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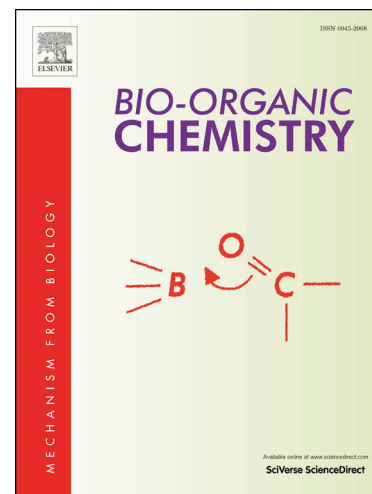
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Design, Synthesis and Evaluation of Benzothiazole Derivatives as Multifunctional Agents

Ernestine Nicaise Djuidje^a, Sabrina Sciabica^b, Raissa Buzzi^a, Valeria Dissette^a, Jan Balzarini^c, Sandra Liekens^c, Elena Serra^{a,e}, Elisa Andreotti^d, Stefano Manfredini^a, Silvia Vertuani^{a,*}, Anna Baldisserotto^a

^aDepartment of Life Sciences and Biotechnology, Master Course in Cosmetic Science and Technologies, University of Ferrara, via L. Borsari 46, 44121 Ferrara, Italy

^bDepartment of Chemical and Pharmaceutical Sciences, University of Ferrara, via F. di Mortara 17-19, 44121 Ferrara, Italy

^cDepartment of Microbiology and Immunology, KU Leuven, University of Leuven, Rega Institute for Medical Research, Laboratory of Virology and Chemotherapy, Leuven B-3000, Belgium

^dDepartment of Life Sciences, University of Modena and Reggio Emilia, Via Giuseppe Campi 287, 41125 Modena, Italy

^eAptuit, An Evotec Company, Via A. Fleming 4, 37135 Verona, Italy

*Corresponding author. Phone: (+39) 0532455294. E-mail: vrs@unife.it

ABSTRACT

Oxidative stress is the product or aetiology of various multifactorial diseases; on the other hand, the development of multifunctional compounds is a recognized strategy for the control of complex diseases. To this end, a series of benzothiazole derivatives was synthesized and evaluated for their multifunctional effectiveness as antioxidant, sunscreen (filter), antifungal and antiproliferative agents. Compounds were easily synthesized via condensation reaction between 2-aminothiophenols and different benzaldehydes. SAR study, particularly in position 2 and 6 of benzothiazoles, led to the identification of **4g** and **4k** as very interesting potential compounds for the design of multifunctional drugs. In particular, compound **4g** is the best blocker of hERG potassium channels expressed in HEK 293 cells exhibiting 60.32% inhibition with $IC_{50} = 4.79 \mu\text{M}$.

Keywords: Benzothiazoles, antioxidant, UV-filter, antifungal, antiproliferative, hERG channels.

1. Introduction

Multifactorial diseases, such as cancer, are caused by a combination of genetic and/or environmental factors and have complex pathological mechanisms. However, it is not always easy to find drugs that can act on different sites. Several approaches, including cocktails of drugs, were developed for the extension of prevention and effective therapy, as well as reduction of the disease complications. Nevertheless, these methodologies were found to be inefficient due to their complex side effects and the unbalance between pharmacokinetic and pharmacodynamic relationships. Nowadays a new approach to the problem consists in the design and development of a single multifunctional chemical entity [1,2].

Environmental factors that can cause multifactorial diseases include exposure to ultraviolet radiation (UVR) or other forms of radiation (e.g. X-rays, gamma rays), pollution and related chemical substance, viruses, bacterial and fungal infections, diet and lifestyle. As stated above, UV radiation (UVB and UVA) can damage cellular components (DNA, RNA, proteins, lipids, trans-urocanic acid and aromatic amino acids) contributing to the development of various skin diseases, including photoaging (accelerated skin and eyes aging) and cancer

[3-5]. Furthermore, absorption of UVB by DNA bases induces the formation of cyclobutane pyrimidine dimers (CPDs) and pyrimidine-pyrimidone (6-4) photoproducts (6-4PPs) [6, 7], while absorption of UVA induces the formation of photo-excited states of chromophores, generating reactive species that damage DNA [8]. Thus, UVA is a potent inducer of a variety of physiological changes characteristic of photoaging including chronic inflammation, immunosuppression and cancer [9]. In general, UV damage are also associated to ROS (reactive oxygen species) production, they are highly unstable and reactive molecules and for this reason they are readily detoxified or neutralized by biological system (enzymatic and non-enzymatic antioxidant). When ROS increase, the antioxidant defences could not be enough to neutralize the free-radical production inducing the oxidative stress. ROS react with lipids, nucleic acids and proteins, altering their cellular functions and contributing to many diseases.

Mycotoxins are very toxic for human and animal cells. Low molecular weight mycotoxins are soluble in lipids and can penetrate into skin creating damage to the cells, resulting specifically aflatoxin, carcinogenic to mankind.

In addition, chronic aflatoxicosis lead to cancer and immune suppression [10]. Cells treated with mycotoxins (i.e. ochratoxin A (OTA) and/or aflatoxin B1 (AFB1) produces high level of ROS and the use of antioxidant agents have shown to be effective in contrasting their cytotoxicity [11].

Therefore, it is important to develop products with the ability to prevent or inhibit the harmful effects of environmental factors described above.

PBSA (2-phenyl-1-H-benzimidazole-5-sulfonic acid) is a heterocyclic sunscreen filter used in the last 20 years in commercial products without any particular drawbacks. It is water-soluble, protects against UVB rays and has a good safety profile. Based on these properties, we recently selected PBSA as a model molecule for sequential modification to obtain potential new sunscreen filters endowed also with other biological properties useful in skin disorders treatment. Completely unattended, the new molecules resulted very potent radical scavenger (useful to counteract UV radiation effects) also endowed with an interesting UV-filter boosting activity when used in combination with conventional UV-sunscreen filters [12].

Inspired by this results, continuing our search for scaffolds for novel multi-active compounds with a good profile of safety, and also to further investigate the above observed effects, we turned our attention to benzothiazoles that are very well represented in several class of drugs such as anti-diabetic [13], Alzheimer [14], antimicrobial [15-21], anthelmintic, anticancer [22-30], analgesic [31, 32], anticonvulsant [33-36], trypanocidal [37], antifungal, neuroprotective [35], anti inflammatory [32] and microsomal triglyceride transfer protein (MTP) inhibitors activity [38]. In addition, among the whole class, 2-aryl benzothiazole is the most investigated due to its wide range of synthetic, industrial and pharmaceutical application as amyloid imaging agents [39], schistosomicidal [40], antioxidant, anticancer agents [41].

On the other hand, phenolic compounds are most reactive and important class of natural compounds, because of their biological and pharmacological properties, especially antioxidant activity by H-atom donation or electron transfer [42, 43]. Considering that benzothiazoles and phenols exhibit multiple pharmacological

properties, including antioxidant effects and skin protection from UVR, their molecular combination could be interesting for the research of new multifunctional compounds.

Taking into consideration the results obtained by us with benzimidazole polyphenols [12], this work focused on the design and synthesis of a series of 2-arylbenzothiazole derivatives which are also isosters of benzimidazole.

By exploiting the structure-activity relationship, an extended investigation on 2-arylbenzothiazole derivatives was developed maintaining the same auxochrome at position 2 and inserting substitutions at the position 6. At this position, we have a proton (-H), a carboxylic acid (-COOH) and a sulfonamide (-SO₂NH₂), these latter are both non-classic isosteres.

2-Phenylbenzothiazole (**4a**) was chosen as reference compound in all the tests performed as it represented the unsubstituted parent compound.

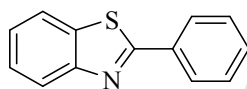


Figure 1. Chemical structure of parent compound (**4a**).

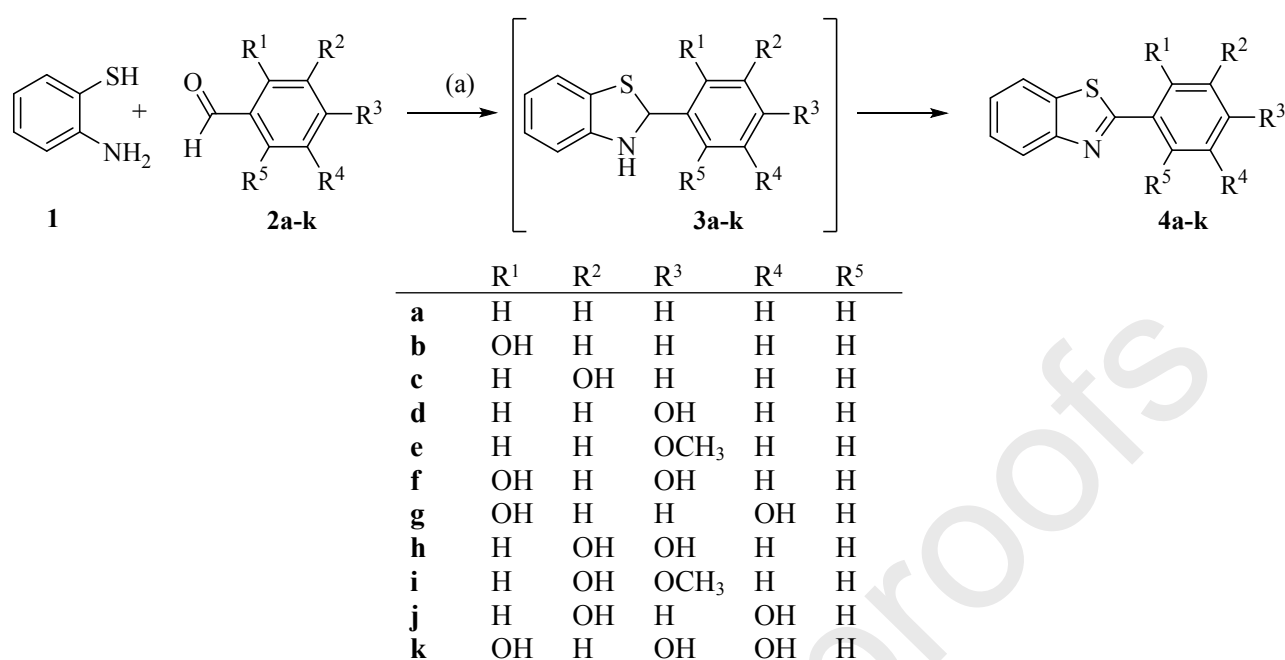
To better discuss the structure-activity relationship, the ten series shown here include both newly synthesized and already known compounds. In order to explore the possibility of a multi-target approach, all synthesized compounds were evaluated for their photoprotective, antioxidant, antifungal and antiproliferative activities.

2. Results and discussion

2.1. Chemistry

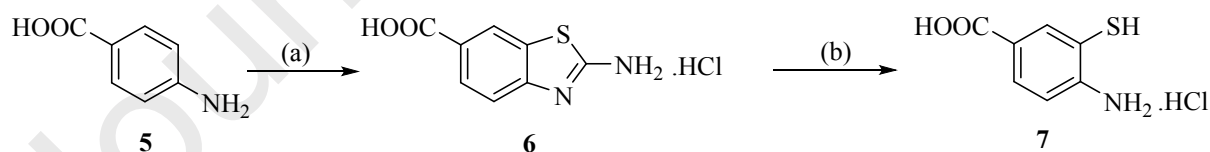
In the literature several methods of synthesis of benzothiazoles had been reported such as the condensation of 2-aminothiophenol with substituted nitriles, carboxylic acids, aldehydes, acyl chlorides or esters [44]. After evaluating these methods, we finally turned our attention to simple and effective procedure which involves condensation of 2-aminothiophenol with aldehydes using sodium hydrosulfite as oxidizing agent. Compounds **4b**, **4e**, and **4f** have been also synthesized under free catalyst conditions.

2-Arylbenzothiazoles unsubstituted on the benzothiazole ring (**4a-k**) were synthesized in one-step via condensation of 2-aminothiophenol (**1**) and benzaldehydes (**2a-k**) in ethanol and sodium hydrosulfite as catalyst (**Scheme 1**). The same reaction, without catalyst, gave a mixture of benzothiazolines (**3a-k**) and benzothiazoles (**4a-k**). Therefore, oxidation of the intermediates **3a-k** was accelerated by Na₂S₂O₄.



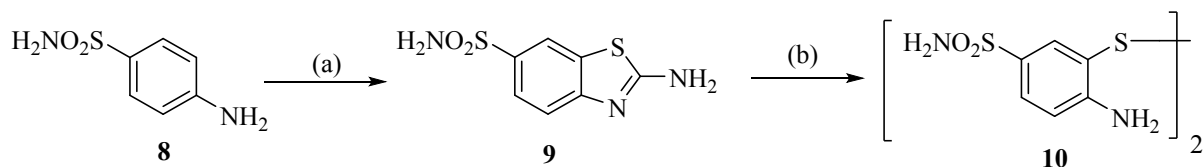
Scheme 1. Synthetic route of compounds 4a-k. Reagents and conditions: (a) EtOH, Na₂S₂O₄ in H₂O, Reflux.

However, the synthesis of 2,6-disubstituted benzothiazole involved several steps. The initial step was the preparation of intermediates 4-amino-3-mercaptopbenzoic acid hydrochloride (**7**) and bis(2-amino-4-benzenesulfonamide)disulfide (**10**) according to a modified literature method [45]. Treatment of 4-aminobenzoic acid with potassium thiocyanate in the presence of bromine in acidic condition at low temperature (0-5°C) gave 2-aminobenzothiazole-5-carboxylic acid hydrochloride (**6**) which was converted into **7** by alkaline hydrolysis (**Scheme 2**).



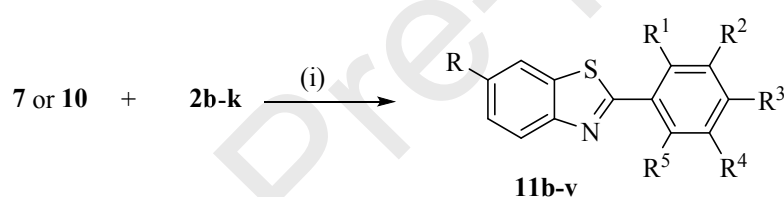
Scheme 2. Synthetic route of 4-amino-3-mercaptopbenzoic acid hydrochloride. Reagents and conditions: (a) MeOH, KSCN, Br₂, -5°C, HCl; (b) KOH, Reflux, 35% HCl.

To optimize the synthetic condition for 2-aminobenzothiazole-6-sulfonamide, two solvents were tried: acetic acid and methanol. Acetic acid was found to be the most suitable reaction media giving a high reaction rate and good yield of the product. Treatment of 2-aminobenzothiazole-6-sulfonamide with aqueous potassium hydroxide gave bis(2-amino-4-benzenesulfonamide)disulfide (**Scheme 3**). Therefore, the thiazole ring was opened but the sulfonamide group did not hydrolyze to a sulfonate.



Scheme 3. Synthetic route of bis(2-amino-4-benzenesulfonamide)disulfide. Reagents and conditions: (a) AcOH, KSCN, Br₂ in AcOH, -5°C, 30% NH₃; (b) KOH, Reflux, 35% HCl.

Subsequently, condensation of **7** or **10** with benzaldehydes (**2b-k**) yielded to the corresponding benzothiazole compounds (**11b-v**), summarized in **Scheme 4**. All reactions were conducted in ethanol with catalyst to evaluate the efficiency of sodium hydrosulfite. For all compounds, the best results were obtained in the presence of catalyst. However, among reactions without catalyst, those with bis(2-amino-4-benzenesulfonamide) disulfide gave corresponding 2-arylbenzothiazoles with good yield whereas reaction with 4-amino-3-mercaptobenzoic acid gave the desired product with yield less than 30%.



	R	R ¹	R ²	R ³	R ⁴	R ⁵		R	R ¹	R ²	R ³	R ⁴	R ⁵
b	COOH	OH	H	H	H	H	m	SO ₂ NH ₂	OH	H	H	H	H
c	COOH	H	OH	H	H	H	n	SO ₂ NH ₂	H	OH	H	H	H
d	COOH	H	H	OH	H	H	o	SO ₂ NH ₂	H	H	OH	H	H
e	COOH	H	H	OCH ₃	H	H	p	SO ₂ NH ₂	H	H	OCH ₃	H	H
f	COOH	OH	H	OH	H	H	q	SO ₂ NH ₂	OH	H	OH	H	H
g	COOH	OH	H	H	OH	H	r	SO ₂ NH ₂	OH	H	H	OH	H
h	COOH	H	OH	OH	H	H	s	SO ₂ NH ₂	H	OH	OH	H	H
i	COOH	H	OH	OCH ₃	H	H	t	SO ₂ NH ₂	H	OH	OCH ₃	H	H
j	COOH	H	OH	H	OH	H	u	SO ₂ NH ₂	H	OH	H	OH	H
k	COOH	OH	H	OH	OH	H	v	SO ₂ NH ₂	OH	H	OH	OH	H

Scheme 4. Synthesis of compounds **11b-v**. Reagents and conditions: (i) EtOH, Na₂S₂O₄ in H₂O, Reflux.

To better analyze the experimental results, the compounds were divided into ten classes (A-J), which differ from the parent compound for the substitutions on the 2-aryl ring (Table S1). In each class, the compounds

present the same substitution on the 2-aryl ring while the substituent at 6 position of the benzothiazole ring change.

2.2. Biological evaluations

2.2.1. Photoprotective activity

Since the Sun Protection Factor (SPF) is related to the UV absorption of the substances, the spectral profile of the synthesized compounds was examined in the first part of this study. Absorption spectra were measured from 290 to 400 nm. The wavelength of maximum absorption (λ_{\max}) was closely related to stereo electronic effects, which can be affected by substituents on the 2-aryl or benzothiazole moiety that alter the electron density of compounds. The spectral absorption data of the non-substituted molecules in position 6, dissolved in DMSO, are shown in Figure 2. The maximum wavelength of **4d** was red-shifted relative to **4c** and can be attributed to the presence of -OH group in para position, which lead the resonance interaction of lone pair of oxygen and π , cloud of 2-aryl and benzothiazole group. This shift increase if -OH is at ortho position (**4b**), due to the presence of the formation of an intramolecular hydrogen bond between nitrogen of benzothiazole and the hydrogen of hydroxy group. Nevertheless, substitution of the para-OH (**4d**) with a para-methoxy (**4e**) does not have significant changes in absorption spectra.

Indeed the absorption spectrum of **4c** is similar to that of 2-arylbenzothiazole (**4a**). This can be explained by the fact that lone electron pair of -OH group in meta position would be delocalized on the 2-aryl ring rather than on the whole entire molecule and, therefore, it has very little effect on the absorption spectrum. However, λ_{\max} of **4j** is slightly higher than that of **4c** since both meta positions are occupied, as confirmed by Joykrishna Dey and al. [46] which demonstrated that red-shift is observed when the number of auxochromic groups increase. The same result is obtained with **4h**, **4i**, and **4g** analogues of **4d**, **4e**, and **4b** respectively, with the second -OH in meta position. The presence of three -OH on the 2-aryl moiety (**4k**) makes the molecule more rigid and promotes the shift at the longest wavelength absorption band compared to the other compounds. Although **4g** has only two hydroxyl groups, its spectrum is similar to that of **4k**, in line with the above mentioned. The presence of an OH in position para has less influence on the shift compared to the other position.

Substitution with an electron withdrawing group (-COOH and -SO₂NH₂) at the position 6 of the benzothiazole moiety showed a bathochromic shift (Figures S1 and S2). This long wavelength absorption band is associated with the intramolecular charge transfer transition due to the transfer of the charge between the electron donor group (phenolic group) to the electron-withdrawing of benzothiazole and neighboring carboxylic or sulfonamidic chromophore. The order of λ_{\max} along the series is -H \ll -SO₂NH₂ < -COOH. Therefore absorption range increase with the trend of the increasing electron-withdrawing ability: this could be mainly due to the significant stabilization of the LUMO leading to a smaller HOMO-LUMO energy space.

In short, beyond the position and the number of -OH on the 2-aryl ring, the presence of an electron-acceptor group at the 6 position of benzothiazole ring, as well as the extent of conjugation, favors the bathochromic shift of the molecule absorption.

Figure 2. Spectral profile of benzothiazole derivatives **4a-k**.

Among the proposed *in vitro* **Abs** methods for the calculation of SPF sun protection, two are the principal: measurement of absorption or the transmission of UV radiation of a thin film of sunscreen agent on the quartz plate or biomimetic membranes and spectrophotometric assay of dilute solutions of sunscreen agent [47-49]. In order to study the synthesized compounds more deeply, both methods were employed. First of all, a pre-screening test was carried out by determination of absorption characteristics of all selected compounds dissolved in DMSO. Then, more promising compounds were introduced in formulation (O/W emulsion) and their transmission spectrum were determined using polymethylmethacrylate (PMMA) plate in order to simulate real human use [50].

2.2.1.1. *In vitro* studies of dilute solutions of target compounds by spectrophotometry

The photoprotective activity is shown in Table 1. Spectral data of samples were collected in the 290-400 nm range and the data were elaborated by the calculator software [51]. The software consent to evaluate COLIPA (The European Cosmetic and Perfumery Association) – SPF, COLIPA – UVA-PF, critical wavelength value (λ_c) (see **Equation 3**, **Equation 4** and **Equation 5**) and UVA/UVB ratio as preliminary theoretical data.

All the synthesized compounds, in general, showed a significant photoprotective activity as compared to photoprotective activity of the lead compound PBSA. Photoprotective activity of the sunscreen against UVB is reported as SPF value. Thereby, among all classes of compounds having a single substituent on the 2-aryl ring, those without substituent at the 6-position of the benzothiazole moiety exhibited good photoprotective profile, followed by compounds having -SO₂NH₂, and finally compounds having -COOH. Only in class **D**, compound bearing -COOH showed the best SPF value.

Moreover, it was evident that the substitution of benzothiazole ring by COOH and SO₂NH₂ had no significant effect on sun protection. Taking all together, we can summarize that the presence of a hydrogen atom in 6 position of benzothiazole ring increases SPF value, therefore the SAR order was -H > -SO₂NH₂ > -COOH. On the contrary, among the compounds having more than one substituent on the 2-aryl ring, those bearing -COOH group showed the best UVB profile except the compound **11f**. However, the order of SPF values according to the substitution on the 2-aryl ring was the following: para > meta > ortho for compounds having -H or -SO₂NH₂ at 6 position of benzothiazole moiety, while compounds with -COOH gave the following order: ortho > meta > para. Compounds of class **B** (para-methoxy substituted on the 2-aryl ring) showed lower value than compounds of class **A**, indicating that para-hydroxy group is better than para-methoxy one, except compound **11d** which showed SPF value slightly lower than that of **11e**.

The introduction of an -OH in position ortho or meta of compounds of class **A** to obtain compounds of classes **E** and **F** respectively, yield to a decrease of SPF values. In the same way, introduction of -OH in meta position of compounds of class **D** (ortho-OH) and class **C** (meta-OH) gave respectively compounds of classes **H** (two -OH in position 2 and 5) and **I** (two -OH in position 3 and 5) with lower SPF. Interestingly, compounds of class **J** with three -OH on the 2-aryl ring showed SPF values similar to those with two -OH. This means that the SPF factor not only depends on the number of -OH on the 2-aryl ring but also on their position. Interestingly, replacing para-hydroxy with para-methoxy (class **F** to **G**) the SPF values increase. Nevertheless, this result is contrary to that previously described (class **A** to **B**). The best SPF value is exhibited by compound **4d** bearing an -OH in para position of 2-aryl ring. Protection offered by a sunscreen product against chronic damages (i.e. tumor formation) induced by UVA rays is indicated by UVA-PF value, which is considered sufficient for protection against UVA by European regulation if its value results $\geq 1/3$ of SPF. Compounds of the same class presented similar UVA-PF values despite they differ in substitution at position 6. Compounds **11h** of class **F** and **11k** of class **J**, characterized both by the presence of -OH in meta and para-position of the phenyl ring, are the best candidate for UVA protection (with UVA-PF ≈ 7), followed by compounds of class **G** (3-OH, 4-OMe). Thus, the effect of the electron donor group (hydroxyl or methoxyl) in the meta and para position of the 2-aryl ring appears to be important for protection against UVA.

Critical wavelength (λ_c) is the parameter that provides information on the broad spectrum of UV-filter. It is classified in five numerical categories: 0 ($\lambda_c < 325$ nm), 1 ($325 \leq \lambda_c \leq 335$), 2 ($335 \leq \lambda_c \leq 350$), 3 ($350 \leq \lambda_c < 370$) and 4 ($\lambda_c \geq 370$). FDA considers that the broad-spectrum sunscreen products should have a $\lambda_c \geq 370$ nm [52]. Under that classification, compounds of classes **H** and **J** have fallen into category “4”, as well as compounds **11f** (class **E**), **11t** (class **G**) and **11u** (class **I**). All synthesized compounds showed $\lambda_c >$ PBSA. Thus, very interestingly, compounds of classes **H**, **J** and compounds **11f**, **11t** and **11u** can be considered potential candidate for broad-spectrum filters.

Finally, we also determined the UVA/UVB absorbance ratio, which is an important parameter to get information on the absorbance through the entire UV spectrum. According to the latest EU recommendation and PBSA data (the UVA / UVB ratio should be at least 1/3), all synthesized compounds have good UVA/UVB values, apart from **11o**, **4e**, **4c** and **11n**.

Classes	Compound	SPF	UVA-PF	UVA/UVB	λ_c (nm)
	PBSA	3.40 \pm 0.17	1.03 \pm 0.08	0.29	322
	4a	8.38 \pm 0.29	0.13 \pm 0.01	1.8	331
A	4d	21.86 \pm 2.06	1.57 \pm 0.02	0.81	353
	11d	6.19 \pm 0.98	2.23 \pm 0.08	0.81	353
	11o	19.41 \pm 1.75	2.35 \pm 0.10	0.32	350
	4e	14.23 \pm 1.43	1.30 \pm 0.04	0.1	335
B	11e	6.54 \pm 0.87	1.88 \pm 0.12	0.65	349
	11p	8.91 \pm 0.45	1.80 \pm 0.09	0.45	347
	4c	10.05 \pm 0.11	1.18 \pm 0.07	0.13	335
C	11c	8.84 \pm 0.64	1.71 \pm 0.02	0.36	346
	11n	9.42 \pm 0.06	1.46 \pm 0.05	0.18	340
	4b	7.56 \pm 0.59	2.57 \pm 0.06	0.86	355
D	11b	18.03 \pm 1.15	4.09 \pm 0.14	0.62	358

	11m	6.59 ± 0.83	3.45 ± 0.22	0.77	360
E	4f	6.62 ± 0.07	3.34 ± 0.13	1.62	356
	11f	3.43 ± 0.49	4.62 ± 0.05	1.61	370
	11q	3.85 ± 0.31	4.41 ± 0.11	1.63	364
F	4h	5.51 ± 0.96	2.33 ± 0.16	0.9	358
	11h	7.42 ± 0.13	7.35 ± 0.41	1.74	367
	11s	5.39 ± 0.09	4.71 ± 0.08	1.40	365
G	4i	9.13 ± 0.34	5.94 ± 0.15	0.42	350
	11i	17.33 ± 1.45	5.95 ± 0.26	1.02	361
	11t	11.87 ± 0.07	5.80 ± 0.33	0.96	380
H	4g	2.82 ± 0.49	2.50 ± 0.17	0.66	381
	11g	7.34 ± 0.49	3.32 ± 0.24	0.40	384
	11r	5.47 ± 0.07	2.76 ± 0.01	0.40	382
I	4j	3.38 ± 0.16	1.52 ± 0.09	0.45	345
	11j	4.45 ± 0.23	1.57 ± 0.07	0.45	353
	11u	3.06 ± 0.06	1.72 ± 0.02	0.95	380
J	4k	3.87 ± 0.012	4.71 ± 0.08	0.86	385
	11k	5.16 ± 0.33	7.50 ± 0.42	1.25	389
	11v	3.65 ± 0.15	4.20 ± 0.19	1.34	384

Table 1. Values of SPF, UVA-PF, UVA/UVB and λ_c in DMSO. Each value is the mean of three independent measurements (mean ± SEM).

2.2.1.2. *In vitro* sun protection factor of the Cosmetic formulation

On the basis of the results obtained in solution, it was decided to continue the investigation on compounds of class **A** and **J**, representative of derivatives with the best UVB profile and broad spectrum, respectively. The 6 molecules synthesized and selected were then inserted at the concentration of 1% in a conventional cosmetic formulation O/W in order to deepen their UV-filter activity. The modified *in vitro* method of Diffey and Robson [47], based on the measurement of transmission of ultraviolet radiation on the PMMA plate, was used. Data of the SPF, UVA-PF, UVA/UVB and λ_c parameters are shown in **Table 2**.

Taking into consideration the spectral data of diluted solutions and cosmetic formulations, we can affirm that the structure-activity relationship is in principle maintained in both cases. Compound **11k**, characterized by the presence of three hydroxy groups in position 2,4,5 of the 2-aryl ring, showed the best SPF value (SPF = 3.91). This value is significant since the formulation contains only 1% of active ingredient. Compounds **4k**, **11k**, and **11v** of class **J** have confirmed their profile and are therefore potential compounds for broad-spectrum UV filters. *In vitro* data of the formulations indicated that the presence of auxochromes influences the photoprotective profile..

Classes	O/W Formulation	SPF	UVA-PF	UVA/UVB	λ_c (nm)
	PBSA	3.02 ± 0.89	1.04 ± 0.06	0.26	333
A	4d	3.2 ± 0.21	1.20 ± 0.11	0.63	350
	11d	1.57 ± 0.09	1.79 ± 0.10	1.12	353
	11o	1.99 ± 0.18	3.59 ± 0.24	1.35	351
J	4k	3.66 ± 0.29	3.24 ± 0.26	0.81	385
	11k	3.91 ± 0.24	3.33 ± 0.11	0.86	388
	11v	2.82 ± 0.19	3.26 ± 0.25	1.15	385

Table 2. Photoprotection results of 1% benzothiazole selected derivatives in O/W formulations. Each value is the mean of three independent measurements (mean \pm SEM).

2.2.1.3. Photostability study

Photostability of selected compounds was evaluated using one of the criteria developed by Garoli et al. [53], based on the measurement of SPF before and after exposition. The loss of sunscreen protection after exposure to sunlight is expressed as the residual effectiveness after exposure (%). The *in vitro* % SPF_{eff.} and % UVA-PF_{eff.} of compounds are calculated according to **Equation 6** and **Equation 7** respectively and are reported in Table 3. A sunscreen was considered photostable if its residual percentage is greater than or equal to 80%. From the results obtained, % SPF_{eff.} and % UVA-PF_{eff.} values of compounds ranged from 94.06 to 100%. Therefore, these compounds are considered photostable with a degradation rate less than 6%. Interestingly, the substitution of the benzothiazole ring in position 6 with -COOH or -SO₂NH₂ increases the photostability of the compounds.

Classes	Compound	%SPF _{eff.}	%UVA-PF _{eff.}
A	4d	94.06 \pm 0.04	98.07 \pm 0.23
	11d	97.45 \pm 0.08	99.85 \pm 0.35
	11o	95.21 \pm 0.73	100.0 \pm 0.04
J	4k	94.28 \pm 3.07	98.64 \pm 2.09
	11k	97.37 \pm 0.22	99.35 \pm 0.17
	11v	98.44 \pm 1.55	99.76 \pm 2.08

Table 3. Evaluation of photostability of 1% benzothiazole selected formulations. Each value is the mean of three independent measurements (mean \pm SEM).

2.2.2. Antioxidant activity

The synthesized compounds were then tested *in vitro* to evaluate the antioxidant activity which, if present, is very important and synergistic with the SPF described above in reducing UV-induced ROS damages. The method employed were DPPH and FRAP assays (**Table 4**).

2.2.2.1. DPPH radical scavenging activities. To better understand the results of the DPPH test, we first evaluated the percentage of inhibition of the DPPH radical of synthesized compounds at the same concentration, namely 1 mg/mL. Compounds of classes **F** (3,4-dihydroxyaryl) and **H** (2,5-dihydroxyaryl) have a percentage of inhibition close to 100%. However, the substitution of -OH with -OCH₃ to obtain class **G** (3-hydroxy, 4-methoxy), lead to a decreases of the percentage of inhibition. Compounds of class **A**, with -OH in para position on the 2-aryl ring, did not exhibit any particular activity with respect to the radical under consideration. In this case, replacing -OH with -OCH₃ to obtain class **B** compounds lead to a considerably increase of the percentage of inhibition compounds **4e** and **11e**. In addition, among compounds of class **D**, only

11m with -OH in ortho position of 2-aryl ring and SO₂NH₂ in 6 position of the benzothiazole ring displayed a good inhibition. Alike, for compounds of class **C** (meta-hydroxy), only compound **11c** with COOH in 6-position on benzothiazole ring has good inhibition. The introduction of methoxy group at position 4 of the 2-aryl ring (compounds of class **G**) drastically reduced the activity in comparison to compounds of class **F**, due to the lack of stabilization.

Based on this first screening, only compounds with good inhibition ($\% \geq 50$) were further investigated for the determination of IC₅₀ values. The results are expressed in $\mu\text{g/mL}$ (Table 4). Structure-activity relationship analysis indicated that various factors such as the nature of substituents in position 6 on benzothiazole ring, the number and also the position of the electron donor group (-OH and -OCH₃) on the 2-aryl ring are very important for the radical scavenging activity. All compounds of class **F** and **H** especially those without electron withdrawing group on the benzothiazole ring (**4h** and **4g**) showed good inhibition, with IC₅₀ values between 6.42 and 11.29 $\mu\text{g/mL}$, respectively. This could be due to the fact that, polyphenols having two -OH groups in the 2,5- and 3,4-positions react twice with DPPH radical to form a very stable conjugated diketone structure, further stabilized the hydrogen bonds between two hydroxy for the 3,4-dihydroxy compounds and between one hydroxy and nitrogen of the benzothiazole ring for the 2,5-dihydroxy compounds. Nevertheless, introduction of methoxy group at position 4 of the 2-aryl ring (compounds of class **G**) drastically reduced the activity in comparison to compounds of class **F** (in term of percentage of inhibition), due to the lack of stabilization above mentioned. Surprisingly, compound **11c** with meta-hydroxy group displayed good activity, while compound 3,5-dihydroxysubstituted on 2-aryl ring have no activity. In addition, compounds of class **J**, although having three -OH group, are less active than their parent compounds (compounds of class **H**). This could be explained by the fact that the presence of the third -OH prevents the resonance.

2.2.2.2. Ferric Reducing Potential. Similar to DPPH results, the molecules with a better antioxidant profile are those of classes **F** and **H** which bear two hydroxy groups respectively at positions 3, 4 and 2, 5- of the 2-aryl ring. The compounds of classes **A** and **D**, having para and ortho hydroxy on the 2-aryl ring respectively, have moderate values. Among compounds of class **C**, **11c** bearing -COOH on the benzothiazole ring have a good activity ($2045.37 \pm 18.68 \mu\text{molT/g}$), instead both **4c** and **11n** have very low values (48.61 ± 0.40 and $17.36 \pm 0.81 \mu\text{molT/g}$ respectively). The substitution of the hydroxyl group in para (class **A**) with a methoxyl (class **B**) did not lead to significant changes in the activity, except for the compound **11d**, whose correspondent **11e** has more than halved activity. Instead the replacement of the para methoxy group (class **G**) with an hydroxyl group (Class **F**) led to a significant increase in activity, indicating that para methoxy substitution is not suitable for this activity.

In summary, considering the above mentioned results, in DPPH and FRAP assays, molecules with better antioxidant profile are compounds of classes **F** and **H**, which bear two hydroxy groups at positions 3, 4 and 2, 5- of the 2-aryl ring.

Classes	Compound	DPPH %Inhibition	DPPH IC ₅₀ (µg/ml)	FRAP µmolT/g
A	4a	43.6 ± 0.11	974.49 ± 4.78	167.01 ± 3.34
	4d	11.60 ± 0.32	-	123.00 ± 5.92
	11d	30.75 ± 0.89	-	402.17 ± 1.95
	11o	31.22 ± 0.45	-	65.60 ± 1.18
B	4e	45.27 ± 0.61	-	206.88 ± 6.64
	11e	71.70 ± 0.58	322.70 ± 6.29	173.83 ± 5.05
	11p	15.48 ± 0.33	-	90.95 ± 3.64
C	4c	16.27 ± 0.06	-	48.61 ± 0.40
	11c	94.64 ± 1.22	28.97 ± 0.56	2045.37 ± 18.68
	11n	22.73 ± 0.69	-	17.36 ± 0.81
D	4b	30.72 ± 0.47	-	264.75 ± 5.84
	11b	18.00 ± 0.36	-	20.60 ± 0.85
	11m	58.22 ± 0.87	-	197.80 ± 5.07
E	4f	72.83 ± 0.58	822.63 ± 8.16	187.64 ± 5.15
	11f	22.37 ± 0.52	-	50.32 ± 1.54
	11q	23.89 ± 0.48	-	91.31 ± 3.25
F	4h	84.65 ± 0.93	7.2 ± 0.11	6021.15 ± 25.84
	11h	94.74 ± 0.66	11.29 ± 0.07	7402.73 ± 22.78
	11s	94.36 ± 0.82	7.96 ± 0.55	5273.01 ± 15.01
G	4i	65.00 ± 0.31	561.62 ± 18.13	3247.66 ± 56.72
	11i	28.02 ± 0.87	-	712.52 ± 10.75
	11t	10.98 ± 0.11	-	65.39 ± 0.35
H	4g	94.32 ± 1.15	6.42 ± 0.28	6268.43 ± 25.16
	11g	96.41 ± 0.89	7.61 ± 0.28	7321.64 ± 34.73
	11r	96.23 ± 0.77	9.09 ± 0.24	7115.91 ± 14.46
I	4j	77.5 ± 4.76	159.55 ± 6.20	273.49 ± 15.38
	11j	57.9 ± 0.82	-	175.98 ± 1.78
	11u	19.93 ± 1.54	-	73.33 ± 1.42
J	4k	23.78 ± 0.82	-	4876.44 ± 34.54
	11k	72.73 ± 3.67	43.01 ± 2.93	1960.24 ± 18.43
	11v	53.20 ± 0.95	186.89 ± 5.46	513.04 ± 2.66

Table 4.
activity of
derivatives.
mean of three
measurements

Antioxidant
benzothiazole
Each value is the
independent
(mean ± SEM).

2.2.3. Antifungal activity

2.2.3.1. Antidermatophytic activity

All synthesized compounds were investigated to determine their antifungal activity against five dermatophytes responsible for the most common dermatomycoses: *Microsporum gypseum*, *Microsporum canis*, *Trichophyton mentagrophytes*, *Trichophyton tonsurans* and *Epidermophyton floccosum*. Inhibition of dermatophytes was evaluated by inhibition growth method in Sabouraud Dextrose Agar (SDA), using DMSO as a solvent. All compounds were tested at a concentration of 100 µg/ml (Tables S2). Compound **4h** showed the highest antifungal activity. It is active against all five dermatophytes, in particular against *M. gypseum*, *T. tonsurans* and *E. floccosum* with a percentage of inhibition close to 100%. Significant activity was observed for compounds **4f** and **4g** against *M. gypseum* and *E. floccosum*. Compound **4c** and **4j** showed good activity against *Microsporum gypseum* and *Epidermophyton floccosum* respectively. In general it can be stated that compounds substituted in position 6 of the benzothiazole ring showed a reduced activity, thus suggesting that electron withdrawing substituent decrease the antifungal activity.

In addition compound **4h**, identified as the most active in the previous screening, was also evaluated for its potency against *M. gypseum*, *T. tonsurans* and *E. floccosum* as reported in Table 5.

	IC ₅₀ µg/ml		
	<i>M. gypseum</i>	<i>T. tonsurans</i>	<i>E. floccosum</i>
4h	26.95 ± 1.04	43.31 ± 1.85	11.63 ± 0.24
Fluconazole	18.5 ± 1.23	19.41 ± 0.87	0.08 ± 0.005

Table 5. IC₅₀ values of **4h** benzothiazole derivate and Fluconazole. Each value is the mean of three measurements (mean ± SEM).

2.2.3.1. Anti-*Candida* Activity

The antifungal activity of the synthesized compounds was also determined against *Candida albicans* (ATCC 10231). Minimal inhibitory concentration (MIC) of the synthesized agents was determined by broth microdilution method using RPMI (Roswell Park Memorial Institute) 1640 + MOPS (3-(N-Morpholino)propanesulfonic acid sodium salt). The concentration of the tested compounds ranged from 0.25 µg·mL⁻¹ to 128 µg·mL⁻¹. The growth in the 96-well microdilution plate was observed visually for turbidity and inhibition was determined by the absence of growth. MIC was determined by the lowest concentration of the sample that prevented the development of turbidity. The results, shown in Table 6, are representative of 24 and 48 hours of incubation arranged in triplicate (only active compounds against *Candida albicans* were shown). Fluconazole was used as referenced compound. Derivative **4h**, **4f**, **4k**, showed good activity. In particular, compounds **4f** and **4k** characterized respectively by the presence of -OH groups in positions 2, 4 and 2, 4, 5 of the 2-aryl ring and no functional group on the benzothiazole ring are more effective with a range of MIC of 4-8 µg/mL at 24 hours.

Compound	MIC µg · ml ⁻¹	
	24 h	48 h
4f	4	32
4h	32	32
4k	8	128
11k	64	-
11v	128	-
Fluconazole	0.5	-

Table 6. Anti-*Candida albicans* activities of benzothiazole derivatives. The average values were taken as the MICs.

2.2.4. Antiproliferative activity

Antiproliferative activities of target compounds against human T cell leukemia cell line (CEM), human cervix carcinoma cells (HeLa), human pancreas carcinoma cells (Mia Paca-2) and human melanoma cells (SK-Mel 5) were evaluated *in vitro* and summarized in Table 7. The results were expressed as IC₅₀ values in μM and normal kidney epithelial cells (HEK 293) were chosen as control of cytotoxicity and/or selectivity.

Among the tested compounds, **4e**, **11n** and **11o** were the most potent against Mia Paca-2 cells with IC₅₀ values of 6.6, 5.5 and 6.5 μM respectively, but inactive against HeLa and CEM cells (IC₅₀ > 50 μM). However, **4e** also displayed good activity against SK-Mel5 cells while **11n** and **11o** were moderately active. Compounds **4g** and **4h** exhibited good antiproliferative activity against CEM and SK-Mel 5 cells with IC₅₀ < 10 μM and a moderate activity against HeLa and Mia Paca-2 (IC₅₀ > 20 μM). **4k** showed the best activity against SK-Mel 5 and it also displayed good inhibitory activity against CEM.

Interestingly, substitution of the para hydroxyl group of compound **4d** with a methoxy group led to compound **4e** endowed with increased antiproliferative activity against Mia Paca-2 cell (52 to 6.6 μM respectively). Similarly, the introduction of -SO₂NH₂ at the 6-position on the benzothiazole ring (**11o**) increase potency. Curiously, in this case, substitution by para-methoxy has the same effect on Mia Paca-2 cell as substitution by -SO₂NH₂ in position 6 on benzothiazole, therefore activity of **4e** is similar to **11o**. The shift of the hydroxyl group along 2-aryl group did not have significant effect on the activity, whereas the introduction of a second or third hydroxyl group (compound **4g**, **4h**, and **4k**) led to an increase in the antiproliferative activity on the CEM and SK-Mel 5 cells.

After introduction of the -SO₂NH₂ group into the benzothiazole skeleton of **4c** to obtain **11n**, an increase in activity against Mia Paca-2 cells was observed but activity against CEM, HeLa and SK-Mel 5 cells decreased. Apart from **4c** and **4d**, the introduction of -SO₂NH₂ into the backbone reduces the activity against Mia Paca-2 cells. All tested compounds showed low antiproliferative activity towards HeLa cell line, except compound **4h** with an inhibition activity less than 30 μM . To our surprise, replacement of the -SO₂NH₂ with -COOH led to almost total loss of activity.

Based on cytotoxicity screening, all unsubstituted compounds on the benzothiazole ring were cytotoxic to normal HEK 293 except compound **4e** and **4i** with para-methoxy group on 2-aryl ring, suggesting the importance of para-methoxy for antiproliferative activity. As well as **4b** and **4k** bearing respectively one and three hydroxy group was not cytotoxic. However electron-withdrawing group -SO₂NH₂ on the benzothiazole ring decreases the cytotoxicity of the target compound except compound **11o** and **11s** characterized by the presence of para-hydroxy group on 2-aryl ring. Compounds bearing -COOH on the benzothiazole ring (**11a-11k**) were inactive against both normal and cancer cells.

The Selectivity Index (SI) was determined by comparing the IC₅₀ values (μM) obtained on the healthy cells (HEK 293) with that obtained on cancer cells (CEM, HeLa, Mia-Paca-2 and SK-Mel 5). From the results presented in Table 7, we observed that compounds **4e**, **11n** and **11o** were respectively 15, 18 and 5 fold more selective toward Mia-Paca-2 cells, while compounds **4g** and **4k** were approximately 6 fold more selective for CEM cells. In addition, compounds **4e**, **4i** and **4k** showed selectivity for SK-Mel 5 cells. Compounds **4k** were 25 fold more selective for SK-Mel 5 cells that could be due to the presence of three hydroxy groups on the 2-

Table 7. Inhibitory effects of benzothiazole derivatives on the proliferation of CEM, HeLa, Mia Paca-2 and SK-Mel 5. Each value is the mean of three independent measurements (mean \pm SEM). SI=IC₅₀ of pure compound in a normal cell line/IC₅₀ of the same pure compound in cancer cell line.

2.2.5. Benzothiazole derivatives block hERG potassium channels expressed in HEK293 cells

In order to have an idea of the possible mechanism of compounds presenting a good antiproliferative profile, we studied the effect of these derivatives on the modulation of human ether-a-go-go related gene (hERG) current. hERG is a gene (KCNH2) that encodes for a protein highly selective for potassium channel (K_v11.1). These protein channels are widely expressed, mainly in the cardiac tissues, neuronal tissues, brain, adrenal gland, thymus, retina and in some cancer cells [54]. Indeed, the ion channels are transmembrane proteins that regulate the flow of ions through biological membranes. In particular, potassium channels represent the most diverse group of ion channels and play an important role in several functions such as muscle excitation, regulation of blood pressure [55], proliferation of several types of cancer, such as prostate, colon, and glioma [56-58]. It has been shown that, hERG potassium channels are overexpressed in many types of human cancers as well as in primary human cancers [59, 60]. In addition, it was shown that over-expression of hERG channels in HEK-293 cells greatly increased the proliferation, tumor cell invasion and metastasis. The blockade or inhibition of hERG in these cells induced both antiproliferative and antimigratory effect [61, 62]. Thus the interest in the study of hERG channels has increased exponentially, mainly because of its involvement in many cellular events such as repolarization and proliferation of cells [63]. Therefore, given their multifunction, these channels are potential therapeutic targets for cancer treatment by application of hERG channel blocking.

One of the most accurate methods for determining the potency of a compound in K⁺ channel inhibition is patch-clamp electrophysiology. In this work we focused on the QPatch automated patch clamp system to evaluate the blockade of hERG potassium channels expressed in HEK293 cells. Three different concentrations (0.1, 1 and 10 μ M) of benzothiazole derivatives were tested and summarized in Table 8. E-4031 (methanesulfonanilide class III antiarrhythmic agent) is the reference compound for this assay. The percent inhibition of the peak tail current was measured, the concentration of drugs needed to yield a 50% blockade of the hERG current (IC₅₀) was obtained by fitting the data to a Hill equation (**Equation 10**).

At the higher tested concentration (10 μ M) **4g** reduced hERG current amplitude to 60.32 ± 2.43 % with IC₅₀ equal to 4,79 μ M, while **4e**, **11c** and **11d** have an inhibition less than 50%. As compared to the reference compound, **4g** is a potential candidate for blocking HERG potassium channels.

Compound	hERG activity channels	
	pIC ₅₀	% Inhibition
4e	< 5	12.09 \pm 5.05
4g	5.32 \pm 0.04	60.32 \pm 2.43
4k	< 5	41.17 \pm 2.94
11c	< 5	20.73 \pm 1.79
11d	< 5	8.07 \pm 4.08
E-4031	7.55 \pm 0.08	96.32 \pm 0.9

Table 8. Inhibition of hERG channels by benzothiazole derivatives tested at 10 μ M. Each value is the mean of three independent measurements (mean \pm SEM).

3. Experimental section

3.1. General

Reagents and solvents were purchased from Sigma-Aldrich and Carlo Erba Reagenti (Italia) and without further purification. Analytical TLC was performed on silica gel plates (Macherey-Nagel Poligram SIL G/UV254 0.20 mm) and visualized by exposure to ultraviolet light (254 nm) and /or used 1% solution of KMnO_4 . Column chromatography was performed using silica gel Macherey-Nagel 60M 230400 mesh. ^1H -NMR and ^{13}C -NMR were registered with Varian spectrometers at 200 MHz and 400 MHz at room temperature. Chemical shifts are measured with respect to tetramethylsilane and relative to residual solvent peaks as an internal standard set to D_2O and $(\text{CD}_3)_2\text{SO}$. The following abbreviations are used to indicate signal multiplicity: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), brs (broad singlet) and dd (doublet of doublets). Molecular weights of the compounds were determined by ESI (Micromass ZMD 2000), and the values are expressed as $[\text{MH}]^+$. UV spectrophotometric analyses were carried out on a UV-VIS spectrophotometer (Shimadzu UV-2600). IR analyses were performed on FT-IR spectrometer Spectrum 100 (Perkin Elmer precisely).

3.2. Chemistry

3.2.1. General Method for the Synthesis of 2-Arylbenzothiazoles Unsubstituted on the Benzothiazole Ring

To a solution of 2-aminothiophenol (0.4 g, 3.2 mmol) and aldehyde (3.2 mmol) in ethanol (3.5 mL) at room temperature was added solution of sodium hydrosulfite (2 eq. in 3 mL H_2O). The mixture was heated at reflux for 12 h. After cooling to room temperature, the solution was concentrated in vacuo. The residue was treated with HCl 2N (15 ml) and filtered to obtain the crude solid, which was recrystallized from appropriate solvent. Compounds **4b**, **4e** and **4f** were synthesized with and without catalyst following the same procedure described above.

3.2.1.1. 2-phenylbenzo[d]thiazole (4a). Yield 60%; white solid; m.p. 98–99°C; UV λ_{max} 298.4 nm. IR (KBr) cm^{-1} : 3446.7, 1589.75, 1474.3, 1316.4, 1217.2, 756.9. ^1H NMR (400MHz, DMSO-d_6): δ (ppm) 8.10 (d, J= 8Hz, 1H, BTZ), 8.01 (d, J= 8Hz, 1H, BTZ), 7.74 (m, 2H, Ar), 7.33 (m, 5H, 2H BTZ, 3H Ar). ^{13}C -NMR (400MHz, DMSO-d_6): δ (ppm) 169.45, 154.4, 143.65, 133.32, 131.87, 130.32, 129.00, 126.76, 124.54, 123.67, 120.09. ESI-M $[\text{M}+\text{H}]^+$: calcd for $\text{C}_{13}\text{H}_9\text{NS}$, 211.28; found 212.20.

3.2.1.2. 2-(2-hydroxyphenyl)benzo[d]thiazole (4b). Yield 80%; yellow solid; m.p. 125-126.4 °C; UV λ_{max} 331.4nm. IR (KBr) cm^{-1} : 3000.5, 1589.1, 1479.8, 1315.2, 1214.4, 724.7. ^1H NMR (400MHz, DMSO-d_6): δ

(ppm) 11.65 (brs, 1H, OH), 8.21-8.18 (2H, m, BTZ), 8.07 (d, J= 8 Hz, 1H, Ar), 7.58 (t, 1H, BTZ), 7.39 (m, 2H, 1H BTZ, 1H Ar), 7.15 (d, J= 8.0Hz, 1H, Ar), 7.01 (t, J= 7.2Hz, 1H, Ar). $^{13}\text{C-NMR}$ (400MHz, DMSO- d_6): δ (ppm) 165.59, 156.65, 151.83, 134.68, 132.95, 128.95, 126.94, 125.56, 122.54, 122.45, 120.25, 118.76, 117.38. ESI-MS $[\text{M}+\text{H}]^+$: calcd for $\text{C}_{13}\text{H}_9\text{NOS}$, 227.28; found 228.17.

3.1.1.3. 2-(3-hydroxyphenyl)benzo[d]thiazole (4c). Yield 71%; yellow solid; m.p. 158-159 °C; UV λ_{max} 299.4 nm. IR (KBr) cm^{-1} : 3251.3, 1598.0, 1453.6, 1317.1, 1231.3, 759.0. $^1\text{H NMR}$ (400MHz, DMSO- d_6): δ (ppm) 9.93(brs, 1H, OH), 8.19 (d, J= 8.0Hz, 1H, BTZ), 8.08 (d, J= 8.0Hz, 1H, BTZ), 7.60-7.39 (m, 3H BTZ, 2H Ar), 6.99 (dd, J= 8.4Hz, J= 1.6Hz, 1H, Ar). $^{13}\text{C-NMR}$ (400MHz, DMSO- d_6): δ (ppm) 167.79, 158.42, 153.94, 134.80, 134.45, 131.03, 127.09, 125.97, 123.30, 122.79, 118.99, 118.54, 113.83. ESI-MS $[\text{M}+\text{H}]^+$: calcd for $\text{C}_{13}\text{H}_9\text{NOS}$, 227.28; found 228.30.

3.1.1.4. 2-(4-hydroxyphenyl)-benzo[d]thiazole (4d). Yield 78%; yellow solid; m.p. 224-225 °C; UV λ_{max} 323.5 nm. IR (KBr) cm^{-1} : 3200.5, 1603.5, 1424.7, 1283.5, 754.4. $^1\text{H NMR}$ (400MHz, DMSO- d_6): δ (ppm) 10.22 (brs, 1H, OH), 8.08 (d, J= 8.0Hz, 1H, BTZ), 7.98-7.96 (m, 3H, BTZ), 7.56-7.38 (m, 2H, Ar), 6.91 (d, J= 8.4Hz, 2H, Ar). $^{13}\text{C-NMR}$ (400MHz, DMSO- d_6): δ (ppm) 167.90, 160.95, 154.166, 134.54, 129.48, 126.87, 125.34, 124.47, 122.73, 122.56, 116.54. ESI-MS $[\text{M}+\text{H}]^+$: calcd for $\text{C}_{13}\text{H}_9\text{NOS}$, 227.28; found 228.18.

3.1.1.5. 2-(4-methoxyphenyl)-benzo[d]thiazole (4e). Yield 82%; white solid; m.p. 118-119 °C; UV λ_{max} 321.4 nm. IR (KBr) cm^{-1} : 2996.0, 2835.9, 1589.1, 1433.6, 1224.4, 1170.7, 751.7. $^1\text{H NMR}$ (400.0 MHz, DMSO- d_6): δ (ppm) 8.24 (d, J= 8.0Hz, 1H, BTZ), 8.18 (d, J= 8.0Hz, 1H, BTZ), 7.90 (d, J= 8.0Hz, 2H, Ar), 7.75 (t, J= 6.4Hz, 1H, BTZ), 7.70 (t, J= 6.4Hz, 1H, BTZ), 7.17 (d, J= 8.4 Hz, 2H, Ar), 3.92 (s, 3H, OCH₃). $^{13}\text{C-NMR}$ (400MHz, DMSO- d_6): δ (ppm) 167.50, 162.23, 154.09, 134.65, 129.33, 126.99, 125.95, 125.57, 122.90, 122.67, 115.21, 55.95. ESI-MS $[\text{M}+\text{H}]^+$: calcd for $\text{C}_{14}\text{H}_{11}\text{NOS}$, 241.06; found 242.27.

3.1.1.6. 2-(2,4-dihydroxyphenyl)-benzo[d]thiazole (4f). Yield 68%; yellow solid; m.p. 212-213 °C; UV λ_{max} 337.0 nm. IR (KBr) cm^{-1} : 3446.7, 1599.8, 1474.3, 1217.2, 756.9. $^1\text{H NMR}$ (400MHz, DMSO- d_6): δ (ppm) 9.22 (brs, 2H, OH), 8.18 (d, J= 8.0Hz, 1H, BTZ), 8.01 (d, J= 8.0Hz, 1H, BTZ), 7.56-7.46 (m, 3H, 2H BTZ, 1H Ar), 6.42 (d, J= 8Hz, 1H, Ar), 6.33(s, 1H, Ar). $^{13}\text{C-NMR}$ (400MHz, DMSO- d_6): δ (ppm) 162.01, 158.65, 151.91, 133.73, 130.48, 126.78, 124.95, 122.31, 121.86, 110.65, 108.89, 103.072. ESI-MS $[\text{M}+\text{H}]^+$: calcd for $\text{C}_{13}\text{H}_9\text{NO}_2\text{S}$, 243.04; found 244.11.

3.1.1.7. 2-(2,5-dihydroxyphenyl)-benzo[d]thiazole (4g). Yield 58%; yellow solid; m.p. 220-221 °C; UV λ_{max} 295.2, 362.8 nm. IR (KBr) cm^{-1} : 3332.8, 1579.8, 1496.0, 1197.2, 756.5. $^1\text{H NMR}$ (400.0 MHz, DMSO- d_6): δ (ppm) 10.82 (brs, 1H, OH), 9.18 (brs, 1H, OH), 8.17 (d, J= 8.0Hz, 1H, BTZ), 8.01 (d, J= 8.0Hz, 1H, BTZ), 7.59-7.38 (m, 2H BTZ, 1H Ar), 6.98-6.82 (m, 2H, Ar). $^{13}\text{C-NMR}$ (400MHz, DMSO- d_6): δ (ppm) 165.47, 151.93, 150.53, 149.68, 134.83, 126.85, 125.43, 122.51, 122.43, 120.73, 118.82, 118.31, 113.51. ESI-MS $[\text{M}+\text{H}]^+$: calcd for $\text{C}_{13}\text{H}_9\text{NO}_2\text{S}$, 243.04; found 244.14.

3.1.1.8. 2-(3,4-dihydroxyphenyl)-benzo[d]thiazole (4h). Yield 70%; yellow solid; m.p. 216-217 °C; UV λ_{max} 329.2 nm. IR (KBr) cm^{-1} : 3488.6, 2916.8, 1599.1, 1474.3, 1260.5, 754.1. $^1\text{H NMR}$ (400MHz, DMSO- d_6): δ (ppm) 9.51 (brs, 2H, OH), 8.12 (d, J= 8.0Hz, 1H, BTZ), 7.98 (d, J= 8.0Hz, 1H, BTZ), 7.51 (m, 2H, BTZ), 7.38 (m, 2H, Ar), 6.88 (d, J= 8.4 Hz, 1H, Ar). $^{13}\text{C-NMR}$ (400MHz, DMSO- d_6): δ (ppm) 168.10, 154.06, 149.40,

146.22, 134.47, 126.88, 125.34, 124.73, 122.52, 119.92, 118.56, 116.54, 114.36. ESI-MS [M+H]⁺: calcd for C₁₃H₉NO₂S, 243.04; found 244.29.

3.1.1.9. 2-(3-hydroxy-4-methoxyphenyl)-benzo[d]thiazole (4i). Yield 65%; white solid; m.p. 184–185°C; UV λ_{max} 326.4 nm. IR (KBr) cm⁻¹: 3252.3, 1597.4, 1474.3, 1122.7, 763.9. ¹H NMR (400MHz, DMSO-d₆): δ (ppm) 9.49 (brs, 1H, OH), 8.08 (d, J= 8.0Hz, 1H, BTZ), 7.98 (d, J= 8.0Hz, 1H, BTZ), 7.56-7.46 (m, 3H, 1H BTZ, 2H Ar), 7.40 (t, J= 7.2Hz, 1H, BTZ), 7.01(d, J= 8.4Hz, 1H, Ar), 3.85 (s, 3H, Ar). ¹³C-NMR (400MHz, DMSO-d₆): δ (ppm) 167.77, 154.04, 151.07, 147.37, 134.62, 126.93, 126.07, 125.50, 122.85, 122.57, 119.62, 113.98, 112.76, 56.12. ESI-MS [M+H]⁺: calcd for C₁₄H₁₁NO₂S, 257.31; found 258.29.

3.1.1.10. 2-(3,5-dihydroxyphenyl)-benzo[d]thiazole (4j). Yield 58%; yellow solid; m.p. 225–226°C; UV λ_{max} 302.0 nm. IR (KBr) cm⁻¹: 3068.7, 1606.9, 1438.3, 1147.9, 755.6. ¹H NMR (400MHz, DMSO-d₆): δ (ppm) 10.01-10.22(brs, 2H, OH), 8.05 (d, J= 8.0Hz, 1H, BTZ), 7.96 (d, J= 8.0Hz, 1H, BTZ), 7.38-7.56 (m, 2H, BTZ), 6.96 (d, J= 2.0Hz, 2H, Ar), 6.39 (s, 1H, Ar). ¹³C-NMR (400MHz, DMSO-d₆): δ (ppm) 167.43, 158.96, 153.35, 134.28, 134.13, 126.45, 125.33, 122.70, 122.16, 105.34, 105.05. ESI-MS [M+H]⁺: calcd for C₁₃H₉NO₂S, 243.04; found 244.19.

3.1.1.11. 2-(2,4,5-trihydroxyphenyl)-benzo[d]thiazole (4k). Yield 51%; grey solid; m.p. 225–226°C; UV λ_{max} 295.6, 365.4 nm. IR (KBr) cm⁻¹: 3068.8, 1603.2, 1437.8, 1150.3, 756.1. ¹H NMR (400MHz, DMSO-d₆): δ (ppm) 9.48 (brs, 3H, OH), 8.16 (d, J= 8.0Hz, 1H, BTZ), 8.01 (d, J= 8.0Hz, 1H, BTZ), 7.64 (m, 2H, BTZ), 6.65 (s, 1H, Ar), 6.32 (s, 1H, Ar). ¹³C-NMR (400MHz, DMSO-d₆): δ (ppm) 166.73, 151.51, 150.86, 150.39, 138.90, 133.29, 126.16, 124.21, 121.70, 121.19, 113.37, 108.68, 103.48. ESI-MS [M+H]⁺: calcd for C₁₃H₉NO₃S, 259.03; found 260.18.

Following the general procedure compounds **6** and **7** were prepared and their analytical and spectra data are in agreement with those reported in literature [45].

3.2.2. 2-aminobenzo[d]thiazole-6-sulfonamide (9). 4-amino-benzenesulfonamide (5.00g, 0.029 mol) and potassium thiocyanate (5.64g, 0.058 mol) were dissolved in glacial acetic acid (45 mL). The mixture was stirred at room temperature for 20 minutes and then a bromine solution (1.49 mL 0.029 mol) in acetic acid glacial (5 mL) was added with a dropping funnel. After the addition, the mixture was kept under stirring at room temperature for 24 h. The precipitated solids were collected by filtration and obtained solid was dissolved in H₂O. NH₃ 30% (about 2 mL) was added to the solution to bring the solution up to a pH8-9 and placed in the refrigerator overnight. The precipitated solids were collected by filtration and the product was washed with H₂O. The white product was dried and purified by recrystallization in EtOH to obtain 3.01g of **9** as a white solid, with a yield of 45%. m.p. > 250 °C. UV λ_{max} 305.90 nm. ¹H NMR (400MHz, DMSO-d₆): δ (ppm) 8.23 (1H, d), 7.96-7.84 (2H, dd), 7.02-6.83 (2H, d). ¹³C-NMR (400MHz, DMSO-d₆): δ (ppm) 166.53, 156.34, 136.10, 125.36, 123.63, 121.59, 115.33. ESI-MS [M+H]⁺: calcd for C₇H₇N₃O₂S₂, 229.28; found 230.09.

3.2.3. bis(2-amino-4-benzenesulfonamide) disulfide (10).

To the 2-amino-benzothiazole-6-sulfonamide (3g, 0.013 mol), a KOH solution (3.95 g of KOH in 5.8 mL of H₂O) was added, which was subsequently refluxed for 8 hours. After cooling, 35% HCl (about 2 mL) was added at -5 °C until PH ≈ 8. The mixture was placed in refrigerator, left overnight and then filtered. The solid product was washed with H₂O and subsequently dried and recrystallized in EtOH to obtain 2.20g (0.0054 mol) of **10** as a yellow solid, with a yield of 32%. m.p. 243-249 °C. UV λ_{max} 355.1 nm. ¹H NMR (400MHz, DMSO-d₆): δ (ppm) 7.59 (1H, d, H₆), 7.39(1H, m), 7.28 (2H, brs), 6.75(1H, m), 6.26 (2H, br). ¹³C-NMR (400MHz, DMSO-d₆): δ (ppm) 153.03, 133.99, 130.72, 129.60, 115.52, 114.59. ESI-MS [M+H]⁺: calcd for C₁₂H₁₄N₄O₄S₄, 405.99; found 406.45.

3.2.4. General Method for the Synthesis of 2-Arylbenzothiazoles Substituted on the Benzothiazole Ring

To a solution of 4-amino-3-mercaptopbenzoic acid (0.49 g, 2.9 mmol) or bis(2-amino-4-benzenesulfonamide) disulfide (2.35 g, 5.8 mmol) and corresponding benzaldehydes in ethanol (10 mL) was added a solution of sodium hydrosulfite (1 eq. in 5 mL H₂O) at room temperature. The mixture was heated at reflux for 36h. After cooling, the solution was concentrated in vacuum. The residue was treated with HCl 2N (15 mL) and filtered to obtain the crude solid, which was recrystallized in MeOH/H₂O.

3.2.4.1. 2-(2-hydroxyphenyl)benzo[d]thiazole-6-carboxylic acid (11b). Yield 71%; yellow solid; m.p. >250 °C; UV λ_{max} 338.5 nm. IR (KBr) cm⁻¹: 3111.6, 1677.8, 1590.6, 1481.8, 1217.7, 1167.1, 739.9. ¹H NMR (400MHz, DMSO-d₆) δ (ppm) 11.40 (br, 1H, COOH), 9.82 (brs, 1H, OH), 8.61 (s, 1H, BTZ), 8.12 (d, J= 8.4Hz, 1H, BTZ), 7.74-7.68 (m, 2H, 1H BTZ, 1H Ar), 7.47 (t, J= 6.8Hz, 1H, Ar), 7.21-7.16 (m, 2H, Ar). ¹³C-NMR (400MHz, DMSO-d₆): δ(ppm) 173.46, 171.10, 153.55, 143.3, 132.48, 131.85, 129.48, 127.48, 127.33, 124.28, 122.50, 119.53, 118.78, 115.88. ESI-MS [M+H]⁺: calcd for C₁₄H₉NO₃S, 271.03; found 272.24.

3.2.4.2. 2-(3-hydroxyphenyl)benzo[d]thiazole-6-carboxylic acid (11c). Yield 55%; yellow solid; m.p. >250°C; UV λ_{max} 301.1 nm. IR (KBr) cm⁻¹: 3110.6, 1676.6, 1582.6, 1481.6, 1217.6, 1142.4, 769.5. ¹H NMR (400MHz, DMSO-d₆): δ (ppm) 11.62 (brs, 1H, -COOH), 9.34 (brs, 1H, OH), 8.49 (s, 1H, BTZ), 8.02 (d, J= 8.4Hz, 1H, BTZ), 7.71 (d, J= 8.4Hz, 1H, BTZ), 7.56 (d, J= 8.4Hz, 1H, Ar), 7.28 (t, 1H, Ar), 7.11 (s, 1H, Ar), 6.95 (d, J= 8.4Hz, 1H, Ar). ¹³C-NMR (400MHz, DMSO-d₆): δ(ppm) 167.38, 167.20, 156.84, 153.86, 146.96, 134.64, 132.67, 128.38, 127.21, 123.68, 121.49, 119.30, 118.58, 116.96. ESI-MS [M+H]⁺: calcd for C₁₄H₉NO₃S, 271.03; found 272.31.

3.2.4.3. 2-(4-hydroxyphenyl)benzo[d]thiazole-6-carboxylic acid (11d). Yield 67%; yellow solid; m.p. >250 °C; UV λ_{max} 334.3 nm. IR (KBr) cm⁻¹: 3259.1, 1680.9, 1587.4, 1418.4, 1240.2, 1167.0, 770.5. ¹H NMR (400MHz, DMSO-d₆): δ (ppm) 10.89 (brs, 1H, OH), 9.78 (brs, 1H, OH), 8.68 (s, 1H, BTZ), 8.05 (d, J= 8.4Hz, 1H, BTZ), 7.72 (d, J= 8.4Hz, 1H, BTZ), 7.59 (d, J= 8.4Hz, 2H Ar), 6.99 (d, J= 8.4 Hz, 2H, Ar). ¹³C-NMR (400MHz, DMSO-d₆): δ(ppm) 177.53, 167.38, 161.56, 157.00, 134.70, 132.53, 131.18, 129.82, 127.88, 127.43, 124.59, 124.12, 122.41, 116.65. ESI-MS [M+H]⁺: calcd for C₁₄H₉NO₃S, 271.03; found 272.20.

3.2.4.4. 2-(4-methoxyphenyl)benzo[d]thiazole-6-carboxylic acid (11e). Yield 82%; yellow solid; m.p. >250 °C; UV λ_{max} 331.9 nm. IR (KBr) cm⁻¹: 2980.8, 1683.0, 1592.8, 1480.2, 1263.2, 1085.4, 712.0. ¹H NMR (400MHz, DMSO-d₆): δ (ppm) 9.87 (brs, 1H, OH), 8.75 (s, 1H, BTZ), 8.17 (d, J= 8.4Hz, 1H, BTZ), 7.87 (d, J= 8.4Hz, 1H, BTZ), 7.14 (m, 4H, Ar) 3.8 (s, 3H, OCH₃). ¹³C-NMR (400MHz, DMSO-d₆): δ(ppm) 179.26,

170.57, 166.81, 162.12, 156.44, 134.28, 129.11, 127.38, 125.11, 124.14, 122.06, 114.77, 65.42. ESI-MS [M+H]⁺: calcd for C₁₅H₁₁NO₃S, 285.32; found 286.27.

3.2.4.5. 2-(2,4-dihydroxyphenyl)benzo[d]thiazole-6-carboxylic acid (11f). Yield 53%; yellow solid; m.p. >250 °C; UV λ_{max} 345.5 nm. IR (KBr) cm⁻¹: 3351.2, 1674.1, 1577.9, 1481.5, 1216.8, 1090.2, 779.9. ¹H NMR (400MHz, DMSO-d₆): δ (ppm) 10.22 (brs, 2H, OH), 8.79 (s, 1H, BTZ), 7.99 (dd, J= 8 Hz, J= 1.6 Hz, 2H, BTZ), 7.79 (d, J= 8.4Hz, 1H, Ar), 6.58 (s, 1H, Ar), 6.39 (d, J= 8.4 Hz, 1H, Ar). ¹³C-NMR (400MHz, DMSO-d₆): δ (ppm) 169.42, 166.99, 162.11, 152.93, 135.48, 133.66, 129.89, 128.16, 122.84, 120.21, 111.51, 108.04, 104.64, 103.29. ESI-MS [M+H]⁺: calcd for C₁₄H₉NO₄S, 287.29; found 288.30.

3.2.4.6. 2-(2,5-dihydroxyphenyl)benzo[d]thiazole-6-carboxylic acid (11g). Yield 75%; yellow solid; m.p. >250 °C; UV λ_{max} 305.9, 371.6 nm. IR (KBr) cm⁻¹: 3440.7, 2533.3, 1676.8, 1596.9, 1493.6, 1288.5, 1180.4, 780.5. ¹H NMR (400MHz, DMSO-d₆): δ (ppm) 13.07 (brs, 1H, OH), 10.82 (s, 1H, OH), 9.19 (s, 1H, OH), 8.71 (d, J= 1.2Hz, 1H, BTZ), 8.07-8.02 (m, 2H, BTZ), 7.67(d, J= 2.8Hz, 1H, Ar), 6.85-6.92 (m, 2H, Ar). ¹³C-NMR (400MHz, DMSO-d₆): δ (ppm), 167.50, 154.77, 149.91, 127.64, 124.52, 122.25, 121.25, 119.14, 118.28, 113.48, 110.00. ESI-MS [M+H]⁺: calcd for C₁₄H₉NO₄S, 287.29; found 288.23.

3.2.4.7. 2-(3,4-dihydroxyphenyl)benzo[d]thiazole-6-carboxylic acid (11h). Yield 69%; yellow solid; m.p. >250 °C; UV λ_{max} 346.2 nm. IR (KBr) cm⁻¹: 3487.4, 1681.8, 1594.1, 1417.7, 1274.7, 1180.8, 770.3. ¹H NMR (400MHz, DMSO-d₆): δ (ppm) 12.90 (brs, 1H, OH), 9.78 (s, 1H, OH), 9.45 (s, 1H, OH), 8.64 (s, 1H, BTZ), 8.02 (m, 2H, 1H BTZ, 1H Ar), 7.55 (d, J= 1.2Hz, 1H, BTZ), 7.43 (d, J= 8.2Hz, 1H, Ar), 6.92 (d, J= 8.2Hz, 1H, Ar). ¹³C-NMR (400MHz, DMSO-d₆): δ (ppm) 171.12, 166.85, 156.46, 149.48, 145.77, 134.12, 127.29, 126.81, 123.99, 123.89, 121.83, 119.77, 116.09, 114.00. ESI-MS [M+H]⁺: calcd for C₁₄H₉NO₄S, 287.23; found 288.23.

3.2.4.8. 2-(3-hydroxy-4-methoxyphenyl)benzo[d]thiazole-6-carboxylic acid (11i). Yield 69%; yellow solid; m.p. >250 °C; UV λ_{max} 341.1 nm. IR (KBr) cm⁻¹: 3547.6, 2956.4, 1702.9, 1587.4, 1477.6, 1217.2, 1142.4, 769.5. ¹H NMR (400MHz, DMSO-d₆): δ (ppm) 9.68 (brs, 1H, OH), 8.72 (s, 1H, BTZ), 8.05 (d, J= 8Hz, 2H, BTZ), 7.58 (d, J= 8Hz, 1H, Ar), 7.50 (s, 1H, Ar) 7.11 (d, J= 8Hz, 1H, Ar), 3.84 (s, 3H, OCH₃). ¹³C-NMR (400MHz, DMSO-d₆): δ (ppm) 171.11, 167.46, 156.74, 151.51, 147.47, 134.72, 127.93, 125.79, 124.52, 122.47, 119.93, 114.09, 112.82, 56.46. ESI-MS [M+H]⁺: calcd for C₁₅H₁₁NO₄S, 301.04; found 302.19.

3.2.4.9. 2-(3,5-dihydroxyphenyl)benzo[d]thiazole-6-carboxylic acid (11j). Yield 59%; yellow solid; m.p. >250 °C; UV λ_{max} 313.2 nm. IR (KBr) cm⁻¹: 3481.4, 1681.0, 1589.8, 1423.2, 1270.1, 1182.9, 772.8. ¹H NMR (400MHz, DMSO-d₆) δ: (ppm) 10.78 (brs, 2H, OH), 8.84 (s, 1H, BTZ), 8.18 (d, J= 8Hz, 1H, BTZ), 7.85 (d, J= 8Hz, 1H, BTZ), 6.95 (s, 2H, Ar), 6.25 (s, 1H, Ar). ¹³C NMR (400MHz, DMSO-d₆): δ (ppm) 170.10, 166.46, 158.73, 157.49, 136.60, 133.72, 133.63, 127.03, 123.92, 122.13, 105.51, 104.57. ESI-MS [M+H]⁺: calcd for C₁₄H₉NO₄S, 287.23; found 288.12.

3.2.4.10. 2-(2,4,5-trihydroxyphenyl)benzo[d]thiazole-6-carboxylic acid (11k). Yield 63%; grey solid; m.p. >250 °C; UV λ_{max} 378.0 nm. IR (KBr) cm⁻¹: 3498.0, 1689.7, 1611.5, 1455.0, 1277.5, 1186.0, 775.3. ¹H NMR (400MHz, DMSO-d₆): δ (ppm) 10.24 (brs, 3H, OH), 8.90 (s, 1H, BTZ), 8.23(d, J= 8Hz, 1H, BTZ), 8.07 (d, J= 8Hz, 1H, BTZ), 6.96 (s, 1H, Ar), 6.19 (s, 1H, Ar). ¹³C-NMR (400MHz, DMSO-d₆): δ (ppm) 170.45,

166.11, 157.14, 155.56, 149.12, 138.45, 135.38, 126.70, 125.76, 122.09, 121.05, 113.41, 108.50, 107.17. ESI-MS $[M+H]^+$: calcd for $C_{14}H_9NO_5S$, 303.02; found 304.23.

3.2.4.11. *2-(2-hydroxyphenyl)benzo[d]thiazole-6-sulfonamide (11m)*. Yield 78%; yellow solid; m.p. >250 °C; UV λ_{max} 335.6 nm. IR (KBr) cm^{-1} : 3316.0, 3242.7, 1584.2, 1483.7, 1312.7, 1151.5, 746.0. 1H NMR (400MHz, DMSO- d_6): δ 9.82 (brs, 1H, OH), 8.48 (s, 1H, BTZ), 7.96 (d, $J=8.2$ Hz, 1H, BTZ), 7.82 (d, $J=8.2$ Hz, 1H, BTZ), 7.64 (d, $J=8.2$ Hz, 1H, Ar), 7.36 (t, 1H, Ar), 7.28 (brs, 2H, NH_2), 7.08-6.95(m, 2H, Ar). ^{13}C NMR (400MHz, DMSO- d_6): δ (ppm) 177.67, 156.83, 154.66, 140.63, 135.33, 133.45, 129.02, 124.35, 122.86, 120.47, 120.04, 119.09, 117.34. ESI-MS $[M+H]^+$: calcd for $C_{13}H_{10}N_2O_3S_2$, 306.01; found 307.19.

3.2.4.12. *2-(3-hydroxyphenyl)benzo[d]thiazole-6-sulfonamide (11n)*. Yield 78%; yellow solid; m.p. >250 °C; UV λ_{max} 299.5 nm. IR (KBr) cm^{-1} : 3312.9, 3206.3, 1584.6, 1461.6, 1300.8, 1290.4, 1152.4, 780.0. 1H NMR (400MHz, DMSO- d_6): δ (ppm) 9.28 (brs, 1H, OH), 8.51 (s, 1H, BTZ), 8.02 (d, $J=8.2$ Hz, 1H, BTZ), 7.88 (d, $J=8.2$ Hz, 1H, BTZ), 7.54 (d, $J=8.2$ Hz, 1H, Ar), 7.38-26 (m, 2H, Ar), 7.18 (brs, 2H, NH_2), 6.83 (d, $J=8.2$ Hz, 1H, Ar). ^{13}C -NMR (400MHz, DMSO- d_6): δ (ppm) 170.95, 157.96, 155.05, 140.75, 134.35, 133.47, 130.59, 124.08, 123.05, 120.55, 119.05, 118.23, 113.45. ESI-MS $[M+H]^+$: calcd for $C_{13}H_{10}N_2O_3S_2$, 306.01; found 307.22.

3.2.4.13. *2-(4-hydroxyphenyl)benzo[d]thiazole-6-sulfonamide (11o)*. Yield 84%; yellow solid; m.p. > 250 °C; UV λ_{max} 331.2 nm. IR (KBr) cm^{-1} : 3337.2, 3230.2, 1605.7, 1478.3, 1303.0, 1273.0, 1147.8, 832.8. 1H NMR (400MHz, DMSO- d_6): δ (ppm) 10.39 (brs, 1H, OH), 8.58 (s, 1H, BTZ), 8.15 (d, $J=8.2$ Hz, 1H, BTZ), 7.94 (d, $J=8.2$ Hz, 1H, BTZ), 7.84 (dd, $J=8.2$ Hz, $J=1.6$ Hz, 2H, Ar), 7.44 (brs, 2H, NH_2), 6.96 (d, $J=8.2$ Hz, 2H, Ar). ^{13}C -NMR (400MHz, DMSO- d_6): δ (ppm) 169.19, 157.72, 152.48, 136.42, 133.16, 129.03, 127.29, 125.95, 123.95, 122.28, 116.56. ESI-MS $[M+H]^+$: calcd for $C_{13}H_{10}N_2O_3S_2$, 306.01; found 307.12.

3.2.4.14. *2-(4-methoxyphenyl)benzo[d]thiazole-6-sulfonamide (11p)*. Yield 80%; yellow solid; m.p. > 250 °C; UV λ_{max} 328.3 nm. IR (KBr) cm^{-1} : 3329.4, 3258.2, 1605.4, 1480.2, 1304.0, 1261.2, 1165.2, 829.9. 1H NMR (400MHz, DMSO- d_6): δ (ppm) 8.59 (s, 1H, BTZ), 8.17 (d, $J=8.2$ Hz, 1H, BTZ), 8.09 (dd, $J=8.2$ Hz, $J=1.6$ Hz, 2H, Ar), 7.97 (d, $J=8.2$ Hz, 1H, BTZ), 7.43 (brs, 2H, NH_2), 7.18 (d, $J=8.2$ Hz, 2H, Ar), 3.83 (s, 3H, $-OCH_3$). ^{13}C -NMR (400MHz, DMSO- d_6): δ (ppm) 171.19, 162.72, 155.81, 140.90, 134.75, 129.70, 125.50, 124.60, 123.14, 120.96, 115.34, 56.02. ESI-MS $[M+H]^+$: calcd for $C_{14}H_{12}N_2O_3S_2$, 320.03; found 321.23.

3.2.4.15. *2-(2,4-dihydroxyphenyl)benzo[d]thiazole-6-sulfonamide (11q)*. Yield 74%; yellow solid; m.p. > 250 °C; UV λ_{max} 344.5 nm. IR (KBr) cm^{-1} : 3338.4, 3230.1, 1592.6, 1474.6, 1340.1, 1296.7, 1150.5, 807.5. 1H NMR (400MHz, DMSO- d_6): δ (ppm) 9.84 (brs, 1H, OH), 9.76 (brs, 1H, OH), 8.50 (s, 1H, BTZ), 8.02 (d, $J=8.2$ Hz, 1H, BTZ), 7.88 (d, $J=8.2$ Hz, 1H, BTZ), 7.52 (d, $J=8.2$ Hz, 1H, Ar), 7.21 (brs, 2H, NH_2), 6.48 (m, 1H, Ar), 6.31 (s, 1H, Ar). ^{13}C NMR (400MHz, DMSO- d_6): δ (ppm) 168.84, 162.47, 153.86, 139.97, 133.98, 130.61, 129.60, 124.24, 122.02, 120.46, 114.59, 109.05, 102.97. ESI-MS $[M+H]^+$: calcd for $C_{13}H_{10}N_2O_4S_2$, 322.01; found 323.14.

3.2.4.16. *2-(2,5-dihydroxyphenyl)benzo[d]thiazole-6-sulfonamide (11r)*. Yield 55%; yellow solid; m.p. > 250 °C; UV λ_{max} 301.0, 369.0 nm. IR (KBr) cm^{-1} : 3347.9, 3263.1, 1601.6, 1496.5, 1342.4, 1215.5.

1155.5, 765.5. ^1H NMR (400MHz, DMSO- d_6): δ (ppm) 10.82 (brs, 1H, OH), 9.2 (brs, 1H, OH), 8.60 (s, 1H, BTZ), 8.19 (d, J = 8.2 Hz, 1H, BTZ), 7.98 (dd, J = 8.2 Hz, J = 1.6 Hz, 1H, BTZ), 7.64 (d, J = 1.6 Hz, 1H, Ar), 7.42 (brs, 2H, NH_2), 6.98- 6.83 (m, 2H, Ar). ^{13}C -NMR (400MHz, DMSO- d_6): δ (ppm) 167.95, 153.67, 150.57, 149.88, 140.51, 135.44, 124.29, 122.78, 121.32, 120.68, 119.14, 118.27, 113.46. ESI-MS $[\text{M}+\text{H}]^+$: calcd for $\text{C}_{13}\text{H}_{10}\text{N}_2\text{O}_4\text{S}_2$, 322.01; found 322.19.

3.2.4.17. *2-(3,4-dihydroxyphenyl)benzo[d]thiazole-6-sulfonamide (11s)*. Yield 69%; yellow solid; m.p. > 250 °C; UV λ_{max} 344.1 nm. IR (KBr) cm^{-1} : 3499.0, 3307.1, 1603.0, 1490.8, 1335.7, 1279.3, 1145.2, 825.2. ^1H NMR (400MHz, DMSO- d_6): δ (ppm) 9.37 (brs, 2H, OH), 8.47 (s, 1H, BTZ), 8.12 (d, J = 8.2Hz, 1H, BTZ), 7.94 (d, J = 8.2Hz, 1H, BTZ), 7.41 (brs, 2H, NH_2), 7.28 (d, J = 8.2Hz, 1H, Ar), 7.03 (s, 1H, Ar), 6.81 (d, J = 8.2Hz, 1H, Ar). ^{13}C NMR (400MHz, DMSO- d_6): δ (ppm) 170.12, 155.36, 149.55, 145.80, 140.09, 134.04, 123.92, 123.74, 122.30, 120.25, 119.77, 116.12, 114.04. ESI-MS $[\text{M}+\text{H}]^+$: calcd for $\text{C}_{13}\text{H}_{10}\text{N}_2\text{O}_4\text{S}_2$, 322.01; found 322.16.

3.2.4.18. *2-(3-hydroxy-4-methoxyphenyl)benzo[d]thiazole-6-sulfonamide (11t)*. Yield 78%; yellow solid; m.p. > 250 °C; UV λ_{max} 354.8 nm. IR (KBr) cm^{-1} : 3329.4, 2957.7, 1605.4, 1486.5, 1313.4, 1270.8, 1169.9, 826.4. ^1H NMR (400MHz, DMSO- d_6): δ (ppm) 9.41 (brs, 1H, OH), 8.48 (s, 1H, BTZ), 8.06 (d, J = 8.1Hz, 1H, BTZ), 7.92 (d, J = 8.1Hz, 1H, BTZ), 7.47 (d, J = 8.1Hz, 1H, Ar), 7.33 (brs, 2H, NH_2), 7.16 (s, 1H, Ar), 6.94 (d, J = 8.1Hz, 1H, Ar), 3.88 (s, 3H, $-\text{OCH}_3$). ^{13}C NMR (400MHz, DMSO- d_6): δ (ppm) 168.05, 155.54, 148.98, 145.92, 142.11, 134.51, 122.43, 122.01, 121.79, 120.00, 118.70, 116.86, 114.04, 56.98. ESI-MS $[\text{M}+\text{H}]^+$: calcd for $\text{C}_{14}\text{H}_{12}\text{N}_2\text{O}_4\text{S}_2$, 336.02; found 337.38.

3.2.4.19. *2-(3,5-dihydroxyphenyl)benzo[d]thiazole-6-sulfonamide (11u)*. Yield 63%; yellow solid; m.p. > 250 °C; UV λ_{max} 354.1 nm. IR (KBr) cm^{-1} : 3499.7, 3312.1, 1623.3, 1492.2, 1338.2, 1278.9, 1144.6, 826.1. ^1H NMR (400MHz, DMSO- d_6): δ (ppm) 9.48 (brs, 2H, OH), 8.39 (s, 1H, BTZ), 8.01 (d, J = 8.1Hz, 1H, BTZ), 7.86 (d, J = 8.1Hz, 1H, BTZ), 7.40 (brs, 2H, NH_2), 6.91 (s, 2H, Ar), 6.35 (s, 1H, Ar). ^{13}C NMR (400MHz, DMSO- d_6): δ (ppm) 166.43, 158.23, 157.17, 137.56, 132.80, 132.02, 127.43, 123.55, 122.37, 105.98, 104.24. ESI-MS $[\text{M}+\text{H}]^+$: calcd for $\text{C}_{13}\text{H}_{10}\text{N}_2\text{O}_4\text{S}_2$, 322.01; found 323.04.

3.2.4.20. *2-(2,4,5-trihydroxyphenyl)benzo[d]thiazole-6-sulfonamide (11v)*. Yield 51%; grey solid; m.p. > 250 °C; UV λ_{max} 353.9nm. IR (KBr) cm^{-1} : 3350.3, 3264.8, 1609.5, 1495.8, 1341.9, 1216.3, 1157.4, 766.8. ^1H NMR (400MHz, DMSO- d_6): δ (ppm) 9.57 (brs, 3H, OH), 8.39 (s, 1H, BTZ), 8.08 (d, J = 8.2Hz, 1H, BTZ), 7.91 (d, J = 8.2Hz, 1H, BTZ), 7.32 (brs, 2H, NH_2), 6.88 (s, 1H, Ar), 6.41 (s, 1H, Ar). ^{13}C -NMR (400MHz, DMSO- d_6): δ (ppm) 166.61, 157.90, 150.80, 149.87, 138.15, 133.86, 126.90, 124.45, 121.84, 121.60, 113.90, 111.24, 105.56. ESI-MS $[\text{M}+\text{H}]^+$: calcd for $\text{C}_{13}\text{H}_{10}\text{N}_2\text{O}_5\text{S}_2$, 338.00; found 339.18.

3.3. Biological Studies

3.3.1. Antioxidant Activity Assays

Free radical Scavenging Activity on DPPH. The following assay is modified procedure described by Wang at al. [64] The methanolic solution of DPPH (1.5 mL) was added to 0.750 mL of tested sample solutions (solubilized in DMSO and diluted in MeOH) at different concentrations (1, 0.5, 0.25, 0.125 and 0.0625

mg/mL). The samples were kept in the dark at room temperature. After 30 min, the absorbance values were measured using UV-VIS spectrophotometer at 517 nm using UV-VIS spectrophotometer and were converted into the percentage antioxidant activity (%) using the **Equation 1**.

Equation 1: DPPH radical-scavenging capacity (%) = $[1 - A_1 / A_0] \times 100\%$

Where A_0 was the absorbance of the control (without sample) and A_1 was the absorbance in the presence of the sample. Linear regression a plot is carried out for calculating the effective concentration of the sample required to scavenge 50% of DPPH free radicals.

Ferric Reducing Antioxidant Power (FRAP) assay. Ferric reducing antioxidant power (FRAP) assay is a widely used method that uses antioxidants as reductants in a redox-linked colorimetric reaction and it is based on the reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) in the presence of TPTZ (2,4,6-tripyridyl-striazine). This reaction is monitored by measuring the change in absorbance at 593 nm. The analysis reagent was prepared according to Benzie [65] procedure by mixing the following solutions in the reported ratio 10/1/1 (v:v:v): i) 0.1 M acetate buffer pH 3.6, ii) TPTZ 10 mmol/L in 40 mmol/ HCl, iii) ferric chloride 20 mmol/L. To 1.9 mL of reagent were added 0.1 mL of sample proper diluted or solvent when blank was performed. FRAP values are obtained by comparing the absorbance change at 593 nm in sample solution with the absorbance of the blank reaction, using a UV-VIS spectrophotometer.

3.3.2. *In vitro* Photoprotection assay

3.3.2.1. Evaluation of filtering parameters of compounds in solution. Sun protection factor evaluation was performed by means of a UV-VIS spectrophotometer SHIMADZU UV-2600 240 V. Test compounds were dissolved in dimethylsulfoxide at the concentration of 0.0015 (\pm 0.000005) % and the absorbance was measured between 290-400 nm using a 1 cm quartz cell at intervals of one 1 nm using a UV-Vis Spectrophotometer. The absorbance at wavelength λ is related to the transmittance ($T(\lambda)$) by the **Equation 2**

Equation 2: $A(\lambda) = -\text{Log}[T(\lambda)]$

Where $T(\lambda)$ is the fraction of incident irradiance transmitted by the sample.

3.3.2.2. *In vitro* evaluation of filtering parameters of formulation. The selected synthesized molecules were included at the concentration of 1% in a topical formulation O/W in order to evaluate their UV -filter activity INCI: aqua, glycerin, Euxyl PE 9010, Xanthan gum, cetareth-12, cetareth-20, Stearic acid, Butylhydroxytoluene, Myritol, PEG-7 glycerylcocoate and sodium solution 10%.

WW5 PMMA plates have been purchased from Schonberg GmbH (Munich, Germany). 32.5 mg of each compound formulation O/W were spread onto 5 x 5 cm² PMMA plates in small droplets of approximately equal mass evenly distributed over the rough surface of the plate. For each product, three plates were prepared and left to dry at room temperature in the dark for 15-30 min before exposure, in order to facilitate the formation of a standard stabilized sunscreen film. After this period, the plates were placed in the light path of the transmittance analyzer. Then the measure of UV transmittance was carried out from 290 to 400 nm on 5 different sites of each plate. The blank was prepared using a plate covered with 32.5 mg of glycerin. This

substrate allows to have a reference spectrum with a transmission close to 100% due to its non-fluorescent and UV transparency.

Moreover, Values of *in vitro* SPF, UVA-PF and Critical Wavelength were calculated according to **Equation 3**, **Equation 4** and **Equation 5** [66] respectively using SPF Calculator Software (SPF Calculator Software (version 2.1), Shimadzu, Milan, Italy).

$$\text{Equation 3: } \text{SPF}_{\text{in vitro}} = \frac{\int_{\lambda=290\text{nm}}^{\lambda=400\text{nm}} E(\lambda) \times I(\lambda) \times d\lambda}{\int_{\lambda=290\text{nm}}^{\lambda=400\text{nm}} E(\lambda) \times I(\lambda) \times 10^{-A_0(\lambda)} \times d\lambda}$$

Where $E(\lambda)$ is the erythema action spectrum, $I(\lambda)$ is the spectral irradiance of the UV source, $A_0(\lambda)$ is the monochromatic absorbance of the test sample before UV exposure, and $d\lambda$ is the wavelength step (1 nm)

$$\text{Equation 4: } \text{UVA} - \text{PF} = \frac{\int_{\lambda=290\text{nm}}^{\lambda=400\text{nm}} P(\lambda) \times I(\lambda) \times d\lambda}{\int_{\lambda=290\text{nm}}^{\lambda=400\text{nm}} P(\lambda) \times I(\lambda) \times 10^{-A(\lambda) \times C} \times d\lambda}$$

Where $P(\lambda)$ is the persistent pigment darkening (PPD) action spectrum. C is the coefficient of adjustment. $A(\lambda)$ is the mean monochromatic absorbance of the test product layer after UV exposure.

The wavelength at which the summed absorbance reaches 90% of total absorbance is defined as the critical wavelength. Therefore (T_λ) were converted into absorbance values $A(\lambda)$ following **Equation 5** and final Critical Wavelength is:

$$\text{Equation 5: } \int_{290\text{nm}}^{\lambda_c} A\lambda \times d\lambda = 0.9 \int_{290\text{nm}}^{400\text{nm}} A\lambda \times d\lambda$$

Where $A(\lambda)$: monochromatic absorbance calculated from transmittance at wavelength λ

3.3.3. Antifungal activity

3.3.3.1. Antidermatophyte activity

Microorganisms. The dermatophytes used were *Epidermophyton floccosum* var. *floccosum* (Netherlands) CBS 358.93 strain; *Trichophyton tonsurans* (Netherlands) CBS 483.76 strain; *Trichophyton mentagrophytes* (Netherlands) CBS 160.66 strain; *Microsporum canis* (Iran) CBS 131110 strain; and *Microsporum gypseum* (Iran) CBS 130948 strain. All dermatophytes were maintained at 4°C as agar slants on Sabouraud dextrose agar (SDA; Sigma-Aldrich SRL, Milano, Italy).

Culture. *In vitro* antifungal activity against five dermatophytes was evaluated using plate growth rate method. Each compound was dissolved in DMSO. Suitable solution adjusted to 0.1% was added to the sterile culture medium (SDA) at 45°C. The final concentration of the medium was 100 $\mu\text{g}\cdot\text{mL}^{-1}$. The mixture was homogenized and transferred to a sterile Petri dish to solidify. Inoculums were previously obtained by transplanting mycelium disks cut from the periphery of a single mother cultures, incubated on thin sheets of

cellophane delicately extended on the agar plates and placed at 26°C until the appearance of some characters that show how the fungus grew. According to the poisoned-food technique [67], inoculums were then transferred to analyzed Petri dish and incubated under growth conditions. The mixed medium (SDA + DMSO) without sample was used as the blank control. Antifungal activity was determined by measuring the diameter of the inhibition zone for seven days. Percentages of growth inhibition were calculated by comparing mean value of diameters of the mycelia in test plates with that of untreated control plates following the **Equation 6**. The percent inhibition of growth was determined as the average of three different experiments.

Equation 6: $I = [(C - T)/C] \cdot 100\%$.

Where, I is the growth inhibition rate (%), C is the extended diameter of the circle mycelium of the control (mm), and T is the extended diameter of the circle during testing (mm).

3.3.3.2. Anti-*Candida albicans* activity

Stock solutions were prepared by dissolving the compounds and Fluconazole in DMSO at a concentration of 12.80 mg/mL. Minimal inhibitory concentration (MIC) of the synthesized agents was performed by broth microdilution method according to the Clinical and Laboratory Standards Institute/National Committee for Clinical Laboratory Standards (CLSI/NCCLS) Approved Standard M27-A3, 2008 [NCCLS]. RPMI 1640 was buffered with 0.165 M MOPS, solution was agitated and brought to pH 6.9 with a 1N NaOH solution. The solution was filtered with 0.45 µm sterilized filter and stocked in fridge. Just before setting up the assay, further dilute of stock solutions was dilute in 1:10 RPMI supplemented with Tween 80 (used as blank). Dilutions, 11 increasing concentrations, ranging from 0.25 to 128 µg·mL⁻¹ of the compounds, were prepared in 96-well plates. The inoculum size was about 2.5 × 10³ cells·mL⁻¹. The plates were incubated at 30 °C for 24–48 h.

3.3.4. Antiproliferative activity

Cell lines. Human cervical carcinoma (HeLa), human CD₄⁺T-lymphoblast (CEM) and human melanoma cells (SK-Mel 5) cells were obtained from ATCC (Middlesex, UK). Human pancreatic carcinoma (Mia-Paca 2) cells were kindly provided by Prof. Anna Karlsson (Karolinska Institute, Stockholm, Sweden). All cell lines were grown in Dulbecco's modified Eagle's medium (DMEM; Gibco, Carlsbad, CA, USA), supplemented with 10% fetal bovine serum (FBS, Gibco), 0.01M HEPES (Gibco) and 1 mM sodium pyruvate (Gibco) in a humidified 5% CO₂ incubator at 37°C.

Cell proliferation. Suspension of CEM cells were seeded in 96-well microtiter plates at 60,000 cells/well in the presence of different concentrations of the compounds. The cells were allowed to proliferate for 48 h or 72 h, respectively, and then counted in a Coulter counter. The 50% inhibitory concentration (IC₅₀) was defined as the compound concentration required to reduce cell proliferation by 50%. HeLa and Mia-Paca2 cells were

seeded in 96-well plates at 15,000 cells/well in the presence of different concentrations of the compounds. After 4 days of incubation, the cells were trypsinized and counted in a Coulter counter).

IC₅₀ Determination. The compounds were dissolved in DMSO at 20 mM (stock solution) and kept in the refrigerator until use. Then, compound dilutions were made in cell culture medium, and serial compound concentrations were tested starting at 100 μM as the highest concentration. The DMSO concentration, present at the highest compound concentration was 0.5% that is at a concentration that did not affect the tumour cell proliferation. The IC₅₀ values were calculated using **Equation 7**.

$$\text{Equation 7: } C1 - [50 - N1\%/N2\% - N1\%] - (C1 - C2)$$

Wherein C1 is the compound concentration that inhibits cell proliferation more than 50%; C2 is the compound concentration that inhibits cell proliferation less than 50%; N1% represents the cell number (in percent of control in the absence of compound) obtained in the presence of C1 and N2% represents the cell number (in percent of control in the absence of compound) obtained in the presence of C2.

3.3.5. HERG expressed in HEK

Cell line. Electrophysiology study was performed using HEK293 cells stably transfected with hERG cDNA.

Reagents and solutions. Test substances were formulated in 0.1% DMSO. Stock solutions were diluted in extracellular solution plus 0.05% Pluronic F-68 (Sigma), to final perfusion concentrations, which were 0.1, 1 and 10 μM. In positive control group, 0.003-0.03-0.3 μM E-4031 (Tocris) was assessed as a test compound. The mM composition of the extracellular solution was: NaCl, KCl, CaCl₂, MgCl₂, D-glucose, N-(2-hydroxyethyl)piperazine-N'-2-(ethanesulfonic acid) (HEPES), pH 7.4 with 1 M NaOH (137:4:1.8:1:10:10 respectively). The intracellular solution composition was (in mM): KCl, MgCl₂, Ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), MgATP, HEPES, pH 7.2 with 1 M KOH (130:1:5:5:10 respectively). The intracellular solution was filtered through a 0.2 μm polycarbonate membrane filter upon formulation. All reagents were purchased from Sigma-Aldrich.

Voltage protocol. The voltage protocol used to evaluate the effect of test substances on hERG channel current was as follows: step from -80 mV to -50 mV for 200 ms, +20 mV for 4.8 s, step to -50 mV for 5 s then step to the holding potential of -80 mV. The step from -80 mV to the test command (+20 mV) results in an outward current (i.e. current flows out of the cell) and the step from the test command (+20 mV) to -50 mV results in the tail current. The pulse pattern was repeated continuously at 15 sec intervals from a holding potential of -80 mV.

The voltage protocol was run and recorded continuously during the experiment. The stability of recording was assessed through initial wash with extracellular solution alone. The vehicle was then applied for 3 min followed by the test substance. The test substance was applied in triplicate to ensure adequate mixing. The standard

combined exposure time was 5 min. Each test substance was tested in at least two cells for every concentration. All experiments were performed at room temperature.

Data analysis. Data acquisition and analyses were performed using the QPatch Assay software. The average of current amplitude values recorded from 4 sequential voltage pulses were used to calculate for each cell the effect of the test substance by calculating the residual current (% control) compared with vehicle pre-treatment and the % of inhibition (**Equation 8**).

The normalized data were plotted against the concentration of compound. The $-\text{Log}$ of concentrations of compounds required to decrease current by 50% (pIC_{50}) were determined by fitting the data to a Hill equation with $n \geq 2$

Equation 8: $I/I_0 = 1/[1+([C]/\text{IC}_{50})^{nH}]$, with $\text{pIC}_{50} = -\log\text{IC}_{50}$

Where I_0 and I are the current amplitudes measured in the absence and presence of test compound, respectively, $[C]$ is the concentration of compound in the external solution and nH is the Hill coefficient.

3.4. Study of photostability

Each UV-filter incorporated in an oil-in-water (O/W) emulsion were spread on PMMA plate and irradiated with a solar simulator applying different UVA-dose equivalent to an effective erythema radiant exposure of 120 kJ/m^2 . The spectral transmittance of thin film of sunscreen was measured before and after sunlight exposure from 290 to 400 nm. The residual percentage of SPF *in vitro* (% SPF_{eff}) and UVA-PF (% UVA- PF_{eff}) were calculated according to **Equations 9** and **10** respectively. In fact, a filter is considered photostable if % SPF_{eff} and % UVA- PF_{eff} are more than or equal to 80.

Equation 9: $\% \text{SPF}_{\text{eff}} = \text{in vitro SPF}_{\text{after}} / \text{in vitro SPF}_{\text{before}} \times 100$

Equation 10: $\% \text{UVA} - \text{PF}_{\text{eff}} = \text{UVA-PF}_{\text{after}} / \text{UVA-PF}_{\text{before}} \times 100$

4. Conclusions

The development of multifunctional compounds is of great importance due to the correlation of biological networks. For this purpose, a series of benzothiazole derivatives was synthesized and investigated for its *in vitro* sunscreen UV-filter capacity, antioxidant, antifungal and antiproliferative activities. Regarding sunscreen UV-filter capacity, compounds **4g**, **4h**, **4k**, **11d**, **11g**, **11o** and **11r** could be considered as broad-spectrum and also UVA-filter compounds since their $\lambda_c \geq 370 \text{ nm}$ and the UVA-PF value of each compound was greater than 1/3 of its SPF value. While compounds **4d** and **4j** were potential compounds for UVB protection. In addition, photostability studies showed that all synthesized compounds are photostable especially **11e** with a degradation rate less than 2%. In terms of antioxidant activity, compounds of classes **F** and **H**, characterized

respectively by the presence of two hydroxyl groups in positions 3,4 and 2,5 of the 2-aryl ring showed good antioxidant profile. According to antifungal activity, compound **4h** showed the highest activity against all five dermatophytes, in particular against *Microsporum gypseum*, *Trichophyton tonsurans* and *Epidermophyton floccosum*. Moreover, significant activity was also observed for compounds **4f** and **4g** against *Microsporum gypseum* and *Epidermophyton floccosum*. With regard to the antiproliferative activity, compounds **4g** and **4k** are the potential compounds against CEM and SK-Mel 5 tumor cells, however, **4k** showed the best activity: it was respectively 6-fold more selective for CEM and 25-fold more selective toward SK-Mel 5 cells. Furthermore, compounds **4e**, **11n** and **11o** were respectively 15, 18 and 5 fold more selective toward Mia-Paca-2 cells. Considering that inhibition of HERG channels reduces proliferation and impairs invasiveness in several cancer cell types, hERG channels expressed in HEK cells can be target receptor for compounds with good anticancer activity. In these regards we may conclude that compound **4g** could be the potential candidate for blocking hERG potassium channels.

Taking into account all the biological evaluation, **4g** and **4k** could be potential compounds for the design of multifunctional drugs because of their various biological activities. In addition, the benzothiazole derivatives could have a possible application as drugs for the treatment of neoplastic diseases including childhood leukemia, pancreatic cancer and melanoma. All together the data obtained have highlighted interesting activities sufficient to propose this scaffold as potential usefulness in the design of multifunctional compounds. Finally, the activity on **Mia-Paca-2**, **SK-Mel5** and **CEM** cells paralleled by hERG channel inhibition warrant further investigation because of the importance in antitumor therapy.

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- Multifunctional compounds are smart strategy for the treatment of multifactorial diseases.
- Benzothiazole derivatives were evaluated for their UV-filtering, antioxidant, antifungal and antiproliferative activities.
- Compounds **4g** and **4k** were potential multifunctional compounds.

Journal Pre-proofs

Declaration of interests

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.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: