SYNTHESIS, BIOLOGICAL EVALUATION AND BINDING STUDIES OF NEW FLAVONE DERIVATIVES AS ADENOSINE A2B RECEPTOR ANTAGONISTS

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ABSTRACT
A series of eleven flavone derivatives were synthesized. The synthesized compounds were characterized structurally by various techniques using spectral analyses. All of the synthesized compounds were subjected to MTT proliferation assay to investigate their in-vitro cytotoxic activity. Among all the studied compounds, compounds, VIi, VIh, VIld and VIk revealed moderate growth inhibitory effect towards the MDA-MB 231 cell line compared to the reference, doxorubicin. These compounds showed cytotoxicity activity with IC50 values ranging from 43.7 to 138 µM in MDA-MB 231 cell line. The results of cytotoxic activity revealed that flavone derivatives with N-aryl acetamide substituted at the 3-position of flavone backbone have better cytotoxic activity. Moreover, the highest activity was observed with compound VIi that has oxy-N-pyriden-2-yl acetamide substituent at the 3-position of flavone backbone followed by compound VIh with IC50 values of 43.7 and 50 µM, respectively. The biological activity results were elucidated by molecular docking studies using the homology model of the human adenosine A2B receptor. As a result, the present study has highlighted that the bicyclic moiety of the compounds attached to hydrogen bond donor-acceptor capability and π-π stacking is an attractive scaffold for obtaining cytotoxic activity.

Keywords
Adenosine A2B Antagonists; Flavone Derivatives; Antitumor Activity; Molecular Docking Studies.

Introduction
G-protein coupled receptors (GPCRs) are one of the most common types of membrane bound receptors. They mediate response to diverse natural ligands. Activation of GPCRs result in either rapid response as activation of ion channels or slower one as intracellular enzyme cascades. These events are responsible for different physiological responses [Congreve et al, 2014]. Therefore, GPCRs are targets in many recent pharmaceutical researches which focused on drug discovery. They are formed from seven membrane spanning α-helices (TM1-7) connected by intracellular (IL1, IL2 and IL3) and extracellular loops (EL1, EL3 and EL3). N-terminal is located extracellular and C-terminal is positioned intracellular and maintain interaction with cytosolic G-protein (Figure 1).
(Figure 1) Schematic diagram of a GPCR with seven TM domains (TM1–TM7), extracellular loops (EL1–EL3) and intracellular loops (ICL1–ICL3).

Adenosine receptors (ARs) comprise a group of G-protein receptors which mediate the physiological actions of adenosine. These receptors are classified according to their differential coupling to adenyll cyclase to regulate cyclic AMP levels. The A₁ and A₃ ARs are coupled to Gi/o proteins, while A₂A and A₂B ARs are coupled to Gs/olf proteins [Fredholm et al., 2011]. A₂B human AR is defined as the “low-affinity” subtype because requires high micromolar concentrations of adenosine to be activated. It couples to Gs proteins, thus stimulating adenylate cyclase and cAMP accumulation, and Gq proteins, resulting in phospholipase C activation [Schulte et al., 2003]. A₂B AR regulates a number of physiological and pathological events [Fredholm et al., 2011].

Adenosine A₂B receptor antagonists are considered as a good therapy in the treatment of cancer [Panjehpour et al., 2005], asthma [Feoktistov et al., 1998, Marx et al., 2001], Alzheimer’s disease [Rosi et al., 2003], and type-II diabetes [Fiebich et al., 2005]. A₂B AR is the least well characterized among the ARs primarily due to the lack of suitable, specific ligands. Furthermore, the xanthine-based agents have now completed clinical trials. However, the xanthine derivatives are of weak affinity and thus, are nonselective at the AR subtypes. Therefore, the discovery and development of selective and potent non-xanthine antagonists for the human adenosine A₂B ligands remains an attractive goal. In view of this, the knowledge of the 3D structure of the adenosine A₂B receptor could be of great benefit in the process of structure-guided drug design. The A₂B receptor encodes a protein of 328 to 332 amino acid residues depending on the species [Pierce et al., 1992]. As a result, a new and improved homology model for the human adenosine A₂B receptor was created and investigated [Sherbiny et al., 2009]. Consequently, virtual screening of potential ligands using the adenosine A₂B receptor model has been accomplished. Some of these hits are related to flavone nucleus, and have been used as a template for ongoing research.

Result and Discussion
Chemistry

The hit compound produced from virtual screening based on homology model of the human A₂B AR has been made by retrosynthetic analysis. Therefore, it was found that we need to synthesize both flavonol (3-hydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one) (IV), and N-phenylacetamide (V) (Chart 1).
Flavonols can be synthesized by different pathways including modified Kostaniki-Robinson reaction [Looker et al., 1978], Baker-Venkatatamaran rearrangement [Looker et al., 1964] and Algar-Flynn-Oymada reaction [Bennett et al., 1996]. The last one was selected as pathway for the synthesis of our flavonol as it is a modular synthetic method using commercially available starting materials and milder conditions which makes it an ideal method for the combinatorial synthesis [Boldi et al., 2006]. It is the synthesis of flavonols via oxidative cyclization of 2-hydroxychalcones with hydrogen peroxide under alkaline conditions (Scheme 1).

Different methods are available for the preparation of chalcones [Meyer et al., 1991] (III). The most convenient method is the Claisen-Schmidt condensation [Smith et al., 1954] of equimolar quantities of aryl methyl ketone with aromatic aldehyde in the presence of base or acid catalyst [Davey et al., 1957, Gharpure et al., 2012] followed by a dehydration to yield chalcones. Therefore, in this study, chalcone (III) was prepared through the reaction of 2-hydroxyacetophenone (I) with 4-methoxybenzaldehyde (II) in the presence of aqueous sodium hydroxide.

Compound (IV) was prepared by oxidative cyclization of 2-hydroxychalcones with hydrogen peroxide under alkaline conditions. This reaction is generally known as Algar-Flynn oxidation [Shah et al., 1955], or Algar-Flynn-Oyamada oxidation [Beutler et al., 1998, Geissman et al., 1948, Gharpure et al., 2012]. The IR spectrum of compound (IV) is characterized by strong absorption band at 1690 cm\(^{-1}\) due to carbonyl ketone stretching, which appeared at low absorption value because of conjugation with the double bond and aromatic system and a broad peak at 3650 cm\(^{-1}\) due to phenolic hydroxyl group. \(^1\)H NMR spectrum of compound (IV) is characterized by the presence of a singlet peak exchangeable with D\(_2\)O at 9.8 ppm due
to OH group in addition to multiplet of eight protons in the aromatic region 7.1-8.3 ppm, singlet of three protons at 3.85 ppm.

2-Chloro-N-aryl acetamides or 3-chloro-N-phenylpropanamide (Vαj) can be synthesized based on the literature survey by reaction of an appropriate amine with chloroacetyl chloride using different solvents (acetone, DMF, chloroform) and catalysts (TEA, pyridine) [Merino et al, 2013, Kumar et al, 2012]. (Vαj) can also be prepared by addition of lithium carbenoids to variously N-functionalized isocyanates [Pace et al, 2013] or by solvent free method [Ghosh et al, 2012]. The selected method was the dissolving of amine in glacial acetic acid containing saturated solution sodium acetate then, chloroacetyl chloride was added [Kumar et al, 2014].

On the other hand, compounds (VIα-k) were prepared in good yield via a nucleophilic substitution reaction and its rate is based on positive charge availability on α carbon of acetanilide which mainly affected by substitution on aromatic ring. Electron withdrawing group increases the rate of the reaction and its yield while electron donating decreases the rate of reaction and its yield. In these compounds modification was carried out by lengthening the linker between oxygen atom at the 3-position of chromenone ring and the N-aryl amide by addition of CH₂ bridge in order to exhibit its effect on the binding of compound to the human adenosine A₂B receptor and thus it's pharmacological activity [Chen et al, 2008].

The structures of this set of compounds were confirmed based on their spectral data, ¹H NMR, ¹³C NMR and mass spectroscopy. The ¹H NMR spectrum revealed the main features of all series is the presence of singlet signal corresponding to three protons at 3.8 ppm due to para methoxy group on exocyclic ring, singlet two protons at 4.5 ppm due to methylene bridge beside carbonyl of acetamide and singlet signal corresponding to one proton at 10.5 ppm representing (-NH) amide which is D₂O exchangeable. The aromatic protons number, splitting, and chemical shifts are vary according to number and type of substitution on N-phenyl ring. ¹³C NMR shows twenty signals due to the presence of ten equivalent carbons and thus it shows a signal at 55 ppm be due to two methoxy groups in para position of both phenyl ring attached to bicyclic moiety and N-acetamide aromatic ring. Furthermore, a signal at 77 ppm due to methylene bridge between oxygen and carbonyl group and signals at 114 ppm and 160 ppm be due to aromatic carbons. Two signals at 167 ppm and 173 ppm be due to carbonyl carbons. The characteristic mass spectral data of compound VIefor example, showing molecular ion peak at 432 m/z and base peak at 281 m/z.

Anti-cancer activity and molecular docking studies

A₂B receptor activation is thought to support tumor growth by stimulating the release of angiogenic factors from vascular smooth muscle, endothelial cells and host immune cells [Dubey et al, 2002, Goel et al, 2011]. The selective expression of high levels of endogenous A₂B receptors coupled to two signaling pathways make MDA-MB-231 cells a suitable model for this human adenosine receptor subtype [Panjehpour et al, 2005]. Thus, the new synthesized compounds eleven were subjected to MTT proliferation assay to investigate their in-vitro cytotoxic activity, in comparison with the activity of the known anticancer agent doxorubicin as a reference drug. The biological results are given in (Table 1) and the results of cytotoxic activity revealed that compound (VII) with IC₅₀ value of 43.7 has the highest activity against MDA-MB 231 cell line (breast cancer).
(Table 1) IC₅₀ values of chromone derivatives on MDA-MB 231 cell line

<table>
<thead>
<tr>
<th>Comp. No.</th>
<th>IC₅₀ (μM)</th>
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<tbody>
<tr>
<td>VIₐ</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>VI₋</td>
<td>363</td>
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<tr>
<td>VI₃</td>
<td>245</td>
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</tr>
<tr>
<td>VI₁₀</td>
<td>288</td>
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<tr>
<td>VI₁¹</td>
<td>138</td>
</tr>
<tr>
<td>Doxorubicin</td>
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</table>

(Chart 2) IC₅₀ values of chromone derivatives on MDA-MB 231 cell line.

The obtained binding mode results of (VIi) with the human adenosine A₂B homology model allowed us to propose that the bicyclic core of (VIi) is stabilized by an aromatic stacking interaction with His251, aliphatic hydrophobic interactions with
Val250, Met272, Met179, Val191, Met182, and Ile276, and a hydrogen bonding interaction with Asn254 (conjugated hydrogen bonding from Asn254 through Asn186 to Gln90 (Figure 2), which stabilized the receptor in inactive state of the receptor. In addition, Glu174 is water mediated interaction with carbonyl moiety of acetamide of compound (VII).

Furthermore, the pyridine nitrogen atom of the (VII) is in proximity to the sidechain hydroxyl group of Thr257. In addition, the pyridine ring is stabilized by hydrophobic interactions with Met179, Met272, and Val85, and the phenyl ring is involved in an aromatic stacking interaction with Phe173 and formed hydrophobic interactions with Trp247, Val191, Leu86, Ile276, Ala64, His280, and Val85. Because of the existence of additional hydrogen bonding and desirable interactions compared with other derivatives, compound (VII) has the highest affinity towards the receptor than other compounds.

(Figure 2) Predicted binding mode of compound VII with A2B homology model. Interactions between H-bonded atoms are indicated by yellow dotted lines. Hydrogen (white), nitrogen (blue), oxygen (red) and sulfur (yellow).

Moreover, the final binding mode results for compounds, VIa, VIb, VIc, VId, VIe, VIf, VIg, and VIh with the A2B homology model of the adenosine receptor follow the general pattern observed for compound VII. As before the hydrogen bonding, hydrophobic, and aromatic stacking interactions are maintained. However, the substitution of flavone backbone with aromatic ring connected with sulphamoyl moiety, aliphatic, and halogen, can increase the affinity towards adenosine A2B receptor (Table 2). In details, substitution with bromine atom (VIc) instead of chlorine atom (VIb) can increase the affinity due to the compound with bromine atom can accommodate more the binding site than chorine atom. In addition, substitution with methyl group (VId) instead of methoxy group (VIe) can increase the affinity due to the hydrophilic moiety of the compound is surrounded by hydrophobic amino acid residues like, Met179 and Leu172. In addition, substitution with two methyl groups as compound VIg could further hampered the interactions due to steric effect induced by two methyl groups.
The docking scores and IC$_{50}$ for all new synthesized compounds.

<table>
<thead>
<tr>
<th>Comp. No.</th>
<th>Docking Score (Kcal/mol)</th>
<th>IC$_{50}$ (uM)</th>
</tr>
</thead>
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<tr>
<td>VI$_a$</td>
<td>-50.78</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>VI$_b$</td>
<td>-80.36</td>
<td>245</td>
</tr>
<tr>
<td>VI$_c$</td>
<td>-80.87</td>
<td>363</td>
</tr>
<tr>
<td>VI$_d$</td>
<td>-90.89</td>
<td>72.4</td>
</tr>
<tr>
<td>VI$_e$</td>
<td>-60.88</td>
<td>912</td>
</tr>
<tr>
<td>VI$_f$</td>
<td>-70.69</td>
<td>512</td>
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<tr>
<td>VI$_g$</td>
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</tr>
<tr>
<td>VI$_h$</td>
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<td>50</td>
</tr>
<tr>
<td>VI$_i$</td>
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</tr>
<tr>
<td>VI$_j$</td>
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<td>288</td>
</tr>
<tr>
<td>VI$_k$</td>
<td>-90.35</td>
<td>138</td>
</tr>
</tbody>
</table>

(Figure 3) Predicted binding mode for compound VI$_{ih}$ with A$_{2B}$ homology model. Interactions between H-bonded atoms are indicated by yellow dotted lines. Hydrogen (white), nitrogen (blue), oxygen (red) and sulfur (yellow)
**Figure 4** Predicted binding mode for compound VIh with A2B homology model. Interactions between H-bonded atoms are indicated by yellow dotted lines. Hydrogen (white), nitrogen (blue), oxygen (red) and sulfur (yellow)

The obtained binding mode for VIk with the homology A2B model proposed that the Asn254 side-chain forms hydrogen bonding interactions with the carbonyl group at the 4-position and the amino group of acetamide of the compound VIk. In addition, the bicyclic core of the compound is located inside the hydrophobic pocket formed by Met182, Leu86, Val250, Val191, His251, Leu86, Val85, and Trp247. The phenyl moiety of the synthesized compound is stabilized by an aromatic stacking interaction with Phe173 and located inside the pocket formed by Ala64, Ile67, Met179, Ile276, and His280. Moreover, the phenyl moiety attached to propanamide moiety of the compound is surrounded by Val253, Thr257, Met272, Met179, and Glu174.
(Figure 5) Predicted binding mode for compound VIk with A2B homology model. Interactions between H-bonded atoms are indicated by yellow dotted lines. Hydrogen (white), nitrogen (blue), oxygen (red) and sulfur (yellow)

However, the substitution with bromine atom VIj can decrease the affinity for the human adenosine A2B receptor, where the differences between the affinities of compounds VIj and VIk could be explained by unfavorable interactions between the compounds and the receptor e.g. the bromine atom is surrounded by unfavorable interactions with polar groups like, Glu174, and Asn266. The results of docking analysis of the synthesized compounds with the A2B homology model display a common binding mode for the synthesized derivatives (Figure 6). Moreover, the structural findings are accompanied by energetic aspects the observed binding energies ΔG for each complex are listed. The experimentally measured values ranged from -50.78 to -120.5 kcal mol⁻¹. As shown in (Table 2), the computed values reflect the overall trend.

(Figure 6) The superposition of the highest active compounds among other compounds (VIa, VIh, VId, VIk) placements with the human A2B adenosine receptor
Conclusion

The present work involves design, synthesis, and pharmacological evaluation of certain new eleven chromenone derivatives as A\textsubscript{2B} receptor antagonists. Thus, a set of new compounds was successfully synthesized and characterized structurally by various techniques using spectral analyses. All of them were subjected to MTT proliferation assay to investigate their \textit{in-vitro} cytotoxic activity. Among all studied compounds, compounds (VIi, VIh, VId, and VIk) revealed moderate inhibitory activity with IC\textsubscript{50} values ranging from 43.7 to 138 µM in MDA-MB 231 cell line. The results of cytotoxic activity revealed that the highest activity was observed with compound VIi that has N-pyriden-2-yl acetamide substituted at 3-position of flavone backbone followed by compound VIh with IC\textsubscript{50} values of 43.7 and 50 µM, respectively. The biological activity results were elucidated by molecular docking studies using the homology model of the human adenosine A\textsubscript{2B} receptor.

Experimental:

All melting points were measured on a Gallenkamp melting point apparatus and were uncorrected. The IR spectra were recorded on a Pye-Unicam SP-3-300 infrared spectrophotometer (potassium bromide dicks) and expressed in wave number (cm\textsuperscript{-1}). \textsuperscript{1}HNMR spectra were run at 300 and 400MHz, on a Varian Mercury VX-300 and Bruker Avance III NMR spectrometer respectively, while \textsuperscript{13}C NMR spectra were run at 75 MHz. TMS was used as an internal standard in deuterated dimethylsulphoxide (DMSO-d6). Chemical shifts (δ) are quoted in ppm. The abbreviations used are as follows: s, singlet; d, doublet; m, multiplet. All coupling constant (J) values are given in hertz. The mass spectra were recorded on Shimadzu GCMS-QP-1000EX mass spectrometer at70 eV. Elemental analyses were performed on CHN analyzer and all compounds were within ± 0.4 of the theoretical values. The reactions were monitored by thin-layer chromatography (TLC) using TLC sheets coated with UV fluorescent silica gel Merck 60 F254 plates and were visualized using UV lamp and different solvents as mobile phases. All reagents and solvents were purified and dried by standard techniques. All the newly synthesized compounds gave satisfactory elemental analysis.

3-Hydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one, (IV).

1-(2-Hydroxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one, (III) (2.5 g, 0.01 mol) was suspended in absolute ethanol (50 mL) then aqueous solution of sodium hydroxide was added (5 mL, 1.25 N) finally (10 mL) of 30% H\textsubscript{2}O\textsubscript{2} was added dropwise to warm solution. Mixture allowed to stir at room temperature. Then the mixture is diluted by cold water, acidified with diluted hydrochloric acid. The precipitated powder is filtrated, washed with water, and crystallized from isopropyl alcohol as puff powder. Yield 60%, and m.p.235\textsuperscript{-}237 °C. IR, KBr, cm\textsuperscript{-1} for compound IV: 3211 (Phenolic O-H Stretch), 1650 (C=O Stretch). \textsuperscript{1}H NMR (DMSO-d\textsubscript{6}) for compound IV: δ 3.85 (s, 3H, -OCH\textsubscript{3}), 7.10 (d, 2H), 7.40 (t, 1H), 7.80 (d, 2H), 8.10 (d, 2H), 8.30 (t, 1H) total protons in aromatic region is eight protons, 9.40 ppm (s, 1H, -OH, D\textsubscript{2}O exchangeable).

2-[(2-(4-Methoxyphenyl)-4-oxo-4H-chromen-3-yl)oxy]-N-substituted phenyl acetamidederivatives, (VI\textsubscript{a-i})

Equimolar of appropriate 2-chloro-N-arylacetamide derivatives (V\textsubscript{a-i}) (1 mol), 3-hydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one (IV) (1 mol, 0.68 g), potassium carbonate (1.5 mol, 0.13 g), and potassium iodide (1 mol, 0.16g.) in acetone (50 mL)
was stirred at room temperature for 15 minute then refluxed, evaporated and residual powder collected and suspended in water (20 mL) then extracted twice by ethyl acetate (2x10 mL). The organic layer was separated and evaporated giving final product which was then crystallized from ethylacetate.

2-(2-(4-Methoxyphenyl)-4-oxo-4H-chromen-3-yloxy)-N-phenylacetamide (VIa)

Yield 60%, and m.p. 190-192 °C. $^1$H NMR (DMSO-d6) δ ppm 3.86 (s, 3H, 4-methoxy-B-phenyl), 4.63 (s, 2H, CO-CH$_2$), 7.08 (t, 1H, N-phenyl-H4), 7.10 (d, 2H, J = 9, 2-phenyl H3, H5), 7.30 (t, 2H, J = 8.9, N-phenyl-H3, H5), 7.50 (t, 3H, J = 9.2, N-phenyl-H2, H6, chromene-N-H6), 7.62 (t, 2H, J = 9.8, 2-phenyl-H2, H6), 7.71 (d, 1H, J = 9, chromene-H8), 7.80 (t,1H, J = 8.7, chromene-H7), 8.10 (d, 1H, J = 8.8, chromene-H5) and 10.32 ppm (s,1H, -NH, D$_2$O-exchangeable). Mass (m/z) 401.38 (M$^+$) (10), 343.3(C$_{22}$H$_{16}$O$_3$) (1), 309(C$_{18}$H$_{13}$O$_3$) (60), 281(C$_{17}$H$_{13}$O$_4$) (100) 239.22(C$_{15}$H$_{11}$O$_2$) (4), 175.16(C$_{10}$H$_9$NO) (14), 132(C$_8$H$_7$NO$_4$) (5), and 93.1(C$_6$H$_6$O) (1)

N-(2,6-Dimethylphenyl)-2-(2-(4-methoxyphenyl)-4-oxo-4H-chromen-3-yloxy)acetamide (VIIb)

Yield 55%, and m.p. 181-183 °C.$^1$H NMR (DMSO-d6) δ ppm 3.86 (s, 3H, 4-methoxy phenyl), 4.64 (s, 2H, O-CH$_2$), 7.11 (d, 2H, J = 9, 2-phenyl H3, H5), 7.33 (d, 2H, J = 8.9, N-phenyl-H3, H5), 7.50 (t, 1H, J = 11, chromene-H6), 7.60 (d, 2H, J = 8.9, 2-phenyl-H2, H6), 7.70 (t, 1H, J = 9, chromene-H7), 7.82 (d, 1H, J = 8.2, chromene-H8), 8.13 (d, 1H, J = 8.6, chromene-H5) and 8.16 (d, 2H, J = 8.1, N-phenyl-H, H6) and 10.50 ppm (s,1H, -NH, D$_2$O-exchangeable). Mass (m/z) 435(M$^+$) (2), 309(C$_{18}$H$_{13}$O$_3$) (95), 281(C$_{17}$H$_{13}$O$_3$) (100), 252(C$_{16}$H$_{11}$O$_3$) (11), 239(C$_{15}$H$_{11}$O$_3$) (30), 211(C$_{14}$H$_{11}$O$_2$) (5), 197(C$_9$H$_8$ClNO$_2$) (6), 175(C$_{10}$H$_9$O$_3$) (7), 127(C$_6$H$_5$ClN) (3), and 76(C$_6$H$_5$) (4).

2-(2-(4-Methoxyphenyl)-4-oxo-4H-chromen-3-yl)oxy)-N-p-tolylacetamide (VIIc)

Yield 35%, and m.p. 164-166 °C.$^1$H NMR (DMSO-d6) δ ppm 3.86 (s, 3H, 4-methoxy phenyl), 4.64 (s, 2H, O-CH$_2$), 7.10 (d, 2H, J = 9, 2-phenyl H3, H5), 7.52 (d, 3H, J = 10, N-phenyl-H2, H6, chromene-H8), 7.64 (d, 2H, J = 9, 2-phenyl H2, H6), 7.71 (t, 1H, J = 9, chromene-H6), 7.80 (t, 1H, J = 8.7, chromene-H7), 8.10 (d, 3H, J = 8.7, N-phenyl-H3, H5, chromene-H5), and 10.50 ppm (s,1H, -NH, D$_2$O-exchangeable). Mass (m/z) 481.3(M$^+$) (5), 309(C$_{18}$H$_{13}$O$_3$) (76), 281(C$_{17}$H$_{13}$O$_4$) (100), 239(C$_{15}$H$_{11}$O$_3$) (28), 175(C$_{10}$H$_9$O$_3$) (11) and 77(C$_6$H$_5$) (9).

N-(4-Bromophenyl)-2-(2-(4-methoxyphenyl)-4-oxo-4H-chromen-3-yl)oxoacetamide (VID)

Yield 70%, and m.p. 133-135 °C.$^1$H NMR (DMSO-d6) δ ppm 2.26 (s, 3H, 4-methyl-N-phenyl), 3.86 (s, 3H, 4-methoxy phenyl), 4.63 (s, 2H, O-CH$_2$), 7.12 (d, 4H, J = 9.2, 2-phenyl H3, H5, N-phenyl-H3, H5), 7.51 (d, 4H, J = 10, 2-phenyl H2, H6, N-phenyl-H2, H6), 7.77 (t, 1H, J = 9, chromene-H6), 7.80 (t, 1H, J = 7.2, chromene-H7) 8.13 (d, 2H, J = 8, chromene-H5, H8) and 10.30 ppm (s,1H, -NH, D$_2$O-exchangeable) Mass (m/z) 415.(M$^+$) (11), 357(C$_{22}$H$_{18}$NO$_3$) (4), 309(C$_{18}$H$_{13}$O$_3$) (100), 281(C$_{17}$H$_{13}$O$_4$) (97), 239(C$_{15}$H$_{10}$O$_3$) (19), 135(C$_8$H$_7$O$_2$) (7), 106(C$_7$H$_8$N) (23), 77(C$_6$H$_5$) (17), and 65(C$_5$H$_3$) (7). Anal. Calc. for: (C$_{25}$H$_{21}$NO$_3$) (M.W. = 415): C: 61.10; H, 3.42; N, 11.88%; found C, 72.47; H, 5.214; N, 3.46%.
N-(4-Chlorophenyl)-2-(2-(4-methoxyphenyl)-4-oxo-4H-chromen-3-yl)acetamide (VIe)

Yield 80%, and m.p. 155-157 °C. $^1$H NMR (DMSO-d$_6$) δ ppm 3.72 (s, 3H, 4-methoxy-N-phenyl), 3.85 (s, 3H, 4-methoxy-2-phenyl), 4.50 (s, 2H, O-CH$_2$), 6.80 (d, 2H, J = 8, 2-phenyl-H3, H5), 7.10 (d, 2H, J = 8, N-phenyl-H3, H5), 7.52 (t, 1H, J = 11, chromenone-H6), 7.55 (d, 2H, J = 8, 2-phenyl-H2, H6), 7.71 (d, 1H, J = 8, chromenone-H8), 7.80 (t, 1H, J = 8, chromenone-H7), 8.13 (d, 3H, J = 8.6, 2-phenyl-H2, H6, chromenone-H5) and 10.55 ppm (s,1H,-NH, D$_2$O-exchangeable) Mass (m/z) 432(M$^+$) (1), 309(C$_{18}$H$_{13}$O$_3$) (99), 281(C$_{17}$H$_{12}$O$_4$) (100), 268(C$_{16}$H$_{12}$O$_4$) (16), 239(C$_{15}$H$_{11}$O$_3$) (31), 211(C$_{14}$H$_{11}$O$_2$) (10), 196(C$_{13}$H$_{8}$O$_2$) (11), 121(C$_{7}$H$_{3}$O$_2$) (10), 92(C$_6$H$_2$O) (10), 77(C$_5$H$_5$) (12), and 65(C$_5$H$_3$) (6).$^{13}$H NMR (DMSO-d$_6$) δ ppm 55, 71, 114, 118, 119, 122, 123, 124, 125, 126, 130, 134, 138, 139, 141, 154, 155, 161, 167 and 173 ppm

N-(4-Methoxyphenyl)-2-(2-(4-methoxyphenyl)-4-oxo-4H-chromen-3-yl)acetamide (VIf)

Yield 50%, and m.p. 187-189 °C. $^1$H NMR (DMSO-d$_6$) δ ppm 3.85 (s, 3H, 4-methoxy-2-phenyl), 4.72 (s, 2H, O-CH$_2$), 7.11 (d, 2H, J = 9, 2-phenyl-H3, H5), 7.20 (s, 2H, -SO$_2$NH$_2$, D$_2$O-exchangeable), 7.41 (t, 1H, J = 9.6, chromenone-H6), 7.73 (t, 1H, J = 10, chromenone-H7), 7.80 (d, 5H, J = 8.4, chromenone-H8, N-phenyl-H2, H6, 2-phenyl-H2, H6), 8.11 (d, 1H, J = 9.6, chromenone-H8), 8.20 (d, 2H, J = 9, N-phenyl-H3, H5), and 10.70 ppm (s,1H,-NH, D$_2$O-exchangeable) $^{13}$H NMR (DMSO-d$_6$) δ ppm 55, 71, 114,118, 119, 122, 123, 124, 125, 126, 130, 134, 138, 139, 141, 154, 155, 161, 167 and 173 ppm Mass (m/z) 480 (M$^+$) (4) 450(C$_{23}$H$_{18}$N$_2$O$_6$S) (1), 376(C$_{17}$H$_{16}$N$_2$O$_6$S) (1), 373(C$_{17}$H$_{13}$N$_2$O$_6$S) (4), 324(C$_{15}$H$_{14}$NO$_3$) (2), 230(C$_{8}$H$_{10}$N$_2$O$_2$S (1), 211(C$_{14}$H$_{12}$O$_2$) (5), 145 (C$_5$H$_2$O$_2$) (8), 135(C$_8$H$_7$O$_2$) (100), 119(C$_7$H$_3$O$_2$) (60) and 92(C$_6$H$_4$O) (2).

2-(2-(4-Methoxyphenyl)-4-oxo-4H-chromen-3-yl)-N-(4-sulfamoylphenyl)acetamide (VIg)

Yield 25%, and m.p. 153-155 °C. $^1$H NMR (DMSO-d$_6$) δ ppm 2.16 (s, 6H, 2,6 dimethyl-N-phenyl), 3.86 (s, 3H, 4-methoxy phenyl), 4.63 (s, 2H, O-CH$_2$), 7.08 (s, 3H, N-phenyl-H3,H4,H5), 7.12 (d, 2H, J = 9.2, 2-phenyl H3, H5), 7.50 (t, 1H, J = 9, chromenone-H6), 7.72 (t, 1H, J = 9.2, chromenone-H8), 7.83 (t, 1H, J = 8.9, chromenone-H7), 8.12 (d, 1H, J = 9.2, chromenone-H5), 8.20 (d, 2H, J = 9.2, 2-phenyl H2,H6) and 9.71 ppm (s,1H, -NH, D$_2$O-exchangeable). Mass (m/z) 429 (M$^+$) (5.4), 309 (C$_{18}$H$_3$O$_3$) (34), 281(C$_{17}$H$_{13}$O$_4$) (100), 239(C$_{16}$H$_{12}$O$_3$) (20), 211 (C$_{15}$H$_{13}$O$_2$) (6), 144(C$_6$H$_7$O$_2$) (13), and 77 (C$_7$H$_5$) (10).

2-(2-(4-Methoxyphenyl)-4-oxo-4H-chromen-3-yl)-N-(naphthalen-2-yl)acetamide (VIIh)

Yield 75%, and m.p. 195-197 °C. $^1$H NMR (DMSO-d$_6$) δ ppm 3.85 (s, 3H, 4-methoxy-2-phenyl), 4.71(s, 2H, O-CH$_2$), 7.13 (d, 2H, J = 9, 2-phenyl-H3, H5), 7.45(t, 1H, -naphthalene-H5), 7.51 (t, 1H, J = 9.5, chromenone-H6), 7.62 (d, 3H, J = 10, naphthalene-H8, 2-phenyl-H2,H6), 7.71 (t, 1H, J = 9, naphthalene-H4), 7.82 (d, 1H, J = 9, naphthalene-H3), 7.91 (d, 2H, J = 9.5, chromenone-H8, naphthalene-H6), 8.00 (t, 1H, J = 9, chromenone-H7),8.10 (d, 2H, J = 9.2, naphthalene-H6, chromenone-H5) 8.43 (d, 1H, J = 9, naphthalene-H2) and 10.40 ppm: (s,1H, -NH, D$_2$O-exchangeable). Mass (m/z) Mass (m/z) 451(M$^+$) (3), 309(C$_{18}$H$_{13}$O$_3$) (100), 281(C$_{17}$H$_{13}$O$_4$) (91), 267(C$_{16}$H$_{11}$O$_4$) (9), 251 (C$_{16}$H$_{11}$O$_3$) (5), 239 (C$_{15}$H$_{11}$O$_3$) (23), 211 (C$_{14}$H$_{11}$O$_2$) (7), 142
N-(5-Chloropyridin-2-yl)-2-(2-(4-methoxyphenyl)-4-oxo-4H-chromen-3-yl)acetamide (VIj)

Yield 60%, and m.p. 160-162 °C. 1H NMR (DMSO-d6) δ ppm 3.85 (s, 3H, 4-methoxy-2-phenyl), 4.70 (s, 2H, O-CH2), 7.11 (d, 2H, J = 9, 2-phenyl-H3, H5), 7.52 (t, 1H, J = 9.5, chromene-H6), 7.73 (d, 3H, J = 10, chromene-H8, 2-phenyl-H2,H6), 7.90 (t, 1H, chromene-H7), 8.00 (d, 2H, chromene-H5, pyridine-H4), 8.13 (d, 1H, pyridine-H5), 8.32 (s, 1H, pyridine-H6), and 10.90 ppm (s,1H, -NH, D2O-exchangeable). 13C NMR (DMSO-d6) δ ppm 55, 70, 114, 115, 118, 122, 123, 124, 125, 126, 130, 134, 13, 139, 146, 149, 154, 155, 161, 167 and 173 ppm. Mass (m/z) 437 (M+), (1), 422(C22H15ClN2O3) (2), 460(C22H15ClN2O4) (1), 324(C15H14NO3) (2), 281 (C17H12O3) (11), 252 (C18H12O3) (6), 242(C15H14O3) (2), 169 (C7H6ClN2O) (91), 135(C8H7O2) (100), 121(C7H5O2) (94), 78 (C6H4) (23). Anal. Calcd for C23H17ClN2O5, (436); C, 63.24; H, 3.92; N, 6.41. Found: C, 63.43; H, 3.94; N, 6.57.

3-[(2-(4-Methoxyphenyl)-4-oxo-4H-chromen-3-yl)oxy]-N-substituted phenylpropanamide derivatives, (VIj&k)

The appropriate 3-chloro-N-phenylpropanamide derivative (VIj&k) (1 mol) was reacted with 3-hydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one (IV) (1 mol. 0.68g) in DMF as solvent. Reaction mixture was refluxed, then the reaction mixture poured onto crushed ice and the solid is collected and washed with water then crystallized from ethyl acetate giving product, VIj&k

N-(4-Bromophenyl)-3-(2-(4-methoxyphenyl)-4-oxo-4H-chromen-3-yl)propanamide (VIIj)

Yield 40%, and m.p. 223-224 °C. 1H NMR (DMSO-d6) δ ppm δ 2.50 (d, 2H, J = 15, -O-CH2), 3.52 (d, 2H, J = 15,-CO-CH2), 3.85 (s, 3H, -OCH3), 7.10 (d, 2H, J = 9, 2-phenyl-H3, H5), 7.41 (d, 3H, J = 9, 2-phenyl-H2, H6, chromene-H8), 7.55 (d, 2H, J = 8.9, N-phenyl-H2,H6), 7.70 (m, 2H, chromene-H6, H7), 8.01 (d, 1H, J = 9, chromene-H5), and 8.10 (d, 2H, J = 9, N-phenyl-H3,H5), and 9.95 ppm (s,1H, -NH, D2O-exchangeable) Mass (m/z) 494 (M+) (2), 479 (C22H17BrNO3) (4), 387(C14H13BrNO4) (1), 414 (C25H20NO5) (1), 338 (C19H16NO3) (1), 295(C15H15O4) (1), 282 (C17H14O4) (4), 122 (C7H6O2) (27), 106 (C5H4O) (7), 98 (C5H6O2) (15), 94(C6H5O) (100), 55 (C4H7) (93) and 42 (C3H5) (7).

N-(4-Methoxypheynyl)-3-(2-(4-methoxyphenyl)-4-oxo-4H-chromen-3-yl)propanamide (VIIk)

Yield 60%, and m.p. 240-242 °C. 1H NMR (DMSO-d6) δ ppm2.50 (d, 2H, J = 15, -O-CH2), 3.50 (d, 2H, J = 15, -CO-CH2), 3.70 (s, 6H, , 4-methoxy-N-phenyl, 4-methoxy-2-phenyl), 6.80 (d, 4H, J = 9, 2-phenyl-H3,H5, N-phenyl-H3,H5), 7.10 (t, 1H, J= 9, chromene-H6), 7.4 (d,4H, , J = 9, N-phenyl-H2,H6, 2-phenyl-H2,H6), 7.70 (t, 1H, J =10, chromene-H7), 8.10 (d,2H, J = 9, chromene-H5,H8) and 9.80 ppm (s,1H, -NH, D2O-exchangeable).Mass (m/z)445 (M+), (1) 337(C19H13NO3) (1), 323 (C9H15O3) (3), 236 (C12H12NO4) (10) 280(C17H13O4) (1), 145 (C9H4O2) (3), 113 (C3H3NO2) (100), 97(C6H6O) (20), and 71 (C3H4O) (27).
Molecular docking procedure

All docking studies were performed using AutoDock program [Morris et al., 1998]. AutoDock is a suit of automated docking tools, which allows flexible ligand docking and freely available under the GNU general public license [The Scripps Research Institute]. The scoring function used is empirically derived, for empirical binding free energy force field that allows the prediction of binding free energies for docked ligands. The protein target needs to be prepared and modeled according to the format requirements of the docking algorithms used. Thus the homology model of the human $A_{2B}$ adenosine receptor [Sherbiny et al., 2009] was used. All bound water ligand were removed from the protein prior to the docking process.

Pharmacological procedure

The effect of compounds on the proliferation of MDA cell line was assessed using MTT proliferation assay. Exponentially growing cells from cell type was trypsinized, counted and seeded at the appropriate densities (2000-1000 cells/0.33 cm$^2$ well) into 96-well microtiter plates. Cells then was incubated in a humidified atmosphere at 37°C for 24 hours. Then, cells were exposed to different concentrations of compounds (0.1, 10, 100, 1000 µM) for 24, 48 and 72 hours. Then the growth media was removed; cells were incubated with 200 µl of 5% MTT solution/well (Sigma Aldrich, MO) and were allowed to metabolize the dye into a colored-insoluble formazan crystal for 2 hours. The remaining MTT solution were discarded from the wells and the formazan crystals were dissolved in 200 µl/well acidified isopropanol for 30 min, covered with aluminum foil and with continuous shaking using a MaxQ 2000 plate shaker (Thermo Fisher Scientific Inc, MI) at room temperature. Absorbance were measured at 570 nm using a Stat Fax® 4200 plate reader (Awareness Technology, Inc., FL). The cell viability were expressed as percentage of control and the concentration that induces 50% of maximum inhibition of cell 9 ($IC_{50}$) was determined using Graph Pad Prism software version 5 (Graph Pad software Inc, CA) [Mosmann et al., 1983, Scudiero et al., 1988].

REFERENCES


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تخليق والتقييم البيولوجي ودراسة الروابط لمشتقات الفلافون الجذيذة لمستقبلات للادينوزين A2B

للسادة الكاترين

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سلسلة من مشتقات الفلافون الجذيذة تم تخليقها، وهذه المشتقات الجديدة تم تميزها هيكليا من قبل مختلف التقنيات الحديثة باستخدام التحليلات الطيفية الدقيقة. كل المركبات المذكورة تم فحصها لتحقيق في الشاش Vli, Vlh, Vld, Vlk السام لهذه المركبات تجاه الخلايا السرطانية. ومن بين المركبات المدرجة مركب VIh الخاصة بسرطان الثدي مقارنة بالخلايا المرجعية MDA-MB231 التي لها تأثير ملحي للنمو تجاه خلايا دوكسيروبين. وظهرت هذه المركبات نشاط سلبي للخلايا مع قيمة IC50 تتراوح من 43.4 إلى 138 مكرومول. نجحت النشاط السام لهذه المركبات MDA-MB231 ميكرومول تجاه خلايا سرطان الثدي. وكشف هذا النتاج عن النشاط الثدي الخاص بمضادات السرطان VIH. وأظهر الانزلاق بين النشاط الثدي والنباتي. وقد أظهرت هذه النتائج أن هذه المركبات ترتبط بسدادات الهيدروجين والترابط من خلال V-P و التي تعتبر ساقالة جديبة للحصول على النشاط السام لهذه المركبات تجاه خلايا سرطان الثدي.