Anti HIV, Antibacterial and Antifungal Potential of a Variety of Heterocyclic Compounds Containing Nitrogen and/or sulphur

S.M. SONDHI*, R.P. VERMA, NIDHI SINGHAL, V.K. SHARMA, C. HUSIU¹, L. VARGIU¹, S. LONGU¹ AND P.LA COLLA¹

Department of Chemistry, University of Roorkee, Roorkee - 247 667 (U.P.) India

¹Dipartimento di Biologia Sperimentale Universita' Degli studi di Cagliari V. Le. Regina Margherita, 45, 09124-Cagliari. Italy

9-Acridinyl imino/amino derivatives (Ia-f, IIa-b, III, IV and V), pyrimido oxazole derivative (VIa), imidazopyrimidine thiones (VIb, VII), pyrimidooxazinethione (VIc), 1-(2-aminoaryl)-6-hydroxy-4,4,6-trimethyl-1,4,5,6-tetrahydropyrimidine-2(3H)-thiones (VIIIa-c), 1-(2-nitroaryl)-6-methoxy-6-methyl-1,4,5,6-tetrahydro pyrimidine-2-(3H) thiones (IXa,b), 1-(2-hydroxy phenyl)-4,4,6-trimethyl-1, 4-dihydropyrimidine-2(3H)-thione (X), condensed tricyclic pyrimidine derivatives (XIa-h) pyrimido anthraquinonimidazole (XII), N,N'-disubstituted thioureas (XIIIa-c), 1,2-dithia-5,8-diazacyclodeca-4,8-diene (XIV), 1,2-dithia-5,8-diazacyclodecane dihydrochloride (XV), 3-(o-aminophenyl)-2-imino-4-phenyl-4-thiazoline (XVI), 9H-imidazolo [1,2-a] benzimidazoles (XVIIa-c), benzimidazole derivative (XVIII), Schiff's bases (XIX, XXa-b), 1-(2-methylamino-4-phenyl thiazole)-2-hydroxy-naphthalene (XXI), compound XXII and acridone derivative XXIII were synthesized by the procedures developed earlier and were screened for anti HIV, antibacterial and antifungal activities. Compounds XVIIb and XVIIc showed antibacterial activity against Streptococcus D at concentrations slightly higher than those of streptomycin (1.6 µM) and compound XV showed mild activity against Salmonella (MIC = 66 µM). When tested against yeast representatives, compound XV was active against C-neoformans (MIC = 22 µM), compounds XV and XXa showed mild activity against Candida at 66 µM but this concentration was cytotoxic for MT-4 cells. Only compound XIa was capable of protecting MT-4 cells from the cytopathic effect induced by HIV -1 (EC50 = 115 µM). All other compounds were found to be inactive.
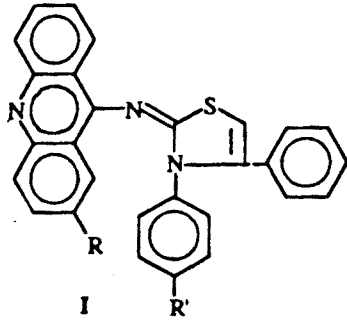
Acquired immune deficiency syndrome (AIDS), a fatal disease, is caused by human immunodeficiency virus (HIV). There are five drugs, 3'-azido-3-deoxythymidine (AZT), 2' 3'-dideoxyinosine (ddi), 2' 3'-dideoxy cytidine (ddc), 3-Tc or lamivudine and 2', 3'-didehydro-3'-deoxythymidine (d4T) available in the market for the treatment of AIDS[1]. Adequate drug levels at the site of replication over extended periods can not be maintained by these drugs due to their relatively short half lives[2-5] and thus fail to stop progression of acquired immunodeficiency syndrome (AIDS) which lead to dementia and other neurological manifestations of HIV infection. The development of an effective drug for the treatment of AIDS continues to be a challenging problem in medicinal research. The drugs mentioned above are nucleoside substrate analogues and often exhibit significant toxic
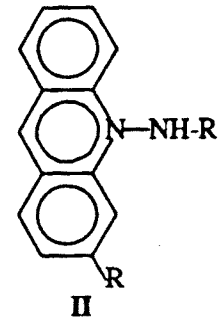
*For correspondence

side effects. Nevirapine[6] (dipyridodiazepinone) which acts by a mechanism distinct from that of nucleoside substrate analogues is expected to avoid clinical toxicities. Tetrahydromidazobenzodiazepinone (TIBO)[7] and 2-thio-9-acridone derivatives[8] are reported to exhibit anti HIV activity. Tempted by the anti HIV activity reported for above mentioned heterocyclic compounds and in search of new lead compounds as anti HIV, antibacterial and antifungal agents we have screened forty six heterocyclic compounds having different types of structures for anti HIV, antibacterial and antifungal activities which we which to report in this paper.

Melting points were determined on JSGW apparatus and are uncorrected. TLC was performed by using silica gel G (Merck) and spots were visualized by iodine vapour or by irradiation with UV light (254 nm). Silica gel (60-120 mesh) was used for column chromatography.
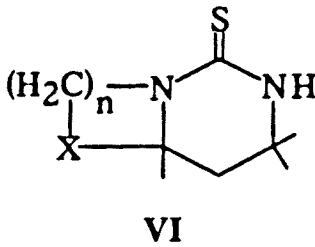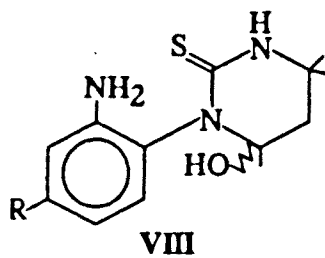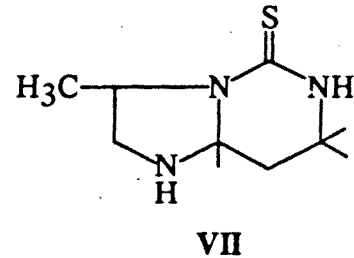
SCHEME

I

Ia. R = H,   R' = H
Ib. R = CH$_3$,   R' = H
Ic. R = H,   R' = Cl
Id. R = H,   R' = CH$_3$
Ie. R = CH$_3$,   R' = CH$_3$
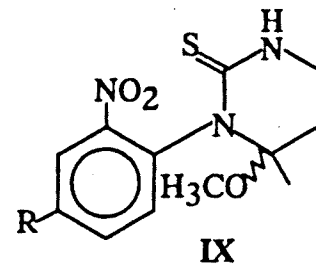If. R = CH$_3$,   R' = Cl

II

IIa, R = H,   R' = 2 - thiazolinyl
IIb, R = CH$_3$,   R' = 2 - thiazolinyl
III, R = CH$_3$,   R' = 2 - pyridinyl
IV, R = H,   R' = 1 - anthraquinonyl
V, R = H,   R' = 2 - anthraquinonyl

VI

VIa . x = O,   n = 2
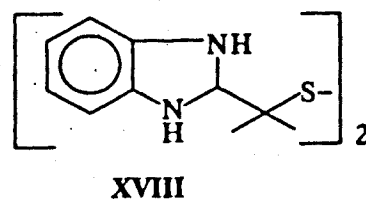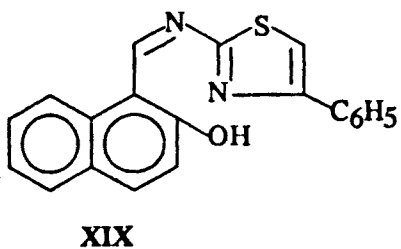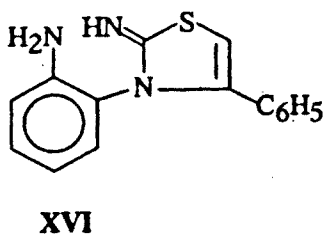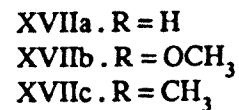VIb . x = NH, n = 2
VIc . x = O,   n = 3

VII

VIII

VIIIa . R = H
VIIIb . R = CH$_3$
VIIIc . R = OCH$_3$

IX

IXa . R = CH$_3$
IXb . R = OCH$_3$

(Contd..)

X

XII

XI

XIa. R=H, R'=OCH₃, R"=H, X=NH
XIb. R=H, R'=CH₃, R"=H, X=NH
XIc. R=NO₂, R'=H, R"=H, X=NH
XId. R=H, R'=H, R"=H, X=NH
XIe. R=H, R'=H, R"=H, X=O
XIf. R=H, R'=H, R"=H, X=S
XIg. R=COC₆H₅, R'=H, R"=H, X=NH
XIh. R=COC₆H₅, R'=H, R"=CH₂COOCH₃, X=NH

XLI

XIIIa. R = C₆H₅, R'=H
XIIIb. R = C₆H₅, R'=OCH₃
XIIIc. R = CH₂-CH=CH₂, R'=H

XV

2 HCl

XIV

XVII

XVIIa. R = H
XVIIb. R = OCH₃
XVIIc. R = CH₃

XVI

XIX

XVIII

(Contd..)

R

XX

XXa. R = OH
XXb. R = COOH

XXI

XXII

XXIII

2-(9-Acridinyl imino)thiazolines[9] (Ia-f), 2-(9-acridinyl amino) thiazolines[9] (II a-b), 2-(9-acridinyl amino) pyridine[9] (III), 1-(9-acridinyl amino) anthraquinone[10] (IV), 2-(9-acridinyl amino) anthraquinone[10] (V), pyrimido oxazole derivative[11] (VIa), imidazopyrimidine thione[12] (VIb), VII) pyrimidoxazine thione[12] (VIc), 1-(2-aminoaryl)-6-hydroxy-4,4, 6-trimethyl-1, 4, 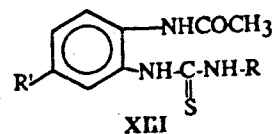5, 6-tetrahydropyrimidine-2-(3H) thiones[13] (VIIIa-c), 1-(2-nitroaryl)-6-methoxy-6-methyl-1, 4, 5, 6-tetrahydropyrimidine-2-(3H) thiones[14] (IXa,b), 1-(2-hydroxyphenyl)-4,4,6-trimethyl-1,4-dihydropyrimidine-2(3H)-thione[11] (X), pyrimido [3,4-a] benzimidazoles[13,11,15] (XIa-d,ζ) pyrimidobenzoxazole[11] (XIe), pyrimido-benzothiazole[11] (XIf), pyrimidobenzimidazole derivative[14] (XIh), pyrimidoanthraquinone imidazole[15] (XII), N, N'-disubstituted thioureas[16] (XIIIa-c), 1, 2-dithia-5, 8-diazacyclodeca-4, 8-diene[17] (XIV), 1,2-dithia-5,8-diazacyclode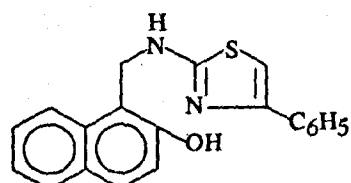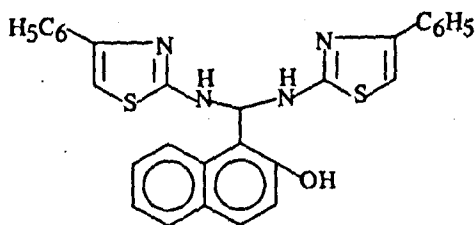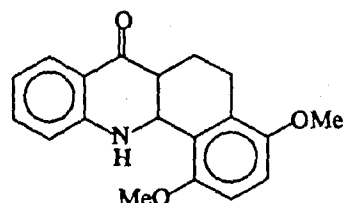cane dihydrochloride[17] (XV), 3-(o-aminophenyl)-2-imino-4-phenyl-4-thiazoline[18] (XVI), 9H-imidazolo [1, 2-a] benzimidazoles[19] (XVIIa-c), benzimidazole derivative[20] (XVIII), Schiff's bases[21] (XIX, XXa-b), 1-(2-methyl amino-4-phenyl thiazole)-2-hydroxy naphthalene[21] (XXI), compound[22] (XXII) and acridone derivative[15] (XXIII) were synthesized according to the procedures reported in literature. (Scheme-1).

Test compounds were dissolved in DMSO at an initial concentration of 0.2 mol and then were serially diluted in culture medium. Cell lines were from American Type Culture Collection (ATCC), bacterial and fungal strains were either clinical isolates (obtained from Clinica Dermosifilopatica, University of Cagliari) or collection strains from ATCC. H9/IIIB, MT-4 and C8166 cells [grown in RPMI 1640 containing 10% foetal calf serum (FCS), 100 UI/ml penicillin G and 100 mg/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). Human immunodeficiency virus type-1 (HIV-1, IIIB strain) was obtained from supernatants of persistently infected H9/IIIB cells. HIV-1 stock solutions had a titre of 5×10^7 cell culture infections dose fifty $(CCID_{50})/ml$.

All the compounds were screened against HIV-1 virus using AZT as a reference drug. Activity against the HIV-1 multiplication in acutely infected cells was based on inhibition of virusinduced cytopathogenicity in MT-4 cells[23]. Briefly, 50 µL of RPMI 10% FCS containing 1×10^4 cells were added to each well of flat-bottomed microtiter trays containing 50 µL of medium and serial dilutions of test compounds 20 µL of an HIV-1 suspension containing 100 $CCID_{50}$ were then added. After a 4d incubation at 37°, the number of viable cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method[24]. The cytotoxicity of

compounds, based on the viability of mockinfected cell as monitored by the MTT method, was evaluated in parallel with their antiviral activity.

The activity of compounds (Ia-f, IIa,b, III, IV, V VIa, X XIa-g, XII And XXIII) against Herpes virus type 1 and 2 and Coxsackie virus were tested in classical plaque reduction assays[25] using guanidine and ACG as reference drugs. Viral and cell growth at each drug concentration was expressed as percentage of untreated controls and the concentrations resulting in 50% (EC_{50}, CC_{50}) growth inhibition was determined by linear regression analysis.

The synthesized compounds (Scheme-1) were screened against Shigella, Salmonella, Staphylococcus and Streptococcus bacteria using streptomycin as a reference drug. Staphylococcus aureus, group D Streptococcus, Shigella and Salmonella sp. were recent clinical isolates. Assays were carried out in nutrient broth, pH 7.2, with an inoculum of 10^3 bacterial cells/tube. Minimum inhibitory concentrations (MIC) were determined after incubation at 37° for 18h in the presence of serial dilutions of test compounds.

Compounds mentioned is Scheme-1 were screened against human pathogenic fungi i.e C. albicans, C. parapsilosis, C. neoformans and A. fumigatus using miconaz as a reference drug. Yeast inocula were obtained by properly diluting cultures incubated at 37° for 30 h in Sabouraud dextrose broth to obtain 5 x 10^3 cells/ml. On the contrary, dermatophyte inocula were obtained from cultures grown at 37° for 5 d in Sabouraud dextrose broth by finely dispersing clumps with a glass homogenizer before diluting to 0.05 OD_{590}/ml. 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°. Growth controls were visually determined after 2 (yeasts) or 3 (dermatophytes). The MIC was defined as the compound concentration at which no macroscopic sign of fungal growth was detected. The minimal germicidal concentration (MGC) was determined by subcultivating in Subouraud dextrose agar samples from cultures with no apparent growth.

Compounds XVIIb and XVIIc showed antibacterial activity against Streptococcus D at concentrations slightly higher than those of streptomycin (1.6 μM) and compound XV was active (Slightly) against salmonella (MIC = 66 μM). When tested against yeast representatives, compound

XV was active against C. neoformans (MIC = 22 μM), compound XV and XXa were found slightly active against Candida at 66 μM but this concentration was cytotoxic for MT-4 cells. Only compound XIa was capable of protecting MT-4 cells from the cytopathic effect induced by HIV-1 (EC_{50} = 115 μM). All other compounds were found to be inactive. Plaque reduction assays revealed that the compounds were inactive against the viruses tested.

From the screening results it is clear that pyrimidobenzimidazole XIa show anti HIV activity, 9H-imidazolo [1,2-a] benzimidazoles XVIIb, c show antibacterial activity, Schiff base XXa show antifungal activity and 1,2-dithia-5,8-diazacyclodecane dihydrochloride (XV) shows antibacterial and antifungal activities. Synthesis and screening of more derivatives of above ring systems may lead to the discovery of more potent compounds which can be studies in detail.

ACKNOWLEDGEMENTS

REFERENCES

1. Kumar, R., Wang, L., Wiebe, L.I. and Knaus, E.E., J. Med. Chem., 1994, 37, 3554.
2. Johnston, M.I. and Hoth, D.F., Science, 1993, 260, 1286.
3. Fauci, A.S., Science, 1988, 239, 617.
4. Price, R.W., Brew, B., Sidtis, J., Rosenblum, M., Scheck, A. and Cleary, P., Science, 1988, 239, 586.
5. Lane, H.C. and Fauci, A.S., In Broder S. Ed; AIDS : Modern Concepts and Therapeutic Challenges, M. Dekker, New York., 1987, 185.
6. Klunder, J.M., Hargrave, K.D., West, M.A., Cullen, E., Pal, K., Behnke, M.L., Kapadia, S.R., Mc Neil, D.W., Wu, J.C., Chow, G.C. and Adams, J., J. Med. Chem. 1992, 35, 1887.
7. Nelson, S., Chem. Ber., 1991, 294.
8. Taraporewala, I.B., Cessac, J.W., Chanh, T.C., Delgado, A.V. and Schinazi, R.F., J. Med. Chem. 1992, 35, 2744.
9. Sondhi, S.M., Singhal, N., Verma, R.P., Raghubir, R., Goel, A.K. and Patnaik, G.K., Indian J. Chem., 1997, 36B, 620.
10. Sondhi, S.M., Sharma, V.K., Singhal, N., Verma, R.P., Shukla, R., Raghubir, R. and Dubey, M.P., Phosphor. Sulfur Silicon, 1999 (In press).
11. Sahu, R.K., Magan, A., Gupta, B., Sondhi, S.M., Srimal, R.C. and Patnaik, G.K., Phosphor. Sulfur Silicon, 1994, 88, 45.
12. Sondhi, S.M., Verma R.P., Singhal, N., Sharma, V.K., Shukla, R. and Patnaik, G.K., Phosphor. Sulfur Sili-

con, 1996, 118, 7.

13. Sondhi, S.M., Magan, A., Sahu, R., Mahesh, V.K., Shukla, R. and Patnaik, G.K., **Synthesis,** 1994, 1175.

14. Sondhi, S.M., Sharma V.K., Singhal, N., Verma, R.P., Shukla, R., Raghubir, R. and Dubey, M.P. **Synthesis** 1999, 878.

15. Sondhi, S.M., Verma, R.P., Sharma, V.K., Singhal, N., Kraus, J.L., Camplo, M. and Chermann, J.C., **Phosphor. Sulfur Silicon,** 1997, 122, 215.

16. Magan A. Sondhi, S.M., Singh P.P. and Ralhan, N.K., **J. Indian Chem. Soc,** 1990, 67, 476.

17. Joshua, A.V., Scott, J.R., Sondhi, S.M., Ball, R.G., and Lown, J.W., **J. Org. Chem.,** 1987, 52, 2447.

18. Vasudeva, S.K., Mahajan, M.P. and Ralhan, N.K., **Indian J. Chem.,** 1973, 11, 1204.

19. Sondhi, S.M., Mahajan, M.P. and Ralhan, N.K., **Indian J. Chem.,** 1979, 17B, 632.

20. Sondhi, S.M., Magan, A., Mahesh, V.K., Srimal, R.C. and Goel, A.K., **Indian J. Chem.,** 1994, 33B, 1144.

21. Sondhi, S.M., Bhatti, A.M., Mahajan, M.P. and Ralhan, N.K., **J. Indian Chem. Soc.,** 1975, 52, 49.

22. Sondhi, S.M., Sharma R.S. and Ralhan, N.K., **Indian J. Chem.,** 1976, 14B, 633

23. Mai, A., Artico, M., Sbardella, G., Quartarone, S., Massa, S., Loi, A. G., De Montis, A., Scintu, F., Putzolu, M. and La Colla, P., **J. Med. Chem.,** 1997, 40, 1447.

24. Pauwels, R., Balzarini, J., Baba, M., Snoeck, R., Scholds, D., Herdewijn, P., Desmyter, J. and De Clercq, E., **J. Virol. Methods,** 1988, 20, 309.

25. Collins, P. and Bauer, D.J. **Ann. N.Y. Acad. Sci.** 1977, 284, 49.