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SYNTHESIS OF NOVEL ELLIPTICINE ANALOGUES AND THEIR INHIBITION OF MOLONEY LEUKAEMIA REVERSE TRANSCRIPTASE

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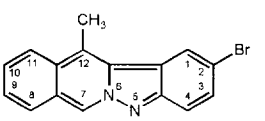
Abstract: Two new ellipticine analogues were synthetized as potential non nucleoside inhibitors of reverse transcriptase and were tested on Moloney leukaemia virus reverse transcriptase *in vitro*. Both derivatives **(9a,b)** showed considerable inhibitory effect; ID_{50} was found to be in the range of 2.8 to 4.5 x 10^{-5} M.

Introduction: There are several compounds which inhibit the replication of retroviruses including HIV and leukaemia viruses. One of the key enzymes is the reverse transcriptase which transcribes the viral RNA into DNA. This enzyme can be inhibited by the class of nucleotide analogues e.g. AZT and by a second class of medicines called non-nucleoside inhibitors[1,2,3]. Since the resistance to retroviral agents continuously emerge in the HIV infected population therefore the evaluation of new types of antiretroviral compounds seems promising. In this paper we describe the synthesis of new nitrogen positional analogues of ellipticine and report on their effective reverse transcriptase inhibition tested with Moloney leukaemia virus.

Synthesis: In the course of our earlier studies on fused tetrazolium salts we have observed[4] that as a result of a nuc-leophile induced ring opening reaction of such systems followed by ring closure, the tetracyclic 2-bromo-12-methyl-indazolo[2,3-b]isoquinoline derivative (1) was obtained, besides other products, in poor yield. As the ring system of **1** differs from that of the potent antitumor compound "ellipticine" **(2)**[5] only in the position of one nitrogen atom, we decided to reinvestigate the synthetic possibilities to **1** and to study its biological effect.



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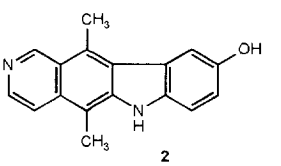

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In order to carry out extensive microbiological or pharmacological studies of such derivatives, elaboration of a synthetic path more economic than that found earlier[4] seemed desirable. In the context of combined directed

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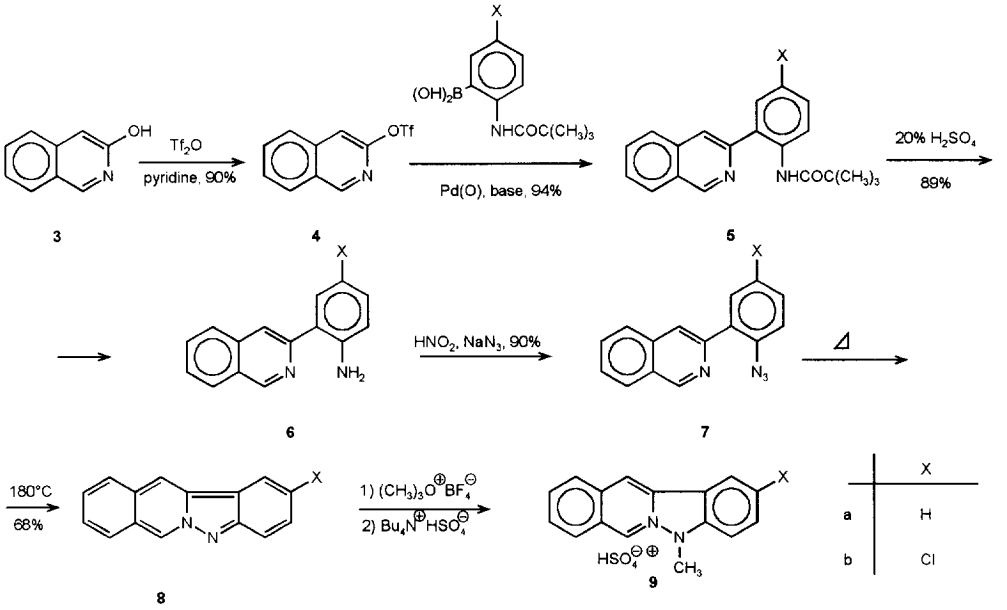
orthometalation - cross coupling strategies we developed a new route to 3-aryl substituted isoquinolines bearing an appropriate functional group for a subsequent ring closure by coupling of o-N-pivaloyl-aminophenyl boronic acids with the easily accessible isoquinoline-3-O-triflate based on the excellent Suzuki protocol.



Thus, 3-hydroxyisoquinoline (**3**)[6] was transformed to a triflate (**4**)[7] which, according to literature data[8], seemed to be suitable for the desired cross-coupling reaction[9] with the appropriate substituted aniline. This conversion (i.e. coupling of **4** with 5-X-2-pivaloyl-aminophenyl boronic acid[10] in the presence of Pd(0), where X = Cl or H) was accomplished in good yield to give **5**[11] which was then hydrolyzed to the free amine **6**[12]. The amine functional group was transformed to an azide in a one-pot procedure through diazotation followed treatment with sodium azide[13] to give **7**[14] which was ring closed to the desired tetracyclic compound **8**[15] by heating, obviously through nitrogen elimination and formation of an intermediate nitrene[16]. In order to achieve better water solubility for the biological tests, both products (**8a,b**) were subjected to methylation by Meerwein's salt to afford N-methyl compounds **9a** and **9b** as hydrogensulfate salts[17].

Results and Discussion[18]: To evaluate the potential of ellipticine analogues **9a** and **9b** for inhibiting reverse transcriptase, the enzyme of Moloney leukaemia virus was incubated in the presence of 2 μl of the enzyme inhibitors. The enzyme activity was inhibited in a concentration dependent manner with an IC value of 18.0 μg (Figure 1.). As these results show both derivatives (**9a,b**) were able to inhibit considerably the reverse transcriptase activity of Moloney leukaemia enzyme with an ID_{50} in the range of 2.8 to 4.5×10^{-5}M. This enzyme inhibitory effect is comparable to that of AZT[19] and may be of practical importance in the future drug design for combination chemotherapy of retroviral infection.

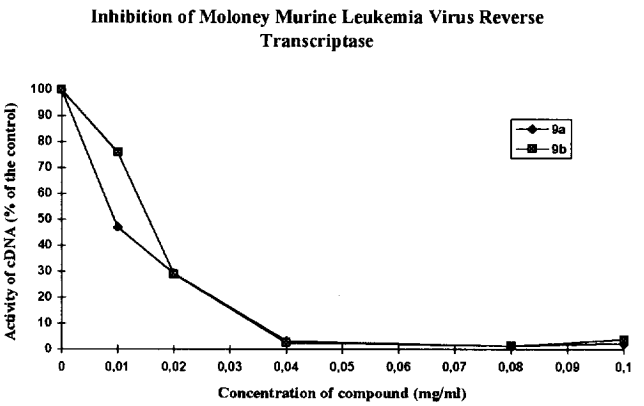


Figure 1. The susceptibility of MoL reverse transcriptase to the inhibition of **9a** and **9b**. Reverse transcriptase assay was performed as described in Materials and methods.

Extension of the new synthetic methodology for these promising indazolo[2,3-b]isoquinolines and related compounds as well as their microbiological studies are in progress.

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7. colorless oil; ^{1}H NMR ($CDCl_3$): δ, 7.59(s, 1H); 7.70 (dt, 1H); 7.80 (dt, 1H); 7.92 (d, 1H); 8.07 (d, 1H); 9.08 (s, 1H).
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11. General procedure of the synthesis of 3-(o-pivaloylamidophenyl)-isoquinolines (**5**): to a solution of tetrakis(triphenylphosphine)palladium(0) (0.2g) in ethylene glycol dimethyl ether (20mL) 3-hydroxiisoquinoline-O-triflate 5mmol (1.4g) was added. After stirring the mixture for 20 minutes under argon 5.5 mmol of the required boronic acid and a solution of 1.3 g of sodium bicarbonate in water (10 mL) were added. The reaction mixture was refluxed for 4 hours under argon. The resulting mixture was treated with 30 mL of water and extracted with ehtyl acetate. The organic layer was evaporated to dryness and subjected to flash chromatography (alumina/ $CHCl_3$:c-hexane 1:1).

2,2-Dimethyl-N-(2-(3-isoquinolyl)phenyl)propanamide (**5a**): This compound was obtained as white needles, yield 94%; m.p.:142°C. ^{1}H NMR ($CDCl_3$): δ 1.31(s, 9H), 7.19(dt, J=7.8Hz, 1.0Hz, 1H), 7.42(dt, J=7.8Hz, 1.8Hz, 1H), 7.65(ddd, J=7.5Hz, 6.5Hz, 1.0Hz, 1H), 7.74(dd, J=7.8Hz, 1.0Hz, 1H) 7.76(ddd, J= 8.0Hz, 7.65Hz, 1.2Hz, 1H), 7.92(dd, J=6.5Hz, 1.0Hz, 1H), 8.03(dd, J=8.0Hz, 1.0Hz, 1H), 8.06(s, 1H), 8.58(dd, J=7.8Hz, 1.8Hz, 1H), 9.30(s, 1H), 12.05(s, 1NH). IR (KBr): 2965, 1669,1583, 1521, 1435, 1311, 761 cm^{-1}.

2,2-Dimethyl-N-(4-chloro-2-(3-isoquinolyl)phenyl)propanamide (**5b**): This compound was obtained as white needles, yield 86%; m.p.: 177°C. ^{1}H NMR ($CDCl_3$): δ 1.30(s, 9H), 7.37(dd, J=8.0Hz, 1.0Hz, 1H), 7.68(ddd, J=7.5Hz, 6.5Hz, 1.0Hz, 1H), 7.72(d, J=1.0Hz, 1H), 7.77(ddd, J=8.0Hz, 7.5Hz, 1.0Hz, 1H), 7.92(dd, J= 6.5Hz, 1.0Hz, 1H), 8.04(dd, J=8.0Hz, 1.0Hz, 1H), 8.06(s, 1H), 8.51(d, J= 8.0Hz, 1H), 9.28(s, 1H), 12.08(s, 1NH). IR (KBr): 2967, 1667, 1577, 1514, 1396, 762 cm^{-1}.

12. General procedure for the hydrolysis of pivaloylamides (**6**): The appropriate pivaloylamido compound (**5**, 1 mmol) was added to a 20% solution of sulfuric acid (10 mL) and refluxed for 2 days. The resulting reaction mixture was neutralized with concentrated ammonia. Extraction with chloroform, afforded the pure amine.

3-(2-Aminophenyl)isoquinoline(**6a**): The reaction of **5a** according to the general procedure gave **6a** as a solid, yield 89%; m.p.: 79°C. ^{1}H NMR ($CDCl_3$) : δ 5.42(s, NH_2), 6.79(dd, J=8.0Hz, 1.0Hz, 1H), 6.84(dt, J=7.0Hz, 1.0Hz, 1H), 7.19(ddd, J=8.0Hz, 7.0Hz, 1.5Hz, 1H), 7.57(dd, J= 7.0Hz, 1.5Hz, 1H), 7.58(dd, J=8.0Hz, 1.0Hz, 1H), 7.69(ddd, J=8.0Hz, 7.0Hz, 1.0Hz, 1H), 7.85(dd, J= 8.0Hz, 1.0Hz, 1H), 7.94(s, 1H), 7.98(dd, J=8.0Hz, 1.0Hz, 1H), 9.28(s,1H). IR (KBr) : 3462, 3358, 3333, 1624, 1611, 1587, 1498, 1463, 892, 766, 755, 752 cm^{-1}.

3-(2-Amino-5-chlorophenyl)isoquinoline (**6b**): The reaction of **5b** according to the general procedure gave **6b** as a solid, yield 91%; m.p.: 107°C. ^{1}H NMR ($CDCl_3$): δ 5.48(s, 2NH), 6.72(d, J=8.0Hz, 1H), 7.14(dd, J= 8.0Hz, 1.0Hz, 1H), 7.54(d, J=1.0Hz, 1H), 7.61(dt, J=8.0Hz, 1.0Hz, 1H), 7.73(dt, J=8.0Hz, 1.0Hz, 1H), 7.88(dd, J=8.0Hz, 1.0Hz, 1H), 7.93(s, 1H), 7.99(dd, J=8.0Hz, 1.0Hz, 1H), 9.28(s, 1H). IR (KBr) : 3467, 3356, 1629, 1604, 1581, 1494, 1482, 881, 757 cm^{-1}.

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14. General procedure for the preparation of 3-(o-azidophenyl)isoquinolines (**7**): A mixture of the appropriate 3(o-aminophenyl)isoquinoline (**6**, 2 mmol) and concentrated hydrochloric acid (10 mL) was cooled at 0°C, then a solution of sodium nitrite (0.28g, 4 mmole) in water (10 mL) was added. The resulting solution was first stirred at 0°C for 2h, then a mixed solution of sodium azide (0.26g, 4 mmole), sodium acetate (2.5g, 30.5 mmole) in water (10 mL) was added. After stirring at this temperature for 1h the solution was neutralized with sodium carbonate, extracted with chloroform. The organic layer was evaporated to dryness and subjected to flash chromatography (alumina, $CHCl_3$:c-hexane 10:1).

3-(2-Azidophenyl)isoquinoline (**7a**): This compound was obtained in 90% yield; m.p.: 68°C.

^{1}H NMR ($CDCl_3$) : δ 7.30(dt, J=7.0Hz, 1.3Hz, 1H), 7.31(dd, J=8.0Hz, 1.0Hz, 1H), 7.45(ddd, J=8.0Hz, 7.0Hz, 1.0Hz, 1H), 7.63(dt, J=8.0Hz, 1.0Hz, 1H), 7.72(dt, J=8.0Hz, 0.8Hz, 1H), 7.81(dd, J=7.0Hz, 1.0Hz, 1H), 7.89(dd, J=8.0Hz, 1.0Hz, 1H), 8.01(dd, J=8.0Hz, 1.0Hz, 1H), 8.06(s, 1H), 9.35(s, 1H). IR (KBr) :2125, 2078, 1281, 750, 732 cm^{-1}.

3-(2-Azido-5-chlorophenyl)isoquinoline (**7b**): This compound was obtained in 91% yield; m.p.:142°C. (decomp.) ^{1}H NMR ($CDCl_3$) : δ 7.27(d, J=8.0Hz, 1H), 7.41(dd, J=8.0Hz, 1.0Hz, 1H), 7.65(dt, J=8.0Hz, 1.0Hz, 1H), 7.75(dt, J=8.0Hz, 1.0Hz, 1H), 7.86(d, J=1.0Hz, 1H), 7.90(dd, J=8.0Hz, 1.0Hz, 1H), 8.02(dd, J=8.0Hz, 1.0Hz, 1H), 8.10(s,1H),9.35(s,1H). IR (KBr) :2129, 2100, 2042, 1489,1303, 876,741 cm^{-1}.

15. General procedure for the synthesis of indazolo-isoquinoline from azides (**8**): The solution of azide (**7**, 1 mmol) in 1,2 dichlorobenzene (10 mL) was heated at 180 °C for 40 minutes. The solvent was removed under vacuum, the crude solid was purified by flash chromatography (alumina/ chloroform). Indazolo[2,3-b]isoquinoline (**8a**): According to the general procedure we obtained as a yellow solid, yield 68%; m.p.: 201-2°C. ^{1}H NMR (DMSO-d6): δ 7.26(ddd, J=8.0Hz, 6.0Hz, 1.0Hz, 1H), 7.54-7.61(m,2H), 7.65(ddd, J=8.0Hz, 6.0Hz, 1.0Hz, 1H), 7.78(dt, J=8.0Hz, 1.0Hz, 1H), 8.08-8.18(m, 2H), 8.44(dt, J=8.0Hz, 1.0Hz, 1H), 9.12(s, 1H), 9.93(s, 1H). IR (KBr) : 1485, 1448, 1351, 866, 733 cm^{-1}.

2-Chloro-indazolo[2,3-b]isoquinoline (**8b**): According to the general procedure we obtained as a yellow solid, yield 72%; m.p.:242-2°C. ^{1}H NMR (DMSO-d6): δ 7.60(m, 2H), 7.63(dd, J=8.0Hz, 1.0Hz, 1H), 7.80(d, J=8.0Hz, 1H), 8.09(dd, J=8.0Hz, 1.0Hz, 1H), 8.17(dd, J=8.0Hz, 1.0Hz, 1H), 8.57(d, J=1.0Hz, 1H), 9.17(s, 1H), 9.95(s, 1H). IR (KBr): 1491,1452, 1350, 1302, 849, 803, 740 cm^{-1}.

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17. General procedure for the methylation of indazoloisoquinolines (preparation of **9a,b**): To a solution of the appropriate indazoloisoquinoline **8,** (1 mmol) in abs. CH_2Cl_2 (20mL) was added 0.24g (1.6 mmol) of Meerwein-salt. The reaction mixture was stirred for 24 hours, then 10mL of ether was added. The

precipitated crystals were filtered off to give a fluoroborate salt. This was transferred to the hydrogensulfate salt by adding tetrabutylammonium hydrogensulfate in acetonitrile, whereupon the product precipitated.

5-Methyl-indazolo[2,3-b]isoquinolinium hydrogensulfate (**9a**): This compound was obtained in 79% yield; m.p.: > 250°C. ^{1}H NMR (CD_3CN+TFA) : δ 4.29(s, 3H), 7.61(dt, J=7.8Hz, 1.6Hz, 1H), 7.79(dd, J=8.0Hz, 1.6Hz, 1H), 7.94(ddd, J=8.0Hz, 7.5Hz, 1.0Hz, 1H), 7.95(ddd, J=8.0Hz, 7.5Hz, 1.0Hz, 1H), 8.01(ddd, J=7.5Hz, 6.5Hz, 1.0Hz, 1H), 8.31(dd, J=6.5Hz, 1.0Hz, 1H), 8.40(dd, J=8.0Hz, 1.0Hz, 1H), 8.48(dd, J=7.8Hz, 1.6Hz, 1H), 9.19(s, 1H), 9.87(s, 1H). IR (KBr) : 3410, 3052, 1494, 1343, 1193, 1047, 847, 745, 581 cm^{-1}.

2-Chloro-5-methylindazolo[2,3-b]isoquinolinium hydrogensulfate (**9b**): This compound was obtained in 62% yield; m.p.: >250°C. ^{1}H NMR (CD_3CN+TFA): δ 4.28(s, 3H), 7.78(d, J=9.0Hz, 1H), 7.92(dd, J=9.0Hz, 2.0Hz, 1H), 7.97(ddd, J=8.0Hz, 7.5Hz, 1.0Hz, 1H), 8.03(ddd, J=8.0Hz, 7.5Hz, 1.0Hz, 1H), 8.33(dd, J=8.0Hz, 1.0Hz, 1H), 8.41(dd, J=8.0Hz, 1.0Hz, 1H), 9.19(s, 1H), 9.84(s, 1H). IR (KBr) : 3443, 2998, 1491, 1482, 1223, 1189, 1009, 879, 571 cm^{-1}.

18. Materials and Methods: **9a, 9b** were assayed for their ability to inhibit theM-MuLV.Reverse Transcriptase, New England BioLabs (isolated from E. coli containing the plasmid pB6B15.23). Enzymatic assay of MuLV Rts were carried out by following the poly(rA)$_n$oligo(dT)$_{12-18}$ (New England BioLabs) directed incorporation of [^{3}H]dTTP (Amersham) into cDNA. The 10x reverse transcriptase buffer contained 500 mM Tris-HCL (pH 8.3), 80 mM $MgCl_2$, 300 mM KCl, and 100 mM DTT. In all experiments the final volume of reaction assay was 20 µl. This contained water, 2µl/10x buffer, 20 µg/ml template-primer, 5 µM dTTP precursor (New England BioLabs), 0,2 µCi triciated precursor, reverse transcriptase and the compounds tested which were administrated in the reaction medium for 10 minutes before adding the RT, in 20µl final volume. The assay was initiated by 5U reverse transcriptase followed by an incubation for 40 min at 37°C. 15 µl of the mixture was then transferred to a DE81 filter paper disc, washed and radioactivity measured by Packard Tri-Carb 4530 liquid scintillation counter. The residual enzymatic activities were calculated relative to the activity in percent when no drug was added.

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(b) The effect of our new derivatives **9a** and **9b** was compared to AZT triphosphate as a reference compound (to give and ID_{50} of 2.10^{-7}) which blocks the enzyme activity at the nucleoside binding site. We assume, however, that our ellipticine analogues are able to block the reverse transcriptase activity at the so called non nucleoside binding site of the enzyme due to sterical fitness.

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