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Cytotoxic and Anti-Inflammatory Active Methylsulfanyltriazoloquinazolines

Rashad A. Al-Salahi[1]*, Amira M. Gamal-Eldeen[3], Amer M. Alanazi[1], Mohamed A. Al-Omar[1], Mohamed A. Marzouk[1,2] and Moustafa M.G. Fouda[4,5]

[1]Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, P. O. Box 2457, Riyadh 11451, Saudi Arabia.
[2] Chemistry of Natural products Group, Center of Excellence for Advanced Sciences, National Research Center, Dokki 12622, Cairo, Egypt.
[3]Cancer Biology Group, Center of Excellence for Advanced Sciences, National Research Center, Dokki 12622, Cairo, Egypt.
[4]Petrochemical Research Chair, Chemistry Department, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia.
[5]Textile Research Division, National Research Center, Dokki 12622, Cairo, Egypt.

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A series of twenty five 2-methylsulfanyl-[1,2,4]triazolo[1,5-*a*]quinazoline derivatives 1–25 was previously synthesized. We have now investigated their cytotoxic effects against hepatocellular Hep-G2 and colon HCT-116 carcinoma cells and effect on the macrophage growth, in addition to their influence of the inflammatory mediators [nitric oxide (NO), tumor necrosis factor-α (TNF-α), prostaglandin E-2 (PGE-2) and in bacterial lipopolysachharide (LPS)-stimulated macrophages]. The findings revealed that compounds 13 and 17 showed the highest cytotoxicity and that 3, 6–8 and 25 are promising multipotent anti-inflammatory agents.

Key words: 1,2,4-Triazoloquinazoline; Antitumor; Hep-G2; HCT-116; Inflammation

Inflammation is defined as part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, chemical irritants and can be classified as either acute or chronic[1]. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes from the blood into the injured tissues. Whereas, prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process. Neutrophils migrate to the inflammatory sites in the very early stage of inflammation under the control of molecules resulted by fast responding mast cells and macrophages prestationed in tissues[1]. Various types of lymphocytes and leukocytes are activated and attracted to the inflamed site by a signaling network including a great number of growth factors, cytokines and chemokines during inflammation progresses[1].

Prostaglandin E2 [2], transforming growth factor-h [3], reactive oxygen and nitrogen intermediates are among molecules responsible for a dual effect in both promoting and suppressing inflammation[4]. A rapid programmed clearance of inflammatory cells (neighboring macrophages, dendritic cells, and backup phagocytes) is required for resolution of inflammation to do this job by inducing apoptosis and conducting phagocytosis[5-10]. The lymphocytes, plasma cells, and macrophages in are the inflammatory foci,

* To whom all correspondence should be addressed.
E-mail: salahi76@yahoo.com

which dominated during chronic inflammation with varying morphology[11]. The cytokines, a great amount of growth factors, reactive oxygen and nitrogen species that generated by macrophages and other inflammatory cells may cause DNA damage[12], and further activation of macrophages may lead to continuous tissue damage[13].

Throughout the epidemiologic studies, it was found that chronic inflammatory diseases are frequently accomplished with increased risk of cancers[1,2,13], and that the development of cancers from inflammation might be a process driven by inflammatory cells. Moreover, a various of mediators, such as cytokines, chemokines, and enzymes, which altogether establish an inflammatory microenvironment, as well[2]. Thus, finding new antiinflammatory agents represents a concrete strategy in fighting not only different inflammatory diseases but also cancer.

The interest in the medicinal chemistry of quinazolinone derivatives was stimulated in the early 1950s with the elucidation of the structure of 3-[β-keto-γ(3-hydroxy-2-piperdyl)-propyl]-4-quinazolone, a quinazolinone alkaloid from the Asian plant *Dichroa febrifuga*, which is an effective ingredient of a traditional Chinese herbal remedy against malaria[14]. In addition, the quinazoline moiety is present in many classes of biologically active compounds, a number of which have been used clinically as antifungal, antibacterial and antiprotozoic drugs[15,16], antituberculotic agents[17–19]. Their broad range of pharmacological properties was reported[20], such as anticancer[21], anti-inflammatory[22], anticonvulsant[23], and antidiuretic activities[24]. On the other hand, 1,2,4-triazoles are associated with diverse pharmacological activities, e.g., analgesic, antiasthmatic, diuretic, anti-hypersensitive, anticholinergic, antibacterial, antifungal and anti-inflammatory activity[25–28]. Combining these two structural features into one molecule has produced new ones with promising biological effects [29–38]. Triazoloquinazoline derivatives are of considerable interest due to their prominent biological properties, such as growth inhibition of *B. subtilis*, *Staphylococcus aureus*, *Candida tropicalis* and *Rickettsia nigricans*[39]. Furthermore, some heterocycles containing quinazoline and triazoloquinazoline moieties were designed to contain a substituted thio functional group that believedto bind an electron-deficient carbon atom and identified as a possible pharmacophore of the anti-tumor and anti-inflammatory activity[40].

MATERIALS AND METHODS

Cell Culture

Several human cell lines were used in testing the anticancer activity, including hepatocellular carcinoma (Hep-G2), colon carcinoma (HCT-116), cervical carcinoma (HeLa), histiocytic lymphoma, and breast adenocarcinoma (MCF-7) (ATCC, Manassas, VA, USA). Murine raw macrophage cell line (RAW 264.7, ATCC, Manassas, VA, USA) was routinely cultured in RPMI 1640 and HCT-116 cells were grown in Mc Coy's medium, while all cells were routinely cultured in DMEM (Dulbeco's Modified Eagle's Medium) at 37 °C in humidified air containing 5% CO2. Media were supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, containing 100 units/mL penicillin G sodium, 100 units/ml streptomycin sulfate, and 250 ng/ml amphotericin B. Monolayer cells were harvested by trypsin/EDTA treatment, while leukemia cells were harvested by centrifugation. RAW 264.7 cells were harvested by gentle scraping. Cells were used when confluence had reached 75%. Compounds were dissolved in 10% dimethyl sulfoxide (DMSO) supplemented with the same concentrations of antibiotics. Compounds dilutions were tested before assays for endotoxin using the Pyrogent® Ultragel clot assay, and they were found endotoxin free. All experiments were repeated four times, unless mentioned otherwise, and the data were represented as (Mean ± SD). Cell culture material was obtained from Cambrex BioScience (Copenhagen, Denmark). Chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA), except as mentioned. This work was carried out at the Center of Excellence for Advanced Sciences, National Research Center, Dokki, Cairo, Egypt.

Cytotoxicity Assay

The cytotoxic effect of the tested compounds on the growth of different human cancer cell lines was estimated by the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay [41], after 24 h of incubation. The yellow tetrazolium salt of MTT was reduced

by mitochondrial dehydrogenases in metabolically active cells to form insoluble purple formazan crystals, which are solubilized by the addition of a detergent. Cells (5 × 104 cells/well) were incubated with various concentrations of the compounds at 37°C in a FBS-free medium, before submitted to MTT assay. The absorbance was measured with microplate reader (BioRad, München, Germany) at 570 nm. The relative cell viability was determined by the amount of MTT converted to the insoluble formazan salt. The data were expressed as the mean percentage of viable cells when compared with untreated cells. The relative cell viability was expressed as the mean percentage of viable cells when compared with the respective untreated cells (control). The half maximal growth inhibitory concentration (IC_{50}) value was calculated from the line equation of the dose-dependent curve of each compound. The results were compared with the cytotoxic activity of paclitaxel, a known anticancer drug.

Macrphage Viability Assay

The effect of different compounds on the viability of RAW 264.7 cells was estimated by MTT assay. RAW 264.7 (5 × 104 cells/well) were incubated for 48 h with 20 µg/ml of the compounds at 37°C, before submitting to MTT assay. The relative cell viability was expressed as the mean percentage of viable cells compared with untreated cells. Treatment of macrophage with 1000 units/ml recombinant macrophage colony-stimulating factor (M-CSF, Pierce, Rockford, IL, USA) was used as positive control.

Nitrite Assay

The accumulation of nitrite, an indicator of nitric oxide (NO) synthesis, was measured by Griess reagent[42]. RAW 264.7 were grown in phenol red-free RPMI-1640 containing 10% FBS. Cells were incubated for 24 h with bacterial lipopolysaccharide (LPS, 1 mg/ml) in the presence or absence of different compounds (20 µg/ml). Fifty microlitres of cell culture supernatant were mixed with 50 ml of Griess reagent and incubated for 10 min. The absorbance was measured spectrophotometrically at 550 nm. A standard curve was plotted using serial concentrations of sodium nitrite. The nitrite content was normalized to the cellular protein content as measured by bicinchoninic acid assay[43].

Determination of Tumor Necrosis Factor-± and Prostaglandin E2

RAW 264.7 cells were incubated for 24 h with compounds without LPS, and in another experiment cells were incubated for 24 h with LPS (1 mg/ml) in the presence or absence of different compounds. TNF-α and prostaglandin E2 (PGE2) were determined in the harvested supernatants using commercial kits (Endogen Inc., Woburn, MA, USA) and (Cayman Chemical, Ann Arbor, MI, USA), respectively, according to the manufacturer protocols.

Statistical Analysis

Data were statistically analyzed using Statistical Package for Social Scientists (SPSS) 10.00 for windows (SPSS Inc., Chicago, IL, USA). The student's unpaired *t*-test as well as the one-way analysis of variance (ANOVA) test followed by the Tukey *post hoc* test was used to detect the statistical significance. A P value of more than 0.05 was considered non-significant.

RESULTS AND DISCUSSION

In our previous papers [37,38,44], we have described the synthetic methodology used to obtain 2-methylsulfanyl-[1,2,4]triazolo[1,5-*a*]quinazolin-5-one and its derivatives 1–25 (Scheme 1).

As a part of our interest in the search for novel cytotoxic and anti-inflammatory agents, we herein report the biological evaluation of our compounds 1–25. Screening of the cytotoxic effects of the tested compounds against various human cancer cell lines (Hep-G2, MCF-7, HCT-116, and HeLa cells) revealed that none of the tested compounds were cytotoxic to both MCF-7 and HeLa cells, as concluded from their high IC_{50} values (>50 µg/ml). On the other hand, the treatment of Hep-G2 cells with 1, 5, 7, 13–19, 24 and 25 led to some cytotoxicity (IC_{50} < 50), with compounds 13 and 17 showing the highest cytotoxic effect and the lower IC_{50} values (9.34 and 19.22 µg/ml). Similarly, 1–5, 7, 13, 14 and 17 exhibited cytotoxicity with (IC_{50} < 50) in the treatment of HCT-116 cells, where compounds 13 and 17 showed the highest cytotoxic effects with the lower IC_{50} values of 11.51 and 17.39 µg/ml, respectively, as shown in Table 1. Although 13 and 17 showed the highest cytotoxic effect against Hep-G2 and HCT-116 cells, attributed

to the presence of fused ring in 13 and 5-ethoxy moiety in 17, which seemed to be essential for the antitumor activity against HCT-116 and Hep-G2, they were less effective as anti-cancer agents than the known drug paclitaxel (Table 1).

Macrophages are the first line of defense in innate immunity against microbial infection. Professional phagocytes engulf and kill microorganisms and present antigens for triggering adaptive immune responses[7]. The growth of macrophages represents a controlling key in that defense system. The data obtained upon macrophage incubation with the compounds for 48 h indicated that all the tested compounds significantly induced the growth of macrophages ($p < 0.01$–$p < 0.001$), up to 4.2-fold of the control growth (Figure 1), except some compounds (4, 9, 11, 13–15, 17, 18, 20 and 25), which produced a non-significant change in the macrophage growth ($p > 0.05$), as shown in Figure 1.

The results indicated that lipopolysachharide (LPS, 100 µg/ml) caused a 1.85-fold increase in nitric oxide production compared to the control. The potent anti-inflammatory drug dexamethasone (50 ng/ml), inhibited the LPS-induced nitric oxide production (5.2 µg/mL with LPS + dexamethasone compared to 25.2 µg/ml in the presence of LPS alone).The tested samples exhibited different extents of anti-inflammatory activity, ranging from strong to weak activity in the order 23 > 24 > 22 > 18 > 12 > 4 and their effect even greater than that of dexamethasone, with highly significant inhibition values ($p < 0.001$) of 95.7, 95.4, 91.0, 90.9, 90.7, 90.4, and 90.1%, respectively, compared to the LPS induced cells (Figure 2).

Table 1. Cytotoxicity (IC_{50}, µg/mL) of different tested compounds against human malignant cell lines after 24 h of incubation.

Cps	Cells			
	Hep-G2	MCF-7	HCT-116	HeLa
1	29.88 ± 3.02	> 50	46.64 ± 0.62	> 50
2	> 50	> 50	29.62 ± 1.94	> 50
3	> 50	> 50	49.83 ± 2.27	> 50
4	> 50	> 50	31.19 ± 1.36	> 50
5	36.41 ± 3.07	> 50	46.58 ± 0.81	> 50
6	> 50	> 50	> 50	> 50
7	42.28 ± 4.69	> 50	> 50	> 50
8	> 50	> 50	> 50	> 50
9	> 50	> 50	> 50	> 50
10	> 50	> 50	> 50	> 50
11	> 50	> 50	> 50	> 50
12	> 50	> 50	> 50	> 50
13	9.34 ± 1.5	> 50	11.51 ± 2.87	> 50
14	31.22 ± 3.33	> 50	41.25 ± 1.93	> 50
15	22.73 ± 3.7	> 50	> 50	> 50
16	25.20 ± 1.96	> 50	> 50	> 50
17	19.22 ± 4.23	> 50	17.39 ± 0.15	> 50
18	22.69 ± 1.81	> 50	> 50	> 50
19	28.29 ± 3.42	> 50	> 50	> 50
20	> 50	> 50	> 50	> 50
21	> 50	> 50	> 50	> 50
22	> 50	> 50	> 50	> 50
23	> 50	> 50	> 50	> 50
24	26.93 ± 2.74	> 50	> 50	> 50
25	42.46 ± 4.11	> 50	> 50	> 50
Paclitaxel	0.51 ± 0.10	0.99 ± 0.20	0.46 ± 0.13	0.54 ± 0.08

The corresponding compounds 9–11, 13–17 have shown potential significant anti-inflammatory effects ($p < 0.01$), compared to that of dexamethasone and the control, which ranged from 75 to 86% inhibition compared to LPS-induced cells, whereas 1, 3, 5, 6, 19–21 were found to possess moderate effects, with an inhibition range of 50–70% with respect to LPS induced cells. Moreover, compounds 2, 7 and 8 have shown a lower effect ranging between 15 and 40% in regard to LPS induced cells. TNF-α may initiate an inflammatory cascade consisting of other inflammatory cytokines, chemokines, growth factors, endothelial adhesion factors and recruiting a variety of activated cells at the site of tissue damage[45]. It is known that TNF-α can induce DNA damage, inhibit DNA repair[46,47], and act as a growth factor for tumor cells[48]. Treatment of macrophages with LPS led to significant increase in the levels of both TNF-α and nitrites in the culture supernatants relative to control levels (Table 2).

The co-treatment of LPS-stimulated macrophages with compounds resulted in potential inhibition of the LPS-stimulated TNF-α secretion ($p < 0.001$, with the following order of efficiency 8 > 3 > 7 > 11 > 2 > 6 > 21 > 25 > 19 > 1 > 23 > 24.

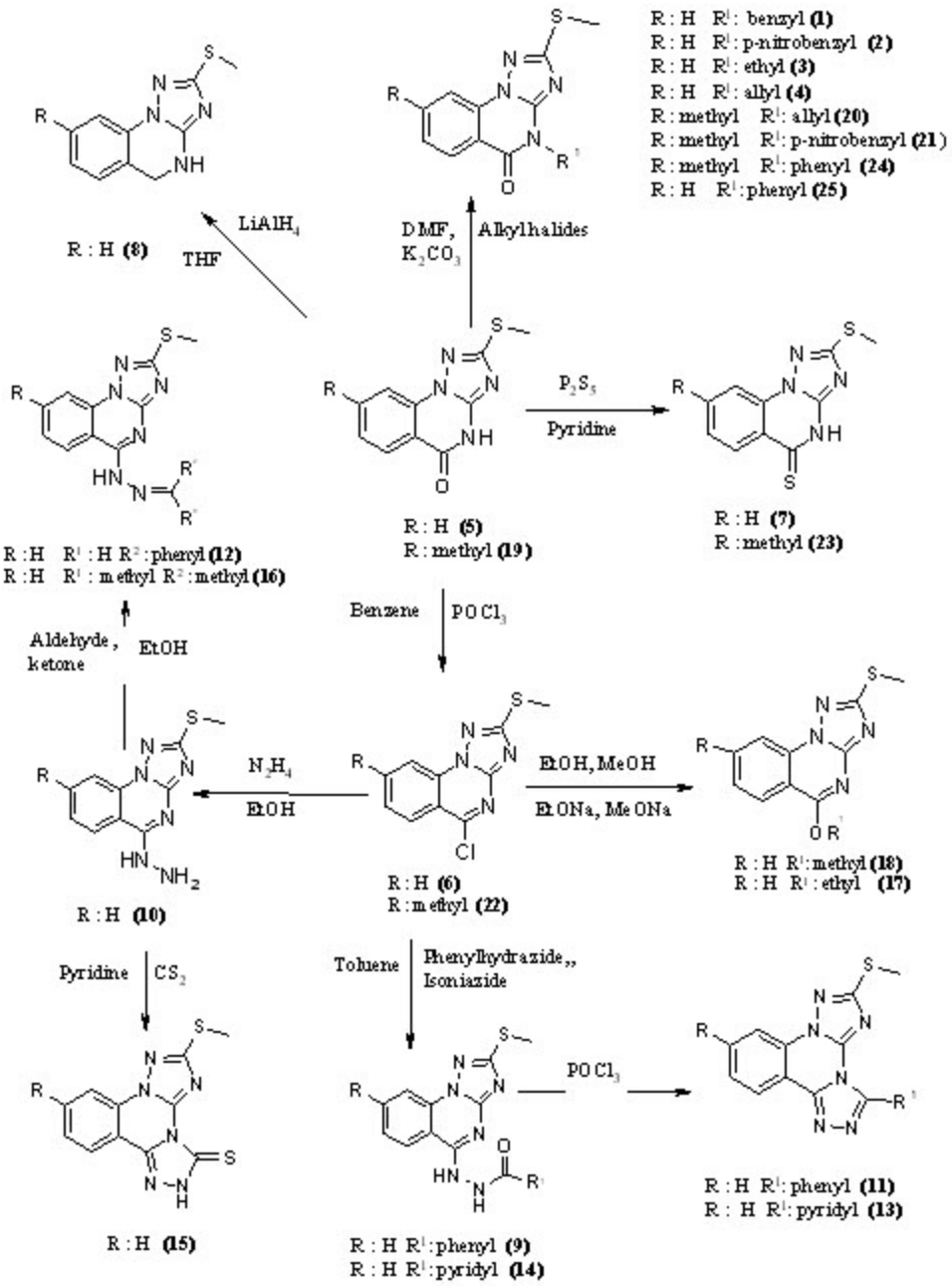
Arachidonic acid is the substrate for cyclogenase to produce prostaglandins (PGs). Interestingly, they can be converted by another enzyme, lipoxygenase, to leukotrienes that are suggested as being another link between inflammation and cancer[49]. The PGs are biologically active derivatives of arachidonic acid and other polyunsaturated fatty acids that are released from

Table 2. Effect of different compounds on the levels of TNF-α and PGE-2 in LPS-stimulated macrophages

Sample	TNF-α (pg/mg protein)	PGE2 (pg/mg protein)
Control	81.2 ± 11.64	34.4 ± 5.06
LPS	5740.6 ± 511.22	3101 ± 110.02[a)]
LPS + 1	1345.8 ± 162.11[a)]	1345.8 ± 162.11[a)]
LPS + 2	904.3 ± 84.45[a)]	1003.8 ± 183.58[a)]
LPS + 3	215.8 ± 24.61[a)]	319.8 ± 34.81[a)]
LPS + 4	5136.9 ± 498.36	3186.8 ± 140.60
LPS + 5	2221.1 ± 325.52	2881.1 ± 323.32
LPS + 6	1041.3 ± 194.21[a)]	1117.7 ± 293.19
LPS + 7	503.2 ± 24.33[a)]	611.7 ± 62.24[a)]
LPS + 8	195.1 ± 22.50[a)]	205.8 ± 23.52[a)]
LPS + 9	3621.5 ± 225.24	3096.2 ± 208.03
LPS + 10	5261.8 ± 488.47	3003. ± 294.21
LPS + 11	809.2 ± 81.47[a)]	2903.7 ± 424.93
LPS + 12	3869.4 ± 381.17	3191.3 ± 292.10
LPS + 13	3009.6 ± 245.70	2548.8 ± 192.98
LPS + 14	5016.7 ± 411.36	2145.1 ± 144.00
LPS + 15	4300.8 ± 398.46	2877.5 ± 305.01
LPS + 16	5096.2 ± 408.33	3221.4 ± 335.24
LPS + 17	2043. ± 194.21	3361.9 ± 185.47
LPS + 18	2403.5 ± 624.33	2804.2 ± 183.71
LPS + 19	1196.3 ± 202.10[a)]	3069.5 ± 391.17
LPS + 20	1546.2 ± 162.78[a)]	3019.4 ± 211.70
LPS + 21	1147.1 ± 184.89[a)]	3016.7 ± 421.60
LPS + 22	1877.7 ± 195.80[a)]	2708.8 ± 278.26
LPS + 23	1500.4 ± 133.24[a)]	1111.5 ± 93.47[a)]
LPS + 24	1666.3 ± 102.78[a)]	1676.2 ± 52.8 [a)]
LPS + 25	1167.2 ± 92.17[a)]	1266.4 ± 94.22[a)]
LPS + DEX	99.9 ± 12.55[a)]	87.11 ± 11.56[a)]

[a)]Significantly different from LPS-stimulated macrophages ($P < 0.05$)

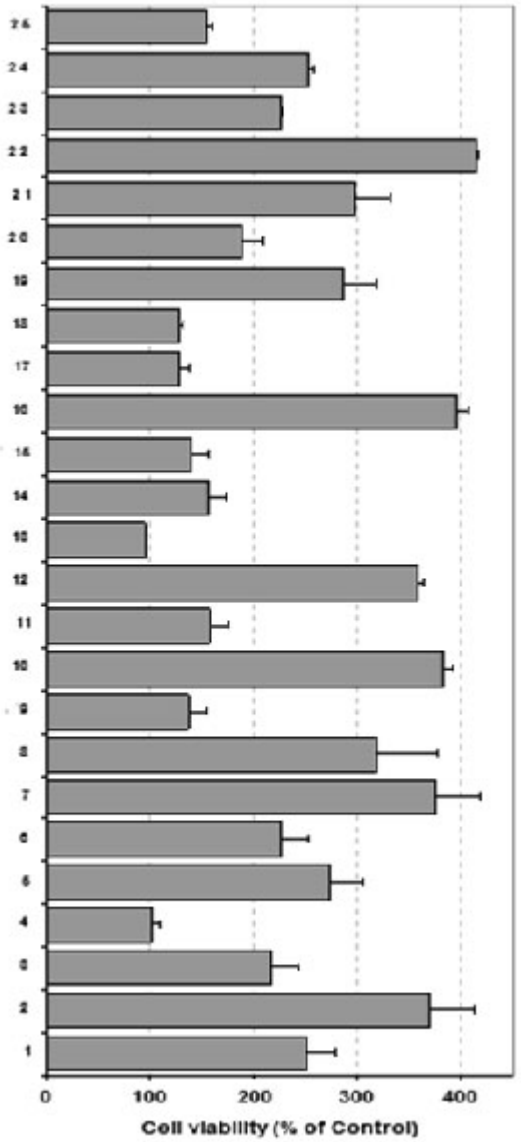
membrane phospholipids by phospholipase A2 [49]. PGE-2 plays a role both in normal physiology and in pathology[49]. The biological actions include inflammation, pain, tumorigenesis, vascular regulation, neuronal functions, female reproduction, gastric mucosal protection, and kidney function[50]. Measurement of PGE-2 by a commercial kit revealed that the treatment with LPS resulted in a dramatic significant increase in PGE-2 levels compared to untreated cells, while the co-treatment with some compounds led to a significant inhibition in the order of 8 > 3 > 7 > 2 >



Scheme 1. Synthesis of 2-methylsulfanyl-[1,2,4]triazolo[1,5-*a*]quinazolin-5-one and its derivatives 1–25

6 > 25 > 1 in this stimulated secretion of PGE-2 ($p < 0.05$, Table 2). This indicates the functionalization of the compounds that increases lipophilic characteristics favorable for increasing their activity.

In conclusions, 2-methylsulfanyl-3-pyridyl-bis-[1,2,4]triazolo[1,5-*a*:4,3-*c*]quinazoline (13) and 2-methylsulfanyl-5-ethoxy-[1,2,4]triazolo[1,5-*a*]quinazoline (17) showed the



Macrophage viability was compared with the induced proliferation by 1,000 units/mL M-CSF (178% of control). The results are represented as the percentage of control untreated cells (Mean ± SD, n = 4).

Fig. 1. The effect of the synthesized compounds (20 µg/mL) on the growth of macrophages

The results were compared with the dexamethasone (50 ng/mL), as an NO inhibitor. The results are represented as the percentage of nitrites inhibition compared to the nitrites level in the LPS-stimulated macrophages (Mean ± SD, n = 4)

Fig. 2. The inhibitory effect of the synthesized compounds (20 µg/mL) on the generation of NO (using nitrites index) from LPS-stimulated macrophages

highest cytotoxic effect on Hep-G2 and HCT-116 cells. It was concluded that the presence of a 5-ethoxy moiety is essential for the antitumor activity against these cell lines. Compound 17 showed IC_{50} values of 19.22 and 17.39 µg/ml, correspondingly, and 13 was the most active one, with IC_{50} = 9.34 µg/ml and 11.51 µg/ml, respectively. It could be assumed that the pyridyl moiety in 13 exhibited better activity than the phenyl analogue 11. The pharmacophoric features for HCT-116 activity could be attributed to the presence of two hydrophobic sites and a hydrogen bond acceptor. Most of the tested compounds significantly induced the growth of macrophages, with up to a 4.2-fold increase compared to growth of the control cells (1–3, 5–8, 10, 12, 16, 19, 21–24). Some tested compounds exhibited strong extents of NO inhibitory activity, as shown in the order 23 > 24 > 22 > 18 > 12 > 4. The co-treatment of LPS-stimulated macrophages resulted in potential inhibition of the LPS-stimulated TNF-± secretion as in the potency order of 8 > 3 > 7 > 11 > 2 > 6 > 21 > 25 > 19 > 1 > 23 > 24. A significant inhibition in the stimulated PGE-2 secretion has been shown (8 > 3 > 7 > 2 > 6 > 25 > 1). These findings indicated that compounds 3, 6–8 and 25 are promising anti-inflammatory agents.

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REFERENCES

1. Lu, H., Ouyang, W., Huang, C. Inflammation, a Key Event in Cancer Development. *Mol. Cancer Res.*, 2006; 4: 221-43.
2. a. Levy, B.D., Clish, C.B., Schmidt, B., Gronert, K., Serhan, C.N. Lipid mediator class switching during acute inflammation: signals in resolution. *Nat. Immunol.*, 2001; **2**: 612–19.
   b. Rastegar, H., Mirzaei, A., Ashtiani, H. R. A., Hedayati, M., Ahmadi, S. The Effect of Extracted Bacterial Salmonella enteritidis Lipopolysaccharide on Inducible Nitric Oxide Synthase in Human Liver Hepatocellular Carcinoma Cell Lines in Induction and Inhibition Conditions. *J. pure Appl. Microbiol*., 2012; **6**: 39-45.
3. Hodge-Dufour, J., Marino, M.W., Horton, M.R. Inhibition of interferon gamma induced interleukin 12 production: A potential mechanism for the anti-inflammatory activities of tumor necrosis factor. *Proc. Natl. Acad. Sci. USA*, 1998; **95**: 13806–11.
4. Nathan, C. Points of control in inflammation. *Nature*, 2002; **420**: 846–52.
5. Savill, J., Wyllie, A.H., Henson, J.E., Walport, M.J., Henson, P.M., Haslett, C. Macrophage phagocytosis of aging neutrophils in inflammation. Programmed cell death in the neutrophil leads to its recognition by macrophages. *J. Clin. Invest*., 1989; **83**: 865–75.
6. Savill, J., Fadok, V.A. Corpse clearance defines the meaning of cell death. *Nature*, 2000; **407**: 784–8.
7. Savill, J., Dransfield, I., Gregory, C., Haslett, C. A blast from the past: Clearance of apoptotic cells regulates immune responses. *Nat. Rev. Immunol*., 2002; **2**: 965–75.
8. Fadok, V.A., Bratton, D.L., Konowal, A., Freed, P.W., Westcott, J.Y., Henson, P.M. Macrophages that have ingested apoptotic cells *in vitro* inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF. *J. Clin. Invest.*, 1998; **101**: 890–8.
9. McDonald, P.P., Fadok, V.A., Bratton, D., Henson, P.M. Transcriptional and translational regulation of inflammatory mediator production by endogenous TGF-beta in macrophages that have ingested apoptotic cells. *J. Immunol*., 1999; **163**: 6164–72.
10. Huynh, M.L.N., Fadok, V.A., Henson, P.M. Phosphatidylserine-dependent ingestion of apoptotic cells promotes TGF-beta1 secretion and the resolution of inflammation. *J. Clin. Invest*., 2002; **109**: 41–50.
11. Philip, M., Rowley, D.A., Schreiber, H. Inflammation as a tumor promoter in cancer induction, *Semin. Cancer Biol*., 2004; **14**: 433–9.
12. Coussens, L.M., Werb, Z. Inflammation and cancer. *Nature*, 2002; **420**: 860–7.
13. Macarthur, M., Hold, G.L., El-Omar, E.M. Inflammation and Cancer II. Role of chronic inflammation and cytokine gene polymorphisms in the pathogenesis of gastrointestinal malignancy. *J. Am. Physiol Gast Liver Physiol*., 2004; **286**: G515–G20.
14. Martin, Y.C., Austel, K.E. (eds.): Paths to Better and Safer Drugs, Modern Drug Research, New York, NY, USA: Marcel Dekker, 1989; pp. 243–73.
15. Roth, H.J., Fenner, H. (eds.): Arzneistoffe, 3rd. edn., Stuttgart,Germany: Deutscher Apotheker

Verlag, 2000; p. 51.
16. Harris, C.R., Thorarensen, A. Advances in the discovery of novel antibacterial agents during the year 2002. *Curr. Med. Chem*., 2004; 11: 2213–43.
17. Andries, K., Verhasselt, P., Guillemont, J., Gohlmann, H.W., Neefs, J.M., Winkler, H., Van Gestel, J., Timmerman, P., Zhu, M., Lee, E. A diarylquinoline drug active on the ATP synthase of Mycobacterium tuberculosis. *Science*, 2005; **307**: 223–6.
18. Vangapandu, S., Jain, M., Jain, R., Kaur, S., Singh, P.P. Ring-substituted quinolines as potential anti-tuberculosis agents. *Bioorg. Med. Chem*., 2004; **12**: 2501–8.
19. Carta, A., Piras, S., Palomba, M., Jabes, D., Molicotti, P., Zanetti, S. Anti-mycobacterial activity of quinolones. Triazoloquinolones a new class of potent anti-mycobacterial agents. *Anti Infective Agents Med. Chem.*, 2008; **7**: 134–47.
20. Padia, J.K., Field, M., Hilton, J., Meecham, K., Pablo, J., Pinnock, R., Roth, B.D., Singh, L., Suman-Chauhan, N., Trivedi, B.K. Novel nonpeptide CCK-B antagonists: Design and development of quinazolinone derivatives as Potent, Selective, and orally active CCKB Antagonists. *J. Med. Chem*., 1998; **41**: 1042–9.
21. Xia, Y., Yang, Z.Y., Hour, M.J., Kuo, S.C., Xia, P., Bastow, K.F., Nakanishi, Y., Nampoothiri, P., Hackl, T., Hamel, E. Antitumor agents. Part 204: Synthesis and biological evaluation of substituted 2-aryl quinazolinones. *Bioorg. Med. Chem. Lett*., 2001; **11**: 1193–6.
22. Kenichi, O., Yoshihisa, Y., Toyonari, O., Toru, I., Yoshio, I. Studies on 4(1H)-quinazolinones. 5. synthesis and antiinflammatory activity of 4(1H)-quinazolinone derivatives. *J. Med. Chem*., 1985; **28**: 568–76.
23. Buchanan, J.G., Sable, H.Z. Selective Organic Transformations, Thygarajan B.S., (ed.): New York, NY, USA, Wiley Interscience, 1972; Volume 2, pp. 1–95.
24. Hamidian, H., Tikdari, A.M., Khabazzadeh, H. Synthesis of new 4(3H)-quinazolinone derivatives using 5(4H)-oxazolones. *Molecules*, 2006; **11**: 377–82.
25. Birendra, N.G., Jiban, C.S.K., Jogendra, N.B. Quinoline-based fused heterocyclic systems are found as potential anticancer. *J. Heterocycl. Chem*., 1984; **21**: 1225–9.
26. Kothari, P.J., Mehlhoff, M.A., Singh, S.P., Parmar, S.S., Stenberg, V.I. Synthesis of some new 5-methyl-2-benzoxazolinone derivatives and investigation on their analgesic-antiinflammatory Activities. *J. Heterocycl. Chem*., 1980; **17**: 1369–72.
27. Sengupta, A.K., Misra, H.K. Studies on potential pesticides 13 Synthesis and evaluation of s(3-substituted-phenoxymethyl-4-aryl/cyclohexyl-4h-1,2,4-triazol-5-yl)-2-mercaptomethyl benzimidazoles for anti-bacterial and insecticidal activities. *J. Indian Chem. Soc*., 1981; **8**: 508.
28. a. Sarmah, S.C., Bahel, S.C. Synthesis of aryloxy/aryl acetyl thiosemicarbazides, substituted 1,3,4-oxadiazoles, 1,3,4-thiadiazoles, 1,2,4-triazoles and related compounds as potential Fungicides. *J. Indian Chem. Soc*., 1982; **59**: 877–80.
b. Kumar, U., Bansal, M., Gupta, S. K., Saharyar, M. Synthesis and Antifungal Activity of 4-amino-5-aryl-1, 2, 4-triazoles. *J. Pure Appl. Microbiol*., 2007; **1**: 341-344.
29. Francis, J.E., Cash, W.D., Psychoyos, S., Ghai, G.; Wenk, P., Friedmann, R.C., Atkins, C., Warren, V., Furness, P., Hyun, T.L. Structure-activity profile of a series of novel triazoloquinazoline adenosine antagonists. *J. Med. Chem*., 1988; **31**: 1014–20.
30. Kim, Y.-C., De Zwart, M., Chang, L., Moro, S., Kuenzel, J., Melman, N., Jzerman, A.P., Jacobson, K.A. Derivatives of the triazoloquinazoline adenosine antagonist (CGS15943) having high potency at the human A2B and A3 receptor subtypes. *J. Med. Chem*., 1998; **41**: 2835–45.
31. Ongini, E., Monoppoli, A., Cacciari, B., Baraldi, P.G. Selective adenosine A2A receptor Antagonists. *Il Farmaco*, 2001; **56**: 87–90.
32. Francis, J.E., Cash, W.D., Barbaz, B.S., Bernard, P.S., Lovell, R.A., Mazzenga, G.C., Friedmann, R.C., Hyun, J.L., Braunwalder, A.F., Loo, P.S. Synthesis and benzodiazepine binding activity of a series of novel [1,2,4]triazolo[1,5-c]quinazolin-5(6H)-ones. *J. Med. Chem*., 1991; **34**: 281–90.
33. Alagarsamy, V., Giridhar, R., Yadav, M.R. Synthesis and pharmacological investigation of novel 1-substituted-4-phenyl-1,2,4-triazolo [4,3-*a*]quinazolin-5(4*H*)-ones as a new class of H1- antihistaminic agents. *Bioorg. Med. Chem. Lett*., 2005; **15**: 1877–80.
34. Alagarsamy, V., Solomon, V.R., Murugan, M. Synthesis and pharmacological investigation of novel 4-benzyl-1-substituted-4*H*-[1,2,4]triazolo [4,3-*a*]quinazolin-5-ones as new class of H1-antihistaminic agents. *Bioorg. Med. Chem*., 2007; **15**: 4009–15.
35. Al-Salahi, R., Geffken, D., Koellner, M. A new series of 2-Alkoxy(aralkoxy)[1,2,4]triazolo[1,5 *a*]quinazolin-5-ones as Adenosine Receptor Antagonists. *Chem. Pharm. Bull*., 2011; **59**:

730–3.
36. Al-Salahi, R., Geffken, D. Synthesis of novel 2-alkoxy(aralkoxy)-4*H*-[1,2,4]triazolo[1,5-*a*]quinazolin-5-ones starting with dialkyl-*N*-cyanoimidocarbonates. *J. Heterocycl Chem*., 2011; **48**: 656–62.
37. Al-Salahi, R., Marzouk, M., Awad, G., Al-Omer M., Ezzeldin, E. Antimicrobial activity of a newly synthesized 2-methylsulfanyl-[1,2,4]triazolo[1,5-a]quinazolin-5-one and its derivatives. *J. Pharm. Pharmacol*., 2013; **65**: 790-7.
38. Al-Salahi R., Geffken, D. Synthesis and reactivity of [1,2,4]triazolo-annelated quinazolines, *Molecules*, 2010; **15**: 7016–34.
39. Jantova, S., Ovadekova, R., Letasiova, S., Spirkova, K., Stankovsky, S. Anti-microbial activity of some substituted triazolo-quinazolines. *Folia Microbiol*., 2005; **50**: 90–4.
40. Al-Omary, M.F., Abou-zeid, L.A., Nagi, M.N., Habib, E.E., Abdel-Aziz, A. El-Azab, A.S., Abdel-Hamide, S.G., Al-Omar, M.A., Al-Obaid, A.M., El-Subbagh, H.I. Non-classical antifolates. Part 2: Synthesis, Biological evaluation, And molecular modeling study of some new 2,6-substituted-quinazolin-4-ones. *Bioorg. Med. Chem*., 2010; **18**: 2849–63.
41. Hansen, M.B., Nielsen, S.E., Berg, K. Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. *J. Immunol. Meth*., 1989; **119**: 203–10.
42. Gerhaeuser, C., Elke Heiss, K.K., Neumann, I., Gamal-Eldeen, A., Knauft, J., Liu, G.-Y., Sitthimonchai, S., Fran, N., Mechanism-based *in vitro* screening of potential cancer chemo-preventive agents. *Mutat. Res*., 2003; **523**(4): 163–72.
43. Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J., Klenk, D.C. Measurement of protein using bicinchoninic acid. *Anal. Biochem*., 1985; **150**: 76–85.
44. Al-Salahi, R., Geffken, D. Synthesis of 2-methylsulfanyl-4*H*-[1,2,4]triazolo[1,5-a]quinazolin 5-one and derivatives. *Synth. Comm*., 2011; **41**: 3512–23.
45. Hong, W.K., Sporn, M.B. Recent advances in chemoprevention of cancer. *Science*, 1997; **278**: 1073–7.
46. Bertram, J.S. The molecular biology of cancer. *Mol. Asp. Med*., 2000; **21**: 167–223.
47. Aggarwal, B.B., Shishodia, S. Molecular targets of dietary agents for prevention and therapy of cancer. *Biochem. Pharmacol*., 2006; **71**: 1397–421.
48. Rooseboom, M., Commandeur, J.N.M., Vermeulen, N.P.E. Enzyme-catalyzed activation of anticancer prodrugs. *Pharmacol. Rev*., 2004; 56: 53–102.
49. Alper, A.E., Taurine, A. Thiazolo[3,2-*a*] benzimidazoles. *Can. J. Chem*., 1967; **45**: 2903–12.
50. Omiecinski, C.J., Hassett, C., Hosagrahara, V. Epoxide hydrolase-polymorphism and role in Toxicology, *Toxicol. Lett*., 2000; **112-113**, 365–70.