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# Study on synthesis and biological activity of a galactosylated piperazinyl porphyrin

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**Abstract**—In order to obtain an carcinoma-selective drug, the synthesis and characterization of 5,10,15,20-tetra[4-(4′-galactosylpiperazinyl)phenyl]porphyrin (**TGPP**) is reported. The biological activity on cancer cells and the pharmacokinetics are also reported as preliminary results showing a very high liver to skin ratio and short retention time in tissues, and thus promising activity in photodynamic therapy.


Recently, promising porphyrin photosensitizers have been developed for photodynamic therapy (PDT).[1–3] Numerous porphyrins have been synthesized and tested for in vitro and in vivo phototoxicity, but reasons for selectivity of the compound remain recondite. Besides the subtle balance between hydrophobicity and hydrophilicity, charge distribution, symmetry and configuration of the molecules were suggested as important factors influencing the selective accumulation and subcellular distribution. The glycosylated porphyrins owing to good solubility in aqueous solution and specific membrane interaction have been applied in cancer PDT as promising photosensitizers.[4–6] In such compounds, the nature and number of carbohydrate residues and hydrophobic substituents linked to the macrocycle allow a large variability in the hydrophilic or hydrophobic characters. Although a number of porphyrin-based photosensitizers have been approved for clinical trials, however, they are not very selective, causing skin sensitivity for some weeks.[7] Moreover, PDT treatment also was not suitable for therapy of the cancer locating at the deeper tissue of the body.[8]

Piperazine-containing compounds are widely used in pharmaceutical industry. Especially, some substituted piperazine compounds have distinctive anticancer activity.[9–14] But so far, all clinical chemical anticancer drugs, including piperazine-containing drugs, the cytotoxicity of drugs on normal cells is caused for the drugs could not efficiently concentrate in tumour tissue. The side effects exist inevitably for patients, the dosages of the drugs have to be reduced in clinical to mitigate the side effects of drugs. But this will prevent the drugs from bringing their efficacy into play and results in the curative effect decreasing. Therefore, it is very promising to synthesize some new compounds as anticancer drugs that can accumulate to higher concentrations in malignant tumours than in normal tissue. It has been reported that nitrogenous heterocycle porphyrins have better anticancer activity than the corresponding nitrogenous heterocycles in the absence of light.[15] We sought to develop an anticancer drug approach that can accumulate in neoplastic tissue to higher concentrations than in surrounding normal tissue.

The asialoglycoprotein receptor (ASGR) is known to be present only on hepatocytes at a high density of 500,000 receptors per cell and retained on several human hepatoma cell lines. This receptor system can not only recognize terminal β-D-galactose or *N*-acetylgalactosamine residues, but can also internalize them within membrane-bound vesicles or endosomes.[16] ASGR are considered a particularly attractive target in many drug carrier studies. The use of such nature molecules with galactosylated or lactosylated residues in targeting drug has resulted in significant targeting efficacy to the liver.[17] Galactose is known as a specific adhesive ligand to ASGR of hepatocyte. Lactobionic acid (LA), bearing a galactosyl group, is usually used as a recognition

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moiety for the hepatocyte-targeting drug.[18,19] It seems a good idea to link a galactose-guided drug moiety to porphyrin ring to avoid or mitigate the side effect of drugs. We now report the synthesis and the biological activity studies of 5,10,15,20-tetra[4-(4′-galactosylpiperazinyl)phenyl]porphyrin (**TGPP**), a structural change which is accompanied by a short retention time in tissues together with a promising activity. Cell uptake and photodynamic properties are compared to those of the structurally related 5,10,15,20-tetra(4-aminophenyl)porphyrin (**TAPP**), a drug which is one of the most potent photosensitizers discovered to date.

**TGPP** is a symmetrical-galactosylated piperazinylporphyrin. The key of the synthesis of **TGPP** is how to introduce galactosylated piperazine to the porphyrin ring. We tried to couple iodoporphyrin with various galactosylated piperazines to synthesize target molecules at room temperature according to Buchwald method.[20] However, the reaction did not proceed, even after stirring over an extended period of time. The coupling of **TAPP** with *N*,*N*′-di(2-bromoethyl)galactosamide at room temperature can obtain the corresponding galactosylated piperazinyl porphyrin, but the components of products are too complicated to purify. **TGPP** is synthesized by the direct condensation of pyrrole with the appropriate benzaldehyde (**3**) under Adler's conditions.[21] Benzaldehydes bearing substituted piperazines were prepared according to the literature.[22–24] *N*-galactosylamino-*N*′-phenylpiperazine was synthesized by the formation of amide bonds between the secondary amino group of the *N*-phenylpiperazine[25] and the carboxylic acid group of lactobionic acid according to the method reported by Bernkop-Schnürch et al.[25] As shown in reaction formula, the carboxylic moieties of lactobionic acid were activated by *N*,*N*′-dicyclohexylcarbodiimide (DCC), forming an intermediate product, which reacted with the secondary amino group of *N*-phenylpiperazine. The efficacy of the purification method for the resulting *N*-galactosylamino-*N*′-phenylpiperazine could be verified by controls which were prepared in exactly the same way as the conjugate but omitting DCC during the coupling reaction, exhibiting a negligible amount of product. In order to optimize the synthesis of *N*-galactosylamino-*N*′-phenylpiperazine, the influence of the coupling reaction time was evaluated. Results demonstrated that the yield increased with increasing reaction time, but when the reaction time lasted more than 72 h, the yield could not increase obviously, indicating that the coupling reaction already came to equilibrium. Other factors that can influence the yield, such as pH of the reaction mixtures and the weight ratio of *N*-phenylpiperazine to lactobionic acid, were already studied, as far as pH of coupling reaction was concerned, alkaline pH would benefit the reaction greater than acidic pH. Therefore, tetramethylethylene diamine solution was selected to the catalyst of the coupling reaction. Our approach to porphyrin-based drugs makes use of a convenient reaction for converting an aldehyde and pyrrole to the corresponding *meso*-substituted porphyrin. The reaction provides a means for converting prefunctionalized benzaldehyde to the corresponding porphyrin. In this paper, the functional groups selected are those drugs that be used in the treatment of cancers. The synthetic route of **TGPP** is summarized in Figure 1. Whilst this work was in progress, a different piperazinylporphyrin[15] has been reported.

The structure of **TGPP** is characterized by elementary analysis, MS, $^1$H NMR, IR and UV–vis.[26] The $^1$H NMR spectra at −2.46 ppm show the characteristic single peak for porphyrin. The molecular ion peak and elementary analysis further confirm its structure.

As expected from the presence of four galactosylated groups, the compound is soluble in polar solvent such as methanol and water. The lipophilic and hydrophilic properties were characterized by the partition coefficient

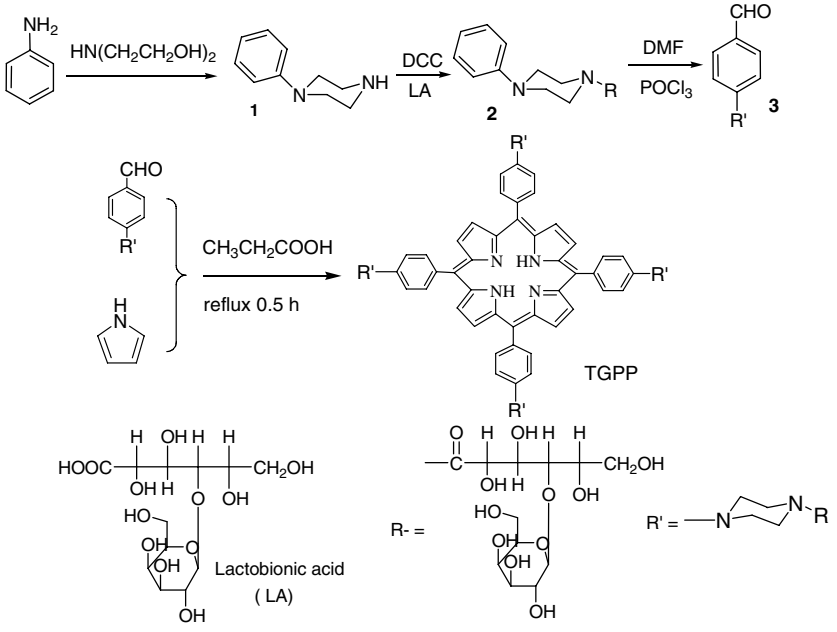


**Figure 1.** The synthesis of **TGPP.**

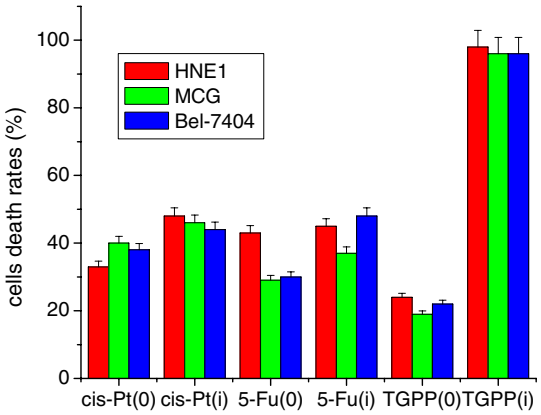


**Figure 2.** Anticancer activity of *cis*-Pt, 5-Fu and **TGPP** in cancer cells (Bel-7404, MCG, and HNE1) determined by MTT assay. Cancer cells were incubated for 18 h in solution (6.25 μg/ml). '0' represented without irradiation, and 'i' represented with irradiation (at 650 nm and 20 J/cm$^2$).

of the compound between the two non-miscible solvents octanol and water. The octanol/water partition ratios were 18 for compound **TGPP** and 42 for **TAPP** indicating that **TGPP** was more hydrophilic than **TAPP**. In order to study the anticancer activity of **TGPP**, with and without irradiation, the cytotoxic effects of **TGPP** and two common anticancer drugs, *cis*-Platinum (*cis*-Pt) and 5-fluorouracil (5-Fu), on three human tumour cell lines, Bel-7404 (a liver cancer cells), MCG (a stomach tumour cells) and HNE1 (a nasopharyngeal carcinoma cancer cells), were determined using the MTT method.[15,27] The results of the pre-screenings are given in Figure 2.

One can see from Figure 2 that the death rates ranged from 24% to 19% for **TGPP**, 40–33% for *cis*-Pt and 43–29% for 5-Fu when cells were incubated for 18 h at 37 °C under an atmosphere of air containing 5% $CO_2$without light. The results showed that the dark toxicity of **TGPP** was weaker to those of *cis*-Pt and 5-Fu in this condition. However, the death rates ranged from 96% to 98% for **TGPP** after 18 h incubation and 20 J/cm$^2$ irradiation (Fig. 2), showing presence of photosensitizing toxicity.

Structurally, **TGPP** consists of two parts: **TAPP** and galactosylated piperazine compound. In order to study whether **TGPP** and two corresponding structure parts (galactosylated piperazine and **TAPP**) have anticancer activity to cancer cell, they were tested in vitro against cancer cell by MTT method in the absence of light.[15] The $ID_{50}$ (50% of cell death) values of the cancer cell to these compounds are listed in Table 1.

Table 1 shows that **TGPP** have smaller $ID_{50}$ values than that of galactosylated piperazine. This indicates that **TGPP** have better anticancer activity than galactosylated piperazine. It is interesting that **TAPP**, which is an anticancer drug too, would be a good carrier for toxic moieties to arrive at improved anticancer drugs. The possible reason might be that **TAPP** with anticancer drugs can intercalate into the base pairs of DNA strongly. The molecular mechanism for the anticancer activities of **TGPP** is being further studied.

**Table 1.** $ID_{50}$ values in μg/ml of **TGPP** and some compounds

| Compound | Bel-7404 | MCG | HNE1 |
|---|---|---|---|
| **TGPP** | 29 | 35 | 33 |
| **TAPP** | 33 | 24 | 28 |
| Galactosylated piperazine | >50 | >50 | >50 |

The distribution of **TGPP** was examined in different selected tissues of tumour-bearing female KM mice. **TGPP** (10 mg/kg) was administered by ip injection. The animals were sacrificed during different points of time (six mice at 24 h and three mice at other points of time), varying between 1 h and 1 week, after administration. The skin, muscle, tumour, and the liver were recovered. About 200 mg of tissues was homogenized in THF. The homogenates were centrifuged at 3000 rev/min for 15 min, and the fluorescence of the supernatants was measured, setting the excitation wavelength at 420 nm and recording the emission spectrum from 500 and 800 nm. Serum samples, isolated from the blood by centrifugation, were diluted with suitable volumes of 500 μl THF and the **TGPP** content was measured by fluorescence. In all the cases, **TGPP** amounts were determined by the interpolation of emission intensity and **TGPP** concentration plotted on a standard curve. The results are shown in Figure 3.

This suggests that **TGPP** is rapidly distributed in tissues and rapidly eliminated. The highest concentrations were observed in the liver at 3 h post-injection treatment; the concentration of **TGPP** decreased rapidly in all tissues after 24 h, and very low sensitizer quantity was detected in these tissues after 144 h. The low quantity detected in the skin could be favourable, as it would cause photosensitization. The higher quantity of **TGPP** in liver can be explained by the presence of four galactosylated moieties which can recognize ASGR. The shorter retention time of **TGPP** in tissues can decrease prolonged cutaneous photosensitivity. Further developments and

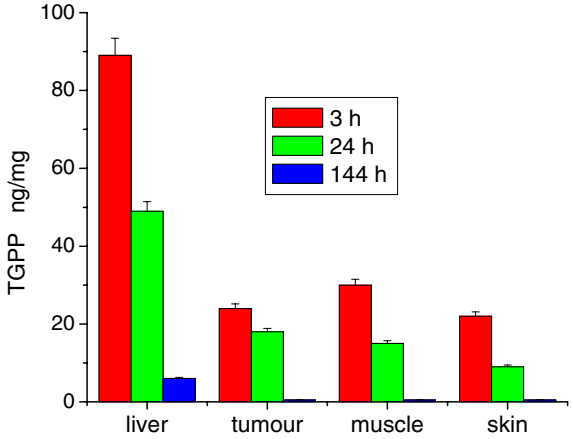


**Figure 3.** Biodistribution of **TGPP** in tumour-bearing mice: Recoveries of **TGPP** from tumour-bearing KM mice injected with 10 mg/kg of drug. Values represent the average of the three experiments.

improvements of this approach are in progress using various sugar units, and more extensive biological studies will be reported elsewhere.

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26. Selected data for 5,10,15,20-tetra[4-(4′-galactosylpiperazinyl)phenyl]porphyrin (**TGPP**): yield 13.5%. $^1$H NMR ($CDCl_3$):$\delta$8.62–8.66 (8H, pyrrolic), 6.97–7.33 (16H, ArH), 2.82–3.57 (32H, N–$CH_2$), 4.22–4.54 (52 H, LA C–H), 2.08 (32H, LA O–H), −2.46 (2H, NH) ppm. UV–vis[$\lambda_{max}$, nm ($\varepsilon \times 10^{-3}$ cm$^{-1}$ mol$^{-1}$ L)] in $CH_2Cl_2$: 427 (204.9), 526 (17.6), 569 (22.1), 614 (19.2), 658 (10.8). MS *m/z*: 2311($M^+$+1). Anal. Calcd for $C_{108}H_{142}N_{12}O_{44}$: C, 56.10; H, 6.15; N, 7.27; O, 30.48%; Found: C, 56.08; H, 6.18; N, 7.25; O, 30.49%. IR (KBr): 3324, 1640, 1606, 1512 cm$^{-1}$.
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