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# Preparation and Biological Evaluation of a [ $^{55}\text{Co}$ ]-2-Acetylpyridine Thiosemicarbazone

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## Abstract

Due to the anti-proliferative properties of cobalt-thiosemicarbazone complexes, the production of [ $^{55}\text{Co}$ ](III)-bis-(2-acetylpyridine thiosemicarbazone) ([ $^{55}\text{Co}$ ](III)[APTS]<sub>2</sub>) was investigated. Co-55 ( $T_{1/2}$ =17.53 h) was produced by 150  $\mu\text{A}$  irradiation of a natural nickel target by 15 MeV protons. The  $^{55}\text{Co}$  was separated from the irradiated target material using a two-step method with a radiochemical yield of >95% followed by radionuclidic and chemical purity control. [ $^{55}\text{Co}$ ](III)chloride was mixed with 2-acetylpyridine thiosemicarbazone for 30 min at room temperature to yield [ $^{55}\text{Co}$ ](III)[APTS]<sub>2</sub> (radiochemical purity > 98% shown by RTLC/HPLC). A specific activity of about 10–20 Ci/mmol was obtained. The final solution was diluted in normal saline to 5% ethanolic solution for biological evaluation. The stability of the final product was checked in the absence and presence of human serum at 37°C to 24 h. The partition co-efficient of the final complex at the pH of 7 was  $1.00 \pm 0.08$ . A significant tumor accumulation (%ID/g; 3.5%) was observed in tumoral tissue 21 h post injection in fibrosarcoma-bearing mice by biodistribution studies. Co-incidence imaging also demonstrated tumor uptake from 21–35 h however at 35 h tumor uptake is more specific and significant.

## Keywords

Cobalt-55 • Thiosemicarbazone • Biodistribution • Tumor imaging • Co-incidence • Fibrosarcoma

## Introduction

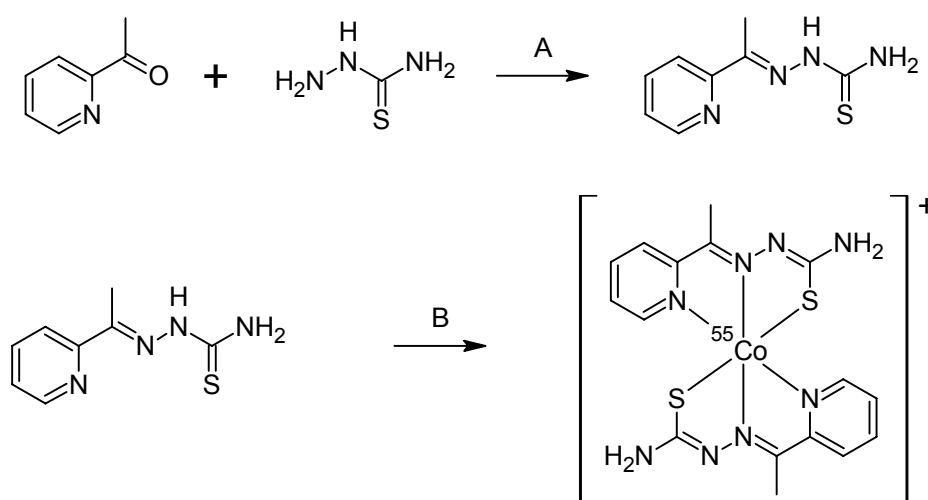
Cobalt thiosemicarbazone complexes exhibit interesting biological properties specially anti-proliferative effects. For instance, furane containing cobalt complexes have demonstrated potent cytotoxicity against the growth of leukemias, lymphomas, human lung, colon, ovary and uterine carcinoma cell cultures [1]. Some pyridoxal thiosemicarbazone cobalt (III) complexes have shown antileukemic activity toward human cell lines U937 and CEM [2]. In another study, the 9,10-phenanthrenequinone cobalt complexes have exhibited antiproliferative activity in the human breast cancer cell-line, T47D [3].

Cobalt offers a selection of radionuclides suitable for imaging as well as tracing techniques [4]. The most commonly used cobalt radionuclide for tracing in long-period studies is  $^{57}\text{Co}$  emitting photons at energy range of 100–200 keV suitable for SPECT detector systems.  $^{55}\text{Co}$  seems a potential radionuclide for positron emission tomography (PET), but emitting high energy photons is a major drawback in imposing unwanted dose to patients (Table 1).

**Tab. 1.** Physical characteristics of Co-55 and Co-57 [4]

Radionuclide	Half-life	Decay Mode	Gamma photon (keV) intensities (%)
$^{55}\text{Co}$	17.53 h	$\beta^+$ (77%), E.C. (23%)	1408.4 (16.88%), 1369.7 (2.92%), 1316.4 (7.09%), 931.3 (75%), 803.4 (1.87%), 477.2 (20.2%) 411.9 (1.07%), 91.9 (1.16%), 511 (154%)
$^{57}\text{Co}$	271.79 d	E.C. (100%)	136.5 (10.68%), 122.1 (85.6), 14.4 (9.16%)

Based on the above mentioned Co-thiosemicarbazones biological effects, it was hypothesized that the production of radiolabeled anti-tumor cobalt-complexes may lead to possible tumor imaging agents.



**Fig. 1.** Production of  $^{55}\text{Co}(\text{III})[\text{APTS}]_2$ ; A: 5% AcOH, 50°C, B:  $^{55}\text{Co}[\text{CoCl}_3]$ ,  $\text{N}_2$ , 25°C

Cobalt-APTS is a well studied complex with characterized structure using spectroscopic and crystallographic methods as well as demonstrating anti-proliferative activity against human cancer cell lines [5].

In continuation of our works on the development of possible radiometal-based imaging agents [6], and vast research works on the pyridine-based metal complexes [2, 7–9], (due to their resemblance to pyridoxal metabolites [10]), it was interesting to develop a possible PET imaging agent by incorporating  $^{55}\text{Co}$  into APTS ligand to prepare a radiolabeled complex, i.e. [ $^{55}\text{Co}$ ](III)[APTS] $_2$  (Figure 1). Preliminary coincidence imaging and post-mortem biodistribution studies in wild-type and fibrosarcoma-bearing animals were also performed.

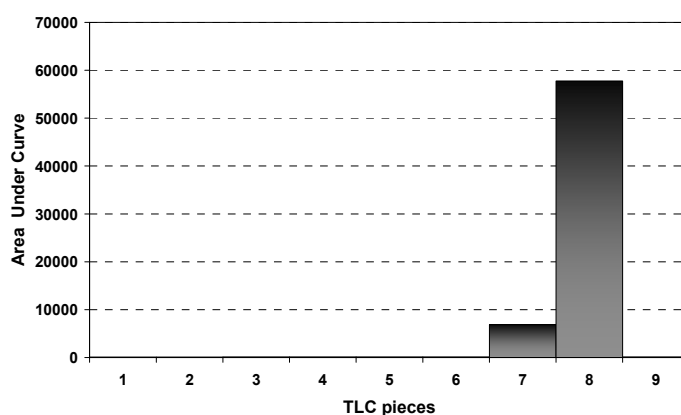
## Results and Discussion

### Cobalt-55 production

Cobalt-55 was produced by bombardment of a 30  $\mu\text{m}$  thick natural nickel target using a 150  $\mu\text{A}$  current of 15–8 MeV protons. The production yield was 270.2  $\mu\text{Ci}/\mu\text{Ah}$  at the end of bombardment. The radiochemical separation was based on a two step no-carrier-added method resulting in a yield of 95%. The radionuclidic purity was higher than 99.3%. The remaining activity was attributed to  $^{57}\text{Co}$ . No nickel ions were detected at a detection limit of 2 ppm.

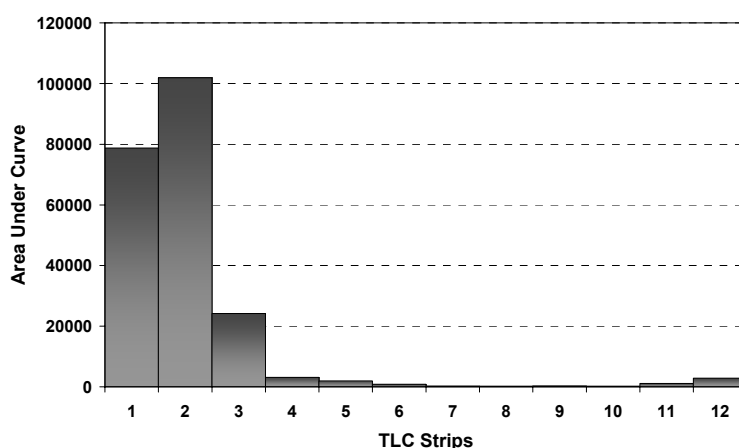
### Radiolabeling of APTS with [ $^{55}\text{Co}$ ]

It has been shown that pyridoxal thiosemicarbazone cobalt complexes form [ $\text{Co}(\text{HL})_2$ ] $_2\text{X}$  in their crystal structure and when dissolved in solution, [ $\text{Co}(\text{HL})_2$ ] $^+$  forms an octahedral cationic complexes. A more polar complex is formed after incorporation of cobalt cation which is lipophilic and carries a positive charge. Uncomplexed  $^{55}\text{Co}$  in the form of  $^{55}\text{Co}^{3+}$  elutes at an  $R_f = 0.8$ .



**Fig. 2.** RTLC of the [ $^{55}\text{Co}$ ]chloride ( $n=5$ ) eluted by ethanol on  $\text{SiO}_2$

The radiochemical yields were higher than 98% in each case ( $n=9$ ) (Figure 2), while the APTS complex moved with an  $R_f$  of 0.1 (Figure 3).



**Fig. 3.** RTLC of the final  $[^{55}\text{Co}](\text{III})[\text{APTS}]_2$  solution eluted by ethanol

In HPLC chromatograms the  $\text{Co}^{3+}$  cation is eluted at 1.2 minutes as a fast washing component (Figure 4), while the free unlabeled ligand elutes at 3.22 minutes (Figure 5) and the labeled complex elutes at 6.28 minutes (Figure 6).

The final radiolabeled complex diluted in normal saline was passed through a 0.22 micron filter (Millipore) to sterilize the product, due to possible thermal instability. The chemical stability of  $[^{55}\text{Co}](\text{III})[\text{APTS}]_2$  was high enough to perform further studies; RTLC of the final product showed no change in stability and the pattern for  $[^{55}\text{Co}](\text{III})[\text{APTS}]_2$  did not change out to 24 hours.

### **Electrophoretic studies**

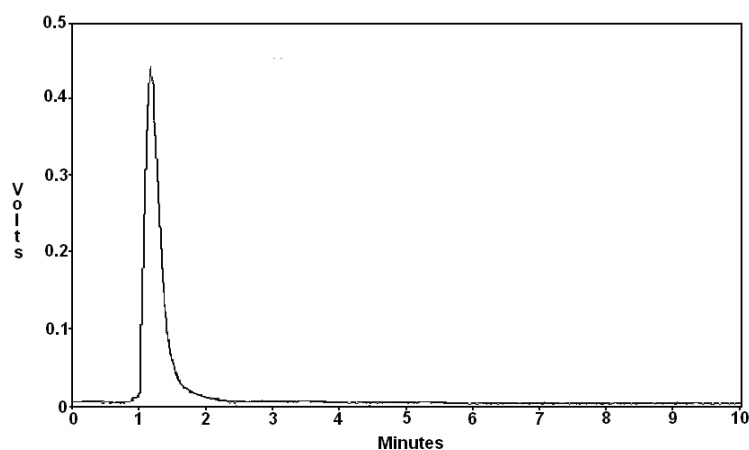
A major component of complex solution migrated to cathode in  $\text{EDTA}^{2-}$  solution demonstrating the total cationic property of the complex. As shown in Fig.1, considering the application of refluxing  $\text{HNO}_3$  to dissolve the Ni target, the most probable cobalt cation would be  $\text{Co}^{3+}$ . Thus, coordination of two nitrogen atoms (2 unpaired electrons in each) in and the two thiol-groups (in anionic form) would result in a positive charge for the complex.

### **Serum Stability Studies**

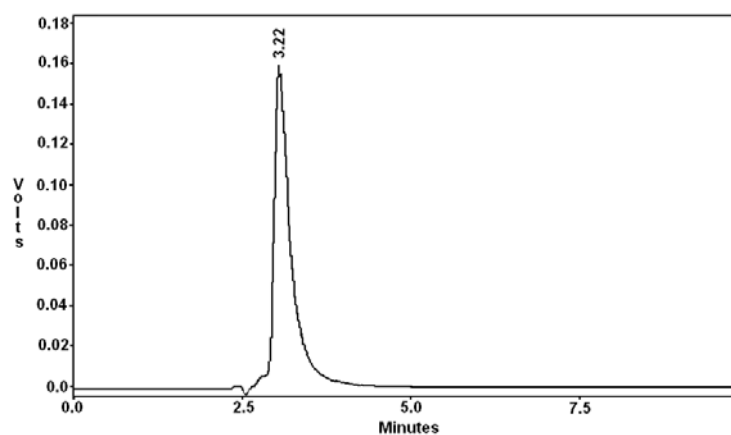
Incubation of  $[^{55}\text{Co}](\text{III})[\text{APTS}]_2$  in freshly prepared human serum for 24 h at  $37^\circ\text{C}$  showed no significant loss of  $^{55}\text{Co}$  from the complex during the course of the studies after RTLC study of the cut-off filter flow-through, and the radiochemical purity of complex remained at 98% for 24 h under physiologic conditions.

### **Partition co-efficient of $[^{55}\text{Co}](\text{III})[\text{APTS}]_2$**

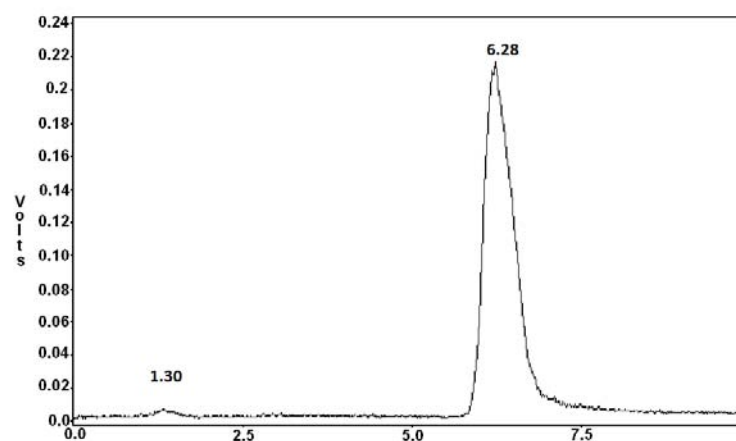
As expected from the RTLC behavior, the lipophilicity of the  $[^{55}\text{Co}](\text{III})[\text{APTS}]_2$  compound was high as determined by the octanol/water partition coefficient ( $P$ ) for the  $^{55}\text{Co}$ -complex and was found to depend on the pH of the preparation. At a pH of 7 (final formulation) the lipophilicity was  $1.00 \pm 0.08$ . The water solubility of the tracer is a bit changed when the pH is out of 5.5–7 range.



**Fig. 4.** HPLC of the final [ $^{55}\text{Co}$ ] $\text{CoCl}_3$  solution eluted by anhydrous ethanol using gamma scintillation detector



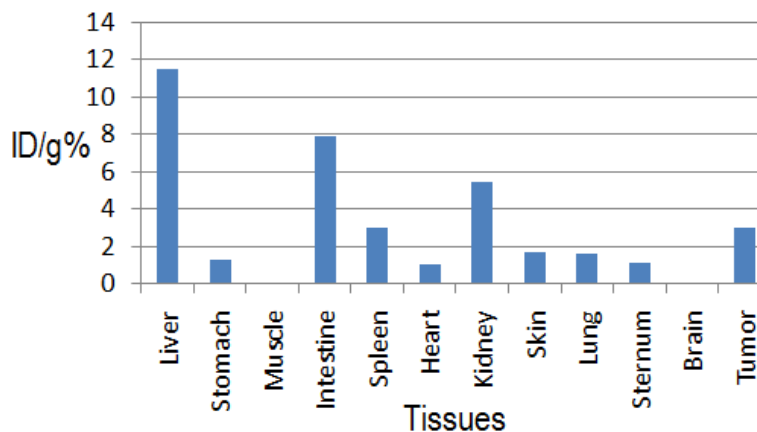
**Fig. 5.** HPLC of APTS ligand used in the radiolabeling (chemical purity > 99%) using ultraviolet detector



**Fig. 6.** HPLC of the final [ $^{55}\text{Co}$ ](III)[APTS] $_2$  used for biological studies (radiochemical purity > 98%) using gamma scintillation

### Biodistribution studies in tumor bearing mice

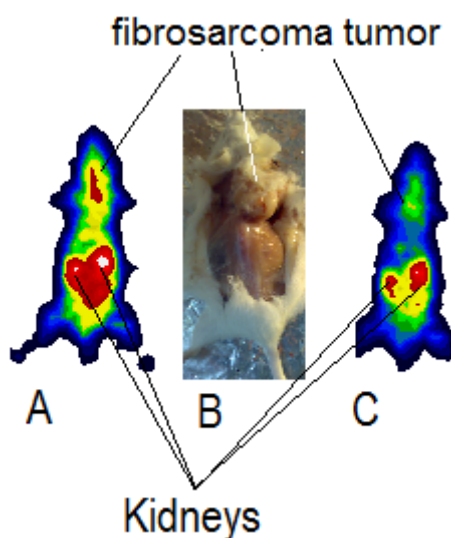
A few hours post-injection, the radioactivity content increased in the kidneys and liver and this pattern remained constant out to 21 hours (Figure 7).



**Fig. 7.** Bio-distribution of  $[^{55}\text{Co}](\text{III})[\text{APTS}]_2$  (100  $\mu\text{Ci}$  IV) in fibrosarcoma-bearing mice 21 h post-injection

Major part uptake of radioactivity accumulated was observed in the reticuloendothelial system including liver and spleen. No significant radioactivity in the tumor at other time points was observed (data not shown) while the best time range for the tracer uptake in tumor showed to be 20–35 h. Intestines exhibited a significant uptake which could be attributed to liver excretion of the tracer or metabolites. A significant tumor/muscle uptake ratio (80.1) was obtained.

### Imaging of fibrosarcoma-bearing mice



**Fig. 8.** Co-incidence images of  $[^{55}\text{Co}](\text{III})[\text{APTS}]_2$  in fibrosarcoma bearing mice 21 h (A), autopsy (B) and 35 h (C) post injection

[ $^{55}\text{Co}$ ](III)[APTS] $_2$  imaging in the fibrosarcoma-bearing mice showed a distinct accumulation of the radiotracer in the tumor tissue around the neck (Figure 8A) 21 h post injection. The retention of the radioactive material in the target organ was further investigated by autopsy study of the tumor position immediately after imaging at 35 h.

Figure 8 demonstrates the tumor uptake after 20 hours, which is significant however at this time point there is also accumulation in urinary tract. After 35 hours (about 2 half lives) tumor is still detectable (Figure 8C), while most of the background activity is removed and tumor is significant.

## Experimental

Production of  $^{55}\text{Co}$  was performed at the AMIRS (Agricultural, Medical and Industrial Research School) 30 MeV cyclotron (Cyclone-30, IBA). All chemicals were of analytical grade and purchased from Merck Chemical Company (Darmstadt, Germany). The ion-exchange resins were provided commercially (Bio-Rad Laboratories, Canada). NMR ( $^1\text{H}$ -NMR) spectra were obtained on a FT-80 Varian instrument (80 MHz) with tetramethylsilane as the internal standard. Infrared spectra were measured on a Perkin-Elmer 781 spectrometer (KBr discs). Radio-thin-layer-chromatography (RTLC) was performed on polymer-backed silica gel (F 1500/LS 254, 20×20 cm, TLC Ready Foil, Schleicher & Schuell<sup>®</sup>, Germany). Analytical HPLC was performed to determine the specific activity using a Shimadzu LC-10AT, connected in line to two detector systems, flow scintillation analyzer (Packard-150 TR) followed by a UV-visible detector (Shimadzu). The HPLC column used was a Si Kromasil 100 A<sup>°</sup>, 5  $\mu\text{m}$  (250×46 mm) from INCHORM WOLF GmbH, Co. Normal saline and sodium acetate used were of high purity and filtered through 0.22  $\mu$  Cativex filters. Radio-chromatography was performed by counting 5-mm portions of the strip using an in-house made scanner equipped with a Canberra<sup>™</sup> high purity germanium (HPGe) detector (model GC1020-7500SL) or after cutting it into pieces counting each 5mm-strip after in a CRC-15R Capintec dose calibrator (NJ, USA). Radionuclide purity was checked using an HPGe detector. All calculations and RTLC counting were performed on the 477.2 keV peak for  $^{55}\text{Co}$ . The oxidation state of cobalt complex was checked by cellulose acetate paper electrophoresis (Gellman) in 0.05N EDTA at 200V for 10 min.

Animal experiments were carried out in compliance with the United Kingdom Biological Council's Guidelines on the Use of Living Animals in Scientific Investigations, 2<sup>nd</sup> edn. Mice weighing 150-200 g were purchased from Razi Institute of Iran. Images were taken using a dual-head SPECT system (SMV, France, Sopha DST-XL).

### *Production of $^{55}\text{Co}$*

The procedure used for the targetry and bombardment for the production of  $^{55}\text{Co}$  was similar to that of  $^{57}\text{Co}$  already reported [11], with the exception of a 30  $\mu\text{m}$  layer of nickel as the target and 15 MeV protons for target irradiation (15–8 MeV on the target). The radiochemical separation process including recovery of the cobalt from the nickel and copper were carried out immediately after the target bombardment. The recovery of cobalt and copper from the nickel was carried out in the same manner as explained above for the radiochemical separation of  $^{57}\text{Co}$  [11]. The only differences were the use of 20 ml of

refluxing warm 7 M HNO<sub>3</sub> in the first step and 20 ml of 4 M HCl was used for the recovery of radiocobalt and radiocopper ions.

The eluent was evaporated to dryness, cooled and dissolved in 25 ml of 0.3 M HCl-94% ethanol and then injected on to an anion exchange resin (AG 1X8, Cl<sup>-</sup> form, 100-200 mesh, h: 5 cm, Ø: 1 cm) pre-equilibrated with 25 ml of 0.3 M HCl-82% ethanol. More than 95% of cobalt ions were recovered as [<sup>55</sup>Co]-CoCl<sub>3</sub> with 25 ml of 0.3 M HCl-72% ethanol through the resin [12]. The whole process took about 4 hours.

### **Quality control of the product**

#### **Radionuclidic purity**

Radionuclidic purity of the products was assayed by gamma spectroscopy of the final samples using an HPGe detector coupled with a Canberra™ multi-channel analyzer. The peaks were observed for 1 h for <sup>55</sup>Co.

#### **Chemical purity**

The production was based on the irradiation of natural nickel electroplated on to a gold-layered backing. Therefore, the presence of nickel cation was detected using visible colorimetric assays. The most important photometric reagents for determining nickel are dioximes, which provide specific and fairly sensitive methods. Dimethylglyoxime reacts with nickel ions in a neutral or ammonia medium forming a pink, flocculent precipitate. Even at 2 ppm of standard nickel concentration, the colored Ni-dimethylglyoxime complex is visible to the naked eye [13]. The amount of gold cation was monitored in the final solution using color formation with acidic rhodamine B reagent, based on a previously reported colorimetric method [14].

### **Preparation of 2-acetylpyridine thiosemicarbazone [ (2E)-2-[1-(Pyridin-2-yl)ethylidene]hydrazinecarbothioamide ]**

This compound was prepared with slight modifications of a previously reported method [15]. A mixture of thiosemicarbazide (182 mg, 2 mmol) in acetic acid solution (5%, prepared with 99% acetic acid and MilliQ-H<sub>2</sub>O) were heated at 50°C with stirring until a transparent solution was formed. Then freshly distilled 2-acetylpyridine (242 mg, 2 mmol) diluted (1:3) with 5% acetic acid was added drop-wise to the mixture over 5 min under a blanket of N<sub>2</sub>. The mixture was stirred while heating for 3–4 h at 50°C. The hot reaction mixture was filtered with two layers of Whatman No.2 filter paper. The filtered precipitate was washed with MilliQ-H<sub>2</sub>O (50 ml), rectified ethanol (25 ml) and finally heated in a vacuum oven overnight at 75°C. The dried powder was refluxed in 80% acetic acid (prepared with MilliQ-H<sub>2</sub>O) for 2 h. The hot mixture was filtered and the precipitate was washed with MilliQ-H<sub>2</sub>O (50 ml), rectified ethanol (25 ml) and heated in an oven overnight at 75°C. Alternatively the powder could be crystallized from hot ethanol to give a brilliant white powder (60%) m.p. 275 °C. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ (ppm) 10.28 (bs, 1H, NH-N<sub>2</sub>), 8.58–8.36 (m, 3H, H<sub>3</sub>&H<sub>6</sub> pyridine & NH-N<sub>4</sub>), 8.1 (bs, 1H, NH-N<sub>4</sub>), 7.88–7.67 (hexlet, 1H, H<sub>5</sub> pyridine), 7.43–7.29 (m, 1H, H<sub>4</sub> pyridine), 2.39 (s, 3H, CH<sub>3</sub>-C=N). IR (CHCl<sub>3</sub>) λ<sub>max</sub> 3208, 3132 (N-H), 1470 (C=N), 1160 (C=S). Mass (electrospray) 194.1 (14%), 172 (4), 157.1 (76), 130 (65), Elemental analysis for C<sub>8</sub>H<sub>10</sub>N<sub>4</sub>S, calcd C, 49.46, H, 5.19, N, 28.84; found C, 49.41, H, 5.21, N, 28.91.



**Preparation of [ $^{55}\text{Co}$ ](III)bis-(2-acetylpyridine thiosemicarbazone)  
[ Bis{N'-[1-(pyridin-2-yl-kN)ethylidene]carbamohydrazonothioato-  
k<sup>2</sup>N',S}( $^{55}\text{Co}$ )cobalt(1+)]**

The acidic solution of [ $^{55}\text{Co}$ ]CoCl<sub>3</sub> (3 mCi in 2.5-3 ml) was transferred to a 5 ml-vial and heated to dryness using a flow of N<sub>2</sub> gas at 50-55°C. Fifty micro µl of 2-acetylpyridine thiosemicarbazone in methanol (1 mg/ml, 240 nmol) was added to the cobalt residue and vortexed at 25°C for 3-5 min and then left at room temperature for 30 min. The resulting mixture was diluted by the addition of normal saline (4.5 ml) and rapidly checked by RTLC and HPLC for radiochemical purity. The final solution was then passed through a 0.22 µm filter and pH adjusted to 5.5–7. The same procedure was used for the preparation of [ $^{55}\text{Co}$ ](III)[APTS]<sub>2</sub>.

**Radiochemical purity of [ $^{55}\text{Co}$ ](III)[APTS]<sub>2</sub>**

**Radio thin layer chromatography**

RTLC was performed using an in-house made radiochromatogram scanner coupled to an HPGe detector. A step motor was installed to permit counting of 0.4 cm segments each 30 seconds through a slot in a shielded chamber. RTLC was performed using absolute ethanol (developing solution) on polymer-backed silica gel. The radiochemical yields were determined by comparison of the activities in the un-complexed  $^{55}\text{Co}$  and the [ $^{55}\text{Co}$ ](III)[APTS]<sub>2</sub> major radio peak.

**High performance liquid chromatography**

HPLC was performed on the final preparation using HPLC grade EtOH as the eluent with at a flow rate of 1.3 ml/min (pressure: 120–140 kgF/cm<sup>2</sup>) for 40 min using a Si Kromasil 100, 5 µm (250×46 mm).

**Paper electrophoresis**

Ionic charge of the cobalt-55 complex in final solution was checked using cellulose acetate paper electrophoresis (Gellman) in 0.05N EDTA at 200V for 10 min. A major component of complex solution migrated to cathode in EDTA<sup>2-</sup> solution.

**Stability of [ $^{55}\text{Co}$ ](III)[APTS]<sub>2</sub> complex in the final product**

A sample of [ $^{55}\text{Co}$ ](III)[APTS]<sub>2</sub> (5 mCi, 2mL) was kept at room temperature for 24 hours and checked by RTLC at various time intervals. A sample (100 µl) was taken from the shaken mixture and diluted 3-4 times with normal saline and passed through a 5KDa cut-off filter (Waters) followed by determination of free cobalt cation content (Rf. 0.8) to [ $^{55}\text{Co}$ ](III)[APTS]<sub>2</sub> (Rf. 0.0) by RTLC using ethanol as eluent.

**Serum stability studies**

In order to perform serum stability studies, 500 µl of freshly prepared human serum was added to 976 µCi of [ $^{55}\text{Co}$ ](III)[APTS]<sub>2</sub> (100-150 µl) and the resulting mixture incubated at 37°C for 24 h. Aliquots (5-µl) were analyzed by RTLC after 2, 4, 8, 16 and 24 hours of incubation, to determine the stability of the complex.

### ***Determination of partition coefficient***

The partition coefficient of the  $[^{55}\text{Co}](\text{III})[\text{APTS}]_2$  was measured following 1 min of vortexing a mixture comprised of 1 ml 1-octanol and 1 ml of isotonic acetate-buffered saline (pH=7) with approximately 100  $\mu\text{Ci}$  of the radiolabeled metal complex (100–150  $\mu\text{l}$ ) at 37°C. Following further incubation for 5 min, the octanol and aqueous phases were sampled and counted in a dose calibrator. A 500  $\mu\text{l}$  sample of the octanol phase from this partitioning was repartitioned two to three times with fresh buffer to ensure that traces of hydrophilic  $^{55}\text{Co}$  impurities did not alter the calculated  $P$  values. The reported ( $\log P$ ) values are the average of the second and third extractions from three to four independent measurements, ( $\log P$ ) values and represent the mean of five measurements.

### ***Animal studies***

#### ***Induction of fibrosarcoma tumors in mice***

Tumor induction performed by the use of poly aromatic hydrocarbon injection in rodents as reported previously [16]. For tumor model preparation, 10 $\mu\text{l}$  of 3-methylcholanthrene solution in extra-virgin olive oil (4 mg/ml) was injected SC to the dorsal area of the mice. After 14–16 weeks the tumor weighed 0.2–0.4 g and was not grossly necrotic. Tumor tissues of some random animals were sent for pathological tests and were diagnosed as fibrosarcoma.

#### ***Biodistribution studies***

A volume (0.1 ml) of the final  $[^{55}\text{Co}](\text{III})[\text{APTS}]_2$  solution containing 100  $\mu\text{Ci}$  activity ( $\leq 2 \mu\text{g}$  APTS in 100  $\mu\text{l}$ ) was injected *via* the dorsal tail vein. The total amount of activity injected into each rat was determined by counting the 1-ml syringe before and after injection in a dose calibrator with fixed geometry. The animals were sacrificed by  $\text{CO}_2$  asphyxiation at selected time intervals (2, 4, 12, 21, 35 h) post injection, tissues (including liver, stomach, muscle, intestine, spleen, heart, kidney, skin, lung, sternum and the tumor) were weighed and their specific activities determined by counting on an HPGe detector to obtain the counts as a percentage of the injected dose per gram of tissues.

#### ***Imaging of $[^{55}\text{Co}](\text{III})[\text{APTS}]_2$ in tumor bearing mice***

Fibrosarcoma-bearing mice were used for tumor imaging when tumors reached a size of 0.5–1  $\text{cm}^3$ , 14–16 weeks after its induction. Images were taken 2–48 hours post injection including (21 and 35 hours) after administration of the radiopharmaceutical in the coincidence mode by a dual-head SPECT system. The mouse-to-high energy septa distance was 12 cm. Images were performed of tumor bearing mice. The useful field of view (UFOV) was 540 mm $\times$ 400 mm. The spatial resolution in the coincidence mode was 10 mm FWHM at the CFOV. Sixty four projections were acquired for 30 seconds per view with a 64 $\times$ 64 matrix.

### **Conclusion**

The methods described herein for the production and radiochemical separation of  $^{55}\text{Co}$  were simple and cost effective, resulting in high production yields and acceptable levels of contamination. Total labeling and formulation of  $[^{55}\text{Co}](\text{III})[\text{APTS}]_2$  took about 40 minutes, with a yield of greater than 99% and a specific activity of  $\approx 10$ –20 Ci/mmol. No significant

amounts of labeled by-products were observed by HPLC analysis of the final preparations. The radio-labeled complex was stable in aqueous solution, and in human serum at 37°C, for at least 24 h and no significant amount of other radioactive species were detected by RTLC/HPLC. The biodistribution of the ligand was checked in tumor bearing mice out to 48 h showing a significant tumor uptake (3%) after 21 h. Co-incidence imaging of the tumor-bearing mice (2–48 h post injection) receiving [ $^{55}\text{Co}$ ](III)[APTS]<sub>2</sub> showed the accumulation of the tracer in the tumoral tissue 20–40 h, while the best time point seem to be 35 h due to background reduction. [ $^{55}\text{Co}$ ](III)[APTS]<sub>2</sub> is a potential tracer, with suitable half life and good chemical stability for tumor diagnostic applications, while further animal studies are required to establish the tumor uptake and best time points in tumoral models.

## Authors' Statements

### Competing Interests

The authors declare no conflict of interest.

### Animal Rights

The institutional and (inter)national guide for the care and use of laboratory animals was followed. See the experimental part for details.

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