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Synthesis and anti-cancer activity of naphthopyrone derivatives

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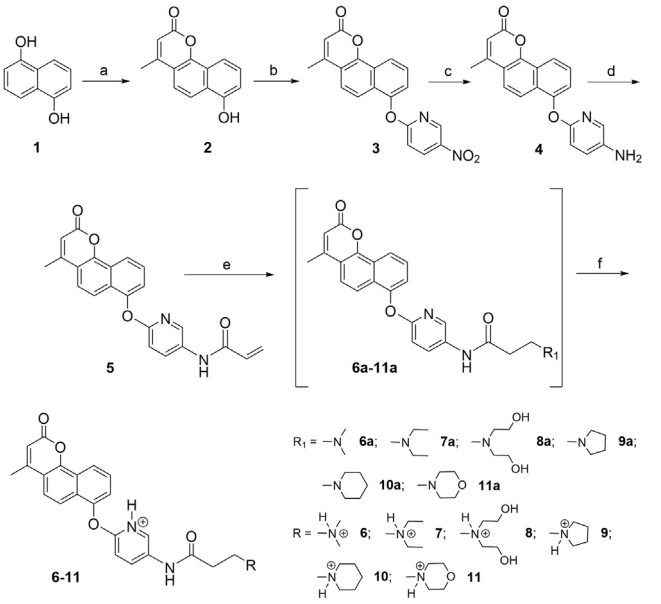
ABSTRACT

Coumarins are naturally evolving molecules that have a wide variety of activities. In medical chemistry and chemical biology, their structural and physicochemical properties make them a suitable scaffold. Herein, we designed and synthesized novel coumarin analogues-7-O-substituted pyridyl-4-methyl naphthopyrone derivatives and initially evaluated their anti-cancer activity. RTCA profiling reveals that compounds **6**, **7** and **8**, in particular **7**, possess stronger in vitro cytotoxicity for A549 cells. All synthesized compounds have been characterized by 1H NMR spectra, elemental analysis, and mass spectrometry.

Introduction

Coumarins are well known as compounds containing a benzo-α-pyrone mother structure, which are considered secondary metabolites in many higher plant species, mostly in leaves, seeds, and roots, especially in the Rutaceae and Umbrelliferae plants [1,2]. The first naturally occurring coumarin was isolated in 1820 [3]. These phytochemical derivatives, some interesting oxygen-containing heterocyclic compounds, are essential fluorescent fluorophores [4] and excellent DNA intercalators [5]. The biological activity of such naturally occurring compounds has become an appealing point of the study. It seems to be a potential resource for developing anti-cancer agents due to their different effects on diseases and less damage to normal cells [6]. For instance, a coumarin-xanthoxyletin can induce S phase arrest and apoptosis in human gastric adenocarcinoma SGC-7901 cells. Rasul et al. observed its inhibitory effects on cells associated with DNA damage [7]. The ability to induce selective DNA damage at the telom-



Scheme 1. Synthesis of the naphthopyrone derivatives **6**–**11**. Reagents and conditions: (a) ethyl acetoacetate, 80% (v/v) H_2SO_4, room temperature (r.t.), 24 h; (b) 2-chloro-5-nitropyridine, K_2CO_3, acetone, reflux, 7 h; (c) zinc powder, CH_3OH, r.t., 1 h; (d) 3-bromopropionyl chloride, Et3N, CH_2Cl_2, ice bath, 0.5 h; (e) ammonia derivatives, K2CO3, KI, MeCN, 12 h; (f) HCl gas, CH_2Cl_2, r.t., 0.5 h.

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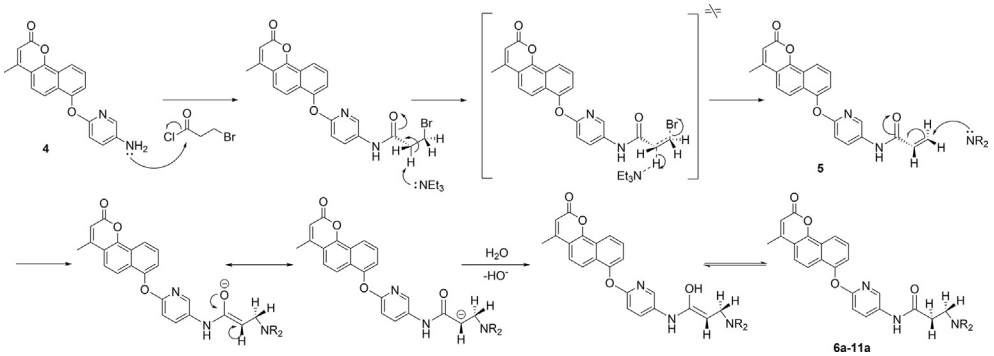

Scheme 2. The proposed mechanism for the formation of naphthopyrone derivatives **6a**-**11a** starting from the compound **4**.

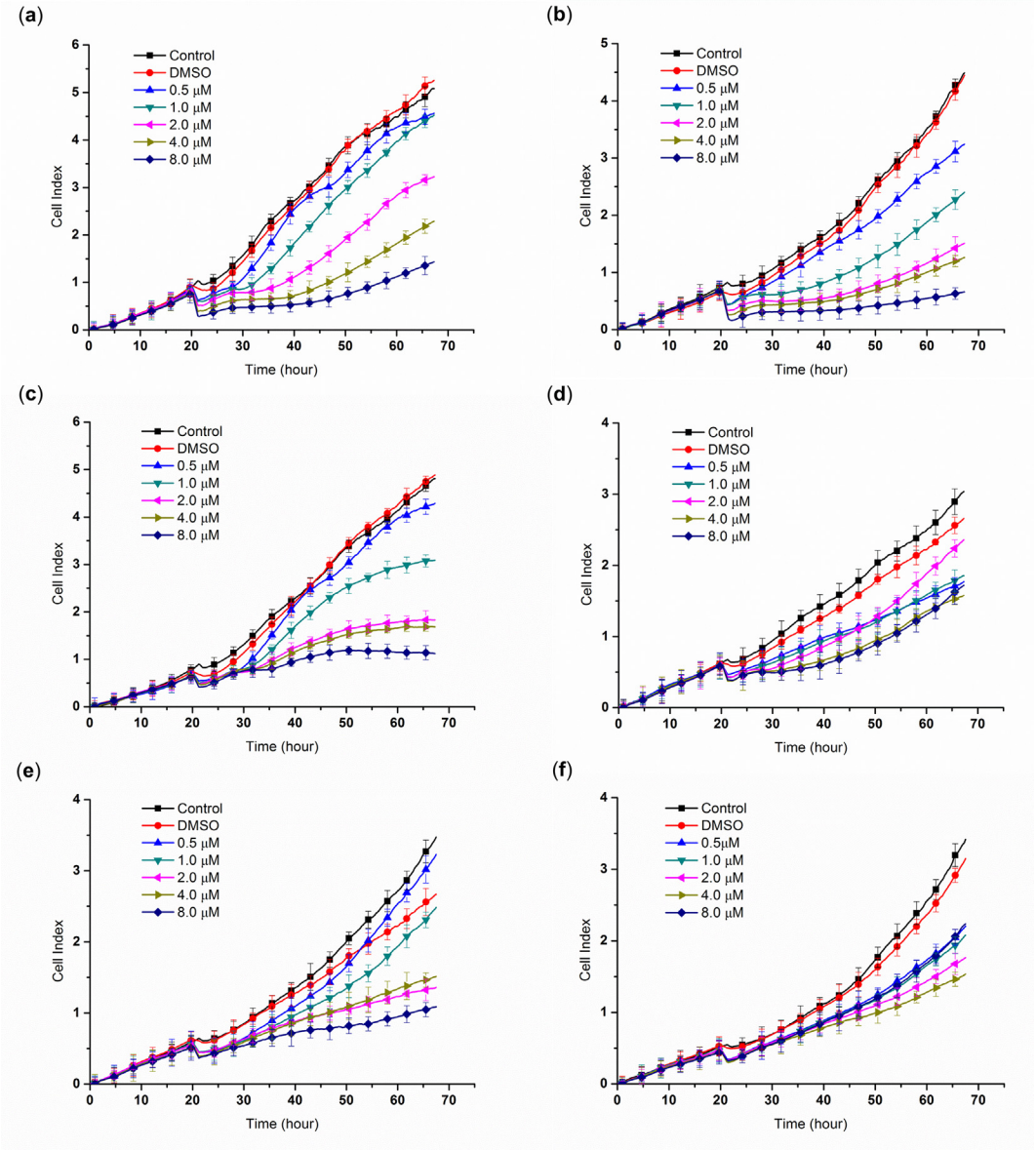

Fig. 1. The cell index curve of A549 cells exposed to different concentrations of compounds **6** (a)-**11** (f). "Control" and "DMSO" denotes RPMI1640 as a negative control and 0.5% DMSO solution in RPMI 1640 as a control, respectively. Each trace is typical of 3 replicates.

eric level and promote apoptosis and senescence of tumor cells is experimentally proven [8]. Coumarins exhibit anti-cancer biological properties, highlighting great therapeutic importance in medicinal chemistry because they display some fascinating pharmacological properties. To date, lots of studies have indicated that coumarins possess a variety of

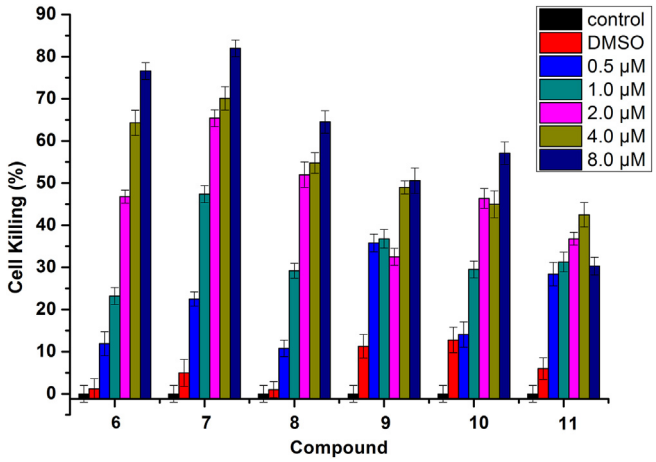

Fig. 2. Apoptosis of A549 cells by RTCA in the presence/absence of naphthopyrone derivatives **6**–**11**. Cell killing rates are calculated based on areas under concentration–time curve (AUC) (Fig. 1). "Control" and "DMSO" denotes RPMI1640 as a negative control and 0.5% DMSO solution in RPMI 1640 as a control, respectively. A549 cells were treated with compounds **6**–**11** with 0, 0.5, 1, 2, 4 and 8 μM for 48 h, respectively. Experiments were completed in 3 replicates.

pharmacological activities, such as: anti-inflammatory [9], antimicrobial [10], anti-viral [11], anti-oxidant [12], antinociceptive [13], anti-tumor [14], antiasthmatic, antidepressant [15], anti-HIV [16], antituberculosis [17], anti-Alzheimer [18], anti-influenza [19], antihyperlipidemic [20]. Thus, a large number of samples are required in the development of drug research and application.

However, obtaining many coumarins only from natural biological resources will result in severe resource scarcity and environmental problems. Therefore, getting them by synthesis is the preferred method to solve the sample source's question; what is more, the synthesis of active natural products and structure optimization can obtain more valuable medicines. In addition, nitrogen-containing heterocycles represent a key structural motif in heterocyclic chemistry and occupy a prominent position for research with ample opportunities to synthesize novel drugs. Hence, in this work, we introduce a substituted pyridine ring at the 7-hydroxy position of naphthopyrone (also named: benzocoumarin), which is coumarin analogues having a good embedding activity for DNA. Six naphthopyrone-pyridine conjugates **6**–**11** (Scheme 1) with positive nitrogen-containing side chains were designed and synthesized. These side chains can effectively improve hydrophilicity and the interaction of compounds with their targets, such as DNA, through the cationic functional side chain groups directing toward DNA grooves and then interacting with negatively charged phosphate groups [21]. The naphthopyrone derivatives were achieved through reactions: firstly, we used the Pechmann condensation reaction to synthesize the backbone **2** in high yield. Secondly, 7-hydroxy naphthopyranone was subjected to nucleophilic substitution reaction to obtain compound **3**. Thirdly, compound **4** was synthesized through **3** being reduced with zinc dust in methanol, and subsequent acylation process gave rise to acrylamide derivatives **5**. Lastly, acrylamide derivatives were reacted with dimethylamine, diaethylamin, diethanolamine, pyrolidine, piperidine, and morpholine, respectively, to obtain corresponding naphthopyrone precursors **6a**-**11a**. Then **6a**-**11a** ere followed by reacting with hydrochloride gas in dichloromethane solution to afford the target compounds **6**–**11**. The kind of naphthopyranone derivatives comprises three parts: naphthopyranone, pyridine ring, and positively charged nitrogen-containing side chain.

Results and discussion

Synthesis

Naphthopyrone derivatives have recently been synthesized in our laboratory and start from 1,5-dihydroxynaphthalene and ethyl acetoacetate through a microwave-assisted Pechmann condensation to obtain **2** according to literature [22]. Then, after nucleophilic substitution reaction took place between 7-hydroxy naphthopyranone (**2**) and 2-chloro-5-nitropyridine to afford **3**, **3** was turned into amino compound **4** in the presence of activating zinc dust. The next step was bimolecular elimination between **4** and 3-bromopropionyl chloride to get an acrylamide derivative (**5**) in the presence of an organic base. Subsequently, 1,4-conjugate additions of secondary amines to the electron-poor alkene (**5**) produced precursors **6a**-**11a**. The mechanism for the formation of naphthopyrone coumarins **5**, **6a**-**11a** (Scheme 2) was summarized. Finally, the target compounds (**6**–**11**) were conveniently prepared by salifying with dry hydrogen chloride gas in dichloromethane. These compounds were characterized (Electronic Supplementary Information (**ESI**), **S1.1**–**1.7**, Figs. S1-S12).

At the beginning of the experiment, we attempted another synthetic route to get target compounds **6**–**11** under examined different conditions (**Scheme S1**, **Table S1**), but failed, because nucleophilic substitution reaction of **2** to electron-poor compounds **15**–**20** did not occur under our experimental conditions. This is

Table 1
The killing effect and IC_{50} value (μM) of compounds **6**–**11** on A549 cells. The values are expressed as mean ± SD (triplicates).

CompoundConcentration (μM)	**6**AUC-48[a](Cell killing[b])	**7**AUC-48(Cell killing)	**8**AUC-48(Cell killing)	**9**AUC-48(Cell killing)	**10**AUC-48(Cell killing)	**11**AUC-48(Cell killing)
DMSO (Control)	139.9 ± 2.6	96.0 ± 1.7	123.6 ± 2.3	69.4 ± 1.6	70.1 ± 1.3	67.2 ± 1.4
0.5	124.7 ± 2.3 (10.9 ± 0.5)	78.3 ± 1.4 (18.4 ± 0.6)	111.4 ± 2.1 (9.87 ± 0.10)	50.2 ± 1.2 (27.7 ± 0.4)	69.1 ± 1.1 (1.43 ± 0.03)	51.2 ± 1.0 (23.8 ± 0.4)
1.0	108.7 ± 2.1 (22.3 ± 0.7)	53.1 ± 1.2 (44.7 ± 0.4)	88.4 ± 1.5 (28.5 ± 0.3)	49.4 ± 1.1 (28.8 ± 0.7)	56.7 ± 1.3 (19.1 ± 0.4)	49.1 ± 0.9 (26.9 ± 0.3)
2.0	75.4 ± 1.1 (46.1 ± 0.6)	34.9 ± 0.5 (63.4 ± 1.7)	60.0 ± 1.1 (51.5 ± 0.4)	52.8 ± 1.0 (23.9 ± 0.4)	43.1 ± 0.7 (38.5 ± 0.5)	45.2 ± 0.7 (32.7 ± 0.4)
4.0	50.5 ± 1.0 (63.9 ± 1.4)	30.2 ± 0.4 (68.5 ± 1.5)	56.4 ± 0.6 (54.4 ± 0.3)	39.9 ± 0.6 (42.5 ± 0.5)	44.2 ± 0.9 (36.9 ± 0.6)	41.1 ± 0.6 (38.8 ± 0.5)
8.0	33.2 ± 0.5 (76.3 ± 1.3)	18.2 ± 0.2 (81.0 ± 1.6)	44.3 ± 0.4 (64.2 ± 1.2)	38.6 ± 0.9 (44.3 ± 0.8)	34.5 ± 0.8 (50.8 ± 1.2)	49.8 ± 0.8 (25.9 ± 0.4)
IC_{50}[c]	2.57 ± 0.12	1.49 ± 0.27	3.06 ± 0.45	18.2 ± 1.0	7.79 ± 0.62	65.0 ± 2.6

[a] AUC-48 (compound): area under concentration–time curve 48 h after adding of compounds (Fig. 1).
[b] Cell killing: 100% × [AUC-48 (Control) – AUC-48 (compound)]/AUC-48 (Control). The Cell killing rate of cells was 0 for the negative control group. AUC-48 (Control): area under concentration–time curve 48 h after addition of 0.5% DMSO solution in RPMI 1640 [23].
[c] IC_{50}: Drug concentration producing 50% cancer cell death.

possibly because 2-position of the pyridine cycle in **15**–**20** possesses less electron deficiency than the pyridine cycle in 2-chloro-5-nitropyridine.

Cytotoxicity

To evaluate the cytotoxicity of naphthopyrone derivatives **6**–**11** to a type of human lung adenocarcinoma cell line (A549, supplied by China General Microbiological Culture Collection Center (CGMCC, Beijing, China)); a real-time cell analysis (RTCA) technique was used to monitor changes of cells dynamically, including cell number, kinetic behavior of cell apoptosis, and so on, exposed to 0, 0.5, 1.0, 2.0, 4.0, and 8.0 μM of examined compounds. A chemically induced decrease in a cell index (CI) value, which corresponds to cell number, shape, and substrate attachment, indicates that fewer cells interact with the microelectrodes due to diminished viability or disruption of the cellular footprint. At the same time, an increased CI can reflect higher cell numbers, increased cell adhesion, or changes in cell morphology (e.g., increased cell/electrode contact area due to cell spreading). Based on experimental curves (CI vs. time), IC50 of drugs inhibiting cell lines can be calculated by RTCA system software. Therefore, this methodology can provide useful analyses for various viable cell activities, drug-induced cellular apoptosis, and kinetics of cytotoxicity responses for drugs. As shown in Fig. 1, cell culture medium alone as a negative control and 0.5% DMSO only as a reference almost did not affect the viability of tested cancer cells.

We found that cancer cells' viability lowered and the time required to induce the cell apoptosis decreased with increasing compounds. Still, it was noteworthy that only little changes with **9** and **11**, especially **11**, took place with increasing their concentration despite the great similarity in structure to other used compounds **6**–**8** and **10**. In any case, however, the apoptosis of cancer cells was dose-dependent. As seen in Fig. 1, exposure to **6**, **7**, and **8** produced quickly decreases in the CI of A549 cells, of which **7** decreased most quickly the CI values at selected concentrations with a minimum IC50 value of 1.49 (±0.27) μM (Fig. 1, Fig. 2, Table 1), suggesting that **7** possesses strongest anti-cancer activity. In addition, it was observed that compounds **6**–**8** with linear alkyl groups, such as methyl, ethyl, hydroxyethyl groups, in three carbon atoms-containing linker tertiary ammonium side chains, displayed strong anti-proliferative ability compared with **9**–**11** with cycloalkyl groups, such as pyrrolidinyl, piperidyl, morpholino groups. This possibly results from the fact that the space which their target provides is more suitable to smaller linear groups, not cycloalkyl groups. Taken together, RTCA profiling reveals that compounds **6**, **7** and **8** possess stronger in vitro cytotoxicity for A549 cells, in particular, **7** with the highest cell killing rate of 81.0 (±1.6) % and the smallest IC50 value of 1.49 (±0.27) μM at 8 μM for A549 cells (Table 1).

Conclusion

We have designed and synthesized six novel 7-O-substituted pyridyl-4-methyl naphthopyrone derivatives **6**–**11**, then examined their cytotoxicity and kinetics of cytotoxicity to A549 cells by RTCA technique. Results reveal that the compound **7** exhibited the strongest ability to promote the apoptosis of A549 cells, suggesting that a bulky substituent in the side chain, particularly ring one, is not beneficial to the anti-cancer activity of these coumarin analogues. Further evaluation of druggability is underway.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tetlet.2021.153111.

References

[1] A. Estévez-Braun, A.G. González, Nat. Prod. Rep. 14 (1997) 465–475.
[2] R. Murray, Fortschritte der Chemie organischer Naturstoffe/Progress in the Chemistry of Organic Natural Products, Springer, 1997, pp. 1–119.
[3] V. Malikov, A. Saidkhodzhaev, K.N. Aripov, Chem. Nat. Compd. 34 (1998) 202–264.
[4] B.-X. Zhang, C.-P. Ge, J. Yao, Y.-P. Liu, H.-C. Xie, J._G. Fang, J. Am. Chem. Soc. 137 (2015) 757–769.
[5] A. Rajendran, M. Endo, Y. Katsuda, K. Hidaka, H. Sugiyama, J. Am. Chem. Soc. 133 (2011) 14488–14491.
[6] H.M. Bilgin, M. Atmaca, B. Obay, S. Özekinci, E. Taşdemir, A. Ketani, Exp. Toxicol. Pathol. 63 (2011) 325–330.
[7] A. Rasul, M. Khan, B. Yu, T.-H. Ma, H. Yang, Asian. Pac. J. Cancer. Prev. 12 (2011) 1219–1223.
[8] S. Cosconati, A. Rizzo, R. Trotta, B. Pagano, S. Iachettini, S. De Tito, I. Lauri, I. Fotticchia, M. Giustiniano, L. Marinelli, C. Giancola, E. Novellino, A. Biroccio, A.J. Randazzo, Med. Chem. 55 (2012) 9785–9792.
[9] S.-J. Lee, U.S. Lee, W.J. Kim, S.K. Moon, Mol. Med. Rep. 4 (2011) 337–341.
[10] D.A. Ostrov, J.A.H. Prada, P.E. Corsino, K.A. Finton, N. Le, T.C. Rowe, Antimicrob. Agents. Chem. 51 (2007) 3688–3698.
[11] J.R. Hwu, M. Kapoor, S.C. Tsay, C.C. Lin, K.C. Hwang, J.C. Horng, I.-C. Chen, F.K. Shieh, P. Leyssen, J. Neyts, Antiviral Res. 118 (2015) 103–109.
[12] I. Kostova, S. Bhatia, P. Grigorov, S. Balkansky, V.S. Parmar, A.K. Prasad, L. Saso, Curr. Med. Chem. 18 (2011) 3929–3951.
[13] P. Anand, B. Singh, N. Singh, Bioorg. Med. Chem. 20 (2012) 1175–1180.
[14] X.-Y. Huang, Z.-J. Shan, H.-L. Zhai, L. Su, X.-Y. Zhang, Chem. Biol. Drug. Des. 78 (2011) 651–658.
[15] J.F. Vasconcelos, M.M. Teixeira, J.M. Barbosa-Filho, M.F. Agra, X.P. Nunes, A.M. Giulietti, R. Ribeiro-dos-Santos, M.B. Soares, Euro. J. Pharma. 609 (2009) 126–131.
[16] Y. Kashman, K.R. Gustafson, R.W. Fuller, J.H. Cardellina, J.B. McMahon, M.J. Currens, R.W. Buckheit Jr, S.H. Hughes, G.M. Cragg, M.R. Boyd, J. Med. Chem. 36 (1993) 1110–1110.
[17] K. Upadhyay, A. Manvar, K. Rawal, S. Joshi, J. Trivedi, R. Chaniyara, A. Shah, Chem. Biol. Drug. Des. 80 (2012) 1003–1008.
[18] S.-S. Xie, X. Wang, N. Jiang, W. Yu, K.-D. Wang, J.-S. Lan, Z.-R. Li, L.-Y. Kong, Eur. J. Med. Chem. 95 (2015) 153–165.
[19] T. Lakshmi, R. Geetha, A. Roy, S. Aravind Kumar, Int. J. Pharm. Sci. Rev. Res. 9 (2011) 136–141.
[20] D. Iyer, U. Patil, Pharm. Biol. 52 (2013) 78–85.
[21] J.Y. Yeh, M.S. Coumar, J.-T. Horng, H.-Y. Shiao, F.-M. Kuo, H.-L. Lee, I.-C. Chen, C.-W. Chang, W.-F. Tang, S.-N. Tseng, C.-J. Chen, S.-R. Shih, J.T.-A. Hsu, C.-C. Liao, Y.-S. Chao, H.-P. Hsieh, J. Med. Chem. 53 (2010) 1519–1533.
[22] J.A. Key, S. Koh, Q.K. Timerghazin, A. Brown, C.W. Cairo, Dyes Pigm. 82 (2009) 196–203.
[23] J. O''. Connell, J. Exp. Med. 184 (1996) 1075–1082.