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Biodistribution of a potential chemotherapeutic, dinuclearbisphosphinogold(I) dithiocarbamate, as determined by its ^{198}Au radiolabelled analogue

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Abstract Dinuclearbisphosphinogold(I) dithiocarbamato, BPDTC, was previously found to have antitumour activity in vitro. ^{198}Au radiolabelled BPDTC (radiochemical yield of 70 ± 6 % and radiochemical purity of >95 %) was used to determine its in vivo biodistribution in Sprague-Dawley rats. Gamma scintigraphs were performed over a period of 48 h and final radioactivity measurements of harvested organs of the test animals after termination was performed at 2, 4 and 48 h. The study successfully showed the biodistribution of the gold complex, with the highest uptake of the compound being observed in the lungs, liver and spleen.

Keywords Radiolabelling · ^{198}Au · Gold(I) · Bisphosphine · Dithiocarbamate · Biodistribution

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Introduction

Research into the application of precious metal containing compounds for medicinal purposes has seen a marked increase in recent years [1]. The clinical success of these compounds, however, seems to be limited to the overall success of cisplatin and its derivatives as anticancer agents as well as the anti-rheumatoid arthritis, gold-containing compound, auranofin. A major hurdle for use of metal containing drugs is the natural occurring reducing agents present in cells, but cisplatin and its derivatives have proven that this can be mediated by the selection of the appropriate ligands [2]. Taking these issues into consideration, the biodistribution and metabolism of metal containing drugs in vivo is an important and contemporary question if better drugs are to be designed.

The assessment of biodistribution through tracer radiolabelling of a metal-containing molecule provides the benefit of easy quantification of distribution in vital organs as well as, in some cases, the real time visualisation of distribution through radionuclide imaging. [3–5] For gold this is possible in the form of the radionuclide, ^{198}Au [2, 6] enabling tracing of the gold itself which is the active centre in these compounds. The γ radiation emitted at 411.8 keV allows the dynamic distribution to be followed through radionuclide imaging. The 2.7 d half-life of ^{198}Au makes it suitable for biological studies as the time frame is of a suitable length to cover synthesis and biological screening at a safe radioactive dose level. Furthermore, with such radiotracers, it is possible to quantify the amount of compound from the radioactivity measured in the organ or location of interest in the body [7].

Dinuclearbisphosphinogold(I) dithiocarbamate, BPDTC, (Fig. 1) was shown to have excellent activities against a panel of cancer cell-lines and proceeded to the in vivo hollow fibre

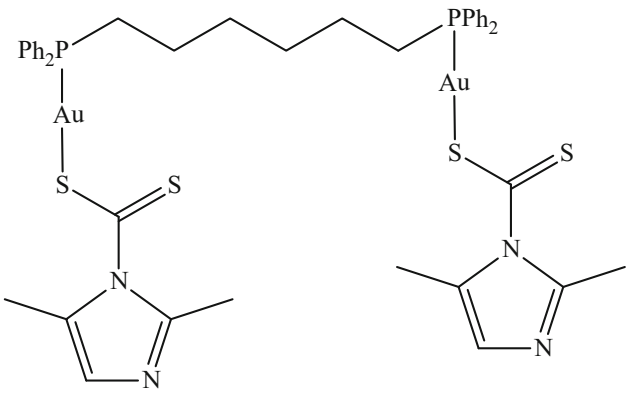

Fig. 1 Dinuclearbisphosphinogold(I) dithiocarbamato, BPDTC

screening stages at the Developmental Therapeutics Program (DTP, NCI, NIH, USA). [8] In a bid to further study the biodistribution of the compound it was identified as a candidate for radiolabelling.

Experimental

General

Bis(diphenylphosphine)hexane (dpph), tetrahydrothiophene (THT) and solvents were used as received from the supplier (Sigma-Aldrich, Germany). The gold powder was supplied by Mintek, South Africa. Potassium 3,5-dimethylpyrazol-1-yldithiocarbamate (L) was synthesised according to previously published methods [8]. Calibration of the gamma camera and well counter apparatus at the University of the free state (UFS) was performed three times, utilising H[^{198}Au]Cl_4 in *aqua regia*. All necessary precautions were taken when working with ^{198}Au.

Preparation of radiolabelled gold

Gold granules (20 mg) were irradiated for 12 min in the Necsa SAFARI-1 research reactor (situated at Pelindaba, South Africa), in the hydraulic position at a thermal flux of 8.0×10^{13} n cm^{-2} s^{-1}. The activity of the ^{198}Au that formed via the (n,γ) reaction was on average 1,573 MBq (42.5 mCi).

Synthesis of radiolabelled BPDTC

The radiosynthesis of BPDTC was based on a method previously described for the synthesis of non-radioactive BPDTC [8]. Radiolabelled ^{198}Au metal (10 mg) was dissolved in 400 µl aqua regia, dried at 120 °C to form the yellow to red H[^{198}Au]$AuCl_4$ salt and cooled. Ethanol (1 ml) was added to dissolve the gold salt. When tetrahydrothiophene (THT) (100 µl) was added to the solution, a white precipitate formed, which upon standing, settled at the bottom, allowing the supernatant to be removed.

To this precipitate, 1,6-bis(diphenylphosphino)hexane (0.5 equivalents) dissolved in dichloromethane (2 ml) was added and stirred for 10 min until all of the precipitate dissolved, whereupon 3,5-dimethylpyrazol-1-yl-dithiocarbamate, dissolved in water (500 µl) was added. The formed biphasic system was stirred until the yellow water layer turned colourless and the colourless dichloromethane layer turned pink. The phases were allowed to separate and the dichloromethane layer was extracted and evaporated to dryness under an argon atmosphere to yield the required product with an average radiochemical yield of 70 ± 6 % (repeated six times). A small amount of the final compound was left to decay to safe radioactive levels, where after the ^{1}H and ^{32}P NMR spectra were obtained.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.62 (*t*, 8H, $J = 8$ Hz, Ph); 7.40 (*q*, 8H, $J = 8$ Hz, Ph); 7.34 (*q*, 4H, $J = 8$ Hz, Ph); 5.81 (*s*, 2H, 4-pz); 2.74 (br s, 4H, $Ph_2P(CH_2)_2(C\underline{H}_2)_2(CH_2)_2PPh_2$); 2.20 (*m*, 12H, $C\underline{H}_3$, 3,5-pz). 1.56 (*m*, 8H, $Ph_2P(C\underline{H}_2)_2(CH_2)_2(C\underline{H}_2)_2PPh_2$).

^{31}P NMR (400 MHz, $CDCl_3$): δ 32.5 (*s*).

In vivo rodent studies

The study was performed after approval by the Ethics Committee of the University of the Free State (UFS), according to the guidelines of the National Code for Animal Use in Research, Education, Diagnosis and Testing of Drugs and Related Substances in South Africa. Six adult female rats (masses 370–416 g) were obtained from the Animal Experimentation Unit of the University of the Free State (UFS). Each rat was injected with [^{198}Au] BPDTC (50–80 ul), dissolved in dimethyl sulphoxide, with activity between 12.0 and 16.8 MBq. The rats were anaesthetised with isoflurane and the radiolabelled compound was administered into the tail vein. The rats were imaged with a gamma camera (Orbiter; Siemens, Erlangen, Germany) fitted with a pinhole collimator. Five minute static images were acquired every hour for 4 h. The animals were immobilised in a restraint for the duration of the dynamic studies. Four animals were terminated using an isoflurane overdose after 4 h, one animal after 2 h and the last animal was terminated after 48 h. The brain, heart, lung, liver, kidney, spleen, stomach, bladder, large and small intestines and tail as well as the femur and muscle tissue from the left hind leg were harvested. Approximately 1 ml of blood and all the urine in the bladder were also collected. The activity of each organ was measured using a well counter (Atom

LabTM; Biodex) set to record events within a 20 % window setting (326–490 keV).

Results and discussion

A range of phosphinogold(I) dithiocarbamate complexes were synthesised and tested for anticancer activity previously [8]. Of the range of gold-containing dithiocarbamate complexes, two showed good selectivity towards cancer cells over normal cells as well as good stability in solution and were sent to the DTP for screening on the 60-cell line. The in vitro and initial in vivo toxicology studies done on the gold-containing BPDTC compound indicate that this compound shows promising anticancer properties. To measure the in vivo distribution of BPDTC, the compound was labelled with ^{198}Au and injected into six rats. The distribution of the [^{198}Au] BPDTC in rats was followed by radionuclide imaging and radioactivity measurements of the harvested organs after termination of the test animals.

Synthesis and radiolabelling

^{198}Au was produced by a (n,γ) reaction on finely divided natural gold granules. The irradiated gold, containing trace amounts of ^{198}Au, was processed for the preparation of radiolabelled BPDTC by dissolving it with aqua regia to form auric acid (H[^{198}Au]$AuCl_4.xH_2O$). This gold(III) compound was reduced to gold(I) by addition of tetrahydrothiophene (THT) producing (THT)[^{198}Au]AuCl which was utilised to synthesise [^{198}Au] BPDTC. The synthesis was successfully executed a number of times and radiochemical yields were calculated to be 70 ± 6 % based on the tracer gold. A small amount of the final compound used in the animal studies was left to decay to negligible/safe radioactive levels after which successful synthesis was confirmed by NMR.

The characterisation of the BPDTC product was not possible by HPLC due to interaction with the mobile phase resulting in blockage of the column, therefore the final product was characterised by ^{1}H and ^{31}P NMR after decay. The radiochemical purity was estimated from the ^{31}P NMR to be >95 % as no other peaks, such as uncomplexed ligand, were observed.

In vivo rodent studies

The biodistribution of ^{198}Au radiolabelled BPDTC was studied by injecting the compound into six rats. The rats were restrained, but were seen to be comfortable, while performing scintigraphs. Imaging results are shown in Figs. 2 and 3. Figure 2 shows the distribution during the first 5 min after injection, while Fig. 3 shows the biodistribution recorded from 1.5 to 48 h after injection.

The scintigraphs showed accumulation of ^{198}Au radiolabelled BPDTC ([^{198}Au] BPDTC) in the heart and lungs within the first 5 min after injection (Fig. 2). The initial high uptake in the lungs was cleared within 6 h (Fig. 3). Accumulation of the BPDTC in the lungs is most likely due to the initial high blood pool values and slow blood clearance of the compound as a pinhole collimator was used at a distance of 10 cm from the animal, making the distinction of organs (such as heart and lungs) difficult. However, Fig. 2c, d clearly shows the two lobes of the lungs. Furthermore upon dissection, distinct spots on the lungs were noted in all rats indicating uptake in the lungs and subsequent damage to this sensitive organ.

The results from the scintigraphy studies are summarised in Fig. 4 which presents the normalised activity in counts per pixel for the same region of interest (ROI) drawn over the liver & heart (combined as indicated before), liver and kidneys. The average for the four rats is given for the scans recorded at 0, 1, 2, 3 and 4 h after injection. There seems to be slow clearance from both the lung and liver while the kidney value remains low indicating the slow clearance from the other organs. The $t_{½}$ of [^{198}Au] BPDTC is >24 h in all the organs. This is considerably longer than that of other radiolabelled metal chelates such as ^{68}Ga-NOTA-conjugate peptides [9]. These ^{68}Ga chelates are excreted efficiently with a blood clearance of $t_{½} = 29$ min and no detectable radioactivity in serum or urine at 4 h p.i. However, the excretion rate of cis-diamminedichloroplatinum(II), (cisplatin) is comparable to [^{198}Au] BPDTC. A recent study showed that approximately 32 % of injected dose (ID) of cisplatin is excreted within 5 h p.i. with trace amounts of cisplatin still being excreted up to 96 h p.i [10, 11]. Cisplatin is known to interact with serum proteins—up to 65–80 % protein plasma binding [12], which affects the rate of excretion from the test subject. It has also been proven for other gold phosphines that interactions with serum proteins occur. In this regard thioredoxin reductase has been identified as a very likely target for gold(I) compounds [13]. In studies related to the elucidation of the possible protein targets it was also found that albumin concentrations have an effect on gold compounds inhibition ability. The general consensus regarding gold containing compounds are that they are relatively easily taken up by proteins [14]. It is therefore likely that [^{198}Au] BPDTC is forming gold-serum protein adducts which are not easily excreted, resulting in a longer $t_{½}$.

After completion of the scintigraphs, four rats were euthanised at 4 h, dissected and the radioactivity of the organs counted. One rat each were euthanised and dissected after 2 and 48 h to determine any changes in the biodistribution over time. Although the latter data cannot be statistically justified the trend from 2 to 4 to 48 h is clear

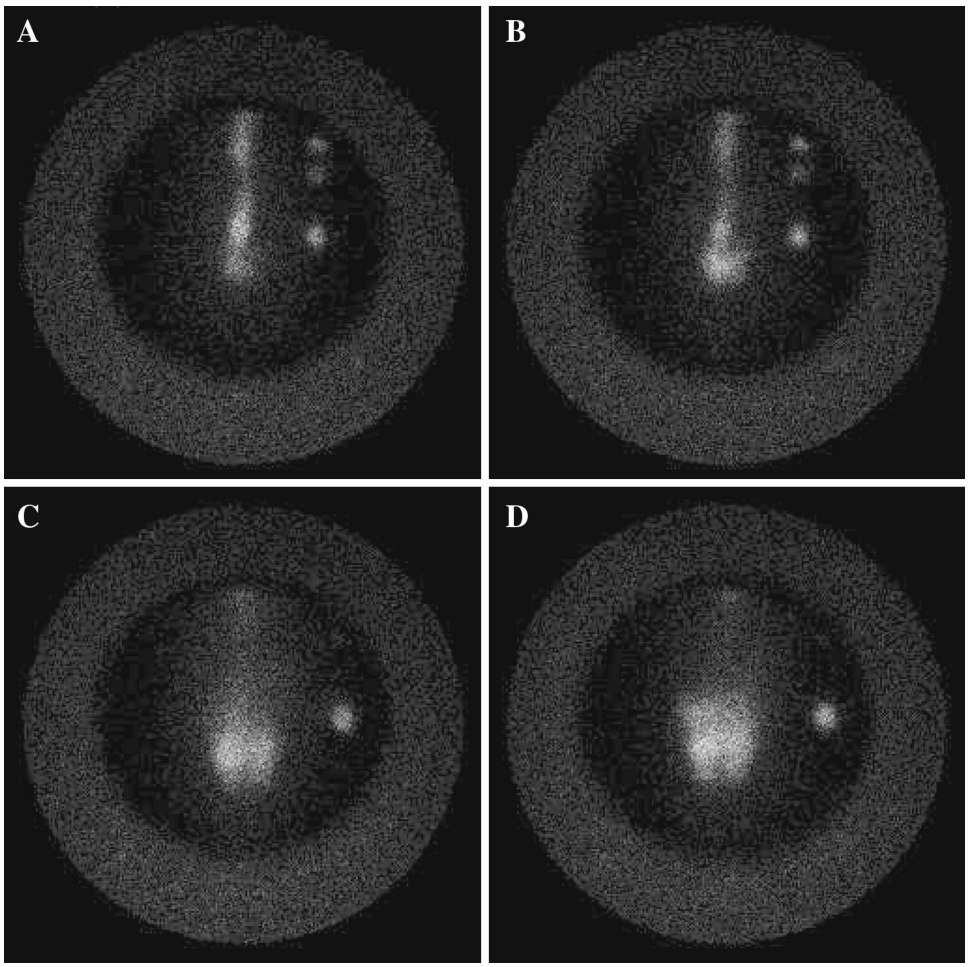

Fig. 2 Posterior images from the first 5 mins after injection. Tail is at the top of the images with three standard point sources on the left side of the rat. **a** ^{198}Au-BPDTC is injected through the tail vain. **b** ^{198}Au-BPDTC reaches the heart. **c** ^{198}Au-BPDTC reaches the lungs. **d** Distribution of ^{198}Au-BPDTC after 5 mins

from Fig. 5 (repeat experiments for 2 and 48 h could not be done due to ethics considerations as the compound had high uptake in the lungs and subsequent damage to this sensitive organ was noted on visual inspection). The average of the percent injected dose per gram of tissue (%ID/g) is given in Table 1. For most organs (except lung and kidney) there is statistically no difference from 4 to 48 h as was also deduced from the scintigraphic images. The large standard deviation of the experimental data for the small and large intestines at 4 h indicates that its possibly due to the low activities measured in these non-target organs but also suggests that those measured values are subject specific and hence do not have much significance. The high percentage standard deviation for the bladder is expected as the mass of the organ is very small leading to high uncertainty in the weight measurement and hence high standard deviation in the %ID/g values.

The biodistribution of [^{198}Au] BPDTC shows that upon injection ^{198}Au accumulates in the lung partly because of slow blood clearance (as the lung is a blood rich organ) but also because of uptake in the lung organ. After 48 h a significant percentage of the injected dose is still in the lungs (refer to Fig. 5) indicating that it is a target organ to some extent. From the lungs it partly clears to other reticuloendothelial organs (liver and spleen). This accumulation is complete by 4 h in these two organs that are clearly the main target organs for BPDTC. The bone, brain and gastrointestinal track are not target organs.

Based on these observations it is unlikely that dinuclearbisphosphinogold(I) dithiocarbamato (on its own) has potential to be used as a chemotherapeutic agent as it will target liver and spleen and partly the lungs. Evidence of the high toxicity of the compound was also found in vivo with spots left on the lungs.

Conclusion

The ^{198}Au-labelled compound, BPDTC, was successfully synthesised as confirmed by NMR spectroscopy and evaluated in vivo. It was shown by radionuclide imaging that the majority of the compound was found to initially reside in the lungs, with partial clearance to the liver and spleen. The blood clearance is slow although at any point in time a

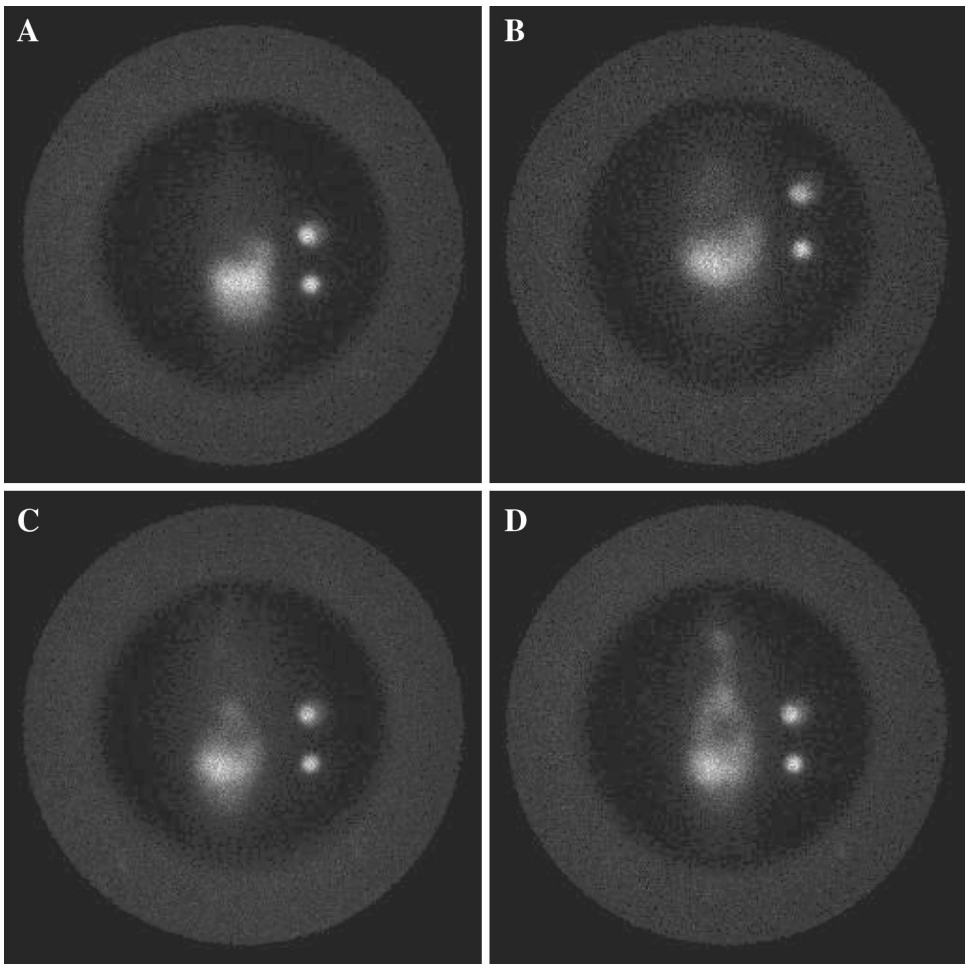

Fig. 3 Posterior images (5 min static recordings) from 1.5 to 48 h after injection of ^{198}Au-BPDTC. Tail is at the top of the images with two standard point sources on the left side of the rat for spatial scaling. **a** 1.5 h after injection. **b** 6 h after injection. **c** 24 h after injection. **d** 48 h after injection

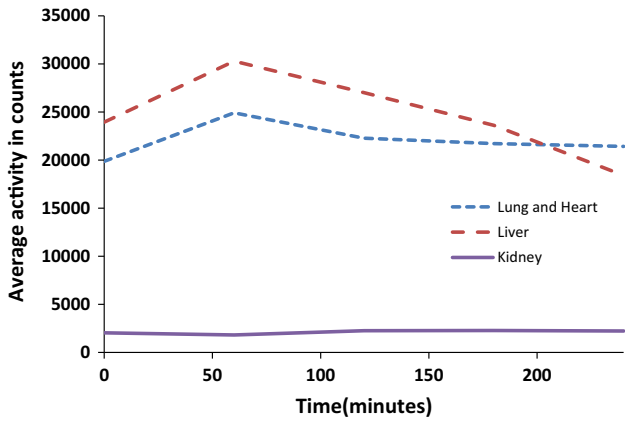

Fig. 4 Mean time activity curves for lung & heart, liver, spleen and kidney versus time in minutes ($n = 4$)

minimal amount of the compound is seen to be circulating in the body, indicating that only a small amount of compound might be available for therapeutic activity. This observation was further confirmed by the radioactive

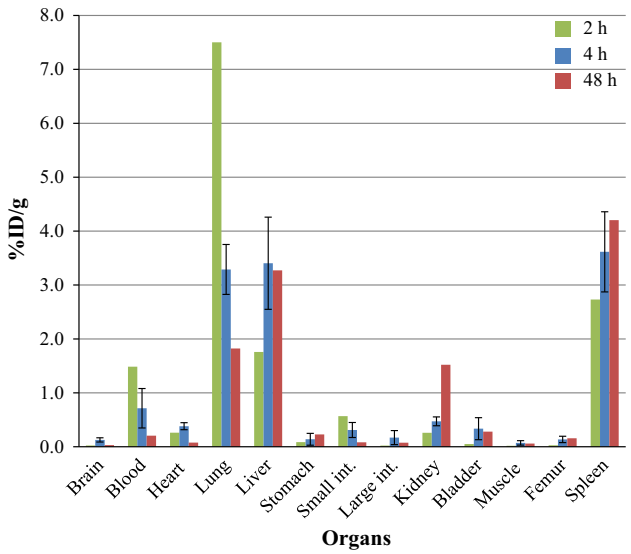

Fig. 5 Biodistribution of ^{198}Au-BPDTC in female Sprague-Dawley rats after 2, 4 and 48 h, as determined by γ-ray emission of ^{198}Au-radiolabelled BPDTC measured in % ID/g

Table 1 Tissue distribution of ^{198}Au-BPDTC in rats

Organ	4 h			2 h	48 h
	Average %ID/g	SD %ID/g	%SD[a]	%ID/g	%ID/g
Brain	0.13	0.04	31	0.0	0.0
Blood	0.71	0.37	51	1.5	0.2
Heart	0.38	0.06	17	0.3	0.1
Lung	3.29	0.46	14	7.5	1.8
Liver	3.40	0.85	25	1.8	3.3
Stomach	0.14	0.11	79	0.1	0.2
Small int.	0.31	0.14	45	0.6	0.1
Large int.	0.17	0.13	77	0.0	0.1
Kidney	0.47	0.08	18	0.3	1.5
Bladder	0.33	0.21	61	0.1	0.3
Muscle	0.07	0.04	58	0.0	0.1
Femur	0.14	0.06	43	0.0	0.2
Spleen	3.62	0.74	21	0.0	4.2

[a] The standard deviation as a fraction of the average %ID/g, shown as a percentage

counting of the vital organs once the animals were terminated and the organs harvested. This experiment shows that the biodistribution of BPDTC is not ideal and further efforts should be made towards the effective distribution of the compound, possibly through the use of a delivery vehicle.

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