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ORIGINAL ARTICLE

Design, synthesis, and molecular docking studies of new [1,2,4] triazolo[4,3-*a*]quinoxaline derivatives as potential A2B receptor antagonists

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Abstract

Many shreds of evidence have recently correlated A2B receptor antagonism with anticancer activity. Hence, the search for an efficient A2B antagonist may help in the development of a new chemotherapeutic agent. In this article, 23 new derivatives of [1,2,4]triazolo[4,3-*a*]quinoxaline were designed and synthesized and its structures were confirmed by different spectral data and elemental analyses. The results of cytotoxic evaluation of these compounds showed six promising active derivatives with IC_{50} values ranging from 1.9 to 6.4 µM on MDA-MB 231 cell line. Additionally, molecular docking for all synthesized compounds was performed to predict their binding affinity toward the homology model of A2B receptor as a proposed mode of their cytotoxic activity. Results of molecular docking were strongly correlated with those of the cytotoxic study. Finally, structure activity relationship analyses of the new compounds were explored.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11030-020-10070-w) contains supplementary material, which is available to authorized users.

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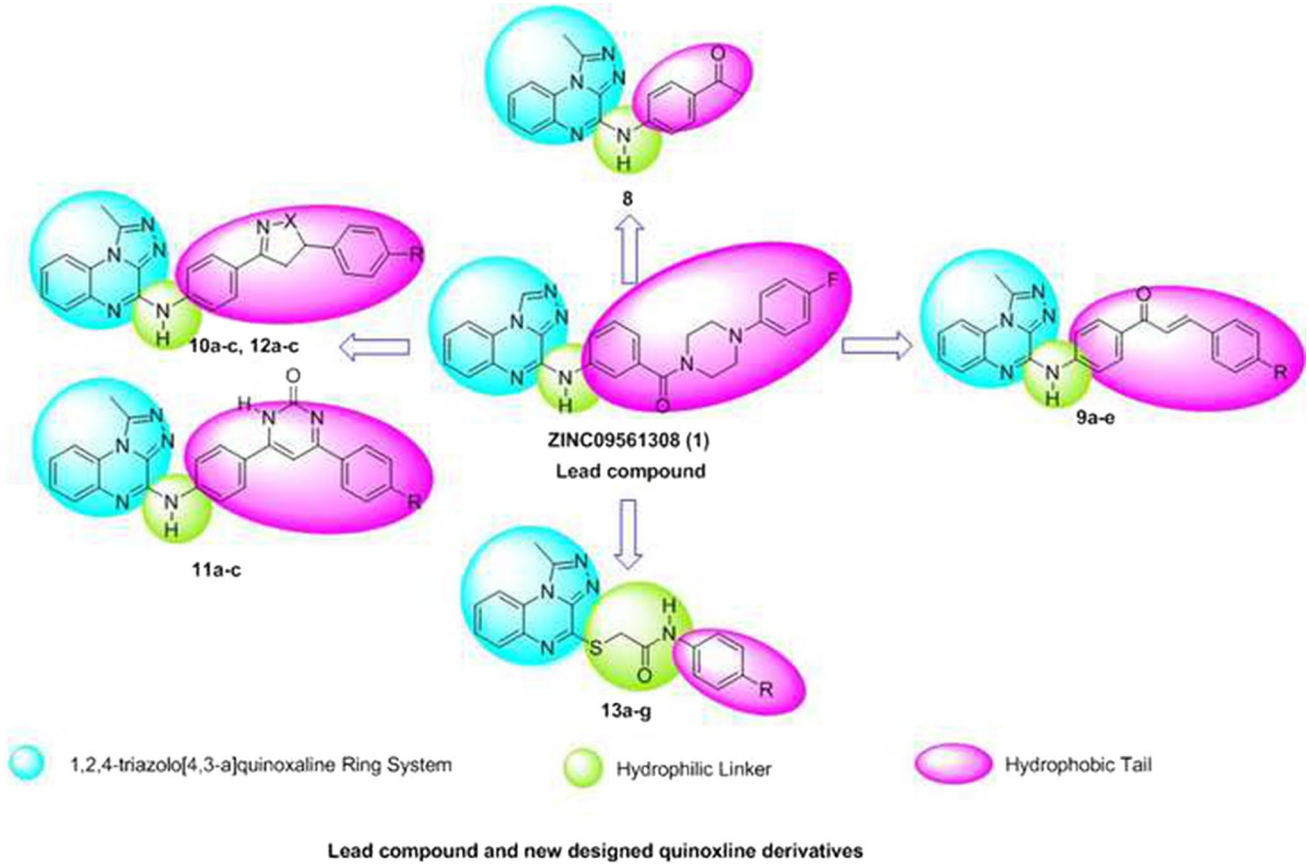
Graphic abstract



Keywords Design · Docking · Quinoxaline · A2B antagonists

Introduction

Adenosine is the metabolite of intracellular and extracellular ADP and ATP and considered as a mediator for a wide range of events under both normal and pathological conditions [1]. There are four subtypes of adenosine receptors known as A1, A2A, A2B, and A3. Each type of these adenosine receptors has its unmatched pharmacological profile [2, 3]. Anticancer activity is one of the medicinal features of adenosine receptors and their four subtypes [4]. Unlike A1, A2A, and A3 adenosine receptors, the A2B subtype requires a high level of adenosine for activation. Thus, the A2B receptors may mediate pathophysiological conditions associated with the cumulative level of adenosine as in the case of tumors and ischemia [1, 5–7]. A2B receptors are expressed in the human microvascular endothelial cells, where they may regulate the angiogenic factors such as basic fibroblast growth factor and vascular endothelial growth factor and subsequently angiogenesis, which is one of the major mechanisms for tumor growth regulation [8]. Also, blockade of human adenosine A2B receptors was reported to play a significant rule in the reduction in metastasis and regulation of oral squamous cell carcinoma [9]. Medicinal chemistry approaches have been applied to all the adenosine receptor subtypes (A1, A2A, A2B, and A3) to create selective antagonists for each [1]. Currently, there is a significant increase in the interest in A2B receptors in different therapeutic areas [10, 11]. Nevertheless, the compendium of potent ligands targeting A2B

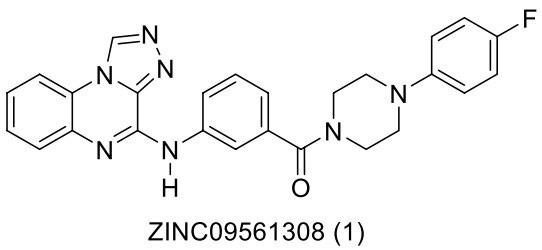


Fig. 1 Reported hit quinoxaline derivatives as potent A2B receptor antagonist

receptors is still rare [12]. An evidence for the expression of A2B adenosine receptors in the human breast adenocarcinoma, 92020424 (MDA-MB-231) cells, has been proved [7]. Consequently, virtual screening of potential ligands using the adenosine A2B receptor homology model has been accomplished. Some of these hits are related to quinoxaline nucleus. (4-([1,2,4]Triazolo[4,3-*a*]quinoxalin-4-ylamino) phenyl)(4-(4-fluorophenyl)piperazin-1-yl)methanone, compound **1** (Fig. 1), has been used as a template for ongoing research [13].

Furthermore, [1,2,4]triazolo[4,3-*a*]quinoxaline is an important pharmacophore ring system, presented in a number of anticancer agents [14, 15]. Over the last few years, our research group was interested in the design and synthesis of novel anticancer heterocycles [16–20]. As an extension to our previous researches and depending on the upper mentioned facts, we are reporting here the design, synthesis, structural confirmation, and docking studies for the novel [1,2,4]triazolo[4,3-*a*]quinoxaline derivatives as potent inhibitors for A2B receptors. The protocol was designed through construction of the [1,2,4]triazolo[4,3-*a*]quinoxaline ring system of the hit compound with chloro-substituent at position 4 for subsequent replacement with either aniline derivatives or another hydrophilic spacer attached with hydrophobic heads. Different linkers and terminals have been inserted to investigate the effect on the cytotoxic activity (Fig. 2). Moreover, a molecular docking study was performed to elucidate the virtual binding mode of the synthesized compounds using the homology model of the human adenosine A2B receptor. Finally, the structure–activity relationship of the synthesized compounds was illustrated based on the results of cytotoxic evaluation against MDA-MB-231 cell lines as a model for A2B adenosine receptor subtype [7].

Results and discussion

Rational design

The core structure of hit compound **1** consists of 1,2,4-triazolo[4,3-a]quinoxaline ring system attached with hydrophilic NH linker at C-4 followed by hydrophobic tail [13]. The predicted binding mode of hit compound shows that the compound targets nine non-conserved amino acid residues, namely Asn273, Leu81, Lys170, Val256, Ala271, Asn266, Lys269, Lys267, and Val250. The tricyclic moiety of the compound is anchored by aromatic stacking interactions with Trp247, Phe173, and His251 and hydrophobic interaction with Val85, Leu86, Met182, Val253, and Ile276 (Fig. 3). These interactions are essential for compound affinity to the receptor.

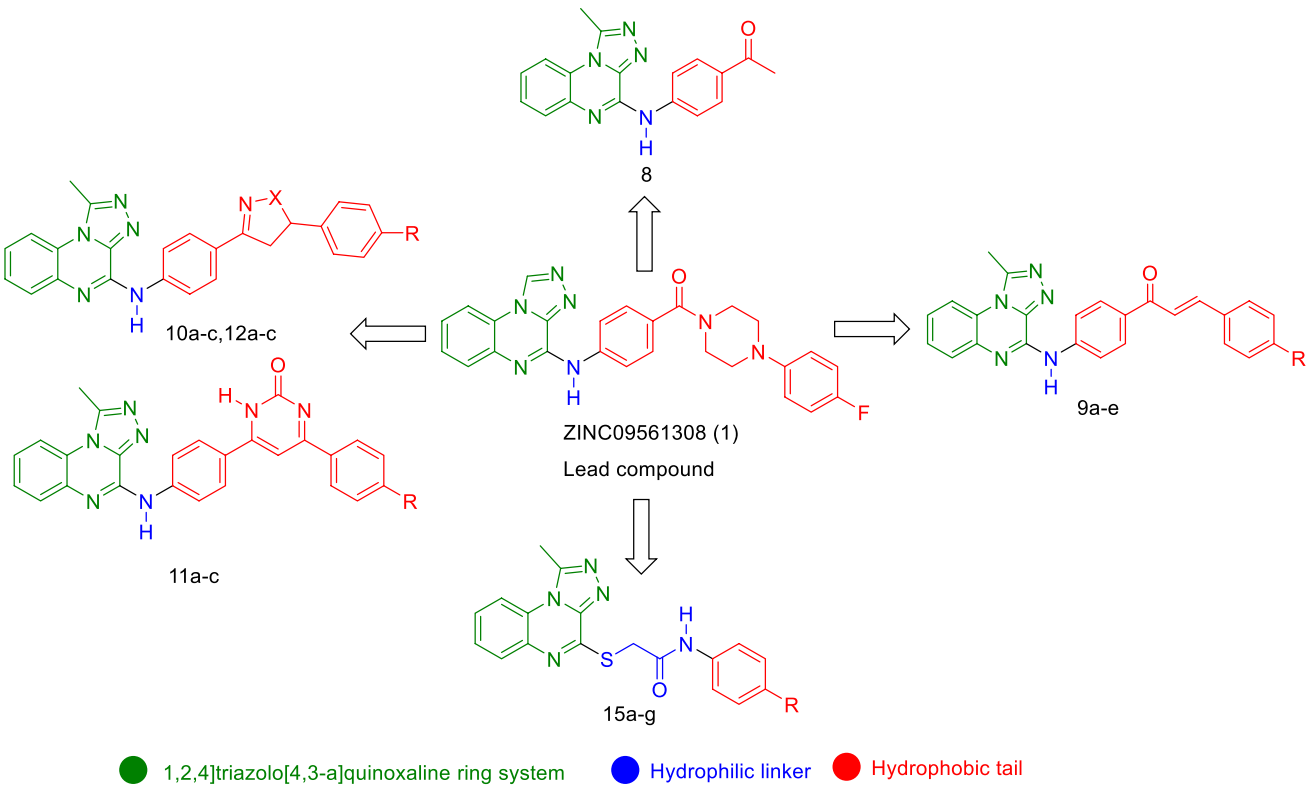


Fig. 2 Lead compound and newly designed quinoxaline derivatives

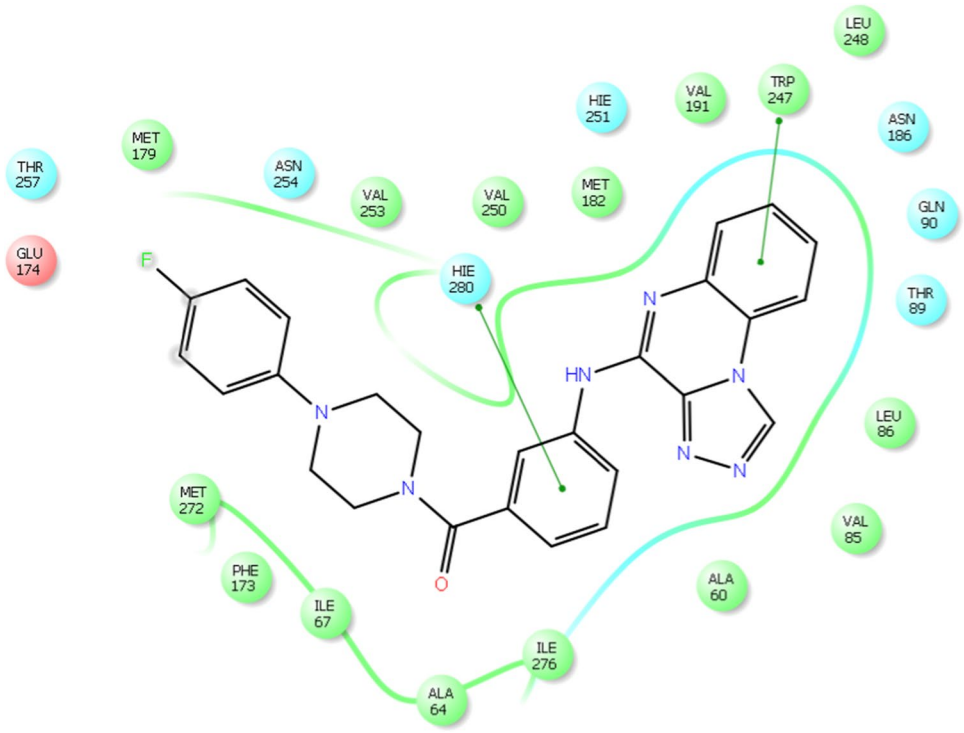


Fig. 3 Predicted binding mode for hit compound 1 with the homology model of the human adenosine A2B receptor [13]

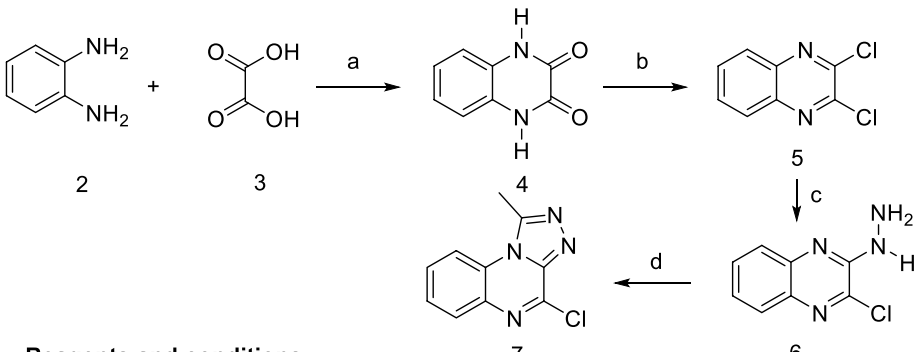
The main objective of the present work was the design and synthesis of new 1,2,4-triazolo[4,3-*a*]quinoxaline derivatives with the same essential structural features of the reported hit compound and almost the same binding mode. There are only two bioisosteric modifications in our designed new derivatives. First is the replacement of the hydrophilic NH linker at C-4 with another hydrophilic one in which both the length and number of hydrogen bond acceptors are increased. The second is the replacement of terminal hydrophobic tail with different hydrophobic moieties including substituted phenyl, heteroaryl, and styryl moieties. This variety of modifications enabled us to study the SAR of these new derivatives as effective anticancer agents with potential A2B antagonistic activity which is one of our major objectives of this work.

Materials and methods

Chemistry

Starting 4-chloro-1-methyl-[1,2,4]triazolo[4,3-*a*]quinoxaline (**7**) was synthesized as outlined in Scheme 1. Oxalic acid in 4N aqueous HCl (50 mL) was allowed to react with *o*-phenylenediamine [21], and the resulting quinoxaline-2,3(1*H*,4*H*)-dione **4** was treated with thionyl chloride in dichloroethane [22] to afford 2,3-dichloroquinoxaline **(5)**.

Scheme 1 Synthetic protocol of starting 4-chloro-1-methyl-[1,2,4]triazolo[4,3-*a*] quinoxaline



Reagents and conditions

a) 4N HCl, reflux, 2 h, 92%; b) $SOCl_2$, DMF, 1,2-DCE, reflux, 2 h, 85%

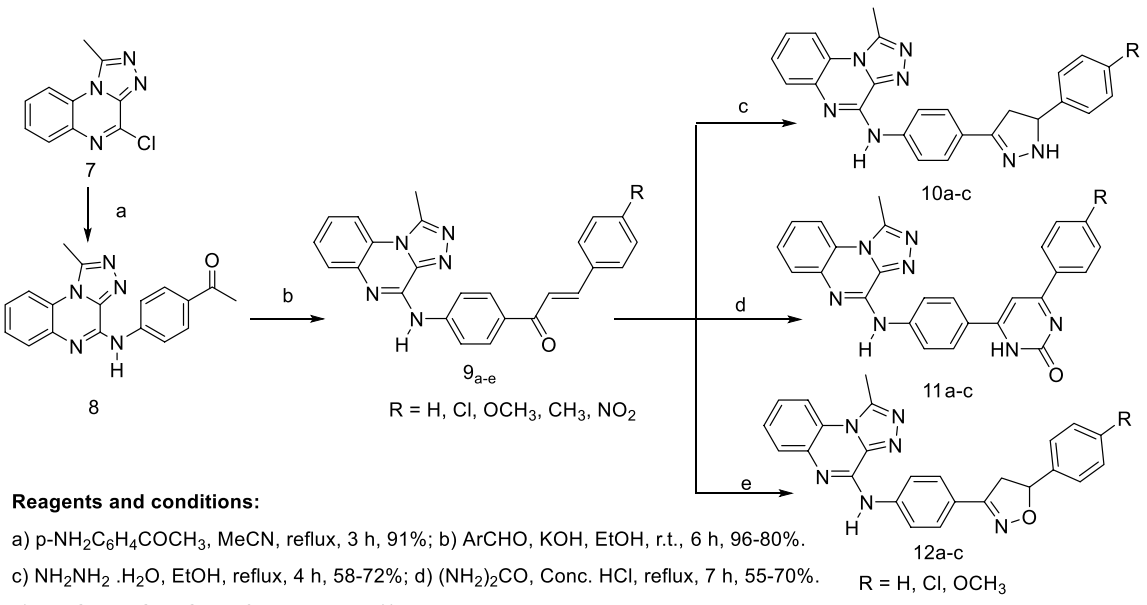
c) NH_2NH_2 .H_2O, EtOH, r.t., 16 h, 90%; d) Ac_2O, reflux, 2 h, 80%

2-Chloro-3-hydrazinylquinoxaline **(6)** was obtained through the stirring of **5** with hydrazine hydrate at room temperature [23]. Subsequent heating compound **6** with acetic anhydride produced our title compound 4-chloro-1-methyl-[1,2,4] triazolo[4,3-*a*]quinoxaline [22].

The obtained 4-chloro-1-methyl-[1,2,4]triazolo[4,3-a] quinoxaline **(7)** was refluxed with *p*-aminoacetophenone in dry acetonitrile to get the acetyl derivative **8** (Scheme 2). The IR spectrum of compound **8** showed two absorption bands at 3417 and 1670 cm^{-1} corresponding to NH and C=O. Claisen–Schmidt condensation [24] of **8** with *p*-substituted benzaldehydes in an ethanolic solution of KOH produced the chalcone derivatives **9a–e**. These two consecutive steps successfully afforded the final products **8** and **9a–e** in satisfying yields, almost over 70% and reasonable purities. The IR spectra of these chalcones are characterized by the presence of C=O stretching bands at 1594–1649 cm^{-1} which appeared at low absorption values because of extended conjugation with the double bond. The absolute geometry of the α, β-unsaturated carbonyl linker was assigned to be in *trans* configuration based on the coupling constants of alkene protons. The ^{1}H NMR spectrum of compound **9c** as a representative example exhibited two doublets, each equivalent to one proton: One doublet at 6.80 ppm corresponds to α proton and another doublet at 7.94 ppm corresponds to β proton. Both have the same coupling constant value of $J = 15$ Hz, which confirms the *trans* configuration.

1,3-Cycloaddition of hydrazine, urea, and hydroxylamine to chalcones **9a–c** produced the corresponding 4,5-dihydro-1*H*-pyrazole **10a–c**, pyrimidin-2(1*H*)-one **11a–c**, and 4,5-dihydroisoxazole derivatives **12a–c**. The IR spectrum of **10a** as a representative example of **10a–e** is characterized by the presence of absorption band at 3349 cm^{-1} corresponding to NH and disappearance of the strong absorption band at 1694 cm^{-1} corresponding to carbonyl group of the starting chalcone. The ^{1}H NMR spectrum exhibited singlet peak at 9.86 ppm corresponding to NH next to phenyl ring which is D_2O exchangeable, singlet peak at 5.59 ppm corresponding to NH proton of pyrazole ring which is also exchangeable with D_2O, triplet peak at 4.84 ppm corresponding to CH proton of pyrazole nucleus, and two doublets at 3.02 and 3.31 ppm corresponding to CH_2 proton of pyrazole ring. The IR spectra of compounds **11a–c** are characterized by the presence of absorption bands at 3449 cm^{-1} corresponding to NH and disappearance of the C=O stretching absorption bands of the starting chalcones. The ^{1}H NMR spectra of this series exhibited two singlet signals at the range of 10.50–8.50 ppm corresponding to NH proton next to quinoxaline ring and the amide NH proton of pyrimidine ring. The IR spectra of isoxazole derivatives **12a–c** are also characterized by the presence of the NH absorption bands at the expected wavenumbers and disappearance of C=O stretching bands. The ^{1}H NMR spectra of these isoxazoles revealed singlet signals at about 9.40 corresponding to NH proton next to quinoxaline ring and triplet signals at 8.50 ppm for CH_2 proton of isoxazole ring.

Alternatively, 4-chloro-1-methyl-[1,2,4]triazolo[4,3-*a*]quinoxaline **(7)** was converted into 1-methyl-[1,2,4] triazolo[4,3-*a*]quinoxaline-4-thiol by the action of thiourea and subsequently to its potassium salt according to the



Scheme 2 Synthetic protocol of target compounds **8–12a–c**

directions of reported procedures [22]. Reaction of the latter with different derivatives of α-chloro-*N*-phenylacetamide [23] gave our target compounds **15a–g**. In general, all reactions proceeded smoothly and final products **10–15** were obtained in relatively good yields as detailed in the "Experimental" part. The IR spectra of these *N*-phenylacetamide derivatives are characterized by the presence of amidic C=O absorption bands at 1661–1698 cm^{-1} in addition to the NH stretching at 3267–3479 cm^{-1} region. The ^{1}H NMR spectra of these compounds showed the diagnostic NHC=O D_2O exchangeable singlet signals at about 10.25 ppm as well as another singlet of the deshielded proton of SCH_2 at 4.45–4.50 ppm (Scheme 3).

Biological evaluation

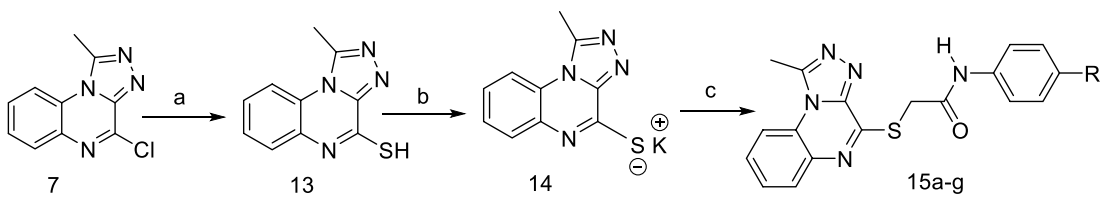
Production of adenosine is a mechanism by which tumors suppress immune surveillance. Activation of host adenosine A2B receptors has been found to inhibit the rejection of solid tumors and promote metastasis. Adenosine A2B receptor activation is expected to support tumor growth by stimulating the release of basic fibroblast growth factor and vascular endothelial growth angiogenic factors from vascular smooth muscle, endothelial cells, and host immune cells [8]. From this point and considering the role of adenosine as a crucial factor in determining the cell progression pathway, the cytotoxic activity has been selected for the pharmacological evaluation of the newly synthesized A2B adenosine receptor antagonists. The selective expression of high levels of endogenous A2B receptors coupled to two signaling pathways makes MDA-MB-231 cells a suitable model for A2B adenosine receptor subtype [7]. The A2B receptor in such cancer cells may serve as a target to control cell growth and proliferation [7].

Experimental

Chemistry

General

Melting points were taken on an electrothermal (Stuart SMP30) apparatus and were uncorrected. IR spectra were recorded on a Pye Unicam SP 1000 IR spectrophotometer at Pharmaceutical Analytical Unit, Faculty of Pharmacy, Al-Azhar University. ^{1}H NMR and ^{13}C NMR spectra were recorded in: (1) DMSO-d_6 at 300/100 MHz on a Varian Mercury VXR-300 NMR spectrometer at NMR Lab, Faculty of Science, Cairo University, (2) DMSO-d_6 and $CDCl_3$ at 400 MHz on a Joel ECA spectrometer at NMR Lab, Microanalytical Unit, Faculty of Pharmacy, Cairo University, (3) $CDCl_3$ at 400 MHz on a Joel ECA spectrometer at NMR Lab, Microanalytical Unit, Faculty of Pharmacy, Cairo University, and (4) DMSO-d_6 at 400 MHz at the Main Chemical Warfare Laboratories, Chemical Warfare Department, Ministry of Defense. Chemical shifts were related to that of the solvent, and TMS was used as an internal standard. Mass spectra and microanalyses were carried out at Regional Center for Mycology Biotechnology, Al-Azhar University, Cairo, Egypt. Progresses of the reactions were monitored by TLC using TLC sheets precoated with UV fluorescent silica gel Merck 60 F254 plates and were visualized using UV lamp and different solvents as mobile phases. Starting reagents, oxalic acid, *o*-phenylenediamine, phosphorus oxychloride, hydrazine hydrate, acetic anhydride, *p*-aminoacetophenone, *p*-substituted benzaldehydes, α-chloroacetyl chloride, and *p*-substituted aniline derivatives were purchased from Aldrich Chemical Company and were used as received. Compounds **5** and **6** were synthesized according to the direction of reported procedures [22].



Reagents and conditions:

a) NH_2CSNH_2, EtOH, reflux,4 h, 65%; b) alc. KOH, EtOH, r.t., 1h; c) $ClCH_2CONHAr$, dioxane, r.t., 1.5 h, 52-72%.

Scheme 3 Synthetic protocol of target compounds **15a–g**

Synthesis of 1-(4-(1-methyl-[1,2,4]triazolo[4,3-*a*] quinoxalin-4-ylamino)phenyl)ethanone (8)

A mixture of 4-chloro-1-methyl-[1,2,4]triazolo[4,3-*a*] quinoxaline (0.5 g, 0.1 mol) and *p*-aminoacetophenone (0.11 mol) in dry acetonitrile was heated under reflux for 3 h, set aside to room temperature, and then filtered to give **8** as faint brown needles and pure enough to be used without further purification. Yield: 91%; m.p. 238–240 °C. IR cm^{-1} (KBr): 3417 (NH), 3132 (C–H aromatic), 2976 (C–H aliphatic), 1786 (C=O). ^{1}H NMR ppm ($CDCl_3$, 400 MHz): 9.61 (s, 1H, NH), 8.25 (d, 2H, Ph-H_2, H_6), 8.01 (d, 1H, QX-H6), 7.94 (d, 1H, QX-H9), 7.86 (t, 1H, QX-H8), 7.65 (t, 1H, QX-H7), 7.18 (d, 2H, Ph-H_3, H_5), 4.21 (s, 3H, $COCH_3$), 2.36 (s, 3H, CH_3). MS (m/z): 317 ($C_{18}H_{15}N_5O$, 100%, M+), 302 ($C_{17}H_{12}N_5O$, 4.21%), 275 ($C_{16}H_{12}N_5$, 36.99%), 198 ($C_{10}H_8N_5$, 1.59%), 183 ($C_{10}H_7N_4$, 2.72%), 131 ($C_8H_7N_2$, 7.14%), 76.1 (C_6H_4, 8.22%), 43.06 (C_2H_3O, 7.06%). Anal. Calcd; C, 68.13; H, 4.76; N, 22.07, found: C, 68.07; H, 4.66; N, 21.93.

General procedure for synthesis of (2*E*)-1-(4-(1-methyl-[1,2,4]triazolo[4,3-*a*] quinoxalin-4-ylamino)phenyl)-3-arylprop-2-en-1-one (9a–e)

A mixture of the appropriate aromatic aldehyde (0.012 mol) and acetyl derivative **8** (0.01 mol) dissolved in ethanol (70 mL) was slowly added to the aqueous solution of potassium hydroxide (0.0128 mol) in water (10 mL). The reaction mixture was stirred on a crushed ice bath for 0.5 h. The temperature is gradually elevated into 20–25 °C with continuous stirring for further 5 h. The mixture was filtered, and the resulting solid was washed with cold water. The product was finally crystallized from ethanol to give the corresponding chalcones **9a–e**.

(2*E*)-1-(4-(1-methyl-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-ylamino)phenyl)-3-phenylprop-2-en-1-one (9a) White solid; yield: 80%; m.p. 230–232 °C. IR cm^{-1} (KBr): 3446 (NH), 3267 (C–H alkenes), 3109 (C–H aromatic), 2970 (C–H aliphatic), 1647 (C=O), 1530 (C=C). ^{1}H NMR ppm ($CDCl_3$, 400 MHz): 9.61 (s, 1H, NH), 8.45 (d, 1H, QX-H6), 8.09 (d, 2H, Ph-H_2, H6), 8.02 (d, 1H, COCH=CH, J = 9.1), 7.93 (t, 1H, QX-H8), 7.87 (t, 1H, QX-H7), 7.74 (d, 1H, QX-H9), 7.66 (d, 1H, COCH=CH, J = 9.1), 7.52 (d, 2H, Ph-H3, H5), 7.38 (d, 2H, Ph-H2, H6), 7.28 (t, 2H, Ph-H3, H5), 6.91 (t, 1H, Ph-H4), 1.57 (s, 3H, CH_3). ^{13}C NMR ppm ($CDCl_3$, 100 MHz): 14.70, 112.09, 120.38, 123.65, 128.01, 128.12, 128.51, 128.80, 129.95, 130.05, 131.18, 132.90, 132.87, 135.28, 136.63, 137.62, 151.05, 146.30, 155.81, 157.89, 189.20. MS (m/z): 317 ($C_{25}H_{19}N_5O$, 100%, M+), 405 ($C_{25}H_{19}N_5O$, 14.67%), 275 ($C_{16}H_{12}N_5$, 100%), Anal. Calcd; C, 74.06; H, 4.72; N, 17.27, found: C, 74.06.98; H, 4.56; N, 17.03.

(2*E*)-1-(4-(1-methyl-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-ylamino)phenyl)-3-(4-chlorophenyl)prop-2-en-1-one (9b) Orange solid; yield: 78%; m.p. 251–253 °C. IR cm^{-1} (KBr): 3454 (NH), 3250 (C–H alkenes), 3094 (C–H aromatic), 2984 (C–H aliphatic), 1600 (C=O), 1525 (C=C). ^{1}H NMR ppm ($CDCl_3$, 400 MHz): 9.91 (s, 1H, NH), 8.44 (d, 1H, QX-H6), 8.07 (d, 2H, Ph-H2, H6), 8.04 (d, 1H, COCH=CH, J = 9.0), 7.91 (t, 1H, QX-H8), 7.85 (t, 1H, QX-H7), 7.72 (d, 1H, QX-H9), 7.62 (d, 1H, COCH=CH–, J = 9.0), 7.50 (d, 2H, Ph-H3, H5), 7.33 (d, 2H, Ph-H2, H6), 6.61 (t, 2H, Ph-H3, H5), 1.61 (s, 3H, CH_3). ^{13}C NMR ppm ($CDCl_3$, 100 MHz): 14.70, 112.09, 120.10, 123.93, 125.90, 128.11, 127.15, 128.44, 129.35, 130.28, 131.54, 132.33, 132.98, 135.71, 136.25, 137.89, 150.52, 155.18, 157.89, 161.59, 187.70. MS (m/z):439 ($C_{25}H_{18}ClN_5O$, 2.97%, M+), 441 ($C_{25}H_{18}ClN_5O$, 0.89%, M + 2), 425 ($C_{24}H_{15}ClN_5O$, 86.53%), 401 ($C_{25}H_{18}N_5O$, 2.35%), 318 ($C_{15}H_{14}N_5O$, 3.21%), 302 ($C_{17}H_{12}N_5O$, 1.16%), 273 ($C_{16}H_{12}N_5$, 0.79%), 257 ($C_{15}H_{12}ClNO$, 15.05%), 138 (C_8H_6Cl, 61.99%), 131 ($C_8H_7N_2$, 9.41%), 111 (C_6H_4Cl, 14.87%), 76.08 (C_6H_4, 13.65%). Anal. Calcd. C, 68.26; H, 4.12; N, 15.92, found: C, 68.29; H, 4.15; N, 15.95.

(2*E*)-1-(4-(1-methyl-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-ylamino)phenyl)-3-(4-methoxyphenyl) prop-2-en-1-one (9c) Faint yellow solid; yield: 80%; m.p. 221–223 °C. IR cm^{-1} (KBr): 3441 (NH), 3215 (C–H alkenes), 3094 (C–H aromatic), 2984 (C–H aliphatic), 1649 (C=O), 1556 (C=C). ^{1}H NMR ppm (DMSO, 400 MHz): 9.86 (s, 1H, NH), 8.30 (d, 1H, QX-H6), 8.18 (d, 2H, Ph-H2, H6), 8.02 (d, 1H, COCH=CH, J = 12.0), 7.95 (t, 1H, QX-H8), 7.83 (t, 1H, QX-H7), 7.69 (d, 1H, QX-H9), 7.60 (d, 1H, COCH=CH, J = 12.0), 7.48 (d, 2H, Ph-H3, H5), 7.41 (d, 2H, Ph-H2, H6), 6.70 (t, 2H, Ph-H3, H5), 3.80 (s, 3H, OCH_3), 2.16 (s, 3H, CH_3). ^{13}C NMR ppm (DMSO, 100 MHz): 14.70, 51.71. 110.62, 120.86, 125.18, 127.29, 128.39, 129.61, 130.23, 131.48, 134.31, 135.33, 136.56, 137.61, 142.24, 145.60, 146.46, 162.48, 189.46. MS (m/z):435.19 ($C_{26}H_{21}N_5O_2$, 0.87%, M+), 420 ($C_{25}H_{18}N_5O_2$, 11.30%), 273 ($C_{16}H_{12}N_5$, 1.73%), 252 ($C_{16}H_{14}NO_2$, 1.75%), 237 ($C_{16}H_{13}O_2$, 6.31%), 198 ($C_{10}H_8N_5$, 1.45%), 183 ($C_{10}H_7N_4$, 3.95%), 133 (C_9H_9O, 65.72%), 131 (C_8H7N, 10.42%), 107 (C_7H_7O, 3.99%), 90 (C_7H_7, 100%), 76.09 (C_6H_4, 27.01%). Anal. Calcd; C, 71.71; H, 4.86; N, 16.08, found: C, 71.73; H, 4.88; N, 16.10.

(2*E*)-1-(4-(1-methyl-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-ylamino)phenyl)-3-(*p*-tolyl)prop-2-en-1-one (9d) Faint yellow solid; yield: 80%; m.p. 225–228 °C. IR cm^{-1} (KBr): 3433 (NH), 3287 (C–H alkenes), 3235 (C–H

aromatic), 2984 (C–H aliphatic), 1594 (C=O), 1538 (C=C). ^{1}H NMR ppm ($CDCl_3$, 100 MHz): 9.92 (s, 1H, NH), 8.59 (d, 1H, QX-H6), 8.08 (d, 2H, Ph-H2, H6), 8.02 (d, 1H, COCH=CH–, J = 10.4), 7.99 (t, 1H, QX-H8), 7.85 (t, 1H, QX-H7), 7.72 (d, 1H, QX-H9), 7.60 (d, 1H, COCH=CH, J = 10.4), 7.49 (d, 2H, Ph-H3, H5), 7.39 (d, 2H, Ph-H2, H6), 6.97 (t, 2H, Ph-H3, H5), 2.28 (s, 3H, CH_3), 1.56 (s, 3H, CH_3). MS (m/z):419.22 ($C_{26}H_{21}N_5O$, 10.32%, M+), 404 ($C_{25}H_{18}N_5O$, 66.59%), 236 ($C_{16}H_{14}NO$, 3.83%), 221 ($C_{16}H_{13}O$, 10.32%), 145 ($C_{10}H_9O$, 44.40%), 131 ($C_8H_7N_2$, 7.32%), 117 (C_9H_9, 59.25%), 115 (C_9H_7, 100%), 91 (C_7H_7, 62.99%), 76.07 (C_6H_4, 13.20%). Anal. Calcd; C, 74.44; H, 5.05; N, 16.70, found: C, 74.47; H, 4.08; N, 16.37.

(2*E*)-1-(4-(1-methyl-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-ylamino)phenyl)-3-(*4*-nitrophenyl)prop-2-en-1-one (9e) Reddish white solid; yield: 69%; m.p. 233–235 °C. IR cm^{-1} (KBr): 3374 (NH), 3242 (C–H alkenes), 3195 (C–H aromatic), 2987 (C–H aliphatic), 1596 (C=O), 1576 (C=C). ^{1}H NMR ppm (DMSO-d_6, 100 MHz): 9.91 (s, 1H, NH), 8.35 (d, 1H, QX-H6), 8.10 (d, 2H, Ph-H2, H6), 8.01 (d, 1H, COCH=CH, J = 9.7), 7.98 (t, 1H, QX-H8), 7.91 (t, 1H, QX-H7), 7.74 (d, 1H, QX-H9), 7.58 (d, 1H, COCH=CH, J = 9.7), 7.45 (d, 2H, Ph-H3, H5), 7.32 (d, 2H, Ph-H2, H6), 7.28 (t, 2H, Ph-H3, H5), 2.61 (s, 3H, CH_3). Anal. Calcd; C, 66.66; H, 4.03; N, 18.66, found: C, 66.82; H, 4.11; N, 18.81.

General procedure for synthesis of *N*-(4-(4,5-dihydro-5-p-tolyl-1*H*-pyrazol-3-yl) aryl)-1-methyl-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-amine (10a–c)

A mixture of the appropriate chalcones **9a–c** (1.0 g, 0.03 mol) and hydrazine hydrate (0.62 g, 0.12 mol) in ethanol (10 mL) was refluxed for 4 h and then left to reach the room temperature for 12 h. The resulting precipitate was filtered out and washed with ethanol. The crude precipitate was crystallized from ethanol to give the corresponding pyrazole derivatives **10a–c**.

***N*-(4-(4,5-dihydro-5-phenyl-1*H*-pyrazol-3-yl)phenyl)-1-methyl-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-amine (10a)** White solid; yield: 65%; m.p. 270–272 °C. IR cm^{-1} (KBr): 3349 (NH), 3020 (C–H aromatic), 2864 (C–H aliphatic); ^{1}H NMR ppm (DMSO-d_6, 400 MHz): 9.86 (s, 1H, NH), 8.58 (d, 1H, QX-H6), 8.35 (d, 1H, QX-H9), 8.05 (d, 2H, Ph-H2, H6), 7.99 (t, 1H, QX-H8), 7.85 (t, 1H, QX-H7), 7.68 (d, 2H, Ph-H3, H5), 7.57 (d, 2H, Ph-H2, H6), 7.42 (t, 1H, Ph-H4), 7.24 (t, 2H, Ph-H3, H5), 5.59 (s, 1H, NH of pyrazole), 4.84 (t, 1H, CH), 3.02 (dd, 1H, CH_2, J = 9.2), 3.31 (dd, 1H, CH_2, J = 9.2), 1.56 (s, 3H, CH_3). ^{13}C NMR ppm (DMSO-d_6, 100 MHz): 12.07, 43.62, 55.07, 111.38, 117.84, 119.27, 120.52, 122.54, 123.63, 124.76, 128.69, 132.01, 133.71, 138.42, 138.41, 144.34, 149.68, 152.89, 163.82. Anal. Calcd; C, 71.58; H, 5.05; N, 23.37, found: C, 71.73; H, 5.12; N, 23.54.

***N*-(4-(5-(4-chlorophenyl)-4,5-dihydro-1*H*-pyrazol-3-yl) phenyl)-1-methyl-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-amine (10b)** White solid; yield: 58%; m.p. 265–267 °C. IR cm^{-1} (KBr): 3402 (NH), 3048 (C–H aromatic), 2972 (C–H aliphatic). ^{1}H NMR ppm ($CDCl_3$, 400 MHz): 9.85 (s, 1H, NH), 8.54 (d, 1H, QX-H6), 8.42 (d, 1H, QX-H9), 8.01 (d, 2H, Ph-H2, H6), 7.99 (t, 1H, QX-H8), 7.88 (t, 1H, QX-H7), 7.65 (d, 2H, Ph-H3, H5), 7.48 (d, 2H, Ph-H2, H6), 7.30 (d, 2H, Ph-H3, H5), 5.29 (s, 1H, NH of pyrazole), 4.79 (t, 1H, CH), 3.77 (dd, 1H, CH_2, J = 9.0), 3.80 (dd, 1H, CH_2, J = 9.0), 1.65 (s, 3H, CH_3). ^{13}C NMR ppm (DMSO-d_6, 100 MHz): 14.20, 43.62, 55.07, 111.38, 117.84, 119.27, 120.52, 122.54, 123.63, 124.76, 128.69, 132.01, 133.71, 138.42, 138.41, 144.34, 149.68, 152.84, 163.83. MS (m/z): 453 ($C_{25}H_{20}ClN_7$, 1.39%, M+), 455 ($C_{25}H_{20}ClN_7$, 0.49%, M+2), 439 ($C_{24}H_{18}ClN_5$, 8.39%), 404 ($C_{24}H_{18}N_7$, 1.38%), 274 ($C_{16}H_{12}N_5$, 6.47%), 270 ($C_{15}H_{13}N_3$, 3.92%), 255 ($C_{15}H_{12}ClN_2$, 2.53%), 198 ($C_{10}H_8N_5$, 2.48%), 184($C_9H_6N_5$, 4.18%), 179 ($C_9H_8ClN_2$, 10.89%), 144 ($C_9H_8N_2$, 16.01%), 131 ($C_8H_7N_2$, 40.34%), 111 (C_6H_4Cl, 40.06%), 90 (C_7H_6, 100%), 76.07 (C_6H_4, 33.05%). Anal. Calcd; C, 65.86; H, 4.86; N, 21.50, found: C, 65.88; H, 4.88; N, 21.52.

***N*-(4-(5-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl) phenyl)-1-methyl-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-amine (10c)** White solid; yield: 72%; m.p. 234–236 °C. IR cm^{-1} (KBr): 3395 (NH), 3020 (C–H aromatic), 2984 (C–H aliphatic). ^{1}H NMR ppm ($CDCl_3$, 400 MHz): 9.85 (s, 1H, NH), 8.65 (d, 1H, QX-H6), 8.52 (d, 1H, QX-H9), 8.31 (d, 2H, Ph-H2, H6), 7.99 (t, 1H, QX-H8), 7.84 (t, 1H, QX-H7), 7.62 (d, 2H, Ph-H3, H5), 7.42 (d, 2H, Ph-H2, H6), 6.62 (d, 2H, Ph-H3, H5), 5.31 (s, 1H, NH of pyrazole), 4.81 (t, 1H, CH), 3.82 (s, 3H, OCH_3), 3.71 (dd, 1H, CH_2, J = 9.0), 3.77 (dd, 1H, CH_2, J = 9.0), 1.67 (s, 3H, CH_3). ^{13}C NMR ppm (DMSO-d_6, 100 MHz): 18.87, 56.55, 64.32, 66.15, 117.71, 118.28, 120.39, 122.95, 123.80, 125.00, 127.31, 129.37, 130.45, 132.21, 134.24, 134.81, 138.89, 139.29, 144.57, 146.98, 150.30, 168.14. Anal. Calcd; C, 69.47; H, 5.16; N, 21.81, found: C, 69.49; H, 5.18; N, 21.83.

General procedure for synthesis of 6-(4-(1-methyl-[1,2,4] triazolo[4,3-*a*]quinoxalin-4-ylamino) aryl)-4-*p*-tolylpyrimidin-2(1*H*)-one (11a–c)

A mixture of the appropriate chalcones **9a–c** (3.23 g, 0.01 mol) and urea (0.6 g, 0.01 mol) was stirred in ethanol (20 mL), and then, hydrochloric acid (2 mL) was added. The mixture was heated under reflux for 7 h. After complete reaction, the solvent was concentrated under reduced pressure

and poured onto ice water (50 mL). The obtained precipitate was filtered, washed with water, and crystallized from ethanol to yield the corresponding pyrimidinones **11a–c**.

6-(4-(1-Methyl-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-ylamino) phenyl)-4-phenylpyrimidin-2(1*H*)-one (11a) Yellowish white solid; yield: 70%, m.p. 294–297 °C. IR cm^{-1} (KBr): 3382 (NH), 3222 (C–H aromatic), 2864 (C–H aliphatic), 1678 (C=O). ^{1}H NMR ppm (DMSO-d_6, 300 MHz): 10.18 (s, 1H, NH), 8.53 (s, 1H, NH pyrimidine), 8.28 (d, 2H, Ph-H2, H6), 7.86 (d, 1H, QX-H6), 7.72 (d, 2H, Ph-H2, H6), 7.68 (d, 1H, QX-H9), 7.54 (t, 1H, QX-H8), 7.36 (t, 1H, QX-H7), 7.33 (t, 1H, Ph-H4), 7.21 (t, 2H, Ph-H3, H5), 6.65 (d, 2H, Ph-H3, H5), 6.63 (s, 1H, pyrimidine-H5), 2.15 (s, 3H, CH_3). ^{13}C NMR ppm (DMSO-d_6, 100 MHz): 18.92, 116.36, 117.64, 123.46, 123.64, 124.68, 126.13, 129.03, 129.14, 131.31, 134.85, 143.11, 146.43, 146.75, 149.21, 159.68, 171.08, 173.49. Anal. Calcd; C, 70.10; H, 4.30; N, 22.01, Found: C, 70.13; H, 4.33; N, 22.04.

6-(4-(1-Methyl-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-ylamino) phenyl)-4-(4-chlorophenyl)pyrimidin-2(1*H*)-one (11b) Reddish white solid; yield: 55%; m.p. 295–299 °C. IR cm^{-1} (KBr): 3403 (NH), 3083 (C–H aromatic), 2975 (C–H aliphatic), 1660 (C=O). ^{1}H NMR ppm (DMSO-d_6, 300 MHz): 10.28 (s, 1H, NH), 8.53 (s, 1H, NH pyrimidine), 8.29 (d, 2H, Ph-H2, H6), 7.86 (d, 1H, QX-H6), 7.83 (d, 2H, Ph-H2, H6), 7.68 (d, 1H, QX-H9), 7.54 (t, 1H, QX-H8), 7.36 (t, 1H, QX-H7), 7.32 (d, 2H, Ph-H3, H5), 6.65 (d, 2H, Ph-H3, H5), 6.60 (s, 1H, pyrimidine-H5), 1.95 (s, 3H, CH_3). MS (m/z):479 ($C_{26}H_{18}ClN_7O$, 1.43%, M+), 481 ($C_{26}H_{18}ClN_7O$, 0.39%, M+2), 464 ($C_{25}H_{16}ClN_7O$, 3.70%), 329 ($C_{18}H_{13}N_6O$, 14.41%), 296 ($C_{16}H_{11}ClN_3O$, 1.06%), 274 ($C_{16}H_{12}N_5$, 1.38%), 205 ($C_{10}H_6ClN_2O$, 12.29%), 199 ($C_{10}H_8N5$, 3.02%), 182($C_{10}H_6N_4$, 14.91%), 150 (C_8H_5ClN, 1.51%), 131 ($C_8H_7N_2$, 23.49%), 76.09 (C_6H_4, 38.51%), 44.07 (CHNO, 100%). Anal. Calcd; C, 65.07; H, 3.78; N, 20.34, found: C, 65.09; H, 3.80; N, 20.36.

6-(4-(1-methyl-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-ylamino) phenyl)-4-(4-methoxyphenyl)pyrimidin-2(1*H*)-one (11c) Yellowish white solid; yield: 70%; m.p. 291–293 °C. IR cm^{-1} (KBr): 3449 (NH), 3120 (C–H aromatic), 2932 (C–H aliphatic), 1680 (C=O). ^{1}H NMR ppm (DMSO-d_6, 300 MHz): 10.49 (s, 1H, NH), 8.53 (s, 1H, NH pyrimidine), 8.47 (d, 2H, Ph-H2, H6), 8.26 (d, 1H, QX-H6), 8.12 (d, 2H, Ph-H2, H6), 7.69 (d, 1H, QX-H9), 7.54 (t, 1H, QX-H8), 7.38 (t, 1H, QX-H7), 7.09 (d, 2H, Ph-H3, H5), 6.59 (d, 2H, Ph-H3, H5), 6.55 (s, 1H, pyrimidine-H5), 4.14 (s, 3H, OCH_3), 1.99 (s, 3H, CH_3). ^{13}C NMR ppm ($CDCl_3$, 100 MHz): 11.92, 55.85, 106.12, 111.32, 112.48, 114.36, 120.91, 124.08, 125.11, 125.95, 127.26, 128.71, 130.28, 135.90, 140.12, 142.09, 149.60, 156.37, 161.51, 162.90, 163.07, 168.29. Anal. Calcd; C, 68.20; H, 4.45; N, 20.62, found: C, 68.08; H, 4.26; N, 20.43.

General procedure for synthesis of *N*-(4-(4,5-dihydro-5-arylisoxazol-3-yl) phenyl)-1-methyl-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-amine (12a–c)

A mixture of the appropriate chalcones **9a–c** (3.23 g, 0.01 mol) and hydroxylamine hydrochloride (0.69 g, 0.01 mol) was stirred in ethanol (20 mL) and then sodium hydroxide (0.8 g, 0.02 mol) was added. The reaction mixture was heated under reflux for 24 h. After the reaction was completed, the solvent was concentrated by evaporation under reduced pressure and then poured onto ice water (50 mL). The obtained precipitate was filtered, washed with copious amount of water, and recrystallized from ethanol to afford the target compounds **12a–c**.

***N*-(4-(4,5-dihydro-5-phenylisoxazol-3-yl)phenyl)-1-methyl-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-amine (12a)** White solid; yield: 50%; m.p. 210–212 °C. IR cm^{-1} (KBr): 3331 (NH), 3022 (C–H aromatic), 2964 (C–H aliphatic). ^{1}H NMR ppm (DMSO-d_6, 300 MHz): 9.38 (s, 1H, NH), 7.94 (d, 1H, QX-H6), 7.87 (d, 1H, QX-H9), 7.77 (d, 2H, Ph-H2, H6), 7.40 (t, 1H, QX-H8), 7.38 (t, 1H, QX-H7), 7.21 (d, 2H, Ph-H3, H5), 7.15 (d, 2H, Ph-H2, H6), 7.09 (t, 1H, Ph-H4), 7.04 (t, 2H, Ph-H3, H5), 5.46 (t, 1H, CH), 3.64 (dd, 1H, CH_2, $J=9.0$), 2.89 (dd, 1H, CH_2, $J=9.0$), 2.19 (s, 3H, CH_3). ^{13}C NMR ppm ($CDCl_3$, 100 MHz): 11.88, 42.15, 84.86, 111.43, 112.30, 120.41, 120.76, 124.80, 125.75, 127.10, 128.09, 128.83, 130.02, 136.05, 142.12, 142.49, 150.08, 156.20, 166.15. Anal. Calcd; C, 71.41; H, 4.79; N, 19.99, found: C, 71.52; H, 4.88; N, 20.03.

***N*-(4-(5-(4-chlorophenyl)-4,5-dihydroisoxazol-3-yl) phenyl)-1-methyl-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-amine (12b)** White solid; yield: 62%; m.p. 259–262 °C. IR cm^{-1} (KBr): 3401 (NH), 3081 (C–H aromatic), 2983 (C–H aliphatic). ^{1}H NMR (DMSO-d_6, 300 MHz): 9.24 (s, 1H, NH), 8.02 (d, 1H, QX-H6), 7.98–7.90 (m, 7H, QX-H9- Ph-H2, H6, QX-H8, QX-H7- Ph-H3, H5), 7.69 (d, 2H, Ph-H2, H6), 7.62 (d, 2H, Ph-H3, H5), 5.85 (t, 1H, CH), 3.62 (dd, 1H, CH_2, $J=9.5$), 2.87 (dd, 1H, CH_2, $J=9.5$), 2.19 (s, 3H, CH_3). ^{13}C NMR ppm (DMSO-d_6, 100 MHz): 18.92, 84.14, 117.71, 118.28, 120.32, 122.90, 123.74, 125.02, 127.30, 129.38, 130.45, 132.21, 134.24, 134.79, 138.88, 139.27, 144.55, 146.99, 150.30, 169.46. MS (m/z): 454 ($C_{25}H_{19}ClN_5O$, 25.92%, M+), 453 ($C_{25}H_{19}ClN_5O$, 4.36%, M+2), 440 ($C_{24}H_{17}ClN_6O$, 0.98%), 286 ($C_{16}H_{12}N_5$, 1.27%), 256 ($C_{15}H_{11}ClNO$, 1.33%), 198 ($C_{10}H_8N_5$, 2.44%), 180 (C_9H_7ClNO, 4.13%), 154 ($C_7H_5ClN_5O$, 10.02%), 141 (C_7H_5ClO, 6.74%), 131 ($C_8H_7N_2$, 9.39%), 111(C_6H_4Cl,

11.59%), 76.09 (C_6H_4, 38.51%). Anal. Calcd; C, 66.01; H, 4.21; N, 18.47, found: C, 66.13; H, 4.29; N, 18.51.

***N*-(4-(4,5-dihydro-5-(4-methoxyphenyl)isoxazol-3-yl) phenyl)-1-methyl-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-amine (12c)** White solid; Yield: 62%; m.p. 221–224 °C. IR cm^{-1} (KBr): 3362 (NH), 3106 (C–H aromatic), 2901 (C–H aliphatic). ^{1}H NMR ppm (DMSO-d_6, 300 MHz): 9.39 (s, 1H, NH), 8.01–7.88 (m, 8H, QX-H6, QX-H9, Ph-H2, H6, QX-H8, QX-H7, Ph-H3, H5), 7.66 (d, 2H, Ph-H2, H6), 6.92 (d, 2H, Ph-H3, H5), 5.25 (t, 1H, CH), 4.19(s, 3H, OCH_3), 3.66 (dd, 1H, CH_2, $J=9.0$), 2.91 (dd, 1H, CH_2, $J=9.0$), 2.19 (s, 3H, CH_3). Anal. Calcd; C, 69.32; H, 4.92; N, 18.60, found: C, 69.48; H, 4.96; N, 18.73.

Synthesis of 1-methyl-[1,2,4]triazolo[4,3-*a*] quinoxaline-4-thiol (13)

A mixture of 4-chloro-1-methyl-[1,2,4]triazolo[4,3-*a*]quinoxaline (5.0 g, 0.0245 mol) and thiourea (1.8 g, 0.0245 mol) in absolute ethanol (150 mL) was refluxed for 4 h, filtered while hot. After cooling, the obtained solid was refluxed with 10% NaOH for 0.5 h and acidified with 4 N HCl. The resulting precipitate was filtered, washed several times with water, and dried to give the target compound as faint yellowish white crystals. Yield: 65%; m.p. 305–308 °C. IR cm^{-1} (KBr): 3433 (NH), 3099 (C–H aromatic), 2976 (C–H aliphatic), 2580 (SH). ^{1}H NMR ppm (DMSO-d_6, 100 MHz): 11.96 (s, 1H, NH), 8.06 (d, 1H, QX-H6), 7.76 (t, 1H, QX-H8), 7.65 (t, 1H, QX-H7), 7.27 (d, 1H, QX-H9), 2.96 (s, 3H, CH_3). MS (m/z): 216 ($C_{10}H_8N_4S$, 1.02%, M+), 201.23 ($C_9H_6N_4S$, 0.71%), 185 ($C_{10}H_{10}N_4$, 2.61%), 131 ($C_8H_7N_2$, 5.37%), 91 (C_6H_5N, 3.46%), 76.06 (C_6H_4, 12.77%), 43.12 (CS, 100%). Anal. Calcd; C, 55.56; H, 3.73; N, 29.91, found: C, 55.61; H, 3.79; N, 29.98.

General procedure for synthesis of 2-(1-methyl-[1,2,4] triazolo[4,3-*a*]quinoxalin-4-ylthio)-*N*-arylacetamide (15a–g)

A mixture of the potassium salt of compound **13** (1.5 g, 0.01 mol) and the appropriate α-chloroacetanilide derivative (0.01 mol) in DMF (20 mL) was heated over a water bath for 3 h. The reaction mixture was then cooled, poured into ice-cooled water (200 mL), and stirred well for 30 m. The separated solid was filtered, washed with water, dried, and finally crystallized from methanol/toluene mixture (1:1).

2-(1-Methyl-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-ylthio)-*N*-phenylacetamide (15a) White solid; yield: 68%; m.p. 272 °C. IR cm^{-1} (KBr): 3270 (NH), 3100 (C–H aromatic), 2948 (C–H aliphatic), 1661 (C=O). ^{1}H NMR ppm (DMSO-d_6, 400 MHz): 10.23 (s, 1H, NH), 7.57 (d, 1H, QX-H6), 7.35 (t, 1H, QX-H8), 7.27 (t, 1H, QX-H7), 7.21 (d, 1H, QX-H9), 7.19 (t, 2H, Ph-H3, H5), 7.12 (t, 1H, Ph-H4), 7.05 (d, 2H, Ph-H2, H6), 4.48 (s, 2H, SCH_2), 2.22 (s, 3H, CH_3). ^{13}C NMR ppm (DMSO-d_6, 100 MHz): 12.30, 56.46, 113.93, 123.34, 123.43, 125.13, 127.80, 133.87, 146.27, 148.84, 149.96, 157.39, 170.12. MS (m/z): 349 ($C_{18}H_{15}N_5OS$, 3.03%, M+), 335 ($C_{17}H_{13}N_5OS$, 7.18%), 256 ($C_{12}H_8NOS$, 5.63%), 229 ($C_{11}H_9N_4S$, 6.80%), 134 ($C_8H_7N_2$, 5.05%), 120 (C_7H_6NO, 1.16%), 93 (C_6H_7N, 100%), 78 (C_6H_6, 27.10%), 76 (C_6H4, 8.03%), 44 (CHNO, 77.17%). Anal. Calcd; C, 61.87; H, 4.33; N, 20.04, found: C, 61.99; H, 4.35; N, 20.16.

2-(1-Methyl-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-ylthio)-*N*-*p*-tolylacetamide (15b) White solid; yield 72%; m.p. 296–298 °C. IR cm^{-1} (KBr):3279 (NH), 3132 (C–H aromatic), 2953 (C–H aliphatic), 1673 (C=O). ^{1}H NMR ppm (DMSO-d_6, 400 MHz): 10.88 (s, 1H, NH), 7.57 (d, 1H, QX-H6), 7.43 (t, 1H, QX-H8), 7.41 (t, 1H, QX-H7), 7.39 (d, 1H, QX-H9), 7.32 (d, 2H, Ph-H3, H5), 7.29 (d, 2H, Ph-H2, H6), 4.62 (s, 2H, SCH_2), 4.02 (s, 3H, CH_3), 2.19 (s, 3H, CH_3). ^{13}C NMR ppm (DMSO-d_6, 100 MHz): 12.02, 22.18, 39.15, 114.14, 120.12, 127.29, 129.04, 129.99, 135.41, 137.15, 142.11, 148.81, 150.50, 152.04, 169.10. Anal. Calcd; C, 62.79; H, 4.71; N, 19.27, found: C, 62.81; H, 4.73; N, 19.32.

2-(1-Methyl-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-ylthio)-*N*-(4-methoxyphenyl)acetamide (15c) Faint violet solid; yield (70%); m.p. 260–262 °C. IR cm^{-1} (KBr):3479 (NH), 3132 (C–H aromatic), 2962 (C–H aliphatic), 1698 (C=O). ^{1}H NMR ppm (DMSO-d_6, 400 MHz): 10.30 (s, 1H, NH), 7.57 (d, 1H, QX-H6), 7.55 (t, 1H, QX-H8), 7.41 (t, 1H, QX-H7), 7.39 (d, 1H, QX-H9), 7.35 (d, 2H, Ph-H3, H5), 7.28 (d, 2H, Ph-H2, H6), 4.49 (s, 2H, CH_2), 4.22 (s, 3H, OCH_3), 2.79 (s, 3H, CH_3). ^{13}C NMR ppm (DMSO-d_6, 100 MHz): 12.15, 39.02, 56.11, 114.18, 115.19, 123.08, 128.40, 130.61, 131.07, 142.20, 150.01, 151.17, 152.34, 159.58, 169.03. Anal. Calcd; C, 60.14; H, 4.52; N, 18.46, found: C, 60.19; H, 4.58; N, 18.49.

2-(1-Methyl-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-ylthio)-*N*-(4-bromophenyl)acetamide (15d) Faint brown solid; yield: 59%; m.p. 258–260 °C. IR cm^{-1} (KBr):3305 (NH), 3122 (C–H aromatic), 2905, (C–H aliphatic), 1664 (C=O); ^{1}H NMR ppm (DMSO-*d6*, 400 MHz): 10.23 (s, 1H, NH), 8.16 (d, 1H, QX-H6), 7.46 (t, 1H, QX-H8), 7.42 (t, 1H, QX-H7), 7.39 (d, 1H, QX-H9), 7.29 (d, 2H, Ph H3, H5), 7.27 (d, 2H, Ph-H2, H6), 4.51 (s, 2H, SCH_2), 2.22 (s, 3H, CH_3). ^{13}C NMR ppm (DMSO-*d6*, 100 MHz): 12. 13, 39.23, 114.18, 121.21, 122.62, 127.29, 128.20, 129.16, 132.02, 137.72, 142.09, 149.58, 150.63, 152.26, 168.27. MS (*m/z*):428 ($C_{18}H_{14}BrN_5OS$, 4.06%, M+), 430 ($C_{18}H_{14}BrN_5OS$, 4.79%, M+2), 412 ($C_{17}H_{12}BrN_5OS$, 1.92%), 384 ($C_{18}H_{14}N_5OS$, 3.20%), 257 ($C_{12}H_9N_4OS$, 8.00%), 243 (C_8H_7BrOS, 4.36%),

229 ($C_{11}H_9N4S$, 40.25%), 197 (C_7H_5BrNO, 7.62%), 171 (C_6H_7BrN, 100%), 131 ($C_8H_7N_2$, 26.90%), 76 (C_6H_4, 32.77%), 44 (CHNO, 93.09%). Anal. Calcd; C, 50.48; H, 3.29; N, 16.35, found: C, 50.66; H, 3.26; N, 16.53.

2-(1-Methyl-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-ylthio)-*N*-(4-chlorophenyl)acetamide (15e) White solid; yield: 62%); m.p. 228–230 °C. IR cm^{-1} (KBr): 3267(NH), 3125 (C–H aromatic), 2950 (CH aliphatic), 1669 (C=O). ^{1}H NMR ppm (DMSO-*d6*, 400 MHz): 10.43 (s, 1H, NH), 8.06 (d, 1H, QX-H6), 7.46 (t, 1H, QX-H8), 7.40 (t, 1H, QX-H7), 7.38 (d, 1H, QXH9), 7.31 (d, 2H, Ph-H3, H5), 7.27 (d, 2H, Ph-H2, H6), 4.49 (s, 2H, SCH_2), 2.22 (s, 3H, CH_3). ^{13}C NMR ppm (DMSO-*d6*, 100 MHz): 12.06, 39.61, 114.01, 120.40, 126.15, 127.12, 129.11, 133.32, 136.61, 142.27, 148.18, 150.17, 152.53, 168.37. MS (*m/z*): 383 ($C_{18}H_{14}ClN_5OS$, 3.54%, M+), 385 ($C_{18}H_{14}ClN_5OS$, 0.98%, M+2), 369 ($C_{17}H_{12}ClN_5OS$, 1.27%), 330 ($C_{16}H_{13}ClN_3OS$, 1.01%), 228 ($C_{11}H_8N_4S$, 0.80%), 183 ($C_{10}H_7N_4$, 4.53%), 168 (C_8H_7ClNO, 3.27%), 154 (C_7H_5ClNO, 11.21%), 127 (C_6H_6ClN, 100%), *Anal. Calcd*; C, 56.32; H, 3.68; N, 18.2, found: C, 56.48; H, 3.75; N, 18.37.

2-(1-Methyl-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-ylthio)-*N*-(4-fluorophenyl)acetamide (15f) Rose red solid; yield: 52%; m.p. 250–253 °C. IR cm^{-1} (KBr): 3274 (NH), 3157 (C–H aromatic), 2964 (C–H aliphatic), 1668 (C=O). ^{1}H NMR ppm (DMSO-d_6, 400 MHz): 10.23 (s, 1H, NH), 7.86 (d, 1H, QX-H6), 7.46 (t, 1H, QX-H8), 7.42 (t, 1H, QX-H7), 7.37 (d, 1H, QX-H9), 7.29 (d, 2H, Ph-H3, H5), 7.07 (d, 2H, Ph-H2, H6), 4.48 (s, 2H, SCH_2), 2.12 (s, 3H, CH_3). ^{13}C NMR ppm (DMSO-d_6, 100 MHz): 12.05, 39.14, 114.40, 115.13, 121.17, 127.17, 128.08, 129.20, 134.11, 142.91, 150.80, 151.06, 152.00, 163.12, 169.02. MS (*m/z*): 367 ($C_{18}H_{14}FN_5OS$, 29.35%, M+), 354 ($C_{17}H_{11}FN_5OS$, 5.23%), 259 ($C_{12}H_{11}N_4OS$, 25.85%), 229 ($C_{11}H_9N4S$, 1.88%), 131 ($C_8H_7N_2$, 59.90%), 76 (C_6H_4, 1.69%), 44 (CHNO, 15.32%). *Anal. Calcd*; C, 58.84; H, 3.84; N, 19.06, found: C, 59.02; H, 3.91; N, 19.24.

2-(1-Methyl-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-ylthio)-*N*-(4-nitrophenyl)acetamide (15g) Yellowish solid; yield: 70%; m.p. 232–235 °C. IR cm^{-1} (KBr): 3274 (NH), 3157 (C–H aromatic), 2964 (CH aliphatic), 1668 (C=O), 1530 & 1366 (N–O). ^{1}H NMR ppm (DMSO-d_6, 400 MHz): 10.23 (s, 1H, NH), 8.12 (d, 1H, QX-H6), 7.49 (t, 1H, QX-H8), 7.44 (t, 1H, QX-H7), 7.39 (d, 1H, QX-H9), 7.67 (d, 2H, Ph-H3, H5), 7.08 (d, 2H, Ph-H2, H6), 4.49 (s, 2H, SCH_2), 2.12 (s, 3H, CH_3). ^{13}C NMR ppm (DMSO-d_6, 100 MHz): 12.05, 38.09, 114.42, 120.02, 124.10, 127.71, 128.18, 129.52, 142.26, 143.57, 144.60, 149.69, 150.61, 151.98, 168.24. Anal. Calcd; C, 54.81; H, 3.58; N, 21.31, found: C, 54.67; H, 3.41; N, 21.19

Table 1 IC_{50} in μM of newly synthesized compounds on MDA-MB-231 using MTT assay

Compound	IC_{50} in μM	Compound	IC_{50} in μM
Doxorubicin	0.1	**11c**	15.5
8	6.4	**12a**	27.5
9a	229	**12b**	144.5
9b	36.3	**12c**	363
9c	213.8	**13**	363
9d	151.4	**15a**	2.9
9e	60.3	**15b**	17.4
10a	128.8	**15c**	15.5
10b	34.7	**15d**	2.7
10c	17.4	**15e**	1.9
11a	162	**15f**	2.8
11b	38.9	**15g**	3.5

Biological evaluation

Human breast adenocarcinoma cells (MDA-MB-231) were allowed to grow in Dulbecco's modified Eagle's medium (DMEM) instead of Rosewell Park Memorial Institute (RPMI 1640) medium. The selected method is 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay which has become one of the most widely used tools in cell biology for measuring the metabolic activity [25, 26]. Exponentially growing cells from MDA-MB-231 cell line were trypsinized, counted, and seeded at the appropriate densities (2000–1000 cells/0.33 cm^2 well) into 96-well microliter plates. Cells then were incubated in a humidified atmosphere at 37 °C for 24 h. After that, cells were exposed to different concentrations of test compounds (0.1, 10, 100, and 1000 μM) for 24, 48, and 72 h. The growth media were removed; cells were incubated with 200 μl of 5% MTT solution (Sigma-Aldrich, MO) and were allowed to metabolize the dye into colored insoluble formazan crystals for 2 h. The remaining MTT solution was discarded from the wells, and the formazan crystals were dissolved in 200 μl acidified isopropanol for 30 min and covered with aluminum foil with continuous shaking using a MaxQ 2000 plate shaker (Thermo Fisher Scientific Inc., MI) at room temperature. Absorbance was measured at 570 nm using a Stat FaxR 4200 plate reader (Awareness Technology, Inc., FL, USA). The cell viability was expressed as a percentage of control, and the concentration that induces 50% of maximum inhibition of cell proliferation (IC_{50}) was determined using GraphPad Prism software version 5 (GraphPad Software Inc., CA) [27]. Results of MTT assay and IC50 in μM of newly synthesized compounds on MDA-MB-231 are represented in Table 1.

Structure activity relationship study

The results of biological evaluation could be summarized as: Low activity of compound **7** reflects the importance of C-4 spacer on the activity. Replacement of C-4 chlorine atom in **7** with thiol group mildly improved the effect. Among all the synthesized derivatives, the best activity was observed with the *N*-arylacetamides **15a–g**. Electron-withdrawing groups attached with the *N*-arylacetamide moiety increase the potency as observed with **15e-g** compared with **15b** and **15c.** Bulky atoms exhibit more steric effects, resulting in the reduction in the activity. Such effect is clear in the comparison of the lower IC_{50} value of **15d** with that of **15e**. In addition, shortening the spacer length at C-4 decreases the activity. Chalcone derivatives **9a–e** show variation of activity depending on the *para* substituents on the aromatic aldehyde. Electron-withdrawing groups increase the activity of compound; e.g., compound **9b** has higher activity than **9c** while unsubstituted aldehyde derivative **9a** shows the lowest activity of this series. Cyclization of chalcones with different binucleophiles results in an increase in the activity in most cases.

Molecular docking

Molecular docking studies were carried out using the program AutoDock Vina [28]. The homology model of A2B receptor (Fig. 4) and the docking protocol described in an earlier report were adapted for this purpose [13]. Water molecules and identified hit were removed. The protein for docking with AutoDock was prepared using ADT, which includes adding polar hydrogen atoms to the protein atoms and assigning Kollman charges afterward. For the ligand, all hydrogen atoms must be present on it to calculate partial atomic charges on the ligand. The protein active site was defined by placing a grid over the center of the identified hit. Hydrogen atoms were also added to the ligand, and Gasteiger charges were assigned. Before a protein is ready for docking simulations, all the necessary grid maps were calculated prior to docking. The grid maps were generated with the help of AutoGrid, which is a program of the AutoDock suite.

Results and discussion

The proposed binding mode of compound **15e** with the homology model of the human adenosine A_{2B} receptor showed that the triazolo moiety of the compound is involved in hydrogen bonding interactions with Asn254. Furthermore, the tricyclic moiety of the compound is anchored by aromatic stacking interaction with Trp247, Phe173, and His251 and hydrophobic interaction with Val85, Leu86, Met182,

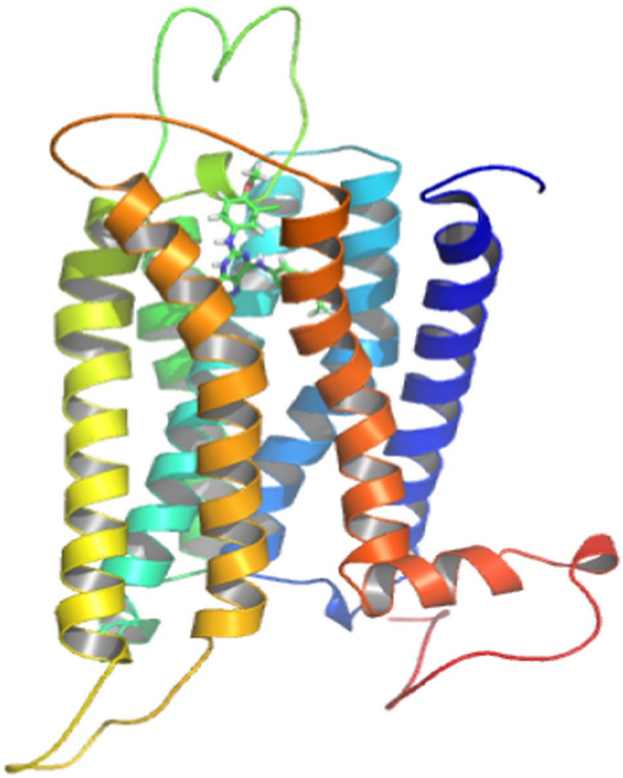


Fig. 4 Homology model of the human adenosine A2B receptor with identified hit (ZINC09074482)

Val253, and Ile276 a pocket that contributes to an increase in the affinity of the ligand (Fig. 5). Besides that, the amino group of the compound formed a water-mediated interaction with Glu174. The phenyl amino is predicted to be involved in hydrophobic interactions with Ile67, Leu172, and Met279. Because of the existence of additional hydrogen bonding and desirable interactions, compound **15e** and related derivatives have a higher affinity toward the receptor than all other derivatives.

The obtained binding mode of compound **15d** with the homology model of A2B receptor follows the general pattern observed for compound **15e** (Fig. 6). As before, the hydrogen bonding, hydrophobic, and aromatic stacking interactions are maintained. Insertion of bromine atom on compound **15e** instead of the chlorine atom is performed to exploit the steric effect of bromine as a bulky atom to occupy the binding site, e.g., steric hindrance effect is more pronounced than chlorine atom which decreases the affinity of this compound.

The absence of a halogen atom in compound **15a** is likely important for decreasing the affinity for the receptor (Table 2). The obtained result of compound **15a** is virtually the same as that of compound **15e**. In addition, deletion of halogen atom can decrease the affinity of the compound

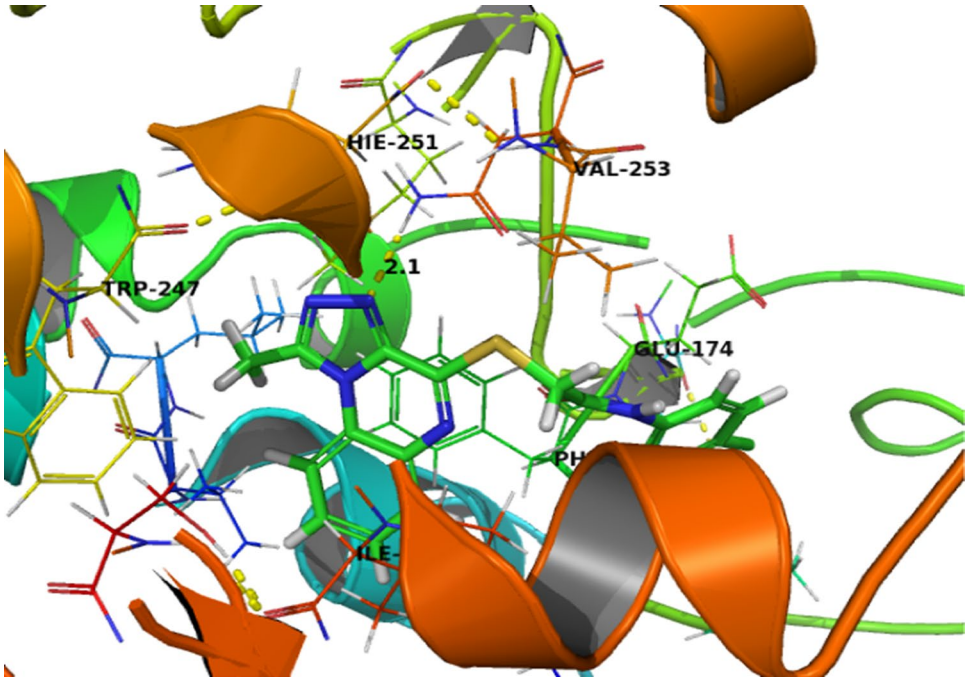


Fig. 5 Predicted binding mode for compound **15e** with the homology model of the human adenosine A2B receptor. Interactions between H-bonded atoms are indicated by dotted lines. Hydrogen (white), nitrogen (blue), oxygen (red), and sulfur (yellow)

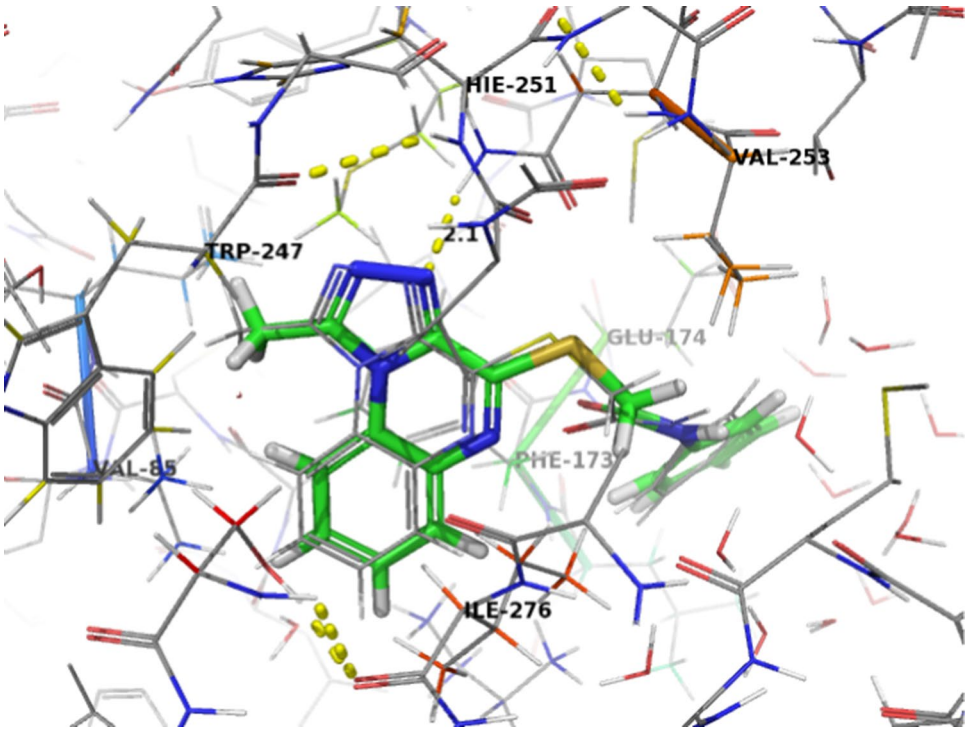


Fig. 6 Predicted binding mode for compound $\mathbf{15_d}$ (aligned with $\mathbf{15_e}$) with the homology model of the human adenosine A2B receptor. Interactions between H-bonded atoms are indicated by dotted lines. Hydrogen (white), nitrogen (blue), oxygen (red), and sulfur (yellow)

because this compound cannot accommodate the binding site (Fig. 7).

Introduction of the nitro group in compound **15g** is likely important for decreasing the affinity for the receptor (Table 2). The obtained result of compound **15g** is virtually the same as that of compound **15e**. In addition, the nitro group of the compound is destabilized by hydrophobic interactions Ile67 and Leu172 (Fig. 8).

Tricyclic or bicyclic ring system and spacers with hydrogen bonding acceptor or donor with aromatic system connected halogen are needed to increase the affinity and selectivity for the human adenosine A_{2B} receptor; thus, reducing the spacer length as in compound **8** led to decreasing the

Table 2 The docking scores and IC_{50} for all synthesized compounds

Compound	IC_{50}	Docking score	Compound	IC_{50}	Docking score
8	6.4	−7.11	$\mathbf{12_a}$	27.5	−6.99
$\mathbf{9_a}$	229	−6.36	$\mathbf{12_b}$	144.5	−6.13
$\mathbf{9_b}$	36	−6.77	$\mathbf{12_c}$	363	−5.01
$\mathbf{9_c}$	213.8	−6.50	**13**	363	−5.88
$\mathbf{9_d}$	151.4	−6.12	$\mathbf{15_a}$	2.9	−8.0
$\mathbf{9_e}$	60.3	−6.15	$\mathbf{15_b}$	17.4	−6.90
$\mathbf{10_a}$	128.8	−6.78	$\mathbf{15_c}$	15.5	−7.00
$\mathbf{10_b}$	34.7	−6.27	$\mathbf{15_d}$	2.7	−7.81
$\mathbf{10_c}$	17.4	−6.81	$\mathbf{15_e}$	1.9	−8.36
$\mathbf{11_a}$	162	−6.78	$\mathbf{15_f}$	2.8	−7.92
$\mathbf{11_b}$	38.9	−7.50	$\mathbf{15_g}$	3.5	−7.39
$\mathbf{11_c}$	15.5	−7.68			

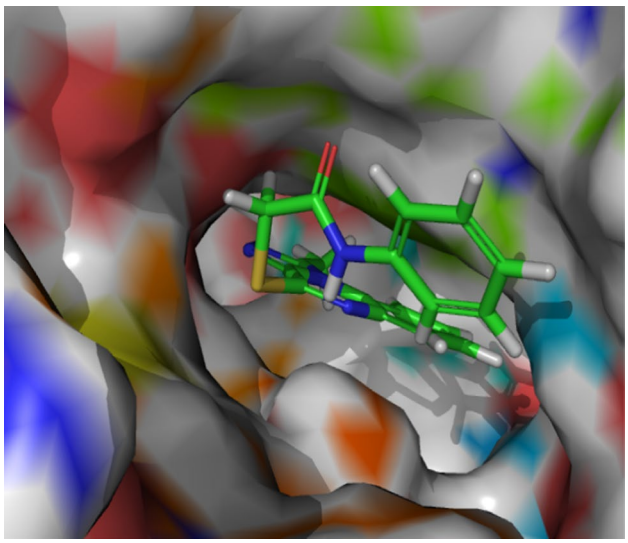


Fig. 7 Predicted binding mode for compound $\mathbf{15_a}$ with the homology model of the human adenosine A2B receptor. As shown, the compound cannot completely accommodate the binding site

affinity where the compound cannot accommodate the binding site. Moreover, the substitution with aminophenyl group (**9**, **10**, **11**, and **12**) instead of sulfur atom and spacer can decrease the affinity for the adenosine A_{2B} receptor where the introduction of phenyl group can delete the hydrogen bonding interactions with Asn254 which is essential for antagonist binding (Fig. 9).

In summary, the obtained results indicated that all the synthesized compounds have a similar position and orientation inside the putative binding site of the homology model of the human adenosine A_{2B} receptor. In addition, the results of the free energy of binding (ΔG) explain that some of these compounds have good binding affinity to

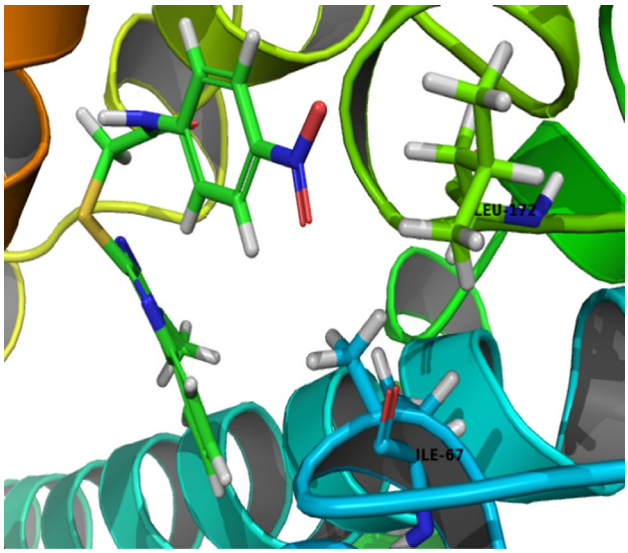


Fig. 8 Predicted binding mode for compound $\mathbf{15_g}$ with the homology model of the human adenosine A2B receptor. As shown, there are unfavorable interactions with hydrophobic groups

the receptor and the computed values reflect the overall trend (Table 2).

Conclusion

The role of human adenosine A2B receptor as a mediator for pathophysiological conditions like tumors and as a regulator for the angiogenic factors renders this receptor of great medicinal interest in tumor chemotherapy. Due to the absence of a 3D A2B target structure, different ligand-based methods were applied for the design of potent A2B inhibitors. Herein, we report the design and synthesis of 23 new 1,2,4-triazolo[4,3-*a*]quinoxaline derivatives as potential inhibitors for A2B adenosine receptor type. Structures of all the new derivatives were confirmed by spectroscopic methods and elemental analyses. The inhibitory effect of these compounds against A2B adenosine receptor was measured depending on its cytotoxic effects against MDA-MB-231 cell lines. Results of cytotoxic evaluation showed six promising active derivatives (**8, 15a, 15d, 15e, 15f,** and **15g**) with IC_{50} values ranging from 1.9 to 6.4 μM on MDA-MB 231 cell line. In addition, docking studies of the synthesized compounds into the binding pocket of the A2B receptor homology model and finally SAR analyses of the new compounds were done. The observed IC_{50} values agreed with the obtained docking scores.

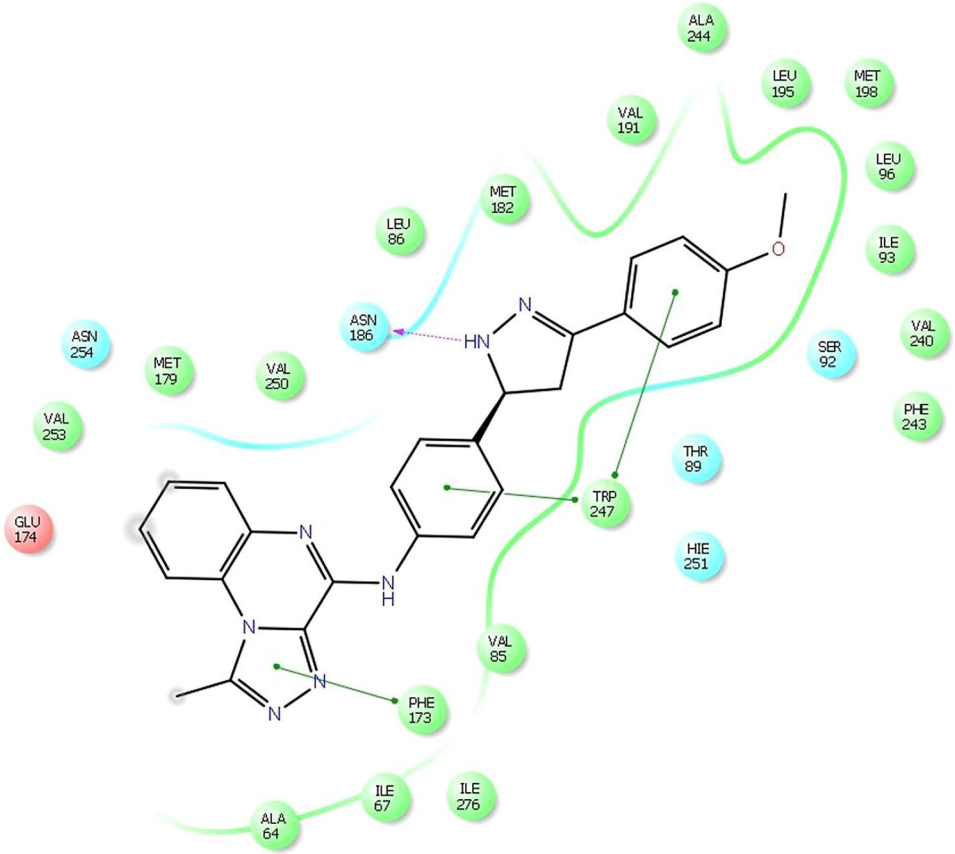


Fig. 9 Predicted binding mode for compound **10c**, with the homology model of the human adenosine A_{2B} receptor. As shown, the hydrogen bonding interaction with Asn254 is absent

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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