**Figure 3.** Some calculated physicochemical properties of the chemical space of macrocycles. A: cLogP over MW scatter plot, B: cLogP over MW box plot, C: Lipinski RO5 radar plot, D: compound distribution based on Lipinski RO5.

**N-(4-Aminobutyl)-3-isocyanopropanamide 3c.** The product was obtained as an oil (60%, 0.464 g).

1H NMR (500 MHz, CD3OD) δ 3.78 (t, J = 6.3 Hz, 2H), 3.26 (t, J = 6.5 Hz, 2H), 2.73 (t, J = 6.5 Hz, 2H), 2.66–2.51 (m, 2H), 1.64–1.51 (m, 4H); 13C NMR (126 MHz, CD3OD) δ 171.6, 156.8, 42.0, 40.3, 39.1, 36.7, 30.3, 27.9.

**N-(2-((2-Aminoethyl)thio)ethyl)-3-(1H-indol-3-yl)-2-isocyanopropanamide 3d.** The product was obtained as an oil (49%, 0.774 g).

1H NMR (500 MHz, CD3OD) δ 7.58 (d, J = 8.0 Hz, 1H), 7.36 (d, J = 8.0 Hz, 1H), 7.20 (s, 1H), 7.10 (t, J = 7.5 Hz, 2H), 4.56 (t, J = 6.6 Hz, 1H), 3.40–3.35 (m, 2H), 3.32 (p, J = 1.6 Hz, 1H), 3.29–3.18 (m, 2H), 2.72 (t, J = 6.6 Hz, 2H), 2.51 (t, J = 6.6 Hz, 2H), 2.32 (t, J = 7.1 Hz, 2H); 13C NMR (126 MHz, methanol-d4) δ 167.1, 158.4, 136.6, 127.1, 123.9, 121.2, 118.6, 118.0, 111.0, 107.7, 58.3, 40.1, 39.1, 33.7, 29.7, 29.5.

**N-(6-Aminohexyl)-2-isocyano-3-phenylpropanamide 3e.** The product was obtained as an oil (56%, 0.591 g).

1H NMR (500 MHz, CDCl3) δ 7.42–7.23 (m, 5H), 3.34 (t, J = 1.7 Hz, 1H), 3.28–3.09 (m, 4H), 2.67 (t, J = 7.2 Hz, 2H), 1.57–1.41 (m, 4H), 1.40–1.31 (m, 4H); 13C NMR (126 MHz, CDCl3) δ 166.3, 158.7, 135.1, 129.1, 128.3, 127.1, 58.3, 40.9, 39.3, 38.9, 31.7, 28.7, 23.3.
1H NMR (500 MHz, CDCl₃) δ 6.96 (t, J = 7.4 Hz, 1H), 7.25 (m, 5H), 6.41 (t, J = 7.2 Hz, 1H). 13C NMR (126 MHz, CDCl₃) δ 176.3, 176.2, 170.7, 153.0, 129.3, 129.2, 127.5, 59.7, 53.4, 46.5, 41.9, 38.4, 36.5, 31.2, 29.4, 28.9, 24.2, 15.8. HRMS (ESI-TOF) m/z: [M + H]^+ Calcd for C₂₃H₃₆N₃O₃S 402.2751; found 402.2750.

2-(1-Benzyl-3-dimethylaminopyridinium-4-yl)-1,4,8-triazacyclododecane-3,7-dione 6f. The product was obtained as a yellow oil (42%, 0.179 g); 1H NMR (500 MHz, DMSO-d₆) δ 8.52 (d, J = 8.2 Hz, 1H), 8.21–8.16 (m, 2H), 7.56–7.52 (m, 2H), 4.67 (s, 1H), 4.21–3.97 (m, 2H), 3.85–3.67 (m, 2H), 3.56 (dd, J = 15.2, 8.4 Hz, 2H), 3.18–2.93 (m, 2H), 2.85 (d, J = 13.4 Hz, 1H), 2.38–2.26 (m, 2H), 1.96–1.84 (m, 1H), 1.54–1.35 (m, 1H). 13C NMR (126 MHz, CDCl₃) δ 173.6, 170.9, 133.6, 129.2, 128.9, 127.6, 73.0, 41.6, 40.8, 38.8, 36.4, 36.0, 29.7, 28.9, 26.2, 25.1, 24.5, 23.1, 21.7. HRMS (ESI-TOF) m/z: [M + H]^+ Calcd for C₁₀H₁₆N₃O; found 144.0673.

3-Isobutyl-4-(2-phenylacetyl)-1,4,8-triazacyclocedane-2,9-dione 6e. The product was obtained as a yellow solid (36%, 0.215 g, mp 164–166 °C); 1H NMR (500 MHz, CDCl₃) δ 7.46–7.29 (m, 3H), 7.29–7.20 (m, 2H), 6.10 (s, 1H), 4.85 (t, J = 7.3 Hz, 1H), 3.77 (2H), 3.73–3.54 (m, 2H), 3.35–3.20 (m, 1H), 3.18–2.96 (m, 2H), 2.57–2.34 (m, 3H), 2.34–2.21 (m, 2H), 2.12 (s, 3H), 2.08–1.92 (m, 2H), 1.96–1.58 (m, 3H), 1.45 (t, J = 11.1 Hz, 2H), 1.36–1.13 (m, 2H). 13C NMR (126 MHz, CDCl₃) δ 173.6, 172.5, 170.7, 153.0, 129.3, 129.2, 127.5, 59.7, 53.4, 46.5, 41.9, 38.4, 36.5, 31.2, 29.4, 28.9, 24.2, 15.8. HRMS (ESI-TOF) m/z: [M + H]^+ Calcd for C₂₃H₂₇N₃O₃Cl 428.2375; found 428.2374.

14-(11H-indol-3-ylmethyl)-6-(2-phenylacetyl)-9-thia-6,12,15-triazaspiro[4.11]hexadecane-13,16-dione 6g. The product was obtained as a yellow solid (53%, 0.274 g, mp 180–182 °C); 1H NMR (500 MHz, CDCl₃) δ 8.84 (d, J = 7.5 Hz, 1H), 8.18 (s, 1H), 7.76–7.25 (m, 7H), 7.34 (t, J = 7.4 Hz, 2H), 7.29 (d, J = 2.6 Hz, 1H), 7.18 (t, J = 7.4 Hz, 3H), 7.15–7.10 (m, 2H), 6.79 (t, J = 6.0 Hz, 1H), 4.67–4.55 (m, 1H), 3.88 (s, 2H), 3.68 (s, 1H), 3.61–3.56 (m, 2H), 3.45 (dd, J = 15.0, 5.4 Hz, 1H), 3.24 (dd, J = 15.0, 8.9 Hz, 1H), 3.06–2.98 (m, 2H), 2.90–2.79 (m, 1H), 2.74–2.61 (m, 1H), 2.56–2.45 (m, 1H), 2.32–2.18 (m, 1H), 1.87–1.77 (m, 1H), 1.58–1.47 (m, 4H), 1.34–1.26 (m, 1H), 1.25–1.15 (m, 1H). 13C NMR (126 MHz, CDCl₃) δ 175.4, 173.8, 171.8, 136.3, 134.9, 129.4, 128.8, 128.7, 128.5, 70.3, 44.2, 43.8, 38.8, 38.1, 35.4, 26.4, 26.1, 25.3. HRMS (ESI-TOF) m/z: [M + H]^+ Calcd for C₂₁H₂₉N₄O₅S 519.2371; found 519.2211.
The Journal of Organic Chemistry

1H, 3.56 (t, J = 6.4 Hz, 1H), 4.26 (s, 1H), 3.83 (d, J = 5.9 Hz, 2H), 3.62–3.54 (m, 2H), 3.32 (q, J = 5.3 Hz, 2H), 3.18–3.11 (m, 1H), 3.07–2.99 (m, 1H), 2.43–2.23 (m, 1H), 2.26–2.19 (m, 1H), 2.15–2.08 (m, 1H), 1.87 (t, J = 7.2 Hz, 2H), 1.81–1.74 (m, 1H), 1.53–1.48 (m, 2H), 1.46–1.42 (m, 1H), 1.37–1.32 (m, 1H), 1.29–1.26 (m, 1H), 1.23 (d, J = 6.0 Hz, 1H), 1.01 (t, J = 6.5 Hz, 1H), 0.90 (d, J = 6.2 Hz, 2H). 13C NMR (126 MHz, CDCl3) δ 173.2, 172.8, 172.4, 134.9, 128.9, 128.7, 127.0, 121.1, 48.9, 41.5, 40.7, 37.5, 37.2, 35.2, 29.1, 28.6, 25.0, 23.8, 23.1, 23.0, 22.1. HRMS (ESI-TOF) m/z: [M + H]+ Calcd for C25H39N3O3SBr 472.0900; found 472.0901.

General Procedure and Analytical Data for the Synthesis of Sulfoxide Macrocycle. Macrocycle 6q (1.0 mmol) was dissolved in 1 mL of DCM, and m-chloroperbenzoic acid (1 equiv) was added. The solution stirred at room temperature for 4 h. After completion of the reaction, the solvent was removed under reduced pressure and the residue was purified using flash chromatography (DCM:MeOH (9:1)).

4-(2-(4-Bromophenyl)acetyl)-1-thia-4,7,13-triazacyclopentadecane-6,12-dione 60. The product was obtained as a white solid (31%, 0.357 g, mp 211–212 °C). 1H NMR (500 MHz, DMSO-d6) δ 7.81 (d, J = 17.2 Hz, 1H), 7.65 (s, 1H), 3.75 (d, J = 16.2, 7.9 Hz, 2H), 7.14 (d, J = 8.0 Hz, 1H), 7.04 (d, J = 8.0 Hz, 1H), 3.95 (d, J = 22.7 Hz, 2H), 3.86–3.72 (m, 2H), 3.54 (d, J = 14.6 Hz, 3H), 3.38 (t, J = 7.6 Hz, 1H), 3.30 (d, J = 7.7 Hz, 1H), 3.27–3.09 (m, 5H), 2.57–2.51 (m, 1H), 2.12 (d, J = 7.3 Hz, 2H), 1.63–1.37 (m, 4H). 13C NMR (126 MHz, DMSO-d6) δ 173.1, 172.0, 168.6, 134.6, 133.2, 130.0, 126.5, 125.4, 118.8, 61.2, 54.5, 51.8, 51.7, 35.7, 35.4, 26.0, 22.0, 17.6. HRMS (ESI-TOF) m/z: [M + H]+ Calcd for C25H39N3O3SBr 472.0900; found 472.0901.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.7b02984.

NMR spectra, crystal structure determinations, and virtual library synthesis (PDF)

CIF data for 6c (CIF)

CIF data for 6e (CIF)

CIF data for 6f (CIF)

**AUTHOR INFORMATION**

**Corresponding Author**

E-mail: a.s.s.domling@rug.nl, www.drugdesign.nl.

**ORCID**

Eman M. M. Abdelraheem: 0000-0002-9008-2729
Justyna Kalinowska-Thulsic: 0000-0001-7714-1651
Shabnam Shaabani: 0000-0001-5546-7140
Alexander Dömling: 0000-0002-9923-8873

**Notes**

The authors declare no competing financial interest.
The work was financially supported by NIH 2R01GM097082-05, the Innovative Medicines Initiative (grant agreement no.115489), and also European Union’s Seventh Framework Programme (FP7/2007-2013) and EFPIA companies’ in-kind contribution. Funding came also from the European Union’s Horizon 2020 research and innovation programme under MSC ITN “Accelerated Early Stage Drug Discovery” (no. 675555), CoFund ALERT (no. 665250). The work was supported by the European Regional Development Fund in the framework of the Polish Innovation Economy Operational Program (contract no. POIG.02.01.00-12-023/08). Eman M. M. Abdelraheem was supported by the Egyptian government.

REFERENCES