Structure of the synthesized Schiff bases was supported by IR, 1H-NMR and Mass spectral studies. In IR spectra, a prominent peak was observed for lactone of coumarins (1), (2), (3) and (4a-m) from 1735-1719 cm⁻¹ ν. In 1H-NMR spectra, the signal due to –N=CH- protons appeared as singlet at 8.98, heteroAr-H(d) proton appeared as singlet at 8.73, heteroAr-H(e) proton appeared as doublet at 8.42, Ar-H(g,g') two protons appeared as doublet at 7.97 (J = 8.27cps), Ar-H(c) proton appeared as doublet at 7.75, Ar-H(b) proton appeared as doublet of doublet at 7.65, Ar-H(h,h') proton appeared as doublet of doublet at 7.51 and Ar-H(a) proton appeared as doublet at 7.27. Molecular ion peak was observed at 445 and base peak at 196. These observations supported the formation of the resulting compound (4a). Out of the fourteen compounds subjected for qualitative antibacterial activity, one of the test compounds (4a), was shown to be active greater than that of test compounds such as (4), (4c), (4d), (4i), (4l) and (4m). All the test compounds were subjected for quantitative antibacterial determination and compounds, such as (4a), showed minimum inhibitory concentration at 147 μg and 141 μg against *Bacillus subtilis* and *Escherichia coli*, respectively when compared to that of the activity against standard drug ampicillin.

**ACKNOWLEDGEMENTS**

The authors thank Prof. B. G. Shivananda, Principal, Al-Ameen College of Pharmacy, Bangalore for support and facilities, Prof. T. N. Guru Row, Department of Solid State and Structural Chemistry Unit, Indian Institute of Science, Bangalore for X-ray powder diffractometer values and Prof. S. Asokan, Department of Instrumentation, Indian Institute of Science, Bangalore for 1H-NMR and mass spectra.

**REFERENCES**

1. Kennedy RO, Thornes RD. Coumarins: Biology, Applications and mode of action Chichester: Wiley and Sons; 1997. p. 155-7.

2. Venugopala KN, Jayashree BS. Synthesis and characterization of carboxamides of 2’-amino-4’-(6-bromo-3-coumarinyl) thiazole for their analgesic and antiinflammatory activity. Indian J Heterocyclic Chem 2003;12:307-10.

3. Venugopala KN, Jayashree BS. Synthesis and characterization of Schiff bases of aminothiazolyl bromocoumarin for their analgesic and antiinflammatory activity. Asian J Chem 2004;16:407-11.

4. Min J, Jiaxing H, Weiyi H, Hongwen H. Synthesis of some new 3-coumarinyl coumarin oximes and related cyclization products derived from 3-acetyl coumarin. Indian J Chem 2001;40:1223-5.

5. Kashman Y, Kirk R, Gustafson RW, Fuller JH 2nd, McMahon JB, Currens MJ, et al. HIV inhibitory natural products. Part 7. The calanolides, a novel HIV-inhibitory class of coumarin derivatives from the tropical rainforest tree, *Calophyllum lanigerum*. J Med Chem 1993;36:1110.

6. Bourinbaiar AS. Tan X, Nagorny R. Inhibitory effect of coumarins on HIV-1 replication and cell-mediated or cell-free viral transmission. Acta Virol 1993;37:241-50.

7. Kavanagh F. Analytical Microbiology. New York: Academic Press; 1963. p. 125-7.

8. Lowdin E, Odenholt-Tornqvist I, Bengtsson S, Cars O. A new method to determine the postantibiotic effect and the effects of subinhibitory antibiotic concentrations. Antimicrob Agents Chemother 1993;37:2200-5.

*Accepted 27 January 2008*  
*Revised 23 October 2007*  
*Received 27 March 2006*  

Indian J. Pharm. Sci., 2008, 70 (1): 88-91  

---

**In Vitro Antiviral Activity of some Novel Isatin Derivatives against HCV and SARS-CoV Viruses**

P. SELVAM*, N. MURGESH¹, M. CHANDRAMOHN², E. DE CLERcq³, E. KEYAERTS³, L. VIJGEN³, P. MAES³, J. NEYTS³  
AND M. V. RANST³

Arulmigu Kalasalingam College of Pharmacy, Krishnankoil - 626 190, ¹Institute of Pharmacology, Madurai Medical College, Madurai - 625 020, India, ²Bharat Ratna Kamarajar Liver Hospital and Research Center, Madurai - 625 001, ³Raga Institute for Medical Research, Katholieke Universiteit-Leuven, Minder broederstraat 10, LeuvenB-3000, Belgium

*Selvam, et al.: In Vitro Antiviral Activity of Isatin Derivatives*

*For correspondence*  
E-mail: periyasamyselvam2001@yahoo.co.in
Isatin (2,3-dioxoindole), a versatile lead molecule for potential bioactive agents, and its derivatives were reported to possess anticancer\(^1\), antibacterial activities\(^2-4\). Methisazone (N-methylisatin-\(\beta\)-thiosemicarbazone) was one of the first clinically used synthetic antiviral agent\(^5\). Isatin derivative were reported for antiviral activity against a variety of pathogens viruses\(^6\) and N,N-disubstitutedthiosemicarbazone derivative of isatin were tested for inhibition of HIV-1 replication\(^7\). Previously we reported synthesis of novel isatin derivatives and evaluated antiviral activity against HIV-1 and HIV-2 in MT-4 cells\(^8\). Significant antiviral activity was observed with these compounds against HIV-1 replication\(^9\).

In view of the broad spectrum activities of isatin derivatives, we aimed at evaluating the antiviral activity of some novel 4-[(1,2-dihydro-2-oxo-3H-indol-3-ylidene)amino]-N(4,6-dimethyl-2-pyrimidinyl)benzenesulphonamide and its derivatives (fig. 1) against pathogenic viruses such as hepatitis C virus (HCV) in human hepatoblastoma cells (Huh 5-2 cells) and Severe Acute Respiratory Syndrome corona virus (SARS-CoV) in Vero cultures.

4-[(1,2-dihydro-2-oxo-3H-indol-3-ylidene)amino]-N(4,6-dimethyl-2-pyrimidinyl)benzenesulphonamide and its derivatives (fig. 1) were prepared by condensing the

\[
\text{R and R}_1 \text{ for SPIII-5Br are Br and H, for SPIII-5Cl are Cl and H, for SPIII-5F are F and H, for SPIII-5H are H and H, for SPIII-Me are CH}_3 \text{ and H and for SPIII-NA are H and COCH}_3 \text{, respectively.}
\]

**TABLE 1: ANTIVIRAL ACTIVITY OF ISATIN DERIVATIVE AGAINST SARS-COV IN VERO E6 CELLS**

| Compound code | EC\(_{50}\) (µg/ml) | CC\(_{50}\) (µg/ml) | Maximum protection (%) (at 125 µg/ml) |
|---------------|-------------------|-------------------|-------------------------------------|
| 5Cl-IS-AC     | >125              | >125              | 0                                   |
| SPIII-5H      | >125              | >125              | 22                                  |
| SPIII-5Cl     | >125              | >125              | 10                                  |
| SPIII-5Br     | >125              | >125              | 2                                   |
| SPIII-5F      | >125              | >125              | 45                                  |
| SPIII-5Me     | >125              | >125              | 12                                  |
| SPIII-NA      | >125              | >125              | 12                                  |

*50% effective concentration required to reduce virus-induced cytopathicity by 50%. 
*50% cytotoxic concentration required to reduce host cell viability by 50%.

In the method adopted for antiviral activity against SARS-CoV in vero cells\(^10\). Vero E6 cells in 96-well tissue culture plates were used confluent. Culture medium was removed and 100 \(\mu\)L of minimum essential medium supplemented with 2% fetal bovine serum containing an appropriate concentration of antiviral compound was added. Inside a biosafety laboratory-3, 25 \(\mu\)L of a SARS-CoV virus solution added. Five concentrations were tested for cytotoxicity of the antiviral compounds. After an incubation period of three days at 37º in 5% CO\(_2\), the inhibition of the cytopathic effect (CPE) by the compounds was measured in a spectrophotometer (at 492) by the reduction by cellular dehydrogenase of the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrzolium (MTS) dye (Cell titer 96 Aqueous One Solution kit, promega) (20 \(\mu\)L MTS for 3h at 37º) in to a water soluble coloured formazan product. The antiviral activity and cytotoxicity of the test compounds are presented in Table 1.

Replication assay undertaken with Huh-5-2 cells\(^11-13\) [a cell line with a persistent HCV replication 1389luc-ubi-neo/NS3-3/5.1; replication with firefly luciferase-lubquitin-neomycine phosphotransferase fusion protein EMCV-IRES driven NS3-5B HCV polyprotein] was cultured in RPMI medium 2 mM glutamine, 1 × non
essential amino acid (Life Technologies, DC); 100 IU/ml penicillin and 100 μg/ml streptomycin and 250 μg/ml G418 (Geneticin, Life Technologies Washington DC). Cells were seeded at a density of 7000 cells per well in 96 well view plate TM (Packard, CA) in medium containing the same compounds as described above, except for G418. Cells were allowed to adhere and proliferate for 24 h. At that time, culture was removed and serial dilution of test compounds were added in culture medium lacking G418. Interferon alfa 2a (500 IU) was added as a positive control. Plates were further incubated at 37° and 5% CO2 for 72 h replication of HCV replicon in Huh-5 cells results in luciferase activity in the cells. Luciferase activity was measured by adding 50 μl of 1 × Gloysis buffer (Promega) for 15 min of following by adding 50 μl Steady-Glo Luciferase assay reagent (promega). Luciferase activity was measured with luminometer and signal in each individual well was expressed as a percentage of the untreated culture. Parallel culture of Huh 5-2 cells, seeded at a density of 7000 cells/well of classical 96-well cell culture plates (Becton-Dickenson) were treated in a similar fashion except that no Glo-lysis buffer or Stady-Glo Luciferase reagent was added. Instead the density of the cluture was measured by means of the MTS method (Promega). The antiviral activity and cytotoxicity of the test compounds are prepared in Table 2.

From the antiviral and cytotoxicity assay it was observed that the compounds 4-[(1,2-dihydro-2-oxo-3H-indol-3-ylidene)amino]-N(4,6-dimethyl-2-pyrimidiny)benzene sulphonamide (SPIII-5H) and bromo derivative (SPIII-Br) inhibits HCV RNA synthesis at the EC50 of 17 and 19 μg/ml respectively while its CC50 for cell growth was 42 μg/ml in Huh 5-2 cells. The isatin lead molecule 5CI-IS-AC did not inhibit the HCV RNA synthesis (EC50 and CC50 more than 50 μg/ml) and the replication of SARS-CoV (maximum protection 0%). SPIII derivative showed 2-45% maximum protection against the replication of SARS-CoV in Vero cells and compound SPIII 5F exhibited 45% maximum protection against the replication of acutely infected SARS-CoV in Vero cell.

The present study was aimed at investigating some novel isatin derivative for antiviral activities against HCV and SARS-CoV to identify potential bioactive agent in the series. From the results of biological activities it appeared that some of the derivatives showed antiviral activity against HCV virus in Huh 5-3 cells.SPIII-5H and bromo derivatives inhibited the synthesis of HCV RNA, but only at a relatively high concentration (17 and 19 μl). Further modification in the series may help in optimizing anti-HCV activity.

REFERENCES

1. Popp FD. Synthesis of potential anti-neoplastic agents, XX: Compounds related to the 3-o-nitrophenylhydrazone of isatin. J Pharm Sci 1969;12:182-4.
2. Varma, RS, Nobles WL, Synthesis and Antiviral and Antibacterial Activity of Certain N-Dialkylaminomethylisatin β-Thiosemicarbazones. J Med Chem 1967;10:972-4.
3. Pandeya SN, Srimath D, De Clercq E, Pannecoque C, Vitrou M. Anti-HIV activity of some Mannich bases of isatin derivatives. Indian J Pharm Sci 1998;60:207-12.
4. Pandeya SN, Srimath D, Nath G, De Clercq E. Synthesis, antibacterial, antifungal and anti-HIV activities of Norfloxacin Mannich bases. Eur J Med Chem 2000:35:249-55.
5. Bauer DJ, Sadler PW. The structure-activity relationships of the antiviral chemotherapeutic activity of isatin β-thiosemicarbazone. Br Pharm Chemother 1960:15:101-10.
6. Wolf ME, Antiviral agents: Burger Medicinal Chemistry, 4th ed., Part-II, New York: John Wiley and Sons; 1979. p. 553.
7. Teitz Y, Ronen D, Vansover A, Stematsky T, Rigg JL. Inhibition of human immunodeficiency virus by N-methylisatin-β-4':4'-diethylthiosemicarbazone and N-allyl isatin-β-4':4'-diallylthiosemicarbazone. Antiviral Res 1994;24:305-14.
8. Pauwels R, Balzarini J, Baba M, Snoeck R, Schols DJ, Herdewijin P, et al. Rapid and automated tetrazolium based colourimetric assay for the detection of anti-HIV compounds. J Virol Methods 1989;20: 309-21.
9. Selvam P, Chandramohan M, De Clercq E, Pannecoque C, Witrou M. Synthesis and anti-HIV activity of 4-[(1,2-dihydro-2-oxo-3H-indol-3-
Physicochemical and Pharmacokinetic Parameters in Drug Selection and Loading for Transdermal Drug Delivery

N. S. CHANDRASHEKAR* AND R. H. SHOBHA RANI
Department of Pharmaceutics, Al-Ameen College of Pharmacy, Hosur Road, Bangalore - 560 027, India

Chandrashekar, et al.: Physicochemical and Pharmacokinetic Parameters for Transdermal Drug Delivery

Skin of an average adult body covers a surface of approximately 2 m² and receives about one-third of the blood circulating through the body. The transdermal route of administration cannot be employed for a large number of drugs. The rationality of drug selection based on pharmacokinetic parameters and physicochemical properties of the drug are the important factors to be considered for deciding its suitability of drug for delivery by transdermal route.

Key words: Missing???

*For correspondance:
E-mail: nschandrashekar@gmail.com

Over the past three decades, developing controlled drug delivery has become increasingly important in the pharmaceutical industry. The pharmacological response, both the desired therapeutic effect and the undesired adverse effect, of a drug is dependent on the concentration of the drug at the site of action, which in turn depends upon the dosage form and the extent of absorption of the drug at the site of action.

Skin of an average adult body covers a surface of approximately 2 m² and receives about one-third of the blood circulating through the body. Skin contains an uppermost layer, epidermis which has morphologically distinct regions; basal layer, spiny layer, stratum granulosum and upper most stratum corneum, it consists of highly cornified (dead) cells embedded in a continuous matrix of lipid membranous sheets. These extracellular membranes are unique in their compositions and are composed of ceramides, cholesterol and free fatty acids. The human skin surface is known to contain, on an average, 10-70 hair follicles and 200-250 sweat ducts on every square centimeters of the skin area. It is one of the most readily accessible organs of the human body. The potential of using the intact skin as the port of drug administration to the human body has been recognised for several decades, but skin is a very difficult barrier to the ingress of materials allowing only small quantities of a drug to penetrate over a period of time.

The transdermal route of administration cannot be employed for a large number of drugs. The objective of this paper is to focus on the rationality of drug selection based on pharmacokinetic parameters and physicochemical properties of the drug.

Physiochemical factors such as solubility, crystallinity, molecular weight <400, polarity, melting point <200, partition coefficient Log P (octanol-water) between –1.0 to 4 must be considered. Biological factor should also be considered such as skin irritation, site of application of the patch e.g. scopolamine patch for motion sickness is applied...