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RESEARCH ARTICLE

Molecular Modeling of Some Novel 4(3h) - Quinazolinone Derivatives

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ABSTRACT

Introduction: Inhibition of soluble epoxide hydrolase (sEH) is considered as a promising target to reduce blood pressure, improve insulin sensitivity, and decrease inflammation. **Material and Method:** In this study, a series of some novel quinazoline-4(3H)- one derivatives (3a-t) with varying steric and electronic properties was designed, synthesized, and evaluated as sEH Inhibitors (sEHI). Most of the synthesized compounds had similar inhibitory activity to the commercial reference inhibitor, 12-(3-adamantan-1-ylureido) dodecanoic acid, and among them, 4-chloro-N-(4-(4-oxo-3,4-dihy- droquinazoline-2-yl)phenyl) benzamide (3g) was identified as the most active sEHI (IC_{50} = 0.5 nM), about two-fold more potent compared to the reference inhibitor. **Conclusion:** The results of molecular modeling followed by biological studies indicate that a quinazolinone ring serves as a suitable scaffold to develop novel small molecule candidates to inhibits EH and the nature of substituent on the amide moiety has a moderate effect on the activity.

Keywords: Quinazolinone Derivatives, Arachidonic Acid, Antibacterial, Hypertension

INTRODUCTION

The arachidonic acid (AA) cascade comprises of a group of metabolic pathways that produce endogenous bioactive lipid mediators, which regulate multiple biological processes such as inflammation, hypertension, and pain. AA is metabolized by different oxygenases including cyclooxygenases, lipoxygenases, and cytochrome P450s. CYP epoxygenase enzymes (including CYP 2C, 2J) transform AA to the anti-inflammatory epoxyeicosatrienoic acids (EETs)[1] which are anti-hypertensive and anti-inflammatory endogens.[2,3] However, EETs are rapidly metabolized to a large extent by soluble epoxide hydrolase (sEH) to the corresponding dihydroxyeicosatrienoic acids (DHETs) with primarily pro-inflammatory properties.[4,5] The degradation of EETs to DHETs can be blocked by sEH Inhibitors (sEHIs) that significantly increase EET concentrations in plasma and tissues to target hypertension and inflammation.[6-8] Stabilization of EETs and blockade of DHETs synthesis are proposed as a therapeutic approach in several pathological disorders and could lead to novel therapies in various animal models of disease.[9] Thus, there is an increasing interest in the development and preclinical evaluation of novels EHIs.

Although several inhibitors of sEH have been identified, no sEHI is available on the market to date. Besides, only a few inhibitors have reached clinical trials among many candidate chemicals. 12-(3-adamantan-1-ylureido) dodecanoic acid (AUDA), AR9281 and GSK2256294 [Figure 1] have proved their potential to inhibit sEH in a number of *in vitro* and *in vivo* studies. A small Phase II a clinical study examined the effect of the well-known sEHI AUDA; IC_{50} = 3.2–100 nM,[10] which is commonly used as experimental sEH reference inhibitor, on the vascular tone.[11] After

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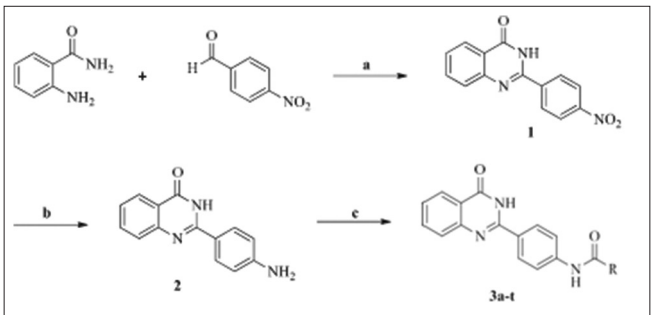
a successful Phase I clinical study,[12] the sEHI AR9281; IC_{50} = 7 nM failedina Phase II a study due to lack of efficacy.[13] Two Phase I clinical studies with GSK2256294, IC_{50} = 27 pM[14] have recently been completed. Many of the sEHI contain a urea group, such as AUDA and AR9281, or anandamide function, such as GSK2256294 as a primary pharmacophore. Quinazolinone scaffold had many pharmaceutical properties including antibacterial, cytotoxicity, and anti-inflammatory activities.[15-18] Based on the suggested pharmacophore models of sEHIs[13,19,20] and in continuance of our previous studies on various heterocyclic compounds with sEHI activity,[21,22] some novel quinazoline-4(3H)-one derivatives were designed and synthesized [Scheme 1], compounds (3a-t) as potent sEHIs. In this series of compounds, the amide group as a primary pharmacophore is placed along a line with a distance of 7 Å in regard to the quinazolinone ring as a secondary pharmacophore [Figure 2] which is completely compatible with the pro-posed model of Merck scientists for sEHI.[13] Quinazolinone ring involves crucial structural features to interact with the active site of sEH through hydrogen and hydrophobic bonds and also improves physical properties of amide based sEHIs. In addition, the quinazoline nucleus is used as a basic framework in a number of biologically active compounds and FDA approved drug molecules.[23] Therefore, in the present study, we synthesized a library of 20 quinazoline-4(3H)-ones and their sEHI activities were evaluated by fluorescence-based human soluble epoxide hydrolase assay kit.



Cmpd.	R	Cmpd.	R
3a	Phenyl	3k	2-Fluorophenyl
3b	4-Methylphenyl	3l	4-Chloro-2-fluorophenyl
3c	3-Methyphenyl	3m	4-Nitrophenyl
3d	4-Methoxyphenyl	3n	3-Nitrophenyl
3e	3-Methoxyphenyl	3o	2-Carboxyphenyl
3f	2-Methoxyphenyl	3p	Benzyl
3g	4-Chlorophenyl	3q	2-Phenyl-3-carboxypropyl
3h	3-Chlorophenyl	3r	2-Carboxyethyl
3i	4-Fluorophenyl	3s	Methyl
3j	3-Fluorophenyl	3t	Chloromethyl

Scheme1.Reagentsandconditions: (a)DMSO,ultrasound,r.t.; (b)sodiumdithionite,DMF-H_2O(9:1),90°C;(c) properacylchloridesoranhydrides,DMF,0°C.

MATERIALS AND METHODS

Chemistry

All chemicals and reagents were purchased from Aldrich or Merck Company with a minimum purity of 97% and were used without further purification. The structures of the synthesized compounds were confirmed by infrared (IR), liquid chromatography–mass spectrometry (LC/MS), ^{1}HNMR, ^{13}CNMR, and elemental analysis. IR spectra were recorded using KBr discs on a Perkin Elmer 843 IR. ^{1}HNMR spectra were obtained with a Bruker Avance II (400 MHz) instrument using DMSO-d_6 as solvent. They are reported as follows: Chemical shifts δ in ppm (multiplicity, coupling constants J in Hz, number of protons, and assignment). Mass spectra were obtained on Agilent 6410 (QQQ) LC/MS

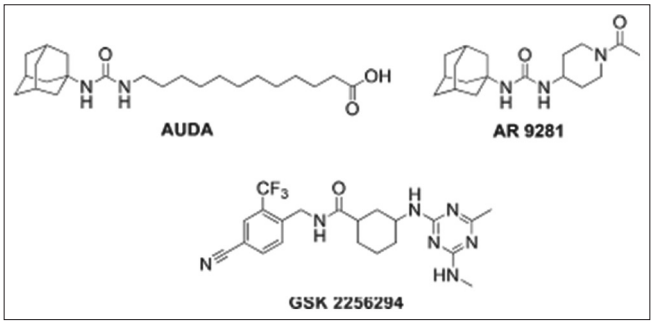


Figure 1: Representatives of known sEH inhibitors.

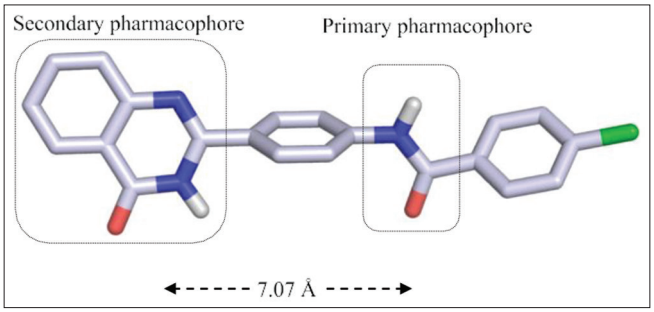


Figure 2: The quinazoline-4(3H)-one scaffold as a secondary pharmacophore is ~7 Å away from the amide group as a primary pharmacophore.

system. Melting points were determined on an Electrothermal 9100 apparatus and are uncorrected.

Synthesis of 2-(4-nitrophenyl) quinazoline-4(3h)-one(1)

Quinazoline the mixture of anthranilamide (10 mmol; 1.0 equiv.) and 4-ni- trobenzaldehyde (12 mmol; 1.2equiv.) in DMSO (15 mL) was kept in an open flask under ultrasound irradiation for 3h. After completion of reaction, ice water was added and the product was filtrated and washed with plenty of water to remove DMSO. Recrystallization in ethanol afforded pure 2-(4-nitrophenyl)quinazoline-4(*3H*)-one1.[24] Yield: 96%, yellow crystalline solid, m.p. 288–289°C, MS (ESI) m/z 267.9 ($[M+H]^+$).

Synthesis of 2-(4-aminophenyl)quinazoline-4(3h)-one(2)

A mixture of 2-(4-nitrophenyl)quinazoline-4(3H)-one 1 (1 mmol) and sodium dithionite (3.5 mmol) in 9:1 DMF-H_2O (3 mL) was taken in a sealed through land heated at 90°C with stirring to get 2-(4-aminophenyl) quinazoline-4(3H)-one(2). The reaction was monitored by TLC, after complete consumption of the starting material; a hot filtration was used to remove sodium dithionite. The solution was then poured in to ice water (10 mL) and the precipitate that formed was collected by filtration, washed with cold water, dried under vacuo and crystallized in EtOH. Yield: 98%, light yellow powder, m.p. 249–251°C, MS(ESI)m/z 238.0($[M+H]^+$).

Synthesis of n-(4-(4-oxo-3,4-dihydroquinazoline-2-yl) phenyl) benzamides (3a-t)

1 mmol of compound 2 was added to corresponding acyl chlorides oranhydrides (1.5 mmol) in DMF (5 mL) at 0°C. The content was kept under argon atmosphere with stirring at 0°C to room temperature. After completion of reaction, water was added to crash out three from solvent. The product was filtered, washed thoroughly with water and recrystallized inmethanol.

N-(4-(4-oxo-3,4-dihydroquinazoline-2-yl)phenyl) benzamide (3a)

Yield: 81%, light yellow powder, m.p. 367–369°C. 1H NMR (400 MHz, DMSO-d_6) δ12.50 (s, 1H, NH-quinazolinone), 10.63 (s, 1H, NH-amide), 8.24 (d, J = 8.8 Hz, 2H, $H_{3,5}$-phenylene), 8.16 (d, J = 8.0 Hz, 1H, H_5-quinazolinone), 8.06 (d, J = 7.6 Hz, 2H, $H_{2,6}$- benzamide), 7.96 (d, J = 8.8 Hz, 2H, $H_{2,6}$-phenylene), 7.84 (t, J = 8.4 Hz, 1H, H_7-quinazolinone), 7.74 (d, J = 8.0 Hz, 1H, H_8- quinazolinone), 7.69 (d, J = 7.6 Hz, 2H, $H_{3,5}$-benzamide) 7.60 (t, J = 8.0 Hz, 1H, H_6-quinazolinone), 7.51 (t, J = 7.6 Hz, 1H, H_6- benzamide).^{13}CNMR(100MHz,DMSO-d_6) δ164.86, 162.73, 152.24, 149.34, 142.29, 137.08, 135.06, 133.74, 132.12, 130.95, 128.89, 127.98, 127.85, 127.09, 126.32, 121.32, 120.26. IR (KBr) 1644, 1663, 3298 cm^{-1}. For $C_{21}H_{15}N_3O_2$ calculated: C73.88, H4.43, N 12.32; found: C 73.93, H 4.42, N 12.29. MS (ESI) *m/z* 342.0 ([M+H]+).

4-methyl-n-(4-(4-oxo-3,4-dihydroquinazoline-2-yl)phenyl) benzamide (3b)

(3b).Yield: 65%, white powder, m.p. 324–326°C. ^{1}HNMR (400 MHz, DMSO-d_6) δ12.47 (s, 1H, NH-quinazolinone), 10.45 (s, 1H, NH-amide), 8.22 (d, J = 8.8 Hz, 2H, $H_{3,5}$- phenylene), 8.16 (d, J = 7.6 Hz, 1H, H_5-quinazolinone), 7.98 (d, J = 8.8 Hz, 2H, $H_{2,6}$- phenylene), 7.92 (d, J = 8.0 Hz, 2H, $H_{2,6}$-benzamide), 7.84 (t, J = 7.6 Hz, 1H, H_7-quinazolinone), 7.74 (d, J = 8.4 Hz, 1H, H_8- quinazolinone), 7.51 (t, J = 7.6Hz, 1H, H_6-quinazolinone),7.37 (d, J = 8.0Hz, 2H, $H_{3,5}$-benzamide), 2.41 (s, 3H, Me).^{13}CNMR (100 MHz, DMSO-d_6) δ166.15, 162.73, 152.30, 149.36, 142.70, 142.41, 135.08, 132.22, 129.46, 128.85, 128.29, 127.85, 127.75, 126.79, 126.33, 121.30, 120.14, 21.52. IR(KBr) 1666, 1679 cm^{-1}. For $C_{22}H_{17}N_3O_2$ calculated: C74.34, H4.82, N11.83; found: C74.31, H4.83, N11.79. MS (ESI) m/z 356.0 ($[M+H]^+$).

3-methyl-n-(4-(4-oxo-3,4-dihydroquinazoline-2-yl)phenyl) benzamide(3c).

Yield:62%,whitepowder,m.p. 301–302°C.^{1}HNMR (400 MHz, DMSO-d_6) δ12.48 (s, 1H, NH-quinazolinone), 10.50 (s, 1H, NH-amide), 8.22 (d, J = 8.8 Hz, 2H, $H_{3,5}$-phenylene), 8.16 (d, J = 7.6 Hz, 1H, H_5-quinazolinone), 8.98 (d, J = 8.8 Hz, 2H, $H_{2,6-}$ phenylene), 7.86–7.78 (m, 3H, H_7-quinazolinone and $H_{2,6}$-benzamide), 7.74 (d, J = 8.0 Hz, 1H, H_8-quinazolinone), 7.51 (t, J = 8.0 Hz, 1H, H_6- quinazolinone), 7.47–7.44 (m, 2H,

$H_{4,5-}$benzamide), 2.42 (s, 3H, Me). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.47, 162.74, 152.29, 149.36, 142.65, 138.27, 135.14, 135.07, 132.89, 128.86, 128.69, 127.85, 127.82, 126.79, 126.33, 125.42, 121.30, 120.12, 21.45. IR (KBr) 1654, 1670, 3304 cm^{-1}. For $C_{22}H_{17}N_3O_2$ calculated: C74.34, H4.82, N 11.83; found: C 74.29, H 4.81, N 11.80. MS (ESI) m/z 356.0 ([M+H]$^+$).

4-methoxy-n-(4-(4-oxo-3,4-dihydroquinazoline-2-yl)phenyl) benzamide (3d)

Yield: 72%, light yellow powder, m.p.318–320°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ12.47 (s, 1H, NH-quinazolinone), 10.39 (s, 1H, NH-amide), 8.22 (d, J = 8.80 Hz, 2H, $H_{3,5}$-phenylene), 8.16 (d, J = 7.24 Hz, 1H, H_5-quinazolinone), 8.01 (d, J =8.84 Hz, 2H, $H_{2,6}$- benzamide), 7.98 (d, J = 8.84 Hz, 2H, $H_{3,5}$-benzamide), 7.84 (t, J=8.00 Hz, 1H, H_7-quinazolinone), 7.74 (d, J = 8.00 Hz, 1H, H_8- quinazolinone), 7.51 (t, J = 8.00Hz, 1H, H_6-quinazolinone), 7.11 (d, J = 8.80 Hz, 2H, $H_{2,6}$-phenylene), 3.86 (s, 3H, OMe). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.67, 162.74, 162.60, 152.31, 149.37, 142.83, 135.07, 130.24, 128.83, 127.84, 127.60, 127.08, 126.76, 126.33, 121.29, 120.09, 114.16, 55.95. IR (KBr) 1645, 1665, 3283 $^{cm-1}$. For $C_{22}H_{17}N_3O_3$ calculated: C71.13, H4.62, N11.32; found: C71.11, H4.59, N11.33. MS (ESI) m/z 371.7 ([M+H]$^+$).

3-methoxy-n-(4-(4-oxo-3,4-dihydroquinazoline-2-yl)phenyl) benzamide (3e)

Yield: 79%, light yellow powder, m.p. 367–369°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ12.44 (s, 1H, NH-quinazolinone), 10.53 (s, 1H, NH-amide), 8.14 (d, J = 8.8 Hz, 2H, $H_{3,5}$-phenylene), 8.07 (d, J = 7.6 Hz, 1H, H_5-quinazolinone), 7.93 (d, J = 8.8 Hz, 2H, $H_{2,6-}$phenylene), 7.76 (t, J =8.0 Hz, 1H, H_7-quinazolinone), 7.67 (d, J = 8.0 Hz, 1H, H_8-quinazolinone), 7.51 (d, J = 8.0 Hz, 1H, H_6- benzamide), 7.47 (s, 1H, H_2-benzamide), 7.45–7.37 (m, 2H, H_6- quinazolinone and H_5-benzamide), 7.10 (d, J = 8 Hz, 1H, H_4- benzamide), 3.78 (s, 3H, OMe). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.09, 162.76, 159.67, 152.49, 149.11, 142.68, 136.45, 135.12, 130.10, 128.91, 127.67, 127.54, 126.87, 126.36, 121.23, 120.53, 120.28, 118.12, 113.49, 55.88. IR (KBr) 1664, 1723, 3355 $^{cm-1}$. For $C_{22}H_{17}N_3O_3$ calculated: C71.13, H4.62, N11.32; found: C71.11, H 4.63, N11.29. MS (ESI) m/z 371.7 ([M+H]$^+$).

2-Methoxy-n-(4-(4-oxo-3,4-dihydroquinazoline-2-yl)phenyl) benzamide (3f)

Yield: 66%, white powder, m.p. 345°C (dec.). ^{1}HNMR (400 MHz, DMSO-d_6) δ12.48 (s, 1H, NH-quinazolinone), 10.43 (s, 1H, NH-amide), 8.21 (d, J = 8.8Hz, 2H, $H_{3,5}$-phenylene), 8.16 (d, J = 7.6 Hz, 1H, H_5-quinazolinone), 7.92 (d, J = 8.8Hz, 2H, $H_{2,6}$- phenylene), 7.84 (t, J = 7.6Hz, 1H, H_7-quinazolinone), 7.74 (d, J = 8.0 Hz, 1H, H_8-quinazolinone), 7.65 (d, J = 7.4Hz, 1H, H_6- benzamide), 7.56–7.49 (m, 2H, H_6-quinazolinone and H_4-benzamide), 7.20 (d, J = 8.4 Hz, 1H, H_3-benzamide), 7.09 (t, J = 7.6Hz, 1H, H_5- benzamide), 3.92 (s, 3H, OMe). ^{13}CNMR (100 MHz, DMSO-d_6) δ 165.45, 162.84, 156.97, 152.32, 149.32, 142.41, 135.05, 132.71, 130.11, 128.97, 127.75, 126.76, 126.33, 125.28, 121.28, 120.99, 119.59, 112.50, 56.39. IR (KBr) 1572, 1665, 3355 $^{cm-1}$. For $C_{22}H_{17}N_3O_3$ calculated: C 71.13, H 4.62, N 11.32; found: C 71.15, H 4.59, N 11.33. MS (ESI) m/z 372 ([M+H]+).

4-chloro-n-(4-(4-oxo-3,4-dihydroquinazoline-2-yl)phenyl) benzamide (3g)

Yield: 93%, white powder, m.p. 381–383°C. ^{1}HNMR (400 MHz, DMSO-d_6) δ12.49 (s, 1H, NH-quinazolinone), 10.60 (s, 1H, NH-amide), 8.23 (d, J = 8.8 Hz, 2H, $H_{3,5}$-phenylene), 8.15 (d, J = 8.0 Hz, 1H, H_5-quinazolinone), 8.04 (d, J = 8.4 Hz, 2H, $H_{2,6-}$benzamide), 7.97 (d, J = 8.8 Hz, 2H, $H_{2,6}$-phenylene), 7.84 (t, J = 8.4 Hz, 1H, H_7-quinazolinone), 7.74 (d, J = 8.0 Hz, 1H, H_8- quinazolinone), 7.36 (d, J = 8.8 Hz, 4H, $H_{3,5}$-benzamide), 7.16 (t, J = 7.6 Hz, 1H, H_6-quinazolinone). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.85, 162.29, 151.38, 147.92, 139.28, 138.55, 130.23, 130.06, 129.10, 129.03, 128.93, 127.01, 125.85, 120.82, 120.08, 117.26, 94.04. IR (KBr) 1655, 1676, 3307 $^{cm-1}$. For$C_{21}H_{14}ClN_3O_2$ calculated: C 67.10, H 3.76, N 11.19; found: C 67.13, H 3.75, N 11.16. MS (ESI) m/z 375.9 ([^{35}M+H]+), 377.9([^{37}M+H]+).

3-chloro-n-(4-(4-oxo-3,4-dihydroquinazoline-2-yl)phenyl) benzamide (3h)

Yield: 89%, light yellow powder, m.p. 314–316°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ12.50 (s, 1H, NH-quinazolinone), 10.64 (s, 1H, NH-amide), 8.24 (d, J = 8.8 Hz, 2H, $H_{3,5}$-phenylene), 8.16 (d, J = 8.8 Hz, 1H, H_5-quinazolinone), 8.05 (s, 1H, H_2-

benzamide), 7.98–7.95 (m, 3H, $H_{2,6}$-phenylene and H_6-benzamide), 7.84 (t, J = 8.4 Hz, H_5-benzamide), 7.75–7.69 (m, 2H, H_8-quinazolinone and H_4-benzamide), 7.61 (t, J=8 Hz, 1H, H_7-quinazolinone), 7.52 (t, J = 8 Hz, 1H, H_6-quinazolinone). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.88, 162.73, 152.25, 149.33, 142.28, 137.08, 135.09, 133.74, 132.14, 130.97, 128.91, 128.16, 127.99, 127.86, 127.10, 126.84, 126.33, 121.32, 120.27. IR(KBr) 1662, 1676, 3306 $^{cm-1}$. For $C_{21}H_{14}ClN_3O_2$ calculated: C 67.10, H 3.76, N 11.19; found: C 67.07, H3.75, N 11.16. MS (ESI) m/z 375.9 ([M+H]+).

4-fluoro-N-(4-(4-oxo-3,4-dihydroquinazoline-2-yl)phenyl) benzamide (3i)

Yield: 88%, white powder, m.p. 356–358°C. ^{1}HNMR (400 MHz, DMSO-d_6) δ12.49 (s, 1H, NH-quinazolinone), 10.56 (s, 1H, NH-amide), 8.23 (d, J = 8.8 Hz, 2H, $H_{3,5}$-phenylene), 8.16 (d, J = 7.8 Hz, 1H, H_5-quinazolinone), 8.09 (dd, J = 8.8, 3.2 Hz, 2H, $H_{3,5}$-benzamide), 7.97 (d, J = 8.8 Hz, 2H, $H_{2,6}$-phenylene), 7.84 (t, J = 7.6 Hz, 1H, H_7-quinazolinone), 7.73 (d, J = 7.6 Hz, 1H, H_8- quinazolinone), 7.51 (t, J = 7.6 Hz, 1H, H_6-quinazolinone), 7.4 (dd, J = 8.8, 0.8 Hz, 2H, $H_{2,6}$-benzamide). ^{13}CNMR (100 MHz, DMSO-d_6) δ 165.21, 163.45, 162.74, 152.26, 149.35, 142.05, 135.05, 131.53, 130.99, 128.87, 127.84, 126.33, 121.31, 120.18, 116.01, 115, 79, 95.87. IR (KBr) 1653, 1674, 3299 $^{cm-1}$. For $C_{21}H_{14}FN_3O_2$ calculated: C70.17, H3.93, N11.70; found: C70.09, H3.92, N11.71. MS (ESI) m/z 360.2 ([M+H]+).

3-fluoro-n-(4-(4-oxo-3,4-dihydroquinazoline-2-yl) phenyl) benzamide (3j)

4-fluoro-N-(4-(4-oxo-3,4-dihydroquinazoline-2-yl)phenyl) benzamide (3i). Yield:88%,whitepowder,m.p.356–358°C.1HNMR (400MHz,DMSO-d_6)δ12.49(s,1H,NH-quinazolinone),10.56(s,1H, NH-amide), 8.23 (d, J = 8.8 Hz, 2H, $H_{3,5}$-phenylene), 8.16 (d, J = 7.8 Hz, 1H, H_5-quinazolinone), 8.09 (dd, J = 8.8, 3.2 Hz, 2H, $H_{3,5}$-benzamide), 7.97 (d, J = 8.8 Hz, 2H, $H_{2,6}$-phenylene), 7.84 (t, J = 7.6 Hz, 1H, H_7-quinazolinone), 7.73 (d, J = 7.6 Hz, 1H, H_8- quinazolinone),7.51(t,J =7.6Hz,1H,H_6-quinazolinone),7.4(dd, J=8.8,0.8Hz,2H,$H_{2,6}$-benzamide).13CNMR(100MHz,DMSO-d_6)δ165.21, 163.45, 162.74, 152.26, 149.35, 142.05, 135.05, 131.53, 130.99, 128.87, 127.84, 126.33, 121.31, 120.18, 116.01, 115, 79,Yield: 81%, white powder, m.p. 320–322°C. ^{1}HNMR (400 MHz, DMSO-d_6) δ 12.50 (s, 1H, NH-quinazolinone), 10.60 (s, 1H, NH-amide), 8.23 (d, J = 8.8 Hz, 2H, $H_{3,5}$-phenylene), 8.16 (d, J = 8.0 Hz, 1H, H_5-quinazolinone), 7.97 (d, J = 8.8 Hz, 2H, $H_{2,6}$-phenylene), 7.86–7.80 (m, 3H, H_7-quinazolinone and $H_{2,6}$- benzamide), 7.73 (d, J = 7.6 Hz, H_8-quinazolinone), 7.62 (dd, J = 8.0, 2.0 Hz, 1H, H_4-benzamide), 7.53–7.46 (m, 2H, H_6- quinazolinone and H_5-benzamide). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.95, 163.61, 162.78, 161.18, 152.29, 142.31, 135.07, 131.19, 131.11, 128.91, 128.12, 126.82, 126.34, 124.51, 121.30, 120.26, 115.21, 114.98, 95.86. IR (KBr) 1667, 1686, 3313 $^{cm-1}$. For $C_{21}H_{14}FN_3O_2$ calculated: C70.17, H3.93, N11.70; found: C70.18, H 3.89, N11.67. MS (ESI) m/z 359.8 ([M+H]+).

2-fluoro-n-(4-(4-oxo-3,4-dihydroquinazoline-2-yl) phenyl) benzamide (3k)

Yield: 70%, white powder, m.p. 291–292°C. ^{1}HNMR (400 MHz, DMSO-d_6) δ 12.50 (s, 1H, NH-quinazolinone), 10.75 (s, 1H, NH-amide), 8.24 (d, J = 8.8 Hz, 2H, $H_{3,5}$-phenylene), 8.16 (d, J = 7.1 Hz, 1H, H_5-quinazolinone), 7.90 (d, J = 8.8 Hz, $H_{2,6}$- phenylene), 7.84 (t, J = 8.4 Hz, 1H, H_4-benzamide), 7.75–7.70 (m, 2H, H_8-quinazolinone and H_6-benzamide), 7.60 (m, 1H, H_3-benzamide), 7.52 (t, J = 7.7 Hz, 1H, H_7-quinazolinone), 7.37 (m, 2H, H_6- quinazolinone and H_5-benzamide). ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.61, 162.74, 160.61, 158.14, 152.22, 149.33, 142.17, 135.08, 130.44, 129.04, 128.14, 127.87, 126.84, 126.33, 125.13, 121.31, 119.67, 116.82, 116.60. IR (KBr) 1653, 1668, 3441 $^{cm-1}$. 129.10, 129.03, 128.93, 127.01, 125.85, 120.82, 120.08, 117.26, 94.04. IR (KBr) 1655, 1676, 3307 $^{cm-1}$. For $C_{21}H_{14}ClN_3O_2$ calculated: C 67.10, H 3.76, N 11.19; found: C 67.13, H 3.75, N 11.16. MS (ESI) m/z 375.9 ([^{35}M+H]+), 377.9 ([^{37}M+H]+).

3-chloro-n-(4-(4-oxo-3,4-dihydroquinazoline-2-yl)phenyl) benzamide (3h)

Yield: 89%, light yellow powder, m.p. 314–316°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.50 (s, 1H, NH-quinazolinone), 10.64 (s, 1H, NH-amide), 8.24 (d, J = 8.8 Hz, 2H, $H_{3,5}$-phenylene), 8.16 (d, J = 8.8 Hz, 1H, H_5-quinazolinone), 8.05 (s, 1H, H_2-benzamide), 7.98–7.95 (m, 3H, $H_{2,6}$-phenylene and

H_6-benzamide), 7.84 (t, J = 8.4 Hz, H_5-benzamide), 7.75–7.69 (m, 2H, H_8-quinazolinone and H_4-benzamide), 7.61 (t, J = 8 Hz, 1H, H_7-quinazolinone), 7.52 (t, J = 8 Hz, 1H, H_6-quinazolinone). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.88, 162.73, 152.25, 149.33, 142.28, 137.08, 135.09, 133.74, 132.14, 130.97, 128.91, 128.16, 127.99, 127.86, 127.10, 126.84, 126.33, 121.32, 120.27. IR (KBr) 1662, 1676, 3306 $^{cm-1}$. For $C_{21}H_{14}ClN_3O_2$ calculated: C 67.10, H 3.76, N 11.19; found: C 67.07, H3.75, N11.16. MS (ESI) m/z 375.9 ([M+H]+).

4-fluoro-n-(4-(4-oxo-3,4-dihydroquinazoline-2-yl) phenyl) benzamide (3i)

Yield: 88%, white powder, m.p. 356–358°C. ^{1}HNMR (400 MHz, DMSO-d_6) δ 12.49 (s, 1H, NH-quinazolinone), 10.56 (s, 1H, NH-amide), 8.23 (d, J = 8.8 Hz, 2H, $H_{3,5}$-phenylene), 8.16 (d, J = 7.8 Hz, 1H, H_5-quinazolinone), 8.09 (dd, J = 8.8, 3.2 Hz, 2H, $H_{3,5}$-benzamide), 7.97 (d, J = 8.8 Hz, 2H, $H_{2,6}$-phenylene), 7.84 (t, J = 7.6 Hz, 1H, H_7-quinazolinone), 7.73 (d, J = 7.6 Hz, 1H, H_8- quinazolinone), 7.51 (t, J = 7.6 Hz, 1H, H_6-quinazolinone), 7.4 (dd, J = 8.8, 0.8 Hz, 2H, $H_{2,6}$-benzamide). ^{13}CNMR (100 MHz, DMSO-d_6) δ 165.21, 163.45, 162.74, 152.26, 149.35, 142.05, 135.05, 131.53, 130.99, 128.87, 127.84, 126.33, 121.31, 120.18, 116.01, 115, 79, 95.87. IR (KBr) 1653, 1674, 3299 $^{cm-1}$. For $C_{21}H_{14}FN_3O_2$ calculated: C70.17, H3.93, N11.70; found: C70.09, H3.92, N11.71. MS (ESI) m/z 360.2 ([M+H]+).

3-fluoro-n-(4-(4-oxo-3,4-dihydroquinazoline-2-yl) phenyl) benzamide (3j)

Yield: 81%, white powder, m.p. 320–322°C. ^{1}HNMR (400 MHz, DMSO-d_6) δ 12.50 (s, 1H, NH-quinazolinone), 10.60 (s, 1H, NH-amide), 8.23 (d, J = 8.8 Hz, 2H, $H_{3,5}$-phenylene), 8.16 (d, J = 8.0 Hz, 1H, H_5-quinazolinone), 7.97 (d, J = 8.8 Hz, 2H, $H_{2,6}$- phenylene), 7.86–7.80 (m, 3H, H_7-quinazolinone and $H_{2,6}$- benzamide), 7.73 (d, J = 7.6 Hz, H_8-quinazolinone), 7.62 (dd, J = 8.0, 2.0 Hz, 1H, H_4-benzamide), 7.53–7.46 (m, 2H, H_6- quinazolinone and H_5-benzamide). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.95, 163.61, 162.78, 161.18, 152.29, 142.31, 135.07, 131.19, 131.11, 128.91, 128.12, 126.82, 126.34, 124.51, 121.30, 120.26, 115.21, 114.98, 95.86. IR (KBr) 1667, 1686, 3313 $^{cm-1}$. For $C_{21}H_{14}FN_3O_2$ calculated: C70.17, H3.93, N11.70; found: C70.18, H 3.89, N11.67. MS (ESI) m/z 359.8 ([M+H]+).

2-fluoro-n-(4-(4-oxo-3,4-dihydroquinazoline-2-yl) phenyl) benzamide (3k)

Yield: 70%, white powder, m.p. 291–292°C. ^{1}HNMR (400 MHz, DMSO-d_6) δ 12.50 (s, 1H, NH-quinazolinone), 10.75 (s, 1H, NH-amide), 8.24 (d, J = 8.8 Hz, 2H, $H_{3,5}$-phenylene), 8.16 (d, J = 7.1 Hz, 1H, H_5-quinazolinone), 7.90 (d, J = 8.8 Hz, $H_{2,6}$- phenylene), 7.84 (t, J = 8.4 Hz, 1H, H_4-benzamide), 7.75–7.70 (m, 2H, H_8-quinazolinone and H_6-benzamide), 7.60 (m, 1H, H_3-benzamide), 7.52 (t, J = 7.7 Hz, 1H, H_7-quinazolinone), 7.37 (m, 2H, H_6- quinazolinone and H_5-benzamide). ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.61, 162.74, 160.61, 158.14, 152.22, 149.33, 142.17, 135.08, 130.44, 129.04, 128.14, 127.87, 126.84, 126.33, 125.13, 121.31, 119.67, 116.82, 116.60. IR (KBr) 1653, 1668, 3441 $^{cm-1}$. $C_{21}H_{14}FN_3O_2$ calculated: C70.17, H3.93, N11.70; found: C70.17, H 3.89, N11.68. MS (ESI) m/z 359.7 ([M+H]+).

4-chloro-2-fluoro-n-(4-(4-oxo-3,4-dihydroquinazoline-2-yl) phenyl)benzamide (3l)

Yield: 56%, yellow powder, m.p. 198–200°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.50 (s, 1H, NH-quinazolinone), 10.78 (s, 1H, NH-amide), 8.23 (d, J = 8.8 Hz, 2H, $H_{3,5}$-phenylene), 8.16 (d, J= 8.0 Hz, 1H, H_5-quinazolinone), 7.88 (d, J = 8.4 Hz, $H_{2,6}$- phenylene), 7.84 (t, J = 7.2 Hz, 1H, H_4-benzamide), 7.78–7.73 (m, 2H, H_8-quinazolinone and H_6-benzamide), 7.67 (d, J = 10 Hz, 1H, H_3-benzamide), 7.52 (t, J = 7.6 Hz, 1H, H_6-quinazolinone), 7.47 (d, J = 10 Hz, 1H, H_5-benzamide). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.64, 160.80, 141.97, 136.81, 135.09, 131.90, 131.84, 129.08, 128.30, 127.86, 126.91, 126.34, 125.45, 124.91, 124.08, 121.29, 119.74, 117.50, 117.24. IR (KBr) 1699, 1700 $^{cm-1}$. For $C_{21}H_{13}ClFN_3O_2$ calculated: C 64.03, H 3.33, N 10.67; found: C 64.10, H3.28, N 10.63. MS (ESI) m/z 394.0 ([M+H]+).

4-nitro-n-(4-(4-oxo-3,4-dihydroquinazoline-2-yl) phenyl) benzamide (3m)

Yield: 79%, yellow crystal, m.p. 372–374°C. ^{1}HNMR (400 MHz, DMSO-d_6) δ 12.54 (s, 1H, NH-quinazolinone), 10.85 (s, 1H, NH-amide), 8.40 (d, J = 8.8 Hz, 2H, $H_{3,5}$-phenylene), 8.24 (m, 4H, $H_{2,3,5,6}$-benzamide), 8.16 (d, J = 7.8 Hz, 1H, H_5-quinazolinone), 7.98 (d, J = 8.8 Hz,

2H, $H_{2,6}$-phenylene), 7.85 (t, J = 7.6 Hz, 1H, H_7- quinazolinone), 7.74 (d, J = 7.6 Hz, 1H, H_8-quinazolinone), 7.52 (t, J = 7.6 Hz, 1H, H_6-quinazolinone). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.72, 162.73, 152.22, 149.76, 149.32, 142.08, 140.75, 135.09, 129.82, 128.96, 128.42, 127.87, 126.87, 126.34, 124.09, 121.33, 120.36. IR (KBr) 1531, 1659, 1683, 3419 cm^{-1}. For $C_{21}H_{14}N_4O_4$ calculated: C 65.27, H 3.65, N 14.51; found: C 65.23, H 3.66, N 14.49. MS (ESI) m/z 387.2 ([M+H]+).

3-nitro-n-(4-(4-oxo-3,4-dihydroquinazoline-2-yl) phenyl) benzamide (3n)

Yield: 84%, yellow crystal, m.p. 280–282°C. ^{1}HNMR (400 MHz, DMSO-d_6) δ 12.58 (s, 1H, NH-quinazolinone), 10.93 (s, 1H, NH-amide), 8.90 (s, 1H, H_2-benzamide), 8.55–8.50 (m, 2H, $H_{4,6}$- benzamide), 8.31 (d, J = 8.8 Hz, 2H, $H_{3,5}$-phenylene), 8.23 (d, J = 7.6 Hz, 1H, H_5-quinazolinone), 8.05 (d, J = 8.8 Hz, 2H, $H_{2,6}$- phenylene), 7.96–7.89 (m, 2H, H_7-quinazolinone and H_5-benzamide), 7.80 (d, J = 8.0 Hz, 1H, H_8-quinazolinone), 7.58 (t, J = 7.6 Hz, 1H, H_6- quinazolinone). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.15, 162.73, 152.23, 149.32, 148.24, 142.08, 136.46, 135.09, 134.79, 130.76, 128.95, 128.39, 127.87, 126.89, 126.34, 123.01, 121.33, 120.43. IR (KBr) 1533, 1667, 1684, 3425 cm^{-1}. For $C_{21}H_{14}N_4O_4$ calculated: C65.27, H3.65, N14.51; found: C65.26, H3.61, N14.48. MS (ESI) m/z 386.7 ([M+H]+).

2-(4-(4-oxo-3,4-dihydroquinazoline-2-yl)phenyl) carbamoyl)benzoic acid (3o)

Yield: 43%, yellow powder, m.p. 380°C (decompose). ^{1}HNMR (400 MHz, DMSO-d_6) δ 13.13 (s, 1H, COOH), 12.48 (s, 1H, NH-quinazolinone), 10.67 (s, 1H, NH-amide), 8.20 (d, J = 8.76 Hz, 2H, $H_{3,5}$-phenylene), 8.15 (d, J = 7.84 Hz, 1H, H_5- quinazolinone), 7.92 (d, J = 7.12 Hz, 1H, H_3-benzamide), 7.86 (d, J = 8.68 Hz, 2H, $H_{2,6}$-phenylene), 7.82 (m, 1H, H_7-quinazolinone), 7.74–7.67 (m, 2H, $H_{5,6}$-benzamide), 7.63–7.58 (m, 2H, H_4-benzamide and H_8-quinazolinone), 7.51 (t, J = 7.84 Hz, 1H, H_6-quinazolinone).^{13}C NMR (100 MHz, DMSO-d_6) δ 168.28, 167.79, 152.30, 149.36, 142.90, 139.11, 135.09, 132.35, 130.28, 130.10, 128.94, 128.27, 127.84, 127.56, 126.33, 121.27, 119.37. IR (KBr) 1684, 1700, 2957, 3317 cm^{-1}. For $C_{22}H_{15}N_3O_4$ calculated: C68.55, H3.93, N10.91; found: C68.57, H3.88, N10.93. MS (ESI) m/z 385.7 ([M+H]+).

N-(4-(4-oxo-3,4-dihydroquinazoline-2-yl) phenyl)-2- phenylacetamide (3p)

Yield: 92%, white powder, m.p. 374–376°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.45 (s, 1H, NH-quinazolinone), 10.50 (s, 1H, NH-amide), 8.17 (d, J = 8.4 Hz, 2H, $H_{3,5}$-phenylene), 8.14 (d, J = 8.0 Hz, 1H, H_5-quinazolinone), 7.83 (t, J = 8.1 Hz, 1H, H_7- quinazolinone), 7.87 (d, J = 8.8 Hz, 2H, $H_{2,6}$-phenylene), 7.71 (d, J = 8.0 Hz, 1H, H_8-quinazolinone), 7.50 (t, J = 7.6 Hz, 1H, H_6- quinazolinone), 7.37–7.32 (m, 4H, $H_{2,3,5,6}$-phenyl), 7.28–7.26 (m, 1H, H_4-phenyl), 3.70 (s, 2H, CH_2Ph). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.08, 162.72, 152.24, 149.34, 142.54, 136.17, 135.06, 129.64, 129.04, 128.82, 127.81, 127.49, 127.10, 126.76, 126.32, 121.26, 119.04, 43.84. IR (KBr) 1667, 1682, 3283 cm^{-1}. For $C_{22}H_{17}N_3O_2$ calculated: C74.34, H4.82, N11.83; found: C74.31, H4.78, N11.81. MS (ESI) m/z 355.8 ([M+H]+).

5-oxo-5-((4-(4-oxo-3,4-dihydroquinazoline-2-yl) phenyl)amino)- 3-phenylpentanoic acid (3q)

Yield: 58%, white powder, m.p. 299–301°C. ^{1}HNMR (400 MHz, DMSO-d_6) δ 12.34 (s, 1H, COOH), 12.01 (s, 1H, NH-quinazolinone), 10.10 (s, 1H, NH-amide) 8.04 (d, J = 8.8 Hz, 3H, $H_{3,5}$-phenylene and H_5-quinazolinone), 7.73 (t, J = 8.4 Hz, 1H, H_7-quinazolinone), 7.63–7.58 (m, 3H, $H_{2,6}$- phenylene and H_8-quinazolinone), 7.40 (t, J = 8.0 Hz, 1H, H_6- quinazolinone), 7.21–7.18 (m, 4H, $H_{2,3,5,6}$-phenyl), 7.11–7.09 (m, 1H, H_4-phenyl), 3.27 (quint, 1H, CH), 2.69–2.46 (m, 4H, methylenes). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.32, 170.37, 162.72, 152.24, 149.34, 13.96, 142.39, 135.06, 128.95, 128.72, 127.89, 127.81, 127.33, 126.89, 126.75, 126.31, 121.25, 118.97, 43.27, 38.56. IR (KBr) 1651, 1681, 2955, 3295 cm$^{-1.}$ For $C_{25}H_{21}N_3O_4$ calculated: C70.23, H4.95, N9.83; found: C70.19, H4.93, N9.89. MS (ESI) m/z 427.8 ([M+H]+).

4-oxo-4-((4-(4-oxo-3,4-dihydroquinazoline-2-yl) phenyl)amino) butanoic acid (3r)

Yield: 61%, white powder, m.p. 361–362°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.44 (s, 1H, COOH), 12.23 (s, 1H, NH-quinazolinone), 10.31 (s, 1H, NH-amide) 8.18 (d, J = 8.8 Hz, 2H, $H_{3,5}$- phenylene), 8.15 (d, J = 8.4 Hz, 1H, H_5-quinazolinone), 7.83 (2, J = 8.0 Hz, 1H, H_7-

quinazolinone), 7.76 (d, J = 8.8 Hz, 2H, $H_{2,6}$- phenylene), 7.72 (d, J = 8.0 Hz, 1H, H_8-quinazolinone), 7.50 (t, J = 8.0 Hz, 1H, H_6-quinazolinone), 2.64 (t, J = 6.0 Hz, 2H, CH_2CH_2COOH), 2.57 (t, J = 6.0 Hz, 2H, CH_2CH_2COOH). ^{13}C NMR (400 MHz, DMSO-d_6) δ 174.29, 171.11, 162.73, 152.26, 149.36, 142.62, 135.02, 129.01, 127.80, 127.19, 126.69, 126.31, 121.25, 118.81, 31.62, 29.13. IR (KBr) 1642, 1709, 3170, 3319 cm^{-1}. For $C_{18}H_{15}N_3O_4$ calculated: C64.07, H4.48, N12.46; found: C64.12, H 4.43, N12.42. MS (ESI) m/z 337.9 ([M+H]+).

N-(4-(4-oxo-3,4-dihydroquinazoline-2-yl)phenyl) acetamide (3s)

Yield: 95%, white powder, m.p. 198–200°C. ^{1}HNMR (400 MHz, DMSO-d_6) δ 12.43 (s, 1H, NH-quinazolinone), 10.25 (s, 1H, NH-amide), 8.175–8.134 (m, 3H, H_5-quinazolinone and $H_{3,5}$-phenylene), 7.82 (t, J = 8.4 Hz, 1H, H_7-quinazolinone), 7.60–7.04 (m, 3H, H_8- quinazolinone and $H_{2,6}$-phenylene), 7.50 (t, J = 8.4 Hz, 1H, H_6- quinazolinone), 2.10 (s, 3H, Me). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.28, 162.73, 152.28, 149.36, 142.66, 135.04, 128.99, 127.81, 127.26, 126.72, 126.31, 121.25, 118.85, 49.07, 24.64. IR (KBr) 1657, 1671, 3283 cm^{-1}. For $C_{16}H_{13}N_3O_2$ calculated: C68.79, H4.69, N15.05; found: C 68.81, H 4.65, N 15.09. MS (ESI) m/z 280.0 ([M+H]+).

2-chloro-n-(4-(4-oxo-3,4-dihydroquinazoline-2-yl)phenyl) acetamide (3t)

Yield: 91%, dark yellow, m.p. 279–281°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.47 (s, 1H, NH-quinazolinone), 10.61 (s, 1H, NH-amide), 8.19–7.51 (m, 8H, aromatic), 4.32 (s, 2H, CH_2Cl). ^{13}CNMR (100 MHz, DMSO-d_6) δ 165.55, 162.77, 152.23, 149.22, 141.78, 135.05, 129.14, 128.12, 127.76, 126.82, 126.33, 121.29, 119.31, 44.08. IR (KBr) 1682, 1684, 3235 cm^{-1}. For $C_{16}H_{12}ClN_3O_2$ calculated: C 61.24, H 3.86, N 13.40; found: C 61.29, H 3.82, N 13.37. MS (ESI) m/z 313.8 ([M+H]+).

Docking studies

The high resolution crystal structure of sEH (PDB code: 3ANS) complexed with 4-cyanoN-([1S, 2R]-2-phenylcyclopropyl) benzamide was retrieved from RCSB Protein Data Bank. The structures of compounds were investigated using the Lamarckian genetic algorithm search method implemented in AutoDock Vina[25] software. The enzyme was kept rigid, and ligands were allowed to be flexible. Polar hydrogens and Kollman united atom partial charges were added to the individual protein atoms. Each ligand structure was energy minimized under MM +method in HyperChem8 software and converted to pdbqt format file using AutoDock Tools 4.0 version1.5.4. A docking grid box was built with 40, 40, and 40 points in 25.8460, 24.0730, and 114.8150 directions in the catalytic site of protein and the number of generations and maximum number of energy evaluations was set to 100 and 2,700,000, respectively. Docking results were clustered with a root mean square deviation of 0.5 Å and evaluated by Pymol software.

In vitro biological activity

Biological evaluation was performed by Cayman fluorescence-based human soluble epoxide hydrolase assay kit (item number 10011671). The enzyme and inhibitors were incubated for 15 min in 25 mM Bis-Tris/HCl buffer (200 μL; pH 7.0) at 30°C. 3-phenylcyano(6-methoxy-2- naphthalenyl) methyl ester-2-oxiraneacetic acid (PHOME) was used as the substrate for assay. Activity was determined by monitoring the appearanceof6-methoxy-2-naphthaldehyde by fluorescence detection with an excitation wavelength of 330 nm and an emission wavelength of 465 nm. The reference inhibitor for assay is AUDA, one of the most effective inhibitors of sEH with IC_{50} value of 1 nM. The test samples and AUDA at concentrations of 0.1, 1, 10, and 50 nM were dissolved in DMSO.

RESULTS AND DISCUSSION

Chemistry

The synthesis of target quinazoline-4(3H)-one derivative (3a-t) was accomplished in three steps. First, 2-(4-nitrophenyl) quinazoline-4(3*H*)- one 1 was synthesized in high yield via condensation reaction of anthranilamide with 4-nitrobenzaldehyde in DMSO under ultrasound IR-radiation for 3h. In the second step, the nitro

group of the intermediate 1 was reduced using sodium dithionite in DMF-water. Finally, the amide compounds 3a-t were obtained in acceptable yield from there - action of amine 2 with various acyl chlorides and anhydrides in DMF [Scheme 1]. The structures of the synthesized compounds were confirmed by IR, LC/MS, ^{1}HNMR, ^{13} CNMR, and elemental analysis.

Docking studies

To investigate the binding modes of the tested compounds, they were fitted into the binding site of the sEH enzyme in a molecular docking simulation. According to Figure 3a, the potent compounds (3b, 3d, 3g, and 3m) in *in vitro* test had similar orientation in the active site of sEH enzyme and the amid group of these compounds as a primary pharmacophore could interact necessary hydrogen bonding with amino acids Asp335, Tyr383, and Tyr466. Furthermore, quinazolinone ring as a secondary pharmacophore placed in the hydrophobic pocket [Figure 3b]. Moreover, the lipophilic segment of synthesized compound, for example, 4-chlorophenyl group of 3g, was located in the hydrophobic grove consisting of Phe267, Leu408, Phe497, and Val498 [Figure 3c].

In vitro biological activity

The inhibitory activity of the synthesized derivatives 3a–t against sEH enzyme was evaluated *in vitro* and presented as IC_{50} values in Table 1. As shown in Table 1, most compounds had sEH inhibitory activity at concentrations of nM and their activities were compared to a known sEHI, AUDA. Six compounds 3b, 3d, 3g, 3m, 3p, and 3q with IC_{50} values of 0.54, 0.78, 0.50, 0.74, 0.80, and 0.73 nM were found to be higher active than AUDA with IC_{50} value of 1 nM in this test system.

Considering to obtained results, placing phenyl ring in R position is essential for sEHI. So that, compounds 3r, 3s, and 3t with aliphatic groups in R position exhibited the lowest inhibitory effects and compounds bearing phenyl ring appeared to be far more active. In addition, all compounds with parasubstituents on phenyl ring had more appropriate effects in comparison with the compounds with similar substituents in other positions. And also, the replacement of the ortho-substituent on phenyl ring with metha-substituent did not exhibit a significant difference in inhibitory activity. Therefore, phenyl ring with parasubstituent in R position enhance inhibitory activity of sEH enzyme and there is no significant difference between electron with drawing and electron donating substituent in this effect.

Overall, these quinazoline-4(3H)-one compounds exhibited strong activity against sEH comparable to AUDA. According to presented SAR studies, The formation of hydrogen bonds between amide group as the primary pharmacophore and Asp335, Tyr383, and Tyr466 in the hydrophilic tunnel of active site is necessary for a strong inhibitory effect and the placement of quinazolinone ring as a secondary pharmacophore in the large cavity of enzyme is a sufficient condition for a strong inhibitory effect. Furthermore, the presence of lipophilic substituents in these inhibitors and its

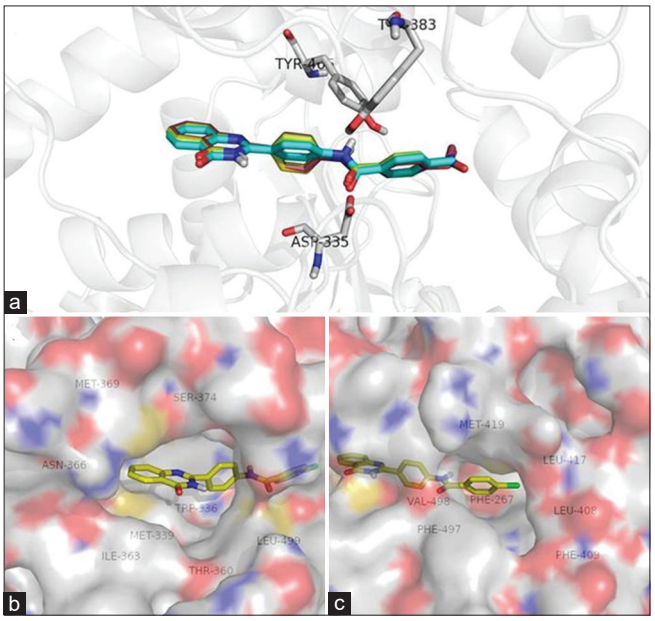
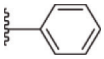
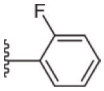
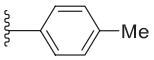
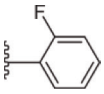
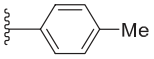
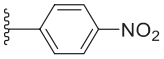
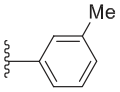
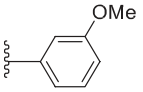
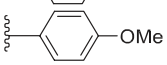
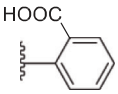
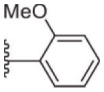
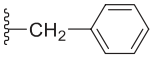
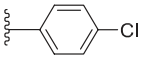
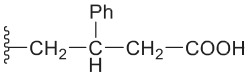
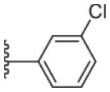
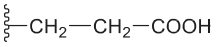
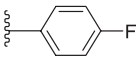
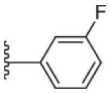
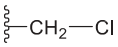
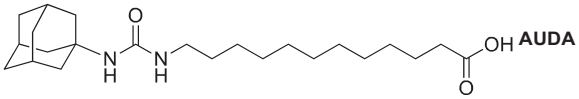


Figure 3: (a) The placement of amide bond of compounds 3b, 3d, 3g and 3m in the hydrophilic tunnel of sEH. (b) The left-hydrophobic cavity of she was filled by quinazolinone ring. (c) The right hydrophobic cavity of sEH was filled by lipophilic substituent (4-chlorophenyl) of inhibitor 3g 95.87. IR(KBr)1653, 1674, 3299 cm−1. For C21H14FN3O2calculated:C70.17,H3.93, N11.70; found: C70.09, H3.92,N11.71.MS (ESI)m/z360.2([M+H]+).

Table 1: Inhibitory activities of the quinazoline-4(3H)-one derivatives

Compound	R	IC_{50}(nM)	Compound	R	IC_{50}(nM)
3a		1.04	**3k**		1.16
3b		0.54	**3l**		2.70
3c		1.04	**3m**		0.74
3d		0.78	**3n**		10.50
3e		2.15	**3o**		1.47
3f		1.19	**3p**		0.80
3g		0.50	**3q**		0.73
3h		1.47	**3r**		10.04
3i		1.00	**3s**		>50
3j		1.04	**3t**		15.20
AUDA			AUDA		

placement in the hydrophobic enzyme cavity could improve the potency of this series of compounds.

CONCLUSION

In this study, we report the development and evaluation of new heterocycles 3a–t containing a quinazolinone scaffold as novel small molecules possessing inhibitory activity against the sEH. Substitution of this series of compounds with a hydrophobic substituted phenyl group was useful for improving inhibition potency (3b, 3d, and 3g). While, compounds with an a liphatic substituent such as 3r, 3s, and 3t led to a significant loss in inhibition. Hence, the SAR results in Table 1 and Figure 3 indicated that quinazolinone moiety, as a well-known and highly used skeleton in approved drugs, is a useful secondary pharmacophore for enhancing inhibition potency of amide-based inhibitors.

REFERENCES

1. Spector AA, Norris AW. Action of epoxyeicosatrienoic acids on cellular function. Am J Physiol Physiol 2007;292:C996-1012.
2. Node K, Huo Y, Ruan X, Yang B, Spiecker M, Ley K, *et al*. Anti-inflammatory properties of cytochrome P450 epoxygenase-derived eicosanoids. Science 1999;285:1276-9.
3. John ID, Xeuying Z, Jorge CH, Christophe C, Bruce HD. Soluble epoxide hydrolase inhibitionl Owers arterial blood pressure in angiotensin II hypertension. Hypertension 2002;39:690-4.
4. Chacos N, Capdevila J, Falck JR, Manna S, Martin-

Wixtrom C, Gill SS, *et al*. The reaction of arachidonic acid epoxides (epox-yeicosatrienoic acids) with a cytosolic epoxide hydrolase. Arch Biochem Biophys 1983;223:639-48.
5. Zeldin DC, Kobayashi J, Falck JR, Winder BS, Hammock BD, Snapper JR, *et al*. Regio-and enantiofacial selectivity of epoxyeicosatrienoic acid hydration by cytosolic epoxide hydrolase. J Biol Chem 1993;268:6402-7.
6. Revermann M, Barbosa-Sicard E, Dony E, Schermuly RT, Morisseau C, Geisslinger G, *et al*. Inhibition of the soluble epoxide hydrolase attenuates monocrotaline-induced pulmonary hypertension in rats. J Hypertens 2009;27:322-31.
7. Ulu A, Davis BB, Tsai HJ, Kim IH, Morisseau C, Inceoglu B, *et al*. Soluble epoxide hydrolase inhibitors reduce the development of atherosclerosis in apolipoprotein e-knockout mouse model. J Cardiovasc Pharmacol 2008;52:314-23.
8. Ghosh S, Chiang PC, Wahlstrom JL, Fujiwara H, Selbo JG, Roberds SL. Oral delivery of 1,3-dicyclohexylurea nanosuspension enhances exposure and lowers blood pressure in hypertensive rats. Basic Clin Pharmacol Toxicol 2008;102:453-8.
9. Morisseau C, Hammock BD. Impact of soluble epoxide hydrolase and epox-yeicosanoids on human health. Annu Rev Pharmacol Toxicol 2013;53:37-58.
10. Jones PD, Wolf NM, Morisseau C, Whetstone P, Hock B, Hammock BD. Fluorescent substrates for soluble epoxide hydrolase and application to inhibition studies. Anal Biochem 2005;343:66-75.
11. Tran L, Kompa AR, Wang BH, Krum H. Evaluation of the effects of urotensin II and soluble epoxide hydrolase inhibitor on skin microvessel tone in healthy controls and heart failure patients. Cardiovasc Ther 2012;30:295-300.
12. Chen D, Whitcomb R, MacIntyre E, Tran V, Do ZN, Sabry J, *et al*. Webb, pharmacokinetics and pharmacodynamics of AR9281, an inhibitor of soluble epoxide hydrolase, in single-and multiple-dose studies in healthy human subjects. J Clin Pharmacol 2012;52:319-28.
13. Shen HC, Hammock BD. Discovery of inhibitors of soluble epoxide hydrolase: A target with multiple potential therapeutic indications. J Med Chem 2012;55:1789-808.
14. Podolin PL, Bolognese BJ, Foley JF, Long E, Peck B, Umbrecht S, *et al*. Callahan, *in vitro* and *in vivo* characterization of a novel soluble epoxide hydrolase inhibitor. Prostaglandins Other Lipid Mediat 2013;104-105:25-31.
15. Gatadi S, Lakshmi TV, Nanduri S. 4(3H)-Quinazolinone derivatives: Promising antibacterial drug leads. Eur J Med Chem 2019;170:157-72.
16. Haikarainen T, Koivunen J, Narwal M, Venkannagari H, Obaji H, Joensuu P, *et al.* Para-substituted 2-phenyl-3, 4-dihydroquinazolin-4-ones as potent and selective tankyrase inhibitors. Chem Med Chem 2013;8:1978-5.
17. Brown JA, Jones KL, Prinjha RK, Witherington J. Assignee Covalent Conjugates of Bet Inhibitors and Alpha Amino Acid Esters, United States Patent Application US 15/559, 518. United Kingdom: Inventors; GlaxoSmithKline Intellectual Property Development Ltd.; 2018.
18. Abdullaha M, Mohammed S, Ali M, Kumar A, Vishwakarma RA, Bharate SB, Discovery of quinazolin-4(3H)-onesas NLRP3 inflammasome inhibitors: Computational design, metal-free synthesis, and *in vitro* biological evaluation. J Org Chem 2019;84:5129-40.
19. Anandan SK, Do ZN, Webb HK, Patel DV, Gless RD. Non-urea functionalityas the primary pharmacophore in soluble epoxide hydrolase inhibitors. Bioorg Med Chem Lett 2009;19:1066-70.
20. Waltenberger B, Garscha U, Temml V, Liers J, Werz J, Schuster D, *et al*. Discovery of potent soluble epoxide hydrolase (sEH) inhibitors by pharmacophore-based virtual screening. J Chem Inf Model 2016;56:747-62.
21. RezaeeZavareh E, Hedayati E, Hoghooghi Rad L, Shahhosseini S, Faizi M, Tabatabai SA. Design, synthesis and biological evaluation of 4-benzamidobenzoic acid hydrazide derivatives as novel soluble epoxide hydrolase inhibitors. Iran J Pharm Res 2014;13:51-9.
22. Zavareh E, Hedayati M, Rad L, Kiani A, Shahhosseini S, Faizi M, *et al*. Design, synthesis and biological evaluation of some oxadiazole derivatives as novel amide-based inhibitors of soluble epoxide hydrolase. Lett Drug Des Discov 2014;11:721-30.
23. Ahmad I. An insight into the therapeutic potential of quinazoline derivatives as anticancer agents. Medchemcomm 2017;8:871.
24. Kim NY, Cheon CH. Synthesis of quinazolinones from anthranilamides and al-dehydes via metal-free aerobic oxidation in DMSO. Tetrahedron Lett 2014;55:2340-4.
25. Trott O, Olson AJ. Software news and update auto dock Vina: Improvingthe speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem 2010;31:455-61.