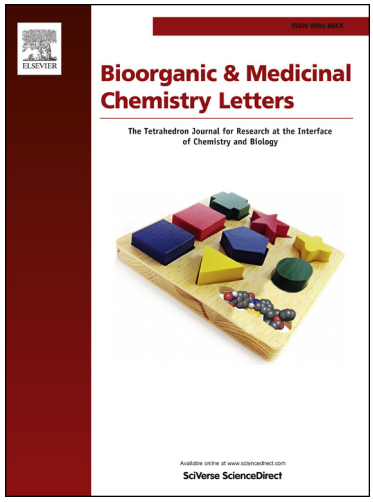


Regioselective synthesis of 6-aryl-benzo[h][1,2,4]-triazolo[5,1-b] quinazoline-7,8-diones as potent antitumoral agents

Liqiang wu, Chong Zhang, Weilin Li

PII: S0960-894X(13)00761-0
DOI: http://dx.doi.org/10.1016/j.bmcl.2013.06.040
Reference: BMCL 20604

To appear in: *Bioorganic & Medicinal Chemistry Letters*

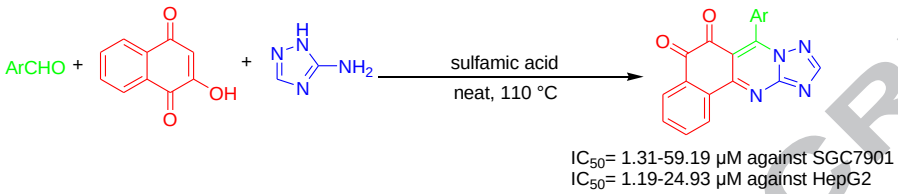
Received Date: 18 January 2013
Revised Date: 13 May 2013
Accepted Date: 13 June 2013

Please cite this article as: wu, L., Zhang, C., Li, W., Regioselective synthesis of 6-aryl-benzo[h][1,2,4]-triazolo[5,1-b] quinazoline-7,8-diones as potent antitumoral agents, *Bioorganic & Medicinal Chemistry Letters* (2013), doi: http://dx.doi.org/10.1016/j.bmcl.2013.06.040

Graphical Abstract

Regioselective synthesis of 6-aryl-benzo[*h*][1,2,4]-triazolo[5,1-*b*] quinazoline-7,8-diones as potent antitumoral agents

Liqiang wu,[a,*] Chong Zhang,[a] Weilin Li[a]



IC_{50}= 1.31-59.19 μM against SGC7901
IC_{50}= 1.19-24.93 μM against HepG2

Regioselective synthesis of 6-aryl-benzo[*h*][1,2,4]-triazolo[5,1-*b*] quinazoline-7,8-diones as potent antitumoral agents

Liqiang wu,* Chong Zhang, Weilin Li

School of Pharmacy, Xinxiang Medical University, Xinxiang 453003, China

ABSTRACT Three-component coupling of aldehyde, 2-hydroxy-1,4-naphthoquinone and 3-amino-1,2,4-triazole has been achieved using a catalytic amount of sulfamic acid under solvent free conditions to produce a novel series of 6-aryl-benzo[*h*][1,2,4]-triazolo[5,1-*b*]quinazoline-7,8-dione derivatives in good yields and with high regioselectivity. These compounds are found to exhibit potent antitumoral properties.

Keywords: Sulfamic acid; 2-Hydroxy-1,4-naphthoquinone; Aldehydes; *Ortho*-naphthoquinone; Antitumor; Solvent-free

*Corresponding author. Tel.: +86 371 3029879; fax: +86 371 3029879; e-mail: wliq1974@163.com

Naphthoquinones constitute a major class of naturally occurring compounds, and have wide range of biological and therapeutic properties such as antioxidant,[1] antifungal,[2] anti-inflammatory,[3] antiallergic,[4] antiviral,[5] and anticancer activity.[6] A variety of naturally occurring and synthetic substituted 1,2-naphthoquinones received a great deal of attention for their anticancer activity. Many derivatives of *ortho*-naphthoquinone are cytotoxic and have been used as antitumor drugs. Among them, some naturally occurring 1,2-naphthoquinones include Lantalucratins,[7] Biflorin,[8] Rhinacanthone,[9] β-lapachone[10] and Mansonones,[11] and synthetic 1,2-naphthoquinones include Salvicin (Figure 1).[12] One of these quinones was β-lapachone, a 1,2-napthaquinone derivative and a natural product, which inhibited tumor growths in rats implanted with W-256 carcinoma.[13] The compound was found to be cytotoxic to many human cancer cell lines through inhibition of DNA repair enzymes.[14] Frydman et al.[15] have recently demonstrated that several 1,2-napthoquinone derivatives can induce DNA-TOPO II-mediated cleavages of DNA in an ATP-independent manner and this effect may be crucial for the cytotoxicity of these compounds. Considering the above reports, development of new and simple synthetic methods for efficient preparation of new *ortho*-naphthoquinones are therefore an interesting challenge.

< **Figure 1**>

Multi-component reactions (MCRs) have received considerable attention because of their wide range of applications in pharmaceutical chemistry for generation of structural diversity and combinatorial libraries for drug discovery.[16] The convergent character, atom economy, ease of one-pot operation, access to structurally diverse libraries of compounds render MCRs greatly advantageous over linear multistep synthesis.[17] Further, it has become imperative both in academia and industry to design catalyst- and solvent-free MCRs, as these processes are rendered green with

diminution of waste, time, manpower and cost.

In recent years, the use of solid acidic catalysts has offered important advantages in organic synthesis, for example, operational simplicity, environmental compatibility, nontoxic, low cost, and ease of isolation. A tremendous upsurge of interest in various chemical transformations processes by catalysts under heterogeneous conditions has occurred. One of those heterogeneous catalysts is sulfamic acid (SA), which makes reaction processes convenient, more economic, and environmentally benign. Owing to the numerous advantages associated with the cheap and nonhazardous catalyst, SA has been explored as a powerful catalyst for various organic transformations.[18] We now report a highly regioselective procedure for the preparation of 6-aryl-benzo[*h*][1,2,4]-triazolo[5,1-*b*quinazoline-7,8-diones along with their antitumor activities against the SGC7901 (stomach cancer cell line) and HepG2 (Human hepatocellular liver carcinoma cell line) using SA as an efficient and versatile catalyst under solvent-free conditions (Scheme 1).

< Scheme 1>

Initially, to achieve suitable conditions for the synthesis of 6-aryl-benzo[*h*][1,2,4]- triazolo[5,1-*b*] quinazoline-7,8-diones, we tested the three-component reaction of benzaldehyde, 2-hydroxy-1,4-naphthoquinone, and 3-amino-1,2,4-triazole as a simple model system at 110 °C under solvent-free conditions using various catalysts (Table 1). As could be seen in Table 1, the best result was obtained with 10 % mol of SA as the catalyst at 110 °C under solvent-free conditions (Table1, entry 3). Using less catalyst resulted in lower yields, whereas higher amounts of catalyst did not affect reaction times and yields. When this reaction was carried out without SA, or $InCl_3$, $FeCl_3$ the yield of the expected product was low (Table 1, entry 1, 6, 7). In the presence of *p*-TsOH, silica sulfuric acid the product was obtained in moderate yield (Table 1, entry 8, 9).

< Table 1>

In order to extend the above reaction (Scheme 1) to a library system, various kinds of arylaldehydes **1** (Table 2) were subjected to react with 2-hydroxy-1,4-naphthoquinone **2** and 3-amino-1,2,4-triazole **3** to give the corresponding 6-aryl-benzo[*h*][1,2,4]-triazolo[5,1-*b*]quinazoline-7,8-diones, and representative examples are shown in Table 2. All of **1** gave the expected products in high yields, either bearing electron-withdrawing groups (such as halide, nitro) or electron-donating groups (such as alkyl group) under the same reaction condition. To further demonstrate the scope and limitation of the substrates, aliphatic aldehydes, such as phenylacetaldehyde, propionaldehyde, *n*-butylaldehyde and *n*-heptaldehyde, were used as reactants to react with 2-hydroxy-1,4-naphthoquinone and 3-amino-1,2,4-triazole. However, the desired products were not found and obtained successfully. The structure of the *ortho*-quinone structures **4** is in full agreement with IR, ^{1}H NMR, ^{13}C NMR, MS and elemental analysis as illustrated below for a representative example (compound **4c**). The ESI-MS spectrum of **4c** displayed a peak at *m/z* 361 for $[M + H]^{+}$, and gave two very close carbonyl resonances at δ 178.6 and 178.2 ppm, which was the very close chemical shifts for the carbonyl ^{13}C NMR signals suggested an *ortho*-quinone structure (in the *para*-quinone **5** the C-7 signal was more deshielded, about appearing about at 182 ppm, and C-12 resonating about at 179 ppm).[19] Two peak at 1685 and 1598cm^{-1} were observed for C=O stretching in the IR spectrum as a broad peak, which was more easily attributable to the *ortho*-quinone moiety. In its ^{1}HNMR spectrum, H-9 and H-12 occur as doublets, respectively, at 8.98 (J = 8.0 Hz) and 8.25 (J = 7.6 Hz) ppm. The H-9 occur about at 9 ppm, more downfield than expected of aromatic protons. This is explicable by the close proximity of these protons to the lone pairs of the neighbouring nitrogens and the consequent anisotropic and van de Waals deshielding. These spectral data are consistent with the structure of **4c**. The formation of isomeric systems **4** and **5** is possible in the reaction (Figure 2). So, we considered it desirable to obtain independent chemical evidence for the presence of *ortho*- or *para*-quinone units in

4. To this end, we reacted **4c** with *o*-phenylenediamine for 30 min under solvent-free conditions, affording compound **6c** in 99% yield, confirming the *ortho*-quinone structure (Scheme 2).[19] The structure of **6c** was fully characterized by spectroscopic data and elemental analysis, The H-5 and H-8 occur as a multiplet at 9.25-9.31ppm, more downfield than expected of aromatic protons. This is explicable by the close proximity of these protons to the lone pairs of the neighbouring nitrogens and the consequent anisotropic and van de Waals deshielding. The lack of any carbonyl signal and the presence of two imine carbon signals at 154.6 and 154.4 ppm in ^{13}C NMR spectrum of **6c**, and the fact that **6c** is formed by the reaction of one molecule of **4c** with one molecule of *o*-phenylenediamine clearly support the structure of **6c**, which, in turn, further corroborates the structure of **4** and the regiochemistry of its formation.

<Table 2>

<Figure 2>

<Scheme 2>

Although the detailed mechanism of the above reaction remains to be fully clarified, the formation of 6-aryl-benzo[*h*][1,2,4]-triazolo[5,1-*b*]quinazoline-7,8-diones could be explained by a reaction sequence presented in Scheme 3. It is conceivable that SA catalyzes the formation of a carbocation in a reversible reaction with the aromatic aldehyde. The higher reactivity of the carbocation compared with the carbonyl species is utilized to facilitate Knoevenagel condensation between arylaldehyde **1** and 2-hydroxy-1,4-naphthoquinone **2** *via* intermediate **7**, and after dehydration olefin **8** is produced. Subsequent Michael-type addition of 3-amino-1,2,4-triazole **3** to the olefin followed by intramolecular nucleophilic cyclization, dehydration, and dehydrogenation affords the corresponding products **4**.[20]

<Scheme 3>

All synthesized compounds were evaluated for their antiproliferative activities against the human gastric carcinoma cell line SCG7901, hepatoma cell line HepG2, and their IC_{50} values in micromolar concentration are represented in Table 3. It is clearly observed that all the tested compounds showed moderate to good antiproliferative activities against the tested cancer cell lines. The compound **4b**, **4g**, **4h**, **4j** showed better antitumor activity against all cancer cell line. The results in Table 3 showed also some important structure-activity relationships (SARs) for this series of derivatives. First, the *ortho*-quinone moiety appeared to have an important effect upon cytotoxicity, compounds **6c** was less potent cytotoxicity, than the corresponding analogues with an *ortho*-quinone moiety. The wide activity range observed for compounds **4a-4j** (IC_{50} from 1.19 to >40 μM) indicated that the nature of substituents at the C-6 position markedly affected the activity profile of these compounds. Substitution of the electron-rich aromatic ring at the C-6 position confered far greater cytotoxicity in comparison with the substitution of the electron-rich aromatic ring at the C-6 position.

<Table 3>

In summary, we have developed a novel method for the synthesis of 6-aryl-benzo[*h*][1,2,4]-triazolo[5,1-*b*]quinazoline-7,8-dione derivatives by means of a three-component reaction between aldehyde, 2-hydroxy-1,4-naphthoquinone and 3-amino-1,2,4-triazole using a catalytic amount of SA under neat conditions. This method is simple and convenient to prepare a wide range of *ortho*-quinone derivatives in a single-step operation which are found to possess interesting antitumor properties.

Acknowledgements

We are pleased to acknowledge the financial support from Xinxiang Medical University

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://

References and Notes

1. Kim, J. P.; Kim, W. G.; Koshino, H.; Jung, J.; Yoo, I. *Phytochemistry* 1996. **43**, 425.
2. (a) Errante, G.; Motta, G. L.; Lagana, C.; Wittebolle, V.; Sarciron M.-E.; Barret, R. *Eur. J. Med. Chem*. **2006**, *41*, 773; (b) Gafner, S.; Wolfender, J. L.; Nianga, M.; Stoeckli-Evans, H.; Hostettmann, K. *Phytochemistry* **1996**, *42*, 1315.
3. Moon, D. O.; Choi, Y. H.; Kim, N. D.; Park, Y. M.; Kim, G. Y. *Int. Immunopharmacol.* **2007**, *7*, 506.
4. Buckle, D. R.; Cantello, B. C. C.; Smith, H.; Smith, R. J.; Spicer, B. A. *J. Med. Chem*. **1977**, *20*, 1059.
5. Tando, V. K.; Singhb, R. V.;. Yadava, D. B. *Bioorg. Med. Chem. Lett*. **2004**, *14*, 2901.
6. (a) Kongkathip, N.; Kongkathip, B.; Siripong, P.; Sangma, C.; Luangkamin, S.; Niyomdecha, M.; Pattanapa, S.; Piyaviriyagul, S.; Kongsaeree, P. *Bioorg. Med. Chem*. **2003**, *11*, 3179; (b) Yamashita, M.; Kaneko, M.; Iida, A.; Tokudab, H; Nishimura, K. *Bioorg. Med. Chem. Lett*. **2007**, *17*, 6417.
7. Hayashi, K.; Chang, F.-R.; Nakanishi, Y.; Bastow, K. H. F.; Cragg, G.; McPhail, A. T.; Nozaki, H.; Lee, K.-H. *J. Nat. Prod*. **2004**, *67*, 990.
8. Vasconcellos, M. C.; Bezerra, D. P.; Fonseca, A. M.; Pereira, M. R.; Lemos, T. L.; Pessoa, O. D.; Pessoa, C.; Moraes, M. O.; Alves, A. P.; Costa-Lotufo. L. V. *Biol. Pharm. Bull*., **2007**, *30*, 1416.
9. (a) Siripong, P.; Kanokmedakul, K.; Piyaviriyakul, S.; Yahuafai, J.; Chanpai, R.; Ruchirawat, S; Oku, N. *J. Trad. Med.* **2006**, 23, 166; (b) Pattanapa, S.; Piyaviriyakul, S.; Kongsaeree, P. *Bioorg. Med. Chem*. **2003**, *11*, 3179.
10. Dubin, M.; Fernandez Villamil, S. H.; Stoppani, A. O. *Medicina* **2001**, *61*, 343.

11. Wang, D.; Xia, M. Y.; Cui, Z.; Tashiro, S. I.; Onodera, S.; Ikejima, *Biol. Pharm Bull*. **2004**. *27* , 1025.

12. Zhang ,J .S.; Ding, J.; Tang, Q. M.; Li, M.; Zhao, M.; Lu, L. J.; Chen, L. J.; Yuan, S. T. *Bioorg Med Chem. Lett*. **1999**, *9*, 2731.

13. (a) Planchon, S. M.; Wuerzberger, S.; Frydman, B. *Cancer Re*s. **1995**, *55*, 3706; (b) Li, C. J.;. Averboukh, I.; Pardee, A. B. *J. Biol. Chem*. **1993**, *268*, 22463.

14. Boothman, D. A.; Trask, D. K.; Pardee, A. B. *Cancer Res*. **1989**, *49*, 605.

15. Frydman, B.; Marton, L. J.; Sun, J. S.; Neder, K.; Witiak, D. T.; Liu, A. A.; Wang, H. M.; Mao, Y.; Wu, H. Y.; Sanders, M. M.; Liu, L. F. *Cancer Res*. **1997**, *57*, 620.

16. Zhu, J.; Bienayme, H. Multi-Component Reactions; Wiley: Weinheim, 2005.

17. (a) Toure, B. B.; Hall, D. G. *Chem. Rev*. **2009,** *109*, 4439; (c) Balme, G.; Bossharth, E.; Monteiro, N. *Eur. J. Org. Chem*. **2003**, 410.

18. (a) Jin, T. S.; Sun, G.; Li, Y. W.; Li, T. S. *Green Chem*. **2002**, *4*, 255; (b) Nagarajan, R.; Magesh, C. J.; Perumal, P. T. *Synthesis* **2004**, 69; (c) Rajitha, B.; SunilKumar, B.; Thirupathi Reddy, Y.; Narsimha Redd, P.; Sreenivasulu, N. *Tetrahedron Lett*. **2005**, *46*, 8691.

19. Lopez-Alvarado, P.; Avendano, C.; Menendez, J. C. *J. Chem. Soc. , Perkin Trans*. *1* **1997**, 229.

20. Jiang,B.; Zhang, G.; Ma, N.; Shi, F.; Tu, S.J.; Kaur, P.; Li, G. *Org. Biomol. Chem*. **2011**, *9*, 3834

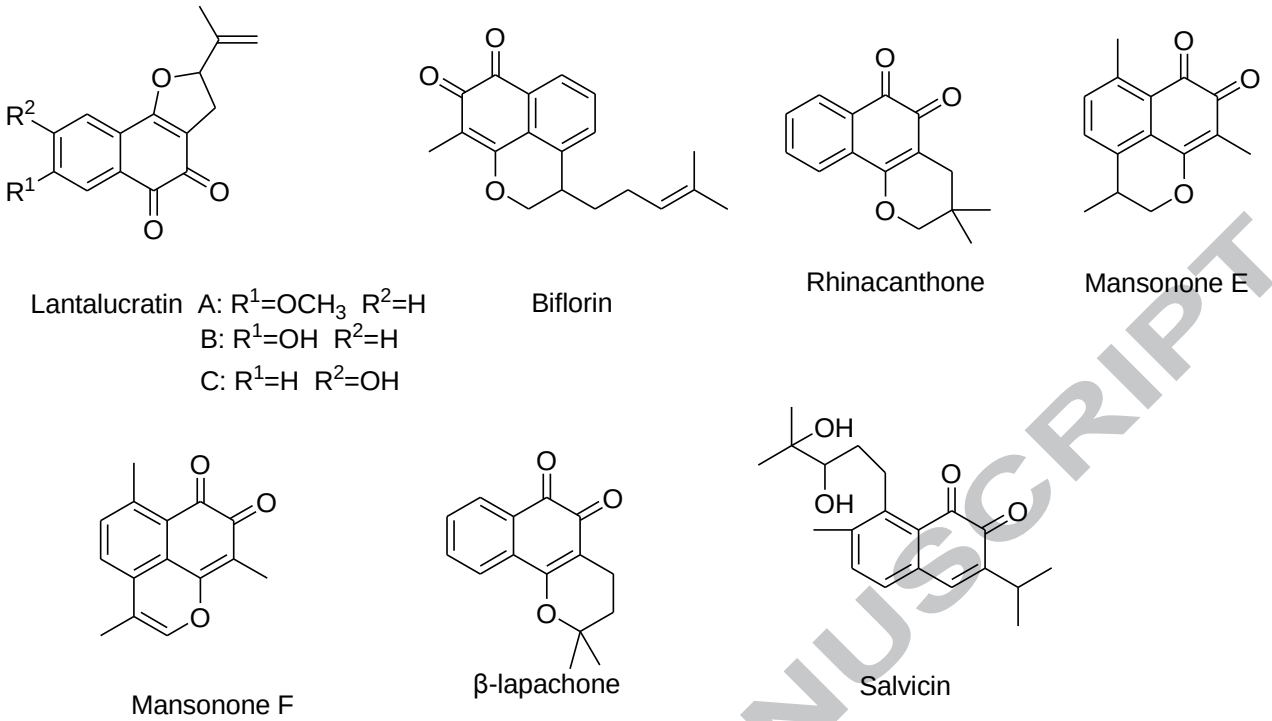
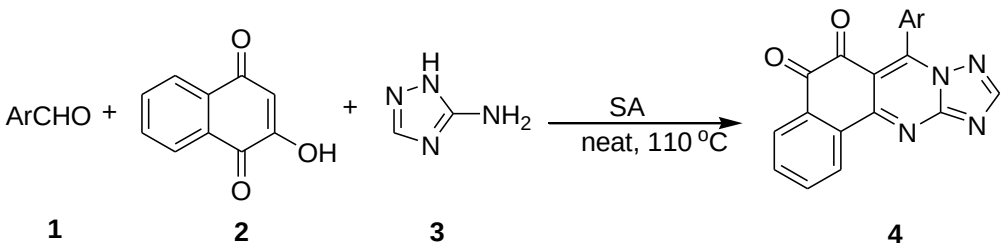


Figure 1. Structures of potent anticancer 1,2-naphthoquinone analogues.



Scheme 1. Synthesis of 6-aryl-benzo[*h*][1,2,4]- triazolo[5,1-*b*]quinazoline-7,8-diones using sulfamic acid as a catalyst.

Table 1. Catalyst optimization for the synthesis 7-phenyl-benzo[*h*][1,2,4]-triazolo[5,1-*b*]quinazoline-7,8-dione[a]

Entry	Catalyst	Mol%	Time/ h	Yield/ %[b]
1	-	-	12	12
2	SA	5	2	78
3	SA	10	1	90
4	SA	15	1	89
5	SA	20	1	90
6	$InCl_3$	10	6	18
7	$FeCl_3$	10	6	23
8	*p*-TsOH	10	3	68
9	silica sulfuric acid	10	3	67

[a] Reaction conditions: benzaldehyde (1 mmol), 2-hydroxy-1,4- naphthoquinone (1 mmol); 3-amino-1,2,4-triazole (1 mmol); 110 °C; neat.

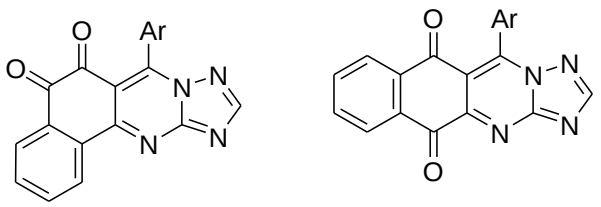
[b] Isolated yield.

Table 2. Preparation of 6-aryl-benzo[*h*][1,2,4]- triazolo[5,1-*b*] quinazoline-7,8-diones[a]

Entry	Ar	Time/ h	Product	Yield/ %[b]
1	C_6H_5	1	**4a**	90
2	2,4-Cl_2-C_6H_3	2	**4b**	86
3	4-Cl-C_6H_4	1.5	**4c**	89
4	4-F-C_6H_4	1.5	**4d**	83
5	3-NO_2-C_6H_4	1.5	**4e**	86
6	3,4,5-$(MeO)_3$-C_6H_2	1	**4f**	92
7	4-MeO-C_6H_4	1	**4g**	90
8	2-thiophenyl	1	**4h**	93
9	4-NO_2-C_6H_4	1	**4i**	87
10	4-Me-C_6H_4	1	**4j**	86

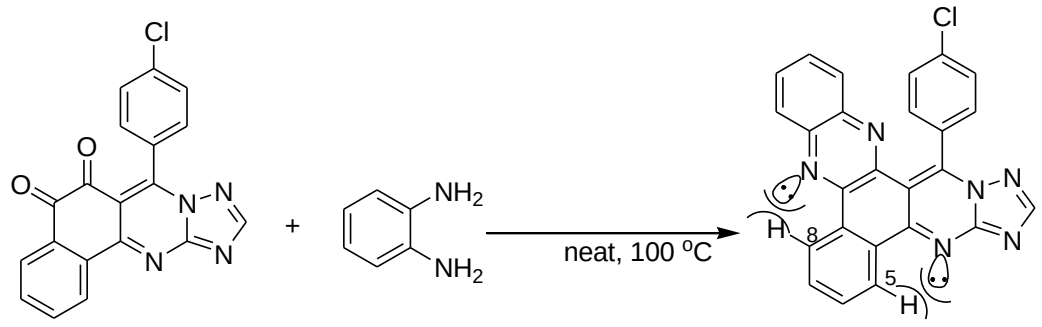
[a] Reaction conditions: aldehyde (1 mmol), 2-hydroxy-1,4-naphthoquinone (1 mmol); 3-amino- 1,2,4-triazole (1 mmol); SA (0.1 mmol); 110 $^{\circ}$C; neat.

[b] Isolated yield.



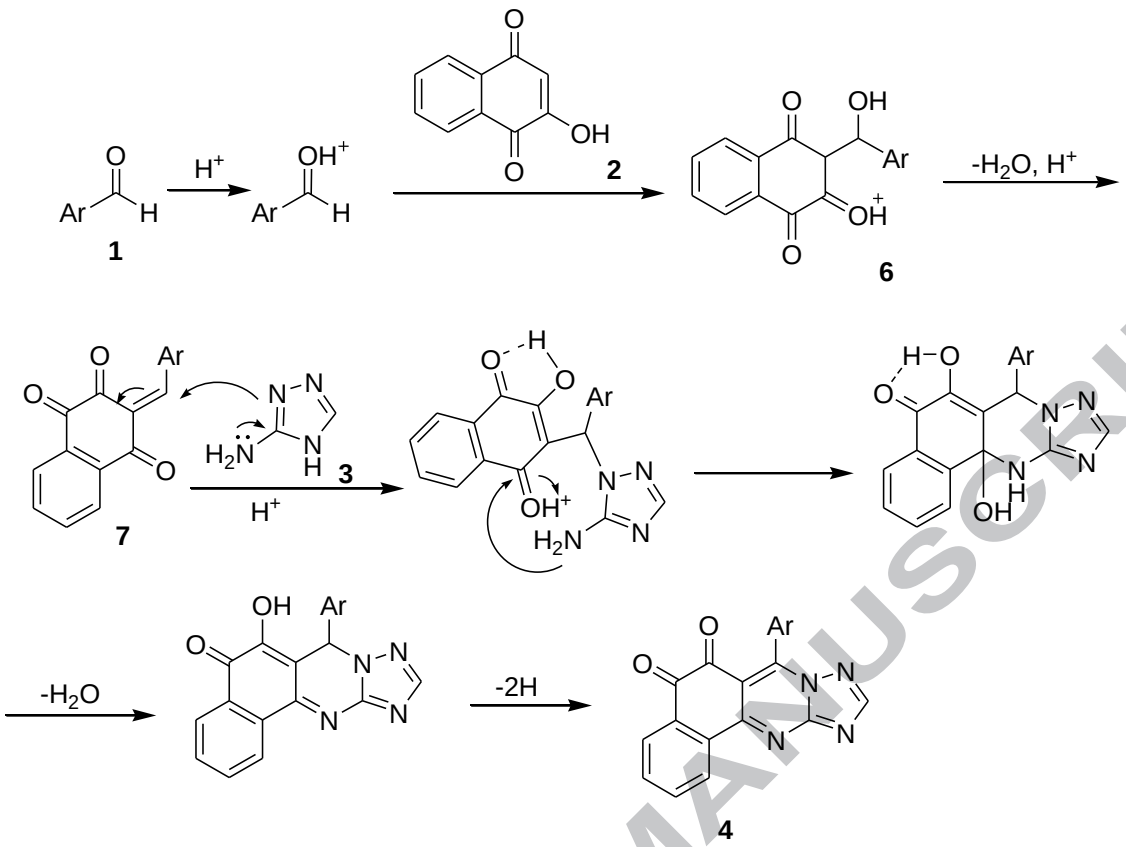
4 **5**

Figure 2. Structures of isomeric systems **4** and **5.**



4c **6c**

Scheme 2. Proof of the *ortho*-quinone structure of **4c**, based on its reaction with *o*-phenylenediamine.



Scheme 3. A plausible mechanistic pathway to explain the SA-catalyzed formation of compounds **4.**

Table 3. Cytotoxic activities of 6-aryl-benzo[*h*][1,2,4]- triazolo[5,1-*b*]quinazoline-7,8-diones.

Compd	IC_{50} (μ*M*)	
	SGC7901	HepG2
4a	2.16 ± 0.04	3.45 ± 0.73
4b	1.83 ± 0.12	2.99 ± 0.10
4c	34.92 ± 2.06	24.93 ± 5.23
4d	6.12 ± 0.13	7.85 ± 0.46
4e	9.88 ± 1.33	15.31 ± 1.41
4f	59.19 ±7.73	17.86 ± 4.33
4g	1.31 ± 0.13	1.71 ± 0.16
4h	1.45 ± 0.24	2.13 ± 0.32
4i	2.65 ± 0.2	4.74 ± 0.72
4j	1.71 ± 0.06	1.19 ± 0.19
6c	> 200	> 200
Doxorubicin	5.72 ± 0.19	1.02 + 0.16