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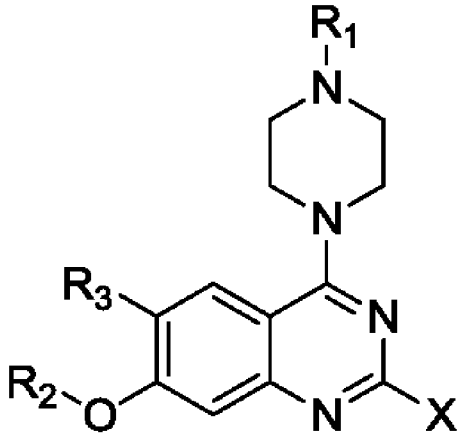
[Continued on next page]

(54) Title: BLOCKING TOLL-LIKE RECEPTOR 9 SIGNALING WITH SMALL MOLECULE ANTAGONIST

(57) Abstract: The present invention relates to small molecule4-(piperazin-1-yl)quinazolin-2-amino compounds with formula (I) useful for inhibiting signalling by certain toll-like receptors (TLRs), especially TLR9. Toll-like receptors (TLRs) are members of the larger family of evolutionarily conserved pattern recognition receptors which are critical first line of defence for self-nonself discrimination by the host immune response. Aberrant TLR9 activation is implicated in autoreactive inflammation in different autoimmune diseases. The invention depicts compounds with formula (I), composition and methods can be used in a number of clinical applications, including as pharmaceutical agents and methods for treating conditions involving unwanted immune activity due to TLR9 activation.



Formula (I)

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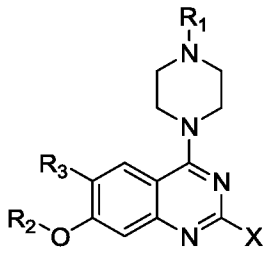
BLOCKING TOLL-LIKE RECEPTOR 9 SIGNALING WITH SMALL MOLECULE ANTAGONIST

FIELD OF INVENTION

The invention relates small molecule antagonist of compounds with formula (I) in free form or in acceptable salt form useful for altering immune function by blocking toll-like receptor 9 signalling.



Formula (I)

BACKGROUND OF THE INVENTION

The innate immunity is comprised of several types of cells including dendritic cells (DC's), macrophages and monocytes, polymorphonuclearcells, natural killer (NK) cells, innate lymphoid cells and natural killer T cells (NKT cells) which detects various pathogens as well asaberrant hostcells with potential for danger to tissue integrity through specialized receptors like toll-like receptors. Toll-like receptors (TLRs) are a family of germline-encoded cell surface pattern recognition molecules containing an pathogen binding ectodomain (ECD) with 19-25 leucine-rich repeats (LRRs), a transmembrane domain and a characteristic cytoplasmic domain called the TIR (Toll/IL -1 receptor) domain. TIR domain is responsible for downstream signalling, whereas LRRs containing 24–29 amino acids are responsible for ligand recognition and binding. TLRs get triggered in response to bacterial and fungal infections (Medzhitov, R;*Nat. Rev. Immunol.* 1, 135-145, 2001) followed by induction of downstream signalling, leading to expression of inflammatory genes like those of the nuclear factor-κB (NF-

κB) family of transcription factors and antimicrobial peptides. There are 11 human and 12 miceTLRs have been identified which recognize different molecular patterns on the pathogens.

Major group of the TLRs are expressed on the cell surface. The leucine-rich repeats in the ectodomains of these molecules bind to unique molecular entities on pathogens (PAMPs), which detect and initiate responses to invading microorganisms (Akira, S; et al. *Annu Rev Immunol.* 21, 335-76, 2003). Another group of TLRs (endosomal TLRs) are located inside the cell within the endosomal-lysosomal compartments, instead of being expressed on the cell surface (Akira, S; et al. *Annu Rev Immunol.* 21, 335-76, 2003). This group comprises of TLR3 (Alexopoulou, L; et al. *Nature*, 413(6857), 732-8, 2001), TLR7 (Hemmi, H; et al. *Nature*, 408(6813), 740-5, 2001; Lund, J. M; et al. *ProcNatlAcadSci U S A.* 101(15), 5598-603, 2004), TLR8 (Heil, F; et al. *Science*, 303(5663), 1526-9, 2004) and TLR9 (Hemmi, H; et al. *Nature*, 408(6813), 740-5, 2001). The endosomal TLRs are specialized for detecting microbial nucleic acids after microbes get phagocytosed and reach the endosomal compartments.

The downstream signalling goes through recruitment of intracellular adaptor molecules such as Myd88 (or the myeloid differentiation primary-response gene 88), TIRAP (or the TIR-domain containing adaptor protein), TRIF (or the TIRAP inducing IFN-beta) and TRAM (or the TRIF-related adaptor molecule). TLR-adaptor molecule interactions in turn recruit other proteins to the signalling complex, which initiates multiple downstream signalling pathways, leading to activation of NFkB or mitogen-activated protein kinases (MAPKs) or recruitment of the IFN regulatory factors (IRFs). These different pathways in turn result in the transcription of genes encoding different cytokines, chemokines, co-stimulatory molecules or other proteins, thereby sculpting the ensuing immune response (Akira, S; et al. *Annu Rev Immunol.* 21, 335-76, 2003).

The intracellular localization of the nucleic acid-recognizing TLRs (TLR3, 7, 8, 9) is one of the mechanisms that prevent their spontaneous activation by circulating host-derived nucleic acids (Barton, G. M; et al. *Nat Immunol.* 7(1):49-56, 2006), however under certain pathological conditions the endogenous nucleic acids can overcome this

regulation. It has been previously shown by us and others that the circulating immune complexes found in sera of patients suffering from systemic lupus erythematosus (SLE) typically contain nucleic acids associated with various proteins such as antibodies, the chromatin-associated protein HMGB1, the antimicrobial peptide LL37, ribonuclear proteins and others (Lande, R; et al. *Nature*, 449(7162), 564-9, 2011; Ganguly, D. et al. *Nat Rev Immunol.* 13(8), 566-77, 2013). Our previous studies have also shown that TLR9, 7 and 8 activation driven by self nucleic acid and LL37 complexes may also play an important pathogenic role in Psoriasis (Lande, R; et al. *Nature*, 449(7162), 564-9, 2007; Ganguly, D. et al. *J Exp Med.* 206(9), 1983-94, 2009). These associated proteins may protect thebound nucleic acid from degradation and/or facilitate their entry into the cell, as is the case for Fc receptor-mediated uptake of antibody-nucleic acid complexes (Leadbetter, F. A; et al. *Nature*, 416(6881), 603-7, 2002;Ganguly, D. et al. *J Exp Med.* 206(9), 1983-94, 2009). Once inside the endolysosomal compartments, the nucleic acid cargo can then stimulate the intracellular TLRs, priming the immune system for a cascade of inflammation inciting cytotoxic and/or humoral response. For example, this cycle of innate immune recognition, generation of autoreactive antibodies, and consequent immune complex formation is believed to play critical role in the pathogenesis of SLE and possibly Sjogren's syndrome (Marshak-Rothstein, A; *Nat Rev Immunol.* 6(11), 823-35, 2006; Lande, R; et al. *Nature*, 449(7162), 564-9, 2011; Ganguly, D. et al. *Nat Rev Immunol.* 13(8), 566-77, 2013), a finding confirmed in animal models treated with TLR7 and TLR9-competitive antagonist oligonucleotides (Barrat, F. J; et al. *Eur J Immunol.* 37(12), 3582-6, 2007; Christensen, S. R; et al. *J Exp Med.* 202(2), 321-31, 2005). TLR-mediated pathological responses to nucleic acids have also been shown to contribute to other pathologies like psoriasis (Lande R et al, Nature, 2007; Ganguly D et al, J Exp Med, 2009), ischemic liver injury (Bamboat, Z. M; et al. *Hepatology*, 51(2), 621-32, 2010) lung infection (Itagaki, K; et al. *Shock*,

36(6), 548-52, 2011), pancreatitis (Hoque, R; et al. *Gastroenterology*, 141(1), 358-69, 2011) and graft-versus-host disease (Calcaterra, C; et al. *J Immunol*. 181(9), 6132-9, 2008).

Hydroxychloroquine and chloroquine are not only used as anti-malarial agents but has been commonly prescribed to treat various clinical contexts of autoreactive inflammation (autoimmune diseases) such as rheumatoid arthritis (RA) and SLE (Wallace, D. J;*Lupus*, 5 Suppl 1, S59-64, 1996). In literature there are several reports of small molecule analogues and derivatives of chloroquine with substituted quinoline and quinazoline scaffold which can inhibit stimulation of the immune system.US. Pat. No.6,221,882; US. Pat. No. 6,479,504; US Pat. No.7,410,975 B2; WO 2008/030455, published Mar 13, 2008; US. Pat. No. US 7,410,975 B2; PCT published application PCT/US03/17733 (WO 03/103586 A2); and PCT published application PCT/US2009/058401 (WO2010/036908).

OBJECTIVES OF THE INVENTION

The main object of the present invention is to provide compounds of general formula I.

Another object of the present invention is to provide a screening method involving human peripheral blood mononuclear cells to screen compounds of general formula I against TLR9.

Yet anotherobjectiveof the present invention is to provide a method for testing TLR9 antagonism of compounds of general formula I, in primary human plasmacytoid dendritic cells (pDCs) purified from human peripheral blood mononuclear cells.

Yet another objective of the present invention is to provide a method for testing TLR9 antagonism of compounds of general formula I a reporter assay method involving a cell line expressing TLR9 to screen compounds of general formula I for TLR9 antagonism.

Yet another objective of the present invention is to correlate the assays results involving human peripheral blood mononuclear cells, human primary pDCs and transfected TLR9 cells.

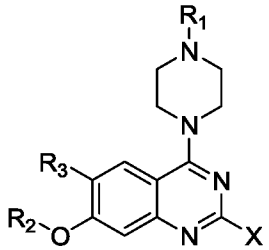
Yet another object of the present invention is to provide composition and methods of compounds of general formula I with TLR9 antagonistic activity that can modulate immune responses.

Yet another object of the present invention is to provide composition and methods of compounds of general formula I that can be used in a number of clinical applications, including as pharmaceutical agents and methods for treating conditions involving untoward immune hyperactivity.

Yet another object of the present invention is to provide composition and methods of compounds of general formula I without considerable cytotoxicity in HepG2 (a hepatic epithelial cell line) and SW480 (an intestinal mucosal epithelial cell line) cells at concentrations below 100 µM.

SUMMARY OF THE INVENTION

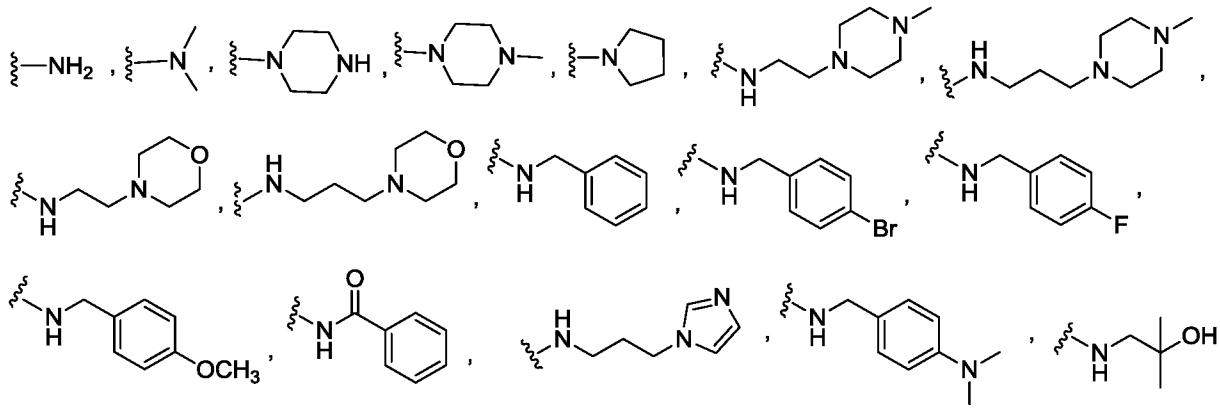
In an embodiment of the present invention, compounds of general formula I is provided.



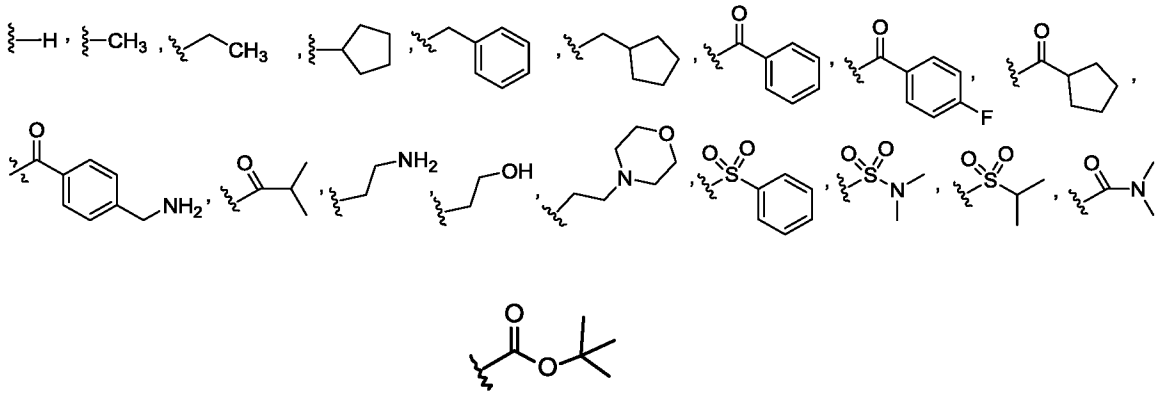
Formula (I)

wherein

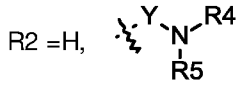
X is independently selected from groups referred to as follows:



wherein R_1 is independently selected from groups referred to as follows:

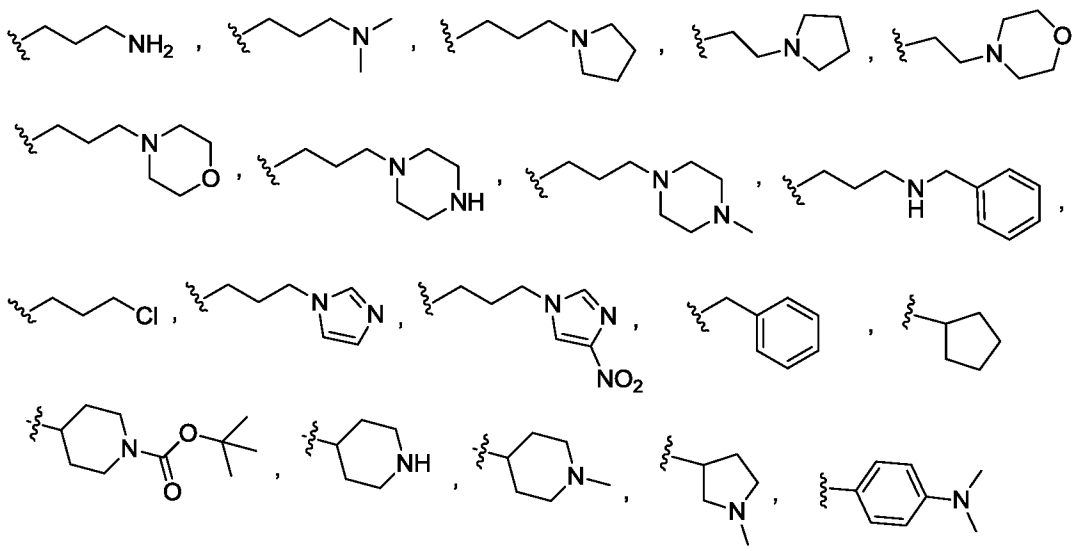


whereinR_2 is a group having structure



Where Y is optionally substituted or unsubstituted C_0 to C_3alkyl; R_5 and R_6 are independently hydrogen or substitutedor unsubstitutedalkyl or R_5 and R_6 is joined to form substituted or unsubstitutedheterocycle.

wherein R_2 is independently selected from groups referred to as follows:



wherein R_3 is independently selected from groups referred to as hydrogen, -OH and $-OCH_3$ groups.

In another embodiment the compounds of general formula 1 is represented by compounds encompassing:

4-(4-(Isopropylsulfonyl)piperazin-1-yl)-6-methoxy-N,N-dimethyl-7-(3-(pyrrolidin-1-yl)propoxy)quinazolin-2-amine**13a(TBP-2-93);**

4-(4-(Dimethylamino)-6-methoxy-7-(3-(pyrrolidin-1yl)propoxy)quinazolin-4-yl)-N,N-dimethylpiperazin-1-sulfonamide**13b(TBP-2-121);**

Cyclopentyl(4-(2-(dimethylamino)-6-methoxy-7-(3-(pyrrolidin-1yl)propoxy)quinazolin-4-yl)piperazine-1yl)methanone**13c(TBP-2-117);**

4-(4-(Cyclopentylmethyl)piperazin-1-yl)-6-methoxy-N,N-dimethyl-7-(3-(pyrrolidin-1yl)propoxy)quinazolin-2-amine **13d(TBP-3-79);**

4-(4-(Cyclopentylpiperazin-1-yl)-6-methoxy-N,N-dimethyl-7-(3-(pyrrolidin-1yl)propoxy)quinazolin-2-amine **13e(TBP-3-73);**

(4-(2-(Dimethylamino)-6-methoxy-7-(3-(pyrrolidin-1-yl)propoxy)quinazoline-4-yl)piperazin-1-yl)(phenyl)methanone **13f(TBP-3-75);**

4-(4-Benzylpiperazin-1-yl)-6-methoxy-N,N-dimethyl-7-(3-(pyrrolidin-1-yl)propoxy)quinazolin-2-amine **13g(TBP-3-67);**

(4-(Aminomethyl)phenyl)(4-(2-(dimethylamino)-6-methoxy-7-(3-(pyrrolidin-1-yl)propoxy)quinazolin-4-yl)piperazin-1-yl)methanone **13h (TBP-3-113);**

6-((4-(4-(2-(Dimethylamino)-6-methoxy-7-(3-(pyrrolidin-1-yl)propoxy)quinazolin-4-yl)piperazine-1-carbonyl)benzyl)amino)-6-oxohexanoic acid **13i (TBP-3-115);**

6-((4-(4-(2-(Dimethylamino)-6-methoxy-7-(3-(pyrrolidin-1-yl)propoxy)quinazolin-4-yl)piperazine-1-carbonyl)benzyl)amino)-6-oxohexanoic acid **13i (TBP-3-115);**

2-(4-(2-(dimethylamino)-6-methoxy-7-(3-(pyrrolidin-1-yl)propoxy)quinazolin-4-yl)piperazin-1-yl)-1-(4-fluorophenyl)ethanone**13j (TBP-4-81);**

t-Butyl-4-(6-methoxy-7-(3-morpholinpropoxy)-2-(pyrrolidin-1-yl)quinazoline-4-yl)piperazine-1-carboxylate **17a(TBP-2-173);**

t-Butyl-4-(6-methoxy-7-(3-(4-methylpiperazin-1-yl)propoxy)-2-(pyrrolidin-1-yl)quinazoline-4-yl)piperazine-1-carboxylate **17b(TBP-2-189);**

t-Butyl-4-(7-(3-(1H-imidazol-1-yl)propoxy)-6-methoxy-2-(pyrrolidin-1-yl)quinazolin-4-yl)piperazine-1-carboxylate **17c(TBP-2-191);**

t-Butyl-4-(7-(3-(1H-imidazol-1-yl)propoxy)-6-methoxy-2-(pyrrolidin-1-yl)quinazoline-4-yl)piperazine-1-carboxylate **17d(TBP-3-47);**

t-Butyl-4-(6-methoxy-2-(pyrrolidin-1-yl)-7-(3-(pyrrolidin-1-yl)propoxy)quinazolin-4-yl)piperazine-1-carboxylate **17e(TBP-2-145);**

4-(3-(6-Methoxy-4-(piperazin-1-yl)-2-(pyrrolidin-1-yl)quinazolin-7-yloxy)propyl)morpholine **18a(TBP-2-179);**

7-(3-(1H-Imidazol-1-yl)propoxy)-6-methoxy-4-(piperazin-1-yl)-2-(pyrrolidin-1-yl)quinazoline **18b(TBP-3-69);**

3-((6-Methoxy-4-(piperazin-1-yl)-2-(pyrrolidin-1-yl)quinazolin-7-yl)oxy)-N,N-dimethylpropan-1-amine **18c(TBP-3-49);**

4-(3-(6-Methoxy-4-(4-(phenylsulfonyl)piperazin-1-yl)-2-(pyrrolidin-1-yl)quinazolin-7-yloxy)propyl)morpholine **19(TBP-2-185);**

4-(4-Benzylpiperazin-1-yl)-6-methoxy-7-(3-(4-methylpiperazin-1-yl)propoxy)-2-(pyrrolidin-1-yl)quinazoline **20(TBP-3-57);**

3-(4-(4-Benzylpiperazin-1-yl)-6-methoxy-2-(pyrrolidin-1-yl)quinazolin-7-yloxy)-N,N-dimethylpropan-1-amine **21a(TBP-3-59);**

(4-(7-(3-(Dimethylamino)propoxy)-6-methoxy-2-(pyrrolidin-1-yl)quinazolin-4-yl)piperazin-1-yl)(phenyl)methanone **21b(TBP-3-71);**

4-(4-Cyclopentylpiperazin-1-yl)-6-methoxy-N-(4-methoxybenzyl)-7-(3-(pyrrolidin-1-yl)propoxy)quinazolin-2-amine **25a(TBP-3-91);**

4-(4-Cyclopentylpiperazin-1-yl)-N-(4-fluorobenzyl)-6-methoxy-7-(3-(pyrrolidin-1-yl)propoxy)quinazolin-2-amine **25b(TBP-3-93);**

4-(4-Cyclopentylpiperazin-1-yl)-6-methoxy-N-(3-(4-methylpiperazin-1-yl)propyl)-7-(3-(pyrrolidin-1-yl)propoxy)quinazolin-2-amine **25c(TBP-3-95);**

N-(3-(1H-Imidazol-1-yl)propyl)-4-(4-cyclopentylpiperazin-1-yl)-6-methoxy-7-(3-(pyrrolidin-1-yl)propoxy)quinazolin-2-amine **25d(TBP-3-97);**

4-(4-Cyclopentylpiperazin-1-yl)-6-methoxy-2-(4-methylpiperazin-1-yl)-7-(3-(pyrrolidin-1-yl)propoxy)quinazoline **25e(TBP-3-99);**

7-(Cyclopentyloxy)-6-methoxy-N,N-dimethyl-4-(piperazin-1-yl)quinazolin-2-amine **27 (TBP-3-149);**

7-(Cyclopentyloxy)-6-methoxy-N,N-dimethyl-4-(4-methylpiperazin-1-yl)quinazolin-2-amine **28a (TBP-4-11);**

1-(4-(7-(Cyclopentyloxy)-2-(dimethylamino)-6-methoxyquinazolin-4-yl)piperazin-1-yl)-2-methylpropan-1-one **28b (TBP-3-155);**

4-(7-(Cyclopentyloxy)-2-(dimethylamino)-6-methoxyquinazolin-4-yl)-N,N-dimethylpiperazine-1-carboxamide **28c (TBP-3-157);**

6-methoxy-N,N-dimethyl-4-(4-methylpiperazin-1-yl)-7-(piperidin-4-yloxy)quinazolin-2-amine**28d (TBP-4-67);**

6-methoxy-N,N-dimethyl-4-(4-methylpiperazin-1-yl)-7-((1-methylpiperidin-4-yl)oxy)quinazolin-2-amine**28e (TBP-4-69);**

6-methoxy-N,N-dimethyl-4-(4-methylpiperazin-1-yl)-7-((1-methylpyrrolidin-3-yl)oxy)quinazolin-2-amine **28f (TBP-4-71);**

7-(4-(dimethylamino)phenoxy)-6-methoxy-N,N-dimethyl-4-(4-methylpiperazin-1-yl)quinazolin-2-amine **28g (TBP-4-73);**

4-(4-cyclopentylpiperazin-1-yl)-6-methoxy-N,N-dimethyl-7-(1-methylpiperidin-4-yloxy)quinazolin-2-amine **28h (TBP-4-77);**

4-(4-cyclopentylpiperazin-1-yl)-6-methoxy-N,N-dimethyl-7-(1-methylpyrrolidin-3-yloxy)quinazolin-2-amine **28i (TBP-4-79);**

7-(Benzyloxy)-6-methoxy-N,N-dimethyl-4-(piperazin-1-yl)quinazolin-2-amine **29a (TBP-2-159);**

(4-(7-(Benzyloxy)-2-(dimethylamino)-6-methoxyquinazolin-4-yl)piperazin-1-yl)(phenyl)methanone **30a (TBP-3-135);**

4-(7-(Benzyloxy)-2-(dimethylamino)-6-methoxyquinazolin-4-yl)-N,N-dimethylpiperazine-1-sulfonamide **30b (TBP-3-137);**

7-(Benzyloxy)-6-methoxy-4-(piperazin-1-yl)-2-(pyrrolidin-1-yl)quinazoline **30c (TBP-2-149);**

(4-(7-(Benzyloxy)-6-methoxy-2-(pyrrolidin-1-yl)quinazolin-4-yl)piperazin-1-yl)(phenyl)methanone**31 (TBP-2-151);**

t-Butyl-4-(7-(benzyloxy)-6-methoxy-2-((4-methoxybenzyl)amino)quinazolin-4-yl)piperazine-1-carboxylate **32a (TBP-3-121);**

t-Butyl-4-(7-(benzyloxy)-2-((2-hydroxy-2-methylpropyl)amino)-6-methoxyquinazolin-4-yl)piperazine-1-carboxylate **32b (TBP-3-145);**

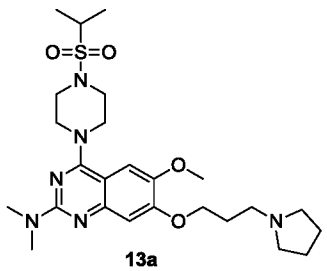
7-(Benzyloxy)-6-methoxy-2-(4-methoxyphenethyl)-4-(piperazin-1-yl)quinazoline**33(TBP-3-123);**

7-(Benzyloxy)-6-methoxy-2-(4-methoxyphenethyl)-4-(4-methylpiperazin-1-yl)quinazoline **34a (TBP-3-127);**

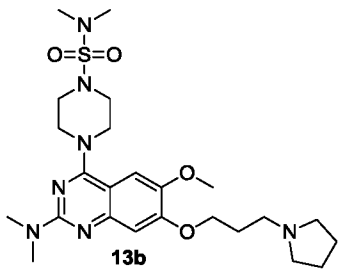
(4-(7-(Benzyloxy)-6-methoxy-2-(4-methoxyphenethyl)quinazolin-4-yl)piperazin-1-yl)(phenyl)methanone **34b (TBP-3-139),**

tert-butyl 4-(2-(dimethylamino)-7-hydroxy-6-methoxyquinazolin-4-yl)piperazine-carboxylate 9 (TBP-2-71),
4-(4-cyclopentylpiperazin-1-yl)-2-(dimethylamino)-6-methoxyquinazolin-7-ol (TBP-4-75),
tert-butyl 4-(7-hydroxy-6-methoxy-2-(pyrrolidin-1-yl)quinazolin-4-yl)piperazine-1-carboxylate 15 (TBP-2-135)
(4-(7-hydroxy-6-methoxy-2-(pyrrolidin-1-yl)quinazolin-4-yl)piperazin-1-yl)(phenyl)methanone (TBP-2-151),
2-(dimethylamino)-6-methoxy-4-(4-methylpiperazin-1-yl)quinazolin-7-ol (TBP-4-9),
2-(dimethylamino)-6-methoxy-4-(piperazin-1-yl)quinazolin-7-ol (TBP-2-169),
tert-butyl 4-(2-(dimethylamino)-6-methoxy-7-(3-morpholinopropoxy)quinazolin-4-yl)piperazine-1-carboxylate (TBP-2-165),
tert-butyl 4-(2-(dimethylamino)-6-methoxy-7-(3-(pyrrolidin-1-yl)propoxy)quinazolin-4-yl)piperazine-1-carboxylate 11 (TBP-2-79),

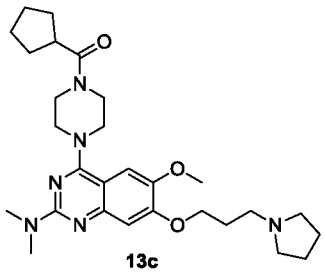
In another embodiment the tructural formulae of general formula 1 is consisting the representative compounds :



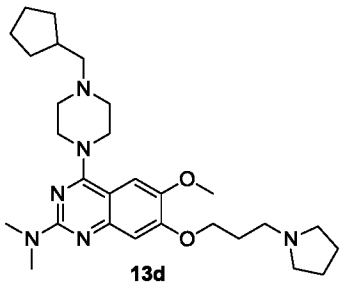
13a
(TBP-2-93)



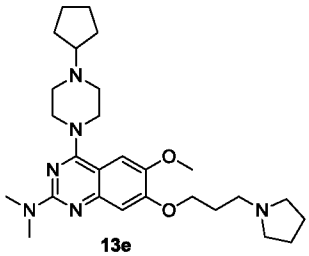
13b
(TBP-2-121)



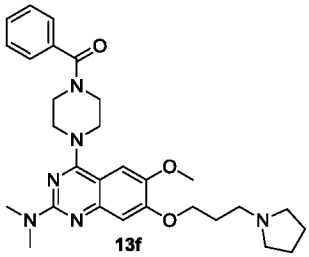
13c
(TBP-2-117)



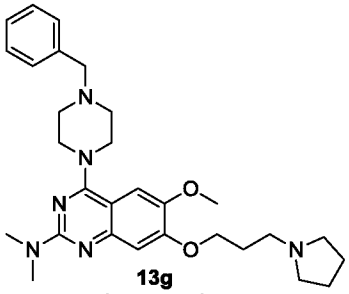
13d
(TBP-3-79)



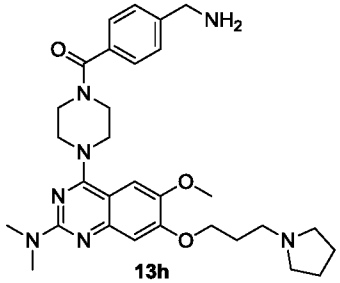
13e
(TBP-3-73)



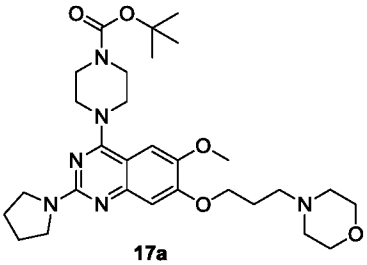
13f
(TBP-3-75)



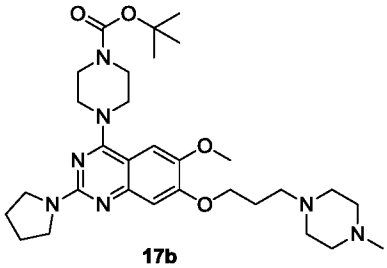
13g
(TBP-3-67)



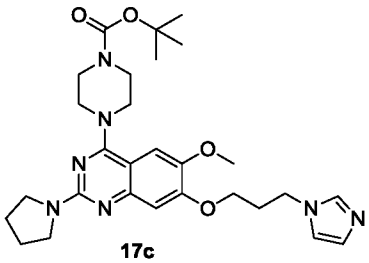
13h
TBP-3-113



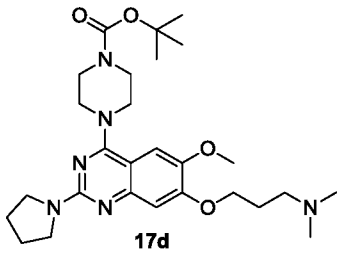
17a
(TBP-2-173)



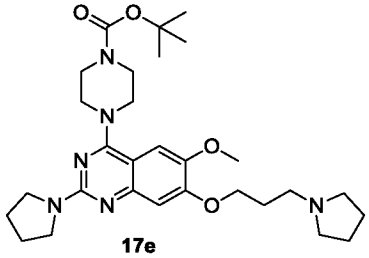
17b
(TBP-2-189)



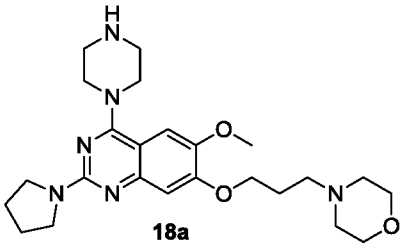
17c
(TBP-2-191)



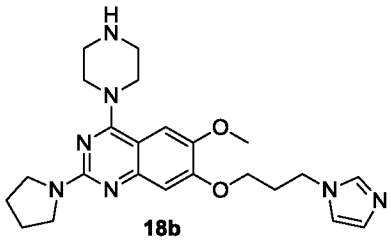
17d
(TBP-3-47)



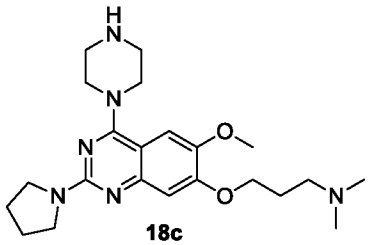
17e
(TBP-2-145)



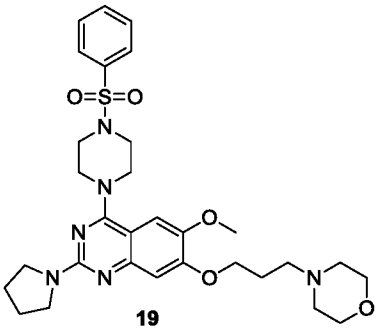
18a
(TBP-2-179)



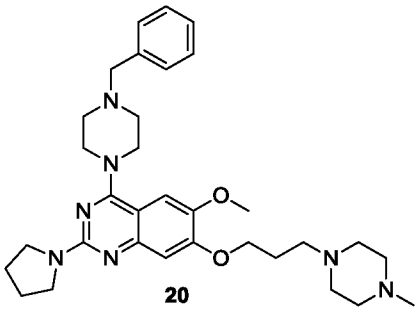
18b
(TBP-3-69)



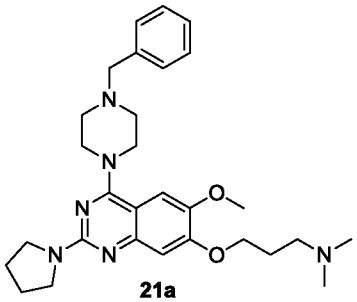
18c
(TBP-3-49)



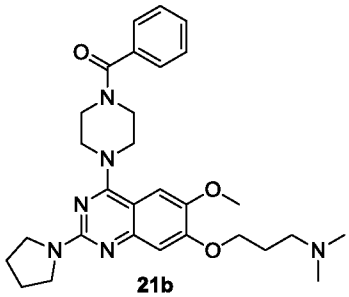
19
(TBP-2-185)



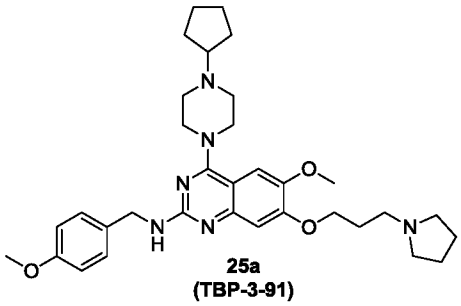
20
(TBP-3-57)



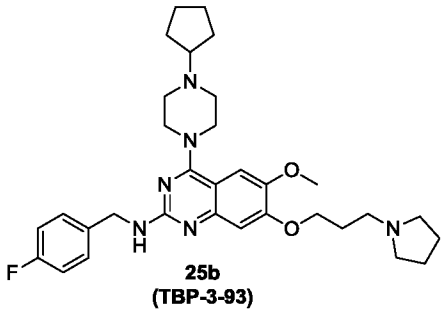
21a
(TBP-3-59)



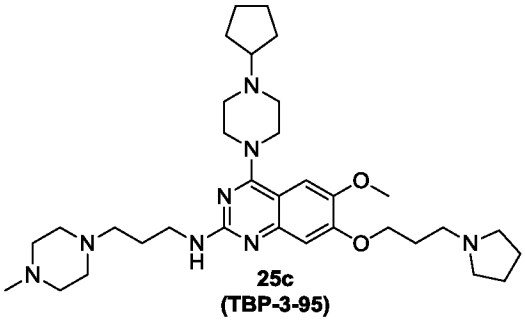
21b
(TBP-3-71)



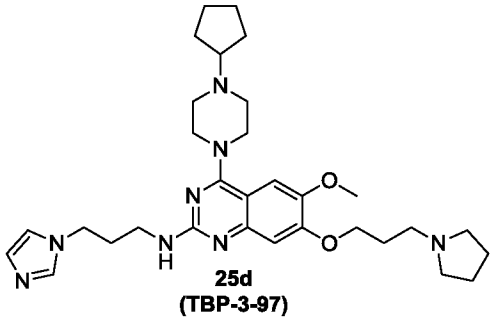
25a
(TBP-3-91)



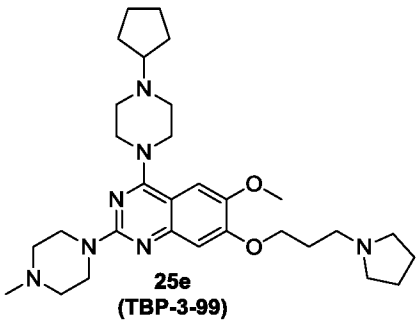
25b
(TBP-3-93)



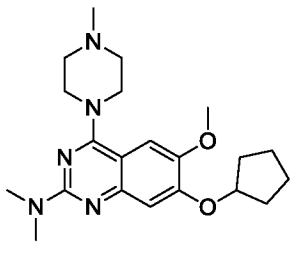
25c
(TBP-3-95)



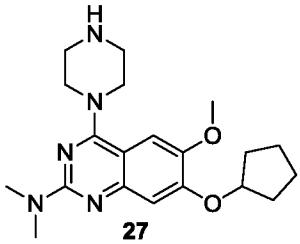
25d
(TBP-3-97)



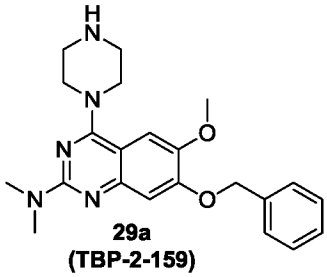
25e
(TBP-3-99)



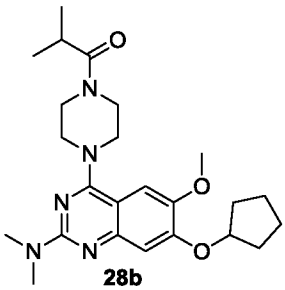
28a
(TBP-4-11)



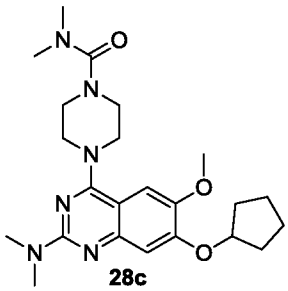
27
(TBP-3-149)



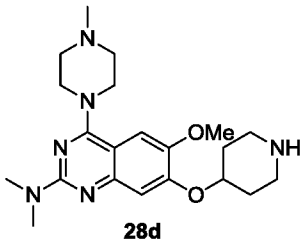
29a
(TBP-2-159)



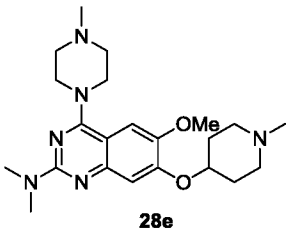
28b
(TBP-3-155)



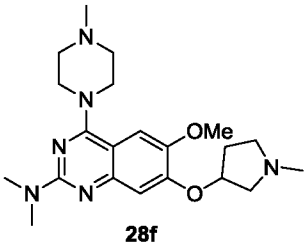
28c
(TBP-3-157)



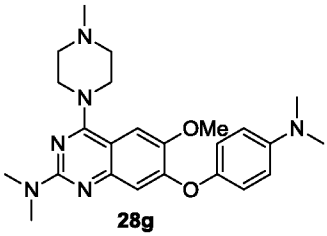
28d
(TBP-4-67)



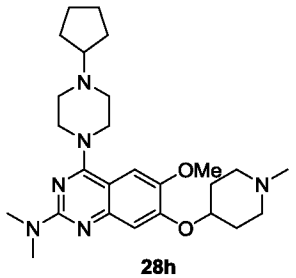
28e
(TBP-4-69)



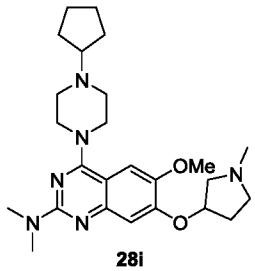
28f
(TBP-4-71)



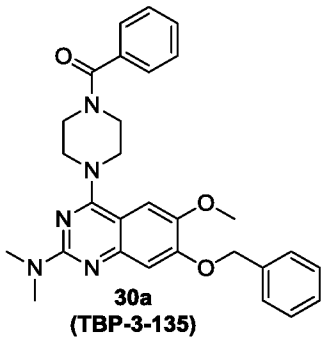
28g
(TBP-4-73)



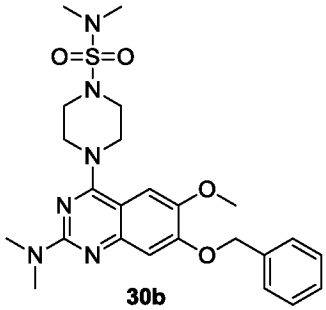
28h
(TBP-4-77)



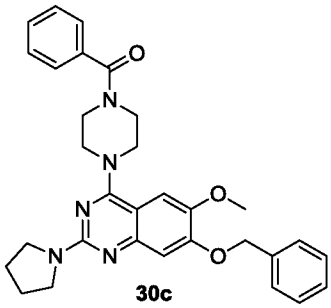
28i
(TBP-4-79)



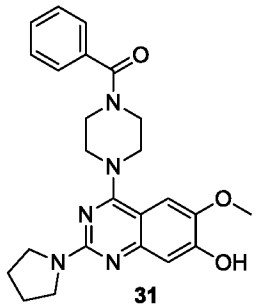
30a
(TBP-3-135)



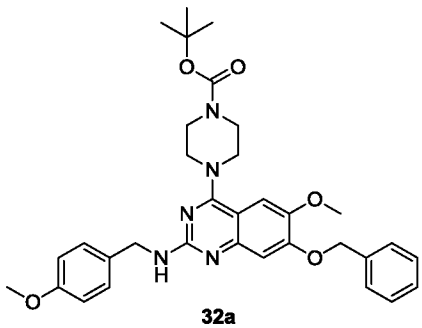
30b
(TBP-3-137)



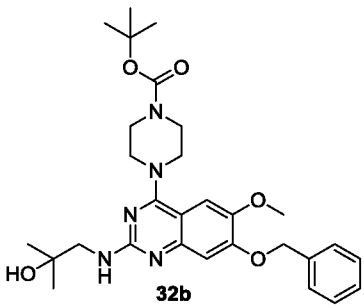
30c
(TBP-2-149)



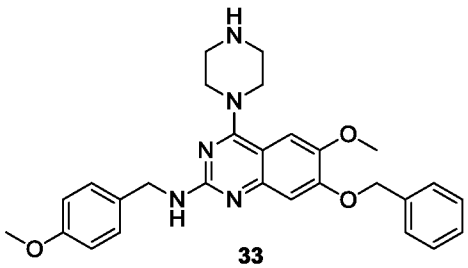
31
(TBP-2-151)



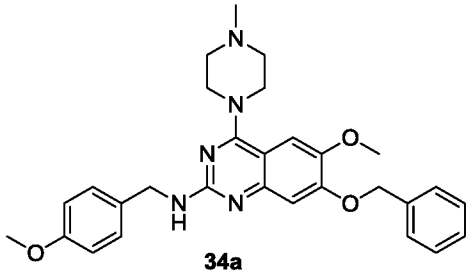
32a
(TBP-3-121)



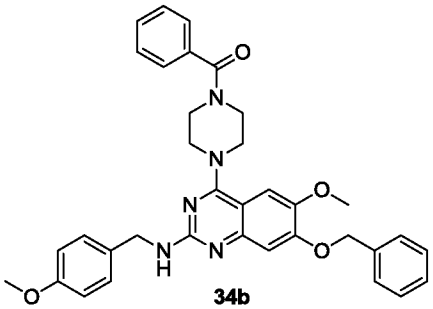
32b
(TBP-3-145)



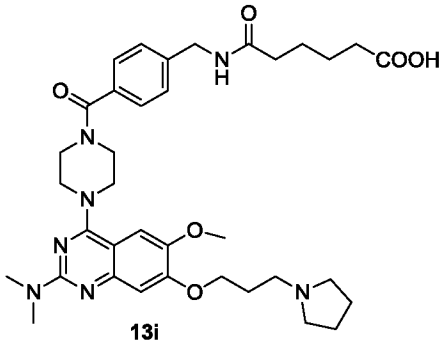
33
(TBP-3-123)



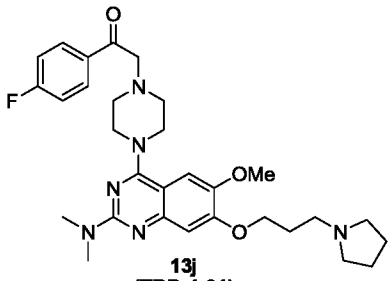
34a
(TBP-3-127)



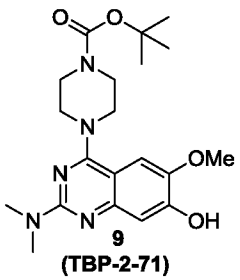
34b
(TBP-3-139)



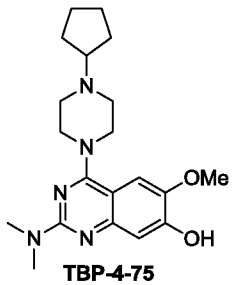
13i
TBP-3-115



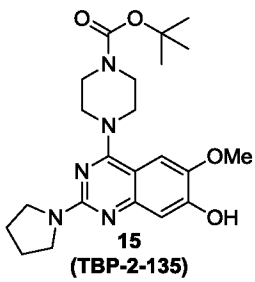
13j
(TBP-4-81)



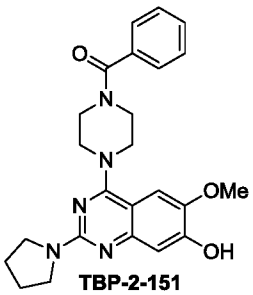
9
(TBP-2-71)

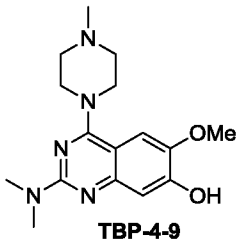


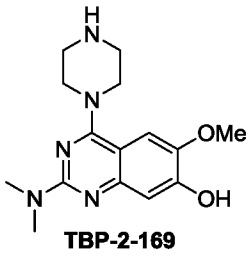
TBP-4-75

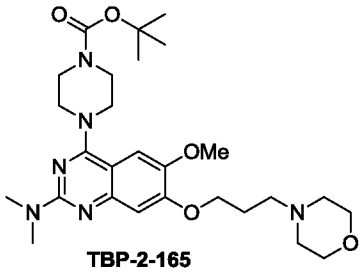


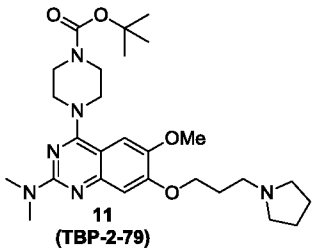
15
(TBP-2-135)


TBP-2-151


TBP-4-9


TBP-2-169


TBP-2-165


11
(TBP-2-79)

In another embodiment the process for the preparation of compounds of formula 1 comprising the steps of:

a. reacting compound of 6 with Boc-piperazxine to obtain compound 7;

b. reacting compound 7 obtained in step a) with amine to obtain compound of formula 8 or 14 or 32;

c. reacting compound 8 or 14 or 32 of step b) either with with hydrogen in presence of Pd/C to obtain compound of 9 or 15 or reacting with TFA to obtain 29a or 29b or 33 ;

d. reacting compound 9 or 15 of step c) either with 1-chloro-3-bromopropane or bromocyclopentane to obtain compound 10 or 26 or 16 ;

e. reacting compound 10 or 16 of step d) with amine to compound 11 or 17;

f. reacting compound 11 or 17 of step e) or compound 26 of step d) or compound 14 of step b) with TFA to obtain 12 or 18 or 27;

g. reacting compound 12obtained in step f) or 29aor 29b of step c) with sulphonly chloride, alkly or aryl carboxylic acid or alkyl halide or aldehyde or reacting compound 33 of step c) with alkyl halide or benzoic acid to obtain the compound of formula 1.

In another embodiment, further comprises, reacting compound 27 of step f) with alkyl halide or acid chloride to obtain compound of formula 1 or compound 31;

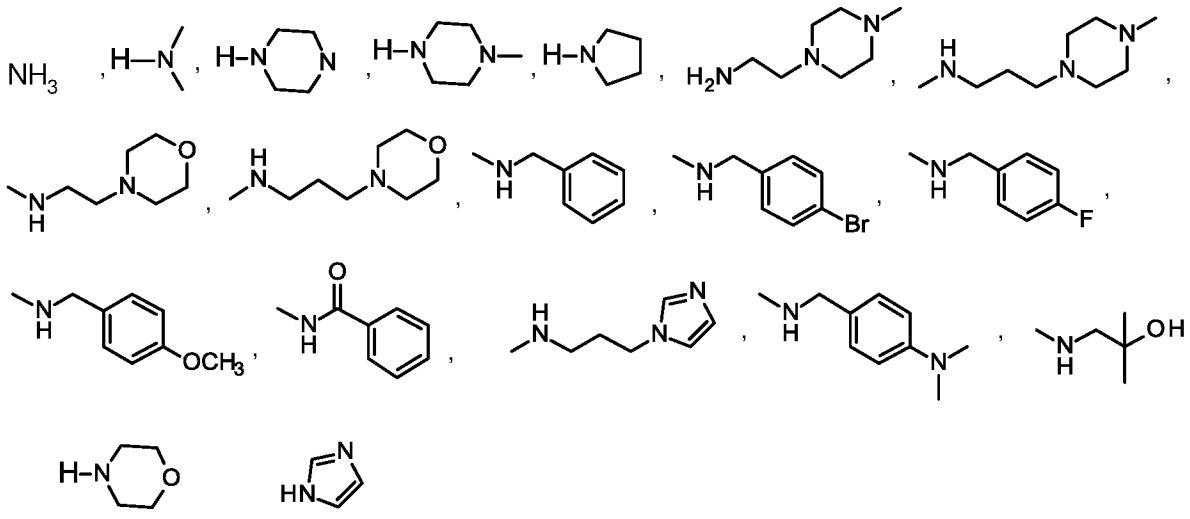
In another embodiment, the compound 31 further reacted with hydrogen in presence of Pd/C to obtain compound of formula 1.

In another embodiment, the process further comprising(i) reacting compound 6 with N-cyclopentylpiperazine or N-methyl piperazine followed by dimethyl amine to obtain an interemediate

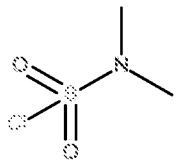
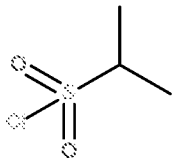
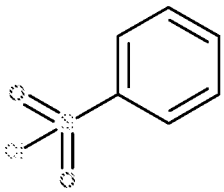
(ii) reacting the intermediate with hydrogen in presence of Pd/C to obtain compound 22 or 9b or 9c; and

(iii)reacting compound 22, 9b or 9c with 1-(3-chloropropyl)pyrrolidine or bromaamine or 4-hydroxy amine to obtain compound of formula 1.

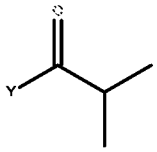
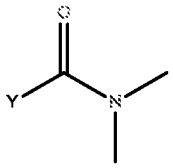
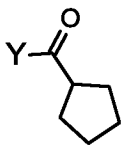
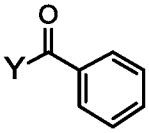
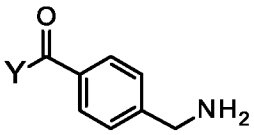
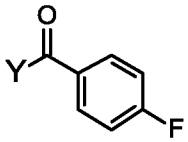
In another embodiment, the amine used in step b) r step e) is selected from the group consisting of,



In another embodiment,the sulphonyl chloride is selected from the group consisting of,

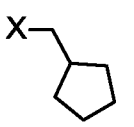




In another embodiment,the alkyl or aryl carboxylic acid or acid chloride is selected from the group consisting of,

Y = OH, Cl

In another embodiment,the alkyl halide is selected from the group consisting of,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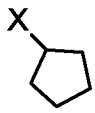
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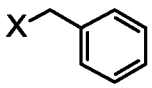
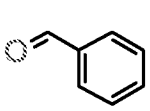
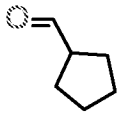


X= halogen

In another embodiment,the alkyl o aryl aldehyde is selected from the group consisting of,

In another embodiment of the present invention, a general screening method is provided involving human peripheral blood mononuclear cells to screen compounds of general formula I against all TLRs.

In another embodiment of the present invention, a method is provided for testing TLR9 antagonism of compounds of general formula I, in primary human plasmacytoid dendritic cells (pDCs) purified from human peripheral blood mononuclear cells.

In yet another embodiment of the present invention, a reporter assay method is provided involving a cell line expressing TLR9 to screen compounds of general formula I for TLR9 antagonism.
Assays results involving human peripheral blood mononuclear cells, human primary pDCs and transfected TLR9 cells are correlating.

In yet another embodiment of the present invention, said compoundswith formula (I) described by the presentinvention affect immune stimulation via interaction with aTLR9.

In yet another embodiment of the present invention, it is believedthat many of the small molecules described by the presentinvention inhibit immune stimulation via TLR9 antagonism.

In yet another embodiment of the present invention, the methods of theinvention are useful whenever it is desirable to alter TLR9mediated signalling in response to a suitable TLR ligand orTLR signalling agonist.

In yet another embodiment of the present invention, it is believed that the said compounds with formula (I) can be useful to inhibit an immune stimulatory nucleic acid associated response in a subject.

In yet another embodiment of the present invention, it is believed that the said compounds with formula (I) shows TLR9 antagonistic activity that can modulate autoreactive inflammation in different autoimmune diseases since aberrant TLR9 activation is implicated in such diseases.

In yet another embodiment of the present invention, it is believed that the said compounds with formula (I)can be used in a number of clinical applications, including as pharmaceutical agents and methods for treating conditions involving unwanted immune activity due to TLR9 activation.

In yet another embodiment of the present invention, the said compounds with formula (I) are without considerable cytotoxicity in HepG2 (a hepatic epithelial cell line) and SW480 (an intestinal mucosal epithelial cell line) cells at concentrations below 100 μM.

In yet another embodiment of the present invention, it is believed that the said compounds with formula (I) can be useful in the treatment of different clinical context of autoreactive inflammation, inflammation, allergy,asthma, graft rejection, and GvHD where aberrant TLR9 activation is present.

In yet another embodiment of the present invention, the small molecules with formula (I) is believed to affect TLRs directly and thus affect TLR-bearing cells, such as antigen-presenting cells (APCs), such agents can be used in conjunction with additional agents which affect non-APC immune cells, such as T lymphocytes (T cells). This will provide immune modulatory intervention at two levels: innate immunity and acquired immunity. The combination intervention is synergistic, since innate immunity is believed to initiate and support acquired immunity.

In one aspect of the invention, a method of affecting TLR mediated signalling in response to a TLR ligand is provided. The method according to this aspect involves detecting TLR9 antagonism of effective amount of a compound of Formula (I) using a reporter cell line that reports nuclear factor kappa B expression downstream of TLR9 signalling.

BRIEF DESCRIPTION OF ACCOMPANYING DRAWING

Fig.1:-Structural evolution of the quinazoline scaffold (FORMULA I) small molecules along with respective TLR9-antagonistic activity. The figure denotes percent interferon alpha production in response to TLR9-agonist ODN2216 from human peripheral blood mononuclear cells in the presence of different doses of the antagonist molecules (0, 0.1, 1, 5, 10μM). Each row represents a single molecule with increasing antagonist concentrations from left to right as shown in the figure. TLR9-antagonist activity of one representative molecule belonging to each structural subset is indicated.

Fig.2: TLR9 inhibition in pDCs by selected compounds with formula (I). The graphs denote dose-dependent reduction in IFN-α production in response to TLR9-agonist ODN2216 from human plasmacytoid dendritic cells (pDC) in the presence of different doses of the antagonist molecules. Each data is derived from two donors. Average values are reported.

Fig.3: TLR9 inhibition inHEK-Blue-hTLR9 reporter cell line by selected compounds with formula (I). The graphs denote dose-dependent inhibition of TLR9 activation in a HEK-Blue-hTLR9 reporter cell line in the presence of different doses of the antagonist molecules, which is represented in terms of decrease in SEAP activity. Data shown are mean of triplicate wells ± SD.

Fig.4: Cytotoxicity based on MTT assay of the identified TLR9 antagonist molecules. HepG2 and SW480 cells were cultured in presence of different concentrations (0.1, 0.5,1, 10, 20 and 100 μM) of different candidate small molecule antagonists for 24 hrs. At 24 hrs MTT assay was performed as described in the text.

Respective absorbance at 570nm is represented. Each line represents a specific small molecule as denoted in the legend.

Table 1 depicts overall structure of the compounds with quinazoline scaffold with formula (I) composition of the Invention

Table 2 depicts IC50 values of the compounds with quinazoline scaffold with formula (I) composition of the Invention.

DETAILED DESCRIPTION OF THE INVENTION

In the present invention the synthesis of compounds of general formula I was prepared as follows.

Intermediate **6** was synthesized through the 5 steps synthetic sequences as shown in Example 1, from commercially available 3-methoxy-4-hydroxybenzonitrile. Benzylation of compound **1** followed by nitration and reduction produces 4-(benzyloxy)-5-methoxy-2-nitrobenzonitrile **4,** which upon amine amide coupling by CDI reagent forms quinazolinedione derivative **5**. On $POCl_3$ treatment **5** was converted to the intermediate 2,4-dichloroquinazoline derivative **6**.

Derivatives **13** were prepared via 7 steps from the dichloroquinazoline intermediate **6** (Example 1**).** On treatment with Bocpiperizine followed by dimethylamine addition **6** was converted to diaminosubstituted quinazoline **8**. On debenzylation by Pd/C and hydrogen gas of **8** followed by SN2 reaction by 1-bromo-3-chloropropane and pyrrolidine substitution at 7-position of quinazoline afforded compound **11**. Boc group deprotection using TFA and subsequent sulphonyl chloride substitution afforded compounds **13a**, **13b** and benzylbromide, bromocyclopentane, bromomethylcyclopentane substitution afforded **13g**, **13e**, **13d**, and carboxylic acid coupling reactions with the corresponding secondary amine using HATU produces the derivatives **13c, 13f**. Compound **7** on substitution by pyrrolidine followed by hydrogenation and SN2 reaction by 1-bromo-3-chloropropane gave compound **16,**

which upon treatmentwith different bases providedcompounds **17** series as shown in **Scheme 3**. Boc groupdeprotection using TFA afforded derivatives **18a**, **18b**, and **18c (Scheme 4).** Compound 6 on treatment with 1-cyclopentylpiperazine followed by hydrogenation and 1-(3-chloropropyl)pyrrolidine attachment provided compound **24**, which upon treatment with different bases at 2-possition in quinazoline scaffold gave the **25** derivative series (**Scheme-6**). Following the above reaction procedure few more derivatives (**28a**, **28b**, **28c**, **30a**, **30b**, **32a**, **32b**, **34a**, **34b**) containing different substitution at the three variable position of quinazoline have been synthesized in **Scheme-7, 8,9** and **10.**

The synthesized compounds of general formula I were screened for toll-like receptor 9 antagonistic activities by a medium throughput biological assay based on toll-like receptor 9 activation in primary human immune cells. Type A and type B unmethylated cytosine-guanine rich DNA oligonucleotides (CpG oligonucleotides) are the bona fide ligands for TLR 9. On activation of TLR9 by CpG oligonucleotides, type I interferons (e.g. IFN-alpha) are released. The synthesized compounds of general formula I was able to alter the release of type I interferons (e.g. IFN-alpha).

Type I interferons(IFN-alpha) production from human peripheral blood mononuclear cells in response to type A CpG oligonucleotides (CpGA) almost exclusively results from TLR9 triggering on the PDCs. Based on this principle the screening assay was designed where we isolated peripheral blood mononuclear cells (PBMCs) from venous blood collected from healthy donors using density gradient centrifugation. The synthesized compounds of general formula I having TLR9 antagonistic activity inhibited IFN-alpha production in this screening assay.

The synthesized compounds of general formula I were screened for toll-like receptor 9 antagonistic activities by a medium throughput biological assay based on toll-like receptor 9 activation in plasmacytoid dendritic cells (pDC) which were isolated from

PBMCs of healthy donors. The synthesized compounds of general formula I having TLR9 antagonistic activity inhibited IFN-alpha production in response to CpGA in this screening assay.

The synthesized compounds of general formula I were screened for toll-like receptor 9 antagonism using a HEK-Blue- hTLR9 Secreted Alkaline Phosphatase (SEAP) reporter assay. The synthesized compounds of general formula I having TLR9 antagonistic activity inhibited TLR9-mediated NF-kB activation in a dose-dependent manner.

The synthesized compounds of general formula I werescreened for cytotoxicity.MTT assay is a colorimetric assay for assessing cell viability. HepG2 (a hepatic epithelial cell line) and SW480 (an intestinal mucosal epithelial cell line) cells were used to check cytotoxicity. The synthesized compounds of general formula I did not show any considerable cytotoxicity at concentrations below 100uM on this assay (Figure 4).

EXPERIMENTAL DETAILS:

The following examples are intended for illustrative purposes only and are not to be construed as being limitations for the invention thereon in any manner. Temperatures are given in degree Celsius. The structure of final products, intermediates and starting materials is confirmed by standard analytical methods, e.g. spectroscopic characterization, e.g., MS, NMR. Abbreviations used are those conventional in the art.

All starting materials, reagents, catalysts, building blocks, acids, bases, dehydrating agent and solvents utilized to synthesize the compounds of the present invention are either commercially available or can be produced by known organic synthesis methods in the art.

Abbreviations

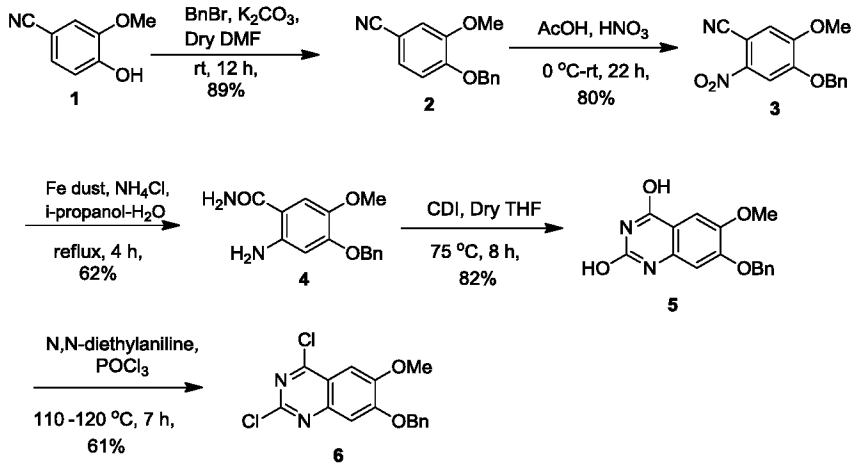
BnBr	Benzylbromide

DMF	N,N-dimethylformamide
AcOH	Acetic acid
CDI	1,1'-Carbonyldiimidazole
$POCl_3$	phosphorous oxychloride
DIPEA	*N*,*N*-Diisopropylethylamine
DCM	Dichloromethane
TFA	Trifluoroacetic acid
DMSO	Dimethyl sulfoxide
Boc	Tert butyl carbamate
THF	Tetrahydrofurane
HATU	1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium-3-oxid hexafluorophosphate
HBTU	2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate)

EXAMPLES

Example 1

SCHEME:1



4-(Benzyloxy)-3-methoxybenzonitrile (2): 4-hydroxy-3-methoxybenzonitrile (10.0 g, 67.11 mmol) and potassium carbonate (19 g, 134 mmol) were taken in DryDMF (20 mL) at 0°C. And then benzyl bromide (9.0 mL, 80.5 mmol) was added to the reaction mixture very slowly. The reaction mixture was stirred 12 h at room temperature, and brine solution (100 mL) was added. The resulting precipitate was collected, washed with water and dried to provide 4-benzyloxy-3-methoxybenzonitrileas a white solid (13.9 g, 89% yield). ^{1}H NMR (300MHz, $CDCl_3$) δ 3.88 (s, 3H), 5.18 (s, 2H),6.88 (d, *J*=8.4 Hz, 1H),7.03 (s, 1H),7.21 (dd, *J*=8.4, 2.0 Hz, 1H),7.32-7.42 (m, 5H). ESI $[M+Na]^+$: 262.25).

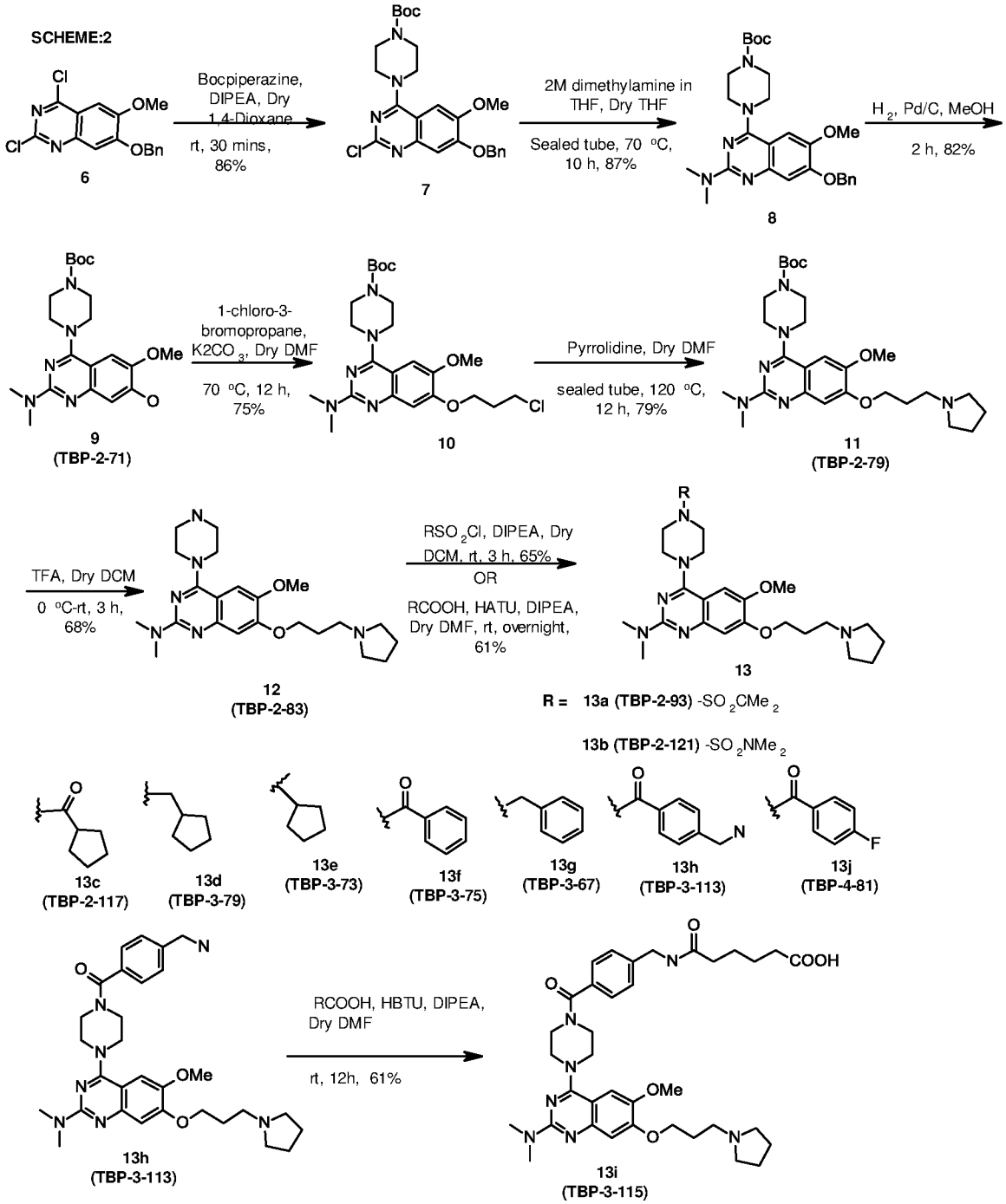
4-(Benzyloxy)-5-methoxy-2-nitrobenzonitrile (3): To an ice cold solution of compound **2** (8.0 g, 33.6 mmol) in 20 mL acetic acid, 69% Nitric acid (126.4 mmol) was added dropwise. Reaction mixture was slowly allowed to come at room temperature and kept in that condition 22 h. The reaction mixture was neutralized with 4N NaOH solution and then residue was extracted with DCM, washed with brine and dried over sodium sulphate. Concentrating DCM part provide compound **3** as a yellow solid (6.8 g, 82% yield).^{1}H NMR (300MHz, $CDCl_3$) δ 4.01 (s, 3H), 5.26 (s, 2H),7.21(s, 1H),7.36-7.42 (m, 5H),7.85 (s. 1H).ESI$[M+Na]^+$: 307.14).

2-Amino-4-(benzyloxy)-5-methoxybenzamide (4): A mixture of compound **3** (5.0 g, 17.6 mmol), iron dust (3.0 g, 54.8 mmol), and ammonium chloride (3.8 g, 70.4 mmol) in isopropyl alcohol-water (2:1) 50 mL was heated to reflux for 4 h. Then, the reaction mixture filtered through celite and the filtrate part was extracted with 5% methanol in DCM. The organic part was dried, concentrated and purified by silica gel chromatography with 10% methanol in DCM to afford compound **4** as light yellow solid (2.7 g, 52% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.89 (s, 3H),5.15 (s, 2H),6.20 (s, 1H),6.88 (s, 1H),7.29-7.51 (m, 5H).FAB $[M+H]^+$: 272.6.

7-(Benzyloxy)-6-methoxyquinazoline-2,4-diol (5): Compound **4** (2.0 g, 61 mmol) was taken in dry THF, to the clean solution CDI (8.05 mmol) was added and the reaction mixture was heated at 75°C. A precipitate formed and the reaction was continued for 8hr for complete consumption of starting material. The precipitate was filtered and washed with THF to afford compound **5** (1.85, 82% yield) as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.86 (s, 3H), 5.14 (s, 2H),6.79 (s, 1H),7.28 (s, 1H),7.28-7.48 (m, 5H). FAB[M+H]$^+$:299.2.

7-(Benzyloxy)-2,4-dichloro-6-methoxyquinazoline (6): Compound **5** (2 g, 6.71 mmol) was taken in 5 mL N,N-diethylaniline, the reaction mixture was cooled to 0°C. Then $POCl_3$ (10 mL) was added very slowly and the reaction mixture was stirred at 110°C-120°C for overnight. The reaction mixture was was neutralized with saturated sodium bicarbonate aqueous solution. The resulting mixture was extracted with chloroform. The organic solvents were dried, concentrated, and purified by silica gel chromatography (hexane to 20% ethyl acetate in hexanes) to give compound **6** as a light yellow solid (1.40 g, 61% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.06 (s, 3H), 5.31 (s, 2H),7.26 (s, 1H),7.30 (s, 1H),7.33-7.51 (m, 5H).FAB[M+H]$^+$:335.2.

Example 2


SCHEME:2
Bocpiperazine, DIPEA, Dry 1,4-Dioxane
rt, 30 mins, 86%
2M dimethylamine in THF, Dry THF
Sealed tube, 70 °C, 10 h, 87%
H2, Pd/C, MeOH
2 h, 82%
1-chloro-3-bromopropane, K2CO3, Dry DMF
70 °C, 12 h, 75%
Pyrrolidine, Dry DMF
sealed tube, 120 °C, 12 h, 79%
TFA, Dry DCM
0 °C-rt, 3 h, 68%
RSO2Cl, DIPEA, Dry DCM, rt, 3 h, 65%
OR
RCOOH, HATU, DIPEA, Dry DMF, rt, overnight, 61%
RCOOH, HBTU, DIPEA, Dry DMF
rt, 12h, 61%
6
7
8
9 (TBP-2-71)
10
11 (TBP-2-79)
12 (TBP-2-83)
13
R = 13a (TBP-2-93) -SO2CMe2
13b (TBP-2-121) -SO2NMe2
13c (TBP-2-117)
13d (TBP-3-79)
13e (TBP-3-73)
13f (TBP-3-75)
13g (TBP-3-67)
13h (TBP-3-113)
13j (TBP-4-81)
13h (TBP-3-113)
13i (TBP-3-115)

***t*-Butyl-4-(7-(benzyloxy-2-chloro-6-methoxyquinazoline-4-yl)piperazine-1-carboxylate (7):** N-bocPiperazine(620 mg, 0.003 mmol) was added to a stirred solution of **6** (1 g, 0.0029 mmol) in dry 1,4-Dioxane and DIPEA (0.7 mL,0.005 mmol). . The solution was stirred for 30 min at room temperature. After adding water a precipitate was formed which was filtered to give compound **7** (1.2g, 86% yield) as white solid (m.p-155-158°C). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.52 (s, 9H),3.63 (m, 4H),3.69 (m, 4H),3.97 (s, 3H),5.27 (s, 2H),7.05 (s, 1H),7.21 (s, 1H),7.33-7.41(m, 3H),7.44-7.47 (m, 2H).EI-HRMS$[M]^+$: Calculated: 484.1877. Found: 484.1877.

***t*-Butyl4-(7-(benzyloxy)-2-(dimethylamino)-6-methoxyquinazolin-4yl)piperazine-1-carboxylate (8):** 2M dimethylamine in THF (1mL, 2mmol)) was added to a solution of compound **7** (250 mg, 0.52 mmol) in dryTHF (2 mL) and the reaction mixture was stirred for 10 h at 75°C in sealed tube. THF was removed under vacuum, the residue then dissolved in ethyl acetate and the organic layer was washed with water and brine, dried and concentrated to give compound **8** (220 mg, 87% yield) as a white solid (m.p-192-195°C). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.52 (s, 9H), 3.21(s, 6H), 3.63 (t,J = 6 Hz, 4H), 3.69 (t,J = 6 Hz, 4H), 3.97 (s, 3H), 5.27 (s, 2H), 6.98 (s, 1H), 7.08 (s, 1H), 7.31-7.38 (m, 3H), 7.41-7.49 (m, 2H). EI-HRMS$[M]^+$: Calculated:493.2689. Found: 494.2757.

***t*-Butyl-4-(2-(dimethylamino)-7-hydroxy-6-methoxyquinazolin-4yl)piperazine-1-carboxylate (9):** 10% Pd/C (50 mg) was added to a solution of compound **8** (500 mg, 0.08 mmol) in MeOH 10 mL followed by the addition of 1 mL DIPEA. A hydrogen balloon was attached and the mixture was stirred at room temperature for 3h. The reaction mixture was filtered through celite and washed with methanol until the filtrate became colourless. The solution was concentrated to provide compound **9** (330 mg, 82% yield) as pale yellow solid (m.p-254-256°C decompose). ^{1}H NMR (300 MHz, $CDCl_3$) δ1.31 (s, 9H), 3.16 (s, 6H), 3.47-3.51 (m, 4H), 3.75 (s, 3H), 3.80-3.83 (m, 4H), 6.86 (s, 1H), 7.29 (s, 1H), EI-HRMS$[M]^+$: Calculated:403.2220. Found: 403.2219.

***t*-Butyl-4-(7-(3-chloropropoxy)-2-(dimethylamino)-6methoxyquinazolin-4yl)piperazine-1-carboxylate (10):** Compound **9** (200 mg, 0.65 mmol) and potassium carbonate (200 mg, 1.43 mmol) was taken in dryDMF (5 mL). The reaction mixture was stirred at room temperature for 30 mins. Then 1-bromo-3-chloropropane (7.2 µL, 0.72 mmol) was added and the mixture was stirred at 110°C for 12 h. The reaction mixture was extracted with ethyl acetate and washed with 50 mL of water followed by brine wash, dried with sodium sulphate and concentrated. The residue was purified by silica gel flash column chromatography, eluting with 60% ethyl acetate in hexane, to give compound **10** (169 mg, 72% yield) as a colourless gummy solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ1.49 (s, 9H), 2.45-2.31 (m, 2H),3.22 (s, 6H), 3.63 (t,*J* = 6 Hz, 4H),3.69 (t,*J* = 6 Hz, 4H),3.79-3.77 (t, *J* = 6 Hz, 2H),3.88 (s, 3H),4.29-4.24 (m, 2H),6.96 (s, 2H), 7.08 (s, 1H). EI-HRMS[M]$^+$: Calculated: 479.2299 Found: 479.2297.

***t*-Butyl-4-(2-(dimethylamino)-6-methoxy-7-(3-(pyrrolidine-1-yl) propoxy) quinazoline-4-yl) piperazine-1-carboxylate (11):** To a solution of compound **10** (150 mg, 0.33 mmol) in 2 mL dryDMF I a sealed tube pyrroline (2.8 µL, 0.34 mmol) was added and the reaction mixture was heated at 90°C for 12 h. The reaction mixture was extracted with ethyl acetate and washed with 50mL of water followed by brine wash; ethyl acetate part was dried with sodium sulphate and concentrated. The residue was purified by silica gel flash column chromatography, eluting with 20% CMA (NH_3: MeOH: $CHCl_3$ = 5:10:85) in chloroform, to give compound **11** as a gummy solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ1.49 (s, 9H),2.45-2.31 (m, 2H),3.22(s, 6H),3.63 (br. s, 4H),3.69 (br. s, 4H),3.79-3.77 (m, 2H),3.88 (s, 3H),4.31 (t,*J* = 6.2 Hz, 2H),7.2 (s, 2H),7.99 (s, 1H). EI-HRMS[M]$^+$: Calculated: 514.3268 Found: 514.3270.

6-Methoxy-N,N-dimethyl-4-(piperazin-1-yl)-7-(3-(pyrrolidin-1yl)propoxy)quinazolin-2-amine (12) : To a solution of compound **11**(100 mg, 0.19 mmol) in 2mL dry DCM 0.5 mL TFA was added at 0°C and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was quenched by adding 2 (N) NaOH solution, then the mixture was extracted with DCM and washed the organic part

with brine, dried over sodium sulphate, concentrating the organic part gives compound **12** as pure colourless solid (70 mg, 67% yield). EI-HRMS$[M]^+$: Calculated: 414.2743 Found: 414.2741

General procedure for the synthesis of compound 13a, 13b: To a solution of compound **12** (50 mg, 0.12 mmol) in 2 mL dryDCM, DIPEA (0.1 mL, 0.24 mmol) and 1.5eqv of sulphonyl chloride were added keeping the reaction mixture in 0°C. Then it was stirred at room temperaturefor 4hrs. Reaction mixture was washed with water and extracted in DCM. Concentrating the organic part gives a mixture. Purification using chloroform-methanol (15%) gives the corresponding compounds as solid.

General procedure for the synthesis of compound 13c, 13f, 13h and 13i: Carboxylic acid (1.0 eqv.) was taken in 2 mL dryDMF followed by the addition of HATU/HBTU (1.5eqv.) and DIPEA (1.5eqv.). The reaction mixture was stirred at room temperature for 30mins. Then compound **12** (50 mg, 0.12 mmol, 1.1eqv.) in dry DMF was added. Reaction mixture at stirred at room temperature for overnight. The reaction mixture was extracted with ethyl acetate and washed with excess of water followed by brine wash; ethyl acetate part was dried with sodium sulphate and concentrated. The residue was purified by flash column to provide compound **13c, 13f, 13h,13i.**

General procedure for the synthesis of compound 13d and 13g: To a solution of compound **12** (80 mg, 0.19 mmol) in dry toluene corresponding aldehydes (cyclopentanecarboxaldehyde and benzaldehyde, 2 eqv.) and activated molecular sieves (4A) were added and the reaction mixture was refluxed for 12 h. Toluene was removed in vacuum and dry DCE was added followed by the addition of Sodium triacetoxyborohydride (2 eqv.). This reaction mixture was stirred at room temperature for 4 h. Saturated $NaHCO_3$ solution was added and extracted with chloroform. The organic layer was washed with brine, dried over sodium sulphate and concentrated. The residue was purified by flash column to provide pure corresponding derivatives.

Procedure for the synthesis of compound 13e: To a solution of compound **12** (80 mg, 0.19 mmol) in 5 mL dry DMF bromocyclopentane (3.1 µL, 0.28mmol) was added followed by the addition of potassium carbonate (58 mg, 0.38mmol) and the reaction mixture was stirred at room temperature for 10 h. 50 mL water was added and extracted with ethylacetate. The organic layer was washed with brine, dried over sodium sulphate and concentrated. The residue was purified by flash column to provide compound **13e** as gummy solid.

4-(4-(Isopropylsulfonyl)piperazin-1-yl)-6-methoxy-N,N-dimethyl-7-(3-(pyrrolidin-1-yl)propoxy)quinazolin-2-amine (13a): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (br. s, 6H), 1.38 (d, *J*= 5.49 Hz, 6H), 2.06 (br. s, 4H), 2.34 (br. s, 2H), 3.23 (br. s, 6H), 3.55 (br. s, 4H), 3.66 (br. s, 5H), 3.88 (br. s, 3H), 4.20 (br. s, 2H), 6.91 (br. s, 1H), 7.00 (br. s, 1H). EI-HRMS$[M]^+$: Calculated: 520.2832. Found: 520.2838.

4-(4-(Dimethylamino)-6-methoxy-7-(3-(pyrrolidin-1yl)propoxy)quinazolin-4-yl)-N,N-dimethylpiperazin-1-sulfonamide (13b):^{1}H NMR (600 MHz, $CDCl_3$) δ 1.73 - 1.75 (m, 2H), 1.82 (br. s., 6H), 2.13 - 2.17 (m, 2H), 2.62 (br. s, 2H), 2.68 - 2.75 (m, 2H), 2.87 (s, 6H), 3.21 (s, 6H), 3.42 - 3.44 (m, 2H), 3.59 - 3.63 (m, 4H), 3.88 (s, 3H), 4.18 - 4.20 (m, 2H), 6.92 (s, 1H), 6.96 (s, 1H). EI-HRMS$[M]^+$: Calculated: 521.2784. Found: 521.2789.

Cyclopentyl-(4-(2-(dimethylamino)-6-methoxy-7-(3-(pyrrolidin-1-yl)propoxy)quinazolin-4-yl)piperazine-1yl)methanone (13c): (35 mg, 55% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.60 (dd, *J*=7.16, 4.52 Hz, 2H), 1.75 (d, *J*=6.03 Hz, 2H), 1.86 (d, *J*=5.27 Hz, 8H), 2.21 (d, *J*=3.77 Hz, 1H), 2.59 (t, *J*=6.40 Hz, 2H), 2.74 (br. s, 2H), 2.81 (br. s, 2H), 2.93 (d, *J*=7.91 Hz, 2H), 3.22 (s, 6H), 3.57 (br. s, 4H), 3.74 (br. s, 2H), 3.83 (br. s, 2H), 3.89 (s, 3H), 4.19 (t, *J*=6.40 Hz, 2H), 6.96 (s, 2H). EI-HRMS$[M]^+$: Calculated: 510.3318. Found: 510.3311.

4-(4-(Cyclopentylmethyl)piperazin-1-yl)-6-methoxy-N,N-dimethyl-7-(3-(pyrrolidin-1yl)propoxy)quinazolin-2-amine (13d): ^{1}H NMR (300 MHz, $CDCl_3$)

δ1.20 - 1.28 (m, 2H), 1.58 (dd, *J*=17.05, 7.25 Hz, 5H), 1.81 (br. s, 6H), 2.13 (d, *J*=6.97 Hz, 2H), 2.34 (d, *J*=7.35 Hz, 2H), 2.56 - 2.63 (m, 8H), 2.66 - 2.71 (m, 2H), 3.16 - 3.26 (m, 6H), 3.51 - 3.63 (m, 4H), 3.91 (s, 3H), 4.17 - 4.22 (m, 2H), 6.94 (br. s, 1H), 6.99 (s, 1H). ESI $[M+H]^+$:497.73.

4-(4-(Cyclopentylpiperazin-1-yl)-6-methoxy-N,N-dimethyl-7-(3-(pyrrolidin-1yl)propoxy)quinazolin-2-amine (13e): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 (d, *J*= 8.29 Hz, 2H), 1.43 - 1.55 (m, 2H), 1.64 (d, *J*= 4.14 Hz, 2H), 1.77 (br. s, 6H), 2.08 - 2.14 (m, 2H), 2.48 (d, *J*= 7.72 Hz, 1H), 2.55 - 2.64 (m, 8H), 2.67 (d, *J*= 7.72 Hz, 2H), 3.14 (s, 6H), 3.54 (d, *J*= 13.75 Hz, 4H), 3.81 (s, 3H), 4.12 (t, *J*=6.50 Hz, 2H), 6.86 (s, 1H), 6.94 (s, 1H). ESI $[M+H]^+$:483.55.

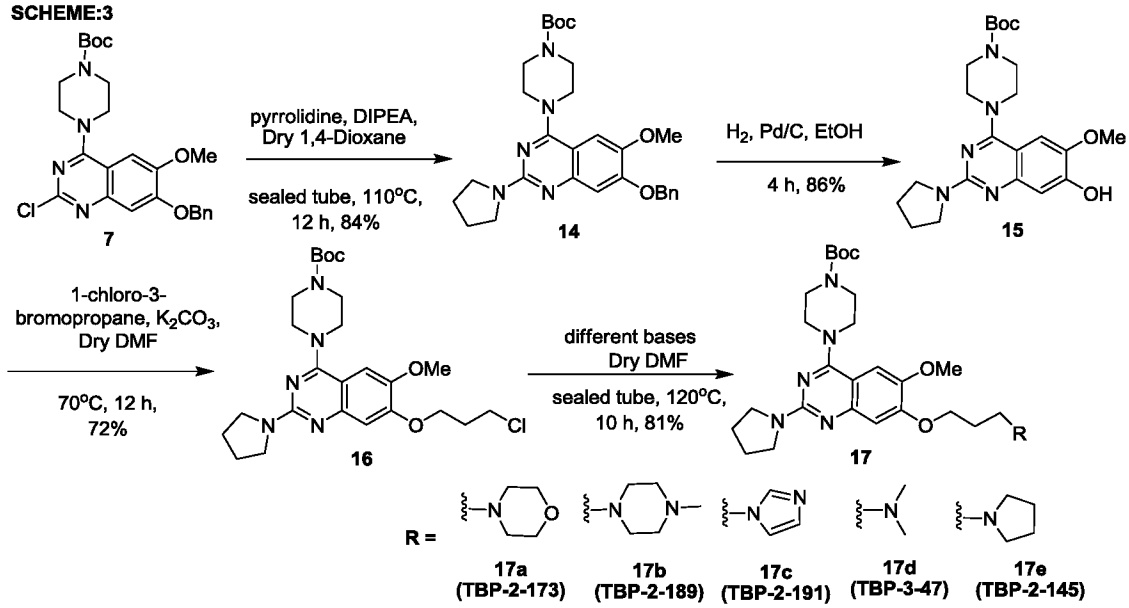
(4-(2-(Dimethylamino)-6-methoxy-7-(3-(pyrrolidin-1-yl)propoxy)quinazoline-4-yl)piperazin-1-yl)(phenyl)methanone (13f): ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.86 (br. s, 4H), 2.15 - 2.19 (m, 2H), 2.75 (br. s, 4H), 2.83 (t, *J*= 7.34 Hz, 2H), 3.19 (s, 6H), 3.45 - 3.70 (m, 8H), 3.86 (s, 3H), 4.18 (t, *J*= 6.38 Hz, 2H), 6.94 (d, *J*= 7.56 Hz, 2H), 7.42 (s, 5H). ESI $[M+H]^+$:519.61.

4-(4-Benzylpiperazin-1-yl)-6-methoxy-N,N-dimethyl-7-(3-(pyrrolidin-1-yl)propoxy)quinazolin-2-amine (13g): ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.79 (t, *J*= 3.23 Hz, 4H), 2.09 - 2.15 (m, 2H), 2.55 (br. s, 4H), 2.62 - 2.67 (m, 6H), 3.20 (s, 6H), 3.58 (s, 2H), 3.58 - 3.63 (m, 4H), 3.86 (s, 3H), 4.17 (t, *J*= 6.71 Hz, 2H), 6.96 (d, *J*= 11.15 Hz, 2H), 7.25 - 7.28 (m, 1H), 7.31 - 7.37 (m, 4H). EI-HRMS$[M]^+$: 504.3198.

(4-(Aminomethyl)phenyl)(4-(2-(dimethylamino)-6-methoxy-7-(3-(pyrrolidin-1-yl)propoxy)quinazolin-4-yl)piperazin-1-yl)methanone(13h): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.81 (t, *J* = 6.6 Hz, 4H), 2.08-2.17 (m, 4H), 2.57 (t, *J* = 6 Hz, 4H), 2.68 (t, *J* = 7.2 Hz, 2H), 3.20 (s, 6H), 3.54-3.64 (m, 6H), 3.87 (s, 3H), 3.93 (d, *J* = 13.8 Hz, 2H), 4.19 (t, *J* = 6.6 Hz, 2H), 6.94 (d, *J* = 6.6 Hz, 2H), 7.36-7.43 (m, 4H). ESI $[M+H]^+$:548.40.

6-((4-(4-(2-(Dimethylamino)-6-methoxy-7-(3-(pyrrolidin-1-yl)propoxy)quinazolin-4-yl)piperazine-1-carbonyl)benzyl)amino)-6-oxohexanoic acid(13i): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.58-1.66 (m, 4H), 1.94 (t, *J* = 6 Hz, 4H), 2.18-2.27 (m, 6H), 3.00 (t, *J* = 9.9 Hz, 6H), 3.18 (s, 6H), 3.59 (br. s, 6H), 3.85 (s, 3H), 4.01 (br. s, 2H), 4.15 (t, *J* = 6 Hz, 2H), 4.43 (d, *J* = 6 Hz, 2H), 6.92 (d, *J* = 6.3 Hz, 2H), 7.29-7.35 (m, 4H), 7.41 (br. s, 1H).ESI $[M+H]^+$:676.19

Example 3



***t*-Butyl-4-(7-(benzyloxy)-6-methoxy-2-(pyrrolidin-1-yl)quinazoline-4-yl)piperazine-1-carboxylate (14):** Pyrrolidine(0.1 mL, 1.24 mmol was added to a solution of compound **7** (500 mg, 1.03 mmol) in dry1,4-Dioxane (10 mL) and the reaction mixture was stirred for 12 h at 110°C. Dioxane was removed under vacuum, the residue then dissolved in ethyl acetate and the organic layer was washed with water and brine, dried and concentrated. The residue was purified by silica gel flash column chromatography, eluting with 30% ethyl acetate in hexane, to give compound to give compound **14** (459 mg, 84% yield) as a brown solid (m.p-188-190°C).^{1}H NMR (600 MHz, $CDCl_3$) δ1.49 (s, 9H), 1.96 (t, *J*= 6.53 Hz, 4H), 3.54 (br. s, 4H), 3.60 - 3.63 (m, 8H), 3.89 (s, 3H), 5.22

(s, 2H), 6.98 (s, 1H), 7.04 (s, 1H), 7.31 (d, *J*= 7.48 Hz, 1H), 7.37 (t, *J*= 7.56 Hz, 2H), 7.46 (d, *J*= 7.56 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ28.40, 37.06,49.59,56.18,70.48,79.99,104.47,105.03,106.94,127.44,128.01,128.58,136.22,145. 17,153.91,154.87, 164.74. EI-HRMS[M]$^+$: Calculated: 519.2846. Found: 519.2845.

***t*-Butyl-4-(7-hydroxy-6-methoxy-2-(pyrrolidin-1-yl)quinazoline-4-yl)piperazine-1-carboxylate (15):** 10% Pd/C (50 mg) was added to a solution of compound **8** (500 mg, 0.96 mmol) in 10Ml ethanol . A hydrogen balloon was attached and the mixture was stirred at room temperature for 3hrs. The reaction mixture was filtered through celite and washed with methanol until the filtrate became colourless. The filtrate was concentrated and purified by column chromatography eluting by 8% methanol in Chloroform to provide compound **15** (345 mg, 82%) as yellowish green solid.^{1}H NMR (300 MHz, $CDCl_3$) δ 1.50 (s, 9H), 1.92 (br. s, 4H), 3.57 (br. s, 4H), 3.61 (br. s, 8H), 3.72 (s, 1H), 3.83 (s, 3H), 6.70 (br. s, 1H), 7.20 (s, 1H);^{13}C NMR (75 MHz, $CDCl_3$) δ25.47,
28.43,49.68,55.60,80.07,103.06,103.36,106.97,144.64,149.46,154.03,154.82,155.49, 164.36. EI-HRMS[M]$^+$: Calculated: 429.2376. Found: 429.2376.

***t*-Butyl-4-(7-(3-chloropropoxy)-6-methoxy-2-(pyrrolidin-1-yl)quinazoline-4-yl)piperazine-1-carboxylate (16):** Compound **15** (300 mg, 0.69 mmol) and potassium carbonate (194 mg, 1.39 mmol) was taken in dryDMF (5mL). The reaction mixture was stirred at room temperature for 30 mins. Then 1-bromo-3-chloropropane (8.0 µL, 0.76 mmol) was added and the mixture was stirred at 110°C for 12 h. The reaction mixture was extracted with ethyl acetate and washed with 50 mL of water followed by brine wash, dried with sodium sulphate and concentrated. The residue was purified by silica gel flash column chromatography, eluting with 2% methanol in chloroform, to give compound **16** (250 mg, 72%) as a colourless gummy solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.50 (s, 9H), 1.97 - 2.02 (m, 4H), 2.33 - 2.39 (m, 2H), 3.60 (br. s, 4H), 3.62 - 3.68 (m, 8H), 3.79 (t, *J*=6.22 Hz, 2H), 3.90 (s, 3H), 4.26 - 4.32 (m, 2H), 6.98 (s, 1H), 7.07 - 7.16 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ56.06, 25.48,

28.35,29.80,31.85,46.54,49.54,85.11,79.81,104.58,105.10,106.45,144.69,151.54,153.75 ,154.78,157.00, 164.71. EI-HRMS[M]$^+$: Calculated: 505.2456. Found: 505.2456.

General procedure for the synthesis of compounds 17 series: To a solution of compound **16** (150 mg, 0.29 mmol) in 2 mL dryDMF in a sealed tube corresponding base 1.1eqv was added and the reaction mixture was heated at 90°C for 10 h. The reaction mixture was extracted with ethyl acetate and washed with 50 mL of water followed by brine wash; ethyl acetate part was dried with sodium sulphate and concentrated. The residue was purified by silica gel flash column chromatography.

***t*-Butyl-4-(6-methoxy-7-(3-morpholinpropoxy)-2-(pyrrolidin-1-yl)quinazoline-4-yl)piperazine-1-carboxylate (17a):** Compound (**17a)** was prepared by the same procedure as **17** as white solid.^{1}H NMR (300 MHz, $CDCl_3$) δ1.49 (s, 9H), 1.98 (t, *J*=6.40 Hz, 6H), 2.05 - 2.11 (m, 3H), 2.47 (br. s, 4H), 2.53 (s, 2H), 3.56 (br. s, 4H), 3.60 - 3.64 (m, 7H), 3.71 - 3.74 (m, 4H), 3.89 (s, 3H), 4.20 (t, *J*= 6.59 Hz, 2H), 6.96 (s, 1H), 7.27 (s, 1H). EI-HRMS[M]$^+$: Calculated: 556.3373 Found: 556.3375.

***t*-Butyl-4-(6-methoxy-7-(3-(4-methylpiperazin-1-yl)propoxy)-2-(pyrrolidin-1-yl)quinazoline-4-yl)piperazine-1-carboxylate (17b):** ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.48 (s, 9H), 1.95 - 1.97 (m, 4H), 2.05 - 2.08 (m, 2H), 2.28 (s, 3H), 2.39 - 2.50 (m, 4H), 2.52 (t, *J*= 7.12 Hz, 4H), 3.53 (br. s, 4H), 3.59 - 3.64 (m, 10H), 3.87 (s, 3H), 4.17 (t, *J*= 6.71 Hz, 2H), 6.95 (s, 1H), 6.97 (s, 1H). EI-HRMS[M]$^+$: Calculated: 569.3690. Found: 569.3694.

***t*-Butyl-4-(7-(3-(1H-imidazol-1-yl)propoxy)-6-methoxy-2-(pyrrolidin-1-yl)quinazolin-4-yl)piperazine-1-carboxylate (17c):** ^{1}H NMR (300 MHz, $CDCl_3$) δ1.47 (s, 9H), 1.96 (br. s, 4H), 2.26 - 2.37 (m, 2H), 3.60 (br. s, 12H), 3.89 (s, 3H), 4.07 (t, *J*= 5.56 Hz, 2H), 4.20 (t, *J*= 6.59 Hz, 2H), 6.93 (br. s, 1H), 6.96 (s, 1H), 7.03 (br. s, 1H), 7.06 - 7.13 (m, 1H), 7.50 (br. s, 1H). ESI [M+H]$^+$:538.46.

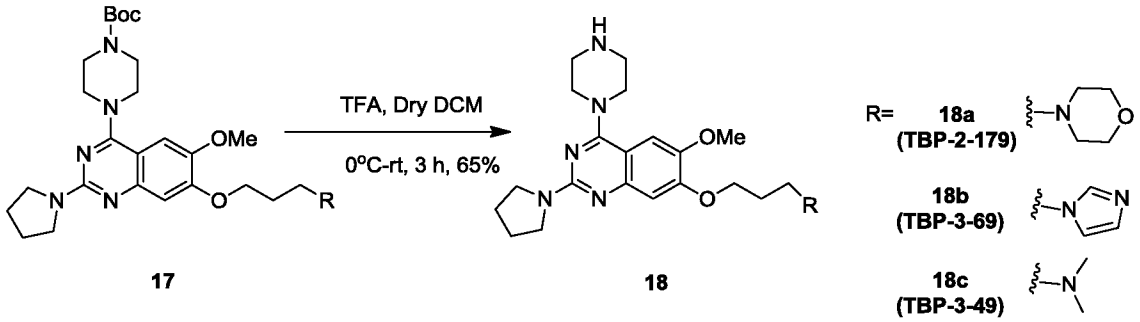
***t*-Butyl-4-(7-(3-(1H-imidazol-1-yl)propoxy)-6-methoxy-2-(pyrrolidin-1-yl)quinazoline-4-yl)piperazine-1-carboxylate (17d):** ^{1}H NMR (300 MHz, $CDCl_3$)

δ1.50 (s, 9H), 2.00 (br. s, 4H), 2.31 - 2.39 (m, 2H), 3.66 (d, *J*= 10.98 Hz, 12H), 3.91 (s, 3H), 4.12 (d, *J*= 5.49 Hz, 2H), 4.22 (t, *J*= 6.49 Hz, 2H), 6.93 - 7.12 (m, 3H), 7.51 (br. s, 2H). ESI $[M+H]^+$:515.71.

***t*-Butyl-4-(6-methoxy-2-(pyrrolidin-1-yl)-7-(3-(pyrrolidin-1-yl)propoxy)quinazolin-4-yl)piperazine-1-carboxylate (17e):** ^{1}H NMR (300 MHz, $CDCl_3$) δ1.49 (s, 9H), 1.92 (br. s, 4H), 1.98 (br. s, 4H), 2.23 - 2.29 (m, 2H),2.81 - 2.94 (m, 8H), 3.30 (br. s, 2H), 3.60 (d, *J*= 4.90 Hz, 4H), 3.65 (br. s, 4H), 3.88 (s, 3H), 4.21 (t, *J*= 6.40 Hz, 2H), 6.96 (s, 1H), 7.12 (s, 1H). ESI $[M+H]^+$:541.66.

Example 4

SCHEME:4



General procedure for Boc-deprotection: To a solution of compound **17** (100 mg, 0.19 mmol) in 2mL dryDCM 0.5 mL TFA was added at 0°C and the reaction mixture was stirred at room temperature for 2 hrs. The reaction mixture was quenched by adding 2 (N) NaOH solution, then the mixture was extracted with DCM and washed the organic part with brine, dried over sodium sulphate, concentrating the organic part gives compound **18.**

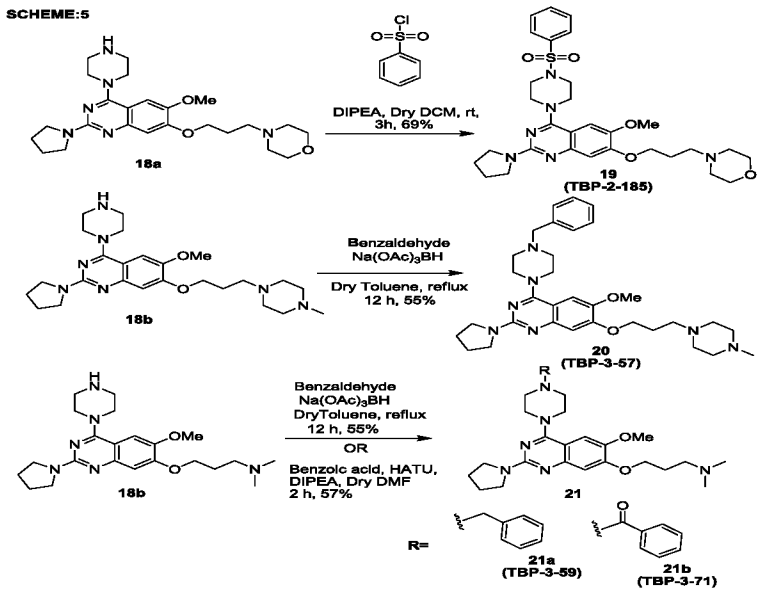
4-(3-(6-Methoxy-4-(piperazin-1-yl)-2-(pyrrolidin-1-yl)quinazolin-7-yloxy)propyl)morpholine (18a): (150 mg, 0.27 mmol) of compound **17a** was taken in 3 mL dry 1,4-dioxane. Then the reaction mixture was cooled to 0°C and 1 ml 4N HCl was added. A pptwas formed which was filtered and the precipitate was the chloride salt

of compound **18**. ^{1}H NMR (300 MHz, CD_3OD) δ2.03 - 2.24 (m, 4H), 2.36 - 2.52 (m, 2H), 3.21 - 3.35 (m, 4H), 3.44 - 3.55 (m, 6H), 3.64 - 3.83 (m, 6H), 3.88 - 3.95 (m, 2H), 3.99 (s, 2H), 4.10 (br. s, 2H), 4.28 (br. s, 3H), 4.37 (t, *J*= 5.27 Hz, 2H), 7.28 (s, 1H), 7.40 (s, 1H). ESI $[M+H]^+$:457.31

7-(3-(1H-Imidazol-1-yl)propoxy)-6-methoxy-4-(piperazin-1-yl)-2-(pyrrolidin-1-yl)quinazoline (18b): ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.97 (t, *J*= 3.19 Hz, 4H), 2.12 - 2.15 (m, 1H), 2.33 (t, *J*= 6.13 Hz, 2H), 3.07 (d, *J*= 5.21 Hz, 2H), 3.57 - 3.59 (m, 4H), 3.62 - 3.65 (m, 4H), 3.87 - 3.90 (m, 2H), 3.91 (s, 3H), 4.07 (t, *J*= 5.87 Hz, 2H), 4.22 (t, *J*= 6.75 Hz, 2H), 6.93 - 6.94 (m, 1H), 6.97 (s, 1H), 7.00 (s, 1H), 7.04 (s, 1H), 7.49 (s, 1H). EI-HRMS $[M]^+$: Calculated 437.2539. Found: 437.2539.

3-((6-Methoxy-4-(piperazin-1-yl)-2-(pyrrolidin-1-yl)quinazolin-7-yl)oxy)-N,N-dimethylpropan-1-amine (18c): ^{1}H NMR (300 MHz, $CDCl_3$) δ1.91-2.55 (m, 4H), 2.04 - 2.13 (m, 2H), 2.26 (s, 6H), 2.45 - 2.50 (m, 2H), 3.06 (d, *J*= 3.66 Hz, 4H), 3.56 (d, *J*= 4.03 Hz, 4H), 3.61 - 3.67 (m, 4H), 3.89 (s, 3H), 4.17 (t, *J*= 6.77 Hz, 2H), 6.99 (s, 1H), 7.01 (s, 1H). EI-HRMS $[M]^+$: Calculated: 414.2743. Found: 414.2743.

Example 5



4-(3-(6-Methoxy-4-(4-(phenylsulfonyl)piperazin-1-yl)-2-(pyrrolidin-1-yl)quinazolin-7-yloxy)propyl)morpholine (19): To a solution of compound **12** (50 mg, 0.12 mmol) in 2 mL dryDCM, DIPEA (0.1 mL, 0.24 mmol) and 1.5eqv of sulphonyl chloride were added keeping the reaction mixture in 0°C. Then it was stirred at room temperature for 4 h. Reaction mixture was washed with water and extracted in DCM. Concentrating the organic part gives a mixture. Purification using chloroform-methanol (15%) gives the corresponding compounds as gummy solid.^{1}H NMR (300 MHz, $CDCl_3$) δ 1.95(br. s, 4H), 2.05 (s, 2H), 2.45(d, *J*=3.96 Hz, 4 H), 2.51(s, 2H), 3.21 (br. s, 4H), 3.55 (br. s, 4H), 3.66 (br. s, 4H), 3.7 (br. s, 4H), 3.83 (s, 3H), 4.17 (s, 2H), 6.82 (s, 1H), 6.95 (s, 1H), 7.61 - 7.54 (m, 3H), 7.80 - 7.77 (m, 2H). EI-HRMS $[M]^+$: Calculated: 596.2781 Found: 596.2785.

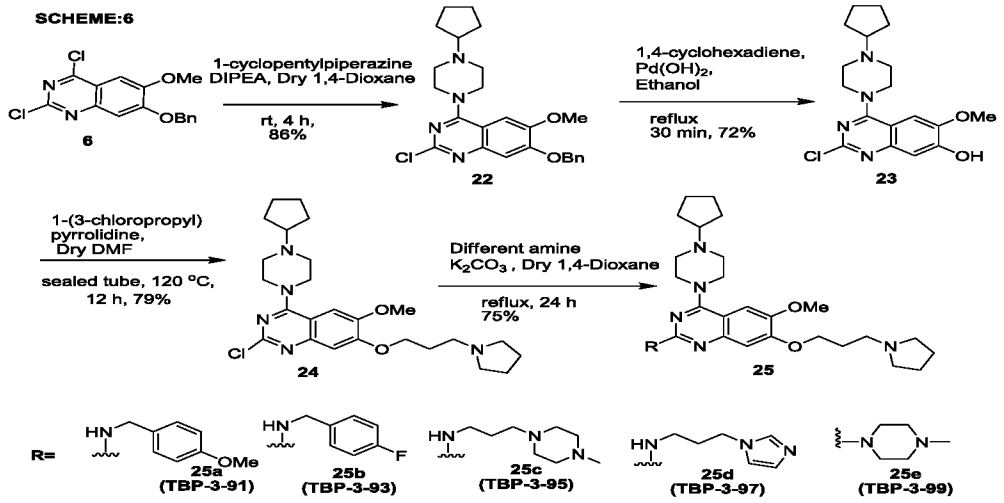
4-(4-Benzylpiperazin-1-yl)-6-methoxy-7-(3-(4-methylpiperazin-1-yl)propoxy)-2-(pyrrolidin-1-yl)quinazoline (20): Compound **20** was prepared by the same procedure as **13d.** ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.96 (t, *J*= 6.40 Hz, 4H), 2.06 (d, *J*= 6.78 Hz, 2H), 2.29 (s, 3H) 2.35 (d, *J*= 7.72 Hz, 4H), 2.56 - 2.50 (m, 6H), 2.66 - 2.61 (m, 4H), 3.65 - 3.57 (m, 10H), 3.87 (s, 3H), 4.17 (t, *J*= 6.69 Hz, 2H), 6.96 (s, 1H), 6.98 (s, 1H), 7.40 - 7.28 (m, 5H). EI-HRMS $[M]^+$: Calculated: 559.3635. Found: 559.3641.

3-(4-(4-Benzylpiperazin-1-yl)-6-methoxy-2-(pyrrolidin-1-yl)quinazolin-7-yloxy)-N,N-dimethylpropan-1-amine (21a): Compound **21a** was prepared by the same procedure as **13g**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.79 (t, *J*= 3.23 Hz, 4H), 2.15 - 2.09 (m, 2H), 2.55 (br. s, 4H), 2.67 - 2.62 (m, 6H), 3.20 (s, 6H), 3.58 (s, 2H),3.63 - 3.58 (m, 4H), 3.86 (s, 3H), 4.17 (t, *J*= 6.71 Hz, 2H), 6.96 (d, *J*= 11.15 Hz, 2H) ,7.28 - 7.25 (m, 1H), 7.37 - 7.31 (m, 4H). EI-HRMS $[M]^+$: Calculated: 504.3213. Found: 504.3198.

(4-(7-(3-(Dimethylamino)propoxy)-6-methoxy-2-(pyrrolidin-1-yl)quinazolin-4-yl)piperazin-1-yl)(phenyl)methanone (21b): Compound**21b** was prepared by the same procedure as **13c** .^{1}H NMR (300 MHz, $CDCl_3$) δ 1.97 (d, *J*= 4.52 Hz, 4H), 2.14 - 2.05 (m, 2H), 2.30 (s, 6H), 2.54 (t, *J*= 7.25 Hz, 2H), 3.62 (d, *J*= 6.22 Hz, 8H), 3.87 (s, 3H),

4.04–3.88 (m, 4H), 4.16 (t, *J*= 6.50 Hz, 2H), 6.94 (s, 1H), 7.01 (s, 1H), 7.43 (s, 5H). ESI $[M+H]^+$:519.61.

Example 6



7-(Benzyloxy)-2-chloro-4-(4-cyclopentylpiperazin-1-yl)-6-methoxyquinazoline (22): 1-cyclopentyl Piperazine(507 mg, 0.003 mmol) was added to a stirred solution of **6** (1 g, 0.0029 mmol) in dry 1,4-Dioxaneand DIPEA (0.7 mL,0.005 mmol). The solution was stirred for 4 h at room temperature. After adding water a precipitate was formed which was filtered to give compound **7** (1.1g, 86% yield) as white solid (m.p-156-159 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39 - 1.50 (m, 2H), 1.58 (dd, *J*=7.72, 4.71 Hz, 2H), 1.68 - 1.75 (m, 2H), 1.88 (br. s, 2H), 2.51 - 2.60 (m, 1H), 2.64 - 2.70 (m, 4H), 3.66 - 3.71 (m, 1H), 3.76 - 3.82 (m, 4H), 3.96 (s, 3H), 5.25 (s, 2H), 7.06 (s, 1H), 7.18 (s, 1H), 7.31 - 7.41 (m, 3H), 7.42 - 7.48 (m, 2H).ESI $[M+H]^+$:453.40.

2-Chloro-4-(4-cyclopentylpiperazin-1-yl)-6-methoxyquinazolin-7-ol (23):$Pd(OH)_2$ (50 mg) was added to a solution of compound **22** (500 mg, 1.12 mol) and 1,4-cyclohexadiene (3mL, 2.21 mol) in20 mLEthanol. Reaction mixture was heated at 70°C for 30min. The reaction mixture was filtered through celite and washed with methanol until the filtrate became colourless. The solution was concentrated to provide compound **23** (300 mg, 72% yield) as pale yellow solid (m.p-135°C). ^{1}H NMR (300 MHz, $CDCl_3$)

δ1.43 - 1.61 (m, 4H), 1.66-1.74 (m, 2H), 1.88 (d, *J*= 7.54 Hz, 2H), 2.57 - 2.65 (m, 1H), 2.65-2.81 (m, 4H), 3.64-3.85(m, 4H), 3.95 (s, 3H), 7.01 (s, 1H), 7.17 (s, 1H). ESI $[M+H]^+$:363.22.

2-Chloro-4-(4-cyclopentylpiperazin-1-yl)-6-methoxy-7-(3-(pyrrolidin-1yl)propoxy)quinazoline (24): Compound **23** (200 mg, 0.55 mmol) and potassium carbonate (153 mg, 1.1 mmol) was taken in dryDMF (5mL). The reaction mixture was stirred at room temperature for 30 min. Then 1-(3-chloropropyl)pyrrolidine (121.8 mg, 0.0.825 mmol) was added and the mixture was stirred at 120°C for 2 h. The reaction mixture was extracted with ethyl acetate and washed with 50 mL of water followed by brine wash, dried with sodium sulphate and concentrated. The residue was purified by flash column chromatography, eluting with 2% methanol in chloroform, to give compound **24** (170 mg, 79%) as a colourless gummy solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37 - 1.51 (m, 2H), 1.52 - 1.64 (m, 2H), 1.66 - 1.76 (m, 2H), 1.83 (d, *J*=3.01 Hz, 4H), 1.88 (br. s, 2H), 2.10 - 2.22 (m, 2H), 2.50 - 2.59 (m, 1H), 2.60 - 2.71 (m, 8H), 2.71 - 2.76 (m, 2H), 3.77 (d, *J*= 4.52 Hz, 4H), 3.93 (s, 3H), 4.19 (t, *J*= 6.40 Hz, 2H), 7.04 (s, 1H), 7.14 (s, 1H). ESI $[M+H]^+$:474.63.

General procedure for the synthesis of compound 25: Compound 24 (80 mg, 0.17 mmol) and K_2CO_3 (93.9 mg, 0.68 mmol) were taken in dry 1,4-dioxane in a sealed tube followed by the addition of corresponding amines (1.1eqv.). Reaction mixture was heated at 120°C for 24 h. 1,4-dioxane was removed under vacuum. The residue was extracted with chloroform and washed with 50 mL of water followed by brine wash, dried with sodium sulphate and concentrated and purified by flash column chromatography, eluting with Chloroform- CMA(Chloroform-methanol-5% NH_3), to give corresponding derivatives.

4-(4-Cyclopentylpiperazin-1-yl)-6-methoxy-N-(4-methoxybenzyl)-7-(3-(pyrrolidin-1-yl)propoxy)quinazolin-2-amine (25a): ^{1}H NMR (600 MHz, $CDCl_3$) δ1.43 - 1.46 (m, 2H), 1.59 (d, *J*= 5.46 Hz, 2H), 1.74 (m, 2H), 1.86 (br. s, 4H), 1.90 (br. s., 2H), 2.16 -

2.20 (m, 2H), 2.55 (dt, *J*= 15.91, 7.94 Hz, 1H), 2.62 - 2.70 (m, 6H), 2.76 (t, *J*= 7.26 Hz, 2H), 2.81 - 2.85 (m, 1H), 3.03 (s, 1H), 3.71 (br. s, 4H),3.81 (s, 3H), 3.90 (s, 3H), 4.09 - 4.12 (m, 1H), 4.21 (t, *J*= 6.33 Hz, 2H), 4.61 (d, *J*= 4.89 Hz, 2H), 6.87 (d, *J*= 8.33 Hz, 2H), 6.97 (s, 1H), 7.00 (s, 1H), 7.32 (d, *J*= 8.21 Hz, 2H). ESI $[M+H]^+$:575.68

4-(4-Cyclopentylpiperazin-1-yl)-N-(4-fluorobenzyl)-6-methoxy-7-(3-(pyrrolidin-1-yl)propoxy)quinazolin-2-amine (25b): ^{1}H NMR (300 MHz, $CDCl_3$) δ1.41 - 1.50 (m, 2H), 1.56 - 1.63 (m, 2H), 1.73 (d, *J*= 4.03 Hz, 2H), 1.76-1.86 (m, 4H), 1.89 (m, 2H), 2.13 - 2.19 (m, 2H),2.55 - 2.62 (m, 4H), 2.66 (d, *J*= 5.49 Hz, 4H), 2.68 - 2.72 (m, 2H), 3.50 (q, *J*= 6.95 Hz, 1H), 3.59-3.69 (m, 4H), 3.91 (s, 3H), 4.20 (t, *J*= 6.59 Hz, 2H), 4.65 (d, *J*= 5.12 Hz, 2H), 6.94 (s, 1H), 6.97 - 7.04 (m, 3H), 7.32 - 7.39 (m, 2H).ESI $[M+H]^+$:563.46.

4-(4-Cyclopentylpiperazin-1-yl)-6-methoxy-N-(3-(4-methylpiperazin-1-yl)propyl)-7-(3-(pyrrolidin-1-yl)propoxy)quinazolin-2-amine (25c):^{1}H NMR (300 MHz, $CDCl_3$) δ1.43 - 1.51 (m, 2H), 1.57 - 1.62 (m, 2H), 1.70 - 1.75 (m, 2H), 1.81 (d, *J*= 4.03 Hz, 4H), 1.84 - 1.91 (m, 2H), 2.10 - 2.19 (m, 4H), 2.31 (s, 3H), 2.52 (dd, *J*= 13.72, 6.40 Hz, 12H), 2.63 - 2.72 (m, 8H), 3.41 (dd, *J*= 11.71, 5.85 Hz, 1H), 3.50 - 3.56 (m, 2H),3.59 - 3.66 (m, 4H), 3.90 (s, 3H), 4.19 (t, *J*= 6.77 Hz, 2H), 6.92 - 6.96 (m, 1H), 7.00 (s, 1H). ESI $[M+H]^+$:595.52.

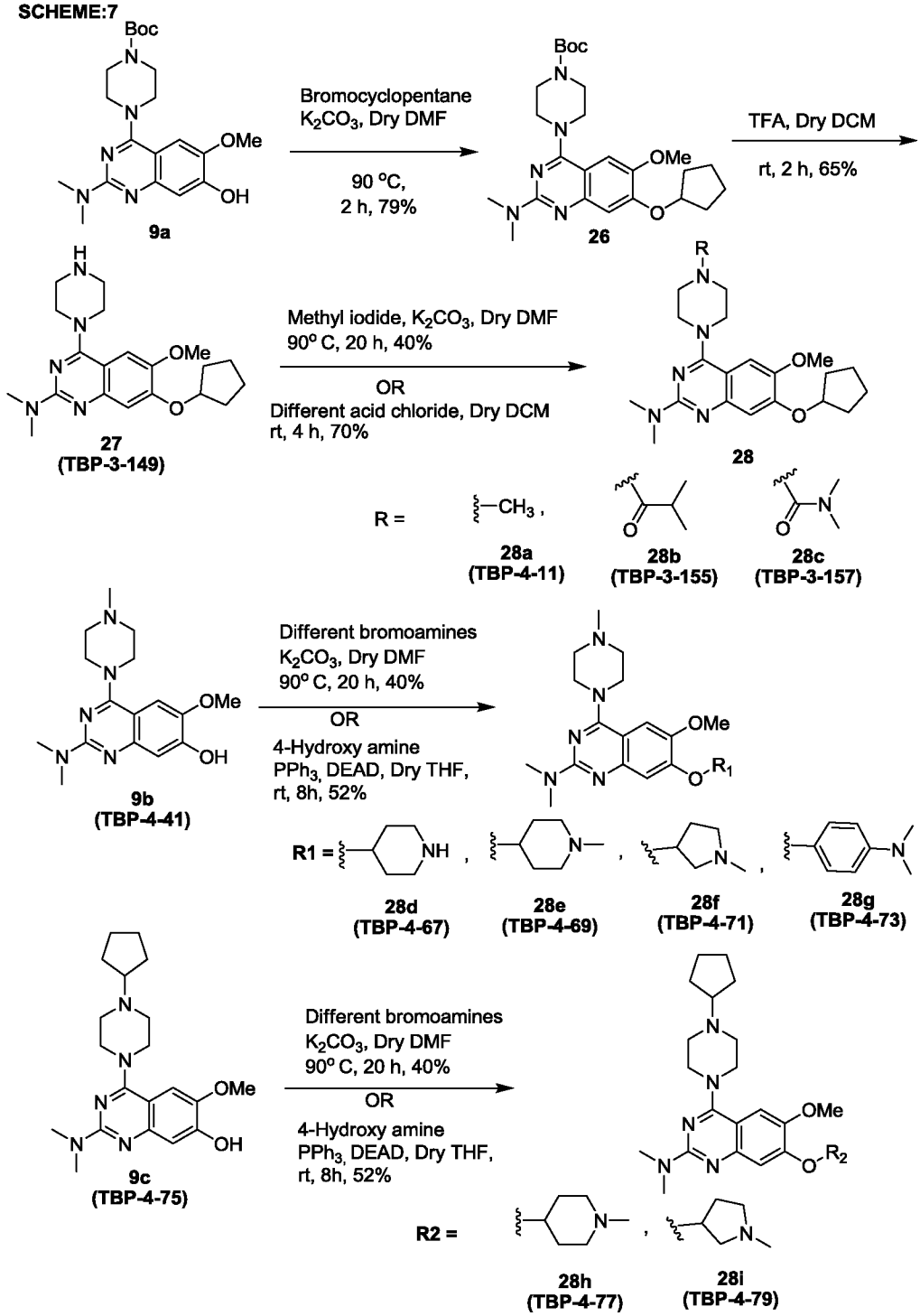
N-(3-(1H-Imidazol-1-yl)propyl)-4-(4-cyclopentylpiperazin-1-yl)-6-methoxy-7-(3-(pyrrolidin-1-yl)propoxy)quinazolin-2-amine (25d):^{1}H NMR (300 MHz, $CDCl_3$) δ1.44 (d, *J*= 8.29 Hz, 2H), 1.60 (m, 2H), 1.73 (m, 2H), 1.83 (m, 4H), 1.91 (m, 2H), 2.07 - 2.18 (m, 4H), 2.30 (d, *J*= 6.03 Hz, 4H), 2.56 - 2.73 (m, 8H), 3.48 (d, *J*= 6.03 Hz, 3H), 3.66 (br. s, 4H), 3.85 - 3.94 (m, 3H), 4.09 (t, *J*= 6.78 Hz, 2H), 4.20 (t, *J*=6.59 Hz, 2H), 6.92 (s, 1H), 6.98 (d, *J*= 6.03 Hz, 2H), 7.06 (s, 1H), 7.53 (s, 1H). ESI $[M+H]^+$:563.40.

4-(4-Cyclopentylpiperazin-1-yl)-6-methoxy-2-(4-methylpiperazin-1-yl)-7-(3-(pyrrolidin-1-yl)propoxy)quinazoline (25e): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38 - 1.50 (m, 2H), 1.51 - 1.63 (m, 2H), 1.65-1.74 (m, 2H), 1.74-1.81 (m, 4H), 1.87-1.93 (m,

2H), 2.12 (dd,*J*= 14.18, 7.04 Hz, 2H), 2.22 - 2.30 (m, 2H), 2.33 (s, 3H), 2.48 (d, *J*= 4.57 Hz, 4H), 2.53 (br. s, 4H), 2.62 (d, *J*= 7.68 Hz, 2H), 2.66 (d, *J*=5.12 Hz, 4H), 2.85 - 2.91 (m, 1H), 3.45 (s, 1H), 3.60 (br. s, 3H), 3.85 (br. s, 2H), 3.87 (s, 3H), 4.16 (t, *J*= 6.59 Hz, 2H), 6.92 (s, 1H), 6.97 (s, 1H). ESI $[M+H]^+$:538.62.

Example 7

SCHEME:7



***t*-Butyl-4-(2-(dimethylamino)-7-hydroxy-6-methoxyquinazolin-4-yl)piperazine-1-carboxylate (26):** Compound**26** was synthesized according to the procedure of compound **10** was synthesized as a yellow solid.^{1}H NMR (300 MHz, $CDCl_3$) δ 1.50 (s, 9H), 1.58-1.66 (m, 2H), 1.81-1.87 (m, 2H), 1.20-1.95 (m, 2H), 2.04-2.08 (m, 2H), 3.23 (s, 6H), 3.52-3.56 (m, 4H), 3.61-3.65 (m, 4H), 3.87 (s, 3H), 4.89-4.94 (m, 1H), 6.96 (s, 2H). ESI $[M+H]^+$:472.37.

7-(Cyclopentyloxy)-6-methoxy-N,N-dimethyl-4-(piperazin-1-yl)quinazolin-2-amine(27): Compound**27** was synthesized in the same way as compound **12** was synthesized.^{1}H NMR (300 MHz, $CDCl_3$) δ 1.62 - 1.67 (m, 2H), 1.81-1.86 (m, 2H),1.90 - 1.97 (m, 2H), 2.04 - 2.08 (m, 2H), 2.98 - 3.00 (m, 1H), 3.12 (t, *J*=9 Hz, 4H), 3.23 (s, 6H), 3.62 (t, *J*=9 Hz, 4H), 3.86 (s, 3H), 6.95 (s, 1H), 7.00 (s, 1H). ESI $[M+H]^+$:372.35.

7-(Cyclopentyloxy)-6-methoxy-N,N-dimethyl-4-(4-methylpiperazin-1-yl)quinazolin-2-amine(28a): Compound**28a** was synthesized in the same way as compound **13a and 13b** was synthesized.^{1}H NMR (300 MHz, $CDCl_3$) δ1.61-1.66 (m, 2H), 1.81-1.86 (m, 2H),1.90-1.97 (m, 2H),2.02-2.11 (m, 2H),2.37 (s, 3H),2.62 (t, *J*=9 Hz, 4H),3.23 (s, 6H),3.65 (t, *J*=9 Hz, 4H),3.87 (s, 3H),4.89-4.94 (m, 1H), 6.97 (s, 1H), 7.00 (s, 1H). ESI $[M+H]^+$:386.43.

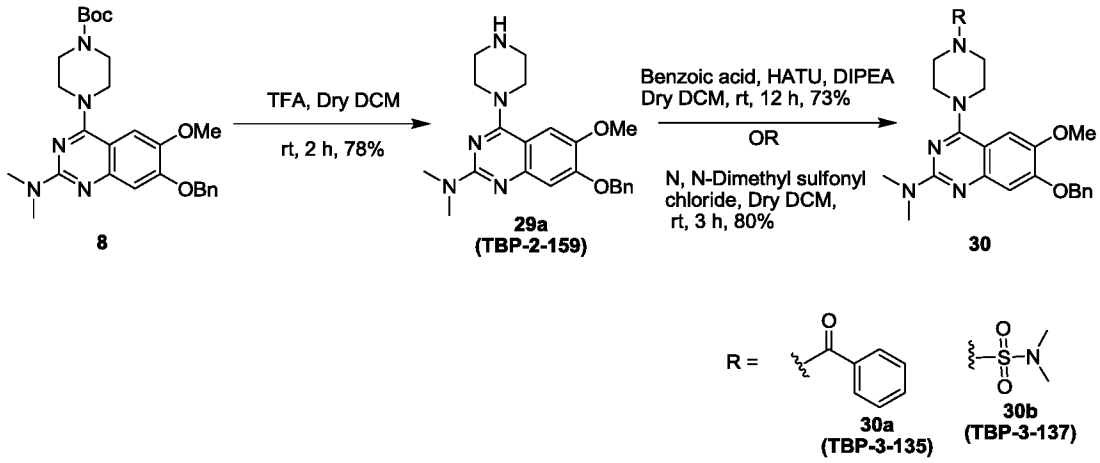
1-(4-(7-(Cyclopentyloxy)-2-(dimethylamino)-6-methoxyquinazolin-4-yl)piperazin-1-yl)-2-methylpropan-1-one(28b): Compound**28b** was synthesized according to the procedure of compound **13a and 13b** was synthesized.^{1}H NMR (300 MHz, $CDCl_3$) δ 1.15 (s, 3H),1.17 (s, 3H),1.61-1.66 (m, 2H),1.81-1.86 (m, 2H),1.90-2.01 (m, 2H),2.01-2.08 (m, 2H),2.80-2.89 (m, 1H),3.22 (s, 6H),3.54-3.60 (m, 4H),3.73 (t,*J*=9 Hz, 2H),3.83 (t, *J*=9 Hz, 2H),3.87 (s, 3H),4.90-4.92 (m, 1H),6.93 (s, 1H), 6.95 (s, 1H). ESI $[M+H]^+$:442.40.

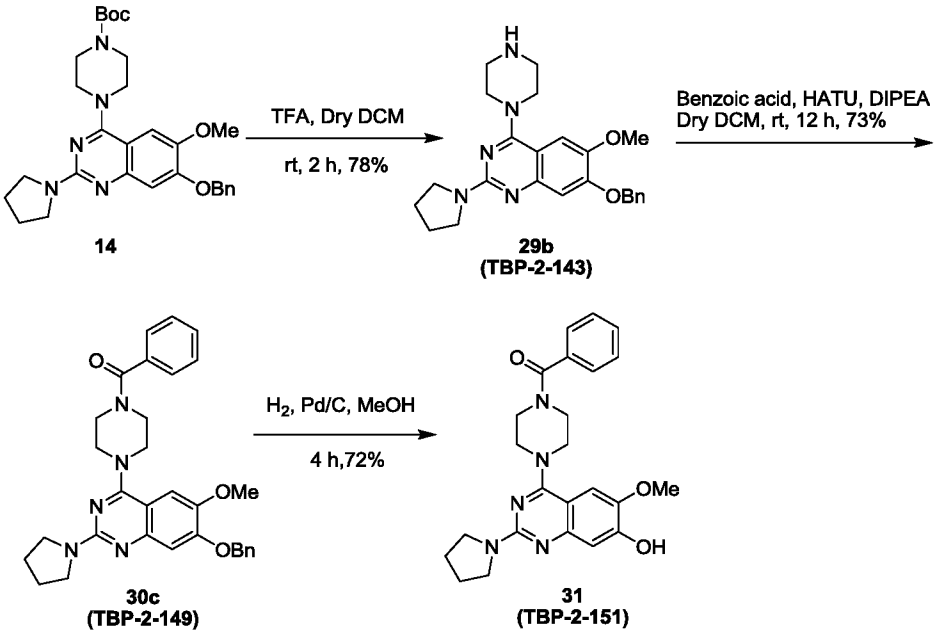
4-(7-(Cyclopentyloxy)-2-(dimethylamino)-6-methoxyquinazolin-4-yl)-N,N-dimethylpiperazine-1-carboxamide(28c): Compound**28c** was synthesized in the same way as compound **13a and 13b** was synthesized.^{1}H NMR (300 MHz, $CDCl_3$) δ 1.61-

1.65 (m, 2H), 1.80-1.87 (m, 2H),1.90-1.97 (m, 2H),2.03-2.07 (m, 2H),2.88 (s, 6H),3.21 (s, 6H),3.41-3.44 (m, 4H),3.55-3.59 (m, 4H),3.86 (s, 3H),4.88-4.93 (m, 1H),6.92 (s, 1H), 6.96 (s, 1H). ESI $[M+H]^+$:443.36.

Example 8

SCHEME:8





7-(Benzyloxy)-6-methoxy-N,N-dimethyl-4-(piperazin-1-yl)quinazolin-2-amine(29a): Compound**29a** was synthesized according to the procedure of compound **12**.^{1}H NMR (300 MHz, $CDCl_3$) δ2.39 (s, 2H),3.13 (t,*J*=9 Hz, 2H),3.23 (s, 6H),3.63 (t, *J*=9 Hz, 4H),3.92 (s, 3H),5.25 (s, 2H),7.00 (s, 1H),7.08 (s, 1H),7.31- 7.43 (m, 3H), 7.50 (d, *J*=9 Hz, 2H). ESI $[M+H]^+$:394.42.

(4-(7-(Benzyloxy)-2-(dimethylamino)-6-methoxyquinazolin-4-yl)piperazin-1-yl)(phenyl)methanone(30a): Compound**30a** was synthesized in the same way as described for compound **13c and 13f**.^{1}H NMR (300 MHz, $CDCl_3$) δ 3.23 (s, 6H),3.68 (br. s, 8H),3.91 (s, 3H),5.25 (s, 2H),6.99 (s, 1H),7.05 (s, 1H), 7.34-7.51 (m, 10H).ESI $[M+H]^+$:498.35.

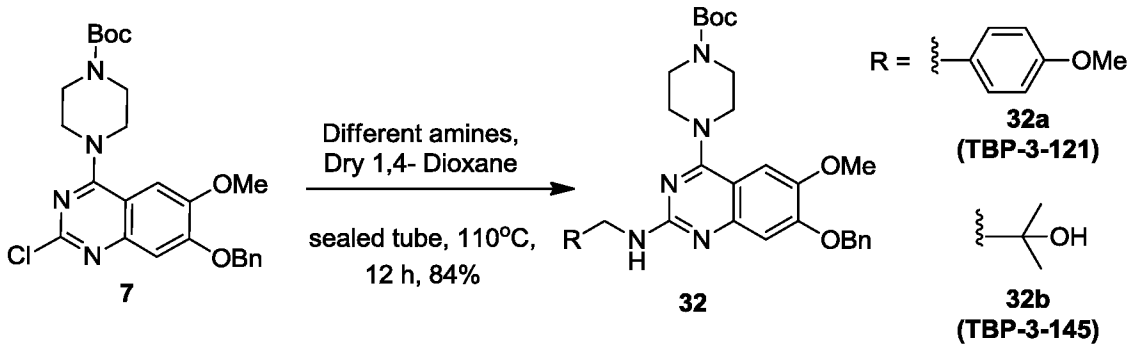
4-(7-(Benzyloxy)-2-(dimethylamino)-6-methoxyquinazolin-4-yl)-N,N-dimethylpiperazine-1-sulfonamide(30b): Compound**30b** was synthesized according to the procedure of compound **13a and 13b**. ^{1}H NMR (300 MHz, $CDCl_3$) δ2.87 (s, 6H),3.21 (s, 6H), 3.44 (t,*J*=9 Hz, 4H),3.62 (t,*J*=9 Hz, 4H),3.90 (s, 3H),5.23 (s, 2H),6.95 (s, 1H),7.02 (s, 1H),7.32-7.41 (m, 3H), 7.46-7.49 (m, 2H).ESI $[M+H]^+$:501.42.

7-(Benzyloxy)-6-methoxy-4-(piperazin-1-yl)-2-(pyrrolidin-1-yl)quinazoline(30c): Compound**30c** was synthesized in the same way as compound **12** was synthesized. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.85-1.88 (m, 2H), 3.17-3.19 (m, 2H),3.47 (t, *J*= 9 Hz, 3H),3.73-3.78 (m, 3H), 4.12 (s, 8H),5.10 (s, 2H),6.88 (s, 1H),7.00 (s, 1H),7.20-7.25 (m, 5H), 7.28-7.33 (m, 5H).ESI $[M+H]^+$:524.47.

(4-(7-(Benzyloxy)-6-methoxy-2-(pyrrolidin-1-yl)quinazolin-4-yl)piperazin-1-yl)(phenyl)methanone(31): Compound**31** was synthesized in the same way as compound **13a and 13b** was synthesized.^{1}H NMR (300 MHz, $CDCl_3$) δ 1.97 (t, *J*= 6 Hz, 4H),3.66 (t, *J*= 6.3 Hz, 12H),3.87 (s, 3H),6.88 (s, 1H), 7.31 (s, 1H), 7.45 (s, 5H). EI-HRMS$[M]^+$: Calculated: 433.2114. Found: 433.2115.

Example 9

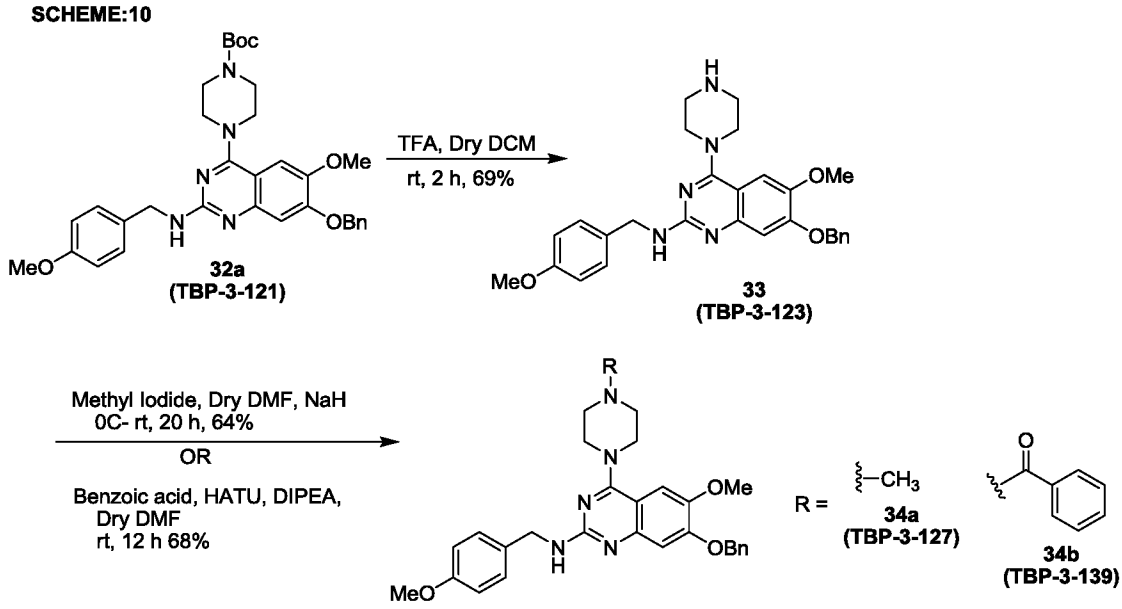
SCHEME:9



***t*-Butyl-4-(7-(benzyloxy)-6-methoxy-2-((4-methoxybenzyl)amino)quinazolin-4-yl)piperazine-1-carboxylate(32a):** Compound**31a** was synthesized in the same way as compound **14** was synthesized.^{1}H NMR (300 MHz, $CDCl_3$) δ 1.5 (s, 9H), 3.56 (d, *J*=6 Hz, 4H),3.61 (d,*J*=6 Hz, 4H),3.81 (s, 3H),3.93 (s, 3H),4.61 (d, *J*=6 Hz, 2H),5.25 (s, 2H),6.86 (s, 1H),6.89 (s, 1H),7.01 (s, 2H),7.30-7.33 (m, 2H),7.36-7.43 (m, 3H), 7.48-7.51 (m, 2H).EI-HRMS $[M]^+$: Calculated 585.2951. Found: 585.2945.

***t*-Butyl-4-(7-(benzyloxy)-2-((2-hydroxy-2-methylpropyl)amino)-6-methoxyquinazolin-4-yl)piperazine-1-carboxylate(32b):** Compound**31b** was synthesized according to the procedure of compound **14**.^{1}H NMR (300 MHz, $CDCl_3$) δ1.26 (s, 6H), 1.47 (s, 9H),3.42 (d, *J*=6 Hz, 2H),3.53 (t,*J*= 9 Hz, 4H),3.62 (t, *J*= 9 Hz, 4H),3.87 (s, 3H),5.16 (s, 2H),6.95 (s, 1H),6.96 (s, 1H),7.30-7.39 (m, 3H), 7.43-7.46 (m, 2H).EI-HRMS $[M]^+$: Calculated 537.2951. Found: 537.2950.

Example 10

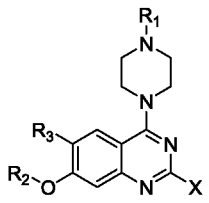


7-(Benzyloxy)-6-methoxy-2-(4-methoxyphenethyl)-4-(piperazin-1-yl)quinazoline(33): Compound**33** was synthesized in the same way as compound **12** was synthesized.^{1}H NMR (300 MHz, $CDCl_3$) δ 3.60 (t, *J*= 9 Hz, 4H), 3.70 (t, *J*= 9 Hz, 4H), 3.80 (s, 3H), 3.92 (s, 3H),4.60 (d, *J*= 6 Hz, 2H),5.24 (s, 2H),6.85 (s, 1H),6.88(s, 1H),7.02 (d, *J*= 6 Hz, 2H),7.29-7.43 (m, 5H), 7.49(d, *J*= 6 Hz, 2H). ESI $[M+H]^+$:486.44.

7-(Benzyloxy)-6-methoxy-2-(4-methoxyphenethyl)-4-(4-methylpiperazin-1-yl)quinazoline(34a): Compound**34a** was synthesized in the same way as described for compound **13a and 13b**.^{1}H NMR (300 MHz, $CDCl_3$) δ2.37 (s, 3H), 2.57 (t, *J*= 9 Hz, 4H), 3.76 (t, *J*= 9 Hz, 4H), 3.80 (s, 3H), 3.91 (s, 3H), 4.60 (d, *J*=6 Hz, 2H), 5.25 (s, 2H), 6.85 (s, 1H), 6.88 (s, 1H), 7.01-7.05 (m,2H),7.29-7.43 (m, 5H), 7.48-7.51 (m, 2H). EI-HRMS $[M]^+$: Calculated 499.2583. Found: 499.2584.

(4-(7-(Benzyloxy)-6-methoxy-2-(4-methoxyphenethyl)quinazolin-4-yl)piperazin-1-yl)(phenyl)methanone(34b): Compound**33b** was synthesized according to the

procedure ofcompound **13c and 13f** .^{1}H NMR (300 MHz, $CDCl_3$) δ 2.72 (br. s, 4H),3.51 (t, *J*=6 Hz, 2H), 3.80 (br. s, 3H), 3.89-3.94 (m, 5H), 4.61 (d, *J*= 6 Hz, 2H), 5.30 (s, 2H), 6.84 (s, 1H), 6.86 (s, 1H), 6.96 (s, 1H), 7.17 (s, 1H), 7.30 (s, 1H), 7.34-7.40 (m, 3H), 7.44-7.49 (m, 5H), 7.51-7.55 (m, 2H), 8.17-8.20 (m, 1H). ESI [M+H]$^+$:509.38.



Formula (I)

Table 1

Quinazoline with formula (I) composition of the Invention

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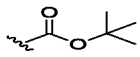
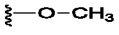
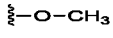
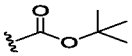
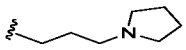
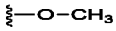
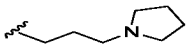
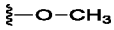
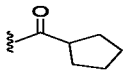
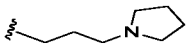
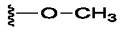
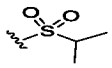
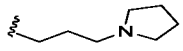
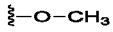
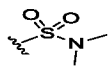
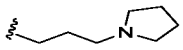
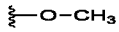
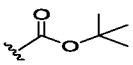
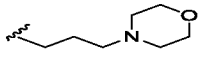
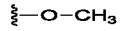
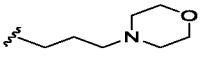
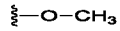
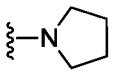
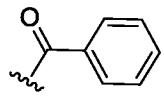
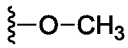
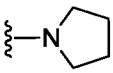
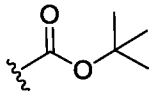
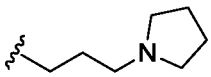
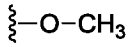
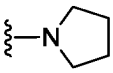
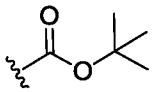
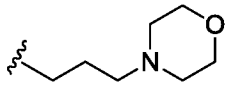
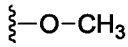
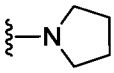
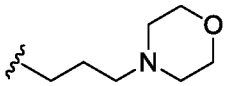
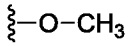
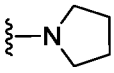
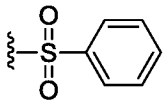
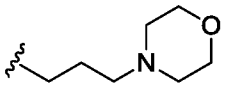
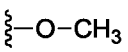
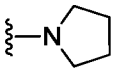
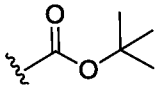
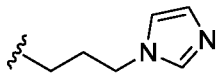
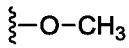
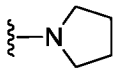
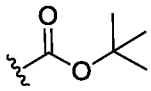
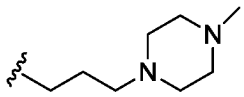
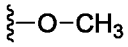
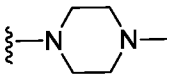
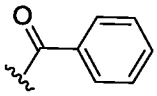
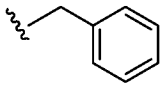
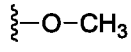
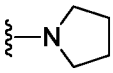
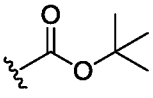
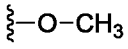
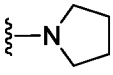
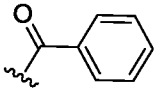
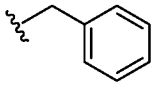
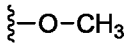
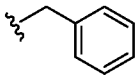
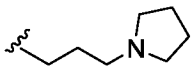
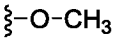
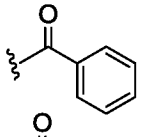
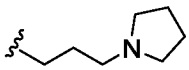
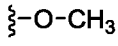
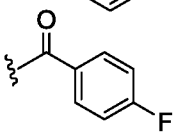
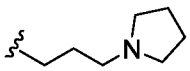
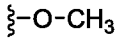
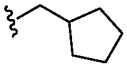
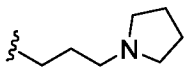
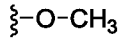
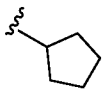
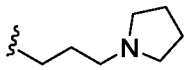
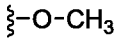
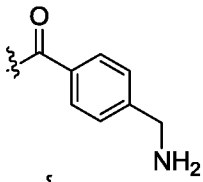
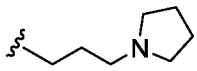
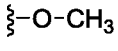
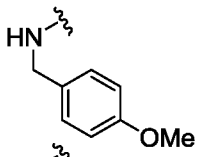
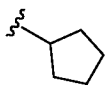
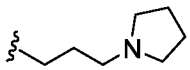
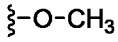
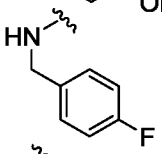
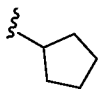
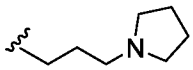
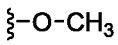
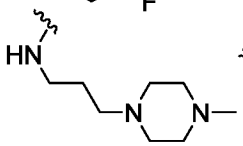
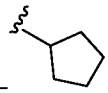
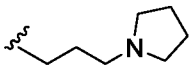
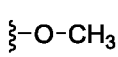
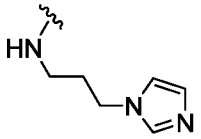
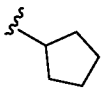
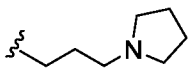
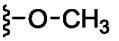
TBP-2-71				
TBP-2-169				
TBP-2-79				
TBP-2-83				
TBP-2-117				
TBP-2-93				
TBP-2-121				
TBP-2-165				
TBP-2-175				

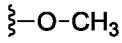
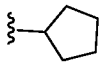
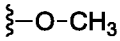
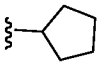
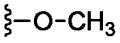
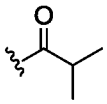
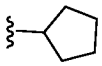
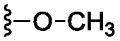
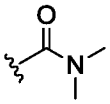
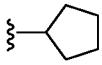
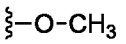
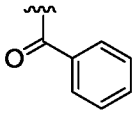
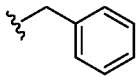
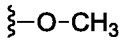
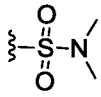
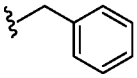
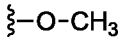
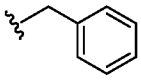
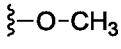
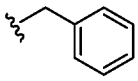
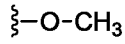
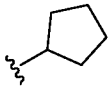
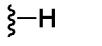
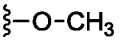
Quinazoline with formula (I) composition of the Invention

ID	X	R1	R2	R3
TBP-2-151				
TBP-2-145				
TBP-2-173				
TBP-2-179				
TBP-2-185				
TBP-2-191				
TBP-2-189				
TBP-1-69				
TBP-2-135				
TBP-2-149				
TBP-3-59				
TBP-3-71				
TBP-3-57				

Quinazoline with formula (I) composition of the Invention

ID	X	R1	R2	R3
TBP-3-67				
TBP-3-75				
TBP-4-81				
TBP-3-79				
TBP-3-73				
TBP-3-113				
TBP-3-91				
TBP-3-93				
TBP-3-95				
TBP-3-97				
TBP-3-99				
TBP-3-69				

Quinazoline with formula (I) composition of the Invention

ID	X	R1	R2	R3
TBP-4-9				
TBP-4-11				
TBP-3-149				
TBP-3-155				
TBP-3-157				
TBP-3-135				
TBP-3-137				
TBP-4-7				
TBP-2-159				
TBP-4-75				
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Quinazoline with formula (I) composition of the Invention

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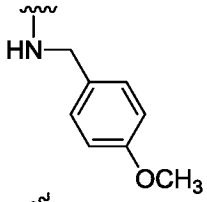
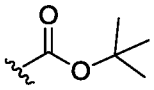
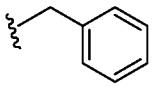
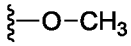
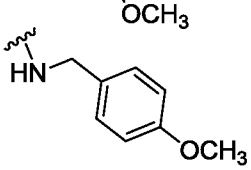
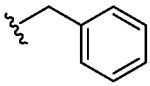
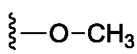
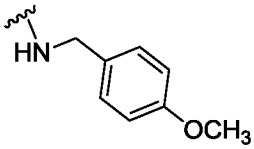
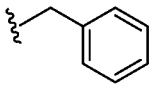
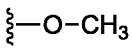
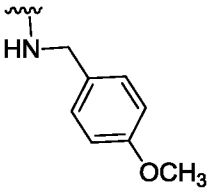
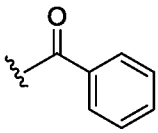
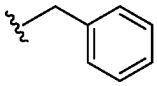
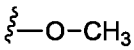
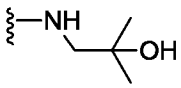
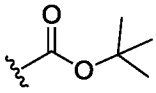
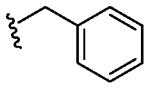
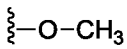
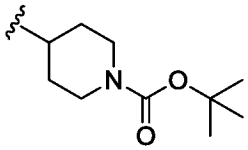
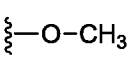
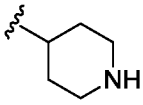
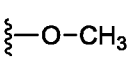
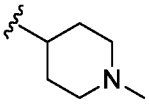
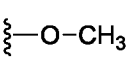
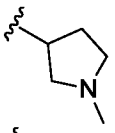
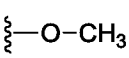
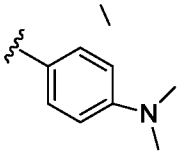
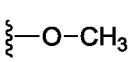
TBP-3-121				
TBP-3-123				
TBP-3-127				
TBP-3-139				
TBP-3-145				
TBP-4-65				
TBP-4-67				
TBP-4-69				
TBP-4-71				
TBP-4-73				

Example 11:

Experimental procedure for screening toll-like receptor 9 antagonistic activity

To screen the synthesized small molecules based on quinazoline scaffolds for toll-like receptor 9 (TLR9) antagonisms, we designed a medium throughput biological assay based on toll-like receptor 9 activation in primary human immune cells. Among the immune cell subsets circulating in the peripheral blood, TLR9 has significant expression in plasmacytoid dendritic cells (PDCs) and B lymphocytes. Among these two cell subsets,plasmacytoid dendritic cells are capable of producing type I interferons (e.g. IFN-alpha) in response to TLR9 ligands. Type A and type B unmethylated cytosine-guanine rich DNA oligonucleotides (CpG oligonucleotides) are the bona fide ligands for TLR9.

Example 12**:**

We established that IFN-alpha production from human peripheral blood mononuclear cells in response to type A CpG oligonucleotides (CpGA) almost exclusively results from TLR9 triggering on the PDCs (data not shown). Based on this principle we designed our screening assay where we isolated peripheral blood mononuclear cells (PBMCs) from venous blood collected from healthy donors using density gradient centrifugation. Then we cultured the PBMCs at 2-3*10^5 cells/200ul/well in a 96 well plate. We added the TLR9 agonist CpGA at 1uM in presence of escalating doses of the synthesized small molecules (0uM, 0.1uM, 1uM, 5uM, 10uM and 20uM). After overnight culture we collected the supernatants from the culture wells and looked for IFN-alpha using enzyme linked immunosorbent assay (ELISA). Molecules having TLR9 antagonistic activity inhibited IFN-alpha production in this screening assay.

Example 13**:**

The structural evolution of the successively synthesized molecules was rationalized using the IFN-alpha inhibition data as depicted in the figures 1. A number of

compounds with formula (I) were found to be efficient at antagonizing TLR9 activation at nanomolar concentration (Figures 1).

Example 14:

Experimental Procedure for TLR9 antagonism in primary human pDC.

To screen the synthesized small molecules based on quinazoline scaffolds for toll-like receptor 9 antagonism, we designed a medium throughput biological assay based on toll-like receptor 9 activation in plasmacytoid dendritic cells (pDC), which were isolated from PBMCs of healthy donors. pDCs were isolated from PBMCs by magnetic immunoselection using anti-BDCA4 microbeads. The isolated pDCs were then cultured at 3*10^4 cells/100µl/well in a 96 well plate. We added the TLR9 agonist CpGA at 500nM in presence of escalating doses of the synthesized small molecules. After overnight culture we collected the supernatants from the culture wells and looked for IFN-alpha using enzyme linked immunosorbent assay (ELISA). Molecules having TLR9 antagonistic activity inhibited IFN-alpha production in this screening assay (Figure 2).

Example 15:

Experimental Procedure for TLR9 Reporter assay

The synthesized small molecules based on quinazoline scaffolds were screened for TLR9 antagonism using a HEK-Blue-hTLR9 Secreted Alkaline Phosphatase (SEAP) reporter assay. Reporter HEK cell lines expressing human TLR9 along with a NF-κB promoter driven secreted embryonic alkaline phosphatase (SEAP) reporter gene were used. 70,000 cells per well were incubated overnight at 37^0C and 5% CO_2 in a 96 well plate in complete DMEM medium supplemented with 100µg/ml Normocin. After incubation, the TLR9 agonist CpGB was added to the wells at a concentration of 1µM in presence of escalating doses of the synthesized small molecules and incubated at 37^0C and 5% CO_2 for 24 hours. After incubation of the HEK cells, supernatants were collected and 20µl of supernatant was added to wells containing 200µl of Quanti-Blue

Detection media. After 2 hours of further incubation, OD values were taken at 620nm in a spectrophotometer. Molecules having TLR9 antagonistic activity inhibited TLR9-mediated NF-kB activation in a dose-dependent manner (Figure 3).

Example 16:

Experimental procedure for screening for cytotoxicity of the identified TLR9 antagonists

MTT assay is a colorimetric assay for assessing cell viability. It is widely used for screening drugs and testing their cytotoxicity. NAD(P)H-dependent cellular oxidoreductase enzymes may, under defined conditions, reflect the number of viable cells present. These enzymes are capable of reducing the tetrazolium dye MTT 3-(4, 5dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide to its insoluble formazan, which has a purple colour. Viable cells with active metabolism convert MTT into a purple coloredformazan product with an absorbance maximum near 570 nm. When cells die, they lose the ability to convert MTT into formazan, thus colour formation serves as a useful and convenient marker of only the viable cells. The exact cellular mechanism of MTT reduction into formazan is not well understood, but likely involves reaction with NADH or similar reducing molecules that transfer electrons to MTT.

Example 17:

To check cytotoxicity of the synthesized TLR9 antagonists, HepG2 (a hepatic epithelial cell line) and SW480 (an intestinal mucosal epithelial cell line) cells were cultured in DMEM Complete media in 96 well plates at density of 30,000 cells per well, making a final volume of 100 µl/well. Treatment with different concentrations (0.1, 0.5,1, 10, 20 and 100 µM) of different candidate small molecule antagonists was added. Plates were incubated for 24 hours at 37deg C and 5% CO2 in incubator. After 24 hrs 50µl of MTT (5mg/ml) was added to each well and further incubated for 1 to 4 hours at 37°C. Then 100µl of DMSO was added to each well and properly mixed to ensure complete solubilisation of formazan crystals. Then absorbance was measured at 570 nm using an ELISA plate reader. None of the identified TLR9 antagonists showed considerable cytotoxicity at concentrations below 100µM on this assay (Figure 4).

ADVANTAGES OF THE INVENTION

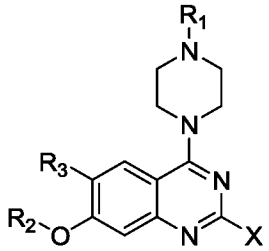
The main advantages of the present invention are:

The synthesized new compounds with general formula (I) of the present invention have several advantages.

1. The invention provides small molecules with general formula (I) which can effect immune stimulation via TLR9 antagonism.
2. The invention provides small molecules with general formula (I) which can inhibit immune stimulation via TLR9 antagonism.
3. The invention provides a medium throughput biological assays results involving human peripheral blood mononuclear cells, isolated human primary pDCs and reporter assay using transfected TLR9 cells to screen compounds with formula (I). All the three assays system was standardized and the results from all three assay systems can be correlated.
4. This invention provides compounds with formula (I) which can be used in a number of clinical contexts of autoreactive inflammation, including as pharmaceutical agents and methods for treating conditions involving unwanted immune activity in response to a suitable TLR ligand or TLR signalling agonist.

We Claim:

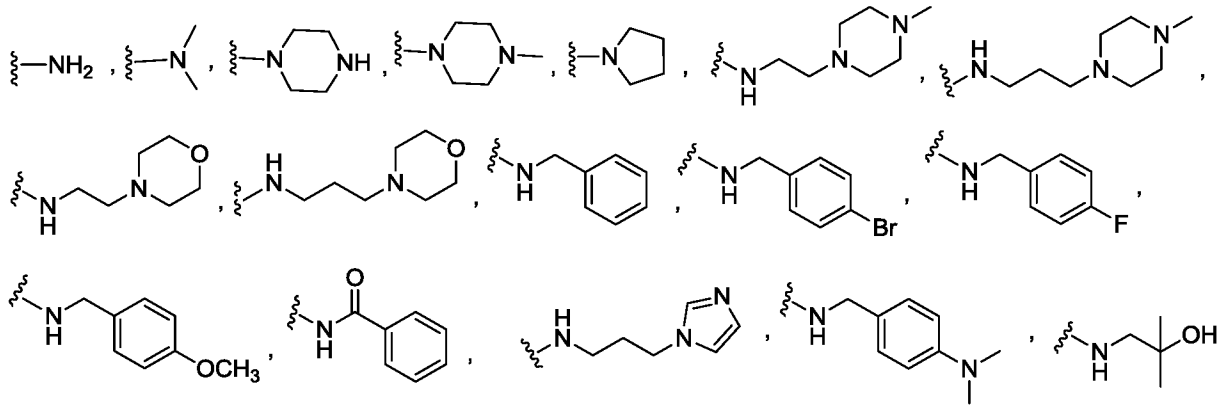
1. A compound of the general formula 1



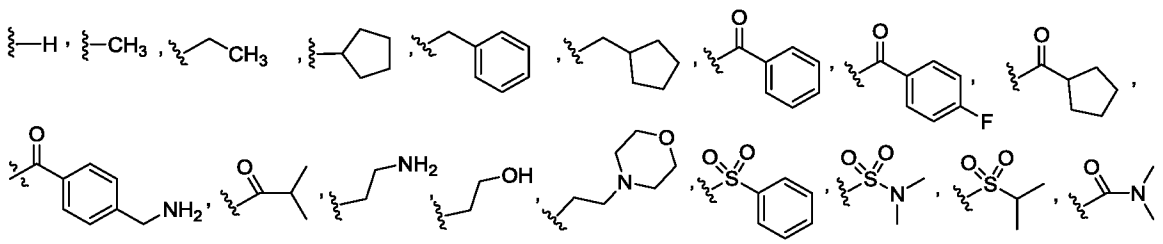
Formula (I)

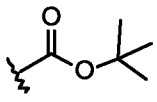
wherein

X is independently selected from groups referred to as follows:

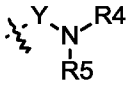


wherein R_1 is independently selected from groups referred to as follows:



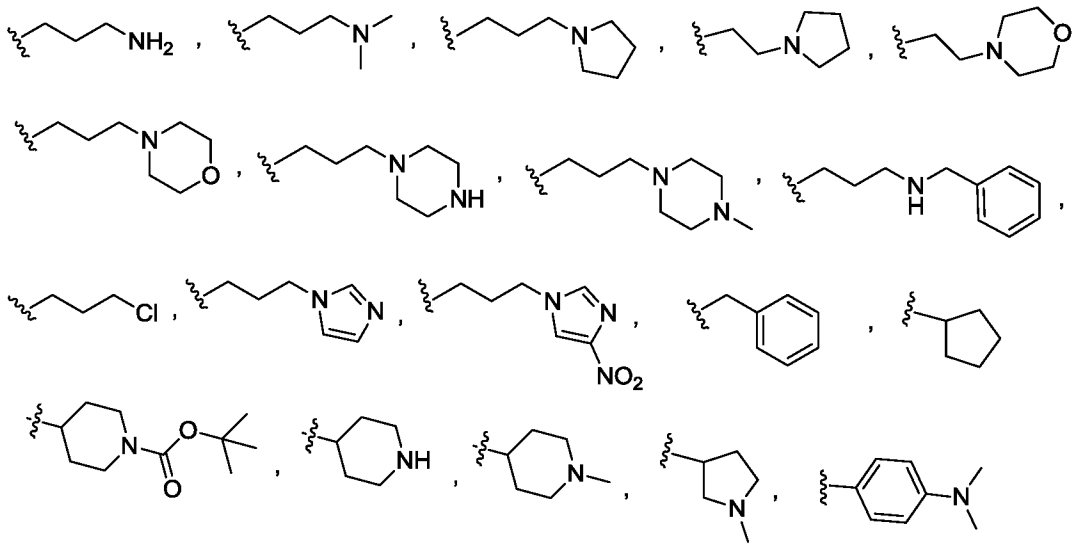


whereinR$_2$ is a group having structure

R2 =H, 

where Y is optionally substituted or unsubstituted C_0 to C_3 alkyl; R_5 and R_6 are independently hydrogen or substituted or unsubstituted alkyl or R_5 and R_6 is joined to form substituted or unsubstituted heterocycle.

wherein R_2 is independently selected from groups referred to as follows:



wherein R_3 is independently selected from groups referred to as hydrogen, -OH and $-OCH_3$ groups.

2. The compounds of general formula 1 as claimed in claim 1, wherein represented compounds encompassing:

4-(4-(Isopropylsulfonyl)piperazin-1-yl)-6-methoxy-N,N-dimethyl-7-(3-(pyrrolidin-1-yl)propoxy)quinazolin-2-amine**13a(TBP-2-93);**

4-(4-(Dimethylamino)-6-methoxy-7-(3-(pyrrolidin-1yl)propoxy)quinazolin-4-yl)-N,N-dimethylpiperazin-1-sulfonamide**13b(TBP-2-121);**

Cyclopentyl(4-(2-(dimethylamino)-6-methoxy-7-(3-(pyrrolidin-1yl)propoxy)quinazolin-4-yl)piperazine-1yl)methanone**13c(TBP-2-117);**

4-(4-(Cyclopentylmethyl)piperazin-1-yl)-6-methoxy-N,N-dimethyl-7-(3-(pyrrolidin-1yl)propoxy)quinazolin-2-amine **13d(TBP-3-79);**

4-(4-(Cyclopentylpiperazin-1-yl)-6-methoxy-N,N-dimethyl-7-(3-(pyrrolidin-1yl)propoxy)quinazolin-2-amine **13e(TBP-3-73);**

(4-(2-(Dimethylamino)-6-methoxy-7-(3-(pyrrolidin-1-yl)propoxy)quinazoline-4-yl)piperazin-1-yl)(phenyl)methanone **13f(TBP-3-75);**

4-(4-Benzylpiperazin-1-yl)-6-methoxy-N,N-dimethyl-7-(3-(pyrrolidin-1-yl)propoxy)quinazolin-2-amine **13g(TBP-3-67);**

(4-(Aminomethyl)phenyl)(4-(2-(dimethylamino)-6-methoxy-7-(3-(pyrrolidin-1-yl)propoxy)quinazolin-4-yl)piperazin-1-yl)methanone **13h (TBP-3-113);**

6-((4-(4-(2-(Dimethylamino)-6-methoxy-7-(3-(pyrrolidin-1-yl)propoxy)quinazolin-4-yl)piperazine-1-carbonyl)benzyl)amino)-6-oxohexanoic acid **13i (TBP-3-115);**

6-((4-(4-(2-(Dimethylamino)-6-methoxy-7-(3-(pyrrolidin-1-yl)propoxy)quinazolin-4-yl)piperazine-1-carbonyl)benzyl)amino)-6-oxohexanoic acid **13i (TBP-3-115);**

2-(4-(2-(dimethylamino)-6-methoxy-7-(3-(pyrrolidin-1-yl)propoxy)quinazolin-4-yl)piperazin-1-yl)-1-(4-fluorophenyl)ethanone**13j (TBP-4-81);**

t-Butyl-4-(6-methoxy-7-(3-morpholinpropoxy)-2-(pyrrolidin-1-yl)quinazoline-4-yl)piperazine-1-carboxylate **17a(TBP-2-173);**

t-Butyl-4-(6-methoxy-7-(3-(4-methylpiperazin-1-yl)propoxy)-2-(pyrrolidin-1-yl)quinazoline-4-yl)piperazine-1-carboxylate **17b(TBP-2-189);**

t-Butyl-4-(7-(3-(1H-imidazol-1-yl)propoxy)-6-methoxy-2-(pyrrolidin-1-yl)quinazolin-4-yl)piperazine-1-carboxylate **17c(TBP-2-191);**

t-Butyl-4-(7-(3-(1H-imidazol-1-yl)propoxy)-6-methoxy-2-(pyrrolidin-1-yl)quinazoline-4-yl)piperazine-1-carboxylate **17d(TBP-3-47);**

t-Butyl-4-(6-methoxy-2-(pyrrolidin-1-yl)-7-(3-(pyrrolidin-1-yl)propoxy)quinazolin-4-yl)piperazine-1-carboxylate **17e(TBP-2-145);**

4-(3-(6-Methoxy-4-(piperazin-1-yl)-2-(pyrrolidin-1-yl)quinazolin-7-yloxy)propyl)morpholine **18a(TBP-2-179);**

7-(3-(1H-Imidazol-1-yl)propoxy)-6-methoxy-4-(piperazin-1-yl)-2-(pyrrolidin-1-yl)quinazoline **18b(TBP-3-69);**

3-((6-Methoxy-4-(piperazin-1-yl)-2-(pyrrolidin-1-yl)quinazolin-7-yl)oxy)-N,N-dimethylpropan-1-amine **18c(TBP-3-49);**

4-(3-(6-Methoxy-4-(4-(phenylsulfonyl)piperazin-1-yl)-2-(pyrrolidin-1-yl)quinazolin-7-yloxy)propyl)morpholine **19(TBP-2-185);**

4-(4-Benzylpiperazin-1-yl)-6-methoxy-7-(3-(4-methylpiperazin-1-yl)propoxy)-2-(pyrrolidin-1-yl)quinazoline **20(TBP-3-57);**

3-(4-(4-Benzylpiperazin-1-yl)-6-methoxy-2-(pyrrolidin-1-yl)quinazolin-7-yloxy)-N,N-dimethylpropan-1-amine **21a(TBP-3-59);**

(4-(7-(3-(Dimethylamino)propoxy)-6-methoxy-2-(pyrrolidin-1-yl)quinazolin-4-yl)piperazin-1-yl)(phenyl)methanone **21b(TBP-3-71);**

4-(4-Cyclopentylpiperazin-1-yl)-6-methoxy-N-(4-methoxybenzyl)-7-(3-(pyrrolidin-1-yl)propoxy)quinazolin-2-amine **25a(TBP-3-91);**

4-(4-Cyclopentylpiperazin-1-yl)-N-(4-fluorobenzyl)-6-methoxy-7-(3-(pyrrolidin-1-yl)propoxy)quinazolin-2-amine **25b(TBP-3-93);**

4-(4-Cyclopentylpiperazin-1-yl)-6-methoxy-N-(3-(4-methylpiperazin-1-yl)propyl)-7-(3-(pyrrolidin-1-yl)propoxy)quinazolin-2-amine **25c(TBP-3-95);**

N-(3-(1H-Imidazol-1-yl)propyl)-4-(4-cyclopentylpiperazin-1-yl)-6-methoxy-7-(3-(pyrrolidin-1-yl)propoxy)quinazolin-2-amine **25d(TBP-3-97);**

4-(4-Cyclopentylpiperazin-1-yl)-6-methoxy-2-(4-methylpiperazin-1-yl)-7-(3-(pyrrolidin-1-yl)propoxy)quinazoline **25e(TBP-3-99);**

7-(Cyclopentyloxy)-6-methoxy-N,N-dimethyl-4-(piperazin-1-yl)quinazolin-2-amine **27 (TBP-3-149);**

7-(Cyclopentyloxy)-6-methoxy-N,N-dimethyl-4-(4-methylpiperazin-1-yl)quinazolin-2-amine **28a (TBP-4-11);**

1-(4-(7-(Cyclopentyloxy)-2-(dimethylamino)-6-methoxyquinazolin-4-yl)piperazin-1-yl)-2-methylpropan-1-one **28b (TBP-3-155);**

4-(7-(Cyclopentyloxy)-2-(dimethylamino)-6-methoxyquinazolin-4-yl)-N,N-dimethylpiperazine-1-carboxamide **28c (TBP-3-157);**

6-methoxy-N,N-dimethyl-4-(4-methylpiperazin-1-yl)-7-(piperidin-4-yloxy)quinazolin-2-amine**28d (TBP-4-67);**

6-methoxy-N,N-dimethyl-4-(4-methylpiperazin-1-yl)-7-((1-methylpiperidin-4-yl)oxy)quinazolin-2-amine**28e (TBP-4-69);**

6-methoxy-N,N-dimethyl-4-(4-methylpiperazin-1-yl)-7-((1-methylpyrrolidin-3-yl)oxy)quinazolin-2-amine **28f (TBP-4-71);**

7-(4-(dimethylamino)phenoxy)-6-methoxy-N,N-dimethyl-4-(4-methylpiperazin-1-yl)quinazolin-2-amine **28g (TBP-4-73);**

4-(4-cyclopentylpiperazin-1-yl)-6-methoxy-N,N-dimethyl-7-(1-methylpiperidin-4-yloxy)quinazolin-2-amine **28h (TBP-4-77);**

4-(4-cyclopentylpiperazin-1-yl)-6-methoxy-N,N-dimethyl-7-(1-methylpyrrolidin-3-yloxy)quinazolin-2-amine **28i (TBP-4-79);**

7-(Benzyloxy)-6-methoxy-N,N-dimethyl-4-(piperazin-1-yl)quinazolin-2-amine **29a (TBP-2-159);**

(4-(7-(Benzyloxy)-2-(dimethylamino)-6-methoxyquinazolin-4-yl)piperazin-1-yl)(phenyl)methanone **30a (TBP-3-135);**

4-(7-(Benzyloxy)-2-(dimethylamino)-6-methoxyquinazolin-4-yl)-N,N-dimethylpiperazine-1-sulfonamide **30b (TBP-3-137);**

7-(Benzyloxy)-6-methoxy-4-(piperazin-1-yl)-2-(pyrrolidin-1-yl)quinazoline **30c (TBP-2-149);**

(4-(7-(Benzyloxy)-6-methoxy-2-(pyrrolidin-1-yl)quinazolin-4-yl)piperazin-1-yl)(phenyl)methanone**31 (TBP-2-151);**

t-Butyl-4-(7-(benzyloxy)-6-methoxy-2-((4-methoxybenzyl)amino)quinazolin-4-yl)piperazine-1-carboxylate **32a (TBP-3-121);**

t-Butyl-4-(7-(benzyloxy)-2-((2-hydroxy-2-methylpropyl)amino)-6-methoxyquinazolin-4-yl)piperazine-1-carboxylate **32b (TBP-3-145);**

7-(Benzyloxy)-6-methoxy-2-(4-methoxyphenethyl)-4-(piperazin-1-yl)quinazoline**33(TBP-3-123);**

7-(Benzyloxy)-6-methoxy-2-(4-methoxyphenethyl)-4-(4-methylpiperazin-1-yl)quinazoline **34a (TBP-3-127);**

(4-(7-(Benzyloxy)-6-methoxy-2-(4-methoxyphenethyl)quinazolin-4-yl)piperazin-1-yl)(phenyl)methanone **34b (TBP-3-139).**

tert-butyl 4-(2-(dimethylamino)-7-hydroxy-6-methoxyquinazolin-4-yl)piperazine-carboxylate 9 (TBP-2-71),

4-(4-cyclopentylpiperazin-1-yl)-2-(dimethylamino)-6-methoxyquinazolin-7-ol (TBP-4-75),

tert-butyl 4-(7-hydroxy-6-methoxy-2-(pyrrolidin-1-yl)quinazolin-4-yl)piperazine-1-carboxylate 15 (TBP-2-135)

(4-(7-hydroxy-6-methoxy-2-(pyrrolidin-1-yl)quinazolin-4-yl)piperazin-1-yl)(phenyl)methanone (TBP-2-151),

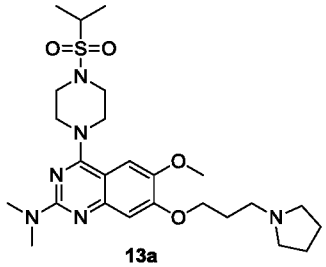
2-(dimethylamino)-6-methoxy-4-(4-methylpiperazin-1-yl)quinazolin-7-ol (TBP-4-9),

2-(dimethylamino)-6-methoxy-4-(piperazin-1-yl)quinazolin-7-ol (TBP-2-169),

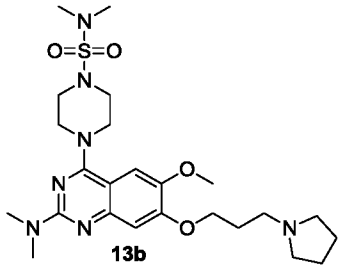
tert-butyl 4-(2-(dimethylamino)-6-methoxy-7-(3-morpholinopropoxy)quinazolin-4-yl)piperazine-1-carboxylate (TBP-2-165),

tert-butyl 4-(2-(dimethylamino)-6-methoxy-7-(3-(pyrrolidin-1-yl)propoxy)quinazolin-4-yl)piperazine-1-carboxylate 11 (TBP-2-79).

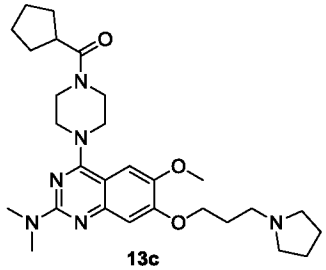
3. The compounds of general formula 1 as claimed in claim 1, wherein The structural formulae of the representative compounds are:



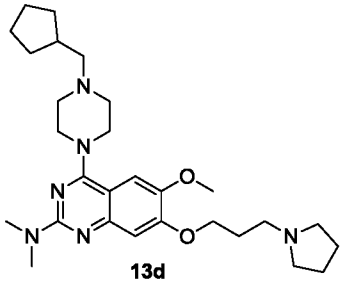
13a
(TBP-2-93)



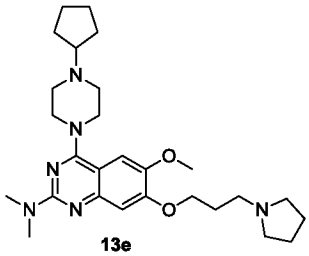
13b
(TBP-2-121)



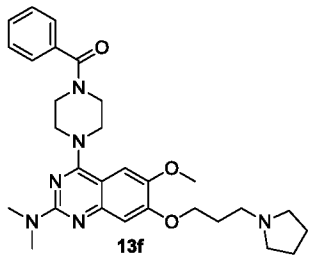
13c
(TBP-2-117)



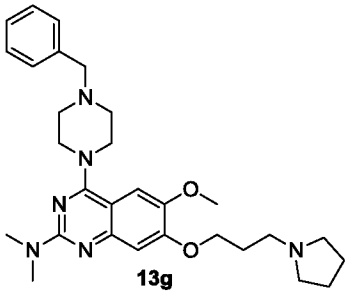
13d
(TBP-3-79)



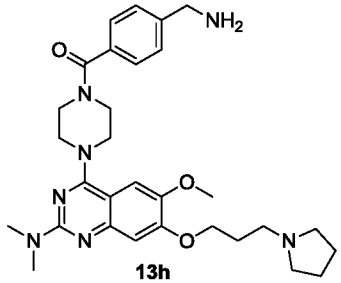
13e
(TBP-3-73)



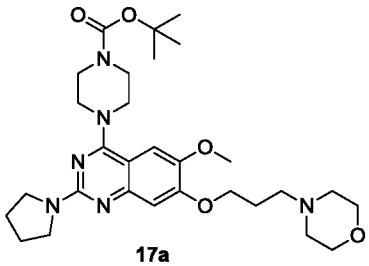
13f
(TBP-3-75)



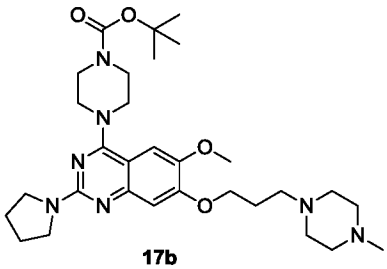
13g
(TBP-3-67)



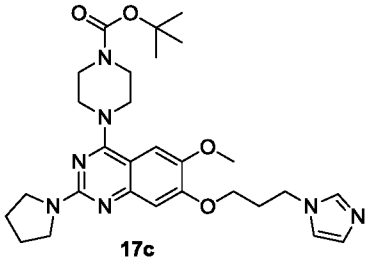
13h
TBP-3-113



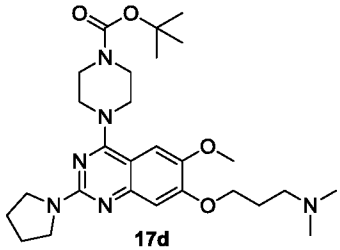
17a
(TBP-2-173)



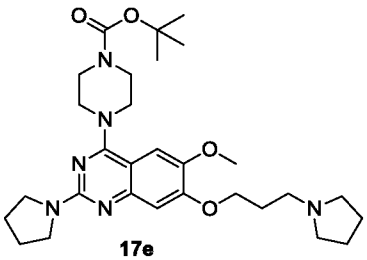
17b
(TBP-2-189)



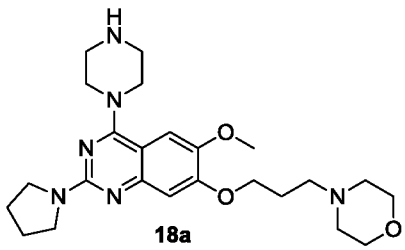
17c
(TBP-2-191)



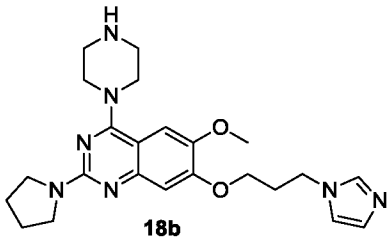
17d
(TBP-3-47)



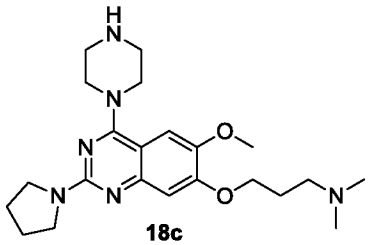
17e
(TBP-2-145)



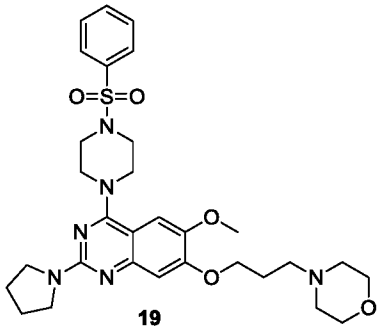
18a
(TBP-2-179)



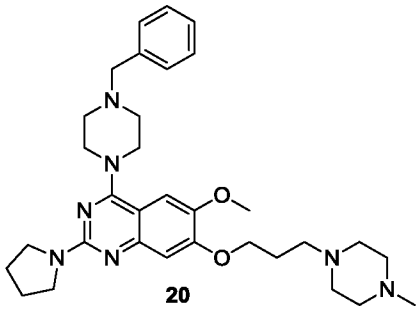
18b
(TBP-3-69)



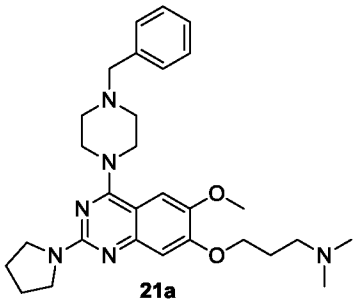
18c
(TBP-3-49)



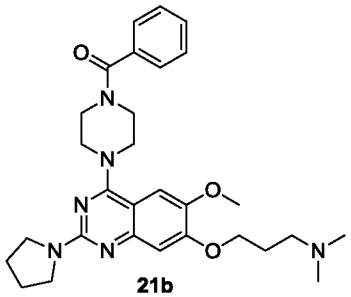
19
(TBP-2-185)



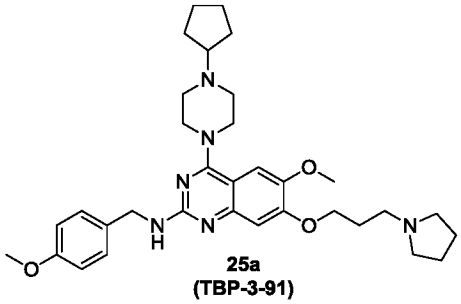
20
(TBP-3-57)



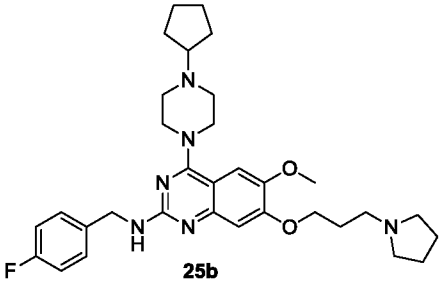
21a
(TBP-3-59)



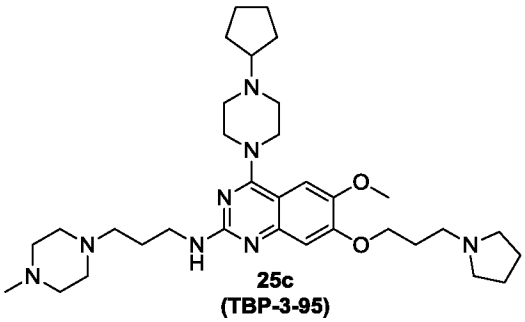
21b
(TBP-3-71)



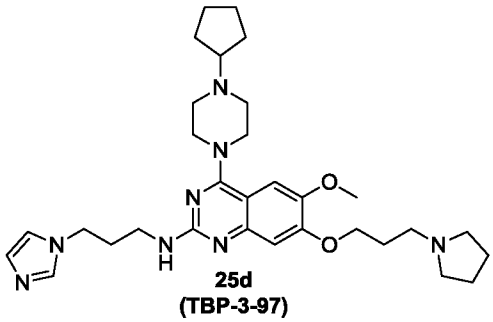
25a
(TBP-3-91)



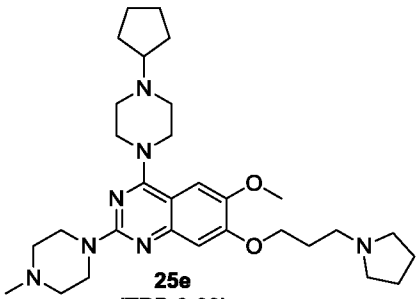
25b
(TBP-3-93)



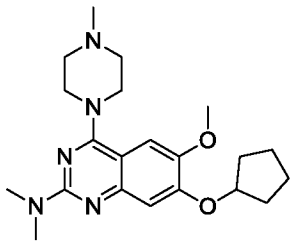
25c
(TBP-3-95)



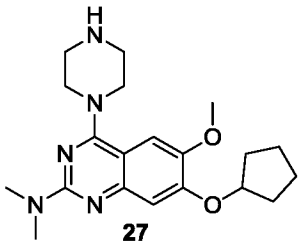
25d
(TBP-3-97)



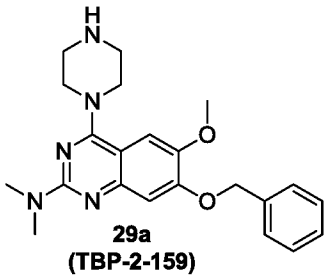
25e
(TBP-3-99)



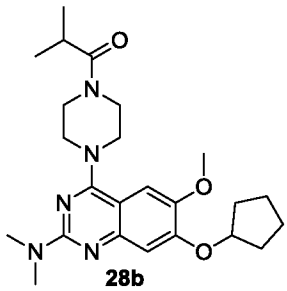
28a
(TBP-4-11)



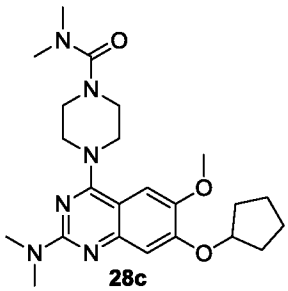
27
(TBP-3-149)



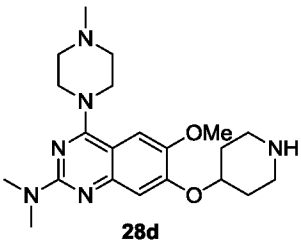
29a
(TBP-2-159)



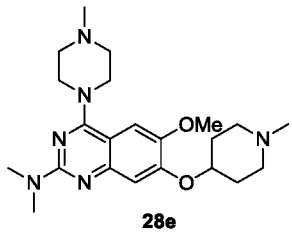
28b
(TBP-3-155)



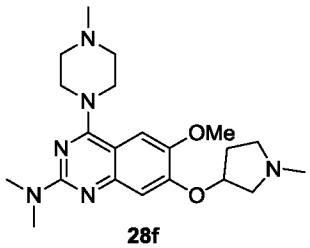
28c
(TBP-3-157)



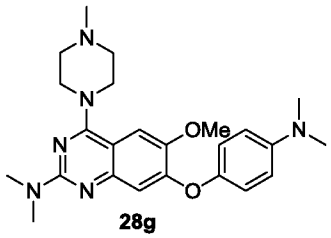
28d
(TBP-4-67)



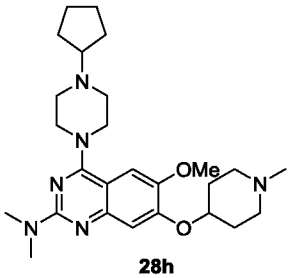
28e
(TBP-4-69)



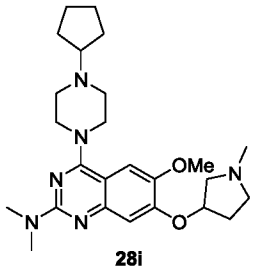
28f
(TBP-4-71)



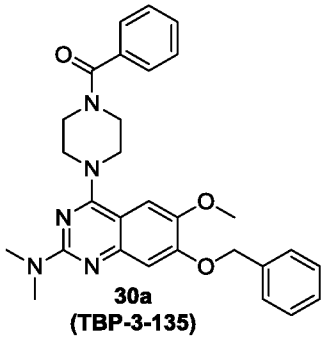
28g
(TBP-4-73)



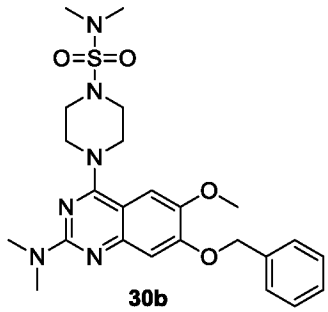
28h
(TBP-4-77)



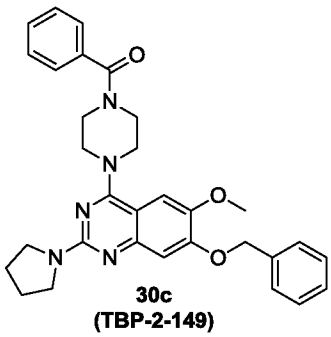
28i
(TBP-4-79)



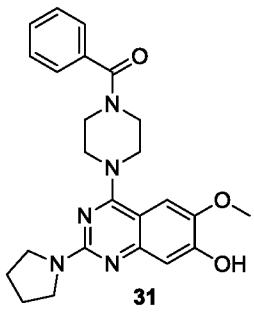
30a
(TBP-3-135)



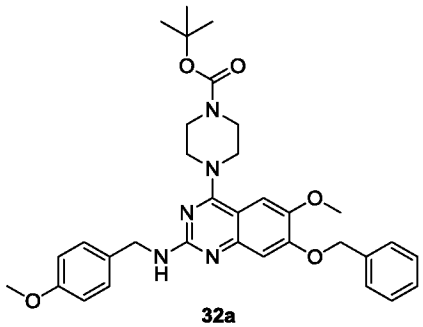
30b
(TBP-3-137)



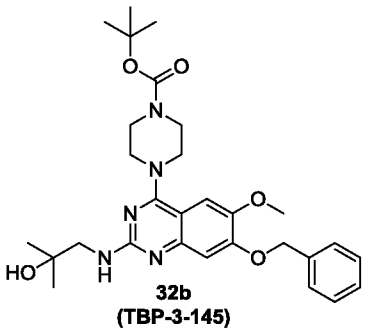
30c
(TBP-2-149)



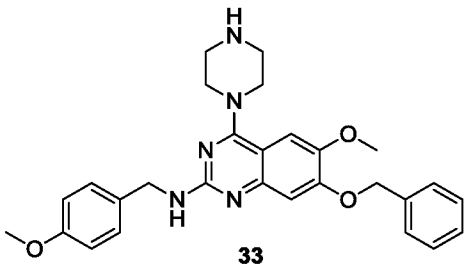
31
(TBP-2-151)



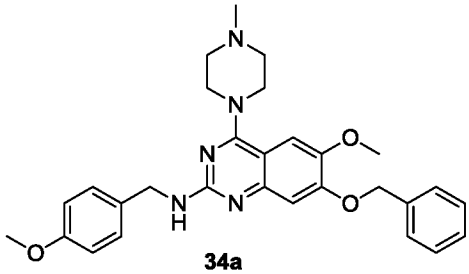
32a
(TBP-3-121)



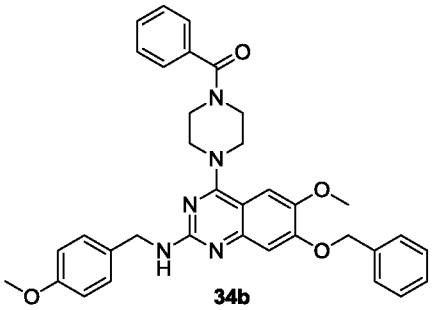
32b
(TBP-3-145)



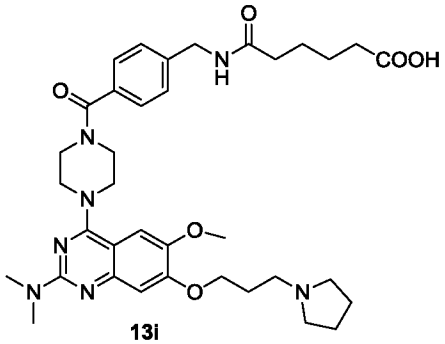
33
(TBP-3-123)



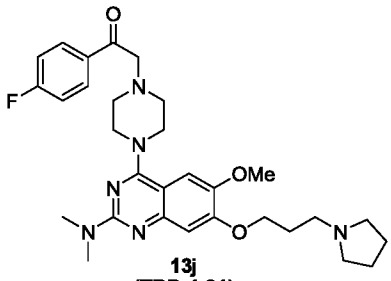
34a
(TBP-3-127)



34b
(TBP-3-139)



13i
TBP-3-115



13j
(TBP-4-81)

4.The process for the preparation of compounds of formula 1 as claimed in claim 1 comprising the steps of:

a. reacting compound of 6 with Boc-piperazxine to obtain compound 7;

b. reacting compound 7 obtained in step a) with amine to obtain compound of formula 8 or 14 or 32;

c. reacting compound 8 or 14 or 32 of step b) either with with hydrogen in presence of Pd/C to obtain compound of 9 or 15 or reacting with TFA to obtain 29a or 29b or 33 ;

d. reacting compound 9 or 15 of step c) either with 1-chloro-3-bromopropane or bromocyclopentane to obtain compound 10 or 26 or 16 ;

e. reacting compound 10 or 16 of step d) with amine to compound 11 or 17;

f. reacting compound 11 or 17 of step e) or compound 26 of step d) or compound 14 of step b) with TFA to obtain 12 or 18 or 27;

g. reacting compound 12obtained in step f) or 29aor 29b of step c) with sulphonly chloride, alkly or aryl carboxylic acid or alkyl halide or aldehyde or reacting compound 33 of step c) with alkyl halide or benzoic acid to obtain the compound of formula 1.

5. The process as claimed in claim 4, further comprises reacting compound 27 of step f) with alkyl halide or acid chloride to obtain compound of formula 1 or compound 31.

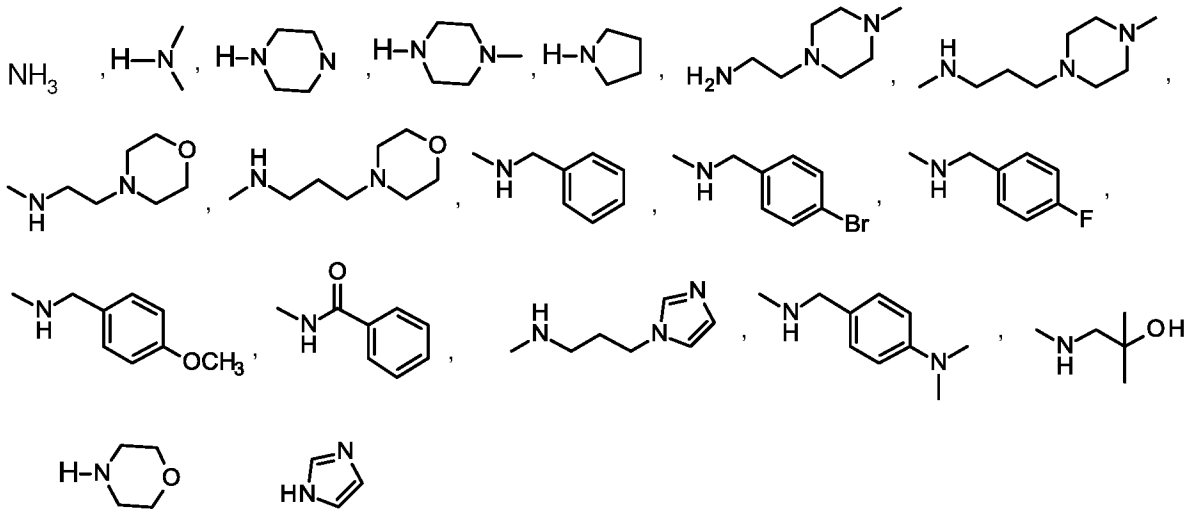
6. The process as claimed in claim 5, wherein compound 31 further reacted with hydrogen in presence of Pd/C to obtain compound of formula 1.

7. The process as claimed in claim 4, further comprising(i) reacting compound 6 with N-cyclopentylpiperazine or N-methyl piperazine followed by dimethyl amine to obtain an interemediate

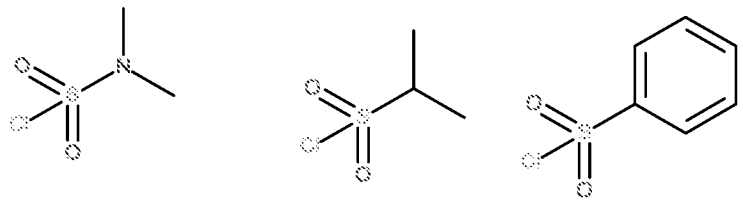
(ii) reacting the intermediate with hydrogen in presence of Pd/C to obtain compound 22 or 9b or 9c; and

(iii)reacting compound 22, 9b or 9c with 1-(3-chloropropyl)pyrrolidine or bromaamine or 4-hydroxy amine to obtain compound of formula 1.

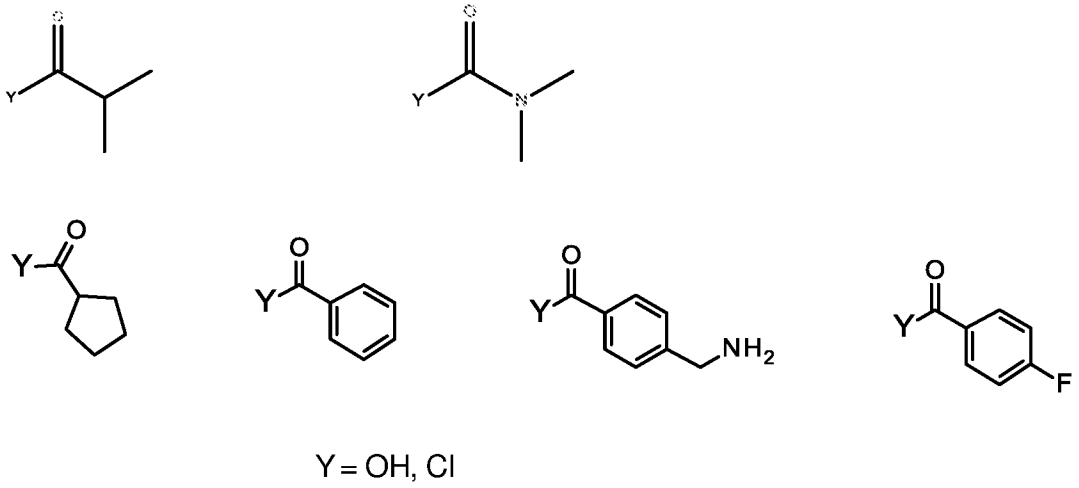
8. The process as claimed in claim 4, wherein amine used in step b) r step e) is selected from the group consisting of,



9. The process as claimed in claim 4, wherein the sulphonyl chloride is selected from the group consisting of,

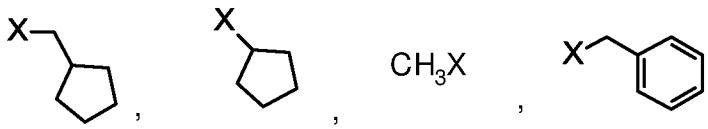


10. The process as claimed in claim 4, wherein the alkyl or aryl carboxylic acid or acid chloride is selected from the group consisting of,



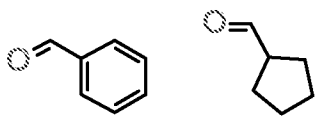
Y = OH, Cl

11. The process as claimed in claim 4, wherein the alkyl halide is selected from the group consisting of,



X= halogen

12. The process as claimed in claim 4, wherein the alkyl o aryl aldehyde is selected from the group consisting of,



13. The compound as claimed in claim 1, wherein the compound is free form or in acceptable salt form.

14. The compound as claimed in claim 1, wherein the compound iscapable of inhibiting immune stimulation mediated through toll-like receptor 9 (TLR9) signalling.

15. A method of affecting toll-like receptor mediated signalling in response to a toll-like receptor ligand using compound of formula (I).

16. The method according claim 16, wherein the method involves detecting toll-like receptor 9 antagonism of effective amount of a compound of Formula (I) using a reporter cell line that reports nuclear factor kappa B expression downstream of toll-like receptor 9 (TLR9) signalling.

17. The compound of formula (I) as claimed in claim 1,wherein compound of formula I affect immune stimulation via interaction with a toll-like receptor 9 (TLR9).

18. The compound of formula (I) as claimed in claim 1,wherein the compound of formula I inhibit immune stimulation via toll-like receptor 9 (TLR9) antagonism.

19. A pharmaceutical composition comprising compound of formula 1.

20. The composition as claimed in claim 19,wherein the composition for use in treating conditions involving unwanted immune activity due to toll-like receptor 9 (TLR9) mediate signalling.

21. The compound as claimed in claim 1,wherein the compounds for use in treatingimmunodeficiency, inflammation, infection, sepsis, allergy, asthma, graft rejection, graft-versus host disease (GvHD) and cancer.

22. The compound as claimed in claim 1, wherein compound isforuseininhibiting an immune stimulatory nucleic acid associated response in a subject.

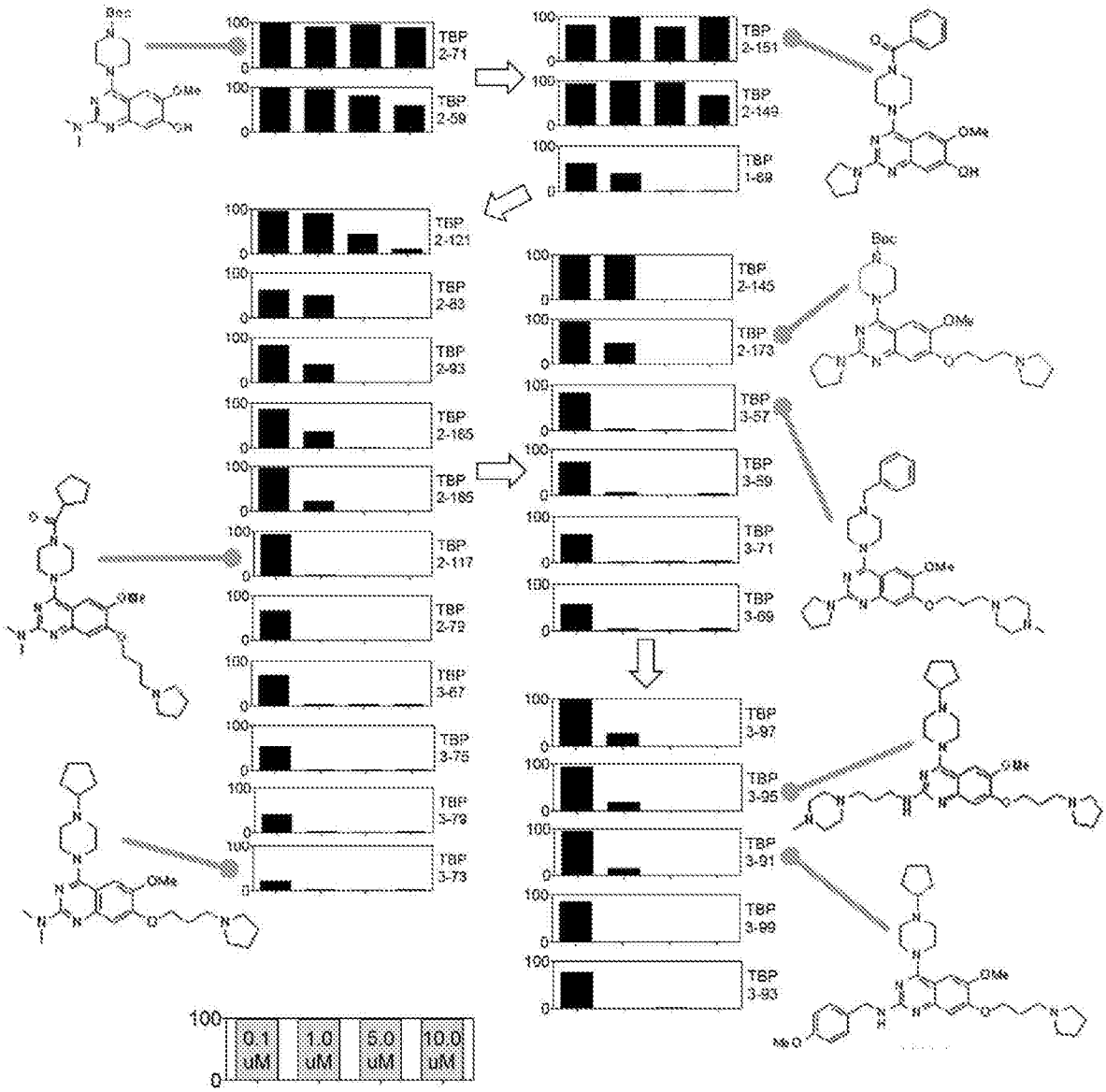

TBP 2-71
TBP 2-59
TBP 2-151
TBP 2-149
TBP 1-88
TBP 2-121
TBP 2-83
TBP 3-83
TBP 2-185
TBP 2-185
TBP 2-117
TBP 2-72
TBP 3-67
TBP 3-75
TBP 3-79
TBP 3-73
TBP 2-145
TBP 2-173
TBP 3-57
TBP 3-59
TBP 3-71
TBP 3-69
TBP 3-97
TBP 3-95
TBP 3-91
TBP 3-99
TBP 3-93
0.1 uM
1.0 uM
5.0 uM
10.0 uM

Fig. 1

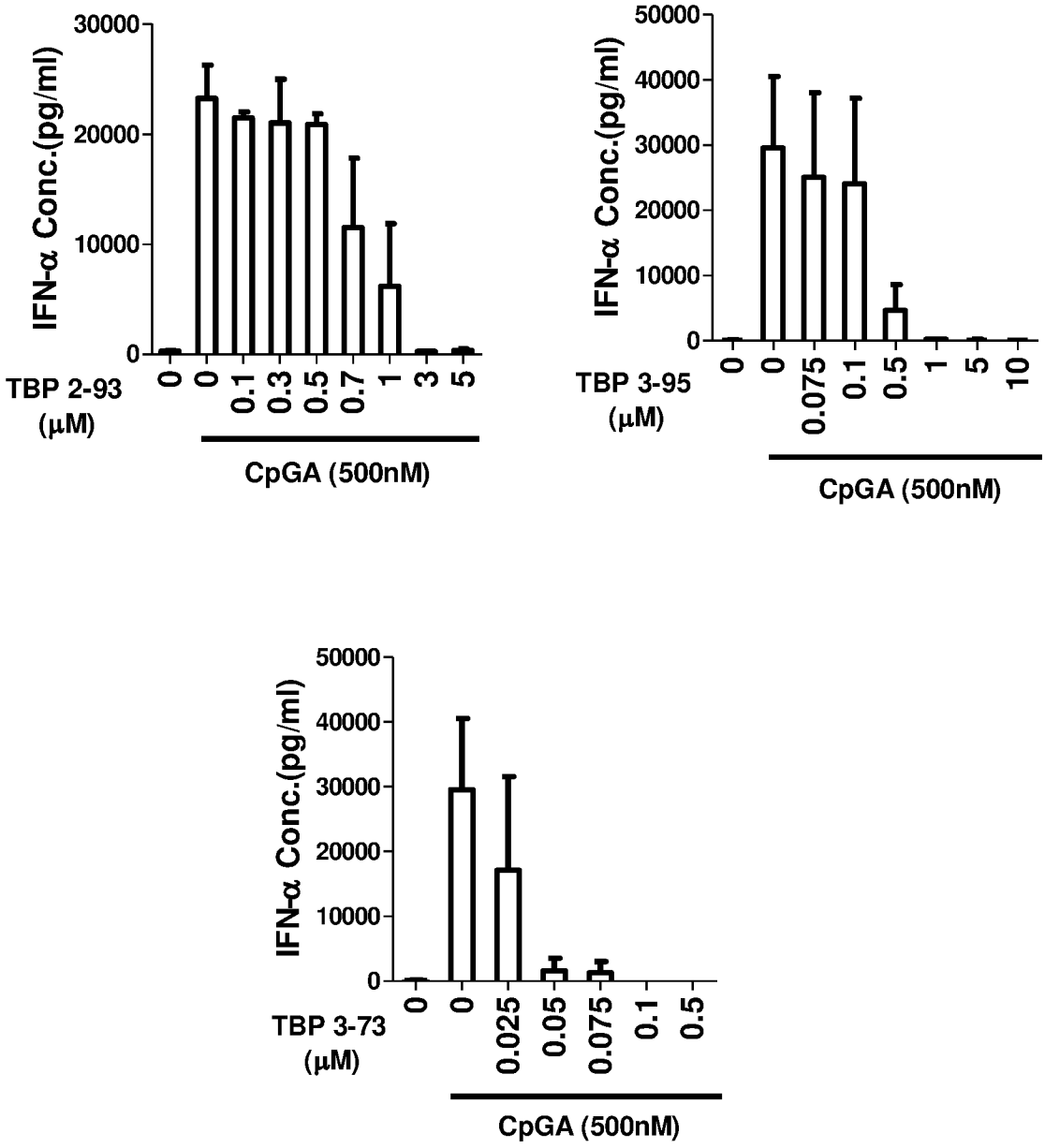

IFN-α Conc.(pg/ml)
30000
20000
10000
0
TBP 2-93
(μM)
0
0
0.1
0.3
0.5
0.7
1
3
5
CpGA (500nM)
IFN-α Conc.(pg/ml)
50000
40000
30000
20000
10000
0
TBP 3-95
(μM)
0
0
0.075
0.1
0.5
1
5
10
CpGA (500nM)
IFN-α Conc.(pg/ml)
50000
40000
30000
20000
10000
0
TBP 3-73
(μM)
0
0
0.025
0.05
0.075
0.1
0.5
CpGA (500nM)

Fig. 2

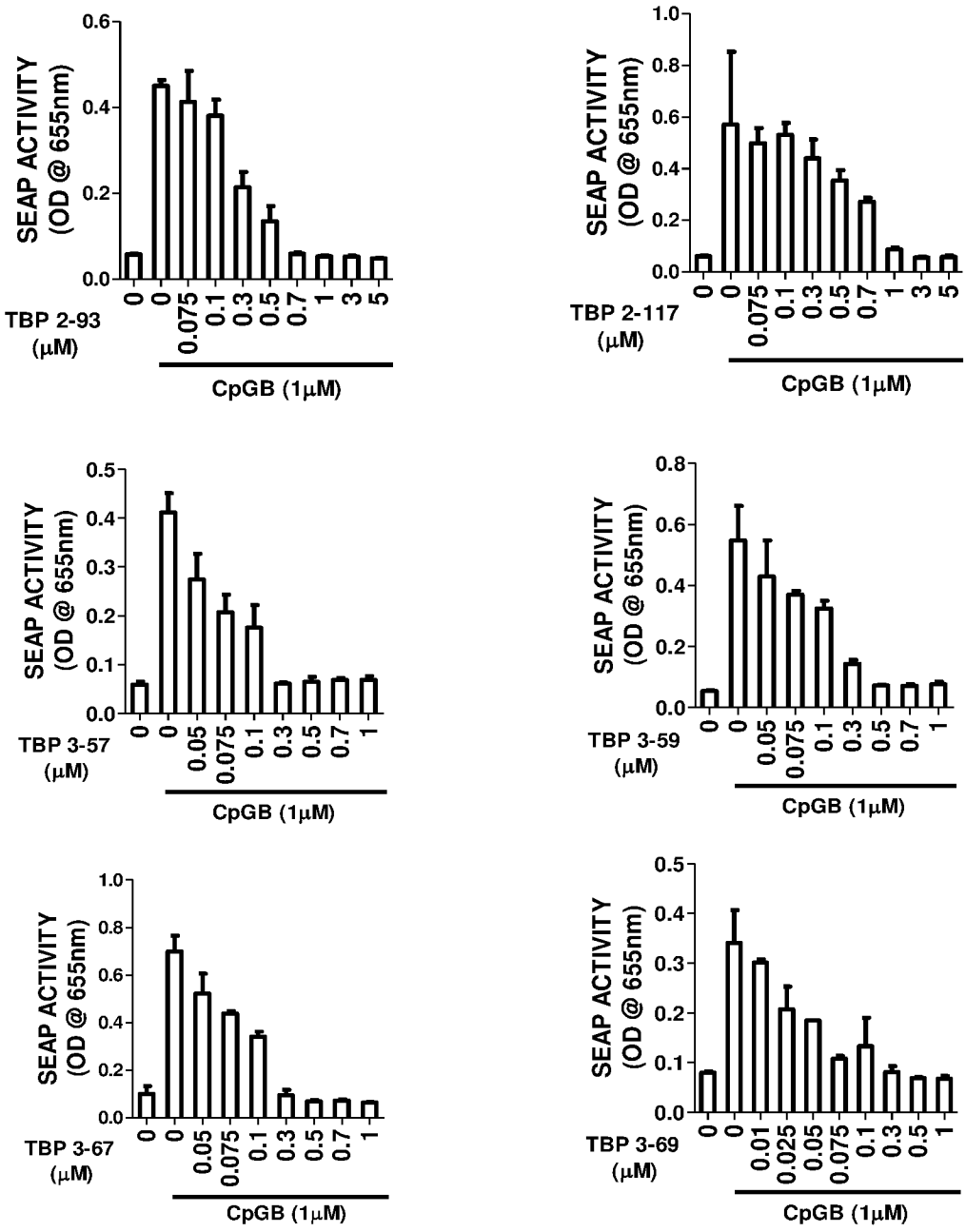

SEAP ACTIVITY (OD @ 655nm)
TBP 2-93 (μM)
0 0 0.075 0.1 0.3 0.5 0.7 1 3 5
CpGB (1μM)
SEAP ACTIVITY (OD @ 655nm)
TBP 2-117 (μM)
0 0 0.075 0.1 0.3 0.5 0.7 1 3 5
CpGB (1μM)
SEAP ACTIVITY (OD @ 655nm)
TBP 3-57 (μM)
0 0 0.05 0.075 0.1 0.3 0.5 0.7 1
CpGB (1μM)
SEAP ACTIVITY (OD @ 655nm)
TBP 3-59 (μM)
0 0 0.05 0.075 0.1 0.3 0.5 0.7 1
CpGB (1μM)
SEAP ACTIVITY (OD @ 655nm)
TBP 3-67 (μM)
0 0 0.05 0.075 0.1 0.3 0.5 0.7 1
CpGB (1μM)
SEAP ACTIVITY (OD @ 655nm)
TBP 3-69 (μM)
0 0 0.01 0.025 0.05 0.075 0.1 0.3 0.5 1
CpGB (1μM)

Fig.3A

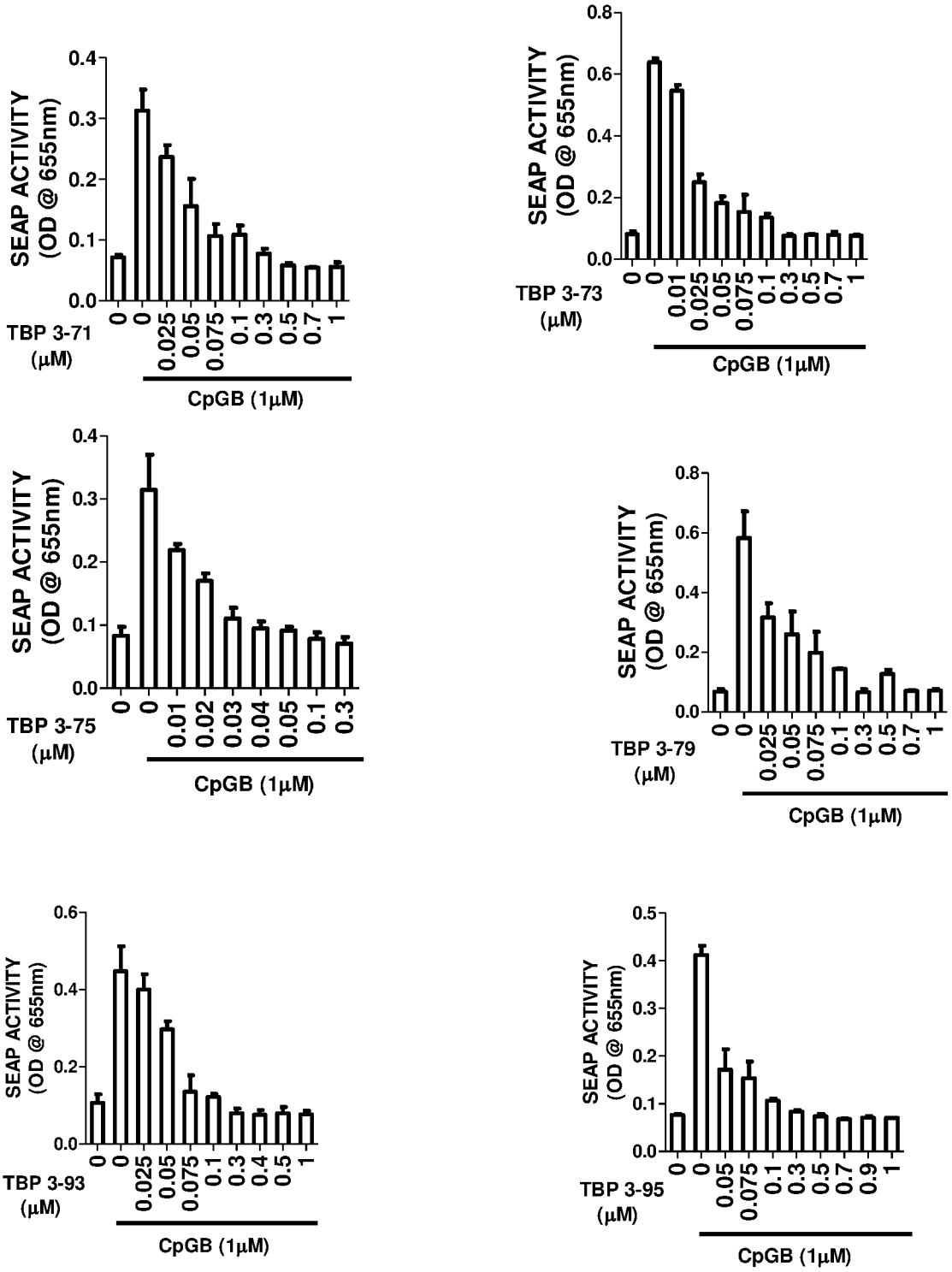

SEAP ACTIVITY (OD @ 655nm)
TBP 3-71 (μM)
0 0 0.025 0.05 0.075 0.1 0.3 0.5 0.7 1
CpGB (1μM)
SEAP ACTIVITY (OD @ 655nm)
TBP 3-73 (μM)
0 0 0.01 0.025 0.05 0.075 0.1 0.3 0.5 0.7 1
CpGB (1μM)
SEAP ACTIVITY (OD @ 655nm)
TBP 3-75 (μM)
0 0 0.01 0.02 0.03 0.04 0.05 0.1 0.3
CpGB (1μM)
SEAP ACTIVITY (OD @ 655nm)
TBP 3-79 (μM)
0 0 0.025 0.05 0.075 0.1 0.3 0.5 0.7 1
CpGB (1μM)
SEAP ACTIVITY (OD @ 655nm)
TBP 3-93 (μM)
0 0 0.025 0.05 0.075 0.1 0.3 0.4 0.5 1
CpGB (1μM)
SEAP ACTIVITY (OD @ 655nm)
TBP 3-95 (μM)
0 0 0.05 0.075 0.1 0.3 0.5 0.7 0.9 1
CpGB (1μM)

Fig. 3B

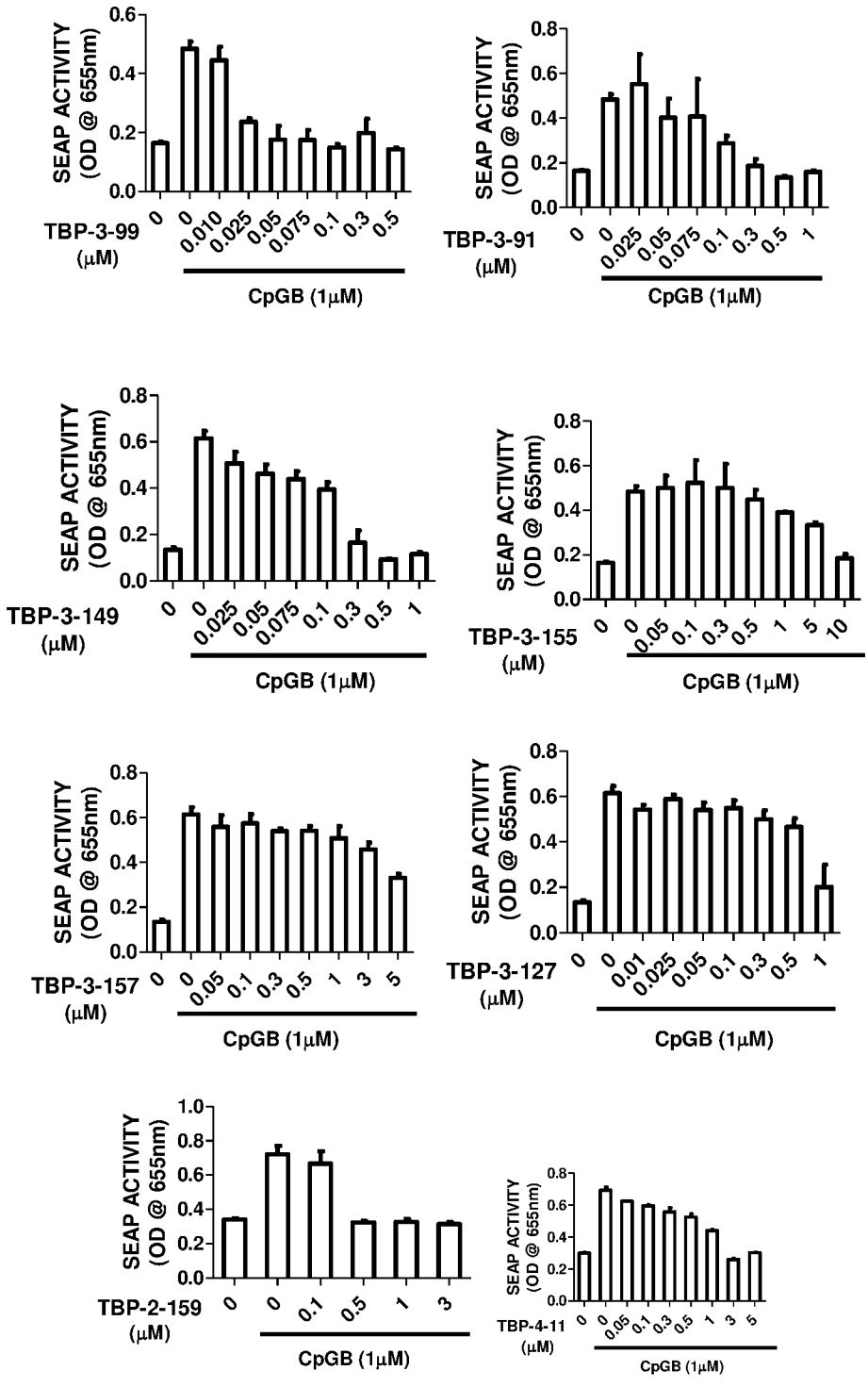

SEAP ACTIVITY (OD @ 655nm)
TBP-3-99 (μM)
0 0 0.010 0.025 0.05 0.075 0.1 0.3 0.5
CpGB (1μM)
TBP-3-91 (μM)
0 0 0.025 0.05 0.075 0.1 0.3 0.5 1
TBP-3-149 (μM)
0 0 0.025 0.05 0.075 0.1 0.3 0.5 1
TBP-3-155 (μM)
0 0 0.05 0.1 0.3 0.5 1 5 10
TBP-3-157 (μM)
0 0 0.05 0.1 0.3 0.5 1 3 5
TBP-3-127 (μM)
0 0 0.01 0.025 0.05 0.1 0.3 0.5 1
TBP-2-159 (μM)
0 0 0.1 0.5 1 3
TBP-4-11 (μM)
0 0 0.05 0.1 0.3 0.5 1 3 5

Fig. 3C

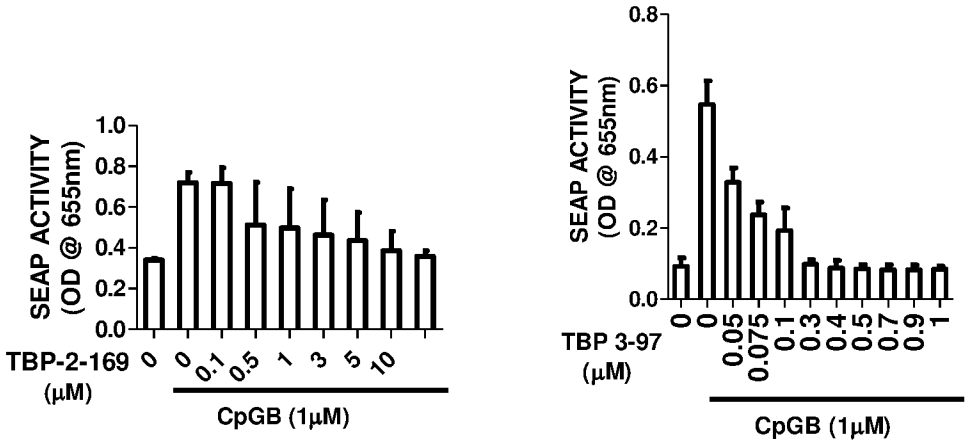

SEAP ACTIVITY
(OD @ 655nm)
1.0
0.8
0.6
0.4
0.2
0.0
TBP-2-169
(μM)
0 0 0.1 0.5 1 3 5 10
CpGB (1μM)
SEAP ACTIVITY
(OD @ 655nm)
0.8
0.6
0.4
0.2
0.0
TBP 3-97
(μM)
0 0 0.05 0.075 0.1 0.3 0.4 0.5 0.7 0.9 1
CpGB (1μM)

Fig. 3D

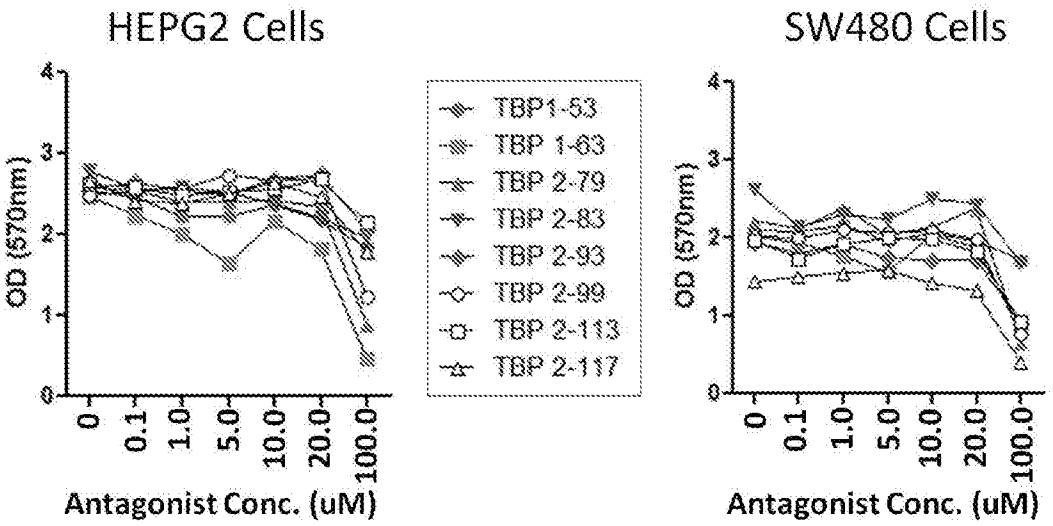

HEPG2 Cells
SW480 Cells
OD (570nm)
0 1 2 3 4
0 0.1 1.0 5.0 10.0 20.0 100.0
Antagonist Conc. (uM)
TBP1-53
TBP 1-63
TBP 2-79
TBP 2-83
TBP 2-93
TBP 2-99
TBP 2-113
TBP 2-117

Fig. 4

MOLECULE ID	IC_{50} (μM)
TBP 2-93 (13a)	0.2820
TBP-2-121 (13b)	3.398
TBP 2-117 (13c)	0.6292
TBP 3-79 (13d)	0.07428
TBP 3-73 (13e)	0.01924
TBP 3-75 (13f)	0.02222
TBP 3-67 (13g)	0.1057
TBP-3-113 (13h)	0.03668
TBP-2-173 (17a)	1.012
TBP-2-145 (17e)	3.001
TBP-3-69 (18b)	0.02544
TBP-2-185 (19)	0.6272
TBP-3-57 (20)	0.08773
TBP-3-59 (21a)	0.1295
TBP-3-71(21b)	0.04707
TBP-3-91 (25a)	0.09100
TBP-3-93 (25b)	0.04142
TBP-3-95 (25c)	0.0939
TBP-3-97 (25d)	0.1886
TBP-3-99 (25e)	0.0176
TBP-3-149 (27)	0.09421
TBP-4-11 (28a)	0.1007
TBP-3-155 (28b)	5.145
TBP-3-157 (28c)	3.450

Fig. 5A (table 2)

TBP-2-159 (29a)	0.1197
TBP-2-149 (30c)	11.38
TBP-3-135 (30a)	8.802
TBP-3-137 (30b)	20.24
TBP-2-151 (31)	20.00
TBP-3-123 (33)	1.121
TBP-3-145 (32b)	32.15
TBP-3-127 (34a)	0.5690

Fig. 5B (table 2)

INTERNATIONAL SEARCH REPORT

International application No
PCT/IN2017/050103

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D403/12 C07D401/12 C07D239/95 A61K31/5377 A61K31/517
A61P37/00 A61P35/00 A61P31/00 A61P29/00
ADD.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6 156 758 A (KUNG PEI-PEI [US] ET AL) 5 December 2000 (2000-12-05) the intermediate compound which is transformed into compound (52) in figure 1 or into compound (61) in figure 3 -----	1,13
Y	WO 2005/007672 A2 (COLEY PHARM GMBH [DE]; COLEY PHARM GROUP INC [US]; LIPFORD GRAYSON B [] 27 January 2005 (2005-01-27) cited in the application the whole document; in particular claims 125-142; p. 28 l. 30 - p. 31 l.3; abstract; examples 8, 24; CMZ 203-76; compounds 220, 229, 212 ----- -/--	1-22

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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Date of the actual completion of the international search	Date of mailing of the international search report
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2008/030455 A2 (COLEY PHARM GROUP INC [US]; LIPFORD GRAYSON B [US]; ZEPP CHARLES M [US) 13 March 2008 (2008-03-13) cited in the application in particular claims 173-178, 190 and 381-385; formula (XV)	1-22
Y	JP 2000 281660 A (SUMITOMO PHARMA) 10 October 2000 (2000-10-10) in particular formulae (6) and (1a); the claims; paragraph [0024]f; paragraph [0043] entries 7 and 9	1-22
Y	US 2009/099165 A1 (HURLEY LAURENCE H [US] ET AL) 16 April 2009 (2009-04-16) in particular the claims	1-22

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IN2017/050103

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 6156758 A	05-12-2000	NONE	
WO 2005007672 A2	27-01-2005	AU 2004257149 A1	27-01-2005
		BR PI0411514 A	01-08-2006
		CA 2528774 A1	27-01-2005
		EA 200600069 A1	25-08-2006
		EP 1635846 A2	22-03-2006
		JP 2007524615 A	30-08-2007
		KR 20060016817 A	22-02-2006
		MX PA05013922 A	24-02-2006
		US 2005119273 A1	02-06-2005
		US 2007232622 A1	04-10-2007
		WO 2005007672 A2	27-01-2005
WO 2008030455 A2	13-03-2008	AU 2007293363 A1	13-03-2008
		CA 2665819 A1	13-03-2008
		EP 2081924 A2	29-07-2009
		US 2008059317 A1	06-03-2008
		US 2010160314 A1	24-06-2010
		WO 2008030455 A2	13-03-2008
JP 2000281660 A	10-10-2000	NONE	
US 2009099165 A1	16-04-2009	US 2009099165 A1	16-04-2009
		US 2011212929 A1	01-09-2011
		US 2013302303 A1	14-11-2013