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Design, synthesis, and biological activity of certain quinazolidione derivatives as potent phosphodiesterase4 inhibitors

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Abstract In this study, a series of 3-butylquinazolidione linked with different substituent to N1 of quinazoline nucleus have been synthesized. Some of the new final compounds tested in vitro for their inhibitory activity against phosphodiesterase 4B which is the enzyme responsible for the hydrolysis of cyclic adenosine mono phosphate, the second messenger involved in the regulation of important cell functions. Compound **7f** (100%) showed inhibition better than rolipram (90%), while the other tested compounds showed moderate activity. Docking study has been done to rationalize the obtained biological results.

Keywords Synthesis ·
Phosphodiesterase4B (PDE4b) inhibitor · Quinazolidiones ·
Docking study

Introduction

Phosphodiesterases (PDEs) are enzymes responsible for the hydrolysis of cyclic adenosine mono phosphate (c-AMP) and cyclic guanosine mono phosphate (c-GMP) which are second messengers involved in the regulation of important cell functions such as secretion, contraction, metabolism, and growth (Potter, 1990). The inhibition of PDEs activity increases cellular levels of the key second messengers c-AMP and c-GMP, thereby activating specific protein phosphorylation cascade that elicit a variety of functional response (Palacios *et al.*, 1995). Strong evidence suggests

that c-AMP play a central role in regulating the function of airway smooth muscle (Torphy, 1998), inflammatory cells (Souness *et al.*, 2000), and immune cells and the c-AMP specific PDE4 is the predominant isoenzyme found in pro-inflammatory cells associated with a number of airways disorders (Barnes, 1999).

There are at least two main reasons for the basis of the rapid development of the chemical, pharmacological and biochemical research in the therapeutic utility of selective PDE4 inhibitors. First, there is a general conviction that the mixed anti-inflammatory and bronchodilator profile of PDE4 inhibitors could allow, through the optimization of first generation prototypes, the discovery of new agents able to compete and, perhaps, to replace corticosteroids which represent the basis of the therapeutic management of asthma. Moreover, PDE4 inhibitors may be beneficial in the treatment of chronic obstructive pulmonary disease (COPD), a major respiratory disease for which pharmacological treatment is still inadequate (Norman, 1999).

Second, new and promising therapeutic applications of PDE4 inhibitors in certain unmet autoimmune diseases, e.g. rheumatoid arthritis, multiple sclerosis, and type 2 diabetes have emerged in recent years (Burnouf *et al.*, 1998).

The PDE4 family is comprised of four primary gene products (PDE4A, PDE4B, PDE4C, and PDE4D) and is highly expressed in neutrophils and monocytes, CNS tissue, and smooth muscles of the lung (Bender and Beavo, 2006; McKenna and Muller, 2006; Zhang *et al.*, 2005). Knockout studies have revealed that PDE4B ablation suppresses TNF- α production, and PDE4D may be responsible for the occurrence of nausea and emesis (Robichaud *et al.*, 2002; Zhang *et al.*, 2005), heart failure, and the risk of arrhythmias (Lehnart *et al.*, 2005).

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From a structural point of view, selective PDE4 inhibitors in the public domain can be divided into three classes: structural analogues of rolipram, structural analogues of nitraquazone, and structures related to xanthines (Fig. 1).

In fact rolipram, since its discovery as a potent and selective PDE 4 inhibitor (Schneider *et al.*, 1986), has represented a useful pharmacological tool for the characterization of this isoenzyme in different tissues, as well as the main template for the synthesis of novel inhibitors. However, severe limiting side effects (nausea, vomiting, headache) precluded the development of rolipram and many promising candidates have been discontinued. Although a number of studies claiming different chemical classes of PDE 4 inhibitors are increasing in recent years, only few detailed studies evaluated the PDE4 inhibition of structural analogues of nitraquazone. These compounds could be devoid of the central side effects of the archetypal rolipram which hampered its development as a drug (Piaz and Giovannoni, 2000).

On this basis, this work was directed to synthesize a hybrid structure containing the quinazoline-2,4-dione nucleus of nitraquazone along with the *n*-butyl side chain of denbufylline. This scaffold is linked with different substituents at N1, which were chosen by selecting the ones with highest scores in a virtual screening study. Moreover, this paper describes the synthesis, PDE4B inhibition evaluation and docking studies for series of quinazolinones structures. Docking results were compared to biological data with the aim of obtaining useful information for the rational design of new PDE4B antagonist.

Methods and materials

Chemistry

All chemicals and reagents were obtained from Aldrich (Sigma-Aldrich, St. Louis, MO, USA), Lancaster (Alfa Aesar, Johnson Company, Ward Hill, MA, USA) and were used without further purification. Reactions were

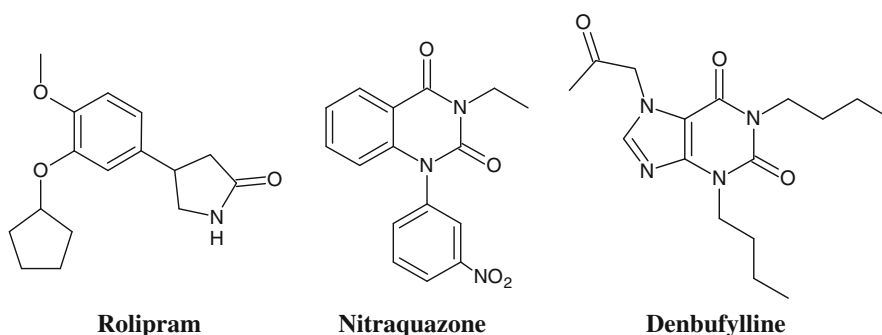
monitored by TLC, performed on silica gel glass plates containing 60 GF-254, and visualization on TLC was achieved by UV light or iodine indicator. IR spectra were determined on Shimadzu IR 435 spectrophotometer (KBr, cm^{-1}). $^1\text{H-NMR}$, $\text{C}^{13}\text{-NMR}$ spectra were recorded on Gemini Varian-VXR-unity (200 MHz), Gemini Varian 500 MHz (Germany) or Bruker UXNMR/XWIN-NMR (300 MHz) instruments. Chemical shifts (δ) are reported in ppm downfield from internal TMS standard. EI-MS Hewlett Packard 5988 spectrometer, Micro analytical Center, Cairo University, Egypt. ESI-MS Quadrupole VG *Quattro* Institute of Pharmacy & Molecular Biotechnology in Neuenheimer Field 364 69120 Heidelberg Germany. Elemental analyses were carried out in the Micro analytical Center, Cairo University, Egypt. Melting points were determined with an electro thermal melting point apparatus, and were uncorrected. On the other hand, 2-amino-*N*-butylbenzamide (**1**) (Clark and Wagner, 1944) was synthesized according to reported procedures. While 3-butylquinazoline-2,4(1*H*, 3*H*)-dione (**3**) was synthesized by a new procedure rather than the reported one (Staiger and Wagner, 1953).

2-Ethoxycarbonylamino-*N*-butylbenzamide (**2**)

A mixture containing 2-amino-*N*-butylbenzamide (**1**) (1.92 g, 0.01 mol) and ethyl chloroformate (28.5 g, 25 ml, 0.26 mol) was heated over steam for 3 h. The reaction mixture was evaporated under reduced pressure. The solid formed crystallized from ethanol/water mixture.

Yield: 80% mp.: 60°C. IR ν_{max} (cm^{-1}) (KBr): 3323 (2 NH), 2958, 2933 (CH aliphatic), 1737 (carbamate C=O), 1662 (C=O). $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ ppm: 0.90(t, 3H, CH_3), 1.31(t, 3H, $J = 7.5$ Hz, OCH_2CH_3), 1.38–1.46(m, 2H, CH_2CH_3), 1.58–1.64(m, 2H, $\text{CH}_2\text{CH}_2\text{CH}_3$), 3.42(q, 2H, N_3CH_2), 4.20(q, 2H, $J = 7.5$ Hz, OCH_2CH_3), 6.24(s, 1H, NH, D_2O exchangeable), 7.01(dd, 1H, Ar- C_5H), 7.40–7.49 (m, 2H, Ar- $\text{C}_3\text{H} + \text{Ar-}\text{C}_4\text{H}$), 8.38(d, 1H, Ar- C_6H), 10.40(s, 1H, NH). ^{13}C NMR (CDCl_3) δ ppm: 13.7 (CH_3), 14.5 (CH_3), 20.2 (CH_2), 31.5 (CH_2), 39.7 ($\text{CH}_2\text{-NH}$), 61.0 (OCH_2),

Fig. 1 Compounds representative of the three chemical classes of PDE 4 inhibitors: rolipram, nitraquazone, and xanthine derivatives (denbufylline)



114.7–139.9 (Ar–C), 153.9 (C=O), 168.8 (C=O). ESIMS m/z (% rel. abundance): 265.21 (M + 1, 100%). Anal. calcd. for C₁₄H₂₀N₂O₃: C, 63.62; H, 7.63; N, 10.60; Found C, 63.92; H, 7.34; N, 10.90.

3-Butylquinazoline-2,4(1H,3H)-dione (3)

A mixture of 2-ethoxycarbonylamino-*N*-butylbenzamide (2) (7.92 g, 0.03 mol) and KOH (3.36 g, 0.06 mol) in absolute ethanol (150 ml) was refluxed over steam for 4 h. The reaction mixture was evaporated under reduced pressure. The residue obtained was dissolved in a minimum amount of water, which was adjusted to pH 7–8 with acetic acid. The precipitated product was crystallized from ethanol/water mixture. Yield: 5.5 g (84%) mp.: 156–157°C (as reported).

3-Butyl-1-(2-chloroethyl) quinazoline-2,4(1H,3H)-dione (4)

A ternary mixture of 3-butylquinazoline-2,4(1H,3H)-dione (3) (2.18 g, 0.01 mol), 1-bromo-2-chloroethane (7.17 g, 4.16 ml, 0.05 mol) and anhydrous K₂CO₃ (6.90 g, 0.05 mol) in dry DMF (30 ml) was stirred at room temperature over night. The mixture was poured onto water and the formed precipitate was filtered, dried, and crystallized from ethanol/water mixture.

Yield: 64% mp.: 83°C. IR ν_{\max} (cm⁻¹) (KBr): 2954, 2931, 2870 (CH aliphatic), 1701 (C=O), 1662 (C=O). ¹HNMR (500 MHz, CDCl₃) δ ppm: 0.95(t, 3H, CH₃), 1.37–1.45(m, 2H, CH₂CH₃), 1.64–1.70(m, 2H, CH₂CH₂CH₃), 3.80(t, 2H, *J* = 7.0 Hz, CH₂Cl), 4.08(t, 2H, N₃CH₂), 4.45(t, 2H, *J* = 7.0 Hz, N₁CH₂), 7.23–7.29(m, 2H, C₆H & C₈H of quinazoline), 7.58(dd, 1H, C₇H of quinazoline), 8.24(d, 1H, C₅H of quinazoline). ¹³C NMR (CDCl₃) δ ppm: 13.8 (CH₃), 20.2 (CH₂), 29.9 (CH₂), 39.6 (CH₂Cl), 41.8 (CH₂-N₃), 44.9 (CH₂-N₁), 113.2–139.6 (Ar–C), 150.7 (C=O), 161.4 (C=O). Anal. calcd. for C₁₄H₁₇ClN₂O₂: C, 59.89; H, 6.10; N, 9.98; Found C, 60.19; H, 6.12; N, 9.93.

General procedure for the synthesis of compounds 5a–e

To the solution of 3-butyl-1-(2-chloroethyl) quinazoline-2,4(1H,3H)-dione (4) (0.84 g, 0.003 mol) in dry acetonitrile (20 ml), anhydrous K₂CO₃ (2.07 g, 0.015 mol) and few specs of KI were added and the mixture was heated under reflux for 30 min. Appropriate amine (0.009 mol) was then added slowly into the reaction mixture. The resulting mixture was heated under reflux for 15 h, cooled and poured onto ice-cold water. The separated solid was filtered, dried, and crystallized from a suitable solvent.

3-Butyl-1-[2-(pyrrolidin-1-yl)ethyl]quinazoline-2,4(1H,3H)-dione (5a)

Yield: 75% mp.: 75°C (ethanol). IR ν_{\max} (cm⁻¹) (KBr): 2958, 2931, 2872 (CH aliphatic), 1699 (C=O), 1654 (C=O). ¹HNMR (300 MHz, CDCl₃) δ ppm: 0.96(t, 3H, CH₃), 1.35–1.47(m, 2H, CH₂CH₃), 1.62–1.72(m, 2H, CH₂CH₂CH₃), 1.80–1.84(m, 4H, C₃ & C₄ of pyrrolidine), 2.64–2.68(m, 4H, C₂ & C₅ of pyrrolidine), 2.79(t, 2H, *J* = 7.5 Hz, N₁CH₂CH₂N), 4.08(t, 2H, N₃CH₂), 4.29(t, 2H, *J* = 7.5 Hz, N₁CH₂), 7.21–7.29(m, 2H, C₆H & C₈H of quinazoline), 7.65(dd, 1H, C₇H of quinazoline), 8.28(d, 1H, C₅H of quinazoline). ESIMS m/z (% rel. abundance): 316.30 (M + 1, 100%). Anal. calcd for C₁₈H₂₅N₃O₂: C, 68.54; H, 7.99; N, 13.32; Found C, 68.60; H, 7.92; N, 12.96.

3-Butyl-1-[2-(morpholin-4-yl)ethyl]quinazoline-2,4(1H,3H)-dione (5b)

Yield: 70% mp.: 102–104°C (ethanol). IR ν_{\max} (cm⁻¹) (KBr): 2962, 2931 (CH aliphatic), 1697 (C=O), 1651 (C=O). ¹HNMR (500 MHz, CDCl₃) δ ppm: 0.95(t, 3H, CH₃), 1.37–1.44(m, 2H, CH₂CH₃), 1.64–1.70(m, 2H, CH₂CH₂CH₃), 2.47–2.53(m, 4H, C₃ & C₅ of morpholine), 2.75(t, 2H, *J* = 7.5 Hz, N₁CH₂CH₂N), 3.65–3.82(m, 4H, C₂ & C₆ of morpholine), 4.08(t, 2H, N₃CH₂), 4.29(t, 2H, *J* = 7.5 Hz, N₁CH₂), 7.22–7.27(m, 2H, C₆H & C₈H of quinazoline), 7.62(dd, 1H, C₇H of quinazoline), 8.25(d, 1H, C₅H of quinazoline). ESIMS m/z (% rel. abundance): 332.27 (M + 1, 100%). Anal. calcd for C₁₈H₂₅N₃O₃: C, 65.23; H, 7.60; N, 12.68; Found C, 65.19; H, 7.56; N, 12.49.

3-Butyl-1-[2-(piperidin-1-yl)ethyl]quinazoline-2,4(1H,3H)-dione (5c)

Yield: 78% mp.: 98–100°C (ethanol). IR ν_{\max} (cm⁻¹) (KBr): 2931, 2862 (CH aliphatic), 1697 (C=O), 1654 (C=O). ¹HNMR (300 MHz, DMSO) δ ppm: 0.89(t, 3H, CH₃), 1.26–1.58(m, 10H, 2CH₂ + 3CH₂ of C³, C⁴, C⁵ piperidine), 2.39–2.52(m, 6H, CH₂ + 2CH₂ of C², C⁶ piperidine), 3.94 (t, 2H, N₃CH₂), 4.20(t, 2H, N₁CH₂), 7.27(dd, 1H, C₆H of quinazoline), 7.45 (d, 1H, C₈H of quinazoline), 7.75(dd, 1H, C₇H of quinazoline), 8.04(d, 1H, C₅H of quinazoline). Anal. calcd for C₁₉H₂₇N₃O₂: C, 69.27; H, 8.26; N, 12.76; Found C, 69.49; H, 8.25; N, 12.77.

3-Butyl-1-[2-(4-phenylpiperazin-1-yl)ethyl]quinazoline-2,4(1H,3H)-dione (5d)

Yield: 69% mp.: 78–84°C (hexane/ethanol). IR ν_{\max} (cm⁻¹) (KBr): 2954, 2927, 2831 (CH aliphatic), 1697 (C=O), 1647 (C=O). ¹HNMR (300 MHz, CDCl₃) δ ppm: 0.95(t, 3H, CH₃), 1.35–1.47(m, 2H, CH₂CH₃),

1.63–1.73(m, 2H, $\text{CH}_2\text{CH}_2\text{CH}_3$), 2.75–2.81(m, 6H, $\text{N}_1\text{CH}_2\text{CH}_2\text{N} + 2\text{CH}_2$ piperazine), 3.22–3.25(m, 4H, 2CH_2 piperazine), 4.09(t, 2H, N_3CH_2), 4.36(t, 2H, N_1CH_2), 6.85–8.25(m, 9H, ArHs). EIMS m/z (% rel. abundance): 406.10 (M^+ , 22.62%), 407.00 ($\text{M} + 1$, 7.31%), 175.05(100%). Anal. calcd for $\text{C}_{24}\text{H}_{30}\text{N}_4\text{O}_2$: C, 70.91; H, 7.44; N, 13.78. Found C, 70.86; H, 7.35; N, 13.66.

3-Butyl-1-[2-(diethylamino)ethyl]quinazoline-2,4(1H,3H)-dione (**5e**)

Yield: 60% mp.: 46–48°C (ethanol). IR ν_{max} (cm^{-1}) (KBr): 2966, 2931 (CH aliphatic), 1701(C=O), 1654 (C=O). ^1H NMR (300 MHz, CDCl_3) δ ppm: 0.96(t, 3H, CH_3), 1.05(t, 6H, $J = 7$ Hz, NCH_2CH_3) 1.35–1.47(m, 2H, CH_2CH_3), 1.63–1.73(m, 2H, $\text{CH}_2\text{CH}_2\text{CH}_3$), 2.64(q, 4H, $J = 7$ Hz, NCH_2CH_3), 2.73(t, 2H, $J = 7.5$ Hz, $\text{N}_1\text{CH}_2\text{CH}_2\text{N}$), 4.09(t, 2H, N_3CH_2), 4.22(t, 2H, $J = 7.5$ Hz, N_1CH_2), 7.23–7.27(m, 2H, C_6H & C_8H of quinazoline), 7.65(dd, 1H, C_7H of quinazoline), 8.24(d, 1H, C_5H of quinazoline). Anal. calcd for $\text{C}_{18}\text{H}_{27}\text{N}_3\text{O}_2$: C, 68.11; H, 8.57; N, 13.24; Found C, 68.23; H, 8.38; N, 13.02.

3-Butyl-1-(3-chloropropyl) quinazoline-2,4(1H,3H)-dione (**6**)

A mixture of 3-butylquinazoline-2,4(1H,3H)-dione (**3**) (2.18 g, 0.01 mol), 1-bromo-3-chloropropane (4.72 g, 2.968 ml, 0.03 mol) and anhydrous K_2CO_3 (6.90 g, 0.05 mol) in dry DMF (30 ml) was stirred over night at room temperature. The mixture was poured onto cold water and the formed precipitate was filtered, dried, and crystallized from ethanol/water mixture.

Yield: 68% mp.: 90°C. IR ν_{max} (cm^{-1}) (KBr): 2960, 2933, 2873, 2862 (CH aliphatic), 1699 (C=O), 1658 (C=O). ^1H NMR (200 MHz, CDCl_3) δ ppm: 0.92(t, 3H, CH_3), 1.31–1.46(m, 2H, CH_2CH_3), 1.45–1.75(m, 2H, $\text{CH}_2\text{CH}_2\text{CH}_3$), 2.16–2.29(m, 2H, $\text{CH}_2\text{CH}_2\text{Cl}$), 3.69(t, 2H, CH_2Cl), 4.08(t, 2H, N_3CH_2), 4.26(t, 2H, N_1CH_2), 7.17–8.26(m, 4H, ArHs). EIMS m/z (% rel. abundance): 294 (M^+ , 22.94%), 296 ($\text{M} + 2$, 9.64%), 132 (100%). Anal. calcd for $\text{C}_{15}\text{H}_{19}\text{ClN}_2\text{O}_2$: C, 61.12; H, 6.50; N, 9.50; Found: C, 61.20; H, 6.40; N, 9.30.

General procedure for the synthesis of compounds **7a–f**

To a solution of 3-butyl-1-(3-chloropropyl) quinazoline-2,4(1H, 3H)-dione (**6**) (0.88 g, 0.003 mol) in dry acetonitrile (20 ml), few specs of KI and anhydrous K_2CO_3 (2.07 g, 0.015 mol) were added. The resulted mixture was refluxed for 30 min. Appropriate amine (0.009 mol) was then added slowly into the reaction mixture and refluxed for 15 h. The mixture was cooled, diluted with water, and

extracted with chloroform. The chloroform layer was washed with water, dried over anhydrous sodium sulfate, and evaporated. The oily product was dissolved in acetone and treated with ethereal hydrochloride. The separated solid was refrigerated for 48 h, filtered and dried.

3-Butyl-1-[3-(piperidin-1-yl)propyl]quinazoline-2,4(1H,3H)-dione hydrochloride (**7a**)

Yield 40% mp.: 210°C. IR ν_{max} (cm^{-1}) (KBr): 3421(NH), 2951, 2808 (CH aliphatic), 1701(C=O), 1654 (C=O). ^1H NMR (300 MHz, $\text{DMSO-}d_6$) δ ppm: 0.90(t, 3H, CH_3), 1.27–1.34(m, 2H, CH_2CH_3), 1.54–1.59(m, 2H, $\text{CH}_2\text{CH}_2\text{CH}_3$), 1.63–1.74(m, 6H, CH_2 of $\text{C}_3, \text{C}_4, \text{C}_5$ of piperidine), 2.05–2.10(m, 2H, $\text{N}_1\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$), 2.82(t, 2H, $\text{N}_1\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$), 3.02–3.12(m, 4H, C_2, C_6 of piperidine), 3.94(t, 2H, N_3CH_2), 4.17(t, 2H, N_1CH_2), 7.30(dd, 1H, C_6H of quinazoline), 7.55 (d, 1H, C_8H of quinazoline), 7.77(dd, 1H, C_7H of quinazoline), 8.07(d, 1H, C_5H of quinazoline), 9.94(s, 1H, NH of HCl salt). Anal. calcd for $\text{C}_{20}\text{H}_{29}\text{N}_3\text{O}_2 \cdot \text{HCl}$: C, 63.22; H, 7.95; N, 11.06; Found C, 63.12; H, 7.87; N, 11.10.

3-Butyl-1-[3-(4-phenylpiperazin-1-yl)propyl] quinazoline-2,4(1H,3H)-dione hydrochloride (**7b**)

Yield 55% mp.: 180°C. IR ν_{max} (cm^{-1}) (KBr): 3487(NH), 2981, 2866 (CH aliphatic), 1697(C=O), 1654 (C=O). ^1H NMR (200 MHz, $\text{DMSO-}d_6$) δ ppm: 0.94(t, 3H, CH_3), 1.24–1.44(m, 2H, CH_2CH_3), 1.54–1.70(m, 2H, $\text{CH}_2\text{CH}_2\text{CH}_3$), 2.00–2.32(m, 2H, $\text{N}_1\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$), 3.10–3.34(m, 4H, 2CH_2 of piperazine) 3.40(t, 2H, $\text{N}_1\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$), 3.70–3.92(m, 4H, 2CH_2 of piperazine), 3.98(t, 2H, N_3CH_2), 4.24(t, 2H, N_1CH_2), 6.88–8.09(m, 4H, ArHs), 11.38(s, 1H, NH of HCl salt, D_2O exchangeable). EIMS m/z (% rel. abundance): 420 (M^+ , 30.36%), 421 ($\text{M} + 1$, 10.19%), 175 (100%). Anal. calcd for $\text{C}_{25}\text{H}_{32}\text{N}_4\text{O}_2 \cdot \text{HCl}$: C, 65.70; H, 7.27; N, 12.25; Found C, 65.67; H, 7.24; N, 12.22.

3-Butyl-1-[3-[4-(4-methoxyphenyl)piperazin-1-yl]propyl]quinazoline-2,4(1H,3H)-dione hydrochloride (**7c**)

Yield (50%) mp.: 152°C. IR ν_{max} (cm^{-1}) (KBr): 3485(NH), 2980, 2858 (CH aliphatic), 1701 (C=O), 1654 (C=O). ^1H NMR (300 MHz, DMSO) δ ppm: 0.89(t, 3H, CH_3), 1.26–1.35(m, 2H, CH_2CH_3), 1.52–1.62(m, 2H, $\text{CH}_2\text{CH}_2\text{CH}_3$), 2.10–2.19(m, 2H, $\text{N}_1\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$), 2.90–3.15(m, 6H, 2CH_2 of piperazine + $\text{N}_1\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$), 3.49–3.57(m, 4H, 2CH_2 of piperazine), 3.68(s, 3H, OCH_3), 3.94(t, 2H, N_3CH_2), 4.19(t, 2H, N_1CH_2), 6.9–6.82 (dd, 4H, ArHs), 7.30(dd, 1H, C_6H of quinazoline), 7.56(d, 1H, C_8H of quinazoline), 7.77(dd, 1H, C_7H

of quinazoline), 8.08(d, 1H, C₅H of quinazoline), 10.72(s, 1H, NH of HCl salt, D₂O exchangeable). EIMS *m/z* (% rel. abundance): 450 (M⁺, 100%), 451 (M + 1, 29.68%). Anal. calcd for C₂₆H₃₄N₄O₃·HCl C, 64.11; H, 7.24; N, 11.50; Found C, 64.13; H, 7.13; N, 11.46.

3-Butyl-1-[3-(diethylamino)propyl]quinazoline-2,4(1H,3H)-dione hydrochloride (**7d**)

Yield (42%) mp.: 127°C. IR ν_{\max} (cm⁻¹) (KBr): 3417(NH), 2954, 2870 (CH aliphatic), 1701 (C=O), 1654 (C=O). ¹HNMR (300 MHz, DMSO) δ ppm: 0.90(t, 3H, CH₃), 1.20(t, 6H, *J* = 7 Hz, NCH₂CH₃), 1.24–1.37(m, 2H, CH₂CH₂CH₃), 1.49–1.61(m, 2H, CH₂CH₂CH₃), 1.99–2.09(m, 2H, N₁CH₂CH₂CH₂N), 3.05(q, 4H, *J* = 7 Hz, NCH₂CH₃), 3.15(t, 2H, N₁CH₂CH₂CH₂N), 3.94(t, 2H, N₃CH₂), 4.18(t, 2H, N₁CH₂), 7.30(dd, 1H, C₆H of quinazoline), 7.61(d, 1H, C₈H of quinazoline), 7.76(dd, 1H, C₇H of quinazoline), 8.07(d, 1H, C₅H of quinazoline), 10.76(s, 1H, NH of HCl salt). ESIMS *m/z* (% rel. abundance): 332.39(M + 1, 100%). Anal. calcd for C₁₉H₂₉N₃O₂·HCl C, 62.02; H, 8.21; N, 11.42; Found C, 62.12; H, 8.43; N, 11.41.

3-Butyl-1-[3-(pyrrolidin-1-yl)propyl]quinazoline-2,4(1H,3H)-dione hydrochloride (**7e**)

Yield (39%) mp.: 142°C. IR ν_{\max} (cm⁻¹) (KBr): 3412(NH), 2956, 2872 (CH aliphatic), 1701 (C=O), 1654 (C=O). ¹HNMR (500 MHz, CDCl₃) δ ppm: 0.91(t, 3H, CH₃), 1.30–1.38(m, 2H, CH₂CH₂CH₃), 1.56–1.63(m, 2H, CH₂CH₂CH₃), 1.99–2.07(m, 2H, C₃H of pyrrolidine), 2.13–2.22(m, 2H, C₄H of pyrrolidine), 2.32–2.38(m, 2H, N₁CH₂CH₂CH₂N), 2.80(t, 2H, N₁CH₂CH₂CH₂N), 3.20–3.24(m, 2H, C₂H of pyrrolidine), 3.74–3.77(m, 2H, C₅H of pyrrolidine), 4.00(t, 2H, N₃CH₂), 4.22(t, 2H, N₁CH₂), 7.20(dd, 1H, C₆H of quinazoline), 7.32 (d, 1H, C₈H of quinazoline), 7.64 (dd, 1H, C₇H of quinazoline), 8.18(d, 1H, C₅H of quinazoline), 12.42(s, 1H, NH of HCl salt). EIMS *m/z* (% rel. abundance): 329 (M⁺, 8.05%), 330 (M + 1, 1.97%), 84 (100%). Anal. calcd for C₁₉H₂₇N₃O₂·HCl C, 62.36; H, 7.71; N, 11.48; Found C, 62.18; H, 7.49; N, 11.46.

3-Butyl-1-[3-(morpholin-4-yl)propyl]quinazoline-2,4(1H,3H)-dione hydrochloride (**7f**)

Yield (45%) mp.: 130°C. IR ν_{\max} (cm⁻¹) (KBr): 3425(NH), 2970, 2870 (CH aliphatic), 1697(C=O), 1654 (C=O). ¹HNMR (300 MHz, CDCl₃) δ ppm: 0.93(t, 3H, CH₃), 1.31–1.43(m, 2H, CH₂CH₂CH₃), 1.57–1.67(m, 2H, CH₂CH₂CH₃), 2.34–2.39(m, 2H, N₁CH₂CH₂CH₂N), 3.14–3.20(m, 6H, C₂, C₆ of morpholine and N₁CH₂CH₂CH₂N), 3.94–4.06(m, 6H,

CH₂ of C₃, C₅ of morpholine and N₃CH₂), 4.25(t, 2H, N₁CH₂), 5.39(s, 1H, NH of HCl salt, D₂O exchangeable), 7.23(dd, 1H, C₆H of quinazoline), 7.36 (d, 1H, C₈H of quinazoline), 7.68(dd, 1H, C₇H of quinazoline), 8.18(d, 1H, C₅H of quinazoline), Anal. calcd for C₁₉H₂₇N₃O₃·HCl C, 59.75; H, 7.39; N, 11.00; Found C, 59.63; H, 7.42; N, 11.10.

1-Benzyl-3-butylquinazoline-2,4(1H,3H)-dione (**8**)

A mixture of 3-butylquinazoline-2,4(1H,3H)-dione (**3**) (0.218 g, 0.001 mol), benzyl chloride (0.25 g, 0.002 mol), anhydrous K₂CO₃ (0.69 g, 0.005 mol) and few specs of KI in dry acetone (15 ml) was stirred and refluxed for 7 h. The reaction mixture was cooled and poured onto cold water. The formed precipitate was filtered, dried and crystallized from ethanol.

Yield: 0.25 g (81%) mp.: 109–111°C. IR ν_{\max} (cm⁻¹) (KBr): 2958 (CH aliphatic), 1701(C=O), 1654(C=O). ¹HNMR (300 MHz, CDCl₃) δ ppm: 0.99(t, 3H, CH₃), 1.38–1.51(m, 2H, CH₂CH₃), 1.66–1.79(m, 2H, CH₂CH₂CH₃), 4.16 (t, 2H, N₃CH₂), 5.38(s, 2H, N₁CH₂), 7.10–8.25(m, 9H, ArHs). Anal. calcd for C₁₉H₂₀N₂O₂ C, 74.00; H, 6.54; N, 9.08; Found C, 74.21; H, 6.43; N, 9.11.

General procedure for the synthesis of compounds **9a–c**

A mixture of 3-butylquinazoline-2,4(1H,3H)-dione (**3**) (0.218 g, 0.001 mol), appropriate phenacyl bromide (0.001 mol) and anhydrous K₂CO₃ (0.69 g, 0.005 mol) in dry DMF (15 ml) was stirred and refluxed for 3 h. The reaction mixture was cooled, poured onto cold water and the solid formed was filtered, dried, and crystallized from ethanol.

3-Butyl-1-(2-oxo-2-phenylethyl)quinazoline-2,4(1H,3H)-dione (**9a**)

Yield: (65%) mp.: 110°C. IR ν_{\max} (cm⁻¹) (KBr): 2956, 2931(CH aliphatic), 1695(C=O), 1625–1665(C=O). ¹HNMR (200 MHz, CDCl₃) δ ppm: 0.96(t, 3H, CH₃), 1.36–1.47(m, 2H, CH₂CH₃), 1.63–1.72(m, 2H, CH₂CH₂CH₃), 4.11(t, 2H, N₃CH₂), 5.62(s, 2H, N₁CH₂), 6.79–8.28(m, 9H, ArHs). EIMS *m/z* (% rel. abundance): 336.20 (M⁺, 12.88%), 337.20 (M + 1, 3.13%), 105.05(100%). Anal. calcd for C₂₀H₂₀N₂O₃: C, 71.41; H, 5.99; N, 8.33; Found C, 71.71; H, 5.79; N, 8.23.

3-Butyl-1-[2-(4-chlorophenyl)-2-oxoethyl]quinazoline-2,4(1H,3H)-dione (**9b**)

Yield: (68%) mp.: 212°C. IR ν_{\max} (cm⁻¹) (KBr): 2958, 2935(CH aliphatic), 1701(C=O), 1650–1675(C=O). ¹HNMR

(200 MHz, CDCl₃) δppm: 0.96(t, 3H, CH₃), 1.36–1.43(m, 2H, CH₂CH₃), 1.63–1.73(m, 2H, CH₂CH₂CH₃), 4.10(t, 2H, N₃CH₂), 5.57(s, 2H, N₁CH₂), 6.79–8.28(m, 8H, ArHs). EIMS *m/z* (% rel. abundance): 370.15 (M⁺, 7.64%), 371.20 (M + 1, 2.18%), 372.20 (M + 2, 2.86%), 132.10(100%). Anal. calcd for C₂₀H₁₉ClN₂O₃ C, 64.78; H, 5.16; N, 7.55; Found C, 63.78 H, 5.00; N, 7.25.

1-[2-(4-Bromophenyl)-2-oxoethyl]-3-butylquinazoline-2,4(1H,3H)-dione (**9c**)

Yield: (71%) mp.: 223°C. IR ν_{\max} (cm⁻¹) (KBr): 2956, 2935 (CH aliphatic), 1703 (C=O), 1635–1670 (C=O). ¹HNMR (500 MHz, CDCl₃) δppm: 0.95(t, 3H, CH₃), 1.38–1.45(m, 2H, CH₂CH₃), 1.66–1.72(m, 2H, CH₂CH₂CH₃), 4.12(t, 2H, N₃CH₂), 5.59(s, 2H, N₁CH₂), 6.82(d, 1H, C₈H of quinazoline), 7.26 (dd, 1H, C₆H of quinazoline), 7.57(dd, 1H, C₇H of quinazoline), 7.65(dd, *J* = 8 Hz, 2H, C₃, C₅ of 4-Br-ph), 7.95(dd, 2H, *J* = 8 Hz, C₂, C₆ of 4-Br-ph), 8.35(d, 1H, C₅H of quinazoline). ¹³C NMR (CDCl₃) δppm: 13.79 (CH₃), 20.21 (CH₂), 29.88 (CH₂), 41.96 (CH₂–N₃), 49.57 (CH₂–N₁), 113.08–139.81 (Ar–C), 151.11(C=O), 161.54(C=O), 191.20(Ph–C=O). Anal. calcd for C₂₀H₁₉BrN₂O₃ C, 57.84; H, 4.61; N, 6.75; Found C, 58.14; H, 4.36; N, 6.68.

Biological activity

Evaluation of PDE4B inhibitory activity

With several new analogs now in hand, we evaluated their inhibitory potency against PDE4B via a purified enzyme fluorescence polarization assay. PDE activity assays were performed in duplicate at 10 μM. Fluorescence intensity is converted to fluorescence polarization using the Magellan6 software. The fluorescence polarization data were analyzed using the computer software, Graphpad Prism. 100 μM solutions of the test compounds were prepared with 10% DMSO in assay buffer and 5 μl of the solution was added to a 50 μl reaction so that the final concentration of DMSO is 1% in all of the reactions. The enzymatic reactions were conducted at room temperature for 60 min in a 50 μl mixture containing PDE assay buffer, 100 nM FAM-cAMP, PDE4B2, and the test compound. After the enzymatic reaction, 100 μl of a binding solution (1:100 dilution of the binding agent with the binding agent diluents) was added to each reaction and the reaction was performed at room temperature for 60 min. Fluorescence intensity was measured at an excitation of 470 nm and an emission of 528 nm using a Tecan Infinite M1000 microplate reader.

The percent activity in the presence of the compound was calculated according to the following equation: % activity = (FP – FP_b)/(FP_t – FP_b) × 100%, where FP is the fluorescence polarization in the presence of the compound. The fluorescence polarizations of PDE4B were then measured in the presence of the synthesized compounds and consequently their % inhibition were calculated.

Molecular docking

All the molecular modeling studies were carried out on an Intel Pentium 1.6 GHz processor, 512 MB memory with Windows XP operating system using Molecular Operating Environment (MOE 2008.10; Chemical Computing Group, Canada) (MOE 2008.10) as the computational software.

All the minimizations were performed with MOE until a RMSD gradient of 0.05 kcal mol⁻¹Å⁻¹ with MMFF94x force-field and the partial charges were automatically calculated.

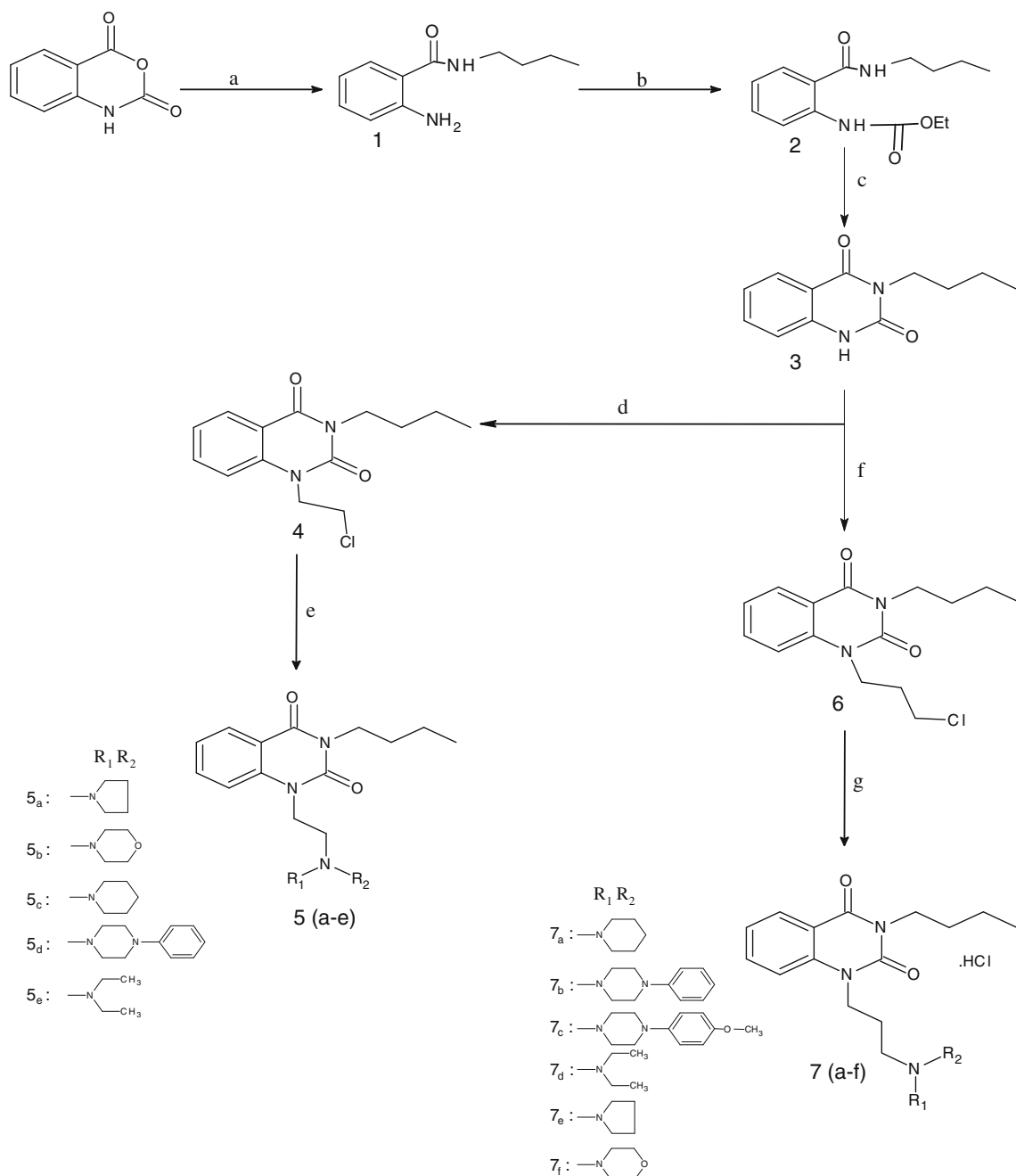
The X-ray crystallographic structure of phosphodiesterase 4B complexed with rolipram (PDB ID: 1RO6) was obtained from the protein data bank available at the RCSB Protein Data Bank, <http://www.pdb.org> with a 2.00 Å resolution

1. Enzyme structures were checked for missing atoms, bonds and contacts.
2. Water molecules were manually deleted.
3. Hydrogens and partial charges were added to the system using Protonate3D application.
4. The active site was generated using the residues within 5 Å near to the rolipram atoms.
5. The ligand molecules were constructed using the builder module and were energy minimized using the MMFF94x force field.
6. All antagonist structures were docked into the active site by using the MOE Dock tool. This method is divided into a number of stages:
 - (a) Conformational analysis of ligands: the algorithm generated conformations from a single 3D conformation by conducting a systematic search. In this way, all combinations of angles were created for each ligand.
 - (b) Placement: a collection of poses was generated from the pool of ligand conformations using Triangle Matcher placement method. Poses were generated by superposition of ligand atom triplets and triplets of points in the receptor binding site in a systematic way
 - (c) Scoring: poses generated by the placement methodology were scored using London dG scoring function implemented in MOE, which

estimates the free energy of binding of the ligand from a given pose. The top 30 poses for each ligand were output in a MOE database. Each resulting ligand pose was then subjected to MMFF94x energy minimization until the RMS gradient of the potential energy was less than $0.05 \text{ kJ mol}^{-1} \text{ \AA}^{-1}$. The minimized docking conformations were then re-scored using London dG scoring methods.

Result and discussion

The starting compound 2-amino-*N*-butylbenzamide **1** was prepared as the reported procedure (Clark and Wagner, 1944). Formylation of **1** with ethyl chloroformate afforded 2-ethoxycarbonylamino-*N*-butylbenzamide **2** which was cyclized to the desired key compound **3**. We report here a new application of the method described by Gaddekar *et al.* (Gaddekar *et al.*, 1964) for the preparation of 3-butylquinazoline-2,4(1*H*),



Scheme 1 Synthetic protocol for title compounds **5a-e** and **7a-f**. *Reagents and conditions*: (a) butyl amine (b) ethyl chloroformate, heated over steam, 3 h (c) KOH, ethanol, heated over steam,

4 h (d) 1-bromo-2-chloroethane, anhydrous K_2CO_3 , dry DMF, rt, overnight (e) HNR_1R_2 (f) 1-bromo-3-chloropropane, anhydrous K_2CO_3 , dry DMF, rt, overnight (g) (1) HNR_1R_2 (2) ethereal HCl

using MOE builder, and the structures were energy minimized using the MMFF94x force field. Validation of the function implemented in MOE was done by docking of the native ligand into its binding site. The docked results were compared to the crystal structure of the bound ligand–protein complex. The RMSD of the docked ligand was 0.32 Å as it seems exactly superimposed on the native bound one (Fig. 2a). These results indicated the high accuracy of the MOE simulation in comparison with the biological methods. Although PDE4 enzyme crystal structure with nitraquazone co-crystallized was absent, a prediction of the binding model for

nitraquazone was done by docking nitraquazone in the active site gorge of PDE4B (Fig. 2b). This binding model was useful in our interpretation to the activities of our synthesized compounds.

Then, we performed docking studies to our synthesized compound and the final docked complexes of ligand–enzyme were selected according to the criteria of interaction energy combined with geometrical matching quality.

The saved pose for the ligand–enzyme complex of the most active compound **7f** (Fig. 3) revealed that several molecular interactions were considered to be responsible

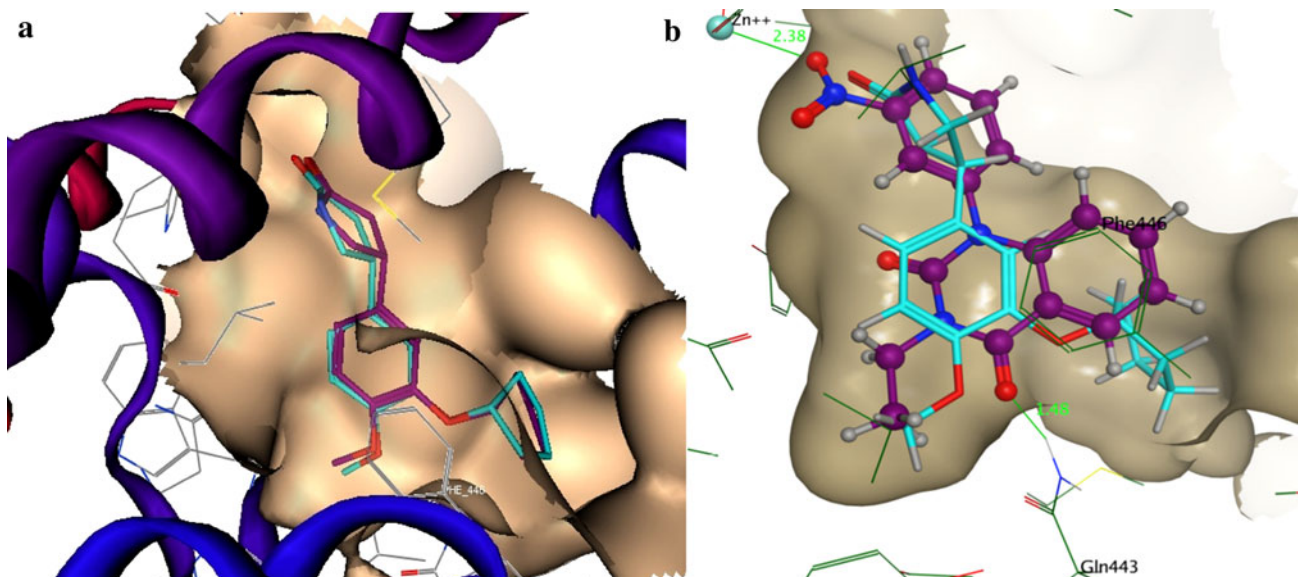


Fig. 2 a The docked rolipram ligand into PDE4B seems superimposed on the native rolipram ligand, RMSD: 0.32 Å. b Predicted binding model of nitraquazone

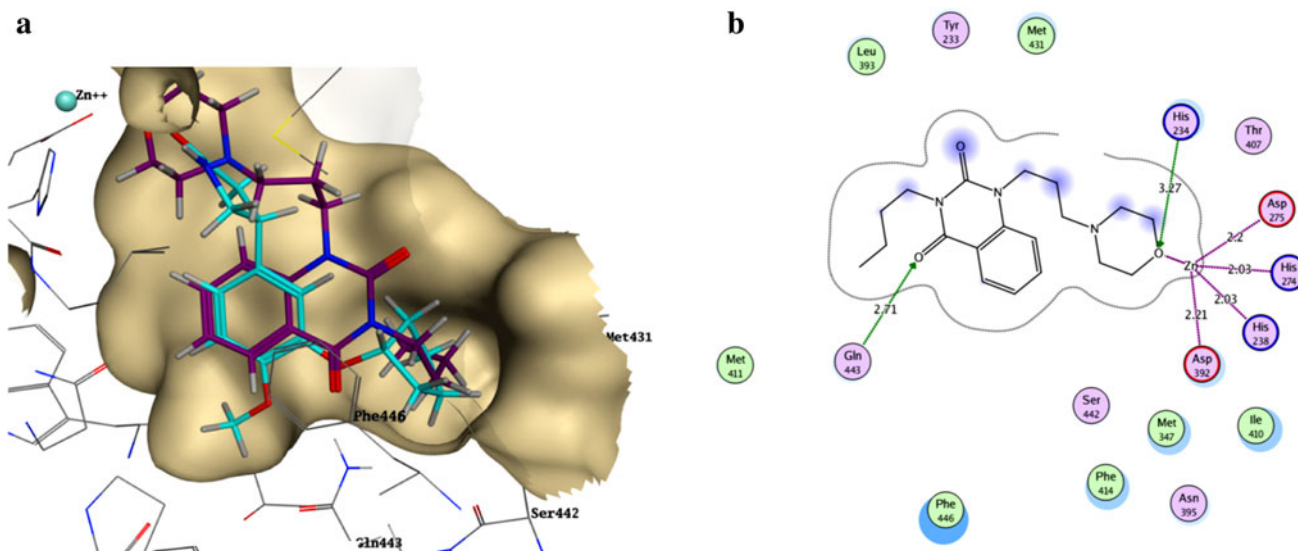


Fig. 3 a Docked conformation alignment of **7f** and its original co-crystallized ligand in PDE4B binding site generated by MOE docking. b Simplified structure of **7f** docked at PDE4B active site showing hydrogen bonds and metal coordination

for the observed affinity: (i) The quinazoline nucleus was sandwiched between the side chains of Phe446 and Ile410 showing great overlapping of benzo of quinazoline with the phenyl ring of rolipram. (ii) Quinazolinone's 4-oxo forms hydrogen bond with the side-chain NH_2 of Gln443 (distance = 2.71 Å). (iii) Unlike nitraquazone *N*-ethyl group, the *N*-butyl group of **7f** was too bulky to be embedded to the rolipram's methoxy group small pocket made up of Tyr403, Tyr233, Thr407, Pro396, Gln443, Asn395, Ile410, and Trp406, instead quinazoline ring of **7f** adopted a flipped position so that its *n*-butyl occupies the rolipram's cyclopentoxy group hydrophobic pocket surrounded by Phe414, Phe446, Met411, Met431, Ser442, and Gln443 (Fig. 4). (iv) Moreover, the morpholine oxygen was weakly hydrogen bond to imidazole's NH of His234 (distance = 3.27 Å) and metal coordinated with the Zn^{2+}

bounded within the active site (distance = 2.37 Å). This metal coordination seemed to be crucial for the high potency of **7f**.

Comparing the active **7f**, with a propyl spacer, to its less active congener **5b**, with an ethyl spacer, shows similar binding interactions except of Zn^{2+} binding and His234 hydrogen bond, which were lost in **5b** as the ethyl spacer was not long enough to allow morpholine's oxygen to reach the Zn^{2+} and His234 in the active site. Although compounds **5d**, **8**, and **9a** showed less inhibitory action to PDE4B than **7f** which was rationalized by the fact that they were unable to bind to Zn^{2+} , these compounds still binds to His234 by π -cation interaction. This explained why compounds **5d**, **8**, and **9a** achieved better inhibitory activity to PDE4B (Fig. 5) than the rest of our compounds which lost both Zn^{2+} and His234 bindings.

Fig. 4 Predicted binding model of nitraquazone and the docked pose of **7f** showing its flipped quinazoline nucleus

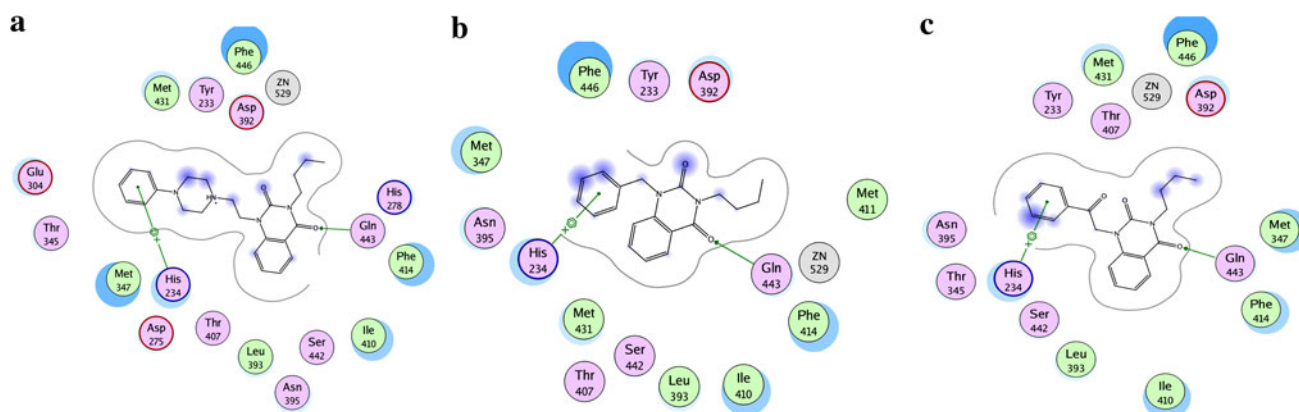
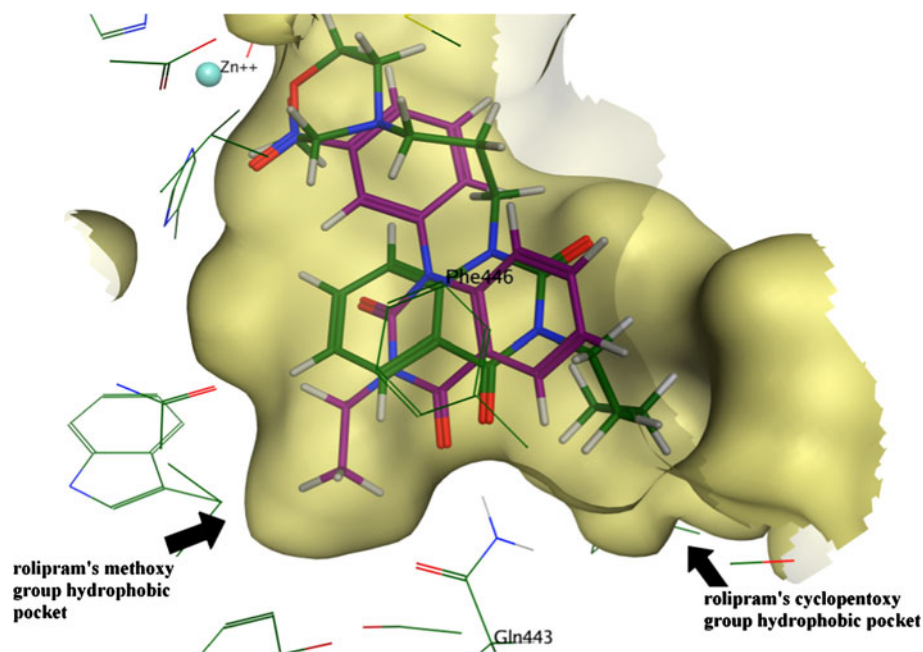


Fig. 5 Schematic view of a compound **5d**, b compound **8** and c compound **9a** docking conformations within PDE4B active site showing π -cation interaction with His234

Conclusion

We observed that the bulkiness of N_3 substituent is important in the orientation of quinazoline nucleus in the active site. Metal coordination with the Zn^{2+} bound within the active site greatly increases activity and may lead to potent PDE4 inhibition. Hydrogen bonds to aminoacids residues (Asp392 and His234) or π -cation interaction with His234 increases activity. Moreover quinazolinone's 2-oxo seems to be unessential for PDE4 binding. These preliminary encouraging results of biological screening of the tested compounds could offer an excellent framework in this field that may lead to discovery of potent PDE4B inhibitors which can be used for treatment of several diseases.

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References

- Barnes PJ (1999) Therapeutic strategies for allergic diseases. *Nature* 402:B31–B38
- Bender AT, Beavo JA (2006) Cyclic nucleotide phosphodiesterases: molecular regulation to clinical use. *Pharmacol Rev* 58:488–520
- Blizzard TA, Marino G, Mrozek H, Fisher MH, Hoogsteen K, Springer JP (1989) Chemical modification of paraherquamide. 1. Unusual reactions and absolute stereochemistry. *J Org Chem* 54:2657–2663
- Burnouf C, Pruniaux MP, Szilagyai CM (1998) Phosphodiesterase 4 inhibitors. In: Bristol JA (ed) *Annual reports in medicinal chemistry*. Academic Press, Michigan, pp 91–109
- Clark RH, Wagner EC (1944) Isatoic anhydride. I. Reactions with primary and secondary amines and with some amides. *J Org Chem* 9:55
- Gaddekar SM, Kotsen AM, Cohen E (1964) Anthranilamides as Intermediates for 3-Substituted Quinazoline-2,4-diones. *J Chem Soc* 4666–4668. <http://pubs.rsc.org/en/content/articlelanding/1964/jr/jr9640004633>
- Lehnart SE, Wehrens XHT, Reiken S, Warriar S, Belevych AE, Harvey RD, Richter W, Jin SLC, Conti M, Marks AR (2005) Phosphodiesterase 4D deficiency in the ryanodine-receptor complex promotes heart failure and arrhythmias. *Cell* 123:25–35
- McKenna JM, Muller GW (2006) Cyclic nucleotide phosphodiesterases. In: Beavo JA, Francis SH, Houslay MD (eds) *Health and disease*. CRC Press, pp 667–670
- Molecular Operating Environment (MOE 2008.10); C.C.G., Inc., 1255 University St., Suite 1600, Montreal, Quebec, Canada H3B 3X3. 2005. <http://www.chemcomp.com>
- Norman P (1999) PDE4 inhibitors. *Exp Opin Ther Patents* 9:1101–1117
- Palacios JM, Beleta J, Segarra V (1995) Second messengers systems as targets for new therapeutic agents: focus on selective phosphodiesterase inhibitors. *Farmacol* 50:819–827
- Piaz VD, Giovannoni MP (2000) Phosphodiesterase 4 inhibitors, structurally unrelated to Rolipram, as promising agents for the treatment of asthma and other pathologies. *Eur J Med Chem* 35:463–480
- Potter BVL (1990) Transmembrane signalling second messenger analogues and inositol phosphates. In: Hansch C, Sammes PG, Taylor JB (eds) *Comprehensive medicinal chemistry*. Pergamon Press, Oxford, pp 102–128
- Robichaud A, Stamatou PB, Jin SLC, Lachance N, MacDonald D, Laliberte F, Liu S, Huang Z, Conti M, Chan CC (2002) Deletion of phosphodiesterase 4D in mice shortens α 2-adrenoceptor-mediated anesthesia, a behavioral correlate of emesis. *J Clin Invest* 110:1045–1052
- Schneider HH, Schmiechen R, Brezinski M, Seidler J (1986) Stereospecific binding of the antidepressant rolipram to brain protein structures. *Eur J Pharmacol* 127:105–115
- Souness JE, Aldous D, Sargent C (2000) Immunosuppressive and anti-inflammatory effects of cyclic AMP phosphodiesterase (PDE) type 4 inhibitors. *Immunopharmacol* 47:127–162
- Staiger RP, Wagner EC (1953) Isatoic anhydride. III. Reactions with primary and secondary amines. *J Org Chem* 18:1427–1439
- Torphy TJ (1998) Action of mediators on airway smooth muscle: functional antagonism as a mechanism for bronchodilator drugs. *Agents Actions* 23(Suppl):S37–S53
- Zhang KYJ, Ibrahim PN, Gillette S, Bollag G (2005) Phosphodiesterase-4 as a potential drug target. *Expert Opin Ther Targets* 9:1283–1305