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Synthesis and *In Vitro* Cytotoxic Activity of Novel [1,3] Dioxolo[4,5-g]Chromen-8-ones as a Chalcone-Like Agent

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Abstract

In this investigation, new structures based on homoisoflavonoids were designed. Homoisoflavonoids are considered as an important class of flavonoids with various biological properties such as cytotoxicity. A new series of benzylidene-6,7-dihydro-8H-[1,3]dioxolo[4,5-g] chromen-8-one derivatives were developed and their cytotoxic activities evaluated for all compounds on three human breast cancer cell lines. Benzo[*d*][1,3]dioxol-5-ol was chosen as a substrate and reacted with 3-bromopropanoic acid to form an intermediate which turns to 6,7-dihydro-*8H*-[1,3]dioxolo[4,5-g]chromen-8-one after Feridel-Crafts reaction with oxalyl chloride. In the end, title compounds were produced by aldol condensation of later compound under acidic condition with aromatic aldehydes in moderate yields. Eight novel derivatives were tested for their activities against all human breast cancer cell lines including MCF-7, T47-D and MDA-MB-231 using MTT assay. After all tests on synthesized products, we can reach to this point that (*E*)-7-(3-bromo-4,5-dimethoxybenzylidene)-6,7-dihydro-*8H*-[1,3] dioxolo[4,5-g]chromen-8-one 4a presents the highest cytotoxicity in all three cell lines as a result of its shorter aliphatic tail in benzylidene moiety.

Keywords: *Cytotoxicity, Chalcone, Homoisoflavonoids, Chromene.*

Introduction

Drug development has been emerged based on the most severe disease which takes the abundance victims. For recent decades, cancer has been considered as the important cause of the substantial fatalities. Regarding the wide variety of treatments for cancer, chemotherapy still plays an important and efficient role in

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handling cancer [1]. Chemotherapy agents are chemical compounds containing selective inhibitors for the proliferation of abnormal cells. The ideal chemotherapy agents eliminate abnormal cells with no or least effects on normal cells [2]. Anticancer agents often deal with target cells with rapid metabolism and proliferation. Chalcones and Chalcone-like (chalconoid) compounds, known as two aromatic rings linked by enone fragment, among the current well-known cytotoxic agents represent an important class of small useful molecules in cancer treatment. The vital part of chalcone prototypes is the double bond of the enone system [3]. Reduced levels of mutagenicity risk as the common side effect of chemotherapy in chalcones have been attributed to their less interact with DNA [4]. Structural modification of chalcones predominantly focused on the replacement of phenyl rings with heterocyclic and polyaromatic groups [5-9].

Homoisoflavonoids, as naturally occurring compounds from flavonoid family with wide spectrum of biological properties like antioxidant [10], antiproliferative [11] and other important effects, have been isolated from plants like *Ophiopogon* [12], *Polygonatum* [13], *Scilla* [14], *Eucomis* [15], and *Muscari* [16]. Several homoisoflavonoids are being successfully isolated from plants and evaluated for their bioactivities [17]. Most of these compounds contain chromanone, chromone, or chromane skeleton.

2-benzylidene-1-tetraone derivatives have been shown cytotoxic activities against human Molt 4/CB leukemia, CEM lymphoma and murine L1210 [18, 19]. Perjesi et al. reported the synthesis and evaluation of cytotoxic activity of 3-benzylidine chroman-4-ones by replacing oxygen instead of 4-methylene group at 2-benzylidine-1-tetralones (Figure 1) [20-22]. In the present work, we would like to report a new series of benzylidene-6,7-dihydro-*8H*-[1,3]dioxolo[4,5-g]chromen-8-one derivatives **4a-h** as a hybrid of both chalcones and homoisoflavonoids and investigate their cytotoxic activities against three human breast lines: MC-F-7, MDA-MB-231, and T-47D using the MTT assay.

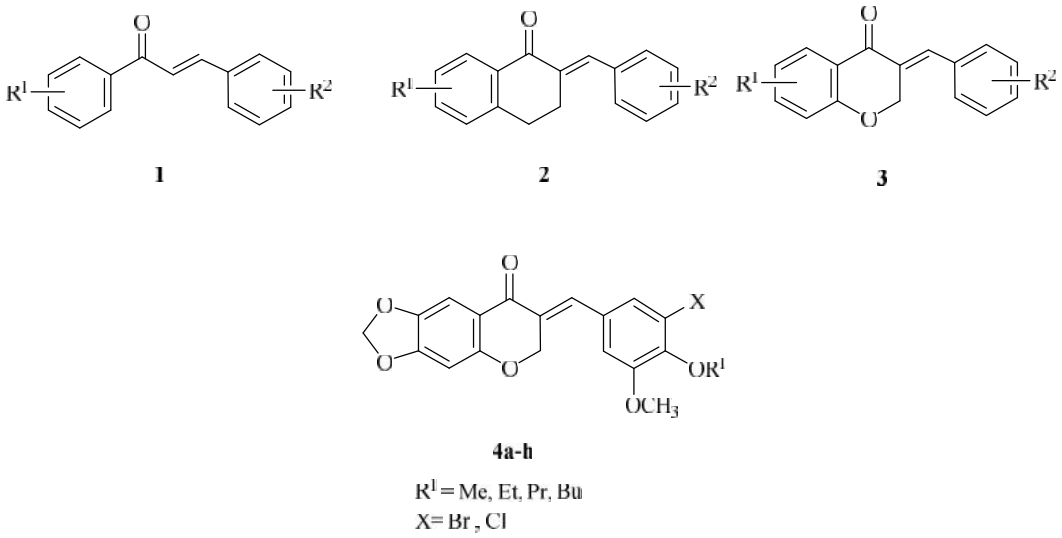
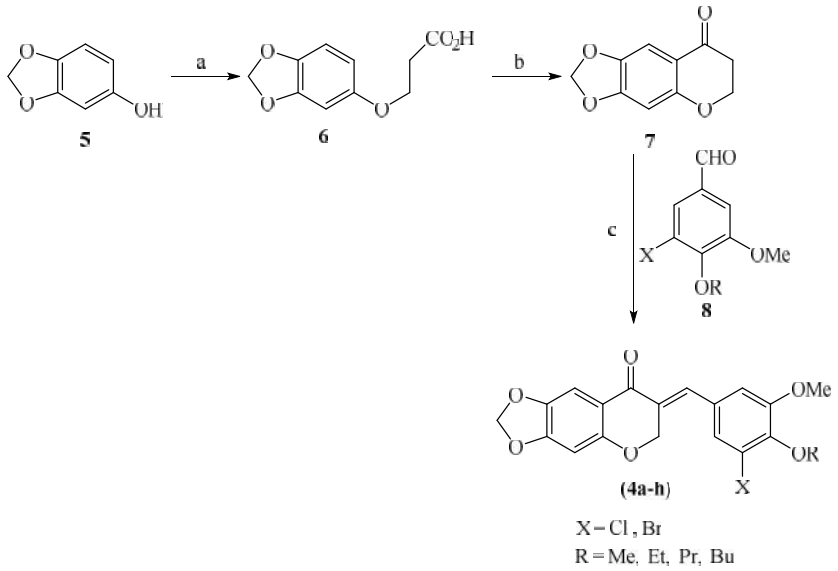


Figure 1. The structures of some chalcone–based agents, compound **4** were designed in this work.

In order to prepare compound 7, benzo[d][1,3] dioxol-5-ol reacted with 3-bromopropanoic acid in the presence of NaOH and Na_2CO_3 under reflux condition. The Friedel-crafts reaction of 3-(benzo[d][1,3]dioxol-5-yloxy)propanoic acid 6 with oxalyl chloride and $SnCl_2$ in benzene gave 6,7-dihydro-*8H*-[1,3]dioxolo[4,5-g] chromen-8-one 7. Then, condensation with aldehyde derivatives gave **4a-h** within 48h as presented in Scheme 1.



Scheme 1. Synthesis of benzylidene-6,7-dihydro-8*H*-[1,3]dioxolo[4,5-*g*]chromen-8-one **(4a-h)**. (a) NaOH, Na_2CO_3, $Br(CH_2)_2COOH$, H_2O, reflux; (b) oxalyl chloride, $SnCl_4$, benzene; (c) HCl (g), EtOH, 48h.

Experimental

General

All chemical reagents were bought from Merck AG (Darmstadt, Germany). The intermediates [1,3]dioxolo[4,5-g]chromen-8-one and poly substituted benzaldehydes were prepared based on previous report [23, 24]. Shimadzu 470 Spectrophotometer was used to acquire IR spectra with KBr (potassium bromide) disk. Bruker 500 MHz Spectrometer (Bruker Bioscience, Billerica, MA, USA) recorded NMR spectra and chemical shift has shown with δ (ppm) using tetramethylsilane (TMS) as internal standard.

General procedure for the synthesis of [1,3] dioxolo-[4,5-g]chromen-8-one derivatives

A solution of 6,7-dihydro-*8H*-[1,3] dioxolo[4,5-g]chromen-8-one (0.5 mmol) and benzaldehyde derivatives (0.7 mmol) in absolute ethanol (3 mL) was reacted by passing dry hydrogen chloride for 2 min in ice bath. After 48h stand condition at room temperature, the precipitated product was subjected to simple filtration, washed with cold ethanol, dried at air and recrystallized from boiling mixture of ethanol and water (80:20)[25].

(E)-7-(3-bromo-4,5-dimethoxybenzylidene)-6,7-dihydro-8H-[1,3]dioxolo[4,5-g]chromen-8-one (4a)

Yield: 48%, oily substance. IR (KBr): 1669 (C=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.70 (s, 1H, benzylidene), 7.39 (s, 1H, H_9 Chroman), 7.04 (s, 1H, Ar), 6.79 (s, 1H, Ar), 6.46 (s, 1H, H_4 Chroman), 6.00 (s, 2H, CH_2), 5.37 (d, 2H, H_2-Chroman, J = 1.4 Hz), 3.86 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3).

(E)-7-(3-bromo-4-ethoxy-5-methoxy-benzylidene)-6,7-dihydro-8H-[1,3]dioxolo [4,5-g] chromen-8-one (4b)

Yield: 30%. IR (KBr): 1612 (C=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.79 (s, 1H, benzylidene), 7.38 (s, 1H, H9 Chroman), 7.04 (s, 1H, Ar), 6.93 (s, 1H, Ar), 6.41 (s, 1H, H_4 Chroman), 6.00 (s, 2H, CH_2), 5.37 (d, 2H, H_2-Chroman, J = 1.6 Hz), 4.13 (q, 2H, OCH_2), 3.84 (s, 3H, OCH_3), 1.43 (t, 3H, CH_3).

(E)-7-(3-bromo-5-methoxy-4-propoxy-benzylidene)-6,7-dihydro-8H-[1,3]dioxolo [4,5-g]chromen-8-one (4c)

Yield: 42%, IR (KBr): 1666 (C=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.77 (s, 1H, benzylidene), 7.26 (s, 1H, H_9 Chroman), 7.04 (s, 1H, Ar), 6.86 (s, 1H, Ar), 6.42 (s, 1H, H_4 Chroman), 6.00 (s, 2H, CH_2), 5.29 (d, 2H, H_2-Chroman, J = 1.8 Hz), 3.99 (q, 2H, OCH_2), 3.84 (s, 3H, OCH_3), 1.85 (m, 2H, CH_2), 1.08 (t, 3H, CH_3).

(E)-7-(3-bromo-4-butoxy-5-methoxy-benzylidene)-6,7-dihydro-8H-[1,3]dioxolo [4,5-g]chromen-8-one (4d)

Yield: 42%, IR (KBr): 1667 (C=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.81 (s, 1H,

benzylidene), 7.28 (s, 1H, H9 Chroman), 7.04 (s, 1H, Ar), 6.79 (s, 1H, Ar), 6.43 (s, 1H, H_4 Chroman), 6.00 (s, 2H, CH_2), 5.29 (d, 2H, H_2-Chroman, J = 1.8 Hz), 4.07-4.03 (q, 2H, OCH_2), 3.91 (s, 3H, OCH_3), 1.76-1.83 (m, 2H, CH_2), 1.50-1.59 (m, 2H, CH_2), 0.09-1.01 (t, 3H, CH_3).

(E)-7-(3-chloro-4,5-dimethoxybenzylidene)-6,7-dihydro-8H-[1,3]dioxolo[4,5-g]chromen-8-one (4e)

Yield: 31%, Yield: 48%, IR (KBr): 1662 (C=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.70 (s, 1H, benzylidene), 7.37 (s, 1H, H_9 Chroman), 7.04 (s, 1H, Ar), 6.79 (s, 1H, Ar), 6.41 (s, 1H, H_4 Chroman), 6.00 (s, 2H, CH_2), 5.29 (d, 2H, H_2-Chroman, J = 1.8 Hz), 3.86 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3).

(E)-7-(3-chloro-4-ethoxy-5-methoxybenzylidene)-6,7-dihydro-8H-[1,3]dioxolo [4,5-g]chromen-8-one (4f)

Yield: 42%, IR (KBr): 1665 (C=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.72 (s, 1H, benzylidene), 7.43 (s, 1H, H_9 Chroman), 7.04 (s, 1H, Ar), 6.81 (s, 1H, Ar), 6.41 (s, 1H, H_4 Chroman), 6.00 (s, 2H, CH_2), 5.34 (d, 2H, H_2-Chroman, J = 1.6 Hz), 4.13 (q, 2H, OCH_2), 3.84 (s, 3H, OCH_3), 1.43 (t, 3H, CH_3).

(E)-7-(3-chloro-5-methoxy-4-propoxybenzylidene)-6,7-dihydro-8H-[1,3]dioxolo [4,5-g]chromen-8-one (4g)

Yield: 35%, IR (KBr): 1667 (C=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.79 (s, 1H, benzylidene), 7.40 (s, 1H, H_9 Chroman), 7.19 (s, 1H, Ar), 7.04 (s, 1H, Ar), 6.41 (s, 1H, H_4 Chroman), 6.00 (s, 2H, CH_2), 5.29 (d, 2H, H_2-Chroman, J = 1.8 Hz), 3.98 (q, 2H, OCH_2), 3.86 (s, 3H, OCH_3), 1.85 (m, 2H, CH_2), 1.08 (t, 3H, CH_3).

(E)-7-(4-butoxy-3-chloro-5-methoxybenzylidene)-6,7-dihydro-8H-[1,3]dioxolo [4,5-g]chromen-8-one (4h)

Yield: 38%, IR (KBr): 1665 (C=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.81 (s, 1H, benzylidene), 7.43 (s, 1H, H_9 Chroman), 7.06 (s, 1H, Ar), 6.8 (s, 1H, Ar), 6.40 (s, 1H, H_4 Chroman), 6.00 (s, 2H, CH_2), 5.29 (d, 2H, H_2-Chroman, J = 1.8 Hz), 3.99 (q, 2H, OCH_2), 3.84 (s, 3H, OCH_3), 1.76-1.85 (m, 2H, CH_2), 1.43-1.46 (m, 2H, CH_2), 0.99 (t, 3H, CH_3).

Cytotoxic assay

Each synthesized compound was tested against 3 human breast cancer cell lines to calculate their cytotoxic activity. MCF-7, T47D and MDA-MB231 were the three human breast cancer lines which were examined by MTT colorimeter assay according to the Masson method to evaluate cytotoxic activity. Each cell lines were obtained from National Cell Bank of Iran Pasteur Institute, Tehran, Iran [26]. Cancer cell lines were grown in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum (Gibco BRL), 100

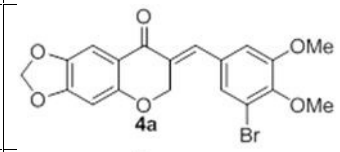
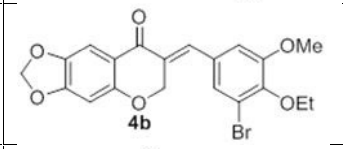
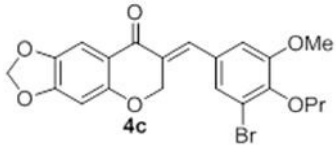
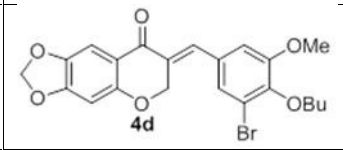
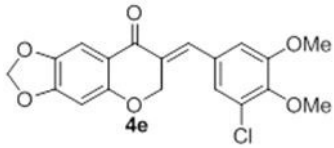
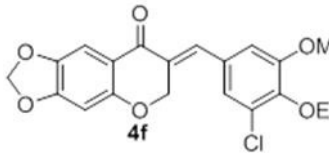
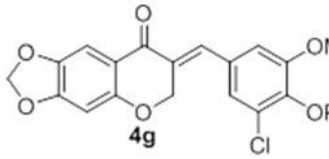
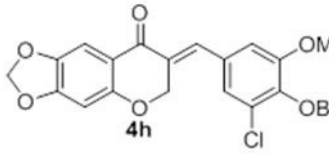
μg/ml streptomycin and 100 U/ml penicillin at 37 oC in a humidified atmosphere with 5% CO_2. 5μl of all compounds, containing various concentrations, was added per well in triplicate after overnight incubating. The incubation took 24 hours as a result the final concentration of DMSO in highest concentration on utilized compound was about 0.1%. In each plate three control wells (cells without test compound) and three blank wells (the medium with 0.1% DMSO) were placed for cells viability. To positive control of cytotoxicity, Etoposide was used. In the next step 200μl phenol red-free medium containing MTT (1 mg/ml) was added to wells, followed by 4 hours incubation. The culture medium was replaced with 100μl of DMSO in the next move. So in the following step the absorbance of each well was measured by using microplate reader at 570 nm. The result was reported with IC50 standard as a comparative source for biological activity.

Results and discussion

The final outcome for each compound has been shown in Table 1. It was confirmed that (E)-7-(3-bromo-4,5-dimethoxybenzylidene)-6,7-dihydro-8H-[1,3]dioxolo[4,5-g]chromen-8-one **4a** shows the highest efficiency against MCF-7, T-47D and MDA-MB-231 cell lines, with 14.5 ±2.3, 2.1 ±0.1 and 8.6 ±0.3 μM values respectively. Whereas, (E)-7-(4-butoxy-3-chloro-5-methoxybenzylidene)-6,7-dihydro-8H-[1,3]dioxolo[4,5-g]chromen-8-one **4h** and **4d** shows the least activity against these cancer cell lines. According to Table 1, the longer aliphatic chain linked to the benzylidene moiety in para position decreased cytotoxicity activity compared to other derivatives. It should be mentioned that **4a** with chlorine substituent in benzylidene moiety shows better cytotoxicity effects in comparison to the same counterpart **4e** containing bromine in meta position. Our derivatives influenced T-47D cell lines more drastically and MCF-7 showed the most resistance in the presence of synthesized compounds.

Since the highest and lowest activity against all cell lines was recorded for 4a and 4h respectively, it came out to this conclusion that O-alkylation of 4-hydroxy moiety diminished the activity especially in MCF-7 cell lines. As a result of longer alkoxy in benzylidene moiety, solubility in water decrease. Finally, the solubility equivalent between oily and water media undermines. So, those derivatives with longer alkoxy groups presented less activity against all cell lines.

Table 1. Cytotoxicity activity (IC_{50}, mM)[a] of compounds **4a-h** against cancer cell lines.

Entry	compound	MCF-7 cell line IC_{50} (μg/ml)	T-47D cell line IC_{50} (μg/ml)	MDA-MB-231 cell line IC_{50} (μg/ml)
1	 **4a**	14.5±2.3	2.1±0.1	8.6±0.3
2	 **4b**	21.6±7.3	12.6±2.3	12.3±2.9
3	 **4c**	>50	18.4±1.3	34.3±6.8
4	 **4d**	>50	>50	>50
5	 **4e**	17.8±1.7	2.2±0.1	12.5±2.1
6	 **4f**	16.1±2.9	8.3±0.5	11.2±0.07
7	 **4g**	>50	21.1±6.7	24.0±9.2
8	 **4h**	>50	>50	>50
9	Doxorubicin	0.002±0.002	0.03±0.002	0.006±0.004

Conclusion

To conclude, We investigated the cytotoxic effect of poly oxygenated 3-benzylidene-8-ones coupled with [1,3]dioxolo group as a new series of chalcon-like agents. Our investigation outcome demonstrates that the presence of [1,3]dioxolo in our derivatives has an unpleasant effect in comparison with

other chalcone analogs. However, chlorine-containing derivatives present an acceptable IC_{50} amounts. 4-methoxy **4a** with the lowest IC_{50} could be a good pattern for structural optimization of [1,3]dioxolo[4,5-g]chromen-8-one core structure.

References

[1] M. Harrison, K. Holen, G. Liu, *Clin. Adv. Hematol. Oncol*., 7, 54 (2009).

[2] S. Eckhardt, *Curr. Med. Chem*., 2, 419 (2002).

[3] P. Hinnen, F.A. Eskens, *Br. J. Cancer*, 96, 1159 (2007).

[4] J.R. Dimmock, D.W. Elias, M.A. Beazely, N.M. Kandepu, *Curr. Med. Chem.,* 6, 1125 (1999).

[5] H.-B. Shi, S.-J. Zhang, Q.-F. Ge, D.-W. Guo, C.-M. Cai, W.-X. Hi, *Bioorg. Med. Chem. Lett*., 20, 6555 (2010).

[6] L. Firoozpour, N. Edraki, M. Nakhjiri, S. Emami, M. Safavi, S.K. Ardestani, M. Khoshneviszadeh, A. Shafiee, A. Foroumadi, *Arch. Pharm. Res.,* 35, 2117 (2012).

[7] M.L. Go, X. Wu, X. Liu, *Curr. Med. Chem*., 12, 483 (2005).

[8] H. Aryapour, G.H. Riazi, A. Foroumadi, S. Ahmadian, A. Shafiee, O. Karima, M. Mahdavi, S. Emami, M. Sorkhi, S. Khodadady, *Med. Chem. Res*., 20, 503 (2011).

[9] Y. Shan, J. Zhang, Z. Liu, M. Wang, Y. Dong, *Curr. Med. Chem*., 18, 523 (2011).

[10] (a) V. Siddaiah, C.V. Rao, S. Venkateswarlu, A.V. Krishnaraju, G.V. Subbaraju, *Bioorg. Med. Chem.,* 14, 2545 (2006); (b) V. Siddaiah, M. Maheswara, C.V. Rao, S. Venkateswarlu, G.V. Subbaraju, *Bioorg. Med. Chem. Lett*., 17, 1288 (2007); (c) V.M. Rao, G.L.V. Damu, D. Sudhakar, V. Siddaiah, C.V. Rao, Arkivoc, 285 (2008).

[11] P. Perjési, U. Das, E. De Clercq, J. Balzarini, M. Kawase, H. Sakagami, J.P. Stables, T. Lorand, Z. Rozmer, J.R. Dimmock, *Eur. J. Med. Chem*., 43, 839 (2008).

[12] N. Li, J.Y. Zhang, K.W. Zeng, L. Zhang, Y.Y. Che, P.F. Tu, *Fitoterapia*, 83, 1042 (2012).

[13] H. Guo, H. Zhao, Y. Kanno, W. Li, Y. Mu, X. Kuang, Y. Inouye, K. Koike, H. Jiang, H. Bai, Bioorg. *Med. Chem. Lett*., 23, 3137 (2013).

[14] M. Bezabih, S.O. Famuyiwa, B.M. Abegaz, *Nat. Prod. Commun*., 4, 1367 (2009).

[15] C. Koorbanally, N.R. Crouch, D.A. Mulholland, *Phytochemistry: Advances in Research*, 69 (2006).

[16] M. Urbancíková, I. Masterová, J. Tóth, *Fitoterapia*, 73, 724 (2002).

[17] (a) J. Mutanyatta, B.G. Matapa, D.D. Shushu, B.M. Abegaz, *Phytochemistry*, 62, 797 (2003); (b) J. Qi, D. Xu, Y.-F. Zhou, M.-J. Qin, B.-Y. Yu, *Rapid Commun. Mass Spectrum*, 24, 2193 (2010).

[18] J.R. Dimmock, N.M. Kandepu, A.J. Zanarali, T.P. Kowalchuk, N. Motagabahalli, J.W. Quail, P.A. Mykytiuk, G.F Audette, L. Prasad, P. Perjési, T.M. Allen, C.L. Santos, J. Szydlowski, E. De Clercq , J. Balzarini, *J. Med.*

Chem., 42,1358 (1999).

[19] J.R. Dimmock, G.A. Zello, E.O. Oloo, J.W. Quail, H.-B. Kraatz, P. Perjési, F. Aradi, K. Takács-Novák, T.M. Allen, C.L. Santos, J. Balzarini, E. De Clercq, J.P. Stables, *J. Med. Chem*., 45, 3103 (2002).

[20] P. Perjési, U. Das, E. De Clercq, J. Balzarini, M. Kawase, H. Sakagami, J.P. Stables, T. Lorand, Z. Rozmer, J.R. Dimmock, *Eur. J. Med. Chem*., 43, 839 (2009).

[21] M. Bezabih, S.O. Famuyiwa, B.M. Abegaz, *Nat. Prod. Commun*., 4, 1367 (2009).

[22] M. Nakhjiri, M. Safavi, E. Alipour, S. Emami, A.F. Atash, M. Jafari-Zavareh, S.K. Ardestani, M. Khoshneviszadeh, A. Foroumadi, A. Shafiee, *Eur. J. Med. Chem*., 50, 113 (2012).

[23] A. Foroumadi, A. Samzadeh-Kermani, S. Emami, G.H. Dehghan, M. Sorkhi, F. Arabsorkhi, M.R. Heidari, M. Abdollahi, A. Shafiee, *Bioorg. Med. Chem. Lett*., 17, 6764 (2007).

[24] J.P. Cueva, G. Giorgioni, R.A. Grubbs, R.B. Chemel, V.J. Watts, D.E. Nichols, *J. Med. Chem.*, 49, 6848 (2006).

[25] S. Noushini, E. Alipour, S. Emami, M. Safavi, S.K. Ardestani, A.R. Gohari, A. Shafiee, A. Foroumadi, *Daru J. Pharm. Sci*., 21, 31 (2013).

[26] T. Mosmann, *J. Immunol. Methods*, 65, 55 (1983).