

Sonochemical Decoration of Graphene Oxide with Magnetic Fe₃O₄@ CuO Nanocomposite for Efficient Click Synthesis of Coumarin-Sugar Based Bioconjugates and Their Cytotoxic Activity

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Abstract

A magnetic nanocomposite of GO-Fe₃O₄@CuO was fabricated via a simple sonochemical technique and successfully utilized for the ultrasound promoted synthesis of regioselective 1,4-disubstituted mono/bis/tris-1,2,3-triazoles (**3a–k**). This catalyst could be separated conveniently from the reaction mixture using an external magnet and reused up to eight consecutive runs without noticeable drop in the desired product yield. Other noteworthy features of this green protocol are negligible metal leaching from the support during reaction, high yield in lesser time, aqueous media and good results with gram scale synthesis. Representative compounds were also screened for their cytotoxic activity against PC-12 cell line using standard MTT assay and flow cytometry. Trifluoromethyl group containing triazole derivative (3f) displayed cytotoxic activity (IC₅₀ 8.3 µg/mL) comparable to the standard drug cisplatin (IC₅₀ 5.8 µg/mL).

Graphic Abstract



Keywords Aqueous click reaction \cdot Graphene oxide-Fe₃O₄@CuO \cdot Ultrasonication \cdot 1,2,3-Triazoles \cdot Cytotoxic activity \cdot PC-12 cell lines

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1 Introduction

Click reactions are considered as a magical trick in a synthetic chemist's tool box since these reactions are characterized by being biorthogonal, modular, wide in scope, show good functional group tolerance with high atom economy, ease of procedure with high yield of pure products which can be fruitfully utilized for creating a combinatorial library of biologically important and diverse molecules [17, 28]. The basic requirement for a reaction to be classified as a "click reaction" is that it must satisfy the following criteria such as (i) Show no sensitivity towards moisture or air, (ii) Generate stereospecific product in high yield, (iii) Should furnish desired product even in the absence of a solvent or in a benign one (iv) Should have easy product isolation protocol [5, 27].

The Huisgen 1,3-dipolar cycloaddition reaction [20, 40] between azide and its coupling partner alkyne is the epitome of click reaction resulting in a straightforward creation of 1,2,3-triazoles. This conventional reaction usually needs high temperature and gives a mixture of 1,4- and 1,5-disubstituted 1,2,3-triazoles. Later on, Sharpless-Fokin [40] and Meldal [50], working independently, in 2002 made it regioselective using Cu(I) catalyst leading to the exclusive formation of 1,4-disubstituted 1,2,3-triazole derivative. Triazoles containing three nitrogen atoms is an important heterocycle which displays, broad spectrum of biological activities, such as antiviral, antibacterial [34], antifungal, antituberculour, anticonvulsive, anti-inflammatory, antitumor [37], cytostatic [3, 38] etc. to name a few with possible applications in DNA modification, bioconjugation [53], drug discovery [28], material science, etc. and still their applications are increasing exponentially. Further, they also play an impressive role as a linker in increasing biological activity because they are stable under typical physiological conditions and form hydrogen bonds which can be suitable for solubility improvement and for binding of biomolecular targets.

Fluorescent triazoles, using sugar scaffold as a core with different fluorogenic units (coumarin, anthracene, 1,8-naph-thalimide) are gaining impetus because of their use in bioimaging [16], biosensing, biolabelling, and as chemosensors for detection of ions [14, 25], small molecules and biomolecules [13]. These fluorophores are easily achieved by installing an azide or alkyne functional group in either a sugar moiety or any fluorogenic framework which could be subsequently "clicked" via CuAAC reaction.

Generally, CuAAC reaction proceeds in different solvents such as tetrahydrofuran (THF), acetonitrile (CH₃CN), dimethyl sulfoxide (DMSO), or mixed solvents but for biological application solely aqueous media is preferred. Cu(II) salts with reducing agent (generally sodium ascorbate) or Cu(I) salts in the presence of a base are commonly used for homogeneous catalytic systems but use of coloured toxic copper salts hinders its practical application and feasibility on a large scale synthesis due to metal contamination of end product and recyclability issues. To overcome aforementioned limitations many heterogeneous supports [42, 43] have been applied as an alternative to homogeneous copper catalysts for CuAAC such as organic polymers [49], zeolites, charcoal [44], alumina, silica [45], clay [10], magnetic materials [19] and polysaccharides [12] which can minimizes the

amount of metal used in the reaction by immobilization and allow recyclability of the catalyst. Besides this they also prevent the formation of bistriazoles and diacetylenes which are formed as byproducts of CuAAC reaction. making these catalyst magnetically separable induces the advantage of easy product recovery by simply use an external magnet and make its reusability feasible in many consecutive cycles [1].

Recently, various carbonaceous nanomaterials like carbon nanotubes, nanofibers, graphene, and graphene oxide have been used as support material in numerous applications such as sensors [18], cell imaging, adsorbent, drug delivery, catalysis [8, 33, 46] etc. Inherently they are endowed with remarkable properties including flexibility, good accessibility, mechanical strength, large surface area, unique interaction with metal particles, physical and electrical properties [36]. Graphene oxide has an array of functional groups such as carboxylic acid, hydroxyl, epoxy (–COOH, –OH, C–O–C) on its surface which allows for easy chemical modification and fabrication of nanocomposites with metals and metal oxides [51].

Several improved methods including microwave and ultrasonic irradiation have been reported to increase the yield of the product. Ultrasound is a powerful technique in organic synthesis in terms of enhanced rate of reaction, formation of pure products, and high yield in less time [2, 6, 7, 35, 41].

In continuation to earlier work of on synthesis of different heterocycles via green and sustainable routes [15, 22-24, 29] herein, we have successfully fabricated magnetically separable catalyst and effectively applied it for synthesis of fluorescent sugar-coumarin based 1,4-disubstituted 1,2,3-triazole derivatives using well known click chemistry under ultrasonication. The results reveal that this magnetically separable catalyst not only exhibits excellent selectivity and activity, but can also be easily recovered by using simple permanent magnet from the reaction mixture and reused eight times without any appreciable drop in its catalytic activity. In addition, high product yield, mild reaction conditions, simple work-up procedure and stability of the catalyst are other attractive features of this method. This work represents the fusion of many pervasive green chemistry themes: magnetically recoverable catalysts, recyclability and aqueous "click reaction".

2 Results and Discussion

2.1 Characterization of GO-Fe₃O₄@CuO Nanocomposite

A semi-heterogeneous nanocatalyst has been developed using ultrasonic irradiation by combining graphene oxide, magnetite and copper oxide which can dispersed homogeneously in aqueous media for obtaining fluorescent 1,2,3-triazole. Initially, graphene oxide was synthesized using modified Hummer's method [21, 30, 56] which was then sonochemically decorated by Fe_3O_4 and CuO nanoparticles on its surface (Fig. 1). Nanocomposite was then characterized by vibrating sample magnetometer (VSM), thermogravimetric analysis (TGA), High resolution transmission electron microscopy (HRTEM), energy dispersive X-ray spectroscopy (EDS), X-ray photoelectron spectroscopy (XPS), and X-ray diffraction (XRD).

Magnetically separation of the catalyst from the reaction mixture depends on the magnetic property of the nanoparticles. Magnetic properties of GO-Fe₃O₄@CuO was investigated using vibrating sample magnetometer (VSM) with the field sweeping from -20,000 to +20,000 Oe at room temperature as shown in Fig. 2. Lower saturation magnetization (Ms) of Fe₃O₄ and GO-Fe₃O₄@CuO nanocomposite are 60 emu g⁻¹ and 50 emu g⁻¹ respectively which clearly revealed magnetic nature. The Ms value of prepared nanocomposite was less compared to pure magnetite. However, the magnetization is still large enough and could be easily exploited for removal of the catalyst from reaction mixture. Figure 3a shows the X-ray diffraction (XRD) pattern of GO, showing diffraction peak at 2 theta = 10.5° which correspond to 002 plane of graphene oxide. The XRD pattern of GO-Fe₃O₄@CuO (Fig. 3b) nanocatalyst show diffraction peaks at 2 theta 30.1° , 35.6° , 43.1° , 53.9° , 57.0° , 62.7° correspond to (220), (311), (400), (422), (551) and (440) diffraction planes of magnetic nanoparticles, respectively (JCPDS card no. 19-0629) [32]. The XRD result for the prepared catalyst indicates that the crystal structure of the Fe₃O₄ core does not change during the immobilization on the surface of GO. The signals of Copper were not observed in XRD pattern, indicating that Cu species is highly dispersed. No characteristic peaks of other impurities were detected.

The TGA thermogram of GO shows a thermal decomposition around 160 °C which is attributed to the vaporization of oxygen containing functional groups which are present on the GO surface [9] (Fig. 3c). Figure 3d presents the TGA curve of graphene oxide GO-Fe₃O₄@CuO, in which first weight loss was 2.3% at 200 °C, which may be assigned to the loss of absorbed residual or water and/or organic solvents used during the preparation of catalyst. An obvious weight loss (8.7 wt%) between 200 and 600 °C is observed, which can be assigned to the decomposition and vaporization of



Fig. 2 VSM magnetization curve of GO-Fe₃O₄@CuO



Fig. 3 XRD analysis of a graphene oxide, b GO-Fe₃O₄@CuO and TGA analysis of c graphene oxide, d GO-Fe₃O₄@CuO

various oxygen-containing functional groups at different positions on the surface of the GO-Fe₃O₄@CuO composite. Composite shows better thermal stability than GO due to the presence of Fe₃O₄@CuO composite on graphene oxide sheets which does not allow easy degradation of the oxygen functionalities [54]. The GO-Fe₃O₄@CuO catalyst possesses good thermal stabilities (up to 600 °C), which meets the demands for potential applications in catalysis.

Transmission electron microscopy (TEM) and high resolution electron microscopy (HRTEM) analysis were also performed for Fe_3O_4 and $GO-Fe_3O_4$ @CuO nanocomposite. TEM images clearly revealed that Fe_3O_4 and CuO nanoparticles were successfully embedded on multilayer graphene oxide sheets. The TEM image in Fig. 4a shows magnetite nanoparticles which are somewhat spherical in shape, with some cubic partials and HRTEM image of Fe_3O_4 (4a, inset) the characteristic lattice fringes with the d-spacing of 0.23 nm corresponds to the [311] plane of Fe_3O_4 . The TEM images shows wrinkled sheets of GO which demonstrate high degree of oxidation [58]. CuO nanoparticles exhibits spindle like structure and Fe_3O_4 almost spherical in shape (Fig. 4b, c). The average width and length of spindle-shaped CuO nanoparticles were about 20 to 40 nm and 80 to 100 nm respectively. The HRTEM image in Fig. 4d shows the characteristic lattice fringes of Fe_3O_4 and CuO nanoparticles about 0.252 nm and 0.3 nm respectively. Furthermore, elemental mapping was also carried out to illustrate the space distributions of the component elements. Figure 4e–h exhibit the individual distribution of elements in prepared nanocomposite. The amount of copper was found 8.33 wt% in composite by using atomic absorption spectroscopy.

Additionally, XPS was performed to acquire further insight into the valence information of the GO-Fe₃O₄@ CuO composite. Full survey spectrum of the sample given in supplementary information (Fig. S1), which indicates the presence of Cu, Fe, O and C without obvious impurities. The C1s spectra can be deconvoluted into three main components corresponding to carbon atoms in different functional groups, which exhibits the presence of C=C at 284.39 eV, C–O at 285.60 eV and C=C–O at 290.24 eV (Fig. 5a). These values are in good agreement with previously reported values [18, 57]. The binding energies of Cu2p_{3/2} and Cu 2p_{1/2} in GO-Fe₃O₄@CuO found to be around 934.5 eV and 954.20 eV respectively indicating the presence of Cu²⁺ (Fig. 5c). The gap between the Cu 2p_{1/2} and 2p_{3/2} level is about 20 eV that is in agreement with the standard



Fig.4 a TEM image of Fe_3O_4 (HRTEM image of Fe_3O_4 inset). b, c TEM images. d HRTEM image and e-h EDS elemental mapping of GO- $Fe_3O_4@CuO$



Fig. 5 XPS analysis. a C1s, b O1s, c Cu2p of the GO-Fe₃O₄@CuO sample

spectrum of CuO [11, 31]. Figure 5c also shows two additional shake-up peaks at 943.29 and 963.00 eV, suggesting the presence of an unfilled Cu 3d shell and thus further confirming the existence of Cu^{2+} in the sample which are positioned at higher binding energies compared to those of the main peaks. The peaks at 710 eV and 723 eV for Fe²⁺ and at 712 eV, 718, 725 characteristic peaks of Fe2p_{1/2} of Fe³⁺.

2.2 Catalytic Performance

Catalytic performance of GO-Fe₃O₄@CuO was assessed in the synthesis of 1,4-disubstituted mono/bis/tris triazole derivatives using sugar azide (1a) and coumarin alkyne (2a) as model substrates. To obtain an optimum reaction profile for GO-Fe₃O₄@CuO mediated triazole synthesis, the effect of different reaction parameters such as amount of catalyst, reaction time, temperature and solvents have been studied.

Click reaction of azide and alkyne to afford regioselective 1,4-disubstituted triazoles are usually catalyzed by using copper salts [52]. To affirm this fact, initially, the model reaction was performed using sugar azide (1a, 1 mmol) and coumarin alkyne (2a, 1.1 mmol) in water without any catalyst under ultrasonication at 45 °C for 80 min. No product formation was observed as revealed by thin-layer chromatography (TLC) monitoring even after 8 h. To ensure that only copper plays a role as catalyst each component (GO, Fe_3O_4 , GO- Fe_3O_4) of the prepared catalyst (GO- Fe_3O_4 @ CuO) under the same conditions cited above were studied (Table 1, entries 1–3). It was observed that only trace amount of product was obtained with GO, Fe₃O₄ GO-Fe₃O₄ which clearly established that copper is essential for CuAAC reaction. This fact was also corroborated when the reaction was performed with prepared catalyst (GO-Fe₃O₄@CuO) which gave 97% yield under identical reaction conditions (Table 1, entry 5). Catalyst was also compared it with the conventional catalysts (CuSO₄.5H₂O/Na ascorbate (1 mol%/5 mol%)) in water, only 40% product yield was observed in 2 h which may be due to the low interaction of initial compounds with water (Table 1, entry 4). Good catalytic activity of prepared catalyst (GO-Fe₃O₄@CuO) can be explained on the basis of hydrophilic nature of GO which has -COOH and -OH groups on its surface making the reaction feasible in aqueous media due to its high dispersity and facile interaction of Cu with organic substrates and also by "Breslow effect" [4].

The productivity of the click reaction is majorly affected by the amount of catalyst so a series of reactions were performed using 1a and 2a by varying the catalyst concentration (2, 4, 6, 8, 10 mg) while keeping other parameters like temperature, solvent, time substrate amount constant. Results are shown in Fig. 1 which revealed that increase in amount of catalyst from 2 to 6 mg (2,4, 6 mg) resulted in improvement of the transformation of the initial reactants to the desired product. A further increase in the catalytic amount (8, 10 mg) did not lead to any significant increase in the conversion. So, the optimal amount of GO-Fe₃O₄@Cu was fixed to 6 mg for the rest of the experiments. In order to observe the effect of temperature, the model reaction was carried out at various temperatures (25, 35, 45, 55 °C) for 80 min under ultrasonication using GO-Fe₃O₄@CuO nanocomposite and the results are given in Fig. 6. Detailed examination of the results revealed that 45 °C was optimum temperature for the maximum conversion of the reactants when the reaction was carried out for 80 min under ultrasonication (Fig. 7). For observing the effect of ultrasonication same reaction was repeated using simple magnetic stirring at 45 °C for 80 min, only 60% conversion was observed. So, ultrasonication is used for rest of the experiments. Excitingly, ultrasonic radiations as an efficient and convenient process, could shorten reaction



Fig. 6 Optimization of amount of catalyst for model reaction



Table 1	Comparative	screening of o	other catalyst and	prepared	catalyst for	the CuAAC reaction
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Entry	Catalyst	Amount	Yield (%) ^a
1	GO	10 mg	<10
2	Fe ₃ O ₄	10 mg	< 10
3	GO-Fe ₃ O ₄	10 mg	<15
4	CuSO ₄ .5H ₂ O/Na ascorbate	2.49 mg/9.9 mg	40 ^b
5	GO-Fe ₃ O ₄ @CuO	10 mg	97

Reagents and conditions peracetylated glucose azide (1a) (1 mmol), coumarin alkyne (2a) (1.1 mmol), in aqueous medium was ultrasonicated in open air at 45 °C for 80 min

^aIsolated yield of product

^bReaction proceeded at high temperature (60 °C) for 2 h



Fig. 7 Effect of temperature on model reaction under ultrasonication



Fig. 8 Effect of solvent on click reaction

time. Also, the yields of the obtained desired product were improved compared with conventional heating.

Subsequently, the activity of the GO supported heterogeneous magnetically separable catalyst was evaluated for the click reaction using different solvents in the presence of the model substrates. The effect of solvents on the synthesis of triazole derivatives is illustrated in Fig. 8. Results indicated that the best catalytic performance was observed when water was employed as solvent. The efficiency of the catalyst also calculated by Turnover Number (TON) and Turnover Frequency methods (TOF). TON can be defined as number of moles of product per mole catalyst and TOF is the simply turn over number per unit time. TON and TOF value were found to be 3200 and 53.33 min⁻¹ respectively (for model reaction).

To further explore the potential of this catalyst for 1,4-disubstituted 1,2,3-triazole synthesis, various alkynes (**2a–k**) and sugar azide (**1a**) were subjected to the click reaction under the above optimized reaction conditions using GO-Fe₃O₄@Cu nanocomposite (Table 2). Analysis of the results showed that all of the substrates produced the expected regioselective 1,4-disubstituted triazole with excellent yield, indicating remarkable catalytic efficacy of the GO-Fe₃O₄ based copper catalyst. The most attractive attributes of the present methodology are that the click reaction is devoid of unwanted products and the catalyst can be recycled for eight consecutive cycles without appreciable drop in its catalytic activity and use

of water as green solvent at ambient temperature under ultrasonication.

Mechanism of copper catalyzed synthesis of 1,2,3-triazoles has been recently been reviewed by Fokin who proposed a revisited mechanism whereby a σ -bound Cu(I)acetylide bearing a π complexed copper atom reacts with an organoazide forming a bridging dicopper µ-acetylide intermediate [40, 55]. Based upon their studies we proposed following mechanism for the formation of 1,2,3-triazoles (Fig. S2). In order to investigate the reusability of the catalyst, the model reaction was performed under the optimized reaction conditions. Upon completion of the reaction, the catalyst was separated by means of an external magnet after each run, washed with ethanol and dried and then subjected to the next run under the same conditions. It is obvious from Fig. S3 that there was no significant decrease in conversion yield % even after eight runs, indicating robustness and reusability of the prepared catalyst. The leaching test of metal (Copper) after recycling was investigated by determining content of the metal in reaction solution using FAAS. Results are revealed that small amount of copper leached from the support to the reaction solution. The loss of insignificant copper metal from the surface of GO may explain decrease yield of compound 3a during consecutive runs. To check the heterogeneous nature of catalyst, a hot filtering experiment was performed for using model reaction under identical reaction conditions. The reaction was stopped after 30 min, and the catalyst was removed by means of simple external magnet. The filtrate was further subjected into reaction vessel and reaction continued for the remaining period of time (30 min). After the reaction time, the mixture was cooled and extracted with ethyl acetate to identify the product and yield. No further conversion was noticed in the filtrate, which clearly supported that the catalysis is heterogeneous in nature. Filtrate was also analyzed by FAAS, it was found that concentration of copper in the filtrate corresponds to negligible catalyst leaching. Subsequently, the recovered catalyst stability was assessed using XRD and TEM analyses. The TEM analysis of the recovered catalyst after the eighth run (Fig. S5) showed that TEM image of recycled catalyst (after the eighth run) was very similar to the fresh catalyst image which revealed that the morphology of the catalyst remains unaltered after the reaction. The XRD pattern (Figure S6) of the reused catalyst showed similar set of peaks to those of the fresh one and no additional peaks were observed, which indicates that the recovered catalyst is highly stable even after the eight consecutive runs.

2.3 Comparison with the Literature Precedents

The efficiency of the GO-Fe₃O₄@CuO magnetically separable catalyst was compared with the previously reported catalytic system for the synthesis of triazole derivatives.

 Table 2
 Scope and versatility of the prepared catalyst using sugar azide (1a) and various alkynes (2a-k) in aqueous media for CuAAC



Entry	Azide	Alkyne	Triazole	Time	Yield ^b	Product	TON/
	(1a)			(min)	%	code	TOF min ⁻¹
1.	Aco Aco N3	2a	Aco Aco Aco Aco Aco Aco Aco Aco Aco Aco	60	96	3a	3200/53.33
2	Aco OAc OAc N3	2b	Aco	60	95	3b	3166/52.76
3	Aco Aco Aco Aco OAc	2c	ACO ACO OAc NN OAc CzHs	60	95	3с	3166/52.76
4	OAc Aco Aco OAc	2d	Aco CAC NAN G	80	85	3d	2833/35.41
5	AcO AcO AcO AcO OAc OAc	2e	Aco Aco Aco OAc N=N OAc	70	90	Зе	3000/42.85
6	Aco Aco Aco OAc	2f	AcO AcO OAc OAc OAc	60	93	3f	3100/51.66
7	Aco Aco Aco OAc N3	2g	ACO OAC ACO OAC	80	85	3g	2833/35.41

Table 2 (continued)



All reactions were carried out using sugar azide (1 mmol), terminal alkynes (1.1 mmol), GO-Fe $_3O_4@Cu$ (6 mg) at 45 °C temperature under ultrasonication

^aIsolated yields

From Table S1, it is evident that the newly synthesized copper catalyst is far more proficient in terms of product yield, time, temperature and cost. Besides, the catalyst can be easily recovered through a simple magnet without filtration, centrifugation or sedimentation techniques and reused for eight consecutive cycles without any significant loss in its catalytic activity making it an interesting alternative for copper catalyzed click promoted triazole synthesis.

3 Cytotoxic Studies

Cytotoxicity of representative compounds (3a, 3b, 3d, 3e, 3f) were tested against rat pheochromocytoma cell line (PC-12) by using standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [48]. Rat pheochromocytoma cell line (PC-12) purchased from National Centre for Cell Science, Pune, India were maintained in RPMI-1640 supplemented with 10% FBS and antibiotic-antimycotic solution. The PC-12 cells in the log phase were seeded in 96-well plates at a concentration of 0.1 mL/well and incubated overnight at 37 °C in a 5% CO₂ CO₂/95%. Working solutions of the compounds $(0.05, 0.5, 5, 50 \text{ and } 500 \text{ }\mu\text{g})$ mL) were prepared from a 10 mg/mL stock solution by serial dilution in culture medium. The working solutions were added to cell suspension (100 µL/well) and further incubated for 48 h. The cell viability was determined by MTT assay. A 50 mg/ml MTT stock was prepared in DMSO and diluted to 5 mg/mL in RPMI-1640. The working solution (5 mg/mL) was added in each well (0.02 ml/well) and incubated for 3 h. Thereafter, the formazon was dissolved by addition of 0.1 ml/well of MTT dissolving solution (0.01 M HCl in 20% w/v sodium dodecyl sulphate) and absorbance at 595 nm was recorded using an ELISA plate reader.

Cytotoxicity of samples 3a-f was determined following 48 h incubation with PC-12 cells. The IC_{50} values of 3a and 3e were > 500 µg/mL while those of 3b, 3d and 3f were 382.8, 73.9 and 8.3 µg/mL, respectively (Fig. 9). The IC_{50} value of 3f was comparable to that of cisplatin (5.8 µg/mL) [26, 47]. Results revealed that compound 3f shows promising results.

Forward scatter (FSC) is an indicator of cell size/volume and an increase in FSC indicates an increase in cell size/volume. As observed in the above Fig. S4A, the FSC distribution in 3a, 3d and 3e was comparable to control suggesting no change in cell size/volume. On the other hand, cells treated with 3b were found to be relatively larger in size while cells exposed to 3f were relatively smaller. Side scatter (SSC) is an indicator of cell granularity and an increase in SSC indicates an increase in granularity (Fig.S4B). SSC distribution in cells treated with all compounds was shifted towards right indicating an increase in granularity. The increase in granularity followed the order: 3e < 3d < 3a < 3f < 3b. Figure 10 is FSC-SSC correlation plots of Control, and sugar coumarin based triazoles (3a, 3b, 3d, 3e, 3f).

The results of FSC and SSC were also confirmed by FSC-SSC correlation plots. Cells treated with 3a constituted of two types of cell populations: the first population subset exhibited FSC-SSC values similar to the control cells while the second population subset (highlighted in black oval) exhibited SSC values greater than control cells. Cells treated



Fig. 9 Cytotoxic activity of sugar coumarin based triazoles (3a, 3b, 3d, 3e, 3f) against PC-12 cells

with 3b also exhibited a population subset with increase in SSC along with an increase in SSC (highlighted in black oval) while a general decrease in FSC along with an increase in SSC was observed in 3f-treated cells. No significant deviations from control cells were observed in cells treated with 3d and 3e.

4 Conclusions

Decoration of GO with Fe_3O_4 and CuO on its surface sonochemically, furnishes a semi-heterogeneous nanocomposite which can be homogeneously dispersed in water, rendering the hydrophobic organic reactants interact fruitfully to create the desired product quantitatively in less time at 45 °C. AAS results revealed that the catalyst is devoid of leaching problem. The facile recovery of prepared catalyst by means of an external magnet allows the catalyst to be recycled eight times without any discernible drop in its catalytic activity. Easy work-up procedure, mild reaction conditions and broad substrate scope are additional attributes of the present methodology. Results of cytotoxic studies of representative compounds revealed that compound having trifluoromethyl group 3f showed best results with IC₅₀ (8.3 µg/mL) comparable to that of cisplatin (5.8 µg/mL) against PC-12 cells.

5 Experimental Section

5.1 Materials and Method

All chemicals were purchased from commercial sources and were used as received. The purity of all the compounds was checked by TLC using silica gel as adsorbent and solvents of increasing polarity as mobile phase. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), RPMI-1640,



Fig. 10 FSC-SSC correlation plots of a control, b 3a, c 3b, d 3d, e 3e, f 3f compounds

fetal bovine serum (FBS), antibiotic-antimycotic solution (100×) were obtained from HiMedia, Mumbai, India. Cisplatin was a gift sample from Venus Remedies Pvt. Ltd., Panchkula, India. Melting points were determined in open glass capillaries using Gallenkamp melting point apparatus and are reported uncorrected. ¹H NMR (400 MHz) and ¹³C (100 MHz) NMR were recorded on a Jeol ECS 400 MHz spectrophotometer using CDCl₃ as a solvent. TMS was taken as an internal standard and chemical shifts are reported in δ ppm. FTIR spectra were recorded on a Perkin Elmer Spectrum 2 spectrophotometer using pressed KBr discs in the region of 4000–400 cm⁻¹. Mass spectra were recorded on a Xevo G2-S Q-Tof spectrometer (Waters, USA), capable of recording high resolution mass spectrum (HRMS) in the ESI (Electrospray Ionization) mode. The visualization of surface morphologies of prepared catalyst (GO-Fe₃O₄-Cu) was done by using a field emission scanning electron microscope (FESEM) Nova Nano FE-SEM 450 (FEI) operating at 20 kV. High resolution transmission electron microscope (HRTEM) images were measured using a Tecnai G² 20 (FEI) S-Twin transmission electron microscope at 200 kV and equipped with an energy-dispersive X-ray detector (EDX). Sample preparation was done by grinding a small amount of sample which was then dispersed in isopropanol solution using an ultrasonic bath. Some drops of the sample were dropped onto the copper grid and isopropanol was evaporated at room temperature. Thermo gravimetric analysis (TGA) was done on a Mettler thermal analyzer in an inert atmosphere at a heating rate of 10 °C/min in the range of 10-800 °C to determine the loading of organic molecules on the surface of magnetite. Magnetization measurement were performed on vibrating sample magnetometer (VSM) (quantum design MPMS) at 300 K. X-ray photoelectron spectroscopy (XPS) measurements recorded in ESCA⁺ omicron nanotechnology oxford instrument. Powder X-ray diffraction (XRD) pattern of the sample was obtained with X-ray Diffractometer (Panalytical X Pert Pro) using Cu K α radiation. Ultrasonication (Elma S 70 H) with 37 kHz output frequency was used for synthesis of desired products. Acetylation of glucose and fructose was carried out by adopting previously reported procedure [39]. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), RPMI-1640, fetal bovine serum (FBS), antibiotic-antimycotic solution $(100 \times)$ were obtained from HiMedia, Mumbai, India. Cisplatin was a gift sample from Venus Remedies Pvt. Ltd., Panchkula, India.

5.2 Synthetic Procedure for Synthesis of GO-Fe₃O₄@ CuO

GO was synthesized using modified Hummer's method [21, 30, 56]. To prepare this composite, Graphene oxide in 25 mL water was taken in a flat bottom flask and ultrasonicate for 60 min for dispersion. Then, 1 mmol Fe²⁺, 2 mmol Fe³⁺ salts and 2 M NaOH were slowly added into a reaction vessel. After the mixture ultrasonicate for 1.5 h, CuNO₃ and 2 M NaOH were added simultaneously and slowly into reaction vessel. After the mixture ultrasonicate for 1.5 h, precipitate was formed which was then separated, washed with deionized water (3 × 10) and ethanol (2 × 10) and dried.

5.3 Typical Procedure for the Synthesis of 1,4-Disubstituted 1,2,3-Triazoles Catalyzed Using the GO-Fe₃O₄@CuO Catalyst (3a-k)

A flat bottom flask containing coumarin alkyne (1.1 mmol, 0.220 g), sugar azide (1 mmol, 0.373 g), water (10 mL) and GO-Fe₃O₄-Cu (6 mg) catalyst was ultrasonicated at 45 °C. After completion of the reaction as monitored through thin layer chromatography (TLC), the catalyst was removed by means of magnet. The reaction mixture was then extracted with ethyl acetate (10 mL×3), and dried over anhydrous Na₂SO₄. Finally, the ¹H NMR and HRMS analysis of the products was done to confirm the structures of the resultant 1,4-disubstituted 1,2,3-triazole derivatives.

5.4 Gram scale Synthesis of 1,4-Disubstituted Triazoles (3a)

In a flat bottom flask, to a mixture of sugar azide (3 mmol, 1.119 g) and coumarin alkyne (3.3 mmol, 0.660 g), GO-Fe₃O₄-Cu catalyst (18 mg) was added in 10 ml water. The reaction mixture was ultrasonicated until TLC show completion of reaction at 45 °C. The catalyst was removed from reaction mixture using an external magnet. The resulting reaction mixture was extracted with ethyl acetate and dried over anhydrous Na₂SO₄. Product was obtained with 93% isolated yields as a white solid.

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