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# Molecular Modelling Analysis of the Metabolism of Topotecan

Fazlul Huq

School of Biomedical Sciences, Faculty of Health Sciences, C42,
The University of Sydney, P.O. Box 170, Lidcombe, NSW 1825, Australia

**Abstract:** Topotecan (TOP) is a water-soluble analogue of camptothecin, approved for use in second line therapy against ovarian carcinoma and small-cell lung cancer. TOP inhibits DNA replication and RNA transcription by stabilizing the cleavable complexes formed between nuclear enzyme topoisomerase I and DNA. There are two general types of topoisomerases: type I and type II. Type I cleaves and separates a single strand of DNA whereas type II cleaves both the DNA strands. Mammalian topoisomerase I is particularly important for supporting movement of replication fork during DNA replication and for relaxing supercoils formed during DNA transcription. The lactone ring undergoes pH-dependent reversible hydrolysis. The primary metabolite of TOP is $N$-desmethyltopotecan (NDMTOP). Both TOP, NDMTOP exist in the lactone ring form at low pH and open ring acid form at high pH. Molecular modelling analyses based on molecular mechanics, semi-empirical (PM3) and DFT (at B3LYP/6-31G* level) calculations show that TOP and all its metabolites have small LUMO-HOMO energy differences so that they would be kinetically labile. The molecular surfaces of TOPO and NDMTOPO have some electron-deficient regions so they may be subject to nucleophilic attacks such as those by glutathione and nucleobases in DNA. The reaction with glutathione would induce cellular toxicity by compromising the antioxidant status of the cell whereas that with nucleobases would cause DNA damage. The lability of TOP and its metabolites means that the rates of such adverse reactions are likely to be significant.

**Key words:** Topotecan, camptothecin, topoisomerase I, ovarian carcinoma, small-cell lung cancer, molecular modelling

## Introduction

Topotecan (TOP, Hycamin™) is a water-soluble analogue of camptothecin, approved for use in second line therapy of patients with ovarian carcinoma and small-cell lung cancer (Arbuck and Takimoto, 1998; Herben *et al.*, 2002). In addition, it has also been used to treat many forms of childhood cancer, including neuroblastoma, medulloblastoma, rhabodomyosarcoma and leukaemia (Furman *et al.*, 2002; Galindo-Rodriguez *et al.*, 2002). TOP inhibits DNA replication and RNA transcription by stabilizing the cleavable complexes formed between nuclear enzyme topoisomerase I and DNA (Rosing *et al.*, 1999). There is an increased interest in topoisomerases as they are found to be targets for naturally occurring anticancer drugs (Tian *et al.*, 2005). Topoisomerases are a group of enzymes, present in all organisms including bacteria, viruses, yeast and humans, that alter topology of DNA (Wang, 1996). There are two general types of topoisomerases: type I and type II. Type I cleaves and separates a single strand of DNA whereas type II cleaves both the DNA strands. Mammalian topoisomerase I is particularly important for supporting movement of replication fork during DNA replication and for relaxing supercoils formed during DNA transcription (Wang, 1996).

The lactone ring undergoes reversible hydrolysis that is found to be pH-dependent. The ring form (TOPR) is more stable at low pH whereas the open ring acid form (TOPO) is more stable at high pH. The drug needs to be in lactone ring form for the stabilization of topoisomerase I complex. TOP undergoes both renal and hepatic elimination (Bai *et al.*, 2003). About 49% of an intravenously administered dose and 20% of an oral dose are excreted in the urine as parent drug. The primary hepatic metabolite of TOP is *N*-desmethyltopotecan. The primary metabolite of TOP is *N*-desmethyltopotecan (NDM-TOP). Like TOP, *N*-desmethyltopotecan also exists in the lactone ring form (NDM-TOPR) at low pH and in the open ring acid form (NDM-TOPO) at high pH.

In this study molecular modelling analyses have been carried out of TOPR, TOPO and the metabolites NDM-TOPR and NDM-TOPO, in order to obtain a better understanding of toxicity due to TPT and its metabolites. The study was carried out in the School of Biomedical Sciences, The University of Sydney during February to June 2006.

**Computational Methods**

The geometries of TOPR, TOPO and the metabolites NDM-TOPR and NDM-TOPO have been optimized based on molecular mechanics (Fig. 1), semi-empirical and DFT calculations, using the molecular modelling program Spartan '04 (Spartan, 2004). Molecular mechanics calculations were carried out using MMFF force field. Semi-empirical calculations were carried out using the routine PM3. DFT calculations were carried out at B3LYP/6-31G* level. In optimization calculations, a RMS gradient of 0.001 was set as the terminating condition. For the optimized structures, single point calculations were carried to give heat of formation, enthalpy, entropy, free energy, dipole moment, solvation energy, energies for HOMO and LUMO. The order of calculations: molecular mechanics
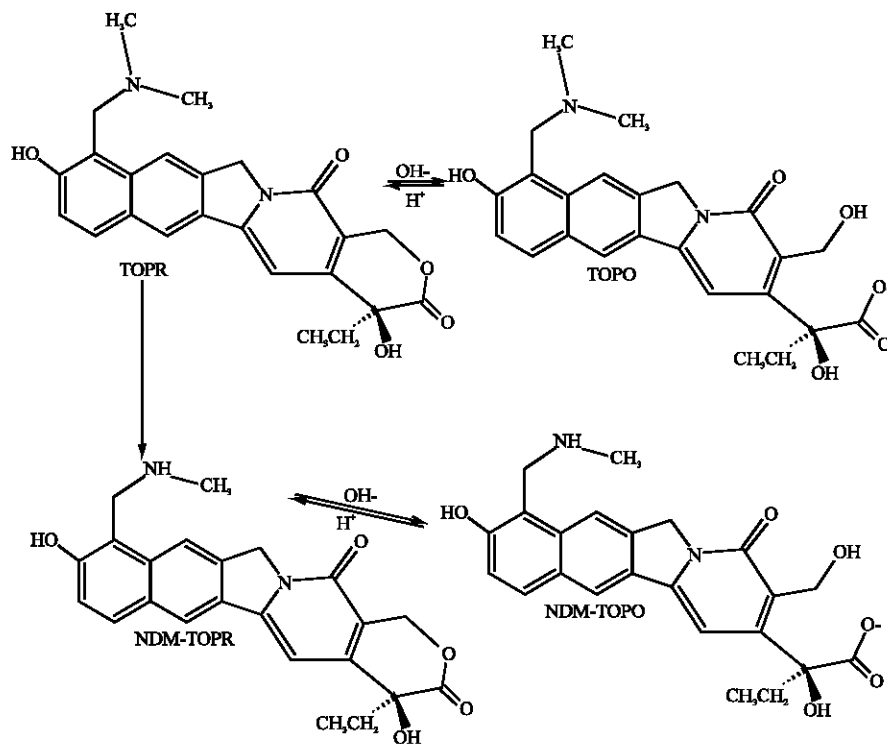


Fig. 1: Metabolic pathways of toptotecan

followed by semi-empirical followed by DFT ensured that the structure was not embedded in a local minimum. To further check whether the global minimum was reached, some calculations were carried out with improvable structures. It was found that when the stated order was followed, structure corresponding to the global minimum or close to that could ultimately be reached in all cases. Although RMS gradient of 0.001 may not be sufficiently low for vibrational analysis, it is believed to be sufficient for calculations associated with electronic energy levels.

## Results and Discussion

Table 1 gives the total energy, heat of formation as per PM3 calculation, enthalpy, entropy, free energy, surface area, volume, dipole moment, energies of HOMO and LUMO as per both PM3 and DFT calculations for TOPR and TOPO and their metabolites NDM-TOPR and NDM-TOPO. Figure 2-5 give the regions of negative electrostatic potential (greyish-white envelopes) in (a) and density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral) in (b) as applied to the optimized structures of TOPR, TOPO, NDM-TOPR and NDM-TOPO. The calculated solvation energies of TOPR, TOPO and their metabolites NDM-TOPR and NDM-TOPO from PM3 calculations in kcal mol$^{-1}$ are respectively -18.21, -29.65, -20.38 and -36.68 and their dipole moments from DFT calculations are 8.8, 8.2, 8.9 and 8.7, respectively.

Large or moderately large solvation energies and dipole moments for TOPR, TOPO, NDM-TOPR and NDM-TOPO indicate that the compounds will be soluble in water so that they would have a high clearance rate.

The calculated LUMO-HOMO energy differences for TOPR, TOPO, NDM-TOPR and NDM-TOPO are found to be low indicating that the compounds would all be kinetically labile.

The molecular surface of TOPR, TOPO, NDM-TOPR and NDM-TOPO are found to abound in neutral (green), negative (yellow and red) regions, indicating the two compounds are more

Table 1: Calculated thermodynamic and other parameters of toptotecan and its metabolites

| Molecule | Calculation type | Total energy (kcal mol$^{-1}$/atomic unit*) | Heat of formation (kcal mol$^{-1}$) | Enthalpy (kcal mol$^{-1}$ K$^{-1}$) | Entropy (cal mol$^{-1}$ K$^{-1}$) | Solvation energy (kcal mol$^{-1}$ K$^{-1}$) |
|---|---|---|---|---|---|---|
| TOPR | PM3 | | -140.63 | 1205.85 | 759.30 | -15.69 |
| | DFT | -1430.69 | | 1207.80 | 758.69 | -20.56 |
| TOPO | PM3 | | -188.47 | 1282.58 | 817.74 | -29.65 |
| | DFT | -1507.08 | | 1284.63 | 816.07 | -31.73 |
| NDM-TOPR | PM3 | | -138.89 | 1131.80 | 731.59 | -17.44 |
| | DFT | -1391.38 | | 1133.27 | 730.85 | -20.05 |
| NDM-TOPO | PM3 | | -190.97 | 1213.04 | 765.56 | -33.11 |
| | DFT | -1467.80 | | 1214.71 | 764.21 | -35.66 |

| Molecule | Calculation type | Free energy (kcal mol$^{-1}$) | Area (Å$^2$) | Volume (Å$^3$) | Dipole moment (debye) | HOMO (eV) | LUMO (eV) | LUMO-HOMO (eV) |
|---|---|---|---|---|---|---|---|---|
| TOPR | PM3 | 979.47 | 419.36 | 409.89 | 6.8 | -8.83 | -1.40 | 7.43 |
| | DFT | 981.71 | 417.72 | 408.59 | 8.8 | -5.84 | -2.15 | 3.79 |
| TOPO | PM3 | 1038.77 | 438.99 | 426.61 | 6.4 | -8.75 | -1.30 | 7.45 |
| | DFT | 1041.44 | 434.01 | 424.88 | 8.2 | -5.53 | -2.00 | 3.53 |
| NDM-TOPR | PM3 | 913.68 | 401.02 | 390.59 | 6.5 | -8.85 | -1.42 | 7.43 |
| | DFT | 915.48 | 397.07 | 388.87 | 8.6 | -5.68 | -2.05 | 3.63 |
| NDM-TOPO | PM3 | 984.79 | 413.55 | 405.66 | 7.4 | -8.87 | -1.41 | 7.46 |
| | DFT | 986.98 | 410.96 | 404.72 | 8.7 | -5.49 | -1.94 | 3.55 |

* in atomic units from DFT calculations

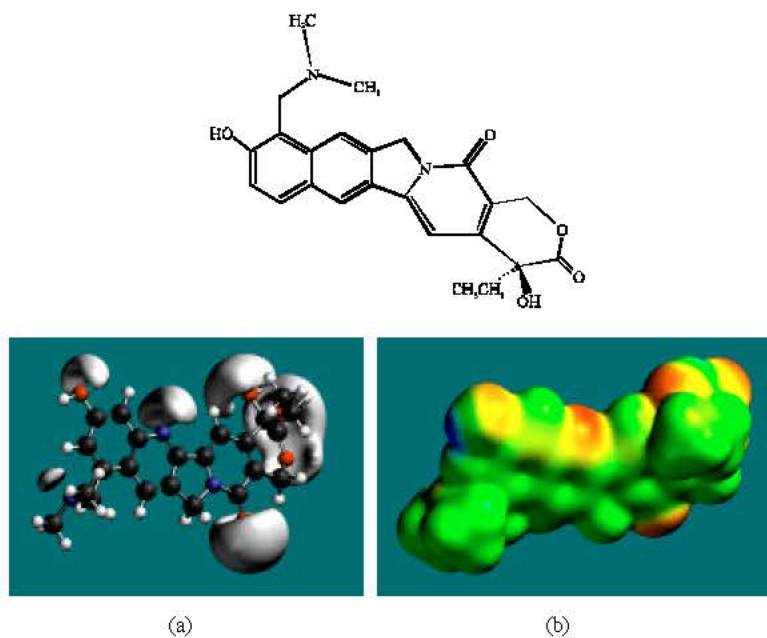(a)                                        (b)

Fig. 2:  Structure of TOPR giving in: (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential) and in (b) surface electric charges (where red indicates negative, blue indicates positive and green indicates neutral)
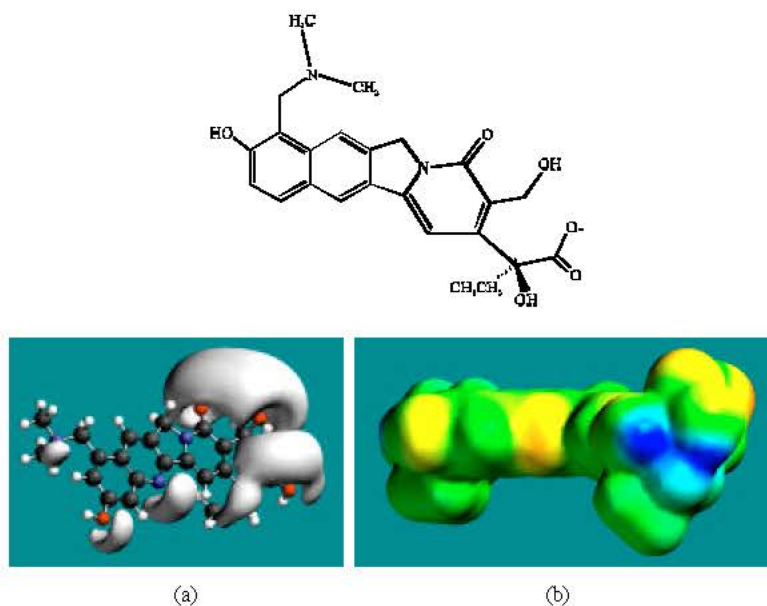


(a)                                        (b)

Fig. 3:  Structure of TOPO giving in: (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential) and in (b) surface electric charges (where red indicates negative, blue indicates positive and green indicates neutral)
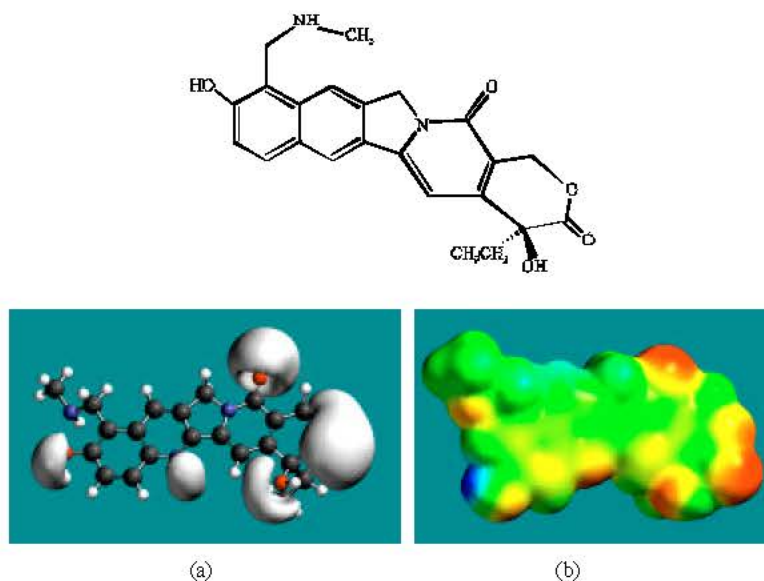
Fig. 4: Structure of NDM-TOPR giving in: (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential) and in (b) surface electric charges (where red indicates negative, blue indicates positive and green indicates neutral)
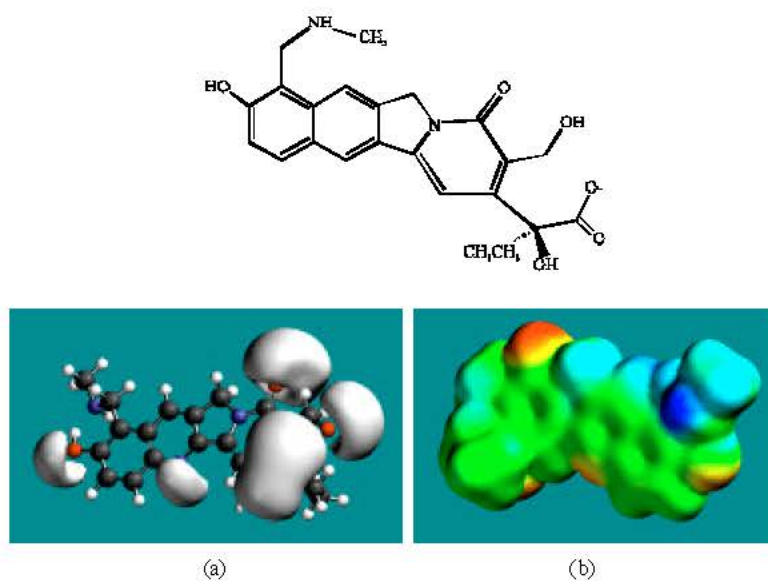


Fig. 5: Structure of NDM-TOPO giving in: (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential) and in (b) surface electric charges (where red indicates negative, blue indicates positive and green indicates neutral)

likely to subject to hydrophobic interaction and electrophilic attack. The presence of one or more positively charged lobes (blue) on the molecular surfaces of TOPO, NDM-TOPR and NDM-TOPO indicates that the compounds may also be subject to nucleophilic attack such as that by glutathione and nucleobases in DNA. Reaction with glutathione results into glutathione depletion that introduces oxidative stress which in turn induces cellular toxicity. Oxidation of nucleobases in DNA causes DNA damage.

When the surface area and volume of TOPR are compared with those of TOPO, NDM-TOPR and NDM-TOPO, it is found that the values for TOPR are distinctly different from those of other compounds, giving support to the idea that TOPO, NDM-TOPR and NDM-TOPO may not associate with topoisomerase I. It was noted earlier that toptotecan needs to be in lactone ring form for the stabilization of topoisomerase I complex that is involved in inhibiting DNA replication.

In the case of TOPR, TOPO, NDM-TOPR and NDM-TOPO, the electrostatic potential is found to be more negative around the various oxygen and nitrogen centers indicating that the positions may be subject to electrophilic attack.

## Conclusions

Molecular modelling analyses based on semi-empirical and DFT calculations show that TOPR, TOPO, NDM-TOPR and NDM-TOPO all have small LUMO-HOMO energy differences so that they would be kinetically labile. The molecular surface of all the compounds abound in neutral and negative regions so they would readily undergo hydrophobic interaction and electrophilic attack, NDM-TOPR and NDM-TOPO also have some positively charged lobe so that they can be subject to some nucleophilic attack. This means that NDM-TOPR and NDM-TOPO can cause glutathione depletion and oxidation of nucleobases in DNA, thus inducing cellular toxicity and DNA damage.

## Abbreviations

TOP   :   Topotecan,
NDMTOP   :   *N*-desmethyltopotecan
DFT   :   Density functional theory
LUMO   :   Lowest unoccupied molecular orbital
HOMO   :   Highest occupied molecular orbital

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## References

Arbuck, S.G. and C.H. Takimoto, 1998. An overview of topoisomerase I-targeting agents. Semin. Hematol., 35: 3-12.

Bai, F., M.N. Kirstein, S.K. Hanna, L.C. Lacono, B. Johnston and C.F. Stewart, 2003. Determination of plasma toptotecan and its metabolite *N*-desmethyl topotecan as both lactone and total form by reversed-phase liquid chromatography with fluorescence detection. J. Chromatogr., B 784: 225-232.

Furman, W.L., C.F. Stewart, M. Kirstein, J.L. Kepner, M.L. Bernstein, F. Kung, T.J. Vietti, C.F. Steuber, D.L. Becton, S. Baruchei and C. Pratt, 2002. Protracted intermittent schedule of topotecan in children with refractory acute leukemia: A pediatric oncology group study. J. Clin. Oncol., 20: 1617-1624.

Galindo-Rodriguez, C., K. Radomski, C.F. Stewart, W. Furman, V.M. Santana and P.J. Houghton, 2000. Clinical use of topoisomerase I inhibitors in anticancer treatment. Med. Pediatr. Oncol., 35: 385-402.

Herben, V.M., B.W.W. Huinink, J.H. Schellens and J.H. Beijnen, 1998. Clinical pharmacokinetics of camptothecin topoisomerase I inhibitors. Pharm. World Sci., 20: 161-172.

Rosing, H., D.M. van Zomerren, E. Doyle, H. ten Bokkel, J.H.M. Schellens, A. Bult and J.H. Beijnen, 1999. Quantification of topotecan and its metabolite *N*-desmethyltopotecan in human plasma, urine and faeces by high-performance liquid chromatographic methods. J. Chromatogr, B 727: 191-203.

Spartan '04, 2004. Wavefunction, Inc. Irvine, CA, USA.

Tian, Q., J. Zhang, T.M.C. Tan, E. Chan, W. Duan and S.Y. Chan *et al.*, 2005. Human multidrug resistance associated protein 4 confers resistance to camptothecins. Pharmaceut. Res., 22: 1837-1853.

Wang, J., 1996. DNA topoisomerases. Annu. Rev. Biochem., 65: 635-692.