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Substituted quinolinones. Part 22. In vitro antimicrobial evaluation of some 4-hydroxy-1-methyl-3-pyrazolinylquinolin-2(1H)-ones as useful antibiotic intermediates

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Abstract Thirty derivatives of 3-pyrazolylquinolinone, in three different types; enaminones **1a–j**, enones **2a–j**, and hydrazonones **3a–j**, have been investigated for their in vitro antimicrobial activity. The tested compounds have been screened against four chosen microorganisms, two bacteria strains (*Staphylococcus aureus,* as Gram-positive bacterium, and *Escherichia coli*, as Gram-negative bacterium), and two fungi strains (*Aspergillus flavus* and *Candida albicans*). Disc plate and serial dilutions techniques were used to evaluate and determine the antimicrobial activity and minimum inhibition concentration of the tested compounds. The results revealed that the enaminone derivatives **1a–j** have desirable antimicrobial activity against both bacteria and fungi groups, and they are promising useful antibiotic intermediates.

Keywords Quinolinone · Pyrazolinone · Bactericide · Fungicide · Antibiotic intermediate

Introduction

Recent decades have seen exploration and great progress in the synthesis of new antimicrobial active reagents for the medication of many infectious microbial

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diseases [1]. The quinolinones group has been one of the most distinguishable successful pharmacophores and efficient therapeutic antibiotics [2–5]. Despite the fact that there is a vast increase in synthesized quinolinones, their therapeutic significance has been restricted due to their relatively toxic side effects and the appearance of medication resistance [6]. This demands exploration and improvement of further efficient wide spectrum antimicrobials with minimal side effects. We have previously described a strategy to synthesize some new 4-hydroxy-1-methyl-3-pyrazolinylquinolin-2(1H)-one derivatives [7].

Quinolinones can enter cells easily via porins and, therefore, are often used to treat intracellular pathogens such as *Legionella pneumophila* and *Mycoplasma pneumoniae*. For many Gram-negative bacteria, DNA gyrase is the target, whereas topoisomerase IV is the target for many Gram-positive bacteria. Quinolinones are successful against some bacteria and fungi, such as *Escherichia coli* which is responsible for some infections and syndromes such as blood hemolytic syndrome, bloody diarrhea, anemia, kidney failure, urinary tract infections, fever, stomach cramps, nausea, and vomiting. Some compounds in this class have been shown to inhibit the synthesis of mitochondrial DNA [8–14]. On the other hand, azoles inhibit the synthesis of sterols in fungi by inhibiting cytochrome P450-dependent 14 α-lanosterol demethylase, which removes the methyl group on C14 of lanosterol, a key intermediate step in the formation of ergosterol in the fungal cell membrane [15].

For the purpose of the examination of the synthesized pyrazolylquinolinones as antimicrobials against tested bacteria and fungi, we classified the 30 derivatives into three different categories. 2-Pyrazolin-5-one core is attached to 4-hydroxy-1-methylquinolin-2-one substrate at position 3 of each and substituted with arylaminomethylene, arylidene, or arylhydrazone at position 4 of the pyrazoline ring. All the compounds contain a substitution at the aryl ring or, instead, a hetaryl substituent is present. Antibacterial and antifungal activities of all 30 compounds were evaluated using both disc diffusion and serial concentrations methods.

Materials and methods

All chemicals and solvents are Analar grade. The synthesized compounds; pyrazolylquinolinone derivatives **1a–j**, **2a–j**, and **3a–j** were prepared in the Department of Chemistry, Faculty of Education, Ain Shams University (Table 1), according to our previous reported methods [7]. Different species of microorganisms were purchased from M.R.C. (Microbial Research Center, Faculty of Agriculture, Ain Shams University, Cairo, Egypt). The tested bacteria are *E. coli* (ATCC 8739) (G −ve) and *Staphylococcus aureus* (ATCC 6538) (G +ve), while the tested fungi are *Aspergillus flavus* (ATCC 28542) and *Candida albicans* (ATCC 10231). The disc diffusion method and serial dilution assay were used to evaluate the minimum inhibition concentrations [16, 17].

Sterilized conditions were controlled using an autoclave (120 °C). Nutrient agar and nutrient broth media or fungous medium (DOX's medium) were prepared by dissolving 2.8 and 0.8 g of the media into 100 mL distilled water, respectively. The

Table 1 Chemical names of the tested pyrazolylquinolinone derivatives

Cpd. No.	*R*	Chemical name
1a	Phenyl	3-[4-(Anilinomethylene)-5-oxo-4,5-dihydro-1H-pyrazol-3-yl]-4-hydroxy-1-methylquinolin-2(1H)-one
1b	4-Tolyl	4-Hydroxy-1-methyl-3-(4-{[(4-methylphenyl)amino]-methylene}-5-oxo-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one
1c	4-Chlorophenyl	3-(4-{[(4-Chlorophenyl)amino]methylene}-5-oxo-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-1-methyl-quinolin-2(1H)-one
1d	4-Nitrophenyl	4-Hydroxy-1-methyl-3-(4-{[(4-nitrophenyl)amino]-methylene}-5-oxo-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one
1e	2-Pyridyl	4-Hydroxy-1-methyl-3-(5-oxo-4-[(pyridin-2-ylamino)methylene]-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one
1f	3-Pyridyl	4-Hydroxy-1-methyl-3-(5-oxo-4-[(pyridin-3-ylamino)methylene]-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one
1g	4-Pyridyl	4-Hydroxy-1-methyl-3-(5-oxo-4-[(pyridin-4-ylamino)methylene]-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one
1h	2-Pyrimidyl	4-Hydroxy-1-methyl-3-(5-oxo-4-[(pyrimidin-2-ylamino)methylene]-4,5-dihydro-1H-pyrazol-3-yl})quinolin-2(1H)-one
1i	2-Thiazolyl	4-Hydroxy-1-methyl-3-(5-oxo-4-[(1,3-thiazol-2-ylamino)methylene]-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one
1j	4-Antipyrenyl	3-{4-[(1,5-Dimethyl-3-oxo-2-phenyl-1,2-dihydro-3H-pyrazol-4-ylamino)methylene]-5-oxo-4,5-dihydro-1H-pyrazol-3-yl}-4-hydroxy-1-methylquinolin-2(1H)-one
2a	Phenyl	3-[4-Benzylidene-5-oxo-4,5-dihydro-1H-pyrazol-3-yl]-4-hydroxy-1-methylquinolin-2(1H)-one
2b	4-Tolyl	4-Hydroxy-1-methyl-3-[4-(4-methylbenzylidene)-5-oxo-4,5-dihydro-1H-pyrazol-3-yl]quinolin-2(1H)-one
2c	4-Chlorophenyl	3-[4-(4-Chlorobenzylidene)-5-oxo-4,5-dihydro-1H-pyrazol-3-yl]-4-hydroxy-1-methylquinolin-2(1H)-one
2d	4-Nitrophenyl	4-Hydroxy-1-methyl-3-[4-(4-nitrobenzylidene)-5-oxo-4,5-dihydro-1H-pyrazol-3-yl]quinolin-2(1H)-one
2e	2-Pyridyl	4-Hydroxy-1-methyl-3-[5-oxo-4-(pyridin-2-ylmethylene)-4,5-dihydro-1H-pyrazol-3-yl]quinolin-2(1H)-one
2f	2-Anisyl	4-Hydroxy-3-[4-(2-methoxybenzylidene)-5-oxo-4,5-dihydro-1*H*-pyrazol-3-yl]-1-methylquinolin-2(1H)-one
2g	3-Anisyl	4-Hydroxy-3-[4-(3-methoxybenzylidene)-5-oxo-4,5-dihydro-1H-pyrazol-3-yl]-1-methylquinolin-2(1H)-one
2h	4-Anisyl	4-Hydroxy-3-[4-(4-methoxybenzylidene)-5-oxo-4,5-dihydro-1H-pyrazol-3-yl]-1-methylquinolin-2(1H)-one
2i	3,4-Dimethoxyphenyl	3-[4-(3,4-Dimethoxybenzylidene)-5-oxo-4,5-dihydro-1H-pyrazol-3-yl]-4-hydroxy-1-methylquinolin-2(1H)-one
2j	1,3-Benzodioxol-5-yl	3-[4-(1,3-Benzodioxol-5-ylmethylene)-5-oxo-4,5-dihydro-1H-pyrazol-3-yl]-4-hydroxy-1-methylquinolin-2(1H)-one
3a	Phenyl	4-Hydroxy-1-methyl-3-(5-oxo-4-phenylhydrazono-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one
3b	4-Tolyl	4-Hydroxy-1-methyl-3-(5-oxo-4-(4-methylphenyl)-hydrazono-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one

Table 1 continued

Cpd. No.	*R*	Chemical name
3c	4-Chlorophenyl	3-(4-(4-Chlorophenyl)hydrazono-5-oxo-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-1-methylquinolin-2(1H)-one
3d	2-Chlorophenyl	3-(4-(2-Chlorophenyl)hydrazono-5-oxo-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-1-methylquinolin-2(1H)-one
3e	3-Chlorophenyl	3-(4-(3-Chlorophenyl)hydrazono-5-oxo-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-1-methylquinolin-2(1H)-one
3f	4-Nitrophenyl	4-Hydroxy-3-(4-(4-nitrophenyl)hydrazono-5-oxo-4,5-dihydro-1H-pyrazol-3-yl)-1-methylquinolin-2(1H)-one
3g	2-Bromophenyl	3-(4-(2-Bromophenyl)hydrazono-5-oxo-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-1-methylquinolin-2(1H)-one
3h	3-Bromophenyl	3-(4-(3-Bromophenyl)hydrazono-5-oxo-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-1-methylquinolin-2(1H)-one
3i	4-Bromophenyl	3-(4-(4-Bromophenyl)hydrazono-5-oxo-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-1-methylquinolin-2(1H)-one
3j	4-Antipyrenyl	3-(4-((1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)hydrazono)-5-oxo-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-1-methylquinolin-2(1H)-one

media were heavily seeded with spore suspension of the tested organisms. Microbial standard suspensions were prepared at a concentration 10^8 CFU/mL. The solutions of the compounds were prepared in DMSO at a concentration of 1 mg/mL.

In the disc plate method, 5–20 μg of stock solutions were transferred to the disc (6 mm in diameter) and located on the nutrient agar. After incubation at 37 °C for 24 h in the case of bacteria and 72 h in the case of fungi, the diameter of growth inhibition was recorded as the average diameter of the inhibition zone of triplicate measurements (Table 2). The DMSO disc was used as control. In this method, 10-μg discs of Tetracyclin and Amphotericin B were used as positive reference standards to determine the sensitivity of the tested bacteria and fungi, respectively.

In the serial dilution method, seven test tubes, containing different concentrations of pyrazolylquinolinone derivatives, were prepared. Each test tube contained liquid media, microbial suspension, and compounds, in addition to two test tubes containing liquid media, microbial suspensions, and the solvent. Concentrations of the azole compounds in these tubes were 3.125, 6.25, 12.5, 25, 50, and 100 μg/mL. Minimum inhibition concentrations (MICs) of the compounds are listed in Table 3.

Results and discussion

As illustrated in Fig. 1, the 30 synthesized compounds were prepared from 4-hydroxy-1-methyl-3-(5-oxo-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one (PQ) according to our previously described method [7]. The pyrazolylquinolinones were divided into three different types; enaminones **1a**–**j**, enones **2a**–**j**, and hydrazonones **3a**–**j** (Fig. 1).

Table 2 Inhibition activity of the pyrazolylquinolinones against test bacteria and fungi

Compound	Inhibition zone diameter (mm)			
	E. coli (ATCC 8739)	*S. aureus* (ATCC 6538)	*A. flavus* (ATCC 28542)	*C. albicans* (ATCC 10231)
1a	22	19	11	10
1b	34	29	20	23
1c	32	28	16	22
1d	28	22	15	17
1e	26	22	21	23
1f	31	27	16	17
1g	28	26	14	17
1h	27	24	05	07
1i	29	26	15	14
1j	26	23	12	14
2a	09	08	06	07
2b	07	08	05	06
2c	10	11	07	08
2d	06	05	00	00
2e	10	12	08	06
2f	15	14	11	13
2g	15	13	07	08
2h	17	15	10	09
2i	17	14	11	13
2j	10	13	12	14
3a	16	17	10	09
3b	17	18	11	10
3c	22	24	08	14
3d	22	20	14	12
3e	20	17	15	14
3f	19	20	10	13
3g	17	18	12	11
3h	27	24	14	15
3i	19	18	11	08
3j	21	20	17	16
Standard[a]	30	26	18	20
Control[b]	00	00	00	00

[a] Standard: antibacterial agent; Tetracyclin and/or antifungal agent; Amphotericin B

[b] Control test: solvent; DMSO without test compounds

All compounds in these three categories were screened and evaluated against four chosen microorganisms, two bacteria strains (*S. aureus,* as Gram-positive bacterium and *E. coli* as Gram-negative bacterium), and two fungi strains (*A. flavus* and *C. albicans*) using disc diffusion and serial dilution methods.

Table 3 Evaluation of minimum inhibition concentration of the pyrazolylquinolinones

Compound	Minimum inhibition concentration (MIC) (μg/ml)			
	E. coli	*S. aureus*	*A. flavus*	*C. albicans*
1a	25	25	>100	>100
1b	3.125	6.25	25	25
1c	6.25	6.25	25	25
1d	12.5	12.5	50	50
1e	12.5	12.5	50	50
1f	6.25	6.25	25	25
1g	12.5	6.25	25	25
1h	12.5	12.5	>100	>100
1i	6.25	6.25	25	50
1j	12.5	12.5	50	50
2a	>100	>100	>100	>100
2b	>100	>100	>100	>100
2c	>100	>100	>100	>100
2d	>100	>100	>100	>100
2e	>100	>100	>100	>100
2f	50	>100	>100	50
2g	50	>100	>100	>100
2h	50	50	>100	>100
2i	50	>100	>100	50
2j	>100	>100	50	50
3a	50	>100	>100	>100
3b	50	50	>100	>100
3c	25	50	50	>100
3d	25	50	50	50
3e	50	12.5	>100	50
3f	50	50	50	50
3g	50	50	50	>100
3h	6.25	12.5	25	50
3i	25	50	>100	>100
3j	25	25	50	50

On the recorded inhibition zones for the tested compounds, it was found that, in general, the 20 tested pyrazolylquinolinones of the types enones **2a–j** and hydrazonones **3a–j** showed low to moderate antibacterial and antifungal activity (inhibition zone against bacteria 5–24 mm. and against fungi 5–14 mm) with a few small exceptions. Generally, the pyrazolylquinolinones of the type enaminones **1a–j** showed significant antibacterial and antifungal activities (highly active). In particular, the enaminones **1b**, **1c**, **1f**, and **1i** are comparable or even more reactive than the standard Tetracyclin towards both Gram-negative and -positive bacteria (inhibition zone 26–34 mm). Interestingly, measuring the minimum inhibition concentration showed that these four derivatives exhibit the lowest MIC values

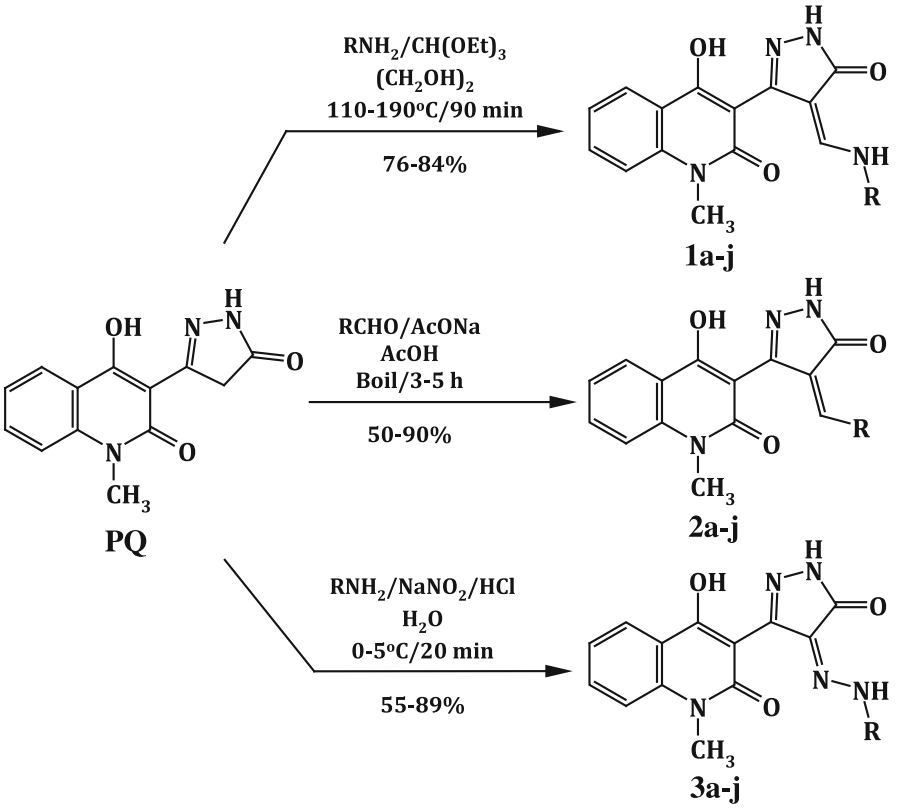

Fig. 1 Preparation of the pyrazolylquinolinone derivatives

confirming their distinguishable antibacterial activity (6.25–12.5 μg/mL). The compounds **1b–f** showed potential fungicidal actitivity against both *A. flavus* and *C. albicans* by means of their inhibition zone diameters and also MIC values (Tables 2, 3).

To make a comparison between the activities of the three groups of compounds, we have taken the first four derivatives from each group, **1a–d**, **2a–d**, and **3a–d** ($R = a$, Ph; b, 4-tolyl; c, 4-ClPh; d, 4-NO_2Ph). Plotting their inhibition zone diameters as indicators of their antimicrobial activities showed that enaminones **1a–d** have nearly threefold activity values in comparison to their related enones **2a–d**. At the same time, the hydrazonones derivatives **3a–d** showed twofold activity values compared to their analogues **2a–d** (Fig. 2).

Interestingly the calculated MM2 parameters calculations, which were obtained using Chem3D Ultra-Version 10 (CambridgeSoft), revealed that there was a good correlation between the antimicrobial activity and torsional energy in these derivatives; **1a**, **2a**, and **3a** have been taken as examples. Thus; MM2 (energy minimization) calculations showed that; in the enaminone **1a**; charges on $N_{azomethine}$ and $O_{pyrazolinone}$ are −0.251 and −0.843, respectively. Comparing these values with those of both the enone **2a** (−0.177 and −0.769) and hydrazonone **3a** (−0.233 and −0.822) revealed that the effect of enaminone as a push–pull electron system on

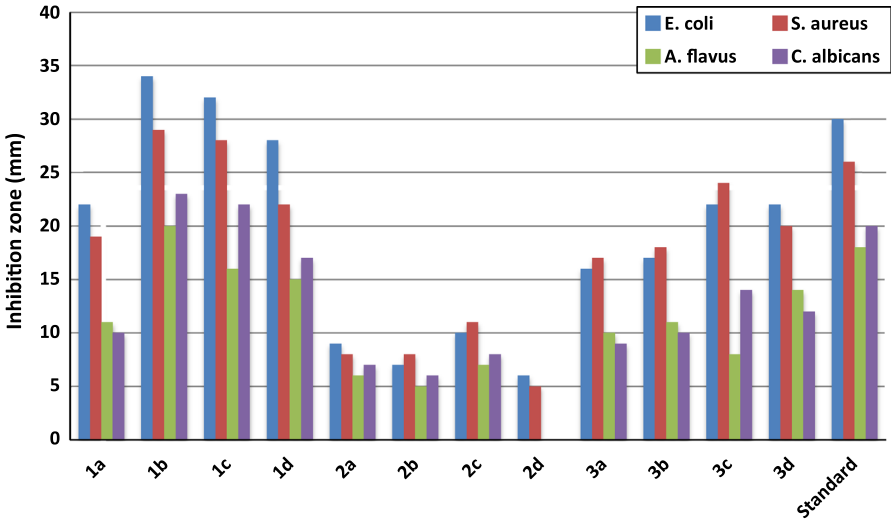

Fig. 2 Comparison of antimicrobial activities of the enaminones **1a–d**, **2a–d**, and **3a–d**

increasing the charge density on both the azomethine N and carbonyl O of the pyrazolinone moiety, a phenomenon which is also present in the hydrazonones but to a lesser extent, due to replacing the carbon of double bond with the more electronegative atom nitrogen. This phenomenon is not standard in the enone derivatives, where these compounds have the pushing moiety around the double bound. Even this structure–activity relationship can lead to expectations about the activity of these categories of compounds, in which insertion of an electron-repealing group at *para* or *ortho* positions of the phenyl group enhances the activity of these derivatives. This is found to be valid in the series of chlorophenyl and bromophenyl derivatives in the group of hydrazonones **3**.

Conclusions

As a general conclusion, the pyrazolylquinolinones proved to be a potent antimicrobial agent. Accordingly, these compounds can be used as useful antibiotic drug intermediates. In vitro cell experiments show that enaminone derivatives of this category of heterocyclic compounds are more active than their enone or hydrazonone analogues. Evaluating the antimicrobial activity of the series of enaminones and hydrazonones reveals that the compounds possessing more activity are those with good push–pull electron systems at the pyrazolinone moiety, in which *para* or *ortho* +M mesomeric effect groups are present.

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References

1. K. Bhandari, N. Srinivas, G.B.S. Keshava, P.K. Shukla, Eur. J. Med. Chem. **44**, 437–447 (2009)
2. Y. Niki, K. Itokawa, O. Okazaki, Antimicrob. Agents Chemother. **42**, 1751–1755 (1998)
3. M. Honma, Nihon Rinsho. **54**, 3125–3129 (1996)
4. T.D. Gootz, J.F. Barrett, J.A. Sutcliffe, Antimicrob. Agents Chemother. **34**, 8–12 (1990)
5. D.V. Ivanov, S.V. Budanov, Antibiot. Khimioter. **51**, 29–37 (2006)
6. D.L. Paterson, Clin. Infect. Dis. **38**(Suppl 4), S341–S345 (2004)
7. M. Abass, E.S. Othman, Synth. Commun. **31**, 3361–3376 (2001)
8. T. Bergan, J. Chemother. **1**, 10–17 (1989)
9. T. Bergan, Diagn. Microbiol. Infect. Dis. **13**, 103–114 (1990)
10. F.J. Castora, F.F. Vissering, M.V. Simpson, Biochim. Biophys. Acta **740**, 417–427 (1983)
11. N. Kaplowitz, Hepatology **41**, 227 (2005)
12. H. Enzmann, C. Wiemann, H.J. Ahr, G. Schlüter, Mutat. Res. **425**, 213–224 (1999)
13. H.E. Holden, J.F. Barett, C.M. Huntington, P.A. Muehlbauer, M.G. Wahrenburg, Environ. Mol. Mutagen. **13**, 238–252 (1989)
14. M.J. Suto, J.M. Domagala, G.E. Roland, G.B. Mailloux, M.A. Cohen, J. Med. Chem. **35**, 4745–4750 (1992)
15. S.A.F. Rostom, H.M.A. Ashour, H.A. Abd-El-Razik, A.H. Abd-El-Fattah, N.N. El-Din, Bioorg. Med. Chem. **17**, 2410–2422 (2009)
16. R.J. Grayer, J.B. Harborne, Phytochemistry **37**, 19–42 (1994)
17. O.N. Irob, M. Moo-Young, W.A. Anderson, Int. J. Pharm. **34**, 87–90 (1996)