Med Chem Res
DOI 10.1007/s00044-014-1010-4

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and selective cytotoxicity of novel biphenyl-based tetrazole derivatives

Mahesh H. Malani · Bharatkumar Z. Dholakiya · Ahmed S. Ibrahim · Farid A. Badria

Received: 14 August 2013 / Accepted: 14 April 2014

Abstract Cancer today represents a significant public health problem worldwide, and the challenge is to produce cost-effective drugs. Recently, biphenyl compounds as well as tetrazole derivatives is known for their potential non-selective anticancer activities. In search of novel selective anticancer agents, a series of newly hybrid molecules was designed and synthesized by combining the structural features of biphenyl and tetrazole moieties. The structures of newly synthesized compounds were characterized using spectroscopic techniques (FT-IR, ^{1}H NMR, ^{13}C NMR, and HMBC). Cytotoxic evaluations of these novels compound on human cancer cell lines showed a significant anticancer activity against more than one tested cell lines. Compounds 5n, 5j, and 5o proved to exhibit the strongest and selective cytotoxic effect on HepG2 and MCF-7 lines. Taken together, this study has led to the development of promising leads for cancer therapy.

Keywords Tetrazole · Cancer · Chemotherapy · Cytotoxity · Antiproliferative

Electronic supplementary material The online version of this article (doi:10.1007/s00044-014-1010-4) contains supplementary material, which is available to authorized users.

M. H. Malani · B. Z. Dholakiya (✉)
Department of Applied Chemistry, S.V. National Institute of Technology, Ichchhanath, Surat 395007, Gujarat, India
e-mail: bharat281173@gmail.com

M. H. Malani
e-mail: maheshmalani.svnit@gmail.com

A. S. Ibrahim
Department of Biochemistry, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt

F. A. Badria
Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt

Introduction

Cancer (a term comprising over 100 types of malignancy) is one of the major burdens of chronic disease in the world. According to Cancer Society statistics, more than one-quarter of all death rates is attributed to cancer. Nevertheless, it is very difficult to cure this disease and to clarify its pathogenesis due to the intricate etiology of cancer. (Cancer Facts & Figs. 2nd Ed., 2011; Colorectal Cancer Facts & Fig., 2011; Canadian Cancer Society., 2012). Chemotherapy is the last chance for many cancer survivors and perhaps the only alternative for patients who have had multiple resections and maximum irradiation. After four decades of the cancer chemotherapy era, research has achieved a great success, and a large number of chemotherapeutic agents have been discovered since that time. However, only a few have earned a solid position in the list of useful drugs due to the associated side effects to varying degrees, and the emergence of drug resistance. Therefore, developing new anticancer drugs with a better potency and specificity against cancer cells has become the hotspot of global media attention.(Choi *et al*., 2008; Rapp *et al*., 2003; Yuancai and Si-shen, 2005).

Nowadays, biphenyl compounds as well as tetrazole derivatives are known for their varied biological activities, such as antibacterial, anti-inflammatory, and recently anticancer activities. Accordingly, a suitable combination of these structural features is a rational strategy that may result in the design of new molecules in which the characteristics of various components are modulated, amplified, or give rise to entirely new properties. Although syntheses

Published online: 07 May 2014

of tetrazole derivatives have been reported since the mid-century, there is still a dearth of efficient processes. The well-known and the most efficient route for such preparation is the [3+2] cycloaddition between hydrazoic acid and cyanide derivatives. (Kiyoto *et al*., 1998) However, hydrazoic acid is highly explosive. As such, the use of sodium azide as a substrate in place of the hydrazoic acid would be practically convenient. Unfortunately, the [3+2] cycloaddition energy barrier would significantly lower with hydrazoic acid than the azide ion. To overcome this energy limitation, synthesis has been designed either to control the hydrazoic acid formation (Sauer *et al*., 1960) or to use a large excess of azide ions in the presence of metal catalysts (Steven and Wittenberger, 1993) or strong Lewis Acids. (Bret and Michael, 1993) Overall, these procedures are undesirable due to the longer reaction times, high temperature, low yields, or non-recoverable catalysts. In the present study, we have described the synthesis of 20 novel hybrid tetrazole and biphenyl derivatives, and their evolution toward tumor growth inhibitory activities over a panel of human cancer cell lines to develop novel, selective, and active anticancer agents.

Experimental

Materials and methods

All reagents were of analytical grade and were used without further purification. Solvents employed were purified by standard procedure before use. Brominated trityl group protected biphenyl tetrazol was prepared as per reported process (Somisetti and Sudhaker, 2011), and sodium azide was purchased from Aldrich, and 4′-methyl-biphenyl-2-carbonitrile was purchased from Acros Organics-India.

Synthesis of 5-(4′-methylbiphenyl-2-yl)-2*H*-tetrazole

Mixture of 19.3 gm (0.1 mol) 4′-methyl-biphenyl-2-carbonitrile **(1)** and 18.8 gm (0.137 mol) triethylamine HCl and 8.9 gm (0.137 mol) sodium azide in 100 ml xylene was charged in a reaction kettle equipment with stirrer, thermometer, and water condenser. Reaction mass was stirred at 28–30 °C for 1.0 h, and then temperature was raised to 138–142 °C (reflux) and maintained for 28 h. Progress of the reaction was monitored on TLC, and after completion of the reaction, reaction mass was cooled to room temperature and 50 ml water was added. pH of reaction mass containing water was adjusted to 10–11 by the addition of 10 % NaOH solution, stirred for 30 min, settled in separating funnel, and the layer was separated. Lower aqueous layer was collected, washed with 25 ml xylene, and the pH was adjusted to 4–5 by 50 % HCl. The aqueous layer was stirred for 60 min, and solid product was separated, filtered and finally washed with water. Drying was carried out at 60–70°c for 12 h, and white-colored powder **(2)** (18.8 gm, 87 % yield) was obtained (Murli Krishna et al., 2011).

Synthesis of trityl protected 5-(4′-methylbiphenyl-2-yl)-2*H*-tetrazole (**3**) compound

Trityl protected 5-(4′-methylbiphenyl-2-yl)-2H-tetrazole compound **(3)** (Scheme 1) was prepared at present work using the reported technique. (Grigor *et al.,* 2001) Mixture of compound **(2)** 15.0 gm (0.0634 mol), triphenyl methanol 17.35 gm (0.066 mol), and 70 ml toluene was charged in three-neck flat-bottom reaction flask. Reaction mass was stirred at 28–30 °C for 15 min, and 2–3 drops of concentrated sulphuric acid were added. Reaction mass was continuously stirred, and the temperature was maintained at 110–112 °C for 2.0 h. Simultaneously, water was removed by azeotropic distillation using toluene as distillation solvent. After completion of reaction, reaction mass was cooled to 28–30 °C, filtered, and dried at 65–70 °C. White solid powder **(3)** (27.3 gm 90 % yield) was obtained.

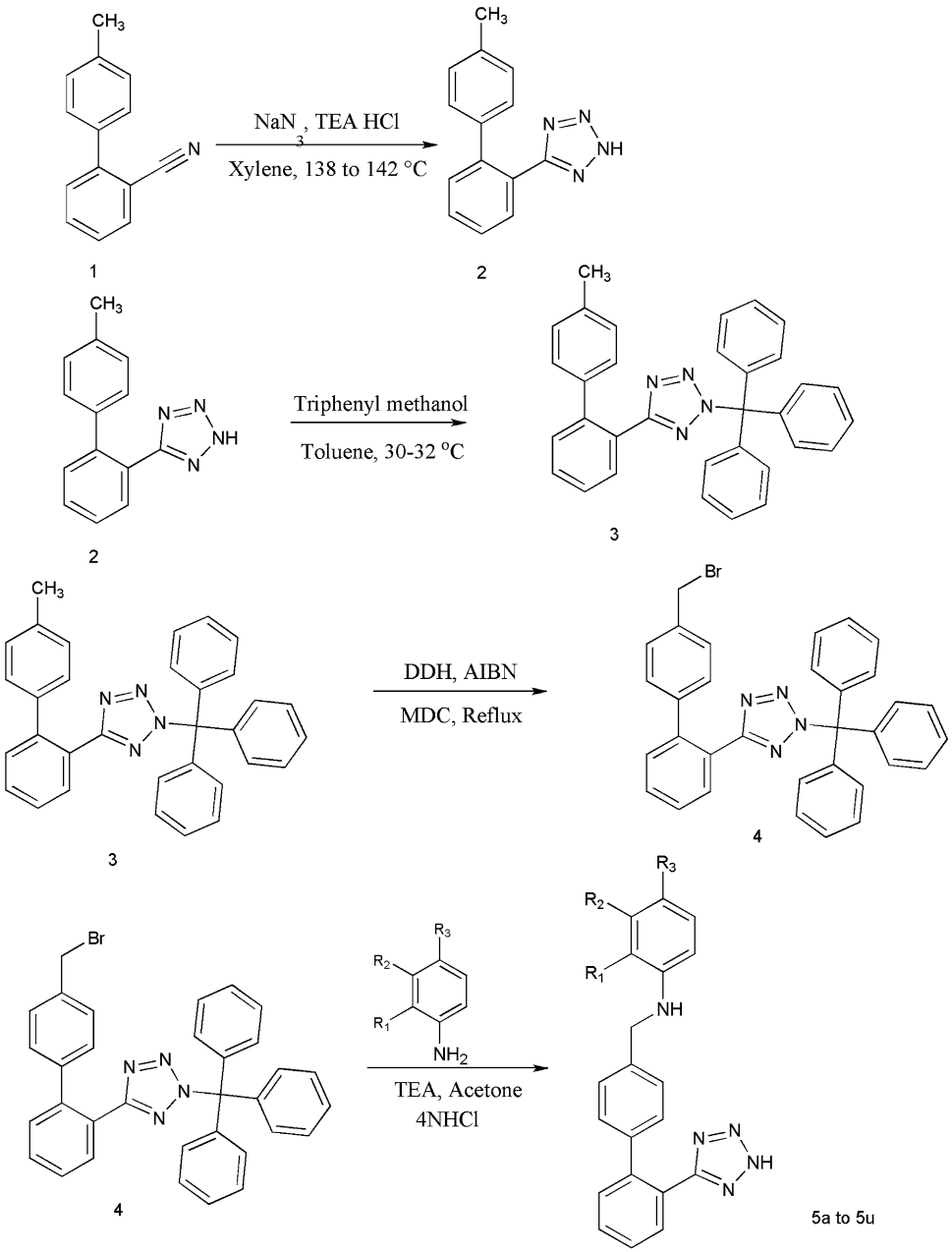
Synthesis of brominated trityl protected 5-(4′-methylbiphenyl-2-yl)-2*H*-tetrazole compound

Brominated trityl protected 5-(4′-methylbiphenyl-2-yl)-2H-tetrazole compound **(4)** was prepared using the reported process.(Somisetti and Sudhaker, 2011) Mixture of compound **(3)** 15.0 gm (0.0313 mol), DBDMH 6.0 gm (0.020 mol), AIBN 1.02 gm (0.0062 mol), and dichloromethane (75 ml) as a solvent was charged in three-neck flat-bottom reaction flask. Reaction mass was continuously stirred, and initially temperature was raised to 28–30 °C and then maintain to reflux for 8–9 h. After completion of the reaction, reaction mass was cooled to 20–22 °C, and 10 ml water was added, stirred and transfer in separating funnel. Lower organic layer from separating funnel was separated and washed with sodium bicarbonate solution followed by washing with water, and treated with sodium sulfate. The organic layer was evaporated to dryness, and white-colored solid product **(4)** (14.79 gm 85 % yield) was obtained.

Synthesis of Amino based tetrazole compounds (**5a–5t**)

Compounds **(5a–5t)** were prepared by addition of compound **(4)** (1.0 mol), substituted aromatic's amine (1.1 mol), and

Scheme 1 Reaction scheme: reaction scheme of synthesized compounds



NaN_3- Sodium azide, **TEAHCl**-Triethyl amine.HCl, **DDH**-1,3,dibromo 5,5 dimethylhydantoin
AIBN- Azo bis isobutyronitrile, **MDC**- Dichloromethane

acetone as a solvent in three-neck flat-bottom reaction flask. Reaction mass was stirred at 28–30 °C for 15–20 min, and tri ethyl amine (1.1 mol) as an acid scavenger was added. Reaction mass was continuously stirred, and the temperature was maintained 55–60 °C for 3.0 h. Progress of the reaction was monitored by TLC. After completion of the reaction, reaction mass was cooled to 28–30 °C, filtered and washed with acetone. De-protection of trityl group was carried out by charging wet cake with methanol in three-neck flat-bottom flask and was stirred at 28–30 °C with addition of 4 N HCl to adjust pH 5–6, and temperature was raised and maintain at 40–45 °C for 1.0 h. The solvent was distilled under vacuum at 40–45 °C temperature, and the solvent was added (water and ethyl acetate). Again, reaction mass was stirred for 10 min and transferred to separating funnel and settled. Top organic layer was separated and treated with sodium sulfate and evaporated to dryness. Solid product **(5a–5t)** having yield 75–95 % was obtained.

Table 1 Physico chemical parameter of synthesized compounds

Sr. no.	Compounds Code	% Yield	Color (solid powder)	Melting Point	Compounds name
1	**5a**	77 %	Off White	198–200 °C	1-[4-({[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}amino)phenyl]ethanone
2	**5b**	95 %	Dirty White to Creamish	167–168 °C	4-({[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}amino)benzenesulfonic acid
3	**5c**	81 %	Light Brown	194–196 °C	4-({[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}amino)phenol
4	**5d**	75 %	White	193–194 °C	*N*-{[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}aniline
5	**5e**	83 %	White to Creamish	210–212 °C	2-({[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}amino)benzoic acid
6	**5f**	78 %	White	165–168 °C	*N*-{[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}naphthalen-1-amine
7	**5g**	79 %	White	172–174 °C	1-phenyl-*N*-{[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}methanamine
8	**5h**	82 %	White	178–180 °C	2-methyl-*N*-{[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}aniline
9	**5i**	84 %	White	184–185 °C	3-methyl-*N*-{[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}aniline
10	**5j**	85 %	White	188–190 °C	4-methyl-*N*-{[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}aniline
11	**5k**	88 %	Light Yellow	177–179 °C	2-nitro-*N*-{[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}aniline
12	**5l**	90 %	Off White	187–189 °C	3-nitro-*N*-{[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}aniline
13	**5m**	92 %	Creamish Powder	192–193 °C	4-nitro-*N*-{[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}aniline
14	**5n**	83 %	White	194–195 °C	2-chloro-*N*-{[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}aniline
15	**5o**	82 %	White	191–193 °C	4-chloro-*N*-{[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}aniline
16	**5p**	79 %	White	197–199 °C	4-fluoro-*N*-{[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}aniline
17	**5q**	77 %	White	201–203 °C	5-methyl-*N*-{[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}-1,3-thiazol-2-amine
18	**5r**	91 %	Yellow Colored	117–122 °C	4-methyl-*N*-{[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}piperazin-1-amine
19	**5s**	86 %	Off White	182–183 °C	*N*-{[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}pyridin-2-amine
20	**5t**	88 %	White	197–198 °C	*N*-{[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}pyridin-3-amine

Biological evaluation of the isolated compounds

Cytotoxicity and antitumor assays

Samples were prepared for assay by dissolving in 50 ml of dimethyl sulfoxide (DMSO), and diluting aliquots into a sterile culture medium at 0.4 mg/ml. These solutions were sub-diluted to 0.02 mg/ml in sterile medium, and the two solutions were used as stocks to test samples at 100, 50, 20, 10, 5, 2, and 1 mg/ml in triplicate in the wells of microtiter plates. The compounds were assayed in triplicate on monolayers grown in Dulbecco's modified Eagle's medium supplemented with 10 % (v/v) fetal calf serum (HyClone Laboratories, Ogden, UT), 60 mg/ml Penicillin G, and 100 mg/ml streptomycin sulfate maintained at 37 °C in a humidified atmosphere containing about 5 % (v/v) CO_2 in air. All medium components were obtained from Sigma Chemical Co., St. Louis, MO, unless otherwise indicated. Subcultures of cells for screening were grown in the wells of microtiter trays (Falcon Microtest III 96-wells trays, Becton–Dickinson Labware, Lincolin Park, NJ) by suspending cells in medium following trypsin–EDTA treatment, counting the suspension with a hemocytometer, diluting in medium containing 10 % calf serum to 2×10^4 cells per 20 ml culture, aliquoting into each well of a tray, and culturing until confluent. Microtiter trays with confluent monolayer cultures of cells were inverted, the medium shaken out, and replaced with serial dilutions of sterile compounds in triplicate. In each tray, the last row of wells was reserved for controls that were not treated with compounds. Trays were cultured for 96 h. Trays were inverted onto a paper towel pad, and the remaining cells rinsed carefully with medium and fixed with 3.7 % (v/v) formaldehyde in saline for at least 20 min. The fixed cells were rinsed with water, and examined visually. The cytotoxic activity is identified as confluent, relatively unaltered monolayers of stained cells treated with the investigated compounds. Cytotoxicity was estimated as the concentration that caused approximately 50 % loss of the monolayer. 5-fluorouracil was used as a positive control.

Results and discussion

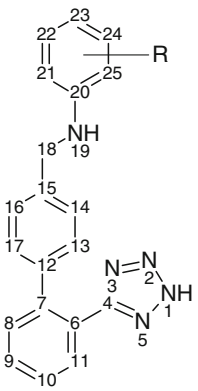
Physicochemical parameters of the compounds are presented in Table 1. All the compounds were colored and

stable in air. They were insoluble in water, but soluble in organic solvents like ethylacetae, ethanol, DMF and DMSO.

FTIR & NMR spectral studies

The important infrared spectral bands and their tentative assignments for the highly yielded biphenyl-based tetrazole compound, 5b, (Supplementary Fig. 1), and its derivatives were recorded as KBr disks, IR spectrum of the compounds 5b showed a characteristic band between 3,417 cm^{-1} confirming the presence of -OH groups of tetrazole compounds and a characteristic band around at 3,336 and 3,227 cm^{-1} confirming the presence of -NH groups of tetrazole and secondary amine respectively. NMR spectral data of compound 5b revealed signals 1.25 δ ppm for -$C\underline{H}_2$NH group, singlet at 4.48 δ ppm for proton of –NH tertazole group, and signals at 7.98 δ ppm for –$\underline{NH}$-CH_2 (Supplementary Fig. 2). The ^{13}C NMR data of compounds 5b revealed signals between 163 δ ppm for –C=N group, at 148 δ ppm for Ar–C–SO_2 group, and at 129, 127, 112, δ ppm for aromatic carbon, ESIMS; M/Z (Supplementary Figs. 3 and 4) (Silverstein and Webster, 1997).

Characterization data of synthesized compounds



*1-[4-({[2′-(2H-tetrazol-5-yl)biphenyl-4-yl] methyl}amino) phenyl]ethanone (**5a**)*

Yield 77 %; m.p. 198–200 °C; ^{1}H NMR (400 MHz, DMSO), 1.27 δ ppm (2H, d, $J = 14.7$ *Hz*, *–CH_2–*NH), 3.4 δ ppm (3H, s, *–CH_3*), 4.46 δ ppm (1H, s, *–NH*, tetrazole), 6.60–7.94 δ ppm (8H, m, *–Ar–H*), 7.99 δ ppm (1H, t, $J = 24.6$ *Hz*, *–NH*CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 44.68 (C-18), 111.5 (C-21,C-25), 126.33 (C-13,C-17), 126.89 (C-8,C-10), 127.06 (C-14,C-16), 128.77 (C-9), 129.15 (C-22,C-24), 134.42 (C-6), 139.69 (C-15), 147.25 (C-20); FTIR (KBr) υ_{max} cm^{-1}: 3338 (–NH tetrazole), 3229 (–NH), 2480 (–C=N), 851 (*p*-disubstituted Ar). MW:-368; ESIMS; M/Z 368.

*4-({[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}amino)benzenesulfonic acid (**5b**)*

Yield 95 %; m.p. 167–168 °C; ^{1}H NMR (400 MHz, DMSO), 1.25 δ ppm (2H, d, $J = 14.6$ *Hz*, *–CH_2–*NH), 3.34 δ ppm (3H, s, -O*CH_3*), 4.48 δ ppm (1H, s, *–NH*, tetrazole), 6.58–7.94 δ ppm (8H, m, *–Ar–H*), 7.98 δ ppm (1H, t, $J = 24.56$ *Hz*, *–NH*CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 46.58 (C-18), 113.5 (C-21,C-25), 128.55 (C-13,C-17), 128.89 (C-8,C-10), 129.06 (C-14,C-16), 131.58 (C-9), 131.96 (C-22,C-24), 136.42 (C-6), 141.59 (C-15), 149.65 (C-20); FTIR (KBr) υ_{max} cm^{-1}: 3417 (-OH), 3336 (–NH tetrazole), 3227 (–NH), 2490 (–C=N), 1028(-S=O), 851 (*p*-disubstituted Ar). MW:-421; ESIMS; M/Z 421.

*4-({[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}amino)phenol (**5c**)*

Yield 81 %; m.p. 194–196 °C; ^{1}H NMR (400 MHz, DMSO), 1.27 δ ppm (2H, d, $J = 14.5$ *Hz*, *–CH_2–*NH), 4.50 δ ppm (1H, s, *–NH*, tetrazole), 6.60–7.96 δ ppm (8H, m, *–Ar–H*), 8.1 δ ppm (1H, t, $J = 24.46$ *Hz*, *–NH*CH_2), 9.1 δ ppm (1H, s, *-OH*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 45.88 (C-18), 112.8 (C-21,C-25), 127.55 (C-13,C-17), 127.92 (C-8,C-10), 128.26 (C-14,C-16), 129.77 (C-9), 129.96 (C-22,C-24), 135.482 (C-6), 140.79 (C-15), 148.68 (C-20); FTIR (KBr) υ_{max} cm^{-1}: 3420 (-OH), 3330 (–NH tetrazole), 3222 (–NH), 2482 (–C=N), 851 (*p*-disubstituted Ar). MW:-343; ESIMS; M/Z 343.

*N-{[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}aniline (**5d**)*

Yield 75 %; m.p. 193–194 °C; ^{1}H NMR (400 MHz, DMSO), 1.30 δ ppm (2H, d, $J = 14.4$ *Hz*, *–CH_2–*NH), 4.52 δ ppm (1H, s, *–NH*, tetrazole), 6.62–7.98 δ ppm (9H, m, *–Ar–H*), 8.2 δ ppm (1H, t, $J = 24.52$ *Hz*, *–NH*CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 44.68 (C-18), 111.4 (C-21,C-25), 126.35 (C-13,C-17), 126.19 (C-8,C-10), 127.16 (C-14,C-16), 128.57 (C-9), 128.86 (C-22,C-24), 134.22 (C-6), 139.69 (C-15), 147.45 (C-20); FTIR (KBr) υ_{max} cm^{-1}: 3340 (–NHtetrazole), 3230 (–NH), 2480 (–C=N), 848 (*p*-disubstituted Ar).MW:-327; ESIMS; M/Z 327.

*2-({[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}amino)benzoic acid (**5e**)*

Yield 83 %; m.p. 210–212 °C; ^{1}H NMR (400 MHz, DMSO), 1.28 δ ppm (2H, d, $J = 14.58$ *Hz*, *–CH_2–*NH), 4.52 δ ppm (1H, s, *–NH*, tetrazole), 6.64–8.0 δ ppm (9H, m, *–Ar–H*), 8.2 δ ppm (1H, t, $J = 24.6$ *Hz*, *–NH*CH_2), 10.2 δ

ppm (1H, s, –*COOH*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 45.88 (C-18), 112.95 (C-21,C-25), 127.85 (C-13,C-17), 128.11 (C-8,C-10), 128.56 (C-14,C-16), 129.97 (C-9), 130.16 (C-22,C-24), 135.82 (C-6), 140.89 (C-15), 148.75 (C-20); FTIR (KBr) υ_{max} cm^{-1}: 3417 (-OH), 3332 (–NH tetrazole), 3220 (–NH), 2482 (–C=N), 1710 (-CO), 846 (*p*-disubstituted Ar). MW:-370; ESIMS; M/Z 370.

*N-{[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}naphthalen-1-amine (**5f**)*

Yield 78 %; m.p. 165–168 °C; ^{1}H NMR (400 MHz, DMSO), 1.23 δ ppm (2H, d, J = *14.7 Hz*, *–CH$_2$–*NH), 4.46 δ ppm (1H, s, *–NH*, tetrazole), 6.56–7.90 δ ppm (11H, m, *–Ar–H*), 7.96 δ ppm (1H, t, J = *24.3 Hz*, *–NH*CH$_2$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 46.78 (C-18), 113.1 (C-21,C-25), 128.15 (C-13,C-17), 128.59 (C-8,C-10), 128.96 (C-14,C-16), 129.17 (C-9), 130.16 (C-22,C-24), 134.12 (C-6), 139.19 (C-15), 147.25 (C-20); FTIR (KBr) υ_{max} cm^{-1}: 3338 (–NH tetrazole), 3225 (–NH), 2478 (–C=N), 845 (*p*-disubstituted Ar). MW:-377; ESIMS; M/Z 377.

*1-phenyl-N-{[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}methanamine (**5g**)*

Yield 79 %; m.p. 172–174 °C; ^{1}H NMR (400 MHz, DMSO), 1.29 δ ppm (2H, d, J = *14.65 Hz*, *–CH$_2$–*NH), 4.52 δ ppm (1H, s, *–NH,* tetrazole), 6.5 δ ppm (1H, *–NH–*CH$_2$), 6.61–7.98 δ ppm (9H, m, *–Ar–H*), 8.1 δ ppm (1H, t, J = *23.9 Hz*, *–NH*CH$_2$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 45.18 (C-18), 112.15 (C-21,C-25), 127.25 (C-13,C-17), 127.19 (C-8,C-10), 128.36 (C-14,C-16), 129.27 (C-9), 129.66 (C-22,C-24), 135.12 (C-6), 140.33 (C-15), 148.45 (C-20); FTIR (KBr) υ_{max} cm^{-1}: 3340 (–NH tetrazole), 3230 (–NH), 2472 (–C=N), 848 (*p*-disubstituted Ar). MW:-341; ESIMS; M/Z 341.

*2-methyl-N-{[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}aniline (**5h**)*

Yield 82 %; m.p. 178–180 °C; ^{1}H NMR (400 MHz, DMSO), 1.22 δ ppm (2H, d, J = *14.56 Hz*, *–CH$_2$–*NH), 3.1 δ ppm (3H, s, *–CH$_3$*), 4.42 δ ppm (1H, s, *–NH*, tetrazole), 6.49–7.80 δ ppm (10H, m, *–Ar–H*), 7.92 δ ppm (1H, t, J = *24.1 Hz*, *–NH*CH$_2$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 46.25 (C-18), 113.5 (C-21,C-25), 128.15 (C-13,C-17), 128.59 (C-8,C-10), 128.86 (C-14,C-16), 129.97 (C-9), 130.46 (C-22,C-24), 136.52 (C-6), 141.69 (C-15), 149.35 (C-20); FTIR (KBr) υ_{max} cm^{-1}: 3332 (–NH tetrazole), 3225 (–NH), 2485 (–C=N), 848 (*p*-disubstituted Ar). MW:-342; ESIMS; M/Z 342.

*3-methyl-N-{[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}aniline (**5i**)*

Yield 84 %; m.p. 184–185 °C; ^{1}H NMR (400 MHz, DMSO), 1.24 δ ppm (2H, d, J = *14.3 Hz*, *–CH$_2$–*NH), 3.13 δ ppm (3H, s, *–CH$_3$*), 4.44 δ ppm (1H, s, *–NH*, tetrazole), 6.51–7.81 δ ppm (10H, m, *–Ar–H*), 7.90 δ ppm (1H, t, J = *24.26 Hz*, *–NH*CH$_2$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 44.28 (C-18), 111.45 (C-21,C-25), 126.55 (C-13, C-17), 126.89 (C-8,C-10), 127.16 (C-14,C-16), 128.77 (C-9), 129.06 (C-22,C-24), 134.22 (C-6), 139.99 (C-15), 147.35 (C-20); FTIR (KBr) υ_{max} cm^{-1}: 3334 (–NH tetrazole), 3228 (-NH), 2488 (–C=N), 852 (*p*-disubstituted Ar). MW:-342; ESIMS; M/Z 342.

*4-methyl-N-{[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}aniline (**5j**)*

Yield 85 %; m.p. 188–190 °C; ^{1}H NMR (400 MHz, DMSO), 1.26 δ ppm (2H, d, J = *14.5 Hz*, *–CH$_2$–*NH), 3.18 δ ppm (3H, s, *–CH$_3$*), 4.46 δ ppm (1H, s, *–NH*, tetrazole), 6.51–7.85 δ ppm (8H, m, *–Ar–H*), 7.94 δ ppm (1H, t, J = *24.15 Hz*, *–NH*CH$_2$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 45.98 (C-18), 112.85 (C-21,C-25), 128.15 (C-13,C-17), 128.59 (C-8,C-10), 128.96 (C-14,C-16), 129.97 (C-9), 129.86 (C-22,C-24), 135.82 (C-6), 141.69 (C-15), 149.35 (C-20); FTIR (KBr) υ_{max} cm^{-1}: 3334 (–NH tetrazole), 3228 (–NH), 2488 (–C=N), 851 (*p*-disubstituted Ar). MW:-342; ESIMS; M/Z 342.

*2-nitro-N-{[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}aniline (**5k**)*

Yield 88 %; m.p. 177–179 °C; ^{1}H NMR (400 MHz, DMSO), 1.25 δ ppm (2H, d, J = *14.6 Hz*, *–CH$_2$–*NH), 4.48 δ ppm (1H, s, *–NH*, tetrazole), 6.58–7.96 δ ppm (10H, m, *–Ar–H*), 7.94 δ ppm (1H, t, J = *24.74 Hz*, *–NH*CH$_2$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 46.68 (C-18), 112.5 (C-21,C-25), 127.95 (C-13,C-17), 128.19 (C-8,C-10), 128.56 (C-14,C-16), 130.17 (C-9), 130.66 (C-22,C-24), 136.22 (C-6), 141.19 (C-15), 149.25 (C-20); FTIR (KBr) υ_{max} cm^{-1}: 3336 (–NH tetrazole), 3227 (–NH), 2485 (–C=N), 1550 (-NO$_2$), 852 (*p*-disubstituted Ar). MW:-372; ESIMS; M/Z 372.

*3-nitro-N-{[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}aniline (**5l**)*

Yield 90 %; m.p. 187–189 °C; ^{1}H NMR (400 MHz, DMSO), 1.27 δ ppm (2H, d, J = *14.2 Hz*, *–CH$_2$–*NH), 4.50 δ ppm (1H, s, *–NH*, tetrazole), 6.60–7.98 δ ppm (10H, m, *–Ar–H*), 7.96 δ ppm (1H, t, J = *24.36 Hz*, *–NH*CH$_2$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 43.88 (C-18), 111.95

Table 2 Antiproliferative activities of target compounds on different cancer cell lines

Compounds	IC_{50} (μM)[a]			
	WI-38	VERO	MCF-7	HEPG-2
5a	92 ± 0.08	80 ± 0.14	70 ± 1.34	67 ± 0.29
5b	85 ± 0.17	88 ± 0.12	71 ± 1.32	60 ± 0.29
5c	92 ± 0.05	89 ± 0.13	55 ± 1.30	67 ± 0.15
5d	88 ± 0.12	84 ± 0.15	86 ± 1.74	66 ± 0.23
5e	97 ± 0.13	90 ± 0.12	60 ± 1.32	56 ± 0.21
5f	82 ± 0.12	70 ± 0.15	64 ± 1.25	70 ± 0.23
5g	95 ± 0.19	88 ± 0.15	78 ± 1.35	63 ± 0.21
5h	40 ± 0.14	39 ± 0.18	58 ± 1.24	52 ± 0.21
5i	110 ± 0.05	95 ± 0.05	30 ± 0.05	25 ± 0.32
5j	115 ± 0.34	95 ± 0.11	20 ± 0.35	15 ± 0.35
5k	105 ± 0.12	85 ± 0.04	30 ± 0.01	25 ± 0.05
5l	80 ± 0.25	90 ± 0.22	35 ± 0.22	30 ± 3.01
5m	75 ± 0.09	85 ± 0.24	40 ± 0.05	30 ± 0.05
5n	105 ± 0.07	125 ± 0.36	25 ± 1.21	10 ± 0.14
5o	100 ± 0.04	105 ± 0.03	25 ± 0.25	20 ± 0.32
5p	90 ± 0.17	90 ± 0.15	50 ± 1.34	40 ± 0.29
5q	60 ± 0.14	56 ± 0.14	50 ± 1.34	47 ± 0.29
5r	23 ± 0.13	19 ± 0.15	30 ± 1.30	41 ± 0.26
5s	80 ± 0.10	85 ± 0.12	70 ± 1.29	50 ± 0.27
5t	78 ± 0.17	69 ± 0.17	80 ± 0.11	94 ± 0.23
5-FU	10 ± 0.05	08 ± 1.02	10 ± 0.48	05 ± 1.36

[a] (IC_{50}, μM): 1–10 (very strong), 11–25 (strong), 26–50 (moderate), 51–100 (weak), 100–200 (very weak), Above 200 (non-cytotoxic)

(C-21,C-25), 126.85 (C-13,C-17), 127.09 (C-8,C-10), 127.66 (C-14,C-16), 128.37 (C-9), 129.06 (C-22,C-24), 133.72 (C-6), 138.89 (C-15), 146.95 (C-20); FTIR (KBr) υ_{max} cm^{-1}: 3332 (–NH tetrazole), 3225 (–NH), 2488 (–C=N), 1555 (-NO_2), 855 (*p*-disubstituted Ar). MW:-372; ESIMS; M/Z 372.

*4-nitro-N-{[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}aniline (**5m**)*

Yield 92 %; m.p. 192–193 °C; ^{1}H NMR (400 MHz, DMSO), 1.22 δ ppm (2H, d, J = *14.4 Hz*, *–CH_2–*NH), 4.46 δ ppm (1H, s, *–NH*, tetrazole), 6.56–7.94 δ ppm (8H, m, *–Ar–H*), 7.92 δ ppm (1H, t, J = *24.28 Hz*, *–NH*CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 46.38 (C-18), 113.15 (C-21,C-25), 128.05 (C-13,C-17), 128.69 (C-8,C-10), 129.06 (C-14,C-16), 130.07 (C-9), 130.86 (C-22,C-24), 136.42 (C-6), 141.69 (C-15), 149.45 (C-20); FTIR (KBr) υ_{max} cm^{-1}: 3334 (–NH tetrazole), 3229 (–NH), 2488 (–C=N), 1558 (-NO_2), 854 (*p*-disubstituted Ar). MW:-372; ESIMS; M/Z 372.

*2-chloro-N-{[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}aniline (**5n**)*

Yield 83 %; m.p. 194–195 °C; ^{1}H NMR (400 MHz, DMSO), 1.24 δ ppm (2H, d, J = *14.55 Hz*, *–CH_2–*NH), 4.50 δ ppm (1H, s, *–NH*, tetrazole), 6.61–7.99 δ ppm (10H, m, *–Ar–H*), 8.1 δ ppm (1H, t, J = *24.16 Hz*, *–NH*CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 45.28 (C-18), 112.5 (C-21,C-25), 127.55 (C-13,C-17), 127.79 (C-8,C-10), 128.26 (C-14,C-16), 129.37 (C-9), 129.66 (C-22,C-24), 135.82 (C-6), 140.89 (C-15), 148.75 (C-20); FTIR (KBr) υ_{max} cm^{-1}: 3338 (–NH tetrazole), 3229 (–NH), 2483 (–C=N), 710 (-C–Cl) 856 (*p*-disubstituted Ar). MW:-362; ESIMS; M/Z 362.

*4-chloro-N-{[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}aniline (**5o**)*

Yield 82 %; m.p. 191–193 °C; ^{1}H NMR (400 MHz, DMSO), 1.26 δ ppm (2H, d, J = *14.52 Hz*, *–CH_2-*NH), 4.52 δ ppm (1H, s, *–NH*, tetrazole), 6.63–7.97 δ ppm (8H, m, *–Ar–H*), 8.12 δ ppm (1H, t, J = *24.52 Hz*, *–NH*CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 46.88 (C-18), 113.45 (C-21,C-25), 128.45 (C-13,C-17), 128.89 (C-8,C-10), 129.06 (C-14,C-16), 129.57 (C-9), 130.06 (C-22,C-24), 136.62 (C-6), 141.49 (C-15), 148.55 (C-20); FTIR (KBr) υ_{max} cm^{-1}: 3336 (–NH tetrazole), 3227 (–NH), 2481 (–C=N), 725 (-C–Cl) 852 (*p*-disubstituted Ar). MW:-362; ESIMS; M/Z 362.

*4-fluoro-N-{[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}aniline (**5p**)*

Yield 79 %; m.p. 197–199 °C; ^{1}H NMR (400 MHz, DMSO), 1.27 δ ppm (2H, d, J = *14.33 Hz*, *–CH_2–*NH), 4.55 δ ppm (1H, s, *–NH,* tetrazole), 6.64–8.1 δ ppm (8H, m, *–Ar–H*), 8.3 δ ppm (1H, t, J = *24.1 Hz*, *–NH*CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 45.73 (C-18), 112.58 (C-21,C-25), 127.35 (C-13,C-17), 127.79 (C-8,C-10), 127.96 (C-14,C-16), 129.77 (C-9), 130.08 (C-22,C-24), 135.62 (C-6), 140.89 (C-15), 148.75 (C-20); FTIR (KBr) υ_{max} cm^{-1}: 3340 (–NH tetrazole), 3233 (–NH), 2486 (–C=N), 1110 (-C–F) 857 (*p*-disubstituted Ar). MW:-345; ESIMS; M/Z 345.

*5-methyl-N-{[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}-1,3-thiazol-2-amine (**5q**)*

Yield 77 %; m.p. 201–203 °C; ^{1}H NMR (400 MHz, DMSO), 1.25 δ ppm (2H, d, J = *14.45 Hz*, *–CH_2–*NH), 2.98 δ ppm (3H, s, *–CH_3)*, 4.46 δ ppm (1H, s, *–NH*, tetrazole), 6.54–7.92 δ ppm (7H, m, *–Ar–H*), 7.96 δ ppm (1H, t, J = *24.22 Hz*, *–NH*CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 44.75 (C-18), 111.3 (C-21,C-25), 126.55 (C-13, C-17), 126.89 (C-8,C-10), 127.26 (C-14,C-16), 128.47

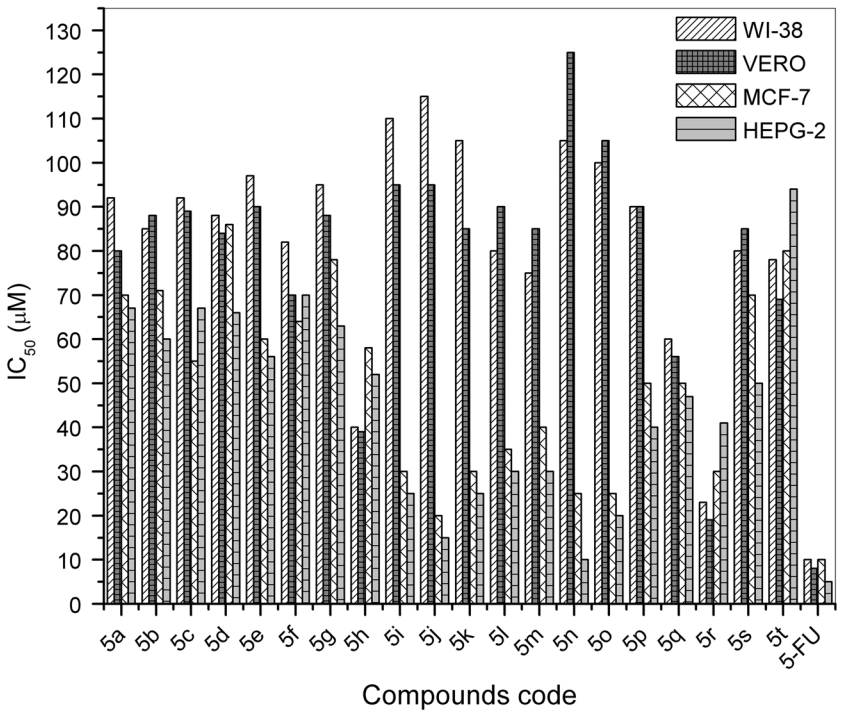


Fig. 1 Comparison of cytotoxic activities of target compounds on different cancer cell lines

(C-9), 128.96 (C-22,C-24), 134.52 (C-6), 139.79 (C-15), 147.65 (C-20); FTIR (KBr) υ_{max} cm^{-1}: 3419 (-OH), 3339 (–NH tetrazole), 3229 (–NH), 2480 (–C=N), 735(-C–S) 848 (*p*-disubstituted Ar). MW:-347; ESIMS; M/Z 347.

*4-methyl-N-{[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}piperazin-1-amine (**5r**)*

Yield 91 %; m.p. 117–122 °C; ^{1}H NMR (400 MHz, DMSO), 1.22 δ ppm (2H, d, $J = 14.1$ *Hz*, $-CH_2$–NH), 3.15 δ ppm (3H, s, $-CH_3$), 4.42 δ ppm (1H, s, *–NH*, tetrazole), 6.48–7.82 δ ppm (6H, m, *–Ar–H*), 7.92 δ ppm (1H, t, $J = 24.46$ *Hz*, *–NH*CH_2), 8.11 δ ppm (4H,m, piperazine ring); ^{13}C NMR (100 MHz, DMSO-d_6): δ 46.88 (C-18), 114.5 (C-21,C-25), 128.45 (C-13,C-17), 128.89 (C-8,C-10), 129.08 (C-14,C-16), 130.17 (C-9), 130.86 (C-22,C-24), 136.42 (C-6), 141.39 (C-15), 149.25 (C-20); FTIR (KBr) υ_{max} cm^{-1}: 3340 (–NH tetrazole), 3230 (–NH), 2470 (–C=N), 855 (*p*-disubstituted Ar). MW:-349; ESIMS; M/Z 349.

*N-{[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}pyridin-2-amine (**5s**)*

Yield 86 %; m.p. 182–183 °C; ^{1}H NMR (400 MHz, DMSO), 1.25 δ ppm (2H, d, $J = 14.2$ *Hz*, $-CH_2$–NH), 4.46 δ ppm (1H, s, *–NH*, tetrazole), 6.52–7.85 δ ppm (6H, m, *–Ar–H*), 7.95 δ ppm (1H, t, $J = 24.52$ *Hz*, *–NH*CH_2), 8.3 δ ppm (4H,m, pyridine ring); ^{13}C NMR (100 MHz, DMSO-d_6): δ 45.58 (C-18), 112.82 (C-21,C-25), 127.35 (C-13,C-17), 127.89 (C-8,C-10), 128.36 (C-14,C-16), 129.27 (C-9), 129.96 (C-22,C-24), 135.82 (C-6), 140.89 (C-15), 148.95 (C-20); FTIR (KBr) υ_{max} cm^{-1}: 3342 (–NH tetrazole), 3228 (–NH), 2468 (–C=N), 848 (*p*-disubstituted Ar). MW:-328; ESIMS; M/Z 328.

*N-{[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}pyridin-3-amine (**5t**)*

Yield 88 %; m.p. 197–198 °C; ^{1}H NMR (400 MHz, DMSO), 1.28 δ ppm (2H, d, $J = 14.36$ *Hz*, $-CH_2$–NH), 4.49 δ ppm (1H, s, *–NH*, tetrazole), 6.55–7.89 δ ppm (6H, m, *–Ar–H*), 7.98 δ ppm (1H, t, $J = 24.68$ *Hz*, *–NH*CH_2), 8.44 δ ppm (4H,m, pyridine ring); ^{13}C NMR (100 MHz, DMSO-d_6): δ 46.68 (C-18), 113.53 (C-21,C-25), 128.45 (C-13,C-17), 128.86 (C-8,C-10), 129.08 (C-14,C-16), 130.17 (C-9), 130.86 (C-22,C-24), 136.52 (C-6), 141.79 (C-15), 149.35 (C-20); FTIR (KBr) υ_{max} cm^{-1}: 3340 (–NH tetrazole), 3225 (–NH), 2462 (–C=N), 845 (*p*-disubstituted Ar). MW:-328; ESIMS; M/Z 328.

Cytotoxicity and antitumor assays

Cytotoxicity was expressed as the concentration that caused a 50 % loss of the cell monolayer (IC_{50}). The

results of our preliminary screening indicated that compounds 5n, 5j, and 5o exhibited a very strong and a selective cytotoxic activity against both HEPG-2 and MCF-7, whereas compounds 5l, 5m, 5p, 5q, and 5r showed a moderate cytotoxic activity against the aforementioned cell lines. Other compounds 5a-h and 5s-t showed a weak cytotoxicity activity (Table 2). Interestingly, compounds 5i and 5k showed a moderate cytotoxic activity against MCF-7 while they had a strong activity against HEPG-2. On the other hand, all tested compounds showed a weak cytotoxic activity against both WI-38 and VERO cell lines (Fig. 1). Subsequently, we may conclude the following structure activity relationships for the promo sing biphenyl-based tetrazole derivatives. The presence of the basic skeleton (tetrazole moiety) is necessary for the broad spectrum of cytotoxic activity toward different cell lines (HEPG-2, WI-38, VERO, and MCF-7). Introducing the halogen group, an electronic negative group, in position 2, 3, or 4 on the benzene ring (e.g., Compounds 5n, 5o, or 5p) showed a strong to moderate cytotoxicity against HEPG-2 and MCF-7 cell lines. Particularly, CL at ortho position gives a very strong cytotoxicity compared to para position. F substitution had a moderate cytotoxic activity compared to chlorine. The presence of NO_2, an electron withdrawing group (strongly deactivating group), in position 2, 3, or 4 of the benzene ring showed a moderate cytotoxicity toward HEPG-2 and MCF-7 cell lines (e.g., Compounds 5k, 5l, or 5m). However, the presence of the electron releasing group, CH_3, in position 2, 3, or 4 showed a strong to moderate cytotoxicity activity toward HEPG-2 and MCF-7 (compounds 5h, 5i, and 5j). Following this further, the presence of electron withdrawing or releasing groups at any subsequent position of the benzene ring in tetrazole motif showed a weak cytotoxicity against WI-38 and VERO. Finally, incorporation of weak electron withdrawing groups, irrespective of their position on benzene ring, did not affect the cytotoxic activity on all tested cell lines.

Conclusion

In conclusion, we have designed hybrid molecules on the basis of the biological significance of biphenyl and tetrazole moieties and evaluated their anticancer activities. Out of a set of 20 molecules, three compounds exhibited a significant and a selective anticancer activity against HEPG-2 as well as MCF-7 that could be used as leads for further investigations in the area of liver and breast cancer therapies.

Acknowledgments We wish to thank Department of Applied Chemistry, SVNIT, Surat for providing laboratory facilities and financial support. We are grateful to Mr. Avtar Singh SAIF Punjab University for spectral analysis.

References

Bret EH, Michael AS (1993) A new method for the preparation of tetrazoles from nitriles using trimethylsilylazide/trimethylaluminum. Tetrahedron Lett 34:8011–8014

Choi SJ, Oh JM, Choy JH (2008) Anticancer drug-layered hydroxide nanohybrids as potent cancer chemotherapy agents. J Phys Chem Solids 69:1528–1532

Grigor YV, Gaponik PN, Koldobskii GI (2001) Tritylation of tetrazole and 5-substituted tetrazoles with triphenylmethanol. Russ J Org Chem 37:1670–1671

Kiyoto K, Toshikazu O, Sunao M, Ryozo OKK, Oga T, Mitsui S, Orita R (1998) Novel synthesis of 5-substituted tetrazoles from nitriles. Synthesis 6:910

Murli Krishna PD, Narayana R,Surendra KN, Yugandar PB (2011) US 0275828 A1

Rapp M, Giraud I, Maurizis JC, Madelmont JC (2003) Synthesis and pharmacokinetic profile of a quaternary ammonium derivative of chlorambucil, a potential anticancer drug for the chemotherapy of chondrosarcoma. Bioorg Med Chem 6:5007–5012

Sauer J, Huisgen R, Sturm HJ (1960) Zur acylierung von 5-aryltetrazolen; ein duplikationsverfahren zur darstellung von polyarylen. Tetrahedron 11:241–251

Silverstein RM, Webster FX (1997) Spectrometric identification of organic compounds, 6th edn. Wiley, New York, pp 79–223

Somisetti NR, Sudhaker KB (2011) An improved and efficient synthesis of Irbesartan, an antihypertensive drug. Opt Commun 4:105–111

Steven J, Wittenberger B (1993) Dialkyltin oxide mediated addition of trimethylsilyl azide to nitriles. A novel preparation of 5-substituted tetrazols. J Org Chem 58:4139–4141

Yuancai D, Si-Shen F (2005) Poly(d, l-lactide-co-glycolide)/montmorillonite nanoparticles for oral delivery of anticancer drugs. Biomaterials 26:6068–6076