Radiomodulatory effect of a non-electrophilic NQO1 inducer identified in a screen of new 6, 8-diiodoquinazolin-4(3H)-ones carrying a sulfonamide moiety

Soliman, Aiten M.; Karam, Heba M.; Mekkawy, Mai H.; Higgins, Maureen; Dinkova-Kostova, Albena; Ghorab, Mostafa M.

Published in: European Journal of Medicinal Chemistry

DOI: 10.1016/j.ejmech.2020.112467

Publication date: 2020

Document Version Publisher's PDF, also known as Version of record

Link to publication in Discovery Research Portal

Citation for published version (APA):
Soliman, A. M., Karam, H. M., Mekkawy, M. H., Higgins, M., Dinkova-Kostova, A., & Ghorab, M. M. (2020). Radiomodulatory effect of a non-electrophilic NQO1 inducer identified in a screen of new 6, 8-diiodoquinazolin-4(3H)-ones carrying a sulfonamide moiety. European Journal of Medicinal Chemistry, 200, [112467]. https://doi.org/10.1016/j.ejmech.2020.112467

General rights
Copyright and moral rights for the publications made accessible in Discovery Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

• Users may download and print one copy of any publication from Discovery Research Portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain.
• You may freely distribute the URL identifying the publication in the public portal.

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Download date: 04. Nov. 2020
Radiomodulatory effect of a non-electrophilic NQO1 inducer identified in a screen of new 6, 8-diiodoquinazolin-4(3H)-ones carrying a sulfonamide moiety

Aiten M. Soliman a, Heba M. Karam a, Mai H. Mekkawy a, Maureen Higgins b, Albena T. Dinkova-Kostova b, c, Mostafa M. Ghorab a, *

a Department of Drug Radiation Research, National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority (EAEA), Nasr City P.O. Box 25, Cairo, 11765, Egypt
b Jacqui Wood Cancer Centre, Division of Cellular Medicine, School of Medicine, University of Dundee, Dundee, DD1 9SY, Scotland, UK
c Department of Pharmacology and Molecular Sciences and Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, 21205, USA

ARTICLE INFO

Article history:
Received 15 April 2020
Received in revised form
13 May 2020
Accepted 13 May 2020
Available online 18 May 2020

Keywords:
Diodoquinazolinone
Sulfonamide
Radiomodulatory
NQO1
Nrf2
Oxidative stress
Docking

ABSTRACT

Fifteen new quinazolinone derivatives bearing benzenesulfonamide moiety with variable acetamide tail were synthesized. The structures assigned to the products were concordant with the microanalytical and spectral data. Compounds 4–18 were screened for their ability to induce the antioxidant enzyme NAD(P)H: quinone oxidoreductase 1 (NQO1) in cells, a classical target for transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2). The 2-((6,8-diiodo-4-oxo-3-(4-sulfamoylphenyl)-3,4-dihydroquinazolin-2-yl)thio)-N-(3,4,5-trimethoxyphenyl) acetamide 15 showed the most potent NQO1 inducer activity in vitro. Compound 15 had low toxicity in mice (LD50 = 500 mg/kg). It also reduced the damaging effects of gamma radiation, as assessed by the levels of Nrf2, NQO1, reactive oxygen species (ROS) and malondialdehyde (MDA) in liver tissues. In addition, compound 15 showed amelioration in the complete blood count of irradiated mice and enhanced survival over a period of 30 days following irradiation. Molecular docking of 15 inside the Nrf2-binding site of Kelch-like ECH associated protein 1 (Keap1), the main negative regulator of Nrf2, showed the same binding interactions as that of the co-crystallized ligand considering the binding possibilities and energy scores. These findings suggest that compound 15 could be considered as a promising antioxidant and radiomodulatory agent.

© 2020 The Authors. Published by Elsevier Masson SAS. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Introduction

The extensive use of radiotherapy and the damage caused to the surrounding normal organs have provoked researchers to find new strategies to protect normal tissues from radiation hazards [1,2]. The risk of injury from radiation can diminish the value of radiotherapy and contribute to complications for long-term cancer survivors [3]. Ionizing radiation interrupts cell functions through radiolysis of water and the production of reactive oxygen species (ROS) or reactive nitrogen species (RNS) [4,5]. Excessive production of ROS and RNS promotes oxidative stress, which can affect all cellular components, including single or double DNA strand breaks [6]. This ROS-mediated toxicity can lead to mutations and consequently cause cardiovascular, neurological toxicities and sexual dysfunction as well as cancer [7–10]. In order to reduce these radiation-induced side effects, radioprotective drugs are used [11]. Also, the use of multi-target antioxidants that act as radioprotectors can help limit normal tissue damage caused by ionizing radiation [12–14].

Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor that regulates the expression of various antioxidant proteins to protect against oxidative damage in the cell [15]. The abundance of Nrf2 is negatively regulated by Kelch-like ECH associated protein 1 (Keap1), a substrate adaptor for a Cullin3/Rbx1 ubiquitin ligase that binds and continuously targets Nrf2 for ubiquitination and proteasomal degradation [16–18]. Under conditions of oxidative stress, redox-sensitive cysteine sensors of Keap1 are modified leading to loss of, its ability to target Nrf2 for degradation;
consequently, Nrf2 transports into the nucleus where it initiates the transcription of its downstream target genes, such as NAD(P)H:quinone oxidoreductase1 (NQO1) [19].

Quinazolinone is a strategic scaffold that has a wide range of pharmacological activities such as antioxidant, anti-inflammatory and anticancer activities [20–23]. Sulfonamides, in addition to their use as antibiotics [24–27], have many pharmacological activities and can be used as antiviral [28], anti-inflammatory [29], antioxidant [30,31], and anticancer agents [32–35]. These versatile pharmacological activities make the two chemical classes excellent candidates for developing new multi-target agents through a slight alteration in the structure that might lead to diversity in the biological activity [20,36,37]. In addition, numerous studies have revealed iodine to be a potent antioxidant with higher potency than that of ascorbic acid [38,39]. Iodine can act as an electron donor that quench ROS, such as OH\textsuperscript* and H\textsubscript{2}O\textsubscript{2} [40], or decreases the damaging effects of ROS, thus increasing the total antioxidant status in human serum [41].

In this context, it seemed of interest to search for new compounds with the ability to scavenge ROS and protect cells. A series of new 6,8-diiodoquinoxalin-4(3H)-one conjugated to benzene-sulfonamide was synthesized by the introduction of the sulfonamide group at the N-3 of quinazolinone with the incorporation of various acetamide terminal aimed at exploring the potential antioxidant and radioprotective activity. The antioxidant potential of the target compounds was first measured using a quantitative and robust NQO1 inducer activity bioassay in cells. Acute toxicity study for the most active compound was then performed in vivo. A non-toxic dose was subsequently selected to investigate the potential protective effect against whole-body gamma irradiation-induced oxidative stress in experimental mice. All groups were observed 30 days after irradiation for survival and weight changes. Additionally, molecular docking was performed inside the Nrf2-binding site of Keap1 to gain insights into the molecular interactions and possible mode of action.

2. Results and discussion

2.1. Chemistry

Scheme 1 shows the synthesis of thioacetamide quinazolinone benzenesulfonamide derivatives 5–18. The starting material 6-(6,8-diido-2-mercaptop-4- oxoquinazolin-3(4H)-yl) benzenesulfonamide 4 was prepared from the reaction of 4-isothiocyanato benzenesulfonamide 2 [42] and 2-amino-3,5-diiodobenzoic acid 3. The coupling of 4 with the 2-chloro-N-substituted acetamide in dry acetone and anhydrous K\textsubscript{2}CO\textsubscript{3} yielded the corresponding 2-(6,8-diido-4-oxo-3-(4-sulamopyryl)phenyl)-3,4-dihydroquinazolin-2-yl(thio)-N-substituted acetamide 5–18. IR spectra of 5–18 displayed additional NH, CH\textsubscript{2} aliphatic and CO bands at their specified regions. \textsuperscript{1}H NMR spectra of 5–18 revealed the acetamide group through the presence of two singlets, one at 4.17–4.31 ppm referring to the CH\textsubscript{2} and the other at 9.66–11.21 ppm attributed to the NH protons with the disappear-ance of SH singlet of 4 at 1.97 ppm. \textsuperscript{13}C NMR of 5–18 exhibited two signals peculiar to the CH\textsubscript{2} and CO carbons. \textsuperscript{1}H NMR spectra of 6–8 displayed singlets at 2.21, 2.28 and 2.30 ppm assigned to the CH\textsubscript{2} group at the ortho, meta and para-positions of the phenyl group. \textsuperscript{13}C NMR of 6–8 showed signals at 16.32, 24.13 and 19.21 ppm for the CH\textsubscript{3} group. \textsuperscript{1}H NMR spectra of 9–11 revealed triplets at 1.31, 1.20 and 1.15 ppm attributed to the CH\textsubscript{3} ethyl and quartet at 2.54, 2.58 and 2.55 ppm referring to the CH\textsubscript{2} ethyl at the ortho, meta and para-positions. \textsuperscript{13}C NMR of 9–11 showed two signals at 14.67, 15.21, 17.12 due to CH\textsubscript{3} ethyl and 24.23, 24.10 and 29.40 due to the CH\textsubscript{3} ethyl groups, respectively. \textsuperscript{1}H NMR spectra of 12 revealed singlet at 3.75 ppm attributed to the OCH\textsubscript{3} protons, while \textsuperscript{13}C NMR of 12 showed a signal at 54.26 ppm due to the OCH\textsubscript{3} carbon. \textsuperscript{1}H NMR spectra of 13 revealed triplet at 1.27 ppm and quartet at 3.97 ppm due to the ethoxy group. \textsuperscript{1}H NMR spectra of 14 revealed a singlet at 3.74 ppm due to the 2OCH\textsubscript{3} protons, while 15 revealed two singlets at 3.70 and 3.81 ppm due to the 3OCH\textsubscript{3} protons. IR of 16–18 showed NO\textsubscript{2} bands.

2.2. Biological activity

2.2.1. In vitro screening

The antioxidant activity of compounds 4–18 was screened using the NQO1 inducer activity assay. The Concentration of the novel compounds to Double the specific enzyme activity of NQO1 (CD value) was used as a measure of inducer potency and results obtained are presented in Fig. 1 & Table 1. Evaluation of the NQO1 inducer activity showed that compounds 4, 5, 8, 9, 11 and 13 were inactive, whereas compounds 5, 6, 7, 10, 12, 14 and 18 had activity; however, CD value was not reached. Compounds 15 (CD = 20 \textmu M), and 17 (CD = 50 \textmu M) showed concentration-dependent inducer activity. These diiodoquinazolinones represent a new chemical class of NQO1 inducers, thus adding to the existing knowledge of the diversity of the many chemical scaffolds that have been reported to induce this antioxidant enzyme. The classical NQO1 inducers are primarily oxidants and electrophiles or other compounds that react (or are metabolized to products that react) and chemically modify cysteine sensors of Keap1 [43]. A new generation of NQO1 inducers is also emerging, that of noncovalent small-molecule modulators of the Keap1–Nrf2 protein-protein interaction [44–46]. Because our diiodoquinazolinones have some common features with the Keap1–Nrf2 protein-protein interaction inhibitors, in this study we tested the potential ability of these compounds to directly disrupt the binding of Keap1 to Nrf2 by molecular modeling (see section 2.3).

2.2.2. In vivo evaluation

2.2.2.1. Determination of toxicity (lethal dose fifty, LD\textsubscript{50}) of compound 15. The most promising compound, 15, was investigated in vivo for acute toxicity (LD\textsubscript{50}) in albino mice, and the value was found to be 500 mg/kg body weight (i.p.). Subsequently, one-tenth of this dose was selected as the therapeutic dose for further evaluation of the potential radioprotective effects of compound 15.

2.2.2.2. Evaluation of the radiomodulatory effect of compound 15 in mice. Four groups of mice were used, the first group served as control, the second group was irradiated at a dose of 7 Gy as a single dose, the third group was injected i.p. with compound 15 only for 5 consecutive days and the last group received compound 15 then exposed to 7 Gy of gamma radiation. After 3 days from irradiation, five mice were checked for liver and hematopoietic system toxicities. The residual mice in all groups were monitored over 30 days to evaluate the survival rate and body weight changes.

2.2.2.2.1. The effect of compound 15 on radiation-induced liver toxicity. Gamma radiation-induced hepatic oxidative stress as shown by a significant increase in hepatic levels of nuclear Nrf2 (1.3-fold), NQO1 (3.2-fold), ROS (1.5-fold) and the lipid peroxidation product malondialdehyde (MDA) (2-fold) as compared to non-irradiated (control) mice. This was in agreement with other studies [2,47].

Iodizing radiation is believed to induce damage through the generation of ROS, resulting in an imbalance in the oxidant/antioxidant ratio in cells [8,48]. In the current experiment, the presence of ROS-mediated damage was confirmed by the increase in MDA levels in irradiated liver, in addition to the increase in the expression of the enzymatic antioxidant system. Moreover, these results
support the notion that Nrf2 is an initial regulator of cellular responses to radiation exposure [49]. Once Nrf2 translocates to the nucleus it induces expression of endogenous antioxidant enzymes, such as NQO1 [50], a flavoprotein involved in cellular protection against oxidative stress [51].

Treatment of non-irradiated mice with compound 15 led to an increase in NQO1 and ROS levels and a decrease in Nrf2, with no significant change in MDA level as compared to normal (non-irradiated) mice (Fig. 2). A significant increase in Nrf2 levels (19%) as well decrease in the levels of NQO1 (30%), ROS (23%) and MDA (28%) was observed in irradiated mice livers treated with compound 15 when compared to the group subjected to radiation alone (Fig. 2). Moreover, treatment with compound 15 improved both survival and body weight of the animals following irradiation.
Additionally, it has been reported that Nrf2 modifies ROS production partly by regulating NQO1 expression [55]. On the other hand, the NQO1 levels were significantly higher than the non-irradiated controls, in agreement with the cell culture results (this study). Notably, the increased levels of ROS in non-irradiated mice treated with compound 15 are consistent with the increased levels of ROS following genetic Nrf2 activation by Keap1 knockdown [54]. Importantly however, the increased ROS production that accompanies NQO1 induction does not lead to damage, as evidenced by the lack of increase in the levels of MDA (this study).

### Table 1

NQO1 inducer activity and CD values of compounds 4–18.

| Conc. (µM) | Compound no. |
|-----------|--------------|
|           | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| 0.313     | NR | NR | NR | NR | NR | 0.99 | 1.03 | NR | NR | 1.01 | NR | 1.02 | NR | 1.01 |
| 0.625     | NR | NR | NR | NR | NR | 0.97 | 1.02 | NR | NR | 1.02 | NR | 1.08 | NR | 1.01 |
| 0.8       | 1.04 | 1.00 | 1.04 | 1.05 | NR | 1.01 | NR | NR | NR | 1.00 | NR | NR | 0.95 | NR |
| 1.25      | NR | NR | NR | NR | NR | 0.99 | 1.05 | NR | NR | 0.99 | NR | 1.10 | 0.99 | NR |
| 1.6       | NR | NR | NR | NR | NR | 0.99 | 1.05 | NR | NR | 0.99 | NR | 1.10 | 0.99 | NR |
| 2.5       | NR | NR | NR | NR | NR | 0.97 | 1.04 | NR | NR | 1.02 | NR | 1.16 | 1.00 | NR |
| 3.125     | 1.01 | 1.06 | 1.06 | 1.05 | NR | 0.97 | NR | NR | NR | 1.06 | NR | NR | 0.83 | NR |
| 5         | NR | NR | NR | NR | NR | 1.01 | 1.04 | NR | NR | 1.00 | NR | 1.24 | 1.06 | NR |
| 6.25      | 1.02 | 1.11 | 1.13 | 1.06 | 0.99 | NR | NR | NR | 0.99 | 0.99 | NR | 1.22 | NR | 0.91 |
| 10        | NR | NR | NR | NR | NR | 1.01 | 1.09 | NR | NR | 0.99 | NR | 1.43 | 1.28 | NR |
| 12.5      | 1.06 | 1.11 | 1.12 | 1.14 | NR | 1.00 | NR | NR | NR | 1.01 | 1.08 | NR | 1.52 | NR | 1.05 |
| 20        | NR | NR | NR | NR | NR | 1.01 | 1.15 | NR | NR | 1.09 | NR | 1.98 | 1.53 | NR |
| 25        | 1.05 | 1.26 | 1.31 | 1.27 | 1.08 | NR | NR | NR | NR | 1.03 | 1.18 | NR | 1.73 | NR |
| 40        | NR | NR | NR | NR | NR | 1.05 | 1.30 | NR | NR | 1.16 | NR | 2.42 | NR | 1.68 |
| 50        | NR | 1.47 | 1.43 | 1.48 | 1.13 | NR | NR | NR | NR | 1.14 | 1.29 | NR | 1.81 | NR |
| CD*       | NR | NR | NR | NR | NR | NR | NR | NR | NR | NR | NR | NR | NR | NR |

NR means not recorded.

a CD values are the averages of three independent experiments, each with eight replicate wells of cells, and SD for each data point was within 5% of the value.
happened after irradiation so it could protect blood components against irradiation [62]. Taken all together, these results demonstrate the protective effect of compound 15 against gamma radiation.

2.3. Molecular docking

Molecular docking was performed to assess the ability of compound 15 to block the Kelch domain of Keap1. Through its Kelch domain, Keap1 binds to Nrf2, promoting its degradation, resulting in low cytoprotective gene levels [63]. The PDB file: 4IQK was obtained from the Protein Data Bank. The binding site of Kelch domain has been reported to have five subpockets: P1, P2, P3, P4 and P5 [64]. P1 and P2 are positively charged pockets that contain the arginine triad (Arg 415, Arg 483 and Arg 380). This triad is crucial for the selectivity of the molecular recognition, together with a
Finally, compounds that adopt the same orientation inside the binding site (Fig. 8).

In summary, a hybridization strategy was adopted using the iodinated quinazolinone scaffold and sulfonamide moieties to produce the 2-((6,8-diido-4-oxo-3-(4-sulfamoylphenyl)-3,4-dihydroquinazolin-2-yl)thio)-N-(substituted) acetamide derivatives 5–18. Different substitutions were introduced to the acetamide group to study the structure-activity relationship. All the compounds were screened for their antioxidant potential using the NQO1 inducer activity assay. The 3,4,5-trimethoxyphenol derivative 15 showed the highest inducer activity in this series (CD = 20 μM) and had low toxicity (LD50 = 500 mg/kg). Treatment of gamma-irradiated mice with compound 15 lowered oxidative stress as evidenced by the lower levels of MDA, ROS and NQO1 in liver. Furthermore, compound 15 ameliorated the complete blood picture of irradiated mice, as well as enhanced the survival of mice caused by oxidative stressors of four different types, namely menadione, tert-butyl hydroperoxide, 4-hydroxynonenal, and peroxynitrite, as well as by exposure to ultraviolet radiation [65,66]. Furthermore, unlike the effects of most direct antioxidants, the indirect antioxidant effect of sulforaphane, which results from Nrf2 activation, persists for several days after sulforaphane is no longer present in the cell culture medium. This is because direct antioxidants, such as ascorbic acid, tocopherols, carotenoids, and polyphenols, which neutralize ROS and other chemical oxidants are consumed in these reactions, whereas Nrf2 activation results in transcriptional upregulation of antioxidant defences, which are mediated by proteins with long half-lives, often several days. The new compounds generated in the current study have an additional advantage in that they are non-electrophilic and are therefore expected to have a broader therapeutic window compared to electrophilic Nrf2 activators. This is supported by the very low toxicity of compound 15 in mice. Taken together, these results demonstrate the powerful effect of Nrf2 activation and induction of NQO1 in protecting cells and animals against high levels of ROS and preventing ROS-mediated damage. This is of particular relevance to protecting the hematopoietic system, which is highly sensitive to ROS.

3. Conclusion

In summary, a hybridization strategy was adopted using the iodinated quinazolinone scaffold and sulfonamide moiety to produce the 2-((6,8-diido-4-oxo-3-(4-sulfamoylphenyl)-3,4-dihydroquinazolin-2-yl)thio)-N-(substituted) acetamide derivatives 5–18. Different substitutions were introduced to the acetamide group to study the structure-activity relationship. All the compounds were screened for their antioxidant potential using the NQO1 inducer activity assay. The 3,4,5-trimethoxyphenol derivative 15 showed the highest inducer activity in this series (CD = 20 μM) and had low toxicity (LD50 = 500 mg/kg). Treatment of gamma-irradiated mice with compound 15 lowered oxidative stress as evidenced by the lower levels of MDA, ROS and NQO1 in liver. Furthermore, compound 15 ameliorated the complete blood picture of irradiated mice, as well as enhanced the survival of mice.
over a period of 30 days post-irradiation. Molecular docking of 15 inside the active site of Keap1 confirmed that it binds in the same manner as that of the co-crystallized ligands. The inducer activity of compound 15 in upregulating NQO1 strongly suggests that it could be used as a lead antioxidant and radiomodulatory agent for further optimization of the quinazolinone scaffold.

4. Materials and methods

4.1. Chemistry

All chemicals were purchased from Sigma-Aldrich and are of AR grade. Melting points were determined in open capillary on a Gallen Kamp melting point apparatus (Sanyo Gallen Kamp, UK). Thin layer chromatography using precoated silica gel plates (Kieselgel 0.25 mm, 60 F254, Merck, Germany) was performed with a solvent system of chloroform/methanol (8:2) to detect the spots by UV light. IR spectra (KBr disc) were recorded using an FT-IR spectrophotometer (Perkin Elmer, USA). NMR spectra were scanned on NMR spectrophotometer (Bruker AXS Inc., Switzerland), operating at 500 MHz for $^1$H and 125.76 MHz for $^{13}$C. Mass spectra were recorded on the ISQ LT Thermo Scientific GCMS model (Massachusetts, USA). Chemical shifts are expressed in δ-values (ppm) relative to TMS as an internal standard, using DMSO-d$_6$ as a solvent. Elemental analyses were done on a model 2400 CHNSO analyser (Perkin Elmer, USA). All the values were within ±0.4% of the theoretical values.

4.1.1. 4-(6, 8-diiodo-2-mercapto-4-oxoquinazolin-3(4H)-yl)benzenesulfonamide (4)

A mixture of 2-amino-3,5-diiodobenzoic acid 3 (3.88 g, 0.01 mol) and 4- isothiocyanobenzenesulfonamide 2 (2.14 g, 0.01 mol) in absolute ethanol (30 mL) containing 3 drops of triethylamine, was refluxed for 3 h. The solid product formed was collected by filtration and crystallized from ethanol to give 4.

4: Yield, 88%; m.p. > 300 °C. IR (KBr, ν cm$^{-1}$): 3311, 3210 (NH$_2$),
3098 (arom.), 1701 (CO), 1618 (CN), 1379, 1160 (SO2). $^1$H NMR (DMSO-d$_6$, $\delta$, ppm): 1.97 (s, 1H), 7.86 (d, 2H, $J = 7$ Hz, AB), 8.06 (d, 2H, $J = 7$ Hz, AB), 8.23 (d, 1H, $J = 2$ Hz), 8.58 (d, 1H, $J = 2$ Hz), 10.05 (s, 2H). $^{13}$C NMR (DMSO-d$_6$, $\delta$, ppm): 86.84, 89.74, 118.49, 118.84, 122.99, 127.24 (2), 133.61, 134.21, 136.20, 144.48, 158.31, 165.76, 176.14. Anal. Calcd. for C$_{14}$H$_9$I$_2$N$_3$O$_3$S$_2$ (584.82): C, 28.73; H, 1.55; N, 7.18. Found: C, 29.02; H, 1.82; N, 7.41.

4.1.2. 3,4-Dihydroquinazolin-sulfonamide derivatives (5-18)

4.1.2.1. General procedure. A mixture of 4 (5.85 g, 0.01 mol) and 2-chloro-N-substituted acetamide derivatives (0.01 mol) in dry acetone (30 mL) and anhydrous K$_2$CO$_3$ (1.38 g, 0.01 mol) was stirred at room temperature for 18 h, filtered and the solid product formed was crystallized from dioxane to give 5-18.

4.1.2.2. 2-((6,8-Diiodo-4-oxo-3-(4-sulfamoylphenyl)-3,4-dihydroquinazolin-2-yl)thio)-N-phenylacetamide (5). 5: Yield, 79%; m.p. > 300°C. IR (KBr, $\nu$ cm$^{-1}$): 3408, 3310, 3231 (NH, NH$_2$), 3079 (arom.), 2945, 2881 (aliph.), 1702, 1679 (2CO), 1620 (CN), 1349, 1170 (SO$_2$). $^1$H NMR (DMSO-d$_6$, $\delta$, ppm): 4.17 (s, 2H), 7.03-7.30 (m, 3H), 7.60-7.83 (m, 4H), 8.02 (s, 2H), 8.09 (d, 2H, $J = 10$ Hz, AB), 8.35 (d, 1H, $J = 2$ Hz), 8.60 (d, 1H, $J = 2$ Hz), 10.12 (s, 1H). $^{13}$C NMR (DMSO-d$_6$, $\delta$, ppm): 29.73, 81.27, 89.61, 118.79 (2), 123.62 (2), 124.33, 127.61, 128.05 (2), 128.80 (2), 133.12, 137.64, 137.90, 139.42, 145.13, 158.92, 163.31, 164.02, 169.81. MS m/z [%]: 454 [M$^+$], 29.82, 719 [M+1, 19.85], 454 [100]. Anal. Calcd. for C$_{22}$H$_{16}$I$_2$N$_4$O$_4$S$_2$ (732.35): C, 37.72; H, 2.48; N, 7.65. Found: C, 38.04; H, 2.68; N, 7.96.

4.1.2.3. 2-((6,8-Diiodo-4-oxo-3-(4-sulfamoylphenyl)-3,4-dihydroquinazolin-2-yl)thio)-N-o-tolylacetamide (6). 6: Yield, 81%; m.p. > 300°C. IR (KBr, $\nu$ cm$^{-1}$): 3403, 3321, 3216 (NH, NH$_2$), 3098 (arom.), 2956, 2891 (aliph.), 1711, 1681 (2CO), 1631 (CN), 1355, 1188 (SO$_2$). $^1$H NMR (DMSO-d$_6$, $\delta$, ppm): 2.21 (s, 3H), 4.30 (s, 2H), 7.10 (ddd, 1H, $J = 8$ & 2.5 Hz), 7.30-7.55 (m, 3H), 7.87 (d, 2H, $J = 8.5$ Hz, AB), 8.01 (s, 2H), 8.05 (d, 2H, $J = 8.5$ Hz, AB), 8.28 (d, 1H, $J = 1.5$ Hz), 8.55 (d, 1H, $J = 1.5$ Hz), 9.66 (s, 1H). $^{13}$C NMR (DMSO-d$_6$, $\delta$, ppm): 16.32, 26.61, 82.13, 91.41, 120.80 (2), 122.81, 123.90, 124.63, 128.72, 128.91 (2), 129.66, 130.81, 131.54, 134.02, 135.25, 135.87, 147.31, 153.82, 159.18, 159.62, 167.53. Anal. Calcd. for C$_{23}$H$_{18}$I$_2$N$_4$O$_4$S$_2$ (732.35): C, 37.72; H, 2.48; N, 7.65. Found: C, 38.04; H, 2.68; N, 7.96.

4.1.2.4. 2-((6,8-Diiodo-4-oxo-3-(4-sulfamoylphenyl)-3,4-dihydroquinazolin-2-yl)thio)-N-(m-tolyl)acetamide (7). 7: Yield, 86%; m.p. > 300°C. IR (KBr, $\nu$ cm$^{-1}$): 3421, 3318, 3207 (NH, NH$_2$), 3095 (arom.), 2978, 2842 (aliph.), 1707, 1675 (2CO), 1625 (CN), 1378, 1145 (SO$_2$). $^1$H NMR (DMSO-d$_6$, $\delta$, ppm): 2.28 (s, 3H), 4.25 (s, 2H), 8.01 (s, 2H), 8.05 (d, 2H, $J = 8.5$ Hz, AB), 8.28 (d, 1H, $J = 1.5$ Hz), 8.55 (d, 1H, $J = 1.5$ Hz), 9.66 (s, 1H). $^{13}$C NMR (DMSO-d$_6$, $\delta$, ppm): 16.32, 26.61, 82.13, 91.41, 120.80 (2), 122.81, 123.90, 124.63, 128.72, 128.91 (2), 129.66, 130.81, 131.54, 134.02, 135.25, 135.87, 147.31, 153.82, 159.18, 159.62, 167.53. Anal. Calcd. for C$_{23}$H$_{18}$I$_2$N$_4$O$_4$S$_2$ (732.35): C, 37.72; H, 2.48; N, 7.65. Found: C, 38.04; H, 2.68; N, 7.96.

Fig. 6. 2D and 3D interaction poses of the N,N0-naphthalene-1,4-diylbis(4-methoxybenzenesulfonamide showing cation-π, π-π interaction and hydrogen bonds with the key amino acids inside the binding pocket.

Fig. 7. 2D and 3D interaction pose of compound 15 showing cation-π, π-π interactions inside the binding pocket of 4IQK.
1H, C₂₄H₂₀I₂N₄O₄S₂ (746.38): C, 38.62; H, 2.70; N, 7.51. Found: C, 38.31; H, 2.44; N, 7.78.

4.1.2.8. 2-((6,8-Diiodo-4-oxo-3-(4-sulfamoylphenyl)-3,4-dihydroquinazolin-2-ylthio)-N-(4-ethoxyphenyl) acetamide (10).

4.1.2.9. 2-((6,8-Diiodo-4-oxo-3-(4-sulfamoylphenyl)-3,4-dihydroquinazolin-2-ylthio)-N-(4-methoxyphenyl) acetamide (12).

4.1.2.10. 2-((6,8-Diiodo-4-oxo-3-(4-sulfamoylphenyl)-3,4-dihydroquinazolin-2-ylthio)-N-(4-ethoxyphenyl) acetamide (13).

4.1.2.11. 2-((6,8-Diiodo-4-oxo-3-(4-sulfamoylphenyl)-3,4-dihydroquinazolin-2-ylthio)-N-(3,5-dimethoxyphenyl) acetamide (14).

4.1.2.12. 2-((6,8-Diiodo-4-oxo-3-(4-sulfamoylphenyl)-3,4-dihydroquinazolin-2-ylthio)-N-(3,4,5-trimethoxyphenyl) acetamide (15).
10 A.M. Soliman et al. / European Journal of Medicinal Chemistry 200 (2020) 112467

Yield, 74%; m.p. > 300 °C (IR (KBr, v cm⁻¹): 3329, 3265, 3203 (NH, NH₂), 3055 (arom.), 2943, 2811 (aliph.), 1683, 1660 (2CO), 1621 (CN), 1530, 1370 (NO₂), 1397, 1155 (SO₂). ¹³C NMR (DMSO-d₆, δ ppm): 23.93 (s, 3H), 4.30 (s, 2H), 7.72 (d, 1H, J = 9.5 Hz), 7.82 (d, 2H, J = 8 Hz, AB), 7.90–0.85 (m, 6H), 8.26 (d, 1H, J = 3 Hz), 8.59 (d, 1H, J = 3 Hz), 10.27 (s, 1H). ¹⁵N NMR (DMSO-d₆, δ ppm): 18.05, 23.74, 83.69, 92.03, 106.32, 120.85, 121.71 (2), 125.83, 126.71, 130.64 (2), 133.72, 134.30, 139.42, 141.42, 141.94, 147.56, 154.02, 160.82, 161.04, 167.42. Anal. Calcd. for C₂₂H₂₁N₇O₆S₂ (524.54): C, 45.42; H, 3.07; N, 14.14. Found: C, 45.75; H, 3.37; N, 14.26.

4.2.1. NQO1 in vitro inducer activity

4.2.1.1. Animals. Eight-week old Swiss albino male mice (20–25 g) were supplied from the breeding unit of the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt. They were housed in the laboratory room for one week prior to the experiment for acclimatization to the lab environment. Water and food were allowed ad libitum. Mice were kept under controlled conditions: room temperature of 25 ± 5 °C, humidity (60 ± 5%), alternating 12 h dark and 12 h light cycle. Animals were treated gently; squeezing, pressure and tough handling were avoided. All animal procedures were performed in accordance with the Ethics Committee for Animal Experiments, Faculty of Pharmacy, Cairo University, which complies with the Guide for the Care and Use of Laboratory Animals issued by the US National Institutes of Health (NIH Publication No. 85-23, revised 2011).

4.2.2. Irradiation process. Mice were exposed to whole-body gamma radiation as a single dose of 7 Gy using Canadian Gamma Cell-40 biological irradiator (¹³⁷Cs) located at the NCRRT, Cairo, Egypt and the dose rate was 0.655 rad/s.

4.2.2.3. Acute toxicity study. The median lethal dose (LD₅₀) of the most promising compound, 15 was determined according to Chinedu et al. [69].

4.2.2.4. Experimental design. Eighty mice were randomly classified into four groups. First (control) group was injected i.p. with 1% DMSO, daily for 5 days. Second (irradiated) group was treated as control, and after 1 h from last DMSO injection, the mice were irradiated at a dose of 7 Gy. Third (Compound 15) group; received 50 mg/kg/day i.p. (1/10 LD₅₀) of compound 15, daily for 5 days.

4.2.2.7. Irradiation procedure. Mice were subjected to whole-body gamma radiation as a single dose of 7 Gy using Canadian Gamma Cell-40 biological irradiator (¹³⁷Cs) located at the NCRRT, Cairo, Egypt and the dose rate was 0.655 rad/s.
Fourth (Compound 15 + Irradiation) group was treated as third group then on the last day, after 1 h of injection, mice exposed to 7 Gy gamma radiation. On the third day, five mice from each group (n = 5) were weighed and anesthetized using urethane (1.2 mg/kg i.p.) [70]. Then the blood samples were collected by cardiac puncture. At that time, they were euthanized by cervical dislocation. Each blood sample was collected into EDTA coated tubes for complete blood picture. Liver tissues were rinsed with ice-cold saline, dried on a filter paper and weighed to calculate the relative liver/body weight ratio. Then, it was homogenized in ice-cold 0.1 M phosphate buffer saline (pH 7.4) and stored at −80 °C till used for subsequent biochemical analysis. The residual of mice in all the groups was monitored on a daily basis for 30 days to check the survival rate, as well as their body weight, were recorded weekly to estimate the changes in body weight.

4.2.2.5. Biochemical parameters investigated in liver homogenate. Liver homogenates were used for measuring the level of Nrf2 using colorimetric cell-based Elisa kit (OKAG00918) Aviva systems biology (San Diego, CA, USA), as well as the level of NAD(P)H:quinone oxidoreductase 1 (NQO1) was measured using an ELISA Kit (OKCD02727) Aviva Systems Biology (San Diego, CA, USA). Liver lipid peroxides were determined by measuring MDA as an indicator according to the method of Yoshioka et al. [71]. The generation of ROS in liver tissues was measured according to a modified technique of Vrablec et al. [72].

4.2.2.6. Hematological analysis. Complete blood count with platelet count was determined using the automated micro-analyzer (BC-2800 Mindray, China).

4.2.2.7. Statistical analysis. Data were analyzed using Prism 5.03 (GraphPad, San Diego, CA, USA) and expressed as means ± standard error. Comparisons between groups were analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni’s multiple comparison test. Survival was analyzed by the Kaplan–Meier method followed by the Mantel–Cox (log rank) test. Body weight changes between groups through 30 days were analyzed by two-way ANOVA followed by Bonferroni’s post test. P < 0.05 was considered to represent statistically significant differences.

4.3. Molecular docking

The molecular modeling studies were fulfilled by the Molecular Operating Environment software (MOE, 10.2008). The receptor was chosen from the protein data bank; 4iQK that represents Keap1 co-crystallized with N,N’-naphthalene-1,4-diylbis(4-methoxybenzenesulfonamide). The protein was prepared for docking by ignoring water in the receptor. Hydrogen atoms were added to the structure with their standard geometry. The co-crystallized ligand was used to determine the binding site. Triangle Matcher placement method and dG scoring function were used for docking. Energy minimizations were performed with an RMSD gradient of 0.1 kcal mol⁻¹Å⁻¹ with the MMFF94X force field and the partial charges were automatically calculated. Validation of the docking protocol was performed by re-docking of the co-crystallized ligands into the active site of Keap1 protein followed by docking of compound 15. The obtained data were used to interpret the ligand-protein interactions at the Nrf2-binding site.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The authors appreciate the staff members of the gamma irradiation unit at the National Center for Radiation Research and Technology (NCRRT) for carrying out the irradiation process. Maureen Higgins and Albena T Dinkova-Kostova are grateful to Cancer Research UK (C20953/A18644) for financial support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2020.112467.

References

[1] D. Citrin, A.P. Cotrim, F. Hyodo, B.J. Baum, M.C. Krishna, J.B. Mitchell, Radioprotectors and mitigators of radiation-induced normal tissue injury, Oncol. 15 (2010) 360–371.
[2] H.M. Karam, O.A. Gharib, The therapeutic role of ziziphus extract on liver injury induced by electromagnetic waves and ionizing radiation as environmental pollutants, J. Nucl. Tech. Appl. Sci. 6 (2018) 207–219.
[3] R.R. Radwan, H.M. Karam, Rosveratrol attenuates intestinal injury in irradiated rats via PI3K/Akt/mTOR signaling pathway, Environ. Toxicol. 35 (2020) 223–230.
[4] A. Hafior, M. Al-Sadoon, Increased antioxidant potential and decreased free radical production in response to mild injection of crude venom, Cerastes cerastes gasperetti, Toxicol. Mech. Methods 18 (2008) 11–16.
[5] T. Shimura, N. Kunugita, Mitochondrial reactive oxygen species-mediated genomic instability in low-dose irradiated human cells through nuclear retention of cytokeratin 18, J Cell Cycle 15 (2016) 1410–1414.
[6] M. Selim, A. Saha, K.K. Mukherjea, Protection of radiation induced DNA damage by a newly developed molybdenum complex, J. Radioanal. Nucl. Chem. 311 (2017) 189–193.
[7] S.M. Abdel Fattah, H.K. Mohmed, M.A.E.H. Mohamed, The potential protective effect of ferulic acid against gamma irradiation induced ovarian failure in rats, Egypt, J. Radiat. Sci. Appl. 32 (2019) 1–12.
[8] A.A. Ibrahim, H.M. Karam, E.A. Shaaban, M.M. Safar, M.F. El-Yamany, MitoQ ameliorates testicular damage induced by gamma irradiation in rats: modulation of mitochondrial apoptosis and steroidogenesis, Life Sci. 232 (2019) 116655.
[9] H.M. Karam, R.R. Radwan, Metformin modulates cardiac endothelial dysfunction, oxidative stress and inflammation in irradiated rats: a new perspective of an antidiabetic drug, Clin. Exp. Pharmacol. Physiol. 46 (2019) 1124–1132.
[10] M. Valko, D. Leibfritz, J. Moncol, M.T. Cronin, M. Mazur, J. Telser, Free radicals and antioxidants in normal physiological functions and human disease, Int. J. Biochem. Cell Biol. 39 (2007) 44–84.
[11] M.Z. Kamran, A. Ranjan, N. Kaur, S. Sur, V. Tandon, Radioprotective agents: strategies and translational advances, Med. Res. Rev. 36 (2016) 461–493.
[12] J.S. Greenberger, Radioprotection, In Vivo 23 (2009) 323–336.
[13] N. Tsutou, Y. Munetaka, O. Toshikho, K. Shunro, Suppression of active oxygen-induced cytotoxicity by flavonoids, Biochem. Pharmacol. 45 (1993) 265–267.
[14] T. Devasagayam, J. Tilak, K. Boloor, K.S. Sane, S.S. Ghaskadbi, R. Lele, Free radicals and antioxidants in human health: current status and future prospects, J. Assoc. Phys. India 52 (2004) 794–804.
[15] T.W. Kestler, N. Wakahayashi, S. Biswal, Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway, Annu. Rev. Pharmacol. Toxicol. 47 (2007) 89–116.
[16] S.B. Cullman, J.D. Gordon, J. Jin, J.W. Harper, J.A. Diehl, The Keap1-8BT protein is an adaptor that bridges Nrf2 to a Cull3-based E3 ligase: oxidative stress sensing by a Cull3-Keap1 ligase, Mol. Cell Biol. 24 (2004) 8477–8486.
[17] A. Kobayashi, M.-I. Kang, H. Okawa, M. Ohtsui, Y. Zenke, T. Chiba, K. Igarashi, M. Yamamoto, Oxidative stress sensor Keap1 functions as an adaptor for Cull3-based E3 ligase to regulate proteasomal degradation of Nrf2, Mol. Cell Biol. 24 (2004) 7130–7139.
[18] B. Zhang, D. Schmoyer, S. Kirov, J. Snoddy, G0Tree Machine (GOTM): a web-based platform for interpreting sets of interesting genes using Gene Ontology hierarchies, BMC Bioinf. 5 (2004) 16–24.
[19] A.T. Dinkova-Kostova, P. Talalay, NAD (P) H: quinone acceptor oxidoreductase 1 (NQO1), a multifunctional antioxidant enzyme and exceptionally versatile cytoprotector, Arch. Biochem. Biophys. 501 (2010) 116–123.
[20] M.S. Alsaied, A.A. Al-Mishari, A.M. Soliman, F.A. Ragab, M.M. Ghorab, Discovery of Benzo [g] quinazolin benzenesulfonamide derivatives as dual EGFR/HER2 inhibitors, Eur. J. Med. Chem. 141 (2017) 84–91.
[21] M. Ghorab, M.S. Alsaied, A.M. Soliman, A.A. Al-Mishari, Benzo [g] quinazolin-based scaffold derivatives as dual EGFR/HER2 inhibitors, J. Enzym. Inhib. Med. Chem. 33 (2018) 67–73.
[22] A. Kumar, P. Sharma, P. Kumari, B.L. Kalal, Exploration of antimicrobial and antioxidant potential of newly synthesized 3-,5-diabstituted quinazoline-4.
