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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Modification of carboxylated multiwall nanotubes with benzotriazole derivatives and study of their anticancer activities

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Abstract In vitro and in vivo results indicate that functionalized carbon nanotubes are promising for the development of unique anticancer drugs. Since therapeutic and pharmacologic agents could functionalize the structure of carbon nanotubes, we report for the first time three-component condensation reactions consisting of multiwalled carbon nanotube–COCl (MWNTCOCl), diazonium sulfonated salt and phenol derivatives to produce novel anticancer agents. The functionalized carboxylated multiwall nanotubes were then characterized by FT-IR, Raman, SEM, TEM, and solubility test. The functionalized MWNTs exhibited good aqueous solubility. Furthermore, the cytotoxic activity of the MWNT–COOH (**A**) as well as its functionalized carboxylated multiwall nanotubes (MWNT-CO-2-(1-hydroxynaphthalen-2-yl)-2*H*-benzo[*d*][1,2,3]triazole-5-sulfonic acid (**B**), MWNT-CO-2-(2-hydroxy-1-nitrosonaphthalen-3-yl)-2*H*-benzo[*d*][1,2,3]triazole-5-sulfonic acid (**C**) and MWNT-CO-2-(2-hydroxy-5-nitrophenyl)-2*H*-benzo[*d*][1,2,3]triazole-5-sulfonic acid (**D**)) was tested against human gastric cancer MKN45 and human Colon cancer SW742. In vitro cytotoxicity studies represented a significant enhancement in the cytotoxic capability of f-MWNTs, suggesting these drugs improve drug antitumor activity. Therefore, the highly versatile functionalized carboxylated multiwall nanotubes could potentially be considered as anticancer candidates.

Keywords Carboxylated multiwall nanotubes · Functionalization · Characterization · Cell line · Antitumor activity

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Introduction

Cancer is a complex disease occurring as a result of a progressive accumulation of genetic aberrations and epigenetic changes that enable escape from normal cellular and environmental controls (Srinivas and Das, 2004). Gastric and colorectal cancers are among the most common malignant cancers with poor prognoses and high mortality rates worldwide (Xiao *et al.*, 2011; Goldberg *et al.*, 2004). Despite recent advances in targeted therapy and improved understanding of the biology and development of the malignancy, progress in the treatment of these cancers has been limited. Chemotherapy is an important therapeutic approach in the treatment of a variety of cancers, but its clinical applications are limited because of severe side effects (Xiao *et al.*, 2011; Safari *et al.*, 2013). Recent studies have shown that heterocyclic compounds containing nitrogen atoms have very promising biological activities such as antibacterial, antiviral and antifungal activity (Swarnkar *et al.*, 2007; Pandey and Negi, 2003; Chaudhary *et al.*, 2010). Benzotriazoles have become the most rapidly expanding group of antifungal and antiviral compounds (Wu *et al.*, 2006; Handratta *et al.*, 2005). More recently, biotechnological applications of CNT are being anticipated in a variety of fields ranging from microfluidics to bioinformatics (Kostarelos *et al.*, 2005). In vitro antitumor

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properties of MWNTs have been investigated against a wide panel of tumor cell lines of human origin and found to be as effective as, or even better than standard anticancer drugs (Iijima, 1991; Bachtold *et al.*, 2000; McEuen *et al.*, 2002; Azizian *et al.*, 2010). Very recent studies have explored the possibilities for the use of CNT in tissue regeneration after damage of the spinal cord, brain or bone tissues and also in drug delivery system (Zhang *et al.*, 2006; Kam *et al.*, 2005; Kostarelos *et al.*, 2005). The chemical modification of carbon nanotubes has received much recent attention in recent decades (Chen *et al.*, 1998; Hamon *et al.*, 1999). Chemical functionalization is a common technique to enhance dispersion stability and biocompatibility of CNTs (Georgakilas *et al.*, 2002). Moreover, these functionalized CNTs are capable of forming chemical covalent or non-covalent bonds with different pharmacologic agents, which makes them ideal candidates for drug delivery systems. The covalent side wall modifications of nanotubes have been well described in several review papers (Gannon, 2007; Liu *et al.*, 2007). Keeping all these factors in mind, we aimed to attach the new synthesized benzotriazole derivatives to the surface of carboxylated multiwall nanotubes, to take advantage of the MWNTs outstanding mechanical properties and to screen the final products for their antitumor activity.

Results and discussion

Solubility of functionalized MWNTs

Functionalized MWNTs were prepared using esterification of MWNT to obtain a homogenous distribution of MWCNTs in solvent. Controlling the degree of dispersion of nanotubes is difficult because of the strong intra-molecular forces that exist between carbon nanotubes which are responsible for the formation of 10–50 nm nanotube bundles. The bundles are difficult to exfoliate since the nanotubes may be hundreds to thousands of nano meters long (Szymczyk *et al.*, 2011). Noticeably, before any biology-related application can even be envisaged, the aqueous solubility of carbon nanotubes has to be resolved. Herein, the existence of extremely large hydrophilic groups containing sulfonic acid in modified CNTs gives good dispersion in water for almost a month, as shown in Fig 1.

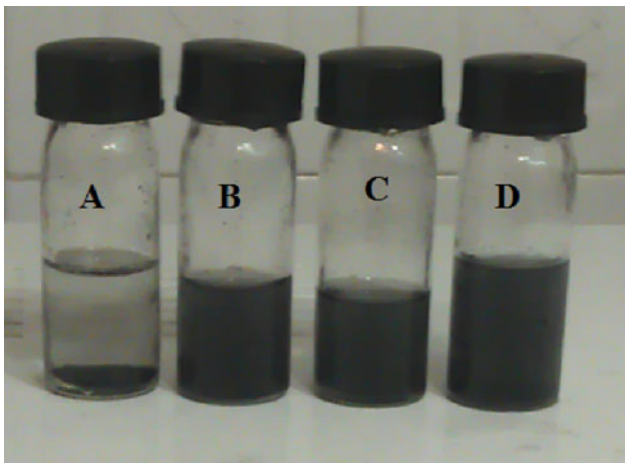


Fig. 1 Solubility test of MWNT–COOH (**A**) and functionalized MWNTs (**B**–**D**)

Spectroscopic studies

Infrared spectroscopy

FT-IR bands related to different groups have been assigned by comparison of the FT-IR spectra of the MWNT–COOH with the functionalized MWNTs (Fig. 2). The two bands indicative of C=O and C–O stretching vibrations give valuable information about the behavior of the attached functional groups to the MWCNTs. In spectrum A, the characteristic absorption peaks of OH, C=O, and C–O appear at 3,733, 1,704 and 1,204 cm^{-1}, respectively. The peak at 1,704 cm^{-1} assignable to a ν (C=O) in MWNT–COOH shifts to higher frequencies of 1,797, 1,719, and 1,718 cm^{-1} in functionalized MWCNTs (**B**–**D**), a result of ester linkage formation. In the spectra of functionalized MWCNTs (**B**–**D**), the bands at 1,111, 1,199, and 1,084 cm^{-1} apparently correspond to the stretching modes of C–O, respectively. The new band at 1,512 cm^{-1} in the spectrum of functionalized MWCNT (**C**) is attributed to the nitroso group. The absorption bands at 1,489 and 1,262 cm^{-1} which are seen in the spectrum of functionalized MWNT (**D**) can be assigned to the nitro group. The appearance of two bands in the range of 2,800–2,900 cm^{-1} are attributed to the C–C sp^3 modes in the functionalized products. In addition, the S–O stretching vibrations at around 1,100–1,250 and 600–690 cm^{-1} are observed in the IR spectra of all functionalized MWNTs (Hu *et al.*, 2009; Holzinger and coworkers, 2002; Zaragoza-Contreras *et al.*, 2009). Therefore, FT-IR spectra confirmed that MWNT–COOH had been successfully modified by esterification. The characteristic IR frequencies (cm^{-1}) of the studied compounds (**A**–**D**) are listed in Table 1.

Raman spectroscopy

Raman spectroscopy is a powerful technique used to characterize structural changes of carbon nanotubes, specifically changes owing to significant sidewall functionalization.

As shown in Fig. 3, the D and G bands of the MWNT at around 1,300 and 1,500 cm^{-1}, attributed to defects,

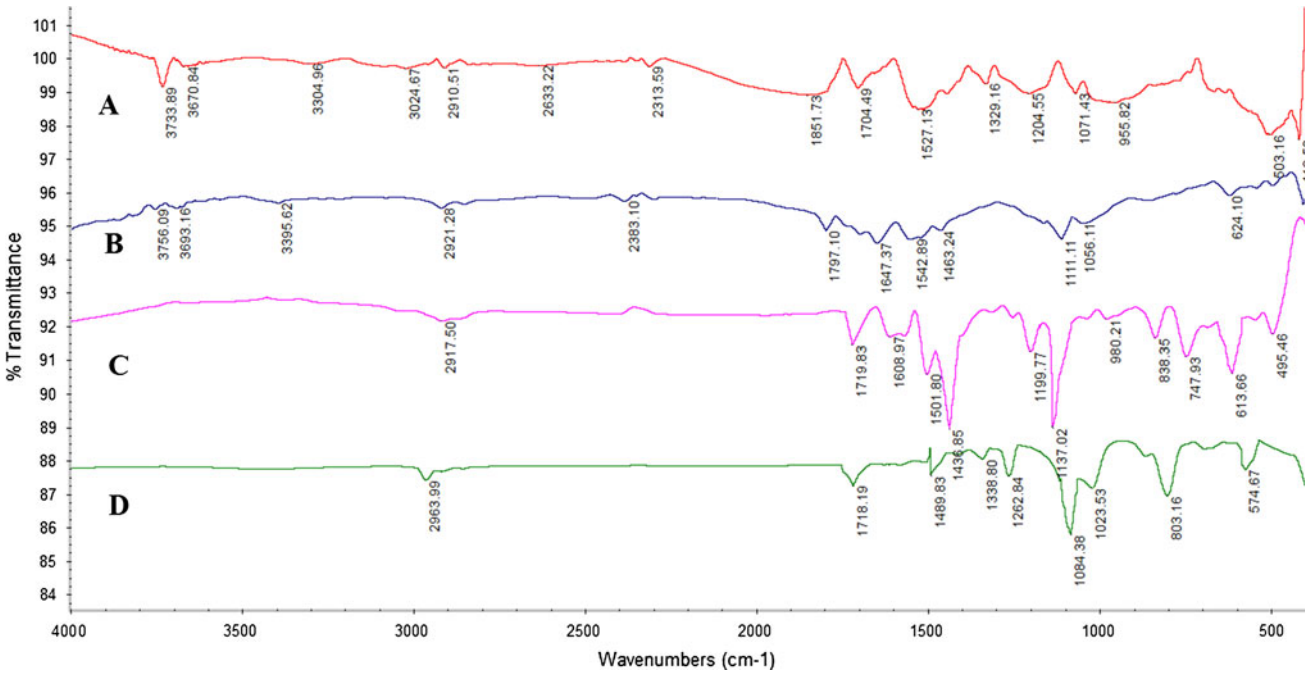


Fig. 2 FT-IR spectra of MWNT–COOH (*a*) and functionalized MWNTs (*b–d*)

Table 1 Diagnostic IR bands of the MWNT–COOH and functionalized MWNTs

Compound	ν (C=O)	ν (C–O)	ν (OH)	ν (NO)	ν (NO_2)	ν (S–O)
MWNT–COOH **A**	1,704	1,204	3,733	_	_	–
Compound **B**	1,797	1,111	_	–	–	1,056,624
Compound **C**	1,719	1,199	_	1,512	–	1,137,613
Compound **D**	1,718	1,084	_	–	14,891,262	1,262,693

disorder-induced peaks, and tangential-mode peaks, can be clearly observed for both MWNT–COOH and functionalized MWNTs. More importantly, D- to G-band intensity ratios (ID/IG) for functionalized MWNTs (**B–D**) are around 1.66, 1.50, 1.62, respectively, which are greater than that for MWNT–COOH (0.50). The increase in intensity of the defect mode at 1,300 cm^{-1} was related to sp^3 hybridization of carbon (Jorio *et al.*, 2004; Dresselhaus *et al.*, 2005). This band is barely observable in MWNT–COOH but is clearly detectable after functionalization, indicating an increase in defects along the nanotube.

SEM and TEM characterization of the modified MWNTs

More direct evidence for the functionalization of MWNTs is manifested by scanning electron microscopy (SEM) images (Zheng *et al.*, 2003). Figure 4 shows SEM micrographs of fracture surfaces of **A** (MWNT–COOH) and **B**, **C**, and **D** (modified nanotubes). The images clearly indicate that compound **A** has a smooth surface but functionalized products have a rough surface. The changes in the morphology of products are remarkable.

Transmission electron microscopy (TEM) is the most powerful tool that reveals the diameters of the single-wall and multiwall CNTs, the number of walls and the distance between the walls. Moreover, the coating on the surface of nanotubes can be observed by the aid of TEM. The TEM image of **A** shows that MWNT–COOH exist as bundles or ropes of MWNT. These bundles dissociate in the modified nanotubes, indicating functionalization prevents MWNT from aggregation and enhances the diameter (Najafi *et al.*, 2006) (Fig. 5).

Pharmacology: antiproliferative activity in vitro

The in vitro cytotoxicities of MWNT–COOH as well as corresponding functionalized MWNTs against tumor cell lines of human gastric cancer MKN45 and human colon cancer (SW742) were determined using the MTT microculture colorimetric assay. This study was designed to evaluate the antitumor activity of MWNT–COOH and

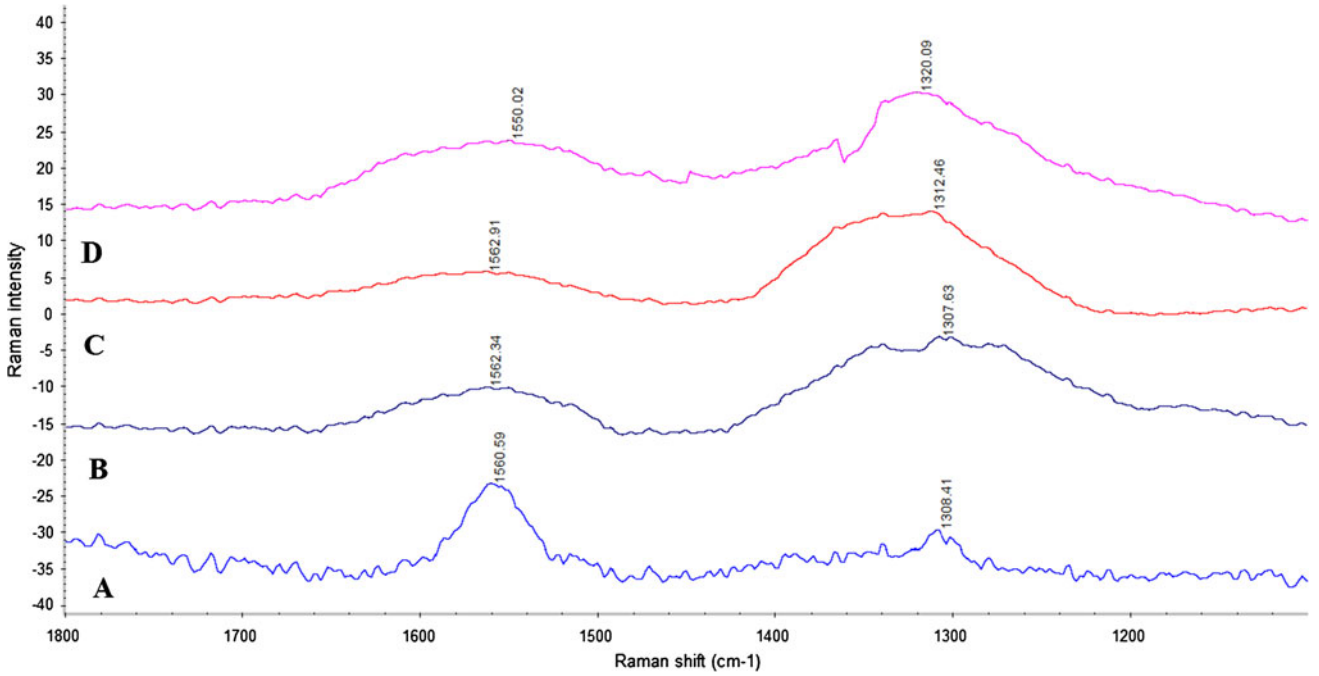


Fig. 3 Raman spectra of MWNT–COOH (*a*) and functionalized MWNTs (*b–d*)

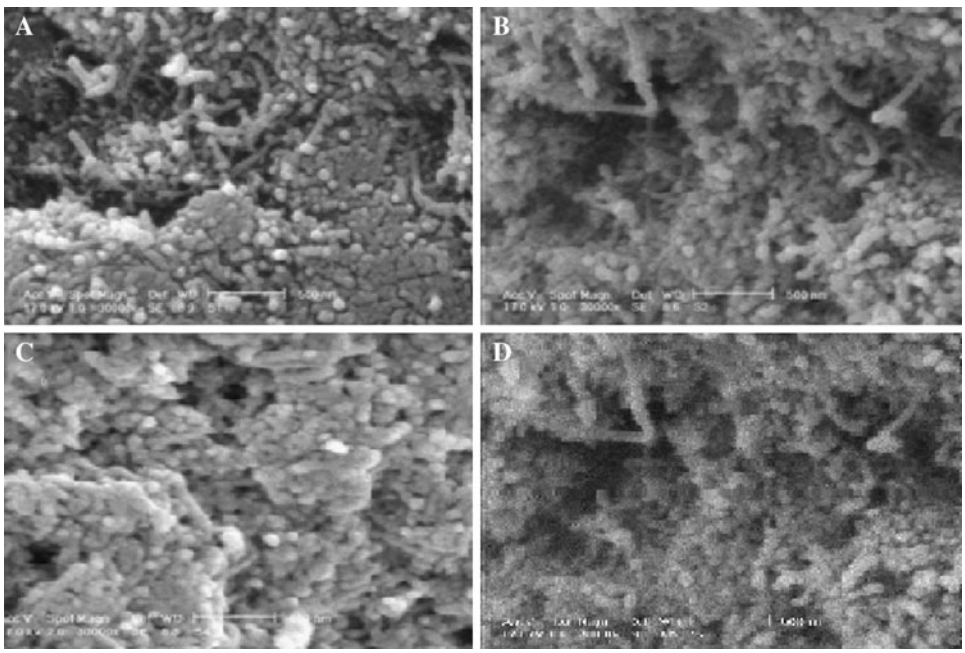


Fig. 4 Scanning electron microscopy (SEM) images for MWNT–COOH (**a**) and functionalized MWNTs (**b–d**)

modified MWNTs to observe the effect of different substituents attached to the surface of MWNTs on tumor cell lines. An improved activity was observed for the functionalized MWNTs of the present investigation. The results of the in vitro cytotoxicity tests are expressed as IC_{50}, the concentration of compound (μg/mL) that inhibits proliferation rate of the tumor cells by 50 % as compared to control untreated cells (Table 2). Furthermore, the screening results are compared with the standard drugs cisplatin and doxorubicin that are in current clinical use as antitumour agents.

From the derived IC_{50} values, it is concluded that the cytotoxic effect of the MWNT–COOH and modified MWNTs on tested cell lines was strictly concentration dependent (Fig. 6).

Estimates based on IC_{50} values shows that MWNT–COOH is less cytotoxic compared to modified MWNTs **(B–D)** and more cytotoxic than cisplatin and doxorubicin

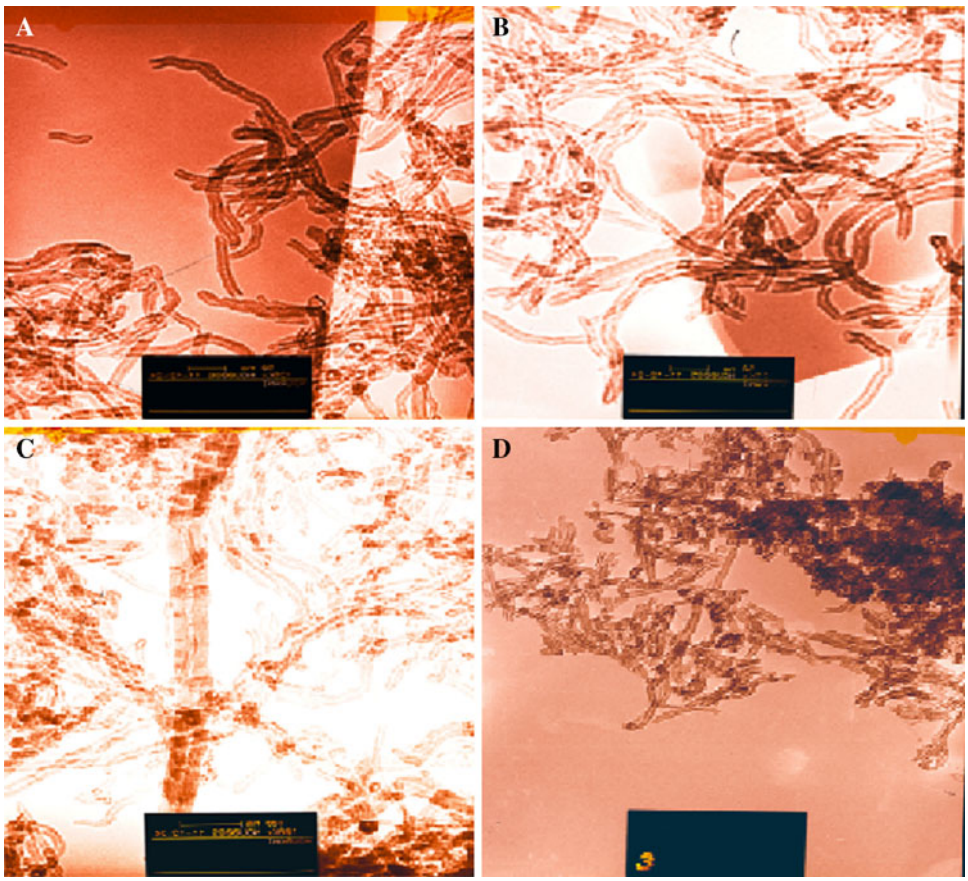


Fig. 5 Transmission electron microscopy (TEM) of MWNT–COOH (**a**) and functionalized MWNTs (**b–d**)

Table 2 IC_{50} (μg/mL) for the 48 h of action of the studied compounds, cisplatin and doxorubicin on MKN-45 and SW742 determined by MTT test

Compound	MKN-45	SW742
MWNT–COOH **A**	0.026	0.002
Compound **B**	0.005	0.001
Compound **C**	0.06	0.001
Compound **D**	0.002	0.001
Cisplatin	5.11	_
Doxorubicin	_	0.45

against the investigated cell lines. Compounds **(A–D)** present lower IC_{50} values than those of cisplatin and doxorubicin, which indicates their higher activity against the tumoral cell lines evaluated. The IC_{50} values of the compounds **B**, **C**, and **D** against SW742 were in a similar range, and were 450 times better than that of the antitumor drug doxorubicin.

Compounds **B** and **D** exhibited higher activity (0.005 and 0.002 μg/mL) than compound **C** (0.06 μg/mL) against MKN-45 cell line.

From those results one can envisage that modification of carboxylated multiwall nanotube led to a significant decrease of the final cytotoxic activity. Further study on the antitumor effects of these compounds is ongoing based on these very positive preliminary results.

Experimental section

Material and physical measurement

All chemicals and reagents were purchased from Merck and Fluka and used without further purification. MWNT–COOH (95 % purity, 20–30 nm; Netrino Co. Ltd) were purchased and used as received. The FT-IR spectra were recorded on a Nexus 870 FT-IR spectrometer using KBr pellets (Thermo Nicolet, Madison, WI). FT-Raman spectra were recorded on 960 ES spectrometer (Thermo Nicolet). SEM was used to study the morphology of the MWNTs and carried out on the XL30 electron microscope (Philips, Amsterdam, Netherlands). TEM images were recorded using JEOL 6400. Elemental analyses of carbon, hydrogen, and nitrogen were performed using a Series II 2400 (Perkin Elmer, Waltham, MA).

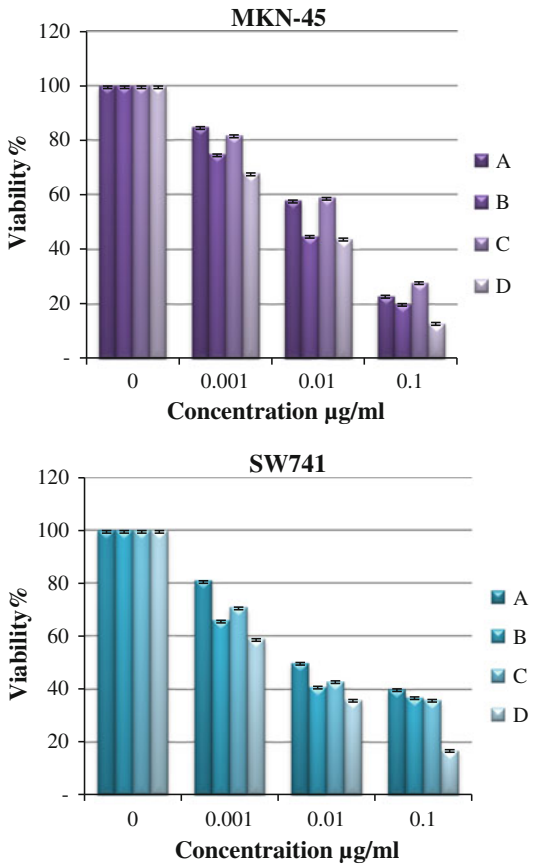


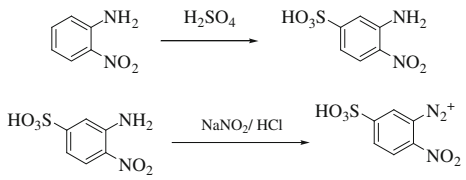
Fig. 6 Cytotoxicity graphs from typical MTT assay showing the effect of MWNT–COOH (**a**) and functionalized MWNTs (**b–d**) on the viability of MKN-45 and SW742

Preparation of 3-amino-4-nitrobenzenesulfonic acid and its diazonium salt

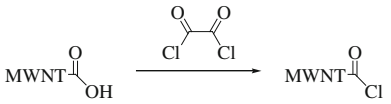
The reaction of 2-nitrobenzenamine with concentrated sulfuric acid for 3 h led to the formation of 3-amino-4-nitrobenzenesulfonic acid (Riggs *et al.*, 2000; Fu *et al.*, 2001; Sun *et al.*, 2002; Czerw *et al.*, 2001). Diazonium salt was then prepared by adding HCl/$NaNO_2$ to 3-amino-4-nitrobenzenesulfonic acid at 0 °C, as shown in Scheme 1.

Preparation of MWCNT–COCl

MWNT–COOH (20 mg) were sonicated in 30 mL of *N*,*N*-dimethylformamide (DMF) in three separate pots for 45 min to give a homogeneous suspension. Oxalyl chloride (1 mL) was added dropwise to any MWNT suspension at 0 °C under a constant flow of nitrogen. The mixture was stirred at 0 °C for 2 h and followed at room temperature for the same duration. Finally, the temperature was increased to 70 °C, and the mixture was stirred overnight to remove excess oxalyl chloride (Scheme. 2).



Scheme 1 Sulfonation of 2-nitrobenzenamine and preparation of diazonium salt



Scheme 2 Preparation of MWCNT–COCl

Esterification of carboxylated multiwall nanotubes

The mixture of diazonium salt (1 mmol) and phenol derivatives (1 mmol) dissolved in DMF was added to the MWNTCOCl suspension followed by stirring for 15 h at 90 °C. 20 % w/w aqueous solution of NaOH was then added dropwise to the reaction mixture within 1 h in the presence of catalyst (Zn powder). The mixture was stirred for 6 h at 95 °C. After cooling to room temperature, the mixture was filtered and washed thoroughly with tetrahydrofuran. Subsequently, the black solid was vacuum-dried at room temperature for 5 h (Scheme. 3).

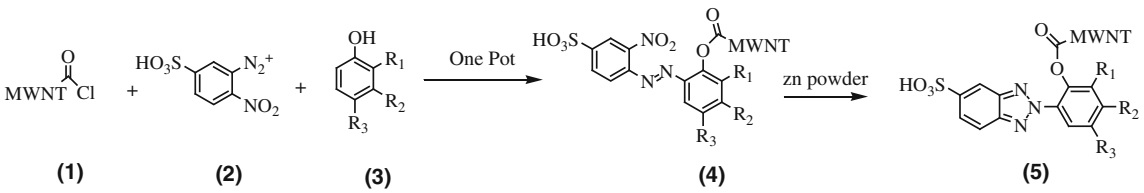
In vitro studies

Preparation of drug solutions

Stock solutions of the studied compounds were prepared in dimethyl sulfoxide (DMSO, Sigma Aldrich) at a concentration of 1,000 µg/mL, sterilized by filtration through Millipore filter, 0.22 lm, before use, and diluted by cell culture medium to various working concentration. Nutrient medium was RPMI-1640 (Gibco BRL, Scotland) supplemented with 10 % fetal bovine serum (FBS-Gibco BRL/Scotland). MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide was dissolved (5 mg/mL) in phosphate buffer saline pH 7.2, and filtered through Millipore filter, 0.22 lm, before use.

Cell line and cell culture

The MKN-45 (human gastric cancer) cells (NCBI C115615, National Cell Bank of Iran) and SW742 (human colon cancer) cells (NCBI C146, National Cell Bank of Iran), were obtained from Pasteur Institute of Iran. Cells were cultured in RPMI-1640 medium supplemented with 10 % heat inactivated fetal calf serum (FCS- Gibco BRL,



5	B	C	D
R_1	phenyl	NO	H
R_2		phenyl	H
R_3	H		NO_2

Scheme 3 Preparation of anti cancer drug candidate by esterification of carboxylated multiwall nanotubes

Scotland) 2 mM L-glutamine (Gibco BRL, Scotland) and antibiotics, including streptomycin (100 µg/mL) and penicillin (100 IU/mL) (Sigma, USA) and incubated at 37 °C in a humidified 5 % CO_2 atmosphere.

Trypan blue exclusion

The loss of membrane integrity, as a morphological characteristic for cell death, was assayed by trypan blue dye exclusion (Moldeus *et al.*, 1978). The alive cells were estimated through a hemocytometer and phase-contrast microscopy.

Cell sensitivity analysis

MKN-45 and SW742 cells were seeded (15,000 cells per well) into 96-wells flat-bottom microtiter plates and incubated for 4 h prior to the addition of filtered 3 different concentrations of the studied compounds. Final concentrations achieved in treated wells were 0.001, 0.01, and 0.1 µg/mL. Each concentration was tested in quadruplicate on each cell line. The final concentrations (<0.1 %) of DMSO, were non-toxic to the cells. Each assay included a blank containing complete medium without cells. The incubation time was 48 h, during the period the control cells showed exponential growth.

Determination of target cell survival

Cell survival was determined by MTT test according to the method of (Mosmann, 1983) and modified by (Ohno and Abe, 1991), which measures the reduction of the yellow tetrazolium salt MTT (3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide; SIGMA) into a purple formazan crystal, mainly by the activity of the mitochondrial enzymes, cytochrome oxidase and succinate dehydrogenase. In brief, cells were incubated for 48 h and then, 20 µL of MTT solution (5 mg/mL) in phosphate buffer saline (1/10 of total volume in a well) was added to wells. Samples were incubated for further 4 h at 37 °C in humidified atmosphere with 5 % CO_2. Supernatants were removed and 100 µL of DMSO was added to the plate as a solvent to each well. The plate was shaken for 15 min by a shaker incubator to dissolve the formazan crystals.

The optical density (OD) value was defined as the absorbance of each individual well, minus the blank value (blank is the mean optical density of the control cells). Finally, the absorbance at 570 nm (test wavelength) and with a reference filter of 630 nm was measured using an ELISA microplate reader (Stat Fax-2100, USA). All experiments were performed three times, and the percentage of cytotoxicity was calculated according to following formula:

$$\%\ \text{Cytotoxicity} = 1 - \frac{\text{Mean absorbance of toxicant treated cells}}{\text{Mean absorbance of negative control}} \times 100$$

$$\%\ \text{Viability} = 100 - \%\ \text{Cytotoxicity}$$

Data analysis

After subtracting the solvent toxicity, the concentration giving 50 % inhibition (IC_{50}) was determined for the test samples by nonlinear regression analysis of curves. Graph

Pad Prism version 4.00 was used to calculate IC_{50}. Mean difference among groups was calculated by paired t test, one-way and repeated measures ANOVA ($p < 0.05$).

Conclusions and outlook

This study is the first of its kind to present esterification of carboxylated multiwall nanotubes with benzotriazole derivatives to modify MWNT–COOH for biomedical studies. The functionalized MWNTs have been characterized by FT-IR and Raman spectroscopies, SEM and TEM. The in vitro cytotoxic activity of the studied compounds has been evaluated against human cancer cell lines: MKN-45 and SW742. The screening results have shown that modified MWNTs have better anticancer activity than MWNT–COOH. The most outstanding results were obtained from the activity of compounds **B**, **C**, and **D** against SW742 cell line, which exhibited much higher cytotoxicity than doxorubicin. Our findings are of considerable value for selecting the most appropriate material and functionalization protocol for in vitro applications. Therefore, these functionalized multiwall carbon nanotubes are envisaged to become very promising candidates for further in vivo tests which will be carried out in the near future.

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References

Azizian J, Tahermansouri H, Biazar E, Heidari S, Chobfrosh Khoei D (2010) Functionalization of carboxylated multiwall nanotubes with imidazole derivatives and their toxicity investigations. Int J Nanomedicine 5:907–914

Bachtold A, Fuhrer MS, Plyasunov S (2000) Scanned probe microscopy of electronic transport in carbon nanotubes. Phys Rev Lett 84:6082–6608

Chaudhary M, Pareek D, Ojha KG, Pareek A (2010) Synthesis and antibacterial activity of 1-(2-Diazo- 6-ethoxybenzothiazolyl) substituted benzene derivatives. Int J Curr Chem 1:175–179

Chen J, Hamon MA, Hu H, Chen Y, Rao AM, Eklund PC, Haddon RC (1998) Solution properties of single-walled carbon. Nanotubes Sci 282:95–98

Czerw R, Guo Z, Ajayan PM, Sun YP, Carroll DL (2001) Organization of polymers onto carbon nanotubes: a route to nanoscale assembly. Nano Lett 1:423–427

Dresselhaus MS, Dresselhaus G, Saito R, Jorio A (2005) Raman spectroscopy of carbon nanotubes. Phys Rep-Rev Sect Phys Lett 409:47–99

Fu K, Huang W, Lin Y, Riddle LA, Carroll DL, Sun YP (2001) Defunctionalization of functionalized carbon nanotubes. Nano Lett 1:439–441

Gannon GI (2007) Carbon nanotube-enhanced thermal destruction of cancer cells in a noninvasive radiofrequency field. Cancer 110:2654–2655

Georgakilas V, Kordatos K, Prato M, Guldi DM, Holzinger M, Hirsch A (2002) Organic functionalization of carbon nanotubes. J Am Chem Soc 124:760–761

Goldberg RM, Sargent DJ, Morton RF, Fuchs CS, Ramanathan RK, Williamson SK, Findlay BP, Pitot HC, Alberts SR (2004) A randomized controlled trial of fluorouracil plus leucovorin, irinotecan, and oxaliplatin combinations in patients with previously untreated metastatic colorectal cancer. J Clin Oncol 22:23–30

Hamon MA, Chen J, Hu H, Chen Y, Itkis ME, Rao AM, Eklund PC, Haddon RC (1999) Dissolution of single-walled carbon nanotubes. Adv Mater 11:834–840

Handratta VD, Vasaitis TS, Njar VCO, Gediyal K, Kataria R, Chopra P, Newman D, Farquhar R, Guo Z, Qiu Y, Brodie AMH (2005) Novel C-17-Heteroaryl Steroidal CYP17 inhibitors/antiandrogens: synthesis, in vitro biological activity, pharmacokinetics, and antitumor activity in the LAPC4 human prostate cancer xenograft mode. J Med Chem 48:2972–2984

Hu CY, Xu YJ, Duo SW, Zhang RF, Li MS (2009) One-step electrodeposited carbon nanotube/zirconia/myoglobin film. J Chin Chem Soc 56:234–238

Iijima S (1991) Helical microtubules of graphitic carbon. Nature 354:56–58

Jorio A, Saito R, Dresselhaus G, Dresselhaus MS (2004) Determination of nanotubes properties by Raman spectroscopy. Phys Eng Sci 362:2311–2336

Kam NW, O'Connell M, Wisdom JA, Dai H (2005) Carbon nanotubes as multifunctional biological transporters and nearinfrared agents for selective cancer cell destruction. J Am Chem Soc 127:12492–12493

Kostarelos K, Lacerda L, Partidos CD, Prato M, Bianco A (2005) Carbon nanotube-mediated delivery of peptides and genes to cells: translating nanobiotechnology to therapeutics. J Drug Del Sci Tech 15:41–47

Liu Z, Sun X, Nakayama-Ratchford N, Dai H (2007) The supramolecular chemistry of organic-inorganic hybrid materials. ACS Nano 1:50–56

McEuen PL, Fuhrer MS, Park H (2002) Single-walled carbon nanotube electronics. IEEE Trans Nanotech 1:78–185

Moldeus T, Hogberg J, Orrhenius S, Fleischer S, Packer L (1978) Isolation and use of liver cells. Meth Enzymol 52:60–71

Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Meth 65:55–63

Najafi E, Kim J, Han S (2006) UV-ozone treatment of multi-walled carbon nanotube for enhanced organic solvent dispersion. J Coll Surf 284(A):373–378

Ohno M, Abe T (1991) Rapid colorimetric assay for the quantification of leukemia inhibitory factor (LIF) and interleukin-6 (IL-6). J Immunol Meth 145:199–203

Pandey VK, Negi HS (2003) Synthesis and biological activity of some new benzophenothiazines. Indian J Chem (B) 42:206–210

Riggs JE, Walker DB, Carroll DL, Sun YP (2000) Optical limiting properties of suspended and solubilized carbon nanotubes. J Phys Chem 104:7071–7076

Safari M, Yuosefi M, Jenkins HA, Bikhof Torbati M, Amanzadeh A (2013) Synthesis, spectroscopic characterization, X-ray structure and in vitro antitumor activities of new triorganotin(IV) complexes with sulphur donor ligand. Med Chem Res. doi: 10.1007/s00044-013-0549-9

Srinivas KVNS, Das B (2004) Iodine catalyzed one-pot synthesis of 3,4-dihydropyrimidin-2(1H)-ones and thiones: a simple and

efficient procedure for the Biginelli reaction. Synthesis 13:2091–2093

Sun YP, Fu K, Lin Y, Huang W (2002) Functionalized carbon nanotubes: properties and applications. Acc Chem Res 35:1096

Swarnkar PK, Kriplani P, Gupta GN, Jha KGO (2007) Synthesis and antibacterial activity of some new phenothiazine derivatives. E J Chem 4:14–20

Szymczyk A, Roslaniec Z, Zenker M, García-Gutiérrez MC, Hernández JJ, Rueda DR, Nogales A, Ezquerra TA (2011) Preparation and characterization of nanocomposites based on COOH functionalized multi-walled carbon nanotubes and on poly (trimethylene terephthalate). eXPRESS. Polymer Lett 5:955–995

Wu CY, King KY, Kuo CJ, Fang JM, Wu YT, Ho MY, Liao CL, Shie JJ, Liang PH, Wong CH (2006) Stable benzotriazole esters as mechanism-based in activators of the severe acute respiratory syndrome 3CL protease. Chem Biol 13:261–268

Xiao XY, Hao M, Yang XY, Ba Q, Li M, Ni SJ, Wang LS, Du X (2011) Licochalcone A inhibits growth of gastric cancer cells by arresting cell cycle progression and inducing apoptosis. Cancer Lett 302:62–75

Zaragoza-Contreras EA, Lozano-Rodrıguez ED, Roman-Aguirre M, Antunez-Flores W, Hernandez-Escobar CA, Sergio G, Aguilar-Elguezabal A (2009) Evidence of multi-walled carbon nanotube fragmentation induced by sonication during nanotube encapsulation via bulk-suspension polymerization. Micron 40:621–627

Zhang Z, Yang X, Zhang Y (2006) Delivery of telomerase reverse transcriptase small interfering RNA in complex with positively charged single-walled nanotubes suppresses tumor growth. Clin Cancer Res 12:4933–4939

Zheng M, Jagota A, Strano MS, Santos AP, Barone P, Chou SG (2003) Structure-based carbon nanotube sorting by sequence-dependent DNA assembly. Science 302:1545–1548