ISSN 1015 • 3918

ANKARA ÜNİVERSİTESİ ECZACILIK FAKÜLTESİ DERGİSİ

JOURNAL OF FACULTY OF PHARMACY OF ANKARA UNIVERSITY

Cilt/Vol : 31
Sayı /No : 4
Yıl/Year: 2002

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(Ankara Ecz, Fak. Derg.)

Sahibi: Prof. Dr. Seçkin ÖZDEN
Editör : Prof. Dr. Feyyaz ONUR

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Ankara Üniversitesi Basımevi
2002

JOURNAL OF FACULTY OF PHARMACY OF ANKARA UNIVERSITY
(J. Fac. Pharm. Ankara)

Published by : Prof. Dr. Seçkin ÖZDEN
Editor : Prof. Dr. Feyyaz ONUR

Journal of Faculty of Pharmacy of Ankara University is an international medium for the publication of original research reports, reviews and short communications on relevant developments in pharmaceutical sciences. This journal is published quarterly. All the articles appeared in this journal are published on the responsibility of the author(s). The manuscript submitted to the journal should not be published previously as a whole or in part and not be submitted elsewhere. The manuscripts should be prepared in accordance with the requirements specified at the end of the issue.

This journal is indexed in Chemical Abstracts (CA), Excerpta Medica Database (EMBASE), Medicinal Aromatic Plants Abstracts (MAPA) and Turkish Medical Index

Web address : www.pharmacy.ankara.edu.tr/journal

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Ankara Üniversitesi Basımevi
2002

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Ankara Ecz. Fak. Derg.
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EVALUATION OF INDOLE-2-CARBOXYLIC ACID ESTERS AS ANTI-HIV AGENTS

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ABSTRACT

The indole esters, which were previously synthesized for inhibition of cyclooxygenase-2 (COX-2), were evaluated for their anti-HIV activities. Among these compounds 4 and 27 were found slightly active against HIV without any cytotoxicity.

Key Words: Indole esters; Anti-HIV activity; Cytotoxicity

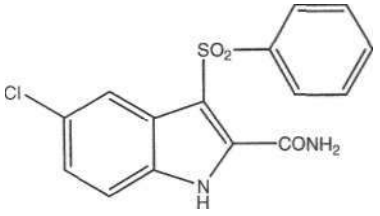
ÖZET

Daha önce siklooksigenaz-2 (COX-2) enziminin inhibisyonu için sentezlenen indol esterleri, anti-HIV aktiviteleri için değerlendirilmiştir. Bileşik 4 ve 27, sitotoksisiteleri olmaksızın az da olsa HIV'ye karşı aktif bulunmuşlardır.

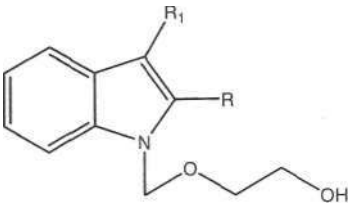
Anahtar Kelimeler: İndol esterleri; Anti-HIV aktivitesi; Sitotoksisite

INTRODUCTION

A number of diverse indole derivatives such as; 5-chloro-3-(phenylsulfonyl)indole-2-carboxamide (L-737,126) (1), 1-(2-hydroxyethoxy)methylindoles (compounds I) (2) and 5-(2-phenyl-3'-indolal)-2-thiohydantoin (compound II) have been shown to have potent anti-HIV activity (human immunodeficiency virus) (3). The structural formula of these compounds are shown in Figure 1. In clinical settings, non-nucleoside class inhibitors of reverse transcriptase (RT) such as nevirapine, have been shown resistant and thus require that this class of inhibitors should be used in combination with other drugs (4). Recent studies were focused on the elimination of the resistance problem against RT mutant. The changing of 2-pyrrolyl substituent to larger rings such as indole on the nevirapine derivatives (compound III) (Figure 1) demonstrated the activity against several clinically relevant forms of HIV-1 reverse transcriptase (5).



L-737,126

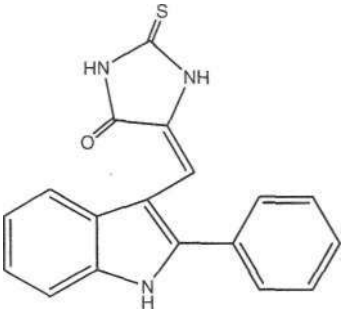


Compounds I

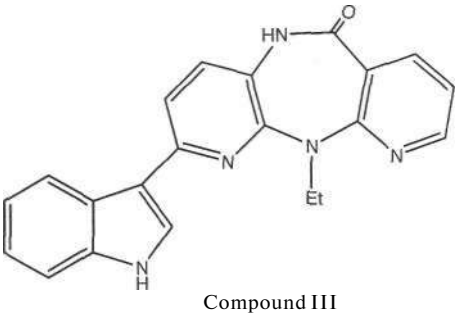
R_1= Ph, R=Ph

R_1= COOEt, R=Me

R_1= COOEt, R=Ph



Compound II



Compound III

Figure 1: Indole-based molecules with anti-HIV activity.

In connection with our research project on indole derivatives, it was noteworthy for evaluating anti-HIV activities of previously synthesized indole esters (6). The formula of tested compounds are shown in Table 1.

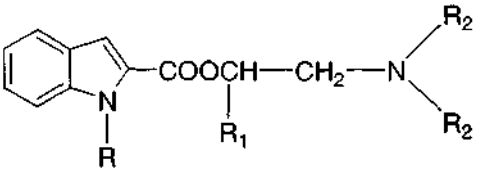


Table 1: Structural formulas of compounds **1-27.**

Compd.	type of salt	R	R1	R_2
1	HC1	CH_2Ph	H	methyl
2	HC1	CH_2Ph	H	ethyl
3	HC1	CH_2Ph	H	pyrrole
4	HC1	CH_2Ph	H	pyrimidine
5	HC1	CH_2Ph	CH_3	methyl
6	CH_3I	CH_2Ph	H	methyl
7	CH_3I	CH_2Ph	H	ethyl
8	CH_3I	CH_2Ph	H	pyrrole
9	CH_3I	CH_2Ph	H	pyrimidine
10	CH_3I	CH_2Ph	CH_3	methyl
11	HC1	Ph	H	methyl
12	HC1	Ph	H	ethyl
13	HC1	Ph	H	pyrrole
14	HC1	Ph	H	pyrimidine
15	HC1	Ph	CH_3	methyl
16	HC1	Ph	H	piperazine
17	CH_3I	Ph	H	methyl
18	CH_3I	Ph	H	ethyl
19	CH_3I	Ph	H	pyrrole
20	CH_3I	Ph	H	pyrimidine
21	CH_3I	Ph	CH_3	methyl
22	HC1	H	H	ethyl
23	CH_3I	H	H	ethyl
24	HC1	H	H	pyrrole
25	HC1	H	H	pyrimidine
26	HC1	H	CH_3	methyl
27	HC1	H	H	piperazine

MATERIALS AND METHODS

Candidate compounds were dissolved in dimethylsulfoxide and diluted 1:100 in cell culture medium before preparing serial half-log_{10} dilutions. T4 lymphocytes (CEM cell line) were added and after a brief interval HIV-1 was added, resulting in a 1:200 final dilution of the compound. Uninfected cells with the compounds served as the toxicity control, and infected and uninfected cells without the compounds served as basic controls. Cultures were incubated at 37°C in a 5% carbon dioxide atmosphere for 6 days. The tetrazolium salt, (XTT) was added to all wells, and cultures were incubated to allow formazon colour development by viable cells. Individual wells were analyzed spectrophotometrically to quantitate formazon production and were viewed by microscopy for detection of viable cells and confirmation of protective activity. Drug-treated virus-infected cells were compared with drug-treated noninfected cells and with other appropriate controls on the same plate. Data were reviewed in comparison with other tests carried on at the same time and the activity was determined.

RESULTS AND DISCUSSION

The procedure used in the National Cancer Institute's test for the agents active against human immunodeficiency virus (HIV) is designed to detect agents acting at any stage of the virus reproductive cycle (7). The assay basically involves the killing of T4 lymphocytes by HIV. Small amounts of HIV are added to cells, and two cycles of virus reproduction are necessary to obtain the required cell killing. Agents that interact with virions, cells or virus gene-products to interfere with viral activities will protect cells from cytolysis. The system is automated in several features to accommodate large numbers of candidate agents and is generally design to detect anti-HIV activity. However, compounds that degenerate or are rapidly metabolized in the culture conditions may not show activity in the screen. All tests are compared with at least one positive AZT-treated control, which was done at the same time under the identical conditions.

The anti-HIV assay of indole esters showed that compounds 4 and 27 were slightly active anti-HIV agents without any cytotoxicity. *In vitro* anti-HIV screening results of compounds 4 and 27 are shown in Figures 2 and 3 and activity against HIV-1 and growth inhibitory properties were placed in Tables 2 and 3 respectively.

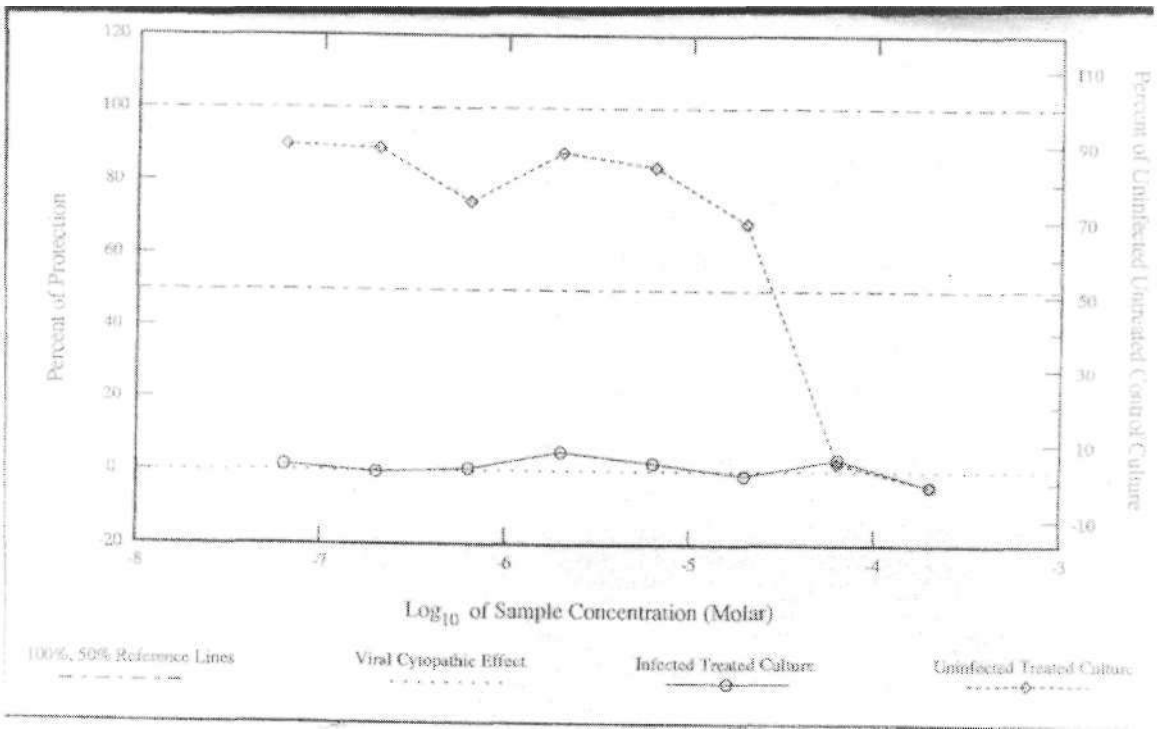


Figure 2: *In vitro* anti-HIV screening of compound 4.

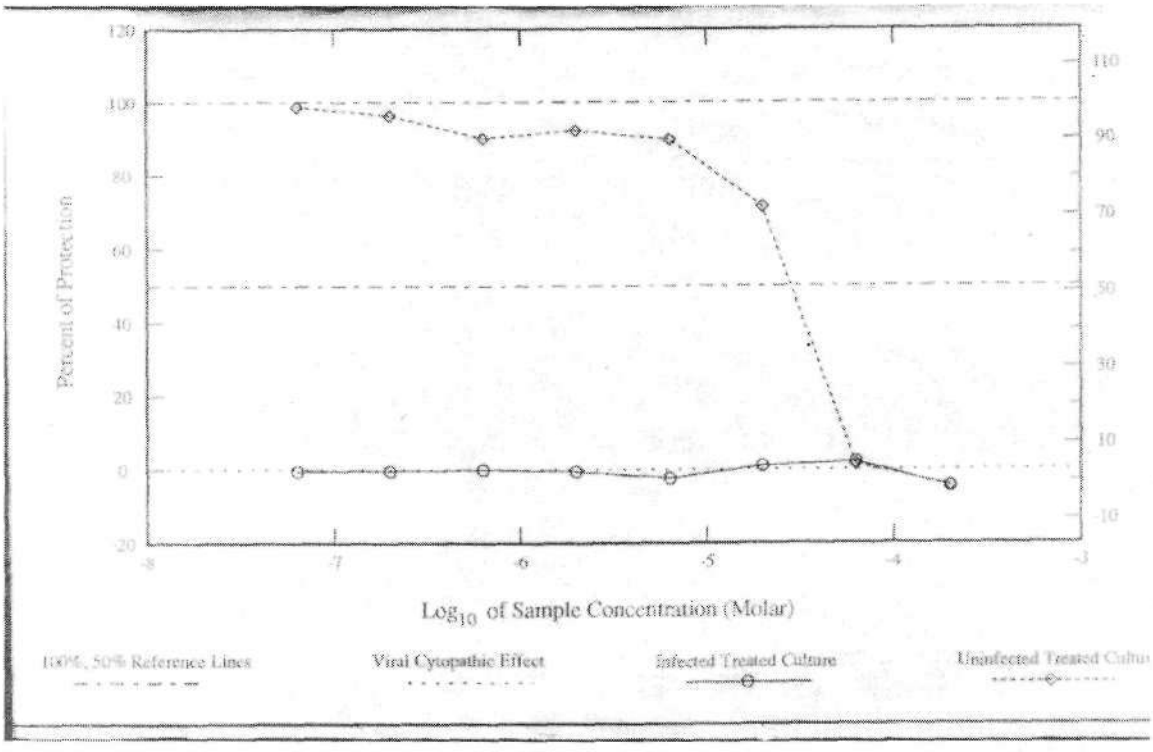


Figure 3: *In vitro* anti-HIV screening of compound **27.**

Table 2: Activity against HIV-1 and growth inhibitory properties of compound 4.

Dose (M)	Percent of protection	Percent of control	
		Infected	Uninfected
6.35×10^{-8}	1.31	4.27	90.08
2.00×10^{-7}	-0.54	2.48	89.18
6.34×10^{-7}	0.44	3.43	75.08
2.00×10^{-6}	4.95	7.80	88.09
6.33×10^{-6}	2.03	4.97	84.32
2.00×10^{-5}	-1.25	1.79	69.42
6.32×10^{-5}	3.21	6.11	6.06
2.00×10^{-4}	-4.06	-0.94	-0.89

Table 3: Activity against HIV-1 and growth inhibitory properties of compound 27.

Dose (M)	Percent of protection	Percent of control	
		Infected	Uninfected
6.35×10^{-8}	-0.44	2.57	98.71
2.00×10^{-7}	-0.40	2.61	96.41
6.34×10^{-7}	-0.04	2.96	90.31
2.00×10^{-6}	-0.59	2.43	92.43
6.33×10^{-6}	-2.41	0.66	89.87
2.00×10^{-5}	0.92	3.89	72.26
6.32×10^{-5}	1.97	4.91	4.25
2.00×10^{-4}	-4.64	-1.50	-1.33

None of the other derivatives was active and did not exhibit a rather strong cytotoxicity. According to the biological activity result, it can be concluded that N-substitution of indole ring is not important for anti-HIV activity since compound 27 does not have substitution on one position of indole structure. Furthermore, the cyclic groups such as pyrrolidine and piperazine, which constitute the side chain of indole-2-carboxylic acid esters, might have increased the anti-HIV activity of the compounds. Despite these results, the series of compounds presented in this work demonstrated the possibility of modifying indole derivatives that show activity against HIV virus.

ACKNOWLEDGMENTS

This work was supported in part by a grant from the Turkish Scientific and Technical Research Institute (SBAG-AYD-108).

We thank to Dr. V.L. Narayanan (Drug synthesis & Chemistry Branch of National Cancer Institute-U.S.A.) for performing anti-HIV activity tests.

REFERENCES

1. **Williams, T.M., Ciccarone, T.M., MacTough, S.C., Rooney, C.S., Balani, S.K., Condra, J.H., Emini, E.A., Goldman, M.E., Greenlee, W.J., Kauffman, L.R., O'Brien, J.A., Sardana, V.V., Schleif, W.A., Theoharides, A.D., Anderson, P.S.** "5-Chloro 3 (phenylsulfonyl)indole-2-carboxamide: A Novel, Non-Nucleoside Inhibitor of HIV-1 Reverse Transcriptase", *J. Med. Chem.* 36, 1291-1294 (1993)

2. **Almerico, A.M., Barraja, P., Diana, P., Cirrincione, G., Mingoia, F., Musiu, C, Perra, G., Putzolu, M., Marongiu, M.E.** "Glycosidopyrroles Part 3. Effect of the benzocondensation on acyclic derivatives: l-(2-hydroxyethoxy)methylindoles as potential antiviral agents", *IL Farmaco,* 53, 409-414 (1998)

3. **Suzen,** S., **Buyukbingol,** E. "Evaluation of anti-HIV activity of 5-(2-phenyl-3'-indolyl)-2-thiohydantoin", *IL Farmaco,* 53, 525-527 (1998)

4. **Murphy, R., Montaner, J.** "Nevirapine: a review of its development, pharmacological profile and potential for clinical use", *Exp. Opin. Invest. Drugs,* 5, 1183-1199 (1996)

5. **Kelly, T.A., McNeil, D.W., Rose, J.M., David, E., Shih, C.-C, Grob, P.M.** Novel non-nucleoside inhibitors of human immunodeficiency virus type 1 reverse transcriptase. 6. 2-indol-3-yl- and 2-azaindol-3-yl-dipyridodiazepinones", *J. Med. Chem.* 40, 2430-2433 (1997)

6. **Olgen, S., Akaho, E., Nebioğlu, D.** "Synthesis and receptor docking studies of /V-substituted indole-2-carboxylic acid esters as a search for COX-2 selective enzyme inhibitors", *Eur. J. Med. Chem.* 36, 747-770 (2001)

7. Weislow, O.W., Kiser, R., Fine, D., Bader, J., Shoemaker, R.H., Boyd, M.R. "New soluble-formazon assay for HIV-1 cytopathic effects: application to high-flux screening of synthetic and natural products for AIDS-antiviral activity", *J. Natl. Cancer Inst.* **81,** 577-586(1988)

Başvuru Tarihi: 12.07.2002

Kabul Tarihi: 05.08.2002