Chemistry Central Journal

RESEARCH ARTICLE

Open Access

CrossMark

Platelet-12 lipoxygenase targeting via a newly synthesized curcumin derivative radiolabeled with technetium-99m

Reem Ibrahim Al-Wabli[1], Tamer Mostafa Mohamed Hafez Sakr[2*], Mohammed Abdou Khedr[3], Adly Abdallah Selim[4], Mohamed Abd El-Motaleb Abd El-Rahman[4] and Wafaa Abdou Zaghary[3*]

Abstract

Background: One of the most popular techniques for cancer detection is the nuclear medicine technique. The present research focuses on Platelet-12-lipoxygenase (P-12-LOX) as a promising target for treating and radio-imaging tumor tissues. Curcumin was reported to inhibit this enzyme via binding to its active site.

Results: A novel curcumin derivative was successfully synthesized and characterized with yield of 74%. It was radiolabeled with the diagnostic radioisotope technetium-99m with 84% radiochemical yield and in vitro stability up to 6 h. The biodistribution studies in tumor bearing mice confirmed the high affinity predicted by the docking results with a free binding energy value of (ΔG -50.10 kcal/mol) and affinity (13.64 pki) showing high accumulation in solid tumor with target/non-target ratio >6.

Conclusion: The newly synthesized curcumin derivative, as a result of a computational study on platelet-12 lipoxygenase, showed its excellent free binding energy (ΔG -50.10 kcal/mol) and high affinity (13.64 pKi). It could be an excellent radio-imaging agent that targeting tumor cells via targeting of P-12-LOX.

Keywords: Platelet-12 lipoxygenase, Curcumin, Technetium-99m, Cancer imaging, Enzyme targeting, Docking

Background

Cancer is the main cause of mortality worldwide and the number of cancer patients is increasing at an alarming rate. Early detection of cancer greatly increases the chances of saving the patient's life [1–3]. One of the most popular techniques for cancer detection is the nuclear medicine technique that is a breakthrough in the cancer imaging procedures [4, 5]. Technetium-99m is the most popular gamma emitting radionuclides used in nuclear medicine due to its perfect characteristics (6.02 h half-life and 140 keV γ-ray energy) and to its low cost and good availability [3–7]. In order to develop a successful radioactive tracer for cancer targeting, a selective organic compound that can differentiate between tumor and normal cells is extensively needed [8]. Curcumin is a natural product isolated from the rhizomes of the Indian *Curcuma longa* plant [9]. The Indian culture used curcumin as a food-flavoring agent, coloring agent and also in medicine as antiseptic, analgesic and antimalarial agent [10]. Researches proved that curcumin possesses other diverse of biological activities including antiviral [11], antibacterial [12], antifungal [13], anti-inflammatory [14], and antioxidant activities [15]. Recently, curcumin has drawn much attention due to its powerful anti-proliferative effect and anticancer activity in multiple cancers including ovarian, pancreatic, breast, melanoma, neck, colon, prostate, and head cancers [16–20]. The anticancer effect is manifested through the induction of apoptosis, growth arrest, and inhibition of the tubulin polymerization [21–23]. Furthermore, studies have shown that curcumin appeared as cytotoxic to cancer cells and cytoprotective to normal cells indicating that curcumin could be used

*Correspondence: Tamer_sakr78@yahoo.com; wzaghary@yahoo.com
[2] Radioactive Isotopes and Generator Department, Hot Labs Center, Egyptian Atomic Energy Authority, P.O. Box 13759, Cairo, Egypt
[3] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Helwan University, Ein Helwan, Cairo 11795, Egypt
Full list of author information is available at the end of the article

Springer Open

Al-Wabli *et al. Chemistry Central Journal* (2016) 10:73

Page 2 of 12

as a selective safe radiotracer [24]. Specific enzyme targeting that is overexpressed in cancer, by using selective radiolabeled inhibitor of this enzyme, could be a great approach for treatment, imaging and diagnosis of cancers. The targeting process is a highly selective step that can be achieved by the computational approach [25]. The high over-expression of the Platelet-12 Lipoxygenase (P-12-LOX) was reported in different cancer tissues [26, 27]. Inhibition of such enzyme is considered to be a promising target for cancer treatment. To date few P-12-LOX inhibitors are known. Curcumin was reported to inhibit P-12-LOX via binding to its active site [28, 29]. The development of a novel curcumin derivative, which possesses higher free binding energy and good affinity to P-12-LOX, was one of the main objectives of this work. This is to select the highly predicted selective inhibitor of P-12-LOX to be synthesized then radiolabeled with technetium-99m followed by its in vivo evaluation as a novel target agent to P-12-LOX receptor in cancer cells.

Experimental
Chemicals
Curcumin and 2,4,6-trimethylbenzoyl chloride were purchased from Sigma-Aldrich, Steinheim, Germany. Analytical grade chemicals were directly used without further purification. All solutions were prepared using deionized water. Technetium-99m was eluted as $^{99m}TcO_4^-$ from $^{99}Mo/^{99m}Tc$ generator, Elutec Brussels, Belgium.

Instruments
Mettler FP 80 melting point apparatus was used to determine the melting points that were uncorrected. Ultrospec-2100 Pro UV visible spectrophotometer was used to record ultraviolet (UV) spectrum. Infrared (IR) spectra were recorded on FT/IR Shimadzu, Fourier transform, Infrared spectrometer/cm scale using KBr disc technique. ^1H NMR and ^{13}C NMR were carried out on Burker AC 500 MHz Spectrometer; chemical shifts are expressed in δ (ppm) downfield from TMS as an internal standard. Accela U-HPLC system coupled to a TSQ Quantum Access MAX triple stage quadrupole mass spectrometer carried out the LC–MS analysis (Thermo Scientific Corporation, USA) that was controlled with Xcalibur software version 2.2. Radioactivity measurements were done using Sodium Iodide (Tl) γ-ray scintillation counter (Scaler Ratemeter SR7, Nuclear Enterprises, Edinburgh, England). Ascending thin layer chromatography (TLC) run on pre-coated (0.25 mm) (GF 254) silica gel plates were used to follow up the reaction and the homogeneity of the compound. Routinely, used developing solvents system was C_6H_6:EtOAc:CHCl$_3$ (5:1:5) (in ratio v/v). UV lamp at 254 nm was used to visualize the spots. Silica gel (60-230 mesh E. Merck) was used after heating at 110 °C for 1 h and

was used for column chromatography separations. Silica gel 60 GF$_{254}$ for TLC was used for coating 20 × 20 cm glass plates for preparative TLC.

Animals
The biological distribution was evaluated in 20–25 g Albino mice.

Software programs
Molecular Operating Environment (MOE) package license was purchased from Chemical Computing Group Inc, Sherbooke St, Montreal, QC, Canada [30].
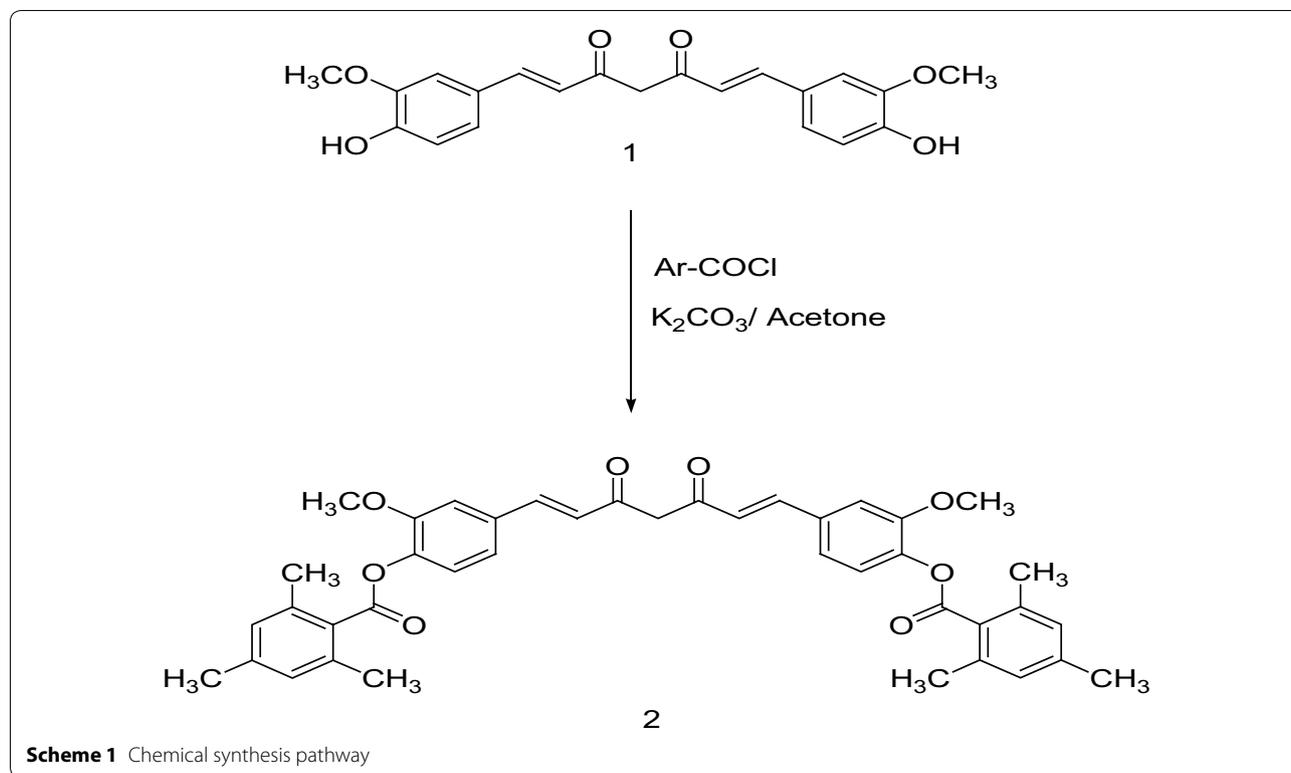
Molecular docking
All compounds were built and saved as MOE. Rigid receptor was used as a docking approach. Receptor and solvent were kept as a "receptor". Triangle matcher was used as a placement method with timeout of 300 s. Two rescoring were computed; rescoring 1 was selected as London dG while rescoring 2 was selected as affinity. Force field was used as a refinement. The best conformation for each compound was kept inside the docking pocket and the affinity (pKi) was computed. The free energy of binding ΔG (kcal/mol) for the proposed derivatives were also recorded.

Synthesis of curcumin derivative (1,7-bis[((4′-(2″,4″,6″-trimethylbenzoyl)oxy)-3′-methoxyphenyl]-1,6-heptandiene-3,5-dione)
To an ice-cold curcumin solution (**1**) (3.68 g, 0.01 mol) in 50 ml dry acetone, 2 g of sodium carbonate was added with stirring for 15 min. The 2,4,6-trimethylbenzoyl chloride (0.025 mol) was added dropwise to the mixture over a period of 30 min. The reaction was then refluxed for 12 h [31]. The reaction mixture was filtered, evaporated then extracted using EtOAc (3 × 30 ml). The combined organic layers were dried using anhydrous $MgSO_4$ and the solvent volume was reduced under reduced pressure to give a yellow precipitate, which was recrystallized from ethanol to give compound (**2**) (Scheme 1) with yield 74%, mp 210–212 °C, molecular formula $C_{41}H_{40}O_8$ and M.Wt. 660.

Preparation of ^{99m}Tc-curcumin derivative complex
Curcumin (150 µg) was dissolved in 1 ml DMSO in 10 ml penicillin vials. Then about 10 mg of $NaBH_4$ was added to each vial with pH adjustment in a range of 6–10 using of 0.1 N sodium hydroxide or 0.1 N Hydrochloric Acid. Followed by the addition of 100 µl of freshly eluted $^{99m}TcO_4^-$ (~200 MBq) to each vial. These reactions were performed for 15 min at room temperature. These procedures were repeated to evaluate the radiochemical yields with varying $NaBH_4$ amounts (5–30 µg), varying

Al-Wabli *et al. Chemistry Central Journal* (2016) 10:73

Page 3 of 12

Scheme 1 Chemical synthesis pathway

curcumin amounts (50–300 μg), on different time scale (5–60 min).

Radiochemical yield assay of ^{99m}Tc-curcumin derivative complex

^{99m}Tc-curcumin derivative complex radiochemical yield and in vitro stability were evaluated by using strips of ascending Whatman paper chromatography (PC). Two strips were used per experiment, on which two drops of the reaction product were placed on origin line at distance of 2 cm from the bottom.

For the determination of the ratio of free ^{99m}TcO$_4^-$ radio-contaminant, acetone was used as a developing solvent for one PC strip, where free ^{99m}TcO$_4^-$ R$_f$ was 1 while ^{99m}Tc-curcumin derivative complex and reduced hydrolyzed technetium colloid species R$_f$ was zero.

Another strip was developed in C$_2$H$_5$OH:H$_2$O:NH$_4$OH mixture (2:5:1, v/v/v) to determine the ratio of the hydrolyzed ^{99m}Tc radio-contaminant, where reduced hydrolyzed technetium colloid R$_f$ is zero while free ^{99m}TcO$_4^-$ and ^{99m}Tc-curcumin derivative complex species R$_f$ is 1.

At the end of the developing process, the strips were dried, cut into 1 cm pieces and counted using the sodium iodide (Tl) γ-ray scintillation counter. Each experiment was repeated three times.

The radiochemical yield percent of ^{99m}Tc-curcumin derivative complex was calculated according to the following equation:

$$\%\text{Radio chemical yield} = 100 - \left(\%\text{Free }^{99m}\text{TcO}_4^- + \%\text{colloid}\right)$$

Biological distribution

The animal ethics committee guided this study was following the guidelines of the Egyptian Atomic Energy Authority. The biological distribution was evaluated in mice bearing solid tumor.

Solid tumor induction in mice

The solid tumor induction was done using Ehrlich ascites carcinoma (EAC) that was derived from a murine mammary carcinoma [32, 33]. The parent tumor line EAC had been derived from 7 days old donor female Swiss Albino mice and diluted with sterile physiological saline solution. To induce a solid tumor, about 0.2 ml solution was I.M. injected in the female Albino mice right thigh for 4–6 days [3, 5, 34].

Biodistribution assay of ^{99m}Tc-curcumin derivative complex

The biodistribution study of ^{99m}Tc-curcumin derivative complex was evaluated at time intervals of 5, 15, 30, 60, 120 and 180 min post injection (p. i) in solid tumor bearing Albino mice (n = 5 mice/time point). Mice were

Al-Wabli *et al. Chemistry Central Journal* (2016) 10:73

Page 4 of 12

separated in groups and supplied with food and water. ^{99m}Tc-curcumin derivative complex was I.V. injected in the mice tail vein.

Firstly, animals were anaesthetized using chloroform, then weighted and sacrificed at different time intervals. All body organs and tissues were separated, collected and washed with saline then weighted. Blood, bone and muscle samples were collected and weighted then were assumed to be 7, 10 and 40% of the total body weight, respectively [3, 35]. The organs radioactivities as well as the background were measured in a well type γ-counter NaI(Tl). In a population of five, the percent injected dose/gram organ or tissue (%ID/g) were calculated. Target (solid tumor) to non-target (normal muscle) ratio (%T/NT) was calculated from %ID/g for solid tumor and normal muscle.

Statistical analysis

Graph Pad Prism version 6.0 software was used to do all the statistical analyses. Statistical analysis was conducted using one-way ANOVA followed by multiple Tukey–Kranes post hoc test at $v < 0.05$ considered for statistical significance.

Results and discussion

Computational selection of the best curcumin derivative

Selectivity plays a major role in drug targeting process. It can help in identifying the most suitable ligand (key) for a specific enzyme (lock). Computational approaches are widely used nowadays to compute the free energy of binding, affinity and other parameters like LogP that have an indication of good fitting and predictive high selectivity. P-12-LOX is overexpressed in many tumor tissues [36]. Arachidonic acid is metabolized by P-12-LOX to produce a hydroxyeicosatetraenoic acid that has been reported to be a main cause of cancer development [37, 38]. Thus, inhibition of P-12-LOX can decrease both cell proliferation and metastasis [39]. Curcumin was reported to inhibit P-12-LOX (66 μmol/l) [28, 29]. Also, a number of synthetic curcuminoids were reported to have a promising P-12-LOX inhibitory activity [40]. Compound E26C, a curcumin derivative with benzofuran moiety, had P-12-LOX inhibitory activity IC50 = 17 μmol/l and showed the best fitting distance 3.3 Å among all the reported curcuminoids (Fig. 1). The discovery of a curcumin derivative that can possess high affinity toward P-12-LOX and can be radiolabeled for tumor imaging was the main objective of this study. Radiolabeling of a highly selective P-12-LOX inhibitor will also ensure high accuracy of tumor cells imaging, as this enzyme is overexpressed in tumor tissues as mentioned before.

The study aimed to propose different curcumin derivatives that possess different substitution on the aromatic ring and that simulate the lead structure (Fig. 1).

A number of curcumin derivatives with a substituted phenyl ring at the same position of the lead derivative were designed (Fig. 2). The computational affinity (pKi), cLogP and free binding energy (ΔG) were computed and compared to those of both curcumin itself and the reported benzofuran derivative (Table 1).
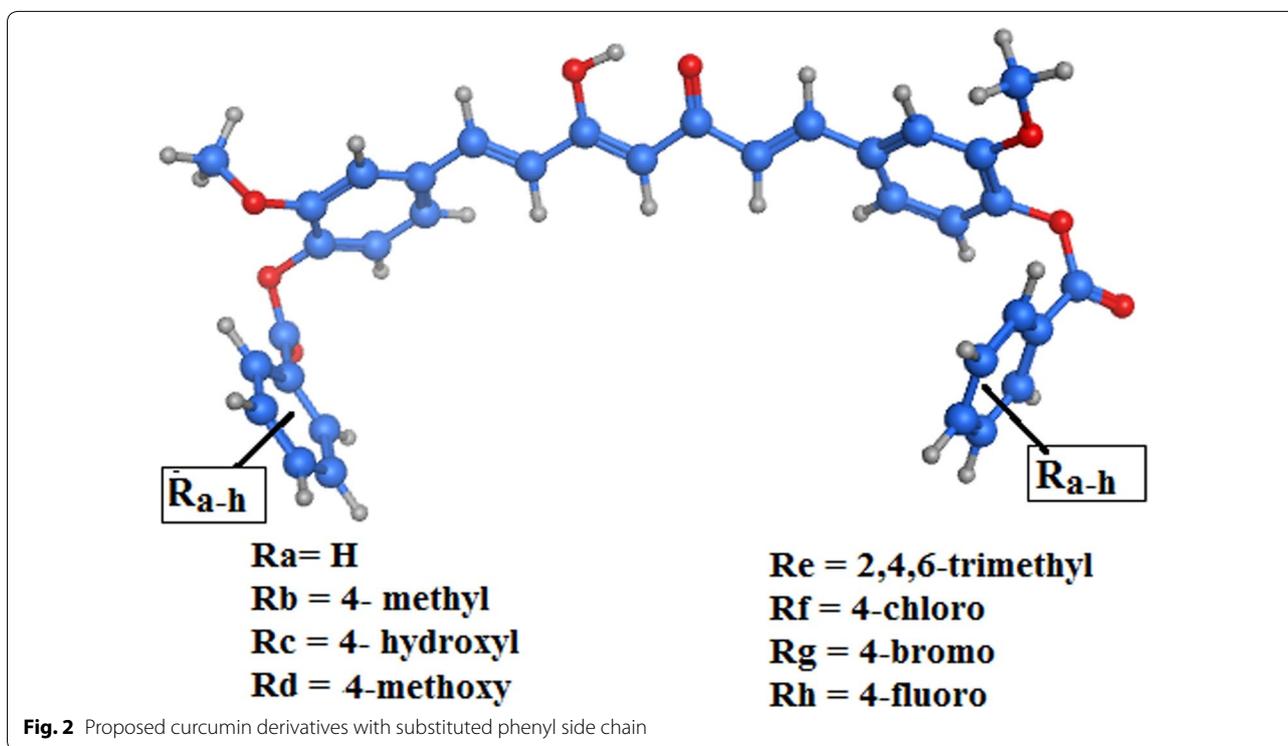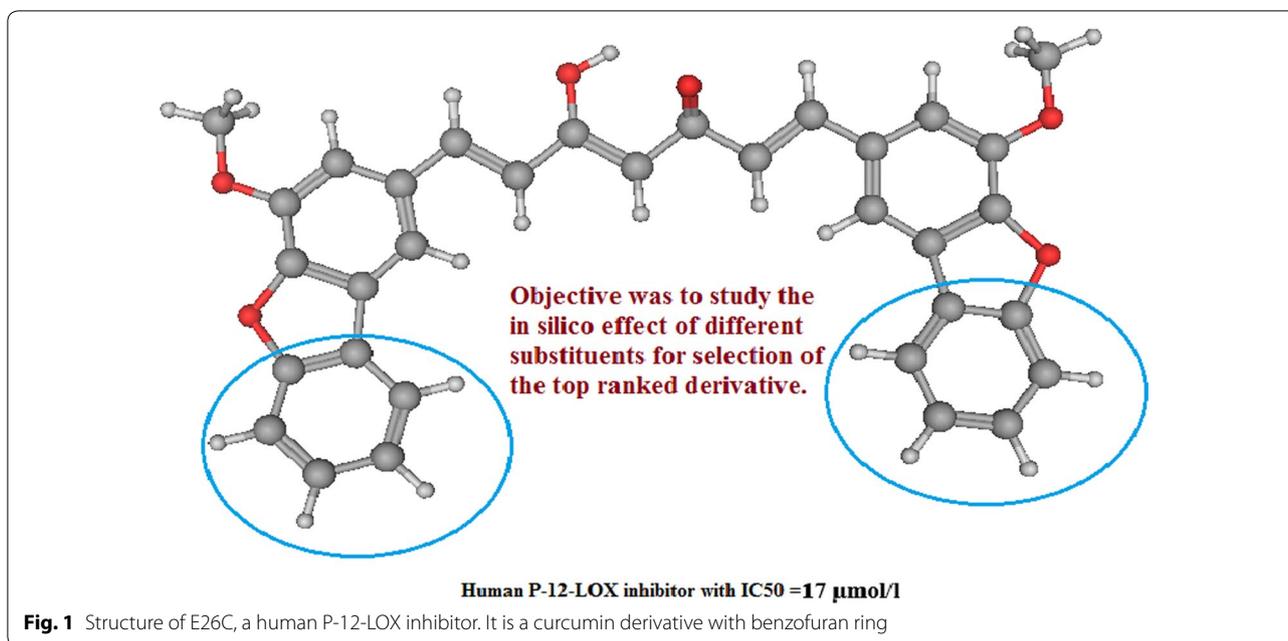
It was clear that E26C had more inhibitory activity than curcumin, it showed higher affinity (pKi) 10.40 and higher cLogP (7.9) as well. Besides, its free binding energy was very less and favorable (−51.65 kcal/mol) than that of curcumin (−30.85 kcal/mol).

As a result, from all the proposed structures, we were looking for the one with higher affinity, less free binding energy and higher cLogP than curcumin and comparing it with E26C as well. The 2,4,6-trimethyl phenyl derivative showed almost the same cLogP (7.7) to that of E26C and with high affinity (13.64) that was higher than both of E26C and curcumin. The 2,4,6-trimethyl phenyl derivative also showed less free binding energy (−50.10 kcal/mol) when compared to other proposed derivatives.

The new curcumin derivative showed better free binding energy that was reflected upon the total potential energy, which was lower toward the most stable state in case of the new derivative when compared to that of curcumin complex. In addition, it had a higher affinity (13.64). The binding of the new compound showed good coordination with the iron metal in the active site that was not achieved by the curcumin itself (Fig. 3).

In accordance, the curcumin derivative with (1,7-Bis[((4′-(2″,4,6″-trimethylbenzoyl)oxy)-3′-methoxyphenyl]-1,6-heptandiene-3,5-dione) was selected to be synthesized and radiolabeled with techniethium-99m.

The proposed chemical complex that may be formed between technetium 99m and the top ranked selected curcumin derivative compound with (1,7-Bis[((4′-(2″,4″,6″-trimethylbenzoyl)oxy)-3′-methoxyphenyl]-1,6-heptandiene-3,5-dione) was compared to curcumin-technetium-99m complex. It was proposed that two molecules of curcumin were complexed with one technetium-99m. While, (1,7-Bis[((4′-(2″,4″,6″-trimethylbenzoyl)oxy)-3′-methoxyphenyl]-1,6-heptandiene-3,5-dione) was complexed with by 1:1 ratio in which the technetium-99m formed a complex with the two carbonyl of the benzoyl moieties in addition to that at the 3,5-dione. In the first case the two curcumin molecules formed a long and wide complex while the 2,4,6-trimethyl benzoyl derivative showed a conformation that

Al-Wabli *et al. Chemistry Central Journal* (2016) 10:73

Page 5 of 12

Fig. 1 Structure of E26C, a human P-12-LOX inhibitor. It is a curcumin derivative with benzofuran ring

Ra= H
Rb = 4- methyl
Rc = 4- hydroxyl
Rd = 4-methoxy

Re = 2,4,6-trimethyl
Rf = 4-chloro
Rg = 4-bromo
Rh = 4-fluoro

Fig. 2 Proposed curcumin derivatives with substituted phenyl side chain

illustrated a well-fitting. When both complexes were docked, curcumin showed an affinity pki of (33.54) that was lower than that of the selected curcumin derivative that achieved an affinity pki of (45.20) to prove that it has more stability than that of curcumin in enzyme binding in the technetium-99m complex form (Fig. 4).

Al-Wabli *et al. Chemistry Central Journal* (2016) 10:73

Page 6 of 12

Table 1 Computed affinity (pKi), cLogP, and ΔG (kcal/mol) for the proposed derivatives

	Compound	Affinity (pKi)	cLogP	ΔG kcal/mol
1	Phenyl	11.38	6.8	−30.54
2	4-Methyl phenyl	11.51	7.49	−40.65
3	4-Hydroxy phenyl	10.37	6.2	−43.40
4	4-Methoxy phenyl	11.50	6.8	−43.45
5	2,4,6-Trimethyl phenyl	*13.64*	*7.7*	*−50.10*
6	4-Chloro phenyl	10.55	8.1	−41.75
7	4-Bromo phenyl	10.72	8.4	−41.95
8	4-Fluoro phenyl	10.24	7.1	−42.15
9	Curcumin	8.66	3.85	−30.85
10	Curcumin derivative (E26C)	*10.40*	*7.9*	*−51.65*

Chemical synthesis of curcumin derivative

The chemical synthesis was achieved in one-step reaction through reacting 2,4,6-trimethylbenzoyl chloride with curcumin according to the method mentioned previously.

IR (KBr) υ (cm^{-1}): 3008 (CH-Ar), 2922, 2854 (CH-aliphatic), 1741 (C=O of ester), 1631 (C=O) 1600 (C=C aromatic), and 1122 (C–O). ^{1}H NMR (CDCl$_3$) δ ppm (500 MHz): 2.35–2.49 (s, 18H, 6 × CH$_3$), 3.94 (s, 6H, 2 × OCH$_3$), 5.91 (s, 1H, H-4), 6.62 (d, 2H, H-2 & H-6, $J = 15.5$ Hz), 6.96 (s, 4H, H-3″ & H-5″), 7.21–7.29 (m, 6H, Ar–H) and 7.68 (d, 2H, H-1 & H-7, $J = 16$ Hz). ^{13}C NMR (DMSO-d_6) δ ppm (125 MHz): 20.0 (4 × CH$_3$), 21.2 (2 × CH$_3$), 55.8 (2 × OCH$_3$), 101.9 (C-4), 111.6 (2 × C-2′), 121.2 (2 × C-6′), 123.3 (2 × C-5′), 124.4 (2 × C-1′), 128.7 (C-2, C-6, 2 × C-3″ & 2 × C-5″), 129.6 (2 × C-1″), 134.1 (2 × C-4″), 136.0 (2 × C-2″ & 2 × C-6″), 140.0 (C-1 & C-7), 141.3 (2 × C-3′), 151.7 (2 × C-4′), 167.6 (2 × C=O of ester) and 183.1 (C-3 & C-5). MS: *m/z* (%) for C$_{41}$H$_{40}$O$_8$: 661 [M$^+$ + 1] (26%).

Factors affecting on radiochemical yield of ^{99m}Tc-curcumin derivative

Effect of reducing agent (NaBH$_4$) amount

The effect of NaBH$_4$ on the % radiochemical yield of ^{99m}Tc-curcumin derivative complex was shown in (Fig. 5). At 5 mg NaBH$_4$, the %^{99m}TcO$_4^-$ was equal to 5.3 ± 0.8% that may be due to insufficient amount of NaBH$_4$ to complete the reduction of ^{99m}TcO$_4^-$ to form ^{99m}Tc-complex. Increasing the amount of NaBH$_4$ to 10 mg, the maximum radiochemical yield (84 ± 1.4%) was revealed. Increasing the NaBH$_4$ greater than 10 mg the radiochemical yield was decreased to 75 ± 1.11% at 30 μg NaBH$_4$.

Effect of pH

As shown in Fig. 6, the radiochemical yield of ^{99m}Tc-curcumin derivative complex was affected by pH change. At pH 6, the radiochemical yield was relatively low (72.1 ± 1.2%). While, the maximum radiochemical yield (84 ± 1.4%) was observed at pH 8, where the curcumin combined all the reduced technetium. When the pH increased above 8, the percent radiochemical yield was slightly decreased.

Effect of curcumin derivative amount

The correlation between the radiochemical yield and the amount of curcumin derivative is shown in Fig. 7. The maximum radiochemical yield of ^{99m}Tc-curcumin derivative complex (84 ± 1.4%) was obtained at 150 μg curcumin. At low curcumin amount (50 μg), the radiochemical yield was low (74.6 ± 1.3%), where the amount of curcumin was insufficient for forming complex with the reduced technetium. The radiochemical yield was increased by increasing the curcumin amount where a maximum radiochemical yield of 84 ± 1.4% was obtained at 150 μg curcumin.

Effect of reaction time

The formation of ^{99m}Tc-curcumin derivative complex was started relatively slowly as the radiochemical yield was 70 ± 1.1% at 5 min reaction time as shown in Fig. 8. The maximum radiochemical yield of ^{99m}Tc-curcumin derivative complex was obtained at 30 min. Also, it remained constant up to 1 h.

## In vitro stability study

The radiochemical yield of the ^{99m}Tc-curcumin derivative complex showed stability for up to 6 h that confirmed its suitability for use during this time period.

Biology study

The distribution of ^{99m}Tc-curcumin derivative complex was studied in solid tumor-bearing mice (%ID/g) at 5, 15, 30, 60, 120 and 180 min post injection. The accumulation of the %ID/g of ^{99m}Tc-curcumin derivative in different body organs and fluids is illustrated in Fig. 9. It was clear that ^{99m}Tc-curcumin derivative didn't accumulate in a specific body organ and was mainly excreted via both the urinary tract and hepatobiliary pathways. The kidneys showed 15.72 ± 3.1% ID/g at 15 min and intestine showed 8.49 ± 1.8% ID/g at 30 min.

The tumor tissue (mouse right leg muscle)/normal tissue (mouse left leg muscle) ratio represents the key factor in the evaluation of the selectivity and sensitivity of

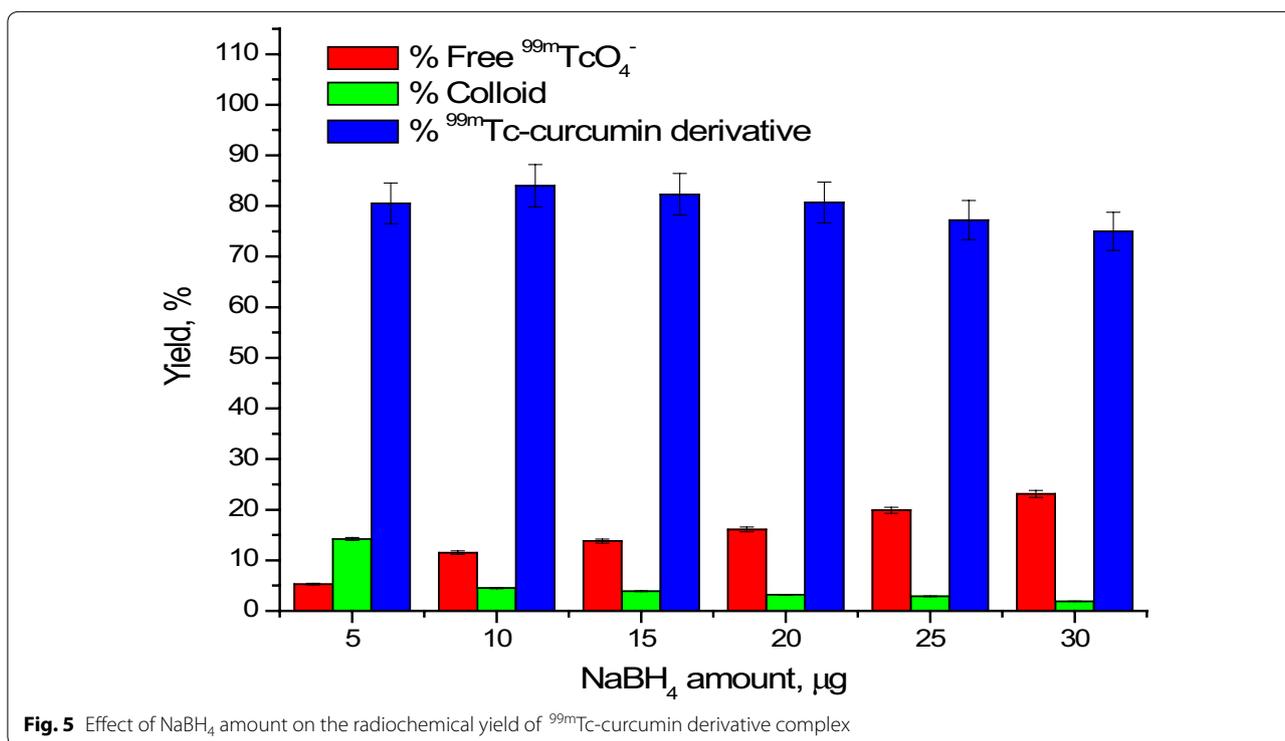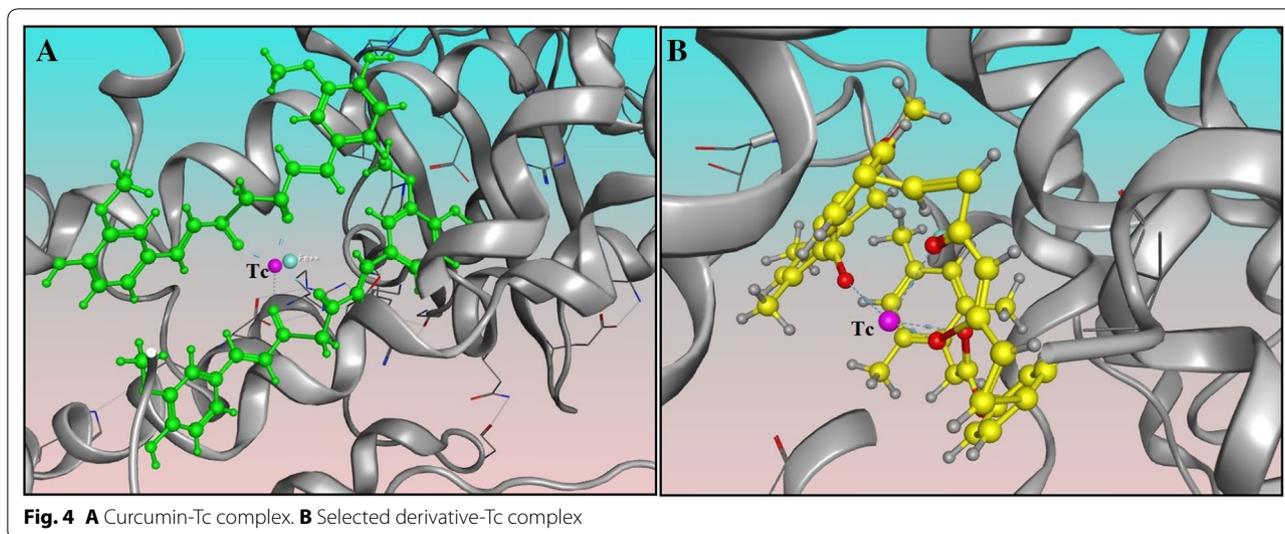Al-Wabli *et al. Chemistry Central Journal* (2016) 10:73

Page 7 of 12
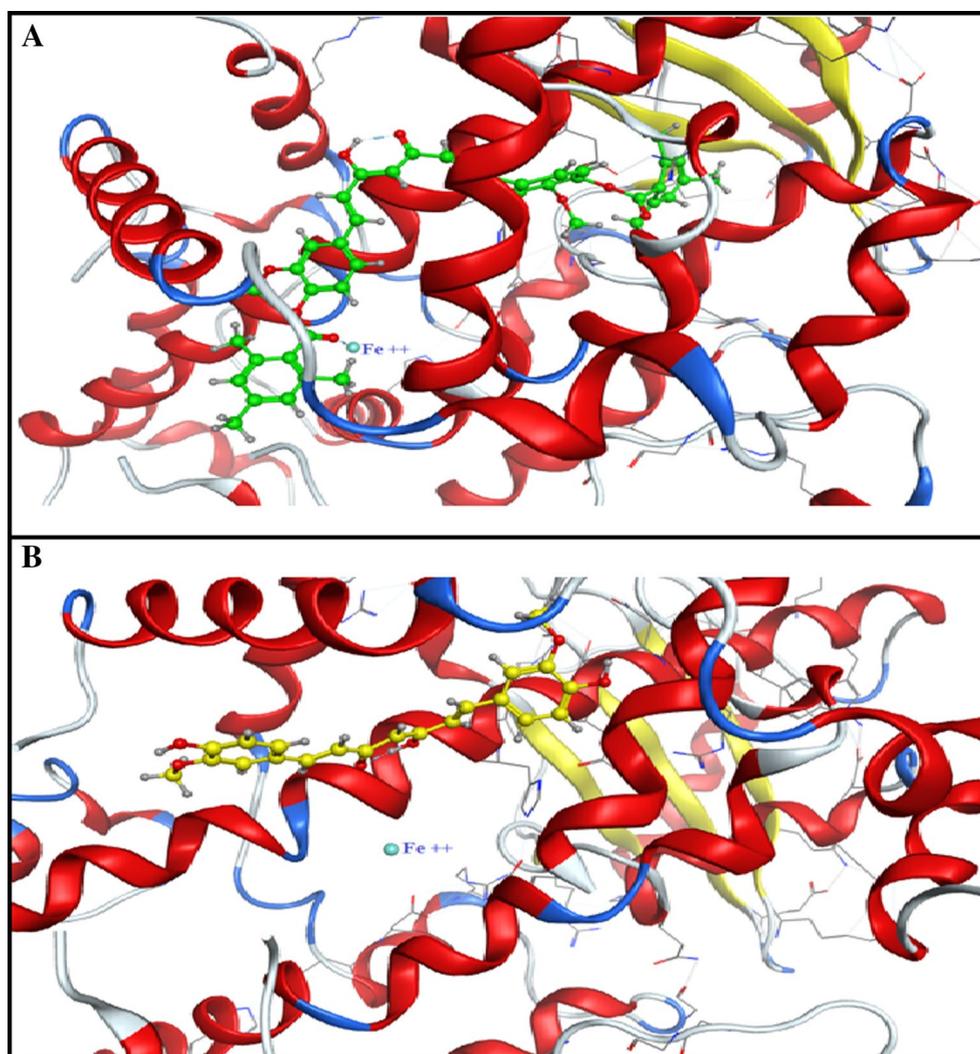
Fig. 3 Predicted binding mode of **A** new curcumin derivative in complex with 12-LOX active site. **B** Curcumin itself in the 12-LOX active site

[99m]Tc-curcumin derivative complex to solid tumor. As shown from Fig. 10, the T/NT ratio of ^{99m}Tc-curcumin derivative complex in solid tumor-bearing mice was ~1.7 at 15 min post injection and increases to its highest value of ~6.01 at 120 min post injection (p.i.) that clearly prove its high selectivity for the tumor cells.

This high preclinical T/NT ratio presents ^{99m}Tc-curcumin derivative complex as a non-invasive probe for solid tumor imaging when compared with many other agents such as: ^{99m}Tc-meropenem (3.5 at 1 h p.i.) [7], ^{99m}Tc-sunitinib (3 at 1 h p.i.) [3], ^{99m}Tc-PyDA (3 at 1 h

p.i.) [5], Radioiodinated anastrozole (4.7 ± 0.06 at 2 h p.i.) [41], radioiodinated epirubicin (5.2 ± 0.09 at 1 h p.i.) [42], ^{99m}Tc-BnAO-NI (2.59, 2 h) [43], [[99m]Tc(CO)$_3$(IDA–PEG3–CB)]$^-$ (3.45, 3 h) [44], [99m]Tc(CO)$_3$-labeled chlorambucil analog (3.2 at 3 h p.i.) [45], ^{99m}Tc-nitride-pyrazolo [1,5-*a*] pyrimidine (2.2 at 1 h p.i.) [46], ^{99m}Tc-DETA (2.47 at 4 h p.i.) [32], ^{99m}Tc-TETA (2.45 at 4 h p.i.) [32], ^{99m}Tc-TEPA (2.91 at 4 h p.i.) [32], ^{99m}Tc-citro-folate (4.3 at 4 h p.i.) [47] and ^{99m}Tc-gemcitabine (4.9 at 2 h p.i.) [48]. All of these present ^{99m}Tc-curcumin derivative complex as a promising solid tumor imaging agent.

Al-Wabli *et al. Chemistry Central Journal* (2016) 10:73

Page 8 of 12

Fig. 4 **A** Curcumin-Tc complex. **B** Selected derivative-Tc complex

**Fig. 5** Effect of NaBH$_4$ amount on the radiochemical yield of ^{99m}Tc-curcumin derivative complex

Conclusion

A promising curcumin derivative with high affinity 13.64 (pKi) and excellent free binding energy ($-$50.10 kcal/mol) was selected for chemical synthesis and radiolabelling to target P-12-LOX. It showed a high radiochemical yield of 84% and in vitro stability up to 6 h. Its high accumulation in solid tumor with target/non-target ratio >6 indicated that it could be an excellent radio-imaging agent that targets tumor cells via selectivity of P-12-LOX.

Al-Wabli *et al. Chemistry Central Journal* (2016) 10:73

Page 9 of 12
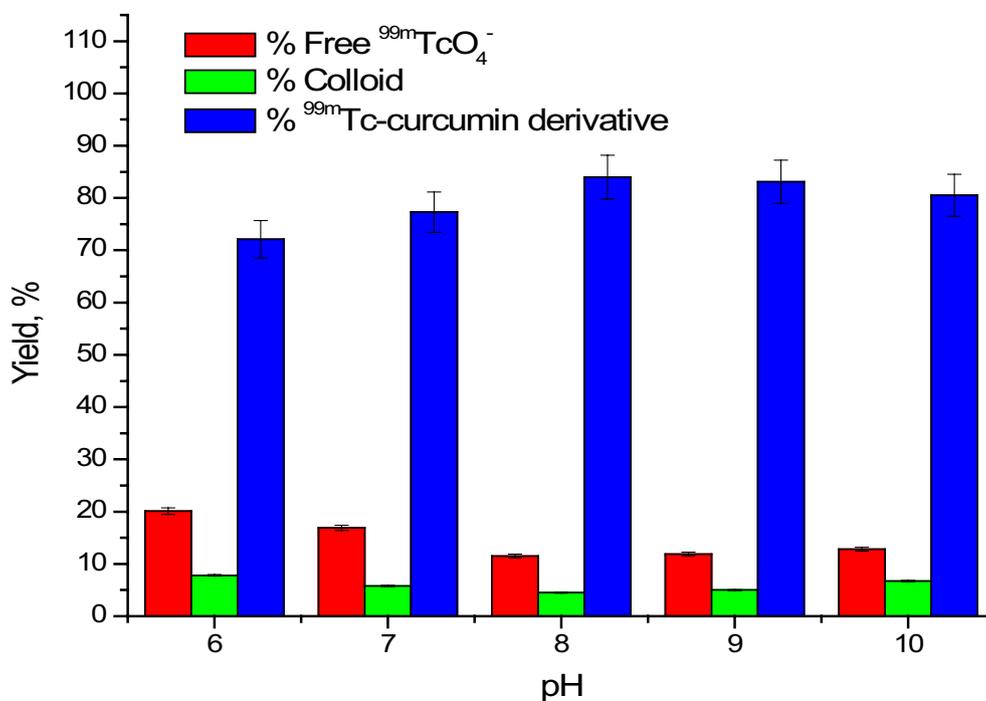
Fig. 6 Effect of pH on the radiochemical yield of ^{99m}Tc-curcumin derivative complex
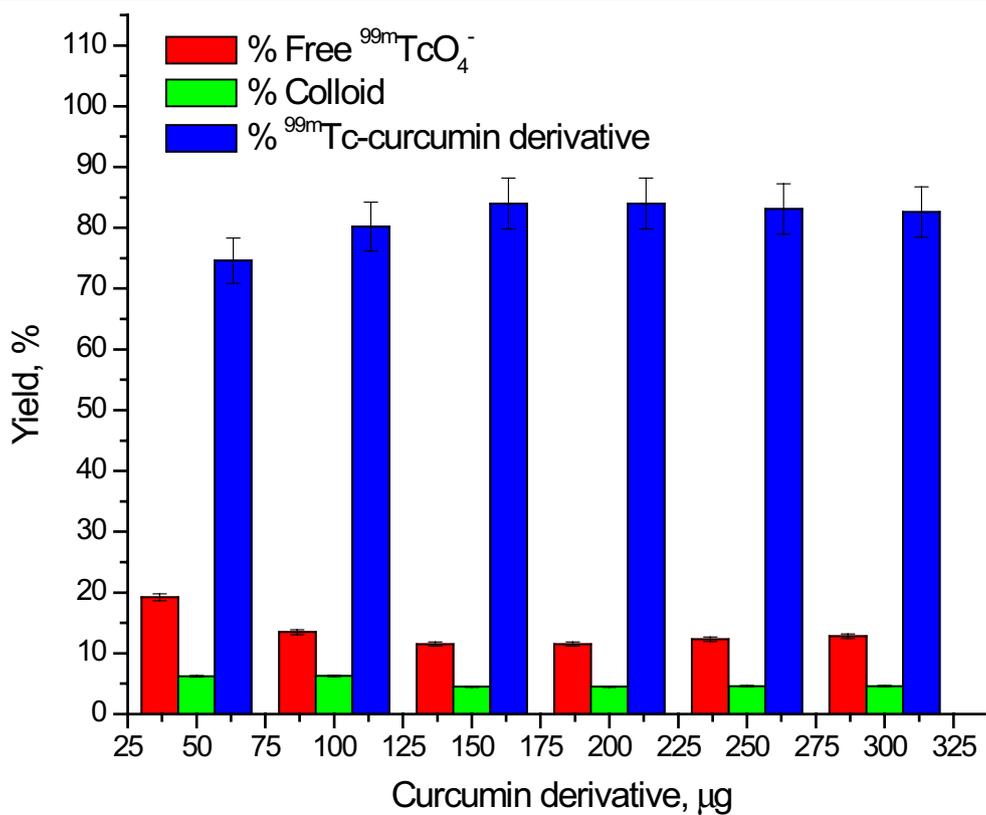
**Fig. 7** Correlation between curcumin derivative amount and the radiochemical yield of ^{99m}Tc-curcumin derivative complex

Al-Wabli *et al. Chemistry Central Journal* (2016) 10:73

Page 10 of 12
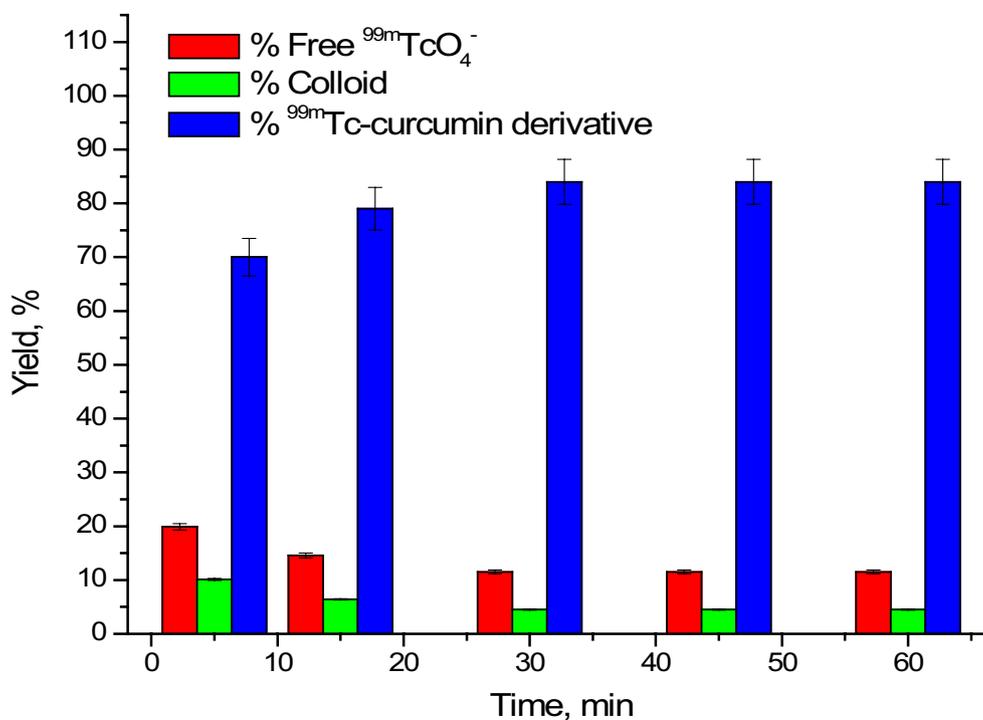
Fig. 8 Effect of reaction time on the radiochemical yield of ^{99m}Tc-curcumin derivative complex
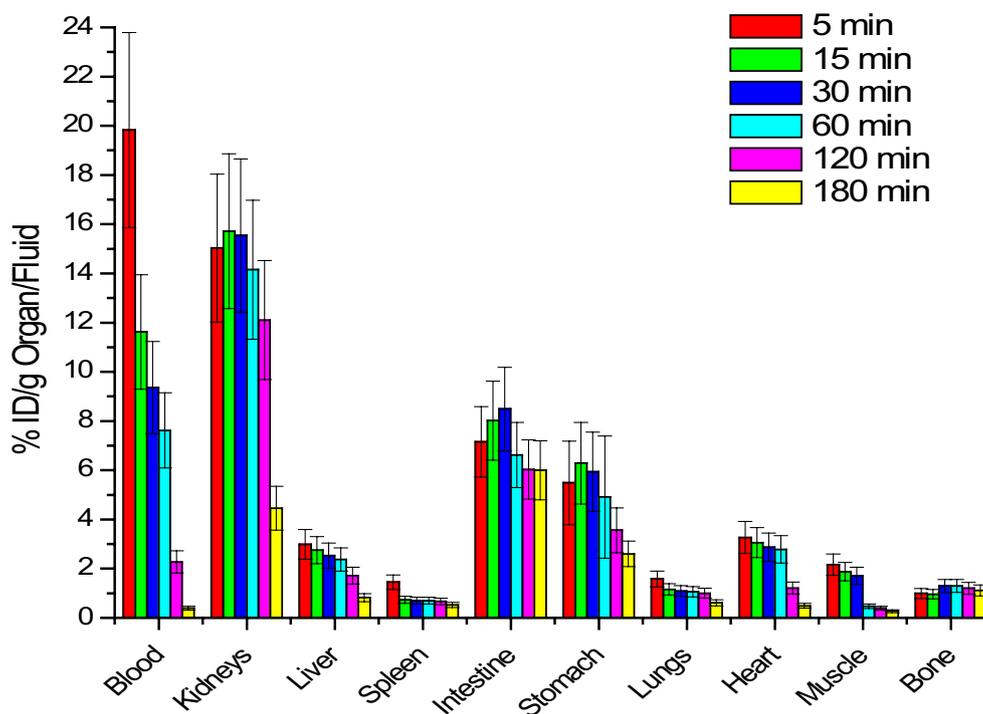
Fig. 9 Biological distribution of ^{99m}Tc-curcumin derivative complex in solid tumor bearing Albino mice

Al-Wabli *et al. Chemistry Central Journal* (2016) 10:73

Page 11 of 12
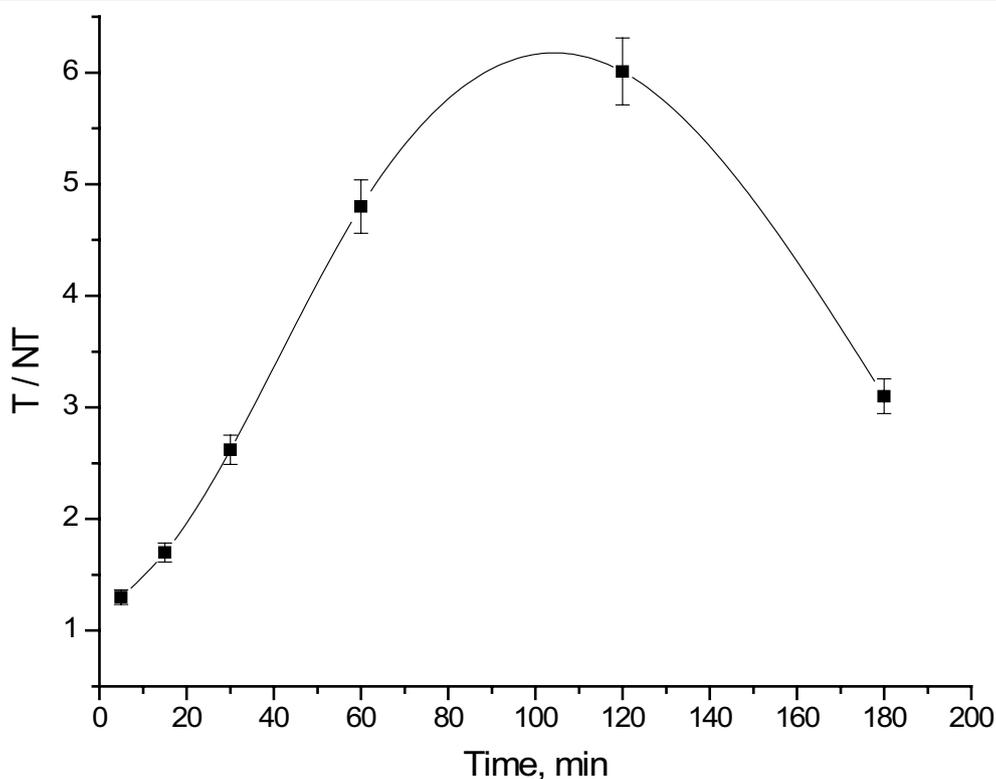
Fig. 10 T/NT of ^{99m}Tc-curcumin derivative complex in solid tumor bearing Albino mice

Author details

[1] Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia. [2] Radioactive Isotopes and Generator Department, Hot Labs Center, Egyptian Atomic Energy Authority, P.O. Box 13759, Cairo, Egypt. [3] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Helwan University, Ein Helwan, Cairo 11795, Egypt. [4] Labeled Compounds Department, Hot Labs Center, Egyptian Atomic Energy Authority, P.O. Box 13759, Cairo, Egypt.

References

1. Badenhorst J, Todd A, Lindsey L, Husband A (2015) Widening the scope for early cancer detection: identification of alarm symptoms by community pharmacies. Int J Clin Pharm 37(3):465–470
2. Ma L, Ji L, Yu Y, Wang J (2015) Novel molecular targets for diagnosis and treatment of hepatocellular carcinoma. Discov Med 19(102):7–14
3. Sakr TM, El-Safoury DM, Awad GAS, Motaleb MA (2013) Biodistribution of ^{99m}Tc-sunitinib as a potential radiotracer for tumor hypoxia imaging. J Label Compd Radiopharm 56:392–395
4. Ibrahim AB, Sakr TM, Khoweysa OMA, Motaleb MA, Abd El-Bary A, El-Kolaly MT (2014) Formulation and preclinical evaluation of ^{99m}Tc-gemcitabine as a novel radiopharmaceutical for solid tumor imaging. J Radioanal Nucl Chem 302(1):179–186
5. Sakr TM, Essa BM, El-Essawy FA, El Mohty AA (2014) Synthesis and biodistribution of ^{99m}Tc-PyDA as a potential marker for tumor hypoxia imaging. Radiochemistry 56(1):76–80
6. Dilworth JR, Parrott SJ (1998) The biomedical chemistry of technetium and rhenium. Chem Soc Rev 27:43–55
7. Sakr TM, Motaleb MA, Ibrahim IT (2012) ^{99m}Tc-meropenem as a potential SPECT imaging probe for tumor hypoxia. J Radioanal Nucl Chem 292(2):705–710
8. Du C, Ying H, Zhou J, Jiang J, Liu C, Chen J, Wang X, Hu C (2014) Prediction of the response to docetaxel-based chemotherapy for locoregionally advanced nasopharyngeal carcinoma: the role of double-phase (99m) Tc-MIBI SPECT/CT. Med Oncol 31:833–842
9. Chattopadhyay I, Biswas K, Bandyopadhyay U, Banerjee RK (2004) Turmeric and curcumin biological actions and medicinal applications. Curr Sci 87:44–50

Al-Wabli *et al. Chemistry Central Journal* (2016) 10:73

Page 12 of 12

10. Ammon HP, Wahl MA (1991) Pharmacology of *Curcuma longa*. Planta Med 57:1–7

11. Chen DY, Shien JH, Tiley L, Chiou SS, Wang SY, Chang TJ, Lee YJ, Chan KW, Hsu WL (2010) Curcumin inhibits influenza virus infection and haemagglutination activity. Food Chem 119:1346–1351

12. Lal J, Gupta SK, Thavaselvamc D, Agarwal DD (2013) Biological activity, design, synthesis and structure activity relationship of some novel derivatives of curcumin containing sulfonamides. Eur J Med Chem 64:579–588

13. Chen J, He ZM, Wang FL, Zhang ZS, Liu X, Zhai DD, Chen WC (2016) Curcumin and its promise as an anticancer drug: an analysis of its anticancer and antifungal effects in cancer and associated complications from invasive fungal infections. Eur J Pharmacol 772:33–42

14. Liu W, Li Y, Yue Y, Zhang K, Chen Q, Wang H, Lu Y, Huang M, Zheng X, Du Z (2015) Synthesis and biological evaluation of curcumin derivatives containing NSAIDs for their anti-inflammatory activity. Bioorg Med Chem Lett 25:3044–3051

15. Sribalan R, Kirubavathi M, Banuppriya G, Padmini V (2015) Synthesis and biological evaluation of new symmetric curcumin derivatives. Bioorg Med Chem Lett 25:4282–4286

16. Raghavan S, Manogaran P, Narasimha KKG, Kuppusami BK, Mariyappan P, Gopalakrishnan A, Venkatraman G (2015) Synthesis and anticancer activity of novel curcumin-quinolone hybrids. Bioorg Med Chem Lett 25:3601–3605

17. Chen QH, Yu K, Zhang X, Chen G, Hoover A, Leon F, Wang R, Subrahmanyam N, Mekuria EA, Rakotondraibe LHA (2015) A new class of hybrid anticancer agents inspired by the synergistic effects of curcumin and genistein: design, synthesis, and anti-proliferative evaluation. Bioorg Med Chem Lett 25:4553–4556

18. Wang R, Zhang X, Chen C, Chen G, Zhong Q, Zhang Q, Zheng S, Wang G, Chen QH (2016) Synthesis evaluation of 1, 7-diheteroarylhepta-1, 4, 6-trien-3-ones as curcumin-based anticancer agents. Eur J Med Chem 110:164–180

19. LoTempio MM, Veena MS, Steele HL, Ramamurthy B, Ramalingam TS, Cohen AN, Chakrabarti R, Srivatsan ES, Wang ES (2005) Curcumin suppresses growth of head and neck squamous cell carcinoma. Clin Cancer Res 11(19):6994–7002

20. Siwak DR, Shishodia S, Aggarwal BB, Kurzrock R (2005) Curcumin-induced antiproliferative and proapoptotic effects in melanoma cells are associated with suppression of I kappaB kinase and nuclear factor kappaB activity and are independent of the B-Raf/mitogen-activated/extracellular signal-regulated protein kinase pathway and the Akt pathway. Cancer 104(4):879–890

21. Magalska A, Sliwinska M, Szczepanowska J, Salvioli S, Franceschi C, Sikora E (2006) Resistance to apoptosis of HCW-2 cells can be overcome by curcumin-orvincristine-induced mitotic catastrophe. Int J Cancer 119:1811–1818

22. Mosieniak G, Adamowicz M, Alster O, Jaskowiak H, Szczepankiewicz AA, Wilczynski GM, Ciechomska IA, Sikora E (2012) Curcumin induces permanent growth arrest of human colon cancer cells: link between senescence and autophagy. Mech Ageing Dev 133:444–455

23. Sharm S, Gupta MK, Saxena AK, Bedi PM (2015) Triazole linked monocarbonyl curcumin-isatin bifunctional hybrids as novel antitublin agents: design, synthesis, biological evaluation and molecular modeling studies. Bioorg Med Chem 23:7165–7180

24. Sood A, Mathew R, Trachtman H (2001) Cytoprotective effect of curcumin in human proximal tubule epithelial cells exposed to shiga toxin. Biochem Biophys Res Commun 283(1):36–41

25. Essa BM, Sakr TM, Khedr MA, El-Essawy FA, El-Mohty AA (2015) 99mTc-Amitrole as a novel selective imaging probe for solid tumor: in silico and preclinical pharmacological study. Eur J Pharm Sci 76:102–109

26. Tang K, Cai Y, Joshi S, Tovar E, Tucker SC, Maddipati KR, Crissman JD, Repaskey WT, Honn KV (2015) Convergence of eicosanoid and integrin biology: 12-lipoxygenase seeks a partner. Mol Cancer 14:111

27. Gondek T, Szajewski M, Szefel J, Aleksandrowicz-Wrona E, Skrzypczak-Jankun E, Jankun J (2014) Evaluation of 12-lipoxygenase (12-LOX) and plasminogrn activator inhibitor 1 (PAI-1) as prognostic markers in prostate cancer. Biomed Res Int 2014:102478

28. Cuendet M, Pezzuto JM (2000) The role of cyclooxygenase and lipoxygenase in cancer chemoprevention. Drug Metabol Drug Interact. 17:109–157

29. Hong J, Bose M, Ju J, Ryu JH, Chen X, Sang S, Lee MJ, Yang CS (2004) Modulation of arachidonic acid metabolism by curcumin and related {beta}-diketone derivatives: effects on cytosolic phospholipase A2, cyclooxygenases, and 5-lipoxygenase. Carcinogenesis 25:1671–1679

30. Molecular Operating Environment (MOE) (2014) 9th edition. Montreal: Chemical Computing Group Inc

31. Al-Wabli R, AboulWafa OM, Youssef K (2012) Synthesis of curcumin and ethyl curcumin bioconjugates as potential antitumor agents. Med Chem Res 21:874–890

32. Sheeja KR, Kuttan G, Kuttan R (1997) Cytotoxic and antitumour activity of Berberin. Amala Res Bull 17:73–76

33. Abu-Zeid M, Hori H, Nagasawa H, Uto Y, Inayama S (2000) Studies of methyl 2-nitroimidazole-1-acetohydroxamate (KIN-804) 2: effect on certain antioxidant enzyme systems in mice bearing ehrlich ascites carcinoma. Biol Pharm Bull 23(2):195–198

34. Wan W, Yang M, Pan S, Yu C, Wu N (2008) [^{99m}Tc]polyamine analogs as potential tumor imaging agent. Drug Dev Res 69(8):520–525

35. Rhodes BA (1974) Considerations in the radiolabeling of albumin. Semin Nucl Med 4(3):281–293

36. Timar J, Raso E, Dome B, Li L, Grignon D, Nie D, Honn KV, Hagmann W (2000) Expression subcellular localization and putative function of platelet-type 12-lipoxygenase in human prostate cancer cell lines of different metastatic potential. Int J Cancer 87:37–43

37. Eling TE, Glasgow WC (1994) Cellular proliferation and lipid metabolism: importance of lipoxygenases in modulating epidermal growth factor dependent mitogenesis. Cancer Metastasis Rev 13:397–410

38. Honn KV, Timar J, Rozhin J, Bazaz R, Sameni M, Ziegler G, Sloane BFA (1994) A lipoxygenase metabolite, 12-(S)- HETE, stimulates protein kinase C-mediated release of cathepsin B from malignant cells. Exp Cell Res 214:120–130

39. Connolly JM, Rose DP (1998) Enhanced angiogenesis and growth of 12- lipoxygenase gene-transfected MCF-7 human breast cancer cells in athymic nude mice. Cancer Lett 132:107–112

40. Jankun J, Aleem AM, Malgorzewicz M, Szkudlarek M, Zavodszky MI, Dewitti DL, Feig M, Selman SH, Skrzypczak-Jankun E (2006) Synthetic curcuminoids modulate the arachidonic acid metabolism of human platelet 12-lipoxygenase and reduce sprout formation of human endothelial cells. Mol Cancer Ther 5(5):1371–1382

41. Badr AB, Sakr TM, Khoweysa OMA, Motaleb MA, Abd El-Bary A, El-Kolaly MT (2015) Radioiodinated anastrozole and epirubicin as potential targeting radiopharmaceuticals for solid tumor imaging. J Radioanal Nucl Chem 303(1):967–975

42. Hsia CC, Huang FL, Hung GU, Shen LH, Chen CL, Wang HE (2010) The biological characterization of ^{99m}Tc-BnAO-NI as a SPECT probe for imaging hypoxia in a sarcoma-bearing mouse model. Appl Radiat Isot 69(4):649–655

43. Wang J, Yang J, Yan Z, Duan X, Tan C, Shen Y, Wu W (2011) Synthesis and preliminary biological evaluation of [^{99m}Tc(CO)$_3$(IDA–PEG3–CB)]$^-$ for tumor imaging. J Radioanal Nucl Chem 287(2):465–469

44. Satpati D, Korde A, Venkatesh M, Banerjee S (2009) Preparation and bioevaluation of a ^{99m}Tc-labeled chlorambucil analog as a tumor targeting agent. Appl Radiat Isot 67(9):1644–1649

45. Ding R, He Y, Xu J, Liu H, Wang X, Feng M, Qi C, Zhang J, Peng C (2012) Preparation and bioevaluation of ^{99m}Tc nitrido radiopharmaceuticals with pyrazolo[1,5-a]pyrimidine as tumor imaging agents. Med Chem Res 21:523–530

46. Altiparmak B, Lambrecht FY, Bayrak E, Durkan K (2010) Design and synthesis of ^{99m}Tc-citro-folate for use as a tumor targeted radiopharmaceuticals. Int J Pharm 400(1–2):8–14

47. Machac J, Krynyckyi B, Kim C (2002) Peptide antibody imaging in lung cancer. Semin Nucl Med 32(4):276–292

48. Badr AB, Sakr TM, Khoweysa MA, Motaleb OMA, Abd El-Bary A, El-Kolaly MT (2014) Formulation and preclinical evaluation of ^{99m}Tc-gemcitabine as a novel radiopharmaceutical for solid tumor imaging. J Radioanal Nucl Chem 302(1):179–186