Anticancer activity of novel 3-(furan-2-yl)pyrazolyl and 3-(thiophen-2-yl)pyrazolyl hybrid chalcones: Synthesis and in vitro studies

Mirna T. Helmy1 | Farid M. Sroor2,3 | Karima F. Mahrous4 | Khaled Mahmoud5 | Hamdi M. Hassanean1 | Fatma M. Saleh1 | Ismail A. Abdelhamid1 | Mohamed A. Mohamed Teleb1

1Department of Chemistry, Faculty of Science, Cairo University, Giza, Egypt
2Organometallic and Organometalloid Chemistry Department, National Research Centre, Cairo, Egypt
3Institut für Anorganische Chemie, Universität Göttingen, Göttingen, Germany
4Cell Biology Department, National Research Centre, Dokki, Egypt
5Pharmacognosy Department, National Research Centre, Dokki, Egypt

Correspondence
Farid M. Sroor, Organometallic and Organometalloid Chemistry Department, National Research Centre, 12622 Cairo, Egypt.
Email: faridsroor@gmx.de and fm.sroor@nrc.sci.eg

Hamdi M. Hassanean and Ismail A. Abdelhamid, Department of Chemistry, Faculty of Science, Cairo University, 12511 Giza, Egypt.
Email: hamdi_251@yahoo.com and ismail_shafy@yahoo.com and ismail_shafy@cu.edu.eg

Abstract
Twelve novel chalcone derivatives were prepared using the Claisen–Schmidt condensation reaction. The reaction of 4-acetyl-5-furan/thiophene-pyrazole derivatives with the corresponding aldehydes afforded the targeted chalcone derivatives in good yields. The newly synthesized chalcones were fully characterized by spectrometric and elemental analyses. The in vitro anticancer activities of the novel compounds were evaluated against four human cancer cell lines: HepG2 (human hepatocellular carcinoma), MCF7 (human Caucasian breast adenocarcinoma), A549 (lung carcinoma), and BJ1 (normal skin fibroblasts). Compound 7g emerged as the most promising compound, with IC50 = 27.7 µg/ml against A549 cells compared to the reference drug doxorubicin (IC50 = 28.3 µg/ml), and IC50 = 26.6 µg/ml against HepG2 cells compared to the reference drug doxorubicin (IC50 = 21.6 µg/ml). The gene expression and DNA damage values and the DNA fragmentation percentages for compound 7g were determined on the lung and liver cell lines. The expression levels of the AMY2A and FOXG1 genes increased significantly (p < 0.01) in the negative samples of lung cancer cells compared with treated cells. Also, the expression values of the PKM and PSPH genes improved significantly (p < 0.01) in the negative samples compared with treated samples of liver cancer cells. The DNA damage values increased significantly (p < 0.01) in treated lung cell line samples (7g) and the positive control. The results showed a significant decrease (p < 0.05) in DNA damage values in the negative samples of liver cancer cells compared to those treated with 7g. However, the DNA fragmentation values increased significantly (p < 0.01) in the treated lung and liver cell line samples compared with the negative control.

KEYWORDS
breast cancer, DNA fragmentation, gene expression, human cancer cell lines, pyrazolyl-chalcones

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2021 The Authors. Archiv der Pharmazie published by Wiley-VCH GmbH on behalf of Deutsche Pharmazeutische Gesellschaft.
1 | INTRODUCTION

Chalcones, which are known as 1,3-diphenylprop-2-en-1-one, exist in various conjugated formulas, where the two rings are linked by the keto-ethylenic system (α,β-unsaturated carbonyl bridge, –CO–CH=CH–). It is assumed that the conjugation between the double bond and the carbonyl group is responsible for the biological activity of these compounds. They can exist in either one of two forms: cis-form or the more thermodynamically stable trans-form. Several studies indicated that some chalcones possess varieties of biological activities including as antioxidant,[2,3] anti-inflammatory,[4,5] antimalarial,[6] antiviral,[7] analgesic,[8] antibacterial,[9,10] antiplatelet,[11] and anticancer agents.[2,12]

Pyrazole and its derivatives are well-known nitrogen-containing heterocycles, and play an important role in medicinal chemistry due to their diverse biological applications including anti-inflammatory,[13,14] anticancer,[15–17] antioxidant,[18–20] anticonvulsant,[21] and antimicrobial activities.[14,20] Besides, pyrazoles have several applications in semiconductors, liquid crystals, and organic light-emitting diodes.[22–26] 3-(Thiophen-2-yl)-1H-pyrazoles,[27–31] and 3-(furan-2-yl)-1H-pyrazoles[32–34] have been found to show interesting biological applications.

Based on these facts and in continuation of our research interest in the preparation of bioactive heterocycles,[35–44] we aimed to synthesize pyrazolyl-chalcones and test their biological activity in vitro as anticancer agents against four human cancer cell lines: HepG2 (human hepatocellular carcinoma), MCF7 (human Caucasian breast adenocarcinoma), A549 (lung carcinoma), and BJ1 (normal skin fibroblast). The gene expression, DNA damage values, and DNA fragmentation percentages for the most promising compounds have been studied on lung and liver cell lines.

Cancer and mortality rates continue to increase rapidly worldwide. Due to the rapid and adaptive nature of cancer development, the resistance and side effects associated with the use of existing drugs pose challenges in the search for additional drugs with the aim of offering more effective therapeutic treatments. Therefore, new therapeutic drugs are always needed to overcome the side effects associated with existing drugs.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

The precursors used in this study, 1-[3-(furan-2-yl)-5-methyl-1-(4-nitrophenyl)-1H-pyrazol-4-yl]ethan-1-one 5a and 1-[5-methyl-1-(4-nitrophenyl)-3-(thiophen-2-yl)-1H-pyrazol-4-yl]ethan-1-one 5b, were prepared with good yields as reported.[45,46] The first step involved the chlorination of the respective N-(4-nitrophenyl)furan-carbohydrazide 1a, or N’-(4-nitrophenyl)thiophene-carbohydrazide 1b, which yielded N-(4-nitrophenyl)furan-2-carboxyrazonoyl chloride 2a or N’-(4-nitrophenyl)thiophene-2-carboxyrazonoyl chloride 2b. Subsequent reaction of 2a,b with acetylacetone 4 in the presence of ethanolic sodium ethoxide solution afforded 5a,b. It is proposed that 2a,b in the presence of sodium ethoxide, are converted into nitrilimine intermediates 3a,b that interact with acetylacetone 4 through [3 + 2] cycloaddition reaction to afford the final products 5a,b (Scheme 1).

Claisen–Schmidt condensation of compounds 5a,b with equimolar amounts of arylaldehydes 6a–f in ethanol in the presence of sodium hydroxide solution yielded pyrazolyl-chalcones 7a–l.
(Scheme 2). The structures of the formed products were confirmed from their spectral data. The $^1$H NMR spectrum of chalcone 7a as a representative example indicated the presence of one singlet signal at $\delta$ 2.62 for one methyl group. Also, the $^1$H NMR spectrum of compound 7a featured two doublet vinyl protons at $\delta$ 6.94 and 7.64 with coupling constant $J$ = 15.9 Hz (which confirms the trans configuration of the two vinyl protons). The two doublets at 7.75 and 8.38 with $J$ = 9 Hz are assigned to $p$-nitrophenyl protons. The composition of 7a was also confirmed on the basis of $^{13}$C NMR, which featured nineteen signals corresponding to 19 different carbon atoms.

2.2 | Anticancer activity

2.2.1 | Primary screening

The synthesized chalcone compounds 7a–l were tested against four human cancer cell lines, namely, HepG2 (human hepatocellular carcinoma), MCF7 (human Caucasian breast adenocarcinoma), A549 (lung carcinoma), and BJ1 (normal skin fibroblast), at 100 µg/ml. The results showed that compounds 7a and 7g possess anticancer activities in the range of 85–100% on HepG2, MCF7, and A549 cell lines. Most of the compounds showed low activity against A549, MCF7, and HepG2 cell lines as shown in Table 1. Compound 7a showed high activity against BJ1 with 100% inhibition while compound 7g has less activity on BJ1 with 43.2% inhibition. Therefore, the promising compounds 7a and 7g were subjected to secondary screening to calculate the IC$_{50}$ and the selectivity index.

2.2.2 | Secondary screening

In terms of IC$_{50}$ values, as shown in Table 2, compound 7g was found to be the most promising compound for HepG2 (human hepatocellular carcinoma cell line) and A549 (lung carcinoma), with IC$_{50}$ values of 64.0 and 66.6 µM, respectively. These results

| TABLE 1 | % Mortality of cancer and normal cell lines at 100 µg/ml |
|----------------|----------------|
| Compound | HepG2 | MCF7 | A549 | BJ1 |
| 7a | 87.2 | 95.3 | 84.5 | 100 |
| 7b | 7.3 | 5.4 | 24.3 | – |
| 7c | 33.8 | 61.4 | 44.3 | – |
| 7d | 23.4 | 21.1 | 13.2 | – |
| 7e | 2.4 | 1.5 | 2.5 | – |
| 7f | 3.4 | 2.4 | 63.5 | – |
| 7g | 94.2 | 51.2 | 100 | 43.2 |
| 7h | 33.4 | 21.4 | ND | – |
| 7i | 2.3 | 21.3 | 59.3 | – |
| 7j | 14.5 | 31.5 | 49.8 | – |
| 7k | 5.6 | 2.5 | 11.3 | – |
| 7l | 4.5 | 3.4 | 41.2 | – |
| DOX | 100 | 100 | 100 | – |
| Negative control | 0 | 0 | 0 | 0 |

SCHEME 2 Synthesis of the targeted pyrazolyl-chalcones 7a–l
show that compound 7g has selectivity on the HepG2 and A549 cell lines.

2.2.3 | Gene expression analysis of lung cancer- and liver cancer-related genes

**Gene expression in the lung cell line**

Gene expression analysis in lung cancer cell lines was performed using lung cancer-related genes, namely, amylase alpha 2A (AMY2A) and forkhead box G1 (FOXG1) genes. The results revealed that the expression levels of AMY2A and FOXG1 genes increased significantly ($p < 0.01$) in the negative samples of the lung cancer cell lines compared with the treated cell lines (Figure 1ab). In contrast, the expression values of AMY2A and FOXG1 genes decreased significantly ($p < 0.05$) in the treated lung cell lines (7g) and the positive control (+ve) lung cell line compared with the negative samples of lung cancer cell lines (cancer cell lines [−ve]). Furthermore, the expression levels of the FOXG1 gene were downregulated in 7g, much lower than those in positive control lung cell lines (+ve).

**Gene expression in the liver cancer cell line**

The results of PKM (pyruvate kinase M1/2) and PSPH (phosphoserine phosphatase) gene expression analysis in liver cancer cell lines revealed that the expression levels of PKM and PSPH genes improved significantly ($p < 0.01$) in the negative samples compared with the treated samples of liver cancer cell lines (Figure 1c.d). A suppression ($p < 0.05$) in the expression levels of PKM and PSPH genes was recorded in the treated (7g) and positive control liver cell lines compared with the negative samples. Furthermore, the expression levels of PKM and PSPH genes were downregulated in 7g, much lower than those in the positive control liver cell lines (+ve).

2.2.4 | DNA damage in the lung and liver cell lines using the comet assay

**DNA damage in the lung cell line**

The results of DNA damage in lung cancer cell lines showed that negative samples of lung cancer cell lines showed a significant decrease ($p < 0.05$) in DNA damage values compared with the treated cell lines (Table 3). However, the DNA damage values increased significantly ($p < 0.01$) in the treated lung cell line samples (7g) and the positive control (+ve) lung cell line. Additionally, the highest values of DNA damage were observed in 7g, much more than those in the positive control (+ve) lung cell line.

**DNA damage in the liver cell line**

The DNA damage in liver cancer cell lines was determined using the comet assay as shown in Table 4. The current results showed a significant decrease ($p < 0.05$) in DNA damage values in negative samples of the liver cancer cell line compared with those treated with 7g (Table 4). In contrast, the DNA damage values increased significantly ($p < 0.01$) in the treated liver cancer cell line samples (7g) and the positive control cell line. Furthermore, the highest values of DNA damage were observed in the cell lines treated with 7g, much more than those in the positive control cell line.

2.2.5 | DNA fragmentation in the lung and liver cancer cell lines

**Measurement of DNA fragmentation in the lung cancer cell line**

In addition to the results of DNA fragmentation in liver cancer cell lines, the results presented in Table 5 and Figure 2a showed that negative samples of liver cancer cell lines showed a significant decrease ($p < 0.01$) in DNA fragmentation rates compared with the treated samples (7g) and positive liver cancer cell lines (+ve). However, the DNA fragmentation values increased significantly ($p < 0.01$) in the treated liver cell line samples compared with the negative control. Moreover, the highest DNA fragmentation values were observed in 7g, much more than those in the positive liver cancer cell line.

**Measurement of DNA fragmentation in the liver cancer cell line**

In addition to the results of DNA fragmentation in liver cancer cell lines, it is clear from the results presented in Table 6 and Figure 2 that negative samples of liver cancer cell lines showed a significant decrease ($p < 0.01$) in DNA fragmentation rates compared with those in treated samples (7g) and positive liver cancer cell lines (+ve). However, the DNA fragmentation values increased significantly ($p < 0.01$) in the treated liver cell line samples compared with the negative control. Moreover, the highest DNA

### Table 2

| Compound | HepG2  | MCF7   | A549   | BJ1    |
|----------|--------|--------|--------|--------|
| 7a       | 117.6 (±3.2) | 64.1 (±1.5) | 98.9 (±4.6) | 24.8 (±2.8) |
| 7b       | ND     | ND     | ND     | ND     |
| 7c       | ND     | ND     | ND     | ND     |
| 7d       | ND     | ND     | ND     | ND     |
| 7e       | ND     | ND     | ND     | ND     |
| 7f       | ND     | ND     | ND     | ND     |
| 7g       | 64.0 (±4.1) | 66.6 (±2.2) | 196.4 (±4.9) |
| 7h       | ND     | ND     | ND     | ND     |
| 7i       | ND     | ND     | ND     | ND     |
| 7j       | ND     | ND     | ND     | ND     |
| 7k       | ND     | ND     | ND     | ND     |
| 7l       | ND     | ND     | ND     | ND     |
| DOX      | 39.7 (±1.2) | 48.0 (±3.8) | 52.1 (±3.4) | 24.8 (±2.7) |
| Negative control | 0     | 0     | 0     | 0     |

Abbreviation: ND, not detected.
fragmentation values were observed in 7g, much more than those in the positive liver cancer cell lines (+ve).

2.3 Structure–activity relationship

As shown in Figure 3, the anticancer activity of the prepared chalcones 7a-1 toward the tested cancer cell lines increased when R1 = R2 = R3 = R4 = R5 = H, as shown in compounds 3-(furan-2-yl) pyrazol-4-yl]chalcone 7a and 3-(thiophen-2-yl)pyrazol-4-yl] chalcone 7g as compared to the other chalcones. In addition, the presence of a furan moiety increased the anticancer activity in compounds 7a and 7c against the MCF7 cell line. On the other hand 3-(thiophen-2-yl)pyrazol-4-yl]chalcone 7g containing the thiophene moiety increased the anticancer activity against HepG2 and A549 cell lines.

| Treatment                  | No. of samples | No. of cells Analyzed | Comets 0 | 1 | 2 | 3 | DNA damaged cells (%) (mean ± SEM) |
|----------------------------|----------------|-----------------------|----------|---|---|---|-----------------------------------|
| Cancer cell line (-ve)     | 4              | 400                   | 44       | 356| 31| 26| 31| 11.00 ± 0.91 b                    |
| 7g                         | 4              | 400                   | 92       | 308| 35| 26| 31| 23.00 ± 1.29 a                    |
| Cancer cell line (+ve)     | 4              | 400                   | 83       | 317| 33| 29| 21| 20.75 ± 0.85 a                    |

*Note: Means with different superscripts (a and b) between locations in the same column are significantly different at p < 0.05.

*Number of cells examined per group.

**Class 0 = no tail; 1 = tail length < diameter of the nucleus; 2 = tail length between 1× and 2× of the diameter of the nucleus; and 3 = tail length >2× of the diameter of the nucleus.
In conclusion, we were able to prepare a novel series of 3-(furan-2-yl)pyrazolyl and 3-(thiophen-2-yl)pyrazolyl chalcones using the Claisen–Schmidt condensation of 1-[3-(furan-2-yl)pyrazol-4-yl]ethan-1-one and 1-[3-(thiophen-2-yl)pyrazol-4-yl]ethan-1-one with the appropriate aldehydes. The in vitro anticancer activities of the novel compounds were evaluated against HepG2, MCF7, A549, and BJ1. Compound 7g yielded IC<sub>50</sub> values of 27.7 and 26.6 µg/ml against A549 and HepG2, respectively, as compared to doxorubicin.

**TABLE 4** Visual score of DNA damage in the liver tumor cell line treated with 7g

| Treatment           | No. of samples | No. of cells Analyzed | Comets | Class ** | DNA damaged cells (%) (mean ± SEM) |
|---------------------|----------------|-----------------------|--------|----------|-----------------------------------|
| Cancer cell line (-ve) | 4              | 400                   | 42     | 358      | 10.50 ± 1.04<sup>b</sup>           |
| 7g                  | 4              | 400                   | 97     | 303      | 24.25 ± 1.38<sup>a</sup>           |
| Cancer cell line (+ve) | 4              | 400                   | 88     | 312      | 22.00 ± 1.29<sup>a</sup>           |

*Note: Means with different superscripts (a and b) between locations in the same column are significantly different at p < 0.05.*

*Number of cells examined per group.

**Class 0 = no tail; 1 = tail length < diameter of the nucleus; 2 = tail length between 1× and 2× of the diameter of the nucleus; and 3 = tail length > 2× of the diameter of the nucleus.

**TABLE 5** DNA fragmentation detected in the lung cancer cell line treated with 7g

| Treatment           | DNA fragmentation (%) (mean ± SEM) | Change | Inhibition |
|---------------------|-----------------------------------|--------|------------|
| Cancer cell line (-ve) | 12.0 ± 0.76<sup>c</sup>         | 0.0    | 0.0        |
| 7g                  | 33.8 ± 1.02<sup>a</sup>          | 21.7   | 37.11      |
| Cancer cell line (+ve) | 27.9 ± 0.59<sup>b</sup>         | 15.9   | 0.0        |

*Note: Means with different superscripts (a, b, c) between treatments in the same column are significantly different at p < 0.05.*

**FIGURE 2** DNA fragmentation detected by agarose gel electrophoresis. (a) Lung cancer cell line and (b) liver cancer cell line treated with 7g. M represents the DNA marker; lane 1 shows the negative cancer cell line (-ve); lane 2 shows 7g; and lane 3 shows positive cancer cell lines (+ve).

**TABLE 6** DNA fragmentation detected in liver cancer cell lines treated with 7g

| Treatment           | DNA fragmentation (%) (mean ± SEM) | Change | Inhibition |
|---------------------|-----------------------------------|--------|------------|
| Cancer cell line (-ve) | 11.1 ± 0.57<sup>b</sup>        | 0.0    | 0.0        |
| 7g                  | 30.6 ± 1.31<sup>a</sup>          | 19.5   | 10.79      |
| Cancer cell line (+ve) | 28.7 ± 0.55<sup>a</sup>         | 17.6   | 0.0        |

*Note: Means with different superscripts (a and b) between locations in the same column are significantly different at p < 0.05.*

3 | CONCLUSIONS

In conclusion, we were able to prepare a novel series of 3-(furan-2-yl)pyrazolyl and 3-(thiophen-2-yl)pyrazolyl chalcones using the Claisen–Schmidt condensation of 1-[3-(furan-2-yl)pyrazol-4-yl]ethan-1-one and 1-[3-(thiophen-2-yl)pyrazol-4-yl]ethan-1-one with the appropriate aldehydes. The in vitro anticancer activities of the novel compounds were evaluated against HepG2, MCF7, A549, and BJ1. Compound 7g yielded IC<sub>50</sub> values of 27.7 and 26.6 µg/ml against A549 and HepG2, respectively, as compared to doxorubicin.
(IC\textsubscript{50} 28.3 and 21.6 \textmu g/ml), respectively. The gene expression, DNA damage values, and DNA fragmentation percentages for compound 7g have been studied on lung and liver cell lines. Furthermore, the expression levels of the AMY2A, FOXG and PKM, PSPH genes were downregulated in 7g, much lower than those in the positive control lung and liver cell lines (+ve), respectively. The highest values of DNA damage were observed in 7g, much more than those in the positive control (+ve) lung and liver cell lines. Furthermore, the highest values of DNA damage were observed in the 7g-treated cell lines, much more than those in the positive control cell line. Moreover, the highest DNA fragmentation value was observed in 7g, much more than those in the positive lung and liver cancer cell lines. Like most drugs, there may be limitations such as side effects and acquired tumor resistance, so further in vivo anticancer studies of our new drugs are recommended in the near future.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

Melting points were measured using a Stuart melting point apparatus, and are uncorrected. The IR spectra were recorded on an FTIR Bruker-vector 22 spectrophotometer as KBr pellets. The 1\textsuperscript{H} and 13\textsuperscript{C} NMR spectra (see the Supporting Information) were measured in CDCl\textsubscript{3} as a solvent on a Varian Gemini NMR spectrometer at 300 MHz using TMS as the internal standard. Chemical shifts are reported as \delta values in ppm. Mass spectra were recorded using a Shimadzu GCMS–QP–1000 EX mass spectrometer in EI (70 eV) mode. The elemental analyses were performed at the Microanalytical Center, Cairo University.

1-(2-Furyl)-2-(4-nitrophenyl)hydrazine 1a\textsuperscript{[47]} 1-(2-thienyl)-2-(4-nitrophenyl)hydrazine 1b\textsuperscript{[45]} N-(4-nitrophenyl)-C-(2-furyl) methanohydrazonoyl chloride 2a\textsuperscript{[48]} N-(4-nitrophenyl)-C-(2-thienyl) methanohydrazonoyl chloride 2b\textsuperscript{[49]} 1-[3-(furan-2-yl)-5-methyl-1-[4-nitrophenyl]-1H-pyrazol-4-yl]ethan-1-one 5a\textsuperscript{[46]} and 1-[5-methyl-1-(4-nitrophenyl)-3-(thiophen-2-yl)-1H-pyrazol-4-yl]ethan-1-one 5b\textsuperscript{[43]} were prepared using the reported procedures.

The InChI codes of the investigated compounds, together with some biological activity data, are provided as the Supporting Information.

4.1.2 | Synthesis of 1-[3-(furan-2-yl)-5-methyl-1-(4-nitrophenyl)-1H-pyrazol-4-yl]-3-phenylprop-2-en-1-one derivatives (7a–f) and 1-[5-methyl-1-(4-nitrophenyl)-3-(thiophen-2-yl)-1H-pyrazol-4-yl]-3-phenylprop-2-en-1-one derivatives (7g–l)

To a stirred mixture of 1-[3-(furan-2-yl)-5-methyl-1-(4-nitrophenyl)-1H-pyrazol-4-yl]ethan-1-one 5a, or 1-[5-methyl-1-(4-nitrophenyl)-3-(thiophen-2-yl)-1H-pyrazol-4-yl]ethan-1-one 5b (0.001 mol), and the appropriate aldehyde 6 (0.001 mol) in ethanol (30 ml), sodium hydroxide solution 20% was added, the reaction mixture was stirred for 6 h at room temperature, and left overnight. The resulting solid product that precipitated was filtered, washed with water, and crystallized from a suitable solvent to yield the corresponding 1-[3-(furan (or thiophene)-2-yl)-5-methyl-1-(4-nitrophenyl)-1H-pyrazol-4-yl]-3-phenylprop-2-en-1-one derivatives 7a–l.

1-[3-(Furan-2-yl)-5-methyl-1-[4-nitrophenyl]-1H-pyrazol-4-yl]-3-phenylprop-2-en-1-one (7a)

Beige crystals, m.p 116–118°C (EtOH), yield (75%); IR (\nu\textsubscript{max}, cm\textsuperscript{-1}) ν 1659 (CO). 1\textsuperscript{H} NMR (300 MHz, CDCl\textsubscript{3}) \delta 2.62 (s, 3H, CH\textsubscript{3}), 6.52–5.53 (m, 1H, Ar-H, J = 4.8 Hz), 6.84 (d, 1H, Ar-H, J = 3.3 Hz), 6.94 (d, 1H, vinyl-H, J = 15.9 Hz), 7.34–7.54 (m, 6H, Ar-H), 7.64 (d, 1H, vinyl-H, J = 15.9 Hz), 7.75 (d, 2H, Ar-H, J = 9 Hz), 8.38 (d, 2H, Ar-H, J = 9 Hz); 13\textsuperscript{C} NMR (75 MHz, CDCl\textsubscript{3}) \delta 125.7, 125.9, 128.2, 128.8, 130.4, 134.4, 143.4, 143.7, 146.02, 146.7, 158.3, 187.2. MS (EI, 70 eV) m/z (%): 399 (M\textsuperscript{+}, 84.08), 371 (100.00), 324 (31.81), 117 (23.81), 103 (35.51), 77 (60.99). Anal.

\textbf{FIGURE 3} Structure–activity relationship study of the prepared chalcones 7a–l
Beige crystals, m.p 166.8°C (EtOH), yield (78%). 1H NMR (300 MHz, CDCl3) δ 2.37 (s, 3H, CH3), 2.61 (s, 3H, CH3), 6.51–6.52 (m, 1H, Ar-H), J = 4.8 Hz), 6.83 (d, 1H, Ar-H, J = 3.3 Hz), 6.91 (d, 1H, vinyl-H, J = 15.9 Hz), 7.16–7.53 (m, 5H, Ar-H), 7.62 (d, 1H, vinyl-H, J = 15.6 Hz), 7.75 (d, 2H, Ar-H, J = 9 Hz); 13C NMR (75 MHz, CDCl3) δ 12.5, 21.3, 111.0, 111.3, 121.1, 124.5, 125.0, 125.3, 128.2, 129.5, 131.6, 140.9, 143.0, 143.2, 143.3, 143.4, 143.6, 146.0, 146.7, 187.3. MS (EI, 70 eV) m/z (%): 413 (M+, 91.28), 385 (100.00), 338 (26.23), 322 (24.31), 250 (23.29), 117 (37.36), 91 (24.02), 76 (28.01). Anal. calcd. for C24H19N3O4 (433.8): C, 63.68; H, 3.72; Cl, 8.17; N, 9.69. Found: C, 63.77; H, 3.89; Cl, 8.31; N, 9.82.

Yellow crystals, m.p 106–108°C (EtOH), yield (78%). 1H NMR (300 MHz, CDCl3) δ 2.64 (s, 3H, CH3), 6.93 (d, 1H, vinyl-H, J = 15.9 Hz), 7.08–7.44 (m, 8H, Ar-H), 7.64 (d, 1H, vinyl-H, J = 15.9 Hz), 7.76 (d, 2H, Ar-H, J = 9 Hz), 8.38 (d, 2H, Ar-H, J = 9 Hz); 13C NMR (75 MHz, CDCl3) δ 12.7, 121.5, 121.8, 124.5, 125.8, 125.9, 128.1, 128.7, 128.8, 130.3, 134.4, 143.3, 143.5, 143.6, 146.7, 187.3. MS (EI, 70 eV) m/z (%): 415 (M+, 100.00), 414 (34.65), 338 (32.96), 324 (18.91), 266 (11.59), 103 (17.91), 91 (14.30), 77 (34.92), 76 (27.02). Anal. calcd. for C24H19ClN3O4 (415.5): C, 66.49; H, 4.12; N, 10.11; S, 7.72. Found: C, 66.41; H, 4.06; N, 10.04; S, 7.65.

Yellow crystals, m.p 156–158°C (CH2CN), yield (80%); IR (νmax, cm⁻¹) v 1652 (CO). 1H NMR (300 MHz, CDCl3) δ 2.59 (s, 3H, CH3), 3.81 (s, 3H, OCH3), 3.84 (s, 3H, OCH3), 6.43–6.86 (m, 4H, Ar-H), 7.01 (d, 1H, vinyl-H, J = 15.9 Hz), 7.37–7.52 (m, 2H, Ar-H), 7.75 (d, 2H, Ar-H, J = 9 Hz), 7.89 (d, 1H, vinyl-H, J = 15.9 Hz), 8.37 (d, 2H, Ar-H, J = 9 Hz); 13C NMR (75 MHz, CDCl3) δ 12.6, 55.2, 55.3, 98.2, 105.3, 111.0, 111.3, 116.5, 121.4, 124.3, 124.6, 125.3, 130.7, 139.4, 142.6, 143.1, 143.5, 143.6, 146.2, 146.7, 160.2, 163.0, 188.1. Anal. calcd. for C25H21N3O5 (429.4): C, 67.31; H, 4.85; N, 8.59.

Yellow crystals, m.p 156–158°C (CH2CN), yield (80%); IR (νmax, cm⁻¹) v 1652 (CO). 1H NMR (300 MHz, CDCl3) δ 2.59 (s, 3H, CH3), 3.81 (s, 3H, OCH3), 3.84 (s, 3H, OCH3), 6.43–6.86 (m, 4H, Ar-H), 7.01 (d, 1H, vinyl-H, J = 15.9 Hz), 7.37–7.52 (m, 2H, Ar-H), 7.75 (d, 2H, Ar-H, J = 9 Hz), 7.89 (d, 1H, vinyl-H, J = 15.9 Hz), 8.37 (d, 2H, Ar-H, J = 9 Hz); 13C NMR (75 MHz, CDCl3) δ 12.6, 55.2, 55.3, 98.2, 105.3, 111.0, 111.3, 116.5, 121.4, 124.3, 124.6, 125.3, 130.7, 139.4, 142.6, 143.1, 143.5, 143.6, 146.2, 146.7, 160.2, 163.0, 188.1. Anal. calcd. for C25H21N3O5 (429.4): C, 67.31; H, 4.85; N, 8.59.
(75 MHz, CDCl₃) δ 12.8, 21.3, 121.6, 124.6, 125.1, 125.3, 126.9, 127.4, 128.3, 128.7, 129.5, 131.7, 133.5, 140.9, 143.3, 143.4, 143.4, 146.7, 146.8, 187.5. Anal. calcd. for C₂₄H₁₉N₃O₃S (429.5): C, 67.12; H, 4.46; N, 9.78; S, 7.46. Found: C, 67.26; H, 4.61; N, 9.71; S, 7.53.

3-(4-Methoxyphenyl)-1-(5-methyl-1-(4-nitrophenyl))-3-(thiophen-2-yl)-1H-pyrazol-4-yl)-prop-2-en-1-one (7j)

Yellow crystals, m.p 174–176°C (dioxane), yield (85%); ¹H NMR (300 MHz, CDCl₃) δ 2.62 (s, 3H, CH₃), 3.77 (s, 3H, OCH₃), 3.84 (s, 3H, OCH₃), 6.42–6.49 (m, 2H, Ar-H), 7.01 (d, 1H, vinyl-H, J = 15.9 Hz), 7.07–7.39 (m, 5H, Ar-H), 7.76 (d, 2H, Ar-H, J = 9 Hz), 7.89 (d, 1H, vinyl-H, J = 15.9 Hz), 8.38 (d, 2H, Ar-H, J = 9 Hz); ¹³C NMR (75 MHz, CDCl₃) δ 12.9, 55.3, 55.4, 98.3, 105.4, 116.6, 122.0, 124.7, 125.3, 126.6, 127.5, 128.8, 131.0, 139.4, 143.1, 143.7, 146.7, 160.4, 163.1, 188.5. MS (EI, 70 eV) m/z (%): 475 (M⁺, 100.00), 445 (M⁺, 100.00), 438 (27.01), 266 (22.32), 121 (73.17), 89 (13.08), 76 (19.99). Anal. calcd. for C₂₅H₂₁N₃O₅S (475.5): C, 63.15; H, 4.45; N, 9.57; S, 7.38. Found: C, 64.54; H, 4.44; N, 9.57; S, 7.38.

3-(2,4-Dimethoxyphenyl)-1-(5-methyl-1-(4-nitrophenyl))-3-(thiophen-2-yl)-1H-pyrazol-4-yl)-prop-2-en-1-one (7k)

Yellow crystals, m.p 172–174°C (CH₂CN), yield (82%). ¹H NMR (300 MHz, CDCl₃) δ 2.62 (s, 3H, CH₃), 3.77 (s, 3H, OCH₃), 3.84 (s, 3H, OCH₃), 6.42–6.49 (m, 2H, Ar-H), 7.01 (d, 1H, vinyl-H, J = 15.9 Hz), 7.07–7.39 (m, 5H, Ar-H), 7.76 (d, 2H, Ar-H, J = 9 Hz), 7.89 (d, 1H, vinyl-H, J = 15.9 Hz), 8.38 (d, 2H, Ar-H, J = 9 Hz); ¹³C NMR (75 MHz, CDCl₃) δ 12.9, 55.3, 55.4, 98.3, 105.4, 116.6, 122.0, 124.7, 125.3, 126.6, 127.5, 128.8, 131.0, 139.4, 143.1, 143.7, 146.7, 160.4, 163.1, 188.5. MS (EI, 70 eV) m/z (%): 475 (M⁺, 100.00), 445 (32.81), 444 (100.00), 398 (12.56), 266 (11.50), 151 (27.05), 117 (17.75), 76 (15.51). Anal. calcd. for C₂₆H₂₃N₂O₅S (475.5): C, 63.15; H, 4.45; N, 8.84; S, 6.74. Found: C, 63.23; H, 4.51; N, 8.75; S, 6.67.

1-(5-Methyl-1-(4-nitrophenyl))-3-(thiophen-2-yl)-1H-pyrazol-4-yl)-3-(3,4,5-trimethoxyphenyl)-prop-2-en-1-one (7l)

Yellow crystals, m.p 190–192°C (CH₂CN), yield (81%). ¹H NMR (300 MHz, CDCl₃) δ 2.66 (s, 3H, CH₃), 3.82 (s, 6H, 2OCH₃), 3.87 (s, 3H, OCH₃), 6.57 (s, 2H, Ar-H), 6.81 (d, 1H, vinyl-H, J = 15.9 Hz), 7.10–7.44 (m, 3H, Ar-H), 7.55 (d, 1H, vinyl-H, J = 15.6 Hz), 7.76 (d, 2H, Ar-H, J = 9 Hz), 8.39 (d, 2H, Ar-H, J = 9 Hz); ¹³C NMR (75 MHz, CDCl₃) δ 12.7, 55.9, 60.7, 105.1, 121.6, 124.6, 125.2, 125.3, 126.9, 127.4, 129.0, 129.8, 133.5, 140.0, 142.7, 143.2, 143.8, 146.7, 146.9, 153.1, 186.8. Anal. calcd. for C₂₆H₂₁N₂O₅S (505.5): C, 61.77; H, 4.59; N, 8.31; S, 6.34. Found: C, 61.98; H, 4.78; N, 8.46; S, 6.45.

### 4.2 | Anticancer evaluation

#### 4.2.1 | Materials and methods

Cell viability was assessed by the mitochondria-dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan. 

**Procedure:** All the following procedures were performed in a sterile area using a laminar flow cabinet biosafety class II level (Baker, SG403INT, Sanford). Cells were suspended in DMEM-F12 medium (for HepG2, MCF7, and A549) beside one normal cell line (BJ1), a 1% antibiotic–antimycotic mixture (10,000 U/ml potassium penicillin, 10,000 µg/ml streptomycin sulfate, and 25 µg/ml amphotericin B), and 1% L-glutamine at 37°C under 5% CO₂.

Cells were cultured for 10 days, and then seeded at a concentration of 10 × 10⁵ cells/well in fresh complete growth medium in 96-well microtiter plastic plates at 37°C for 24 h under 5% CO₂ using a water-jacketed carbon dioxide incubator (Sheldon, TC2323). Media were aspirated, fresh medium (without serum) was added, and cells were incubated either alone (negative control) or with different concentrations of the sample to yield a final concentration of (100–50–25–12.5–6.25–3.125–0.78 and 1.56 µg/ml). After 48 h of incubation, the medium was aspirated and 40 µl of MTT salt (2.5 µg/ml) was added to each well and incubated for another four hours at 37°C under 5% CO₂. To stop the reaction and dissolve the formed crystals, 200 µl of 10% sodium dodecyl sulfate (SDS) in deionized water was added to each well and incubated overnight at 37°C. A positive control composed of 100 µg/ml was used as a known cytotoxic natural agent that yields 100% lethality under the same conditions.

The absorbance was then measured using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350) at 595 nm and a reference wavelength of 620 nm. Statistical significance was tested between the samples and the negative control (cells with vehicle) using an independent t-test using the SPSS 11 program. DMSO was the vehicle used for the dissolution of plant extracts, and its final concentration on the cells was less than 0.2%. The percentage of change in viability was calculated according to the formula: ((Reading of extract/Reading of negative control) - 1) × 100. A Probit analysis was carried out for the determination of IC₅₀ using the SPSS 11 program.

In the present study, the degree of selectivity of the synthetic compounds is expressed as SI = IC₅₀ of a pure compound in a normal cell line/IC₅₀ of the same pure compound in the cancer cell line, where IC₅₀ is the concentration required to kill 50% of the cell population.

#### 4.2.2 | Gene expression analysis

**RNA isolation and reverse transcription (RT) reaction**

The RNasy Mini Kit (Qiagen) supplemented with the DNsae (Qiagen) digestion step was used to isolate total RNA from lung and liver cancer cell lines according to the manufacturer's protocol. Isolated total RNA was treated with one unit of RQ1 RNase-free DNase (Invitrogen) to digest DNA residues, resuspended in DEPC-treated water, and quantified photospectrometrically at 260 nm.
The purity of total RNA was assessed by the 260/280 nm ratio, which was between 1.8 and 2.1. Additionally, integrity was assured by ethidium bromide-stain analysis of 28S and 18S bands by formaldehyde-containing agarose gel electrophoresis. Aliquots were used immediately for RT; otherwise, they were stored at −80°C.

Complete Poly(A)^+ RNA isolated from lung and liver cell lines was reverse-transcribed into cDNA in a total volume of 20 μl using the RevertAid™ First Strand cDNA Synthesis Kit (Fermentas). An amount of total RNA (5 μg) was used with a master mix in total of 20 μl in Eppendorf tubes. The master mix consisted of 50 mM MgCl2, 10× RT buffer (50 mM KCl; 10 mM Tris-HCl; pH 8.3), 10 mM of each dNTP, 50 μM oligo-dT primer, 20 IU ribonuclease inhibitor (50 kDa recombinant enzyme to inhibit RNase activity), and 50 IU MuLV reverse transcriptase. The mixture of each sample was centrifuged for 30 s at 1000g and transferred to the thermocycler. The RT reaction was carried out at 25°C for 10 min, followed by 1 h at 42°C, and completed with a denaturation step at 99°C for 5 min. Afterward, the reaction tubes containing RT preparations were flash-cooled in an ice chamber until use for cDNA amplification using a quantitative real-time polymerase chain reaction (qRT-PCR).

**Real-time quantitative PCR (qPCR)**

Determination of the lung and liver cell line cDNA copy number was carried out using the StepOne™ Real-Time PCR system from applied Biosystems (Thermo Fisher Scientific). PCRs were set up in 25 μl reaction mixtures containing 12.5 μl of 1× SYBR® Premix Ex Taq™ (TakaRa, Biotech. Co. Ltd.), 0.5 μl of 0.2 μM sense primer, 0.5 μl of 0.2 μM antisense primer, 6.5 μl of distilled water, and 5 μl of the cDNA template. The reaction program included three steps. The first step was performed at 95°C for 3 min. The second step consisted of 40 cycles, in which each cycle was divided into three steps: (a) at 95°C for 15 s; (b) at 55°C for 30 s; and (c) at 72°C for 30 s. The third step consisted of 71 cycles, which started at 60°C and then increased by about 0.5°C every 10 s up to 95°C. At the end of each qRT-PCR, a melting curve analysis was performed at 95°C to check the quality of the primers used. Each experiment included a distilled water control. The sequences of the specific primers of the lung (ISL1 and MALL genes, Watanabe et al.)[52] and liver (ASNS and ACLY genes, Saur et al.)[53] cancer-related genes were designed and are listed in Table 7. At the end of each qPCR, a melting curve analysis was performed at 95°C to check the quality of the used primers. The relative quantification of the target to the reference was performed using the 2^−ΔΔCT method (Yang et al.).[54]

### Table 7

| Gene     | Primer sequence        | GenBank (accession no.) |
|----------|------------------------|-------------------------|
| AMY2A    | F: CATTGTTGTGCAGGTTCA  | NM_000699.4             |
|          | R: ACCCGGCCATTACAAAGTA |                         |
| FOXG1    | F: CCCTGCCCTGTAGCTTTA  | NM_005249.5             |
|          | R: ACGTACAGAAATGGGAGGGG|                         |
| PKM      | F: GCACACGTATCTGCCTG   | NM_001206798.3          |
|          | R: TCCAGGAATGTGTCAGCCAT|                         |
| PSPH     | F: AGCAGCTGGAGAAAGGAGAA| NM_001370522.1          |
|          | R: AAGACGCCTTTGAAAGGCAC|                         |
| β-Actin  | F: CATGGATCTCTGGCATC   | HQ154074.1              |
|          | R: CACACAGATCTCCGCT    |                         |

Abbreviations: AMY2A, amylase alpha 2A; FOXG1, forkhead box G1; PKM, pyruvate kinase M1/2; PSPH, phosphoserine phosphatase.

**4.2.4 DNA fragmentation assay**

The DNA fragmentation assay in lung and liver cancer cell lines was performed in accordance with the method of Yawata et al.[57] with some modifications. Briefly, after 24 h of exposure of lung and liver cancer cell lines to the tested substances in different Petri dishes (60 × 15 mm, Greiner), the cells were trypsinized, suspended, homogenized in 1 ml of medium, and centrifuged (10 min at 800 rpm). Low-molecular-weight genomic DNA was extracted as described in Yawata et al.[57] Approximately 1 × 10⁶ cells were plated and treated with the tested substances in various treatments. All the cells (including floating cells) were harvested temperature agarose and rapidly pipetted onto a precoated microscope slide. Samples were lysed for 4 h at 50°C in 0.5% SDS and 30 mM ethylenediaminetetraacetic acid (EDTA), pH 8.0. After rinsing overnight at room temperature in Tris/borate/EDTA buffer, pH 8.0, samples were electrophoresed for 25 min at 60 V/cm and then stained with propidium iodide. Slides were viewed using a fluorescence microscope with a CCD camera, and 150 individual comet images were analyzed from each sample for tail moment, DNA content, and percentage DNA in the tail. For each sample, about 100 cells were examined to determine the percentage of cells with DNA damage that appear like comets. The nonoverlapping cells were randomly selected and were visually assigned a score on an arbitrary scale of 0–3 (i.e., Class 0 = no detectable DNA damage and no tail; Class 1 = tail with a length less than the diameter of the nucleus; Class 2 = tail with a length between 1× and 2× the nuclear diameter; and Class 3 = tail longer than 2× the diameter of the nucleus) based on perceived comet tail length migration and the relative proportion of DNA in the nucleus.[56]
by trypsinization and washed with Dulbecco’s phosphate-buffered saline. Cells were lysed with the lysis buffer containing 10 mM Tris (pH 7.4), 150 mM NaCl, 5 mM EDTA, and 0.5% Triton X-100 for 30 min on ice. Lysates were vortexed and cleared by centrifugation at 10,000×g for 20 min. Fragmented DNA in the supernatant was extracted with an equal volume of a neutral phenol/chloroform/isoamyl alcohol mixture (25:24:1) and analyzed electrophoretically on 2% agarose gels containing 0.1 μg/ml ethidium bromide.

4.3 Statistical Analysis

The obtained data were analyzed using one-way analysis of variance using the software of the Statistical Analysis System (SAS, 1982). The 2^ΔΔCT method was used for relative quantification in qPCR data analysis and to calculate the relative gene expression. Significant differences between treatments (groups) were determined using the least significant digit multiple-range test (hoc test) at a level of p < 0.05. Data are expressed as mean ± standard error of the mean (SEM); there were at least four replicates.

ACKNOWLEDGMENTS

This work was supported by the National Research Centre (NRC) and Cairo University. F. M. Sroor would like to express his special thanks to the Ministry of Higher Education and Scientific Research (MHESR) of Egypt for a postdoctoral fellowship. F. M. Sroor thanks Georg-August-Universität Göttingen for open access support. Open Access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interests.

ORCID

Farid M. Sroor http://orcid.org/0000-0003-1283-2157
Hamdi M. Hassaneen http://orcid.org/0000-0003-0963-4192
Ismail A. Abdelhamid http://orcid.org/0000-0003-1220-8370

REFERENCES

[1] S. R. Lemes, C. R. eSilva, J. H. Véras, L. Chen-Chen, R. S. Lima, C. N. Perez, M. A. Montes de Sousa, P. R. de Melo, Reis, N. J. da Silva Junior, Drug Chem. Toxicol. 2018, 43, 383.
[2] S. Shenvi, K. Kumar, K. S. Hatti, K. Rijesh, L. Diwakar, G. C. Reddy, Eur. J. Med. Chem. 2013, 62, 435.
[3] B. P. Bandgar, S. S. Gawande, R. G. Bodade, N. M. Gawande, C. N. Khobragade, Bioorg. Med. Chem. 2009, 17, 8168.
[4] A. A. Bekhit, T. Abdel-Aziem, Bioorg. Med. Chem. 2004, 12, 1935.
[5] H.-K. Hsieh, L.-T. Tsao, J.-P. Wang, C.-N. Lin, J. Pharm. Pharmacol. 2000, 52, 163.
[6] R. Li, G. L. Kenyon, F. E. Cohen, X. Chen, B. Gong, J. N. Dominguez, E. Davidson, G. Kurzban, R. E. Miller, E. O. Nuzum, P. J. Rosenthal, J. H. McKerrow, J. Med. Chem. 2002, 38, 5031.
[7] J. C. Onyilagha, B. Malhotra, M. Elder, C. J. French, G. H. N. Towers, Can J. Plant Pathol. 1997, 19, 133.
[8] M. R. Heidari, A. Forouamadi, A. Amirabadi, A. Samzadeh-Kermani, B. S. Azimzadeh, A. Eskandarizadeh, Ann. N. Y. Acad. Sci. 2009, 1171, 399.
[9] A. M. Asiri, S. A. Khan, Molecules 2011, 16, 523.
[10] M. F. Mohamed, M. S. Mohamed, S. A. Shouman, M. M. Fathi, I. A. Abdelhamid, Appl. Biochem. Biotechnol. 2012, 168, 1153.
[11] C.-N. Lin, H.-K. Hsieh, H.-H. Ko, M.-F. Hsu, H.-C. Lin, Y.-L. Chang, M.-I. Chung, J.-J. Kang, J.-P. Wang, C.-M. Teng, Drug Dev. Res. 2001, 53, 9.
[12] K. V. Sashidhara, A. Kumar, M. Kumar, J. Sarkar, S. Sinha, Bioorg. Med. Chem. Lett. 2010, 20, 7205.
[13] A. A. Farghaly, A. A. Bekhit, J. Young Park, Arch. Pharm. (Weinheim) 2000, 333, 53.
[14] B. V. Kendre, M. G. Landge, S. R. Bhusare, Arabian J. Chem. 2019, 12, 2091.
[15] N. H. Metwally, M. A. Badawy, D. S. Okpy, Drug Res. (Kaunas) 2014, 64, 955.
[16] R. Alam, D. Wahi, R. Singh, D. Sinha, V. Tandon, A. Grover, Rahisuddin, Bioorg. Chem. 2016, 69, 77.
[17] C. B. Sangani, J. A. Makawana, X. Zhang, S. B. Teraiya, L. Lin, H.-L. Zhu, Eur. J. Med. Chem. 2014, 76, 549.
[18] H. A. Sallam, A. S. Elgubbi, E. A. E. El-Helw, Synth. Commun 2020, 50, 2066.
[19] M. Bellam, M. Gundiluru, S. Sarva, S. Chadive, V. R. Netala, V. Kartte, S. R. Cirandur, Chem. Heterocycl. Compds. 2017, 53, 173.
[20] Dinesha S. V. Madhu L. N., Nagaraja G. K., Monatsh. Chem. 2015, 146, 1547.
[21] D. Kaushik, S. A. Khan, G. Chawla, S. Kumar, Eur. J. Med. Chem. 2010, 45, 3943.
[22] F. Karci, F. Karci, A. Demircal, M. Yama, J. Mol. Liq. 2013, 187, 302.
[23] J. Burschka, F. Kessler, M. K. Nazeeruddin, M. Grätzel, Chem. Mater. 2013, 25, 2986.
[24] P.-T. Chou, Y. Chi, Chemistry 2007, 13, 380.
[25] U. M. Kauhanka, M. M. Kauhanka, Liq. Cryst. 2006, 33, 121.
[26] M. Wang, J. Zhang, J. Liu, C. Xu, H. Ju, J. Lumin. 2002, 99, 79.
[27] C. Yamali, H. Sakagami, Y. Uesawa, K. Kurosaki, K. Satoh, Y. Masuda, S. Yokose, A. Ece, S. Bua, A. Angeli, C. T. Supuran, H. I. Gul, Eur. J. Med. Chem. 2021, 217, 113351.
[28] R. Li, X. Chen, R. S. Lima, J. H. McKerrow, E. Davidson, G. Kurzban, R. E. Miller, E. O. Nuzum, P. J. Rosenthal, J. H. McKerrow, J. Med. Chem. 2002, 38, 5031.
[29] J. C. Onyilagha, B. Malhotra, M. Elder, C. J. French, G. H. N. Towers, Can J. Plant Pathol. 1997, 19, 133.
[30] M. R. Heidari, A. Forouamadi, A. Amirabadi, A. Samzadeh-Kermani, B. S. Azimzadeh, A. Eskandarizadeh, Ann. N. Y. Acad. Sci. 2009, 1171, 399.
[31] A. M. Asiri, S. A. Khan, Molecules 2011, 16, 523.
[32] I. A. Abdelhamid, A. M. Abdelmoniem, F. M. Sroor, M. A. Ramadan, S. A. S. Ghouslan, Synlett 2020, 31, 895.
