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Synthesis and antimicrobial properties of 3-aryl-1-(1,1′-biphenyl-4-yl)-2-(1H-imidazol-1-yl)propanes as 'carba-analogues' of the *N*-arylmethyl-*N*-[(1,1′-biphenyl)-4-ylmethyl])-1H-imidazol-1-amines, a new class of antifungal agents

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Abstract

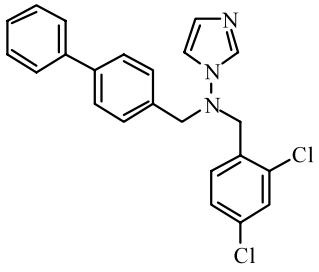
A new series of 3-phenyl-1-(1,1′-biphenyl-4-yl)-2-(1H-imidazol-1-yl)propane derivatives **2a**–**l** (related to the antifungal bifonazole) was synthesized and tested for antimicrobial activity. A number of substituents on the phenyl ring were chosen to compare the relative biological properties with those of corresponding aza-analogues, previously described by us. The in vitro antifungal activities of the newly synthesized azoles were tested against several pathogenic fungi responsible for human disease. Test pathogens included representatives of yeasts (*Candida albicans*, *Candida parapsilosis*, *Criptococcus neoformans*), dermathophytes (*Tricophyton verrucosum*, *Tricophyton rubrum*, *Microsporum gypseum*) and moulds (*Aspergillus fumigatus*). Bifonazole and miconazole were used as reference drugs. Title compounds were prepared by alkylation of 1-biphenyl-4-yl-2-imidazol-1-yl-ethanone with the proper arylmethyl halide and subsequent reduction of corresponding ketones applying the Huang–Minlon modification of the Wolff–Kishner reaction.

Keywords: Imidazole antifungal agents; Aminoimidazole isosters; Bifonazole analogues

1. Introduction

Treatment of fungal infections is an area of increasing concern. This is because of the limited number of antifungal agents available for use and the emergence of clinically resistant pathogens [1]. These infections are mostly caused by *Candida albicans* but there is a growing proportion of strains with reduced susceptibility to commonly used antifungals [2]. The past decade has seen some encouraging developments in azole antifungal therapy and there are three investigational triazoles currently undergoing evaluation: the structure of posaconazole [3] is strictly related to ketoconazole, while voriconazole [4] and ravuconazole [5] resemble fluconazole.

In searching for a completely new class of antifungal agents, recently we focused our attention on *N*-azolylamine derivatives [6] and found compound *N*-(biphenyl-4-yl)methyl-*N*-(2,4-dichlorophenyl)methyl-1H-imidazol-1-ylamine (**1**) to be the most active of the series against yeasts and dermatophytes, with potency and selectivity comparable to those of miconazole [7].



We report now the synthesis and the biological activity of the carba-analogues **2a**–**l** (Fig. 1) of pre-

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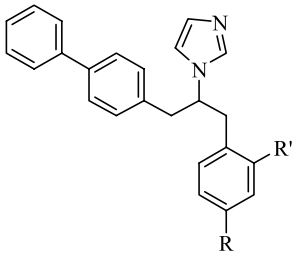


2	R	R'
a	H	H
b	Cl	Cl
c	F	F
d	H	Cl
e	H	F
f	H	Me
g	H	OMe
h	Cl	H
i	F	H
j	Me	H
k	OMe	H
l	^{t}Bu	H

Fig. 1. Newly synthesized derivatives **2a**–**l**.

viously synthesized compounds containing the *N*-imidazolylamine portion, to explore the real importance of this new pharmacophoric moiety.

It is noteworthy to observe that these structures are homologous to antifungal drug bifonazole and its derivatives (e.g. lombazole). To our knowledge, no biological data have as yet been reported for structures homologous to bifonazole, where the aryl and/or biphenyl moiety are separated by a methylene bridge from the chiral carbon. M.R. Cuberes et al. performed the synthesis of 1-(1-biphenyl-4-yl-2-phenyl-ethyl)- and 1-[2-biphenyl-4-yl-1-(2,4-dichloro-phenyl)-ethyl]-1H-imidazole [8], but antimicrobial activities of these compounds were not described. In this work we considered not fundamental to deal with the preparation/separation of enantiomers for subsequent biological evaluation, since M. Botta et al. have noticed the lack of stereoselectivity for the two enantiomers of bifonazole and related compounds [9].

2. Chemistry

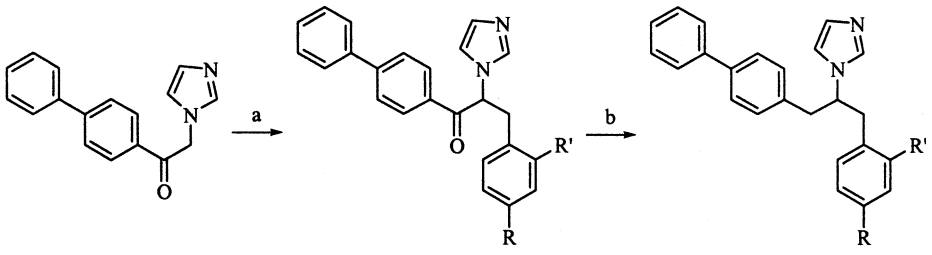
As shown in Scheme 1, the synthesis of carba-analogues (**2a**–**l**) was accomplished by reduction of the corresponding ketones (**3a**–**l**) with hydrazine hydrate and KOH in ethylene glycol, applying the Huang–Minlon modification of the Wolff–Kishner reaction [10]. Compounds (**3a**–**l**) were prepared by alkylation of 1-biphenyl-4-yl-2-imidazol-1-yl-ethanone (**4**) [11] with the proper arylmethyl halide in THF and in the presence of sodium hydride [12].

3. Biology

The title compounds were evaluated in vitro against several pathogenic fungi responsible for human disease. Test pathogens included representatives of yeasts (*Candida albicans*, *Candida parapsilosis*, *Criptococcus neoformans*), dermathophytes (*Tricophyton verrucosum*, *Tricophyton rubrum*, *Microsporum gypseum*) and moulds (*Aspergillus fumigatus*). Bifonazole and miconazole were used as reference drugs. Antibacterial activity against representative Gram positive bacteria (*Staphylococcus aureus*) and Gram negative bacteria (*Salmonella* spp.) was also evaluated and streptomycin was used as reference drug. Furthermore, title compounds were tested for in vitro cytotoxicity in a lymphoid cell line (MT-4). Cytotoxicity evaluation was performed in order to determine whether test compounds were endowed with selective antimicrobial activity.

4. Results and discussion

As shown in Table 1, all the carba-analogues **2a**–**l** displayed a fairly good antimycotic activity against yeasts and dermatophytes, generally exceeding that of bifonazole. This response was slightly influenced by the substituents on the phenyl ring and a structure activity relationship study was not crucial. It is notable to observe that unsubstituted compound **2a** proved to be the most effective in this series, followed by 2,4-difluoroderivative **2c**, in contrast to the corresponding counterpart in the set of *N*-azolylamines, which was devoid of activity [7b]. No in vitro cytotoxity was found when these derivatives were tested in the lymphoid cell line; the compound dose required to reduce the viability of MT-4 cells by 50% was superior to 100 μM. In both



4 **3a-l** **2a-l**

Scheme 1. Reagents: (a) NaH, THF, halide-CH_2-Ar; (b) ethylene glycol, hydrazine hydrate, KOH.

Table 1
In vitro antifungal activity of compounds **2a**–**l**

No.	R	CC_{50} MT-4 [a]	MIC [b]/MFC [c]					
			C. parapsilosis	*C. neoformans*	*C. albicans*	*T. verrucosum*	*M. gypseum*	*T. rubrum*
1	2,4-Cl	48	6.2/6.2	2.5/7.4	6.2/6.2	2.4/7.4	7.4/22	0.8/2.5
2a	H	> 100	0.9/5.5	5.5/5.5	1.6/ > 100	0.4/3.1	33/33	33/33
2b	2,4-Cl	> 100	5.5/33	5.5/33	8/40	5.5/33	33/ > 100	33/33
2c	2,4-F	> 100	3.2/5.5	5.5/33	≥ 5.5/19	5.5/33	33/33	5.5/5.5
2d	2-Cl	> 100	19/33	5.5/33	19.2/19	5.5/33	33/33	5.5/5.5
2e	2-F	> 100	3.2/19	5.5/33	≥ 5.5/19	5.5/33	33/33	5.5/5.5
2f	2-Me	> 100	33/33	33/33	33/ > 100	5.5/33	33/33	5.5/5.5
2g	2-MeO	82	3.2/5.5	5.5/33	≥ 5.5/19	5.5/5.5	33/33	5.5/5.5
2h	4-Cl	49	19.2/19	5.5/33	19.2/33	5.5/33	33/33	5.5/5.5
2i	4-F	96	3.2/5.5	5.5/33	5.5/19	5.5/33	33/33	5.5/5.5
2j	4-Me	> 100	19/33	33/33	19/33	5.5/33	33/33	5.5/33
2k	4-MeO	13	19/33	5.5/33	33/ > 100	5.5/33	33/33	5.5/33
2l	4-^{t}Bu	87	≥ 33/ > 100	33/33	66.5/ > 100	33/33	33/33	33/33
Bifonazole		> 100	> 100	66/ > 100	83/ > 100	7.4/22	66/66	2.5/7.4
Miconazole		18	7.8/7.8	0.8/1.6	7.0/7.0	0.4/1.6	1.6/3.1	< 0.4/0.8

[a] Compound concentration (μM) required to reduce the viability of mock-infected MT-4 cells by 50%, as determined by the MTT method.
[b] Minimum inhibitory concentration (μM).
[c] Minimum fungicidal concentration (μM).

series, the *para* substitution with a bulky group, like *tert*-butyl in **2l**, led to a decrease of activity, more evident in the case of *N*-azolylamines. Also intermediate ketones **3a**–**l** were submitted to antifungal assays and *para*-fluoroderivate **3i** exhibited activity comparable to that of bifonazole. No activity was detected against the mould strain *Aspergillus fumigatus*.

Title compounds were also evaluated for antibacterial activity (data not shown), but none showed significant activity.

In conclusion, these findings suggest that the *N*-imidazolylamine moiety is not determinant for antimycotic activity, even if the *N*-(biphenyl-4-yl)methyl-*N*-(2,4-dichlorophenyl)methyl-1H-imidazol-1-ylamine (**1**) remains the most active derivative within these two series. With respect to bifonazole, the introduction of two methylene bridges between the aromatic rings and the chiral carbon directly linked to imidazole N_1 is profitable for antimicrobial properties. These results may signify a cooperative role of methylene linkers in modulating the interaction with the enzyme by the coordination bond of imidazole N_3 lone pair to the iron ion of the porphyrin system of the cytochrome P450 14-α-lanosterol demethylase. This assumption may seem in contrast with the hypothesis of A. Tafi et al.. In a molecular modeling study of azole antifungal agents belonging to chemically diverse families related to bifonazole, they found that compounds which have an extra CH_2 group between the imidazole and the aromatic residues were less active because their aromatic residues are extended away from the favoured region of interaction [13].

5. Experimental

5.1. Chemistry

Melting points were determined in open capillary tubes with a Büchi apparatus and are uncorrected. Elemental analyses (C, H, N) were performed by Dr Emilio Cebulec at the Chemistry Department of the University of Trieste. IR spectra were obtained on a JASCO FT/IR-200 spectrophotometer (KBr). The ^{1}H-NMR spectra were determined on a Varian 200 instrument. Chemical shifts are given in δ values downfield from TMS as internal standard and coupling constants (J) are expressed in hertz. Mass spectra data were determined on a V6-Micromass 7070H mass spectrometer. Silica gel chromatography was performed using Merck silica gel 60 (0.015–0.040 mm). Petroleum ether refers to petroleum ether (40–60 °C).

5.1.1. General procedure for the preparation of 3-Aryl-1-(1,1′-biphenyl-4-yl)-2-(1H-imidazol-1-yl)propan-1-ones (**3a**–**l**)

A solution of *N*-(4-phenyl)phenacylimidazole [1-(1,1′-biphenyl-4-yl)-2-(1*H*-imidazol-1-yl)ethanone] (1.00 g, 3.81 mmol) in anhydrous THF (30 ml) was added to a suspension of NaH (100 mg, 4.20 mmol) in the same solvent (20 ml). The mixture was stirred for 1 h and a solution of the appropriate halide (3.81 mmol) in anhydrous THF (20 ml) was added dropwise. After 3 hours of stirring at 50 °C, the reaction mixture was quenched by the addition of methanol (5 ml) and evaporated. The residue was partitioned between water and diethyl ether. The organic extract was washed with a

saturated sodium chloride solution, dried over Na_2SO_4 and evaporated. The crude product was purified by silica gel column chromatography using ethyl acetate as eluent. After crystallization (ligroin/benzene) all compounds were obtained as white crystals. Yields, melting points, analytical and spectroscopic data are reported in Table 2.

5.1.2. General procedure for the preparation of 3-Aryl-1-(1,1′-biphenyl-4-yl)-2-(1H-imidazol-1-yl)propanes (**2a–l**)

A mixture of the appropriate 3-aryl-1-(1,1′-biphenyl-4-yl)-2-(1*H*-imidazol-1-yl)propan-1-one (5.64 mmol), hydrazine hydrate (1.28 ml), ethylene glycol (50 ml) and potassium hydroxide (2.68 g, 46.2 mmol) was heated at 185–190 °C for 3 h. The reaction mixture was cooled, poured into 500 ml water, acidified to pH 1.0 with concentrated hydrochloric acid and extracted with chloroform (3 × 50 ml). The organic extract was washed with a saturated sodium chloride solution (50 ml), dried over Na_2SO_4 and evaporated. The crude product was purified by silica gel column chromatography using ethyl acetate as eluent.

Compounds **2a–l** were obtained as colorless glasses. Trituration with petroleum ether followed by recrystallization (benzene–ligroin), gave compound **2f** as white solid. Compounds **2a–e**, **g–l** were crystallized (ethanol–diethyl ether) as oxalate salts. Yields, melting points, analytical and spectroscopic data are reported in Table 3.

5.2. Microbiology

5.2.1. Compounds

Test compounds were dissolved in DMSO at an initial concentration of 100 mM and then were serially diluted in culture medium.

5.2.2. Cells

Eukariotic cell lines were from American Type Culture Collection (ATCC). MT-4 cells (grown in RPMI 1640 containing 10% fetal calf serum, 100 UI/ml penicillin G and 100 μg/ml streptomycin) were used for anti-HIV-1 assays. Cell cultures were checked periodically for the absence of mycoplasma contamination with a Myco Tect Kit (Gibco). Bacterial and fungal strains were either clinical isolates obtained from Clinica Dermosifilopatica, University of Cagliari (*Staphylococcus aureus* and *Salmonella* spp.) or collection strains purchased from ATCC.

5.2.3. Antibacterial assays

Assays were carried out in nutrient broth, pH 7.2, with an inoculum of 10^3 cells/ml. Minimum inhibitory concentrations (MICs) were determined after 18 hours incubation at 37 °C in the presence of serial dilutions of

Table 2
Yields, physical, analytical and spectroscopic data of compounds **3a–l**

No.	Formula [a]	Yield (%)	M.p. (°C)	IR C=O	MS *m/z* [M^+]	^{1}H NMR: δ (ppm)
a	$C_{24}H_{20}N_2O$	52	120–121	1681	352	3.24 (dd, J = 14.1, 9.3, 1H, C*H*H); 3.48 (dd, J = 14.1, 5.2, 1H, C*H*H); 5.76 (dd, J = 9.3, 5.2, 1H, C*H*); 6.95–8.00 (m, 17H, Ar–H).
b	$C_{24}H_{18}N_2OCl_2$	50	129–130	1672	421	3.24 (dd, J = 14.3, 9.5, 1H, C*H*H); 3.64 (dd, J = 14.3, 5.5, 1H, C*H*H); 5.91 (dd, J = 9.5, 5.5, 1H, C*H*); 6.73–8.06 (m, 15H, Ar–H).
c	$C_{24}H_{18}N_2OF_2$	58	151	1681	388	3.16 (dd, J = 14.4, 9.8, 1H, C*H*H); 3.61 (dd, J = 14.4, 5.3, 1H, C*H*H); 6.00 (dd, J = 9.8, 5.3, 1H, C*H*); 6.62–8.02 (m, 15H, Ar–H).
d	$C_{24}H_{19}N_2OCl$	65	106–108	1673	386	3.27 (dd, J = 14.2, 9.8, 1H, C*H*H); 3.61 (d, J = 14.2, 5.5, 1H, C*H*H); 6.00 (dd, J = 9.8, 5.5, 1H, C*H*); 6.82–8.04 (m, 16H, Ar–H).
e	$C_{24}H_{19}N_2OF$	49	111	1677	370	3.19 (dd, J = 14.3, 9.8, 1H, C*H*H); 3.61 (dd, J = 14.3, 5.1, 1H, C*H*H); 5.90 (dd, J = 9.8, 5.1, 1H, C*H*); 6.78–8.05 (m, 16H, Ar–H).
f	$C_{25}H_{22}N_2O$	51	95	1674	366	2.21 (s, 3H, CH_3) 3.25 (dd, J = 14.3, 9.3, 1H, C*H*H); 3.43 (dd, J = 14.3, 5.4, 1H, C*H*H); 5.71 (dd, J = 9.3, 5.4, 1H, C*H*); 6.84–7.95 (m, 16H, Ar–H).
g	$C_{25}H_{22}N_2O_2$	30	100–102	1685	382	3.09 (dd, J = 13.9, 9.9, 1H, C*H*H); 3.61 (dd, J = 13.9, 4.4, 1H, C*H*H); 3.91 (s, 3H, OCH_3) 5.99 (dd, J = 9.9, 4.4, 1H, C*H*); 6.70–8.11 (m, 16H, Ar–H).
h	$C_{24}H_{19}N_2OCl_2$	47	125–126	1682	386	3.21 (dd, J = 14.0, 9.5, 1H, C*H*H); 3.43 (dd, J = 14.0, 5.1, 1H, C*H*H); 5.71 (dd, J = 9.5, 5.1, 1H, C*H*); 6.87–7.98 (m, 16H, Ar–H).
i	$C_{24}H_{19}N_2OF$	54	136–138	1677	370	3.22 (dd, J = 14.2, 9.4, 1H, C*H*H); 3.43 (dd, J = 14.2, 5.1, 1H, C*H*H); 5.71 (dd, J = 9.4, 5.1, 1H, C*H*); 6.83–7.98 (m, 16H, Ar–H).
j	$C_{25}H_{22}N_2O$	52	128	1677	366	2.27 (s, 3H, CH_3) 3.20 (dd, J = 14.1, 9.4, 1H, C*H*H); 3.44 (dd, J = 14.1, 5.0, 1H, C*H*H); 5.73 (dd, J = 9.4, 5.0, 1H, C*H*); 6.81–8.01 (m, 16H, Ar–H).
k	$C_{25}H_{22}N_2O_2$	14	96–98	1672	382	3.18 (dd, J = 14.2, 9.2, 1H, C*H*H); 3.42 (dd, J = 14.2, 5.1, 1H, C*H*H); 3.74 (s, 3H, OCH_3); 5.71 (dd, J = 9.2, 5.1, 1H, C*H*); 6.70–8.00 (m, 16H, Ar–H).
l	$C_{28}H_{28}N_2O$	56	119	1674	408	1.15 (s, 9H, $C(CH_3)_3$,) 3.22(dd, J = 14.3, 9.1, 1H, C*H*H); 3.44 (dd, J = 14.3, 5.1, 1H, C*H*H); 5.76 (dd, J = 9.1, 5.1, 1H, C*H*); 6.85–8.00 (m, 16H, Ar–H).

[a] Analytical results for C, H, N were within ±0.4% of the calculated values.

Table 3
Yields, physical, analytical and spectroscopic data of compounds **2a–l**

No.	Formula [a]	Yield (%)	M.p. (°C)	MS *m/z* [M^+]	^{1}H NMR: δ (ppm)
a	$C_{24}H_{22}N_2 \cdot C_2H_2O_4$	31	103–105	338	2.96–3.24 (m, 4H, CH_2); 4.23–4.45 (m, 1H, CH); 6.90–7.60 (m, 17H, Ar–H).
b	$C_{24}H_{20}N_2Cl_2 \cdot C_2H_2O_4$	30	158–160	407	3.01 (dd, $J = 13.9$, 10.0, 1H, C*H*H); 3.17 (d, $J = 7.3$, 2H, CH_2); 3.36 (dd, $J = 13.9$, 4.4, 1H, C*H*H); 4.35–4.56 (m, 1H, CH); 6.69–7.60 (m, 15H, Ar–H).
c	$C_{24}H_{20}N_2F_2 \cdot C_2H_2O_4$	31	84–86	374	2.90–3.30 (m, 4H, CH_2); 4.32–4.48 (m, 1H, CH); 6.62–7.57 (m, 15H, Ar–H).
d	$C_{24}H_{21}N_2Cl \cdot C_2H_2O_4$	29	128–131	372	3.00–3.43 (m, 4H, CH_2); 4.40–4.58 (m, 1H, CH); 6.80–7.62 (m, 16H, Ar–H).
e	$C_{24}H_{21}N_2F \cdot C_2H_2O_4$	35	78–80	356	2.97–3.36 (m, 4H, CH_2); 4.38–4.54 (m, 1H, CH); 6.82–7.57 (m, 16H, Ar–H).
f	$C_{25}H_{24}N_2$	34	95–97	352	2.14 (s, 3H, CH_3); 2.93–3.27 (m, 4H, CH_2); 4.23–4.40 (m, 1H, CH); 6.78–7.58 (m, 16H, Ar–H).
g	$C_{25}H_{24}N_2O \cdot C_2H_2O_4$	34	115–118	368	2.94–3.32 (m, 4H, CH_2); 3.78 (s, 3H, OCH_3) 4.40–4.54 (m, 1H, CH); 6.75–7.60 (m, 16H, Ar–H).
h	$C_{24}H_{21}N_2Cl \cdot C_2H_2O_4$	37	171–173	372	2.96–3.18 (m, 4H, CH_2); 4.28–4.41 (m, 1H, CH); 6.80–7.58 (m, 16H, Ar–H).
i	$C_{24}H_{21}N_2F \cdot C_2H_2O_4$	35	85–87	356	2.96–3.20 (m, 4H, CH_2); 4.23–4.37 (m, 1H, CH); 6.80–7.62 (m, 16H, Ar–H).
j	$C_{25}H_{24}N_2 \cdot C_2H_2O_4$	41	87–90	352	2.28 (s, 3H, CH_3); 2.94–3.22 (m, 4H, CH_2); 4.22–4.42 (m, 1H, CH); 6.78–7.66 (m, 16H, Ar–H).
k	$C_{25}H_{24}N_2O \cdot C_2H_2O_4$	33	85–89	368	2.93–3.22 (m, 4H, CH_2); 3.74 (s, 3H, OCH_3); 4.20–4.37 (m, 1H, CH); 6.68–7.60 (m, 16H, Ar–H).
l	$C_{28}H_{30}N_2 \cdot C_2H_2O_4$	36	167–170	394	1.27 (s, 9H, $C(CH_3)_3$); 2.90–3.22 (m, 4H, CH_2); 4.28–4.45 (m, 1H, CH); 6.90–7.60 (m, 16H, Ar–H).

[a] Analytical results for C, H, N were within $\pm 0.4\%$ of the calculated values.

test compounds. Minimal bactericidal concentrations (MBCs) were determined by subcultivating in Triptose-agar the samples from cultures with no apparent growth.

5.2.4. Antimycotic assays

Yeast inocula were obtained by properly diluting cultures incubated at 37 °C for 24 hours in Sabouraud dextrose broth to obtain 5×10^3 cells/ml. On the contrary, dermatophyte and mould inocula were obtained from cultures grown at 37 °C for 5 days in Sabouraud broth by finely dispersing clumps with a glass homogenizer before diluting to 0.05 OD (590 nm). Then, 20 μl of the above suspensions were added to each well of flat-bottomed microtiter trays containing 80 μl of medium with serial dilutions of test compounds and were incubated at 37 °C. Growth controls were visually determined after 1 days (yeasts and moulds) or 3 days (dermatophites). MIC was defined as the compound concentration at which no macroscopic sign of fungal growth was detected. The minimal fungicidal concentration (MFC) was determined by subcultivating in Sabouraud dextrose agar samples from cultures with no apparent growth.

The cytotoxicity evaluation of compounds was based on the viability of MT-4 cells, as monitored by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method [14].

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