Synthesis and Antitumor Activity of 1,2,4-Triazolo[1,5-*a*]quinazolines

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Cytotoxicity of 22 triazoloquinazoline compounds was evaluated *in vitro* against medulloblastoma (Daoy), hepatocellular carcinoma (HepG2) and melanoma (SK-MEL28) cell lines. This study showed that compounds **17**, **18** and **21** exhibited remarkable *in vitro* cytotoxicity against all tested cell lines. Moreover, it was found that compounds **10**, **17**, **6**, **18**, **21**, **7** and **19** are active against Daoy cell line with IC₅₀ values of 1.29, 2.93, 5.53, 6.14, 6.59, 9.71 and 19 µg/mL, respectively, as compared to that of the reference drug dasatinib (7.26 µg/mL). The HepG2 cell line was affected by compounds **17**, **6**, **21**, **19** and **18** with IC₅₀ values of 4.52, 11.33, 14.69, 16.96 and 24.49 µg/mL, respectively, relative to that of dasatinib (8.21 µg/mL). In addition, compounds **17**, **18** and **21** have shown significant antiproliferative activity against SK-MEL28 with IC₅₀ values of 3.88, 13.85 and 14.96, respectively, relative to an IC₅₀ value of 23.83 µg/mL of the reference drug. It is worth mentioning that compounds **6**, **10**, **17**, **18** and **21** are more potent than the reference drug against Daoy cell. In the same manner, **17**, **18** and **21** revealed higher cytotoxicity than dasatinib against SK-MEL28 cells. Notably, compound **17** was also more potent than the reference drug against HepG2 cells. In the term of selectivity, compound **10** was found to possess the highest selectivity, as it was active only against Daoy cells. These compounds could be useful as templates for further development through their structural modification to design more potent antitumor agents.

Keywords: Antitumor, 1,2,4-Triazolo[1,5-*a*]quinazolines, Daoy, HepG2, SK-MEL28.

INTRODUCTION

Development of anticancer molecule is always a fascinating challenge in the field of cancer chemotherapy. Many recent research programmes are ongoing globally to identify and design new lead compounds. Cancer is continuing to be a major health problem in developed as well as undeveloped countries¹⁻⁹. Although major advances have been made in the chemotherapeutic management of some patients, discovering and designing of new lead structures still employed as one of the most urgent research areas in contemporary medicinal chemistry. Although a numerous number of biologically active compounds have been investigated, however many of them are not suitable for therapeutic use due to their toxic, carcinogenic and mutagenic properties. Nowadays, it is possible to make structural modifications of active compounds, in order to synthesize new derivatives with improvement of therapeutic activity and reduction of their toxicity¹⁰. In our previous work, we have reported the synthesis of different 1,2,4-triazolo[1,5-*a*]quinazolines types and evaluated their biological activities as antimicrobial, antiviral, cytotoxic, antiinflammatory and adenosine antagonist agents¹¹⁻¹⁶.

In spite of various triazoloquinazolines having been prepared and their significance studied, the investigation of

1,2,4-triazoloquinazoline moiety as anticancer is relatively unexplored. This work has done in continuation of a programme a part of our interest in the search for anticancer agents. Herein we report the biological activity of the synthesized compounds (**1-22**) as anticancer agents.

EXPERIMENTAL

Uncorrected melting points were determined on a Mettler FP 62 apparatus using an open glass capillaries. The IR (KBr, ν_{max}, cm⁻¹) spectra were recorded on a Perkin Elmer FT-IR Spectrum BX system. NMR spectra were recorded on a Bruker AMX 500 spectrometer in DMSO-*d*₆ and reported as δ ppm values relative to TMS at 500 and 125 MHz for ¹H and ¹³C NMR, respectively. Mass spectra were measured on an Agilent 6410 TSQ system connected to Agilent 1200 HPLC interface (samples were infused in MeOH). Follow up of the reactions and checking the purity of compounds was made by TLC on DC-Mikroartenpolygram SIL G/UV254, from the Macherey-Nagel Firm, Duren Thickness: 0.25 mm. Column chromatography was conducted on silica gel (ICN Silica 100-200, active 60 Å).

Preparation of compounds 21 and 22: At room temperature, an amount of 1.2 mmol from potassium carbonate was

Scheme-I: Synthesized triazoloquinazolines (1-22)

added portion-wise to a solution of **17** (1 mmol) in DMF (5 mL) over a period of 10 min. After stirring for 20 min, the appropriate 3-arylthioquinazolin-4-ones (1.3 mmol) was added in portion-wise and the reaction mixture was stirred for 18 h at room temperature. The mixture was poured into ice/water and then the precipitate was filtered off, washed with water and dried. Analytically pure products **21** and **22** were obtained after recrystallization from DMF.

3-(4-Bromophenyl)-6-methyl-2-(2-methylsulfanyl-1,2,4-triazolo[1,5-a]quinazolin-5-ylsulfanyl)-3H-quinazolin-4-one (21): White amorphous powder; (yield: 50 %), m.p. 290 °C IR (KBr, ν_{max} , cm^{-1}): 1697 (C=O). 1H NMR (DMSO- d_6): δ ppm 8.36 (1H, br d, $J = 7.5$ Hz, H-7'), 8.21 (2H, m, H-9/8), 8.04 (1H, br s, H-5'), 7.99 (2H, d, $J = 8$ Hz, H-2''/6''), 7.60 (2H, br s, H-6/7), 7.52 (1H, d, $J = 8.5$ Hz, H-8'), 7.44 (2H, d, $J = 8$ Hz, H-3''/5''), 2.73 (3H, s, S-CH₃), 2.42 (3H, s, CH₃-6'); ^{13}C NMR (DMSO- d_6): δ ppm 170.5 (C-2'), 161.7 (C-2), 160.3 (C-4'), 154.2 (C-5), 151.4 (C-9a), 148.1 (C-8a), 138.3 (C-6'), 136.2 (C-3a), 136.0 (C-8), 133.4 (C-7'), 132.1 (C-1'), 128.5 (C-3''/5''), 127.1 (C-6), 126.5 (C-5'), 126.3 (C-8'), 126.2 (C-5a), 123.4 (C-2''/6''), 120.5 (C-4'a), 117.8 (C-4''), 116.4 (C-7), 115.3 (C-9), 20.7 (CH₃-6'), 13.7 (-S-CH₃); EI-MS, m/z (%): 560 (M^{+} , 78). Anal. calcd. for C₂₅H₁₇N₆OS₂Br (560.01).

3-Benzyl-6-methyl-2-(2-methylsulfanyl-1,2,4-triazolo[1,5-a]quinazolin-5-ylsulfanyl)-3H-quinazolin-4-one (22): White amorphous powder; (yield: 52 %), m.p. 270 °C IR (KBr, ν_{max} , cm^{-1}): 1721 (C=O). 1H NMR (DMSO- d_6): δ ppm 8.28 (1H, br d, $J = 8$ Hz, H-7'), 8.19 (1H, br d, $J = 8$ Hz, H-9), 8.13 (1H, br

t, $J = 8$ Hz, H-8), 8.08 (1H, br s, H-5'), 7.73 (2H, br s, H-6/7), 7.58 (1H, d, $J = 8.5$ Hz, H-8'), 7.14 (2H, d, $J = 8.5$ Hz, H-2''/6''), 7.06 (3H, br s, H-3'', 4'', 5''), 5.50 (2H, s, -CH₂-Ar), 2.65 (3H, s, -S-CH₃), 2.51 (3H, s, CH₃-6'); ^{13}C NMR (DMSO- d_6): δ ppm 164.6 (C-2'), 161.5 (C-2), 160.5 (C-4'), 154.5 (C-5), 151.8 (C-9a), 148.4 (C-8a), 144.8 (C-1'), 138.6 (C-6'), 136.4 (C-3a), 136.2 (C-8), 133.7 (C-7'), 128.1 (C-3''/5''), 127.3 (C-6), 126.8 (C-2''/6'', 5'), 126.6 (C-4''), 126.2 (C-8'), 126.1 (C-5a), 120.3 (C-4'a), 116.7 (C-7), 115.1 (C-9), 49.1 (-CH₂-Ar), 20.9 (CH₃-6'), 13.5 (-S-CH₃). EI-MS, m/z (%): 496 (M^{+} , 72). Anal. calcd. for C₂₆H₂₀N₆OS₂ (413.11).

(3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) (MTT) was purchased from Sigma Aldrich (St Louis, MO, USA). DMEM/high glucose FBS and penicillin/streptomycin were obtained from Hyclone Laboratories (Logan, UT, USA).

Cell lines: Three tumor cell lines were tested in this study, namely human medulloblastoma (Daoy), human hepatocellular carcinoma (HepG2) and human melanoma (SK-Mel-28). HepG2 cells were cultured in DMEM/high glucose supplemented with 10 % FBS, 2 mM L-glutamine and 1 % penicillin/streptomycin. Daoy and SK-Mel-28 were cultured in DMEM/F12 supplemented with 10 % FBS, 2 mM L-glutamine and 1 % penicillin/streptomycin.

Screening of antiproliferative activity of compounds by MTT assay: The new compounds were evaluated at the Cell Culture Laboratory, College of Pharmacy, King Saud University, in a primary three cell line-one concentration

(25 µg/mL) anticancer assay against the previously mentioned cell lines. The cytotoxic effect of the newly synthesized compounds were evaluated by testing the capacity of the reducing enzymes present in viable cells to convert MTT to formazan crystals as previously described¹⁷, with some modifications. Briefly, cells cultured in complete medium were seeded into 96-well microtiter plates (in quintuplicates) with 2×10^4 cells per well and incubated at 37 °C under a humidified atmosphere of 5 % CO₂ for 24 h. The cell medium in test wells were then changed to serum free medium (SFM) containing 25 µg/mL of the tested compounds, while the cell medium in control wells were changed to serum free medium containing an equivalent volume of solvent (dimethyl sulfoxide). After incubation at 37 °C for 24 h, serum free medium in control and test wells were replaced by 100 µL/well of MTT; 0.5 mg/mL in phosphate-buffered saline (PBS) and incubated at 37 °C for an additional 3 h. MTT solution were removed and the purple formazan crystals formed at the bottom of the wells were dissolved using 100 µL isopropyl alcohol/well with shaking for 1 h at room temperature. The absorbance at 549 nm was read on a microplate reader (ELX 800; Bio-Tek Instruments, Winooski, VT, USA). Cell death was estimated with the following formula¹⁸:

$$\% \text{ Specific death} = \frac{A(\text{untreated cells}) - A(\text{treated cells})}{A(\text{untreated cells})} \times 100$$

The dose response curves of the compounds effecting ≥ 50 % inhibition in one-dose prescreening for each cell line were established with concentrations of 25, 12.5, 6.25, 3.125, 1.56 and 0.78 µg/mL and the concentrations causing 50 % cell growth inhibition (IC₅₀) were calculated. The cytotoxic activity of the anticancer drug dasatinib, a potent multi-targeted kinase inhibitor of BCR-ABL and SRC family kinases¹⁹, against the three cell lines was examined at the same concentrations of tested compounds and utilized as a standard for comparative purposes.

RESULTS AND DISCUSSION

In previous papers²⁰⁻²⁴, we have described the synthetic routes for the triazoloquinazoline derivatives **1-20** (Scheme-I). The corresponding **21** and **22** were prepared by reaction of compound **17** with appropriate 3-aryl(aryl)-thioxoquinazolin-4-ones in dimethylformamide at room temperature. The structures of **21** and **22** were established on the basis of IR, MS and confirmed by ¹H- and ¹³C NMR spectral data (splitting pattern, δ - and *J*-values and comparison with literature of structural related compounds). As it was explained in previous article²⁴, the 1,2,4-triazolotricyclic main structural nucleus was proved by the assignment of its four one proton ¹H-signals as two dd (or br d) resonances with *J*_{ortho} (7.5-8.5 Hz) and *J*_{meta} (1-2 Hz) assignable for H-9 and H-6 and two td (or br t) resonances with *J*_{ortho} and *J*_{meta} for H-8 and H-7, respectively. Moreover, ¹³C NMR spectra proved the main tricyclic moiety through characteristic nine resonances including the most downfield key signal of C-2 assigned at ≈ 165 ppm in 2-phenoxy derivatives that was observed relatively upfield at ≈ 160 -161 in case of 2-methylsulfonyl function and in **20** and **21** at about 160.5 ppm due to the stronger -R and -I (deshielding) effect of *O*-phenoxy than -SO₂ or S-CH₃ (**20** & **21**)²⁴. The presence of one

S-CH₃ in the structures of **21** and **22** was proved by the singlet at 2.73 and 2.65 ppm together with their own ¹³C-signals as the most upfield characteristic position at 13.7 and 13.5. Another key ¹³C-signal was C-5 that interpreted at $\delta \approx 154$ ppm due to the presence of 5-S-quinazolin-4-one function (**21**, **22**). As well, methylquinazolin-4-one moiety was deduced from its AMX-spin coupling system for H-7', H-5' and H-8' at about 8.3 (br d, *J* = 8 Hz), 8 (1H, br s), 7.5 (1H, d, *J* = 8.5 Hz), respectively. This moiety was interpreted in the ¹³C-spectrum in the form of nine resonances including three characteristic signals at 170.5 (C-2'), 160.3 (C-4') and 20.7 (CH₃-6') in case of **21**, which assigned at 164.6 (C-2'), 160.5 (C-4') and 20.9 (CH₃-6') in **22**. The difference between the NMR spectra of both compounds was summarized in the form of presence the characteristic signals for *N*-*p*-bromophenyl in case of **21** and *N*-benzyl in the structure of **22**. Final confirmation of their structures was obtained from the comparison of all ¹H and ¹³C resonances with our assigned data for structural related triazoloquinazoline derivatives^{14,24}.

The *in vitro* antitumor activity of compounds **1-22** was evaluated by testing their cytotoxic effects using the MTT assay against Medulloblastoma (Daoy), Hepatocellular carcinoma (HepG2) and Melanoma (SK-MEL28) cells¹⁷. The IC₅₀-values represented the drug concentration (µg/mL) required to inhibit cell growth by 50 %, are summarized in Table-1 as a common parameter for cytotoxicity of the investigated compounds. As indicated in Table-1, some target molecules have higher or comparable cytotoxicity with that of Dasatinib against the cancer cell lines examined, with IC₅₀ values in the range of 1.29-14.96 µg/mL. Compounds **6**, **10**, **17**, **18** and **21** have shown the highest cytotoxicity against Medulloblastoma with the IC₅₀ values of 5.53, 1.29, 2.93, 6.14 and 6.59 µg/mL in regards to the reference drug IC₅₀ (7.26 µg/mL). For hepatocellular carcinoma (HepG2) cell line, the most active compound (**17**) was investigated to exhibit higher inhibition concentration and a lower IC₅₀ with respect to Dasatinib. The target products **17**, **18** and **21** were found to possess the highest activity against Melanoma (SK-MEL28) with their IC₅₀ values of 3.88, 13.85 and 14.96, respectively. Compound **19** was exhibited activity against Medulloblastoma (Daoy), Hepatocellular carcinoma (HepG2) cells but not more potent than the respected reference drug. Similarly, compound **6** was less potent than Dasatinib against hepatocellular carcinoma (HepG2) cell. Throughout our study we have noticed that structure modifications in the parent compounds (**1-4**) have led to remarkable cytotoxicity depending on the structure change and examined cell lines. In compounds **7-12**, regioselective *N*-alkylation of lactam has demonstrated noticeable activity such as compound **7** and **10** that displayed a significant IC₅₀ values (Table-1) in comparison with their parents and Dasatinib as well. However, **10** represents one of the most populated compound obtained during this study. Furthermore, conversion of lactam in compounds **2** and **4** into an imidoyl chloride function (**17**, **18**) has influenced positively cytotoxic effect and more significant on the activity profiles, this may be attributed to the increase of the lipophilicity. Whereas, further chemical transformation of chlorine in **16** into ethoxy (**19**) was offered advantageous in the activity. The *S*-arylation of **17** with *N*-(4-bromophenyl)thioxoquinazoline to form **21** has led to decrease the activity and in case

of **22** (N-benzyl) it was completely abolished. However the same behaviour in case of compound **20** has demonstrated with respect to **16**. Thionation product **6** has showed high significant and remarkable cytotoxic activity in regard to its parent **2**; this could be attributed to the enhancing of the lipophilicity comparable to that of parent **2**.

TABLE-1
in vitro CYTOTOXICITY OF COMPOUNDS
AGAINST CANCER CELL LINES

Cpd. Nr.	Daoy ¹		HepG2 ²		SK-MEL28 ³	
	Inhibition* (%)	IC ₅₀ **	Inhibition* (%)	IC ₅₀ **	Inhibition* (%)	IC ₅₀ **
1	13.99	nt [#]	30	nt [#]	0	nt [#]
2	0	nt [#]	9.94	nt [#]	0	nt [#]
3	14.52	nt [#]	0	nt [#]	19.82	nt [#]
4	17.92	nt [#]	15.71	nt [#]	0	nt [#]
5	21.24	nt [#]	43.7	nt [#]	12.63	nt [#]
6	75.13	5.53	54.03	11.33	36.4	nt [#]
7	59.48	9.71	14.62	nt [#]	42.96	nt [#]
8	31.3	nt [#]	22.21	nt [#]	27.21	nt [#]
9	0	nt [#]	9.94	nt [#]	0	nt [#]
10	62.78	1.29	43.5	nt [#]	37.2	nt [#]
11	18.42	nt [#]	8	nt [#]	0	nt [#]
12	47.57	nt [#]	24.5	nt [#]	0	nt [#]
13	7.46	nt [#]	14.72	nt [#]	13.27	nt [#]
14	48.92	nt [#]	28.23	nt [#]	38.46	nt [#]
15	48.28	nt [#]	16.5	nt [#]	0	nt [#]
16	36.37	nt [#]	9.87	nt [#]	0	nt [#]
17	93.91	2.93	90.87	4.52	93.16	3.88
18	94	6.14	51.68	24.49	91.36	13.85
19	58.23	19.08	57.86	16.96	29.03	nt [#]
20	28.92	nt [#]	14.63	nt [#]	11.24	nt [#]
21	94.08	6.59	84.67	14.69	91.87	14.96
22	47.57	nt [#]	24.5	nt [#]	0	nt [#]
D	76.6	7.26	72.03	8.21	51.95	23.83

*Per cent inhibition of cell survival at 25 µg/mL, relative to control (DMSO-treated cells); ** IC₅₀ is expressed as µg/ml; # nt: Not tested; D = Dasatinib; ¹Medulloblastoma cell line. ²Hepatocellular carcinoma cell line. ³Melanoma cell line

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REFERENCES

1. B.S.P. Sandor Eckhardt, *Curr. Med. Chem. Anticancer Agents*, **2**, 419 (2002).
2. J.C. Medina, B. Shan, H. Beckmann, R.P. Farrell, D.L. Clark, R. Marc Learned, D. Roche, A. Li, V. Baichwal, C. Case, P.A. Baeuerle, T. Rosen and J.C. Jaen, *Bioorg. Med. Chem. Lett.*, **8**, 2653 (1998).
3. A.A.M. Abdel-Aziz, *Eur. J. Med. Chem.*, **42**, 614 (2007).
4. H.S. Hwang, E.Y. Moon, S.K. Seong, C.H. Choi, C.H. Chung, S.H. Jung and S. Yoon, *J. Anticancer Res.*, **19**, 5087 (1999).
5. H.-Y. Park Choo, M. Kim, S.K. Lee, S. Woong Kim and I. Kwon Chung, *Bioorg. Med. Chem.*, **10**, 517 (2002).
6. S.T. Al-Rashood, I.A. Aboldahab, M.N. Nagi, L.A. Abouzeid, A.A.M. Abdel-Aziz, S.G. Abdel-hamide, K.M. Youssef, A.M. Al-Obaid and H.I. El-Subbagh, *Bioorg. Med. Chem.*, **14**, 8608 (2006).
7. A.M. Al-Obaid, S.G. Abdel-Hamide, H.A. El-Kashef, A.A.-M. Abdel-Aziz, A.S. El-Azab, H.A. Al-Khamees and H.I. El-Subbagh, *Eur. J. Med. Chem.*, **44**, 2379 (2009).
8. F.A.M. Al-Omary, L.A. Abou-zeid, M.N. Nagi, E.-S.E. Habib, A.A.-M. Abdel-Aziz, A.S. El-Azab, S.G. Abdel-Hamide, M.A. Al-Omar, A.M. Al-Obaid and H.I. El-Subbagh, *Bioorg. Med. Chem.*, **18**, 2849 (2010).
9. F. Barlesi, C. Tchouhadjian, C. Doddoli, P. Villani, L. Greillier, J.-P. Kleisbauer, P. Thomas and P. Astoul, *Fundam. Clin. Pharmacol.*, **19**, 385 (2005).
10. I. Kostova, *Curr. Med. Chem. Anticancer Agents*, **5**, 29 (2005).
11. R.A. Al-Salahi, M.A. Al-Omar, I. Alswaidan, M. Marzouk, W.M. El-Senousy and A.E.E. Amr, *Res. Chem. Intermed.*; doi:10.1007/s11164-013-1177-1.
12. R.A. Al-Salahi, M. Marzouk, G. Awad, M. Al-Omar and E. Ezzeldin, *J. Pharm. Pharmacol.*, **65**, 790 (2013).
13. R.A. Al-Salahi, D. Geffken and M. Koellner, *Chem. Pharm. Bull. (Tokyo)*, **59**, 730 (2011).
14. R.A. Al-Salahi, I. Alswaidan, M.A. Al-Omar and M.S. Marzouk, *Life Sci. J.*, **10**, 2018 (2013).
15. R.A. Al-Salahi, I. Alswaidan, M.A. Al-Omar and M.S. Marzouk, *Life Sci. J.*, **10**, 2164 (2013).
16. R.A. Al-Salahi, A.M. Gamal-Eldeen, A.M. Alanazi, M.A. Al-Omar, M.A. Marzouk and M.M.G. Fouda, *J. Pure Appl. Microbiol.*, **7**, 189 (2013).
17. C. Kohle, O.A. Badary, K. Nill, B.S. Bock-Hennig and K.W. Bock, *Biochem. Pharmacol.*, **69**, 1397 (2005).
18. T. Abdollahi, N.M. Robertson, A. Abdollahi and G. Litwack, *Cancer Res.*, **63**, 4521 (2003).
19. L.J. Lombardo, F.Y. Lee, P. Chen, D. Norris, J.C. Barrish, K. Behnia, S. Castaneda, L.A.M. Cornelius, J. Das, A.M. Doweyko, C. Fairchild, J.T. Hunt, I. Inigo, K. Johnston, A. Kamath, D. Kan, H. Klei, P. Marathe, S. Pang, R. Peterson, S. Pitt, G.L. Schieven, R.J. Schmidt, J. Tokarski, M.-L. Wen, J. Wityak and R.M. Borzilleri, *J. Med. Chem.*, **47**, 6658 (2004).
20. R. Al-Salahi and D. Geffken, *J. Heterocycl. Chem.*, **48**, 656 (2011).
21. R. Al-Salahi and D. Geffken, *Heterocycles*, **81**, 1843 (2010).
22. R. Al-Salahi and D. Geffken, *Molecules*, **15**, 7016 (2010).
23. R. Al-Salahi and D. Geffken, *Synth. Commun.*, **41**, 3512 (2011).
24. R. Al-Salahi, K.-E. El-Tahir, I. Alswaidan, N. Lolak, M. Hamidaddin and M. Marzouk, *Chem. Cent. J.*, **8**, 3 (2014).