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An efficient and convenient microwave-assisted chemical synthesis of (thio)xanthones with additional in vitro and in silico characterization

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ABSTRACT

Xanthones and their thio-derivatives are a class of pleiotropic compounds with various reported pharmacological and biological activities. Although these activities are mainly determined in laboratory conditions, the class itself has a great potential to be utilized as promising chemical scaffold for the synthesis of new drug candidates. One of the main obstacles in utilization of these compounds was related to the difficulties in their chemical synthesis. Most of the known methods require two steps, and are limited to specific reagents not applicable to a large number of starting materials. In this paper a new and improved method for chemical synthesis of xanthones is presented. By applying a new procedure, we have successfully obtained these compounds with the desired regioselectivity in a shorter reaction time (50 s) and with better yield (>80%). Finally, the preliminary in vitro screenings on different bacterial species and cytotoxicity assessment, as well as in silico activity evaluation were performed. The obtained results confirm potential pharmacological use of this class of molecules.

1. Introduction

Many plants are known to have pharmacological properties and have been used in traditional medicine for the treatment of numerous ailments and diseases since ancient times. Plants have also been used for religious purposes and in mystical rituals.[1] Although these plants have exhibited healing effects in some populations and in many conditions, the clear pharmacological bases of these actions still remain to be fully elucidated. Since pharmaceutical companies are on a continual quest for new active compounds, their focus has recently shifted to the utilization of natural biodiversity of traditional medicinal plants' constituents by applying virtual and biological screening approaches to detect their therapeutic potentials.[2]

The main objective of this paper is to present the synthesis optimization of the naturally occurring compounds that is, xanthones. Synthetic variants can be optimized further to enhance their biological activity and improve their selectivity and safety. The obtained compounds can be considered as new promising hits for subsequent research and development of novel pharmacophores.[3] Xanthones are a group of heterocyclic compounds containing a dibenzo-γ-pyrone skeleton (Fig. 1).

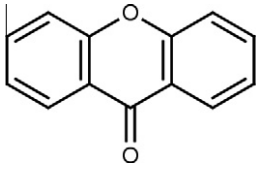

Figure 1. Xanthones as heterocyclic compounds with dibenzo-γ-pyrone scaffold.

These secondary metabolites are found in higher plant families such as *Guttiferae* and *Gentianaceae*[4,5] and they also occur in fungi, lichens and ferns.[6] The growing interest in the natural and synthetic xanthones is due to their pharmacological and biological functions:[7–10] antibacterial, anti-inflammatory and modulators of glucose metabolism,[11] anticancer[12] and antiviral.[9] Their antioxidant potential makes them feasible for utilization as nutritional

Abbreviation: MW, microwave.

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supplements in order to prevent premature ageing and ameliorate conditions in chronic inflammatory diseases.[9]

Thioxanthones, the synthetic analogues of xanthones not found in nature, have also been found to exhibit pharmacologic characteristics[13–22] such as antihistaminic, antiparasitic, neuroleptic, and antiproliferative properties.[22] Hydroxyl thioxanthones are particularly useful as heat and ultraviolet stabilizers for polyolefins.[19] Acetylation of hydroxyxanthones and thioxanthones can further broaden their biological activities.[23] Monohydroxyxanthones, for example, 1-hydroxyxanthone, are reported in the literature as monoamine oxidase inhibitors,[24,25] α-glucosidase inhibitors[26,27] and exhibit antioxidant properties.[28] Of the dihydroxyxanthones, 1,3-dihydroxyxanthone possesses antimalarial activity,[29] antihypertensive activity[30] and can inhibit α-glucosidase.[26,27] The same has been reported for 1,3-diacetoxyxanthone, while this compound also inhibits Na/K-ATPase.[31] Likewise, its analogue, 1,6-dihydroxyxanthone, possesses antihypertensive activity[30] and can inhibit α-glucosidase.[26,27] Trihydroxyxanthones, like 1,3,5-trihydroxyxanthone, has shown anticancer[32] and antimalarial activity.[29] The same compound tested in vitro exhibited antiviral activity on HIV-1-infected MT-4 cells and MDCK cells infected with influenza virus.[33] Trihydroxyxanthones have also been shown to be monoamine oxidase[24,25] and Na/K-ATPase inhibitors.[31] Finally, 1,3,7-trihydroxyxanthone also shows anticancer activity[32] and possesses monoamine oxidase,[24,25] α-glucosidase[26,27] and fatty acid synthase inhibitory activity.[34]

Synthetically, xanthones are usually obtained by chemical synthesis from benzophenones or diaryl ethers under harsh reaction conditions and/or in the presence of strong acids or toxic metals.[35]

Literature has offered a range of methods with varying yields.[36–41] Base catalyzed cyclisation of a substituted benzophenone precursor to obtain some polymethoxyxanthones has also been reported, but the yields were low.[37] The improvement in yield was achieved by heating the same precursor for 72 h in the presence of tetramethylammonium hydroxide/pyridine/H_2O.[42] This reaction was also carried out applying microwave (MW) irradiation, significantly shortening the time of reaction to 13 min.[43] Different methods for the synthesis of thioxanthones and substituted thioxanthones have also been described.[13,15–20]

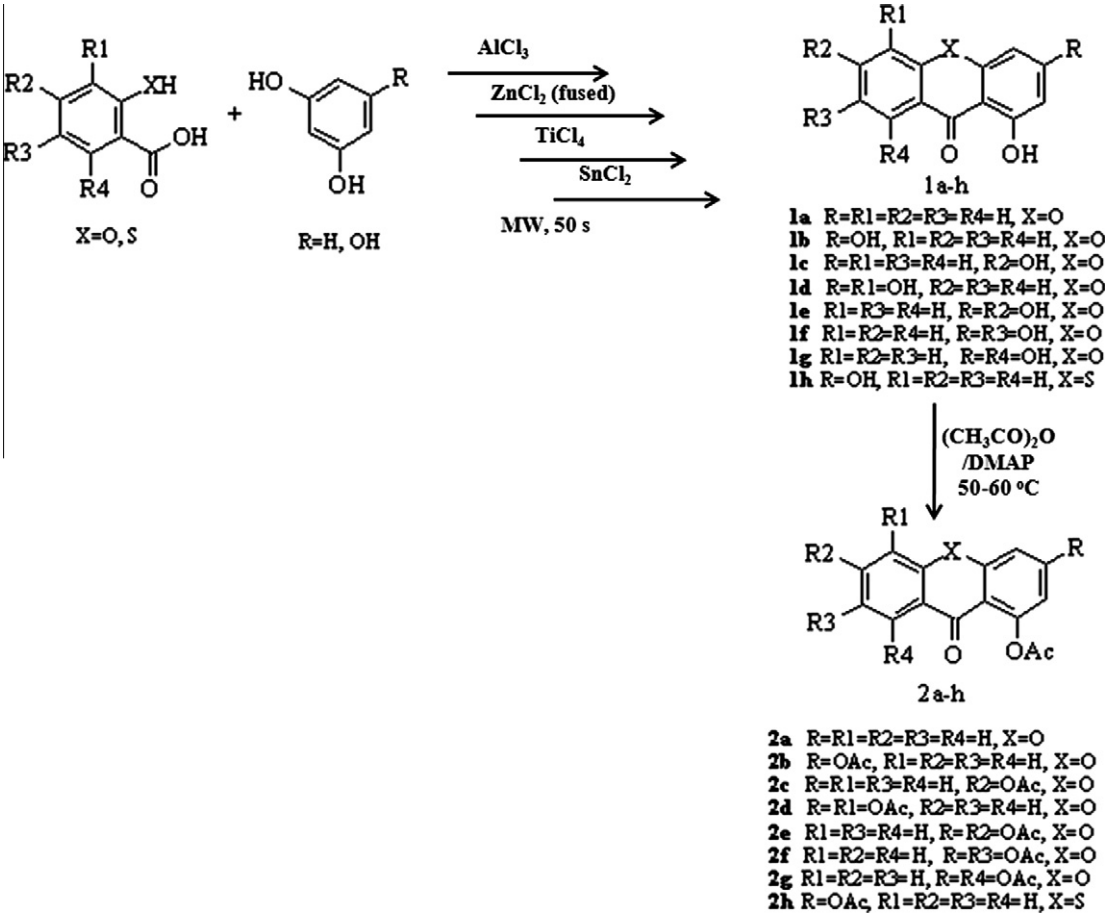
All methods mentioned above suffer from one of the following pitfalls: low yields, long reaction times, the use of large amounts of concentrated sulfuric acid, and lack of regiochemical control in the ring closure step. Moreover, most of these methods require two steps, are limited to specific benzoic acids or benzene derivatives having electron-withdrawing groups and are not applicable to a large number of starting materials.

Bearing all this in mind and taking into consideration the pharmacological relevance of xanthones, thioxanthones and their acetyl derivatives, we have developed and reported here a simple methodology to obtain hydroxyxanthones and hydroxythioxanthones in a short time using MW irradiation. Moreover, this paper also includes the in vitro screening on different bacterial species and cytotoxicity assessment as well as preliminary in silico evaluation of their potential biological targets.

2. Results and discussion

2.1. Synthesis

Herein we report the modified MW synthesis of hydroxy xanthones and thioxanthones (Scheme 1).



Scheme 1. Xanthone synthesis.

Table 1
Microwave assisted synthesis of xanthones/thioxanthones using different Lewis acids.

Compounds	Reagents	Yield (%)	Time (s)
1-Hydroxyxanthone (1a)	$AlCl_3$	64	50
	$ZnCl_2$	77	50
	$TiCl_4$	79	50
	$SnCl_2$	80	50
1,3-Dihydroxyxanthone (1b)	$AlCl_3$	68	50
	$ZnCl_2$	76	50
	$TiCl_4$	77	50
	SnCl	82	50
1,6-Dihydroxyxanthone (1c)	AlCl	63	50
	$ZnCl_2$	71	50
	$TiCl_4$	76	50
	$SnCl_2$	81	50
1,3,5-Trihydroxyxanthone (1d)	$AlCl_3$	65	50
	$ZnCl_2$	75	50
	$TiCl_4$	78	50
	$SnCl_2$	83	50
1,3,6-Trihydroxyxanthone (1e)	$AlCl_3$	66	50
	$ZnCl_2$	73	50
	$TiCl_4$	76	50
	$SnCl_2$	81	50
1,3,7-Trihydroxyxanthone (1f)	AlCl	65	50
	$ZnCl_2$	77	50
	$TiCl_4$	76	50
	$SnCl_2$	84	50
1,3,8-Trihydroxyxanthone (1g)	$AlCl_3$	64	50
	$ZnCl_2$	75	50
	$TiCl_4$	77	50
	nCl_2	80	50
1,3-Dihydroxythioxanthone (1h)	$AlCl_3$	70	50
	$ZnCl_2$	74	50
	$TiCl_4$	69	50
	$SnCl_2$	82	50

In the modified synthesis, a homogenous mixture of resorcinol/phloroglucinol, substituted salicylic acids and a Lewis acid $AlCl_3$/$ZnCl_2$/$TiCl_4$/$SnCl_2$, was subjected to MW irradiation using CEM Discover Model 908010 at a power of 200 W, with continuous stirring to obtain the required xanthone(s) with good yield (Table 1) without the formation of any side products, which was confirmed by TLC and HPTLC. The use of a Lewis acid under microwave irradiation reduces the reaction time from 13 min[43] to just 50 s. Of all the Lewis acids, SnCl2 was found to be the most efficient with xanthone yields of 80–84%. Compounds **1a**–**h** were then acetylated separately using acetic anhydride in DMAP under dry conditions to obtain acetyl derivatives **2a**–**h** in quantitative yield. This general method has also been successfully extended to the synthesis of thioxanthones, as illustrated here by the compounds 1 h and 2 h.

2.2. In vitro biological screening

The synthesized compounds have been assessed experimentally in terms of their antibacterial potency as well as cytotoxicity, the two major concerns associated with potential preventive or curative drugs that are intended for prolonged use.

The determined antibacterial and cytotoxic activities are presented in the Table 2. The majority of compounds showed no effects on the growth of the microorganisms tested. However, several compounds exhibited weak antibacterial activity on *M. catarrhalis*, *S. aureus* and *H.influenzae*. In order to improve antibacterial activity of these few compounds, the exact mode of action should be clarified and the compounds have to be further derivatised.

The cytotoxic activity was assessed on a HepG2 (hepatocytes) and Jurkat (T lymphocytes) cell lines that are used to determine potential inhibitory effects on cell metabolism. The test measures cellular metabolic activity by assessing NADH levels, thus indicating whether the compounds impair any of the key metabolic pathways. An encouraging result was obtained in that none of the compounds tested exhibited any significant inhibition on the HepG2 or Jurkat within 24 h, suggesting that the compounds could be used for further derivatisation in order to optimize their biological activities.

2.3. In silico analysis

All compounds were additionally subjected to a similarity search for potential biological activity in order to identify their additional 'hidden' values and to create conditions for further exploration of their pharmacological effects. Such an approach is currently widely accepted by the pharmaceutical industry.[44]

By combining computational and biological methods, positive and reliable hits could be detected in a very short time frame by

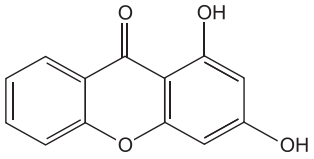
Table 2
The results of compound antibacterial screening are shown as follows and are expressed as minimum inhibitory concentrations (MICs) in μg/mL. The compound cytotoxicity results are expressed as IC_{50} values in μM.

Compound #	Antibacterial activity MIC (μg/mL)						Cytotoxicity IC_{50} (μM)	
	*S. aureus** *ATCC 29213*	*S. pneumoniae** *ATCC 49619*	*S. pyogenes** *ATCC 700294*	*M. catarrhalis*** *ATCC 23246*	*H. influenzae*** *ATCC 49247*	*E. coli*** *ATCC25922*	HepG2 ATCC HB-8065	Jurkat ATCC TIB-152
1a	>128	>128	>128	128	>128	>128	>100	>100
2a	>128	>128	>128	128	>128	>128	>100	78
1b	64	>128	>128	16	64	>128	>100	>100
2b	128	>128	>128	32	128	>128	>100	>100
1c	32	>128	>128	8	>128	>128	93	75
2c	64	>128	>128	16	128	>128	90	>100
1d	>128	>128	>128	16	>128	>128	>100	>100
2d	>128	>128	>128	>128	>128	>128	>100	92
1e	64	>128	>128	2	32	>128	>100	>100
2e	64	>128	>128	4	128	>128	>100	>100
1f	128	>128	>128	128	>128	>128	>100	>100
2f	>128	>128	>128	>128	>128	>128	>100	>100
1g	8	128	>128	8	16	>128	90	83
2g	128	>128	>128	128	>128	>128	93	62
1h	16	>128	>128	8	16	>128	>100	>100
2h	>128	>128	>128	64	>128	>128	>100	>100
Azithromycin (standard)	1	<0.125	<0.125	<0.125	1	2		

* Gram-positive bacterial species.
** Gram-negative bacterial species.

Table 3
PASS predicted human activity spectra for compound 1b (at Pa > 0.8)[a]. Similar activities have been provided for other synthetised xanthones.

Compd**1b**

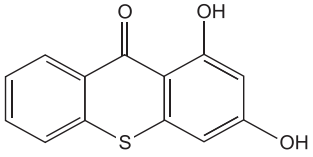


Pa	Pi	**Biological targets**	Pa	Pi	**Biological targets**
0.953	0.002	Chlordecone reductase inhibitor	0.834	0.002	Glycerol dehydrogenase (NADP+) inhibitor
0.921	0.001	Quercetin 2,3-dioxygenase inhibitor	0.834	0.009	CYP2B6 inhibitor
0.915	0.003	Alcohol dehydrogenase (NADP+) inhibitor	0.829	0.005	27-Hydroxycholesterol 7alpha-monooxygenase inhibitor
0.915	0.004	CYP1A substrate	0.827	0.009	CYP2B6 substrate
0.910	0.004	CYP1A1 substrate	0.824	0.003	UGT1A7 substrate
0.909	0.004	CYP1 substrate	0.818	0.002	Cystathionine beta-synthase inhibitor
0.899	0.003	NOS-2 expression inhibitor	0.816	0.003	Carbonyl reductase (NADPH) inhibitor
0.895	0.003	Kinase inhibitor	0.802	0.003	Beta-carotene 15,15′-monooxygenase inhibitor
0.888	0.003	CYP1A inhibitor	0.801	0.005	UGT1A substrate
0.888	0.003	CYP1A2 inhibitor			
0.888	0.004	CYP1A2 substrate			**Pharmacological effects**
0.880	0.002	CYP1A inducer	0.941	0.002	Membrane permeability inhibitor
0.878	0.004	UGT1A9 substrate	0.968	0.002	Membrane integrity agonist
0.871	0.003	15-Oxoprostaglandin 13-reductase inhibitor	0.879	0.004	Antineurotoxic
0.863	0.003	Cholestanetriol 26-monooxygenase inhibitor	0.856	0.004	Mucomembranous protector
0.848	0.002	CYP1A1 inducer	0.844	0.004	Vasoprotector
0.844	0.004	UGT1A6 substrate			
0.838	0.001	Protein kinase C zeta inhibitor			

[a] The predicted activity spectrum is presented in PASS by the list of activities with the probabilities 'to be active' (Pa) and 'to be inactive' (Pi) calculated for each activity.[48,49] The list is arranged in descending order of Pa–Pi; therefore, more probable activities are at the top of the list. The probability Pa reflects the similarity of a molecule under prediction with the structures of molecules, which are the most typical in a sub-set of 'actives' in the structure–activity relationship (SAR) PASS base.

Table 4
PASS predicted human activity spectra for a representative thioxanthone derivate 1 h (at Pa >0.5)[a].

Compd**1h**



Pa	Pi	**Biological targets**	Pa	Pi	**Pharmacological effects**
0.705	0.009	Thioredoxin inhibitor	0.859	0.005	Antineurotoxic
0.553	0.005	Phosphatidylinositol 3-kinase gamma inhibitor	0.781	0.004	Insulin and insulin analogs
0.543	0.005	Quercetin 2,3-dioxygenase inhibitor	0.776	0.009	Fibrinolytic
0.541	0.007	Thioredoxin-disulfide reductase inhibitor	0.629	0.009	Antihelmintic (Nematodes)
0.540	0.008	FMO3 substrate	0.625	0.003	Postmenopausal disorders treatment
0.539	0.004	CF transmembrane conductance regulator agonist	0.541	0.008	Antidote, cyanide
0.505	0.005	Sulfotransferase substrate	0.536	0.005	Keratolytic
0.501	0.001	Estrogen beta receptor agonist	0.512	0.005	Antihelmintic
0.500	0.004	SULT1A3 substrate			

[a] The meanings of parameters are described in the footnote of Table 3.

using small amounts of reagents. In this way, the whole early stage drug discovery process known as 'hit generation' can be performed utilizing minimal resources and funds, and any duplication work can be avoided.[45]

Considering simple structural characteristics and lipophilicity, the synthesized hydroxy xanthones/thioxanthones are drug-like molecules satisfying the Rule of Five.[46] According to the calculated lipophilicity *c* log *P* coefficients, all analyzed molecules have moderate lipophilicity. In addition, all considered polyphenolic derivatives are weakly acidic compounds (ACD (Advanced Chemistry Development, www.acdlabs.com) with pK_a values ~7 as calculated using ChEMBL (www.ebi.ac.uk/chembl/). This can affect their ADME properties like binding to plasma protein human serum albumin.[47]

Regarding anti-infective activities, only antihelmintic activity (Pa >0.6) of studied thioxanthones were predicted by PASS[48,49] in accordance with reported experimental observations (Tables 3 and 4). According to PASS predictions the most plausible targets of hydroxylated xanthones are oxidoreductases, particularly CYP450 isoforms (Pa >0.9), similar to other planar polyphenols such as quercetin. Other targets pointed out by program PASS may be involved in glucose and lipids metabolism and homeostasis, for example, chlordecone reductase and UDP-glucuronosyltransferase. All xanthones have been predicted to be membrane integrity agonists and permeability inhibitors (Pa >0.9). The predicted membrane activity is in accordance with previously experimentally detected activities on the membrane localized targets that is, lysosomal α-glucosidase[50] and mitochondrial monoamine oxidases.[24] In addition to metabolic targets, PASS predictions (Pa >0.8) indicate that all compounds could bind to kinases such as protein kinase C zeta and PI3-kinase subunit gamma.

PASS predicted fewer activities for hydroxy thioxanthone derivatives in comparison with the xanthone analogs. This is due to their lower structural similarity with the compounds with known activities used in the PASS training set.[49] In that respect, the studied thioxanthones can be considered the 'newest' chemical entities. They were not predicted to have CYP450 activities, but they do also target enzymes related to xenobiotic metabolism. Thioxantones have been suggested as chemopreventive agents (predicted with Pa = 0.500, Pi = 0.019).[22] According to the Molinspiration predictions, acetyl derivatives appeared to be potential nuclear receptor ligands.

3. Conclusions

A series of (poly)hydroxyl xanthones and their acetyl derivatives have been successfully synthesized in moderate to high yields by using the microwave approach along with Lewis acids $AlCl_3$, $ZnCl_2$, $TiCl_4$ or $SnCl_2$ during just 50 s. Out of the four explored Lewis acids, $SnCl_2$ was found to be most efficient with xanthone yields of 80–84%.

The (poly)hydroxyl xanthones and their thio-analogs shown no significant HepG2 or Jurkat cytotoxicity and majority have no antibacterial activities on 6 bacterial strains tested. The in silico predictions based on the program PASS indicate that these xanthone derivatives possess the general potential to target proteins with redox activity and metal ions as co-factors, included in lipid and glucose metabolism. Therefore, further structural modification of the synthesized xanthones is a good and feasible approach to increase their activities towards specific targets.

4. Materials and methods

4.1. General procedures for the synthesis of hydroxyxanthones

To an equimolar mixture of phenolic acids and phenol derivatives, anhydrous $AlCl_3$, $ZnCl_2$, $TiCl_4$ or $SnCl_2$ was added. The reaction mixture was heated at 140 °C for 50 s in CEM microwave. The contents were poured into ice and extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulphate and the solvent was removed under reduced pressure. The product thus obtained was purified by silica gel column chromatography.

4.2. General procedures for the synthesis of acetoxyxanthones

To a solution of hydroxyxanthone/thioxanthones (0.2 mmol) in acetic anhydride (5–8 mL), DMAP was added in catalytic amount. The mixture was stirred at 60 °C for 4–5 h. The contents were then poured into ice and extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulphate and the solvent was removed under reduced pressure to obtain the desired acetoxy xanthones. Since this is a simple acetylation reaction and all the hydroxyl groups present in the molecule get acetylated under these conditions, there are no issues regarding regioselectivity of this reaction.

All the above compounds were characterized by comparing their melting points as well as their ^1H NMR data with those already reported in the literature.[27,51,52]

4.3. Antibacterial activity

Bacterial strains, *Staphylococcus aureus* (ATCC 29213), *Streptococcus pneumoniae* (ATCC 49619), *Streptococcus pyogenes* (ATCC 700294), *Moraxella catarrhalis* (ATCC 23246), *Haemophilus influenzae* (ATCC 49247) and *Escherichia coli* (ATCC 25922), were purchased from ATCC and used for evaluation of antibacterial activity of the compounds.

Antibacterial activity was determined by the standard broth microdilution method with azithromycin as comparator. Minimum inhibitory concentrations (MICs) were established according to guidelines of the Clinical Laboratory Standards Institute,[53] except that for Streptococcus medium, lysed blood was substituted with 5% horse serum. Double dilutions of tested compounds in 96-well microtitre plates were prepared in a 128–0.5 μg/mL concentration range. Bacteria were grown on appropriate agar plates (Becton Dickinson, USA)—Columbia agar with 5% sheep blood for streptococci, Mueller-Hinton chocolate agar for *H. influenzae* and Mueller–Hinton agar for staphylococci. Inocula were prepared by direct colony suspension method and plates inoculated with 5×104 CFU/well. Results were determined by visual inspection after 20–22 h incubation at 37 °C in ambient air.

4.4. Cytotoxic activity

A HepG2 human hepatocellular carcinoma cell line (ATCC HB-8065) and Jurkat human leukemic T cell lymphoblast cell line (ATCC TIB-152) were purchased from ATCC and maintained in complete RPMI 1640 medium (Sigma, R7388) supplemented with 10% Fetal Bovine Serum (Sigma, R7524) at 37 °C in 5% CO_2 atmosphere.

A cytotoxicity assay was performed using MTS CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega, G3580).[44] Double dilutions of tested compounds in 96-well microtiter plates were prepared in 100–0.2 μM concentration range. 5×10^4 cells were added per well and incubated overnight at 37 °C in 5% CO_2 atmosphere. Fifteen microliter of MTS reagent was dispensed per well. Plates were incubated for 1 h at 37 °C in 5% CO_2 atmosphere and absorbance recorded at 490 nm using a 96-well Wallac Victor2 plate reader. Results were analyzed in GraphPad Prism software.

4.5. In silico analysis

Simple structural characteristics and physicochemical properties were calculated using the Internet servers of Molinspiration [http://www.molinspiration.com/] and the ChEMBL database [https://www.ebi.ac.uk/chembl/].

Tentative predictions of biological targets and activities were made by web-services using methods based on the identification of substructure features typical for active molecules which are available publicly at the Molinspiration [www.molinspiration.com/] and PASS[48,49] [www.pharmaexpert.ru/PASSOnline/services.php] web pages.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.03.074.

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