Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2012, 4(8):3991-4000

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Synthesis, anti-bacterial, anti-asthmatic and anti-diabetic activities of novel 3-substituted quinazolin-4-ones using 1-Butyl-3-methyl-imidazoliumtetrafluoro borate [BMIM$^+$][BF4$^-$] as a green, efficient and reusable catalyst under solvent free conditions

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ABSTRACT

A convenient, one-pot synthesis of 3-substitutedquinazolinone derivatives by the reaction of anthranalic acid, ortho formates and aryl or alkyl amines in presence of 1-Butyl-3-methylimidazolium tetrafluoroborate [BMIM$^+$][BF4$^-$] as a green, efficient reusable catalyst has been described. The reaction proceeded within few minutes with excellent yields. The simplicity of the experimental procedure as well as the re-usability of the catalyst are the significant advantages of this protocol. All the compounds synthesized were screened for their anti-bacterial, anti-asthmatic and anti-diabetic activities.

Key words: 4(3H)-quinazolinones, [BMIM$^+$][BF4$^-$], Heterogeneous Catalyst, Solvent-Free Conditions.

INTRODUCTION

In regards to importance of Quinazoline derivatives, especially (3H)-4-quinazolinones have gained more importance in recent years because of their biological activities such as anti-inflammatory, anti-malarial, anti-cancer, anti-convulsant, anti-hypertensive, anti-Parkinsonin, analgesic activities [1-7]. Several bioactive natural products containing quinazolinone skeleton have also been reported from natural sources [8-11]. Some of these compounds were reported as anti-hyperlipidemic active compounds [12]. The common method for the preparation of quinazolinones involves the amidation of 2-aminobenzoic acid or 2-aminobenzonitrile followed by oxidative ring closure under basic conditions [13-16] and aza wittig reactions of α-azido substituted aromatic imides [17, 18] There are different one-pot synthesis of these compounds have also been reported [19-30]. However most of these methods have significant drawbacks such as harsh reaction conditions, long reaction times, low yields, difficult work-up procedures, expensive reagents and difficulty in recovery and reusability of the catalysts. Therefore there is need for development of simple and efficient catalyst to prepare (3H)-quinazolinones.

EXPERIMENTAL SECTION

General

Melting points were measured on a Buchi 510 apparatus and are uncorrected. The IR spectra were recorded on a Perkin-Elmer RX 1 FT-IR spectrophotometer, the ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra on a

Brucker-400 MHz spectrometer and the mass spectra on a API-2000, LCMS-MS system. Column chromatography was performed over silica gel (BDH 100-200 mesh) and TLC with silica gel GF 254.

General Procedure for the preparation of 4-(3H)-Quinazolinones.
To a mixture of anthranillic acid (**1**) (1 mmol), Alkylorthoester (**2**) (1.2 mmol) and an amine derivative (**3**) (1.2 mmol), [$BMIM^+$][$BF4^-$], (100 mg) was added. The mixture was stirred with heating at 110 ^oC for appropriate time (**Table-2**). TLC monitored the reaction. After completion of the reaction, 10 ml of Chloroform was added to the reaction mixture, layers separated and the product purified appropriately either by crystallization or by silica-gel column chromatography to obtain the pure products.

Compound 4a: 3-PHENYL-3H-QUINAZOLIN-4-ONE
Melting range: 136-138^{0}C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.33 (1H, s), 8.19 (1H, d, *J*= 8.0 Hz), 7.85 (1H, t, *J*=8.0 Hz), 7.72 (1H, dd, *J*=8.0, 1.9 Hz), 7.59-7.49 (5H, m). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 160.3, 148.0, 147.4, 137.9, 134.9, 129.6, 127.8, 126.8, 122.2. , I.R (KBr) cm^{-1}: 1671, 1595, 1498, 1323, 1262, MS *m/z:* 223 $[M+H]^{+.}$

Compound 4b: 3-(2-FLUORO PHENYL)-3H-QUINAZOLIN-4-ONE
Melting range: 119-121.5^{0}C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.39 (1H, s), 8.22 (1H, dd, *J*=7.9, 1.9 Hz), 7.85 (1H, td, *J*=8.0, 1.3 Hz), 7.78 (1H, d, *J*=8.0 Hz), 7.64 (1H, t, *J*=8.0 Hz), 7.62-7.38 (4H, m); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 159.7, 158.7, 156.2, 148.0, 147.4, 135.3, 131.8, 130.3, 128.0, 126.7, 125.6, 125.3, 121.9, 116.8, 116.6; I.R (KBr) cm^{-1}: 3054.8, 1674.7, 1500, 1472, 1311, 1265, 771; MS *m/z*: 241.4 $[M+H]^{+.}$

Compound 4c: 3-(2-CHLORO PHENYL)-3H-QUINAZOLIN-4-ONE
Melting range: 160.1-163.2^{0}C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.31 (1H, s), 8.20 (1H, dd, *J*= 8.0, 2.0 Hz), 7.85 (1H, t, *J* =8.0 Hz), 7.78-7.70 (3H, m), 7.61-7.56 (3H, m); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 159.8, 148.1, 147.3, 135.3, 131.8, 131.0, 130.4, 128.8, 128.0, 127.8, 126.8, 122.1; I.R (KBr) cm^{-1}: 3062.5, 1676.7, 1607.2, 1472.6, 1302, 1266; MS *m/z:* 157.3 $[M+H]^{+.}$

Compound 4d: 3-(3-CHLORO PHENYL)-3H-QUINAZOLIN-4-ONE
Melting range: 164.7-166.3 ^{0}C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.31 (1H, s), 8.21 (1H, dd, *J* = 8.0, 2.0 Hz), 8.19 (1H, t, *J*=8.0 Hz), 7.91-7.70 (3H, m), 7.62-7.56 (3H, m); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 159.8, 148.1, 147.3, 135.3, 131.8, 130.9, 128.8, 128.0, 127.8, 126.8, 122.09; I.R (KBr) cm^{-1}: 3072, 1678, 1610, 1469, 1311, 1248; MS *m/z:* 157.3$[M+H]^{+.}$.

Compound 4e: 3-(3, 4-DICHLORO PHENYL)-3H-QUINAZOLIN-4-ONE
Melting range: 213.7-215.4^{0}C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.36 (1H, s), 8.20 (1H, dd, *J*=8.0, 2.0 Hz), 7.97 (1H, d, *J*= 1.9 Hz), 7.89-7.84 (2H, m), 7.74 (1H, dd, *J*=8.0, 2.0 Hz), 7.62-7.59 (2H, m); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 160.2, 147.9, 146.9, 137.6, 135.2, 132.0, 131.3, 130.2, 128.5, 127.7, 126.8, 122.1; I.R (KBr) cm^{-1}: 3067, 1677, 1610, 1470, 1305, 1245; MS *m/z:* 291$[M+H]^{+.}$

Compound 4f: 3-(4-BROMO PYRIDINE)-3H-QUINAZOLIN-4-ONE
Melting range: 211.8-214.6^{0}C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.81 (1H, d, *J*=1.9 Hz), 8.56 (1H, s), 8.33 (1H, dd, *J*=8.0, 2.0 Hz), 8.23 (1H, d, *J*=8.0 Hz), 7.91-7.75 (3H, m), 7.63 (1H, t, *J*= 8.0 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 159.9, 150.2, 148.8, 147.6, 145.7, 141.4, 135.5, 128.1, 127.8, 126.9, 124.3, 121.9, 120.4; I.R (KBr) cm^{-1}: 3060, 1680, 1614, 1576, 1474, 1371, 1306, 1255; MS *m/z:* 302 $[M+H]^{+.}$

Compound 4g: 3-(4-BROMO PHENYL)-3H-QUINAZOLIN-4-ONE
Melting range: 186.4-187.9^{0}C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.28 (1H, s), 8.20 (1H, dd, *J*=8.0, 2.0 Hz), 7.93-7.87 (2H, m), 7.77 (1H, d, *J*=8.0 Hz), 7.70 (1H, m), 7.63-7.50 (3H, m); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 159.8, 148.1, 147.3, 137.0, 135.3, 133.5, 131.7, 129.3, 128.0, 127.8, 126.8; I.R (KBr) cm^{-1}: 3061, 1677, 1606, 1470, 1301, 1265. 122.2; MS *m/z:* 301 $[M+H]^{+.}$

Compound 4h: 3-(4-METHYL PHENYL)-3H-QUINAZOLIN-4-ONE
Melting range: 139.8-143.2^{0}C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.30 (1H, s), 8.19 (1H, d, *J*=8.0 Hz), 7.87 (1H, t, *J*=8.0 Hz), 7.73 (1H, d, *J*=8.0 Hz), 7.59 (1H, t, *J*=8.0 Hz), 7.41-7.34 (4H, m), 2.49 (3H, s); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 160.4, 148.1, 147.6, 138.6, 1354.4, 134.9, 130.0, 127.6, 126.7, 122.2, 21.0; IR (KBr) cm^{-1}: 1690, 1600, 1515, 1472, 1260; MS *m/z:* 237 $[M+H]^{+.}$

Compound 4i: 3-(3-METHYL PHENYL)-3H-QUINAZOLIN-4-ONE
Melting range: 125.5-128.1^{0}C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.30 (1H, s), 8.18 (1H, d, *J*=8.0 Hz), 7.87 (1H, t, *J*=8.0 Hz), 7.84 (1H, d, *J*=8.0 Hz), 7.72 (1H, t, *J*=8.0 Hz), 7.59 (1H, t, *J*=8.0 Hz), 7.43-7.30 (3H, m), 2.37 (3H, s); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 160.3, 148.0, 147.4, 139.2, 137.8, 135.0, 129.7, 128.2, 127.6, 124.8, 122.2, 21.1; I.R (KBr) cm^{-1}: 1671, 1608, 1470, 1260; MS *m/z:* 237[M+H]$^{+.}$

Compound 4j: **3-(4-METHOXY PHENYL)-3H-QUINAZOLIN-4-ONE**
Melting range: 155.1-156.8 ^{0}C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.33 (1H, s), 8.19 (1H, d, *J*=8.0 Hz), 7.83 (1H, t, *J*=8.0 Hz), 7.64 (1H, d, *J*=8.0 Hz), 7.48 (1H, t, *J*=8.0 Hz), 7.44 (1H, t, *J*=8.0 Hz), 7.18-7.03 (3H, m), 3.80 (3H, s); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 160.2, 160.1, 148.0, 147.4, 139.0, 135.0, 130.3, 127.6, 122.2, 115.0, 113.7, 55.8; I.R (KBr) cm^{-1}: 3050, 1686, 1596, 1459, 1308, 1260; MS *m/z:* 253[M+H]$^{+.}$

Compound 4k: 3-(4-CARBOXY PHENYL)-3H-QUINAZOLIN-4-ONE
Melting range: Above 260^{0}C**;** ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 13.1 (1H, brs), 8.31 (1H, s), 8.18 (1H, dd, *J*=8.0, 1.8 Hz), 8.05 (1H, dd, *J*=7.8, 1.8 Hz), 7.92-7.85 (1H, t, *J*= 8.0 Hz), 7.82-7.71 (2H, m), 7.69-7.52 (3H, m); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 166.2, 160.6, 148.3, 147.5, 137.5, 133.8, 131.4, 130.1, 129.5, 127.6, 126.7, 122.2. I.R (KBr) cm^{-1}: 3368, 3072, 1694, 1601, 1480, 1313, 1263; MS *m/z:* 267[M+H]$^{+.}$

Compound 4l: 3-(4-BENZOIC ACID METHYL ESTER)-3H-QUINAZOLIN-4-ONE
Melting range: 199.6-201.3^{0}C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.40 (1H, s), 8.22 (1H, dd, *J*=8.0, 2.0 Hz), 8.13 (1H, d, *J*= 8.0 Hz), 7.89 (1H, t, *J* = 8.0 Hz), 7.79-7.70 (3H, m), 7.62 (1H, m), 4.38-4.35 (2H, q, *J*= 7.0 Hz), 1.36-1.32 (3H, t, *J*=7.0 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 165.4, 160.1, 147.9, 146.9, 141.8, 135.2, 130.3, 128.2, 127.7, 126.8, 122.1, 61.4, 14.4; I.R (KBr) cm^{-1}: 3359, 2903, 1692, 1596, 1473, 1369, 1280, 1109; MS *m/z:* 295 [M+H]$^{+.}$

Compound 4n: 6-FLUORO-3-PHENYL-3H-QUINAZOLIN-4-ONE
Melting range: 230.1-204.3 ^{0}C; ^{1}H-NMR (400 MHz, CDCl3) δ: 8.10 (1H, s), 8.0 (1H, d, *J*= 8.0 Hz), 7.90(1H, t, *J*=8.0 Hz), 7.78 (1H, dd, *J*=8.0, 1.9 Hz), 7.59-7.27 (5H, m); ^{13}C-NMR (100 MHz, CDCl3) δ: 159.94, 145.31, 144.44, 137.15, 130.0, 129.6, 129.1, 126.84, 123.1, 122.0; I.R (KBr) cm^{-1}: 3055, 1676.17,1596, 1486.88, 1400, 1329, 1268, 1192.48, 748.34; MS *m/z:* 241.3 [M+H]$^{+.}$

Compound 4o: 6-CHLORO -3-PHENYL- 3 H-QUINAZOLIN-4-ONES
Melting range: 177.8-179.9 ^{0}C; ^{1}H-NMR (400 MHz, CDCl3) δ: 8.33 (1H, s), 8.19 (1H, d, *J*= 8.0 Hz), 7.85 (1H, t, *J*=8.0 Hz), 7.72 (1H, dd, *J*=8.0, 1.9 Hz), 7.59-7.49 (5H, m); ^{13}C-NMR (100 MHz, CDCl3) δ: 159.65, 146.27, 146.19, 137.0, 134.92, 129.65, 129.16, 126.82, 126.44, 123.36; I.R (KBr) cm^{-1}: 3042.56, 1679.0, 1594.79, 1473.71, 1393.73, 1268.0, 1182.71, 750.4; MS *m/z:* 257.3 [M+H]$^{+.}$

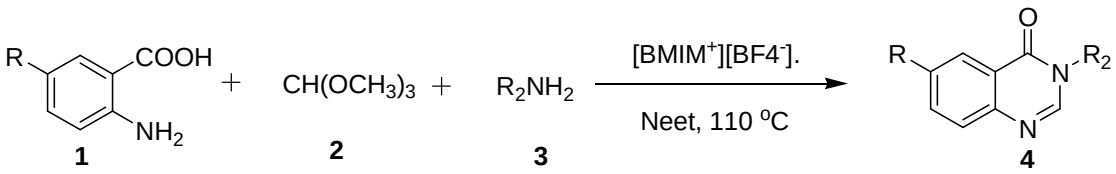
Compound 4p: 6-BROMO -3-PHENYL- 3 H-QUINAZOLIN-4-ONES
Melting range: 178.6-180.2 ^{0}C;^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.39 (1H, s), 8.25 (1H, d, *J*= 8.0 Hz), 8.0 (1H, t, *J*=8.0 Hz), 7.69 (1H, dd, *J*=8.0, 1.9 Hz), 7.59-7.53 (5H, m); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 159.30, 148.16, 147.10, 137.7, 129.63, 129.29, 128.83, 127.79, 123.94, 120.22; I.R (KBr) cm^{-1}: 3065.6, 1674.75, 1592, 1466, 1399, 1268.32, 1175.6, 749.19; MS *m/z:* 301.0 [M] $^{+.}$

RESULTS AND DISCUSSION

The growing awareness of numerous issues related to sustainable environment with respect to environment pollution reduction led the search for more friendly forms of heterogeneous catalyst to use for more and more of reactions. The heterogeneous catalysts have gained greater importance in recent years because of their ease of handling, simple work-up procedures, enhanced reaction rates, recovery and reuse of the catalysts. It also gained significant preference due to economical and environmental considerations. In this regard, Ionic liquids are emerging as a green reaction media (catalyst+ solvent). The use of ionic liquids as reaction medium may offer a convenient solution to both the solvent emission and catalytic recycling problem [31, 32, 33, 34].Room temperature ionic liquids (RTILs) have attracted great research interest due to the potential commercial and environmental advantages they offer over existing liquids. RTIL's are comprised of ions that allow them to potentially behave in a very different manner to conventional molecular liquids when they are used as solvents [31, 35-37]. To keep economic and environmetal important in mind, we have used highly 1-Butyl-3-methylimidazolium tetrafluoroborate [BMIM$^+$][BF4$^-$] as a green, efficient reusable catalyst for the one pot synthesis of 4(3H)-quinazolines **4** derivatives from the reaction of

anthranalic acid derivatives **1**, with orthoformates (aryl or alkyl) **2**, and amines derivatives **3** (aryl or alkyl) at room temperature under solvent free conditions (Scheme I & Table-1). The catalyst can easily be prepared from 1-Butyl 3-methylimidazolium chloride following the literature procedure [38].

Further the supported catalyst an easily to handle and they are considerable less toxic, non-corrosive free powder. The ease of filtrating and recovering the catalyst from the reaction system was significant advantages.



Scheme-I

Table-1: Preparation of Quinazolinone derivatives catalyzed by [BMIM$^+$][BF4$^-$].

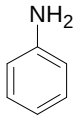
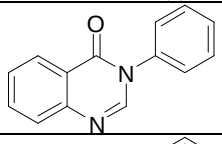
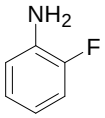
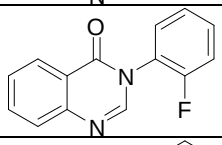
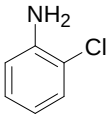
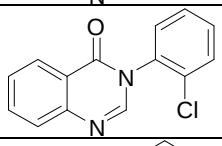
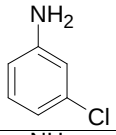
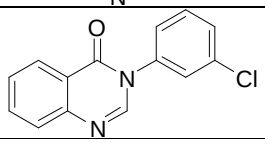
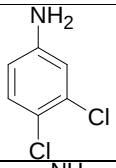
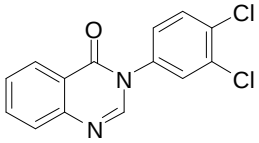
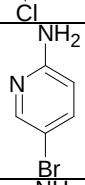
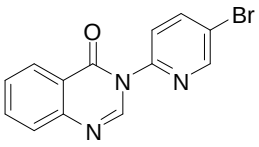
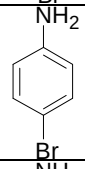
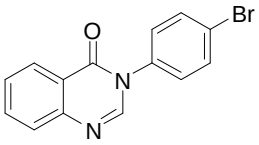
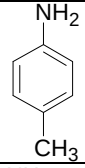
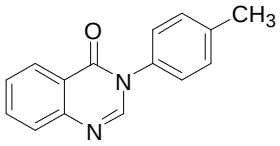
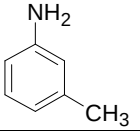
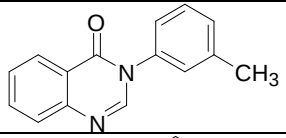
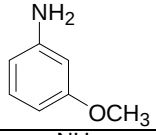
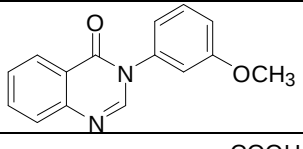
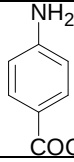
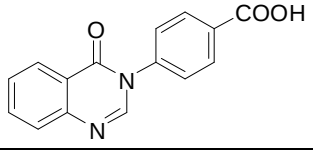
Entry	R^1	Quinazolinone **(4)**, R^2
a	H	Phenyl
b	H	2-Fluoro phenyl
c	H	2-Chloro phenyl
d	H	3-Chloro phenyl
e	H	3,4-Dichloro phenyl
f	H	3-Bromo pyridine
g	H	4-Bromo phenyl
h	H	4-Methyl phenyl
i	H	3-Methylphenyl
j	H	3-Methoxy phenyl
k	H	4-Carboxyphenyl
l	H	4-Benzoic acid methyl ester
m	H	4-Butyl
n	F	Phenyl
o	Cl	Phenyl
p	Br	Phenyl

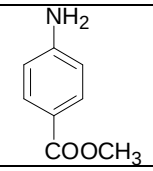
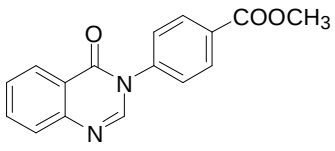
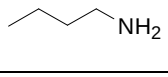
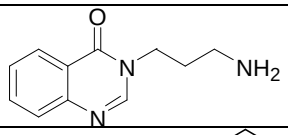
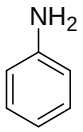
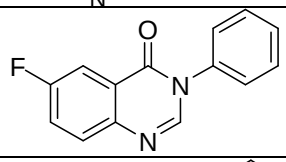
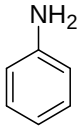
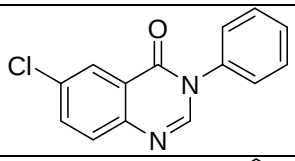
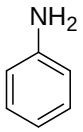
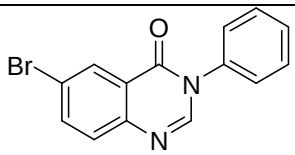
We prepared various 4(3H)-quinazolinones **4** using anthranalic acid derivatives (**1)** with different substituted aryl and alkyl amines (**3**). The reaction procedure is simple and proceeded at room temperature within few minutes (6-25 min) in excellent yields after addition of the catalyst. The anilines containing both electrons withdrawing as well as electron donating groups proceeds the smoothly. The reaction with amine having electron withdrawing groups (entry k, l) required reflux conditions and prolonged reaction times (20, 25 min). The rate of the reaction was tested with aliphatic amines and gave good yields of products in same reaction conditions (entry m). The reusability of the recovered catalyst was tested with the reaction of 1, 2 and 4-methyl aniline (entry h)**.** The first time when the fresh catalyst was used the yield of the product 3-p-tolylquinazolin-4(3H)-one was 97%, while with the recovered catalyst in three subsequent recycles the yields were mentioned in the Table-2.

Biological Activity

All the compounds prepared herein were screened for their potential biological activities such as, anti-bacterial, anti-asthmatic and anti-diabetic activities. The anti-bacterial activity was carried out using both Staphylococcus aureus (Gram positive) and Salmonella typhimurium (Gram negative) bacteria. The compounds were added to the medium as dimethyl sulfoxide solutions. No inhibition zone was observed in controls (i.e., for DMSO). The concentrations used were as follows: 500, 200, 100, 10, 1.0 and 0.1 mg/ml. Minimum Inhibitory Concentration values were determined after incubation at 37 _C for 48 h and was determined using tube dilution method according to the standard procedure [39]. Cephalexin was used as the anti-bacterial standard and dimethyl sulfoxide was used both as a solvent and as a control (see **Tables 3 and 4**). The anti-asthmatic activity studies were carried out using Phosphodiesterase IV enzyme (PDE-IV) [40] and the primary screening of the compounds was done at 1 nM concentration using human PDE-IV enzyme, where Rolipram and Ariflo were used as standard compounds. (**Table 5**).

Table-2: Preparation of Quinazolinone derivatives catalyzed by $HBF_4.SiO_2$[a]

Entry	Amine **(3)**	Quinazolinone **(4)**	Time (min)	Yield (%)
a			6	94
b			10	91
c			15	89
d			15	93
e			10	91
f			20	92
g			10	96
h			15	97
i			20	94
j			15	90
k			20	1[b]

l			25	79[b]
m			10	92
n			20	81[b]
o			25	79[b]
p			25	79[b]

[a]*The structures of the products were established from their spectral (IR, [1]H, [13]C NMR and MS) data.*
[b]*The reactions were conducted at reflux conditions.*

The anti-diabetic activity screening was carried out with dipeptidylpeptidase (DPP-IV) [41] enzyme and the primary screening of the compounds was carried out at 300 nM concentration using recombination human DPP-IV enzyme by the use of 1-(2-amino-3,3-dimethylbutanoyl) pyrrolidine-2-carbonitrile as the standard compound at 100 nM. (see **Table 4**). Similarly, the PTP-1B (in-house compound, also for anti-diabetic) activity was done using the test compounds at 30 mM with the standard compound N-[5-[N-acetyl-4-[N-(2-carboxyphenyl)-N-(2-hydroxyoxalyl) amino]-3-ethyl-DL-phenylalanylamino] pentanoyl]-L-methionine at a concentration of 0.3 mM (**Table 4**).

Protocol for PDE-IV inhibition assay

Phosphodiesterase IV enzyme converts [3H] cAMP to the corresponding [3H] 50-AMP in proportion to the amount of Phosphodiesterase IV present. The [3H] 50-AMP then was quantitatively converted to free [3H] adenosine and phosphate by the action of snake venom 50-nucleotidase hence the amount of [3H] adenosine liberated is proportional to Phosphodiesterase IV activity.

The assay was performed at 34 _C in a 200 ml total reactionmixture. The reaction mixture contained 25 mM of tris buffer, 10 mM MgCl2, 1 mM cAMP (cold) and [3H] cAMP (0.1 mCi). Stock solutions of the compounds to be investigated wereprepared in dimethyl sulfoxide in concentrations such that the dimethyl sulfoxide content in the test samples did not exceed 0.05% by volume to avoid affecting the Phosphodiesterase IV activity. Compounds were then added in the reaction mixture (25 ml/tube). The assay was initiated by addition of enzyme mix (75 ml) and the mixture was incubated for 20 min at 34 _C. The reaction was stopped by boiling the tubes for 2 min at 100 _C in a water bath. After cooling on ice for 5 min and addition of 50 mg 50-nucleotidase snake venom from Crotalus atrox, incubation was carried out again for 20 min at 34 _C. The unreacted substrate was separated from (3H) adenosine by addition of Dowex AG 1X-8 (400 ml), which was pre-equilibrated in (1:1) water:ethanol. Reaction mixturewas then thoroughly mixed, placed on ice for 15 min, vortexedand centrifuged at 14,000 rpm. for 2 min. After centrifugation, a sample of the supernatant (150 ml) was taken and added in 24-well optiplates containing scintillant (1 ml) and mixed well.The samples in the plates were then determined for radioactivity in a Top Counter and the Phosphodiesterase IV activitywas calculated. Phosphodiesterase IV enzyme was present in quantities that yield <30% total hydrolysis of substrate (linearassay conditions). Rolipram and Cilomilast were used as standards in all assays.

Protocol for the DPP-IV assay

DPP-IV inhibition measurement in vitro: DPP-IV activity was determined by the cleavage rate of 7-amino-4-methyl coumarin (AMC) from synthetic substrate Gly-Pro-AMC. In brief, the assay was conducted by adding 10 ng of human recombinant dipeptidyl peptidase IV enzyme (DPP-IV, available commercially from R&D Systems) in 50 ml of the assay buffer(25 mM tris, pH 7.4, 140 mM NaCl, 10 mM KCl, 1% BSA) to 96-well black flat bottom microtiter plates. The reaction was initiated by adding 50 ml of 100 mM substrate Gly-Pro-AMC. The incubation was carried out in the kinetic mode at 30 _C for 30 min. Fluorescence was measured using Fluorostar at the excitation filter of 380 nm and the emission filter of 460 nm. Test compounds and solvent controls were added as 1 ml additions. Test compounds were dissolved in DMSO and tested at 300 nM concentration. Percentage inhibition was calculated with respect to the solvent control sample (no test compound added). Dipeptidyl peptidase (i.e. antidiabetic).

Protocol for PTB-1B assay

In-house generated human recombinant enzyme: w35 ng in assay.
Paranitrophenyle phosphate (SRL144916): 25 mM.
Buffer: Hepes 25 mM, 3 mM DTT, 0.15 M NaOH, 1 mM
EDTA, pH 7.4.
Dilution buffer (for enzyme): 2_ reaction buffer (3 mM DTT).
DMSO (Calbiochem).
Test compound in DMSO.
DMSO concentration not to exceed 1% in the assay.

Protocol

	Blank	**Control**	**Test**
DMSO	1 µl	1 µl	-
Compound	-	-	1 µl
Buffer	89 µl	88 µl	88 µl
Enzyme	-	1 µl	1 µl
PNPP	10 µl	10 µl	10 µl

Incubate and continuously monitor at 30^0C for 30 minutes at 405 nm

NaOH	100 µl	100 µl	100 µl

Read at 405 nm.

Evaluation of the study observation:

Calculations: Activity = % of control; % Inhibition = 100- activity

Table-3: Antibacterial activity of compounds against Staphylococcus *aureus*.

Compound No.	**Concentration**						
	0.1µg/ ml	**1µg/ml**	**10µg/ml**	**100µg/ml**	**200µg/ml**	**500µg/ml**	**APP.MIC**
4a	++	++	++	P	--	--	200 µg/ml
4b	++	++	+	P	--	--	200 µg/ml
4c	++	++	++	+	P	--	500 µg/ml
4d	++	++	++	++	+	--	500 µg/ml
4e	++	++	++	+	P	--	500 µg/ml
4f	++	++	+	+	P	--	500 µg/ml
4g	++	++	+	P	P	--	500 µg/ml
4h	++	++	+	+	P	--	500 µg/ml
4i	++	++	++	++	+	--	500 µg/ml
4j	++	++	++	++	+	--	500 µg/ml
4k	++	++	++	++	P	--	500 µg/ml
4l	++	++	+	+	--	--	200 µg/ml
4m	++	++	+	P	--	--	200 µg/ml
4n	++	++	++	+	--	--	200 µg/ml
4o	++	++	++	+	P	--	500 µg/ml
4p	++	+	+	P	--	--	200 µg/ml
Cephalexin	++	++	--	--	--	--	10 µg/ml

Table-4: Antibacterial activity of compounds against Salmonella *typhimurium*

Compound No.	Concentration						
	0.1µg/ ml	**1µg/ml**	**10µg/ml**	**100µg/ml**	**200µg/ml**	**500µg/ml**	**APP.MIC**
4a	++	++	++	+	--	--	200 µg/ml
4b	++	++	+	P	--	--	200 µg/ml
4c	++	++	++	P	--	--	200 µg/ml
4d	++	++	++	++	+	--	500 µg/ml
4e	++	++	+	+	P	--	500 µg/ml
4f	++	++	+	+	P	--	500 µg/ml
4g	++	++	++	+	P	--	500 µg/ml
4h	++	++	+	P	P	--	500 µg/ml
4i	++	++	++	++	P	--	500 µg/ml
4j	++	++	++	++	+	--	500 µg/ml
4k	++	++	++	+	P	--	500 µg/ml
4l	++	++	++	P	--	--	200 µg/ml
4m	++	++	+	P	--	--	200 µg/ml
4n	++	++	++	P	--	--	200 µg/ml
4o	++	++	++	+	P	--	500 µg/ml
4p	++	++	+	P	--	--	200 µg/ml
Cephalexin	++	++	+	P	--	--	200µg/ml

Total Inhibition, no growth of organism = --
Poor growth compared to control = *P*
Medium growth compared to controls = +
Confluent growth, no inhibition = ++

Table-5: Anti-diabetic & Anti- asthmatic activity screening results of compounds

Compound No	**PTP1B** (30µM) % Inhibition	**PDE-IV** (1µM) % Inhibition	**DPP-IV** (0.3µM) % Inhibition
4a	1.64	3.40	0
4b	2.42	0	0
4c	8.42	22.29	9
4d	2.2	13.52	0
4e	0	8.91	0
4f	0	19.45	3
4g	29	18.22	0
4h	27.67	16.50	0
4i	5.84	16.25	0
4j	17.43	17.21	0
4k	0	16.88	0
4l	0	22.56	0
4m	0	18.20	0
4n	0	15.84	0
4o	7.38	17.28	0
4p	0.15	19.85	5

Standard Compound Assay:

PTP 1B: N-[5-[Acetyl]-4-[N-(2-carboxypheyl)-N-(2-hydroxalyl)amino]-3-ethyl-DL-phenylalanylamino]pentanoyl]-L-methionine is used as a standard in all assays and shows percentage inhibition of 49.09 % at a concentration of 0.3 µM.

PDE IV: Rolipram and Cilomilast were used as a standard in all assays. Rolipram shows percentage inhibition 67.41% at a concentration of 2µM. Cilomilast shows percentage inhibition 45.28% at a concentration of 0.075 µM.

DPP IV*: 1-(2-amino-3, 3-dimethylbutyryl)pyrrolidine-2-carbonitrile is used as a standard in all assays and shows percentage inhibition of 96 % at a concentration of 0.1 µM.*

In conclusion we have developed a simple and efficient synthesis of 3-substituted-quinqzolin-4-ones by coupling of anthranalic acid, orthoformates and aryl or alkyl amines using 1-Butyl-3-methylimidazolium tetrafluoroborate as a green, efficient and reusable catalyst under solvent free conditions. The simple experimental procedure,

environmentally clean technology, comprising and ease of handling, fast reaction conditions, with excellent yields, the reusability of the catalyst, low cost and eco-friendly nature are notable advantages of the present protocol.

Acknowledgements
The author Radhika Gadiparthi is grateful to Head, Department of Chemistry, Osmania University for providing laboratory facilities. Authors Ashok Dongamanti and Radhika Gadiparthi are also thankful to Coordinator, OU-DST-PURSE for financial support.

REFERENCES

[1]Y. Kurogi; Y. Inoue; K. Tsutsumi.; S. Nakamura; K. Nagao; H.Yohsitsugu.; Y.J Tsuda *Med. Chem.*, **1996**, 39, 1433-1437.
[2] J. B Dienei; F.Dowalo; H. V.Hoeven; P.Bender; B. Love, *J. Med. Chem.*, **1973**, 16, 633-637.
[3] R. O.Dempcy,; E. B Skibo, *Bioorg. Med. Chem.Lett.* **1993**, **1**, 39-42.
[4] K.Tereshima,; H. Shimamura; A. Kawase; Y. Tanaka; T. Tanimura; T. Kamisaki; Y.Ishizuka,; M.Sato, *Chem. Pharm. Bull.* , **1995**, **43,** 2021-2023.
[5] N. J. Liverton; D. J. Armstrong; D. A. Claremon; D. C. Remy; J. J. Baldwin; R. J. Lynch; G.Zhang; R.Gould, *Bioorg. Med. Chem. Lett.*, **1998**, 8, 483-486.
[6] S. Kobayashi; M.Ueno; R.Suzuki; H Ishitani. *Tetrahedron Lett.* **1999,** 40, 2175-2178.
[7] S.L. Cao; Y.P. Feng; Y.Y. Jiang; S.Y. Liu; G.Y. Ding; R.T. Li, *Bioorg. Med. Chem. Lett.* **2005,** 15, 1915-1917.
[8] F.Ablondi; S. Gordon; J. Morton, II; J. H. Williams, *J. Org. Chem*, **1952,** 17, 14-18 .
[9] J. B.Koepfly; J. F.Mead; J. A.Brockman, J. *Am. Chem. Soc.* **1947**, 69, 1837.
[10] S. Kobayashi; M.Ueno; R.Suzuki; H Ishitani. *Tetrahedron Lett*. **1999**, 40, 2175-2178.
[11] C. S.Jang; F. Y.Fu; C. Y.Wang; K. C.Huang; G.Lu; T. C.Thou, *Science*, **1946,** 103, 59.
[12] F. M.Refaie; A. Y. Esmat; S. M. Abdel-Gawad; A. M. Ibrahim; M. A. Mohamed *Lipids Health Dis*. **2005**, 4, 22.
[13] M. T. Bogert; W. F. Hand, *J. Am. Chem. Soc.* **1902** , 24, 1031-1050.
[14] M. T.Bogert; W. F Hand; *J. Am. Chem. Soc.* **1903**, 25, 935.
[15]E. C.Taylor; R. J.Knopf; Borror, A. L; *J. Am. Chem. Soc.* **1960**, 82, 3152-3157.
[16]H. Kotsuki; H.Sakai; H.Morimoto; H Suenaga; *Synlett*, **1999**, 1993-1995.
[17]H.Takeuchi; S. Haguvara; S. Eguchi, *Tetrahedron*, **1989**, 45, 6375-6386.
[18]H.Takeuchi; S.Haguvara; S. Eguchi, J. *Org. Chem.* **1991**,, 56, 1535-1537,
[19]G.Rabillound; B. Sillion, J. *Heterocycl. Chem.* 17, **1980**, 1065-1068,
[20]M. Okabe; R.-C Sun. *Tetrahedron*, **1995**, 51, 1861-1866,
[21]B. L. Chenard; W. M. Welch; J. F.Blake; T. W. Butler; A. Reinhold; F. E. Ewing;
F. S.Menniti,; M. J. Pagnozzi, *J. Med. Chem.* **2001**, 44, 1710-1717.
[22]D. J.Connolly; P. J. Guiry, *Synlett*, **2001**, 1707-1710.
[23]L.Wang; J.Xia; F.Qin; C. Qian; J. Sun, *Synthesis*, **2003**, 1241-1247.
[24]S. Xue; J. McKenna; W.C. Shineh; O. Repic, *J. Org. Chem.* **2004**, *69*, 6474.
[25]R.J. Abdel-Jalil, ; W. Voelter; M. Saeed, *Tetrahedron Lett.* **2004**, 45, 3475-3476.
[26]B. Das; R. Banerjee, J. *Chem. Lett.*, **2004**, 33, 960-961.
[27]A.R. Khosropour; M.B. Iraj; H. Ghorbankhani, *Tetrahedron letters*, **2006**, 47, 3561-3564.
[28]M. Narasimhulu; K.Chinni Mahesh, T. Srikanth Reddy, K. Rajesh and Y. Venkateswarlu, Tetrahedran Letters, **2006**, 47, 4381-4383.
[29]Asit K.Chankraborti, J. Molecular Catalysis A. **2007**, 277, 266.
[30]S. Hamid Reza, M. Fahimeh Shahrekipoor, J.Molecular Catalysis A, **2007**, 272,142-151.
[31]T, Welton, Chem. Rev. **1999**, 99, 2071.
[32]M.J. Earle, P.B. McCormac, K.R. Seddon, Chem. Commun. **1998**, 2245.
[33]F. Liu, M.B. Abrams, R.T. Baker, W. Tumas, Chem. Commun. **2001**, 5433.
[34]E.D. Bates, R.D. Mayton, I. Ntai, J.H. Davis, J. Am. Chem. Soc. **2002**, 124, 926.
[36] P. Wasserscheid, W. Keim, Angew. Chem. Int. Ed. **2000**, 39, 3772.
[37] C. Gordan, Appl. Catal. A: Gen. **2001**, 222, 101.
[38]T. Joseph, S. Sahoo, S.B.Halligudi,Journal of Molecular Catalysis A: Chemical **2005,** 234, 107–110.
[39]Frankel, S.;Reitman, S.; Sonnenwirth, A. C.; Gradwol's Clinical Laboratory Methods and Diagonosis' , A textbook on a laboratory procedure and their interpretation C. V. Mosby Company ,Germany, 7th edition 2 (**1970**)1406.

[40] John, E. S.; David, A.; Carol, S. Immunopharmacology., 47 (**2000**),127-162.
[41] Senten, K.; Veken, P.V.V.; Meester, I. D.; Lambeir, A.M.; Scharpe.S.; Haemers.A.; Augustyns, K., J. Med.Chem., 46 (**2003**), 5005-5014.