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MEDICINAL CHEMISTRY RESEARCH

SYMPOSIUM PAPER

Design, synthesis and in vitro PDE4 inhibition activity of certain quinazolinone derivatives for treatment of asthma

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Abstract In this study, a novel series of quinazolinone derivatives analogue to nitraquazone structure were synthesized. The compounds tested for their inhibitory activity against phosphodiesterase 4B revealed that compound **6d** shows promising inhibitory activity comparable to that of Rolipram, whereas compounds **6a** and **6c** exhibited moderate inhibitory activity.

Keywords Phosphodiesterase 4 (PDE4) inhibitors · Quinazolinone · Asthma · Synthesis

Introduction

Asthma is one of the most common chronic diseases (Barnes, 1992) worldwide and antiasthmatic medications are widely prescribed. Despite advancements in treatment, the incidences of asthma, asthma-related deaths and hospitalizations for asthma have increased significantly during the past decade. Asthma is characterized by a reversible airway obstruction, ongoing cellular inflammation and nonspecific hyper-responsiveness to a variety of challenges. Both acute and long-term manifestations of asthma are believed to be a consequence of various inflammatory mediators released by activated inflammatory and immune cells (Holgate, 1988; Reed, 1988; Adelroth *et al*., 1990; Djukanovic *et al*., 1990). Therefore, the ability to suppress activation of these cells is an essential property for a compound to have a therapeutic effect on asthma. Four major classes of compounds are currently used in the treatment of asthma, including bronchodilators (particularly *â*-adrenoceptor agonists), immunosuppressive agents (corticosteroids), antiallergic agents for prophylactic use and xanthines (e.g. theophylline) (Vassallo and Lipsky, 1998), which appear to possess bronchodilator and antiinflammatory as well as immunomodulator properties. Newer drugs include leukotriene antagonists such as montelukast (Reques and Rodriguez, 1999). To date, much research has been directed towards the discovery of new antiasthmatic agents with high selectivity and efficacy and a reduced side-effect profile.

Phosphodiesterase enzymes (PDEs) (Butcher and Sutherland, 1962), involved in the intracellular degradation of cAMP and cGMP to their corresponding 5′-monophosphate counterparts, have received a considerable amount of attention as molecular targets for the treatment of asthma. Intracellular cyclic AMP (cAMP) and cyclic GMP (cGMP) are ubiquitous intracellular second messengers which play a prominent role in the regulation of important cellular functions such as secretion, contraction, metabolism and growth. PDEs have been classified into 11 major families (PDE1-11) with respect to their substrate sensitivity, Ca^2/calmodulin requirement and inhibitor selectivity (Torphy, 1998; Fisher *et al*., 1998; Crocker and Townley, 1999; Juilfs *et al*., 1999; Lanfear and Robas, 1999). PDE4 is a cAMP-specific enzyme localized in airway smooth muscles in immune and inflammatory cells. Thus, the elevation of cAMP levels by PDE4 inhibition represents a useful strategy for the development of new antiasthmatic and antiinflammatory drugs.

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 (Crespo *et al*., 1998) (Fig. 1).

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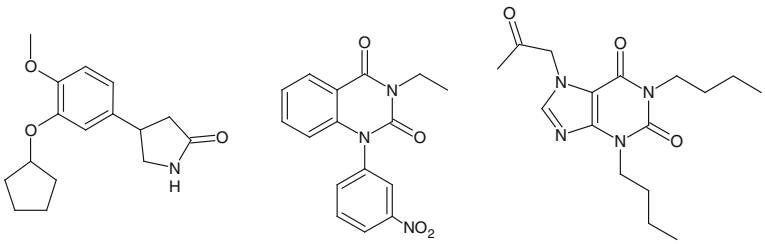


Fig. 1 Compounds representative of the three chemical classes of PDE4 inhibitors: Rolipram, nitraquazone and xanthine derivatives (denbufylline)

In 1984, nitraquazone, the first selective PDE type 4 having quinazolin-2,4-dione, was synthesized (Glaser and Traber, 1984). Nitraquazone appears to be very attractive model for the synthesis of novel PDE4 inhibitors potentially useful for the treatment of asthma and chronic obstructive pulmonary disease. Structural analogues of nitraquazone could be devoid of the central side-effects (nausea, vomiting and headache) of the archetypal Rolipram which hampered its development as a drug (Piaz and Giovannoni, 2000).

Although the number of studies claiming different chemical classes of PDE4 inhibitors is increasing in recent years, only few detailed studies evaluated the PDE4 inhibition of structural analogues of nitraquazone. On this basis, the rational design of our compounds was based on a hybrid structure of nitraquazone and denbufylline. A common pharmacophore using flexible alignment module implemented in MOE software was done for nitraquazone and denbufylline (Fig. 2), which simultaneously searches the conformation space and the space of alignments of those molecules using stochastic search procedure. Therefore, compounds obeying this pharmacophore, containing a flat aromatic area of quinazoline, hydrogen bond acceptor at position 4, hydrophobic part at position 3, aromatic region at positions 1 or 2 of the quinazoline-4-one nucleus, were synthesized; hoping that the new derivatives will have potent PDE4 inhibition.

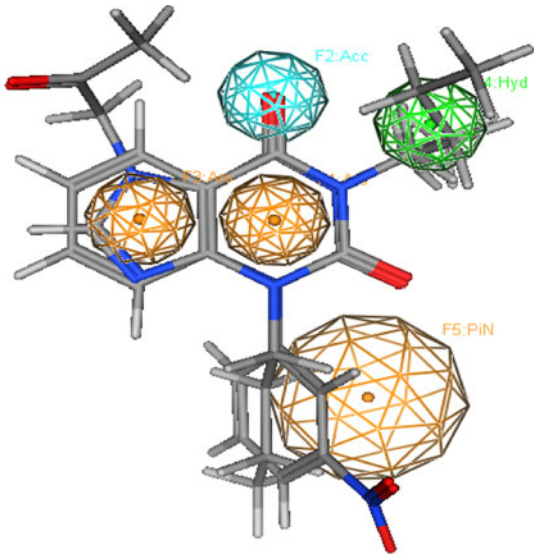


Fig. 2 Flexible alignment of nitraquazone and denbufylline using MOE 2008.10 software

Methods and materials

Chemistry

All chemicals and reagents were obtained from Aldrich (Sigma-Aldrich, St. Louis, MO, USA) and 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}HNMR 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 at the Micro analytical Center, Cairo University, Egypt. Melting points were determined with an Electro thermal melting point apparatus, and were uncorrected. 2-Amino-*N*-butylbenzamide (**1**) (Clark and Wagner, 1944) and 3-butyl-2-hydrazinoquinazolin-4(3H)-one (**4**) (Kottke *et al*., 1990) was synthesized according to reported procedures. 3-Butyl-2-thioxo-2, 3-dihydroquinazolin-4(1H)-one (**3**) (Shafik *et al*., 1979), was synthesized by a new procedure rather than the reported one.

2-Aryl-3-butyl-2,3-dihydroquinazolin-4(1H)-ones (**2a–2f**)

Equimolar amount of 2-amino-*N*-butylbenzamide (**1**) (0.57 g, 0.003 mol) and an appropriate aromatic aldehyde

(0.003 mol) in toluene (40 ml) containing *p*-toluenesulphonic acid (0.05 g, 0.2 mmol) was refluxed under Dean-Stark trap for 4 h. The solution was filtered while hot and the filtrate was evaporated under reduced pressure and the residue was crystallized from benzene.

*3-Butyl-2-(3-hydroxyphenyl)-2,3-dihydroquinazolin-4(1H)-one (**2a**)*

Yield: 84%; mp. 176–178°C. IR (cm^{-1}): 3311 (NH), 3280 (OH) 3066, 3057 (CH aromatic), 2962, 2926 (CH aliphatic), 1616 (C=O). ^{1}HNMR (300 MHz, DMSO); 0.85 (t, 3H, CH_3), 1.22–1.30 (m, 2H, $C\underline{H}_2CH_3$), 1.44–1.54 (m, 2H, $C\underline{H}_2CH_2CH_3$), 2.69–2.74 (m, 1H, NCH_2), 3.80–3.87 (m, 1H, NCH_2), 5.72 (s, 1H, C_2H quinazoline), 6.61–7.65 (m, 9H, ArHs + NH, D_2O exchangeable), 9.44 (s, 1H, OH, D_2O exchangeable). Anal. Calcd for $C_{18}H_{20}N_2O_2$: C, 72.95; H, 6.80; N, 9.45; Found: C, 73.10; H, 6.71; N, 9.41.

*3-Butyl-2-(3-fluorophenyl)-2,3-dihydroquinazolin-4(1H)-one (**2b**)*

Yield: 80%; mp. 130–132°C. IR (cm^{-1}): 3300 (NH), 2956, 2937 (CH aliphatic), 1627 (C=O) ^{1}HNMR (300 MHz, $CDCl_3$): 0.91 (t, 3H, CH_3), 1.27–1.43 (m, 2H, $C\underline{H}_2CH_3$), 1.54–1.65 (m, 2H, $C\underline{H}_2CH_2CH_3$), 2.72–2.81 (m, 1H, NCH_2), 3.97–4.06 (m, 1H, NCH_2), 4.73 (s, 1H, NH, D_2O exchangeable), 5.72 (s, 1H, C_2H quinazoline), 6.52–7.96 (m, 8H, ArHs). Anal. Calcd for $C_{18}H_{19}FN_2O$: C, 72.46; H, 6.41; N, 9.39; Found: C, 72.65; H, 6.38; N, 9.35.

*3-Butyl-2-(4-fluorophenyl)-2,3-dihydroquinazolin-4(1H)-one (**2c**)*

Yield: 75%; mp. 170–172°C. IR (cm^{-1}): 3304 (NH), 3070, 3001 (CH aromatic), 2956, 2939 (CH aliphatic), 1631 (C=O). ^{1}HNMR (200 MHz, $CDCl_3$): 0.77 (t, 3H, CH_3), 1.10–1.28 (m, 2H, $C\underline{H}_2CH_3$), 1.36–1.55 (m, 2H, $C\underline{H}_2CH_2CH_3$), 2.55–2.75 (m, 1H, NCH_2), 3.78–3.94 (m, 1H, NCH_2), 4.74 (s, 1H, NH, D_2O exchangeable), 5.65 (s, 1H, C_2H quinazoline), 6.45–7.84 (m, 8H, ArHs). EIMS (% rel. abundance): 298.10 (M^+, 14.11%), 299 (M + 1, 3.14%), 203.10 (100%). Anal. Calcd for $C_{18}H_{19}FN_2O$: C, 72.46; H, 6.41; N, 9.39; Found: C, 72.70; H, 6.41; N, 9.35.

*3-Butyl-2-[4-(dimethylamino)phenyl]-2,3-dihydroquinazolin-4(1H)-one (**2d**)*

Yield: 80%; mp. 176–178°C. IR (cm^{-1}): 3305 (NH), 2958, 2933 (CH aliphatic), 1629 (C=O). ^{1}HNMR (300 MHz, $CDCl_3$): 0.87 (t, 3H, CH_3), 1.26–1.31 (m, 2H, $C\underline{H}_2CH_3$), 1.52–1.57 (m, 2H, $C\underline{H}_2CH_2CH_3$), 2.73–2.84 (m, 1H, NCH_2), 2.96 (s, 6H, $N(CH_3)_2$), 3.86–3.96 (m, 1H, NCH_2), 4.42 (s, 1H, NH, D_2O exchangeable), 5.68 (s, 1H, C_2H quinazoline), 6.50–7.96 (m, 8H, ArHs). EIMS (% rel. abundance): 323 (M^+, 60.19%), 324.10 (M + 1, 15.15%), 121.10 (100%). Anal. Calcd for $C_{20}H_{25}N_3O$: C, 74.27; H, 7.79; N, 12.99; Found: C, 74.45; H, 7.69; N, 13.04.

*3-Butyl-2-(3-nitrophenyl)-2,3-dihydroquinazolin-4(1H)-one (**2e**)*

Yield: 87%; mp. 114–116°C. IR (cm^{-1}): 3300 (NH), 3089, 3066 (CH aromatic), 2968, 2937 (CH aliphatic), 1627 (C=O), 1537, 1350 (NO_2). ^{1}HNMR (500 MHz, $CDCl_3$): 0.90 (t, 3H, CH_3), 1.31–1.39 (m, 2H, $C\underline{H}_2CH_3$), 1.55–1.66 (m, 2H, $C\underline{H}_2CH_2CH_3$), 2.75–2.81 (m, 1H, NCH_2), 4.08–4.13 (m, 1H, NCH_2), 4.90 (s, 1H, NH, D_2O exchangeable), 5.85 (s, 1H, C_2H quinazoline), 6.60 (d, 1H, C_8H of quinazoline), 6.88 (dd, 1H, C_6H of quinazoline), 7.25 (dd, 1H, C_7H of quinazoline), 7.52 (dd, 1H, C_5H–3-$ArNO_2$), 7.71 (d, 1H, C_5H of quinazoline), 7.95 (d, 1H, C_6H–3-$ArNO_2$), 8.19 (d, 1H, C_4H–3-$ArNO_2$), 8.22 (s, 1H, C_2H–3-$ArNO_2$). ^{13}CNMR ($CDCl_3$): 13.76 (CH_3), 20.10 (CH_2), 29.92 (CH_2), 45.01 (CH_2–N_3), 70.83 (C_2), 114.89–148(Ar-C), 162.75 (C=O). Anal. Calcd for $C_{18}H_{19}N_3O_3$: C, 66.45; H, 5.88; N, 12.91; Found: C, 66.75; H, 5.99; N, 12.86.

*3-Butyl-2-(4-hydroxy-3-methoxyphenyl)-2,3-dihydroquinazolin-4(1H)-one (**2f**)*

Yield: 78%; mp. 142–144°C. IR (cm^{-1}): 3396 (NH), 3160 (OH), 2958, 2927 (CH aliphatic), 1625 (C=O). ^{1}HNMR (300 MHz, $CDCl_3$): 0.88 (t, 3H, CH_3), 1.27–1.38 (m, 2H, $C\underline{H}_2CH_3$), 1.45–1.54 (m, 2H, $C\underline{H}_2CH_2CH_3$), 2.74–2.83 (m, 1H, NCH_2), 3.81 (s, 3H, OCH_3), 3.84–3.97 (m, 1H, NCH_2), 4.71 (s, 2H, NH + OH, D_2O exchangeable), 5.69 (s, 1H, C_2H quinazoline), 6.52–7.95 (m, 7H, ArHs). EIMS (% rel. abundance): 326.15 (M^+, 15.59%), 327.05 (M + 1, 3.65%), 203.10 (100%). Anal. Calcd for $C_{19}H_{22}N_2O_3$: C, 69.92; H, 6.79; N, 8.58; Found: C, 70.03; H, 6.65; N, 8.31.

3-Butyl-2-thioxo-2,3-dihydroquinazolin-4(1H)-one (**3**)

A solution of 2-amino-*N*-butylbenzamide (**1**) (9.61 g, 0.05 mol) in ethanol (50 ml) was treated with KOH (3.36 g, 0.06 mol) in water (10 ml) and CS_2 (7.56 g, 6.0 ml, 0.1 mol). The solution was heated under reflux for 8 h. After reflux, the reaction mixture was cooled, poured into diluted HCl and the solid product was crystallized from aqueous ethanol. Yield: 6.0 g (51%); mp. 175–176°C (Shafik *et al*., 1979).

2-[2-(Arylidene)hydrazinyl]-3-butyl-quinazolin-4(3*H*)-ones (**5a**–**5d**)

A solution of 3-butyl-2-hydrazinoquinazolin-4(1*H*)-one (**4**) (0.51 g, 0.0022 mol), appropriate aromatic aldehyde (0.0022 mol) in absolute ethanol (20 ml) and few drops of acetic acid was refluxed for 30 h. The reaction mixture was concentrated; few drops of water were added and cooled. The precipitate formed was filtered, dried and crystallized from aqueous ethanol.

*3-Butyl-2-[2-(3-hydroxybenzylidene)hydrazinyl] quinazolin-4(3H)-one (**5a**)*

Yield: 60%; mp. 220–222°C. IR (cm^{-1}): 3377 (OH), 3354 (NH), 2966, 2951 (CH aliphatic), 1670 (C=O), 1612 (C=N). ^{1}HNMR (300 MHz, $CDCl_3$): 0.98 (t, 3H, CH_3), 1.41–1.49 (m, 2H, $C\underline{H}_2CH_3$), 1.70–1.83 (m, 2H, $CH_2\underline{CH}_2CH_3$), 4.24 (t, 2H, NCH_2), 5.79 (s, 1H, OH, D_2O exchangeable), 6.8–8.1 (m, 8H, ArHs), 8.48 (s, 1H, N=CH), 9.39 (s, 1H, NH, D_2O exchangeable). EIMS m/z (% rel. abundance): 337.30 (M + 1, 100%). Anal. Calcd for $C_{19}H_{20}N_4O_2$: C, 67.84; H, 5.99; N, 16.66; Found: C, 68.09; H, 6.03; N, 16.52.

*3-Butyl-2-{2-[4-(dimethylamino)benzylidene]hydrazinyl} quinazolin-4(3H)-one (**5b**)*

Yield: 80%; mp. 140–142°C. IR (cm^{-1}): 3402 (NH), 2954 (CH aliphatic), 1670 (C=O), 1612 (C=N). ^{1}HNMR (300 MHz, $CDCl_3$): 0.99 (t, 3H, CH_3), 1.44–1.51 (m, 2H, $C\underline{H}_2CH_3$), 1.67–1.72 (m, 2H, $C\underline{H}_2CH_2CH_3$), 3.08 (s, 6H, $N(CH_3)_2$), 3.70 (t, 2H, NCH_2), 6.7–8.2 (m, 8H, ArHs), 8.89 (s, 1H, N=CH), 9.40 (s, 1H, NH, D_2O exchangeable). Anal. Calcd for $C_{21}H_{25}N_5O$: C, 69.39; H, 6.93; N, 19.27; Found: C, 69.65; H, 6.80; N, 19.00.

*3-Butyl-2-[2-(3-nitrobenzylidene)hydrazinyl]quinazolin-4(3H)-one (**5c**)*

Yield: 75%; mp. 168–170°C. IR (cm^{-1}): 3452 (NH), 3070 (CH aromatic), 2954, 2931 (CH aliphatic), 1670 (C=O), 1635 (C=N), 1531, 1350 (NO_2). ^{1}HNMR (300 MHz, $CDCl_3$): 0.97 (t, 3H, CH_3), 1.42–1.50 (m, 2H, $C\underline{H}_2CH_3$), 1.69–1.75 (m, 2H, $C\underline{H}_2CH_2CH_3$), 4.24 (t, 2H, NCH_2), 7.3–8.6 (m, 8H, ArHs), 8.61 (s, 1H, N=CH), 9.40 (s, 1H, NH, D_2O exchangeable). EIMS (% rel. abundance): 365.05 (M^+, 37.66%), 366.20 (M + 1, 12.37%), 186.95(100%). Anal. Calcd for $C_{19}H_{19}N_5O_3$: C, 62.46; H, 5.24; N, 19.17; Found: C, 62.65; H, 5.30; N, 18.94.

*3-Butyl-2-[2-(4-hydroxy-3-methoxybenzylidene) hydrazinyl]quinazolin-4(3H)-one (**5d**)*

Yield: 55%; mp. 170–172°C. IR (cm^{-1}): 3417 (OH), 3367 (NH), 3062 (CH aromatic), 2958, 2931 (CH aliphatic), 1681 (C=O), 1627 (C=N). ^{1}HNMR (300 MHz, DMSO): 0.82 (t, 3H, CH_3), 1.20–1.23 (m, 2H, $C\underline{H}_2CH_3$), 1.35–1.40 (m, 2H, $C\underline{H}_2CH_2CH_3$), 3.73 (s, 3H, OCH_3), 3.86 (t, 2H, NCH_2), 5.61(s, 1H, OH, D_2O exchangeable), 6.80–7.95 (m, 7H, ArHs), 8.80 (s, 1H, =CH), 9.25 (s, 1H, NH, D_2O exchangeable). EIMS (% rel. abundance): 366.20 (M^+, 50.70%), 367.20 (M + 1, 12.36%), 174.10 (100%). Anal. Calcd for $C_{20}H_{22}N_4O_3$: C, 65.56; H, 6.05; N, 15.29; Found: C, 65.55; H, 6.12; N, 15.08.

1-Aryl-4-butyl [1,2,4]triazolo[4,3-a] quinazolin-5(4H)-ones (**6a**–**6d**)

To a solution of the appropriate schiffs bases **5a**–**5d** (0.001 mol) in ethanol (20 ml) was added a solution of ferric chloride (2 M, 1 ml) and the mixture was refluxed for 30 min. After reflux, the solution was left overnight at room temperature then poured onto cold water. The solid separated was filtered, washed with water, dried and finally crystallized from aqueous ethanol.

*4-Butyl-1-(3-hydroxyphenyl)[1,2,4]triazolo [4,3-a]quinazolin-5(4H)-one (**6a**)*

Yield: 90%; mp. 238–240°C. IR (cm^{-1}): 3425 (OH), 3070, 3043 (CH aromatic), 2951, 2939 (CH aliphatic), 1685 (C=O), 1597 (C=N). ^{1}HNMR (300 MHz, $CDCl_3$): 1.00 (t, 3H, CH_3), 1.47–1.54 (m, 2H, $C\underline{H}_2CH_3$), 1.81–1.96 (m, 2H, $C\underline{H}_2CH_2CH_3$), 4.39 (t, 2H, NCH_2), 4.70 (s, 1H, OH, D_2O exchangeable), 7.02–8.40 (m, 8H, ArHs). EIMS (% rel. abundance): 334.15 (M^+, 24.72%), 335.05 (M + 1, 6.15%), 277 (100%). Anal. Calcd for $C_{19}H_{18}N_4O_2$: C, 68.25; H, 5.42; N, 16.76; Found: C, 68.40; H, 5.41; N, 16.67.

*4-Butyl-1-[4-(dimethylamino)phenyl][1,2,4]triazolo [4,3-a]quinazolin-5(4H)-one (**6b**)*

Yield: 87%; mp. 180–182°C. IR (cm^{-1}): 2954 (CH aliphatic), 1697 (C=O), 1625 (C=N). ^{1}HNMR (300 MHz, $CDCl_3$): 0.93 (t, 3H, CH_3), 1.34–1.39 (m, 2H, $C\underline{H}_2CH_3$), 1.81–1.88 (m, 2H, $C\underline{H}_2CH_2CH_3$), 3.08 (s, 6H, $N(CH_3)_2$), 4.28 (t, 2H, NCH_2), 6.81–8.48 (m, 8H, ArHs). EIMS (% rel. abundance): 361 (M^+, 3.26%), 63 (100%). Anal. Calcd for $C_{21}H_{23}N_5O$: C, 69.78; H, 6.41; N, 19.38; Found: C, 70.07; H, 6.40; N, 19.19.

*4-Butyl-1-(3-nitrophenyl)[1,2,4]triazolo[4,3-a]quinazolin-5(4H)-one (**6c**)*

Yield: 85%; mp. 180–182°C. IR (cm^{-1}): 2962, 2931 (CH aliphatic), 1705 (C=O), 1627 (C=N), 1523, 1354 (NO_2). ^{1}HNMR (300 MHz, $CDCl_3$): 0.94 (t, 3H, CH_3), 1.33–1.42 (m, 2H, $C\underline{H}_2CH_3$), 1.83–1.91 (m, 2H, $C\underline{H}_2CH_2CH_3$), 4.27 (t, 2H, NCH_2), 7.39–8.69 (m, 8H, ArHs). EIMS (% rel. abundance): 363 (M^+, 57.71%), 363.95 (M + 1, 17.98%), 307(100%). Anal. Calcd for $C_{19}H_{17}N_5O_3$: C, 62.80; H, 4.71; N, 19.27; Found: C, 63.10; H, 4.84; N, 19.16.

*4-Butyl-1-(4-hydroxy-3-methoxyphenyl)[1,2,4]triazolo [4,3-a]quinazolin-5(4H)-one (**6d**)*

Yield: 85%; mp. 190–192°C. IR (cm^{-1}): 3410 (OH), 2958, 2931 (CH aliphatic), 1693 (C=O), 1627(C=N). ^{1}HNMR (300 MHz, $CDCl_3$): 0.92 (t, 3H, CH_3), 1.33–1.40 (m, 2H, $C\underline{H}_2CH_3$), 1.82–1.89 (m, 2H, $C\underline{H}_2CH_2CH_3$), 4.01 (s, 3H, OCH_3), 4.28 (t, 2H, NCH_2), 6.03 (s, 1H, OH, D_2O exchangeable), 7.08–8.49 (m, 7H, ArHs). Anal. Calcd for $C_{20}H_{20}N_4O_3$: C, 65.92; H, 5.53; N, 15.38; Found: C, 66.20; H, 5.61; N, 15.30.

Biological activities

Materials

PDE assay kit is used in the assay (Catalog number 60300).

PDE4B inhibition activity assay

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 diluent) 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 = $(FP - FP_b)/(FP_t - FP_b) \times 100\%$, where FP is the fluorescence polarization in the presence of the compound. The % PDE4B inhibition of the synthesized compounds was consequently calculated (Table 1; Fig. 7).

Table 1 Inhibitory effects of the compounds and rolipram on PDE4B2 enzyme

Compound (10 μM)	Mean % inhibition
No compound	0
Rolipram	90
2d	7
2e	15
2f	15
5a	11
5b	19
6a	44
6b	6
6c	44
6d	62

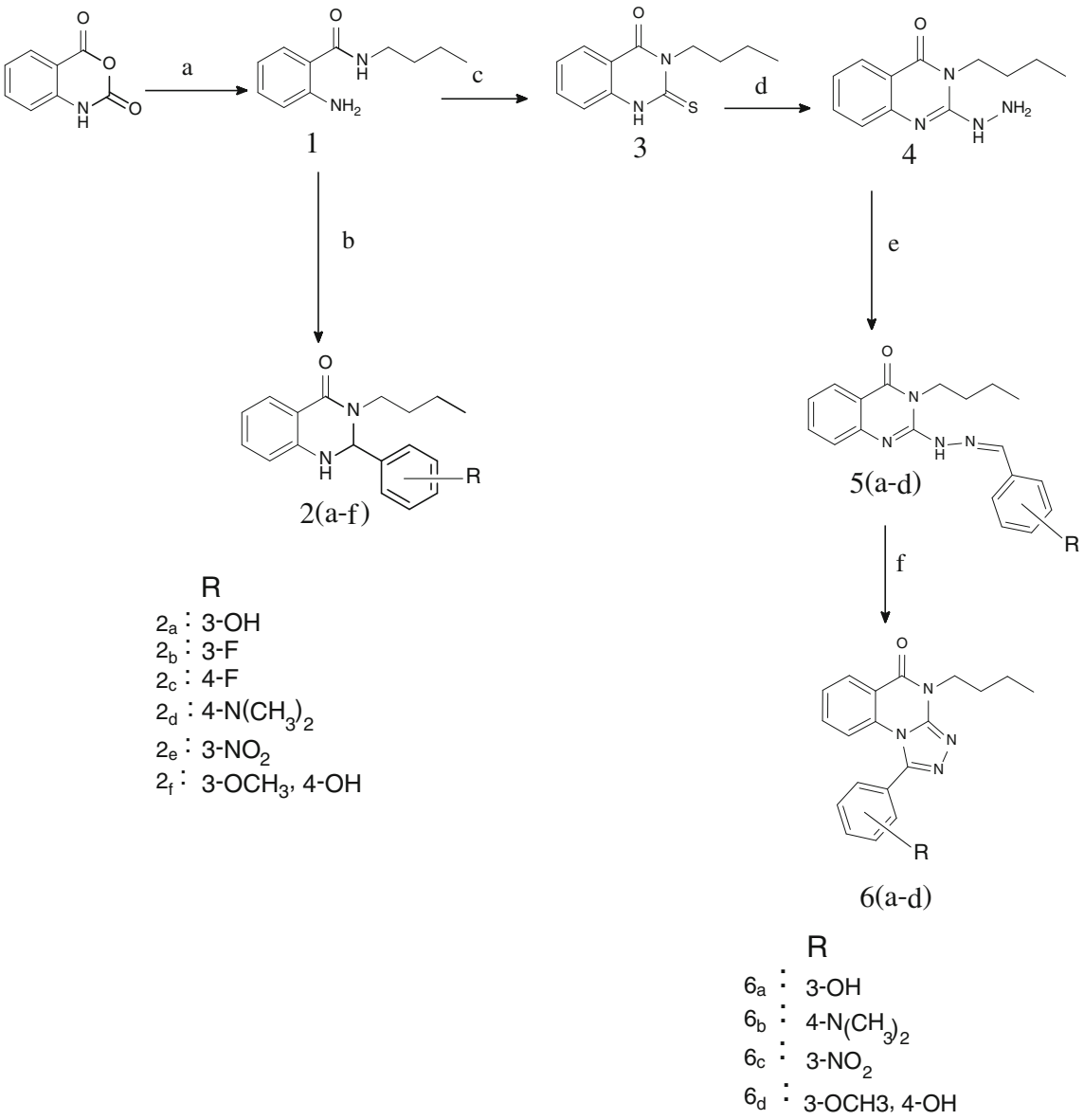
Results and discussion

To prepare the target quinazoline derivatives, reactions presented in Scheme 1 were performed. The starting material 2-amino-*N*-butylbenzamide (**1**) was prepared as reported procedure (Clark and Wagner, 1944). Reacting **1** with appropriate substituted benzaldehyde in the presence of *p*-toluenesulphonic acid as a catalyst using Dean-Stark to trap water formed in the reaction afforded 2-aryl-3-butyl-2,3-dihydroquinazolin-4(1H)-one **2a**–**2f**).

^{1}HNMR proved the presence of a singlet peak around δ 5.7 ppm assignable to C_2H of the quinazolinone nucleus. In addition, an interesting observation was the appearance of geminal hydrogens of NCH_2 at different chemical shifts (2.75 and 3.94) (Chen *et al*., 2008). Geminal hydrogens at NCH_2 was proved by COSY and HMQC spectra of compound (**2e**) which showed that these two hydrogens are coupled to each other (Fig. 3) and are attached to the same carbon (Fig. 4).

This observation may be resides on the fact that the two hydrogens are diastereomeric protons due to the presence of asymmetric center (C_2). Moreover, the limited free rotation of N_3–C bond results in different environments for the protons (Rodrıguez-Franco *et al*., 2000; Tormena *et al*., 2002; Elmaaty and Castle, 2005). The restricted rotation was supported by constructing models of these compounds using MOE builder (Fig. 5) and plotting the conformational energy for each dihedral angle through N_3–C bond using dihedral energy plot module implemented in MOE. The result showed that this conformer lies in a deep valley in which high energy barriers must be overcome in order to rotate (i.e. change its dihedral angle) the N_3–C bond (Fig. 6).

On the other hand, compound **1** was allowed to react with carbon disulphide to give 3-butyl-2-thioxo-2,



Scheme 1 Synthetic protocol for title compounds. (*a*) butyl amine; (*b*) substituted benzaldehydes, *p*-toluenesulphonic acid; (*c*) CS_2, KOH; (*d*) NH_2NH_2, reflux 30 h; (*e*) substituted benzaldehydes, ethanol, acetic acid; (*f*) $FeCl_3$, ethanol

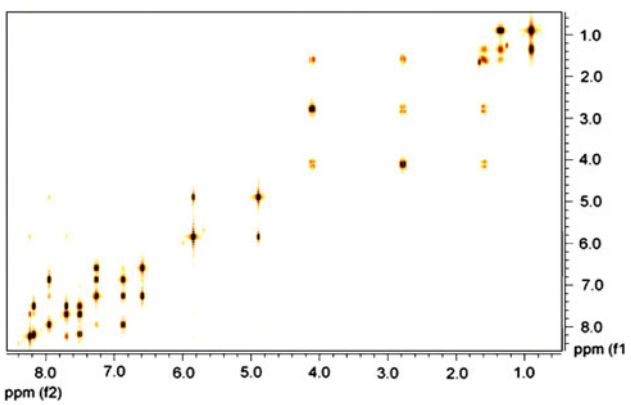


Fig. 3 Correlation spectroscopy (COSY) of compound (**2e**)

3-dihydroquinazolin-4(1H)-one (**3**) which upon treatment with hydrazine hydrate gave 3-butyl-2-hydrazinoquinazolin-4(3H)-one (**4**). Condensation of compound **4** with the respective aromatic aldehyde was conducted in ethanol containing a catalytic amount of glacial acetic acid to give the novel Schiff's base **5a**–**5d**. The long duration of reaction (30 h) required might be because of the presence of bulky butyl group at position three which might have reduced the reactivity of quinazoline ring system at C-2 position. ^{1}HNMR revealed, in each case, three characteristic signals in the regions δ 0.97–4.24, 8.48–8.89 and 9.25–9.40 ppm corresponding to the butyl, azomethine (–N=CH–) and hydrazone (–NH–N=C) protons, respectively.

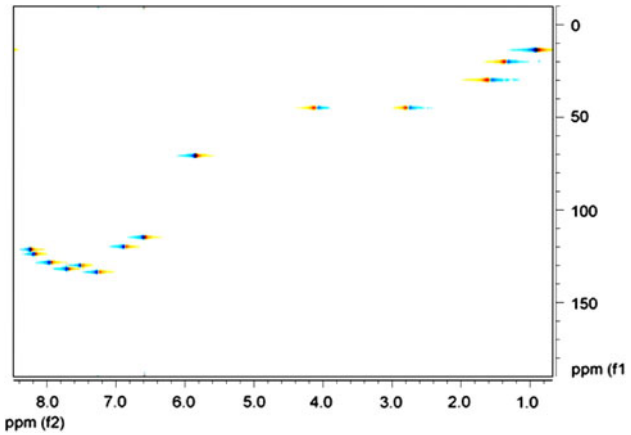


Fig. 4 Heteronuclear multiple quantum coherence (HMQC) of compound (**2e**)

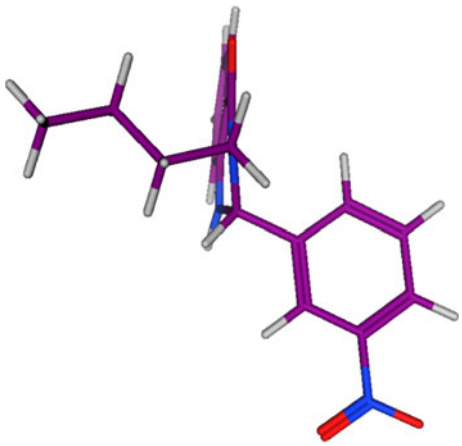


Fig. 5 3D model of the most stable conformer of compound **2e**

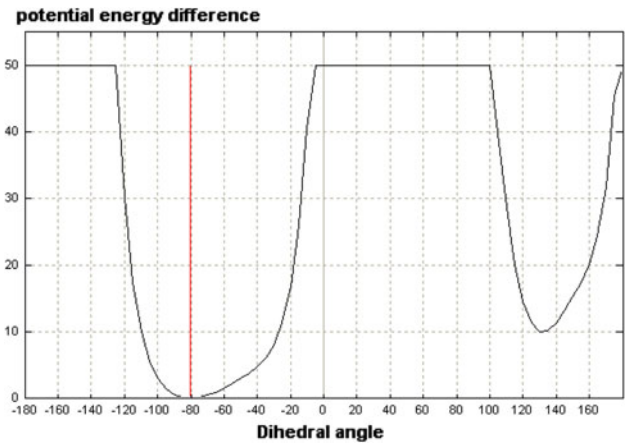


Fig. 6 Dihedral energy plot of N_3–C bond of compound **2e**. This was obtained using the dihedral energy plot implemented in MOE 2008.10. The dihedral angle energy of kcal/mol was based on the energy of the most stable conformation

In addition to this, oxidative cyclization of the schiffs base **5a**–**5d** using ferric chloride yielded 1-aryl-4-butyl [1,2,4]triazolo[4,3-a] quinazolin-5(4H)-one (**6a**–**6d**). ^{1}HNMR spectra showed the absence of both the azomethine proton (–N=CH) and hydrazone –NH–N=C proton signals. Both elemental analysis and mass spectrum of each compound revealed that it has two hydrogens less than the respective hydrazone.

Molecular docking studies of the synthesized compounds were performed in order to rationalize the obtained biological results (Fig. 7) as well as to help us in understanding the various interactions between the ligands and enzyme active site in details.

The X-ray crystallographic structure of PDE4B complexed with Rolipram (PDB: 1RO6) was used in our docking studies. All water molecules in the experimental structure were removed. Hydrogen atoms were added and the protonation states of the amino acid residues were assigned using the Protonate 3D algorithm. Ligand molecules were modelled 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. 8a). 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. 8b). This binding model was useful in our interpretation to the activities our synthesized compounds.

Next, 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 presence of a Zn binding group was important in the lead compounds, **6a**, **6c** and **6d** (Fig. 9), which showed high

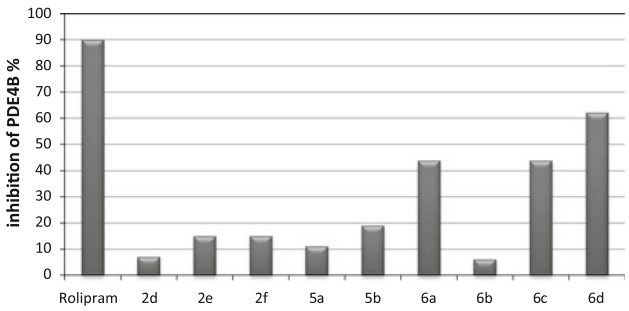


Fig. 7 Graph for the inhibitory effects of the compounds on PDE4B2 enzyme

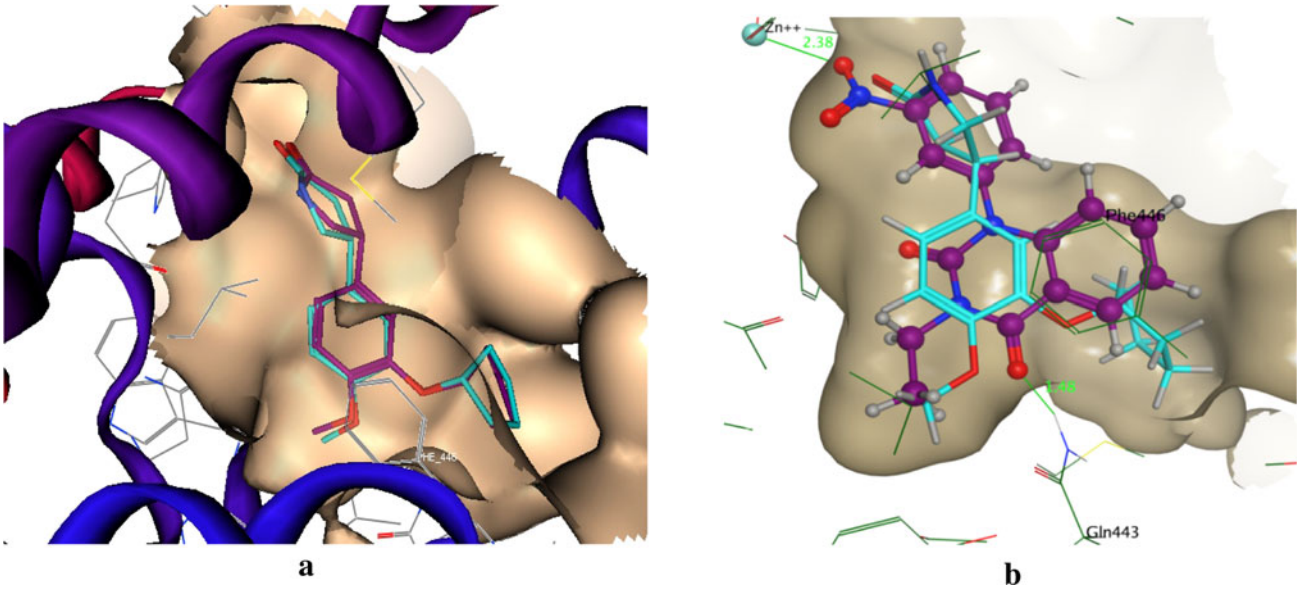


Fig. 8 **a** The docked Rolipram ligand into PDE4B seems superimposed on the native Rolipram ligand, RMSD: 0.32 Å. **b** Predicted binding model of nitraquazone

inhibition against PDE4. The absence of Zn binding group in **6b** was therefore responsible of its low inhibitory activity.

Although the aryl moieties of **2a**–**2f** contain groups that can bind with Zn^{2+} or form hydrogen bonds with amino acid residues (Asp392, His234 and His278). Unfortunately, these aryls were not oriented towards the Zn^{2+} and the amino acids residues (Asp392, His234 and His278) but, on the contrary, they were directed in the opposite direction and partly exposed to the active site surface. This finding gives a reasonable explanation to the poor PDE4 activities of these derivatives (Fig. 10).

Conclusions

From the common pharmacophore for nitraquazone related compounds, a series of novel heteroaromatic compounds have been designed, synthesized and evaluated as PDE4 inhibitors. Rolipram was selected as a reference standard. The biological results reported in Table 1 confirm that compound **6d** showed promising inhibitory activity comparable to that of Rolipram, whereas compound **6a** and **6c** exhibited moderate inhibitory activity. Finally, the rest of the tested compounds exhibited poor inhibition.

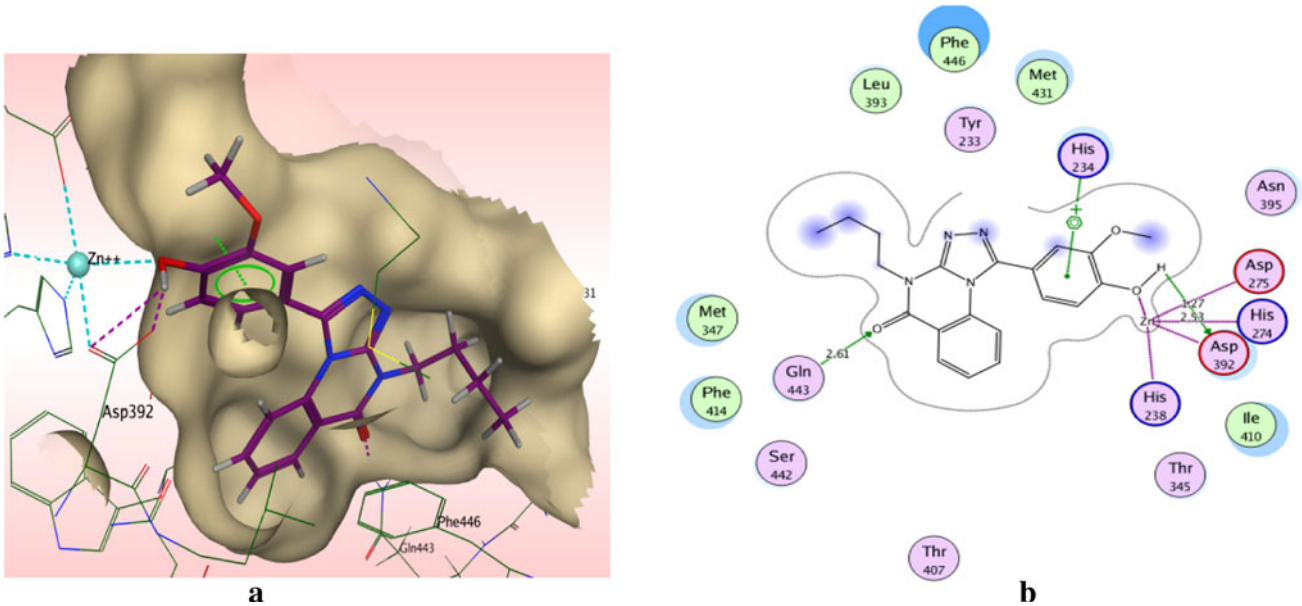


Fig. 9 **a** Proposed binding mode of **6d** in PDE4B active site generated by MOE docking. **b** Simplified 2D ligand interaction of **6d**

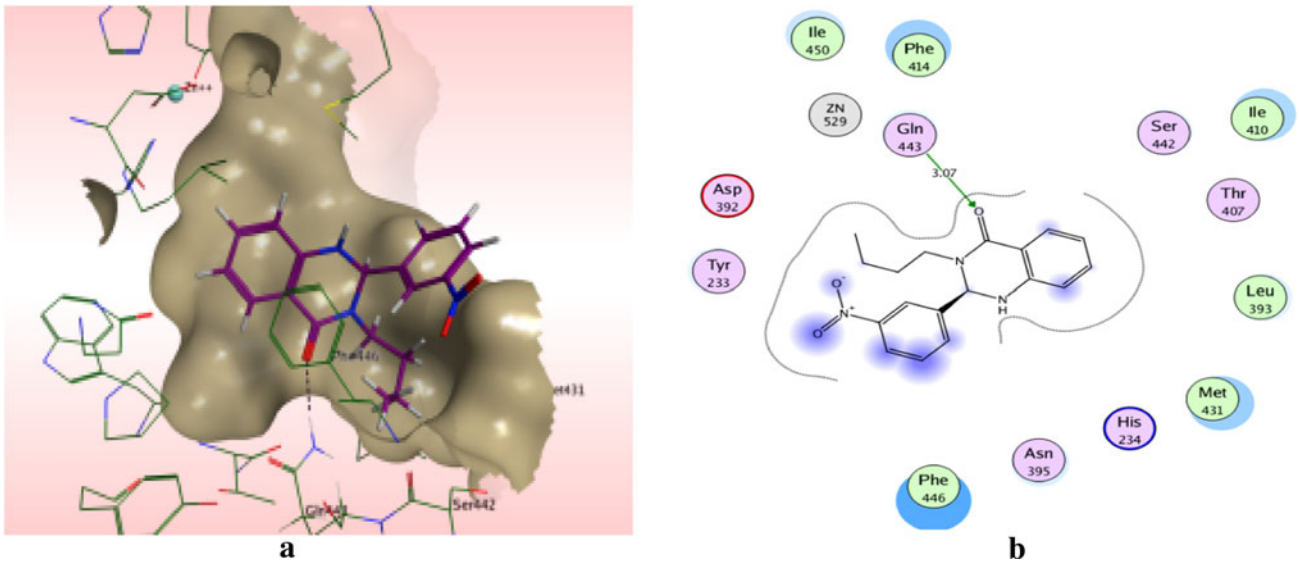


Fig. 10 **a** Docked poses of **2e** in PDE4B binding site generated by MOE docking. **b** Simplified 2D ligand interaction of **2e**

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