European Journal of Medicinal Chemistry 45 (2010) 1739–1745

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and theoretical studies on energetics of novel N- and O- perfluoroalkyl triazole tagged thienopyrimidines – Their potential as adenosine receptor ligands

B. Sirisha [a], B. Narsaiah [a,*], T. Yakaiah [a], G. Gayatri [b], G. Narahari Sastry [b], M. Raghu Prasad [c], A. Raghuram Rao [d]

[a] Fluoroorganic Division, Indian Institute of Chemical Technology, Tarnaka, Hyderabad 500607, India
[b] Molecular Modeling Division, Indian Institute of Chemical Technology, Tarnaka, Hyderabad 500607, India
[c] Vishnu College of Pharmacy, Bhimavaram, India
[d] University Institute of Pharmaceutical Sciences and UGC-CAS, Punjab University, Chandigarh, India

ARTICLE INFO

Article history:
Received 24 September 2009
Received in revised form
1 December 2009
Accepted 31 December 2009
Available online 14 January 2010

Keywords:
Propargyl bromide
Thienopyrimidines
Perfluoroalkyl triazole
Sharpless conditions
Cycloaddition
Regioisomers

ABSTRACT

A series of novel N- and O- perfluoroalkyl triazole tagged thienopyrimidines **6a–c** and **7a–d** was synthesized in two steps from thienopyrimidin-4-ones **2** through O- and N-propargylated regioisomers **3a–i** and **4a–i** respectively. Compound **2** was reacted with propargyl bromide to form O- and N-propargylated regioisomers **3** and **4** in definite proportions. Each regioisomer was separated and independently subjected to [3 + 2] cycloaddition using perfluoroalkyl azides through Click reaction under Sharpless conditions and obtained exclusively *anti* product in each case. The formation of two regioisomers in the first step and single *anti* addition product in the next step could be explained based on computational studies carried out at B3LYP/6-31G(d) level of theory. Results of Fukui function indices at the reactive centers are in accordance with the observations. On evaluation of the synthesized molecules for their binding affinities towards adenosine receptors, 4**d** and 4**f** were found to be selective to A_1 over A_{2A} receptors.

© 2010 Elsevier Masson SAS. All rights reserved.

1. Introduction

The bioisosteric replacement of benzene ring with π-excess thiophene has been an established ligand-based drug design strategy to optimize various lead structures. Diverse functionalized thiophene derivatives were reported as adenosine receptor ligands for the past twenty years [1–3]. Thiophene fused pyrimidines called thienopyrimidines exhibit DHFR inhibitory [4] CDK4 inhibitory [5], anti-inflammatory [6] activities. Several quinazolines and thienopyrimidines were reported from our laboratory as antihistaminic [7] antibacterial [8] bronchodilatory [9], anti-inflammatory agents [10] and as adenosine receptor ligands [11].

Based on the importance and in continuation to our investigations, we report the synthesis of novel derivatives with thienopyrimidine nucleus. Since five-membered rings with more number of nitrogens like tetrazole, triazole and imidazole is found to be the best ranked substitutions in antagonizing a wide variety of macromolecular targets [12], we attempted to synthesize O, N- propargylated thienopyrimidines and 3/4-triazolyl thienopyrimidines. Such a non-classic isosteric modification of triazolo thienopyrimidines is done to evaluate them for adenosine binding activity. Perfluorinated alkyl chain was selected to increase the lipophilicity of molecules in order to cross the blood brain barrier. The synthesis of title compounds was performed using Cu(I)-catalyzed Huisgen [3 + 2] dipolar cycloaddition reaction between an organic azide and an alkyne commonly known as click chemistry. This reaction has several applications in chemistry, biology and materials science. It has enabled demanding bio-conjugations involving more number of steps and has been used in activity-based protein profiling (ABPP) of crude proteome homogenates for selective labeling of modified bacterial cell walls and in the synthesis of novel biologically active compounds [13–15]. Initially the synthesis of O-/N-propargylated thienopyrimidines was carried out and the variation in the product yield is correlated with the substituent at the 2nd position. Position and orientation of attack of reagent on the transition state of the staring material was predicted with the help of chemoinformatics to elucidate the observed synthetic trends. Further, existence of two reactive nitrogens in perfluoroalkyl azide in reaction with alkyne can lead to the formation of 1, 4 and 1, 5-disubstituted alkyl [1–3] triazoles through their *syn* and *anti* additions. But, formation of only 1,4-disubstituted triazole in considerable yields along with

* Corresponding author. Tel.: +91 40 27193630; fax: +91 40 27193185.
E-mail address: narsaiah_1984@yahoo.co.in (B. Narsaiah).

0223-5234/$ – see front matter © 2010 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2009.12.075

remaining amount of starting material in the experimental studies led us to use density functional theory to unravel the experimental observations. Molecular modeling studies were carried out and the reactants, products and transition states were optimized at B3LYP/6–31G* level of theory as B3LYP with 6–31G* basis set is proved to be better in modeling the reactions [16]. IRC calculations were performed to characterize the transition states. Fukui function indices were calculated using the following equations

$$f_k^+ = [q_k(N+1) - q_k(N)] \text{ for nucleophilic attack} \quad (1)$$

$$f_k^- = \left[q^k(N) - q_k(N-1)\right] \text{ for electrophilic attack} \quad (2)$$

$$f_k^\bullet = [q_k(N+1) - q_k(N-1)] \text{ for free radical attack} \quad (3)$$

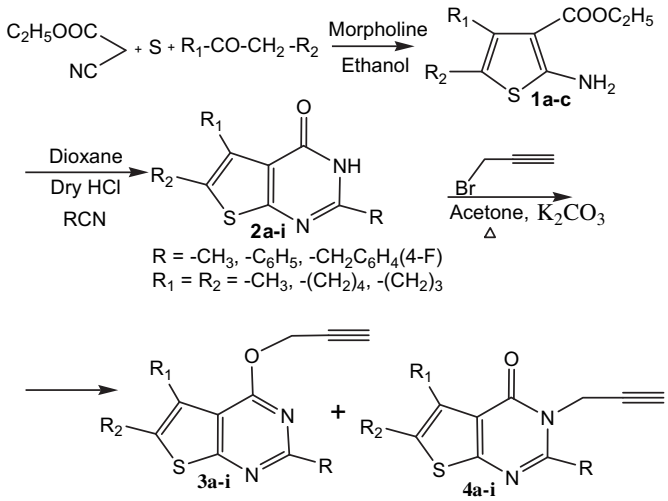
All the calculations were performed using G03W program [17]. The charge densities were obtained using AIM2000 program [18].

2. Results and discussion

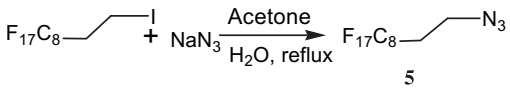
2.1. Chemistry

The initial step involves the synthesis of thiophene 2-amino-3-carboxylic acid ethyl ester **1** from cyanoethylacetate, ketone and sulfur in ethanol using morpholine as base [19]. Then preparation of thieno [2,3-*d*] pyrimidin-4(3H)-ones **2** from compound 1 and alkyl/aryl nitrile in dioxane using dry HCl [20]. The thieno [2,3-*d*] pyrimidin-4(3*H*)-ones **2** were reacted with propargyl bromide in acetone using potassium carbonate as a base to yield two products namely O- and N-propargylated thieno pyrimidines **3** and **4** in definite proportions [21] as outlined in Scheme 1. The formation of each regioisomer depends on the bulkiness/electron-withdrawing or donating nature of substituents present. The O-propargylated product **3** is formed in major when R is aromatic, while N-propargylated compound **4** is major when –R is aliphatic. In the second step, synthesis of various perfluoroalkyl azides **5** was performed as outlined in Scheme 2 [22]. All the products were separated through column chromatography and characterized based on their difference in polarity.

Further the O- and N-propargylated thienopyrimidines **3** and **4** were independently reacted with perfluoroalkyl azide **5** in THF, using copper (I) iodide as catalyst [23] and resulted in exclusively



Scheme 1. Synthesis of O- and N- propargylated thienopyrimidines **3** and **4.**



Scheme 2. Synthesis of perfluorinated alkylazides **5.**

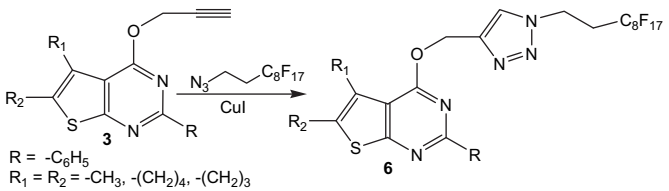
1,4-disubstituted-1,2,3-triazole derivatives **6** and **7**, respectively according to Schemes 3 and 4. The reaction is considered to take place via 1,3-dipolar cycloaddition of perfluoroalkyl azide to alkyne through a preformed copper acetylide complex formation [24,25]. The percentage yields of products tabulated in Tables 1 and 2. The cycloaddition reaction was also performed in DMSO in order to see the influence of solvent on the formation of products and found to give better yields.

2.2. Modeling studies

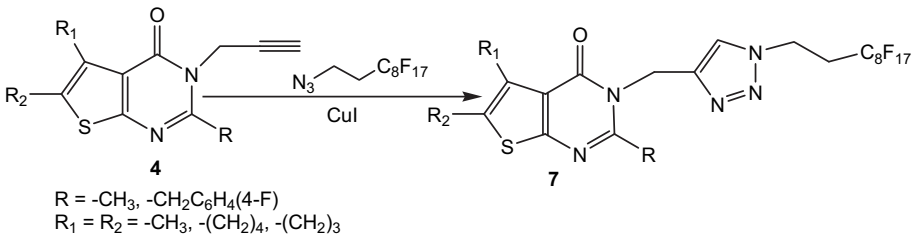
Molecular modeling studies were carried out in order to understand the observed synthetic trends. As the nature of substituent (–R_1 and –R_2 groups) seems to be inconsequential with respect to the observed yields of **3a–i** and **4a–i**, it was felt logical to replace them with H in all computational calculations.

From the experimental trends, the R group is mainly found to influence the site of attack of nucleophile which would result in formation of O- and N- propargylated thienopyrimidines **3** and **4** from thienopyrimidines **2**. Computational calculations were performed on thienopyrimidine **2** by taking R as H, –CH_3 or –C_6H_5, as representatives of unsubstituted, aliphatic (alkyl) and aromatic (aryl) groups respectively. Both gas phase and solvent calculations have been carried out on thienopyrimidine in order to examine the solvent dependency. The activation and reaction energies (in kcal/mol) for both gas and solvent phases are illustrated in Table 3. IRC calculations were performed to characterize the transition states which further confirmed the authenticity of transition states.

Initially the solvent effects on the reactivity were studied, followed by the comparison of reactivity of CH_3 and C_6H_5 substituted compounds with that of unsubstituted (H as substituent) compounds which is followed by the explanation for the site selective addition at N and O sites. From the data (Table 3) it can be understood that the addition of solvent decreases the activation energies and reaction energies (except for H substituted compounds). The H substituted compounds show increase in both activation and reaction energies compared to the CH_3 and C_6H_5 substituted compounds. Since variation in the yields seems to be mainly due to the aliphatic and aromatic groups, further studies were carried out based on the site selectivity at N and O positions. Also from the table, it can be inferred that the N-propargylated thienopyrimidine (**4**) with methyl substitution is found to be stable, both kinetically and thermodynamically. Replacement of –R with –C_6H_5 suggested the formation of kinetically stable O-propargylated thienopyrimidine and thermodynamically stable N-propargylated thienopyrimidines. However the difference between the activation energies for **2-A–Me** and **2-B–Me**, and **2-A–Ph** and **2-B–Ph** regioisomers in gas/solvent phases are 0.0/0.4 and 3.8/3.0



Scheme 3. Synthesis of O- perfluoroalkyl triazole tagged thienopyrimidines **6.**



Scheme 4. Synthesis of N- perfluoroalkyl triazole tagged thienopyrimidines **7.**

respectively. So, further studies are carried out in order to look at the regioselectivity. The next section discusses the AIM analysis which is followed by explaining the reactivity based on Fukui function indices. These studies give a better understanding about the factors responsible for the formation of a particular regioisomer.

The electronic charge densities (ρ) and hydrogen bond lengths (in Å) between 'N–H' and 'O–H' for **3** and **4** respectively are depicted in Fig. 1. The hydrogen bond lengths and charge density values for 'O–H' and 'N–H' bonds are considered in order to see whether the stabilizing factors in such cases can be attributed to hydrogen bonding. From the Fig. 1, it can be inferred that, the hydrogen bond 'N–H' stabilizes **2-A–Ph–TS** compared to **2-B–Ph–TS**, while the hydrogen bond between "O–H" stabilizes **2-B–Me–TS** compared to **2-A–Me–TS**.

The Fukui function indices for the reacting sites were calculated as they have been proved to be better in explaining the regioselectivity [26,27]. Fukui function indices are the local reactivity descriptors introduced by Parr and coworkers based on the frontier orbital concept given by Fukui [28–31]. Since the thienopyrimidine exists in lactim and lactam forms, the reactant was considered based on the product formation. In the case of O-propargylated, lactim form and N-propargylated product, lactam form was considered. Also as the thienopyrimidine acts as a nucleophile, f_k^- values on the reactive centers were considered. The reactants along with the Fukui function indices values are depicted in Fig. 2. From the figure, it can be observed that in the case of 'CH_3' substitution, the reactive site 'N' (0.112) is preferred to 'O' (0.106) while in the case of 'C_6H_5' substitution, 'O' is preferred (0.458) to 'N' (0.044). From the above computational studies, it can be clearly understood that when the –R is aromatic, O-propargylated product is feasible while when R group is aliphatic, N-propargylated product is feasible.

Table 1
Percentage yields of propargylated thienopyrimidines **3** and **4**.

Cmpd	R1	R2	R	% Yield
3a	$-(CH_2)_4-$		CH_3	8.5
4a	$-(CH_2)_4-$		CH_3	76.7
3b	$-(CH_2)_4-$		C_6H_5	88.18
4b	$-(CH_2)_4-$		C_6H_5	4.4
3c	$-(CH_2)_4-$		p-F–C_6H_4–CH_2	15.17
4c	$-(CH_2)_4-$		p-F–C_6H_4–CH_2	75.89
3d	CH_3	CH_3	CH_3	7.11
4d	CH_3	CH_3	CH_3	80.16
3e	CH_3	CH_3	C_6H_5	82.31
4e	CH_3	CH_3	C_6H_5	6.09
3f	CH_3	CH_3	p-F–C_6H_4–CH_2	4.0
4f	CH_3	CH_3	*p*-F–C_6H_4–CH_2	70.79
3g	$-(CH_2)_3-$		CH_3	7.6
4g	$-(CH_2)_3-$		CH_3	80.45
3h	$-(CH_2)_3-$		C_6H_5	92.02
4h	$-(CH_2)_3-$		C_6H_5	4.38
3i	$-(CH_2)_3-$		*p*-F–C_6H_4–CH_2	8.88
4i	$-(CH_2)_3-$		*p*-F–C_6H_4–CH_2	71.04

Further [3 + 2] cycloaddition reaction between N- and O-propargylated thienopyrimidines, and alkyl azides was analyzed. Theoretical studies on such molecules using Cu as catalyst along with the mechanism is well established [25]. The '$C_2H_4C_8F_{17}$' group on the azide nitrogen is replaced with 'CH_3' and the results obtained are illustrated in Table 3. The theoretical results clearly suggested the formation of *anti* product over *syn*, both kinetically and theromodynamically which were in accordance with experimental results. This is assumed to be mainly due to the steric repulsions arising from the bulky alkyl group attached to the azide.

2.3. Pharmacological studies

Representative compounds were selected and tested for their ability to displace [^{3}H] 2-chloro-N6-cyclohexyladenosine ([^{3}H]CCPA) from A_1ARs and [^{3}H]MSX-2 from A_{2A}AR in rat cortical and striatal membranes, respectively [11]. The A_1 and A_{2A} receptor binding affinities of compounds are expressed as K_i values of radioligand binding (at 1.0 μM concentration) and presented in Table 4.

All the compounds evaluated were found to bind with adenosine A_1 and A_{2A} receptors with moderate efficiency. The compounds **4d**, **4f**, **4h** were selectively bind to A_1 receptor. The compound **4a** was found to be potent in binding with A_1 and A_{2A} receptor but with lack of selectivity. The substitution at second position was found to influence the binding, where selectivity towards A_{2A} receptor was increased with aryl group (**4e**) and reduced with alkyl and aralkyl substitutions (**4d**, **4f**). Except **4d**, **4f**, **4h**, all other molecules evaluated were showed better binding profiles when compared to standard DPCPX.

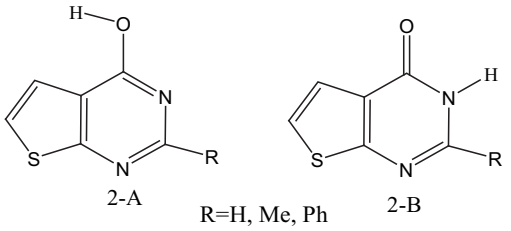
3. Conclusion

The formation of O- and N-propargylated thienopyrimidines in definite proportions was established based on substitution present in 2nd position. Further [3 + 2] cycloaddition of **3** or **4** with perfluorinated azides resulted in the exclusive formation of '*anti*' product. The formation of products was analyzed both kinetically and thermodynamically at B3LYP/6-31G* level of theory. Fukui function indices values and charge critical densities were in accordance with the observed experimental trends. Representative compounds were found to be selective for A_1 over A_{2A} receptors on

Table 2
Percentage yields of perfluoroalkyl triazole tagged thienopyrimidines **6** and **7**.

Cmpd	R1	R2	R	% Yield
6a	$-(CH_2)_4-$		C_6H_5	59.33
6b	CH_3	CH_3	C_6H_5	42.45
6c	$-(CH_2)_3-$		C_6H_5	52.93
7a	$-(CH_2)_4-$		CH_3	69.44
7b	CH_3	CH_3	CH_3	40.22
7c	CH_3	CH_3	*p*-F–C_6H_4–CH_2	45.00
7d	$-(CH_2)_3-$		CH_3	41.66

Table 3
Activation and reaction energies of the compounds studied. Values in parenthesis are obtained using PCM model, acetone is used as solvent.



	$\Delta E^{\ddagger}$ (K. cal)	ΔE_r (K. cal)
2-A–H	23.7(21.7)	−64.7 (−129.3)
2-A–Me	23.6 (5.5)	−63.49 (−20.4)
2-A–Ph	23.1 (6.1)	−63.09 (−19.9)
2-B–H	22.5(17.1)	−74.0 (−140.0)
2-B–Me	23.6(5.1)	−71.81 (−30. 6)
2-B–Ph	26.9(9.1)	−65.52 (−23.3)
3 + RN_3-syn	16.8	−71.1
3 + RN_3-anti	16.5	−73.3
4 + RN_3-syn	17.1	−70.7
4 + RN_3-anti	16.7	−75.9

evaluation of their adenosine binding affinities which offers a clue to design more potent ligands with improved selectivity.

4. Experimental section

4.1. General

Melting points were recorded on Casia-siamia (VMP-AM) melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer FT-IR 240-C spectro photometer using KBr optics. ^{1}H NMR spectra were recorded on Gemini varian 200 MHz, Bruker AV 300 MHz and Unity 400 MHz spectrometer in DMSO-d_6 or CDCl3 using TMS as an internal standard. Electron impact (EI) and chemical ionization mass spectra were recorded on

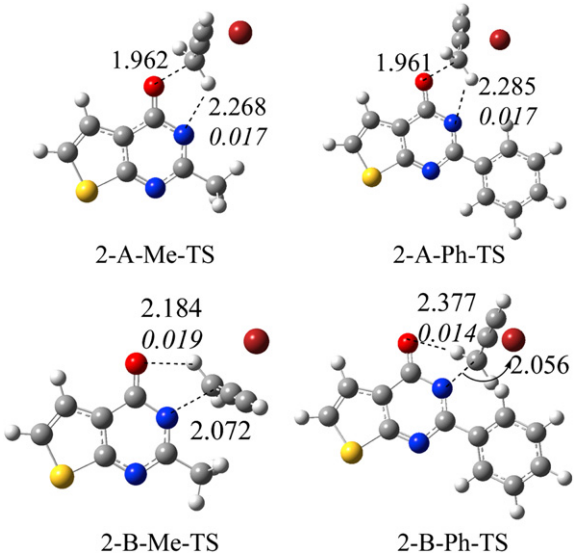


Fig. 1. Bond distances (normal) in Å and electronic charge densities in *amu* on the 'N···H' and 'O···H'; bonds for the alkynyl thienopyrimidine transition states Transition state is represented as 'TS'.

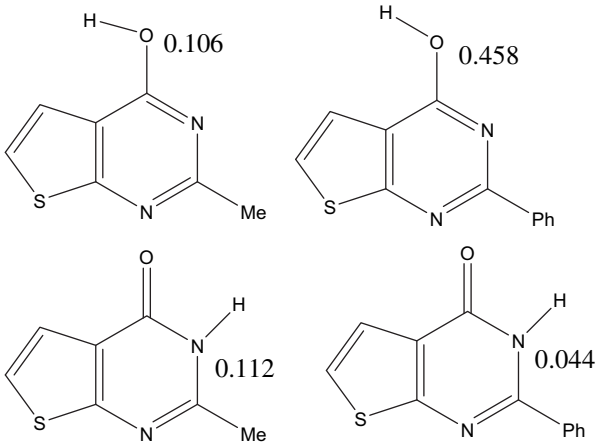


Fig. 2. Reactants along with their fukui function indices

a VG 7070 H instrument at 70 eV. All reactions were monitored by thin layer chromatography (TLC) on precoated silica gel 60 F254 (mesh); Spots were visualized with UV light. Merck silica gel (100–200 mesh) was used for chromatography. CHN analyses were recorded on a Vario EL analyser.

4.2. Synthesis

Preparation of 2-amino-4,5-substituted thiophene-3-carboxylic acid ethyl ester (**1**) [19]
Preparatin of 2, 5, 6 substituted thienopyrimidin-4-one (**2**) [20].
Preparation of propargylated thieno [2,3-*d*] pyrimidines (**3**) and thieno [2,3-*d*] pyrimidin-4(3H)ones (**4**).

4.2.1. General procedure

Compound **2** (3.0 mmol) and potassium carbonate (6.0 mmol) was taken in acetone (20 mL) and propargyl bromide (6.0 mmol) was added drop-wise. The reaction mixture was heated under reflux for 5 h, concentrated *in vacuo* and the residue was washed with *n*-hexane to remove excess propargyl bromide followed by treated with ice-cold water. The separated solid product was collected by filtration. The O- and N- propargylated products **3** and **4** were separated by column chromatography using *n*-hexane:ethylacetate.

4.2.2. 2-Methyl-4-(prop-2-yn-1-yloxy)-5,6,7,8-tetrahydro[1] benzothieno[2,3-d]pyrimidine (**3a**)

White solid, yield: 8.5% (65 mg), mp:135 °C, IR (KBr): 2116 (–C≡C–) cm^{-1}; ^{1}H NMR ($CDCl_3$): 1.86–1.96 (m, 4H, $2CH_2$), 2.42–2.46 (s, 1H, –C≡CH), 2.66 (s, 3H, CH_3), 2.78–2.86 (m, 2H, CH_2), 2.92–

Table 4
Radioligand binding data of the molecules with A_1 receptor (rat brain cortical membranes) and A_{2A} receptor (rat brain striaital membranes).

Cmpd	A_1[^{3}H]CCPA	A_{2A}[^{3}H] MSX-2
	K_i ± SEM [nM]	
3b	(−10 ± 4) (n = 4)	(3 ± 6) (n = 3)
3h	(−3 ± 7) (n = 3)	(−10 ± 9) (n = 4)
4a	(0 ± 3) (n = 4)	(7 ± 10) (n = 3)
4c	(21 ± 5) (n = 4)	(14 ± 6) (n = 3)
4d	(6 ± 4) (n = 4)	3790 ± 370 (n = 3)
4e	(−1 ± 2) (n = 3)	(29 ± 6) (n = 4)
4f	(4 ± 8) (n = 4)	7620 ± 2930 (n = 3)
4g	(6 ± 5) (n = 4)	(18 ± 13) (n = 3)
4h	(5 ± 3) (n = 4)	9700 ± 640 (n = 3)
7a	(−14 ± 7) (n = 4)	(−8 ± 3) (n = 4)
DPCPX	0.5 + 0.2	157 + 6.0

3.02 (m, 2H, CH_2), 5.10 (s, 2H, OCH_2); EIMS, *m/z*: 259 ($M^+ + 1$); Anal. Calcd. for $C_{14}H_{14}N_2OS$: C, 65.09; H, 5.46; N, 10.84%. Found: C, 64.84; H, 5.68; N, 11.02%.

*4.2.3. 2-Methyl-3-prop-2-yn-1-yl-5, 6, 7, 8-tetrahydro [1] benzothieno[2,3-d]pyrimidin-4(3H)-one (**4a**)*

White solid, yield: 76.7% (595 mg), mp: 195 °C; IR (KBr): 1679 (C═O), 2116 (–C≡C–) cm^{-1}; ^1H NMR ($CDCl_3$): 1.78–1.92(m, 4H, $2CH_2$), 2.22–2.26 (s, 1H, –C≡CH), 2.68(s, 3H, CH_3), 2.72–2.78(m, 2H, CH_2), 2.91–3.2 (m, 2H, CH_2), 4.85 (s, 2H, NCH_2); EIMS, *m/z*: 259 ($M^+ + 1$); Anal. Calcd. for $C_{14}H_{14}N_2OS$: C, 65.09; H, 5.46; N, 10.84%. Found: C, 65.27; H, 5.21; N, 11.06%.

*4.2.4. 2-Phenyl-4- (prop-2-yn-1-yloxy)-5,6,7,8-tetrahydro [1]benzothieno [2,3-d]pyrimidine (**3b**)*

White solid, yield: 88.18% (845 mg), mp:121 °C, IR (KBr): 2118 (–C≡C–) cm^{-1}; ^1H NMR ($CDCl_3$): 1.84–1.98 (m, 4H, $2CH_2$), 2.42–2.46 (s, 1H, –C≡CH), 2.82–2.90 (m, 2H, CH_2), 2.84–3.3 (m, 2H, CH_2), 5.24 (s, 2H, OCH_2), 7.40–7.49 (m, 3H, Ar–H), 8.41–8.49 (m, 2H, Ar–H); EIMS, *m/z*: 321 ($M^+ + 1$); Anal. Calcd. for $C_{19}H_{16}N_2OS$: C, 71.22; H, 5.03; N, 8.74%. Found: C, 71.39; H, 4.87; N, 8.99%.

*4.2.5. 2-Phenyl-3-prop-2-yn-1-yl-5,6,7,8-tetrahydro [1] benzothieno [2,3-d] pyrimidin-4(3H)-one (**4b**)*

White solid, yield: 4.4% (45 mg), mp:175 °C, IR (KBr):1672 (C═O), 2116 (–C≡C–) cm^{-1}; ^1H NMR ($CDCl_3$): 1.78–1.90 (m, 4H, $2CH_2$), 2.22–2.26 (s, 1H, –C≡CH), 2.72–2.78 (m, 2H, CH_2), 2.91–3.2 (m, 2H, CH_2), 4.74 (s, 2H, NCH_2), 7.51–7.59 (m, 3H, Ar–H), 7.72–7.78 (m, 2H, Ar–H); EIMS, *m/z*: 321 ($M^+ + 1$); Anal. Calcd. for $C_{19}H_{16}N_2OS$: C, 71.22; H, 5.03; N, 8.74%. Found: C, 71.05; H, 4.73; N, 9.0%.

*4.2.6. 2-(4-Fluorobenzyl)-4-(prop-2-yn-1-yloxy)-5, 6, 7, 8-tetrahydro [1]benzothieno[2,3-d] pyrimidine (**3c**)*

White solid, yield: 15.17% (160 mg), mp: 146 °C, IR (KBr): 2116 (–C≡C–) cm^{-1}; ^1H NMR ($CDCl_3$): 1.32–1.38(m, 2H, CH_2), 1.38–1.41(s, 1H, –C≡CH), 1.81–1.93 (m, 4H, $2CH_2$), 2.78–2.85 (m, 2H, CH_2), 4.10 (s, 2H, CH_2), 5.04 (s, 2H, OCH_2), 6.85–6.98 (m, 2H, Ar–H), 7.22 (s, 1H, Ar–H), 7.28–7.36 (m, 1H, Ar–H); EIMS, *m/z*: 353 ($M^+ + 1$); Anal. Calcd. for $C_{20}H_{17}FN_2OS$: C, 68.16; H, 4.86; N, 7.85%. Found: C, 68.38; H, 5.04; N, 7.7%.

*4.2.7. 2-(4-Fluorobenzyl)-3-prop-2-yn-1-yl-5,6,7, 8-tetrahydro[1]benzothieno [2,3-d] pyrimidin -4(3H) – one (**4c**)*

White solid, yield: 75.89% (800 mg), mp: 167 °C, IR (KBr): 1620 (C═O), 2116 (–C≡C–) cm^{-1}; ^1H NMR ($CDCl_3$): 1.78–1.92 (m, 4H, $2CH_2$), 2.21–2.28 (s, 1H, –C≡CH), 2.72–2.86 (m, 2H, CH_2), 2.91–3.06 (m, 2H, CH_2), 4.30 (s, 2H, CH_2), 4.76 (s, 2H, NCH_2), 6.92–7.08 (m, 2H, Ar–H), 7.18–7.31 (m, 2H, Ar–H); EIMS, *m/z*: 353 ($M^+ + 1$); Anal. Calcd. for $C_{20}H_{17}FN_2OS$: C, 68.16; H, 4.86; N, 7.85%. Found: C, 68.05; H, 5.11; N, 8.12%.

*4.2.8. 2,5,6-Trimethyl-4- (prop-2-yn-1-yloxy)thieno[2,3-d]pyrimidine (**3d**)*

White solid, yield: 7.11% (50 mg), mp: 129 °C, IR(KBr):2116 (–C≡C–) cm^{-1}; ^1H NMR ($CDCl_3$): 2.38–2.48 (m, 7H, –C≡CH, $2CH_3$), 2.62 (s, 3H, CH_3), 5.10 (s, 2H, OCH_2); EIMS, *m/z*: 233 ($M^+ + 1$); Anal. Calcd. for $C_{12}H_{12}N_2OS$: C, 62.04; H, 5.21; N, 12.06%. Found: C, 62.3; H, 5.04; N, 11.76%.

*4.2.9. 2,5,6-Trimethyl-3-prop-2-yn-1-ylthieno[2,3-d]pyrimidin-4(3H)-one (**4d**)*

White solid, yield: 80.16% (560 mg), mp: 153 °C, IR (KBr): 1620 (C═O), 2116 (–C≡C–) cm^{-1}; ^1H NMR ($CDCl_3$): 2.21–2.26 (s, 1H, –C≡CH), 2.36 (s, 3H, CH_3), 2.44 (s, 3H, CH_3), 2.68 (s, 3H, CH_3), 4.86 (s, 2H, NCH_2); EIMS, *m/z*: 233($M^+ + 1$); Anal. Calcd. for $C_{12}H_{12}N_2OS$: C, 62.04; H, 5.21; N, 12.06%. Found: C, 61.88; H, 5.03; N, 12.24%.

*4.2.10. 5,6-Dimethyl-2-phenyl-4-(prop-2-yn-1-yloxy) thieno [2,3-d]pyrimidine (**3e**)*

White solid, yield: 82.31% (720 mg), mp: 153 °C, IR(KBr): 2119 (–C≡C–) cm^{-1}; ^1H NMR ($CDCl_3$): 2.42–2.51 (m, 7H, –C≡CH, $2CH_3$), 5.24 (m, 2H, OCH_2), 7.38–7.47 (m, 3H, Ar–H), 8.42–8.48 (m, 2H, Ar–H); EIMS, *m/z*: 295 ($M^+ + 1$); Anal. Calcd. for $C_{17}H_{14}N_2OS$: C, 69.36; H, 4.7; N, 9.52%. Found: C, 69.54; H, 5.01; N, 9.27%.

*4.2.11. 5, 6-Dimethyl-2-phenyl-3-prop-2-yn-1-ylthieno [2, 3-d] pyrimidin-4 (3H)-one (**4e**)*

White solid, yield: 6.1% (55 mg), m.p. 185 °C, IR (KBr):1643 (C═O), 2116 (–C≡C–) cm^{-1}; ^1H NMR ($CDCl_3$): 2.31–2.36 (s, 1H, –C≡CH), 2.46 (s, 3H, CH_3), 2.56 (s, 3H, CH_3), 4.68 (m, 2H, NCH_2), 7.51–7.59 (m, 3H, Ar–H), 7.72–7.78 (m, 2H, Ar–H); EIMS, *m/z*: 295 ($M^+ + 1$); Anal. Calcd. for $C_{17}H_{14}N_2OS$: C, 69.36; H, 4.79; N, 9.52%. Found: C, 69.20; H, 5.02; N, 9.68%.

*4.2.12. 2-(4-Fluorobenzyl) -5,6-dimethyl-4- (prop-2-yn-1-yloxy) thieno [2,3-d] pyrimidine (**3f**)*

White solid, yield: 4% (30 mg), mp: 105 °C, IR (KBr): 2119 (–C≡C–) cm^{-1}; ^1H NMR ($CDCl_3$):), 2.44–2.56 (m, 7H, -C≡CH, $2CH_3$), 4.10 (s, 2H, CH_2), 5.34 (s, 2H, OCH_2), 7.02 (m, 2H, Ar–H), 7.28 (m, 2H, Ar–H) EIMS, *m/z*: 327 ($M^+ + 1$); Anal. Calcd. for $C_{18}H_{15}FN_2OS$: C, 66.24; H, 4.63; N, (8.58) %. Found: C, 66.49; H, 4.47; N, 8.75%.

*4.2.13. 2-(4-Fluorobenzyl)-5,6-dimethyl-3-prop-2-yn-1-ylthieno[2,3-d]pyrimi din-4(3H)-one (**4f**)*

White solid, yield: 70.8% (695 mg), mp: 128 °C, IR (KBr):1670 (C═O), 2116 (–C≡C–) cm^{-1}; ^1H NMR ($CDCl_3$): 2.28–2.32 (s, 1H, –C≡CH), 2.42 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 4.38 (s, 2H, CH_2), 4.75 (s, 2H, NCH_2), 7.02–7.10 (m, 2H, Ar–H), 7.25–7.35 (m, 2H, Ar–H); EIMS, *m/z*: 327 ($M^+ + 1$); Anal. Calcd. for $C_{18}H_{15}FN_2OS$: C, 66.24; H, 4.63; N, 8.58%. Found: C, 65.99; H, 4.85; N, 8.76%.

*4.2.14. 2-Methyl-4-(prop-2-yn-1-yloxy)-6,7-dihydro-5H-cyclopenta[4,5]thieno[2,3-d] pyrimidine (**3g**)*

White solid, yield: 7.6% (55 mg), mp: 118 °C, IR (KBr): 2116 (–C≡C–) cm^{-1};^1H NMR ($CDCl_3$): 2.40–2.44 (s, 1H, –C≡CH), 2.46–2.51 (m, 2H, CH_2), 2.66(s, 3H, CH_3), 2.98–3.08 (m, 4H, $2CH_2$), 5.12 (s, 2H, OCH_2); EIMS, *m/z*: 245 ($M^+ + 1$); Anal. Calcd. for $C_{13}H_{12}N_2OS$: C, 63.91; H, 4.95; N, 11.47%. Found: C, 64.14; H, 4.79; N, 11.68%.

*4.2.15. 2-Methyl-3-prop-2-yn-1-yl-3,5,6,7-tetrahydro-4H-cyclopenta[4,5]thieno[2,3-d]pyrimidin-4-one (**4g**)*

White solid, yield: 84.45% (620 mg), mp: 210 °C, IR (KBr):1670 (C═O), 2116 (–C≡C–) cm^{-1}; ^1H NMR ($CDCl_3$): 2.28–2.32 (s, 1H, –C≡CH), 2.42–2.56 (m, 2H, CH_2), 2.47 (s, 2H, CH_2), 2.72 (s, 3H, CH_3), 2.78–2.98 (m, 2H, CH_2), 4.88 (s, 2H, NCH_2); EIMS, *m/z*: 245 ($M^+ + 1$); Anal. Calcd. for $C_{13}H_{12}N_2OS$: C, 63.91; H, 4.95; N, 11.47%. Found: C, 63.75; H, 5.18; N, 11.65%.

*4.2.16. 2-Phenyl-4-(prop-2-yn-1-yloxy)-6,7-dihydro-5H-cyclopenta[4,5]thieno[2,3-d]pyrimidine (**3h**)*

White solid, yield: 92.02% (845 mg), mp 159 °C, IR (KBr):2116 (–C≡C–); ^1H NMR ($CDCl_3$): 2.44–2.46 (s, 1H, –C≡CH), 2.46–2.52 (m, 2H, CH_2), 2.98–3.11 (m, 4H, $2CH_2$), 5.25 (s, 2H, OCH_2), 7.38–7.48 (m, 3H, Ar–H), 8.43–8.52 (m, 2H, Ar–H); EIMS, *m/z*: 307 ($M^+ + 1$); Anal. Calcd. for $C_{18}H_{14}N_2OS$: C, 70.56; H, 4.61; N, 9.14%. Found: C, 70.26; H, 4.87; N, 8.97%.

*4.2.17. 2-Phenyl-3-prop-2-yn-1-yl-3,5,6,7-tetrahydro-4H-cyclopenta[4,5]thieno[2,3-d]pyrimidine-4-one (**4h**)*

White solid, yield: 4.38% (40 mg), mp: 205 °C, IR (KBr): 1672 (C═O), 2116 (–C≡C–); ^{1}H NMR ($CDCl_3$): 2.28–2.33 (s, 1H, –C≡CH), 2.47–2.58 (m, 2H, CH_2), 2.82–3.0 (m, 2H, CH_2), 3.08–3.18 (m, 2H, CH_2) 4.68 (s, 2H, NCH_2), 7.49–7.56 (m, 3H, Ar–H), 7.69–7.77 (m, 2H, Ar–H); EIMS, *m/z*: 307 ($M^+ + 1$); Anal. Calcd. for $C_{18}H_{14}N_2OS$: C, 70.56; H, 4.61; N, 9.14%. Found: C, 70.74; H, 4.36; N, 9.36%.

*4.2.18. 2-(4-Fluorobenzyl)-4-(prop-2-yn-1-yloxy)-6,7-dihydro-5H-cyclopenta[4,5]thieno[2,3-d] pyrimidine (**3i**)*

White solid, yield: 8.88%, (90 mg), mp: 120 °C, IR (KBr):2119 (–C≡C–) cm^{-1}; ^{1}H NMR ($CDCl_3$): 2.30–2.32 (s, 1H, –C≡CH), 2.50–2.54 (m, 2H, CH_2), 2.80–3.0 (m, 2H, CH_2), 3.06–3.14(m, 2H, CH_2) 4.10(s, 2H, CH_2), 5.4(s, 2H, OCH_2), 7.04–7.2(m, 2H, Ar–H), 7.28 (m, 2H, Ar–H); EIMS, *m/z*: 339 ($M^+ + 1$); Anal. Calcd. for $C_{19}H_{15}FN_2OS$: C, 67.44; H, 4.47; N, 8.28%. Found: C, 67.16; H, 4.72; N, 8.46%.

*4.2.19. 2-(4-Fluorobenzyl)-3-prop-2-yn-1-yl-3,5,6,7-tetrahydro-4H-cyclopenta [4,5] thieno [2,3-d] pyrimidin-4-one (**4i**)*

White solid, yield: 71.04% (720mg), mp: 140 °C, IR (KBr):1670 (C═O), 2116 (–C≡C–) cm^{-1}; ^{1}H NMR ($CDCl_3$): 2.28–2.32 (s, 1H, –C≡CH), 2.50–2.56(m, 2H, CH_2), 2.82–3.0(m, 2H, CH_2), 3.06–3.14(m, 2H, CH_2), 4.10(s, 2H, CH_2), 4.52(s, 2H, NCH_2), 7.04–7.2(m, 2H, Ar–H), 7.28 (m, 2H, Ar–H); EIMS, *m/z*: 339 ($M^+ + 1$); Anal. Calcd. for $C_{19}H_{15}FN_2OS$: C, 67.44; H, 4.47; N, 8.28%. Found: C, 67.27; H, 4.65; N, 8.12%.

Preparation of 10-azido-1,1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8-heptadeca fluorodecane (**5**) [22]

Preparation of 1H–1,2,3-triazol-4-yl thieno[2,3-*d*]pyrimidines (**6**) and 1H-1,2,3-triazol-4-yl thieno[2,3-*d*]pyrimidin-4(3H) ones (**7**).

4.2.20. General procedure

The propargylated thieno [2,3-*d*] pyrimidines **3** or thieno [2,3-*d*] pyrimidin-4-ones **4** (0.001 mol) and copper (I) iodide (0.001 mol) was taken in dry THF (5 mL) and cooled to 0 °C. Perfluoroalkylazide **5** (0.0015 mol) in THF (3 mL) was added dropwise to the reaction mixture over period of 20 min under stirring and continued for 12 h at room temperature. The reaction mixture was concentrated *in vacuo* and poured into the crushed ice. The crude triazolyl thienopyrimidine product was collected by extraction with ethyl acetate. The product was purified by passing through a column packed with silica gel using n-hexane and ethylacetate as eluents.

*4.2.21. 2-Phenyl-4{1-(3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10, 10-heptadecafluorodecyl)-1H-1,2,3-tria zol-4-yl)methyl}oxy)-5,6,7,8-tetrahydro[1] benzothieno[2,3-d]pyrimidine (**6a**)*

White solid, yield: 59.33% (479 mg); mp: 160 °C; IR (KBr): 1569 (C═N), 1508 (C═C), 1250 (C–O–C) cm^{-1}; ^{1}H NMR ($CDCl_3$): 1.85–1.98 (m, 4H, $2CH_2$), 2.80–2.86 (m, 4H, $2CH_2$), 2.92–3.02 (m, 2H, CH_2), 4.55–4.68 (t, 2H, NCH_2), 5.85 (s, 2H, OCH_2), 7.4–7.52 (m, 3H, Ar–H), 7.7(s, 1H, –C═CH), 8.48–8.55(m, 2H, Ar–H); EIMS, *m/z*: 810 ($M^+ + 1$); Anal. Calcd. for $C_{29}H_{20}F_{17}N_5OS$: C, 43.03; H, 2.49; N, 8.65%. Found: C, 43.19; H, 2.07; N, 8.88%.

*4.2.22. 2-Phenyl-4-{1-(3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10, 10- heptadeca fluorodecyl)-1H-1,2,3-triazol-4-yl)methyl}oxy)-5,6-dimethyl thieno[2,3-d] pyrimidine (**6b**)*

White solid, yield: 42.45% (332 mg), mp: 202 °C; IR (KBr): 1560 (C═N), 1490(C═C), 1260 (C–O–C) cm^{-1}; ^{1}H NMR ($CDCl_3$): 2.42–2.51 (m, 6H, $2CH_3$), 2.73–2.9 (m, 2H, CH_2), 4.60–4.65 (t, 2H, NCH_2), 5.82 (s, 2H, OCH_2), 7.40–7.50 (m, 3H, Ar–H), 7.65 (s, 1H, –C═CH), 8.45–8.52 (m, 2H, Ar–H); EIMS, *m/z*: 784 ($M^+ + 1$); Anal. Calcd. for $C_{27}H_{18}F_{17}N_5OS$: C, 41.39; H, 2.32; N, 8.94%. Found: C, 41.22; H, 2.16; N, 9.19%.

*4.2.23. 2-Phenyl-4{1-(3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10heptadeca fluoro decyl)-1H-1, 2, 3-triazol-4-yl)methyl}oxy)-6,7-dihydro-5H-cyclopenta[4,5] thieno[2,3-d]pyrimidine (**6c**)*

White solid, yield: 52.93% (420 mg), mp: 198.6 °C; IR (KBr): 1572 (C═N), 1501 (C═C), 1255 (C–O–C) cm^{-1}: ^{1}H NMR ($CDCl_3$): 2.47–2.58 (m, 2H, CH_2), 2.75–2.98(m, 2H, CH_2), 3.02–3.12 (m, 4H, $2CH_2$), 4.60–4.69(t, 2H, NCH_2), 5.85(s, 2H, OCH_2), 7.45–7.56 (m, 3H, Ar–H), 7.70(s, 1H, –C═CH), 8.52–8.58(m, 2H, Ar–H); EIMS, *m/z*: 796 ($M^+ + 1$); Anal. Calcd. for $C_{28}H_{18}F_{17}N_5OS$: C, 42.27; H, 2.28; N, 8.80%. Found: C, 42.45; H, 2.50; N, 8.55%.

*4.2.24. 2-Methyl-3-{1-(3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10, 10-heptadeca fluoro decyl)-1H-1,2,3-triazol-4-yl)methyl}-5,6,7, 8-tetrahydro [1]benzothieno [2,3-d]pyrimidin-4(3H)-one (**7a**)*

White solid, yield: 69.44% (518 mg), mp: 189 °C; IR(KBr):1620 (C═O), 1551 (C═N), 1456 (C═C) cm^{-1}; ^{1}H NMR ($CDCl_3$): 1.8–1.95 (m, 4H, $2CH_2$), 2.8 (s, 3H, CH_3), 2.85–2.90 (m, 4H, $2CH_2$), 2.95 (s, 2H, CH_2), 4.57–4.68 (m, 2H, NCH_2), 5.30 (s, 2H, NCH_2), 7.8(s, 1H, –C═CH); EIMS, *m/z*: 748 ($M^+ + 1$); Anal. Calcd. for $C_{24}H_{18}F_{17}N_5OS$: C, 38.56; H, 2.43; N, 9.37%. Found: C, 38.86; H, 2.26; N, 9.63%.

*4.2.25. 2,5,6-Trimethyl-3-{1-(3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10, 10- heptadecafluorodecyl)-1H-1,2,3-triazol-4-yl) methyl}thieno [2,3-d] pyrimidin-4(3H)one (**7b**)*

White solid, yield: 40.22% (289 mg), mp: 176 °C, IR (KBr): 1672 (C═O), 1545 (C═N), 1430 (C═) cm^{-1}; ^{1}H NMR ($CDCl_3$): 2.38 (s, 2H, CH_2), 2.46 (s, 3H, CH_3), 2.85 (s, 6H, $2CH_3$), 4.55–4.68 (m, 2H, NCH_2), 5.30 (s, 2H, NCH_2), 7.8(s, 1H, –C═CH); EIMS, *m/z*: 722 ($M^+ + 1$); Anal. Calcd. for $C_{22}H_{16}F_{17}N_5OS$: C, 36.63; H, 2.24; N, 9.71%. Found: C, 36.81; H, 1.99; N, 9.49%.

*4.2.26. 2-(4-Fluorobenzyl)-5,6-dimethyl-3-{1-(3,3,4,4,5,5,6,6,7,7,8,8, 9,9,10,10,10- heptadeca fluorodecyl)-1H-1,2,3-triazol-4-yl)methyl}-thieno [2,3-d] pyrimidin-4(3H)-one (**7c**)*

White solid, yield: 45% (366 mg), mp: 139 °C; IR (KBr): 1660 (C═O), 1530 (C═N), 1470 (C═C) cm^{-1}; ^{1}H NMR ($CDCl_3$): 2.40 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 2.72–2.9 (m, 2H, CH_2), 4.52 (s, 2H, CH_2), 4.65–4.7(t, 2H, NCH_2), 5.15 (s, 2H, NCH_2), 7.0–7.18 (m, 2H, Ar–H), 7.35–7.42 (m, 2H, Ar–H), 7.8 (s, 1H, –C═CH); EIMS, *m/z*: 816 ($M^+ + 1$); Anal. Calcd for $C_{28}H_{19}F_{18}N_5OS$: C, 41.24; H, 2.35; N, 8.59%. Found: C, 41.49; H, 2.19; N, 8.76%.

*4.2.27. 2-Methyl-3-{1-(3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10, 10-heptadecafluorodecyl)-1H-1,2,3-tria zol-4-yl)methyl}-3,5,6, 7-tetrahydro-4H-cyclopenta [4,5] thieno [2,3-d] pyrimidin-4-one (**7d**)*

White solid, yield: 41.66% (305 mg), mp: 186 °C, IR (KBr):1670 (C═O), 1543 (C═N), 1460 (C═C) cm^{-1}; ^{1}H NMR ($CDCl_3$): 2.50 (s, 3H, CH_3), 2.85–2.90 (m, 4H, $2CH_2$), 2.95 (s, 2H, CH_2), 3.0–3.12 (m, 2H, CH_2), 4.58–4.65 (t, 2H, NCH_2), 5.3 (s, 2H, NCH_2), 7.85 (s, 1H, –C═CH); EIMS, *m/z*: 734 ($M^+ + 1$); Anal. Calcd. for $C_{23}H_{16}F_{17}N_2OS$: C, 37.66; H, 2.20; N, 9.55%. Found: C, 37.45; H, 2.02; N, 9.73%.

4.3. Pharmacological studies

4.3.1. Radioligand binding studies

4.3.1.1. Materials. [^{3}H] CCPA was obtained from NEN Life Science (48.6 Ci/mmol) and [^{3}H] MSX-2 from Amersham (85 Ci/mmol).

4.3.1.2. Membrane preparations. Frozen rat brains were obtained from Pel Freez, Rogers, AR, USA. Rat brains were dissected to obtain cortical membrane preparations for A_1 and striatal membrane preparations for A_{2A} assay as described [11].

4.3.1.3. Radioligand binding assays. Binding assays for A_1 and A_{2A} were performed essentially as described in the literature. Stock solutions of the compounds were prepared in dimethyl sulphoxide (DMSO) and the final concentration of DMSO in the assay was 2.5%. The radioligand concentrations were [^{3}H] CCPA: 0.5 nM (rat A_1) and [^{3}H] MSX-2: 1.0 mM (rat A_{2A}).

Drug solution (50 μL) was added into a 96-well plate followed by 50 μL of radioligand solution and 100 μL (30 μg/vial) of protein suspension of rat brain cortex (A_1) or rat brain striatal membranes (A_{2A}) and incubated at room temperature for 90/30 min. CADO (2-chloroadenosine)/NECA [5′-(*N*-ethylcarbamoyl)adenosine] was used for nonspecific binding for A_1 and A_{2A} AR binding studies, respectively. Incubation was terminated by rapid filtration over Whatman GF/B filters using a Brandell cell harvester (Brandell, Gaithersburg, MD). To the filter plate, scintillation liquid was added (40 μl/vail – Microscent-20) and incubated at room temperature for 10 h and radioactivity was measured by a scintillation counter. Those compounds whose percentage inhibition was found to be more than 20% at a test concentration of 1 μM were taken and a full concentration – inhibition curve was determined.

4.3.2. Data analysis

Data were analyzed using Graph Pad PRISM version 3.0 (San Diego, CA). For nonlinear regression analysis, the Cheng–Prushoff equation and a K_D value of 0.5 nM for [^{3}H] CCPA at rat A_1ARs was used to calculate K_i values from EC_{50} values.

Acknowledgments

Authors wish to thank Dr. J.S. Yadav, Director, IICT, Hyderabad, India for his constant encouragement and CSIR, India for the research fellowships to two of them (GG and TY). We thankfully acknowledge the cooperation extended by Prof. Dr. Christa E. Mueller, University of Bonn, Germany for providing facilities and guidance for radioligand binding studies.

References

[1] A. Michiel van Rhee, S.M. Siddiqi, N. Melman, D. Shi, W.L. Padgett, J.W. Daly, K.A. Jacobson, J. Med. Chem. 39 (1996) 398–406.
[2] P.G. Baraldi, US005939432A, 1999.
[3] R. Romagnoli, P.G. Baraldi, A.R. Moorman, M.A. Iaconinoto, M.D. Carrion, C.L. Cara, M.A. Tabrizi, D. Preti, F. Fruttarolo, S.P. Baker, K. Varani, P.A. Borea, Bioorg. Med. Chem. Lett. 16 (2006) 5530–5533.
[4] I.O. Donkor, H. Li, S.F. Queener, Eur. J. Med. Chem. 38 (2003) 605–611.
[5] T. Horiuchi, J. Chiba, K. Uoto, T. Soga, Bioorg. Med. Chem. Lett. 19 (2009) 305–308.
[6] B.A. Abdel-rahman, A.R. El-Gazzarhoda, N. Hussein Hend, Hafez, Acta Pharm. 57 (2007) 395–411.
[7] B. Shireesha, K. Uma Shankar, A. Raghuram Rao, K.S. Rajan, M. Raghuprasad, Int. J. Pharma. Sci. Nanotechnol. 1 (2008) 136–143.
[8] M.R. Prasad, D.P. Kishore, Chem. Pharm. Bull. 55 (2007) 776–779.
[9] K.S. Rajan, M.R. Prasad, D. Harikrishna, M.C. Prabhakar, D. Rama Krishna, A.R. Rao, Open Med. Chem. J. 2 (2008) 101–111.
[10] B. Shireesha, B. Narsaiah, A. Raghuram Rao, S. Ramakrishna, Madhusudhana, P.V. Diwan, T. Yakaiah, Thienopyridines as pharmacologicaly active agents, Indian Patent application No. 10/DEL/2009 dated 5/01/2009.
[11] M. Raghu Prasad, A. Raghuram Rao, P. Shantan Rao, K.S. Rajan, S. Meena, K. Madhavi, Eur. J. Med. Chem. 43 (2007) 614–620.
[12] J.W. Lee, B. Ku Kim, S.H. Jin, Bull. Korean Chem. Soc. 26 (2005) 833–836.
[13] H.C. Kolb, M.G. Finn, K.B. Sharpless, Angew. Chem. Int. Ed. 40 (2001) 2004–2021.
[14] V.V. Rostovtsev, L.G. Green, V.V. Fokin, K.B. Sharpless, Angew. Chem. Int. Ed. 41 (2002) 2596–2599.
[15] H.C. Kolb, K.B. Sharpless, Drug Discov. Today 8 (2003) 1128–1137.
[16] J.K. Mishra, G. Panda, J.S. Rao, G.N. Sastry, Tetrahedron Lett. 47 (2006) 3357–3360.
[17] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, et al., Gaussian 03, Revision C.02. Gaussian, Inc., Wallingford CT,, 2004.
[18] F. Biegler-König, J. Schönbohm, AIM2000, version 2.0.
[19] K. Gewald, E. Schinke, H. Böttcher, Chem. Ber. 99 (1966) 94–100.
[20] M.R. Prasad, K.S. Rajan, K. Mogilaiah, P.S. Rao, A.R.R. Rao, Synthesis 14 (2001) 2119–2123.
[21] P. Mani Chandrika, T. Yakaiah, G. Gayatri, K. Pranay Kumar, B. Narsaiah, U.S.N. Murthy, A. Raghu Ram Rao, Eur. J. Med. Chem. 45 (2010) 78–84.
[22] A. Gheorghe, E. Cuevas-Yañez, J. Horn, W. Bannwarth, B. Narsaiah, O. Reiser, Syn. Lett. 17 (2006) 2767–2770.
[23] A. Gheorghe, A. Matsuno, O. Reiser, Adv. Synth. Catal. 348 (2006) 1016–1020.
[24] C.W. Tornoe, C. Christensen, M. Meldal, J. Org. Chem. 67 (2002) 3057–3064.
[25] F. Himo, T. Lovell, R. Hilgraf, V.V. Rostovtsev, L. Noodleman, K.B. Sharpless, V.V. Fokin, J. Am. Chem. Soc. 127 (2005) 210–216.
[26] G. Gayatri, G.N. Sastry, J. Chem. Sci. 117 (2005) 573–582.
[27] G. Gayatri, G.N. Sastry, J. Phys. Chem. A. 113 (2009) 12013–12021.
[28] K. Fukui, Science 218 (1982) 747–754.
[29] R.G. Parr, W. Yang, J. Am. Chem. Soc. 106 (1984) 4049–4050.
[30] W. Yangs, W.J. Mortier, J. Am. Chem. Soc. 108 (1986) 5708–5711.
[31] P. Geerlings, F.D. Proft, W. Langenaeker, Chem. Rev. 103 (2003) 1793–1874.