Biological Activity of some benzothiazolopyrazolines and Their Photoproducts: A Comparative Study

Veena Pareek[#], Pradeep Paliwal, Archana Kushwaha, S R Jetti, Shubha Jain

[#]*School of Studies in Chemistry & Biochemistry, Vikram University, Ujjain-MP Pincode-456010*

Abstract- **A series of benzothiazolopyrazolines were prepared and photolysed by UV radiation using benzophenone as photosensitizer. The photolysis of these pyrazoline derivatives gave the rearranged pyrazolines. The substrate and rearranged photoproducts were evaluated for their antibacterial, antifungal and antitubercular activity. The biological activity of pyrazolines and their photoproducts were compared. In some cases substrates were found to be more active against bacterial and fungal strain than their photoproducts and vice versa.**

Keywords- **Benzothiazolopyrazolines, Chalcones, Photoproducts, Photolysis, Biological activity.**

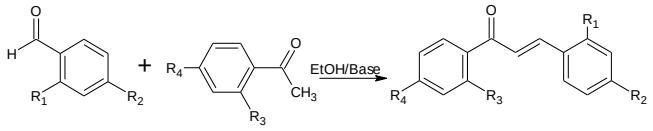
I. INTRODUCTION

Pyrazolines are important two nitrogen-containing five-membered heterocyclic compounds. Several pyrazoline derivatives possess important pharmacological activities and are useful material in drug research; therefore they have attracted the attention of medicinal chemists. [1] Several procedures have been developed for their synthesis. [2]-[4] A number of pyrazolines have been found to possess important bioactivities, viz. central nervous system,[5] antimicrobial and antimycotic, [6] [7] immunosuppressive,[8] etc. As far as the different pyrazoline isomers are concerned, 2-pyrazoline derivatives became the most frequently studied pyrazolines. The chemistry and biological study of heterocyclic compounds has been an interesting field for a long time in medicinal chemistry. Benzothiazole is one of the most important heterocyclic that has received overwhelming response owing to its diversified molecular design and remarkable optical and electronic properties. [9][10] Benzothiazolopyrazoline is N-substituted benzothiazole pyrazoline. Benzothiazole derivatives possess a wide spectrum of biological applications such as antitumor, antimicrobial, schictosomicidal, anti-inflammatory, anticonvulsants, antidiabetic, antipsychotic and diuretic etc. [11] In continuation of our work on the study of biological activity of heterocyclic compounds, here in we report the biological activity of some benzothiazolopyrazolines and their rearranged photoproducts.

II. EXPERIMENTAL

Synthesis of Substituted Chalcones

Substituted chalcones were prepared by the base catalysed Claisen Schmidt condensation of substituted aryl aldehydes and substituted acetophenones using reported procedure. [12] The synthesis of chalcones were identified with the help of their m.p.

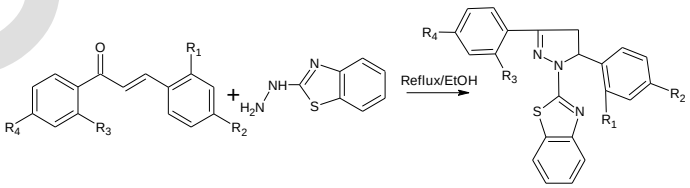


A **B** **1a-j**

R_1= H/OH, R_2= H/Cl/OMe, R3= H/Cl, R_4= H/OMe/NO_2

Synthesis of Benzothiazolopyrazolines

The pyrazoline derivatives have been synthesized using substituted chalcones and 2-hydrazinobenzothiazole (*Across Chem*) as follows using the standard procedure reported earlier.[13]



1a-j **2** **V201-210**

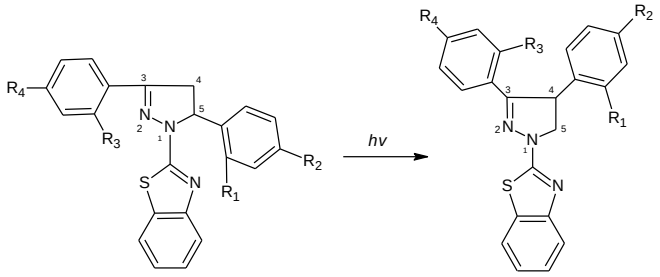
Scheme-I Synthesis of Benzothiazolopyrazolines

Table - 1

Photolysis of Benzothiazolopyarzolines

1.5 gms of benzothiazolopyarzoline (V201-210) was taken in 250 mL beaker and dissolved in 2:1 $CHCl_3$:EtOH. Benzophenone (0.01gm) was added as a photosensitizer. Then the solution was irradiated in an immersion well photoreactor with low pressure mercury vapor lamp placed inside the immersion well. The mixture was maintained at room temperature by constant water circulation. TLC of the reaction mixture was taken after every one hour. After 10hrs the TLC showed two spots corresponding to the reactant and the expected product. After 30-45hrs of irradiation, spot of the substrate disappeared, then the irradiation was stopped. The

reaction mixture was concentrated on water bath under reduced pressure and was kept overnight at room temperature. The solid obtained was filtered, washed with water, dried and recrystallized from ethanol to give pure product (**V301-310**). The physical data of the products is given in **table 2**.



V201-210 **V301-310**

Scheme - II

The reactions were carried out in neutral and alkaline medium also but no change in TLC was observed even after prolonged irradiation.

Table - 2

III. BIOLOGICAL ACTIVITY

Antibacterial and antifungal activities of the compounds were determined by sensitivity test procedure and their minimum inhibitory concentration (MIC) was determined by disc diffusion method.

Sensitivity Test Procedure

The sensitivity test of the bacterial strains was done by agar-diffusion techniques.[14] Stock solution at a concentration of 10mg/mL of each compound was prepared in dimethyl sulphoxide (DMSO). 20 µL of each of the solution was adsorbed on sterile 6 mm Whattmann filter paper (#4) discs and kept for few hours under vacuum to remove the solvent.

Grown cultures were stored in TSB (Tripticase Soya Broth) oxide maintaining the pH 7.3 in 0.5% agar at 4°C in the screw capped tubes. Trypticase soya agar (DIFCO) plates were inoculated with standard suspension of different bacterial cultures containing 10^7 c.f.u/mL using sterile cotton swabs to obtain a confluent lawn. The prepared discs were placed on the surface at different positions and plates were incubated at 37±2°C for 24 hrs. The results were recorded in tables.

Determination of Minimum Inhibitory Concentration

The titled compounds were tested for minimum inhibitory concentration studies. The MIC was determined using disc diffusion technique[15] by preparing discs containing 10-300 µg/mL of each compound and proceeded as per protocol presented in earlier reports.[16]

Determination of Antitubercular Activity

The pyrazoline derivatives and their photoproduct were screened for their antitubercular activity against Mycobacterium tuberculosis $H_{37}R_V$ strain using Lowenstien-Jensen medium method (L-J medium)[17] [18] at different concentration of newly synthesized products.

Procedure

The contents were mixed with water one by one, slowly. The pH was maintained at 6.9±0.3 at 25°C. Prepared L-J medium was preserved at 4°C. After the preparation of L-J medium, 10mg of each product was dissolved in 10mL of Dimethyl sulfoxide (DMSO) to get a concentration of 1000µg/mL. Further dilutions were made with DMSO to get different concentrations such as 100, 10, and 1 µg/mL. 0.8ml of each concentration was taken into a sterile *Bijou* bottle containing six 3 mm glass beads and 4 ml of sterile distilled water for the study. To this, 7.2ml of L-J medium was added. Then the bottle was labeled and set for solidification in an inssipator for 2 hrs at 45°C. After solidification, a sweep from mycobacterium tuberculosis $H_{37}R_V$ strain culture was discharged with the help of nichrome wire loop with a 3mm external diameter, and was inoculated on the surface of L-J medium containing photoproduct. The inoculated medium was incubated at 37°C for 4 weeks. At the end of 4 weeks readings were taken and recorded (**Table 5**).

IV. RESULTS & DISCUSSION

All the photoproducts have been characterized with the help of spectral data as follows:
The IR (KBr) of the products show absorptions at 2966 cm^{-1} (aliph. CH str.), 2922 cm^{-1} (C-H str.), 1668 cm^{-1} (C=N str.), 1601 cm^{-1} (C=C str.)etc. ^1H NMR ($CDCl_3$) of the products show following signals: δ 7.05-7.82 (aromatic protons), δ 5.6 (CH proton of pyrazoline ring) and δ 4.2 (CH_2 protons of pyrazoline ring). ^{13}C NMR ($CDCl_3$) of the products show important signals at δ 113-118, 152 (carbon atoms of pyrazoline ring) δ120 to 150 (carbon atoms of aromatic rings), and δ 162 (S-C=N, thiazole ring). LCMS spectrum of all the photoproducts shows molecular ion peaks corresponding to their molecular weights.

Comparative Study of Biological Activities

A. Antibacterial

3,5-diphenyl-1-benzothiazolopyrazoline **V201** and its photoproduct (3,4-diphenyl-1-benzothiazolopyrazoline **V301**) both had no activity against *E. coli*, *Citrobacter frundii, and Salmonella paratyphy A*. The substrate V201 showed less activity than its photoproduct against *Klebsiella Sp.* and *Bacillus subtilis*. With respect to *Streptococcus pyogene*s the substrate showed no activity while its photoproduct was found to be good activity. V201 did not show any activity against *Pseudomonas* but its photoproduct exhibited good activity.

V202 showed no activity against *Staphylcoccus aureus, Salmonella paratyphi A, Pseudomonas and Streptococus pyogenes.* It exhibited moderate activity with respect to *Bacillus subtilis* and excellent activity against *Klebsiella and E. coli.* Its photoproduct **V302** showed excellent activity against *E. coli. and Salmonella paratyphy A* and very good activity against *Citrobacter frundii* and *C. hoffmani* and almost no activity against *Klebsiella sp., Pseudomonas*.

V203 and its photoproduct both were found to be least active against *E. coli*. Substrate V203 showed nil activity but its photoproduct showed excellent activity against *Staphylococcus*. With respect to *Citrobacter frundii* and *Klebsiella sp*. both the substrate and its photoproduct showed antibacterial activity but the substrate showed little more activity than its photoproduct. The photoproduct of V203 showed greater activity against *Bacillus subtilis*, *Streptococcus pyogenes* and *Pseudomonas* as compared to that of substrate.

V204 showed good antibacterial activity against *E. coli*, *Citrobacter frundii* and *Bacillus substilis*. Its photoproduct showed greater activity against the same bacterial strains than substrate. Substrate V204 showed little more activity than its photoproduct against *Bacillus substilis*, but V204 and its photoproduct both did not exhibited any activity against *Pseudomonas, Staphylococcus* and *Salmonella paratyphy*.

Both **V205** and its photoproduct both showed nil activity against *Salmonella paratyphy A, E. coli., Pseudomonas, Citrobacter frundii* and *Streptococcus pyogenes. Against Klebsiella sp. The substrate exhibited no activity but its photoproduct was found to be moderately active. With respect to bacillus subtilis both substrate and its photoproduct exhibited equal activity against S aurus the* substrate was found to be little more active than its photoproduct. Substrate V205 showed very good activity against *C. hoffmani* but its photoproduct exhibited nil activity.

V206 and its product are found to be effective in inhibiting growth of *E. coli, Streptococcus* and *Salmonella paratyphy A*. V206 and its photoproduct both were found to be active against *Citrobacter frundii, C. hoffmani* and *Bacillus subtilis* but their photoproduct exhibited comparatively more active than substrate. Compound V206 showed good activity with respect to *Klebsiella sp.* but its photoproduct showed no activity. **V206** and its photoproduct both show nil activity against *Salmonella paratyphy*, *E. coli*, *Pseudomonas* and *Streptococcus pyogen*.

V207 and its photoproduct both are found to be inactive against *Citrobacter frundii*, *Streptococcus pyogenes* and *Salmonella paratyphy A.* **V207** showed no activity against pseudomonas and staphylococcus but its photoproduct showed good activity against these bacterial strains. Against *bacillus subtillis* both substrate and photoproduct were fund to show good activity but its photoproduct show slight more activity than the substrate.

V208 and its photoproduct both are found to be inactive against *Klebsiella sp., Citrobacter frundii* and *Salmonella paratyphy A.* **V208** exhibited similar activity with respect to *Streptococcus pyogenes, C. hoffmani* and *staphylococcus*. Its photoproduct also showed activity but lesser than the substrate. Substrate **V208** against *Bacillus subtilis* showed poor activity but its photoproduct showed excellent activity.

V207, V208 and their photoproducts all show nil activity against *Salmonella paratyphy A, Citrobacter, Pseudomonas* and *Streptococcus pyogen*.

Against *E. coli* **V209** did not show any activity but its photoproduct showed very good activity. **V209** and its photoproduct both were found to be inactive against *Klebsiella, Citrobacter frundii*, *Streptococcus pyogenes, Pseudomonas* and *Salmonella paratyphy A* but both showed very good activity against *Bacillus subtilis*.

Substrate **V210** showed excellent activity against *E. coli* but its photoproduct showed comparatively poor activity against the same bacteria. Against *staphylococcus* substrate and its photoproduct showed very good activity but the photoproduct was comparatively more active.

V209, V210 and their photoproducts all show nil activity against *Salmonella paratyphy, C. hoffmani, Pseudomonas* and *Streptococcus pyogen*.

All substrates and their photoproducts show very good activity against *Bacillus subtilis*. **V203** and its photoproduct both shows highest active against all bacterial strains tested. Only V203 and its photoproduct shows activity against salmonella paratyphy but remaining substrates and their photoproducts are inactive.

B. Antifungal

Table 4: Comparative antifungal activity of the substrates and their photoproducts

Table - 4

V201 showed maximum activity against *Candida albicans* and *Alterneria alterneria*, moderate activity and almost no activity towards *Aspergillus niger*, but the photoproduct **V301** exhibited almost no activity against all tested fungi.

V202 showed nil activity against all tested fungi like *Candida albicans*, *Aspergillus niger*, Alterneria alterneria and *Candida tropicalis* but photoproduct **V302** exhibited very good activity against all tested fungi.

V203 as well as its photoproduct **V303** showed good activity against all tested fungi like *Candida albicans, Aspergillus niger* and *Candida tropicalis* except V303 showed moderate activity against *Alterneria alterneria*.

V204 and its photoproduct **V304** showed good to moderate activity against all tested fungi like *Candida albicans*, *Aspergillus niger* and *Alterneria alterneria*. V204 showed excellent activity against *Candida tropicalis* but its photoproduct did not.

When **V205** and its photolysed product were tested against four strains of fungi i.e. *Candida albicans*, *Aspergillus niger*, *Alternaria alternari* and *Candida tropicalis* substrate only showed very good activity against *Aspergillus niger* and *Candida tropicalis.* Its product showed no activity rest of the tested fungi except *Alternaria alternaria*.

V206 and its photoproduct showed no activity against all the tested fungi except **V206** shows very good activity against *Candida albicans*.

V207 showed only activity against *Aspergillus niger* but its photoproduct showed good activity against all tested fungi.

V208 showed very good activity against *Candida albicans* but its photoproduct very good activity against all tested fungi.

V209 and its photoproduct did not show any activity against all the tested fungi except *Candida albicans*.

V210 showed excellent activity against *Alternaria alternaria* but its photoproduct showed lesser activity. With respect to *Aspergillus niger* the substrate showed moderate activity but its photoproduct did not. Substarate V210 showed nil activity against candia albicans and candida tropricalis but its photoproduct showed good activity against the same.

C. Antitubercular

Table 5: Comparative antitubercular activities of compounds and their photoproducts against *Mycobacterium tuberculosis* $H_{37}R_V$.

Reactant	Tuberculosis growth	Photoproduct	Tuberculosis growth
V201	Positive	**V301**	**Negative**
V202	Positive	**V302**	Positive
V203	**Negative**	**V303**	**Negative**
V204	Positive	**V304**	**Negative**
V205	Positive	**V305**	**Negative**
V206	Positive	**V306**	Positive
V207	**Negative**	**V307**	Positive
V208	Positive	**V308**	Positive
V209	Positive	**V309**	Positive
V210	**Negative**	**V310**	**Negative**

Negative: inhibition, Positive: No inhibition

V201, **V204** and **V205** showed no antitubercular activity but their photo product showed activity against Mycobacterium tuberculosis. **V207** showed antitubercular activity but its photo product showed less activity against Mycobacterium tuberculosis. Both substrates **V203** & **V210** and their photoproducts showed antitubercular activity. The other substrates and their photoproducts showed no antitubercular activity.

V. CONCLUSION

The present study shows that the 3,5-diphenyl and rearranged 3,4-diphenyl benzothiazolopyrazolines show different activities against the microorganisms. Chlorine containing derivatives of pyrazoline and their photoproducts exhibited significant activity. In some cases the photoproducts were more active while in others substrates were more active, indicating that the rearrangement either increases or decreases the antimicrobial activity of pyrazolines.

REFERENCES

[1]. Elguero, J. In *Comprehensive Heterocyclic Chemistry II*, Katritzky, A. R.; Rees, C. W.; Scriven E. F. V., Eds., Pergamon Press: Oxford, **1996**; Vol. 3, p. 1.
[2]. Lévai, A. *Khim. Geterotsikl. Soedin.*, **1997**, 747.
[3]. Lévai, A. *J. Heterocycl. Chem.*, **2002**, *39*, 1.
[4]. Brown, R. E; Shavrel, Jr., J. *US Patent* **1972**, 3,624,102; *Chem. Abstr*. **1972**, *76*, 59618.
[5]. Ramalingham, K.; Thyvekikakath, G. X.; Berlin, K. D.; Chesnut, R. W.; Brown, R. A.; Durham, N. N.; Ealick, S. E., van der Helm, D., *J. Med. Chem.* **1977**, *20*, 847.
[6]. Nauduri, D.; Reddy, G. B. S., *Chem. Pharm. Bull*., **1998**, *46*, 1254.
[7]. Lombardino, J. G.; Otternes, I. G., *J. Med. Chem.*, **1981**, *24*, 830.
[8]. Karthikeyan M. S.; Holla B. S.; Kumari N. S., *Eur. J. Med. Chem*., **2007**, 42, 30-36.
[9]. Yadav, P. and Senthilkumar, G., *Int. J. Pharm. Drug Res*., **2011**, 3(1), 01-07.
[10]. Khokra, S.; Arora, K.; Mehta, H.; Aggarwal, A. and Yadav M., *Int. J. Pharm. Res.*, **2011**, 2(6), 1356-1377.
[11]. Shrivastava V. K. A., Chandra R. and Kumar A., *Ind. J. Chem*., **2002**, 41B, 2371-2375.
[12]. Babu V H, Sridevi C, Joseph A, Srinivasan K K; *Ind. J. Pharm Sc*., **2008**, 69, 470.
[13]. Sharma V. and Sharma K. V., *E. J. Chem.*, **2009**, 6(2), 348.
[14]. Khan K A, Khan S A, Khalid S M, Ahmed A, Siddiqui B S, Saleem R, Siddiqui S, Faizi S; *Drug Res.* , **1994**, 44, 972.
[15]. Baur A W, Kirby W M, Sherris J C, Tink M; *J. Clin. Pathology*, **1966**, *45*, 493.
[16]. Mcleod L C; Pharmacological experiments an intact preparations; E&S Livingstone, *Edinburgh*, 63, **1996**.
[17]. Lowenstien; Zentralbl. Bakteiol Parasitenkd Infektionskr; *Hyg. Abt*., **1931**, 1 Orig, 120, 127.
[18]. Jensen; Zentralbl. Bakteiol Parasitenkd Infektionskr; *Hyg. Abt*., **1932**, 1 Orig, 125, 222.

Table-1 Physical data of synthesized pyrazolines

Product	R_1	R_2	R_3	R_4	MP °C
3,5-diphenyl-1-benzothia-zolopyrazoline **[V201]**	H	H	H	H	173
3-phenyl,5-(2'-hydroxyphenyl)-1-benzothiazolopyrazoline **[V202]**	OH	H	H	H	225
3-phenyl, 5-(4'-chlorophenyl)-1-benzothiazolopyrazoline **[V203]**	H	Cl	H	H	145
3-(4-methoxyphenyl)-5-phenyl-1-benzothiazolopyrazoline **[V204]**	H	H	H	OCH_3	148
3-(4-methoxyphenyl)-5-(2'-hydroxyphe-nyl)-1-benzothia-zolopyrazoline **[V205]**	OH	H	H	OCH_3	250-252
3-(4-methoxyphenyl)-5-(4'-chlorophe-nyl)-1-benzothia-zolopyrazoline **[V206]**	H	Cl	H	OCH_3	125
3-(4-methoxyphenyl)-5-(4'-methoxy-phenyl)-1-benzothia-zolopyrazoline **[V207]**	H	OCH_3	H	OCH_3	120-122
3-(4-nitrophenyl)-5-(4'-chlorophenyl)-1-benzothia-zolopyrazoline **[V208]**	H	Cl	H	NO_2	143
3-(4-nitrophenyl)-5-(4'-methoxyphenyl)-1-benzothia-zolopyrazoline **[V209]**	H	OCH_3	H	NO_2	189
3-(4-chlorophenyl)-5-(4'-chlorophenyl)-1-benzothia-zolopyrazoline **[V210]**	H	Cl	H	Cl	154-155

Table-2 : Photoproduts of substituted benzothiazolopyrazolines **(V301-310)**

Product	Time (hrs)	Yield	Mp (°C)	Elemental Analysis	
				Calculated	Observed
V 301	38	1.01	64	C 74.34; H 4.82; N 11.82	C 74.25; H 4.56; N 11.68
V 302	45	0.91	152	C 71.14; H 4.61; N 11.37	C 71.01; H 4.47; N 11.46
V 303	40	0.99	96	C 67.77; H 4.14; Cl 9.09; N 10.78	C 67.61; H 4.21 Cl 9.15; N 10.86
V 304	35	1.10	81	C 71.66; H 4.97; N10.9	C 71.53; H 4.89; N 10.81
V 305	42	0.92	144	C 68.81; H 4.77; N 10.47	C 68.69; H 4.70; N 10.39
V 306	37	0.99	115	C 65.78; H4.32; Cl 8.44; N 10.01	C 65.60; H4.23; Cl 8.31; N 9.95
V 307	32	1.21	72	C 69.37; H 5.09; N 10.11	C 69.26; H 4.88; N 10.01
V 308	36	1.00	132	C 60.76; H 3.48; N 12.88; Cl 8.15	C 60.65; H 3.40; N 12.78; Cl 8.22
V 309	34	1.15	154	C 64.17; H 4.21; N 13.01	C 64.29; H 4.12; N 12.87
V 310	38	0.99	102	C 62.27; H 3.56; Cl 16.71; N 9.90	C 62.10; H 3.43; Cl 16.56; N 9.81

Table 3: Comparative antibacterial study of substrate and their photoproducts

Bacteria			Substrate/ **Photoproduct**		
Gram – ve bacteria	V201/**V301**	V202/**V302**	V203/**V303**	V204/**V304**	V205/**V305**
S. paratyphi A	8/**10**	10/**24**	18/**24**	10/**8**	6/**10**
E. coli	6/**8**	26/**30**	10/**13**	14/**22**	12/**6**
Klebsiella sp.	10/**18**	30/**10**	18/**14**	12/**22**	6/**14**
Pseudomonas A.	8/**18**	10/**08**	18/**24**	10/**8**	6/**10**
C.freundii	6/**6**	10/**24**	26/**14**	23/**24**	6/**11**
Gram +ve bacteria					
S. aureus	10/**18**	10/**16**	8/**24**	10/**8**	18/**16**
B. subtilis	13/**18**	14/**15**	18/**24**	20/**16**	14/**14**
S. pyogenes	8/**18**	10/**08**	18/**24**	10/**8**	6/**10**
S. faecalis	8/**12**	20/**18**	30/**32**	22/**24**	6/**10**
C.hoffmanii	12/**22**	24/**20**	24/**28**	20/**22**	16/**10**
Bacteria			Substrate/ **Photoproduct**		
Gram – ve bacteria	V206/**V306**	V207/**V307**	V208/**V308**	V209/**V309**	V210/**V310**
S. paratyphi A	10/**06**	08/**10**	08/**10**	06/**10**	10/**06**
E. coli	10/**06**	18/**24**	12/**24**	10/**18**	25/**18**
Klebsiella sp.	22/**06**	18/**14**	08/**11**	10/**10**	22/**24**
Pseudomonas A.	10/**06**	08/**14**	16/**14**	06/**10**	11/**06**
C.freundii	20/**18**	08/**10**	08/**10**	06/**08**	22/**18**
Gram +ve bacteria					
S. aureus	10/**06**	10/**14**	08/**14**	18/**10**	14/**16**
B. subtilis	14/**20**	14/**16**	18/**18**	15/**15**	12/**20**
S. pyogenes	10/**06**	10/**10**	08/**14**	06/**10**	10/**06**
S. faecalis	12/**14**	20/**16**	12/**16**	10/**12**	20/**22**
C.hoffmanii	14/**20**	20/**18**	16/**12**	08/**08**	10/**10**

Table 4: Comparative antifungal activity of the substrates and their photoproducts

Fungi / Substrate/ **Photoproduct**	**Candida albicans**	**Aspergillus niger**	**Alterneria alterneria**	**Candida tropicalis**
V201/ **V301**	15/**06**	8/**8**	18/**9**	12/**6**
V202/ **V302**	10/**19**	11/**13**	10/**15**	10/**18**
V203/ **V303**	19/**20**	19/**15**	16/**10**	13/**21**
V204/ **V304**	7/**10**	10/**10**	10/**11**	16/**10**
V205/ **V305**	6/**10**	19/**10**	6/**18**	15/**10**
V206/ **V306**	23/**7**	8/**8**	8/**10**	10/**7**
V207/ **V307**	8/**14**	16/**21**	7/**12**	8/**23**
V208/ **V308**	21/**16**	8/**24**	13/**17**	6/**17**
V209/ **V309**	17/**8**	6/**8**	6/**10**	10/**8**
V210/ **V310**	11/**16**	14/**10**	25/**20**	10/**14**