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Original article

Synthesis, antitumor activity and SAR study of novel [1,2,4]triazino[4,5-*a*] benzimidazole derivatives

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ABSTRACT

A series of novel 1,2,3,4-tetrahydro[1,2,4]triazino[4,5-*a*]benzimidazoles carrying variety of aryl and heteroaryl groups at position 1 were synthesized. The newly synthesized compounds were tested *in vitro* on human breast adenocarcinoma cell line (MCF7). Some of the test compounds showed potent antitumor activity, especially compound **3c** [1-(2-chlorophenyl) derivative] which displayed the highest activity among the test compounds.

1. Introduction

Breast cancer (BC) is the most frequent cause of cancer among women in both developed and undeveloped societies. It is also the primary cause of cancer death among women worldwide. Its incidence has been rising in several developing countries over the past few years [1–5].

Currently, many anticancer drugs are used clinically and indeed many of these compounds are used successfully for the treatment of several neoplastic diseases such as leukemia or testicular cancer. However, their effect on solid tumors such as BC has been poor. Since the response of solid tumors to available anticancer chemotherapy has been reduced, new drugs with improved efficacy are desired.

Benzimidazole derivatives represent one of the chemical classes that showed potent anticancer activity especially against breast cancer cell line (MCF7) [6–10]. Benzimidazole nucleus was used as biomimetic of guanine residues and many benzimidazole derivatives were reported to selectively inhibit endothelial cell growth and suppress angiogenesis both *in vitro* and *in vivo* [11].

Recently, many fused benzimidazoles were reported to exhibit promising anticancer activity [12–19]. Several publications reported the synthesis and biological activities of [1,2,4]triazino[2,3-*a*] benzimidazoles as well as [1,2,4]triazino[4,3-*a*]benzimidazoles [12–16]. However, little work had been published on the biological activity of [1,2,4]triazino[4,5-*a*]benzimidazoles [13,17–19].

In 2008, Styskala et al. [17] reported the anticancer activity of 2-aryl-1,2-dihydro[1,2,4]triazino[4,5-*a*]benzimidazol-1-one derivatives **I** (Fig. 1) against several cell lines including breast cancer cell line (MCF7). The authors stated that the synthesized compounds showed preferential activity against solid tumors compared to leukemia cells. They also studied the effect of the 2-aryl substituents and reported that compounds with the lipophilic substituents phenyl, 4-methylphenyl and 4-methoxyphenyl were highly active, while, substitutions with 4-chlorophenyl or 4-nitrophenyl groups substantially decreased the cytotoxic potency [17]. Since then, no further study of the anticancer activity or the effect of substituents on other positions of this tricyclic nucleus had been published.

Encouraged by these findings, we aim in this study to synthesize the novel tricyclic 1,2,3,4-tetrahydro[1,2,4]triazino[4,5-*a*]benzimidazole derivatives as a new cytotoxic lead structure. The aim also involves studying the effect of the different aryl and heteroaryl groups at position 1 of the nucleus on the anticancer activity against breast cancer cell line (MCF7).

2. Results and discussion

2.1. Chemistry

The target compounds depicted in Scheme 1 were obtained by reacting the starting material 2-(chloromethyl)-1*H*-benzimidazole

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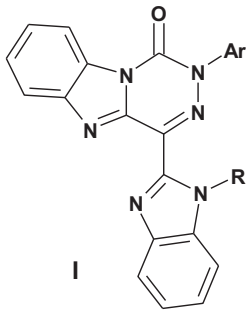


I

Ar = C_6H_5, 4-$CH_3C_6H_4$, 4-$CH_3OC_6H_4$, 4-ClC_6H_4, 4-$NO_2C_6H_4$

R= H, $COOC_2H_5$

Fig. 1. Structure of previously synthesized potent antitumor [1,2,4]triazino[4,5-*a*] benzimidazoles.

(**1**) with 4 molar equivalent of hydrazine hydrate (99%) for 45 min to give 2-(hydrazinylmethyl)-1*H*-benzimidazole (**2**) in 75% yield.

The formation of compound **2** was confirmed by ^{1}H NMR that showed two exchangeable singlet signals at δ 4.38 and 10.38 ppm corresponding to NH_2 and NH protons, respectively. Moreover, the mass spectrum of compound **2** displayed molecular ion peak at *m*/*z* 162 (M^+).

Searching the literature revealed that no previous work had been published on the cyclization reactions of 2-(hydrazinylmethyl)-1*H*-benzimidazole to afford the tricyclic [1,2,4]triazino [4,5-*a*]benzimidazole.

In this work, 2-(hydrazinylmethyl) derivative **2** was subjected to cycloaddition condensation reaction using different aryl and heteroaryl aldehydes in ethanol and triethylamine to give 1-aryl-1,2,3,4-tetrahydro[1,2,4]triazino[4,5-*a*]benzimidazoles **3a**–**s**.

^{1}H NMR spectra of compounds **3a**–**s** revealed the disappearance of the NH_2 signal at δ 4.38 and the appearance of two exchangeable singlet signals at δ 10–12 ppm corresponding to two NH protons. Besides, a singlet signal appeared at δ 4–5 ppm corresponding to CH proton at position 1.

The mass spectra of compounds **3a**–**s** showed the corresponding molecular ion peaks and most of them were characterized by the presence of peaks due to $[ArCHNH]^+$.

A possible mechanism for the formation of compounds **3a**–**s** was outlined in Fig. 2.

2.2. In vitro anticancer screening

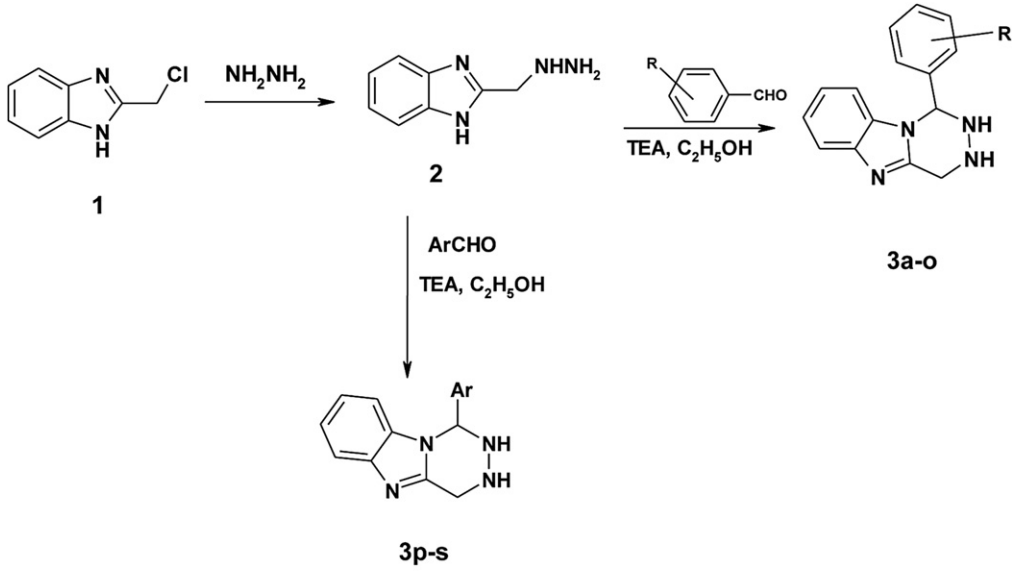
All the synthesized tricyclic derivatives were evaluated for their *in vitro* cytotoxic activity against human breast cancer cell line, MCF7, using Sulforhodamine-B stain (SRB) assay [20].

The relationship between surviving fraction of MCF7 and drug concentration was plotted and the response parameter IC_{50} was calculated. IC_{50} value corresponds to the concentration required for 50% inhibition of cell viability. The IC_{50} of the test compounds are shown in Table 1 and the results are represented graphically in Fig. 3.

Structurally, the test compounds differ only in the nature of the aryl group at position 1 of the tricyclic nucleus, being (substituted phenyl) in compounds **3a**–**o** and heteroaryl in compounds **3p**–**s**.

The obtained data revealed that most of the synthesized compounds showed potent antitumor activity. The best result was obtained by compound **3c** [R = 2-Cl]. Whilst, compound **3h** [R = 3-OH] exhibited the least cytotoxic activity among the test compounds.

Regarding the 1-(substituted phenyl) derivatives **3a**–**o**, better result was obtained by the 2-substituted derivatives (**3c**, **3g**, **3l** and **3n**). While, the 3-substituted derivatives (**3h** and **3m**) demonstrated the least antitumor activity against MCF7 cell line. Besides, better results were obtained by electron withdrawing groups especially at the 2 position of the benzene nucleus (**3l** and **3n**) and also by halides [Br and Cl] (**3b**–**3d**).



R= H, 4-Br, 2-Cl, 4-Cl, 4-$N(CH_3)_2$, 4-F, 2-OH, 3-OH, 3-CH_3O-4-OH, 2-CH_3O, 4-CH_3O, 2-NO_2, 3-NO_2, 2-CF_3, 3,4,5-$(CH_3O)_3$

Ar= 3-indolyl, 2-pyridyl, 4-pyridyl, 2-thienyl

Scheme 1.

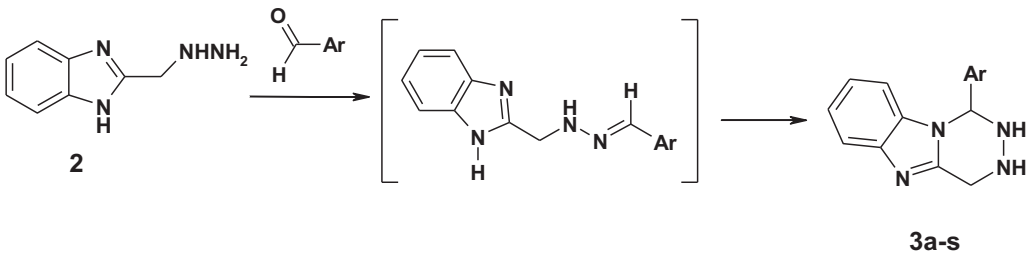


Fig. 2. A possible mechanism for the formation of compound **3a**–**s**.

On the other hand, the 1-(heteroaryl) derivatives **3p**–**s** showed better antitumor activities compared to the unsubstituted phenyl derivative **3a**. The six membered pyridine derivatives (**3q** and **3r**) showed better antitumor activity than the five membered thienyl or the indole derivative. Within the pyridyl derivatives, the 4-pyridyl derivative **3r** exhibited better cytotoxic activity than the 2-pyridyl one **3q**.

The results of the anticancer screening suggested the following general structural requirements for the anti-proliferative action of 1-aryl-1,2,3,4-tetrahydro[1,2,4]triazino[4,5-*a*]benzimidazoles on MCF7 cell line:

1. An *ortho*-substitution on the aromatic nucleus at position 1 with electron withdrawing groups like CF_3 and NO_2 or halogens especially Cl enhanced the anticancer activity.
2. Substitutions at the *meta*-position reduced the anti-proliferative activity.
3. The heteroaryl groups exhibited similar anticancer activity compared to the substituted phenyl groups.

3. Conclusion

The aim of the present study was to synthesize novel 1,2,3,4-tetrahydro[1,2,4]triazino[4,5-*a*]benzimidazoles carrying different aryl and heteroaryl groups at position 1 in order to examine the effect of the substitution at position 1 on the antitumor activity. The newly synthesized compounds were tested *in vitro* on human breast adenocarcinoma cell line (MCF7). Some of the test compounds showed potent antitumor activity, especially compound **3c** [1-(2-chlorophenyl) derivative] which displayed the highest activity among the test compounds. The results of the anticancer screening suggested the following general conclusions: 1) An *ortho*-substitution on the aromatic nucleus at position 1 with electron withdrawing groups like CF_3 and NO_2 or halogens especially Cl enhanced the anticancer activity. 2) Substitutions at the *meta*-position reduced the anti-proliferative activity. 3) The heteroaryl groups exhibited similar anticancer activity compared to the substituted phenyl groups. Up to our knowledge, this is the first published work on the synthesis and the antitumor activity of 1-aryl-1,2,3,4-tetrahydro[1,2,4]triazino [4,5-*a*]benzimidazoles.

Table 1
Results of *in vitro* cytotoxic activity of the synthesized compounds on human breast adenocarcinoma cell line (MCF7).

Compound no.	IC_{50} in μM[a]	Compound no.	IC_{50} in μM[a]
3a	0.0752	**3k**	0.0639
3b	0.0547	**3l**	0.0505
3c	0.0390	**3m**	0.0711
3d	0.0545	**3n**	0.0518
3e	0.0713	**3o**	0.0529
3f	0.0746	**3p**	0.0702
3g	0.0583	**3q**	0.0501
3h	0.0835	**3r**	0.0450
3i	0.0766	**3s**	0.0675
3j	0.0703		

[a] The values given are means of three experiments.

4. Experimental part

4.1. General

Melting points were determined using a Griffin apparatus and were uncorrected. IR spectra were recorded on Shimadzu IR 435 spectrophotometer and values were represented in cm^{-1}. ^1H NMR and ^{13}C NMR were carried out on Varian Gemini 300 MHz spectrophotometer, Cairo University, Cairo, Egypt, using TMS as an internal standard and chemical shifts were recorded in ppm on δ scale. The electron impact (EI) mass spectra were recorded on Hewlett Packard 5988 spectrometer, Microanalytical center, Cairo University, Cairo, Egypt. Analytical thin layer chromatography (TLC) on silica gel plates containing UV indicator was employed routinely to follow the course of reactions and to check the purity of products. All reagents and solvents were purified and dried by standard techniques.

4.1.1. 2-(Hydrazinylmethyl)-1H-benzimidazole (**2**)

2-(Chloromethyl)-1*H*-benzimidazole (**1**) (3.33 g, 20 mmol) and hydrazine hydrate (99%, 4 mL, 80 mmol) were heated under reflux for 45 min. The solid formed on hot was cooled, triturated with ethanol (95%, 20 mL), filtered, dried and crystallized from ethanol. Yield: 75%; mp: 197–198 °C; IR (cm^{-1}): 3302, 3190 (NH/NH_2), 2920, 2893 (CH aliphatic); ^1H NMR (DMSO-d_6) δ ppm 3.91 (s, 2H, CH_2), 4.38 (br s, 2H, NH_2, D_2O exchangeable), 6.48–6.84 (m, 4H, Ar–***H***), 9.27 (s, 1H, NH, D_2O exchangeable), 10.38 (s, 1H, NH, D_2O exchangeable); MS *m*/*z*: 162 [M^+, 6.58%], 161 [($M^+ - 1$), 64.55%], 133 [100%].

4.1.2. General procedure for the synthesis of 1-aryl-1,2,3,4-tetrahydro[1,2,4]triazino[4,5-a]benzimidazoles ***3a–s***

A mixture of 2-(hydrazinylmethyl)-1*H*-benzimidazole (**2**) (0.25 g, 1.5 mmol) and the appropriate aldehyde (1.5 mmol) in absolute ethanol (10 mL) and triethylamine (1 mL) were heated under reflux for 12 h. The separated solid was filtered, dried and crystallized from acetic acid.

*4.1.2.1. 1-Phenyl-1,2,3,4-tetrahydro[1,2,4]triazino[4,5-a]benzimidazole (**3a**).* Yield: 86%; mp: 253–254 °C; IR (cm^{-1}): 3371, 3186 (NH), 2918, 2870 (CH aliphatic); ^1H NMR (DMSO-d_6) δ ppm 3.98 (s, 2H, CH_2), 4.48 (s, 1H, CH), 6.48–8.23 (m, 9 H, Ar–H), 10.41 (s, 1H, NH, D_2O exchangeable), 11.58 (s, 1H, NH, D_2O exchangeable); MS *m*/*z*: 250 [M^+, 9.94%], 249 [$(M-1)^+$, 11.60%], 173 [$(M-C_6H_5)^+$, 10.50%], 160 [$(M-C_6H_5CH)^+$, 62.43%], 133 [100%], 105 [$C_6H_5CHNH^+$, 7.37%], 90 [$C_6H_5CH^+$, 11.79%], 77 [$C_6H_5^+$, 37.94%].

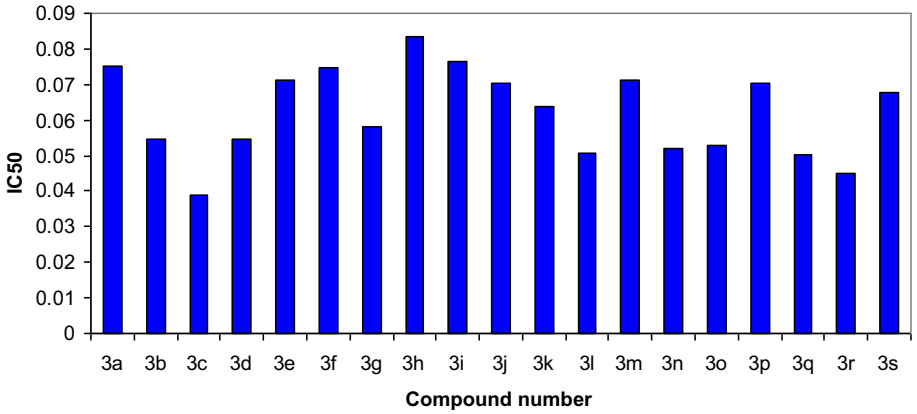


Fig. 3. IC_{50} of the synthesized compounds in μM on the human breast adenocarcinoma cell line (MCF7).

*4.1.2.2. 1-(4-Bromophenyl)-1,2,3,4-tetrahydro[1,2,4]triazino[4,5-a] benzimidazole (**3b**).* Yield: 79%; mp: 268–269 °C; IR (cm^{-1}): 3429, 3190 (NH), 2924, 2854 (CH aliphatic); ^1H NMR (DMSO-d_6) δ ppm 3.96 (s, 2H, CH_2), 4.48 (s, 1H, CH), 6.45–8.20 (m, 8 H, Ar–H), 10.40 (s, 1H, NH, D_2O exchangeable), 11.63 (s, 1H, NH, D_2O exchangeable); MS *m/z*: 330 [$(M + 2)^+$, 6.86%], 328 [M^+, 4.62%], 221 [100%], 185 [$^{81}BrC_6H_4CHNH^+$, 4.41%], 183 [$^{79}BrC_6H_4CHNH^+$, 3.50%], 157 [$^{81}BrC_6H_4^+$, 8.52%], 155 [$^{79}BrC_6H_4^+$, 8.08%], 76 [$C_6H_4^+$, 20.04%].

*4.1.2.3. 1-(2-Chlorophenyl)-1,2,3,4-tetrahydro[1,2,4]triazino[4,5-a] benzimidazole (**3c**).* Yield: 72%; mp: 266–267 °C; IR (cm^{-1}): 3448, 3190 (NH), 2920, 2854 (CH aliphatic); ^1H NMR (DMSO-d_6) δ ppm 4.92 (s, 1H, CH), 5.35 (s, 2H, CH_2), 7.14–8.59 (m, 8 H, Ar–H), 12.00 (s, 1H, NH, D_2O exchangeable), 12.21 (s, 1H, NH, D_2O exchangeable); MS *m/z*: 286 [$(M+2)^+$, 0.54%], 284 [M^+, 2.29%], 173 [$(M-ClC_6H_4)^+$, 1.20%], 160 [$(M-ClC_6H_4CH)^+$, 1.64%], 145 [$(M-ClC_6H_4CHNH)^+$, 1.74%], 69 [100%].

*4.1.2.4. 1-(4-Chlorophenyl)-1,2,3,4-tetrahydro[1,2,4]triazino[4,5-a] benzimidazole (**3d**).* Yield: 83%; mp: 271–272 °C; IR (cm^{-1}): 3271, 3190 (NH), 2912, 2858 (CH aliphatic); ^1H NMR (DMSO-d_6) δ ppm 3.97 (s, 2H, CH_2), 4.54 (s, 1H, CH), 6.47–8.53 (m, 8 H, Ar–H), 10.41 (s, 1H, NH, D_2O exchangeable), 11.81 (s, 1H, NH, D_2O exchangeable); MS *m/z*: 286 [$(M + 2)^+$, 2.28%], 284 [M^+, 7.52%], 249 [$(M-Cl)^+$, 12.26%], 165 [100%], 160 [$(M-ClC_6H_4CH)^+$, 6.55%], 141 [$^{37}ClC_6H_4CHNH^+$, 6.97%], 139 [$^{35}ClC_6H_4CHNH^+$, 18.57%], 113 [$^{37}ClC_6H_4^+$, 26.58%], 111 [$^{35}ClC_6H_4^+$, 79.25%], 76 [$C_6H_4^+$, 17.84%].

*4.1.2.5. 1-(4-Dimethylaminophenyl)-1,2,3,4-tetrahydro[1,2,4]triazino [4,5-a]benzimidazole (**3e**).* Yield: 81%; mp: 237–238 °C; IR (cm^{-1}): 3200, 3178 (NH), 2897, 2854 (CH aliphatic); ^1H NMR (DMSO-d_6) δ ppm 3.33 (s, 6H, CH_3), 3.98 (s, 2H, CH_2), 4.42 (s, 1H, CH), 6.44–8.07 (m, 8 H, Ar–H), 10.39 (s, 1H, NH, D_2O exchangeable), 11.26 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ ppm 40.2 (CH_3), 53.0 (C-4), 72.0 (C-1), 114.7, 117.8, 121.2, 122.8, 126.5, 128.2, 129.3, 134.8, 144.2, 151.2 (aromatic carbons); MS *m/z*: 293 [M^+, 1.60%], 148 [$(CH_3)_2NC_6H_4CHNH^+$, 21.02%], 133 [$(CH_3)_2NC_6H_4CH^+$, 100%].

*4.1.2.6. 1-(4-Flourophenyl)-1,2,3,4-tetrahydro[1,2,4]triazino[4,5-a] benzimidazole (**3f**).* Yield: 84%; mp: 235–236 °C; IR (cm^{-1}): 3383, 3190 (NH), 2985, 2920 (CH aliphatic); ^1H NMR (DMSO-d_6) δ ppm 3.97 (s, 2H, CH_2), 4.47 (s, 1H, CH), 6.45–8.23 (m, 8 H, Ar–H), 10.40 (s, 1H, NH, D_2O exchangeable), 11.58 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ ppm 52.6 (C-4), 72.0 (C-1), 111.1, 115.6, 115.9, 117.9, 122.8, 127.0, 129.0, 130.6, 146.0 (aromatic carbons); MS *m/z*: 268 [M^+, 0.93%], 123 [$FC_6H_4CHNH^+$, 17.98%], 108 [$FC_6H_4CH^+$, 10.19%], 95 [$FC_6H_4^+$, 61.72%], 76 [$C_6H_4^+$, 5.11%], 57 [100%].

*4.1.2.7. 1-(2-Hydroxyphenyl)-1,2,3,4-tetrahydro[1,2,4]triazino[4,5-a] benzimidazole (**3g**).* Yield: 70%; mp: 280–281 °C; IR (cm^{-1}): 3352, 3221, 3182 (NH/OH), 2924, 2854 (CH aliphatic); ^1H NMR (DMSO-d_6) δ ppm 3.97 (s, 2H, CH_2), 4.45 (s, 1H, CH), 6.56–8.44 (m, 8 H, Ar–H), 10.06 (s, 1H, OH, D_2O exchangeable), 10.40 (s, 1H, NH, D_2O exchangeable), 11.50 (s, 1H, NH, D_2O exchangeable); MS *m/z*: 266 [M^+, 1.97%], 121 [$HOC_6H_4CHNH^+$, 5.05%], 106 [$HOC_6H_4CH^+$, 5.00%], 64 [100%].

*4.1.2.8. 1-(3-Hydroxyphenyl)-1,2,3,4-tetrahydro[1,2,4]triazino[4,5-a] benzimidazole (**3h**).* Yield: 51%; mp: 273–274 °C; IR (cm^{-1}): 3421, 3309, 3194 (NH/OH), 2924, 2850 (CH aliphatic); ^1H NMR (DMSO-d_6) δ ppm 3.98 (s, 2H, CH_2), 4.47 (s, 1H, CH), 6.57–8.44 (m, 8 H, Ar–H), 10.06 (s, 1H, OH, D_2O exchangeable), 10.40 (s, 1H, NH, D_2O exchangeable), 11.78 (s, 1H, NH, D_2O exchangeable); MS *m/z*: 266 [M^+, 0.18%], 121 [$HOC_6H_4CHNH^+$, 12.79%], 106 [$HOC_6H_4CH^+$, 2.29%], 93 [$HOC_6H_4^+$, 14.37%], 76 [$C_6H_4^+$, 3.68%], 58 [100%].

*4.1.2.9. 1-(4-Hydroxy-3-methoxyphenyl)-1,2,3,4-tetrahydro[1,2,4]triazino[4,5-a]benzimidazole (**3i**).* Yield: 20%; mp: 247–248 °C; IR (cm^{-1}): 3417, 3275, 3217 (NH/OH), 2927, 2839 (CH aliphatic); ^1H NMR (DMSO-d_6) δ ppm 3.83 (s, 3H, CH_3), 4.83 (s, 1H, CH), 5.31 (s, 2H, CH_2), 6.80–8.08 (m, 7 H, Ar–H), 9.55 (s, 1H, OH, D_2O exchangeable), 11.65 (s, 1H, NH, D_2O exchangeable), 12.21 (s, 1H, NH, D_2O exchangeable); MS *m/z*: 296 [M^+, 54.07%], 295 [$(M - 1)^+$, 38.52%], 294 [$(M - 2)^+$, 46.67%], 151 [4–OH–3-$CH_3OC_6H_3CHNH^+$, 42.22%], 109 [100%].

*4.1.2.10. 1-(2-Methoxyphenyl)-1,2,3,4-tetrahydro[1,2,4]triazino[4,5-a]benzimidazole (**3j**).* Yield: 83%; mp: 233–234 °C; IR (cm^{-1}): 3394, 3209 (NH), 2924, 2854 (CH aliphatic); ^1H NMR (DMSO-d_6) δ ppm 3.86 (s, 3H, OCH_3), 4.87 (s, 1H, CH), 5.32 (s, 2H, CH_2), 6.81–8.53 (m, 8 H, Ar–H), 10.36 (s, 1H, NH, D_2O exchangeable), 11.78 (br s, 1H, NH, D_2O exchangeable); MS *m/z*: 280 [M^+, 8.29%], 135 [$CH_3OC_6H_4CHNH^+$, 100%].

*4.1.2.11. 1-(4-Methoxyphenyl)-1,2,3,4-tetrahydro[1,2,4]triazino[4,5-a]benzimidazole (**3k**).* Yield: 87%; mp: 250–251 °C; IR (cm^{-1}): 3437, 3190 (NH), 2924, 2839 (CH aliphatic); ^1H NMR (DMSO-d_6) δ ppm 3.80 (s, 3H, OCH_3), 4.88 (s, 1H, CH), 5.31 (s, 2H, CH_2), 7.00–8.01 (m, 8 H, Ar–H), 11.69 (s, 1H, NH, D_2O exchangeable), 12.20 (s, 1H, NH,

D_2O exchangeable); MS *m/z*: 280 [M^+, 2.50%], 279 [$(M-1)^+$, 12.09%], 173 [$(M-CH_3OC_6H_4)^+$, 15.16%], 160 [$(M-CH_3OC_6H_4CH)^+$, 11.52%], 135 [$CH_3OC_6H_4CHNH^+$, 6.91%], 133 [$CH_3OC_6H_4CN^+$, 100%], 120 [$CH_3OC_6H_4CH^+$, 7.29%], 107 [$CH_3OC_6H_4^+$, 9.60%], 76 [$C_6H_4^+$, 5.18%].

*4.1.2.12. 1-(2-Nitrophenyl)-1,2,3,4-tetrahydro[1,2,4]triazino[4,5-a] benzimidazole (**3l**).* Yield: 73%; mp: 246–247 °C; IR (cm^{-1}): 3275, 3190 (NH), 2924, 2854 (CH aliphatic), 1523, 1342 (NO_2); ^1H NMR (DMSO-d_6) δ ppm 3.96 (s, 2H, CH_2), 4.48 (s, 1H, CH), 6.47–8.38 (m, 8 H, Ar–H), 10.41 (s, 1H, NH, D_2O exchangeable), 11.84 (s, 1H, NH, D_2O exchangeable); MS *m/z*: 295 [M^+, 0.11%], 150 [$NO_2C_6H_4CHNH^+$, 1.02%], 135 [$NO_2C_6H_4CH^+$, 35.13%], 76 [$C_6H_4^+$, 46.60%], 57 [100%].

*4.1.2.13. 1-(3-Nitrophenyl)-1,2,3,4-tetrahydro[1,2,4]triazino[4,5-a] benzimidazole (**3m**).* Yield: 78%; mp: 283–284 °C; IR (cm^{-1}): 3336, 3190 (NH), 2920, 2854 (CH aliphatic), 1523, 1350 (NO_2); ^1H NMR (DMSO-d_6) δ ppm 3.96 (s, 2H, CH_2), 4.48 (s, 1H, CH), 6.45–8.00 (m, 8 H, Ar–H), 10.41 (s, 1H, NH, D_2O exchangeable), 11.63 (s, 1H, NH, D_2O exchangeable); MS *m/z*: 295 [M^+, 52.94%], 173 [$(M-NO_2C_6H_4)^+$, 60.73%], 150 [$NO_2C_6H_4CHNH^+$, 55.88%], 76 [$C_6H_4^+$, 55.88%], 57 [100%].

*4.1.2.14. 1-[(2-Triflouromethyl)phenyl]-1,2,3,4-tetrahydro[1,2,4]triazino[4,5-a]benzimidazole (**3n**).* Yield: 74%; mp: 248–249 °C; IR (cm^{-1}): 3213, 3155 (NH), 2920, 2858 (CH aliphatic); ^1H NMR (DMSO-d_6) δ ppm 3.96 (s, 2H, CH_2), 4.52 (s, 1H, CH), 6.49–8.58 (m, 8 H, Ar–H), 10.41 (s, 1H, NH, D_2O exchangeable), 11.81 (s, 1H, NH, D_2O exchangeable); MS *m/z*: 318 [M^+, 0.18%], 158 [$F_3CC_6H_4CH^+$, 4.20%], 145 [$F_3CC_6H_4^+$, 12.20%], 133 [100%].

*4.1.2.15. 1-(3,4,5-Trimethoxyphenyl)-1,2,3,4-tetrahydro[1,2,4]triazino [4,5-a]benzimidazole (**3o**).* Yield: 95%; mp: 223–224 °C; IR (cm^{-1}): 3228, 3159 (NH), 2939, 2839 (CH aliphatic); ^1H NMR (DMSO-d_6) δ ppm 3.69 (s, 3H, OCH_3), 3.82 (s, 6H, OCH_3 two groups), 3.98 (s, 2H, CH_2), 4.49 (s, 1H, CH), 6.47–8.15 (m, 6 H, Ar–H), 10.40 (s, 1H, NH, D_2O exchangeable), 11.54 (s, 1H, NH, D_2O exchangeable); MS *m/z*: 340 [M^+, 3.04%], 221 [100%], 195 [$(CH_3O)_3C_6H_2CHNH^+$, 10.12%], 180 [$(CH_3O)_3C_6H_2CH^+$, 13.61%], 160 [$(M-(CH_3O)_3C_6H_2CH)^+$, 5.30%].

*4.1.2.16. 1-(1H-Indol-3-yl)-1,2,3,4-tetrahydro[1,2,4]triazino[4,5-a] benzimidazole (**3p**).* Yield: 90%; mp: 262–263 °C; IR (cm^{-1}): 3290, 3197, 3155 (NH), 2931, 2897 (CH aliphatic); ^1H NMR (DMSO-d_6) δ ppm 3.96 (s, 2H, CH_2), 4.44 (s, 1H, CH), 6.39–8.30 (m, 9 H, Ar–H), 10.33 (s, 1H, NH, D_2O exchangeable), 11.15 (s, 1H, NH, D_2O exchangeable), 11.48 (s, 1H, NH, D_2O exchangeable); MS *m/z*: 289 [M^+, 65.82%], 288 [$(M-1)^+$, 73.42%], 213 [100%].

*4.1.2.17. 1-(Pyridin-2-yl)-1,2,3,4-tetrahydro[1,2,4]triazino[4,5-a]benzimidazole (**3q**).* Yield: 67%; mp: 208–209 °C; IR (cm^{-1}): 3336, 3201 (NH), 2939, 2870 (CH aliphatic); ^1H NMR (DMSO-d_6) δ ppm 3.89 (s, 2H, CH_2), 4.43 (s, 1H, CH), 6.39–8.52 (m, 8 H, Ar–H), 10.32 (s, 1H, NH, D_2O exchangeable), 11.68 (s, 1H, NH, D_2O exchangeable); MS *m/z*: 251 [M^+, 21.53%], 160 [$(M-C_5H_4NCH)^+$, 22.34%], 145 [$(M-C_5H_4NCHNH)^+$, 16.89%], 133 [100%], 106 [$C_5H_4NCHNH^+$, 25.07%], 91 [$C_5H_4NCH^+$, 31.06%], 78 [$C_5H_4N^+$, 41.96%].

*4.1.2.18. 1-(Pyridin-4-yl)-1,2,3,4-tetrahydro[1,2,4]triazino[4,5-a]benzimidazole (**3r**).* Yield: 92%; mp: 216–217 °C; IR (cm^{-1}): 3421, 3194 (NH), 2970, 2850 (CH aliphatic); ^1H NMR (DMSO-d_6) δ ppm 4.08 (s, 2H, CH_2), 4.58 (s, 1H, CH), 6.48–8.63 (m, 8 H, Ar–H), 10.21 (s, 1H, NH, D_2O exchangeable), 11.84 (s, 1H, NH, D_2O exchangeable); MS *m/z*: 251 [M^+, 5.20%], 221 [100%], 160 [$(M-C_5H_4NCH)^+$, 9.90%], 106 [$C_5H_4NCHNH^+$, 9.27%], 91 [$C_5H_4NCH^+$, 11.06%], 78 [$C_5H_4N^+$, 17.00%].

*4.1.2.19. 1-(Thiophen-2-yl)-1,2,3,4-tetrahydro[1,2,4]triazino[4,5-a]benzimidazole (**3s**).* Yield: 73%; mp: 252–253 °C; IR (cm^{-1}): 3200, 3194 (NH), 2947, 2850 (CH aliphatic); ^1H NMR (DMSO-d_6) δ ppm 3.97 (s, 2H, CH_2), 4.37 (s, 1H, CH), 6.43–8.44 (m, 7 H, Ar–H), 10.39 (s, 1H, NH, D_2O exchangeable), 11.51 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ ppm 53.0 (C-4), 70.5 (C-1), 117.9, 118.4, 122.8, 126.8, 127.8, 128.8, 130.9, 134.7, 138.7, 142.0 (aromatic carbons); MS *m/z*: 256 [M^+, 1.60%], 133 [100%], 111 [$C_4H_3SCHNH^+$, 3.52%], 96 [$C_4H_3SCH^+$, 16.90%].

4.2. Biological testing

4.2.1. Materials and methods

The human breast adenocarcinoma cell line (MCF7) was obtained as a gift from NCI, MD, USA.

All chemicals and solvents were purchased from Sigma–Aldrich.

4.2.2. Measurement of potential cytotoxicity

The cytotoxic activity of the newly synthesized compounds was examined *in vitro* on human breast adenocarcinoma cell line (MCF7) using Sulforhodamine-B stain (SRB) assay applying the method of Skehan et al. [20].

Cells were plated in 96-multiwell plate (10^4 cells/well) for 24 h before treatment with the test compounds to allow attachment of the cells to the wall of the plate. Test compounds were dissolved in DMSO and diluted with saline to the appropriate volume. Different concentrations of the test compound (5, 12.5, 25 and 50 μg/mL) were added to the cell monolayer. Triplicate wells prepared for each individual dose. Monolayer cells were incubated with the test compound for 48 h at 37 °C in atmosphere of 5% CO_2. After 48 h, cells were fixed with trichloroacetic acid, washed with water and stained for 30 min with 0.4% (wt/vol) Sulforhodamine-B stain dissolved with 1% acetic acid. Excess stain was removed by four washes with 1% acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was measured in ELISA reader. The relation between surviving fraction and compound concentration was plotted and IC_{50} [the concentration required for 50% inhibition of cell viability] was calculated for each compound and results are given in Table 1 and represented graphically in Fig. 3.

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